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(54) Title: MANGANESE-BASED CHELATE CONJUGATES FOR MOLECULAR MR IMAGING

(57) Abstract: Provided herein are examples of metal chelating ligands that have high affinity for manganese. The resultant metal complexes can be used as MRI contrast agents, and can be functionalized with moieties that bind to or cause relaxivity change in the presence of biochemical targets.

Manganese-Based Chelate Conjugates for Molecular MR Imaging

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 62/204,519, filed on August 13, 2015, and U.S. Provisional Application Serial No. 62/356,732, filed on June 30, 2016, the contents of which are herein incorporated by reference in their entireties.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under Grant No. R01 CA161221 awarded by the National Institutes of Health. The Government has certain rights in the invention.

TECHNICAL FIELD

This invention relates to metal chelating ligands, and more particularly to manganese chelate complexes of these ligands that can be used as contrast agents for magnetic resonance imaging.

BACKGROUND

Many the metal chelating ligands currently used in magnetic resonance (MR) are polyaminopolycarboxylate metal binding chelating ligands designed for chelation of lanthanide(III) ions like gadolinium(III). All commercially available MR contrast formulations contain metal-chelate compounds of gadolinium(III). However, the last decade has seen a rise in awareness of gadolinium(III) induced toxicity in patients receiving gadolinium(III) containing MR contrast formulations. This toxicity is manifested as nephrogenic systemic fibrosis (NSF). Patients experiencing chronic kidney disease are particularly susceptible to gadolinium(III) induced NSF, and MR contrast formulations are generally not administered to patients suffering severe chronic kidney disease. To avoid

the potential for gadolinium(III) associated toxicity, it would therefore be useful to identify MR contrast agents that do not contain gadolinium(III).

An effective contrast agent must contain several features. It should have high relaxivity to generate image contrast. Relaxivity is the ability of the metal chelate to relax water protons, and is defined as the change in the relaxation rate of water divided by the millimolar concentration of the chelate. Relaxivity depends on many molecular factors. For high relaxivity, it is advantageous to use a metal ion with a high spin quantum number; it is advantageous to have one or more water molecules directly bonded to the metal ion; and the bonded water molecule should undergo very fast chemical exchange with other water molecules in the solvent. Most metal ions are toxic at the concentrations required to provide MR contrast. Therefore the metal ion should be chelated by a multidentate ligand with sufficient stability to prevent the metal ion from being released in the body in significant amounts.

It would also be valuable to identify metal chelating ligands that form high-relaxivity compounds that either bind to, or change relaxivity, in the presence of biochemical targets. Compounds that bind to biochemical targets would enable detection of the targeted protein, enzyme, or cell at a delayed phase, after the compound in unbound form has been cleared via excretion. Compounds that change relaxivity in the presence of biochemical target will provide a change in MRI signal intensity at the locus of the target. The ability to detect change in biochemical processes with MRI would provide a non-invasive means to stage or monitor the progression of disease states such as cancer, inflammation, fibrosis, and thrombosis. Such compounds and imaging methods would also provide a non-invasive means to track therapeutic response.

SUMMARY

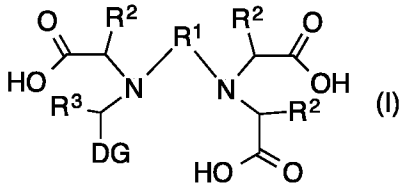
This disclosure is based on one or more modifications of a chelating ligand to either bind to a biochemical target, or enhance the relaxivity of a resultant metal chelate in the presence of a biochemical target, or to provide additional stability of a resultant metal chelate to prevent metal ion dissociation in the body. These modifications include changing

the donor groups (functional groups that directly coordinate to the metal ion), introducing groups that organize water in the second coordination sphere (e.g., by hydrogen bonding), introducing groups that facilitate water exchange on and off the metal ion, introducing groups that slow down molecular tumbling either by increased molecular weight or by targeting the metal chelate to a macromolecule (e.g., a protein), and introducing groups that support or promote a change in metal oxidation state. The donor groups can include a number of functionalities to exploit high relaxivity mechanisms, including, by way of example, enhancing relaxivity via binding to a macromolecular target, or changing oxidation state in response to enzyme activity.

Metal chelates prepared with the chelating ligands can be examined with techniques including relaxivity measurements at different magnetic fields and temperatures, and variable temperature ^{17}O NMR measurements.

Finally, chelating ligands may be useful for preparing diagnostic and/or therapeutic compositions of radioactive metal ions.

Provided herein is a compound of Formula (I):



(I)

or a pharmaceutically acceptable salt thereof,

wherein:

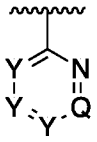
R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

each R^2 and R^3 is independently selected from the group consisting of H, CO_2H, (C_1-C_6 alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10}

aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and [L]-[TBM];

each R^4 and R^5 is independently selected from the group consisting of H, C_1-C_6 alkyl, and [L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

DG is selected from the group consisting of:


(II)
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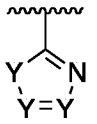
(III)
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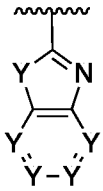
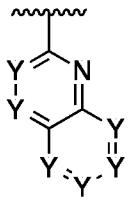

(IV)
;

and


(V)

or any constitutional isomers of Formulas IV and V, wherein

each Y is independently CH, CZ, N, O, S or NR^4;

Q is CH, CZ, N, O, S or NR^4;

each Z is independently selected from the group consisting of H, OH, OR^4, CO_2R^4, -(C_1-$_6$ alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl,

cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

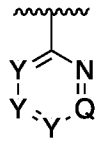
L is a linker;

TBM is a target binding moiety; and

each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM];

wherein if Q is CH or CCOOH and all Y are CH, than at least one of R^2 or R^3 is not H.

In some embodiments, DG is:



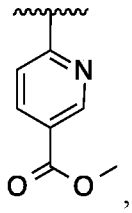
(II)

.

In some embodiments, Q is CH. In some embodiments, Y is CH. In some embodiments, at least one Y is CZ, wherein Z is selected from the group consisting of CO_2R^4, C_1-C_6 alkyl, and OR^4. In some embodiments, one Y is CZ, wherein Z is selected from the group consisting of CO_2R^4, C_1-C_6 alkyl, and OR^4, and all other Y are CH. In some embodiments, each R^4 is H or C_1-C_6 alkyl, wherein the alkyl is optionally substituted by 1, 2, 3, or 4 OH groups.

In some embodiments, R^2 and R^3 is H.

In some embodiments, DG is selected from the group consisting of:



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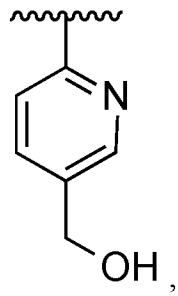

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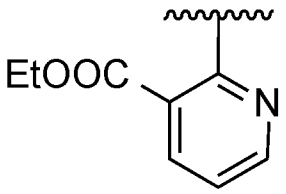

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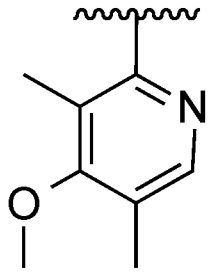

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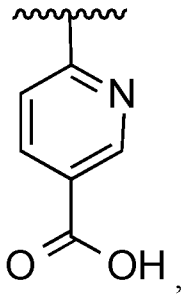

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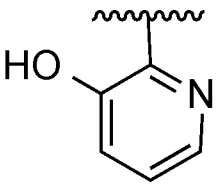

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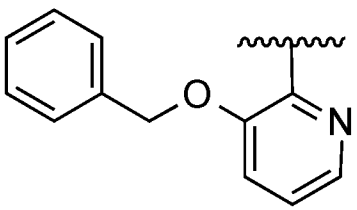
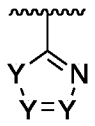
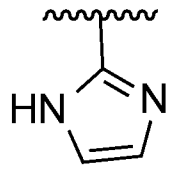


.

In some embodiments, DG is


(III)

. For example, wherein at least one Y is N, O, S or NR^4. In some embodiments, one Y is NR^4 and the remaining Y are CH. For example, DG is selected from the group consisting of: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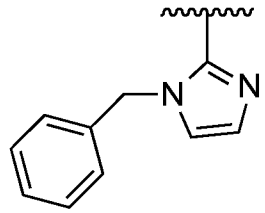
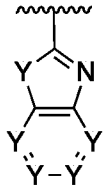
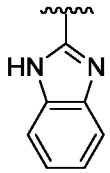

,

In some embodiments, DG is:


(IV)

. In some embodiments, at least one Y is N, O, S or NR^4. For example, one Y is NR^4 and the remaining Y are CH. In some embodiments, DG is: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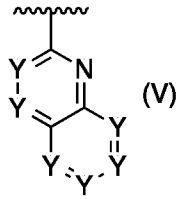
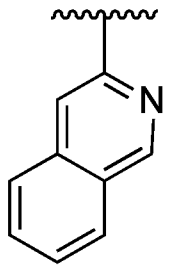
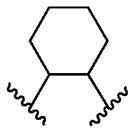
In some embodiments, DG is:


(V)

. For example, DG is:



In some embodiments, R^1 is C_3-C_{10} cycloalkylene. For example, R^1 is a C_6 cycloalkylene. In some embodiments, R^1 is:

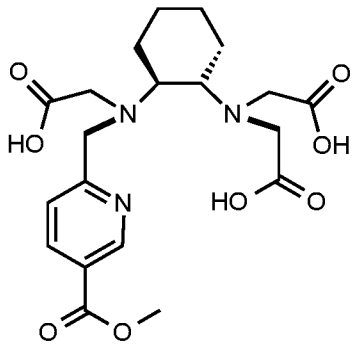
 . In some embodiments, R^1 is a C_1-C_6 alkylene. For example, R^1 is a C_2 alkylene. In some embodiments, R^1 is:

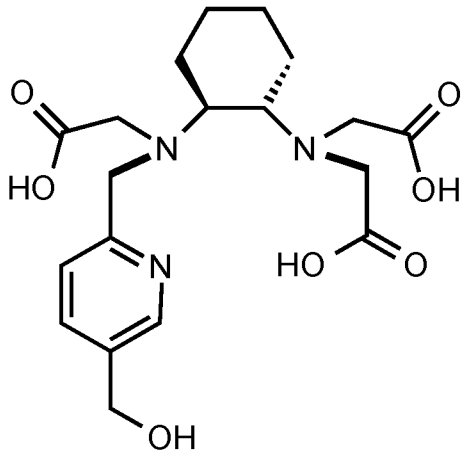
 .

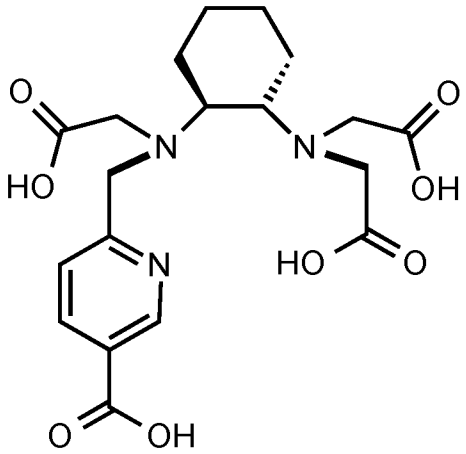
In some embodiments, R^2 is H.

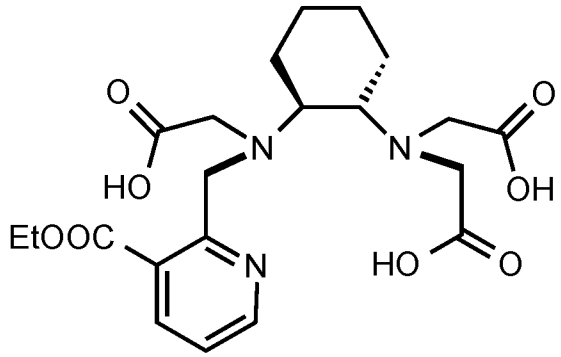
In some embodiments, R^3 is H.

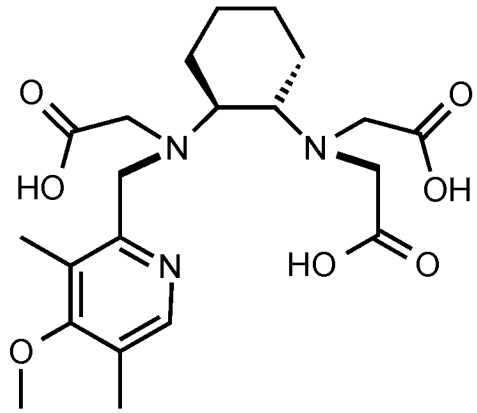
In some embodiments, the compound of Formula (I) is selected from the group consisting of:

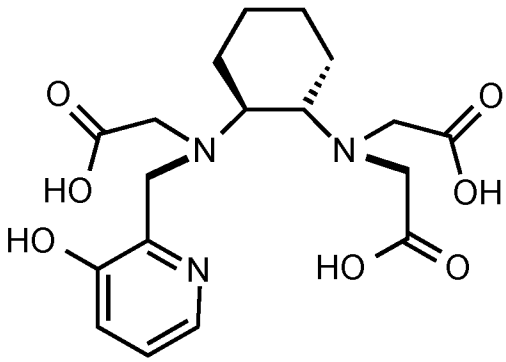


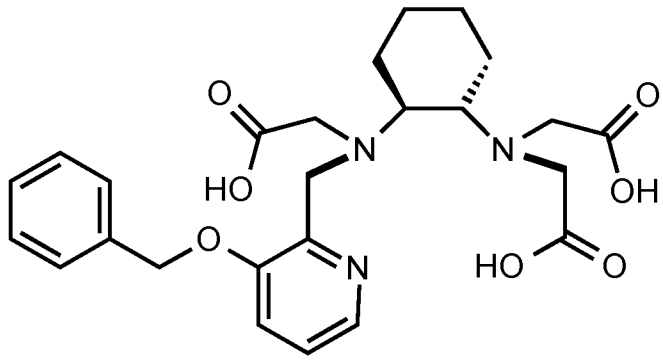


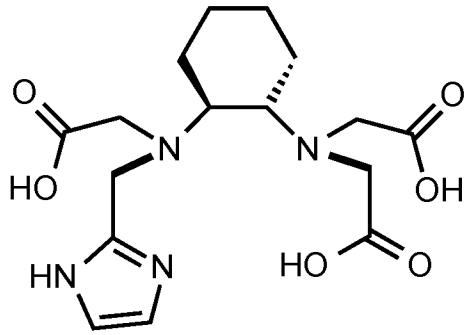


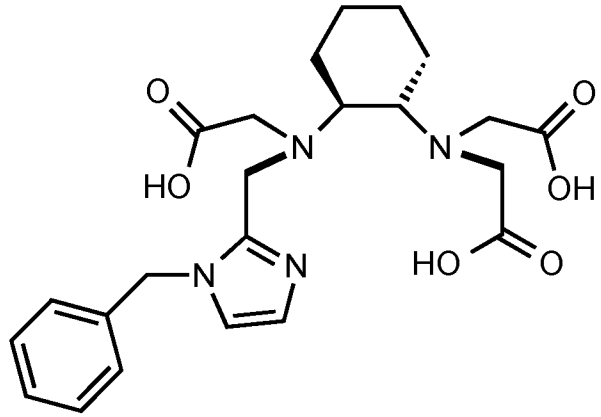


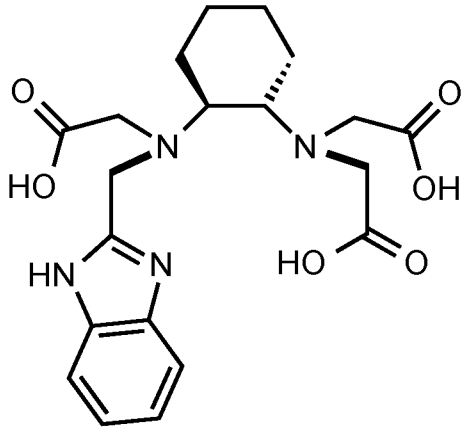












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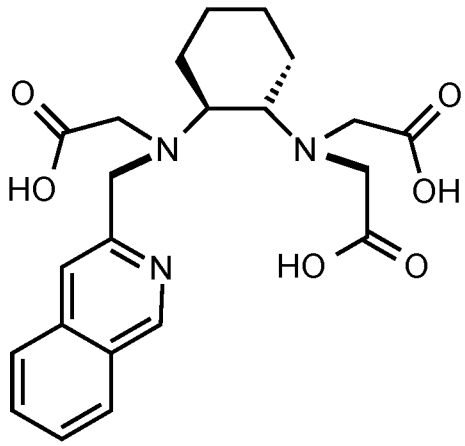
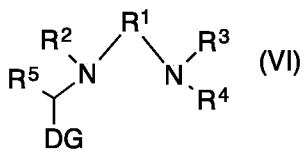


or a pharmaceutically acceptable salt thereof.

Also provided herein are compounds of Formula (VI):



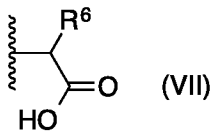
(VI)

or a pharmaceutically acceptable salt thereof,

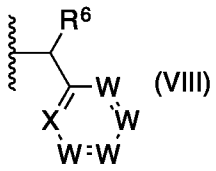
wherein:

R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

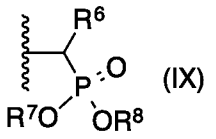
R^2, R^3, and R^4 are independently selected from the group of compounds of formula:



(VII) ;

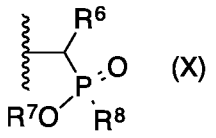


(VIII) ;



(IX) ;

and



(X)

R^5 and R^6 are independently selected from the group consisting of H, CO_2H, (C_1-C_6 alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7, $C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and [L]-[TBM];

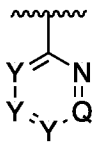
X is CZ, N, O, S or NR^7;

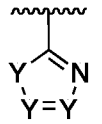
each W is independently CH, CZ, N, O, S or NR^7;

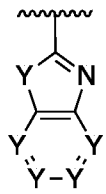
each Z is independently selected from H, OH, OR^4, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

each R^7 and R^8 are independently selected from the group consisting of H, C_1-C_6 alkyl, and -[L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

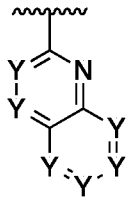
DG is selected from the group consisting of:


(XI)
;


(XII)
;


(XIII)
;

and


(XIV)

or any constitutional isomers of Formulas XIII-XIV,

wherein each Y is independently CH, CZ^1, N, O, S, or NR^7;

Q is independently CH, CZ^1, N, O, S, or NR^7;

each Z^1 is independently selected from H, OH, OR^4, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

L is a linker;

TBM is a target binding moiety; and

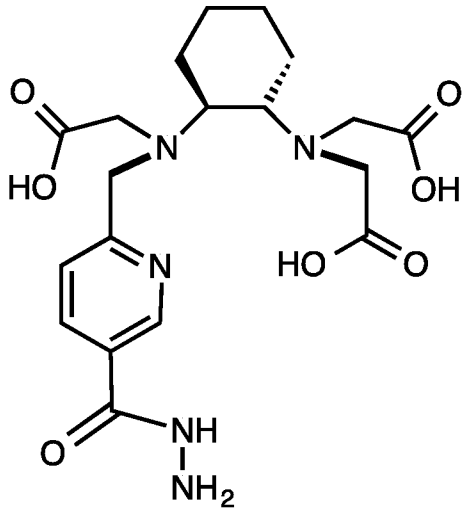
each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM];

wherein if Q is CH or CCOOH, all Y are CH, and all of R^2, R^3, and R^4 are formula VII, than at least one of R^5 or R^6 is not H; and

if one of R^2, R^3, or R^4 is formula VIII, and all of R^5 and R^6 are H, than the aromatic ring component of formula VIII (i.e. the ring containing X and W) must be different than DG.

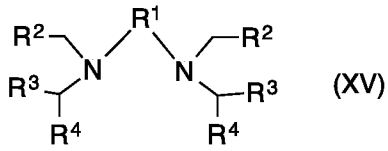
In some embodiments, R^1 is 1,2-cyclohexylene, R^2, R^3, and R^4 are formula VII, R^5 and R^6 are H, and DG is formula XI and one Y is [L]-[TBM], where [L] is –C(O)- and [TBM] is $–NHNH_2$.

In some embodiments, the compound of Formula (VI) is:



or a pharmaceutically acceptable salt thereof.

Further provided herein is a compound of Formula (XV):



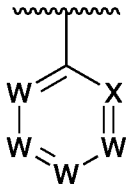
(XV)

or a pharmaceutically acceptable salt thereof,

wherein:

R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

each R^2, R^3, and R^4 are independently selected from the group consisting of CO_2H, $(C(O)NR^5R^6$, CH_2NHCOR^5, $C(O)N(OH)R^5$, $C(O)NHSO_2R^5$, $CH_2NHSO_2R^5$, $N(OH)C(O)R^5$, $P(R^5)O_2R^6$, and $PO_3R^5R^6$, and compounds of formula:



(XVI)

;

wherein X is CZ, N, O, S, or NR^5;

each W is independently CH, CZ, N, O, S, or NR^5;

each Z is independently selected from H, OH, OR^4, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^5R^6$, CH_2NHCOR^5, $C(O)N(OH)R^5$, $C(O)NHSO_2R^5$, $CH_2NHSO_2R^5$, $N(OH)C(O)R^5$, $P(R^5)O_2R^6$, $PO_3R^5R^6$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

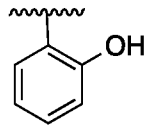
each R^5 and R^6 are independently selected from the group consisting of H, C_1-C_6 alkyl, and -[L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

L is a linker;

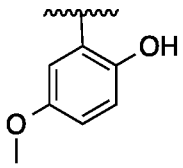
TBM is a target binding moiety; and

each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM].

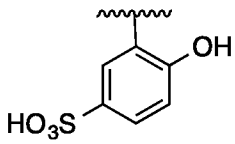
In some embodiments, R^1 is 1,2-ethylene, R^2 is COOH, R^3 is Formula XVI wherein X is N and all W are CH, and R^4 is selected from a compound Formula XVI. For example, R^4 is:



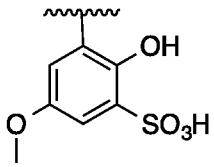
. In some embodiments, R^4 is:



. In some embodiments, R^4 is:

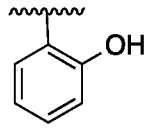


. In some embodiments, R^4 is:

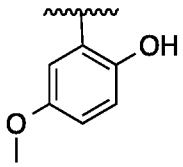


.

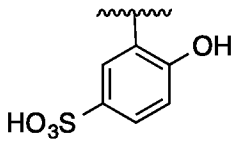
In some embodiments, R^1 is 1,2-ethylene, R^2 is COOH, R^3 is COOH, and R^4 is selected from compound Formula XVI. For example, R^4 is:



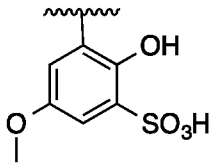
. In some embodiments, R^4 is:



. In some embodiments, R^4 is:

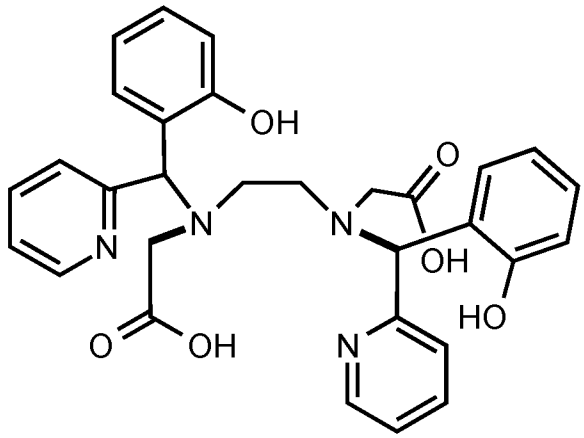


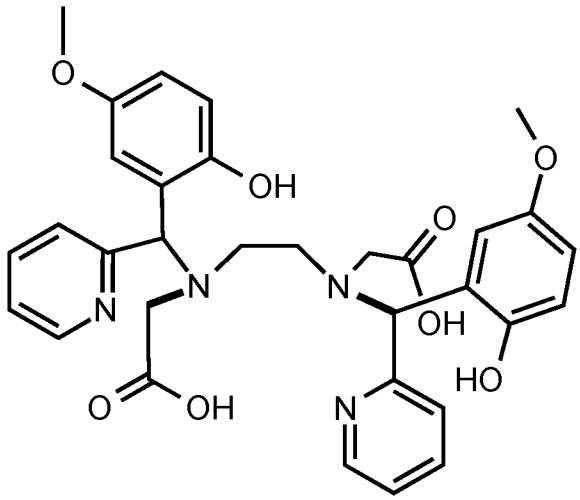
. In some embodiments, R^4 is:

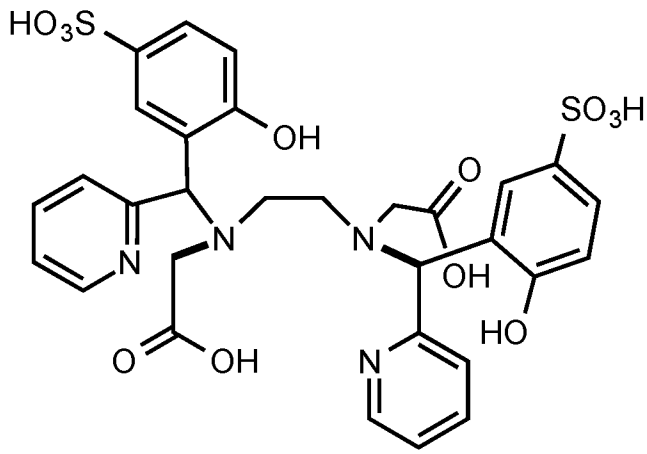


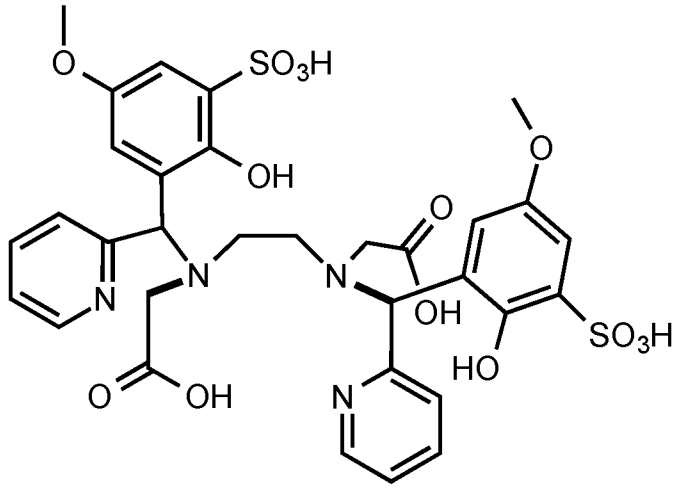
.

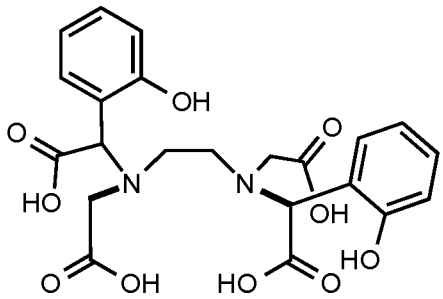
In some embodiments, the compound of Formula (XV) is selected from the group consisting of:

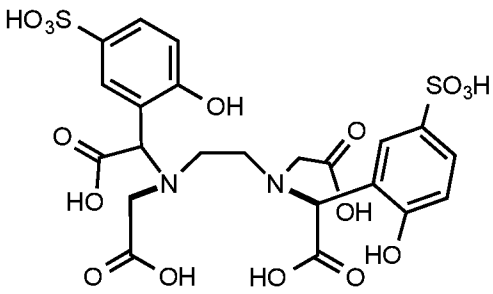

OH
O
N
N
N
OH
HO
O
OH
N

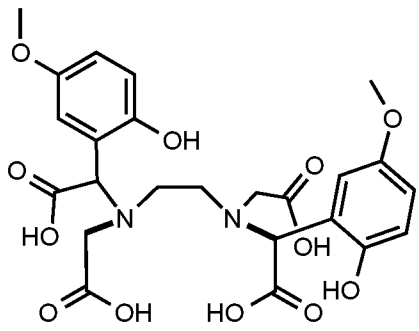

O
OH
O
N
N
N
OH
HO
O
OH
N


HO3S
OH
SO3H
O
N
N
N
OH
HO
O
OH
N

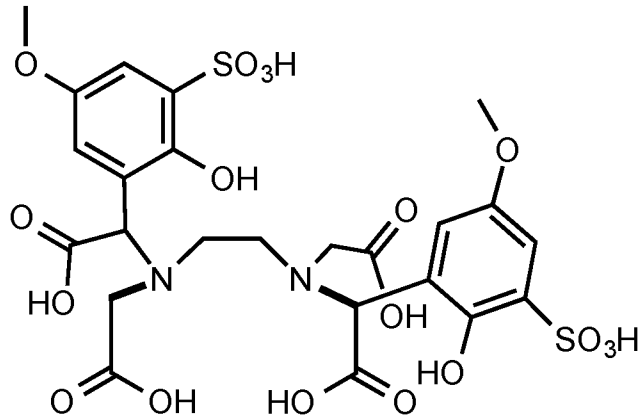






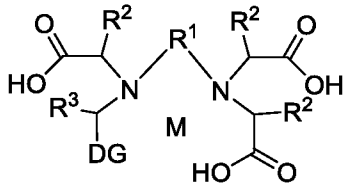


, and



or a pharmaceutically acceptable salt thereof.

Also provided herein are compounds of Formula (XVII):



or a pharmaceutically acceptable salt thereof,

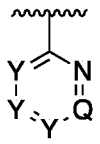
wherein:

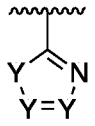
R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

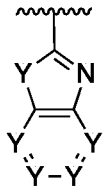
each R^2 and R^3 are independently selected from the group consisting of H, CO_2H, (C_1-C_6 alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and [L]-[TBM];

each R^4 and R^5 is independently selected from the group consisting of H, C_1-C_6 alkyl, and [L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

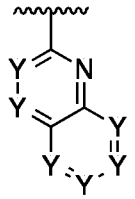
DG is selected from the group consisting of:


(II)
;


(III)
;


(IV)
;

and


(V)

or any constitutional isomers of Formulas IV and V, wherein

each Y is independently CH, CZ, N, O, S, or NR^4;

Q is CH, CZ, N, O, S, or NR^4;

each Z is independently selected from the group consisting of H, OH, OR^4, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

L is a linker;

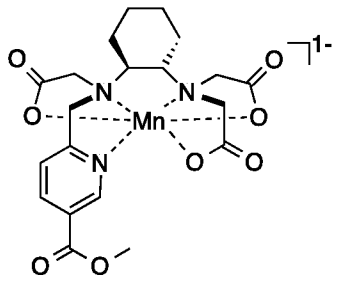
TBM is a target binding moiety;

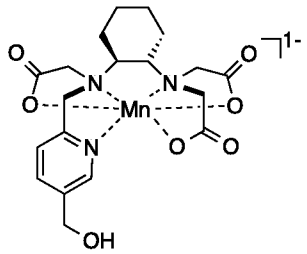
each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM]; and

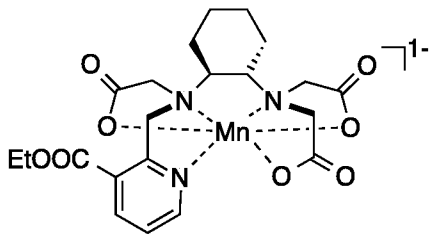
M is selected from the group consisting of Gd(III), Fe(III), Mn(II), Mn(III), Cr(III), Cu(II), Cu(III), Dy(III), Ho(III), Er(III), Pr(III), Eu(II), Eu(III), Nd(III), La(III), Lu(III), Sm(III), Tb(III), Tb(IV), Tm(III), Y(III), In(III), Ga(III), Tc(III), Tc(IV), Tc(V), Re(III), Re(IV), Re(V), Bi(III), and Yb(III);

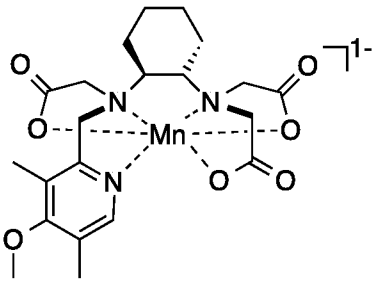
wherein, if Q is CH or CCOOH and all Y are CH, than at least one of R^2 or R^3 is not H..

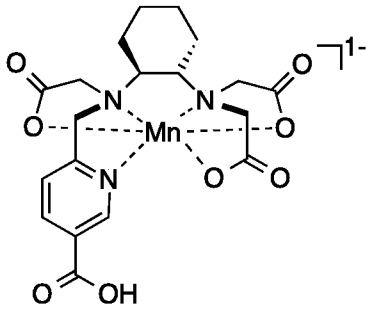
In some embodiments, the compound of Formula (XVII) is selected from the group consisting of:

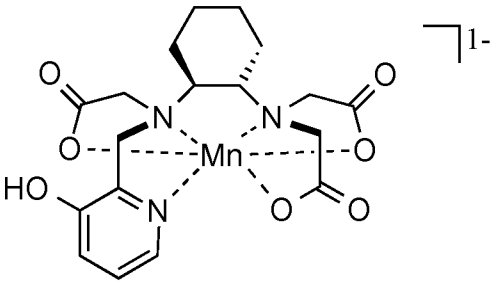


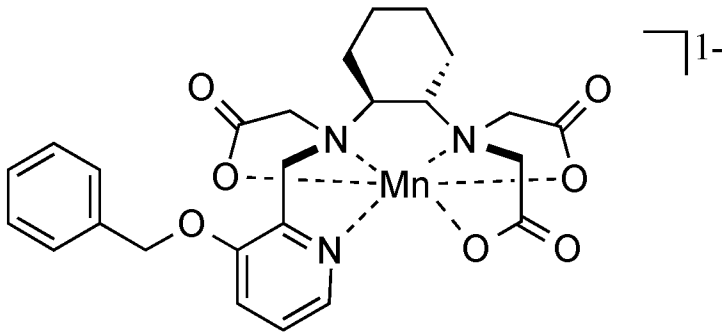


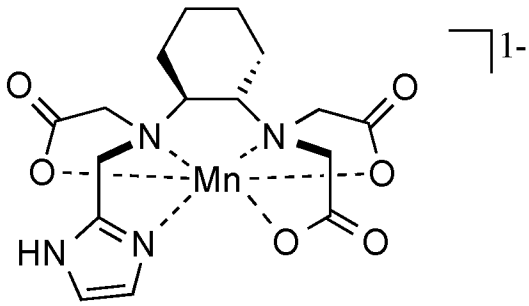


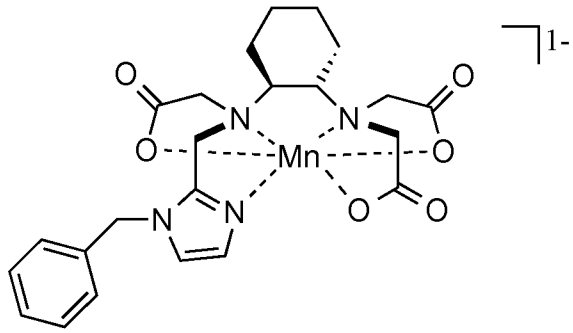


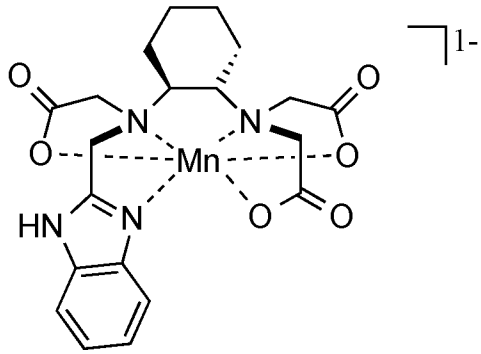




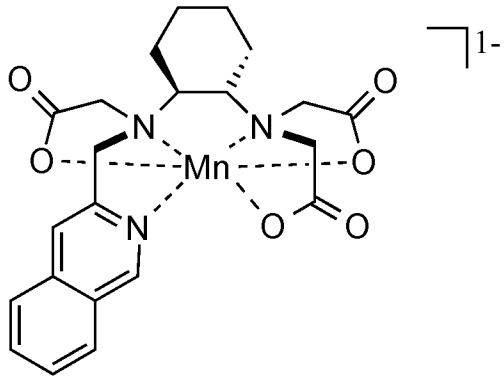






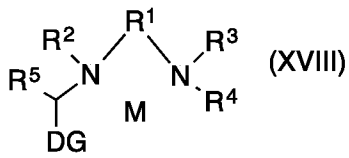


, and



or a pharmaceutically acceptable salt thereof.

Further provided herein are compounds of Formula (XVIII):



(XVIII)

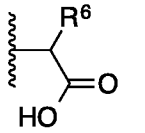
or a pharmaceutically acceptable salt thereof,

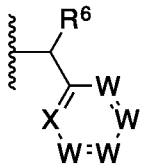
wherein:

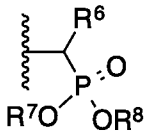
R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene,

(C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

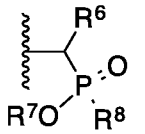
R^2, R^3, and R^4 are independently selected from the group of compounds of formula:


(VII)
;


(VIII)
;


(IX)
;

and


(X)

R^5 and R^6 are independently selected from the group consisting of H, CO_2H, (C_1-C_6 alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7, $C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and [L]-[TBM];

X is CZ, N, O, or S, or NR^7;

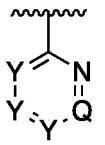
each W is independently CH, CZ, N, O, S, or NR^7;

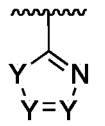
each Z is independently selected from H, OH, OR^4, CO_2H, $C_{1\text{-}6}CO_2H$, -($C_{1\text{-}6}$ alkyl)CO_2H, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7,

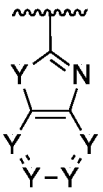
$C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

each R^7 and R^8 are independently selected from the group consisting of H, C_1-C_6 alkyl, and -[L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

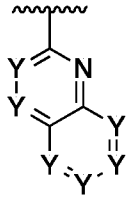
DG is selected from the group consisting of:


(XI)
;


(XII)
;


(XIII)
;

and


(XIV)

or any constitutional isomers of Formulas XIII-XIV,

wherein each Y is independently CH, CZ^1, N, O, S, or NR^7;

Q is independently CH, CZ^1, N, O, S, or NR^7;

each Z^1 is independently selected from H, OH, OR^7, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7, $C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl,

heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

L is a linker;

TBM is a target binding moiety;

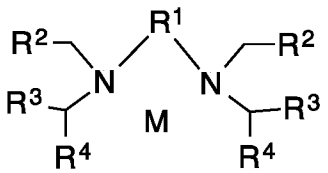
each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM]; and

M is selected from the group consisting of Gd(III), Fe(III), Mn(II), Mn(III), Cr(III), Cu(II), Cu(III), Dy(III), Ho(III), Er(III), Pr(III), Eu(II), Eu(III), Nd(III), La(III), Lu(III), Sm(III), Tb(III), Tb(IV), Tm(III), Y(III), In(III), Ga(III), Tc(III), Tc(IV), Tc(V), Re(III), Re(IV), Re(V), Bi(III), and Yb(III); and

wherein if Q is CH or CCOOH, all Y are CH, and all of R^2, R^3, and R^4 are formula VII, than at least one of R^5 or R^6 is not H; and

if one of R^2, R^3, or R^4 is formula VIII, and all of R^5 and R^6 are H, than the aromatic ring component of formula VIII (i.e. the ring containing X and W) must be different than DG.

