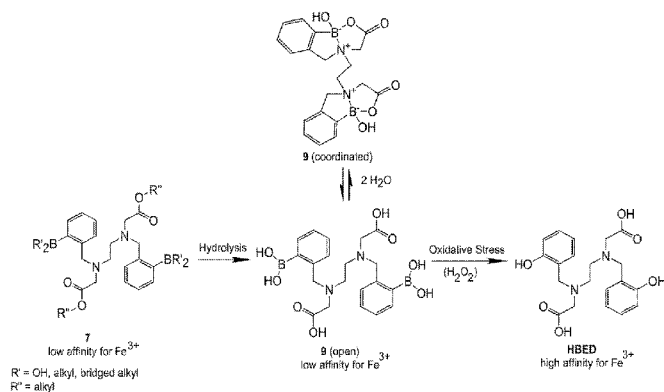


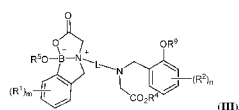
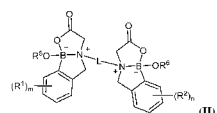
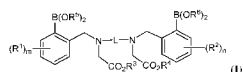


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[Continued on next page]

(54) **Title:** HBED PRODRUGS AND USE THEREOF

(57) **Abstract:** The present invention provides N,N'-bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED) prodrugs of Formula (I), (II), and (III), and pharmaceutically acceptable salts, tautomers, stereoisomers, solvates, hydrates, and polymorphs for treatment or prevention of diseases that are associated with oxidative stress and/or excess metal (e.g. iron). The present invention further provides HBED prodrugs of Formula (I), (II), and (III), and pharmaceutically acceptable salts, tautomers, stereoisomers, solvates, hydrates, and polymorphs for the treatment and/or prevention of macular degeneration. The provided HBED prodrugs may have improved oral availability and/or brain penetration compared to HBED.



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HBED PRODRUGS AND USES THEREOF

RELATED APPLICATIONS

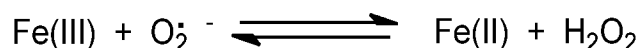
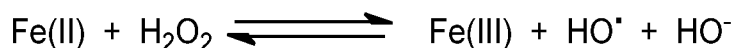
[0001] The present application claims priority under 35 U.S.C § 119(e) to U.S. provisional patent applications, U.S.S.N. 62/351,151, filed June 16, 2016, and U.S.S.N. 62/212,985, filed September 1, 2015, both of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Nearly every organism requires iron. Iron is essential as a cofactor for proteins involved in biological processes such as DNA synthesis (*e.g.*, ribonucleotide reductase), cellular metabolism (*e.g.*, aconitase and cytochrome *c*), oxygen transport (*e.g.*, hemoglobin and myoglobin), neurotransmitter synthesis (*e.g.*, tyrosine hydroxylase), hydrogen peroxide decomposition (*e.g.*, catalase and peroxidases), and biotransformation (*e.g.*, cytochrome P-450s) (Crichton, 2001). Due to the low solubility of Fe(III) hydroxide ($K_{sp} \approx 1 \times 10^{-38}$) (Raymond and Carrano, 1979), the predominant form of the metal in the biosphere, organisms have developed sophisticated systems to take up, transport, and store iron.

[0003] However, in humans, there is no regulated mechanism for excretion of iron from the body (Byrne et al., 2013; Anderson and McLaren, 2012). Excess intracellular iron will enter the labile iron pool (LIP), a redox active and chelatable pool of iron that is purported to be composed of Fe^{2+} and Fe^{3+} loosely bound to small organic molecules (*e.g.* phosphate, citrate, phospholipid head groups, polypeptides, etc.) (Jacobs, 1976; Kakhlon and Cabantchik, 2002; Kruszewski, 2003). Similarly, a pool of redox active labile plasma iron (LPI) has been recognized (Esposito *et al.*, 2003). Both pools of iron can be considered non-transferrin bound iron (NTBI) (Brissot *et al.*, 2012), and are capable of catalyzing the formation of reactive oxygen species (ROS) via the well-known Fenton reaction. Shown in Scheme (i), the Fenton reaction produces highly reactive hydroxyl radicals from the one-electron reduction of hydrogen peroxide by ferrous iron (Fenton, 1894; Fenton, 1896). Fe^{2+} can then be regenerated by cellular reductants (*e.g.*, glutathione, ascorbic acid) or superoxide anion, and is available to react once again in the Fenton reaction to produce more toxic hydroxyl radicals. These radicals can damage lipids, proteins, and DNA, and may ultimately lead to cell death (Halliwell and Gutteridge, 1999).

Scheme (i)



[0004] Excess iron and oxidative stress have been implicated in the etiology and pathogenesis of a variety of diseases and conditions (for reviews, see Jomova and Valko, 2011; Fleming and Ponka, 2012).

[0005] Systemic iron overload can be caused by a genetic mutation, as is the case in hereditary hemochromatosis, or can be a consequence of required blood transfusions, as seen in β -thalassemia, aplastic anemia, sickle cell anemia, myelodysplasia, and Diamond-Blackfan anemia. Diseases associated with a more focal accumulation of labile iron include neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, and neurodegeneration with brain iron accumulation; age-related macular degeneration; and skin photoaging. Subjects with these conditions may benefit from iron chelation therapy. Iron chelators may also be useful for treating cancer, diabetes, inflammatory disorders, cardiovascular diseases, anthracycline cardiotoxicity, and viral infections (Pace and Leaf, 1995; van Asbeck *et al.*, 2001; Mandas *et al.*, 2009).

[0006] Neurodegenerative diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD), are neurodegenerative syndromes for which at present no cure is available. Both diseases are widespread neurodegenerative disorders and affect approximately 0.5% and 4-8%, respectively, of the population over the age of 50 years, creating an increasing economic burden for society (Meissner *et al.*, 2011). Numerous studies including *in vivo*, *in vitro*, and relevant animal models have shown a linkage between hydroxyl and oxygen free radicals production and neurodegenerative diseases and disorders, such as Parkinson's disease and Alzheimer's disease. Iron accumulation in neurodegenerative diseases is a common feature, and it has been shown to play a pivotal role in the process of neurodegeneration as well as age-related macular degeneration (AMD) (Hahn *et al.*, 2003).

[0007] In Parkinson's disease, the brain's defensive mechanisms against the formation of oxygen free radicals are defective. In the substantia nigra of Parkinsonian brains, there are reductions in activities of antioxidant enzymes. Iron concentrations are found significantly elevated in Parkinsonian substantia nigra pars compacta and within the melanized dopamine neurons. Recent studies have shown that significant accumulations of iron in white matter tracts and neurons throughout the brain but especially in the substantia

nigra pars compacta precede the onset of neurodegeneration and movement disorder symptoms (Stankiewicz *et al.*, 2007).

[0008] Macular degeneration is a medical condition that usually affects older adults and results in a loss of vision in the macula because of damage to the retina. Maculas affected by age-related macular degeneration were found to contain increased chelatable iron in the retinal pigment epithelium.

[0009] Iron chelators and radical scavengers may provide potent neuroprotective activity in animal models of neurodegeneration. However, the major problem with these compounds is that they do not cross the brain blood barrier (BBB). For example, desferrioxamine is one of the most highly potent neuroprotective agents in animal models of Parkinson's disease. However, desferrioxamine does not cross the BBB and must be injected centrally (WO 2004/041151).

[0010] There are three chelators currently approved by the FDA for the treatment of iron overload: desferrioxamine B (DFO), deferasirox, and deferiprone. Unfortunately, DFO is poorly absorbed from the gastrointestinal tract, has a short plasma half-life, and does not cross the blood brain barrier. It must be administered by continuous subcutaneous infusion. Deferasirox and deferiprone, while orally active, have some potential associated toxicities. Exjade® (deferasirox) has a boxed warning of possible renal toxicity, hepatic toxicity, and gastrointestinal hemorrhage. Ferriprox® (deferiprone) has a boxed warning of neutropenia and agranulocytosis. All three chelators may also be suboptimal for the treatment of conditions and diseases that require localized chelation therapy, as they are strong chelators and thus pose a risk of purloining iron and other metals from proteins and processes dependent upon these metals. Therefore, there is a strong need for the development of iron chelators with more desirable properties (*e.g.*, improved neuroprotective activity, good transport properties through cell membranes including the blood brain barrier, optimal oral uptake, optimal or sufficient oral uptake and pharmacokinetic behavior, acceptable toxicity, suitability for chronic use, and site specificity) for a better treatment and/or prevention of diseases associated with excess iron and oxidative stress.

SUMMARY OF THE INVENTION

[0011] N,N'-bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED) is an iron chelator that was first successfully synthesized in 1967 (L'Eplattenier *et al.*, 1967; see also US 3,632,637 and US 3,758,540, each of which is incorporated herein by reference). This hexadentate ligand strongly chelates Fe³⁺ (log *K* = 39.68), and also has a lower affinity

for other physiologically-important metals (*e.g.* $\log K = 21.38$ and 18.37 for Cu^{2+} and Zn^{2+} , respectively). HBED showed promise in rodent models of iron overload, even when orally administered (for a review, see Grady and Hershko, 1990). For example, when HBED was orally administered to hypertransfused rats, it was 70% as effective as desferrioxamine (DFO) given by intraperitoneal injection (Grady and Jacobs, 1981). When HBED was administered by intraperitoneal injection, total iron excretion was 2.5x that of DFO.

[0012] However, a study by Peter *et al.* (1994) using iron-loaded *Cebus apella* primates (tufted capuchin) showed results significantly different from those obtained in the rodent studies. When these primates were dosed orally with HBED, it was ~10x less efficient in removing iron than DFO administered subcutaneously. HBED also performed poorly in a small phase 1 clinical trial in humans (Grady *et al.*, 1994). Six patients with β -thalassemia were given DFO subcutaneously during the first phase of the study, followed by administration of HBED orally during the second phase of the study. Results showed that total iron excretion due to HBED chelation was only 17.8% of that due to DFO. The results of these studies suggest that in primates and in humans, HBED is poorly absorbed from the gastrointestinal tract, probably because of its charged nature at physiological pH.

[0013] In an effort to increase the bioavailability of HBED, the carboxylic acid moieties were masked with simple esters. Great success was observed in rodent studies. Grady and Jacobs (1981) found that dimethyl HBED (dmHBED), when administered orally to hypertransfused rats, was 4x more effective than DFO given parenterally. Likewise, using a hypertransfused mouse model, Pitt *et al.* (1986) found that the bis esters (dimethyl, diethyl, diisopropyl, dipentyl, dibenzyl) were orally active. See also US 4,528,196, incorporated herein by reference.

[0014] Once again, although the bis esters of HBED were orally-active chelators in rodent models, in the *Cebus* primate model of iron overload, dmHBED administered orally was only one-third as efficient as a similar dose of DFO administered subcutaneously, although it was 3x as efficient as HBED administered orally (Peter *et al.*, 1994). Administered subcutaneously, dmHBED was much *less* efficient than HBED administered subcutaneously (Bergeron *et al.*, 1998). Pitt *et al.* (1986) found the rate of chemical hydrolysis of dmHBED to be “immeasurably slow” at pH 7.5. A half-life of 108 days at pH 7.5 was extrapolated from the hydrolysis rate obtained at pH 10.5 in 50% aqueous MeOH at 38 °C. Thus, the hydrolysis of these simple esters of HBED was expected to occur by the actions of serum/tissue esterases *in vivo*. The inability of *Cebus* esterases to catalyze the hydrolysis of the HBED diesters is commonly cited as the cause for the differences observed

between rodents and primates in the effectiveness of HBED esters (Faller *et al.*, 2000). Testing in the *Cebus* primate model of iron overload is considered a good predictor of the behavior of chelators in humans.

[0015] Given the above-described limitations of HBED and other derivatives, prodrugs of HBED with more desirable properties (*e.g.*, optimal or sufficient oral uptake, good transport properties through cell membranes including the blood brain barrier, ability to convert to the parent compound chemically and/or enzymatically, *etc.*) are needed for a better treatment and/or prevention of diseases associated with oxidative stress and iron overload. Additionally, for diseases where there is a localized accumulation of excess iron or a localized need for chelation, it would be beneficial for a prodrug to preferentially convert to HBED only at those sites where chelation is most needed, thereby minimizing the risk of sequestering iron and other metals that are necessary for normal physiological processes.

[0016] In one aspect, the present invention provides pharmaceutically acceptable salts, tautomers, stereoisomers, solvates, hydrates, and polymorphs of HBED prodrugs for the treatment and/or prevention of a pathological condition in a subject. In certain embodiments, the pathological condition is responsive to the chelation or sequestration of a metal. In certain embodiments, the metal is iron (*e.g.*, Fe(III)). In certain embodiments, the pathological condition is metal overload. In certain embodiments, the pathological condition is iron overload. In certain embodiments, the pathological condition is metal poisoning (*e.g.*, iron poisoning). In certain embodiments, the pathological condition is oxidative stress, transfusional iron overload, thalassemia, primary hemochromatosis, secondary hemochromatosis, diabetes, liver disease, heart disease, cancer, radiation injury, neurological or neurodegenerative disorder (*e.g.*, Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, neurodegeneration with brain iron accumulation), macular degeneration, closed head injury, irritable bowel disease, stroke, and reperfusion injury. In certain embodiments, the pathological condition is an infectious disease (*e.g.*, HIV and malaria). In certain embodiments, the pathological condition is aging.

[0017] The present invention provides novel HBED prodrugs, pharmaceutically acceptable salts, tautomers, stereoisomers, solvates, hydrates, and polymorphs thereof. In certain embodiments, the HBED prodrugs have one or both phenolic hydroxyl groups of HBED masked as boronic acids or boronic esters. In certain embodiments, the HBED prodrugs have one or both phenolic hydroxyl groups of HBED masked as boronic acids or boronic esters and one or both carboxylic acid groups of HBED masked as esters. Masking the phenolic hydroxyl groups of HBED with boronic acids or esters appears to promote the

chemical hydrolysis of carboxylate esters at physiological pH. Masking the phenolic hydroxyl groups of HBED with boronic acids or esters alone or in combination with masking the carboxylic acid groups as esters would be predicted to decrease the affinity of these compounds for iron and other metals in conditions and tissues where metal levels are normal and chelation is not needed. In certain embodiments, the provided prodrugs can be activated upon hydrolysis. In certain embodiments, the provided prodrugs can be activated upon oxidation. In certain embodiments, the provided prodrugs can be activated upon hydrolysis and oxidation. The carboxylate esters can be converted to carboxylic acids upon chemical and/or enzymatic hydrolysis. The boronic acids or boronic esters can be converted to the corresponding phenolic hydroxyls upon oxidation (*e.g.*, oxidative stress). Thus, the provided prodrugs may preferentially be fully converted to HBED in tissues where chelation is needed most, by the very conditions prevailing at these sites (*e.g.*, oxidative stress), so that disruption of systemic homeostasis of iron and other physiologically-important metals may be avoided.

[0018] The provided HBED prodrugs may show superior physicochemical, pharmacokinetic, pharmacodynamic, and/or toxicological properties (such as greater solubility, permeability, and bioavailability; improved distribution, absorption, metabolism, and iron-clearing efficiency; and reduced clearance, excretion, and toxicity) compared with the parent compound HBED and/or other HBED analogs. In certain embodiments, the provided HBED prodrugs can be converted to HBED *in vivo*. In certain embodiments, the provided HBED prodrugs have improved oral availability compared to HBED. In certain embodiments, the provided HBED prodrugs have improved brain penetration.

[0019] In still another aspect, the invention provides methods of using the inventive compounds, or pharmaceutical compositions thereof, for the treatment and/or prevention of a pathological condition in a subject. In certain embodiments, the pathological condition is responsive to the chelation or sequestration of a metal. In certain embodiments, the metal is iron (*e.g.*, Fe(III)). In certain embodiments, the pathological condition is metal overload (*e.g.*, iron overload, aluminum overload, chromium overload, magnesium overload, calcium overload, strontium overload, nickel overload, manganese overload, cobalt overload, copper overload, zinc overload, silver overload, sodium overload, potassium overload, cadmium overload, mercury overload, lead overload, molybdenum overload, tungsten overload, or actinide overload (*e.g.*, uranium overload)). In certain embodiments, the pathological condition is iron overload. In certain embodiments, the pathological condition is metal poisoning (*e.g.*, iron poisoning, aluminum poisoning, thallium poisoning, chromium poisoning, magnesium poisoning, calcium poisoning, strontium poisoning, nickel poisoning,

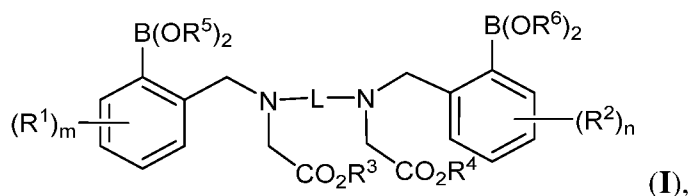
manganese poisoning, cobalt poisoning, copper poisoning, zinc poisoning, silver poisoning, sodium poisoning, potassium poisoning, cadmium poisoning, mercury poisoning, lead poisoning, antimony poisoning, molybdenum poisoning, tungsten poisoning, lanthanide poisoning (*e.g.*, cerium poisoning), or actinide poisoning (*e.g.*, uranium poisoning). In certain embodiments, the pathological condition is oxidative stress, transfusional iron overload, thalassemia, primary hemochromatosis, secondary hemochromatosis, diabetes, liver disease, heart disease, cancer, radiation injury, neurological or neurodegenerative disorder (*e.g.*, Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, and neurodegeneration with brain iron accumulation), macular degeneration, closed head injury, irritable bowel disease, stroke, and reperfusion injury. In certain embodiments, the pathological condition is an infectious disease (*e.g.*, HIV and malaria). In certain embodiments, the pathological condition is aging. In certain embodiments, the methods of treatment and/or prevention include administering to the subject a therapeutically or prophylactically effective amount of a compound of the invention, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, or a pharmaceutical composition thereof.

[0020] The invention also provides methods of using the provided HBED prodrugs, or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, isotopically enriched forms, or polymorphs thereof, and cosmetic compositions thereof, for improving skin appearance. The invention also provides methods of using the provided HBED prodrugs, or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, isotopically enriched forms, or polymorphs thereof, and cosmetic compositions thereof, for treating and/or preventing skin aging. The invention also provides methods of using the provided HBED prodrugs, or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, isotopically enriched forms, or polymorphs thereof, and cosmetic compositions thereof, for treating and/or preventing skin photoaging. The invention also provides methods of using the provided HBED prodrugs, or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, isotopically enriched forms, or polymorphs thereof, and cosmetic compositions thereof, for treating and/or preventing skin cancer. In certain embodiments, the methods of improving skin appearance include administering to the subject a therapeutically or prophylactically effective amount of a compound of the invention, or a cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, or a cosmetic composition thereof. In certain embodiments, the methods of preventing and/or treating skin aging, skin photoaging, and/or skin cancer include administering to the subject a therapeutically or prophylactically effective amount of a compound of the invention, or a

cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, or a cosmetic composition thereof.

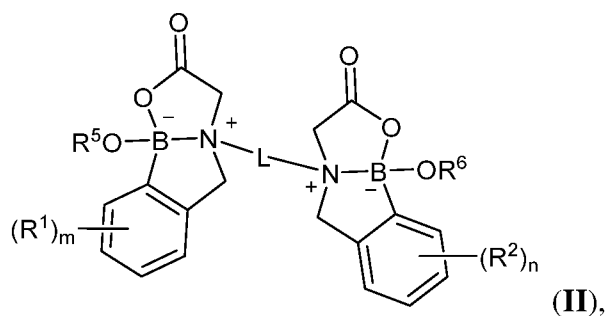
[0021] Without wishing to be bound by any particular theory, the inventive compounds, after conversion to HBED, are thought to chelate iron and/or other metals (*e.g.*, aluminum, thallium, chromium, magnesium, calcium, strontium, nickel, manganese, cobalt, copper, zinc, silver, sodium, potassium, cadmium, mercury, lead, antimony, molybdenum, tungsten, a lanthanide (*e.g.*, cerium), or an actinide (*e.g.*, uranium)). Further provided by the invention are kits, containing one or more HBED prodrugs as described herein, or pharmaceutically acceptable salts, tautomers, stereoisomers, solvates, hydrates, or polymorphs thereof, or pharmaceutical compositions thereof, for treating and/or preventing a pathological condition described herein.

[0022] In one aspect of the present invention, provided are compounds of Formula (I):



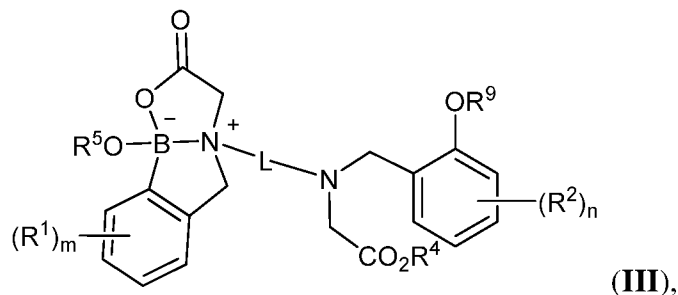
and pharmaceutically or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, or polymorphs thereof, wherein R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , m , and n are as described herein.

[0023] In another aspect of the present invention, provided are compounds of Formula (II):



and pharmaceutically or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, or polymorphs thereof, wherein R^1 , R^2 , L , R^5 , R^6 , m , and n are as described herein.

[0024] In one aspect of the present invention, provided are compounds of Formula (III):



and pharmaceutically or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, or polymorphs thereof, wherein R^1 , R^2 , L , R^4 , R^5 , R^9 , m , and n are as described herein.

[0025] In another aspect, the present invention provides pharmaceutical compositions comprising a compound as described herein, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, and optionally a pharmaceutically acceptable excipient. The pharmaceutical compositions of the invention may include a therapeutically or prophylactically effective amount of the compound as described herein.

[0026] The present invention also provides cosmetic or personal care compositions comprising a compound as described herein, or a cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, and optionally a cosmetically acceptable carrier. The cosmetic or personal care composition may include a therapeutically or prophylactically effective amount of the compound as described herein.

[0027] In still another aspect, the invention provides methods and kits for using the provided compounds, or pharmaceutical compositions thereof, for the treatment and/or prevention of a pathological condition in a subject. In certain embodiments, the pathological condition is responsive to the chelation or sequestration of a metal. In certain embodiments, the metal is iron (*e.g.*, Fe(III)). In certain embodiments, the pathological condition is metal overload. In certain embodiments, the pathological condition is iron overload. In certain embodiments, the pathological condition is metal poisoning (*e.g.*, iron poisoning). In certain embodiments, the pathological condition is oxidative stress, transfusional iron overload, thalassemia, primary hemochromatosis, secondary hemochromatosis, diabetes, liver disease, heart disease, cancer, radiation injury, neurological or neurodegenerative disorder (*e.g.*, Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, and neurodegeneration with brain iron accumulation), macular degeneration, closed head injury, irritable bowel disease, stroke, and reperfusion injury. In certain embodiments, the pathological condition is an

infectious disease (*e.g.*, HIV and malaria). In certain embodiments, the pathological condition is aging.

[0028] The inventive kits include a first container containing a therapeutically effective amount of a compound of the invention, or a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, or a pharmaceutical or cosmetic composition thereof; and instructions for administering the compound to the subject to treat and/or prevent the pathological condition. A kit may include multiple unit dosages, for example, for multiple days of treatment. A kit may also include packaging information describing the use of the compound or composition, or prescribing information for the subject or a health care professional. Such information may be required by a regulatory agency such as the U.S. Food and Drug Administration (FDA). The kit may also optionally include a device for administration of the compound or composition, for example, a dropper for ocular administration or a syringe for parenteral administration.

[0029] The details of one or more embodiments of the invention are set forth herein. Other features, objects, and advantages of the invention will be apparent from the Detailed Description, Examples, and Claims.

DEFINITIONS

[0030] Definitions of specific functional groups and chemical terms are described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in *Organic Chemistry*, Thomas Sorrell, University Science Books, Sausalito, 1999; Smith and March *March's Advanced Organic Chemistry*, 5th Edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd Edition, Cambridge University Press, Cambridge, 1987.

[0031] It is to be understood that compounds that have the same molecular formula but differ in the nature or sequence of bonding of their atoms or the arrangement of their atoms in space are termed "isomers." Isomers that differ in the arrangement of their atoms in space are termed "stereoisomers." Stereoisomers that are not mirror images of one another are termed "diastereomers," and those that are non-superimposable mirror images of each other are termed "enantiomers". When a compound has an asymmetric center, for example, a

carbon atom of the compound is bonded to four different groups, a pair of enantiomers is possible. An enantiomer can be characterized by the absolute configuration of its asymmetric center and is described by the *R*- and *S*-sequencing rules of Cahn and Prelog, or by the manner in which the molecule rotates plane polarized light and designated as dextrorotatory or levorotatory (*i.e.*, as (+) or (-)-isomers respectively). A chiral compound can exist as either individual enantiomer or as a mixture thereof. A mixture containing equal proportions of the enantiomers is called a “racemic mixture.” For example, the compounds described herein can be in the form of an individual enantiomer, diastereomer or geometric isomer, or can be in the form of a mixture of stereoisomers, including racemic mixtures and mixtures enriched in one or more stereoisomer. Isomers can be isolated from mixtures by methods known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts; or preferred isomers can be prepared by asymmetric syntheses. See, for example, Jacques *et al.*, *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen *et al.*, *Tetrahedron* 33:2725 (1977); Eliel, *Stereochemistry of Carbon Compounds* (McGraw–Hill, NY, 1962); Wilen, *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972). The invention additionally encompasses compounds described herein as individual isomers substantially free of other isomers, and alternatively, as mixtures of various isomers.

[0032] Where an isomer/enantiomer is preferred, it may, in some embodiments, be provided substantially free of the corresponding enantiomer, and may also be referred to as “optically enriched” or “enantiomerically enriched.” “Optically enriched” and “enantiomerically enriched” means that a provided compound is made up of a significantly greater proportion of one enantiomer. In certain embodiments, a compound of the present invention is made up of at least about 70% by weight of a preferred enantiomer. In certain embodiments, a compound of the present invention is made up of at least about 80% by weight of a preferred enantiomer. In certain embodiments, a compound of the present invention is made up of at least about 90% by weight of a preferred enantiomer. In other embodiments the compound is made up of at least about 95%, 98%, or 99% by weight of a preferred enantiomer. Preferred enantiomers may be isolated from racemic mixtures by any method known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts or prepared by asymmetric syntheses. See, for example, Jacques *et al.*, *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen *et al.*, *Tetrahedron* 33:2725

(1977); Eliel, *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); Wilen, *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972).

[0033] Unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the depicted structures that differ only in the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by ^{13}C or ^{14}C are within the scope of this invention. Such compounds are useful, for example, as analytical tools, as probes in biological assays, or as therapeutic agents in accordance with the present invention.

[0034] When a range of values is listed, it is intended to encompass each value and sub-range within the range. For example “C₁₋₆” is intended to encompass, C₁, C₂, C₃, C₄, C₅, C₆, C₁₋₆, C₁₋₅, C₁₋₄, C₁₋₃, C₁₋₂, C₂₋₆, C₂₋₅, C₂₋₄, C₂₋₃, C₃₋₆, C₃₋₅, C₃₋₄, C₄₋₆, C₄₋₅, and C₅₋₆.

[0035] The terms “purified,” “substantially purified,” and “isolated” refer to a compound useful in the present invention being free of other, dissimilar compounds with which the compound is normally associated in its natural state, so that the compound comprises at least 0.5%, 1%, 5%, 10%, 20%, 50%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% of the mass, by weight, of a given sample or composition. In one embodiment, these terms refer to the compound comprising at least 95%, 98%, 99%, or 99.9% of the mass, by weight, of a given sample or composition.

[0036] The term “acyl” refers to a group having the general formula $-\text{C}(=\text{O})\text{R}^{\text{X1}}$, $-\text{C}(=\text{O})\text{OR}^{\text{X1}}$, $-\text{C}(=\text{O})-\text{O}-\text{C}(=\text{O})\text{R}^{\text{X1}}$, $-\text{C}(=\text{O})\text{SR}^{\text{X1}}$, $-\text{C}(=\text{O})\text{N}(\text{R}^{\text{X1}})_2$, $-\text{C}(=\text{S})\text{R}^{\text{X1}}$, $-\text{C}(=\text{S})\text{N}(\text{R}^{\text{X1}})_2$, and $-\text{C}(=\text{S})\text{S}(\text{R}^{\text{X1}})$, $-\text{C}(=\text{NR}^{\text{X1}})\text{R}^{\text{X1}}$, $-\text{C}(=\text{NR}^{\text{X1}})\text{OR}^{\text{X1}}$, $-\text{C}(=\text{NR}^{\text{X1}})\text{SR}^{\text{X1}}$, and $-\text{C}(=\text{NR}^{\text{X1}})\text{N}(\text{R}^{\text{X1}})_2$, wherein R^{X1} is hydrogen; halogen; substituted or unsubstituted hydroxyl; substituted or unsubstituted thiol; substituted or unsubstituted amino; substituted or unsubstituted acyl, cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched alkyl; cyclic or acyclic, substituted or unsubstituted, branched or unbranched alkenyl; substituted or unsubstituted alkynyl; substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, mono- or di- aliphaticamino, mono- or di- heteroaliphaticamino, mono- or di- alkylamino, mono- or di- heteroalkylamino, mono- or di-arylamino, or mono- or di-heteroarylamino; or two R^{X1} groups taken together form a 5- to 6-membered heterocyclic

ring. Exemplary acyl groups include aldehydes ($-\text{CHO}$), carboxylic acids ($-\text{CO}_2\text{H}$), ketones, acyl halides, esters, amides, imines, carbonates, carbamates, and ureas. Acyl substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (*e.g.*, aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted).

[0037] The term “acyloxy” refers to a “substituted hydroxyl” of the formula ($-\text{OR}^i$), wherein R^i is an optionally substituted acyl group, as defined herein, and the oxygen moiety is directly attached to the parent molecule.

[0038] The term “aliphatic” includes both saturated and unsaturated, nonaromatic, straight chain (*i.e.*, unbranched), branched, acyclic, and cyclic (*i.e.*, carbocyclic) hydrocarbons, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, “aliphatic” is intended herein to include, but is not limited to, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties. Thus, the term “alkyl” includes straight, branched and cyclic alkyl groups. An analogous convention applies to other generic terms such as “alkenyl”, “alkynyl”, and the like. Furthermore, the terms “alkyl”, “alkenyl”, “alkynyl”, and the like encompass both substituted and unsubstituted groups. In certain embodiments, “aliphatic” is used to indicate those aliphatic groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1–20 carbon atoms. Aliphatic group substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (*e.g.*, aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted).

[0039] The term “alkyl” refers to saturated, straight- or branched-chain hydrocarbon radicals derived from a hydrocarbon moiety containing between one and twenty carbon atoms by removal of a single hydrogen atom. In some embodiments, the alkyl group employed in

the invention contains 1–20 carbon atoms. In another embodiment, the alkyl group employed contains 1–15 carbon atoms. In another embodiment, the alkyl group employed contains 1–10 carbon atoms. In another embodiment, the alkyl group employed contains 1–8 carbon atoms. In another embodiment, the alkyl group employed contains 1–5 carbon atoms. Examples of alkyl radicals include, but are not limited to, methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *iso*-butyl, *sec*-butyl, *sec*-pentyl, *iso*-pentyl, *tert*-butyl, *n*-pentyl, neopentyl, *n*-hexyl, *sec*-hexyl, *n*-heptyl, *n*-octyl, *n*-decyl, *n*-undecyl, dodecyl, and the like, which may bear one or more substituents. Alkyl group substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (*e.g.*, aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted).

[0040] The term “alkenyl” denotes a monovalent group derived from a straight- or branched-chain hydrocarbon moiety having at least one carbon-carbon double bond by the removal of a single hydrogen atom. In certain embodiments, the alkenyl group employed in the invention contains 2–20 carbon atoms. In some embodiments, the alkenyl group employed in the invention contains 2–15 carbon atoms. In another embodiment, the alkenyl group employed contains 2–10 carbon atoms. In still other embodiments, the alkenyl group contains 2–8 carbon atoms. In yet other embodiments, the alkenyl group contains 2–5 carbons. Alkenyl groups include, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, and the like, which may bear one or more substituents. Alkenyl group substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (*e.g.*, aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted).

[0041] The term “alkynyl” refers to a monovalent group derived from a straight- or branched-chain hydrocarbon having at least one carbon-carbon triple bond by the removal of

a single hydrogen atom. In certain embodiments, the alkynyl group employed in the invention contains 2–20 carbon atoms. In some embodiments, the alkynyl group employed in the invention contains 2–15 carbon atoms. In another embodiment, the alkynyl group employed contains 2–10 carbon atoms. In still other embodiments, the alkynyl group contains 2–8 carbon atoms. In still other embodiments, the alkynyl group contains 2–5 carbon atoms. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl, and the like, which may bear one or more substituents. Alkynyl group substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (*e.g.*, aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted).

[0042] Exemplary carbon atom substituents include, but are not limited to, halogen, $-\text{CN}$, $-\text{NO}_2$, $-\text{N}_3$, $-\text{SO}_2\text{H}$, $-\text{SO}_3\text{H}$, $-\text{OH}$, $-\text{OR}^{\text{aa}}$, $-\text{ON}(\text{R}^{\text{bb}})_2$, $-\text{N}(\text{R}^{\text{bb}})_2$, $-\text{N}(\text{R}^{\text{bb}})_3^+\text{X}^-$, $-\text{N}(\text{OR}^{\text{cc}})\text{R}^{\text{bb}}$, $-\text{SH}$, $-\text{SR}^{\text{aa}}$, $-\text{SSR}^{\text{cc}}$, $-\text{C}(=\text{O})\text{R}^{\text{aa}}$, $-\text{CO}_2\text{H}$, $-\text{CHO}$, $-\text{C}(\text{OR}^{\text{cc}})_2$, $-\text{CO}_2\text{R}^{\text{aa}}$, $-\text{OC}(=\text{O})\text{R}^{\text{aa}}$, $-\text{OCO}_2\text{R}^{\text{aa}}$, $-\text{C}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{OC}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{NR}^{\text{bb}}\text{C}(=\text{O})\text{R}^{\text{aa}}$, $-\text{NR}^{\text{bb}}\text{CO}_2\text{R}^{\text{aa}}$, $-\text{NR}^{\text{bb}}\text{C}(=\text{O})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{C}(=\text{NR}^{\text{bb}})\text{R}^{\text{aa}}$, $-\text{C}(=\text{NR}^{\text{bb}})\text{OR}^{\text{aa}}$, $-\text{OC}(=\text{NR}^{\text{bb}})\text{R}^{\text{aa}}$, $-\text{OC}(=\text{NR}^{\text{bb}})\text{OR}^{\text{aa}}$, $-\text{C}(=\text{NR}^{\text{bb}})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{OC}(=\text{NR}^{\text{bb}})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{NR}^{\text{bb}}\text{C}(=\text{NR}^{\text{bb}})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{C}(=\text{O})\text{NR}^{\text{bb}}\text{SO}_2\text{R}^{\text{aa}}$, $-\text{NR}^{\text{bb}}\text{SO}_2\text{R}^{\text{aa}}$, $-\text{SO}_2\text{N}(\text{R}^{\text{bb}})_2$, $-\text{SO}_2\text{R}^{\text{aa}}$, $-\text{SO}_2\text{OR}^{\text{aa}}$, $-\text{OSO}_2\text{R}^{\text{aa}}$, $-\text{S}(=\text{O})\text{R}^{\text{aa}}$, $-\text{OS}(=\text{O})\text{R}^{\text{aa}}$, $-\text{Si}(\text{R}^{\text{aa}})_3$, $-\text{OSi}(\text{R}^{\text{aa}})_3$, $-\text{C}(=\text{S})\text{N}(\text{R}^{\text{bb}})_2$, $-\text{C}(=\text{O})\text{SR}^{\text{aa}}$, $-\text{C}(=\text{S})\text{SR}^{\text{aa}}$, $-\text{SC}(=\text{S})\text{SR}^{\text{aa}}$, $-\text{SC}(=\text{O})\text{SR}^{\text{aa}}$, $-\text{OC}(=\text{O})\text{SR}^{\text{aa}}$, $-\text{SC}(=\text{O})\text{OR}^{\text{aa}}$, $-\text{SC}(=\text{O})\text{R}^{\text{aa}}$, $-\text{P}(=\text{O})(\text{R}^{\text{aa}})_2$, $-\text{P}(=\text{O})(\text{OR}^{\text{cc}})_2$, $-\text{OP}(=\text{O})(\text{R}^{\text{aa}})_2$, $-\text{OP}(=\text{O})(\text{OR}^{\text{cc}})_2$, $-\text{P}(=\text{O})(\text{N}(\text{R}^{\text{bb}})_2)_2$, $-\text{OP}(=\text{O})(\text{N}(\text{R}^{\text{bb}})_2)_2$, $-\text{NR}^{\text{bb}}\text{P}(=\text{O})(\text{R}^{\text{aa}})_2$, $-\text{NR}^{\text{bb}}\text{P}(=\text{O})(\text{OR}^{\text{cc}})_2$, $-\text{NR}^{\text{bb}}\text{P}(=\text{O})(\text{N}(\text{R}^{\text{bb}})_2)_2$, $-\text{P}(\text{R}^{\text{cc}})_2$, $-\text{P}(\text{OR}^{\text{cc}})_2$, $-\text{P}(\text{R}^{\text{cc}})_3^+\text{X}^-$, $-\text{P}(\text{OR}^{\text{cc}})_3^+\text{X}^-$, $-\text{P}(\text{R}^{\text{cc}})_4$, $-\text{P}(\text{OR}^{\text{cc}})_4$, $-\text{OP}(\text{R}^{\text{cc}})_2$, $-\text{OP}(\text{R}^{\text{cc}})_3^+\text{X}^-$, $-\text{OP}(\text{OR}^{\text{cc}})_2$, $-\text{OP}(\text{OR}^{\text{cc}})_3^+\text{X}^-$, $-\text{OP}(\text{R}^{\text{cc}})_4$, $-\text{OP}(\text{OR}^{\text{cc}})_4$, $-\text{B}(\text{R}^{\text{aa}})_2$, $-\text{B}(\text{OR}^{\text{cc}})_2$, $-\text{BR}^{\text{aa}}(\text{OR}^{\text{cc}})$, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, hetero C_{1-10} alkyl, hetero C_{2-10} alkenyl, hetero C_{2-10} alkynyl, C_{3-10} carbocyclyl, 3-14 membered heterocyclyl, C_{6-14} aryl, and 5-14 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups; wherein X^- is a counterion;

or two geminal hydrogens on a carbon atom are replaced with the group =O, =S, =NN(R^{bb})₂, =NNR^{bb}C(=O)R^{aa}, =NNR^{bb}C(=O)OR^{aa}, =NNR^{bb}S(=O)₂R^{aa}, =NR^{bb}, or =NOR^{cc};

each instance of R^{aa} is, independently, selected from C₁₋₁₀ alkyl, C₁₋₁₀ perhaloalkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, heteroC₁₋₁₀ alkyl, heteroC₂₋₁₀ alkenyl, heteroC₂₋₁₀ alkynyl, C₃₋₁₀ carbocyclyl, 3-14 membered heterocyclyl, C₆₋₁₄ aryl, and 5-14 membered heteroaryl, or two R^{aa} groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

each instance of R^{bb} is, independently, selected from hydrogen, -OH, -OR^{aa}, -N(R^{cc})₂, -CN, -C(=O)R^{aa}, -C(=O)N(R^{cc})₂, -CO₂R^{aa}, -SO₂R^{aa}, -C(=NR^{cc})OR^{aa}, -C(=NR^{cc})N(R^{cc})₂, -SO₂N(R^{cc})₂, -SO₂R^{cc}, -SO₂OR^{cc}, -SOR^{aa}, -C(=S)N(R^{cc})₂, -C(=O)SR^{cc}, -C(=S)SR^{cc}, -P(=O)(R^{aa})₂, -P(=O)(OR^{cc})₂, -P(=O)(N(R^{cc})₂)₂, C₁₋₁₀ alkyl, C₁₋₁₀ perhaloalkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, heteroC₁₋₁₀ alkyl, heteroC₂₋₁₀ alkenyl, heteroC₂₋₁₀ alkynyl, C₃₋₁₀ carbocyclyl, 3-14 membered heterocyclyl, C₆₋₁₄ aryl, and 5-14 membered heteroaryl, or two R^{bb} groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups; wherein X⁻ is a counterion.

each instance of R^{cc} is, independently, selected from hydrogen, C₁₋₁₀ alkyl, C₁₋₁₀ perhaloalkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, heteroC₁₋₁₀ alkyl, heteroC₂₋₁₀ alkenyl, heteroC₂₋₁₀ alkynyl, C₃₋₁₀ carbocyclyl, 3-14 membered heterocyclyl, C₆₋₁₄ aryl, and 5-14 membered heteroaryl, or two R^{cc} groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

each instance of R^{dd} is, independently, selected from halogen, -CN, -NO₂, -N₃, -SO₂H, -SO₃H, -OH, -OR^{ee}, -ON(R^{ff})₂, -N(R^{ff})₂, -N(R^{ff})₃⁺X⁻, -N(OR^{ee})R^{ff}, -SH, -SR^{ee}, -SSR^{ee}, -C(=O)R^{ee}, -CO₂H, -CO₂R^{ee}, -OC(=O)R^{ee}, -OCO₂R^{ee}, -C(=O)N(R^{ff})₂, -OC(=O)N(R^{ff})₂, -NR^{ff}C(=O)R^{ee}, -NR^{ff}CO₂R^{ee}, -NR^{ff}C(=O)N(R^{ff})₂, -C(=NR^{ff})OR^{ee}, -OC(=NR^{ff})R^{ee}, -OC(=NR^{ff})OR^{ee}, -C(=NR^{ff})N(R^{ff})₂, -OC(=NR^{ff})N(R^{ff})₂, -NR^{ff}C(=NR^{ff})N(R^{ff})₂, -NR^{ff}SO₂R^{ee}, -SO₂N(R^{ff})₂, -SO₂R^{ee}, -SO₂OR^{ee}, -OSO₂R^{ee}, -S(=O)R^{ee}, -Si(R^{ee})₃, -OSi(R^{ee})₃, -C(=S)N(R^{ff})₂, -C(=O)SR^{ee}, -C(=S)SR^{ee}, -SC(=S)SR^{ee}, -P(=O)(OR^{ee})₂, -P(=O)(R^{ee})₂, -OP(=O)(R^{ee})₂, -OP(=O)(OR^{ee})₂, C₁₋₆ alkyl, C₁₋₆ perhaloalkyl,

C₂₋₆ alkenyl, C₂₋₆ alkynyl, heteroC₁₋₆alkyl, heteroC₂₋₆alkenyl, heteroC₂₋₆alkynyl, C₃₋₁₀ carbocyclyl, 3-10 membered heterocyclyl, C₆₋₁₀ aryl, 5-10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups, or two geminal R^{dd} substituents can be joined to form =O or =S; wherein X⁻ is a counterion;

each instance of R^{ee} is, independently, selected from C₁₋₆ alkyl, C₁₋₆ perhaloalkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, heteroC₁₋₆ alkyl, heteroC₂₋₆alkenyl, heteroC₂₋₆ alkynyl, C₃₋₁₀ carbocyclyl, C₆₋₁₀ aryl, 3-10 membered heterocyclyl, and 3-10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups;

each instance of R^{ff} is, independently, selected from hydrogen, C₁₋₆ alkyl, C₁₋₆ perhaloalkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, heteroC₁₋₆alkyl, heteroC₂₋₆alkenyl, heteroC₂₋₆alkynyl, C₃₋₁₀ carbocyclyl, 3-10 membered heterocyclyl, C₆₋₁₀ aryl and 5-10 membered heteroaryl, or two R^{ff} groups are joined to form a 3-10 membered heterocyclyl or 5-10 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups; and

each instance of R^{gg} is, independently, halogen, -CN, -NO₂, -N₃, -SO₂H, -SO₃H, -OH, -OC₁₋₆ alkyl, -ON(C₁₋₆ alkyl)₂, -N(C₁₋₆ alkyl)₂, -N(C₁₋₆ alkyl)₃⁺X⁻, -NH(C₁₋₆ alkyl)₂⁺X⁻, -NH₂(C₁₋₆ alkyl) ⁺X⁻, -NH₃⁺X⁻, -N(OC₁₋₆ alkyl)(C₁₋₆ alkyl), -N(OH)(C₁₋₆ alkyl), -NH(OH), -SH, -SC₁₋₆ alkyl, -SS(C₁₋₆ alkyl), -C(=O)(C₁₋₆ alkyl), -CO₂H, -CO₂(C₁₋₆ alkyl), -OC(=O)(C₁₋₆ alkyl), -OCO₂(C₁₋₆ alkyl), -C(=O)NH₂, -C(=O)N(C₁₋₆ alkyl)₂, -OC(=O)NH(C₁₋₆ alkyl), -NHC(=O)(C₁₋₆ alkyl), -N(C₁₋₆ alkyl)C(=O)(C₁₋₆ alkyl), -NHCO₂(C₁₋₆ alkyl), -NHC(=O)N(C₁₋₆ alkyl)₂, -NHC(=O)NH(C₁₋₆ alkyl), -NHC(=O)NH₂, -C(=NH)O(C₁₋₆ alkyl), -OC(=NH)(C₁₋₆ alkyl), -OC(=NH)OC₁₋₆ alkyl, -C(=NH)N(C₁₋₆ alkyl)₂, -C(=NH)NH(C₁₋₆ alkyl), -C(=NH)NH₂, -OC(=NH)N(C₁₋₆ alkyl)₂, -OC(NH)NH(C₁₋₆ alkyl), -OC(NH)NH₂, -NHC(NH)N(C₁₋₆ alkyl)₂, -NHC(=NH)NH₂, -NH₂SO₂(C₁₋₆ alkyl), -SO₂N(C₁₋₆ alkyl)₂, -SO₂NH(C₁₋₆ alkyl), -SO₂NH₂, -SO₂C₁₋₆ alkyl, -SO₂OC₁₋₆ alkyl, -OSO₂C₁₋₆ alkyl, -SOC₁₋₆ alkyl, -Si(C₁₋₆ alkyl)₃, -OSi(C₁₋₆ alkyl)₃ -C(=S)N(C₁₋₆ alkyl)₂, C(=S)NH(C₁₋₆ alkyl), C(=S)NH₂, -C(=O)S(C₁₋₆ alkyl), -C(=S)SC₁₋₆ alkyl, -SC(=S)SC₁₋₆ alkyl, -P(=O)(OC₁₋₆ alkyl)₂, -P(=O)(C₁₋₆ alkyl)₂, -OP(=O)(C₁₋₆ alkyl)₂, -OP(=O)(OC₁₋₆ alkyl)₂, C₁₋₆ alkyl, C₁₋₆ perhaloalkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, heteroC₁₋₆alkyl, heteroC₂₋₆

₆alkenyl, heteroC₂₋₆alkynyl, C₃₋₁₀ carbocyclyl, C₆₋₁₀ aryl, 3-10 membered heterocyclyl, 5-10 membered heteroaryl; or two geminal R^{gg} substituents can be joined to form =O or =S; wherein X⁻ is a counterion.

[0043] The term “halo” or “halogen” refers to fluorine (fluoro, -F), chlorine (chloro, -Cl), bromine (bromo, -Br), or iodine (iodo, -I).

[0044] The term “hydroxyl” or “hydroxy” refers to the group -OH. The term “substituted hydroxyl” or “substituted hydroxyl,” by extension, refers to a hydroxyl group wherein the oxygen atom directly attached to the parent molecule is substituted with a group other than hydrogen, and includes groups selected from -OR^{aa}, -ON(R^{bb})₂, -OC(=O)SR^{aa}, -OC(=O)R^{aa}, -OCO₂R^{aa}, -OC(=O)N(R^{bb})₂, -OC(=NR^{bb})R^{aa}, -OC(=NR^{bb})OR^{aa}, -OC(=NR^{bb})N(R^{bb})₂, -OS(=O)R^{aa}, -OSO₂R^{aa}, -OSi(R^{aa})₃, -OP(R^{cc})₂, -OP(R^{cc})₃⁺X⁻, -OP(OR^{cc})₂, -OP(OR^{cc})₃⁺X⁻, -OP(=O)(R^{aa})₂, -OP(=O)(OR^{cc})₂, and -OP(=O)(N(R^{bb}))₂, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein.

[0045] The term “amino” refers to a group of the formula (-NH₂). A “substituted amino” refers either to a mono-substituted amine (-NHR^h) or a disubstituted amine (-NR^h)₂, wherein the R^h substituent is any substituent as described herein that results in the formation of a stable moiety (*e.g.*, a suitable amino protecting group; aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, amino, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted). In certain embodiments, the R^h substituents of the disubstituted amino group(-NR^h)₂ form a 5- to 6-membered heterocyclic ring.

[0046] The term “alkoxy” refers to a “substituted hydroxyl” of the formula (-ORⁱ), wherein Rⁱ is an optionally substituted alkyl group as defined herein, and the oxygen moiety is directly attached to the parent molecule.

[0047] The term “alkylthioxy” refers to a “substituted thiol” of the formula (-SR^r), wherein R^r is an optionally substituted alkyl group as defined herein, and the sulfur moiety is directly attached to the parent molecule.

[0048] The term “alkylamino” refers to a “substituted amino” of the formula (-NR^h)₂, wherein R^h is, independently, a hydrogen or an optionally substituted alkyl group as defined herein, and the nitrogen moiety is directly attached to the parent molecule.

[0049] The term “aryl” refer to stable aromatic mono- or polycyclic ring system having 3–20 ring atoms, of which all the ring atoms are carbon, and which may be substituted or unsubstituted. In certain embodiments of the present invention, “aryl” refers to a mono, bi, or tricyclic C₄–C₂₀ aromatic ring system having one, two, or three aromatic rings which include, but not limited to, phenyl, biphenyl, naphthyl, and the like, which may bear one or more substituents. Aryl substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (*e.g.*, aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted).

[0050] The term “arylalkyl” refers to an aryl substituted alkyl group, wherein the terms “aryl” and “alkyl” are defined herein, and wherein the aryl group is attached to the alkyl group, which in turn is attached to the parent molecule. Exemplary arylalkyl groups are benzyl and phenethyl.

[0051] The term “aryloxy” refers to a “substituted hydroxyl” of the formula (–ORⁱ), wherein Rⁱ is an optionally substituted aryl group as defined herein, and the oxygen moiety is directly attached to the parent molecule.

[0052] The term “arylamino,” refers to a “substituted amino” of the formula (–NR^h₂), wherein R^h is, independently, a hydrogen or an optionally substituted aryl group as defined herein, and the nitrogen moiety is directly attached to the parent molecule.

[0053] The term “arylthioxy” refers to a “substituted thiol” of the formula (–SR^r), wherein R^r is an optionally substituted aryl group as defined herein, and the sulfur moiety is directly attached to the parent molecule.

[0054] The term “heteroaliphatic” refers to an aliphatic moiety, as defined herein, which includes both saturated and unsaturated, nonaromatic, straight chain (*i.e.*, unbranched), branched, acyclic, cyclic (*i.e.*, heterocyclic), or polycyclic hydrocarbons, which are optionally substituted with one or more functional groups, and that contain one or more oxygen, sulfur, nitrogen, phosphorus, or silicon atoms, *e.g.*, in place of carbon atoms. In certain embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or more substituents. As will be appreciated by one of ordinary skill in the art, “heteroaliphatic” is intended herein to include, but is not

limited to, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, and heterocycloalkynyl moieties. Thus, the term “heteroaliphatic” includes the terms “heteroalkyl,” “heteroalkenyl,” “heteroalkynyl,” and the like. Furthermore, the terms “heteroalkyl,” “heteroalkenyl,” “heteroalkynyl,” and the like encompass both substituted and unsubstituted groups. In certain embodiments, “heteroaliphatic” is used to indicate those heteroaliphatic groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1–20 carbon atoms. Heteroaliphatic group substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (*e.g.*, aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, sulfinyl, sulfonyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted).

[0055] The term “heteroalkyl” refers to an alkyl moiety, as defined herein, which contain one or more oxygen, sulfur, nitrogen, phosphorus, or silicon atoms, *e.g.*, in place of carbon atoms.

[0056] The term “heteroalkenyl” refers to an alkenyl moiety, as defined herein, which contain one or more oxygen, sulfur, nitrogen, phosphorus, or silicon atoms, *e.g.*, in place of carbon atoms.

[0057] The term “heteroalkynyl” refers to an alkynyl moiety, as defined herein, which contain one or more oxygen, sulfur, nitrogen, phosphorus, or silicon atoms, *e.g.*, in place of carbon atoms.

[0058] The term “heteroalkylamino” refers to a “substituted amino” of the formula ($-NR^h$), wherein R^h is, independently, a hydrogen or an optionally substituted heteroalkyl group, as defined herein, and the nitrogen moiety is directly attached to the parent molecule.

[0059] The term “heteroalkyloxy” refers to a “substituted hydroxyl” of the formula ($-OR^i$), wherein R^i is an optionally substituted heteroalkyl group, as defined herein, and the oxygen moiety is directly attached to the parent molecule.

[0060] The term “heteroalkylthioxy” refers to a “substituted thiol” of the formula ($-SR^r$), wherein R^r is an optionally substituted heteroalkyl group, as defined herein, and the sulfur moiety is directly attached to the parent molecule.

[0061] The term “heterocyclic,” “heterocycles,” or “heterocyclyl” refers to a cyclic heteroaliphatic group. A heterocyclic group refers to a non-aromatic, partially unsaturated or fully saturated, 3- to 12-membered ring system, which includes single rings of 3 to 8 atoms in size, and bi- and tri-cyclic ring systems which may include aromatic five- or six-membered aryl or heteroaryl groups fused to a non-aromatic ring. These heterocyclic rings include those having from one to three heteroatoms independently selected from oxygen, sulfur, and nitrogen, in which the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. In certain embodiments, the term heterocyclic refers to a non-aromatic 5-, 6-, or 7-membered ring or polycyclic group wherein at least one ring atom is a heteroatom selected from O, S, and N (wherein the nitrogen and sulfur heteroatoms may be optionally oxidized), and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms. Heterocyclyl groups include, but are not limited to, a bi- or tri-cyclic group, comprising fused five, six, or seven-membered rings having between one and three heteroatoms independently selected from the oxygen, sulfur, and nitrogen, wherein (i) each 5-membered ring has 0 to 2 double bonds, each 6-membered ring has 0 to 2 double bonds, and each 7-membered ring has 0 to 3 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to an aryl or heteroaryl ring. Exemplary heterocycles include azacyclopropanyl, azacyclobutanyl, 1,3-diazatidinyl, piperidinyl, piperazinyl, azocanyl, thiaranyl, thietanyl, tetrahydrothiophenyl, dithiolanyl, thiacyclohexanyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropuranyl, dioxanyl, oxathiolanyl, morpholinyl, thioxanyl, tetrahydronaphthyl, and the like, which may bear one or more substituents. Substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (*e.g.*, aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, sulfinyl, sulfonyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted).

[0062] The term “heteroaryl” refer to stable aromatic mono- or polycyclic ring system having 3-20 ring atoms, of which one ring atom is selected from S, O, and N; zero, one, or two ring atoms are additional heteroatoms independently selected from S, O, and N; and the

remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms. Exemplary heteroaryls include, but are not limited to pyrrolyl, pyrazolyl, imidazolyl, pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, tetrazinyl, pyrolozyl, indolyl, quinolyl, isoquinolyl, benzoimidazolyl, indazolyl, quinolyl, isoquinolyl, quinolizyl, cinnolyl, quinazolyl, phthalazyl, naphthridyl, quinoxalyl, thiophenyl, thianaphthenyl, furanyl, benzofuranyl, benzothiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, oxazolyl, isoxazolyl, oxadiazolyl, and the like, which may bear one or more substituents. Heteroaryl substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (*e.g.*, aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, sulfinyl, sulfonyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted).

[0063] The term “heteroarylamino” refers to a “substituted amino” of the $(-NR^h)$, wherein R^h is, independently, hydrogen or an optionally substituted heteroaryl group, as defined herein, and the nitrogen moiety is directly attached to the parent molecule.

[0064] The term “heteroaryloxy” refers to a “substituted hydroxyl” of the formula $(-OR^i)$, wherein R^i is an optionally substituted heteroaryl group, as defined herein, and the oxygen moiety is directly attached to the parent molecule.

[0065] The term “heteroarylthioxy” refers to a “substituted thiol” of the formula $(-SR^r)$, wherein R^r is an optionally substituted heteroaryl group, as defined herein, and the sulfur moiety is directly attached to the parent molecule.

[0066] The term “imino” refers to a group of the formula $(=NR^r)$, wherein R^r corresponds to hydrogen or any substituent as described herein, that results in the formation of a stable moiety (for example, a suitable amino protecting group; aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, amino, hydroxyl, alkylaryl, arylalkyl, and the like, each of which may or may not be further substituted). In certain embodiments, imino refers to $=NH$ wherein R^r is hydrogen.

[0067] The term “nitro” refers to a group of the formula $(-NO_2)$.

[0068] The term “oxo” refers to a group of the formula $(=O)$.

[0069] A “protecting group” is well known in the art and include those described in detail in *Greene’s Protective Groups in Organic Synthesis*, P. G. M. Wuts and T. W. Greene, 4th edition, Wiley-Interscience, 2006, the entirety of which is incorporated herein by reference.

[0070] Suitable “amino-protecting groups” (also referred to as “nitrogen protecting groups”) include methyl carbamate, ethyl carbamate, 9-fluorenylmethyl carbamate (Fmoc), 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl carbamate, 2,7-di-*t*-butyl-[9-(10,10-dioxo-10,10,10-tetrahydrothioxanthyl)]methyl carbamate (DBD-Tmoc), 4-methoxyphenacyl carbamate (Phenoc), 2,2,2-trichloroethyl carbamate (Troc), 2-trimethylsilylethyl carbamate (Teoc), 2-phenylethyl carbamate (hZ), 1-(1-adamantyl)-1-methylethyl carbamate (Adpoc), 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate (DB-*t*-BOC), 1,1-dimethyl-2,2,2-trichloroethyl carbamate (TCBOC), 1-methyl-1-(4-biphenyl)ethyl carbamate (Bpoc), 1-(3,5-di-*t*-butylphenyl)-1-methylethyl carbamate (*t*-Bumeoc), 2-(2'- and 4'-pyridyl)ethyl carbamate (Pyoc), 2-(*N,N*-dicyclohexylcarboxamido)ethyl carbamate, *t*-butyl carbamate (BOC or Boc), 1-adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1-isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4-nitrocinnamyl carbamate (Noc), 8-quinolyl carbamate, *N*-hydroxypiperidinyll carbamate, alkylthio carbamate, benzyl carbamate (Cbz), *p*-methoxybenzyl carbamate (Moz), *p*-nitrobenzyl carbamate, *p*-bromobenzyl carbamate, *p*-chlorobenzyl carbamate, 2,4-dichlorobenzyl carbamate, 4-methylsulfinylbenzyl carbamate (MsZ), 9-anthrylmethyl carbamate, diphenylmethyl carbamate, 2-methylthioethyl carbamate, 2-methylsulfonylethyl carbamate, 2-(*p*-toluenesulfonyl)ethyl carbamate, [2-(1,3-dithianyl)]methyl carbamate (Dmoc), 4-methylthiophenyl carbamate (Mtpc), 2,4-dimethylthiophenyl carbamate (Bmpc), 2-phosphonioethyl carbamate (Peoc), 2-triphenylphosphonioisopropyl carbamate (Ppoc), 1,1-dimethyl-2-cyanoethyl carbamate, *m*-chloro-*p*-acyloxybenzyl carbamate, *p*-(dihydroxyboryl)benzyl carbamate, 5-benzisoxazolylmethyl carbamate, 2-(trifluoromethyl)-6-chromonylmethyl carbamate (Tcroc), *m*-nitrophenyl carbamate, 3,5-dimethoxybenzyl carbamate, *o*-nitrobenzyl carbamate, 3,4-dimethoxy-6-nitrobenzyl carbamate, phenyl(*o*-nitrophenyl)methyl carbamate, phenothiazinyl-(10)-carbonyl derivative, *N'*-*p*-toluenesulfonylaminocarbonyl derivative, *N'*-phenylaminothiocarbonyl derivative, *t*-amyl carbamate, *S*-benzyl thiocarbamate, *p*-cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, *p*-decyloxybenzyl carbamate, 2,2-dimethoxycarbonylvinyl

carbamate, *o*-(*N,N*-dimethylcarboxamido)benzyl carbamate, 1,1-dimethyl-3-(*N,N*-dimethylcarboxamido)propyl carbamate, 1,1-dimethylpropynyl carbamate, di(2-pyridyl)methyl carbamate, 2-furanylmethyl carbamate, 2-iodoethyl carbamate, isoboryn carbamate, isobutyl carbamate, isonicotinyl carbamate, *p*-(*p'*-methoxyphenylazo)benzyl carbamate, 1-methylcyclobutyl carbamate, 1-methylcyclohexyl carbamate, 1-methyl-1-cyclopropylmethyl carbamate, 1-methyl-1-(3,5-dimethoxyphenyl)ethyl carbamate, 1-methyl-1-(*p*-phenylazophenyl)ethyl carbamate, 1-methyl-1-phenylethyl carbamate, 1-methyl-1-(4-pyridyl)ethyl carbamate, phenyl carbamate, *p*-(phenylazo)benzyl carbamate, 2,4,6-tri-*t*-butylphenyl carbamate, 4-(trimethylammonium)benzyl carbamate, 2,4,6-trimethylbenzyl carbamate, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3-phenylpropanamide, picolinamide, 3-pyridylcarboxamide, *N*-benzoylphenylalanyl derivative, benzamide, *p*-phenylbenzamide, *o*-nitrophenylacetamide, *o*-nitrophenoxyacetamide, acetoacetamide, (*N'*-dithiobenzoyloxycarbonylamino)acetamide, 3-(*p*-hydroxyphenyl)propanamide, 3-(*o*-nitrophenyl)propanamide, 2-methyl-2-(*o*-nitrophenoxy)propanamide, 2-methyl-2-(*o*-phenylazophenoxy)propanamide, 4-chlorobutanamide, 3-methyl-3-nitrobutanamide, *o*-nitrocinnamide, *N*-acetylmethionine derivative, *o*-nitrobenzamide, *o*-(benzoyloxymethyl)benzamide, 4,5-diphenyl-3-oxazolin-2-one, *N*-phthalimide, *N*-dithiasuccinimide (Dts), *N*-2,3-diphenylmaleimide, *N*-2,5-dimethylpyrrole, *N*-1,1,4,4-tetramethyldisilylazacyclopentane adduct (STABASE), 5-substituted 1,3-dimethyl-1,3,5-triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridone, *N*-methylamine, *N*-allylamine, *N*-[2-(trimethylsilyl)ethoxy]methylamine (SEM), *N*-3-acetoxypentylamine, *N*-(1-isopropyl-4-nitro-2-oxo-3-pyrroline-3-yl)amine, quaternary ammonium salts, *N*-benzylamine, *N*-di(4-methoxyphenyl)methylamine, *N*-5-dibenzosuberylamine, *N*-triphenylmethylamine (Tr), *N*-[(4-methoxyphenyl)diphenylmethyl]amine (MMTr), *N*-9-phenylfluorenylamine (PhF), *N*-2,7-dichloro-9-fluorenylmethylethylamine, *N*-ferrocenylmethylamino (Fcm), *N*-2-picolylamino *N'*-oxide, *N*-1,1-dimethylthiomethylethylamine, *N*-benzylideneamine, *N*-*p*-methoxybenzylideneamine, *N*-diphenylmethylethylamine, *N*-[(2-pyridyl)mesityl]methylethylamine, *N*-(*N'*,*N'*-dimethylaminomethylene)amine, *N*,*N'*-isopropylidenediamine, *N*-*p*-nitrobenzylideneamine, *N*-salicylideneamine, *N*-5-chlorosalicylideneamine, *N*-(5-chloro-2-hydroxyphenyl)phenylmethylethylamine, *N*-cyclohexylideneamine, *N*-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine, *N*-borane derivative, *N*-diphenylborinic acid derivative, *N*-[phenyl(pentacarbonylchromium- or

tungsten)carbonyl]amine, *N*-copper chelate, *N*-zinc chelate, *N*-nitroamine, *N*-nitrosoamine, amine *N*-oxide, diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, *o*-nitrobenzenesulfenamide (Nps), 2,4-dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2-nitro-4-methoxybenzenesulfenamide, triphenylmethylsulfenamide, 3-nitropyridinesulfenamide (Npys), *p*-toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6-trimethyl-4-methoxybenzenesulfonamide (Mtr), 2,4,6-trimethoxybenzenesulfonamide (Mtb), 2,6-dimethyl-4-methoxybenzenesulfonamide (Pme), 2,3,5,6-tetramethyl-4-methoxybenzenesulfonamide (Mte), 4-methoxybenzenesulfonamide (Mbs), 2,4,6-trimethylbenzenesulfonamide (Mts), 2,6-dimethoxy-4-methylbenzenesulfonamide (iMds), 2,2,5,7,8-pentamethylchroman-6-sulfonamide (Pmc), methanesulfonamide (Ms), β -trimethylsilylethanesulfonamide (SES), 9-anthracenesulfonamide, 4-(4',8'-dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenaclysulfonamide.

