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(54) Title: PENICILLAMINE CONJUGATES AND PARTICLES AND FORMULATIONS THEREOF

(57) Abstract: Conjugates of an active agent attached to a targeting moiety via a penicillamine linker, and particles comprising such conjugates have been designed. Such conjugates and particles can provide increased stability and improved temporospatial delivery of the active agent, improved biodistribution and penetration in tumor, and/or decreased toxicity. Methods of making the conjugates, the particles, and the formulations thereof are provided. Methods of administering the formulations to a subject in need thereof are provided, for example, to treat or prevent cancer.

PENICILLAMINE CONJUGATES AND PARTICLES AND FORMULATIONS THEREOF

REFERENCED TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application No. 62/343,445, filed May 31, 2016, the contents of which are herein incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The invention generally relates to the field of targeting ligands, conjugates thereof, and particles for drug delivery. More particularly, the invention relates to the use conjugates comprising penicillamine linkers.

BACKGROUND OF THE INVENTION

[0003] Developments in nanomedicine are generally directed towards improving the pharmaceutical properties of the drugs and, in some cases, enhancing the targeted delivery in a more cell-specific manner. Several cell-specific drugs have been described, and include monoclonal antibodies, aptamers, peptides, and small molecules. Despite some of the potential advantages of such drugs, a number of problems have limited their clinical application, including size, stability, manufacturing cost, immunogenicity, poor pharmacokinetics and other factors. Nanoparticulate drug delivery systems are attractive for systemic drug delivery because they may be able to prolong the half-life of a drug in circulation, reduce non-specific uptake of a drug, and improve accumulation of a drug at tumors, e.g., through an enhanced permeation and retention (EPR) effect. There are limited examples of therapeutics formulated for delivery as nanoparticles, which include DOXIL® (liposomal encapsulated doxyrubicin) and ABRAXANE® (albumin bound paclitaxel nanoparticles).

[0004] 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 that 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 **Cmax**. Accordingly, there is a need in the art for improved drug targeting and delivery and to design drugs with deeper solid tumor penetration. Further, there is a need for improving stability of the drugs.

SUMMARY OF THE INVENTION

[0005] Applicants have created molecules that are conjugates of a targeting moiety attached to an active agent, e.g., a cancer therapeutic agent with a linker, wherein the linker comprises a penicillamine group or derivative thereof. Linkers comprising a penicillamine group or derivative are hereinafter referred to as penicillamine linkers. Furthermore, such conjugates can be encapsulated into particles. The conjugates and particles are useful for delivering active agents such as tumor cytotoxic agents to cells.

[0006] The conjugates include a targeting ligand and an active agent connected by a linker, wherein the conjugate in some embodiments has the formula:

(X—Y—Z)

wherein X is a targeting moiety; Y is a penicillamine linker; and Z is an active agent.

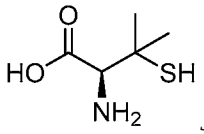
[0007] In one aspect of the invention, a method of reducing proliferation, increasing apoptosis, or increasing arrest of cells is provided. The method comprises administering a conjugate to the cells, wherein the conjugate comprises an active agent coupled to a targeting moiety by a penicillamine linker.

[0008] In another aspect of the invention, a method of treating a tumor, reducing volume of a tumor or delivering an active agent to a tumor in a subject is provided. The method comprises administering a conjugate to the subject, wherein the conjugate comprises an active agent coupled to a targeting moiety by a penicillamine linker.

[0009] In yet another aspect of the invention, a method of treating neuroendocrine cancers is provided, wherein the neuroendocrine cancer is selected from small cell lung cancer (SCLC), pheochromocytoma, neuroblastoma, ganglioneuroma, paraganglioma, carcinoids, gastrinoma, glucagonoma, vasoactive intestinal polypeptide-secreting tumor, pancreatic polypeptide-secreting tumor, nonfunctioning gastroenteropancreatic tumors, meduallary thyroid cancer, Merkel cell tumor of the skin, pituitary adenoma, and pancreatic cancer. The method comprises administering a conjugate to the cells, wherein the conjugate comprises an active agent coupled to a targeting moiety by a penicillamine linker.

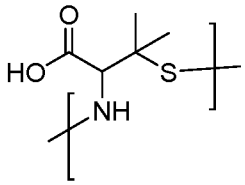
DETAILED DESCRIPTION OF THE INVENTION

[0010] Applicants have designed conjugates comprising a targeting moiety attached to an active agent with a penicillamine linker to deliver the active agent to a disease tissue target. Penicillamine is an a-amino acid metabolite of penicillin and has no antibiotic properties. Penicillamine as used herein may be *JJ*-penicillamine shown below, or *J* -penicillamine. In WO 2007/022493, the contents of which are incoroporated herein by reference in their entirety, Leamon et al. designed a compound comprising folate connected to vinca alkaloid with penicillamine.

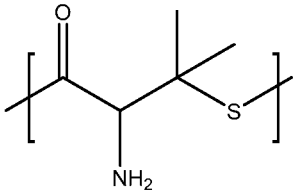


JJ-penicillamine

[0011] The active agent may be attached through a disulfide bond incorporating the sulfur atom on the penicillamine linker. The targeting moiety may be attached to the N-terminus or C-terminus of the penicillamine linker, or the penicillamine residue may be part of the targeting ligand. Some of the non-limiting exmaples are shown below:

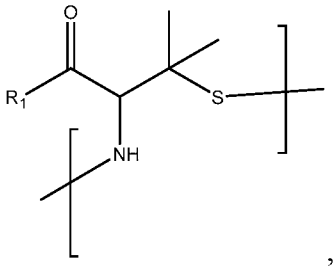
a conjugate (A) comprising 

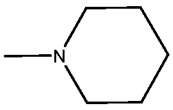
group; or

a conjugate (B) comprising 

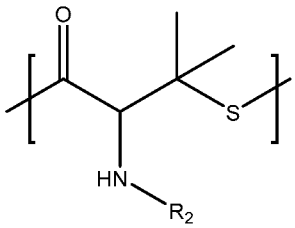
group.

[0012] The penicillamine linker may be substituted at any position. For example, the -OH group of conjugate (A) may be substituted with group R1:



, wherein R1 may be any suitable chemical group, such as -NH2 or substituted -NH2, an alkyl group (e.g., **-CH3** group) or a substituted alkyl group (e.g., an alkoxy group), a cyclic group or a substituted cyclic group, a heterocyclic group (e.g. a piperidine group 

) or a substituted heterocyclic group. The -NH2 group of conjugate (B) may be substituted with group R2:



, wherein R2 may be any suitable chemical group, such as an alkyl group (e.g., **-CH3** group) or a substituted alkyl group (e.g., an oxyalkyl group), a cyclic group or a substituted cyclic group, a heterocyclic group or a substituted heterocyclic group.

[0013] Linking the active agent through a disulfide bond formed by the penicillamine residue can result in compounds with greater stability than corresponding conjugates linked through cysteine residues, as the gem-dimethyl group adjacent to the sulfur stabilizes the disulfide bond.

[0014] Use of penicillamine as an agent to create hindered disulfide bonds can yield advantages over other reagents used to create hindered disulfide bonds such as N-succinimidyl 4-methyl-4-(2-pyridyldithio)pentanoate (SMPP) (see Kellogg, et. al., Bioconj. Chem., 22:717-727, 2011), used to conjugate a hindered disulfide bond at lysine residues. The penicillamine residue can be incorporated in a peptide sequence, either at the N-terminus, C-terminus, or within the targeting sequence as necessary.

[0015] Including a targeting moiety in the conjugates 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.

[0016] 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 or thrombopenia may also be metrics of toxicity.

[0017] Pharmacologic indicators of toxicity include elevated AST/ALT levels, neurotoxicity, kidney damage, GI damage and the like.

[0018] In addition, the toxicity of a conjugate containing a targeting moiety linked to an active agent for cells that do not bind to the targeting moiety is predicted to be decreased compared to the toxicity of the active agent alone. Without committing to any particular theory, applicants believe that this feature is because the ability of the conjugated active agent to enter a cell is decreased compared the ability to enter a cell of the active agent alone. Accordingly, the conjugates comprising an active agent and particles containing the conjugates as described herein generally have decreased toxicity for cells that do not bind to the targeting moiety and at least the same or increased toxicity for cells that bind to the targeting moiety compared to the active agent alone.

[0019] It is an object of the invention to provide improved compounds, such as increased stability, compositions, and formulations for temporospatial drug delivery.

[0020] It is further an object of the invention to provide methods of making improved compounds, compositions, and formulations for temporospatial drug delivery.

[0021] It is also an object of the invention to provide methods of administering the improved compounds, compositions, and formulations to individuals in need thereof.

I. Conjugates

[0022] Conjugates include an active agent or prodrug thereof attached to a targeting moiety by a penicillamine linker. The conjugates can be a conjugate between a single active agent and a single targeting moiety, e.g., a conjugate having

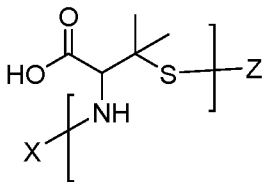
the structure X-Y-Z, where X is the targeting moiety, Y is the penicillamine linker, and Z is the active agent.

[0023] In some embodiments, the conjugate contains more than one targeting moiety, more than one linker, more than one active agent, or any combination thereof, wherein at least one linker is a penicillamine linker. The conjugate can have any number of targeting moieties, linkers, and active agents, wherein at least one linker is a penicillamine linker. The conjugate can have the structure X-Y-Z-Y-X, (X-Y)n-Z, X-(Y-Z)n, X-Y-Zn, (X-Y-Z)n, (X-Y-Z-Y)n-Z, Xn-Y-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, wherein at least one linker is a penicillamine linker. 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, wherein at least one linker is a penicillamine linker.

[0024] 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, wherein at least one linker is a penicillamine linker.

[0025] 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, wherein at least one linker is a penicillamine 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, wherein at least one linker is a penicillamine linker.

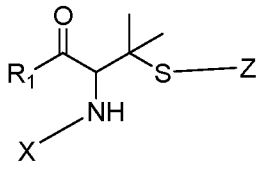
[0026] In some embodiments, the conjugate has a structure of A1:



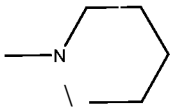
A1,

wherein X is a targeting moiety and Z is an active agent. The linker moiety of A1 may

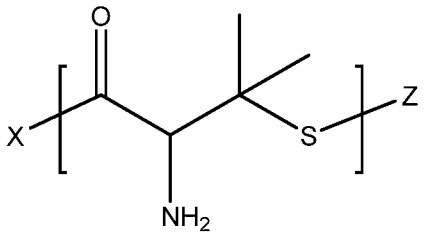
be substituted with any chemical group. For example, the conjugate may have a structure of A2:



A2,

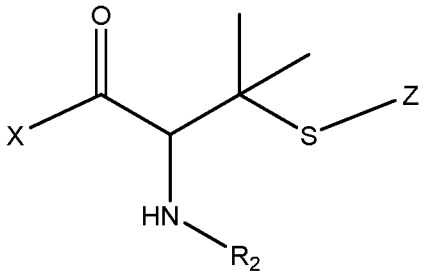
wherein X is a targeting moiety, Z is an active agent, and R1 may be any suitable chemical group, such as $-NH_2$ or substituted $-NH_2$, an alkyl group (e.g., $-CH_3$ group) or a substituted alkyl group (e.g., an alkoxy group), a cyclic group or a substituted cyclic group, a heterocyclic group (e.g. a piperidine group ) or a substituted heterocyclic group.

[0027] In some embodiments, the conjugate has a structure of B1:



B1,

wherein X is a targeting moiety and Z is an active agent. The linker moiety of B1 may be substituted with any chemical group. For example, the conjugate may have a structure of B2:



B2,

wherein X is a targeting moiety, Z is an active agent, and R2 may be any suitable chemical group, such as an alkyl group (e.g., $-CH_3$ group) or a substituted alkyl group (e.g., an oxyalkyl group), a cyclic group or a substituted cyclic group, a heterocyclic group or a substituted heterocyclic group.

A. Active Agents

[0028] A conjugate as described herein contains at least one active agent (a first active agent). The conjugate can contain more than one active agent, that can be the same or different from the first active agent. The active agent can be a therapeutic, prophylactic, diagnostic, or nutritional agent. A variety of active agents are known in the art and may be used in the conjugates described herein. The active agent can be a protein or peptide, small molecule, nucleic acid or nucleic acid molecule, lipid, sugar, glycolipid, glycoprotein, lipoprotein, or combination thereof. In some embodiments, the active agent is an antigen, an adjuvant, radioactive, an imaging agent (e.g., a fluorescent moiety) or a polynucleotide. In some embodiments, the active agent is an organometallic compound. In some embodiments, the active agent is selected from a maytansinoid or derivative such as mertansine (DM1) or DM4, cabazitaxel, SN-38, or doxorubicin.

Anti-cancer agents

[0029] In some embodiments, the active agent can be a cancer therapeutic. Cancer therapeutics include, for example, death receptor agonists such as the TNF-related apoptosis-inducing ligand (TRAIL) or Fas ligand or any ligand or antibody that binds or activates a death receptor or otherwise induces apoptosis. Suitable death receptors include, but are not limited to, TNFR1, Fas, DR3, DR4, DR5, DR6, LTpR and combinations thereof.

[0030] Cancer therapeutics such as chemotherapeutic agents, cytokines, chemokines, and radiation therapy agents can be used as active agents. Chemotherapeutic agents include, for example, alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumor agents. Such agents typically affect cell division or DNA synthesis and function. Additional examples of therapeutics that can be used as active agents include monoclonal antibodies and the tyrosine kinase inhibitors e.g. imatinib mesylate, which directly targets a molecular abnormality in certain types of cancer (e.g., chronic myelogenous leukemia, gastrointestinal stromal tumors).

[0031] Chemotherapeutic agents include, but are not limited to cisplatin, carboplatin, oxaliplatin, mechlorethamine, cyclophosphamide, chlorambucil, vincristine, vinblastine, vinorelbine, vindesine, taxol and derivatives thereof, irinotecan, topotecan, amsacrine, etoposide, etoposide phosphate, teniposide,

epipodophyllotoxins, trastuzumab, cetuximab, and rituximab, bevacizumab, and combinations thereof. Any of these may be used as an active agent in a conjugate.

[0032] In certain embodiments, the active agent is a small molecule having a molecular weight preferably < about 5 kDa, more preferably < about 4 kDa, more preferably about 3 kDa, most preferably < about 1.5 kDa or < about 1 kDa.

[0033] The small molecule active agents used in this invention (e.g. antiproliferative (cytotoxic and cytostatic) agents) include cytotoxic compounds (e.g., broad spectrum), angiogenesis inhibitors, cell cycle progression inhibitors, PBK/m-TOR/AKT pathway inhibitors, MAPK signaling pathway inhibitors, kinase inhibitors, protein chaperones inhibitors, HDAC inhibitors, PARP inhibitors, Wnt/Hedgehog signaling pathway inhibitors, RNA polymerase inhibitors and proteasome inhibitors. The small molecule active agents in some embodiments the active agent is an analog, derivative, prodrug, or pharmaceutically acceptable salt thereof.

[0034] Broad spectrum cytotoxins include, but are not limited to, DNA-binding or alkylating drugs, microtubule stabilizing and destabilizing agents, platinum compounds, and topoisomerase I or II inhibitors.

[0035] Exemplary DNA-binding or alkylating drugs include, CC-1065 and its analogs, anthracyclines (doxorubicin, epirubicin, idarubicin, daunorubicin) and its analogs, alkylating agents, such as calicheamicins, dactinomycins, mitomycins, pyrrolobenzodiazepines, trioxacarcins and the like. Non-limiting examples of trioxacarcins include Trioxacarcins DC-45-A2, DC-45-A1, A, D, C7"-epi-C, and C disclosed in Nicolaou et al., *JACS*, vol. 138:31 18 (2016), and trioxacarcin A, DC-45-A1 and structural analogues disclosed in Fig. 1 of Magauer et al., *Nature Chemistry*, vol. 5:886 (2013), the contents of each of which are incorporated herein by reference in their entirety.

[0036] Exemplary doxorubicin analogs include nemorubicin metabolite or analog drug moiety disclosed in US 20140227299 to Cohen et al., the contents of which are incorporated herein by reference in their entirety.

[0037] Exemplary CC-1065 analogs include duocarmycin SA, duocarmycin CI, duocarmycin C2, duocarmycin B2, DU-86, KW-2189, bizelesin, seco-adozelesin, and those described in U.S. Patent Nos. 5,475,092; 5,595,499; 5,846,545; 6,534,660; 6,586,618; 6,756,397 and 7,049,316. Doxorubicin and its analogs include PNU-159682 and those described in U.S. Patent No. 6,630,579 and nemorubicin metabolite

or analog drugs disclosed in US 20140227299 to Cohen et al., the contents of which are incorporated herein by reference in their entirety.

[0038] Calicheamicins include those described in U.S. Patent Nos. 5,714,586 and 5,739,1 16. Duocarmycins include those described in U.S. Patent Nos. 5,070,092; 5,101,038; 5,187,186; 6,548,530; 6,660,742; and 7,553,816 B2; and Li et al., Tet Letts., 50:2932 - 2935 (2009). Pyrrolobenzodiazepines include SG2057 and those described in Denny, Exp. Opin. Ther. Patents., 10(4):459-474 (2000), Anti-Cancer Agents in Medicinal Chemistry, 2009, 9, 1-31; WO 201 1/130613 Al; EP 2 789 622 Al; Blood 2013, 122, 1455; J. Antimicrob. Chemother. 2012, 67, 1683-1696; Cancer Res. 2004, 64, 6693-6699; WO 2013041606; US 8481042; WO 2013177481; WO 201 1130613; WO201 1130598, Angew. Chem. Int. Ed. 2017, 56, 462 - 488, the contents of each of which are incorporated herein by reference in their entirety.

[0039] Exemplary microtubule stabilizing and destabilizing agents include taxane compounds, such as paclitaxel, docetaxel, cabazitaxel; maytansinoids, auristatins and analogs thereof, tubulysin A and B derivatives, vinca alkaloid derivatives, epothilones, PM060184 and cryptophycins.

[0040] Exemplary maytansinoids or maytansinoid analogs include maytansinol and maytansinol analogs, maytansine or DM1 and DM4 are those described in U.S. Patent Nos. 5,208,020; 5,416,064; 6,333.410; 6,441,163; 6,716,821; RE39,151 and 7,276,497. In certain embodiments, the cytotoxic agent is a maytansinoid, another group of anti-tubulin agents (ImmunoGen, Inc.; see also Chari et al., 1992, Cancer Res. 52: 127-131), maytansinoids or maytansinoid analogs. Examples of suitable maytansinoids include maytansinol and maytansinol analogs. Suitable maytansinoids are disclosed in U.S. Patent Nos. 4,424,219; 4,256,746; 4,294,757; 4,307,016; 4,313,946; 4,315,929; 4,331,598; 4,361,650; 4,362,663; 4,364,866; 4,450,254; 4,322,348; 4,371,533; 6,333,410; 5,475,092; 5,585,499; and 5,846,545.

[0041] Exemplary auristatins include auristatin E (also known as a derivative of dolastatin-10), auristatin EB (AEB), auristatin EFP (AEFP), monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), auristatin F and dolastatin. Suitable auristatins are also described in U.S. Publication Nos. 2003/0083263, 201 1/0020343, and 201 1/0070248; PCT Application Publication Nos. WO 09/1 1753 1, WO 2005/08171 1, WO 04/010957; WO02/088172 and WO01/24763, and U.S. Patent Nos. 7,498,298; 6,884,869; 6,323,315; 6,239,104; 6,124,431; 6,034,065; 5,780,588; 5,767,237; 5,665,860; 5,663,149; 5,635,483; 5,599,902;5,554,725; 5,530,097;

5,521,284; 5,504,191; 5,410,024; 5,138,036; 5,076,973; 4,986,988; 4,978,744; 4,879,278; 4,816,444; and 4,486,414, the disclosures of which are incorporated herein by reference in their entirety.

[0042] Exemplary tubulysin compounds include compounds described in U.S. Patent Nos. 7,816,377; 7,776,814; 7,754,885; U.S. Publication Nos. 201 1/0021568; 2010/004784; 2010/0048490; 2010/00240701; 2008/0176958; and PCT Application Nos. WO 98/13375; WO 2004/005269; WO 2008/138561; WO 2009/002993; WO 2009/055562; WO 2009/012958; WO 2009/026177; WO 2009/134279; WO 2010/033733; WO 2010/034724; WO 201 1/017249; WO 201 1/057805; the disclosures of which are incorporated by reference herein in their entirety.

[0043] Exemplary vinca alkaloids include vincristine, vinblastine, vindesine, and navelbine (vinorelbine). Suitable Vinca alkaloids that can be used in the present invention are also disclosed in U.S. Publication Nos. 2002/0103136 and 2010/0305149, and in U.S. Patent No. 7,303,749 Bl, the disclosures of which are incorporated herein by reference in their entirety.

[0044] Exemplary epothilone compounds include epothilone A, B, C, D, E and F, and derivatives thereof. Suitable epothilone compounds and derivatives thereof are described, for example, in U.S. Patent Nos. 6,956,036; 6,989,450; 6,121,029; 6,1 17,659; 6,096,757; 6,043,372; 5,969,145; and 5,886,026; and WO 97/19086; WO 98/08849; WO 98/22461; WO 98/25929; WO 98/38192; WO 99/01 124; WO 99/02514; WO 99/03848; WO 99/07692; WO 99/27890; and WO 99/28324; the disclosures of which are incorporated herein by reference in their entirety.

[0045] Exemplary cryptophycin compounds are described in U.S. Patent Nos. 6,680,3 11 and 6,747,021, the disclosures of which are incorporated herein by reference in their entirety.

[0046] Exemplary platinum compounds include cisplatin (PLATINOL®), carboplatin (PARAPLATIN®), oxaliplatin (ELOX ATINE®), iproplatin, ormaplatin, and tetraplatin.

[0047] Exemplary topoisomerase I inhibitors include camptothecin, camptothecin, derivatives, camptothecin analogs and non-natural camptothecins, such as, for example, CPT-1 1 (irinotecan), SN-38, topotecan, 9-aminocamptothecin, rubitecan, gimatecan, karenitecin, silatecan, lurtotecan, exatecan, diflomotecan, belotecan, lurtotecan and S39625. Other camptothecin compounds that can be used in the present

invention include those described in, for example, J. Med. Chem., 29:2358-2363 (1986); J. Med. Chem., 23:554 (1980); J. Med. Chem., 30: 1774 (1987).

[0048] Exemplary topoisomerase II inhibitors include azonafide and etoposide.

[0049] Additional agents acting on DNA include Lurbinectedin (PM0 1183), Trabectedin (also known as ecteinascidin 743 or ET-743) and analogs as described in WO 20010771 1, WO 2003014127.

[0050] Angiogenesis inhibitors include, but are not limited to, MetAP2 inhibitors.

[0051] Exemplary MetAP2 inhibitors include fumagillol analogs, meaning any compound that includes the fumagillin core structure, including fumagillamine, that inhibits the ability of MetAP-2 to remove NFh-terminal methionines from proteins as described in Rodeschini et al., /. Org. Chem., 69, 357-373, 2004 and Liu, et al., Science 282, 1324-1327, 1998. Non-limiting examples of "fumagillol analogs" are disclosed in /. Org. Chem., 69, 357, 2004; J.Org. Chem., 70, 6870, 2005; European Patent Application 0 354 787; /. Med. Chem., 49, 5645, 2006; Bioorg. Med. Chem., 11, 5051, 2003; Bioorg. Med. Chem., 14, 91, 2004; Tet. Lett. 40, 4797, 1999; W099/61432; U.S. Patent Nos. 6,603,812; 5,789,405; 5,767,293; 6,566,541; and 6,207,704.

[0052] Exemplary cell cycle progression inhibitors include CDK inhibitors such as BMS-387032 and PD0332991; Rho-kinase inhibitors such as GSK429286; checkpoint kinase inhibitors such as AZD7762; aurora kinase inhibitors such as AZD1 152, MLN8054 and MLN8237; PLK inhibitors such as BI 2536, BI6727 (Volasertib), GSK461364, ON-01910 (Estybon); and KSP inhibitors such as SB 743921, SB 715992 (ispinesib), MK-0731, AZD8477, AZ3146 and ARRY-520.

[0053] Exemplary PI3K/m-TOR/AKT signaling pathway inhibitors include phosphoinositide 3-kinase (PI3K) inhibitors, GSK-3 inhibitors, ATM inhibitors, DNA-PK inhibitors and PDK-1 inhibitors.

[0054] Exemplary PI3 kinase inhibitors are disclosed in U.S. Patent No. 6,608,053, and include BEZ235, BGT226, BKM120, CALIOI, CAL263, demethoxyviridin, GDC-0941, GSK615, IC871 14, LY294002, Palomid 529, perifosine, PF-04691502, PX-866, SAR245408, SAR245409, SF1 126, Wortmannin, XL147, XL765, GSK2126458 (Omipalisib), GDC-0326, GDC-0032 (Taselisib, RG7604), PF-05212384 (Gedatolisib, PKI-587), BAY 80-6946 (copanlisib), PF-04691502, PF-04989216, PI-103, PKI-402 VS-5584 (SB2343), GDC-0941, NVP-BEZ235 (Dactoslisib), BGT226, NVP-BKM120 (Buparlisib), NVP-BYL719 (alpelisib),

GSK2636771, AMG-319, GSK2269557, PQR309, PWT143, TGR-1202 (RP5264), PX-866, GDC-0980 (apitolisib), AZD8835, MLN1 117, DS-7423, ZSTK474, CUDC-907, IPI-145 (INK-1 197, Duvelisib), AZD8186, XL147 (SAR245408), XL765 (SAR245409), CAL-101 (Idelalisib, GS-1 101), GS-9820 (Acalisib) and KA2237.

[0055] Exemplary AKT inhibitors include, but are not limited to, AT7867, MK-2206, Perifosine, GSK690693, Ipatasertib, AZD5363, TIC10, Afuresertib, SC79, AT13148, PHT-427, A-674563, and CCT128930.

[0056] Exemplary MAPK signaling pathway inhibitors include MEK, Ras, JNK, B-Raf and p38 MAPK inhibitors.

[0057] Exemplary MEK inhibitors are disclosed in U.S. Patent No. 7,517,994 and include GDC-0973, GSK1 120212, MSC1936369B, AS703026, R05 126766 and R04987655, PD0325901, AZD6244, AZD 8330 and GDC-0973.

[0058] Exemplary B-raf inhibitors include CDC-0879, PLX-4032, and SB590885.

[0059] Exemplary B p38 MAPK inhibitors include BIRB 796, LY2228820 and SB202190

[0060] Receptor tyrosine kinases (RTK) are cell surface receptors which are often associated with signaling pathways stimulating uncontrolled proliferation of cancer cells and neoangiogenesis. Many RTKs, which over express or have mutations leading to constitutive activation of the receptor, have been identified, including, but not limited to, VEGFR, EGFR, FGFR, PDGFR, EphR and RET receptor family receptors. Exemplary RTK specific targets include ErbB2, FLT-3, c-Kit, c-Met, and HIF.

[0061] Exemplary inhibitors of ErbB2 receptor (EGFR family) include but not limited to AEE788 (NVP-AEE 788), BIBW2992 (Afatinib), Lapatinib, Erlotinib (Tarceva), and Gefitinib (Iressa).

[0062] Exemplary RTK inhibitors targeting more than one signaling pathway (multitargeted kinase inhibitors) include AP24534 (Ponatinib) that targets FGFR, FLT-3, VEGFR-PDGFR and Bcr-Abl receptors; ABT-869 (Linifanib) that targets FLT-3 and VEGFR- PDGFR receptors; AZD2171 that targets VEGFR-PDGFR, Flt-1 and VEGF receptors; CHR-258 (Dovitinib) that targets VEGFR-PDGFR, FGFR, Flt-3, and c-Kit receptors.

[0063] Exemplary kinase inhibitors include inhibitors of the kinases ATM, ATR, CHK1, CHK2, WEE1, and RSK.

[0064] Exemplary protein chaperone inhibitors include HSP90 inhibitors. Exemplary HSP90 inhibitors include Ganetespib, 17AAG derivatives, BIIB021, BIIB028, SNX-5422, NVP-AUY-922, and KW-2478.

[0065] Exemplary HDAC inhibitors include Belinostat (PXD101), CUDC-101, Doxinostat, ITF2357 (Givinostat, Gavinostat), JNJ-26481585, LAQ824 (NVP-LAQ824, Dacinostat), LBH-589 (Panobinostat), MC1568, MGCD0103 (Mocetinostat), MS-275 (Entinostat), PCI-24781, Pyroxamide (NSC 696085), SB939, Trichostatin A, and Vorinostat (SAHA).

[0066] Exemplary PARP inhibitors include neratinib (HKI-272), iniparib (BSI 201), olaparib (AZD-2281), ABT-888 (Veliparib), rucaparib (AG014699, CEP 9722, niraparib (MK-4827), KU-0059436 (AZD2281), talazoparib (BMN-673), 3-aminobenzamide, A-966492, E7016, BGB-290 and AZD2461

[0067] Exemplary Wnt/Hedgehog signaling pathway inhibitors include vismodegib (RG3616/GDC-0449), cyclopamine (11-deoxojervine) (Hedgehog pathway inhibitors), and XAV-939 (Wnt pathway inhibitor).

[0068] Exemplary RNA polymerase inhibitors include amatoxins. Exemplary amatoxins include a- amanitins, β- amanitins, γ- amanitins, ε-amanitins, amanullin, amanullic acid, amaninamide, amanin, and proamanullin. Other amanitin compounds that can be used in the present invention include those described in, for example WO2014135282, WO20 16 142049, and EP2872479 the contents of each of which are incorporated herein by reference in their entirety.

[0069] Exemplary proteasome inhibitors include bortezomib, carfilzomib, ONX 0912, CEP- 18770, and MLN9708.

[0070] In one embodiment, the drug of the invention is a non-natural camptothecin compound, vinca alkaloid, kinase inhibitor (e.g., PI3 kinase inhibitor (GDC-0941 and PI- 103)), MEK inhibitor, KSP inhibitor, RNA polymerse inhibitor, PARP inhibitor, docetaxel, paclitaxel, doxorubicin, duocarmycin, tubulysin, auristatin or a platinum compound. In specific embodiments, the drug is a derivative of SN-38, vindesine, vinblastine, PI- 103, AZD 8330, auristatin E, auristatin F, a duocarmycin compound, tubulysin compound, or ARRY-520.

[0071] In another embodiment, the drug used in the invention is a combination of two or more drugs, such as, for example, PI3 kinases and MEK inhibitors; broad spectrum cytotoxic compounds and platinum compounds; PARP inhibitors and platinum compounds; broad spectrum cytotoxic compounds and PARP inhibitors.

[0072] The active agent can be a cancer therapeutic. The cancer therapeutics may include death receptor agonists such as the TNF-related apoptosis-inducing ligand (TRAIL) or Fas ligand or any ligand or antibody that binds or activates a death receptor or otherwise induces apoptosis. Suitable death receptors include, but are not limited to, TNFRl, Fas, DR3, DR4, DR5, DR6, LTpR and combinations thereof.

[0073] The active agent can be a DNA minor groove binders such as lurbectidin and trabectidin.

[0074] The active agent can be E3 ubiquitin ligase inhibitors, adeubiquitinase inhibitors or an NFkB pathway inhibitor.

[0075] The active agent can be a phopsphatase inhibitors including inhibitors of PTP1B, SHP2, LYP, FAP-1, CD45, STEP, MKP-1, PRL, LMWPTP or CDC25.

[0076] The active agent can be an inhibitor of tumor metabolism, such as an inhibitor of GAPDH, GLUT1, HK II, PFK, GAPDH, PK, LDH orMCTs

[0077] The active agent can target epigenetic targets including EZH2, MIX, DOT 1-like protein (DOT1L), bromodomain-containing protein 4 (BRD4), BRD2, BRD3, NUT, ATAD2, or SMYD2.

[0078] The active agent can target the body's immune system to help fight cancer, including moecules targeting IDOl, ID02, TDO, CD39, CD73, A2A antagonists, STING activators, TLR agonists (TLR 1-13), ALK5, CBP/EP300 bromodomain, ARGl, ARG2, iNOS, PDE5, P2X7, P2Y1 1, COX2, EP2 Receptor, or EP4 receptor.

[0079] The active agent can target Bcl-2, IAP, or fatty acid synthase.

[0080] In some embodiments, the active agent can be 20-epi-l,25 dihydroxyvitamin D3, 4-ipomeanol, 5-ethynyluracil, 9-dihydrotaxol, abiraterone, acivicin, aclarubicin, acodazole hydrochloride, acronine, acylfulvene, adecypenol, adozelesin, aldesleukin, all-tk antagonists, altretamine, ambamustine, ambomycin, ametantrone acetate, amidox, amifostine, aminoglutethimide, aminolevulinic acid, amrubicin, amsacrine, anagrelide, anastrozole, andrographolide, angiogenesis inhibitors, antagonist D, antagonist G, antarelix, anthramycin, anti-dorsalizing morphogenetic protein-1, antiestrogen, antineoplaston, antisense oligonucleotides, aphidicolin glycinate, apoptosis gene modulators, apoptosis regulators, apurinic acid, ARA-CDP-DL-PTBA, arginine deaminase, asparaginase, asperlin, asulacrine, atamestane, atrimustine, axinastatin 1, axinastatin 2, axinastatin 3, azacitidine, azasetron, azatoxin, azatyrosine, azetepa, azotomycin, baccatin III derivatives, balanol, batimastat, benzochlorins, benzodepa, benzoylstaurosporine, beta lactam

derivatives, beta-alethine, betaclamycin B, betulinic acid, BFGF inhibitor, bicalutamide, bisantrene, bisantrene hydrochloride, bisaziridinylspermine, bisnafide, bisnafide dimesylate, bistratene A, bizelesin, bleomycin, bleomycin sulfate, BRC/ABL antagonists, breflate, brequinar sodium, bropirimine, budotitane, busulfan, buthionine sulfoximine, cabazitaxel, cactinomycin, calcipotriol, calphostin C, calusterone, camptothecin, camptothecin derivatives, canarypox IL-2, capecitabine, caracemide, carbetimer, carboplatin, carboxamide-amino-triazole, carboxyamidotriazole, carest M3, carmustine, earn 700, cartilage derived inhibitor, carubicin hydrochloride, carzelesin, casein kinase inhibitors, castano spermine, cecropin B, cedefingol, cetrorelix, chlorambucil, chlorins, chloroquinoxaline sulfonamide, cicaprost, cirolemycin, cisplatin, cis-porphyrin, cladribine, clomifene analogs, clotrimazole, collismycin A, collismycin B, combretastatin A4, combretastatin analog, conagenin, crambescidin 816, crisnatol, crisnatol mesylate, cryptophycin 8, cryptophycin A derivatives, curacin A, cyclopentanthraquinones, cyclophosphamide, cycloplatam, cypemycin, cytarabine, cytarabine ocfosfate, cytolytic factor, cytostatin, dacarbazine, dacliximab, dactinomycin, daunorubicin hydrochloride, decitabine, dehydrodidemnin B, deslorelin, dexifosfamide, dexormaplatin, dexrazoxane, dexverapamil, dezaguanine, dezaguanine mesylate, diaziquone, didemnin B, didox, diethylnorspermine, dihydro-5-azacytidine, dioxamycin, diphenyl spiromustine, docetaxel, docosanol, dolasetron, doxifluridine, doxorubicin, doxorubicin hydrochloride, droloxifene, droloxifene citrate, dromostanolone propionate, dronabinol, duazomycin, duocarmycin SA, ebselen, ecomustine, edatrexate, edelfosine, edrecolomab, eflornithine, eflornithine hydrochloride, elemene, elsamitrucin, emitefur, enloplatin, enpromate, epipropidine, epirubicin, epirubicin hydrochloride, epristeride, erbulozole, erythrocyte gene therapy vector system, esorubicin hydrochloride, estramustine, estramustine analog, estramustine phosphate sodium, estrogen agonists, estrogen antagonists, etanidazole, etoposide, etoposide phosphate, etoprine, exemestane, fadrozole, fadrozole hydrochloride, fazarabine, fenretinide, filgrastim, finasteride, flavopiridol, flezelastine, floxuridine, fluasterone, fludarabine, fludarabine phosphate, fluorodaunorunicin hydrochloride, fluorouracil, flurocitabine, forfenimex, formestane, fosquidone, fostriecin, fostriecin sodium, fotemustine, gadolinium texaphyrin, gallium nitrate, galocitabine, ganirelix, gelatinase inhibitors, gemcitabine, gemcitabine hydrochloride, glutathione inhibitors, hepsulfam, heregulin, hexamethylene

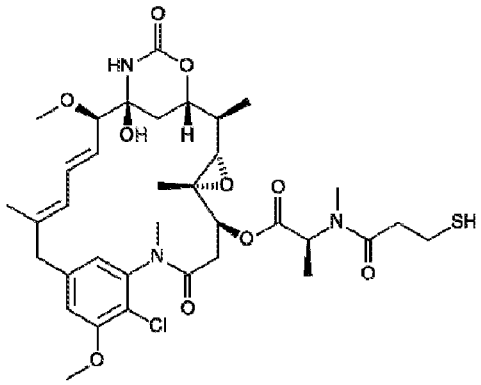
bisacetamide, hydroxyurea, hypericin, ibandronic acid, idarubicin, idarubicin hydrochloride, idoxifene, idramantone, ifosfamide, ilmofosine, ilomastat, imidazoacridones, imiquimod, immunostimulant peptides, insulin-like growth factor-1 receptor inhibitor, interferon agonists, interferon alpha-2A, interferon alpha-2B, interferon alpha-Nl, interferon alpha-N3, interferon beta-IA, interferon gamma-IB, interferons, interleukins, iobenguane, iododoxorubicin, iproplatin, irinotecan, irinotecan hydrochloride, iroplact, irsogladine, isobengazole, isohomohalicondrin B, itasetron, jasplakinolide, kahalalide F, lamellarin-N triacetate, lanreotide, larotaxel, lanreotide acetate, leinamycin, lenograstim, lentinan sulfate, leptolstatin, letrozole, leukemia inhibiting factor, leukocyte alpha interferon, leuprolide acetate, leuprolide/estrogen/progesterone, leuprorelin, levamisole, liarozole, liarozole hydrochloride, linear polyamine analog, lipophilic disaccharide peptide, lipophilic platinum compounds, lissoclinamide 7, lobaplatin, lombricine, lometrexol, lometrexol sodium, lomustine, lonidamine, losoxantrone, losoxantrone hydrochloride, lovastatin, loxoribine, lurtotecan, lutetium texaphyrin, lysofylline, lytic peptides, maitansine, mannostatin A, marimastat, masoprocol, maspin, matrilysin inhibitors, matrix metalloproteinase inhibitors, maytansine, maytansinoid, mertansine (DM1), mechlorethamine hydrochloride, megestrol acetate, melengestrol acetate, melphalan, menogaril, merbarone, mercaptopurine, meterelin, methioninase, methotrexate, methotrexate sodium, metoclopramide, metoprine, meturedepa, microalgal protein kinase C inhibitors, MIF inhibitor, mifepristone, miltefosine, mirimostim, mismatched double stranded RNA, mitindomide, mitocarcin, mitocromin, mitogillin, mitoguazone, mitolactol, mitomalcin, mitomycin, mitomycin analogs, mitonafide, mitosper, mitotane, mitotoxin fibroblast growth factor-saporin, mitoxantrone, mitoxantrone hydrochloride, mofarotene, molgramostim, monoclonal antibody, human chorionic gonadotrophin, monophosphoryl lipid a/myobacterium cell wall SK, mopidamol, multiple drug resistance gene inhibitor, multiple tumor suppressor 1-based therapy, mustard anticancer agent, mycaperoxide B, mycobacterial cell wall extract, mycophenolic acid, myriaporone, n-acetyldinaline, nafarelin, nagrestip, naloxone/pentazocine, napavin, naphterpin, nartograstim, nedaplatin, nemorubicin, neridronic acid, neutral endopeptidase, nilutamide, nisamycin, nitric oxide modulators, nitroxide antioxidant, nitrullyn, nocodazole, nogalamycin, n-substituted benzamides, 06-benzylguanine, octreotide, okicenone, oligonucleotides, onapristone, ondansetron, oracin, oral cytokine inducer, ormaplatin, osaterone, oxaliplatin,

oxaunomycin, oxisuran, paclitaxel, paclitaxel analogs, paclitaxel derivatives, palauamine, palmitoylrhizoxin, pamidronic acid, panaxytriol, panomifene, parabactin, pazelliptine, pegaspargase, peldesine, peliomycin, pentamustine, pentosan polysulfate sodium, pentostatin, pentrozole, peplomycin sulfate, perflubron, perfosfamide, perillyl alcohol, phenazinomycin, phenylacetate, phosphatase inhibitors, picibanil, pilocarpine hydrochloride, pipobroman, piposulfan, pirarubicin, piritrexim, piroxantrone hydrochloride, placetin A, placetin B, plasminogen activator inhibitor, platinum(IV) complexes, platinum compounds, platinum-triamine complex, plicamycin, plomestane, porfimer sodium, porfiromycin, prednimustine, procarbazine hydrochloride, propyl bis-acridone, prostaglandin J2, prostatic carcinoma antiandrogen, proteasome inhibitors, protein A-based immune modulator, protein kinase C inhibitor, protein tyrosine phosphatase inhibitors, purine nucleoside phosphorylase inhibitors, puromycin, puromycin hydrochloride, purpurins, pyrazofurin, pyrazoloacridine, pyridoxylated hemoglobin polyoxy ethylene conjugate, RAF antagonists, raltitrexed, ramosetron, RAS farnesyl protein transferase inhibitors, RAS inhibitors, RAS-GAP inhibitor, retelliptine demethylated, rhenium RE 186 etidronate, rhizoxin, riboprine, ribozymes, RII retinamide, RNAi, rogletimide, rohitukine, romurtide, roquinimex, rubiginone Bl, ruboxyl, safingol, safingol hydrochloride, saintopin, sarcnu, sarcophytol A, sargramostim, SDI 1 mimetics, semustine, senescence derived inhibitor 1, sense oligonucleotides, siRNA, signal transduction inhibitors, signal transduction modulators, simtrazene, single chain antigen binding protein, sizofiran, sobuzoxane, sodium borocaptate, sodium phenylacetate, solverol, somatomedin binding protein, sonermin, sparfosate sodium, sparfosic acid, sparsomycin, spicamycin D, spirogermanium hydrochloride, spiromustine, spiroplatin, splenopentin, spongistatin 1, squalamine, stem cell inhibitor, stem-cell division inhibitors, stipiamide, streptonigrin, streptozocin, stromelysin inhibitors, sulfinosine, sulofenur, superactive vasoactive intestinal peptide antagonist, suradista, suramin, swainsonine, synthetic glycosaminoglycans, talisomycin, tallimustine, tamoxifen methiodide, tauromustine, tazarotene, tecogalan sodium, tegafur, tellurapyrylium, telomerase inhibitors, teloxantrone hydrochloride, temoporfin, temozolomide, teniposide, teroxirone, testolactone, tetrachlorodecaoxide, tetrazomine, thaliblastine, thalidomide, thiamiprine, thiocoraline, thioguanine, thiotepa, thrombopoietin, thrombopoietin mimetic, thymalfasin, thymopoietin receptor agonist, thymotrinan, thyroid stimulating hormone, tiazofurin, tin ethyl

etiopurpunn, tirapazamine, titanocene dichlonde, topotecan hydrochloride, topsentin, toremifene, toremifene citrate, totipotent stem cell factor, translation inhibitors, trestolone acetate, tretinoin, triacetyluridine, triciribine, triciribine phosphate, trimetrexate, trimetrexate glucuronate, triptorelin, tropisetron, tubulozole hydrochloride, turosteride, tyrosine kinase inhibitors, tyrphostins, UBC inhibitors, ubenimex, uracil mustard, uredepa, urogenital sinus-derived growth inhibitory factor, urokinase receptor antagonists, vapreotide, variolin B, velaresol, veramine, verdins, verteporfin, vinblastine sulfate, vincristine sulfate, vindesine, vindesine sulfate, vinepidine sulfate, vinglycinate sulfate, vinleurosine sulfate, vinorelbine, vinorelbine tartrate, vinrosidine sulfate, vinxaltine, vinzolidine sulfate, vitaxin, vorozole, zanoterone, zeniplatin, zilascorb, zinostatin, zinostatin stimalamer, or zorubicin hydrochloride.

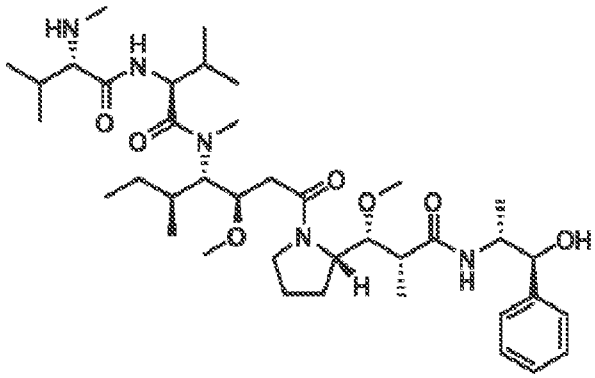
[0081] The active agent can be an inorganic or organometallic compound containing one or more metal centers. In some examples, the compound contains one metal center. The active agent can be, for example, a platinum compound, a ruthenium compound (e.g., *trans*-[$RuCl_2$ (**DMSO)4],** or *trans*-[RuCl4(imidazole) 2, etc.), cobalt compound, copper compound, or iron compounds.

[0082] In some embodiments, the active agent is a small molecule. In some embodiments, the active agent is a small molecule cytotoxin. In one embodiment, the active agent is cabazitaxel, or an analog, derivative, prodrug, or pharmaceutically acceptable salt thereof. In another embodiment, the active agent is mertansine (DM1) or DM4, or an analog, derivative, prodrug, or pharmaceutically acceptable salt thereof. DM1 or DM4 inhibits the assembly of microtubules by binding to tubulin. Structure of DM1 is shown below:



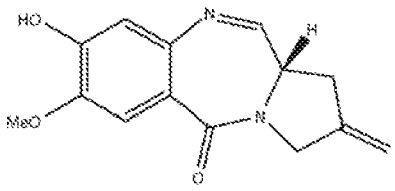
(DM1).