Also provided herein are compounds of Formula (XIX):



(XIX)

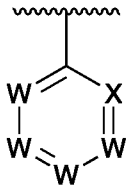
or a pharmaceutically acceptable salt thereof,

wherein:

R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered

heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

each R^2, R^3, and R^4 are independently selected from the group consisting of CO_2H, $(C(O)NR^5R^6$, CH_2NHCOR^5, $C(O)N(OH)R^5$, $C(O)NHSO_2R^5$, $CH_2NHSO_2R^5$, $N(OH)C(O)R^5$, $P(R^5)O_2R^6$, and $PO_3R^5R^6$, and compounds of formula:



(XVI)

;

wherein X is CZ, N, O, or S. or NR^4;

each W is independently CH, CZ, N, O, S, or NR^4;

each Z is independently selected from H, OH, OR^4, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^5R^6$, CH_2NHCOR^5, $C(O)N(OH)R^5$, $C(O)NHSO_2R^5$, $CH_2NHSO_2R^5$, $N(OH)C(O)R^5$, $P(R^5)O_2R^6$, $PO_3R^5R^6$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

each R^5 and R^6 are independently selected from the group consisting of H, C_1-C_6 alkyl, and -[L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

L is a linker;

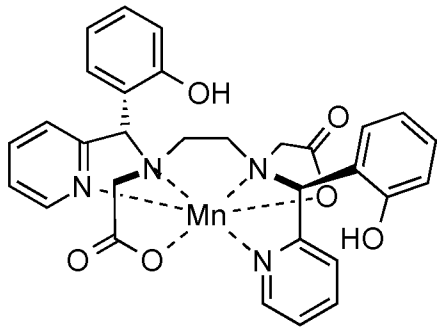
TBM is a target binding moiety;

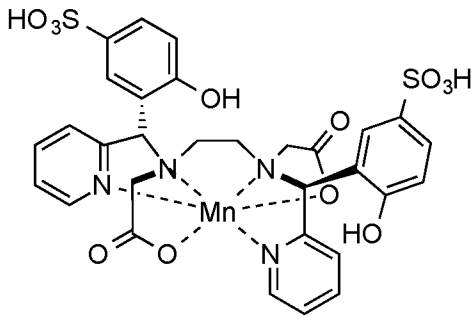
each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4}

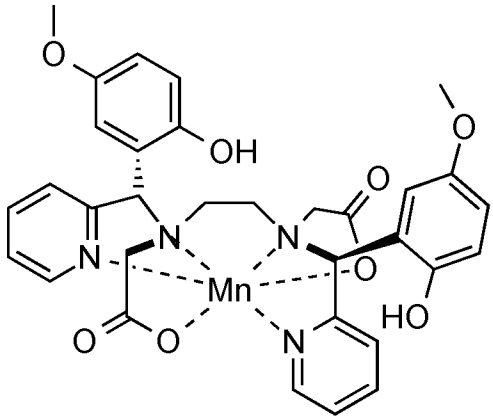
alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM]; and

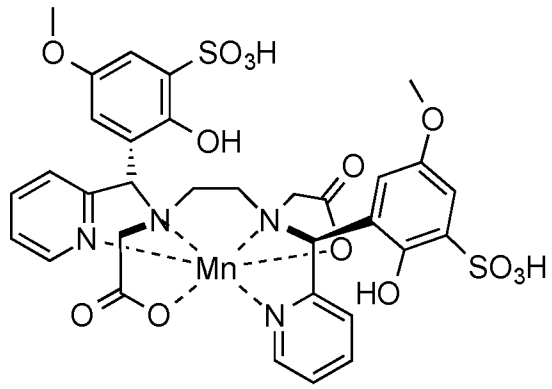
M is selected from the group consisting of Gd(III), Fe(III), Mn(II), Mn(III), Cr(III), Cu(II), Cu(III), Dy(III), Ho(III), Er(III), Pr(III), Eu(II), Eu(III), Nd(III), La(III), Lu(III), Sm(III), Tb(III), Tb(IV), Tm(III), Y(III), In(III), Ga(III), Tc(III), Tc(IV), Tc(V), Re(III), Re(IV), Re(V), Bi(III), and Yb(III).

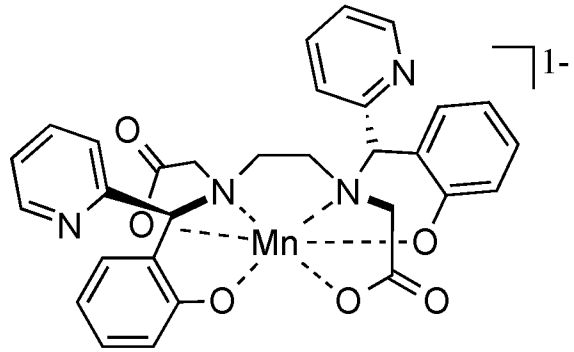
In some embodiments, the compound of Formula (XVIII) is selected from the group consisting of:

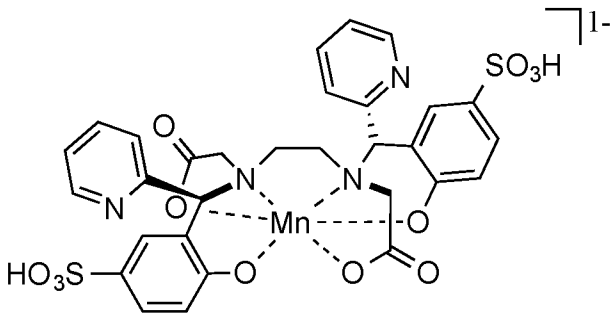


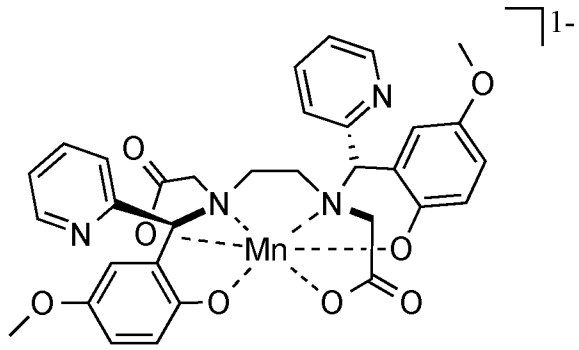


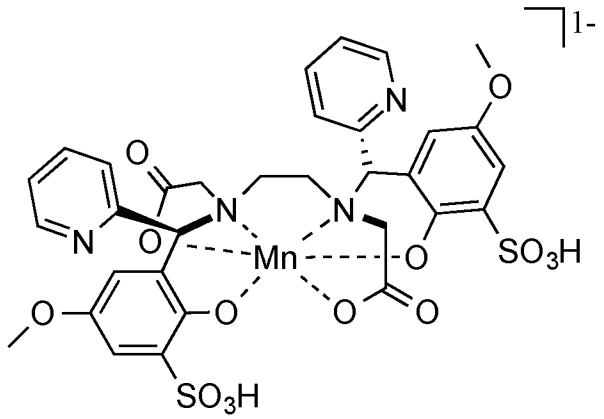


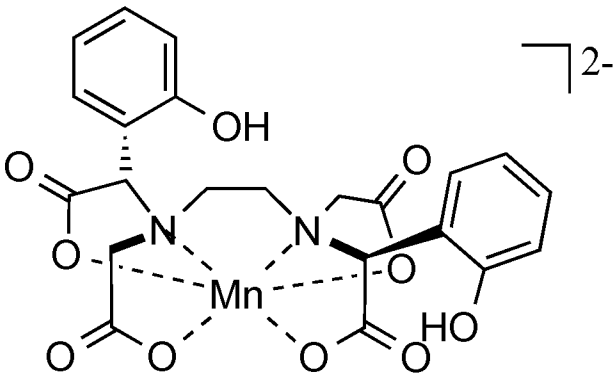


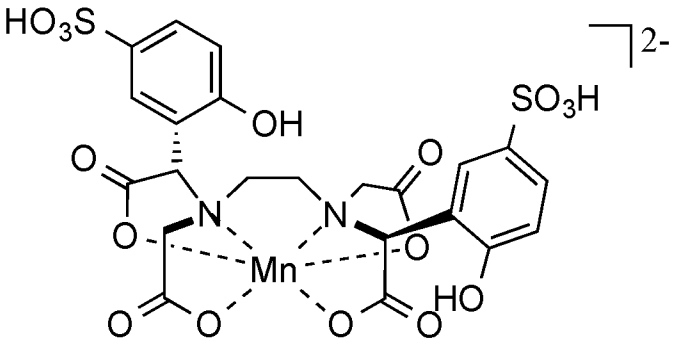


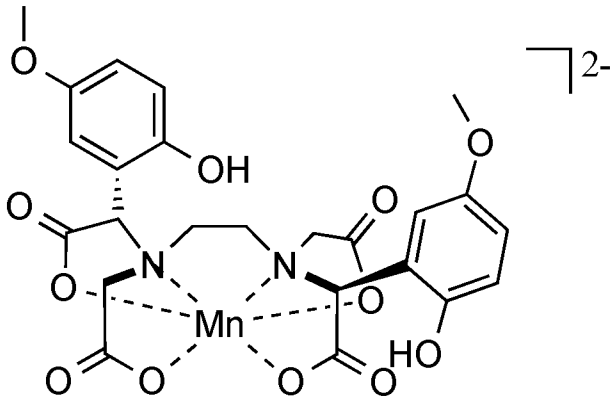


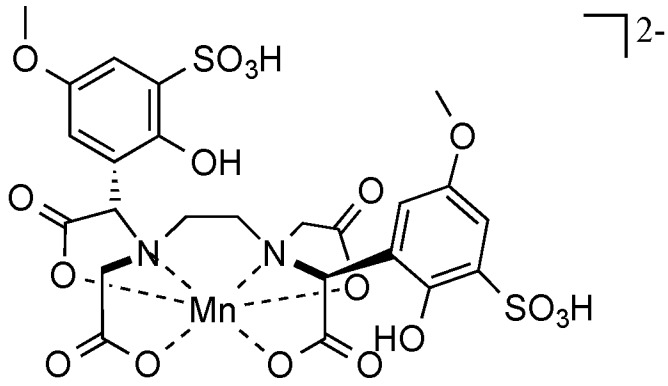



1-
N
O
O
N
N
O
Mn
O
SO3H
O
O
O
O
SO3H

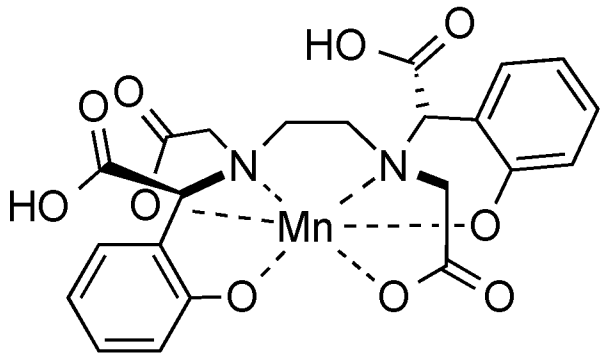

2-
OH
O
O
N
N
O
O
Mn
O
O
O
O
HO


HO3S
2-
SO3H
OH
O
O
N
N
O
O
Mn
O
O
O
O
HO

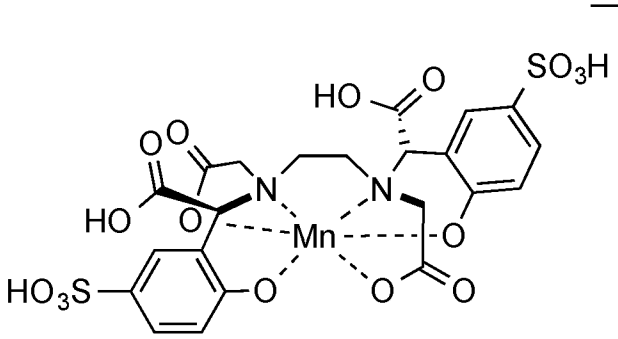

O
2-
OH
O
O
N
N
O
O
Mn
O
O
O
O
HO



2-



1-



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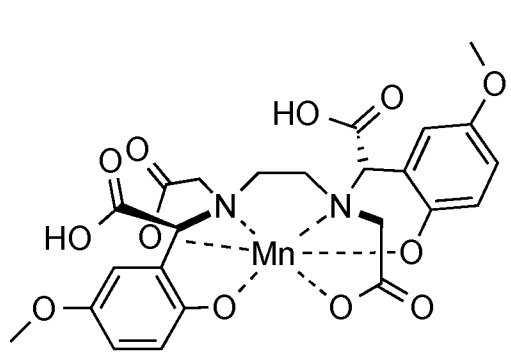

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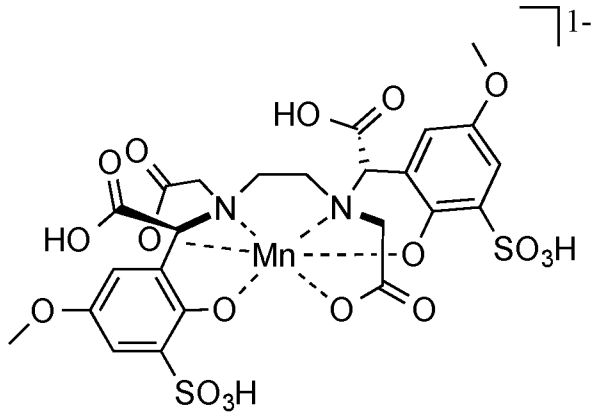
, and



or a pharmaceutically acceptable salt or any corresponding stereoisomer thereof..

This disclosure further provides a compound of Formula (A):

$$(D^1)_a—(L^1)_b—TBM—(L^2)_c—(D^2)_d$$

(A)

or a pharmaceutically acceptable salt thereof,

wherein:

TBM is a target binding moiety;

each D^1 is independently a metal chelate of any one of claims 44-48;

each D^2 is independently a metal chelate of any one of claims 44-48;

L^1 is a linker;

L^2 is a linker;

a is an integer from 0 to 4;

b is 0 or 1;

wherein if a is 0, b is 0;

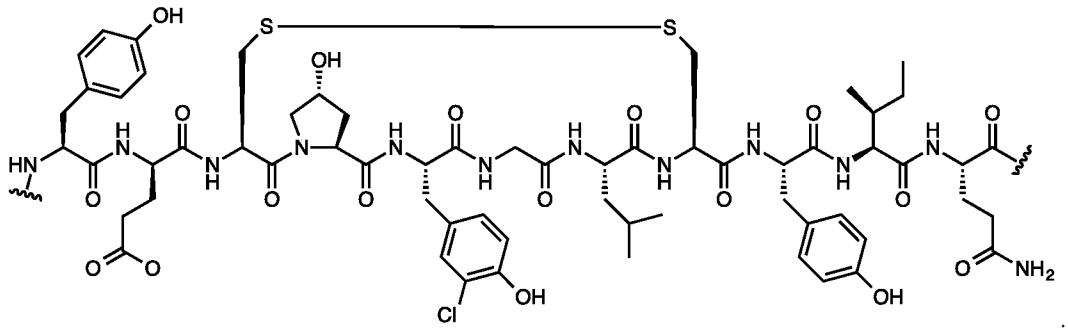
c is 0 or 1;

d is an integer from 0 to 4;

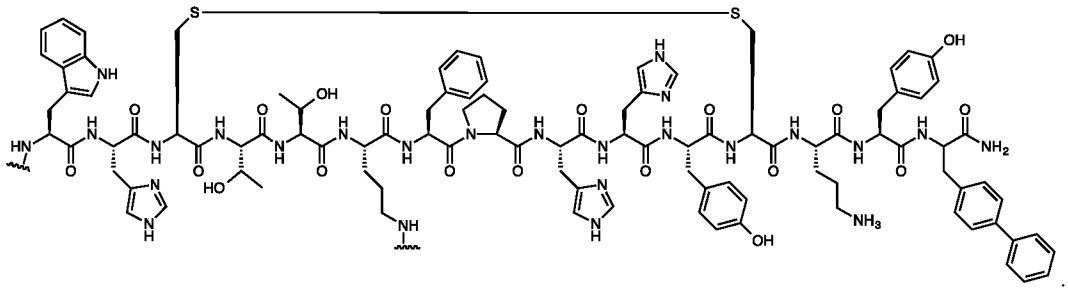
wherein if d is 0, c is 0;

wherein at least one of a and d is an integer from 1 to 4.

In some embodiments, the [TBM] is:

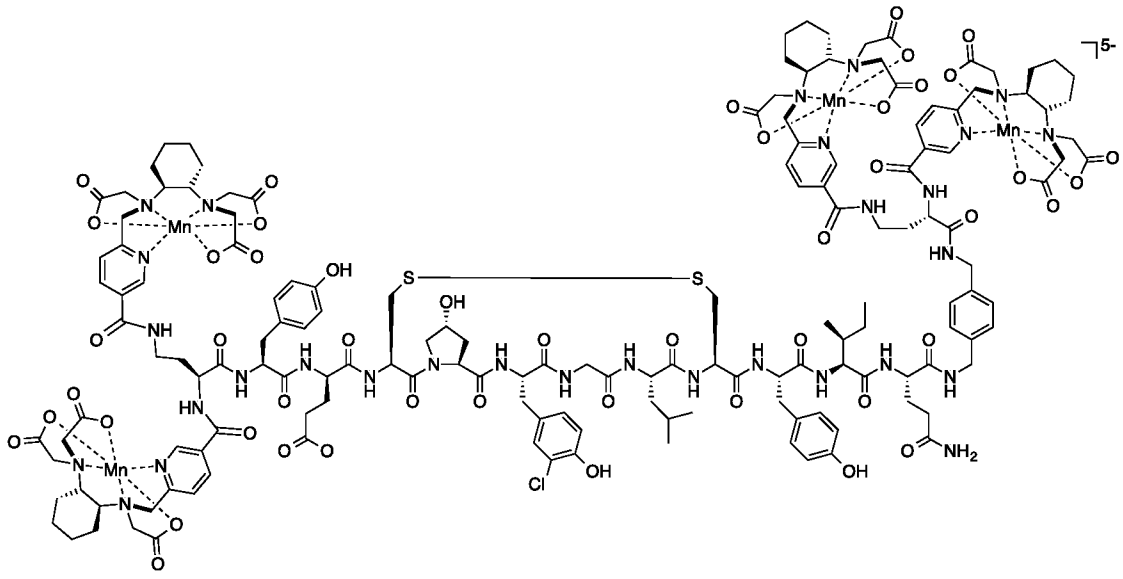


In some embodiments, the [TBM] is:

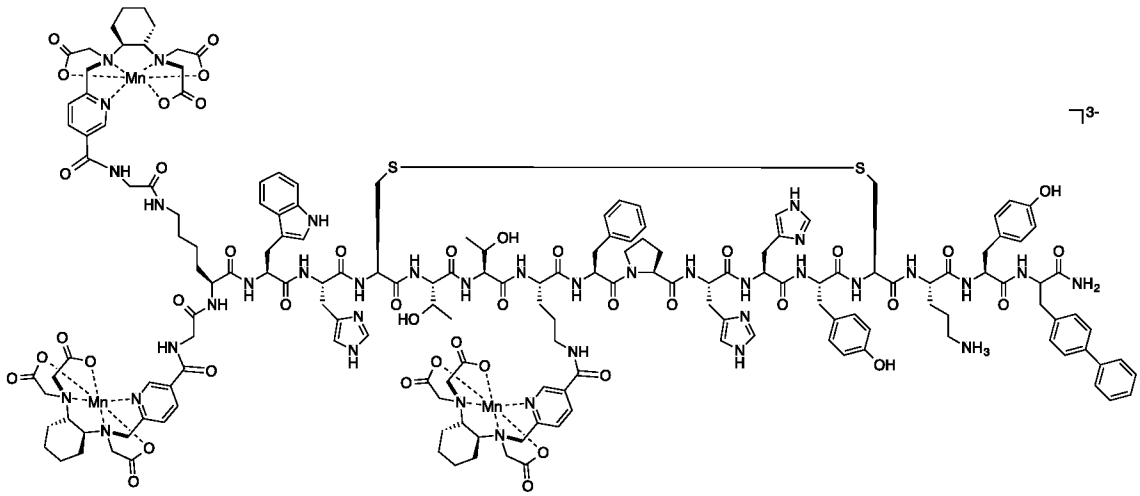


In some embodiments, D^1 and D^2 are a compound according to claim 1, wherein R^1 is 1,2-cyclohexylene, R^2 is H, R^3 is H, DG is Formula II, Q is CH, the Y positioned α- to Q is C-[L]-TBM, and all other Y are CH, and L is –C(O)-.

In some embodiments, the compound is selected from the group consisting of:



and



Also provided herein is a method of magnetic resonance (MR) imaging a patient, the method comprising: a) administering to the patient an effective amount of a compound comprising a metal chelate as provided herein; and b) acquiring an MR image of the patient. For example, a method for imaging a tumor in a patient is provided herein, the method comprising: a) administering to the patient an effective amount of a compound comprising a metal chelate as provided herein; and b) acquiring an MR image of the tumor. In some embodiments, the method can include a method for imaging a blood clot in a patient, the method comprising: a) administering to the patient an effective amount of a compound comprising a metal chelate as provided herein; and b) acquiring an MR image of the blood clot. In some embodiments, the method can include a method for imaging a brain lesion in a patient, the method comprising: a) administering to the patient an effective amount of a compound comprising a metal chelate as provided herein; and b) acquiring an MR image of the brain lesion.

Further provided herein is a method for detecting the presence or absence of disrupted blood-brain-barrier in a patient, the method comprising: a) administering to the patient an effective amount of a compound comprising a metal chelate as provided herein, wherein M is Mn(II); b) acquiring a first MR image of the brain of the patient; c) acquiring a second MR image of the brain of the patient; and comparing the images. In some embodiments, a method for detecting the presence or absence of arterial stenosis in a patient is provided, the method comprising: a) administering to the patient an effective amount of a compound comprising a metal chelate as provided herein, wherein M is

Mn(II); b) acquiring a first MR image of the arteries of a patient; c) acquiring a second MR image of the arteries of patient immediately after injection of the compound; and d) comparing the images.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the methods, materials, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 shows that fibrin targeting compound **20** possesses high affinity for the soluble fibrin degradation product comprising the complex of a D-dimer domain and a E domain of the protein that is termed DD(E). Fluorescence polarization anisotropy of fluorescein labeled fibrin binding peptide in DD(E) solution as a function of added Mn-FBP (filled circles) or a known fibrin binding molecule EP2104R (open circles) to determine Ki to DD(E) is shown.

FIG. 2 shows that the Mn ions in compound **20** have one rapidly exchanging water ligand. $H_2{}^{17}O$ transverse relaxivity in the presence of compound **20** (open circles) as a function of temperature is shown. Solid lines are fits to the data.

FIG. 3 shows MR imaging of carotid artery thrombosis in a rat model with compound **20**. Axial T_1-weighted images before (A, C) and 35 minutes after intravenous administration of compound **20** (B, D) at 1.5T. (C) and (D) are expanded regions from (A) and (B), respectively showing the common carotid arteries. Compound **20** generates

marked signal enhancement in the ipsilateral vessel (open arrow, D) after compound **20** injection, but not in contralateral vessel (filled arrow, D) or in the vessel prior to Mn-FBP injection (C). (E) Hematoxylin and Eosin stained sections of contralateral (left) and ipsilateral (right) carotid arteries showing occlusive thrombus in the injured vessel; scale bar = 300 μm.

FIG. 4 shows the quantitation of the MR imaging data with compound **20**. (A) and (B) show normalized signal-to-noise ratio (nSNR) of the thrombus (closed circles), contralateral vessel region (open circles), and muscle (closed diamonds) following administration of compound **20** and EP-2104R, respectively, showing persistently enhanced thrombus with each probe and washout of signal from background tissue. (C) and (D) contrast-to-noise ratio (CNR) of thrombus-to-muscle (closed circles) and contralateral vessel region-to-muscle (open circles) following administration of compound **20** and EP2104R, respectively, showing large and persistently high CNR for the thrombus with each probe. N=4 for each probe, error bars represent standard error of the mean.

FIG. 5 shows blood clearance of compound **20** as a function of time (N=4),

FIG. 6 shows relaxivity change as a function of time for the peroxidase reactive compound **20** in the presence of hydrogen peroxide without (closed circles) or with (open circles) horseradish peroxidase. A 7-fold in relaxivity is observed within 3 min of peroxidase exposure.

FIG. 7 shows that the conversion of compound **33** to compound **34** occurs cleanly and without byproducts. The top trace is compound **33** and the bottom trace is compound **33** (largely converted to compound **34**) after treatment with hydrogen peroxide and horseradish peroxide.

FIG. 8 shows relaxivity change (1.41 T, 37 °C) as a function of time for the thiol reactive compound **33** while incubating in human blood plasma without (closed circles) or with 5 mol. equiv. L-cysteine (open circles).

DETAILED DESCRIPTION

1. Definitions

Commonly used chemical abbreviations that are not explicitly defined in this disclosure may be found in The American Chemical Society Style Guide, Second Edition; American Chemical Society, Washington, DC (1997), "2001 Guidelines for Authors" J. Org. Chem. *66(1)*, 24A (2001), and "A Short Guide to Abbreviations and Their Use in Peptide Science" J. Peptide. Sci. *5*, 465-471 (1999).

The terms "chelating ligand," "chelating moiety," and "chelate moiety" are used interchangeably and refer to any polydentate ligand that is capable of coordinating a metal ion, either directly or after removal of protecting groups, or is a reagent, with or without suitable protecting groups, that is used in the synthesis of a MR contrast agent and comprises substantially all of the atoms that ultimately will coordinate the metal ion of the final metal complex. The terms "chelate" or "metal chelate" refer to the actual metal-ligand complex. It is understood that the polydentate ligand can eventually be coordinated to a medically useful or diagnostic metal ion.

The term "specific binding affinity" as used herein, refers to the capacity of a contrast agent to be taken up by, retained by, or bound to a particular or target biological component to a greater degree as compared to other non-targeted biological components. Contrast agents that have this property are said to be "targeted" to the "target" component. Contrast agents that lack this property are said to be "non-specific" or "non-targeted" agents. The binding affinity of a binding group for a target is expressed in terms of the equilibrium dissociation constant "K_d."

The term "relaxivity" as used herein, refers to the increase in either of the MR quantities $1/T_1$ or $1/T_2$ per millimolar (mM) concentration of paramagnetic ion or contrast agent, which quantities may be different if the contrast agent contains a multiplicity of paramagnetic ions, wherein T_1 is the longitudinal or spin-lattice relaxation time, and T_2 is the transverse or spin-spin relaxation time of water protons or other imaging or spectroscopic nuclei, including protons found in molecules other than water. Relaxivity is expressed in units of $mM^{-1}s^{-1}$.

The terms "target binding" and "binding" for purposes herein refer to non-covalent interactions of a contrast agent with a target. These non-covalent interactions are independent from one another and may be, *inter alia*, hydrophobic, hydrophilic, dipole-

dipole, pi-stacking, hydrogen bonding, electrostatic associations, or Lewis acid-base interactions.

Coordination of metal ions by water and other ligands is often regarded in terms of coordination spheres (see e.g., D. T. Richens, The Chemistry of Aqua Ions, John Wiley and Sons, New York, 1997, Chapter 1). The first or primary coordination sphere represents all the ligands directly bonded to the metal ion and is defined by the ligands. There is a second coordination sphere where water molecules and counterions bond to the groups in the first coordination sphere via hydrogen bonding and electrostatic interactions. Tertiary and subsequent coordination spheres are typically termed "bulk water" or "bulk solvent". The distinctions between these spheres are both spatial and temporal. The first coordination sphere is typically well-defined and the time that a water or other ligand spends in the first coordination sphere is longer than in other coordination spheres. The second sphere is less well-defined, but the waters here have a longer lifetime than the typical diffusion time of water. Beyond the second sphere water diffuses freely.

As used herein, all references to “Mn(II)” or “manganese(II)” mean the Mn(II) paramagnetic metal ion; all references to “Mn(III)” or “manganese(III)” mean the Mn(III) paramagnetic metal ion.As used herein, “alkyl” means a branched, or straight chain chemical group containing only carbon and hydrogen, such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, n-pentyl, iso-pentyl, sec-pentyl and neo-pentyl. Alkyl groups can either be unsubstituted or substituted with one or more substituents. In some embodiments, alkyl groups include 1 to 9 carbon atoms (for example, 1 to 6 carbon atoms, 1 to 4 carbon atoms, or 1 to 2 carbon atoms).

As used herein, "alkenyl" means a straight or branched chain chemical group containing only carbon and hydrogen and containing at least one carbon-carbon double bond, such as ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, and the like. In various embodiments, alkenyl groups can either be unsubstituted or substituted with one or more substituents. Typically, alkenyl groups will comprise 2 to 9 carbon atoms (for example, 2 to 6 carbon atoms, 2 to 4 carbon atoms, or 2 carbon atoms).

As used herein, “alkynyl” means a straight or branched chain chemical group containing only carbon and hydrogen and containing at least one carbon-carbon triple bond,

such as ethynyl, 1-propynyl, 1-butynyl, 2-butynyl, and the like. In various embodiments, alkynyl groups can either be unsubstituted or substituted with one or more substituents. Typically, alkynyl groups will comprise 2 to 9 carbon atoms (for example, 2 to 6 carbon atoms, 2 to 4 carbon atoms, or 2 carbon atoms).

As used herein, "alkylene" means a bivalent branched, or straight chain chemical group containing only carbon and hydrogen, such as methylene, ethylene, n-propylene, iso-propylene, n-butylene, iso-butylene, sec-butylene, tert-butylene, n-pentylene, iso-pentylene, sec-pentylene and neo-pentylene. Alkylene groups can either be unsubstituted or substituted with one or more substituents. Alkylene groups can be saturated or unsaturated (e.g., containing -C=C- or -C≡C- subunits), at one or several positions. In some embodiments, alkylene groups include 1 to 9 carbon atoms (for example, 1 to 6 carbon atoms, 1 to 4 carbon atoms, or 1 to 2 carbon atoms).

As used herein, "alkenylene" means a bivalent branched, or straight chain chemical group containing only carbon and hydrogen and containing at least one carbon-carbon double bond, such as ethenylene, 1-propenylene, 2-propenylene, 2-methyl-1-propenylene, 1-butenylene, 2-butenylene, and the like. In various embodiments, alkenylene groups can either be unsubstituted or substituted with one or more substituents. Typically, alkenylene groups will comprise 2 to 9 carbon atoms (for example, 2 to 6 carbon atoms, 2 to 4 carbon atoms, or 2 carbon atoms).

As used herein, "alkynylene" means a bivalent branched, or straight chain chemical group containing only carbon and hydrogen and containing at least one carbon-carbon triple bond, such as ethynylene, 1-propynylene, 1-butynylene, 2-butynylene, and the like. In various embodiments, alkynylene groups can either be unsubstituted or substituted with one or more substituents. Typically, alkynylene groups will comprise 2 to 9 carbon atoms (for example, 2 to 6 carbon atoms, 2 to 4 carbon atoms, or 2 carbon atoms).

As used herein, "cycloalkyl" or "carbocyclyl" means a cyclic ring system containing only carbon atoms in the ring system backbone, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cyclohexenyl. Carbocyclyls may include multiple fused rings. Carbocyclyls may have any degree of saturation provided that at least one ring in the ring system is not aromatic. Carbocyclyl groups can either be unsubstituted or substituted with

one or more substituents. In some embodiments, carbocyclyl groups include 3 to 10 carbon atoms, for example, 3 to 6 carbon atoms.

As used herein, "cycloalkylene" means a bivalent cyclic ring system containing only carbon atoms in the ring system backbone, such as cyclopropylene, cyclobutylene, cyclopentylene, cyclohexylene, and cyclohexenylene. Cycloalkylenes may include multiple fused rings. Cycloalkylenes may have any degree of saturation provided that at least one ring in the ring system is not aromatic. Cycloalkylene groups can either be unsubstituted or substituted with one or more substituents. In some embodiments, cycloalkylene groups include 3 to 10 carbon atoms, for example, 3 to 6 carbon atoms.

As used herein, "aryl" means a mono-, bi-, tri- or polycyclic group with only carbon atoms present in the ring backbone having 5 to 14 ring atoms, alternatively 5, 6, 9, or 10 ring atoms; and having 6, 10, or 14 pi electrons shared in a cyclic array; wherein at least one ring in the system is aromatic. Aryl groups can either be unsubstituted or substituted with one or more substituents. Examples of aryl include fluorenyl, phenyl, naphthyl, tetrahydronaphthyl, 2,3-dihydro-1H-indenyl, and others. In some embodiments, the aryl is phenyl.

As used herein, "arylene" means a bivalent mono-, bi-, tri- or polycyclic group with only carbon atoms present in the ring backbone having 5 to 14 ring atoms, alternatively 5, 6, 9, or 10 ring atoms; and having 6, 10, or 14 pi electrons shared in a cyclic array; wherein at least one ring in the system is aromatic. Arylene groups can either be unsubstituted or substituted with one or more substituents. Examples of arylene include fluorenylene, phenylene, naphthylene, tetrahydronaphthyl, 2,3-dihydro-1H-indenylene, and others. In some embodiments, the aryl is phenylene.

As used herein, "arylalkylene" means an aryl-alkylene- group in which the aryl and alkylene moieties are as previously described. In some embodiments, arylalkylene groups contain a C_{1-4}alkylene moiety. Exemplary arylalkylene groups include benzyl and 2-phenethyl.

As used herein, the term "heteroaryl" means a mono-, bi-, tri- or polycyclic group having 5 to 14 ring atoms, alternatively 5, 6, 9, or 10 ring atoms; and having 6, 10, or 14 pi electrons shared in a cyclic array; wherein at least one ring in the system is aromatic,

and at least one ring in the system contains one or more heteroatoms independently selected from the group consisting of N, O, and S. Heteroaryl groups can either be unsubstituted or substituted with one or more substituents. Examples of heteroaryl include thienyl, pyridinyl, furyl, oxazolyl, oxadiazolyl, pyrrolyl, imidazolyl, triazolyl, thiodiazolyl, pyrazolyl, isoxazolyl, thiadiazolyl, pyranyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, thiazolyl benzothienyl, benzoxadiazolyl, benzofuranyl, benzimidazolyl, benzotriazolyl, cinnolinyl, indazolyl, indolyl, isoquinolinyl, isothiazolyl, naphthyridinyl, purinyl, thienopyridinyl, pyrido[2,3-*d*]pyrimidinyl, pyrrolo[2,3-*b*]pyridinyl, quinazolinyl, quinolinyl, thieno[2,3-*c*]pyridinyl, pyrazolo[3,4-*b*]pyridinyl, pyrazolo[3,4-*c*]pyridinyl, pyrazolo[4,3-*c*]pyridine, pyrazolo[4,3-*b*]pyridinyl, tetrazolyl, chromane, 2,3-dihydrobenzo[*b*][1,4]dioxine, benzo[*d*][1,3]dioxole, 2,3-dihydrobenzofuran, tetrahydroquinoline, 2,3-dihydrobenzo[*b*][1,4]oxathiine, and others. In some embodiments, the heteroaryl is selected from thienyl, pyridinyl, furyl, pyrazolyl, imidazolyl, pyranyl, pyrazinyl, and pyrimidinyl.

As used herein, the term "heteroarylene" means a bivalent mono-, bi-, tri- or polycyclic group having 5 to 14 ring atoms, alternatively 5, 6, 9, or 10 ring atoms; and having 6, 10, or 14 pi electrons shared in a cyclic array; wherein at least one ring in the system is aromatic, and at least one ring in the system contains one or more heteroatoms independently selected from the group consisting of N, O, and S. Heteroarylene groups can either be unsubstituted or substituted with one or more substituents.

As used herein, "halo", "halide" or "halogen" is a chloro, bromo, fluoro, or iodo atom radical. In some embodiments, a halo is a chloro, bromo or fluoro. For example, a halide can be fluoro.

As used herein, "haloalkyl" means a hydrocarbon substituent, which is a linear or branched, alkyl, alkenyl or alkynyl substituted with one or more chloro, bromo, fluoro, and/or iodo atom(s). In some embodiments, a haloalkyl is a fluoroalkyls, wherein one or more of the hydrogen atoms have been substituted by fluoro. In some embodiments, haloalkyls are of 1 to about 3 carbons in length (e.g., 1 to about 2 carbons in length or 1 carbon in length). The term "haloalkylene" means a diradical variant of haloalkyl, and such

diradicals may act as spacers between radicals, other atoms, or between a ring and another functional group.

As used herein, "heterocyclyl" means a nonaromatic cyclic ring system comprising at least one heteroatom in the ring system backbone. Heterocyclyls may include multiple fused rings. Heterocyclyls may be substituted or unsubstituted with one or more substituents. In some embodiments, heterocycles have 5-7 members. In six membered monocyclic heterocycles, the heteroatom(s) are selected from one to three of O, N or S, and wherein when the heterocycle is five membered, it can have one or two heteroatoms selected from O, N, or S. Examples of heterocyclyl include azirinyl, aziridinyl, azetidinyl, oxetanyl, thietanyl, 1,4,2-dithiazolyl, dihydropyridinyl, 1,3-dioxanyl, 1,4-dioxanyl, 1,3-dioxolanyl, morpholinyl, thiomorpholinyl, piperazinyl, pyranyl, pyrrolidinyl, tetrahydrofuryl, tetrahydropyridinyl, oxazinyl, thiazinyl, thiinyl, thiazolidinyl, isothiazolidinyl, oxazolidinyl, isoxazolidinyl, piperidinyl, pyrazolidinyl imidazolidinyl, thiomorpholinyl, and others. In some embodiments, the heterocyclyl is selected from azetidinyl, morpholinyl, piperazinyl, pyrrolidinyl, and tetrahydropyridinyl.

As used herein, "monocyclic heterocyclyl" means a single nonaromatic cyclic ring comprising at least one heteroatom in the ring system backbone. Heterocyclyls may be substituted or unsubstituted with one or more substituents. In some embodiments, heterocycles have 5-7 members. In six membered monocyclic heterocycles, the heteroatom(s) are selected from one to three of O, N or S, and wherein when the heterocycle is five membered, it can have one or two heteroatoms selected from O, N, or S. Examples of heterocyclyl include azirinyl, aziridinyl, azetidinyl, oxetanyl, thietanyl, 1,4,2-dithiazolyl, dihydropyridinyl, 1,3-dioxanyl, 1,4-dioxanyl, 1,3-dioxolanyl, morpholinyl, thiomorpholinyl, piperazinyl, pyranyl, pyrrolidinyl, tetrahydrofuryl, tetrahydropyridinyl, oxazinyl, thiazinyl, thiinyl, thiazolidinyl, isothiazolidinyl, oxazolidinyl, isoxazolidinyl, piperidinyl, pyrazolidinyl imidazolidinyl, thiomorpholinyl, and others.

