SSTR-TARGETED CONJUGATES AND FORMULATIONS THEREOF

REFERENCED TO RELATED APPLICATIONS

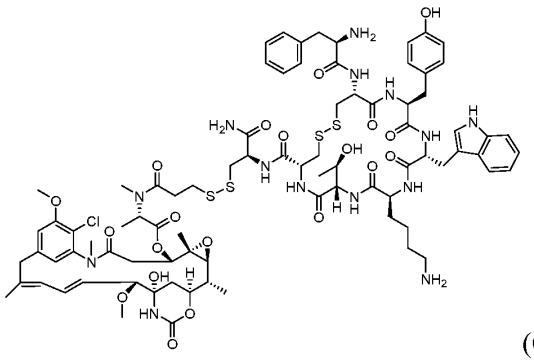
[0001] The present application claims priority to U.S. Provisional Patent Application No. 62/866,134, filed June 25, 2019, entitled SSTR-TARGETED CONJUGATES AND FORMULATIONS THEREOF, the contents of which are herein incorporated by reference in their entirety.

BACKGROUND

[0002] The development of nanotechnologies for effective delivery of drugs or drug candidates to specific diseased cells and tissues, e.g., to cancer cells, in specific organs or tissues, in a temporospatially regulated manner potentially can overcome or ameliorate therapeutic challenges, such as systemic toxicity. However, while targeting of the delivery system may preferentially deliver drug to a site where therapy is needed, the drug released from the nanoparticle may not for example, remain in the region of the targeted cells in efficacious amounts or may not remain in the circulation in a relatively non-toxic state for a sufficient amount of time to decrease the frequency of treatment or permit a lower amount of drug to be administered while still achieving a therapeutic effect. Antibody drug conjugates comprise an antibody and a cytotoxic payload have been designed. However, the size of antibodies limits solid tumor penetration compared to smaller targeting ligands (*see* Xiang et al., *Theranostics,* vol.5(10):1083-1097 (2015), the contents of which are incorporated herein by reference in their entirety). Smaller targeting ligands also penetrate solid tumors faster, which is important for payloads that require a high tumor C_{max}. Accordingly, there is a need in the art for improved drug targeting and delivery and to design drugs with deeper solid tumor penetration.

SUMMARY OF THE DISCLOSURE

[0003] In one aspect of the disclosure, a pharmaceutical composition comprising a conjugate, wherein the conjugate comprises an active agent coupled to a somatostatin receptor (SSTR) targeting moiety by a linker, and wherein the active agent is mertansine (DM1). In one embodiment, the pharmaceutical composition comprising



(Conjugate **57**), acetate buffer, mannitol, and solutol. The acetate buffer may have a strength of at least 30mM or at least 40mM. The mannitol may have a concentration of about 5%. The solutol may have a concentration of about 2%.

[0004] Another aspect of the disclosure provides a method of treating tumor comprising administering Conjugate **57** or a pharmaceutically acceptable salt thereof to a subject in need thereof, wherein the dose of Conjugate **57** is based on the body surface area (BSA) of the subject, and wherein the dose of Conjugate **57** is 8.8 mg/m2 or less than 8.8 mg/m2. The tumor may be a neuroendocrine tumor (NET), gastroenteropancreatic (GEP) tumor, gastrointestinal (GI) tumor, pancreatic cancer, lung cancer (small cell lung cancer (SCLC) or large cell neuroendocrine carcinoma (LCNEC) of the lung), prostate cancer, or thymus neuroendocrine tumor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] Fig. 1A shows the correlation of AUC with body surface area. Fig. 1B shows the correlation of Cmax with body surface area.

DETAILED DESCRIPTION

[0006] At least five somatostatin receptors subtypes have been characterized, and tumors can express various receptor subtypes. (e.g., see Shaer et al., Int. 3. Cancer 70:530-537, 1997). Naturally occurring somatostatin and its analogs exhibit differential binding to receptor subtypes. Applicants have exploited this feature to create novel conjugates to improve targeting of a conjugate comprising an active agent to a disease tissue target. Such targeting can, for example, improve the amount of active agent at a site and decrease active agent toxicity to the subject. As used herein, “toxicity” refers to the capacity of a substance or composition to be harmful or poisonous to a cell, tissue organism or cellular environment. Low toxicity refers to a reduced capacity of a substance or composition to be harmful or poisonous to a cell,

tissue organism or cellular environment. Such reduced or low toxicity may be relative to a standard measure, relative to a treatment or relative to the absence of a treatment.

[0007] It is an object of the disclosure to provide improved compounds, compositions, and formulations for temporospatial drug delivery.

[0008] It is further an object of the disclosure to provide methods of making improved compounds, compositions, and formulations for temporospatial drug delivery.

[0009] It is also an object of the disclosure to provide methods of administering the improved compounds, compositions, and formulations to individuals in need thereof.

I. Conjugates

[0010] Conjugates include an active agent or prodrug thereof attached to a targeting moiety, e.g., a molecule that can bind to an SSTR, by a linker. The conjugates can be a conjugate between a single active agent and a single targeting moiety, e.g., a conjugate having the structure X-Y-Z where X is the targeting moiety, Y is the linker, and Z is the active agent.

[0011] In some embodiments the conjugate contains more than one targeting moiety, more than one linker, more than one active agent, or any combination thereof. The conjugate can have any number of targeting moieties, linkers, and active agents. The conjugate can have the structure X-Y-Z-Y-X, $(X\text{-}Y)_n\text{-}Z$, $X\text{-}(Y\text{-}Z)_n$, $X\text{-}Y\text{-}Z_n$, $(X\text{-}Y\text{-}Z)_n$, $(X\text{-}Y\text{-}Z\text{-}Y)_n\text{-}Z$ where X is a targeting moiety, Y is a linker, Z is an active agent, and n is an integer between 1 and 50, between 2 and 20, for example, between 1 and 5. Each occurrence of X, Y, and Z can be the same or different, e.g., the conjugate can contain more than one type of targeting moiety, more than one type of linker, and/or more than one type of active agent.

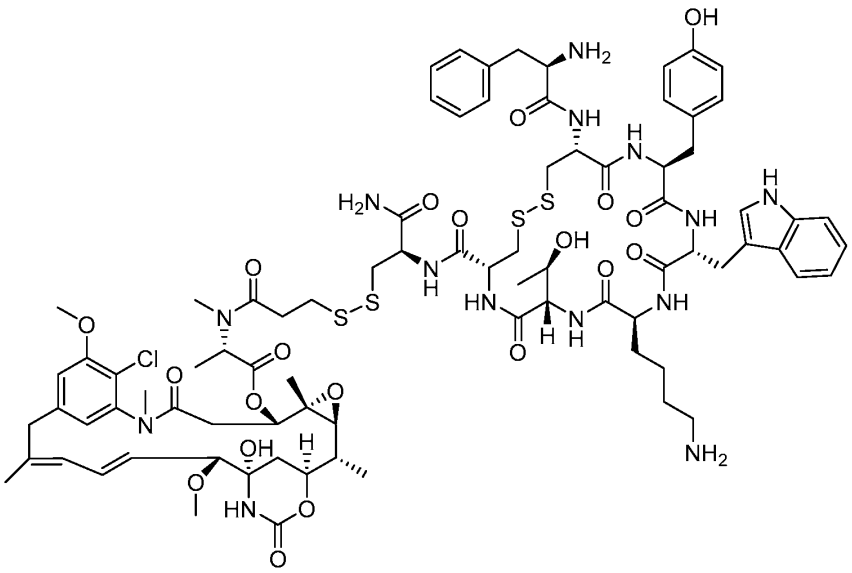
[0012] The conjugate can contain more than one targeting moiety attached to a single active agent. For example, the conjugate can include an active agent with multiple targeting moieties each attached via a different linker. The conjugate can have the structure X-Y-Z-Y-X where each X is a targeting moiety that may be the same or different, each Y is a linker that may be the same or different, and Z is the active agent.

[0013] The conjugate can contain more than one active agent attached to a single targeting moiety. For example the conjugate can include a targeting moiety with

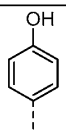
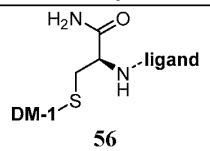
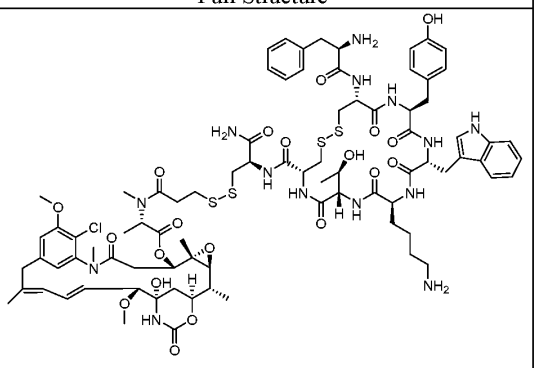
multiple active agents each attached via a different linker. The conjugate can have the structure Z-Y-X-Y-Z where X is the targeting moiety, each Y is a linker that may be the same or different, and each Z is an active agent that may be the same or different.