[0083] In some embodiments, the active agent Z is Monomethyl auristatin E (MMAE), or an analog, derivative, prodrug, or pharmaceutically acceptable salt thereof. Structure of MMAE is shown below:

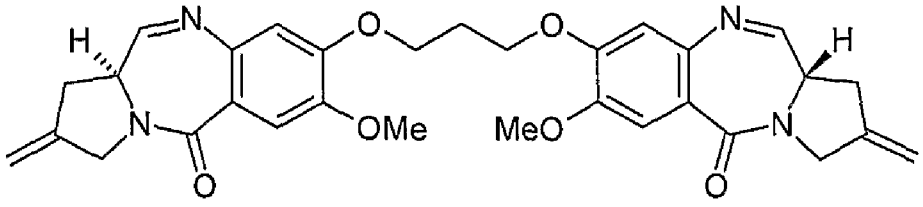


(MMAE).

[0084] In some embodiments, the active agent Z is a sequence-selective DNA minor-groove binding crosslinking agent. For example, Z may be pyrrolobenzodiazepine (PBD), a PBD dimer, or an analog, derivative, prodrug, or pharmaceutically acceptable salt thereof. Pyrrolobenzodiazepines or pyrrolo[2,l-c][l, ^benzodiazepines (PBDs) are a family of sequence-selective DNA minor-groove binding agents. The first example of a PBD monomer is the natural product anthramycin. Synthetic PBDs have been developed by attaching non-covalent minor-groove binding components to the C8-position of the PBD aromatic-ring. Monomeric PBD units have been joined together to afford PBD dimers. An example of PBD dimer is SJG-136). PBD analogs and dimers include, but not limited to, any PBD-based payload disclosed in Mantaj et al., *Angew. Chem. Int. Ed*, vol. 56:462 (2017), the contents of which are incorporated herein by reference in their entirety, such as GWL-78, KMR-28-39, DSB-120, SJG-136 (also known as SG2000, NSC 694501 or BN2629) in Fig. 1 of Mantaj et al. Structures of PBD and a PBD dimer are shown below:

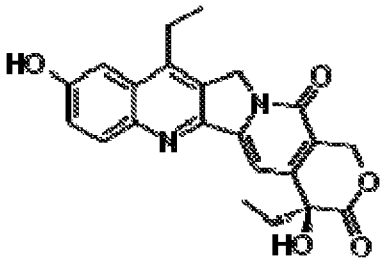


(PBD),



(PBD dimer).

[0085] In some embodiments, the active agent Z is a topoisomerase I inhibitor, such as camptothecin, irinotecan, SN-38, or an analog, derivative, prodrug, or pharmaceutically acceptable salt thereof.



SN-38 (7-Ethyl- 10-hydroxy-camptothecin)

[0086] Any cytotoxic moiety disclosed in WO2013 158644, WO2015038649, WO2015066053, WO20151 16774, WO2015134464, WO2015143004, WO201 5184246, the contents of each of which are incorporated herein by reference in their entirety, such as bendamustine, VDA, doxorubicin, pemetrexed, vorinostat, lenalidomide, docetaxel, 17-AAG, 5-FU, abiraterone, crizotinib, KW-2189, BUMB2, DC1, CC-1065, adozelesin, or derivatives/analogs thereof, may be used as an active agent in conjugates of the present invention.

[0087] In certain embodiments, the active agent of the conjugate comprises a predetermined molar weight percentage from about 1% to about 10%, or about 10% to about 20%, or about 20% to about 30%, or about 30% to about 40%, or about 40% to about 50%, or about 50% to about 60%, or about 60% to about 70%, or about 70% to about 80%, or about 80% to about 90%, or about 90% to about 99% such that the sum of the molar weight percentages of the components of the conjugate is 100%. The amount of active agent(s) of the conjugate may also be expressed in terms of proportion to the targeting ligand(s). For example, the present teachings provide a ratio of active agent to ligand of about 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4; 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10.

B. Targeting Moieties

[0088] The conjugates contain one or more targeting moieties and/or targeting ligands. Targeting ligands or moieties can be peptides, antibody mimetics, nucleic acids (e.g., aptamers), polypeptides (e.g., antibodies), glycoproteins, small molecules, carbohydrates, or lipids. The targeting moiety, X, can be a peptide such as somatostatin, octreotide, LHRH, an EGFR-binding peptide, RGD-containing peptides, a protein scaffold such as a fibronectin domain, an aptide or bipodal peptide, a single domain antibody, a stable scFv, or a bispecific T-cell engagers, nucleic acid (e.g., aptamer), polypeptide (e.g., antibody or its fragment), glycoprotein, small molecule, carbohydrate, or lipid. The targeting moiety, X can be an aptamer being either RNA or DNA or an artificial nucleic acid; small molecules; carbohydrates such as mannose, galactose and arabinose; vitamins such as ascorbic acid, niacin, pantothenic acid, carnitine, inositol, pyridoxal, lipoic acid, folic acid (folate), riboflavin, biotin, vitamin B12, vitamin A, E, and K; a protein or peptide that binds to a cell-surface receptor such as a receptor for thrombospondin, tumor necrosis factors (TNF), annexin V, interferons, cytokines, transferrin, GM-CSF (granulocyte-macrophage colony-stimulating factor), or growth factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), (platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and epidermal growth factor (EGF).

[0089] In some embodiments, the targeting moiety is a protein scaffold. The protein scaffold may be an antibody-derived protein scaffold. Non-limiting examples include single domain antibody (dAbs), nanobody, single-chain variable fragment (scFv), antigen-binding fragment (Fab), Avibody, minibody, CH2D domain, Fcab, and bispecific T-cell engager (BiTE) molecules. In some embodiments, scFv is a stable scFv, wherein the scFv has hyperstable properties. In some embodiments, the nanobody may be derived from the single variable domain (VHH) of camelidae antibody.

[0090] In some embodiments, the protein scaffold may be a nonantibody-derived protein scaffold, wherein the protein scaffold is based on nonantibody binding proteins. The protein scaffold may be based on enginnered Kunitz domains of human serine protease inhibitors (e.g., LAC1-D1), DARPins (designed ankyrin repeat domains), avimers created from multimerized low-density lipoprotein receptor class A (LDLR-A), anticalins derived from lipocalins, knottins constructed from cysteine-rich

knottin peptides, affibodies that are based on the Z-domain of staphylococcal protein A, adnectins or monobodies and pronectins based on the 10^{th} or 14^{th} extracellular domain of human fibronectin III, Fynomers derived from SH3 domains of human Fyn tyrosine kinase, or nanofitins (formerly Affitins) derived from the DNA binding protein Sac7d.

[0091] In some embodiments, the protein scaffold may be any protein scaffold disclosed in Mintz and Crea, *BioProcess*, vol.1 1(2):40-48 (2013), the contents of which are incorporated herein by reference in their entirety. Any of the protein scaffolds disclosed in Tables 2-4 of Mintz and Crea may be used as a targeting moiety of the conjugate of the invention.

[0092] In some embodiments, the protein scaffold may be based on a fibronectin domain. In some embodiments, the protein scaffold may be based on fibronectin type III (FN3) repeat protein. In some embodiments, the protein scaffold may be based on a consensus sequence of multiple FN3 domains from human Tenascin-C (hereinafter "Tenascin"). Any protein scaffold based on a fibronectin domain disclosed in U S Pat. No. 8569227 to Jacobs et al., the contents of which are incorporated herein by reference in their entirety, may be used as a targeting moiety of the conjugate of the invention.

[0093] In some embodiments, the targeting moiety or targeting ligand may be any molecule that can bind to luteinizing-hormone-releasing hormone receptor (LITRFIR). Such targeting ligands can be peptides, antibody mimetics, nucleic acids (e.g., aptamers), polypeptides (e.g., antibodies), glycoproteins, small molecules, carbohydrates, or lipids. In some embodiments, the targeting moiety is LHRH or a LHRH analog.

[0094] Luteinizing-hormone-releasing hormone (LHRH), also known as gonadotropin-releasing hormone (GnRH) controls the pituitary release of gonadotropins (LH and FSH) that stimulate the synthesis of sex steroids in the gonads. LHRH is a 10-amino acid peptide that belongs to the gonadotropin-releasing hormone class. Signaling by LHRH is involved in the first step of the hypothalami c-pituitary-gonadal axis. An approach in the treatment of hormone-sensitive tumors directed to the use of agonists and antagonists of LHRH (A.V. Schally and A.M. Comaru-Schally. Sem. Endocrinol., 5 389-398, 1987) has been reported. Some LHRH agonists, when substituted in position 6, 10, or both are much more active than LHRH

and also possess prolonged activity. Some LHRH agonists are approved for clinical use, e.g., Leuprolide, triptorelin, nafarelin and goserelin.

[0095] Some human tumors are hormone dependent or hormone-responsive and contain hormone receptors. Certain of these tumors are dependent on or responsive to sex hormones or growth factors, or have components that are dependent or responsive to such hormones. Mammary carcinomas contain estrogen, progesterone, glucocorticoid, LHRH, EGF IGF-I and somatostatin receptors. Peptide hormone receptors have been detected in acute leukaemia, prostate-, breast-, pancreatic, ovarian-, endometrial cancer, colon cancer and brain tumors (M.N. Pollak, et al., Cancer Lett. 38 223-230 1987; F. Pekonen, et al., Cancer Res., 48 1343-1347, 1988; M. Fekete, et al., J Clin.Lab. Anal. 3 137-147, 1989; G. Emons, et al., Eur. J. Cancer Oncol., 25215-221 1989). It has been found (M. Fekete, et al., Endocrinology. 124 946-955. 1989; M Fekete, et al. Pancreas 4521-528, 1989) that both agonistic and antagonistic analog of LHRH bind to human breast cancer cell membranes, and also to the cell membranes of pancreatic cancer. It has been demonstrated that biologically active peptides such a melanotropin (MSH), epidermal growth factor, insulin and agonistic and antagonist analogs of LHRH (L Jennes, et. al., Peptides 5 215-220, 1984) are internalized b their target cells by endocytosis.

[0096] The conjugates of the invention can employ any of the large number of known molecules that recognize the LHRH receptor, such as known LHRH receptor agonists and antagonists. In some embodiments, the LHRH analog portion of the conjugate contains between 8 and 18 amino acids.

[0097] Examples of LHRH binding molecules useful in the present invention are described herein. Further non-limiting examples are analogs of pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2, leuprolide, triptorelin, nafarelin, buserelin, goserelin, cetrorelix, ganirelix, azaline-B, degarelix and abarelix.

[0098] Methods for synthesizing LHRH peptides and analogs are well documented and are within the ability of a person of ordinary skill in the art as exemplified in the references listed supra. Further synthetic procedures are provided in the following examples. The following examples also illustrate methods for synthesizing the targeted cytotoxic compounds of the present invention. Specific targeting of therapeutic or cytotoxic agents allows selective destruction of a tumor expressing a receptor specific for a biologically active peptide. For example, a tumor expressing a LHRH receptor includes a neoplasm of the lung, breast, prostate, colon, brain,

gastrointestinal tract, neuroendocrine axis, liver, or kidney (see Schaer et al., Int. J. Cancer, 70:530-537, 1997; Chave et al., Br. J. Cancer 82(1): 124-130, 2000; Evans et al., Br. J. Cancer 75(6):798-803, 1997).

[0099] In some embodiments, the targeting moiety, e.g., LHRH analog, used in the invention is hydrophilic, and is therefore water soluble. In some embodiments, such targeted constructs are used in treatment paradigms in which this feature is useful, e.g., compared to conjugates comprising hydrophobic analogs. Hydrophilic analogs described herein can be soluble in blood, cerebrospinal fluid, and other bodily fluids, as well as in urine, which may facilitate excretion by the kidneys. This feature can be useful, e.g., in the case of a composition that would otherwise exhibit undesirable liver toxicity. The invention also discloses specific hydrophilic elements (e.g., incorporation of a PEG linker, and other examples in the art) for incorporation into peptide analogs, allowing modulation of the analog's hydrophilicity to adjust for the chemical and structural nature of the various conjugated cytotoxic agents.

[00100] In some embodiments, the targeting moiety is an antibody mimetic such as a monobody, e.g., an ADNECTIN™ (Bristol-Myers Squibb, New York, New York), an Affibody® (Affibody AB, Stockholm, Sweden), Affilin (Navigo Proteins, Halle, Germany), nanofitin (affitin, such as those described in WO 2012/085861, an Anticalin™, an avimers (avidity multimers), a DARPin™, a Fynomer™, Centyrin™, a Humabody®, Kunitz domain or an Abdurin peptide. In certain cases, such mimetics are artificial peptides or proteins with a molar mass of about 3 to 20 kDa. Nucleic acids and small molecules may be antibody mimetic.

[00101] In one example, the targeting moiety is an Abdurin peptide. It is an engineered antibody domain molecule comprising at least one protein-binding domain derived from a CH2 domain or CH2-like domain of an immunoglobulin (such as IgG, IgA, IgD), or a CH3 domain or CH3-like domain of IgE or IgM, comprising at least one mutation. The mutation may be an N-terminal truncation of at least one amino acid and/or a C-terminal truncation of at least one amino acid. The molecular weight of an Abdurin peptide is usually less than about 15 kD. Any peptide disclosed in US8580927, US9527903, US9156917, US20130189247, or US20150353943, the contents of each of which are incorporated herein by reference in their entirety, may be used as the targeting moiety in the present disclosure,

[00102] In another example, a targeting moiety can be an aptamer, which is generally an oligonucleotide (e.g., DNA, RNA, or an analog or derivative thereof)

that binds to a particular target, such as a polypeptide. In some embodiments, the targeting moiety is a polypeptide (e.g., an antibody that can specifically bind a tumor marker). In certain embodiments, the targeting moiety is an antibody or a fragment thereof. In certain embodiments, the targeting moiety is an Fc fragment of an antibody.

[00103] In another example, a targeting moiety may be a non-immunoreactive ligand. For example, the non-immunoreactive ligand may be insulin, insulin-like growth factors I and II, lectins, apoprotein from low density lipoprotein, etc. as disclosed in US 20140031535 to Jeffrey, the contents of which are incorporated herein by reference in their entirety. Any protein or peptide comprising a lectin disclosed in WO2013 181454 to Radin, the contents of which are incorporated herein by reference in their entirety, may be used as a targeting moiety.

[00104] In another example, the conjugate of the invention may target a hepatocyte intracellularly and a hepatic ligand may be used as a targeting moiety. Any hepatic ligand disclosed in US 200301 19724 to Ts'o et al., the contents of which are incorporated herein by reference in their entirety, such as the ligands in Fig. 1, may be used. The hepatic ligand specifically binds to a hepatic receptor, thereby directing the conjugate into cells having the hepatic receptor.

[00105] In another example, a targeting moiety may interact with a protein that is overexpressed in tumor cells compared to normal cells. The targeting moiety may bind to a chaperonin protein, such as Hsp90, as disclosed in US 20140079636 to Chimmanamada et al., the contents of which are incorporated herein by reference in their entirety. The targeting moiety may be an Hsp90 inhibitor, such as ganetespib or a ganetespib analog (e.g., 4-(5-hydroxy-4-(l-(2-(piperidin-4-yl)ethyl)-lH-indol-5-yl)-4H- 1,2,4-triazol-3 -yl)-6-isopropylbenzene- 1,3-diol), geldanamycins, macbecins, tripterins, tanespimycins, and radicicols.

[00106] In another example, the targeted construct may have a terminal half-life of longer than about 72 hours and a targeting moiety may be selected from Table 1 or 2 of US 20130165389 to Schellenberger et al., the contents of which are incorporated herein by reference in their entirety. The targeting moiety may be an antibody targeting delta-like protein 3 (DLL3) in disease tissues such as lung cancer, pancreatic cancer, skin cancer, etc., as disclosed in WO2014125273 to Hudson, the contents of which are incorporated herein by reference in their entirety. The targeting moiety may also any targeting moiety in WO2007137170 to Smith, the contents of which are

incorporated herein by reference in their entirety. The targeting moiety binds to glypican-3 (GPC-3) and directs the conjugate to cells expressing GPC-3, such as hepatocellular carcinoma cells.

[00107] In some embodiments, a target of the targeting moiety may be a marker that is exclusively or primarily associated with a target cell, or one or more tissue types, with one or more cell types, with one or more diseases, and/or with one or more developmental stages. In some embodiments, a target can comprise a protein (e.g., a cell surface receptor, transmembrane protein, glycoprotein, etc.), a carbohydrate (e.g., a glycan moiety, glycocalyx, etc.), a lipid (e.g., steroid, phospholipid, etc.), and/or a nucleic acid (e.g., a DNA, RNA, etc.).

[00108] In another embodiment, targeting moieties may be peptides for regulating cellular activity. For example, the targeting moiety may bind to Toll Like Receptor (TLR). It may be a peptide derived from vaccinia virus A52R protein such as a peptide comprising SEQ ID No. 13 as disclosed in US 7557086, a peptide comprising SEQ ID No. 7 as disclosed in US 8071553 to Hefeneider, et al., or any TLR binding peptide disclosed in WO 2010141845 to McCoy, et al., the contents of each of which are incorporated herein by reference in their entirety. The A52R derived synthetic peptide may significantly inhibit cytokine production in response to both bacterial and viral pathogen associated molecular patterns, and may have application in the treatment of inflammatory conditions that result from ongoing toil-like receptor activation.

[00109] In another embodiment, targeting moieties many be amino acid sequences or single domain antibody fragments for the treatment of cancers and/or tumors. For example, targeting moieties may be an amino acid sequence that binds to Epidermal Growth Factor Receptor 2 (HER2). Targeting moieties may be any HER2-binding amino acid sequence described in US 201 10059090, US8217140, and US 8975382 to Revets, et al., the contents of each of which are incorporated herein by reference in their entirety. The targeting moiety may be a domain antibody, a single domain antibody, a VHH, a humanized VHH or a camelized VH.

[00110] In another embodiment, targeting moieties may be peptidomimetic macrocycles for the treatment of disease. For example, targeting moieties may be peptidomimetic macrocycles that bind to the growth hormone-releasing hormone (GHRH) receptor, such as a peptidomimetic macrocycle comprising an amino acid sequence which is at least about 60% identical to GHRH 1-29 and at least two

macrocycle-forming linkers as described in US20130123169 to Kawahata et al, the contents of which are incorporated herein by reference in their entirety. In another embodiment, the peptidomimetic macrocycle targeting moiety may be prepared by introducing a cross-linker between two amino acid residues of a polypeptide as described in US 20120149648 and US 20130072439 to Nash et al., the contents of each of which are incorporated herein by reference in their entirety. Nash et al. teaches that the peptidomimetic macrocycle may comprise a peptide sequence that is derived from the BCL-2 family of proteins such as a BH3 domain. The peptidomimetic macrocycle may comprise a BID, BAD, BIM, BIK, NOXA, PUMA peptides.

[00111] In another embodiment, targeting moieties may be polypeptide analogues for transport to cells. For example, the polypeptide may be an Angiopep-2 polypeptide analog. It may comprising a polypeptide comprising an amino acid sequence at least 80% identical to SEQ ID No.97 as described in US 20120122798 to Castaigne et al., the contents of which are incorporated herein by reference in their entirety. Additionally, polypeptides may transport to cells, such as liver, lung, kidney, spleen, and muscle, such as Angiopep-4b, Angiopep-5, Angiopep-6, and Angiopep-7 polypeptide as described in EP 2789628 to Beliveau et al., the contents of each of which are incorporated herein by reference in their entirety.

[00112] In another embodiment, targeting moieties may be homing peptides to target liver cells in vivo. For example, the melittin delivery peptides that are administered with RNAi polynucleotides as described in US 8501930 Rozema, et al., the contents of which are incorporated herein by reference in their entirety, may be used as targeting moieties. In addition, delivery polymers provide membrane penetration function for movement of the RNAi polynucleotides from the outside the cell to inside the cell as described in US 83 13772 to Rozema et al., the contents of each of which are incorporated herein by reference in their entirety. Any delivery peptide disclosed by Rozema et al. may be used as targeting moeities.

[00113] In another embodiment, targeting moieties may be structured polypeptides to target and bind proteins. For example, polypeptides with sarcosine polymer linkers that increase the solubility of structured polypeptides, as described in WO 2013050617 to Tite, et al., the contents of which are incorporated herein by reference in their entirety, may be used as targeting moieties. Additionally, polypeptide with variable binding activity produced by the methods described in WO 2014140342 to

Stace, et al., the contents of which are incorporated herein by reference in their entirety. The polypeptides may be evaluated for the desired binding activity.

[00114] In another embodiment, modifications of the targeting moieties affect a compound's ability to distribute into tissues. For example, a structure activity relationship analysis was completed on a low orally bioavailable cyclic peptide and the permeability and clearance was determined as described in Rand, AC, et al., *Medchemcomm.* 2012, 3(10): 1282-1289, the contents of which are incorporated herein by reference in their entirety. Any of the cyclic peptide disclosed by Rand et al., such as *N*-methylated cyclic hexapeptides, may be used as targeting moieties.

[00115] In another embodiment, targeting moieties may be a polypeptide which is capable of internalization into a cell. For example, targeting moieties may be an Alphabody capable of internalization into a cell and specifically binding to an intracellular target molecule as described in US 20140363434 to Lasters, et al., the contents of which are incorporated herein by reference in their entirety. As taught by Lasters et al., an 'Alphabody' or an 'Alphabody structure' is a self-folded, single-chain, triple-stranded, predominantly alpha-helical, coiled coil amino acid sequence, polypeptide or protein. The Alphabody may be a parallel Alphabody or an anti-parallel Alphabody. Moreover, targeting moieties may be any Alphabody in the single-chain Alphabody library used for the screening for and/or selection of one or more Aiphabodies that specifically bind to a target molecule of interest as described in WO 2012092970 to Desmet et al., the contents of which are incorporated herein by reference in their entirety.

[00116] In another embodiment, targeting moieties may consist of an affinity-matured heavy chain-only antibody. For example, targeting moieties may be any **VH** heavy chain-only antibodies produced in a transgenic non-human mammal as described in US 20090307787 to Grosveld et al., the contents of which are incorporated herein by reference in their entirety.

[00117] In another embodiment, targeting moieties may bind to the hepatocyte growth factor receptor "HGFr" or "cMet". For example, targeting moieties may be a polypeptide moiety that is conjugated to a detectable label for diagnostic detection of cMet as described in US 9000124 to Dransfield et al., the contents of which are incorporated herein by reference in their entirety. Additionally, targeting moieties may bind to human plasma kallikrein and may comprise BPTI-homologous Kunitz domains, especially LACI homologues, to bind to one or more plasma (and/or tissue)

kallikreins as described in WO 1995021601 to Markland et al., the contents of which are incorporated herein by reference in their entirety.

[00118] In another embodiment, targeting moieties are evolved from weak binders and anchor-scaffold conjugates having improved target binding and other desired pharmaceutical properties through control of both synthetic input and selection criteria. Any target binding element identified in US 20090163371 to Stem et al., the contents of which are incorporated herein by reference in their entirety, may be used as a targeting moiety. Moreover, targeting moieties may be macrocyclic compounds that bind to inhibitors of apoptosis as described in WO 2014074665 to Borzilleri et al., the contents of which are incorporated herein by reference in their entirety.

[00119] In another embodiment, targeting moieties may comprise pre-peptides that encode a chimeric or mutant lantibiotic. For example, targeting moieties may be pre-tide that encode a chimera that was accurately and efficiently converted to the mature lantibiotic, as demonstrated by a variety of physical and biological activity assays as described in US5861275 to Hansen, the contents of which are incorporated herein by-reference in their entirety. The mixture did contain an active minor component with a biological activity.

[00120] In another embodiment, targeting moieties may comprise a leader peptide of a recombinant manganese superoxide dismutase (rMnSOD-Lp). For example, rMnSOD-Lp which delivers cisplatin directly into tumor cells as described in Borrelli, A., et al., *Chem Biol Drug Des.* 2012, 80(1):9-16, the contents of which are incorporated herein by reference in their entirety, may be used a targeting moiety.

[00121] In another embodiment, the targeting moiety may be an antibody for the treatment of glioma. For example, an antibody or antigen binding fragment which specifically binds to JAMM-B or JAM-C as described in US8007797 to Dietrich et al., the contents of which are incorporated herein by reference in their entirety, may be used as a targeting moiety. JAMs are a family of proteins belonging to a class of adhesion molecules generally localized at sites of cell-cell contacts in tight junctions, the specialized cellular structures that keep ceil polarity and serve as barriers to prevent the diffusion of molecules across intercellular spaces and along the basolateral-apical regions of the plasma membrane.

[00122] In another embodiment, the targeting moiety may be a target interacting modulator. For example, nucleic acid molecules capable of interacting with proteins associated with the Human Hepatitis C virus or corresponding peptides or mimetics

capable of interfering with the interaction of the native protein with the HIV accessor}' protein as described in WO 201 1015379 and US 8685652, the contents of each of which are incorporated herein by reference in their entirety, may be used as a targeting moiety.

[00123] In another embodiment, the targeting moiety may bind with biomolecules. For example, any cystine-knot family small molecule polycyclic molecular scaffolds were designed as peptidomsmetics of FSH and used as peptide-vaccine as described in US7863239 to Timmerman, the contents the contents of which are incorporated herein by reference in their entirety, may be used as targeting moieties.

[00124] In another embodiment, the targeting moiety may bind to integrin and thereby block or inhibit integrin binding. For example, any highly selective disulfide-rich dimer molecules which inhibit binding of a4ß7 to the mucosal addressin cell adhesion molecule (MAdCAM) as described in WO 2014059213 to Bhandari, the contents of which are incorporated herein by reference in their entirety, may be used as a targeting moiety. Any inhibitor of specific integrins-ligand interactions may be used as a targeting moiety. The conjugates comprising such target moieties may be effective as anti-inflammatory agents for the treatment of various autoimmune diseases.

[00125] In another embodiment, the targeting moiety may comprise novel peptides. For example, any cyclic peptide or mimetic that is a serine protease inhibitor as described in WO 2013 172954 to Wang et al., the contents of which are incorporated herein by reference in their entirety, may be used as a targeting moiety. Additionally, targeting moieties may comprise a targeting peptide that is used in the reduction of cell proliferation and the treatment of cancer. For example, a peptide composition inhibiting the trpv6 calcium channel as described in US 201203 161 19 to Stewart, the contents of which are incorporated herein by reference in their entirety, may be used as a targeting moiety.

[00126] In another embodiment, the targeting moiety may comprise a cyclic peptide. For example, any cyclic peptides exhibit various types of action *in vivo,* as described in US20100168380 and WO 20081 17833 to Suga et al., and WO 2012074129 to Higuchi et al., the contents of each of which are incorporated herein by reference, may be used as targeting moieties. Such cyclic peptide targeting moieties have a stabilized secondary structure and may inhibit biological molecule

interactions, increase cell membrane permeability and the peptide's half-life in blood serum.

[00127] In another embodiment, the targeting moiety may consist of a therapeutic peptide. For example, peptide targeting moieties may be an AP-1 signaling inhibitor, such as a peptide analog comprising SEQ ID No. 104 of US8946381B2 to Fear that is used for the treatment of wounds, a peptide comprising SEQ ID No. 108 in US8822409B2 to Milech, et al. that is used to treat acute respiratory distress syndrome (ARDS), or a neuroprotective AP-1 signaling inhibitory peptide that is a fusion peptide comprising a protein transduction domain having the amino acid sequence of SEQ ID NO: 1 and a peptide having the sequence of SEQ ID NO: 54 as described in US8063012 to Watt, the contents of each of which are incorporated herein by reference in their entirety. In another example, the targeting moiety may be any biological modulator isolated from biodiverse gene fragment libraries as described in US7803765 and EP 1754052 to Watt, any inhibitor of c-Jun dimerization as described in EP 160 1766 and EP 1793 841 to Watt, any peptide inhibitors of CD40L signaling as described in US8802634 and US20130266605 to Watt, or any peptide modulators of cellular phenotype as described in US201 102181 18 to Watt, the contents of each of which are incorporated herein by reference in their entirety.

[00128] In another embodiment, the targeting moiety may consist of a characterized peptide. For example, any member of the screening libraries created from bioinformatic source data to theoretically predict the secondary structure of a peptide as described in EP 1987 178 to Watt et al., any peptide identified from peptide libraries that are screened for antagonism or inhibition of other biological interactions by a reverse hybrid screening method as described by EP1268842 to Hopkins, et al., the contents of each of which are incorporated herein by reference in their entirety, may be used as a targeting moiety. Additionally, targeting moieties may be cell-penetrating peptides. For example, any cell-penetrating peptides linked to a cargo that are capable of passing through the blood brain barrier as described by US20140141452A1 to Watt, et al., the contents of which are incorporated herein by reference, may be used a targeting moiety.

[00129] In another embodiment, the targeting moiety may comprise a LHRH antagonist, agonist, or analog. For example, the targeting moiety may be Cetrorelix, a decapeptide with a terminal acid amide group (AC-D-Nal(2)-D-pCl-Phe-D-Pal(3)-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH2) as described in US 4800191, US 6716817,

US 6828415, US 6867191, US 7605121, US 7718599, US 7696149 (Zentaris Ag), or pharmaceutically active decapeptides such as SB-030, SB-075 (cetrorelix) and SB-088 disclosed in EP 0 299 402 (Asta Pharma), the contents of each of which are incorporated herein by reference in their entirety. In another example, the targeting moiety may be LHRH analogues such as D-/L-Mel (4-[bis(2-chloroethyl)amino]-D/L-phenylalanine), cyclopropanealkanoyl, aziridine-2-carbonyl, epoxyalkyl, 1,4-naphthoquinone-5-oxycarbonyl-ethyl, doxorubicinyl (Doxorubicin, DOX), mitomicinyl (Mitomycin C), esperamycinyl or methotrexoyl, as disclosed in US 6214969 to Janaky et al., the contents of which are incorporated herein by reference in their entirety.

[00130] In another embodiment, the targeting moiety may be any cell-binding molecule disclosed in US 7741277 or US 7741277 to Guenther et al. (Aeterna Zentaris), the contents of which are incorporated herein by reference in their entirety, such as octamer peptide, nonamer peptide, decamer peptide, luteinizing hormone releasing hormone (LHRH), [D-Lys6]-LHRH, LHRH analogue, LHRH agonist, Triptorelin ([D-Trp6]-LHRH), LHRH antagonist, bombesin, bombesin analogue, bombesin antagonist, somatostatin, somatostatin analogue, serum albumin, human serum albumin (HSA). These cell-binding molecules may be conjugated with disorazoles.

[00131] In another embodiment, targeting moieties may bind to growth hormone secretagogue (GHS) receptors, including ghrelin analogue ligands of GHS receptors. For example, targeting moieties may be any triazole derivatives with improved receptor activity and bioavailability properties as ghrelin analogue ligands of growth hormone secretagogue receptors as describe by US8546435 to Aicher, at al. (Aeterna Zentaris), the contents of which are incorporated herein by reference in their entirety.

[00132] In some embodiments, the targeting moiety X is an aptide or bipodal peptide. X may be any D-Aptamer-Like Peptide (D-Aptide) or retro-inverso Aptide which specifically binds to a target comprising: (a) a structure stabilizing region comprising parallel, antiparallel or parallel and antiparallel D-amino acid strands with interstrand noncovalent bonds; and (b) a target binding region I and a target binding region II comprising randomly selected n and m D-amino acids, respectively, and coupled to both ends of the structure stabilizing region, as disclosed in US Pat. Application No. 20140296479 to Jon et al., the contents of which are incorporated herein by reference in their entirety. X may be any bipodal peptide binder (BPB)

comprising a structure stabilizing region of parallel or antiparallel amino acid strands or a combination of these strands to induce interstrand non-covalent bonds, and target binding regions I and II, each binding to each of both termini of the structure stabilizing region, as disclosed in US Pat. Application No. 20120321697 to Jon et al., the contents of which are incorporated herein by reference in their entirety. X may be an intracellular targeting bipodal-peptide binder specifically binding to an intracellular target molecule, comprising: (a) a structure-stabilizing region comprising a parallel amino acid strand, an antiparallel amino acid strand or parallel and antiparallel amino acid strands to induce interstrand non-covalent bonds; (b) target binding regions I and II each binding to each of both termini of the structure-stabilizing region, wherein the number of amino acid residues of the target binding region I is n and the number of amino acid residues of the target binding region II is m; and (c) a cell-penetrating peptide (CPP) linked to the structure-stabilizing region, the target binding region I or the target binding region II, as disclosed in US Pat. Application No. 20120309934 to Jon et al., the contents of which are incorporated herein by reference in their entirety. X may be any bipodal peptide binder comprising a β-hairpin motif or a leucine-zipper motif as a structure stabilizing region comprising two parallel amino acid strands or two antiparallel amino acid strands, and a target binding region I linked to one terminus of the first of the strands of the structure stabilizing region, and a target binding region II linked to the terminus of the second of the strands of the structure stabilizing region, as disclosed in US Pat. Application No. 201 10152500 to Jon et al., the contents of which are incorporated herein by reference in their entirety. X may be any bipodal peptide binder targeting KPI as disclosed in WO2014017743 to Jon et al, any bipodal peptide binder targeting cytokine as disclosed in WO201 1132939 to Jon et al., any bipodal peptide binder targeting transcription factor as disclosed in WO201 132941 to Jon et al., any bipodal peptide binder targeting G protein-coupled receptor as disclosed in WO201 1132938 to Jon et al., any bipodal peptide binder targeting receptor tyrosine kinase as disclosed in WO201 1132940 to Jon et al., the contents of each of which are incorporated herein by reference in their entireties. X may also be bipodal peptide binders targeting cluster differentiation (CD7) or an ion channel.

[00133] In another embodiment, the targeting moiety may be a bicyclic peptide or a modified bicyclic peptide, as disclosed in WO20 15063465, EP2464727, WO2013050617, WO2016067035, EP233518, US20140249292, US20140256596,

EP2970954, US 9518081, EP2393520, US20 160046928, US20160031939, or US20 160046673 (Bicycle Therapeutics), the contents of which are incorporated heren by reference in their entirety.

[00134] In some embodiments, the target, target cell or marker is a molecule that is present exclusively or predominantly on the surface of malignant cells, e.g., a tumor antigen. In some embodiments, a marker is a prostate cancer marker. In some embodiments the target can be an intra-cellular protein.

[00135] In some embodiments, a marker is a breast cancer marker, a colon cancer marker, a rectal cancer marker, a lung cancer marker, a pancreatic cancer marker, a ovarian cancer marker, a bone cancer marker, a renal cancer marker, a liver cancer marker, a neurological cancer marker, a gastric cancer marker, a testicular cancer marker, a head and neck cancer marker, an esophageal cancer marker, or a cervical cancer marker.

[00136] The targeting moiety directs the conjugates to specific tissues, cells, or locations in a cell. The target can direct the conjugate in culture or in a whole organism, or both. In each case, the targeting moiety binds to a receptor that is present on the surface of or within the targeted cell(s), wherein the targeting moiety binds to the receptor with an effective specificity, affinity and avidity. In other embodiments, the targeting moiety targets the conjugate to a specific tissue such as the liver, kidney, lung or pancreas. The targeting moiety can target the conjugate to a target cell such as a cancer cell, such as a receptor expressed on a cell such as a cancer cell, a matrix tissue, or a protein associated with cancer such as tumor antigen. Alternatively, cells comprising the tumor vasculature may be targeted. Targeting moieties can direct the conjugate to specific types of cells such as specific targeting to hepatocytes in the liver as opposed to Kupffer cells. In other cases, targeting moieties can direct the conjugate to cells of the reticular endothelial or lymphatic system, or to professional phagocytic cells such as macrophages or eosinophils.

[00137] In some embodiments the target is member of a class of proteins such as receptor tyrosine kinases (RTK) including the following RTK classes: RTK class I (EGF receptor family) (ErbB family), RTK class II (Insulin receptor family), RTK class III (PDGF receptor family), RTK class IV (FGF receptor family), RTK class V (VEGF receptors family), RTK class VI (HGF receptor family), RTK class VII (Trk receptor family), RTK class VIII (Eph receptor family), RTK class IX (AXL receptor family), RTK class X (LTK receptor family), RTK class XI (TIE receptor family),

RTK class XII (ROR receptor family), RTK class XIII (DDR receptor family), RTK class XIV (RET receptor family), RTK class XV (KLG receptor family), RTK class XVI (RYK receptor family) and RTK class XVII (MuSK receptor family).

[00138] In some embodiments the target is a serine or threonine kinase, G-protein coupled receptor, methyl CpG binding protein, cell surface glycoprotein, cancer stem cell antigen or marker, carbonic anhydrase, cytolytic T lymphocyte antigen, DNA methyltransferase, an ectoenzyme, a glycosylphosphatidylinositol-anchored co-receptor, a glypican-related integral membrane proteoglycan, a heat shock protein, a hypoxia induced protein, a multi drug resistant transporter, a Tumor-associated macrophage marker, a tumor associated carbohydrate antigen, a TNF receptor family member, a transmembrane protein, a tumor necrosis factor receptor superfamily member, a tumour differentiation antigen, a zinc dependent metallo-exopeptidase, a zinc transporter, a sodium-dependent transmembrane transport protein, a member of the SIGLEC family of lectins, or a matrix metalloproteinase.

[00139] Other cell surface markers are useful as potential targets for tumor-homing therapeutics, including, for example HER-2, HER-3, EGFR, the folate receptor and neurotensin receptors (NTSR1 orNTSR2).

[00140] In other embodiments, the targeting moiety binds a target such as CD19, CD70, CD56, PSMA, alpha integrin, CD22, CD138, EphA2, AGS-5, Nectin-4, HER2, GPMNB, CD74 and Le.

[00141] In some embodiments, the target is a protein listed in Table A.

Table A. Non-limiting examples of proteins that may be targeted

5T4	CD64	GPIIb/IIIa receptors	PDGFRbeta
A20/TNFAIP3	CD68	GPR161/RE2	P-glycoprotein
ABCB5	CD70	Guanylyl cyclase receptor C	Podoplanin
ABCG2	CD80	HA-CD44v3	PON1
AFP	CD86	HER2/ERBB2	PRAME
ALCAM/CD166	CD90	HIF1alpha	PSAM
ALDH1A1	CD96	HIF-2	PTEN
Apelin J Receptor	CEACAM-5/cd66e	HLA-DR	RAAG12
APN/CD13	CEACAM-6	Hsp90	RON
AXL	c-KIT	IGE receptor	sialyl-Le(x)
B7H4	c-Maf	IGF-1R	sialyl-Le(x)
BCMA	c-Met	IL-1 alpha	sialyl-Tn
BCRP/ABCG2	Cripto/TDGF-1	IL-11R	Sigma Receptor/Pgrmc1
BMI-1	CSFR	IL-1R	SLC34A2
CA9	CXCR1	IL-23R	SLC44A4
CAIX	CXCR1	IL-2R	SLITRK6
mmp	CXCR4	IL-3 R	SOX2

CanAg	disialylgalactosylgloboside	IL-4R	STAT-3
CD117	DLL4	IL-6 R	STEAP-1
CD11a	DNMT1	Indegrin alpha 6	STRO-1
CD11b	DNMT3A	iNOS	Tenasin-C
CD136	DNMT3B	Insulin receptor	TF antigen
CD138	DNMT3L	L1CAM	TIM-3
CD14	EDB (Fibronectin extra domain B)	LGR5	Tissue Factor (CD142)
CD15	EGFR VIII	LIV-1 (SLC39A6), Zip6	Tn antigen
CD152 (CTLA-4)	E-NPP3/CD203c	LRP	TNFR
CD172A	Epcam/TROP1	MAGE-A3	TRAIL-R1
CD19	EphA1	MBD1	TRAIL-R2
CD20	EphA2	MBD2	Transferrin receptor
CD204	ERBB3	MBD4	TRK-A
CD206	FAP	Mesothelin	TRK-B
CD22	FGFR1	Metadherin/MTDH/AEG-1	Trop-2/EGP-1
CD24	FGFR2	MICL	UHRF1
CD25	FGFR3	MMP-2	UHRF2
CD26	FGFR4	MMP-9	VEGFR1
CD27 (CD70L)	Fibronectin	MRP1	VEGFR2
CD28	Folate receptor	Muc-1	VEGFR3
CD3	FRb	MUC16/CA-125	ZBTB33
CD30	Galbg4	Mushai-1	ZBTB4
CD33	GD2 ganglioside	NaPi2b	EphA3
CD34	GD3 ganglioside	Nectin-4	EphA4
CD38	GLI-1	Nestin	EphA5
CD40	GLI-2	Neurotensin receptor 1	EphA6
CD41	globo-H	NF2	EphA7
CD44	GLUT1	Notch1	EphA8
CD45	Glycoprotein NMB	Notch2	EphB1
CD45.1	glycosphingolipid P_1	Notch3	EphB2
CD45.2	GM2 ganglioside	Notch4	EphB3
CD47/IAP	GP130	Ovastacin	EphB4
CD52	GPC3 Glypican-3	PDGFRalpha	EphB5
EphB6	GRP78		

[00142] In certain embodiments, the targeting moiety or moieties of the conjugate are present at a predetermined molar weight percentage from about 1% to about 10%, or about 10% to about 20%, or about 20% to about 30%, or about 30% to about 40%, or about 40% to about 50%, or about 50% to about 60%, or about 60% to about 70%, or about 70% to about 80%, or about 80% to about 90%, or about 90% to about 99% such that the sum of the molar weight percentages of the components of the conjugate is 100%. The amount of targeting moieties of the conjugate may also be expressed in terms of proportion to the active agent(s), for example, in a ratio of ligand to active agent of about 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4; 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10.

SSTR-binding targeting moieties

[00143] In one embodiment, targeting ligands (also refered to as targeting moieties) as described herein include any molecule that can bind one or more SSTRs, e.g., human SSTR1, SSTR2, SSTR3, SSTR4, or SSTR5. Such targeting ligands can be peptides, antibody mimetics, nucleic acids (e.g., aptamers), polypeptides (e.g., antibodies), glycoproteins, small molecules, carbohydrates, or lipids. In some embodiments, the targeting moiety is somatostatin or a somatostation analog.

[00144] The conjugates of the invention can employ any somatostatin analog that binds somatostatin receptor. In some embodiments, the somatostatin analog portion of the conjugate contains between 8 and 18 amino acids, and includes the core sequence: cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys] (SEQ ID NO:l) or cyclo[Cys-Tyr-D-Trp-Lys-Thr-Cys] (SEQ ID NO. 2). For example, the C-terminus of the analog is Thr-NH2.

[00145] In one embodiment, the targeting moiety binds preferably to SSTR2. Therefore, the conjugate comprising the targeting moiety binds preferably to SSTR2. The binding of the conjugate to SSTR2 is stronger than the binding of the conjugate to SSTR1, SSTR3, SSTR4 or SSTR5.

[00146] In some embodiments, the conjugates as described herein have low membrane permeability. Membrane permeability may be low in both the apical to basolateral direction and the basolateral to apical direction. Not wiling to be bound by any theory, low membrane permeability enhances selective uptake by SSTRs by decreasing non-specific permeability. Low permeability leads to decreased uptake in cells that do not express SSTR2, leading to lower toxicity to non-SSTR2 expressing cells. Membrane permeability may be determined by any method known in the art. For example, it may be determined by measuring apparent permeability (Papp) in Caco-2 monolayers.

[00147] In some embodiments, the targeting moiety, X, may be selected from somatostatin, octreotide, octreotate, lanreotide, lutathera (^{177}Lu-DOTATATE), ^{90}Y-DOTATOC, Tyr^3-octreotate (TATE), vapreotide, cyclo(AA-Tyr-DTrp-Lys-Thr-Phe) where AA is α-N-Me lysine or N-Me glutamic acid, pasireotide, lanreotide, seglitide, or any other example of somatostatin receptor binding ligands. In some embodiments, the targeting moiety is a somatostatin receptor binding moiety that binds to somatostatin receptors 2 and/or 5. In some embodiments, X binds to the linker moiety Y at the C-terminal. In some embodiments, X binds to the linker moiety Y at the N-

terminal. In some embodiments, the targeting moiety X comprises at least one D-Phe residue and the phenyl ring of the D-Phe residue of the targeting moiety X has been replaced by a linker-containing moiety.

[00148] Methods for synthesizing somatostatin peptides and analogs are well documented and are within the ability of a person of ordinary skill in the art as exemplified in the references listed *supra*. Further synthetic procedures are provided in the following examples. The following examples also illustrate methods for synthesizing the targeted cytotoxic compounds of the present invention. Specific targeting of therapeutic or cytotoxic agents allows selective destruction of a tumor expressing a receptor specific for a biologically active peptide. For example, a tumor expressing a somatostatin receptor includes a neoplasm of the lung, breast, prostate, colon, brain, gastrointestinal tract, neuroendocrine axis, liver, or kidney (see Schaer et al., Int. J. Cancer, 70:530-537, 1997; Chave et al., Br. J. Cancer 82(1): 124-130, 2000; Evans et al., Br. J. Cancer 75(6):798-803, 1997).

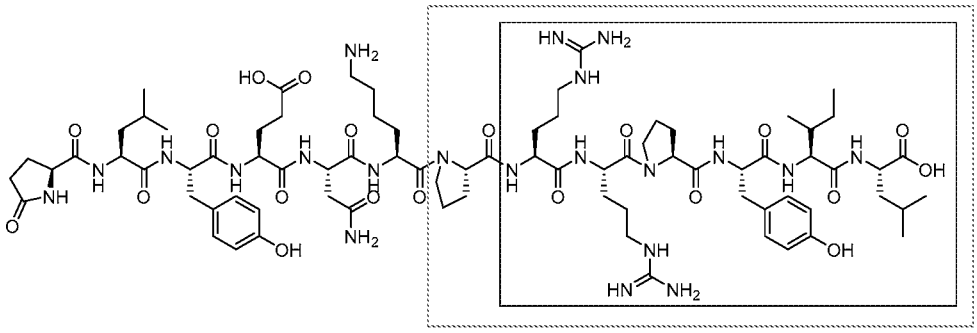
[00149] In some embodiments, the targeting moiety has therapeutic features, e.g., the targeting moiety is cytotoxic or anti-angiogenic. In some embodiments, a targeting moiety has some increased affinity for tumor vasculature, or angiogenic blood vessels, e.g., those that over-express somatostatin receptors (see Denzler and Reubi, Cancer 85:188-198, 1999; Gulec et al., J. Surg. Res. 97(2): 131-137, 2001; Woltering et al., J. Surg. Res. 50:245, 1991).