The term "substituted" refers to moieties having substituents replacing a hydrogen on one or more non-hydrogen atoms of the molecule. It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that

the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. Substituents can include, for example, –(C_{1-9} alkyl) optionally substituted with one or more of hydroxyl, -NH_2, -NH(C_{1-3} alkyl), and –N(C_{1-3} alkyl)$_2$; -(C_{1-9} haloalkyl); a halide; a hydroxyl; a carbonyl [such as -C(O)OR, and -C(O)R]; a thiocarbonyl [such as -C(S)OR, -C(O)SR, and -C(S)R]; –(C_{1-9} alkoxyl) optionally substituted with one or more of halide, hydroxyl, -NH_2, -NH(C_{1-3} alkyl), and –N(C_{1-3} alkyl)$_2$; -OPO(OH)$_2$; a phosphonate [such as -PO(OH)$_2$ and -PO(OR')$_2$]; -OPO(OR')R"; -NRR'; -C(O)NRR'; -C(NR)NR'R"; -C(NR')R"; a cyano; a nitro; an azido; -SH; -S-R; -OSO$_2$(OR); a sulfonate [such as -SO$_2$(OH) and -SO$_2$(OR)]; -SO$_2$NR'R"; and -SO$_2$R; in which each occurrence of R, R' and R" are independently selected from H; –(C_{1-9} alkyl); C_{6-10} aryl optionally substituted with from 1-3R'''; 5-10 membered heteroaryl having from 1-4 heteroatoms independently selected from N, O, and S and optionally substituted with from 1-3 R'''; C_{3-7} carbocyclyl optionally substituted with from 1-3 R'''; and 3-8 membered heterocyclyl having from 1-4 heteroatoms independently selected from N, O, and S and optionally substituted with from 1-3 R'''; wherein each R''' is independently selected from –(C_{1-6} alkyl), –(C_{1-6} haloalkyl), a halide (e.g., F), a hydroxyl, -C(O)OR, -C(O)R, –(C_{1-6} alkoxyl), -NRR', -C(O)NRR', isothiocyanyl, and a cyano, in which each occurrence of R and R' is independently selected from H and –(C_{1-6} alkyl). In some embodiments, the substituent is selected from –(C_{1-6} alkyl), -(C_{1-6} haloalkyl), a halide (e.g., F), a hydroxyl, -C(O)OR, -C(O)R, –(C_{1-6} alkoxyl), -NRR', -C(O)NRR', and a cyano, in which each occurrence of R and R' is independently selected from H and –(C_{1-6} alkyl).

As used herein, pseudohalides or pseudohalo groups are groups that behave substantially similar to halides. Such compounds can be used in the same manner and treated in the same manner as halides. Pseudohalides include, but are not limited to, cyanide, cyanate, thiocyanate, selenocyanate, trifluoromethoxy, and azide.

As used herein, "sulfinyl" or "thionyl" refers to -S(O)-. As used herein, "sulfonyl" or "sulfuryl" refers to -S(O)$_2$-. As used herein, "sulfo" refers to -S(O)$_2$O-.

As used herein, "phosphinate" refers to –P(R)O$_2$H, and "phosphinate ester" refers to –P(R)O$_2$R'. As used herein, "phosphonate" refers to –PO$_3$H$_2$, and "phosphonate ester"

refers to -PO_3RR'. As used herein, "phosphodiester" refers to –OPO_3R-, and "alkylphosphodiester" refers to –OPO_3RR'. In the above groups, R is H or alkyl, and R' is alkyl.

The term "mammal" is used in its usual biological sense. Thus, it specifically includes humans, cattle, horses, monkeys, dogs, cats, mice, rats, cows, sheep, pigs, goats, and non-human primates, but also includes many other species.

"Patient" as used herein, means a human or a non-human mammal, e.g., a dog, a cat, a mouse, a rat, a cow, a sheep, a pig, a goat, a non-human primate, or a bird, e.g., a chicken, as well as any other vertebrate or invertebrate. In some embodiments, the patient is a human.

Design of Chelating Ligands

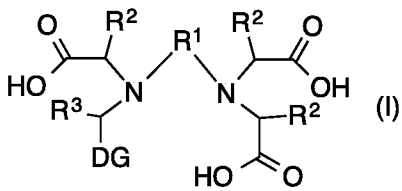
Provided herein are chelating ligands useful for preparing metal chelates having high relaxivity. In some embodiments, chelating ligands can be used to prepare non-specific metal chelates having high relaxivity. In some embodiments, the chelating ligands can be modified to incorporate one or more target binding moieties (TBMs). Chelating ligands having one or more target binding moieties can allow the chelating ligands (and metal chelates) to be targeted to one or more sites *in vivo*. Chelating ligands and metal chelates can be modified to incorporate self-assembling moieties (SAMs), which are groups that promote the self-assembly of the chelates into micelles, liposomes, emulsions, etc. In some embodiments, chelating ligands and metal chelates are also useful as luminescent probes for use in high-throughput, multiplex, and/or real-time detection and analysis of biological molecules (e.g., immunoassays or real-time PCR applications).

Chelating ligands described herein are based on derivatives of diamine functionalized backbones. Derivatives are prepared by modifying the amine moieties of the scaffold with from one to three R groups and one or more heterocycle based donor groups ("DG"). The carbon that links the backbone amine and DG can be functionalized with an additional "R" group. Typically, R groups and DGs are able to coordinate a metal ion. R groups and DGs can be selected for their ability to enhance the relaxivity of the

chelating ligand when in a metal chelate form and/or to promote a specific oxidation state of the metal ion. Relaxivity may be enhanced, for example, by a DG's effect on the water exchange rate of a metal chelate; its ability to decrease the electronic relaxation rate of the metal ion; its ability to prevent anion coordination (e.g., by electrostatic repulsion); or its ability to trap a biochemically generated oxidation state. In some embodiments, a DG can also incorporate a second sphere moiety (SSM), a group that increases relaxivity by its ability to coordinate a second sphere of water (e.g., by hydrogen bonding). In some embodiments, a DG can also incorporate a TBM, optionally through a linker (L) as discussed below. Relaxivity can be further enhanced by binding to the target of the TBM or by forming self-assembled systems such as liposomes.

A variety of chelating ligands can be prepared.

In some embodiments, the chelating ligand is a compound of Formula (I):



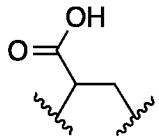
(I)

or a pharmaceutically acceptable salt thereof,

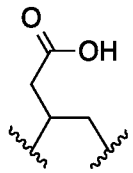
R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups.In some embodiments, the R^1 moiety is bound to the adjacent nitrogens through adjacent atoms, such as adjacent carbon atoms (e.g., binding through the 1 and 2 carbons on ethylene) or through atoms separated by a single methylene (e.g., binding through the 1 and 3 carbons on propylene). Similar binding may be observed with the cyclic moieties of R^1, for example, binding through adjacent carbon atoms (e.g., binding through the 1 and 2 carbons on cyclohexylene).

In some embodiments, R^1 is selected from the group consisting of a substituted or unsubstituted alkylene, such as 1,3-propylene or 1,2-ethylene, as shown below:

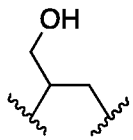
or 2,3-propylene-1-carboxylate, as shown below,



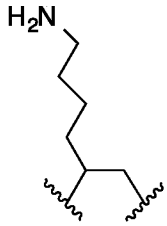
or 3,4-butylene-1-carboxylic acid, as shown below,



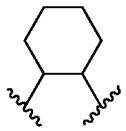
or 1-hydroxy-3,4-butylene, as shown below,



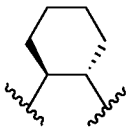
or 1-amino-5,6-hexylene, as shown below,



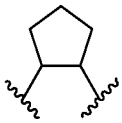
substituted or unsubstituted cycloalkylene, such as those shown below, for example, cis- or trans-1,2-cyclohexylene, as shown below,



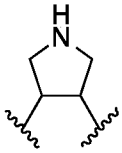
or trans-1,2-cyclohexylene, as shown below,



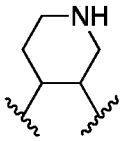
or cis- or trans-1,2-cyclopentylene, as shown below,



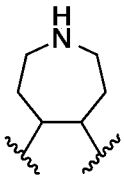
substituted or unsubstituted monocyclic heterocyclyl, such as 2,5-dihydro-1*H*-pyrrolene, as shown below



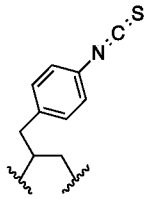
1,2,3,6-tetrahydropyridinene, as shown below,



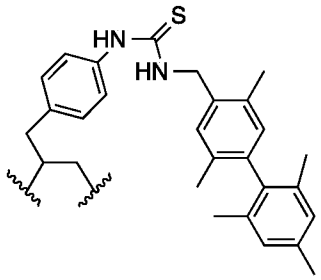
2,3,6,7-tetrahydro-1*H*-azepinene, as shown below,



and any corresponding isomers of the substituted or unsubstituted monocyclic heterocyclyl compounds, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, and substituted or unsubstituted arylalkylene, such as 1-propylene-4-isothiocyanatobenzene, as shown below,



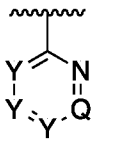
In some embodiments, R^1 can be substituted with [L]-[TBM], such as 1-propylene-benzene functionalized with a thiourea (–NH-C(S)-NH-) [L] and the albumin targeting substituted arylalkylene TBM, 2,2',4,4',5,6'-hexamethyl-1,1'-biphenyl, as shown below,



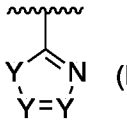
each R^2 and R^3 is independently selected from the group consisting of H, CO_2H, (C_1-C_6alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and [L]-[TBM];

each R^4 and R^5 are independently selected from the group consisting of H, substituted or unsubstituted C_1-C_6 alkyl, and [L]-[TBM]; and

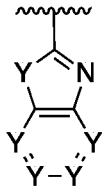
DG is selected from the group consisting of:


(II)

;

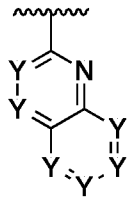

(III)

;


(IV)

;

and


(V)

or any constitutional isomers of Formulas IV and V, wherein

Y is CH, CZ, N, O, S or NR^4;

each Q is independently CH, CZ, N, O, S, or NR^4; and

Z is selected from the group consisting of H, OH, OR^4, CO_2R^4, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

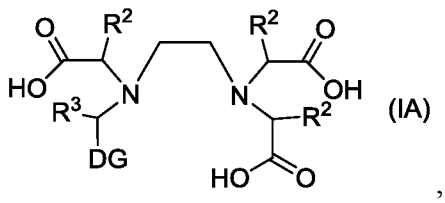
L is a linker;

TBM is a target binding moiety; and

each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, isothiocyanyl, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, wherein the phenyl may be substituted or unsubstituted, and -[L]-[TBM];

In some embodiments, if Q is CH, CCOOH, or CCH_2-(4-nitrobenzylsulfonamide) and all Y are CH, than at least one of R^2 or R^3 is not H.

In some embodiments, a compound of Formula (I) is a compound of Formula (IA):

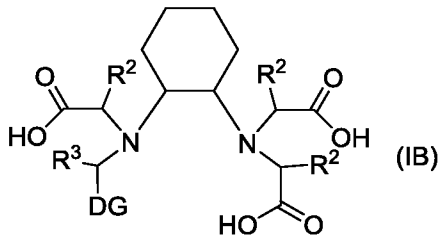


(IA)

,

or a pharmaceutically acceptable salt thereof, wherein R^2, R^3, and DG are as defined for Formula (I).

In some embodiments, a compound of Formula (I) is a compound of Formula (IB):

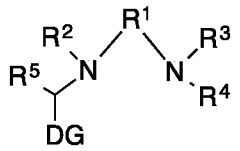


(IB)

,

or a pharmaceutically acceptable salt thereof, wherein R^2, R^3, and DG are as defined for Formula (I).

In some embodiments, the chelating ligand is a compound of Formula (VI):



(VI)

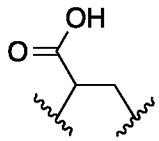
or a pharmaceutically acceptable salt thereof,

R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups.

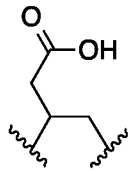
In some embodiments, the R^1 moiety is bound to the adjacent nitrogens through adjacent carbon atoms (e.g., binding through the 1 and 2 carbons on ethylene) or through carbons separated by a single methylene (e.g., binding through the 1 and 3 carbons on propylene). Similar binding may be observed with the cyclic moieties of R^1, for example, binding through adjacent carbon atoms (e.g., binding through the 1 and 2 carbons on cyclohexylene).

In some embodiments, R^1 is selected from the group consisting of a substituted or unsubstituted alkylene, such as 1,3-propylene or 1,2-ethylene, as shown below, :

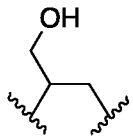
or 2,3-propylene-1-carboxylate, as shown below,



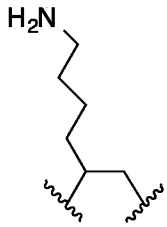
or 3,4-butylene-1-carboxylic acid, as shown below,



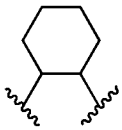
or 1-hydroxy-3,4-butylene, as shown below,



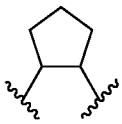
or 1-amino-5,6-hexylene, as shown below,



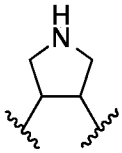
such as cis- or trans-1,2-cyclohexylene, as shown below,



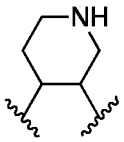
or cis- or trans-1,2-cyclopentylene, as shown below,



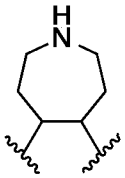
substituted or unsubstituted monocyclic heterocyclyl, such as 2,5-dihydro-1*H*-pyrrolene, as shown below



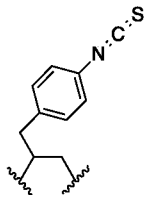
1,2,3,6-tetrahydropyridinene, as shown below,



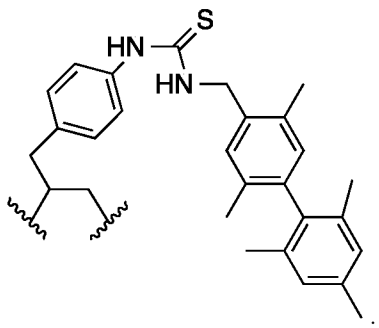
2,3,6,7-tetrahydro-1*H*-azepinene, as shown below,



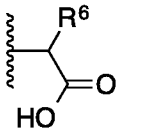
and any corresponding isomers of the substituted or unsubstituted monocyclic heterocyclyl compounds, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, and substituted or unsubstituted arylalkylene, such as 1-propylene-4-isothiocyanatobenzene, as shown below,

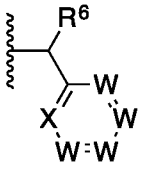


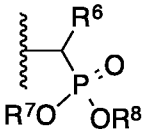
In some embodiments, R^1 can be substituted with [L]-[TBM], such as 1-propylene-benzene functionalized with a thiourea (–NH-C(S)-NH-) [L] and the albumin targeting substituted arylalkylene TBM, 2,2',4,4',5,6'-hexamethyl-1,1'-biphenyl, as shown below,



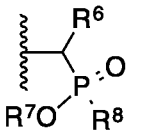
R^2, R^3, and R^4 are independently selected from the group of compounds of formula:


(VII)
;


(VIII)
;


(IX)
;

or


(X)

wherein each R^5 and R^6 are independently selected from the group consisting of is independently selected from the group consisting of H, CO_2H, (C_1-C_6alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7, $C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and [L]-[TBM];

X is CZ, N, O, S or NR^4;

W is CH, CZ, N, O, S, or NR^4; and

each Z is independently selected from H, OH, OR^7, CO_2R^7, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7, $C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

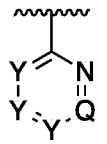
L is a linker;

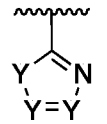
TBM is a target binding moiety; and

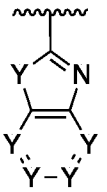
each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM];

each R^7 and R^8 are independently selected from the group consisting of H, substituted or unsubstituted C_1-C_6 alkyl, and [L]-[TBM]; and

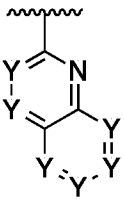
DG is selected from the group consisting of:


(XI)
;


(XII)
;


(XIII)
;

and


(XIV)

or any constitutional isomers of Formulas XIII-XIV,

wherein each Y is independently CH, CZ^1, N, O, S, or NR^4

each Q is independently CH, CZ^1, N, O, S, or NR^4; and

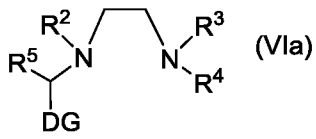
each Z^1 is independently selected from the group consisting of H, OH, OR^7, CO_2R^7, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl,

C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7, $C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups..

In some embodiments, if Q is CH, CCOOH, or CCH_2-(4-nitrobenzylsulfonamide), all Y are CH, and all of R^2, R^3, and R^4 are formula VII, than at least one of R^5 or R^6 is not H.

In some embodiments, if one of R^2, R^3, or R^4 is formula VIII, and all of R^5 and R^6 are H, than the aromatic ring component of formula VIII (i.e. the ring containing X and W) must be different than DG.

In some embodiments, a compound of Formula (VI) is a compound of Formula (XVa):

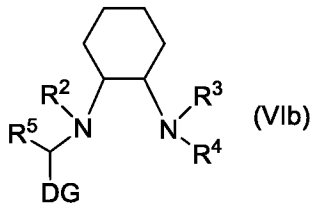


(VIa)

,

or a pharmaceutically acceptable salt thereof, wherein R^2, R^3, R^4, R^5 and DG [4] are as defined for Formula (VI).

In some embodiments, a compound of Formula (VI) is a compound of Formula (XVb):

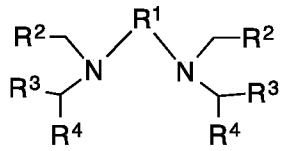


(VIb)

,

or a pharmaceutically acceptable salt thereof, wherein R^2, R^3, R^4, R^5 and DG are as defined for Formula (VI).

In some embodiments, the chelating ligand is a compound of Formula XV



(XV)

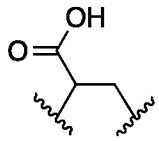
or a pharmaceutically acceptable salt thereof,

R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups.

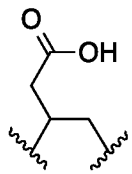
In some embodiments, the R^1 moiety is bound to the adjacent nitrogens through adjacent carbon atoms (e.g., binding through the 1 and 2 carbons on ethylene) or through carbons separated by a single methylene (e.g., binding through the 1 and 3 carbons on propylene). Similar binding may be observed with the cyclic moieties of R^1, for example, binding through adjacent carbon atoms (e.g., binding through the 1 and 2 carbons on cyclohexylene).

In some embodiments, R^1 is selected from the group consisting of a substituted or unsubstituted alkylene, such as 1,3-propylene or 1,2-ethylene, as shown below, :

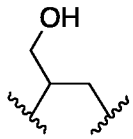
or 2,3-propylene-1-carboxylate, as shown below,



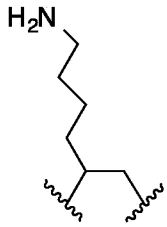
or 3,4-butylene-1-carboxylic acid, as shown below,



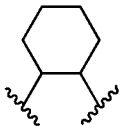
or 1-hydroxy-3,4-butylene, as shown below,



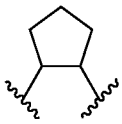
or 1-amino-5,6-hexylene, as shown below,



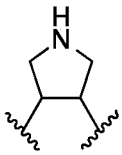
such as cis- or trans-1,2-cyclohexylene, as shown below,



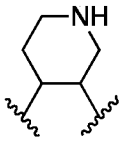
or cis- or trans-1,2-cyclopentylene, as shown below,



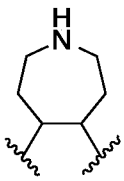
substituted or unsubstituted monocyclic heterocyclyl, such as 2,5-dihydro-1*H*-pyrrolene, as shown below



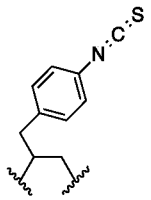
1,2,3,6-tetrahydropyridinene, as shown below,



2,3,6,7-tetrahydro-1*H*-azepinene, as shown below,

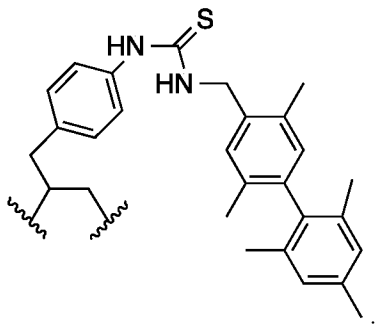


and any corresponding isomers of the substituted or unsubstituted monocyclic heterocyclyl compounds, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, and substituted or unsubstituted arylalkylene, such as 1-propylene-4-isothiocyanatobenzene, as shown below,



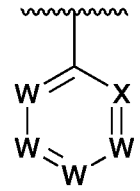
.

In some embodiments, R^1 can be substituted with [L]-[TBM], such as 1-propylene-benzene functionalized with a thiourea (–NH-C(S)-NH-) [L] and the albumin targeting substituted arylalkylene TBM, 2,2',4,4',5,6'-hexamethyl-1,1'-biphenyl, as shown below,



.

each R^2, R^3, and R^4 are independently selected from the group consisting of CO_2H, $(C(O)NR^5R^6$, CH_2NHCOR^5, $C(O)N(OH)R^5$, $C(O)NHSO_2R^5$, $CH_2NHSO_2R^5$, $N(OH)C(O)R^5$, $P(R^5)O_2R^6$, and $PO_3R^5R^6$, and compounds of formula



(XVI)

;

wherein X is a CZ, N, O, S, or NR^4;

each W is independently CH, CZ, N, O, S, or NR^4; and

each Z is independently selected from H, OH, OR^5, CO_2R^5, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered

heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^5R^6$, CH_2NHCOR^5, $C(O)N(OH)R^5$, $C(O)NHSO_2R^5$, $CH_2NHSO_2R^5$, $N(OH)C(O)R^5$, $P(R^5)O_2R^6$, $PO_3R^5R^6$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

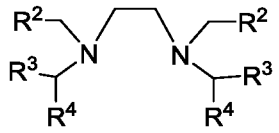
L is a linker;

TBM is a target binding moiety; and

each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM];and

each R^5 and R^6 are independently selected from the group consisting of H, substituted or unsubstituted C_1-C_6 alkyl, and [L]-[TBM].

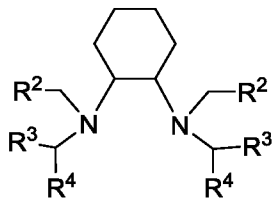
In some embodiments, a compound of Formula (XV) is a compound of Formula (XVa):


(XVa)

,

or a pharmaceutically acceptable salt thereof, wherein R^2, R^3, and R^4 are as defined for Formula (XV).

In some embodiments, a compound of Formula (XV) is a compound of Formula (XVb):

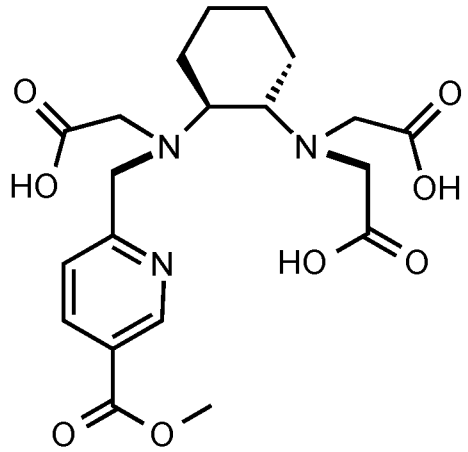

(XVb)

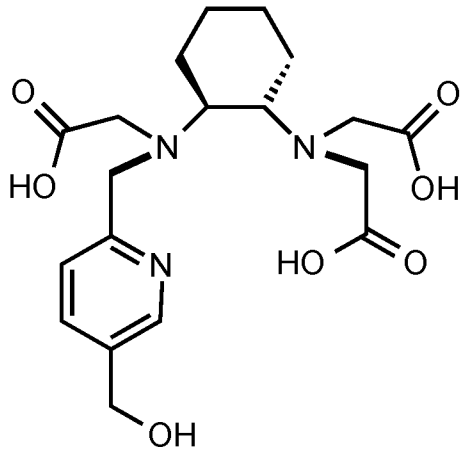
,

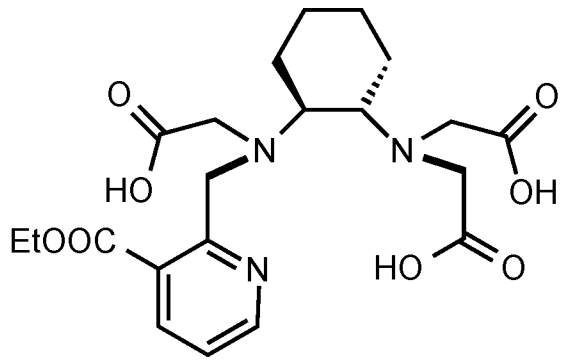
or a pharmaceutically acceptable salt thereof, wherein R^2, R^3, and R^4 are as defined for Formula (XV).

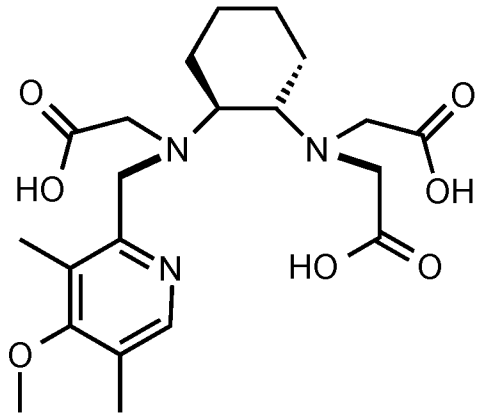
In some embodiments, the DG has been modified to target blood plasma proteins such as serum albumin.

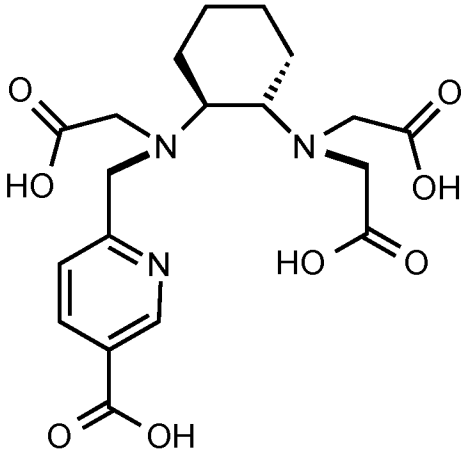
Non-limiting examples of chelating ligands as provided herein include:

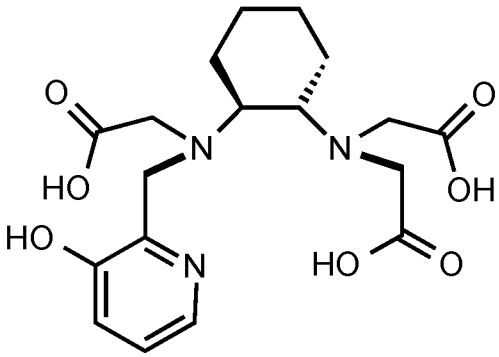


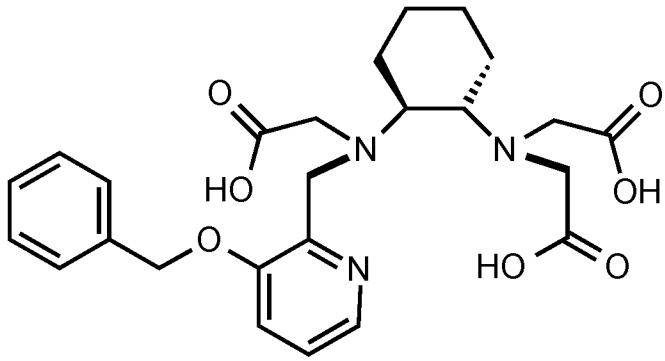


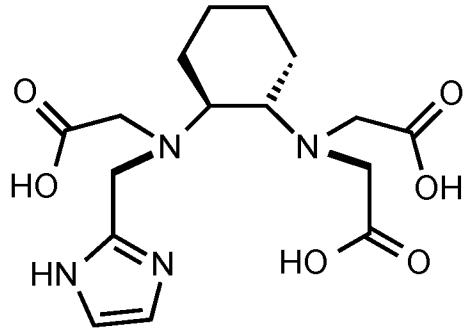


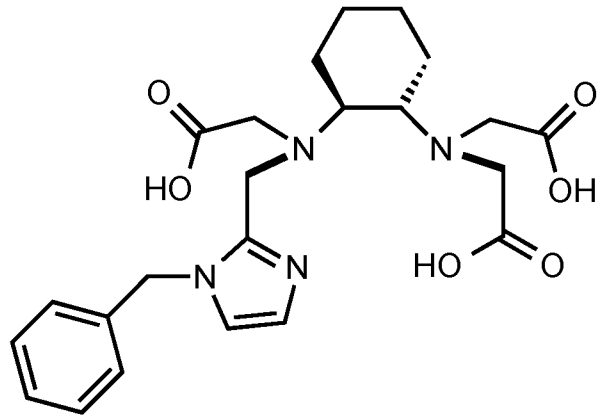


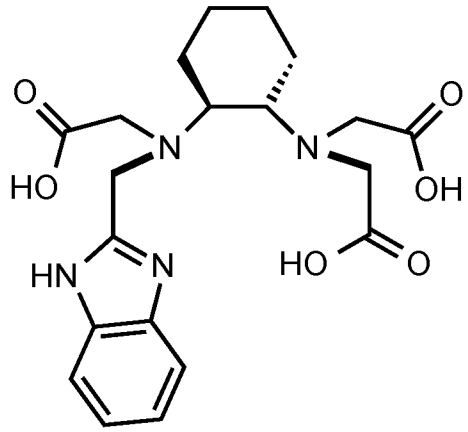




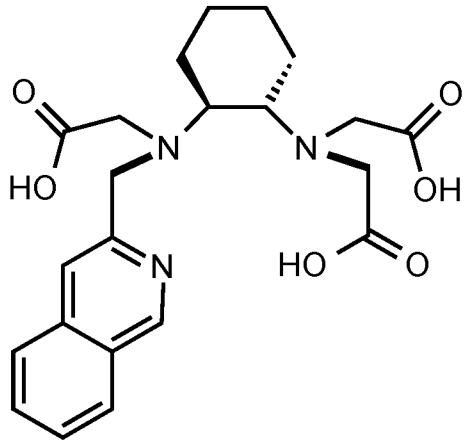






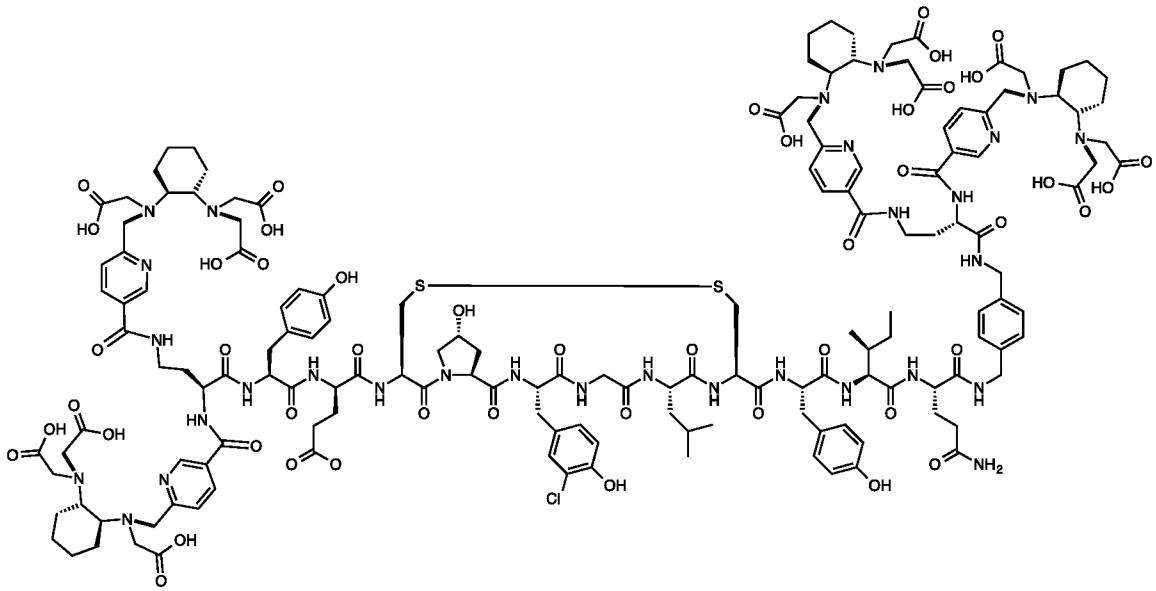


, and



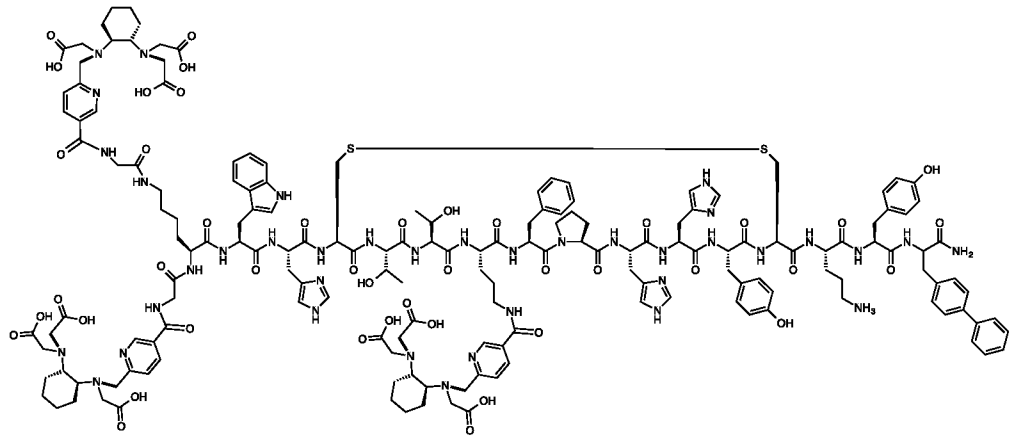
or a pharmaceutically acceptable salt thereof.

In another example embodiment, one or more (e.g., 2, 3, 4, 5, or 6) chelating ligands are linked to a TBM that has high affinity for fibrin. For example, such a compound can have the formula shown below:



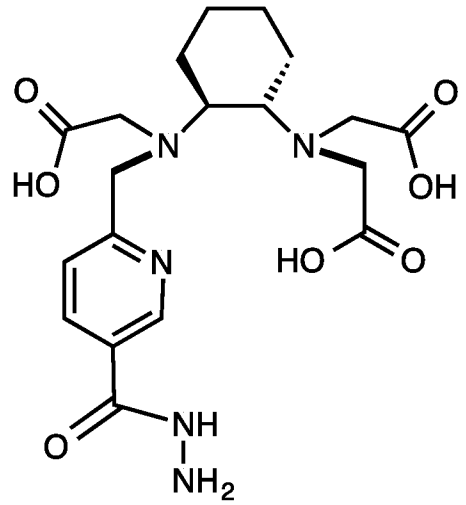
or a pharmaceutically acceptable salt thereof.

In another embodiment, one or more (e.g., 2, 3, 4, 5, or 6) chelating ligands are linked to a TBM that has high affinity for collagen. For example, one such compound can have the formula shown below:



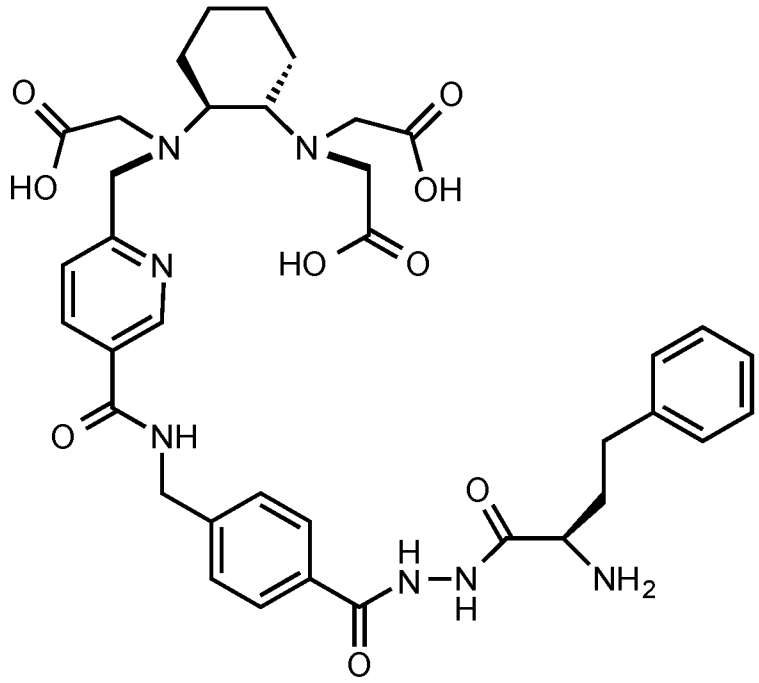
or a pharmaceutically acceptable salt thereof.

In another embodiment, the chelating ligand linked to a TBM that has high affinity for protein carbonyls, such as hydrazides. For example, one chelating ligand has the formula shown below



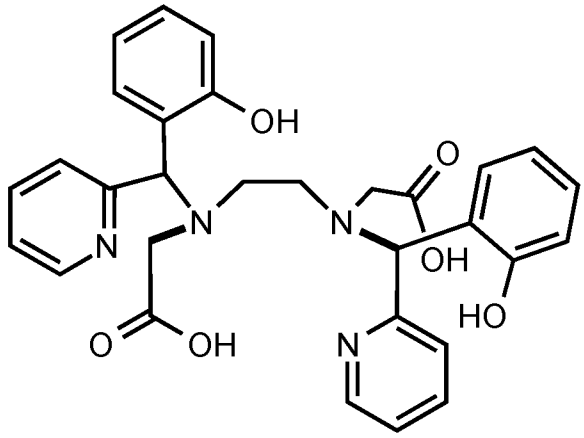
or a pharmaceutically acceptable salt thereof.