[0014] In some embodiments, the conjugate is selected from any conjugate that comprises DM1 (mertansine) as an active agent, a somatostatin receptor ligand as a targeting moiety, and a linker, wherein the linker binds to the C-terminus of the somatostatin receptor ligand, and wherein the somatostatin receptor ligand is a derivative of octreotide such as Tyr3-Octreotate (TATE). Non-limiting examples of the conjugates are disclosed in Table 2 of PCT Application No. PCT/US15/38569 (WO2016/004048) filed June 30, 2015, the contents of which are incorporated herein by reference.

[0015] In some embodiments, the conjugate is Conjugate **57**.



57

R	Ar1	Ar2	Linker*	Full Structure
H			 56	 57

II. Formulations

[0016] In some embodiments, compositions are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase "active ingredient" generally refers to the conjugate to be delivered as described herein.

[0017] 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 any other animal, e.g., to non-human animals, e.g. non-human mammals. 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 merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[0018] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and

then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0019] A pharmaceutical composition in accordance with the disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is 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.

[0020] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the disclosure 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%, e.g., between .5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

[0021] The conjugates of the present disclosure can be formulated using one or more excipients to: (1) increase stability; (2) permit the sustained or delayed release (e.g., from a depot formulation of the monomaleimide); (3) alter the biodistribution (e.g., target the monomaleimide compounds to specific tissues or cell types); (4) alter the release profile of the monomaleimide compounds *in vivo*. Non-limiting examples of the excipients include any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, and preservatives. Excipients of the present disclosure may also include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, hyaluronidase, nanoparticle mimics and combinations thereof. Accordingly, the formulations of the disclosure may include one or more excipients, each in an amount that together increases the stability of the monomaleimide compounds.

[0022] In some embodiments, the pharmaceutical composition comprises the conjugate of the present disclosure has a pH of about 4.0 to about 5.0. In some embodiments, the pharmaceutical composition comprises acetate buffer (sodium acetate and acetic acid) having a pH of about 4.0 to about 4.8. In some embodiments,

the pharmaceutical composition further comprises mannitol and polyoxyl 15 hydroxystearate.

[0023] In one embodiment, a composition for solution for injection is provided. The solution comprises Conjugate **57**, mannitol, Polyoxyl 15 Hydroxystearate, and aqueous acetate buffer. Each dosage unit contains 2.5 mg/mL of Conjugate **57** (free-base), 50 mg/mL (5% in weight percentage) mannitol, 20 mg/mL (2% in weight percentage) Polyoxyl 15 Hydroxystearate and pH 4.0 – 4.8 acetate buffer in a stoppered 10 mL clear glass vial. The clear glass vial is stopped with 20 mm FluroTec® gray lyo stoppers, and sealed with 20 mm dark blue flip-off seals. Prior to administration, the solution is diluted with 5% Mannitol Injection USP. The resulting diluted composition can be infused intravenously.

Excipients

[0024] Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference in its entirety) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this disclosure.

[0025] In some embodiments, a pharmaceutically acceptable excipient is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use in humans and for veterinary use. In some embodiments, an excipient is approved by United States Food and Drug Administration. In some embodiments, an excipient is pharmaceutical grade. In some embodiments, an excipient meets the standards of the United States Pharmacopoeia

(USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[0026] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, 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. Such excipients may optionally be included in pharmaceutical compositions.

[0027] Exemplary diluents include, but are not limited to, 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, etc., and/or combinations thereof.

[0028] Exemplary granulating and/or dispersing agents include, but are not limited to, 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, etc., and/or combinations thereof.

[0029] Exemplary surface active agents and/or emulsifiers include, but are not limited to, 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 [TWEENn®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, PLUORINC®F 68, POLOXAMER®188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, etc. and/or combinations thereof.

[0030] Exemplary binding agents include, but are not limited to, starch (e.g. cornstarch and starch paste); gelatin; sugars (e.g. sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol,); 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; etc.; and combinations thereof.

[0031] Exemplary preservatives may include, but are not limited to, antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and/or other preservatives. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and/or sodium sulfite. Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and/or trisodium edetate. Exemplary antimicrobial preservatives

include, but are not limited to, 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/or thimerosal. Exemplary antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and/or sorbic acid. Exemplary alcohol preservatives include, but are not limited to, ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and/or phenylethyl alcohol. Exemplary acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and/or phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluened (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/or EUXYL®.

[0032] Exemplary buffering agents include, but are not limited to, 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, etc., and/or combinations thereof.

[0033] Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behanate,

hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, etc., and combinations thereof.

[0034] Exemplary oils include, but are not limited to, 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 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/or combinations thereof.

[0035] Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

Administration

[0036] The conjugates of the present disclosure may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited to enteral, gastroenteral, epidural, oral, transdermal, epidural (peridural), intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), epicutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavernous injection, (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), insufflation (snorting), sublingual, sublabial,

enema, eye drops (onto the conjunctiva), or in ear drops. In specific embodiments, compositions may be administered in a way which allows them cross the blood-brain barrier, vascular barrier, or other epithelial barrier.

[0037] The formulations described herein contain an effective amount of conjugates in a pharmaceutical carrier appropriate for administration to an individual in need thereof. The formulations may be administered parenterally (e.g., by injection or infusion). The formulations or variations thereof may be administered in any manner including enterally, topically (e.g., to the eye), or via pulmonary administration. In some embodiments the formulations are administered topically.

Dosing

[0038] The present disclosure provides methods comprising administering conjugates as described herein to a subject in need thereof. Conjugates as described herein may be administered to a subject using any amount and any route of administration effective for preventing or treating or imaging a disease, disorder, and/or condition (e.g., a disease, disorder, and/or condition relating to working memory deficits). The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like.

[0039] Compositions in accordance with the disclosure 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 disclosure may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[0040] In some embodiments, compositions in accordance with the present disclosure may be administered at dosage levels sufficient to deliver from about 0.0001 mg/kg to about 100 mg/kg, from about 0.001 mg/kg to about 0.05 mg/kg, from about 0.005 mg/kg to about 0.05 mg/kg, from about 0.001 mg/kg to about 0.005 mg/kg, from about 0.05 mg/kg to about 0.5 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, 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, or 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, diagnostic, prophylactic, or imaging effect. The desired dosage may 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 some embodiments, the desired dosage may be delivered using multiple administrations (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used.

[0041] In some embodiments, Conjugate **57** and/or its pharmaceutically acceptable salt is administered at a dosage of between about 1 mg to about 50mg, such as about 1 mg, 2 mg, 4 mg, 6 mg, 8 mg, 10 mg, 12 mg, 14 mg, 16 mg, 18 mg, 20 mg, 22 mg, 24 mg, 26 mg, 28 mg, 30 mg, 32 mg, 34 mg, 36 mg, 38 mg, 40 mg, 42 mg, 44 mg, 46 mg, 48 mg, or 50 mg. In some embodiments, Conjugate **57** and/or its pharmaceutically acceptable salt is administered at a dosage of from about 18 mg to about 50 mg or about 25 mg to about 50 mg. In some embodiments, Conjugate **57** and/or its pharmaceutically acceptable salt is administered at a dosage of about 25 mg. In some embodiments, Conjugate **57** and/or its pharmaceutically acceptable salt is administered at a dosage of 25 mg.

[0042] The concentration of the conjugates of the present disclosure may be between about 0.01 mg/mL to about 50 mg/mL, about 0.1 mg/mL to about 25 mg/mL, about 0.5 mg/mL to about 10 mg/mL, or about 1 mg/mL to about 5 mg/mL in the pharmaceutical composition.

[0043] As used herein, a “split dose” is the division of single unit dose or total daily dose into two or more doses, e.g, two or more administrations of the single unit dose. As used herein, a “single unit dose” is a dose of any therapeutic administed in one

dose/at one time/single route/single point of contact, i.e., single administration event. As used herein, a "total daily dose" is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose. In one embodiment, the monomaleimide compounds of the present disclosure are administed to a subject in split doses. The monomaleimide compounds may be formulated in buffer only or in a formulation described herein.

Dosage Forms

[0044] A pharmaceutical composition described herein can be formulated into a dosage form described herein, such as a topical, intranasal, intratracheal, or injectable (e.g., intravenous, intraocular, intravitreal, intramuscular, intracardiac, intraperitoneal, subcutaneous).

III. Methods of Using the Conjugates

[0045] The conjugates as described herein can be administered to treat any hyperproliferative disease, metabolic disease, infectious disease, or cancer, as appropriate. The formulations can be used for immunization. Formulations may be administered by injection, orally, or topically, typically to a mucosal surface (lung, nasal, oral, buccal, sublingual, vaginally, rectally) or to the eye (intraocularly or transocularly).