[0071] A “hydroxyl protecting group” (also referred to as an “oxygen protecting group”) is well known in the art and includes those described in detail in Greene (1999). Suitable hydroxyl protecting groups include methyl, methoxymethyl (MOM), methylthiomethyl (MTM), *t*-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), *p*-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (*p*-AOM), guaiacolmethyl (GUM), *t*-butoxymethyl, 4-pentenylloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, *t*-butyl, allyl, *p*-chlorophenyl, *p*-methoxyphenyl, 2,4-dinitrophenyl, benzyl, *p*-methoxybenzyl, 3,4-dimethoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, *p*-halobenzyl, 2,6-dichlorobenzyl, *p*-cyanobenzyl, *p*-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl *N*-oxido,

diphenylmethyl, *p,p'*-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α -naphthylmethyl, *p*-methoxyphenyldiphenylmethyl, di(*p*-methoxyphenyl)methyl, tri(*p*-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4',4''-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylhexylsilyl, *t*-butyldimethylsilyl (TBDMS), *t*-butyldiphenylsilyl (TBDPS), tribenzylsilyl, tri-*p*-xylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), *t*-butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantate, crotonate, 4-methoxycrotonate, benzoate, *p*-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl) ethyl carbonate (Psec), 2-(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate, alkyl allyl carbonate, alkyl *p*-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl *p*-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl *o*-nitrobenzyl carbonate, alkyl *p*-nitrobenzyl carbonate, alkyl *S*-benzyl thiocarbonate, 4-ethoxy-1-naphthyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, *o*-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (*E*)-2-methyl-2-butenate, *o*-(methoxycarbonyl)benzoate, α -naphthoate, nitrate, alkyl *N,N,N',N'*-tetramethylphosphorodiamidate, alkyl *N*-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfonate, sulfate, methanesulfonate (mesylate), benzylsulfonate, and tosylate (Ts). For protecting 1,2- or 1,3-diols, protecting groups include methylene acetal, ethylidene acetal, 1-*t*-butylethylidene ketal, 1-phenylethylidene ketal, (4-

methoxyphenyl)ethylidene acetal, 2,2,2-trichloroethylidene acetal, acetonide, cyclopentylidene ketal, cyclohexylidene ketal, cycloheptylidene ketal, benzylidene acetal, *p*-methoxybenzylidene acetal, 2,4-dimethoxybenzylidene ketal, 3,4-dimethoxybenzylidene acetal, 2-nitrobenzylidene acetal, methoxymethylene acetal, ethoxymethylene acetal, dimethoxymethylene ortho ester, 1-methoxyethylidene ortho ester, 1-ethoxyethylidene ortho ester, 1,2-dimethoxyethylidene ortho ester, α -methoxybenzylidene ortho ester, 1-(*N,N*-dimethylamino)ethylidene derivative, α -(*N,N'*-dimethylamino)benzylidene derivative, 2-oxacyclopentylidene ortho ester, di-*t*-butylsilylene group (DTBS), 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene) derivative (TIPDS), tetra-*t*-butoxydisiloxane-1,3-diylidene derivative (TBDS), cyclic carbonates, cyclic boronates, ethyl boronate, and phenyl boronate.

[0072] The term “pharmaceutically acceptable salt” refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and other animals without undue toxicity, irritation, allergic response, and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66, 1–19, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. The salts can be prepared during the final isolation and purification of the compounds or separately by reacting the appropriate compound in the form of the free base with a suitable acid. Representative acid addition salts include acetate, adipate, alginate, L-ascorbate, aspartate, benzoate, benzenesulfonate (besylate), bisulfate, butyrate, camphorate, camphorsulfonate, citrate, digluconate, formate, fumarate, gentisate, glutarate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hippurate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isethionate), lactate, maleate, malonate, DL-mandelate, mesitylenesulfonate, methanesulfonate, naphthylenesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphonate, picrate, pivalate, propionate, pyroglutamate, succinate, sulfonate, tartrate, L-tartrate, trichloroacetate, trifluoroacetate, phosphate, glutamate, bicarbonate, *para*-toluenesulfonate (*p*-tosylate), and undecanoate. Also, basic groups in the compounds disclosed herein can be quaternized with methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides; dimethyl, diethyl, dibutyl, and diamyl sulfates; decyl, lauryl, myristyl, and steryl chlorides, bromides, and iodides; and benzyl and phenethyl bromides. Examples of acids which can be employed to form

therapeutically acceptable salts include inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, and phosphoric acid; and organic acids such as oxalic acid, maleic acid, succinic acid, and citric acid. "Basic addition salts" refer to salts derived from appropriate bases, these salts including alkali metal, alkaline earth metal, and quaternary amine salts. Hence, the present invention contemplates sodium, potassium, magnesium, and calcium salts of the compounds disclosed herein, and the like. Basic addition salts can be prepared during the final isolation and purification of the compounds, often by reacting a carboxyl group with a suitable base such as the hydroxide, carbonate, or bicarbonate of a metal cation or with ammonia or an organic primary, secondary, or tertiary amine. The cations of therapeutically acceptable salts include lithium, sodium (by using, *e.g.*, NaOH), potassium (by using, *e.g.*, KOH), calcium (by using, *e.g.*, Ca(OH)₂), magnesium (by using, *e.g.*, Mg(OH)₂ and magnesium acetate), zinc, (by using, *e.g.*, Zn(OH)₂ and zinc acetate), and aluminum, as well as nontoxic quaternary amine cations such as ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, diethylamine, ethylamine, tributylamine, pyridine, *N,N*-dimethylaniline, *N*-methylpiperidine, *N*-methylmorpholine, dicyclohexylamine, procaine, dibenzylamine, *N,N*-dibenzylphenethylamine, 1-phenamine, and *N,N*-dibenzylethylenediamine. Other representative organic amines useful for the formation of base addition salts include ethylenediamine, ethanolamine, diethanolamine, piperidine, piperazine, choline hydroxide, hydroxyethyl morpholine, hydroxyethyl pyrrolidone, imidazole, *n*-methyl-*D*-glucamine, *N,N'*-dibenzylethylenediamine, *N,N'*-diethylethanolamine, *N,N'*-dimethylethanolamine, triethanolamine, and tromethamine. Basic amino acids (*e.g.*, 1-glycine and 1-arginine) and amino acids which may be zwitterionic at neutral pH (*e.g.*, betaine (*N,N,N*-trimethylglycine)) are also contemplated. In certain embodiments, the pharmaceutically acceptable salt is a mono salt, *i.e.* the ration of a compound as described herein versus an acid counterion ratio is 1:1. In certain embodiments, the pharmaceutically acceptable salt is a bis salt, *i.e.* the ration of a compound as described herein versus an acid counterion ratio is 1:2. In certain embodiments, the pharmaceutically acceptable salt is a mono-mesylate salt. In certain embodiments, the pharmaceutically acceptable salt is a bis-mesylate salt.

[0073] The term "tautomer" refers to a particular isomer of a compound in which a hydrogen and double bond have changed position with respect to the other atoms of the molecule. For a pair of tautomers to exist there must be a mechanism for interconversion. Examples of tautomers include keto-enol forms, imine-enamine forms, amide-imino alcohol forms, amidine-aminidine forms, nitroso-oxime forms, thio ketone-enethiol forms, *N*-nitroso-

hydroxyazo forms, nitro-*aci*-nitro forms, lactam-lactim forms, ketene-ynol forms, enamine-enamine forms, and pyridione-hydroxypyridine forms.

[0074] The term “polymorphs” refers to a crystalline form of a compound (or a salt, hydrate, or solvate thereof) in a particular crystal packing arrangement. All polymorphs have the same elemental composition. Different crystalline forms usually have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability, and solubility. Recrystallization solvent, rate of crystallization, storage temperature, and other factors may cause one crystal form to dominate. Various polymorphs of a compound can be prepared by crystallization under different conditions.

[0075] The term “solvate” refers to forms of the compound that are associated with a solvent, usually by a solvolysis reaction. This physical association may include hydrogen bonding. Conventional solvents include water, methanol, ethanol, acetic acid, DMSO, THF, diethyl ether, dichloromethane, and the like. The compounds of the invention may be prepared, *e.g.*, in crystalline form, and may be solvated. Suitable solvates include pharmaceutically acceptable solvates and further include both stoichiometric solvates and non-stoichiometric solvates. In certain instances, the solvate will be capable of isolation, for example, when one or more solvent molecules are incorporated in the crystal lattice of a crystalline solid. “Solvate” encompasses both solution-phase and isolable solvates. Representative solvates include hydrates, ethanolates, methanolates, and dichloromethane associates. In certain embodiments, the solvate is a compound as described herein with one to five solvent molecules incorporated in the crystal lattice of a crystalline solid. In certain embodiments, the solvate is Compound **8a** with one to five solvent molecules incorporated in the crystal lattice of a crystalline solid. In certain embodiments, the solvate is Compound **8a** with one to five dichloromethane molecules incorporated in the crystal lattice of a crystalline solid. In certain embodiments, the solvate is Compound **8a** with one dichloromethane molecule incorporated in the crystal lattice of a crystalline solid. In certain embodiments, the solvate is Compound **8a** with two dichloromethane molecules incorporated in the crystal lattice of a crystalline solid. In certain embodiments, the solvate is Compound **8a** with three dichloromethane molecules incorporated in the crystal lattice of a crystalline solid. In certain embodiments, the solvate is Compound **8a** with four dichloromethane molecules incorporated in the crystal lattice of a crystalline solid. In certain embodiments, the solvate is Compound **8a** with five dichloromethane molecules incorporated in the crystal lattice of a crystalline solid.

[0076] The term “hydrate” refers to a compound which is associated with water. Typically, the number of the water molecules contained in a hydrate of a compound is in a definite ratio to the number of the compound molecules in the hydrate. Therefore, a hydrate of a compound may be represented, for example, by the general formula $R \cdot xH_2O$, wherein R is the compound and wherein x is a number greater than 0. A given compound may form more than one type of hydrates, including, *e.g.*, monohydrates (x is 1), lower hydrates (x is a number greater than 0 and smaller than 1, *e.g.*, hemihydrates ($R \cdot 0.5H_2O$)), and polyhydrates (x is a number greater than 1, *e.g.*, dihydrates ($R \cdot 2H_2O$) and hexahydrates ($R \cdot 6H_2O$)).

[0077] The term “prodrug” refers to a first compound (*e.g.*, a compound of any one of Formulae (I)-(III)) that has cleavable group(s) and is converted by hydrolysis, oxidation, hydrolysis and oxidation, or other transformations to a second compound (*e.g.*, a compound of Formula (IV)) that is different from the first compound and is pharmaceutically active *in vivo*. Prodrugs include acid derivatives well known to practitioners of the art, such as, for example, esters prepared by reaction of the parent acid (*e.g.* carboxylic acid, boronic acid) with a suitable alcohol, or amides prepared by reaction of the parent acid compound with a substituted or unsubstituted amine. Simple aliphatic or aromatic esters, from acidic groups pendant on the compounds of this invention, are exemplary prodrugs and can be converted to the parent acids by hydrolysis. In some cases, it is desirable to prepare a double ester type prodrug such as an (acyloxy)alkyl ester or ((alkoxycarbonyl)oxy)alkyl ester. Prodrugs also include compounds whereby a phenolic hydroxyl group is synthetically masked by a boronic acid or ester. These prodrugs can be converted to the parent phenols by oxidation by reactive oxygen or nitrogen species. In certain embodiments, the prodrug is a double prodrug, which contains two different cleavable groups (*e.g.*, two different classes of cleavable groups), for example, a boronic acid and a carboxylate ester, or two boronic acids and two carboxylate esters. In certain embodiments, the boronic acids may be further derivatized to boronic esters by reaction with an alcohol, and these boronic esters may be converted by hydrolysis to the parent boronic acids.

[0078] The term “subject” refers to any animal. In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a human (*e.g.*, a man, a woman, or a child). The human may be of either sex and may be at any stage of development. In certain embodiments, the subject has been diagnosed with the condition or disease to be treated. In other embodiments, the subject is at risk of developing the condition or disease. In certain embodiments, the subject is an experimental animal (*e.g.*, mouse, rat, rabbit, dog, pig, or

primate). The experimental animal may be genetically engineered. In certain embodiments, the subject is a domesticated animal (*e.g.*, dog, cat, bird, horse, cow, goat, sheep).

[0079] The terms “administer,” “administering,” or “administration” refer to implanting, absorbing, ingesting, injecting, or inhaling an inventive compound, or a pharmaceutical composition thereof.

[0080] The terms “treatment,” “treat,” and “treating” refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a “pathological condition” (*e.g.*, a disease, disorder, or condition, or one or more signs or symptoms thereof) described herein. In some embodiments, treatment may be administered after one or more signs or symptoms have developed or have been observed. In other embodiments, treatment may be administered in the absence of signs or symptoms of the disease or condition. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (*e.g.*, in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example, to delay or prevent recurrence.

[0081] The terms “condition,” “disease,” and “disorder” are used interchangeably.

[0082] An “effective amount” of a compound of the present invention or a pharmaceutical composition thereof refers to an amount sufficient to elicit the desired biological response, *i.e.*, treating the condition. As will be appreciated by those of ordinary skill in this art, the effective amount of a compound of the invention may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of the compound, the condition being treated, the mode of administration, and the age and health of the subject. An effective amount encompasses therapeutically and prophylactically effective amounts.

[0083] A “therapeutically effective amount” of a compound of the present invention or a pharmaceutical composition thereof is an amount sufficient to provide a therapeutic benefit in the treatment of a condition, *e.g.*, iron overload, or to delay or minimize one or more symptoms associated with the condition. A therapeutically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment of the condition. The term “therapeutically effective amount” can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of the condition, and/or enhances the therapeutic efficacy of another therapeutic agent.

[0084] A “prophylactically effective amount” of a compound of the present invention is an amount sufficient to prevent a condition, *e.g.*, iron overload, or one or more symptoms associated with the condition or prevent its recurrence. A prophylactically effective amount

of a compound means an amount of a therapeutic agent, alone or in combination with other agents, which provides a prophylactic benefit in the prevention of the condition. The term “prophylactically effective amount” can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of another prophylactic agent.

[0085] “Chelation,” “chelating,” “sequestration,” or “sequestering” is the formation or presence of two or more separate coordinate bonds between a polydentate (multiple-bonded) compound and a single central atom. The polydentate compound is typically an organic compound and referred to as a “chelator,” “chelant,” “chelating agent,” “sequestrator,” “sequestering agent,” or “ligand.” The central atom is usually a metal atom or metal ion (*e.g.*, a metal atom or metal ion described herein, such as iron (*e.g.*, Fe(III)), Al(III), chromium (*e.g.*, Cr(VI)), and uranium (*e.g.*, U(VI)), *etc.*). The chelator may form a stable complex with the central atom through coordinate bonds, inactivating the central atom so that the central atom is less likely to react with other molecules or atoms.

[0086] The term “reactive oxygen species” or “ROS” refers to molecules or ions formed by the incomplete reduction of oxygen. Reactive oxygen species include superoxide anion ($O_2^{\bullet -}$), peroxides such as hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), and hypochlorous acid (HClO). These molecules are typically chemically reactive. Reactive oxygen species may be formed by any number of mechanisms (*e.g.*, enzymatically, by ionizing radiation, by reaction oxygen with a metal). In certain embodiments, the reactive oxygen species are formed by the reduction of oxygen by an iron ion, such as Fe^{+2} .

[0087] The term “reactive nitrogen species (RNS)” refers to molecules derived from nitric oxide (NO) and superoxide ($O_2^{\bullet -}$). In some embodiments, RNS are produced via the enzymatic activity of inducible nitric oxide synthase 2 (NOS2) and NADPH oxidase respectively. In certain embodiments, RNS is peroxynitrite.

[0088] The term “oxidative stress condition” as used herein may be any condition that can be treated by the compounds and methods of the invention (and including disorders attributable to iron and copper induced oxidative stress) including but not limited to cancer, neurodegenerative disease, cardiovascular disease, inflammatory disease, diabetes, ischemia, stroke, and iron chelation therapy subjects in general, *etc.* A subject in need of oxidative stress reduction can have one or more of the following conditions: decreased levels of reducing agents, increased levels of reactive oxygen species, mutations in or decreased levels of antioxidant enzymes (*e.g.*, Cu/Zn superoxide dismutase, Mn superoxide dismutase, glutathione reductase, glutathione peroxidase, thioredoxin, thioredoxin peroxidase, DT-diaphorase), mutations in or decreased levels of metal-binding proteins (*e.g.*, transferrin,

ferritin, ceruloplasmin, albumin, metallothionein), mutated or overactive enzymes capable of producing superoxide (*e.g.*, nitric oxide synthase, NADPH oxidases, xanthine oxidase, NADH oxidase, aldehyde oxidase, dihydroorotate dehydrogenase, cytochrome c oxidase), and radiation injury.

[0089] The term “cancer” refers to a class of diseases characterized by the development of abnormal cells that proliferate uncontrollably and have the ability to infiltrate and destroy normal body tissues. See, *e.g.*, *Stedman’s Medical Dictionary*, 25th ed.; Hensyl ed.; Williams & Wilkins: Philadelphia, 1990. Exemplary cancers include, but are not limited to, acoustic neuroma; adenocarcinoma; adrenal gland cancer; anal cancer; angiosarcoma (*e.g.*, lymphangiosarcoma, lymphangioendotheliosarcoma, hemangiosarcoma); appendix cancer; benign monoclonal gammopathy; biliary cancer (*e.g.*, cholangiocarcinoma); bladder cancer; breast cancer (*e.g.*, adenocarcinoma of the breast, papillary carcinoma of the breast, mammary cancer, medullary carcinoma of the breast); brain cancer (*e.g.*, meningioma, glioblastomas, glioma (*e.g.*, astrocytoma, oligodendroglioma, medulloblastoma); bronchus cancer; carcinoid tumor; cervical cancer (*e.g.*, cervical adenocarcinoma); choriocarcinoma; chordoma; craniopharyngioma; colorectal cancer (*e.g.*, colon cancer, rectal cancer, colorectal adenocarcinoma); connective tissue cancer; epithelial carcinoma; ependymoma; endotheliosarcoma (*e.g.*, Kaposi’s sarcoma, multiple idiopathic hemorrhagic sarcoma); endometrial cancer (*e.g.*, uterine cancer, uterine sarcoma); esophageal cancer (*e.g.*, adenocarcinoma of the esophagus, Barrett’s adenocarcinoma); Ewing’s sarcoma; ocular cancer (*e.g.*, intraocular melanoma, retinoblastoma); familial hypereosinophilia; gall bladder cancer; gastric cancer (*e.g.*, stomach adenocarcinoma); gastrointestinal stromal tumor (GIST); germ cell cancer; head and neck cancer (*e.g.*, head and neck squamous cell carcinoma, oral cancer (*e.g.*, oral squamous cell carcinoma), throat cancer (*e.g.*, laryngeal cancer, pharyngeal cancer, nasopharyngeal cancer, oropharyngeal cancer)); hematopoietic cancers (*e.g.*, leukemia such as acute lymphocytic leukemia (ALL) (*e.g.*, B-cell ALL, T-cell ALL), acute myelocytic leukemia (AML) (*e.g.*, B-cell AML, T-cell AML), chronic myelocytic leukemia (CML) (*e.g.*, B-cell CML, T-cell CML), and chronic lymphocytic leukemia (CLL) (*e.g.*, B-cell CLL, T-cell CLL)); lymphoma such as Hodgkin lymphoma (HL) (*e.g.*, B-cell HL, T-cell HL) and non-Hodgkin lymphoma (NHL) (*e.g.*, B-cell NHL such as diffuse large cell lymphoma (DLCL) (*e.g.*, diffuse large B-cell lymphoma), follicular lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), mantle cell lymphoma (MCL), marginal zone B-cell lymphomas (*e.g.*, mucosa-associated lymphoid tissue (MALT) lymphomas, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell

lymphoma), primary mediastinal B-cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma (*i.e.*, Waldenström's macroglobulinemia), hairy cell leukemia (HCL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma and primary central nervous system (CNS) lymphoma; and T-cell NHL such as precursor T-lymphoblastic lymphoma/leukemia, peripheral T-cell lymphoma (PTCL) (*e.g.*, cutaneous T-cell lymphoma (CTCL) (*e.g.*, mycosis fungoides, Sezary syndrome), angioimmunoblastic T-cell lymphoma, extranodal natural killer T-cell lymphoma, enteropathy type T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, and anaplastic large cell lymphoma); a mixture of one or more leukemia/lymphoma as described above; and multiple myeloma (MM)), heavy chain disease (*e.g.*, alpha chain disease, gamma chain disease, mu chain disease); hemangioblastoma; hypopharynx cancer; inflammatory myofibroblastic tumors; immunocytic amyloidosis; kidney cancer (*e.g.*, nephroblastoma *a.k.a.* Wilms' tumor, renal cell carcinoma); liver cancer (*e.g.*, hepatocellular cancer (HCC), malignant hepatoma); lung cancer (*e.g.*, bronchogenic carcinoma, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), adenocarcinoma of the lung); leiomyosarcoma (LMS); mastocytosis (*e.g.*, systemic mastocytosis); muscle cancer; myelodysplastic syndrome (MDS); mesothelioma; myeloproliferative disorder (MPD) (*e.g.*, polycythemia vera (PV), essential thrombocytosis (ET), agnogenic myeloid metaplasia (AMM) *a.k.a.* myelofibrosis (MF), chronic idiopathic myelofibrosis, chronic myelocytic leukemia (CML), chronic neutrophilic leukemia (CNL), hypereosinophilic syndrome (HES)); neuroblastoma; neurofibroma (*e.g.*, neurofibromatosis (NF) type 1 or type 2, schwannomatosis); neuroendocrine cancer (*e.g.*, gastroenteropancreatic neuroendocrine tumor (GEP-NET), carcinoid tumor); osteosarcoma (*e.g.*, bone cancer); ovarian cancer (*e.g.*, cystadenocarcinoma, ovarian embryonal carcinoma, ovarian adenocarcinoma); papillary adenocarcinoma; pancreatic cancer (*e.g.*, pancreatic adenocarcinoma, intraductal papillary mucinous neoplasm (IPMN), Islet cell tumors); penile cancer (*e.g.*, Paget's disease of the penis and scrotum); pinealoma; primitive neuroectodermal tumor (PNT); plasma cell neoplasia; paraneoplastic syndromes; intraepithelial neoplasms; prostate cancer (*e.g.*, prostate adenocarcinoma); rectal cancer; rhabdomyosarcoma; salivary gland cancer; skin cancer (*e.g.*, squamous cell carcinoma (SCC), keratoacanthoma (KA), melanoma, basal cell carcinoma (BCC)); small bowel cancer (*e.g.*, appendix cancer); soft tissue sarcoma (*e.g.*, malignant fibrous histiocytoma (MFH), liposarcoma, malignant peripheral nerve sheath tumor (MPNST), chondrosarcoma, fibrosarcoma, myxosarcoma); sebaceous gland carcinoma; small intestine cancer; sweat gland carcinoma; synovioma; testicular cancer (*e.g.*, seminoma, testicular embryonal carcinoma); thyroid cancer (*e.g.*,

papillary carcinoma of the thyroid, papillary thyroid carcinoma (PTC), medullary thyroid cancer); urethral cancer; vaginal cancer; and vulvar cancer (*e.g.*, Paget's disease of the vulva). In certain embodiments, the cancer is skin cancer, lung cancer, colon cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, liver cancer, leukemia, or lymphoma.

[0090] The term “neurological disease” refers to any disease of the nervous system, including diseases that involve the central nervous system (brain, brainstem and cerebellum), the peripheral nervous system (including cranial nerves), and the autonomic nervous system (parts of which are located in both central and peripheral nervous system). Neurodegenerative diseases refer to a type of neurological disease marked by the loss of nerve cells, including, but not limited to, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, tauopathies (including frontotemporal dementia), and Huntington's disease. Examples of neurological diseases include, but are not limited to, headache, stupor and coma, dementia, seizure, sleep disorders, trauma, infections, neoplasms, neuro-ophthalmology, movement disorders, demyelinating diseases, spinal cord disorders, and disorders of peripheral nerves, muscle and neuromuscular junctions. Addiction and mental illness, include, but are not limited to, bipolar disorder and schizophrenia, are also included in the definition of neurological diseases. Further examples of neurological diseases include acquired epileptiform aphasia; acute disseminated encephalomyelitis; adrenoleukodystrophy; agenesis of the corpus callosum; agnosia; Aicardi syndrome; Alexander disease; Alpers' disease; alternating hemiplegia; Alzheimer's disease; amyotrophic lateral sclerosis; anencephaly; Angelman syndrome; angiomas; anoxia; aphasia; apraxia; arachnoid cysts; arachnoiditis; Arnold-Chiari malformation; arteriovenous malformation; Asperger syndrome; ataxia telangiectasia; attention deficit hyperactivity disorder; autism; autonomic dysfunction; back pain; Batten disease; Behcet's disease; Bell's palsy; benign essential blepharospasm; benign focal; amyotrophy; benign intracranial hypertension; Binswanger's disease; blepharospasm; Bloch Sulzberger syndrome; brachial plexus injury; brain abscess; brain injury; brain tumors (including glioblastoma multiforme); spinal tumor; Brown-Sequard syndrome; Canavan disease; carpal tunnel syndrome (CTS); causalgia; central pain syndrome; central pontine myelinolysis; cephalic disorder; cerebral aneurysm; cerebral arteriosclerosis; cerebral atrophy; cerebral gigantism; cerebral palsy; Charcot-Marie-Tooth disease; chemotherapy-induced neuropathy and neuropathic pain; Chiari malformation; chorea; chronic inflammatory demyelinating polyneuropathy (CIDP); chronic pain; chronic regional pain syndrome; Coffin Lowry syndrome; coma, including persistent vegetative state; congenital

facial diplegia; corticobasal degeneration; cranial arteritis; craniosynostosis; Creutzfeldt-Jakob disease; cumulative trauma disorders; Cushing's syndrome; cytomegalic inclusion body disease (CIBD); cytomegalovirus infection; dancing eyes-dancing feet syndrome; Dandy-Walker syndrome; Dawson disease; De Morsier's syndrome; Dejerine-Klumpke palsy; dementia; dermatomyositis; diabetic neuropathy; diffuse sclerosis; dysautonomia; dysgraphia; dyslexia; dystonias; early infantile epileptic encephalopathy; empty sella syndrome; encephalitis; encephaloceles; encephalotrigeminal angiomas; epilepsy; Erb's palsy; essential tremor; Fabry's disease; Fahr's syndrome; fainting; familial spastic paralysis; febrile seizures; Fisher syndrome; Friedreich's ataxia; frontotemporal dementia and other "tauopathies"; Gaucher's disease; Gerstmann's syndrome; giant cell arteritis; giant cell inclusion disease; globoid cell leukodystrophy; Guillain-Barre syndrome; HTLV-1 associated myelopathy; Hallervorden-Spatz disease; head injury; headache; hemifacial spasm; hereditary spastic paraplegia; hereditary ataxia polyneuritis; herpes zoster oticus; herpes zoster; Hirayama syndrome; HIV-associated dementia and neuropathy (see also neurological manifestations of AIDS); holoprosencephaly; Huntington's disease and other polyglutamine repeat diseases; hydranencephaly; hydrocephalus; hypercortisolism; hypoxia; immune-mediated encephalomyelitis; inclusion body myositis; incontinentia pigmenti; infantile; phytanic acid storage disease; Infantile Refsum disease; infantile spasms; inflammatory myopathy; intracranial cyst; intracranial hypertension; Joubert syndrome; Kearns-Sayre syndrome; Kennedy disease; Kinsbourne syndrome; Klippel Feil syndrome; Krabbe disease; Kugelberg-Welander disease; kuru; Lafora disease; Lambert-Eaton myasthenic syndrome; Landau-Kleffner syndrome; lateral medullary (Wallenberg) syndrome; learning disabilities; Leigh's disease; Lennox-Gastaut syndrome; Lesch-Nyhan syndrome; leukodystrophy; Lewy body dementia; lissencephaly; locked-in syndrome; Lou Gehrig's disease (aka motor neuron disease or amyotrophic lateral sclerosis); lumbar disc disease; Lyme disease-neurological sequelae; Machado-Joseph disease; macrencephaly; megalencephaly; Melkersson-Rosenthal syndrome; Meniere's disease; meningitis; Menkes disease; metachromatic leukodystrophy; microcephaly; migraine; Miller Fisher syndrome; mini-strokes; mitochondrial myopathies; Mobius syndrome; monomelic amyotrophy; motor neuron disease; moyamoya disease; mucopolysaccharidoses; multi-infarct dementia; multifocal motor neuropathy; multiple sclerosis and other demyelinating disorders; multiple system atrophy with postural hypotension; muscular dystrophy; myasthenia gravis; myelinoclastic diffuse sclerosis; myoclonic encephalopathy of infants; myoclonus; myopathy; myotonia congenital; narcolepsy; neurofibromatosis; neuroleptic malignant syndrome; neurological manifestations

of AIDS; neurological sequelae of lupus; neuromyotonia; neuronal ceroid lipofuscinosis; neuronal migration disorders; Niemann-Pick disease; O'Sullivan-McLeod syndrome; occipital neuralgia; occult spinal dysraphism sequence; Ohtahara syndrome; olivopontocerebellar atrophy; opsoclonus myoclonus; optic neuritis; orthostatic hypotension; overuse syndrome; paresthesia; Parkinson's disease; paramyotonia congenita; paraneoplastic diseases; paroxysmal attacks; Parry Romberg syndrome; Pelizaeus-Merzbacher disease; periodic paralyses; peripheral neuropathy; painful neuropathy and neuropathic pain; persistent vegetative state; pervasive developmental disorders; photic sneeze reflex; phytanic acid storage disease; Pick's disease; pinched nerve; pituitary tumors; polymyositis; porencephaly; Post-Polio syndrome; postherpetic neuralgia (PHN); postinfectious encephalomyelitis; postural hypotension; Prader-Willi syndrome; primary lateral sclerosis; prion diseases; progressive; hemifacial atrophy; progressive multifocal leukoencephalopathy; progressive sclerosing poliodystrophy; progressive supranuclear palsy; pseudotumor cerebri; Ramsay-Hunt syndrome (Type I and Type II); Rasmussen's Encephalitis; reflex sympathetic dystrophy syndrome; Refsum disease; repetitive motion disorders; repetitive stress injuries; restless legs syndrome; retrovirus-associated myelopathy; Rett syndrome; Reye's syndrome; Saint Vitus Dance; Sandhoff disease; Schilder's disease; schizencephaly; septo-optic dysplasia; shaken baby syndrome; shingles; Shy-Drager syndrome; Sjogren's syndrome; sleep apnea; Soto's syndrome; spasticity; spina bifida; spinal cord injury; spinal cord tumors; spinal muscular atrophy; stiff-person syndrome; stroke; Sturge-Weber syndrome; subacute sclerosing panencephalitis; subarachnoid hemorrhage; subcortical arteriosclerotic encephalopathy; sydenham chorea; syncope; syringomyelia; tardive dyskinesia; Tay-Sachs disease; temporal arteritis; tethered spinal cord syndrome; Thomsen disease; thoracic outlet syndrome; tic douloureux; Todd's paralysis; Tourette syndrome; transient ischemic attack; transmissible spongiform encephalopathies; transverse myelitis; traumatic brain injury; tremor; trigeminal neuralgia; tropical spastic paraparesis; tuberous sclerosis; vascular dementia (multi-infarct dementia); vasculitis including temporal arteritis; Von Hippel-Lindau Disease (VHL); Wallenberg's syndrome; Werdnig-Hoffman disease; West syndrome; whiplash; Williams syndrome; Wilson's disease; and Zellweger syndrome. In certain embodiments, the neurodegenerative disease is Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Friedreich's ataxia, or neurodegeneration with brain iron accumulation (NBIA).

[0091] The term “neurodegeneration with brain iron accumulation” or “NBIA” refers to a group of inherited neurologic disorders characterized by abnormal accumulation of iron

in the basal ganglia. NBIA includes, but is not limited to, pantothenate kinase-associated neurodegeneration (NBIA1), neuroferritinopathy, and aceruloplasminemia.

[0092] The term “macular degeneration” refers to a disease that affects the retina of a subject. In macular degeneration, cells in the macular region begin to die, which results in blind spots and distorted vision. In certain embodiments, macular degeneration is dry macular degeneration. In certain embodiments, macular degeneration is wet macular degeneration. Dry macular degeneration occurs when the photosensitive cells of the macula slowly break down and drusen form and accumulate under the retina between the retinal pigmented epithelium (RPE) layer and the Bruch’s membrane. Dry macular degeneration is usually accompanied by a blurring or spotty loss of clear, straight-ahead vision. In certain embodiments, dry macular degeneration may advance and cause loss of vision without turning into the wet form of the disease. In certain embodiments, dry macular degeneration may change into the wet form of macular degeneration. Wet macular degeneration occurs when abnormal blood vessels grow behind the macula as RPE and photoreceptor cells die. The Bruch’s membrane begins to break down, usually near drusen deposits, and new blood vessels grow. These vessels can leak fluid and blood, resulting scarring of and severe damage to the macula.

[0093] The term “photoaging” refers to premature aging of the skin caused by repeated exposure to ultraviolet radiation (UVR) from the sun and/or artificial UV sources. When skin is directly exposed to UVR, cutaneous intracellular labile iron levels increase because of the release of iron from iron-binding proteins (*e.g.* ferritin). This released iron can catalyze ROS generation, causing cutaneous damage.

DETAILED DESCRIPTION OF THE DRAWINGS

[0094] *Figure 1* shows exemplary synthesis of compounds **7a-f**, their corresponding dimesylate salts **8a-c,e**, and compound **9**.

[0095] *Figure 2* provides the crystal structure of the dimesylate salt of N,N’-bis-(2-boronic acid pinacol ester benzyl)ethylenediamine-N,N’-diacetic acid methyl ester (**8a**), shown as a stick diagram.

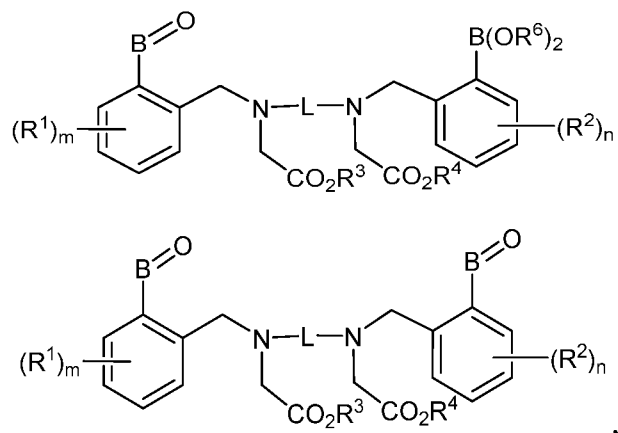
[0096] *Figure 3* shows the ¹H NMR spectrum of compound **9** in DMSO-d₆. Two diastereomers were distinguishable.

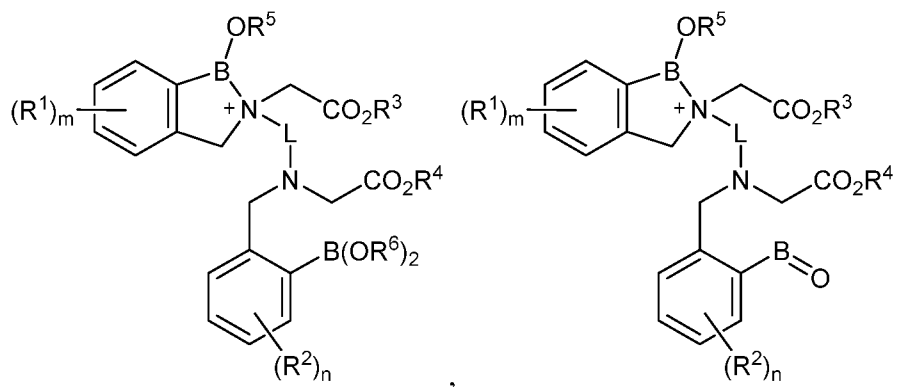
[0097] *Figure 4* shows the expected scheme for the hydrolysis of prodrugs **7a-c** to compound **9** (shown in both open and coordinated forms). Four rate constants may be measured. At physiological pH, the carboxylate anion is expected to predominate. The

amino nitrogens may or may not be protonated. The boron atoms may or may not be coordinated to the amino nitrogens and/or to the carboxylate esters during the hydrolysis.

[0098] *Figure 5* shows a table containing the ions ($M + H$) observed by LC-MS during the hydrolysis of N,N'-bis(2-boronic acid pinacol ester benzyl)ethylenediamine-N,N'-diacetic acid methyl ester dimesylate (**8a**) at room temperature in both CH_3CN/H_2O (50:50) and a 25:75 solution of MeOH : pH 7.4 N-methylmorpholine buffer.

[0099] *Figure 6* shows plots pertaining to the LC-MS-observed hydrolysis of Compound **8a** in a 25:75 solution of MeOH : pH 7.41 50 mM N-methylmorpholine buffer. Over the course of 42 hours, m/z 455.2 and 437.2, both dehydrated species of N,N'-bis(2-boronic acid benzyl)ethylenediamine-N,N'-diacetic acid methyl ester (see *Figure 5* for corresponding structures), decreased in a first order manner (top left graph). Plots of the natural log of peak integration vs. time for both ions yielded straight lines with the slopes of the linear fits equal to k_3 , the rate constant for the hydrolysis of the first carboxylate ester (top right graph). The average of these two values ($k_3 = 1.37 \times 10^{-3} \text{ min}^{-1}$) corresponds to a half-life of 8.4 hours. During the hydrolysis, the intermediate “mono” ions, m/z 441.2 and 423.2 (see *Figure 5* for corresponding structures), were observed to form from the hydrolysis of the first carboxylate ester and then subsequently decay as the second carboxylate ester was hydrolyzed (bottom left graph). Only the dehydrated species were detected. It is to be understood that the dehydrated species may be of one of the following formulae:





or a salt thereof.

[00100] Hydrolysis of the second carboxylate ester yielded m/z 409.3 (see Figure 5 for structure), corresponding to compound **9**. The plot of its appearance was slightly s-shaped (bottom right graph). By using k_3 and $t_{\max}$, which is the time necessary to reach the maximum peak area of the mono intermediates, k_4 was able to be solved ($k_4 = 1.22 \times 10^{-3} \text{ min}^{-1}$). The rate constant for the hydrolysis of the second ester, k_4 , corresponds to a half-life of 9.5 hours. There is little difference between the rates of hydrolysis of the first and second carboxylate esters.

[00101] Figures 7A-7B show a representative example of the hydrolysis of prodrugs **8a-c** in MEM at 37 °C, monitored by UV spectrophotometry. In Figure 7A, the absorbance at 270 nm (and 277 nm) decreases over the course of the hydrolysis of 150 μM **8a** in MEM. In Figure 7B, the final spectrum of **8a** ($t = 15 \text{ h}$) matches the spectrum of an independently-prepared sample of **9**, the product of complete hydrolysis. Both spectra have a $\lambda_{\max}$ at 263 nm, while **8a** at time 0 had a $\lambda_{\max}$ at 270 nm.

[00102] Figure 8 shows pseudo first-order rate constants (k_{obs}) and half-lives ($t_{1/2}$) of **8a-c** in 50% MeOH/50% 30 mM pH 7.5 phosphate buffer (PB) and in minimum essential medium (MEM) at 37 °C. All hydrolyses were performed in triplicate.

[00103] Figure 9 shows the reaction scheme for the oxidation of compound **9** to HBED under pseudo first-order conditions of excess H_2O_2 .

[00104] Figure 10 shows a representative plot of the oxidation of **9** (m/z 409.3) in pH 7.4 N-methylmorpholine buffer using excess H_2O_2 , as monitored by LC-MS. As **9** disappears, a mono-oxidized intermediate m/z 399.4 forms and then is further oxidized to HBED (m/z 389.3). Structures for the molecular ions can be found in Figure 9.

[00105] Figure 11 includes UV spectra showing a representative example of the oxidation of a hydrolyzed solution of **8a** in MEM at 37 °C (solid lines). Here, a solution of **8a** post-hydrolysis (17 h), verified by UV to be **9**, was reacted with 200x excess H_2O_2 . The

spectrum of the oxidized product matched that of HBED in MEM containing 200x excess H_2O_2 (dotted line).

[00106] *Figure 12* is a plot of k_{obs} versus H_2O_2 concentration. The slope of the linear fit gives the second-order rate constant ($k = 1.82 \text{ M}^{-1} \text{ min}^{-1}$) for the overall oxidation of the product of **8a** hydrolysis (*i.e.*, **9**) to HBED in MEM at 37 °C, as monitored by UV.

[00107] *Figure 13* shows the reaction scheme for the H_2O_2 oxidation of prodrug **8a** to dimethyl HBED dimesylate in DMSO-d_6 . The reaction, followed by NMR, was complete in ~2 h.

[00108] *Figure 14* shows ^1H NMR spectra of prodrug **8a** in DMSO-d_6 before (blue peaks) and after (maroon peaks) the addition of 10x excess H_2O_2 . Over the course of the reaction (~2 h), the aromatic peaks shifted upfield and the boronic acid pinacol ester singlet at 1.30 ppm disappeared, suggesting that the aryl boronic acid pinacol esters of **8a** were oxidized to give the phenolic hydroxyl groups of dimethyl HBED dimesylate salt.

[00109] *Figure 15* shows UV/Vis spectra of HBED, compound **9**, EDTA, HBED + **9**, and HBED + EDTA with 30 μM ferric ammonium citrate (FAC) in pH 7.5 phosphate buffer at 23 °C. After a 22 h equilibration period, a 3.3-fold excess of **9** (300 μM) was unable to compete with HBED (90 μM) for binding to iron. In contrast, a 3.3-fold excess of EDTA (300 μM) decreased the amount of Fe-HBED chelate by 83%, as evidenced by a decrease in absorbance at 481 nm.

[00110] *Figure 16* shows UV/Vis spectra of 300 μM **9**, 100 μM EDTA, and 300 μM **9** + EDTA (various concentrations) in 25:75 MeOH/pH 7.5 phosphate buffer at 23 °C with 100 μM copper (II) sulfate pentahydrate. The solutions were equilibrated for 24 h. The absorbance at 410 nm suggests that **9** weakly interacts with copper. However, addition of 20, 60, and 100 μM EDTA decreases the A_{410} by ~17%, 57%, and 94%, respectively, indicating that **9**, even in excess, does not compete with EDTA for copper chelation.

[00111] *Figures 17A-17B* show the protection of ARPE-19 cells against H_2O_2 -induced death by prodrugs **8a-c**, **9**, and HBED. Proliferating cells were pretreated with compounds for 15 h in MEM containing FBS, and then challenged with 300 μM (*Figure 17A*) or 500 μM (*Figure 17B*) H_2O_2 for 8 h. Cell viability was determined by crystal violet assay.

[00112] *Figure 18* shows the viability of proliferating ARPE-19 cells exposed to the indicated concentrations of HBED, **8b**, and **8c** for 24 h, as determined by crystal violet assay.

[00113] *Figure 19* shows exemplary activation pathways of compounds **7** and **9**.

[00114] *Figure 20* shows an activation pathway for the HBED double prodrugs.

[00115] *Figure 21* shows the coordination of the designated nitrogen atom of **9** to boron when the boronic acid is either in the plane of or perpendicular to the phenyl ring. Coordination while the boronic acid is in the plane of the phenyl ring is unfavorable due to steric interactions.

[00116] *Figure 22* shows UV spectra acquired during the hydrolysis of double prodrug **8a** at 37 °C in pH 7.4 phosphate buffer containing 50% MeOH. Over the course of the hydrolysis, the absorbance at 270/277 nm decreased (solid lines). The final spectrum acquired matched the spectrum of an authentic sample of prodrug **9** (dotted line).

[00117] *Figure 23* shows the ^{13}C NMR spectrum of **9** in DMSO-*d*₆.

[00118] *Figure 24* shows the ^{11}B NMR spectrum of **9** in DMSO-*d*₆.

[00119] *Figure 25* shows the cell viability of ARPE-19 cells after exposure to double prodrugs **8a-c**, prodrug **9**, or HBED. After cells were grown to 100% confluence in growth medium (DMEM:F12 with 10% FBS), the medium was removed and the cells were treated with various concentrations of prodrug or HBED in MEM for 23 h. Percent cell viability was measured using an MTT assay and is presented as the mean $\pm$ 1 standard deviation ($n = 6$) for each treatment.

[00120] *Figure 26* shows the effect of DMSO on the viability of ARPE-19 cells. DMSO (0.15-1.5% by vol) in MEM was applied to confluent cells. Percent cell viability was measured after 23 h using an MTT assay, and is presented as the mean $\pm$ 1 standard deviation for each DMSO treatment ($n = 6$). Statistical significance relative to the negative control where cells received no DMSO is indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

[00121] *Figure 27* shows a schematic of a Franz diffusion cell.

[00122] *Figure 28* shows the H₂O₂ kill curve for ARPE-19 cells. After cells were grown to 100% confluence in growth medium (DMEM:F12 with 10% FBS), the medium was removed, and MEM was applied. After 15 h, various concentrations of H₂O₂ were added to the wells, and the plates were incubated for another 8 h. Cell viability was determined by an MTT assay, and is reported as the average of triplicate wells $\pm$ 1 standard deviation for each concentration of H₂O₂ applied.

[00123] *Figure 29* shows the protection of ARPE-19 cells against 500 μM H₂O₂ by double prodrugs **8a-c**, prodrug **9**, and HBED. After cells were grown to 100% confluence in growth medium (DMEM:F12 with 10% FBS), the medium was removed and the cells were pretreated with various concentrations of prodrug or HBED in MEM for 15 h. This was followed by an 8 h challenge with 500 μM H₂O₂. Percent protection was calculated from the

results of an MTT cell viability assay and is reported as the mean $\pm$ 1 standard deviation (n = 6) for each concentration of drug applied.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

[00124] Excess iron and oxidative stress have been implicated in the etiology and pathogenesis of a variety of diseases and conditions (for reviews, see Jomova and Valko, 2011; Fleming and Ponka, 2012). Systemic iron overload can be caused by a genetic mutation, as is the case in hereditary hemochromatosis, or can be a consequence of required blood transfusions, as seen in β -thalassemia, aplastic anemia, sickle cell anemia, myelodysplasia, and Diamond-Blackfan anemia. Diseases associated with a more focal accumulation of labile iron include neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, and neurodegeneration with brain iron accumulation; age-related macular degeneration; and skin photoaging. Subjects with these conditions may benefit from iron chelation therapy. Iron chelators may also be useful for treating cancer, diabetes, inflammatory disorders, cardiovascular diseases, anthracycline cardiotoxicity, and viral infections (Pace and Leaf, 1995; van Asbeck *et al.*, 2001; Mandas *et al.*, 2009).

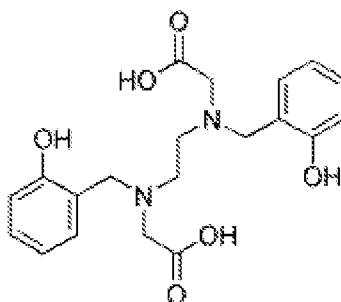
[00125] HBED is a strong, hexadentate chelator that has shown limited effectiveness as an orally-active iron chelator in primates and humans due to its poor physicochemical properties. Therefore, prodrugs of HBED with more desirable properties (*e.g.*, optimal or sufficient oral uptake, good transport properties through cell membranes including the blood brain barrier, ability to convert to the parent compound chemically and/or enzymatically, etc.) are needed for use in the treatment and/or prevention of diseases associated with oxidative stress and iron overload. Additionally, for diseases where there is a localized accumulation of excess iron or a localized need for chelation, it would be beneficial for a prodrug to preferentially convert to HBED only at those sites where chelation is most needed, thereby minimizing the risk of sequestering iron and other metals that are necessary for normal physiological processes.

[00126] Provided herein are compounds, compositions, kits, and methods for treating a pathological condition in a subject. In certain embodiments, the pathological condition is responsive to the chelation or sequestration of a metal. In certain embodiments, the metal is iron (*e.g.*, Fe(III)). In certain embodiments, the pathological condition is metal overload (*e.g.*, iron overload). In certain embodiments, the pathological condition is metal poisoning (*e.g.*, iron poisoning). In certain embodiments, the pathological condition is oxidative stress,

transfusional iron overload, thalassemia, primary hemochromatosis, secondary hemochromatosis, diabetes, liver disease, heart disease, cancer, radiation injury, neurological or neurodegenerative disorder (*e.g.*, Parkinson's disease, Alzheimer's disease, neurodegeneration with brain iron accumulation, and Friedreich's ataxia), macular degeneration, closed head injury, irritable bowel disease, stroke, and reperfusion injury. In certain embodiments, the pathological condition is an infectious disease (*e.g.*, HIV and malaria). In certain embodiments, the pathological condition is aging. In certain embodiments, provided herein are compounds, compositions, kits, and methods for treating a pathological condition with HBED prodrugs such as a compound of any one of Formulae (I)-(III). In certain embodiments, the provided HBED prodrugs are HBED prodrugs with one or both of the phenolic hydroxyl groups masked as boronic acids or boronic esters. In certain embodiments, the provided HBED prodrugs are HBED prodrugs with one or both of the phenolic hydroxyl groups masked as boronic acids or boronic esters, and one or both of the carboxylic acids masked as carboxylate esters. The provided compounds can be converted to HBED *in situ* by hydrolysis and/or oxidation. Without wishing to be bound by any particular theory, the compounds of any one of Formulae (I)-(III) have little or low affinity to chelate iron. Upon hydrolysis and/or oxidation, the compounds of Formulae (I)-(III) have strong affinity to chelate iron and prevent it from participating in the generation of reactive oxygen species. Upon hydrolysis and/or oxidation, the compounds of Formulae (I)-(III) may act as free radical scavengers thereby limiting the damage of reactive oxygen species or other radicals.

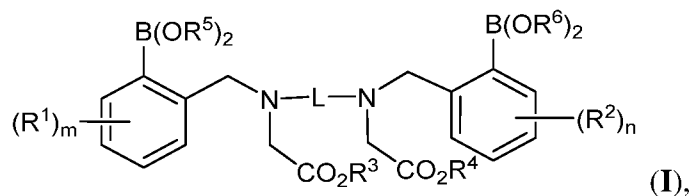
Compounds

[00127] N,N'-bis(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED) is a compound known to be an iron chelator and useful as a source of iron in plant nutrition (see U.S Patent No. 3,758,540).



Structure of HBED

[00128] In one aspect of the present invention, provided are compounds of Formula (I):



and pharmaceutically or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, or polymorphs thereof, wherein:

each of m and n is independently 0, 1, 2, 3, or 4;

each instance of R^1 and R^2 is independently hydrogen, halogen, -CN, -NO₂, -N₃, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, optionally substituted amino group, or optionally substituted acyl;

each of R^3 and R^4 is independently hydrogen, optionally substituted alkyl, or an oxygen protecting group;

each instance of R^5 and R^6 is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group; or two R^5 are taken together with the intervening atoms to form optionally substituted heterocyclyl; or two R^6 are taken together with the intervening atoms to form optionally substituted heterocyclyl; and

L is optionally substituted C₁₋₈ alkylene.

[00129] In the compounds of Formula (I), each instance of R^5 is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group; or two R^5 are taken together with the intervening atoms to form optionally substituted heterocyclyl. In certain embodiments, each instance of R^5 is different. In certain embodiments, both instances of R^5 are the same. In certain embodiments, both instances of R^5 are hydrogen. In certain embodiments, each instance of R^5 is independently optionally substituted alkyl. In certain embodiments, each instance of R^5 is independently optionally substituted C₁₋₆ alkyl. In certain embodiments, each instance of R^5 is independently unsubstituted C₁₋₆ alkyl (*e.g.*, methyl or ethyl). In certain embodiments, each instance of R^5 is independently substituted C₁₋

₆ alkyl. In certain embodiments, each instance of R⁵ is independently an oxygen protecting group. In certain embodiments, one instance of R⁵ is hydrogen. In certain embodiments, one instance of R⁵ is optionally substituted alkyl. In certain embodiments, one instance of R⁵ is optionally substituted C₁₋₆ alkyl. In certain embodiments, one instance of R⁵ is unsubstituted C₁₋₆ alkyl (*e.g.*, methyl or ethyl). In certain embodiments, one instance of R⁵ is substituted C₁₋₆ alkyl. In certain embodiments, one instance of R⁵ is an oxygen protecting group. In certain embodiments, one instance of R⁵ is hydrogen and one instance of R⁵ is optionally substituted alkyl. In certain embodiments, one instance of R⁵ is hydrogen and one instance of R⁵ is an oxygen protecting group. In certain embodiments, one instance of R⁵ is unsubstituted alkyl (*e.g.*, methyl or ethyl) and one instance of R⁵ is substituted alkyl. In certain embodiments, two R⁵ are taken together with the intervening atoms to form an optionally substituted heterocyclyl ring. In certain embodiments, two R⁵ are taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclyl ring. In certain embodiments, two R⁵ are taken together with the intervening atoms to form an optionally substituted 6-membered heterocyclyl ring.

[00130] In the compounds of Formula (I), each instance of R⁶ is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group; or two R⁶ are taken together with the intervening atoms to form an optionally substituted heterocyclyl moiety. In certain embodiments, each instance of R⁶ is different. In certain embodiments, both instances of R⁶ are the same. In certain embodiments, both instances of R⁶ are hydrogen. In certain embodiments, each instance of R⁶ is independently optionally substituted alkyl. In certain embodiments, each instance of R⁶ is independently optionally substituted C₁₋₆ alkyl. In certain embodiments, each instance of R⁶ is independently unsubstituted C₁₋₆ alkyl (*e.g.*, methyl or ethyl). In certain embodiments, each instance of R⁶ is independently substituted C₁₋₆ alkyl. In certain embodiments, each instance of R⁶ is independently an oxygen protecting group. In certain embodiments, one instance of R⁶ is hydrogen. In certain embodiments, one instance of R⁶ is optionally substituted alkyl. In certain embodiments, one instance of R⁶ is optionally substituted C₁₋₆ alkyl. In certain embodiments, one instance of R⁶ is unsubstituted C₁₋₆ alkyl (*e.g.*, methyl or ethyl). In certain embodiments, one instance of R⁶ is substituted C₁₋₆ alkyl. In certain embodiments, one instance of R⁶ is an oxygen protecting group. In certain embodiments, one instance of R⁶ is hydrogen and one instance of R⁶ is optionally substituted alkyl. In certain embodiments, one instance of R⁶ is hydrogen and one instance of R⁶ is an

oxygen protecting group. In certain embodiments, one instance of R^6 is unsubstituted alkyl (*e.g.*, methyl or ethyl) and one instance of R^6 is substituted alkyl. In certain embodiments, two R^6 are taken together with the intervening atoms to form an optionally substituted heterocyclic ring. In certain embodiments, two R^6 are taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring. In certain embodiments, two R^6 are taken together with the intervening atoms to form an optionally substituted 6-membered heterocyclic ring.

[00131] In the compounds of Formula (I), R^5 and R^6 are different. In the compounds of Formula (I), R^5 and R^6 are the same. In the compounds of Formula (I), all instances of R^5 and R^6 are hydrogen. In certain embodiments, both instances of R^5 are hydrogen, and each instance of R^6 is independently optionally substituted alkyl. In certain embodiments, both instances of R^5 are hydrogen, and each instance of R^6 is independently optionally substituted C_{1-6} alkyl. In certain embodiments, both instances of R^5 are hydrogen, and each instance of R^6 is independently unsubstituted C_{1-6} alkyl (*e.g.*, methyl or ethyl). In certain embodiments, both instances of R^5 are hydrogen, and each instance of R^6 is independently substituted C_{1-6} alkyl. In certain embodiments, both instances of R^5 are hydrogen, and each instance of R^6 is independently an oxygen protecting group. In certain embodiments, both instances of R^5 are hydrogen and two R^6 are taken together with the intervening atoms to form an optionally substituted heterocyclic ring. In certain embodiments, both instances of R^5 are hydrogen, and two R^6 are taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring. In certain embodiments, both instances of R^5 are hydrogen, and two R^6 are taken together with the intervening atoms to form an optionally substituted 6-membered heterocyclic ring. In certain embodiments, each instance of R^5 and R^6 is independently optionally substituted alkyl. In certain embodiments, each instance of R^5 and R^6 is independently optionally substituted C_{1-6} alkyl. In certain embodiments, each instance of R^5 and R^6 is independently unsubstituted C_{1-6} alkyl (*e.g.*, methyl or ethyl). In certain embodiments, each instance of R^5 and R^6 is independently substituted C_{1-6} alkyl. In certain embodiments, each instance of R^5 and R^6 is independently an oxygen protecting group. In certain embodiments, each instance of R^5 and R^6 are taken together with the intervening atoms to form an optionally substituted heterocyclic ring. In certain embodiments, each instance of R^5 is taken together with the intervening atoms to form an optionally substituted heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted heterocyclic ring. In certain embodiments, each instance of R^5 and R^6 are taken together with the intervening atoms to form an optionally substituted 5-

membered heterocyclic ring. In certain embodiments, each instance of R^5 is taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring. In certain embodiments, each instances of R^5 and R^6 are taken together with the intervening atoms to form an optionally substituted 6-membered heterocyclic ring. In certain embodiments, each instance of R^5 is taken together with the intervening atoms to form an optionally substituted 6-membered heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted 6-membered heterocyclic ring.

[00132] In the compounds of Formula (I), R^3 is hydrogen, optionally substituted alkyl, or an oxygen protecting group. In certain embodiments, R^3 is hydrogen. In certain embodiments, R^3 is optionally substituted alkyl. In certain embodiments, R^3 is optionally substituted C_{1-6} alkyl. In certain embodiments, R^3 is unsubstituted C_{1-6} alkyl. In certain embodiments, R^3 is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl. In certain embodiments, R^3 is substituted C_{1-6} alkyl. In certain embodiments, R^3 is optionally substituted heteroarylalkyl, optionally substituted heterocyclylalkyl, optionally substituted -alkyl- $C(=O)N(R^{N1})_2$, optionally substituted -alkyl- $N(R^{N1})C(=O)$ -alkyl, optionally substituted alkyl- $C(=O)O$ -alkyl, or optionally substituted alkyl- $OC(=O)$ -alkyl, wherein each instance of R^{N1} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or a nitrogen protecting group. In certain embodiments, R^3 is an oxygen protecting group (*e.g.*, silyl, TBDPS, TBDMS, TIPS, TES, TMS, MOM, THP, *t*-Bu, Bn, allyl, acetyl, pivaloyl, or benzoyl).

[00133] In the compounds of Formula (I), R^4 is hydrogen, optionally substituted alkyl, or an oxygen protecting group. In certain embodiments, R^4 is hydrogen. In certain embodiments, R^4 is optionally substituted alkyl. In certain embodiments, R^4 is optionally substituted C_{1-6} alkyl. In certain embodiments, R^4 is unsubstituted C_{1-6} alkyl. In certain embodiments, R^4 is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl. In certain embodiments, R^4 is substituted C_{1-6} alkyl. In certain embodiments, R^4 is optionally substituted heteroarylalkyl, optionally substituted heterocyclylalkyl, optionally substituted -alkyl- $C(=O)N(R^{N1})_2$, optionally substituted -alkyl- $N(R^{N1})C(=O)$ -alkyl, optionally substituted alkyl- $C(=O)O$ -alkyl, or optionally substituted alkyl- $OC(=O)$ -alkyl, wherein each instance of R^{N1} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl,

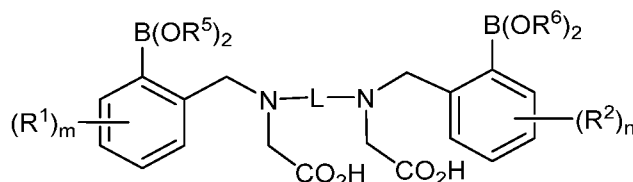
optionally substituted heterocyclyl, optionally substituted heteroaryl, or a nitrogen protecting group. In certain embodiments, R⁴ is an oxygen protecting group (*e.g.*, silyl, TBDPS, TBDMS, TIPS, TES, TMS, MOM, THP, *t*-Bu, Bn, allyl, acetyl, pivaloyl, or benzoyl).

[00134] In certain embodiments of Formula (I), R³ and R⁴ are different. In the compounds of Formula (I), R³ and R⁴ are the same. In certain embodiments of Formula (I), R³ and R⁴ are hydrogen. In certain embodiments of Formula (I), R³ is hydrogen, and R⁴ is optionally substituted alkyl. In certain embodiments of Formula (I), R³ is hydrogen, and R⁴ is optionally substituted C₁₋₆ alkyl. In certain embodiments of Formula (I), R³ is hydrogen, and R⁴ is unsubstituted C₁₋₆ alkyl. In certain embodiments of Formula (I), R³ is hydrogen, and R⁴ is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl. In certain embodiments, R³ is hydrogen, and R⁴ is substituted C₁₋₆ alkyl. In certain embodiments of Formula (I), R⁴ is hydrogen, and R³ is optionally substituted alkyl. In certain embodiments of Formula (I), R⁴ is hydrogen, and R³ is optionally substituted C₁₋₆ alkyl. In certain embodiments of Formula (I), R⁴ is hydrogen, and R³ is unsubstituted C₁₋₆ alkyl. In certain embodiments of Formula (I), R⁴ is hydrogen, and R³ is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl. In certain embodiments, R⁴ is hydrogen, and R³ is substituted C₁₋₆ alkyl. In certain embodiments of Formula (I), each of R³ and R⁴ is independently optionally substituted alkyl. In certain embodiments of Formula (I), each of R³ and R⁴ is independently optionally substituted C₁₋₆ alkyl. In certain embodiments of Formula (I), each of R³ and R⁴ is independently unsubstituted C₁₋₆ alkyl. In certain embodiments of Formula (I), each of R³ and R⁴ is independently methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl. In certain embodiments of Formula (I), each of R³ and R⁴ is independently substituted C₁₋₆ alkyl.

[00135] In certain embodiments of Formula (I), both instances of R⁵ are hydrogen; each instance of R⁶ is independently optionally substituted alkyl; and each of R³ and R⁴ is independently hydrogen or optionally substituted alkyl. In certain embodiments, each instance of R⁵ and R⁶ is independently optionally substituted alkyl; and each of R³ and R⁴ is independently optionally substituted alkyl. In certain embodiments, each instance of R⁵ and R⁶ is independently optionally substituted C₁₋₆ alkyl; and each of R³ and R⁴ is independently optionally substituted C₁₋₆ alkyl. In certain embodiments, each instance of R⁵ and R⁶ and each of R³ and R⁴ is independently optionally substituted alkyl. In certain embodiments, each instance of R⁵ and R⁶ is independently optionally substituted alkyl; and each of R³ and R⁴ is hydrogen. In certain embodiments of Formula (I), each instance of R⁵ and R⁶ is independently optionally substituted alkyl; and each of R³ and R⁴ is independently hydrogen or optionally substituted alkyl. In certain embodiments of Formula (I), each instance of R⁵ and R⁶ is

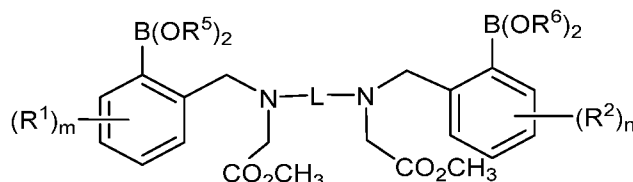
hydrogen; and each of R^3 and R^4 is independently hydrogen or optionally substituted alkyl. In certain embodiments of Formula (I), each instance of R^5 is taken together with the intervening atoms to form an optionally substituted heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted heterocyclic ring; and each of R^3 and R^4 is independently optionally substituted alkyl. In certain embodiments of Formula (I), each instance of R^5 is taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring; and each of R^3 and R^4 is independently optionally substituted alkyl. In certain embodiments of Formula (I), each instance of R^5 is taken together with the intervening atoms to form an optionally substituted heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted heterocyclic ring; and each of R^3 and R^4 is independently optionally substituted C_{1-6} alkyl. In certain embodiments of Formula (I), each instance of R^5 is taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring; and each of R^3 and R^4 is independently optionally substituted C_{1-6} alkyl. In certain embodiments of Formula (I), each instance of R^5 is taken together with the intervening atoms to form an optionally substituted heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted heterocyclic ring; and each of R^3 and R^4 is independently C_{1-6} alkyl. In certain embodiments of Formula (I), each instance of R^5 is taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring; and each of R^3 and R^4 is independently C_{1-6} alkyl. In certain embodiments of Formula (I), each instance of R^5 is taken together with the intervening atoms to form an optionally substituted heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted heterocyclic ring; and each of R^3 and R^4 is independently methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl. In certain embodiments of Formula (I), each instance of R^5 is taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring and each instance of R^6 is taken together with the intervening atoms to form an optionally substituted 5-membered heterocyclic ring; and each of R^3 and R^4 is independently methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl.

[00136] In certain embodiments, the provided compounds are of the following formula:



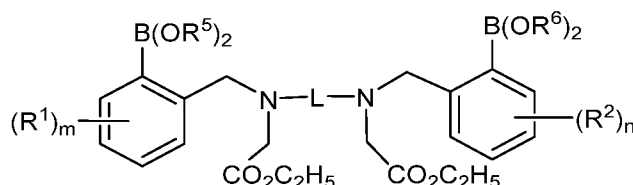
and a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, optionally wherein each instance of R⁵ and R⁶ is independently optionally C₁₋₆ substituted alkyl.