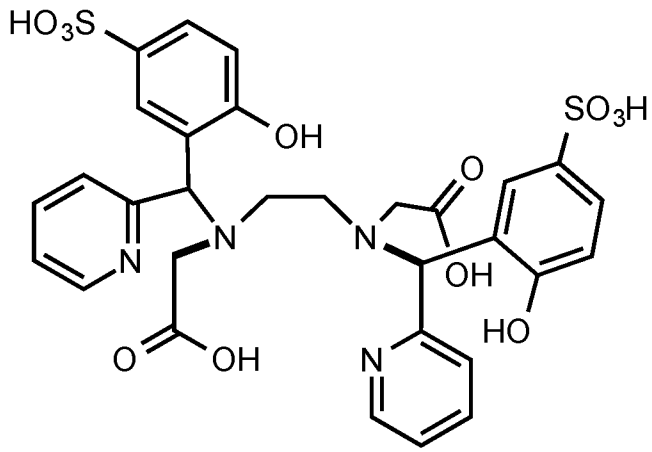
In another embodiment, the chelating ligand linked to a TBM that has high affinity for elastin. For example, one chelating ligand has the formula shown below

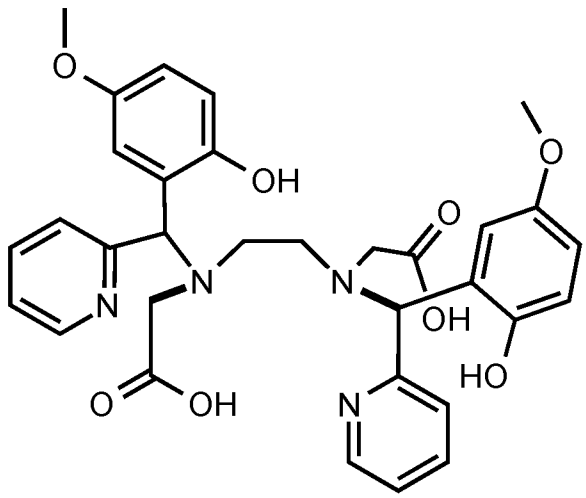


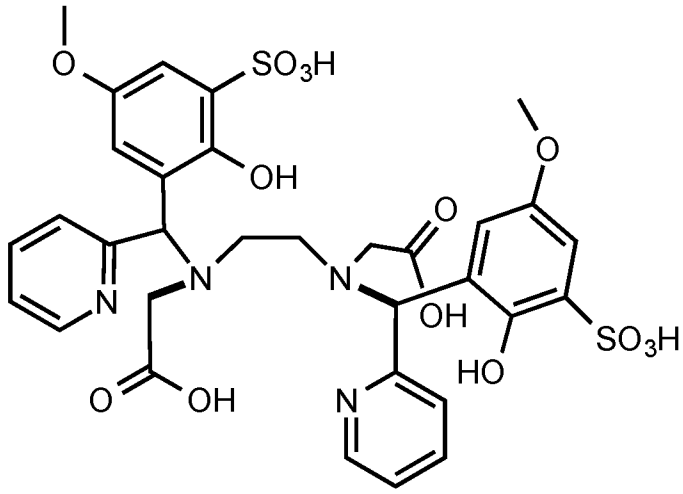
or a pharmaceutically acceptable salt thereof.

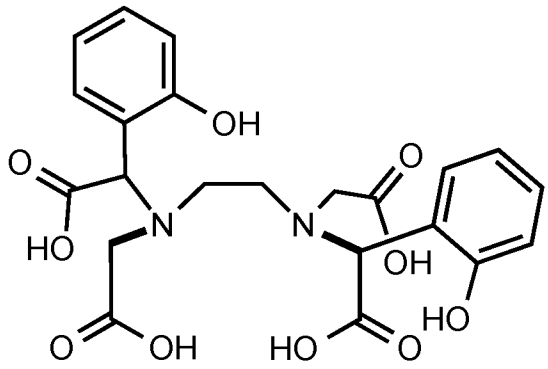
In another embodiment, the chelating ligand has DG and R that favor binding of Mn(II), but also ancillary R that favor binding of Mn(III), wherein the R groups favoring Mn(II) in each chelate or chelating ligand are different from those that favor Mn(III). For example, when M=Mn, R^3 groups that favor Mn(II) can be independently selected from COOH, PO_3H_2, or formula XVI where X = N, while R^4 groups that favor Mn(III) can be independently selected from COOH, PO_3H_2, or formula XVI where X = C(OH). Non-limiting examples of such compounds include:

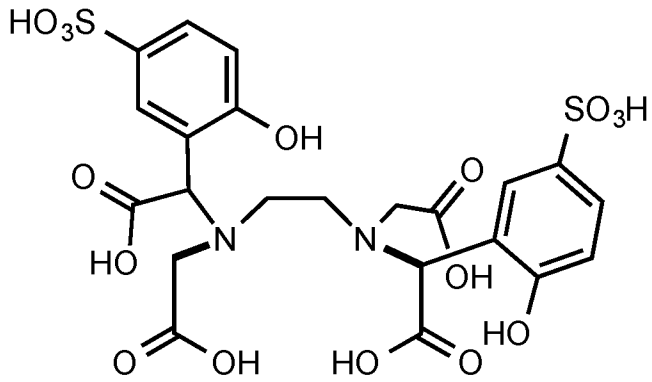


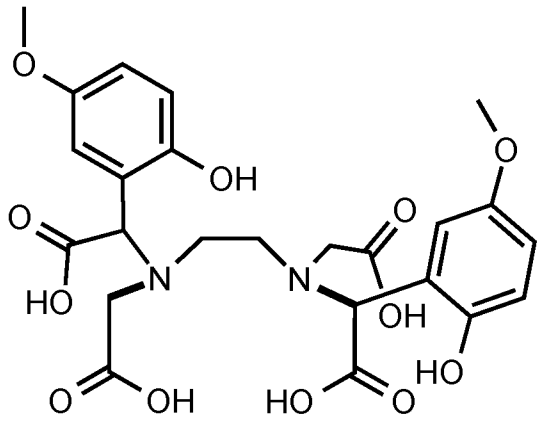




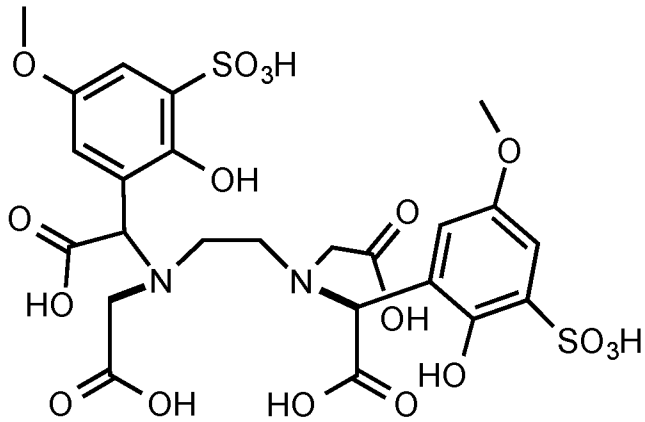








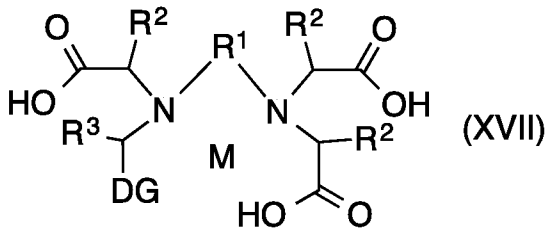
, and



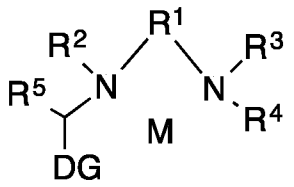
or a pharmaceutically acceptable salt thereof.

The synthetic protocols used to the prepare chelating ligands are general and can be broadly extended to include additional embodiments.

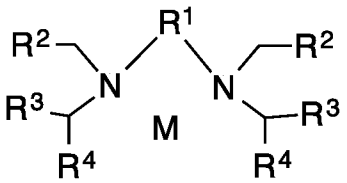
In some embodiments, the chelating ligand is coordinated to a metal to form a compound of general formula as follows:



(XVII)

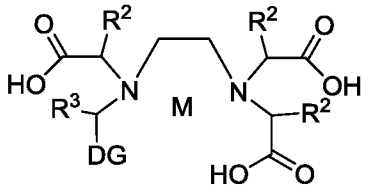


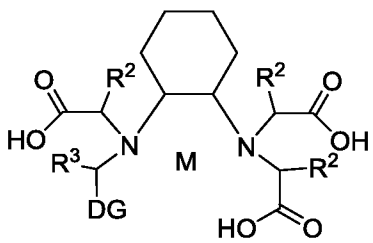
(XVIII)

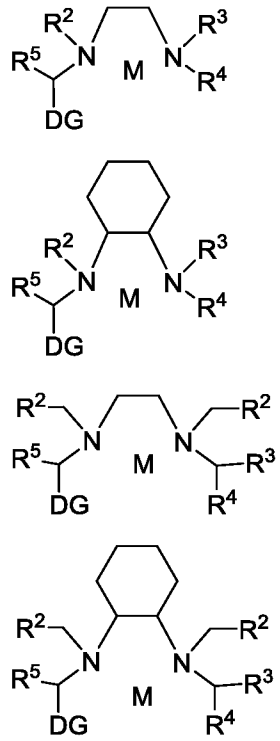


(XIX)

or a pharmaceutically acceptable salt thereof, wherein all moieties are as defined above. For example, the chelating ligand coordinated to a metal can form a caompound of general formula:





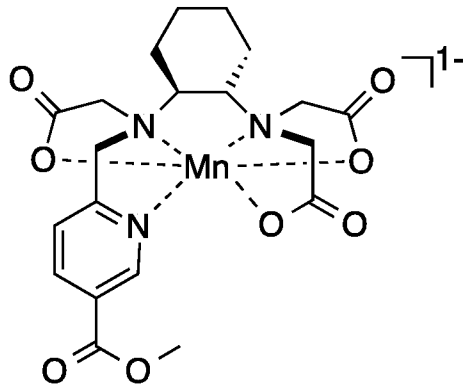


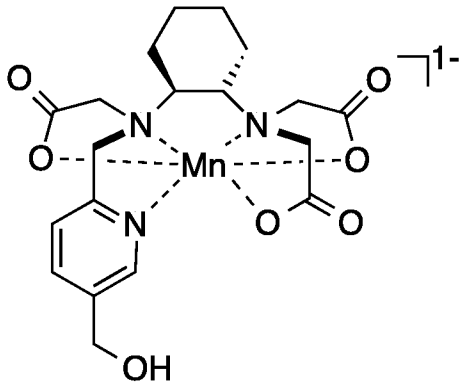
or a pharmaceutically acceptable salt thereof.

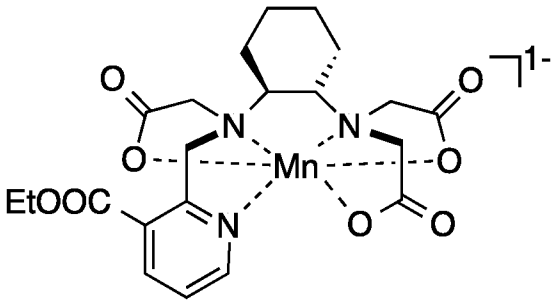
In such embodiments, M can be a stable or unstable isotope selected from Gd(III), Fe(III), Mn(II), Mn(III), Cr(III), Cu(II), Cu(III), Dy(III), Ho(III), Er(III), Pr(III), Eu(II), Eu(III), Nd(III), La(III), Lu(III), Sm(III), Tb(III), Tb(IV), Tm(III), Y(III), In(III), Ga(III), Tc(III), Tc(IV), Tc(V), Re(III), Re(IV), Re(V), Bi(III), and Yb(III). In some embodiments, M is Mn(II) or Mn(III).

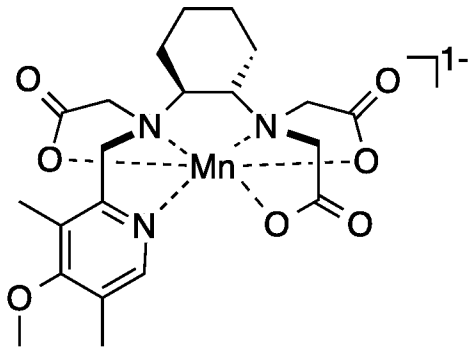
In some embodiments, the DG of the chelate-metal complex has been modified to target blood plasma proteins such as serum albumin.

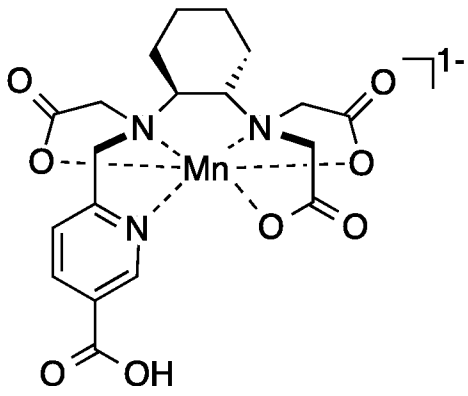
Non-limiting examples of metal chelates as provided herein include:

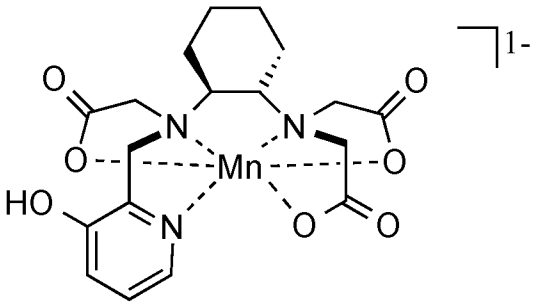


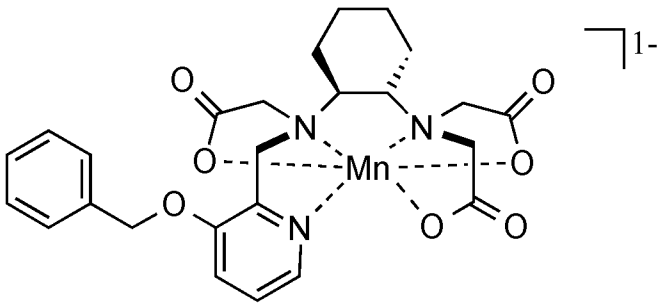


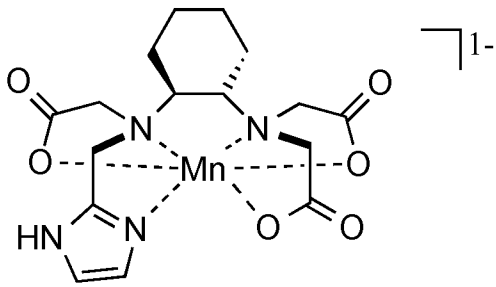


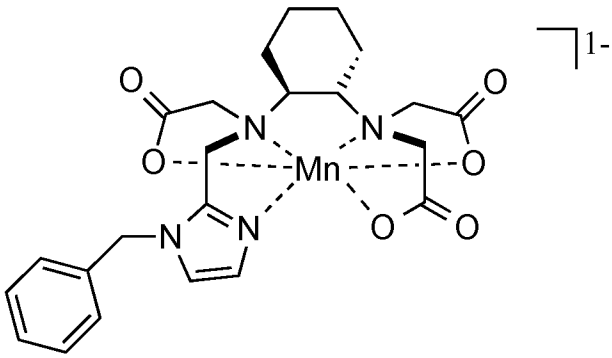


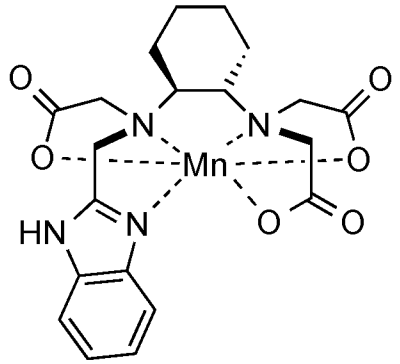



HO
N
N
Mn
O
O
O
O
O
O
O
N
1-

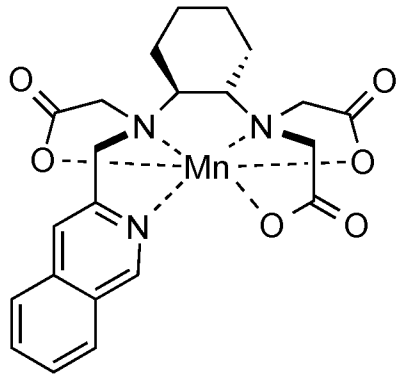

O
N
N
Mn
O
O
O
O
O
O
N
1-


HN
N
N
Mn
O
O
O
O
O
O
O
1-


N
N
N
Mn
O
O
O
O
O
O
O
1-

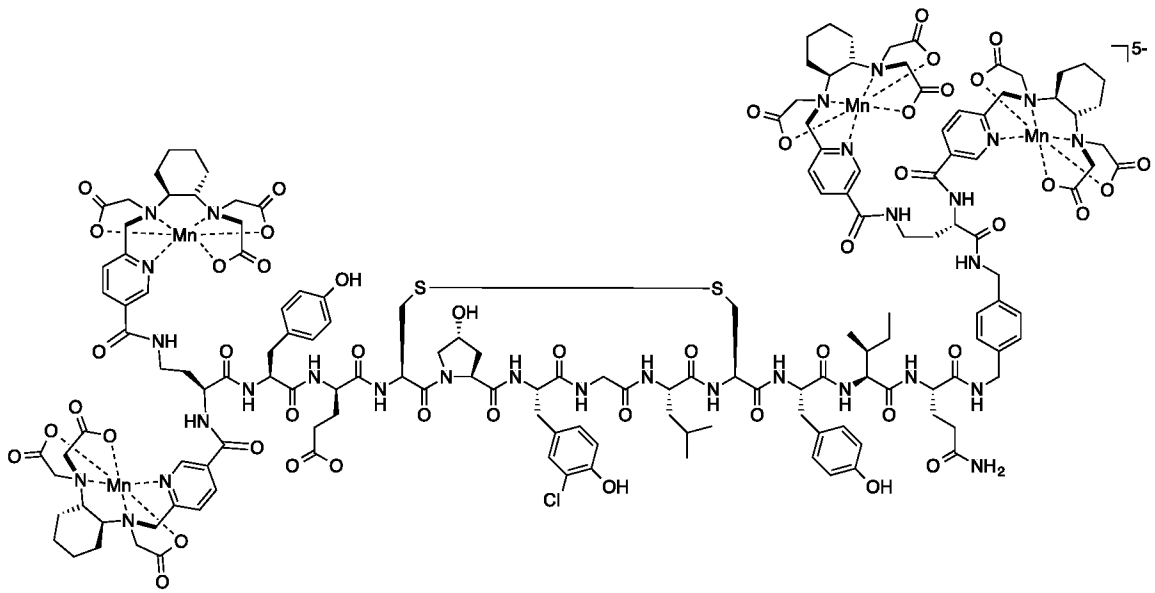


, and



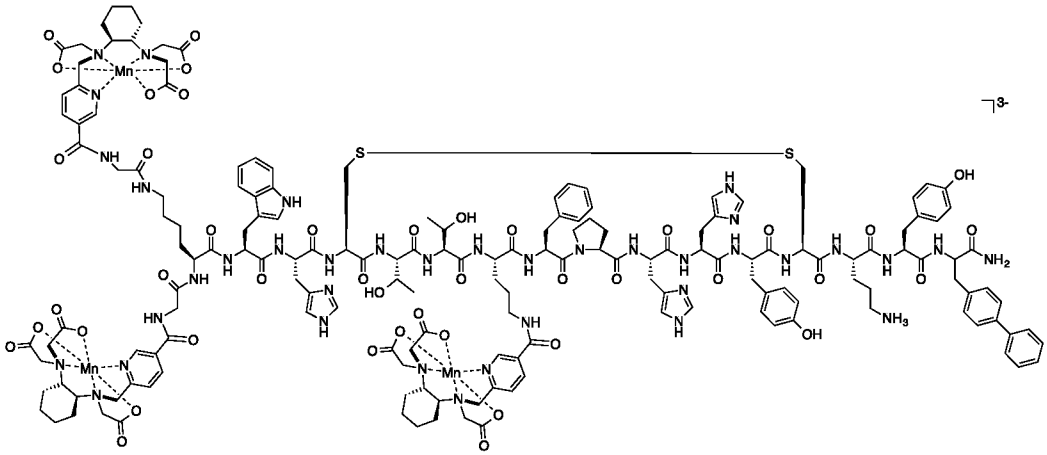
or a pharmaceutically acceptable salt thereof.

In another example embodiment, one or more (e.g., 2, 3, 4, 5, or 6) chelate-metal complexes are linked to a TBM that has high affinity for fibrin. For example, one such chelate-metal complex has the formula shown below



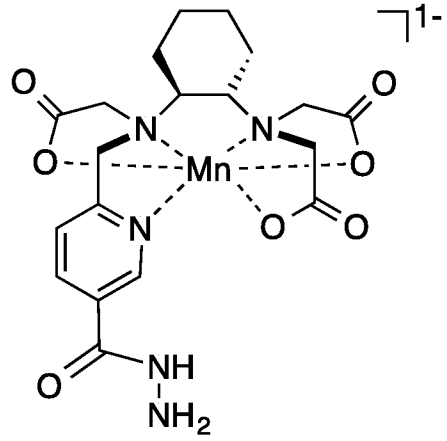
or a pharmaceutically acceptable salt thereof.

In another embodiment, one or more (e.g., 2, 3, 4, 5, or 6) chelate-metal complexes are linked to a TBM that has high affinity for collagen. For example, one chelate-metal complex has the formula shown below:



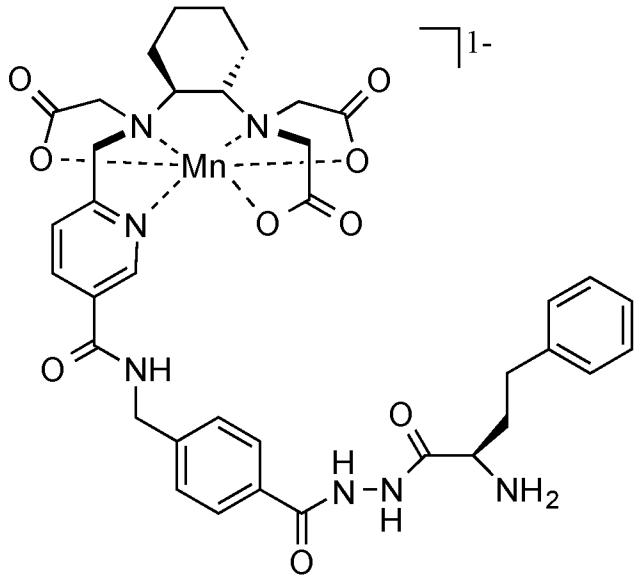
or a pharmaceutically acceptable salt thereof.

In another embodiment, the chelating ligand linked to a TBM that has high affinity for protein carbonyls, such as hydrazides. For example, one chelating ligand has the formula shown below



or a pharmaceutically acceptable salt thereof.

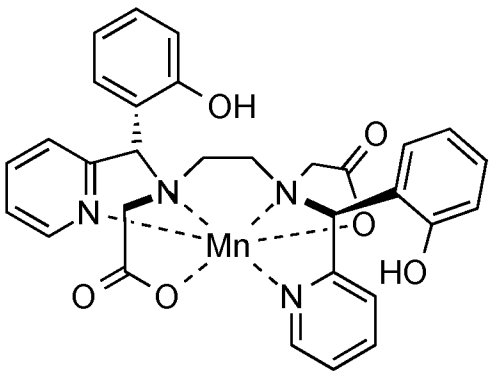
In another embodiment, the chelating ligand linked to a TBM that has high affinity for elastin. For example, one chelating ligand has the formula shown below



or a pharmaceutically acceptable salt thereof.

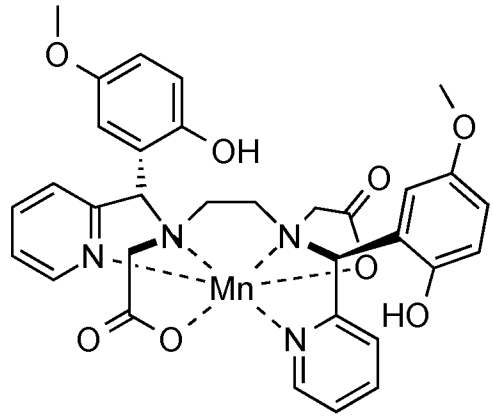
In another embodiment, the chelate-metal complex has DG and R that favor binding of Mn(II), but also ancillary R that favor binding of Mn(III), wherein the R groups favoring Mn(II) in each chelate or chelating ligand are different from those that favor Mn(III). For example in formula XIX, when M=Mn, R groups that favor Mn(II) can be independently selected from COOH, PO_3H_2, or formula XVI where X = N, while those that favor Mn(III) can be independently selected from formula XVI where X = C(OH). Upon oxidation of Mn(II) to Mn(III), the R groups that favor Mn(III) bind to the oxidized Mn(III) ion. Switching from Mn(II) to Mn(III) results in decreased relaxivity.

In one example embodiment, the chelate-metal complex has the formula shown below



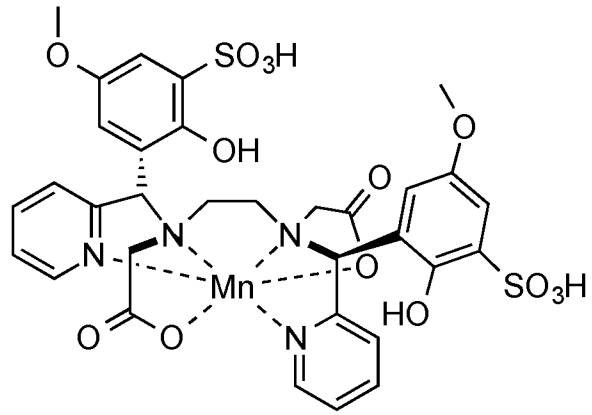
or a pharmaceutically acceptable salt thereof.

In another example embodiment, the chelate-metal complex has the formula shown below



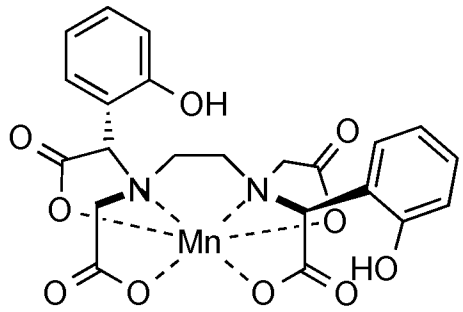
or a pharmaceutically acceptable salt thereof

In another example embodiment, the chelate-metal complex has the formula shown below



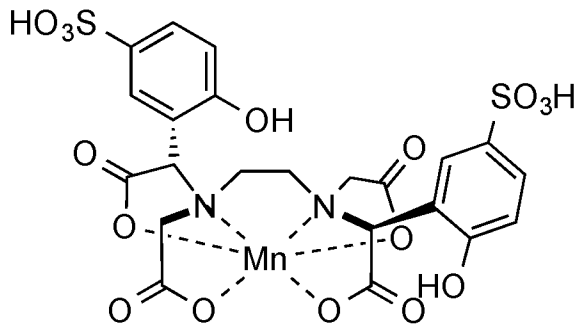
or a pharmaceutically acceptable salt thereof

In another example embodiment, the chelate-metal complex has the formula shown below



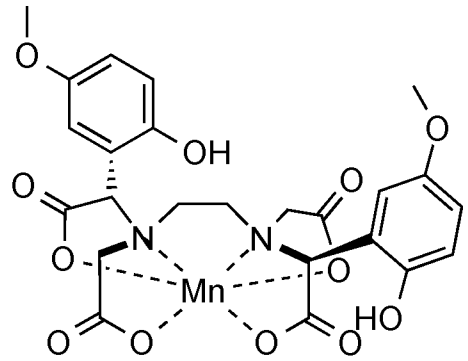
or a pharmaceutically acceptable salt thereof

In another example embodiment, the chelate-metal complex has the formula shown below



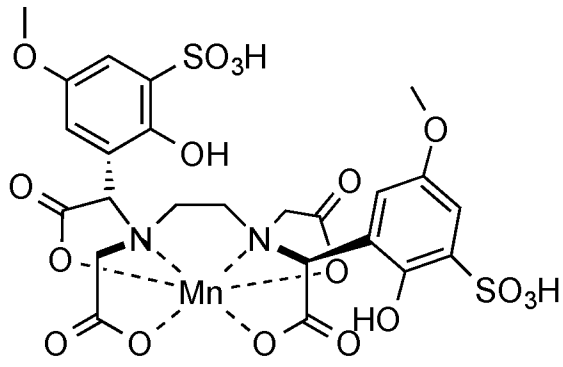
or a pharmaceutically acceptable salt thereof

In another example embodiment, the chelate-metal complex has the formula shown below



or a pharmaceutically acceptable salt thereof

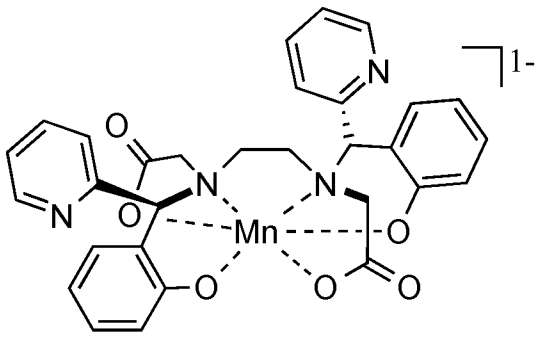
In another example embodiment, the chelate-metal complex has the formula shown below



or a pharmaceutically acceptable salt thereof

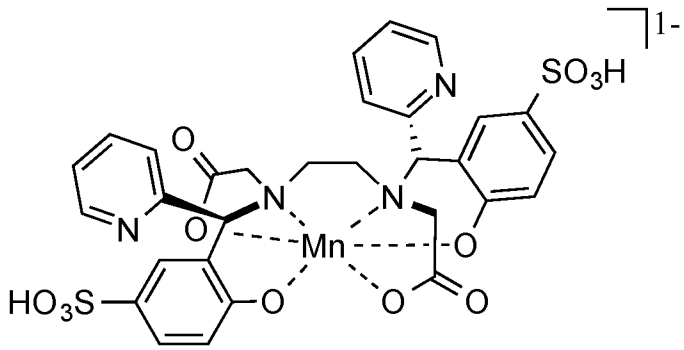
In a related fashion, the chelate-metal complex has DG and R that favor binding of Mn(III), but also ancillary R that favor binding of Mn(II), wherein the R groups favoring Mn(III) in each chelate or chelating ligand are different from those that favor Mn(II). For example, when M=Mn, R^4 groups that favor Mn(III) can be independently selected from formula XVI where X = C(OH), while R^3 groups that favor Mn(II) can be independently selected from COOH, PO_3H_2, or formula XVI where X = N. Upon reduction of Mn(III) to Mn(II), the R groups that favor Mn(II) bind to the reduced Mn(II) ion. Switching from Mn(III) to Mn(II) results in increased relaxivity.

In one example embodiment, the chelate-metal complex has the formula shown below



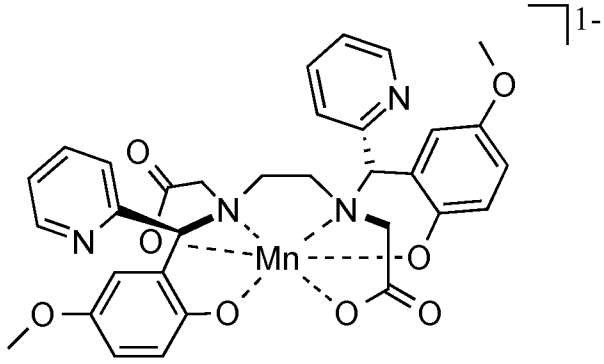
or a pharmaceutically acceptable salt thereof.

In another example embodiment, the chelate-metal complex has the formula shown below



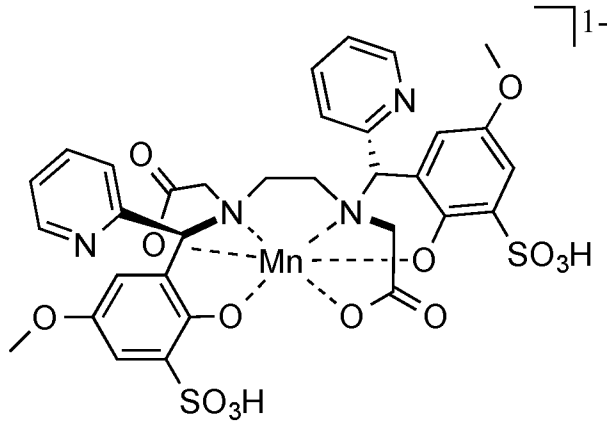
or a pharmaceutically acceptable salt thereof

In another example embodiment, the chelate-metal complex has the formula shown below



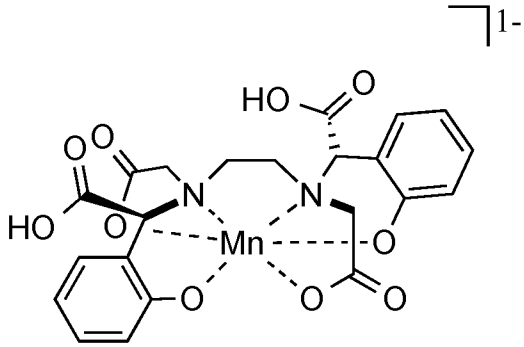
or a pharmaceutically acceptable salt thereof

In another example embodiment, the chelate-metal complex has the formula shown below



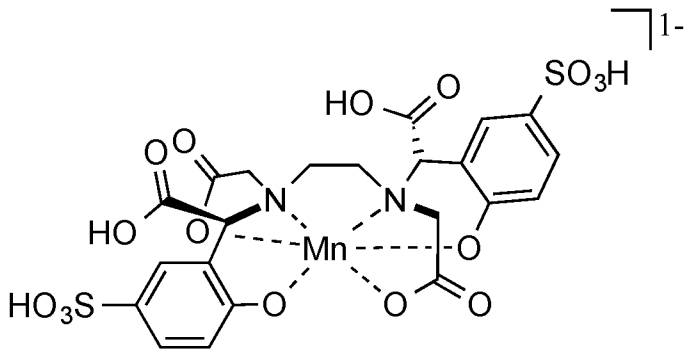
or a pharmaceutically acceptable salt thereof

In another example embodiment, the chelate-metal complex has the formula shown below



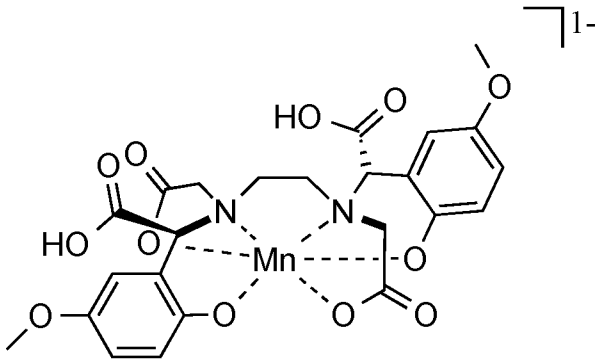
or a pharmaceutically acceptable salt thereof

In another example embodiment, the chelate-metal complex has the formula shown below



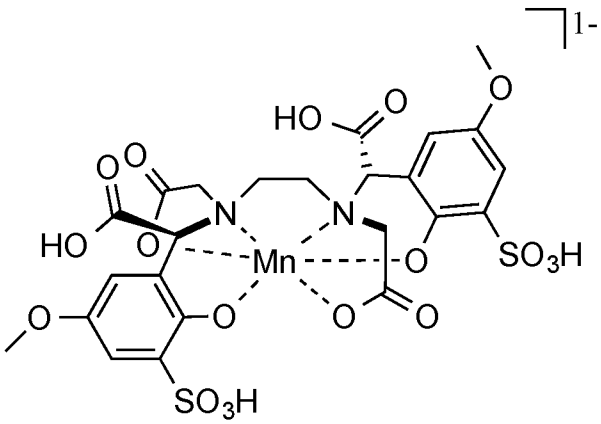
or a pharmaceutically acceptable salt thereof

In another example embodiment, the chelate-metal complex has the formula shown below



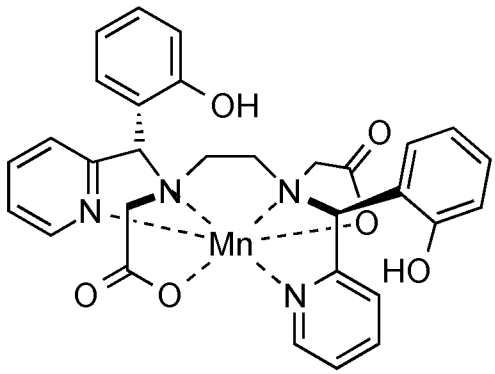
or a pharmaceutically acceptable salt thereof

In another example embodiment, the chelate-metal complex has the formula shown below



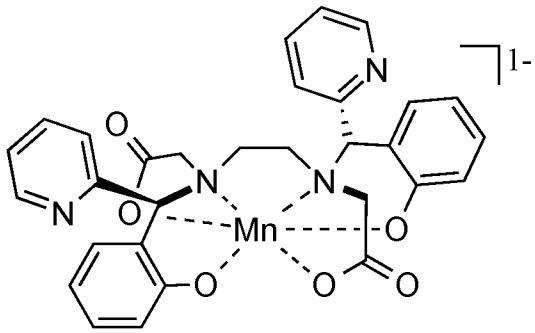
or a pharmaceutically acceptable salt thereof.

Chelate-metal complexes that can toggle between the Mn(II) and Mn(III) oxidation states can be turned "on" and "off" in response to biochemical stimuli. For example, the compound can be a compound in which the Mn ion is in the Mn(II) oxidation state, as shown in the formula below



or a pharmaceutically acceptable salt thereof.

The compound Mn(II) complex, upon reduction, can be a compound in which the Mn ion is in the Mn(III) oxidation state:



or a pharmaceutically acceptable salt thereof. Such contrast agents that can change their relaxivity and signal generating properties in response to an oxidizing or reducing stimulus can be used to detect regions of oxidative stress occurring in the body, for instance due to myocardial ischemia in the context of acute coronary syndrome or myocardial infarction; or in stroke; or in inflammation; or in nonalcoholic steatohepatitis; or in inflammatory bowel disease; or in multiple sclerosis; or in high risk atherosclerotic plaque; or in other diseases characterized by tissue or organ ischemia; or in diseases of chronic inflammation such as rheumatoid arthritis or lupus. Proliferating cancer cells

require a more reducing environment than normal cells and thus these contrast agents may be useful in distinguishing cancerous tissue from normal tissue, or be used to stage the aggressiveness of a specific cancer.

The synthetic protocols used to the prepare chelating ligands are general and can be broadly extended to include additional embodiments

Targeting Groups

Chelating ligands may be modified to incorporate one or more Target Binding Moieties (TBM), as indicated above. TBMs can include peptides, nucleic acids, or small organic molecules, examples of which are provided below. TBMs allow chelating ligands and metal chelates to be bound to targets *in vivo*. Typically, a TBM has an affinity for a target. For example, the TBM can bind its target with a dissociation constant of less than 10 μM, or less than 5 μM, or less than 1 μM, or less than 100 nM. In some embodiments, the TBM has a specific binding affinity for a specific target relative to other physiologic targets. For example, the TBM may exhibit a smaller dissociation constant for fibrin relative to its dissociation constant for collagen. Some TBMs do not necessarily adhere to a target, but promote a change in relaxivity of the contrast agent in the presence of a specific target. For example, a TBM may promote a change in relaxivity in the presence of reactive oxygen species (ROS) generated by peroxidase enzymes.