[0046] In various embodiments, methods for treating a subject having a cancer are provided, wherein the method comprises administering a therapeutically-effective amount of the conjugates, as described herein, to a subject having a cancer, suspected of having cancer, or having a predisposition to a cancer. According to the present disclosure, cancer embraces any disease or malady characterized by uncontrolled cell proliferation, e.g., hyperproliferation. Cancers may be characterized by tumors, e.g., solid tumors or any neoplasm.

[0047] In some embodiments, the conjugates of the present teachings have been found to inhibit cancer and/or tumor growth. They may also reduce, including cell proliferation, invasiveness, and/or metastasis, thereby rendering them useful for the treatment of a cancer.

[0048] In some embodiments, the conjugates of the present teachings may be used to prevent the growth of a tumor or cancer, and/or to prevent the metastasis of a tumor

or cancer. In some embodiments, compositions of the present teachings may be used to shrink or destroy a cancer.

[0049] In some embodiments, the conjugates provided herein are useful for inhibiting proliferation of a cancer cell. In some embodiments, the conjugates provided herein are useful for inhibiting cellular proliferation, e.g., inhibiting the rate of cellular proliferation, preventing cellular proliferation, and/or inducing cell death. In general, the conjugates as described herein can inhibit cellular proliferation of a cancer cell or both inhibiting proliferation and/or inducing cell death of a cancer cell. In some embodiments, cell proliferation is reduced by at least about 25%, about 50%, about 75%, or about 90% after treatment with conjguates of the present disclosure compared with cells with no treatment. In some embodiments, cell cycle arrest marker phospho histone H3 (PH3 or PHH3) is increased by at least about 50%, about 75%, about 100%, about 200%, about 400% or about 600% after treatment with conjguates of the present disclosure compared with cells with no treatment. In some embodiments, cell apoptosis marker cleaved caspase-3 (CC3) is increased by at least 50%, about 75%, about 100%, about 200%, about 400% or about 600% after treatment with conjguates of the present disclosure compared with cells with no treatment.

[0050] Furthermore, in some embodiments, conjugates of the present disclosure are effective for inhibiting tumor growth, whether measured as a net value of size (weight, surface area or volume) or as a rate over time, in multiple types of tumors.

[0051] In some embodiments, the size of a tumor is reduced by about 60 % or more after treatment with conjugates of the present disclosure. In some embodiments, the size of a tumor is reduced by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 100%, by a measure of weight, and/or area and/or volume.

[0052] The cancers treatable by methods of the present teachings generally occur in mammals. Mammals include, for example, humans, non-human primates, dogs, cats, rats, mice, rabbits, ferrets, guinea pigs, horses, pigs, sheep, goats, and cattle. In various embodiments, the cancer is lung cancer, breast cancer, e.g., mutant BRCA1 and/or mutant BRCA2 breast cancer, non-BRCA-associated breast cancer, colorectal

cancer, ovarian cancer, pancreatic cancer, colorectal cancer, bladder cancer, prostate cancer, cervical cancer, renal cancer, leukemia, central nervous system cancers, myeloma, and melanoma.

[0053] In some embodiments, the cancer is a neuroendocrine cancer such as but not limited to small cell lung cancer (SCLC), adrenal medullary tumors (e.g., pheochromocytoma, neuroblastoma, ganglioneuroma, or paraganglioma), gastroenteropancreatic neuroendocrine tumors (e.g., carcinoids, gastrinoma, glucagonoma, vasoactive intestinal polypeptide-secreting tumor, pancreatic polypeptide-secreting tumor, or nonfunctioning gastroenteropancreatic tumors), meduallary thyroid cancer, Merkel cell tumor of the skin, pituitary adenoma, and pancreatic cancer. The somatostain receptor SSTR2 is over expressed on 50-90% of neuroendocrine cancers. In some embodiments, the neuroendocrine cancer is a primary neuroendocrine cancer. In some embodiments, the neuroendocrine cancer is a neuroendocrine metastatsis. Neuroendocrine metastatis may be in liver, lung, bone, or brain of a subject. In certain embodiments, the cancer is brain cancer, human lung carcinoma, ovarian cancer, pancreatic cancer or colorectal cancer.

[0054] In one embodiment, the conjugates as described herein or formulations containing the conjugates as described herein are used to treat small cell lung cancer. About 12%-15% of patients having lung cancer have small cell lung cancer. Survival in metastatic small cell lung caner is poor. Survival rate is below 5% five years after diagnosis. US incidence of small cell lung cancer is about 26K-30K. Among these patients, about 40%-80% are SSTR2 positive.

[0055] In some embodiments, the conjugates as described herein or formulations containing the conjugates as described herein are used to treat paitents with tumors that express or over-express the somatostatn receptor. Such patients can be identified with any method known in the art, such as but not limted to using a radionuclide imaging agent, a radiolabeled somatostatin analog imaging agent, SSTR scintigraphy or SSTR positron emission tomography (PET). In one embodiment, ^{111}Indium (Indium111)-labeled pentetreotide scintigraphy (OctreoScan^{TM}) is used to identify patients with SSTR-expressing tumors. In another embodiment, a 68Ga conjugate such as 68Ga-DOTA-TATE, 68Ga-DOTA-TOC, or 68Ga-DOTA-NOC is used in PET imaging to identify patients with SSTR-expressing tumors. Patients who show

positive scan results detected with Indium111-labeled pentetreotide scintigraphy are treated with conjguates of the present disclosure.

[0056] In one embodiment, the conjugates as described herein or formulations containing the conjugates as described herein are used to treat pateints having a histologically proven locally advanced or metastatic high grade neuroendocrine carcinoma (NEC). In some embodiments, the patients may have small cell and large cell neuroendocrine carcinoma of unknown primary or any extrapulmonary site. In some embodiments, the pateints may have well differentiated G3 neuroendocrine neoplasms if Ki-67>30%. In some embodiments, the pateints may have neuroendocrine prostate cancer (de novo or treatment-emergent) of prostate if small cell or large cell histology. In some embodiments, the patients may have mixed tumors, e.g. mixed adenoneuroendocrine carcinoma (MANEC) or mixed squamous or acinar cell NEC if the high grade (small or large cell) NEC component comprises >50% of the original sample or subsequent biopsy. In some embodiments, the patients may have castrate resistant prostate cancer (CRPC). In some embodiments, patients may be selected or stratified by having, or not having, any of the foregoing conditions.

[0057] In some embodiments, Conjugate **57** or its pharmaceutically acceptable salt is administered to patients diagnosed with neuroendocrine tumors (NETs), pancreatic cancer, gastrointestinal (GI) cancer (such as small intestine cancer, stomach cancer, rectum cancer, ileum cancer, colon cancer, small bowel cancer, large bowel cancer, gastric cancer, etc.), lung cancer (such as large-cell neuroendocrine carcinoma (LCNEC) of the lung, small cell lung cancer (SCLC), etc.), or pheochromocytoma. In some embodiments, patients treated may have, or not have, been diagnosed with any of the foregoing conditions prior to such treatment.

[0058] In some embodiments, the patients have a metastatic cancer. In some embodiments, the patients have metastasis to lymph nodes, liver, lung, peritoneum, back, bone, soft tissues outside of uterus, kidney, or vertebral column. In some embodiments, patients treated may have, or not have, been diagnosed with any of the foregoing conditions prior to such treatment.

[0059] In some embodiments, the patients have had prior cancer treatment therapies. In some embodiments, the patients have previously been treated with lancreotide, mTOR kinase inhibitor, Lutathera (a lutetium-177 (Lu-177) labeled

somatostatin analogue peptide), sunitinib, cyclophosphamide, vincristine, dacarbazine, octreotide, carbo, streptozocin, a FOLFIRI therapy (a combination therapy comprising folinic acid (e.g., leucovorin), fluorouracil (5-FU), and irinotecan (e.g., Camptosar))

[0060] In some embodiments, the patients are male. In some embodiments, the patients are female. In some embodiments, the patients are at least 18 years old. In some embodiments, the patients are at least 40 years old. In some embodiments, the pateints are at least 60 years old.

[0061] A feature of conjugates of the present disclosure is relatively low toxicity to an organism while maintaining efficacy at inhibiting, e.g. slowing or stopping tumor growth. As used herein, "toxicity" refers to the capacity of a substance or composition to be harmful or poisonous to a cell, tissue organism or cellular environment. Low toxicity refers to a reduced capacity of a substance or composition to be harmful or poisonous to a cell, tissue organism or cellular environment. Such reduced or low toxicity may be relative to a standard measure, relative to a treatment or relative to the absence of a treatment. For example, conjugates of the present disclosure may have lower toxicity than the active agent moiety Z administered alone. For conjugates comprsing DM1, their toxicity is lower than DM1 administered alone.