[00137] In certain embodiments, the provided compounds are of the following formula:



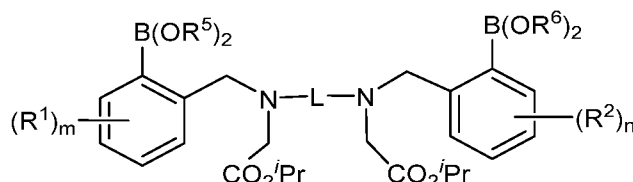
and a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, optionally wherein each instance of R⁵ and R⁶ is independently optionally C₁₋₆ substituted alkyl.

[00138] In certain embodiments, the provided compounds are of the following formula:



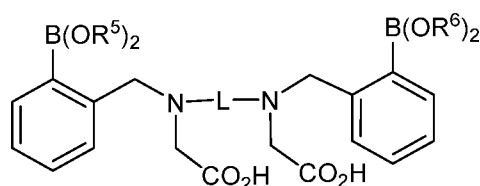
and a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, optionally wherein each instance of R⁵ and R⁶ is independently optionally C₁₋₆ substituted alkyl.

[00139] In certain embodiments, the provided compounds are of the following formula:



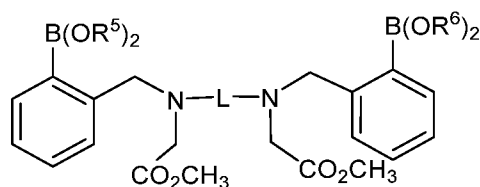
and a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, optionally wherein each instance of R^5 and R^6 is independently optionally C_{1-6} substituted alkyl.

[00140] In certain embodiments, the provided compounds are of the following formula:



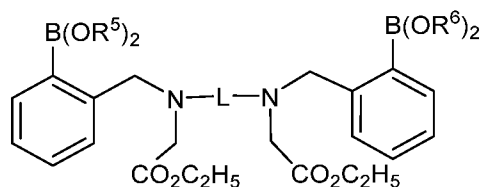
and a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, optionally wherein each instance of R^5 and R^6 is independently optionally C_{1-6} substituted alkyl.

[00141] In certain embodiments, the provided compounds are of the following formula:



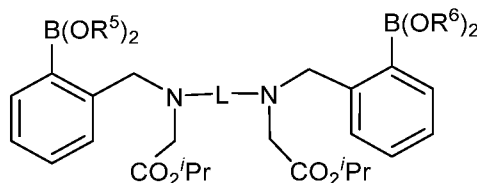
and a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, optionally wherein each instance of R^5 and R^6 is independently optionally C_{1-6} substituted alkyl.

[00142] In certain embodiments, the provided compounds are of the following formula:



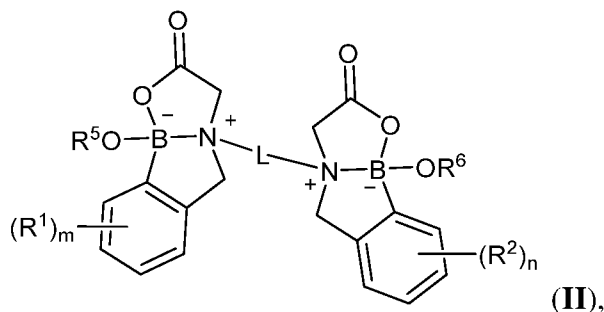
and a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, optionally wherein each instance of R^5 and R^6 is independently optionally C_{1-6} substituted alkyl.

[00143] In certain embodiments, the provided compounds are of the following formula:



and a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, optionally wherein each instance of R^5 and R^6 is independently optionally C_{1-6} substituted alkyl.

[00144] In another aspect of the present invention, provided are compounds of Formula (II):



and pharmaceutically or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, or polymorphs thereof, wherein:

each of m and n is independently 0, 1, 2, 3, or 4;

each instance of R^1 and R^2 is independently hydrogen, halogen, -CN, -NO₂, -N₃, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, optionally substituted amino, or optionally substituted acyl;

each of R^5 and R^6 is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group; and

L is optionally substituted C_{1-8} alkylene.

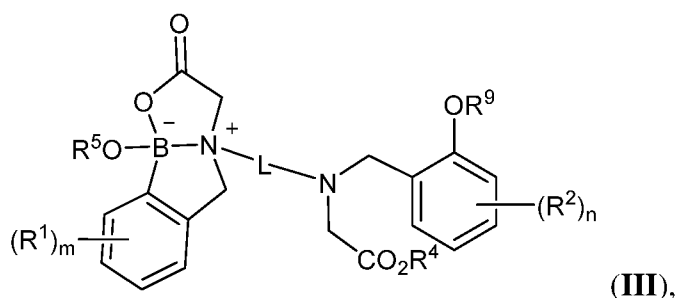
[00145] In the compounds of Formula (II), R^5 is independently hydrogen, optionally substituted alkyl, or an oxygen protecting group. In certain embodiments, R^5 is hydrogen. In certain embodiments, R^5 is optionally substituted alkyl. In certain embodiments, R^5 is optionally substituted C_{1-6} alkyl. In certain embodiments, R^5 is unsubstituted C_{1-6} alkyl. In

certain embodiments, R^5 is methyl or ethyl. In certain embodiments, R^5 is substituted C_{1-6} alkyl. In certain embodiments, R^5 is an oxygen protecting group.

[00146] In the compounds of Formula (II), R^6 is independently hydrogen, optionally substituted alkyl, or an oxygen protecting group. In certain embodiments, R^6 is hydrogen. In certain embodiments, R^6 is optionally substituted alkyl. In certain embodiments, R^6 is optionally substituted C_{1-6} alkyl. In certain embodiments, R^6 is unsubstituted C_{1-6} alkyl. In certain embodiments, R^6 is methyl or ethyl. In certain embodiments, R^6 is substituted C_{1-6} alkyl. In certain embodiments, R^6 is an oxygen protecting group.

[00147] In certain embodiments of Formula (II), R^5 and R^6 are different. In certain embodiments, R^5 and R^6 are the same. In certain embodiments, R^5 and R^6 are hydrogen. In certain embodiments, each of R^5 and R^6 is independently optionally substituted alkyl. In certain embodiments, each of R^5 and R^6 is independently optionally substituted C_{1-6} alkyl. In certain embodiments, each of R^5 and R^6 is independently unsubstituted C_{1-6} alkyl. In certain embodiments, each of R^5 and R^6 is independently methyl or ethyl. In certain embodiments, R^5 is hydrogen, and R^6 is optionally substituted alkyl. In certain embodiments, R^5 is hydrogen, and R^6 is substituted C_{1-6} alkyl. In certain embodiments, R^5 is hydrogen, and R^6 is unsubstituted C_{1-6} alkyl. In certain embodiments, R^5 is hydrogen, and R^6 is methyl or ethyl. In certain embodiments, R^5 is hydrogen, and R^6 is substituted C_{1-6} alkyl.

[00148] In yet another aspect of the present invention, provided are compounds of Formula (III):



and pharmaceutically or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, or polymorphs thereof, wherein:

each of m and n is independently 0, 1, 2, 3, or 4;

each instance of R^1 and R^2 is independently hydrogen, halogen, $-CN$, $-NO_2$, $-N_3$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, optionally substituted amino group, or optionally substituted acyl;

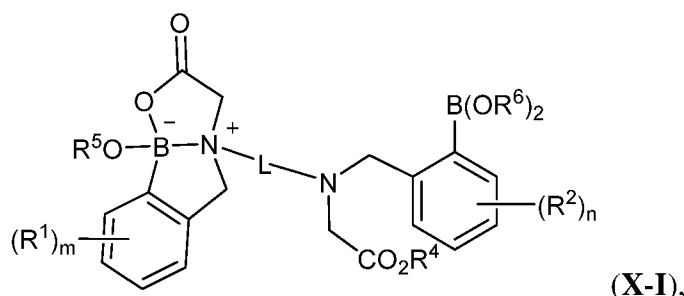
R^4 is hydrogen, optionally substituted alkyl, or an oxygen protecting group;

R^5 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group;

R^9 is hydrogen, optionally substituted alkyl, or an oxygen protecting group; and

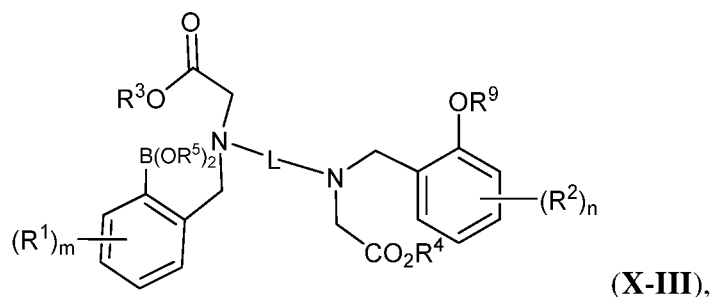
L is optionally substituted C_{1-8} alkylene.

[00149] In one aspect of the present invention, provided are compounds of Formula (X-I):



and pharmaceutically or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, or polymorphs thereof, wherein L, R^1 , R^2 , R^4 , R^5 , R^6 , m, and n are as defined herein.

[00150] In one aspect of the present invention, provided are compounds of Formula (X-III):



and pharmaceutically or cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, or polymorphs thereof, wherein:

each of m and n is independently 0, 1, 2, 3, or 4;

each instance of R^1 and R^2 is independently hydrogen, halogen, -CN, -NO₂, -N₃, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, optionally substituted amino group, or optionally substituted acyl;

each instance of R^3 and R^4 is independently hydrogen, optionally substituted alkyl, or an oxygen protecting group;

each instance of R^5 is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group; or two R^5 are taken together with the intervening atoms to form optionally substituted heterocyclyl;

R^9 is independently hydrogen, optionally substituted alkyl, or an oxygen protecting group; and

L is optionally substituted C_{1-8} alkylene.

[00151] In the compounds of Formula (X-III), R^3 is hydrogen, optionally substituted alkyl, or an oxygen protecting group. In certain embodiments, R^3 is hydrogen. In certain embodiments, R^3 is optionally substituted alkyl. In certain embodiments, R^3 is optionally substituted C_{1-6} alkyl. In certain embodiments, R^3 is unsubstituted C_{1-6} alkyl. In certain embodiments, R^3 is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl. In certain embodiments, R^3 is substituted C_{1-6} alkyl. In certain embodiments, R^3 is optionally substituted heteroarylalkyl, optionally substituted heterocyclalkyl, optionally substituted -alkyl- $C(=O)N(R^{N1})_2$, optionally substituted -alkyl- $N(R^{N1})C(=O)$ -alkyl, optionally substituted alkyl- $C(=O)O$ -alkyl, or optionally substituted alkyl- $OC(=O)$ -alkyl, wherein each instance of R^{N1} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an nitrogen protecting group. In certain embodiments, R^3 is an oxygen protecting group.

[00152] In the compounds of Formulae (III), (X-I), and (X-III), R^4 is hydrogen, optionally substituted alkyl, or an oxygen protecting group. In certain embodiments, R^4 is hydrogen. In certain embodiments, R^4 is optionally substituted alkyl. In certain embodiments, R^4 is optionally substituted C_{1-6} alkyl. In certain embodiments, R^4 is unsubstituted C_{1-6} alkyl. In certain embodiments, R^4 is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl. In certain embodiments, R^4 is substituted C_{1-6} alkyl. In certain embodiments, R^4 is optionally substituted heteroarylalkyl, optionally substituted heterocyclalkyl, optionally substituted -alkyl- $C(=O)N(R^{N1})_2$, optionally substituted -alkyl- $N(R^{N1})C(=O)$ -alkyl, optionally substituted alkyl- $C(=O)O$ -alkyl, or optionally substituted alkyl- $OC(=O)$ -alkyl, wherein each instance of R^{N1} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl,

optionally substituted heterocyclyl, optionally substituted heteroaryl, or an nitrogen protecting group. In certain embodiments, R⁴ is an oxygen protecting group.

[00153] In the compounds of Formulae (III), (X-I), and (X-III), R⁵ is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group. In certain embodiments, R⁵ is hydrogen. In certain embodiments, R⁵ is optionally substituted alkyl. In certain embodiments, R⁵ is optionally substituted C₁₋₆ alkyl. In certain embodiments, R⁵ is unsubstituted C₁₋₆ alkyl. In certain embodiments, R⁵ is methyl or ethyl. In certain embodiments, R⁵ is substituted C₁₋₆ alkyl. In certain embodiments, R⁵ is an oxygen protecting group.

[00154] In the compounds of Formula (X-I), each instance of R⁶ is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group; or two R⁶ are taken together with the intervening atoms to form an optionally substituted heterocyclic moiety. In certain embodiments, each instance of R⁶ is different. In certain embodiments, both instances of R⁶ are the same. In certain embodiments, both instances of R⁶ are hydrogen. In certain embodiments, each instance of R⁶ is independently optionally substituted alkyl. In certain embodiments, each instance of R⁶ is independently optionally substituted C₁₋₆ alkyl. In certain embodiments, each instance of R⁶ is independently unsubstituted C₁₋₆ alkyl (*e.g.*, methyl or ethyl). In certain embodiments, each instance of R⁶ is independently substituted C₁₋₆ alkyl. In certain embodiments, each instance of R⁶ is independently an oxygen protecting group. In certain embodiments, one instance of R⁶ is hydrogen. In certain embodiments, one instance of R⁶ is optionally substituted alkyl. In certain embodiments, one instance of R⁶ is optionally substituted C₁₋₆ alkyl. In certain embodiments, one instance of R⁶ is unsubstituted C₁₋₆ alkyl (*e.g.*, methyl or ethyl). In certain embodiments, one instance of R⁶ is substituted C₁₋₆ alkyl. In certain embodiments, one instance of R⁶ is an oxygen protecting group. In certain embodiments, one instance of R⁶ is hydrogen and one instance of R⁶ is optionally substituted alkyl. In certain embodiments, one instance of R⁶ is hydrogen and one instance of R⁶ is an oxygen protecting group. In certain embodiments, one instance of R⁶ is unsubstituted alkyl (*e.g.*, methyl or ethyl) and one instance of R⁶ is substituted alkyl. In certain embodiments, two R⁶ are taken together with the intervening atoms to form an optionally substituted heterocyclic ring. In certain embodiments, two R⁶ are taken together with the intervening

atoms to form an optionally substituted 5-membered heterocyclic ring. In certain embodiments, two R⁶ are taken together with the intervening atoms to form an optionally substituted 6-membered heterocyclic ring.

[00155] In the compounds of Formulae (III) and (X-III), R⁹ is independently hydrogen, optionally substituted alkyl, or an oxygen protecting group. In certain embodiments, R⁹ is hydrogen. In certain embodiments, R⁹ is optionally substituted alkyl. In certain embodiments, R⁹ is optionally substituted C₁₋₆ alkyl. In certain embodiments, R⁹ is unsubstituted C₁₋₆ alkyl. In certain embodiments, R⁹ is methyl or ethyl. In certain embodiments, R⁹ is substituted C₁₋₆ alkyl. In certain embodiments, R⁹ is an oxygen protecting group.

[00156] In any one of Formulae (I), (II), (III), (X-I), and (X-III), linker L is optionally substituted C₁₋₈ alkylene. In certain embodiments, L is substituted C₁₋₈ alkylene. In certain embodiments, L is unsubstituted C₁₋₈ alkylene. In certain embodiments, L is -(CH₂)_e-, wherein e is an integer of 1 to 8, inclusive. In certain embodiments, L is -CH₂-. In certain embodiments, L is -(CH₂)₂-. In certain embodiments, L is -(CH₂)₃-. In certain embodiments, L is -(CH₂)₄-. In certain embodiments, L is -(CH₂)₅-. In certain embodiments, L is -(CH₂)₆-. In certain embodiments, L is -(CH₂)₇-. In certain embodiments, L is -(CH₂)₈-.

[00157] In any one of Formulae (I), (II), (III), (X-I), and (X-III), m is 0, 1, 2, 3, or 4. In certain embodiments, m is 0. In certain embodiments, m is 1. In certain embodiments, m is 2. In certain embodiments, m is 3. In certain embodiments, m is 4.

[00158] In any one of Formulae (I), (II), (III), (X-I), and (X-III), n is 0, 1, 2, 3, or 4. In certain embodiments, n is 0. In certain embodiments, n is 1. In certain embodiments, n is 2. In certain embodiments, n is 3. In certain embodiments, n is 4.

[00159] In certain embodiments, m and n are different. In certain embodiments, m and n are the same. In certain embodiments, m and n are 0. In certain embodiments, m is 0; and n is 1, 2, 3, or 4. In certain embodiments, n is 0; and m is 1, 2, 3, or 4. In certain embodiments, each of m and n is independently 1, 2, 3, or 4.

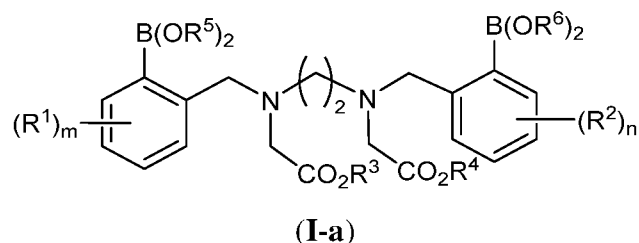
[00160] In any one of Formulae (I), (II), (III), (X-I), and (X-III), each instance of R¹ is independently hydrogen, halogen, -CN, -NO₂, -N₃, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, an optionally substituted amino group, or optionally substituted acyl. In certain embodiments, at least one instance of R¹ is hydrogen. In certain embodiments, at least one instance of R¹ is halogen, -CN, -NO₂, -N₃, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted

carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, an optionally substituted amino group, or optionally substituted acyl. In certain embodiments, at least one instance of R^1 is halogen. In certain embodiments, at least one instance of R^1 is F. In certain embodiments, at least one instance of R^1 is Cl. In certain embodiments, at least one instance of R^1 is Br. In certain embodiments, at least one instance of R^1 is I. In certain embodiments, at least one instance of R^1 is optionally substituted alkyl. In certain embodiments, at least one instance of R^1 is optionally substituted C_{1-6} alkyl. In certain embodiments, at least one instance of R^1 is unsubstituted C_{1-6} alkyl. In certain embodiments, at least one instance of R^1 is methyl or ethyl. In certain embodiments, at least one instance of R^1 is optionally substituted alkenyl. In certain embodiments, at least one instance of R^1 is optionally substituted alkynyl. In certain embodiments, at least one instance of R^1 is optionally substituted carbocyclyl. In certain embodiments, at least one instance of R^1 is optionally substituted aryl. In certain embodiments, at least one instance of R^1 is optionally substituted heterocyclyl. In certain embodiments, at least one instance of R^1 is optionally substituted heteroaryl. In certain embodiments, at least one instance of R^1 is optionally substituted alkoxy. In certain embodiments, at least one instance of R^1 is an optionally substituted amino group. In certain embodiments, at least one instance of R^1 is optionally substituted acyl (*e.g.*, acetyl).

[00161] In any one of Formulae (I), (II), (III), (X-I), and (X-III), each instance of R^2 is independently hydrogen, halogen, -CN, -NO₂, -N₃, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, an optionally substituted amino group, or optionally substituted acyl. In certain embodiments, at least one instance of R^2 is hydrogen. In certain embodiments, at least one instance of R^2 is halogen, -CN, -NO₂, -N₃, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, an optionally substituted amino group, or optionally substituted acyl. In certain embodiments, at least one instance of R^2 is halogen. In certain embodiments, at least one instance of R^2 is F. In certain embodiments, at least one instance of R^2 is Cl. In certain embodiments, at least one instance of R^2 is Br. In certain embodiments, at least one instance of R^2 is I. In certain embodiments, at least one instance of R^2 is optionally substituted alkyl. In certain embodiments, at least one instance of R^2 is optionally substituted C_{1-6} alkyl. In certain embodiments, at least one instance of R^2 is

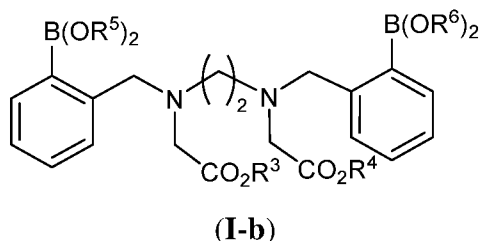
unsubstituted C₁₋₆ alkyl. In certain embodiments, at least one instance of R² is methyl or ethyl. In certain embodiments, at least one instance of R² is optionally substituted alkenyl. In certain embodiments, at least one instance of R² is optionally substituted alkynyl. In certain embodiments, at least one instance of R² is optionally substituted carbocyclyl. In certain embodiments, at least one instance of R² is optionally substituted aryl. In certain embodiments, at least one instance of R² is optionally substituted heterocyclyl. In certain embodiments, at least one instance of R² is optionally substituted heteroaryl. In certain embodiments, at least one instance of R² is optionally substituted alkoxy. In certain embodiments, at least one instance of R² is an optionally substituted amino group. In certain embodiments, at least one instance of R² is optionally substituted acyl (*e.g.*, acetyl).

[00162] In certain embodiments, the compound of Formula (I) is of Formula (I-a):



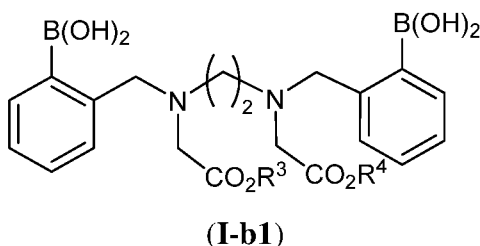
or a pharmaceutically or cosmetically acceptable salt thereof, wherein R¹, R², R³, R⁴, R⁵, R⁶, m, and n are as described herein.

[00163] In certain embodiments, the compound of Formula (I) is of Formula (I-b):



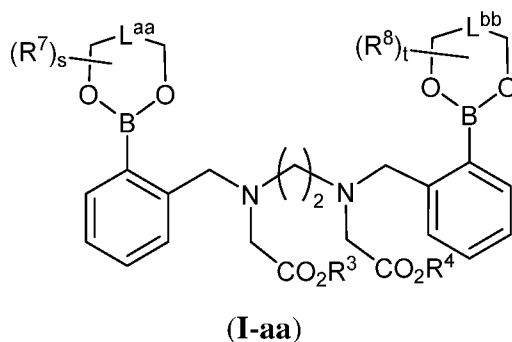
or a pharmaceutically or cosmetically acceptable salt thereof, wherein R³, R⁴, R⁵, and R⁶ are as described herein.

[00164] In certain embodiments, the compound of Formula (I) is of Formula (I-b1):



or a pharmaceutically or cosmetically acceptable salt thereof, wherein R³ and R⁴ are as described herein.

[00165] In certain embodiments, the compound of Formula (I) is of Formula (I-aa):



or a pharmaceutically or cosmetically acceptable salt thereof,

wherein:

each of L^{aa} and L^{bb} is independently a bond, optionally substituted alkylene, or optionally substituted heteroalkylene;

each instance of R^7 and R^8 is independently hydrogen, halogen, or optionally substituted alkyl;

or L^{aa} and one instance of R^7 are joined to form an optionally substituted aryl ring;

or two instances of R^7 are joined to form an optionally substituted aryl ring;

or L^{bb} and one instance of R^8 are joined to form an optionally substituted aryl ring;

or two instances of R^8 are joined to form an optionally substituted aryl ring; and

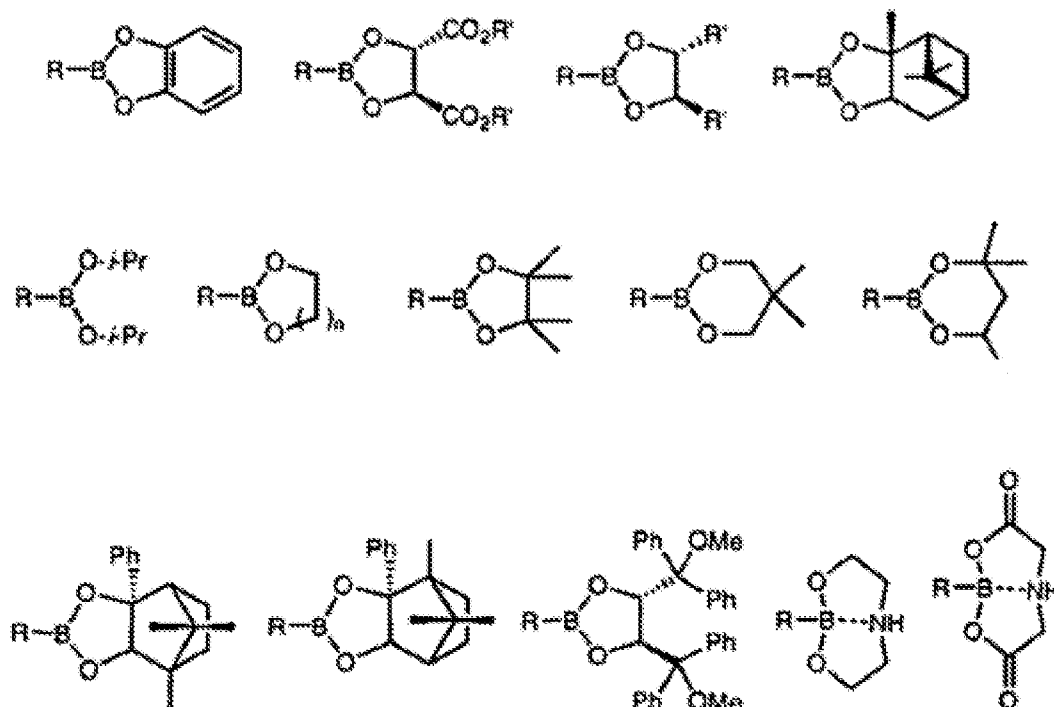
each of s and t is independently 0, 1, 2, 3, or 4.

[00166] In certain embodiments, L^{aa} is a bond. In certain embodiments, L^{aa} is optionally substituted alkylene. In certain embodiments, L^{aa} is optionally substituted C_{1-6} alkylene. In certain embodiments, L^{aa} is unsubstituted C_{1-6} alkylene. In certain embodiments, L^{aa} is $-(CH_2)_{1-6}-$. In certain embodiments, L^{aa} is substituted C_{1-6} alkylene.

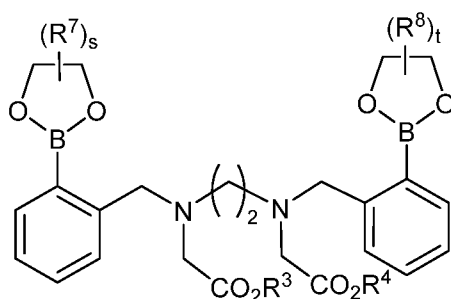
[00167] In certain embodiments, L^{bb} is a bond. In certain embodiments, L^{bb} is optionally substituted alkylene. In certain embodiments, L^{bb} is optionally substituted C_{1-6} alkylene. In certain embodiments, L^{bb} is unsubstituted C_{1-6} alkylene. In certain embodiments, L^{bb} is $-(CH_2)_{1-6}-$. In certain embodiments, L^{bb} is substituted C_{1-6} alkylene.

[00168] In certain embodiments, L^{aa} and L^{bb} are the same. In certain embodiments, L^{aa} and L^{bb} are different. In certain embodiments, each of L^{aa} and L^{bb} is independently optionally substituted alkylene. In certain embodiments, each of L^{aa} and L^{bb} is independently optionally substituted heteroalkylene. In certain embodiments, L^{aa} is optionally substituted alkylene and L^{bb} is optionally substituted heteroalkylene.

[00169] It is to be understood that the $-B(OR^5)_2$ and $-B(OR^6)_2$ moieties encompass any types of boronic acids and boronic esters. Exemplified boronic acids and boronic esters are shown below:



[00170] In certain embodiments, the compound of Formula (I) is of Formula (I-b2):



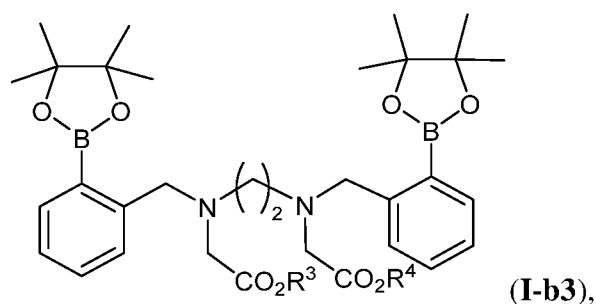
(I-b2)

or a pharmaceutically or cosmetically acceptable salt thereof, wherein R^3 and R^4 are as described herein; wherein each instance of R^7 and R^8 is independently hydrogen, halogen, or optionally substituted alkyl; and

each of s and t is independently 0, 1, 2, 3, or 4. In certain embodiments, s is 0, 1, 2, 3, or 4. In certain embodiments, s is 0. In certain embodiments, s is 1. In certain embodiments, s is 2. In certain embodiments, s is 3. In certain embodiments, s is 4. In certain embodiments, t is 0, 1, 2, 3, or 4. In certain embodiments, t is 0. In certain embodiments, t is 1. In certain embodiments, t is 2. In certain embodiments, t is 3. In certain embodiments, t is 4. In certain embodiments, s and t are different. In certain embodiments, s and t are the same. In certain

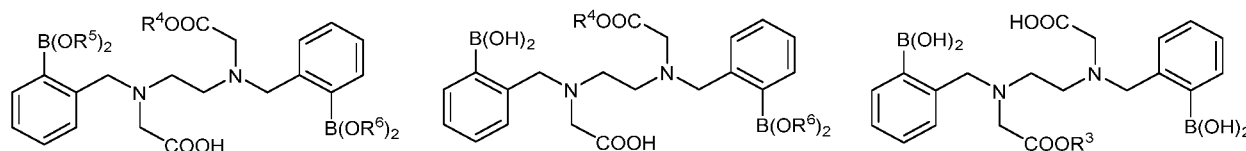
embodiments, s and t are 0. In certain embodiments, s is 0 and t is 1, 2, 3, or 4. In certain embodiments, t is 0 and s is 1, 2, 3, or 4. In certain embodiments, each of s and t is independently 1, 2, 3, or 4. In certain embodiments, at least one instance of R^7 is hydrogen. In certain embodiments, at least one instance of R^7 is halogen. In certain embodiments, at least one instance of R^7 is F. In certain embodiments, at least one instance of R^7 is Cl. In certain embodiments, at least one instance of R^7 is Br. In certain embodiments, at least one instance of R^7 is I. In certain embodiments, at least one instance of R^7 is optionally substituted alkyl. In certain embodiments, at least one instance of R^7 is optionally substituted C_{1-6} alkyl. In certain embodiments, at least one instance of R^7 is unsubstituted C_{1-6} alkyl. In certain embodiments, at least one instance of R^7 is methyl or ethyl. In certain embodiments, at least one instance of R^8 is hydrogen. In certain embodiments, at least one instance of R^8 is halogen. In certain embodiments, at least one instance of R^8 is F. In certain embodiments, at least one instance of R^8 is Cl. In certain embodiments, at least one instance of R^8 is Br. In certain embodiments, at least one instance of R^8 is I. In certain embodiments, at least one instance of R^8 is optionally substituted alkyl. In certain embodiments, at least one instance of R^8 is optionally substituted C_{1-6} alkyl. In certain embodiments, at least one instance of R^8 is unsubstituted C_{1-6} alkyl. In certain embodiments, at least one instance of R^8 is methyl or ethyl. In certain embodiments, L^{aa} and one instance of R^7 are joined to form an optionally substituted aryl ring (*e.g.*, optionally substituted phenyl ring). In certain embodiments, two instances of R^7 are joined to form an optionally substituted aryl ring (*e.g.*, optionally substituted phenyl ring). In certain embodiments, L^{bb} and one instance of R^8 are joined to form an optionally substituted aryl ring (*e.g.*, optionally substituted phenyl ring). In certain embodiments, two instances of R^8 are joined to form an optionally substituted aryl ring (*e.g.*, optionally substituted phenyl ring).

[00171] In certain embodiments, the compound of Formula (I) is of the formula:

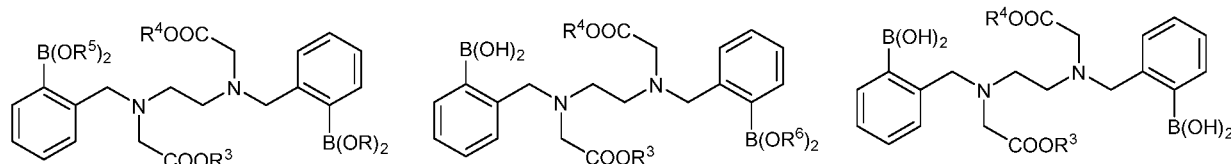


or a pharmaceutically or cosmetically acceptable salt thereof, wherein R^3 and R^4 are as described herein. In certain embodiments of Formula (I), one of R^3 and R^4 is not hydrogen. In certain embodiments of Formula (I), R^3 is hydrogen and R^4 is optionally substituted alkyl or

an oxygen protecting group. In certain embodiments of Formula (I), R^4 is hydrogen and R^3 is optionally substituted alkyl or an oxygen protecting group. In certain embodiments, one or both nitrogens may be coordinated to boron. In certain embodiments, the carboxylic acid may be coordinated to boron. In certain embodiments, one or both nitrogens and the carboxylic acid may be coordinated to boron. In certain embodiments, coordination may lead to the loss of one or more molecules of HOR^5 or HOR^6 (e.g., H_2O if $R^5=R^6=H$) from the compound. In certain embodiments, compounds of Formula (I) are double prodrugs that can be unmasked by hydrolysis and oxidative stress to the active chelator HBED. In certain embodiments, the compound of Formula (I) is of one of the following formulae:

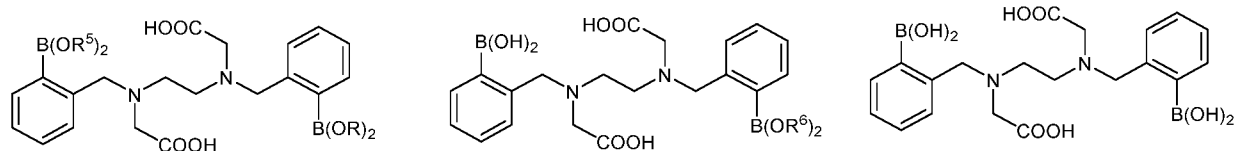


In certain embodiments of Formula (I), each of R^3 and R^4 is independently optionally substituted alkyl or an oxygen protecting group. In certain embodiments, one or both nitrogens may be coordinated to boron. In certain embodiments, coordination may lead to the loss of one or more molecules of HOR^5 or HOR^6 (e.g., H_2O if $R^5=R^6=H$) from the compound. In certain embodiments, the compound of Formula (I) is of one of the following formulae:

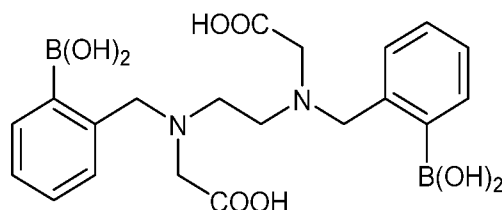


[00172] In certain embodiments of Formula (I), both R^3 and R^4 are hydrogen. In certain embodiments, the compounds of Formula (I), wherein both R^3 and R^4 are hydrogen, are prodrugs that can be unmasked by oxidative stress to the active chelator HBED.

In certain embodiments, one or both nitrogens may be coordinated to boron. In certain embodiments, one or both carboxylic acids may be coordinated to boron. In certain embodiments, one or both nitrogens and one or both carboxylic acids may be coordinated to boron. In certain embodiments, coordination may lead to loss of one or more molecules of HOR^5 or HOR^6 (e.g., H_2O if $R^5=R^6=H$) from the compound. In certain embodiments, the compound of Formula (I) is of one of the following formulae:

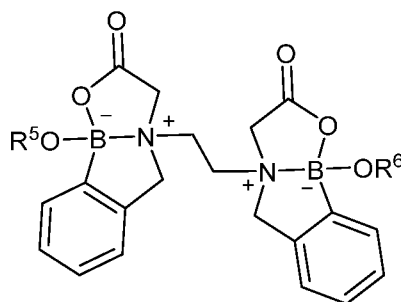


In certain embodiments, the compound of Formula (I) is of the dehydrated form of the formula:



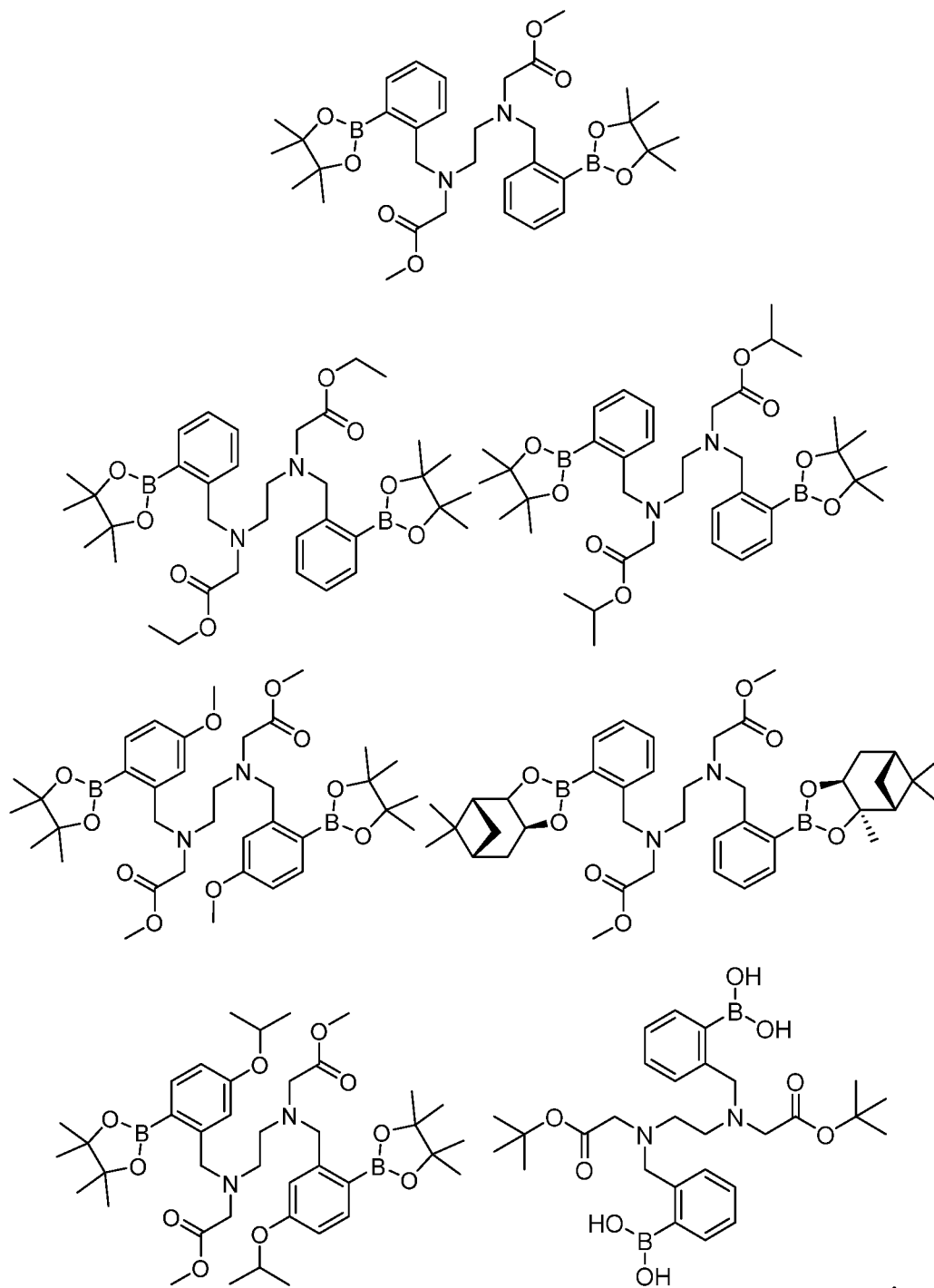
[00173] In certain embodiments, one or both nitrogens may be coordinated to boron. In certain embodiments, one or both carboxylic acids may be coordinated to boron. In certain embodiments, one or both nitrogens and one or both carboxylic acids may be coordinated to boron. In certain embodiments, coordination may lead to loss of one or more molecules of HOR^5 or HOR^6 (e.g., H_2O if $\text{R}^5=\text{R}^6=\text{H}$) from the compound.

[00174] In certain embodiments, the compound of Formula (I) is of a



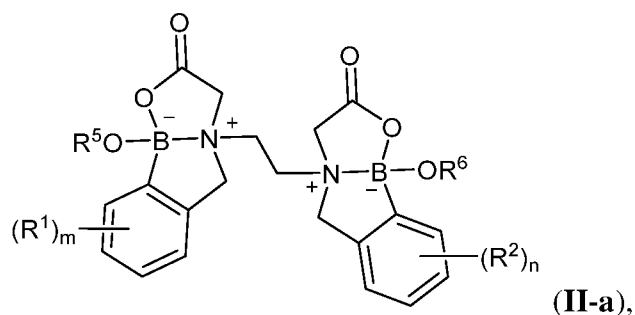
coordinated/dehydrated form and of the formula:

[00175] In certain embodiments, the compound of Formula (I) is of one of the following formulae:



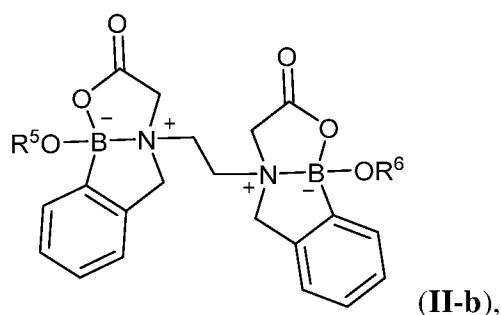
or a pharmaceutically or cosmetically acceptable salt.

[00176] In certain embodiments, the compound of Formula (II) is of Formula (II-a):



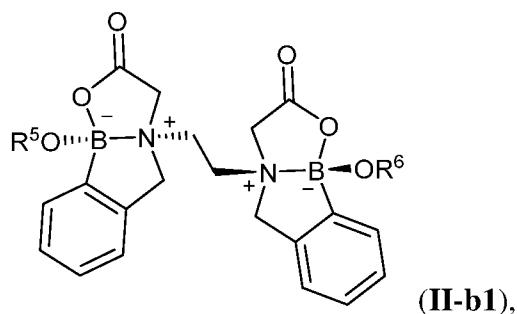
or a pharmaceutically or cosmetically acceptable salt thereof, wherein R^1 , R^2 , R^5 , R^6 , m , and n are as defined herein.

[00177] In certain embodiments, the compound of Formula (II) is of Formula (II-b):



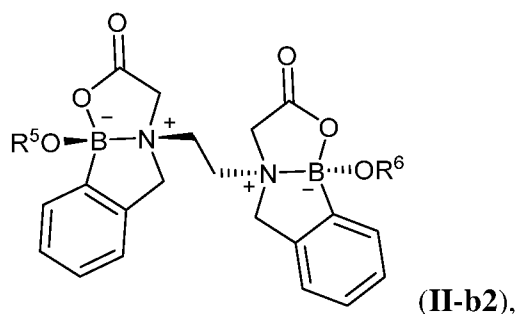
or a pharmaceutically or cosmetically acceptable salt thereof, wherein R^5 and R^6 are as defined herein.

[00178] In certain embodiments, the compound of Formula (II) is of Formula (II-b1):



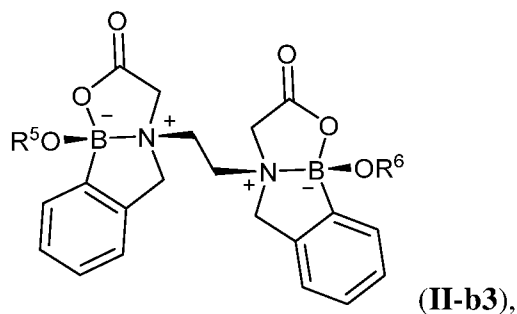
or a pharmaceutically or cosmetically acceptable salt thereof, wherein R^5 and R^6 are as defined herein.

[00179] In certain embodiments, the compound of Formula (II) is of Formula (II-b2):



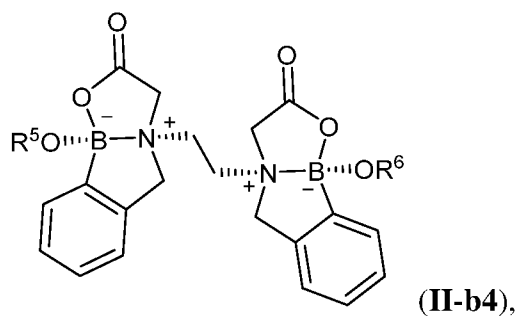
or a pharmaceutically or cosmetically acceptable salt thereof, wherein R^5 and R^6 are as defined herein.

[00180] In certain embodiments, the compound of Formula (II) is of Formula (II-b3):



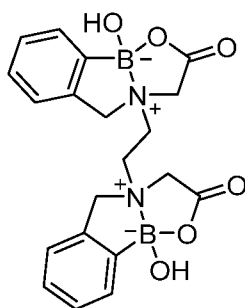
or a pharmaceutically or cosmetically acceptable salt thereof, wherein R^5 and R^6 are as defined herein.

[00181] In certain embodiments, the compound of Formula (II) is of Formula (II-b4):



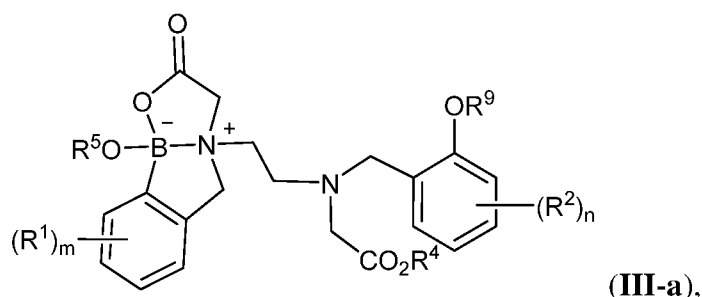
or a pharmaceutically or cosmetically acceptable salt thereof, wherein R^5 and R^6 are as defined herein.

[00182] In certain embodiments, the compound of Formula (II) is of the following formula:



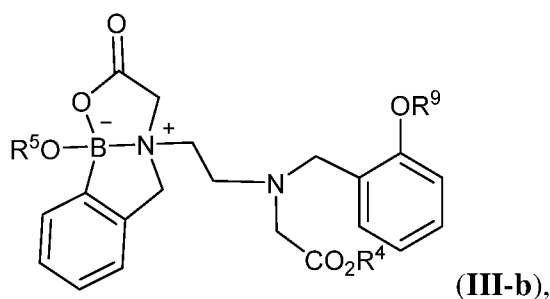
or a pharmaceutically or cosmetically acceptable salt thereof.

[00183] In certain embodiments, the compound of Formula (III) is of Formula (III-a):



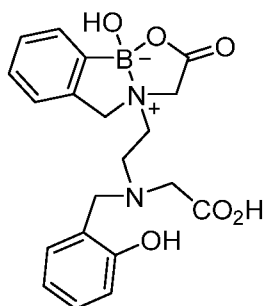
or a pharmaceutically or cosmetically acceptable salt thereof, wherein R^1 , R^2 , R^4 , R^5 , R^9 , m , and n are as defined herein.

[00184] In certain embodiments, the compound of Formula (III) is of Formula (III-b):



or a pharmaceutically or cosmetically acceptable salt thereof, wherein R^4 , R^5 , and R^9 are as defined herein.

[00185] In certain embodiments, the compound of Formula (III) is of the following formula:



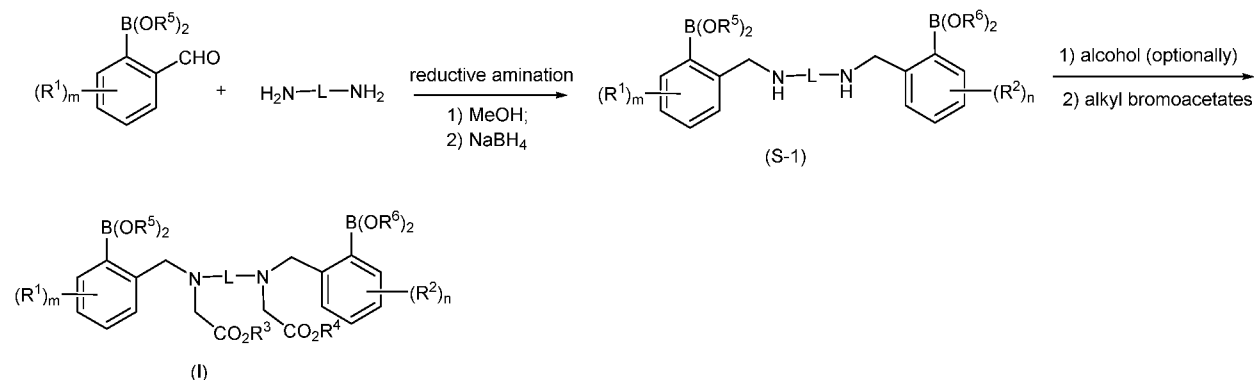
or a pharmaceutically or cosmetically acceptable salt thereof.

[00186] It is to be understood that the inventive compounds may exist in different coordination forms depending on the chelation between B and N and/or O. In certain embodiments, the inventive compounds may exist in a mixture of different coordination forms. In certain embodiments, the inventive compounds may exist in a dehydrated form of any of the compounds as described herein. In certain embodiments, the inventive compounds may exist in a mixture of un-dehydrated and dehydrated forms of any of the compounds as described herein. In certain embodiments, the inventive compounds may exist in a mixture of

dehydrated forms and different coordination forms of any of the compounds as described herein.

[00187] The compounds as described herein can be prepared from the standard organic synthesis known in the art. In certain embodiments, the compounds of Formula (I) can be synthesized from *Scheme 1*.

Scheme 1



[00188] Reductive amination of a phenyl aldehyde and a diamine provides an intermediate of Formula (S-1). The compound of Formula (S-1) can react with an alcohol (*e.g.*, pinacol) to form the corresponding boronic esters. To attach the acetate esters, the compound of Formula (S-1) or its boronic esters react with alkyl bromoacetates to generate a compound of Formula (I). In certain embodiments, the compound of Formula (S-1) or its boronic esters react with alkyl iodoacetates to generate a compound of Formula (I). It is to be understood that the bromo- or iodo- group of the haloacetate can be replaced by another leaving group as defined herein.

[00189] The provided HBED prodrugs may have superior physiochemical, pharmacokinetic, pharmacodynamic, and/or toxicological properties (such as greater solubility, permeability, and bioavailability; improved distribution, absorption, metabolism, and iron-clearing efficiency; and reduced clearance, excretion, and toxicity) compared with the parent compound HBED and/or other HBED analogs. Masking the phenolic hydroxyl groups of HBED as boronic acids or esters appears to promote the chemical hydrolysis of carboxylate esters at physiological pH. Masking the phenolic hydroxyl groups of HBED as boronic acids or esters alone or in combination with masking the carboxylic acid groups as carboxylate esters would be predicted to decrease the affinity of these compounds for iron and other metals in conditions and tissues where metal levels are normal and chelation is not needed.

[00190] Activation of the provided prodrugs may be achieved by hydrolysis and/or oxidation. Hydrolysis of the provided prodrugs with the masked carboxylate esters may give the corresponding carboxylic acids. Oxidation of the boronic acids or boronic esters of the provided prodrugs may give the corresponding phenolic hydroxyls. In certain embodiments, reactive oxygen species can oxidatively convert the boronic acids or boronic esters to the corresponding phenol and boric acid. Reactive oxygen species (ROS) refer to chemically reactive molecules containing oxygen. Examples include oxygen ions and peroxides. Hydrogen peroxide is often found in conditions of oxidative stress. In certain embodiments, hydrogen peroxide can oxidatively convert the boronic acids or boronic esters to the corresponding phenol and boric acid. In certain embodiments, peroxyxynitrite can oxidatively convert the boronic acids or boronic esters to the corresponding phenol and boric acid.

[00191] In some embodiments, a compound of Formula (I) is hydrolyzed and/or oxidized to a compound of Formula (II) and/or Formula (III). Under oxidative conditions (*e.g.*, oxidative stress in PD), the compound of Formula (II) or Formula (III) is oxidized to HBED or an analog thereof, which can chelate labile iron and prevent further oxidative damage caused by reactive oxygen species generated by the Fenton reaction. In this way, the provided prodrugs may preferentially be fully converted to HBED only in the tissues where chelation is needed most, by the very conditions prevailing at these sites (*e.g.*, oxidative stress), so that the global disruption of metal homeostasis can be avoided.

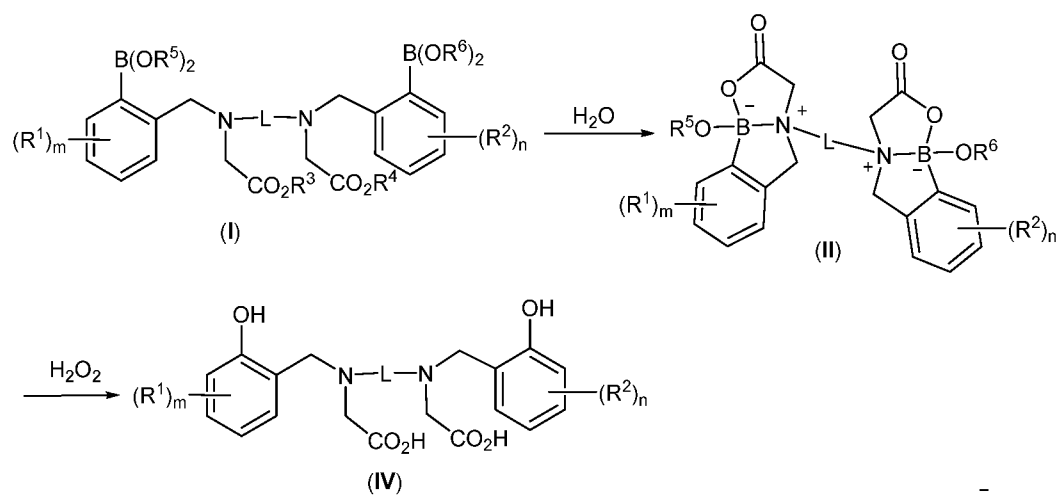
[00192] In some embodiments, the provided prodrugs can be unmasked by chemical and/or enzymatic hydrolysis and subsequently oxidized to HBED or an analog thereof. In some embodiments, the provided prodrugs can be unmasked by chemical hydrolysis and subsequently oxidized by H₂O₂ to HBED or an analog thereof. In some embodiments, the provided prodrugs can be unmasked by chemical hydrolysis and subsequently oxidized by H₂O₂ to HBED or an analog thereof at physiological pH. In some embodiments, the H₂O₂ is generated from oxidative stress *in vivo*.

[00193] As used herein, physiological pH refers to the optimum pH for the body of a subject to survive and maintain homeostasis. In certain embodiments, physiological pH is from about 7.0 to about 7.6. In certain embodiments, physiological pH is from about 7.1 to about 7.5. In certain embodiments, the physiological pH is about 7.4.

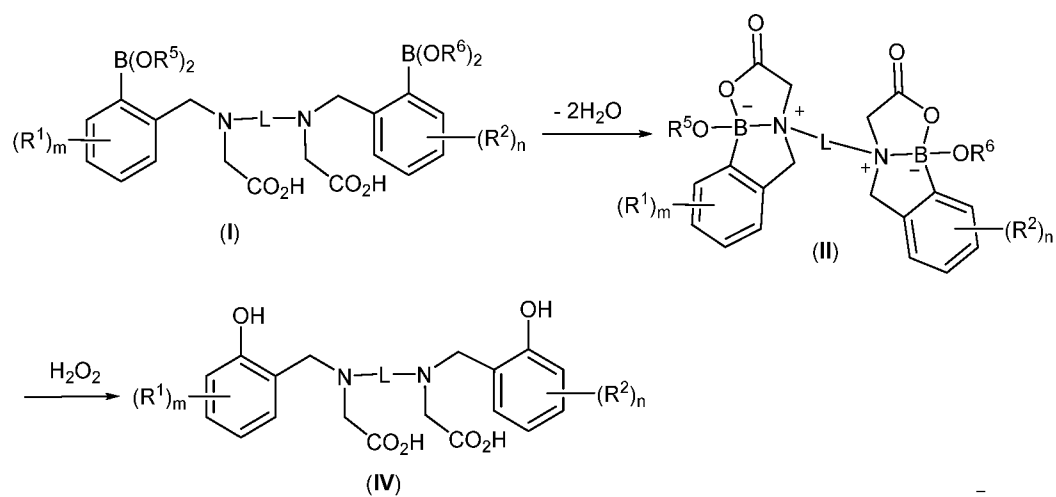
[00194] Exemplary hydrolysis and oxidation of the compounds of Formula (I), (II), and (III) are shown in *Scheme 2*.

Scheme 2

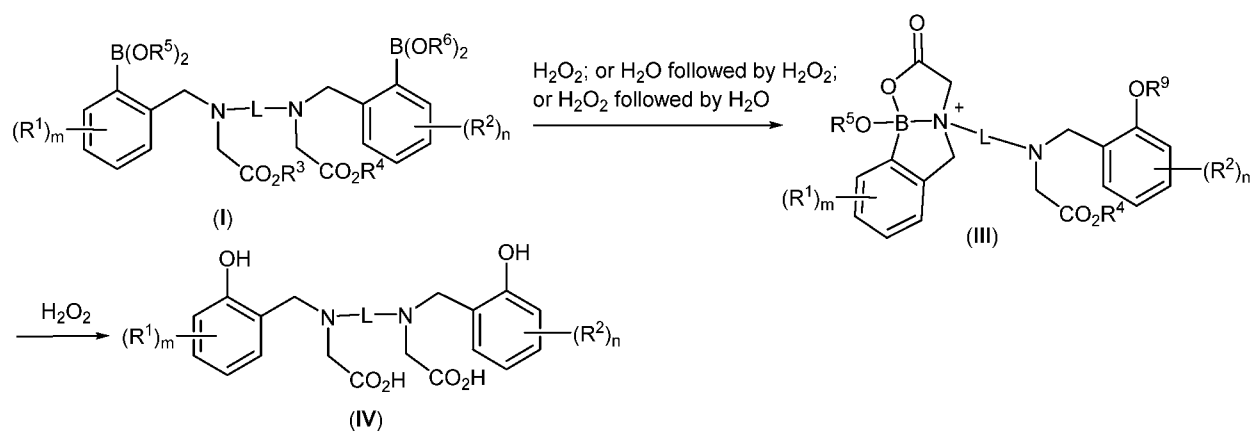
A



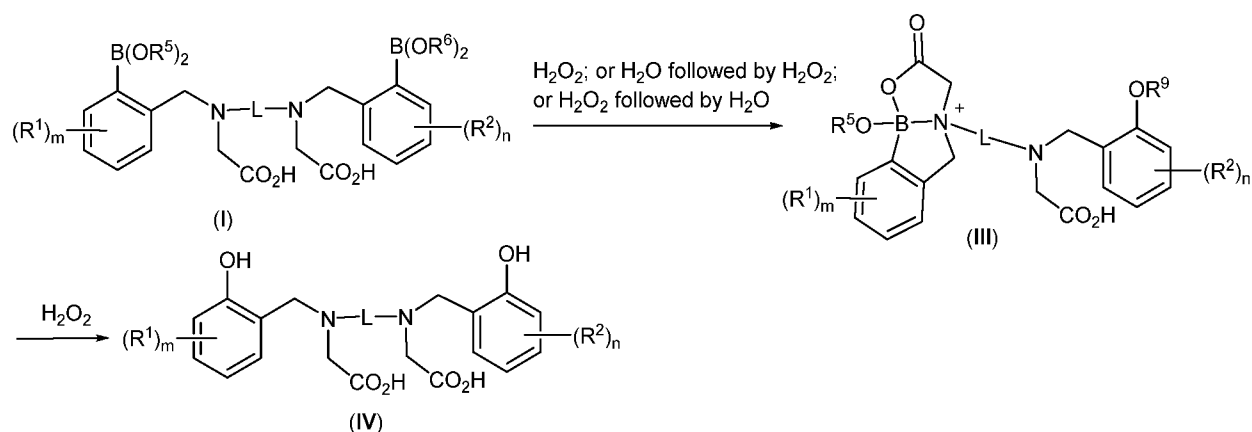
B



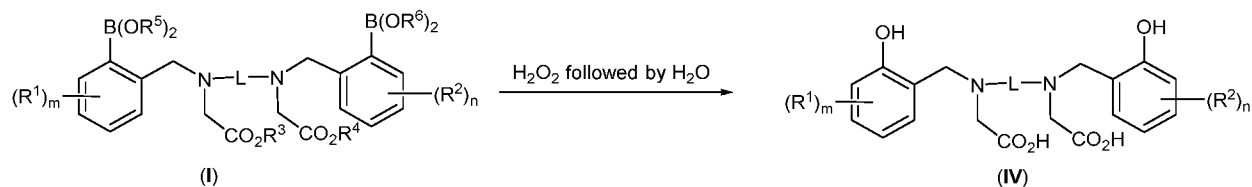
C



D



E



Methods of Preparing the Compounds described herein

[00195] In another aspect, provided are methods of preparing the compounds described herein. In certain embodiments, a method of preparing the compounds described herein is a method described in the Examples section of the present disclosure. In certain embodiments, a method of preparing the compounds described herein is a method shown in Scheme 1. In certain embodiments, a method of preparing the compounds described herein is a method shown in Scheme 2.

Compositions, Kits, and Administration

[00196] The present invention provides pharmaceutical compositions comprising a compound of any one of Formula (I)-(III), and pharmaceutically acceptable salts, tautomers, stereoisomers, solvates, hydrates, isotopically enriched derivatives, and polymorphs thereof, and optionally a pharmaceutically acceptable excipient. In certain embodiments, the compound of the present invention, or a pharmaceutically acceptable salt thereof, is provided in an effective amount in the pharmaceutical composition. In certain embodiments, the effective amount is a therapeutically effective amount. In certain embodiments, the effective amount is a prophylactically effective amount.

[00197] In certain embodiments, the provided pharmaceutical compositions are useful in a pathological condition in a subject. In certain embodiments, the pathological condition is responsive to the chelation or sequestration of a metal. In certain embodiments, the metal is iron (*e.g.*, Fe(III)). In certain embodiments, the metal is aluminum, thallium, chromium, magnesium, calcium, strontium, nickel, manganese, cobalt, copper, zinc, silver, sodium, potassium, cadmium, mercury, lead, antimony, molybdenum, tungsten, a lanthanide (*e.g.*, cerium), or an actinide (*e.g.*, uranium). In certain embodiments, the metal is a trivalent metal. In certain embodiments, the metal is a monovalent, divalent, tetravalent, pentavalent, or hexavalent metal. In certain embodiments, the subject is a human. In certain embodiments, the pathological condition is metal overload (*e.g.*, iron overload, aluminum overload, chromium overload, magnesium overload, calcium overload, strontium overload, nickel overload, manganese overload, cobalt overload, copper overload, zinc overload, silver overload, sodium overload, potassium overload, cadmium overload, mercury overload, lead overload, molybdenum overload, tungsten overload, or actinide overload (*e.g.*, uranium overload)). In certain embodiments, the pathological condition is iron overload. In certain embodiments, the pathological condition is metal poisoning (*e.g.*, iron poisoning, aluminum poisoning, thallium poisoning, chromium poisoning, magnesium poisoning, calcium poisoning, strontium poisoning, nickel poisoning, manganese poisoning, cobalt poisoning, copper poisoning, zinc poisoning, silver poisoning, sodium poisoning, potassium poisoning, cadmium poisoning, mercury poisoning, lead poisoning, antimony poisoning, molybdenum poisoning, tungsten poisoning, lanthanide poisoning (*e.g.*, cerium poisoning), or actinide poisoning (*e.g.*, uranium poisoning)). In certain embodiments, the pathological condition is oxidative stress, transfusional iron overload, thalassemia, primary hemochromatosis, secondary hemochromatosis, diabetes, liver disease, heart disease, cancer, radiation injury, neurological or neurodegenerative disorder (*e.g.*, Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, and neurodegeneration with brain iron accumulation), macular degeneration, closed head injury, irritable bowel disease, stroke, and reperfusion injury. In certain embodiments, the pathological condition is an infectious disease (*e.g.*, HIV and malaria). In certain embodiments, the pathological condition is aging. In certain embodiments, the methods of treatment and/or prevention include administering to the subject a therapeutically or prophylactically effective amount of a compound of the invention, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, or a pharmaceutical compositions thereof.

[00198] Pharmaceutical compositions described herein can be prepared by any method known in the art of pharmacology. In general, such preparatory methods include the steps of bringing the compound of the present invention (the “active ingredient”) into association with a carrier or excipient, and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping, and/or packaging the product into a desired single- or multi-dose unit. In certain embodiments, the pharmaceutical composition comprises a compound lyophilized in the presence of an equimolar or excess amount of an alcohol (*e.g.*, mannitol).

[00199] Pharmaceutical compositions can be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. A “unit dose” is a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00200] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[00201] Pharmaceutically acceptable excipients used in the manufacture of provided pharmaceutical compositions include inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and perfuming agents may also be present in the composition.

[00202] Exemplary diluents include calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, and mixtures thereof.

[00203] Exemplary granulating and/or dispersing agents include potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose, and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose,

pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, and mixtures thereof.

[00204] Exemplary surface active agents and/or emulsifiers include natural emulsifiers (*e.g.*, acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (*e.g.*, bentonite (aluminum silicate) and Veegum (magnesium aluminum silicate)), long chain amino acid derivatives, high molecular weight alcohols (*e.g.*, stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (*e.g.*, carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (*e.g.*, carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (*e.g.*, polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan (Tween 60), polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60), sorbitan tristearate (Span 65), glyceryl monooleate, sorbitan monooleate (Span 80)), polyoxyethylene esters (*e.g.*, polyoxyethylene monostearate (Myrj 45), polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and Solutol), sucrose fatty acid esters, polyethylene glycol fatty acid esters (*e.g.*, Cremophor™), polyoxyethylene ethers, (*e.g.*, polyoxyethylene lauryl ether (Brij 30)), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, Pluronic F-68, Poloxamer188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, and/or mixtures thereof.