[00150] In some embodiments, the targeting moiety, e.g., somatostatin analog, used in the invention is hydrophilic, and is therefore water soluble. In some embodiments, such conjugates and particles containing such conjugates are used in treatment paradigms in which this feature is useful, e.g., compared to conjugates comprising hydrophobic analogs. Hydrophilic analogs described herein can be soluble in blood, cerebrospinal fluid, and other bodily fluids, as well as in urine, which may facilitate excretion by the kidneys. This feature can be useful, e.g., in the case of a composition that would otherwise exhibit undesirable liver toxicity. The invention also discloses specific hydrophilic elements (e.g., incorporation of a PEG linker, and other examples in the art) for incorporation into peptide analogs, allowing modulation of the analog's hydrophilicity to adjust for the chemical and structural nature of the various conjugated cytotoxic agents, e.g., conjugate 6 *infra*.

NTSR1-binding targeting moieties

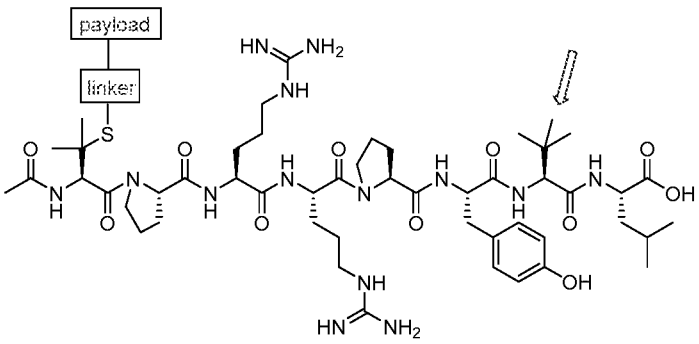
[00151] Neurotensin is a neuropeptide involved in dopamine signaling and thermoregulation. Neurotensin receptor 1 (NTSRl) is normally expressed only in the brain and colon, but some cancers can overexpress NTSRl. NTSRl is expressed in majority of pancreatic cancers, and has high expression in subsets of NSCLC and ductal breast carcinomas. NTSRl is involved in the growth of expressing cancer cells, and NTSRl expression correlates with poor prognosis.

[00152] The natural ligand (neurotensin) and its analogs have very high affinity for the receptor, internalize rapidly, and degrade within the cell after internalization. Neurotensin is a 13-amino acid peptide with six C-terminal amino acids as the targeting domain for NTSRl .



Neurotensin (6 and 7 C-terminal amin acids circled)

[00153] In some embodiments, the targeting moiety comprises the targeting domain of neurotensin or derivative thereof, e.g., six or seven C-terminal amino acids of neurotensin. The targeting moiety may futher comprise a linking amino acid which attaches the targeting domain of neurotensin to a variety of releasable linkers. The targeting domain of neurotensin may be modified to increase stability. For example, an isoleucine group on isoleucine residue may be replaced with tert-leucine for greater stability. A targeting moiety comprise seven C-terminal amino acids of neurotensin with tert-leucine modification is shown below:

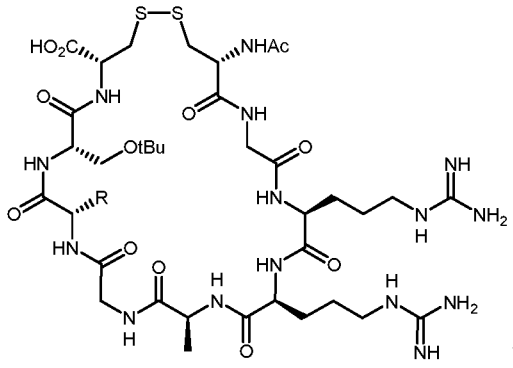


An example of NTSRl -binding conjugate

IL-1lRa-binding targeting moieties

[00154] ILl IRa is an important cytokine receptor that is part of a multimeric complex comprising the ubiquitously expressed gpl30R subunit. The complex triggers intracellular signaling and engagement of Stat3, which once activated, promotes cell survival and proliferation as well as immune responses associated with inflammatory diseases and tumor progression. IL-1 IRa links oxidative stress and compensatory proliferation, regulates autoimmune demyelination and the invasion and proliferation of cancer cells. Overexpression of IL-1 IRa indicates a poor long-term prognosis in cancer patients. IL-1 IRa is an established molecular target in primary tumors of bone, such as osteosarcoma, and in secondary bone metastases from solid tumors, such as prostate cancer. It is related to breast cancer development and progression and may play a significant role in the bone metastasis of human breast cancer. It has limited expression in healthy tissues.

[00155] Direct screening of combinatorial peptide libraries in patients has allowed the identification of ligands that target biochemical differences in the endothelium of blood vessels. For example, a mimic motif of ILl 1 (displaying the cyclic nonapeptide CGRRAGGSC) isolated from prostate biopsies binds specifically to IL-1 IRa. The cyclic nonapeptide or its derivatives may be used as a targeting moiety in the conjugates of the present invention. Its structure is shown below.



R=H or Me

C. Linkers

[00156] The conjugates contain one or more linkers attaching the active agents and targeting moieties, wherein at least one linker is a penicillamine linker. The penicillamine linker comprises a penicillamine group or derivative thereof. The linker, Y, is bound to one or more active agents and one or more targeting ligands to form a conjugate. The linker Y is attached to the targeting moiety X and the active agent Z by functional groups independently selected from an ester bond, disulfide, amide,

acylhydrazone, ether, carbamate, carbonate, and urea. The bond between the penicillamine linker Y and the targeting moiety X and/or the active agnet Z may be cleavable. Alternatively, the linker can be attached to either the targeting ligand or the active drug by a non-cleavable group such as provided by the conjugation between a thiol and a maleimide, an azide and an alkyne.

[00157] In some embodiments, the linker comprises a cleavable functionality that is cleavable. The cleavable functionality may be hydrolyzed *in vivo* or may be designed to be hydrolyzed enzymatically, for example by Cathepsin B. A "cleavable" linker, as used herein, refers to any linker which can be cleaved physically or chemically. Examples for physical cleavage may be cleavage by light, radioactive emission or heat, while examples for chemical cleavage include cleavage by re- dox-reactions, hydrolysis, pH-dependent cleavage or cleavage by enzymes.

[00158] In some embodiments, the active agent is selected from Non-limiting examples of conjugates of the present invention include the following compoumds: a maytansinoid or derivative such as mertansine (DM1) or DM4, cabazitaxel, SN-38, or doxorubicin. In some embodiments, the targeting moiety is selected from a SSTR-binding group, a NTSRl-binding group, or an IL1 IRa-binding group.

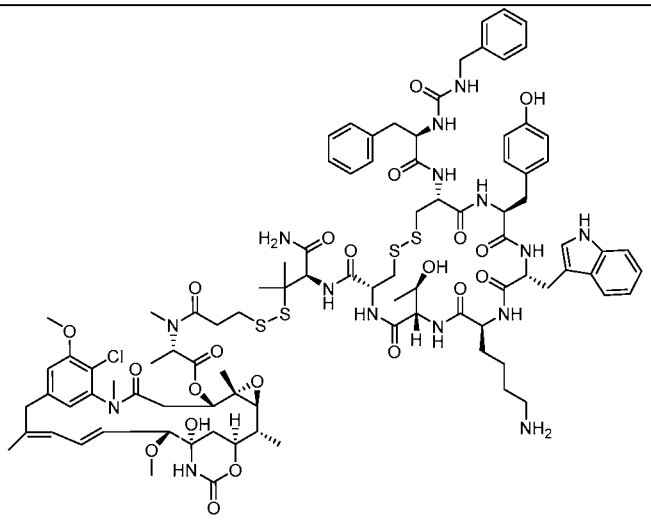
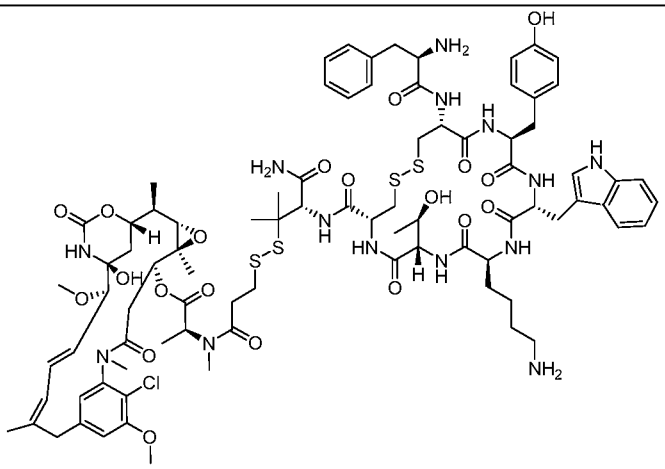
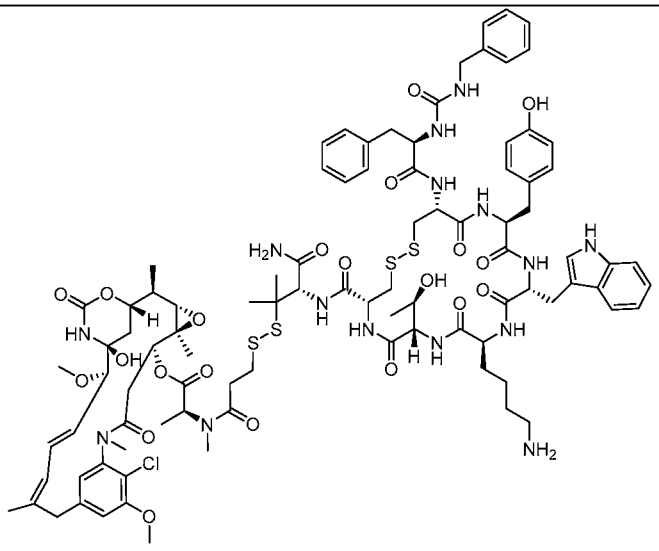
[00159] In some embodiments, the active agent Z is selected from DM1, DM4, cabazitaxel, SN-38, or doxorubin, and the targeting moiety X is a somatostatin receptor binding agent. X may be selected from somatostatin, cyclo(AA-Tyr-DTrp-Lys-Thr-Phe), octreotide, vapreotide or TATE. In some embodiments, the active agent Z is connected to the C-terminus of X with the linker Y. In some embodiments, the active agent Z is connected to the N-terminus of X with the linker Y. In some embodiments, the active agent Z is connected to X with the linker Y, wherein the targeting moiety X comprises at least one D-Phe residue and the phenyl ring of the D-Phe residue has been replaced by a group containing linker Y.

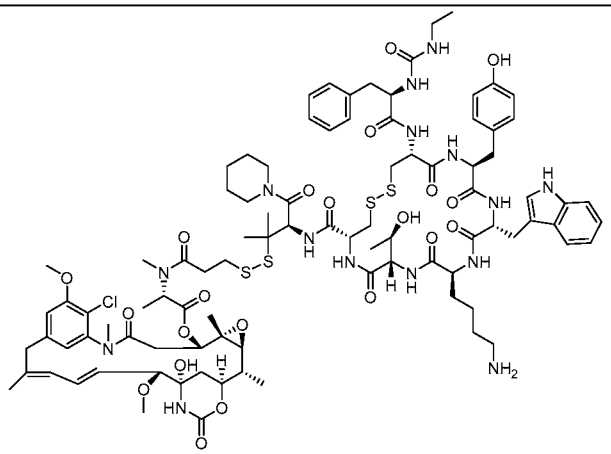
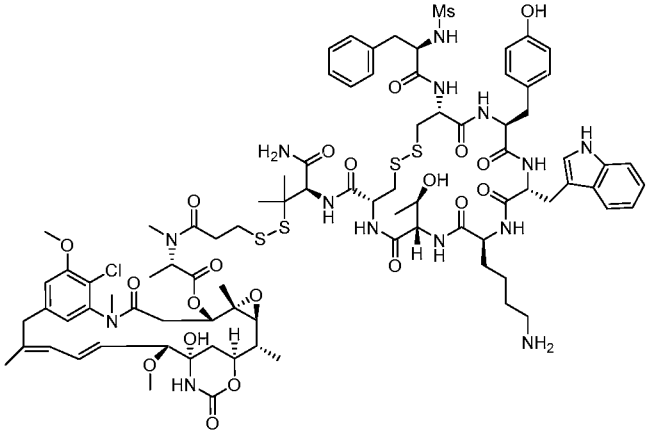
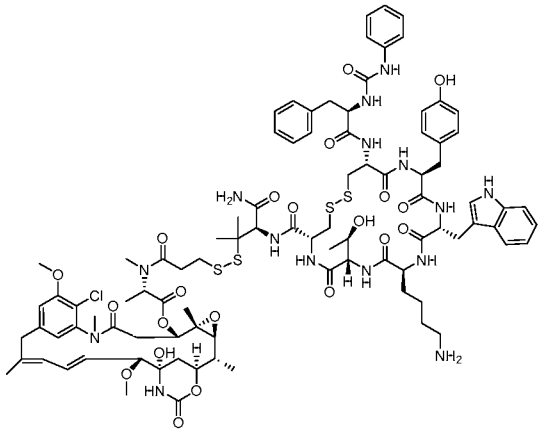
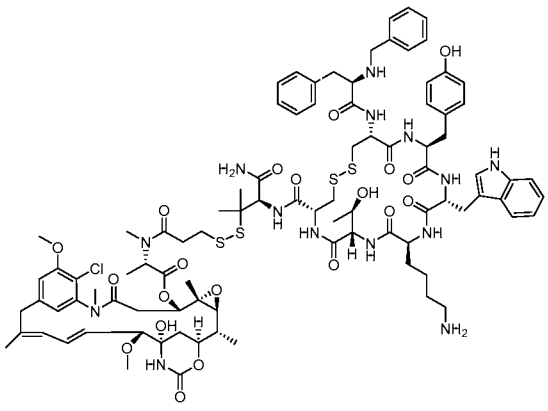
[00160] In some embodiments, the active agent Z is selected from DM1, DM4, cabazitaxel, SN-38, or doxorubin, and the targeting moiety X is a NTSR1 binding agent. X may comprise the targeting domain of neurotensin.

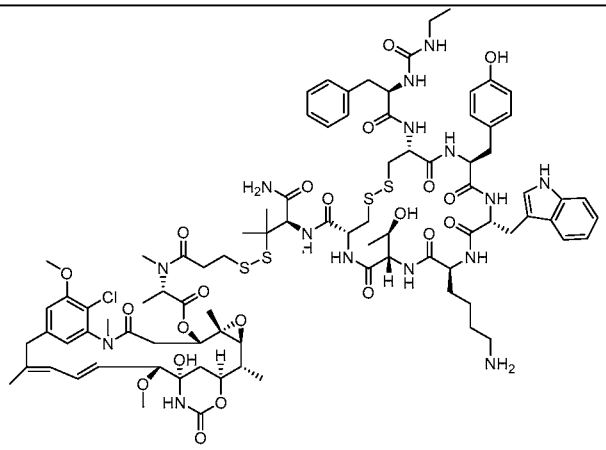
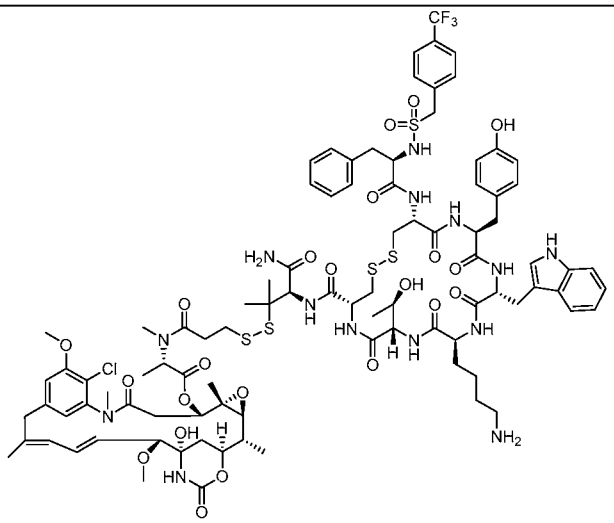
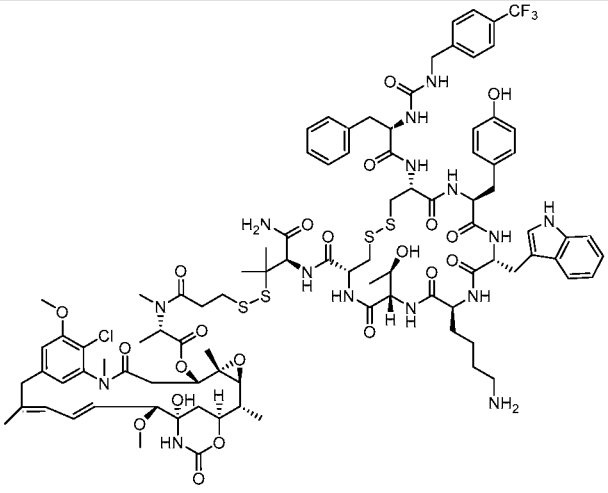
[00161] In some embodiments, the active agent Z is selected from DM1, DM4, cabazitaxel, SN-38, or doxorubin, and the targeting moiety X is a IL1 IRa binding agent. X may comprise IL1 1 or derivative thereof.

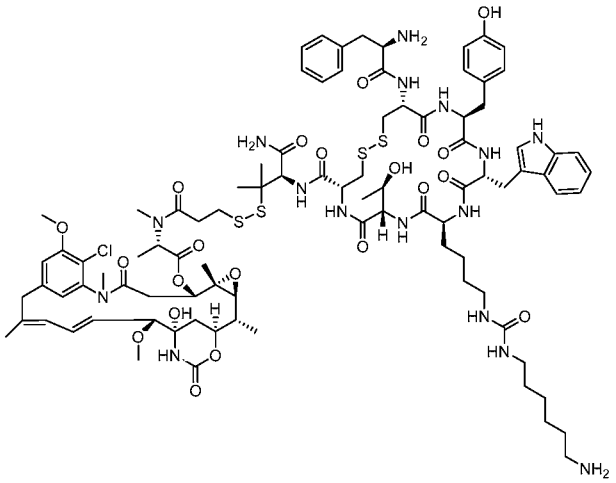
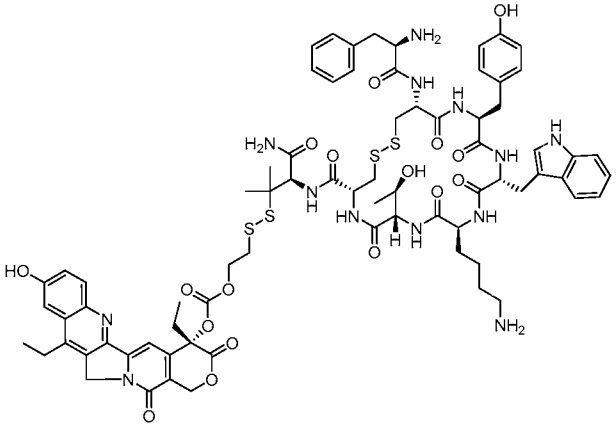
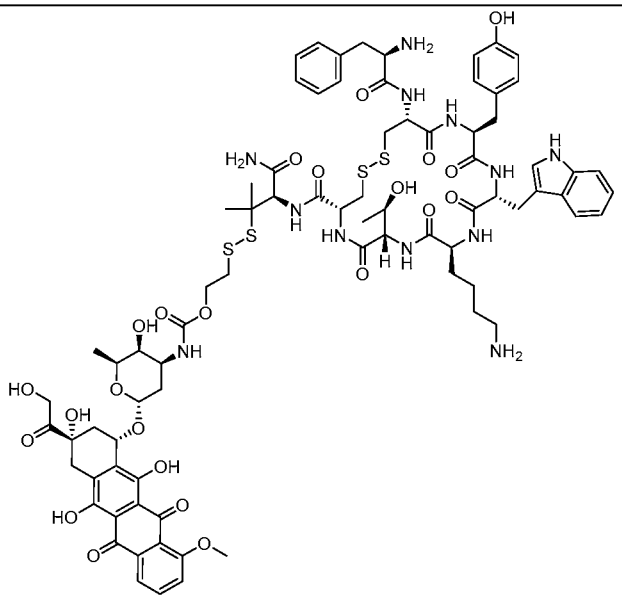
[00162] Non-limiting examples of conjugates of the present invention are included in Table 1.

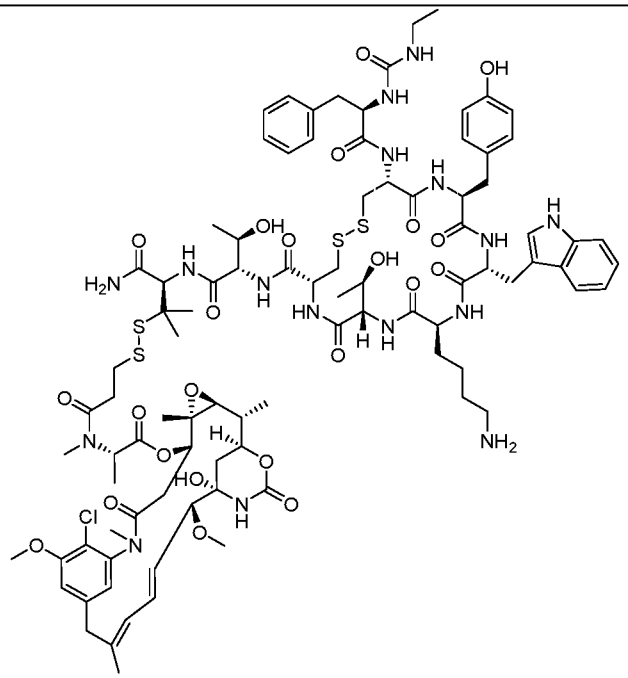
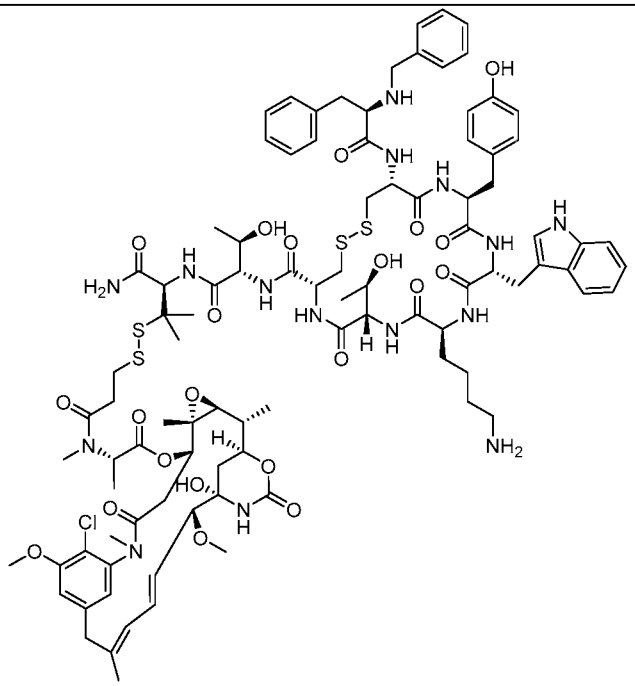
Table 1. Conjugates with Penicillamine Linkers

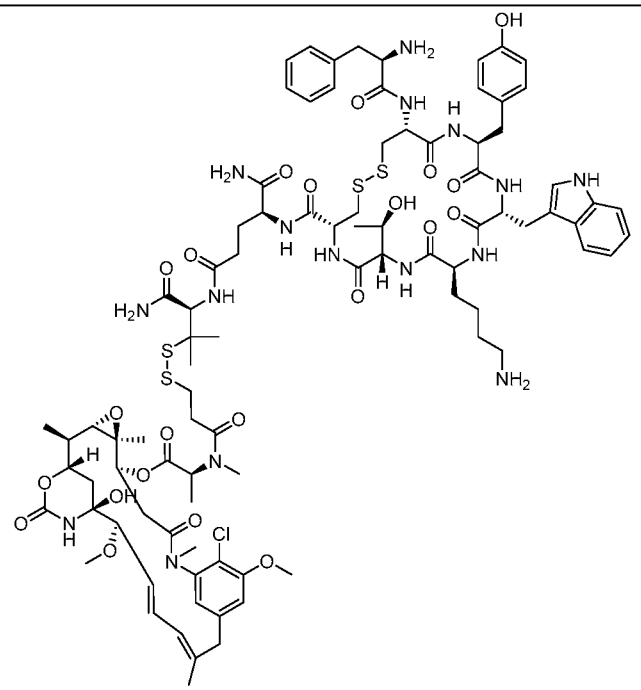
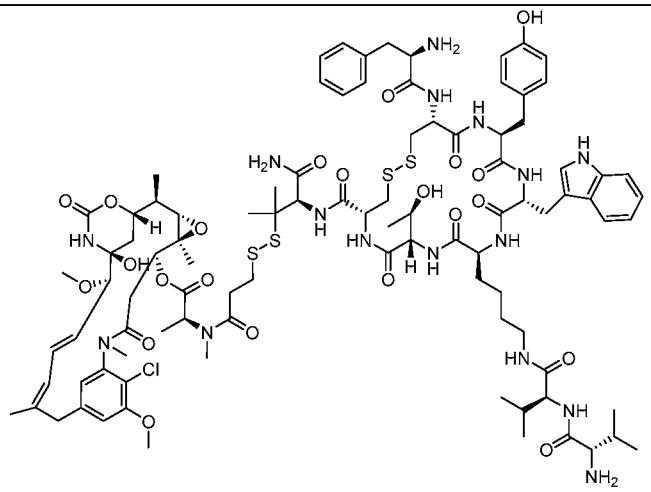
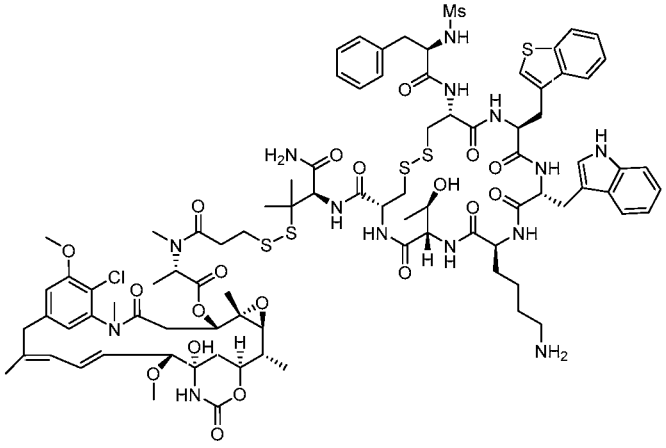
Target	Active Agent	Structure	Cmpd No.
SSTR2	DM1		**102**
SSTR2	DM1		**103**
SSTR2	DM1		**104**

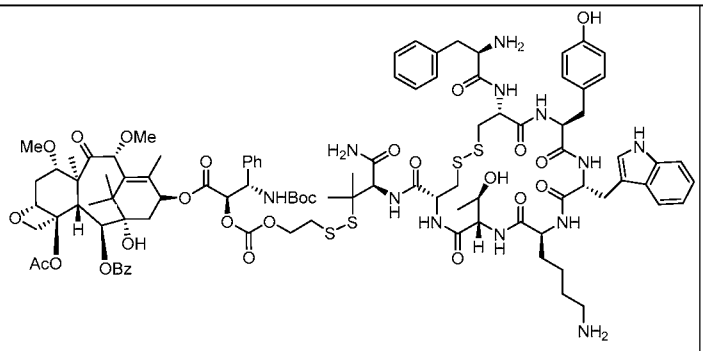
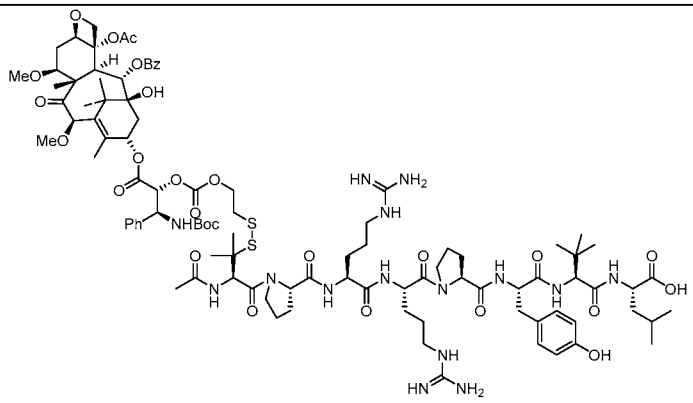
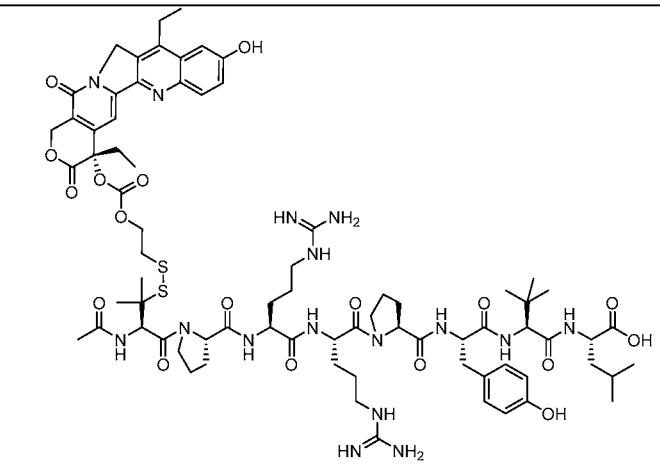
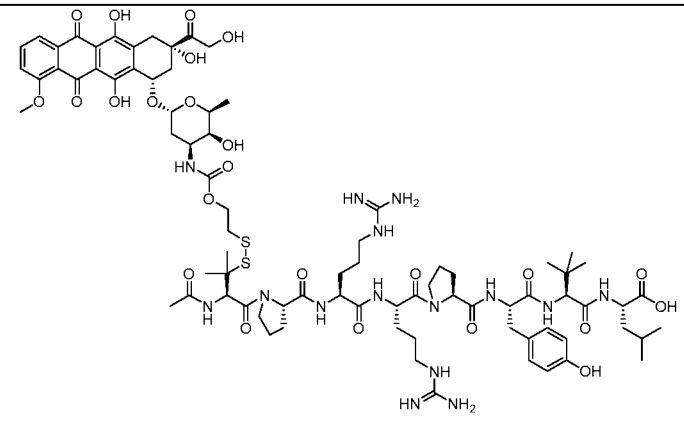
SSTR2	DM1		**105**
SSTR2	DM1		**111**
SSTR2	DM1		**112**
SSTR2	DM1		**113**

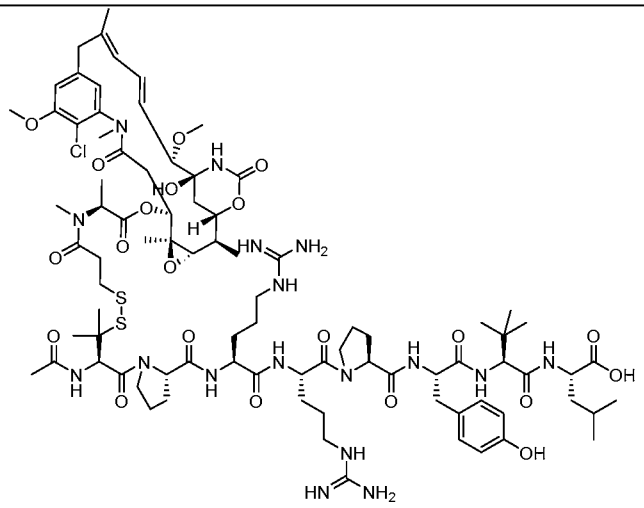
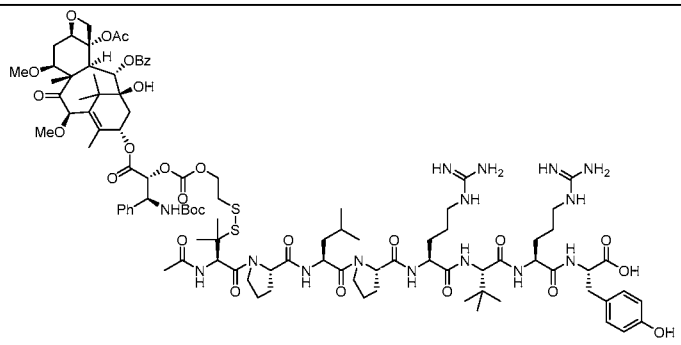
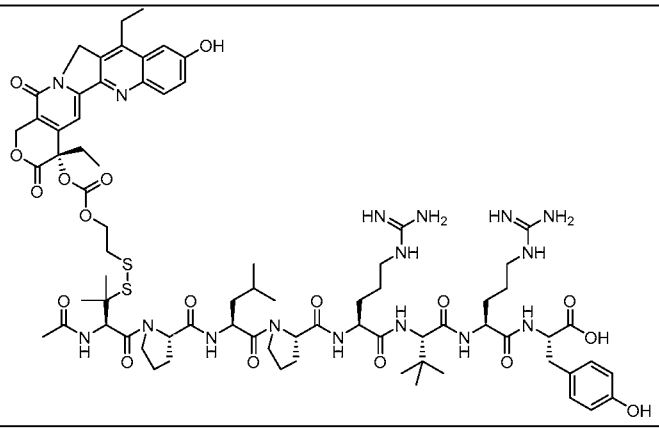
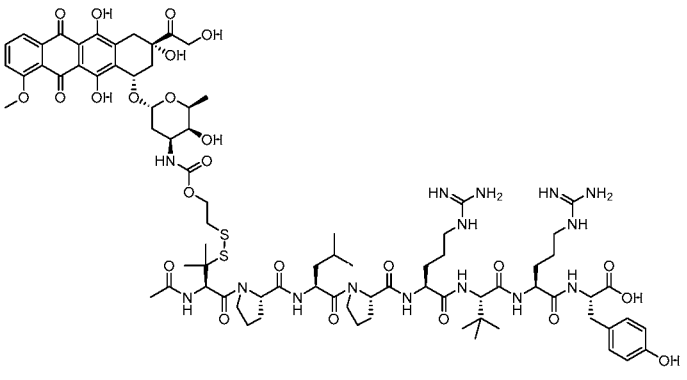
SSTR2	DM1		**114**
SSTR2	DM1		**115**
SSTR2	DM1		**116**

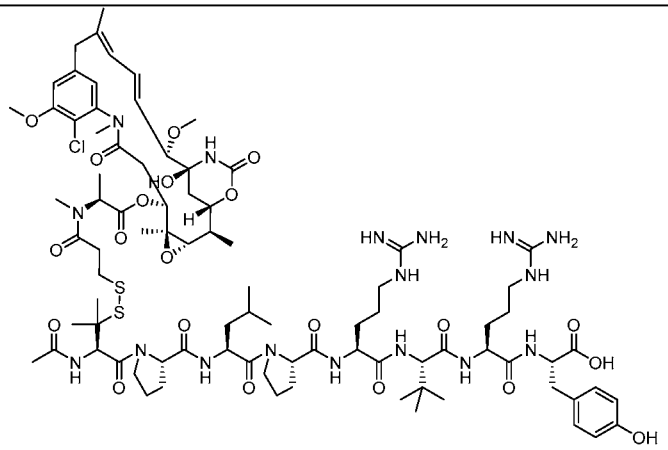
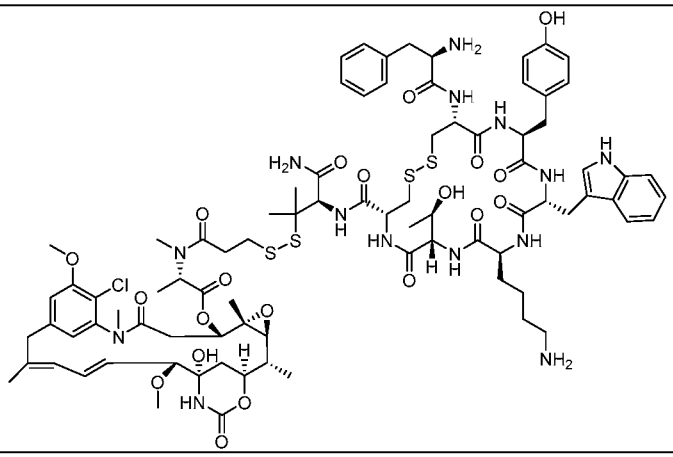
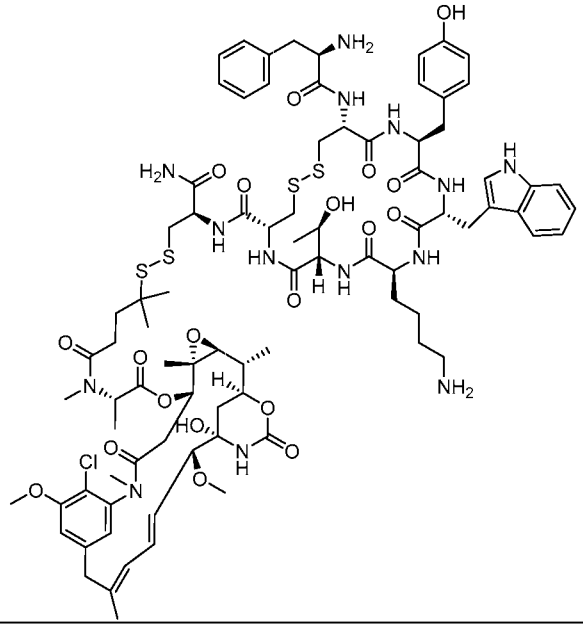
SSTR2 non-targeting control	DM1		**117**
SSTR2	SN-38		**118**
SSTR2	doxorubicin		**119**

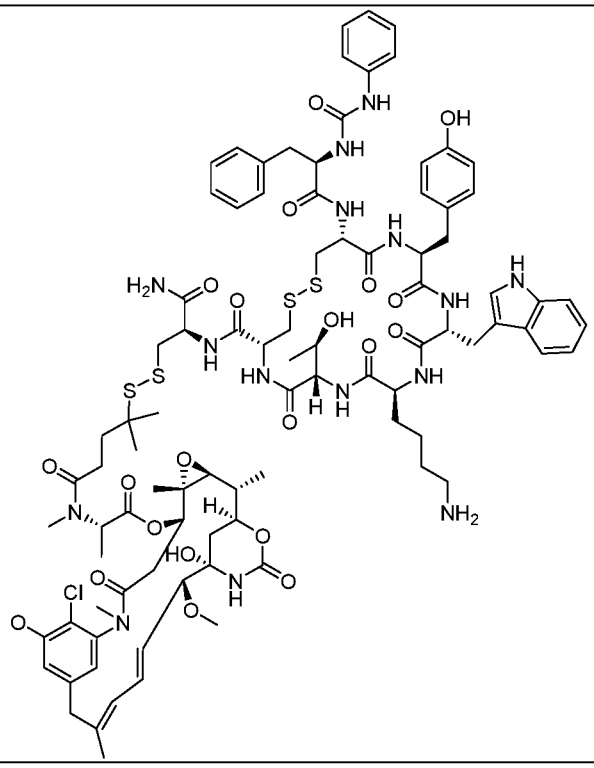
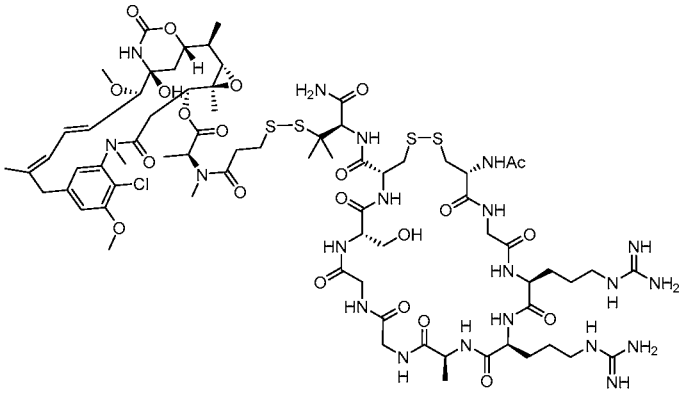
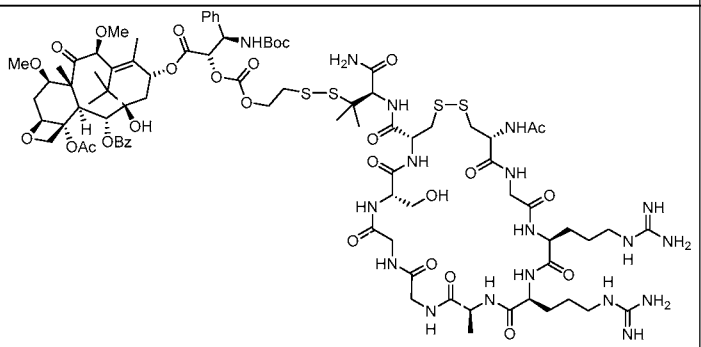
SSTR2	DM1		**120**
SSTR2	DM1		**121**

SSTR2	DM1		**122**
SSTR2 non-targeting control	DM1		**124**
SSTR2	DM1		**125**

SSTR2	Cabazitaxel		**126**
NTSR1	Cabazitaxel		**127**
NTSR1	SN-38		**128**
NTSR1	doxorubicin		**129**

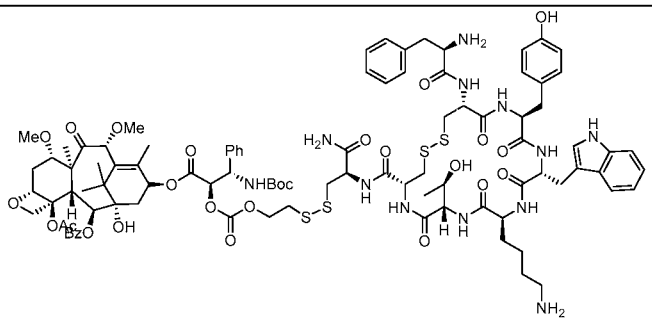
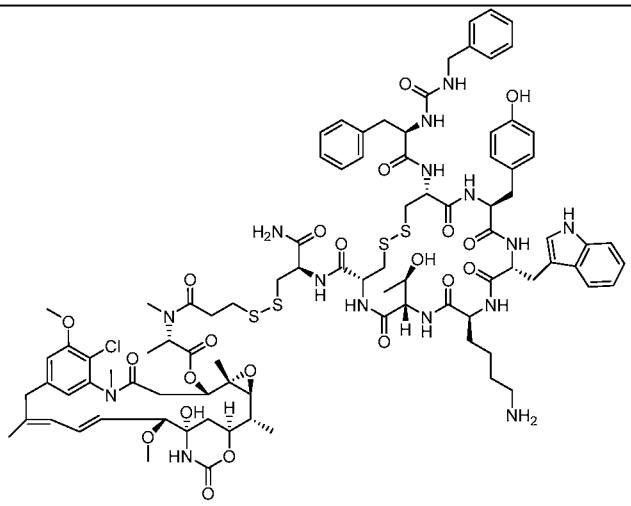
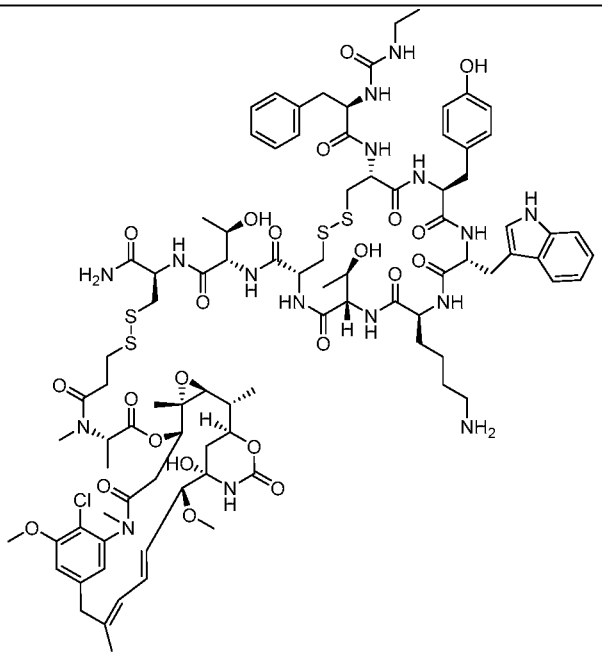
NTSR1	DM1		**130**
NTSR1 scrambled control	cabazitaxel		**131**
NTSR1 scrambled control	SN-38		**132**
NTSR1 scrambled control	doxorubicin		**133**

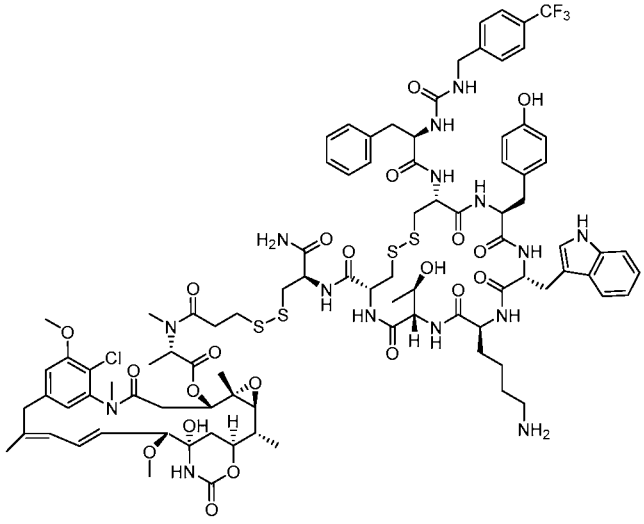
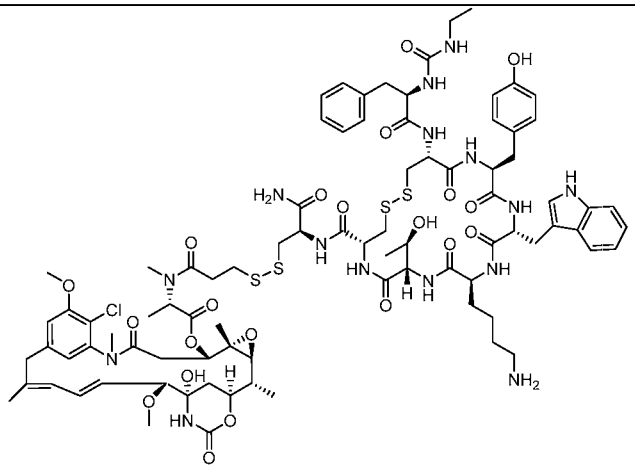
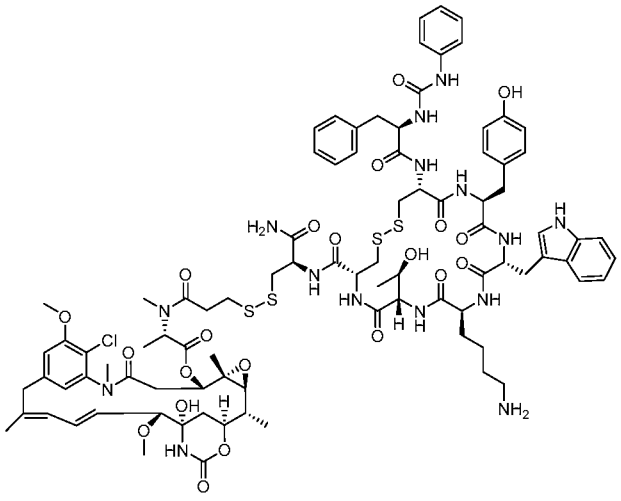
NTSR1 scrambled control	DM1		**134**
SSTR2	DM1		**135**
SSTR2	DM4		**137**