[0062] Toxicity may further be measured relative to a subject's weight loss where weight loss over 15%, over 20% or over 30% of the body weight is indicative of toxicity. Other metrics of toxicity may also be measured such as patient presentation metrics including lethargy and general malaiase. Neutropenia, thrombopenia, white blood cell (WBC) count, complete blood cell (CBC) count may also be metrics of toxicity. Pharmacologic indicators of toxicity include elevated aminotransferases (AST/ALT) levels, neurotoxicity, kidney damage, GI damage and the like. In one embodiment, conjugates of the present disclosure do not cause a significant change of a subject's body weight. The body weight loss of a subject is less about 30%, about 20%, about 15%, about 10%, or about 5% after treatment with conjguates of the present disclosure. In another embodiment, conjugates of the present disclosure do not cause a significant increase of a subject's AST/ALT levels. The AST or ALT level of a subject is increased by less than about 30%, about 20%, about 15%, about 10%, or about 5% after treatment with conjugates of the present disclosure. In yet another embodiment, conjugates of the present disclosure do not cause a significant change of

a subject's CBC or WBC count after treatment with conjugates of the present disclosure. The CBC or WBC level of a subject is decreased by less than about 30%, about 20%, about 15%, about 10%, or about 5% after treatment with conjugates of the present disclosure.

[0063] In some embodiments, Conjugate **57** is administered to a patient and any one or more of white blood cells (WBC), red blood cells (RBC), hemoglobin, platelets, neutrophils, lymphocytes, blood urea nitrogen (BUN), creatinine, glucose, albumin, total protein, calcium levels, magnesium levels, alkaline phosphatase, total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase, lipase, international normalized ratio (INR), the prothrombin time (PT), and/or activated partial thromboplastin time (aPTT) of the patient are measured.

[0064] In some embodiments, the treatment-related adverse effects (AE) of a pharmaceutical composition comprising Conjugate **57** may include nausea, fatigue, increased alanine aminotransferase, constipation, diarrhea, increased aspartate aminotransferase, pyrexia, abdominal distension, abdominal pain, anaemia, arthralgia, increased blood alkaline phosphatase, increased blood creatinine, decreased appetite, dyspepsia, hypertension, hypoalbuminaemia, hypotension, insomnia, increased lipase, pain in extremity, paraesthesia, pelvic pain, and/or urinary tract infection.

[0065] In some embodiments, less than 30% of the patient population has any one or more treatment-related adverse effects. In some embodiments, a single patient experiences treatment-related adverse effects in less 30% of the whole treatment time.

[0066] In some embodiments, the patients treated with Conjguate **57** have lower, reduced or no circulating tumor cells.

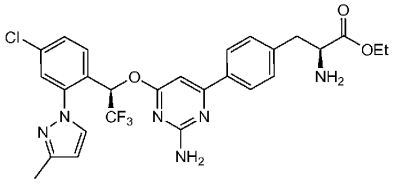
[0067] In some embodiments, Conjugate **57** has a half life of about 1.8 hours in a patient. In some embodiments, Conjugate **57** has a half life of about 3.3 hours in a patient. In some embodiments, Conjugate **57** has a clearance of 19.1 L/h.

[0068] In some embodiments, conjugates of the present disclosure are combined with at least one addtional active agent. The active agent may be any suitable drug. It may be selected from any active agent described herein such as a drug for treating cancer. It may also be a cancer symptom relief drug. Non-limiting examples of symptom relief drugs include: octreotide or lanreotide; interferon, cypoheptadine or any other antihistamines. In some embodiments, conjugates of the present disclosure do not have drug-drug interference with the additional active agent. In one

embodiment, conjugates of the present disclosure do not inhibit cytochrome P450 (CYP) isozymes. CYP isozymes may include CYP3A4 Midazolam, CYP3A4 Testosterone, CYP2C9, CYP2D6, CYP1A2, CYP2C8, CYP2B6, and CYP2C19. The additional active agent may be administered concomitantly with conjguates of the present disclosure.

[0069] In some embodiments, the additional active agent may not bind to any somatostatin receptor. In one embodiment, the additional active agent is a cancer symptom relief drug. The symptom relief drug may reduce diarrhea or the side effects of chemotherapy or radiation therapy. In one example, conjugates of the present disclosure may be combined with a symptom relief drug for carcinoid symdrome, such as telotristat or telotristat etiprate (LX1032, Lexicon®). Telotristat etiprate is telotristat's crystalline hippurate salt as disclosed in WO2013059146 to Chen et al., the contents of which are incorporated herein by reference in their entirety. Telotristat, its salts and crystalline forms can be obtained by methods known in the art (*see* US 7709493 to Devasagayaraj et al., the contents of which are incorporated herein by reference in their entirety). Any other compound disclosed in US 7709493 may be combined with conjugates of the present disclosure.

Telotristat:



[0070] In another example, conjugates of the present disclosure may be combined with a moderate dose of chemotherapy agents such as mitomycin C, vinblastine and cisplatin (*see* Ellis et al., *Br J Cancer*, vol.71(2): 366–370 (1995), the contents of which are incorporated herein by reference in their entirety).

[0071] The conjugates as described herein or formulations containing the conjugates as described herein can be used for the selective tissue delivery of a therapeutic, prophylactic, or diagnostic agent to an individual or patient in need thereof. For example, DM1 conjugates of the present disclosure are used to deliver DM1 to selective tissues. These tissues may be tumor tissues. Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several

divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic.

IV. Kits and Devices

[0072] The disclosure provides a variety of kits and devices for conveniently and/or effectively carrying out methods of the present disclosure. Typically kits will comprise sufficient amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments.

[0073] In one embodiment, the present disclosure provides kits for inhibiting tumor cell growth in vitro or in vivo, comprising a conjugate of the present disclosure or a combination of conjugates of the present disclosure, optionally in combination with any other active agents.

[0074] The kit may further comprise packaging and instructions and/or a delivery agent to form a formulation composition. The delivery agent may comprise a saline, a buffered solution, or any delivery agent disclosed herein. The amount of each component may be varied to enable consistent, reproducible higher concentration saline or simple buffer formulations. The components may also be varied in order to increase the stability of the conjugates in the buffer solution over a period of time and/or under a variety of conditions.

[0075] The present disclosure provides for devices which may incorporate conjugates of the present disclosure. These devices contain in a stable formulation available to be immediately delivered to a subject in need thereof, such as a human patient. In some embodiments, the subject has cancer.

[0076] Non-limiting examples of the devices include a pump, a catheter, a needle, a transdermal patch, a pressurized olfactory delivery device, iontophoresis devices, multi-layered microfluidic devices. The devices may be employed to deliver conjugates of the present disclosure according to single, multi- or split-dosing regiments. The devices may be employed to deliver conjugates of the present disclosure across biological tissue, intradermal, subcutaneously, or intramuscularly.

V. Definitions

[0077] The term "compound", as used herein, is meant to include all stereoisomers, geometric isomers, tautomers, and isotopes of the structures depicted. In the present application, compound is used interechangably with conjugate. Therefore, conjugate, as used herein, is also meant to include all stereoisomers, geometric isomers, tautomers, and isotopes of the structures depicted.

[0078] The compounds described herein can be asymmetric (e.g., having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

[0079] Compounds of the present disclosure also include tautomeric forms. Tautomeric forms result from the swapping of a single bond with an adjacent double bond and the concomitant migration of a proton. Tautomeric forms include prototropic tautomers which are isomeric protonation states having the same empirical formula and total charge. Examples prototropic tautomers include ketone – enol pairs, amide – imidic acid pairs, lactam – lactim pairs, amide – imidic acid pairs, enamine – imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, such as, 1H- and 3H-imidazole, 1H-, 2H- and 4H- 1,2,4-triazole, 1H- and 2H- isoindole, and 1H- and 2H-pyrazole. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution.

[0080] Compounds of the present disclosure also include all of the isotopes of the atoms occurring in the intermediate or final compounds. "Isotopes" refers to atoms having the same atomic number but different mass numbers resulting from a different number of neutrons in the nuclei. For example, isotopes of hydrogen include tritium and deuterium.

[0081] The compounds and salts of the present disclosure can be prepared in combination with solvent or water molecules to form solvates and hydrates by routine methods.

[0082] The terms "subject" or "patient", as used herein, refer to any organism to which the conjugates may be administered, e.g., for experimental, therapeutic, diagnostic, and/or prophylactic purposes. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, guinea pigs, cattle, pigs, sheep, horses, dogs, cats, hamsters, lamas, non-human primates, and humans).

[0083] The terms "treating" or “preventing”, as used herein, can include preventing a disease, disorder or condition from occurring in an animal that may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having the disease, disorder or condition; inhibiting the disease, disorder or condition, e.g., impeding its progress; and relieving the disease, disorder, or condition, e.g., causing regression of the disease, disorder and/or condition. Treating the disease, disorder, or condition can include ameliorating at least one symptom of the particular disease, disorder, or condition, even if the underlying pathophysiology is not affected, such as treating the pain of a subject by administration of an analgesic agent even though such agent does not treat the cause of the pain.

