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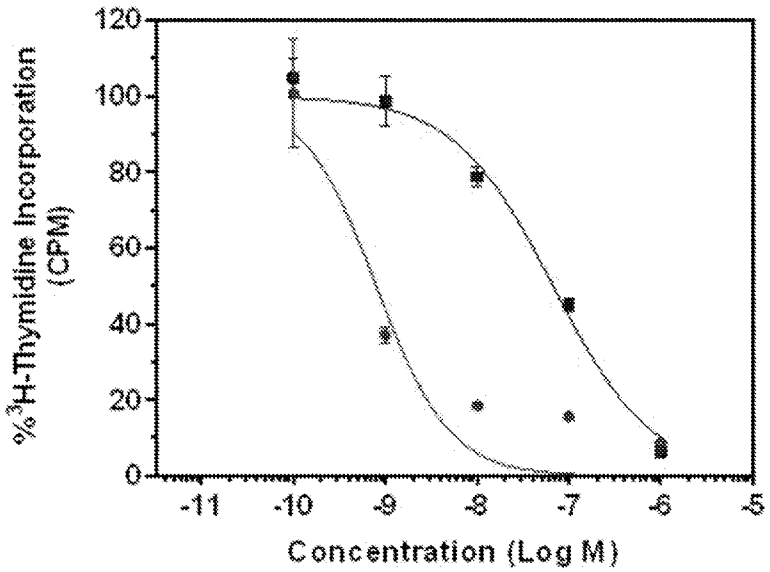
(54) Title: PBD CONJUGATES FOR TREATING DISEASES

FIG. 1



(57) Abstract: The present disclosure relates to pyrrolobenzodiazepine (PBD) prodrugs and conjugates thereof. The present disclosure also relates to pharmaceutical compositions of the conjugates described herein, methods of making and methods of using the same.

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PBD CONJUGATES FOR TREATING DISEASES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 62/314,688, filed March 29, 2016, U.S. Provisional Application Serial No. 62/323,282, filed April 15, 2016, and U.S. Provisional Application Serial No. 62/396,409, filed September 19, 2016, in which all of which are incorporated herein by reference in their entirety.

TECHNICAL FIELD

The present disclosure relates to pyrrolobenzodiazepine (PBD) prodrugs and conjugates thereof. The present disclosure also relates to pharmaceutical compositions of the conjugates described herein, methods of making and methods of using the same.

BACKGROUND

The mammalian immune system provides a means for the recognition and elimination of pathogenic cells, such as tumor cells, and other invading foreign pathogens. While the immune system normally provides a strong line of defense, there are many instances where pathogenic cells, such as cancer cells, and other infectious agents evade a host immune response and proliferate or persist with concomitant host pathogenicity. Chemotherapeutic agents and radiation therapies have been developed to eliminate, for example, replicating neoplasms. However, many of the currently available chemotherapeutic agents and radiation therapy regimens have adverse side effects because they lack sufficient selectivity to preferentially destroy pathogenic cells, and therefore, may also harm normal host cells, such as cells of the hematopoietic system, and other non-pathogenic cells. The adverse side effects of these anticancer drugs highlight the need for the development of new therapies selective for pathogenic cell populations and with reduced host toxicity.

Researchers have developed therapeutic protocols for destroying pathogenic cells by targeting cytotoxic compounds to such cells. Many of these protocols utilize toxins conjugated to antibodies that bind to antigens unique to or overexpressed by the pathogenic cells in an attempt to minimize delivery of the toxin to normal cells. Using this approach, certain immunotoxins have been developed consisting of antibodies directed to specific antigens on pathogenic cells, the antibodies being linked to toxins such as ricin, Pseudomonas exotoxin, Diptheria toxin, and tumor necrosis factor. These immunotoxins target pathogenic cells, such as tumor cells, bearing the specific antigens recognized by the antibody (Olsnes, S., *Immunol.*

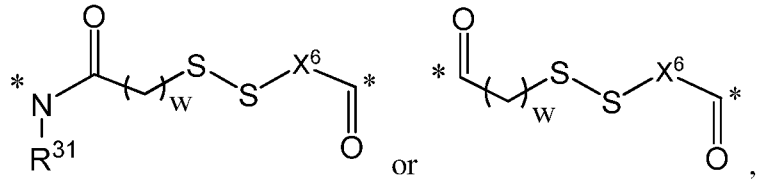
Today, 10, pp. 291-295, 1989; Melby, E.L., *Cancer Res.*, 53(8), pp. 1755-1760, 1993; Better, M.D., PCT Publication Number WO 91/07418, published May 30, 1991).

Another approach for targeting populations of pathogenic cells, such as cancer cells or foreign pathogens, in a host is to enhance the host immune response against the pathogenic cells to avoid the need for administration of compounds that may also exhibit independent host toxicity. One reported strategy for immunotherapy is to bind antibodies, for example, genetically engineered multimeric antibodies, to the surface of tumor cells to display the constant region of the antibodies on the cell surface and thereby induce tumor cell killing by various immune-system mediated processes (De Vita, V.T., *Biologic Therapy of Cancer*, 2d ed. Philadelphia, Lippincott, 1995; Soulillou, J.P., U.S. Patent 5,672,486). However, these approaches have been complicated by the difficulties in defining tumor-specific antigens.

Folate plays important roles in nucleotide biosynthesis and cell division, intracellular activities which occur in both malignant and certain normal cells. The folate receptor has a high affinity for folate, which, upon binding the folate receptor, impacts the cell cycle in dividing cells. As a result, folate receptors have been implicated in a variety of cancers (e.g., ovarian, endometrial, lung and breast) which have been shown to demonstrate high folate receptor expression. In contrast, folate receptor expression in normal tissues is limited (e.g., kidney, liver, intestines and placenta). This differential expression of the folate receptor in neoplastic and normal tissues makes the folate receptor an ideal target for small molecule drug development. The development of folate conjugates represents one avenue for the discovery of new treatments that take advantage of differential expression of the folate receptor. There is a great need for the development of folate conjugates, methods to identify folate receptor positive cancers, and methods to treat patients with folate receptor positive cancers.

SUMMARY

In one embodiment (referred to herein as embodiment 1), the present disclosure provides a conjugate, or a pharmaceutically acceptable salt thereof, comprising a binding ligand (B), one or more linkers (L), at least one releasable group, a first drug (D^1) and a second drug (D^2), wherein B is covalently attached to at least one L, at least one L is covalently attached to at least one of the first drug or the second drug, at least one of the first drug or the second drug is a PBD, and the one or more linkers comprises at least one releasable linker (L^r) of the formula



wherein

each R^{31} and $R^{31'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{32}$, $-OC(O)R^{32}$, $-OC(O)NR^{32}R^{32'}$, $-OS(O)R^{32}$, $-OS(O)_2R^{32}$, $-SR^{32}$, $-S(O)R^{32}$, $-S(O)_2R^{32}$, $-S(O)NR^{32}R^{32'}$, $-S(O)_2NR^{32}R^{32'}$, $-OS(O)NR^{32}R^{32'}$, $-OS(O)_2NR^{32}R^{32'}$, $-NR^{32}R^{32'}$, $-NR^{32}C(O)R^{33}$, $-NR^{32}C(O)OR^{33}$, $-NR^{32}C(O)NR^{33}R^{33'}$, $-NR^{32}S(O)R^{33}$, $-NR^{32}S(O)_2R^{33}$, $-NR^{32}S(O)NR^{33}R^{33'}$, $-NR^{32}S(O)_2NR^{33}R^{33'}$, $-C(O)R^{32}$, $-C(O)OR^{32}$ or $-C(O)NR^{32}R^{32'}$;

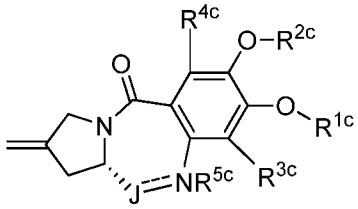
each X^6 is independently selected from the group consisting of $-C_1$-C_6 alkyl-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-, $-C_1$-C_6 alkyl-O-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-O-, $-C_1$-C_6 alkyl-$NR^{31'}$- and $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-$NR^{31'}$-, wherein each hydrogen atom in $-C_1$-C_6 alkyl-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-, $-C_1$-C_6 alkyl-O-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-O-, $-C_1$-C_6 alkyl-$NR^{31'}$- or $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-$NR^{31'}$ is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{34}$, $-OC(O)R^{34}$, $-OC(O)NR^{34}R^{34'}$, $-OS(O)R^{34}$, $-OS(O)_2R^{34}$, $-SR^{34}$, $-S(O)R^{34}$, $-S(O)_2R^{34}$, $-S(O)NR^{34}R^{34'}$, $-S(O)_2NR^{34}R^{34'}$, $-OS(O)NR^{34}R^{34'}$, $-OS(O)_2NR^{34}R^{34'}$, $-NR^{34}R^{34'}$, $-NR^{34}C(O)R^{35}$, $-NR^{34}C(O)OR^{35}$, $-NR^{34}C(O)NR^{35}R^{35'}$, $-NR^{34}S(O)R^{35}$, $-NR^{34}S(O)_2R^{35}$, $-NR^{34}S(O)NR^{35}R^{35'}$, $-NR^{34}S(O)_2NR^{35}R^{35'}$, $-C(O)R^{34}$, $-C(O)OR^{34}$ or $-C(O)NR^{34}R^{34'}$; and

each R^{32}, $R^{32'}$, R^{33}, $R^{33'}$, R^{34}, $R^{34'}$, R^{35} and $R^{35'}$ are independently selected from the group consisting of H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, and 5- to 7-membered heteroaryl;

each w is independently an integer from 1 to 4; and

each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 1, at least one of the first drug or the second drug is a PBD of the formula



wherein

J is –C(O)-, –CR^{13c}= or –($CR^{13c}R^{13c'}$)-;

R^{1c}, R^{2c} and R^{5c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -C(O)R^{6c}, -C(O)OR^{6c} and -C(O)$NR^{6c}R^{6c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{7c}, -OC(O)R^{7c}, -OC(O)$NR^{7c}R^{7c'}$, -OS(O)R^{7c}, -$OS(O)_2R^{7c}$, -SR^{7c}, -S(O)R^{7c}, -$S(O)_2R^{7c}$, -$S(O)_2OR^{7c}$, -S(O)$NR^{7c}R^{7c'}$, -$S(O)_2NR^{7c}R^{7c'}$, -$OS(O)NR^{7c}R^{7c'}$, -$OS(O)_2NR^{7c}R^{7c'}$, -$NR^{7c}R^{7c'}$, -NR^{7c}C(O)R^{8c}, -NR^{7c}C(O)OR^{8c}, -NR^{7c}C(O)$NR^{8c}R^{8c'}$, -NR^{7c}S(O)R^{8c}, -$NR^{7c}S(O)_2R^{8c}$, -NR^{7c}S(O)$NR^{8c}R^{8c'}$, -$NR^{7c}S(O)_2NR^{8c}R^{8c'}$, -C(O)$R^{7c}$, -C(O)$OR^{7c}$ or -C(O)$NR^{7c}R^{7c'}$; or when J is –CR^{13c}=, R^{5c} is absent; provided that at least one of R^{1c}, R^{2c} or R^{5c} is a covalent bond to the rest of the conjugate;

R^{3c} and R^{4c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -CN, -NO_2, -NCO, -OR^{9c}, -OC(O)R^{9c}, -OC(O)$NR^{9c}R^{9c'}$, -OS(O)R^{9c}, -$OS(O)_2R^{9c}$, -SR^{9c}, -S(O)R^{9c}, -$S(O)_2R^{9c}$, -S(O)$NR^{9c}R^{9c'}$, -$S(O)_2NR^{9c}R^{9c'}$, -$OS(O)NR^{9c}R^{9c'}$, -$OS(O)_2NR^{9c}R^{9c'}$, -$NR^{9c}R^{9c'}$, -NR^{9c}C(O)R^{10c}, -NR^{9c}C(O)OR^{10c}, -NR^{9c}C(O)$NR^{10c}R^{10c'}$, -NR^{9c}S(O)R^{10c}, -$NR^{9c}S(O)_2R^{10c}$, -NR^{9c}S(O)$NR^{10c}R^{10c'}$, -$NR^{9c}S(O)_2NR^{10c}R^{10c'}$, -C(O)$R^{9c}$, -C(O)$OR^{9c}$ and -C(O)$NR^{9c}R^{9c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{11c}, -OC(O)R^{11c}, -OC(O)$NR^{11c}R^{11c'}$, -OS(O)R^{11c}, -$OS(O)_2R^{11c}$, -SR^{11c}, -S(O)R^{11c}, -$S(O)_2R^{11c}$, -S(O)$NR^{11c}R^{11c'}$, -$S(O)_2NR^{11c}R^{11c'}$, -$OS(O)NR^{11c}R^{11c'}$, -$OS(O)_2NR^{11c}R^{11c'}$, -$NR^{11c}R^{11c'}$, -NR^{11c}C(O)R^{12c}, -NR^{11c}C(O)OR^{12c}, -NR^{11c}C(O)$NR^{12c}R^{12c'}$, -NR^{11c}S(O)R^{12c}, -$NR^{11c}S(O)_2R^{12c}$, -NR^{11c}S(O)$NR^{12c}R^{12c'}$, -$NR^{11c}S(O)_2NR^{12c}R^{12c'}$, -C(O)$R^{11c}$, -C(O)$OR^{11c}$ or -C(O)$NR^{11c}R^{11c}$;

each R^{6c}, $R^{6c'}$, R^{7c}, $R^{7c'}$, R^{8c}, $R^{8c'}$, R^{9c}, $R^{9c'}$, R^{10c}, $R^{10c'}$, R^{11c}, $R^{11c'}$, R^{12c} and $R^{12c'}$ is independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

R^{13c} and $R^{13c'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{11c}$, $-OC(O)R^{11c}$, $-OC(O)NR^{11c}R^{11c'}$, $-OS(O)R^{11c}$, $-OS(O)_2R^{11c}$, $-SR^{11c}$, $-S(O)R^{11c}$, $-S(O)_2R^{11c}$, $-S(O)NR^{11c}R^{11c'}$, $-S(O)_2NR^{11c}R^{11c'}$, $-OS(O)NR^{11c}R^{11c'}$, $-OS(O)_2NR^{11c}R^{11c'}$, $-NR^{11c}R^{11c'}$, $-NR^{11c}C(O)R^{12c}$, $-NR^{11c}C(O)OR^{12c}$, $-NR^{11c}C(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)R^{12c}$, $-NR^{11c}S(O)_2R^{12c}$, $-NR^{11c}S(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)_2NR^{12c}R^{12c'}$, $-C(O)R^{11c}$, $-C(O)OR^{11c}$ and $-C(O)NR^{11c}R^{11c}$.

In some aspects of embodiment 1, each releasable group comprises at least one cleavable bond. In some aspects of embodiment 1, each cleavable bond is broken under physiological conditions. In some aspects of embodiment 1, the conjugate further comprises a releasable group that is not disulfide bond. In some aspects of embodiment 1, the releasable group that is not disulfide bond is a group within the structure of at least one of D^1 or D^2. In some aspects of embodiment 1, one of D^1 or D^2 is a PBD pro-drug, and the releasable group is a group within the structure of the PBD pro-drug. In some aspects of embodiment 1, the one or more linkers (L) are independently selected from the group consisting of AA, L^1, L^2, L^3 and L^r, and combinations thereof.

In another embodiment (referred to herein as embodiment 2), the present disclosure provides a conjugate, or a pharmaceutically acceptable salt thereof, comprising a binding ligand (B), one or more linkers (L), at least one releasable group, a first drug (D^1) and a second drug (D^2), wherein B is covalently attached to at least one L, at least one L is covalently attached to at least one of the first drug or the second drug, and at least one of the first drug or the second drug is a PBD.

In some aspects of embodiment 2, each releasable group comprises at least one cleavable bond. In some aspects of embodiment 2, each cleavable bond is broken under physiological conditions. In some aspects of embodiment 2, the conjugate comprises at least one releasable group that is not disulfide bond. In some aspects of embodiment 2, the releasable group is a group within the structure of at least one of D^1 or D^2. In some aspects of embodiment 2, one of D^1 or D^2 is a PBD pro-drug, and the releasable group is a group within the structure of the PBD pro-drug. In some aspects of embodiment 2, at least one releasable group is a disulfide bond. In some aspects of embodiment 2, the one or more linkers (L) are independently selected from the group consisting of AA, L^1, L^2, L^3 and L^r, and combinations thereof.

In one aspect, the present disclosure provides conjugates comprising a binding ligand, a linker and a drug, having the formula B-$(AA)_{z1}$-L^2-$(L^3)_{z2}$-$(AA)_{z3}$-$(L^1)_{z4}$-$(L^4)_{z5}$-D^1-L^5-D^2, B-$(AA)_{z10}$-L^2-D^2, B-$(AA)_{z11}$-L^2-D^1-L^5-D^1-L^2-$(AA)_{z12}$-B or B-L^1-AA-L^1-AA-L^1-L^2-$(L^3)_{z6}$-$(L^4)_{z7}$-$(AA)_{z8}$-$(L^4)_{z9}$-D^1-L^5-D^2, wherein each of B, AA, L^1, L^2, L^3, L^4, L^5, D^1, D^2, z1, z2, z3, z4, z5, z6, z7, z8, z9, z10, z11 and z12 are defined as described herein; or a pharmaceutically acceptable salt thereof.

In another embodiment, the disclosure provides pharmaceutical compositions comprising a therapeutically effective amount of the conjugates described herein, or a pharmaceutically acceptable salt thereof, and at least on excipient.

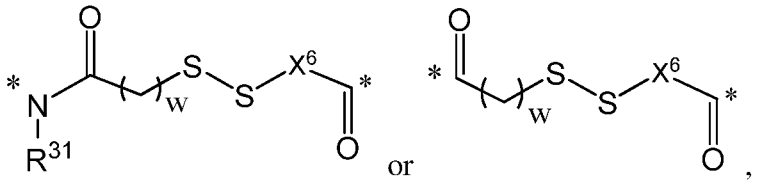
In another embodiment, the disclosure provides a method of treating abnormal cell growth in a mammal, including a human, the method comprising administering to the mammal a therapeutically effective amount of any of the conjugates or compositions described herein. In some aspects of these embodiemts, the abnormal cell growth is cancer. In some aspects of these embodiemts, the cancer is folate receptor positive triple negative breast cancer. In some aspects of these embodiemts, the cancer is folate receptor negative triple negative breast cancer. In some aspects of these embodiemts, the cancer is ovarian cancer. In some aspects of these embodiemts, the method further comprises concurrently treatment with anti-CTLA-4 treatment. In some aspects of these embodiemts, the method further comprises concurrently treatment with anti-CTLA-4 treatment for the treatment of ovarian cancer.

In another embodiment, the disclosure provides a conjugate, or a pharmaceutically acceptable salt thereiof, as described herein for use in a method of treating cancer in a patient. In some aspects, the method comprises administering to the patient a therapeutically effective amount of any of the conjugates described herein. In some aspects of these embodiemts, the cancer is folate receptor positive triple negative breast cancer. In some aspects of these embodiemts, the cancer is folate receptor negative triple negative breast cancer. In some aspects of these embodiemts, the cancer is ovarian cancer. In some aspects of these embodiemts, the method further comprises concurrently treatment with anti-CTLA-4 treatment. In some aspects of these embodiemts, the method further comprises concurrently treatment with anti-CTLA-4 treatment for the treatment of ovarian cancer.

The conjugates of the present disclosure can be described as embodiments in any of the following enumerated clauses. It will be understood that any of the embodiments described herein can be used in connection with any other embodiments described herein to the extent that the embodiments do not contradict one another.

1. A conjugate, or a pharmaceutically acceptable salt thereof, comprising a binding ligand (B), one or more linkers (L), at least one releasable group, a first drug (D^1) and a second

drug (D^2), wherein B is covalently attached to at least one L, at least one L is covalently attached to at least one of the first drug or the second drug, at least one of the first drug or the second drug is a PBD, and the one or more linkers comprises at least one releasable linker (L^r) of the formula



wherein

each R^{31} and $R^{31'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{32}$, $-OC(O)R^{32}$, $-OC(O)NR^{32}R^{32'}$, $-OS(O)R^{32}$, $-OS(O)_2R^{32}$, $-SR^{32}$, $-S(O)R^{32}$, $-S(O)_2R^{32}$, $-S(O)NR^{32}R^{32'}$, $-S(O)_2NR^{32}R^{32'}$, $-OS(O)NR^{32}R^{32'}$, $-OS(O)_2NR^{32}R^{32'}$, $-NR^{32}R^{32'}$, $-NR^{32}C(O)R^{33}$, $-NR^{32}C(O)OR^{33}$, $-NR^{32}C(O)NR^{33}R^{33'}$, $-NR^{32}S(O)R^{33}$, $-NR^{32}S(O)_2R^{33}$, $-NR^{32}S(O)NR^{33}R^{33'}$, $-NR^{32}S(O)_2NR^{33}R^{33'}$, $-C(O)R^{32}$, $-C(O)OR^{32}$ or $-C(O)NR^{32}R^{32'}$;

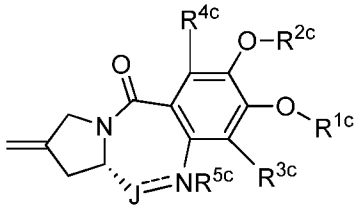
each X^6 is independently selected from the group consisting of $-C_1$-C_6 alkyl-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-, $-C_1$-C_6 alkyl-O-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-O-, $-C_1$-C_6 alkyl-$NR^{31'}$- and $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-$NR^{31'}$-, wherein each hydrogen atom in $-C_1$-C_6 alkyl-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-, $-C_1$-C_6 alkyl-O-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-O-, $-C_1$-C_6 alkyl-$NR^{31'}$- or $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-$NR^{31'}$ is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{34}$, $-OC(O)R^{34}$, $-OC(O)NR^{34}R^{34'}$, $-OS(O)R^{34}$, $-OS(O)_2R^{34}$, $-SR^{34}$, $-S(O)R^{34}$, $-S(O)_2R^{34}$, $-S(O)NR^{34}R^{34'}$, $-S(O)_2NR^{34}R^{34'}$, $-OS(O)NR^{34}R^{34'}$, $-OS(O)_2NR^{34}R^{34'}$, $-NR^{34}R^{34'}$, $-NR^{34}C(O)R^{35}$, $-NR^{34}C(O)OR^{35}$, $-NR^{34}C(O)NR^{35}R^{35'}$, $-NR^{34}S(O)R^{35}$, $-NR^{34}S(O)_2R^{35}$, $-NR^{34}S(O)NR^{35}R^{35'}$, $-NR^{34}S(O)_2NR^{35}R^{35'}$, $-C(O)R^{34}$, $-C(O)OR^{34}$ or $-C(O)NR^{34}R^{34'}$; and

each R^{32}, $R^{32'}$, R^{33}, $R^{33'}$, R^{34}, $R^{34'}$, R^{35} and $R^{35'}$ are independently selected from the group consisting of H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, and 5- to 7-membered heteroaryl;

each w is independently an integer from 1 to 4; and

each * represents a covalent bond to the rest of the conjugate.

2. The conjugate of clause 1, wherein at least one of the first drug or the second drug is a PBD of the formula



wherein

J is –C(O)-, –CR^{13c}= or –($CR^{13c}R^{13c'}$)-;

R^{1c}, R^{2c} and R^{5c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -C(O)R^{6c}, -C(O)OR^{6c} and -C(O)N$R^{6c}R^{6c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{7c}, -OC(O)R^{7c}, -OC(O)N$R^{7c}R^{7c'}$, -OS(O)R^{7c}, -O$S(O)_2R^{7c}$, -SR^{7c}, -S(O)R^{7c}, -$S(O)_2R^{7c}$, -$S(O)_2OR^{7c}$, -S(O)N$R^{7c}R^{7c'}$, -$S(O)_2$N$R^{7c}R^{7c'}$, -OS(O)N$R^{7c}R^{7c'}$, -O$S(O)_2$N$R^{7c}R^{7c'}$, -N$R^{7c}R^{7c'}$, -NR^{7c}C(O)R^{8c}, -NR^{7c}C(O)OR^{8c}, -NR^{7c}C(O)N$R^{8c}R^{8c'}$, -NR^{7c}S(O)R^{8c}, -N$R^{7c}$$S(O)_2R^{8c}$, -N$R^{7c}$S(O)N$R^{8c}R^{8c'}$, -N$R^{7c}$$S(O)_2NR^{8c}R^{8c'}$, -C(O)$R^{7c}$, -C(O)O$R^{7c}$ or -C(O)N$R^{7c}R^{7c'}$; or when J is –CR^{13c}=, R^{5c} is absent; provided that at least one of R^{1c}, R^{2c} or R^{5c} is a covalent bond to the rest of the conjugate;

R^{3c} and R^{4c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -CN, -NO_2, -NCO, -OR^{9c}, -OC(O)R^{9c}, -OC(O)N$R^{9c}R^{9c'}$, -OS(O)R^{9c}, -O$S(O)_2R^{9c}$, -SR^{9c}, -S(O)R^{9c}, -$S(O)_2R^{9c}$, -S(O)N$R^{9c}R^{9c'}$, -$S(O)_2$N$R^{9c}R^{9c'}$, -OS(O)N$R^{9c}R^{9c'}$, -O$S(O)_2$N$R^{9c}R^{9c'}$, -N$R^{9c}R^{9c'}$, -NR^{9c}C(O)R^{10c}, -NR^{9c}C(O)OR^{10c}, -NR^{9c}C(O)N$R^{10c}R^{10c'}$, -NR^{9c}S(O)R^{10c}, -N$R^{9c}$$S(O)_2R^{10c}$, -N$R^{9c}$S(O)N$R^{10c}R^{10c'}$, -N$R^{9c}$$S(O)_2NR^{10c}R^{10c'}$, -C(O)$R^{9c}$, -C(O)O$R^{9c}$ and -C(O)N$R^{9c}R^{9c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{11c}, -OC(O)R^{11c}, -OC(O)N$R^{11c}R^{11c'}$, -OS(O)R^{11c}, -O$S(O)_2R^{11c}$, -SR^{11c}, -S(O)R^{11c}, -$S(O)_2R^{11c}$, -S(O)N$R^{11c}R^{11c'}$, -$S(O)_2$N$R^{11c}R^{11c'}$, -OS(O)N$R^{11c}R^{11c'}$, -O$S(O)_2$N$R^{11c}R^{11c'}$, -N$R^{11c}R^{11c'}$, -NR^{11c}C(O)R^{12c},

$-NR^{11c}C(O)OR^{12c}$, $-NR^{11c}C(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)R^{12c}$, $-NR^{11c}S(O)_2R^{12c}$, $-NR^{11c}S(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)_2NR^{12c}R^{12c'}$, $-C(O)R^{11c}$, $-C(O)OR^{11c}$ or $-C(O)NR^{11c}R^{11c}$;

each R^{6c}, $R^{6c'}$, R^{7c}, $R^{7c'}$, R^{8c}, $R^{8c'}$, R^{9c}, $R^{9c'}$, R^{10c}, $R^{10c'}$, R^{11c}, $R^{11c'}$, R^{12c} and $R^{12c'}$ is independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

R^{13c} and $R^{13c'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{11c}$, $-OC(O)R^{11c}$, $-OC(O)NR^{11c}R^{11c'}$, $-OS(O)R^{11c}$, $-OS(O)_2R^{11c}$, $-SR^{11c}$, $-S(O)R^{11c}$, $-S(O)_2R^{11c}$, $-S(O)NR^{11c}R^{11c'}$, $-S(O)_2NR^{11c}R^{11c'}$, $-OS(O)NR^{11c}R^{11c'}$, $-OS(O)_2NR^{11c}R^{11c'}$, $-NR^{11c}R^{11c'}$, $-NR^{11c}C(O)R^{12c}$, $-NR^{11c}C(O)OR^{12c}$, $-NR^{11c}C(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)R^{12c}$, $-NR^{11c}S(O)_2R^{12c}$, $-NR^{11c}S(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)_2NR^{12c}R^{12c'}$, $-C(O)R^{11c}$, $-C(O)OR^{11c}$ and $-C(O)NR^{11c}R^{11c}$.

3. The conjugate of clause 1 or 2, or a pharmaceutically acceptable salt thereof, wherein each releasable group comprises at least one cleavable bond.

4. The conjugate of clause 3, or a pharmaceutically acceptable salt thereof, wherein each cleavable bond is broken under physiological conditions.

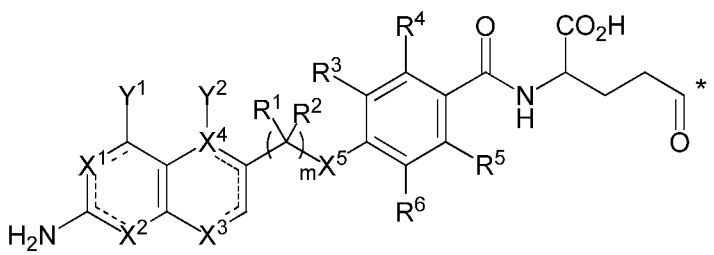
5. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, further comprising a releasable group that is not disulfide bond.

6. The conjugate of clause 5, or a pharmaceutically acceptable salt thereof, wherein the releasable group that is not disulfide bond is a group within the structure of at least one of D^1 or D^2.

7. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein one of D^1 or D^2 is a PBD pro-drug, and the releasable group is a group within the structure of the PBD pro-drug.

8. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein the one or more linkers (L) are independently selected from the group consisting of AA, L^1, L^2, L^3 and L^r, and combinations thereof.

9. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein B is of the formula



wherein

R^1 and R^2 in each instance are independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -OR^7, -SR^7 and -$NR^7R^{7'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl is independently optionally substituted by halogen, –OR^8, -SR^8, -$NR^8R^{8'}$, -C(O)R^8, -C(O)OR^8 or -C(O)$NR^8R^{8'}$;

R^3, R^4, R^5 and R^6 are each independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -CN, -NO_2, -NCO, -OR^9, -SR^9, –$NR^9R^{9'}$, -C(O)R^9, -C(O)OR^9 and -C(O)$NR^9R^{9'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl is independently optionally substituted by halogen, –OR^{10}, -SR^{10}, -$NR^{10}R^{10'}$, -C(O)R^{10}, -C(O)OR^{10} or -C(O)$NR^{10}R^{10'}$;

each R^7, $R^{7'}$, R^8, $R^{8'}$, R^9, $R^{9'}$, R^{10} and $R^{10'}$ is independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl or C_2-C_6 alkynyl;

X^1 is –NR^{11}-, =N-, -N=, -C(R^{11})= or =C(R^{11})-;

X^2 is –$NR^{11'}$- or =N-;

X^3 is –$NR^{11''}$-, -N= or -C($R^{11'}$)=;

X^4 is –N= or –C=;

X^5 is NR^{12} or $CR^{12}R^{12'}$;

Y^1 is H,–OR^{13}, –SR^{13} or –$NR^{13}R^{13'}$ when X^1 is -N= or -C(R^{11})=, or Y^1 is =O when X^1 is -NR^{11}-, =N- or =C(R^{11})-;

Y^2 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, -C(O)R^{14}, -C(O)OR^{14}, -C(O)$NR^{14}R^{14'}$ when X^4 is -C=, or Y^2 is absent when X^4 is –N=;

R^{11}, $R^{11'}$, $R^{11''}$, R^{12}, $R^{12'}$, R^{13}, $R^{13'}$, R^{14} and $R^{14'}$ are each independently selected from the group consisting of H, C_1-C_6 alkyl, -C(O)R^{15}, -C(O)OR^{15} and -C(O)$NR^{15}R^{15'}$;

R^{15} and $R^{15'}$ are each independently H or C_1-C_6 alkyl; and

m is 1, 2, 3 or 4;

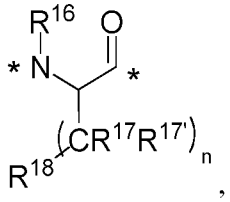
wherein * represents a covalent bond to the rest of the conjugate.

10. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein the one or more linkers (L) comprises at least one AA selected from the group consisting of L-lysine, L-asparagine, L-threonine, L-serine, L-isoleucine, L-methionine, L-proline, L-histidine, L-glutamine, L-arginine, L-glycine, L-aspartic acid, L-glutamic acid, L-alanine, L-valine, L-phenylalanine, L-leucine, L-tyrosine, L-cysteine, L-tryptophan, L-phosphoserine, L-sulfo-cysteine, L-arginosuccinic acid, L-hydroxyproline, L-phosphoethanolamine, L-sarcosine, L-taurine, L-carnosine, L-citrulline, L-anserine, L-1,3-methyl-histidine, L-alpha-amino-adipic acid, D-lysine, D-asparagine, D-threonine,

D-serine, D-isoleucine, D-methionine, D-proline, D-histidine, D-glutamine, D-arginine, D-glycine, D-aspartic acid, D-glutamic acid, D-alanine, D-valine, D-phenylalanine, D-leucine, D-tyrosine, D-cysteine, D-tryptophan, D-citrulline and D-carnosine.

11. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein wherein the one or more linkers (L) comprises at least one AA selected from the group consisting of L-arginine, L-aspartic acid, L-cysteine, D-arginine, D-aspartic acid, and D-cysteine.

12. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein, when the one or more linkers (L) comprises a first spacer linker (L^1), the first spacer linker is of the formula



,

wherein

R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -$C(O)R^{19}$, -$C(O)OR^{19}$ and -$C(O)NR^{19}R^{19'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl, -OR^{20}, -$OC(O)R^{20}$, -$OC(O)NR^{20}R^{20'}$, -$OS(O)R^{20}$, -$OS(O)_2R^{20}$, -SR^{20}, -$S(O)R^{20}$, -$S(O)_2R^{20}$, -$S(O)NR^{20}R^{20'}$, -$S(O)_2NR^{20}R^{20'}$, -$OS(O)NR^{20}R^{20'}$, -$OS(O)_2NR^{20}R^{20'}$, -$NR^{20}R^{20'}$, -$NR^{20}C(O)R^{21}$, -$NR^{20}C(O)OR^{21}$, -$NR^{20}C(O)NR^{21}R^{21'}$, -$NR^{20}S(O)R^{21}$, -$NR^{20}S(O)_2R^{21}$, -$NR^{20}S(O)NR^{21}R^{21'}$, -$NR^{20}S(O)_2NR^{21}R^{21'}$, -$C(O)R^{20}$, -$C(O)OR^{20}$ or -$C(O)NR^{20}R^{20'}$;

each R^{17} and $R^{17'}$ is independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{22}, -$OC(O)R^{22}$, -$OC(O)NR^{22}R^{22'}$, -$OS(O)R^{22}$, -$OS(O)_2R^{22}$, -SR^{22}, -$S(O)R^{22}$, -$S(O)_2R^{22}$, -$S(O)NR^{22}R^{22'}$, -$S(O)_2NR^{22}R^{22'}$, -$OS(O)NR^{22}R^{22'}$, -$OS(O)_2NR^{22}R^{22'}$, -$NR^{22}R^{22'}$, -$NR^{22}C(O)R^{23}$, -$NR^{22}C(O)OR^{23}$, -$NR^{22}C(O)NR^{23}R^{23'}$, -$NR^{22}S(O)R^{23}$, -$NR^{22}S(O)_2R^{23}$, -$NR^{22}S(O)NR^{23}R^{23'}$, -$NR^{22}S(O)_2NR^{23}R^{23'}$, -$C(O)R^{22}$, -$C(O)OR^{22}$, and -$C(O)NR^{22}R^{22'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -OR^{24}, -$OC(O)R^{24}$, -$OC(O)NR^{24}R^{24'}$, -$OS(O)R^{24}$, -$OS(O)_2R^{24}$, -SR^{24},

$-S(O)R^{24}$, $-S(O)_2R^{24}$,
$-S(O)NR^{24}R^{24'}$, $-S(O)_2NR^{24}R^{24'}$, $-OS(O)NR^{24}R^{24'}$, $-OS(O)_2NR^{24}R^{24'}$, $-NR^{24}R^{24'}$, $-NR^{24}C(O)R^{25}$, $-NR^{24}C(O)OR^{25}$, $-NR^{24}C(O)NR^{25}R^{25'}$, $-NR^{24}S(O)R^{25}$, $-NR^{24}S(O)_2R^{25}$, $-NR^{24}S(O)NR^{25}R^{25'}$, $-NR^{24}S(O)_2NR^{25}R^{25'}$, $-C(O)R^{24}$, $-C(O)OR^{24}$ or $-C(O)NR^{24}R^{24'}$; or R^{17} and $R^{17'}$ may combine to form a C_4-C_6 cycloalkyl or a 4- to 6- membered heterocycle, wherein each hydrogen atom in C_4-C_6 cycloalkyl or 4- to 6- membered heterocycle is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{24}$, $-OC(O)R^{24}$, $-OC(O)NR^{24}R^{24'}$, $-OS(O)R^{24}$, $-OS(O)_2R^{24}$, $-SR^{24}$, $-S(O)R^{24}$, $-S(O)_2R^{24}$, $-S(O)NR^{24}R^{24'}$, $-S(O)_2NR^{24}R^{24'}$, $-OS(O)NR^{24}R^{24'}$, $-OS(O)_2NR^{24}R^{24'}$, $-NR^{24}R^{24'}$, $-NR^{24}C(O)R^{25}$, $-NR^{24}C(O)OR^{25}$, $-NR^{24}C(O)NR^{25}R^{25'}$, $-NR^{24}S(O)R^{25}$, $-NR^{24}S(O)_2R^{25}$, $-NR^{24}S(O)NR^{25}R^{25'}$, $-NR^{24}S(O)_2NR^{25}R^{25'}$, $-C(O)R^{24}$, $-C(O)OR^{24}$ or $-C(O)NR^{24}R^{24'}$;

R^{18} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{26}$, $-OC(O)R^{26}$, $-OC(O)NR^{26}R^{26'}$, $-OS(O)R^{26}$, $-OS(O)_2R^{26}$, $-SR^{26}$, $-S(O)R^{26}$, $-S(O)_2R^{26}$,
$-S(O)NR^{26}R^{26'}$, $-S(O)_2NR^{26}R^{26'}$, $-OS(O)NR^{26}R^{26'}$, $-OS(O)_2NR^{26}R^{26'}$, $-NR^{26}R^{26'}$, $-NR^{26}C(O)R^{27}$, $-NR^{26}C(O)OR^{27}$, $-NR^{26}C(O)NR^{27}R^{27'}$, $-NR^{26}C(=NR^{26''})NR^{27}R^{27'}$, $-NR^{26}S(O)R^{27}$, $-NR^{26}S(O)_2R^{27}$, $-NR^{26}S(O)NR^{27}R^{27'}$, $-NR^{26}S(O)_2NR^{27}R^{27'}$, $-C(O)R^{26}$, $-C(O)OR^{26}$ and $-C(O)NR^{26}R^{26'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, $-(CH_2)_pOR^{28}$, $-(CH_2)_p(OCH_2)_qOR^{28}$, $-(CH_2)_p(OCH_2CH_2)_qOR^{28}$, $-OR^{29}$, $-OC(O)R^{29}$, $-OC(O)NR^{29}R^{29'}$, $-OS(O)R^{29}$, $-OS(O)_2R^{29}$, $-(CH_2)_pOS(O)_2OR^{29}$, $-OS(O)_2OR^{29}$, $-SR^{29}$, $-S(O)R^{29}$, $-S(O)_2R^{29}$,
$-S(O)NR^{29}R^{29'}$, $-S(O)_2NR^{29}R^{29'}$, $-OS(O)NR^{29}R^{29'}$, $-OS(O)_2NR^{29}R^{29'}$, $-NR^{29}R^{29'}$, $-NR^{29}C(O)R^{30}$, $-NR^{29}C(O)OR^{30}$, $-NR^{29}C(O)NR^{30}R^{30'}$, $-NR^{29}S(O)R^{30}$, $-NR^{29}S(O)_2R^{30}$, $-NR^{29}S(O)NR^{30}R^{30'}$, $-NR^{29}S(O)_2NR^{30}R^{30'}$, $-C(O)R^{29}$, $-C(O)OR^{29}$ or $-C(O)NR^{29}R^{29'}$;

each R^{19}, $R^{19'}$, R^{20}, $R^{20'}$, R^{21}, $R^{21'}$, R^{22}, $R^{22'}$, R^{23}, $R^{23'}$, R^{24}, $R^{24'}$, R^{25}, $R^{25'}$, R^{26}, $R^{26'}$, $R^{26''}$, R^{29}, $R^{29'}$, R^{30} and $R^{30'}$ is independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, or 5- to 7-membered heteroaryl is independently optionally substituted by halogen, -OH, -SH, $-NH_2$ or $-CO_2H$;

R^{27} and $R^{27'}$ are each independently selected from the group consisting of H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_3-C_6 cycloalkyl, -$(CH_2)_p$(sugar), -$(CH_2)_p(OCH_2CH_2)_q$-(sugar) and -$(CH_2)_p(OCH_2CH_2CH_2)_q$(sugar);

R^{28} is a H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl or sugar;

n is 1, 2, 3, 4 or 5;

p is 1, 2, 3, 4 or 5;

q is 1, 2, 3, 4 or 5; and

each * represents a covalent bond to the rest of the conjugate.

13. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein when the one or more linkers (L) comprises at least one second spacer linker (L^2), each second spacer linker is independently selected from the group consisting of C_1-C_6 alkyl, -OC_1-C_6 alkyl, -SC_1-C_6 alkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -$NR^{36}(CR^{36'}R^{36''})_r$-S-(succinimid-1-yl)-, -$(CR^{36'}R^{36''})_r$C(O)$NR^{36}$-, -$(CR^{39}R^{39'})_r$C(O)-, -$(CR^{39}R^{39'})_r$OC(O)-, -S$(CR^{39}R^{39'})_r$OC(O)-, -C(O)$(CR^{39}R^{39'})_r$-, -C(O)O$(CR^{39}R^{39'})_r$-, -$NR^{39}$C(O)$(CR^{39'}R^{39''})_r$-, -$NR^{39}$C(O)$(CR^{39'}R^{39''})_r$S-, -$(CH_2)_rNR^{39}$-, -$NR^{39}(CH_2)_r$-, -$NR^{39}(CH_2)_r$S-, -$NR^{39}(CH_2)_rNR^{39'}$-, -$(OCR^{39}R^{39'}CR^{39}R^{39'})_r$C(O)-, -$(OCR^{39}R^{39'}CR^{39}R^{39'}CR^{39}R^{39'})_r$C(O)-, -OC(O)$(CR^{44}R^{44'})_t$-, -C(O)$(CR^{44}R^{44'})_t$-, -$NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_t$-, -$CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tNR^{42}$-, -$NR^{42}$$C_6$-$C_{10}$ aryl(C_1-C_6 alkyl)OC(O)-, -C(O)$CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tNR^{42}$-, -$NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_t$C(O)-, and -$NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(CR^{44}=CR^{44'})_t$-;

wherein

each R^{36}, $R^{36'}$ and $R^{36''}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, -C(O)R^{37}, -C(O)OR^{37} and -C(O)$NR^{37}R^{37'}$ wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{37}, -OC(O)R^{37}, -OC(O)$NR^{37}R^{37'}$, -OS(O)R^{37}, -$OS(O)_2R^{37}$, -SR^{37}, -S(O)R^{37}, -$S(O)_2R^{37}$, -S(O)$NR^{37}R^{37'}$, -$S(O)_2NR^{37}R^{37'}$, -OS(O)$NR^{37}R^{37'}$, -$OS(O)_2NR^{37}R^{37'}$, -$NR^{37}R^{37'}$, -NR^{37}C(O)R^{38}, -NR^{37}C(O)OR^{38}, -NR^{37}C(O)$NR^{38}R^{38'}$, -NR^{37}S(O)R^{38}, -$NR^{37}S(O)_2R^{38}$, -NR^{37}S(O)$NR^{38}R^{38'}$, -$NR^{37}S(O)_2NR^{38}R^{38'}$, -C(O)$R^{37}$, -C(O)O$R^{37}$ or -C(O)$NR^{37}R^{37'}$;

R^{37}, $R^{37'}$, R^{38} and $R^{38'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

each R^{39} and $R^{39'}$ is independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{40}$, $-OC(O)R^{40}$, $-OC(O)NR^{40}R^{40'}$, $-OS(O)R^{40}$, $-OS(O)_2R^{40}$, $-SR^{40}$, $-S(O)R^{40}$, $-S(O)_2R^{40}$, $-S(O)NR^{40}R^{40'}$, $-S(O)_2NR^{40}R^{40'}$, $-OS(O)NR^{40}R^{40'}$, $-OS(O)_2NR^{40}R^{40'}$, $-NR^{40}R^{40'}$, $-NR^{40}C(O)R^{41}$, $-NR^{40}C(O)OR^{41}$, $-NR^{40}C(O)NR^{41}R^{41'}$, $-NR^{40}S(O)R^{41}$, $-NR^{40}S(O)_2R^{41}$, $-NR^{40}S(O)NR^{41}R^{41'}$, $-NR^{40}S(O)_2NR^{41}R^{41'}$, $-C(O)R^{40}$, $-C(O)OR^{40}$ and $-C(O)NR^{40}R^{40'}$;

R^{40}, $R^{40'}$, R^{41} and $R^{41'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, and 5- to 7-membered heteroaryl; and

R^{42} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{45}$, $-OC(O)R^{45}$, $-OC(O)NR^{45}R^{45'}$, $-OS(O)R^{45}$, $-OS(O)_2R^{45}$, $-SR^{45}$, $-S(O)R^{45}$, $-S(O)_2R^{45}$, $-S(O)NR^{45}R^{45'}$, $-S(O)_2NR^{45}R^{45'}$, $-OS(O)NR^{45}R^{45'}$, $-OS(O)_2NR^{45}R^{45'}$, $-NR^{45}R^{45'}$, $-NR^{45}C(O)R^{46}$, $-NR^{45}C(O)OR^{46}$, $-NR^{45}C(O)NR^{46}R^{46'}$, $-NR^{45}S(O)R^{46}$, $-NR^{45}S(O)_2R^{46}$, $-NR^{45}S(O)NR^{46}R^{46'}$, $-NR^{45}S(O)_2NR^{46}R^{46'}$, $-C(O)R^{45}$, $-C(O)OR^{45}$ or $-C(O)NR^{45}R^{45'}$,

each R^{43}, $R^{43'}$, R^{44} and $R^{44'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{47}$, $-OC(O)R^{47}$, $-OC(O)NR^{47}R^{47'}$, $-OS(O)R^{47}$, $-OS(O)_2R^{47}$, $-SR^{47}$, $-S(O)R^{47}$, $-S(O)_2R^{47}$, $-S(O)NR^{47}R^{47'}$, $-S(O)_2NR^{47}R^{47'}$, $-OS(O)NR^{47}R^{47'}$, $-OS(O)_2NR^{47}R^{47'}$, $-NR^{47}R^{47'}$, $-NR^{47}C(O)R^{48}$, $-NR^{47}C(O)OR^{48}$, $-NR^{47}C(O)NR^{48}R^{48'}$, $-NR^{47}S(O)R^{48}$, $-NR^{47}S(O)_2R^{48}$, $-NR^{47}S(O)NR^{48}R^{48'}$, $-NR^{47}S(O)_2NR^{48}R^{48'}$, $-C(O)R^{47}$, $-C(O)OR^{47}$ or $-C(O)NR^{47}R^{47'}$;

R^{45}, $R^{45'}$, R^{46}, $R^{46'}$, R^{47}, $R^{47'}$, R^{48} and $R^{48'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

r in each instance is an integer from 1 to 40; and

t is in each instance is an integer from 1 to 40.

14. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein when the one or more linkers (L) comprises at least one third spacer linker

(L^3), each third spacer linker is independently selected from the group consisting of C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, -$(CR^{49}R^{49'})_uC(O)$-, -$CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'})_u$-, -$CH_2CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'}CR^{49}R^{49'})_u$-, -$CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'})_uC(O)$- and -$CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'}CR^{49}R^{49'})_uC(O)$-,

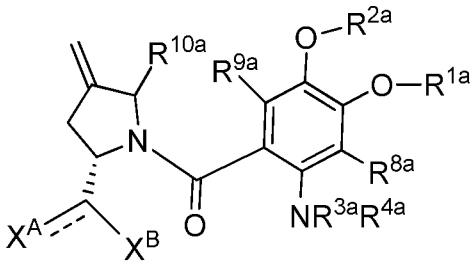
wherein

each R^{49} and $R^{49'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{50}, -$OC(O)R^{50}$, -$OC(O)NR^{50}R^{50'}$, -$OS(O)R^{50}$, -$OS(O)_2R^{50}$, -SR^{50}, -$S(O)R^{50}$, -$S(O)_2R^{50}$, -$S(O)NR^{50}R^{50'}$, -$S(O)_2NR^{50}R^{50'}$, -$OS(O)NR^{50}R^{50'}$, -$OS(O)_2NR^{50}R^{50'}$, -$NR^{50}R^{50'}$, -$NR^{50}C(O)R^{51}$, -$NR^{50}C(O)OR^{51}$, -$NR^{50}C(O)NR^{51}R^{51'}$, -$NR^{50}S(O)R^{51}$, -$NR^{50}S(O)_2R^{51}$, -$NR^{50}S(O)NR^{51}R^{51'}$, -$NR^{50}S(O)_2NR^{51}R^{51'}$, -$C(O)R^{50}$, -$C(O)OR^{50}$ or -$C(O)NR^{50}R^{50'}$;

R^{50}, $R^{50'}$, R^{51} and $R^{51'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

u is in each instance 0, 1, 2, 3, 4 or 5.

15. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein the first drug is of the formula



wherein

X^A is -OR^{6a}, =N-OR^{5a} or -$NR^{5a}R^{6a}$-, provided that when the hash bond is a pi-bond, X^A is =NR^{5a};

X^B is H or OR^{7a};

R^{1a}, R^{2a}, R^{3a} and R^{4a} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -$C(O)R^{11a}$, -$C(O)OR^{11a}$, and -$C(O)NR^{11a}R^{11a'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered

heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{11a}$, $-OC(O)R^{11a}$, $-OC(O)NR^{11a}R^{11a'}$, $-OS(O)R^{11a}$, $-OS(O)_2R^{11a}$, $-SR^{11a}$, $-S(O)R^{11a}$, $-S(O)_2R^{11a}$, $-S(O)NR^{11a}R^{11a'}$, $-S(O)_2NR^{11a}R^{11a'}$, $-OS(O)NR^{11a}R^{11a'}$, $-OS(O)_2NR^{11a}R^{11a'}$, $-NR^{11a}R^{11a'}$, $-NR^{11a}C(O)R^{12a}$, $-NR^{11a}C(O)OR^{12a}$, $-NR^{11a}C(O)NR^{12a}R^{12a'}$, $-NR^{11a}S(O)R^{12a}$, $-NR^{11a}S(O)_2R^{12a}$, $-NR^{11a}S(O)NR^{12a}R^{12a'}$, $-NR^{11a}S(O)_2NR^{12a}R^{12a'}$, $-C(O)R^{11a}$, $-C(O)OR^{11a}$ or $-C(O)NR^{11a}R^{11a'}$; or R^{1a} is a bond; or R^{4a} is a bond;

R^{5a}, R^{6a} and R^{7a} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-C(O)R^{13a}$, $-C(O)OR^{13a}$ and $-C(O)NR^{13a}R^{13a'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{14a}$, $-OC(O)R^{14a}$, $-OC(O)NR^{14a}R^{14a'}$, $-OS(O)R^{14a}$, $-OS(O)_2R^{14a}$, $-SR^{14a}$, $-S(O)R^{14a}$, $-S(O)_2R^{14a}$, $-S(O)NR^{14a}R^{14a'}$, $-S(O)_2NR^{14a}R^{14a'}$, $-OS(O)NR^{14a}R^{14a'}$, $-OS(O)_2NR^{14a}R^{14a'}$, $-NR^{14a}R^{14a'}$, $-NR^{14a}C(O)R^{15a}$, $-NR^{14a}C(O)OR^{15a}$, $-NR^{14a}C(O)NR^{15a}R^{15a'}$, $-NR^{14a}S(O)R^{15a}$, $-NR^{14a}S(O)_2R^{15a}$, $-NR^{14a}S(O)NR^{15a}R^{15a'}$, $-NR^{14a}S(O)_2NR^{15a}R^{15a'}$, $-C(O)R^{14a}$, $-C(O)OR^{14a}$ or $-C(O)NR^{14a}R^{14a'}$; wherein R^{6a} and R^{7a} taken together with the atoms to which they are attached optionally combine to form a 3- to 7-membered heterocycloalkyl or a 3- to 7-membered heterocycloalkyl fused to a 6-membered aryl ring, or R^{5a} and R^{6a} taken together with the atoms to which they are attached optionally combine to form a 3- to 7-membered heterocycloalkyl or 5- to 7-membered heteroaryl, wherein each hydrogen atom in 3- to 7-membered heterocycloalkyl or 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{16a}$, $-OC(O)R^{16a}$, $-OC(O)NR^{16a}R^{16a'}$, $-OS(O)R^{16a}$, $-OS(O)_2R^{16a}$, $-SR^{16a}$, $-S(O)R^{16a}$, $-S(O)_2R^{16a}$, $-S(O)NR^{16a}R^{16a'}$, $-S(O)_2NR^{16a}R^{16a'}$, $-OS(O)NR^{16a}R^{16a'}$, $-OS(O)_2NR^{16a}R^{16a'}$, $-NR^{16a}R^{16a'}$, $-NR^{16a}C(O)R^{17a}$, $-NR^{16a}C(O)CH_2CH_2$-, $-NR^{16a}C(O)OR^{17a}$, $-NR^{16a}C(O)NR^{17a}R^{17a'}$, $-NR^{16a}S(O)R^{17a}$, $-NR^{16a}S(O)_2R^{17a}$, $-NR^{16a}S(O)NR^{17a}R^{17a'}$, $-NR^{16a}S(O)_2NR^{17a}R^{17a'}$, $-C(O)R^{16a}$, $-C(O)OR^{16a}$ or $-C(O)NR^{16a}R^{16a'}$, and wherein when R^{5a} and R^{6a} taken together with the atoms to which they are attached form a 5- to 7-membered heteroaryl, one hydrogen atom in 5- to 7-membered heteroaryl is optionally a bond, or when R^{6a} and R^{7a} taken together with the atoms to which they are attached optionally combine to form a 3- to 7-membered heterocycloalkyl fused to a 6-membered aryl, one hydrogen atom in the 6-membered aryl ring is optionally a bond; or R^{5a} is a bond;

R^{8a} and R^{9a} are each independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -CN, -NO_2, -NCO, -OR^{18a}, -$OC(O)R^{18a}$, -$OC(O)NR^{18a}R^{18a'}$, -$OS(O)R^{18a}$, -$OS(O)_2R^{18a}$, -SR^{18a}, -$S(O)R^{18a}$, -$S(O)_2R^{18a}$, -$S(O)NR^{18a}R^{18a'}$, -$S(O)_2NR^{18a}R^{18a'}$, -$OS(O)NR^{18a}R^{18a'}$, -$OS(O)_2NR^{18a}R^{18a'}$, -$NR^{18a}R^{18a'}$, -$NR^{18a}C(O)R^{19a}$, -$NR^{18a}C(O)OR^{19a}$, -$NR^{18a}C(O)NR^{19a}R^{19a'}$, -$NR^{18a}S(O)R^{19a}$, -$NR^{18a}S(O)_2R^{19a}$, -$NR^{18a}S(O)NR^{19a}R^{19a'}$, -$NR^{18a}S(O)_2NR^{19a}R^{19a'}$, -$C(O)R^{18a}$, -$C(O)OR^{18a}$ and -$C(O)NR^{18a}R^{18a'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{20a}, -$OC(O)R^{20a}$, -$OC(O)NR^{20a}R^{20a'}$, -$OS(O)R^{20a}$, -$OS(O)_2R^{20a}$, -SR^{20a}, -$S(O)R^{20a}$, -$S(O)_2R^{20a}$, -$S(O)NR^{20a}R^{20a'}$, -$S(O)_2NR^{20a}R^{20a'}$, -$OS(O)NR^{20a}R^{20a'}$, -$OS(O)_2NR^{20a}R^{20a'}$, -$NR^{20a}R^{20a'}$, -$NR^{20a}C(O)R^{21a}$, -$NR^{20a}C(O)OR^{21a}$, -$NR^{20a}C(O)NR^{21a}R^{21a'}$, -$NR^{20a}S(O)R^{21a}$, -$NR^{20a}S(O)_2R^{21a}$, -$NR^{20a}S(O)NR^{21a}R^{21a'}$, -$NR^{20a}S(O)_2NR^{21a}R^{21a'}$, -$C(O)R^{20a}$, -$C(O)OR^{20a}$ or -$C(O)NR^{20a}R^{20a'}$;

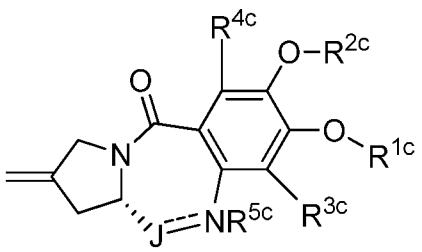
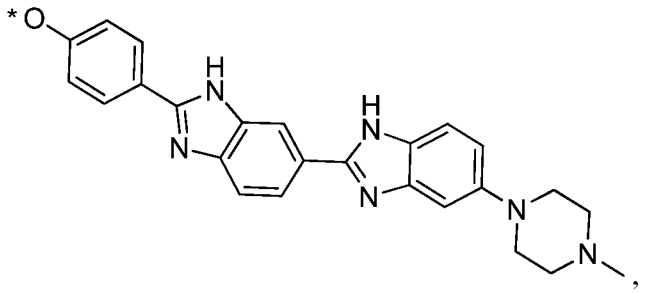
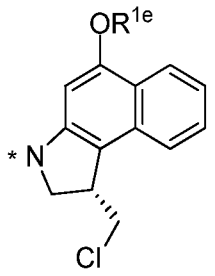
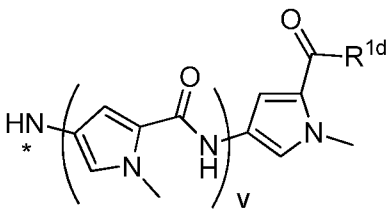
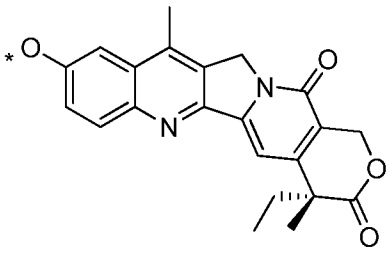
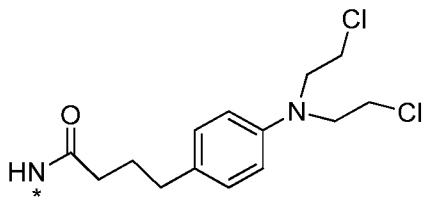
R^{10a} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{22a}, -$OC(O)R^{22a}$, -$OC(O)NR^{22a}R^{22a'}$, -$OS(O)R^{22a}$, -$OS(O)_2R^{22a}$, -SR^{22a}, -$S(O)R^{22a}$, -$S(O)_2R^{22a}$, -$S(O)NR^{22a}R^{22a'}$, -$S(O)_2NR^{22a}R^{22a'}$, -$OS(O)NR^{22a}R^{22a'}$, -$OS(O)_2NR^{22a}R^{22a'}$, -$NR^{22a}R^{22a'}$, -$NR^{22a}C(O)R^{23a}$, -$NR^{22a}C(O)OR^{23a}$, -$NR^{22a}C(O)NR^{23a}R^{23a'}$, -$NR^{22a}S(O)R^{23a}$, -$NR^{22a}S(O)_2R^{23a}$, -$NR^{22a}S(O)NR^{23a}R^{23a'}$, -$NR^{22a}S(O)_2NR^{23a}R^{23a}$, -$C(O)R^{22a}$, -$C(O)OR^{23a}$ and -$C(O)NR^{22a}R^{22a'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{24a}, -$OC(O)R^{24a}$, -$OC(O)NR^{24a}R^{24a'}$, -$OS(O)R^{24a}$, -$OS(O)_2R^{24a}$, -SR^{24a}, -$S(O)R^{24a}$, -$S(O)_2R^{24a}$, -$S(O)NR^{24a}R^{24a'}$, -$S(O)_2NR^{24a}R^{24a'}$, -$OS(O)NR^{24a}R^{24a'}$, -$OS(O)_2NR^{24a}R^{24a'}$, -$NR^{24a}R^{24a'}$, -$NR^{24a}C(O)R^{25a}$, -$NR^{24a}C(O)OR^{25a}$, -$NR^{24a}C(O)NR^{25a}R^{25a'}$, -$NR^{24a}S(O)R^{25a}$, -$NR^{24a}S(O)_2R^{25a}$, -$NR^{24a}S(O)NR^{25a}R^{25a'}$, -$NR^{24a}S(O)_2NR^{25a}R^{25a'}$, -$C(O)R^{24a}$, -$C(O)OR^{24a}$ or -$C(O)NR^{24a}R^{24a'}$; and

each R^{11a}, $R^{11a'}$, R^{12a}, $R^{12a'}$, R^{13a}, $R^{13a'}$, R^{14a}, $R^{14a'}$, R^{15a}, $R^{15a'}$, R^{16a}, $R^{16a'}$, R^{17a}, $R^{17a'}$, R^{18a}, $R^{18a'}$, R^{19a}, $R^{19a'}$, R^{20a}, $R^{20a'}$, R^{21a}, $R^{21a'}$, R^{22a}, $R^{22a'}$, R^{23a}, $R^{23a'}$, R^{24a}, $R^{24a'}$, R^{25a} and $R^{25a'}$ is independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_{13} cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, and 5- to 7-membered heteroaryl; and

provided that at least two of R^{1a}, R^{4a}, R^{5a} are a bond; or when R^{5a} and R^{6a} taken together with the atoms to which they are attached optionally combine to form a 5- to 7-membered heteroaryl, one hydrogen atom in 5- to 7-membered heteroaryl is a bond and one of R^{1a} or R^{4a} is a bond.

16. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein the first drug is covalently attached to the second drug by a third spacer linker (L^3).

17. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein the second drug is selected from the group consisting of

and

wherein

J is –C(O)-, $–CR^{13c}=$ or $–(CR^{13c}R^{13c'})$-;

R^{1c}, R^{2c} and R^{5c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-C(O)R^{6c}$, $-C(O)OR^{6c}$ and $-C(O)NR^{6c}R^{6c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{7c}$, $-OC(O)R^{7c}$, $-OC(O)NR^{7c}R^{7c'}$, $-OS(O)R^{7c}$, $-OS(O)_2R^{7c}$, $-SR^{7c}$, $-S(O)R^{7c}$, $-S(O)_2R^{7c}$, -

$S(O)_2OR^{7c}$, $-S(O)NR^{7c}R^{7c'}$, $-S(O)_2NR^{7c}R^{7c'}$, $-OS(O)NR^{7c}R^{7c'}$, $-OS(O)_2NR^{7c}R^{7c'}$, $-NR^{7c}R^{7c'}$, $-NR^{7c}C(O)R^{8c}$, $-NR^{7c}C(O)OR^{8c}$, $-NR^{7c}C(O)NR^{8c}R^{8c'}$, $-NR^{7c}S(O)R^{8c}$, $-NR^{7c}S(O)_2R^{8c}$, $-NR^{7c}S(O)NR^{8c}R^{8c'}$, $-NR^{7c}S(O)_2NR^{8c}R^{8c'}$, $-C(O)R^{7c}$, $-C(O)OR^{7c}$ or $-C(O)NR^{7c}R^{7c'}$; or when J is $-CR^{13c}=$, R^{5c} is absent; provided that at least one of R^{1c}, R^{2c} or R^{5c} is a covalent bond to the rest of the conjugate;

R^{3c} and R^{4c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -CN, $-NO_2$, -NCO, $-OR^{9c}$, $-OC(O)R^{9c}$, $-OC(O)NR^{9c}R^{9c'}$, $-OS(O)R^{9c}$, $-OS(O)_2R^{9c}$, $-SR^{9c}$, $-S(O)R^{9c}$, $-S(O)_2R^{9c}$, $-S(O)NR^{9c}R^{9c'}$, $-S(O)_2NR^{9c}R^{9c'}$, $-OS(O)NR^{9c}R^{9c'}$, $-OS(O)_2NR^{9c}R^{9c'}$, $-NR^{9c}R^{9c'}$, $-NR^{9c}C(O)R^{10c}$, $-NR^{9c}C(O)OR^{10c}$, $-NR^{9c}C(O)NR^{10c}R^{10c'}$, $-NR^{9c}S(O)R^{10c}$, $-NR^{9c}S(O)_2R^{10c}$, $-NR^{9c}S(O)NR^{10c}R^{10c'}$, $-NR^{9c}S(O)_2NR^{10c}R^{10c'}$, $-C(O)R^{9c}$, $-C(O)OR^{9c}$ and $-C(O)NR^{9c}R^{9c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{11c}$, $-OC(O)R^{11c}$, $-OC(O)NR^{11c}R^{11c'}$, $-OS(O)R^{11c}$, $-OS(O)_2R^{11c}$, $-SR^{11c}$, $-S(O)R^{11c}$, $-S(O)_2R^{11c}$, $-S(O)NR^{11c}R^{11c'}$, $-S(O)_2NR^{11c}R^{11c'}$, $-OS(O)NR^{11c}R^{11c'}$, $-OS(O)_2NR^{11c}R^{11c'}$, $-NR^{11c}R^{11c'}$, $-NR^{11c}C(O)R^{12c}$, $-NR^{11c}C(O)OR^{12c}$, $-NR^{11c}C(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)R^{12c}$, $-NR^{11c}S(O)_2R^{12c}$, $-NR^{11c}S(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)_2NR^{12c}R^{12c'}$, $-C(O)R^{11c}$, $-C(O)OR^{11c}$ or $-C(O)NR^{11c}R^{11c}$;

each R^{6c}, $R^{6c'}$, R^{7c}, $R^{7c'}$, R^{8c}, $R^{8c'}$, R^{9c}, $R^{9c'}$, R^{10c}, $R^{10c'}$, R^{11c}, $R^{11c'}$, R^{12c} and $R^{12c'}$ is independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

R^{13c} and $R^{13c'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{11c}$, $-OC(O)R^{11c}$, $-OC(O)NR^{11c}R^{11c'}$, $-OS(O)R^{11c}$, $-OS(O)_2R^{11c}$, $-SR^{11c}$, $-S(O)R^{11c}$, $-S(O)_2R^{11c}$, $-S(O)NR^{11c}R^{11c'}$, $-S(O)_2NR^{11c}R^{11c'}$, $-OS(O)NR^{11c}R^{11c'}$, $-OS(O)_2NR^{11c}R^{11c'}$, $-NR^{11c}R^{11c'}$, $-NR^{11c}C(O)R^{12c}$, $-NR^{11c}C(O)OR^{12c}$, $-NR^{11c}C(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)R^{12c}$, $-NR^{11c}S(O)_2R^{12c}$, $-NR^{11c}S(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)_2NR^{12c}R^{12c'}$, $-C(O)R^{11c}$, $-C(O)OR^{11c}$ and $-C(O)NR^{11c}R^{11c}$;

R^{1d} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{2d}$, $-SR^{2d}$ and $-NR^{2d}R^{2d'}$,

R^{2d} and $R^{2d'}$ are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is optionally substituted by $-OR^{3d}$, $-SR^{3d}$, and $-NR^{3d}R^{3d'}$;

R^{3d} and $R^{3d'}$ are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

R^{1e} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{2e}$, $-OC(O)R^{2e}$, $-OC(O)NR^{2e}R^{2e'}$, $-OS(O)R^{2e}$, $-OS(O)_2R^{2e}$, $-SR^{2e}$, $-S(O)R^{2e}$, $-S(O)_2R^{2e}$, $-S(O)NR^{2e}R^{2e'}$, $-S(O)_2NR^{2e}R^{2e'}$, $-OS(O)NR^{2e}R^{2e'}$, $-OS(O)_2NR^{2e}R^{2e'}$, $-NR^{2e}R^{2e'}$, $-NR^{2e}C(O)R^{3e}$, $-NR^{2e}C(O)OR^{3e}$, $-NR^{2e}C(O)NR^{3e}R^{3e'}$, $-NR^{2e}S(O)R^{3e}$, $-NR^{2e}S(O)_2R^{3e}$, $-NR^{2e}S(O)NR^{2e}R^{2e'}$, $-NR^{2e}S(O)_2NR^{3e}R^{3e'}$, $-C(O)R^{2e}$, $-C(O)OR^{2e}$ or $-C(O)NR^{2e}R^{2e}$;

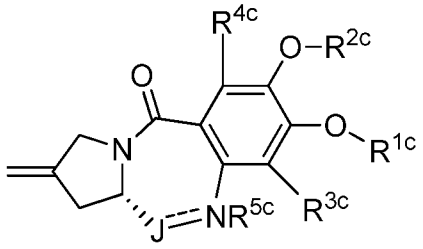
each R^{2e}, $R^{2e'}$, R^{3e} and $R^{3e'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is optionally substituted by $-OR^{4e}$, $-SR^{4e}$ or $-NR^{4e}R^{4e'}$;

R^{4e} and $R^{4e'}$ are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

v is 1, 2 or 3; and

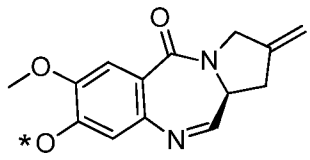
each * represents a covalent bond to the rest of the conjugate.

18. The conjugate of any one of the preceding clauses, wherein the second drug is of the formula



or a pharmaceutically acceptable salt thereof.

19. The conjugate of any one of the preceding clauses, wherein the second drug is of the formula



wherein * represents a covalent bond to the rest of the conjugate.

20. The conjugate of clause 8, having the formula B-$(L^1)_{z1}$-$(AA)_{z2}$-$(L^1)_{z3}$-$(AA)_{z4}$-$(L^1)_{z5}$-$(AA)_{z6}$-$(L^2)_{z7}$-$(L^r)_{z8}$-$(L^2)_{z9}$-D-L^3-D-$(L^2)_{y9}$-$(L^r)_{y8}$-$(L^2)_{y7}$-$(AA)_{y6}$-$(L^1)_{y5}$-$(AA)_{y4}$-$(L^1)_{y3}$-$(AA)_{y2}$-$(L^1)_{y1}$-X,

wherein

z1 is an integer from 0 to 2, z2 is an integer from 0 to 3, z3 is an integer from 0 to 2, z4 is an integer from 0 to 3, z5 is an integer from 0 to 2, z6 is an integer from 0 to 3, z7 is an integer from 0 to 8, z8 is 1, z9 is an integer from 0 to 8, y1 is an integer from 0 to 2, y2 is an integer from 0 to 3, y3 is an integer from 0 to 2, y4 is an integer from 0 to 3, y5 is an integer from 0 to 2, y6 is 0 or 1, y7 is an integer from 0 to 8, y8 is 0 or 1; y9 is an integer from 0 to 8;

each D is independently D^1 or D^2;

X is H or B;

each B is independently a binding ligand;

each AA is independently an amino acid;

each L^1 is independently a first spacer linker;

each L^2 is independently a second spacer linker;

each L^3 is independently a third spacer linker; and

each L^r is independently a releasable linker;

or a pharmaceutically acceptable salt thereof.

21. The conjugate of clause 20, or a pharmaceutically acceptable salt thereof, wherein y1 is 0, y2 is 0, y3 is 0, y4 is 0, y5 is 0, y6 is 0, y7 is 0, y8 is 0, y9 is 0 and X is H.

22. The conjugate of clause 20 or 21, or a pharmaceutically acceptable salt thereof, wherein z1 is 0, z2 is 2, z3 is 0, z4 is 1, z5 is 0 and z6 is 1.

23. The conjugate of clause 20 or 21, or a pharmaceutically acceptable salt thereof, wherein z1 is 0, z2 is 2, z3 is 0, z4 is 2, z5 is 0 and z6 is 1.

24. The conjugate of clause 20 or 21, or a pharmaceutically acceptable salt thereof, wherein z1 is 1, z2 is 1, z3 is 1, z4 is 1, z5 is 1 and z6 is 1.

25. The conjugate of clause 20 or 21, or a pharmaceutically acceptable salt thereof, wherein z1 is 1, z2 is 1, z3 is 1, z4 is 1, z5 is 1 and z6 is 0.

26. The conjugate of clause 20, or a pharmaceutically acceptable salt thereof, wherein z1 is 0, z2 is 2, z3 is 0, z4 is 1, z5 is 0, z6 is 1, y1 is 0, y2 is 2, y3 is 0, y4 is 1, y5 is 0 and y6 is 1.

27. The conjugate of clause 20, or a pharmaceutically acceptable salt thereof, wherein z1 is 0, z2 is 2, z3 is 0, z4 is 2, z5 is 0, z6 is 1, y1 is 0, y2 is 2, y3 is 0, y4 is 2, y5 is 0 and y6 is 1.

28. The conjugate of clause 26 or 27, or a pharmaceutically acceptable salt thereof, wherein y7 is 1.

29. The conjugate of clause 28, or a pharmaceutically acceptable salt thereof, wherein y8 is 0.

30. The conjugate of clause 29, or a pharmaceutically acceptable salt thereof, wherein y9 is 0.

31. The conjugate of clause 26 or 27, or a pharmaceutically acceptable salt thereof, wherein y7 is 0.

32. The conjugate of clause 31, or a pharmaceutically acceptable salt thereof, wherein y8 is 1.

33. The conjugate of clause 32, or a pharmaceutically acceptable salt thereof, wherein y9 is 0.

34. The conjugate of clause 26 or 27, or a pharmaceutically acceptable salt thereof, wherein y8 is 0.

35. The conjugate of any one of clauses 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 6.

36. The conjugate of any one of clauses 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 5.

37. The conjugate of any one of clauses 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 4.

38. The conjugate of any one of clauses 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 3.

39. The conjugate of any one of clauses 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 2.

40. The conjugate of any one of clauses 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 1.

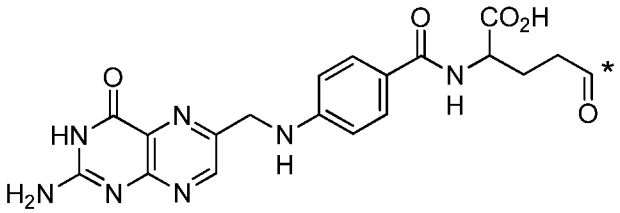
41. The conjugate of any one of clauses 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 0.

42. The conjugate of any one of clauses 20 to 41, or a pharmaceutically acceptable salt thereof, wherein z9 is 2.

43. The conjugate of any one of clauses 20 to 41, or a pharmaceutically acceptable salt thereof, wherein z9 is 1.

44. The conjugate of any one of clauses 20 to 41, or a pharmaceutically acceptable salt thereof, wherein z9 is 0.

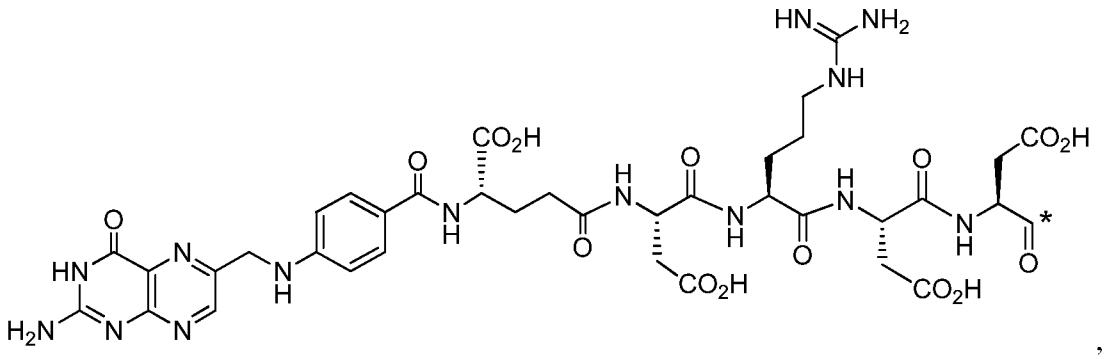
45. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, wherein B is of the formula



,

or a pharmaceutically acceptable salt thereof.

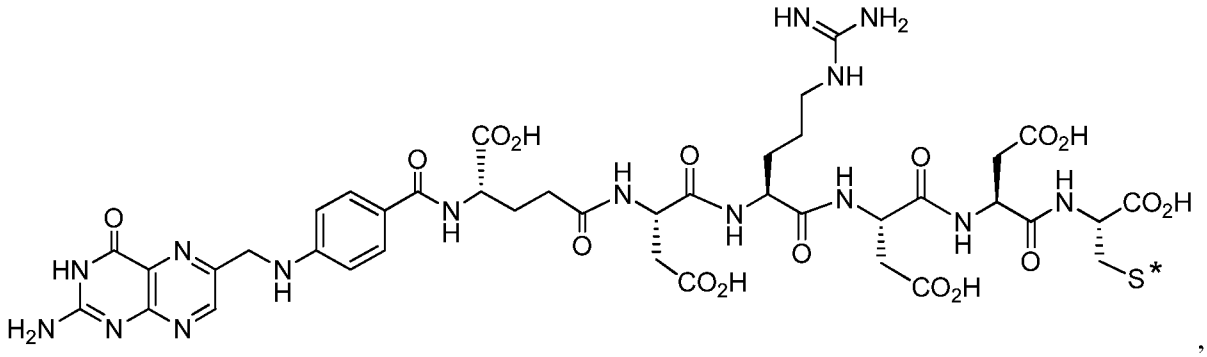
46. The conjugate of any one of clauses 1 to 22, or a pharmaceutically acceptable salt thereof, comprising the formula



,

wherein * represents a covalent bond to the rest of the conjugate.

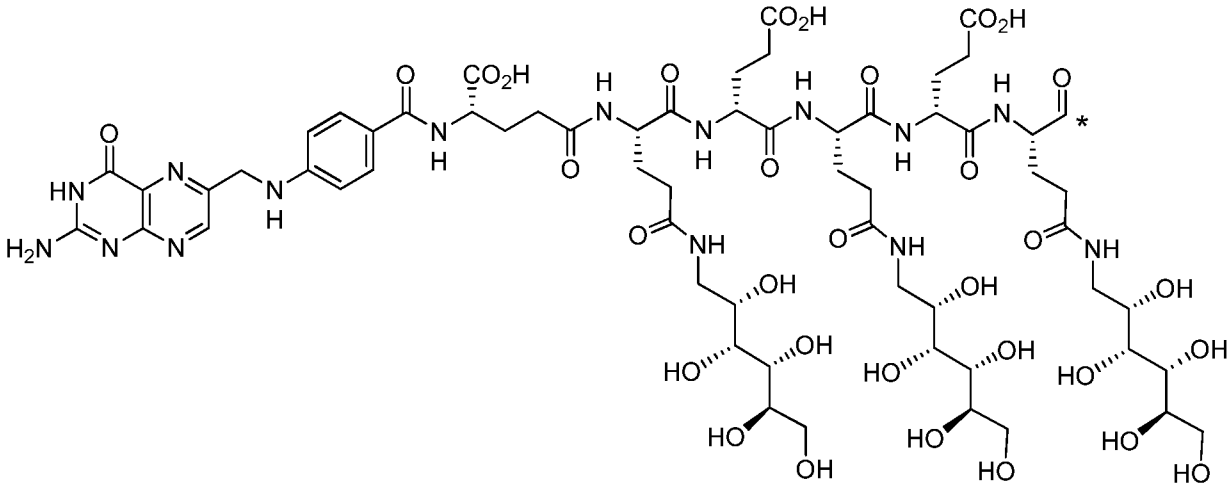
47. The conjugate of any one of clauses 1 to 21 or 23, or a pharmaceutically acceptable salt thereof, comprising the formula



,

wherein * represents a covalent bond to the rest of the conjugate.

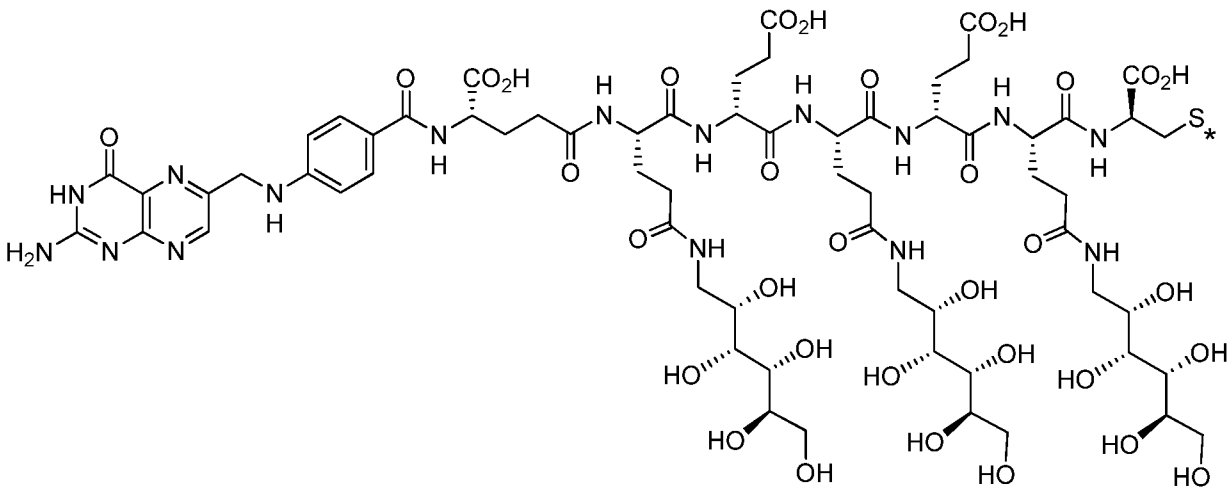
48. The conjugate of any one of clauses 1 to 21 or 25, or a pharmaceutically acceptable salt thereof, comprising the formula



,

wherein * represents a covalent bond to the rest of the conjugate.

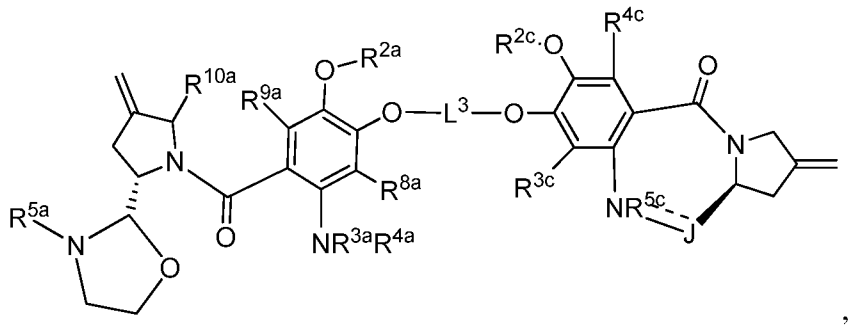
48. The conjugate of any one of clauses 1 to 21 or 24, or a pharmaceutically acceptable salt thereof, comprising the formula



,

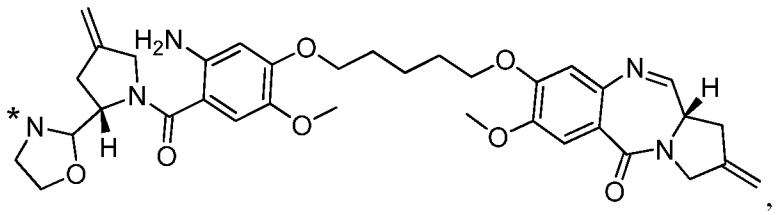
wherein * represents a covalent bond to the rest of the conjugate.

49. The conjugate of any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, comprising the formula



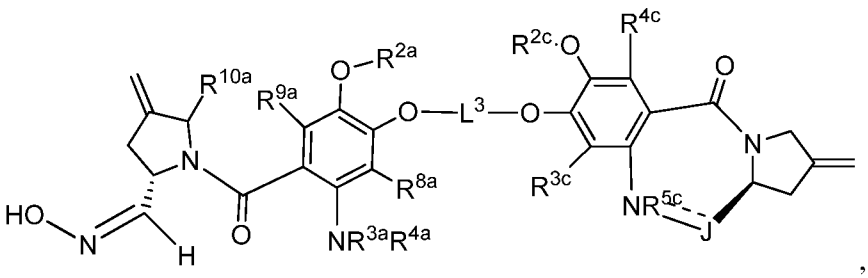
wherein R^{5a} is a covalent bond to the rest of the conjugate.

50. The conjugate of clause 49, any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, comprising the formula



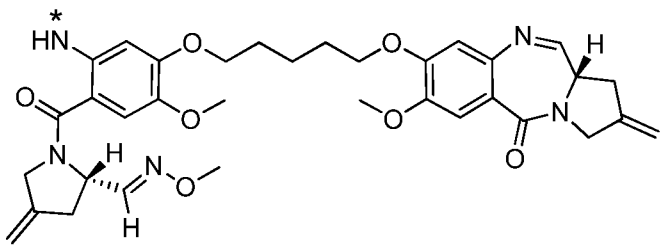
wherein * represents a covalent bond to the rest of the conjugate.

51. The conjugate of any one of clauses 1 to 48, or a pharmaceutically acceptable salt thereof, comprising the formula



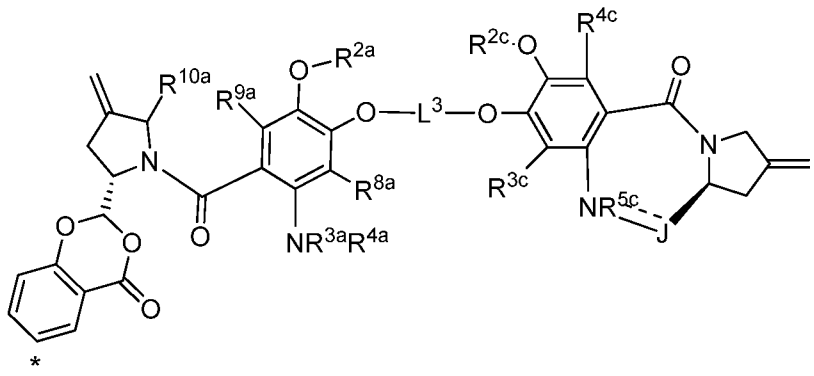
wherein R^{4a} is a covalent bond to the rest of the conjugate.

52. The conjugate of clause 51, or a pharmaceutically acceptable salt thereof, comprising the formula



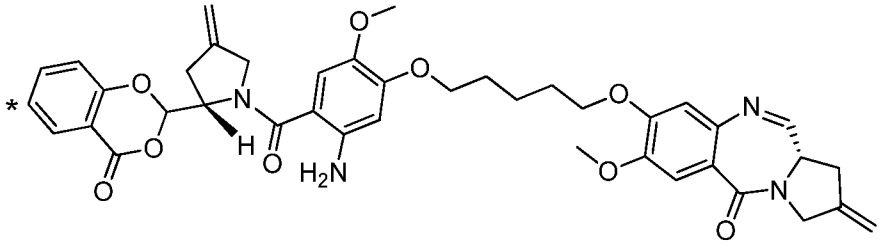
wherein * represents a covalent bond to the rest of the conjugate.

53. The conjugate of any one of clauses 1 to 48, or a pharmaceutically acceptable salt thereof, comprising the formula



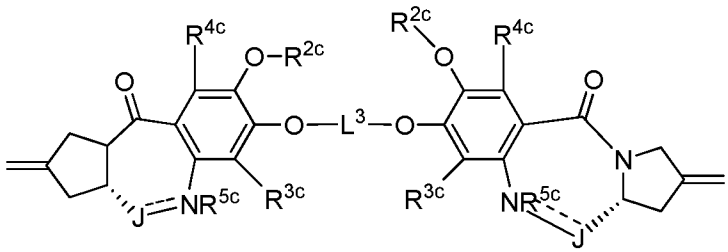
wherein * represents a covalent bond to the rest of the conjugate.

54. The conjugate of clause 53, or a pharmaceutically acceptable salt thereof, comprising the formula



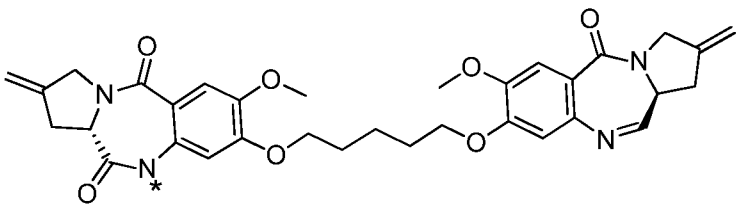
wherein * represents a covalent bond to the rest of the conjugate.

55. The conjugate of any one of clauses 1 to 48, or a pharmaceutically acceptable salt thereof, comprising the formula

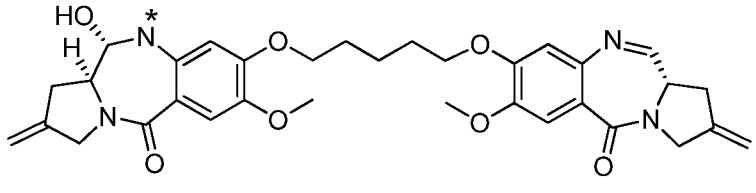


wherein at least one R^{5c} is a covalent bond to the rest of the conjugate.