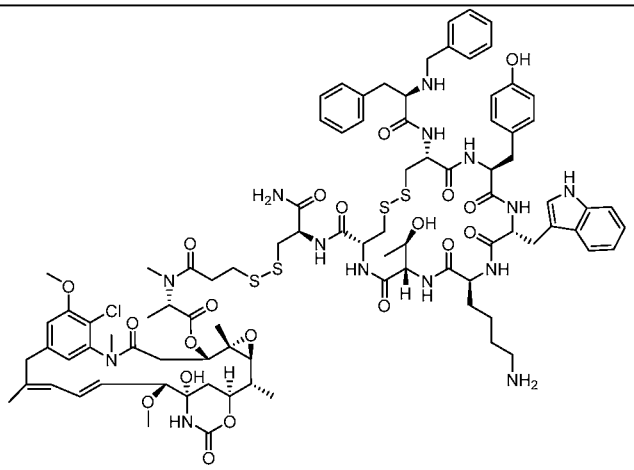
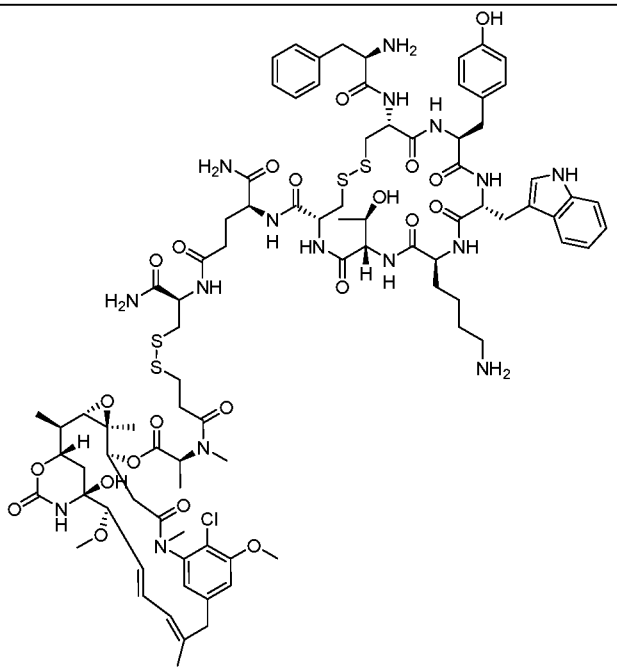
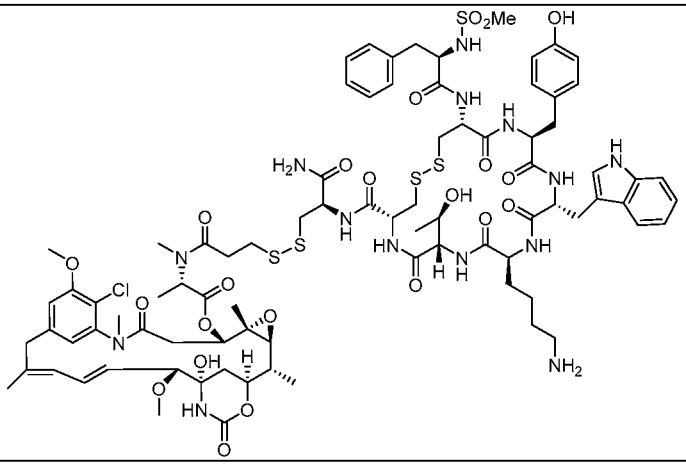
SSTR2	DM-4		**138**
IL-11Rα	DM1		**139**
IL-11Rα	cabazitaxel		**140**

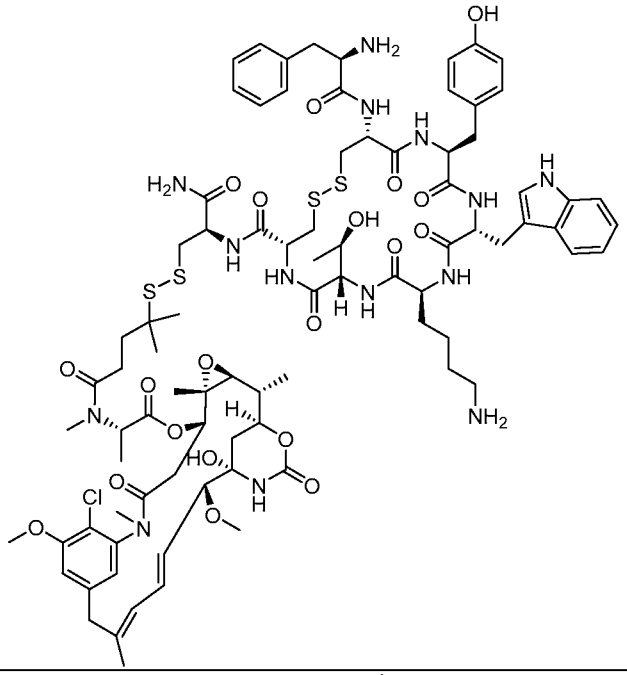
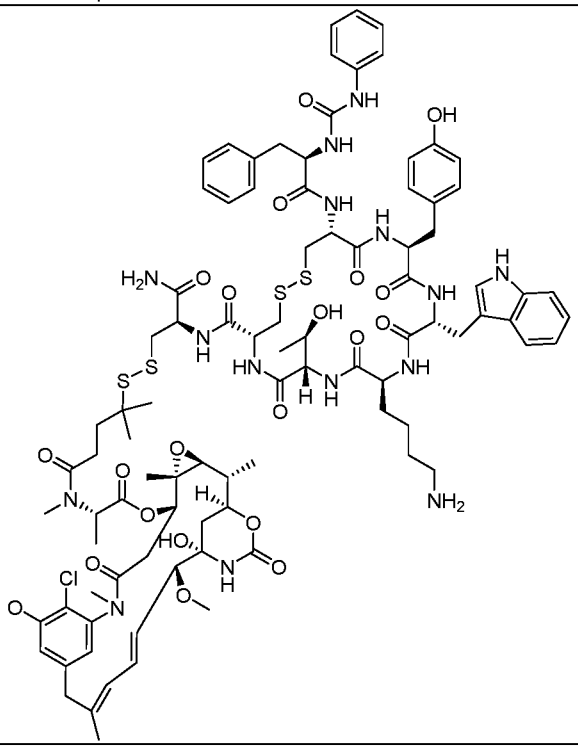
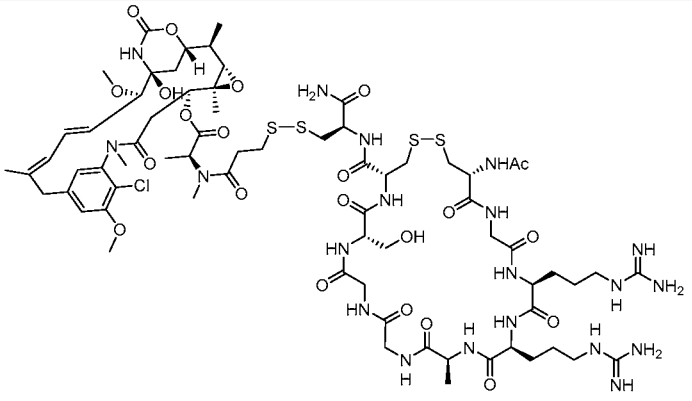
[00163] Analogous conjugates linked through cysteine linkers are included in Table 2.

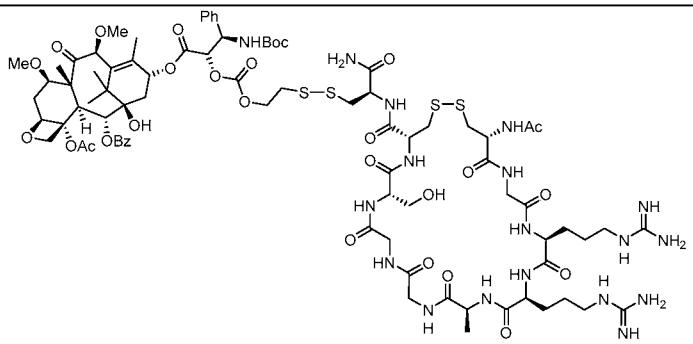
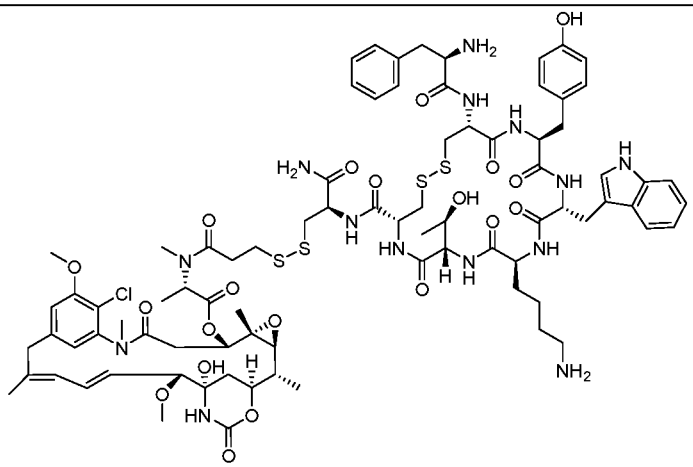
Table 2. Conjugates with Cysteine Linkers

Target	Active Agent	Structure	Cmpd No.
SSTR2	Cabazitaxel		**100**
SSTR2	DM1		**101**
SSTR2	DM1		**106**

SSTR2	DM1		**107**
SSTR2	DM1		**108**
SSTR2	DM1		**109**

SSTR2	DM1		**110**
SSTR2	DM1		**123**
SSTR2	DM1		**136**

SSTR2	DM4		**137**
SSTR2	DM-4		**138**
IL-11Rα	DM1		**141**

IL-11Rα	cabazitaxel		**142**
SSTR2	DM1		**200**

II. Particles

[00164] Another aspect of the invention is particles containing one or more conjugates. Particles can be polymeric particles, lipid particles, solid lipid particles, inorganic particles, or combinations thereof (e.g., lipid stabilized polymeric particles). In some embodiments, the particles are polymeric particles or contain a polymeric matrix. The particles can contain any of the polymers described herein or derivatives or copolymers thereof. The particles generally contain one or more biocompatible polymers. The polymers can be biodegradable polymers. The polymers can be hydrophobic polymers, hydrophilic polymers, or amphiphilic polymers. In some embodiments, the particles contain one or more polymers having an additional targeting moiety attached thereto.

[00165] The size of the particles can be adjusted for the intended application. The particles can be nanoparticles or microparticles. The particle can have a diameter of about 10 nm to about 10 microns, about 10 nm to about 1 micron, about 10 nm to about 500 nm, about 20 nm to about 500 nm, or about 25 nm to about 250 nm. In some embodiments, the particle is a nanoparticle having a diameter from about 25 nm

to about 250 nm. It is understood by those in the art that a plurality of particles will have a range of sizes and the diameter is understood to be the median diameter of the particle size distribution.

[00166] In various embodiments, a particle may be a nanoparticle, i.e., the particle has a characteristic dimension of less than about 1 micrometer, where the characteristic dimension of a particle is the diameter of a perfect sphere having the same volume as the particle. The plurality of particles can be characterized by an average diameter (e.g., the average diameter for the plurality of particles). In some embodiments, the diameter of the particles may have a Gaussian-type distribution. In some embodiments, the plurality of particles have an average diameter of less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 50 nm, less than about 30 nm, less than about 10 nm, less than about 3 nm, or less than about 1 nm. In some embodiments, the particles have an average diameter of at least about 5 nm, at least about 10 nm, at least about 30 nm, at least about 50 nm, at least about 100 nm, at least about 150 nm, or greater. In certain embodiments, the plurality of the particles have an average diameter of about 10 nm, about 25 nm, about 50 nm, about 100 nm, about 150 nm, about 200 nm, about 250 nm, about 300 nm, about 500 nm, or the like. In some embodiments, the plurality of particles have an average diameter between about 10 nm and about 500 nm, between about 50 nm and about 400 nm, between about 100 nm and about 300 nm, between about 150 nm and about 250 nm, between about 175 nm and about 225 nm, or the like. In some embodiments, the plurality of particles have an average diameter between about 10 nm and about 500 nm, between about 20 nm and about 400 nm, between about 30 nm and about 300 nm, between about 40 nm and about 200 nm, between about 50 nm and about 175 nm, between about 60 nm and about 150 nm, between about 70 nm and about 130 nm, or the like. For example, the average diameter can be between about 70 nm and 130 nm. In some embodiments, the plurality of particles have an average diameter between about 20 nm and about 220 nm, between about 30 nm and about 200 nm, between about 40 nm and about 180 nm, between about 50 nm and about 170 nm, between about 60 nm and about 150 nm, or between about 70 nm and about 130 nm. In one embodiment, the particles have a size of 40 to 120 nm with a zeta potential close to 0 mV at low to zero ionic strengths (1 to 10 mM), with zeta potential values between + 5 to - 5 mV, and a zero/neutral or a small -ve surface charge.

A. Conjugates

[00167] The particles contain one or more conjugates as described above. The conjugates can be present on the interior of the particle, on the exterior of the particle, or both. The particles may comprise hydrophobic ion-pairing complexes or hydrophobic ion-pairs formed by one or more conjugates described above and counterions.

[00168] Hydrophobic ion-pairing (HIP) is the interaction between a pair of oppositely charged ions held together by Coulombic attraction. HIP, as used here in, refers to the interaction between the conjugate of the present invention and its counterions, wherein the counterion is not H^+ or HO^- ions. Hydrophobic ion-pairing complex or hydrophobic ion-pair, as used herein, refers to the complex formed by the conjugate of the present invention and its counterions. In some embodiments, the counterions are hydrophobic. In some embodiments, the counterions are provided by a hydrophobic acid or a salt of a hydrophobic acid. In some embodiments, the counterions are provided by bile acids or salts, fatty acids or salts, lipids, or amino acids. In some embodiments, the counterions are negatively charged (anionic). Non-limited examples of negative charged counterions include the counterions sodium sulfosuccinate (AOT), sodium oleate, sodium dodecyl sulfate (SDS), human serum albumin (HSA), dextran sulphate, sodium deoxycholate, sodium cholate, anionic lipids, amino acids, or any combination thereof. Without wishing to be bound by any theory, in some embodiments, HIP may increase the hydrophobicity and/or lipophilicity of the conjugate of the present invention. In some embodiments, increasing the hydrophobicity and/or lipophilicity of the conjugate of the present invention may be beneficial for particle formulations and may provide higher solubility of the conjugate of the present invention in organic solvents. Without wishing to be bound by any theory, it is believed that particle formulations that include HIP pairs have improved formulation properties, such as drug loading and/or release profile. Without wishing to be bound by any theory, in some embodiments, slow release of the conjugate of the invention from the particles may occur, due to a decrease in the conjugate's solubility in aqueous solution. In addition, without wishing to be bound by any theory, complexing the conjugate with large hydrophobic counterions may slow diffusion of the conjugate within a polymeric matrix. In some emobodiments, HIP occurs without covalent conjuatation of the counterion to the conjugate of the present invention.

[00169] Without wishing to be bound by any theory, the strength of HIP may impact the drug load and release rate of the particles of the invention. In some embodiments, the strength of the HIP may be increased by increasing the magnitude of the difference between the pKa of the conjugate of the present invention and the pKa of the agent providing the counterion. Also without wishing to be bound by any theory, the conditions for ion pair formation may impact the drug load and release rate of the particles of the invention.

[00170] In some embodiments, any suitable hydrophobic acid or a combination thereof may form a HIP pair with the conjugate of the present invention. In some embodiments, the hydrophobic acid may be a carboxylic acid (such as but not limited to a monocarboxylic acid, dicarboxylic acid, tricarboxylic acid), a sulfinic acid, a sulfenic acid, or a sulfonic acid. In some embodiments, a salt of a suitable hydrophobic acid or a combination thereof may be used to form a HIP pair with the conjugate of the present invention. Examples of hydrophobic acids, saturated fatty acids, unsaturated fatty acids, aromatic acids, bile acid, polyelectrolyte, their dissociation constant in water (pKa) and logP values were disclosed in WO2014/043,625, the contents of which are incorporated herein by reference in their entirety. The strength of the hydrophobic acid, the difference between the pKa of the hydrophobic acid and the pKa of the conjuagate of the present invention, logP of the hydrophobic acid, the phase transition temperature of the hydrophobic acid, the molar ratio of the hydrophobic acid to the conjugate of the present invention, and the concentration of the hydrophobic acid were also disclosed in WO2014/043,625, the contents of which are incorporated herein by reference in their entirety.

[00171] In some embodiments, particles of the present invention comprising a HIP complex and/or prepared by a process that provides a counterion to form HIP complex with the conjugate may have a highter drug loading than particles without a HIP complex or prepared by a process that does not provide any counterion to form HIP complex with the conjugate. In some embodiments, drug loading may increase 50%, 100%, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, or 10 times.

[00172] In some embodiments, the particles of the invention may retain the conjugate for at least about 1 minute, at least about 15 minutes, at least about 1 hour, when placed in a phosphate buffer solution at 37°C.

[00173] In some embodiments, the weight percentage of the conjugate in the particles is at least about 0.05%, 0.1%, 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50%> such that the sum of the weight percentages of the components of the particles is 100%>. In some embodiments, the weight percentage of the conjugate in the particles is from about 0.5% to about 10%>, or about 10%> to about 20%, or about 20% to about 30%, or about 30% to about 40%, or about 40% to about 50%, or about 50% to about 60%, or about 60% to about 70%, or about 70% to about 80%, or about 80% to about 90%, or about 90% to about 99% such that the sum of the weight percentages of the components of the particles is 100%>.

[00174] In some instances, a conjugate may have a molecular weight of less than about 50,000 Da, less than about 40,000 Da, less than about 30,000 Da, less than about 20,000 Da, less than about 15,000 Da, less than about 10,000 Da, less than about 8,000 Da, less than about 5,000 Da, or less than about 3,000 Da. In some cases, the conjugate may have a molecular weight of between about 1,000 Da and about 50,000 Da, between about 1,000 Da and about 40,000 Da, in some embodiments between about 1,000 Da and about 30,000 Da, in some embodiments bout 1,000 Da and about 50,000 Da, between about 1,000 Da and about 20,000 Da, in some embodiments between about 1,000 Da and about 15,000 Da, in some embodiments between about 1,000 Da and about 10,000 Da, in some embodiments between about 1,000 Da and about 8,000 Da, in some embodiments between about 1,000 Da and about 5,000 Da, and in some embodiments between about 1,000 Da and about 3,000 Da. The molecular weight of the conjugate may be calculated as the sum of the atomic weight of each atom in the formula of the conjugate multiplied by the number of each atom. It may also be measured by mass spectrometry, NMR, chromatography, light scattering, viscosity, and/or any other methods known in the art. It is known in the art that the unit of molecular weight may be g/mol, Dalton (Da), or atomic mass unit (amu), wherein 1 g/mol = 1 Da = 1 amu.

B. Polymers

[00175] The particles may contain one or more polymers. Polymers may contain one more of the following polyesters: homopolymers including glycolic acid units, referred to herein as "PGA", and lactic acid units, such as poly-L-lactic acid, poly-D-lactic acid, poly-D,L-lactic acid, poly-L-lactide, poly-D-lactide, and poly-D,L-lactide, collectively referred to herein as "PLA", and caprolactone units, such as poly(s-

caprolactone), collectively referred to herein as "PCL"; and copolymers including lactic acid and glycolic acid units, such as various forms of poly(lactic acid-co-glycolic acid) and poly(lactide-co-glycolide) characterized by the ratio of lactic acid:glycolic acid, collectively referred to herein as "PLGA"; and polyacrylates, and derivatives thereof. Exemplary polymers also include copolymers of polyethylene glycol (PEG) and the aforementioned polyesters, such as various forms of PLGA-PEG or PLA-PEG copolymers, collectively referred to herein as "PEGylated polymers". In certain embodiments, the PEG region can be covalently associated with polymer to yield "PEGylated polymers" by a cleavable linker.

[00176] The particles may contain one or more hydrophilic polymers. Hydrophilic polymers include cellulosic polymers such as starch and polysaccharides; hydrophilic polypeptides; poly(amino acids) such as poly-L-glutamic acid (PGS), gamma-polyglutamic acid, poly-L-aspartic acid, poly-L-serine, or poly-L-lysine; polyalkylene glycols and polyalkylene oxides such as polyethylene glycol (PEG), polypropylene glycol (PPG), and poly(ethylene oxide) (PEO); poly(oxyethylated polyol); poly(olefinic alcohol); polyvinylpyrrolidone); poly(hydroxyalkylmethacrylamide); poly(hydroxyalkylmethacrylate); poly(saccharides); poly(hydroxy acids); poly(vinyl alcohol);polyoxazoline; and copolymers thereof.

[00177] The particles may contain one or more hydrophobic polymers. Examples of suitable hydrophobic polymers include polyhydroxyacids such as poly(lactic acid), poly(glycolic acid), and poly(lactic acid-coglycolic acids); polyhydroxyalkanoates such as poly3-hydroxybutyrate or poly4-hydroxybutyrate; polycaprolactones; poly(orthoesters); polyanhydrides; poly(phosphazenes); poly(lactide-co-caprolactones); polycarbonates such as tyrosine polycarbonates; polyamides (including synthetic and natural polyamides), polypeptides, and poly(amino acids); polyesteramides; polyesters; poly(dioxanones); poly(alkylene alkylates); hydrophobic polyethers; polyurethanes; polyetheresters; polyacetals; polycyanoacrylates; polyacrylates; polymethylmethacrylates; polysiloxanes; poly(oxyethylene)/poly(oxypropylene) copolymers; polyketals; polyphosphates; polyhydroxyvalerates; polyalkylene oxalates; polyalkylene succinates; poly(maleic acids), as well as copolymers thereof.

[00178] In certain embodiments, the hydrophobic polymer is an aliphatic polyester. In some embodiments, the hydrophobic polymer is poly(lactic acid), poly(glycolic acid), or poly(lactic acid-co-glycolic acid).

[00179] The particles can contain one or more biodegradable polymers. Biodegradable polymers can include polymers that are insoluble or sparingly soluble in water that are converted chemically or enzymatically in the body into water-soluble materials. Biodegradable polymers can include soluble polymers crosslinked by hydolyzable cross-linking groups to render the crosslinked polymer insoluble or sparingly soluble in water.

[00180] Biodegradable polymers in the particle can include polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and copolymers thereof, alkyl cellulose such as methyl cellulose and ethyl cellulose, hydroxyalkyl celluloses such as hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, and hydroxybutyl methyl cellulose, cellulose ethers, cellulose esters, nitro celluloses, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, polymers of acrylic and methacrylic esters such as poly (methyl methacrylate), poly(ethylmethacrylate), poly(butylmethacrylate), poly(isobutylmethacry late), poly(hexlmethacrylate), poly(isodecylmethacrylate), poly(lauryl methacrylate), poly (phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), poly(vinyl acetate, poly vinyl chloride polystyrene and polyvinylpyrrolidone, derivatives thereof, linear and branched copolymers and block copolymers thereof, and blends thereof. Exemplary biodegradable polymers include polyesters, poly(ortho esters), poly(ethylene imines), poly(caprolactones), poly(hydroxyalkanoates), poly(hydroxyvalerates), polyanhydrides, poly(acrylic acids), polyglycolides, poly(urethanes), polycarbonates, polyphosphate esters, polyphosphazenes, derivatives thereof, linear and branched copolymers and block copolymers thereof, and blends thereof. In some embodiments the particle contains biodegradable polyesters or polyanhydrides such as poly(lactic acid), poly(glycolic acid), and poly(lactic-co-glycolic acid).

[00181] The particles can contain one or more amphiphilic polymers. Amphiphilic polymers can be polymers containing a hydrophobic polymer block and a hydrophilic polymer block. The hydrophobic polymer block can contain one or more of the

hydrophobic polymers above or a derivative or copolymer thereof. The hydrophilic polymer block can contain one or more of the hydrophilic polymers above or a derivative or copolymer thereof. In some embodiments the amphiphilic polymer is a di-block polymer containing a hydrophobic end formed from a hydrophobic polymer and a hydrophilic end formed of a hydrophilic polymer. In some embodiments, a moiety can be attached to the hydrophobic end, to the hydrophilic end, or both. The particle can contain two or more amphiphilic polymers.

C. Lipids

[00182] The particles may contain one or more lipids or amphiphilic compounds. For example, the particles can be liposomes, lipid micelles, solid lipid particles, or lipid-stabilized polymeric particles. The lipid particle can be made from one or a mixture of different lipids. Lipid particles are formed from one or more lipids, which can be neutral, anionic, or cationic at physiologic pH. The lipid particle, in some embodiments, incorporates one or more biocompatible lipids. The lipid particles may be formed using a combination of more than one lipid. For example, a charged lipid may be combined with a lipid that is non-ionic or uncharged at physiological pH.

[00183] The particle can be a lipid micelle. Lipid micelles for drug delivery are known in the art. Lipid micelles can be formed, for instance, as a water-in-oil emulsion with a lipid surfactant. An emulsion is a blend of two immiscible phases wherein a surfactant is added to stabilize the dispersed droplets. In some embodiments the lipid micelle is a microemulsion. A microemulsion is a thermodynamically stable system composed of at least water, oil and a lipid surfactant producing a transparent and thermodynamically stable system whose droplet size is less than 1 micron, from about 10 nm to about 500 nm, or from about 10 nm to about 250 nm. Lipid micelles are generally useful for encapsulating hydrophobic active agents, including hydrophobic therapeutic agents, hydrophobic prophylactic agents, or hydrophobic diagnostic agents.

[00184] The particle can be a liposome. Liposomes are small vesicles composed of an aqueous medium surrounded by lipids arranged in spherical bilayers. Liposomes can be classified as small unilamellar vesicles, large unilamellar vesicles, or multi-lamellar vesicles. Multi-lamellar liposomes contain multiple concentric lipid bilayers. Liposomes can be used to encapsulate agents, by trapping hydrophilic agents in the aqueous interior or between bilayers, or by trapping hydrophobic agents within the bilayer.

[00185] The lipid micelles and liposomes typically have an aqueous center. The aqueous center can contain water or a mixture of water and alcohol. Suitable alcohols include, but are not limited to, methanol, ethanol, propanol, (such as isopropanol), butanol (such as «-butanol, isobutanol, *sec*-butanol, *tert*-butanol, pentanol (such as amyl alcohol, isobutyl carbinol), hexanol (such as 1-hexanol, 2-hexanol, 3-hexanol), heptanol (such as 1-heptanol, 2-heptanol, 3-heptanol and 4-heptanol) or octanol (such as 1-octanol) or a combination thereof.

[00186] The particle can be a solid lipid particle. Solid lipid particles present an alternative to the colloidal micelles and liposomes. Solid lipid particles are typically submicron in size, i.e. from about 10 nm to about 1 micron, from 10 nm to about 500 nm, or from 10 nm to about 250 nm. Solid lipid particles are formed of lipids that are solids at room temperature. They are derived from oil-in-water emulsions, by replacing the liquid oil by a solid lipid.

[00187] Suitable neutral and anionic lipids include, but are not limited to, sterols and lipids such as cholesterol, phospholipids, lysolipids, lysophospholipids, sphingolipids or pegylated lipids. Neutral and anionic lipids include, but are not limited to, phosphatidylcholine (PC) (such as egg PC, soy PC), including 1,2-diacyl-glycero-3-phosphocholines; phosphatidylserine (PS), phosphatidylglycerol, phosphatidylinositol (PI); glycolipids; sphingophospholipids such as sphingomyelin and sphingoglycolipids (also known as 1-ceramidyl glucosides) such as ceramide galactopyranoside, gangliosides and cerebrosides; fatty acids, sterols, containing a carboxylic acid group for example, cholesterol; 1,2-diacyl-sn-glycero-3-phosphoethanolamine, including, but not limited to, 1,2-dioleylphosphoethanolamine (DOPE), 1,2-dihexadecylphosphoethanolamine (DHPE), 1,2-distearoylphosphatidylcholine (DSPC), 1,2-dipalmitoyl phosphatidylcholine (DPPC), and 1,2-dimyristoylphosphatidylcholine (DMPC). The lipids can also include various natural (e.g., tissue derived L-a-phosphatidyl: egg yolk, heart, brain, liver, soybean) and/or synthetic (e.g., saturated and unsaturated l,2-diacyl-s«-glycero-3-phosphocholines, l-acyl-2-acyl-s«-glycero-3-phosphocholines, 1,2-diheptanoyl-SN-glycero-3-phosphocholine) derivatives of the lipids.

[00188] Suitable cationic lipids include, but are not limited to, N-[l-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium salts, also references as TAP lipids, for example methylsulfate salt. Suitable TAP lipids include, but are not limited to, DOTAP (dioleoyl-), DMTAP (dimyristoyl-), DPTAP (dipalmitoyl-), and DSTAP

(distearoyl-). Suitable cationic lipids in the liposomes include, but are not limited to, dimethyldioctadecyl ammonium bromide (DDAB), 1 ,2-diacyloxy-3-trimethylammonium propanes, N-[l-(2,3-dioloyloxy)propyl]-N,N-dimethyl amine (DODAP), 1 ,2-diacyloxy-3-dimethylammonium propanes, N-[l-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 1 ,2-dialkyloxy-3-dimethylammonium propanes, dioctadecylamidoglycylspermine (DOGS), 3 -[N-(N',N'-dimethylamino-ethane)carbamoyl]cholesterol (DC-Choi); 2,3-dioleoyloxy-N-(2-(sperminecarboxamido)-ethyl)-N,N-dimethyl- 1-propanaminium trifluoro-acetate (DOSPA), β-alanyl cholesterol, cetyl trimethyl ammonium bromide (CTAB), diCi4-amidine, N-ferf-butyl-N'-tetradecyl-3-tetradecylamino-propionamidine, N-(alpha-trimethylammonioacetyl)didodecyl-D-glutamate chloride (TMAG), ditetradecanoyl-N-(trimethylammonio-acetyl)diethanolamine chloride, 1 ,3-dioleoyloxy-2-(6-carboxy-spermyl)-propylamide (DOSPER), and N , N , N' , N'-tetramethyl- , N'-bis(2-hydroxylethyl)-2,3-dioleoyloxy-l ,4-butanediammonium iodide. In one embodiment, the cationic lipids can be l-[2-(acyloxy)ethyl]2-alkyl(alkenyl)-3-(2-hydroxyethyl)-imidazolinium chloride derivatives, for example, l-[2-(9(Z)-octadecenoyloxy)ethyl]-2-(8(Z)-heptadecenyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), and l-[2-(hexadecanoyloxy)ethyl]-2-pentadecyl-3-(2-hydroxyethyl)imidazolinium chloride (DPTIM). In one embodiment, the cationic lipids can be 2,3-dialkyloxypropyl quaternary ammonium compound derivatives containing a hydroxyalkyl moiety on the quaternary amine, for example, 1 ,2-dioleoyl-3-dimethyl-hydroxy ethyl ammonium bromide (DORI), 1 ,2-dioleyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide (DORIE), 1 ,2-dioleyloxypropyl-3-dimetyl-hydroxypropyl ammonium bromide (DORIE-HP), 1 ,2-dioleyl-oxy-propyl-3-dimethyl-hydroxybutyl ammonium bromide (DORIE-HB), 1 ,2-dioleyloxypropyl-3-dimethyl-hydroxypentyl ammonium bromide (DORIE-Hpe), 1 ,2-dimyristyloxypropyl-3-dimethyl-hydroxylethyl ammonium bromide (DMRIE), 1 ,2-dipalmityloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide (DPRIE), and 1 ,2-disteryloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DSRIE).

[00189] Suitable solid lipids include, but are not limited to, higher saturated alcohols, higher fatty acids, sphingolipids, synthetic esters, and mono-, di-, and triglycerides of higher saturated fatty acids. Solid lipids can include aliphatic alcohols having 10-40, for example, 12-30 carbon atoms, such as cetostearyl alcohol. Solid lipids can include higher fatty acids of 10-40, for example, 12-30 carbon atoms, such

as stearic acid, palmitic acid, decanoic acid, and behenic acid. Solid lipids can include glycerides, including monoglycerides, diglycerides, and triglycerides, of higher saturated fatty acids having 10-40, for example, 12-30 carbon atoms, such as glyceryl monostearate, glycerol behenate, glycerol palmitostearate, glycerol trilaurate, tricaprin, trilaurin, trimyristin, tripalmitin, tristearin, and hydrogenated castor oil. Suitable solid lipids can include cetyl palmitate, beeswax, or cyclodextrin.

[00190] Amphiphilic compounds include, but are not limited to, phospholipids, such as 1,2 distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), diarachidoylphosphatidylcholine (DAPC), dibehenoylphosphatidylcholine (DBPC), ditricosanoylphosphatidylcholine (DTPC), and dilignoceroylphatidylcholine (DLPC), incorporated at a ratio of between 0.01-60 (weight lipid/w polymer), for example, between 0.1-30 (weight lipid/w polymer). Phospholipids that may be used include, but are not limited to, phosphatidic acids, phosphatidyl cholines with both saturated and unsaturated lipids, phosphatidyl ethanolamines, phosphatidylglycerols, phosphatidylserines, phosphatidylinositols, lysophosphatidyl derivatives, cardiolipin, and β-acyl-y-alkyl phospholipids. Examples of phospholipids include, but are not limited to, phosphatidylcholines such as dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipentadecanoylphosphatidylcholine dilauroylphosphatidylcholine, dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), diarachidoylphosphatidylcholine (DAPC), dibehenoylphosphatidylcho- line (DBPC), ditricosanoylphosphatidylcholine (DTPC), dilignoceroylphatidylcholine (DLPC); and phosphatidylethanolamines such as dioleoylphosphatidylethanolamine or 1-hexadecyl-2-palmitoylglycerophos-phoethanolamine. Synthetic phospholipids with asymmetric acyl chains (e.g., with one acyl chain of 6 carbons and another acyl chain of 12 carbons) may also be used.

D. Additional Active Agents

[00191] The particles can contain one or more additional active agents in addition to those in the conjugates. The additional active agents can be therapeutic, prophylactic, diagnostic, or nutritional agents as listed above. The additional active agents can be present in any amount, e.g. from about 0.5% to about 90%, from about 0.5% to about 50%, from about 0.5% to about 25%, from about 0.5% to about 20%, from about 0.5% to about 10%, or from about 5% to about 10% (w/w) based upon the weight of

the particle. In one embodiment, the agents are incorporated in an about 0.5% to about 10% loading w/w.

E. Additional Targeting Moieties

[00192] The particles can contain one or more targeting moieties targeting the particle to a specific organ, tissue, cell type, or subcellular compartment in addition to the targeting moieties of the conjugate. The additional targeting moieties can be present on the surface of the particle, on the interior of the particle, or both. The additional targeting moieties can be immobilized on the surface of the particle, e.g., can be covalently attached to polymer or lipid in the particle. In some embodiments, the additional targeting moieties are covalently attached to an amphiphilic polymer or a lipid such that the targeting moieties are oriented on the surface of the particle.

III. Formulations

[00193] 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 or particles comprising the conjugates to be delivered as described herein.

[00194] 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.

[00195] 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.

[00196] A pharmaceutical composition in accordance with the invention 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.

[00197] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100%, e.g., between .5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

[00198] The conjugates or particles of the present invention 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 invention 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 invention may include one or more excipients, each in an amount that together increases the stability of the monomaleimide compounds.

Excipients

[00199] 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 invention.

[00200] 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.

[00201] 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.

[00202] 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.

[00203] 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.

[00204] 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.

[00205] 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.

[00206] 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®.

[00207] 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.

[00208] 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.

[00209] 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.

[00210] 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

[00211] The conjugates or particles of the present invention 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.

[00212] The formulations described herein contain an effective amount of conjugates or particles 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.

A. **Parenteral Formulations**

[00213] The conjugates or particles can be formulated for parenteral delivery, such as injection or infusion, in the form of a solution, suspension or emulsion. The formulation can be administered systemically, regionally or directly to the organ or tissue to be treated.

[00214] Parenteral formulations can be prepared as aqueous compositions using techniques is known in the art. Typically, such compositions can be prepared as injectable formulations, for example, solutions or suspensions; solid forms suitable for using to prepare solutions or suspensions upon the addition of a reconstitution medium prior to injection; emulsions, such as water-in-oil (w/o) emulsions, oil-in-water (o/w) emulsions, and microemulsions thereof, liposomes, or emulsomes.

[00215] The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, one or more polyols (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), oils, such as vegetable oils (e.g., peanut oil, corn oil, sesame oil, etc.), and combinations thereof. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. In some cases, an isotonic agent is included, for example, one or more sugars, sodium chloride, or other suitable agent known in the art.

[00216] Solutions and dispersions of the conjugates or particles can be prepared in water or another solvent or dispersing medium suitably mixed with one or more pharmaceutically acceptable excipients including, but not limited to, surfactants, dispersants, emulsifiers, pH modifying agents, and combinations thereof.

[00217] Suitable surfactants may be anionic, cationic, amphoteric or nonionic surface active agents. Suitable anionic surfactants include, but are not limited to, those containing carboxylate, sulfonate and sulfate ions. Examples of anionic surfactants include sodium, potassium, ammonium of long chain alkyl sulfonates and alkyl aryl sulfonates such as sodium dodecylbenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium dodecylbenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium bis-(2-ethylthioxyl)-sulfosuccinate; and alkyl sulfates such as sodium lauryl sulfate. Cationic surfactants include, but are not limited to, quaternary ammonium compounds such as benzalkonium chloride, benzethonium chloride, cetrimonium bromide, stearyl dimethylbenzyl ammonium chloride, polyoxyethylene and coconut amine. Examples of nonionic surfactants include ethylene glycol monostearate, propylene glycol myristate, glyceryl monostearate, glyceryl stearate, polyglyceryl-4-oleate, sorbitan acylate, sucrose acylate, PEG- 150 laurate, PEG-400 monolaurate, polyoxyethylene monolaurate, polysorbates, polyoxyethylene octylphenylether, PEG-1000 cetyl ether, polyoxyethylene tridecyl ether, polypropylene glycol butyl ether, Poloxamer® 401, stearoyl monoisopropanolamide, and polyoxyethylene hydrogenated tallow amide. Examples of amphoteric surfactants include sodium N-dodecyl -P-alanine, sodium N-lauryl -P-iminodipropionate, myristoamphoacetate, lauryl betaine and lauryl sulfobetaine.

[00218] The formulation can contain a preservative to prevent the growth of microorganisms. Suitable preservatives include, but are not limited to, parabens,

chlorobutanol, phenol, sorbic acid, and thimerosal. The formulation may also contain an antioxidant to prevent degradation of the active agent(s) or particles.

[00219] The formulation is typically buffered to a pH of 3-8 for parenteral administration upon reconstitution. Suitable buffers include, but are not limited to, phosphate buffers, acetate buffers, and citrate buffers. If using 10% sucrose or 5% dextrose, a buffer may not be required.

[00220] Water soluble polymers are often used in formulations for parenteral administration. Suitable water-soluble polymers include, but are not limited to, polyvinylpyrrolidone, dextran, carboxymethylcellulose, and polyethylene glycol.

[00221] Sterile injectable solutions can be prepared by incorporating the conjugates or particles in the required amount in the appropriate solvent or dispersion medium with one or more of the excipients listed above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized conjugates or particles into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those listed above. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation include vacuum-drying and freeze-drying techniques that yield a powder of the particle plus any additional desired ingredient from a previously sterile-filtered solution thereof. The powders can be prepared in such a manner that the particles are porous in nature, which can increase dissolution of the particles. Methods for making porous particles are known in the art.

[00222] Pharmaceutical formulations for parenteral administration can be in the form of a sterile aqueous solution or suspension of conjugates or particles formed from one or more polymer-drug conjugates. Acceptable solvents include, for example, water, Ringer's solution, phosphate buffered saline (PBS), and isotonic sodium chloride solution. The formulation may also be a sterile solution, suspension, or emulsion in a nontoxic, parenterally acceptable diluent or solvent such as 1,3-butanediol.

[00223] In some instances, the formulation is distributed or packaged in a liquid form. Alternatively, formulations for parenteral administration can be packed as a solid, obtained, for example by lyophilization of a suitable liquid formulation. The solid can be reconstituted with an appropriate carrier or diluent prior to administration.

[00224] Solutions, suspensions, or emulsions for parenteral administration may be buffered with an effective amount of buffer necessary to maintain a pH suitable for ocular administration. Suitable buffers are well known by those skilled in the art and some examples of useful buffers are acetate, borate, carbonate, citrate, and phosphate buffers.

[00225] Solutions, suspensions, or emulsions for parenteral administration may also contain one or more tonicity agents to adjust the isotonic range of the formulation. Suitable tonicity agents are well known in the art and some examples include glycerin, sucrose, dextrose, mannitol, sorbitol, sodium chloride, and other electrolytes.

[00226] Solutions, suspensions, or emulsions for parenteral administration may also contain one or more preservatives to prevent bacterial contamination of the ophthalmic preparations. Suitable preservatives are known in the art, and include polyhexamethylenebiguanidine (PHMB), benzalkonium chloride (BAK), stabilized oxychloro complexes (otherwise known as Purite®), phenylmercuric acetate, chlorobutanol, sorbic acid, chlorhexidine, benzyl alcohol, parabens, thimerosal, and mixtures thereof.

[00227] Solutions, suspensions, or emulsions for parenteral administration may also contain one or more excipients known art, such as dispersing agents, wetting agents, and suspending agents.

B. **Mucosal Topical Formulations**

[00228] The conjugates or particles can be formulated for topical administration to a mucosal surface Suitable dosage forms for topical administration include creams, ointments, salves, sprays, gels, lotions, emulsions, liquids, and transdermal patches. The formulation may be formulated for transmucosal transepithelial, or transendothelial administration. The compositions contain one or more chemical penetration enhancers, membrane permeability agents, membrane transport agents, emollients, surfactants, stabilizers, and combination thereof. In some embodiments, the conjugates or particles can be administered as a liquid formulation, such as a solution or suspension, a semi-solid formulation, such as a lotion or ointment, or a solid formulation. In some embodiments, the conjugates or particles are formulated as liquids, including solutions and suspensions, such as eye drops or as a semi-solid formulation, to the mucosa, such as the eye or vaginally or rectally.

[00229] "Surfactants" are surface-active agents that lower surface tension and thereby increase the emulsifying, foaming, dispersing, spreading and wetting

properties of a product. Suitable non-ionic surfactants include emulsifying wax, glyceryl monooleate, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polysorbate, sorbitan esters, benzyl alcohol, benzyl benzoate, cyclodextrins, glycerin monostearate, poloxamer, povidone and combinations thereof. In one embodiment, the non-ionic surfactant is stearyl alcohol.

[00230] "Emulsifiers" are surface active substances which promote the suspension of one liquid in another and promote the formation of a stable mixture, or emulsion, of oil and water. Common emulsifiers are: metallic soaps, certain animal and vegetable oils, and various polar compounds. Suitable emulsifiers include acacia, anionic emulsifying wax, calcium stearate, carbomers, cetostearyl alcohol, cetyl alcohol, cholesterol, diethanolamine, ethylene glycol palmitostearate, glycerin monostearate, glyceryl monooleate, hydroxpropyl cellulose, hypromellose, lanolin, hydrous, lanolin alcohols, lecithin, medium-chain triglycerides, methylcellulose, mineral oil and lanolin alcohols, monobasic sodium phosphate, monoethanolamine, nonionic emulsifying wax, oleic acid, poloxamer, poloxamers, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene stearates, propylene glycol alginate, self-emulsifying glyceryl monostearate, sodium citrate dehydrate, sodium lauryl sulfate, sorbitan esters, stearic acid, sunflower oil, tragacanth, triethanolamine, xanthan gum and combinations thereof. In one embodiment, the emulsifier is glycerol stearate.

[00231] Suitable classes of penetration enhancers are known in the art and include, but are not limited to, fatty alcohols, fatty acid esters, fatty acids, fatty alcohol ethers, amino acids, phospholipids, lecithins, cholate salts, enzymes, amines and amides, complexing agents (liposomes, cyclodextrins, modified celluloses, and diimides), macrocyclics, such as macrocylic lactones, ketones, and anhydrides and cyclic ureas, surfactants, N-methyl pyrrolidones and derivatives thereof, DMSO and related compounds, ionic compounds, azone and related compounds, and solvents, such as alcohols, ketones, amides, polyols (e.g., glycols). Examples of these classes are known in the art.

Dosing

[00232] The present invention provides methods comprising administering conjugates or particles containing the conjugate as described herein to a subject in need thereof. Conjugates or particles containing the 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.

[00233] Compositions in accordance with the invention are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present invention 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.

[00234] In some embodiments, compositions in accordance with the present invention 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.

[00235] The concentration of the conjugates or particles of the present invention 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.

[00236] 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 invention 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

[00237] 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).