[0084] A “target”, as used herein, shall mean a site to which targeted constructs bind. A target may be either *in vivo* or *in vitro*. In certain embodiments, a target may be cancer cells found in leukemias or tumors (e.g., tumors of the brain, lung (small cell and non-small cell), ovary, prostate, breast and colon as well as other carcinomas and sarcomas). In still other embodiments, a target may refer to a molecular structure to which a targeting moiety or ligand binds, such as a hapten, epitope, receptor, dsDNA fragment, carbohydrate or enzyme. A target may be a type of tissue, e.g., neuronal tissue, intestinal tissue, pancreatic tissue, liver, kidney, prostate, ovary, lung, bone marrow, or breast tissue.

[0085] The “target cells” that may serve as the target for the method or conjugates, are generally animal cells, e.g., mammalian cells. The present method may be used to modify cellular function of living cells *in vitro*, i.e., in cell culture, or *in vivo*, in which the cells form part of or otherwise exist in animal tissue. Thus, the target cells may include, for example, the blood, lymph tissue, cells lining the alimentary canal, such as the oral and pharyngeal mucosa, cells forming the villi of the small intestine, cells

lining the large intestine, cells lining the respiratory system (nasal passages/lungs) of an animal (which may be contacted by inhalation of the subject disclosure), dermal/epidermal cells, cells of the vagina and rectum, cells of internal organs including cells of the placenta and the so-called blood/brain barrier, etc. In general, a target cell expresses at least one type of SSTR. In some embodiments, a target cell can be a cell that expresses an SSTR and is targeted by a conjugate described herein, and is near a cell that is affected by release of the active agent of the conjugate. For example, a blood vessel expressing an SSTR that is in proximity to a tumor may be the target, while the active agent released at the site will affect the tumor.

[0086] The term "therapeutic effect" is art-recognized and refers to a local or systemic effect in animals, particularly mammals, and more particularly humans caused by a pharmacologically active substance. The term thus means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease, disorder or condition in the enhancement of desirable physical or mental development and conditions in an animal, e.g., a human.

[0087] The term “modulation” is art-recognized and refers to up regulation (i.e., activation or stimulation), down regulation (i.e., inhibition or suppression) of a response, or the two in combination or apart. The modulation is generally compared to a baseline or reference that can be internal or external to the treated entity.

[0088] “Parenteral administration”, as used herein, means administration by any method other than through the digestive tract (enteral) or non-invasive topical routes. For example, parenteral administration may include administration to a patient intravenously, intradermally, intraperitoneally, intrapleurally, intratracheally, intraossiously, intracerebrally, intrathecally, intramuscularly, subcutaneously, subjunctivally, by injection, and by infusion.

[0089] “Topical administration”, as used herein, means the non-invasive administration to the skin, orifices, or mucosa. Topical administration can be delivered locally, i.e., the therapeutic can provide a local effect in the region of delivery without systemic exposure or with minimal systemic exposure. Some topical formulations can provide a systemic effect, e.g., via adsorption into the blood stream of the individual. Topical administration can include, but is not limited to, cutaneous and transdermal administration, buccal administration, intranasal administration,

intravaginal administration, intravesical administration, ophthalmic administration, and rectal administration.

[0090] "Enteral administration", as used herein, means administration via absorption through the gastrointestinal tract. Enteral administration can include oral and sublingual administration, gastric administration, or rectal administration.

[0091] "Pulmonary administration", as used herein, means administration into the lungs by inhalation or endotracheal administration. As used herein, the term "inhalation" refers to intake of air to the alveoli. The intake of air can occur through the mouth or nose.

[0092] The terms "sufficient" and "effective", as used interchangeably herein, refer to an amount (e.g., mass, volume, dosage, concentration, and/or time period) needed to achieve one or more desired result(s). A "therapeutically effective amount" is at least the minimum concentration required to effect a measurable improvement or prevention of at least one symptom or a particular condition or disorder, to effect a measurable enhancement of life expectancy, or to generally improve patient quality of life. The therapeutically effective amount is thus dependent upon the specific biologically active molecule and the specific condition or disorder to be treated. Therapeutically effective amounts of many active agents, such as antibodies, are known in the art. The therapeutically effective amounts of compounds and compositions described herein, e.g., for treating specific disorders may be determined by techniques that are well within the craft of a skilled artisan, such as a physician.

[0093] The terms "bioactive agent" and "active agent", as used interchangeably herein, include, without limitation, physiologically or pharmacologically active substances that act locally or systemically in the body. A bioactive agent is a substance used for the treatment (e.g., therapeutic agent), prevention (e.g., prophylactic agent), diagnosis (e.g., diagnostic agent), cure or mitigation of disease or illness, a substance which affects the structure or function of the body, or pro-drugs, which become biologically active or more active after they have been placed in a predetermined physiological environment.

[0094] The term "prodrug" refers to an agent, including a small organic molecule, peptide, nucleic acid or protein, that is converted into a biologically active form *in vitro* and/or *in vivo*. Prodrugs can be useful because, in some situations, they may be easier to administer than the parent compound (the active compound). For example, a

prodrug may be bioavailable by oral administration whereas the parent compound is not. The prodrug may also have improved solubility in pharmaceutical compositions compared to the parent drug. A prodrug may also be less toxic than the parent. The term "biocompatible", as used herein, refers to a material that along with any metabolites or degradation products thereof that are generally non-toxic to the recipient and do not cause any significant adverse effects to the recipient. Generally speaking, biocompatible materials are materials which do not elicit a significant inflammatory or immune response when administered to a patient.

[0095] The term "biodegradable" as used herein, generally refers to a material that will degrade or erode under physiologic conditions to smaller units or chemical species that are capable of being metabolized, eliminated, or excreted by the subject. The degradation time is a function of composition and morphology. Degradation times can be from hours to weeks.

[0096] The term "pharmaceutically acceptable", as used herein, refers to compounds, materials, compositions, and/or dosage forms that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio, in accordance with the guidelines of agencies such as the U.S. Food and Drug Administration. A "pharmaceutically acceptable carrier", as used herein, refers to all components of a pharmaceutical formulation that facilitate the delivery of the composition *in vivo*. Pharmaceutically acceptable carriers include, but are not limited to, diluents, preservatives, binders, lubricants, disintegrators, swelling agents, fillers, stabilizers, and combinations thereof.

[0097] The term "molecular weight", as used herein, generally refers to the mass or average mass of a material. If a polymer or oligomer, the molecular weight can refer to the relative average chain length or relative chain mass of the bulk polymer. In practice, the molecular weight of polymers and oligomers can be estimated or characterized in various ways including gel permeation chromatography (GPC) or capillary viscometry. GPC molecular weights are reported as the weight-average molecular weight (M_w) as opposed to the number-average molecular weight (M_n). Capillary viscometry provides estimates of molecular weight as the inherent viscosity

determined from a dilute polymer solution using a particular set of concentration, temperature, and solvent conditions.

[0098] The term “small molecule”, as used herein, generally refers to an organic molecule that is less than 2000 g/mol in molecular weight, less than 1500 g/mol, less than 1000 g/mol, less than 800 g/mol, or less than 500 g/mol. Small molecules are non-polymeric and/or non-oligomeric.

[0099] The term “hydrophilic”, as used herein, refers to substances that have strongly polar groups that readily interact with water.

[0100] The term “hydrophobic”, as used herein, refers to substances that lack an affinity for water; tending to repel and not absorb water as well as not dissolve in or mix with water.

[0101] The term “lipophilic”, as used herein, refers to compounds having an affinity for lipids.

[0102] The term “amphiphilic”, as used herein, refers to a molecule combining hydrophilic and lipophilic (hydrophobic) properties. “Amphiphilic material” as used herein refers to a material containing a hydrophobic or more hydrophobic oligomer or polymer (e.g., biodegradable oligomer or polymer) and a hydrophilic or more hydrophilic oligomer or polymer.

[0103] The term "targeting moiety", as used herein, refers to a moiety that binds to or localizes to a specific locale. The moiety may be, for example, a protein, nucleic acid, nucleic acid analog, carbohydrate, or small molecule. The locale may be a tissue, a particular cell type, or a subcellular compartment. In some embodiments, a targeting moiety can specifically bind to a selected molecule.

[0104] The term “reactive coupling group”, as used herein, refers to any chemical functional group capable of reacting with a second functional group to form a covalent bond. The selection of reactive coupling groups is within the ability of those in the art. Examples of reactive coupling groups can include primary amines (-NH_2) and amine-reactive linking groups such as isothiocyanates, isocyanates, acyl azides, NHS esters, sulfonyl chlorides, aldehydes, glyoxals, epoxides, oxiranes, carbonates, aryl halides, imidoesters, carbodiimides, anhydrides, and fluorophenyl esters. Most of these conjugate to amines by either acylation or alkylation. Examples of reactive coupling groups can include aldehydes (-COH) and aldehyde reactive linking groups such as hydrazides, alkoxyamines, and primary amines. Examples of reactive

coupling groups can include thiol groups (-SH) and sulfhydryl reactive groups such as maleimides, haloacetyls, and pyridyl disulfides. Examples of reactive coupling groups can include photoreactive coupling groups such as aryl azides or diazirines. The coupling reaction may include the use of a catalyst, heat, pH buffers, light, or a combination thereof.