[00205] Exemplary binding agents include starch (*e.g.*, cornstarch and starch paste), gelatin, sugars (*e.g.*, sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol, *etc.*), natural and synthetic gums (*e.g.*, acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (Veegum), and larch arabogalactan), alginates, polyethylene oxide, polyethylene glycol, inorganic calcium salts, silicic acid, polymethacrylates, waxes, water, alcohol, and/or mixtures thereof.

[00206] Exemplary preservatives include antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives.

[00207] Exemplary antioxidants include alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and sodium sulfite.

[00208] Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA) and salts and hydrates thereof (*e.g.*, sodium edetate, disodium edetate, trisodium edetate, calcium disodium edetate, dipotassium edetate, and the like), citric acid and salts and hydrates thereof (*e.g.*, citric acid monohydrate), fumaric acid and salts and hydrates thereof, malic acid and salts and hydrates thereof, phosphoric acid and salts and hydrates thereof, and tartaric acid and salts and hydrates thereof. Exemplary antimicrobial preservatives include benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and thimerosal.

[00209] Exemplary antifungal preservatives include butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and sorbic acid.

[00210] Exemplary alcohol preservatives include ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and phenylethyl alcohol.

[00211] Exemplary acidic preservatives include vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and phytic acid.

[00212] Other preservatives include tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, Glydant Plus, Phenonip, methylparaben, Germall 115, Germaben II, Neolone, Kathon, and Euxyl. In certain embodiments, the preservative is an anti-oxidant. In other embodiments, the preservative is a chelating agent.

[00213] Exemplary buffering agents include citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium

chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and mixtures thereof.

[00214] Exemplary lubricating agents include magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, and mixtures thereof.

[00215] Exemplary natural oils include almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary synthetic oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and mixtures thereof.

[00216] Liquid dosage forms for oral and parenteral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredients, the liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (*e.g.*, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can include adjuvants such as

wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents. In certain embodiments for parenteral administration, the conjugates of the invention are mixed with solubilizing agents such as Cremophor™, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and mixtures thereof.

[00217] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions can be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

[00218] The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00219] In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This can be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

[00220] Compositions for rectal or vaginal administration are typically suppositories which can be prepared by mixing the conjugates of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active ingredient.

[00221] While it may be possible for the compounds disclosed herein, or pharmaceutically acceptable salts, tautomers, stereoisomers, solvates, hydrates, or polymorphs thereof, to be administered orally as they are, it is also possible to present them as a pharmaceutical formulation or dosage. Solid dosage forms for oral administration

include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active ingredient is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or (a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, (b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, (c) humectants such as glycerol, (d) disintegrating agents such as agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, (e) solution retarding agents such as paraffin, (f) absorption accelerators such as quaternary ammonium compounds, (g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, (h) absorbents such as kaolin and bentonite clay, and (i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may comprise buffering agents.

[00222] Solid compositions of a similar type can be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type can be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

[00223] The active ingredient can be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active ingredient can be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may comprise, as is normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may comprise buffering agents. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or

preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner.

Examples of embedding compositions which can be used include polymeric substances and waxes.

[00224] Dosage forms for topical and/or transdermal administration of a compound of this invention may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants and/or patches. Generally, the active ingredient is admixed under sterile conditions with a pharmaceutically acceptable carrier or excipient and/or any needed preservatives and/or buffers as can be required. Additionally, the present invention contemplates the use of transdermal patches, which often have the added advantage of providing controlled delivery of an active ingredient to the body. Such dosage forms can be prepared, for example, by dissolving and/or dispensing the active ingredient in the proper medium. Alternatively or additionally, the rate can be controlled by either providing a rate controlling membrane and/or by dispersing the active ingredient in a polymer matrix and/or gel.

[00225] Suitable devices for use in delivering intradermal pharmaceutical compositions described herein include short needle devices. Intradermal compositions can be administered by devices which limit the effective penetration length of a needle into the skin. Jet injection devices which deliver liquid vaccines to the dermis *via* a liquid jet injector and/or *via* a needle which pierces the stratum corneum and produces a jet which reaches the dermis are suitable. Ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis are suitable. Alternatively or additionally, conventional syringes can be used in the classical mantoux method of intradermal administration.

[00226] Formulations suitable for topical administration include, but are not limited to, liquid and/or semi-liquid preparations such as liniments, lotions, oil in water and/or water in oil emulsions such as creams, ointments and/or pastes, and/or solutions and/or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient can be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

[00227] Low boiling propellants generally include liquid propellants having a boiling point of below 65 °F at atmospheric pressure. Generally the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w)

of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic and/or solid anionic surfactant and/or a solid diluent (which may have a particle size of the same order as particles comprising the active ingredient).

[00228] Pharmaceutical compositions of the invention formulated for pulmonary delivery may provide the active ingredient in the form of droplets of a solution and/or suspension. Such formulations can be prepared, packaged, and/or sold as aqueous and/or dilute alcoholic solutions and/or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization and/or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, and/or a preservative such as methylhydroxybenzoate. The droplets provided by this route of administration may have an average diameter in the range from about 0.1 to about 200 nanometers.

[00229] Formulations described herein as being useful for pulmonary delivery are useful for intranasal delivery of a pharmaceutical composition of the invention. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 to 500 micrometers. Such a formulation is administered by rapid inhalation through the nasal passage from a container of the powder held close to the nares.

[00230] Formulations for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition of the invention can be prepared, packaged, and/or sold in a formulation for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may contain, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising the active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

[00231] A pharmaceutical composition of the invention can be prepared, packaged, and/or sold in a formulation for ophthalmic administration. Such formulations may, for

example, be in the form of eye drops including, for example, a 0.1/1.0% (w/w) solution and/or suspension of the active ingredient in an aqueous or oily liquid carrier or excipient. Such drops may further comprise buffering agents, salts, and/or one or more other of the additional ingredients described herein. Other ophthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form and/or in a liposomal preparation. Ear drops and/or eye drops are contemplated as being within the scope of this invention.

[00232] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with ordinary experimentation.

[00233] Compounds provided herein are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject or organism will depend upon a variety of factors including the disease being treated and the severity of the disorder; the activity of the specific active ingredient employed; the specific composition employed; the age, body weight, general health, sex, and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific active ingredient employed; the duration of the treatment; drugs used in combination or coincidental with the specific active ingredient employed; and like factors well known in the medical arts.

[00234] The compounds and compositions provided herein can be administered by any route, including enteral (*e.g.*, oral), parenteral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subcutaneous, intraventricular, transdermal, interdermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, and/or drops), mucosal, nasal, bucal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. Specifically contemplated routes are oral administration, intravenous administration (*e.g.*, systemic intravenous injection), regional administration *via* blood and/or lymph supply, and/or direct

administration to an affected site. The inventive compounds and compositions may also be mixed with blood *ex vivo*, and the resulting mixture may be administered (*e.g.*, intravenously) to a subject. In general the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (*e.g.*, its stability in the environment of the gastrointestinal tract), and/or the condition of the subject (*e.g.*, whether the subject is able to tolerate oral administration).

[00235] The exact amount of a compound required to achieve an effective amount will vary from subject to subject, depending, for example, on species, age, and general condition of a subject, severity of the side effects or disorder, identity of the particular compound, mode of administration, and the like. The desired dosage can be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dosage can be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations).

[00236] In certain embodiments, an effective amount of a compound for administration one or more times a day to a 70 kg adult human may comprise about 0.0001 mg to about 3000 mg, about 0.0001 mg to about 2000 mg, about 0.0001 mg to about 1000 mg, about 0.001 mg to about 1000 mg, about 0.01 mg to about 1000 mg, about 0.1 mg to about 1000 mg, about 1 mg to about 1000 mg, about 1 mg to about 100 mg, about 10 mg to about 1000 mg, or about 100 mg to about 1000 mg, of a compound per unit dosage form.

[00237] In certain embodiments, the compounds of the invention may be at dosage levels sufficient to deliver from about 0.001 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, preferably from about 0.1 mg/kg to about 40 mg/kg, preferably from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, and more preferably from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect.

[00238] It will be appreciated that dose ranges as described herein provide guidance for the administration of provided pharmaceutical compositions to an adult. The amount to be administered to, for example, a child or an adolescent can be determined by a medical practitioner or person skilled in the art and can be lower or the same as that administered to an adult.

[00239] It will be also appreciated that a compound or composition, as described herein, can be administered in combination with one or more additional therapeutically active

agents. The compounds or compositions can be administered in combination with additional therapeutically active agents that improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body. It will also be appreciated that the therapy employed may achieve a desired effect for the same disorder, and/or it may achieve different effects.

[00240] The compound or composition can be administered concurrently with, prior to, or subsequent to, one or more additional therapeutically active agents. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. It will further be appreciated that the additional therapeutically active agent utilized in this combination can be administered together in a single composition or administered separately in different compositions. The particular combination to employ in a regimen will take into account compatibility of the inventive compound with the additional therapeutically active agent and/or the desired therapeutic effect to be achieved. In general, it is expected that additional therapeutically active agents utilized in combination be utilized at levels that do not exceed the levels at which they are utilized individually. In some embodiments, the levels utilized in combination will be lower than those utilized individually.

[00241] Exemplary additional therapeutically active agents include, but are not limited to, anti-cancer agents, anti-diabetic agents, anti-inflammatory agents, immunosuppressant agents, a pain-relieving agent, anti-Parkinsonism agents, anti-Alzheimer agents, anti-aging agent, and/or sunscreen agents. Therapeutically active agents include small organic molecules such as drug compounds (*e.g.*, compounds approved by the U.S. Food and Drug Administration as provided in the Code of Federal Regulations (CFR)), peptides, proteins, carbohydrates, monosaccharides, oligosaccharides, polysaccharides, nucleoproteins, mucoproteins, lipoproteins, synthetic polypeptides or proteins, small molecules linked to proteins, glycoproteins, steroids, nucleic acids, DNAs, RNAs, nucleotides, nucleosides, oligonucleotides, antisense oligonucleotides, lipids, hormones, vitamins, and cells.

[00242] The present invention provides cosmetic compositions comprising a compound of any one of Formula (I)-(III), and cosmetically acceptable salts, tautomers, stereoisomers, solvates, hydrates, isotopically enriched derivatives, and polymorphs thereof, and optionally a cosmetically acceptable excipient. In certain embodiments, the compound of the present invention, or a cosmetically acceptable salt thereof, is provided in an effective amount in the cosmetic composition. In certain embodiments, the effective amount is a prophylactically effective amount. In certain embodiments, the provided cosmetic compositions are useful in improving skin appearance. In certain embodiments, the provided

cosmetic compositions are useful in the prevention and/or treatment of skin aging. In certain embodiments, the provided cosmetic compositions are useful in the prevention and/or treatment of skin photoaging. In certain embodiments, the provided cosmetic compositions are useful in the prevention and/or treatment of skin cancer.

[00243] The provided cosmetic compositions may be formulated with other cosmetically acceptable components, and vehicles, *e.g.*, emulsions or serums, into a composition for topical application to the skin. The compositions may include other ingredients, such as, for example, alkylene oxide copolymer, emulsifiers, sunscreens, thickeners, botanicals, film formers, pH adjusters, fragrances, and preservatives. The compositions are topically applied to the skin in effective amounts, by which is meant an amount sufficient to achieve a measurable improvement in skin health or reduction in one or more dermatological signs of aging with daily (once, twice, etc.) administration, typically for a period of at least one week or more. Such signs of skin aging include without limitation, the following: (a) treatment, reduction, and/or prevention of fine lines or wrinkles; (b) reduction of skin pore size; (c) improvement in skin thickness, plumpness, and/or tautness; (d) improvement in skin smoothness, suppleness and/or softness; (e) improvement in skin tone, radiance, and/or clarity; (f) improvement in procollagen, and/or collagen production; (g) improvement in maintenance and remodeling of elastin; (h) improvement in skin texture and/or promotion of retexturization; (i) improvement in skin barrier repair and/or function; (j) improvement in appearance of skin contours; (k) restoration of skin luster and/or brightness; (l) replenishment of essential nutrients and/or constituents in the skin; (m) improvement of skin appearance decreased by aging and/or menopause; (n) improvement in skin moisturization; (o) increase in skin elasticity and/or resiliency; (p) treatment, reduction, and/or prevention of skin sagging; (q) improvement in skin firmness; and/or (r) reduction of pigment spots and/or mottled skin (s) improvement of optical properties of skin by light diffraction or reflection.

[00244] Also encompassed by the invention are kits (*e.g.*, pharmaceutical packs). The kits provided may comprise an inventive pharmaceutical or cosmetic composition or compound and a container (*e.g.*, a vial, ampule, bottle, syringe, and/or dispenser package, or other suitable container). In some embodiments, provided kits may optionally further include a second container comprising a pharmaceutical or cosmetic excipient for dilution or suspension of an inventive pharmaceutical or cosmetic composition or compound. In some embodiments, the inventive pharmaceutical or cosmetic composition or compound provided in the first container and the second container are combined to form one unit dosage form.

[00245] Thus, in another aspect, provided are kits for treating and/or preventing a pathological condition of a subject. In certain embodiments, the kits include a first container comprising a compound of the present invention, or a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, or composition thereof; and an instruction for administering the compound, or a pharmaceutically or cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, or composition thereof, to the subject to treat and/or prevent the pathological condition. In certain embodiments, the kits of the present invention include one or more additional approved therapeutic agents for use as a combination therapy. In certain embodiments, the instruction includes a notice in the form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use, or sale for human administration.

Methods of Treatment and Uses

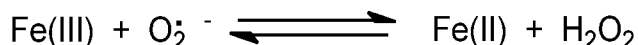
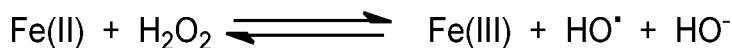
[00246] The compounds of the invention and pharmaceutical compositions thereof are useful in the treatment and/or prevention of a pathological condition in a subject. In certain embodiments, the pathological condition is responsive to the chelation or sequestration of a metal. In certain embodiments, the metal is iron (*e.g.*, Fe(III)). In certain embodiments, the metal is aluminum, thallium, chromium, magnesium, calcium, strontium, nickel, manganese, cobalt, copper, zinc, silver, sodium, potassium, cadmium, mercury, lead, antimony, molybdenum, tungsten, a lanthanide (*e.g.*, cerium), or an actinide (*e.g.*, uranium). In certain embodiments, the metal is a trivalent metal. In certain embodiments, the metal is a monovalent, divalent, tetravalent, pentavalent, or hexavalent metal. In certain embodiments, the subject is a human. In certain embodiments, the pathological condition is metal overload (*e.g.*, iron overload, aluminum overload, chromium overload, magnesium overload, calcium overload, strontium overload, nickel overload, manganese overload, cobalt overload, copper overload, zinc overload, silver overload, sodium overload, potassium overload, cadmium overload, mercury overload, lead overload, molybdenum overload, tungsten overload, or actinide overload (*e.g.*, uranium overload)). In certain embodiments, the pathological condition is iron overload. In certain embodiments, the pathological condition is metal poisoning (*e.g.*, iron poisoning, aluminum poisoning, thallium poisoning, chromium poisoning, magnesium poisoning, calcium poisoning, strontium poisoning, nickel poisoning, manganese poisoning, cobalt poisoning, copper poisoning, zinc poisoning, silver poisoning, sodium poisoning, potassium poisoning, cadmium poisoning, mercury poisoning, lead

poisoning, antimony poisoning, molybdenum poisoning, tungsten poisoning, lanthanide poisoning (*e.g.*, cerium poisoning), or actinide poisoning (*e.g.*, uranium poisoning). In certain embodiments, the pathological condition is oxidative stress, transfusional iron overload, thalassemia, primary hemochromatosis, secondary hemochromatosis, diabetes, liver disease, heart disease, cancer, radiation injury, neurological or neurodegenerative disorder (*e.g.*, Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, and neurodegeneration with brain iron accumulation), macular degeneration (*e.g.*, age-related macular degeneration), closed head injury, irritable bowel disease, stroke, and reperfusion injury. In certain embodiments, the pathological condition is an infectious disease (*e.g.*, HIV and malaria). In certain embodiments, the pathological condition is aging. In certain embodiments, the methods of treatment and/or prevention of a pathological condition include administering to the subject a therapeutically or prophylactically effective amount of a compound of the invention, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, or a pharmaceutical composition thereof.

[00247] In another aspect, in certain embodiments, the compounds of the invention and cosmetic compositions thereof are useful in improving skin appearance. In certain embodiments, the provided compounds and cosmetic compositions thereof are useful in the prevention and/or treatment of skin aging. In certain embodiments, the provided compounds and cosmetic compositions thereof are useful in the prevention and/or treatment of skin photoaging. In certain embodiments, the provided compounds and cosmetic compositions thereof are useful in the prevention and/or treatment of skin cancer. In certain embodiments, the methods of improving skin appearance include administering to the subject a therapeutically or prophylactically effective amount of a compound of the invention, or a cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, or a cosmetic composition thereof. In certain embodiments, the methods of preventing and/or treating skin aging, skin photoaging, and/or skin cancer include administering to the subject a therapeutically or prophylactically effective amount of a compound of the invention, or a cosmetically acceptable salt, tautomer, stereoisomer, solvate, hydrate, or polymorph thereof, or a cosmetic composition thereof.

[00248] The etiology and pathogenesis of the aforementioned pathological conditions and skin conditions may involve free iron and the generation of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, hypochlorous acid, and hydroxyl radicals, and other longer lived, free radicals. Free iron is known to contribute to the formation of reactive oxygen species. For example, Fe^{+2} ions in biological systems react with

oxygen species to produce highly reactive hydroxyl radicals via the Fenton reaction (see scheme below). The hydroxyl radical is a highly effective oxidizing agent, reacting at a diffusion-controlled rate with most organic species, such as nucleic acids, proteins, and lipids. Furthermore, superoxide anions or a biological reductant (*e.g.*, ascorbic acid) can reduce the resulting Fe^{+3} ion back to Fe^{+2} for continued peroxide reduction, thus a problematic cycle.



[00249] Without wishing to be bound by any particular theory, the compounds of the invention, after conversion to HBED, are thought to chelate or sequester a metal, and, in certain embodiments, the pathological condition is responsive to the chelation or sequestration of the metal. In certain embodiments, the metal is iron (*e.g.*, Fe(II) or Fe(III)), aluminum, thallium (*e.g.*, Tl(I) or Tl(III)), chromium (*e.g.*, Cr(III) or Cr(VI)), magnesium, calcium, strontium, nickel (*e.g.*, Ni(II)), manganese (*e.g.*, Mn(II)), cobalt (*e.g.*, Co(II) or Co(III)), copper (*e.g.*, Cu(I) or Cu(II)), zinc, silver (*e.g.*, Ag(I)), sodium, potassium, cadmium (*e.g.*, Cd(II)), mercury (*e.g.*, Hg(I) or Hg(II)), lead (*e.g.*, Pb(II) or Pb(IV)), antimony (*e.g.*, Sb(III) or Sb(V)), molybdenum (*e.g.*, Mo(III) or Mo(VI)), tungsten (*e.g.*, W(VI)), a lanthanide (*e.g.*, cerium, such as Ce(III) or Ce(IV)), or an actinide (*e.g.*, uranium, such as U(VI)). In certain embodiments, the metal is a trivalent metal. In certain embodiments, the metal is iron (*e.g.*, Fe(III)). In certain embodiments, the metal is aluminum. In certain embodiments, the metal is Tl(III), Cr(III), Co(III), Sb(III), Mo(III), or Ce(III). In certain embodiments, the metal is a monovalent metal (*e.g.*, Tl(I), Cu(I), Ag(I), Na(I), K(I), or Hg(I)). In certain embodiments, the metal is a divalent metal (*e.g.*, Fe(II), Mg(II), Ca(II), Sr(II), Ni(II), Mn(II), Co(II), Cu(II), Zn(II), Cd(II), Hg(II), or Pb(II)). In certain embodiments, the metal is a tetravalent metal (*e.g.*, Pb(IV) or Ce(IV)). In certain embodiments, the metal is a pentavalent metal (*e.g.*, Sb(V)). In certain embodiments, the metal is a hexavalent metal (*e.g.*, Cr(VI), Mo(VI), W(VI), or U(VI)).

[00250] In certain embodiments, the subject administered the inventive compound or pharmaceutical composition is a mammal. In certain embodiments, the subject is a human. In certain embodiments, the subject is a domesticated animal, such as a dog, cat, cow, pig, horse, sheep, or goat. In certain embodiments, the subject is a companion animal such as a dog or cat. In certain embodiments, the subject is a livestock animal such as a cow, pig,

horse, sheep, or goat. In certain embodiments, the subject is a zoo animal. In another embodiment, the subject is an experimental animal such as a rodent or non-human primate.

[00251] The inventive compounds, pharmaceutical compositions, and methods may also be useful for the treatment and/or prevention of infectious diseases in a subject. Infectious diseases are typically caused by microbial pathogens (*e.g.*, viruses, bacteria, parasites (*e.g.*, protozoa and multicellular parasites), and fungi) into the cells (“host cells”) of a subject (“host”). Iron is an oxidant as well as a nutrient for many microorganisms. To survive and replicate, microbial pathogens must acquire iron from their host. Highly virulent microbial strains usually possess powerful mechanisms for obtaining iron from their host. Depriving the pathogenic microbes of iron may inhibit their activities and may be useful for the treatment and/or prevention of the infectious diseases caused by microbes. In certain embodiments, the pathological condition that is treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is a viral infection (*e.g.*, HIV). In certain embodiments, the pathological condition is a bacterial infection. In certain embodiments, the pathological condition is a parasitic infection. In certain embodiments, the pathological condition is a protozoan infection. In certain embodiments, the pathological condition is malaria. Malaria is typically caused by parasites of the genus *Plasmodium* (phylum *Apicomplexa*), including, but not limited to, the species *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*, and *P. knowlesi*. In certain embodiments, the pathological condition is a multicellular-parasitic infection. In certain embodiments, the pathological condition is a fungal infection.

[00252] In certain embodiments, methods are provided herein that are useful in the treatment and/or prevention of metal overload in a subject. The amount of free metal (*e.g.*, a trivalent metal, such as iron(III) or aluminum) may be elevated in the subject (*e.g.*, in the serum or in a cell), such as when there is insufficient storage capacity for the metal or an abnormality in the metal storage system that leads to metal release. In certain embodiments, the metal overload is iron overload (*e.g.*, Fe(III) overload or Fe(II) overload).

[00253] Iron overload conditions or diseases can be characterized by global iron overload or focal iron overload. Global iron overload conditions generally involve an excess of iron in multiple tissues or excess iron located throughout an organism. Global iron overload conditions can result from excess uptake of iron by a subject, excess storage and/or retention of iron, from, for example, dietary iron or blood transfusions. One global iron overload condition is primary hemochromatosis, which is typically a genetic disorder. A second global iron overload condition is secondary hemochromatosis, which is typically the

result of receiving multiple (chronic) blood transfusions. Blood transfusions are often required for subjects suffering from thalassemia or sickle cell anemia. A type of dietary iron overload is referred to as Bantu siderosis, which is associated with the ingestion of homebrewed beer with high iron content. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is global iron overload. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is focal iron overload. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is primary hemochromatosis. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is secondary hemochromatosis. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is Bantu siderosis.

[00254] In focal iron overload conditions, the excess iron is limited to one or a few cell types or tissues or a particular organ. Alternatively, symptoms associated with the excess iron are limited to a discrete organ, such as the heart, lungs, liver, pancreas, kidneys, or brain. It is believed that focal iron overload can lead to neurological or neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, Huntington's disease, neurodegeneration with brain iron accumulation (*e.g.*, pantothenate kinase-associated neurodegeneration (NBIA1), neuroferritinopathy, and aceruloplasminemia), amyotrophic lateral sclerosis, and multiple sclerosis. Pathological conditions that benefit from metal chelation or sequestration are often associated with deposition of the metal in the tissues of a subject. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is a neurological or neurodegenerative disorder. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is a neurological disorder. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is a neurodegenerative disorder. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is Parkinson's disease. In certain embodiments, the pathological condition that

may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is Alzheimer's disease. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is Huntington's disease. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is neurodegeneration with brain iron accumulation. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is amyotrophic lateral sclerosis. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is multiple sclerosis. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is Friedreich's ataxia.

[00255] While humans have a highly efficient iron management system in which they absorb and excrete about 1 mg of iron daily, there is no conduit for the excretion of excess metal. Transfusion-dependent anemias, like thalassemia, lead to a build up of iron in the liver, heart, pancreas, and elsewhere resulting in (i) liver disease that may progress to cirrhosis (Angelucci *et al.*, 2000; Bonkovsky *et al.*, 2000; Peitrangelo, 2002, (ii) diabetes related both to iron-induced decreases in pancreatic beta -cell secretion and to increases in hepatic insulin resistance (Cario *et al.*, 2004; Wojcik *et al.*, 2002, and (iii) heart disease. Relative excess iron has been associated with increased risk of heart disease. Cardiac failure is still the leading cause of death in thalassemia major and related forms of transfusional iron overload (Brittenham, 2000; Brittenham *et al.*, 1994; Zurlo *et al.*, 1989). There is a strong correlation between serum ferritin levels, inflammatory biomarkers such as C-reactive protein and interleukin-1, and mortality in a subset of patients with peripheral arterial disease; phlebotomy and iron chelation has been used to mitigate that risk. Treatment with an iron chelator would reduce iron stores, reduce serum ferritin and potentially reduce the incidence of heart disease and stroke. In certain embodiments, the pathological condition that is treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is transfusional iron overload. In certain embodiments, the pathological condition that is treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is transfusion-dependent anemia. In certain embodiments, the pathological condition that is treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is thalassemia. In certain embodiments, the

pathological condition that is treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is a liver disease (*e.g.*, hepatitis B, hepatitis C, and liver cirrhosis). In certain embodiments, the pathological condition that is treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is a heart disease (*e.g.*, cardiomyopathy, coronary heart disease, inflammatory heart disease, ischemic heart disease, valvular heart disease, hypertensive heart disease, and atherosclerosis). In certain embodiments, the pathological condition that is treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is a pancreas disease. In certain embodiments, the pathological condition that is treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is diabetes.

[00256] Moreover, the compounds, pharmaceutical compositions, and methods of the present invention may be useful in the treatment and/or prevention of metal overload where the metal is not iron. All metals described herein are contemplated for chelation by the inventive compounds. In certain embodiments, the metal is aluminum. In certain embodiments, the metal is Tl(III), Cr(III), Co(III), Sb(III), Mo(III), or Ce(III). In certain embodiments, the metal is a monovalent metal (*e.g.*, Tl(I), Cu(I), Ag(I), Na(I), K(I), or Hg(I)). In certain embodiments, the metal is a divalent metal (*e.g.*, Fe(II), Mg(II), Ca(II), Sr(II), Ni(II), Mn(II), Co(II), Cu(II), Zn(II), Cd(II), Hg(II), or Pb(II)). In certain embodiments, the metal is a tetravalent metal (*e.g.*, Pb(IV) or Ce(IV)). In certain embodiments, the metal is a pentavalent metal (*e.g.*, Sb(V)). In certain embodiments, the metal is a hexavalent metal (*e.g.*, Cr(VI), Mo(VI), W(VI), or U(VI)).

[00257] In certain embodiments, the metal overload is aluminum overload, chromium overload, magnesium overload, calcium overload, strontium overload, nickel overload, manganese overload, cobalt overload, copper overload, zinc overload, silver overload, sodium overload, potassium overload, cadmium overload, mercury overload, lead overload, molybdenum overload, tungsten overload, or actinide overload (*e.g.*, uranium overload). In certain embodiments, the metal overload is trivalent metal overload. In certain embodiments, the metal overload is aluminum overload. In certain embodiments, the metal overload is Cr(III) overload, Mo(III) overload, or Co(III) overload). In certain embodiments, the metal overload is monovalent metal overload (*e.g.*, Cu(I) overload, Ag(I) overload, Na(I) overload, K(I) overload, or Hg(I) overload). In certain embodiments, the metal overload is divalent metal overload (*e.g.*, Mg(II) overload, Ca(II) overload, Sr(II) overload, Ni(II) overload, Mn(II) overload, Co(II) overload, Cu(II) overload, Zn(II) overload, Cd(II) overload, Hg(II)

overload, or Pb(II) overload). In certain embodiments, the metal overload is tetravalent metal overload (*e.g.*, Pb(IV) overload). In certain embodiments, the metal overload is pentavalent metal overload. In certain embodiments, the metal overload is hexavalent metal overload (*e.g.*, Cr(VI) overload, Mo(VI) overload, W(VI) overload, or U(VI) overload).

[00258] The inventive compounds, pharmaceutical compositions, and methods may also be useful in treating and/or preventing metal poisoning in a subject. Metal poisoning may be caused by metal toxicity to a subject. For example, metals with little or no endogenous function may find their way into the body of a subject and cause damage. Heavy metal ions such as Hg(II) can replace ions such as Zn(II) in metalloproteins and render them inactive, resulting in serious acute or chronic toxicity that can end in a patient's death or in birth defects. Even more significantly, radioactive isotopes of the lanthanide (*e.g.*, cerium) and actinide (*e.g.*, uranium) series can cause grave illness in an individual exposed to them by mouth, air, or skin contact. Such exposure could result not only from the detonation of a nuclear bomb or a "dirty bomb" composed of nuclear waste, but also from the destruction of a nuclear power facility. In certain embodiments, the metal poisoning is iron poisoning, aluminum poisoning, thallium poisoning, chromium poisoning, magnesium poisoning, calcium poisoning, strontium poisoning, nickel poisoning, manganese poisoning, cobalt poisoning, copper poisoning, zinc poisoning, silver poisoning, sodium poisoning, potassium poisoning, cadmium poisoning, mercury poisoning, lead poisoning, antimony poisoning, molybdenum poisoning, tungsten poisoning, lanthanide poisoning (*e.g.*, cerium poisoning), or actinide poisoning (*e.g.*, uranium poisoning). In certain embodiments, the metal poisoning is iron poisoning (*e.g.*, Fe(II) poisoning or Fe(III) poisoning). In certain embodiments, the metal poisoning is aluminum poisoning. In certain embodiments, the metal poisoning is trivalent metal poisoning (*e.g.*, Fe(III) poisoning, Al(III) poisoning, Tl(III) poisoning, Cr(III) poisoning, Co(III) poisoning, Sb(III) poisoning, Mo(III) poisoning, or Ce(III) poisoning). In certain embodiments, the metal poisoning is monovalent metal poisoning (*e.g.*, Tl(I) poisoning, Cu(I) poisoning, Ag(I) poisoning, Na(I) poisoning, K(I) poisoning, or Hg(I) poisoning). In certain embodiments, the metal poisoning is divalent metal poisoning (*e.g.*, Fe(II) poisoning, Mg(II) poisoning, Ca(II) poisoning, Sr(II) poisoning, Ni(II) poisoning, Mn(II) poisoning, Co(II) poisoning, Cu(II) poisoning, Zn(II) poisoning, Cd(II) poisoning, Hg(II) poisoning, or Pb(II) poisoning). In certain embodiments, the metal poisoning is tetravalent metal poisoning (*e.g.*, Pb(IV) or Ce(IV) poisoning). In certain embodiments, the metal poisoning is pentavalent metal poisoning (*e.g.*, Sb(V) poisoning). In certain

embodiments, the metal poisoning is hexavalent metal poisoning (*e.g.*, Cr(VI) poisoning, Mo(VI) poisoning, W(VI) poisoning, or U(VI) poisoning).

[00259] The compounds, pharmaceutical compositions, and methods of the invention are also useful in treating and/or preventing oxidative stress in a subject. In a subject who suffers from oxidative stress and thus needs oxidative stress reduction, the iron released from red blood cells of the subject may react with oxygen species produced by inflammatory cells such as neutrophils to produce hydroxyl radicals that cause cell and tissue injury. Chelation and removal of the unmanaged iron may prevent or impede these harmful reactions and, therefore, reduce oxidative stress. A subject in need of oxidative stress reduction can have one or more of the following conditions: decreased levels of reducing agents, increased levels of reactive oxygen species, mutations in or decreased levels of antioxidant enzymes (*e.g.*, Cu/Zn superoxide dismutase, Mn superoxide dismutase, glutathione reductase, glutathione peroxidase, thioredoxin, thioredoxin peroxidase, DT-diaphorase), mutations in or decreased levels of metal-binding proteins (*e.g.*, transferrin, ferritin, ceruloplasmin, albumin, metallothionein), mutated or overactive enzymes capable of producing superoxide (*e.g.*, nitric oxide synthase, NADPH oxidases, xanthine oxidase, NADH oxidase, aldehyde oxidase, dihydroorotate dehydrogenase, cytochrome c oxidase), and radiation injury. Increased or decreased levels of reducing agents, reactive oxygen species, and proteins are determined relative to the amount of such substances typically found in healthy persons. A subject in need of oxidative stress reduction can be suffering from an ischemic episode. Ischemic episodes can occur when there is mechanical obstruction of the blood supply, such as from arterial narrowing or disruption. Myocardial ischemia, which can give rise to angina pectoris and myocardial infarctions, results from inadequate circulation of blood to the myocardium, usually due to coronary artery disease. Ischemic episodes in the brain that resolve within 24 hours are referred to as transient ischemic attacks. A longer-lasting ischemic episode, a stroke, involves irreversible brain damage, where the type and severity of symptoms depend on the location and extent of brain tissue whose access to blood circulation has been compromised. A subject at risk of suffering from an ischemic episode typically suffers from atherosclerosis, other disorders of the blood vessels, increased tendency of blood to clot, or heart disease.

[00260] A subject in need of oxidative stress reduction can be suffering from inflammation. Inflammation is a fundamental pathologic process consisting of a complex of cytologic and chemical reactions that occur in blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by a physical, chemical, or biologic agent.

Inflammatory disorders are characterized by inflammation that lasts for an extended period (*i.e.*, chronic inflammation) or that damages tissue. Such inflammatory disorders can affect a wide variety of tissues, such as respiratory tract, joints, bowels, and soft tissue. The compounds or pharmaceutical compositions of the invention can be used to treat these pathological conditions. Not wishing to be bound by any theory, it is believed that the compounds of the invention derive their ability to reduce oxidative stress through various mechanisms. In one mechanism, the compound binds to a metal, particularly a redox-active metal (*e.g.*, iron), and fills all of the coordination sites of the metal. When all of the metal coordination sites are filled, it is believed that oxidation and/or reducing agents have a diminished ability to interact with the metal and cause redox cycling. In another mechanism, the compound stabilizes the metal in a particular oxidation state, such that it is less likely to undergo redox cycling. In yet another mechanism, the compound itself has antioxidant activity (*e.g.*, free radical scavenging, scavenging of reactive oxygen or nitrogen species). Desferrithiocin and desazadesferrithiocin, and their derivatives and analogs, are known to have intrinsic antioxidant activity, as described in U.S. Application Publication No. 2004/0044220, published March 4, 2004 and now abandoned; U.S. Application Publication No. 2004/0132789 and now abandoned, published July 8, 2004; International PCT Application Publication No. WO2004/017959, published March 4, 2004; U.S. Application Publication No. 2005/0234113, published October 20, 2005 and now abandoned; U.S. Application Publication No. 2008/0255081, published October 16, 2008 and now abandoned; U.S. Application Publication No. 2003/0236417, published December 25, 2003 and now abandoned; U.S. Patent Application, U.S.S.N. 61/576,920, filed December 16, 2011; U.S. Patent Application, U.S.S.N. 61/576,913, filed December 16, 2011; and U.S. Patent Nos.: 6,083,966, 6,559,315, 6,525,080, 6,521,652, 7,126,004, 7,531,563, and 8,008,502; each of which is incorporated herein by reference. HBED is also known to have intrinsic antioxidant activity (Samuni *et al.*, 2001). The compounds of the invention can be used to treat these pathological conditions. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is oxidative stress. In certain embodiments, the compounds, pharmaceutical compositions, and methods of the present invention are useful in the reduction of oxidative stress. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is radiation injury. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the

invention is an ischemic episode. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is stroke. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is inflammation.

[00261] In certain embodiments, the provided compounds, pharmaceutical compositions, and methods of the invention are useful to treat or prevent a neurodegenerative disease. Neurodegenerative diseases refer to a type of neurological disease marked by the loss of nerve cells, including, but not limited to, Alzheimer's disease, Parkinson's disease, Friedreich's ataxia, neurodegeneration with brain iron accumulation, amyotrophic lateral sclerosis, tauopathies (including frontotemporal dementia), and Huntington's disease. In certain embodiments, the neurodegenerative disease is Parkinson's disease. In certain embodiments, the neurodegenerative disease is Alzheimer's disease. In certain embodiments, the neurodegenerative disease is Friedreich's ataxia. In certain embodiments, the neurodegenerative disease is neurodegeneration with brain iron accumulation. In certain embodiments, the compounds, pharmaceutical compositions, and methods as described herein can be used to prevent the onset of a neurodegenerative disease, *e.g.* to prevent the progression of an early neurodegenerative disease to advanced forms of a neurodegenerative disease.

[00262] Macular degeneration refers to a family of diseases that are characterized by a progressive loss of central vision associated with abnormalities of Bruch's membrane, the choroid, the neural retina and/or the retinal pigment epithelium. Exemplary macular degeneration includes, but is not limited to age-related macular degeneration (AMD), geographic atrophy (GA), Stargardt disease, and retinitis pigmentosa. In certain embodiments, the macular degeneration is age-related macular degeneration (AMD). In certain embodiments, the macular degeneration is dry AMD. In certain embodiments, the macular degeneration is wet AMD. In certain embodiments, the macular degeneration is developed in a subject who has developed Alzheimer's disease. In certain embodiments, the macular degeneration is developed in a subject who is at risk of developing Alzheimer's disease. In certain embodiments, subjects who are currently asymptomatic but are at risk of developing a symptomatic macular degeneration related disorder are suitable for administration with a compound or a pharmaceutical composition as described herein. The methods of treating or preventing macular degeneration include, but are not limited to, methods of treating or preventing one or more symptoms or aspects of macular degeneration

selected from formation of ocular drusen, inflammation of the eye or eye tissue, loss of photoreceptor cells, loss of vision (including loss of visual acuity or visual field), neovascularization (including CNV), retinal detachment, photoreceptor degeneration, retinal pigment epithelium (RPE) degeneration, retinal degeneration, chorioretinal degeneration, cone degeneration, retinal dysfunction, retinal damage in response to light exposure, damage of the Bruch's membrane, and/or loss of RPE function. In certain embodiments, the compounds and pharmaceutical compositions as described herein can be used, *inter alia*, to prevent the onset of macular disease, *e.g.*, to prevent the progression of early AMD to advanced forms of AMD including neovascular AMD or geographic atrophy, to slow and/or prevent progression of geographic atrophy, to treat or prevent macular edema from AMD or other conditions (such as diabetic retinopathy, uveitis, or post surgical or non-surgical trauma), to prevent or reduce the loss of vision from AMD, and to improve vision lost due to pre-existing, early or advanced AMD.

[00263] The compound being used in the treatment may have the ability to cross the blood brain barrier. In certain embodiments, when the subject has been diagnosed with a neurodegenerative disease, the compound being used in the treatment can pass through the blood brain barrier. In certain embodiments, when the subject has been diagnosed with Parkinson's disease, the compound used in the treatment can pass through the blood brain barrier. The compound being used in the treatment may have the ability to cross the blood-retinal barrier. In certain embodiments, when the subject has been diagnosed with macular degeneration, the compound used in the treatment can pass through the blood-retinal barrier.

[00264] Moreover, the present invention may be useful in treating a subject after the subject has been diagnosed with having a neurodegenerative disease or macular degeneration, or a subject who is susceptible to having a neurodegenerative disease or macular degeneration may be administered a compound of the invention or composition thereof to prevent or minimize the neurodegenerating effects.

[00265] The compounds of the invention and pharmaceutical compositions thereof are expected to be useful in the treatment of head injury, particularly those involving bleeding into the brain or other parts of the central nervous system. Without wishing to be bound by any particular theory, the compounds of the invention are thought to chelate the iron from red blood cells of the blood resulting from the head injury, thereby preventing iron ions from generating reactive oxygen species. In the case of head injury resulting in bleeding into the central nervous system where the vasculature has been compromised, a compound being used may or may not have the ability to cross the blood brain barrier. In certain embodiments, the

compound being used to treat a head injury in a subject is able to cross the blood brain barrier. In other embodiments, the compounds are not able to cross the blood brain barrier.

[00266] Head injuries come in various forms and result from various causes. In certain embodiments, the injury is an injury to the head that penetrates the skull. In other embodiments, the head injury being treated is a closed head injury, which does not penetrate the skull. Closed head injuries results from a variety of causes including accidents including vehicular accidents, falls, and assaults. Types of closed head injuries include concussions, brain contusions, diffuse axonal injury, and hematoma. In certain embodiments, the closed head injury being treated in the present invention includes closed head injuries that result in blood outside the blood vessels of the brain.

[00267] The local accumulation of iron from the bleeding is thought to contribute to after effects associated with closed head injury. By assisting the clearance of iron from the brain the effects of the bleeding are minimized.

[00268] In the treatment of closed head injury, the compound of the invention or a pharmaceutical composition thereof may be administered systemically, for example, parenterally or orally. In certain embodiments, the compound or composition is administered orally. In other embodiments, the compound or composition is administered parenterally (*e.g.*, intravenously).

[00269] Reactive oxygen species have been implicated in the pathogenesis of inflammatory bowel disease (IBD). Grisham *et al.*, 1988; Allgayer, 1991; Yamada *et al.*, 1991; Babbs, 1992. The present invention provides for the treatment or preventon of IBD. DFO, an iron chelator, has been discovered to prevent acetic acid-induced colitis in rats, an animal model of IBD. See, *e.g.*, U.S. Patent Application, U.S.S.N. 61/576,920, filed December 16, 2011; U.S. Patent Application, U.S.S.N. 61/576,913, filed December 16, 2011; Bergeron *et al.*, 2003. The compounds used in the inventive treatment are thought to prevent or eliminate the generation of reactive oxygen species or other longer-lived, more stable radicals that may be responsible for the tissue damage and inflammation seen in subjects with IBD. Another possible mechanism of action of the compounds useful in the invention is the chelation of metal, such as iron, which may contribute to the generation of reactive oxygen species, such as hydroxyl radicals and hydrogen peroxide, that cause cell damage.

[00270] The present invention may also be useful in treating a subject diagnosed with IBD. The treatment may be used to treat the subject long term or may be used to treat a subject with a flare up of IBD. A therapeutically effective amount of a compound of the invention or pharmaceutical composition thereof is administered to a subject in need thereof

to treat IBD. In certain embodiments, treatment with a compound of the invention leads to reduced levels of reactive oxygen species in the intestines, specifically the intestinal mucosa. The compound or composition thereof may be administered to a subject once or multiple times in the treatment of IBD.

[00271] In the treatment of IBD, the compound of the invention or a pharmaceutical composition thereof may be administered systemically, for example, parenterally or orally. In certain embodiments, the compound or composition is administered orally. In other embodiments, the compound or composition is administered parenterally (*e.g.*, intravenously). In certain embodiments, the compound or a composition is administered rectally.

[00272] The methods of the present invention are also useful in the treatment and/or prevention of stroke. The inventive treatment typically leads to a better and/or faster recovery from stroke. The stroke being treated may be either an ischemic stroke or a hemorrhagic stroke. In the treatment of an ischemic stroke, a compound of the invention or a pharmaceutical composition thereof is administered to a subject to prevent or minimize the damage due to reperfusion injury after the blood supply to the affected part of the brain is restored. The compound is thought to prevent the generation of reactive oxygen species by either chelating iron responsible for the generation of such species and/or quenching such radical species when they do occur. In hemorrhagic stroke, the compound is thought to work by similar mechanisms although the sequestering of iron from the blood in the brain is probably the predominate mechanism by which the inventive treatment works. The mechanism of action of the compound of the invention is similar to that in the treatment of head injury.

[00273] The compound being used in the treatment may have the ability to cross the blood brain barrier. In certain embodiments, when the subject has been diagnosed with an ischemic stroke, the compound used in the treatment can pass through the blood brain barrier.

[00274] Moreover, the present invention may be useful in treating a subject after the subject has been diagnosed with having a stroke, or a subject who is susceptible to having a stroke may be administered a compound of the invention or composition thereof to prevent or minimize the stroke's effects. In certain embodiments, the compound is administered as quickly as possible after a subject has been diagnosed with having a stroke. In certain embodiments, the compound is administered to the subject while the stroke is still occurring. In certain embodiments, the compound or a composition thereof is administered to a subject who has a history of strokes or is susceptible to having a stroke because of the subject's

underlying medical condition. The compound or composition thereof may be administered once or multiple times in the treatment of stroke.

[00275] In the treatment of stroke the compound of the invention or a pharmaceutical composition thereof may be administered systemically, for example, parenterally or orally. In certain embodiments, the compound or composition is administered orally. In other embodiments, the compound or composition is administered parenterally (*e.g.*, intravenously).

[00276] The present invention also provides for the treatment of reperfusion injury. Reperfusion injury may occur in any area of the body where the blood supply has been compromised. In certain embodiments, the reperfusion injury being treated occurs in the heart. In other embodiments, the reperfusion injury occurs in the brain, for example, as discussed above in the context of a stroke. The inventive treatment minimizes reperfusion injury once the blood supply to the affected organ or tissue is restored. In the treatment and/or prevention of reperfusion injury, a compound of the present invention or pharmaceutical composition thereof is administered to a subject who is suffering from ischemia of a tissue or organ. Without wishing to be bound by any particular theory, the compound of the invention is thought to prevent the generation of reactive oxygen species by either chelating iron responsible for the generation of such species and/or quenching such radical species when they do occur.

[00277] The present invention may be useful in treating a subject after the subject has been diagnosed with ischemia of a particular organ or tissue. A therapeutically effective amount of a compound of the invention or composition thereof is administered to a subject to prevent or minimize reperfusion injury. In certain embodiments, the compound is administered as quickly as possible after a subject has been diagnosed with ischemia. In certain embodiments, the compound is administered to the subject at risk of ischemia. In certain embodiments, the compound or a composition thereof is administered to a subject who is about to undergo a procedure that may lead to ischemia of an organ or tissue (*e.g.*, cardiac surgery). In certain embodiments, the compound or a composition thereof is used to prevent reperfusion injury in a transplanted organ. In certain embodiments, the compound or composition thereof is used to perfuse an isolated organ being prepared for donation. The compound or composition thereof may be administered to a subject once or multiple times in the treatment of reperfusion injury.

[00278] In the prevention or treatment of reperfusion injury, the compound of the invention or a pharmaceutical composition thereof may be administered systemically, for

example, parenterally or orally. In certain embodiments, the compound or composition is administered orally. In other embodiments, the compound or composition is administered parenterally (*e.g.*, intravenously). In certain embodiments, the compound or a composition is administered locally to the organ or tissue suffering from ischemia.

[00279] The inventive compounds, or pharmaceutical compositions thereof, may also be useful in the treatment and/or prevention of a neoplastic disease or preneoplastic condition. A neoplastic disease (*i.e.*, neoplasm) is characterized by an abnormal tissue that grows by cellular proliferation more rapidly than normal tissue. The abnormal tissue continues to grow after the stimuli that initiated the new growth cease. Neoplasms show a partial or complete lack of structural organization and functional coordination with the normal tissue, and usually form a distinct mass of tissue that may be benign or malignant. A malignant neoplastic disease is also known as cancer. Neoplasms can occur, for example, in a wide variety of tissues including brain, skin, mouth, nose, esophagus, lungs, stomach, pancreas, liver, bladder, ovary, uterus, testicles, colon, and bone, as well as the immune system (lymph nodes) and endocrine system (thyroid gland, parathyroid glands, adrenal gland, thymus, pituitary gland, pineal gland). Cancer cells have a higher requirement for iron than normal cells as they rapidly proliferate. Therefore, depleting iron from rapidly dividing cancer cells through the implementation of iron chelators results in cell cycle arrest and apoptosis (Kalinowski and Richardson, 2005; Yu *et al.*, 2012). In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is a benign neoplastic disease. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is cancer. In certain embodiments, the pathological condition that may be treated and/or prevented by the compounds, pharmaceutical compositions, and methods of the invention is acoustic neuroma; adenocarcinoma; adrenal gland cancer; anal cancer; angiosarcoma (*e.g.*, lymphangiosarcoma, lymphangioendotheliosarcoma, hemangiosarcoma); appendix cancer; benign monoclonal gammopathy; biliary cancer (*e.g.*, cholangiocarcinoma); bladder cancer; breast cancer (*e.g.*, adenocarcinoma of the breast, papillary carcinoma of the breast, mammary cancer, medullary carcinoma of the breast); brain cancer (*e.g.*, meningioma, glioblastomas, glioma (*e.g.*, astrocytoma, oligodendroglioma), medulloblastoma); bronchus cancer; carcinoid tumor; cervical cancer (*e.g.*, cervical adenocarcinoma); choriocarcinoma; chordoma; craniopharyngioma; colorectal cancer (*e.g.*, colon cancer, rectal cancer, colorectal adenocarcinoma); connective tissue cancer; epithelial carcinoma; ependymoma;

endotheliosarcoma (*e.g.*, Kaposi's sarcoma, multiple idiopathic hemorrhagic sarcoma); endometrial cancer (*e.g.*, uterine cancer, uterine sarcoma); esophageal cancer (*e.g.*, adenocarcinoma of the esophagus, Barrett's adenocarcinoma); Ewing sarcoma; eye cancer (*e.g.*, intraocular melanoma, retinoblastoma); familial hypereosinophilia; gall bladder cancer; gastric cancer (*e.g.*, stomach adenocarcinoma); gastrointestinal stromal tumor (GIST); germ cell cancer; head and neck cancer (*e.g.*, head and neck squamous cell carcinoma, oral cancer (*e.g.*, oral squamous cell carcinoma), throat cancer (*e.g.*, laryngeal cancer, pharyngeal cancer, nasopharyngeal cancer, oropharyngeal cancer)); hematopoietic cancers (*e.g.*, leukemia such as acute lymphocytic leukemia (ALL) (*e.g.*, B-cell ALL, T-cell ALL), acute myelocytic leukemia (AML) (*e.g.*, B-cell AML, T-cell AML), chronic myelocytic leukemia (CML) (*e.g.*, B-cell CML, T-cell CML), and chronic lymphocytic leukemia (CLL) (*e.g.*, B-cell CLL, T-cell CLL)); lymphoma such as Hodgkin lymphoma (HL) (*e.g.*, B-cell HL, T-cell HL) and non-Hodgkin lymphoma (NHL) (*e.g.*, B-cell NHL such as diffuse large cell lymphoma (DLCL) (*e.g.*, diffuse large B-cell lymphoma), follicular lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), mantle cell lymphoma (MCL), marginal zone B-cell lymphomas (*e.g.*, mucosa-associated lymphoid tissue (MALT) lymphomas, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma), primary mediastinal B-cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma (*i.e.*, Waldenström's macroglobulinemia), hairy cell leukemia (HCL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma and primary central nervous system (CNS) lymphoma; and T-cell NHL such as precursor T-lymphoblastic lymphoma/leukemia, peripheral T-cell lymphoma (PTCL) (*e.g.*, cutaneous T-cell lymphoma (CTCL) (*e.g.*, mycosis fungoides, Sezary syndrome), angioimmunoblastic T-cell lymphoma, extranodal natural killer T-cell lymphoma, enteropathy type T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, and anaplastic large cell lymphoma); a mixture of one or more leukemia/lymphoma as described above; and multiple myeloma (MM)), heavy chain disease (*e.g.*, alpha chain disease, gamma chain disease, mu chain disease); hemangioblastoma; hypopharynx cancer; inflammatory myofibroblastic tumors; immunocytic amyloidosis; kidney cancer (*e.g.*, nephroblastoma *a.k.a.* Wilms' tumor, renal cell carcinoma); liver cancer (*e.g.*, hepatocellular cancer (HCC), malignant hepatoma); lung cancer (*e.g.*, bronchogenic carcinoma, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), adenocarcinoma of the lung); leiomyosarcoma (LMS); mastocytosis (*e.g.*, systemic mastocytosis); muscle cancer; myelodysplastic syndrome (MDS); mesothelioma; myeloproliferative disorder (MPD) (*e.g.*, polycythemia Vera (PV), essential thrombocytosis

(ET), agnogenic myeloid metaplasia (AMM) *a.k.a.* myelofibrosis (MF), chronic idiopathic myelofibrosis, chronic myelocytic leukemia (CML), chronic neutrophilic leukemia (CNL), hypereosinophilic syndrome (HES)); neuroblastoma; neurofibroma (*e.g.*, neurofibromatosis (NF) type 1 or type 2, schwannomatosis); neuroendocrine cancer (*e.g.*, gastroenteropancreatic neuroendocrine tumor (GEP-NET), carcinoid tumor); osteosarcoma (*e.g.*, bone cancer); ovarian cancer (*e.g.*, cystadenocarcinoma, ovarian embryonal carcinoma, ovarian adenocarcinoma); papillary adenocarcinoma; pancreatic cancer (*e.g.*, pancreatic adenocarcinoma, intraductal papillary mucinous neoplasm (IPMN), Islet cell tumors); penile cancer (*e.g.*, Paget's disease of the penis and scrotum); pinealoma; primitive neuroectodermal tumor (PNT); plasma cell neoplasia; paraneoplastic syndromes; intraepithelial neoplasms; prostate cancer (*e.g.*, prostate adenocarcinoma); rectal cancer; rhabdomyosarcoma; salivary gland cancer; skin cancer (*e.g.*, squamous cell carcinoma (SCC), keratoacanthoma (KA), melanoma, basal cell carcinoma (BCC)); small bowel cancer (*e.g.*, appendix cancer); soft tissue sarcoma (*e.g.*, malignant fibrous histiocytoma (MFH), liposarcoma, malignant peripheral nerve sheath tumor (MPNST), chondrosarcoma, fibrosarcoma, myxosarcoma); sebaceous gland carcinoma; small intestine cancer; sweat gland carcinoma; synovioma; testicular cancer (*e.g.*, seminoma, testicular embryonal carcinoma); thyroid cancer (*e.g.*, papillary carcinoma of the thyroid, papillary thyroid carcinoma (PTC), medullary thyroid cancer); urethral cancer; vaginal cancer; or vulvar cancer (*e.g.*, Paget's disease of the vulva).

[00280] A preneoplastic condition precedes the formation of a benign or malignant neoplasm. A precancerous lesion typically forms before a malignant neoplasm. Preneoplastic conditions include, but are not limited to, photodermatitis, x-ray dermatitis, tar dermatitis, arsenic dermatitis, lupus dermatitis, senile keratosis, Paget disease, condylomata, burn scar, syphilitic scar, fistula scar, ulcer cruris scar, chronic ulcer, varicose ulcer, bone fistula, rectal fistula, Barrett esophagus, gastric ulcer, gastritis, cholelithiasis, kraurosis vulvae, nevus pigmentosus, Bowen dermatosis, xeroderma pigmentosum, erythroplasia, leukoplakia, Paget disease of bone, exostoses, ecchondroma, osteitis fibrosa, leontiasis ossea, neurofibromatosis, polyposis, hydatidiform mole, adenomatous hyperplasia, and struma nodosa. The compounds, pharmaceutical compositions, and methods of the present invention can be used to treat and/or prevent these preneoplastic conditions.

[00281] Imaging or examining one or more organs, tissues, tumors, or a combination thereof can be conducted after a metal salt of a compound of the invention is administered to a subject. The methods of imaging and examining are intended to encompass various instrumental techniques used for diagnosis, such as x-ray methods (including CT scans and

conventional x-ray images), magnetic imaging (magnetic resonance imaging, electron paramagnetic resonance imaging) and radiochemical methods. Typically, the metal salts used in imaging or examining serve as a contrast agent. Therefore in one embodiment the metal complexes or metal salts of compounds of the present invention can be used as contrast agents for example in imaging or examining one or more organs, for example, the gastrointestinal tract. Metals that can serve as contrast agents include gadolinium, iron, manganese, chromium, dysprosium, technetium, scandium, barium, aluminum and holmium, preferably as trications. Radioactive metal salts can be made from isotopes including ^{241}Am , ^{51}Cr , ^{60}Co , ^{57}Co , ^{58}Co , ^{64}Cu , ^{153}Gd , ^{67}Ga , ^{198}Au , ^{113m}In , ^{111}In , ^{59}Fe , ^{55}Fe , ^{197}Hg , ^{203}Hg , ^{99m}Tc , ^{201}Tl , and ^{169}Yb , again preferably when the metal is present as a trivalent cation.

EXAMPLES

Experimental

Materials and Instrumentation

[00282] All solvents were purchased from Fisher Scientific or Sigma Aldrich. Reaction solvents were ACS certified and LC-MS solvents were Optima grade. Solvents noted as “dry” were obtained following storage over 3 Å molecular sieves (Acros Organics), except for DMSO, which was purchased (extra dry over molecular sieves, Acros Organics). LC-MS solvents were filtered using Magna 0.22 micron nylon membrane filters (GVS Maine). Syringe filtration was performed using Magna 0.45 micron nylon membrane filters (GVS Maine) or Whatman Puradisc 25 AS 0.2 micron polyethersulfone membrane filters (GE Healthcare Life Sciences). The following chemicals were used: ammonium iron(III) citrate (Acros Organics), copper(II) sulfate pentahydrate (99+%, Acros Organics), crystal violet solution (0.41%, Protocol, Fisher Scientific), diisopropylethylamine (peptide synthesis grade, Fisher Scientific), ethyl bromoacetate (98%, Sigma Aldrich), ethylenediamine (anhydrous, Fisher Scientific), ethylenediaminetetraacetic acid disodium dihydrate (Sigma Aldrich), formic acid (Optima LC/MS, Fisher Scientific), 2-formyl-4-methoxyphenylboronic acid (97%, Sigma Aldrich), 2-formylphenylboronic acid (97%, Matrix Scientific), hydrochloric acid (TraceMetal grade, Fisher Scientific), hydrogen peroxide (50 wt% solution in water, stabilized, Acros Organics), isopropyl bromoacetate (99%, Sigma Aldrich), methanesulfonic acid (99%, Sigma Aldrich), methyl bromoacetate (99%, Acros Organics), N-methylmorpholine (99.5%, Sigma Aldrich), paraformaldehyde (Fisher Scientific), pinacol (99%, Acros Organics), (1R,2R,3S,5R)-(-)-pinanediol (99%, Sigma Aldrich), and sodium borohydride ($\geq 98\%$, Sigma Aldrich). The mono-hydrochloride salt of HBED was

generously donated by PPC ADOB (Poland) and recrystallized from boiling 85% EtOH. Reactions were monitored by thin-layer chromatography (Whatman UV₂₅₄ aluminum-backed silica gel). NMR spectra were recorded on a Varian Mercury-Plus 400 MHz spectrometer at ambient temperature, a Bruker Avance III 500 MHz spectrometer at 27 °C, and a Bruker Avance III 600 MHz spectrometer at 27 °C. The chemical shifts of ¹H and ¹³C spectra are expressed in ppm and J values in Hz. CDCl₃ and DMSO-d₆ spectra were referenced to the TMS internal standard, while CD₃OD spectra were referenced to the solvent residual peak at 3.31 ppm. The splitting of proton resonances in the reported ¹H spectra is defined as: s = singlet, d = doublet, t = triplet, sep = septet, dd = doublet of doublets, m = multiplet, br = broad, app = apparent. The ¹¹B NMR spectra were referenced to an external standard of boron trifluoride diethyl etherate (0.0 ppm) in CDCl₃ and chemical shifts were reported in ppm. Background from the borosilicate glass in the probe and NMR tubes was minimized by first obtaining spectra of empty NMR tubes and then subtracting them from the corresponding [tube-matched] spectra of samples. Melting points (MP) were determined on a Mel-temp melting point apparatus. Elemental analysis (EA) was performed by Atlantic Microlab, Inc. (Norcross, GA). UV/Visible spectra were recorded on two Shimadzu spectrophotometers, models 2550 (equipped with a CPS controller) and 2700 (equipped with a TCC controller), using 1-cm quartz cuvettes. Mass spectra were recorded on a 3200 Qtrap LC/MS/MS system (AB Sciex, USA) in electrospray ionization (ESI) mode. High-resolution mass spectra were recorded on an Orbitrap XL in positive ESI mode (Thermo Finnigan, USA). LC-MS was performed using a Prominence SIL-20 autosampler (Shimadzu, Japan), LC-20AD pumps (Shimadzu, Japan), and a Prominence SPD-M20A photo diode array detector (Shimadzu, Japan), connected to the 3200 Qtrap LC/MS/MS system in ESI mode with positive polarity.

Synthesis and Characterization

N,N'-bis-(2-boronic acid benzyl)ethylenediamine (**3a**, Figure 1)

[00283] Procedure modified from Gray and Houston (2002) and Gray *et al.* (2003). To a stirred solution of ethylenediamine (**2**, 2.31 g, 0.038 mol) in 250 mL dry methanol (MeOH) at room temperature was added 2-formylphenylboronic acid (**1a**, 11.51 g, 0.077 mol), giving a bright-yellow solution containing minimal light-colored precipitate. After 2 h, 3 equiv. sodium borohydride (4.36 g, 0.12 mol) was added in 3 batches, 15 min. apart, to the reaction flask immersed in an ice bath. The reaction flask was removed from the ice bath 10 min. after the final sodium borohydride addition and was stirred for an additional 50 min. at room

temperature. The faintly-yellow reaction mixture containing fine, white precipitate was concentrated on the rotary evaporator (35 °C) and then further dried *in vacuo* to afford a cream-colored solid.

[00284] To this solid was added 450 mL dichloromethane (DCM), and the mixture was stirred vigorously for 1 h before the boron salts were removed by vacuum filtration. The white, filtered solid was washed with 100 mL DCM and discarded after ¹H NMR in CD₃OD confirmed that it did not contain a significant amount of **3a**. The slightly yellow filtrate was concentrated via rotary evaporator (35 °C) and *in vacuo* to afford a cream-colored solid.

[00285] To this solid was added 195 mL 1 M NaOH. After vigorous swirling, most of the solid dissolved. Further precipitation of white solid occurred when the ice-cooled mixture was adjusted to a final pH of 8.29 with concentrated HCl. The suspension was vacuum filtered and washed with 4 x 20 mL cold H₂O. The white, powdery solid was dried *in vacuo* at room temperature (~2 days) and at 70 °C (12 h) to give 10.34 g of the title compound **3** in ~87% yield. ¹H (500 MHz, CD₃OD): δ 7.42-7.44 (m, 2 H, Ph-H), 7.17-7.24 (m, 6 H, Ph-H), 4.10 (s, 4 H, Ph-CH₂-NHR), 3.22 (s, 4 H, RNH-CH₂-CH₂). ¹³C (APT, 100 MHz, CD₃OD,): δ 142.77 (Ph quaternary), 131.47 (Ph), 128.49 (Ph), 127.65 (Ph), 123.86 (Ph), 55.10 (Ph-CH₂-NHR), 45.94 (RNH-CH₂-CH₂). ¹¹B (161 MHz, CD₃OD): δ 9.84. MP: 145-150 °C (dehydration), 212-218 °C (melted). EA Found: C, 63.13, 63.25; H, 6.49, 6.34; N, 9.21, 9.15. Calc. for C₁₆H₂₂B₂N₂O₄: C, 58.59; H, 6.76; N, 8.54. MS (*m/z*): 311.2 (M + H - H₂O; Calc: 311.17).

N,N'-bis-(2-boronic acid-5-methoxy benzyl)ethylenediamine (**3b**, Figure 1)

[00286] A similar procedure to the one described above for **3a** was followed, but replacing 2-formylphenylboronic acid (**1a**) with 2-formyl-4-methoxyphenylboronic acid (**1b**) and omitting the dichloromethane trituration step. A pale-tan powder was isolated in 59% yield. ¹H (400 MHz, CD₃OD): δ 7.32 (d, 2 H, J = 8.0, Ph-H), 6.81 (dd, 2 H, J = 8.0, 2.2, Ph-H), 6.77 (d, 2 H, J = 2.2, Ph-H), 4.07 (s, 4 H, Ph-CH₂-NHR), 3.78 (s, 6 H, OCH₃), 3.20 (s, 4 H, RNH-CH₂-CH₂).

N,N'-bis-(2-boronic acid pinacol ester benzyl)ethylenediamine (**5a**, Figure 1)

[00287] In a round-bottom flask equipped with a stir bar was added compound **3a** (1.00 g), pinacol (**4a**, 0.77 g), and 80 mL toluene. A Dean-Stark trap was attached and filled with toluene. The suspension was refluxed for 22 h. The white suspension was cooled for 1 h and then concentrated at 55 °C on the rotary evaporator. The off-white solid (**5a**) was

further dried *in vacuo* and used in the next step without further purification. ^1H (400 MHz, CDCl_3): δ 7.685 (br d, 2 H, $J = 5.8$, Ph-H), 7.23-7.30 (m, Ph-H), 7.13 (d, 2 H, $J = 7.2$, Ph-H), 3.96 (s, 4 H, Ph-CH₂-NHR), 2.77 (s, 4 H, NHR-CH₂-CH₂), 1.31 (s, 24 H, CH₃). ^{13}C (APT, 100 MHz, CDCl_3): δ 144.48 (Ph quaternary), 134.90 (Ph), 130.51 (Ph), 128.39 (Ph), 127.32 (Ph), 83.15 (BO-C-R₃), 53.97(Ph-CH₂-NHR), 46.85 (RNH-CH₂-CH₂), 26.70 (CH₃). MP: 211.5-217.5 °C. MS (m/z): 493.6 (M + H; Calc: 492.34).