56. The conjugate of clause 55, or a pharmaceutically acceptable salt thereof, comprising the formula



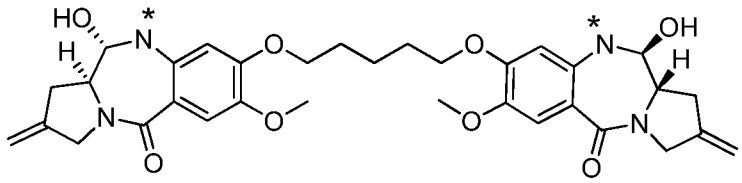
or



,

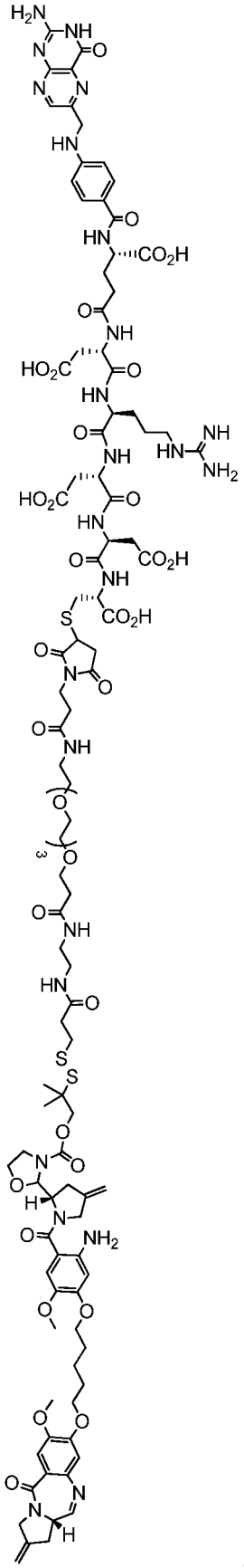
wherein * represents a covalent bond to the rest of the conjugate.

57. The conjugate of clause 55, or a pharmaceutically acceptable salt thereof, comprising the formula



wherein * represents a covalent bond to the rest of the conjugate.

58. A conjugate selected from the group consisting of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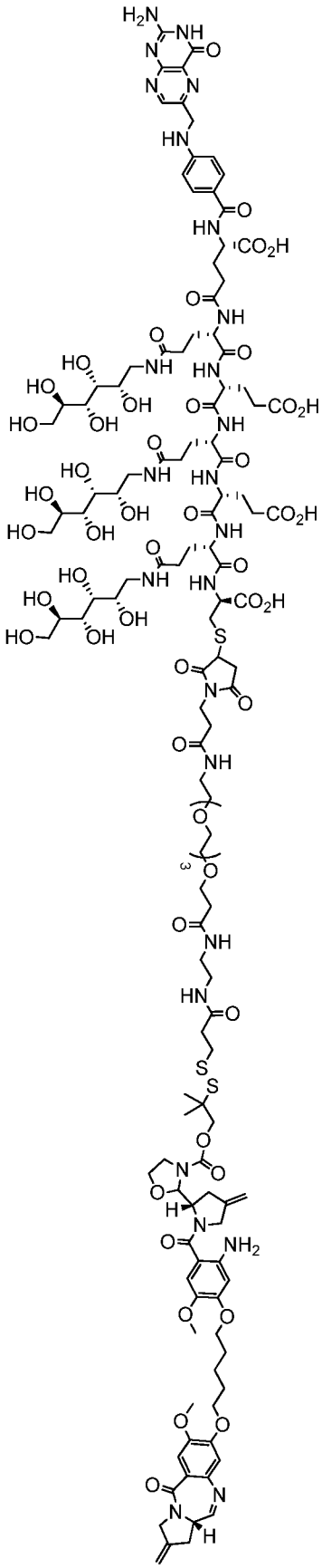
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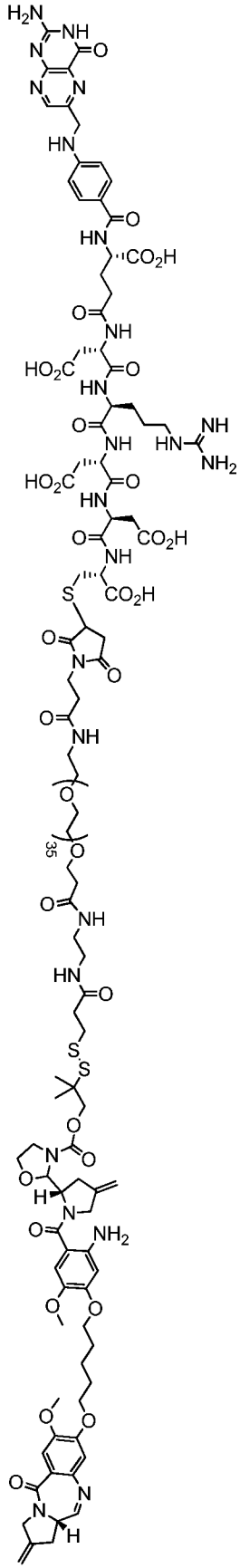
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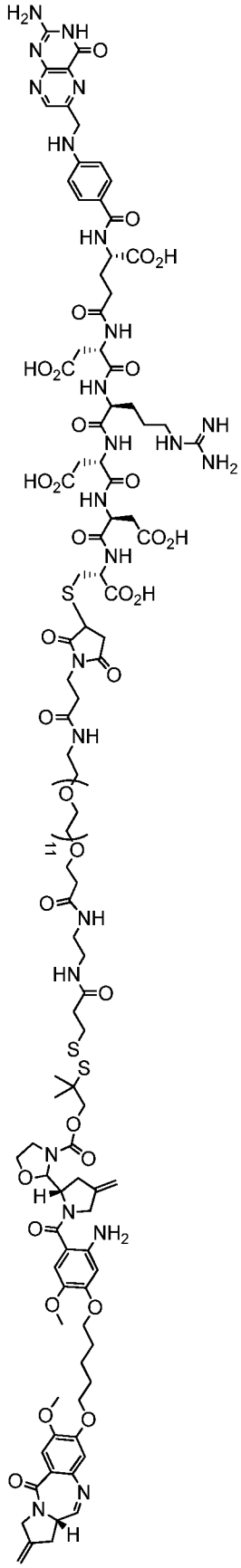
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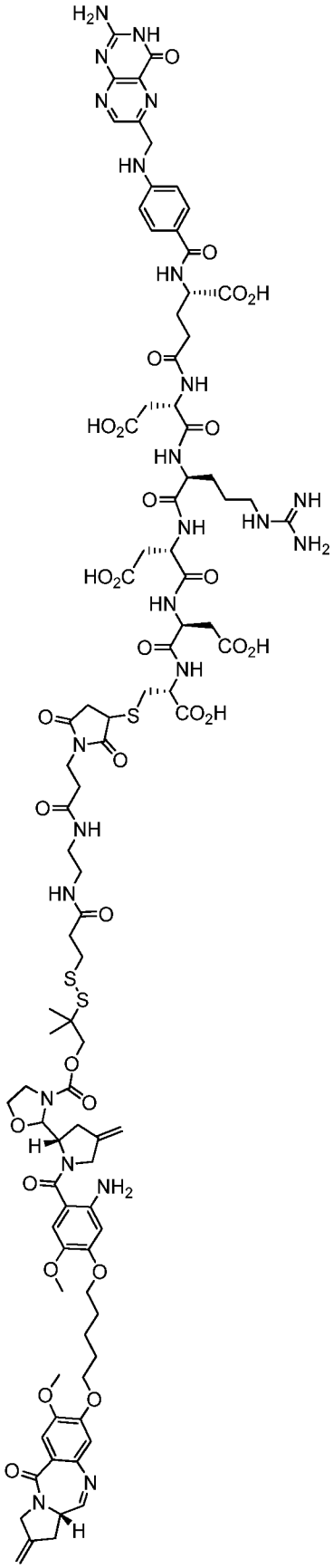

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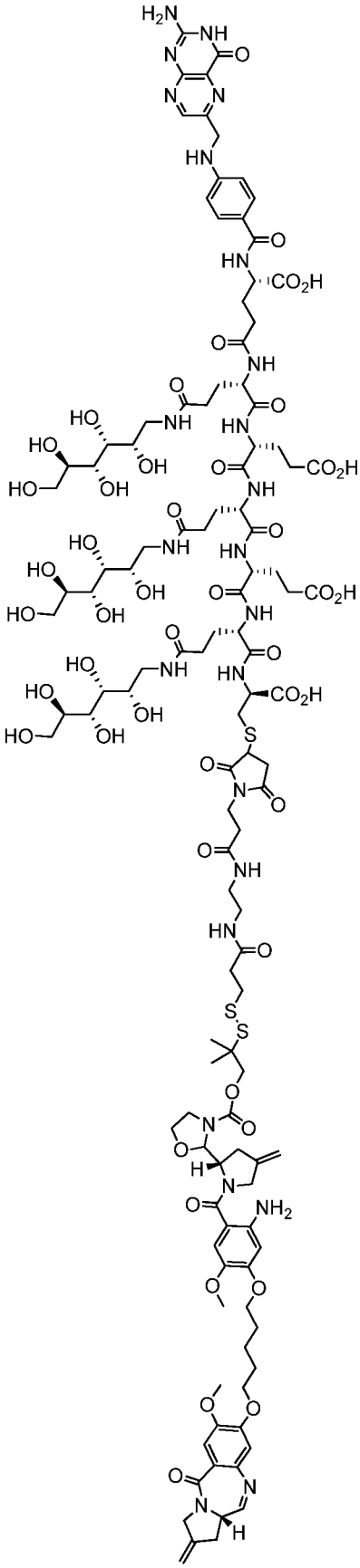

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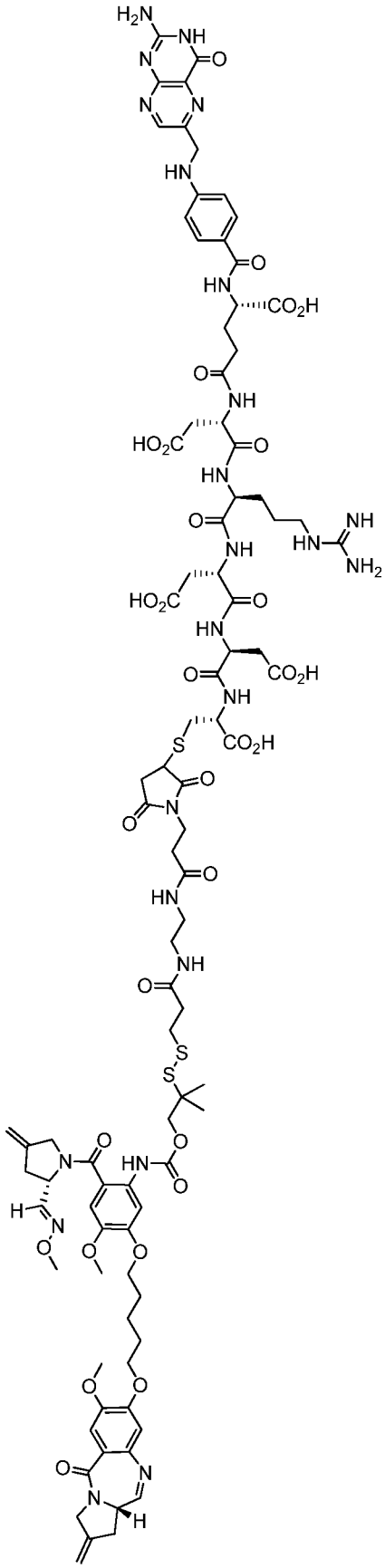

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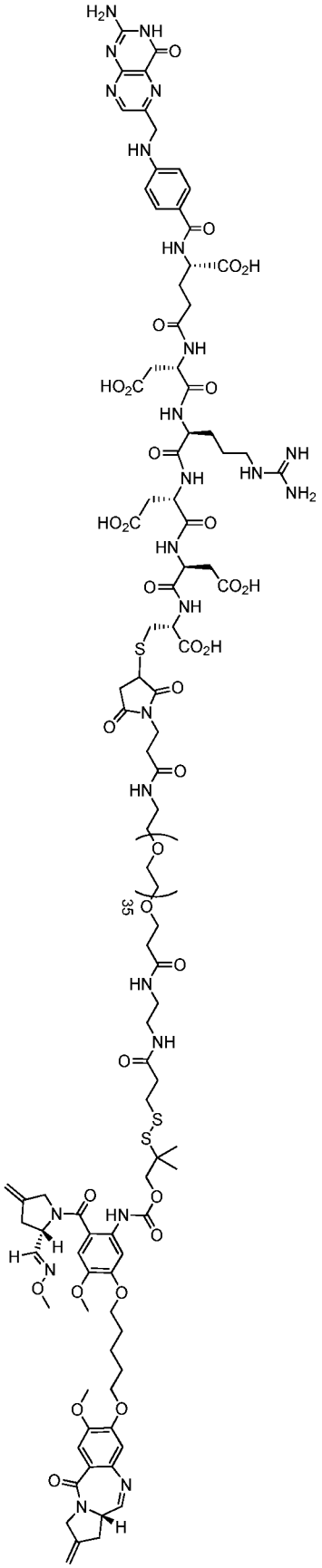

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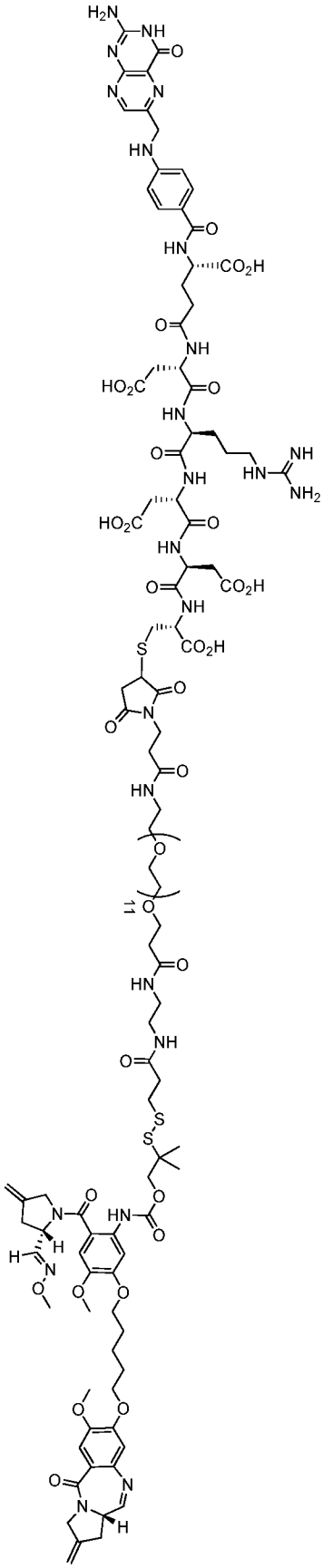

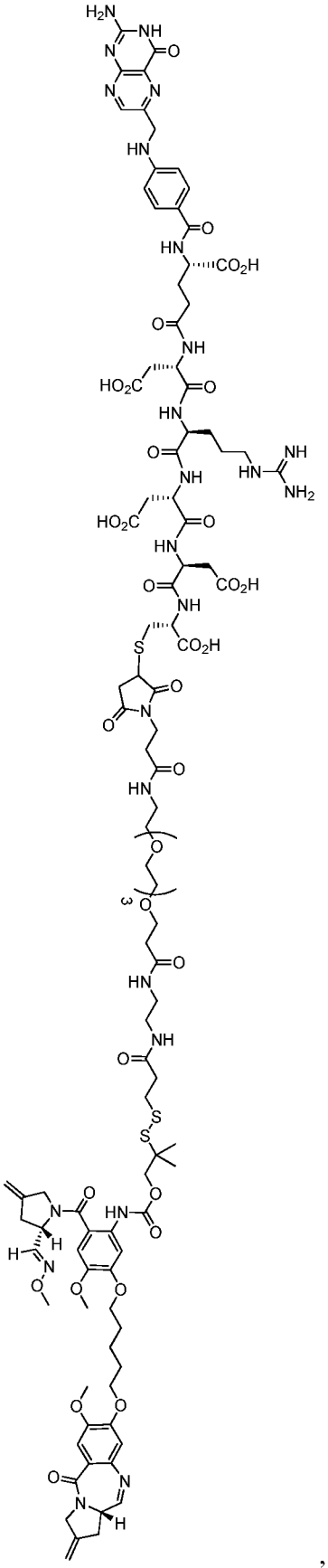

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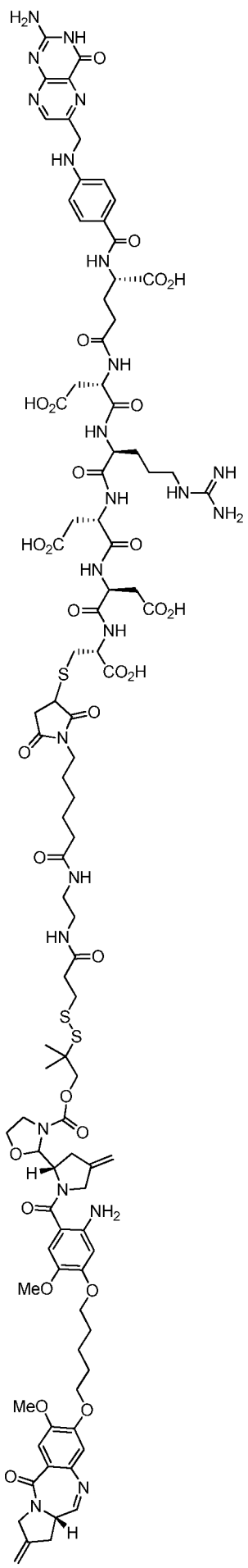

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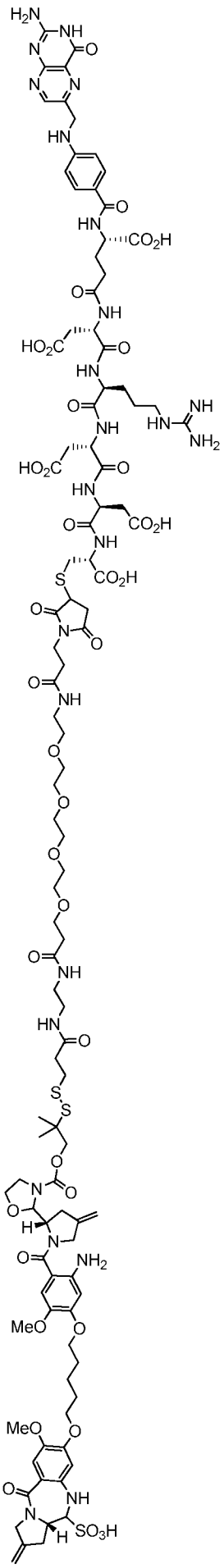

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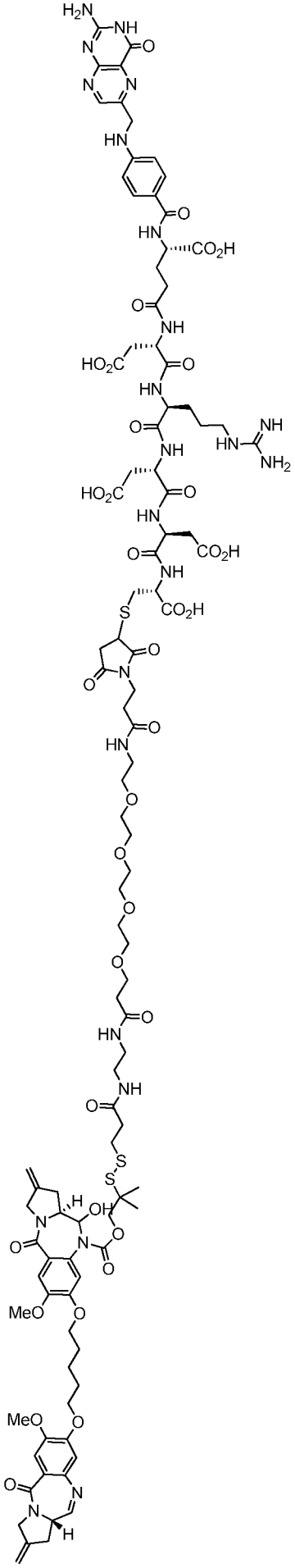

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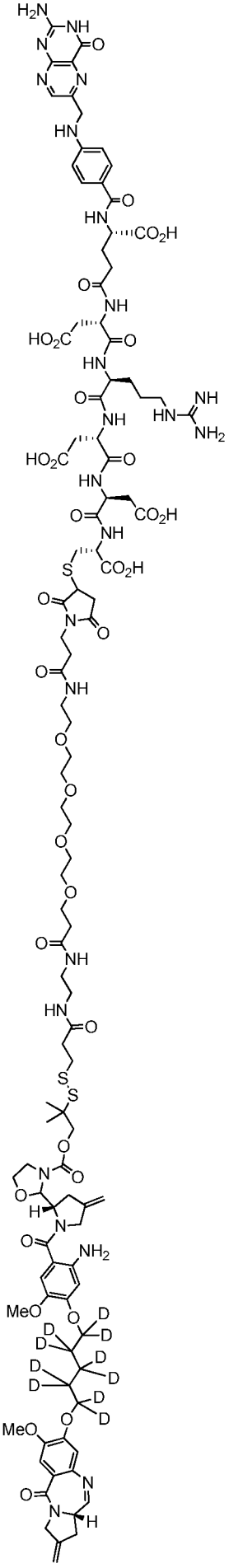

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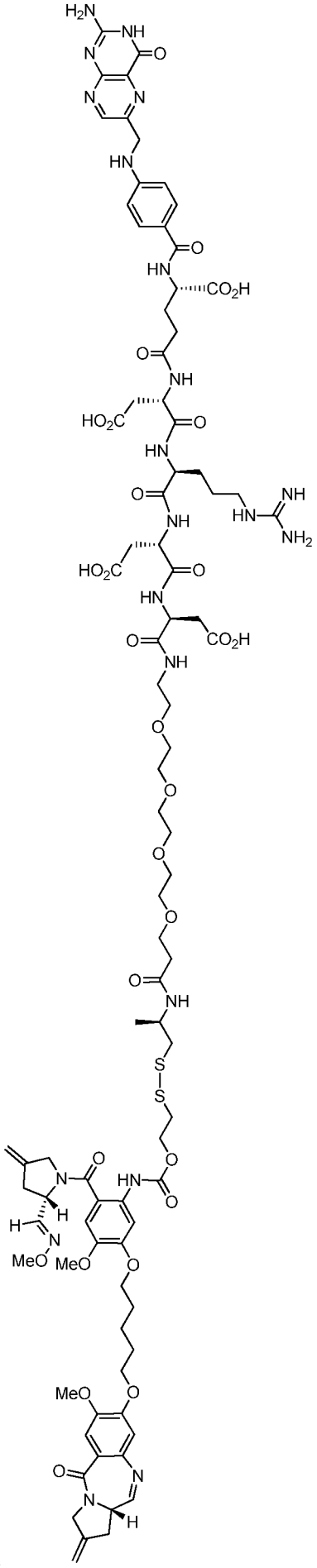
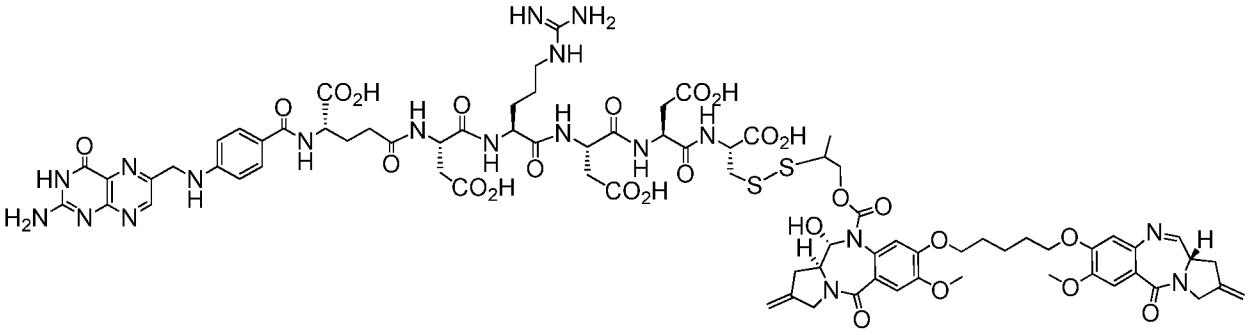


,

or a pharmaceutically acceptable salt thereof.

59. A conjugate selected from the group consisting of



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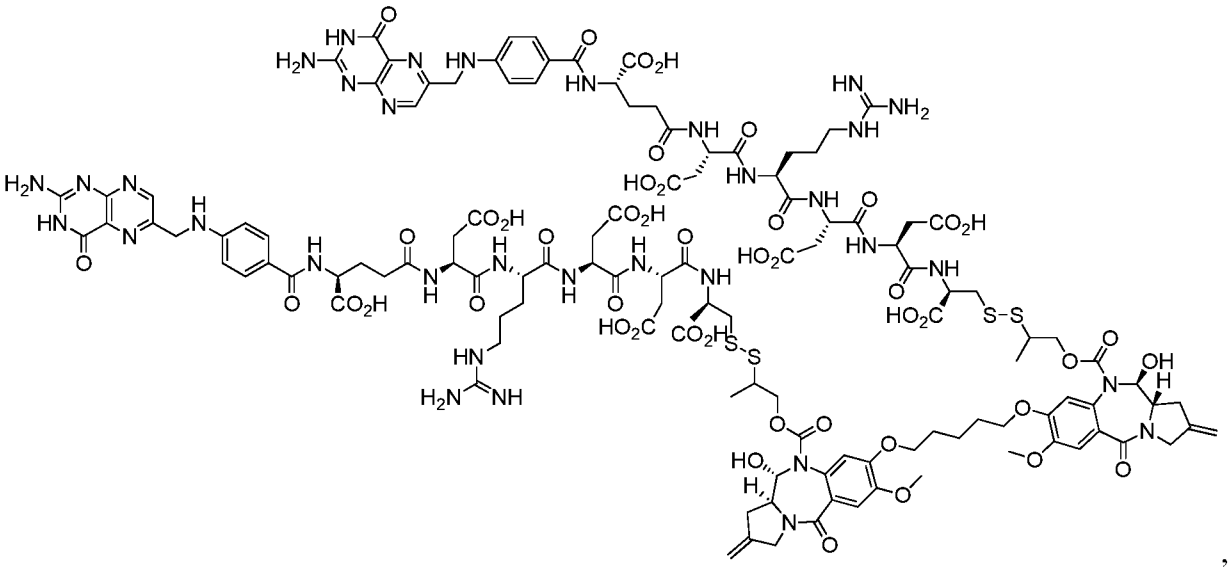

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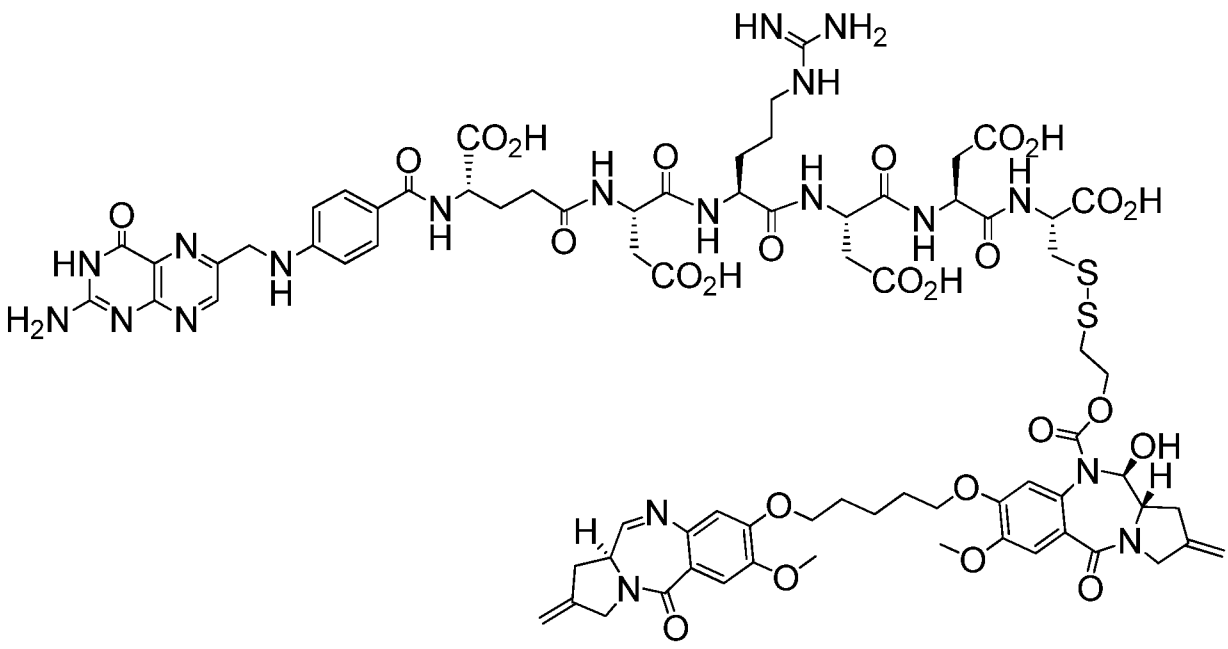

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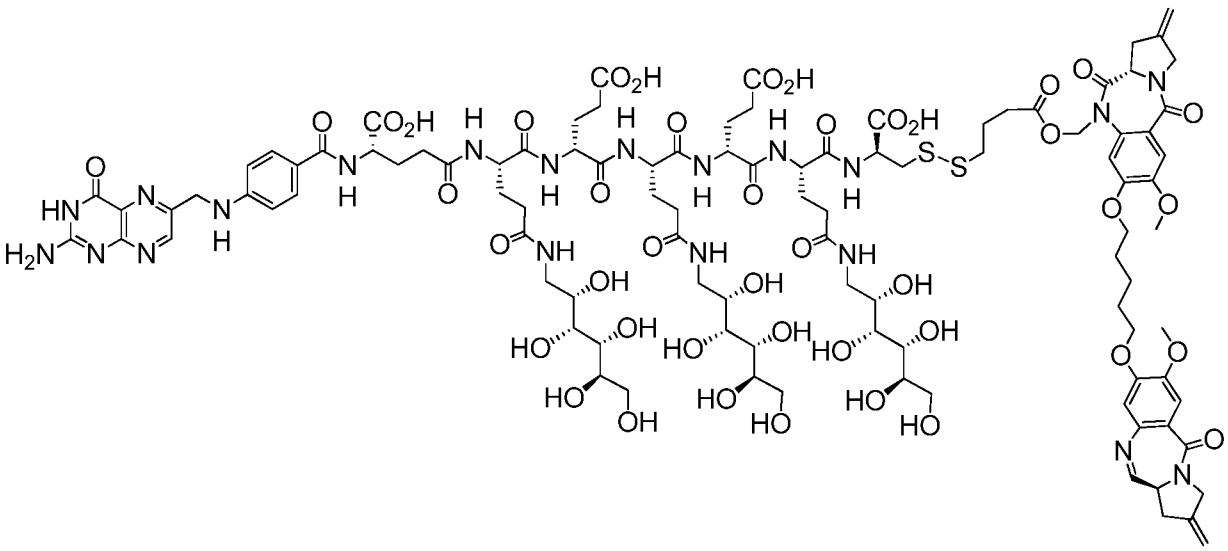

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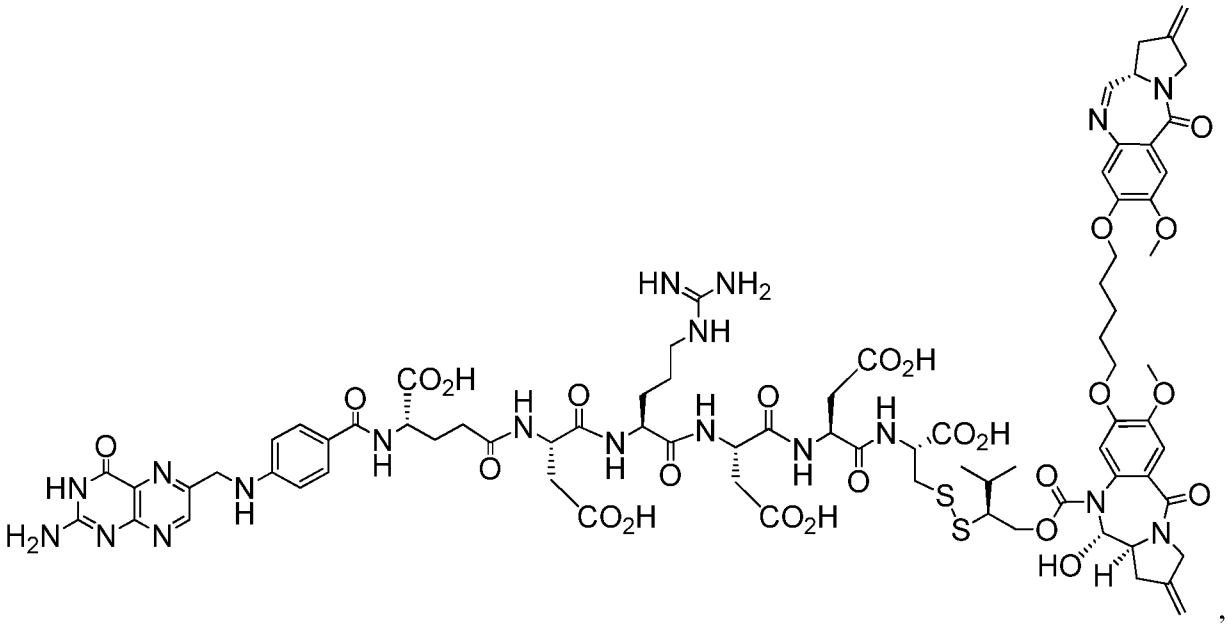
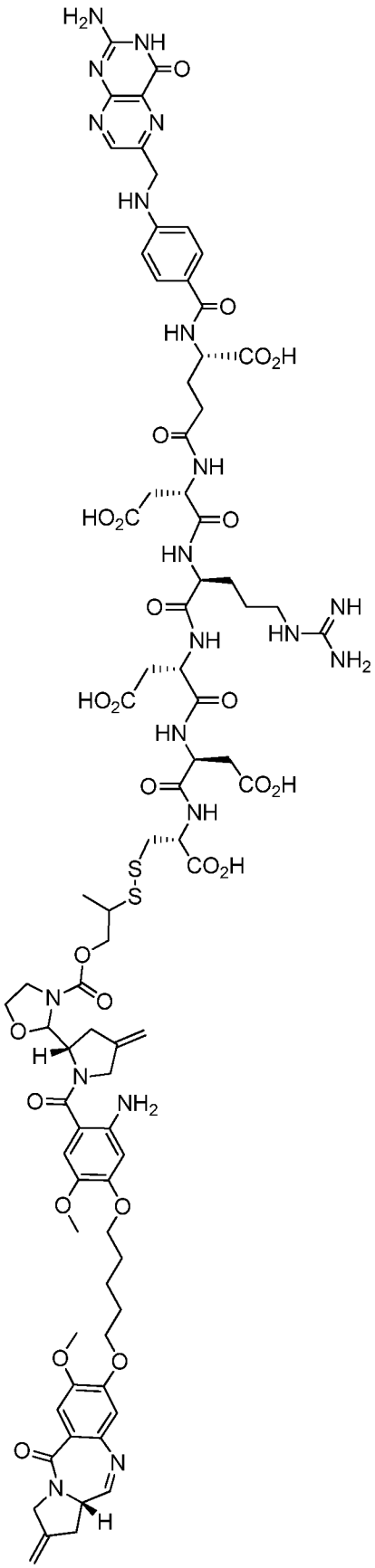
and



,

or a pharmaceutically acceptable salt thereof.

60. A conjugate selected from the group consisting of



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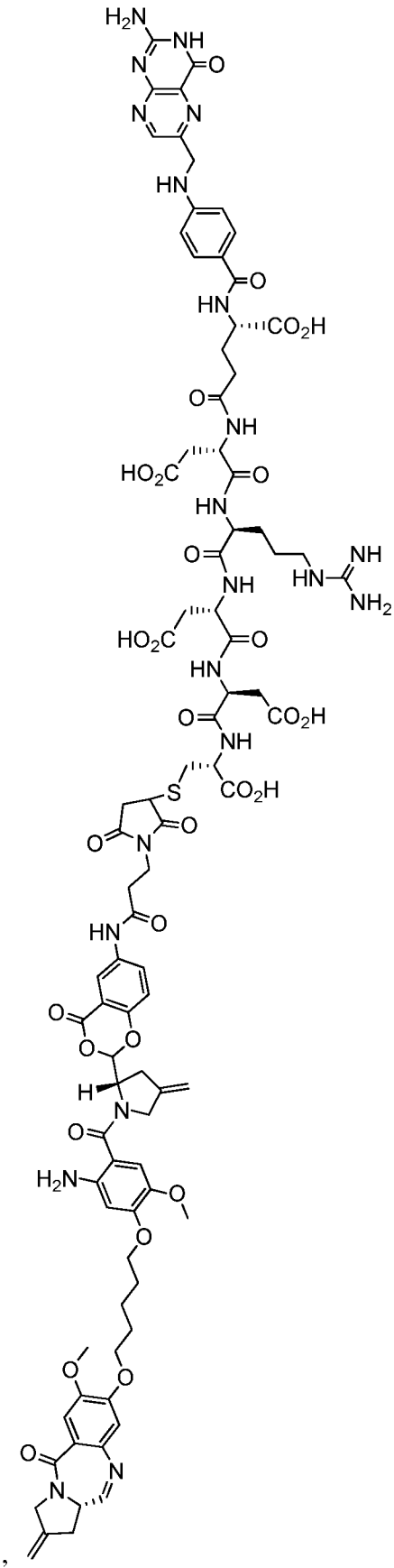

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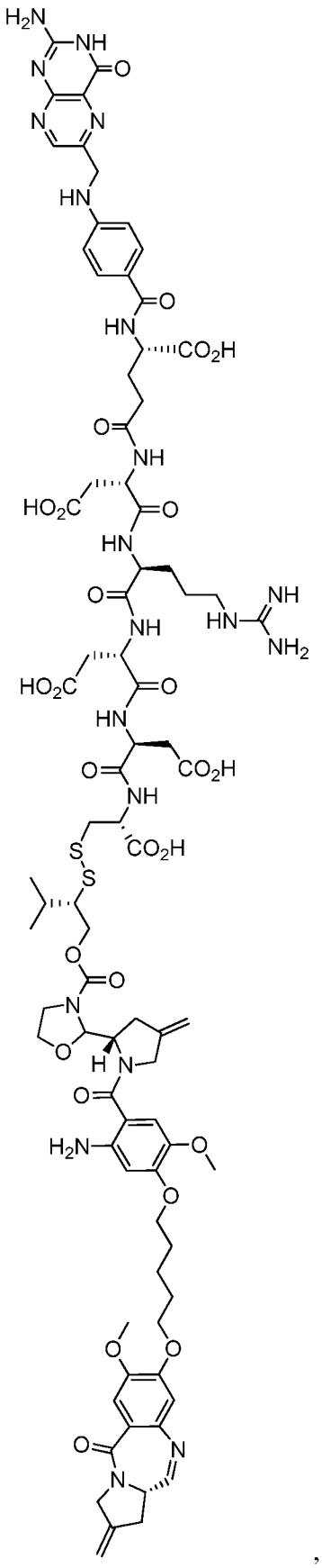

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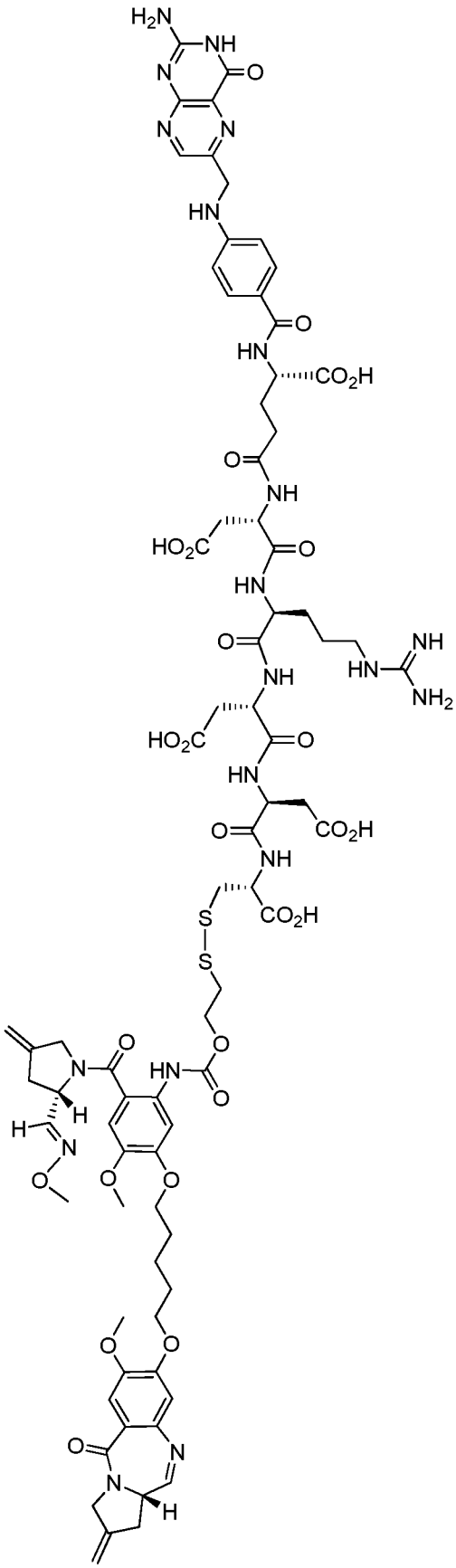

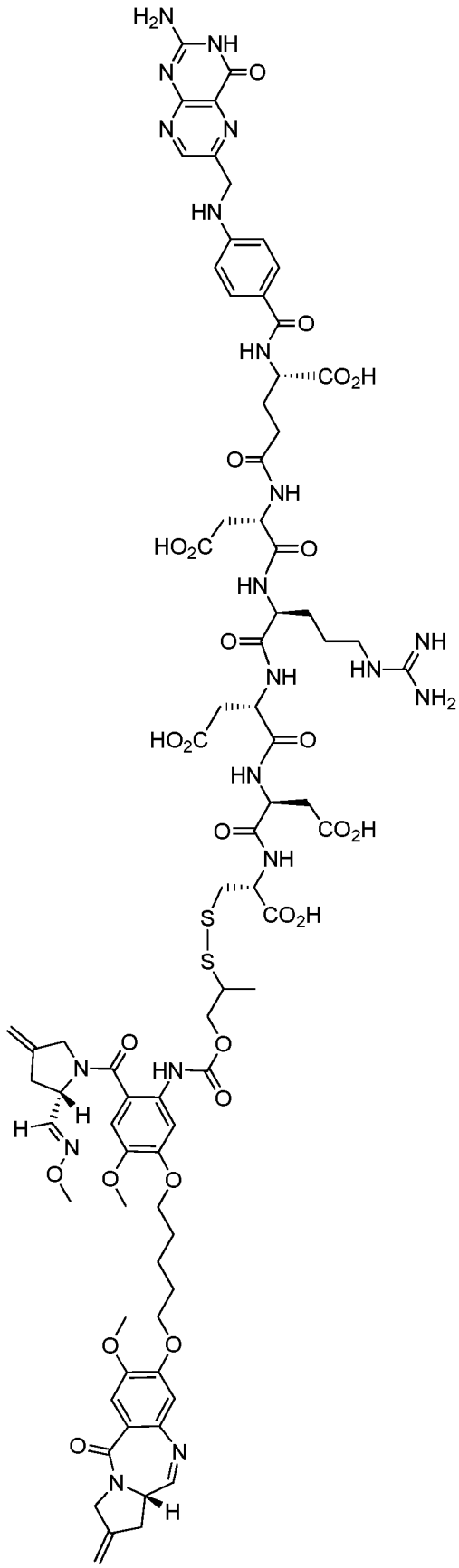

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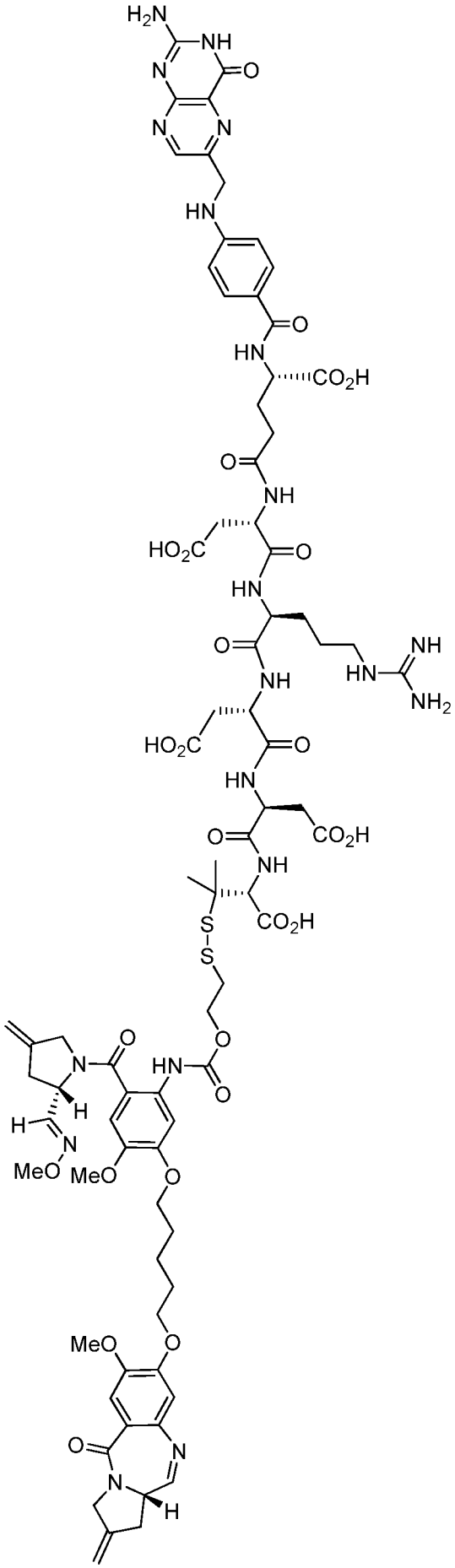

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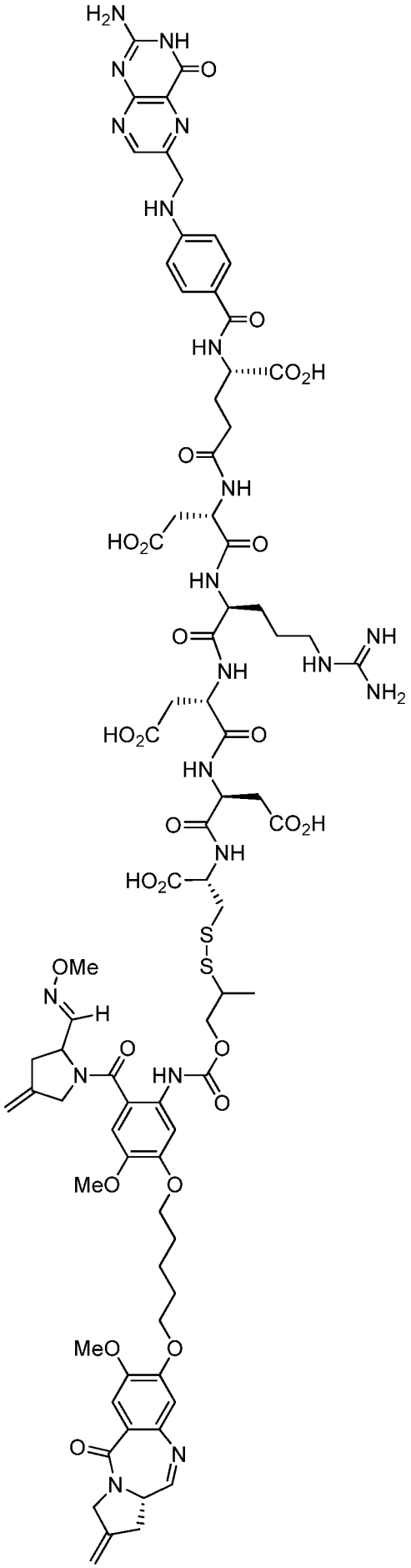

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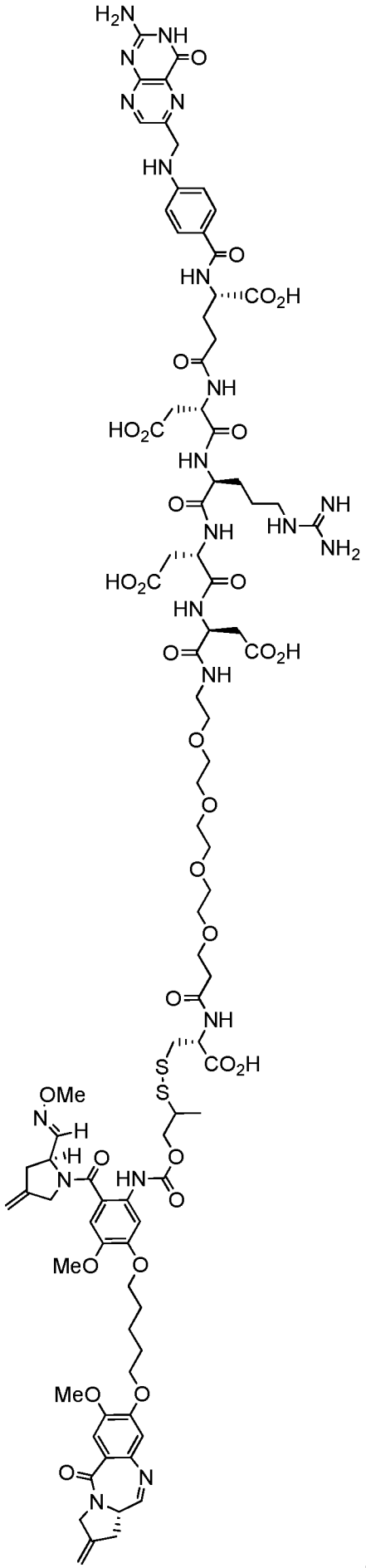

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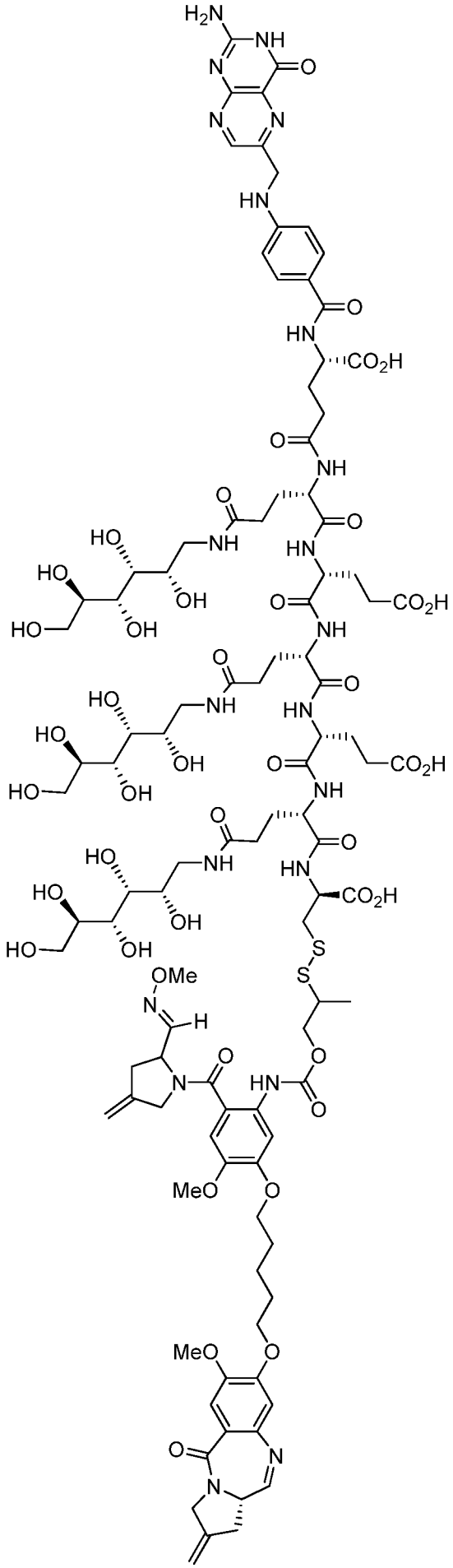

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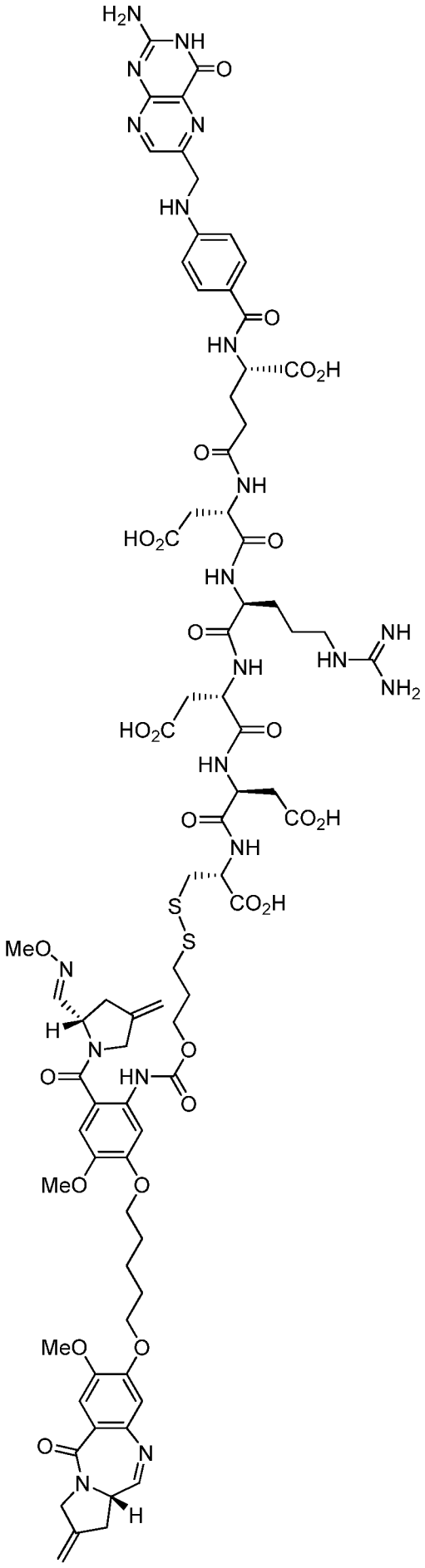


,

or a pharmaceutically acceptable salt thereof.

61. A pharmaceutical composition comprising a therapeutically effective amount of a conjugate according to any one of the preceding clauses, or a pharmaceutically acceptable salt thereof, and optionally at least one pharmaceutically acceptable excipient.

62. A method of treating abnormal cell growth in a patient, comprising

a. administering to the patient a therapeutically effective amount of a conjugate, or a pharmaceutically acceptable salt thereof, or pharmaceutical composition, of any one of the preceding clauses.

63. The method of clause 62, wherein the abnormal cell growth is cancer.

64. The method of clause 63. wherein the cancer is selected from the group consisting of lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, triple negative breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma and pituitary adenoma.

65. Use of a conjugate according to any one of clauses 1 to 60 in the preparation of a medicament for the treatment of cancer.

66. A conjugate according to any one of clauses 1 to 60 for use in a method of treating cancer in a patient.

67. The conjugate of clause 66, where the method comprises administering to the patient a therapeutically effective amount of a conjugate, or a pharmaceutically acceptable salt thereof.

68. The conjugate of clause 67, wherein the cancer is selected from the group consisting of lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, triple negative breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the

urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma and pituitary adenoma.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 9** (●) and with **Conjugate 9** and excess folate (■).

FIG. 2A is a chart that shows that **Conjugate 9** (■) dosed at 1 μmol/kg SIW for two weeks decreased KB tumor size in test mice compared to untreated control (●). The dotted line indicates the last dosing day.

FIG. 2B is a chart that shows % weight change for test mice dosed at 1 μmol/kg **Conjugate 9** SIW for two weeks (■) compared to untreated control (●).

FIG. 3 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 1** (●) and with **Conjugate 1** and excess folate (■).

FIG. 4A is a chart that shows that **Conjugate 1** dosed at 0.5 μmol/kg SIW for two weeks (●) decreased KB tumor size in test mice compared to untreated control (▲). The dotted line indicates the last dosing day.

FIG. 4B is a chart that shows % weight change for test mice dosed at 0.5 μmol/kg **Conjugate 1** SIW for two weeks (●) compared to untreated control (▲).

FIG. 5 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 2** (●) and with **Conjugate 2** and excess folate (■).

FIG. 6A is a chart that shows that **Conjugate 2** dosed at 0.5 μmol/kg SIW for two weeks (■) decreased KB tumor size in test mice compared to untreated control (●). The dotted line indicates the last dosing day.

FIG. 6B is a chart that shows % weight change for test mice dosed at 0.5 μmol/kg **Conjugate 2** SIW for two weeks (■) compared to untreated control (●).

FIG. 7 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 5** (●) and with **Conjugate 5** and excess folate (■).

FIG. 8A is a chart that shows that **Conjugate 5** dosed at 0.5 μmol/kg SIW for two weeks (▲) decreased KB tumor size in test mice compared to untreated control (■). The dotted line indicates the last dosing day.

FIG. 8B is a chart that shows % weight change for test mice dosed at 0.5 μmol/kg

Conjugate 5 SIW for two weeks (▲) compared to untreated control (■).

FIG. 9 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 3** (●) and with **Conjugate 3** and excess folate (■).

FIG. 10A is a chart that shows that **Conjugate 3** dosed at 0.5 μmol/kg SIW for two weeks (▼) decreased KB tumor size in test mice compared to untreated control (●). The dotted line indicates the last dosing day.

FIG. 10B is a chart that shows % weight change for test mice dosed at 0.5 μmol/kg **Conjugate 3** SIW for two weeks (▼) compared to untreated control (●).

FIG. 11 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 12** (▲) and with **Conjugate 12** and excess folate (●).

FIG. 12 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 4** (●) and with **Conjugate 4** and excess folate (■).

FIG. 13A is a chart that shows that each **Conjugate 12** dosed at 0.5 μmol/kg SIW for two weeks (▲) and **Conjugate 4** dosed at 0.5 μmol/kg SIW for two weeks (◆) decreased KB tumor size in test mice compared to untreated control (●). The dotted line indicates the last dosing day.

FIG. 13B is a chart that shows % weight change for test mice dosed at 0.5 μmol/kg **Conjugate 12** SIW for two weeks (▲) and test mice dosed at 0.5 μmol/kg **Conjugate 4** SIW for two weeks (◆) compared to untreated control (●).

FIG. 14 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 16** (●) and with **Conjugate 16** and excess folate (■).

FIG. 15A is a chart that shows that **Conjugate 16** dosed at 0.5 μmol/kg SIW for two weeks (●) decreased KB tumor size in test mice compared to untreated control (Δ). The dotted line indicates the last dosing day.

FIG. 15B is a chart that shows % weight change for test mice dosed at 0.5 μmol/kg **Conjugate 16** SIW for two weeks (●) compared to untreated control (Δ).

FIG. 16 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 6** (●) and with **Conjugate 6** and excess folate (■).

FIG. 17A is a chart that shows that **Conjugate 6** dosed at 0.5 μmol/kg SIW for two weeks (▼) decreased KB tumor size in test mice compared to untreated control (●). The dotted line indicates the last dosing day.

FIG. 17B is a chart that shows % weight change for test mice dosed at 0.5 μmol/kg

Conjugate 6 SIW for two weeks (▼) compared to untreated control (●).

FIG. 18 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 15** (●) and with **Conjugate 15** and excess folate (■).

FIG. 19A is a chart that shows that **Conjugate 15** dosed at 0.5 μmol/kg SIW for two weeks (◆) decreased KB tumor size in test mice compared to untreated control (●). The dotted line indicates the last dosing day.

FIG. 19B is a chart that shows % weight change for test mice dosed at 0.5 μmol/kg **Conjugate 15** SIW for two weeks (◆) compared to untreated control (●).

FIG. 20 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 7** (●) and with **Conjugate 7** and excess folate (■).

FIG. 21A is a chart that shows that **Conjugate 7** dosed at 0.5 μmol/kg SIW for two weeks (■) decreased KB tumor size in test mice compared to untreated control (●). The dotted line indicates the last dosing day.

FIG. 21B is a chart that shows % weight change for test mice dosed at 0.5 μmol/kg **Conjugate 7** SIW for two weeks (■) compared to untreated control (●).

FIG. 22 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 8** (●) and with **Conjugate 8** and excess folate (■).

FIG. 23A is a chart that shows that **Conjugate 8** dosed at 0.2 μmol/kg SIW for two weeks (■) decreased KB tumor size in test mice compared to untreated control (●). The dotted line indicates the last dosing day.

FIG. 23B is a chart that shows % weight change for test mice dosed at 0.2 μmol/kg **Conjugate 8** SIW for two weeks (■) compared to untreated control (●).

FIG. 24 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 18** (●) and with **Conjugate 18** and excess folate (■).

FIG. 25 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 19** (●) and with **Conjugate 19** and excess folate (■).

FIG. 26 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 20** (●) and with **Conjugate 20** and excess folate (■).

FIG. 27A is a chart that shows that each **Conjugate 18** dosed at 0.5 μmol/kg SIW for two weeks (■), **Conjugate 19** dosed at 0.5 μmol/kg SIW for two weeks (▲), and **Conjugate 20** dosed at 0.5 μmol/kg SIW for two weeks (▼) decreased KB tumor size in test mice compared to untreated control (●). The dotted line indicates the last dosing day.

FIG. 27B is a chart that shows % weight change for test mice dosed at 0.5 μmol/kg **Conjugate 18** SIW for two weeks (■), test mice dosed at 0.5 μmol/kg **Conjugate 19** SIW for two weeks (▲), and test mice dosed at 0.5 μmol/kg **Conjugate 20** SIW for two weeks (▼) compared to untreated control (●).

FIG. 28 is a chart that shows the relative binding affinity of **Conjugate 1** toward the folate receptor. The experiment shows that the relative binding affinity of **Conjugate 1** was ~4.2-fold lower than that of folic acid. (■) folic acid (Control); (●) **Conjugate 1**.

FIG. 29 is a graph that shows that intact **Conjugate 1** is not able to crosslink DNA while the reduced form (treated with DTT) releases the active PBD molecule, which can then crosslink with DNA. (■) **Conjugate 1** plus DTT; (◆)**Conjugate 1** alone.

FIG. 30 is a chart that shows the percentage of ^{3}H-thymidine incorporated into MDA-MB231cells treated with **Conjugate 1** (●) and with **Conjugate 1** and excess folate (■).

FIG. 31 is a chart showing that mice bearing paclitaxel resistant KB tumors dosed at 0.5 μmol/kg SIW for two weeks with **Conjugate 5** (▲) had decreased tumor size compared to untreated control (■). The dotted line indicates the last dosing day. n = 5, **Conjugate 5** {0,1,4} as {partial response, complete response, cure}.

FIG. 32 is a chart showing that mice bearing platinum resistant KB tumors dosed at 0.5 μmol/kg SIW for two weeks with **Conjugate 5** (■), and dosed at 2.0 μmol/kg BIW for two weeks with EC1456 (▼) had decreased tumor size compared to untreated control (●). The dotted line indicates the last dosing day. n = 4, **Conjugate 5** {0,0,4}; EC1446 {0,2,2} as {partial response, complete response, cure}.

FIG. 33 is a chart showing that mice bearing ST502 TNBC PDX tumors dosed at 0.3 μmol/kg BIW for two weeks with **Conjugate 5** (▲) had decreased tumor size compared to untreated control (■), while mice dosed at 2.0 μmol/kg BIW for two weeks with EC1456 (●) did not have decreased tumor size compared to untreated control (■). The dotted line indicates the last dosing day. n = 7, **Conjugate 5** {0,0,7}as {partial response, complete response, cure}.

FIG. 34 is a chart showing that mice bearing ST070 ovarian PDX tumors dosed at 0.5 μmol/kg SIW for two weeks with **Conjugate 5** (●) had decreased tumor size compared to untreated control (■), while mice dosed at 4.0 μmol/kg SIW for two weeks with EC1456 (▲) or dosed at 15.0 mg/kg SIW for two weeks with paclitaxel (▼) did not have decreased tumor size. The dotted line indicates the last dosing day. n = 7, **Conjugate 5** {0,0,7}as {partial response, complete response, cure}.

FIG. 35 is a chart that shows the relative binding affinity of **Conjugate 5** toward the folate receptor. The experiment shows that the relative binding affinity of **Conjugate 5** was ~1.9-fold lower than that of folic acid. (■) folic acid (Control); (●) **Conjugate 5**.

FIG. 36 is a graph that shows that intact **Conjugate 5** is not able to crosslink DNA while the reduced form (treated with DTT) releases the active PBD molecule, which can then crosslink with DNA. (●) **Conjugate 5** plus DTT; (■) **Conjugate 1** without DTT.

FIG. 37A is a chart that shows that **Conjugate 5** dosed at 0.1 μmol/kg SIW for two weeks (■) and **Conjugate 5** dosed at 0.15 μmol/kg SIW for two weeks (▲) decreased KB tumor size in test rats compared to untreated control (●). The dotted line indicates the last dosing day.

FIG. 37B is a chart that shows % weight change for test rats dosed at 0.1 μmol/kg **Conjugate 5** SIW for two weeks (■) and test mice dosed at 0.15 μmol/kg **Conjugate 5** SIW for two weeks (▲) compared to untreated control (●).

FIG. 38 is a chart that shows that **Conjugate 5** dosed at 0.27 μmol/kg BIW for two weeks (●) decreased TNBC PDX tumor size in test mice compared to untreated control (■), whereas erubulin mesylate dosed at 1.0 μmol/kg SIW for two weeks (▲) did not decrease TNBC PDX tumor size.

FIG. 39 is a chart that shows that **Conjugate 5** dosed at 0.27 μmol/kg BIW for two weeks (●) produced partial response in Endometrial PDX tumor size in test mice compared to untreated control (■), whereas paclitaxel dosed at 15.0 mg/kg SIW for two weeks (▲) did not produce a partial response.

FIG. 40 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 22** (●) and with **Conjugate 20** and excess folate (■).

FIG. 41 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 24** (●) and with **Conjugate 20** and excess folate (■).

FIG. 42 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 25** (●) and with **Conjugate 20** and excess folate (■).

FIG. 43 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 26** (●) and with **Conjugate 20** and excess folate (■).

FIG. 44 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 27** (●) and with **Conjugate 20** and excess folate (■).

FIG. 45 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells

treated with **Conjugate 28** (●) and with **Conjugate 20** and excess folate (■).

FIG. 46 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 31** (●) and with **Conjugate 20** and excess folate (■).

FIG. 47 is a chart that shows the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 32** (●) and with **Conjugate 20** and excess folate (■).

FIG. 48A is a chart that shows that **Conjugate 17** dosed at 0.3 μmol/kg SIW (▼) {0,2,3}, decreased KB tumor size in test mice compared to untreated control (●) {0,0,0}.

FIG. 48B is a chart that shows % weight change for test mice dosed at 0.3 μmol/kg **Conjugate 17** (▼) compared to untreated control (●).

FIG. 49A is a chart that shows that **Conjugate 22** dosed at 0.3 μmol/kg SIW for two weeks (▲) {2,1,2} decreased KB tumor size in test mice compared to untreated control (●) {0,0,0}. The dotted line indicates the last dosing day.

FIG. 49B is a chart that shows % weight change for test mice dosed at 0.3 μmol/kg **Conjugate 22** SIW for two weeks (▲) compared to untreated control (●).

FIG. 50A is a chart that shows that **Conjugate 24** dosed at 0.3 μmol/kg SIW for two weeks (■) {0,0,5} decreased KB tumor size in test mice compared to untreated control (●) {0,0,0}. The dotted line indicates the last dosing day.

FIG. 50B is a chart that shows % weight change for test mice dosed at 0.3 μmol/kg **Conjugate 24** SIW for two weeks (■) compared to untreated control (●).

FIG. 51A is a chart that shows that **Conjugate 26** dosed at 0.3 μmol/kg SIW for two weeks (■) {3,0,2} decreased KB tumor size in test mice compared to untreated control (●) {0,0,0}. The dotted line indicates the last dosing day.

FIG. 51B is a chart that shows % weight change for test mice dosed at 0.3 μmol/kg **Conjugate 26** SIW for two weeks (■) compared to untreated control (●).

FIG. 52A is a chart that shows that **Conjugate 27** dosed at 0.3 μmol/kg SIW for two weeks (■) {1,4,0} decreased KB tumor size in test mice compared to untreated control (●) {0,0,0}. The dotted line indicates the last dosing day.

FIG. 52B is a chart that shows % weight change for test mice dosed at 0.3 μmol/kg **Conjugate 27** SIW for two weeks (■) compared to untreated control (●).

FIG. 53A is a chart that shows that **Conjugate 28** dosed at 0.3 μmol/kg SIW for two weeks (▲) {0,0,5} decreased KB tumor size in test mice compared to untreated control (●) {0,0,0}. The dotted line indicates the last dosing day.

FIG. 53B is a chart that shows % weight change for test mice dosed at 0.3 μmol/kg **Conjugate 28** SIW for two weeks (▲)compared to untreated control (●).

FIG. 54A is a chart that shows that **Conjugate 30** dosed at 0.3 μmol/kg SIW for two weeks (■) {0,0,3} decreased KB tumor size in test mice compared to untreated control (●) {0,0,0}. The dotted line indicates the last dosing day.

FIG. 54B is a chart that shows % weight change for test mice dosed at 0.3 μmol/kg **Conjugate 30** SIW for two weeks (■) compared to untreated control (●).

FIG. 55A is a chart that shows that **Conjugate 32** dosed at 0.3 μmol/kg SIW for two weeks (○) {0,5,0} decreased KB tumor size in test mice compared to untreated control (●) {0,0,0}. The dotted line indicates the last dosing day.

FIG. 55B is a chart that shows % weight change for test mice dosed at 0.3 μmol/kg **Conjugate 32** SIW for two weeks (○) compared to untreated control (●).

FIG. 56 is a chart showing a potent dose-dependent inhibition of cell proliferation with relative IC_{50} values of ~0.52 (72 h), 0.61 (96 h), and 0.17 (120 h) in ID8-CI15 ovarian cancer cells treated with **Conjugate 5**.

FIG. 57 is a graph showing that **Conjugate 5** demonstrated a potent activity at all concentrations tested (1 nM, 10 nM and 100 nM) after a 2 h exposure and 9-day chase. The anti-tumor activity of **Conjugate 5** was significantly reduced in the presence of excess amount of folic acid at both 1 nM and 10 nM concentrations.

FIG. 58 is a graph showing functional FR levels were measured on the IGROV1 human ovarian cancer cells: (a) hHLA+ CD45- ascites cancer cells [FR+ = 6.04%; (b) ascites F480+ CD11+ macs [FR+ = 52.6%]; (c) IGROV cell line control [FR+ = 98.5%].

FIG. 59A is chart showing the presence of CD4+ and CD8+ T cells quantitated in total peritoneal cells of the immunocompetent C57BL6 mice at 7 day intervals post IP injection of the mouse ovarian cell line, ID8-CL15 (FIG. 59A). The CD45+ CD3e+ CD8+ CD4- T cells (■) slowly increased in number from day 7 to day 42 post implantation. The CD45+ CD3e+ CD4+ CD8- T cells (▲) also increased in number from day 7 to day 35.

FIG. 59B is a chart showing CD45- non bone-marrow derived ascites cells from ID8-CL15 implanted mice expressed very little functional FR (see FIG. 59B (■)), whereas ascites macrophages expressed a significant amount of a functional FR (see FIG. 59B (●)).

FIG. 59C is a graph showing ascites macrophages expressed a significant amount of a functional FR.

FIG. 60A is a chart that shows that **Conjugate 5** dosed at 100 nmol/kg BIW, 6 doses, first dose at day 7 (▲) increased survival time in test mice compared to untreated control (●)

and anti-CTLA-5 alone dosed at 250 μg/dose BIW, 5 doses, and comparable to a significantly higher dose of comparator compound EC1456 (▼) 2000nmol/kg BIW, 6 doses, first dose at day 7. FIG. 60A also shows that **Conjugate 5** dosed with anti-CTLA-5, initiated at day 11, (○) increased survival time in test mice compared to all other test animals. The dotted line indicates the last dosing day.

FIG. 60B is a chart that shows % weight change for test mice dosed with **Conjugate 5** (▲),**Conjugate 5** + anti-CTLA-5 (■), EC1456 (▼) and anti-CTLA-5 (○) compared to untreated control (●).

DEFINITIONS

As used herein, the term "alkyl" includes a chain of carbon atoms, which is optionally branched and contains from 1 to 20 carbon atoms. It is to be further understood that in certain embodiments, alkyl may be advantageously of limited length, including C_1-C_{12}, C_1-C_{10}, C_1-C_9, C_1-C_8, C_1-C_7, C_1-C_6, and C_1-C_4, Illustratively, such particularly limited length alkyl groups, including C_1-C_8, C_1-C_7, C_1-C_6, and C_1-C_4, and the like may be referred to as "lower alkyl." Illustrative alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, 3-pentyl, neopentyl, hexyl, heptyl, octyl, and the like. Alkyl may be substituted or unsubstituted. Typical substituent groups include cycloalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, mercapto, alkylthio, arylthio, cyano, halo, carbonyl, oxo, (=O), thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, nitro, and amino, or as described in the various embodiments provided herein. It will be understood that "alkyl" may be combined with other groups, such as those provided above, to form a functionalized alkyl. By way of example, the combination of an "alkyl" group, as described herein, with a "carboxy" group may be referred to as a "carboxyalkyl" group. Other non-limiting examples include hydroxyalkyl, aminoalkyl, and the like.

As used herein, the term "alkenyl" includes a chain of carbon atoms, which is optionally branched, and contains from 2 to 20 carbon atoms, and also includes at least one carbon-carbon double bond (i.e. C=C). It will be understood that in certain embodiments, alkenyl may be advantageously of limited length, including C_2-C_{12}, C_2-C_9, C_2-C_8, C_2-C_7, C_2-C_6, and C_2-C_4. Illustratively, such particularly limited length alkenyl groups, including C_2-C_8, C_2-C_7, C_2-C_6, and C_2-C_4 may be referred to as lower alkenyl. Alkenyl may be unsubstituted, or substituted as described for alkyl or as described in the various embodiments provided herein. Illustrative

alkenyl groups include, but are not limited to, ethenyl, 1-propenyl, 2-propenyl, 1-, 2-, or 3-butenyl, and the like.

As used herein, the term "alkynyl" includes a chain of carbon atoms, which is optionally branched, and contains from 2 to 20 carbon atoms, and also includes at least one carbon-carbon triple bond (i.e. C≡C). It will be understood that in certain embodiments alkynyl may each be advantageously of limited length, including C_2-C_{12}, C_2-C_9, C_2-C_8, C_2-C_7, C_2-C_6, and C_2-C_4. Illustratively, such particularly limited length alkynyl groups, including C_2-C_8, C_2-C_7, C_2-C_6, and C_2-C_4 may be referred to as lower alkynyl. Alkenyl may be unsubstituted, or substituted as described for alkyl or as described in the various embodiments provided herein. Illustrative alkenyl groups include, but are not limited to, ethynyl, 1-propynyl, 2-propynyl, 1-, 2-, or 3-butynyl, and the like.

As used herein, the term "aryl" refers to an all-carbon monocyclic or fused-ring polycyclic groups of 6 to 12 carbon atoms having a completely conjugated pi-electron system. It will be understood that in certain embodiments, aryl may be advantageously of limited size such as C_6-C_{10} aryl. Illustrative aryl groups include, but are not limited to, phenyl, naphthalenyl and anthracenyl. The aryl group may be unsubstituted, or substituted as described for alkyl or as described in the various embodiments provided herein.

As used herein, the term "cycloalkyl" refers to a 3 to 15 member all-carbon monocyclic ring, an all-carbon 5-member/6-member or 6-member/6-member fused bicyclic ring, or a multicyclic fused ring (a "fused" ring system means that each ring in the system shares an adjacent pair of carbon atoms with each other ring in the system) group where one or more of the rings may contain one or more double bonds but the cycloalkyl does not contain a completely conjugated pi-electron system. It will be understood that in certain embodiments, cycloalkyl may be advantageously of limited size such as C_3-C_{13}, C_3-C_6, C_3-C_6 and C_4-C_6. Cycloalkyl may be unsubstituted, or substituted as described for alkyl or as described in the various embodiments provided herein. Illustrative cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclopentadienyl, cyclohexyl, cyclohexenyl, cycloheptyl, adamantyl, norbornyl, norbornenyl, 9*H*-fluoren-9-yl, and the like.

As used herein, the term "heterocycloalkyl" refers to a monocyclic or fused ring group having in the ring(s) from 3 to 12 ring atoms, in which at least one ring atom is a heteroatom, such as nitrogen, oxygen or sulfur, the remaining ring atoms being carbon atoms. Heterocycloalkyl may optionally contain 1, 2, 3 or 4 heteroatoms. Heterocycloalkyl may also have one of more double bonds, including double bonds to nitrogen (e.g. C=N or N=N) but does not contain a completely conjugated pi-electron system. It will be understood that in certain embodiments, heterocycloalkyl may be advantageously of limited size such as 3- to 7-

membered heterocycloalkyl, 5- to 7-membered heterocycloalkyl, and the like. Heterocycloalkyl may be unsubstituted, or substituted as described for alkyl or as described in the various embodiments provided herein. Illustrative heterocycloalkyl groups include, but are not limited to, oxiranyl, thianaryl, azetidinyl, oxetanyl, tetrahydrofuranyl, pyrrolidinyl, tetrahydropyranyl, piperidinyl, 1,4-dioxanyl, morpholinyl, 1,4-dithianyl, piperazinyl, oxepanyl, 3,4-dihydro-2H-pyranyl, 5,6-dihydro-2H-pyranyl, 2H-pyranyl, 1, 2, 3, 4-tetrahydropyridinyl, and the like.

As used herein, the term "heteroaryl" refers to a monocyclic or fused ring group of 5 to 12 ring atoms containing one, two, three or four ring heteroatoms selected from nitrogen, oxygen and sulfur, the remaining ring atoms being carbon atoms, and also having a completely conjugated pi-electron system. It will be understood that in certain embodiments, heteroaryl may be advantageously of limited size such as 3- to 7-membered heteroaryl, 5- to 7-membered heteroaryl, and the like. Heteroaryl may be unsubstituted, or substituted as described for alkyl or as described in the various embodiments provided herein. Illustrative heteroaryl groups include, but are not limited to, pyrrolyl, furanyl, thiophenyl, imidazolyl, oxazolyl, thiazolyl, pyrazolyl, pyridinyl, pyrimidinyl, quinolinyl, isoquinolinyl, purinyl, tetrazolyl, triazinyl, pyrazinyl, tetrazinyl, quinazolinyl, quinoxalinyl, thienyl, isoxazolyl, isothiazolyl, oxadiazolyl, thiadiazolyl, triazolyl, benzimidazolyl, benzoxazolyl, benzthiazolyl, benzisoxazolyl, benzisothiazolyl and carbazoloyl, and the like.

As used herein, "hydroxy" or ""hydroxyl" refers to an -OH group.

As used herein, "alkoxy" refers to both an -O-(alkyl) or an -O-(unsubstituted cycloalkyl) group. Representative examples include, but are not limited to, methoxy, ethoxy, propoxy, butoxy, cyclopropyloxy, cyclobutyloxy, cyclopentyloxy, cyclohexyloxy, and the like.

As used herein, "aryloxy" refers to an -O-aryl or an -O-heteroaryl group. Representative examples include, but are not limited to, phenoxy, pyridinyloxy, furanyloxy, thienyloxy, pyrimidinyloxy, pyrazinyloxy, and the like, and the like.

As used herein, "mercapto" refers to an -SH group.

As used herein, "alkylthio" refers to an -S-(alkyl) or an -S-(unsubstituted cycloalkyl) group. Representative examples include, but are not limited to, methylthio, ethylthio, propylthio, butylthio, cyclopropylthio, cyclobutylthio, cyclopentylthio, cyclohexylthio, and the like.

As used herein, "arylthio" refers to an -S-aryl or an -S-heteroaryl group. Representative examples include, but are not limited to, phenylthio, pyridinylthio, furanylthio, thienylthio, pyrimidinylthio, and the like.

As used herein, "halo" or "halogen" refers to fluorine, chlorine, bromine or iodine.

As used herein, “trihalomethyl” refers to a methyl group having three halo substituents, such as a trifluoromethyl group.

As used herein, “cyano” refers to a -CN group.

As used herein, “sulfinyl” refers to a -S(O)R" group, where R" is any R group as described in the various embodiments provided herein, or R" may be a hydroxyl group.

As used herein, “sulfonyl” refers to a -$S(O)_2$R" group, where R" is any R group as described in the various embodiments provided herein, or R" may be a hydroxyl group.

As used herein, “S-sulfonamido” refers to a -$S(O)_2$NR"R" group, where R" is any R group as described in the various embodiments provided herein.

As used herein, “N-sulfonamido” refers to a -NR"$S(O)_2$R" group, where R" is any R group as described in the various embodiments provided herein.

As used herein, “O-carbamyl” refers to a -OC(O)NR"R" group, where R" is any R group as described in the various embodiments provided herein.

As used herein, “N-carbamyl” refers to an R"OC(O)NR"- group, where R" is any R group as described in the various embodiments provided herein.

As used herein, “O-thiocarbamyl” refers to a -OC(S)NR"R" group, where R" is any R group as described in the various embodiments provided herein.

As used herein, “N-thiocarbamyl” refers to a R"OC(S)NR"- group, where R" is any R group as described in the various embodiments provided herein.

As used herein, “amino” refers to an -NR"R" group, where R" is any R group as described in the various embodiments provided herein.

As used herein, “C-amido” refers to a -C(O)NR"R" group, where R" is any R group as described in the various embodiments provided herein.

As used herein, “N-amido” refers to a R"C(O)NR"- group, where R" is any R group as described in the various embodiments provided herein.

As used herein, “nitro” refers to a –NO_2 group.

As used herein, “bond” refers to a covalent bond.

As used herein, “optional” or “optionally” means that the subsequently described event or circumstance may but need not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. For example, “heterocycle group optionally substituted with an alkyl group” means that the alkyl may but need not be present, and the description includes situations where the heterocycle group is substituted with an alkyl group and situations where the heterocycle group is not substituted with the alkyl group.

As used herein, “independently” means that the subsequently described event or circumstance is to be read on its own relative to other similar events or circumstances. For example, in a circumstance where several equivalent hydrogen groups are optionally substituted by another group described in the circumstance, the use of “independently optionally” means that each instance of a hydrogen atom on the group may be substituted by another group, where the groups replacing each of the hydrogen atoms may be the same or different. Or for example, where multiple groups exist all of which can be selected from a set of possibilities, the use of “independently” means that each of the groups can be selected from the set of possibilities separate from any other group, and the groups selected in the circumstance may be the same or different.

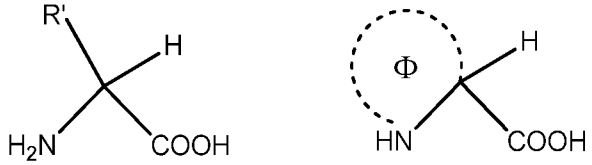
As used herein, the term “pharmaceutically acceptable salt” refers to those salts which counter ions which may be used in pharmaceuticals. Such salts include:

(1) acid addition salts, which can be obtained by reaction of the free base of the parent conjugate with inorganic acids such as hydrochloric acid, hydrobromic acid, nitric acid, phosphoric acid, sulfuric acid, and perchloric acid and the like, or with organic acids such as acetic acid, oxalic acid, (D) or (L) malic acid, maleic acid, methane sulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, tartaric acid, citric acid, succinic acid or malonic acid and the like; or

(2) salts formed when an acidic proton present in the parent conjugate either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, trimethamine, N-methylglucamine, and the like.

Pharmaceutically acceptable salts are well known to those skilled in the art, and any such pharmaceutically acceptable salt may be contemplated in connection with the embodiments described herein

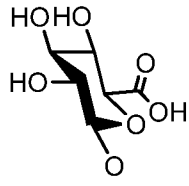
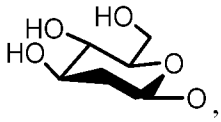
As used herein, "amino acid" (a.k.a. “AA”) means any molecule that includes an alpha-carbon atom covalently bonded to an amino group and an acid group. The acid group may include a carboxyl group. "Amino acid" may include molecules having one of the formulas:



wherein R’ is a side group and Φ includes at least 3 carbon atoms. "Amino acid" includes stereoisomers such as the D-amino acid and L-amino acid forms. Illustrative amino acid groups include, but are not limited to, the twenty endogenous human amino acids and their derivatives,

such as lysine (Lys), asparagine (Asn), threonine (Thr), serine (Ser), isoleucine (Ile), methionine (Met), proline (Pro), histidine (His), glutamine (Gln), arginine (Arg), glycine (Gly), aspartic acid (Asp), glutamic acid (Glu), alanine (Ala), valine (Val), phenylalanine (Phe), leucine (Leu), tyrosine (Tyr), cysteine (Cys), tryptophan (Trp), phosphoserine (PSER), sulfo-cysteine, arginosuccinic acid (ASA), hydroxyproline, phosphoethanolamine (PEA), sarcosine (SARC), taurine (TAU), carnosine (CARN), citrulline (CIT), anserine (ANS), 1,3-methyl-histidine (ME-HIS), alpha-amino-adipic acid (AAA), beta- alanine (BALA), ethanolamine (ETN), gamma-amino-butyric acid (GABA), beta-amino- isobutyric acid (BAIA), alpha-amino-butyric acid (BABA), L-allo-cystathionine (cystathionine- A; CYSTA-A), L-cystathionine (cystathionine-B; CYSTA-B), cystine, allo-isoleucine (ALLO- ILE), DL-hydroxylysine (hydroxylysine (I)), DL-allo-hydroxylysine (hydroxylysine (2)), ornithine (ORN), homocystine (HCY), and derivatives thereof. It will be appreciated that each of these examples are also contemplated in connection with the present disclosure in the D-configuration as noted above. Specifically, for example, D-lysine (D-Lys), D-asparagine (D-Asn), D-threonine (D-Thr), D-serine (D-Ser), D-isoleucine (D-Ile), D-methionine (D-Met), D-proline (D-Pro), D-histidine (D-His), D-glutamine (D-Gln), D-arginine (D-Arg), D-glycine (D-Gly), D-aspartic acid (D-Asp), D-glutamic acid (D-Glu), D-alanine (D-Ala), D-valine (D-Val), D-phenylalanine (D-Phe), D-leucine (D-Leu), D-tyrosine (D-Tyr), D-cysteine (D-Cys), D-tryptophan (D-Trp), D-citrulline (D-CIT), D-carnosine (D-CARN), and the like. In connection with the embodiments described herein, amino acids can be covalently attached to other portions of the conjugates described herein through their alpha-amino and carboxy functional groups (i.e. in a peptide bond configuration), or through their side chain functional groups (such as the side chain carboxy group in glutamic acid) and either their alpha-amino or carboxy functional groups. It will be understood that amino acids, when used in connection with the conjugates described herein, may exist as zwitterions in a conjugate in which they are incorporated.

As used herein, "sugar" refers to carbohydrates, such as monosaccharides, disaccharides, or oligosaccharides. In connection with the present disclosure, monosaccharides are preferred. Non-limiting examples of sugars include erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, mannose, galactose, ribulose, fructose, sorbose, tagatose, and the like. It will be undertsood that as used in connection with the present disclosure, sugar includes cyclic isomers of amino sugars, deoxy sugars, acidic sugars, and combinations thereof. Non-limiting examples of such sugars include, galactosamine, glucosamine, deoxyribose, fucose, rhamnose, glucuronic acid, ascorbic acid, and the like. In some embodiments, sugars for use in connection with the present disclosure include

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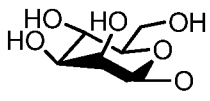


As used herein, "prodrug" refers to a compound that can be administered to a subject in a pharmacologically inactive form which then can be converted to a pharmacologically active form through a normal metabolic process, such as hydrolysis of an oxazolidine. It will be understood that the metabolic processes through which a prodrug can be converted to an active drug include, but are not limited to, one or more spontaneous chemical reaction(s), enzyme-catalyzed chemical reaction(s), and/or other metabolic chemical reaction(s), or a combination thereof. It will be appreciated that understood that a variety of metabolic processes are known in the art, and the metabolic processes through which the prodrugs described herein are converted to active drugs are non-limiting. A prodrug can be a precursor chemical compound of a drug that has a therapeutic effect on a subject.

As used herein, the term "releasable group" refers to a bond or bonds that can be broken ("a cleavable bond" or "cleavable bonds") under physiological conditions, such as a pH-labile, acid-labile, base-labile, oxidatively labile, metabolically labile, biochemically labile, or enzyme-labile bond. It will be appreciated that such physiological conditions resulting in bond breaking do not necessarily include a biological or metabolic process, and instead may include a standard chemical reaction, such as a hydrolysis reaction, for example, at physiological pH, or as a result of compartmentalization into a cellular organelle such as an endosome having a lower pH than cytosolic pH.

It will be appreciated that a releasable group can connect two adjacent atoms within a releasable linker and/or connect other linkers (e.g. AA, L^1, L^2, L^3, etc), B and/or D, as described herein. Alternatively, a releasable group can form part of a drug or a prodrug, D, and/or connect a drug or pro-drug, D, to other linkers (e.g. AA, L^1, L^2, L^3, etc), B and/or D, as described herein. In the case where a releasable group connects two adjacent atoms within a releasable linker, following breakage of the cleavable bond, such releasable linker is broken into two or more fragments. Alternatively, in the case where a releaseable group connects a linker (e.g. AA, L^1, L^2, L^3, etc) to another moiety, such as another linker, a drug or binding ligand, then such releasable linker becomes separated from such other moiety following breaking of the cleavable bond or cleavable bonds. Alternatively, in the case where a releaseable group is within a drug or prodrug, D, that is connected to a linker, another drug or a binding ligand, then following breaking of the cleavable bond or cleavable bonds, such linker, drug or binding ligand becomes separated from such drug or prodrug having the releaseable group within.

The lability of the releasable group can be adjusted by, for example, substituents at or near the cleavable bond, such as including alpha-branching adjacent to a cleavable disulfide bond, increasing the hydrophobicity of substituents on silicon in a moiety having silicon-oxygen bond that may be hydrolyzed, homologating alkoxy groups that form part of a ketal or acetal that may be hydrolyzed, and the like.