[0105] The term "protective group", as used herein, refers to a functional group that can be added to and/or substituted for another desired functional group to protect the desired functional group from certain reaction conditions and selectively removed and/or replaced to deprotect or expose the desired functional group. Protective groups are known to the skilled artisan. Suitable protective groups may include those described in Greene and Wuts, Protective Groups in Organic Synthesis, (1991). Acid sensitive protective groups include dimethoxytrityl (DMT), tert- butylcarbamate (tBoc) and trifluoroacetyl (tFA). Base sensitive protective groups include 9-fluorenylmethoxycarbonyl (Fmoc), isobutyrl (iBu), benzoyl (Bz) and phenoxyacetyl (pac). Other protective groups include acetamidomethyl, acetyl, tert-amyloxycarbonyl, benzyl, benzyloxycarbonyl, 2-(4-biphεnylyl)-2-propy!oxycarbonyl, 2- bromobenzyloxycarbonyl, tert-butyl7 tert-butyloxycarbonyl, l-carbobenzoxamido-2,2.2- trifluoroethyl, 2,6-dichlorobenzyl, 2-(3,5-dimethoxyphenyl)-2-propyloxycarbonyl, 2,4- dinitrophenyl, dithiasuccinyl, formyl, 4-methoxybenzenesulfonyl, 4-methoxybenzyl, 4- methylbenzyl, o-nitrophenylsulfenyl, 2-phenyl-2-propyloxycarbonyl, α-2,4,5- tetramethylbenzyloxycarbonyl, p-toluenesulfonyl, xanthenyl, benzyl ester, N- hydroxysuccinimide ester, p-nitrobenzyl ester, p-nitrophenyl ester, phenyl ester, p- nitrocarbonate, p-nitrobenzylcarbonate, trimethylsilyl and pentachlorophenyl ester.

[0106] The term "activated ester", as used herein, refers to alkyl esters of carboxylic acids where the alkyl is a good leaving group rendering the carbonyl susceptible to nucleophilic attack by molecules bearing amino groups. Activated esters are therefore susceptible to aminolysis and react with amines to form amides. Activated esters contain a carboxylic acid ester group $-CO_2R$ where R is the leaving group.

[0107] The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl

(alicyclic) groups, alkyl-substituted cycloalkyl groups, and cycloalkyl-substituted alkyl groups.

[0108] In some embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chains, C_3-C_{30} for branched chains), 20 or fewer, 12 or fewer, or 7 or fewer. Likewise, in some embodiments cycloalkyls have from 3-10 carbon atoms in their ring structure, e.g., have 5, 6 or 7 carbons in the ring structure. The term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having one or more substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents include, but are not limited to, halogen, hydroxyl, carbonyl (such as a carboxyl, alkoxycarbonyl, formyl, or an acyl), thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), alkoxyl, phosphoryl, phosphate, phosphonate, a hosphinate, amino, amido, amidine, imine, cyano, nitro, azido, sulfhydryl, alkylthio, sulfate, sulfonate, sulfamoyl, sulfonamido, sulfonyl, heterocyclyl, aralkyl, or an aromatic or heteroaromatic moiety.

[0109] Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, or from one to six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths. In some embodiments, alkyl groups are lower alkyls. In some embodiments, a substituent designated herein as alkyl is a lower alkyl.

[0110] It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include halogen, hydroxy, nitro, thiols, amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), $-CF_3$, -CN and the like. Cycloalkyls can be substituted in the same manner.

[0111] The term “heteroalkyl”, as used herein, refers to straight or branched chain, or cyclic carbon-containing radicals, or combinations thereof, containing at least one heteroatom. Suitable heteroatoms include, but are not limited to, O, N, Si, P, Se, B,

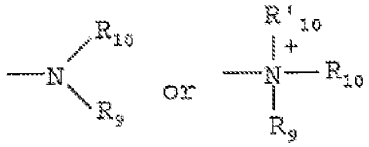
and S, wherein the phosphorous and sulfur atoms are optionally oxidized, and the nitrogen heteroatom is optionally quaternized. Heteroalkyls can be substituted as defined above for alkyl groups.

[0112] The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In some embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, and -S-alkynyl. Representative alkylthio groups include methylthio, and ethylthio. The term “alkylthio” also encompasses cycloalkyl groups, alkene and cycloalkene groups, and alkyne groups. “Arylthio” refers to aryl or heteroaryl groups. Alkylthio groups can be substituted as defined above for alkyl groups.

[0113] The terms "alkenyl" and "alkynyl", refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

[0114] The terms "alkoxyl" or "alkoxy" as used herein refers to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, and tert-butoxy. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, and -O-alkynyl. Aroxy can be represented by –O-aryl or O-heteroaryl, wherein aryl and heteroaryl are as defined below. The alkoxy and aroxy groups can be substituted as described above for alkyl.

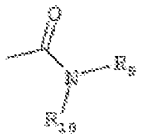
[0115] The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formula:



wherein R_9, R_{10}, and R'_{10} each independently represent a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R_8$ or R_9 and R_{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R_8 represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is

zero or an integer in the range of 1 to 8. In some embodiments, only one of R_9 or R_{10} can be a carbonyl, e.g., R_9, R_{10} and the nitrogen together do not form an imide. In still other embodiments, the term “amine” does not encompass amides, e.g., wherein one of R_9 and R_{10} represents a carbonyl. In additional embodiments, R_9 and R_{10} (and optionally R'_{10}) each independently represent a hydrogen, an alkyl or cycloalkly, an alkenyl or cycloalkenyl, or alkynyl. Thus, the term "alkylamine" as used herein means an amine group, as defined above, having a substituted (as described above for alkyl) or unsubstituted alkyl attached thereto, i.e., at least one of R_9 and R_{10} is an alkyl group.

[0116] The term "amido" is art-recognized as an amino-substituted carbonyl and includes a moiety that can be represented by the general formula:



wherein R_9 and R_{10} are as defined above.

[0117] “Aryl”, as used herein, refers to C_5-C_{10}-membered aromatic, heterocyclic, fused aromatic, fused heterocyclic, biaromatic, or bihetereocyclic ring systems. Broadly defined, “aryl”, as used herein, includes 5-, 6-, 7-, 8-, 9-, and 10-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as “aryl heterocycles” or “heteroaromatics”. The aromatic ring can be substituted at one or more ring positions with one or more substituents including, but not limited to, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino (or quaternized amino), nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, $-CF_3$, -CN; and combinations thereof.

[0118] The term “aryl” also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (i.e., “fused rings”) wherein at least one of the rings is aromatic, e.g., the other cyclic ring

or rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocycles. Examples of heterocyclic rings include, but are not limited to, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazolinyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolinyl, carbazolyl, 4aH carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2*H*,6*H*-1,5,2-dithiazinyl, dihydrofuro[2,3 b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, 1*H*-indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, 3*H*-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxindolyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4*H*-quinolizinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, tetrazolyl, 6*H*-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl and xanthenyl. One or more of the rings can be substituted as defined above for “aryl”.

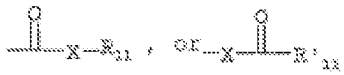
[0119] The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

[0120] The term "carbocycle", as used herein, refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.

[0121] “Heterocycle” or “heterocyclic”, as used herein, refers to a cyclic radical attached via a ring carbon or nitrogen of a monocyclic or bicyclic ring containing 3-10 ring atoms, for example, from 5-6 ring atoms, consisting of carbon and one to four heteroatoms each selected from the group consisting of non-peroxide oxygen, sulfur, and N(Y) wherein Y is absent or is H, O, (C_1-C_{10}) alkyl, phenyl or benzyl, and optionally containing 1-3 double bonds and optionally substituted with one or more substituents. Examples of heterocyclic rings include, but are not limited to,

benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazolinyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolinyl, carbazolyl, 4a*H*-carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2*H*,6*H*-1,5,2-dithiazinyl, dihydrofuro[2,3-*b*]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, 1*H*-indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, 3H-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxepanyl, oxetanyl, oxindolyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4*H*-quinolizinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydropyranyl, tetrahydroquinolinyl, tetrazolyl, 6*H*-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl and xanthenyl. Heterocyclic groups can optionally be substituted with one or more substituents at one or more positions as defined above for alkyl and aryl, for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphate, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF3, and -CN.

[0122] The term "carbonyl" is art-recognized and includes such moieties as can be represented by the general formula:



wherein X is a bond or represents an oxygen or a sulfur, and R_{11} represents a hydrogen, an alkyl, a cycloalkyl, an alkenyl, an cycloalkenyl, or an alkynyl, R'_{11} represents a hydrogen, an alkyl, a cycloalkyl, an alkenyl, an cycloalkenyl, or an alkynyl. Where X is an oxygen and R_{11} or R'_{11} is not hydrogen, the formula

represents an "ester". Where X is an oxygen and R_{11} is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R_{11} is a hydrogen, the formula represents a "carboxylic acid". Where X is an oxygen and R'_{11} is hydrogen, the formula represents a "formate". In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiocarbonyl" group. Where X is a sulfur and R_{11} or R'_{11} is not hydrogen, the formula represents a "thioester." Where X is a sulfur and R_{11} is hydrogen, the formula represents a "thiocarboxylic acid." Where X is a sulfur and R'_{11} is hydrogen, the formula represents a "thioformate." On the other hand, where X is a bond, and R_{11} is not hydrogen, the above formula represents a "ketone" group. Where X is a bond, and R_{11} is hydrogen, the above formula represents an "aldehyde" group.