TBMs can be synthesized and conjugated to the chelating ligands by methods well-known in the art, including standard peptide and nucleic acid synthesis methods. (see, e.g., WO 01/09188, WO 01/08712, and U.S. Pat. Nos. 6,406,297 and 6,515,113, all of which are incorporated by reference in their entireties). In some embodiments, a TBM is covalently bound to the chelating ligand. For example, the TBM can be covalently bound to the chelating ligand through an optional Linker (L). As indicated in the structures above, a TBM can be anywhere on a chelating ligand. For example, the TBM can be bound, optionally via an L, to any Rs or DG. In some embodiments, incorporating multiple TBMs onto the chelating ligand can result in higher affinity and avidity for the target. Chelating ligands will typically contain 1 – 4 (L-TBM) units, for example a

chelating ligand can be bound to one L-TBM, or a chelating ligand can be bound to two L-TBM units, or a chelating ligand can be bound to three L-TBM units, or a chelating ligand can be bound to four L-TBM units. In other embodiments it may be preferable to bind multiple chelating ligands to one or more L-TBM units in order to increase the MR signal generating capability of the contrast agent. In such embodiments a contrast agent will typically contain 1 – 4 (L-TBM) units bound to 2 – 8 chelating ligands, for example two chelating ligands can be bound to one L-TBM, or three chelating ligands can be bound to one L-TBM, or four chelating ligands can be bound to one L-TBM, or six chelating ligands can be bound to one L-TBM, or eight chelating ligands can be bound to one L-TBM.

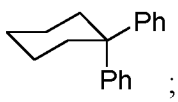
Chelating ligands having a TBM can be assayed for relaxivity values (as the metal chelate) in the presence or absence of the target, e.g., when the TBM is bound or unbound to the target, respectively. Typically, a metal chelate having a TBM will exhibit a higher relaxivity when bound to a target because of the RIME effect (see, e.g., U.S. Pat. Nos. 4,899,755 and 4,880,008, both of which are incorporated by reference in their entireties).

Typical targets include human serum albumin (HSA), fibrin, an extracellular component of myocardium (e.g., collagen, elastin, and decorin), extracellular enzymes secreted in inflammation or cancer (e.g. peroxidase or protease enzymes), or an extracellular component of a lesion (e.g., hyaluronic acid, heparin, chondroitin sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, versican, biglycan, dysregulated thiol/ disulfide composition).

TBMs for binding to HSA are well known in the art, and can include a variety of hydrophobic or amphiphilic moieties. For example, a TBM for binding HSA can have one of the following formulas:

$(CH_2)_nCH_3$;

$(CH_2)_nPh$;



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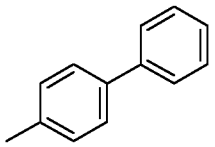
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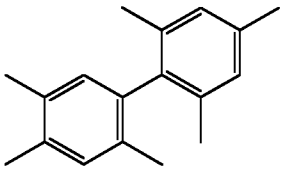
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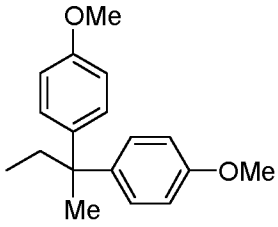

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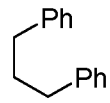

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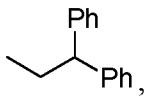

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where n is 2 to 20 and Ph is phenyl (see, for example, WO 96/23526).

Useful TBMs for binding fibrin are described in U.S. Pat. Application Ser. No. 10/209,183, entitled PEPTIDE-BASED MULTIMERIC TARGETED CONTRAST AGENTS, filed July 30, 2002, which is incorporated by reference in its entirety. For example, fibrin binding peptides may be chosen from the cyclic, disulfide bridged peptide, C-P*-Y*-X-L-C (SEQ ID NO:1) where P* is proline or its derivative 4-hydroxyproline, Y* is tyrosine or its non-natural derivative substituted at the 3-position with a moiety from the group of F, Cl, Br, I, or NO_2, and X is either glycine or D- or L-aspartic acid. As another example peptides can be chosen from, X_1-X_2-C-P*-Y*-X_3-L-C-X_4-X_5-X_6 (SEQ ID NO:2) where X_1 is selected from W, S, F, Y, or substituted Y or substituted F; X_2 is selected from E, H, dH, S; X_3 is selected from G, D, dD; X_4 is selected from H, F, Y, and W; X_5 is selected from I, L, V, and N; and X_6 is selected from N, Q, I, L, V, or X_6 is not present.

TBMs for binding an extracellular component of a lesion include peptides having affinity for Hyaluranic Acid (HA). Peptides that have affinity for HA are known. For

example, peptides that bind to HA from a random 12-mer phage peptide library have been isolated (see e.g., Mummert, M., Mohamedzadeh, M., Mummert, D., Mizumoto, N., and Takashima, A. J., *Exp. Med.* (**2000**) 769-779, which is incorporated by reference in its entirety). One of these peptides, GAHWQFNALTVR (SEQ ID. NO:3), binds to HA with Kd~1 μM. As described herein, all peptides are written from their N to their C terminus. Other HA binding peptides include TSYGRPALLPAA (SEQ ID NO:4), MDHLAPTRFRPAI (SEQ ID NO:5), TLRAIWPMWMSS (SEQ ID NO:6), and IPLTANYQGDFT (SEQ ID NO:7).

In addition, peptides having affinity for HA can include a consensus binding motif found in many HA-binding peptides, including RHAMM, CD44, and the link protein. The consensus motif can be $B(X)_7$, where B is a basic residue (e.g., Lys, His or Arg) and X is a non-acidic residue.

In other embodiments, a lesion-targeting peptide can have affinity for heparin, and can include a heparin-binding motif found in heparin-binding proteins. Heparin-binding motifs for inclusion in the peptides include XBBXBX or XBBBXXBX, where B is a basic residue (e.g., Lys, His, or Arg) and X is a non-acidic residue. For example, the heparin-binding peptide ACQWHRVSVRWG (SEQ ID NO:8) conforms to the XBBXXXBX sequence (see e.g., Nielsen, P.K., Gho, Y.S., Hoffman, M.P., Watanabe, H., Makino, M., Nomizu, M., and Yamada, Y. J. Biol. Chem. (2000) 275, 14517-14523, which is incorporated by reference in its entirety). Finally, the heparin sulfate / heparin interacting protein sequence (HIP) motif can also be included in a peptide. One example of such a motif is CRPKAKAKAKAKDQTK (SEQ ID NO:9).

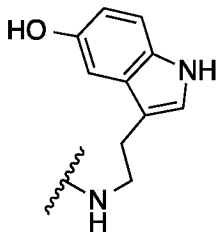
In other embodiments, a lesion-targeting peptide can have affinity for proteolyzed fragments of transmembrane proteins such as receptor protein tyrosine phosphatase (PTPμ) (see e.g., Burden-Gully, S. M., Zhou, Z., Craig, S. E. L., Lu, Z.-R., Brady-Kalnay, S. M. Transl. Oncol. (2013) 6, 329-337). One example of such a motif is CGEGDDFNWEQVNTLTKPTSD (SEQ ID NO:10).

In other embodiments, a lesion-targeting peptide can have affinity for fibronectin (see e.g. Zhou, Z., Qutaish, M. Han, Z. Schur, R. M., Liu, Y., Wilson, D. L., and Lu, Z.-

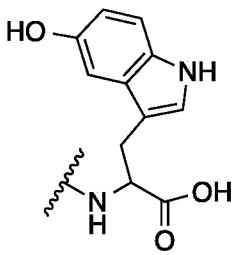
R. Nat. Commun. (2015) 6, 7984-7994). One example of such a motif is CREKA (SEQ ID NO:11).

Useful TBMs for targeting fibrin include fibrin binding peptides to allow for specific imaging of fibrin (e.g., thrombi, solid tumors, and atherosclerotic plaques) within a mammal. Any peptide capable of binding fibrin may be used. For example, the peptides disclosed in WO 2008/071679, U.S. Patent Nos. 6,984,373; 6,991,775; and 7,238,341 and U.S. Patent Application No. 2005/0261472 may be used, all of which are incorporated by reference in their entireties. A peptide can be from about 2 to about 25 amino acids in length (e.g., about 3 to about 20, about 5 to about 18, about 8 to about 15, and about 10 to about 14).

Useful TBMs for targeting enzymes secreted in inflammation and cancer include 5-hydroxytryptamine, shown below



and 5-hydroxytryptophan, shown below,



which are oxidized and subsequently oligomerize in the presence of ROS generated by peroxidase enzymes such as myeloperoxidase (see e.g. Shazeeb, M. S., Xie, Y., Gupta, S., and Bogdanov, A. A. Jr., Mol. Imaging. (2012) 11, 433-443, which is incorporated by reference in its entirety). The oligomerization results in larger, more slowly tumbling chelates that have higher relaxivity.

In other embodiments, ROS targeting moieties can include DGs or R groups that can be oxidized by biological oxidizing agents such as oxygen, hydrogen peroxide, superoxide, peroxidase enzymes, hypochlorous acid, or disulfides. In the absence of the

oxidizing agent, the DG or R group favors the formation of a metal chelate with the metal ion in a high-valent oxidation state such as Mn(III). When the DG or R group is oxidized, then the oxidized DG or R group now favors the formation of a metal chelate with the metal ion in a low-valent oxidation state such as Mn(II). Switching from Mn(III) to Mn(II) results in increased relaxivity.

In other embodiments, metal-chelates that comprise R groups and DGs that favor, and are bound to, metal ions in low-valent oxidation states such as Mn(II) can include ancillary, non-coordinating R groups that favor binding to high-valent metal oxidation states such as Mn(III). Such ancillary R groups can bind to and trap Mn(III) generated from oxidation of Mn(II) by biological oxidizing agents such as oxygen, hydrogen peroxide, superoxide, peroxidase enzymes, hypochlorous acid, or disulfides. Switching from Mn(II) to Mn(III) results in decreased relaxivity.

In other embodiments, metal-chelates that comprise R groups and DGs that favor, and are bound to, metal ions in high-valent oxidation states such as Mn(III) can include ancillary, non-coordinating R groups that favor binding to low-valent metal oxidation states such as Mn(II). Such ancillary R groups can bind to and trap Mn(II) generated from reduction of Mn(II) by biological reducing agents such as thiols, ascorbic acid, mitochondria, superoxide, reductase enzymes, NADH, or NADPH. Switching from Mn(III) to Mn(II) results in increased relaxivity.

Useful TBMs for targeting enzymes secreted in inflammation and cancer can include peptidic substrates for protease enzymes (see Jastrzebska, B., Lebel, R., Therriault, H., McIntyre, J. O., Escher, E., Guerin, B., Paquette, B., Neugebauer, W. A., and Lepage, M. J. Med. Chem. (2009) 52, 1576-1581, which is incorporated by reference in its entirety). Activity of the protease enzyme on the peptidic substrate can effect a change in solubility of the chelate-metal complex. Decreased solubility results in in vivo retention at the site of the biochemical target.

Useful TBMs for targeting collagen include peptides derived from the propolypeptide of von Willebrand factor, which is known to bind collagen. As used herein, all peptides are written from the N to C terminus. Additionally, peptides

containing two or more cysteine residues can form disulfide bonds under non-reducing conditions. A peptide for targeting collagen can include the following general formula: X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10} (SEQ ID NO:12) where X_1 can be W, C, or A; X_2 can be R, C, or A; X_3 can be E, C, A, K, or T; X_4 can be P, C, or A; X_5 can be D, G, S, C, or A; X_6 can be F, R, C, or A; X_7 can be C, M, or A; X_8 can be A, E, or C; X_9 can be L, M, R, C, or A; and X_{10} can be S, N, G, L, C, or A; where no more than 3 of X_1-X_{10} are C or A, independently, and where the total number of C and A residues in X_1-X_{10} is a maximum of 4. For example, a peptide can have the following sequences: WREPSFCALS (SEQ ID NO:13); WREPSFMALS (SEQ ID NO:14); and WREPGFCALS (SEQ ID NO:15).

Another example of a peptide that binds collagen has the following general formula: X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13} (SEQ ID NO:16) where X_1 can be W, C, or A; X_2 can be R, C, or A; X_3 can be E, C, A, K, or T; X_4 can be P, C, or A; X_5 can be D, G, S, C, or A; X_6 can be F, R, C, or A; X_7 can be C, M, or A; X_8 can be A, E, or C; X_9 can be L, M, R, C, or A; X_{10} can be S, N, G, L, C, or A; X_{11} can be C, M, or A; X_{12} can be P, A, or C; and where X_{13} can be K, Q, P, H, G, C, or A; where no more than 4 of X_1-X_{13} are C or A, independently, and where the total number of C and A residues in X_1-X_{13} is a maximum of 5.

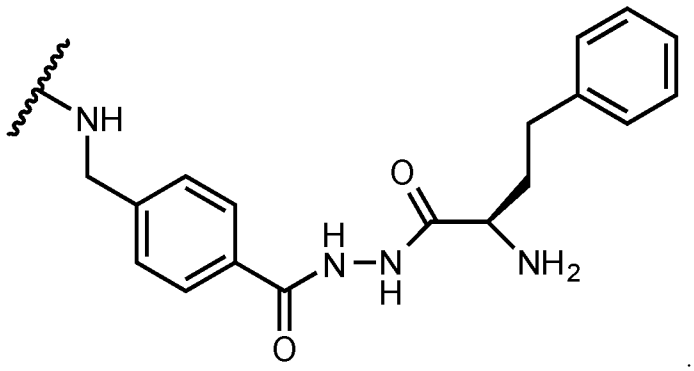
A peptide for binding collagen can also have the following general formula: X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15} (SEQ ID NO:17) where X_1 can be V, I, C, or A; X_2 can be A, G, R, D, or C; X_3 can be W, C, or A; X_4 can be R, C, or A; X_5 can be E, C, A, K, or T; X_6 can be P, C, or A; X_7 can be D, G, S, C, or A; X_8 can be F, R, C, or A; X_9 can be C, M, or A; X_{10} can be E, A, or C; X_{11} can be L, C, A, M, or R; X_{12} can be S, C, A, N, G., or L; X_{13} can be C, M, or A; X_{14} can be P, A, or C; and X_{15} can be K, Q, P, H, G, C, or A; where no more than 4 of X_1-X_{15} are C or A, independently, and where the total number of C and A residues in X_1-X_{15} is a maximum of 6.

Further peptides for targeting collagen can be found in U.S. Patent 8,034,898, entitled "Methods of Collagen Imaging", filed December 29, 2006, which is incorporated by reference in its entirety. For example, collagen binding peptides can be selected from cyclic, disulfide bridged peptides W-H-C-X_1-T-X_2-F-P-H-H-Y-C (SEQ ID NO:18)where

X_1 is selected from Y, T, or S, and X_2 can be any amino acid. Other peptides for targeting collagen can be identified by modifying (e.g., mutating, truncating, lengthening) the peptides described above.

Useful TBMs for binding folate receptors, vitronectins, alpha-v-beta-3 and alpha-v-beta-5 integrins, RGD peptides for MMP targets, porphyrins, and phosphonates are described in WO 2004/112839, filed June 17, 2004, which is incorporated by reference in its entirety, and references therein.

Useful TBMs for binding elastin include the following formula:



Self-Assembling Moieties

Magnetic resonance imaging of low concentration targets can be limited by the relaxivity of the contrast agent. Different strategies have been employed to assemble many chelates together to improve the sensitivity of the contrast agent including covalent and non-covalent assembly of chelating ligands and metal chelates. The non-covalent approach involves using a group that can interact with itself or similar groups to form an assembly of molecules. Chelating ligands may be modified to incorporate one or more Self-Assembling Moieties (SAM), as indicated above. SAMs can include lipids, long chain alkyl or substituted alkyl groups, perfluorocarbons, peptides, nucleic acids, or small organic molecules. SAMs allow chelating ligands and metal chelates to associate with themselves to form larger aggregates, particles, or assemblies.

SAMs can be synthesized and conjugated to chelating ligands by methods well known in the art, including standard peptide and nucleic acid synthesis methods; see, e.g., WO 01/09188, WO 01/08712, and U.S. Pat. Nos. 6,406,297 and 6,515,113, all of which

are incorporated by reference in their entirety. Typically, a SAM is covalently bound to a chelating ligand, and can be covalently bound to a chelating ligand through an optional Linker (L). As indicated in the structures above, a SAM may be anywhere on a chelating ligand. For example, the SAM may be bound, optionally via an L, to any Rs or DG.

Chelating ligands having a SAM can be assayed for relaxivity values (as the metal chelate) at or above a critical self-assembly concentration. At very low concentrations the chelate may exist in predominantly monomeric, unassembled form; above a critical self-assembly concentration the chelate may exist predominantly in the self-assembled form. Typically, a metal chelate having a SAM will exhibit a higher relaxivity when in the self-assembled form because of the RIME effect (see, e.g., U.S. Pat. Nos. 4,899,755 and 4,880,008, both of which are incorporated by reference in their entirety).

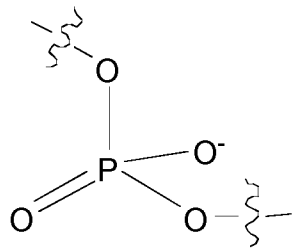
SAMs can include lipids and lipid-like groups capable of forming micelles (see e.g., Nicolle, G.M., Toth, E., Eisenwiener, K.P., Macke, H.R., and Merbach, A.E. *J Biol Inorg Chem.* **2002** 7:757-69, which is incorporated by reference in its entirety) or liposomes (see e.g., Mulder, W.J., Strijkers, G.J., van Tilborg, G.A., Griffioen, A.W., and Nicolay, K., NMR *Biomed.* **2006** 19:142-64, which is incorporated by reference in its entirety). SAMs can also facilitate incorporation into mixed liposomes or emulsions (see e.g., U.S. Patent No. 6,869,591, which is incorporated by reference in its entirety)

SAMs can also be perfluoroalkyl groups that promote self-assembly (see e.g., U.S. Patent No. 6,916,461 and WO 2003/0232012, both of which are incorporated by reference in their entirety). Alternatively, peptides can also form self-assemblies (see e.g., WO 2004/0204561, which is incorporated by reference in its entirety, and peptide sequences disclosed therein).

Linkers

In some embodiments, the TBM is covalently bound to the chelating ligand through a linker (L). L can include, for example, a linear, branched or cyclic peptide sequence. In one embodiment, an L can include the linear dipeptide sequence G-G (glycine-glycine). In embodiments where the TBM includes a peptide, the L can cap the N-terminus of the TBM peptide, the C-terminus, or both N- and C- termini, as an amide

moiety. Other exemplary capping moieties include sulfonamides, ureas, thioureas and carbamates. Linkers can also include linear, branched, or cyclic alkanes, alkenes, or alkynes, and phosphodiester moieties. L may be substituted with one or more functional groups, including ketone, ester, amide, ether, carbonate, sulfonamide, or carbamate functionalities. Specific linkers contemplated include $-(O-(CH_2)_2-O)n$, where n = 1-20,000, and more specifically where n = 1 - 6; NH-CO-NH-; $-CO-(CH_2)_n-NH-$, where n = 1 to 10; dpr; dab; -NH-Ph-; $-NH-(CH_2)_n-$, where n = 1 to 10; -CO-NH-; $-(CH_2)_n-NH-$, where n = 1 to 10; $-CO-(CH_2)_n-NH-$, where n = 1 to 10; -CS-NH-, and



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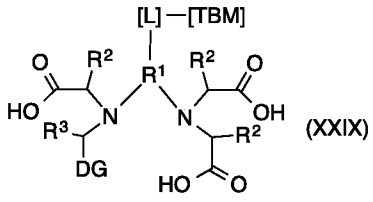
In some embodiments, L is linked to the chelate via any R or DG. For example, a chelate may have the general formula:



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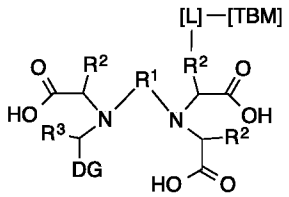

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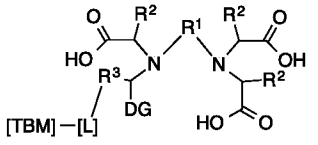

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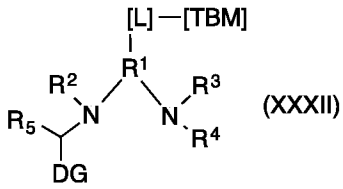

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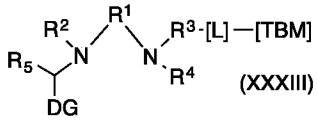
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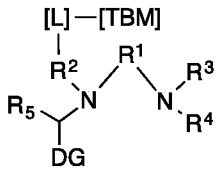
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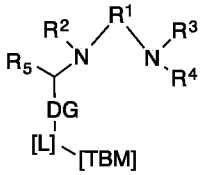
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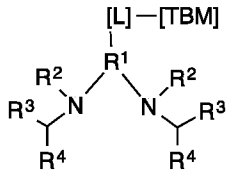
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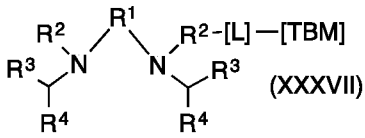
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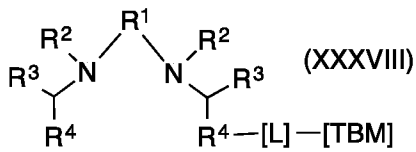

(XXXVIII)

or a pharmaceutically acceptable salt thereof.

Additional examples of linkers and synthetic methodologies for incorporating them into chelating ligands, particularly chelating ligands comprising peptides, are set forth in WO 01/09188, WO 01/08712, WO 2004/112839, U.S. Pat. Application Ser. No. 10/209,183, entitled "Peptide-Based Multimeric Targeted Contrast Agents," filed July 30, 2002, and U.S. Pat. Application Ser. No. 11/618,564, entitled "COLLAGEN BINDING PEPTIDES," filed December 29, 2006, all of which are incorporated by reference in their entireties.

Properties of Chelating Ligands and Metal Chelates

Chelating ligands are capable of binding one or more metal ions to result in a metal chelate. Metal chelates can be prepared by methods well known in the art; see e.g., WO 96/23526, U.S. Pat. Nos. 6,406,297 and 6,515,113, all of which are incorporated by reference in their entireties, and Examples, below.

Metal chelates can include a metal ion with an atomic number of 21-29, 40, 42, or 57-83. For example, metal chelates can include a stable or unstable isotope selected from Gd(III), Fe(III), Mn(II), Mn(III), Cr(III), Cu(II), Cu(III), Dy(III), Ho(III), Er(III), Pr(III), Eu(II), Eu(III), Nd(III), La(III), Lu(III), Sm(III), Tb(III), Tb(IV), Tm(III), Y(III), In(III), Ga(III), Tc(III), Tc(IV), Tc(V), Re(III), Re(IV), Re(V), Bi(III), or Yb(III). The metal ion can be paramagnetic. Typically, because of the chemical nature and number of DGs on the chelating ligands, the metal ion is tightly bound by the chelating ligand, and physiologically compatible metal chelates can be made. The formation constant, K_f, of a chelating ligand for a metal ion is an indicator of binding affinity, and is typically discussed with reference to a log K_f scale. Physiologically compatible metal chelates can have a log K_f ranging from 10 to about 25, i.e. K_f ranges from 10^{10} to 10^{25} M^{-1}. For Mn(II) metal chelates, the log K_f should be greater than 12. Methods for measuring K_f are well known in the art; see, e.g., Martell, A.E. and Motekaitis, R.J., Determination and Use of Stability Constants, 2d Ed., VCH Publishers, New York (1992), which is incorporated by reference in its entirety.

The relaxivity values of metal chelates can also be assessed. If the metal chelate incorporates a TBM, the relaxivity can be measured in the presence and absence of the target molecule. Methods for measuring relaxivity are well known in the art; see e.g., WO 96/23526, which is incorporated by reference in its entirety.

One challenge in identifying new high relaxivity chelates is the fact that for most metal chelates, the relaxivity observed is limited by the tumbling rate (rotational diffusion) of the chelate. This is well documented in reviews such as R.B. Lauffer, *Chem. Rev.* **1987**, 87:901-27 and P. Caravan *et al.*, *Chem. Rev.* **1999**, 99:2293-2352, which is incorporated by reference in its entirety. The effect of many of the parameters listed above (e.g., water residency time and second sphere effect) are not pronounced for

low (<1500 Da) molecular weight metal complexes. When metal chelates tumble slowly, either by binding to large molecules like proteins, linking to polymeric structures, or self-assembling into large aggregates, the effect of these other parameters can be observed. Therefore, one way to identify metal chelates capable of very high relaxivities is to screen the chelates for relaxivity under conditions where rotational diffusion is slow.

One way to do this is to incorporate into each chelate that is to be tested, a common TBM or SAM group. By comparing chelates that have a common TBM in the presence of the target protein (e.g., albumin), it is possible to rank the chelates in order of highest to lowest relaxivity and determine which donor groups and SSMs are the most favorable combination. The high relaxivity chelates identified in this way can be further modified to incorporate a different TBM.

Metal chelates can also be evaluated for the mean residence time of water molecule(s) in the first (or higher) coordination sphere(s). The mean residence time of water molecules is the inverse of the water exchange rate and is dependent on temperature. The mean residence time of water in the coordination sphere of the metal chelates at 37 °C can be between 1 and 100 ns. In some embodiments, the mean residence time of water in the coordination sphere of the metal chelates at 37 °C is between 3 and 30 ns. ^{17}O NMR can be used to evaluate the mean residence time of water molecules. See, e.g., Example 7, below.

Luminescence lifetime measurements can be used to evaluate the number of water molecules bound to a metal chelate. Methods for measuring luminescence lifetimes are known in the art, and typically include monitoring emissive transitions of the chelate at particular wavelengths for lifetime determination, followed by fitting of luminescence decay data. Luminescence lifetime measurements are also useful for evaluating the suitability of the metal chelates as luminescent probes. Alternatively ^{17}O NMR can be used for Mn(II) chelates (see Gale EM, Zhu J, Caravan P. Direct Measurement of the Mn(II) Hydration State in Metal Complexes and Metalloproteins through ^{17}O NMR Line Widths. J Am Chem Soc 2013; 135:18600–18608, which is incorporated by reference in its entirety).

Use of Chelating Ligands and Metal Chelates

Chelating ligands can be used to prepare metal chelates, as described above, for diagnostic purposes. For example, metal chelates prepared with Mn(II) can be useful as contrast agents in MR imaging. Contrast agents incorporating a TBM can bind a target and therefore can be particularly useful in targeted MR applications, e.g., to image blood flow, clots, lesions, or the myocardium. In some embodiments, at least about 10% (e.g., at least about 50%, about 80%, about 90%, about 92%, about 94%, or about 96%) of the contrast agent can be bound to the desired target at physiologically relevant concentrations of contrast agent and target. The extent of binding of a contrast agent to a target can be assessed by a variety of equilibrium binding methods, e.g., ultrafiltration methods; equilibrium dialysis; affinity chromatography; or competitive binding inhibition or displacement of probe compounds.

Contrast agents can exhibit high relaxivity as a result of target binding, which can lead to better MR image resolution. In some embodiments, the increase in relaxivity upon binding is at least about 1.5-fold (e.g., at least a 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold increase in relaxivity) as compared to the chelate-metal complex not bound to the target or existing in a different oxidation state. For example, a targeted contrast agents can have a 7 to 8-fold, 9 to 10-fold, or even greater than 10-fold increase in relaxivity as compared to the chelate-metal complex not bound to the target or existing in a different oxidation state. In some embodiments, the relaxivity of an MRI contrast agent as provided herein at 60 MHz and 37 °C is at least 8 $mM^{-1}s^{-1}$ per paramagnetic metal ion (e.g., at least 10, 15, 20, 25, 30, 35, 40, or 60 $mM^{-1}s^{-1}$ per paramagnetic metal ion). For example, the contrast agents provided herein can have a relaxivity greater than 10 $mM^{-1}s^{-1}$ at 60 MHz and 37° C.

Metal chelates of lanthanides can also be useful as luminescent probes. Luminescent metal chelate probes can be useful in a variety of assays, e.g., to detect, separate, and/or quantify chemical and biological analytes in research and diagnostic applications, including high-throughput, real-time, and multiplex applications. For example, probes incorporating a TBM can bind to a target analyte of interest, and can have long luminescent lifetimes (e.g., greater than 0.1 μs, or 100 μs, or 1 ms), thereby

improving sensitivity and applicability of various assay formats. See, generally, U.S. Pat. Nos. 6,406,297 and 6,515,113, for a description of assays suitable for inclusion of luminescent metal chelate probes, both of which are incorporated by reference in their entireties. Luminescent metal chelate probes are particularly useful in immunoassays and real-time PCR detection assays.

Use of MRI Contrast Agents

MRI contrast agents may be used in the same manner as conventional MRI contrast agents. For example, an effective amount of the contrast agent is administered to a patient (e.g., an animal, such as a human) and an MR image of the patient is acquired. In embodiments having a TBM, a contrast-enhancing imaging sequence that preferentially increases a contrast ratio of a magnetic resonance signal of the target having a contrast agent bound thereto relative to the magnetic resonance signal of background blood or tissue can be used. These techniques include, but are not limited to, black blood angiography sequences that seek to make blood dark, such as fast spin echo sequences; flow-spoiled gradient echo sequences; and out-of-volume suppression techniques to suppress in-flowing blood. These methods also include flow independent techniques that enhance the difference in contrast due to the T_1 difference between contrast-enhanced target and blood and tissue, such as inversion-recovery prepared or saturation-recovery prepared sequences that will increase the contrast between the target and background tissues. Methods of preparation for T_2 techniques may also prove useful. Finally, preparations for magnetization transfer techniques may also improve contrast with contrast agents.

Methods may be used that involve the acquisition and/or comparison of contrast-enhanced and non-contrast images and/or the use of one or more additional contrast agents. The additional contrast agents can exhibit affinity for a target. Exemplary methods as set forth in U.S. Pat. Application Ser. No. 09/778,585, entitled MAGNETIC RESONANCE ANGIOGRAPHY DATA, filed February 7, 2001 and U.S. Pat. Application Ser. No. 10/209,416, entitled SYSTEMS AND METHODS FOR TARGETED MAGNETIC RESONANCE IMAGING OF THE VASCULAR SYSTEM, filed July 30, 2002, both of which are incorporated by reference in their entireties.

Contrast agents can be formulated as a pharmaceutical composition in accordance with routine procedures. As used herein, the contrast agents can include pharmaceutically acceptable derivatives thereof. "Pharmaceutically acceptable" means that the agent can be administered to an animal without unacceptable adverse effects. A "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, ester, salt of an ester, or other derivative of a contrast agent or compositions that, upon administration to a recipient, is capable of providing (directly or indirectly) a contrast agent or an active metabolite or residue thereof. Other derivatives are those that increase the bioavailability when administered to a mammal (e.g., by allowing an orally administered compound to be more readily absorbed into the blood) or that enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system) thereby increasing the exposure relative to the parent species. Pharmaceutically acceptable salts of the contrast agents include counter ions derived from pharmaceutically acceptable inorganic and organic acids and bases known in the art, including, without limitation, sodium, calcium, and N-methyl-glucamine.

Pharmaceutical compositions can be administered by any route, including both oral and parenteral administration. Parenteral administration includes, but is not limited to, subcutaneous, intravenous, intraarterial, interstitial, intrathecal, and intracavity administration. When administration is intravenous, pharmaceutical compositions may be given as a bolus, as two or more doses separated in time, or as a constant or non-linear flow infusion. Thus, contrast agents can be formulated for any route of administration.

Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent, a stabilizing agent, and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients will be supplied either separately, e.g., in a kit, or mixed together in a unit dosage form, for example, as a dry lyophilized powder or water free concentrate. The composition may be stored in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent in activity units. Where the composition is administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade "water for

injection," saline, or other suitable intravenous fluids. Where the composition is to be administered by injection, an ampule of sterile water for injection or saline may be provided so that the ingredients may be mixed prior to administration. Pharmaceutical compositions comprise the contrast agents and pharmaceutically acceptable salts thereof, with any pharmaceutically acceptable ingredient, excipient, carrier, adjuvant or vehicle.

In some embodiments, a contrast agent is administered to the patient in the form of an injectable composition. The method of administering a contrast agent is can include parenterally, meaning intravenously, intra-arterially, intrathecally, interstitially or intracavitarilly. Pharmaceutical compositions can be administered to mammals including humans in a manner similar to other diagnostic or therapeutic agents. The dosage to be administered, and the mode of administration will depend on a variety of factors including age, weight, sex, condition of the patient and genetic factors, and will ultimately be decided by medical personnel subsequent to experimental determinations of varying dosage followed by imaging as described herein. In general, dosage required for diagnostic sensitivity or therapeutic efficacy will range from about 0.001 to 50,000 μg/kg, preferably between 0.01 to 25.0 μg/kg of host body mass.

EXAMPLES

Methods.

General. All chemicals and solvents were purchased commercially and used without further purification.

NMR. NMR spectra were recorded on a 500 MHz Varian spectrometer at 25 °C unless otherwise noted. Chemical shifts are reported in δ (ppm). For ^{1}H and ^{13}C NMR spectra, the residual solvent peaks were used as internal reference except for ^{13}C NMR recorded in D_2O where dioxane was used as the internal references (Fulmer, *Organometallics* **2010**, *29*, 2176.).[1] Relaxivity measurements were performed on a Bruker mq60 Minispec, 1.41 T and 37 °C. Longitudinal (T_1) relaxation was acquired via an inversion recovery experiment using 10 inversion times of duration ranging between 0.05 × T_1 and 5 × T_1; tranverse (T_2) relaxation was measured using a Carl-Purcell-Meiboom-Gill spin-echo experiment. Relaxivity ($r_{1,2}$) was determined from the slope of a plot of

$1/T_{1,2}$ vs. [Mn] for at least 4 concentrations. The transverse (T_2) relaxation times of ^{17}O were acquired at 11.7 T from the full-width at half-height of the $H_2{}^{17}O$ signal (Gale, Carvan, *J. Am. Chem. Soc.* **2013**, *135*, 18600). ^{17}O T_2 relaxivity (r_2^O) was calculated by dividing the Mn-imparted increase in $1/T_2$ relative to neat H_2O (pH 3) by the Mn concentration in mM. 0.7-1.0 mL NMR samples were enriched with a 10 μL of 18% $H_2{}^{17}O$.

Relaxivity in the presence of fibrin clots. The measurements were performed as previously described (Carvan, *J. Am. Chem. Soc.* **2008**, *130*, 6025). Briefly, $CaCl_2$ was added to a solution of compound **20,** shown below, thrombin and human fibrinogen to trigger fibrin formation (final concentrations of $CaCl_2$, fibrinogen and thrombin were 10 mM, 10 mg/mL and 0.6 U/mL, respectively). The resultant fibrin gels were incubated for 20 min at 37 ºC before measurement.

HPLC methods. Liquid chromatography-mass spectrometry (LC-MS) was performed using an Agilent 1100 Series apparatus with an LC/MSD trap and Daly conversion dynode detector with UV detection at 220, 254, and 280 nm. The methods used on this system are as follows: (A1) Kromasil C18 column (100 × 4.6 mm); eluent C: 90 % MeCN/10 % 10 mM ammonium acetate, D: 10 mM ammonium acetate; gradient 5 % C to 95 % C over 14 min; flow rate 0.8 mL/min. Reverse-phase semi-preparative purification was performed on the Rainin Dynamax HPLC system with UV detection from 220 to 280 nm using a Phenomenex C18 or C5 column (250 x 21.8 cm). The mobile phase A was water with 0.1% TFA added; mobile phase B was MeCN with 0.1% TFA added; mobile phase C was 50 mM ammonium acetate buffer, pH 6.5; mobile phase D was a mixture of 5 % 50 mM ammonium acetate buffer, pH 6.5 and 95% MeCN. The methods used for purification are as follows: (P1) starting from 95% A/ 5% B, the fraction of B increased to 70 % over 23 min. The column was washed with 95 % B for 2 min and then ramped to 5 % B. The system was re-equilibrated at 5% B for 3 min, (P2) starting from 95% C/ 5% D, the fraction of D increased to 70 % over 23 min. The column was washed with 95 % D for 2 min and then ramped to 5 % B. The system was re-equilibrated at 5% B for 3 min, (P3) starting from 95% A/ 5% B, the fraction of B increased to 95 % over 23 min. The column was washed with 95 % B for 2 min and then ramped to 5 % B. The system was re-equilibrated at 5% B for 3 min, (P4) starting from 95% C/ 5% D, the fraction of D increased

to 95 % over 23 min. The column was washed with 95 % D for 2 min and then ramped to 5 % D. The system was re-equilibrated at 5% B for 3 min, (P5) starting from 80% A/ 20% B, the fraction of B increased to 95 % over 23 min. The column was washed with 95 % B for 2 min and then ramped to 5 % B. The system was re-equilibrated at 5% B for 3 min, (P6) starting from 95% A/ 5% B, the fraction of B increased to 60 % over 40 min. The column was washed with 95 % B for 2 min and then ramped to 5 % B. The system was re-equilibrated at 5% B for 3 min, (P7) starting from 95% C/ 5% D, the fraction of D increased to 60 % over 23 min. The column was washed with 95 % D for 2 min and then ramped to 5 % D. The system was re-equilibrated at 5% D for 3 min, (P8) starting from 95% C/ 5% D, the fraction of D increased to 60 % over 40 min. The column was washed with 95 % D for 2 min and then ramped to 5 % D. The system was re-equilibrated at 5% D for 3 min, (P9) starting from 95% C/ 5% D, the fraction of D increased to 40 % over 23 min. The column was washed with 95 % D for 2 min and then ramped to 5 % D. The system was re-equilibrated at 5% D for 3 min.

DD(E) binding assay. The affinity of the probes was assessed using a DD(E) fluorescence polarization displacement assay that was described previously.[5] The displacement of a tetramethylrhodamine labeled peptide (TRITC-Tn6) from DD(E) was detected by observing the corresponding change in fluorescence anisotropy. The K_d of the TRITC-Tn6 probe was determined by titrating it with the DD(E) protein and fitting the resultant fluorescence data as described previously (Caravan, *Bioconjugate Chem.* **2012**, *23*, 548). This experiment was performed at room temperature using a concentration of TRITC-Tn6 of 0.1 μM in the following assay buffer: Tris base (50 mM), NaCl (100 mM), $CaCl_2$ (2 mM), Triton X- 100 (0.01%), pH = 7.8. The anisotropy measurements were made using a TECAN Infinity F200 Pro plate reader equipped with the appropriate filter set for tetramethylrhodamine (excitation 535 nm, emission 590 nm).

Mn quantification in tissues and blood. Metal concentrations were determined using an Agilent 8800-QQQ ICP-MS system. All samples were diluted with 0.1 % Triton X-100 in 5 % nitric acid. A linear calibration curve for each metal ranging from 0.1 ppb to 200 ppb was generated daily for the quantification.