As used herein, the term "therapeutically effective amount" refers to an amount of a drug or pharmaceutical agent that elicits the biological or medicinal response in a subject (i.e. a tissue system, animal or human) that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes, but is not limited to, alleviation of the symptoms of the disease or disorder being treated. In one aspect, the therapeutically effective amount is that amount of an active which may treat or alleviate the disease or symptoms of the disease at a reasonable benefit/risk ratio applicable to any medical treatment. In another aspect, the therapeutically effective amount is that amount of an inactive prodrug which when converted through normal metabolic processes to produce an amount of active drug capable of eliciting the biological or medicinal response in a subject that is being sought.

It is also appreciated that the dose, whether referring to monotherapy or combination therapy, is advantageously selected with reference to any toxicity, or other undesirable side effect, that might occur during administration of one or more of the conjugates described herein. Further, it is appreciated that the co-therapies described herein may allow for the administration of lower doses of conjugates that show such toxicity, or other undesirable side effect, where those lower doses are below thresholds of toxicity or lower in the therapeutic window than would otherwise be administered in the absence of a cotherapy.

As used herein, "administering" includes all means of introducing the conjugates and compositions described herein to the host animal, including, but are not limited to, oral (po), intravenous (iv), intramuscular (im), subcutaneous (sc), transdermal, inhalation, buccal, ocular, sublingual, vaginal, rectal, and the like. The conjugates and compositions described herein may be administered in unit dosage forms and/or formulations containing conventional nontoxic pharmaceutically-acceptable carriers, adjuvants, and/or vehicles.

As used herein "pharmaceutical composition" or "composition" refers to a mixture of one or more of the conjugates described herein, or pharmaceutically acceptable salts, solvates, hydrates thereof, with other chemical components, such as pharmaceutically acceptable excipients. The purpose of a pharmaceutical composition is to facilitate administration of a conjugate to a subject. Pharmaceutical compositions suitable for the delivery of conjugates described and methods for their preparation will be readily apparent to those skilled in the art.

Such compositions and methods for their preparation may be found, for example, in 'Remington's Pharmaceutical Sciences', 19th Edition (Mack Publishing Company, 1995).

A "pharmaceutically acceptable excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a conjugate such as a diluent or a carrier.

DETAILED DESCRIPTION

In each of the foregoing and each of the following embodiments, it is to be understood that the formulae include and represent not only all pharmaceutically acceptable salts of the conjugates, but also include any and all hydrates and/or solvates of the conjugate formulae. It is appreciated that certain functional groups, such as the hydroxy, amino, and like groups form complexes and/or coordination conjugates with water and/or various solvents, in the various physical forms of the conjugates. Accordingly, the above formulae are to be understood to include and represent those various hydrates and/or solvates. It is also to be understood that the non-hydrates and/or non-solvates of the conjugate formulae are described by such formula, as well as the hydrates and/or solvates of the conjugate formulae.

The conjugates described herein can be expressed by the generalized descriptors B, L and D, where B is a cell surface receptor binding ligand (a.k.a. a "binding ligand"), L is a linker that may include a releasable group, L can be described by one or more of the linker groups AA, L^1, L^2, L^3, or L^r as defined herein, and D represents one or more drugs (D^1 and D^2). In the embodiments described herein, it will be appreciated that B is covalently attached to a linker (L) that comprises one or more (for example from 1 to 20) linker from one or more linker groups AA, L^1, L^2, L^3, or L^r, which linker (L) is covalently attached to one or more drugs (D^1 or D^2), and when the conjugate contauins two drugs D^1 or D^2, the drugs D^1 and D^2 can be covalently attached to one another by one or more of AA, L^1, L^2 and L^3, provided that one of D^1 or D^2 in the conjugate is a PBD drug.

The conjugates described herein in connection with embodiment 1 can be described by various general structures including but not limited to $B-(L^1)_{z1}-(AA)_{z2}-(L^1)_{z3}-(AA)_{z4}-(L^1)_{z5}-(AA)_{z6}-(L^2)_{z7}-(L^r)_{z8}-(L^2)_{z9}-D-L^3-D-(L^2)_{y9}-(L^r)_{y8}-(L^2)_{y7}-(AA)_{y6}-(L^1)_{y5}-(AA)_{y4}-(L^1)_{y3}-(AA)_{y2}-(L^1)_{y1}-X$, wherein z1 is an integer from 0 to 2, z2 is an integer from 0 to 3, z3 is an integer from 0 to 2, z4 is an integer from 0 to 3, z5 is an integer from 0 to 2, z6 is an integer from 0 to 3, z7 is an integer from 0 to 8, z8 is 1, z9 is an integer from 0 to 8, y1 is an integer from 0 to 2, y2 is an integer from 0 to 3, y3 is an integer from 0 to 2, y4 is an integer from 0 to 3, y5 is an integer from 0 to 2, y6 is 0 or 1, y7 is an integer from 0 to 8, y8 is 0 or 1; y9 is an integer from 0 to 8; each D is independently D^1 or D^2; X is H or B; each B is independently a binding ligand; each

AA is independently an amino acid; each L^1 is independently a first spacer linker; each L^2 is independently a second spacer linker; each L^3 is independently a third spacer linker; and each L^r is independently a releasable linker. The conjugates described herein can also be described by any of the formulae

$B-(AA)_{z2}-(L^2)_{z7}-L^r-D^1-L^3-D^2$,

$B-(AA)_4-(L^2)_4-L^r-D^1-L^3-D^2$,

$B-(AA)_4-(L^2)_5-L^r-D^1-L^3-D^2$,

$B-(AA)_5-(L^2)_4-L^r-D^1-L^3-D^2$,

$B-(AA)_5-(L^2)_5-L^r-D^1-L^3-D^2$,

$B-(AA)_4-L^r-D^1-L^3-D^2$,

$B-(L^1)_{z1}-(AA)_{z2}-(L^1)_{z3}-(AA)_{z4}-(L^1)_{z5}-(L^2)_{z7}-L^r-D^1-L^3-D^2$,

$B-(L^1)_{z1}-(AA)_{z2}-(L^1)_{z3}-(AA)_{z4}-(L^1)_{z5}-(AA)_{z6}-(L^2)_{z7}-L^r-D^1-L^3-D^2$,

$B-L^1-AA-L^1-AA-L^1-(L^2)_{z7}-L^r-D^1-L^3-D^2$,

$B-L^1-AA-L^1-AA-L^1-AA-(L^2)_{z7}-L^r-D^1-L^3-D^2$,

$B-L^1-AA-L^1-AA-L^1-(L^2)_4-L^r-D^1-L^3-D^2$,

$B-L^1-AA-L^1-AA-L^1-(L^2)_5-L^r-D^1-L^3-D^2$,

$B-L^1-AA-L^1-AA-L^1-AA-(L^2)_3-L^r-D^1-L^3-D^2$,

$B-(AA)_{z2}-(L^2)_{z7}-(L^r)_{z8}-D-L^3-D-L^r-(L^2)_{y7}-(AA)_{y2}-B$,

$B-L^1-AA-L^1-AA-L^1-(L^2)_{z7}-(L^r)_{z8}-D-L^3-D-L^r-(L^2)_{y7}-L^1-AA-L^1-AA-L^1-B$ and

$B-L^1-AA-L^1-AA-L^1-AA-(L^2)_{z7}-(L^r)_{z8}-D-L^3-D-L^r-(L^2)_{y7}-AA-L^1-AA-L^1-AA-L^1-B$,

wherein B, AA, L^1, L^2, L^3, L^r, D^1, D^2 z1, z2, z3, z4, z5, z6, z7 and y7 are as defined herein.

The conjugates described herein in connection with embodiment 2 can be described by various general structures including but not limited to $B-(L^1)_{z1}-(AA)_{z2}-(L^1)_{z3}-(AA)_{z4}-(L^1)_{z5}-(AA)_{z6}-(L^2)_{z7}-(L^r)_{z8}-(L^2)_{z9}-D-L^3-D-(L^2)_{y9}-(L^r)_{y8}-(L^2)_{y7}-(AA)_{y6}-(L^1)_{y5}-(AA)_{y4}-(L^1)_{y3}-(AA)_{y2}-(L^1)_{y1}-X$, wherein z1 is an integer from 0 to 2, z2 is an integer from 0 to 3, z3 is an integer from 0 to 2, z4 is an integer from 0 to 3, z5 is an integer from 0 to 2, z6 is an integer from 0 to 3, z7 is an integer from 0 to 8, z8 is 0 or 1, z9 is an integer from 0 to 8, y1 is an integer from 0 to 2, y2 is an integer from 0 to 3, y3 is an integer from 0 to 2, y4 is an integer from 0 to 3, y5 is an integer from 0 to 2, y6 is 0 or 1, y7 is an integer from 0 to 8, y8 is 0 or 1; y9 is an integer from 0 to 8; each D is independently D^1 or D^2; X is H or B; each B is independently a binding ligand; each AA is independently an amino acid; each L^1 is independently a first spacer linker; each L^2 is independently a second spacer linker; each L^3 is independently a third spacer linker; and each L^r is independently a releasable linker. The conjugates described herein can also be described by any of the formulae

$B-(AA)_{z2}-(AA)_{z4}-(L^2)_{z7}-L^r-D^1-L^3-D^2$,

$B\text{-}(AA)_{z2}\text{-}(AA)_{z4}\text{-}(L^2)_{z7}\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_4\text{-}(L^2)_4\text{-}L^r\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_4\text{-}(L^2)_5\text{-}L^r\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_4\text{-}(L^2)_7\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_4\text{-}(L^2)_6\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_5\text{-}(L^2)_4\text{-}L^r\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_5\text{-}(L^2)_5\text{-}L^r\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_5\text{-}(L^2)_7\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_5\text{-}(L^2)_6\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_4\text{-}L^r\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_5\text{-}L^2\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(L^1)_{z1}\text{-}(AA)_{z2}\text{-}(L^1)_{z3}\text{-}(AA)_{z4}\text{-}(L^1)_{z5}\text{-}(L^2)_{z7}\text{-}(L^r)_{z8}\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(L^1)_{z1}\text{-}(AA)_{z2}\text{-}(L^1)_{z3}\text{-}(AA)_{z4}\text{-}(L^1)_{z5}\text{-}(AA)_{z6}\text{-}(L^2)_{z7}\text{-}(L^r)_{z8}\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}(L^2)_{z7}\text{-}(L^r)_{z8}\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}(L^2)_{z7}\text{-}(L^r)_{z8}\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}(L^2)_4\text{-}L^r\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}(L^2)_5\text{-}L^r\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}(L^2)_6\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}(L^2)_7\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}(L^2)_3\text{-}L^r\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}(L^2)_5\text{-}D^1\text{-}L^3\text{-}D^2$,

$B\text{-}(AA)_{z2}\text{-}(AA)_{z4}\text{-}(L^2)_{z7}\text{-}(L^r)_{z8}\text{-}D\text{-}L^3\text{-}D\text{-}(L^r)_{y8}\text{-}(L^2)_{y7}\text{-}(AA)_{y2}\text{-}B$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}(L^2)_{z7}\text{-}(L^r)_{z8}\text{-}D\text{-}L^3\text{-}D\text{-}(L^r)_{y8}\text{-}(L^2)_{y7}\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}B$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}(L^2)_{z7}\text{-}(L^r)_{z8}\text{-}D\text{-}L^3\text{-}D\text{-}(L^r)_{y8}\text{-}(L^2)_{y7}\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}B$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}(L^2)_{z7}\text{-}D\text{-}L^3\text{-}D\text{-}(L^2)_{y7}\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}B$,

$B\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}(L^2)_{z7}\text{-}D\text{-}L^3\text{-}D\text{-}(L^2)_{y7}\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}AA\text{-}L^1\text{-}B$,

wherein B, AA, L^1, L^2, L^3, L^r, D^1, D^2 z1, z2, z3, z4, z5, z6, z7, z8, y7 and y8 are as defined herein.

The Binding Ligand

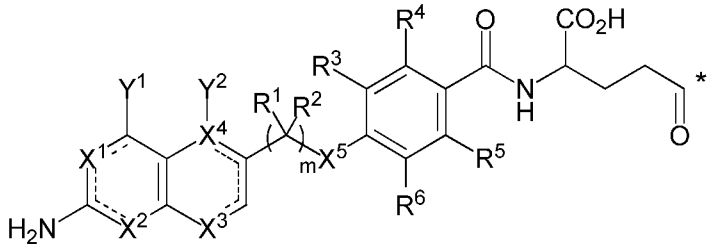
It will be appreciated that any of the descriptions of binding ligands provided herein can be used independently in connection with either embodiment 1 or embodiment 2. Specifically, neither embodiment 1 nor embodiment 2 requires any particular restriction on the identity of the binding ligand.

As used herein, the term cell surface receptor binding ligand (aka a “binding ligand”), generally refers to compounds that bind to and/or target receptors that are found on cell

surfaces, and in particular those that are found on, over-expressed by, and/or preferentially expressed on the surface of pathogenic cells. Illustrative ligands include, but are not limited to, vitamins and vitamin receptor binding compounds.

Illustrative vitamin moieties include carnitine, inositol, lipoic acid, pyridoxal, ascorbic acid, niacin, pantothenic acid, folic acid, riboflavin, thiamine, biotin, vitamin B_{12}, and the lipid soluble vitamins A, D, E and K. These vitamins, and their receptor-binding analogs and derivatives, constitute the targeting entity covalently attachment to the linker. Illustrative biotin analogs that bind to biotin receptors include, but are not limited to, biocytin, biotin sulfoxide, oxybiotin, and the like).

In some embodiments, the B is folate or derivative thereof. In some embodiments, the B is of the formula I



wherein

R^1 and R^2 in each instance are independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -OR^7, -SR^7 and -$NR^7R^{7'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl is independently optionally substituted by halogen, –OR^8, -SR^8, -$NR^8R^{8'}$, -C(O)R^8, -C(O)OR^8 or -C(O)$NR^8R^{8'}$;

R^3, R^4, R^5 and R^6 are each independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -CN, -NO_2, -NCO, -OR^9, -SR^9, –$NR^9R^{9'}$, -C(O)R^9, -C(O)OR^9 and -C(O)$NR^9R^{9'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl is independently optionally substituted by halogen, –OR^{10}, -SR^{10}, -$NR^{10}R^{10'}$, -C(O)R^{10}, -C(O)OR^{10} or -C(O)$NR^{10}R^{10'}$;

each R^7, $R^{7'}$, R^8, $R^{8'}$, R^9, $R^{9'}$, R^{10} and $R^{10'}$ is independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl or C_2-C_6 alkynyl;

X^1 is –NR^{11}-, =N-, -N=, -C(R^{11})= or =C(R^{11})-;

X^2 is –$NR^{11'}$- or =N-;

X^3 is –$NR^{11''}$-, -N= or -C($R^{11'}$)=;

X^4 is –N= or –C=;

X^5 is NR^{12} or $CR^{12}R^{12'}$;

Y^1 is H,–OR^{13}, –SR^{13} or –$NR^{13}R^{13'}$ when X^1 is -N= or -C(R^{11})=, or Y^1 is =O when X^1 is -NR^{11}-, =N- or =C(R^{11})-;

Y^2 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, -C(O)R^{14}, -C(O)OR^{14}, -C(O)$NR^{14}R^{14'}$ when X^4 is -C=, or Y^2 is absent when X^4 is –N=;

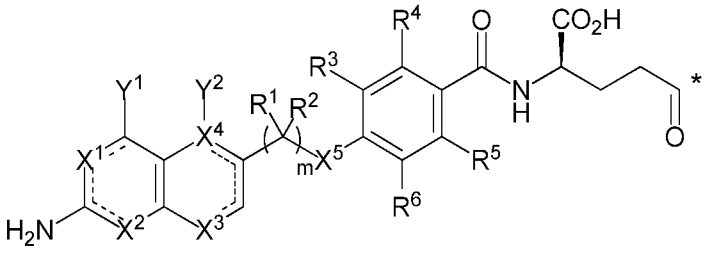
R^{11}, $R^{11'}$, $R^{11''}$, R^{12}, $R^{12'}$, R^{13}, $R^{13'}$, R^{14} and $R^{14'}$ are each independently selected from the group consisting of H, C_1-C_6 alkyl, -C(O)R^{15}, -C(O)OR^{15} and -C(O)$NR^{15}R^{15'}$;

R^{15} and $R^{15'}$ are each independently H or C_1-C_6 alkyl;

m is 1, 2, 3 or 4; and

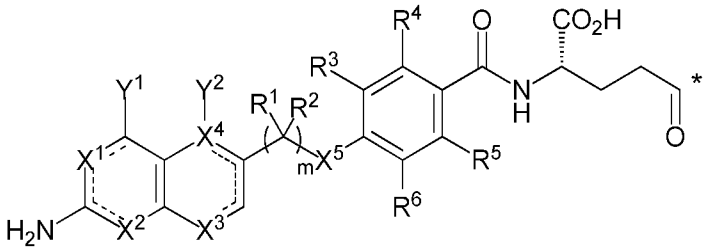
* is a covalent bond to the rest of the conjugate.

It will be appreciate that when B is described according to the formula I, that both the D- and L- forms are contemplated. In some embodiments, B is of the formula Ia or Ib



or

Ia

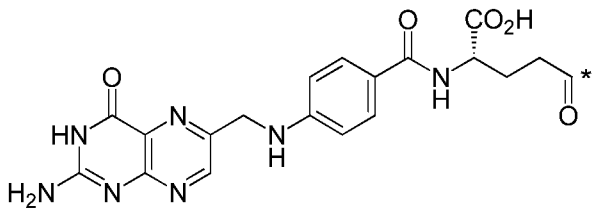


Ib

where each of R^1, R^2, R^3, R^4, R^5, R^6, Y^1, Y^2, X^1, X^2, X^3, X^4, X^5, m and * are as defined for the formula I.

In some embodiments described herein, R^1 and R^2 are H. In some embodiments described herein, m is 1. In some embodiments described herein, R^3 is H. In some embodiments described herein, R^4 is H. In some embodiments described herein, R^5 is H. In some embodiments described herein, R^6 is H. In some embodiments described herein, R^3, R^4, R^5 and R^6 are H. In some embodiments described herein, X^1 is –NR^{11}, and R^{11} is H. In some embodiments described herein, X^2 is =N-. In some embodiments described herein, X^3 is –N=. In some embodiments described herein, X^4 is –N=. In some embodiments described herein, X^1 is –NR^{11}, and R^{11} is H; X^2 is =N-; X^3 is –N=; and X^4 is –N=. In some embodiments described herein, X^5 is NR^{12}, and R^{12} is H. In some embodiments, Y^1 is =O. In some embodiments, Y^2 is

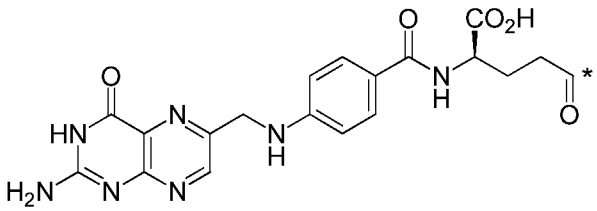
absent. In some embodiments, B is of the formula Ic



Ic

wherein * is defined for formula I.

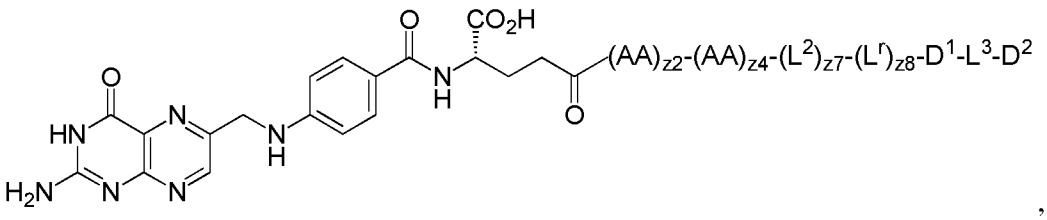
In some embodiments, B is of the formula Id



Id

wherein * is defined for formula I.

It will be appreciated that in certain embodiments, the conjugates described herein can be represented by the exemplary formulae



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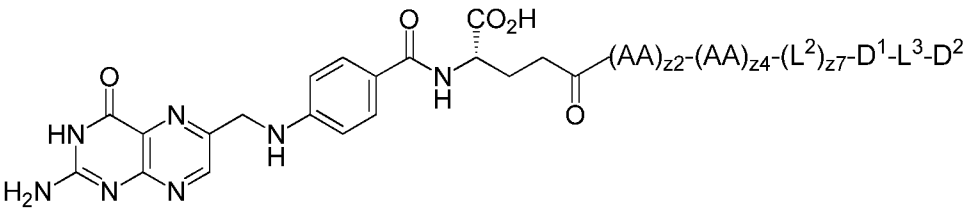

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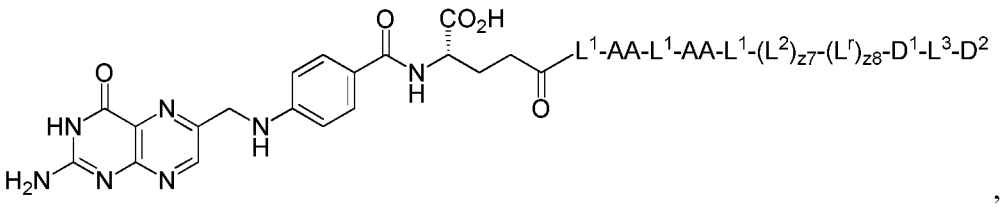

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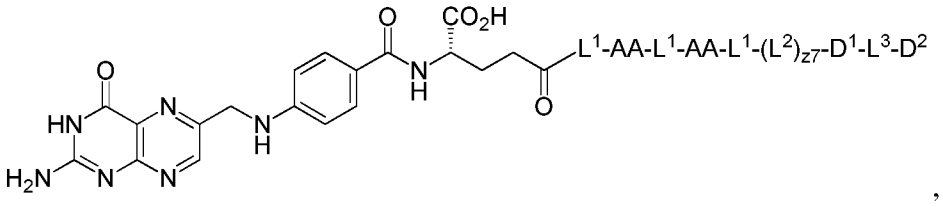

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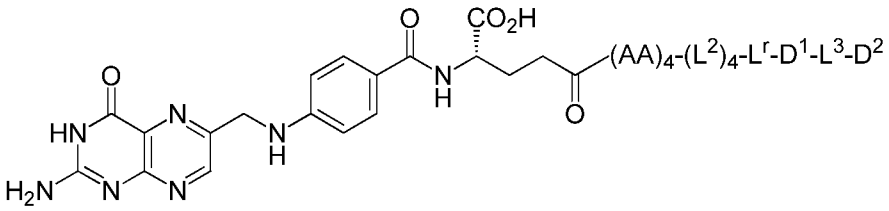
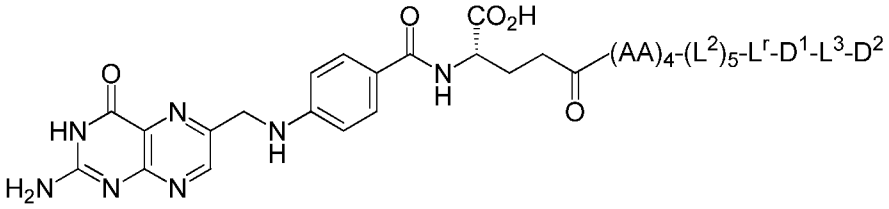
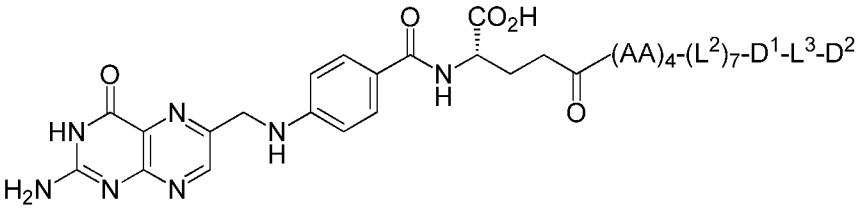

O
CO2H
(AA)4-(L2)4-Lr-D1-L3-D2
HN
H2N
N
N
N
N
H

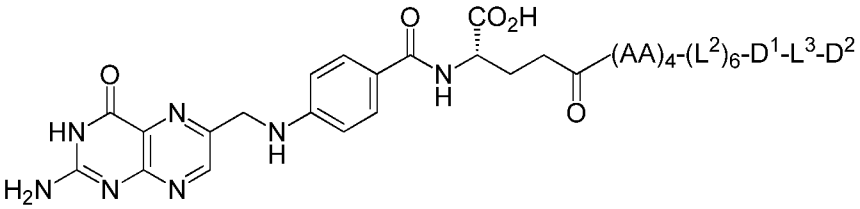
,


O
CO2H
(AA)4-(L2)5-Lr-D1-L3-D2
HN
H2N
N
N
N
N
H

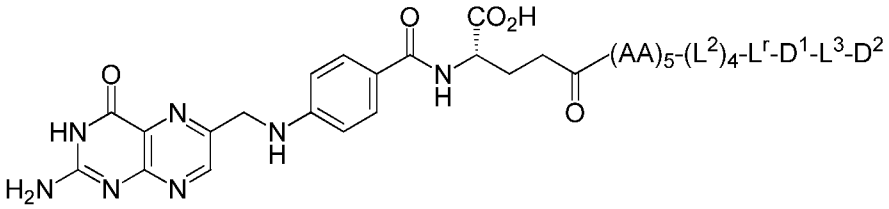
,


O
CO2H
(AA)4-(L2)7-D1-L3-D2
HN
H2N
N
N
N
N
H

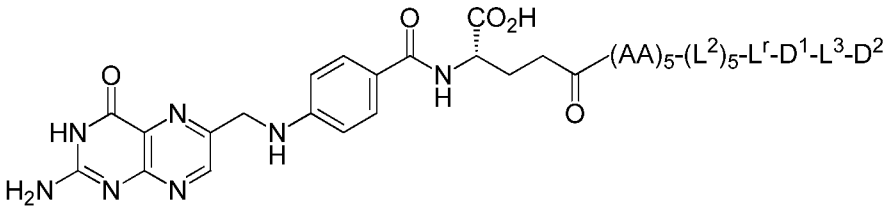
,


O
CO2H
(AA)4-(L2)6-D1-L3-D2
HN
H2N
N
N
N
N
H

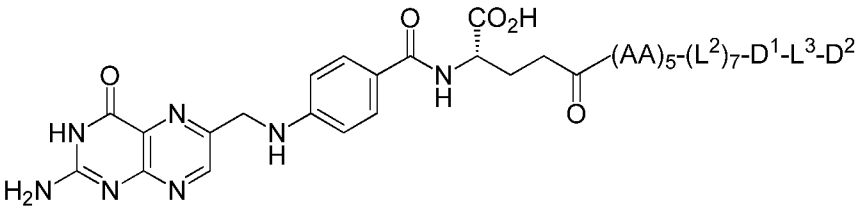
,


O
CO2H
(AA)5-(L2)4-Lr-D1-L3-D2
HN
H2N
N
N
N
N
H

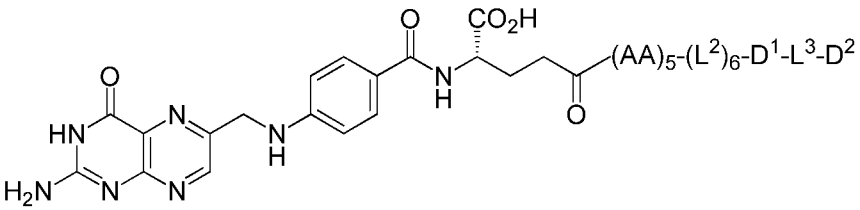
,


O
CO2H
(AA)5-(L2)5-Lr-D1-L3-D2
HN
H2N
N
N
N
N
H

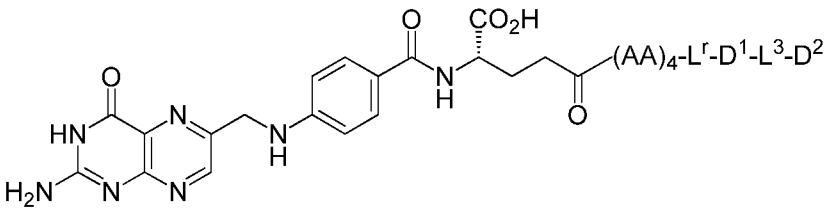
,


O
CO2H
(AA)5-(L2)7-D1-L3-D2
HN
H2N
N
N
N
N
H

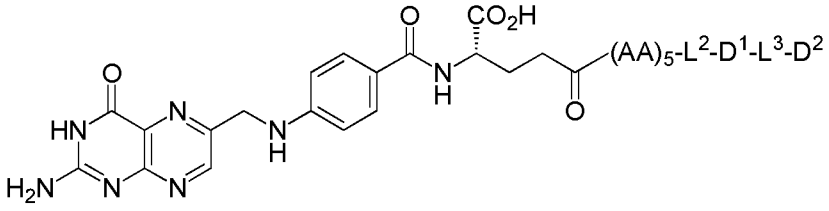
,


O
CO2H
(AA)5-(L2)6-D1-L3-D2
HN
H2N
N
N
N
N
H

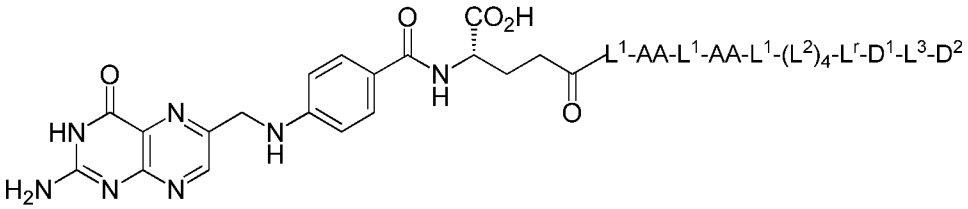
,


O
CO2H
(AA)4-Lr-D1-L3-D2
O
N
H
O
HN
N
N
H
H2N
N
N

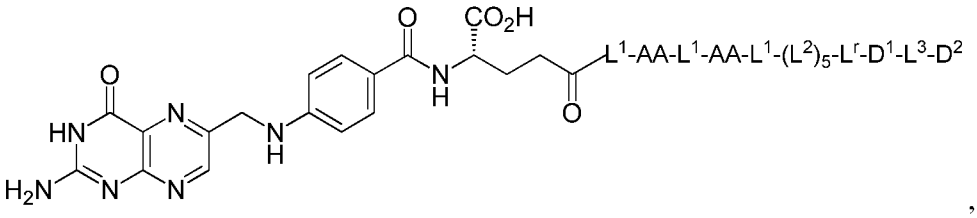
,


O
CO2H
(AA)5-L2-D1-L3-D2
O
N
H
O
HN
N
N
H
H2N
N
N

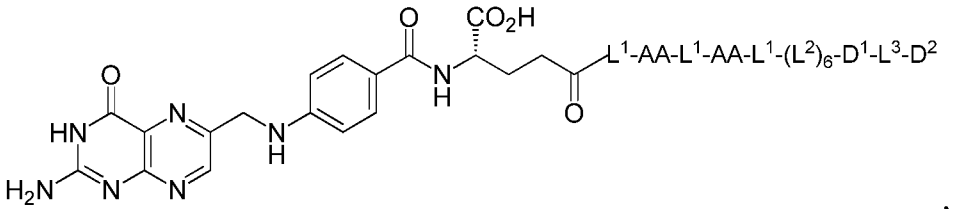
,


O
CO2H
L1-AA-L1-AA-L1-(L2)4-Lr-D1-L3-D2
O
N
H
O
HN
N
N
H
H2N
N
N

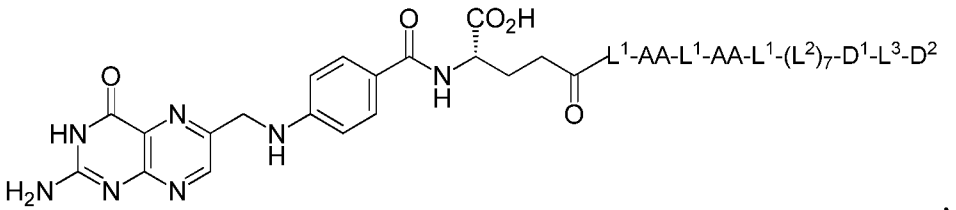
5 ,


O
CO2H
L1-AA-L1-AA-L1-(L2)5-Lr-D1-L3-D2
O
N
H
O
HN
N
N
H
H2N
N
N

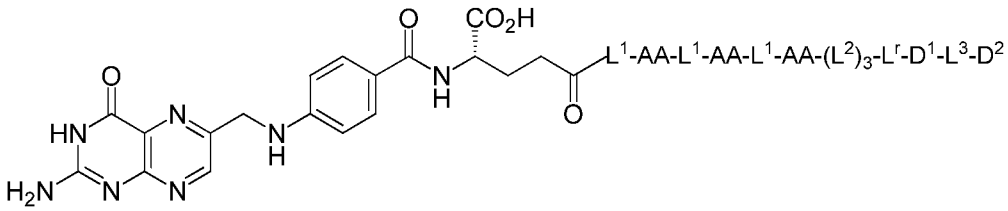
,


O
CO2H
L1-AA-L1-AA-L1-(L2)6-D1-L3-D2
O
N
H
O
HN
N
N
H
H2N
N
N

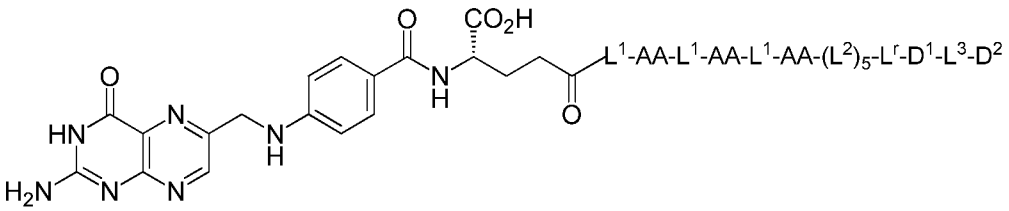
,


O
CO2H
L1-AA-L1-AA-L1-(L2)7-D1-L3-D2
O
N
H
O
HN
N
N
H
H2N
N
N

,


O
CO2H
L1-AA-L1-AA-L1-AA-(L2)3-Lr-D1-L3-D2
O
N
H
O
HN
N
N
H
H2N
N
N

or

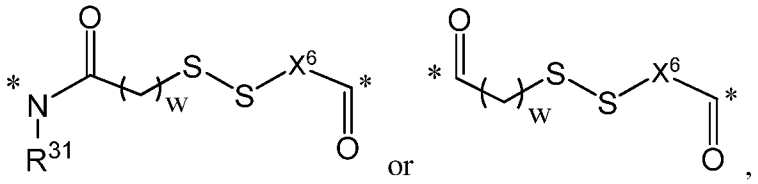


or a pharmaceutically acceptable salt thereof.

The Linker (L)

The linker (L) for connecting B, D^1 and or D^2, in the conjugates described herein can be any linker as defined herein comprising one or more of groups AA, L^1, L^2, L^3, and/or L^r.

It will be appreciated that any of the descriptions of linkers AA, L^1, L^2 and L^3 provided herein can be used independently in connection with either embodiment 1 or embodiment 2. Specifically, neither embodiment 1 nor embodiment 2 requires any particular restriction on the identity of the binding ligand. With respect to the linker L^r, it will be appreciated that at least one L^r of the formula



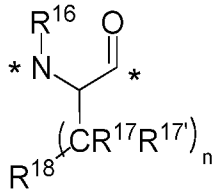
is included in the conjugates described by embodiment 1. However, it will be appreciated that independent of embodiment 1, any of the linkers described herein can be present or not present in conjugates described within embodiment 2. Specifically, embodiment 2 places no particular restriction on the identity of L^r.

AA is an amino acid as defined herein. In certain embodiments, AA is a naturally occurring amino acid. In certain embodiments, AA is in the L-form. In certain embodiments, AA is in the D-form. It will be appreciated that in certain embodiments, the conjugates described herein will comprise more than one amino acid as portions of the linker, and the amino acids can be the same or different, and can be selected from a group of amino acids. It will be appreciated that in certain embodiments, the conjugates described herein will comprise more than one amino acid as portions of the linker, and the amino acids can be the same or different, and can be selected from a group of amino acids in D- or L-form. In some embodiments, each AA is independently selected from the group consisting of L-lysine, L-asparagine, L-threonine, L-serine, L-isoleucine, L-methionine, L-proline, L-histidine, L-glutamine, L-arginine, L-glycine, L-aspartic acid, L-glutamic acid, L-alanine, L-valine, L-phenylalanine, L-leucine, L-tyrosine, L-cysteine, L-tryptophan, L-phosphoserine, L-sulfo-cysteine, L-arginosuccinic acid, L-hydroxyproline, L-phosphoethanolamine, L-sarcosine, L-taurine, L-carnosine, L-citrulline, L-anserine, L-1,3-methyl-histidine, L-alpha-amino-adipic

acid, D-lysine, D-asparagine, D-threonine, D-serine, D-isoleucine, D-methionine, D-proline, D-histidine, D-glutamine, D-arginine, D-glycine, D-aspartic acid, D-glutamic acid, D-alanine, D-valine, D-phenylalanine, D-leucine, D-tyrosine, D-cysteine, D-tryptophan, D-citrulline and D-carnosine.

In some embodiments, each AA is independently selected from the group consisting of L-asparagine, L-arginine, L-glycine, L-aspartic acid, L-glutamic acid, L-glutamine, L-cysteine, L-alanine, L-valine, L-leucine, L-isoleucine, L-citrulline, D-asparagine, D-arginine, D-glycine, D-aspartic acid, D-glutamic acid, D-glutamine, D-cysteine, D-alanine, D-valine, D-leucine, D-isoleucine and D-citrulline. In some embodiments, each AA is independently selected from the group consisting of Asp, Arg, Glu and Cys. In some embodiments, z2 is 2, z4 is 2, and the sequence of AAs is -Asp-Arg-Asp-Asp-. In some embodiments, z2 is 2, z4 is 2, and z6 is 1, and the sequence of AAs is -Asp-Arg-Asp-Asp-Cys. In some embodiments, z2 is 2, z4 is 3, and the sequence of AAs is -Asp-Arg-Asp-Asp-Cys.

L^1 can be present or absent in the conjugates described herein. When L^1 is present, L^1 can be any group covalently attaching portions of the linker to the binding ligand, portions of the linker to one another, or to D^1, or to D^2. It will be understood that the structure of L^1 is not particularly limited in any way. It will be further understood that L^1 can comprise numerous functionalities well known in the art to covalently attach portions of the linker to the binding ligand, portions of the linker to one another, or to D^1, or to D^2, including but not limited to, alkyl groups, ether groups, amide groups, carboxy groups, sulfonate groups, alkenyl groups, alkynyl groups, cycloalkyl groups, aryl groups, heterocycloalkyl, heteroaryl groups, and the like. In some embodiments, L^1 is a linker of the formula II



II

wherein

R^{16} is selected from the group consisting of H, D, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, $-C(O)R^{19}$, $-C(O)OR^{19}$ and $-C(O)NR^{19}R^{19'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl, $-OR^{20}$, $-OC(O)R^{20}$, $-OC(O)NR^{20}R^{20'}$, $-OS(O)R^{20}$, $-OS(O)_2R^{20}$, $-SR^{20}$, $-S(O)R^{20}$, $-S(O)_2R^{20}$, $-S(O)NR^{20}R^{20'}$, $-S(O)_2NR^{20}R^{20'}$, $-OS(O)NR^{20}R^{20'}$, $-OS(O)_2NR^{20}R^{20'}$, $-NR^{20}R^{20'}$, $-NR^{20}C(O)R^{21}$, $-NR^{20}C(O)OR^{21}$, -

$NR^{20}C(O)NR^{21}R^{21'}$, $-NR^{20}S(O)R^{21}$, $-NR^{20}S(O)_2R^{21}$, $-NR^{20}S(O)NR^{21}R^{21'}$, $-NR^{20}S(O)_2NR^{21}R^{21'}$, $-C(O)R^{20}$, $-C(O)OR^{20}$ or $-C(O)NR^{20}R^{20'}$;

each R^{17} and $R^{17'}$ is independently selected from the group consisting of H, D, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{22}$, $-OC(O)R^{22}$, $-OC(O)NR^{22}R^{22'}$, $-OS(O)R^{22}$, $-OS(O)_2R^{22}$, $-SR^{22}$, $-S(O)R^{22}$, $-S(O)_2R^{22}$, $-S(O)NR^{22}R^{22'}$, $-S(O)_2NR^{22}R^{22'}$, $-OS(O)NR^{22}R^{22'}$, $-OS(O)_2NR^{22}R^{22'}$, $-NR^{22}R^{22'}$, $-NR^{22}C(O)R^{23}$, $-NR^{22}C(O)OR^{23}$, $-NR^{22}C(O)NR^{23}R^{23'}$, $-NR^{22}S(O)R^{23}$, $-NR^{22}S(O)_2R^{23}$, $-NR^{22}S(O)NR^{23}R^{23'}$, $-NR^{22}S(O)_2NR^{23}R^{23'}$, $-C(O)R^{22}$, $-C(O)OR^{22}$, and $-C(O)NR^{22}R^{22'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, $-OR^{24}$, $-OC(O)R^{24}$, $-OC(O)NR^{24}R^{24'}$, $-OS(O)R^{24}$, $-OS(O)_2R^{24}$, $-SR^{24}$, $-S(O)R^{24}$, $-S(O)_2R^{24}$, $-S(O)NR^{24}R^{24'}$, $-S(O)_2NR^{24}R^{24'}$, $-OS(O)NR^{24}R^{24'}$, $-OS(O)_2NR^{24}R^{24'}$, $-NR^{24}R^{24'}$, $-NR^{24}C(O)R^{25}$, $-NR^{24}C(O)OR^{25}$, $-NR^{24}C(O)NR^{25}R^{25'}$, $-NR^{24}S(O)R^{25}$, $-NR^{24}S(O)_2R^{25}$, $-NR^{24}S(O)NR^{25}R^{25'}$, $-NR^{24}S(O)_2NR^{25}R^{25'}$, $-C(O)R^{24}$, $-C(O)OR^{24}$ or $-C(O)NR^{24}R^{24'}$; or R^{17} and $R^{17'}$ may combine to form a C_4-C_6 cycloalkyl or a 4- to 6- membered heterocycle, wherein each hydrogen atom in C_4-C_6 cycloalkyl or 4- to 6- membered heterocycle is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{24}$, $-OC(O)R^{24}$, $-OC(O)NR^{24}R^{24'}$, $-OS(O)R^{24}$, $-OS(O)_2R^{24}$, $-SR^{24}$, $-S(O)R^{24}$, $-S(O)_2R^{24}$, $-S(O)NR^{24}R^{24'}$, $-S(O)_2NR^{24}R^{24'}$, $-OS(O)NR^{24}R^{24'}$, $-OS(O)_2NR^{24}R^{24'}$, $-NR^{24}R^{24'}$, $-NR^{24}C(O)R^{25}$, $-NR^{24}C(O)OR^{25}$, $-NR^{24}C(O)NR^{25}R^{25'}$, $-NR^{24}S(O)R^{25}$, $-NR^{24}S(O)_2R^{25}$, $-NR^{24}S(O)NR^{25}R^{25'}$, $-NR^{24}S(O)_2NR^{25}R^{25'}$, $-C(O)R^{24}$, $-C(O)OR^{24}$ or $-C(O)NR^{24}R^{24'}$;

R^{18} is selected from the group consisting of H, D, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{26}$, $-OC(O)R^{26}$, $-OC(O)NR^{26}R^{26'}$, $-OS(O)R^{26}$, $-OS(O)_2R^{26}$, $-SR^{26}$, $-S(O)R^{26}$, $-S(O)_2R^{26}$, $-S(O)NR^{26}R^{26'}$, $-S(O)_2NR^{26}R^{26'}$, $-OS(O)NR^{26}R^{26'}$, $-OS(O)_2NR^{26}R^{26'}$, $-NR^{26}R^{26'}$, $-NR^{26}C(O)R^{27}$, $-NR^{26}C(O)OR^{27}$, $-NR^{26}C(O)NR^{27}R^{27'}$, $-NR^{26}C(=NR^{26''})NR^{27}R^{27'}$, $-NR^{26}S(O)R^{27}$, $-NR^{26}S(O)_2R^{27}$, $-NR^{26}S(O)NR^{27}R^{27'}$, $-NR^{26}S(O)_2NR^{27}R^{27'}$, $-C(O)R^{26}$, $-C(O)OR^{26}$ and $-C(O)NR^{26}R^{26'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, $-(CH_2)_pOR^{28}$, $-(CH_2)_p(OCH_2)_qOR^{28}$, $-(CH_2)_p(OCH_2CH_2)_qOR^{28}$, $-OR^{29}$, $-OC(O)R^{29}$,

$-OC(O)NR^{29}R^{29'}$, $-OS(O)R^{29}$, $-OS(O)_2R^{29}$, $-(CH_2)_pOS(O)_2OR^{29}$, $-OS(O)_2OR^{29}$, $-SR^{29}$, $-S(O)R^{29}$, $-S(O)_2R^{29}$, $-S(O)NR^{29}R^{29'}$, $-S(O)_2NR^{29}R^{29'}$, $-OS(O)NR^{29}R^{29'}$, $-OS(O)_2NR^{29}R^{29'}$, $-NR^{29}R^{29'}$, $-NR^{29}C(O)R^{30}$, $-NR^{29}C(O)OR^{30}$, $-NR^{29}C(O)NR^{30}R^{30'}$, $-NR^{29}S(O)R^{30}$, $-NR^{29}S(O)_2R^{30}$, $-NR^{29}S(O)NR^{30}R^{30'}$, $-NR^{29}S(O)_2NR^{30}R^{30'}$, $-C(O)R^{29}$, $-C(O)OR^{29}$ or $-C(O)NR^{29}R^{29'}$;

each R^{19}, $R^{19'}$, R^{20}, $R^{20'}$, R^{21}, $R^{21'}$, R^{22}, $R^{22'}$, R^{23}, $R^{23'}$, R^{24}, $R^{24'}$, R^{25}, $R^{25'}$, R^{26}, $R^{26'}$, $R^{26''}$, R^{29}, $R^{29'}$, R^{30} and $R^{30'}$ is independently selected from the group consisting of H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, or 5- to 7-membered heteroaryl is independently optionally substituted by halogen, -OH, -SH, $-NH_2$ or $-CO_2H$;

R^{27} and $R^{27'}$ are each independently selected from the group consisting of H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_3-C_6 cycloalkyl, $-(CH_2)_p$(sugar), $-(CH_2)_p(OCH_2CH_2)_q$-(sugar) and $-(CH_2)_p(OCH_2CH_2CH_2)_q$(sugar);

R^{28} is a H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl or sugar;

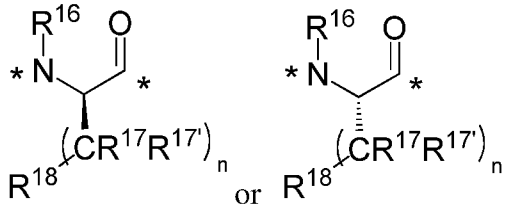
n is 1, 2, 3, 4 or 5;

p is 1, 2, 3, 4 or 5;

q is 1, 2, 3, 4 or 5; and

* is a covalent bond.

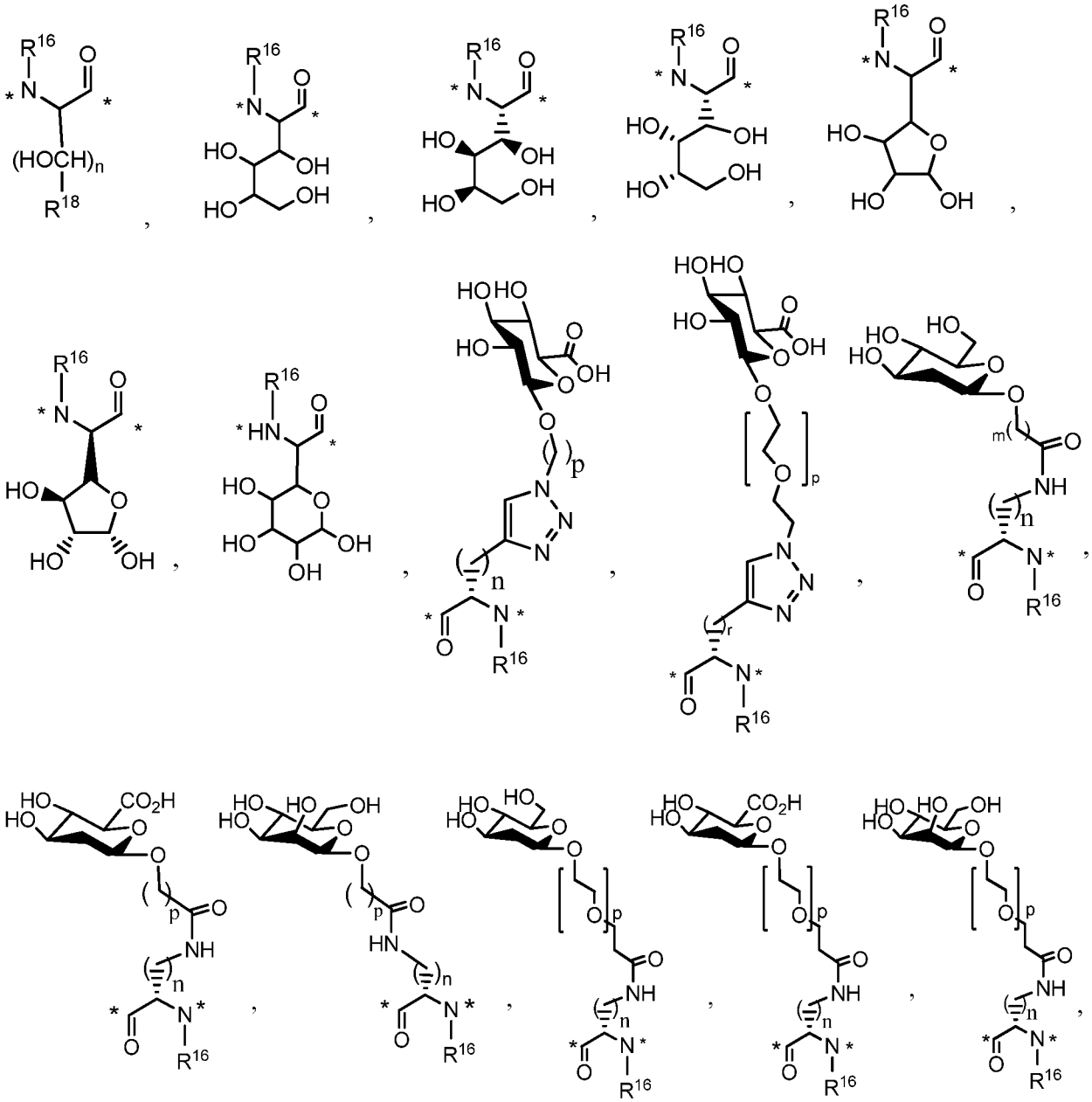
It will be appreciated that when L^1 is described according to the formula II, that both the R- and S- configurations are contemplated. In some embodiments, L^1 is of the formula IIa or IIb



IIa IIb

where each of R^{16}, R^{17}, $R^{17'}$, R^{18}, n and * are as defined for the formula II.

In some embodiments, each L^1 is selected from the group consisting of



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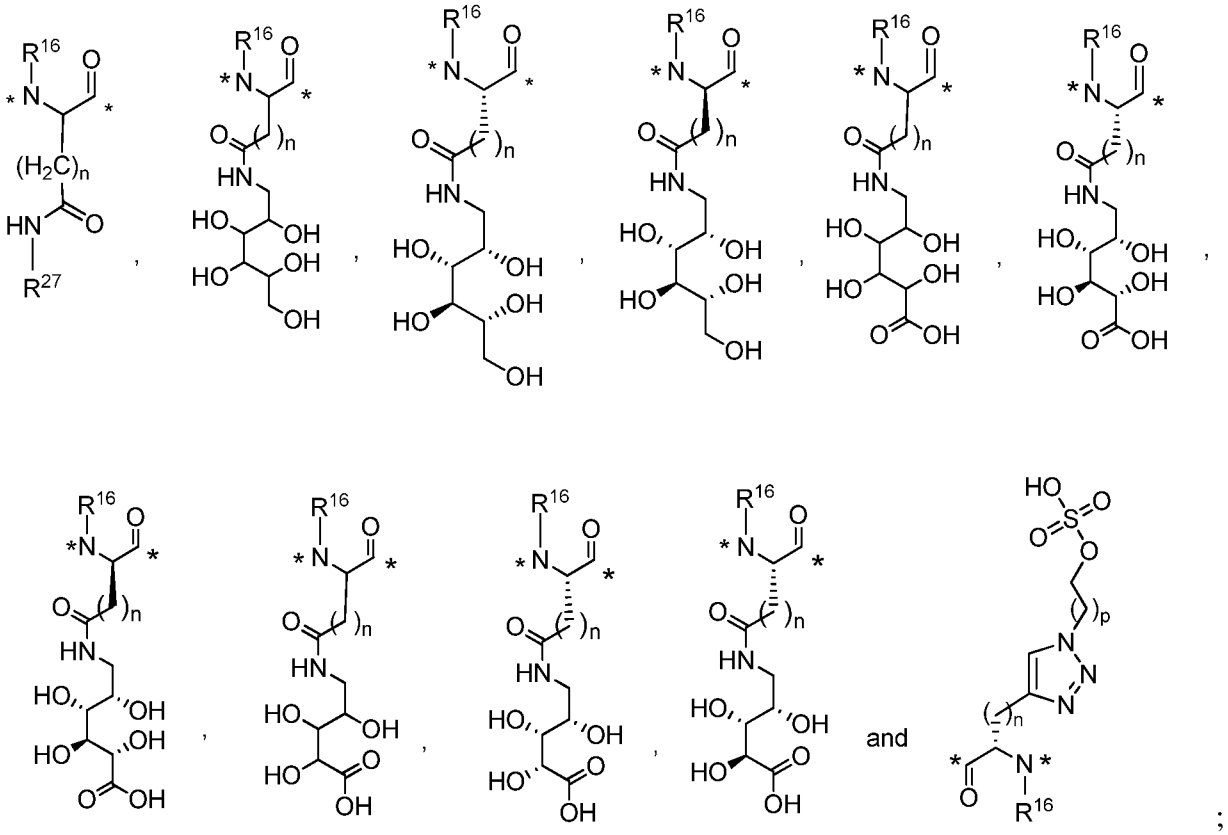


and combinations thereof,

wherein

R^{16} is selected from the group consisting of H, D, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -$C(O)R^{19}$, -$C(O)OR^{19}$ and -$C(O)NR^{19}R^{19'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl, -OR^{20}, -$OC(O)R^{20}$, -$OC(O)NR^{20}R^{20'}$, -$OS(O)R^{20}$, -$OS(O)_2R^{20}$, -SR^{20}, -$S(O)R^{20}$, -$S(O)_2R^{20}$, -$S(O)NR^{20}R^{20'}$, -$S(O)_2NR^{20}R^{20'}$, -$OS(O)NR^{20}R^{20'}$, -$OS(O)_2NR^{20}R^{20'}$, -$NR^{20}R^{20'}$, -$NR^{20}C(O)R^{21}$, -$NR^{20}C(O)OR^{21}$, -$NR^{20}C(O)NR^{21}R^{21'}$, -$NR^{20}S(O)R^{21}$, -$NR^{20}S(O)_2R^{21}$, -$NR^{20}S(O)NR^{21}R^{21'}$, -$NR^{20}S(O)_2NR^{21}R^{21'}$, -$C(O)R^{20}$, -$C(O)OR^{20}$ or -$C(O)NR^{20}R^{20'}$;

R^{18} is selected from the group consisting of H, D, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{26}, -$OC(O)R^{26}$, -$OC(O)NR^{26}R^{26'}$, -$OS(O)R^{26}$, -$OS(O)_2R^{26}$, -SR^{26}, -$S(O)R^{26}$, -$S(O)_2R^{26}$, -$S(O)NR^{26}R^{26'}$, -$S(O)_2NR^{26}R^{26'}$, -$OS(O)NR^{26}R^{26'}$, -$OS(O)_2NR^{26}R^{26'}$, -$NR^{26}R^{26'}$, -$NR^{26}C(O)R^{27}$, -$NR^{26}C(O)OR^{27}$, -$NR^{26}C(O)NR^{27}R^{27'}$, -$NR^{26}C(=NR^{26''})NR^{27}R^{27'}$, -$NR^{26}S(O)R^{27}$, -$NR^{26}S(O)_2R^{27}$, -$NR^{26}S(O)NR^{27}R^{27'}$, -$NR^{26}S(O)_2NR^{27}R^{27'}$, -$C(O)R^{26}$, -$C(O)OR^{26}$ and -$C(O)NR^{26}R^{26'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5-

to 7-membered heteroaryl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, $-(CH_2)_pOR^{28}$, $-(CH_2)_p(OCH_2)_qOR^{28}$, $-(CH_2)_p(OCH_2CH_2)_qOR^{28}$, $-OR^{29}$, $-OC(O)R^{29}$, $-OC(O)NR^{29}R^{29'}$, $-OS(O)R^{29}$, $-OS(O)_2R^{29}$, $-(CH_2)_pOS(O)_2OR^{29}$, $-OS(O)_2OR^{29}$, $-SR^{29}$, $-S(O)R^{29}$, $-S(O)_2R^{29}$, $-S(O)NR^{29}R^{29'}$, $-S(O)_2NR^{29}R^{29'}$, $-OS(O)NR^{29}R^{29'}$, $-OS(O)_2NR^{29}R^{29'}$, $-NR^{29}R^{29'}$, $-NR^{29}C(O)R^{30}$, $-NR^{29}C(O)OR^{30}$, $-NR^{29}C(O)NR^{30}R^{30'}$, $-NR^{29}S(O)R^{30}$, $-NR^{29}S(O)_2R^{30}$, $-NR^{29}S(O)NR^{30}R^{30'}$, $-NR^{29}S(O)_2NR^{30}R^{30'}$, $-C(O)R^{29}$, $-C(O)OR^{29}$ or $-C(O)NR^{29}R^{29'}$;

each each R^{19}, $R^{19'}$, R^{20}, $R^{20'}$, R^{21}, $R^{21'}$, R^{26}, $R^{26'}$, $R^{26''}$, R^{29}, $R^{29'}$, R^{30} and $R^{30'}$ is independently selected from the group consisting of H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, or 5- to 7-membered heteroaryl is independently optionally substituted by halogen, -OH, -SH, $-NH_2$ or $-CO_2H$;

R^{27} and $R^{27'}$ are each independently selected from the group consisting of H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_3-C_6 cycloalkyl, $-(CH_2)_p$(sugar), $-(CH_2)_p(OCH_2CH_2)_q$-(sugar) and $-(CH_2)_p(OCH_2CH_2CH_2)_q$(sugar);

R^{28} is H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl or sugar;

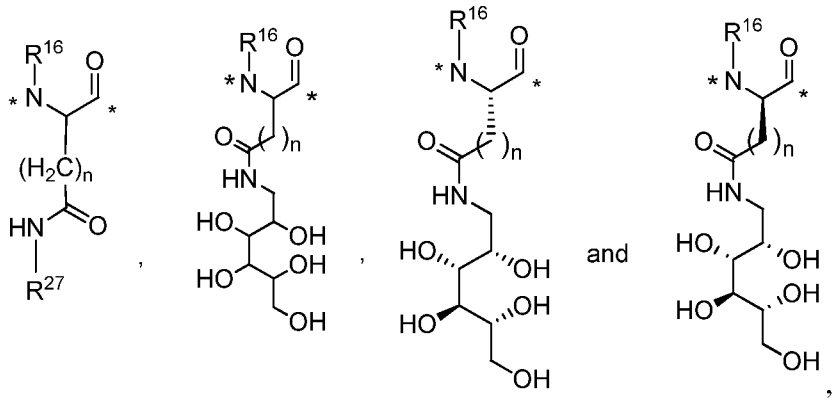
n is 1, 2, 3, 4 or 5;

p is 1, 2, 3, 4 or 5;

q is 1, 2, 3, 4 or 5; and

each * represent a covalent bond to the rest of the conjugate.

In some embodiments, each L^1 is selected from the group consisting of



wherein R^{16} is defined as described herein, and each * represent a covalent bond to the rest of the conjugate.

In some embodiments, R^{16} is H. In some embodiments, R^{18} is selected from the group consisting of H, 5- to 7-membered heteroaryl, $-OR^{26}$, $-NR^{26}C(O)R^{27}$, $-NR^{26}C(O)NR^{27}R^{27'}$,

$-NR^{26}C(=NR^{26''})NR^{27}R^{27'}$, and $-C(O)NR^{26}R^{26'}$, wherein each hydrogen atom 5- to 7-membered heteroaryl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, $-(CH_2)_pOR^{28}$, $-(CH_2)_p(OCH_2)_qOR^{28}$, $-(CH_2)_p(OCH_2CH_2)_qOR^{28}$, $-OR^{29}$, $-OC(O)R^{29}$, $-OC(O)NR^{29}R^{29'}$, $-OS(O)R^{29}$, $-OS(O)_2R^{29}$, $-(CH_2)_pOS(O)_2OR^{29}$, $-OS(O)_2OR^{29}$, $-SR^{29}$, $-S(O)R^{29}$, $-S(O)_2R^{29}$, $-S(O)NR^{29}R^{29'}$, $-S(O)_2NR^{29}R^{29'}$, $-OS(O)NR^{29}R^{29'}$, $-OS(O)_2NR^{29}R^{29'}$, $-NR^{29}R^{29'}$, $-NR^{29}C(O)R^{30}$, $-NR^{29}C(O)OR^{30}$, $-NR^{29}C(O)NR^{30}R^{30'}$, $-NR^{29}S(O)R^{30}$, $-NR^{29}S(O)_2R^{30}$, $-NR^{29}S(O)NR^{30}R^{30'}$, $-NR^{29}S(O)_2NR^{30}R^{30'}$, $-C(O)R^{29}$, $-C(O)OR^{29}$ or $-C(O)NR^{29}R^{29'}$;

each R^{26}, $R^{26'}$, $R^{26''}$, R^{29}, $R^{29'}$, R^{30} and $R^{30'}$ is independently selected from the group consisting of H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, or 5- to 7-membered heteroaryl is independently optionally substituted by halogen, -OH, -SH, $-NH_2$ or $-CO_2H$;

R^{27} and $R^{27'}$ are each independently selected from the group consisting of H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_3-C_6 cycloalkyl, $-(CH_2)_p$(sugar), $-(CH_2)_p(OCH_2CH_2)_q$-(sugar) and $-(CH_2)_p(OCH_2CH_2CH_2)_q$(sugar);

R^{28} is a H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl or sugar;

n is 1, 2, 3, 4 or 5;

p is 1, 2, 3, 4 or 5;

q is 1, 2, 3, 4 or 5; and

each * represent a covalent bond to the rest of the conjugate.

In some embodiments, R^{18} is selected from the group consisting of H, 5- to 7-membered heteroaryl, $-OR^{26}$, $-NR^{26}C(O)R^{27}$, $-NR^{26}C(O)NR^{27}R^{27'}$, $-NR^{26}C(=NR^{26''})NR^{27}R^{27'}$, and $-C(O)NR^{26}R^{26'}$, wherein each hydrogen atom 5- to 7-membered heteroaryl is independently optionally substituted by $-(CH_2)_pOR^{28}$, $-OR^{29}$, $-(CH_2)_pOS(O)_2OR^{29}$ and $-OS(O)_2OR^{29}$;

each R^{26}, $R^{26'}$, $R^{26''}$ and R^{29} is independently H or C_1-C_7 alkyl, wherein each hydrogen atom in C_1-C_7 alkyl is independently optionally substituted by halogen, -OH, -SH, $-NH_2$ or $-CO_2H$;

R^{27} and $R^{27'}$ are each independently selected from the group consisting of H, $-(CH_2)_p$(sugar), $-(CH_2)_p(OCH_2CH_2)_q$(sugar) and $-(CH_2)_p(OCH_2CH_2CH_2)_q$(sugar);

R^{28} is H or sugar;

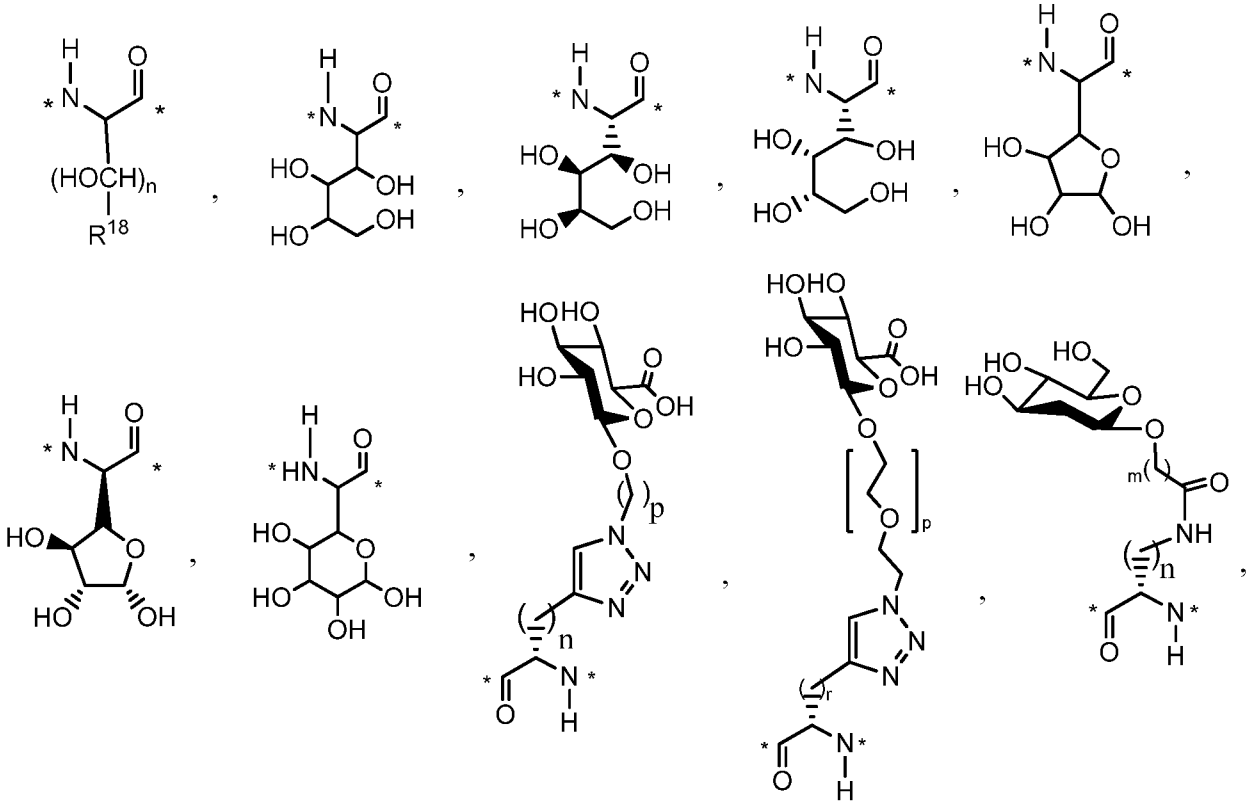
n is 1, 2, 3, 4 or 5;

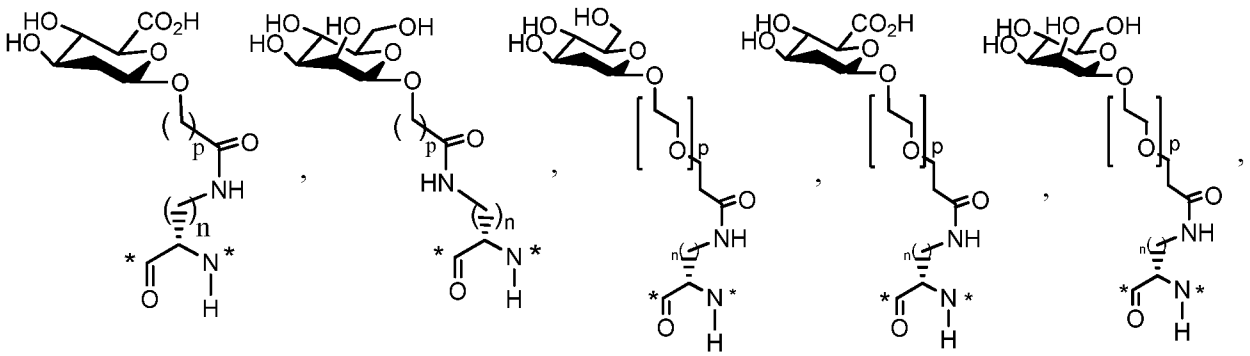
p is 1, 2, 3, 4 or 5;

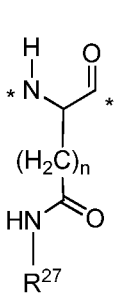
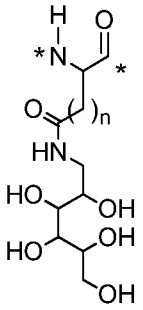
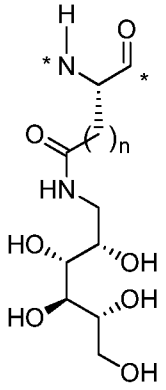
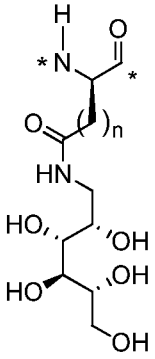
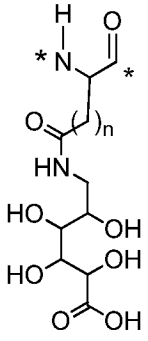
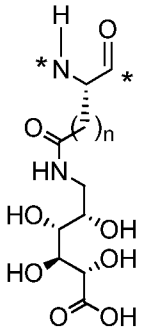
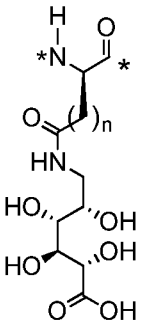
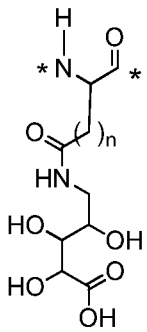
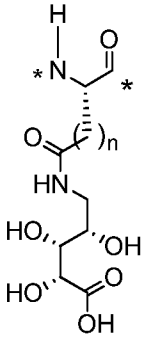
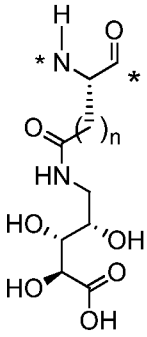
q is 1, 2, 3, 4 or 5; and

each * represent a covalent bond to the rest of the conjugate.

In some embodiments, each L^1 is selected from the group consisting of



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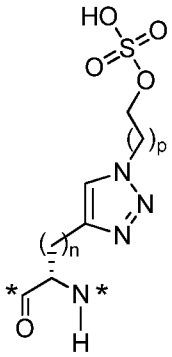

;

and combinations thereof,

wherein

R^{18} is selected from the group consisting of H, D, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{26}$, $-OC(O)R^{26}$, $-OC(O)NR^{26}R^{26'}$, $-OS(O)R^{26}$, $-OS(O)_2R^{26}$, $-SR^{26}$, $-S(O)R^{26}$, $-S(O)_2R^{26}$, $-S(O)NR^{26}R^{26'}$, $-S(O)_2NR^{26}R^{26'}$, $-OS(O)NR^{26}R^{26'}$, $-OS(O)_2NR^{26}R^{26'}$, $-NR^{26}R^{26'}$, $-NR^{26}C(O)R^{27}$, $-NR^{26}C(O)OR^{27}$, $-NR^{26}C(O)NR^{27}R^{27'}$, $-NR^{26}C(=NR^{26''})NR^{27}R^{27'}$, $-NR^{26}S(O)R^{27}$, $-NR^{26}S(O)_2R^{27}$, $-NR^{26}S(O)NR^{27}R^{27'}$, $-NR^{26}S(O)_2NR^{27}R^{27'}$, $-C(O)R^{26}$, $-C(O)OR^{26}$ and $-C(O)NR^{26}R^{26'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, $-(CH_2)_pOR^{28}$, $-(CH_2)_p(OCH_2)_qOR^{28}$, $-(CH_2)_p(OCH_2CH_2)_qOR^{28}$, $-OR^{29}$, $-OC(O)R^{29}$, $-OC(O)NR^{29}R^{29'}$, $-OS(O)R^{29}$, $-OS(O)_2R^{29}$, $-(CH_2)_pOS(O)_2OR^{29}$, $-OS(O)_2OR^{29}$, $-SR^{29}$, $-S(O)R^{29}$, $-S(O)_2R^{29}$, $-S(O)NR^{29}R^{29'}$, $-S(O)_2NR^{29}R^{29'}$, $-OS(O)NR^{29}R^{29'}$, $-OS(O)_2NR^{29}R^{29'}$, $-NR^{29}R^{29'}$, $-NR^{29}C(O)R^{30}$, $-NR^{29}C(O)OR^{30}$, $-NR^{29}C(O)NR^{30}R^{30'}$, $-NR^{29}S(O)R^{30}$, $-NR^{29}S(O)_2R^{30}$, $-NR^{29}S(O)NR^{30}R^{30'}$, $-NR^{29}S(O)_2NR^{30}R^{30'}$, $-C(O)R^{29}$, $-C(O)OR^{29}$ or $-C(O)NR^{29}R^{29'}$;

each R^{26}, $R^{26'}$, $R^{26''}$, R^{29}, $R^{29'}$, R^{30} and $R^{30'}$ is independently selected from the group consisting of H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to

7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, or 5- to 7-membered heteroaryl is independently optionally substituted by halogen, -OH, -SH, $-NH_2$ or $-CO_2H$;

R^{27} and $R^{27'}$ are each independently selected from the group consisting of H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_3-C_6 cycloalkyl, $-(CH_2)_p$(sugar), $-(CH_2)_p(OCH_2CH_2)_q$-(sugar) and $-(CH_2)_p(OCH_2CH_2CH_2)_q$(sugar);

R^{28} is a H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl or sugar;

n is 1, 2, 3, 4 or 5;

p is 1, 2, 3, 4 or 5;

q is 1, 2, 3, 4 or 5; and

each * represent a covalent bond to the rest of the conjugate.

In some embodiments, R^{18} is selected from the group consisting of H, 5- to 7-membered heteroaryl, $-OR^{26}$, $-NR^{26}C(O)R^{27}$, $-NR^{26}C(O)NR^{27}R^{27'}$, $-NR^{26}C(=NR^{26''})NR^{27}R^{27'}$, and $-C(O)NR^{26}R^{26'}$, wherein each hydrogen atom 5- to 7-membered heteroaryl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, $-(CH_2)_pOR^{28}$, $-(CH_2)_p(OCH_2)_qOR^{28}$, $-(CH_2)_p(OCH_2CH_2)_qOR^{28}$, $-OR^{29}$, $-OC(O)R^{29}$, $-OC(O)NR^{29}R^{29'}$, $-OS(O)R^{29}$, $-OS(O)_2R^{29}$, $-(CH_2)_pOS(O)_2OR^{29}$, $-OS(O)_2OR^{29}$, $-SR^{29}$, $-S(O)R^{29}$, $-S(O)_2R^{29}$, $-S(O)NR^{29}R^{29'}$, $-S(O)_2NR^{29}R^{29'}$, $-OS(O)NR^{29}R^{29'}$, $-OS(O)_2NR^{29}R^{29'}$, $-NR^{29}R^{29'}$, $-NR^{29}C(O)R^{30}$, $-NR^{29}C(O)OR^{30}$, $-NR^{29}C(O)NR^{30}R^{30'}$, $-NR^{29}S(O)R^{30}$, $-NR^{29}S(O)_2R^{30}$, $-NR^{29}S(O)NR^{30}R^{30'}$, $-NR^{29}S(O)_2NR^{30}R^{30'}$, $-C(O)R^{29}$, $-C(O)OR^{29}$ or $-C(O)NR^{29}R^{29'}$;

each R^{26}, $R^{26'}$, $R^{26''}$, R^{29}, $R^{29'}$, R^{30} and $R^{30'}$ is independently selected from the group consisting of H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, or 5- to 7-membered heteroaryl is independently optionally substituted by halogen, -OH, -SH, $-NH_2$ or $-CO_2H$;

R^{27} and $R^{27'}$ are each independently selected from the group consisting of H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_3-C_6 cycloalkyl, $-(CH_2)_p$(sugar), $-(CH_2)_p(OCH_2CH_2)_q$-(sugar) and $-(CH_2)_p(OCH_2CH_2CH_2)_q$(sugar);

R^{28} is a H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl or sugar;

n is 1, 2, 3, 4 or 5;

p is 1, 2, 3, 4 or 5;

q is 1, 2, 3, 4 or 5; and

each * represent a covalent bond to the rest of the conjugate.

In some embodiments, R^{18} is selected from the group consisting of H, 5- to 7-membered heteroaryl, $-OR^{26}$, $-NR^{26}C(O)R^{27}$, $-NR^{26}C(O)NR^{27}R^{27'}$, $-NR^{26}C(=NR^{26''})NR^{27}R^{27'}$ and $-C(O)NR^{26}R^{26'}$, wherein each hydrogen atom 5- to 7-membered heteroaryl is independently optionally substituted by $-(CH_2)_pOR^{28}$, $-OR^{29}$, $-(CH_2)_pOS(O)_2OR^{29}$ and $-OS(O)_2OR^{29}$;

each R^{26}, $R^{26'}$, $R^{26''}$ and R^{29} is independently H or C_1-C_7 alkyl, wherein each hydrogen atom in C_1-C_7 alkyl is independently optionally substituted by halogen, -OH, -SH, $-NH_2$ or $-CO_2H$;

R^{27} and $R^{27'}$ are each independently selected from the group consisting of H, $-(CH_2)_p$(sugar), $-(CH_2)_p(OCH_2CH_2)_q$(sugar) and $-(CH_2)_p(OCH_2CH_2CH_2)_q$(sugar);

R^{28} is H or sugar;

n is 1, 2, 3, 4 or 5;

p is 1, 2, 3, 4 or 5;

q is 1, 2, 3, 4 or 5; and

each * represent a covalent bond to the rest of the conjugate.

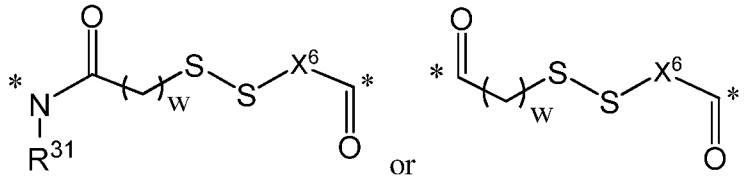
In some embodiments of the conjugates described herein, L^1 is present. In some embodiments of the conjugates described herein, L^1 is absent. In some embodiments, z1 is 0. In some embodiments, z3 is 0. In some embodiments, z5 is 0. In some embodiments, z1 is 0, z3 is 0 and z5 is 0. In some embodiments, z1 is 1. In some embodiments, z3 is 1. In some embodiments, z5 is 1. In some embodiments, z1 is 1, z3 is 1 and z5 is 1.

L^r is a releasable linker. As used described herein, a "releasable linker" refers to a linker that includes at least one cleavable bond that can be broken under physiological conditions, such as a pH-labile, acid-labile, base-labile, oxidatively labile, metabolically labile, biochemically labile, or enzyme-labile bond.

It will be appreciated that a releasable linker includes a cleavable bond that can connect two adjacent atoms within the releasable linker. The lability of the cleavable bond can be adjusted by, for example, substituents at or near the cleavable bond, such as including alpha-branching adjacent to a cleavable disulfide bond, increasing the hydrophobicity of substituents on silicon in a moiety having silicon-oxygen bond that may be hydrolyzed, homologating alkoxy groups that form part of a ketal or acetal that may be hydrolyzed, and the like.

Illustrative releasable linkers described herein include linkers that include hemiacetals and sulfur variations thereof, acetals and sulfur variations thereof, hemiaminals, aminals, disulfides, hydrazines, and the like.

In connection with embodiemt 1, at least one L^r of the formula



is included in the conjugates described by embodiment 1, wherein

each R^{31} and $R^{31'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{32}$, $-OC(O)R^{32}$, $-OC(O)NR^{32}R^{32'}$, $-OS(O)R^{32}$, $-OS(O)_2R^{32}$, $-SR^{32}$, $-S(O)R^{32}$, $-S(O)_2R^{32}$, $-S(O)NR^{32}R^{32'}$, $-S(O)_2NR^{32}R^{32'}$, $-OS(O)NR^{32}R^{32'}$, $-OS(O)_2NR^{32}R^{32'}$, $-NR^{32}R^{32'}$, $-NR^{32}C(O)R^{33}$, $-NR^{32}C(O)OR^{33}$, $-NR^{32}C(O)NR^{33}R^{33'}$, $-NR^{32}S(O)R^{33}$, $-NR^{32}S(O)_2R^{33}$, $-NR^{32}S(O)NR^{33}R^{33'}$, $-NR^{32}S(O)_2NR^{33}R^{33'}$, $-C(O)R^{32}$, $-C(O)OR^{32}$ or $-C(O)NR^{32}R^{32'}$;

each X^6 is independently selected from the group consisting of $-C_1$-C_6 alkyl-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-, $-C_1$-C_6 alkyl-O-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-O-, $-C_1$-C_6 alkyl-$NR^{31'}$- and $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-$NR^{31'}$-, wherein each hydrogen atom in $-C_1$-C_6 alkyl-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-, $-C_1$-C_6 alkyl-O-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-O-, $-C_1$-C_6 alkyl-$NR^{31'}$- or $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-$NR^{31'}$ is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{34}$, $-OC(O)R^{34}$, $-OC(O)NR^{34}R^{34'}$, $-OS(O)R^{34}$, $-OS(O)_2R^{34}$, $-SR^{34}$, $-S(O)R^{34}$, $-S(O)_2R^{34}$, $-S(O)NR^{34}R^{34'}$, $-S(O)_2NR^{34}R^{34'}$, $-OS(O)NR^{34}R^{34'}$, $-OS(O)_2NR^{34}R^{34'}$, $-NR^{34}R^{34'}$, $-NR^{34}C(O)R^{35}$, $-NR^{34}C(O)OR^{35}$, $-NR^{34}C(O)NR^{35}R^{35'}$, $-NR^{34}S(O)R^{35}$, $-NR^{34}S(O)_2R^{35}$, $-NR^{34}S(O)NR^{35}R^{35'}$, $-NR^{34}S(O)_2NR^{35}R^{35'}$, $-C(O)R^{34}$, $-C(O)OR^{34}$ or $-C(O)NR^{34}R^{34'}$;

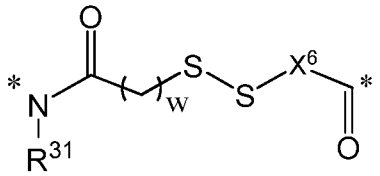
each R^{32}, $R^{32'}$, R^{33}, $R^{33'}$, R^{34}, $R^{34'}$, R^{35} and $R^{35'}$ are independently selected from the group consisting of H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, and 5- to 7-membered heteroaryl;

each w is independently an integer from 1 to 4; and

each * represents a covalent bond to the rest of the conjugate.

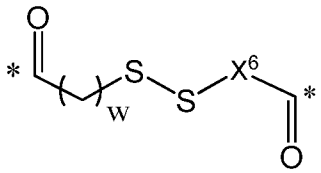
In some embodiments, R^{31} is H. In some embodiments, R^{36} is H. In some embodiments, X^6 is C_1-C_6 alkyl. In some embodiments, X^6 is C_1-C_6 alkyl. C_6-C_{10} aryl-(C_1-C_6 alkyl).

In some aspects of embodiment 1, L^r is of the formula



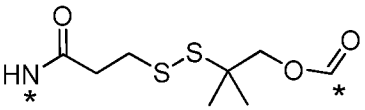
wherein R^{31}, X^6 and w are as described herein, and each * represents a covalent bond to the rest of the conjugate..

In some aspects of embodiment 1, L^r is of the formula



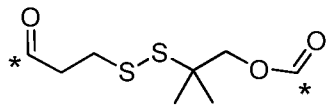
wherein X^6 and w are as described herein, and each * represents a covalent bond to the rest of the conjugate..

In some aspects of embodiment 1, L^r is of the formula



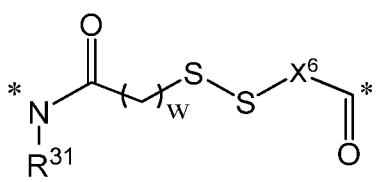
wherein each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 1, L^r is of the formula



wherein each * represents a covalent bond to the rest of the conjugate.

In connection with embodiment 2, L^r can be present or absent, and when present, L^r can be selected from the group consisting of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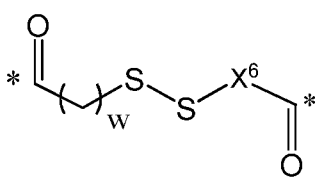
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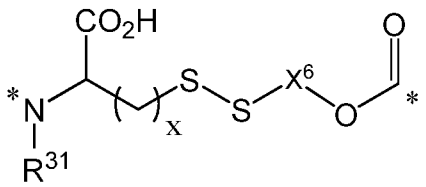
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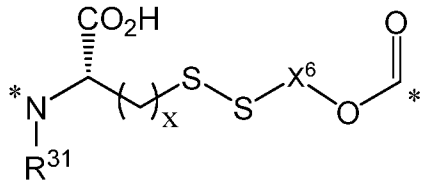
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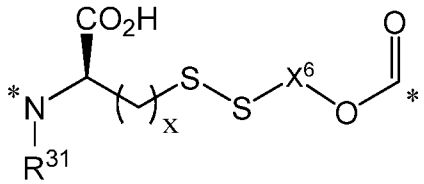
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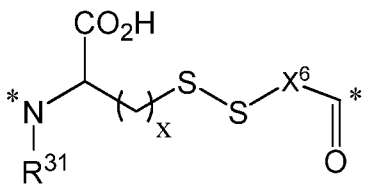
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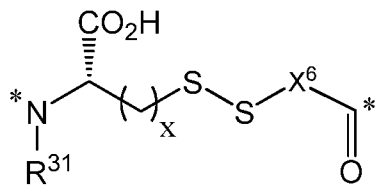
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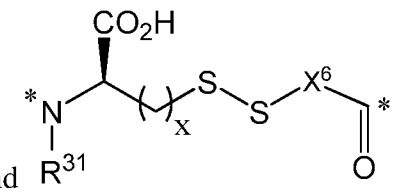

,

wherein

each R^{31} and $R^{31'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{32}$, $-OC(O)R^{32}$, $-OC(O)NR^{32}R^{32'}$, $-OS(O)R^{32}$, $-OS(O)_2R^{32}$, $-SR^{32}$, $-S(O)R^{32}$, $-S(O)_2R^{32}$, $-S(O)NR^{32}R^{32'}$, $-S(O)_2NR^{32}R^{32'}$, $-OS(O)NR^{32}R^{32'}$, $-OS(O)_2NR^{32}R^{32'}$, $-NR^{32}R^{32'}$, $-NR^{32}C(O)R^{33}$, $-NR^{32}C(O)OR^{33}$, $-NR^{32}C(O)NR^{33}R^{33'}$, $-NR^{32}S(O)R^{33}$, $-NR^{32}S(O)_2R^{33}$, $-NR^{32}S(O)NR^{33}R^{33'}$, $-NR^{32}S(O)_2NR^{33}R^{33'}$, $-C(O)R^{32}$, $-C(O)OR^{32}$ or $-C(O)NR^{32}R^{32'}$;

each X^6 is independently selected from the group consisting of $-C_1$-C_6 alkyl-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-, $-C_1$-C_6 alkyl-O-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-O-, $-C_1$-C_6 alkyl-$NR^{31'}$- and $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-$NR^{31'}$-, wherein each hydrogen atom in $-C_1$-C_6 alkyl-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-, $-C_1$-C_6 alkyl-O-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-O-, $-C_1$-C_6 alkyl-$NR^{31'}$- or $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-$NR^{31'}$ is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{34}$, $-OC(O)R^{34}$, $-OC(O)NR^{34}R^{34'}$, $-OS(O)R^{34}$, $-OS(O)_2R^{34}$, $-SR^{34}$, $-S(O)R^{34}$, $-S(O)_2R^{34}$, $-S(O)NR^{34}R^{34'}$, $-S(O)_2NR^{34}R^{34'}$, $-OS(O)NR^{34}R^{34'}$, $-OS(O)_2NR^{34}R^{34'}$, $-NR^{34}R^{34'}$, $-NR^{34}C(O)R^{35}$, $-NR^{34}C(O)OR^{35}$, $-NR^{34}C(O)NR^{35}R^{35'}$, $-NR^{34}S(O)R^{35}$, $-NR^{34}S(O)_2R^{35}$, $-NR^{34}S(O)NR^{35}R^{35'}$, $-NR^{34}S(O)_2NR^{35}R^{35'}$, $-C(O)R^{34}$, $-C(O)OR^{34}$ or $-C(O)NR^{34}R^{34'}$;

each R^{32}, $R^{32'}$, R^{33}, $R^{33'}$, R^{34}, $R^{34'}$, R^{35} and $R^{35'}$ are independently selected from the group consisting of H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, and 5- to 7-membered heteroaryl;

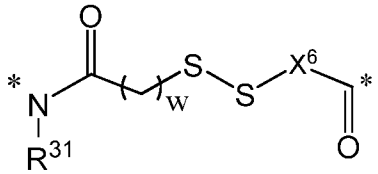
each w is independently an integer from 1 to 4;

each x is and integer from 1 to 3; and

each * represents a covalent bond to the rest of the conjugate.

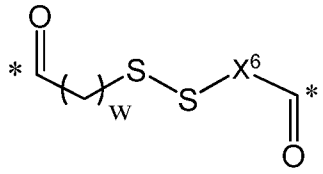
In some embodiments, R^{31} is H. In some embodiments, R^{36} is H. In some embodiments, X^6 is C_1-C_6 alkyl. In some embodiments, X^6 is C_1-C_6 alkyl. C_6-C_{10} aryl-(C_1-C_6 alkyl).