[0123] The term “monoester” as used herein refers to an analog of a dicarboxylic acid wherein one of the carboxylic acids is functionalized as an ester and the other carboxylic acid is a free carboxylic acid or salt of a carboxylic acid. Examples of monoesters include, but are not limited to, to monoesters of succinic acid, glutaric acid, adipic acid, suberic acid, sebacic acid, azelaic acid, oxalic and maleic acid.

[0124] The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Examples of heteroatoms are boron, nitrogen, oxygen, phosphorus, sulfur and selenium. Other useful heteroatoms include silicon and arsenic.

[0125] As used herein, the term "nitro" means $-NO_2$; the term "halogen" designates -F, -Cl, -Br or -I; the term "sulfhydryl" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means $-SO_2-$.

[0126] The term “substituted” as used herein, refers to all permissible substituents of the compounds described herein. In the broadest sense, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, but are not limited to, halogens, hydroxyl groups, or any other organic groupings containing any number of carbon atoms, for example, 1-14 carbon atoms, and optionally include one or more heteroatoms such as oxygen, sulfur, or nitrogen grouping in linear, branched, or cyclic structural formats. Representative substituents include alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted

heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, aminoacid, peptide, and polypeptide groups.

[0127] Heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. It is understood that "substitution" or "substituted" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, i.e., a compound that does not spontaneously undergo transformation, for example, by rearrangement, cyclization, or elimination.

[0128] In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein. The permissible substituents can be one or more and the same or different for appropriate organic compounds. The heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms.

[0129] In various embodiments, the substituent is selected from alkoxy, aryloxy, alkyl, alkenyl, alkynyl, amide, amino, aryl, arylalkyl, carbamate, carboxy, cyano, cycloalkyl, ester, ether, formyl, halogen, haloalkyl, heteroaryl, heterocyclyl, hydroxyl, ketone, nitro, phosphate, sulfide, sulfinyl, sulfonyl, sulfonic acid, sulfonamide, and thioketone, each of which optionally is substituted with one or more suitable substituents. In some embodiments, the substituent is selected from alkoxy, aryloxy, alkyl, alkenyl, alkynyl, amide, amino, aryl, arylalkyl, carbamate, carboxy, cycloalkyl, ester, ether, formyl, haloalkyl, heteroaryl, heterocyclyl, ketone, phosphate, sulfide, sulfinyl, sulfonyl, sulfonic acid, sulfonamide, and thioketone, wherein each of the alkoxy, aryloxy, alkyl, alkenyl, alkynyl, amide, amino, aryl, arylalkyl, carbamate, carboxy, cycloalkyl, ester, ether, formyl, haloalkyl, heteroaryl, heterocyclyl, ketone,

phosphate, sulfide, sulfinyl, sulfonyl, sulfonic acid, sulfonamide, and thioketone can be further substituted with one or more suitable substituents.

[0130] Examples of substituents include, but are not limited to, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, thioketone, ester, heterocyclyl, –CN, aryl, aryloxy, perhaloalkoxy, aralkoxy, heteroaryl, heteroaryloxy, heteroarylalkyl, heteroaralkoxy, azido, alkylthio, oxo, acylalkyl, carboxy esters, carboxamido, acyloxy, aminoalkyl, alkylaminoaryl, alkylaryl, alkylaminoalkyl, alkoxyaryl, arylamino, aralkylamino, alkylsulfonyl, carboxamidoalkylaryl, carboxamidoaryl, hydroxyalkyl, haloalkyl, alkylaminoalkylcarboxy, aminocarboxamidoalkyl, cyano, alkoxyalkyl, perhaloalkyl, arylalkyloxyalkyl, and the like. In some embodiments, the substituent is selected from cyano, halogen, hydroxyl, and nitro.

[0131] The term “copolymer” as used herein, generally refers to a single polymeric material that is comprised of two or more different monomers. The copolymer can be of any form, for example, random, block, or graft. The copolymers can have any end-group, including capped or acid end groups.

[0132] The terms "polypeptide," "peptide" and "protein" generally refer to a polymer of amino acid residues. As used herein, the term also applies to amino acid polymers in which one or more amino acids are chemical analogs or modified derivatives of corresponding naturally-occurring amino acids or are unnatural amino acids. The term "protein", as generally used herein, refers to a polymer of amino acids linked to each other by peptide bonds to form a polypeptide for which the chain length is sufficient to produce tertiary and/or quaternary structure. The term “protein” excludes small peptides by definition, the small peptides lacking the requisite higher-order structure necessary to be considered a protein.

[0133] The terms "nucleic acid," "polynucleotide," and "oligonucleotide" are used interchangeably to refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. These terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogs of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate

backbones). In general and unless otherwise specified, an analog of a particular nucleotide has the same base-pairing specificity; i.e., an analog of A will base-pair with T. The term “nucleic acid” is a term of art that refers to a string of at least two base-sugar-phosphate monomeric units. Nucleotides are the monomeric units of nucleic acid polymers. The term includes deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the form of a messenger RNA, antisense, plasmid DNA, parts of a plasmid DNA or genetic material derived from a virus. An antisense nucleic acid is a polynucleotide that interferes with the expression of a DNA and/or RNA sequence. The term nucleic acids refers to a string of at least two base-sugar-phosphate combinations. Natural nucleic acids have a phosphate backbone. Artificial nucleic acids may contain other types of backbones, but contain the same bases as natural nucleic acids. The term also includes PNAs (peptide nucleic acids), phosphorothioates, and other variants of the phosphate backbone of native nucleic acids.

[0134] A "functional fragment" of a protein, polypeptide or nucleic acid is a protein, polypeptide or nucleic acid whose sequence is not identical to the full-length protein, polypeptide or nucleic acid, yet retains at least one function as the full-length protein, polypeptide or nucleic acid. A functional fragment can possess more, fewer, or the same number of residues as the corresponding native molecule, and/or can contain one or more amino acid or nucleotide substitutions. Methods for determining the function of a nucleic acid (e.g., coding function, ability to hybridize to another nucleic acid) are well-known in the art. Similarly, methods for determining protein function are well-known. For example, the DNA binding function of a polypeptide can be determined, for example, by filter-binding, electrophoretic mobility shift, or immunoprecipitation assays. DNA cleavage can be assayed by gel electrophoresis. The ability of a protein to interact with another protein can be determined, for example, by co-immunoprecipitation, two-hybrid assays or complementation, e.g., genetic or biochemical. See, for example, Fields et al. (1989) Nature 340:245-246; U.S. Patent No. 5,585,245 and PCT WO 98/44350.

[0135] As used herein, the term “linker” refers to a carbon chain that can contain heteroatoms (e.g., nitrogen, oxygen, sulfur, etc.) and which may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 atoms long.

Linkers may be substituted with various substituents including, but not limited to, hydrogen atoms, alkyl, alkenyl, alkynl, amino, alkylamino, dialkylamino, trialkylamino, hydroxyl, alkoxy, halogen, aryl, heterocyclic, aromatic heterocyclic, cyano, amide, carbamoyl, carboxylic acid, ester, thioether, alkylthioether, thiol, and ureido groups. Those of skill in the art will recognize that each of these groups may in turn be substituted. Examples of linkers include, but are not limited to, pH-sensitive linkers, protease cleavable peptide linkers, nuclease sensitive nucleic acid linkers, lipase sensitive lipid linkers, glycosidase sensitive carbohydrate linkers, hypoxia sensitive linkers, photo-cleavable linkers, heat-labile linkers, enzyme cleavable linkers (e.g., esterase cleavable linker), ultrasound-sensitive linkers, and x-ray cleavable linkers.

[0136] The term "pharmaceutically acceptable counter ion" refers to a pharmaceutically acceptable anion or cation. In various embodiments, the pharmaceutically acceptable counter ion is a pharmaceutically acceptable ion. For example, the pharmaceutically acceptable counter ion is selected from citrate, malate, acetate, oxalate, chloride, bromide, iodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)). In some embodiments, the pharmaceutically acceptable counter ion is selected from chloride, bromide, iodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, citrate, malate, acetate, oxalate, acetate, and lactate. In particular embodiments, the pharmaceutically acceptable counter ion is selected from chloride, bromide, iodide, nitrate, sulfate, bisulfate, and phosphate.