Estimates of albumin binding. Measurements were performed on a series of

solutions ranging between 150-300 μM chelate-metal complex in either 4.5% wt/v BSA or bovine blood plasma. 150 μL of each solution was placed within a Millipore Ultra Free MC 5 kDa cutoff filtration vessel and ~10 μL of the solution was forced through the filter by centrifugation. Mn content in each unfiltered solution and filtrate were quantified by ICP-MS. The percentage of $[Mn(PyC3A)(H_2O)]^-$ bound to albumin was estimated from the difference in Mn concentrations between unfiltered solution and filtrate.

Rat model of carotid artery thrombosis. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (*Guide for the care and use of laboratory animals* Bethesda, MD, 1985.) and were approved by the Institutional Animal Care and Use Committee at Massachusetts General Hospital. Adult male Wistar rats (N =4; weight, 200–300 g; Charles River Laboratories) were used for this study. Arterial thrombosis was induced by application of 25% w/v $AlCl_{3(aq)}$ to the vessel outer wall. Under isoflurane anesthesia, the right common carotid artery was exposed, and a small strip of filter paper soaked in the $AlCl_3$ solution was applied. Injury was performed 1–2 cm proximal to carotid bifurcation by the same investigator to minimize variability. The femoral artery was catheterized using PE-50 tubing (Fisher Scientific) for probe injection. Probes, either compound **20** or gadolinium containing control compound EP-2104R, were injected 30 min after thrombus formation. Each rat was injected with 0.01 mmol/kg probe (0.04 mmol/kg based on metal ion). For blood draw experiments, rats were catheterized in the femoral vein and artery for injection and sampling, respectively. Blood was drawn at 2, 5, 10, 15, 30, 60 and 120 min, than 24h after injection and collected in heparinized vials. Immediately after collection a portion of the blood was centrifuged for 10 min at 5000 rpm and the plasma separated and weighed, diluted 2-fold with PBS buffer and injected onto the analytical HPLC column.

MR imaging of thrombosis in rats. Imaging was performed on a human whole-body 1.5T system (Avanto, Siemens Healthcare, Erlangen, Germany) with a custom-built transmit-receive coil. Animals were anesthetized with isoflurane (1–2%) for the duration of the experiment. Catheters were placed in the femoral vein and the femoral artery for blood draws and contrast administration, respectively. First, the head and neck were visualized with T_1-weighted images in sagittal, coronal, and axial planes, followed by a 3D

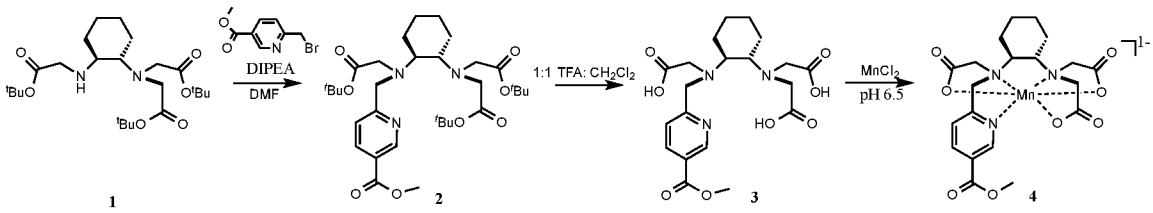
TOF angiogram acquired transversely. Next molecular imaging was performed at baseline with two different sequences: 3D T_1-weighted gradient echo (GRE) and 2D T_1-weighted dark-blood fast spin echo (DB-TSE). After all pre-contrast scans were completed, 0.01 mmol/kg of the imaging probe, either EP-2104R or **18**, was injected as a bolus via the femoral artery. The molecular imaging sequences used at baseline were repeated for 60 minutes following contrast delivery. The TOF angiogram was acquired with the following parameters: 3D T_1-weighted gradient echo sequence, TR/TE/flip angle=26 ms/5.75 ms/25°, in-plane FOV = 85x85 mm, matrix=320×320, 58 slices, slice thickness = 0.47 mm, voxel size = 0.3 x 0.3 x 0.47 mm, 1 average, and acquisition time = 4:18 minutes. The GRE sequence for molecular imaging had identical parameters to the TOF angiogram, but smaller head-to-foot coverage (48 slices) and longer TR (35 ms) resulting in a scan time of 6:34 minutes. In addition, inferior saturation was performed to null inflowing arterial blood. DB-TSE was performed with TR/TE = 800 ms/20 ms, in-plane FOV = 85x85 mm, matrix=320×320, 11 slices, slice thickness = 2 mm, voxel size = 0.3 x 0.3 x 2 mm, echo train length = 11, 1 average, and acquisition time = 4 minutes. GRE and DB-TSE had overlapping volumetric coverage and were acquired with axial orientation. Images were analyzed in Matlab (Version R2104a, MathWorks, Natick, MA) by drawing ROIs and measuring mean SI of the clot, contralateral artery, and adjacent muscle. Noise was quantified as the standard deviation (SD) of the signal measured in the air outside the animal. SNR was calculated as described above for each tissue type. We also calculated contrast-to-noise ratios for the clot and the contralateral artery relative to muscle: CNR = $(SI_{tissue} - SI_{adj\ muscle})/SD_{air}$. SNR and CNR were estimated at baseline (SNR_{pre} and CNR_{pre}) from pre-contrast images and at various time points after contrast injection (SNR_{post} and CNR_{post}). Normalized SNR (nSNR) values were obtained by dividing SNR at each time point by SNR_{pre}. Unpaired Student's t-Test was used for statistical analysis where $p < 0.05$ was considered as significant.

Histology. Carotid arteries were harvested 90 min after generation of induction of thrombosis, carefully rinsed in phosphate buffer, embedded in OCT mounting media (Tissue-Tek) and snap-frozen in -45 °C isopentane. Arteries were cryosectioned in 20 μM slices and processed for Hemotoxylin and Eosin staining according to the standard

protocol. Images were acquired using a Nikon TE-2000 microscope (40x magnification).

Example 1: Synthesis of chelating ligands that form protein binding Mn(II) complexes.

A. Synthesis of N-((5-methoxycarbonyl)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetriacetate (PyC3A-5-methyl ester) (**4**).



Scheme 1

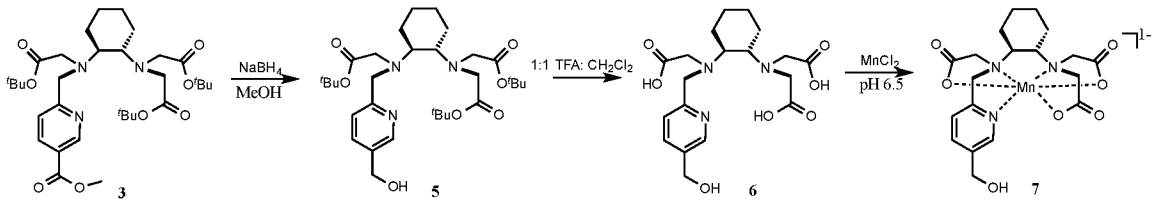
***N*-((5-methoxycarbonyl)-pyridin-2-yl)methyl-*N,N',N'-trans*-l,2-cyclohexylenediaminetri-*tert*-butylacetate (2).** 6-(bromomethyl)-methylnicatinoate (0.450 g, 1.95 mmol) was added to **3** (1.00 g, 2.01 mmol), potassium iodide (0.243 g, 1.46 mmol), and diisopropylethylamine (0.468 g, 3.62 mmol) stirring in 4 mL DMF. After 3h, the reaction was diluted to 100 mL with Et_2O, washed with satd. $Na_2CO_{3(aq)}$, copious water and brine before drying over Na_2SO_4 and concentration to a brown oil. The crude product was purified by flash chromatography (basic alumina, hexane:EtOAc, 0% to 20% EtOAc) and as 1.02 g (1.68 mmol, 84%) **6** was isolated as a clear colored oil. ^{1}H NMR (500 MHZ, $CDCl_3$, δ from protio solvent): 9.07 (s, 1H), 8.24 (d, 1H), 8.03 (d, 1H), 4.20 (d, 1H), 3.04 (s, 3H), 3.86 (d, 1H), 3.53-3.30 (m, 6H), 2.73 (br t, 1H), 2.58 (br t, 1H), 2.05 (br m, 2H), 1.69 (br m, 2H), 1.65 (br m, 2H), 1.45 (s, 18H), 1.43 (s, 9H), 1.26-1.09 (m, 4H). ^{13}C NMR (125.7 MHZ, $CDCl_3$, δ from solvent): 171.8, 171.7, 166.4, 166.3, 149.9, 137.5, 124.3, 123.7, 80.7, 80.6, 63.7, 62.0, 56.4, 54.0, 53.0, 52.4, 28.3, 26.0, 25.9. ESI-MS: *m/z* = 606.4 $[M+H]^+$; calcd: 606.4.

***N*-((5-methoxycarbonyl)-pyridin-2-yl)methyl-*N,N',N'-trans*-l,2-cyclohexylenediaminetriacetate (PyC3A-5-methyl ester) (3).** A batch of **2** (0.390 g, 0.645 mmol) was stirred in 5 mL 1:1 TFA:CH_2Cl_2 for 16h. The reaction mixture was

concentrated to en vacuo and purified by RP-HPLC using C18 column and method P1 to yield **3** (0.184 g, 0.421 mmol, 65% yield) as a white solid. ^{1}H NMR (500 MHZ, D_2O, 70 °C, δ from protio solvent): 9.83 (br s, 1H), 9.42 (br s, 1H), 8. 68 (br s, 1H), 5.04-4.80 (br m, 2H), 4.61 (br s, 3H), 4.47-4.27 (br m, 6H), 3.98 (br s, 1h), 3.74 (br s, 1h), 2.86-2.77 (br m, 2H), 2.46 (br s, 2H), 2.09-2.01 (br m, 2H), 1.89 (br s, 2H). ^{13}C NMR (125.7 MHZ, $CDCl_3$, 70 °C): 173.5, 170.7, 165.3 (one carboxylate *C*=O resonance was not resolved, likely due to coincidental overlay with another resonance), 157.0, 146.0, 145.0, 128.7, 127.1, 64.6, 62.9, 54.1, 53.0, 52.4, (one carboxylate *C*H_2 resonance was not resolved, likely due to coincidental overlay with another resonance), 24.7, 24.6, 24.5, 24.3. ESI-MS: *m/z* = 438.2 $[M+H]^+$; calcd: 438.2.

Na[Mn(PyC3A-5-methyl ester)] (4). A batch of **3** (0.182 g, 0.416 mmol) was dissolved in 10 mL H_2O and the pH adjusted to 6.5 with ammonium acetate. $MnCl_2$•$4H_2O$ (0.066 g, 0.130 mmol) were added and the pH re-adjusted to 6.5. The reaction mixture was purified via RPLC using the C18 column and method P2 to yield **4** (0.140 g, 0.273 mmol, 66% yield) as white solids. ESI-MS: *m/z* = 491.1 $[M+2H]^+$; calcd: 491.1.

B. Synthesis of N-((5-hydroxymethyl)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetriacetate (PyC3A-5-hydroxymethyl) (7).



Scheme 2

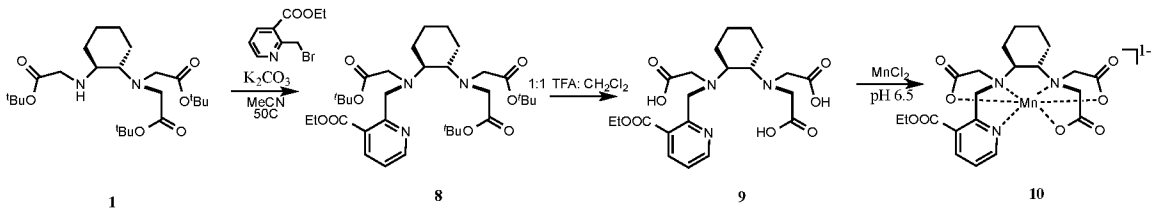
***N*-((5-hydroxymethyl)-pyridin-2-yl)methyl-*N,N',N'-trans*-l,2-cyclohexylenediaminetri-*tert*-butylacetate (5)**. A batch of **2** (0.200 g, 0.331 mmol) was stirred in 20 mL MeOH at RT and $NaBH_4$ (0.562 g, 14.9 mmoL) added portionwise over the course of 24h. The reaction was monitored by HPLC. At completion, 10 mL water was added and the reaction mixture was concentrated to dryness en vacuo. Crude **5** was carried

directly through to the next step without further workup or NMR characterization. ESI-MS: *m/z* = 578.4 $[M+H]^+$; calcd: 578.4.

***N*-((5-hydroxymethyl)-pyridin-2-yl)methyl-*N,N',N'-trans*-l,2-cyclohexylenediaminetriacetate (PyC3A-6-hydroxymethyl) (6)**. Crude **5** was stirred in 6 mL 1:1 TFA:CH_2Cl_2 for 16h. The reaction mixture was concentrated en vacuo and then purified by RP-HPLC using the C18 column and method P1 to yield **6** (0.036 g, 0.082 mmol, 27 % yield) as a white solid. ^{1}H NMR (500 MHZ, D_2O, 70 °C, δ from protio solvent): 9.32 (br s, 1H), 9.07 (br s, 1H), 8.65 (br s, 1H), 5.42 (br m, 2H), 4.92-4.71 (br m, 3H), 4.47-4.27 (br m, 3H), 4.16-4.07 (br m, 3H), 3.61 (br s, 1H). 2.83-2.78 (br m, 2H), 2.46 (br s, 2H), 2.04 (br s, 2H), 1.89 (br s, 2H). ^{13}C NMR (125.7 MHZ, $CDCl_3$, 70 °C): 176.8, 171.5 (one carboxylate *C*=O resonance was not resolved, likely due to coincidental overlay with another resonance), 153.7, 147.7, 163.0, 162.4, 129.6, 67.2, 63.8, 62.4, (one carboxylate CH_2 resonance was not resolved, likely due to coincidental overlay with another resonance), 55.3, 54.3, 26.5, 26.5, 26.4, 26.2. ESI-MS: *m/z* = 410.2 $[M+H]^+$; calcd: 410.2.

Na[Mn(PyC3A-5-hydroxymethyl)] (7). A batch of **6** (0.036 g, 0.082 mmol) was dissolved in 4 mL H_2O and the pH adjusted to 6.5 by addition of ammonium acetate. $MnCl_2$•$4H_2O$ (0.014 g, 0.070 mmol) were added and the pH re-adjusted to 6.5. The reaction mixture was purified using the C18 column and method P2 to yield **7** (0.040 g, 0.082 mmol, 100 yield) as white solids. ESI-MS: *m/z* = 463.1 $[M+2H]^+$; calcd: 463.1.

C. Synthesis of N-((3-ethoxycarbonyl)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetriacetate (PyC3A-3-ethyl ester) (**10**)



1 8 9 10

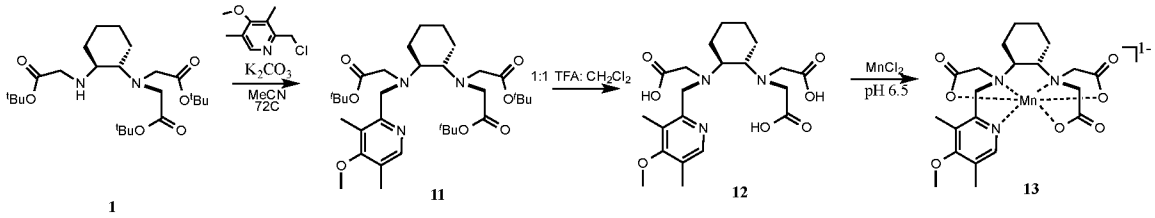
Scheme 3

N-((3-ethoxycarbonyl)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetri-*tert*-butylacetate (8). A batch of 0.042 g **1** (0.092 mmol) was combined with 0.152 g ethyl 2-(bromomethyl)nicotinate (0.661 mmol) and 0.120 g K_2CO_3 (8.70 mmol) in 5 mL MeCN and heated to 50 °C for 1h. The crude reaction mixture was then concentrated to dryness and purified by flash chromatography (silica gel, 30:70 hexane:EtOAc). **8** was isolated as a crude oil with impurities and carried directly through to the next reaction without characterization.

N-((3-ethoxycarbonyl)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetriacetate ((PyC3A-3-methylester) (9). 0.092 g (0.148 mmol) **8** was dissolved in 2 mL TFA and stirred for 16h. The TFA was removed en vacuo, the crude product taken up in CH_2Cl_2 and concentrated en vacuo. The product was purified by RP-HPLC using the C18 column and method P3 to yield 24 mg (0.053 mmol, 36% yield). ^{1}H NMR (500 MHZ, D_2O, δ from protio solvent): 8.67 (d, 1H), 8.41 (d, 1H), 7.47 (t, 1H), 4.90 (br s, 1H), 4.33 (q, 2H), 4.16 (d, 1H), 3.80-3.30 (m, 6H), 3.08 (br m, 2H), 2.28 (br m, 1H), 2.11 (br m, 1H), 1.84 (br m, 1H), 1.76 (br m, 1H), 1.57 (br m, 1H), 1.35 (t, 3H), 1.35-1.21 (m, 3H).). ^{1}H NMR (125.7 MHZ, d_6-DMSO): 174.7, 169.6, 166.4, 154.0, 152.6, 144.1, 126.4, 124.8, 66.3, 63.2, 61.9, 54.1, 50.4, 26.2, 26.2, 15.9, 25.6, 14.6. ESI-MS: *m/z* = 452.2 $[M+H]^+$; calcd: 452.1.

Na[Mn(PyC3A-3-ethyl ester)] (10). A batch of **9** (0.024 g, 0.053 mmol) and the pH adjusted to 6.5. $MnCl_2 \cdot 4H_2O$ (0.015 g, 0.076 mmol) were combined in 1.5 mL water and the pH adjusted to 6.5. The reaction mixture was purified by RP-HPLC using C18 column and method P4 to yield **10** (0.020 g, 0.031 mmol, 58% yield) as white solids. ESI-MS: *m/z* = 505.0 $[M+2H]^+$; calcd: 505.1.

D. Synthesis of N-((4-methoxy-3,5-dimethyl)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetriacetate (PyC3A-2-methoxy,3,5-dimethyl) (**13**)



Scheme 4

N-((4-methoxy-3,5-dimethyl)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetri-*tert*-butylacetate (11). A batch of **1** (0.127 g, 0.278 mmol) was combined with 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine (0.052 g, 0.280 mmol) and K_2CO_3 (0.103, 0.746 mmol) in 10 mL MeCN and heated to 72 °C for 24h. The crude reaction mixture was then concentrated to dryness. **11** was isolated as a crude oil with impurities and carried directly through to the next reaction without characterization.

N-((4-methoxy-3,5-dimethyl)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetriacetate (PyC3A-2-methoxy,3,5-dimethyl) (12). Crude **11** was stirred in 1 mL TFA for 16h, then concentrated to a yellow-colored oil and purified by RP-HPLC using the C18 column and method P3. **12** was isolated as a white solid (0.022 g, 0.050 mmol, 18% yield from **1**). ESI-MS: *m/z* = 438.2 $[M+H]^+$; calcd: 438.2.

Na[Mn(PyC3A-4-methoxy-3.5-dimethyl) (13). A batch of **12** (0.022 g, 0.050 mmol) and the pH adjusted to 6.5. $MnCl_2 \cdot 4H_2O$ (0.012 g, 0.061 mmol) were combined in 1.2 mL water and the pH adjusted to 6.5. The reaction mixture was purified by RP-HPLC using the C18 column and method P4 yield **13** (0.021 g, 0.041 mmol, 82 % yield) as white solids. ESI-MS: *m/z* = 491.1 $[M+2H]^+$; calcd: 491.1.

N'-(6-methyl)nicatinoyl-,N',N'',N''trans-l,2-cyclohexylenediaminetri-^{t}Bu-acetate (14): Lithium hydroxide (0.044 g, 1.84 mmol) and **2** (1.02 g, 1.69 mmol) were combined in 16 mL of 1:1 THF:H_2O and stirred for 3h at RT. The reaction was then concentrated to dryness and purified by preparative RP-HPLC using the C18 column and method P5. Fractions containing pure product were freeze dried to yield product as a white solid (0.670 g, 1.13 mmol, 67%). ^{1}H NMR (500 MHZ, D_2O, δ from protio solvent, stirred over K_2CO_3(s)): 9.09 (s, 1H), 8.15 (d, 1H), 7.07 (d, 1H), 3.95 (d, 1H), 3.63 (d, 1H), 3.28 (d, 1H), 3.14-3.06 (m, 5H), 2.33 (br t, 2H), 1.94 (m, 2H), 1.68 (m, 2H), 1.43 (9H), 1.38

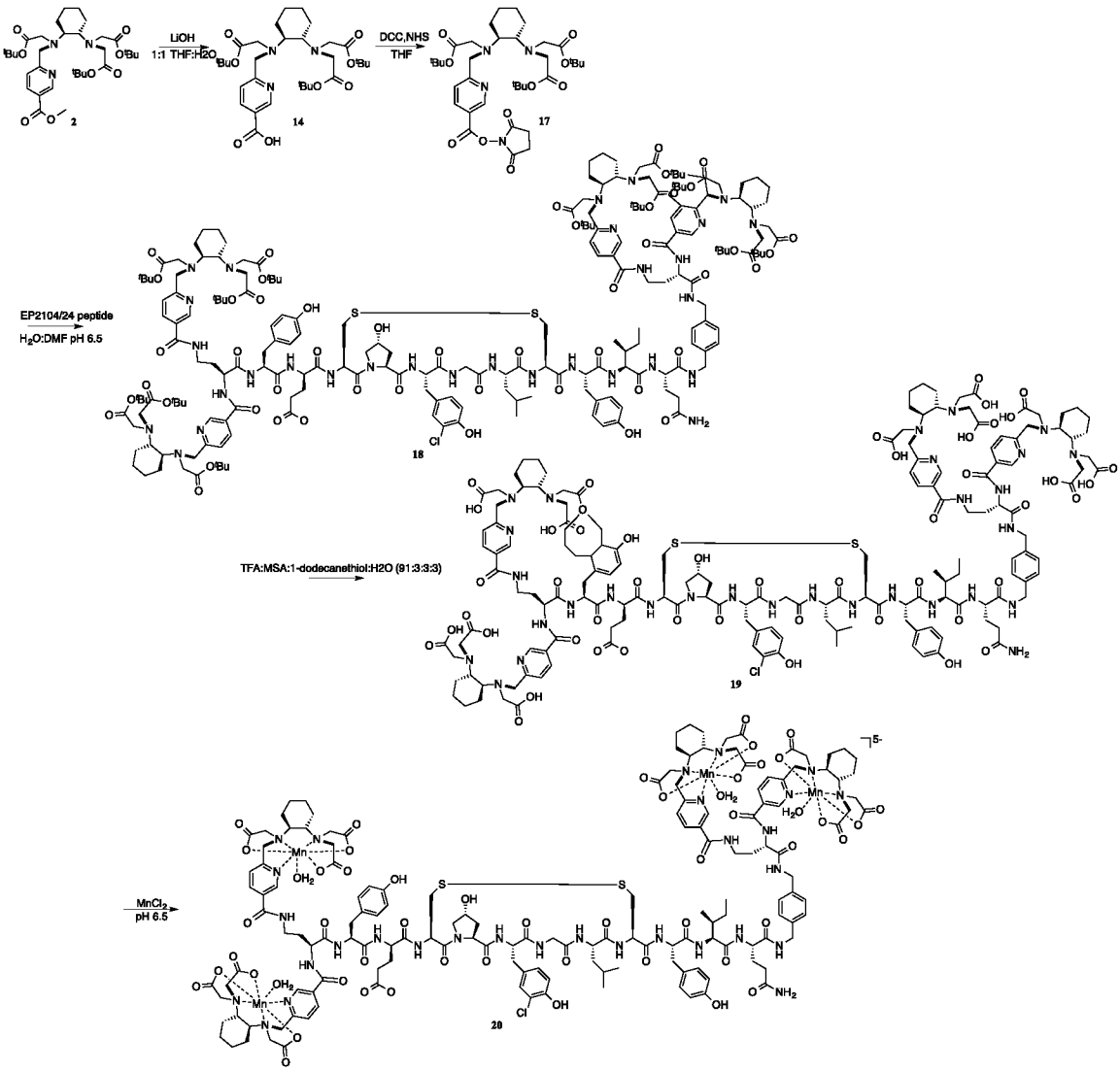
(18H), 1.18-0.99 (m, 4H). ^{13}C NMR (125.7 MHZ, $CDCl_3$, δ from protio solvent, stirred over K_2CO_3(s))): 172.2, 171.9, 170.8, 158.2, 151.1, 1380, 131.1, 122.9, 81.8, 81.5, 62.1, 59.5, 55.9, 53.0, 52.6, 29.8, 28.1, 28.0, 25.9, 25.7, 25.4, 24.8. ESI-MS: *m/z* = 592.3 $[M+H]^+$; calcd: 592.4.

E. Synthesis of N-((5-carboxylic acid)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetriacetate (PyC3A-5-carboxylic acid) (**15**).

N-((5-carboxylic acid)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetriacetate (PyC3A-5-carboxylic acid) (15). A batch of **14** (0.140 g, 0.237 mmol) was stirred in 5 mL TFA for 16h, then concentrated to yield **15** as a TFA adduct (0.12 g, 0.223 mmol, 94 % yield) as a white solid. ESI-MS: *m/z* = 424.2 $[M+H]^+$; calcd: 424.2.

Na[Mn(PyC3A-5-carboxylic acid) (16). To a batch of **15** (0.12 g, 0.223 mmol) adjusted to pH 6.5 was added with $MnCl_2 \cdot 4H_2O$ (0.047 g, 0.237 mmol) and the pH re-adjusted to pH 6.5. A portion of the reaction mixture was purified using method P7 to yield pure **15**. ESI-MS: *m/z* = 477.0 $[M+2H]^+$; calcd: 477.1.

Example 2: Synthesis of Fibrin-targeted chelate-metal complexes of Mn(II).



Scheme 5

***N*-(6-methyl)-N-hydoxysuccinimidylnicatinoyl-,*N*,*N*',*N*'-*trans*-l,2-cyclohexylenediaminetri-*tert*-butylacetate (^tBu-PyC3A-NHS) (17)**: (0.471 g, 0.797 mmol) **14** was stirred with dicyclohexylcarbodiimide (0.167 g, 0.809 mmol) and N-hydroxysuccinimide (0.109 g, 0.947 mmol) in 10 mL THF. A white precipitate formed within seconds. LC-MS confirmed full conversion after 16 h stirring. The precipitate was removed by filtration and the clear mother liquor concentration to a pale, colorless oil. The product can be carried directly through to the next step, or purified by RP-HPLC using the

C18 column and method P5. ^{1}H NMR (500 MHZ, D_2O, δ from protio solvent): 9.15 (s, 1H), 8.32 (d, 1H), 8.21 (d, 1H), 4.33 (d, 1H), 4.15 (d, 1H), 3.55-3.25 (m, 6H), 2.91 (s, 4H), 2.73 (t, 1H), 2.50 (t, 1H), 1.91 (m, 2H), 1.81 (m, 2H), 1.45 (18H), 1.38 (9H), 1.18-1.02 (m 4H). ^{13}C NMR (125.7 MHZ, $CDCl_3$, δ from protio solvent): 171.5, 169.3, 169.2, 161.3, 150.3, 138.2, 124.29, 119.7, 108.0, 98.5, 80.7, 80.6 (two coinicidental peaks), 68.6, 63.9, 62.2, 54.0, 52.8, 49.3, 34.0, 28.2 (two coincidental peaks), 29.3, 25.9, 25.9 (two coincidental peaks), 25.8, 25.7. ESI-MS: *m/z* = 689.3 $[M+H]^+$; calcd: 689.4.

^{t}Bu-protected FBP-CyP3A$_4$ (18): The oil was taken up in 2 mL DMF and added to *L*-2,4-diamino-N-butyramide-[Tyr-dGlu-Cys-Hyp-Typ(3-Cl)-Leu-Cys-Ile-Gln (3→8) disulfide]-1-(4-[(*L*-2,4-diamino-butyrylamino)-methyl]-benzylamide (EP2104/24: 0.211 g, 0.123 mmol)) and 4-dimethylaminopyridine (0.016g, 0.131 mmol) stirring in 2 mL DMF. The reaction was adjusted to pH 6.5 with DIPEA and stirred at RT. After 16 h stirring, 250 mL satd. NaCl solution was added to the reaction mixture dropwise to precipitate white solids. ^{t}Bu-protected FBP-CyP3A$_4$ was purified by RP-HPLC using the C5 column and method P6. Fractions containing product were freeze dried to yield product as a white solid (0.177g, 0.0441 mmol, 35 %). ESI-MS: *m/4z* = 1004.0 $[M+4H]^{4+}$; calcd. 1004.0. *m/3z* = 1338.4 $[M+3H]^{3+}$; calcd. 1338.4. *m/2z* = 2007.6 $[M+2H]^{2+}$; calcd. 2007.6.

FBP-CyP3A$_4$ (19): Compound **18** (0.177g, 44.1 µmol) was stirred in 5 mL of a 91:3:3:3 mixture of TFA: methane sulfonic acid: n-dodecanethiol: water for 90 min before dilution with 50 mL Et_2O. The flocculent white solids were centrifuged to a solid pellet and the supernatant decanted. After several washes with Et_2O the solids were dried to yield pure product as a white powder (0.147 g, 44.0 µmol, 100%). ESI-MS: *m/3z* = 1113.6 $[M+3H]^{3+}$; calcd. 1113.8. ESI-MS: *m/z* = 1670.1 $[M+2H]^{3+}$; calcd. 1670.2.

Mn-FBP (20): The pH of a 10 mL solution of **19** (0.018 g, 5.4 µmol) adjusted to pH 6.5 was added $MnCl_2$•$4H_2O$ (0.0053 g, 27.0 µmol). The solution was re-adjusted to pH 6.5 and purified by RP-HPLC using the C5 column and method P7. Fractions containing product were freeze dried to yield pure product as a while powder (0.0080 g, 2.2 µmol, 41%). ESI-MS: *m/3z* = 1184.6 $[M+8H]^{3+}$; calcd. 1184.4. *m/2z* = 1776.4 $[M+6H]^{2+}$; calcd. 1776.5.

Example 3: Synthesis of collagen-targeted chelate-metal complexes of Mn(II). **^{t}Bu-protected CBP-PyC3A$_4$ (21)**. To a batch of EP-3533 peptide (see Caravan, P., Biplab, D., Dumas, S., Epstein, F. H., Helm, P. A., Jacques, V., Koerner, S., Kolodziej, A., Shen, L., Sun, W.-C., Zhang, Z. *Angew. Chem. Int. Ed.* **2007**, *46*, 8171) (0.154 g, 0.067 mmol) stirring in 3 mL DMF/ 1 mL H_2O adjusted to pH 9 with DIPEA (wet pH paper), was added **17** (0.280g, 0.407 mmol) portionwise. After 16h stirring, the solution was diluted with 100 mL water and freeze dried to a crude white residue. Compound **21** was carried through to the next step without further purification. ESI-MS: *m/3z* = 1337.0 $[M+3H]^{3+}$; calcd. 1337.0 **CBP-PyC3A$_4$ (22)**. Compound **21** was stirred in 4 mL 91:3:3:3 TFA:methanesulfonic acid:1-dodecanethiol:H_2O for 2h. The reaction mixture was then added to 100 mL cold Et_2O to precipitate the product. The solids were collected in the bottom of a 50 mL conical tubes by centrifugation and separated by decantation. Compound **22** was carried through to the next step without further purification. ESI-MS: *m/3z* = 1168.0 $[M+3H]^{3+}$; calcd. 1167.8

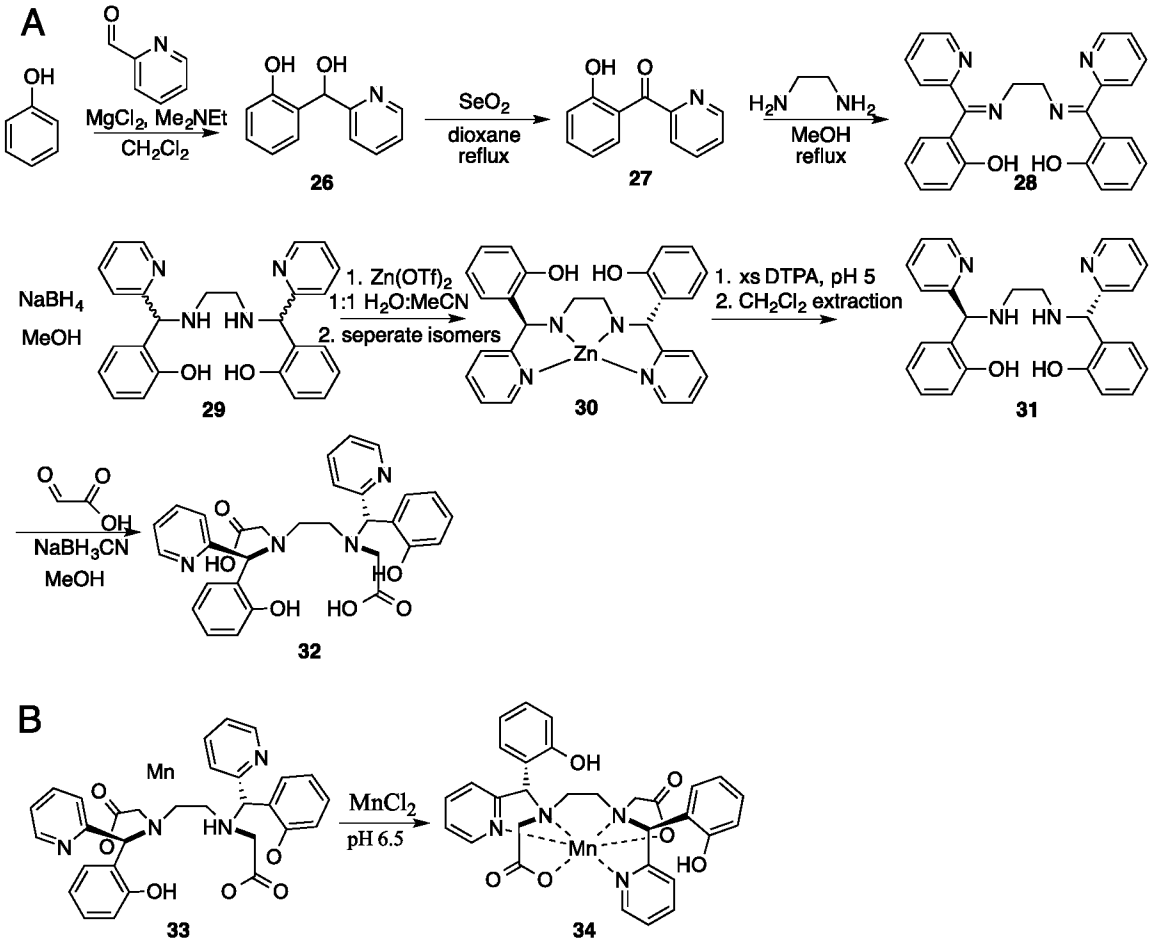
Mn-CBP (23). A batch of $MnCl_2 \cdot 4H_2O$ (0.047 g, 0.237 mmol) was added to crude **22** to form **23** which was then purified by HPLC. ESI-MS: *m/6z* = 1221.0 $[M+6H]^{3+}$; calcd. 1221.1.

Example 4: Synthesis of oxidized collagen-targeted chelate-metal complexes of Mn(II).

N-((5-hydrazide)-pyridin-2-yl)methyl-N,N',N'-trans-l,2-cyclohexylenediaminetriacetate (PyC3A-5-hydrazide) (24). A batch of **17** (0.180 g, 0.262 mmol) and *tert*-butylcarbazate (0.070 g, 0.606 mmol) were stirred in 5 mL MeOH/ 2 mL THF. The pH of the reaction mixture was adjusted to ~9 (wet pH paper). After 2h stirring, the reaction mixture was concentrated to dryness and taken up in 5 mL of 6M HCl. After 2h stirring, the reaction mixture was concentrated to white solids and taken up in H_2O. Any solids were removed by filtration. Small aliquots of this reaction mixture were removed and purified by method P7 to yield **24** as white powder. ESI-MS: *m/z* = 438.2 $[M+H]^+$; calcd: 438.2.

Na[Mn(PyC3A-5-hydrazide)] (25). Compound **25** was prepared in situ. To a solution of **24** in pH 7.4 Tris buffer (50 mM) was titrated $MnCl_2 \cdot 4H_2O$. Chelation of Mn was monitored by HPLC. ESI-MS: m/z = 491.2 $[M+2H]^+$; calcd: 491.1.

Example 5: Synthesis of a peroxidase reactive complex



Scheme 6

2-((hydroxy(pyridin-2-yl)methyl)phenol (26). A batch of 10.4 g (111 mmol) phenol in 50 mL CH_2Cl_2 was stirred with 18.4 g (193 mmol) magnesium chloride and 8.42 g (115 mmol) N,N-dimethylethylamine for 30 min. To this mixture, 5.85 g (55.0 mmol) 2-pyridinecarboxaldehyde in 25 mL CH_2Cl_2 was added dropwise over the course of 3h. After completion of the addition, the resultant heterogenous, bright orange mixture was stirred

at RT for 72 h. 100 mL H_2O was added to the reaction, and the mixture was titrated with conc. HCl to pH 1, than the pH adjusted to 9 via addition of conc. Na_2CO_3. The organic layer was separated, and the aqueous portion washed again with 100 mL CH_2Cl_2. The organic portions were pooled, dried over Na_2SO_4 and concentrated to an orange-colored syrup. The product was purified by flash chromatography (silica gel, hexane:EtOAc, 0-60% EtOAc) to yield 7.08 g (35.2 mmol, 64 % yield) **26** as a white solid. ^{1}H NMR (500 MHZ, $CDCl_3$, δ from TMS): 8.47 (d, 1H), 7.72 (t, 1H), 7.45 (d, 1H), 7.21 (d, 1H), 7.21 (m, 2H), 6.95 (d, 1H), 6.89 (t, 1H), 6.96 (s, 1H). ^{13}C NMR (125.7 MHZ, CD_3Cl, δ from TMS): 161.7, 155.3, 147.6, 138.2, 129.5, 128.3, 127.0, 123.0, 120.4, 120.2, 118.5, 74.4. ESI-MS: *m/z* = 202.0 $[M+H]^+$; calcd.: 202.0.

2-picoloylphenol (27). To a batch of 1.41 g (7.01 mmol) 2-(hydroxy(pyridin-2-yl)methyl)phenol in 40 mL dioxane was added 0.418 g (3.77 mmol) selenium(IV) dioxide. The mixture was heated to reflux and stirred for 90 min. The reaction was then cooled and black insoluble were removed filtration through celite and the mother liquor concentrated to a yellow-green oil. The oil was purified by flash chromatography (silica gel, hexane:EtOAc, 0-70% EtOAc) and 1.190 g (0.597 mmol, 85.2%) were isolated as a bright yellow oil.