In some aspects of embodiment 2, L^r is of the formula



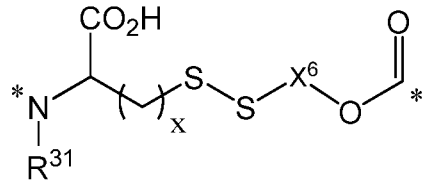
wherein R^{31}, X^6 and w are as described herein, and each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^r is of the formula



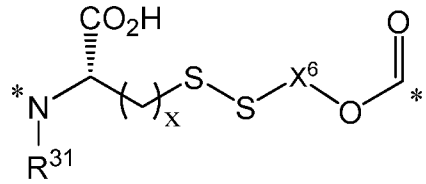
wherein X^6 and w are as described herein, and each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^r is of the formula



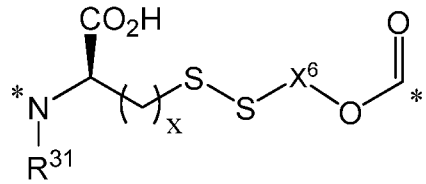
wherein R^{31}, X^6 and x are as described herein, and each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^r is of the formula



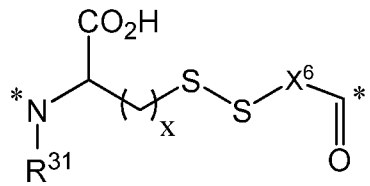
wherein R^{31}, X^6 and x are as described herein, and each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^r is of the formula



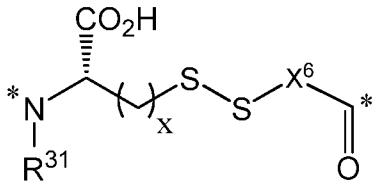
wherein R^{31}, X^6 and x are as described herein, and each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^r is of the formula



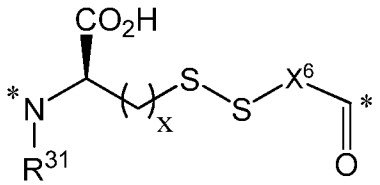
wherein R^{31}, X^6 and x are as described herein, and each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^r is of the formula



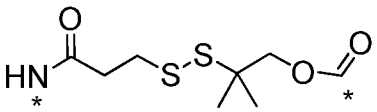
wherein R^{31}, X^6 and x are as described herein, and each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^r is of the formula



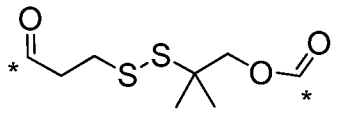
wherein R^{31}, X^6 and x are as described herein, and each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^r is of the formula



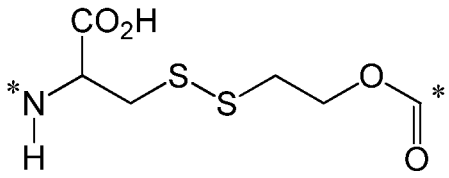
wherein each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^r is of the formula



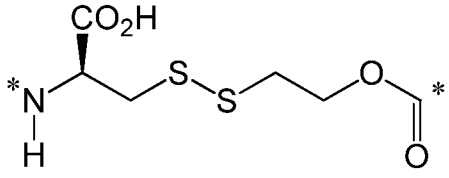
wherein each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^2 is of the formula



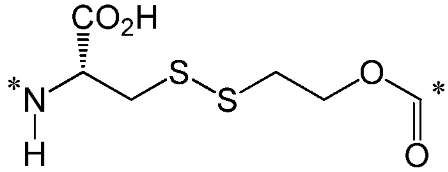
wherein each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^2 is of the formula



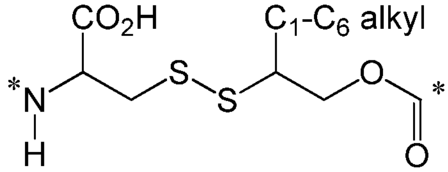
wherein each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^2 is of the formula



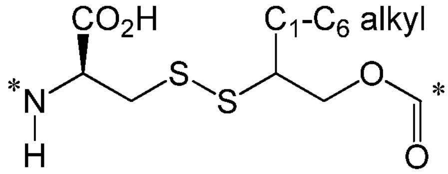
wherein each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^2 is of the formula



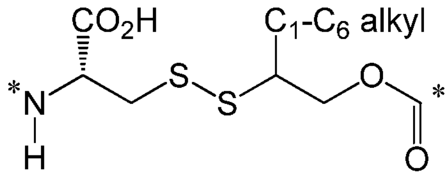
wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, C_1-C_6 alkyl is methyl, ethyl, or isopropyl.

In some aspects of embodiment 2, L^2 is of the formula



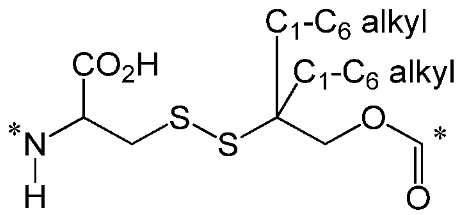
wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, C_1-C_6 alkyl is methyl, ethyl, or isopropyl.

In some aspects of embodiment 2, L^2 is of the formula



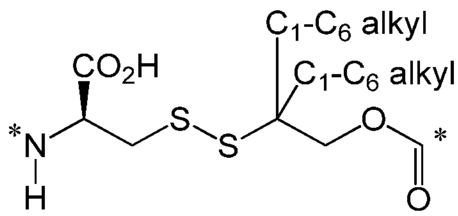
wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, C_1-C_6 alkyl is methyl, ethyl, or isopropyl.

In some aspects of embodiment 2, L^2 is of the formula



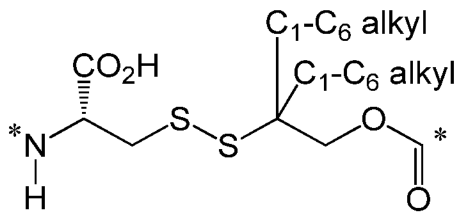
wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, each C_1-C_6 alkyl is methyl.

In some aspects of embodiment 2, L^2 is of the formula



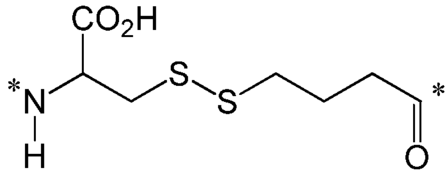
wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, each C_1-C_6 alkyl is methyl.

In some aspects of embodiment 2, L^2 is of the formula



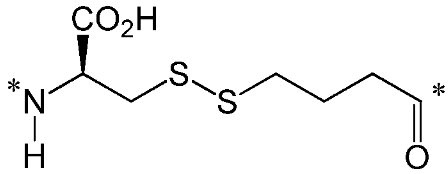
wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, each C_1-C_6 alkyl is methyl.

In some aspects of embodiment 2, L^2 is of the formula



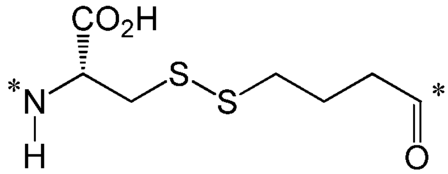
wherein each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^2 is of the formula



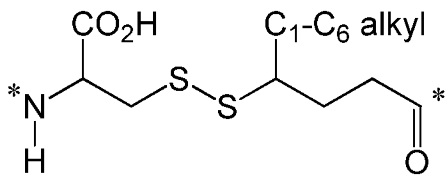
wherein each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^2 is of the formula



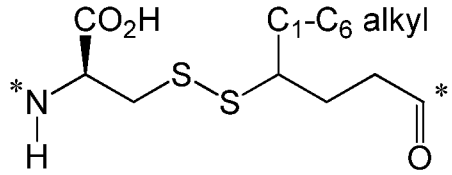
wherein each * represents a covalent bond to the rest of the conjugate.

In some aspects of embodiment 2, L^2 is of the formula



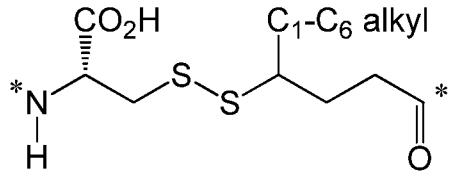
wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, C_1-C_6 alkyl is methyl, ethyl, or isopropyl.

In some aspects of embodiment 2, L^2 is of the formula



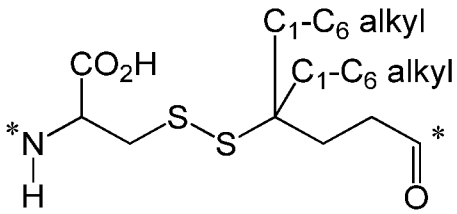
wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, C_1-C_6 alkyl is methyl, ethyl, or isopropyl.

In some aspects of embodiment 2, L^2 is of the formula



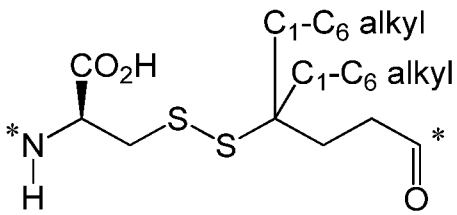
wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, C_1-C_6 alkyl is methyl, ethyl, or isopropyl.

In some aspects of embodiment 2, L^2 is of the formula



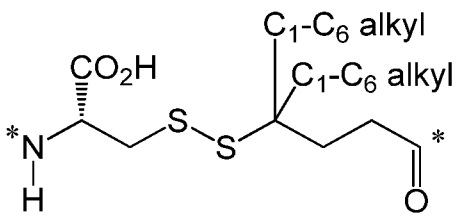
wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, each C_1-C_6 alkyl is methyl.

In some aspects of embodiment 2, L^2 is of the formula



wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, each C_1-C_6 alkyl is methyl.

In some aspects of embodiment 2, L^2 is of the formula



wherein each * represents a covalent bond to the rest of the conjugate. In some aspects, each C_1-C_6 alkyl is methyl.

L^2 can be present or absent in the conjugates described herein. When L^2 is present, L^2 can be any group covalently attaching portions of the linker to the binding ligand, portions of the linker to one another, or to D^1, or to D^2. It will be understood that the structure of L^2 is not particularly limited in any way. It will be further understood that L^2 can comprise numerous functionalities well known in the art to covalently attach portions of the linker to the binding ligand, portions of the linker to one another, or to D^1, or to D^2, including but not limited to, alkyl groups, ether groups, amide groups, carboxy groups, sulfonate groups, alkenyl groups, alkynyl groups, cycloalkyl groups, aryl groups, heterocycloalkyl, heteroaryl groups, and the like. In some embodiments, L^2 is selected from the group consisting of C_1-C_6 alkyl, -OC_1-C_6 alkyl, -SC_1-C_6 alkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-NR^{36}(CR^{36'}R^{36''})_x$-S-(succinimid-1-yl)-, $-(CR^{36'}R^{36''})_rC(O)NR^{36}$-, $-(CR^{39}R^{39'})_rC(O)$-, $-(CR^{39}R^{39'})_rOC(O)$-, $-S(CR^{39}R^{39'})_rOC(O)$-, $-C(O)(CR^{39}R^{39'})_r$-, $-C(O)O(CR^{39}R^{39'})_r$-, $-NR^{39}C(O)(CR^{39'}R^{39''})_r$-, $-NR^{39}C(O)(CR^{39'}R^{39''})_rS$-, $-(CH_2)_rNR^{39}$-, $-NR^{39}(CH_2)_r$-, $-NR^{39}(CH_2)_rS$-, $-NR^{39}(CH_2)_rNR^{39'}$-, $-(OCR^{39}R^{39'}CR^{39}R^{39'})_rC(O)$-, $-(OCR^{39}R^{39'}CR^{39}R^{39'}CR^{39}R^{39'})_rC(O)$-, $-OC(O)(CR^{44}R^{44'})_t$-, $-C(O)(CR^{44}R^{44'})_t$-, $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_t$-, $-CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tNR^{42}$-, $-NR^{42}C_6$-C_{10} aryl(C_1-C_6 alkyl)OC(O)-, $-C(O)CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tNR^{42}$-, $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tC(O)$-, and $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(CR^{44}{=}CR^{44'})_t$-;

wherein

each R^{36}, $R^{36'}$ and $R^{36''}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, $-C(O)R^{37}$, $-C(O)OR^{37}$ and $-C(O)NR^{37}R^{37'}$ wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{37}$, $-OC(O)R^{37}$, $-OC(O)NR^{37}R^{37'}$, $-OS(O)R^{37}$, $-OS(O)_2R^{37}$, $-SR^{37}$, $-S(O)R^{37}$, $-S(O)_2R^{37}$, $-S(O)NR^{37}R^{37'}$, $-S(O)_2NR^{37}R^{37'}$, $-OS(O)NR^{37}R^{37'}$, $-OS(O)_2NR^{37}R^{37'}$, $-NR^{37}R^{37'}$, $-NR^{37}C(O)R^{38}$, $-NR^{37}C(O)OR^{38}$, $-NR^{37}C(O)NR^{38}R^{38'}$, $-NR^{37}S(O)R^{38}$, $-NR^{37}S(O)_2R^{38}$, $-NR^{37}S(O)NR^{38}R^{38'}$, $-NR^{37}S(O)_2NR^{38}R^{38'}$, $-C(O)R^{37}$, $-C(O)OR^{37}$ or $-C(O)NR^{37}R^{37'}$;

R^{37}, $R^{37'}$, R^{38} and $R^{38'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

each R^{39} and $R^{39'}$ is independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered

heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{40}$, $-OC(O)R^{40}$, $-OC(O)NR^{40}R^{40'}$, $-OS(O)R^{40}$, $-OS(O)_2R^{40}$, $-SR^{40}$, $-S(O)R^{40}$, $-S(O)_2R^{40}$, $-S(O)NR^{40}R^{40'}$, $-S(O)_2NR^{40}R^{40'}$, $-OS(O)NR^{40}R^{40'}$, $-OS(O)_2NR^{40}R^{40'}$, $-NR^{40}R^{40'}$, $-NR^{40}C(O)R^{41}$, $-NR^{40}C(O)OR^{41}$, $-NR^{40}C(O)NR^{41}R^{41'}$, $-NR^{40}S(O)R^{41}$, $-NR^{40}S(O)_2R^{41}$, $-NR^{40}S(O)NR^{41}R^{41'}$, $-NR^{40}S(O)_2NR^{41}R^{41'}$, $-C(O)R^{40}$, $-C(O)OR^{40}$ and $-C(O)NR^{40}R^{40'}$;

R^{40}, $R^{40'}$, R^{41} and $R^{41'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, and 5- to 7-membered heteroaryl; and

R^{42} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{45}$, $-OC(O)R^{45}$, $-OC(O)NR^{45}R^{45'}$, $-OS(O)R^{45}$, $-OS(O)_2R^{45}$, $-SR^{45}$, $-S(O)R^{45}$, $-S(O)_2R^{45}$, $-S(O)NR^{45}R^{45'}$, $-S(O)_2NR^{45}R^{45'}$, $-OS(O)NR^{45}R^{45'}$, $-OS(O)_2NR^{45}R^{45'}$, $-NR^{45}R^{45'}$, $-NR^{45}C(O)R^{46}$, $-NR^{45}C(O)OR^{46}$, $-NR^{45}C(O)NR^{46}R^{46'}$, $-NR^{45}S(O)R^{46}$, $-NR^{45}S(O)_2R^{46}$, $-NR^{45}S(O)NR^{46}R^{46'}$, $-NR^{45}S(O)_2NR^{46}R^{46'}$, $-C(O)R^{45}$, $-C(O)OR^{45}$ or $-C(O)NR^{45}R^{45'}$,

each R^{43}, $R^{43'}$, R^{44} and $R^{44'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{47}$, $-OC(O)R^{47}$, $-OC(O)NR^{47}R^{47'}$, $-OS(O)R^{47}$, $-OS(O)_2R^{47}$, $-SR^{47}$, $-S(O)R^{47}$, $-S(O)_2R^{47}$, $-S(O)NR^{47}R^{47'}$, $-S(O)_2NR^{47}R^{47'}$, $-OS(O)NR^{47}R^{47'}$, $-OS(O)_2NR^{47}R^{47'}$, $-NR^{47}R^{47'}$, $-NR^{47}C(O)R^{48}$, $-NR^{47}C(O)OR^{48}$, $-NR^{47}C(O)NR^{48}R^{48'}$, $-NR^{47}S(O)R^{48}$, $-NR^{47}S(O)_2R^{48}$, $-NR^{47}S(O)NR^{48}R^{48'}$, $-NR^{47}S(O)_2NR^{48}R^{48'}$, $-C(O)R^{47}$, $-C(O)OR^{47}$ or $-C(O)NR^{47}R^{47'}$;

R^{45}, $R^{45'}$, R^{46}, $R^{46'}$, R^{47}, $R^{47'}$, R^{48} and $R^{48'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

r in each instance is an integer from 1 to 40; and

t is in each instance is an integer from 1 to 40.

In some aspects of the conjugates described herein in connection with either embodiment 1 or embodiment 2, L^2 is present. In some aspects of the conjugates described herein in connection with either embodiment 1 or embodiment 2, L^2 is absent.

With respect to embodiment 1: In some aspects, z7 is 0. In some aspects, z7 is 1. In some aspects, z7 is 2. In some aspects, z7 is 3. In some aspects, z7 is 4. In some aspects, z7 is 5. In some aspects, z7 is 6. In some aspects, z7 is 7.

With respect to embodiment 2: In some aspects, z7 is 0. In some aspects, z7 is 1. In some aspects, z7 is 2. In some aspects, z7 is 3. In some aspects, z7 is 4. In some aspects, z7 is 5. In some aspects, z7 is 6. In some aspects, z7 is 7.

In some aspects of embodiment 1, at least one L^2 is a PEG linker. In some aspects, at least one L^2 is $-(OCR^{39}R^{39'}CR^{39}R^{39'})_rC(O)-$, r is 4, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, at least one L^2 is $-(OCR^{39}R^{39'}CR^{39}R^{39'})_rC(O)-$, r is 12, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, at least one L^2 is $-(OCR^{39}R^{39'}CR^{39}R^{39'})_rC(O)-$, r is 36, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_t-$, t is 4, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_t-$, t is 12, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_t-$, t is 36, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tC(O)-$, t is 4, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tC(O)-$, t is 12, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tC(O)-$, t is 36, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H.

In some aspects of embodiment 2, at least one L^2 is a PEG linker. In some aspects, at least one L^2 is $-(OCR^{39}R^{39'}CR^{39}R^{39'})_rC(O)-$, r is 4, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, at least one L^2 is $-(OCR^{39}R^{39'}CR^{39}R^{39'})_rC(O)-$, r is 12, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, at least one L^2 is $-(OCR^{39}R^{39'}CR^{39}R^{39'})_rC(O)-$, r is 36, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_t-$, t is 4, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_t-$, t is 12, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_t-$, t is 36, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tC(O)-$, t is 4, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tC(O)-$, t is 12, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H. In some aspects, at least one L^2 is $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tC(O)-$, t is 36, and each R^{42}, R^{43}, $R^{43'}$, R^4, and $R^{44'}$ is H.

With respect to embodiment 1:

In some aspects, at least one L^2 is $-(CR^{39}R^{39'})_rC(O)-$. In some aspects, L^2 is $-(CR^{39}R^{39'})_rC(O)-$, r is 5, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, L^2 is $-(CR^{39}R^{39'})_rC(O)-$, r is 4, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, L^2 is $-(CR^{39}R^{39'})_rC(O)-$, r is 3, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, L^2 is $-(CR^{39}R^{39'})_rC(O)-$, r is 2, each R^{39} is H, and each $R^{39'}$ is H.

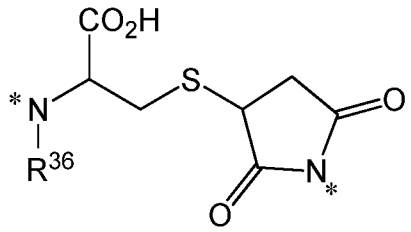
In some aspects, at least one L^2 is $-(CR^{36'}R^{36''})_rC(O)NR^{36}-$. In some aspects, L^2 is $-(CR^{36'}R^{36''})_rC(O)NR^{36}-$, r is 5, each R^{36}, R^{36}, $R^{36''}$ is H. In some aspects, L^2 is $-(CR^{36'}R^{36''})_rC(O)NR^{36}-$, r is 4, each R^{36}, R^{36}, $R^{36''}$ is H. In some aspects, L^2 is $-(CR^{36'}R^{36''})_rC(O)NR^{36}-$, r is 3, each R^{36}, R^{36}, $R^{36''}$ is H. In some aspects, L^2 is $-(CR^{36'}R^{36''})_rC(O)NR^{36}-$, r is 2, each R^{36}, R^{36}, $R^{36''}$ is H.

In some aspects, at least one L^2 is $-S(CR^{39}R^{39'})_rOC(O)-$. In some aspects, r is 4. In some aspects, r is 3. In some aspects, r is 2. In some aspects, at least one L^2 is $-NR^{39}C(O)(CR^{39'}R^{39''})_rS-$. In some aspects, at least one L^2 is $-NR^{39}C(O)(CR^{39'}R^{39''})_rS-$, r is 4, and each of R^{39}, $R^{39'}$ and $R^{39''}$ is H. In some aspects, at least one L^2 is $-NR^{39}C(O)(CR^{39'}R^{39''})_rS-$, r is 3, and each of R^{39}, $R^{39'}$ and $R^{39''}$ is H. In some aspects, at least one L^2 is $-NR^{39}C(O)(CR^{39'}R^{39''})_rS-$, r is 2, and each of R^{39}, $R^{39'}$ and $R^{39''}$ is H.

In some aspects, at least one L^2 is $-(CH_2)_rNR^{39}-$, r is 5 and R^{39} is H. In some aspects, at least one L^2 is $-(CH_2)_rNR^{39}-$, r is 4 and R^{39} is H. In some aspects, at least one L^2 is $-(CH_2)_rNR^{39}-$, r is 3 and R^{39} is H. In some aspects, at least one L^2 is $-(CH_2)_rNR^{39}-$, r is 2 and R^{39} is H.

In some aspects, at least one L^2 is $-NR^{39}(CH_2)_r-$, r is 5 and R^{39} is H. In some aspects, at least one L^2 is $-NR^{39}(CH_2)_r-$, r is 4 and R^{39} is H. In some aspects, at least one L^2 is $-NR^{39}(CH_2)_r-$, r is 3 and R^{39} is H. In some aspects, at least one L^2 is $-NR^{39}(CH_2)_r-$, r is 2 and R^{39} is H.

In some aspects, at least one L^2 is



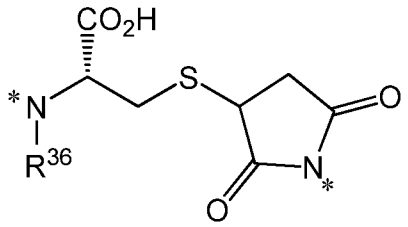
R^{36} is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{37}$, $-OC(O)R^{37}$, $-OC(O)NR^{37}R^{37'}$, $-OS(O)R^{37}$, $-OS(O)_2R^{37}$, $-SR^{37}$, $-S(O)R^{37}$, -

$S(O)_2R^{37}$, $-S(O)NR^{37}R^{37'}$, $-S(O)_2NR^{37}R^{37'}$, $-OS(O)NR^{37}R^{37'}$, $-OS(O)_2NR^{37}R^{37'}$, $-NR^{37}R^{37'}$, $-NR^{37}C(O)R^{38}$, $-NR^{37}C(O)OR^{38}$, $-NR^{37}C(O)NR^{38}R^{38'}$, $-NR^{37}S(O)R^{38}$, $-NR^{37}S(O)_2R^{38}$, $-NR^{37}S(O)NR^{38}R^{38'}$, $-NR^{37}S(O)_2NR^{38}R^{38'}$, $-C(O)R^{37}$, $-C(O)OR^{37}$ or $-C(O)NR^{37}R^{37'}$;

R^{37}, $R^{37'}$, R^{38} and $R^{38'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

* is a covalent bond. In some embodiments, R^{36} is H.

In some aspects, at least one L^2 is

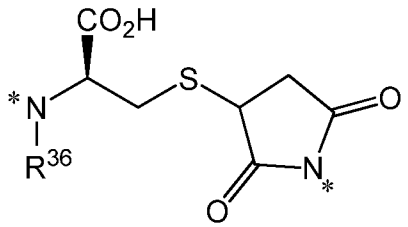


R^{36} is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{37}$, $-OC(O)R^{37}$, $-OC(O)NR^{37}R^{37'}$, $-OS(O)R^{37}$, $-OS(O)_2R^{37}$, $-SR^{37}$, $-S(O)R^{37}$, $-S(O)_2R^{37}$, $-S(O)NR^{37}R^{37'}$, $-S(O)_2NR^{37}R^{37'}$, $-OS(O)NR^{37}R^{37'}$, $-OS(O)_2NR^{37}R^{37'}$, $-NR^{37}R^{37'}$, $-NR^{37}C(O)R^{38}$, $-NR^{37}C(O)OR^{38}$, $-NR^{37}C(O)NR^{38}R^{38'}$, $-NR^{37}S(O)R^{38}$, $-NR^{37}S(O)_2R^{38}$, $-NR^{37}S(O)NR^{38}R^{38'}$, $-NR^{37}S(O)_2NR^{38}R^{38'}$, $-C(O)R^{37}$, $-C(O)OR^{37}$ or $-C(O)NR^{37}R^{37'}$;

R^{37}, $R^{37'}$, R^{38} and $R^{38'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

* is a covalent bond. In some embodiments, R^{36} is H.

In some aspects, at least one L^2 is



R^{36} is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered

heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{37}, -$OC(O)R^{37}$, -$OC(O)NR^{37}R^{37'}$, -$OS(O)R^{37}$, -$OS(O)_2R^{37}$, -SR^{37}, -$S(O)R^{37}$, -$S(O)_2R^{37}$, -$S(O)NR^{37}R^{37'}$, -$S(O)_2NR^{37}R^{37'}$, -$OS(O)NR^{37}R^{37'}$, -$OS(O)_2NR^{37}R^{37'}$, -$NR^{37}R^{37'}$, -$NR^{37}C(O)R^{38}$, -$NR^{37}C(O)OR^{38}$, -$NR^{37}C(O)NR^{38}R^{38'}$, -$NR^{37}S(O)R^{38}$, -$NR^{37}S(O)_2R^{38}$, -$NR^{37}S(O)NR^{38}R^{38'}$, -$NR^{37}S(O)_2NR^{38}R^{38'}$, -$C(O)R^{37}$, -$C(O)OR^{37}$ or -$C(O)NR^{37}R^{37'}$;

R^{37}, $R^{37'}$, R^{38} and $R^{38'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

* is a covalent bond. In some embodiments, R^{36} is H.

With respect to embodiment 2:

In some aspects, at least one L^2 is –$(CR^{39}R^{39'})_rC(O)$-. In some aspects, L^2 is -$(CR^{39}R^{39'})_rC(O)$-, r is 5, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, L^2 is -$(CR^{39}R^{39'})_rC(O)$-, r is 4, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, L^2 is -$(CR^{39}R^{39'})_rC(O)$-, r is 3, each R^{39} is H, and each $R^{39'}$ is H. In some aspects, L^2 is -$(CR^{39}R^{39'})_rC(O)$-, r is 2, each R^{39} is H, and each $R^{39'}$ is H.

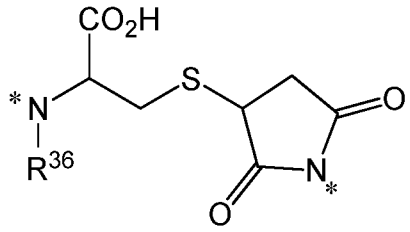
In some aspects, at least one L^2 is -$(CR^{36'}R^{36''})_rC(O)NR^{36}$-. In some aspects, L^2 is -$(CR^{36'}R^{36''})_rC(O)NR^{36}$-, r is 5, each R^{36}, R^{36}, $R^{36''}$ is H. In some aspects, L^2 is -$(CR^{36'}R^{36''})_r$-$C(O)NR^{36}$-, r is 4, each R^{36}, R^{36}, $R^{36''}$ is H. In some aspects, L^2 is -$(CR^{36'}R^{36''})_rC(O)NR^{36}$-, r is 3, each R^{36}, R^{36}, $R^{36''}$ is H. In some aspects, L^2 is -$(CR^{36'}R^{36''})_rC(O)NR^{36}$-, r is 2, each R^{36}, R^{36}, $R^{36''}$ is H.

In some aspects, at least one L^2 is -$S(CR^{39}R^{39'})_rOC(O)$-. In some aspects, r is 4. In some aspects, r is 3. In some aspects, r is 2. In some aspects, at least one L^2 is -$NR^{39}C(O)(CR^{39'}R^{39''})_rS$-. In some aspects, at least one L^2 is -$NR^{39}C(O)(CR^{39'}R^{39''})_rS$-, r is 4, and each of R^{39}, $R^{39'}$ and $R^{39''}$ is H. In some aspects, at least one L^2 is -$NR^{39}C(O)(CR^{39'}R^{39''})_rS$-, r is 3, and each of R^{39}, $R^{39'}$ and $R^{39''}$ is H. In some aspects, at least one L^2 is -$NR^{39}C(O)(CR^{39'}R^{39''})_rS$-, r is 2, and each of R^{39}, $R^{39'}$ and $R^{39''}$ is H.

In some aspects, at least one L^2 is –$(CH_2)_rNR^{39}$-, r is 5 and R^{39} is H. In some aspects, at least one L^2 is –$(CH_2)_rNR^{39}$-, r is 4 and R^{39} is H. In some aspects, at least one L^2 is –$(CH_2)_rNR^{39}$-, r is 3 and R^{39} is H. In some aspects, at least one L^2 is –$(CH_2)_rNR^{39}$-, r is 2 and R^{39} is H.

In some aspects, at least one L^2 is -$NR^{39}(CH_2)_r$-, r is 5 and R^{39} is H. In some aspects, at least one L^2 is -$NR^{39}(CH_2)_r$-, r is 4 and R^{39} is H. In some aspects, at least one L^2 is -$NR^{39}(CH_2)_r$-, r is 3 and R^{39} is H. In some aspects, at least one L^2 is -$NR^{39}(CH_2)_r$-, r is 2 and R^{39} is H.

In some aspects, at least one L^2 is

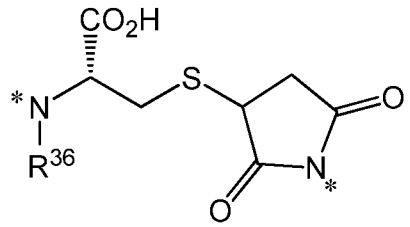


R^{36} is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{37}$, $-OC(O)R^{37}$, $-OC(O)NR^{37}R^{37'}$, $-OS(O)R^{37}$, $-OS(O)_2R^{37}$, $-SR^{37}$, $-S(O)R^{37}$, $-S(O)_2R^{37}$, $-S(O)NR^{37}R^{37'}$, $-S(O)_2NR^{37}R^{37'}$, $-OS(O)NR^{37}R^{37'}$, $-OS(O)_2NR^{37}R^{37'}$, $-NR^{37}R^{37'}$, $-NR^{37}C(O)R^{38}$, $-NR^{37}C(O)OR^{38}$, $-NR^{37}C(O)NR^{38}R^{38'}$, $-NR^{37}S(O)R^{38}$, $-NR^{37}S(O)_2R^{38}$, $-NR^{37}S(O)NR^{38}R^{38'}$, $-NR^{37}S(O)_2NR^{38}R^{38'}$, $-C(O)R^{37}$, $-C(O)OR^{37}$ or $-C(O)NR^{37}R^{37'}$;

R^{37}, $R^{37'}$, R^{38} and $R^{38'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

* is a covalent bond. In some embodiments, R^{36} is H.

In some aspects, at least one L^2 is

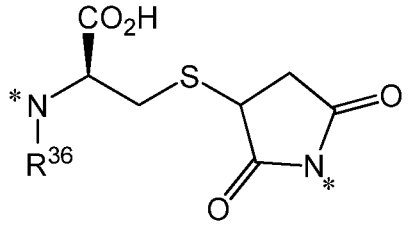


R^{36} is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{37}$, $-OC(O)R^{37}$, $-OC(O)NR^{37}R^{37'}$, $-OS(O)R^{37}$, $-OS(O)_2R^{37}$, $-SR^{37}$, $-S(O)R^{37}$, $-S(O)_2R^{37}$, $-S(O)NR^{37}R^{37'}$, $-S(O)_2NR^{37}R^{37'}$, $-OS(O)NR^{37}R^{37'}$, $-OS(O)_2NR^{37}R^{37'}$, $-NR^{37}R^{37'}$, $-NR^{37}C(O)R^{38}$, $-NR^{37}C(O)OR^{38}$, $-NR^{37}C(O)NR^{38}R^{38'}$, $-NR^{37}S(O)R^{38}$, $-NR^{37}S(O)_2R^{38}$, $-NR^{37}S(O)NR^{38}R^{38'}$, $-NR^{37}S(O)_2NR^{38}R^{38'}$, $-C(O)R^{37}$, $-C(O)OR^{37}$ or $-C(O)NR^{37}R^{37'}$;

R^{37}, $R^{37'}$, R^{38} and $R^{38'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

* is a covalent bond. In some embodiments, R^{36} is H.

In some aspects, at least one L^2 is



R^{36} is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{37}$, $-OC(O)R^{37}$, $-OC(O)NR^{37}R^{37'}$, $-OS(O)R^{37}$, $-OS(O)_2R^{37}$, $-SR^{37}$, $-S(O)R^{37}$, $-S(O)_2R^{37}$, $-S(O)NR^{37}R^{37'}$, $-S(O)_2NR^{37}R^{37'}$, $-OS(O)NR^{37}R^{37'}$, $-OS(O)_2NR^{37}R^{37'}$, $-NR^{37}R^{37'}$, $-NR^{37}C(O)R^{38}$, $-NR^{37}C(O)OR^{38}$, $-NR^{37}C(O)NR^{38}R^{38'}$, $-NR^{37}S(O)R^{38}$, $-NR^{37}S(O)_2R^{38}$, $-NR^{37}S(O)NR^{38}R^{38'}$, $-NR^{37}S(O)_2NR^{38}R^{38'}$, $-C(O)R^{37}$, $-C(O)OR^{37}$ or $-C(O)NR^{37}R^{37'}$;

R^{37}, $R^{37'}$, R^{38} and $R^{38'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

* is a covalent bond. In some embodiments, R^{36} is H.

It will be appreacited that L^3 can any linker covalently attaching D^1 to D^2. Specifically, the structure of L^3 is not particularly limited in any way in connection with either embodiment 1 or embodiment 2. It will be further understood that L^3 can comprise numerous functionalities well known in the art to covalently attach D^1 to D^2, including but not limited to, alkyl groups, ether groups, amide groups, carboxy groups, sulfonate groups, alkenyl groups, alkynyl groups, cycloalkyl groups, aryl groups, heterocycloalkyl, heteroaryl groups, and the like. In some embodiments, L^3 is selected from the group consisting of C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, $-(CR^{49}R^{49'})_uC(O)-$, $-CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'})_u-$, $-CH_2CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'}CR^{49}R^{49'})_u-$, $-CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'})_uC(O)-$ and $-CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'}CR^{49}R^{49'})_uC(O)-$,

wherein

each R^{49} and $R^{49'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered

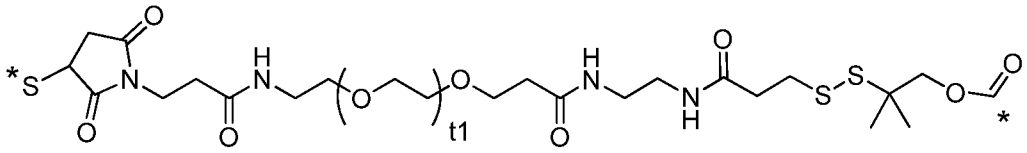
heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{50}, -$OC(O)R^{50}$, -$OC(O)NR^{50}R^{50'}$, -$OS(O)R^{50}$, -$OS(O)_2R^{50}$, -SR^{50}, -$S(O)R^{50}$, -$S(O)_2R^{50}$, -$S(O)NR^{50}R^{50'}$, -$S(O)_2NR^{50}R^{50'}$, -$OS(O)NR^{50}R^{50'}$, -$OS(O)_2NR^{50}R^{50'}$, -$NR^{50}R^{50'}$, -$NR^{50}C(O)R^{51}$, -$NR^{50}C(O)OR^{51}$, -$NR^{50}C(O)NR^{51}R^{51'}$, -$NR^{50}S(O)R^{51}$, -$NR^{50}S(O)_2R^{51}$, -$NR^{50}S(O)NR^{51}R^{51'}$, -$NR^{50}S(O)_2NR^{51}R^{51'}$, -$C(O)R^{50}$, -$C(O)OR^{50}$ or -$C(O)NR^{50}R^{50'}$;

R^{50}, $R^{50'}$, R^{51} and $R^{51'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

u is in each instance 0, 1, 2, 3, 4 or 5.

In some embodiments, L^3 is C_1-C_6 alkyl. In some embodiments, L^3 is –$(CR^{49}R^{49'})_uC(O)$-, wherein each R^{49} and $R^{49'}$ is H, and u is 3. In some embodiments, L^3 is –$(CR^{49}R^{49'})_uC(O)$-, wherein each R^{49} and $R^{49'}$ is H, and u is 4. In some embodiments, L^3 is –$(CR^{49}R^{49'})_uC(O)$-, wherein each R^{49} and $R^{49'}$ is H, and u is 5.

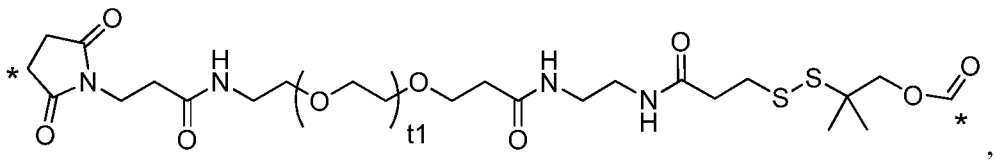
In some embodiments, the linker comprises the formula



,

wherein t1 if an integer from 0 to 39, and each * represents a covaltent bond to the rest of the conjugate.

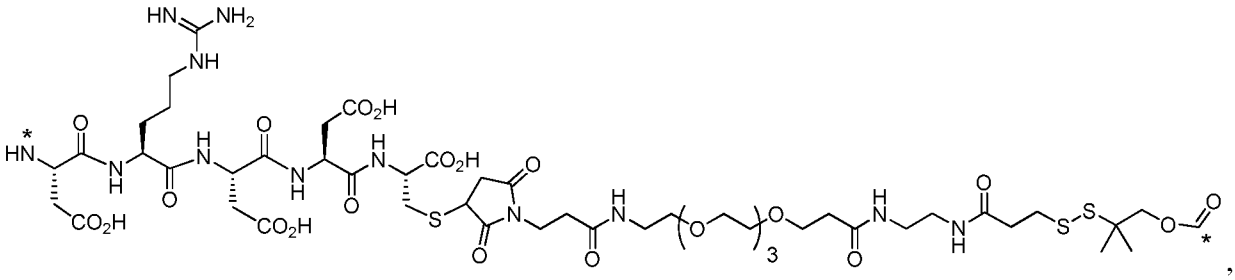
In some embodiments, the linker comprises the formula



,

wherein t1 if an integer from 0 to 39, and each * represents a covaltent bond to the rest of the conjugate.

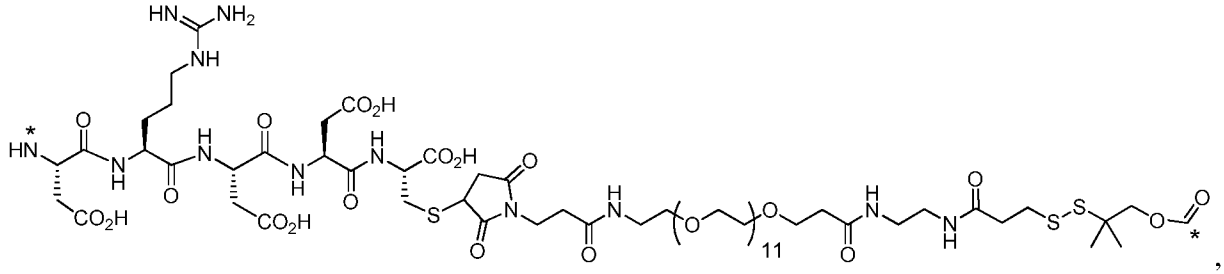
In some embodiments, the linker is of the formula



,

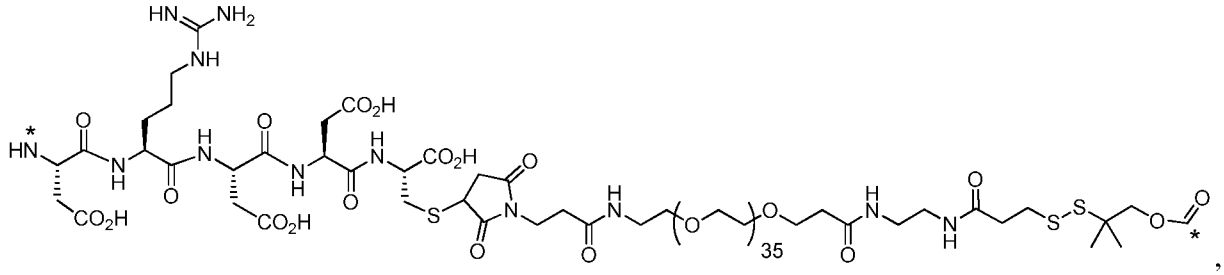
wherein each * represents a covaltent bond to the rest of the conjugate.

In some embodiments, the linker is of the formula



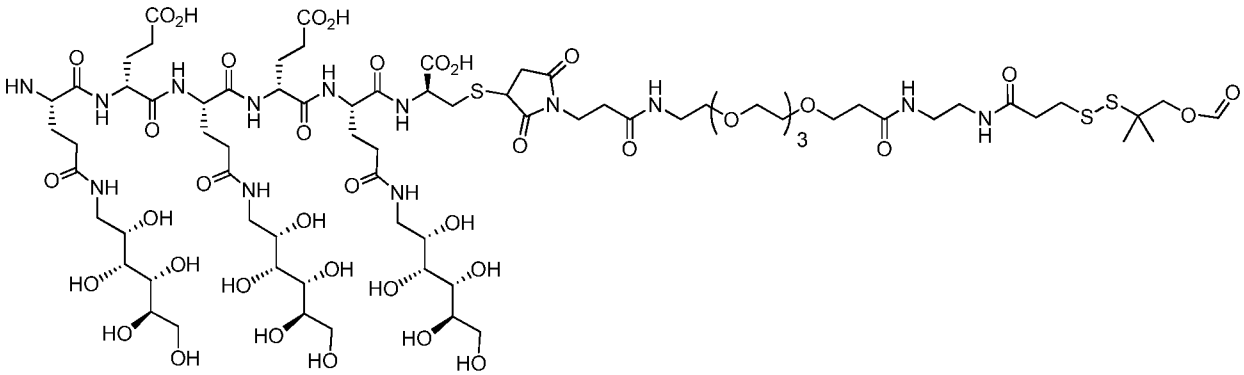
wherein each * represents a covalent bond to the rest of the conjugate.

In some embodiments, the linker is of the formula



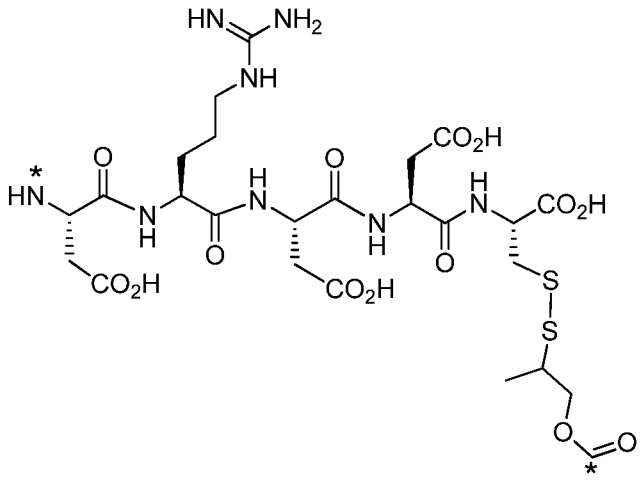
wherein each * represents a covalent bond to the rest of the conjugate.

In some embodiments, the linker is of the formula



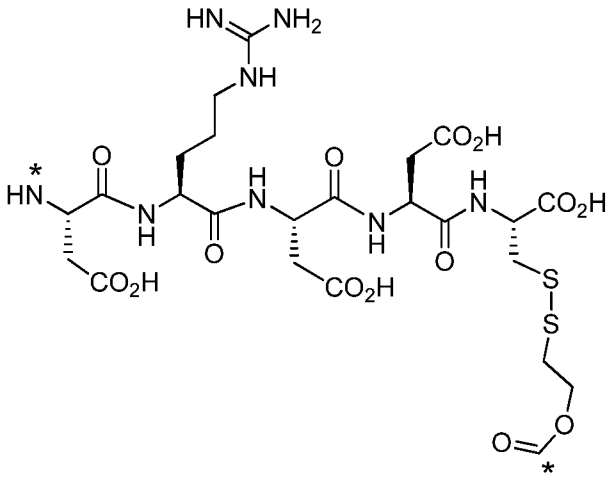
wherein each * represents a covalent bond to the rest of the conjugate.

In some embodiments, the linker is of the formula



wherein each * represents a covalent bond to the rest of the conjugate.

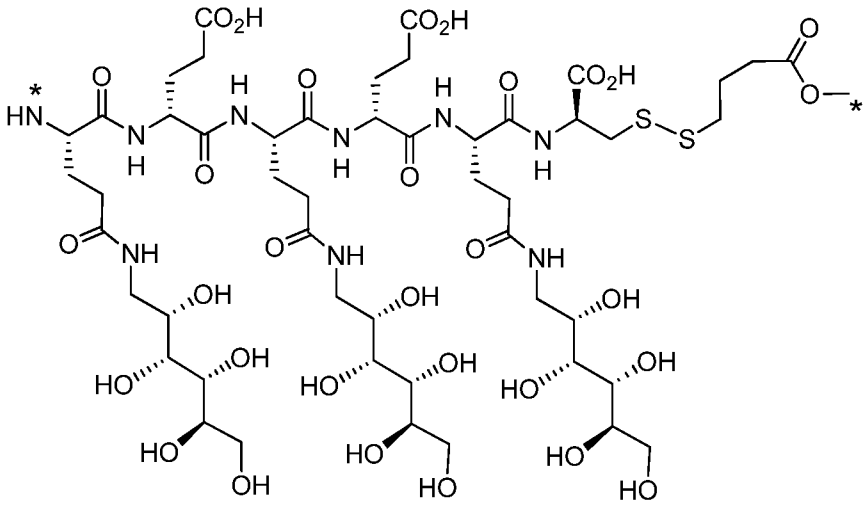
In some embodiments, the linker is of the formula



,

wherein each * represents a covaltent bond to the rest of the conjugate.

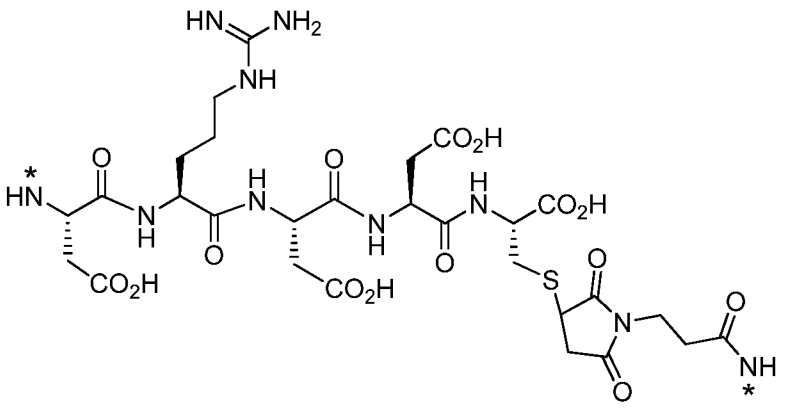
In some embodiments, the linker is of the formula



,

wherein each * represents a covaltent bond to the rest of the conjugate.

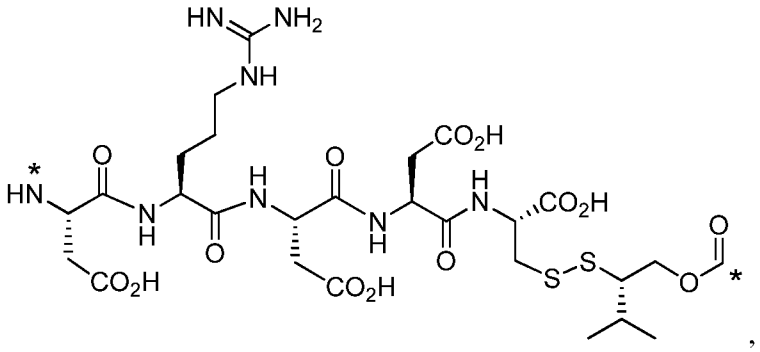
In some embodiments, the linker comprises the formula



,

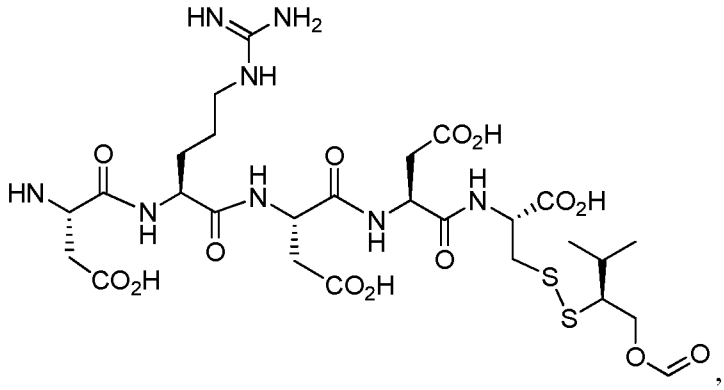
wherein each * represents a covaltent bond to the rest of the conjugate.

In some embodiments, the linker is the formula



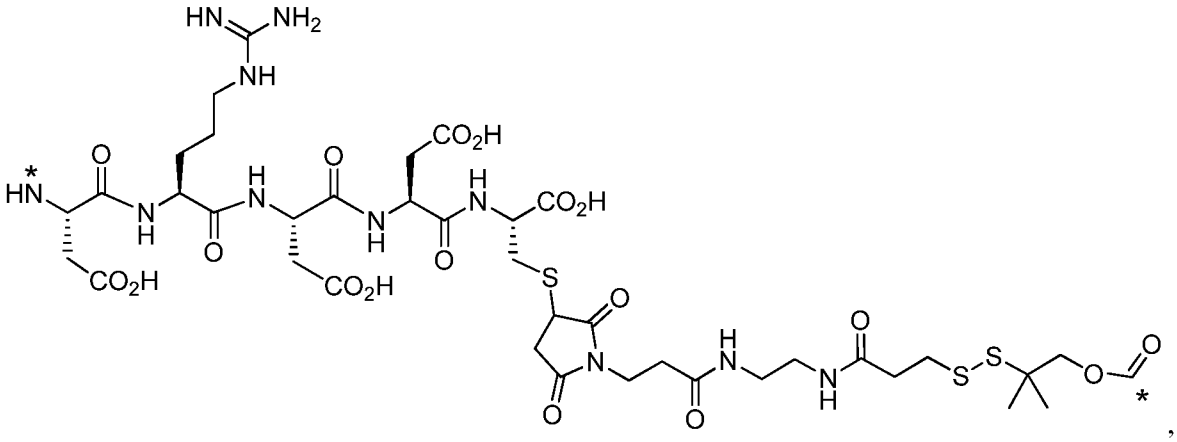
wherein each * represents a covalent bond to the rest of the conjugate.

In some embodiments, the linker is the formula



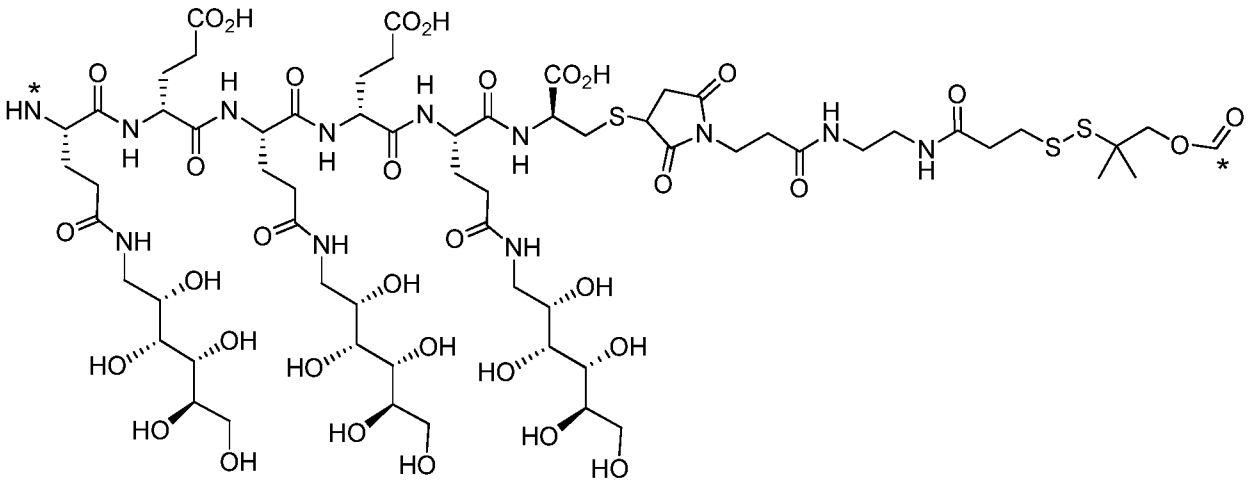
wherein each * represents a covalent bond to the rest of the conjugate.

In some embodiments, the linker is of the formula



wherein each * represents a covalent bond to the rest of the conjugate.

In some embodiments, the linker is of the formula



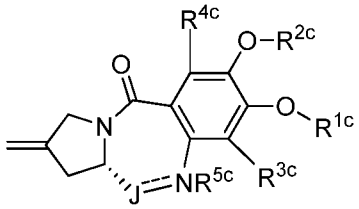
,

wherein each * represents a covaltent bond to the rest of the conjugate.

Drugs

The conjugates described herein comnprise the drugs D^1 and/or D^2, covalently attached to one or more linker portions of the linkers described herein, with the proviso that at least one drug D^1 or D^2 is a pyrrolobenzodiazepine (also referedn to herein as a PBD). In some embodiments, both D^1 and D^2 are PBD drugs. In some embodiments, the drug comprises the formula $-D^1-L^3-D^2$. In some embodiments, Drug comprises the structure $-D^1-L^3-D^2-$. In some embodiments, one of D^1 or D^2 is a PBD drug, and the other of D^1 or D^2 is a pyrrolobenzodiazepine pro-drug (also referred to herin as a PBD pro-drug or pro-PBD). It will be understood that such PBD prodrugs undergo conversion to a therapeutically active PBD compound through processes in the body after delivery of a conjugate as decribed herein. In some embodiments, at least one of the drugs incorporated into conjugates decribed herein is a PBD prodrug as described herein. It will be appreciated that the drugs are not particularly limited in any way with respect each either embodiment 1 or embodiment 2, with the proviso that at least one of D^1 or D^2 is a PBD. Accoridngly, the description of drugs for use in connection with the present teachings apply equally to both embodiment 1 and embodiment 2.

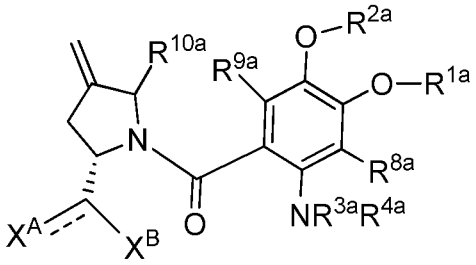
In some embodiments, the first drug or the second drug is a PBD of the formula



wherein

J, R^{1c}, R^{2c}, R^{3c}, R^{4c} and R^{5c} are each defined as described herein.

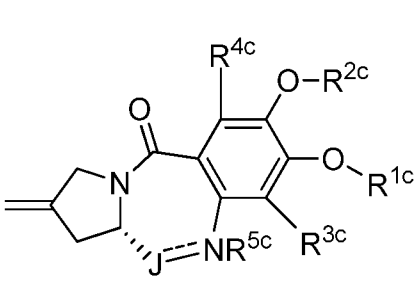
In some embodiemtns, the first drug is of the formula



wherein

X^A, X^B, R^{1a}, R^{2a}, R^{3a}, R^{4a}, R^{8a}, R^{9a} and R^{10a} are as defined herein.

In some embodiemtns, the second drug is selected from the group consisting of



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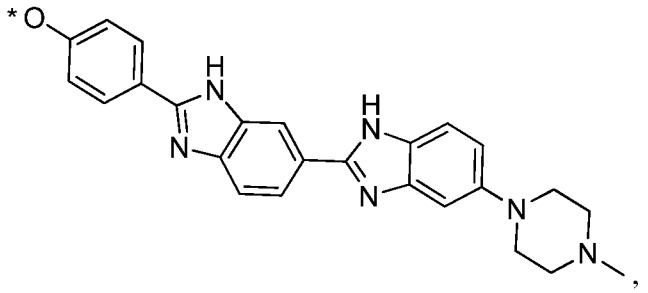

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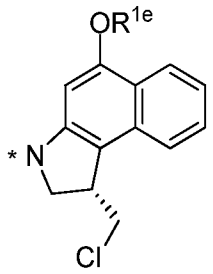

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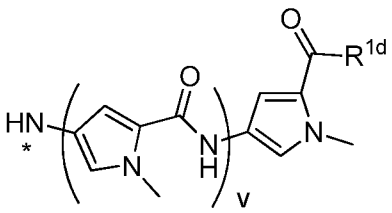

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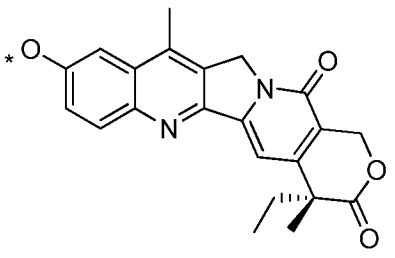
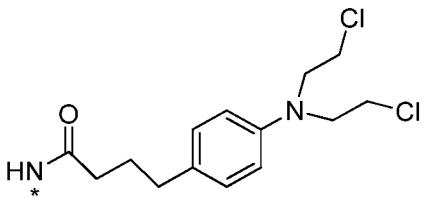


and



wherein

J is –C(O)-, $-CR^{13c}=$ or $-(CR^{13c}R^{13c'})-$;

R^{1c}, R^{2c} and R^{5c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-C(O)R^{6c}$, $-C(O)OR^{6c}$ and $-C(O)NR^{6c}R^{6c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{7c}$, $-OC(O)R^{7c}$, $-OC(O)NR^{7c}R^{7c'}$, $-OS(O)R^{7c}$, $-OS(O)_2R^{7c}$, $-SR^{7c}$, $-S(O)R^{7c}$, $-S(O)_2R^{7c}$, $-S(O)_2OR^{7c}$, $-S(O)NR^{7c}R^{7c'}$, $-S(O)_2NR^{7c}R^{7c'}$,

-OS(O)$NR^{7c}R^{7c'}$, -$OS(O)_2NR^{7c}R^{7c'}$, -$NR^{7c}R^{7c'}$, -$NR^{7c}C(O)R^{8c}$, -$NR^{7c}C(O)OR^{8c}$, -$NR^{7c}C(O)NR^{8c}R^{8c'}$, -$NR^{7c}S(O)R^{8c}$, -$NR^{7c}S(O)_2R^{8c}$, -$NR^{7c}S(O)NR^{8c}R^{8c'}$, -$NR^{7c}S(O)_2NR^{8c}R^{8c'}$, -$C(O)R^{7c}$, -$C(O)OR^{7c}$ or -$C(O)NR^{7c}R^{7c'}$; or when J is –CR^{13c}=, R^{5c} is absent; provided that at least one of of R^{1c}, R^{2c} or R^{5c} is a covalent bond to the rest of the conjugate;

R^{3c} and R^{4c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -CN, -NO_2, -NCO, -OR^{9c}, -$OC(O)R^{9c}$, -$OC(O)NR^{9c}R^{9c'}$, -$OS(O)R^{9c}$, -$OS(O)_2R^{9c}$, -SR^{9c}, -$S(O)R^{9c}$, -$S(O)_2R^{9c}$, -$S(O)NR^{9c}R^{9c'}$, -$S(O)_2NR^{9c}R^{9c'}$, -$OS(O)NR^{9c}R^{9c'}$, -$OS(O)_2NR^{9c}R^{9c'}$, -$NR^{9c}R^{9c'}$, -$NR^{9c}C(O)R^{10c}$, -$NR^{9c}C(O)OR^{10c}$, -$NR^{9c}C(O)NR^{10c}R^{10c'}$, -$NR^{9c}S(O)R^{10c}$, -$NR^{9c}S(O)_2R^{10c}$, -$NR^{9c}S(O)NR^{10c}R^{10c'}$, -$NR^{9c}S(O)_2NR^{10c}R^{10c'}$, -$C(O)R^{9c}$, -$C(O)OR^{9c}$ and -$C(O)NR^{9c}R^{9c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{11c}, -$OC(O)R^{11c}$, -$OC(O)NR^{11c}R^{11c'}$, -$OS(O)R^{11c}$, -$OS(O)_2R^{11c}$, -SR^{11c}, -$S(O)R^{11c}$, -$S(O)_2R^{11c}$, -$S(O)NR^{11c}R^{11c'}$, -$S(O)_2NR^{11c}R^{11c'}$, -$OS(O)NR^{11c}R^{11c'}$, -$OS(O)_2NR^{11c}R^{11c'}$, -$NR^{11c}R^{11c'}$, -$NR^{11c}C(O)R^{12c}$, -$NR^{11c}C(O)OR^{12c}$, -$NR^{11c}C(O)NR^{12c}R^{12c'}$, -$NR^{11c}S(O)R^{12c}$, -$NR^{11c}S(O)_2R^{12c}$, -$NR^{11c}S(O)NR^{12c}R^{12c'}$, -$NR^{11c}S(O)_2NR^{12c}R^{12c'}$, -$C(O)R^{11c}$, -$C(O)OR^{11c}$ or -$C(O)NR^{11c}R^{11c}$;

each R^{6c}, $R^{6c'}$, R^{7c}, $R^{7c'}$, R^{8c}, $R^{8c'}$, R^{9c}, $R^{9c'}$, R^{10c}, $R^{10c'}$, R^{11c}, $R^{11c'}$, R^{12c} and $R^{12c'}$ is independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

R^{13c} and $R^{13c'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{11c}, -$OC(O)R^{11c}$, -$OC(O)NR^{11c}R^{11c'}$, -$OS(O)R^{11c}$, -$OS(O)_2R^{11c}$, -SR^{11c}, -$S(O)R^{11c}$, -$S(O)_2R^{11c}$, -$S(O)NR^{11c}R^{11c'}$, -$S(O)_2NR^{11c}R^{11c'}$, -$OS(O)NR^{11c}R^{11c'}$, -$OS(O)_2NR^{11c}R^{11c'}$, -$NR^{11c}R^{11c'}$, -$NR^{11c}C(O)R^{12c}$, -$NR^{11c}C(O)OR^{12c}$, -$NR^{11c}C(O)NR^{12c}R^{12c'}$, -$NR^{11c}S(O)R^{12c}$, -$NR^{11c}S(O)_2R^{12c}$, -$NR^{11c}S(O)NR^{12c}R^{12c'}$, -$NR^{11c}S(O)_2NR^{12c}R^{12c'}$, -$C(O)R^{11c}$, -$C(O)OR^{11c}$ and -$C(O)NR^{11c}R^{11c}$;

R^{1d} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{2d}, -SR^{2d} and -$NR^{2d}R^{2d'}$,

R^{2d} and $R^{2d'}$ are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is optionally substituted by $-OR^{3d}$, $-SR^{3d}$, and $-NR^{3d}R^{3d'}$;

R^{3d} and $R^{3d'}$ are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

R^{1e} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{2e}$, $-OC(O)R^{2e}$, $-OC(O)NR^{2e}R^{2e'}$, $-OS(O)R^{2e}$, $-OS(O)_2R^{2e}$, $-SR^{2e}$, $-S(O)R^{2e}$, $-S(O)_2R^{2e}$, $-S(O)NR^{2e}R^{2e'}$, $-S(O)_2NR^{2e}R^{2e'}$, $-OS(O)NR^{2e}R^{2e'}$, $-OS(O)_2NR^{2e}R^{2e'}$, $-NR^{2e}R^{2e'}$, $-NR^{2e}C(O)R^{3e}$, $-NR^{2e}C(O)OR^{3e}$, $-NR^{2e}C(O)NR^{3e}R^{3e'}$, $-NR^{2e}S(O)R^{3e}$, $-NR^{2e}S(O)_2R^{3e}$, $-NR^{2e}S(O)NR^{2e}R^{2e'}$, $-NR^{2e}S(O)_2NR^{3e}R^{3e'}$, $-C(O)R^{2e}$, $-C(O)OR^{2e}$ or $-C(O)NR^{2e}R^{2e}$;

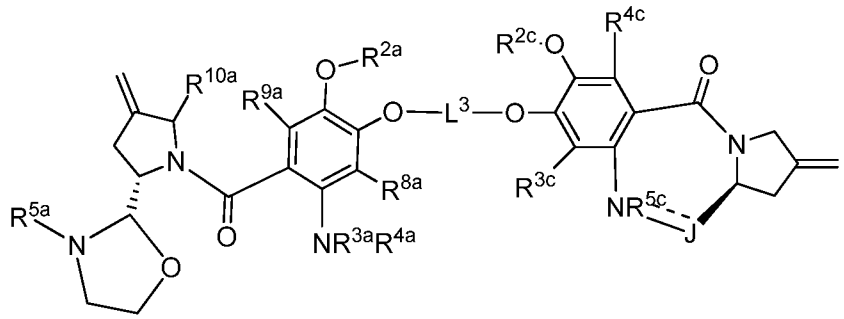
each R^{2e}, $R^{2e'}$, R^{3e} and $R^{3e'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is optionally substituted by $-OR^{4e}$, $-SR^{4e}$ or $-NR^{4e}R^{4e'}$;

R^{4e} and $R^{4e'}$ are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

v is 1, 2 or 3; and

each * represents a covalent bond to the rest of the conjugate.

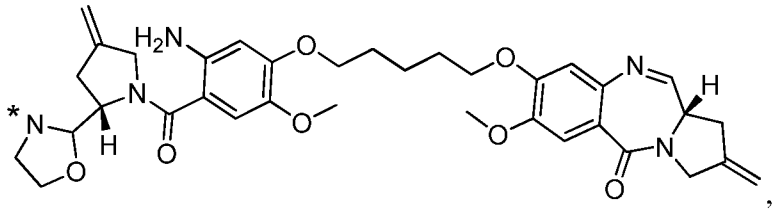
In some embodiments, the drug comprises the formula



,

wherein R^{5a} is a covalent bond to the rest of the conjugate.

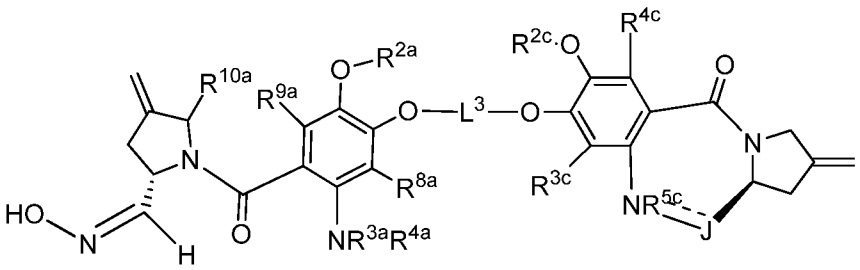
In some embodiments, the drug comprises the formula



,

wherein * represents a covalent bond to the rest of the conjugate.

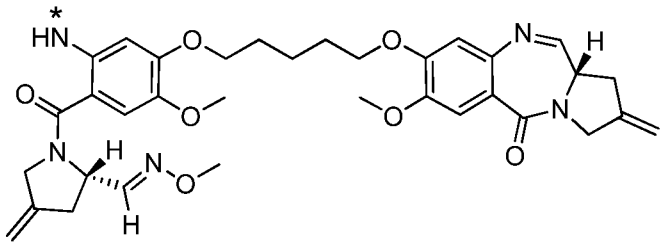
In some embodiments, the drug comprises the formula



,

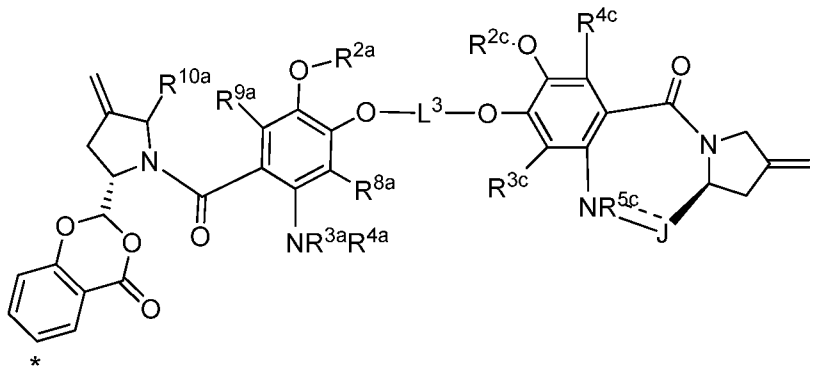
wherein R^{4a} is a covalent bond to the rest of the conjugate.

In some embodiments, the drug comprises the formula



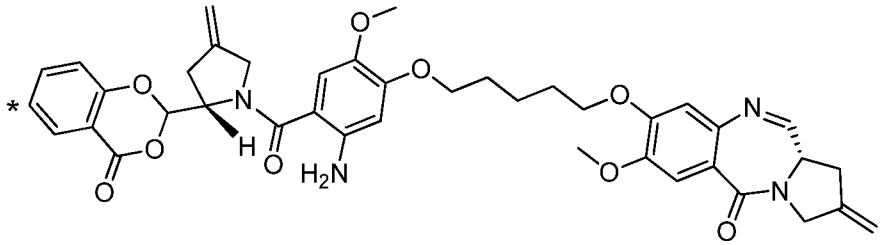
wherein * represents a covalent bond to the rest of the conjugate.

In some embodiments, the drug comprises the formula



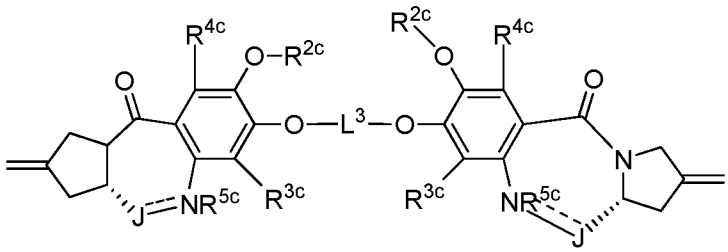
wherein * represents a covalent bond to the rest of the conjugate.

In some embodiments, the drug comprises the formula



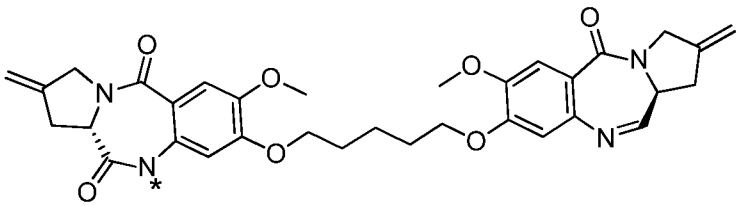
wherein * represents a covalent bond to the rest of the conjugate.

In some embodiments, the drug comprises the formula

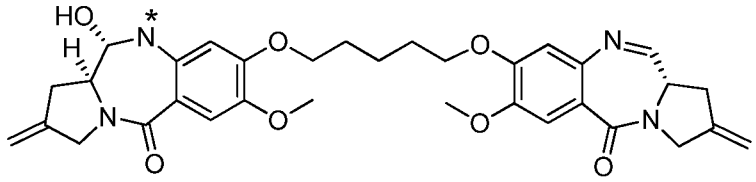


wherein at least one R^{5c} is a covalent bond to the rest of the conjugate.

In some embodiments, the drug comprises the formula



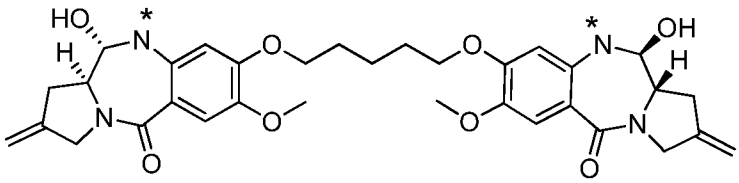
or



,

wherein * represents a covalent bond to the rest of the conjugate.

In some embodiments, the drug comprises the formula



wherein * represents a covalent bond to the rest of the conjugate.

The conjugates described herein can be used for both human clinical medicine and veterinary applications. Thus, the host animal harboring the population of pathogenic cells and treated with the conjugates described herein can be human or, in the case of veterinary applications, can be a laboratory, agricultural, domestic, or wild animal. The conjugates described hereincan be applied to host animals including, but not limited to, humans, laboratory animals such rodents (e.g., mice, rats, hamsters, etc.), rabbits, monkeys, chimpanzees, domestic animals such as dogs, cats, and rabbits, agricultural animals such as cows, horses, pigs, sheep, goats, and wild animals in captivity such as bears, pandas, lions, tigers, leopards, elephants, zebras, giraffes, gorillas, dolphins, and whales.

The conjugate, compositions, methods, and uses described herein are useful for treating diseases caused at least in part by populations of pathogenic cells, which may cause a variety of pathologies in host animals. As used herein, the term "pathogenic cells" or "population of pathogenic cells" generally refers to cancer cells, infectious agents such as bacteria and viruses, bacteria- or virus-infected cells, inflammatory cells, activated macrophages capable of causing a disease state, and any other type of pathogenic cells that uniquely express, preferentially express, or overexpress cell surface receptors or cell surface anitgens that may be bound by or targeted by the conjugates described herein. Pathogenic cells can also include any cells causing a disease state for which treatment with the conjugates described herein results in reduction of the symptoms of the disease. For example, the pathogenic cells can be host cells that are pathogenic under some circumstances such as cells of the immune system that are responsible for graft versus host disease, but not pathogenic under other circumstances.

Thus, the population of pathogenic cells can be a cancer cell population that is tumorigenic, including benign tumors and malignant tumors, or it can be non-tumorigenic. The cancer cell population can arise spontaneously or by such processes as mutations present in the germline of the host animal or somatic mutations, or it can be chemically-, virally-, or radiation-induced. The conjugates described herein can be utilized to treat such cancers as carcinomas, sarcomas, lymphomas, Hodgekin's disease, melanomas, mesotheliomas, Burkitt's lymphoma, nasopharyngeal carcinomas, leukemias, and myelomas; including associated cancers resistant to treatment modalities, such as therapeutic agents. Resistant cancers include but are not limited to paclitaxel resiatent cancers, and platinum resistant cancers, such as those cancers resistant to

platinum drugs, such as cisplatin, carboplatin, oxaplatin, nedaplatin, and the like. The cancer cell population can include, but is not limited to, oral, thyroid, endocrine, skin, gastric, esophageal, laryngeal, pancreatic, colon, bladder, bone, ovarian, cervical, uterine, breast, testicular, prostate, rectal, kidney, liver, stomach and lung cancers. In some embodiments, the cancer cell population prpoduces a cancer, such as lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, triple negative breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma and pituitary adenoma.

In some embodiemts, the cancer is folate receptor positive triple negative breast cancer. In some embodiemts, the cancer is folate receptor negative triple negative breast cancer. In some embodiemts, the cancer is ovarian cancer. In some embodiemts, the method further comprises concurrently treatment with anti-CTLA-4 treatment. In some embodiemts, the method further comprises concurrently treatment with anti-CTLA-4 treatment for the treatment of ovarian cancer.

The disclosure includes all pharmaceutically acceptable isotopically-labelled conjugates, and their Drug(s) incorporated therein, wherein one or more atoms are replaced by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number which predominates in nature.

Examples of isotopes suitable for inclusion in the conjugates, and their Drug(s) incorporated therein, include isotopes of hydrogen, such as ^{2}H and ^{3}H, carbon, such as ^{11}C, ^{13}C and ^{14}C, chlorine, such as ^{36}Cl, fluorine, such as ^{18}F, iodine, such as ^{123}I and ^{125}I, nitrogen, such as ^{13}N and ^{15}N, oxygen, such as ^{15}O, ^{17}O and ^{18}O, phosphorus, such as ^{32}P, and sulfur, such as ^{35}S.

Certain isotopically-labelled conjugates, and their Drug(s) incorporated therein, for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The radioactive isotopes tritium, *i.e.* ^{3}H, and carbon-14, *i.e.* ^{14}C, are

particularly useful for this purpose in view of their ease of incorporation and ready means of detection.

Substitution with heavier isotopes such as deuterium, *i.e.* ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements, and hence may be preferred in some circumstances.

Substitution with positron emitting isotopes, such as ^{11}C, ^{18}F, and ^{13}N, can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. Isotopically-labeled conjugates, and their Drug(s) incorporated therein, can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying Examples using an appropriate isotopically-labeled reagents in place of the non-labeled reagent previously employed.

The conjugates and compositions described herein may be administered orally. Oral administration may involve swallowing, so that the conjugate or composition enters the gastrointestinal tract, or buccal or sublingual administration may be employed by which the conjugate or composition enters the blood stream directly from the mouth.

Formulations suitable for oral administration include solid formulations such as tablets, capsules containing particulates, liquids, or powders, lozenges (including liquid-filled), chews, multi- and nano-particulates, gels, solid solution, liposome, films, ovules, sprays and liquid formulations.

Liquid formulations include suspensions, solutions, syrups and elixirs. Such formulations may be employed as fillers in soft or hard capsules and typically comprise a carrier, for example, water, ethanol, polyethylene glycol, propylene glycol, methylcellulose, or a suitable oil, and one or more emulsifying agents and/or suspending agents. Liquid formulations may also be prepared by the reconstitution of a solid, for example, from a sachet.

The conjugates and compositions described herein may also be used in fast-dissolving, fast-disintegrating dosage forms such as those described in Expert Opinion in Therapeutic Patents, 11 (6), 981-986, by Liang and Chen (2001). For tablet dosage forms, depending on dose, the conjugate may make up from 1 weight % to 80 weight % of the dosage form, more typically from 5 weight % to 60 weight % of the dosage form. In addition to the conjugates and compositions described herein, tablets generally contain a disintegrant. Examples of disintegrants include sodium starch glycolate, sodium carboxymethyl cellulose, calcium carboxymethyl cellulose, croscarmellose sodium, crospovidone, polyvinylpyrrolidone, methyl cellulose, microcrystalline cellulose, lower alkyl-substituted hydroxypropyl cellulose, starch, pregelatinised starch and sodium alginate. Generally, the disintegrant will comprise from 1 weight % to 25 weight %, preferably from 5 weight % to 20 weight % of the dosage form.

Binders are generally used to impart cohesive qualities to a tablet formulation. Suitable binders include microcrystalline cellulose, gelatin, sugars, polyethylene glycol, natural and synthetic gums, polyvinylpyrrolidone, pregelatinised starch, hydroxypropyl cellulose and hydroxypropyl methylcellulose. Tablets may also contain diluents, such as lactose (monohydrate, spray-dried monohydrate, anhydrous and the like), mannitol, xylitol, dextrose, sucrose, sorbitol, microcrystalline cellulose, starch and dibasic calcium phosphate dihydrate.

Tablets may also optionally comprise surface active agents, such as sodium lauryl sulfate and polysorbate 80, and glidants such as silicon dioxide and talc. When present, surface active agents may comprise from 0.2 weight % to 5 weight % of the tablet, and glidants may comprise from 0.2 weight % to 1 weight % of the tablet.

Tablets also generally contain lubricants such as magnesium stearate, calcium stearate, zinc stearate, sodium stearyl fumarate, and mixtures of magnesium stearate with sodium lauryl sulphate. Lubricants generally comprise from 0.25 weight % to 10 weight %, preferably from 0.5 weight % to 3 weight % of the tablet.

Other possible ingredients include anti-oxidants, colorants, flavoring agents, preservatives and taste-masking agents. Exemplary tablets contain up to about 80% drug, from about 10 weight % to 25 about 90 weight % binder, from about 0 weight % to about 85 weight % diluent, from about 2 weight % to about 10 weight % disintegrant, and from about 0.25 weight % to about 10 weight % lubricant.

Tablet blends may be compressed directly or by roller to form tablets. Tablet blends or portions of blends may alternatively be wet-, dry-, or melt-granulated, melt congealed, or extruded before tableting. The final formulation may comprise one or more layers and may be coated or uncoated; it may even be encapsulated. The formulation of tablets is discussed in Pharmaceutical Dosage Forms: Tablets, Vol. 1, by H. Lieberman and L. Lachman (Marcel Dekker, New York, 1980).

Consumable oral films for human or veterinary use are typically pliable water-soluble or water-swellable thin film dosage forms which may be rapidly dissolving or mucoadhesive and typically comprise a conjugate as described herein, a film-forming polymer, a binder, a solvent, a humectant, a plasticizer, a stabilizer or emulsifier, a viscosity-modifying agent and a solvent. Some components of the formulation may perform more than one function.

Solid formulations for oral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release. Suitable modified release formulations for the purposes of the disaclosure are described in US Patent No.6,106,864. Details of other suitable release technologies such as high energy dispersions and osmotic and coated particles are to be

found in Pharmaceutical Technology On-line, 25(2), 1-14, by Verma et al (2001). The use of chewing gum to achieve controlled release is described in WO 00/35298.

The conjugates described herein can also be administered directly into the blood stream, into muscle, or into an internal organ. Suitable means for parenteral administration include intravenous, intraarterial, intraperitoneal, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular and subcutaneous.

Suitable devices for parenteral administration include needle (including micro-needle) injectors, needle-free injectors and infusion techniques. Parenteral formulations are typically aqueous solutions which may contain excipients such as salts, carbohydrates and buffering agents (preferably to a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water.

The preparation of parenteral formulations under sterile conditions, for example, by lyophilisation, may readily be accomplished using standard pharmaceutical techniques well known to those skilled in the art. The solubility of conjugates described herein used in the preparation of parenteral solutions may be increased by the use of appropriate formulation techniques, such as the incorporation of solubility-enhancing agents.

Formulations for parenteral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release. Thus conjugates described herein can be formulated as a solid, semi-solid, or thixotropic liquid for administration as an implanted depot providing modified release of the active compound. Examples of such formulations include drug-coated stents and poly(lactic-coglycolic)acid (PGLA) microspheres. The conjugates described herein can also be administered topically to the skin or mucosa, that is, dermally or transdermally. Typical formulations for this purpose include gels, hydrogels, lotions, solutions, creams, ointments, dusting powders, dressings, foams, films, skin patches, wafers, implants, sponges, fibres, bandages and microemulsions. Liposomes may also be used. Typical carriers include alcohol, water, mineral oil, liquid petrolatum, white petrolatum, glycerin, polyethylene glycol and propylene glycol. Penetration enhancers may be incorporated - see, for example, J. Pharm Sci, 88 (10), 955-958 by Finnin and Morgan (October 1999). Other means of topical administration include delivery by electroporation, iontophoresis, phonophoresis, sonophoresis and microneedle or needle-free *(e.g.* Powderject™, Bioject™, *etc.)* injection.

Formulations for topical administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release. The conjugates described herein can also be

administered intranasally or by inhalation, typically in the form of a dry powder (either alone, as a mixture, for example, in a dry blend with lactose, or as a mixed component particle, for example, mixed with phospholipids, such as phosphatidylcholine) from a dry powder inhaler or as an aerosol spray from a pressurized container, pump, spray, atomizer (preferably an atomizer using electrohydrodynamics to produce a fine mist), or nebulizer, with or without the use of a suitable propellant, such as 1,1,1,2-tetrafluoroethane or 1,1,1,2,3,3,3-heptafluoropropane. For intranasal use, the powder may comprise a bioadhesive agent, for example, chitosan or cyclodextrin. The pressurized container, pump, spray, atomizer, or nebulizer contains a solution or suspension of the conjugates(s) of the present disclosure comprising, for example, ethanol, aqueous ethanol, or a suitable alternative agent for dispersing, solubilizing, or extending release of the active, a propellant(s) as solvent and an optional surfactant, such as sorbitan trioleate, oleic acid, or an oligolactic acid. Prior to use in a dry powder or suspension formulation, the conjugate is micronized to a size suitable for delivery by inhalation (typically less than 5 microns). This may be achieved by any appropriate comminuting method, such as spiral jet milling, fluid bed jet milling, supercritical fluid processing to form nanoparticles, high pressure homogenization, or spray drying. Capsules (made, for example, from gelatin or hydroxypropylmethylcellulose), blisters and cartridges for use in an inhaler or insufflator may be formulated to contain a powder mix of the conjugate described herein, a suitable powder base such as lactose or starch and a performance modifier such as Iso-leucine, mannitol, or magnesium stearate.

The lactose may be anhydrous or in the form of the monohydrate, preferably the latter. Other suitable excipients include dextran, glucose, maltose, sorbitol, xylitol, fructose, sucrose and trehalose. A typical formulation may comprise a conjugate of the present disclosure, propylene glycol, sterile water, ethanol and sodium chloride. Alternative solvents which may be used instead of propylene glycol include glycerol and polyethylene glycol.

The conjugates described here can be combined with soluble macromolecular entities, such as cyclodextrin and suitable derivatives thereof or polyethylene glycol-containing polymers, in order to improve their solubility, dissolution rate, taste-masking, bioavailability and/or stability for use in any of the aforementioned modes of administration.

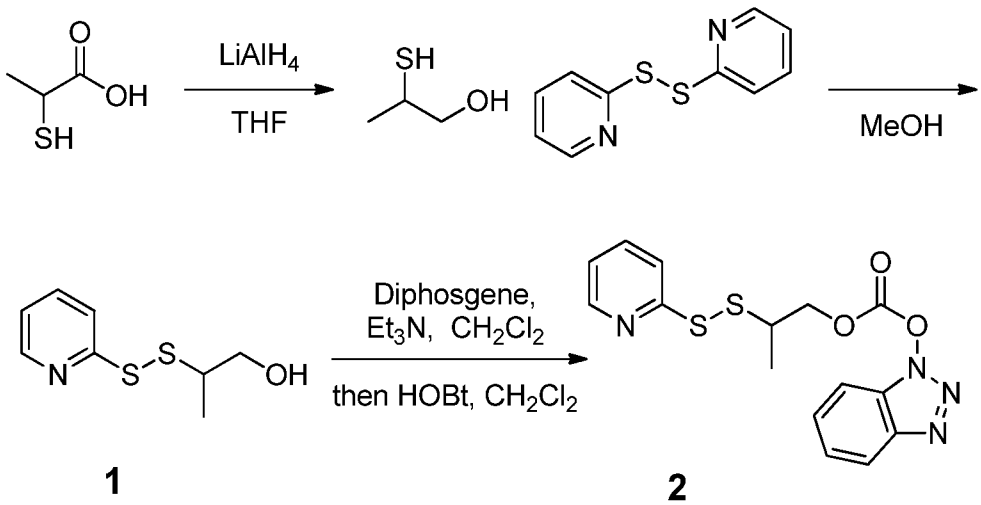
Drug-cyclodextrin complexes, for example, are found to be generally useful for most dosage forms and administration routes. Both inclusion and non-inclusion complexes may be used. As an alternative to direct complexation with the drug, the cyclodextrin may be used as an auxiliary additive, *i.e.* as a carrier, diluent, or solubilizer. Most commonly used for these purposes are alpha-, beta- and gamma-cyclodextrins, examples of which may be found in International Patent Applications Nos. WO 91/11172, WO 94/02518 and WO 98/55148.

Inasmuch as it may desirable to administer a combination of active compounds, for example, for the purpose of treating a particular disease or condition, it is within the scope of the present disclosure that two or more pharmaceutical compositions, at least one of which contains a conjugate as described herein, may conveniently be combined in the form of a kit suitable for co-administration of the compositions. Thus the kit of the present disclosure comprises two or more separate pharmaceutical compositions, at least one of which contains a conjugate as described herein, and means for separately retaining said compositions, such as a container, divided bottle, or divided foil packet. An example of such a kit is the familiar blister pack used for the packaging of tablets, capsules and the like. The kit of the present disclosure is particularly suitable for administering different dosage forms, for example parenteral, for administering the separate compositions at different dosage intervals, or for titrating the separate compositions against one another. To assist compliance, the kit typically comprises directions for administration and may be provided with a so-called memory aid.

EXAMPLES

CHEMICAL EXAMPLES

It is to be understood that the conjuagtes described herein were prepared according to the processes described herein and/or conventional processes. Illustratively, the stereocenters of the conjugates described herein may be substantially pure (*S*), the substantially pure (*R*), or any mixture of (*S*) and (*R*) at any asymmetric carbon atom, and each may be used in the processes described herein. Similarly, the processes described in these illustrative examples may be adapted to prepare other conjuagtes described herein by carrying out variations of the processes described herein with routine selection of alternative starting materials and reagents. It is also to be understood that radicals of these examples are included in the PBD prodrugs, poly-PBD prodrugs, mixed PBDs, and conjugates described herein.



1 **2**

Example 1: Prepararion of Compound 2.

Step 1: Preparation of 2-Thiopropanol.

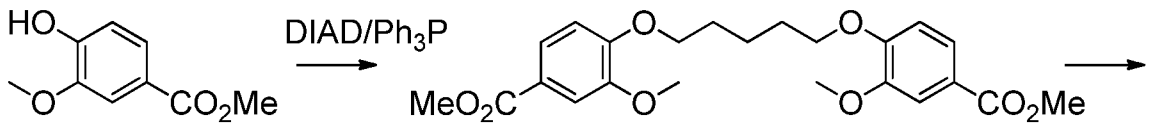
2-Mercaptopropionic acid (1 mL, 11.27 mmol) in anhydrous THF (35 mL) was treated with 2 M $LiAlH_4$ in THF (11.3 mL, 22.5 mmol) and heated at reflux for 2 h. The reaction mixture was cooled to 0°C. 2 N HCl was added dropwise while maintaining an internal temperature below 30°C until the evolution of bubbles ceased. The reaction mixture was stirred for 1 h and filtered through a pad of Celite. The filtrate was concentrated *in vacuo* and used without further purification.