[0137] The term "pharmaceutically acceptable salt(s)" refers to salts of acidic or basic groups that may be present in compounds used in the present compositions. Compounds included in the present compositions that are basic in nature are capable of forming a variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions, including but not limited to sulfate, citrate,

malate, acetate, oxalate, chloride, bromide, iodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Compounds included in the present compositions that include an amino moiety may form pharmaceutically acceptable salts with various amino acids, in addition to the acids mentioned above. Compounds included in the present compositions, that are acidic in nature are capable of forming base salts with various pharmacologically acceptable cations. Examples of such salts include alkali metal or alkaline earth metal salts and, particularly, calcium, magnesium, sodium, lithium, zinc, potassium, and iron salts.

[0138] If the compounds described herein are obtained as an acid addition salt, the free base can be obtained by basifying a solution of the acid salt. Conversely, if the product is a free base, an addition salt, particularly a pharmaceutically acceptable addition salt, may be produced by dissolving the free base in a suitable organic solvent and treating the solution with an acid, in accordance with conventional procedures for preparing acid addition salts from base compounds. Those skilled in the art will recognize various synthetic methodologies that may be used to prepare non-toxic pharmaceutically acceptable addition salts.

[0139] A pharmaceutically acceptable salt can be derived from an acid selected from 1-hydroxy-2-naphthoic acid, 2,2-dichloroacetic acid, 2-hydroxyethanesulfonic acid, 2-oxoglutaric acid, 4-acetamidobenzoic acid, 4-aminosalicylic acid, acetic acid, adipic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, camphoric acid, camphor-10-sulfonic acid, capric acid (decanoic acid), caproic acid (hexanoic acid), caprylic acid (octanoic acid), carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, isethionic, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, mucic, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, nicotinic acid, nitric acid, oleic acid, oxalic acid,

palmitic acid, pamoic acid, pantothenic, phosphoric acid, proprionic acid, pyroglutamic acid, salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, tartaric acid, thiocyanic acid, toluenesulfonic acid, trifluoroacetic, and undecylenic acid.

[0140] The term "bioavailable" is art-recognized and refers to a form of the subject disclosure that allows for it, or a portion of the amount administered, to be absorbed by, incorporated to, or otherwise physiologically available to a subject or patient to whom it is administered.

[0141] It will be appreciated that the following examples are intended to illustrate but not to limit the present disclosure. Various other examples and modifications of the foregoing description and examples will be apparent to a person skilled in the art after reading the disclosure without departing from the spirit and scope of the disclosure, and it is intended that all such examples or modifications be included within the scope of the appended claims. All publications and patents referenced herein are hereby incorporated by reference in their entirety.

EXAMPLES

EXAMPLE 1: Synthesis, HPLC analysis and membrane permeation of the conjugates

[0142] Synthesis and HPLC analysis of the compounds described herein were carried out with methods disclosed in the Examples A, 1-7, and 14 of PCT Application No. PCT/US15/38569 (WO2016/004048) filed June 30, 2015, the contents of which are incorporated herein by reference.

EXAMPLE 2: Improved Formulation of Conjugate **57**

[0143] Conjugate **57** is a free flowing powder. Previously studies have found the stability of Conjugate **57** is dependent on the pH of the solution. After screening various buffers including citrate and phosphate buffers, acetate buffer was found to provide the most stability to Conjugate **57** at a pH range of 4.0 to 4.8.

[0144] Conjugate **57** was previously formated in the following vehicle: 10 mM acetate buffer with 5% mannitol and 2% solutol (Polyoxyl 15 Hydroxystearate, Kolliphor HS 15). However, a big pH change was observed between the pH of the

vehicle and the pH of the solution after Conjugate **57** was added. This is indicative of unsufficient bufering capacity of the vehicle and it may cause some difficulty in commercial scale manufacturing of Conjugate **57** compositions for clinical use. Therefore, there is a need to improve the buffering capacity of the Conjugate **57** formualtion.

[0145] In this study, the components of the vehicle buffer were tweaked to find an optimized vehicle buffering capacity. It was found acetate buffer concentrationis critical to minimize pH change and maintain buffering capacity. Acetate buffers with various concentrations were prepared and tested.

Experimental Procedure

[0146] Acetate buffers were prepared by mixing 100 mM solutions of acetic acid and sodium acetate trihydrate (100 mM stock buffer), followed by dilution of the stock buffer with water: a). Preparation of 100 mM sodium acetate trihydrate stock solution: sodium acetate trihydrate, 0.82048 g, was dissolved in 100 mL of WFI (Water for injections); b). Preparation of 100 mM Acetic acid stock solution: acetic acid, 0.6042 g. was dissolved in 100 mL of WFI; c). Preparation of 100 mM sodium acetate trihydrate stock solution; d). Mix 92 mL of 100 mM acetic acid stock solution and 8 mL of 100 mM sodium acetate trihydrate stock solution; and e). Various concentrations of the acetate buffer were prepared according the following dilution schedule:

Acetate buffer strength, mM	Volume of 100 mM buffer, mL	Volume of water added, mL	Dilution factor
10	5	45	10
20	10	40	5
30	15	35	3
40	20	30	2.5

[0147] Conjugate **57** was then added and dissolved. Solution pH was checked.

Results

[0148] As showin in the table below, 10mM or 20mM of acetate buffer could not achieve a minimal pH charge. Suprisingly, 30mM of acetate buffer provided a much smaller pH change after the addition of Conjugate **57** to the vehicle. 40mM of acetate buffer worked even better to privde minimal changes in pH after the addition of Conjugate **57** to the vehicle.

Table 1. pH of Vehicle and pH after Conjugate **57** addition

Vehicle	pH of Vehicle	pH after Conjugate **57** Addition	pH Difference
10 mM buffer with 5% mannitol & 2% solutol	3.828	4.354	0.526
20 mM buffer with 5% mannitol & 2% solutol	3.755	4.159	0.404
30 mM buffer with 5% mannitol & 2% solutol	4.046	4.21	0.164
40 mM buffer with 5% mannitol & 2% solutol	4.053	4.132	0.079

[0149] The higher concentration buffers provided minimal pH change, indicating that they can provide better buffering capacity and better pH control for Conjugate **57** formulation.

[0150] Two additional experiments were carried out with adjustments of the 30mM and 40mM buffered vehicles. When the starting pH of these vehicles were adjusted to 4.2, the addition of Conjugate **57** resulted in a small change in pH and a final pH in the desired range.

Table 2. pH of Vehicle and pH after Conjugate **57** addition

Vehicle	pH of Vehicle	pH after Conjugate **57** Addition	pH Difference
30 mM buffer with 5% mannitol & 2% solutol	4.221	4.301	0.081
40 mM buffer with 5% mannitol & 2% solutol	4.199	4.376	0.081

EXAMPLE 3: Optimizing Dosing Schedule of Conjugate **57**

[0151] In previous clincial studies, Conjugate **57** was administered via IV on an every 3 week cycle (3-week on followed by 1-week off). The dose of Conjugate **57** may be 1.0 mg, 2.0 mg, 4.0 mg, 8 mg, 12 mg, 15 mg or MTD, which has been determined to be18 mg. However, from the data collected from clinical studies, it was found that Conjugate **57** exposure correlateds with body surface area (BSA). BSA (meters squared), as used herein, can be calculated as the following: the square root of patient height in centimeters times patient weight in kilograms divided by 3600. As shown in Fig. 1A and Fig. 1B, a trend was evident between dose normalized exposure ($AUC_{0\text{-}8}$ and C_{max}) vs BSA for Conjugate **57**. Further, although progressive disease (PD) was observed equally across all does levels, maximum benefit was observed at

dose levels at 8.8 mg/m^2 or less. The majority of toxicity observed was at dose levels greater than 8.8 mg/m^2. Based on these unexpected results, that starting dose of Conjuate **57** for patients was changed from 15 mg to 8.8 mg/m^2 or less than 8.8 mg/m^2. Patients received dose levels such as 8.8 mg/m^2, 8.75 mg/m^2, 8.6 mg/m^2, 8.4 mg/m^2, 8.3 mg/m^2, 7.9 mg/m^2, 7.5 mg/m^2, and 6.7 mg/m^2.

[0152] There was no negative effect on efficacy when the dose levels were changed from flat doses to doses based on body surface area. The risk of over exposure of high Cmax levels in smaller pateints (<1.6m^2) was reduced. The risk of early discontinuation due to intolerability was reduced and the duration of theray was increased.

[0153] The scope of the present disclosure is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[0154] 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 disclosure 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 disclosure 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.

[0155] It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of" is thus also encompassed and disclosed.

[0156] Where ranges are given, endpoints are included. Furthermore, it is to be understood that 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 subrange within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[0157] In addition, it is to be understood that any particular embodiment of the present disclosure that falls within the prior art may be explicitly excluded from any one or more of the claims. Since 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 compositions of the disclosure can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[0158] All cited sources, for example, references, publications, databases, database entries, and art cited herein, are incorporated into this application by reference, even if not expressly stated in the citation. In case of conflicting statements of a cited source and the instant application, the statement in the instant application shall control.

[0159] Section and table headings are not intended to be limiting.