N,N'-bis-(2-boronic acid pinacol ester-5-methoxy benzyl)ethylenediamine (**5b**, Figure 1)

[00288] A similar procedure to the one described above for **5a** was followed, but replacing **3a** with **3b**. A white solid was isolated and used in the next step without further purification. ^1H (400 MHz, CDCl_3): δ 7.63 (d, 2 H, $J = 8.16$, Ph-H), 6.80 (dd, 2 H, $J = 8.1$, 2.4, Ph-H), 6.70 (d, 2 H, $J = 2.40$, Ph-H), 3.93 (s, 4 H, Ph-CH₂-NHR), 3.80 (s, 6 H, Ph-OCH₃), 2.77 (s, 4 H, NHR-CH₂-CH₂), 1.29 (s, 24 H, pinacol CH₃). ^{13}C (APT, 100 MHz, CDCl_3): δ 160.95 (Ph quaternary), 145.45 (Ph quaternary), 135.41 (Ph), 112.53 (Ph), 112.24 (Ph), 81.84 (BO-C-R₃), 55.29 (OCH₃), 52.70 (CH₂), 45.72 (CH₂), 25.35 (pinacol CH₃). MP: 167-170 °C.

N,N'-bis-(2-boronic acid pinanediol ester benzyl)ethylenediamine (**5c**, Figure 1)

[00289] A similar procedure to the one described above for **5a** was followed, but replacing pinacol (**4a**) with (1R,2R,3S,5R)-(-)-pinanediol (**4b**). A white, crystalline solid was isolated and used in the next step without further purification. ^1H (400 MHz, CDCl_3): δ 7.71 (d, 2 H, $J = 7.1$), 7.14-7.31 (m, with toluene impurity), 4.30 (d, 2 H, $J = 8.1$), 4.01 (d, 2 H, $J = 13.7$), 3.87 (d, 2 H, $J = 13.7$), 2.69-2.84 (m, 4 H), 2.38 (m, with toluene impurity), 2.22 (m, 2 H), 2.09 (t, 2 H, $J = 5.5$), 1.95 (m, 4 H), 1.51 (d, 2 H, $J = 10.5$), 1.45 (s, 6 H), 1.30 (s, 6 H), 0.88 (s, 6 H).

N,N'-bis-(2-boronic acid pinacol ester benzyl)ethylenediamine-*N,N'*-diacetic acid esters (**7a-c**, Figure 1)

[00290] **General procedure:** Into a round-bottom flask equipped with a stir bar was added **5a** from the previous step (~0.0032 mol, 1.0 equiv) and 100 mL dry acetonitrile (ACN), followed by 1.16 g (0.0090 mol, 2.79 equiv) diisopropylethylamine (DIPEA) and another 50 mL dry ACN. The flask containing an off-white suspension was then equipped with a condenser and drying tube, and heated to 82 °C. Next, 2.03 equiv. of methyl bromoacetate (**6a**), ethyl bromoacetate (**6b**), or isopropyl bromoacetate (**6c**) was added all at

once to the reaction mixture. The mixture was refluxed for 20.5 h, during which time the off-white suspension became gold-hued and contained fine, light-colored precipitate. After cooling for 1 hour, the mixture was concentrated at 40 °C on the rotary evaporator to afford a soft solid, which was further dried *in vacuo*. This crude product was then extracted with 250 mL dry diethyl ether for 2 hours, followed by vacuum filtration. The faintly peach-colored, crystalline solid was washed with 50 mL dry ether and then discarded. The pale-yellow filtrate was concentrated at 35 °C on the rotary evaporator and then further dried *in vacuo* to yield an oil, which was used in the next step without further purification.

[00291] Methyl ester (7a): Yellow-orange oil containing a small amount of light-colored precipitate. ^1H (400 MHz, CDCl_3): δ 7.68 (d, 2 H, $J = 7.2$, Ph-H), 7.30-7.38 (m, 4 H, Ph-H), 7.20 (dt, 2 H, $J = 7.3$, 1.2, Ph-H), 3.94 (s, 4 H, Ph-CH₂-NR₂), 3.61 (s, 6 H, OCH₃), 3.36 (s, 4H, R₂N-CH₂-COOR), 2.72 (s, 4 H, R₂N-CH₂-CH₂-NR₂), 1.31 (s, 24 H, CH₃ pinacol). ^{13}C (APT, 100 MHz, CDCl_3): δ 172.33 (C=O), 145.44 (Ph quaternary), 135.32 (Ph), 130.43 (Ph), 129.36 (Ph), 126.33 (Ph), 83.58 (BO-C-R₃), 58.15 (Ph-CH₂-NR₂), 54.37 (R₂N-CH₂-COOR), 51.18 (R₂N-CH₂-CH₂-NR₂), 51.12 (COO-CH₃), 25.05 (CH₃ pinacol). ^{11}B NMR (193 MHz, CDCl_3): δ 31.48. MS (m/z): 637.3 (M + H; Calc: 637.38).

[00292] Ethyl ester (7b): Yellow-orange oil containing a small amount of light-colored precipitate. ^1H (400 MHz, CDCl_3): δ 7.68 (d, 2 H, $J = 7.3$, Ph-H), 7.36 (d, 2 H, $J = 7.5$, Ph-H), 7.31 (t, 2 H, $J = 7.4$ Hz, Ph-H), 7.20 (t, 2 H, $J = 7.2$, Ph-H), 4.08 (q, 4 H, $J = 7.1$, O-CH₂-CH₃), 3.95 (s, 4 H, Ph-CH₂-NR₂), 3.34 (s, 4 H, R₂N-CH₂-COOR), 2.73 (s, 4 H, R₂N-CH₂-CH₂-NR₂), 1.31 (s, 24 H, CH₃ pinacol), 1.22 (t, 6 H, $J = 7.1$, O-CH₂-CH₃).

[00293] Isopropyl ester (7c): Yellow-orange oil. ^1H (400 MHz, CDCl_3): δ 7.68 (d, 2 H, $J = 7.1$, Ph-H), 7.37 (d, 2 H, $J = 7.4$, Ph-H), 7.31 (t, 2 H, $J = 7.3$, Ph-H), 7.19 (t, 2 H, $J = 7.1$, Ph-H), 4.98 (sep, 2 H, $J = 6.2$, O-CH-(CH₃)₂), 3.96 (s, 4 H, Ph-CH₂-NR₂), 3.31 (s, 4 H, R₂N-CH₂-COOR), 2.73 (s, 4 H, R₂N-CH₂-CH₂-NR₂), 1.30 (s, 24 H, CH₃ pinacol), 1.20 (d, 12 H, $J = 6.2$, O-CH₂-(CH₃)₂). ^{13}C (APT, 100 MHz, CDCl_3): δ 171.40 (C=O), 145.65 (Ph quaternary), 135.30 (Ph), 130.37 (Ph), 129.19 (Ph), 126.19 (Ph), 83.50 (BO-C-R₃), 67.43 (COO-CH), 58.09 (Ph-CH₂-NR₂), 54.85 (R₂N-CH₂-COOR), 51.18 (R₂N-CH₂-CH₂-NR₂), 25.01 (CH₃ pinacol), 22.04 (O-CH₂-(CH₃)₂). ^{11}B NMR (193 MHz, CDCl_3): δ 31.58. MS (m/z): 693.3 (M + H; Calc: 693.45).

N,N'-bis-(2-boronic acid pinacol ester-5-methoxy benzyl)ethylenediamine-*N,N'*-diacetic acid methyl ester (**7d**, Figure 1)

[00294] A similar procedure to the one described above for **7a** was followed, but replacing *N,N'*-bis-(2-boronic acid pinacol ester benzyl)ethylenediamine (**5a**) with *N,N'*-di-(2-boronic acid pinacol ester-5-methoxy benzyl)ethylenediamine (**5b**). A yellow-orange oil containing light-colored precipitate was obtained. NMR suggested it contained two products, one of which was the title compound. ¹H (400 MHz, CDCl₃): δ 7.65 (d, 2 H, J = 8.2, Ph-H), 7.04 (d, 2 H, J = 2.6, Ph-H), 6.72 (dd, 2 H, J = 8.2, 2.6), 3.96 (s, 4 H, CH₂), 3.77 (s, 6 H, OCH₃), 3.63 (s, 6 H, OCH₃), 3.39 (s, 4 H, CH₂), 2.77 (s, 4 H, CH₂), 1.30 (s, 24 H, pinacol CH₃).

N,N'-bis-(2-boronic acid pinanediol ester benzyl)ethylenediamine-*N,N'*-diacetic acid methyl ester (**7e**, Figure 1)

[00295] A similar procedure to the one described above for **7a** was followed, with some modifications. Methyl bromoacetate (**6a**) was first converted to methyl iodoacetate by reacting **6a** (0.0037 mol, 2.2 equiv) with sodium iodide (0.0036 mol, 2.11 equiv) overnight at room temperature in 45 mL dry ACN. DIPEA (0.0049 mol, 2.91 equiv) and *N,N'*-di-(2-boronic acid pinanediol ester benzyl)ethylenediamine (**5c**, 0.0017 mol, 1.0 equiv) were then added, and the mixture was refluxed overnight. The reaction mixture was concentrated and extracted with diethyl ether to give a yellow-brown oil. This material was purified by flash column chromatography (silica gel, 0-30% gradient of ethyl acetate in hexanes) to give the title compound as a yellow oil in 66% yield. ¹H (400 MHz, CDCl₃): δ 7.67 (d, 2 H, J = 7.3), 7.32 (m, 4 H), 7.21 (m, 2 H), 4.34 (d, 2 H, J = 8.3), 3.98 (d, 2 H, J = 13.5), 3.91 (d, 2 H, J = 13.5), 3.62 (s, 6 H), 3.35 (s, 4 H), 2.75 (m, 4 H), 2.37 (m, 2 H), 2.21 (m, 2 H), 2.09 (m, 2 H), 1.91 (m, 4 H), 1.42 (s, 6 H), 1.30 (m, 8 H), 0.88 (s, 6 H).

N,N'-bis-(2-boronic acid benzyl)ethylenediamine-*N,N'*-diacetic acid tert-butyl ester (**7f**, Figure 1)

[00296] Into a round-bottom flask equipped with a stir bar was added *tert*-butyl chloroacetate (**6d**, 0.002 mol, 2 equiv) in 10 mL dry ACN, followed by NaI (0.002 mol, 2 equiv). The pale-yellow suspension containing white precipitate was protected from light and stirred at room temperature for 3.5 h. Next, to this suspension was added DIPEA (0.0029 mol, 2.86 equiv), followed 10 min later by *N,N'*-bis(2-boronic acid benzyl)ethylenediamine (**3a**, 0.00097 mol, 0.95 equiv). The flask containing an off-white suspension was then

equipped with a condenser and drying tube, and refluxed for 18 h. After cooling for 1 h, the suspension was concentrated at 40 °C on the rotary evaporator to afford a cream solid, which was further dried *in vacuo*. This crude product was then extracted with 50 mL dry diethyl ether for 2 hours, followed by vacuum filtration. The filtrate was concentrated at 35 °C on the rotary evaporator and then further dried *in vacuo* to give the title compound as a pale-yellow solid (lightweight crystalline shards) in approximately 15% yield. ¹H (400 MHz, MeOD, major product): δ 7.1-7.46 (m, 8 H), 3.94 (s, 4 H), 3.26 (s, 4 H), 3.01 (s, 4 H), 1.46 (s, 18 H).

N,N'-bis(2-boronic acid pinacol ester-5-methoxy benzyl)ethylenediamine-*N,N'*-diacetic acid isopropyl ester (**7g**, Figure 1)

[00297] A similar procedure to the one described above for **7c** was followed, but replacing *N,N'*-bis(2-boronic acid pinacol ester benzyl)ethylenediamine (**5a**) with *N,N'*-di-(2-boronic acid pinacol ester-5-methoxy benzyl)ethylenediamine (**5b**). A pale-yellow, cloudy oil was obtained. ¹H (400 MHz, CDCl₃): δ 7.66 (d, 2 H, J = 8.2, Ph-H), 7.08 (d, 2 H, J = 2.6, Ph-H), 6.73 (dd, 2 H, J = 8.3, 2.5, Ph-H), 4.98 (sep, 2 H, J = 6.2, O-CH-(CH₃)₂), 3.98 (s, 4 H, Ph-CH₂-NR₂), 3.77 (s, 6 H, OCH₃), 3.35 (s, 4 H, R₂N-CH₂-COOR), 2.78 (s, 4 H, R₂N-CH₂-CH₂-NR₂), 1.29 (s, 24 H, CH₃ pinacol), 1.20 (d, 12 H, J = 6.3, O-CH-CH₃)₂. ¹³C (APT, 100 MHz, CDCl₃): δ 171.50 (C=O), 161.78 (Ph quaternary), 148.46 (Ph quaternary), 137.45 (Ph), 114.17 (Ph), 111.97 (Ph), 83.39 (BO-C-R₃), 67.50 (COO-CH), 57.86 (Ph-CH₂-NR₂), 55.10 (Ph-OCH₃), 55.00 (R₂N-CH₂-COOR), 51.64 (R₂N-CH₂-CH₂-NR₂), 24.96 (CH₃ pinacol), 22.00 (O-CH₂-(CH₃)₂).

Conversion of Compounds 7a-c,e to Dimesylate Salts (8a-c,e, Figure 1)

[00298] **General procedure:** To a stirring solution of **7a-c,e** (1.0 equiv) in dry diethyl ether at room temperature was added methanesulfonic acid (1.9 equiv) dropwise over ~5 min. After 2 h, the precipitate was collected via vacuum filtration, washed with dry ether, and dried *in vacuo*.

[00299] **8a:** Required recrystallization from dry DCM (55 mL) with dry diethyl ether (55 mL) to give a white solid (~59% yield over 3 steps). ¹H NMR (500 MHz, CDCl₃): δ 7.92 (dd, 2 H, J = 7.4, 1.2, Ph-H), 7.77 (d, 2 H, J = 7.6, Ph-H), 7.52 (dt, 2 H, J = 7.6, 1.5, Ph-H), 7.43 (dt, 2 H, J = 7.4, 0.9, Ph-H), 4.90 (s, 4 H, CH₂), 4.22 (s, 4 H, CH₂), 4.11 (s, 4 H, CH₂), 3.66 (s, 6 H, COOCH₃), 2.79 (s, 6H, SO₃CH₃), 1.41 (s, 24 H, CH₃ pinacol). ¹³C NMR (APT, 100 MHz, CDCl₃): δ 166.24 (C=O), 137.08 (Ph), 134.44 (Ph quaternary), 132.69 (Ph),

132.25 (Ph), 129.53 (Ph), 84.96 (BO-C-R₃), 59.42 (CH₂), 53.08 (COO-CH₃), 52.54 (CH₂), 50.33 (CH₂), 39.30 (SO₃CH₃), 24.83 (CH₃ pinacol). ¹¹B NMR (161 MHz, CDCl₃): δ 30.39. MP: 158.5-160.5 °C. EA Found: C, 51.88; H, 6.90; N, 3.48. Calc for C₃₆H₅₈B₂N₂O₁₄S₂: C, 52.18, H, 7.06, N, 3.38. MS (*m/z*): 637.4 (M + H, minus 2 SO₃CH₃; Calc: 637.38).

[00300] 8b: Off-white solid (~77% yield over 3 steps). ¹H NMR (500 MHz, CDCl₃): δ 7.92 (dd, 2 H, J = 7.4, 1.1, Ph-H), 7.79 (d, 2 H, J = 7.6, Ph-H), 7.52 (dt, 2 H, J = 7.6, 1.4, Ph-H), 7.43 (dt, 2 H, J = 7.4, 1.0, Ph-H), 4.92 (s, 4 H, CH₂), 4.22 (s, 4 H, CH₂), 4.15 (s, 4 H, CH₂), 4.08 (q, 4 H, J = 7.1, O-CH₂-CH₃), 2.79 (s, 6 H, SO₃CH₃), 1.41 (s, 24 H, CH₃ pinacol), 1.19 (t, 6 H, J = 7.1, O-CH₂-CH₃). ¹³C NMR (APT, 100 MHz, CDCl₃): δ 165.77 (C=O), 137.07 (Ph), 134.46 (Ph quaternary), 132.81 (Ph), 132.22 (Ph), 129.51 (Ph), 84.96 (BO-C-R₃), 62.63 (COO-CH₂-CH₃), 59.43 (CH₂), 52.66 (CH₂), 50.40 (CH₂), 39.32 (SO₃CH₃), 24.83 (CH₃ pinacol), 13.87 (COO-CH₂-CH₃). ¹¹B NMR (161 MHz, CDCl₃): δ 31.35. MP: 140-143 °C. EA Found: C, 53.08; H, 7.40; N, 3.26. Calc. for C₃₈H₆₂B₂N₂O₁₄S₂ + 0.14 C₄H₁₀O (ether impurity): C, 53.42; H, 7.37; N, 3.23. MS (*m/z*): 665.5 (M + H, minus 2 SO₃CH₃; Calc: 665.41).

[00301] 8c: White solid (~81% yield over 3 steps). ¹H (400 MHz, CDCl₃): δ 7.93 (dd, 2 H, J = 7.3, 0.9, Ph-H), 7.81 (d, 2 H, J = 7.6, Ph-H), 7.52 (dt, 2 H, J = 7.5, 1.1, Ph-H), 7.43 (t, 2 H, J = 7.2, Ph-H), 4.96 (s, 4 H, CH₂), 4.89 (sep, 2 H, J = 6.2, O-CH₂-(CH₃)₂), 4.20 (s, 8 H, CH₂), 2.81 (s, 6 H, SO₃CH₃), 1.42 (s, 24 H, CH₃ pinacol), 1.16 (d, 12 H, J = 6.2, O-CH₂-(CH₃)₂). ¹³C NMR (APT, 100 MHz, CDCl₃): δ 165.30 (C=O), 137.11 (Ph), 134.50 (Ph quaternary), 132.86 (Ph), 132.18 (Ph), 129.48 (Ph), 84.95 (BO-C-R₃), 71.04 (COO-CH), 59.37 (CH₂), 52.80 (CH₂), 50.46 (CH₂), 39.34 SO₃CH₃), 24.83 (CH₃ pinacol), 21.50 (O-CH₂-(CH₃)₂), 21.50 (O-CH₂-(CH₃)₂). ¹¹B NMR (161 MHz, CDCl₃): δ 31.6. MP: 83-94 °C. EA Found: C, 54.11; H, 7.66; N, 3.18. Calc. for C₄₀H₆₆B₂N₂O₁₄S₂: C, 54.30; H, 7.52; N, 3.17. HRMS (*m/z*): 693.3946 (M + H, minus 2 SO₃CH₃; Calc: 693.4452).

[00302] 8e: White solid (72% yield). ¹H (400 MHz, CDCl₃): δ 7.94 (d, 2 H, J = 7.4), 7.68 (d, 2 H, J = 7.6), 7.52 (t, 2 H, J = 7.6), 7.44 (t, 2 H, J = 7.6), 4.98 (d, 2 H, J = 12.6), 4.86 (d, 2 H, J = 12.6), 4.63 (d, 2 H, J = 8.4), 4.09-4.24 (m, 8 H), 3.69 (s, 6 H), 2.78 (s, 6 H), 2.42 (m, 2 H), 2.14-2.30 (m, 4 H), 2.04 (d, 2 H, J = 15), 1.96 (br s, 2 H), 1.56 (s, 6 H), 1.32 (s, 6 H), 1.18 (d, 2 H, J = 11.1), 0.90 (s, 6 H).

Synthesis of Compound 9 (Figure 1)

[00303] General Procedure: To **8a-c** was added H₂O, and the solution (pH 3-4 by litmus) was allowed to stir at room temperature. White precipitate began to form within a

few hours. After several days, the white suspension was vacuum filtered, and the filtered solid was washed with cold water and dried *in vacuo*. The filtrate was monitored for additional precipitate for several days, and was re-filtered as necessary. The filtered solids were then combined and recrystallized from DMSO with water to yield a snow-white solid, which was dried *in vacuo*. The yield for the synthesis of the title compound from **8c** was 46% after subtraction of DMSO impurity. ^1H (500 MHz, DMSO- d_6 , mixture of diastereomers, Figure 3): δ 7.35-7.40 (m, 4 H, Ph-H), 7.18-7.28 (m, 8 H, Ph-H), 7.12 (app d, 4 H, J = 7.12, Ph-H), 5.18 (s, 2 OH), 5.16 (s, 2 OH), 4.455 (d, 2 H, J = 15.4, CH₂), 4.385 (d, 2 H, J = 15.4, CH₂), 4.14-4.17 (overlapping doublets, 2 H + 2 H, J = 15.35, CH₂), 4.00 (d, 2 H, J = 16.9, CH₂), 3.935 (d, 2 H, J = 16.9, CH₂), 3.62 (overlapping doublets, 4 H, J = 16.9, CH₂), 3.32 (m, hidden by H₂O peak, presumed 4 H, N-CH₂-CH₂-N), 3.24 (m, 4 H, N-CH₂-CH₂-N). ^{13}C (100 MHz, DMSO- d_6): δ 169.65, 169.63 (C=O), 139.86, 139.79 (quaternary Ph), 129.55, 129.52 (Ph), 127.80 (Ph), 126.94 (Ph), 122.78 (Ph), 61.90, 61.76 (CH₂), 57.51, 57.37 (CH₂), 48.69 (N-CH₂-CH₂-N). ^{11}B (161 MHz, DMSO- d_6): δ 14.33. MP: > 325 °C (formed a pellet and began browning at 285 °C). EA Found: C, 57.98; H, 5.60; N, 6.69. Calc. for C₂₀H₂₂B₂N₂O₆ + 0.149 C₂H₆OS (DMSO impurity): C, 58.09; H, 5.50; N, 6.68. HRMS (m/z): 408.1788 (M^{++} ; Calc: 408.1664). MS (m/z): 408.4 (M^{++}), 409.3 ($\text{M} + \text{H}$; Calc: 409.17).

Crystallization and X-Ray Data Collection

[00304] Compound **9** failed to yield crystalline material/single crystals after several efforts.

[00305] Crystals suitable for x-ray crystallography of compound **8a**, the dimesylate salt of N,N'-bis-(2-boronic acid pinacol ester benzyl)ethylenediamine-N,N'-diacetic acid methyl ester, were successfully grown by heating **8a** in dichloromethane until all solid had dissolved, followed by cooling to room temperature. Crystals began to form less than 16 hours after sitting at room temperature.

[00306] X-ray crystallography was performed by Dr. Khalil A. Abboud, Department of Chemistry, University of Florida. X-ray intensity data were collected at 100 K on a Bruker DUO diffractometer using MoK α radiation ($\lambda = 0.71073 \text{ \AA}$) and an APEXII CCD area detector.

[00307] Raw data frames were read by the program SAINT and integrated using 3D profiling algorithms. The resulting data were reduced to produce hkl reflections and their intensities and estimated standard deviations. The data were corrected for Lorentz and

polarization effects, and numerical absorption corrections were applied based on indexed and measured faces.

[00308] The structure was solved and refined in SHELXTL6.1 (Bruker-AXS, Wisconsin, USA), using full-matrix least-squares refinement. The non-H atoms were refined with anisotropic thermal parameters, and all of the H atoms were calculated in idealized positions and refined riding on their parent atoms. The asymmetric unit consists of a half cation and one counterion and a dichloromethane solvent molecule. The Cl and H atoms of the solvent are disordered and refined in two parts. The amine proton was obtained from a Difference Fourier map and refined freely. In the final cycle of refinement, 5529 reflections (of which 4832 are observed with $I > 2\sigma(I)$) were used to refine 309 parameters and the resulting R_1 , wR_2 and S (goodness of fit) were 2.85%, 7.48% and 1.056, respectively. The refinement was carried out by minimizing the wR_2 function using F^2 rather than F values. R_1 is calculated to provide a reference to the conventional R value but its function is not minimized.

Aqueous Solubilities

[00309] The molar absorptivities of prodrugs **8a** and **8b** were determined at 270 nm in 100 mM pH 7.4 sodium phosphate buffer. For each prodrug, three stock solutions of known concentrations were prepared in dry DMSO. A 30 μ L aliquot of each stock solution was added to 2970 μ L phosphate buffer and stirred for 30 min. The ensuing solution was analyzed by UV spectrophotometry. With absorbance (A), concentration (c) and path length known, ϵ_{270} ($L \text{ mol}^{-1} \text{ cm}^{-1}$) was calculated using Beer's law:

$$A_{270} = c * \epsilon_{270} * \ell$$

[00310] The aqueous solubilities of prodrugs **8a-c** were determined in triplicate in 100 mM pH 7.4 phosphate buffer. Each prodrug (10-11 mg) was stirred at room temperature in 5 mL phosphate buffer for 30 min. Each suspension was filtered through a 0.2 micron polyethersulfone membrane filter. An aliquot (2970 μ L) of the filtrate (pH 7.3) was immediately diluted with 30 μ L DMSO and analyzed by UV spectrophotometry. The concentration of each compound was determined using Beer's law (above) and the previously-measured molar absorptivities, except for compound **8c**, where the concentration was calculated using the molar absorptivity of **8a**.

[00311] The lower limit of aqueous solubility of HBED-HCl was investigated by adding 6 mL of 100 mM pH 7.4 phosphate buffer to a test tube containing 0.0259 g HBED-HCl (10 mM). The solid quickly dissolved, but the buffering capacity was exceeded, so the

pH was adjusted to 7.34 from 7.0 using 1 M NaOH. The solution was further stirred for 4 h and remained clear during that time.

Lipid Solubilities

[00312] The molar absorptivity (ϵ) of **7a** was determined at 274 nm in hexanes. Three stock solutions of **7a** were prepared by carefully transferring known amounts of oil from vials to volumetric flasks using hexanes. The stock solutions were then further diluted and analyzed by UV spectrophotometry. With absorbance (A), concentration (c , corrected for a 2.18 wt% diethyl ether impurity) and path length known, ϵ_{274} ($\text{L mol}^{-1} \text{cm}^{-1}$) was calculated using Beer's law:

$$A_{274} = c * \epsilon_{274} * \ell$$

[00313] The solubility of **7a** in mineral oil was ascertained at room temperature in quadruplicate by stirring 132-155 mg **7a** in 1 mL light mineral oil for 24 h, with 3-4.5 of those hours under house vacuum to remove residual ether. After the turbid emulsions were centrifuged for ~15 min at 3000 RPM, a 10 μL aliquot of each supernatant was diluted 301-fold with hexanes and analyzed by UV. The concentration of **7a** in each supernatant was determined using Beer's law (above) and the above-measured ϵ_{274} . Solubility was reported as the mean $\pm$ 1 standard deviation.

[00314] The solubility of HBED-HCl in mineral oil was also investigated in a similar manner as described above for **7a**, with the following modifications: 13-16 mg of HBED-HCl was stirred in quadruplicate for 24 h without applying a vacuum; each suspension was filtered through a 0.2 micron polyethersulfone membrane filter (Whatman Puradisc); and 300 μL of filtrate was diluted 9.7-fold with isopropanol before acquiring a UV spectrum.

Hydrolysis of Prodrugs **8a-c** to **9**

Hydrolysis by LC-MS

[00315] **Compound Optimization.** Since postulated hydrolysis intermediates could not be independently synthesized and optimized, compound **8a** was dissolved in 50% H_2O /50% ACN and directly infused into the mass spectrometer. Over the course of a day, the mass spectrum was monitored for ions with the distinctive ~50%:100%:20% isotopic distribution pattern that was calculated for this type of compound containing 2 boron atoms. Declustering potential (DP), entrance potential (EP), and collision cell entrance potential (CEP) were optimized for each ion. Because of the rapid hydrolysis of the pinacol ester, the parent ion (m/z 637.4) and the parent ion minus one pinacol (m/z 555.3) had to be optimized

in 100% ACN. Figure 5 shows the ions (M + H) that were monitored and their corresponding structures.

[00316] Hydrolysis Conditions. The rate of hydrolysis of **8a** to **9** was determined at ambient temperature. A 3.2 mM stock solution of compound **8a** was prepared in dry ACN. A 50 mM stock solution of N-methylmorpholine (NMM) buffer was prepared, filtered, and adjusted to pH 7.41 using formic acid. To ensure all species remained dissolved throughout the reaction, the hydrolysis was carried out in 25:75 MeOH:NMM buffer (v/v), which gave a solution with an apparent pH of ~7.14 and a final NMM concentration of 38 mM. The hydrolysis (n = 1) was initiated by adding a portion of **8a** stock (39.80 μ M, 0.53% ACN by volume) to the 25:75 MeOH:NMM buffer. Aliquots were removed from the reaction solution via autosampler every 60 minutes for 42 hours.

[00317] LC-MS Conditions. Some separation of reaction species was achieved by liquid chromatography using a Luna C₈ (50 x 4.6 mm, 5 μ m) column (Phenomenex, USA), connected to a guard cartridge. The column was at ambient temperature during the chromatographic runs. The sample volume was 25 μ L. The mobile phase consisted of 0.1% formic acid in H₂O (solvent A, pH 2.7) and 0.1% formic acid in MeOH (solvent B) at a flow rate of 0.5 mL/min. The one-hour program was as follows: 40% B (0-5 min), 40% to 100% B (linear gradient, 5-20 min), 100% B (20-45 min), 100% to 40% B (linear gradient, 45-47 min), 40% B (47-60 min).

[00318] Reaction species were detected using quadrupole 1 multiple ion (Q1MI) monitoring in positive electrospray ionization mode, since this technique does not require complete separation of compounds on the column for peak integration. The optimized source parameters were as follows: 35 psi (curtain gas), 5500 (IonSpray voltage), 650 °C (temperature), 60 psi (nebulizer gas), 55 psi (turbo gas), 5 (vertical probe). The peaks on the extracted ion chromatogram corresponding to the molecular ions observed during the hydrolysis were integrated using Analyst v1.4.2 software (AB Sciex, USA).

[00319] Reaction Kinetics. The integrated rate equations for a series first-order reaction are given in Equations 1, 2, and 3, where [A] represents the concentration of the reactant, [B] the concentration of the intermediate, and [C] the concentration of the final product.

$$[A] = [A]_0 \exp(-k_{AB}t) \quad (1)$$

$$[B] = \frac{k_{AB}[A]_0}{k_{BC}-k_{AB}} (\exp(-k_{AB}t) - \exp(-k_{BC}t)) \quad (2)$$

$$[C] = [A]_0 \left[1 - \frac{1}{k_{BC}-k_{AB}} (k_{BC} \exp(-k_{AB}t) - k_{AB} \exp(-k_{BC}t)) \right] \quad (3)$$

[00320] Thus, a plot of the natural log of the peak area obtained for A ions (m/z 455.2 and 437.2) versus time yielded a straight line, where the negative slope of the linear fit was the observed rate constant (k_{AB}) for the decay of A to B. Using k_{AB} and t_m , the time necessary to reach the maximum concentration of B (m/z 441.2 and 423.2), Equation 4 was solved for k_{BC} , the observed rate constant for the conversion of B to C, by employing the Solver function in Microsoft Excel.

$$t_m = \frac{\ln\left(\frac{k_{AB}}{k_{BC}}\right)}{k_{AB} - k_{BC}} \quad (4)$$

Hydrolysis by UV

[00321] The rates of hydrolysis of prodrugs **8a-c** to **9** were determined by UV at 37 °C in phenol red-free minimum essential medium (MEM, Gibco) supplemented with 2 mM L-glutamine (Corning), and in a 50:50 (v/v) solution of MeOH and 30 mM pH 7.5 sodium phosphate buffer (PB).

[00322] For hydrolysis studies carried out in MEM, fresh stock solutions of **8a** (100mM), **8b** (100 mM), and **8c** (30 mM) were prepared in dry DMSO one day prior to the experiment. MEM supplemented with glutamine was equilibrated overnight at 37 °C in an atmosphere of humidified 5% CO₂. Each hydrolysis was initiated by adding an aliquot of compound stock to a tube containing medium at 37 °C, such that the final concentrations of **8a**, **8b**, and **8c** were approximately 150, 100, and 50 μM, respectively. Each suspension was stirred until all solid had dissolved, and was then transferred to three pre-warmed, stoppered cuvettes. Excess solution containing **8a** was returned to the incubator for use later in oxidation experiments (see Oxidation by UV). Spectra were obtained every 15-30 minutes until no spectral changes were detected. Absorbance at 277 nm was monitored (see below), and pseudo first-order rate constants (k_{obs}) were obtained from the negative slopes of the linear fits of $\ln(A_t - A_\infty)$ versus time in minutes, where A_t is absorbance at time t and A_∞ is the absorbance upon reaction completion. Mean k_{obs} was reported $\pm$ 1 standard deviation. Half-lives were calculated by dividing k into 0.693. The pH of the medium remained stable (pH ~7.4) for the duration of the reaction.

[00323] Hydrolysis studies were carried out in a 50:50 (v/v) solution of MeOH and 30 mM pH 7.5 PB at 37 °C for **8a-c** and in a 30 mM pH 3.1 sodium citrate buffer containing 50% MeOH at 23 °C for **8a**. Fresh 13-18 mM solutions of **8a-c** were prepared in dry ACN every 6 days as necessary, and were diluted to 150-200 μM (1.2% ACN by volume) in the appropriate buffer system. Spectra were taken every 30 minutes (90 minutes for **8c**)

immediately after compound addition until very limited or no spectral changes were detected. While the λ_{max} of **8a-c** was 270 nm, the decrease in absorbance at 277 nm was used instead to follow the hydrolyses. *Total* decrease in absorbance during each reaction was larger at the latter wavelength, so monitoring this instead of the λ_{max} wavelength reduced the influence of instrument variation toward the end of the reactions, when the change in absorbance was nearing the limit of the spectrophotometer's detection. Once a hydrolysis reaction was near completion, absorbance values at the wavelengths monitored began to repeat over a significant number of time points. To prevent these repeat values from affecting the accuracy of the rate constant determination, they were handled in the following manner: all time points where the absorbance reading was the same were averaged, with the exception of the first 7 time points, which were never averaged for repeat absorbance values. Thus, the negative slope of the linear fit of $\ln(A_t - A_\infty)$ vs. *mean* time in minutes gave the pseudo first-order rate constant (k_{obs}). Each experiment was conducted in triplicate., and the mean k_{obs} was reported ± 1 standard deviation. Half-lives were calculated as described above. The pH of all samples was measured upon completion of hydrolysis to confirm buffer stability.

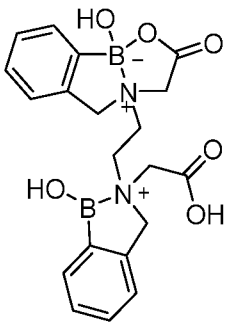
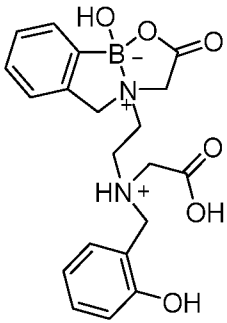
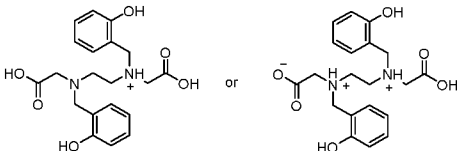
Oxidation of Prodrug **9** by H_2O_2

Oxidation by LC-MS

[00324] Compound Optimization. Stock solutions of **9** and mono-hydrochloride HBED were prepared in DMSO and diluted to 18 μM with 100 mM pH 7.42 N-methylmorpholine (NMM) buffer. Each solution was then directly infused into the mass spectrometer, and parameters were optimized for m/z 409.3 (**9**) and m/z 389.3 (HBED). Ion structures ($\text{M} + \text{H}$) and parameters are listed in Table 1.

[00325] In order to optimize for any intermediates formed during the oxidation reaction, a 16 μM solution of **9** was prepared in 100 mM pH 7.42 NMM buffer with 127x excess H_2O_2 . Over 60 minutes of direct infusion of this reaction solution, m/z 409.3 (reactant) decreased, m/z 389.3 (product) increased, and a third species with an m/z of 399.4 increased and then subsequently decreased. This latter ion, with an isotopic pattern of ~25%:100%:22% that indicated only one boron atom was present in the molecule, was the "mono" oxidation intermediate N,N'-(2-boronic acid, 2'-hydroxy benzyl)ethylenediamine-N,N'-diacetic acid + H^+ - 1 H_2O (Table 1).

Table 1. Structures, optimized parameters, and retention times of ions $[M + H]$ monitored by LC-MS during the oxidation of **9** to HBED.

Structure	m/z Predicted	m/z Experimental	DP	EP	CEP	Retention Time (min)
	409.17	409.3	53	7.5	27	3.75
	399.17	399.4	53	4.6	24	3.64
	389.17	389.3	42	8	17	3.73

[00326] Reaction Conditions. The rate of oxidation of **9** to HBED was determined under pseudo first-order conditions of excess H_2O_2 at ambient temperature. A 5.88 mM stock solution of **9** was prepared in DMSO. A 100 mM NMM buffer was prepared, filtered, and adjusted to pH 7.4 using formic acid. A 62.6 mM stock solution of H_2O_2 was prepared in this NMM buffer using 50 wt% solution H_2O_2 in H_2O , and was refrigerated when not in use. Samples were prepared by diluting an aliquot of **9** stock to a final concentration of 59 μM with NMM buffer, with samples containing 1% DMSO. Oxidation was then initiated by

addition of an aliquot of H₂O₂ stock (0.29-3.5 mM, equaling ~5-59x molar excess of H₂O₂). Aliquots were removed from each sample via autosampler every 30-33 minutes (for samples with 5x or 12x excess H₂O₂) or 11 minutes (for samples with 25x, 43x, or 59x excess H₂O₂) until the peak area of *m/z* 389.3 (HBED) no longer increased. The oxidation reaction was repeated 3-6 times for each concentration of H₂O₂.

[00327] The rate of oxidation of **9** to HBED was also determined in 10 mM phosphate buffer (PB). The buffer was prepared using monobasic sodium phosphate monohydrate and dibasic sodium phosphate heptahydrate, and was titrated to pH 7.4 using formic acid. A 13.4 mM stock solution of H₂O₂ was prepared in this PB using 50 wt% solution H₂O₂ in H₂O, and was stored at room temperature. Oxidation samples were prepared and analyzed as described in the preceding paragraph. The oxidation reaction was run 1-2 times for each concentration of H₂O₂.

[00328] LC-MS Conditions. Very minor separation of reaction species was achieved by liquid chromatography using a Luna C₈ (50 x 4.6 mm, 5 μm) column (Phenomenex, USA), connected to a guard cartridge. The column was at ambient temperature during the chromatographic runs. The sample volume was 25 μL. The isocratic mobile phase, flowing at a rate of 0.5 mL/min, contained 40:60 v/v MeOH:H₂O, with 0.1% formic acid. The time program was 11 minutes.

[00329] Reaction species were detected using quadrupole 1 multiple ion (Q1MI) in electrospray ionization mode with positive polarity, since this technique does not require separation of compounds on the column for peak integration. The optimized source parameters were as follows: 30 psi (curtain gas), 5500 (IonSpray voltage), 700 °C (temperature), 60 psi (nebulizer gas), 70 psi (turbo gas), 3 (vertical probe). The peaks on the extracted ion chromatogram corresponding to the molecular ions observed during the oxidation were integrated using Analyst v1.4.2 software (AB Sciex, USA). Retention times are given in Table 1.

[00330] Reaction Kinetics. The integrated rate equations for a series first-order reaction are given by Equations 1, 2, and 3 (see Hydrolysis by LC-MS), where [A] represents the concentration of the reactant, [B] the concentration of the intermediate, and [C] the concentration of the final product.

[00331] The natural log of the peak area obtained for A (*m/z* 409.3) versus time yielded a straight line, where the negative slope of the linear fit was the observed rate constant (*k*_{AB}) for the decay of A to B. In a case where *k*_{AB} >> *k*_{BC} (i.e. the second step is rate determining), Equation 3 reduces to Equation 5:

$$[C] = \{1 - \exp(-k_2 t)\}[A]_0 \quad (5)$$

[00332] Therefore, $\ln(A_\infty - A_t)$ for C (HBED, m/z 389.3), where A_∞ is the peak area at the final time point and A_t is the peak area at time t , was plotted against time. The negative slope of the linear fit was the observed rate constant (k_{BC}) for the decay of B to C.

[00333] The slopes of the lines through the plots of k_{AB} and k_{BC} vs. H_2O_2 concentration provided the second-order rate constants k ($M^{-1} \text{ min}^{-1}$) for the oxidation of **9** (m/z 409.3) to the mono intermediate (m/z 399.4) and for the oxidation of the mono intermediate to HBED (m/z 389.3), respectively.

Oxidation by UV

[00334] Seventeen hours after initial preparation, the solution of hydrolyzed **8a** in MEM from above (Hydrolysis by UV) was removed from the incubator and transferred to cuvettes. To these samples at 37 °C was added 10 μ L of a stock solution of H_2O_2 prepared in H_2O , to give a final concentration of H_2O_2 (7.5-30 mM) that was in 50-200x excess of compound concentration. Spectra were acquired every 75-240 seconds, depending on H_2O_2 concentration. Data were collected until no further spectral changes were detected. The increase in absorbance at 273 nm was used to monitor the reaction. The negative slope of the linear fit of $\ln(A_\infty - A_t)$ vs. mean time in minutes (see Hydrolysis by UV for explanation of mean time), where A_t is absorbance at time t and A_∞ is the absorbance upon reaction completion, gave the pseudo first-order rate constant (k_{obs}). Oxidations were performed in triplicate for each H_2O_2 concentration. The slope of the line through the plot of mean k_{obs} vs $[H_2O_2]$ provided the second-order rate constant, k ($M^{-1} \text{ min}^{-1}$) for the macro oxidation of **9** to HBED.

Oxidation of Prodrug **8a by H_2O_2**

Oxidation by NMR

[00335] A sample was prepared containing ~6 mM compound **8a** and ~60 mM H_2O_2 in DMSO- d_6 . The reaction was monitored at ambient temperature by NMR for 25 hours.

Relative Binding Affinity of Prodrug **9 for Iron and Copper**

Affinity for Iron (Fe^{3+})

[00336] The relative affinity of **9** for iron was determined by a competition experiment between **9** and HBED in 20 mM pH 7.5 sodium phosphate buffer, and these results were

compared to a competition experiment between ethylenediaminetetraacetic acid (EDTA) and HBED. Stock solutions of **9** and HBED-HCl were prepared in DMSO and MeOH, respectively. Stocks of ferric ammonium citrate (FAC) and EDTA were prepared in H₂O. Five solutions were made containing the following: HBED only (90 μM), **9** only (300 μM), EDTA only (300 μM), HBED + **9**, and HBED + EDTA. All solutions contained 30 μM FAC. The solutions were allowed to equilibrate for 22 h at 23 °C before spectra were acquired. The differences between spectra in absorbance at 481 nm, indicative of an HBED-Fe chelate, were noted.

Affinity for Copper (Cu²⁺)

[00337] The relative affinity of **9** for copper was determined by a competition experiment between **9** and EDTA in a 25:75 solution of MeOH:sodium phosphate buffer (20 mM, pH 7.5). This buffer system was used to ensure all species stayed in solution throughout the experiment, and had a pH of 7.95. Stock solutions of **9** and EDTA were prepared as described above, and a stock solution of copper (II) sulfate pentahydrate was prepared in H₂O. Solutions were made containing 100 μM copper, 300 μM **9**, and 0, 20, 60, or 100 μM EDTA. A solution containing only copper and 100 μM EDTA was also prepared. These solutions were allowed to equilibrate for 24 h at 23 °C before spectra were acquired. The differences between spectra in absorbance at 410 nm, indicative of an interaction between **9** and copper, were noted.

Cell Culture Studies

General

[00338] The spontaneously immortalized human retinal pigment epithelial cell line ARPE-19 was purchased from American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured in 1:1 DMEM:F12 medium containing glutamine (Corning) and supplemented with 10% heat-inactivated fetal bovine serum (FBS, Corning) in an atmosphere of humidified 5% CO₂. The cells were subcultivated twice weekly using trypsin 0.25% solution with 0.1% EDTA (Hyclone).

Cytoprotection Studies using Proliferating Cells and Crystal Violet Staining

[00339] Cells were washed 2 x 10 mL with serum-free minimum essential medium (MEM, Corning), trypsinized, seeded into 24-well plates (Corning) at a density of 40,000 cells in MEM containing 10% FBS, and allowed to attach for 24 h. Stock solutions of **8a**, **8b**,

and HBED (all 100 mM), **8c** (30 mM) and **9** (10 mM) were made in dry DMSO. Stocks of **8a-c** were made fresh less than 1 h before use. Five concentrations of each compound were prepared in MEM containing 10% FBS that was pre-equilibrated to pH 7.4 and cooled to RT before addition of compounds. Before serial dilution, the highest concentrations of **8a-c** were stirred at RT for 15 minutes (**8a**) or 30 minutes (**8b, c**). The medium was removed from the wells and the compounds were applied (1 mL each well) at concentrations such that the amount of DMSO in wells did not exceed 0.15% by volume, with the exception of studies using **9**, in which cells were exposed to up to 1.5% DMSO. The plates were incubated for 15 h (pretreatment period).

[00340] Following the pretreatment period, cells were challenged with either 300 μ M or 500 μ M H₂O₂. To do so, a 300.6 mM stock solution of H₂O₂ in H₂O, kept in the refrigerator between experiments, was further diluted to make 50.5 mM and 30.3 mM solutions. These solutions, prepared fresh for each experiment, were immediately filter-sterilized and applied to wells as 10 μ L doses. Wells not dosed with H₂O₂ were instead dosed with 10 μ L of H₂O. Plates were gently agitated to mix the H₂O₂, and returned to the incubator. Each plate contained 3 empty wells, 3 high control wells (0.15% DMSO in 10% FBS/MEM only), 3 low control wells (0.15% DMSO in 10% FBS/MEM + H₂O₂), and triplicates of each concentration of compound + H₂O₂. All experiments were performed at least two times, on separate occasions.

[00341] After an 8 h challenge, plates were rocked five times to dislodge any weakly-attached cells. The medium was removed from the wells, and cells were fixed (4% w/v paraformaldehyde in phosphate-buffered saline, 30 minutes) and stained (0.1% crystal violet in H₂O, 30 min). Empty ("blank") wells were also fixed and stained. After the stain was removed, the wells of each plate were washed 7 times with H₂O and air dried. The dye was then resolubilized in methanol (1 mL each well), and the absorbance at 590 nm was measured on a BioTek Eon microplate spectrophotometer.

[00342] To analyze the crystal violet assay results, the blank-subtracted absorbance values were used to calculate % protection as follows:

% Protection = [(experimental value – low control) / (high control – low control)] x 100
Values were averaged and reported $\pm$ 1 standard deviation.

Cytoprotection Studies using Confluent Cells and MTT Assay

[00343] Cells in DMEM:F12 with FBS were seeded into 96-well plates at a density of 12,000 cells/100 μ L/well. The cells were allowed to grow for 3 days to 100% confluence.

Then, the medium was carefully removed from the wells and 100 μ L warm, FBS-free and phenol red-free minimum essential medium (MEM, Gibco) supplemented with 2 mM glutamine (Corning) was quickly added. The plates were returned to the incubator while the stock solutions were prepared.

[00344] Stocks solutions of **8a-c** were made fresh less than 1 h before use. Stock solutions of **8a**, **8b**, and HBED (all 100 mM), **8c** (30 mM) and **9** (10 mM) were prepared in dry DMSO and then further diluted to 150 μ M (**8a**, **8b**, **9**, HBED) or 45 μ M (**8c**) in pre-equilibrated MEM (free of FBS and phenol red, supplemented with 2 mM glutamine) that was cooled to RT prior to use. Before further serial dilution by a factor of 1.5, MEM containing the highest concentrations of **8a-c** were stirred at RT for 15 minutes (**8a**) or 30 minutes (**8b**, **8c**). The plates were then removed from the incubator, the wash MEM was carefully aspirated out of the wells, and the compounds were applied (100 μ L per well). The concentrations applied were such that the amount of DMSO in wells did not exceed 0.15% by volume, with the exception of wells containing **9**, to which cells were exposed to a maximum of 1.5% DMSO. One compound was applied to each plate, which was set up to simultaneously carry out a cytoprotection study and a cell viability study. Each plate contained 3 positive control wells, 3 negative control wells, 3 cell-free wells (blanks), and 6 wells per concentration of compound. All control and cell-free wells received 100 μ L MEM. The plates were returned to the incubator for 15 h.

[00345] Following this pretreatment period, the cells were challenged with H_2O_2 to assess whether the prodrugs or HBED could provide cytoprotection. For each experiment, a 10.5 mM stock solution of H_2O_2 was freshly prepared in H_2O and filter sterilized. Five microliters (500 μ M) H_2O_2 was then applied to 3 of the 6 treatment wells for each concentration of compound, as well as to the positive control wells. An equal volume of H_2O was applied to the negative control wells and to the cell-free wells. To maintain a proper control-matched experiment for the cell viability study, the remaining 3 treatment wells for each concentration of compound also received 5 μ L H_2O . The plates were gently rocked to mix the H_2O_2 and H_2O , and then returned to the incubator for 8 h. Thereafter, cell viability was determined by the MTT assay described below and percent cytoprotection was calculated from these results. All cytoprotection and cell viability experiments were performed two times, on separate occasions.

*Cell Viability Studies**Crystal Violet Staining*

[00346] Plates were prepared as described above for cytoprotection studies using crystal violet staining, except seeding density was 25,000 cells per well. The preparation of stock solutions and application of compounds were as described above. Each plate contained 3 empty wells, 3 high control wells (0.15% DMSO in 10% FBS/MEM only), and triplicates of each concentration of compound. All experiments were performed at least two times, on separate occasions. Plates were incubated for 24 h and then fixed, stained, washed, and read as described above. Cell viability in the presence of 0-1.5% DMSO was also determined in this manner (experiment performed one time).

[00347] To analyze the crystal violet assay results, the blank-subtracted absorbance values were used to calculate % viability as follows:

$$\% \text{ Viability} = (\text{experimental value} / \text{high control}) \times 100$$

Values were averaged and reported $\pm$ 1 standard deviation.

MTT Assay

[00348] Cell viability was determined using an MTT assay, which is a colorimetric assay that measures cellular metabolic capacity. NADH-dependent reductases of viable cells cleave the tetrazolium ring of thiazolyl blue tetrazolium bromide (MTT), forming insoluble, purple formazan. The amount of formazan produced will increase as the number of viable cells increases. These purple crystals are then solubilized and the resulting purple solution is measured spectrophotometrically. Absorbance is proportional to the number of viable cells. It was predetermined in cell-free wells that neither prodrugs, HBED, nor H₂O₂, at their highest doses, were able to reduce MTT. The experimental design for these cell viability studies using MTT can be found above (Cytoprotection Studies Using MTT Assay).

[00349] A solution of MTT was freshly prepared at a concentration of 5 mg/mL in MEM (free of FBS and phenol red; supplemented with glutamine). In the dark, 10 μ L of MTT solution was added to every occupied well of every plate. The plates were returned to the incubator for 2 h, at which point 100 μ L of solubilization solution, consisting of 10% triton X-100 plus 0.1 N HCl in dry isopropanol, was added to the wells. Each plate was covered with Parafilm and lid, and mixed by gyratory shaker for 10-12 hours in the dark. The plates were then inspected under a microscope to ensure that all formazan was dissolved before the absorbance of each well at 570 nm and 690 nm was measured on a BioTek Eon microplate spectrophotometer. For each well, the background absorbance at 690 nm was

subtracted from the 570 nm measurement; from this absorbance value was then subtracted the averaged absorbance value from the cell-free wells.

[00350] Using these background- and blank-subtracted absorbance values, percent protection for each well was then calculated as follows:

$$\% \text{ Protection} = [(\text{exptl.} - \text{avg. positive control}) / (\text{avg. negative control} - \text{avg. positive control})] \times 100$$

[00351] In this way, the positive control protection was set to 0% and the negative control protection was set to 100%. Percent protection for triplicate wells were averaged and reported ± 1 standard deviation.

[00352] Also using the background- and blank-subtracted absorbance values, percent viability for each well was calculated as follows:

$$\% \text{ Viability} = (\text{exptl.} / \text{avg. negative control}) \times 100$$

[00353] Percent viability for triplicate wells were averaged and reported ± 1 standard deviation.

H₂O₂ and DMSO Kill Curves

[00354] Cells in DMEM-F12 with FBS were seeded into 96-well plates (Corning) at a density of 12,000 cells/100 μL /well. The cells were allowed to grow for 3 days to 100% confluence. The medium was then carefully removed from the wells and the cells were washed once with 100 μL warm, FBS-free and phenol red-free minimum essential medium (MEM, Gibco) supplemented with 2 mM glutamine (Corning).

[00355] To obtain an H₂O₂ kill curve, the wash MEM was removed and 100 μL fresh MEM (free of FBS and phenol red, supplemented with glutamine) was added to the wells. The plate was returned to the incubator for 15 h. After this time, a 14.7 mM stock solution of H₂O₂ was freshly prepared in H₂O, filter sterilized, and serially diluted with sterile H₂O. Five microliters of each concentration of H₂O₂ was added to triplicate wells. Triplicate negative control wells and cell-free wells all received 5 μL H₂O. The plate was returned to the incubator for 8 h, after which cell viability was determined by the MTT assay described above.

[00356] To obtain a DMSO kill curve, an aliquot of DMSO (1.5% v/v) was added to MEM (free of FBS and phenol red, supplemented with glutamine), and this solution was serially diluted. The wash MEM was removed and 100 μL of each concentration of DMSO was added to triplicate wells. Triplicate negative control wells and cell-free wells instead received 100 μL MEM. The plate was returned to the incubator for 23 h, after which cell

viability was determined by the MTT assay described above. The experiment was performed two times, on separate days.

Passive Diffusion Assay

[00357] The diffusion cell experiments were performed using a Franz diffusion cell (Figure 27) employing 0.010 inch thick silicone membranes (Silmax®, Pillar Surgical, La Jolla, CA). The donor vehicle was mineral oil. The receptor phase (20 mL/4.9 cm²) was 50 mM pH 7.4 phosphate buffer. During the experiments, the receptor phases were maintained at 32 °C with a circulating water bath and stirred using magnetic stir bars.

[00358] The passive diffusion of **7a** and HBED-HCl was investigated. The donor phase containing **7a** was prepared by adding 0.8757 g of the prodrug to 17.514 mL mineral oil (5% w/v, 79 mM). It was stirred for 20 h before first use, and remained stirring while the experiment was in progress. A donor phase containing 79 mM HBED-HCl was also prepared in mineral oil. It was stirred for 24 h prior to application, and remained a suspension throughout the experiment.

[00359] Prior to the first donor applications, the silicone membranes were kept in contact with the receptor phase for either 4 h (membranes receiving HBED-HCl) or 14 h (membranes receiving **7a**). The receptor phase of each cell was then replaced with fresh phosphate buffer, and a 1 mL aliquot of donor phase was applied to each membrane (n = 3 and 4 for HBED-HCl and **7a**, respectively). The donor chambers were sealed with Parafilm. The sampling procedure for each cell was as follows: a sample (~4 mL) was removed from the receptor chamber, and then the donor phase was removed. The receptor phase was replaced with new buffer to ensure sink conditions were maintained, and then the donor phase was (re)applied. For diffusion cells to which **7a** was applied, samples were withdrawn from each receptor chamber after 3, 6, 9, and 12 h, and new donor phase from the stirring stock was applied after each sampling. For diffusion cells to which HBED-HCl was applied, a sample was removed from the receptor phase at 23.5 and 41 h, and the same donor suspension was reapplied after the first sampling. Receptor phase samples from diffusion cells to which HBED-HCl was applied were immediately analyzed by UV. HBED-HCl was not detected in any of the samples. Receptor phase samples from diffusion cells to which **7a** was applied were stored at 32 °C for 24 h before being analyzed by UV. This time period was sufficient to allow any **7a** present in the samples to hydrolyze to prodrug **9**. As such, the absorbance at 263 nm (λ_{max} of **9**, $\epsilon = 610 \text{ L mol}^{-1} \text{ cm}^{-1}$) was measured for each sample, from which concentration was then calculated.

[00360] After this application period, the membranes were washed 2 times with ethanol to remove the donor phases and the receptor phases were changed. The membranes were then leached with methanol in the donor phase for 72 h to remove any residual compound in the membrane. Leaching was considered complete when the UV spectrum of a sample from the receptor phase showed no measurable absorptions due to HBED-HCl, **7a**, or **9**.

[00361] After leaching, a second application was made to the membranes to ensure that they had not been damaged by the first application. This second application consisted of a 1 mL aliquot of a 400 mg/6 mL suspension of theophylline in propylene glycol. Receptor phase samples were collected 3-4 times, at 24 h intervals, and then analyzed by UV. The absorbance at λ_{max} of 272 nm was recorded ($\epsilon = 10,200 \text{ L mol}^{-1} \text{ cm}^{-1}$), from which concentration was calculated. The same donor suspensions were reapplied after each sampling.

[00362] The flux for **7a** was determined by plotting the cumulative amount of **9** (μmol) detected in receptor phase samples against time (h) for each replicate. The slope of the linear fit of each plot divided by receptor volume (20 mL) provided the flux value. Each flux value was then multiplied by 1.46, which was the ratio of the solubility of **7a** in mineral oil (115 mM) versus the concentration of **7a** applied to diffusion cells (79 mM), to give the maximum flux value for each replicate. These were converted to their logarithm and then averaged. The log maximum flux for **7a** was reported ± 1 standard deviation. The log maximum flux of theophylline was determined in a similar manner, except no multiplier was used because theophylline was applied as a suspension. The log flux values calculated from this second application were all slightly less than the literature value of -2.68, suggesting that none of the membranes were damaged by the applications of **7a** or HBED-HCl (Wasdo *et al.*, 2008).

Results

Synthesis and Characterization

[00363] The synthetic scheme for the synthesis of prodrugs **7a-g** is given in Figure 1. Zhang *et al.* (2012) reported the synthesis of **3a** in 90% yield by reacting 2-bromomethylphenylboronic acid with ethylenediamine (**2**) in dry acetonitrile (ACN) in the presence of a non-nucleophilic base. Several attempts by this author to reproduce their synthesis were unsuccessful. Insufficient precipitation of **3a** during the reaction made this method unsuitable.

[00364] Instead, **3a** was obtained by first synthesizing and isolating the imine formed from the reaction of 2-formylphenylboronic acid (**1a**) and ethylenediamine (**2**) in pH 4.3 sodium acetate buffer (Germain and Knapp, 2008). The imine was obtained consistently and with excellent yields (>70%) from this protocol. It was then reduced in ethanol with sodium borohydride, followed by the base/acid workup described above, to obtain **3a** as a precipitate. Although **3a** was obtained in seemingly good yields and was in fact successfully used to synthesize some batches of **5a** and **5c**, some ^{11}B NMR spectra showed additional boron peaks. While this could have been due to different coordination states of the boron atoms in compound **3a**, contamination with boric acid (pK_a 9.23) or its salt could not be ruled out since the boron salts were not removed by dichloromethane (DCM) before the base/acid precipitation step.

[00365] Thus, to remove this uncertainty of contamination, N,N'-bis-(2-boronic acid benzyl)ethylenediamine (**3a**) was alternatively synthesized via reductive amination using a procedure modified from Gray and Houston (2002) and Gray *et al.* (2003). Rather than recrystallizing the organic concentrate with DCM/hexanes after trituration per the reference protocol, the concentrate was instead treated with a base/acid workup as described earlier. The ^1H NMR and ^{13}C NMR of **3a** were in agreement with the shifts reported in the same solvent (CD_3OD) by Gray *et al.* (2003) and Zhang *et al.* (2012), respectively. However, it should be noted that, based on the protocol used by Gray *et al.* (2003), where **3a** was synthesized in methanol followed by workup using only aprotic solvents, the authors most likely isolated N,N'-bis-(2-boronic acid methanol ester benzyl)ethylenediamine rather than the free boronic acid. The difference would be difficult to discern in CD_3OD . The methanol then likely exchanged with glycerol when they performed fast atom bombardment mass spectrometry, giving the diglycerol adduct (m/z 441.1) that they reported. By the same token, the NMR data provided here for **3a** likely represents the methanol ester formed *in situ* (Hall, 2011).

[00366] Compound **3b** was synthesized from 2-formyl-4-methoxyphenylboronic acid (**1b**) and ethylenediamine (**2**) in the same manner as **3a**, except the dichloromethane trituration step was unsuccessful. When attempted, the suspension clogged the filter paper. This was presumably caused by **3b** present as precipitate. The solid and filtrate were recombined, concentrated, and subjected to the NaOH/HCl workup to give **3b** as a precipitate.

[00367] The characterization of free boronic acids is difficult. Although the elemental analysis of **3a** was unsatisfactory, NMR and MS suggest that the desired compound was

synthesized. The discrepancy in the elemental analysis most certainly resulted from dehydration of the boronic acid moiety(ies) in **3a** to boron oxide(s) when **3a** was dried *in vacuo* with moderate heating (Snyder *et al.*, 1938). Under these conditions, boronic acids can form cyclotrimeric anhydrides (boroxines) and oligomeric acyclic analogues (Hall, 2011). Indeed, the elemental analysis values observed for C, H, and N are consistent with a loss of slightly greater than one molecule of H₂O from each molecule of **3a** (Calc. for C₁₆H₂₀B₂N₂O₃: C, 62.00; H, 6.50; N: 9.04).

[00368] Similarly, boronic acids are subject to gas-phase dehydration and anhydride formation in the ion source of a mass spectrometer (Hall, 2011), making it difficult to see the parent ion. ESI mass spectrometric analysis of **3a** in positive mode showed M + H minus one or two H₂O. However, M + H itself was not detected.

[00369] Determination of accurate melting points of free boronic acids is hindered by dehydration and decomposition (Dennis and Shelton, 1930; Santucci and Gilman, 1958). As Santucci and Gilman noted, “In the case of compounds of the acid type, there is usually observed a first fusion or effervescence corresponding to the loss of water, followed by resolidification and melting of the resulting anhydride at a higher temperature.” Thus, the 142-148 °C melting point of compound **3a** reported by Zhang *et al.* (2012) is instead most likely a dehydration point. In fact, this inventor observed that a sample of **3a** condensed into a glassy-looking pellet at 145-150 °C, followed by the apparent “expansion” of the pellet into a more web-like form at 165 °C. The sample gradually browned thereafter, and melted into a brown liquid at 212-218 °C. To test whether decomposition had occurred, the sample was allowed to cool to room temperature, whereupon it appeared to have returned to a solid state based on a milky appearance. It then re-melted at approximately 215-217 °C, suggesting no decomposition (besides dehydration) had occurred. No effervescence at lower temperatures was observed during the re-melt.

[00370] The synthesis of N,N'-bis-(2-boronic acid pinacol ester benzyl)ethylenediamine (**5a**) from **3a** and pinacol (**4a**) proceeded unremarkably in toluene. The chemical shift of free pinacol in CDCl₃ is 1.24 ppm, whereas the chemical shift of the pinacol peak in the ¹H NMR of **5a** was 1.31 ppm. This difference, along with the appropriate integration, suggested that **5a** was formed successfully. It must be noted that given the dehydrated state of compound **3a**, the accurate determination of the reactant quantities of **3a** and **4a** that was needed was somewhat difficult. Ultimately, reaction quantities were based on the assumption that the molecular weight of **3a** was 309.97 g/mol (one H₂O subtracted), rather than 327.98 g/mol. As such, 1.0 g of **3a** was considered to equal 0.0032 mol (1 equiv),

and this was reacted with 2.04 equiv pinacol (0.0065 mol). This assumption was maintained during subsequent synthetic steps requiring **5a** as starting material, since **5a** was used without further purification.

[00371] When **3b** was reacted with pinacol (**4a**) in the same manner as above, the ^1H NMR of the isolated white solid suggested N,N'-bis-(2-boronic acid pinacol ester-5-methoxy benzyl)ethylenediamine (**5b**) had been made. The pinacol CH_3 peak was at 1.29 ppm.

[00372] The synthesis of the diacetic acid esters **7a-c** proceeded smoothly by refluxing the corresponding alkyl bromoacetates with compound **5a** and DIPEA in dry ACN overnight, followed by an ether extraction. All esters were obtained as oils. In an attempt to develop a method for purifying these compounds, silica gel column chromatography was performed on a batch of **7c**. Some fractions contained a singlet at 1.23 ppm that actually surpassed the height (and integration) of the singlet at 1.31 ppm. The former peak was attributed to free pinacol, and the latter peak was attributed to the boronic acid pinacol ester. As such, it appeared that the boronic acid pinacol ester was susceptible to hydrolysis on the column to its corresponding free pinacol and boronic acid. Hydrolysis of the pinacol esters was not expected to be quite so facile based on the earlier literature. For example, Bowie and Musgrave (1963) assessed the hydrolysis of cyclic esters by measuring their increases in weight when exposed to air saturated with water vapor, and found the pinacol ester of phenylboronic acid to be unaffected. However, in line with the findings here, facile hydrolysis was also noticed by Leed *et al.* (2011), by Kielar *et al.* (2012), and by Achilli *et al.* (2013).