Step 2: Prepararion of Compound 1.

2-Mercaptopropanol was dissolved in MeOH (10 mL) and added dropwise to a solution of 2,2'-dipyridyl disulfide (3.00 g, 14.0 mmol) in MeOH (10 mL). The reaction mixture was stirred for 30 min at room temperature and then concentrated under vacuum. The residue was dissolved in 3 mL of CH_2Cl_2 and purified via silica chromatography (0 - 40% EtOAc/pet. ether) to yield the desired product as a colorless oil, (332.7 mg, 17% over two steps); LC/MS (ESI-QMS): m/z = 202 (M + H).

Step 3: Prepararion of Compound 2.

Compound 1 (111 mg, 0.549 mmol) and Et_3N (76.5 µL, 0.549 mmol) were dissolved in CH_2Cl_2 (15 mL) and added dropwise to a solution of diphosgene (36.5 µL, 0.302 mmol) in CH_2Cl_2 (0.5 mL) at 0°C. The reaction mixture was stirred for 30 min at 0°C and monitored by TLC (40% EtOAc/pet. ether). A solution of 1-Hydroxybenzotriazole hydrate (74.2 mg, 0.549 mmol) in CH_2Cl_2 (2 mL) followed by Et_3N (41.2 µL, 0.544 mmol) was added to the reaction mixture at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. After the reaction was carried out to completion, reaction mixture was concentrated and purified via silica chromatography (0 - 40% EtOAc/Pet. ether). The desired product was obtained as a white solid (116.7 mg, 59% over two steps); LC/MS (ESI-QMS): m/z = 363 (M + H), ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (m, 1H), 8.22 (d, J = 8.31 Hz, 1H), 8.01 (d, J = 8.80 Hz, 1H), 7.76 (m, 1H), 7.65 (td, J = 7.83, 1.60 Hz, 1H), 7.56 (t, J = 7.82 Hz, 1H), 7.08 (m, 1H), 4.69 (dd, J = 11.25, 5.87 Hz, 1H), 4.58 (dd, J = 11.25, 6.84 Hz, 1H), 3.45 (m, 1H), 1.49 (d, J = 7.33 Hz, 3H).



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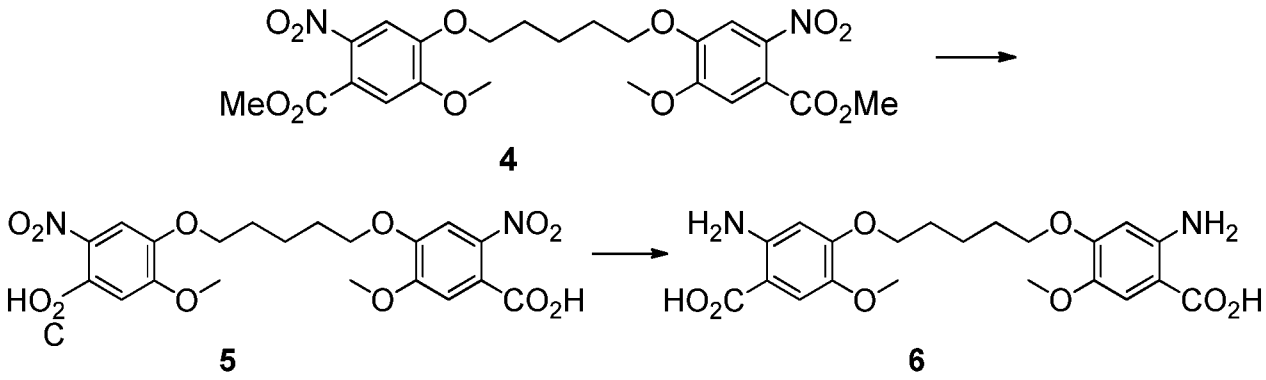


Example 4: Preparation of Compound 6.

Step 1: Preparation of Compound 3.

Methyl vanillate (2.18g, 11.98 mmol) and Ph_3P (4.71 g, 17.97 mmol) in THF (20 mL) was cooled to 0°C and to which was added DIAD (2.59 mL, 13.18 mmol) dropwise. The reaction was stirred at 0°C for 1 hr. 1,5-petanediol (0.6 mL, 5.75 mmol) in THF (20 mL) was added over 30 min. The reaction was stirred overnight and prESIpitate formed and was collected with filtration. The filtrate was concentrated to form more solid. The solid was combined and triturated with MeOH (5 mL) to give qite clean product **Compound 3** 1.74 g in yield of 70%. ^{1}H NMR ($CDCl_3$, δ in ppm): 7.66(m 2H), 7.62(m, 2H), 6.87(m, 2H), 4.10(m, 4H), 3.89(m, 12H), 1.95(m, 4H), 1.69(m, 2H). ^{13}C NMR: 166.88, 152.50, 148.86, 132.12, 132.04, 131.88, 128.52, 128.42, 123.50, 122.55, 112.35, 111.46, 68.67, 56.03, 51.93, 28.73, 22.52, 21.92.

Step 2: Preparation of Compound 4.

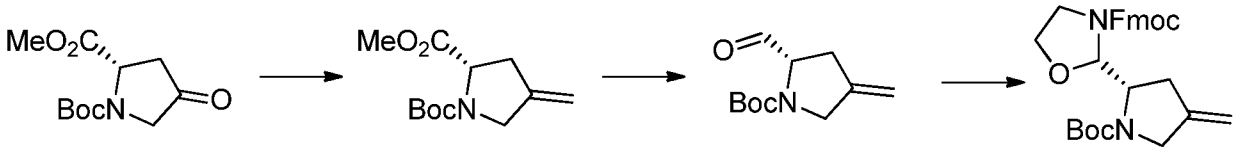
Compound 3 (201.2 mg, 0.465 mmol) in Ac_2O (1.2 mL) was cooled to 0 °C and then $Cu(NO_3)_2{\cdot}3H_2O$ (280.3 mg, 1.16 mmol) was added slowly and after 1 hr, the ice-bath was removed. The reaction was stirred at r.t. for 4 hrs. The reaction was poured into ice water and stirred for 1 h till yellow precipitate formed and was collected with filtration. The solid was washed with more cold water (2 mL, 3 x) and air-dried. 198.4 mg of **Compound 4** was obtained in yield of 82%. LCMS: $[M+NH_4]^+$ m/z =540.

Step 3: Preparation of Compund 5.

Compound 4 (198.4 mg) was dissolved in THF (2 mL) and treated with aq. NaOH (2 mL, 1 M) and heated to 40° C for 3 hrs. The solvent was removed in vacuo. The aqueous phase was acidified to pH 1 with concentrated HCl to form precipitate, which was collected by filtration and was washed with H_2O (1 mL, 3 x). The solid was air-dried to give the acid 187.7 mg of **Compound 5** in quantitative yield. LCMS: $[M+NH_4]^+$ m/z =512.

Step 4: Preparation of Compound 6

Acid **Compound 5** was dissolved in 0.5 M aq. NaOH (6 mL) and hydrogenation was carried out with Pd/C (10%, 4.82 mg) under H_2 (45 PSI) in the hydrogenation parr. The reaction was shook for 5 hrs and the filtered through a pad of celite and the filtrate was adjusted to pH 2-3 with concentrated HCl while stirring. The formed precipitate was isolated by filtration and washed with H_2O (1 mL, 3 x). The solid was dried in a desiccator with the presence of P_2O_5 under high vacuum overnight. **Compound 6** was obtained 34.2 mg as a brown solid in the yield of 81%. LCMS: $[M-H]^-$ m/z =433.



7 **8**

Example 3: Preparation of Compound 8.

Step 1: Preparation of Compound 7.

(S)-1-tert-butyl 2-methyl 4-oxopyrrolidine-1,2-dicarboxylate was converted to **Compound 7** by Wittig reaction: Ph_3PCH_3Br (917.8 mg, 2.57 mmol) in THF (30 mL) was treated with KO^{t}Bu (1 M in THF, 2.57 µL, 2.57 mmol) at 0°C by dropwise addition. The reaction was kept at room temperature for 2 hrs. Into the stirred solution was added the ketone (250 mg, 1.028 mmol) in THF 20 mL) at 0-10°C. The reaction was then stirred at room temperature for onvernight. The reaction was quenched with H_2O/EtOAc (1:1, 40 mL) after most of the THF was removed *in vacuo*. The aq. phase was extracted with EtOAc (20 mL, 3 x) and the organic phase was washed with H_2O, followed by brine, and dried over anhydrous Na_2SO_4 and concentrated. The residue was purified with CombiFlash in 0-50% EtOAc/p-ether to afford the **Compound 7** 77.2 mg, in yield of 31%. LCMS: $[M-Boc+H]^+$ m/z =142.

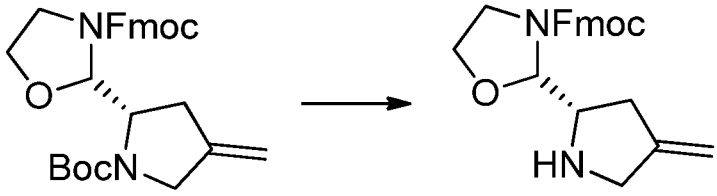
Step 2: Preparation of aldehyde intermediate.

(S)-1-tert-butyl 2-methyl 4-methylenepyrrolidine-1,2-dicarboxylate (353.2 mg, 1.46 mmol) in DCM/toluene (1:3, 9.8 mL) was treated with Dibal (1 M in toluene, 2 eq, 2.92 mmol) dropwise at -78°C under argon. The reaction was stirred at -78°C for ca. 4hrs. Then the reaction was quenched with addition of 60 µL of MeOH at -78°C followed by 5% HCl (.5 mL) and EtOAc (18 mL). The cold bath was removed and the reaction was stirred for 30 min. The EtOAc layer was separated and washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give the crude aldehyde intermediate.

Step 3: Preparation of Compound 8.

The crude aldehyde was redissolved in dry DCM (10 mL) and treated with ethanolamine (106 µL, 1.75 mmol) in the presence of anhydrous $MgSO_4$ (5 mmol, mg) at r.t. (room

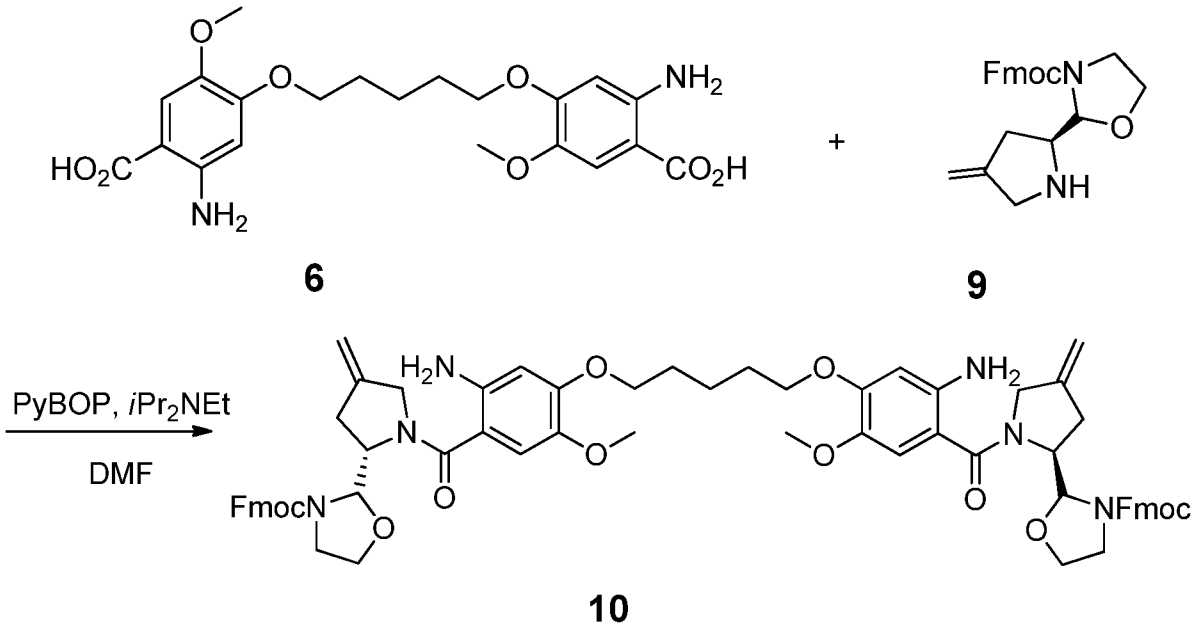
temperature) under Ar. The reaction was stirred for 1 hr. Then into this reaction mixture was added FmocCl (755.4 mg, 2.92 mmol) and TEA (611 µL, 4.38 mmol) and the reaction was stirred for overnight at r.t. under Ar. The reaction was purified with CombiFlash in 0-50% EtOAc/petroleum ether to provide **Compound 8** 334.2 mg, 46% for 3 steps. LCMS: $[M+H]^+$ m/z =477. ^1H NMR (CD_3OD, δ in ppm):7.81(d, J=7.5Hz, 2H), 7.60(d, J=7Hz, 2H), 7.40(m, 2H), 7.32(m, 2H), 4.96(br, 2H), 4.60(br,1H), 4.23(t, J=5.5 Hz, 1H), 3.97(br, 2H), 3.73(br, m, 3H), 2.50(br, 2H), 1.47(s, 1H), 1.39(s, 9H).



8 **9**

Example 4: Preparation of Compound 9.

Compound 8 was deprotected in TFA/DCM (1:1) at r.t. for 30 min, the solvent was removed *in vacuo*. The product (**Compound 9**) was used for the coupling reaction with **Compound 6** without further purification. LCMS: $[M+H]^+$ m/z =377.



6 **9**

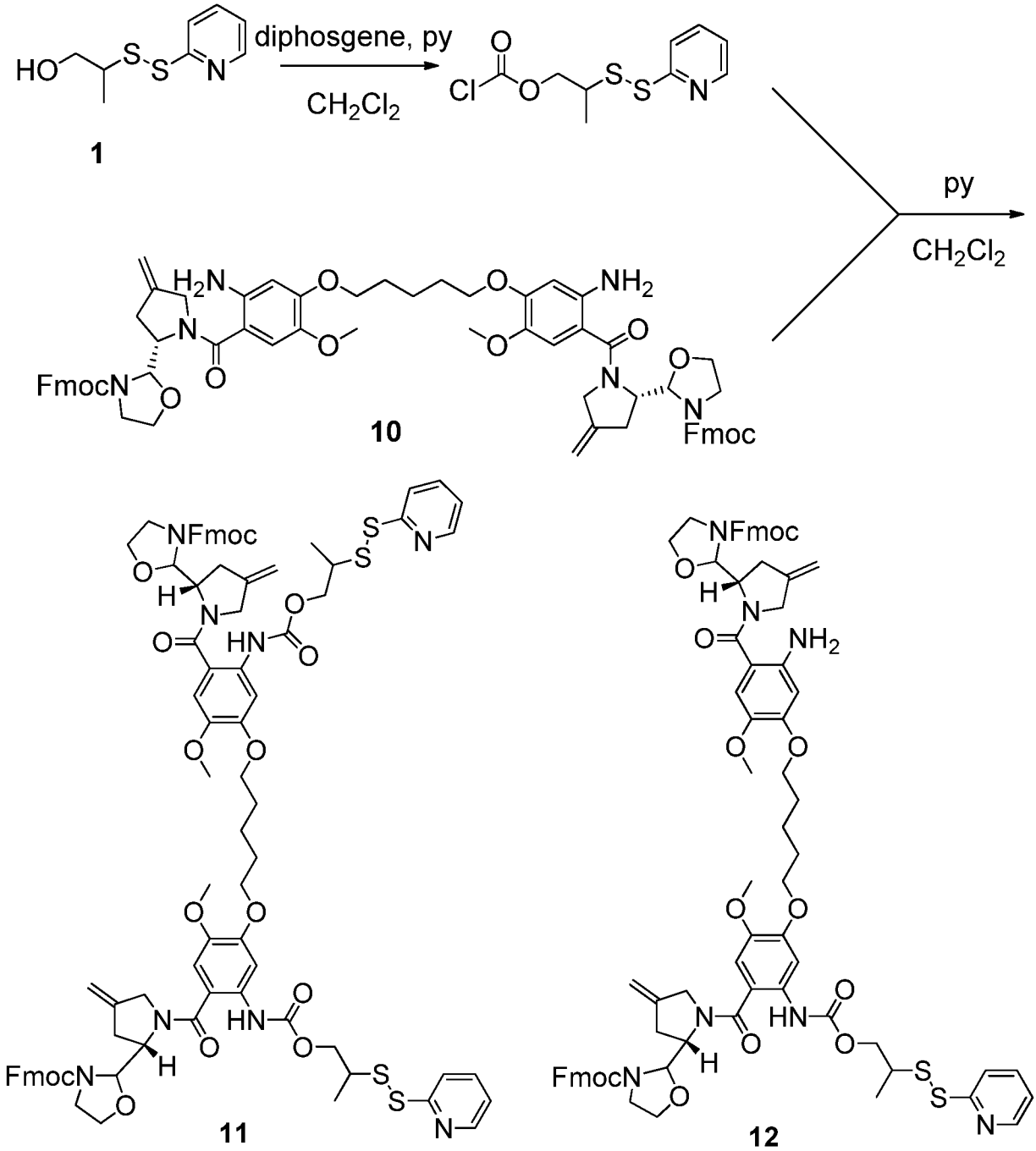
10

Example 5: Preparation of Compound 10.

Under argon, **Compound 6** (482 mg, 1.11 mmol), **Compound 9** (878 mg, 2.33 mmol), and PyBOP (1.21 g, 2.33 mmol) were dissolved in DMF (12 mL) and treated with $^iPr_2NEt$ (773 µL, 4.34 mmol) at room temperature. The reaction was completed within 1 h and purified by preparative HPLC (10 - 100% ACN/50 mM NH_4HCO_3 buffer, pH7). The product was extracted from the buffer solution with CH_2Cl_2 and concentrated under reduced pressure to afford **Compound 10** (556 mg, 44%); LC/MS (ESI-QMS) *m/z*= 1151.96 $(M+H)^+$, ^1H NMR

(500 MHz, DMSO-d6 w/ 2 drops D_2O) δ 7.84 (d, J=6.5Hz, 2H), 7.80 (d, J=8.0Hz, 2H), 7.39 (t, J=7.5Hz, 2H), 7.32 (t, J=7.0Hz, 2H), 6.24 (s, 2H), 4.80-5.14 (m, 2H), 3.80-4.20 (m, 6H), 3.52-3.68 (m, 4H), 3.51 (s, 6H), 3.35 (m, 2H), 2.96 (m, 1H), 2.55 (t, J=6.0Hz, 1H), 2.48 (m, 2H), 1.74 (br, 2H), 1.50 (br, 2H).

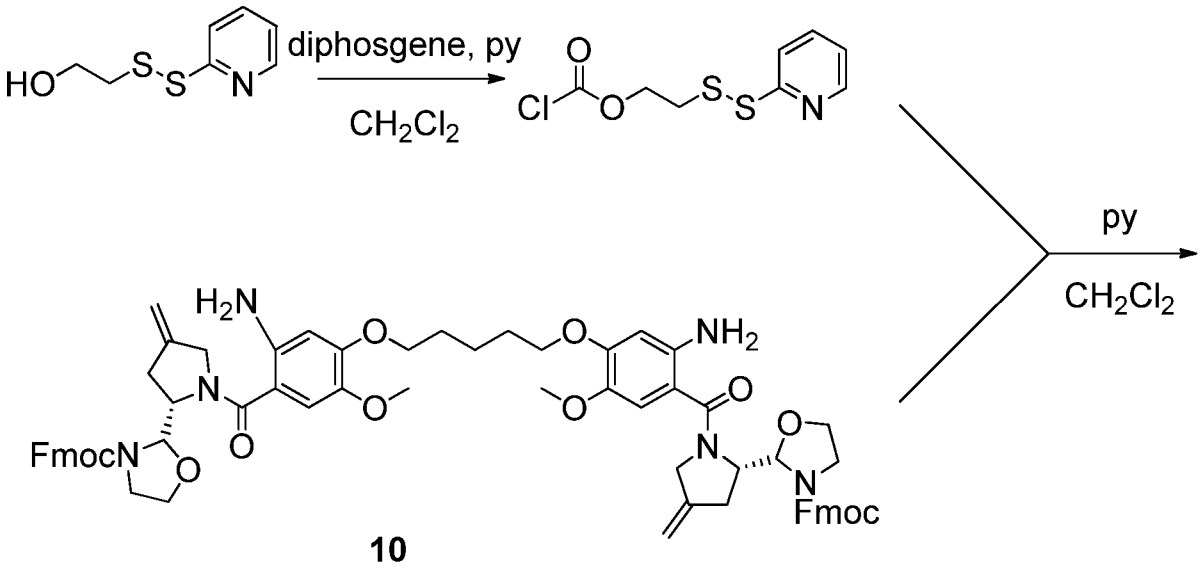
Example 6: Preparation of Compound 11 and 12.



A solution of **Compound 1** (18.9 mg, 0.094 mmol) and pyridine (15.2 µL, 0.190 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise to a solution of diphosgene (6.23 µL, 0.052 mmol) in CH_2Cl_2 (0.2 mL) at 0°C. The reaction mixture was allowed to stir for 15 - 30 min. The resulting chloroformate solution was slowly transferred to a solution of **Compound 10** (108.1

mg, 0.94 mmol) in CH_2Cl_2 (0.5 mL) at 0°C. The reaction was stirred for an additional 15 min and then quenched with water (0.5 mL). The organic phase was removed, and the product was extracted further with EtOAc (3 mL x 3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was further purified via silica chromatography (0 - 100% EtOAc/pet. ether) to provide **Compound 11** (23.2 mg, 15%) and **Compound 12** (43.2 mg, 34%). **Compound 11**: LC/MS: (ESI-QMS): *m/z* = = 1607 (M + H), ^{1}H NMR (500 MHz, $CDCl_3$ + one drop of CD_3OD) δ 8.39 (d, J =3.91 Hz, 2H), 7.78 (d, J = 7.82 Hz, 2H), 7.66 (m, 9H), 7.45 (m, 4H), 7.32 (t, J = 7.34 Hz, 3H), 7.24 (m, 5H), 7.14 (br, 1H), 7.05 (t, J = 5.86 Hz, 2H), 4.94(br, 6H), 4.30 (br, 2H), 4.13 (m, 6H), 3.95 (br, 6H), 3.89 (br, 2H), 3.62 (m, 8H), 3.50 (br, 2H), 3.31 (m, 2H), 3.17 (br, 6H), 2.60 (br, 2H), 1.85 (s, br, 4H), 1.58 (s, br, 2H), 1.30 (m, 6H); **Compound 12**: LC/MS: (ESI-QMS): *m/z* = 1380 (M + H), ^{1}H NMR (500 MHz, $CDCl_3$ + one drop of CD_3OD) δ 8.33 (br, 1H), 7.70 (m, 6H), 7.60 (d, J = 1.46 Hz, 2H), 7.49 (m, 3H), 7.32 (m, 4H), 7.25 (m, 5H), 7.00 (m, 1H), 6.6-6.9 (br, 2H), 4.95 (br, 6H), 4.31 (br, 4H), 3.9-4.2 (m, 12H), 3.54 (m, 10H), 3.50 (br, 2H), 3.32 (m, 1H), 3.20 (m, 1H), 2.90 (br, 3H), 2.60 (m, br, 4H), 1.82 (, br, 4H), 1.58 (br, 2H), 1.30 (m, br, 3H).

Example 7: Preparation of Compound 13 and 14.



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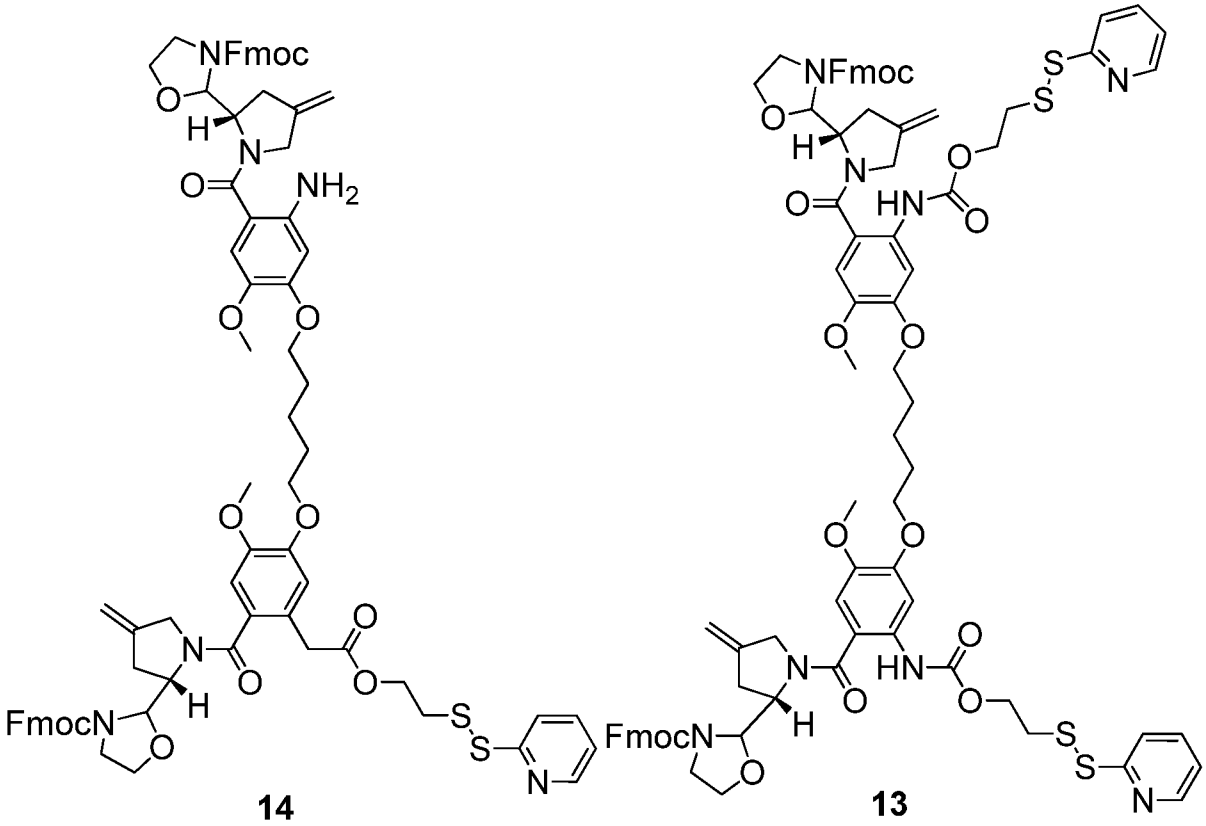
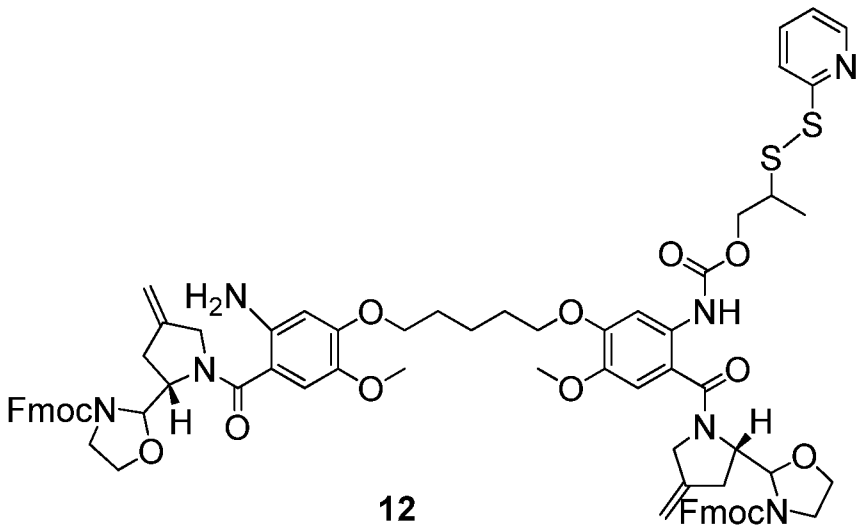
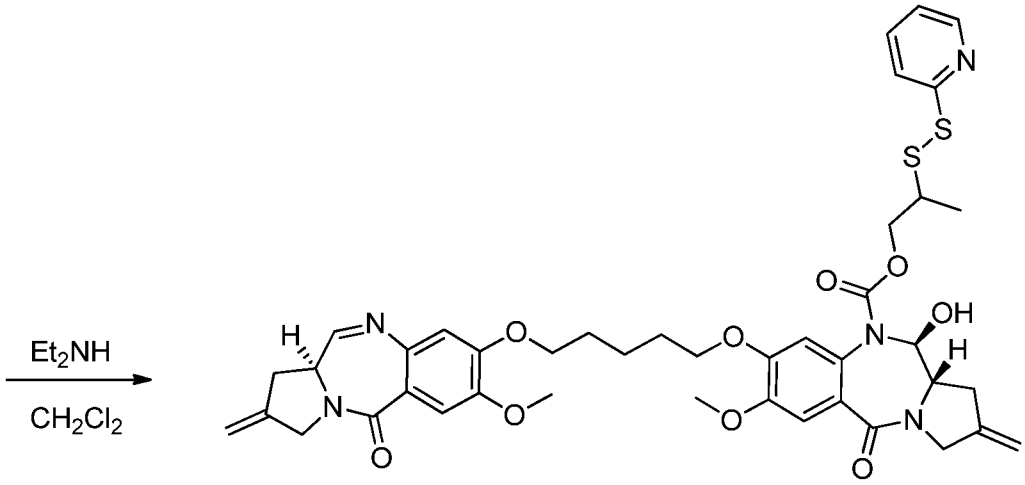


Compound 13 and Compound 14 were synthesized by following the procedure for **Compound 12** and **Compound 11** from 2-(2-Pyridyldithio)ethanol in lieu of **Compound 1**. **Compound 14**: LC/MS (ESI-QMS): *m/z* = 1364 (M + H); **Compound 13**: LC/MS (ESI-QMS): *m/z* = 1578 (M + H).

Example 8: Preparation of Compound 15





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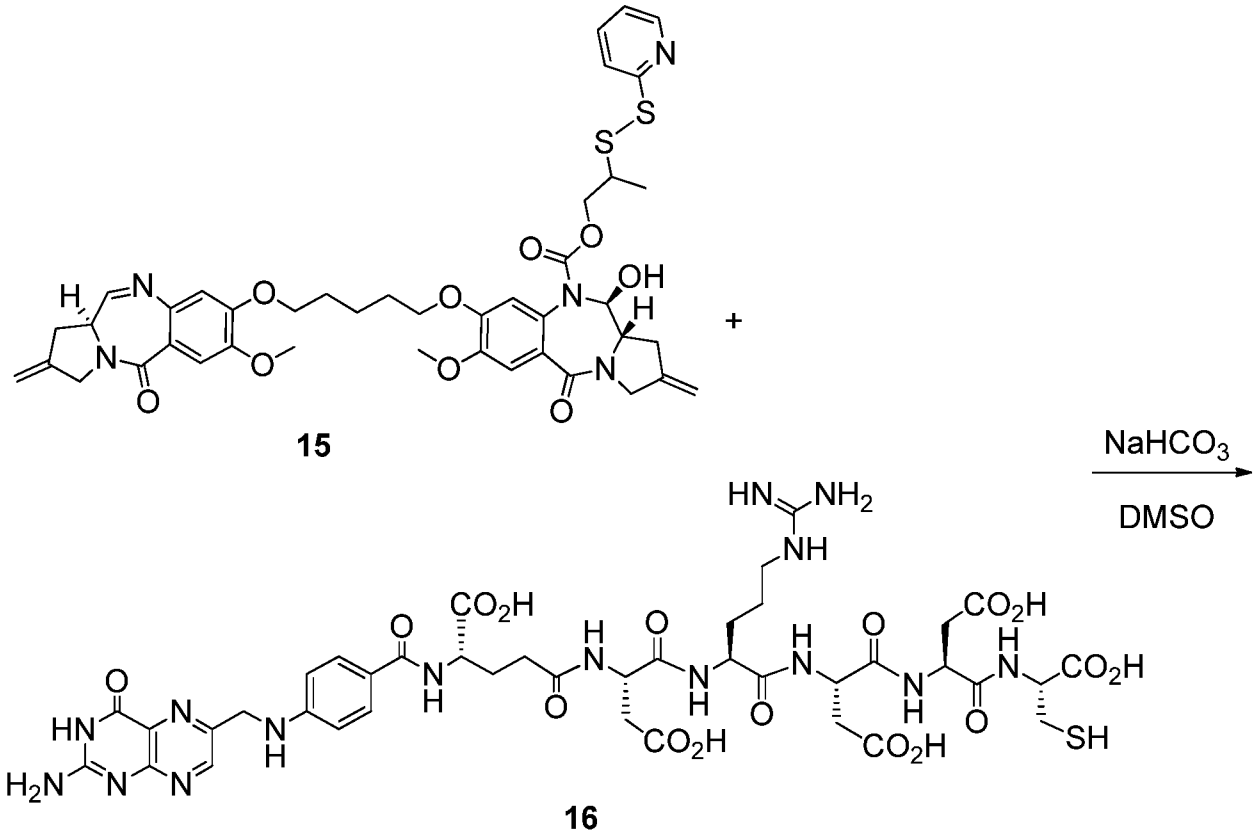
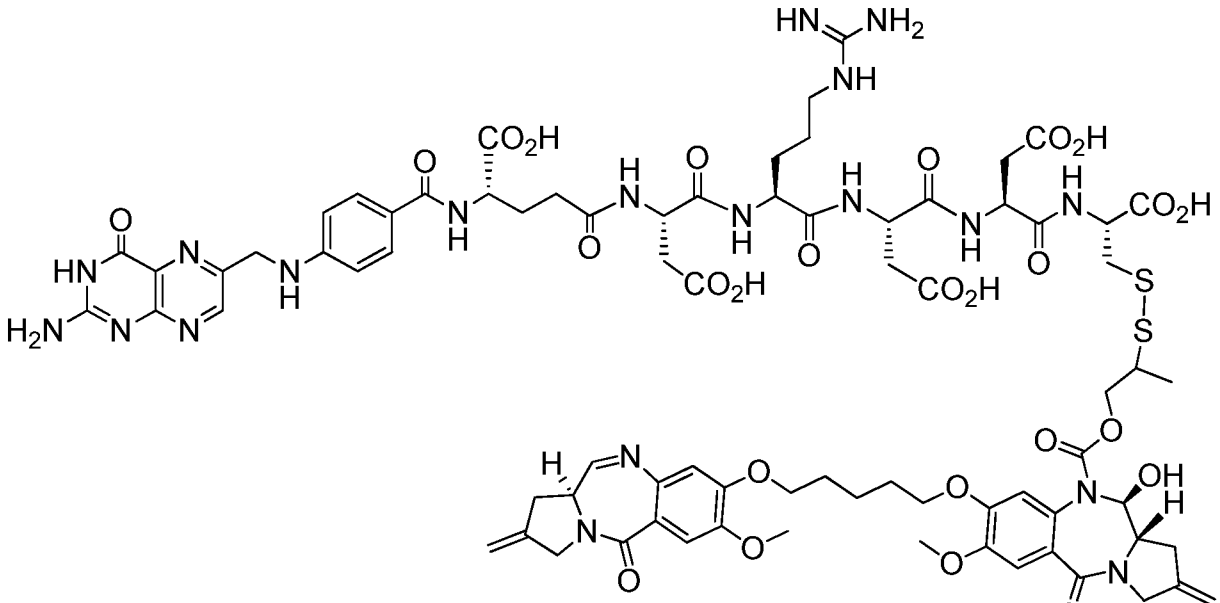
Compound 12 (12.0 mg, 0.0087 mmol) was dissolved in CH_2Cl_2 (1 mL) and treated with diethylamine (0.25 mL, 2.42 mol) at room temperature under argon. The reaction was stirred for 30 min and concentrated *in vacuo*. The crude product **Compound 15** was used without any further purification; LC/MS (ESI-QMS): *m/z* = 830 (M + H).

Example 9: Preparation of Conjugate 1.



15 **16**



Conjugate 1

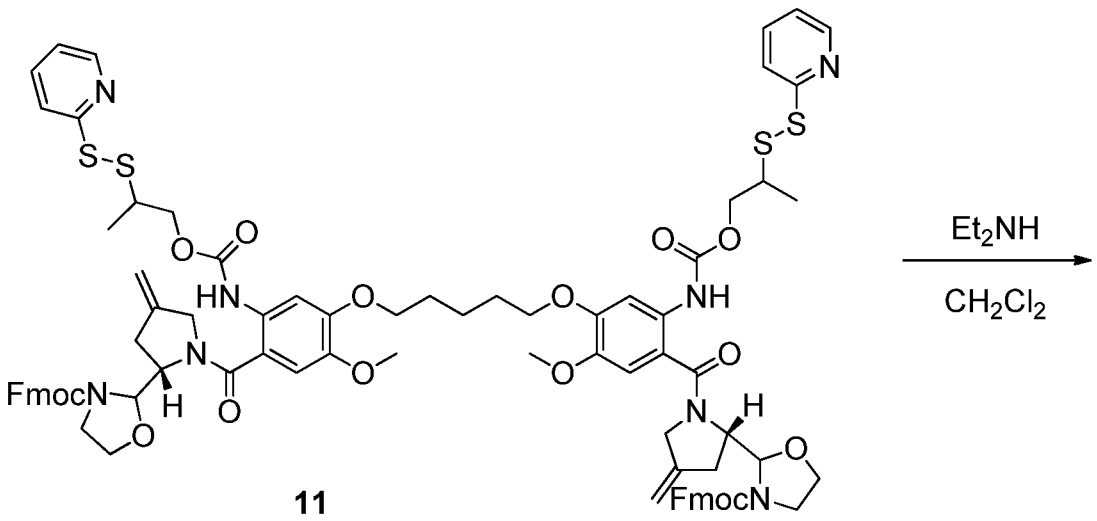
Step 1: Preparation of Compound 16.

Compound 16 is obtainable by the methods disclosed in PCT/US2011/037134 (WO2011146707), incorporated herein by reference.

Step 2: Preparation of Conjugate 1.

Compound 16 (11.4 mg, 0.011 mmol) was dissolved in water (0.5 mL) and the pH was adjusted to 7 with saturated $NaHCO_3$. **Compound 15** (0.0087 mmol) in DMSO (0.2 mL) was added to the reaction mixture and stirred for 1 h at room temperature under argon. The reaction was purified via preparative HPLC (10 - 100% MeCN/0.1% TFA) to yield **Conjugate 1** (1.5 mg, 10% over two steps): LC/MS (ESI-QMS): *m/z* = 1765 (M + H), 883 (M + 2H).

Example 10: Preparation of Compound 18.



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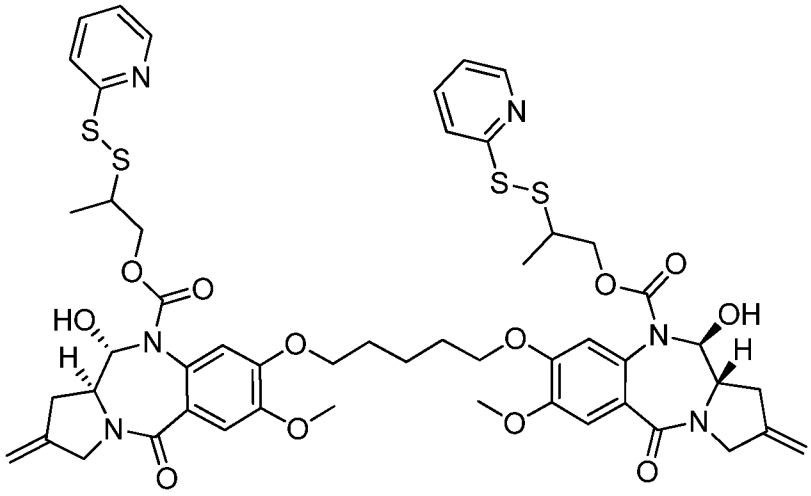

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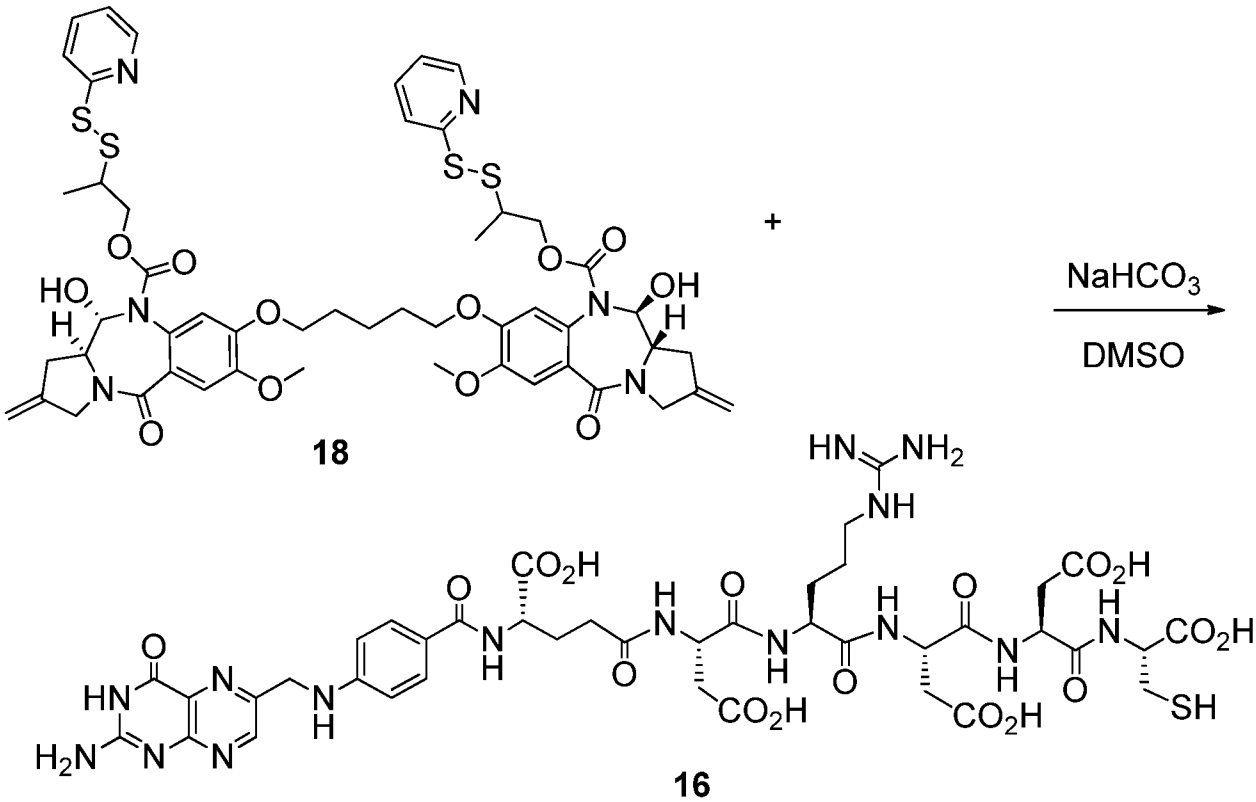
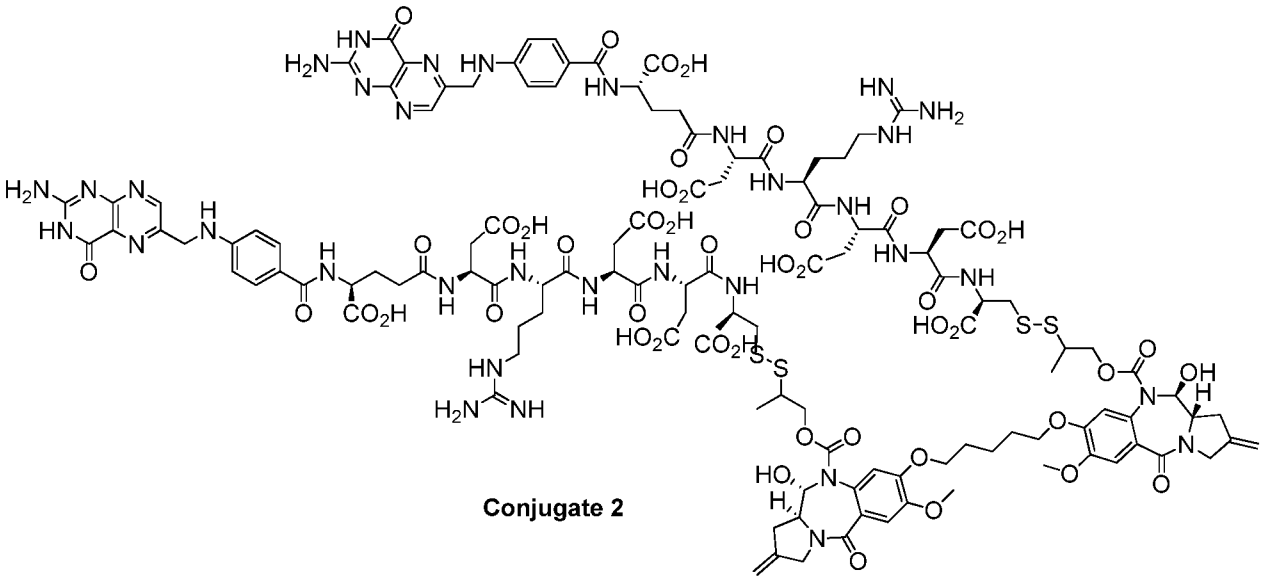
Compound 18 was synthesized by following the procedure for **Compound 15** from **Compound 11** in lieu of **Compound 12:** LC/MS (ESI-QMS): *m/z* = 1075 (M + H).

Example 11: Preparation of Conjugate 2.



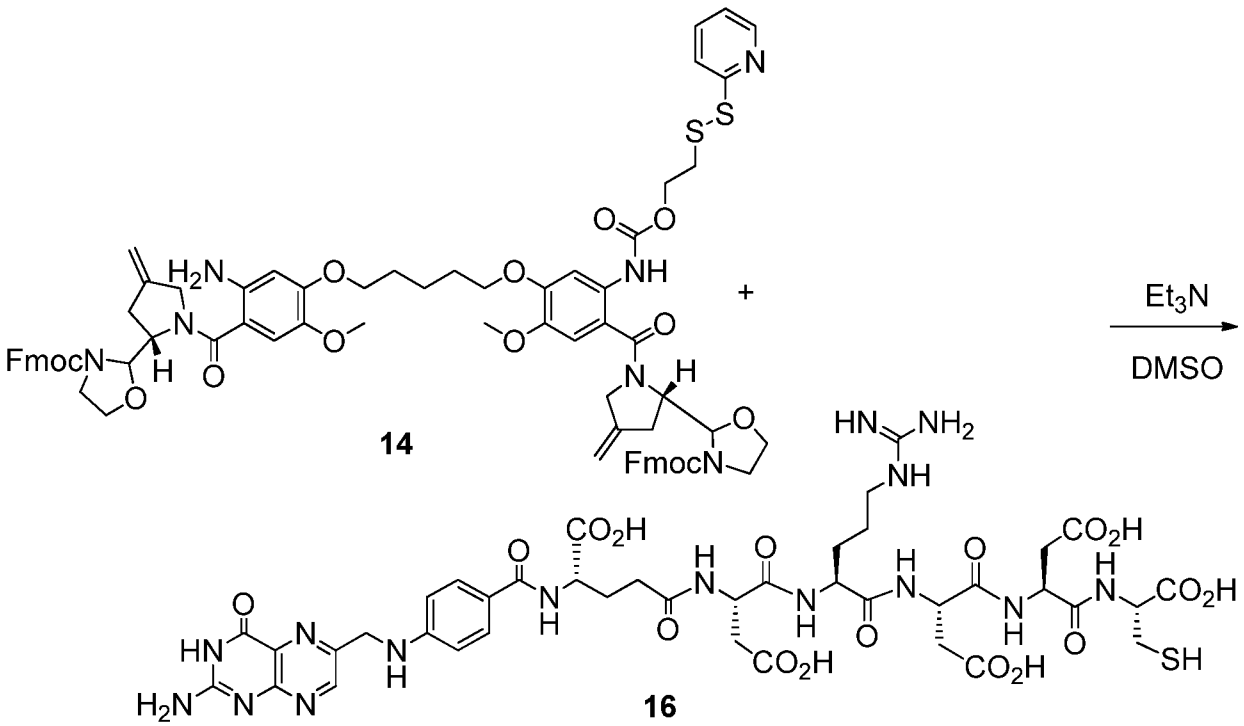
18 **16**



Conjugate 2

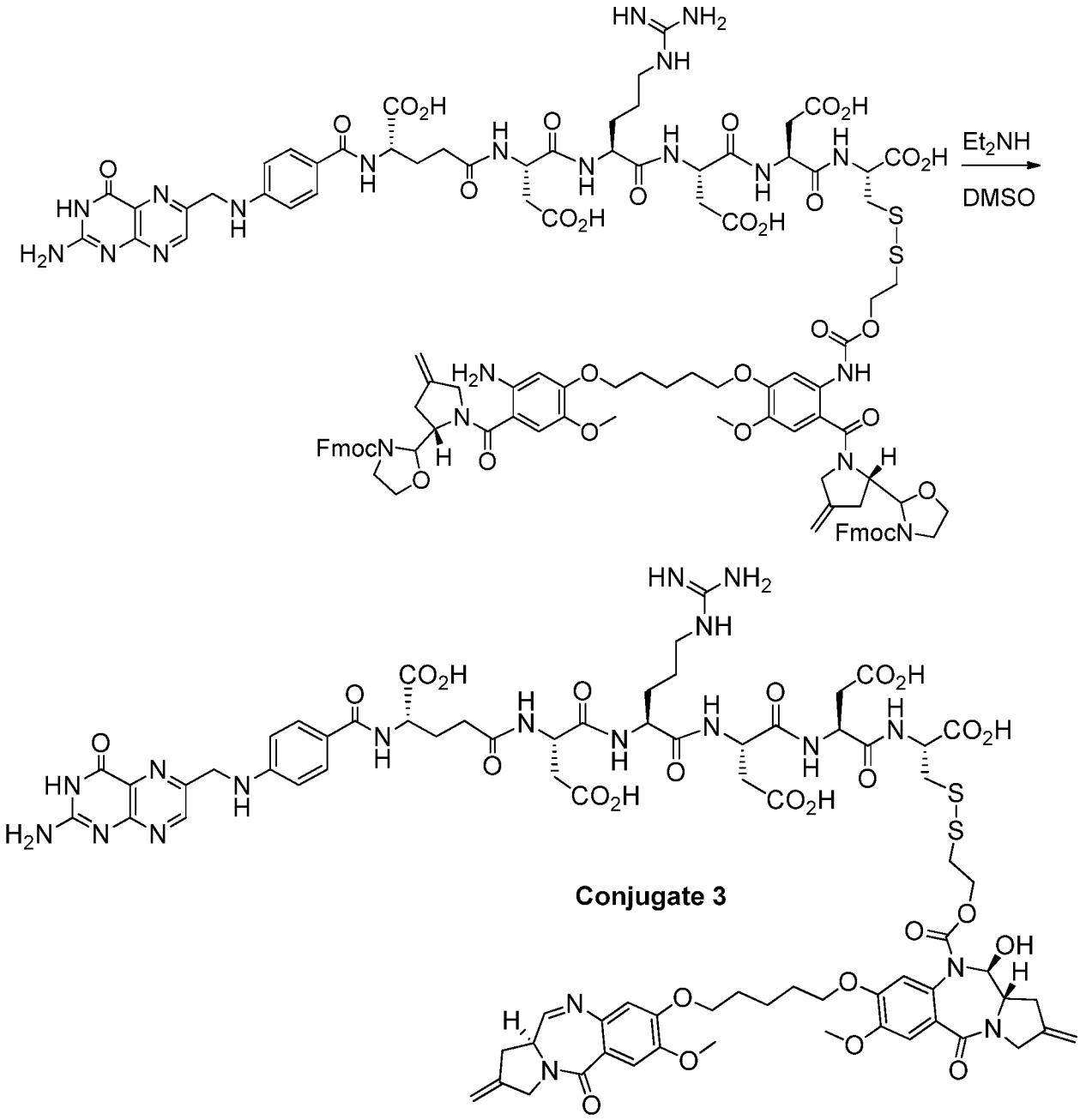
Conjugate 2 (9.6 mg, yield 34% over two steps) was synthesized by following the procedure for **Compound 17** from **Compound 18** in lieu of **Compound 15:** LC/MS (ESI-QMS): *m/z* = = 983 (M + 3H), ^{1}H NMR (500 MHz, DMSO-d_6 + drops of D_2O) δ 8.60 (s, 2H), 7.56 (d, J = 6.60 Hz, 4H), 7.01 (s, 2H), 6.82 (br, 2H), 6.60 (d, J = 7.70 Hz, 4H), 5.37 (s, 2H), 5.09 (br, 4H), 4.85 (d, J = 8.17 Hz, 2H), 4.48 (m, 12H), 4.0-4.3 (m, br, 12H), 3.70 (s, 10H), 3.40 (m, br, 8H), 3.00 (br, 10H), 2.80 (m, 8H), 2.63 (m, 4H), 2.10 (br, 8H), 1.92 (br, 4H), 1.85 (s, 6H), 1.74 (br, 6H), 1.45 (m, br, 14H), 1.12 (m, br, 8H), 0.90 (br, 6H).

Example 12: Preparation of Conjugate 3.



14

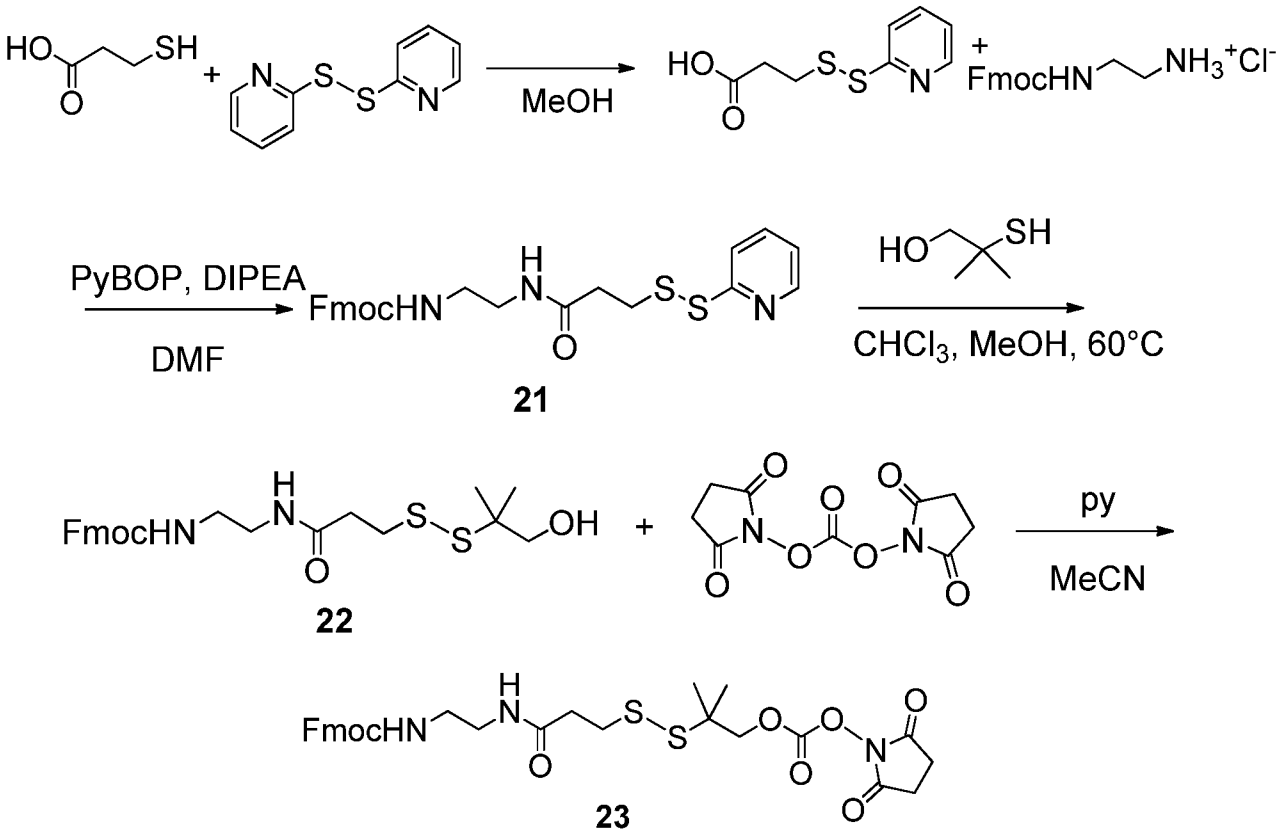
16



A solution of **Compound 14** (11.2 mg, 0.00820 mmol) in DMSO (0.2 mL) was added to a solution of **Compound 16** (8.58 mg, 0.0082 mmol) in DMSO (0.3 mL) at room temperature under argon. The reaction was treated with Et_3N (6.8 µL, 0.049 mmol), and stirred for 1 h. Diethylamine (0.2 mL) was then added, and the reaction mixture was allowed to stir for an additional 30 min before the crude material was purified via preparative HPLC (10 - 100% $MeCN/NH_4HCO_3$ buffer, pH 7.4) to yield the desired product (4.8 mg, yield 33% over two steps): LC/MS (ESI-QMS): *m/z* = = 876 (M + 2H), ^1H NMR (500 MHz, D_2O) δ 8.44 (m, 1H), 7.49 (d, J = 8.07 Hz, 2H), 6.98 (m, 2H), 6.75 (br, 1H), 6.55 (d, J = 8.44 Hz, 2H), 6.38 (br, 1H), 6.02 (br, 1H), 5.5 (m, 1H), 5.08 (s, 4H), 4.95 (m, 2H), 4.58 (m, 3H), 4.49 (m, 3H), 4.35 (br, 4H), 3.95 (m, 4H), 3.80 (m, 3H), 3.70 (m, 5H), 3.66 (s, 2H), 3.62 (s, 2H), 3.5 (m, 2H), 3.10 (br,

1H), 2.82 (m, br, 6H), 2.50 (m, 4H), 2.29 (m, 3H), 2.03 (m,br, 2H), 1.91 (m, br, 2H), 1.75 (br, 1H), 1.62 (br, 6H), 1.39 (br, 6H).

Example 13: Preparation of Compound 23.



Step 1: Preparation of 3-(2-Pyridyldithio)propionic acid.

2,2'-dipyridyl disulfide (8.70 g, 39.5 mmol) was dissolved in MeOH (150 mL) and purged with argon for 20 minutes. 3-Mercaptopropionic acid (2.10 g, 19.8 mmol) was dissolved in MeOH (35 mL) and purged under argon for 15 min. The 3-mercaptopropionic acid solution was added slowly to the 2,2'-dipyridyl disulfide solution using an addition funnel. The reaction was monitored by LC/MS, and after complete consumption of 3-mercaptopropionic acid, the reaction mixture was concentrated and loaded onto a 120 g C18 column. The purification was carried out with MeCN/H_2O (0 - 100%). The fractions were analyzed on LC/MS, and fractions containing the desired product were combined and evaporated under reduced pressure. An oil phase was observed on the bottom of the flask during concentration. This oily residue was separated from the aqueous phase and dried under high vacuum to yield the desired product as colorless solid (2.4 g). The aqueous phase was extracted with EtOAc in order to separate additional product. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to yield the desired product (0.5g). 3-(2-Pyridyldithio)propionic acid was isolated as a white solid (2.9 g, 68%); LC/MS (ESI-QMS): *m/z* = 216.25 (M + H), ^{1}H NMR (CD_3OD): 8.39 (m, 1H), 7.84 (m, 1H), 7.79 (m, 1H), 7.21 (m,

1H), 4.87 (br, 1H), 3.03 (t, J = 6.8 Hz, 2H), 2.70 (t, J = 6.8 Hz, 2H). ^{13}C NMR (CD_3OD): 173.53, 159.82, 148.97, 137.74, 120.99, 119.81, 33.50, 32.96.

Step 2: Preparation of Compound 21.

To a solution of *N*-Fmoc-ethylenediamine hydrochloride (500 mg, 1.57 mmol), 3-(2-Pyridyldithio)propionic acid (338 mg, 1.57 mmol), and $^{i}Pr_2NEt$ (839 uL, 4.71 mmol) in DMF (7.85 mL) was added PyBOP (950 mg, 1.57 mmol) in one portion. The reaction mixture was stirred for 5 min at room temperature and then concentrated under high vacuum. Water was added to the crude mixture (50 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic layers were dried over sodium sulfate, filtered, and evaporated to dryness to yield a pale yellow oil. The product was further purified via silica chromatography (0 - 80% EtOAc/pet. ether). The product was isolated as a white solid with 86% purity according to HPLC (633 mg, 84.1%): LC/MS (ESI-QMS): *m/z* = 480.56 (M+H), ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, J = 4.9, 1H), 7.75 (d, J = 7.3, 2H), 7.59 (m, 3H), 7.40 (t, J = 7.3, 2H), 7.30 (t, J = 7.3, 2H), 7.09 (t, J = 5.9, 1H), 6.98 (s, 1H), 4.56 (d, J = 6.8, 2H), 4.17 (t, J = 6.8, 1H), 3.43 (m, 2H), 3.40 (m, 2H), 3.08 (t, J = 6.4, 2H), 2.60 (t, J = 6.4, 2H).

Step 3: Preparation of Compound 22.

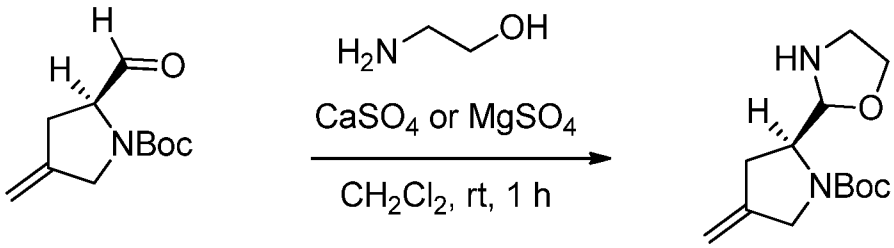
In a dry flask, **Compound 21** (318 mg, 0.664 mmol, 1.0 equiv.) and 2-mercapto-2-methyl-propan-1-ol (92 mg, 0.863 mmol, 1.3 equiv.) were dissolved in $CHCl_3$:MeOH (1:3, 20 mL). The reaction mixture was stirred for 4 h at 60°C and monitored until completion by LC/MS. The solvent was removed under reduced pressure to yield an oily residue, followed addition of water and subsequent extractions with EtOAc (3x). The organic extracts were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The product was further purified using silica gel chromatography (CH_2Cl_2/MeOH, 0 - 4%) to yield **Compound 22** (285 mg, 90%): LC/MS (ESI-QMS): *m/z* = 475.18 (M+H), ^{1}H NMR (500 MHz $CDCl_3$) δ 7.78 (d, J = 7.3 Hz, 2H), 7.67 (d, J = 7.3 Hz, 2H), 7.40 (dd, J = 14.7, 7.9 Hz, 2H), 7.32 (dd, J = 14.7, 7.9 Hz, 2H), 6.38 (s, 1H), 5.35 (s, 1H), 4.40 (d, J = 6.9 Hz, 2H), 4.21 (dd, J = 13.7, 6.8 Hz, 1H), 3.47 (s, 2H), 3.42-3.31 (m, 4H), 2.82 (t, J = 6.9 Hz, 2H), 2.58 (t, J = 6.9 Hz, 2H), 1.25 (s, 6H).

Step 4: Preparation of Compound 23.

To a suspension of **Compound 22** (0.552 mg, 1.16 mmol) in dry MeCN (12 mL) under argon was added N,N'-disuccinimidyl carbonate (0.358 g, 1.40 mmol) and pyridine (0.118 mL, 1.45 mmol) respectively. The reaction was allowed to stir at for 15 h room temperature in which the reaction turned into clear solution. LC/MS analysis confirmed that the reaction went to completion. The reaction mixture was concentrated and purified via silica chromatography (0 - 5% CH_2Cl_2/MeOH)to yield **Compound 23** (0.68 g, 95%): LC/MS (ESI-QMS): m/z =

616.24 (M + H), ^{1}H NMR (500 MHz, CD3OD) δ 7.79 (d, J1= 7.5 Hz, 2H), 7.64 (d, J1= 7.0 Hz, 2H), 7.38 (dd, J1= 8.0 Hz, J2= 7.5 Hz, 2H), 7.30 (dd, J1= 7.0 Hz, J2= 7.5 Hz, 2H), 4.33 (d, J1= 7.0 Hz, 2H), 4.28 (s, 2H), 4.19 (t, J1= 7.0 Hz, J2= 6.5 Hz, 1H), 3.20-3.30 (m, 4H), 2.91 (t, J1= 7.0 Hz, J2= 7.0 Hz, 2H), 2.80 (s, 4H), 2.56 (t, J1= 7.5 Hz, J2= 7.5 Hz, 2H), 1.31 (s, 6H); ^{13}C NMR (125 MHz, CD3OD) δ 172.41, 169.81(2C), 157.60, 151.59, 143.92 (2C), 141.19 (2C), 127.37 (2C), 126.74 (2C), 124.79 (2C), 119.53 (2C), 75.90, 66.40, 48.39 (2C), 39.83, 39.05, 35.58, 35.12, 24.98 (2C), 23.05 (2C).

Example 14: Preparation of Compound 26.



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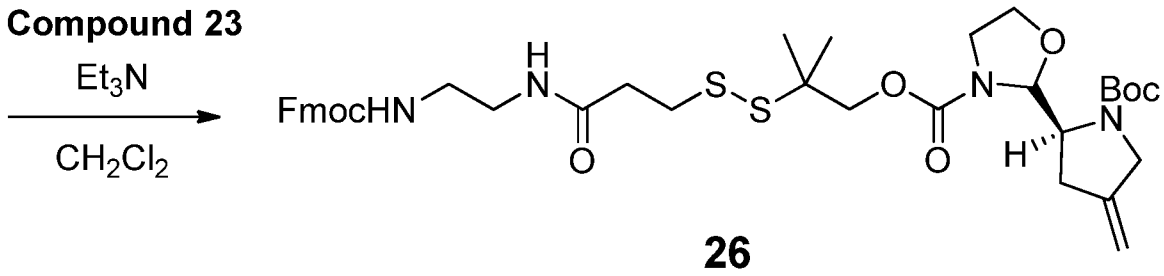
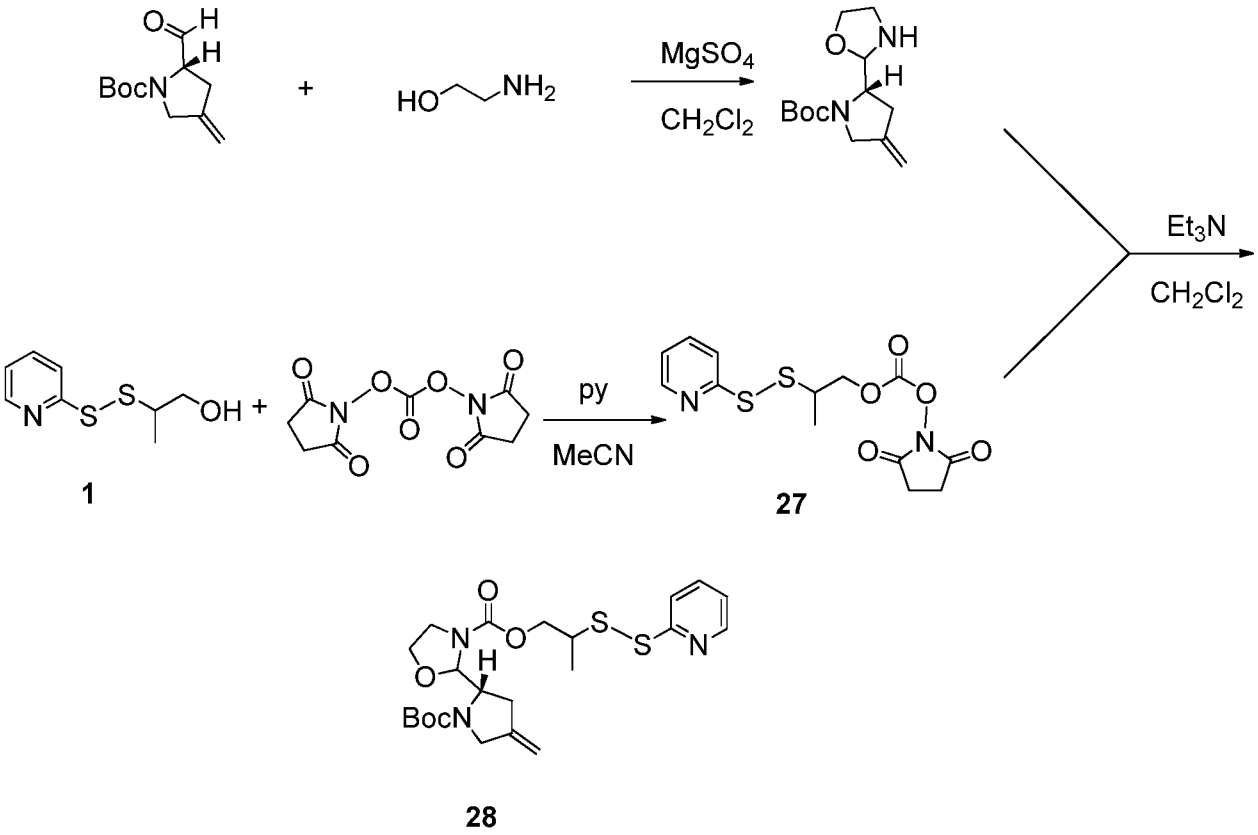


26

To a solution of the *N*-Boc-4-methylene-L-prolinal (44.36 mg, 0.2099 mmol) in dry CH_2Cl_2 (1 mL) was added anhydrous $CaSO_4$ (22 mg, 0.16 mmol) and ethanolamine (10.56 µL, 0.1750 mmol) respectively. The reaction was allowed to stir for 1 h at room temperature. In another flask, **Compound 23** (108 mg, 0.180 mmol) was dissolved in dry CH_2Cl_2 (1 mL). The previous pyrrolidine solution was filtered and slowly added to the **Compound 23** solution. Et_3N (0.037 mL, 0.26 mmol) was added to the reaction mixture, and the resulting mixture was monitored via LC/MS. After stirring for 2h, the reaction mixture was diluted with CH_2Cl_2, washed with sat. $NH_4Cl_{(aq)}$, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was further purified silica chromatography (0 - 10% CH_2Cl_2/MeOH) to yield pure **Compound 26** (83 mg, 63%): LC/MS (ESI-QMS): *m/z* = 755.38 (M + H), ^{1}H NMR (500 MHz, CD_3OD) *δ* 7.79 (d, J1= 8.0 Hz, 2H), 7.64 (d, J1= 7.5 Hz, 2H), 7.38 (dd, J1= 7.5 Hz, J2= 7.5 Hz, 2H), 7.30 (dd, J1= 7.5 Hz, J2= 7.5 Hz, 2H), 5.13-5.20 (m*, 1H), 4.88-5.05 (m*, 2H), 4.36-4.60 (m*, 1H), 4.33 (d, J1= 7.0 Hz, 2H), 4.20 (t, J1= 7.0 Hz, J2= 7.0 Hz, 1H), 3.98-4.10 (m*, 3H), 3.72-3.94 (m*, 4H), 3.36-3.50 (m*, 1H), 3.18-3.30 (m*, 4H), 2.91 (t, J1= 7.5 Hz, J2= 7.0 Hz, 2H), 2.70-2.40 (m*, 2H), 2.54 (t, J1= 7.0 Hz, J2= 7.0 Hz, 2H), 1.40-1.50 (m*, 9H), 1.26-1.38 (m*, 6H).

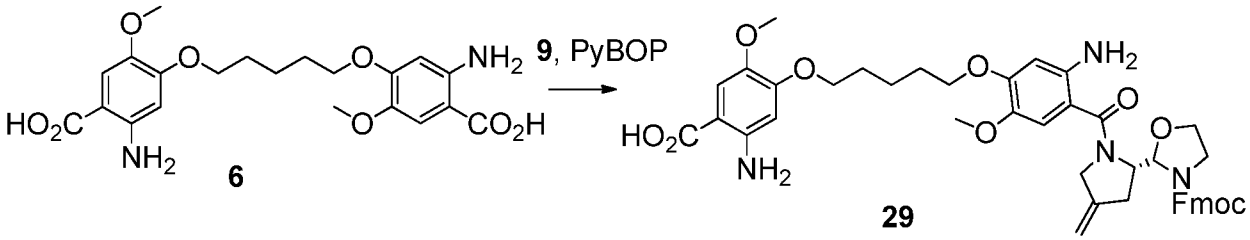
* Due to diasteromeric and/or rotameric nature of the compound

Example 15: Preparation of Compound 28.



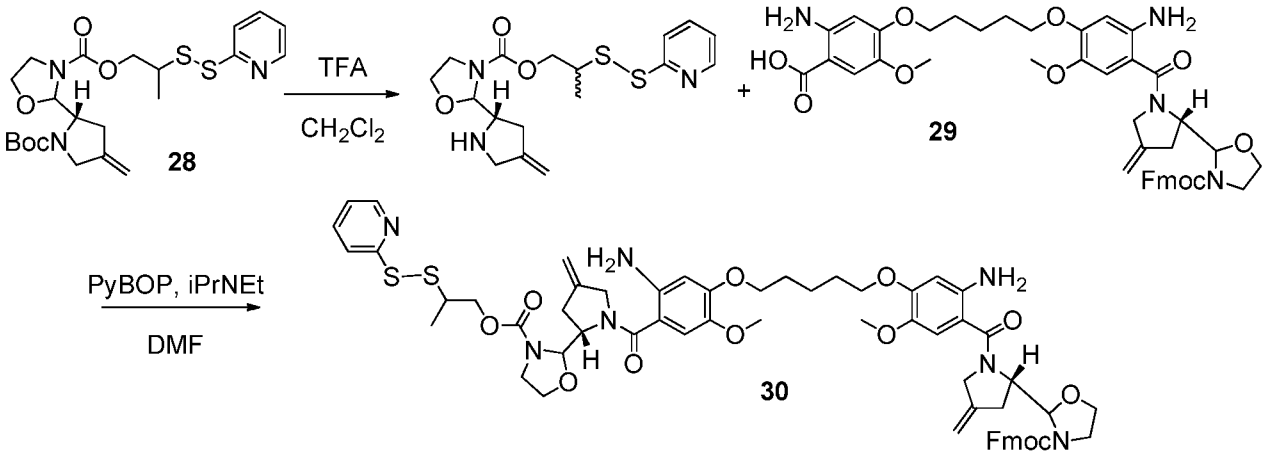
Compound 28 was synthesized by following the procedure for **Compound 26** from **Compound 27** in lieu of **Compound 22**: LC/MS (ESI-QMS): *m/z* = 482 (M + H).

Example 16: Preparation of Compound 29.



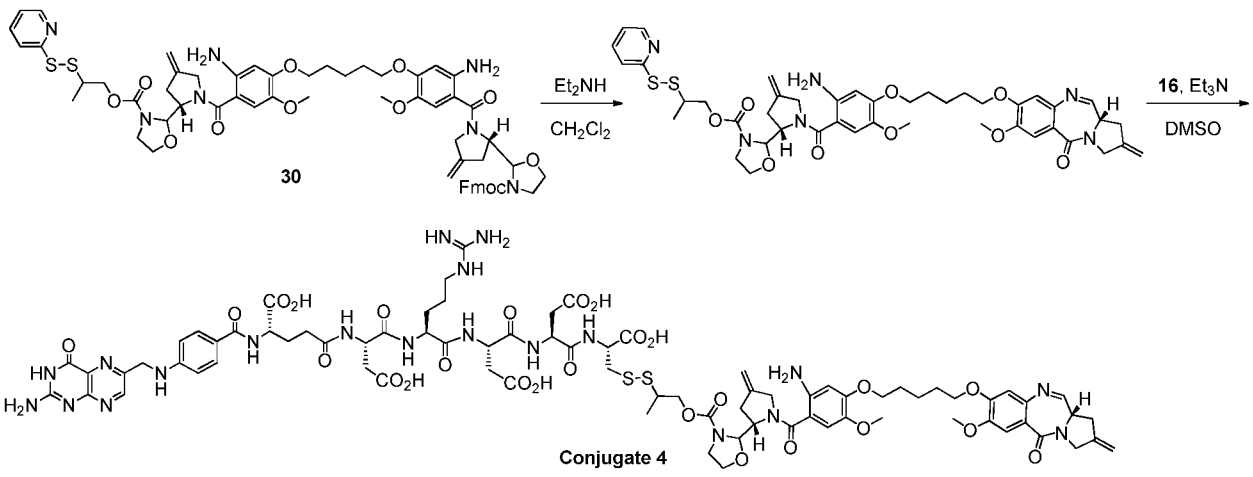
Compound 6 (42.0 mg, 0.097 mmol), **Compound 9** (0.053 mmol), and PyBOP (29.0 mg, 0.056 mmol) were dissolved in DMF/DCM (0.5 mL/0.5 mL) and treated with DIPEA (74 µL, 0.43 mmol) at r.t. under Ar. The reaction was completed within 1hr, then loaded onto CombiFlash column in 0-20% MeOH/DCM to afford the pure product **Compound 29** (25.5 mg, 60%). LCMS: $[M+H]^+$ m/z =793.

Example 17: Preparation of Compound 30.



Compound 28 (26.1 mg, 0.0551 mmol) was added to TFA/CH_2Cl_2 (0.5 mL/0.5 mL) at and stirred for 30 min at room temperature. Then the solvent was removed *in vacuo*, and the residue was dissolved in CH_2Cl_2 (0.5 mL) and added to a solution of **Compound 29** (43.6 mg, 0.0551 mmol) in DMF (0.3 mL). The reaction mixture was treated with PyBOP (47.77 mg, 0.0918 mmol) and $^{i}Pr_2NEt$ (31.98 μL, 0.184 mmol). The reaction was stirred at room temperature under argon for 2 h. The reaction mixture was then concentrated and purified via silica chromatography (0 - 10% MeOH/ CH_2C_2) to yield **Compound 30** (55.2mg, 86%): LC/MS (ESI-QMS): *m/z* = 1157 (M + H).

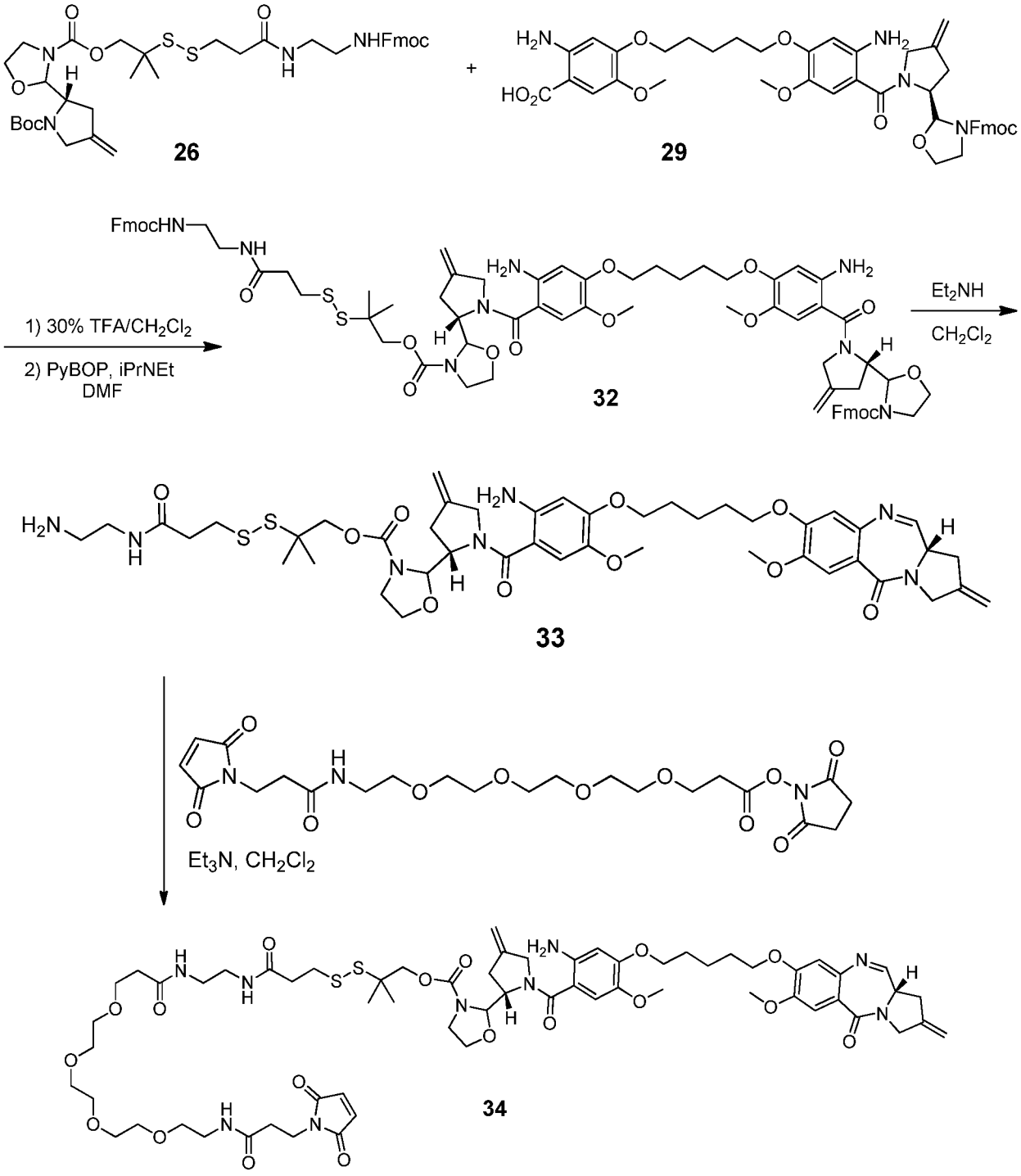
Example 18: Preparation of Conjugate 4.



Compound 30 (27.6 mg, 0.0239 mmol) was dissolved in CH_2Cl_2 (0.5 mL) and treated with diethylamine (0.15 mL, 1.4 mmol) at room temperature under argon for 3h. The reaction mixture was evaporated to dryness and dissolved in DMSO (0.5 mL). The resulted solution was added to the solution of **Compound 16** (25.0 mg, 239 mmol) and Et_3N (20 μL, 140 mmol) in DMSO (2 mL) at room temperature under argon for 1. The product was purified with preparative HPLC (10 - 100% MeCN/NH_4HCO_3 buffer pH 7.4) to yield **Conjugate 4** (8.1 mg, yield 19% over two steps). LC/MS (ESI-QMS): *m/z* = 905 (M + 2H), ^{1}H NMR (500 MHz, D_2O

+ one drop of DMSO-d_6, the major fraction, the selected data) δ 8.59 (br, s, 1H), 7.55 (br, 2H), 7.03 (br, 1H), 6.65 (br, m, 3H), 6.50 (m, br, 1H), 6.35 (br, 1H), 5.00 (m, 6H).

Example 19: Preparation of Compound 32.



Step 1: Preparation of Compound 32.

In a flask, **Compound 26** (95.0 mg, 0.126 mmol) was dissolved in 30% TFA/CH_2Cl_2 (10 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. Upon complete removal of the Boc protecting group, the solvent was removed under reduced pressure, and the crude residue was left under high vacuum for 3 h. In a dry flask, the

crude TFA salt and **Compound 29** (100 mg, 0.126 mmol) were dissolved in dry DMF (2.5 mL) under argon. To the reaction mixture was added PyBOP (131 mg, 0.252 mmol) and $^{i}Pr_2NEt$ (67 µl, 0.378 mmol) subsequently. After 3h, the reaction was quenched by the addition of sat. $NH_4Cl_{(aq)}$ and extracted with EtOAc (3x). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified using silica gel chromatography (0 - 8% MeOH/CH_2Cl_2) to yield **Compound 32** (153 mg, 84.9%): LC/MS (ESI-QMS): *m/z* = 1429.78 (M + H), ^{1}H NMR (500 MHz $CDCl_3$) δ Pivotal signals: δ 7.75-7.66 (m, 4H), 7.58-7.47 (m, 4H), 7.75-7.66 (m, 4H), 7.39-7.31 (m, 4H), 7.29-7.22 (m, 4H), 7.02-6.51 (m, 4H), 5.31-5.14 (m, 1H), 5.04-4.74 (m, 5H), 1.28-1.12 (m, 6H).

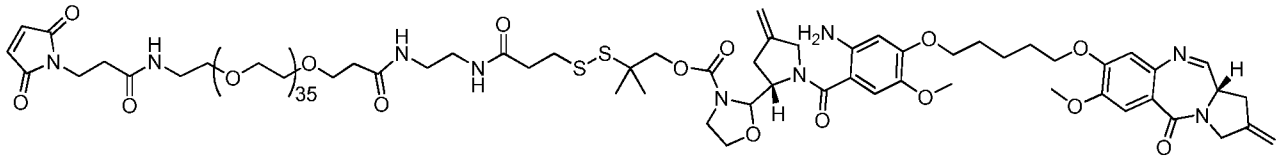
Step 2: Preparation of Compound 33.

Compound 32 (80.0 mg, 0.0559 mmol) in CH_2Cl_2 (2 mL) was treated with diethylamine (0.5 mL) at room temperature under argon. The reaction mixture was stirred for 1 h and concentrated *in vacuo*. The product **Compound 33** was used in the next step without further purification: LC/MS (ESI-QMS): *m/z* = 924, 925 (M + H).

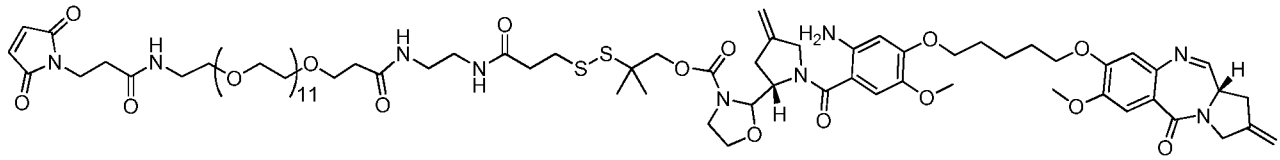
Step 3: Preparation of Compound 34.

Compound 33 (0.0559 mmol) and Mal-PEG4-NHS ester (38.7 mg, 0.0754 mmol) in CH_2Cl_2 (3.5 mL) was treated with Et_3N (7.8 µL, 0.0559 mmol) at room temperature under argon. The reaction was monitored via LC/MS and went to completion within 3 h. The solvent was removed *in vacuo*, and the crude product **Compound 34** was dissolved in DMSO (2 mL) for the conjugation. LC/MS (ESI-QMS): *m/z* = 1323 (M +H).

Example 20: Preparation of Compound 35 and 36.



35



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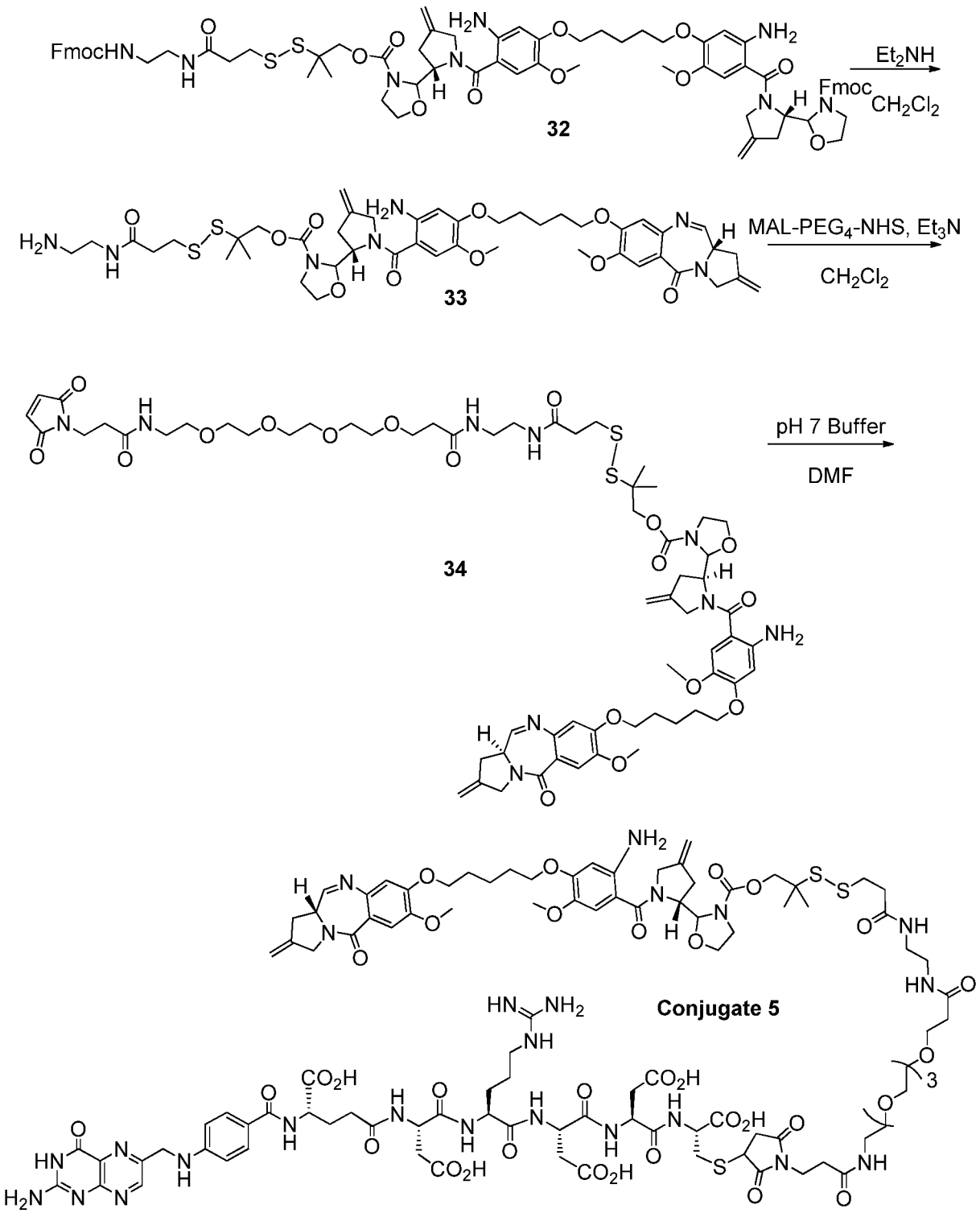
Compound 35 and **Compound 36** were synthesized in the same method as for **Compound 34**. **Compound 35**: LC/MS (ESI-QMS): *m/z* = 1367 (M + 2H); **Compound 36**: LC/MS (ESI-QMS): *m/z* = 838.7 (M + 2H), 1676 (M + H). Mal-PEG4-NHS ester, Mal-PEG12-NHS ester, and Mal-PEG36-NHS ester were obtained from Quanta BioDesign Ltd.