2,2'-((1*Z*,1'*Z*)-(ethane-1,2-diylbis(azanylylidene))bis(pyridin-2-ylmethanylylidene))diphenol (28). To a batch of 0.168 g (2.80 mmol) ethylene diamine in 40 mL MeOH was added 1.190 g (5.98 mmol) **27**. The reaction was heated to reflux and stirred 30 min, then cooled to RT and stirred for 16h. The resultant yellow solids were isolated via filtration and dried en vacuo, yielding 0.942 g (2.22 mmol, 79.6% yield) **28** isolated as a yellow solid. ^{1}H NMR (500 MHZ, $CDCl_3$, δ from TMS): 12.36 (s, 1H), 8.75 (d, 1H), 8.13 (d, 1H), 7.95 (m, 2H), 7.53 (m, 2H), 7.07 (d, 1H), 6.92 (t, 1H). ^{13}C NMR (125.7 MHZ, CD_3Cl, δ from TMS): 197.3, 163.6, 155.5, 148.4, 137.6, 136.8, 134.5, 126.3, 124.7, 119.3, 119.0, 118.6. ESI-MS: *m/z* = 200.0 $[M+H]^+$; calcd.: 200.1.

(+/-) (R,R/S,S) 2,2'-((ethane-1,2-diylbis(azanediyl))bis(pyridin-2-ylmethylene))diphenol (31). To a batch of 2.90 g (6.87 mmol) **28** stirring in 50 mL MeOH was added 0.758 g (20.0 mmol) sodium borohydride. Effervescence was observed as the color of the solution bleached from yellow to a clear beige. After 1h, the reaction was

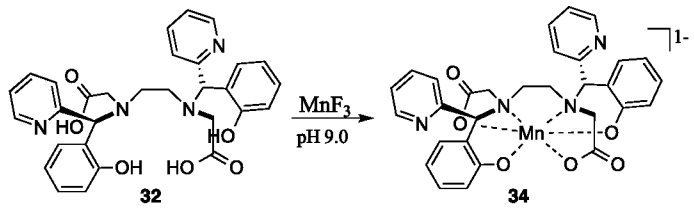
quenched with 10 mL water and MeOH was removed en vacuo. The reaction was then partitioned between 50 mL each satd. $NaHCO_{3\,(aq)}$ and CH_2Cl_2. The layers were separated and the aqueous portion washed with another 100 mL CH_2Cl_2. The oganic portions were pooled, dried over Na_2SO_4 and concentrated to 2.92 g of **29** of yellow oil (6.85 mmol, 100% conversion to racemic diamine). The oil was taken up in 30 mL MeOH/ 10 mL H_2O and 2.490 g $Zn(OTf)_2$ (6.85 mmol) were added. The solution was adjusted to pH 6.5 by addition of solid ammonium acetate and stirred for 16h before concentration to dryness.

The Zn chelated R,R/S,S and R,S/S,R isomers (**30**) were separated by RP-HPLC using the C18 column and method P7. A batch of 0.075 mmol (0.154 mmol) R,R/S,S isomer was dissolved 200 mM diethelynetriaminepentaacetic acid and the pH adjusted to 5.0. After 2h stirring, the pH was adjusted to >8.0 and **31** was extracted with CH_2Cl_2, dried over Na_2SO_4 and dried en vacuo pure product isolated at 0.066 g (0.154 mmol). ^1H NMR (500 MHZ, $CDCl_3$, δ from TMS): 8.57 (d, 2H), 7.60 (t, 2H), 7.21 (m, 4H), 7.03 (m, 4H), 6.84 (m, 4H), 4.94 (s, 2H), 2.94 (s, 4H).

Janus HBED/BPED "JED" (32). To a batch of 0.082 g (0.192 mmol) **31** in 20 mL MeOH was added 1.03 g (12.3 mmol) $NaHCO_3$ and 0.889 g (9.66 mmol) glyoxylic acid monohydrate). A batch of 0.119g (1.89 mmol) sodium cyanoborohydride was added portionwise over the course of 8h. After stirring for 16h at RT, the reaction mixture was concentrated to dryness and purified by RP-HPLC using the C18 column and preparative method P8. Pure **32** was isolated as 0.104g (0.192 mmol, 100% yield) of a white solid. ESI-MS: *m/z* = 543.0 $[M+H]^+$; calcd.: 543.2.

Mn(II)-JED (33): To a batch of 0.104 g (0.192 mmol) **32** stirring in 4 mL each H_2O:MeCN was added 0.037 g (0.187 mmol) $MnCl_2 \cdot H_2O$ and the solution adjusted to pH 6.5 by addition of solid ammonium acetate. The reaction mixture was purified by RP-HPLC using the C18 column and method P7. Pure **33** was isolated as 0.041 g (0.069 mmol, 35.9% yield) white solids. ESI-MS: *m/z* = 596.0 $[M+H]^+$; calcd.: 596.2.

Example 6: Synthesis of a thiol reactive complex.



32 34

Scheme 7

Mn(III)-JED (34): To a batch of 0.053 g (0.098 mmol) **32** stirring in 10 mL H_2O at pH 9.1 was added 0.009 g (0.080 mmol) of MnF_3 and the solution adjusted to pH 6.5. The reaction mixture was purified by RP-HPLC using the C18 column and method P9. Pure **34** was isolated as 0.018 mmol (0.029, 36.3% yield) brown solids. ESI-MS: *m/z* = 595.0 $[M+2H]^+$; calcd.: 595.1.

Example 7: Relaxivity of Mn(II) complexes that target proteins at 1.41T, 37 °C. Incorporation of lipophilic functionality into the chelate-metal complexes promotes binding to plasma proteins such as serum albumin. This binding provides a change in relaxivity measured in human blood plasma compared to that measured in PBS. In most cases, a large (40-170%) increase in relaxivity (r_1 or r_2) is observed (Table 1).

Chelate-metal complex	**r_1 in PBS ($mM^{-1}s^{-1}$)**	**r_2 in PBS ($mM^{-1}s^{-1}$)**	**r_1 in human blood plasma ($mM^{-1}s^{-1}$)**	**r_2 in human blood plasma ($mM^{-1}s^{-1}$)**
4	3.1	6.9	2.1	7.8
7	2.1	3.5	5.7	12.4
10	2.7	5.6	5.8	16.8
13	2.5	4.8	3.5	8.9
25	3.4	8.7	5.2	14.6

Table 1 shows the relaxivity of four protein binding Mn(II)-based chelate-metal complexes.

Example 8. 18 has high affinity for the soluble fibrin degradation product DD(E). The affinity of the probes was assessed using the DD(E) fluorescence polarization displacement assay (see methods, above). The displacement of a tetramethylrhodamine labeled derivative of the fibrin binding peptide (termed TRITC-Tn6) from DD(E) as a function of **18** or EP-2104R concentration was detected by observing the corresponding change in fluorescence anisotropy (Fig 1). The K_d of the TRITC-Tn6 probe was determined by titrating it with the DD(E) protein and fitting the resultant fluorescence data as described by Kolodziej (*Bioconjugate Chem.* 53:548-556). **18** binds DD(E) with K_d = 110 nM; we recorded K_d = 240 nM for EP-2104R in the same DD(E) preparation.

Example 9. The Mn of 20 has a rapidly exchanging inner sphere water co-ligand. The presence of a rapidly exchanging water co-ligand (rate of exchange = 10^8 s^{-1}) was established by monitoring the temperature dependence of $H_2{}^{17}O$ transverse relaxivity of **20** (Fig 2) (see methods, above).

Example 10. 20 exhibits high-relaxivity in the presence of fibrin. Relaxivity values of **20** were recorded in pH 7.4 Tris buffer, bovine blood plasma, 4.5% wt/v BSA, human fibrinogen and human fibrin gel at 1.4T, 37 °C (Table 2). **18** exhibits greater relaxivity in the presence of fibrin gel compared to human fibrinogen or to abundant plasma proteins

conditions	r_1 ($mM^{-1}s^{-1}$)
PBS	8.7
Blood Plasma	10.7
Bovine Serum Albumin	11.4
Fibrinogen	9.5
Fibrin	13.5

Table 2 shows the T_1-relaxivity of **20** measured in pH 7.4 Tris buffer, bovine blood plasma, 4.5% wt/v BSA, human fibrinogen and human fibrin gel at 1.4T, 37 C°.

Example 11. 20 detects carotid artery thrombosis. Compound **20** provides visualization of the arterial thrombus with high conspicuity and the imaging is supported by ex vivo histology (Fig 3). Surprisingly, **20** provides equivalent thrombus nSNR and thrombus-to-muscle contrast to noise ratio (CNR) to the Gd based fibrin imaging probe EP-2104R that is known to have even higher relaxivity (J. Am. Chem. Soc. 2008, 130:6025) (Fig 4). This data further highlights the efficacy of Mn to generate MR contrast.

Example 12. Intravenously injected Mn-FBP is wholly cleared by 24h. Compound **20** clears form the blood with a half-life of 22.6±6.8 min (Fig 5). The only significant increase in Mn over baseline levels were found in the kidney and muscle where 0.37±0.14 and 0.02±0.02 percent of the injected dose per gram remain, respectively (Table 3).

	Endogenous Mn		**24h p.i**		**P**
Tissue	**nmol Mn/g**	**Std. dev.**	**nmol Mn/g**	**Std dev**	*** < 0.05**
Lung	4.48	2.62	4.90	0.51	0.76
Kidney	17.84	5.39	47.39	14.93	0.01*
Brain	7.27	0.52	13.78	6.85	0.11
Liver	50.75	2.97	54.88	7.84	0.36
Heart	7.72	1.01	8.84	0.80	0.13
Spleen	6.21	3.53	12.90	5.76	0.09
Muscle	1.77	0.42	4.30	1.58	0.02*
Bone	7.97	0.57	7.32	2.21	0.59
Blood	1.03	0.83	0.34	0.10	0.15

Table 3 shows endogenous Mn levels in male Wistar rats (N=4) and Mn levels 24h after intravenous injection of 0.01 mmol/kg compound **20** (N=4). Statistically significant differences (*, P<0.05) were observed in kidney and muscle and represent 0.37±0.14 and 0.02±0.02 percent of the injected dose per gram tissue, respectively.

Example 13. The relaxivity difference between compounds 33 and 34 is very large and field independent. The relaxivity of the compounds **33** and **34** were measured in water and human blood plasma (Table 4). The relaxivity changes observed between toggling between **33** and **34** oxidation states are 660%, 900%, 500%, and 400% for conditions A-D, respectively.

	r1 ($mM^{-1}s^{-1}$)			
Compound	**Water**		**Human blood plasma**	
	1.41 T	11.7 T	1.41 T	11.7 T
33	0.5	0.5	0.9	0.5
34	3.3	2.5	8.1	1.9

Table 4 shows that the Mn(II)-based **26** demonstrates much higher relaxivity than its Mn(III)-based sister complex **27** and that this relaxivity difference is largely independent of applied magnetic field.

Example 14. Compound 33 is rapidly converted to compound 34 by peroxidase enzymes with 7-fold relaxivity change. The The relaxivity of compound **33** in PBS (1.41T, 37 °C) was measured in the presence of a steady state concentration of hydrogen peroxide generated by 10U/mL glucose oxidase + 8 mM glucose (Figure 6). Measurements were performed in the absence or presence of horseradish peroxidase. The filled and open

circles depict relaxivity in the absence and presence of the peroxidase enzyme, respectively. Solid lines represent fits to the data.

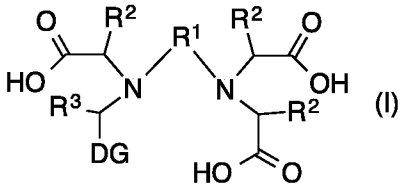
Figure 7 shows HPLC traces taken after the 1h incubation period. The top trace HPLC trace corresponds to that of **33**, the bottom trace corresponds to that of after 1h incubation with hydrogen peroxide and peroxidase. **34** is the only product formed.

Example 15. In human blood plasma, compound 34 can be converted to compound 33 by addition of L-cysteine. The r_1 as a function of time (1.4T, 37 °C) of **34** in human blood plasma without and with 5 mol. equivalent L-cysteine is depicted by the filled and open circles, respectively (Fig 8). Solid lines represent fits to the data. Addition of L-cysteine triggers rapid conversion of compound **34** to compound **33**, this causes a large increase in r_1.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS

1. A compound of Formula (I):



(I)

or a pharmaceutically acceptable salt thereof,

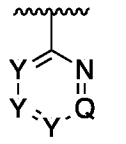
wherein:

R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

each R^2 and R^3 is independently selected from the group consisting of H, CO_2H, (C_1-C_6 alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and [L]-[TBM];

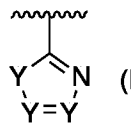
each R^4 and R^5 is independently selected from the group consisting of H, C_1-C_6 alkyl, and [L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

DG is selected from the group consisting of:



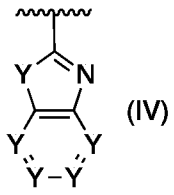
(II)

;



(III)

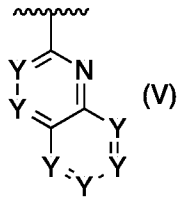
;



(IV)

;

and



(V)

or any constitutional isomers of Formulas IV and V, wherein

each Y is independently CH, CZ, N, O, S or NR^4;

Q is CH, CZ, N, O, S or NR^4;

each Z is independently selected from the group consisting of H, OH, OR^4, CO_2R^4, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

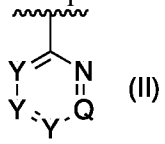
L is a linker;

TBM is a target binding moiety; and

each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM];

wherein if Q is CH or CCOOH and all Y are CH, than at least one of R^2 or R^3 is not H.

2. The compound of claim 1, wherein DG is:


(II)

3. The compound of any one of claims 1-2, wherein Q is CH.

4. The compound of any one of claims 1-3, wherein Y is CH.

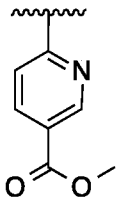
5. The compound of any one of claims 1-4, wherein at least one Y is CZ, wherein Z is selected from the group consisting of CO_2R^4, C_1-C_6 alkyl, and OR^4.

6. The compound of any one of claims 1-5, wherein one Y is CZ, wherein Z is selected from the group consisting of CO_2R^4, C_1-C_6 alkyl, and OR^4, and all other Y are CH.

7. The compound of any one of claims 1-6, wherein each R^4 is H or C_1-C_6 alkyl, wherein the alkyl is optionally substituted by 1, 2, 3, or 4 OH groups.

8. The compound of any one of claims 1-7 wherein R^2 and R^3 is H.

9. The compound of any one of claims 1-8, wherein DG is selected from the group consisting of: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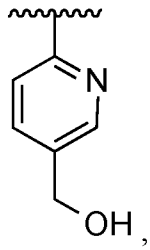
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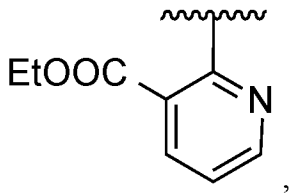

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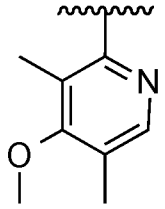
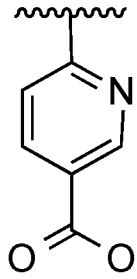


,



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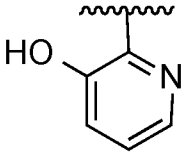
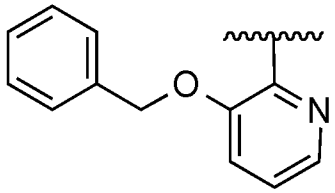
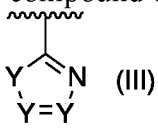


, and



.

10. The compound of claim 1, wherein DG is



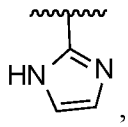
(III)

.

11. The compound of claim 10, wherein at least one Y is N, O, S or NR^4.

12. The compound of any one of claims 10-11, wherein one Y is NR^4 and the remaining Y are CH.

13. The compound of any one of claims 1-8, wherein DG is selected from the group consisting of:



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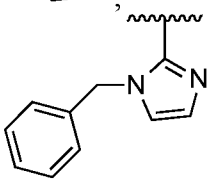
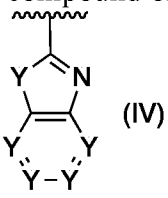


,

14. The compound of claim 1, wherein DG is:



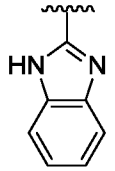
(IV)

.

15. The compound of claim 14, wherein at least one Y is N, O, S or NR^4.

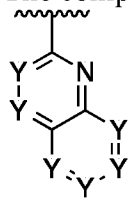
16. The compound of claim 14 or 15, wherein one Y is NR^4 and the remaining Y are CH.

17. The compound of any one of claims 14-16, wherein DG is:



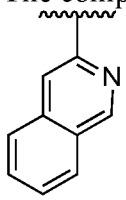
.

18. The compound of claim 1, wherein DG is:



(V)

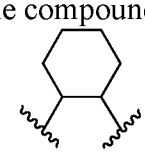
19. The compound of claim 18, wherein DG is:



20. The compound of any one of claims 1-19 wherein R^1 is C_3-C_{10} cycloalkylene.

21. The compound of any one of claims 1-19, wherein R^1 is a C_6 cycloalkylene.

22. The compound of any one of claims 1-19,wherein R^1 is:

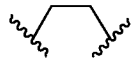


.

23. The compound of any one of claims 1-19, wherein R^1 is a C_1-C_6 alkylene.

24. The compound of any one of claims 1-19, wherein R^1 is a C_2 alkylene.

25. The compound of any one of claims 1-19 wherein R^1 is:

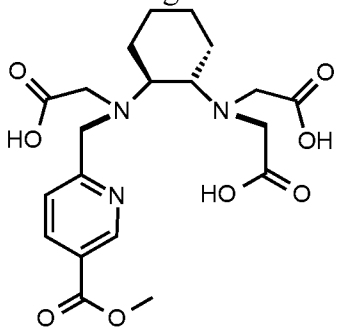


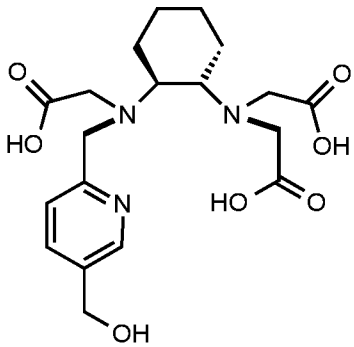
.

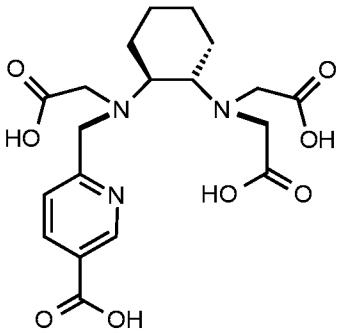
26. The compound of any one of claims 1-25, wherein R^2 is H.

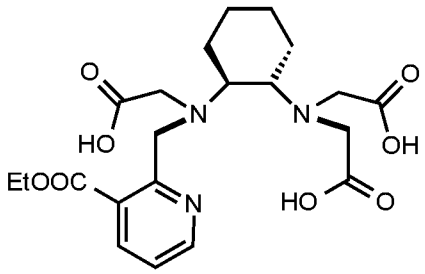
27. The compound of any one of claims 1-26, wherein R^3 is H.

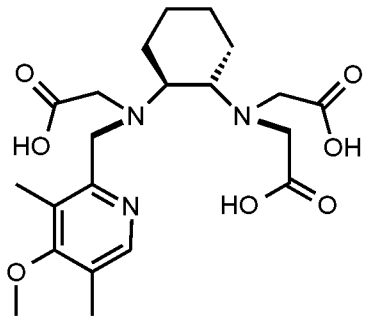
28. The compound of claim 1, wherein the compound is selected from the group consisting of:

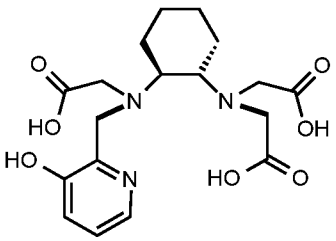


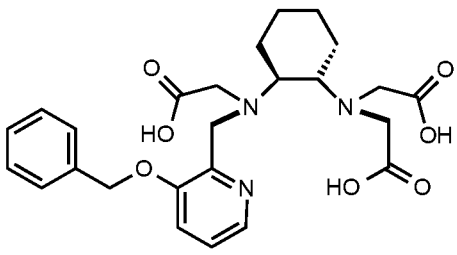


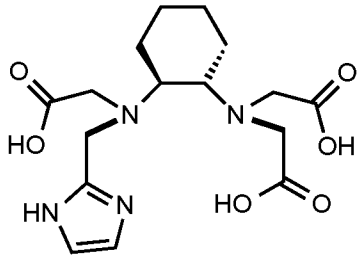
124 

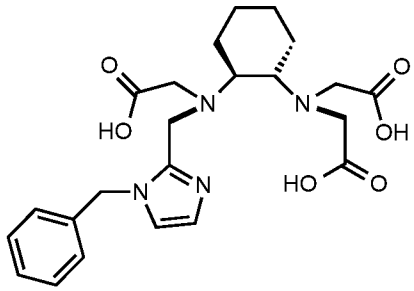
125 

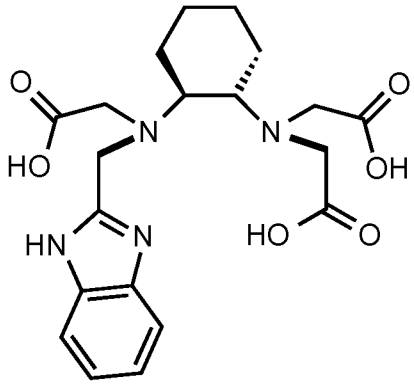
126 

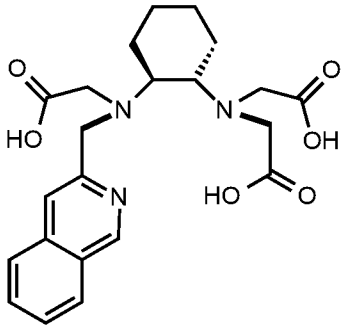
127 

128 

129 

130 

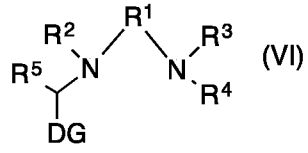
131  , and

132 

133 or a pharmaceutically acceptable salt thereof.

134

135 29. A compound of Formula (VI):

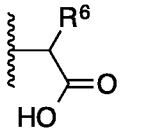
136  (VI)

or a pharmaceutically acceptable salt thereof,

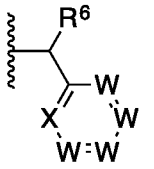
wherein:

R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

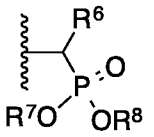
R^2, R^3, and R^4 are independently selected from the group of compounds of formula:


(VII)

;

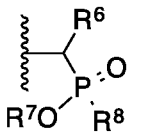

(VIII)

;


(IX)

;

and


(X)

R^5 and R^6 are independently selected from the group consisting of H, CO_2H, (C_1-C_6 alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7, $C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and [L]-[TBM];

X is CZ, N, O, S or NR^7;

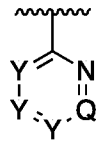
each W is independently CH, CZ, N, O, S or NR^7;

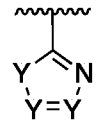
each Z is independently selected from H, OH, OR^4, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered

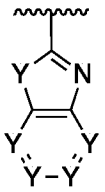
heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

each R^7 and R^8 are independently selected from the group consisting of H, C_1-C_6 alkyl, and -[L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

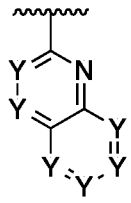
DG is selected from the group consisting of:


(XI)
;


(XII)
;


(XIII)
;

and


(XIV)

or any constitutional isomers of Formulas XIII-XIV,

wherein each Y is independently CH, CZ^1, N, O, S, or NR^7;

Q is independently CH, CZ^1, N, O, S, or NR^7;

each Z^1 is independently selected from H, OH, OR^4, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and -[L]-

[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

L is a linker;

TBM is a target binding moiety; and

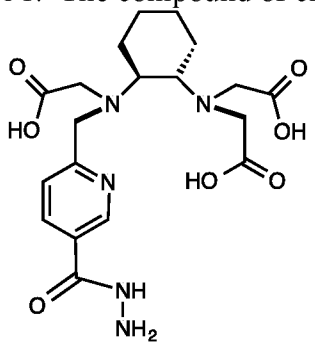
each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM];

wherein if Q is CH or CCOOH, all Y are CH, and all of R^2, R^3, and R^4 are formula VII, than at least one of R^5 or R^6 is not H; and

if one of R^2, R^3, or R^4 is formula VIII, and all of R^5 and R^6 are H, than the aromatic ring component of formula VIII (i.e. the ring containing X and W) must be different than DG.

30. The compound of claim 29, wherein R^1 is 1,2-cyclohexylene, R^2, R^3, and R^4 are formula VII, R^5 and R^6 are H, and DG is formula XI and one Y is [L]-[TBM], where [L] is –C(O)- and [TBM] is $–NHNH_2$.

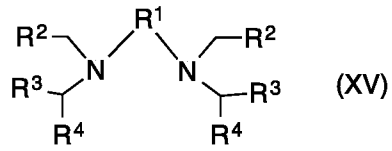
31. The compound of claim 29, wherein the compound is:



or a pharmaceutically acceptable salt thereof.

206

207 32. A compound of Formula (XV):



(XV)

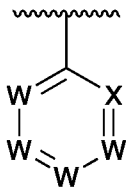
208

209 or a pharmaceutically acceptable salt thereof,

210 wherein:

211 R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10
212 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-
213 C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene),
214 wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene
215 are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and
216 wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

217 each R^2, R^3, and R^4 are independently selected from the group consisting of CO_2H,
218 ($C(O)NR^5R^6$, CH_2NHCOR^5, $C(O)N(OH)R^5$, $C(O)NHSO_2R^5$, $CH_2NHSO_2R^5$,
219 $N(OH)C(O)R^5$, $P(R^5)O_2R^6$, and $PO_3R^5R^6$, and compounds of formula:



(XVI)

220 ;

221 wherein X is CZ, N, O, S, or NR^5;

222 each W is independently CH, CZ, N, O, S, or NR^5;

223 each Z is independently selected from H, OH, OR^4, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6
224 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered
225 heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^5R^6$, CH_2NHCOR^5, $C(O)N(OH)R^5$,
226 $C(O)NHSO_2R^5$, $CH_2NHSO_2R^5$, $N(OH)C(O)R^5$, $P(R^5)O_2R^6$, $PO_3R^5R^6$, and -[L]-
227 [TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and
228 heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X
229 groups;

each R^5 and R^6 are independently selected from the group consisting of H, C_1-C_6 alkyl, and -[L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

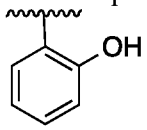
L is a linker;

TBM is a target binding moiety; and

each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM].

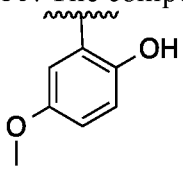
33. The compound of claim 32, wherein R^1 is 1,2-ethylene, R^2 is COOH, R^3 is Formula XVI wherein X is N and all W are CH, and R^4 is selected from a compound Formula XVI.

34. The compound of claim 33, wherein R^4 is:



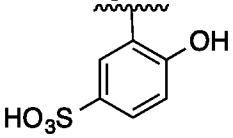
.

35. The compound of claim 33, wherein R^4 is:



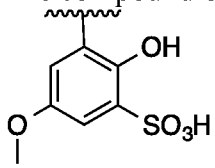
.

36. The compound of claim 33, wherein R^4 is:



.

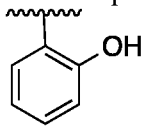
37. The compound of claim 33, wherein R^4 is:



.

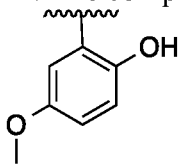
38. The compound of claim 32, wherein R^1 is 1,2-ethylene, R^2 is COOH, R^3 is COOH, and R^4 is selected from compound Formula XVI.

39. The compound of claim 38, wherein R^4 is:



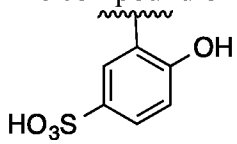
.

40. The compound of claim 38, wherein R^4 is:



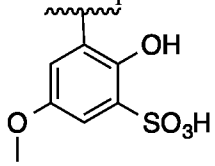
.

41. The compound of claim 38, wherein R^4 is:



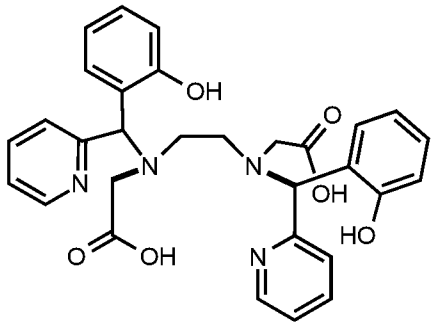
.

42. The compound of claim 38, wherein R^4 is:

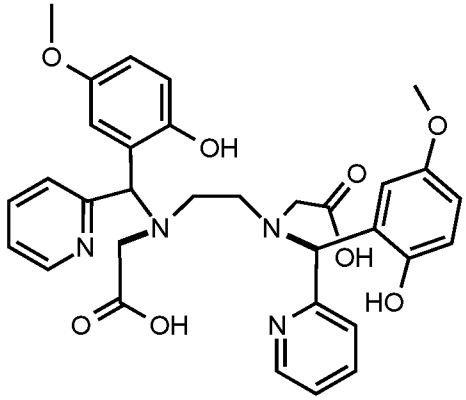


.

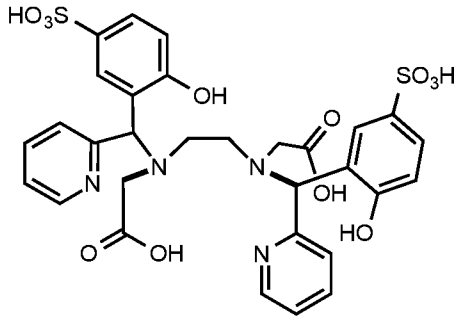
43. The compound of claim 32, wherein the compound is selected from the group consisting of:



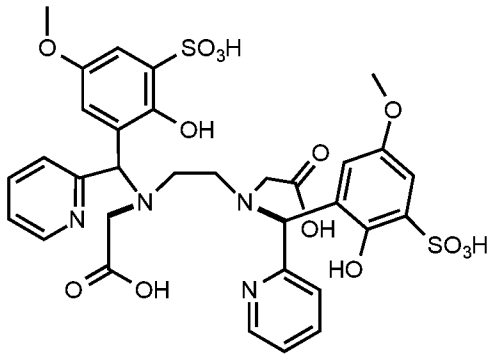
277



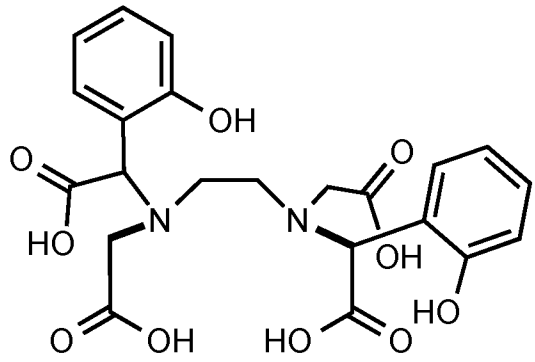
278

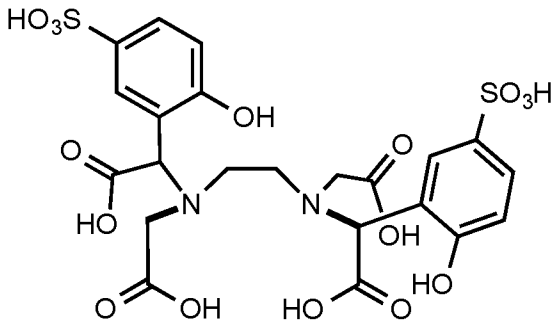


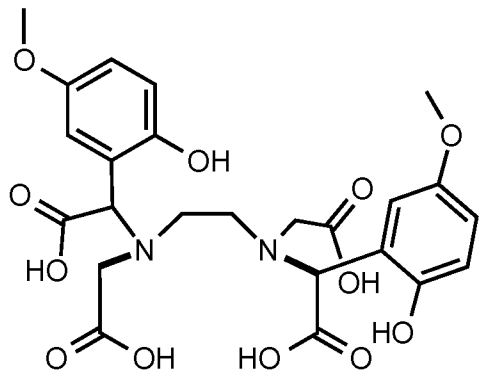
279

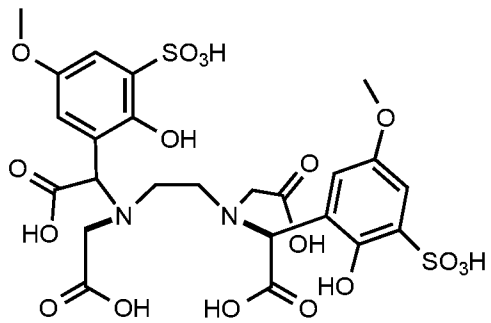


280

281 

282 

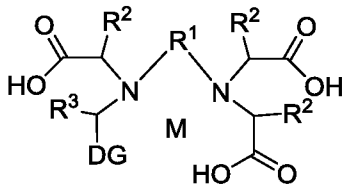
283  , and

284 

285 or a pharmaceutically acceptable salt thereof.

286

287 44. A compound of Formula (XVII):



or a pharmaceutically acceptable salt thereof,

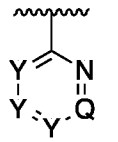
wherein:

R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

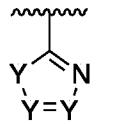
each R^2 and R^3 are independently selected from the group consisting of H, CO_2H, (C_1-C_6 alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and [L]-[TBM];

each R^4 and R^5 is independently selected from the group consisting of H, C_1-C_6 alkyl, and [L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

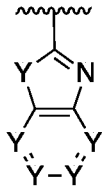
DG is selected from the group consisting of:


(II)

;

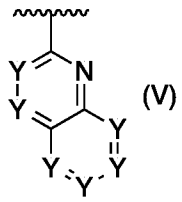

(III)

;


(IV)

;

and



(V)

or any constitutional isomers of Formulas IV and V, wherein

each Y is independently CH, CZ, N, O, S, or NR^4;

Q is CH, CZ, N, O, S, or NR^4;

each Z is independently selected from the group consisting of H, OH, OR^4, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^4R^5$, CH_2NHCOR^4, $C(O)N(OH)R^4$, $C(O)NHSO_2R^4$, $CH_2NHSO_2R^4$, $N(OH)C(O)R^4$, $P(R^4)O_2R^5$, $PO_3R^4R^5$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

L is a linker;

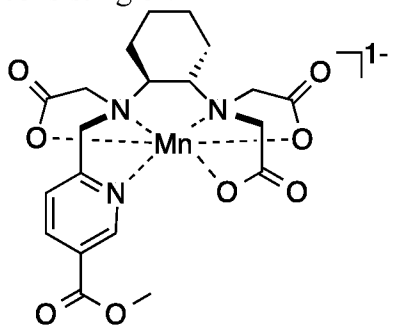
TBM is a target binding moiety;

each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM]; and

M is selected from the group consisting of Gd(III), Fe(III), Mn(II), Mn(III), Cr(III), Cu(II), Cu(III), Dy(III), Ho(III), Er(III), Pr(III), Eu(II), Eu(III), Nd(III), La(III), Lu(III), Sm(III), Tb(III), Tb(IV), Tm(III), Y(III), In(III), Ga(III), Tc(III), Tc(IV), Tc(V), Re(III), Re(IV), Re(V), Bi(III), and Yb(III);

wherein, if Q is CH or CCOOH and all Y are CH, than at least one of R^2 or R^3 is not H..

336 45. The compound of claim 44, wherein the compound is selected from the group
337 consisting of:



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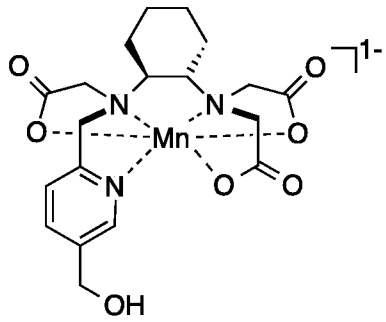

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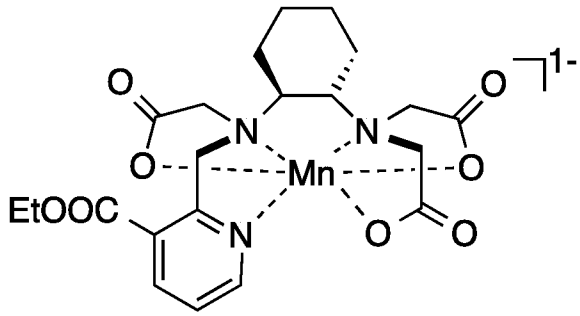

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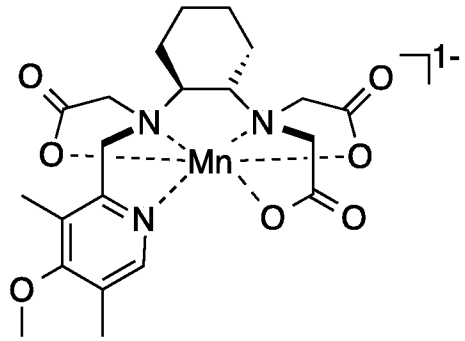

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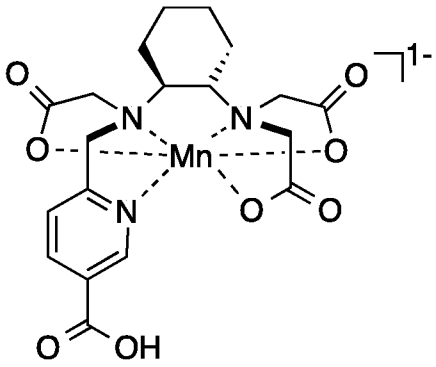

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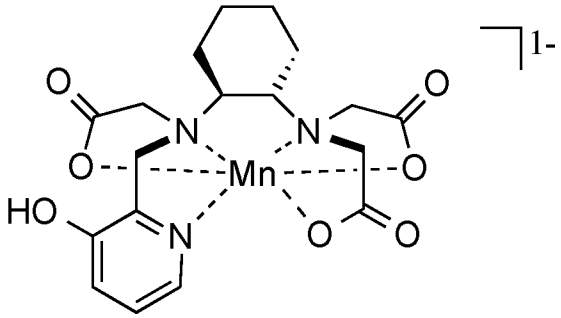

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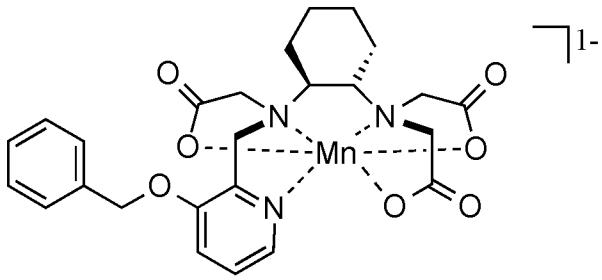

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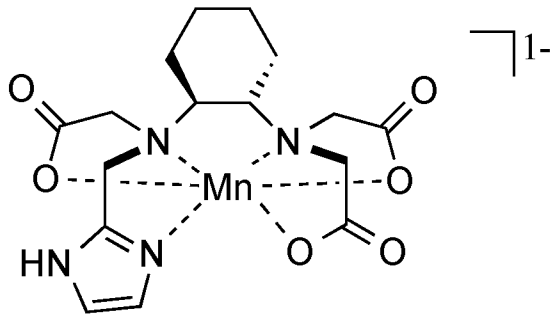
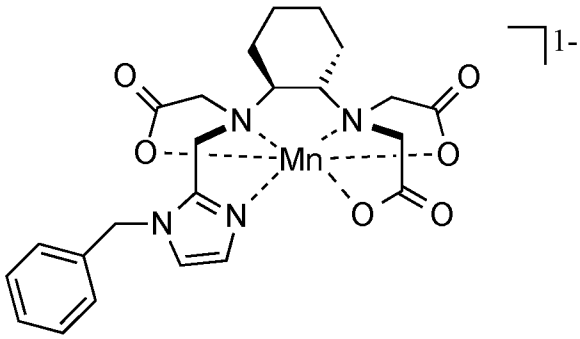
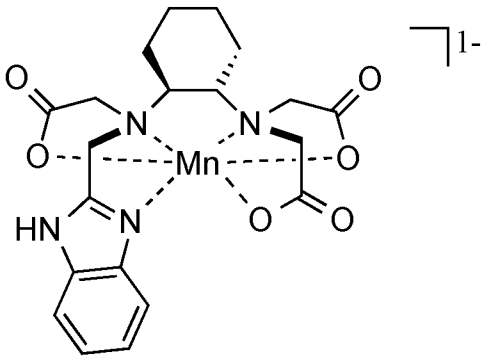
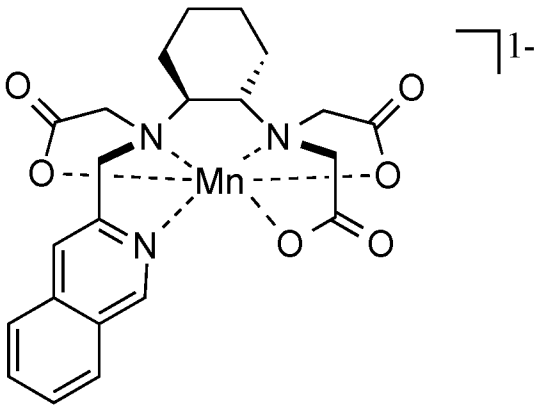


346 

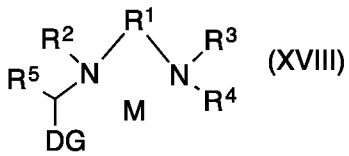
347 
, and

348 

349 or a pharmaceutically acceptable salt thereof.