[00373] The syntheses of **7d** and **7e** were more complicated. To make **7e**, **5c** (0.44 g, 1 equiv) was initially reacted with **6a** (2.12 equiv) in 40 mL of refluxing ACN with DIPEA (2.85 equiv) for 22 h. The synthesis of **7d** was carried out in a similar manner by reacting **5b** with **6a**. ^1H NMR of both of the concentrated crudes indicated the presence of 2 major products, one of which was the desired product. The second product in both crudes seemed to be unsymmetrical. For example, both NMRs contained two triplets at 2.54 and 3.16 ppm, indicating that the two sets of methylene protons of the ethylenediamine moiety were no longer in the same chemical environments. In both instances, the unsymmetrical second species was present in an amount nearly equimolar to that of the desired bis compound. In the case of **7e**, the crude material was successfully purified by flash column chromatography to give pure **7e** ($R_f \approx 0.5$, 7:3 HX:EA), with no indication that hydrolysis of the pinanediol esters was occurring.

[00374] In an effort to improve the yield of **7e**, the reaction was repeated with the following modifications: the amount of solvent was halved, the reaction time was reduced to 17 h, and methyl bromoacetate (**6a**) was first converted to methyl iodoacetate via the Finkelstein reaction before reaction with **5c**. The unsymmetrical product was significantly reduced in the crude NMR, and the yield of **7e** after chromatographic purification was doubled to 66%. Most likely, it was the conversion of the alkylating agent to one with a slightly better leaving group (I⁻ instead of Br⁻) that brought about the cleaner reaction. It may have offset the unfavorableness of the alkylation caused by the steric hindrance of the pinanediol moieties.

[00375] While esterification of free boronic acids allows for more straightforward compound characterization, prodrugs of the general form **7** can be made with free boronic acids. For example, N,N'-bis-(2-boronic acid benzyl)ethylenediamine-N,N'-diacetic acid tert-butyl ester (**7f**) was prepared by first converting *tert*-butyl chloroacetate (**6d**) to the corresponding *tert*-butyl iodoacetate using the Finkelstein reaction, followed by reaction with N,N'-bis-(2-boronic acid benzyl)ethylenediamine (**3a**) to give the desired compound as the major product.

[00376] To improve the aqueous dissolution of compounds of the general form **7**, and as a means to purify **7a-c** without column chromatography, **7a-c,e** were converted to their corresponding dimesylate salts, **8a-c,e**, by precipitation from ether after addition of methanesulfonic acid. A benefit to salt formation is increased ease of handling, as compounds **8a-c,e** were all solids. Due to overly-rapid precipitation from ether upon formation, **8a** required recrystallization.

[00377] Compound **9** was synthesized by hydrolyzing **8a-c** in [unbuffered] H₂O for several days and collecting the subsequent precipitate. Minor impurities present in the crude precipitate (*e.g.* pinacol) were removed by recrystallization from DMSO with H₂O. Although the recrystallized material was thoroughly washed and dried under reduced pressure, the last traces of DMSO could not be removed. The DMSO impurity was accounted for in the calculated elemental analysis and yield.

[00378] Evidence for the structure of **9** is as follows. ¹³C NMR of **9** (Figure 23) revealed splitting of peaks assigned to the carbonyl, quaternary aromatic, CH aromatic (only 1 of 4 split), benzylic methylene, and N-acetic acid methylene carbons, which suggested that more than one species was present in solution. The boron NMR of **9** (Figure 24) showed one peak at 14.33 ppm, which was indicative of boron in a tetrahedral environment, coordinated to nitrogen (see Leed *et al.*, 2011; Zhu *et al.*, 2006; and references cited therein). Given that

neither the ^1H NMR nor the ^{13}C NMR spectra contained any signals corresponding to pinacol or the methanesulfonate counteranion, it can be postulated that, at some point, they hydrolyzed and dissociated, respectively, in water. Additionally, the spectra lacked peaks corresponding to the carboxylate esters of **8a-c**, indicating that the esters also hydrolyzed. Thus, from this information, an open form of **9** can be postulated, where **9** contains two boronic acids and two carboxylic acids (Figure 20). N-B coordination can create chiral centers on both the boron and nitrogen atoms if each atom has four different substituents. While in free base form, the nitrogens are capable of rapidly interconverting between *R* and *S* configurations and can, in theory, coordinate to the boronic acid when the boronic acid is either perpendicular to or in the plane of the phenyl ring (Figure 21). However, coordination to the boronic acid while it is in the plane of the phenyl ring is unfavorable due to steric interactions between the OH attached to boron and the substantially-sized substituents attached to the nitrogen. Thus, to avoid these steric interactions, the boronic acid is postulated to be perpendicular to the plane of the phenyl ring when nitrogen coordinates. N-B dative bond formation “freezes” the nitrogen in either an *R* or *S* configuration, whichever it was at the time of coordination to form a fused 5-membered ring on each side of the molecule. Although the presence of carboxylate coordination cannot be indisputably proven by the analytical techniques used here, there is literature precedent for this type of structure where a nitrogen and a carboxylate oxygen are both coordinated to an aryl boron (Mancilla and Contreras, 1986; Mancilla and Contreras, 1987). Additionally, ^1H NMR, elemental analysis, and mass spectrometry all suggested the loss of one hydroxide from each boron atom of RTT, implying that it was displaced by another ligand. The coordinated carboxylate withdraws electron density from the boron atom, which will cause the N-B bond to strengthen in compensation (Mancilla and Contreras, 1986). However, in solution, a hydrated, open form of **9** may also exist in equilibrium with the coordinated form (Figure 20).

[00379] The coordination of nitrogen in *R* or *S* configuration will essentially direct the carboxylate to preferentially add to the *cis* face of the ring because *trans* coordination strains the resulting five-membered ring. Thus, if nitrogen coordinates in the *R* configuration, carboxylate coordination is directed such that boron is also in the *R* configuration. Likewise, the coordination of nitrogen in the *S* configuration, followed by *cis* coordination of the carboxylate to boron, will cause boron to have an *S* configuration. If carboxylate coordination displaced one hydroxyl group on each boronic acid and formed another 5-membered ring, this would result in a fused 3-ring system on each side of the molecule (Figure 20). In total, there are four possible stereoisomers of **9** (Figure 3), two of which are identical (meso)

compounds. This leaves three isomeric species: two enantiomers and their diastereomer. It should be noted that the isomeric outcome just described would be the same if the carboxylate coordinates first, followed by the nitrogen.

[00380] Turning to the ^1H NMR of **9** (Figure 3) the spectrum distinguishes between two diastereomers with slightly different chemical shifts, in a ~50:50 mixture. The apparent doublet at 3.62 ppm is actually two doublets (one for each diastereomer) that happen to have the same chemical shift. Generalized isomer assignments can be made for some of the peaks (Figure 3) based on other batches/spectra of **9** where the ratio of isomers was not 50:50.

Crystallization and X-Ray Data Collection

[00381] The crystal structure of prodrug **8a**, the dimesylate salt of N,N'-bis-(2-boronic acid pinacol ester benzyl)ethylenediamine-N,N'-diacetic acid methyl ester, was solved. Crystal data and structure refinement (Table 2), atomic coordinates (Table 3), bond lengths and angles (Table 4), anisotropic displacement parameters (Table 5), hydrogen coordinates (Table 6), and hydrogen bonds (Table 7) are shown below.

Table 2. Crystal structure data of compound **8a**.

Identification code	face	
Empirical formula	C38 H62 B2 Cl4 N2 O14 S2	
Formula weight	998.44	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P $\bar{1}$	
Unit cell dimensions	a = 7.6022(5) Å b = 11.6542(7) Å c = 14.6482(9) Å	α = 84.9960(10)° β = 76.2240(10)° γ = 73.1720(10)°
Volume	1206.25(13) Å ³	
Z	1	
Density (calculated)	1.374 Mg/m ³	
Absorption coefficient	0.395 mm ⁻¹	
F(000)	526	
Crystal size	0.44 x 0.26 x 0.23 mm ³	
Theta range for data collection	1.83 to 27.50°	
Index ranges	-9 ≤ h ≤ 9, -15 ≤ k ≤ 14, -19 ≤ l ≤ 19	
Reflections collected	12273	
Independent reflections	5529 [R(int) = 0.0210]	
Completeness to theta = 27.50°	99.9 %	
Absorption correction	Integration	
Max. and min. transmission	0.9140 and 0.8443	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	5529 / 0 / 309	
Goodness-of-fit on F ²	1.056	
Final R indices [I > 2σ(I)]	R1 = 0.0285, wR2 = 0.0748 [4832]	

R indices (all data)	R1 = 0.0334, wR2 = 0.0773
Largest diff. peak and hole	0.385 and -0.420 e.Å ⁻³

$$R1 = \sum(|F_o| - |F_c|) / \sum|F_o|$$

$$wR2 = [\sum[w(F_o^2 - F_c^2)^2] / \sum[w(F_o^2)^2]]^{1/2}$$

$$S = [\sum[w(F_o^2 - F_c^2)^2] / (n-p)]^{1/2}$$

$$w = 1/[\sigma^2(F_o^2) + (m \cdot p)^2 + n \cdot p], p = [\max(F_o^2, 0) + 2 \cdot F_c^2]/3, m \text{ \& } n \text{ are constants.}$$

Table 3. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) of the crystal structure of compound **8a**.

	x	y	z	U(eq)
S1	283(1)	-929(1)	3577(1)	13(1)
O1	-6080(1)	-2262(1)	8009(1)	15(1)
O2	-5253(1)	-4088(1)	8746(1)	17(1)
O3	-5941(1)	-3480(1)	4674(1)	18(1)
O4	-3894(1)	-2461(1)	3905(1)	17(1)
O5	-965(1)	261(1)	3493(1)	27(1)
O6	2150(1)	-941(1)	3661(1)	20(1)
O7	-557(1)	-1675(1)	4304(1)	23(1)
N1	-3848(1)	-1495(1)	5553(1)	10(1)
B1	-4721(2)	-3322(1)	8042(1)	13(1)
C1	-7815(2)	-2389(1)	8662(1)	16(1)
C2	-7028(2)	-3414(1)	9342(1)	16(1)
C3	-8980(2)	-2722(1)	8069(1)	26(1)
C4	-8857(2)	-1190(1)	9122(1)	24(1)
C5	-6515(2)	-2970(1)	10161(1)	24(1)
C6	-8249(2)	-4258(1)	9691(1)	25(1)
C11	-2752(2)	-3715(1)	7344(1)	12(1)
C12	-1624(2)	-4880(1)	7481(1)	15(1)
C13	107(2)	-5358(1)	6883(1)	18(1)
C14	758(2)	-4670(1)	6129(1)	18(1)
C15	-288(2)	-3503(1)	5988(1)	16(1)
C16	-2038(2)	-3020(1)	6586(1)	12(1)
C17	-3056(2)	-1720(1)	6435(1)	12(1)
C18	-5208(2)	-2205(1)	5563(1)	13(1)
C19	-4918(2)	-2708(1)	4606(1)	13(1)
C20	-5922(2)	-3982(1)	3801(1)	24(1)
C21	-4701(2)	-161(1)	5466(1)	12(1)
C22	625(2)	-1640(1)	2507(1)	23(1)
C23	-4485(2)	-755(1)	1907(1)	25(1)
Cl1	-3104(1)	-2050(1)	1267(1)	40(1)
Cl2	-6057(1)	212(1)	1271(1)	30(1)
Cl1'	-3190(30)	-2230(30)	1398(18)	104(8)
Cl2'	-5880(30)	-20(30)	1189(15)	111(9)

U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Table 4. Bond lengths [Å] and angles [°] in the crystal structure of compound **8a**.

S1-O5	1.4498(9)
S1-O6	1.4499(9)
S1-O7	1.4606(9)
S1-C22	1.7655(13)
O1-B1	1.3680(15)
O1-C1	1.4714(14)
O2-B1	1.3690(15)
O2-C2	1.4643(13)
O3-C19	1.3328(14)
O3-C20	1.4469(14)
O4-C19	1.2012(14)
N1-C18	1.4967(14)
N1-C21	1.5080(14)
N1-C17	1.5237(13)
B1-C11	1.5659(17)
C1-C4	1.5175(17)
C1-C3	1.5222(17)
C1-C2	1.5615(16)
C2-C6	1.5181(17)
C2-C5	1.5196(17)
C11-C12	1.4073(16)
C11-C16	1.4108(16)
C12-C13	1.3855(17)
C13-C14	1.3867(17)
C14-C15	1.3888(17)
C15-C16	1.3969(16)
C16-C17	1.5124(15)
C18-C19	1.5088(15)
C21-C21#1	1.523(2)
C23-Cl2'	1.677(18)
C23-Cl1	1.7666(16)
C23-Cl2	1.7700(16)
C23-Cl1'	1.84(2)
O5-S1-O6	114.21(6)
O5-S1-O7	113.14(6)
O6-S1-O7	111.81(5)
O5-S1-C22	106.01(6)
O6-S1-C22	105.47(6)
O7-S1-C22	105.28(6)
B1-O1-C1	107.02(9)
B1-O2-C2	107.11(9)
C19-O3-C20	116.10(10)
C18-N1-C21	113.16(8)
C18-N1-C17	112.18(8)
C21-N1-C17	107.28(8)
O1-B1-O2	113.50(10)
O1-B1-C11	126.16(10)
O2-B1-C11	120.30(10)
O1-C1-C4	108.25(10)
O1-C1-C3	106.34(10)
C4-C1-C3	110.32(11)
O1-C1-C2	102.13(9)
C4-C1-C2	114.92(10)
C3-C1-C2	114.05(10)
O2-C2-C6	108.43(10)

O2-C2-C5	106.48(10)
C6-C2-C5	110.45(11)
O2-C2-C1	102.43(9)
C6-C2-C1	114.78(10)
C5-C2-C1	113.50(10)
C12-C11-C16	117.74(10)
C12-C11-B1	116.20(10)
C16-C11-B1	126.04(10)
C13-C12-C11	121.96(11)
C12-C13-C14	119.28(11)
C13-C14-C15	120.41(11)
C14-C15-C16	120.42(11)
C15-C16-C11	120.14(10)
C15-C16-C17	117.96(10)
C11-C16-C17	121.75(10)
C16-C17-N1	113.58(9)
N1-C18-C19	110.92(9)
O4-C19-O3	126.06(11)
O4-C19-C18	124.70(10)
O3-C19-C18	109.22(9)
N1-C21-C21#1	110.68(11)
Cl2'-C23-Cl1	102.7(11)
Cl2'-C23-Cl2	9.1(11)
Cl1-C23-Cl2	111.64(8)
Cl2'-C23-Cl1'	107.3(10)
Cl1-C23-Cl1'	8.7(10)
Cl2-C23-Cl1'	116.4(7)

Symmetry transformations used to generate equivalent atoms:
#1 -x-1,-y,-z+1

Table 5. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) of of the crystal structure of compound **8a**. The anisotropic displacement factor exponent takes the form: $-2\pi^2 [h^2 a^{*2} U^{11} + \dots + 2 h k a^* b^* U^{12}]$.

	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
S1	10(1)	14(1)	12(1)	1(1)	0(1)	-3(1)
O1	12(1)	15(1)	15(1)	3(1)	0(1)	-2(1)
O2	15(1)	14(1)	15(1)	2(1)	2(1)	-1(1)
O3	22(1)	17(1)	21(1)	-2(1)	-7(1)	-9(1)
O4	14(1)	18(1)	16(1)	-4(1)	-1(1)	-2(1)
O5	23(1)	19(1)	26(1)	1(1)	2(1)	6(1)
O6	16(1)	25(1)	21(1)	-2(1)	-3(1)	-10(1)
O7	14(1)	31(1)	22(1)	11(1)	0(1)	-8(1)
N1	10(1)	9(1)	10(1)	0(1)	-1(1)	-2(1)
B1	15(1)	13(1)	12(1)	-1(1)	-4(1)	-4(1)
C1	12(1)	17(1)	16(1)	2(1)	0(1)	-4(1)
C2	14(1)	15(1)	14(1)	0(1)	2(1)	-2(1)
C3	21(1)	33(1)	28(1)	4(1)	-10(1)	-11(1)
C4	18(1)	18(1)	28(1)	0(1)	4(1)	0(1)
C5	28(1)	29(1)	14(1)	-1(1)	-4(1)	-5(1)
C6	21(1)	21(1)	28(1)	5(1)	3(1)	-8(1)
C11	13(1)	12(1)	11(1)	-1(1)	-4(1)	-3(1)
C12	17(1)	13(1)	14(1)	3(1)	-3(1)	-4(1)
C13	16(1)	13(1)	20(1)	2(1)	-4(1)	0(1)
C14	13(1)	18(1)	18(1)	0(1)	0(1)	0(1)
C15	15(1)	17(1)	14(1)	3(1)	-2(1)	-4(1)
C16	13(1)	12(1)	12(1)	0(1)	-5(1)	-2(1)

C17	14(1)	12(1)	10(1)	2(1)	-4(1)	-3(1)
C18	12(1)	12(1)	14(1)	1(1)	-2(1)	-5(1)
C19	10(1)	9(1)	18(1)	-1(1)	-6(1)	1(1)
C20	28(1)	21(1)	27(1)	-6(1)	-13(1)	-7(1)
C21	12(1)	8(1)	13(1)	0(1)	-3(1)	-2(1)
C22	32(1)	20(1)	19(1)	-1(1)	-12(1)	-7(1)
C23	20(1)	37(1)	20(1)	-7(1)	-1(1)	-10(1)
Cl1	28(1)	49(1)	42(1)	-27(1)	-8(1)	0(1)
Cl2	32(1)	37(1)	24(1)	6(1)	-10(1)	-14(1)
Cl1'	145(17)	108(12)	56(7)	-16(7)	-21(7)	-25(9)
Cl2'	167(19)	57(8)	93(10)	4(6)	-10(8)	-24(7)

Table 6. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) of the crystal structure of compound **8a**.

	x	y	z	U(eq)
H1	-2880(20)	-1702(13)	5069(11)	21(4)
H3A	-9225	-2102	7582	39
H3B	-10180	-2786	8471	39
H3C	-8286	-3494	7771	39
H4A	-9266	-596	8644	36
H4B	-8017	-926	9419	36
H4C	-9962	-1274	9600	36
H5A	-5810	-3654	10488	37
H5B	-7669	-2546	10599	37
H5C	-5736	-2422	9925	37
H6A	-7655	-4872	10110	37
H6B	-8386	-4644	9154	37
H6C	-9495	-3803	10034	37
H12A	-2062	-5353	7998	18
H13A	840	-6149	6990	21
H14A	1926	-5000	5706	21
H15A	190	-3029	5483	19
H17A	-2171	-1230	6393	14
H17B	-4103	-1453	6988	14
H18A	-6513	-1684	5752	15
H18B	-5030	-2870	6031	15
H20A	-7060	-4247	3872	35
H20B	-4804	-4669	3642	35
H20C	-5888	-3372	3298	35
H21A	-3768	263	5508	14
H21B	-5811	103	5991	14
H22A	1187	-1180	1986	34
H22B	-592	-1683	2418	34
H22C	1469	-2453	2525	34
H23A	-3645	-320	2052	30
H23B	-5214	-995	2510	30
H23C	-5225	-930	2530	30
H23D	-3612	-315	2007	30

Table 7. Hydrogen bonds [$\text{\AA}$ and $^\circ$] in the crystal structure of compound **8a**

D-H...A	d(D-H)	d(H...A)	d(D...A)	$\angle(\text{DHA})$
N1-H1...O7	0.881(16)	1.862(16)	2.6892(13)	155.5(14)

Symmetry transformations used to generate equivalent atoms:

#1 -x-1, -y, -z+1

[00382] The crystal structure of **8a** is shown as a stick diagram in Figure 2. Two dichloromethane molecules were trapped in the crystal structure (not shown), one on each side. As expected, no B-N bond was present since the nitrogens were protonated. Each protonated nitrogen (N1, N1A) was hydrogen bonded to a methanesulfonate counteranion at O7/O7A. Interestingly, the boronic acid pinacol ester appeared to be slightly rotated out of the plane of the phenyl ring. Additionally, while a trigonal planar boron species would be expected to show bond angles of approximately 120° , the crystal structure of **8a** revealed 3 different bond angles: 113.50° (O1-B1-O2), 126.16° (O1-B1-C11), and 120.30° (O2-B1-C11).

Lipid and Aqueous Solubilities

Table 8. Molar absorptivities (ϵ), aqueous solubilities (S_{AQ}), and half-lives for hydrolysis ($t_{1/2}$) of prodrugs **8a-c**.

Prodrug	ϵ_{270} ($\text{L mol}^{-1} \text{cm}^{-1}$) ^a	S_{AQ} (μM) ^a	$t_{1/2}$ (h) ^b
8a	1019 ± 87	618 ± 78	$3.8 \pm 0.1, 0.04 \pm 0.003^e$
8b	1066 ± 80	171 ± 17	5.9 ± 0.3
8c	ND ^c	54 ± 7^d	26.3 ± 1.0

^a Determined in pH 7.4 phosphate buffer at RT.^b Determined at 37°C in pH 7.5 phosphate buffer containing 50% MeOH, unless otherwise noted.^c Not determined.^d Calculated using ϵ_{270} of $1019 \text{ L mol}^{-1} \text{cm}^{-1}$.^e Determined at 23°C in pH 3.1 sodium citrate buffer containing 50% MeOH.

[00383] In order to access the interior of a cell to express its activity, a new drug or the prodrug of that new drug must be able to passively diffuse through the cell membrane. One physicochemical property that may facilitate passive diffusion is adequate lipid solubility. Double prodrugs **7b** and **7c**, the free bases of **8b** and **8c**, were not available in sufficient

quantities to determine their lipid solubilities. However, double prodrug **7a**, the free base of **8a**, was found to possess a high solubility in mineral oil, a lipid-like solvent: 115 ± 3.5 mM. In contrast, the parent chelator, HBED, was insoluble as its monohydrochloride salt in mineral oil. This lack of lipid solubility was anticipated since HBED lacks oral availability due to its polar functional groups and to its ionization at physiological pH. Aqueous solubility is also important for passive diffusivity. HBED-HCl displayed substantial aqueous solubility (>10 mM) at pH 7.4 in phosphate buffer, while the double prodrugs **8a**, **8b**, and **8c** had more moderate aqueous solubilities of 618 μ M, 171 μ M, and 54 μ M, respectively (Table 8). Due to the rapid hydrolysis of the boronic pinacol esters of **8a-c**, their aqueous solubilities are probably reflective of the corresponding boronic acid species present in solution. Taken together, these solubility studies suggest that the membrane permeability of the double prodrugs may be improved compared to that of HBED because they possess both good lipid and good aqueous solubilities, while HBED lacks lipid solubility (Sloan, 1992).

Hydrolysis of Prodrugs **8a-c** to **9**

[00384] Double prodrugs **8a-c** all hydrolyzed to give prodrug **9**, the intermediate in the conversion pathway to the active chelator HBED (Figure 20). Experiments were then carried out to determine the rates at which this hydrolysis occurred under various conditions.

Hydrolysis by LC-MS

[00385] The hydrolysis of the prodrugs **8a-c** was anticipated to be more complex than an $A \rightarrow B$ reaction because with the expected hydrolysis of two pinacol boronic esters and two carboxylic acid esters, four rate constants can be measured, in theory (Figure 4).

[00386] The hydrolysis of **8a** (methyl ester) to **9** was carried out in a solution of 25% MeOH and 75% pH 7.41 50 mM N-methylmorpholine (NMM) buffer, and was monitored by LC-MS (Q1MI) over 42 hours. The pH of the hydrolysis solution remained stable over the course of the reaction. Although many molecular ions ($M + H$) were observed during compound optimization in H_2O/ACN (see Figure 5), not all of them appeared during the actual LC-MS run, in part because the sodium atoms adducted to some ions were most likely replaced by protons from the acidic mobile phase. Additionally, facile dehydration of the boronic acid moieties probably occurred to a *greater* extent under the much higher temperature used during LC-MS than it did during compound optimization.

[00387] Thus, the molecular ions actually observed by the LC-MS method were as follows: m/z 555.3 (**8a** minus 1 pinacol), 455.2 (**8a** minus 2 pinacol and 1 H_2O), 437.2 (**8a** minus 2 pinacol and 2 H_2O), 441.2 (**8a** minus 2 pinacol, 1 H_2O , and 1 methyl), 423.2 (**8a**

minus 2 pinacol, 2 H₂O, and 1 methyl), and 409.3 (compound **9**, product of full pinacol and methyl ester hydrolysis). The corresponding structures are given in Figure 5. m/z 637.38 (2 pinacol esters intact) was not observed, and although m/z 555.3 (1 pinacol ester intact) gave a peak at 14.3 minutes during the “t=0” injection, it was no longer visible in the second injection an hour later. These findings support the rapid hydrolysis of the boronic acid pinacol esters at physiological pH. Therefore, k_1 and k_2 were not able to be determined.

[00388] Over the course of 42 hours, peaks 455.2 and 437.2, both dehydrated species of N,N'-bis-(2-boronic acid benzyl)ethylenediamine-N,N'-diacetic acid methyl ester, decreased in a first-order manner (Figure 6, top left graph). These two species are represented by “A” in Equation 1 given above, and a plot of the natural log of peak integration versus time yielded a straight line with the slope of the linear fit equal to k_3 (Figure 6, top right graph). The rate constant obtained from the decay of m/z 455.2 ($1.22 \times 10^{-3} \text{ min}^{-1}$) is slightly different from the one obtained from the decay of m/z 437.2 ($1.51 \times 10^{-3} \text{ min}^{-1}$), so the average was taken ($1.37 \times 10^{-3} \text{ min}^{-1}$), which corresponds to a half-life of 8.44 hours.

[00389] Hydrolysis of one of the two methyl esters of m/z 455.2 and 437.2 gave the intermediate “mono” ions m/z 441.2 and 423.2, respectively, which are represented by “B” in Equation 2 above. The appearance of these ions, and their subsequent decline as the second ester was hydrolyzed, is shown in Figure 6, bottom left graph. Their t_m values – the time necessary to reach their maximum concentrations – were very similar (759 min for m/z 441.2 and 789 min for m/z 423.2) and were averaged to give 774 min.

[00390] The plot of the appearance of compound **9** (m/z 409.3) over time was slightly s-shaped (Figure 6, bottom right graph), which is expected if k_3 is not considerably different from k_4 (House, 1997). **9** is represented by “C” in Equation 3 above. By inputting k_3 (k_{AB} , $1.37 \times 10^{-3} \text{ min}^{-1}$) and t_m (774 min) into Equation 4, k_4 (k_{BC}) was solved using the Solver function in Microsoft Excel. A rate constant of $1.22 \times 10^{-3} \text{ min}^{-1}$ ($t_{1/2} = 9.5 \text{ h}$) was obtained, which is extremely close to the [averaged] value of k_3 . Therefore, it can be concluded that there is little difference between the rates of hydrolysis of the first and second ester groups.

Hydrolysis by UV

[00391] Since **8b** and **8c** have lower aqueous solubilities than **8a** (see Aqueous Solubilities, above), the rates of hydrolysis of all three compounds were measured at 37 °C using 50% MeOH as a co-solvent to ensure fast and easy dissolution. A 30 mM pH 7.5 phosphate buffer (PB) was prepared for these hydrolyses with 50% MeOH, giving this buffer

a final pH of 8.5. The results are summarized in Figure 8. While the half-lives of **8a** (3.84 h) and **8b** (5.89 h) were fairly similar, the half-life of **8c** (26.3 h) was significantly longer. This could be because the branched isopropyl groups of **8c** sterically hinder the attack of water on the carbonyl carbons of the esters more so than do the short-chain methyl and ethyl groups of **8a** and **8b**.

[00392] Hydrolysis was not dependent upon the presence of MeOH in the solution; **8a** was also found to hydrolyze in pH 7.4 phosphate buffer without MeOH with a half-life of 4.2 ± 0.2 h at 23 °C (not shown; $n = 2$). Interestingly, when the hydrolysis of **8a** was measured at 23 °C in pH 3.1 citrate buffer containing 50% MeOH, it was found to be very rapid ($t_{1/2} = 2.4 \pm 0.2$ min). The faster rate of hydrolysis was perhaps due to general acid catalysis. Spectra obtained during a typical hydrolysis, shown for **8a**, are given in Figure 22. Over time, absorbance at 270 and 277 nm decreased with first-order kinetics, and the final spectrum matched that of an authentic sample of prodrug **9** (Figure 22). No distinction between the hydrolysis of the first and second carboxylate esters of **8a-c** were apparent, so the half-lives of the prodrugs (Table 8) are macro half-lives.

[00393] *In vitro* studies (Cell Culture Studies, below) to evaluate the protective effects of prodrugs **8a-c** against H₂O₂-induced cell death were carried out in minimum essential medium (MEM). Therefore, it was very important to both confirm, and measure the rate of, hydrolysis of **8a-c** to **9** in this medium at 37 °C. To avoid UV interference, MEM was purchased without phenol-red, and had to be supplemented with glutamine. Fetal bovine serum was not added to the medium. Figure 7A shows the conversion of 150 μ M **8a** to **9** as a representative example of a typical hydrolysis of **8a-c**. Absorbance at 270 nm (and 277 nm) decreased during the hydrolysis, and a new λ_{max} developed at 263 nm. The formation of **9** was verified by comparing the final hydrolysis spectrum to a spectrum of a synthesized sample of **9**; the spectra were well matched (Figure 7B).

[00394] The pseudo-first order rate constants and corresponding half-lives of **8a-c** in MEM at 37 °C are given in Figure 8. Once again, **8a** and **8b** had similar – but short - half-lives of less than one hour in MEM, while the half-life of **8c** was approximately 3x longer. This increase in half-life of **8c** may again be attributed to the increased steric hindrance of its isopropyl esters.

[00395] Overall, these studies demonstrate that the double prodrugs of HBED undergo chemical hydrolysis near physiological pH, providing support for the first step in the proposed activation pathway of these prodrugs (Figure 20). Further investigation into the mechanism of hydrolysis of the carboxylate esters is needed. Hydrolysis may be

intramolecularly catalyzed by the boronic acids present in the double prodrugs after pinacol ester hydrolysis. While catalysis of hydrolysis of carboxylate esters by boronic acids built in to the compound has not been previously reported in the literature, several studies have shown that ester hydrolysis can be catalyzed by the addition of boric acid to the system [(Capon and Gosh, 1966); (Tanner and Bruice, 1967); and (Okuyama *et al.*, 1981)].

Oxidation of Prodrug **9** by H₂O₂

Oxidation by LC-MS

[00396] The reaction scheme for the oxidation of prodrug **9** to HBED under pseudo first-order conditions of excess H₂O₂ at ambient temperature is shown in Figure 9. Reactions were monitored by LC-MS, which allowed for the formation and decay of the “mono” intermediate, N,N'-bis-(2-boronic acid, 2'-hydroxy benzyl)ethylenediamine-N,N'-diacetic acid, to be observed in this A → B → C series reaction. The rate of oxidation was determined in 100 mM pH 7.4 N-methylmorpholine (NMM) buffer using 5-59x excess H₂O₂.

[00397] A representative plot of peak area vs. time for *m/z* 409.3 (**9**), 399.4 (mono intermediate), and 389.3 (HBED) is given in Figure 10. Since the disappearance of **9** was quite fast, an accurate peak area for *m/z* 409.3 was obtained for only the first 5-9 time points, depending on H₂O₂ concentration. One boron of *m/z* 409.3 (**9**) was quickly oxidized to the corresponding phenol to generate the mono intermediate *m/z* 399.4. The intermediate *m/z* 399.4 then gradually decayed as the second boron was oxidized to generate *m/z* 389.3, HBED. The peak area of HBED decreased slightly at the end of the oxidation; it is not known whether this decrease was due to the spectrometer losing sensitivity over time or due to HBED reacting further to form another species.

[00398] Plots of ln peak area vs. time for *m/z* 409.3 (**9**) and 389.3 (HBED) using various concentrations of H₂O₂ were all linear. With the exception of two, every *r*² value was > 0.98. The observed pseudo-first order rate constant (*k*_{obs}) for the oxidation of **9** to the mono intermediate, which was obtained by averaging *k*_{obs} from all samples within a concentration, was linearly dependent on the concentration of hydrogen peroxide. The second-order rate constant (*k*) obtained from the slope of this straight line is 30.8 M⁻¹ min⁻¹. Likewise, the second-order rate constant for the formation of HBED from the mono intermediate is 10.3 M⁻¹ min⁻¹.

[00399] In addition to determining the observed pseudo-first order rate constants for the disappearance of **9** and the formation of HBED at different H₂O₂ concentrations, *k*_{obs} was measured for the disappearance of the mono intermediate at different H₂O₂ concentrations.

This was done by plotting $\ln$ peak area vs. time for m/z 399.4 beginning at the time point where the peak of **9** was no longer able to be integrated. Interestingly, k obtained from the slope of the linear fit of k_{obs} vs. H_2O_2 concentration was $6.3 \text{ M}^{-1} \text{ min}^{-1}$. In theory, the rate constant for the disappearance of the mono intermediate should equal the rate constant for the formation of HBED in an $\text{A} \rightarrow \text{B} \rightarrow \text{C}$ reaction. The fact that HBED was formed faster than the mono intermediate was oxidized suggests that either some of **A** (**9**) was oxidizing directly to **C** (HBED), or that there was another intermediate formed from the oxidation of **9** that was not noticed during LC-MS optimization.

[00400] The above set of experiments to determine the second-order rate constant for the oxidation of **9** in pH 7.4 NMM buffer were confounded by the fact that H_2O_2 can oxidize NMM to the corresponding N-oxide (Ma *et al.*, 2008). NMM N-oxide may then, in turn, oxidize **9** (Kabalka and Hedgecock Jr., 1975), possibly at a different rate than oxidation by H_2O_2 . As noted above in the experimental section, the stock solution of H_2O_2 was prepared in NMM buffer.

[00401] In order to determine whether the potential reaction between NMM and H_2O_2 affected the rate of oxidation of **9**, a brief LC-MS experiment was conducted in a manner similar to that described above, but replacing NMM buffer with a 10 mM pH 7.4 phosphate buffer (for both the reaction buffer and the H_2O_2 stock buffer). Only single or duplicate samples were run for each H_2O_2 concentration. Due to ion suppression, m/z 409.3 (**9**) peaks were difficult to integrate past 2-4 time points. Therefore, the k_{obs} values from these linearized plots were not accurate enough to report. The second-order rate constants for the disappearance of 399.4 (mono intermediate) and formation of HBED, respectively, were $7.6 \text{ M}^{-1} \text{ min}^{-1}$ ($r^2 = 0.95$) and $9.7 \text{ M}^{-1} \text{ min}^{-1}$ ($r^2 = 0.92$), which were close to the values obtained when oxidation was carried out in NMM buffer ($6.3 \text{ M}^{-1} \text{ min}^{-1}$ and $10.3 \text{ M}^{-1} \text{ min}^{-1}$). It should also be noted that, similar to what was seen in the NMM buffer oxidations, peak area of HBED decreased slightly at the end of each reaction in phosphate buffer.

[00402] These experiments indicate that after the double prodrugs of the general form **7** hydrolyze to prodrug **9**, **9** can then undergo oxidation by H_2O_2 to give HBED (Figure 20). This required second step in the activation pathway of the double prodrugs before an active chelator is released should not only prevent indiscriminant metal sequestration until full activation is achieved, but may also provide the prodrugs with site-specificity. In a highly oxidative environment, such as in tissues where excess labile iron is contributing to free radical production, **9** may be preferentially converted to HBED. HBED, in turn, should prevent further Fenton chemistry by scavenging the harmful iron.

Oxidation by UV

[00403] Although monitoring the oxidation of prodrug **9** by LC-MS was a useful way of observing the reaction pathway and kinetics, N-methylmorpholine buffer is not an ideal representation of physiological conditions. Since *in vitro* studies (Cell Culture Studies, below) to evaluate the protective effects of prodrugs **8a-c** (and **9**) against H₂O₂-induced cell death were carried out in minimum essential medium (MEM), it was very important to verify the subsequent oxidation of the hydrolysis product (i.e. prodrug **9**) of prodrugs **8a-c** in order to confirm that under the cell culture conditions used, prodrugs **8a-c** were being unmasked to HBED via Pathway A in Figure 19. Thus, the oxidation of a hydrolyzed solution of prodrug **8a** in phenol free-MEM supplemented with glutamine, which was confirmed above (Hydrolysis by UV) to contain prodrug **9**, was monitored by UV at 37 °C using various concentrations of H₂O₂ (50-200x excess). UV spectra showing a representative oxidation reaction (solid lines) are shown in Figure 11. In the presence of 200x excess H₂O₂, absorbance at 273 nm increased over time, and the final spectrum of the oxidized product matched that of HBED in MEM containing 200x excess H₂O₂. For each concentration of H₂O₂ used, the negative slope of the linear fit of $\ln(A_{\infty}-A_t)$ vs. mean time in minutes gave the pseudo first-order rate constant (k_{obs}). A second-order rate constant (k) of 1.82 M⁻¹ min⁻¹ for the overall oxidation of the product of **8a** hydrolysis (i.e. **9**) to HBED in MEM at 37 °C was obtained from the linear fit of the plot of k_{obs} versus H₂O₂ concentration (Figure 12).

Oxidation of Prodrug 8a by H₂O₂*Oxidation by NMR*

[00404] The H₂O₂ oxidation of prochelator **8a** to [the mesylate salt of] dimethyl HBED (Figure 13) in DMSO-d₆ was monitored by NMR at ambient temperature. The reaction was complete in a little over two hours, with clean conversion to dimethyl HBED. As Figure 14 shows, the aromatic peaks shifted significantly upfield upon oxidation of the aryl boronic acid moieties to phenolic OHs. The boronic acid pinacol ester singlet at 1.30 ppm seen in the parent prochelator spectrum disappeared during the oxidation, and was replaced by a singlet at 1.16 ppm. This latter singlet was presumably the pinacol ester of *boric* acid because free pinacol appears at 1.069 ppm in DMSO-d₆. Although a reference spectrum of a salt of dimethyl HBED could not be found, the aromatic chemical shifts were well matched to those reported by Wilson (1988) for the dihydrochloride salt of HBED. Additionally, aliphatic chemical shifts were well matched to the dihydrochloride salt of a dimethyl HBED derivative

that has methyl groups ortho and para to the phenols (Wilson, 1988). This oxidation experiment, while carried out in DMSO-d₆, provides evidence that the prodrugs of the invention have the potential to be activated via Pathway B of Figure 19.

Relative Binding Affinity of Prodrug 9 for Iron and Copper

[00405] One of the advantages of the prodrugs of the present invention is that their affinity for iron and other metals would be expected to be little or none in conditions and tissues where metal levels are normal and chelation is not needed. Thus, disruption of systemic homeostasis of iron and other physiologically-important metals may be avoided. This is achieved by masking the phenolic hydroxyl groups of HBED with boronic acids or esters alone or in combination with masking the carboxylic acid groups as esters. Since prodrug 9 only has the phenolic hydroxyl groups of HBED masked with boronic acids, it could still potentially be a tetradentate chelator due to the presence of its two amino nitrogens and two carboxylate oxygens. Thus, it was necessary to verify that compound 9, the product of hydrolysis of compounds 7a-c (or dimesylate salts 8a-c), was a weak chelator. Additionally, Swamy *et al.*, 2008, found that the boronic acid moieties of a bis phenylboronic acid-conjugated probe participated in the selective chelation of copper ions. Therefore, if 9 can be present in an open (hydrated) form in solution (Figure 20), its boronic acids could also serve as chelating ligands. To ascertain whether 9 was active as a chelator, the relative affinity of 9 for iron and copper was investigated.

Affinity for Iron

[00406] The relative affinity of prodrug 9 for iron was determined by UV/vis in a competition experiment with HBED at 23 °C in pH 7.5 phosphate buffer. Under these conditions, a solution of Fe-HBED chelate was light pink in color and had an absorbance at 481 nm (A_{481}), whereas solutions of 9 or EDTA in the presence of iron were colorless and did not absorb at this wavelength (Figure 15). Therefore, A_{481} was monitored to detect any change in the amount of iron chelated by HBED upon addition of 9 or EDTA. After a 22 h equilibration period, it was determined that a 3.3-fold excess of prodrug 9 (300 μ M) was unable to compete with HBED (90 μ M) for iron (30 μ M ferric ammonium citrate). The spectrum of compound 9 + HBED + iron was unchanged from that of HBED + iron (Figure 15). By contrast, the presence of a 3.3-fold excess of ethylenediaminetetraacetic acid (EDTA), a chelator with a lower affinity for iron than HBED, was able to decrease the amount of Fe-HBED chelate by 83%, as evidenced by the decrease in absorbance at 481 nm

in the spectrum of EDTA + HBED + iron (0.088) compared to the spectrum of HBED + iron (0.015) and by the change in color of the solution from light pink to almost colorless (Figure 15). Since **9**, even in excess, failed to compete with HBED for iron, **9** either has little-to-no affinity for iron or at least is a much weaker chelator than EDTA ($\log K = 25.0$) (Martell and Smith, 1974).

Affinity for Copper

[00407] The relative affinity of prodrug **9** for copper was determined by a UV/vis competition experiment with ethylenediaminetetraacetic acid (EDTA) at 23 °C in 25:75 MeOH/pH 7.4 phosphate buffer. As Figure 16 shows, the spectrum of a solution of **9** (300 μM) + copper (100 μM , as copper (II) sulfate pentahydrate) has an absorbance at 410 nm, which suggests that prodrug **9** has some affinity for or interaction with copper. However, the addition of 20, 60, and 100 μM EDTA decreases the A_{410} by ~17%, 57%, and 94%, respectively, indicating that excess **9** does not compete with EDTA for copper chelation. Additionally, the solution grew lighter in color with increasing EDTA concentration. The solutions of 100 μM copper + 100 μM EDTA with or without 300 μM **9** were both faintly blue in color. These results suggested that excess **9** could not compete with EDTA ($\log K = 18.7$) for copper chelation (Martell and Smith, 1974). Thus, the interaction between prodrug **9** and copper must be a very weak one.

[00408] Collectively, the results of these affinity studies indicate that **9** has, at best, a weak affinity for iron and copper, and is therefore expected to pose little risk for off-target chelation of these metals (Figure 20). Thus, **9** can still be considered a prodrug of HBED and requires further activation before the strong chelator HBED is liberated.

Cell Culture Studies

Cytoprotective Effects using Proliferating Cells and Crystal Violet Dye Measurements

[00409] Since prodrugs **8a-c** and **9** were found to be successfully activated to HBED by hydrolysis and H_2O_2 oxidation in buffers and medium, it was next necessary to determine whether they could be activated in a cell culture system to afford cells protection against H_2O_2 -induced death. Presumably, protective capabilities would require that the prodrugs be able to enter cells and be activated to HBED in a timely manner. Non-confluent ARPE-19 cells were pretreated with prodrugs **8a-c**, **9**, and HBED in MEM containing FBS for 15 h before either 300 μM or 500 μM H_2O_2 was applied. After 8 h, the cells were fixed and then

stained with crystal violet dye. The absorbance of the resolubilized dye is proportional to cell number. Figures 17A-17B show the results of these studies.

[00410] Under these conditions, HBED generally provided better protection than did the prodrugs, especially at lower concentrations. This may be because of the time required by the prodrugs to convert to HBED. After the 15 h pretreatment period, prodrugs **8a-c** should be fully hydrolyzed to prodrug **9**, given that their half-lives in cell culture medium (MEM) were approximately 41-137 min, depending on the prodrug. However, once H₂O₂ is applied, prodrug **9** must still be oxidized to HBED, during which time the H₂O₂ may already be damaging cells to some extent. In contrast, when H₂O₂ is applied to cells pretreated with HBED, the HBED is instantaneously able to supply protection in the form of chelation because no unmasking is necessary.

[00411] Another factor to consider in these studies is whether the protection of cells by prodrugs **8a-c** and **9** was due to chelation of iron by the formed HBED, preventing Fenton chemistry, or due to the consumption of H₂O₂ during prodrug conversion to HBED. At a concentration of 44 μ M, **8c**, which contains isopropyl esters and has the longest hydrolysis half-life in MEM ($t_{1/2}$ = 137 min), provided cells with 48% protection against 500 μ M H₂O₂. At this concentration, only 88 μ M H₂O₂ would have been consumed by the conversion to HBED. Therefore, it can be postulated that a significant portion of the protection provided by **8c** was due to chelation by the newly-formed HBED, and not by the conversion process itself. Compounds **8a** and **8b** did not protect as well as **8c** at this concentration (44 μ M). Possibly, this difference is because **8a** and **8b** hydrolyze more rapidly in MEM to give **9** than does **8c**. Compound **8c** may be able to penetrate cells better because it stays intact longer, and this better penetration may afford cells better protection. Indeed, prodrugs **8a** and **8b** protected cells to a similar extent as prodrug **9**.

[00412] *Cytotoxicity Effects using Proliferating Cells and Crystal Violet Dye Measurements*

[00413] As mentioned above, one of the advantages of the prodrugs of the present invention is that their affinity for iron and other metals would be expected to be little or none in conditions and tissues where metal levels are normal and chelation is not needed. Thus, disruption of systemic homeostasis of iron and other physiologically-important metals may be avoided. This advantage was evident when looking at the number of ARPE-19 cells present after treatment for 24 h with HBED, **8b**, and **8c** in MEM containing FBS (Figure 18). At a concentration of 150 μ M, HBED decreased cell number by ~22%, while **8b** at this

concentration did not decrease cell number. Since proliferating cells were used in these cell culture experiments, this decrease in cell number (compared to controls) by HBED may be because this strong chelator pilfers iron from ribonucleotide reductase and other iron-containing proteins involved in growth and survival.

Cytoprotective Effects Using Confluent Cells and MTT Assay Measurements

[00414] Another set of experiments was conducted using ARPE-19 cells that were grown to 100% confluence in growth medium so that the confounding factor of chelator-induced growth inhibition could be avoided (see above). Additionally, the test compounds were applied in medium that did not contain fetal bovine serum, since iron-containing transferrin and ferritin contained in serum could also confound the results. A more sensitive assay – the MTT assay – was also used in this set of experiments to determine cell viability.

[00415] Confluent cells were pretreated with **8a-c**, **9**, or HBED (as its HCl salt) in MEM for 15 h so that all compounds would have sufficient time to enter the cells if they are membrane permeable, and so that the double prodrugs would also have time to hydrolyze. The cells were then treated for 8 h with a lethal dose of 500 μM of H_2O_2 , which decreased cell viability by approximately 79% in a preliminary kill curve experiment (Figure 28). Percent protection afforded by the compounds was calculated from the results of an MTT cell viability assay. The results of these cytoprotection studies are given in Figure 29.

Cytoprotection was dose dependent for all compounds tested. At their highest concentrations of 150 μM , **8a**, **8b**, **9**, and HBED all provided moderate protection to cells from the lethal dose of H_2O_2 relative to cells exposed to H_2O_2 but given no prodrug or HBED (62-68% protection, $p < 0.001$ by t-test). In contrast, despite applying at lower concentrations due to solubility limitations, **8c** gave the highest cytoprotection: at only 44 μM , **8c** afforded 84% protection against H_2O_2 relative to cells exposed to H_2O_2 but given no prodrug or HBED ($p < 0.001$). Additionally, while neither **8a**, **8b**, nor **9** showed enhanced protection over that of HBED at any equivalent dose, **8c** significantly out-protected HBED at concentrations of 13, 20, 30, and 44 μM ($p < 0.001$). At 44 μM , **8c** provided 3-fold higher protection to cells compared to HBED. **8c** was also not toxic to cells at this dose (Figure 25).

[00416] From the exceptional performance of **8c**, three points can be made. The first is that **8c** may gain entry into cells better than **8a** or **8b** because it has the slowest hydrolysis – its isopropyl carboxylate esters remain intact the longest – in MEM. Second, **8c** has enhanced membrane permeability compared to HBED. Presumably, 44 μM of **8c** was converted by hydrolysis and oxidation to 44 μM HBED. If **8c** and HBED had similar

membrane permeability, **8c** would have provided protection similar to that of HBED, or even less protection because the prodrugs are not able to instantly protect the cells upon exposure to H_2O_2 ; they first must be oxidized. Since **8c** provided superior protection over HBED, it must have superior membrane permeability. The third point that can be made is that the primary mode of protection of **8c** is not consumption of H_2O_2 during its unmasking. The oxidation of 44 μM of **8c** (presumably present as **9** after the 15 h pretreatment period) by H_2O_2 will consume a total of 88 μM of H_2O_2 , leaving 412 μM of the 500 μM dose of H_2O_2 to challenge the cells. According to Figure 28, exposure to a similar amount of H_2O_2 (417 μM) decreased cell viability by ~71%. But in the presence of 44 μM of **8c**, cell viability was only decreased by 22% (not shown). Therefore, while cytoprotection by **8c** may have been slightly improved by the consumption of H_2O_2 , it is more likely that the primary mechanism of protection by **8c** against H_2O_2 -induced cell death is from chelation of free iron upon the activation of **8c** to HBED.

Cytotoxicity Measurements Using Confluent Cells and MTT Assay Measurements

[00417] The cytotoxicities of the prodrugs and HBED after 23 h (corresponding to the total length of the cytoprotection experiments) were investigated using confluent ARPE-19 cells. The results are given in Figure 25. Prodrug **9** significantly decreased cell viability at all concentrations tested, in a dose-dependent manner. Viability ranged from 85% at the highest dose of 150 μM ($p < 0.01$) to 94% at the lowest dose of 20 μM ($p < 0.01$). The relatively poor solubility of **9** in DMSO required that the stock solution not exceed 10 mM. As such, cells were exposed to 1.5-0.20% DMSO as the solution of **9** in MEM was serially diluted 1.5-fold from 150 μM to 20 μM . Some of the cytotoxicity of **9** may therefore be explained by the relatively high percentage of DMSO to which the cells were exposed (Figure 26).

[00418] At 150 μM , their highest and most cytoprotective dose, **8a**, **8b**, and HBED all showed some cytotoxic effects (Figure 25), leading to decreases in cell viabilities to 93% ($p < 0.05$), 91% ($p < 0.001$), and 78% ($p < 0.001$), respectively, compared to controls not treated with compound. At a dose of 44 μM , HBED was less toxic to cells, but still significantly decreased cell viability to 91% ($p < 0.001$) (Figure 25). In contrast, the highest and most cytoprotective dose of **8c**, 44 μM , did not cause any loss of cell viability (Figure 25).

[00419] It should be noted that while HBED and **8a** dose-dependently decreased cell viability, viability in the presence of **8b** remained slightly lowered regardless of the concentration of **8b** (Figure 25). Also, viability seemed to decrease and then increase, in a U-

shaped manner, as the concentration of **8c** increased (Figure 25). Since the decreases in cell viability were slight, it may be that they were due to experimental variability or error. It is also surprising that cell viability was significantly decreased by 4% upon exposure to only 0.15% DMSO ($p < 0.05$) (Figure 26). Again, this decrease may have been artifactual.

[00420] In summary, it has been shown herein that selected double prodrugs of HBED can be activated under physiologically-relevant conditions *in vitro* to give HBED, the parent iron chelator, and can ultimately provide cells with protection against oxidative stress promoted by the application of exogenous H_2O_2 . The studies suggest that at least one double prodrug, **8c**, has improved membrane permeability over HBED that results in better cytoprotection. Therefore, these double prodrugs may prove useful in improving the oral bioavailability of HBED and its delivery to specific tissues where an iron chelator is needed to curtail Fenton chemistry. This has implications for a broad range of diseases that are promoted by oxidative stress.

Passive Diffusion Results

[00421] Passive diffusion studies of **7a** and HBED-HCl using Franz diffusion cells (Figure 27) equipped with silicone membranes further support the improved membrane permeability of the double prodrugs. Silicone membranes, which are lipid-like, have been validated as a surrogate for flux across a biological membrane: namely, the skin (Sloan *et al.*, 2013). Mineral oil was used as the donor vehicle in which the compounds were dissolved so that **7a** would remain intact in the donor phase throughout the experiment. Use of a protic solvent would most likely have led to its decomposition. The receptor chambers contained phosphate buffer. All receptor phase samples taken from diffusion cells to which **7a** was applied were allowed to hydrolyze to **9** before analysis because **7a** and **9** do not have sufficiently different UV spectra to enable one to calculate the amount of each in a mixed sample. The amount of **9** in receptor phase samples presumably corresponds to the amount of **7a** that was able to diffuse intact through the silicone membranes before subsequently hydrolyzing in the receptor chambers. **7a** showed excellent diffusion across the silicone membranes, having a maximum flux of $0.43 \pm 0.04 \mu\text{mol}/\text{cm}^2/\text{h}$ ($\log \text{flux} = -0.36$). By contrast, no flux was observed for HBED-HCl through the silicone membranes from a mineral oil vehicle. Once again, this suggests that the double prodrugs may have better membrane permeability than the parent chelator.

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EQUIVALENTS AND SCOPE

[00422] In the claims articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[00423] Furthermore, the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, and descriptive terms from one or more of the listed claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Where elements are presented as lists, *e.g.*, in Markush group format, each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements and/or features, certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements and/or features. For purposes of simplicity, those embodiments have not been specifically set forth *in haec verba* herein. It is also noted that

the terms “comprising” and “containing” are intended to be open and permits the inclusion of additional elements or steps. Where ranges are given, endpoints are included. Furthermore, unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or sub-range within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

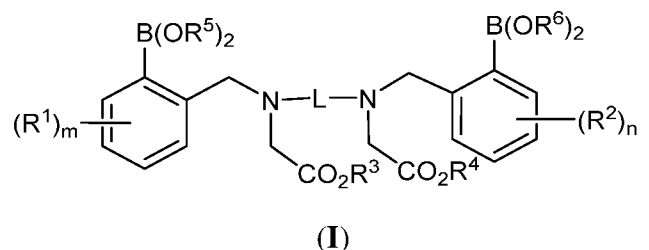
[00424] This application refers to various issued patents, published patent applications, journal articles, and other publications, all of which are incorporated herein by reference. If there is a conflict between any of the incorporated references and the instant specification, the specification shall control. In addition, any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Because such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the invention can be excluded from any claim, for any reason, whether or not related to the existence of prior art.

[00425] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments described herein. The scope of the present embodiments described herein is not intended to be limited to the above Description, but rather is as set forth in the appended claims. Those of ordinary skill in the art will appreciate that various changes and modifications to this description may be made without departing from the spirit or scope of the present invention, as defined in the following claims.

CLAIMS

What is claimed is:

1. A compound of Formula (I):



or a pharmaceutically or cosmetically acceptable salt thereof,

wherein:

each of m and n is independently 0, 1, 2, 3, or 4;

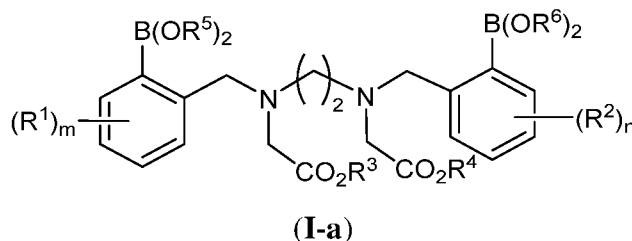
each instance of R¹ and R² is independently hydrogen, halogen, -CN, -NO₂, -N₃, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, optionally substituted amino group, or optionally substituted acyl;

each of R³ and R⁴ is independently hydrogen, optionally substituted alkyl, or an oxygen protecting group;

each instance of R⁵ and R⁶ is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group; or two R⁵ are taken together with the intervening atoms to form optionally substituted heterocyclyl; or two R⁶ are taken together with the intervening atoms to form optionally substituted heterocyclyl; and

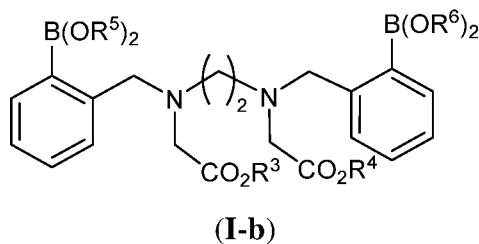
L is optionally substituted C₁₋₈ alkylene.

2. The compound of claim 1, wherein the compound is of Formula (I-a):



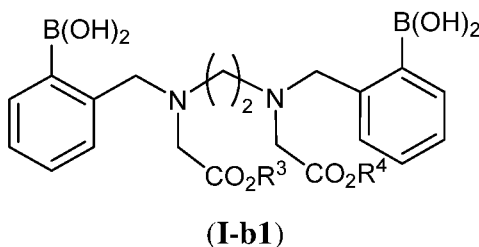
or a pharmaceutically or cosmetically acceptable salt thereof.

3. The compound of claim 1, wherein the compound is of Formula **(I-b)**:



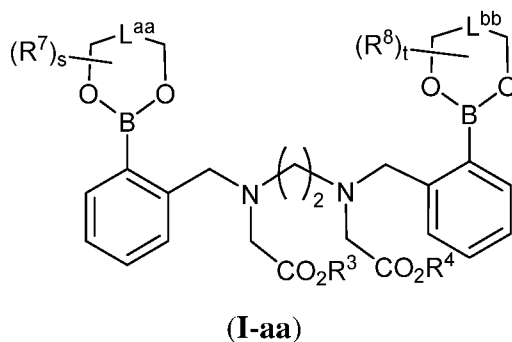
or a pharmaceutically or cosmetically acceptable salt thereof.

4. The compound of claim 1, wherein the compound is of Formula **(I-b1)**:



or a pharmaceutically or cosmetically acceptable salt thereof.

- 4a. The compound of claim 1, wherein the compound is of Formula **(I-aa)**:



or a pharmaceutically or cosmetically acceptable salt thereof,

wherein:

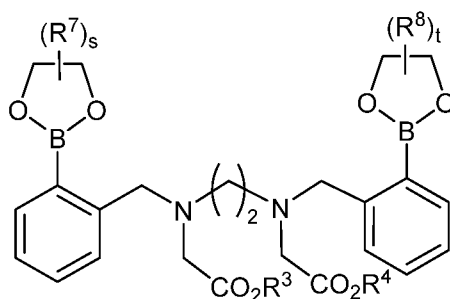
each of L^{aa} and L^{bb} is independently a bond, optionally substituted alkylene, or optionally substituted heteroalkylene;

each instance of R^7 and R^8 is independently hydrogen, or optionally substituted alkyl;

and

each of s and t is independently 0, 1, 2, 3, or 4.

5. The compound of claim 1, wherein the compound is of Formula **(I-b2)**:



(I-b2)

or a pharmaceutically or cosmetically acceptable salt thereof;

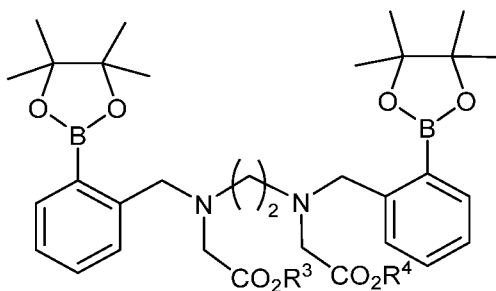
wherein:

each instance of R^7 and R^8 is independently hydrogen or optionally substituted alkyl;

and

each of s and t is independently 0, 1, 2, 3, or 4.

6. The compound of claim 5, wherein the compound is of Formula **(I-b3)**:

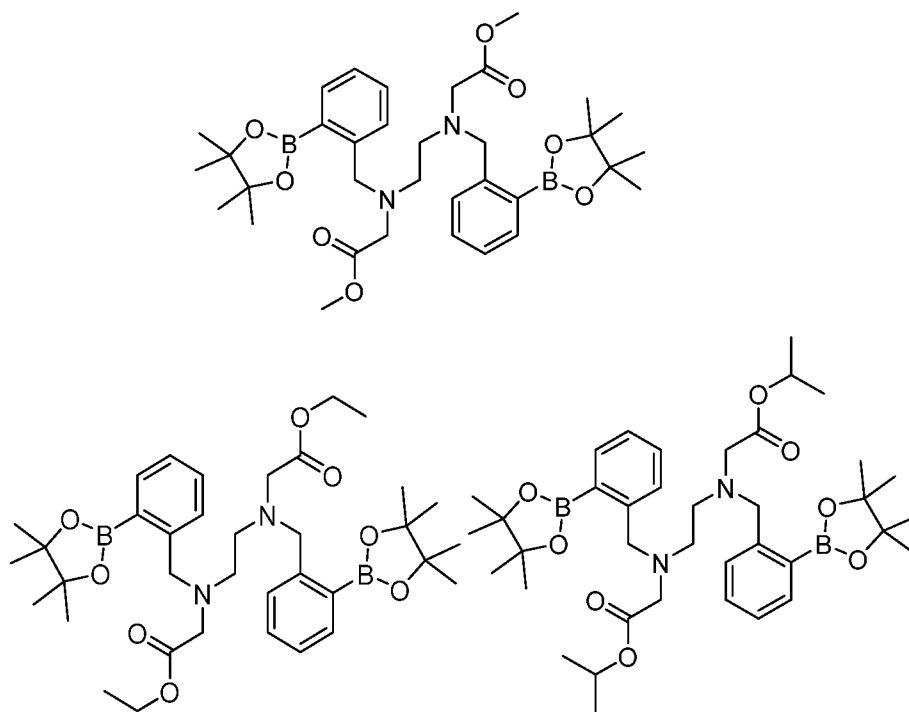


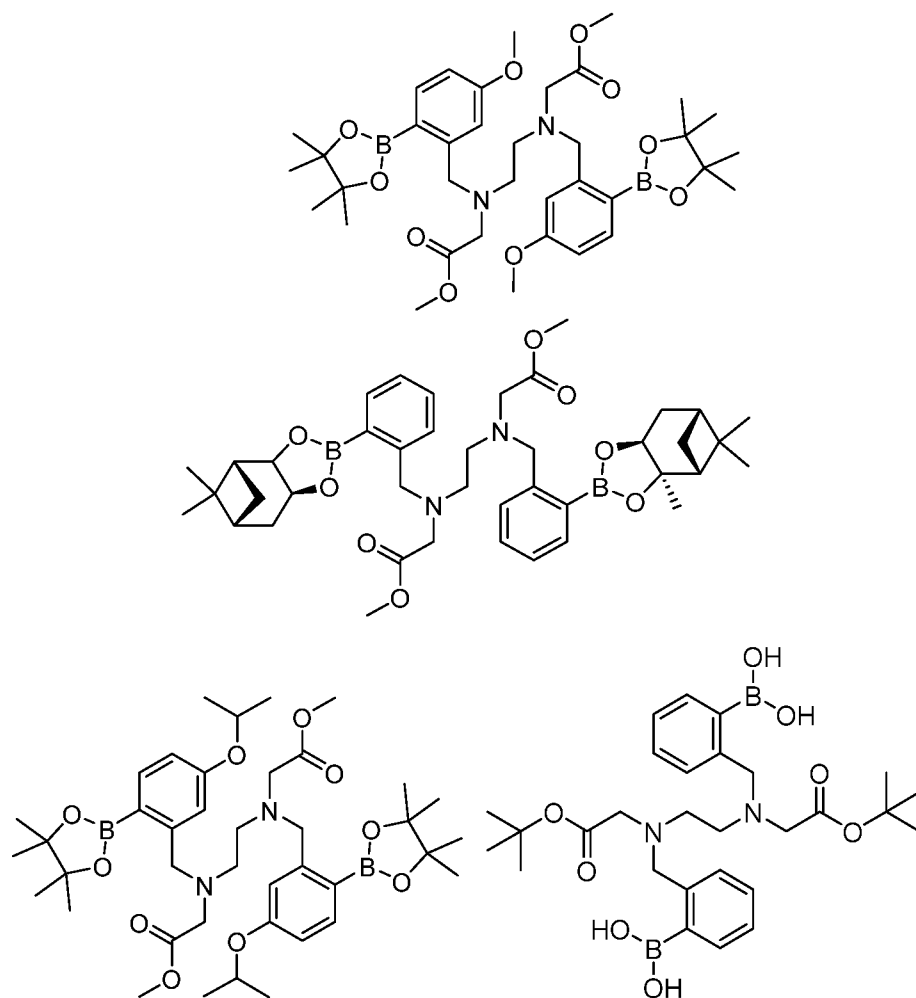
(I-b3)

or a pharmaceutically or cosmetically acceptable salt thereof.

7. The compound of any one of claims 1-6, wherein R^3 and R^4 are the same.
8. The compound of any one of claims 1-6, wherein R^3 and R^4 are different.
9. The compound of any one of claims 1-6, wherein R^3 is hydrogen.
10. The compound of any one of claims 1-6, wherein R^3 is optionally substituted alkyl.
11. The compound of any one of claims 1-6, wherein R^3 is unsubstituted alkyl.

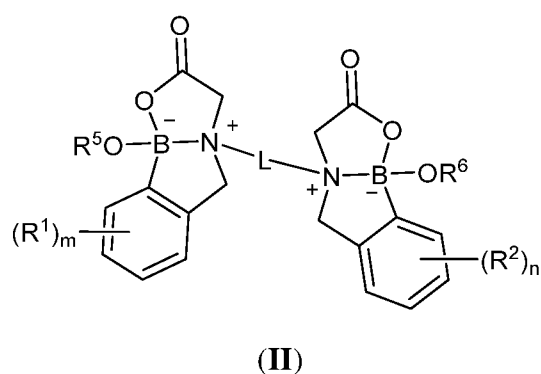
12. The compound of claim 11, wherein R^3 is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl.
13. The compound of any one of claims 1-12, wherein R^4 is hydrogen.
14. The compound of any one of claims 1-12, wherein R^4 is optionally substituted alkyl.
15. The compound of any one of claims 1-12, wherein R^4 is unsubstituted alkyl.
16. The compound of claim 15, wherein R^4 is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl.
17. The compound of claim 1, wherein the compound is of one of the following formulae:





or a pharmaceutically or cosmetically acceptable salt thereof.