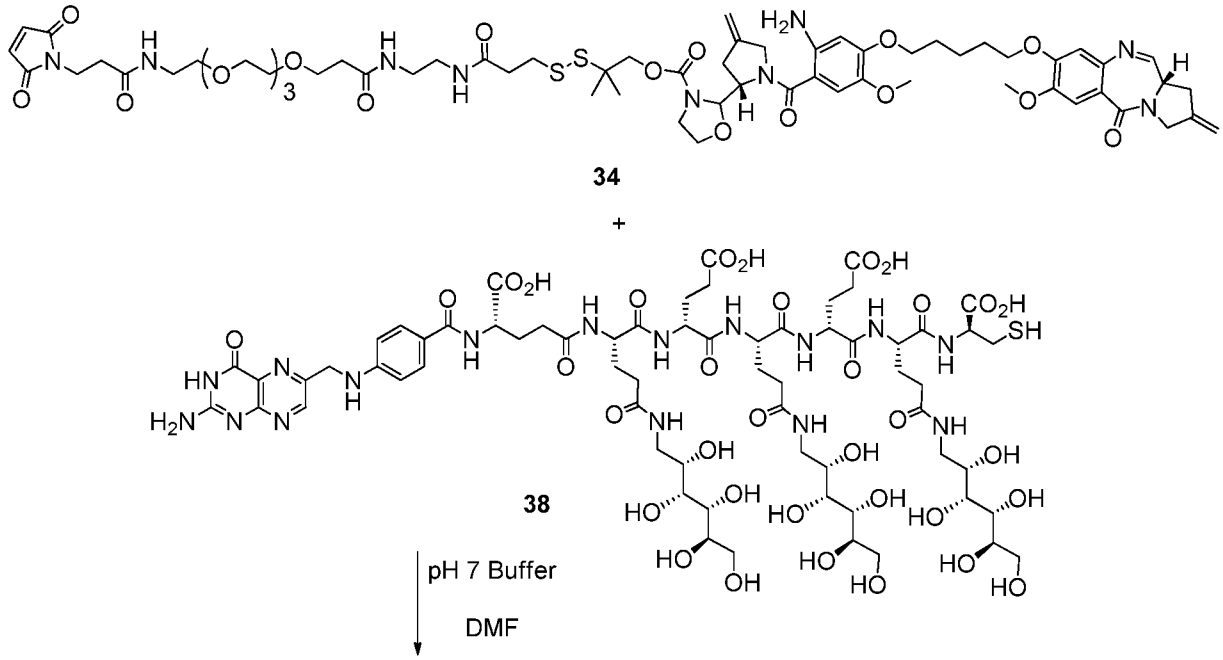
Example 21: Preparation of Conjugate 5.

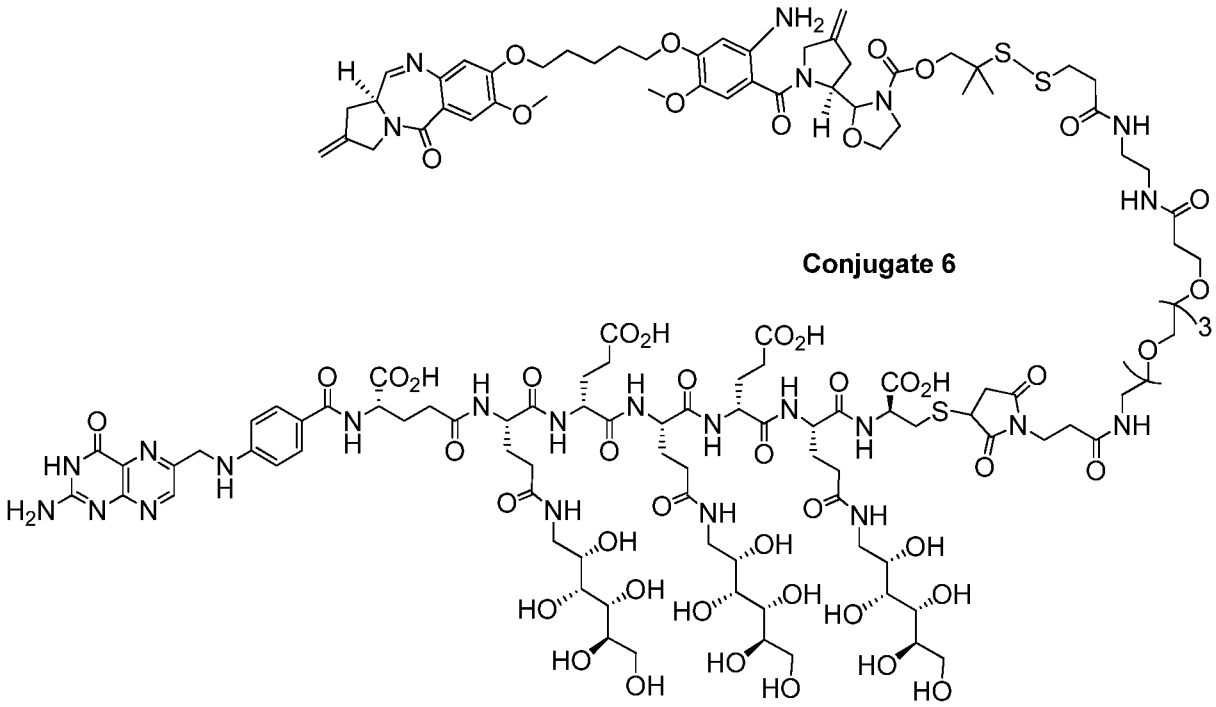


Compound 32 (23 mg, 0.016 mmol) and diethylamine (0.25 mL, 2.4 mmol) were dissolved in CH_2Cl_2 (0.6 mL), and the reaction mixture was stirred at room temperature under argon for 3 h. The reaction was monitored via LC/MS and after complete consumption of **Compound 32**, the solvent was removed under reduced pressure. The resulting residue was co-evaporated with CH_2Cl_2 twice and dried under high vacuum for 15 minutes. The resulting residue was dissolved in CH_2Cl_2 (0.5 mL), and Mal-PEG4-NHS ester (10.9mg, 0.021 mmol) and Et_3N (3.0 μL, 0.021 mmol) were added. The reaction was stirred at room temperature under

argon and monitored via LC/MS for production of **Compound 34** (*m/z* = 1323 and 662). After 1 h, the reaction mixture was evaporated, and the resulting residue was dissolved in DMF (2 mL). The solution was purged with argon. **Compound 16** (22 mg, 0.021 mmol) was dissolved in pH 7 buffer (2 mL, 50 mM NH_4HCO_3), purged with argon, and added to the above **Compound 34** solution. The reaction was stirred at room temperature while purging with argon. The reaction was monitored via LC/MS for the production of **Conjugate 5** (*m/z* = 791). After 2 hours, purification via preperative HPLC (10 – 100% MeCN/50 mM NH_4HCO_3 pH 7 buffer) yielded two sets of isomers: 1.9 mg of 1st set of isomers with a shorter retention time and 7.4 mg of 2nd set of isomers with a longer retention time. The desired product was obtained in a yield of 24% over three steps: LC/MS (ESI-QMS): *m/z* = 791.25 (M + 3H), Major Product: ^{1}H NMR (DMSO-D6, selected data): 8.61 (s, 1H), 7.72 (d, NH), 7.55 (d, J = 8.8 Hz, 2H), 7.30 (s, NH), 7.15 (s, ArH), 7.01 (s, ArH), 6.81 (s, NH), 6.60 (d, J = 8.8 Hz, 2H+1H overlapped), 6.54 (s, ArH), 6.34 (s, N=CH), 6.32 (s, ArH), 5.11+5.06 (m, 2 H), 4.96 + 4.92 + 4.85 (m, 3H), 3.66 + 3.62 (s+s, 3 H), 3.61 (s, 3H), 3.55 (t, 3H), 3.35(t, 3H), 1.21(s, br, 6H). Minor Product: ^{1}H NMR (DMSO-D6, selected data): 8.61 (s, 1H), 7.72 (d, NH), 7.55 (d, J = 8.8 Hz, 2H), 7.29 (s, NH), 7.15 (s, ArH), 7.01 (s, ArH), 6.80 (s, NH), 6.60 (d, J = 8.8 Hz, 2H+1H overlapped), 6.53 (s, ArH), 6.32 (s, N=CH), 6.31 (s, ArH), 5.11+ 5.06 (m, 2H), 4.94 – 4.85 (m, 3 H), 3.66 + 3.62 (s+s, 3 H), 3.61 (s, 3H), 3.55 (t, 3H), 3.35(t, 3H), 1.20(s, br, 6H).

Example 22: Preparation of Conjugate 6.

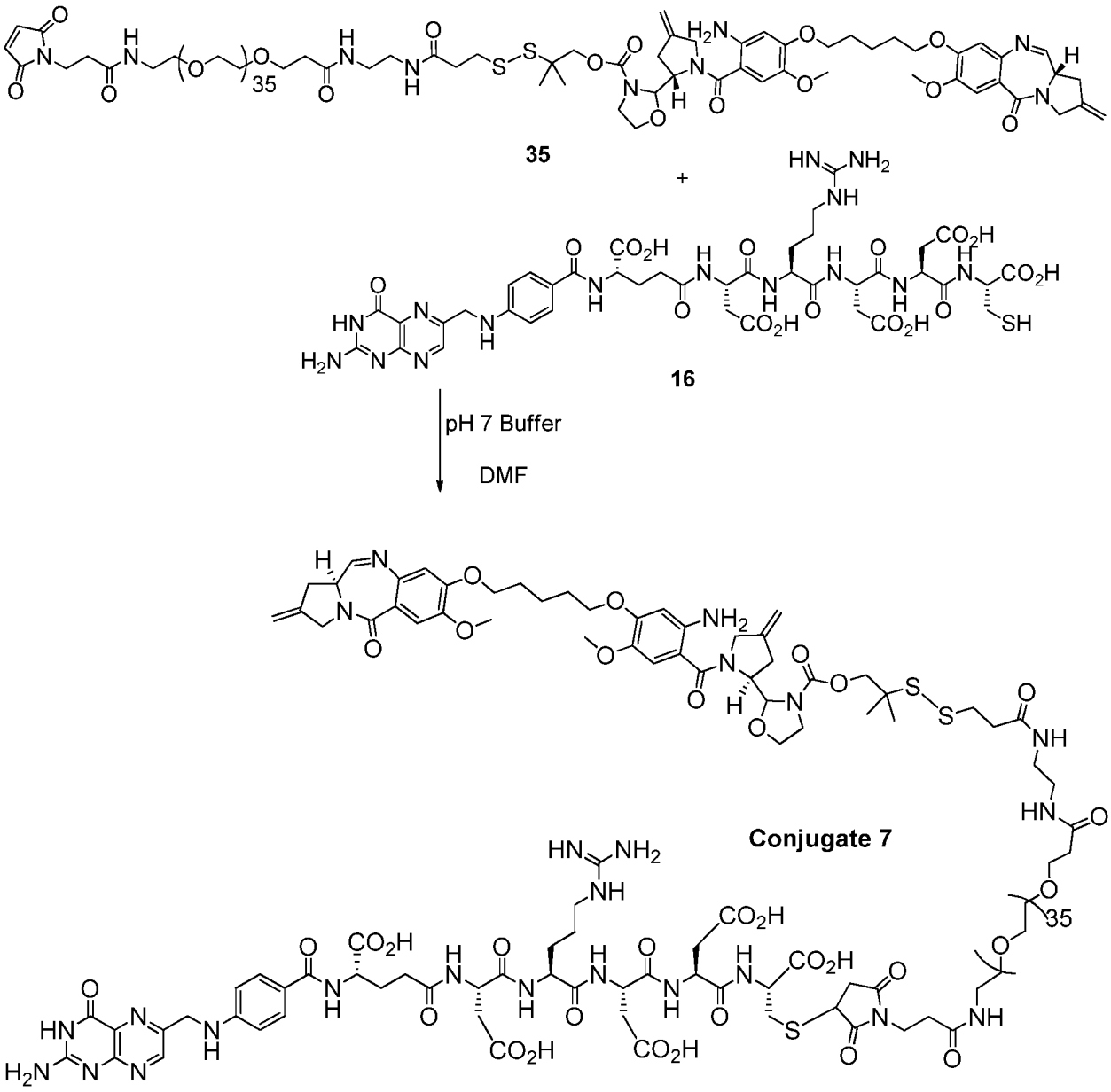


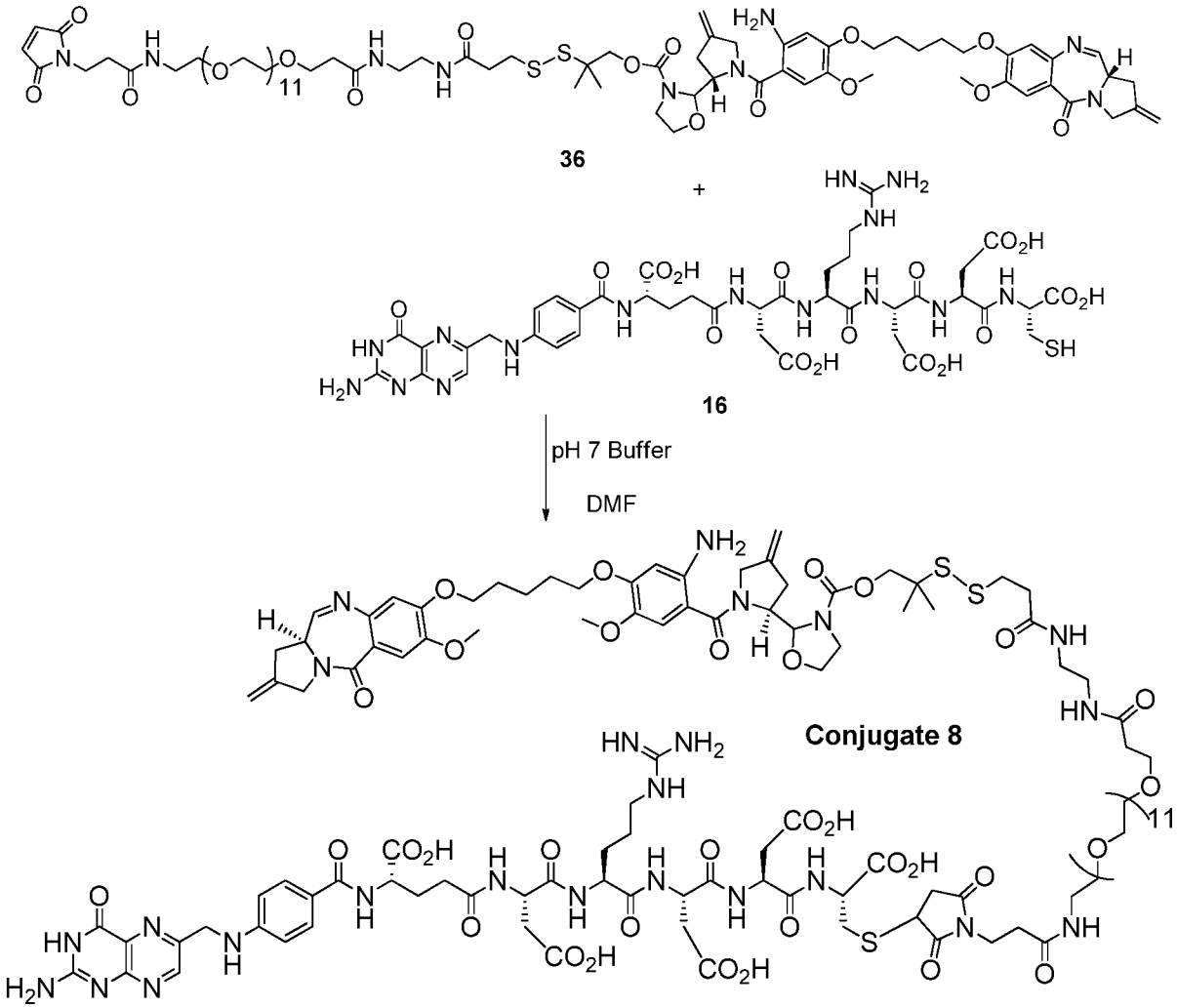


Conjugate 6

Conjugate 6 was synthesized by following the procedure for **Conjugate 5** from **Compound 34** in lieu of **Compound 32**: LC/MS (ESI-QMS): *m/z* = 1502 (M + 2H), 1001 (M + 3H): ^{1}H NMR (500 MHz, DMSO-d_6 + drops of D_2O) δ The major fraction: 8.61 (s, 1H), 7.58 (d, J = 8.32 Hz, 2H), 7.12 (s, 1H), 7.00 (s, 1H), 6.61 (d, J = 8.31 Hz, 2H), 6.50 (s, 1H), 6.30 (m, 2H), 5.00 (m, 6H), 4.50 (m, 3H), 4.13 (m, br, 13H), 3.63 (s, 3H), 3.59 (m, 8H), 3.51 (m, 11H), 3.43 (m br, 15H), 3.35 (m, 9H), 3.20 (m, br, 5H), 3.15 (m, br, 3H), 3.03 (m, br, 9H), 2.80 (br, 4H), 2.61 (br, 2H), 2.40 (br, m, 6H), 2.26 (m, 4H), 2.10 (m, br, 11H), 1.90 (m, br, 8H), 1.74 (br m, 9H), 1.50 (br, 3H), 1.20 (m, br, 10H), The minor fraction: 8.60 (s, 1H), 7.59 (d, J = 8.31 Hz, 2H), 7.11 (s, 1H), 7.00 (s, 1H), 6.62 (d, J = 8.31 Hz, 2H), 6.50 (s, 1H), 6.29 (m, 2H), 5.08 (m, 2H), 4.90 (m, 4H), 4.50 (m, 3H), 4.00 (m, 12H), 3.65 (s, 3H), 3.59 (m, 8H), 3.53 (m, 12H), 3.49 (m, br, 17H), 3.35 (m, 10 H), 3.20 (br, m, 6H),, 3.10 (m, br, 3H), 3.08 (m, br, 10H), 2.78 (br,m, 4H), 2.39 (m, br, 5H), 2.25 (br, m, 5H), 2.15 (br, 6H), 2.10 (br, 7H), 1.93 (br, m, 5H), 1.85 (s, 5H), 1.73 (br, m, 7H), 1.50 (br, 3H), 1.25 (br, m, 8H).

Example 23: Preparation of Conjugate 7 and Conjugate 8.

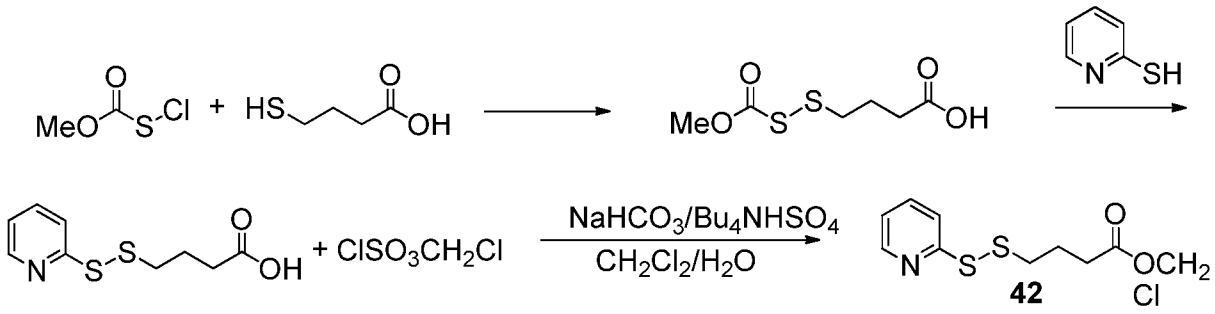

35
16
pH 7 Buffer
DMF
Conjugate 7



Conjugate 7 and **Conjugate 8** were synthesized by following the procedure for **Conjugate 5** from **Compound 35 and Compound 36** respectively in lieu of **Compound 32**.

Conjugate 7: LC/MS (ESI-QMS): *m/z* = 1260 (M + 3H), ^{1}H NMR (500 MHz, DMSO-d_6 + drops of D_2O, the major fraction) δ 8.61 (s, 1H), 7.51 (d, J = 8.31 Hz, 2H), 7.12 (s, 1H), 7.00 (s, 1H), 6.60 (d, J = 8.32 Hz, 2H), 6.50 (s, 1H), 6.32 (m, 2H), 5.00 (m, br, 6H), 4.50 (m, br, 7H), 4.00 (m, br, 20H), 3.60 (m, 4H), 3.50 (br, 134H), 3.30 (m, 2H), 3.13 (m, 2H), 3.05 (s, br, 5H), 2.95 (m, 1H), 2.80 (m, 3H), 2.62 (s, 2H), 2.39 (m, 5H), 2.24 (m, 5H), 2.04 (m, 2H), 1.89 (m, 2H), 1.79 (m, 4H), 1.67 (m, 1H), 1.50 (br, m, 4H), 1.20 (m, 8H) **Conjugate 8**: LC/MS (ESI-QMS): *m/z* = 908 (M + 3H), ^{1}H NMR (500 MHz, DMSO-d_6 + drops of D_2O, the major fraction) δ 8.61 (s, 1H), 7.58 (d, J = 8.31 Hz, 2H), 7.12 (s, 1H), 7.00 (s, 1H), 6.62 (d, J = 8.80 Hz, 2H), 6.50 (s, 1H), 6.30 (m, 2H), 5.00 (m, 6H), 4.50 (m, 5H), 4.35 (m, 1H), 4.15 (m, 8H), 3.65 (s, 3H), 3.60 (m, 5H), 3.55 (m, 5H), 3.47 (s, br, 52H), 3.35 (m, 4H), 3.03 (m, 9H), 2.80 (br, m, 5H), 2.60 (br, m, 5H), 2.40 (m, 6H), 2.27 (m, 5H), 2.13 (br, 2H), 1.90 (m, br, 3H), 1.75 (m, br, 6H), 1.60 (br, m, 7H), 1.20 (br, m, 8H).

Example 24: Preparation of Compound 42.



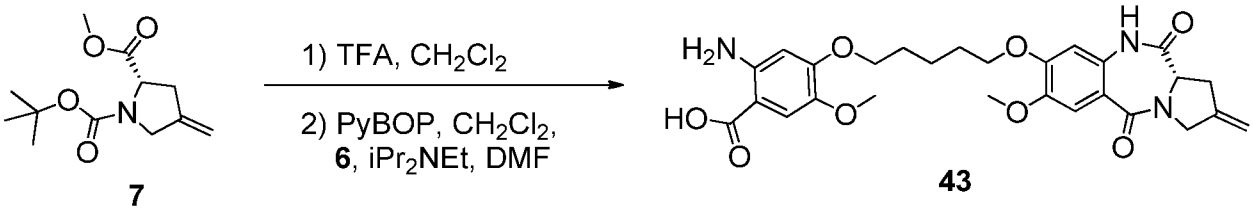
Step 1: Preparation of 4-(Pyridin-2-yldisulfanyl)butanoic acid.

A solution of 2.16 g (18.0 mmol) 4-mercaptobutyric acid in 4 mL THF was added to a solution of 2.33 g (18.4 mmol) methoxycarbonylsulfenyl chloride in 4 mL THF at 0°C. The reaction mixture was stirred at 0°C for 30 min. Then 2.10 g (18.9 mmol) of 2-mercaptopyridine was added to the reaction mixture at 0°C. The resulting reaction mixture was allowed to warm to room temperature. The reaction was monitored by LC/MS. After the reaction was complete, the solvent was evaporated and the residue was dissolved in dichloromethane. Purification with CH_2Cl_2/methanol on Combiflash provided product with impurity. The fractions containing the desired product were combined and concentrated under vacuum. The resulting yellow oil was dissolved in CH_2Cl_2 and purified with silica chromatography (petroleum ether/EtOAc) to afford 1.00 g of 4-(pyridin-2-yldisulfanyl)butanoic acid (24%). LC/MS (ESI-QMS): *m/z* = 230.27 (M + H).

Step 2: Preparation of Compound 42.

458 mg (2 mmol) of 4-(pyridin-2-yldisulfanyl)butanoic acid was mixed with $NaHCO_3$ (672 mg, 8 mmol) and Bu_4NHSO_4 (68 mg, 0.2 mmol) in 8 mL H_2O/8 mL CH_2Cl_2. The mixture was stirred vigorously at 0°C for 10 min. Then the solution of 396 mg (2.4 mmol) of chloromethyl chlorosulfate in 2 mL CH_2Cl_2 was added to the above mixture. The reaction mixture was stirred vigorously and warmed up to room temperature. The reaction was monitored with LC/MS. After 2 hours, the organic layer was separated. The aqueous layer was washed with additional CH_2Cl_2. The organic solution was combined and washed with brine and dried over Na_2SO_4. The salt was filtered and the solvent was removed. Purification with petroleum ether/EtOAc on silica chromatography gave 300 mg of chloromethyl ester **Compound 42** (54%). LC/MS (ESI-QMS): *m/z* = 278.23 (M + H): ^{1}H NMR (500 MHz, CDCl3) δ 8.45 (m, 1 H), 7.64 (m, 1H), 7.08 (m, 1H), 5.68 (s, 2H), 2.84 (m, 2H), 2.55 (m, 2H), 2.07 (m, 2H). ^{13}C NMR (500 MHz, CDCl3) δ 170.85, 159.85, 149.73, 136.97, 120.75, 119.87, 68.60, 37.54, 32.26, 23.52.

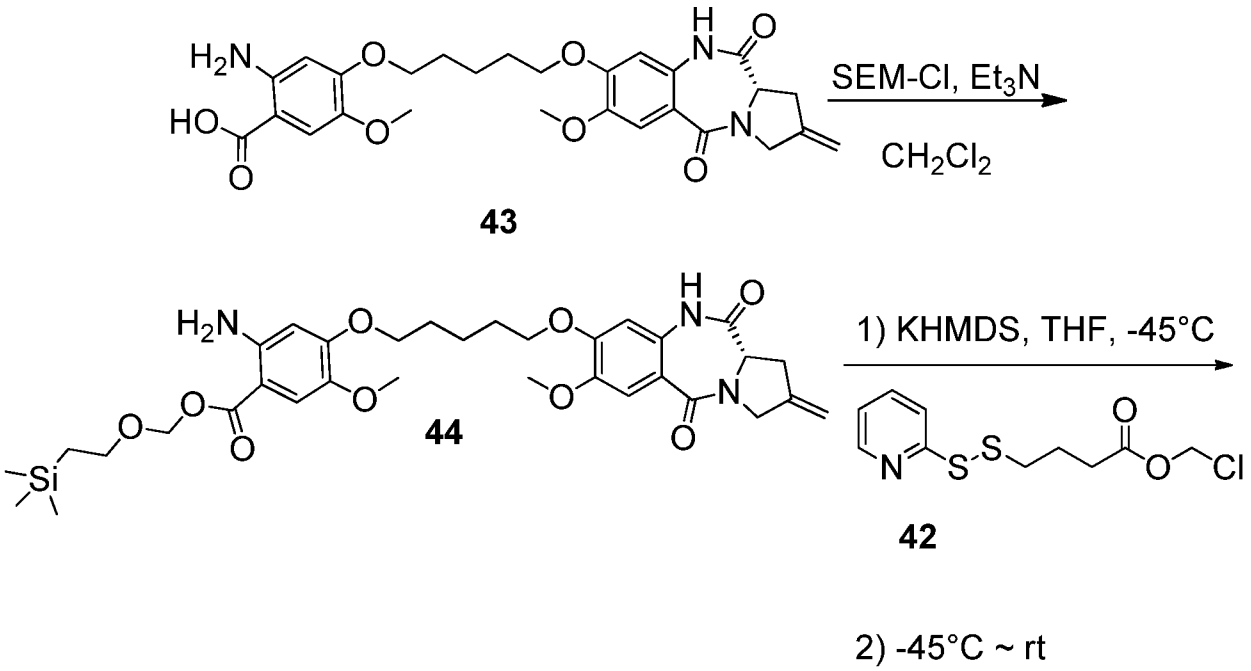
Example 25: Preparation of Compound 43.



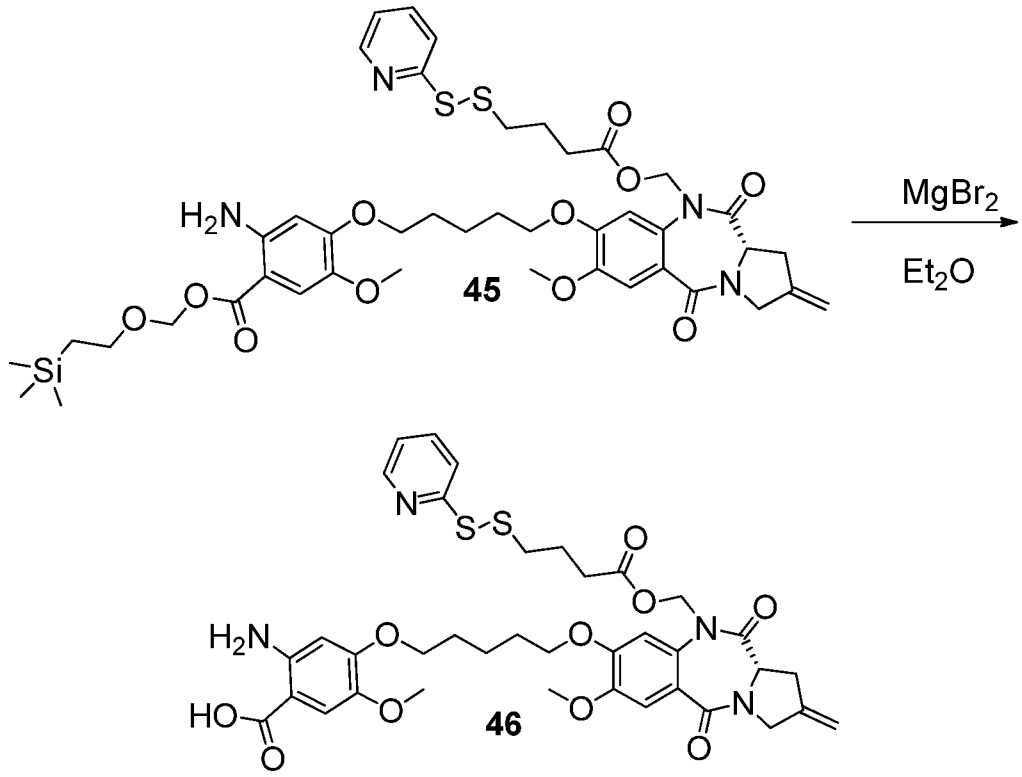
7 43

A solution of **Compound 7** (35.3 mg, 0.146 mmol) in TFA (0.50 mL) and CH_2Cl_2 (0.75 mL) was stirred at ambient temperature for 30 min. The reaction mixture was concentrated under reduced pressure, co-evaporated with DCM (1 mL × 3), and dried under vacuum for 1 h. The residue was dissolved with PyBOP (76.0 mg, 1.00 equiv.) in anhydrous CH_2Cl_2 (3.0 mL) and the resulting solution was transferred into a solution of **Compound 6** (63.4 mg, 1.0 equiv.) in anhydrous DMF (3.0 mL). After addition of iPr_2NEt (0.20 mL, 7.9 equiv.), the reaction mixture was stirred at ambient temperature under argon for 90 min and loaded directly onto a CombiFlash system (silica gel column) eluting with 0-10% MeOH in CH_2Cl_2 to produce 37.5 mg **Compound 43** as a white solid. LC/MS (ESI-QMS): *m/z* = 524.29 (M + H).

Example 26: Preparation of Compound 46.



43 44 42



Step 1: Preparation of Compound 44.

2-(Trimethylsilyl)ethoxymethyl chloride (90.0 µL, 0.508 mmol) and Et_3N (50.0 µL, 0.359 mmol) were added in tandem to a solution of **Compound 43** (86.7 mg, 0.165 mmol) in anhydrous CH_2Cl_2 (7.0 mL). After stirring at room temperature under argon for 2.5 h, the reaction mixture was concentrated under reduced pressure and purified via silica chromatography (0 - 70% EtOAc/pet. ether) to yield **Compound 44** as a white solid (50.1 mg, 46.3%): LC/MS: (ESI-QMS): *m/z* = 656.53 (M + H), ^{1}H NMR (500 MHz, 298 K, DMSO-*d6*) δ 10.258 (s, 1H), 7.236 (s, 1H), 7.153 (s, 1H), 6.706 (s, 1H), 6.452 (s, 2H), 6.380 (s, 1H), 5.078 (s, 2H), 4.260 (m, 2H), 4.022 (m, 2H), 3.977 (m, 3H), 3.763 (m, 5H), 3.682 (s, 3H), 3.214 (d, *J* = 15.0 Hz, 1H), 2.785 (m, 1H), 1.797 (m, 4H), 1.578 (m, 2H), 0.924 (t, *J* = 3.0 Hz, 2H).

Step 2: Preparation of Compound 45.

0.5 M KHMDS in toluene (135 µL, 68.4 µmol) was added dropwise to a solution of **Compound 44** (37.4 mg, 57.0 µmol) in anhydrous THF (2.5 mL) at -45°C. The reaction mixture was stirred at -45°C under argon for 15 min, after which a solution of **Compound 42** (23.0 mg, 79.8 µmol) in anhydrous THF (0.50 mL) was added. The reaction mixture was allowed to warm to room temperature and stirred under argon for 30 min. The reaction was then quenched with MeOH (0.5 mL), concentrated under reduced pressure, and purified via silica chromatography (0 - 80% EtOAc/pet. ether) to yield **Compound 45** as a white solid (31.5 mg, 61.6%): LC/MS: (ESI-QMS): *m/z* = 898.28 (M +H), ^{1}H NMR (500 MHz, 298 K, DMSO-*d6*) δ 8.411 (d, *J* = 1.5 Hz, 1H), 7.788 (t, *J* = 2.5 Hz, 1H), 7.719 (d, *J* = 2.5 Hz, 1H), 7.197 (m,

3H), 7.015 (s, 1H), 6.447 (s, 2H), 6.372 (s, 1H), 5.950 (d, J = 10.0 Hz, 1H), 5.576 (d, J = 10.0 Hz, 1H), 5.096 (d, J = 13.0 Hz, 2H), 4.386 (d, J = 9.0 Hz, 1H), 4.176 (m, 2H), 3.951 (m, 4H), 3.815 (s, 3H), 3.775 (m, 2H), 3.636 (s, 3H), 3.161 (m, 1H), 2.818 (m, 2H), 2.412 (m, 2H), 1.811 (m, 4H), 1.560 (m, 2H), 0.912 (t, J = 3.0 Hz, 2H).

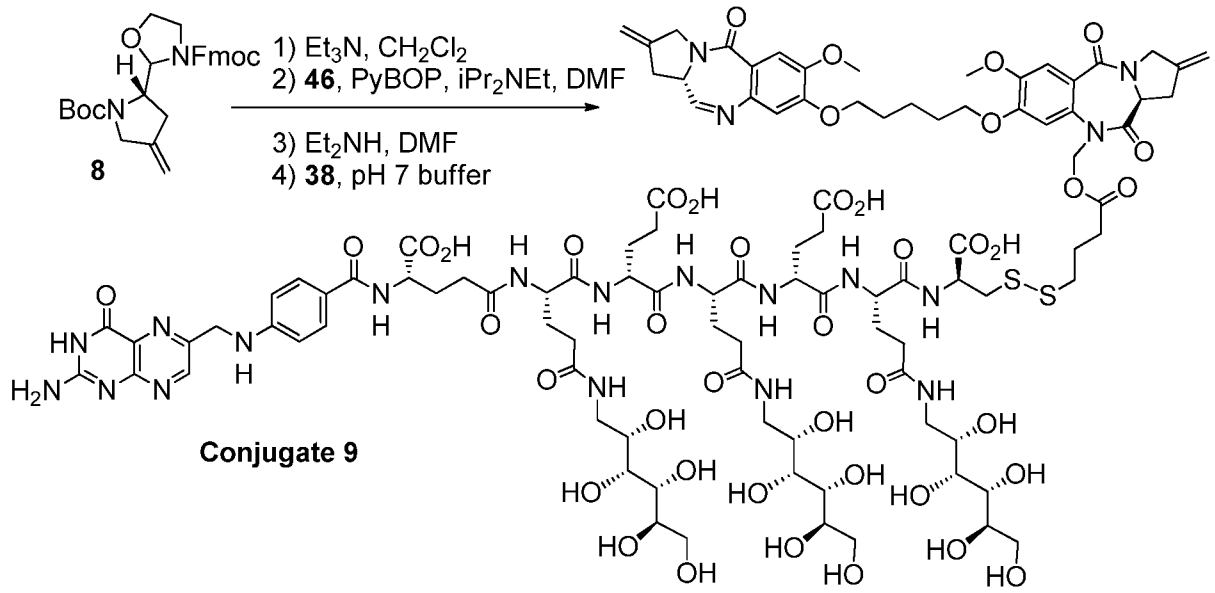
Step 3: Preparation of Compound 46.

A suspension of **Compound 45** (30.1 mg, 33.6 μmol) and $MgBr_2$ (12.4 mg, 67.2 μmol) in anhydrous Et_2O (2.0 mL) was stirred at ambient temperature under argon for 3 min. The reaction mixture was then diluted with anhydrous CH_2Cl_2 (5.0 mL), stirred at room temperature under argon for an additional 60 min, and concentrated under reduced pressure. The resulting residue was dissolved in a pre-mixed solution of formic acid (12.7 μL) in MeOH (9.5 mL), stirred at room temperature for 5 min, and loaded directly onto a preparative HPLC column for purification (10 – 100% MeCN/50 mM NH_4HCO_3 buffer, pH 7.0) to afford **Compound 46** as a white solid (13.5 mg, 52.4%): LC/MS: (ESI-QMS): m/z = 767.20 (M + H), ^{1}H NMR (500 MHz, 298 K, DMSO-*d6*) δ 8.412 (d, J = 1.5 Hz, 1H), 7.795 (t, J = 2.5 Hz, 1H), 7.722 (d, J = 2.5 Hz, 1H), 7.196 (m, 3H), 7.016 (s, 1H), 6.303 (s, 1H), 5.949 (d, J = 10.5 Hz, 1H), 5.579 (d, J = 11.0 Hz, 1H), 5.095 (d, J = 12.5 Hz, 2H), 4.387 (d, J = 9.5 Hz, 1H), 4.208 (d, J = 16.0 Hz, 1H), 4.095 (m, 1H), 4.022 (m, 2H), 3.922 (m, 2H), 3.815 (s, 3H), 3.619 (s, 3H), 3.161 (d, J = 16.5 Hz, 1H), 2.785 (m, 2H), 2.450 (m, 2H), 1.827 (m, 4H), 1.556 (m, 2H).

Example 27: Preparation of Compound 38.

Compound 38 is obtainable by the methods disclosed in PCT/US2013/065079 (WO2014062697), incorporated herein by reference.

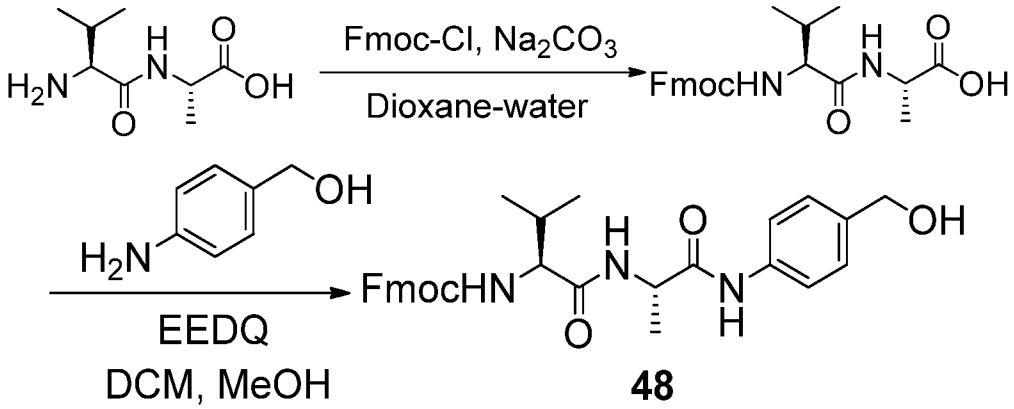
Example 28: Preparation of Conjugate 9



Conjugate 9

TFA (0.10 mL) was added to a solution of **Compound 8** (3.7 mg, 7.67 μmol) in anhydrous CH_2Cl_2 (0.40 mL). The reaction mixture was stirred at room temperature under argon for 30 min and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (1 mL × 3) and dried under high vacuum for 1 h. The crude residue was dissolved in anhydrous CH_2Cl_2 (1.0 mL) and transferred into a solution of **Compound 46** (4.5 mg, 5.9 μmol) and PyBOP (3.7 mg, 7.1 μmol) in anhydrous DMF (1.0 mL). To the solution was then added $^{i}Pr_2NEt$ (10.3 μL, 59 μmol), and the reaction mixture was stirred at room temperature under argon for an additional 100 min. The CH_2Cl_2 was removed from the reaction mixture *in vacuo* after which diethylamine (0.10 mL) was added. The reaction mixture was stirred at room temperature under argon for 15 min and further diluted with DMF (3.5 mL). A solution of **Compound 38** (11.6 mg, 6.5 μmol) in 50 mM NH_4HCO_3 buffer, pH 7.0 (4.5 mL) was then added. The reaction mixture was stirred at room temperature under argon for 20 min and purified via preparative HPLC 10 – 100% MeCN/50 mM NH_4HCO_3 buffer, pH7) to yield **Conjugate 9** as a fluffy yellow solid (4.6 mg, 32% over three steps): LC/MS: (ESI-QMS): *m/z* = 1206.43 (M + H), Selective ^{1}H NMR (500 MHz, 298 K, DMSO-*d6* with D_2O exchange) δ 8.602 (s, 1H), 7.588 (d, J = 8.5 Hz, 2H), 7.148 (s, 1H), 6.945 (s, 1H), 6.623 (d, J = 8.5 Hz, 2H), 5.914 (d, J = 10.5 Hz, 1H), 5.501 (d, J = 10.5 Hz, 1H), 5.076 (b, 3H), 4.938 (d, J = 9.0 Hz, 1H).

Example 29: Preparation of Compound 48.

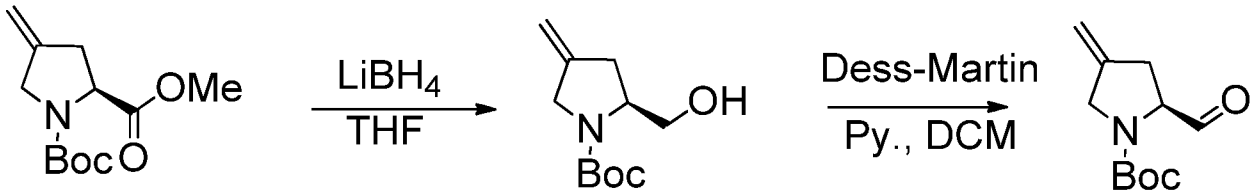


48

To a solution of Val-Ala-OH (1 g, 5.31 mM) in water (40 ml) was added Na_2CO_3 (1.42 g, 13.28 mM) and cooled to 0°C before dioxane (40 mL) was added. A solution of Fmoc-Cl (1.44 g, 5.58 mM) in dioxane (40 mL) was added dropwise over 10 min at 0°C. The reaction mixture was stirred at 0°C for 2h. Then the reaction mixture was allowed to stir at RT for 16 h. Dioxane was removed under vacuum, the reaction mixture diluted with water (450 mL), pH was adjusted to 2 using 1N HCl and extracted with EtOAc (3 x 250 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated under reduced pressure and dried to yield Fmoc-Val-Ala-OH. This product was suspended in dry DCM (25 ml), PABA (0.785 g, 6.38 mM) and EEDQ (1.971 g, 7.97mM) were added. The resulting mixture was

treated under Argon with methanol until a clear solution was obtained. The reaction was stirred overnight and filtered. The filtrate was washed with diethyl ether (4x) and dried under high vacum to yield **Compound 48** (1.85 g, 68%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.79 (d, J_1= 8.0 Hz, 2H), 7.65 (t, J_1= 7.0 Hz, J_2= 7.5 Hz, 2H), 7.54 (d, J_1= 8.0 Hz, 2H), 7.38 (t, J_1= 7.5 Hz, J_2= 7.5 Hz, 2H), 7.33-7.24 (m, 4H), 4.54 (s, 2H), 4.48 (q, J_1= 14.0 Hz, J_2= 7.0 Hz, 1H), 4.42-4.32 (m, 2H), 4.22 (t, J_1= 7.0 Hz, J_2= 6.5 Hz, 1H), 3.94 (d, J_1= 7.0 Hz, 1H), 2.07 (m, 1H), 1.43 (d, J_1= 7.5 Hz, 3H), 0.97 (d, J_1= 7.0 Hz, 3H), 0.95 (d, J_1= 7.0 Hz, 3H); LCMS (ESI): $(M + H)^+$ = Calculated for $C_{30}H_{33}N_3O_5$, 516.24; found 516.24.

Example 30: Preparation of Compound 25.



24 **25**

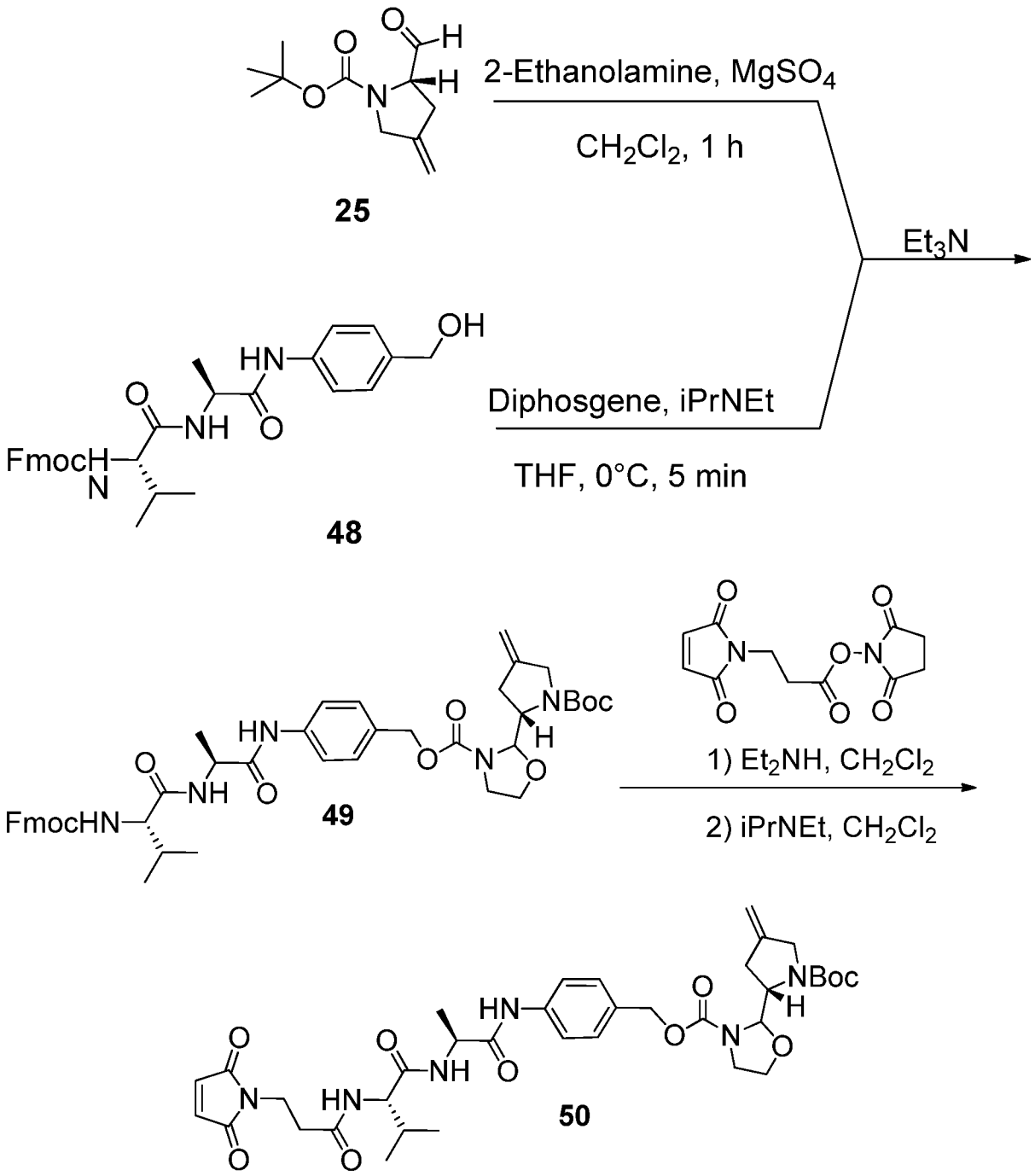
Step 1: Preparation of Compound 24.

To a mixture of 1-(tert-butyl) 2-methyl (S)-4-methylenepyrrolidine-1,2-dicarboxylate (Compound 7) (0.5 g, 2.07 mmol) in THF (10 mL) was added $LiBH_4$ (67.7 mg, 3.11 mmol) in portions at 0°C under argon. The mixture was allowed to warm to room temperature over 2.5 hours. It was cooled to 0°C and quenched with H_2O. The mixture was extracted with EtOAc (3x30 mL) and the organic phase was washed with H_2O, brine sequentially and dried over anhydrous $MgSO_4$. It was filtered and concentrated *in vacuo*. The crude product **Compound 24** was used in next step without further purification.

Step 1: Preparation of Compound 25.

To a mixture of **Compound 24** and pyridine (0.84 ml, 10.35 mmol) in dichloromethane (8 ml) was added Dess-Martin periodinane (1.2 g, 2.90 mmol) at 0°C. It was stirred at room temperature for 2 hours. The crude product was purified with CombiFlash in 0-40% EtOAc/p-ether to afford 0.26 g of **Compound 25** in 59.3 % yield. ^{1}H NMR (500 MHz, $CDCl_3$) (rotamers): δ 9.56 and 9.49 (s, 1H), 5.03 (m, 2H), 4.35-4.20 (m, 1H), 4.13-4.02 (m, 2H), 2.86-2.71 (m, 1H), 2.67-2.64 (m, 1H), 1.49 and 1.44 (s, 9H).

Example 31: Preparation of Compound 50.



Step 1: Preparation of Compound 49.

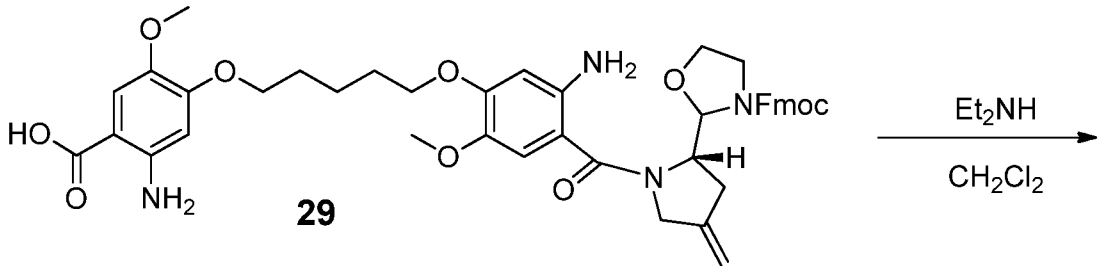
A suspension of **Compound 25** (288 mg, 1.35 mmol), 2-ethanolamine (45 μL, 0.749 mmol), and $MgSO_4$ (200 mg) in anhydrous CH_2Cl_2 (5.0 mL) was stirred at room temperature under argon for 1 h. The reaction mixture was passed through a sintered glass frit, and the filtrate was added to a pre-mixed solution of **Compound 48** (386 mg, 0.749 mmol), diphosgene (55.0 μL, 0.457 mmol), and $^{i}Pr_2NEt$ (270 μL, 1.57 mmol) in anhydrous THF (20 mL) at 0°C. To the solution was added Et_3N (105 μL, 0.749 mmol), and the reaction mixture was stirred at 0°C under argon for 5 min. The reaction mixture was allowed to warm to room temperature and stirred under argon for an additional 25 min. The solution was then concentrated under

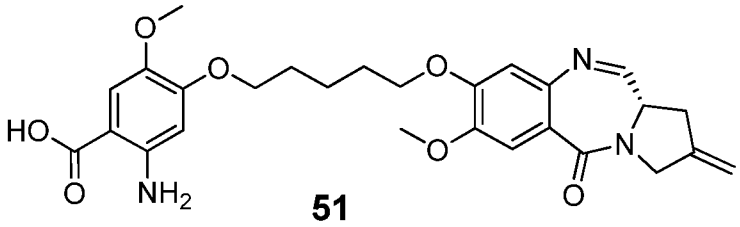
reduced pressure and purified via silica chromatography (0 - 70%EtOAc/pet. ether) to yield **Compound 49** as a white solid (195 mg, 32.7%): LC/MS: (ESI-QMS): *m/z* = 796.47 (M + H).

Step 2: Preparation of Compound 50.

Diethylamine (0.50 mL) was added to a solution of **Compound 49** (62.3 mg, 78.3 μmol) in CH_2Cl_2 (2.0 mL). The reaction mixture was stirred at room temperature under argon for 2.5 h and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (2 mL × 3), dried under high vacuum for 30 min, and dissolved in anhydrous CH_2Cl_2 (3.0 mL). To the solution was added in tandem maleimidopropionic acid NHS ester (25.0 mg, 94.2 μmol) and $^{i}Pr_2NEt$ (50.0 μL, 0.290 mmol). The reaction mixture was stirred at room temperature under argon for 1.5 h, concentrated under reduced pressure, and purified via silica chromatography (0 - 100% EtOAc/pet. ether) to yield **Compound 50** as a white solid (53.5 mg, 94.2%): LC/MS: (ESI-QMS): *m/z* = 743.85 (M +H), ^{1}H NMR (500 MHz, 298 K, DMSO-*d6*) δ 9.894 (s, 1H), 8.166 (d, *J* = 8.5 Hz, 1H), 8.025 (d, *J* = 8.5 Hz, 1H), 7.599 (d, *J* = 8.5 Hz, 2H), 7.322 (b, 2H), 6.995 (s, 2H), 4.998 (m, 5H), 4.378 (m, 1H), 4.249 (m, 1H), 4.126 (t, *J* = 8.0 Hz, 1H), 3.977-3.594 (m, 6H), 2.466 (m, 2H), 1.932 (m, 1H), 1.367 (m, 12H), 0.858 (m, 6H).

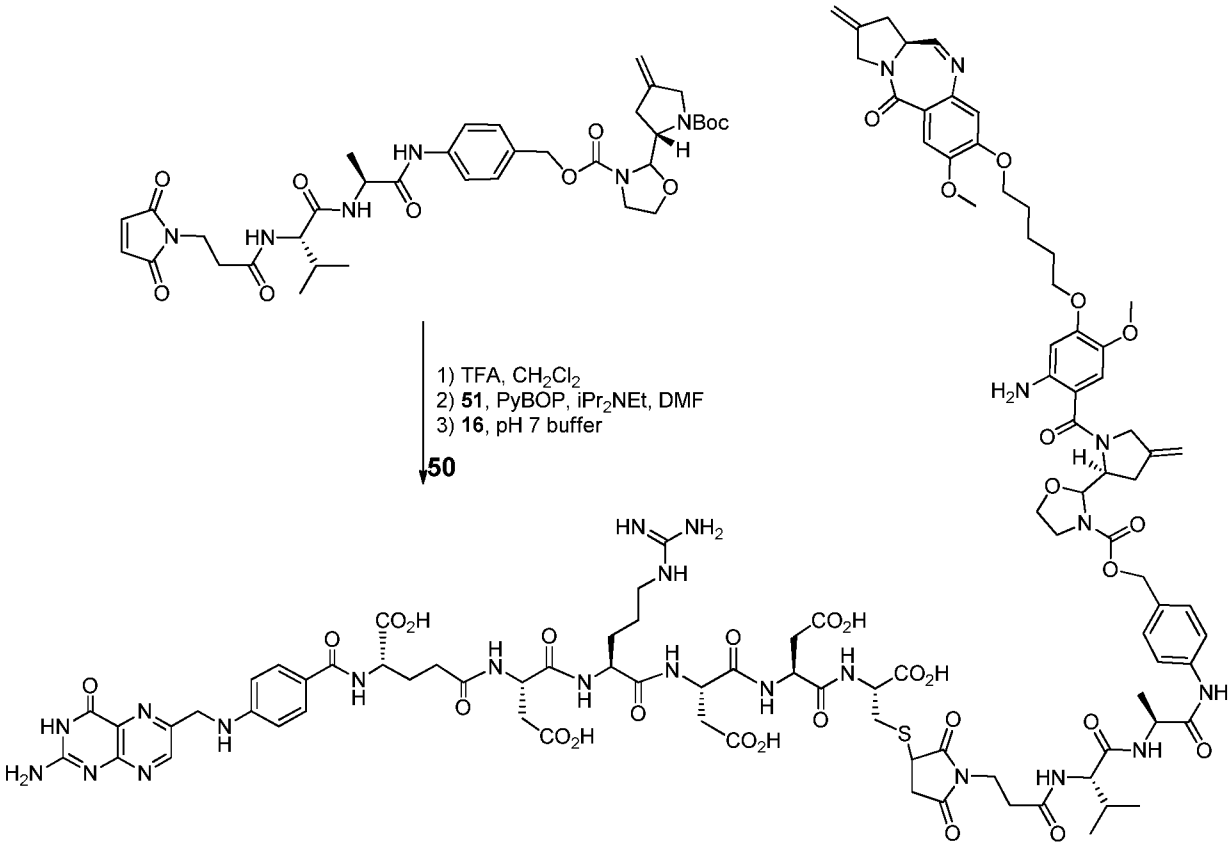
Example 32: Preparation of Compound 51.





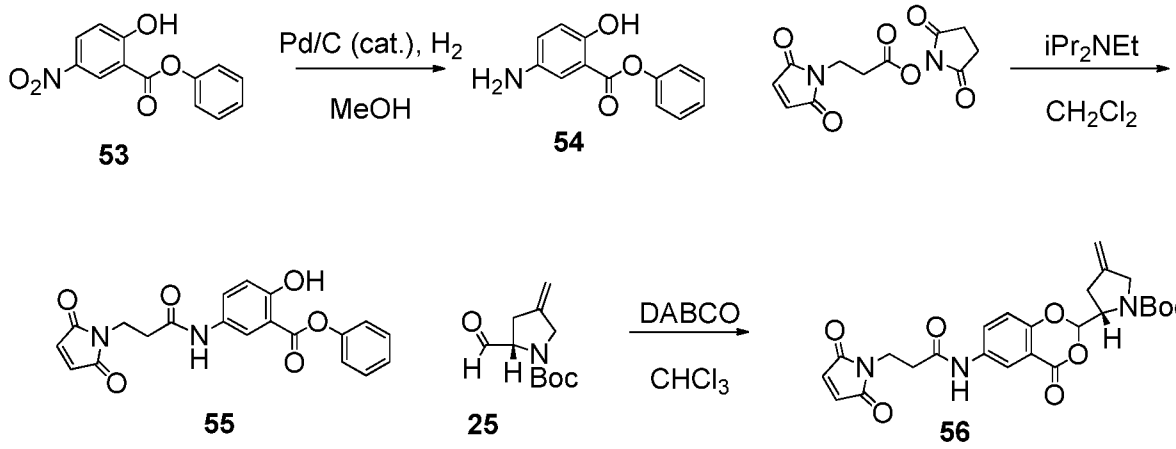
A solution of **Compound 29** (105 mg, 0.132 mmol) and diethylamine (1.0 mL) in anhydrous CH_2Cl_2 (3.0 mL) was stirred at room temperature under argon for 90 min. The reaction mixture was concentrated under reduced pressure and dried under high vacuum to yield crude **Compound 51** as a light brown solid (39.5 mg). The crude material was used without further purification. LC/MS: (ESI-QMS): *m/z* = 510.41 (M + H).

Example 33: Preparation of Conjugate 10.



A solution of **Compound 50** (7.5 mg, 10 μmol) in anhydrous TFA/CH_2Cl_2 (0.35 mL/1.0 mL) was stirred at room temperature under argon for 35 min, after which the reaction mixture was concentrated under reduced pressure. The resulting residue was co-evaporated with CH_2Cl_2 (2 mL × 3), and dried under high vacuum for 1 h. A pre-mixed solution of **Compound 51** (5.3 mg, 10 μmol) and PyBOP (5.7 mg, 11 μmol) in anhydrous DMF (3.0 mL) was then added to the crude residue. To the solution $^{i}Pr_2NEt$ (8.7 μL, 50 μmol) was added, and the reaction mixture was stirred at room temperature under argon for 30 min. The solution was diluted with DMF (1.5 mL) and added a pre-mixed solution of **Compound 16** (12.5 mg, 12 μmol) in 50 mM NH_4HCO_3 buffer, pH 7.0 (4.5 mL). The reaction mixture was stirred at room temperature under argon for 15 min and purified via preparative HPLC (10 – 100% MeCN/50 mM NH_4HCO_3 buffer, pH 7.0 to yield **Conjugate 10** as a fluffy yellow solid (7.9 mg, 37%): LC/MS: (ESI-QMS): *m/z* = 1080.16 (M + H), . Selective ^{1}H NMR (500 MHz, 298 K, DMSO*d6*) δ 8.671 (b, 1H), 7.637 (b, 2H), 7.478 (b, 2H), 7.102 (b, 4H), 6.782 (b, 4H), 6.603 (b, 1H), 6.411 (b, 1H).

Example 34: Preparation of Compound 56.



53 54 55 25 56

Step 1: Preparation of Compound 54.

Pd/C (10% w/w, 7.1 mg) was added to a solution of **Compound 53** (Sigma-Aldrich; 57.8 mg, 0.223 mmol) in MeOH (3.0 mL) under argon. The headspace was evacuated and purged with hydrogen gas. The reaction mixture was stirred under hydrogen for 85 min. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure and dried under vacuum to yield **Compound 54** as a light brown solid (50.1 mg, 98.0%). The crude product was used without further purification: LC/MS: (ESI-QMS): m/z = 230.38 (M + H), ^{1}H NMR (500 MHz, 298 K, DMSO-*d6* with D_2O exchange) δ 7.454 (t, J = 4.0 Hz, 2H), 7.316 (t, J = 4.0 Hz, 1H), 7.233 (m, 3H), 6.918 (dd, J = 9.0 Hz, 3.0 Hz, 1H), 6.786 (d, J = 9.0 Hz, 1H).

Step 2: Preparation of Compound 55.

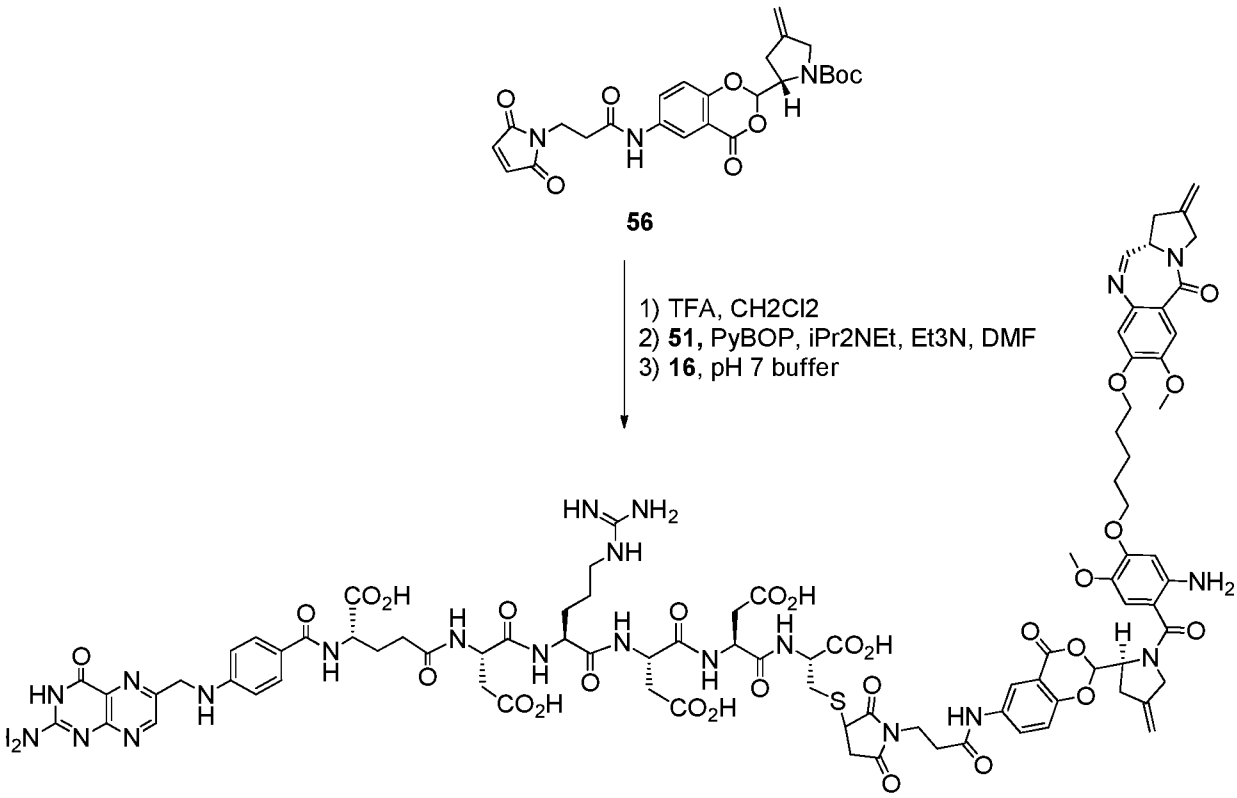
A solution of **Compound 54** (49.5 mg, 0.216 mmol), maleimidopropionic acid NHS ester (115 mg, 0.432 mmol), and ^{i}Pr$_2$NEt (200 µL, 1.17 mol) in anhydrous CH_2Cl_2 (5.0 mL) was stirred at room temperature under argon for 2 h and purified via silica chromatography (0 - 70% EtOAc/pet. ether) to yield **Compound 55** as an impure mixture (40.1mg). The mixture was further purified via silica chromatography (0 - 2% MeOH/CH_2Cl_2) to afford **Compound 55** as a white solid (21.5 mg, 26.2%): LC/MS: (ESI-QMS): m/z = 381.54 (M + H), ^{1}H NMR (500 MHz, 298 K, DMSO-*d6*) δ 8.236 (d, J = 2.5 Hz, 1H), 7.698 (dd, J = 9.0, 2.5 Hz, 1H), 7.500 (m, 2H), 7.334 (m, 3H), 7.021 (s, 2H), 7.006 (d, J = 9.0 Hz, 1H), 3.710 (t, J = 7.0 Hz, 2H), 2.559 (t, J = 6.5 Hz, 2H).

Step 3: Preparation of Compound 56.

A solution of **Compound 55** (85.0 mg, 0.223 mmol), **Compound 25** (95.0 mg, 0.446), and DABCO (80.1 mg, 0.714 mmol) in anhydrous $CHCl_3$ (0.75 mL) was stirred at room temperature under argon for 6 h and purified via silica chromatography (0 - 2% MeOH/CH_2Cl_2) to yield impure **Compound 56** as a white solid (57.1 mg). The crude product was used without further purification. LC/MS: (ESI-QMS): m/z = 496.44 (M + H), ^{1}H NMR (500 MHz, 298 K,

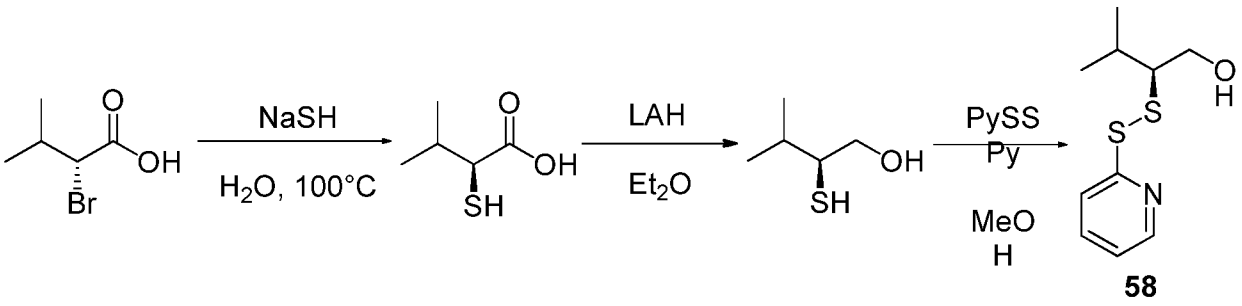
CD_3OD) δ 8.094 (b, 1H), 7.769 (m, 1H), 7.067 (d, J = 9.5 Hz, 1H), 6.818 (s, 2H), 5.900 (d, J = 58.0 Hz, 1H), 4.569 (s, 2H), 4.203 (b, 1H), 4.173 (d, J = 15.0 Hz, 1H), 4.027 (m, 1H), 3.867 (t, J = 6.5 Hz, 2H), 2.908 (b, 2H), 2.653 (t, J = 6.5 Hz, 2H).

Example 35: Preparation of Conjugate 11



A solution of **Compound 56** (16.9 mg, 34.0 μmol) in anhydrous TFA/CH_2Cl_2 (0.20 mL/1.0 mL) was stirred at room temperature under argon for 90 min and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (1.5 mL × 3) and dried under vacuum for 1 h. A pre-mixed solution of **Compound 51** (19.1 mg, 37.4 μmol) and PyBOP (21.2 mg, 40.8 μmol) in anhydrous DMF (3.0 mL) was added to the crude residue. To the solution was added $^{i}Pr_2NEt$ (30.0 μL, 170 μmol), and the reaction mixture was stirred at room temperature under argon for 30 min. Et_3N (15.0 μL,102 μmol) was added to the reaction mixture and stirred at room temperature under argon for an additional 60 min. The solution was the diluted with DMF (1.5 mL) and to which was added and a pre-mixed solution of **Compound 16** (43.1 mg, 40.8 μmol) in 50 mM NH_4HCO_3 buffer, pH 7.0, (4.5 mL). After stirring at room temperature under argon for 35 min, the reaction mixture was filtered, and the filtrate was purified via preparative HPLC (10 – 100%, MeCN/50 mM NH_4HCO_3 buffer, pH 7.0 to yield **Conjugate 11** as a fluffy yellow solid (3.1 mg, 4.7% over three steps): LC/MS: (ESI-QMS): m/z = 1934.06 (M + H), Selective ^{1}H NMR (500 MHz, 298 K, DMSO*d6*) δ 8.611 (s, 1H), 8.125 (b, 1H), 7.598 (b, 4H), 7.102 (b, 4H), 6.617 (b, 4H), 6.513 (s, 1H), 6.361 (s, 1H), 6.289 (b, 1H).

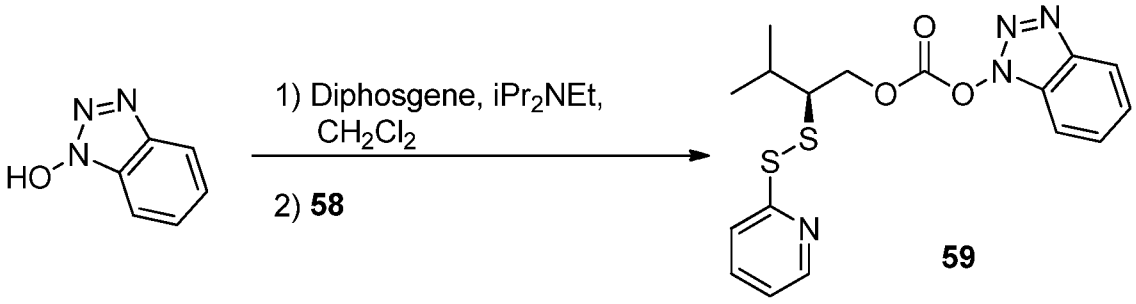
Example 36: Preparation of Compound 58.



58

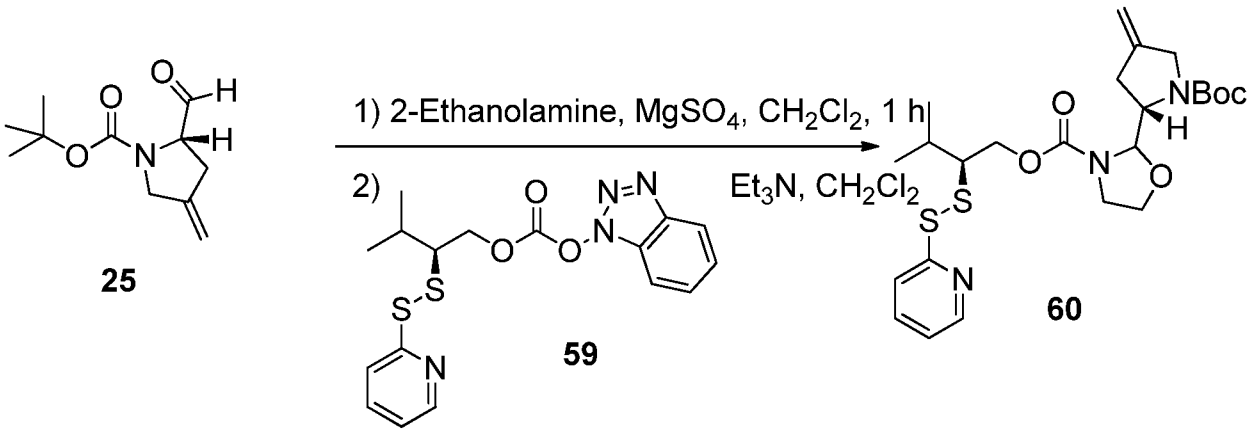
Water (4.5 mL) was added to a glass vial containing (*R*)-(+)-2-bromo-3-methylbutyric acid (958 mg, 5.29 mmol) and $NaHS \cdot XH_2O$ (1.01 g), and the vial was capped immediately. The resulting solution was stirred for 2 min at ambient temperature and for 3.5 h at 100°C. After allowing the reaction mixture to cool to ambient temperature, the cap of the vial was opened, and the solution was flushed with argon for 5 min. The solution was acidified (pH ~2) with 2.0 N HCl and extracted with diethyl ether (35 mL × 2). The organic layers were separated, combined, dried over Na_2SO_4, and filtered. The filtrate was added to a suspension of LAH (600 mg, 3.00 equiv.) in anhydrous diethyl ether (10 mL). After stirring at ambient temperature under argon for 30 min, the reaction mixture was cooled in an ice-bath and quenched with 1.0 N HCl (28 mL) at 0°C. The ice-bath was removed and the reaction mixture stirred at ambient temperature under argon for 15 min. The top clear solution of the reaction mixture was poured into a solution of aldrithiol (1.16 g, 1.00 equiv.) in MeOH (50 mL). The remaining gel-like material from the LAH reduction was washed with diethyl ether (50 mL) and added to the aldrithiol solution. Saturated aqueous $NaHCO_3$ solution (50 mL) was added to the aldrithiol solution until the pH reached ~7.5 and the reaction mixture was stirred at ambient temperature under argon for 1.5 h. The solution was then filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to yield an oily residue, which was further purified by a CombiFlash system (silica gel column) eluting with 0 - 10% EtOAc in petroleum ether to yield 615 mg **Compound 58** as a white solid: LC/MS: (ESI-QMS): *m/z* = 230.07 (M + H), ^{1}H NMR (500 MHz, 298 K, $CDCl_3$) δ 8.502 (d, J = 5.0 Hz, 1H), 7.662 (m, 1H), 7.569 (t, J = 7.5 Hz, 1H), 7.155 (m, 1H), 3.839 (m, 1H), 3.635 (m, 1H), 2.739 (m, 1H), 1. 809 (m, 1H), 1.106 (d, J = 7.0 Hz, 3H), 1.070 (d, J = 7.0 Hz, 3H).

Example 37: Preparation of Compound 59.



A solution of hydroxybenzotriazole (229 mg, 2.0 equiv.) in anhydrous CH_2Cl_2 (15 mL) was added slowly to a stirred solution of diphosgene (0.12 mL, 1.2 equiv.) in anhydrous CH_2Cl_2 (3.0 mL) at ambient temperature. To the resulting solution was added iPr_2NEt (0.75 mL, 5.0 equiv.). After stirring at ambient temperature under argon for 3 min, a solution of **Compound 58** (196 mg, 0.855 mmol) in anhydrous CH_2Cl_2 (5.0 mL) was added to the reaction mixture. The reaction mixture was then stirred at ambient temperature under argon for 1 h, quenched with water (50 µL), stirred at ambient temperature for 5 min, and loaded directly onto a CombiFlash system for purification (Silica gel column) (Gradient 0-60% EtOAc in petroleum ether.) to afford 202 mg **Compound 59** as a glass-like solid: LC/MS: (ESI-QMS): *m/z* = 391.06 (M + H), ^{1}H NMR (500 MHz, 298 K, $CDCl_3$) δ 8.390 (d, J = 1.0 Hz, 1H), 8.232 (d, J = 8.5 Hz, 1H), 8.026 (d, J = 7.5 Hz, 1H), 7.777 (m, 2H), 7.662 (m, 1H), 7.556 (t, J = 7.5 Hz, 1H), 7.042 (m, 1H), 4.767 (m, 2H), 3.193 (m, 1H), 2.267 (m, 1H), 1.189 (d, J = 7.0 Hz, 3H), 1.148 (d, J = 7.0 Hz, 3H).

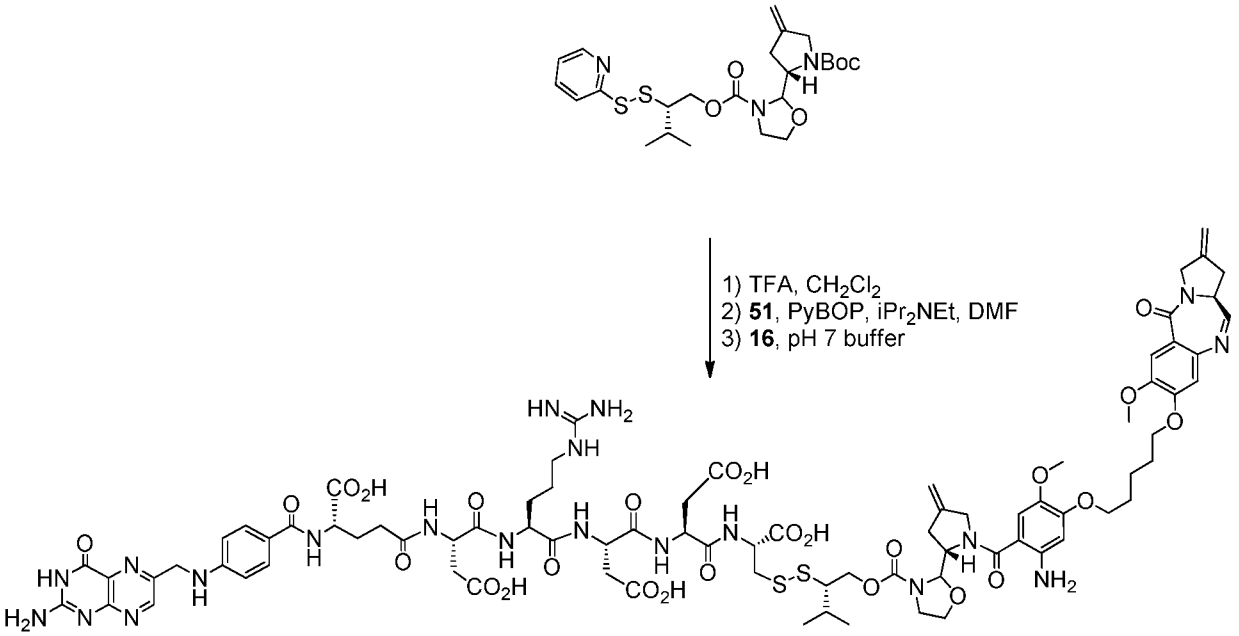
Example 38: Preparation of Compound 60.



A suspension of **Compound 25** (20.0 mg, 95.0 µmol), 2-ethanolamine (4.2 µL, 71.3 µmol), and $MgSO_4$ (90 mg) in anhydrous CH_2Cl_2 (0.35 mL) was stirred at room temperature under argon for 2 h. The reaction mixture was diluted with anhydrous CH_2Cl_2 (0.75 mL) and filtered through a sintered glass frit, and the filtrate was transferred to a small vial containing **Compound 59** (37.0 mg, 95.0 µmol). To the resulting solution was added Et_3N (15.0 µL, 105 µmol). The reaction mixture was then stirred at room temperature under argon for 25 min and

purified via silica chromatography (0 - 35%, EtOAc/pet. ether) to yield **Compound 60** as a light beige solid (22.0 mg, 45.4%): LC/MS: (ESI-QMS): *m/z* = 510.61 (M + H), ^{1}H NMR (500 MHz, 298 K, CD_2Cl_2) δ 8.415 (d, J = 4.0 Hz, 1H), 7.749 (b, 1H), 7.665 (t, J = 8.0 Hz, 1H), 7.098 (m, 1H), 5.109 (m, 1H), 4.936 (s, 1H), 4.905 (s, 1H), 4.315-3.776 (m, 10H), 3.378-3.254 (m, 1H), 3.019 (m, 1H), 2.704-2.387 (m, 2H), 2.115 (b, 1H), 1.408 (b, 9H), 1.113-1.044 (m, 6H). MS^+ (ESI *m/z*) calculated for $C_{24}H_{36}N_3O_5S_2$: 510.20; found 510.61.

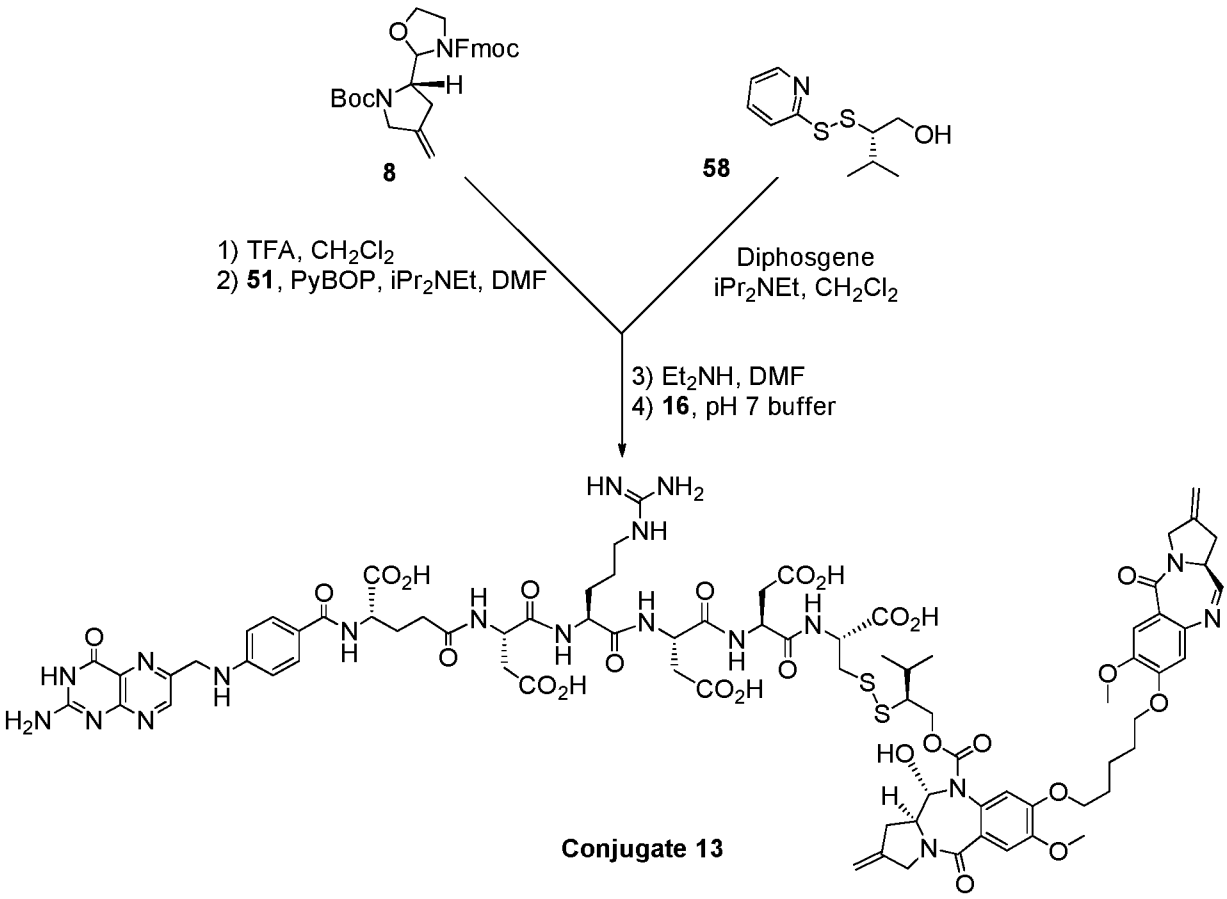
Example 39: Preparation of Conjugate 12.



A solution of **Compound 60** (7.6 mg, 15.0 µmol) in anhydrous TFA/CH_2Cl_2 (0.15 mL/0.75 mL) was stirred at room temperature under argon for 1.5 h and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (1 mL × 3), and dried under high vacuum for 1 h. A pre-mixed solution of **Compound 51** (8.4 mg, 16.5 µmol) and PyBOP (8.5 mg, 16.5 µmol) in anhydrous DMF (2.0 mL) was added to the crude residue. To the solution was then added ^{i}Pr$_2$NEt (15.6 µL, 90 µmol) and the reaction mixture was stirred at room temperature under argon for 1 h. The solution was diluted with DMF (1.5 mL) and to which was added a pre-mixed solution of **Compound 16** (17.2 mg, 16.5 µmol) in 50 mM NH_4HCO_3 buffer, pH 7.0 (4.5 mL). The resulting cloudy solution was stirred at room temperature for 20 min, and then at 65°C for 30 min. The reaction mixture was allowed to cool to room temperature, filtered, and purified via preparative HPLC (10 – 100%, MeCN/50 mM NH_4HCO_3 buffer, pH 7.0) to yield **Conjugate 12** as a fluffy yellow solid (6.1 mg, 22% over three steps): LC/MS: (ESI-QMS): *m/z* = 1834.18 (M + H), Selective ^{1}H NMR (500 MHz, 298

K, D_2O) δ 8.699 (b, 1H), 7.690 (b, 2H), 7.418 (b, 1H), 7.236 (b, 1H), 7.146 (b, 1H), 6.798 (b, 2H), 6.553 (b, 1H), 6.403 (b, 1H).

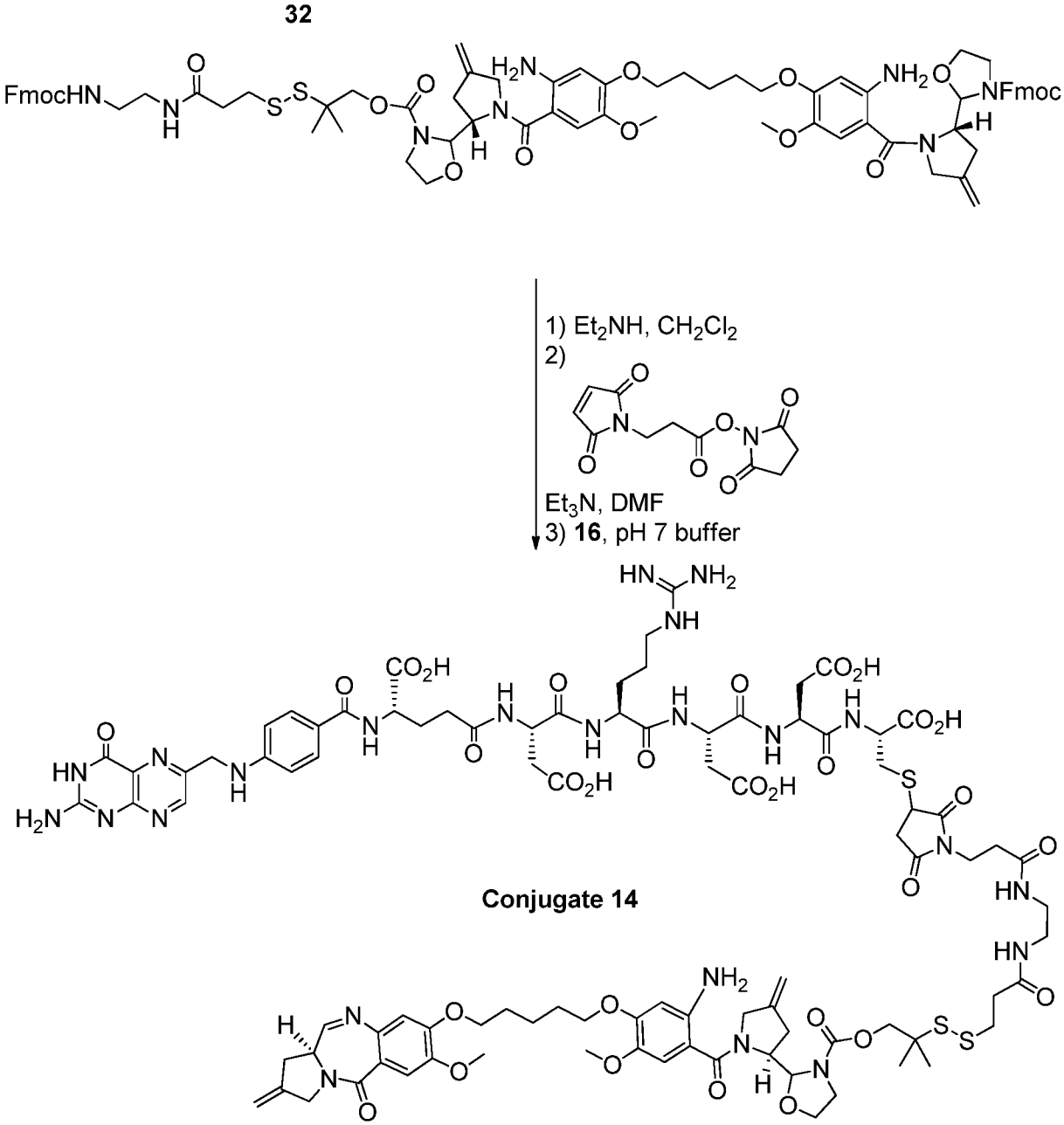
Example 40: Preparation of Conjugate 13.