Liquid dosage forms

[00238] Liquid dosage forms for parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art including, but not limited to, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. In certain embodiments for parenteral administration, compositions may be mixed with solubilizing agents such as CREMOPHOR®, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

Injectable

[00239] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art and may include suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed include, but are not limited to, water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

[00240] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00241] In order to prolong the effect of an active ingredient, it may be desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the monomaleimide compounds then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered monomaleimide compound may be accomplished by dissolving or suspending the monomalimide in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the monomaleimide compunds in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of monomaleimide compounds to polymer and the nature of the particular polymer employed, the rate of monomaleimide compound release can be controlled. Examples of other biodegradable polymers include, but are not limited to, poly(orthoesters) and poly(anhydrides). Depot injectable formulations may be prepared by entrapping the monomaleimide compounds in liposomes or microemulsions which are compatible with body tissues.

Pulmonary

[00242] Formulations described herein as being useful for pulmonary delivery may also be used for intranasal delivery of a pharmaceutical composition. Another

formulation suitable for intranasal administration may be a coarse powder comprising the active ingredient and having an average particle from about 0.2 μιη to 500 μιη. Such a formulation may be administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

[00243] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may, for example, contain about 0.1% to 20% (w/w) active ingredient, where the balance may comprise an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of any additional ingredients described herein.

[00244] General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference in its entirety).

Coatings or Shells

[00245] Solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

IV. Methods of Making Particles

[00246] In various embodiments, a method of making the particles includes providing a conjugate; providing a base component such as PLA-PEG or PLGA-PEG for forming a particle; combining the conjugate and the base component in an organic solution to form a first organic phase; and combining the first organic phase with a first aqueous solution to form a second phase; emulsifying the second phase to form an emulsion phase; and recovering particles. In various embodiments, the emulsion phase is further homogenized.

[00247] In some embodiments, the first phase includes about 5 to about 50% weight, e.g. about 1 to about 40% solids, or about 5 to about 30% solids, e.g. about 5%, 10%, 15%, and 20%, of the conjugate and the base component. In certain embodiments, the first phase includes about 5% weight of the conjugate and the base component. In various embodiments, the organic phase comprises acetonitrile, tetrahydrofuran, ethyl acetate, isopropyl alcohol, isopropyl acetate, dimethylformamide, methylene chloride, dichloromethane, chloroform, acetone, benzyl alcohol, TWEEN® 80, SPAN® 80, or a combination thereof. In some embodiments, the organic phase includes benzyl alcohol, ethyl acetate, or a combination thereof.

[00248] In various embodiments, the aqueous solution includes water, sodium cholate, ethyl acetate, or benzyl alcohol. In various embodiments, a surfactant is added into the first phase, the second phase, or both. A surfactant, in some instances, can act as an emulsifier or a stabilizer for a composition disclosed herein. A suitable surfactant can be a cationic surfactant, an anionic surfactant, or a nonionic surfactant. In some embodiments, a surfactant suitable for making a composition described herein includes sorbitan fatty acid esters, polyoxyethylene sorbitan fatty acid esters and polyoxyethylene stearates. Examples of such fatty acid ester nonionic surfactants are the TWEEN® 80, SPAN® 80, and MYJ® surfactants from ICI. SPAN® surfactants include C12-C18 sorbitan monoesters. TWEEN® surfactants include poly(ethylene oxide) C12-C18 sorbitan monoesters. MYJ® surfactants include poly(ethylene oxide) stearates. In certain embodiments, the aqueous solution also comprises a surfactant (e.g., an emulsifier), including a polysorbate. For example, the aqueous solution can include polysorbate 80. In some embodiments, a suitable surfactant includes a lipid-based surfactant. For example, the composition can include 1,2-dihexanoyl-sn-glycero-3 -phosphocholine, 1,2-diheptanoyl-sn-glycero-3 -

phosphocholine, PEGlyated l,2-distearoyl-sn-glycero-3-phosphoethanolamine (including PEG5000-DSPE), PEGlyated l,2-dioleoyl-sn-glycero-3-phosphoethanolamine (including l,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-5000] (ammonium salt)).

[00249] Emulsifying the second phase to form an emulsion phase may be performed in one or two emulsification steps. For example, a primary emulsion may be prepared, and then emulsified to form a fine emulsion. The primary emulsion can be formed, for example, using simple mixing, a high pressure homogenizer, probe sonicator, stir bar, or a rotor stator homogenizer. The primary emulsion may be formed into a fine emulsion through the use of e.g. a probe sonicator or a high pressure homogenizer, e.g. by pass(es) through a homogenizer. For example, when a high pressure homogenizer is used, the pressure used may be about 4000 to about 8000 psi, about 4000 to about 5000 psi, or 4000 or 5000 psi.

[00250] Either solvent evaporation or dilution may be needed to complete the extraction of the solvent and solidify the particles. For better control over the kinetics of extraction and a more scalable process, a solvent dilution via aqueous quench may be used. For example, the emulsion can be diluted into cold water to a concentration sufficient to dissolve all of the organic solvent to form a quenched phase. Quenching may be performed at least partially at a temperature of about 5°C or less. For example, water used in the quenching may be at a temperature that is less that room temperature (e.g. about 0 to about 10 °C, or about 0 to about 5 °C).

[00251] In various embodiments, the particles are recovered by filtration. For example, ultrafiltration membranes can be used. Exemplary filtration may be performed using a tangential flow filtration system. For example, by using a membrane with a pore size suitable to retain particles while allowing solutes, micelles, and organic solvent to pass, particles can be selectively separated. Exemplary membranes with molecular weight cut-offs of about 300-500 kDa (-5-25 nm) may be used.

[00252] In various embodiments, the particles are freeze-dried or lyophilized, in some instances, to extend their shelf life. In some embodiments, the composition also includes a lyoprotectant. In certain embodiments, a lyoprotectant is selected from a sugar, a polyalcohol, or a derivative thereof. In some embodiments, a lyoprotectant is selected from a monosaccharide, a disaccharide, or a mixture thereof. For example, a

lyoprotectant can be sucrose, lactulose, trehalose, lactose, glucose, maltose, mannitol, cellobiose, or a mixture thereof.

[00253] Methods of making particles containing one or more conjugates are provided. The particles can be polymeric particles, lipid particles, or combinations thereof. The various methods described herein can be adjusted to control the size and composition of the particles, e.g. some methods are best suited for preparing microparticles while others are better suited for preparing particles. The selection of a method for preparing particles having the descried characteristics can be performed by the skilled artisan without undue experimentation.

1. *Polymeric Particles*

[00254] Methods of making polymeric particles are known in the art. Polymeric particles can be prepared using any suitable method known in the art. Common microencapsulation techniques include, but are not limited to, spray drying, interfacial polymerization, hot melt encapsulation, phase separation encapsulation (spontaneous emulsion microencapsulation, solvent evaporation microencapsulation, and solvent removal microencapsulation), coacervation, low temperature microsphere formation, and phase inversion nanoencapsulation (PEST). A brief summary of these methods is presented below.

1. Spray Drying

[00255] Methods for forming polymeric particles using spray drying techniques are described in U.S. Patent No. 6,620,617. In this method, the polymer is dissolved in an organic solvent such as methylene chloride or in water. A known amount of one or more conjugates or additional active agents to be incorporated in the particles is suspended (in the case of an insoluble active agent) or co-dissolved (in the case of a soluble active agent) in the polymer solution. The solution or dispersion is pumped through a micronizing nozzle driven by a flow of compressed gas, and the resulting aerosol is suspended in a heated cyclone of air, allowing the solvent to evaporate from the microdroplets, forming particles. Microspheres/nanospheres ranging between 0.1 10 microns can be obtained using this method.

2. Interfacial Polymerization

[00256] Interfacial polymerization can also be used to encapsulate one or more conjugates and/or active agents. Using this method, a monomer and the conjugates or active agent(s) are dissolved in a solvent. A second monomer is dissolved in a second

solvent (typically aqueous) which is immiscible with the first. An emulsion is formed by suspending the first solution through stirring in the second solution. Once the emulsion is stabilized, an initiator is added to the aqueous phase causing interfacial polymerization at the interface of each droplet of emulsion.

3. Hot Melt Microencapsulation

[00257] Microspheres can be formed from polymers such as polyesters and polyanhydrides using hot melt microencapsulation methods as described in Mathiowitz et al., Reactive Polymers, 6:275 (1987). In some embodiments employing this method, polymers with molecular weights between 3,000-75,000 daltons are used. In this method, the polymer first is melted and then mixed with the solid particles of one or more active agents to be incorporated that have been sieved to less than 50 microns. The mixture is suspended in a non-miscible solvent (like silicon oil), and, with continuous stirring, heated to 5°C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decanting with petroleum ether to produce a free flowing powder.

4. Phase Separation Microencapsulation

[00258] In phase separation microencapsulation techniques, a polymer solution is stirred, optionally in the presence of one or more active agents to be encapsulated. While continuing to uniformly suspend the material through stirring, a nonsolvent for the polymer is slowly added to the solution to decrease the polymer's solubility. Depending on the solubility of the polymer in the solvent and nonsolvent, the polymer either precipitates or phase separates into a polymer rich and a polymer poor phase. Under proper conditions, the polymer in the polymer rich phase will migrate to the interface with the continuous phase, encapsulating the active agent(s) in a droplet with an outer polymer shell.

a. Spontaneous Emulsion Microencapsulation

[00259] Spontaneous emulsification involves solidifying emulsified liquid polymer droplets formed above by changing temperature, evaporating solvent, or adding chemical cross-linking agents. The physical and chemical properties of the encapsulant, as well as the properties of the one or more active agents optionally incorporated into the nascent particles, dictates suitable methods of encapsulation. Factors such as hydrophobicity, molecular weight, chemical stability, and thermal stability affect encapsulation.

b. Solvent Evaporation Microencapsulation

[00260] Methods for forming microspheres using solvent evaporation techniques are described in Mathiowitz et al., J. Scanning Microscopy, 4:329 (1990); Beck et al., Fertil. Steril., 31:545 (1979); Beck et al., Am. J. Obstet. Gynecol. 135(3) (1979); Benita et al., J. Pharm. Sci., 73:1721 (1984); and U.S. Patent No. 3,960,757. The polymer is dissolved in a volatile organic solvent, such as methylene chloride. One or more active agents to be incorporated are optionally added to the solution, and the mixture is suspended in an aqueous solution that contains a surface active agent such as poly(vinyl alcohol). The resulting emulsion is stirred until most of the organic solvent evaporated, leaving solid microparticles/nanoparticles. This method is useful for relatively stable polymers like polyesters and polystyrene.

c. Solvent Removal Microencapsulation

[00261] The solvent removal microencapsulation technique is primarily designed for polyanhydrides and is described, for example, in WO 93/21906. In this method, the substance to be incorporated is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent, such as methylene chloride. This mixture is suspended by stirring in an organic oil, such as silicon oil, to form an emulsion. Microspheres that range between 1-300 microns can be obtained by this procedure. Substances which can be incorporated in the microspheres include pharmaceuticals, pesticides, nutrients, imaging agents, and metal compounds.

5. Coacervation

[00262] Encapsulation procedures for various substances using coacervation techniques are known in the art, for example, in GB-B-929 406; GB-B-929 40 1; and U.S. Patent Nos. 3,266,987, 4,794,000, and 4,460,563. Coacervation involves the separation of a macromolecular solution into two immiscible liquid phases. One phase is a dense coacervate phase, which contains a high concentration of the polymer encapsulant (and optionally one or more active agents), while the second phase contains a low concentration of the polymer. Within the dense coacervate phase, the polymer encapsulant forms nanoscale or microscale droplets. Coacervation may be induced by a temperature change, addition of a non-solvent or addition of a micro-salt (simple coacervation), or by the addition of another polymer thereby forming an interpolymer complex (complex coacervation).

6. Low Temperature Casting of Microspheres

[00263] Methods for very low temperature casting of controlled release particles are described in U.S. Patent No. 5,019,400. In this method, a polymer is dissolved in a solvent optionally with one or more dissolved or dispersed active agents. The mixture is then atomized into a vessel containing a liquid non solvent at a temperature below the freezing point of the polymer substance solution which freezes the polymer droplets. As the droplets and non solvent for the polymer are warmed, the solvent in the droplets thaws and is extracted into the non solvent, resulting in the hardening of the microspheres.

7. Phase Inversion Nanoencapsulation (PIN)

[00264] Particles can also be formed using the phase inversion nanoencapsulation (PIN) method, wherein a polymer is dissolved in a "good" solvent, fine particles of a substance to be incorporated, such as a drug, are mixed or dissolved in the polymer solution, and the mixture is poured into a strong non solvent for the polymer, to spontaneously produce, under favorable conditions, polymeric microspheres, wherein the polymer is either coated with the particles or the particles are dispersed in the polymer. See, e.g., U.S. Patent No. 6,143,21 1. The method can be used to produce monodisperse populations of nanoparticles and microparticles in a wide range of sizes, including, for example, about 100 nanometers to about 10 microns.

[00265] Advantageously, an emulsion need not be formed prior to precipitation. The process can be used to form microspheres from thermoplastic polymers.

8. Emulsion methods

[00266] In some embodiments, a particle is prepared using an emulsion solvent evaporation method. For example, a polymeric material is dissolved in a water immiscible organic solvent and mixed with a drug solution or a combination of drug solutions. In some embodiments a solution of a therapeutic, prophylactic, or diagnostic agent to be encapsulated is mixed with the polymer solution. The polymer can be, but is not limited to, one or more of the following: PLA, PGA, PCL, their copolymers, polyacrylates, the aforementioned PEGylated polymers. The drug molecules can include one or more conjugates as described above and one or more additional active agents. The water immiscible organic solvent, can be, but is not limited to, one or more of the following: chloroform, dichloromethane, and acyl acetate. The drug can be dissolved in, but is not limited to, one or more of the following: acetone, ethanol, methanol, isopropyl alcohol, acetonitrile and Dimethyl sulfoxide (DMSO).

[00267] An aqueous solution is added into the resulting polymer solution to yield emulsion solution by emulsification. The emulsification technique can be, but not limited to, probe sonication or homogenization through a homogenizer.

9. Nanoprecipitation

[00268] In another embodiment, a conjugate containing nanoparticle is prepared using nanoprecipitation methods or microfluidic devices. The conjugate containing polymeric material is mixed with a drug or drug combinations in a water miscible organic solvent, optionally containing additional polymers. The additional polymer can be, but is not limited to, one or more of the following: PLA, PGA, PCL, their copolymers, polyacrylates, the aforementioned PEGylated polymers. The water miscible organic solvent, can be, but is not limited to, one or more of the following: acetone, ethanol, methanol, isopropyl alcohol, acetonitrile and dimethyl sulfoxide (DMSO). The resulting mixture solution is then added to a polymer non-solvent, such as an aqueous solution, to yield nanoparticle solution.

10. Microfluidics

[00269] Methods of making particles using microfluidics are known in the art. Suitable methods include those described in U.S. Patent Application Publication No. 2010/0022680 Al. In general, the microfluidic device comprises at least two channels that converge into a mixing apparatus. The channels are typically formed by lithography, etching, embossing, or molding of a polymeric surface. A source of fluid is attached to each channel, and the application of pressure to the source causes the flow of the fluid in the channel. The pressure may be applied by a syringe, a pump, and/or gravity. The inlet streams of solutions with polymer, targeting moieties, lipids, drug, payload, etc. converge and mix, and the resulting mixture is combined with a polymer non-solvent solution to form the particles having the desired size and density of moieties on the surface. By varying the pressure and flow rate in the inlet channels and the nature and composition of the fluid sources particles can be produced having reproducible size and structure.

11. *Lipid Particles*

[00270] Methods of making lipid particles are known in the art. Lipid particles can be lipid micelles, liposomes, or solid lipid particles prepared using any suitable method known in the art. Common techniques for created lipid particles encapsulating an active agent include, but are not limited to high pressure homogenization

techniques, supercritical fluid methods, emulsion methods, solvent diffusion methods, and spray drying. A brief summary of these methods is presented below.

1. High pressure homogenization (HPH) methods

[00271] High pressure homogenization is a reliable and powerful technique, which is used for the production of smaller lipid particles with narrow size distributions, including lipid micelles, liposomes, and solid lipid particles. High pressure homogenizers push a liquid with high pressure (100-2000 bar) through a narrow gap (in the range of a few microns). The fluid can contain lipids that are liquid at room temperature or a melt of lipids that are solid at room temperature. The fluid accelerates on a very short distance to very high velocity (over 1000 Km/h). This creates high shear stress and cavitation forces that disrupt the particles, generally down to the submicron range. Generally 5-10% lipid content is used but up to 40% lipid content has also been investigated.

[00272] Two approaches of HPH are hot homogenization and cold homogenization, work on the same concept of mixing the drug in bulk of lipid solution or melt.

a. Hot homogenization:

[00273] Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase is obtained by a high-shear mixing. HPH of the pre-emulsion is carried out at temperatures above the melting point of the lipid. A number of parameters, including the temperature, pressure, and number of cycles, can be adjusted to produce lipid particles with the desired size. In general, higher temperatures result in lower particle sizes due to the decreased viscosity of the inner phase. However, high temperatures increase the degradation rate of the drug and the carrier. Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size due to high kinetic energy of the particles.

b. Cold homogenization

[00274] Cold homogenization has been developed as an alternative to hot homogenization. Cold homogenization does not suffer from problems such as temperature-induced drug degradation or drug distribution into the aqueous phase during homogenization. The cold homogenization is particularly useful for solid lipid particles, but can be applied with slight modifications to produce liposomes and lipid micelles. In this technique the drug containing lipid melt is cooled, the solid lipid

ground to lipid microparticles and these lipid microparticles are dispersed in a cold surfactant solution yielding a pre-suspension. The pre-suspension is homogenized at or below room temperature, where the gravitation force is strong enough to break the lipid microparticles directly to solid lipid nanoparticles.

2. Ultrasonication/high speed homogenization methods

[00275] Lipid particles, including lipid micelles, liposomes, and solid lipid particles, can be prepared by ultrasonication/high speed homogenization. The combination of both ultrasonication and high speed homogenization is particularly useful for the production of smaller lipid particles. Liposomes are formed in the size range from 10 nm to 200 nm, for example, 50 nm to 100 nm, by this process.

3. Solvent evaporation methods

[00276] Lipid particles can be prepared by solvent evaporation approaches. The lipophilic material is dissolved in a water-immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent, particles dispersion is formed by precipitation of the lipid in the aqueous medium. Parameters such as temperature, pressure, choices of solvents can be used to control particle size and distribution. Solvent evaporation rate can be adjusted through increased/reduced pressure or increased/reduced temperature.

4. Solvent emulsification-diffusion methods

[00277] Lipid particles can be prepared by solvent emulsification-diffusion methods. The lipid is first dissolved in an organic phase, such as ethanol and acetone. An acidic aqueous phase is used to adjust the zeta potential to induce lipid coacervation. The continuous flow mode allows the continuous diffusion of water and alcohol, reducing lipid solubility, which causes thermodynamic instability and generates liposomes

5. Supercritical fluid methods

[00278] Lipid particles, including liposomes and solid lipid particles, can be prepared from supercritical fluid methods. Supercritical fluid approaches have the advantage of replacing or reducing the amount of the organic solvents used in other preparation methods. The lipids, active agents to be encapsulated, and excipients can be solvated at high pressure in a supercritical solvent. The supercritical solvent is most commonly CO2, although other supercritical solvents are known in the art. To increase solubility of the lipid, a small amount of co-solvent can be used. Ethanol is a common co-solvent, although other small organic solvents that are generally regarded

as safe for formulations can be used. The lipid particles, lipid micelles, liposomes, or solid lipid particles can be obtained by expansion of the supercritical solution or by injection into a non-solvent aqueous phase. The particle formation and size distribution can be controlled by adjusting the supercritical solvent, co-solvent, non-solvent, temperatures, pressures, etc.

6. Microemulsion based methods

[00279] Microemulsion based methods for making lipid particles are known in the art. These methods are based upon the dilution of a multiphase, usually two-phase, system. Emulsion methods for the production of lipid particles generally involve the formation of a water-in-oil emulsion through the addition of a small amount of aqueous media to a larger volume of immiscible organic solution containing the lipid. The mixture is agitated to disperse the aqueous media as tiny droplets throughout the organic solvent and the lipid aligns itself into a monolayer at the boundary between the organic and aqueous phases. The size of the droplets is controlled by pressure, temperature, the agitation applied and the amount of lipid present.

[00280] The water-in-oil emulsion can be transformed into a liposomal suspension through the formation of a double emulsion. In a double emulsion , the organic solution containing the water droplets is added to a large volume of aqueous media and agitated, producing a water-in-oil-in-water emulsion. The size and type of lipid particle formed can be controlled by the choice of and amount of lipid, temperature, pressure, co-surfactants, solvents, etc.

7. Spray drying methods

[00281] Spray drying methods similar to those described above for making polymeric particle can be employed to create solid lipid particles. Typically, this method is used with lipids with a melting point above 70°C.

[00282] In some embodiments, conjugates of the present invention may be encapsulated in polymeric particles using a single oil in water emulsion method. As a non-limiting example, the conjugate and a suitable polymer or block copolymer or a mixture of polymers/block copolymers, are dissolved in organic solvents such as, but not limited to, dichloromethane (DCM), ethyl acetate (EtAc) or choloform to form the oil phase. Co-solvents such as, but not limited to, dimethyl formamide (DMF), acetonitrile (CAN) or benzyl alcohol (BA) may be used to control the size of the particles and/or to solubilize the conjugate. Polymers used in the formulation may

include, but not limited to, PLA97-b-PEG5, PLA35-b-PEG5 and PLA16-b-PEG5 copolymers.

[00283] In some embodiments, particle formulations may be prepared by varying the lipophilicity of conjugates of the present invention. The lipophilicity may be varied by using hydrophobic ion-pairs or hydrophobic ion-paring (HIP) of the conjugates with different counterions. HIP alters the solubility of the conjugates of the present invention. The aqueous solubility may drop and the solubility in organic phases may increase.

[00284] Any suitable agent may be used to provide counterions to form HIP complex with the conjugate of the present invention. In some embodiments, the HIP complex may be formed prior to formulation of the particles.

V. Methods of Using the Conjugates and Particles

[00285] The conjugates or particles 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).

[00286] 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 or particles, as described herein, to a subject having a cancer, suspected of having cancer, or having a predisposition to a cancer. According to the present invention, 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.

[00287] In some embodiments, the cancer is a solid tumor. Large drug molecules have limited penetration in solid tumors. The penetration of large drug molecules is slow. On the other hand, small molecules such as conjugates of the present invention may penetrate solid tumors rapidly and more deeply. Regarding penetration depth of the drugs, larger molecules penetrate less, despite having more durable pharmacokinectics. Small molecules such as conjugates of the present invention penetrate deeper. Dreher et al. (Dreher et al., *JNCI,* vol.98(5):335 (2006), the contents of which are incorporated herein by reference in their entirety) studied penetration of

dextrans with different sizes into a tumor xenograft. As summarized in Fig. 6 and Table 1 of Dreher, Dextrans with a molecular weight of 3.3kDa or lOkDa showed rapid deep penetration into the tumor tissue (>35um from the vascular surface of the tumor). However, 40kDa, 70kDa or 2mDa sized dextrans penetrated much less than the 3.3kDa or lOkDa dextran. The 70kDa dextran reached only about 15um from the vascular surface of the tumor. Conjugates of the present invention have a molecule weight comparable to the 3.3kDa and lOkDa dextrans, while antibody drug conjugates have a molecule weight at least as big as the 70kDa dextran. Therefore, conjugates of the present invention may penetrate deep and rapidly into the core/center of the solid tumor.

[00288] In one embodiment, conjugates of the present invention reach at least about 25 μπι, about 30 μπι, about 35 μπι, about 40 μπι, about 45 μπι, about 50 μπι, about 75 μπι, about 100 μπι, about 150 μπι, about 200 μπι, about 250 μπι, about 300 μπι, about 400 μπι, about 500 μπι, about 600 μπι, about 700 μπι, about 800 μπι, about 900 μπι, about 1000 μπι, about 1100 μπι, about 1200 μπι, about 1300 μπι, about 1400 μιη or about 1500 μιη into the solid tumor from the vascular surface of the tumor. Zero distance is defined as the vascular surface of the tumor, and every distance greater than zero is defined as the distance measured in three dimensions to the nearest vascular surface.

[00289] In another embodiment, conjugates of the present invention penetrate to the core of the tumor. "Core" of the tumor, as used herein, refers to the central area of the tumor. The distance from any part of the core area of the tumor to the vascular surface of the tumor is between about 30% to about 50% of the length or width of the tumor. The distance from any part of the core area of the tumor to the center point of the tumor is less than about 20% of the length or width of the tumor. The core area of the tumor is roughly the center 1/3 of the tumor.

[00290] In another embodiment, conjugates of the present invention conjugates of the present invention penetrate to the middle of the solid tumor. "Middle" of the tumor, as sued herein, refers to the middle area of the tumor. The distance from any part of the middle area of the tumor to the vascular surface of the tumor is between about 15%, and about 30%> of the length or the width of the tumor. The distance from any part of the middle area of the tumor to the center point of the tumor is between about 20%, to about 35% of the length or width of the tumor. The middle area of the tumor is roughly between the center 1/3 of the tumor and the outer 1/3 of the tumor.

[00291] In some embodiments, the subject may be otherwise free of indications for treatment with the conjugates or particles. In some embodiments, methods include use of cancer cells, including but not limited to mammalian cancer cells. In some instances, the mammalian cancer cells are human cancer cells.

[00292] In some embodiments, the conjugates or particles 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.

[00293] In some embodiments, the conjugates or particles 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.

[00294] In some embodiments, the conjugates or particles provided herein are useful for inhibiting proliferation of a cancer cell. In some embodiments, the conjugates or particles 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 or particles 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 or particles of the present invention 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 or particles of the present invention 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 or particles of the present invention compared with cells with no treatment.

[00295] Furthermore, in some embodiments, conjugates or particles of the present invention 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.

[00296] In some embodiments the size of a tumor is reduced by about 60 % or more after treatment with conjugates or particles of the present invention. 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.

[00297] 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. 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.

[00298] 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.

[00299] In one embodiment, the conjugates or particles as described herein or formulations containing the conjugates or particles 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.

[00300] In some embodiments, the conjugates or particles as described herein or formulations containing the conjugates or particles as described herein are used to

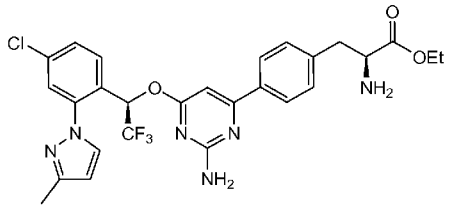
treat paitents with tumors that express or over-express somatostatn receptor, NTSR1, or lLHRa.

[00301] A feature of conjugates or particles of the present invention 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 or particles of the present invention may have lower toxicity than the active agent moiety Z administered alone. For conjugates comprsing DM1, their toxicity is lower than DM1 administered alone.

[00302] 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 or particles of the present invention 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 or particles of the present invention. In another embodiment, conjugates or particles of the present invention 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 or particles of the present invention. In yet another embodiment, conjugates or particles of the present invention do not cause a significant change of a subject's CBC or WBC count after treatment with conjugates or particles of the present invention. 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 or particles of the present invention.

[00303] In some embodiments, conjugates or particles of the present invention 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 or particles of the present invention do not have drug-drug interference with the additional active agent. In one embodiment, conjugates or particles of the present invention 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 or particles of the present invention.

[00304] 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 or particles of the present invention 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 or particles of the present invention.

Telotristat: 

[00305] In another example, conjugates or particles of the present invention 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).

[00306] The conjugates or particles as described herein or formulations containing the conjugates or particles 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 or particles of the present invention 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.

[00307] In various embodiments, a conjugate contained within a particle is released in a controlled manner. The release can be *in vitro* or *in vivo.* For example, particles can be subject to a release test under certain conditions, including those specified in the U.S. Pharmacopeia and variations thereof.

[00308] In various embodiments, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20% of the conjugate contained within particles is released in the first hour after the particles are exposed to the conditions of a release test. In some embodiments, less that about 90%, less than about 80%, less than about 70%, less than about 60%, or less than about 50% of the conjugate contained within particles is released in the first hour after the particles are exposed to the conditions of a release test. In certain embodiments, less than about 50% of the conjugate contained within particles is released in the first hour after the particles are exposed to the conditions of a release test.

[00309] With respect to a conjugate being released *in vivo,* for instance, the conjugate contained within a particle administered to a subject may be protected from a subject's body, and the body may also be isolated from the conjugate until the conjugate is released from the particle.

[00310] Thus, in some embodiments, the conjugate may be substantially contained within the particle until the particle is delivered into the body of a subject. For example, less than about 90%, less than about 80%, less than about 70%, less than

about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 1%, of the total conjugate is released from the particle prior to the particle being delivered into the body, for example, a treatment site, of a subject. In some embodiments, the conjugate may be released over an extended period of time or by bursts (e.g., amounts of the conjugate are released in a short period of time, followed by a periods of time where substantially no conjugate is released). For example, the conjugate can be released over 6 hours, 12 hours, 24 hours, or 48 hours. In certain embodiments, the conjugate is released over one week or one month.

VI. Kits and Devices

[00311] The invention provides a variety of kits and devices for conveniently and/or effectively carrying out methods of the present invention. 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.

[00312] In one embodiment, the present invention provides kits for inhibiting tumor cell growth in vitro or in vivo, comprising a conjugate and/or particle of the present invention or a combination of conjugates and/or particles of the present invention, optionally in combination with any other active agents.

[00313] 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 and/or particles in the buffer solution over a period of time and/or under a variety of conditions.

[00314] The present invention provides for devices which may incorporate conjugates and/or particles of the present invention. 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.

[00315] 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 and/or particles of the present invention according to single, multi- or

split-dosing regiments. The devices may be employed to deliver conjugates and/or particles of the present invention across biological tissue, intradermal, subcutaneously, or intramuscularly.

VII. Definitions

[00316] 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.

[00317] 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.

[00318] 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.

[00319] 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.

[00320] 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.

[00321] The terms "subject" or "patient", as used herein, refer to any organism to which the particles 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).

[00322] 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.

[00323] 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.

[00324] The "target cells" that may serve as the target for the method or conjugates or particles, 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 invention), 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.

[00325] 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.

[00326] 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.

[00327] "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.

[00328] "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.

[00329] "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.

[00330] "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.

[00331] 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.

[00332] 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.

[00333] 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. A prodrug may be converted into the parent drug by various mechanisms, including enzymatic processes and metabolic hydrolysis. Harper, N.J. (1962) Drug Latentiation in Jucker, ed. *Progress in Drug Research,* 4:221-294; Morozowich et al. (1977) Application of Physical Organic Principles to Prodrug Design in E. B. Roche ed. *Design of Biopharmaceutical Properties through Prodrugs and Analogs,* APhA; Acad. Pharm. Sci.; E. B. Roche, ed. (1977) *Bioreversible Carriers in Drug in Drug Design, Theory and Application,* APhA; H. Bundgaard, ed. (1985) *Design of Prodrugs,* Elsevier; Wang et al. (1999) Prodrug approaches to the improved delivery of peptide drug, Curr. Pharm. Design. 5(4):265-287; Pauletti et al. (1997) Improvement in peptide bioavailability: Peptidomimetics and Prodrug Strategies, Adv. Drug. Delivery Rev. 27:235-256; Mizen et al. (1998). The Use of Esters as Prodrugs for Oral Delivery of β-Lactam antibiotics, Pharm. Biotech. 11:345-365; Gaignault et al. (1996) Designing Prodrugs and Bioprecursors I. Carrier Prodrugs, Pract. Med. Chem. 671-696; M. Asgharnejad (2000). Improving Oral Drug Transport Via Prodrugs, in G. L. Amidon, P. I. Lee and E. M. Topp, Eds., *Transport Processes in Pharmaceutical Systems,* Marcell Dekker, p. 185-218; Balant et al. (1990) Prodrugs for the improvement of drug absorption via different routes of administration, *Eur. J. DrugMetab. Pharmacokinet.,* 15(2): 143-53; Balimane and Sinko (1999). Involvement of multiple transporters in the oral absorption of nucleoside analogues, *Adv. Drug Delivery Rev.,* 39(l-3):183-209; Browne (1997). Fosphenytoin (Cerebyx), *Clin. Neuropharmacol* . 20(1): 1-12; Bundgaard (1979). Bioreversible derivatization of drugs—principle and applicability to improve the therapeutic effects of drugs, *Arch. Pharm. Chemi.* 86(1): 1-39; H. Bundgaard, ed. (1985) *Design of Prodrugs,* New York: Elsevier; Fleisher et al. (1996) Improved oral drug delivery: solubility limitations overcome by the use of prodrugs, *Adv. Drug Delivery Rev.* 19(2): 115-130; Fleisher et al. (1985) Design of prodrugs for improved gastrointestinal absorption by intestinal enzyme targeting, *Methods Enzymol.* 112: 360-81; Farquhar D, et al. (1983) Biologically Reversible Phosphate-Protective Groups, *J. Pharm. Sci.,* 72(3): 324-325; Han, H.K. et al. (2000) Targeted prodrug design to optimize drug delivery, *AAPS PharmSci.,* 2(1): E6; Sadzuka Y. (2000) Effective prodrug liposome and conversion to active metabolite, *Curr. DrugMetab.,* 1(1):3 1-48; D.M. Lambert (2000) Rationale and applications of lipids as prodrug carriers, *Eur. J. Pharm. Sci.,* 11 Suppl. 2:SI5-27;

Wang, W. et al. (1999) Prodmg approaches to the improved delivery of peptide drugs. *Curr. Pharm. Des.,* 5(4):265-87.

[00334] 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.

[00335] 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.

[00336] 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.

[00337] 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.

[00338] 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.

[00339] The term "hydrophilic", as used herein, refers to substances that have strongly polar groups that readily interact with water.

[00340] 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.

[00341] The term "lipophilic", as used herein, refers to compounds having an affinity for lipids.

[00342] 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.

[00343] 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.

[00344] 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.

[00345] 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-biph8nylyl)-2-propy!oxycarbonyl, 2- bromobenzyloxycarbonyl, tert-butyh tert-butyloxycarbonyl, 1-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, a-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.

[00346] 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.

[00347] 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.

[00348] 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.

[00349] 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.

[00350] 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.

[00351] 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.

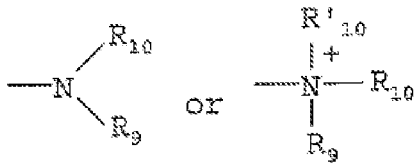
[00352] The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In some embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, and -S-alkynyl. Representative alkylthio

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.

[00353] 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.

[00354] 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-aiyl or O-heteroaiyl, wherein aryl and heteroaryl are as defined below. The alkoxy and aroxy groups can be substituted as described above for alkyl.

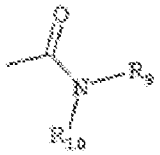
[00355] The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formula:



wherein R9, Rio, and Rio each independently represent a hydrogen, an alkyl, an alkenyl, -(CH2)m-Rs or R9 and Rio taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R8 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 R9 or Rio can be a carbonyl, e.g., R9, Rio 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 R9 and Rio represents a carbonyl. In additional embodiments, R9 and Rio (and optionally R'io) 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 Rio is an alkyl group.

[00356] 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 Rio are as defined above.

[00357] "Aryl", as used herein, refers to Cs-Cio-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, -CF3, -CN; and combinations thereof.

[00358] 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*-l,5,2-dithiazinyl, dihydrofuro[2,3 b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, *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*-l,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".

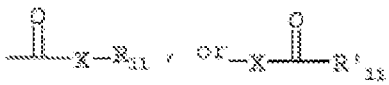
[00359] The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

[00360] The term "carbocycle", as used herein, refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.

[00361] "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, (Ci-Cio) 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*-l,5,2-dithiazinyl, dihydrofuro[2,3-£]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, l*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*-l,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.

[00362] 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'n represents a hydrogen, an alkyl, a cycloalkyl, an alkenyl, an cycloalkenyl, or an alkynyl. Where X is an oxygen and R_{11} or R'n 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'n 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'n is not hydrogen, the formula represents a "thioester." Where X is a sulfur and Rn is hydrogen, the formula represents a "thiocarboxylic acid." Where X is a sulfur and R'n is hydrogen, the formula represents a "thioformate." On the other hand, where X is a bond, and Rn 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.

[00363] 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.

[00364] 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.

[00365] As used herein, the term "nitro" means -NO2; the term "halogen" designates -F, -CI, -Br or -I; the term "sulfhydryl" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means -SO2-.

[00366] 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, C3-C20 cyclic, substituted C3-C20 cyclic, heterocyclic, substituted heterocyclic, aminoacid, peptide, and polypeptide groups.

[00367] 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.

[00368] 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.

[00369] 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.

[00370] 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.

[00371] 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.

[00372] The term "mean particle size", as used herein, generally refers to the statistical mean particle size (diameter) of the particles in the composition. The diameter of an essentially spherical particle may be referred to as the physical or hydrodynamic diameter. The diameter of a non-spherical particle may refer to the hydrodynamic diameter. As used herein, the diameter of a non-spherical particle may refer to the largest linear distance between two points on the surface of the particle. Mean particle size can be measured using methods known in the art such as dynamic light scattering. Two populations can be said to have a "substantially equivalent mean particle size" when the statistical mean particle size of the first population of particles is within 20% of the statistical mean particle size of the second population of particles; for example, within 15%, or within 10%.

[00373] The terms "monodisperse" and "homogeneous size distribution", as used interchangeably herein, describe a population of particles, microparticles, or nanoparticles all having the same or nearly the same size. As used herein, a monodisperse distribution refers to particle distributions in which 90% of the distribution lies within 5% of the mean particle size.

[00374] 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.

[00375] 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.

[00376] 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.

[00377] 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.

[00378] 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.

[00379] 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., l,l'-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.

[00380] 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.

[00381] A pharmaceutically acceptable salt can be derived from an acid selected from l-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, dodecyl sulfuric 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,

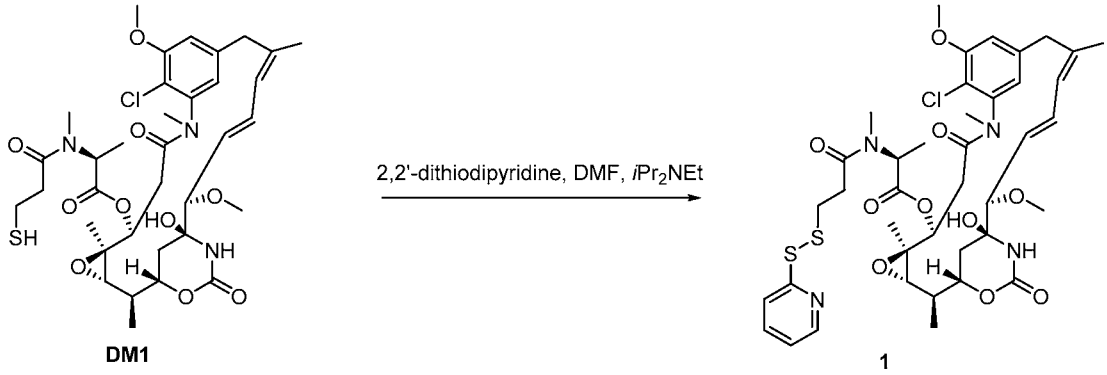
malonic acid, mandelic acid, methanesulfonic acid, mucic, naphthalene- 1,5-disulfonic acid, naphthalene-2-sulfonic acid, nicotinic acid, nitric acid, oleic acid, oxalic acid, palmitic acid, pamoic acid, pantothenic, phosphoric acid, proprionic acid, pyroglutamic acid, salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, tartaric acid, thiocyanic acid, toluenesulfonic acid, trifluoroacetic, and undecylenic acid.

[00382] The term "bioavailable" is art-recognized and refers to a form of the subject invention 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.

[00383] It will be appreciated that the following examples are intended to illustrate but not to limit the present invention. 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 invention, 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

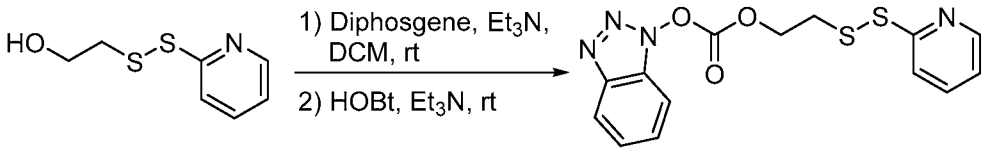
EXAMPLE 1: Synthesis of the conjugates



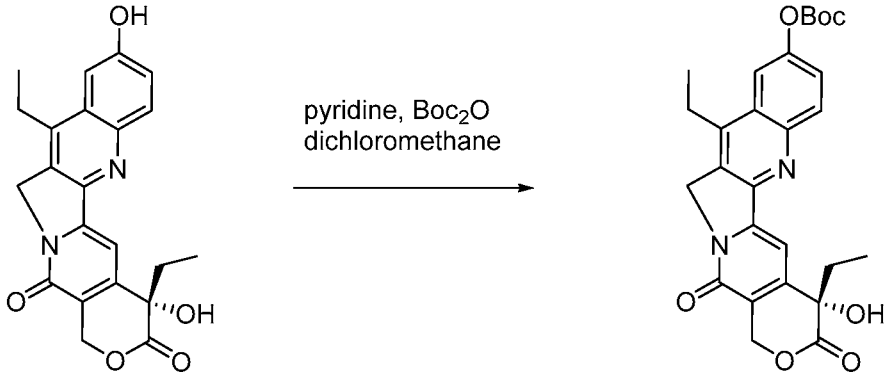
DM1 1

[00384] To a solution of 2,2' -dithiodipyri dine (1.24 g, 5.65 mmol) in DMF (8 mL) and diisopropylethylamine (1 mL) was added a solution of DM1 (417 mg, 0.565 mmol) in 2 mL DMF, dropwise over 5 min. The reaction was stirred at room temperature for another 30 min, and the reaction mixture loaded onto a C18 Isco gold

column. Eluting with 25% to 85% acetonitrile in water provided **1** (287 mg, 0.339 mmol, 60% yield). LCMS M/Z: 847.3 [M + 1].

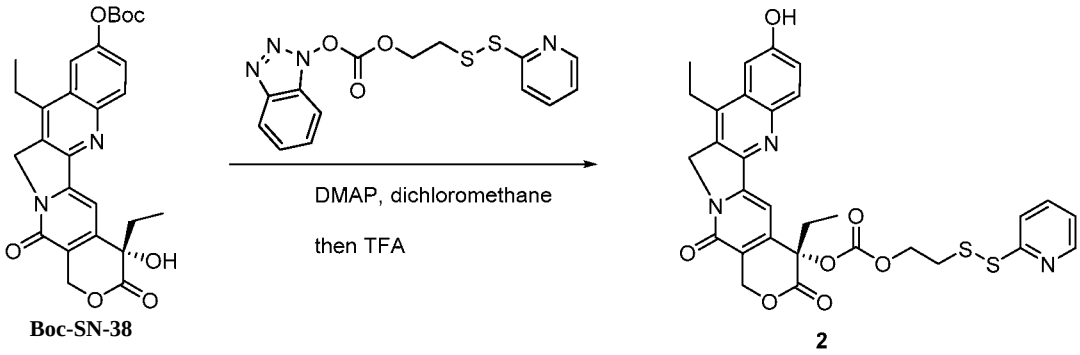


[00385] To a solution of 2-(2-pyridinyldithio)ethanol (2.00 g, 10.7 mmol) in dichloromethane (20 mL) was added diphosgene (1.06 g, 5.4 mmol) and triethylamine (1.08 g, 10.7 mmol) dropwise subsequently at 0 oC, and the mixture was stirred at room temperature for 4 hours. Then HOBt (1.44 g, 10.7 mmol) was added to the reaction mixture, followed by the addition of more triethylamine (1.08 g, 10.7 mmol). After stirring at room temperature overnight, the reaction mixture was concentrated to dryness in vacuo and the residue was dissolved in acetonitrile (20 mL), which was added to H20 (40 mL) to precipitate the solid. The product was collected by filtration, and dried resulting in 2-(2-pyridinyldithio)ethanol HOBt carbonate as a white solid (2.60 g, 7.46 mmol, 70% yield). 1H NMR (400 MHz, CDC13): δ 8.46 (d, J = 6.0 Hz, 1H), 8.22 (d, J = 8.8 Hz, 1H), 8.02 (d, J = 8.8 Hz, 2H), 7.80-7.62 (m, 1H), 7.71-7.63 (m, 2H), 7.58-7.54 (m, 1H), 7.1 1-7.08 (m, 1H), 4.82 (t, J = 6.4 Hz, 1H), 3.27 (t, J = 6.4 Hz, 1H). LCMS M/Z: 349 (M + 1).



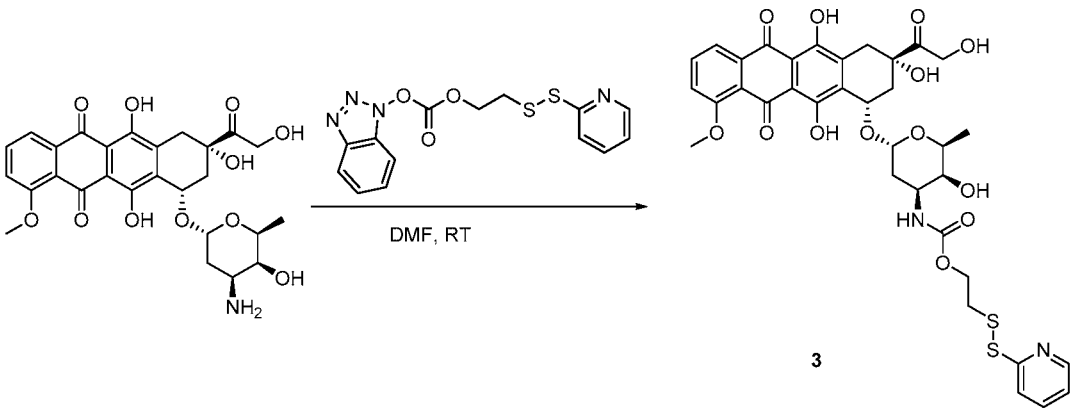
Boc-SN-38

[00386] To a solution of SN-38 (1.64 g, 4.18 mmol) in dichloromethane (15 mL) and pyridine (3.04 mL, 37.6 mmol) was added di-tert-butyl dicarbonate (1.82 g, 8.36 mmol). The reaction was stirred at room temperature for 24 h, and all solvent was removed under vacuum. The remaining residue was redissolved in dichloromethane (20 mL), and this solution loaded onto an 80 g silica gel column. Eluting with 0% to 10% methanol in dichloromethane provided **Boc-SN-38** (1.98 g, 4.02 mmol, 96% yield).