18. A compound of Formula (II):



or a pharmaceutically or cosmetically acceptable salt thereof, wherein:

each of m and n is independently 0, 1, 2, 3, or 4;

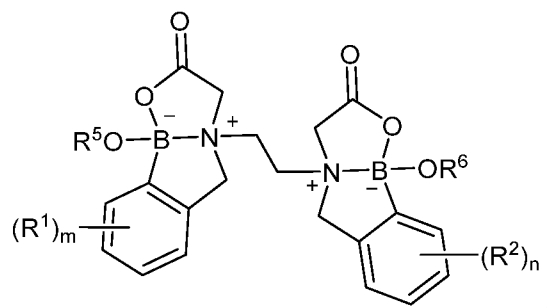
each instance of R¹ and R² is independently hydrogen, halogen, -CN, -NO₂, -N₃, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted

heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, optionally substituted amino group, or optionally substituted acyl;

each of R^5 and R^6 is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group; and

L is optionally substituted C_{1-8} alkylene.

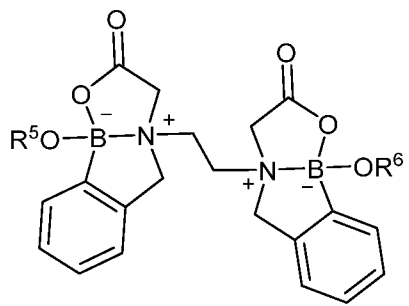
19. The compound of claim 18, wherein the compound is of Formula (II-a):



(II-a)

or a pharmaceutically or cosmetically acceptable salt thereof.

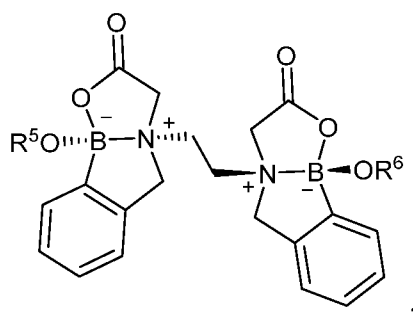
20. The compound of claim 18, wherein the compound is of Formula (II-b):



(II-b)

or a pharmaceutically or cosmetically acceptable salt thereof.

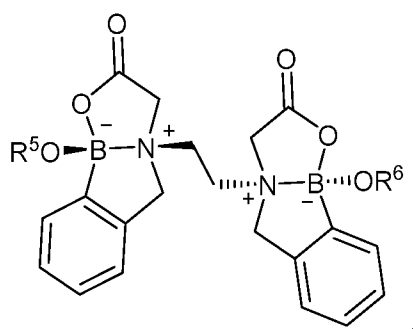
21. The compound of claim 18, wherein the compound is of Formula (II-b1):



(II-b1)

or a pharmaceutically or cosmetically acceptable salt thereof.

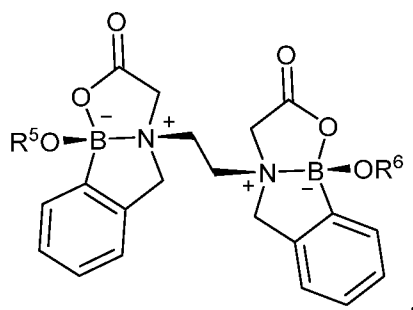
22. The compound of claim 18, wherein the compound is of Formula (II-b2):



(II-b2)

or a pharmaceutically or cosmetically acceptable salt thereof.

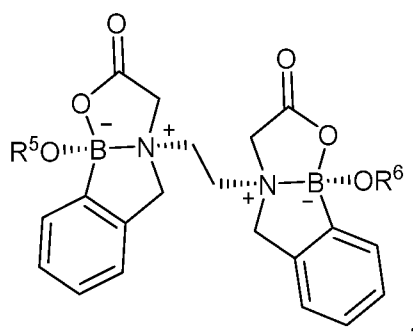
23. The compound of claim 18, wherein the compound is of Formula (II-b3):



(II-b3)

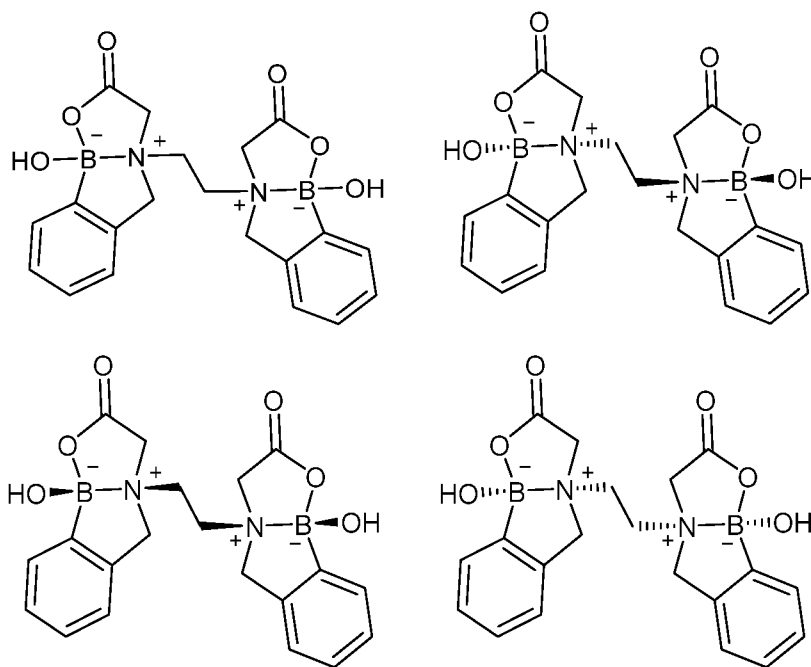
or a pharmaceutically or cosmetically acceptable salt thereof.

24. The compound of claim 18, wherein the compound is of Formula (II-b4):

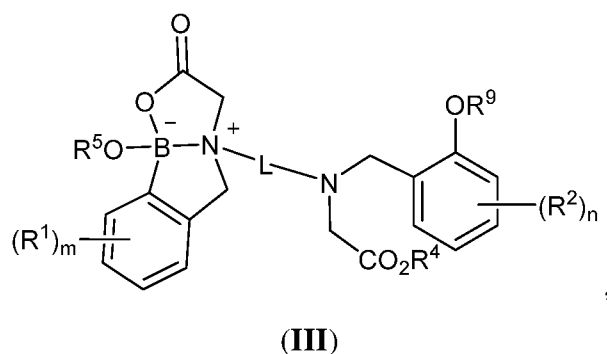


or a pharmaceutically or cosmetically acceptable salt thereof.

25. The compound of any one of claims 18-24, wherein R^5 and R^6 are the same.
26. The compound of any one of claims 18-24, wherein R^5 and R^6 are different.
27. The compound of any one of claims 18-24, wherein R^5 is hydrogen.
28. The compound of any one of claims 18-24, wherein R^6 is hydrogen.
29. The compound of claim 18, wherein the compound is of the following formulae:



30. A compound of Formula (III):



or a pharmaceutically or cosmetically acceptable salt thereof, wherein:

each of m and n is independently 0, 1, 2, 3, or 4;

each instance of R¹ and R² is independently hydrogen, halogen, -CN, -NO₂, -N₃, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, optionally substituted alkoxy, optionally substituted amino group, or optionally substituted acyl;

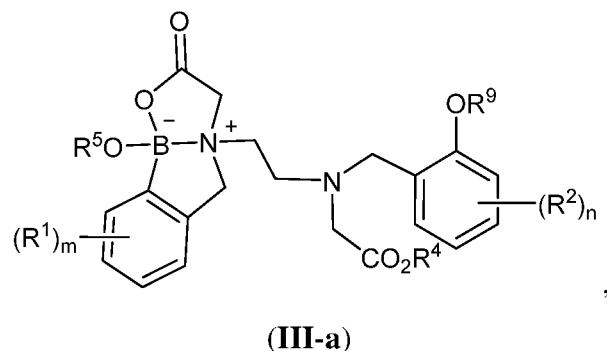
R⁴ is hydrogen, optionally substituted alkyl, or an oxygen protecting group;

R⁵ is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or an oxygen protecting group;

R⁹ is hydrogen, optionally substituted alkyl, or an oxygen protecting group; and

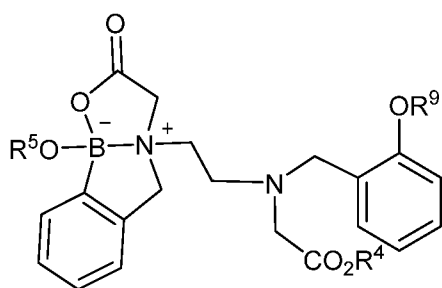
L is optionally substituted C₁₋₈ alkylene.

31. The compound of claim 30, wherein the compound is of Formula (III-a):



or a pharmaceutically or cosmetically acceptable salt thereof.

32. The compound of claim 30, wherein the compound is of Formula (III-b):

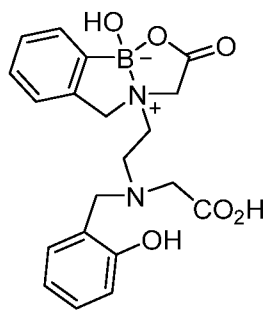


(III-b)

or a pharmaceutically or cosmetically acceptable salt thereof.

33. The compound of any one of claims 30-32, wherein R^9 is hydrogen.
34. The compound of any one of claims 30-33, wherein R^5 is hydrogen.
35. The compound of any one of claims 30-34, wherein R^4 is hydrogen.
36. The compound of any one of claims 30-34, wherein R^4 is optionally substituted alkyl.
37. The compound of any one of claims 30-34, wherein R^4 is unsubstituted alkyl.
38. The compound of claim 37, wherein R^4 is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, or *t*-butyl.

39. The compound of claim 30, wherein the compound is of the formula:



or a pharmaceutically or cosmetically acceptable salt thereof.

40. A pharmaceutical composition comprising an effective amount of a compound of any one of claims 1-39, or a pharmaceutically acceptable salt thereof, and optionally a pharmaceutically acceptable excipient.
41. The pharmaceutical composition of claim 40, wherein the pharmaceutical composition is for use in treating or preventing a pathological condition selected from the group consisting of metal overload, oxidative stress, transfusional iron overload, thalassemia, primary hemochromatosis, secondary hemochromatosis, diabetes, liver disease, heart disease, cancer, radiation injury, neurological or neurodegenerative disorder, macular degeneration, closed head injury, irritable bowel disease, stroke, reperfusion injury, metal poisoning, and an infectious disease.
42. The pharmaceutical composition of claim 41, wherein the metal overload is iron overload, aluminum overload, copper overload, zinc overload, lanthanide overload, or actinide overload.
43. The pharmaceutical composition of claim 41, wherein the metal poisoning is iron poisoning, aluminum poisoning, copper poisoning, zinc poisoning, lanthanide poisoning, or actinide poisoning.
44. The pharmaceutical composition of claim 41, wherein the pathological condition is iron overload, transfusional iron overload, thalassemia, primary hemochromatosis, or secondary hemochromatosis.
45. The pharmaceutical composition of claim 41, wherein the pathological condition is oxidative stress.
46. The pharmaceutical composition of claim 41, wherein the pathological condition is diabetes or liver disease.
47. The pharmaceutical composition of claim 41, wherein the pathological condition is heart disease.

48. The pharmaceutical composition of claim 41, wherein the pathological condition is cancer.
49. The pharmaceutical composition of claim 41, wherein the pathological condition is a neurodegenerative disorder.
50. The pharmaceutical composition of claim 49, wherein the neurodegenerative disorder is Parkinson's disease.
51. The pharmaceutical composition of claim 49, wherein the neurodegenerative disorder is Alzheimer's disease.
52. The pharmaceutical composition of claim 49, wherein the neurodegenerative disorder is Friedreich's ataxia or neurodegeneration with brain iron accumulation.
53. The pharmaceutical composition of claim 41, wherein the pathological condition is macular degeneration.
54. The pharmaceutical composition of claim 41, wherein the pathological condition is closed head injury.
55. The pharmaceutical composition of claim 41, wherein the pathological condition is irritable bowel disease.
56. The pharmaceutical composition of claim 41, wherein the pathological condition is stroke.
57. The pharmaceutical composition of claim 41, wherein the pathological condition is reperfusion injury.
58. A cosmetic composition comprising an effective amount of a compound of any one of claims 1-39, or a cosmetically acceptable salt thereof, and optionally a cosmetically acceptable excipient.

59. The cosmetic composition of claim 58, wherein the composition is suitable for topical administration.
60. The cosmetic composition of claim 58 or 59, wherein the cosmetic composition is for use in improving skin appearance and treating and/or preventing skin aging, skin photoaging, and/or skin cancer.
61. The cosmetic composition of claim 60, wherein the cosmetic composition is for use in treating and/or preventing skin photoaging.
62. A kit comprising a compound of any one of claims 1-39, or a pharmaceutically or cosmetically acceptable salt thereof, or a pharmaceutical composition of claim 40, or a cosmetic composition of claim 58 or 59; and instructions for administering the compound, the pharmaceutically or cosmetically acceptable salt thereof, or the pharmaceutical or cosmetic composition.
63. A method of treating a pathological condition selected from the group consisting of iron overload, aluminum overload, copper overload, zinc overload, lanthanide overload, actinide overload, oxidative stress, transfusional iron overload, thalassemia, primary hemochromatosis, secondary hemochromatosis, diabetes, liver disease, heart disease, cancer, radiation injury, neurological or neurodegenerative disorder, macular degeneration, closed head injury, irritable bowel disease, reperfusion injury, stroke, metal poisoning, and an infectious disease, in a subject, the method comprising administering to the subject a therapeutically or prophylactically effective amount of a compound of any one of claims 1-39, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition of claim 40.
64. The method of claim 63, wherein the metal overload is iron overload, aluminum overload, copper overload, zinc overload, lanthanide overload, or actinide overload.
65. The method of claim 63, wherein the metal poisoning is iron poisoning, aluminum poisoning, copper poisoning, zinc poisoning, lanthanide poisoning, or actinide poisoning.

66. The method of claim 63, wherein the pathological condition is iron overload, transfusional iron overload, thalassemia, primary hemochromatosis, or secondary hemochromatosis.
67. The method of claim 63, wherein the pathological condition is oxidative stress.
68. The method of claim 63, wherein the pathological condition is diabetes or liver disease,
69. The method of claim 63, wherein the pathological condition is heart disease.
70. The method of claim 63, wherein the pathological condition is cancer.
71. The method of claim 63, wherein the pathological condition is a neurodegenerative disorder.
72. The method of claim 71, wherein the neurodegenerative disorder is Parkinson's disease.
73. The method of claim 71, wherein the neurodegenerative disorder is Alzheimer's disease.
74. The method of claim 71, wherein the neurodegenerative disorder is Friedreich's ataxia or neurodegeneration with brain iron accumulation.
75. The method of claim 63, wherein the pathological condition is macular degeneration.
76. The method of claim 63, wherein the pathological condition is closed head injury.
77. The method of claim 63, wherein the pathological condition is irritable bowel disease.
78. The method of claim 63, wherein the pathological condition is stroke.
79. The method of claim 63, wherein the pathological condition is reperfusion injury.

80. A method of improving skin appearance and treating and/or preventing skin aging, skin photoaging, and/or skin cancer, the method comprising administering to the subject a therapeutically or prophylactically effective amount of a compound of any one of claims 1-39, or a cosmetically acceptable salt thereof, or a cosmetic composition of claim 58 or 59.
81. The method of claim 80, wherein the method is for use in treating and/or preventing skin photoaging.
82. The method of any one of claims 63-79, wherein the compound or pharmaceutical composition is administered orally.
83. The method of any one of claims 63-79, wherein the compound or pharmaceutical composition is administered intravenously.
84. The method of claim 75, wherein the compound or pharmaceutical composition is administered ophthalmically.
85. The method of any one of claims 80-81, wherein the compound or cosmetic composition is administered topically.

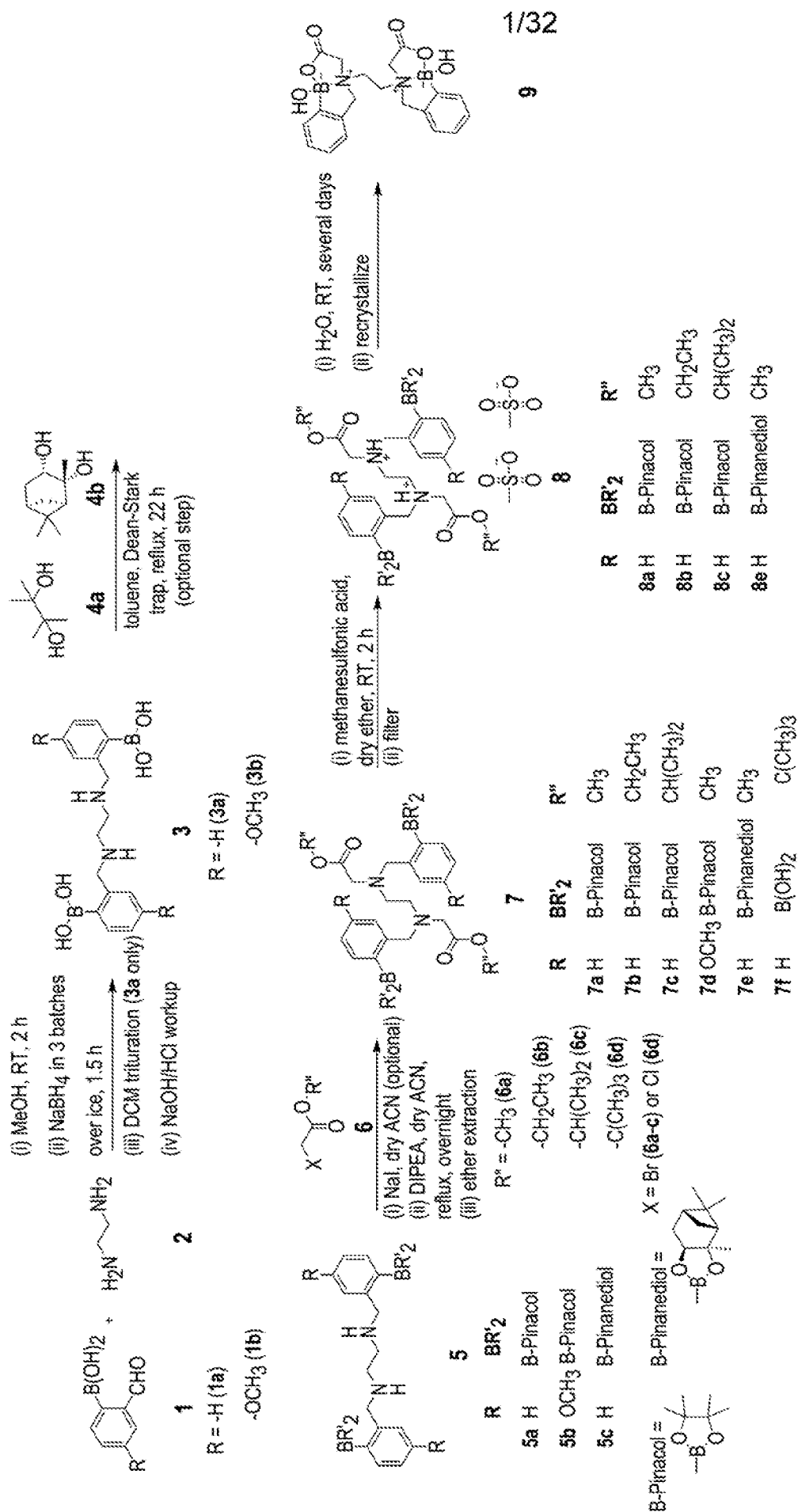


Figure 1

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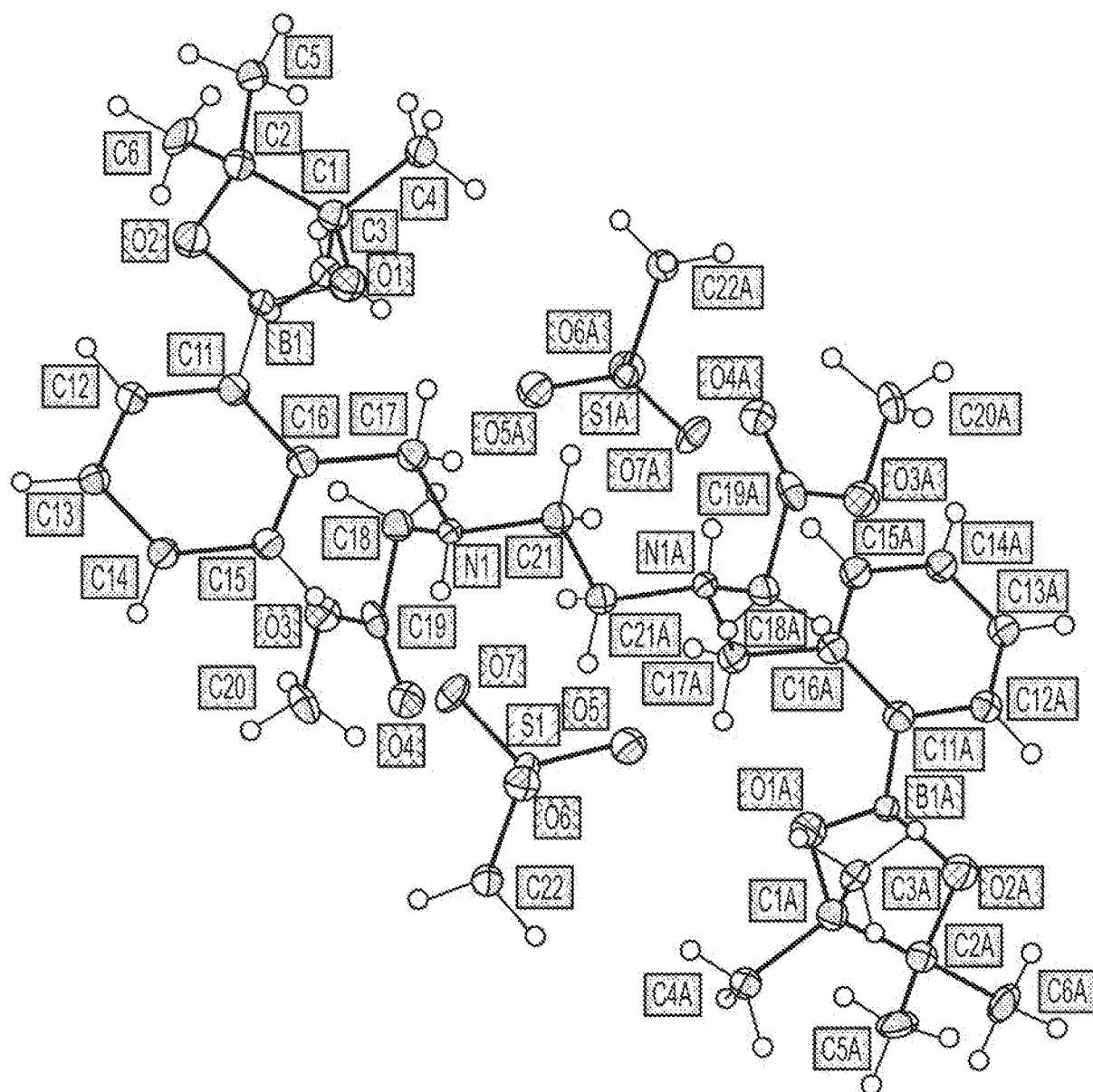
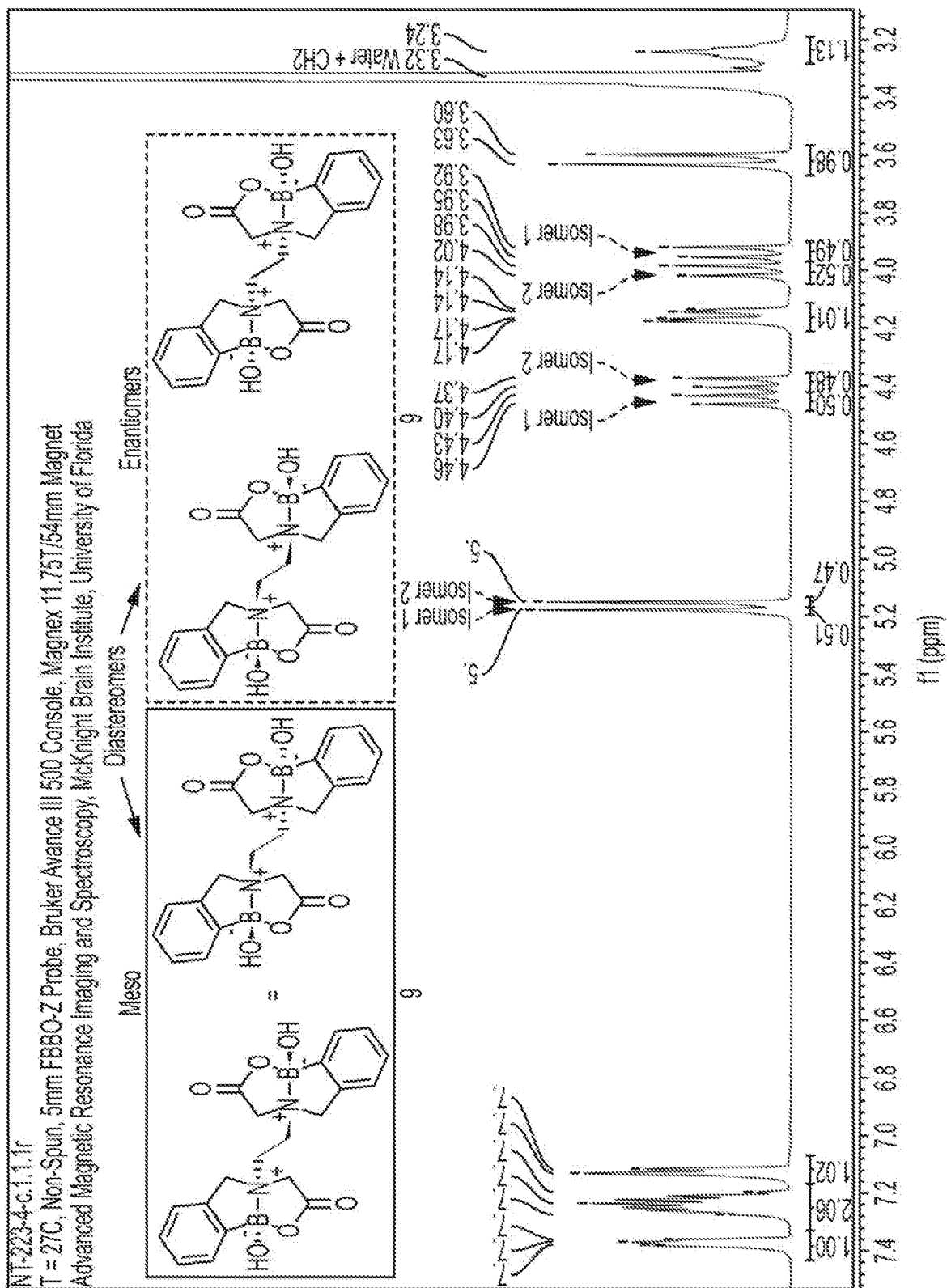


Figure 2



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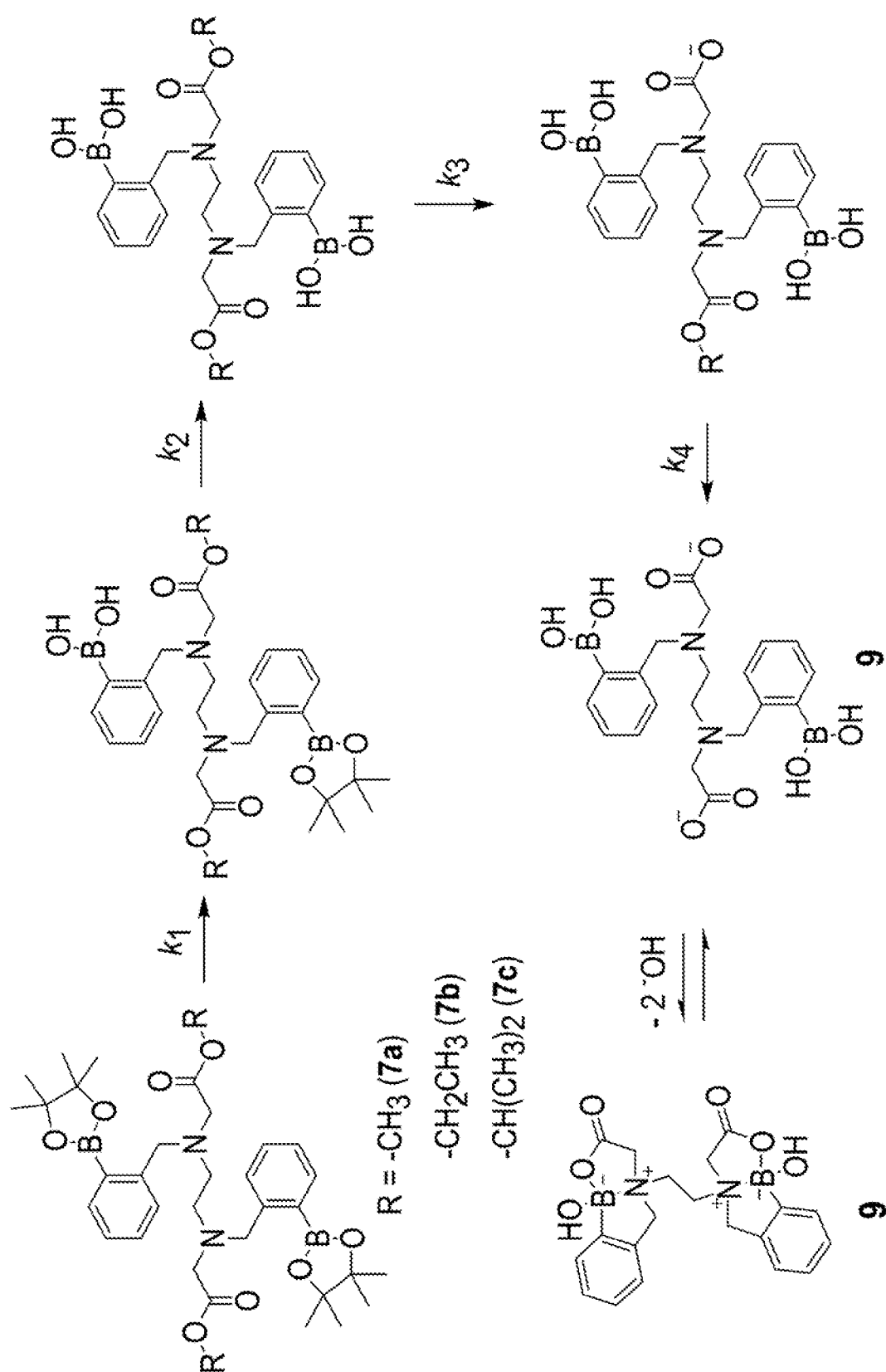


Figure 4

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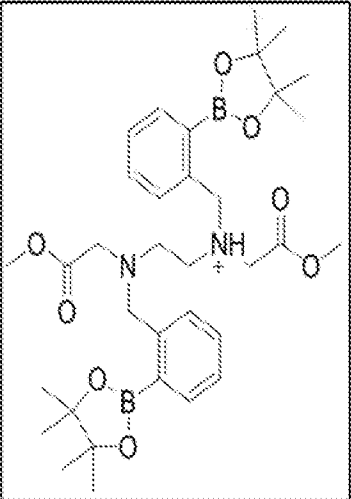
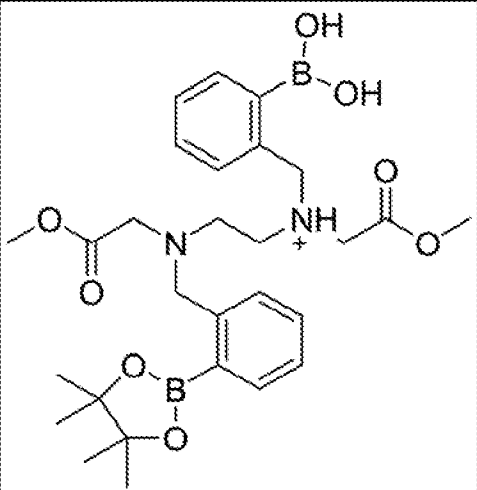
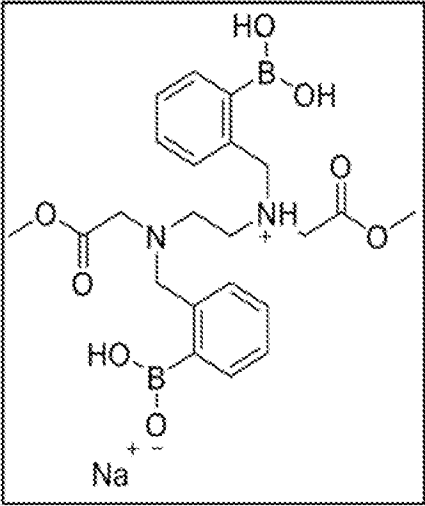
Structure(s)	<i>m/z</i> predicted	<i>m/z</i> experimental
	637.38	637.4
	555.30 (minus 1 pinacol)	555.3
	495.21 (minus 2 pinacol, plus 1 Na)	495.2

Figure 5

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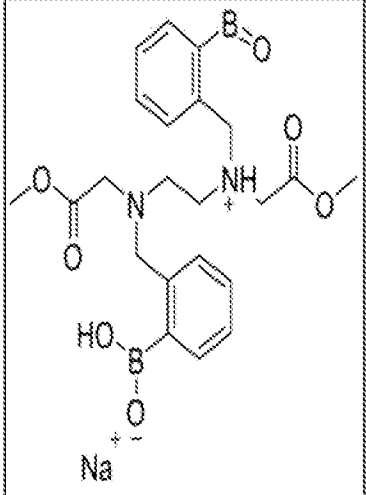
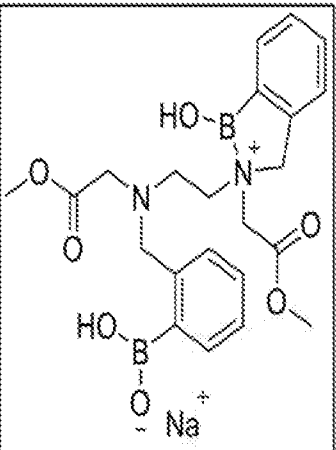
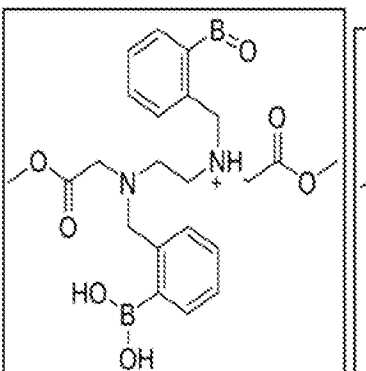
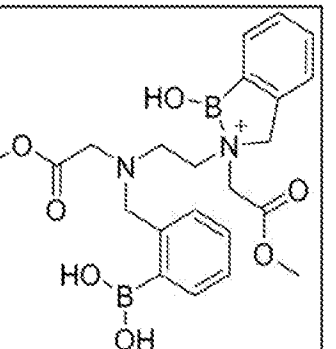
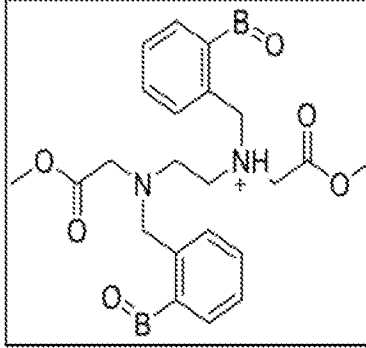
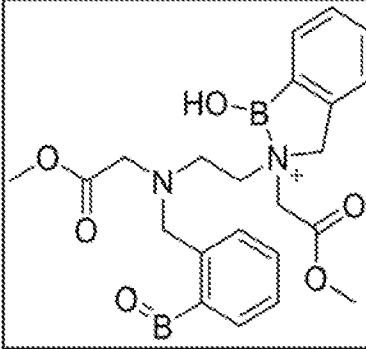
Structure(s)	<i>m/z</i> predicted	<i>m/z</i> experimental
 	477.20 (minus 2 pinacol, minus 1 H ₂ O, plus 1 Na)	477.2
 	455.22 (minus 2 pinacol, minus 1 H ₂ O)	455.2
 	437.20 (minus 2 pinacol, minus 2 H ₂ O)	437.2

Figure 5 (Continued)

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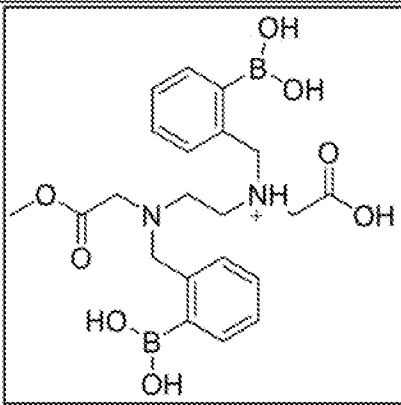
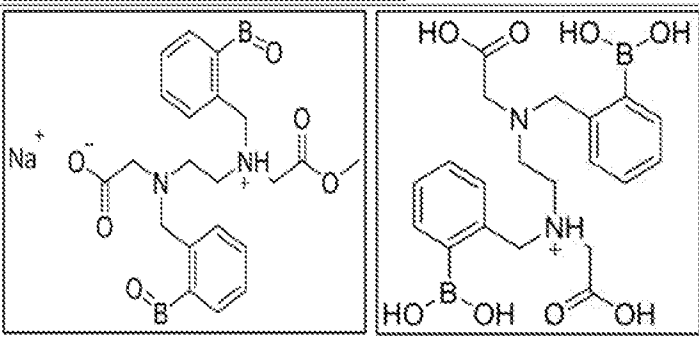
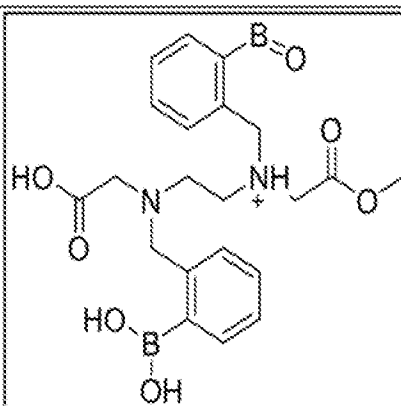
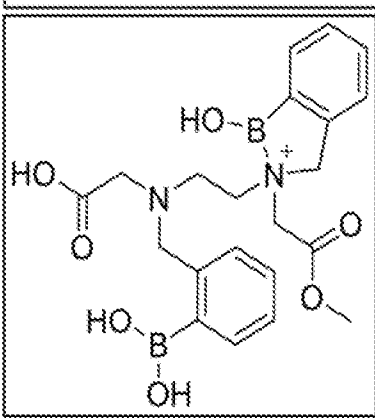
Structure(s)	<i>m/z</i> predicted	<i>m/z</i> experimental
	459.21 (minus 2 pinacol, minus 1 methyl)	459.3
	445.17 (minus 2 pinacol, minus 2 H2O, minus 1 methyl, plus 1 Na) *or* 445.19 (minus 2 pinacol, minus 2 methyl)	445.2
		
	441.20 (minus 2 pinacol, minus 1 H2O minus 1 methyl)	441.2

Figure 5 (Continued)

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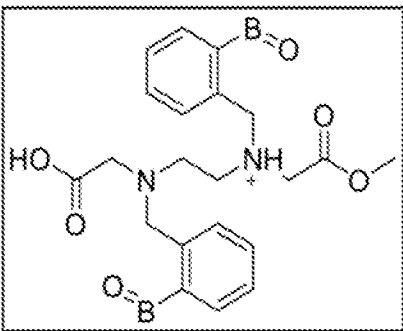
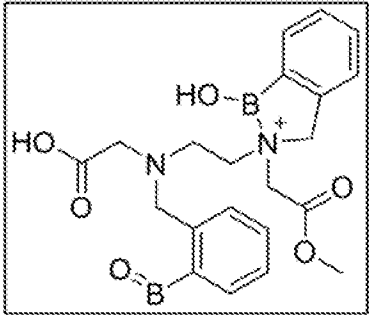
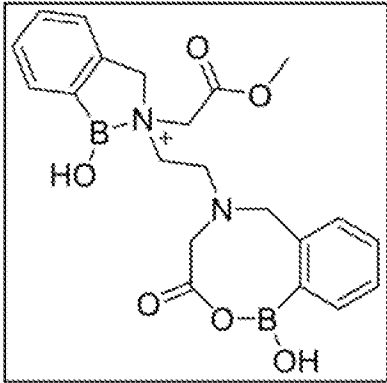
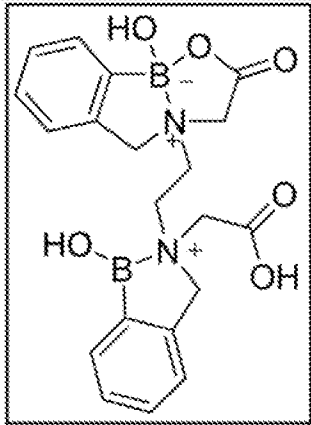
Structure(s)	<i>m/z</i> predicted	<i>m/z</i> experimental
		
		
	423.19 (minus 2 pinacol, minus 2 H ₂ O, minus 1 methyl)	423.2
	409.17 (minus 2 pinacol, minus 2 H ₂ O, minus 2 methyl)	409.3

Figure 5 (Continued)

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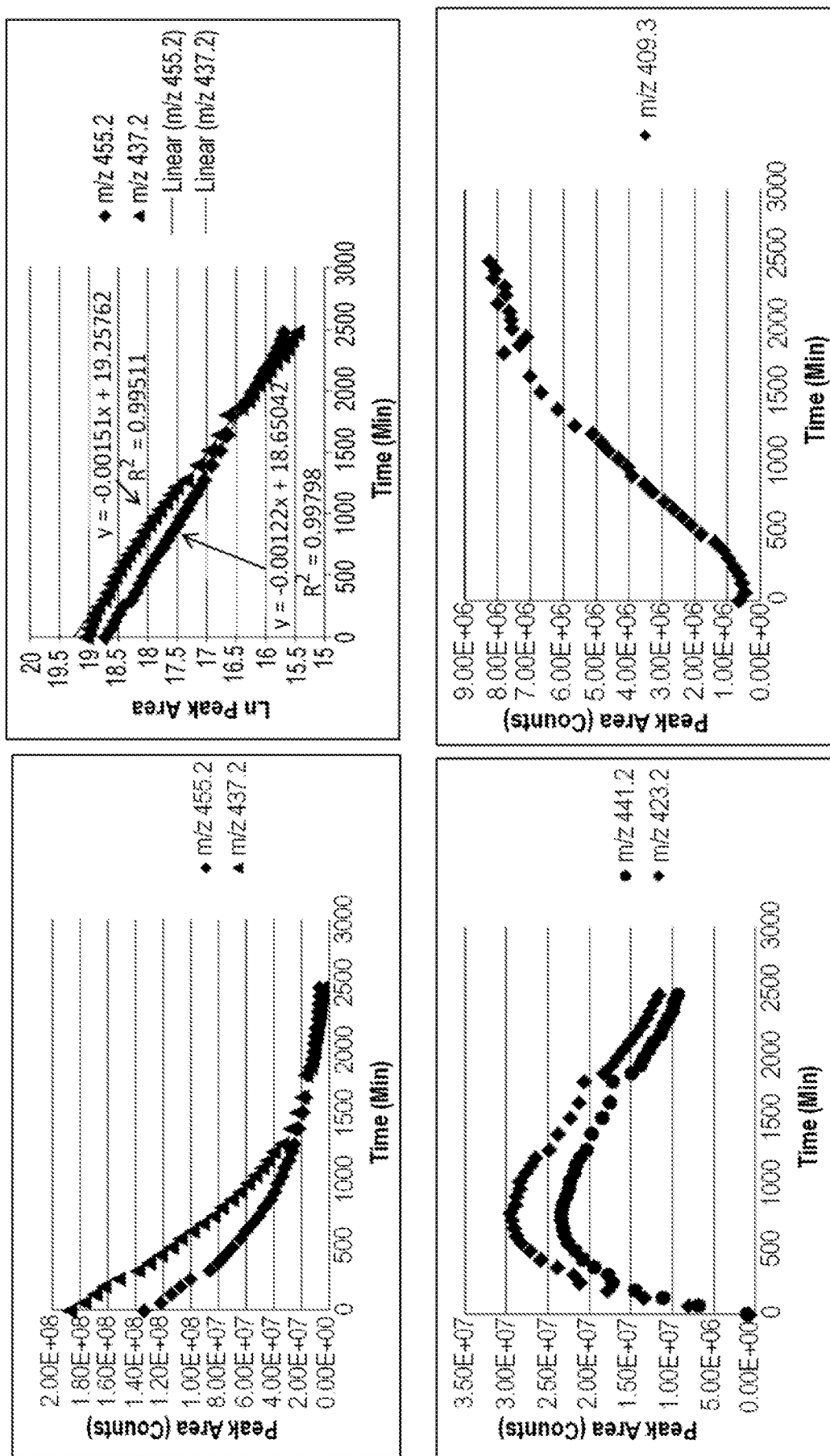


Figure 6

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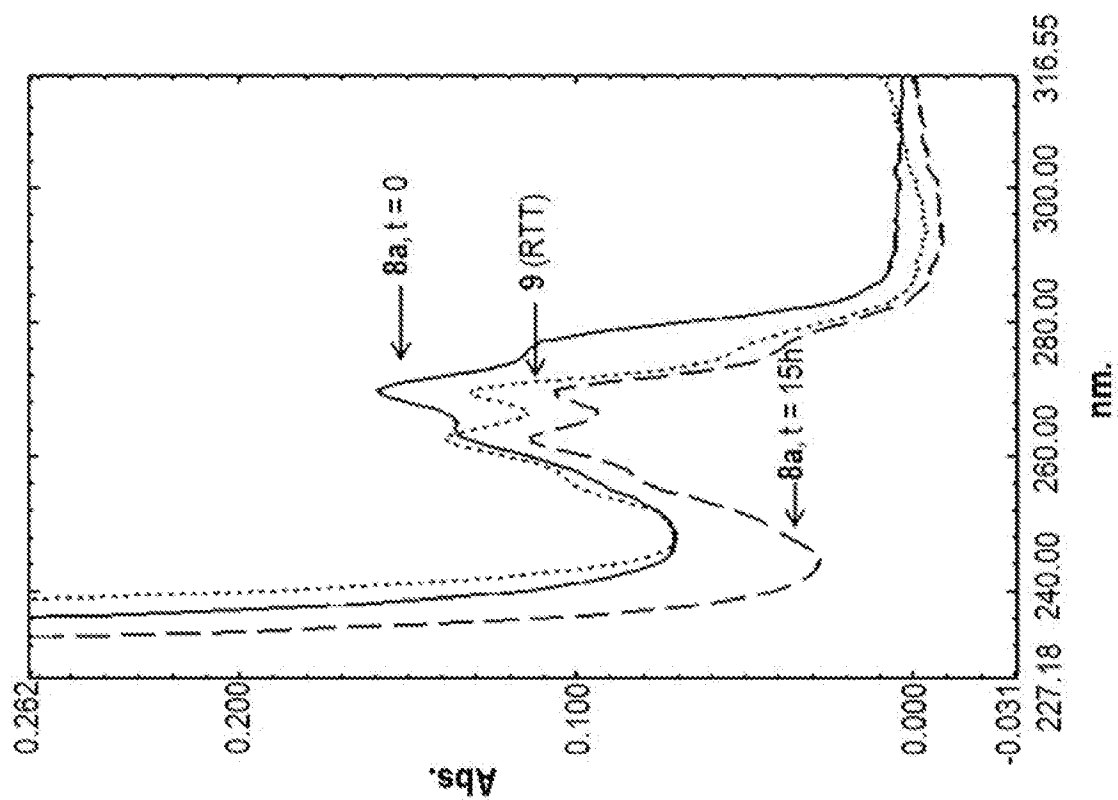


Figure 7B

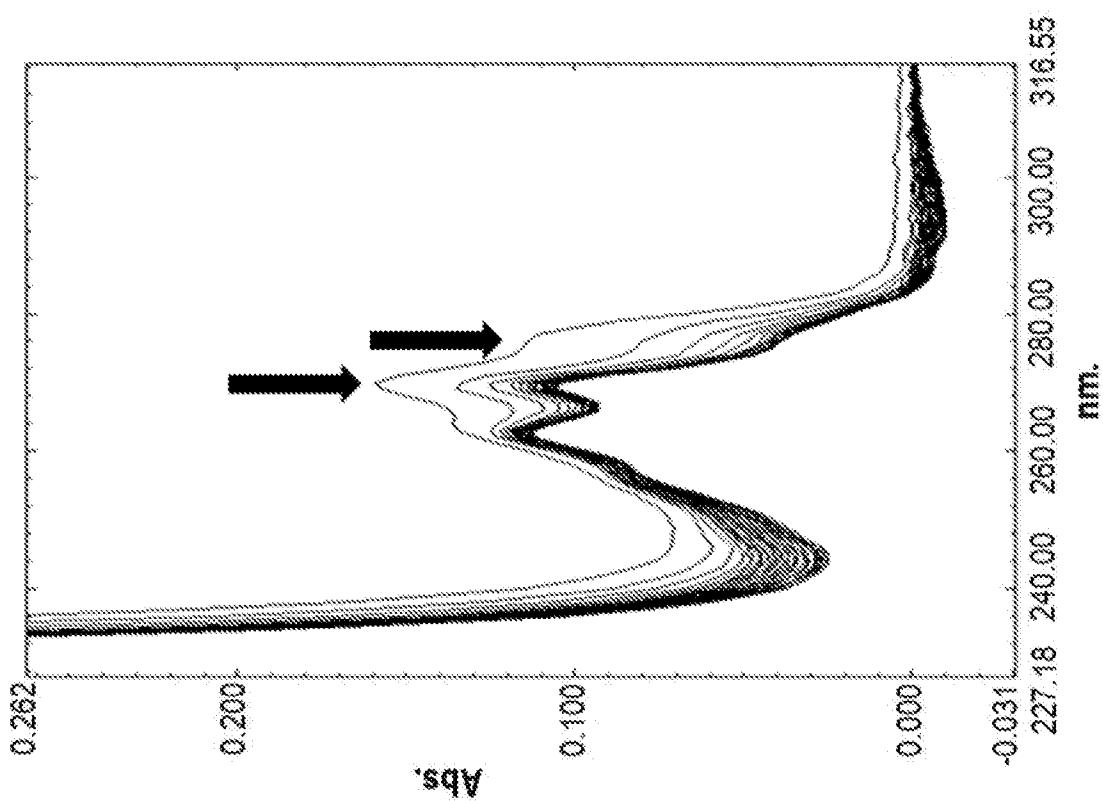


Figure 7A

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Prodrug	<i>MeOH/PB (50:50)</i>		<i>MEM</i>	
	$k_{\text{obs}} \pm 1 \text{ SD}$ ($\times 10^{-3} \text{ min}^{-1}$)	$t_{1/2} \text{ (h)}$	$k_{\text{obs}} \pm 1 \text{ SD}$ ($\times 10^{-3} \text{ min}^{-1}$)	$t_{1/2} \text{ (h)}$
8a	3.01 ± 0.10	3.84	16.7 ± 3.0	0.69
8b	1.96 ± 0.09	5.89	14.3 ± 1.9	0.81
8c	0.44 ± 0.02	26.3	5.1 ± 1.4	2.28

Figure 8

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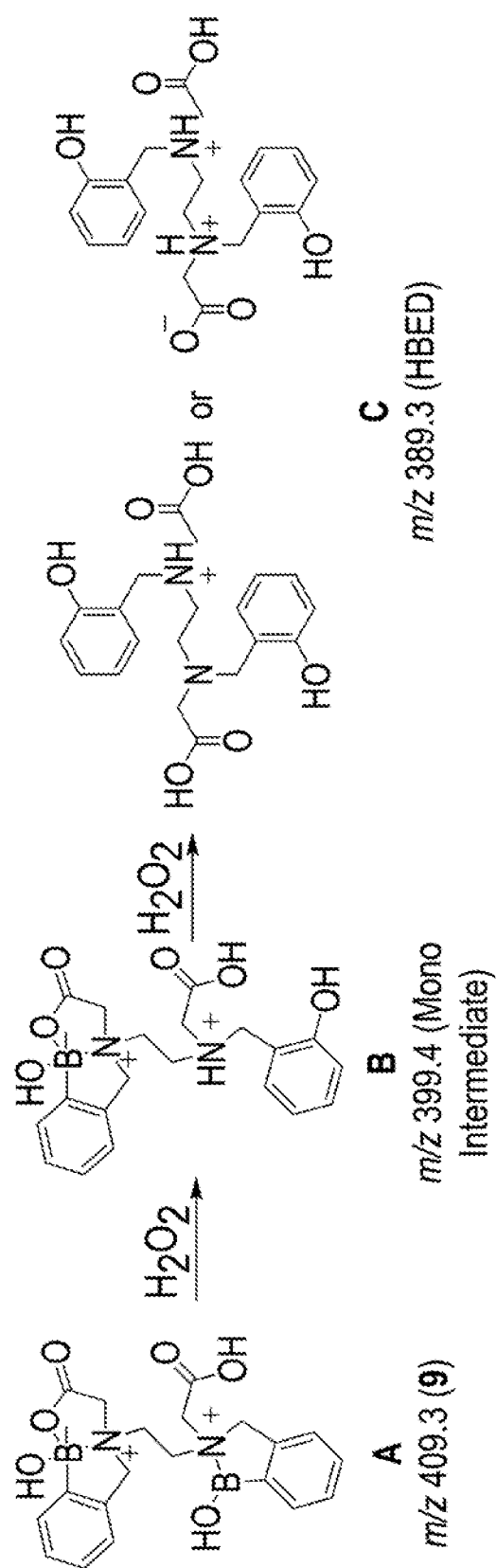


Figure 9

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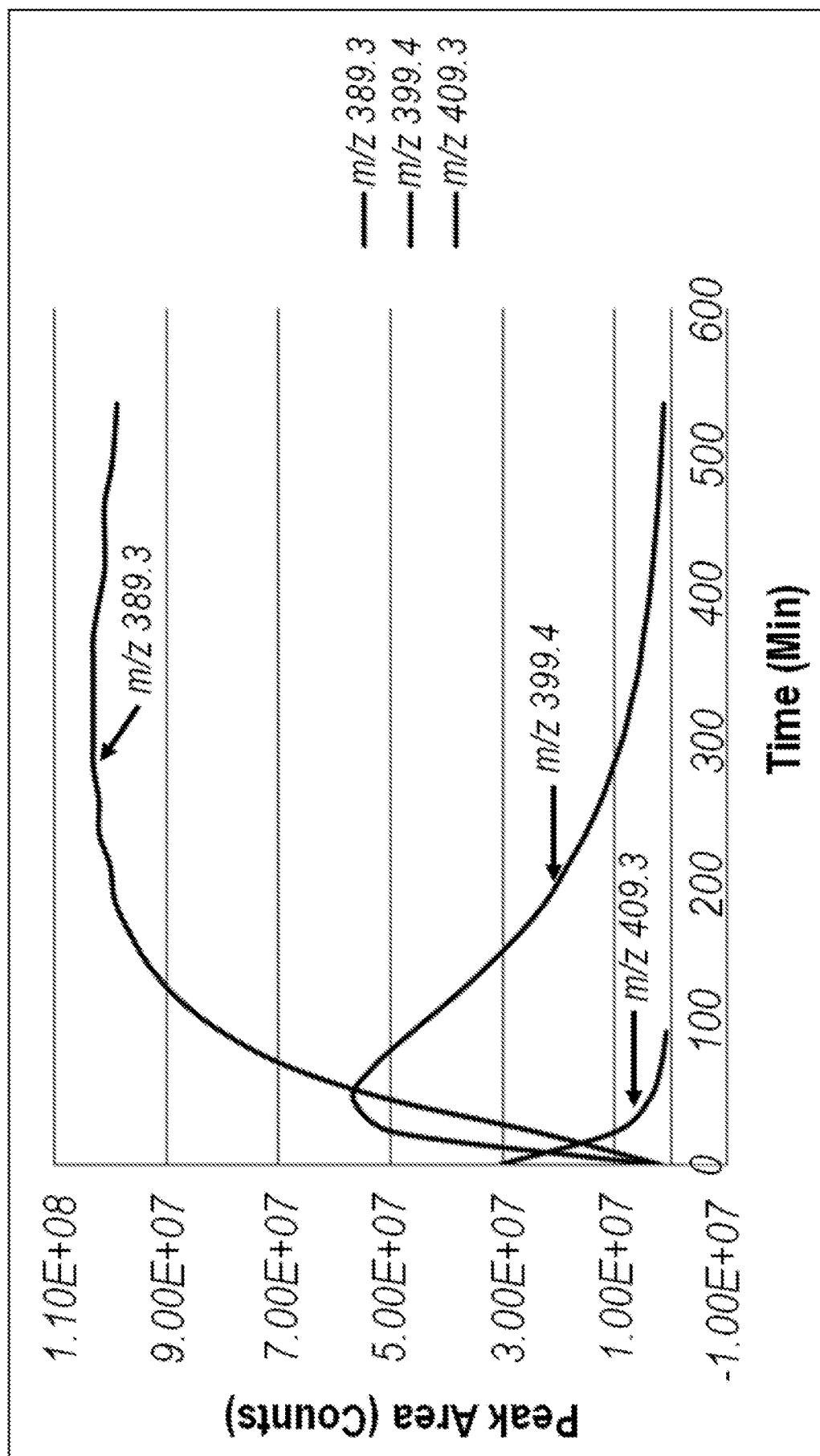


Figure 10

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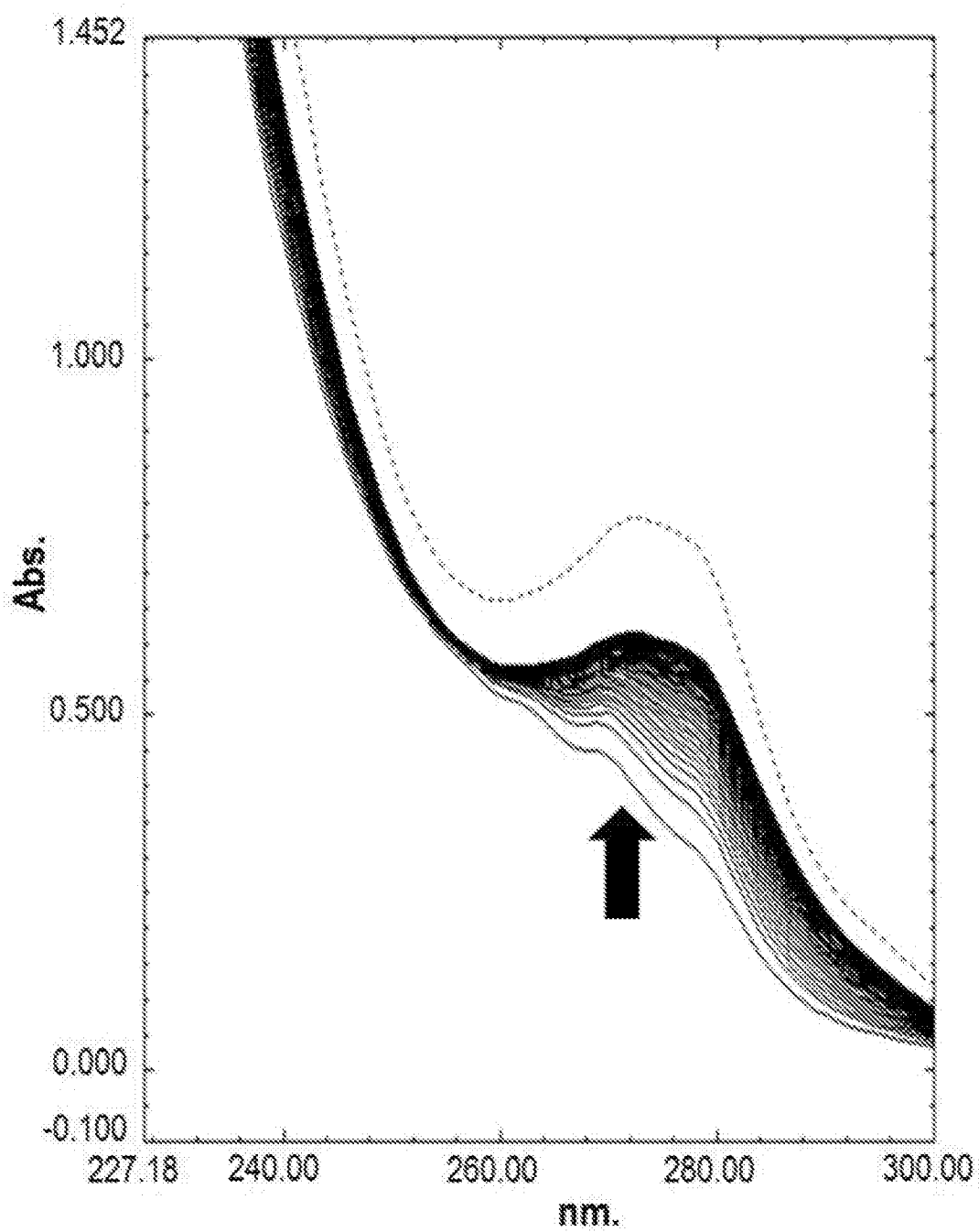


Figure 11

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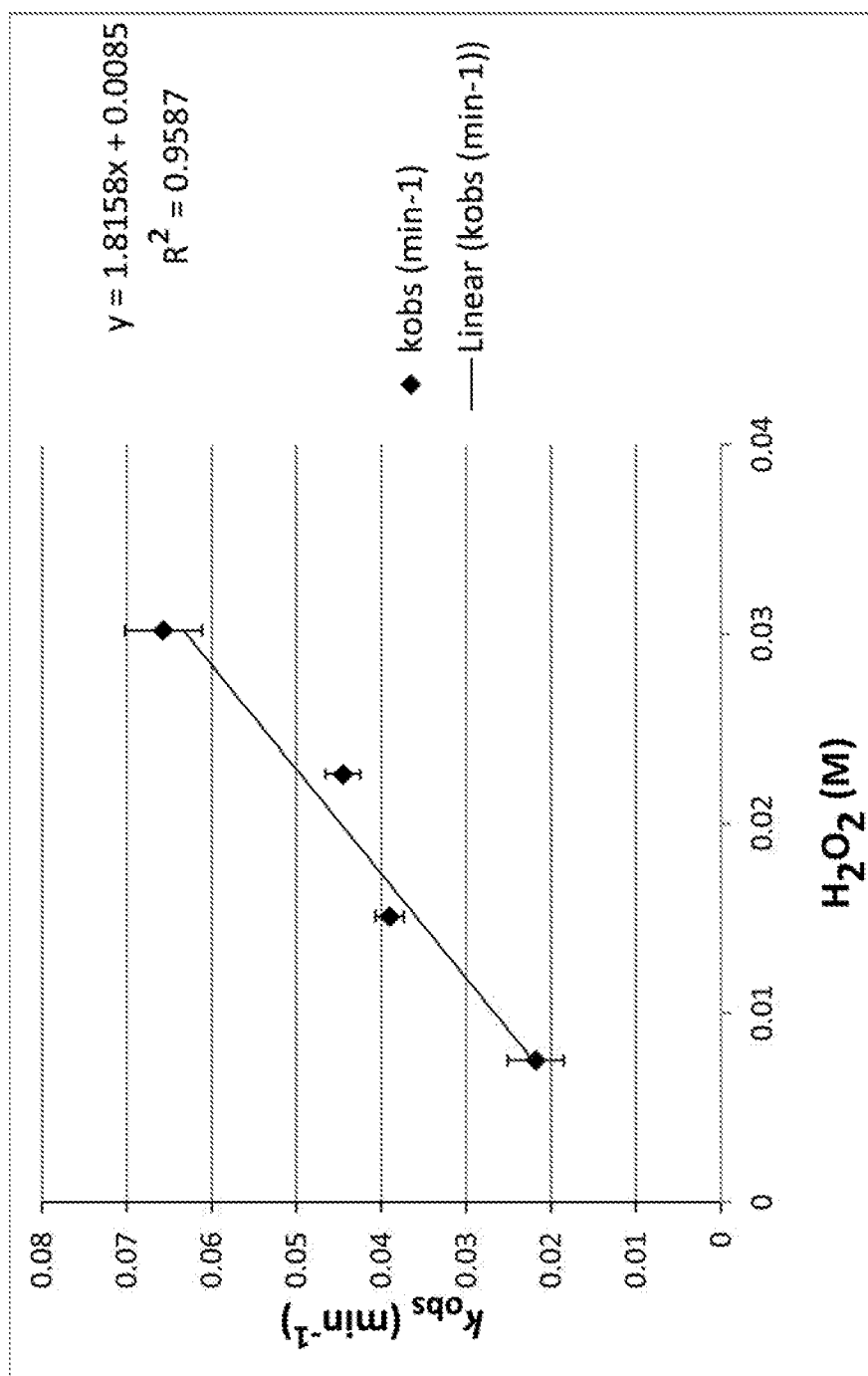