A solution of **Compound 8** (43.9 mg, 92.1 µmol) in anhydrous TFA/CH_2Cl_2 (0.15 mL/0.85 mL) was stirred at room temperature under argon for 30 min and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (1.5 mL × 3), dried under high vacuum for 1 h, and dissolved in anhydrous CH_2Cl_2 (1.5 mL). To the solution was added a pre-mixed solution of **Compound 51** (44.6 mg, 87.5 µmol) and PyBOP (47.8 mg, 92.1 µmol) in anhydrous DMF (1.5 mL). To the solution was added $^{i}Pr_2NEt$ (80.0 µL, 460 µmol). The reaction mixture was stirred at room temperature under argon for 70 min and purified via silica chromatography (0 - 5% MeOH/CH_2Cl_2) to yield 39.9 mg of a beige solid containing mostly the desired product based on LC/MS analysis. In a separate flask, diphosgene (5.3 µL, 44.0 µmol) and $^{i}Pr_2NEt$ (45.0 µL,259 µmol) were added in tandem to a solution of **Compound 58** (10.1 mg, 44.0 µmol) in anhydrous CH_2Cl_2 (0.75 mL). The reaction mixture was stirred at room temperature under argon for 15 min, concentrated under reduced pressure, and concentrated under vacuum for 1 h. To the resulting residue was added a solution of the crude product (22.1 mg, 25 µmol) from the previous step in anhydrous CH_2Cl_2 (1.0 mL). The

reaction mixture was stirred at room temperature under argon for 25 min, concentrated under reduced pressure, and concentrated under vacuum for 1 h. The residue was dissolved in anhydrous DMF (3.0 mL). Two-thirds of the volume was transferred to a glass vial and to which was added diethylamine (0.50 mL). The reaction mixture was stirred at room temperature under argon for 25 min, diluted with DMF (2.5 mL), and added to a pre-mixed solution of **Compound 16** (25.0 mg,23.9 μmol) in 50 mM NH_4HCO_3 buffer, pH 7.0 (4.5 mL). After stirring at 65°C for 1 h, the reaction mixture was cooled to room temperature and filtered. The filtrate was purified via preparative HPLC (10 – 100%, MeCN/50 mM NH_4HCO_3 buffer, pH 7.0) to afford **Conjugate 13** as a fluffy yellow solid (0.8 mg, 1% over three steps): LC/MS: (ESI-QMS): *m/z* = 1808.43 (M + H).

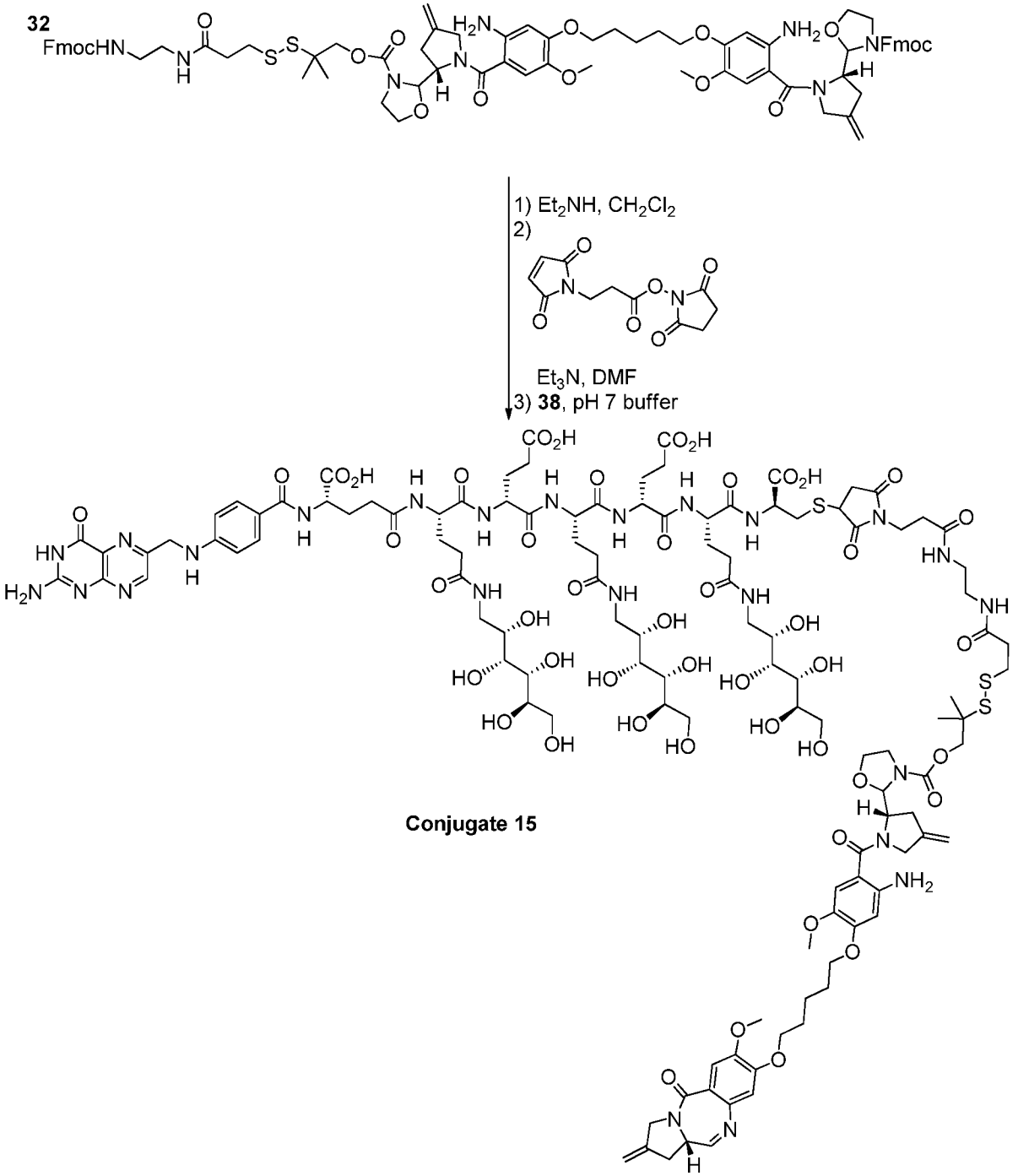
Example 41: Preparation of Conjugate 14.



Conjugate 14

Diethylamine (0.50 mL) was added to a solution of **Compound 32** (52.0 mg, 36.4 μmol) in anhydrous CH_2Cl_2 (1.0 mL). The reaction mixture was stirred at room temperature under argon for 100 min and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (2 mL × 3), dried under high vacuum for 1 h, and dissolved in anhydrous DMF (2.0 mL). To the solution was added in tandem maleimidopropionic acid NHS ester (10.7 mg, 40.0 μmol) and Et_3N (10.1 μL, 72.8 μmol). The reaction mixture was stirred at room temperature under argon for 1 h, diluted with DMF (2.5 mL), and to which was added a solution of **Compound 16** (49.5 mg,47.3 μmol) in 50 mM NH_4HCO_3 buffer, pH 7.0 (5.0 mL). The reaction mixture was stirred at room temperature under argon for 15 min and purified via preparative HPLC (10 – 100%, MeCN/50 mM NH_4HCO_3 buffer, pH 7.0 to yield **Conjugate 14** as a fluffy yellow solid (33.5 mg, 43.4%): LC/MS: (ESI-QMS): *m/z* = 1061.58 (M + 2H), Selective ^{1}H NMR (500 MHz, 298 K, DMSO-*d6* with D_2O exchange) δ 8.554 (b, 1H), 7.484 (d, J = 8.5 Hz, 2H), 7.023 (s, 1H), 6.979 (s, 1H), 6.586 (d, J = 8.5 Hz, 2H), 6.457 (s, 1H), 6.325 (s, 1H), 6.165 (s, 1H).

Example 42: Preparation of Conjugate 15.

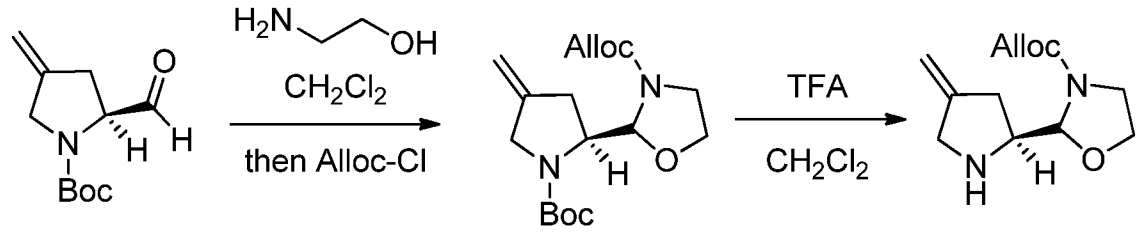


Conjugate 15

Diethylamine (0.50 mL) was added to a solution of **Compound 32** (26.3 mg, 18.4 μmol) in anhydrous CH_2Cl_2 (1.0 mL). The reaction mixture was stirred at room temperature under argon for 165 min and then concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (2 mL × 3), dried under high vacuum for 1 h, and dissolved in anhydrous DMF (2.0 mL). To the solution was added maleimidopropionic acid NHS ester (5.4 mg, 20 μmol) and Et_3N (5.1 μL, 53 μmol) in tandem. The reaction mixture was stirred at room temperature under argon for 55 min, diluted with DMF (2.5 mL), and to which was added a solution of **Compound 38** (40.1 mg,23.9 μmol) in 50 mM NH_4HCO_3 buffer, pH 7.0, (4.5 mL). The reaction mixture was stirred at room temperature under argon for 15 min and purified via

preparative HPLC (10 – 100%, MeCN/50 mM NH_4HCO_3 buffer, pH 7.0 to yield **Conjugate 15** as a fluffy yellow solid (20.8 mg, 41.0%): LC/MS: (ESI-QMS): *m/z* = 1376.11 (M + 2H), Selective ^{1}H NMR (500 MHz, 298 K, D_2O) δ 8.684 (b, 1H), 7.675 (b, 2H), 7.133 (s, 1H), 6.764 (b, 3H), 6.574 (b, 1H), 6.499 (b, 1H).

Example 43: Preparation of Compound 66.



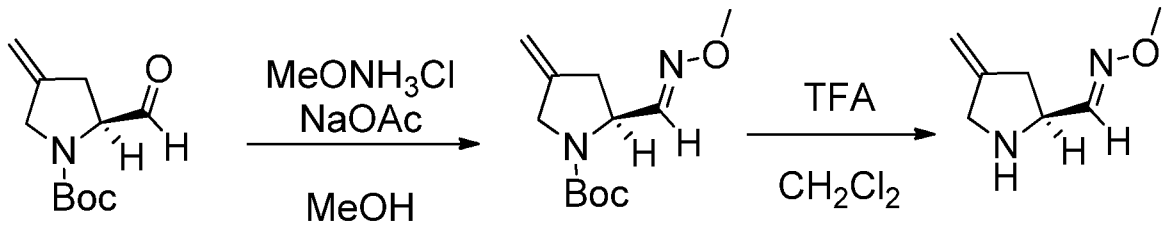
Step 1: Preparation of Compound 65.

A mixture of **Compound 25** (0.108 g, 0.51 mmol), ethanolamine (32.8 mg, 0.54 mmol) and 4Å molecular sieves in CH_2Cl_2 (5 mL) was stirred at room temperature for 3 hours. To the reaction mixture was added allyl chloroformate (57 µl, 0.54 mmol) at room temperature for 3h. The reaction mixture was concentrated *in vacuo*, and the crude residue was purified via silica chromatography (0 - 50% EtOAc/pet. ether) to afford **Compound 65** (0.14 g, 82%): ^{1}H NMR (500 MHz, $CDCl_3$) δ δ 5.94 (m, 1H), 5.31 (m, 1H), 5.24 (d, *J* = 10.5 Hz, 2H), 4.96 (m, 2H), 4.60 (d, *J* = 10.5 Hz, 2H), 4.15-4.06 (m, 2H), 3.88-3.82 (m, 4H), 3.52-3.28 (m, 1H), 2.64 (m, 1H), 2.54-2.42 (m, 1H), 1.44 (s, 9H).

Step 2: Preparation of Compound 66.

A mixture of **Compound 65** (0.14 g, 0.41 mmol) in 20 % TFA/CH_2Cl_2 solution (2 mL) was stirred at room temperature for 4 h. It was concentrated *in vacuo*. The crude product **Compound 66** was used without further purification.

Example 44: Preparation of Compound 68.



Step 1: Preparation of Compound 67.

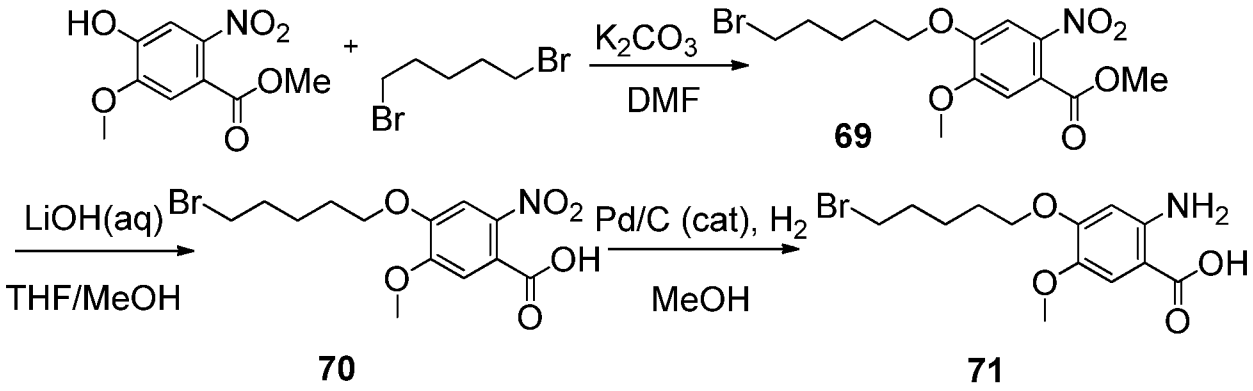
A mixture of **Compound 25** (0.193 g, 0.91 mmol), methoxyamine hydrochloride (76.3 mg, 0.91 mmol) and sodium acetate (0.3 g, 3.64 mmol) in MeOH (6 mL) was stirred at room

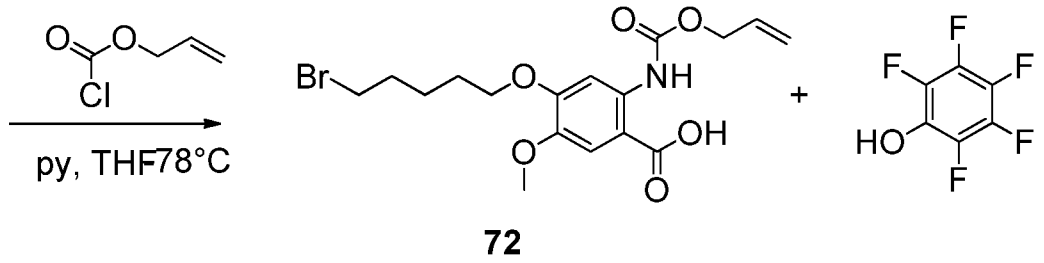
temperature overnight. The reaction was quenched with water and extracted with EtOAc (3 x 30 mL). The combined organic phases were washed with H_2O and brine sequentially, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was further purified via silica chromatography (0 - 50% EtOAc/pet. ether) to afford the **Compound 67** (0.15 g, 69%): ^1H NMR (500 MHz, $CDCl_3$), (E/Z isomers) δ 7.25 (s, 1H), 6.65 (s, 1H), 5.01-4.92 (m, 2H), 4.49 (m, 2H), 4.06-3.91 (m, 2H), 3.87 (s, 3H), 3.82 (s, 3H), 2.92 (m, 1H), 2.83 (m, 1H), 2.61 (d, J = 15.5 Hz, 1H), 2.46 (dd, J_1 = 4.5 Hz, J_2 = 2 Hz, 1H), 1.46 (s, 9H).

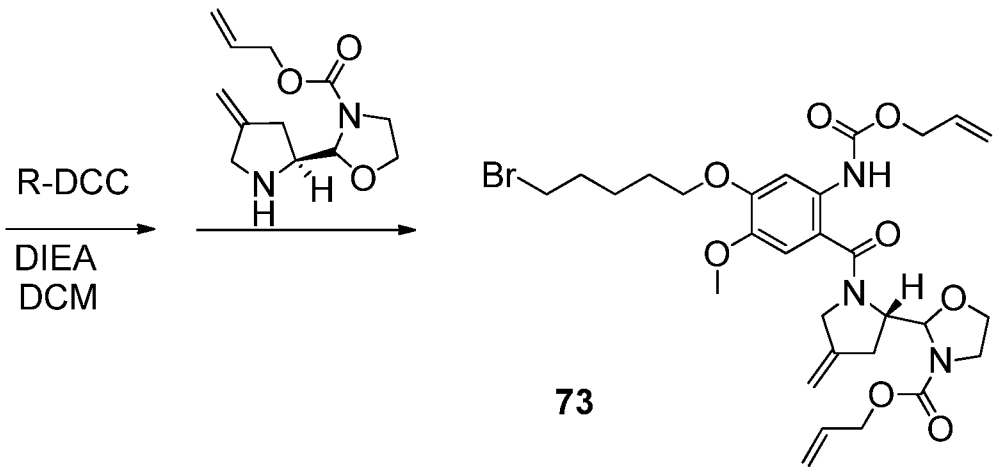
Step 2: Preparation of Compound 68:

A mixture of **Compound 67** (0.15 g, 0.62 mmol) in 20% TFA/CH_2Cl_2 solution was stirred at room temperature and monitored by TLC. After 4 h the solvent was removed under reduced pressure. The product **Compound 68** was used without further purification.

Example 45: Preparation of Compound 73.







Step 1: Preparation of Compound 69.

To a mixture of methyl 4-hydroxy-5-methoxy-2-nitrobenzoate (0.34 g, 1.5 mmol) and potassium carbonate (0.21g 1.5 mmol) in DMF (8 mL) was added 1, 5-dibromopentane (1.72 g, 7.5 mmol) at room temperature under argon. The mixture was stirred room temperature overnight and then concentrated *in vacuo*. The crude product was purified via silica chromatography (0 - 50% EtOAc/pet. ether) to afford **Compound 69 (0.52 g**, 92%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.07 (s, 1H), 4.10 (t, J =6.5 Hz, 2H), 3.95 (s, 3H), 3.91 (s, 3H), 3.44(t, J = 6 Hz, 2H), 1.93 (m, 4H), 1.67 (M, 2H).

Step 2: Preparation of Compound 70.

To a mixture of **Compound 69** (0.52 g, 1.38 mmol) in THF/MeOH/H_2O (3 mL/1 mL/1 mL) was added 1 M $LiOH_{(aq)}$ (6.9 mL, 6.9 mmol) at room temperature. The reaction was monitored via LC/MS and after complete consumption of **Compound 69**, the reaction mixture was adjusted to pH 7 with 1M $HCl_{(aq)}$ solution. The product was extracted with EtOAc (3 x 50 mL), dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo* and the crude product was purified via silica chromatography (0 - 50% EtOAc/pet. ether) to afford the product as yellow solid: LC/MS: (ESI-QMS): m/z = 364.25 (M + 2H) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.20 (s, 1H), 4.10 (t, J =6.5 Hz, 2H), 3.98 (s, 3H), 3.45 (t, J = 6.5 Hz, 2H), 1.99-1.89 (m, 4H), 1.69-1.64 (m, 2H).

Step 3: Preparation of Compound 71.

A mixture of **Compound 70** (0.43 g, 1.3 mmol) and 10 % Pd/C in MeOH/EtOAc (5 mL/5 mL) was stirred under hydrogen atmosphere at room temperature for 3 h. The reaction mixture was then filtered through a plug of Celite, and the filtrated was concentrated *in vacuo* to give the product as dark brown solid. The crude product was used without further purification. LC/MS: (ESI-QMS): m/z = 334.42 (M + 2H).

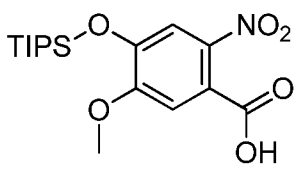
Step 4: Preparation of Compound 72.

To a mixture of **Compound 71** (0.154 g, 0.46 mmol) and pyridine (56.2 µl, 0.70 mmol) in THF (6 mL) was added ally chloroformate (61 mg, 0.51 mmol) at -78°C under argon. The mixture was allowed to warm to room temperature overnight. The mixture was concentrated *in vacuo* and the crude product was purified with silica chromatography (0 - 80% EtOAc/pet. ether) to afford **Compound 72** (0.13 g, 68%) as white solid: LC/MS: (ESI-QMS): m/z = 418.37 (M + 2H) ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.34 (s, 1H), 8.15 (s, 1H), 7.52 (d, J = 2Hz, 1H), 6.0 (m, 1H), 5.4 (d, J = 17 Hz, 1H), 5.28 (d, J = 10.5 Hz, 2H), 4.68 (d, , J = 5 Hz, 2H), 4.13 (dt, J_1 = 7.5 Hz, J_2 = 8 Hz, 2H), 3.78 (s, 3H), 3.44 (td, J_1 = 6.5 Hz, J_2 = 1.5 Hz, 2H), 1.91 (m, 3H), 1.64 (m, 1H), 1.46-1.37 (m, 2H), 0.92 (td, J_1 = 7.5 Hz, J_2 = 1.5 Hz, 2H).

Step 5: Preparation of Compound 73.

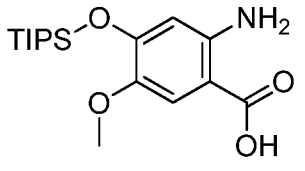
A mixture of **Compound 72** (10 mg, 0.024 mmol), DCC loaded resin (2.3 mmol/g) (52 mg, 0.12 mmol) and pentafluorophenol (4.86 mg, 0.0264 mmol) in CH_2Cl_2 (1 mL) was stirred under argon at room atmosphere for 1 h. The reaction mixture was filtered through a sintered glass frit and concentrated *in vacuo*. The crude residue was dissolved in CH_2Cl_2 (1 mL) and **Compound 66** (5.7 mg, 0.024 mmol) and $^{i}Pr_2NEt$ (12.6 µl, 0.072 mmol) in CH_2Cl_2 (1 mL) at room temperature under argon. The mixture was stirred at room temperature for 3 h. The crude product was purified via silica chromatography (0 - 60% EtOAc/pet. ether): LC/MS: (ESI-QMS): *m/z* = 638.68 (M + 2H), ^{1}H NMR (500 MHz, $CDCl_3$) (mixture of diastereomers) δ 8.94 (s, 1H), 7.83 (s, 2H), 7.04 (s, 1H), 5.94 (m, 2H), 5.83 (m, 2H), 5.33 (dd, J_1 = 17 Hz, J_2 = 1 Hz, 3H), 5.28 (m, 2H), 5.23 (d, , J = 10 Hz, 4H), 5.11-4.97 (m, 9H), 4.67-4.56 (m, 8H), 4.51-4.39 (m, 4H), 4.20(m, 3H), 4.14-4.05(m, 7H), 3.97 (s, 3H), 3.95 (s, 3H), 3.43 (t, J = 7 Hz, 4H), 2.69 (m, 2H), 2.60 (m, 2H), 1.97-1.83 (m, 9H), 1.62 (m, 6H), 1.25 (td, J_1 = 7.5 Hz, J_2 = 1.5 Hz, 2H).

Example 46: Preparation of Compound 78.

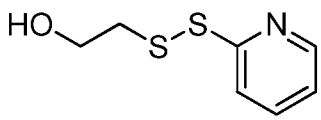


Pd/C, H2

MeOH/EtOAc

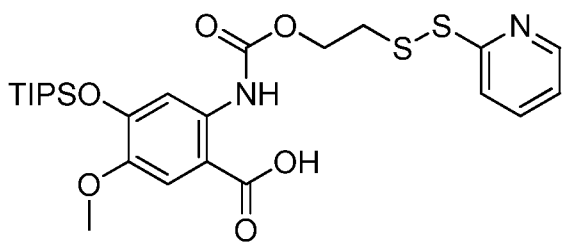


74 **75**

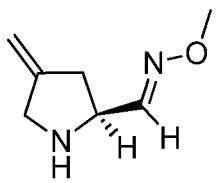


triphosgene, py

CH_2Cl_2

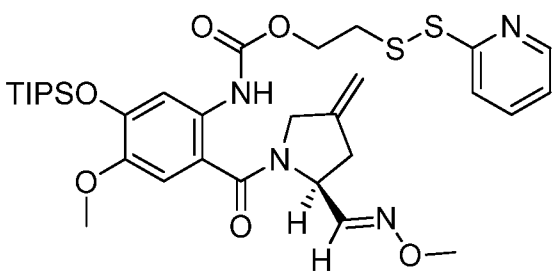


76

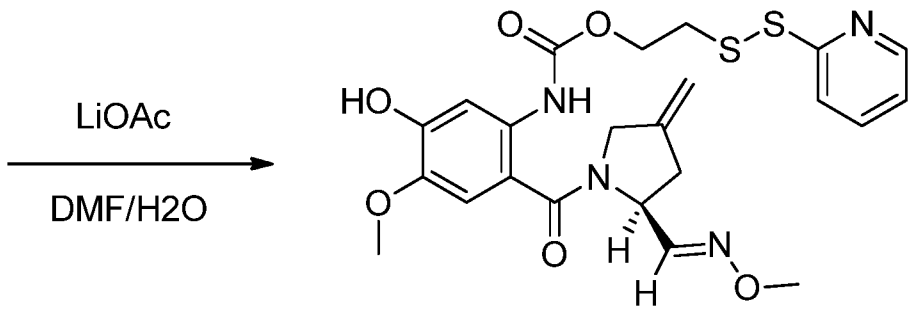


PyBop

DIEA, DMSO



77



78

Step 1: Preparation of Compound 75.

A mixture of **Compound 74** (0.410 g, 1.11 mmol) and 10 % Pd/C (5 %) in MeOH/EtOAc (5 mL/5 mL) was stirred under hydrogen atmosphere at room temperature for 3 h. The reaction mixture filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to give the product as yellow solid. The crude product was used without further purification: LC/MS: (ESI-QMS): *m/z* = 340.26 (M + H), ^{1}H NMR (500 MHz, CDCl3): δ 7.34 (s, 1H), 6.28 (s, 1H), 3.75 (s, 3H), 1.27 (m, 3H), 1.10(s, 9H), 1.08 (s, 9H).

Step 2: Preparation of Compound 76.

To a mixture of 2-(pyridin-2-yldisulfanyl)ethanol (65.2 mg, 0.35 mmol) and pyridine (61.9 µl, 0.77 mmol) in CH_2Cl_2 (1 mL) was added a solution of triphosgene (37.2 mg, 0.13 mmol) in CH_2Cl_2 (1 mL)at under argon. The mixture was stirred at 0°C for 2 h, and then transferred to a mixture of **Compound 75** (0.10 g, 0.29 mmol) and pyridine (51.6 µl, 0.64 mmol) in CH_2Cl_2 (1 mL) at 0°C. The reaction mixture was allowed to slowly warm to room temperature. After stirring for 3 h, the mixture was concentrated *in vacuo* and the crude product was purified via silica chromatography (0 - 60% EtOAc/pet. ether): LC/MS: (ESI-QMS): *m/z* = 553.62 (M + H), ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.38 (s, 1H), 8.76 (d, *J* = 4.5 Hz, 2H), 8.47 (m, 2H), 8.0 (s, 1H), 7.75-7.71 (m, 1H), 7.69-7.61 (m, 1H), 7.53 (s, 1H), 7.08-7.05 (m, 1H), 4.41 (m, 2H), 3.81 (s, 3H), 3.12-3.05 (m, 2H), 1.33-1.28 (m, 3H), 1.16 (s, 9H), 1.10 (s, 9H).

Step 3: Preparation of Compound 77.

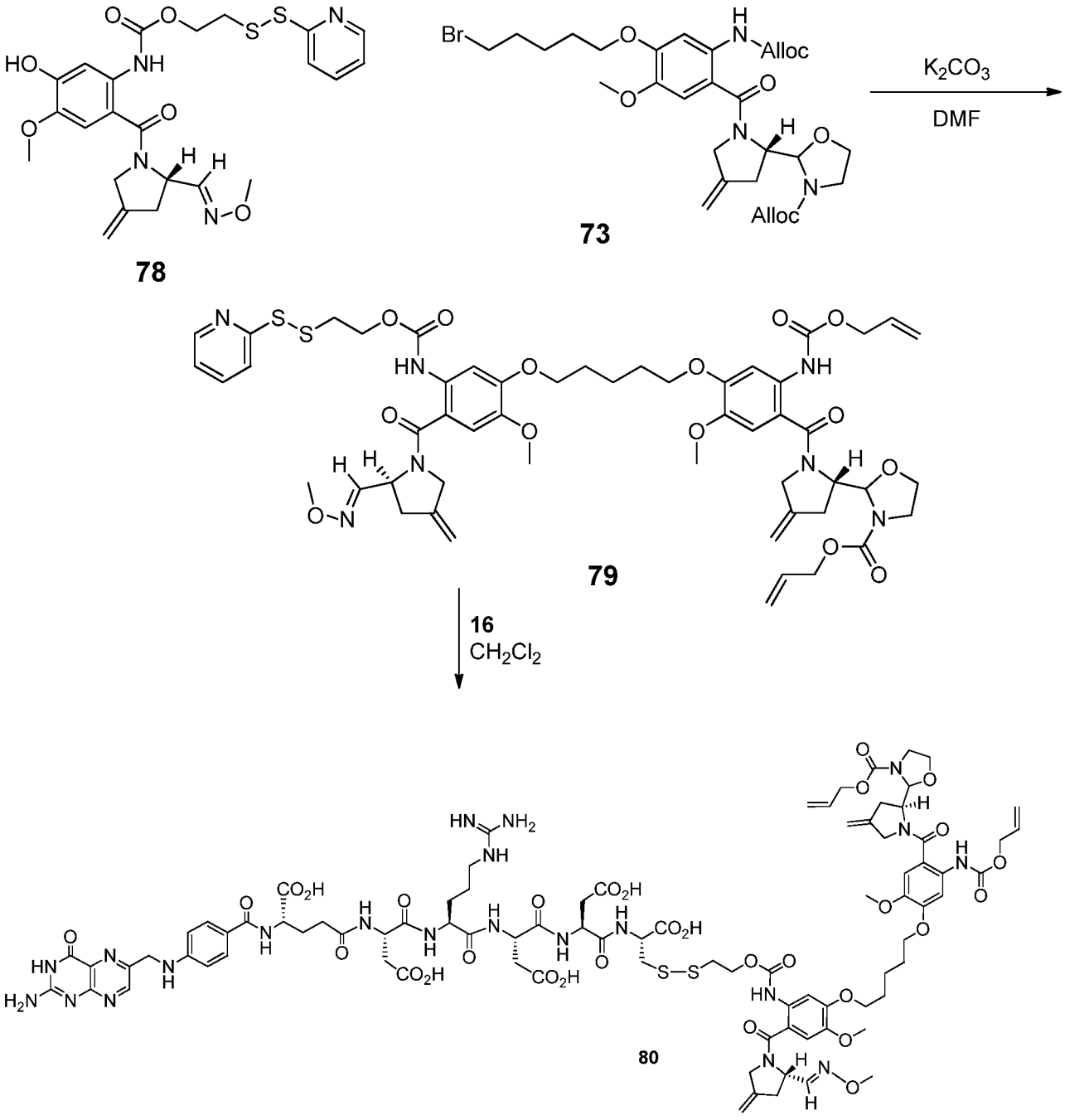
A mixture of **Compound 76** (50.0 mg, 0.0900 mmol), **Compound 68** (12.7 mg, 0.0900 mmol), PyBOP (70.2 mg, 0.140 mmol) and ^{i}Pr$_2$NEt (78.6 µL, 0.450 mmol) in DMSO (1 mL) was stirred at room temperature for 3 h under argon. The crude product was purified via silica chromatography (0 - 60% EtOAc/pet. ether): LC/MS: [(ESI-QMS): *m/z* = 675.77 (M + H)

Step 4: Preparation of Compound 78.

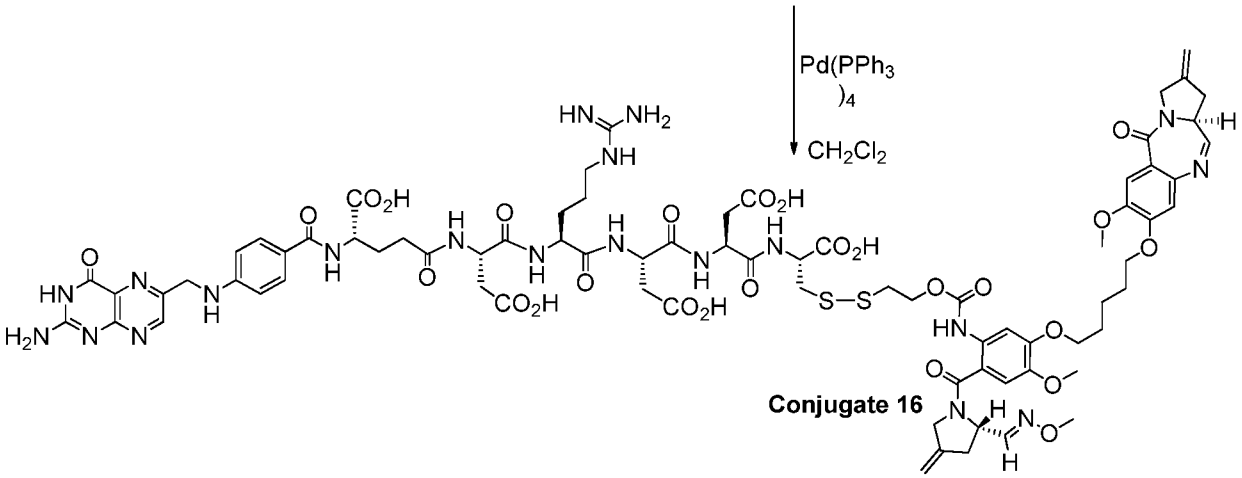
To a mixture of **Compound 77** (9.3 mg, 0.014 mmol) in DMF/H_2O (1 mL, 50:1 DMF/H_2O) was added lithium acetate (0.92 mg, 0.014 mmol) at room temperature. The

reaction mixture was stirred at room temperature for 5 h. The mixture was concentrated *in vacuo* and the crude product was purified with preparative HPLC (10 to 100% MeCN/20 mM NH_4HCO_3 buffer, pH 7.4) to yield pure **Compound 78**: LC/MS: (ESI-QMS): *m/z* = 519.57 (M + H), ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, J = 4.5 Hz, 1H), 7.95 (m, 2H), 7.69 (m, 1H), 7.34 (s, 1H), 7.18 (dd, J_1 = 6.5 Hz, J_2 = 5 Hz, 1H), 6.84 (s, 1H), 6.77 (d, J = 6 Hz, 1H), 5.06-4.99 (m, 2H), 4.38-4.35 (m, 2H), 4.14 (m, 2H), 3.88-3.85 (m, 2H), 3.10 (t, , J = 6.5 Hz, 2H), 2.89-2.84 (m, 2H).

Example 47: Preparation of Conjugate 16.



78 73 79 80



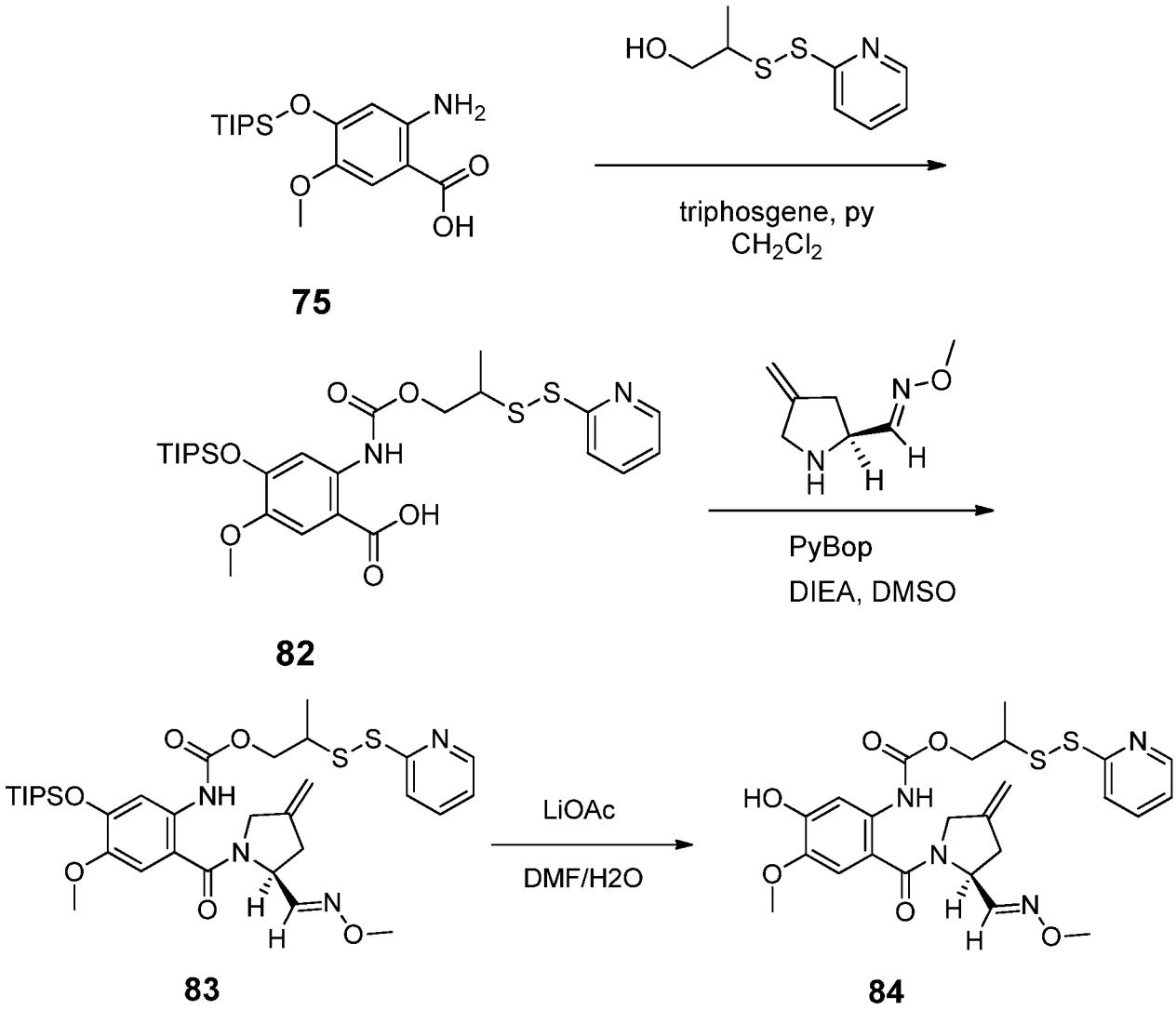
Step 1: Preparation of Compound 79.

A mixture of **Compound 73** (7.6 mg, 0.015 mmol), **Compound 78** (9.3 mg, 0.015 mmol) and potassium carbonate (4.1 mg, 0.030 mmol) in DMF (1 mL) was stirred at 50°C overnight under argon. The crude product was purified via preparative HPLC (10 to 100% MeCN/20 mM NH_4HCO_3 buffer, pH 7.4): LC/MS: (ESI-QMS): *m/z* = 1075.13 (M + H).

Step 2: Preparation of Conjugate 16.

To a mixture of **Compound 79** (24.6 mg, 0.023 mmol) and Et_3N (15.9 µl, 0.115 mmol) in DMSO (0.8 mL) was added **Compound 16** (24.1 mg, 0.023 mmol) in MeOH (0.5 mL) at room temperature under argon. The mixture was stirred at room temperature for 3 h and then concentrated under high vacuum. CH_2Cl_2 (1 mL) was added to the crude residue followed by pyrrolidine (4.8 µl, 0.058 mmol) and $Pd(PPh_3)_4$ (1.33 mg, 0.0012 mmol) The reaction mixture was stirred at room temperature under argon for 4 h. The crude product was purified via preparative HPLC (10 - 100% MeCN/20 mM NH_4HCO_3 buffer, pH 7.4) to yield pure **Conjugate 16**: LC/MS: (ESI-QMS): *m/z* = 890 (M + H).

Example 48: Preparation of Compound 84.



Step 1: Preparation of Compound 82.

Compound 82 was synthesized by following the procedure for **Compound 76** from **Compound 75**: LC/MS: (ESI-QMS): *m/z* = 553.62 (M + H), ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.59 (s, 1H), 8.39 (s, 1H), 7.94 (s, 1H), 7.76 (d, J = 7.4, 1H), 7.59 (t, J = 7.8, 1H), 7.53 (s, 1H). 6.99 – 6.94 (m, 1H), 4.36 (d, J = 5.8, 1H), 3.78 (s, 3H), 3.26 – 3.18 (m, 1H), 2.61 (s, 3H), 1.34 (d, J = 1.34, 2H), 1.31 – 1.20 (m, 3H), 1.26 (d, J = 6.8, 18H).

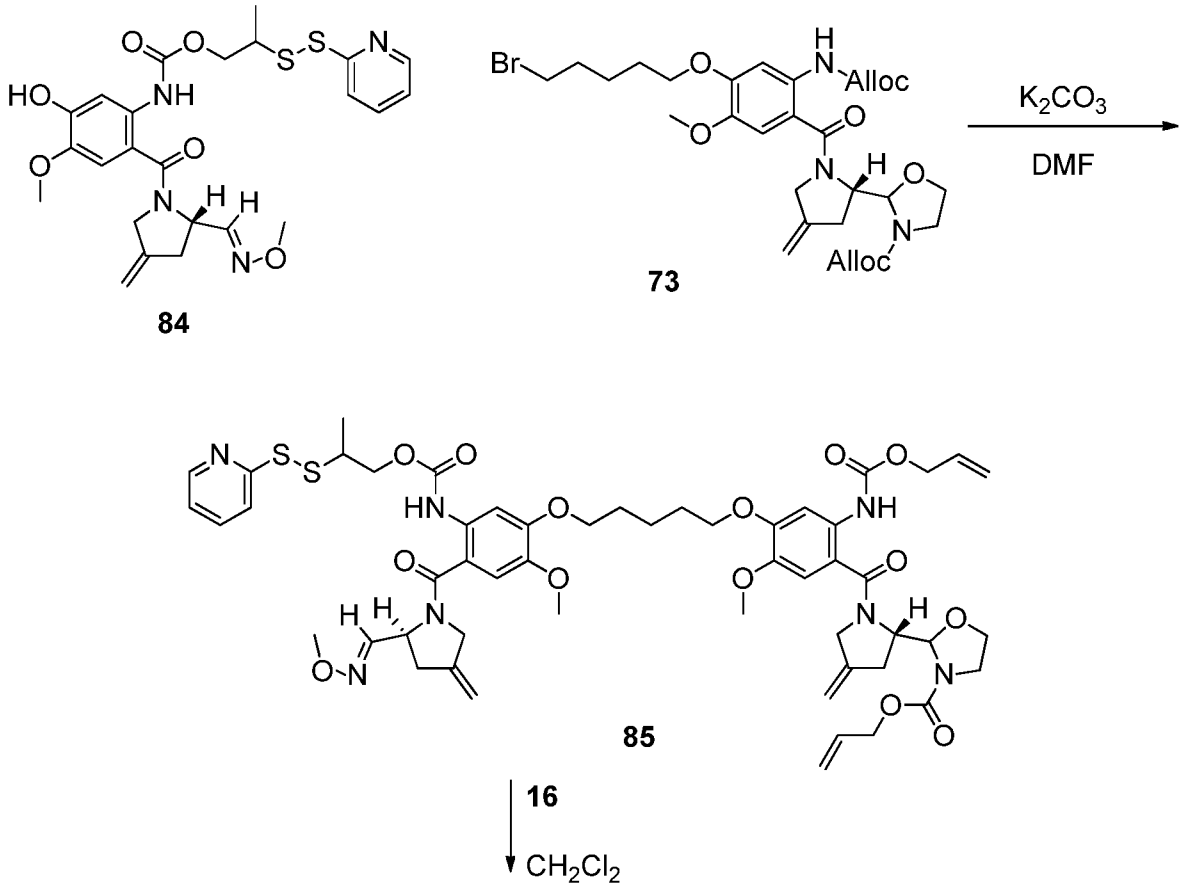
Step 1: Preparation of Compound 83.

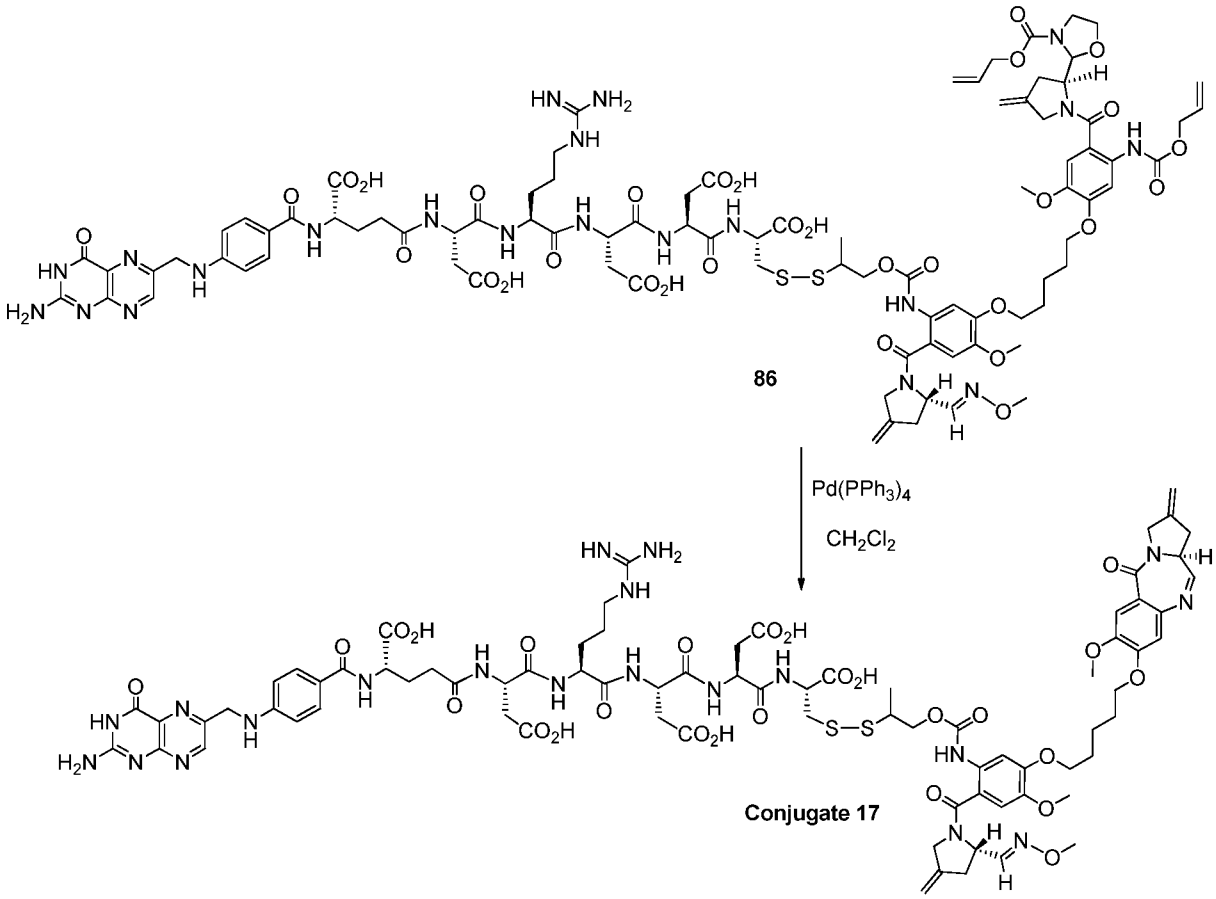
Compound 83 was synthesized by following the procedure for **Compound 77** from **Compound 82** in lieu of **Compound 76**: LC/MS: [(ESI-QMS): *m/z* = 675.77 (M + H), ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.76 (d, J = 7.4, 1H), 7.72 - 7.68 (m, 1H), 7.65 (t, J = 7.9, 1H), 7.07 – 7.04 (m, 1H). 6.84 (s, 1H), 5.06 (s, 1H), 5.01 (s, br, 1H), 4.28 – 4.21 (m, 2H), 4.18 – 4.11 (m, 2H), 3.83 (s, 3H), 3.77 (s, 3H), 3.27 – 3.19 (m, 1H), 2.89 – 2.82 (m, 1H), 1.38 (m, 4H), 1.11 (d, J = 7.4, 18H).

Step 1: Preparation of Compound 84.

Compound 84 was synthesized by following the procedure for **Compound 78** from **Compound 83** in lieu of **Compound 77:** LC/MS: (ESI-QMS): *m/z* = 519.57 (M + H), ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (d, J = 4.9 Hz, 1H), 7.76 – 7.72 (m, 1H), 7.72 - 7.67 (m, 1H), 7.63 (t, J = 7.3, 1H), 7.45 (s, 1H), 7.05 (dd, J_1 = 7.3 Hz, J_2 = 7.3 Hz, 1H), 7.03 (s, 1H), 6.77 (d, J = 6 Hz, 1H), 5.05 (s, 1H), 5.01 (s, br, 1H), 4.28-4.21 (m, 2H), 4.16 – 4.08 (m, 2H), 3.85, (s, 3H) 3.81 (s, 3H), 3.32 (dd, J_1 = 13.2, J_2 =5.9, 1H), 2.87-2.79 (m, 2H), 1.34 (d, J =2.9, 3H).

Example 49: Preparation of Conjugate 17.





Step 1: Preparation of Compound 85.

Compound 85 was synthesized by following the procedure for **Compound 79** from **Compound 84** in lieu of **Compound 78:** LC/MS: (ESI-QMS): *m/z* = 1088.46 (M + H).

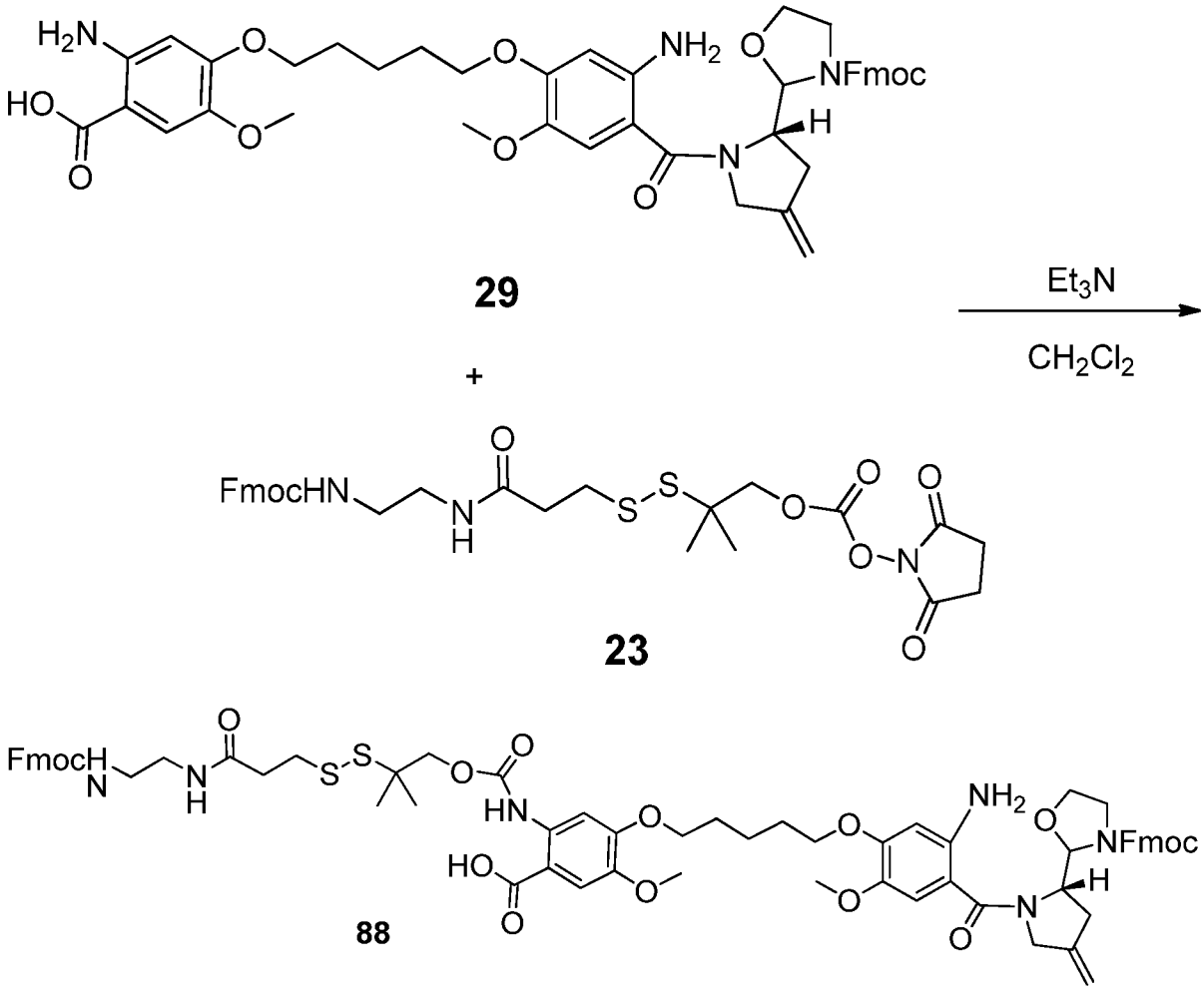
Step 2: Preparation of Compound 86.

Compound 86 was synthesized by following the procedure for **Compound 80** from **Compound 85** in lieu of **Compound 79**: LC/MS: (ESI-QMS): *m/z* = 1011.84 (M + 2H), 675.12 (M + 3H).

Step 3: Preparation of Conjugate 17.

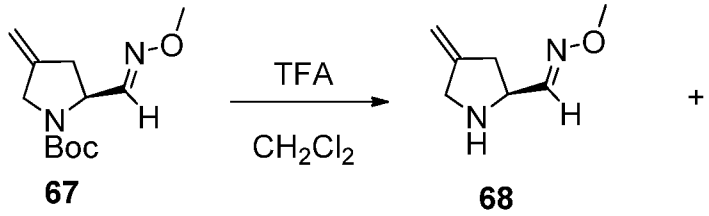
Conjugate 17 was synthesized by following the procedure for **Conjugate 16** from **Compound 86** in lieu of **Compound 80**: LC/MS: (ESI-QMS): *m/z* = 897.82 (M + 2H), 598.63 (M + 3H).

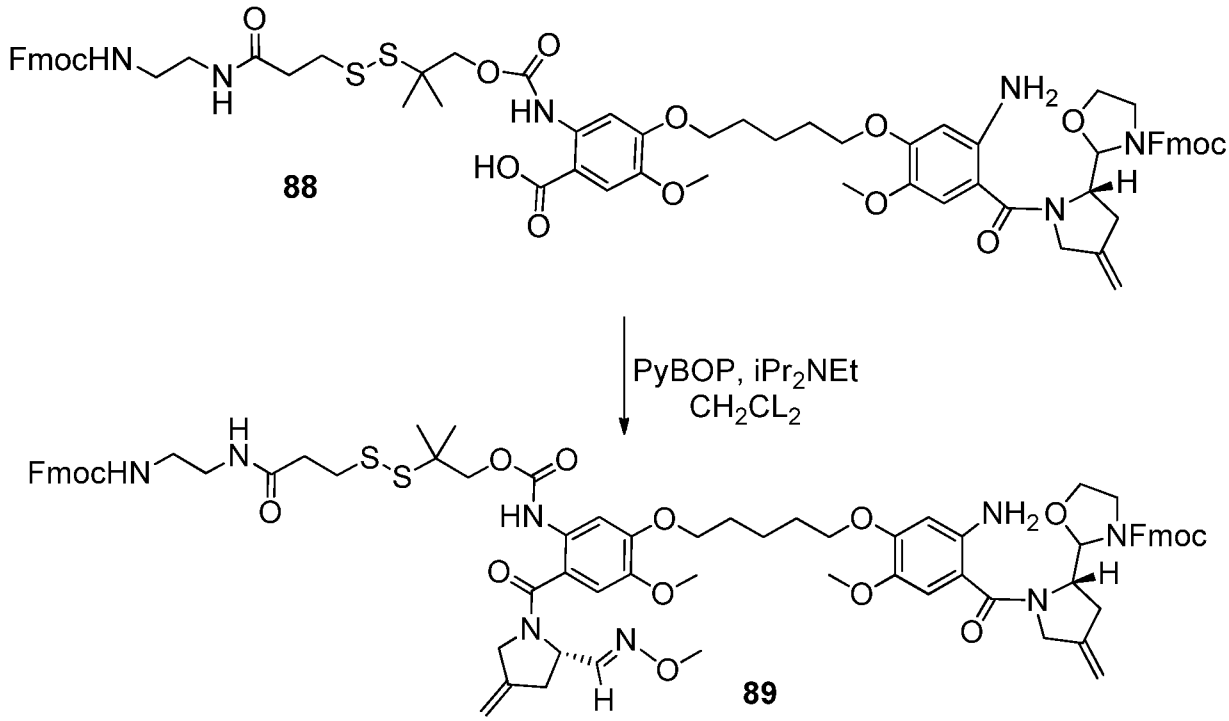
Example 50: Preparation of Compound 88.



Et_3N (12.5 µL, 89.3 µmol) was added to a solution of **Compound 29** (32.3 mg, 40.6 µmol) and **Compound 23** (25.1 mg, 40.6 µmol) in anhydrous CH_2Cl_2 (1.5 mL). The reaction mixture was stirred at room temperature under argon for 3 h and then purified via silica chromatography (0 - 10%, MeOH/CH_2Cl_2) to yield **Compound 88** as a white solid (42.6 mg, 81.1%): (ESI-QMS): *m/z* = 1294.31 (M + H), Selective ^{1}H NMR (500 MHz, 298 K, CD_2Cl_2) δ 7.765 (b, 4H), 7.584 (b, 4H), 7.487 (b, 2H), 7.386 (b, 4H), 7.305 (b, 6H).

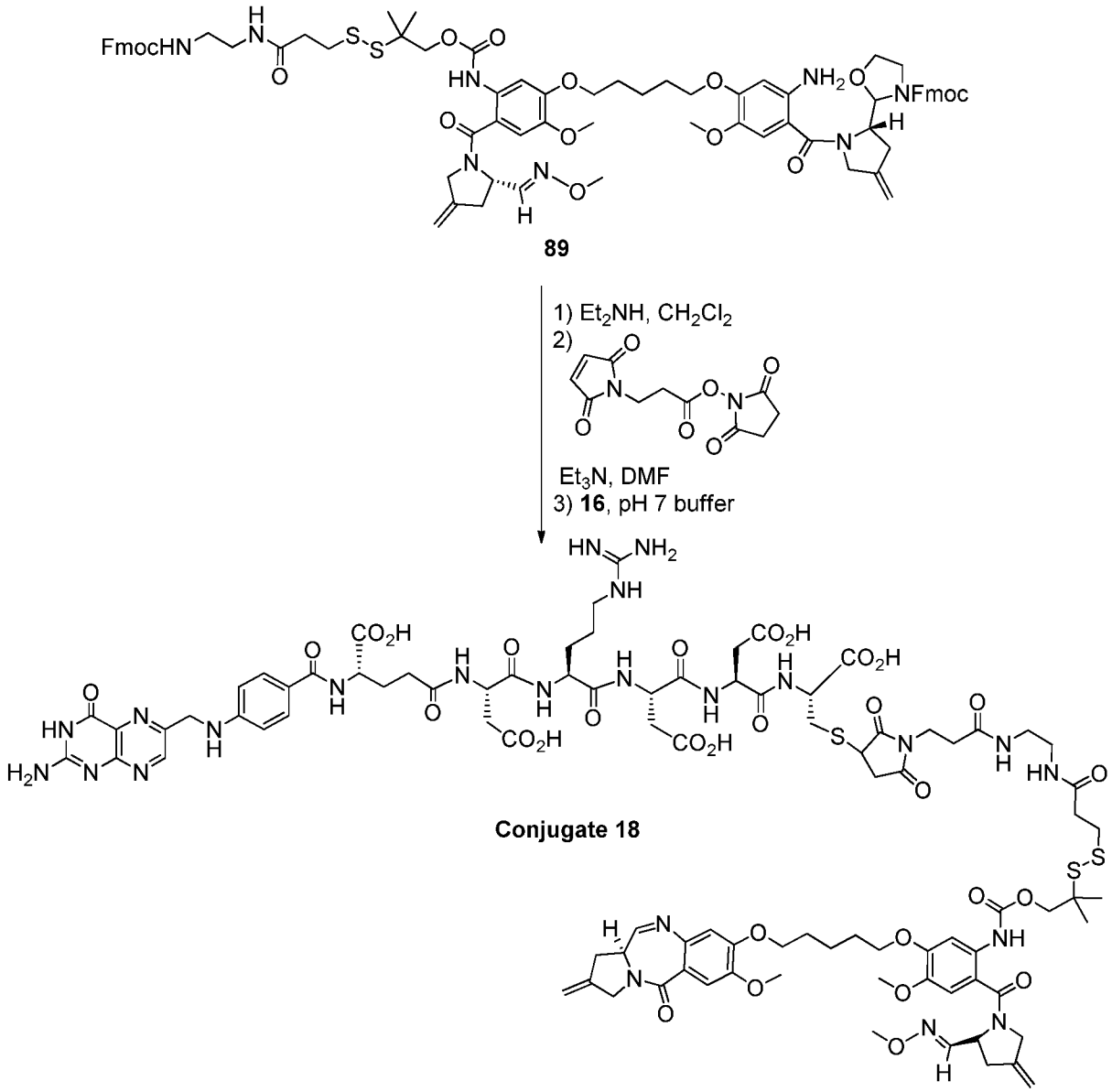
Example 51: Preparation of Compound 89.





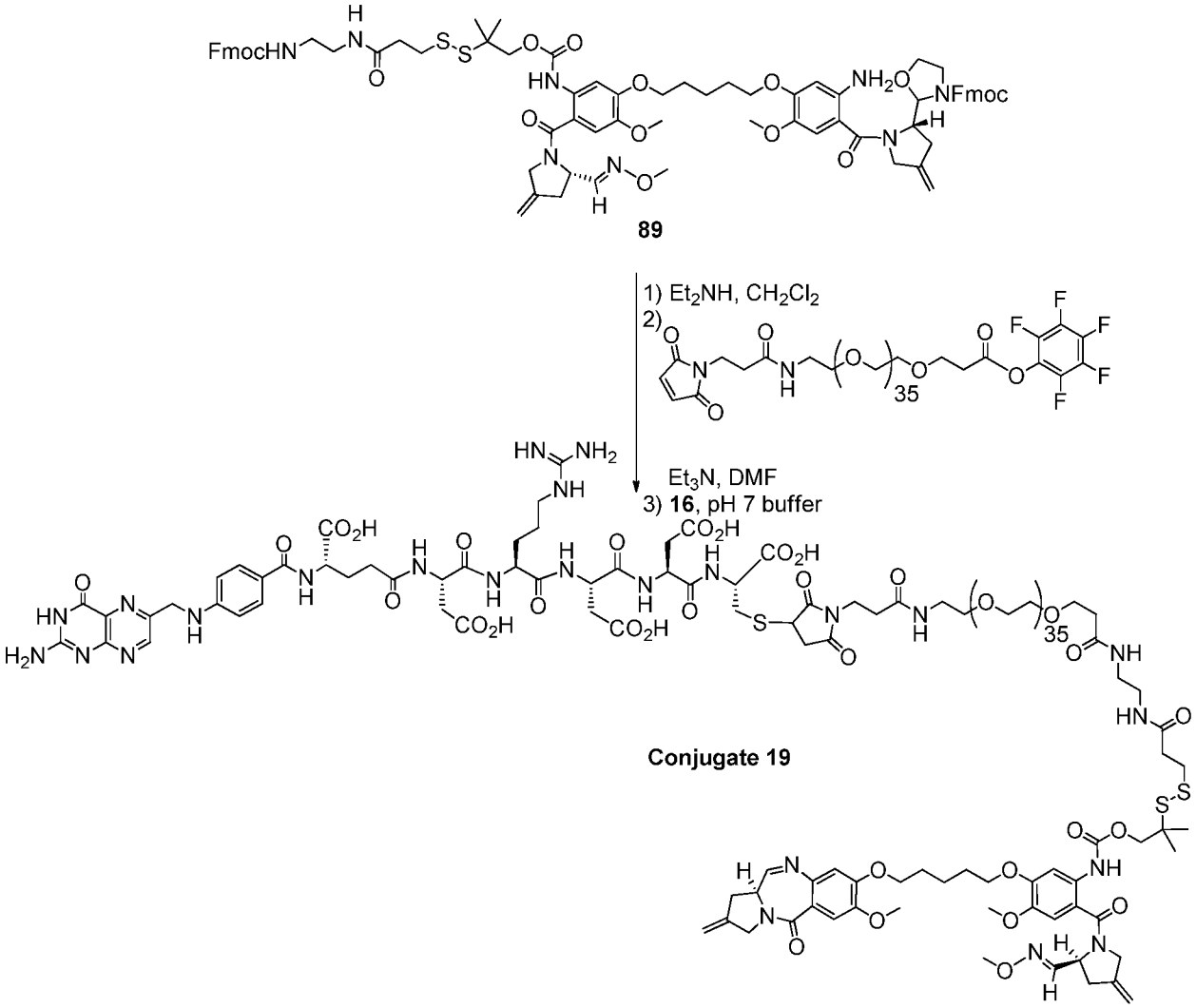
TFA (0.50 mL) was added to a solution of **Compound 67** (10.1 mg, 40.2 μmol) in anhydrous CH_2Cl_2 (0.50 mL). The reaction mixture was stirred at room temperature under argon for 35 min and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (1 mL × 3), and dried under high vacuum for 1 h. To the residue was added a solution of **Compound 88** (40.0 mg, 30.9 μmol) and PyBOP (17.7 mg,34.0 μmol) in anhydrous CH_2Cl_2 (1.0 mL) and $^{i}Pr_2NEt$ (30.0 μL, 5.5 170 μmol) in tandem. The reaction mixture was stirred at room temperature under argon for 1 h and purified via silica chromatography (0 - 10% MeOH/CH_2Cl_2) to yield **Compound 89** as a beige solid (40.8 mg). The purity of the product was about 50 - 60% based on LC/MS analysis and was used without further purification. LC/MS: (ESI-QMS): *m/z* = 1416.31 (M + H).

Example 52: Preparation of Conjugate 18.



Diethylamine (0.75 mL) was added to a solution of **Compound 89** (40.1 mg, 28.3 μmol) in anhydrous CH_2Cl_2 (1.0 mL). The reaction mixture was stirred at room temperature under argon for 3 h and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (1.5 mL × 3), dried under high vacuum for 1 h, and dissolved in anhydrous DMF (2.0 mL). To the solution was added maleimidopropionic acid NHS ester (8.3 mg, 31.1 μmol) and Et_3N (8.0 μL, 57 μmol) in tandem. The reaction mixture was stirred at room temperature under argon for 1 h, diluted with DMF (2.5 mL), and to which was added a solution of **Compound 16** (38.5 mg,36.8 μmol) in 50 mM NH_4HCO_3 buffer, pH 7.0 (5.0 mL). The reaction mixture was stirred at room temperature under argon for 15 min and purified via preparative HPLC (10 – 100%, MeCN/50 mM NH_4HCO_3 buffer, pH 7.0 to yield **Conjugate 18** as a fluffy yellow solid (18.3 mg, 30.7% over three steps): LC/MS: (ESI-QMS): *m/z* = 1052.55 (M + 2H), Selective ^{1}H NMR (500 MHz, 298 K, DMSO-*d6* with D_2O exchange) δ 8.607 (s, 1H), 7.569 (d, J = 8.5 Hz, 2H), 7.003 (s, 1H), 6.865 (b, 1H), 6.625 (d, J = 8.5 Hz, 2H).

Example 53: Preparation of Conjugate 19.

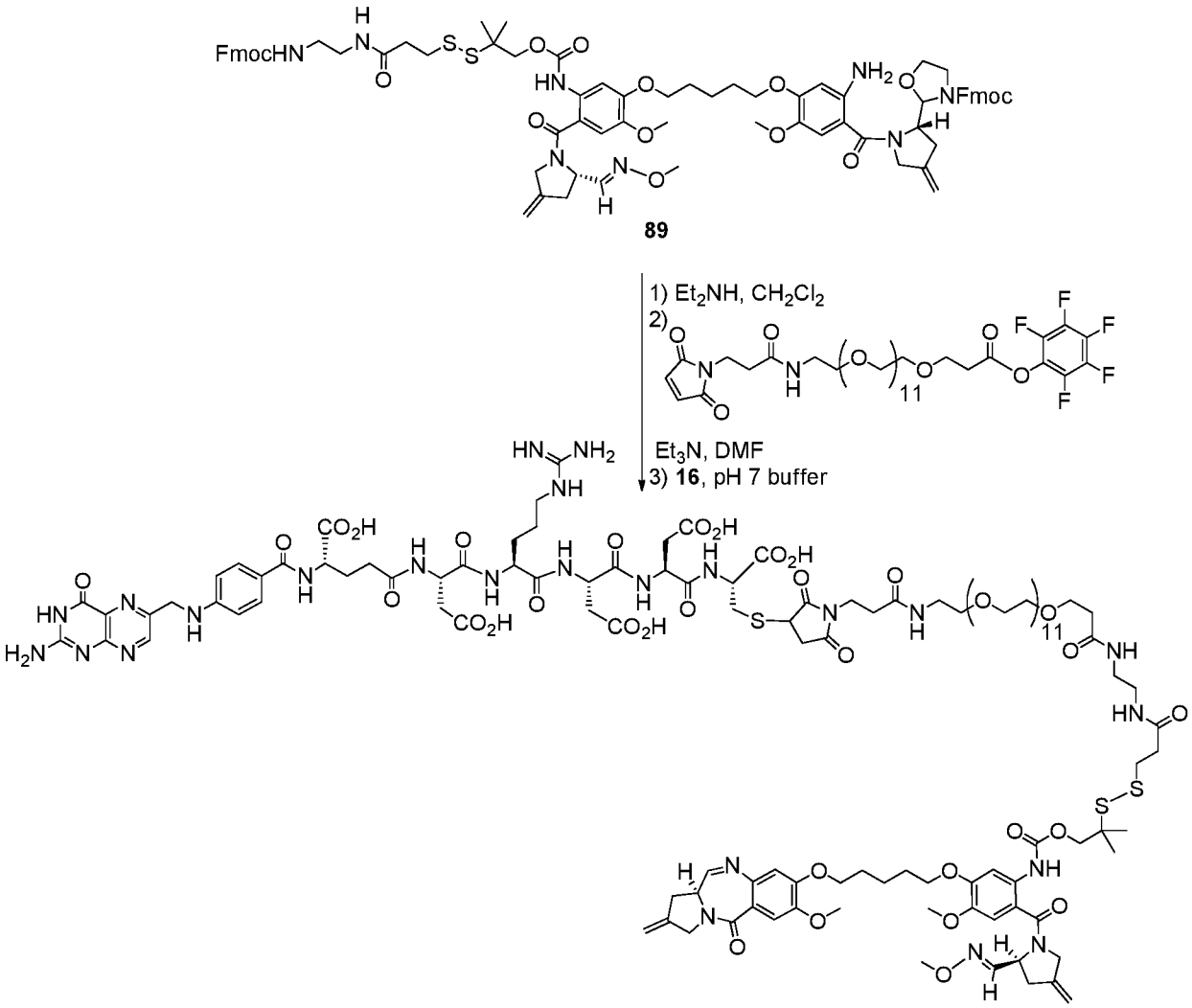


Mal-dPEG$_4$-TFP ester, Mal-dPEG$_{12}$-TFP ester, and Mal-dPEG$_{36}$-TFP ester were obtained from Quanta BioDesign Ltd.

Diethylamine (0.75 mL) was added to a solution of **Compound 89** (50.2 mg, 35.5 μmol) in anhydrous CH_2Cl_2 (0.75 mL). The reaction mixture was stirred at room temperature under argon for 2.5 h and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (1 mL × 3), dried under high vacuum for 1 h, and dissolved in anhydrous DMF (2.0 mL). To the solution was added MAL-dPEG$_{36}$-TFP ester (70.0 mg, 35.5 μmol) and Et_3N (10.0 μL, 71 μmol) in tandem. The reaction mixture was stirred at room temperature under argon for 45 min, diluted with DMF (2.5 mL), and to which was added a solution of **Compound 16** (50.1 mg, 46.2 μmol) in 50 mM NH_4HCO_3 buffer, pH 7.0 (5.0 mL). The reaction mixture was then stirred at room temperature under argon for 15 min and purified via preparative HPLC (10 – 100%, MeCN/50 mM NH_4HCO_3 buffer, pH 7.0) to afford **Conjugate 19** as a fluffy yellow solid (19.1 mg, 14.3 % over three steps): LC/MS: (ESI-QMS): *m/z* =

11880.76 (M + 3H), Selective ^{1}H NMR (500 MHz, 298 K, DMSO-*d6* with D_2O exchange) δ 8.624 (s, 1H), 7.578 (d, J = 8.5 Hz, 2H), 6.888 (b, 1H), 6.523 (d, J = 8.5 Hz, 2H).

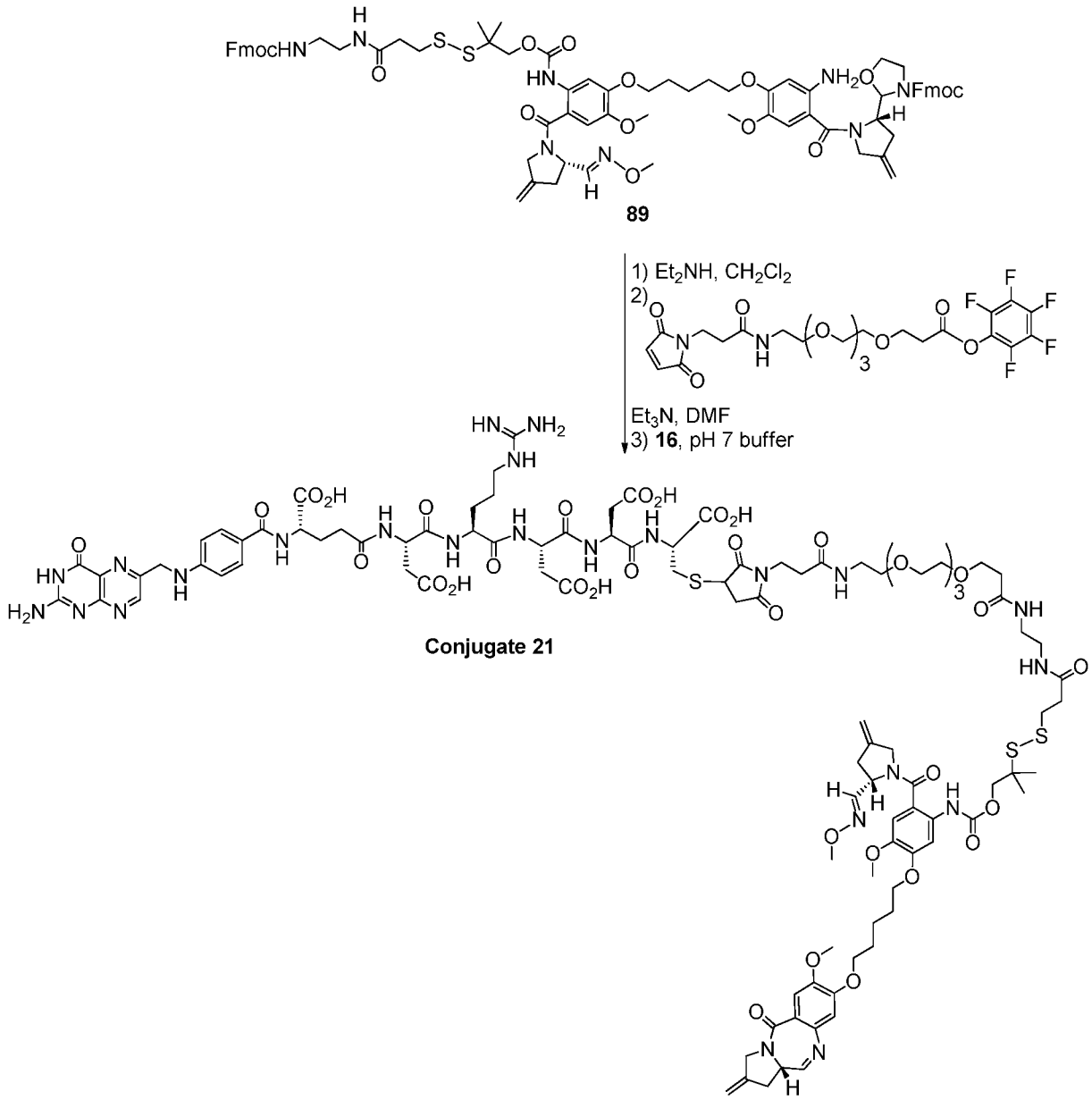
Example 54: Preparation of Conjugate 20.



Diethylamine (0.75 mL) was added to a solution of **Compound 89** (51.7 mg, 36.5 µmol) in anhydrous CH_2Cl_2 (0.75 mL). The reaction mixture was stirred at room temperature under argon for 2.5 h and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (1 mL × 3), dried under high vacuum for 1 h, and dissolved in anhydrous DMF (2.0 mL). To the solution was added MAL-dPEG$_{12}$- TFP ester (34.8 mg, 40.2 µmol) and Et_3N (11.2 µL, 73 µmol) in tandem. The reaction mixture was stirred at room temperature under argon for 45 min, diluted with DMF (2.5 mL), and to which was added a solution of **Compound 16** (53.5 mg, 51.1 µmol) in 50 mM NH_4HCO_3 buffer, pH 7.0 (5.0 mL). The reaction mixture was stirred at room temperature under argon for 15 min and purified via preparative HPLC (10 – 100%, MeCN/50 mM NH_4HCO_3 buffer, pH 7.0) to yield **Conjugate 20** as a fluffy yellow solid (6.1 mg, 6.2% over three steps): LC/MS: (ESI-QMS): *m/z* = 1354.57

(M + 2H), Selective ^{1}H NMR (500 MHz, 298 K, DMSO-*d6* with D_2O exchange) δ 8.709 (b, 1H), 7.666 (b, 2H), 7.158 (s, 1H), 7.059 (s, 1H), 7.012 (s, 1H), 6.930 (s, 1H), 6.764 (b, 2H).

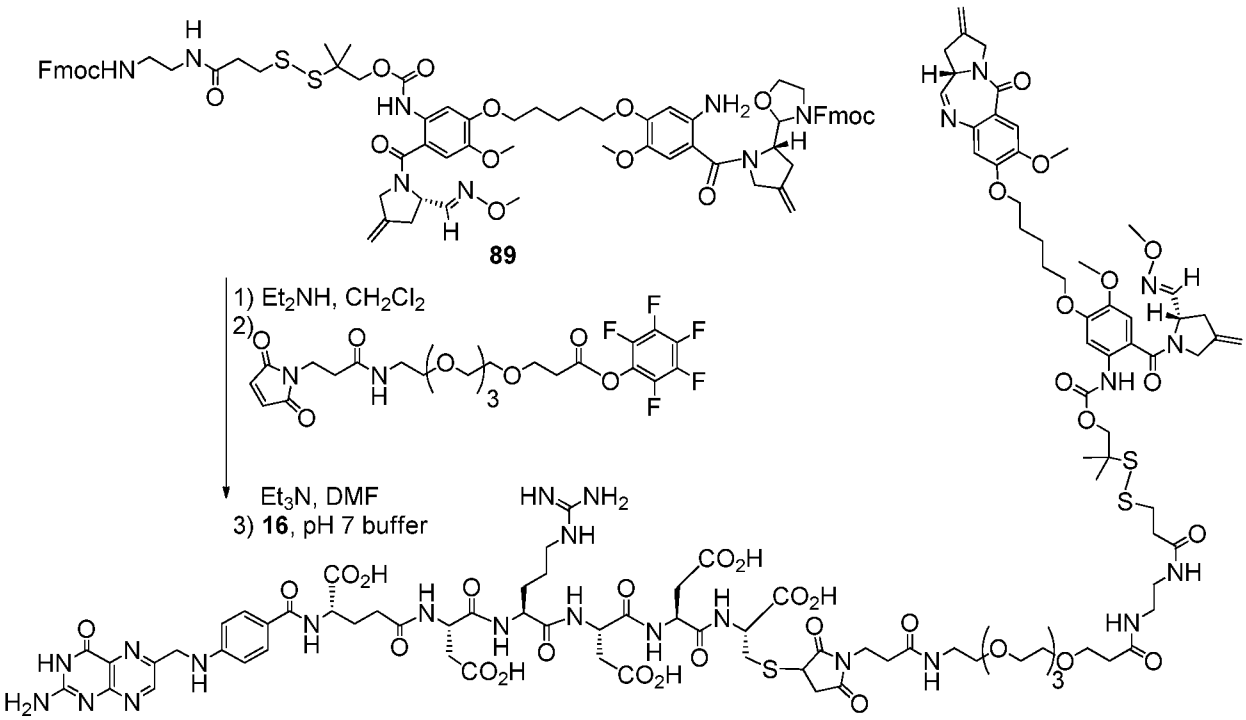
Example 55: Preparation of Conjugate 21.



Diethylamine (0.70 mL) was added to a solution of **Compound 89** (55.0 mg, 38.9 µmol) in anhydrous CH_2Cl_2 (0.70 mL). The reaction mixture was stirred at room temperature under argon for 2.5 h and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (1 mL × 3), dried under high vacuum for 1 h, and dissolved in anhydrous DMF (2.0 mL). To the solution was added MAL-dPEG$_4$-TFP ester (23.9 mg, 46.7 µmol) and Et_3N (12.0 µL, 85.6 µmol) in tandem. The reaction mixture was then stirred at room temperature under argon for 45 min, diluted with DMF (2.5 mL), and to which was added a solution of **Compound 16** (56.9 mg,54.5 mmol) in 50 mM NH_4HCO_3 buffer, pH 7.0 (5.0 mL). The reaction mixture was stirred at room temperature under argon for 15 min and purified via

preparative HPLC (10 – 100% MeCN/50 mM NH_4HCO_3 buffer, pH 7.0) to afford **Conjugate 21** as a fluffy yellow solid (18.0 mg, 19.7% over three steps): LC/MS: (ESI-QMS): *m/z* = 1176.17 (M + 2H), Selective ^{1}H NMR (500 MHz, 298 K, DMSO-*d6* with D_2O exchange) δ 8.619 (s, 1H), 7.577 (d, J = 8.5 Hz, 2H), 7.045 (s, 1H), 7.014 (s, 1H), 6.883 (b, 1H), 6.629 (d, J = 8.5 Hz, 2H).

Example 55: Preparation of Conjugate 21.



Diethylamine (0.70 mL) was added to a solution of **Compound 89** (55.0 mg, 38.9 µmol) in anhydrous CH_2Cl_2 (0.70 mL). The reaction mixture was stirred at room temperature under argon for 2.5 h and concentrated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (1 mL × 3), dried under high vacuum for 1 h, and dissolved in anhydrous DMF (2.0 mL). To the solution was added MAL-dPEG$_4$-TFP ester (23.9 mg, 46.7 µmol) and Et_3N (12.0 µL, 85.6 µmol) in tandem. The reaction mixture was then stirred at room temperature under argon for 45 min, diluted with DMF (2.5 mL), and to which was added a solution of **Compound 16** (56.9 mg,54.5 mmol) in 50 mM NH_4HCO_3 buffer, pH 7.0 (5.0 mL). The reaction mixture was stirred at room temperature under argon for 15 min and purified via preparative HPLC (10 – 100% MeCN/50 mM NH_4HCO_3 buffer, pH 7.0) to afford **Conjugate 21** as a fluffy yellow solid (18.0 mg, 19.7% over three steps): LC/MS: (ESI-QMS): *m/z* = 1176.17 (M + 2H), Selective ^{1}H NMR (500 MHz, 298 K, DMSO-*d6* with D_2O exchange) δ 8.619 (s, 1H), 7.577 (d, J = 8.5 Hz, 2H), 7.045 (s, 1H), 7.014 (s, 1H), 6.883 (b, 1H), 6.629 (d, J = 8.5 Hz, 2H).

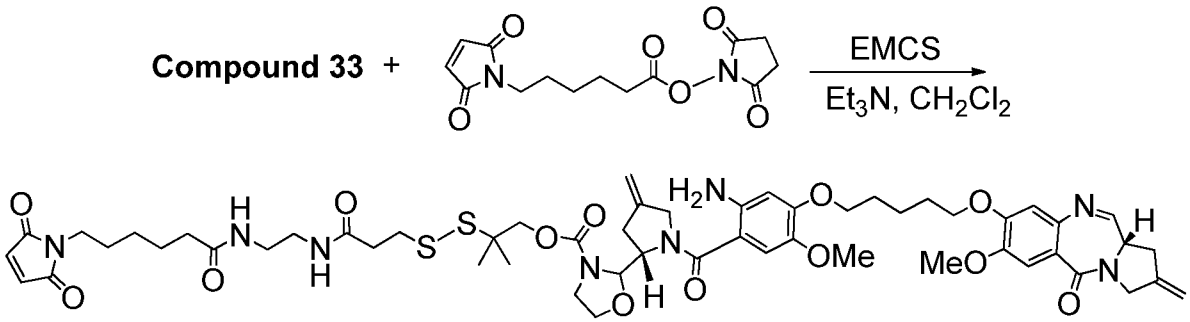
Example 56: Preparation of Conjugate 22.

Step 1: Preparation of Compound 90:

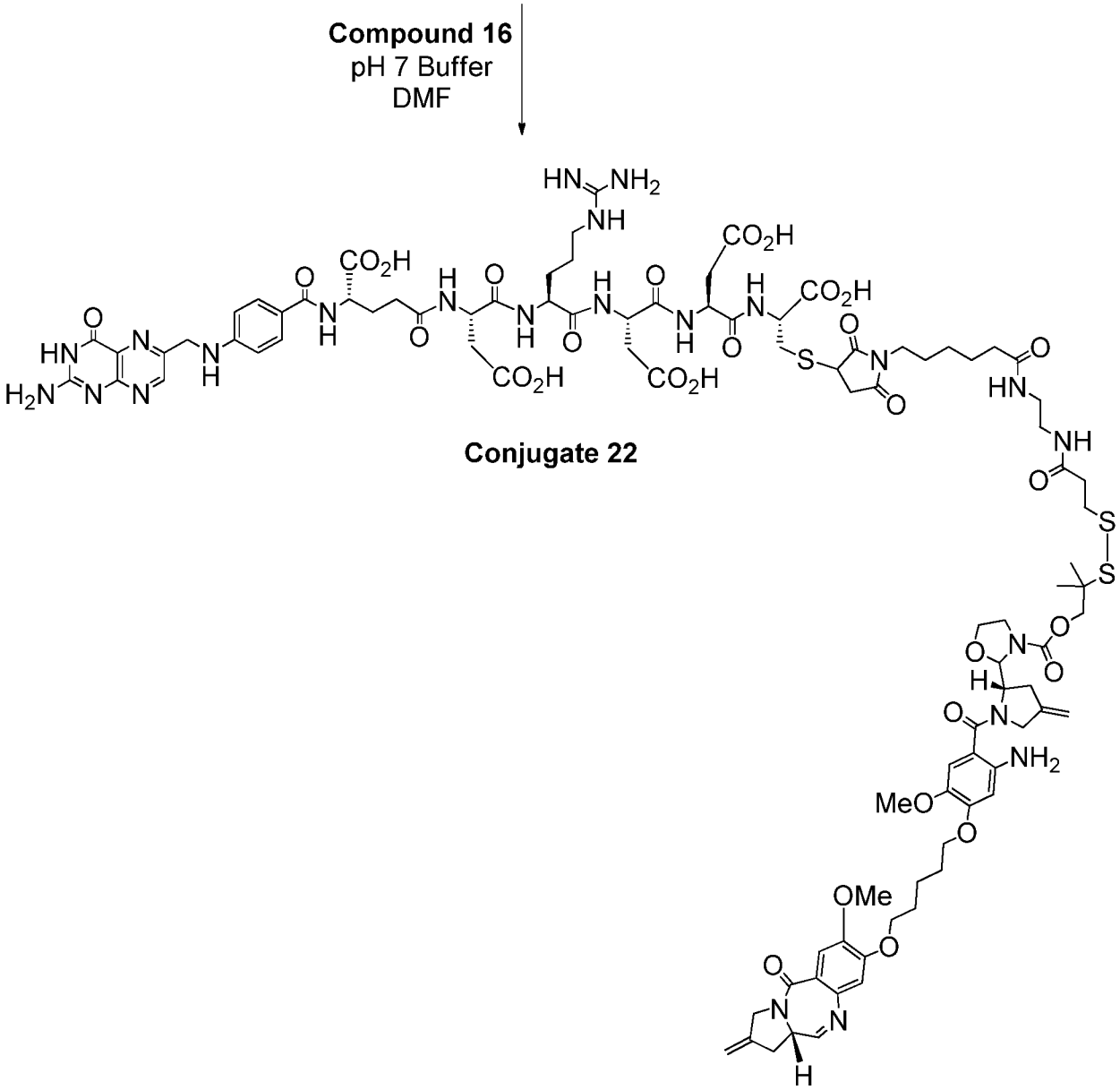
Compound 90 was synthesized by following the same procedure as for the preparation of **Compound 34** from EMCS in lieu of Mal-PEG4-NHS ester: LC/MS (ESI-QMS): *m/z* = 1117 (M + H).