Boc-SN-38 **2**

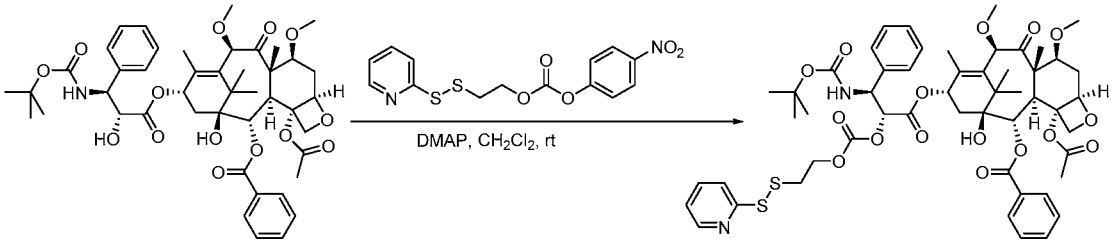
[00387] A vial was charged with **Boc-SN-38** (87.0 mg, 0.177 mmol) and **2-(2-**pyridinyldithio)ethanol HOBt carbonate (89.0 mg, 0.510 mmol). Dichloromethane (2 mL) and DMAP (43.2 mg, 0.353 mmol) were added. The reaction was stirred at room temperature for 6 h. Additional 2-(2-pyridinyldithio)ethanol HOBt carbonate (89.0 mg, 0.510 mmol) was added, and the reaction stirred for another 18 h. The reaction was diluted with heptane (2 mL), and the reaction mixture loaded directly onto a 24 g silica gel column. Elution with 0% to 100% ethyl acetate in heptane provided the desired product, along with an inseparable impurity. This mixture was dried, and dissolved in trifluoroacetic acid (3 mL). The solution was stirred at room temperature for 5 min, and the solvent removed under vacuum. The remaining residue was redissolved in DMF (3 mL), and this solution loaded onto a 50 g CI8 column. The column was flushed with 100 mM aqueous ammonium acetate (75 mL), then a gradient of 5% to 95% acetonitrile in water with 0.1% AcOH was run. The product-containing fractions were collected, the solvent removed, and the remaining material lyophilized from 3:1 acetonitrile:water to give **2** (68.0 mg, 0.1 12 mmol, 63% yield). LCMS M/Z = 606.2 (M + 1).



3

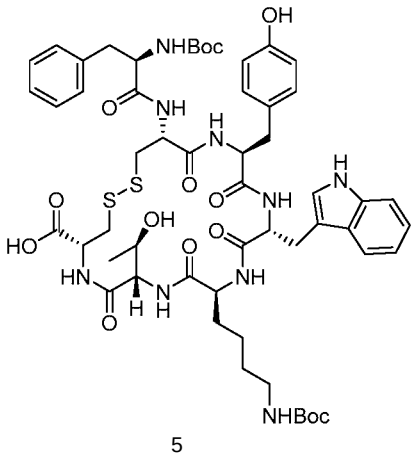
[00388] To a solution of doxorubicin (543 mg, 1.00 mmol) and 2-(2-pyridinyldithio)ethanol HOBt carbonate (348 mg, 10.0 mmol) in DMF (10 mL) was added diisopropylethylamine (258 mg, 2.0 mmol) dropwise, and the reaction mixture

was stirred at room temperature for 2 hours. The solution was concentrated to dryness and the residue was purified by silica gel chromatography (DCM/MeOH: 10/1, Rf = 0.5) to give **3** as a red solid (500 mg, 66% yield). 1H NMR (400 MHz, CDC13): δ 13.99 (s, 1H), 13.27 (s, 1H), 8.41 (d, J = 3.2 Hz, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.82-7.72 (m, 2H), 7.67-7.64 (m, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.1 1-7.07 (m, 1H), 5.52 (s, 1H), 5.31 (d, J = 1.6 Hz, 1H), 5.06 (d, J = 9.2 Hz, 1H), 4.76 (d, J = 3.2 Hz, 2H), 4.58 (s, 1H), 4.46-4.40 (m, 1H), 4.20-4.09 (m, 5H), 3.86-3.80 (m, 1H), 3.64-3.62 (m, 1H), 3.29 (dd, J = 19.2 Hz, 1.6 Hz, 1H), 3.06-2.89 (m, 5H), 2.35 (d, J = 14.8 Hz, 1H), 2.17 (dd, J = 14.8 Hz, 4.0 Hz, 1H), 1.82 (d, J = 8.0 Hz, 2H), 1.31 (d, J = 6.4 Hz, 3H). LCMS M/Z: 757 (M + 1).



4

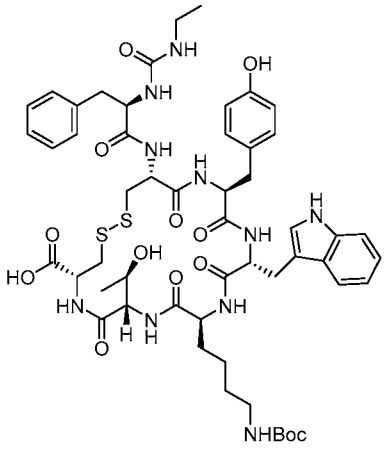
[00389] To a solution of cabazitaxel (2.00 g, 2.40 mmol) and 2-(2-pyridinyldithio)ethanol p-nitrophenyl carbonate (915 mg, 2.60 mmol) in dichloromethane (48 mL) was added DMAP (439 mg, 3.60 mmol). The solution was stirred at room temperature overnight, then washed with 0.IN HCl (3 x 20 mL), brine (50 mL), and dried with sodium sulfate. The solvent was removed in vacuo, the the remaining residue purified by silica gel chromatography (2:1 petroleum ethenethyl acetate) to give **4** (2.50 g, 2.38 mmol, 99% yield). LCMS m/z: 1049 (M + 1).



5

[00390] Fmoc-cystine(Trt) was loaded onto 2-chlorotrityl resin (3.6 g, 1 mmol/g). Iterative deprotection with 4:1 DMF:piperidine and coupling subsequently with

Fmoc-threonine(tBu), Na-Fmoc-Ns-Boc-lysine, Na-Fmoc-N ^{in}-Boc-D-tryptophan, Fmoc-tyrosine(tBu), Fmoc-cysteine(Trt), and Fmoc-D-phenylalanine, and a final DMF:piperidine deprotection provided the protected linear peptide on resin. The resin was washed with dichloromethane (3 x 40 mL), then cleaved with tnfluoroacetic acid (40 mL), water (2 mL), and triisopropylsilane (2 mL). The resin was stirred in the deprotection cocktail for 30 min, drained, the resin washed with dichloromethane (40 mL), and the deprotection/washing sequence repeated once more. The collected washings were concentrated *in vacuo,* and the remaining residue was dissolved in THF (70 mL). 30% hydrogen peroxide (0.65 mL) was added, followed by adding saturated sodium carbonate until reaction pH measured 7.0 (25 mL saturated sodium bicarbonate). Di-tert-butyl dicarbonate (2.50 g, 11.46 mmol) was added, and the reaction stirred at room temperature for 16 h. Acetic acid (25 mL) was added, and all solvents were removed *in vacuo.* The remaining residue was dissolved in 3:1 DMF:water, and this loaded onto a 100 g C18 column. Elution with 5% to 80% acetonitrile in water with 0.1% AcOH gave **5** (2.20 g, 1.92 mmol, 54% yield). LCMS M/Z: 1048.5 [M - Boc + 1].

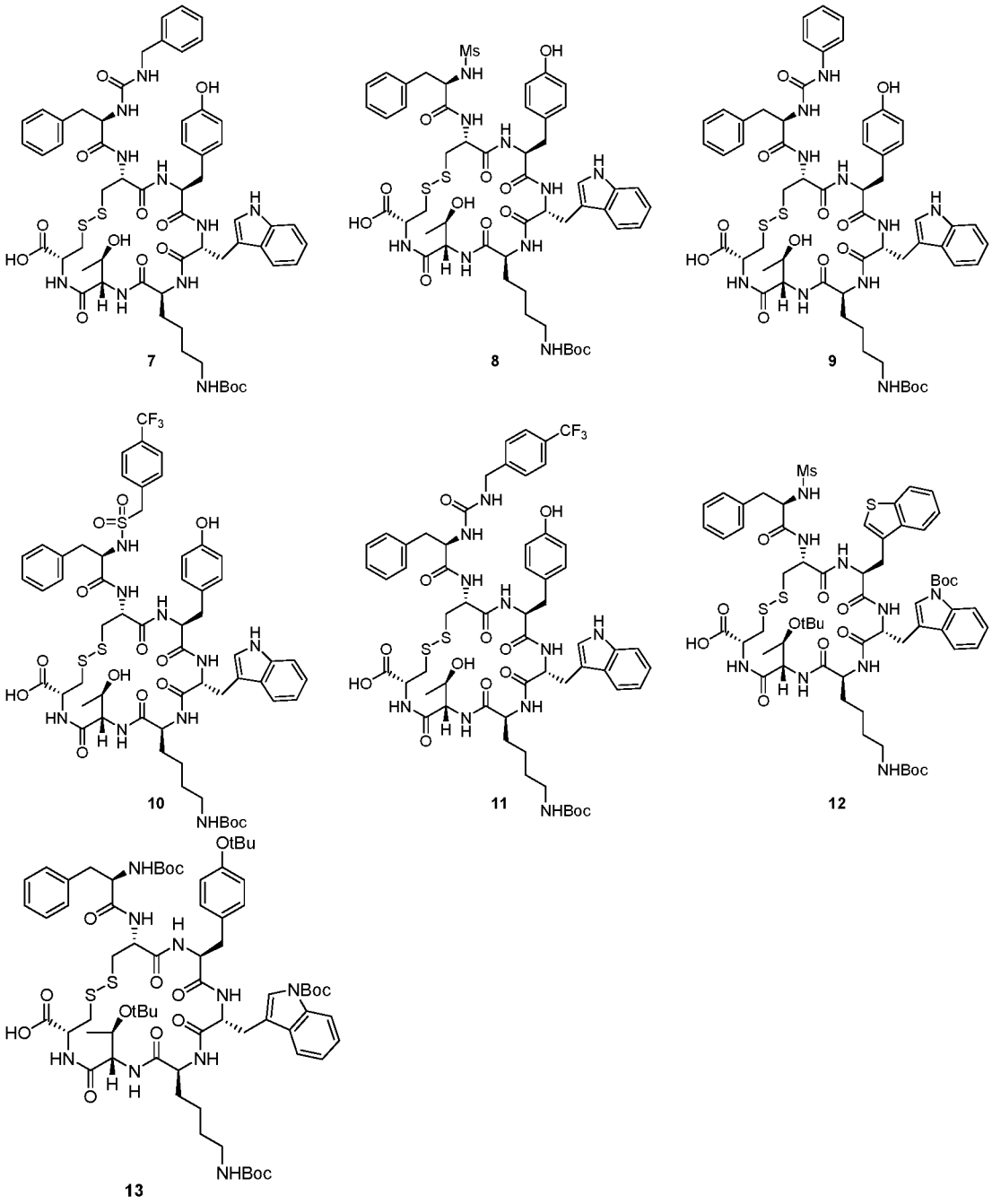


6

[00391] Fmoc-cystine(Trt) was loaded onto 2-chlorotrityl resin (875 mg, 1 mmol/g). Iterative deprotection with 4:1 DMF:piperidine and coupling subsequently with Fmoc-threonine(tBu), Na-Fmoc-Ns-Boc-lysine, Na-Fmoc-N ^{in}-Boc-D-tryptophan, Fmoc-tyrosine(tBu), Fmoc-cysteine(Trt), and Fmoc-D-phenylalanine, deprotection, and two treatments each with ethyl isocyanate (0.69 mL, 8.8 mmol) and triethylamine (3.0 mL) in DMF (20 mL) for 2 h gave the protected linear peptide. The resin was washed with dichloromethane (3 x 40 mL), then cleaved with trifluoroacetic acid (20 mL), water (1 mL), and triisopropylsilane (1 mL). The resin was stirred in the deprotection cocktail for 30 min, drained, the resin washed with dichloromethane (20

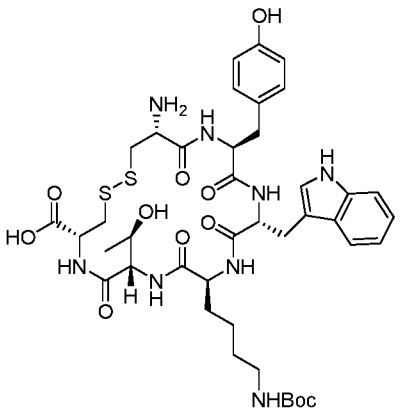
mL), and the deprotection/washing sequence repeated once more. The collected washings were concentrated *in vacuo*, and the remaining residue was dissolved in THF (50 mL). 30% hydrogen peroxide (0.32 mL) was added, followed by adding saturated sodium carbonate until reaction pH measured 8.0 (12 mL saturated sodium bicarbonate). Di-tert-butyl dicarbonate (1.94 g, 8.91 mmol) was added, and the reaction stirred at room temperature for 16 h. Acetic acid (25 mL) was added, and all solvents were removed *in vacuo*. The remaining residue was dissolved in 3:1 DMF:water, and this loaded onto a 100 g C18 column. Elution with 5% to 80% acetonitrile in water with 0.1% AcOH gave **6** (43 1 mg, 0.385 mmol, 44% yield). LCMS M/Z: 1019.6 [M - Boc + 1].

[00392] Compounds **7-12** were made in an analogous manner:



[00393] Fmoc-cystine(Trt) was loaded onto 2-chlorotrityl resin (30.0 g, 1 mmol/g theoretical loading). Iterative deprotection with 4:1 DMF:piperidine and coupling subsequently with Fmoc-threonine(tBu), Na-Fmoc-Ns-Boc-lysine, Na-Fmoc-N ^{in}-Boc-D-tryptophan, Fmoc-tyrosine(tBu), Fmoc-cysteine(Trt), and Boc-D-phenylalanine to give 90.2 g of the protected linear peptide (60.2 g total peptide loaded, 0.369 mmol/g loading of final protected resin). A portion of this linear peptide (10.0 g, 3.69 mmol) was taken, and DMF (25 mL) and pyridine (1.16 g, 14.7 mmol) were added. A solution of iodine (1.87 g, 7.36 mmol) in DMF (25 mL) was added, and the resin stirred for 20 minutes at room temperature. The resin was drained,

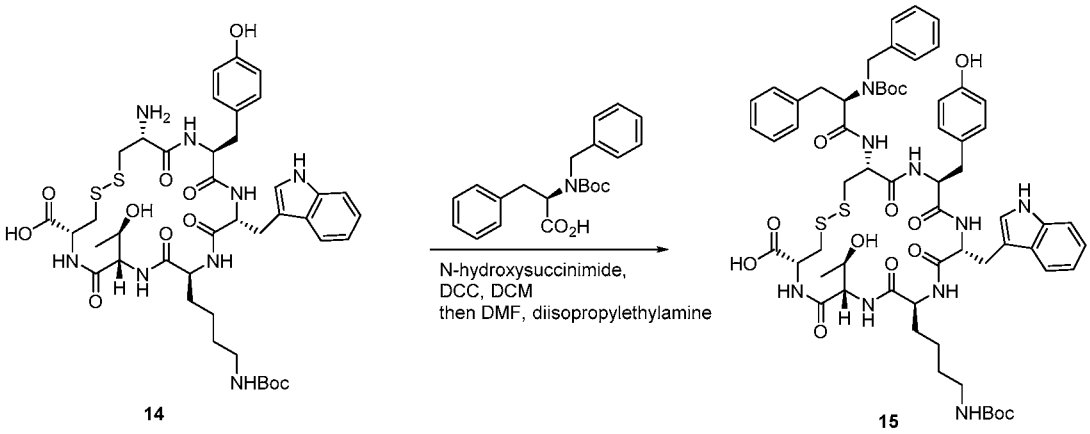
washed with DMF (25 mL), and additional DMF (25 mL) and pyridine (1.16 g, 14.7 mmol) were added. A solution of iodine (1.87 g, 7.36 mmol) in DMF (25 mL) was added, the resin stirred for 20 minutes at room temperature. The resin was drained, washed with DMF (2 x 25 mL) and dichloromethane (4 x 20 mL). The resin was then treated with 4:1 dichloromethane:hexafluoroisopropanol (60 mL) for 1 h. The dichloromethane: hexafluoroisopropanol solution was collected, the resin washed with dichloromethane (25 mL), and treated again with 4:1 dichloromethane:hexafluoroisopropanol (60 mL) for 1 h. The dichloromethane: hexafluoroisopropanol solution was collected, the resin washed with dichloromethane (25 mL), and the combined dichloromethane:FIFIP solutions, and dichloromethane washings, were dried *in vacuo.* The remaining solid was loaded onto an 80 g silica gel column. Elution with 0% to 10% methanol in dichloromethane provided **13** (2.97 g, 2.18 mmol, 59% yield). LCMS M/Z = 1360 [M + 1].



14

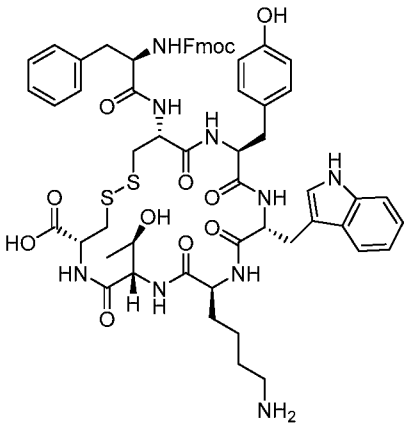
[00394] Fmoc-cystine(Trt) was loaded onto 2-chlorotrityl resin. Iterative deprotection with 4:1 DMF:piperidine and coupling subsequently with Fmoc-threonine(tBu), Na-Fmoc-Ns-Boc-lysine, Na-Fmoc-N ^{in}-Boc-D-tryptophan, Fmoc-tyrosine(tBu), and Fmoc-cysteine(Trt) provided the Fmoc-capped linear peptide. The resin was washed with dichloromethane (3 x 40 mL), then cleaved with 90:5:5 trifluoroacetic acid, water, and triisopropylsilane. The resin was stirred in the deprotection cocktail for 30 min, drained, the resin washed with dichloromethane, and the deprotection/washing sequence repeated once more. The collected washings were concentrated *in vacuo,* and the remaining residue was dissolved in THF. 30% hydrogen peroxide was added, followed by adding saturated sodium carbonate until reaction pH measured 8.0. Di-tert-butyl dicarbonate was added, and the reaction stirred at room temperature for 16 h. The mixture was extracted with ethyl acetate

four times, the combined organic layers dried with sodium sulfate, and the solvent removed *in vacuo.* The remaining residue was dissolved in 4:1 DMF:diethylamine, stirred at room temperature for 30 min, then most of the diethylamine was removed *in vacuo.* The remaining solution was purified by reverse phase chromatography give **14**.



14 15

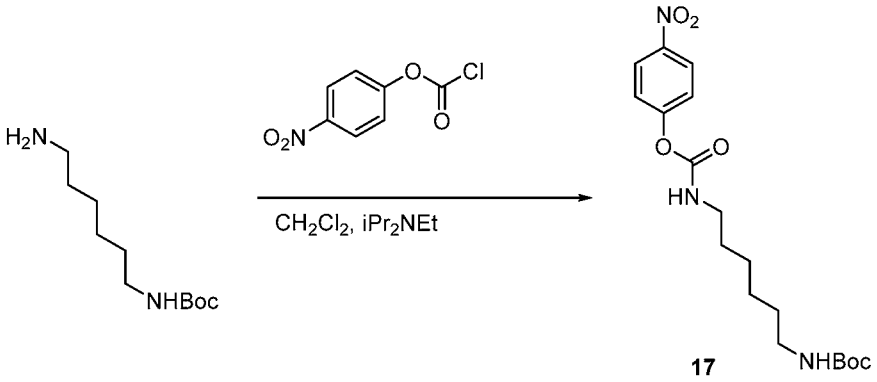
[00395] To a solution of N-benzyl-N-Boc-D-phenylalanine (100 mg, 0.28 1 mmol) in dichloromethane (10 mL) was added N-hydroxysuccinimide (38.9 mg, 0.337 mmol) and DCC (69.7 mg, 0.338 mmol). The reaction was stirred at room temperature for 2 h, and filtered. The filtrate was concentrated *in vacuo,* redissolved in dichloromethane (5 mL), filtered again, and the solvent removed *in vacuo* to give the crude NHS ester. This ester was added to a solution of **14** (140 mg, 0.155 mmol) in DMF (2 mL) and diisopropylethylamine. The reaction was stirred at room temperature for 16 h, then purified by reverse phase chromatography to give **15** (51.0 mg, 0.0412 mmol, 27% yield). LCMS M/Z = 1138.4 [M + 1 - Boc].



16

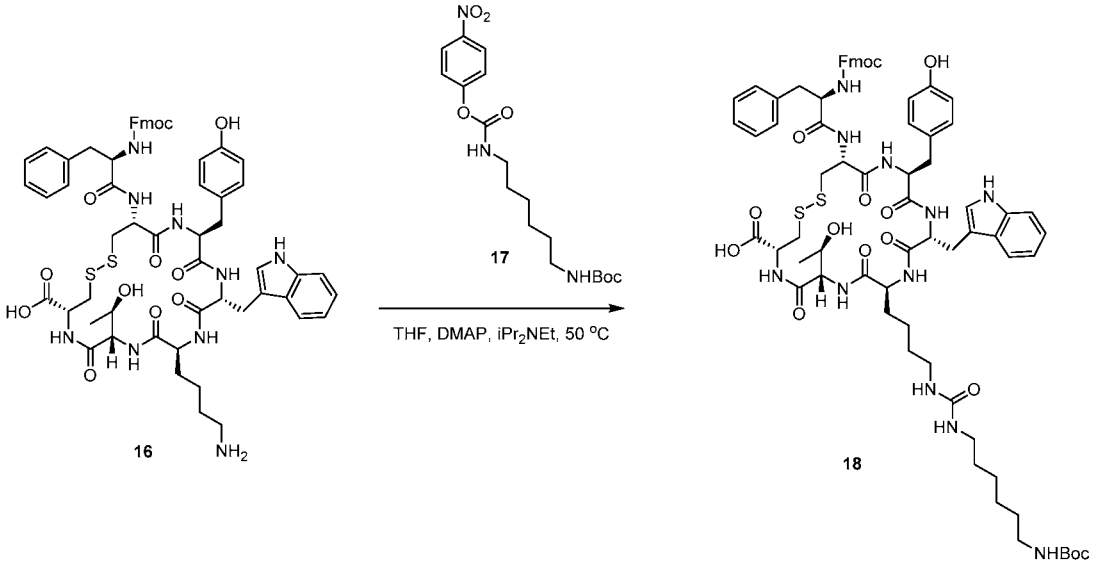
[00396] Fmoc-cystine(Trt) was loaded onto 2-chlorotrityl resin. Iterative deprotection with 4:1 DMF:piperidine and coupling subsequently with Fmoc-

threonine(tBu), Na-Fmoc-Ns-Boc-lysine, Na-Fmoc-N ^{in}-Boc-D-tryptophan, Fmoc-tyrosine(tBu), Fmoc-cysteine(Trt), and Fmoc-D-phenylalanine provided the protected linear peptide. 2.00 g of this resin (0.338 mmol/g loading, 0.672 mmol) was treated with trifluoroacetic acid (20 ml), water (0.50 mL) and triisopropylsilane (0.50 mL) for 20 min. The deprotection cocktail was drained into a flask, and the remaining resin treated again with trifluoroacetic acid (20 ml), water (0.50 mL) and triisopropylsilane (0.50 mL) for 1 h. The deprotection cocktail was drained into a flask, the resin washed with dichloromethane (3 x 40 mL), and the dichloromethane washings drained into a flask. The collected solvent was concentrated *in vacuo*, and the remaining residue dissolved in THF (30 mL) and water (10 mL). 30% hydrogen peroxide (0.30 mL) was added, and saturated sodium bicarbonate was added until the pH reached 8.0 (7.0 mL added). The reaction was stirred at room temperature for 1 h, the solvents removed *in vacuo*, and the remaining residue dissolved in 3:1 DMF:water, and loaded onto a 100 g C18 column. Elution with 5% to 85% acetonitrile in water with 0.1% AcOH provided **16** as the acetate salt (375 mg, 0.305 mmol, 45% yield). LCMS M/Z = 1170.5 (M + 1).

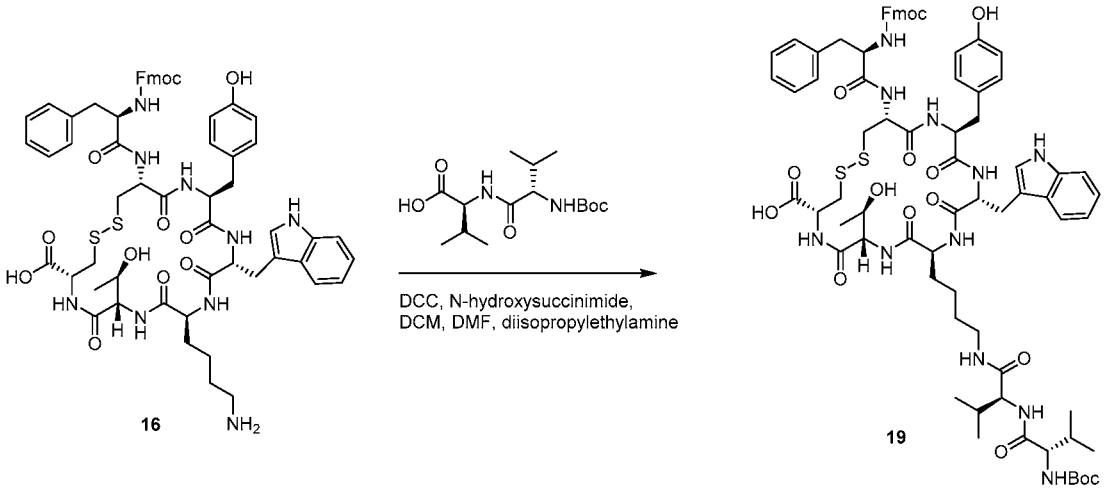


17

[00397] To a solution of para-nitrophenylchloroformate (200 mg, 1.00 mmol) in dichloromethane (5 mL) was added a solution of mono-Boc-l,6-hexanediamine (113 mg, 0.500 mmol) in dichloromethane (3 mL) and diisopropylethylamine (0.20 mL). The reaction was stirred at room temperature for 2 h, then most of the dichloromethane removed *in vacuo* until total reaction volume = 2 mL. This solution was loaded onto a 24 g silica gel column. Elution with 0% to 70% ethyl acetate in heptane provided **17** (102 mg, 0.268 mmol, 54% yield).

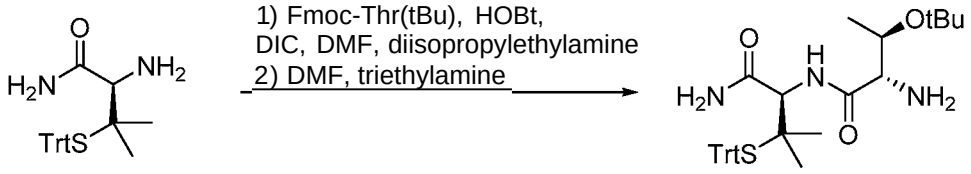


[00398] To a solution of **16** (93.2 mg, 0.0796 mmol), **17** (91.1 mg, 0.239 mmol), and DMAP (29.2 mg, 0.239 mmol) in THF (3 mL) was added diisopropylethylamine (0.30 mL). The reaction was stirred at 50 °C for 2 h. A solution of 1M ammonia in methanol (1 mL) was added, the reaction stirred at 50 °C for another 5 min, and all solvents were removed in vacuo. The remaining material was dissolved in DMF (2 mL) and AcOH (1 mL), loaded onto a 50 g C18 column, and elution with 5% to 80% acetonitrile in water with 0.1% AcOH provided **18** (61.2 mg, 0.0433 mmol, 54% yield).



[00399] To a solution of Boc-Val-Val (69.6 mg, 0.220 mmol) in dichloromethane (5 mL) was added DCC (49.5 mg, 0.240 mmol) and N-hydroxysuccinimide (27.6 mg, 0.240 mmol). The reaction was stirred for 3 h at room temperature, then all solvent removed *in vacuo.* To the remaining residue was added a solution of **16** (234 mg, 0.200 mmol) in DMF (5 mL). The reaction was stirred at room temperature for 16 h,

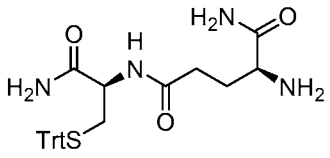
then purified by preparative HPLC (acetonitrile/water with 0.05% TFA modifier) to provide **19** (200 mg, 0.136 mmol, 68% yield). LCMS M/Z = 1468.5 [M + 1].



20

[00400] To a solution of S-trityl-L-penicillamine amide (1.20 g, 3.07 mmol) in DMF (20 ml) was added HOBt (829 mg, 6.14 mmol), DIC (775 mg, 6.14 mmol), Fmoc-Thr(tBu) (1.28 g, 3.22 mmol), and diisopropylethylamine (1.07 mL, 6.14 mmol). The reaction was stirred at room temperature for 16 h, then purified by reverse phase chromatography (water/acetonitrile with 10 mM ammonium bicarbonate buffer) to give DThr(tBu)-Pen(STrt)-NH $_2$ (1.94 g, 2.52 mmol, 82% yield). This material was dissolved in DMF (20 mL) and triethylamine (2 mL). The reaction was stirred at room temperature for 16 h, then purified by reverse phase chromatography (water/acetonitrile with 10 mM ammonium bicarbonate buffer) to give **20** (870 mg, 1.59 mmol, 63% yield).

[00401] Compounds **21-22** were made in an analogous manner:



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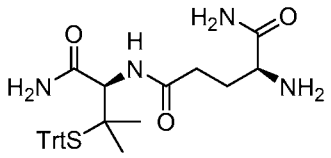
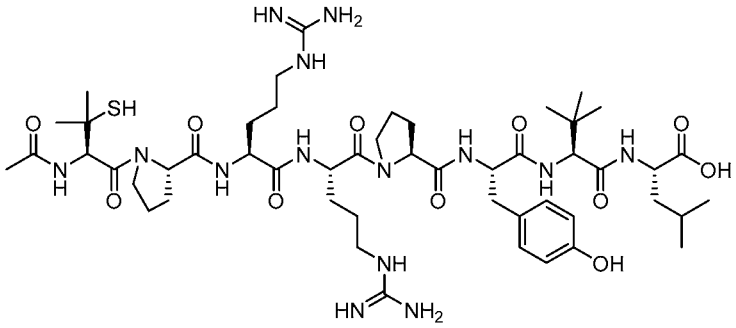


22

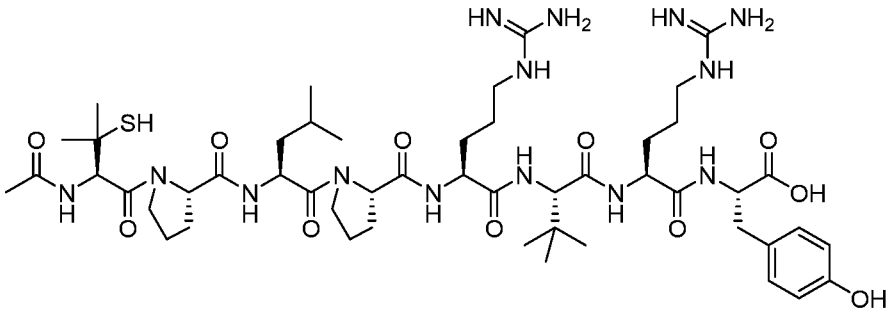


23

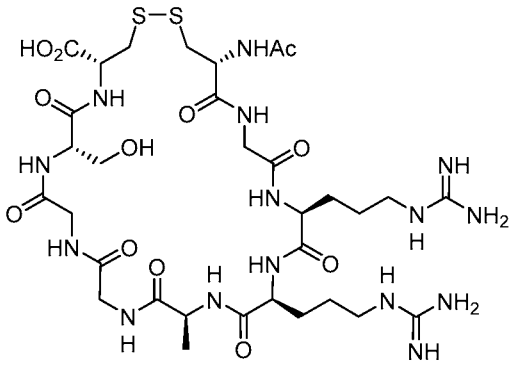
[00402] Fmoc-leucine was loaded onto 2-chlorotrityl resin (4.0 g, 0.5 mmol/g, 2 mmol loading). Iterative deprotection with 4:1 DMF:piperidine and coupling with 3 equiv each HBTU, N-methyl morpholine, and in sequence, Fmoc-tert-leucine, Fmoc-tyrosine(tBu), Fmoc-proline, Fmoc-arginine(Pbf), Fmoc-arginine(Pbf), Fmoc-proline, Fmoc-penicillamine(Trt), and finally acetic anhydride provided the protected linear

peptide. The resin was treated with trifluoroacetic acid (95 ml), water (2.5 mL), EDT (2.5 mL), and triisopropylsilane (2.5 mL) for 2 h. The deprotection cocktail was drained into a flask, ether (800 mL) added, and the resulting precipitate was filtered, the solid collected, and purified by reverse phase chromatography to give **23** as the bis-TFA salt (1.18 g, 0.897 mmol, 45% yield).

[00403] Compound **24** was made in an analogous manner:



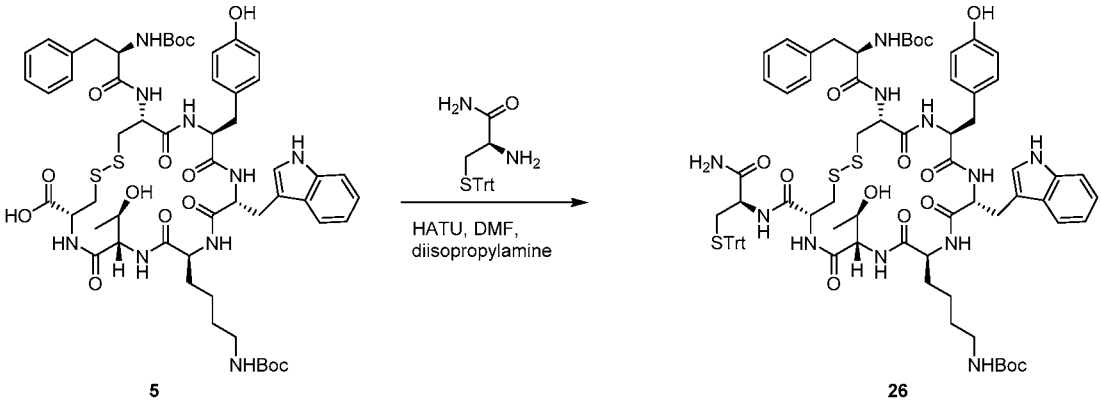
24



25

[00404] Fmoc-cysteine(Trt) was loaded onto 2-chlorotrityl resin (7.27 g, 0.344 mmol/g loading, 2.5 mmol). Iterative deprotection with 4:1 DMF:piperidine and coupling with 3 equiv each FIBTU and N-methyl morpholine and, in sequence, Fmoc-serine(tBu), Fmoc-glycine, Fmoc-glycine, Fmoc-alanine, Fmoc-arginine(Pbf), Fmoc-arginine(Pbf), Fmoc-glycine, Fmoc-cysteine(Trt), and finally capping with acetic anhydride provided the linear peptide. Treating the resin with 94/3/3 TFA/EDT/water (110 mL) for 2 h, and then pouring the deprotection cocktail into diethyl ether (800 mL) provided a suspension. The suspension was centrifuged, the solid washed twice with diethyl ether, and the crude material was dissolved in 1:1 acetonitrile:water (2 L). A solution of iodine in methanol (0.1 M) was added dropwise with vigorous stirring, until the solution began to turn yellow. The mixture was stirred for another 3 min, and saturated sodium bisulfite added until the yellow color vanished. The acetonitrile was removed *in vacuo*, and the remaining solution was lyophilized to give

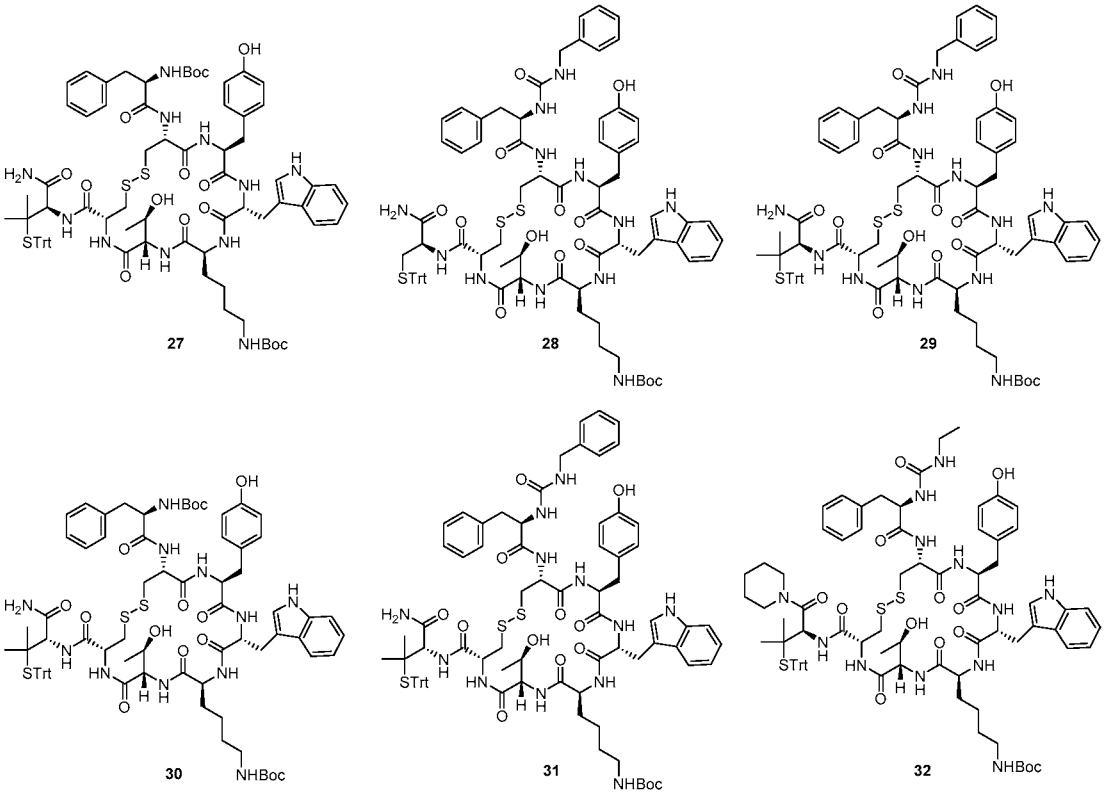
crude cyclized peptide. The peptide was purified by preparative HPLC to provide **25.** LCMS M/Z = 906.4 [M + 1].



5 26

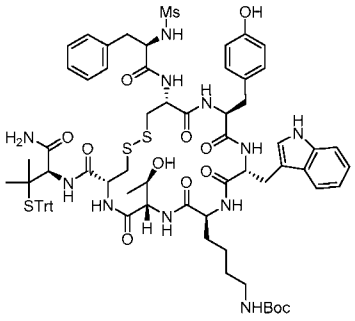
[00405] To a solution of **5** (1.15 g, 1.00 mmol), S-trityl L-cysteine amide (1.09 g, 3.00 mmol), and HATU (1.14 g, 3.00 mmol) in DMF (15 mL) was added diisopropylethylamine (1.00 mmol, 5.7 mmol). The reaction was stirred overnight at room temperature, then the reaction mixture loaded onto a 100 g C18 column. Eluting with 50% to 95% acetonitnle in water with 0.1% AcOH provided **26** (1.24 g, 0.83 1 mol, 83% yield).

[00406] Compounds **27-46** were made in an analogous manner:



27 28 29

30 31 32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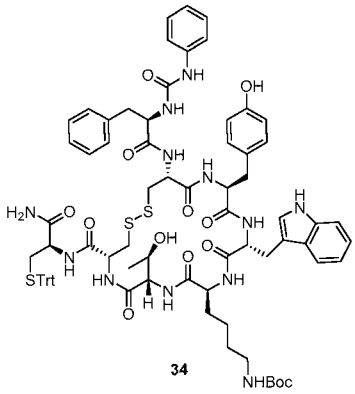
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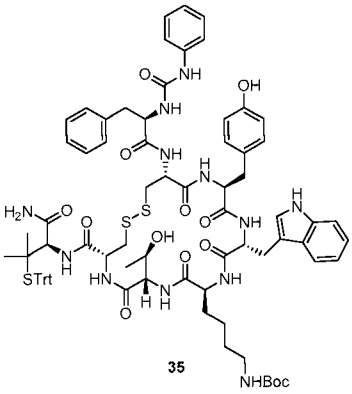
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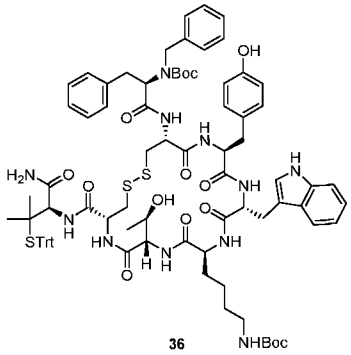
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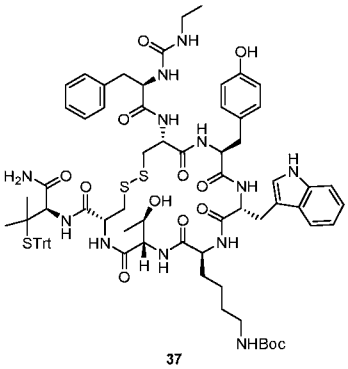
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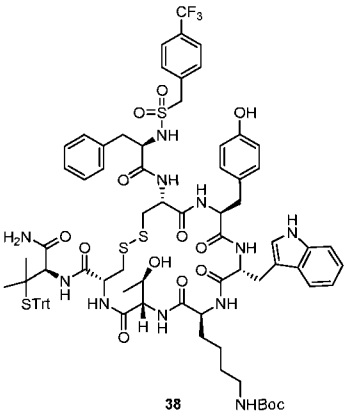
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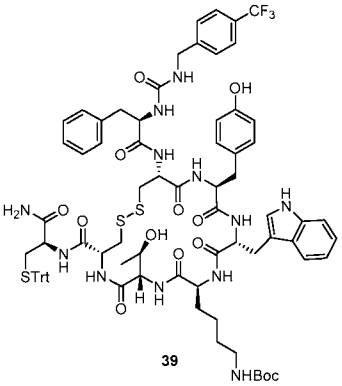
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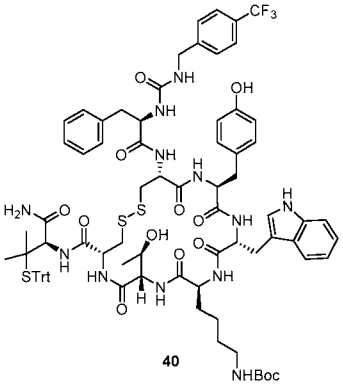
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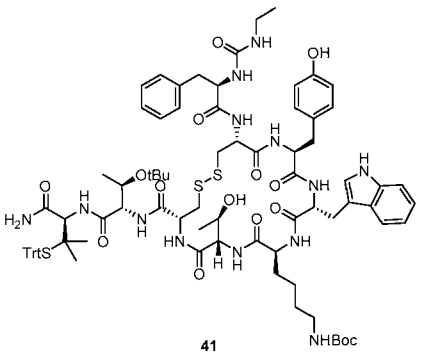
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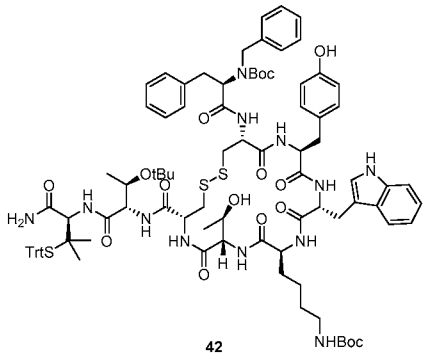

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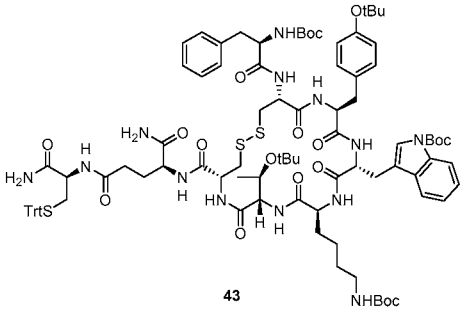

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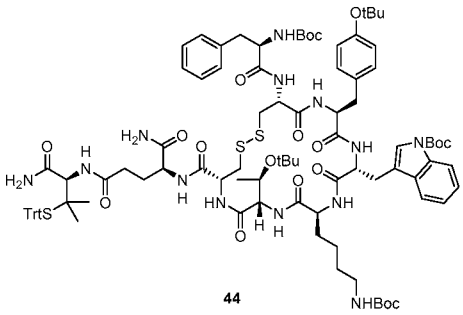

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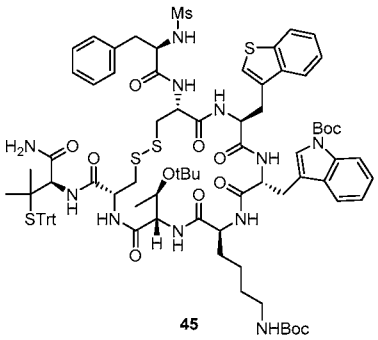
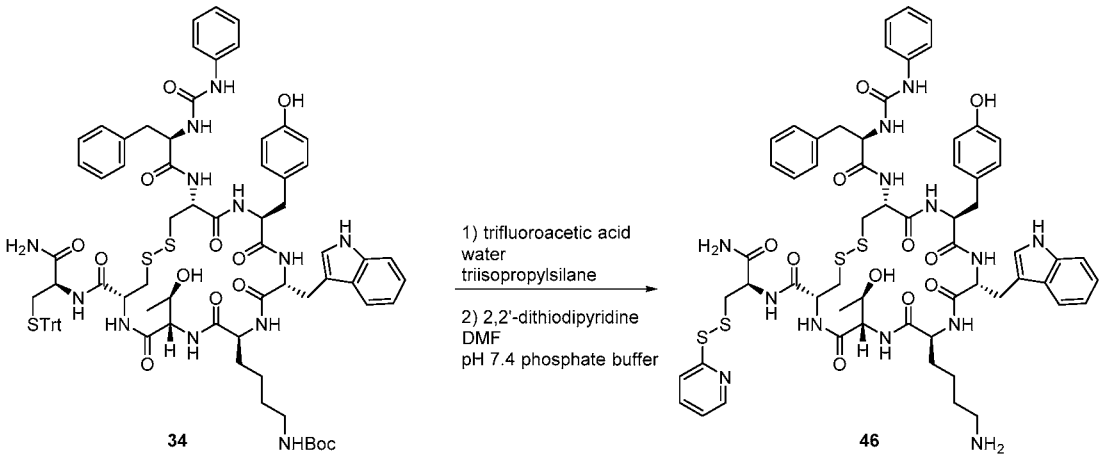


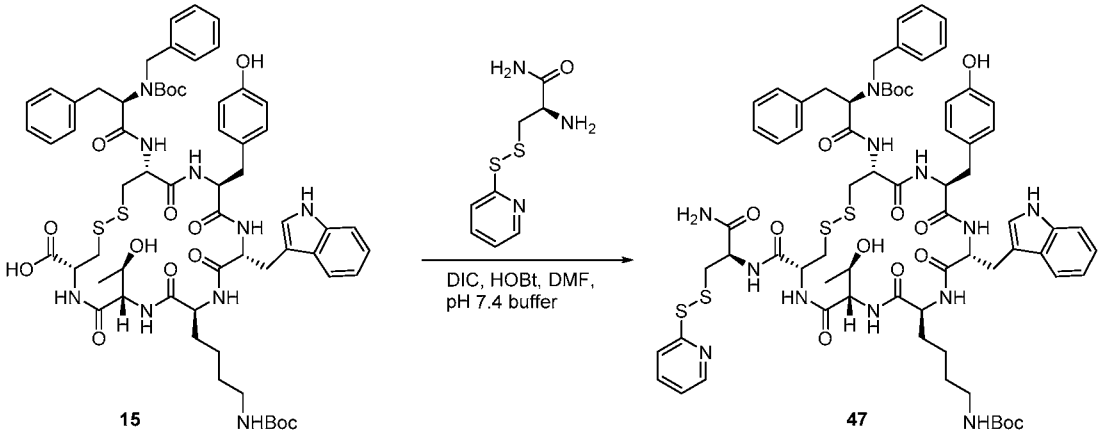
45



34 46

[00407] A vial was charged with **34** (102 mg, 0.0675 mmol). Water (0.10 mL), triisopropylsilane (0.10 mL) and trifluoroacetic acid (4.0 ml) were added. The reaction mixture was stirred for 10 min, and LCMS shows complete deprotection. LCMS M/Z = 585.3 [(M + 2) / 2]. All solvent was removed *in vacuo*, and to the remaining residue was added a solution of 2,2' -dithiodipyri dine (119 mg, 0.540

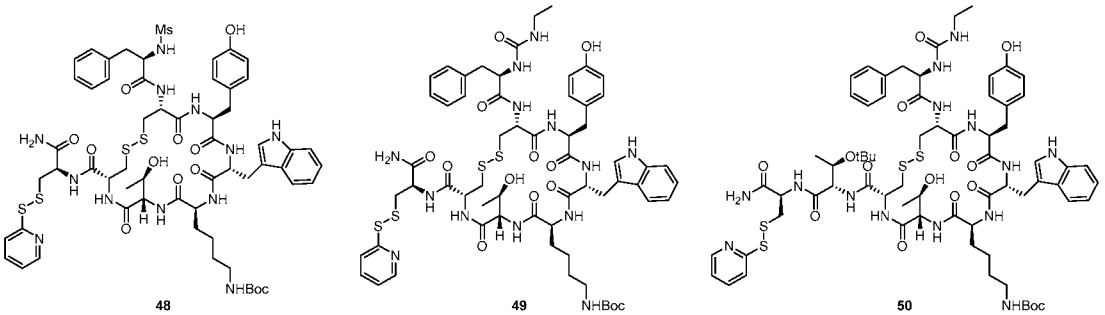
mmol) in DMF (4 mL). pH 7.4 phosphate buffer was adde dropwise to the mixture over 5 min, and the reaction stirred at room temperature for another 30 min. LCMS shows complete conversion to **46.** The reaction was acidifed with acetic acid (1 mL), and water (4 mL) was added. The reaction mixture was loaded onto a 50 g C18 Isco column. Elution with 5% to 40% acetonitrile in water with 0.1% AcOH gave **46** as the acetate salt (74.7 mg, 0.0584 mmol, 87% yield). LCMS M/Z = 639.8 [(M + 2) / 2].