Figure 12

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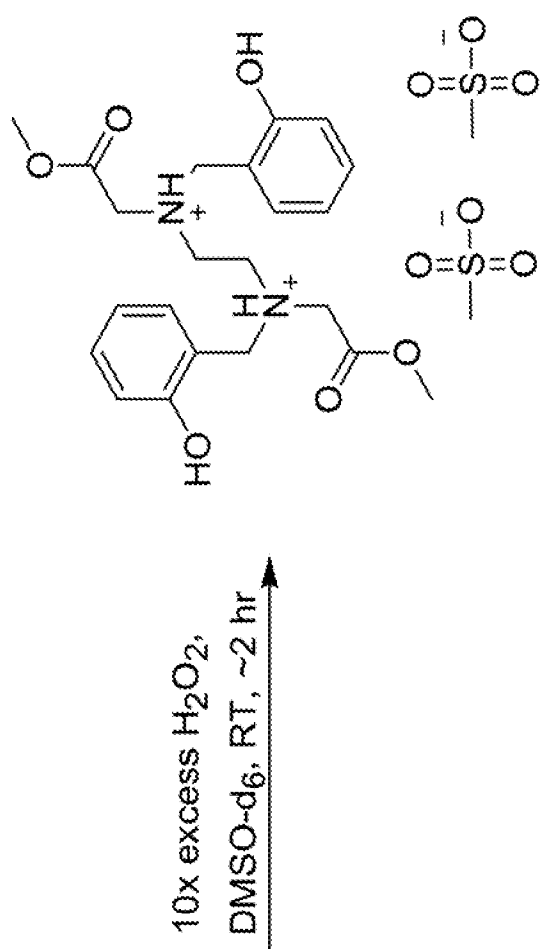
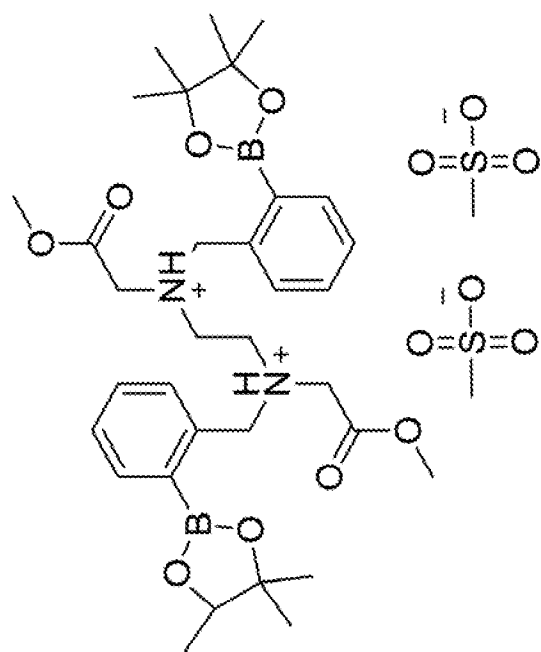


Figure 13



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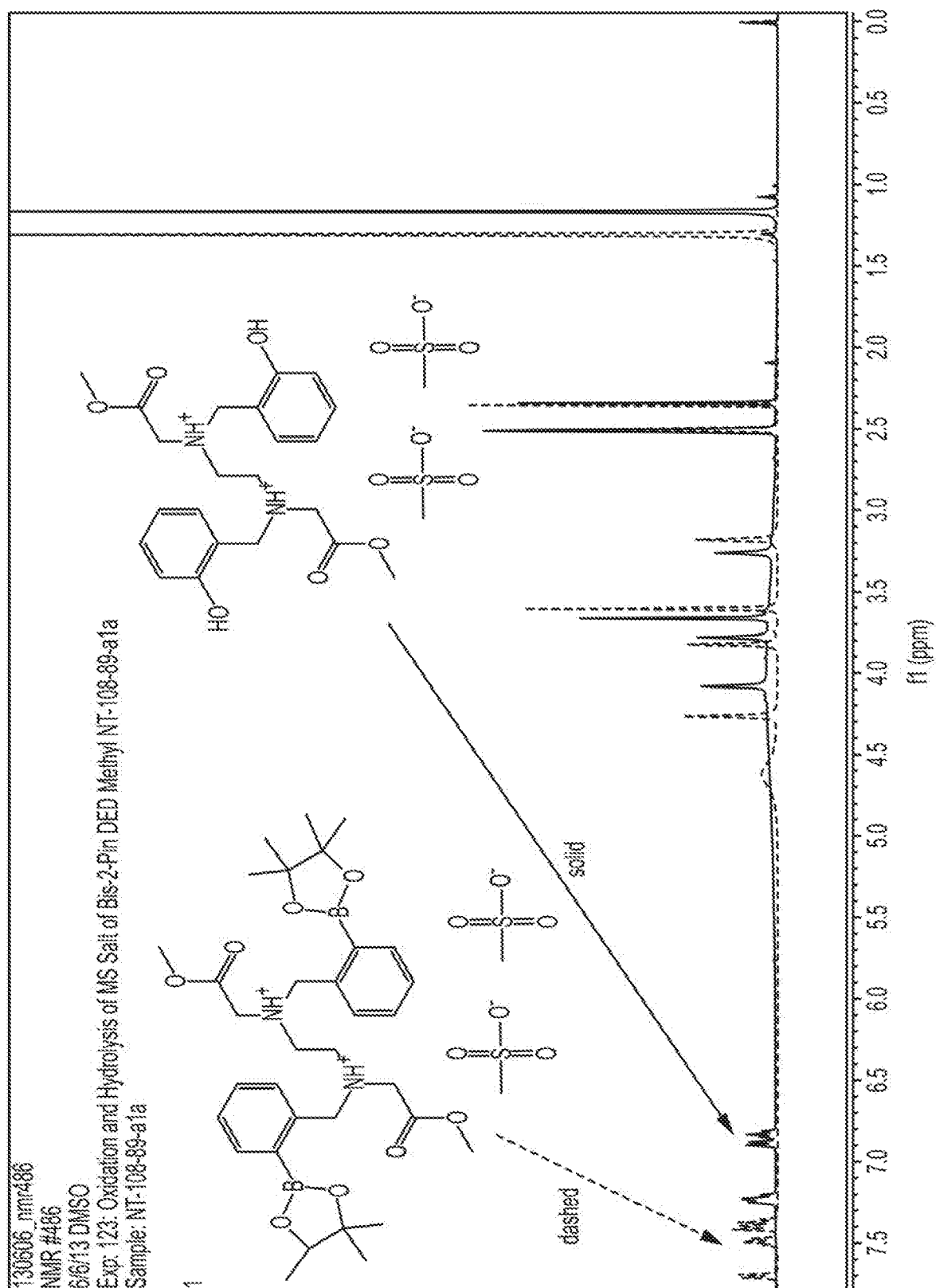


Figure 14

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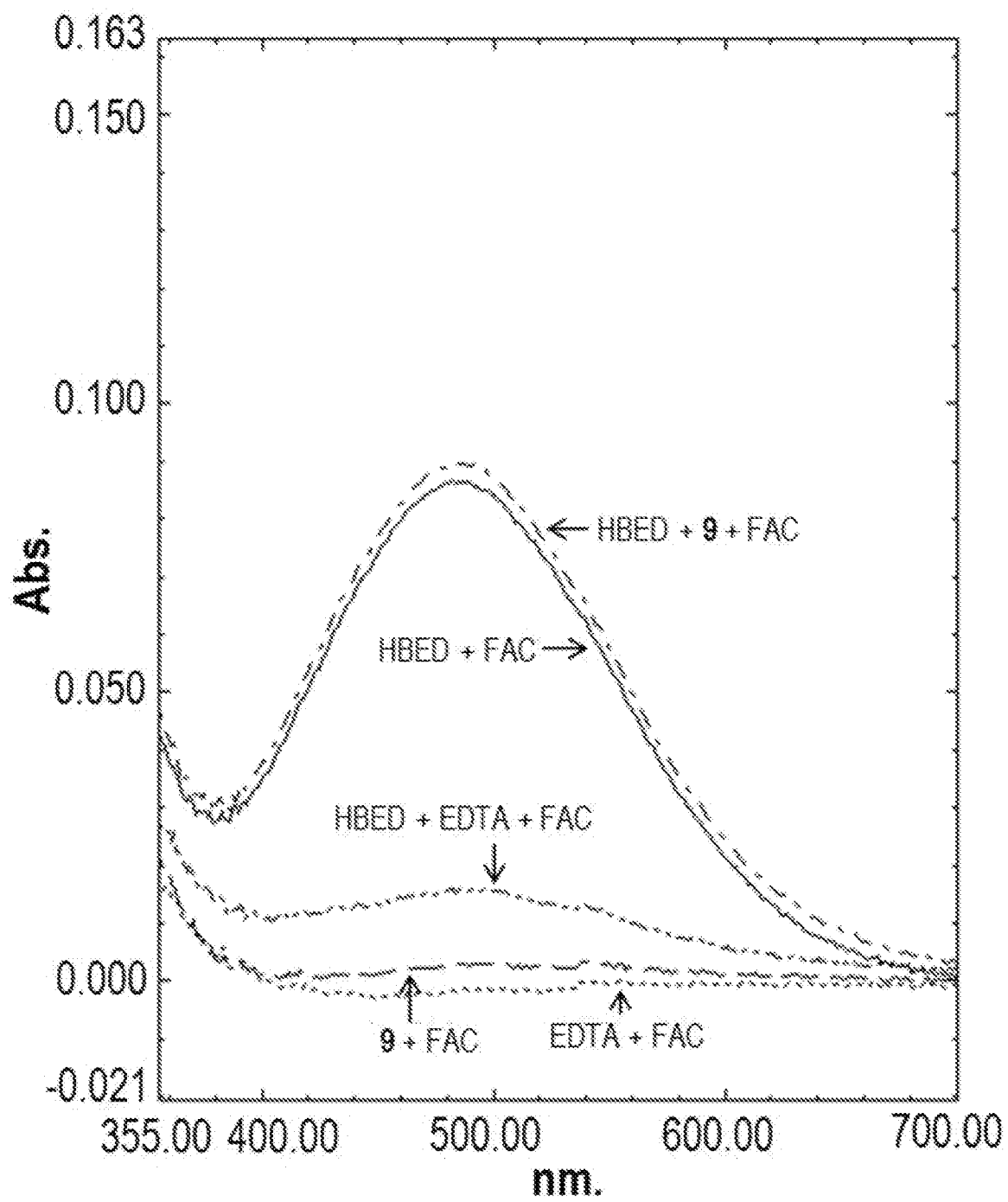


Figure 15

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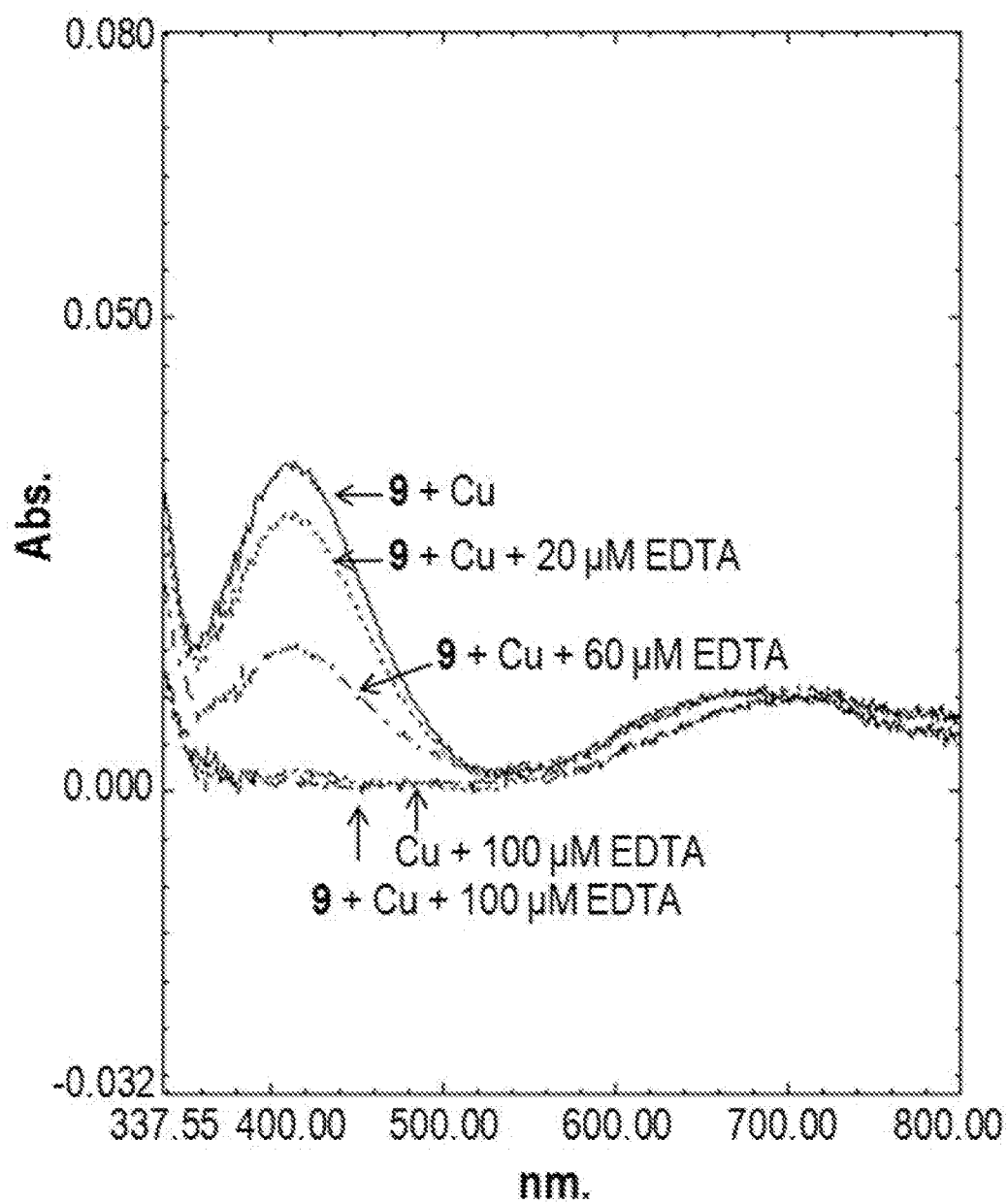
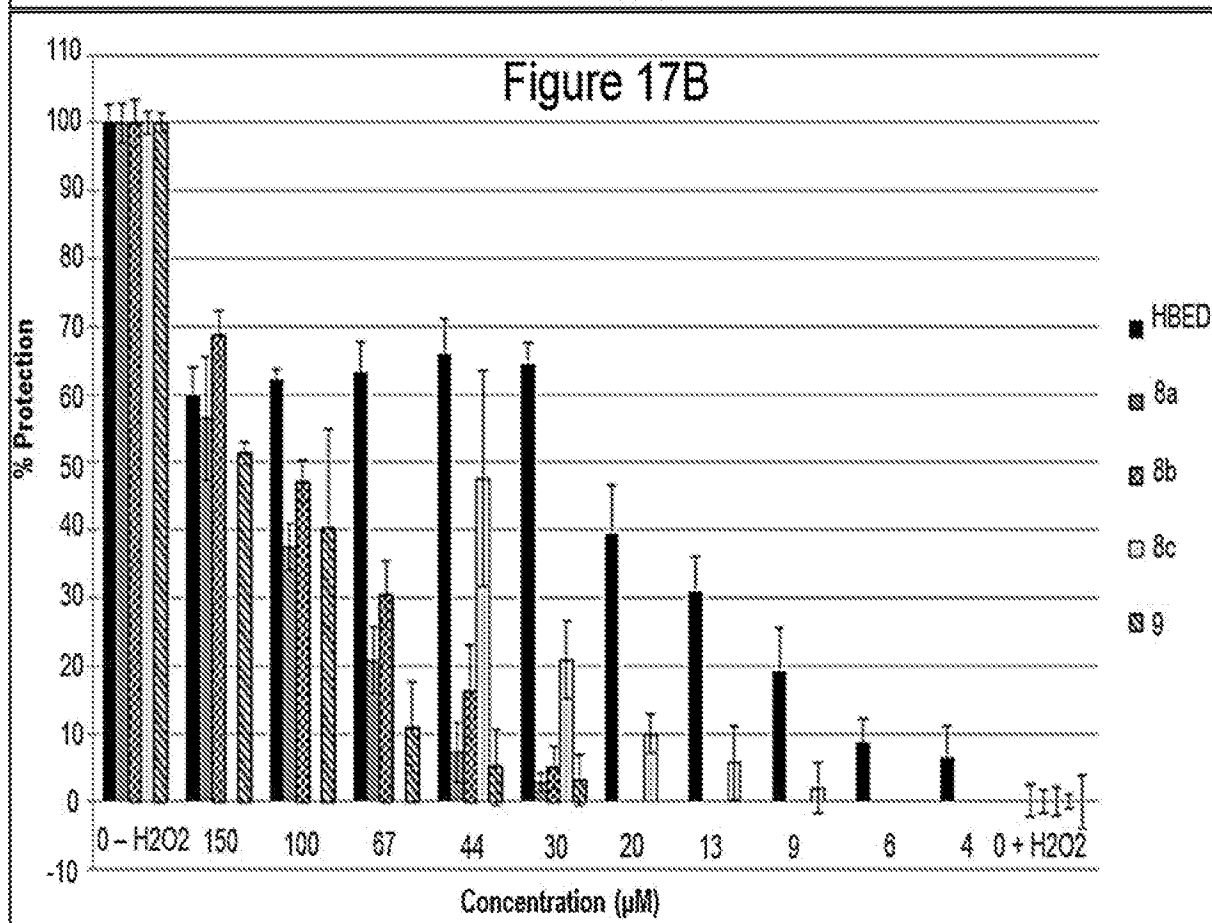
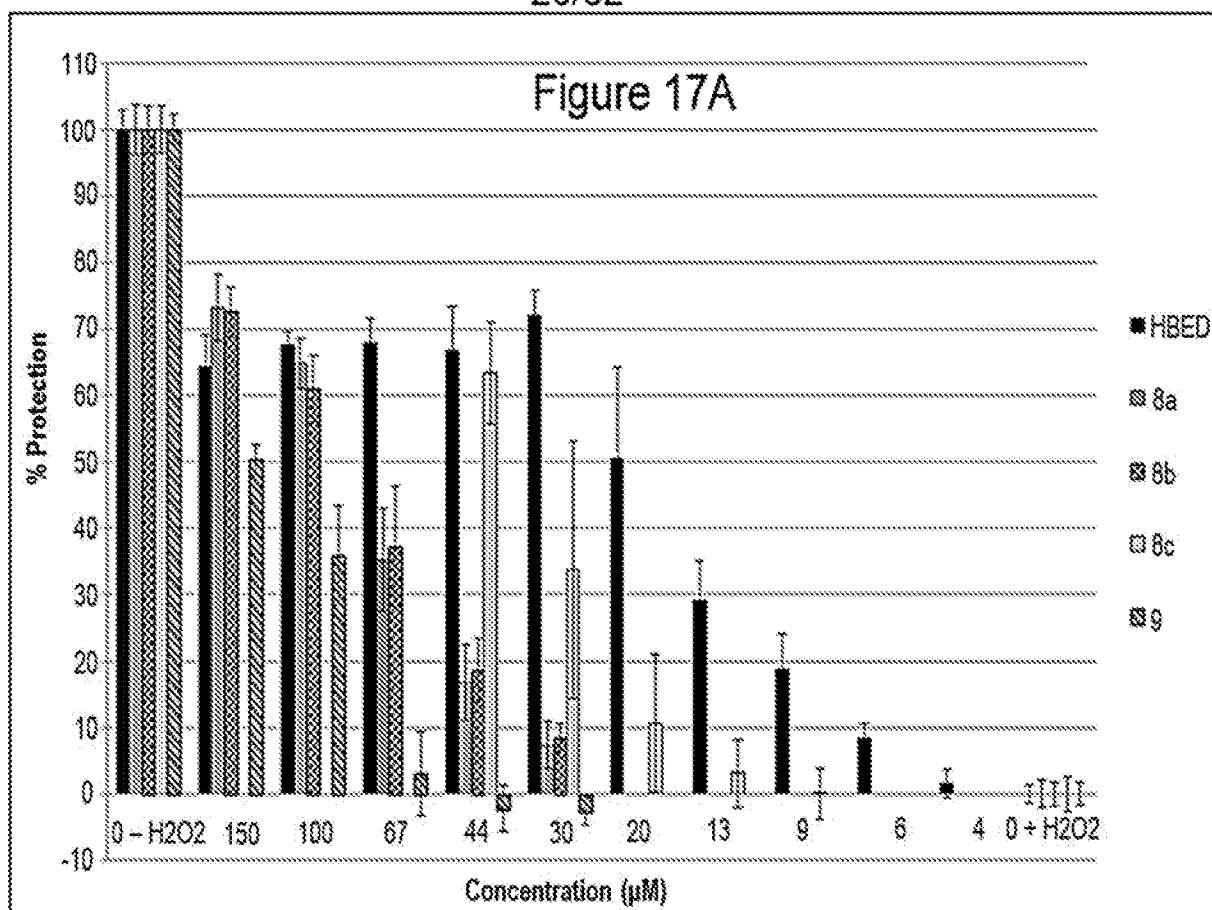


Figure 16

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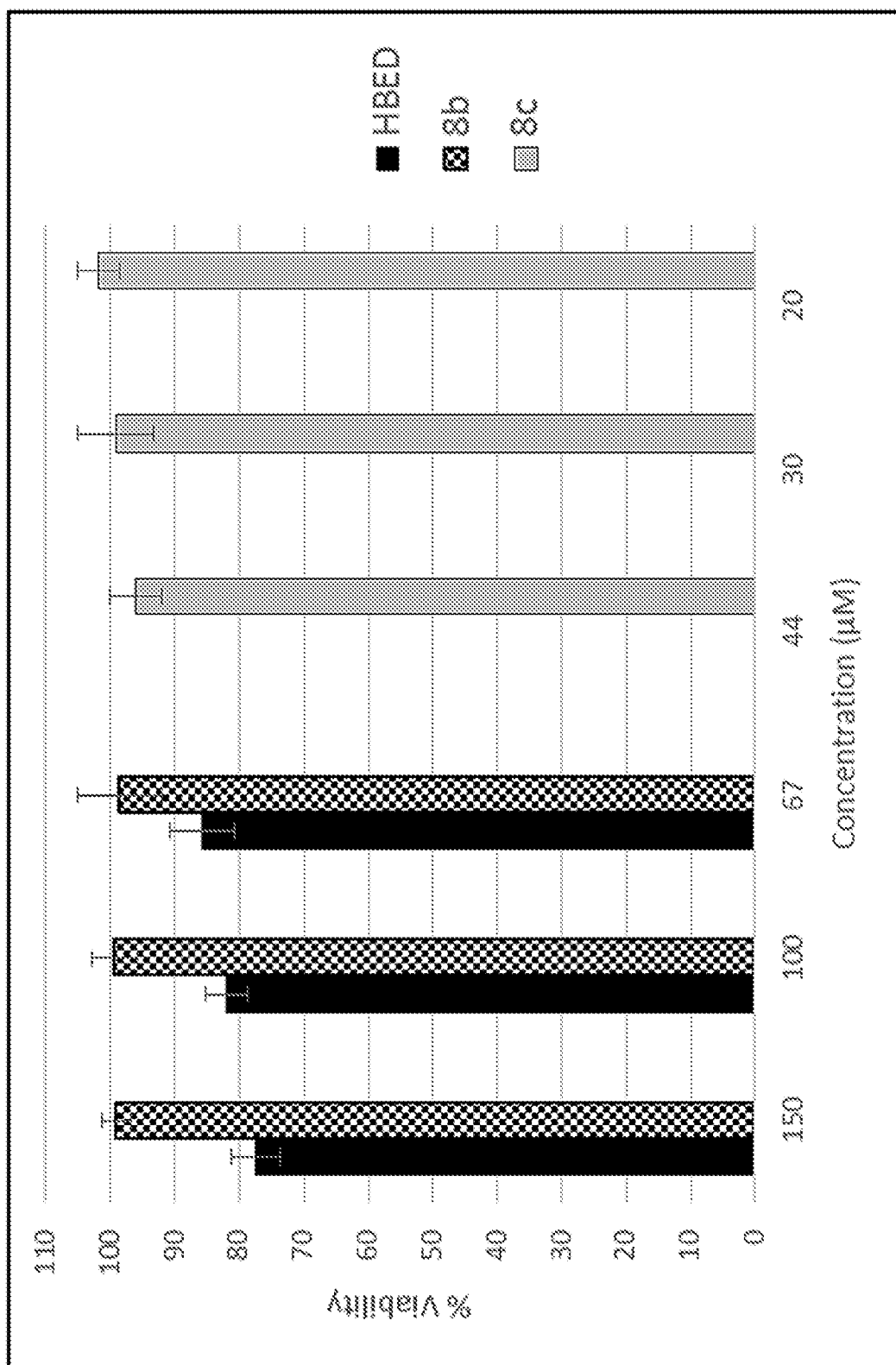


Figure 18

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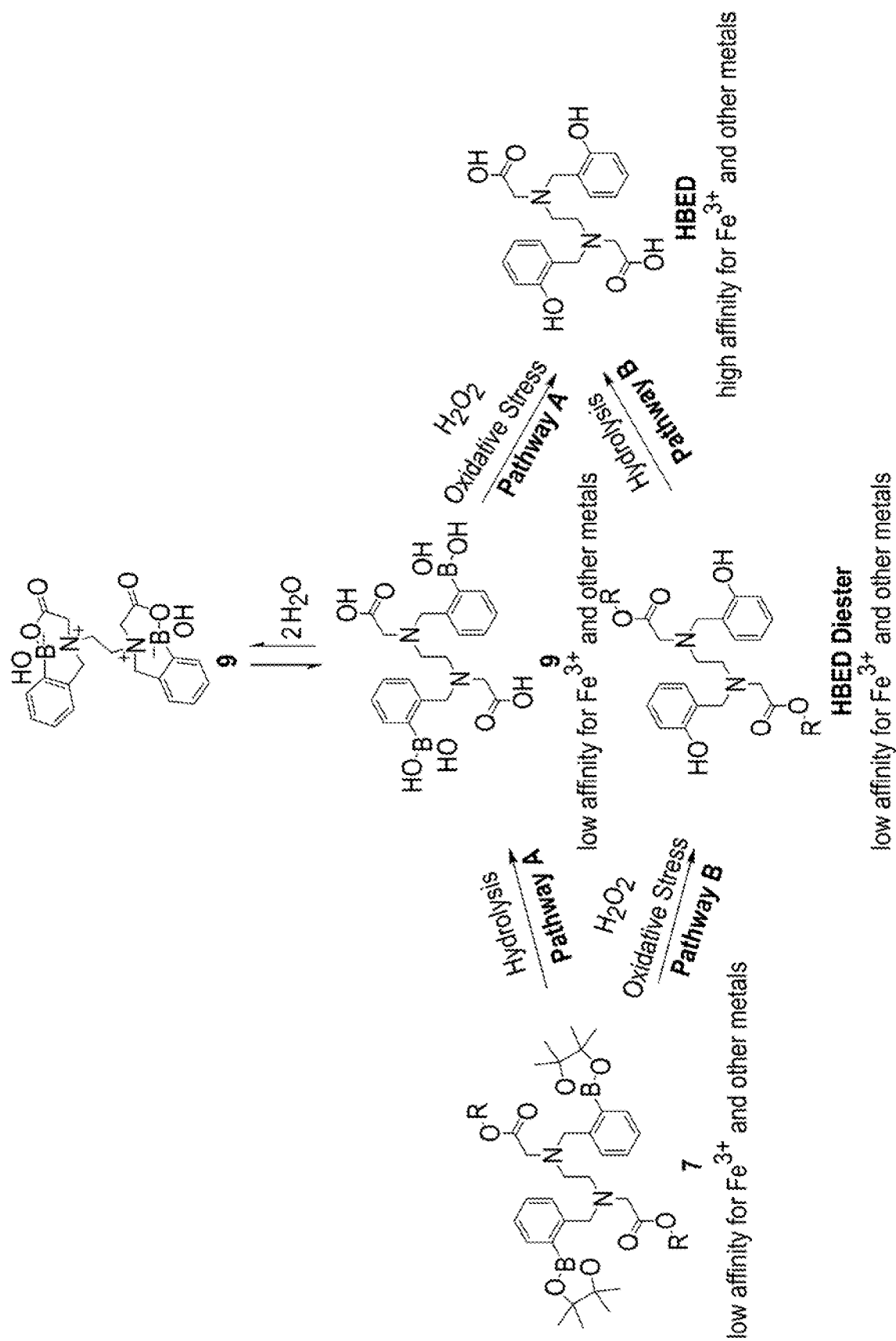


Figure 19

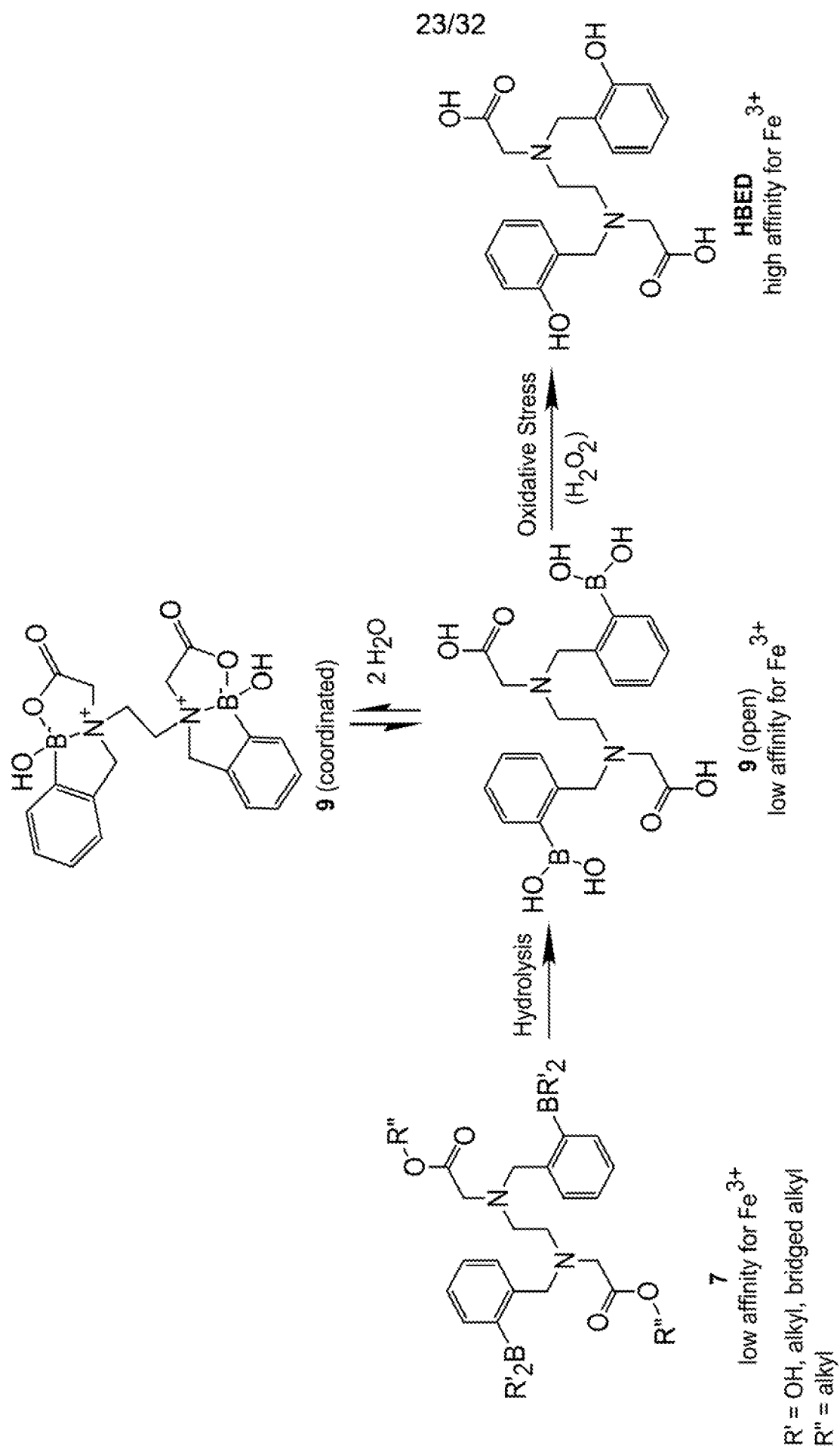


Figure 20

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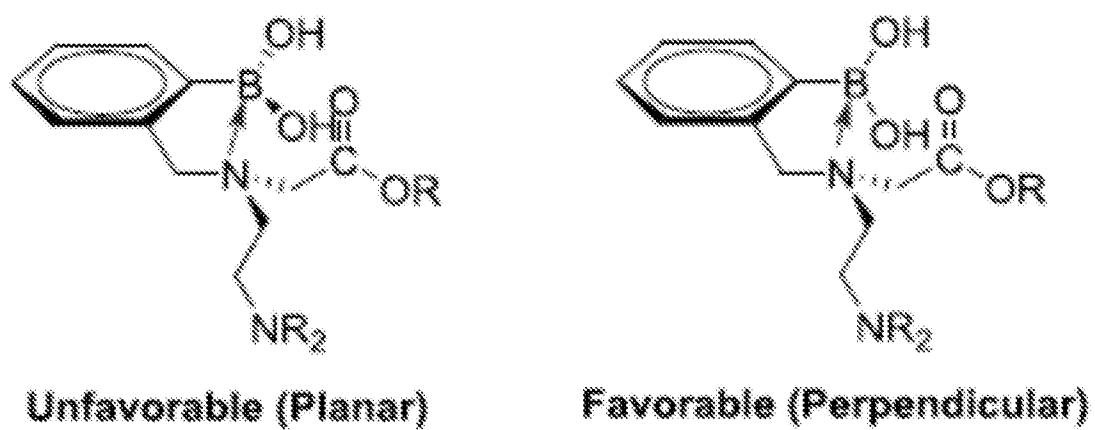


Figure 21

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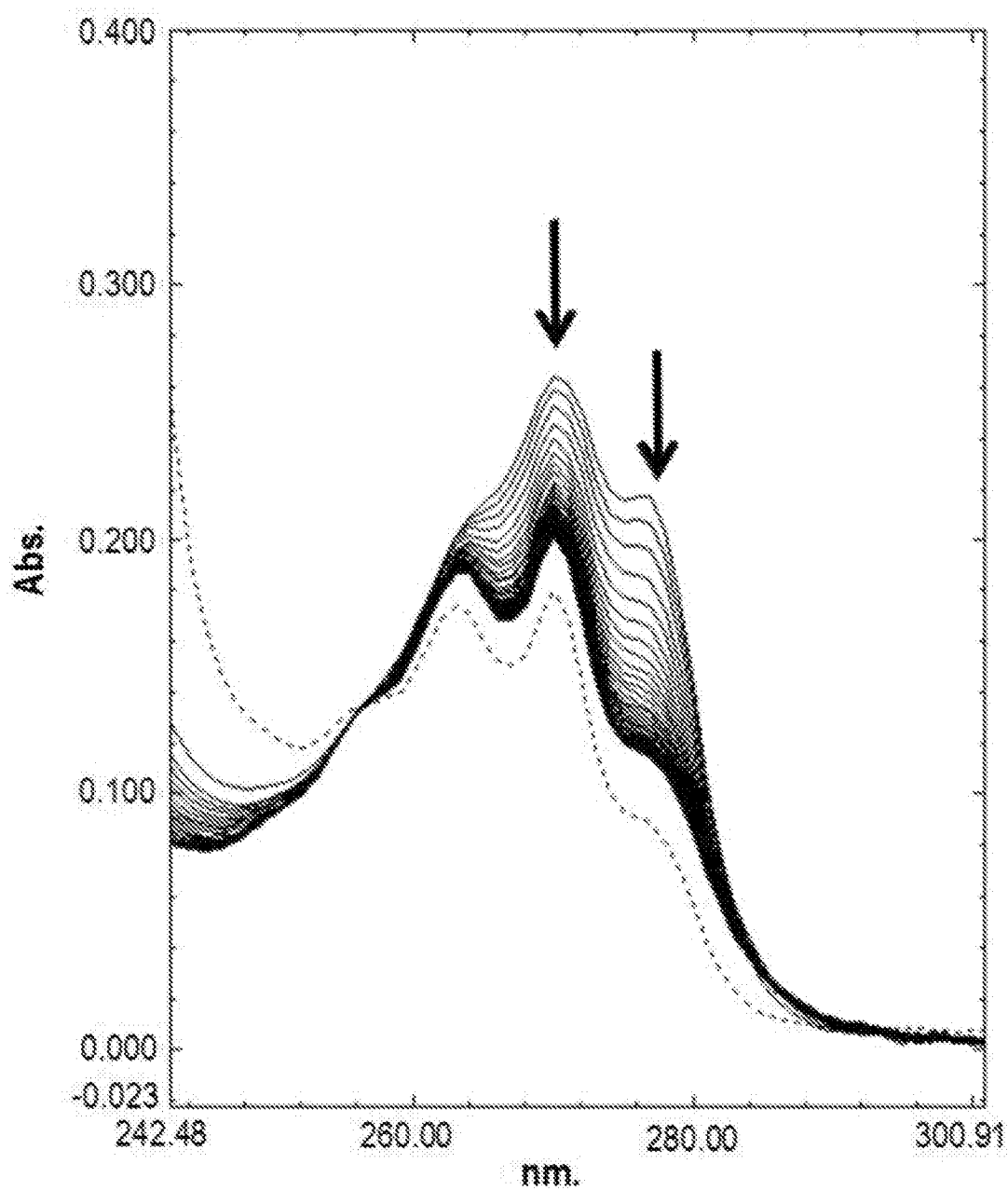


Figure 22

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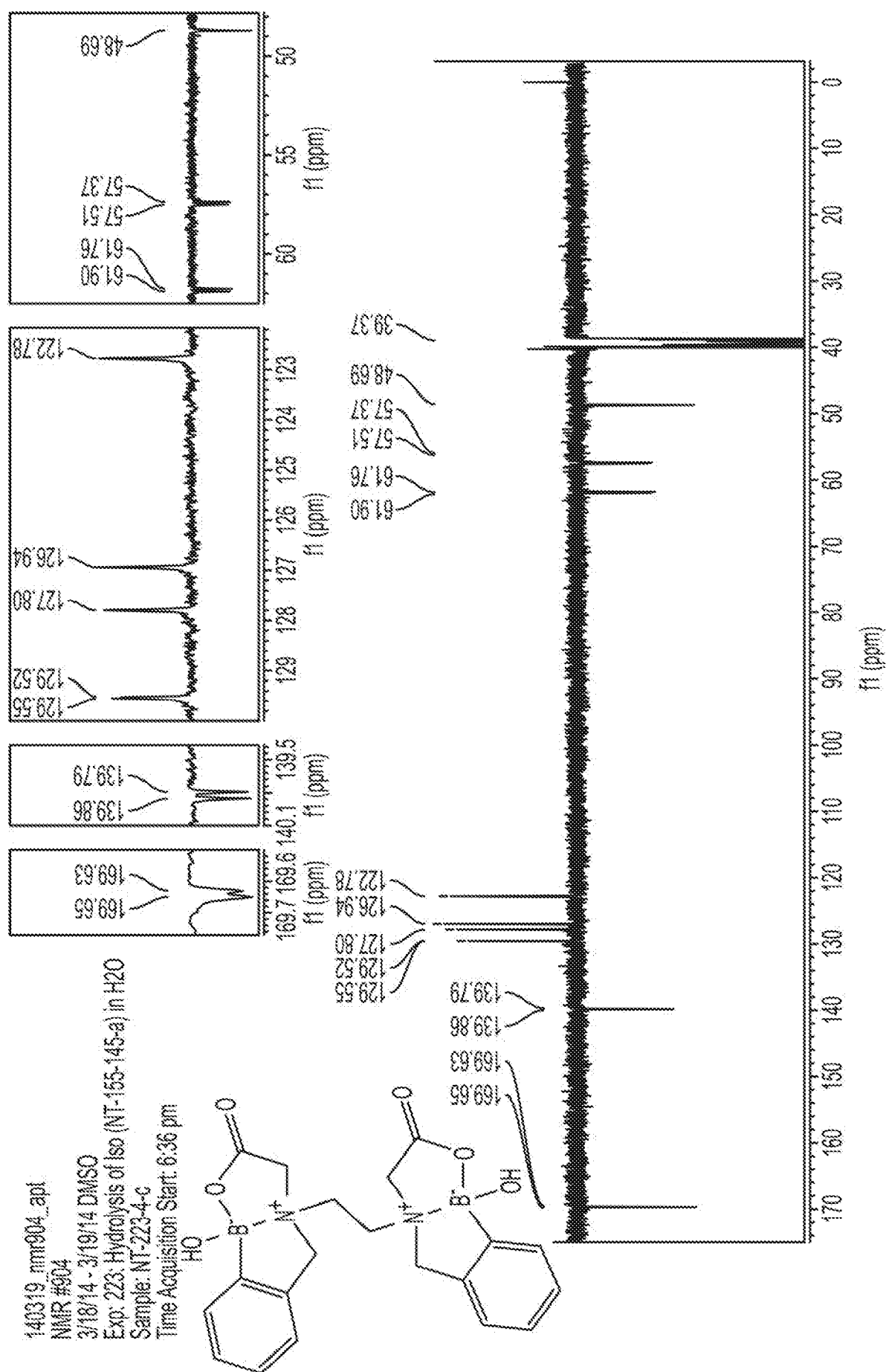


Figure 23

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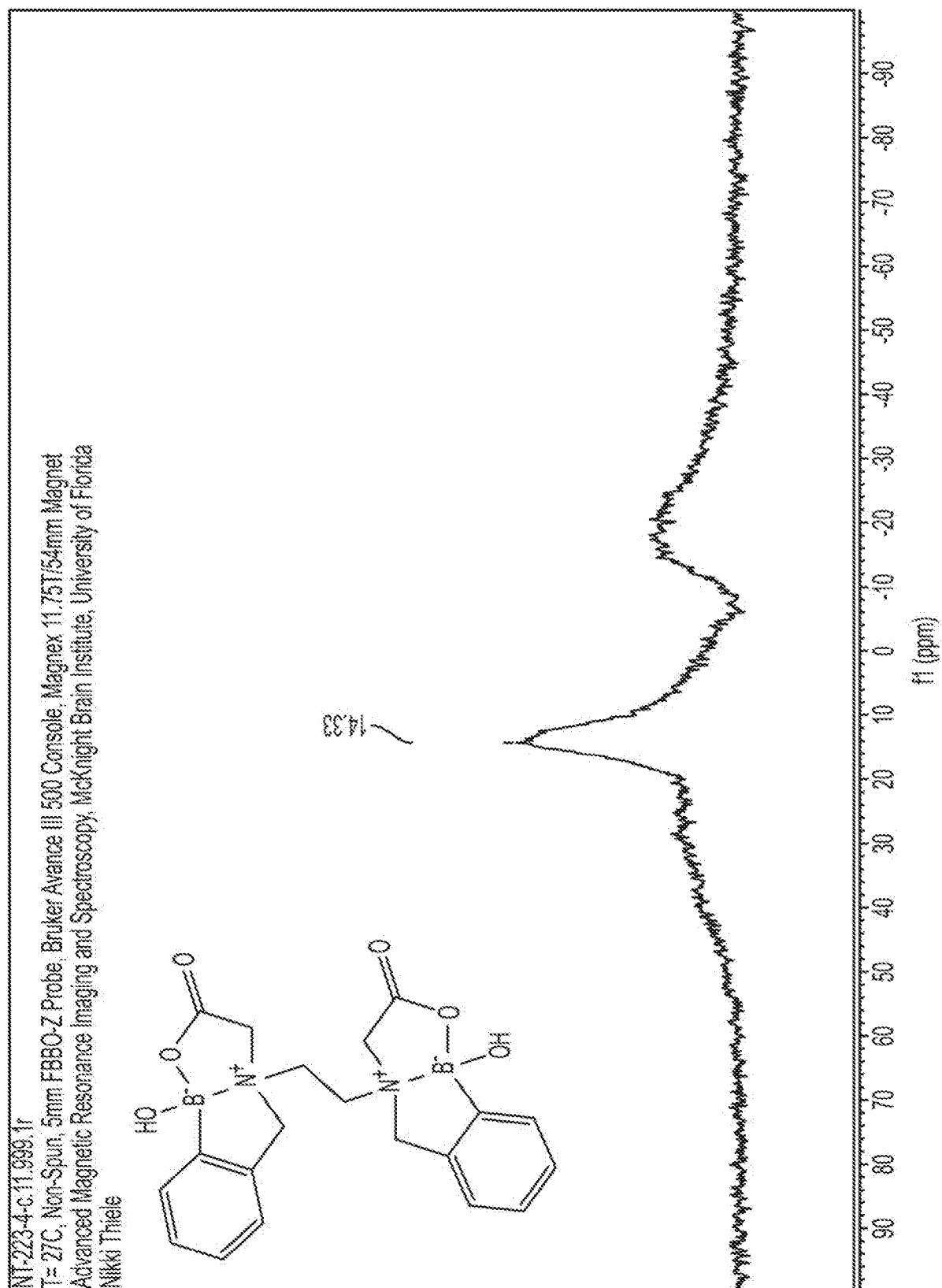


Figure 24

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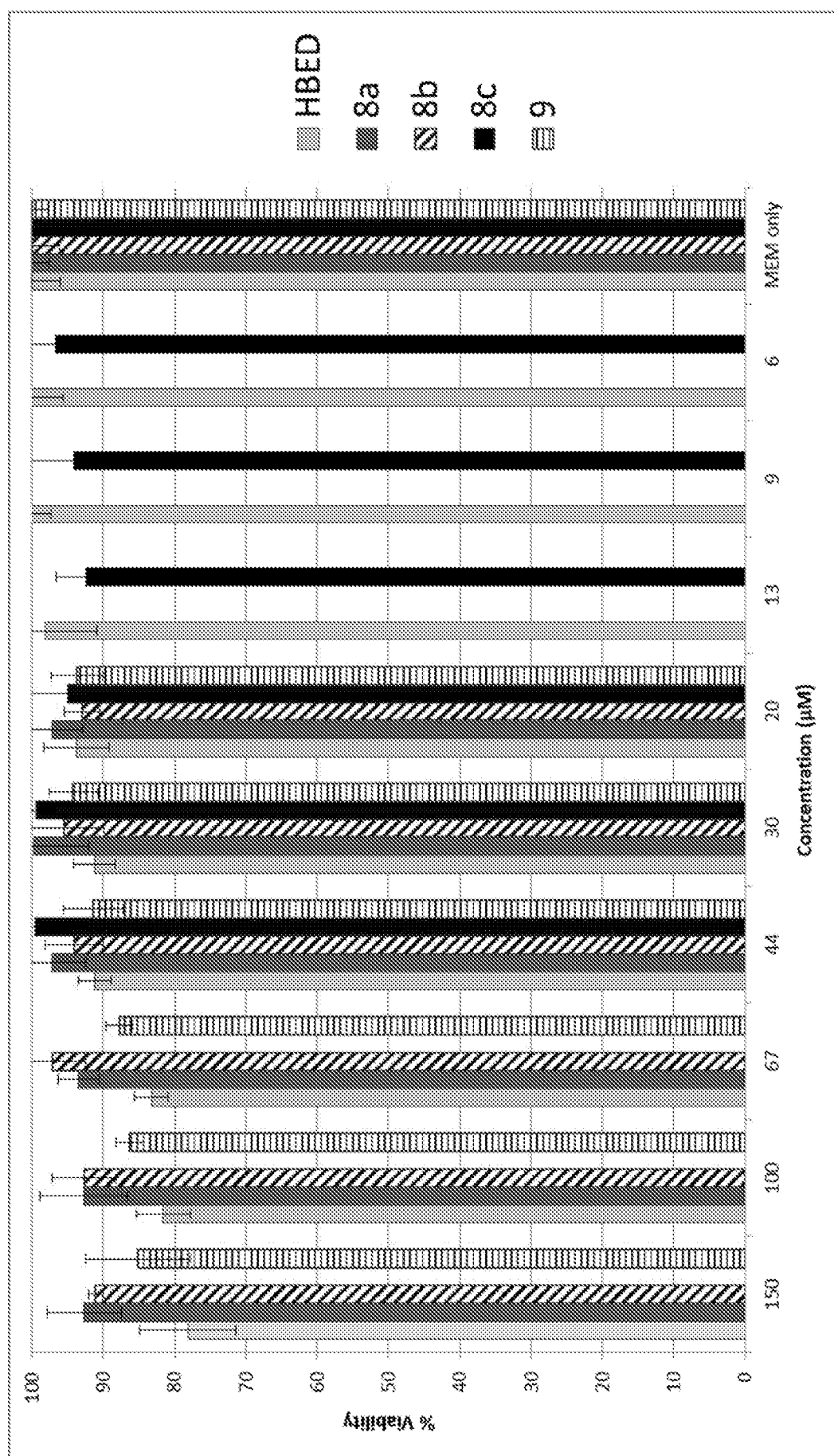


Figure 25

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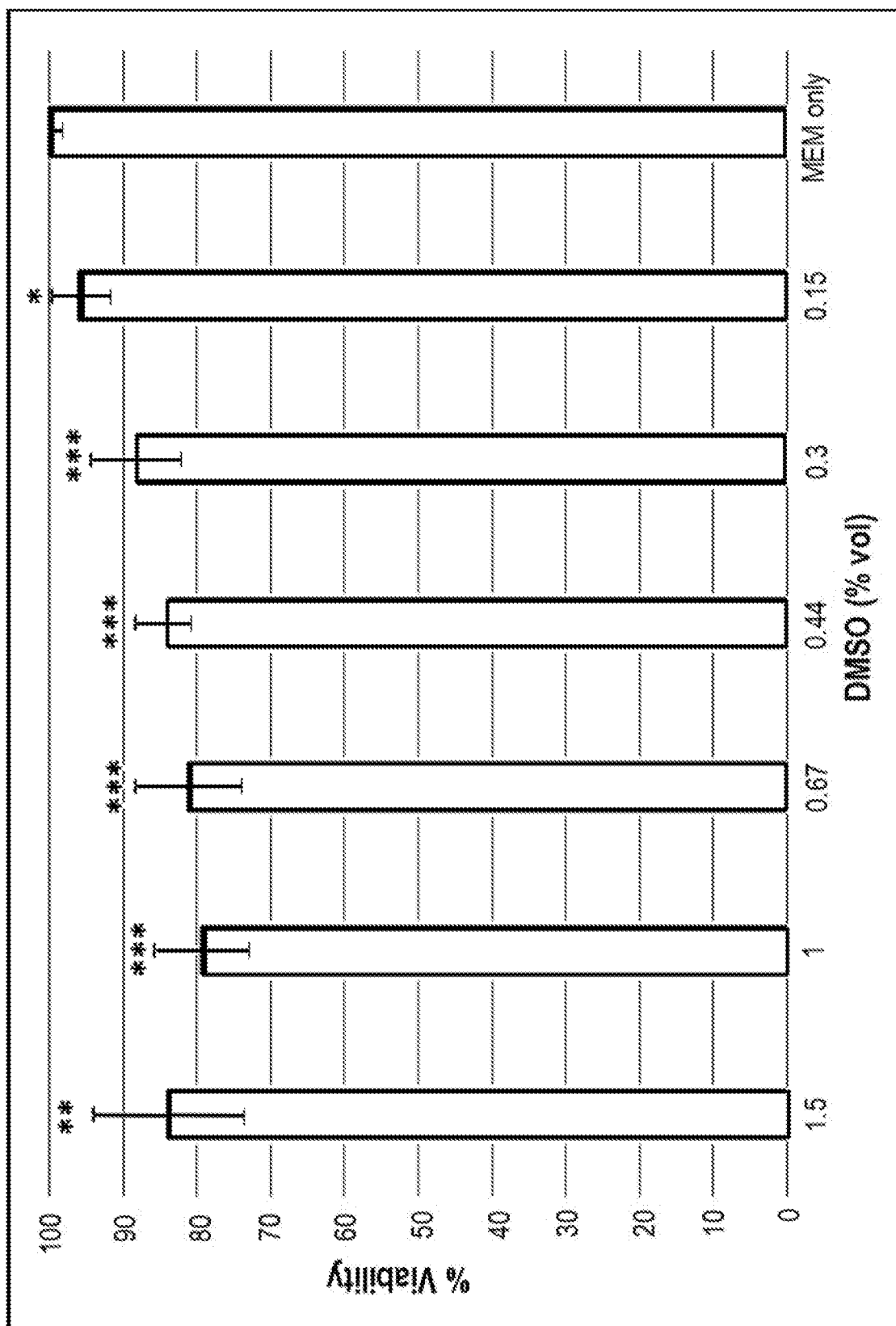


Figure 26

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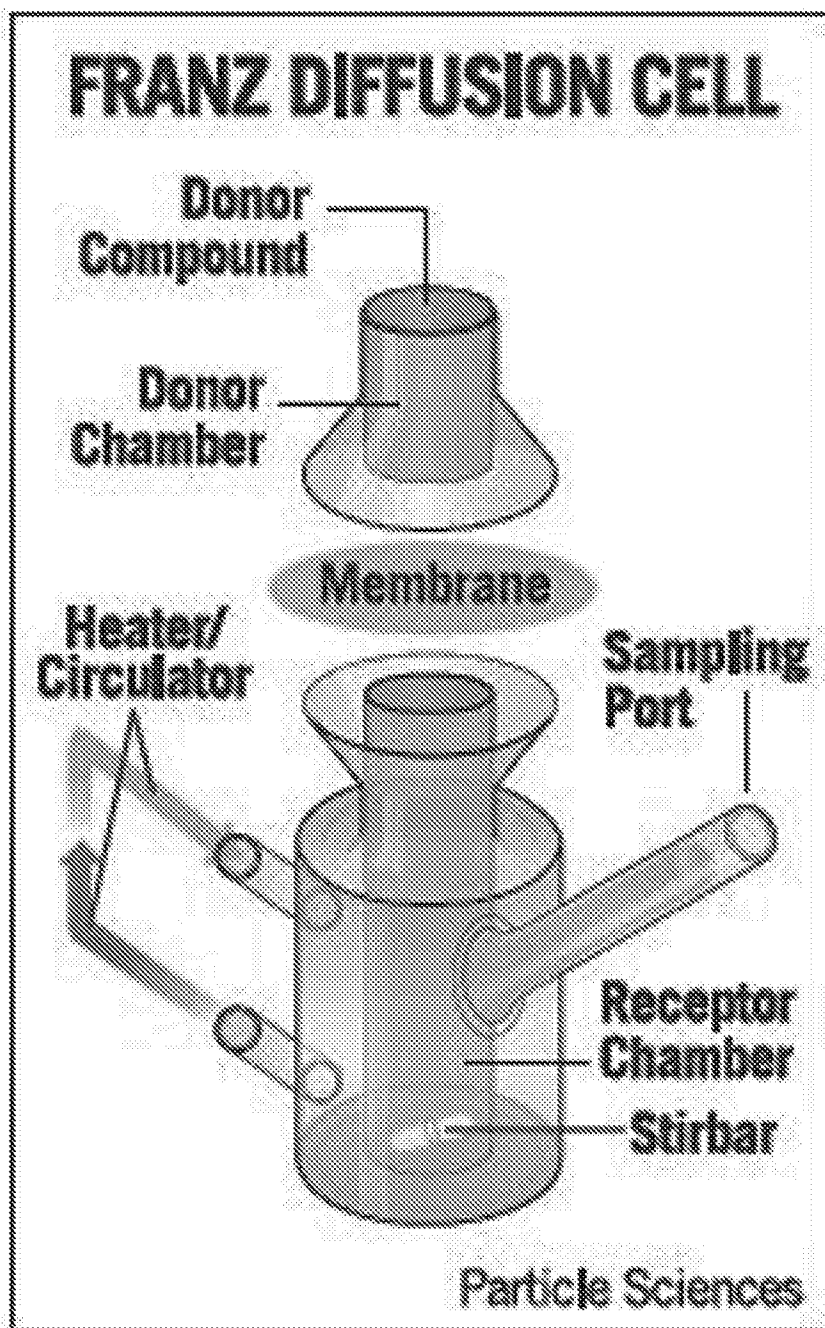


Figure 27

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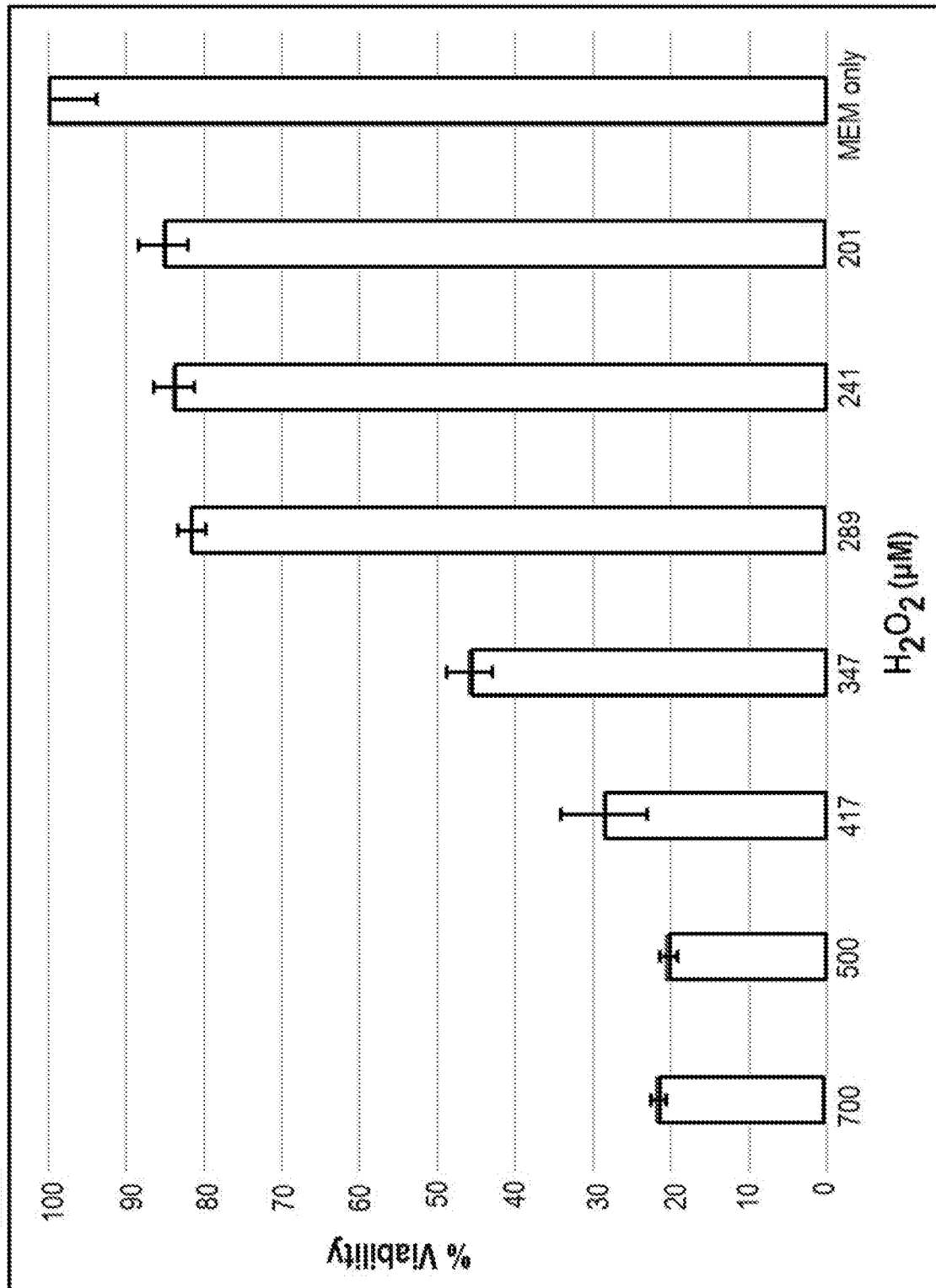


Figure 28

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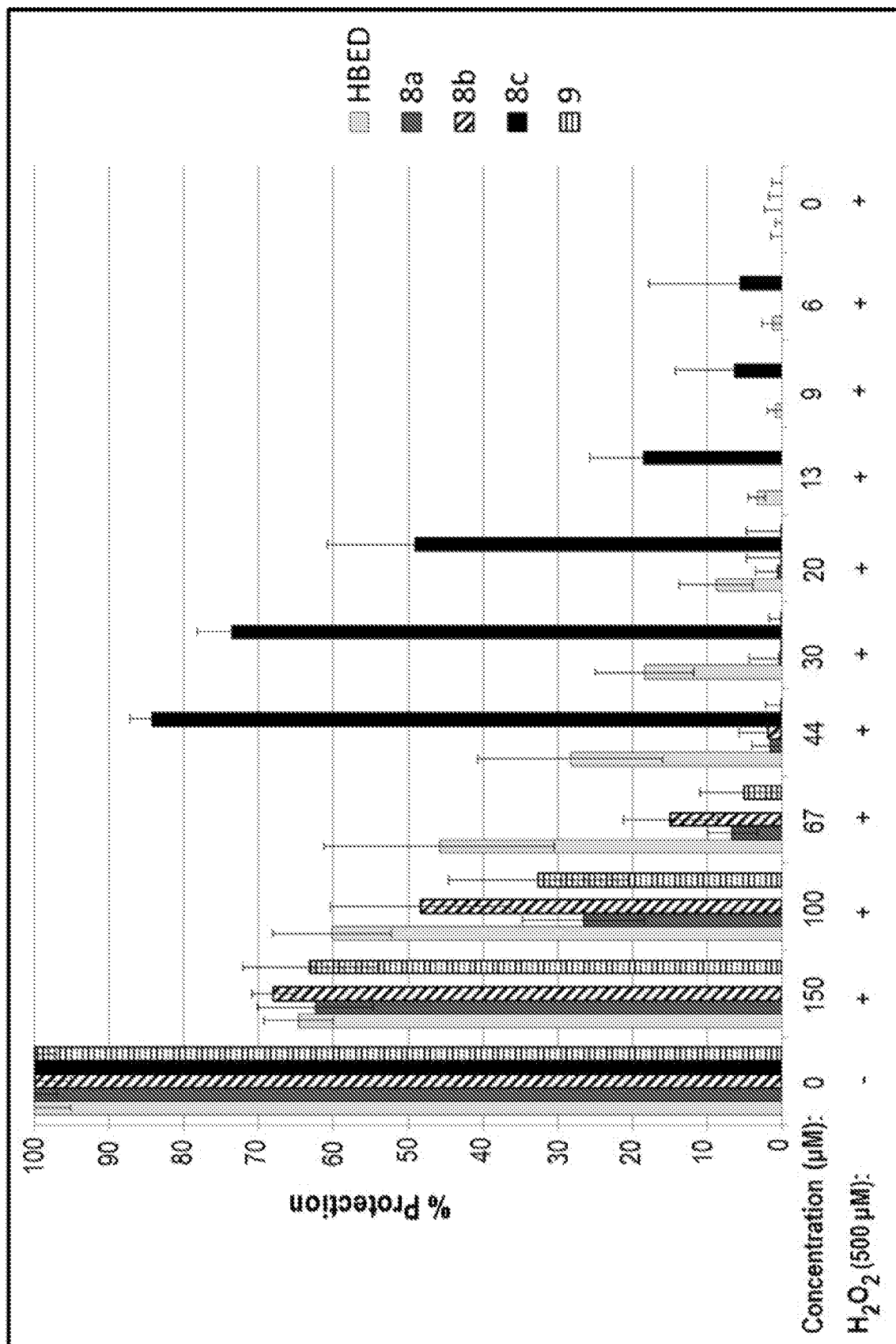


Figure 29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/49689

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/198; A61K 31/69 (2016.01)

CPC - A61K31/195; A61K31/198

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)

CPC: A61K31/195; A61K31/198

IPC(8): A61K 31/198; A61K 31/69 (2016.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/566; 562/451 (See Search Words Below)

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

PATBASE: Full-text = AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO

Google: Scholar/Patents: hydroxybenzyl ethylenediamine diacetic acid hbcd prodrug boron cyclic boronate esters tetrahedral phenylboronate pinanediol pinanediol phenyl phenylboronate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y — A	FALLER et al. 'Improving the Oral Bioavailability of the Iron Chelator HBED by Breaking the Symmetry of the Intramolecular H-Bond Network', Journal of Medicinal Chemistry, 2000, Vol 43, pp 1467-1475. pg 1468, Scheme 1; pg 1470, Figure 1	1-4, 4a, 5-12, 17 ----- 18-33; 39
Y — A	WO 2012/123076 A1 (RUPRECHT-KARLS-UNIVERSITAT Heidelberg) 20 September 2012 (20.09.2012) pg 5, para 1 to pg 6, para 3; pg 14, para 1; pg 19, para 2; pg 22, Compound E; pg 29, para 3	1-4, 4a, 5-12, 17 ----- 18-33; 39
A	MANCILLA et al. 'ASYMMETRIC SYNTHESIS OF NEW BICYCLIC PHENYLBORONIC ESTERS CONTAINING CONFIGURATIONALLY STABLE CHIRAL NITROGEN AND BORON', Journal of Organometallic Chemistry, 1987, Vol 321, pp 191-198. pg 192, Scheme 1	18-33; 39
A	HALL, editor. Structure, properties and preparation of Boronic Acid Derivatives in Organic Synthesis, Medicine and Materials, Second Edition., 2011, pp 1-133. pg 6, Figure 1.4, Compound 12; pg 17m, figure 1.9, compound 44l; pg 72, Figure 1.32, compound 55	18-33; 39



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

11 October 2016

Date of mailing of the international search report

22 NOV 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 16/49689

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.: 13-16, 34-38 and 40-85
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.