350

351 46. A compound of Formula (XVIII):

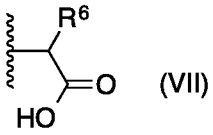
352 
(XVIII)

353 or a pharmaceutically acceptable salt thereof,

354 wherein:

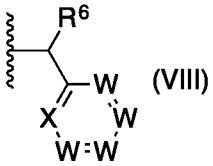
R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene), wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

R^2, R^3, and R^4 are independently selected from the group of compounds of formula:



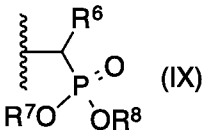
(VII)

;



(VIII)

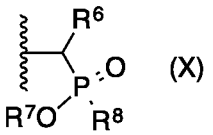
;



(IX)

;

and



(X)

R^5 and R^6 are independently selected from the group consisting of H, CO_2H, (C_1-C_6 alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7, $C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and [L]-[TBM];

X is CZ, N, O, or S, or NR^7;

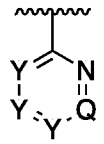
each W is independently CH, CZ, N, O, S, or NR^7;

each Z is independently selected from H, OH, OR^4, CO_2H, $C_{1\text{-}6}CO_2H$, -($C_{1\text{-}6}$ alkyl)CO_2H, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7, $C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and -[L]-

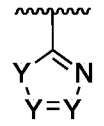
[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

each R^7 and R^8 are independently selected from the group consisting of H, C_1-C_6 alkyl, and -[L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

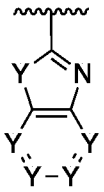
DG is selected from the group consisting of:


(XI)

;

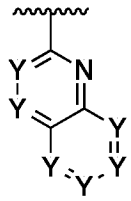

(XII)

;


(XIII)

;

and


(XIV)

or any constitutional isomers of Formulas XIII-XIV,

wherein each Y is independently CH, CZ^1, N, O, S, or NR^7;

Q is independently CH, CZ^1, N, O, S, or NR^7;

each Z^1 is independently selected from H, OH, OR^7, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^7R^8$, CH_2NHCOR^7, $C(O)N(OH)R^7$, $C(O)NHSO_2R^7$, $CH_2NHSO_2R^7$, $N(OH)C(O)R^7$, $P(R^7)O_2R^8$, $PO_3R^7R^8$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and

heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

L is a linker;

TBM is a target binding moiety;

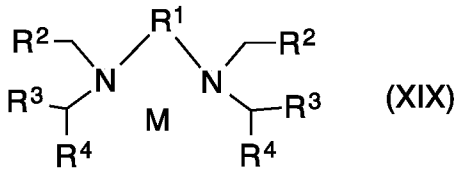
each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM]; and

M is selected from the group consisting of Gd(III), Fe(III), Mn(II), Mn(III), Cr(III), Cu(II), Cu(III), Dy(III), Ho(III), Er(III), Pr(III), Eu(II), Eu(III), Nd(III), La(III), Lu(III), Sm(III), Tb(III), Tb(IV), Tm(III), Y(III), In(III), Ga(III), Tc(III), Tc(IV), Tc(V), Re(III), Re(IV), Re(V), Bi(III), and Yb(III); and

wherein if Q is CH or CCOOH, all Y are CH, and all of R^2, R^3, and R^4 are formula VII, than at least one of R^5 or R^6 is not H; and

if one of R^2, R^3, or R^4 is formula VIII, and all of R^5 and R^6 are H, than the aromatic ring component of formula VIII (i.e. the ring containing X and W) must be different than DG.

47. A compound of Formula (XIX):



(XIX)

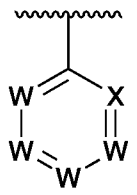
or a pharmaceutically acceptable salt thereof,

wherein:

R^1 is selected from the group consisting of a C_2-C_6 alkylene, a C_3-C_{10} cycloalkylene, 4-10 membered heterocycloalkylene, C_6-C_{10} arylene, 5-10 membered heteroarylene, (C_1-C_6)dialkyl)(C_6-C_{10} arylene), and (C_1-C_6)dialkyl(5-10 membered heteroarylene),

wherein the alkylene, cycloalkylene, heterocycloalkylene, arylene, and heteroarylene are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups, and wherein R^1 is bound to the adjacent nitrogens via the 1,2 or 1,3 positions on R^1;

each R^2, R^3, and R^4 are independently selected from the group consisting of CO_2H, $(C(O)NR^5R^6$, CH_2NHCOR^5, $C(O)N(OH)R^5$, $C(O)NHSO_2R^5$, $CH_2NHSO_2R^5$, $N(OH)C(O)R^5$, $P(R^5)O_2R^6$, and $PO_3R^5R^6$, and compounds of formula:



(XVI)

;

wherein X is CZ, N, O, or S. or NR^4;

each W is independently CH, CZ, N, O, S, or NR^4;

each Z is independently selected from H, OH, OR^4, CO_2H, -(C_{1-6} alkyl)CO_2H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_4-C_6 cycloalkyl, C_6-C_{10} aryl, 5-6 membered heterocyclyl, 5-6 membered heteroaryl, $C(O)NR^5R^6$, CH_2NHCOR^5, $C(O)N(OH)R^5$, $C(O)NHSO_2R^5$, $CH_2NHSO_2R^5$, $N(OH)C(O)R^5$, $P(R^5)O_2R^6$, $PO_3R^5R^6$, and -[L]-[TBM], wherein the alkyl, alkeny, alkynyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

each R^5 and R^6 are independently selected from the group consisting of H, C_1-C_6 alkyl, and -[L]-[TBM], wherein the alkyl is optionally substituted by 1, 2, 3, or 4 independently selected R^X groups;

L is a linker;

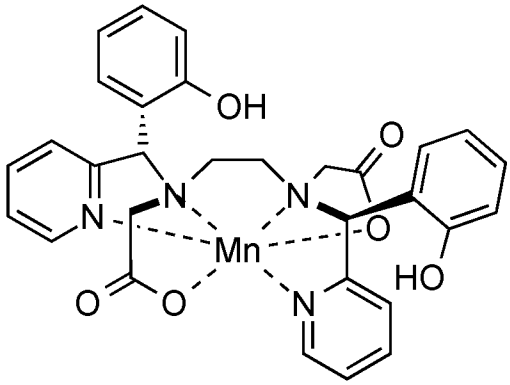
TBM is a target binding moiety;

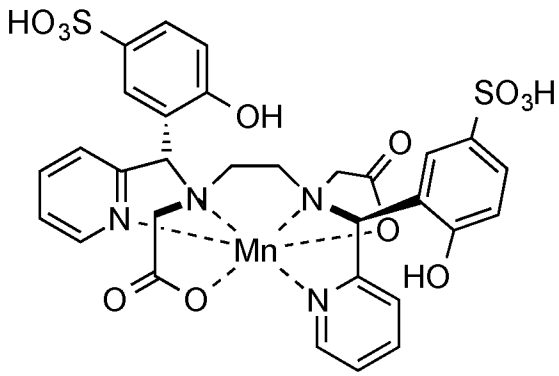
each R^X is independently selected from the group consisting of OH, SH, CN, NO_2, halo, pseudohalo, amino, thionyl, sulfinyl, sulfonyl, sulfo, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 aminoalkyl, di(C_1-C_4 alkyl)amino, C_1-C_4 alkylamine, phosphinate, phosphinate ester, phosphonate, phosphonate ester, phosphodiester, C_{1-4} alkylphosphodiester, C_3-

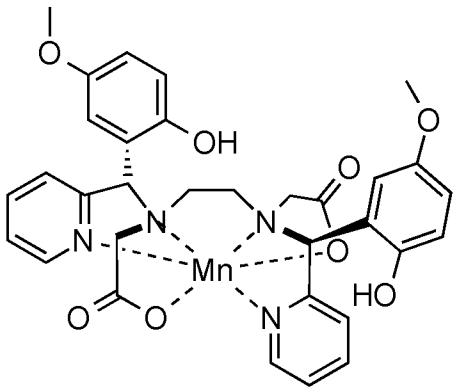
C_6 cycloalkyl, phenyl, 5-6 membered heteroaryl, 5-6 membered heterocycloalkyl, (C_1-C_4 alkyl)phenyl, and -[L]-[TBM]; and

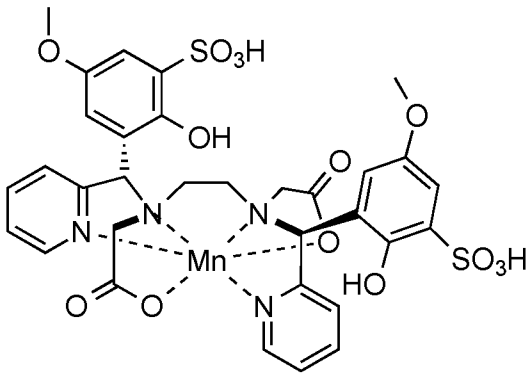
M is selected from the group consisting of Gd(III), Fe(III), Mn(II), Mn(III), Cr(III), Cu(II), Cu(III), Dy(III), Ho(III), Er(III), Pr(III), Eu(II), Eu(III), Nd(III), La(III), Lu(III), Sm(III), Tb(III), Tb(IV), Tm(III), Y(III), In(III), Ga(III), Tc(III), Tc(IV), Tc(V), Re(III), Re(IV), Re(V), Bi(III), and Yb(III).

48. The compound of claim 47, wherein the compound is selected from the group consisting of:









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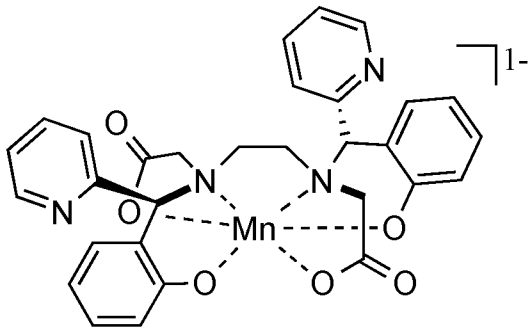
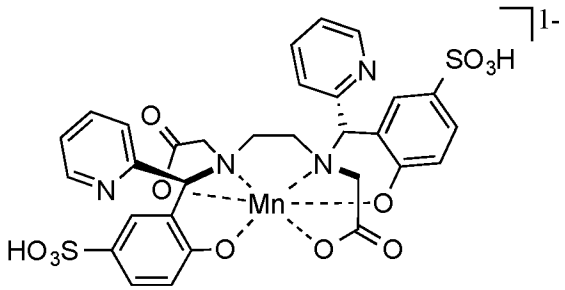


465



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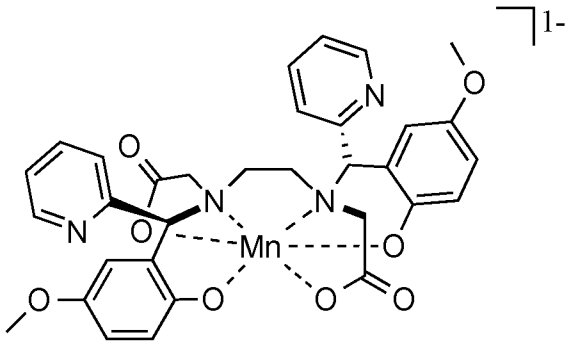

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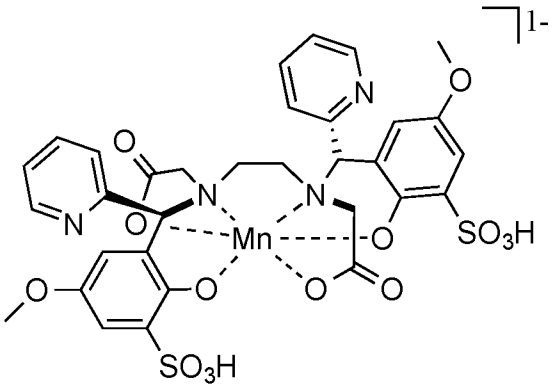

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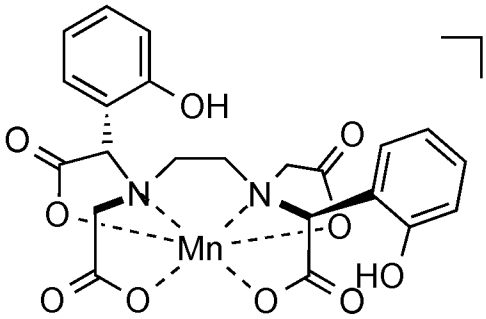

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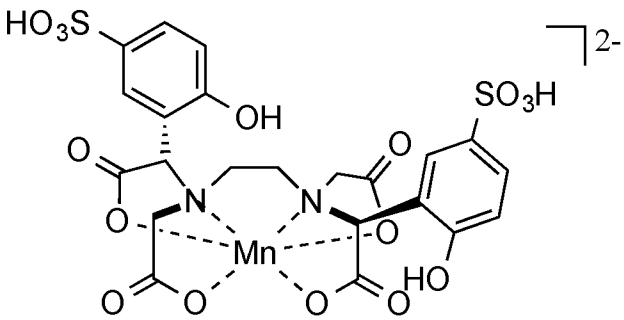

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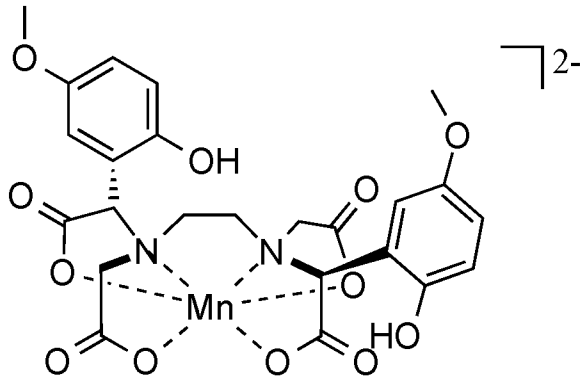
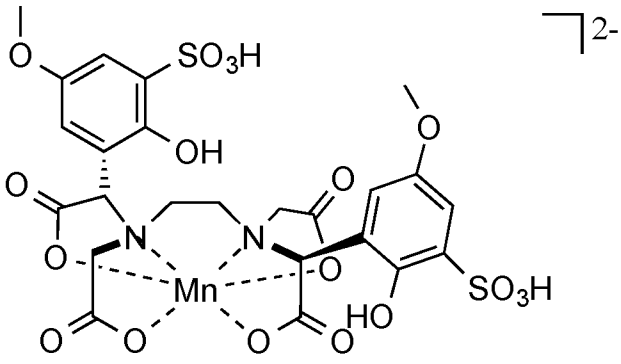
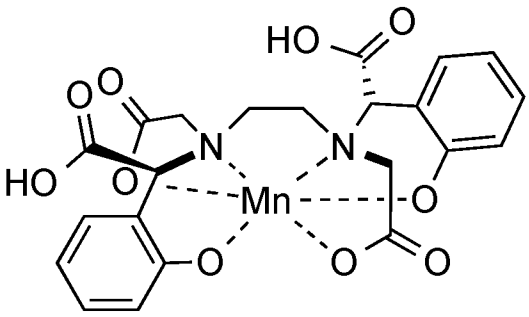
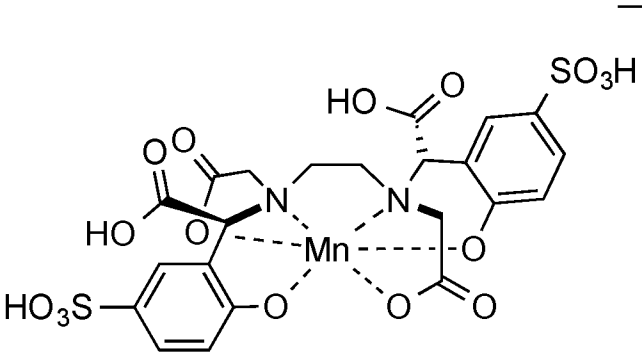
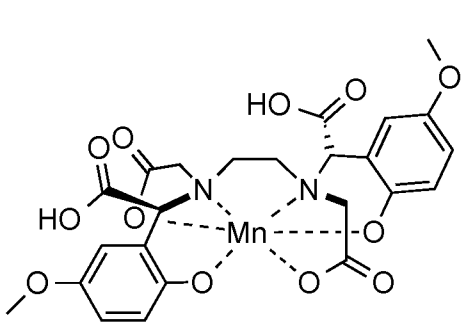
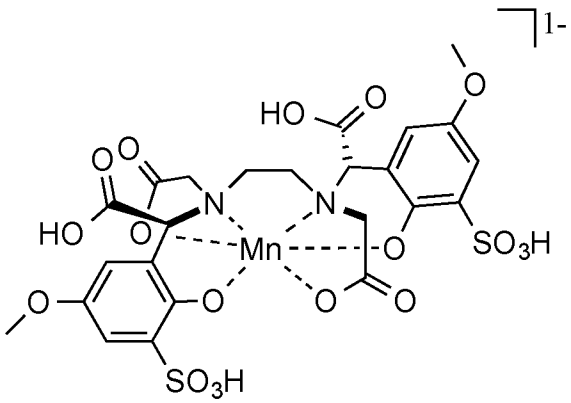


472 

473 

474 

475  , and



or a pharmaceutically acceptable salt or any corresponding stereoisomer thereof.

49. A compound of Formula (A):

$$(D^1)_a—(L^1)_b—TBM—(L^2)_c—(D^2)_d$$

(A)

or a pharmaceutically acceptable salt thereof,

wherein:

TBM is a target binding moiety;

each D^1 is independently a metal chelate of any one of claims 44-48;

each D^2 is independently a metal chelate of any one of claims 44-48;

L^1 is a linker;

L^2 is a linker;

a is an integer from 0 to 4;

b is 0 or 1;

wherein if a is 0, b is 0;

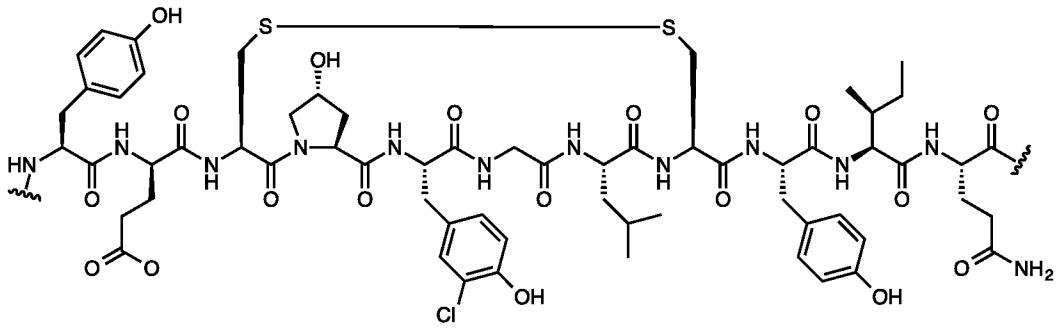
c is 0 or 1;

d is an integer from 0 to 4;

wherein if d is 0, c is 0;

wherein at least one of a and d is an integer from 1 to 4.

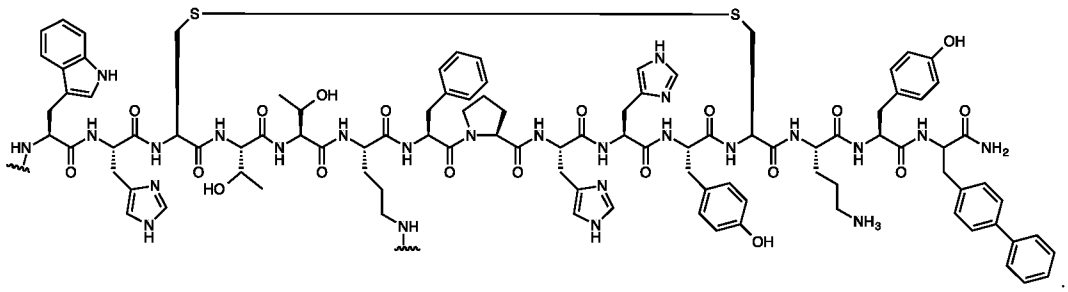
50. The compound from claim 49 where [TBM] is:



498

499 51. The compound from claim 49 where [TBM] is:

500



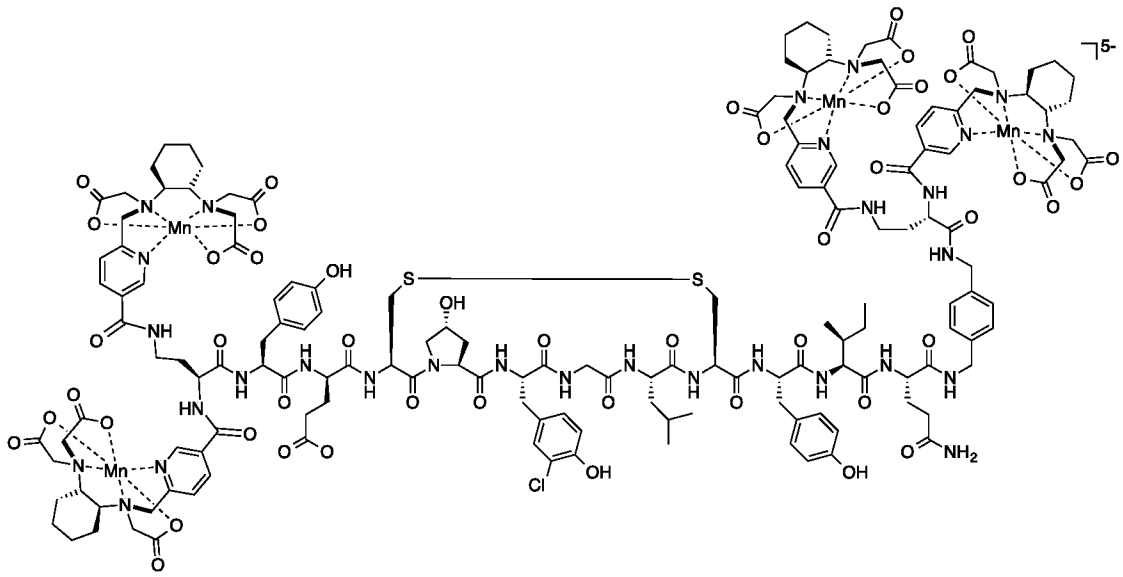
501

502

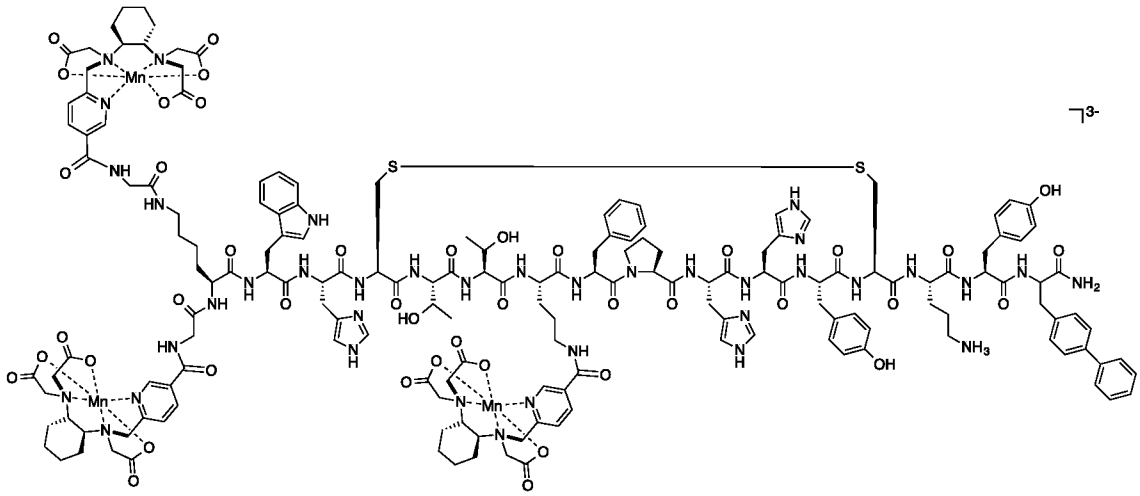
503 52. The compound of claim 49, wherein D^1 and D^2 are a compound according to claim 1,
504 wherein R^1 is 1,2-cyclohexylene, R^2 is H, R^3 is H, DG is Formula II, Q is CH, the Y
505 positioned α- to Q is C-[L]-TBM, and all other Y are CH, and L is –C(O)-.

506

507 53. The compound of claim 49, where the compound is selected from the group
508 consisting of:



and



54. A method of magnetic resonance (MR) imaging a patient, the method comprising:

a) administering to the patient an effective amount of a compound of any one of claims 44-53; and

b) acquiring an MR image of the patient.

55. A method for imaging a tumor in a patient, the method comprising:

a) administering to the patient an effective amount of a compound of any one of claims 44-53; and

b) acquiring an MR image of the tumor in the patient.

56. A method for imaging a blood clot in a patient, the method comprising:

a) administering to the patient an effective amount of a compound of any one of claims 44-53; and

b) acquiring an MR image of the blood clot in the patient.

57. A method for imaging a brain lesion in a patient, the method comprising:

a) administering to the patient an effective amount of a compound of any one of claims 44-53; and

b) acquiring an MR image of the brain lesion in the patient.

58. A method for detecting the presence or absence of disrupted blood-brain-barrierin a patient, the method comprising:

a) administering to the patient an effective amount of a compound of any one of claims 44-53, wherein M is Mn(II);

b) acquiring a first MR image of the brain of the patient;

c) acquiring a second MR image of the brain of the patient; and

d) comparing the images.

59. A method for detecting the presence or absence of arterial stenosis in a patient, the method comprising:

a) administering to the patient an effective amount of a compound of any one of claims 44-53, wherein M is Mn(II);

b) acquiring a first MR image of the arteries of a patient;

c) acquiring a second MR image of the arteries of patient immediately after injection of the compound; and

d) comparing the images.

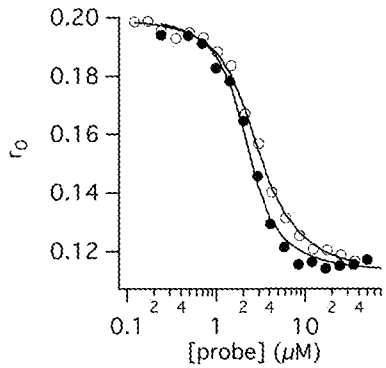


Figure 1

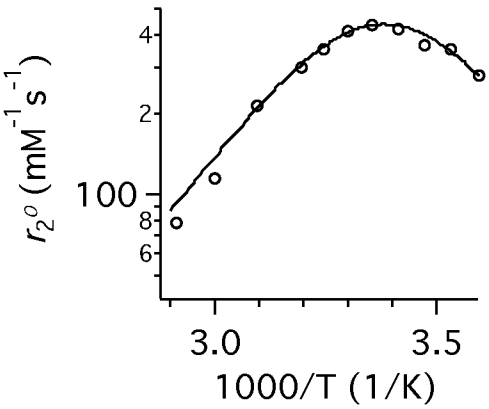


Figure 2

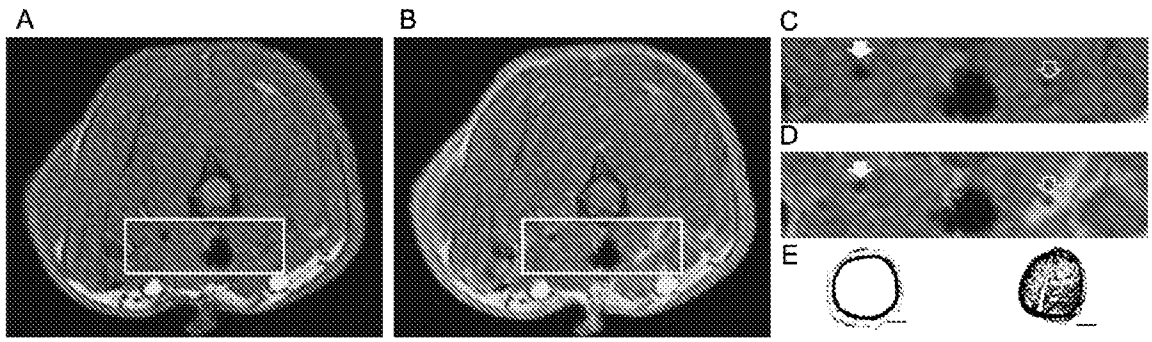


Figure 3

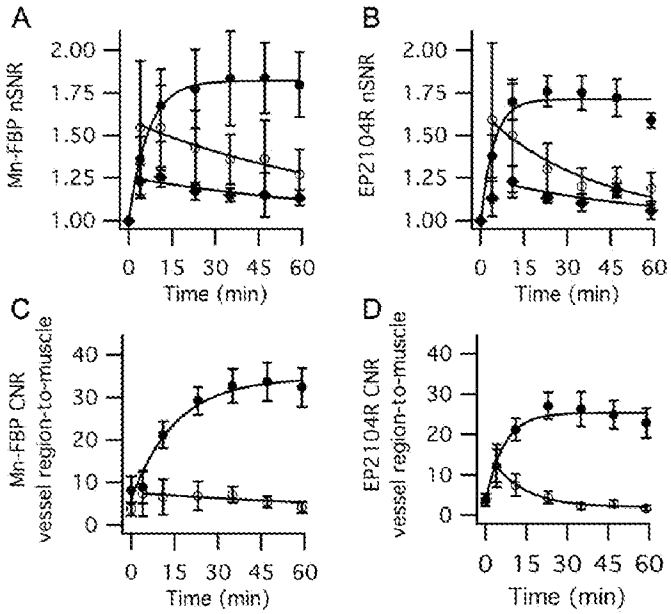


Figure 4

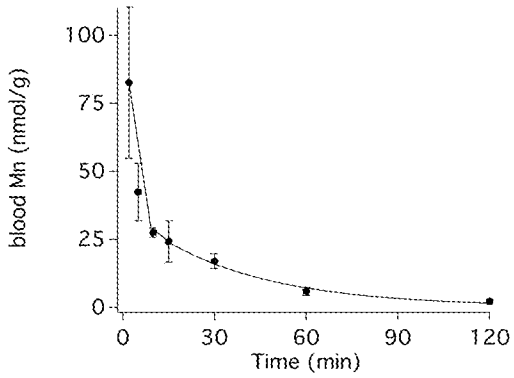


Figure 5

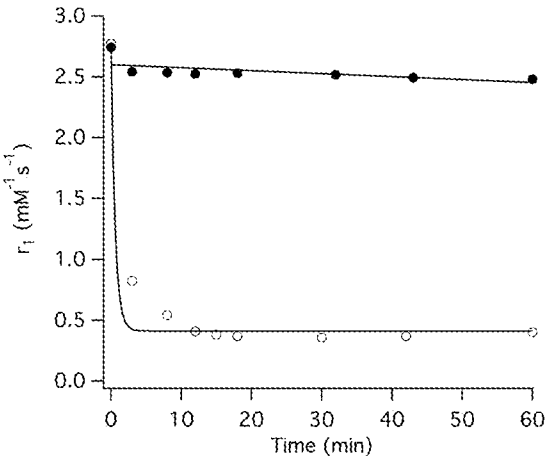


Figure 6

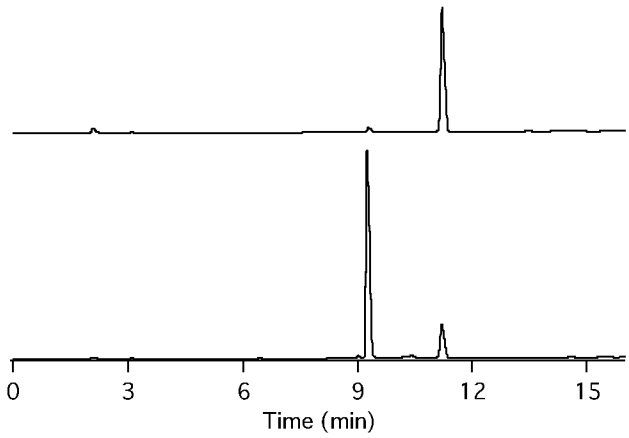


Figure 7

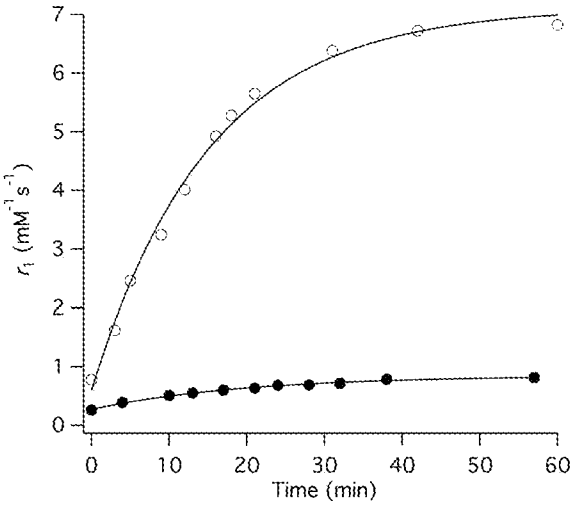


Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/46874

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 49/06; C07F 13/00 (2016.01)
CPC - A61K49/06; A61B5/0035; C07F13/005; A61K49/103
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 49/06; C07F 13/00 (2016.01)
CPC: A61K49/06; A61B5/0035; C07F13/005; A61K49/103

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/9.3; 556/45; 424/639

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Scholar, PubWEST
imaging, contrast agent, manganese, complex, chelating, ethylenediaminetriacetate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,022,490 A (HERMANT et al.) 08 February 2000 (08.02.2000) col 3, ln 10-23; col 3, ln 44-45	1-3, 29
Y	US 2008/0227962 A1 (MAZZANTI) 18 September 2008 (18.09.2008) para [0028]-[0030], [0105], [0107]-[0108]	1-3, 29
A	WO 2014/107722 A1 (THE GENERAL HOSPITAL CORPORATION) 10 July 2014 (10.07.2014) Entire Document	1-3, 29
A	US 4,622,420 A (MEARES et al.) 11 November 1986 (11.11.1986) Entire Document	1-3, 29
A	US 4,889,931 A (ROCKLAGE et al.) 26 December 1989 (26.12.1989) Entire Document	1-3, 29

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

* 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
29 November 2016	28 DEC 2016
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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/46874

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.: 4-9, 13, 17, 20-27 and 54-59
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:
--Please see attached sheet--

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.:
1-3 and 29

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 16/46874

Continuation of Box.No.III - Lack of Unity:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: Claims 1-3, 10-12, 14-16, 18-19 and 28-48, directed to a compound of Formula (I) or a pharmaceutically acceptable salt thereof. The compound of Formula (I) will be searched to the extent that it encompasses the first species of claim 1, wherein R1 is C2-alkylene; each of R2 and R3 is H; DG is formula (II), wherein Q is CH and Y is selected from CH and CZ, such that the compound meets the proviso of claim 1. It is believed that claims 1-3 and 29 read on this first-named invention, and thus these claims will be searched without fee to the extent that they encompass the pharmaceutical composition described above. Applicant is invited to elect additional compounds of formula (I), wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected composition. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be a compound of Formula (I) represented by the first-listed compound in claim 28, wherein R1 is C6-cycloalkylene; each R2 and R3 is H; DG is formula (II) where Q is CH and Y is selected from CH and CZ, where Z is C(=O)O- (i.e claims 1-3, 28-29).

Group II: Claims 49-53, directed to a conjugate containing a compound of Formula (I) and a targeting moiety.

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique compound of Formula (I), which is not required by any other invention of Group I+.

Group II includes the technical feature of a conjugate containing a targeting moiety, not required by Group I+.

Common technical features:

The inventions of Group I+ share the technical feature of a compound having the core structure of Formula (I).

Groups I+ and II also share the technical feature of a compound of Formula (I).

This shared technical feature, however, is not a contribution over the prior art as being anticipated by WO 2014/107722 A1 to The General Hospital Corporation (hereinafter 'General Hospital') discloses a compound of Formula (I), wherein R1 is a C6-cycloalkylene; each R2 and R3 is H; DG is the moiety of formula (II), wherein Q is CH; each Y is selected from CH (Figure 38, compound 40).

As said compound was known in the art at the time of the invention, this cannot be considered a special technical feature which would otherwise unify the inventions of Group I+ or those of Groups I+ and II.

Groups I+ and II, thus lack unity under PCT Rule 13.

Note reg. item 4: Claims 4-9, 13, 17, 20-27 and 54-59 are unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a). These claims are therefore, not included in the above analysis.