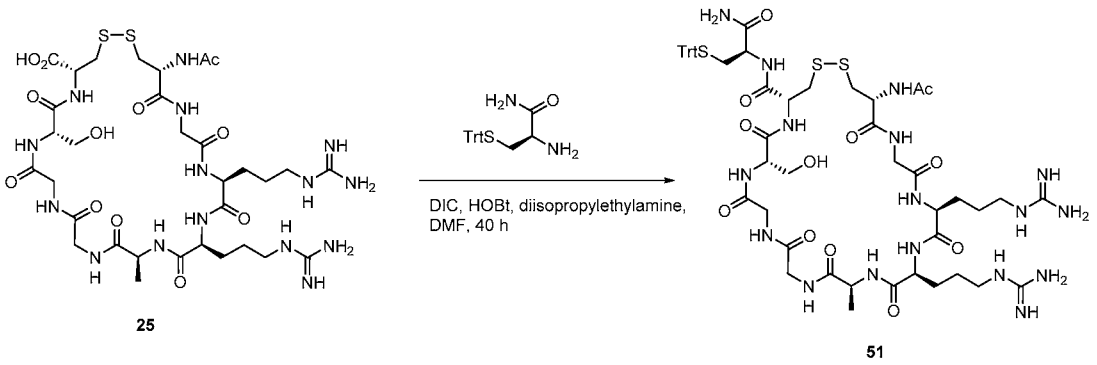
15 47

[00408] To a solution of **15** (25.0 mg, 0.0202 mmol) in DMF (2 mL) was added (S)-cysteine amide-S-(pyridine-2-ylsulfanyl) (9.3 mg, 0.040 mmol), DIC (5.1 mg, 0.040 mmol) and HOBt (8.2 mg, 0.061 mmol). pH 7.4 phosphate buffer (0.50 mL) was added, and the solution stirred at room temperature for 16 h. The resulting reaction mixture was purified by reverse phase chromatography (water/acetonitrile with 0.05% TFA modifier) to give **47** (11.5 mg, 0.00761 mmol, 38% yield). LCMS M/Z = 1449.4 [M + 1].

[00409] Compounds **48**-**50** were made in an analogous manner:



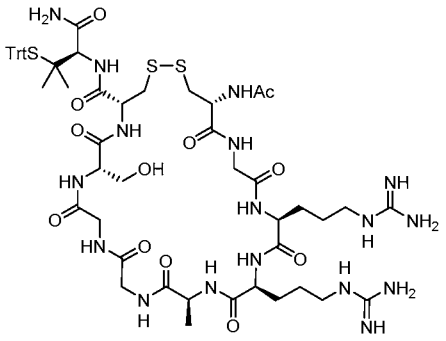
48 49 50



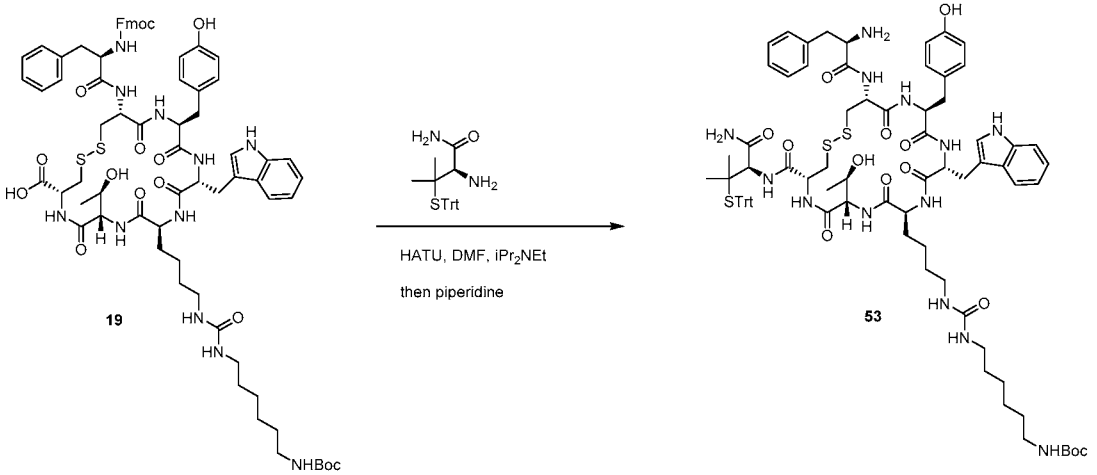
25 51

[00410] A vial was charged with **25** (246 mg, 0.271 mmol), S-trityl cysteine amide (174 mg, 0.406 mmol), diisopropylcarbodiimide (68 mg, 0.54 mmol), HOBt (73 mg, 0.54 mmol), and DMF (5 mL) and diisopropylethylamine (105 mg, 0.813 mmol) were added. The reaction was stirred at room temperature for 40 h, and the resulting reaction mixture purified by preparative HPLC to give **51** (78.1 mg, 0.0625 mmol, 23% yield). LCMS M/Z = 1250.3 [M + 1].

[00411] Compound **52** was made in an analogous manner:



52

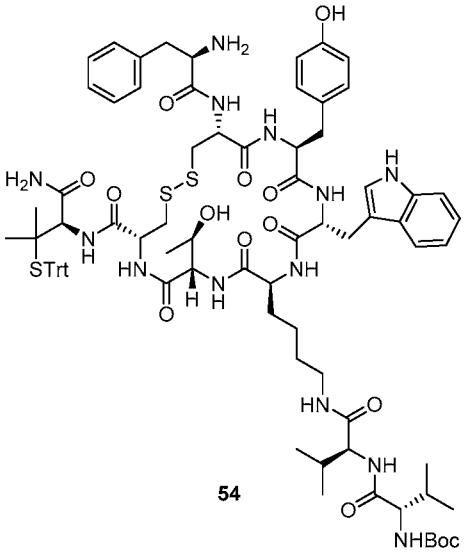


19 53

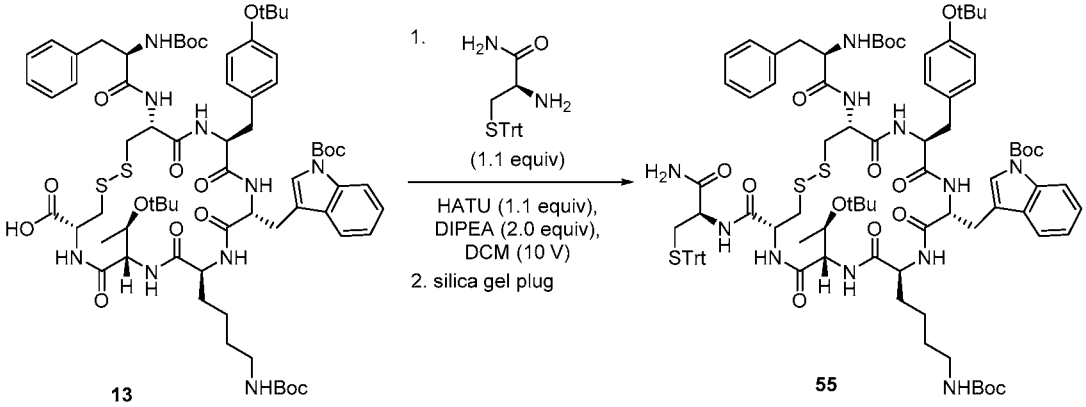
[00412] To a solution of **19** (130 mg, 0.0920 mmol), S-trityl penicillamine amide (108 mg mg, 0.276 mmol) and HATU (105 mg, 0.276 mmol) in DMF (10 mL) was added diisopropylethylamine (0.20 mL). The reaction was stirred at room temperature

for 24 h, then piperidine (3 mL) was added. The reaction was stirred at room temperature for 3 h, then the reaction mixture was concentrated *in vacuo* until total reaction volume was about 4 mL, and the reaction mixture was loaded onto a 50 g C18 column. Elution with 5% to 80% acetonitnle in water with 0.1% AcOH provided **53** as the acetate salt (108 mg, 0.0665 mmol, 72% yield).

[00413] Compound **54** was made in an analogous manner:



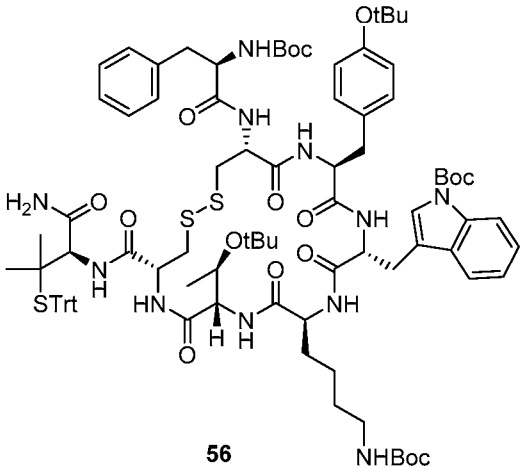
54



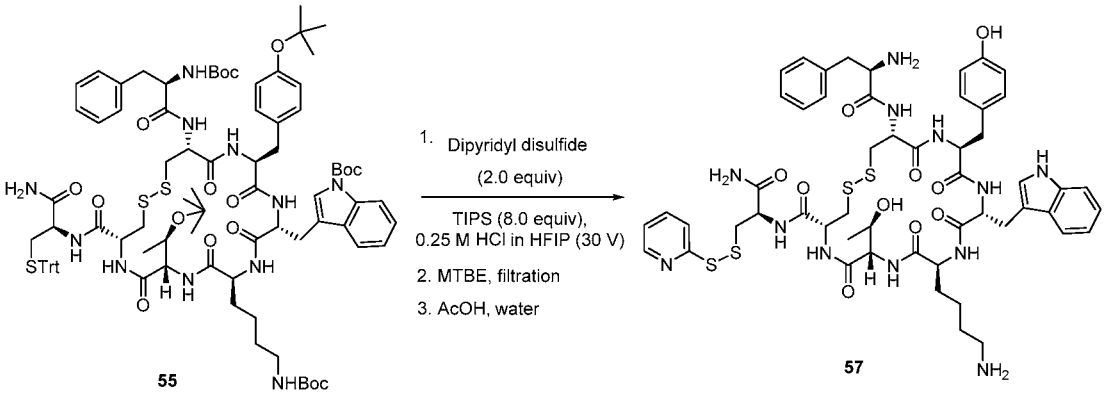
13 **55**

[00414] A flask was charged with **13** (2.73 g, 2.01 mmol) and (2*R*)-2-amino-3-tritylsulfanyl-propanamide (728.60 mg, 2.01 mmol). Dichloromethane (27.00 mL) was added followed by diisopropylethylamine (519.54 mg, 4.02 mmol) and HATU (840.69 mg, 2.21 mmol). After 1.5 h, conversion was complete by HPLC/MS. Silica gel (27 g) was charged in a fritted glass funnel. 40:60 MTBE/dichloromethane was used to wet silica. The DCM solution was added on top of the silica gel, then eluted with 40:60 MTBE/dichloromethane (250 mL), followed by 2% isopropanol in 40:60 MTBE/dichloromethane (250 mL). The filtrate was evaporated to yield **55** (3.16 g, 92% yield). LCMS M/Z: 1705 [M + 1].

[00415] Compound **56** was made in an analogous manner.

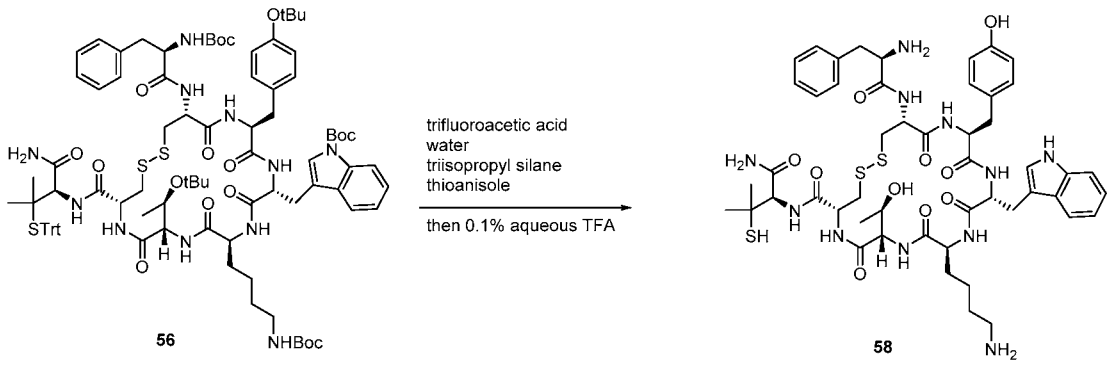


56



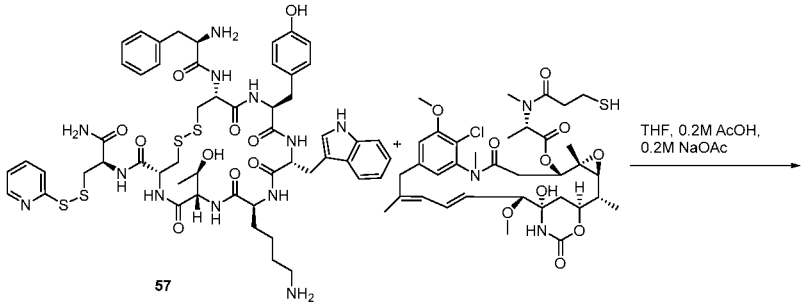
55 **57**

[00416] **55** (500.00 mg, 293.23 umol) was charged in a flask with 2,2'-dithiodipyridine (129.85 mg, 589.39 umol). Triisopropylsilane (386.81 mg, 2.44 mmol) was added, followed by hexafluoroisopropanol (8 mL), then 0.5 M HC1 in hexafluoroisopropanol (8 mL). After 2 h, LCMS showed complete conversion to deprotected product with carboxyl group on the indole. LCMS M/Z = 1204 [M + 1]. Solution was added slowly to MTBE (30 mL) and the precipitate formed was filtered, then washed with MTBE (30 mL). The solid was dissolved in 1 M AcOH (8 mL) and the solution was stirred at RT for 2 h. LC/MS showed complete conversion of the carboxylated material to **57.** The crude mixture was directly injected on a 100 g C18 Isco gold column. Column was flushed with 360 mL (3 volumes) 100 mM ammonium acetate, then re-equilibrated with 5% acetonitrile in water with 0.1% AcOH (2 volumes) and eluted using gradient of 5% to 30% acetonitrile in water with 0.1% AcOH for 24 mins. Pure fractions were lyophilized to give **57** as the bisacetate salt (250 mg, 0.195 mmol, 67% yield). LCMS M/Z: 1160 [M + 1].



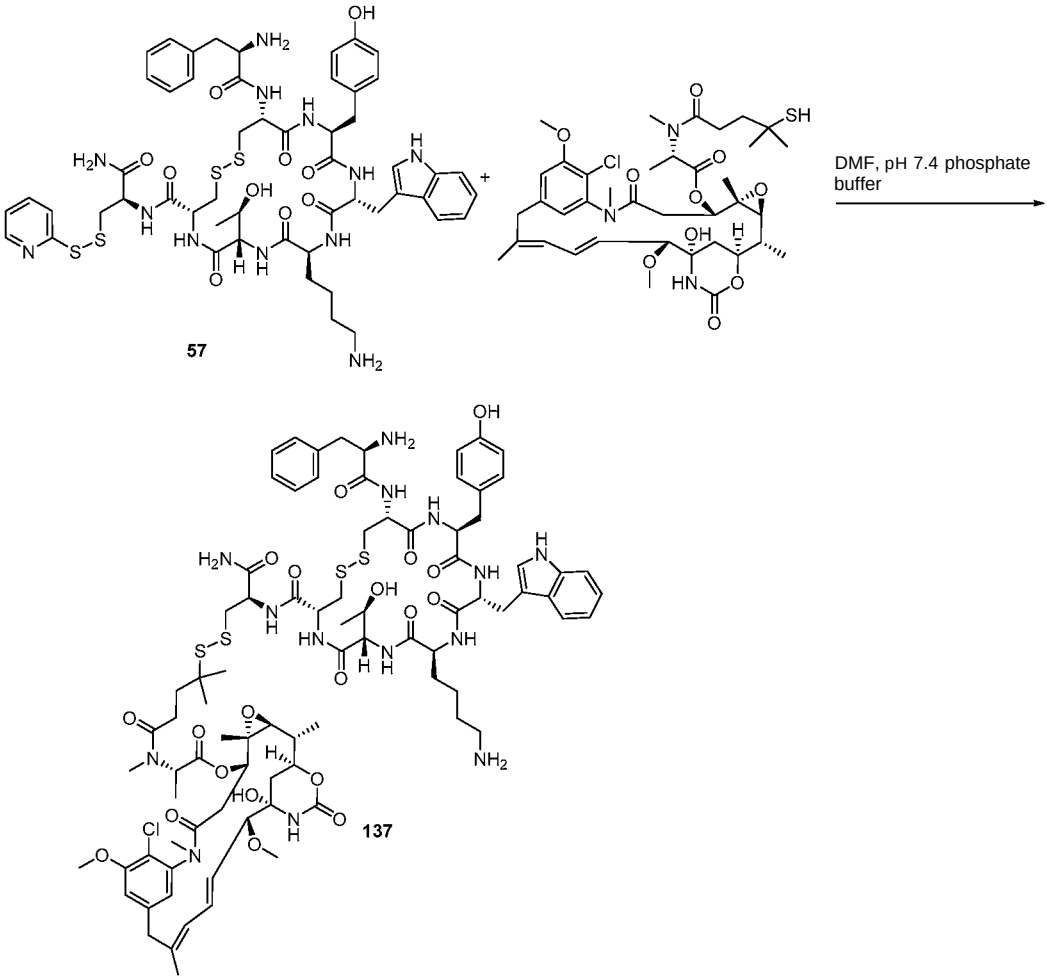
56 58

[00417] A vial was charged with **56** (590 mg, 0.340 mmol), and a mixture of water (0.3 mL), thioanisole (0.3 mL), triisopropylsilane (0.3 mL) and trifluoroacetic acid (5.1 mL) was added. The reaction was stirred until everything went into solution, then stirred for an additional 5 min. LCMS shows complete conversion to deprotected product with a carboxyl group remaining on the indole ring. LCMS M/Z = 1122 (M + 1). The reaction mixture was poured into 1:1 heptane:MTBE (50 mL), and the resulting suspension stirred at room temperature for 10 min. The suspension was filtered, the remaining solid washed with 1:1 heptane:MTBE (50 mL), and the solid was redissolved in 0.1% aqueous TFA (25 mL). This solution was stirred at room temperature for 4 h, after which LCMS shows complete conversion to **58.** The reaction mixture was loaded directly onto a 50 g C18 Isco gold column. Elution with 5% to 50% acetonitrile in water with 0.1% TFA provided **58** as the bis-trifluoroacetate salt (362 mg, 0.277 mmol, 81% yield). LCMS M/Z = 1078 (M + 1).



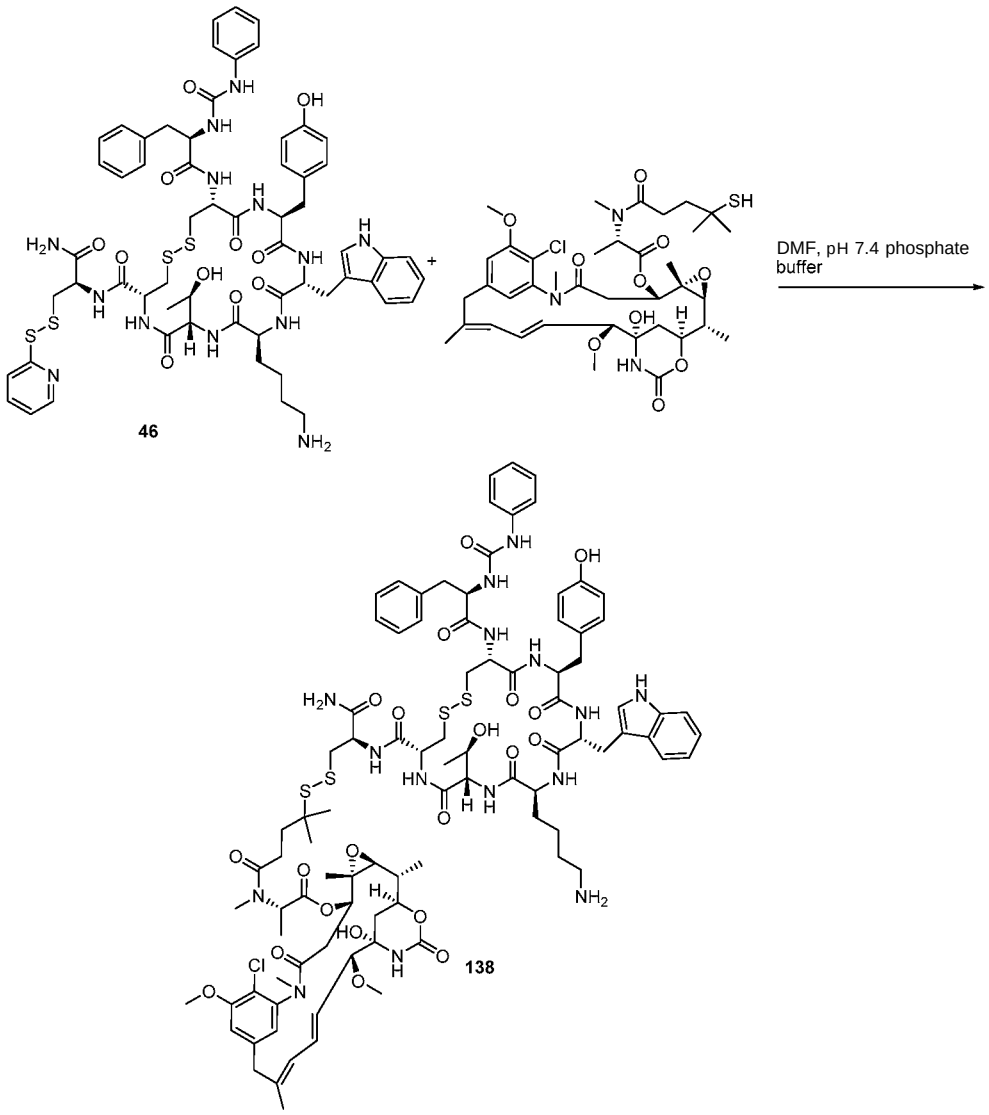
57 200

[00418] A flask was charged with 57 bis-acetate salt (210 mg, 0.164 mmol) and this was dissolved in THF (4 mL) and 0.2M AcOH (3.6 mL) and 0.2M NaOAc (0.4 mL). To the reaction mixture was added a solution of DM-1 (124 mg, 0.167 mmol) in THF (4 mL). The reaction was stirred at room temperature for 1 h. All solvents were removed in vacuo, with bath temperature at 35 °C, and at 10 mbar for 45 min to remove all water. The remaining residue was dissolved in 1 mL DMF, and this was diluted with 3 mL aqueous 1% acetic acid. This solution was loaded onto a 50 g RediSep Rf Gold C18 column, and a gradient of 5% to 40% acetonitrile in water with 0.1% AcOH was run. Product-containing fractions were dried *in vacuo* to yield **200** bis-acetate salt (262 mg, 0.137 mmol, 84% yield). LCMS M/Z: 893.7 [(M + 2) / 2].

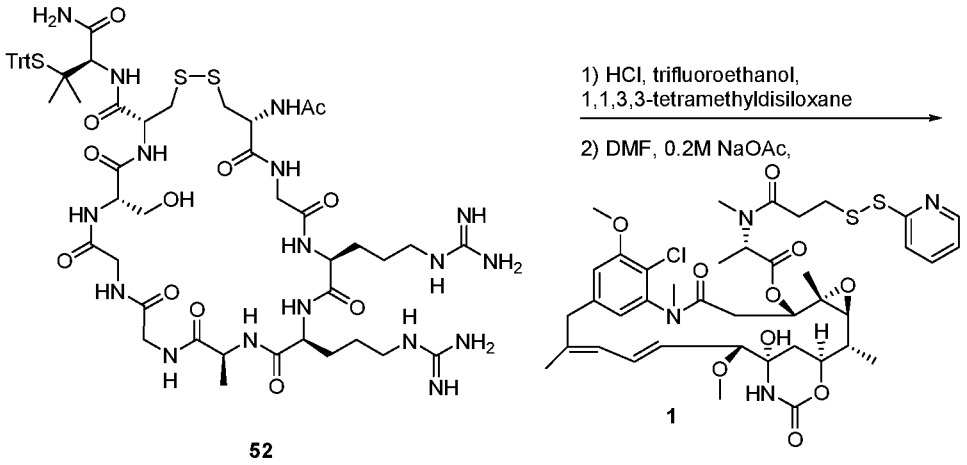


[00419] A vial was charged with 57 (133 mg, 0.114 mmol), and a solution of DM-4 (99.4 mg, 0.127 mmol) in DMF (4 mL) was added. While stirring, pH 7.4 phosphate buffer (4 mL) was added dropwise over 5 min. After stirring for another 5 min at room temperature, LCMS shows complete consumption of 57. The reaction was acidified by adding acetic acid (1 mL), and the resulting reaction mixture loaded directly onto a 50 g C18 Isco Gold column. Elution with 5%> to 50%> acetonitrile in

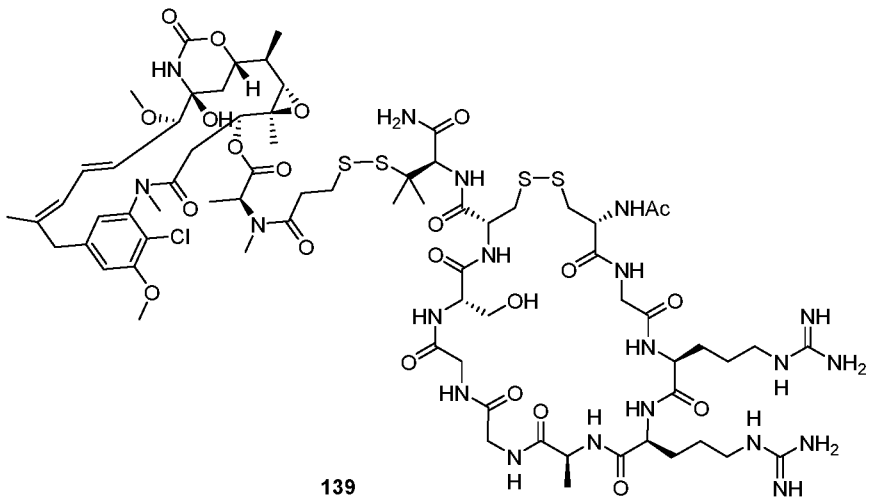
water with 0.1% AcOH provided **137** bis-acetate salt (142 mg, 0.0729 mmol, 64% yield). LCMS M/Z = 914.8 [(M + 2) / 2].



[00420] A vial was charged with **46** (74.7 mg mg, 0.0584 mmol), and a solution of DM-4 (63.0 mg, 0.807 mmol) in DMF (4 mL) was added. While stirring, pH 7.4 phosphate buffer (4 mL) was added dropwise over 5 min. After stirring for another 5 min at room temperature, LCMS shows complete consumption of **46.** The reaction was acidified by adding acetic acid (1 mL), and the resulting reaction mixture loaded directly onto a 50 g C18 Isco Gold column. Elution with 5% to 50% acetonitrile in water with 0.1% AcOH provided **138** acetate salt (62.9 mg, 0.0313 mmol, 54% yield). LCMS M/Z = 974.3 [[(M + 2) / 2].



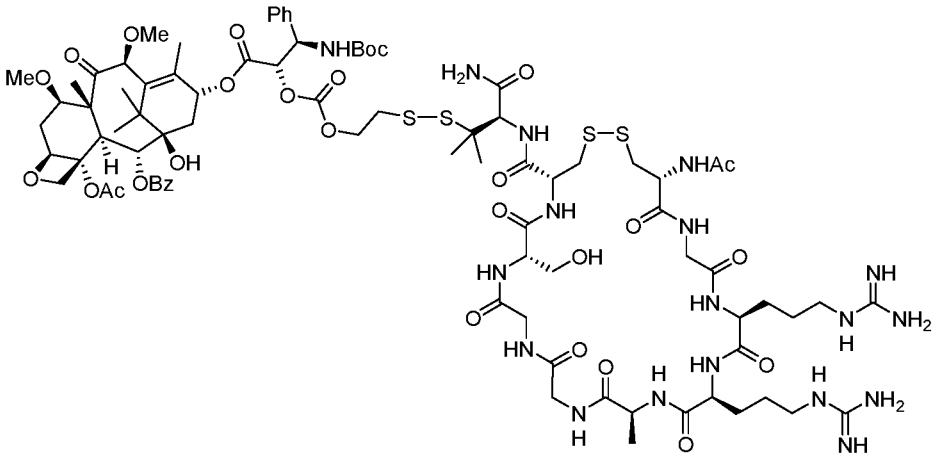
52 1



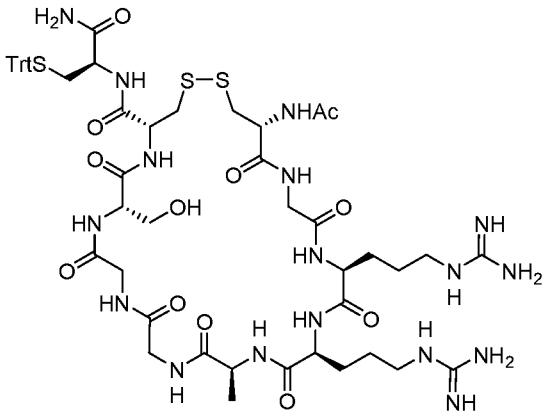
139

[00421] A vial was charged with **52** (12.8 mg, 10.0 μηιοϊ), and this was dissolved in trifluoroethanol (0.1 mL) and 1,1,3,3-tetramethyldisiloxane (13 μL). A solution of 12N HCI (13 μL) in trifluoroethanol (0. 1 mL) was added, and the reaction stirred at room temperature for 15 min. All solvent was removed *in vacuo,* and the remaining residue dissolved in DMF (0.6 mL). This solution was added to **1** (12.7 mg, 15.0 μηοϊ), and 0.2M sodium acetate (0.6 mL) was added. The reaction stirred at room temperature for 1 h, and LCMS shows conversion to the desired product. The crude reaction mixture was purified by preparative HPLC (5% to 95% acetonitrile in water with 0.1% TFA) to give **139** as the bis-TFA salt (14.0 mg, 7.00 μηοϊ, 70% yield). LCMS M/Z = 888 [(M + 2) / 2].

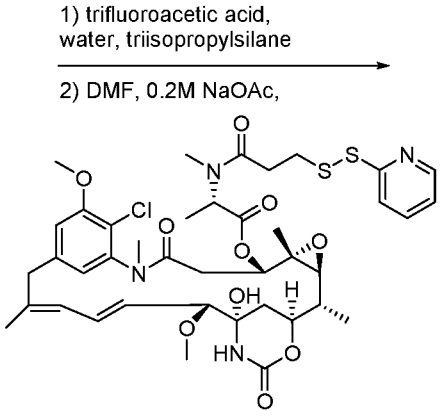
[00422] Compound **140** was made in an analogous manner:



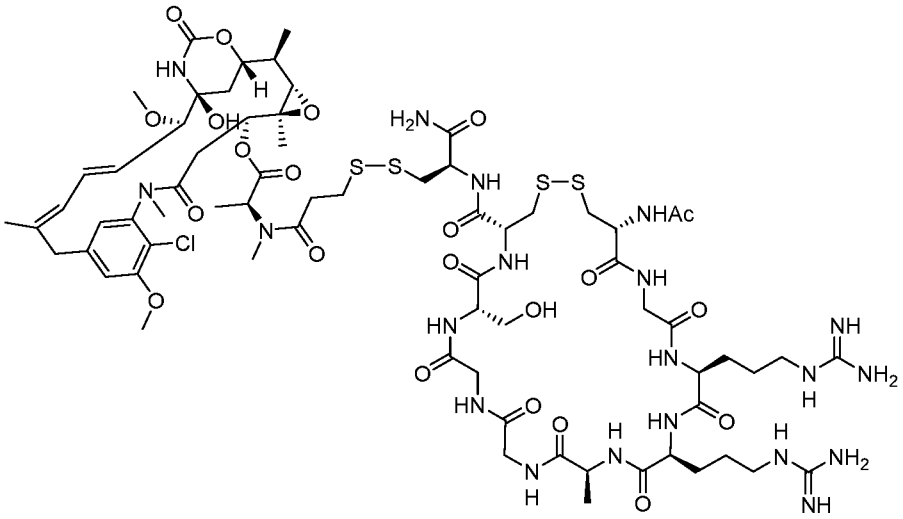
140



51



1

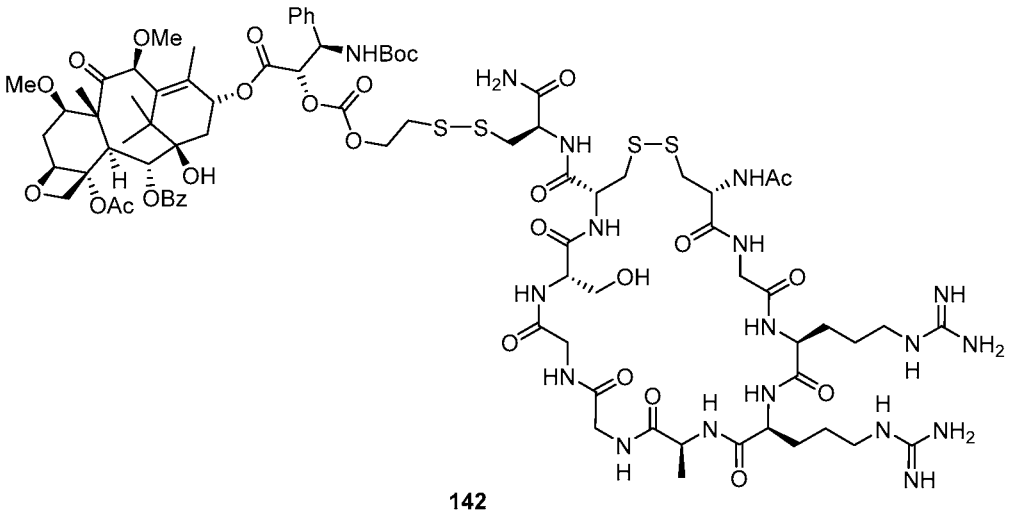


141

[00423] A vial was charged with **51** (15.0 mg, 8.55 μηιοϊ). Trifluoroacetic acid (315 μL), water (8 μL) and tnisopropylsilane (8 μL) were added, and the reaction stirred at room temperature for 20 min. All solvent was removed *in vacuo,* and the remaining

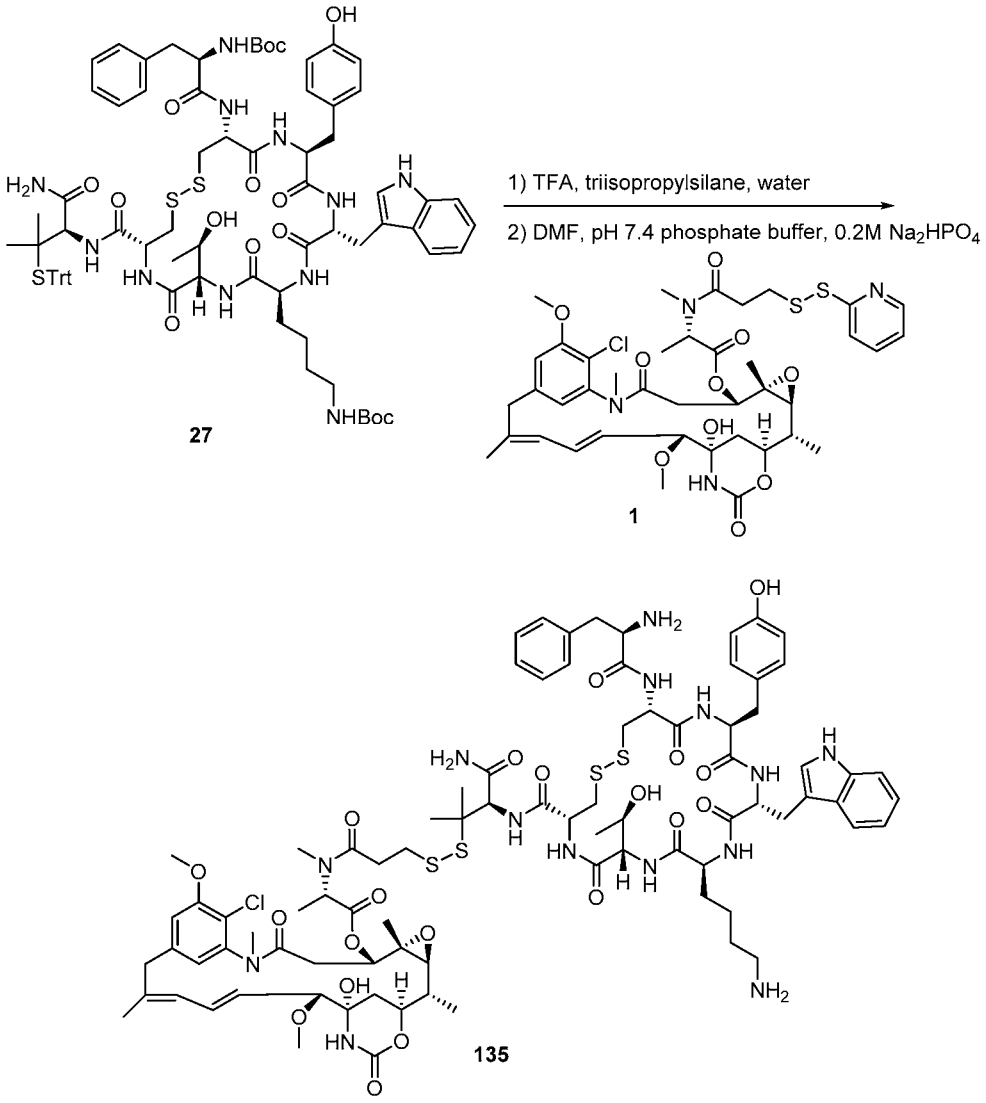
residue dissolved in DMF (1.0 mL). This solution was added to **1** (10.9 mg, 12.8 μηιοϊ), and 0.2M sodium acetate (1.5 mL) was added. The reaction was stirred at room temperature for 1 h, and LCMS shows complete conversion to the desired product. The crude reaction mixture was purified by preparative HPLC (5% to 95% acetonitrile in water with 0.1% TFA) to give **141** as the bis-TFA salt (7.0 mg, 4.0 μηιοϊ, 46% yield).

[00424] Compound **142** was made in an analogous manner:



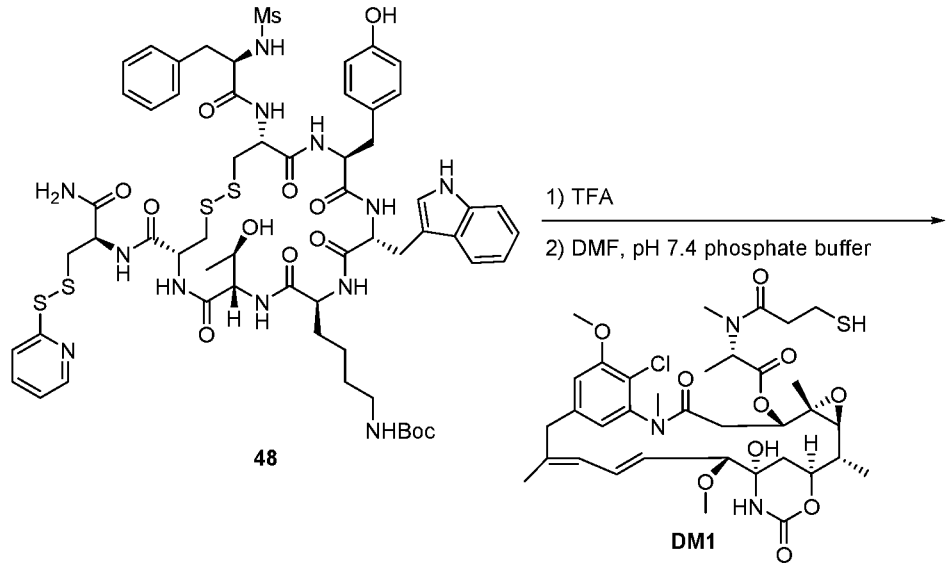
142

Conjugation procedure A:



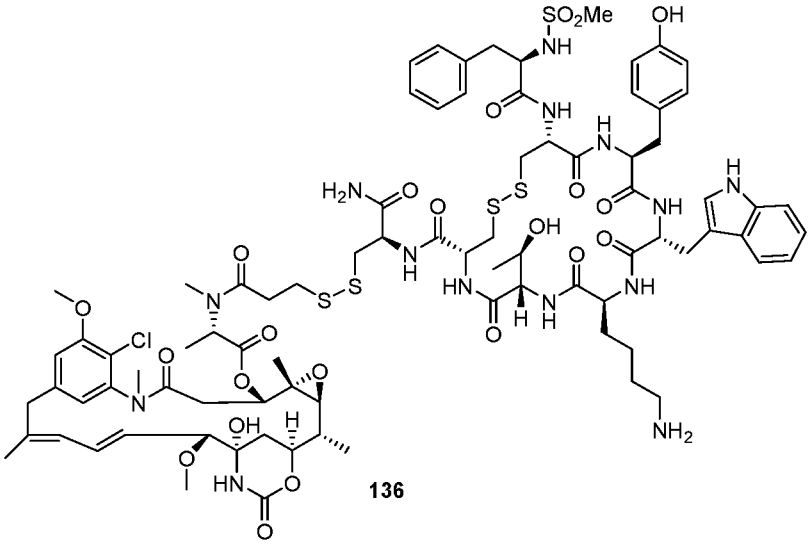
[00425] A vial was charged with **27** (556 mg, 0.366 mmol), and water (0.05 mL), triisopropylsilane (0.05 mL) and trifluoroacetic acid (2.0 mL) were added. The reaction was stirred at room temperature for 10 min, and all solvents were removed *in vacuo.* To the remaining residue was added a solution of **1** (387 mg, 0.457 mmol) in DMF (4 mL). To this solution was then added pH 7.4 phosphate buffer (4.0 mL) and 0.2M **Na_2HP04** (0.60 mL) dropwise, in that order, over 5 min. The reaction was stirred at room temperature for 1 h, and the reaction judged complete by LCMS. The reaction was acidified by adding 3 mL AcOH, and the crude reaction mixture loaded onto a 50 g C18 Isco column. Elution with 5% to 55% acetonitrile in water with 0.1% AcOH provided **135** as the bis-acetate salt (300 mg, 0.165 mmol, 45% yield). LCMS M/Z = 907.7 [(M + 2) / 2].

Conjugation procedure B:



48

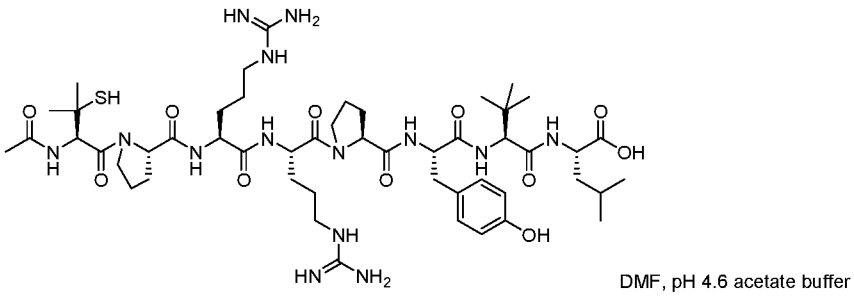
DM1



136

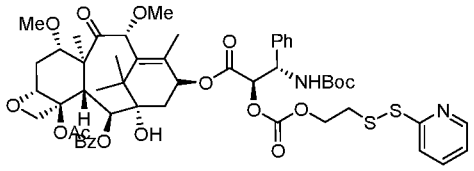
[00426] A vial was charged with **48** (14.5 mg, 10.8 μηιοϊ), and trifluoroacetic acid (2.0 mL) was added. The solution was stirred at room temperature for 5 min, and all solvent was removed *in vacuo.* To the remaining residue was added a solution of **DM1** (16.0 mg, 21.7 μηοϊ) in DMF (2.0 mL). pH 7.4 phosphate buffer (2.0 mL) was then added dropwise over 5 min, and the reaction stirred at room temperature for 30 min. The reaction was judged complete by LCMS. The reaction was acidified by adding acetic acid (0.50 mL), and the crude reaction mixture loaded onto a 24 g C18 Isco column. Eluting with 5% to 75% acetonitnle in water with 0.1% AcOH provided **136** as the bis-acetate salt (7.6 mg, 3.9 μηοϊ, 36% yield). LCMS M/Z = 932.7 [(M + 2) / 2].

Conjugation procedure C:

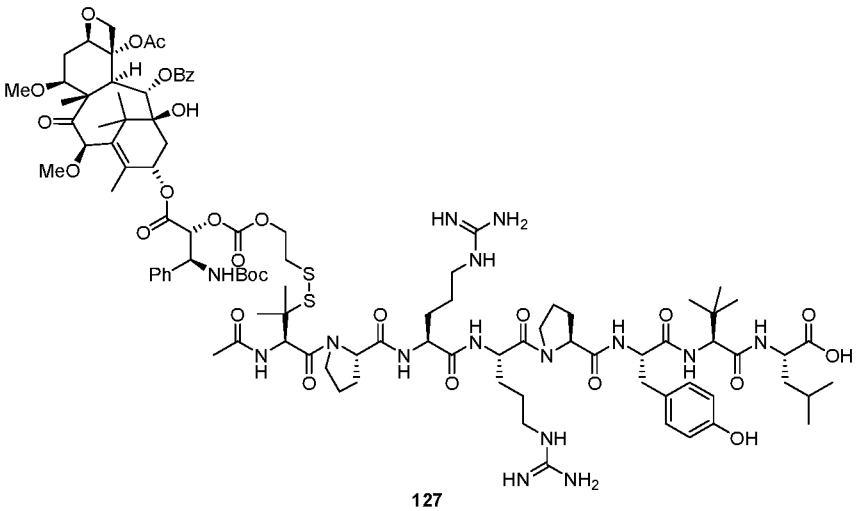


23

+



4

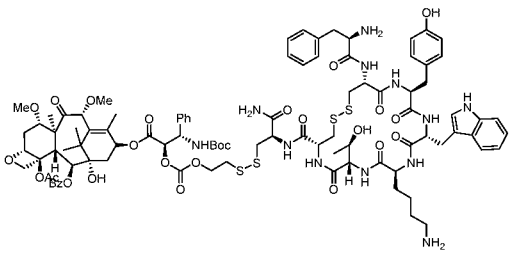
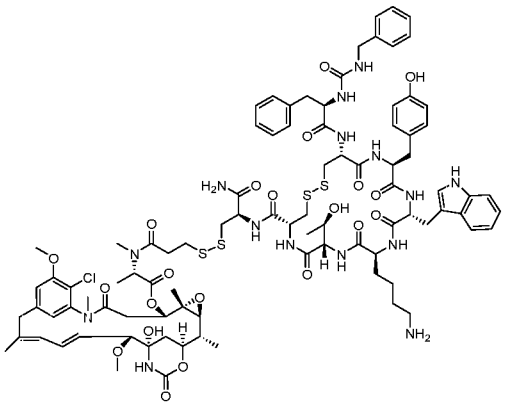
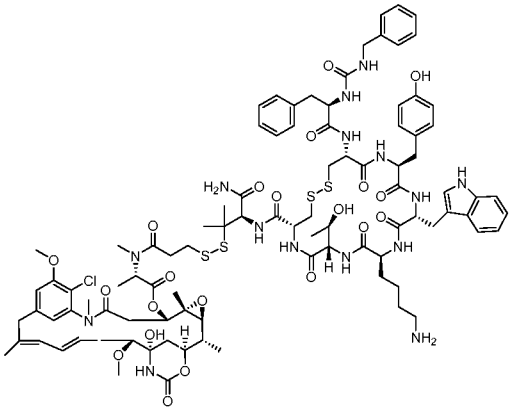
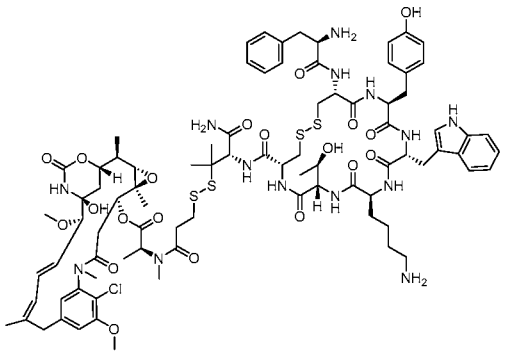


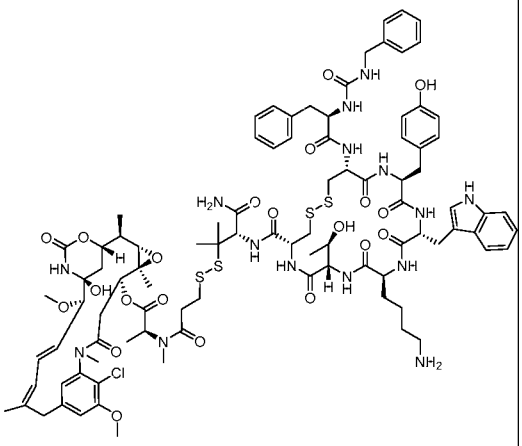
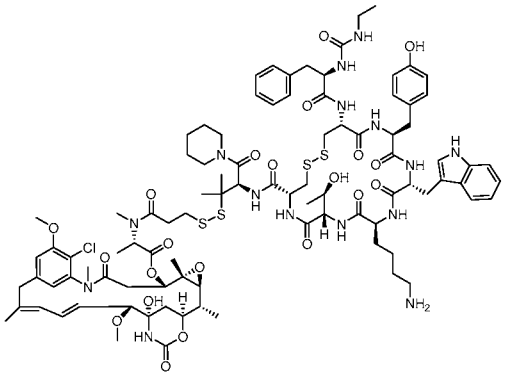
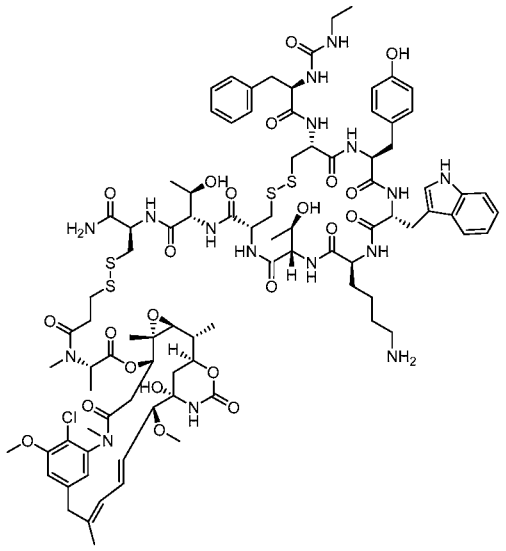
127

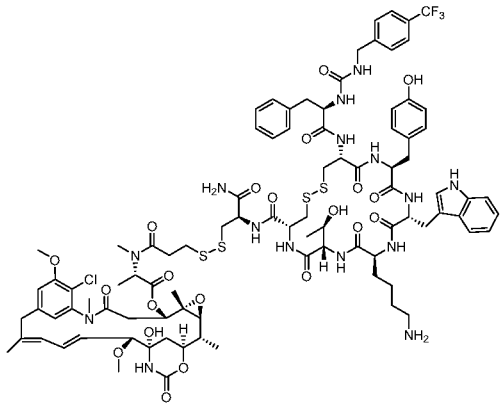
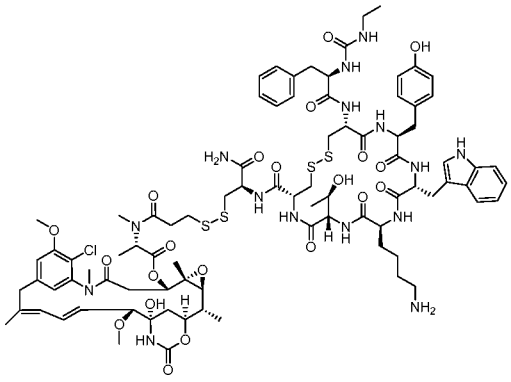
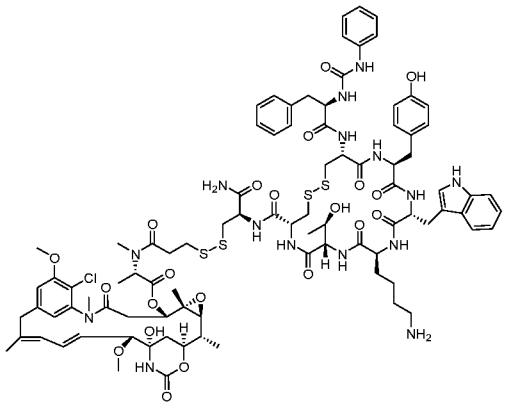
[00427] A solution of **4** (20.0 mg, 19.0 mmol) in DMF (2 mL) was added to a vial charged with **23** (10.0 mg, 9.20 mmol). pH 4.6 acetate buffer (2.0 mL) was added, and the reaction stirred at room temperature for 1 h. Reaction is judged complete by LCMS, and the reaction mixture purified by preparative HPLC (5% to 95% acetonitrile in water with 0.1% TFA) to give **127** as the bis-TFA salt (13.0 mg, 5.77 mmol, 62% yield). LCMS M/Z = 1013.3 [(M + 2) / 2].

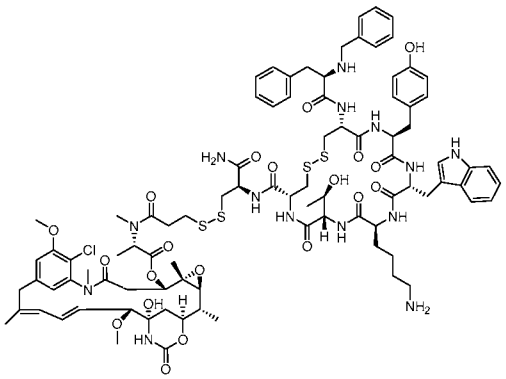
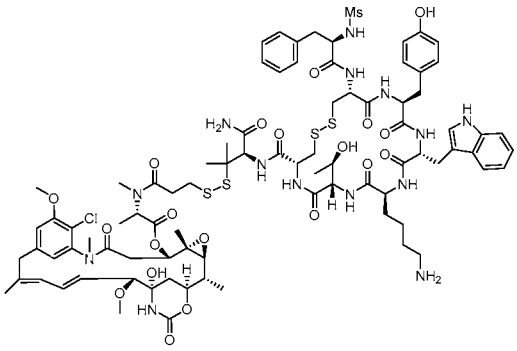
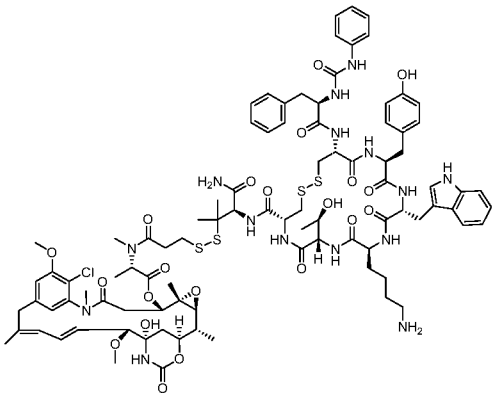
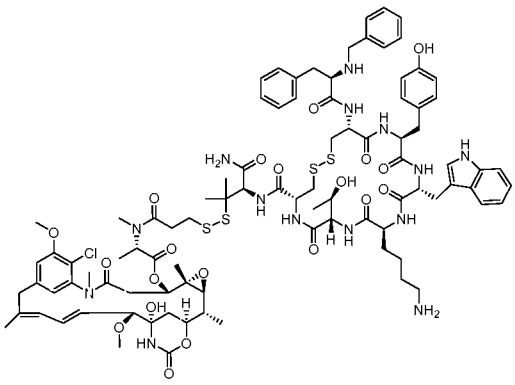
[00428] The following compounds were made by one of the above conjugation procedures:

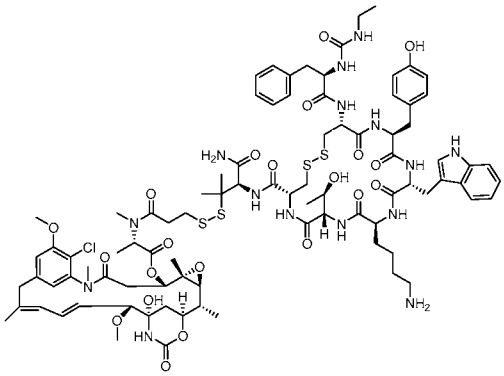
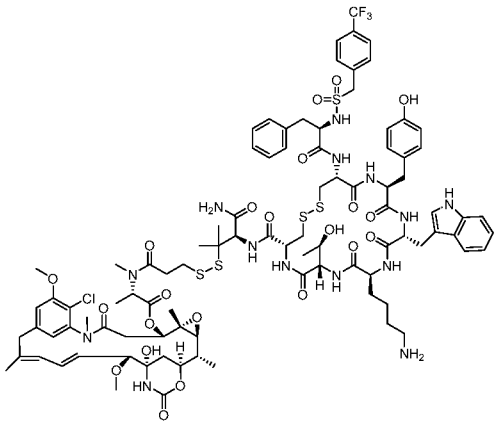
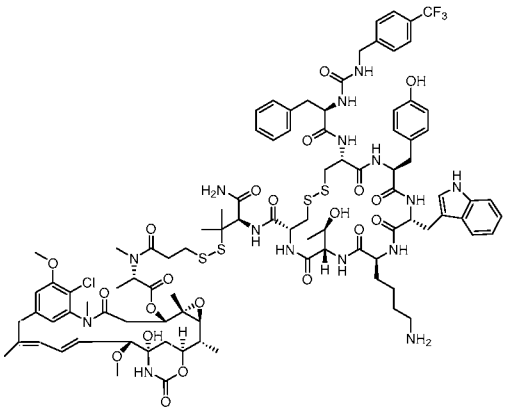
Precursor #	Conjugation method	Payload	Structure	Cmpd.	LCMS M/Z

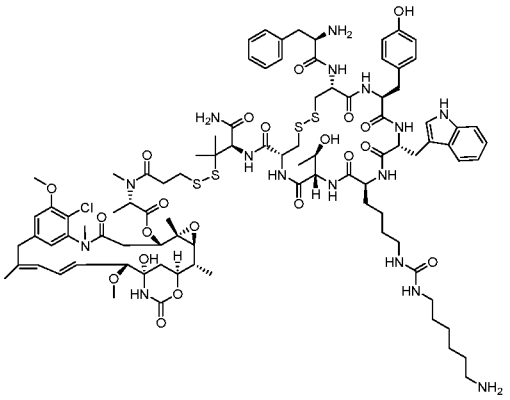
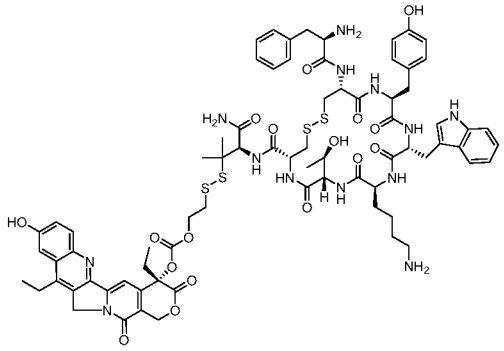
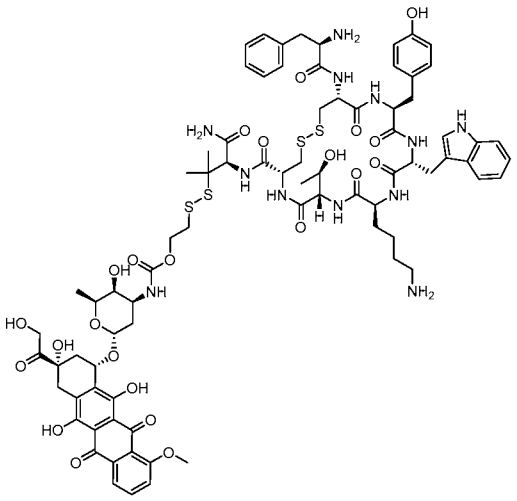
26	A	**4**		**100**	994.8 [(M + 2) / 2]
28	A	**1**		**101**	960.3 [(M + 2) / 2]
29	A	**1**		**102**	974.2 [(M + 2) / 2]
30	A	**1**		**103**	907.7 [(M + 2) / 2]

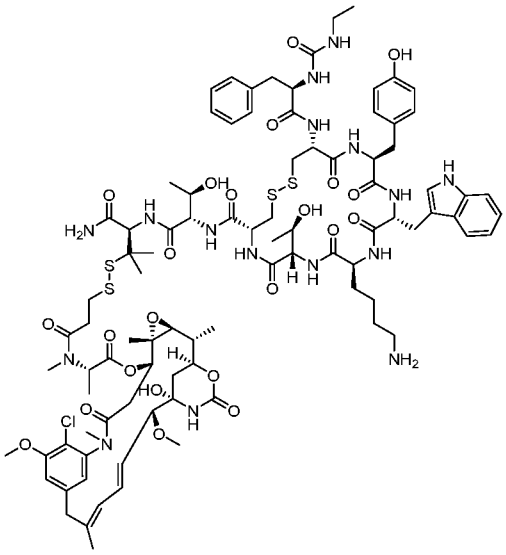
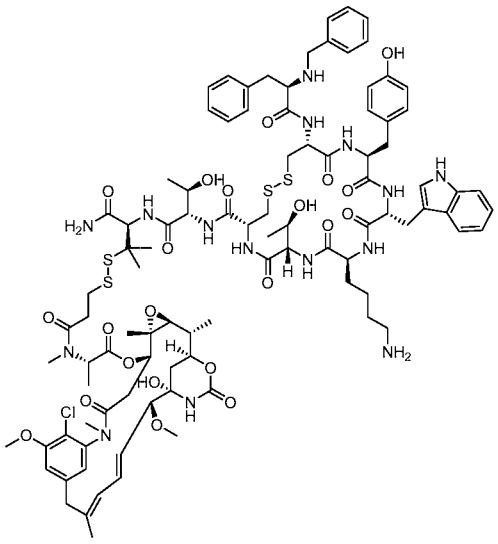
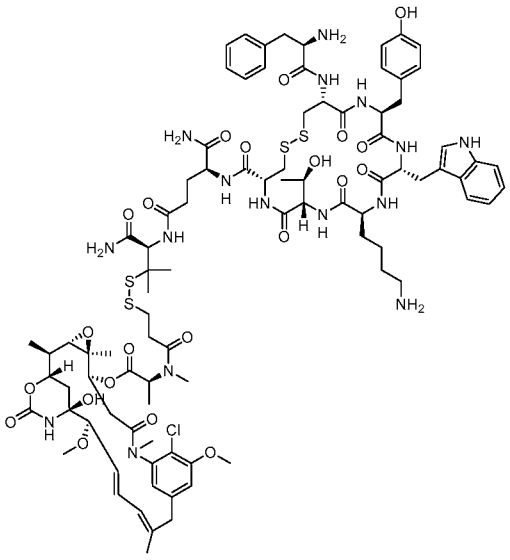
31	A	**1**		**104**	974.3 [(M + 2) / 2]
32	A	**1**		**105**	977.5 [(M + 2) / 2]
50	B	**DM1**		**106**	979.7 [(M + 2) / 2]

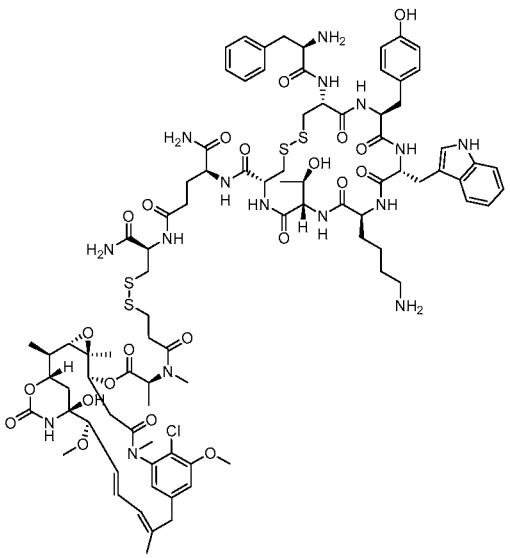
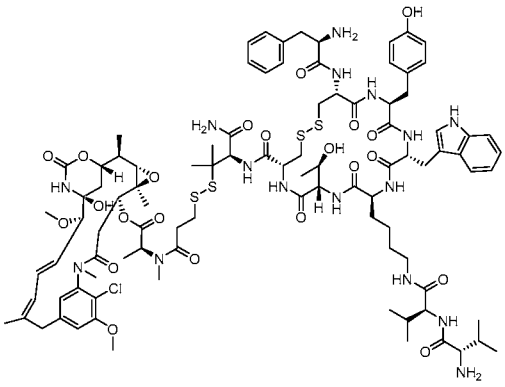
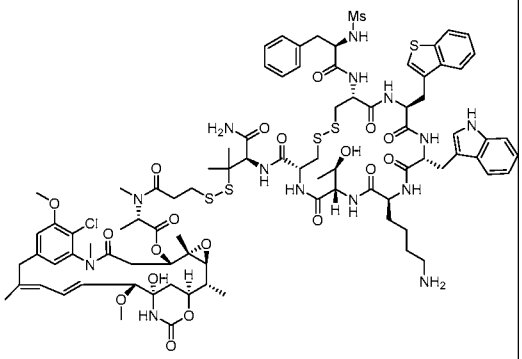
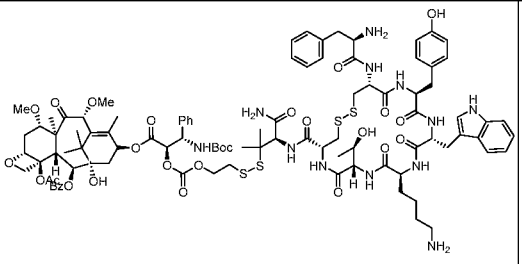
39	A	**1**		**107**	995 [(M + 2) / 2]
49	B	**DM1**		**108**	929 [(M + 2) / 2]
34	A	**1**		**109**	952.9 [(M + 2) / 2]

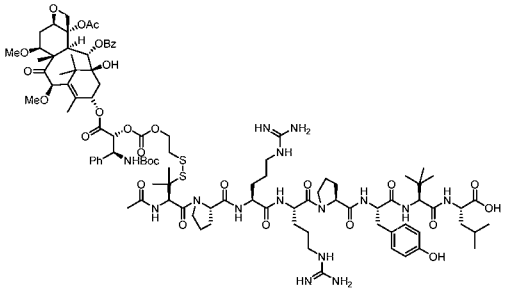
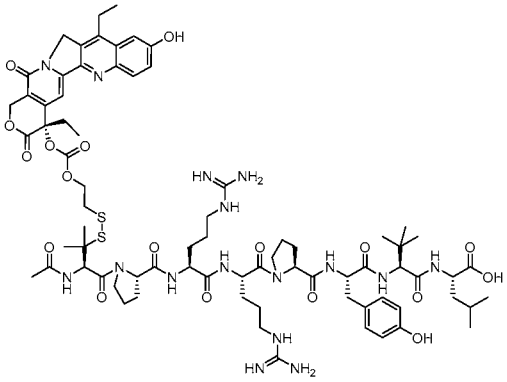
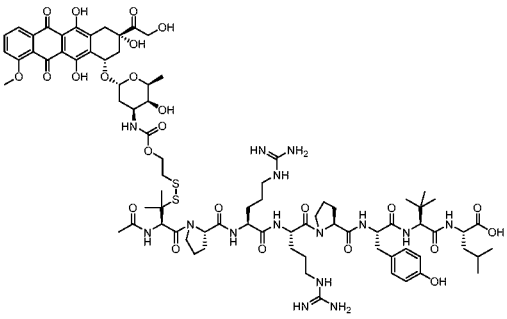
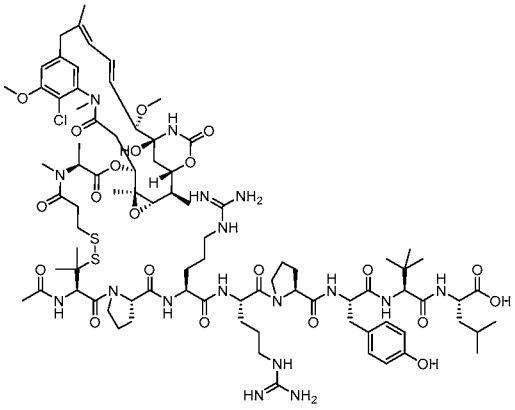
47	B	**DM1**		**110**	938.5 [(M + 2) / 2]
33	A	**1**		**111**	946.7 [(M + 2) / 2]
35	A	**1**		**112**	967.3 [(M + 2) / 2]
36	A	**1**		**113**	952.8 [(M + 2) / 2]

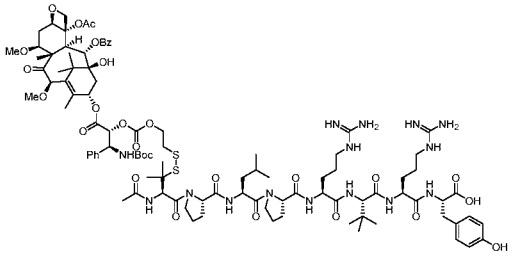
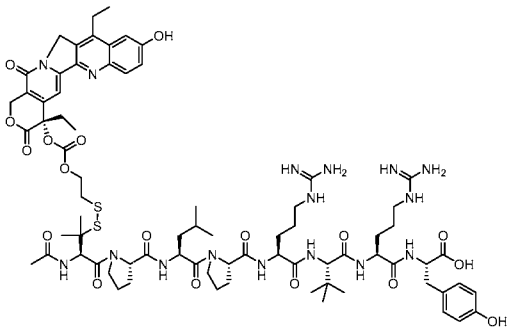
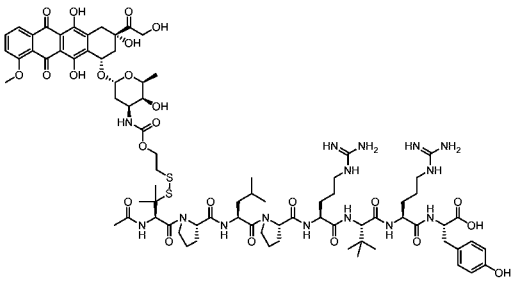
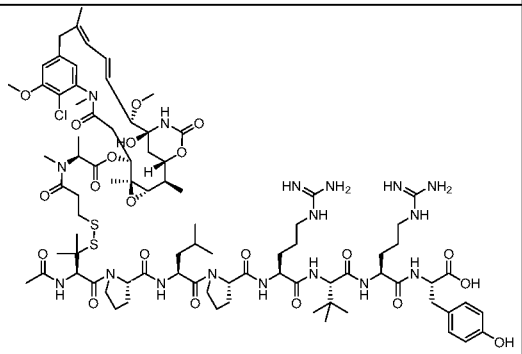
37	A	**1**		**114**	943.3 [(M + 2) / 2]
38	A	**1**		**115**	1018.7 [(M + 2) / 2]
40	A	**1**		**116**	1008.3 [(M + 2) / 2]

53	A	**1**		**117**	978.8 [(M + 2) / 2]
27	A	**2**		**118**	787.0 [(M + 2) / 2]
27	A	**3**		**119**	862.5 [(M + 2) / 2]

41	A	**1**		**120**	993.8 [(M + 2) / 2]
42	A	**1**		**121**	1003.3 [(M + 2) / 2]
44	A	**1**		**122**	971.7 [(M + 2) / 2]

43	A	**1**		**123**	957.8 [(M + 2) / 2]
54	A	**1**		**124**	1006.8 [(M + 2) / 2]
45	A	**1**		**125**	966.3 [(M + 2) / 2]
27	C	**4**		**126**	1008.5 [(M + 2) / 2]

23	C	**4**		**127**	1013.3 [(M + 2) / 2]
23	C	**2**		**128**	791.6 [(M + 2) / 2]
23	C	**3**		**129**	867.2 [(M + 2) / 2]
23	C	**1**		**130**	912.3 [(M + 2) / 2]

24	C	**4**		**131**	1013.3 [(M + 2) / 2]
24	C	**2**		**132**	791.6 [(M + 2) / 2]
24	C	**3**		**133**	867.2 [(M + 2) / 2]
24	C	**1**		**134**	912.3 [(M + 2) / 2]

EXAMPLE 2: Ki of conjugates for somatostatin receptor

[00429] Two conjugates were assessed in an *in vitro* assay evaluating binding to the somatostatin receptor 2 (SSTR2). A radioligand-receptor binding assay was conducted at Eurofins Panlabs (Taiwan) to determine the affinity of conjugates described herein to the SSTR2. The assay measures binding of radiolabeled ligand, [^{125}I] labeled somatostatin, to human SSTR2 using membrane preparations from SSTR2 expressing CHO-Kl cells. Membranes were incubated with radiolabeled

somatostatin (0.03 nM) in the presence of conjugate/compound starting at a dose of either 10 μM (for compounds with a capping group on Lys^5, 117 and 143) or 10 nM (all other compounds) using 5x serial dilutions to obtain a 6-pt curve. After a four hour incubation, membranes were filtered and washed 3x and counted to determine the remaining [^{125}I] somatostatin bound to the receptor. IC_{50} values were determined by a non-linear, least squares regression analysis using MathIQTM (ID Business Solutions Ltd., UK). The K_i values were calculated using the equation of Cheng and Prusoff (Cheng and Prusoff, Biochem. Pharmacol. 22:3099-3108, 1973) using the observed IC_{50} of the tested conjugate/compound, the concentration of radioligand employed in the assay, and the historical values for the K_i of the ligand obtained at Eurofins.

Table 3. *SSTR2 K_is for selected conjugates*

Compound	SSTR2 K_i (pM)
SST14	4.8
200	17
135	21
101	57
102	25
103	790
104	3150
136	180
106	27
107	160
108	70
109	42
110	75
111	35
112	34
113	20
114	18
115	41
116	77
117	69000
118	36
119	19
120	62
121	99

122	180
123	45
124	3980000
125	98

[00430] These data demonstrate the ability of conjugates to bind to SSTR2, and that compounds with a capping group on Lys^5, 117 and 124 have greatly inhibited SSTR2 binding affinity.

EXAMPLE 3: Inhibition of NCI-H524 cell proliferation by conjugates

[00431] Conjugates were assessed in an *in vitro* assay evaluating inhibition of cell proliferation. NCI-H524 (ATCC) human lung cancer cells were plated in 96 well, V-bottomed plates (Costar) at a concentration of 5,000 cells/well and 24 hours later were treated with compound for 6 hours and further incubated 66 hours. Compound starting dose was 20 μM and three fold serial dilutions were done for a total of ten points. After 6 hours of treatment, cells were spun down, the drug containing media was removed, and fresh complete medium was added and used to resuspend the cells, which were spun again. After removal of the wash media, the cells were resuspended in complete medium, then transferred into white walled, flat bottomed 96 well plates. Cells were further incubated for an additional 66 hours to measure inhibition of cell proliferation. Octreotide alone had no significant effect on cell proliferation. Proliferation was measured using CellTiter Glo reagent using the standard protocol (Promega) and a Glomax multi + detection system (Promega). Percent proliferation inhibition was calculated using the following formula: % inhibition = (control-treatment)/ control *100. Control is defined as vehicle alone. IC_{50} curves were generated using the nonlinear regression analysis (four parameter) with GraphPad Prism 6. IC_{50} values for representative compounds with and without octreotide competition were measured and were shown in Table 4.

Table 4. *NCI-H524 IC50s for selected conjugates*

Conjugate	H524 IC_{50} (nM)	H524 IC_{50} + 100 uM octreotide (nM)	Assay shift[1]
200	67	274	4.1
100	395	569	1.4
135	13	545	41.0
101	6.9	31	4.5
102	4.7	181	38.5

103	270	467	1.7
104	208	815	3.9
105	265	462	1.7
136	13	83	6.4
106	0.78	10	12.8
107	3.7	93	25. 1
108	1.3	12	9.2
109	1.7	115	67.6
110	7.2	20	2.8
111	3.35	246	73.4
112	6.08	232	38. 1
113	20	331	17.0
114	6.5	340	52.3
115	14	331	23.6
116	17	262	15.4
117	408	652	1.6
137	78	348	4.5
138	7.7	317	41.2
118	5200	1700	0.3
119	2722	3377	1.2
120	185	516	2.8
121	8.5	48	5.6
122	19	353	18.6
123	0.3	195	650.0
124	90	250	2.8
125	8	230	28.8

[1] - Assay shift is the ratio of ICso with octreotide to the ICso without octreotide

[00432] These data demonstrate the ability of these conjugates to kill SSTR2-expressing cells in a receptor-dependent manner, and that conjugates with impaired SSTR2-binding affinity (117 and 124) show <3-fold assay shift.

EXAMPLE 4: Inhibition of IMR-32 cell proliferation by conjugates

[00433] Conjugates were assessed in an *in vitro* assay evaluating inhibition of cell proliferation. IMR-32(ATCC), human neuroblastoma, cells were plated in 96 well, flat-bottomed plates (Costar) at a concentration of 4,000 cells/well. Cells were then incubated at 37°C with 5% CO2 for 24hrs. After 24hrs, cells were treated with either 100 μM Octreotide or 0.01% DMSO, and incubated for lhr at 37°C with 5% CO2. Compounds were then added at a starting dose of 20 μM and three fold serial dilutions were done for a total of ten points. Following compound addition cells were incubated at 37°C with 5% CO2 for 6hrs. After 6 hours of treatment, the drug

containing media was removed, and fresh complete medium was added. This process was completed a total of 3 times in order to ensure drug containing media was removed and cells were thoroughly washed. Cells were further incubated for an additional 66 hours to measure inhibition of cell proliferation. Octreotide alone had no significant effect on cell proliferation. Proliferation was measured by CellTiter Glo reagent using the standard protocol (Promega) and a Glomax multi + detection system (Promega). Percent proliferation inhibition was calculated using the following formula: % inhibition = (control -treatment)/ control *100. Control is defined as vehicle alone. ICso curves were generated using the nonlinear regression analysis (four parameter) with GraphPad Prism 6. ICso values for representative compounds with and without octreotide competition were measured and were shown in Table 5.

***Table** 5. IMR-32 IC50s for selected conjugates*

Penicillamine conjugate	H524 IC_{50} (nM)	H524 IC_{50} + 100 uM octreotide (nM)	Assay shift[1]
135	0.03	9.8	327
117	29	35	1.2

Cysteine conjugate	H524 IC_{50} (nM)	H524 IC_{50} + 100 uM octreotide (nM)	Assay shift[1]
200	0.75	7.1	9.5

[1] - Assay shift is the ratio of ICso with octreotide to the ICso without octreotide

[00434] These data demonstrate the ability of these conjugates to kill SSTR2-expressing cells in a receptor-dependent manner, that penicillamine conjugate 135 shows greater receptor dependent cell killing than the corresponding cysteine conjugate 200, and that a conjugate with impaired SSTR2 binding affinity, 117, does not show receptor-dependent cell killing.

EXAMPLE 5: Rat plasma stability of conjugates

***Table** 6. Rat plasma stability of conjugates*

Penicillamine conjugate	Rat plasma $t_{1/2}$ (h)	Cysteine conjugate	Rat plasma $t_{1/2}$ (h)
135	18.2	200	3.2
102	49.8	101	8.15

[00435] These data demonstrate that penicillamine-linked conjugates 135 and 102 have greater stability in rat plasma than the corresponding cysteine-linked conjugate 200 and 101, respectively.

EXAMPLE 6: Rat liver microsome stability of conjugates

***Table** 7. Rat liver microsome stability of conjugates*

Penicillamine conjugate	Rat liver microsomes $t_{1/2}$ (min)	Cysteine conjugate	Rat liver microsomes $t_{1/2}$ (min)
135	178	200	34.1
102	283	101	25.8

[00436] These data demonstrate that penicillamine-linked conjugates 135 and 102 have greater stability in rat microsomes than the corresponding cysteine-linked conjugates 200 and 101, respectively.

EXAMPLE 7: Mouse plasma PK

***Table 8.** Mouse plasma PK AUC*

Compound	Mouse plasma PK AUC, 0-inf (uM/umol/kg*hr)
200	6.07
135	11.5

[00437] These data demonstrate that penicillamine-linked conjugate 135 has superior mouse plasma PK than corresponding cysteine-linked conjugate 200.

[00438] The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[00439] In the claims, articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[00440] 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.

[00441] 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 invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00442] In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. 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 invention can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[00443] 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.

[00444] Section and table headings are not intended to be limiting.

CLAIMS

We claim:

1. A conjugate comprising an active agent coupled to a targeting moiety by a linker, wherein the linker comprises a penicillamine group or a derivative thereof, and the active agent is attached to the penicillamine group or derivative thereof by a disulfide bond.

2. The conjugate of claim 1 comprising a formula selected from the group consisting of X-Y-Z, X-Y-Z-Y-X, X-(Y-Z)n, (X-Y)n-Z, X-Y-Zn, Xn-Y-Z, and (X-Y-Z-Y)n-Z;

wherein X is the targeting moiety,
Y is the linker,
Z is the active agent, and
n is an integer between 2 and 1,000.

3. The conjugate of claim 2 comprising the formula X-Y-Z;
wherein X is the targeting moiety,
Y is the linker, and
Z is the active agent.

4. The conjugate of claim 1, wherein the active agent is selected from DM1, DM4, cabazitaxel, SN-38, or doxorubicin.

5. The conjugate of claim 1, wherein the targeting moiety does not bind to a vitamin receptor.

6. The conjugate of claim 5, wherein the targeting moiety does not bind to a folate receptor.

7. The conjugate of claim 1, wherein the active agent is not a vinca alkaloid.

8. The conjugate of claim 1, wherein the targeting moiety is attached to the N-terminus or C-terminus of the penicillamine group or derivative thereof.

9. The conjugate of claim 8, wherein the targeting moiety binds to a somatostatin receptor (SSTR), a neurotensin receptor (NTSR), or IL-1 IRa.

10. The conjugate of claim 9, wherein the targeting moiety binds to SSTR2.

11. The conjugate of claim 10, wherein the target moiety is selected from the group consisting of somatostatin, octreotide, octreotate, lanreotide, lutathera (^{177}Lu-DOTATATE), ^{90}Y-DOTATOC, Tyr^3-octreotate (TATE), vapreotide, cyclo(AA-Tyr-DTrp-Lys-Thr-Phe) where AA is α-N-Me lysine or N-Me glutamic acid, pasireotide, lanreotide, and seglitide.

12. The conjugate of claim 9, wherein the targeting moiety binds to NTSR1.

13. The conjugate of claim 12, wherein the targeting moiety comprises neurotension or derivative thereof.

14. The conjugate of claim 13, wherein the targeting moiety comprises the targeting domain of neurotensin.

15. The conjugate of claim 14, wherein the targeting moiety comprise the 7 C-terminal amino acids of neurotensin.

16. The conjugate of claim 9, wherein the targeting moiety binds to IL-1 IRa.

17. The conjugate of claim 16, wherein the targeting moiety comprises IL-1 1 or derivative thereof.

18. The conjugate of claim 1, wherein the linker further comprises a cleavable group.

19. The conjugate of claim 1, wherein the conjugate has a molecular weight of less than 50,000 Da.

20. The conjugate of claim 19, wherein the conjugate has a molecular weight of between about 1000 Da and about 5000 Da.

21. The conjugate of claim 1, wherein the conjugate is selected from the compounds in Table 1.

22. A particle comprising the conjugate of any of claims 1-21.

23. The particle of claim 22, wherein the particle comprises at least one polymeric matrix.

24. The particle of calim 23, wherein the polymeric matrix comprises one or more polymers selected from the group consisting of hydrophobic polymers, hydrophilic polymers, and copolymers thereof.

25. The particle of claim 24, wherein the hydrophobic polymers are selected from the group consisting of polyhydroxyacids, polyhydroxyalkanoates, olycaprolactones, poly(orthoesters), polyanhydrides, poly(phosphazenes), poly(lactide-co-caprolactones), polycarbonates, polyesteramides, polyesters, and copolymers thereof.

26. The particle of claim 24, wherein the hydrophilic polymers are selected from the group consisting of polyalkylene glycols, polyalkylene oxides, poly(oxyethylated polyol), poly(olefinic alcohol), polyvinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(hydroxy acids), poly(vinyl alcohol), and copolymers thereof.

27. The particle of claim 23, wherein the polymeric matrix comprises one or more polymers selected from the group consisting of poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), poly(ethylene oxide), poly(ethylene glycol), poly(propylene glycol), and copolymers thereof.

28. The particle of claim 22, wherein the particle has a diameter between 10 nm and 5000 nm.

29. The particle of claim 28, wherein the particle has a diameter between 30-70 nm, 70 nm - 120 nm, 120-200 nm, 200-5000 nm, or 500 - 1000 nm.

30. The particle of claim 22, wherein the conjugate is fully or particially encapsulated in the particle.

31. The particle of claim 22, wherein the conjugate is present in an amount between 0.05% and 50 % (w/w) based upon the weight of the particle.

32. A pharmaceutical formulation comprising the conjugate of any of claims 1-21 and at least one pharmaceutically acceptable excipient.

33. A pharmaceutical formulation comprising the particle of any of claims 22-31 and at least one pharmaceutically acceptable excipient.

34. A method of treating a subject in need thereof comprising administering a therapeutically effective amount of the formulation of claim 32 or claim 33.

35. The method of claim 34, wherein the subject has cancer.

36. The method of claim 35, wherein the cancer is selected from lung cancer, breast cancer, colorectal cancer, ovarian cancer, pancreatic cancer, colorectal cancer, bladder cancer, prostate cancer, cervical cancer, renal cancer, leukemia, central nervous system cancers, myeloma, melanoma, and a neuroendocrine cancer.

37. The method of claim 36, wherein the neuroendocrine caner is selected from small cell lung cancer (SCLC), adrenal medullary tumors, pheochromocytoma, neuroblastoma, ganglioneuroma, or paraganglioma, gastroenteropancreatic neuroendocrine tumors, carcinoids, gastrinoma, glucagonoma, vasoactive intestinal polypeptide-secreting tumor, pancreatic polypeptide-secreting tumor, nonfunctioning gastroenteropancreatic tumors, meduallary thyroid cancer, Merkel cell tumor of the skin, pituitary adenoma, and pancreatic cancer.

38. A method of inhibiting cell proliferation comprising administering the conjugate of claim 1.

39. The method of claim 38, wherein the cell is a cancer cell.

40. The method of claim 39, wherein the cancer cell is selected from lung cancer cell, breast cancer cell, colorectal cancer cell, ovarian cancer cell, pancreatic cancer cell, colorectal cancer cell, bladder cancer cell, prostate cancer cell, cervical cancer cell, renal cancer cell, leukemia cell, central nervous system cancer cell, myeloma cell, melanoma cell, and a neuroendocrine cancer cell.