Step 2: Preparation of Conjugate 22:

Conjugate 22 was prepared axccording to the procedure described above for **Conjugate 5** by reacting **Compound 90** with **Compound** 16 instead of Compound 34. Yield: 9% for 3 steps. LC/MS (ESI-QMS), $(M+2H)^{2+}$: 1082.

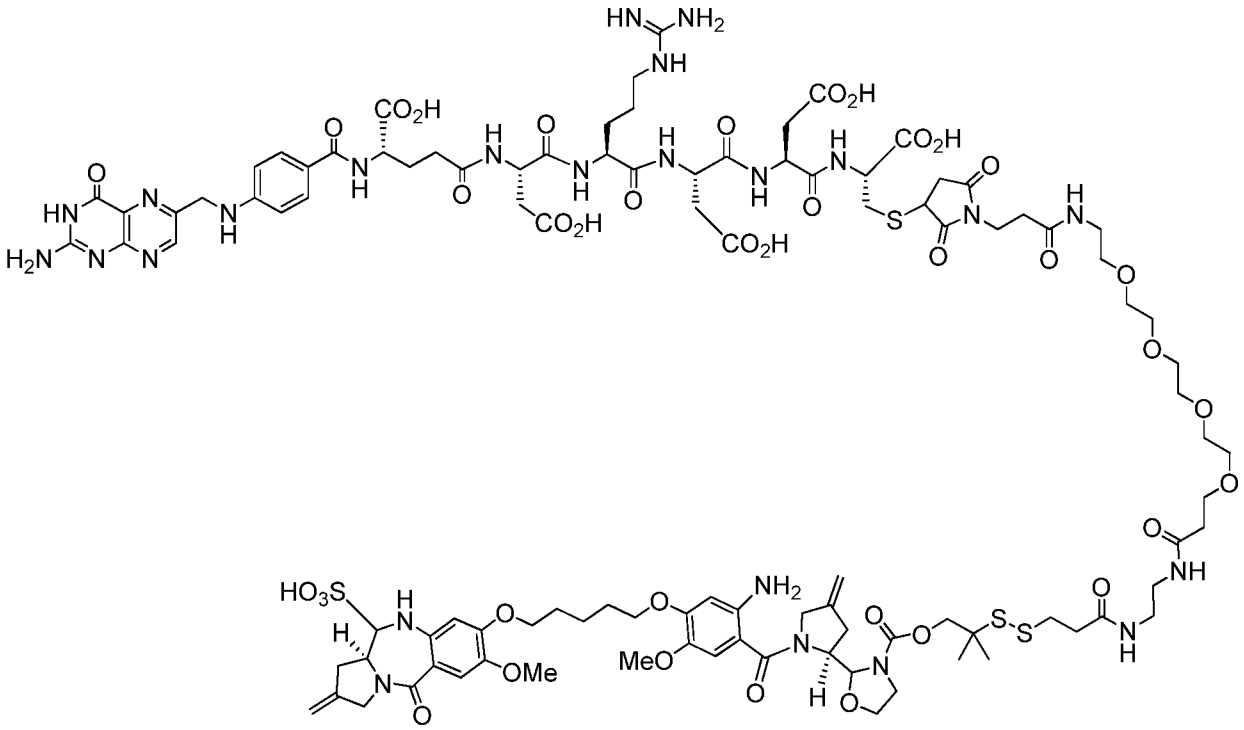


Compound 90



Conjugate 22

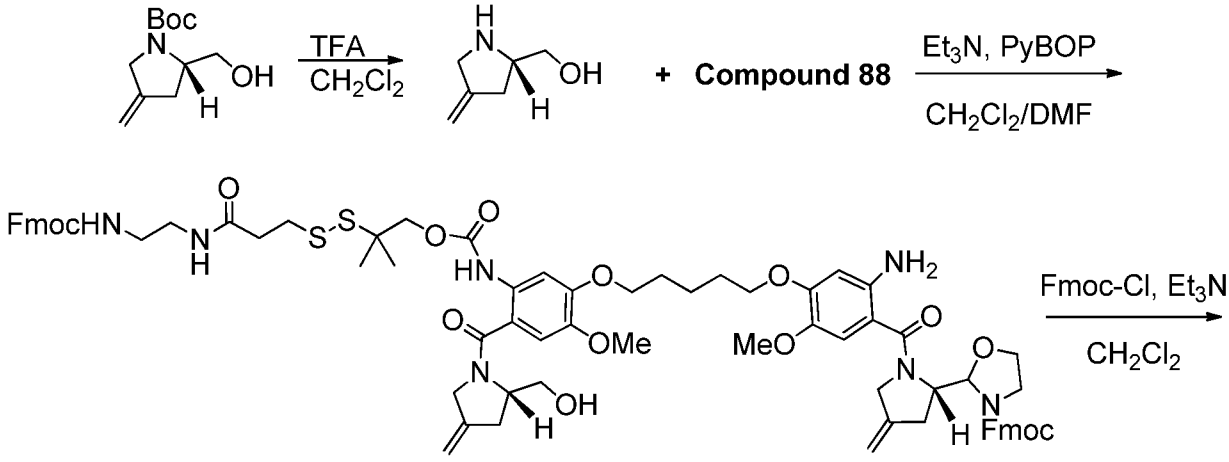
Example 57: Preparation of Conjugate 23.



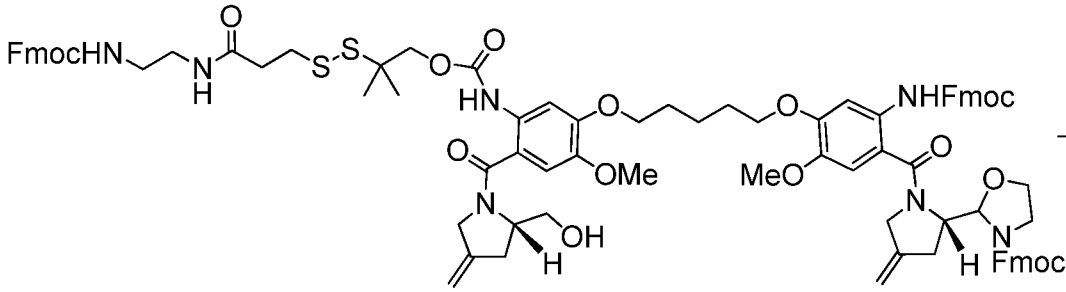
Conjugate 23

To the solution of **Conjuagte 5** (5.7 mg, 0.0024 mmol) in DMSO (0.4 mL) and water (0.4 mL) was added $NaHSO_3$ (0.37 mg, 0.0036 mmol) and the reaction was stirred for 2h. The reaction was then purified with prep-HPLC in 10 - 100% CH_3CN/NH_4HCO_3 buffer (pH 7.4, 50 mM) to provide **Conjugate 23** (2.8 mg, 48% yield). LC/MS (ESI-QMS): $(M+2H)^{2+}$: 1226.

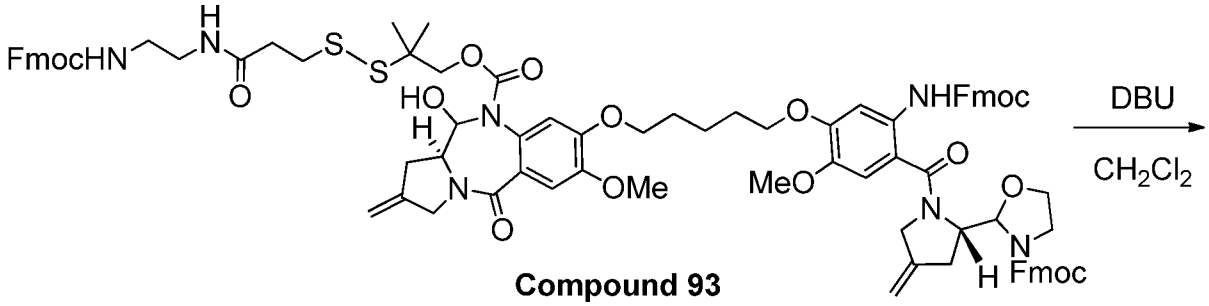
Example 58: Preparation of Conjugate 24.



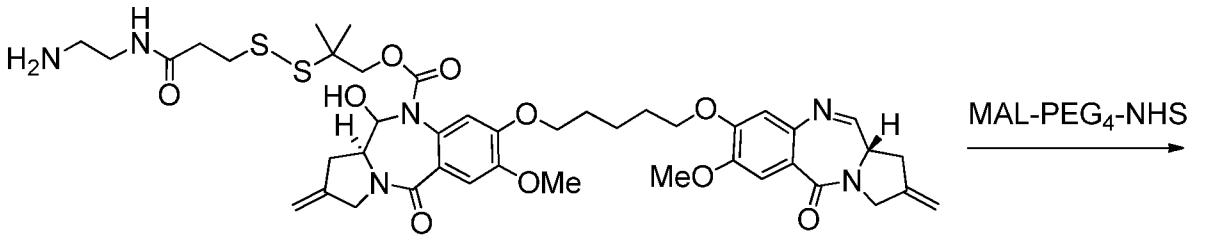
Compound 91



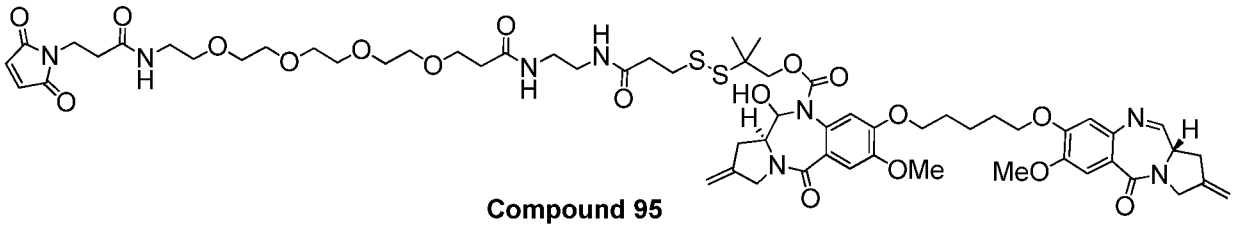
Compound 92



Compound 93



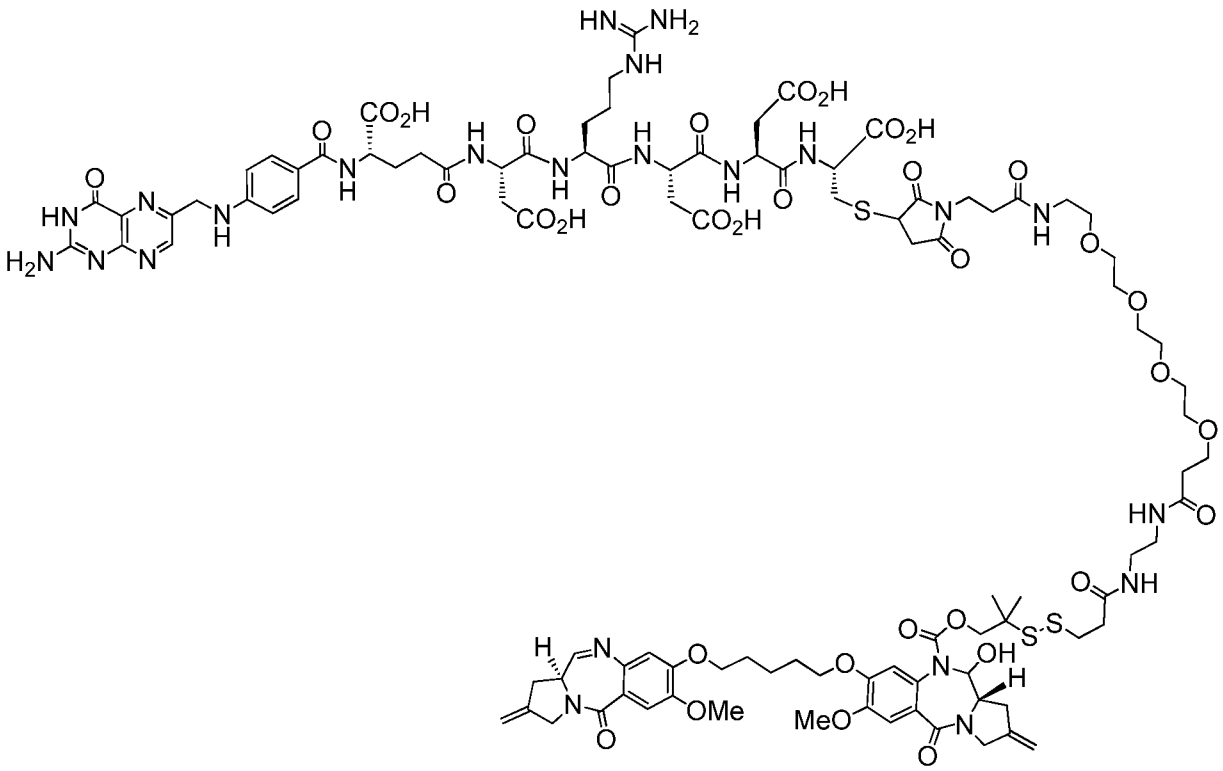
Compound 94



Compound 95

Compound 16

pH 7 Buffer
DMSO, H_2O



Conjugate 24

Step 1: Preparation of Compound 91

Boc-protected prolinol derivatitive (6.72 mg, 0.0315 mmol) was added to TFA/CH_2Cl_2 (0.5 mL/0.5 mL) and stirred for 30 min at room temperature. The solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (1.0 mL), and added to a solution of **Compound 88** (40.74 mg, 0.0315 mmol) and Et_3N (8.8 µl, 0.063 mmol) in DMF (0.5 mL). The reaction mixture was treated with PyBOP (18.0 mg, 0.0347 mmol) at stirred for 1h at room temperature. The desired product was isolated via silica chromatography in 0 - 10% CH_3OH/CH_2Cl_2, yielding 41.0 mg **Compound 91** (94%). LC/MS (ESI-QMS): $(M+H)^+$:1389.

Step 2: Preparation of Compound 92

FmocCl (11.46 mg, 0.0444 mmol) was added to a stirring solution of **Compound 91** (20.5 mg, 0.0148mmol) in CH_2Cl_2 (0.5 mL). The reaction mixture was then treated with Et_3N (2.1 uL, 0.0148 mmol) and stirred for 5h. The desired product was purified via silica chromatography with 0 - 10% CH_3OH/CH_2Cl_2 to yield 14.2 mg of **Compound 92** (60%): LC/MS (ESI-QMS): $(M+H)^+$:1611.

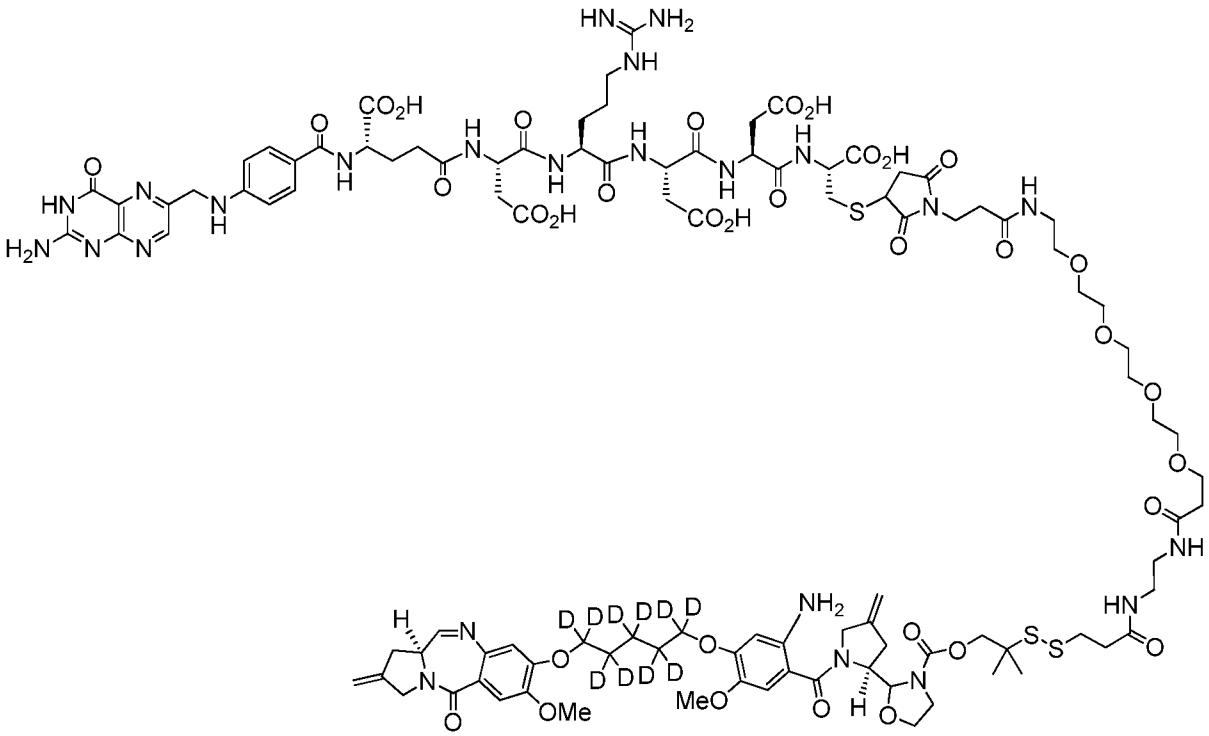
Step 3: Preparation of Compound 93

Compound 92 (14.3 mg, 0.00888 mmol) was added to a solution of Dess-Martin-periodinane (5.65 mg, 0.0133 mmol) in CH_2Cl_2 (0.5 mL). The reaction mixture was stirred at room temperature for 2h. The desired product was purified via silica chromatography with 0 - 10% CH_3OH/CH_2Cl_2 to yield 17.7 mg of **Compound 93**: LC/MS (ESI-QMS): $(M+H)^+$:1609.

Step 4: Preparation of Conjugate 24

Compound 93 (17.7 mg, 0.0128 mmol) in CH_2Cl_2 (0.3 mL) was treated with DBU (1.9 µL, 0.0128 mmol) for 30 min at room temperature. The reaction mixture was then neutralized with AcOH (0.7 µL, 0.0128 mmol). **Compound 94** was observed via LC/MS: LC/MS (ESI-QMS): $(M+H)^+$: 881. Mal-PEG_4-NHS ester (6.6 mg, 0.0128 mmol) was then added to the crude mixture of **Compound 94** to give **Compound 95**, $(M+H)^+$: 1280. The reaction mixture was then concentrated to dryness under high vacuum. The crude residue of **Compound 95** was dissolved with a solution of **Compound 16** (13.4 mg, 0.0128 mmol) in DMSO/PBS buffer (0.3 mL/0.3 mL). The desired product was purified with prep-HPLC in 10 - 100% CH_3CN/NH_4HCO_3 (pH7.4, 50.0 mM) to yield 1.5 mg of **Conjugate 24** (5% for 4 steps): LC/MS (ESI-QMS): $(M+2H)^{2+}$: 1163.

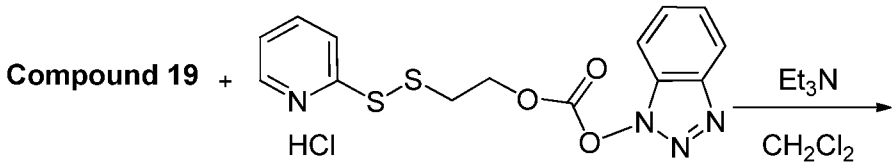
Example 59: Preparation of Conjugate 25.



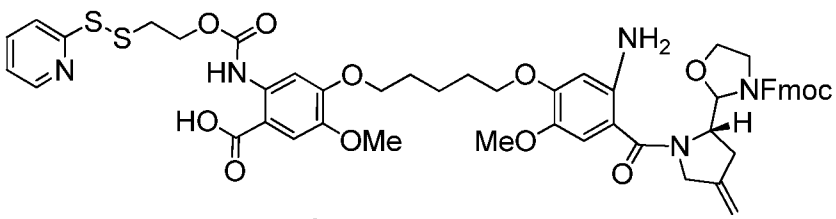
Conjugate 25

Conjugate 25 was synthesized by following the procedure for **Conjugate 5** from **d-10 Compound 6** in lieu of **Compound 6**: LC/MS (ESI-QMS): $(M+3H)^{3+}$: 794. ^1H NMR (500 MHz, 9:1 DMSO-d6:D_2O) δ 8.62 (s, 1H), 7.58 (d, J = 8.5 Hz, 2H), 7.13 (s, 1H), 7.01 (s, 1H), 6.63 (d, J = 8.5 Hz, 3H), 6.51 (s, 1H), 6.32 (s, 1H), 5.09 (s, 1H), 5.06 (s, 1H), 4.99 (s, 1H), 4.94 (d, J = 8.5 Hz, 2H), 4.89 (s, 1H), 4.53 (m, 2H), 4.47 (m, 3H), 4.38 (m, 1H)4.23 (m, 1H), 4.0-4.2 (m, 4H), 3.99 (s, 2H), 3.83 (m, 6H), 3.66 (s, 3H), 3.61 (m, 4H), 3.55 (m, 6H), 3.35 (m, 3H), 3.13 (m 10H), 2.83 (m, 6H), 2.63 (m 3H), 2.41 (m, 8H), 2.28 (m, 5H), 2.14 (b, 3H), 1.80-1.95 (b, 6H), 1.59 (m,b, 2H), 1.30-1.50 (m, b, 4H), 1.22 (b, 6H).

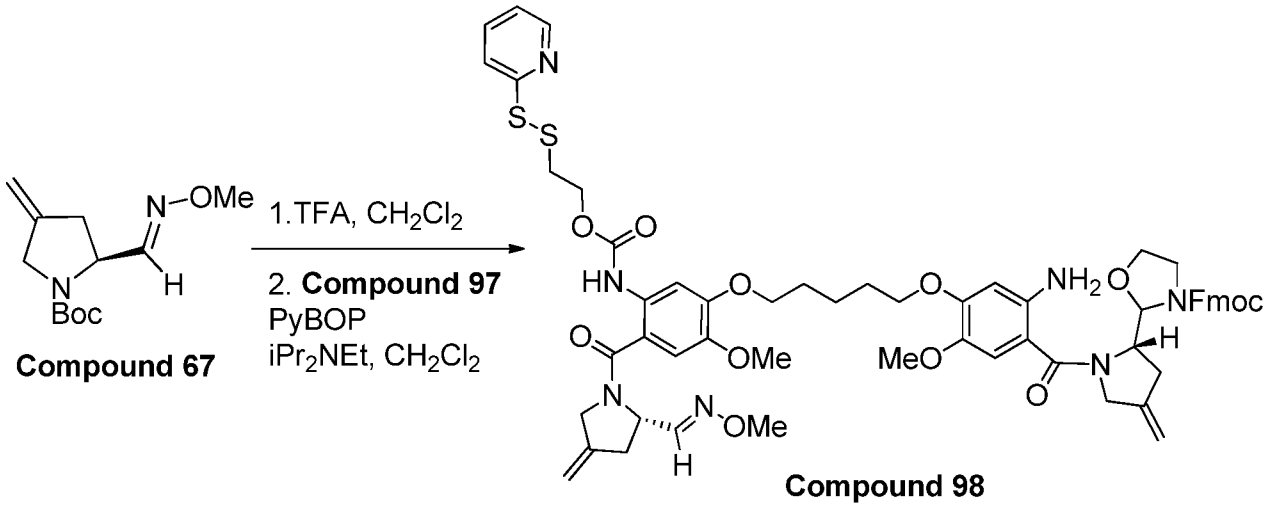
Example 60: Preparation of Conjugate 26.



Compound 96

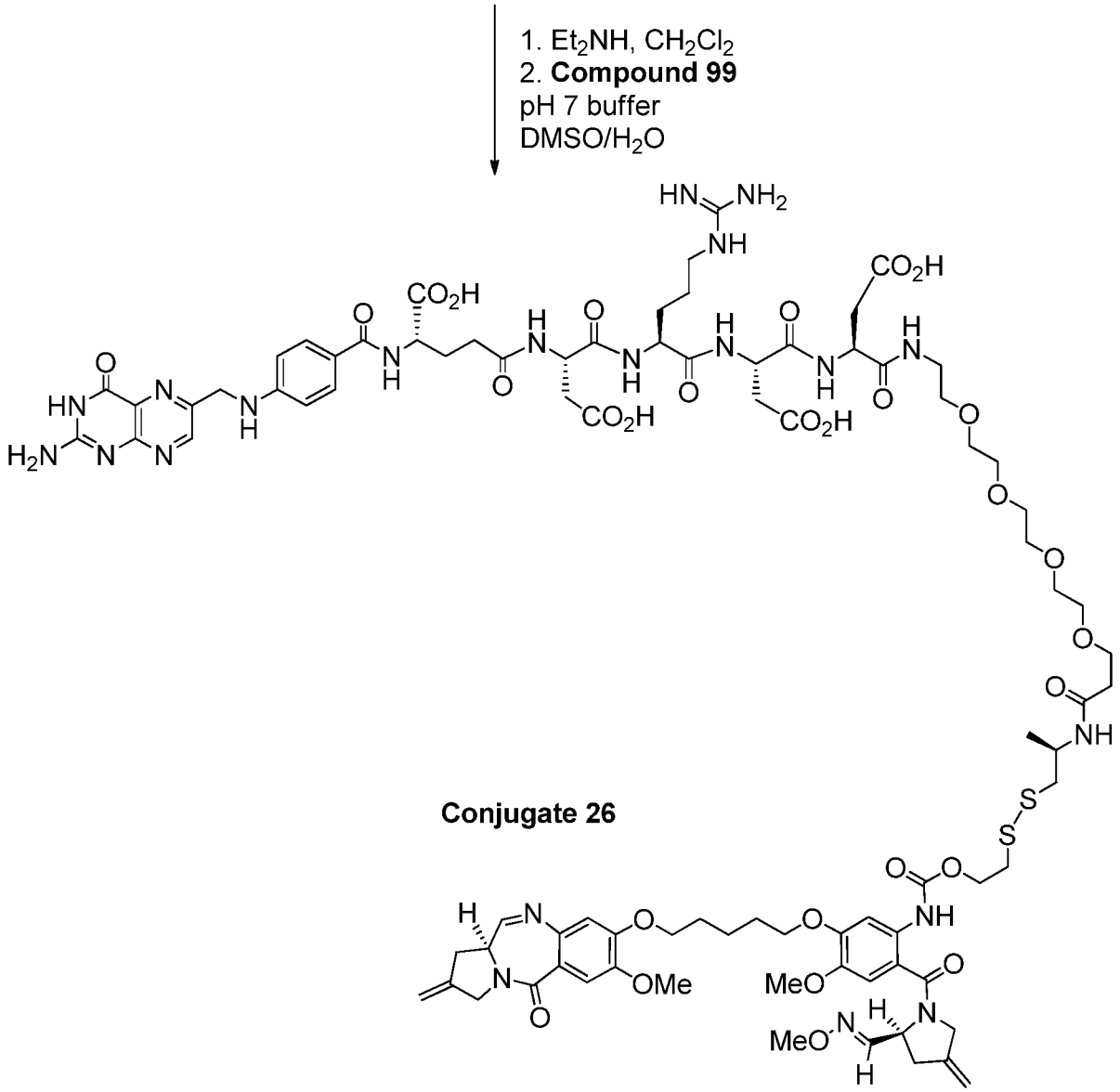


Compound 97



Compound 67

Compound 98



Conjugate 26

Step 1: Preparation of Compound 96

Compound 96 was prepared according to the procedure described for **Compound 2**, except 2-mercaptoethanol was used in lieu of 2-thiopropanol.

Step 2: Preparation of Compound 97

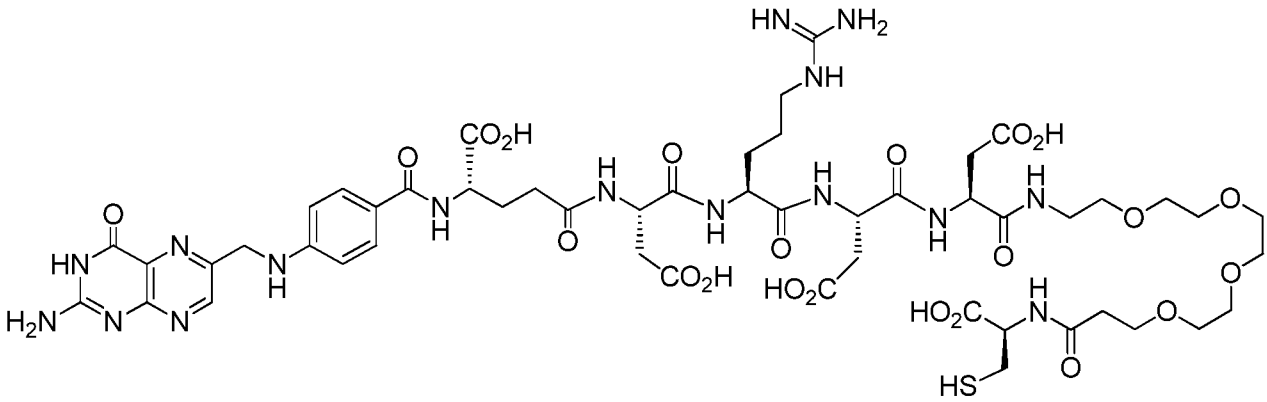
Et_3N (36.8 µL, 2.1 equiv) is added to a solution of **Compound 19** (99.8 mg, 126 µmol) and Compound 96 (50.9 mg, 1.05 equiv) in anhydrous CH_2Cl_2 (1.5 mL). After stirring at ambient temperature under argon for 25 min, the reaction mixture is loaded directly onto a CombiFlash system for purification (Silica gel column. Eluting with 0 - 10% CH_3OH in CH_2Cl_2.) to yield 95.1 mg **Compound 97** (mixture of two stereoisomers) as a light brown solid: (ESI-QMS): m/z = 1006.80 (M + H).

Step 3: Preparation of Compound 98

TFA (0.60 mL) is added to a solution of **Compound 67** (48.5 mg, 1.2 equiv) in anhydrous CH_2Cl_2 (1.5 mL). The reaction mixture is stirred at ambient temperature under

argon for 30 min and concentrated under reduced pressure. The residue is co-evaporated with CH_2Cl_2 (2 mL×3), concentrated under reduced pressure, dried under vacuum for 1 h, redissolved in anhydrous CH_2Cl_2 (1.5 mL), and transferred into a solution of **Compound 97** (159 mg, 158 μmol) and PyBOP (86.3 mg, 1.05 equiv) in anhydrous CH_2Cl_2 (3.5 mL). To the solution is added iPr$_2$NEt (150 μL, 5.5 equiv). The reaction mixture is stirred at ambient temperature under argon for 1 h and loaded directly onto a CombiFlash system for purification (Silica gel column. Eluting with 0 - 10% CH_3OH in CH_2Cl_2.) to give 125 mg **Compound 98** (a mixture of stereoisomers) as a light brown solid: (ESI-QMS): m/z = 1128.94 (M + H).

Step 4: Preparation of Compound 99



Compound 99 was synthesized by solid phase in five steps starting from H-Cys(4-methoxytrityl)-2-chlorotrityl-Resin.

	mmol	equiv	MW	amount
H-Cys(4-methoxytrityl)-2-chlorotrityl-Resin (loading 0.63mmol/g)	0.5			794mg
Fmoc-NH-PEG4-COOH (Dissolve in 15ml DMF)	0.5	2	487.6	487.6
Fmoc-Asp(OtBu)-OH (Dissolve in 15ml DMF)	0.5	2	411.5	411.5
Fmoc-Asp(OtBu)-OH (Dissolve in 15ml DMF)	0.5	2	411.5	411.5
Fmoc-Arg(Pbf)-OH (Dissolve in 15ml DMF)	0.5	2	648	648
Fmoc-Asp(OtBu)-OH (Dissolve in 15ml DMF)	0.5	2	411.5	411.5
Fmoc-Glu-OtBu (Dissolve in 15ml DMF)	0.5	2	425.5	425.5
N^{10}TFA Pteroic Acid (dissolve in 15ml DMF)	0.5	1.25	408	255
iPr$_2$NEt	0.5	4	129	258mg
PyBOP	0.5	2	520	520mg

Coupling steps:

In a peptide synthesis vessel was added the resin, amino acid solution, iPr_2NEt, and PyBOP. Argon was bubbled through the solution for 1 h and then washed 3X with DMF and IPA. A solution of 20% piperdine in DMF for FMOC deprotection was added, 2X (10min), before each amino acid coupling. This was continued to complete all seven coupling steps.

Cleavage step:

25 mL of cleavage reagent (92.5% TFA, 2.5% H_2O, 2.5% Triisopropylsaline, 2.5% (1.34ml) ethandithiol) was added to the peptide synthesis vessel and Argon was bubbled for 1.5 h, drain, and wash 3X with cleavage reagent reagent. The reaction mixture was concentrated under reduced pressure until 10ml remained. The product was triturated in ethyl ether and centrifuge. The resulting pellet was dried under high vacuum.

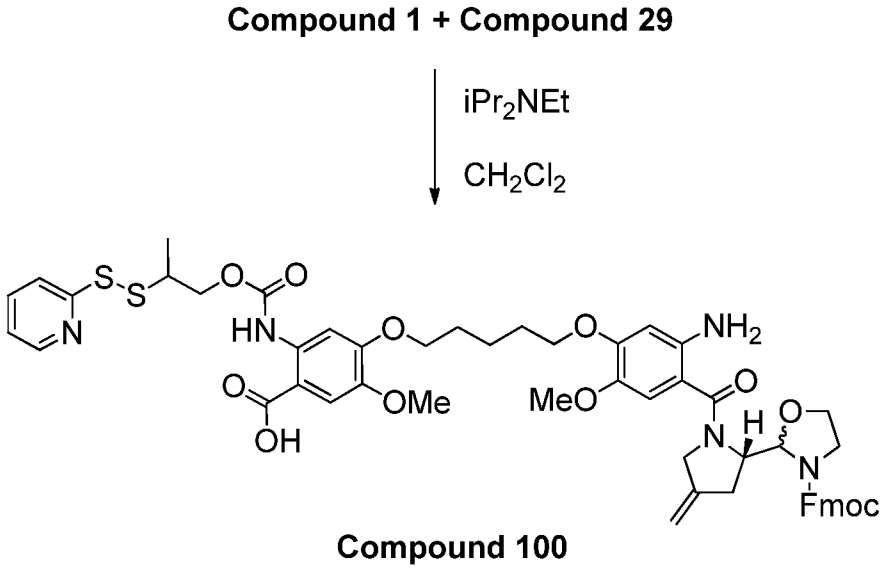
Deprotection step:

Crude protected **Compound 99** was added to 10ml water. The pH adjusted to 9.3 and maintained for 1 h using potassium carbonate. After 1 h the solution was adjusted to pH 5 with 1N HCl. The reaction mixture was load directly onto a C18 reverse phase column and purified via with 0 – 35% CH_3CN/50 mM NH_4HCO_3 buffer, pH 7.0 to yield 413 mg **Compound 99** as a fluffy yellow solid.

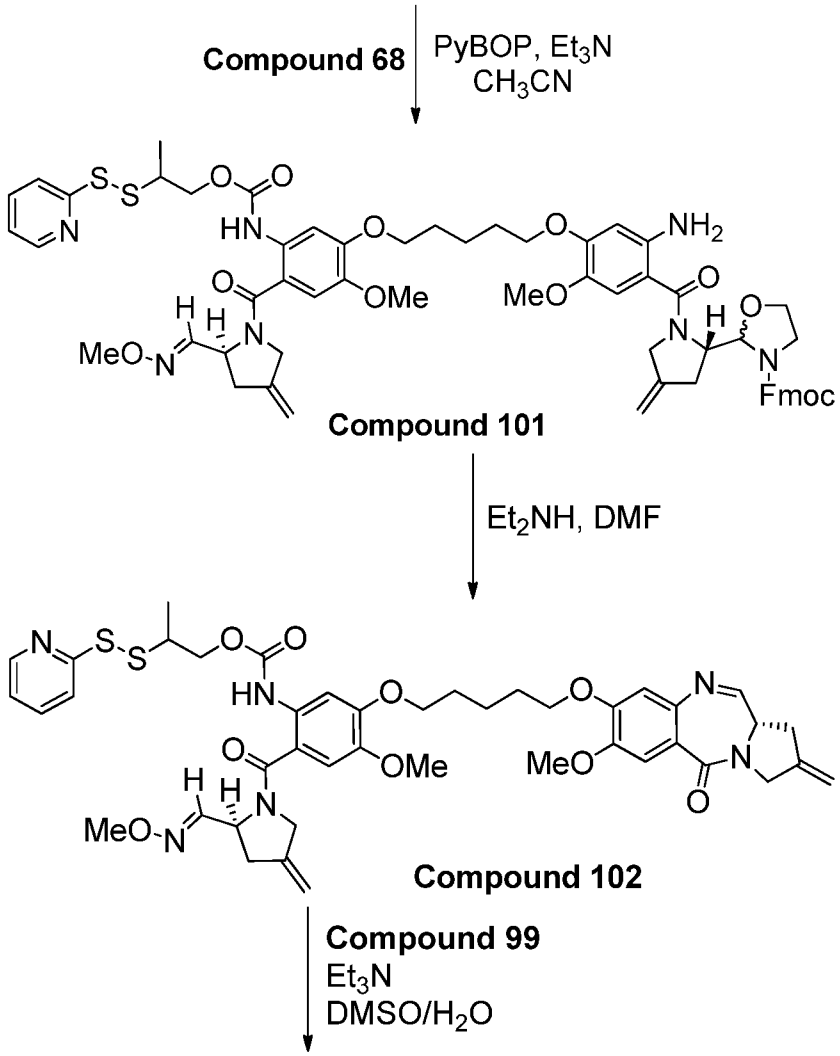
Step 5: Preparation of Conjugate 26

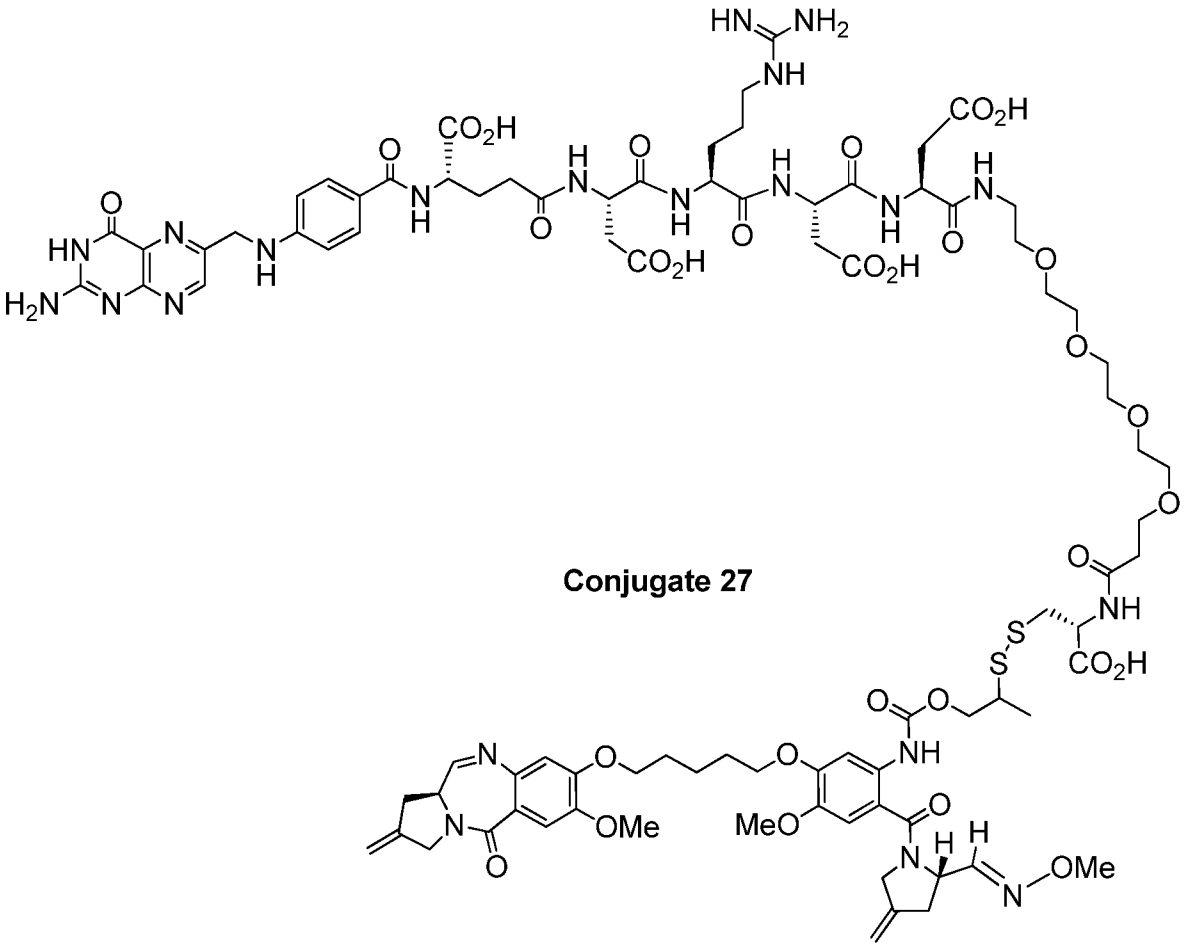
Conjugate 26 was synthesized by following the procedure for **Conjugate 16** using **Compound 98** and **Compound 99**. **Conjugate 26** was isolated as a mixture of two stereoisomers: (ESI-QMS): m/z = 1020.19 $(M - 2H)^{2-}$.

Example 61: Preparation of Conjugate 27



Compound 100


Compound 68
PyBOP, Et3N
CH3CN
Compound 101
Et2NH, DMF
Compound 102
Compound 99
Et3N
DMSO/H2O



Conjugate 27

Step 1: Preparation of Compound 100

iPr_2NEt (24 mL, 0.139 mmol) and **Compound 1** (42 mg, 0.116 mmol) were added to a stirring solution of **Compound 29** (92 mg, 0.116 mmol) in CH_2Cl_2 (1.16 mL). The reaction mixture was stirred for 2h at room temperature. The progress of the reaction was monitored via LC/MS. The mixture was then concentrated and loaded directly to a silica gel column, and purified with 0 – 10% CH_3OH in CH_2Cl_2. 87 mg (85.0 %) of desired product was collected as a white solid: LC/MS (ESI-QMS): *m/z* = 1020.19 (M + H).

Step 2: Preparation os Compound 101

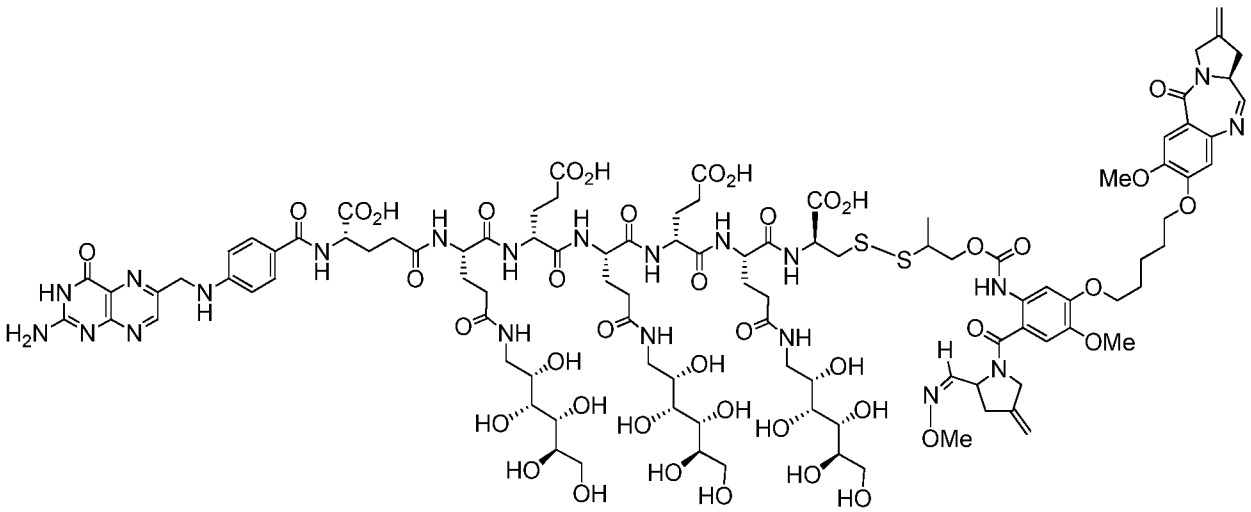
A solution of **Compound 67** (26 mg, 0.109 mmol) in anhydrous 50% TFA in CH_2Cl_2 (1.0 mL) was stirred at room temperature under argon for 30 min, after which the reaction mixture was concentrated under reduced pressure. The resulting residue was co-evaporated with CH_2Cl_2 (2 mL × 3), and dried under high vacuum for 1 h to provide crude **Compound 68**. The residue was dissolved is CH_3CN (1 mL), and Et_3N (27 mL, 0.197 mmol) and **Compound 100** (100 mg, 0.0986 mmol) were added. The reaction was allowed to stir for 5 min before PyBOP (56 mg, 0.109 mmol) was added. After stirring for 30 min the reaction mixture was concentrated under reduced pressure, and the crude residue was purified via silica

chromatography (0 – 10% CH_3OH in CH_2Cl_2). 82.9 mg (72.2%) of desired product was collected as a white solid: LC/MS (ESI-QMS): *m/z* = 1141.35 (M + H).

Step 3: Preparation of Conjugate 27

Compound 101 (8.5 mg, 9.6 μmol was dissolved in 5% Et2NH in DMF (1 mL). The reaction mixture was stirred for 3 h. The reaction was monitored via LC/MS, and after the complete conversion of **Compound 101** to **Compound 102**, a solution of **Compound 99** (15.0 mg, 14.4 μmol) dissolved in DMSO (400 μl) and H_2O (100 μl) was added followed by Et_3N (2.6 μl, 19.2 μmol). The reaction mixture was stirred for an additional 1 h at room temperature. The reaction mixture was then filtered through a 0.45 micron PTFE membrane. Purification via preparative HPLC (10 – 100% MeCN/50 mM NH_4HCO_3 pH 7 buffer) yielded 5.6 mg (32.5 % over two steps) of **Conjugate 27** as a yellow powder: LC/MS (ESI-QMS): *m/z* = 1020.98 $(M + 2H)^{2+}$.

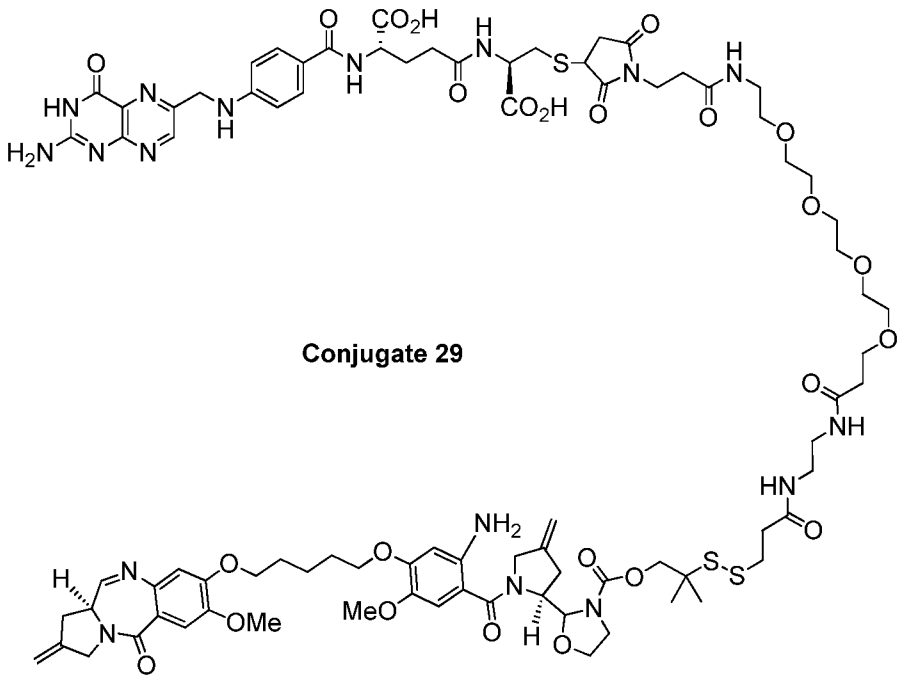
Example 62: Preparation of Conjugate 28



Conjugate 28

Compound 101 (8.5 mg, 9.6 μmol) was dissolved in 5% Et2NH in DMF (1 mL). The reaction mixture was stirred for 3 h. The reaction was monitored via LC/MS, and after the complete conversion of **Compound 101** to **Compound 102**, a solution of **Compound 38** (24.6 mg, 14.4 μmol) dissolved in DMSO (400 μL) and H_2O (100 μL) was added followed by Et_3N (2.6 ml, 19.2 μmol). The reaction mixture was stirred for an additional 1 h at room temperature. The reaction mixture was then filtered through a 0.45 micron PTFE membrane. Purification via preperative HPLC (10 – 100% MeCN/50 mM NH_4HCO_3 pH 7 buffer) provided 6.3 mg (27.0% over two steps) of desired product as a yellow powder LC/MS (ESI-QMS): *m/z* = 1214.43 (M + H).

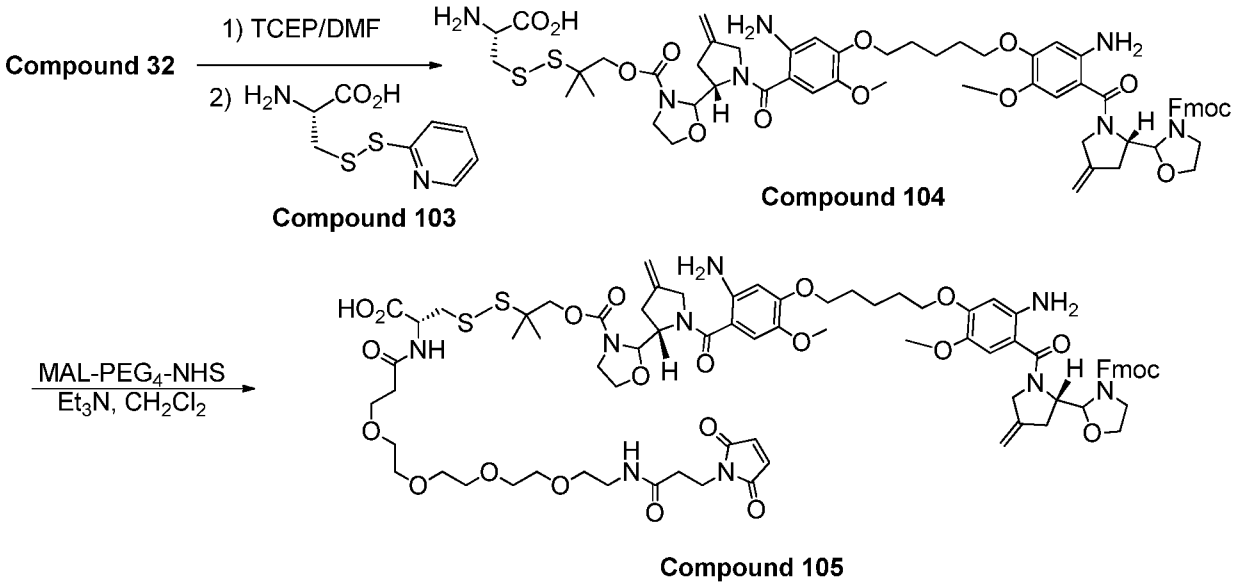
Example 63: Preparation of Conjugate 29



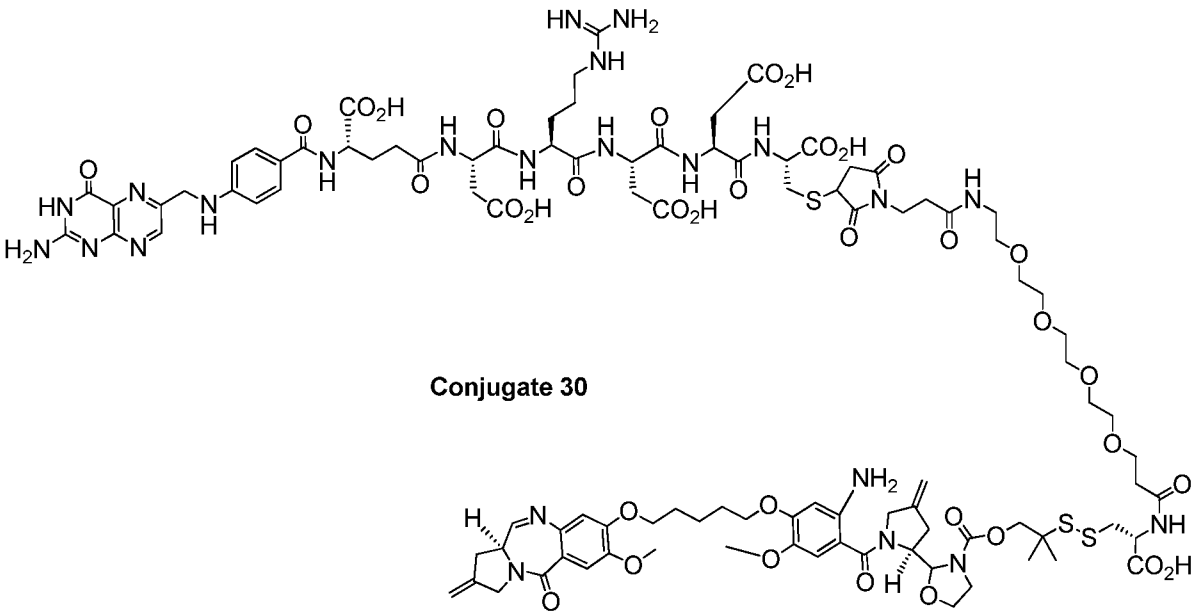
Conjugate 29

Conjugate 29 was synthesized by following the procedure for **Conjugate 5** starting from N^{10}-trifluoroacetyl protected folate-containing peptidyl fragment N^{10}-TFA-Pte-Glu-Cys-OH as described in USPN 7601332, incorporated herein by reference for the preparation of that compound, in lieu of **EC119.** LC/MS (ESI-QMS): $(M+2H)^{2+}$: 1084, $(M+3H)^{3+}$: 723.

Example 64: Preparation of Conjugate 30



1. **Compound 16**
DMSO, PBS Buffer
2. Et_2NH, DMSO



Conjugate 30

Step 1: Preparation of Compound 104

Compound 32 (49.1 mg, 0.034 mmol) was dissolved in DMF (1.2 mL) and treated with 0.5M TECP (74.8 μL, 0.0374 mmol). The reaction was stirred for 20 min at room temperature. **Compound 103** (9.5 mg, 0.040 mmol), prepared according to the procedure described for **Compound 1** except that cysteine was used in place of 2-mercaptopropanol, was added to the reaction mixture and stirred for an additional 1 h. The crude mixture was loaded directly on to a C18 reverse column and purified with 0 – 50% CH_3CN in H_2O) to yield 9 mg of the desired product **Compound 104** (22% yield over two steps): LC/MS (ESI-QMS): *m/z* = 1180 $(M + H)^{1+}$.

Step 2: Preparation of Compound 105

Compound 104 (4.2 mg, 0.0036 mmol) was added to a solution of Maleimide-PEG-NHS Ester (2.01 mg, 0.0039 mmol, available from Sigma-Aldrich) and Et_3N (0.54 μL, 0.0039 mmol) in CH_2Cl_2 (0.5 mL). The reaction mixture was stirred for 30 min at room temperature and then concentrated to dryness.

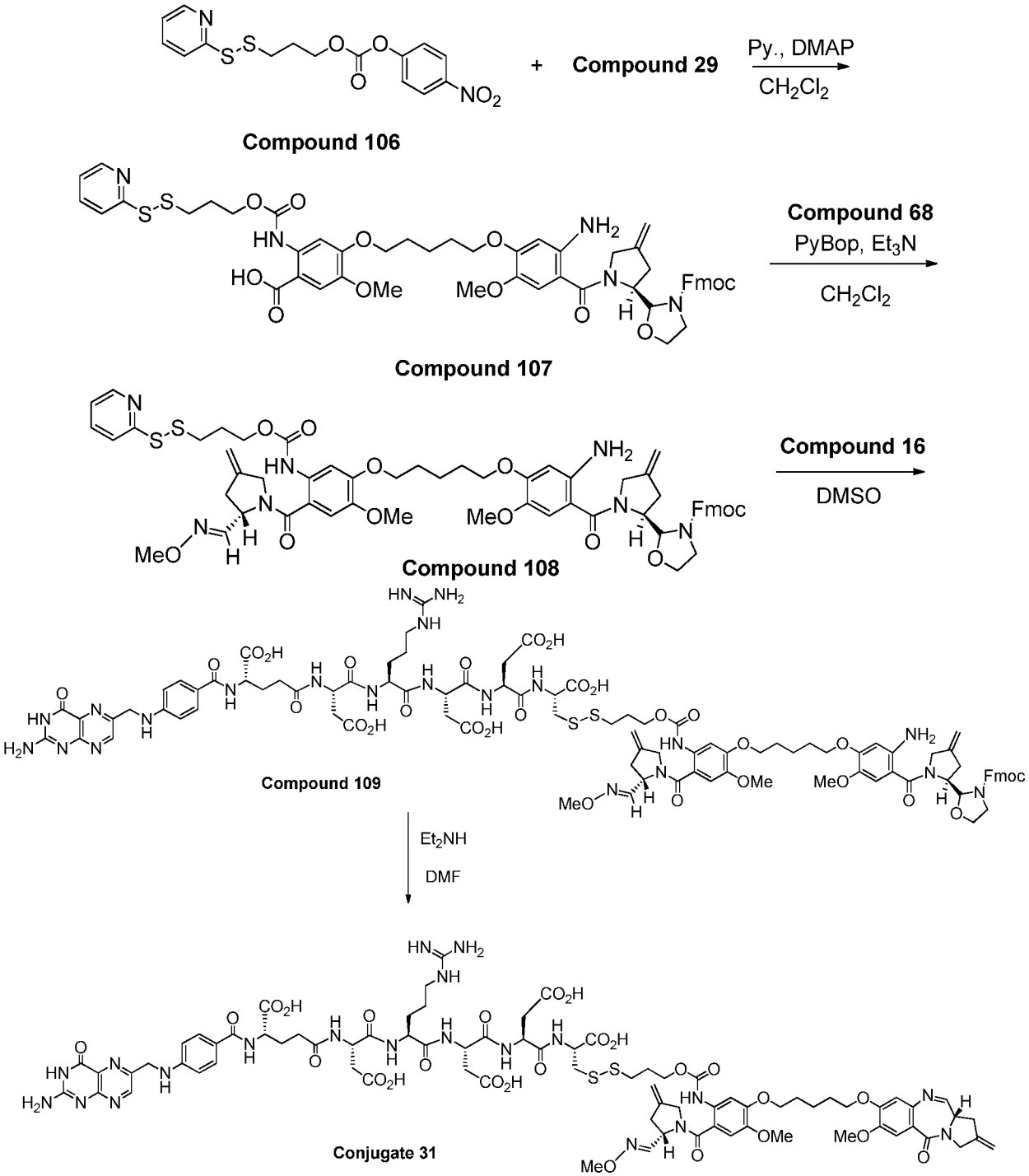
Step 3: Preparation of Conjugate 30

The crude residue of **Compound 105** was carried forward without further purification. **Compound 105** residue was dissolved in DMSO (0.3 mL) and to it was added a solution of EC119 (4.1 mg, 0.00396 mmol) in pH 7.4 PBS buffer (0.5 mL and DMSO (0.5 mL). Et_3N (3.0 μL, 0.0216 mmol) was added to the reaction mixture and stirred for 30 min at room temperature. The crude product was purified by prep-HPLC (10 to 100% acetonitrile in 50 mM NH_4HCO_3, pH 7.4) to yield the desired product: LC/MS (ESI-QMS): *m/z* = 1313 $(M + 2H)^{2+}$.

The product of the preceding step (5.0 mg, 0.0019 mmol) was dissolved in DMSO (0.5 mL), and Et_2NH (0.25 mL) was added. The reaction mixture was stirred for 30 min at room

temperature. The crude product was purified by prep-HPLC (10 to 100% acetonitrile in 50 mM NH_4HCO_3, pH 7.4) to yield 3.44 mg of the desired product **Conjugate 30** (77% yield): LC/MS (ESI-QMS): m/z = 1180 $(M + 2H + H_2O)^{2+}$.

Example 65: Preparation of Conjugate 31



Step 1: Preparation of Compound 106

Compound 106 was prepared according to the procedure described for **Compound 59**, except 3-mercaptopropanol was used in place of 2-mercapto-3-methylbutan-1-ol, and para-nitrophenol was used in place of hydroxybenzotriazole.

Step 2: Preparation of Compound 107

A mixture of **Compound 106** (11.0 mg, 0.03 mmol), **Compound 29** (20.0 mg, 0.025 mmol), pyridine (6.1 µl, 0.075mmol) and DMAP (0.3 mg, 0.003 mmol) in CH_2Cl_2 was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo*. The crude product was purified by Combiflash in 0 - 20% CH_3OH/CH_2Cl_2 to afford 8.1 mg of **Compound 107**: (ESI-QMS): *m/z* = 1020.85 (M + H).

Step 3: Preparation of Compound 108

To a mixture of **Compound 107** (26.5 mg, 0.026 mmol), **Compound 68** (3.64 mg, 0.026 mmol) and PyBOP (16.2 mg, 0.031 mmol) in CH_2Cl_2 (1 ml) was added Et_3N (18 µl, 0.13 mmol) at room temperature. The reaction mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure. The crude product was purified by Combiflash in 0 - 20% CH_3OH/CH_2Cl_2 to afford 11.5 mg of **Compound 108**: (ESI-QMS): *m/z* = 1142.98 (M + H).

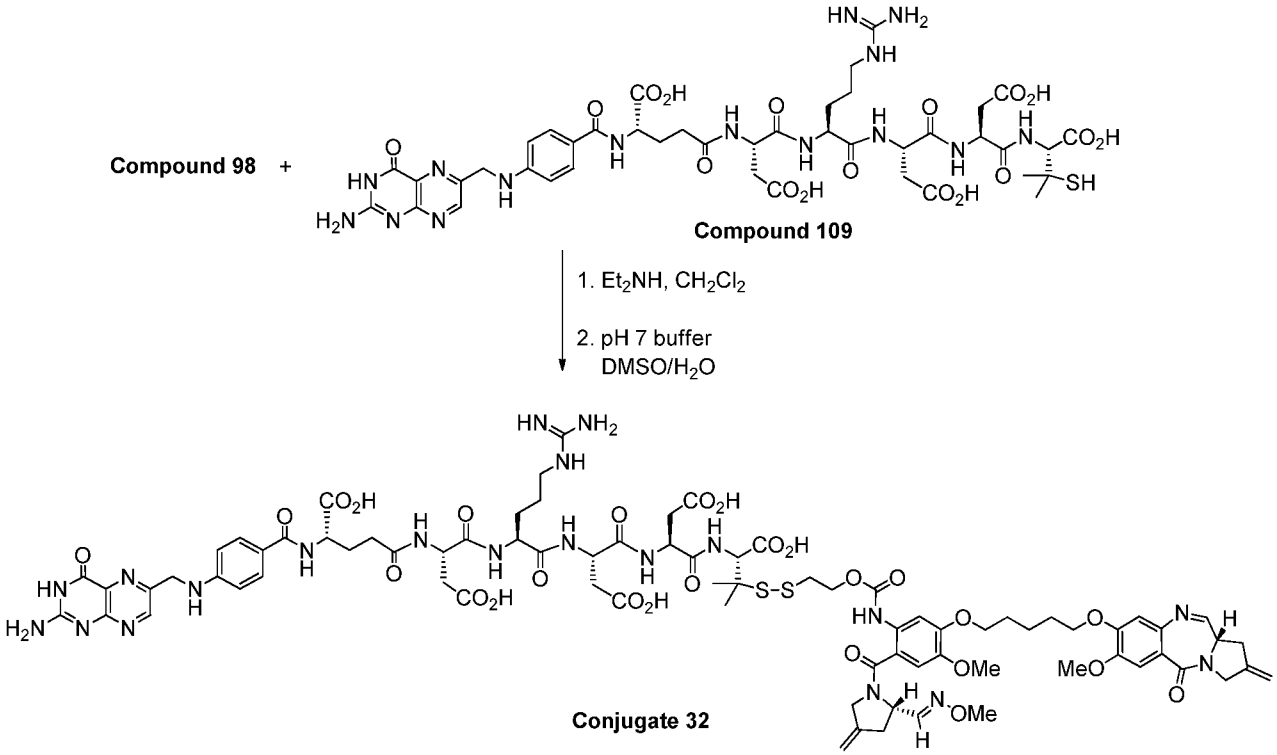
Step 4: Preparation of Compound 109

A mixture of **Compound 108** (11.5 mg, 0.01mmol) and **Compound 16** (10.5 mg, 0.01 mmol) in DMSO (1 ml) was stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo*. The crude product was purified by prep-HPLC HPLC (10 to 100% acetonitrile in 20 mM NH_4HCO_3, pH 7.4) to yield pure **Compound 109**: (ESI-QMS): *m/z* = 1041.28 $(M + 2H)^{2+}$.

Step 5: Preparation of Conjugate 31

To a mixture of **Compound 109** (8 mg, 0.004 mmol) in DMF (1 ml) was added Et_2NH (6 µl, 0.058 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 h. The crude product was purified by prep-HPLC HPLC (10 to 100% acetonitrile in 20 mM NH_4HCO_3, pH 7.4) to yield 4.5 mg of pure **Conjugate 31**: (ESI-QMS): *m/z* = 1794.99 $(M + 2H)^{2+}$.

Example 66: Preparation of Conjugate 32



Compound 109 was prepared according the procedure described for **Compound 99**, except that the coupling step using Fmoc-NH-PEG4-COOH was omitted. **Conjugate 32** was isolated as a mixture of stereoisomers: (ESI-QMS): *m/z* = 1809.35 (M + H).

BIOLOGICAL EXAMPLES

General.

The following abbreviations are used herein: partial response (PR); complete response (CR), once weekly (SIW), biweekly (M/F) (BIW), three times per week (M/W/F) (TIW). A PR is observed where tumor volume, as defined herein, decreases from a previous high during the observation period, though regrowth may occur. A CR is observed where tumor volume, as defined herein, decreases to zero during the observation period, though regrowth may occur. A cure is observed where tumor volume, as defined herein, decreases to zero, and does not regrow during the observation period.

METHOD 1. Inhibition of Cellular DNA Synthesis.

The conjugates described herein were evaluated using an *in vitro* cytotoxicity assay that predicted the ability of the drug to inhibit the growth of the corresponding targeted cells, such as, but not limited to the following

Cell Line	
KB	Human cervical carcinoma
NCl/ADR-RES-Cl_2	Human ovarian carcinoma
IGROV1	Human ovarian adenocarcinoma
MDA-MB-231	Human breast adenocarcinoma (triple negative)
A549	Human lung carcinoma
H23	Human lung adenocarcinoma
HepG2	Human hepatocellular carcinoma
AN3CA	Human endometrial adenocarcinoma

It is to be understood that the choice of cell type can be made on the basis of the susceptibility of those selected cells to the drug that forms the conjugate, and the relative expression of the cell surface receptor or target antigen. The test conjugates were conjugates of a cell surface receptor or target antigen binding compound and PBD prodrugs, poly-PBD prodrugs, and mixed PBDs, as described herein. The test cells were exposed to varying concentrations of the conjugates, and optionally also in the absence or presence of at least a 100-fold excess of the unconjugated cell surface receptor or target antigen binding compound for competition studies to assess activity as being specific to the cell surface receptor or target antigen.

METHOD 2: *In Vitro* Folate Receptor Specific Activity Assay of Folate conjugates.

KB cells were seeded in individual 24-well Falcon plates and allowed to form nearly confluent monolayers overnight in folate free Roswell Park Memorial Institute (FFRPMI)/Heat-Inactivated Fetal Calf Serum (HIFCS). Thirty minutes prior to the addition of folate-conjugate, spent medium was aspirated from all wells and replaced with either fresh FFRPMI or FFRPMI supplemented with 100 μM folic acid. Each well then received 1 mL of medium containing

increasing concentrations of folate-conjugate (3 wells per sample). Cells were pulsed for 2 h at 37°C, rinsed 4 times with 0.5 mL of medium and then chased in 1 mL of fresh medium up to 72 h. Spent medium was aspirated from all wells and replaced with fresh medium containing 5 μCi/mL of ^{3}H-thymidine. Following a 2 h incubation at 37°C, cells were washed 3 times with 0.5 mL of PBS and then treated with 0.5 mL of ice-cold 5% trichloroacetic acid per well. After 15 min, the trichloroacetic acid was aspirated and the cells solubilized by the addition of 0.5 mL of 0.25 N sodium hydroxide for 15 min at room temperature. Four hundred and fifty μL of each solubilized sample were transferred to scintillation vials containing 3 mL of Ecolume scintillation cocktail and counted in a liquid scintillation counter. Final results were expressed as the percentage of ^{3}H-thymidine incorporation relative to untreated controls. For conjugates described herein, dose-dependent cytotoxicity was generally measurable, and in most cases, the IC_{50} values (concentration of drug conjugate required to reduce ^{3}H-thymidine incorporation into newly synthesized DNA by 50%) were in the picomolar to low nanomolar range.

EXAMPLE 1: Conjugate 9 *in vitro* activity.

In FIG. 1, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 9** (●) and with **Conjugate 9** and excess folate (■) is shown. The IC_{50} value was 0.8 nM without excess folate and 67 nM with excess folate.

EXAMPLE 2: Conjugate 1 *in vitro* activity.

In FIG. 3, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 1** (●) and with **Conjugate 1** and excess folate (■) is shown. The IC_{50} value was 0.02 nM without excess folate and 10 nM with excess folate.

EXAMPLE 3: Conjugate 2 *in vitro* activity.

In FIG. 5, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 2** (●) and with **Conjugate 2** and excess folate (■) is shown. The IC_{50} value was 0.14 nM without excess folate and 16 nM with excess folate.

EXAMPLE 4: Conjugate 5 *in vitro* activity.

In FIG. 7, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 5** (●) and with **Conjugate 5** and excess folate (■) is shown.

EXAMPLE 5: Conjugate 3 *in vitro* activity.

In FIG. 9, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 3** (●) and with **Conjugate 3** and excess folate (■) is shown. The IC_{50} value was 39 pM without excess folate and 3 nM with excess folate.

EXAMPLE 6: Conjugate 12 *in vitro* activity.

In FIG. 11, the percentage of ^{3}H-thymidine incorporated into KB cells treated with

Conjugate 12 (▲) and with **Conjugate 12** and excess folate (●) is shown. The IC_{50} value was 0.05 nM without excess folate and 8 nM with excess folate.

EXAMPLE 7: Conjugate 4 *in vitro* activity.

In FIG. 12, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 4** (●) and with **Conjugate 4** and excess folate (■) is shown. The IC_{50} value was 49 pM without excess folate and 6 nM with excess folate.

EXAMPLE 9: Conjugate 16 *in vitro* activity.

In FIG. 14, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 16** (●) and with **Conjugate 16** and excess folate (■) is shown. The IC_{50} value was 70 pM without excess folate and 5 nM with excess folate.

EXAMPLE 10: Conjugate 6 *in vitro* activity.

In FIG. 16, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 6** (●) and with **Conjugate 6** and excess folate (■) is shown. The IC_{50} value was 48 pM without excess folate and 3 nM with excess folate.

EXAMPLE 11: Conjugate 15 *in vitro* activity.

In FIG. 18, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 15** (●) and with **Conjugate 15** and excess folate (■) is shown. The IC_{50} value was 81 pM without excess folate and 2 nM with excess folate.

EXAMPLE 12: Conjugate 7 *in vitro* activity.

In FIG. 20, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 7** (●) and with **Conjugate 7** and excess folate (■) is shown. The IC_{50} value was 0.13 nM without excess folate and 5 nM with excess folate.

EXAMPLE 13: Conjugate 8 *in vitro* activity.

In FIG. 22, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 8** (●) and with **Conjugate 8** and excess folate (■) is shown. The IC_{50} value was 55 pM without excess folate and 0.3 nM with excess folate.

EXAMPLE 14: Conjugate 18 *in vitro* activity.

In FIG. 24, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 18** (●) and with **Conjugate 18** and excess folate (■) is shown. The IC_{50} value was 65 pM without excess folate and 2 nM with excess folate.

EXAMPLE 15: Conjugate 19 *in vitro* activity.

In FIG. 25, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 19** (●) and with **Conjugate 19** and excess folate (■) is shown. The IC_{50} value was

77 pM without excess folate and 3.8 nM with excess folate.

EXAMPLE 16: Conjugate 20 *in vitro* activity.

In FIG. 26, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 20** (●) and with **Conjugate 20** and excess folate (■) is shown. The IC_{50} value was 40 pM without excess folate and 0.7 nM with excess folate.

EXAMPLE 17: Conjugate 22 *in vitro* activity.

In FIG. 40, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 20** (●) and with **Conjugate 22** and excess folate (■) is shown. The IC_{50} value was .14 nM without excess folate and 1.4 nM with excess folate.

EXAMPLE 18: Conjugate 24 *in vitro* activity.

In FIG. 41, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 24** (●) and with **Conjugate 24** and excess folate (■) is shown. The IC_{50} value was 79 pM without excess folate and 1.8 nM with excess folate.

EXAMPLE 19: Conjugate 25 *in vitro* activity.

In FIG. 42, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 25** (●) and with **Conjugate 25** and excess folate (■) is shown. The IC_{50} value was 85 pM without excess folate and 20 nM with excess folate.

EXAMPLE 20: Conjugate 26 *in vitro* activity.

In FIG. 43, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 26** (●) and with **Conjugate 26** and excess folate (■) is shown. The IC_{50} value was 28 pM without excess folate and 1.6 nM with excess folate.

EXAMPLE 21: Conjugate 27 *in vitro* activity.

In FIG. 44, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 27** (●) and with **Conjugate 27** and excess folate (■) is shown. The IC_{50} value was 91 pM without excess folate and 6.1 nM with excess folate.

EXAMPLE 22: Conjugate 28 *in vitro* activity.

In FIG. 45, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 28** (●) and with **Conjugate 28** and excess folate (■) is shown. The IC_{50} value was 56 pM without excess folate and 3.4 nM with excess folate.

EXAMPLE 23: Conjugate 31 *in vitro* activity.

In FIG. 46, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 31** (●) and with **Conjugate 31** and excess folate (■) is shown. The IC_{50} value was 647 pM without excess folate.

EXAMPLE 24: Conjugate 32 *in vitro* activity.

In FIG. 47, the percentage of ^{3}H-thymidine incorporated into KB cells treated with **Conjugate 32** (●) and with **Conjugate 32** and excess folate (■) is shown. The IC_{50} value was 2 nM without excess folate and 57 nM with excess folate.

EXAMPLE 25: Relative affinity assay

FR-positive KB cells were seeded in 24-well Falcon plates and allowed to form adherent monolayers (>90% confluent) overnight in FFRPMI/HIFCS. Spent incubation medium was replaced with FFRPMI supplemented with 10% HIFCS and containing 100 nmol/L of [^{3}H]FA in the absence and presence of increasing concentrations of unlabeled FA or the test conjugate. Cells were incubated for 1 h at 37°C and then rinsed thrice with 0.5 mL PBS. Five hundred microliters of 1% SDS in PBS were added to each well; after 5 min, cell lysates were collected, transferred to individual vials containing 5 mL of scintillation cocktail, and then counted for radioactivity.

Cells exposed to only the [^{3}H]FA in FFRPMI (no competitor) were designated as negative controls, whereas cells exposed to the [^{3}H]FA plus 1 mmol/L unlabeled FA served as positive controls. Disintegrations per minute (DPM) measured in the latter samples (representing nonspecific binding of label) were subtracted from the DPM values from all samples. Notably, relative affinities were defined as the inverse molar ratio of compound required to displace 50% of [^{3}H]FA bound to FR on KB cells, and the relative affinity of FA for the FR was set to 1.

Results for **Conjugate 1** are shown in FIG. 28. The resuls show that linkage of a large drug molecule does not radically alter the vitamin's intrinsic binding affinity to its receptor.

Results for **Conjugate 5** are shown in FIG. 35. The resuls show that linkage of a large drug molecule does not radically alter the vitamin's intrinsic binding affinity to its receptor.

EXAMPLE 26: DNA crosslinking assay of **Conjugate 1** or **Conjugate 5**

Conjugate 1:

Calf thymus DNA (CT-DNA) was combined with increasing concentrations of **Conjugate 1** (0.14 to 33.3 μM) or **Conjugate 1** +/- DTT. CT-DNA + Melphalan was used as a positive control and CT-DNA + DMSO was used as a negative control. These solutions were incubated at 37 °C for 2 hours. The solutions were then mixed with ethidium bromide and incubated for 2 hours at room temperature. Fluorescence (Ex: 535 nm, Em: 605 nm) from these samples was measured on the Fluoroskan II fluorimeter. Next, the samples were heated to 104

°C for 5 minutes, cooled on ice for 5 minutes, kept at RT for 15 minutes and fluorescence measured. % crosslinking of each sample was calculated using the fluorescence values from the positive and negative controls. Results are shown in FIG. 29.

Conjugate 5:

Calf thymus DNA (CT-DNA) was combined with increasing concentrations of **Conjugate 5** (1.1 to 75 μM) or **Conjugate 5** +/- DTT. These solutions were incubated at 37 °C for 2 hours. The solutions were then mixed with ethidium bromide and incubated for 2 hours at room temperature. Fluorescence (Ex: 535 nm, Em: 605 nm) from these samples was measured on the Fluoroskan II fluorimeter. Next, the samples were heated to 104 °C for 5 minutes, cooled on ice for 5 minutes, kept at RT for 15 minutes and fluorescence measured. % crosslinking of each sample was calculated using the fluorescence values from the positive and negative controls. Results are shown in FIG. 36.

Example 27: *In Vitro* analysis of **Conjugate 1** in MDA-MB231 cells.

MDA-MB231 (human breast cancer) cells were seeded in 12-well Falcon plates and allowed to form nearly confluent monolayers overnight in FFRPMI/HIFCS. Designated wells received medium containing 100 μM folic acid (nontoxic FR blocker) and were used to determine the targeting specificity. Each well then received increasing concentrations of **Conjugate 1** (n=4). Cells were pulsed for 2 h at 37°C, rinsed with medium, and then chased in fresh medium up to 72 h. Spent medium was aspirated and replaced with medium containing [^{3}H]thymidine. Following a 2 h incubation, cells were washed with PBS and then treated with 5% trichloroacetic acid. The trichloroacetic acid was aspirated and cells were solubilized in 0.25 N sodium hydroxide. Each solubilized sample were transferred to scintillation vials containing Ecolume scintillation cocktail and counted in a liquid scintillation counter. Final results were expressed as the percentage of [^{3}H]thymidine incorporation relative to untreated controls and IC_{50} were values calculated using GraphPad Prism software. The cell killing activity of **Conjugate 1** was found to be concentration dependent with an IC_{50} of 0.28 nM on MDA-MB-231 cells. The significant reduction in activity of **Conjugate 1** in the presence of an excess of free folate indicates that the observed cytotoxic activity was folate receptor mediated. Results are shown in FIG. 30.

METHOD 3: Antitumor activity in large KB tumor model.

Female Balb/c *nu/nu* mice were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. KB tumor cells were inoculated subcutaneously at the right flank of each mouse. Mice were dosed after the tumors reached an

average of 100 and 180 mm^3 through the lateral tail vein under sterile conditions in a volume of 200 mL of phosphate-buffered saline (PBS).

Growth of each s.c. tumor was followed by measuring the tumor two times per week. Tumors were measured in two perpendicular directions using Vernier calipers, and their volumes were calculated as 0.5 x L x W^2, where L = measurement of longest axis in mm and W = measurement of axis perpendicular to L in mm.

METHOD 4: Toxicity as Measured by Weight Loss.

The percentage weight change of the test animals was determined on selected days post-tumor inoculation (PTI), and during dosing. The results were graphed.

EXAMPLE 28: Conjugate 9 *in vivo* activity against tumors.

As shown in FIG. 2A, **Conjugate 9** (■) dosed at 1 µmol/kg SIW for two weeks decreased KB tumor size in test mice compared to untreated control (●). Treatment with 1 µmol/kg of **Conjugate 9**, once a week for two weeks produced minimal anti-tumor activity with 0% PRs. Change in weight is shown in FIG. 2B for mice dosed with **Conjugate 9** SIW for two weeks (■) compared to untreated control (●).

EXAMPLE 29: Conjugate 1 *in vivo* activity against tumors.

As shown in FIG. 4A, **Conjugate 1** dosed at 0.5 µmol/kg SIW for two weeks (●) decreased KB tumor size in test mice compared to untreated control (▲). Treatment with 0.5 µmol/kg of **Conjugate 1**, once a week for two weeks produced maximal anti-tumor activity with 100% cures. Change in weight is shown in FIG. 4B for mice dosed with **Conjugate 1** SIW for two weeks (●) compared to untreated control (▲).

EXAMPLE 30: Conjugate 2 *in vivo* activity against tumors.

As shown in FIG. 6A, **Conjugate 2** dosed at 0.5 µmol/kg SIW for two weeks (■) decreased KB tumor size in test mice compared to untreated control (●). **Conjugate 2** was highly active with 100% cures. Change in weight is shown in FIG. 6B for test mice dosed at 0.5 µmol/kg **Conjugate 2** SIW for two weeks (■) compared to untreated control (●).

EXAMPLE 31: Conjugate 5 *in vivo* activity against tumors.

As shown in FIG. 8A, **Conjugate 5** dosed at 0.5 µmol/kg SIW for two weeks (▲) decreased KB tumor size in test mice compared to untreated control (■). Treatment with 0.5

μmol/kg of **Conjugate 5**, once a week for two weeks also produced maximal anti-tumor activity with 100% cures. Change in weight is shown in FIG. 8B for test mice dosed at 0.5 μmol/kg **Conjugate 5** SIW for two weeks (▲) compared to untreated control (■).

EXAMPLE 32: Conjugate 3 *in vivo* activity against tumors.

As shown in FIG. 10A, **Conjugate 3** dosed at 0.5 μmol/kg SIW for two weeks (▼) decreased KB tumor size in test mice compared to untreated control (●). Treatment with 0.5 μmol/kg of **Conjugate 3**, once a week for two weeks produced 100% complete responses but mice had to be euthanized on day 48 due to toxicity. Change in weight is shown in FIG. 10B for test mice dosed at 0.5 μmol/kg **Conjugate 3** SIW for two weeks (▼) compared to untreated control (●).

EXAMPLE 33: Conjugate 12 and **Conjugate 4** *in vivo* activity against tumors.

As shown in FIG. 13A, each **Conjugate 12** dosed at 0.5 μmol/kg SIW for two weeks (▲) and **Conjugate 4** dosed at 0.5 μmol/kg SIW for two weeks (◆) decreased KB tumor size in test mice compared to untreated control (●). **Conjugate 4** was highly active with 100% cures at 0.5 μmol/kg, once a week for two weeks. At a similar dosing regimen, **Conjugate 12** produced 100% PR's, but mice had to be euthanized on day 40 due to toxicity. Change in weight is shown in FIG. 13B for test mice dosed at 0.5 μmol/kg **Conjugate 12** SIW for two weeks (▲) and test mice dosed at 0.5 μmol/kg **Conjugate 4** SIW for two weeks (◆) compared to untreated control (●).

EXAMPLE 34: Conjugate 16 *in vivo* activity against tumors.

As shown in FIG. 15A, **Conjugate 16** dosed at 0.5 μmol/kg SIW for two weeks (●) decreased KB tumor size in test mice compared to untreated control (▲). Treatment with 0.5 μmol/kg of **Conjugate 16**, once a week for two weeks produced 40% complete responses and 60% cures. Change in weight is shown in FIG. 15B for test mice dosed at 0.5 μmol/kg **Conjugate 16** SIW for two weeks (●) compared to untreated control (▲).

EXAMPLE 35: Conjugate 6 *in vivo* activity against tumors.

As shown in FIG. 17A, **Conjugate 6** dosed at 0.5 μmol/kg SIW for two weeks (▼) decreased KB tumor size in test mice compared to untreated control (●).Treatment with 0.5 μmol/kg of **Conjugate 6**, once a week for two weeks produced 50% complete responses and

50% cures. Change in weight is shown in FIG. 17B for test mice dosed at 0.5 μmol/kg **Conjugate 6** SIW for two weeks (▼) compared to untreated control (●).

EXAMPLE 36: Conjugate 15 *in vivo* activity against tumors.

As shown in FIG. 19A, **Conjugate 15** dosed at 0.5 μmol/kg SIW for two weeks (◆) decreased KB tumor size in test mice compared to untreated control (●). **Conjugate 15** was highly active with 100% cures at just one 0.5 μmol/kg dose. Change in weight is shown in FIG. 19B for test mice dosed at 0.5 μmol/kg **Conjugate 15** SIW for two weeks (◆) compared to untreated control (●).

EXAMPLE 37: Conjugate 7 *in vivo* activity against tumors.

As shown in FIG. 21A, **Conjugate 7** dosed at 0.5 μmol/kg SIW for two weeks (■) decreased KB tumor size in test mice compared to untreated control (●). **Conjugate 7** was highly active with 100% cures at 0.5 μmol/kg, once a week for two weeks. Change in weight is shown in FIG. 21B for test mice dosed at 0.5 μmol/kg **Conjugate 7** SIW for two weeks (■) compared to untreated control (●).

EXAMPLE 38: Conjugate 8 *in vivo* activity against tumors.

As shown in FIG. 23A, **Conjugate 8** dosed at 0.2 μmol/kg SIW for two weeks (■) decreased KB tumor size in test mice compared to untreated control (●). **Conjugate 8** was highly active with 100% cures at only 0.2 μmol/kg, once a week for two weeks. Change in weight is shown in FIG. 23B for test mice dosed at 0.2 μmol/kg **Conjugate 8** SIW for two weeks (■) compared to untreated control (●).

EXAMPLE 39: Conjugate 18, **Conjugate 19**, and **Conjugate 20** *in vivo* activity against tumors.

As shown in FIG. 27A, each of **Conjugate 18** dosed at 0.5 μmol/kg SIW for two weeks (■), **Conjugate 19** dosed at 0.5 μmol/kg SIW for two weeks (▲), and **Conjugate 20** dosed at 0.5 μmol/kg SIW for two weeks (▼) decreased KB tumor size in test mice compared to untreated control (●). Change in weight is shown in FIG. 27B for test mice dosed at 0.5 μmol/kg **Conjugate 18** SIW for two weeks (■), test mice dosed at 0.5 μmol/kg **Conjugate 19** SIW for two weeks (▲), and test mice dosed at 0.5 μmol/kg **Conjugate 20** SIW for two weeks

(▼) compared to untreated control (●).

EXAMPLE 40: Conjugate 5 *in vivo* activity against paclitaxel resistant tumors.

Mice were maintained and tumor volumes were measures according to Method 3.

KB-PR10 (paclitaxel resistant) tumor cells were inoculated subcutaneously at the right flank of each mouse. Mice were dosed through the lateral tail vein under sterile conditions in a volume of 200 µL of phosphate-buffered saline (PBS).

As shown in FIG. 31, **Conjugate 5** dosed at 0.5 µmol/kg SIW for two weeks (▲) decreased paclitacel resistant KB tumor size in test mice compared to untreated control (■).

EXAMPLE 41: Conjugate 5 *in vivo* activity against platinum resistant tumors.

Mice were maintained and tumor volumes were measures according to Method 3.

KB-CR2000 (platin resistant) tumor cells were inoculated subcutaneously at the right flank of each mouse. Mice were dosed through the lateral tail vein under sterile conditions in a volume of 200 µL of phosphate-buffered saline (PBS).

As shown in FIG. 32, **Conjugate 5** dosed at 0.5 µmol/kg SIW for two weeks (■) and EC1456 dosed at 2.0 µmol/kg BIW for two weeks (▼) decreased paclitacel resistant KB tumor size in test mice compared to untreated control (●).

EXAMPLE 42: Conjugate 5 *in vivo* activity against triple negative breast tumors.

Mice were maintained and tumor volumes were measures according to Method 3.

Primary human TNBC model ST502 (2-4 mm in diameter) or primary human TNBC model ST738 (2-4 mm in diameter) were inoculated subcutaneously at the right flank of each mouse. Mice were randomized into experimental groups of 7 mice each and test articles were injected through the lateral tail vein under sterile conditions in a volume of 200 µL of phosphate-buffered saline (PBS).

As shown in FIG. 33, **Conjugate 5** dosed at 0.3 µmol/kg BIW for two weeks (▲) decreased TNBC PDX tumor size in test mice compared to untreated control (■), whereas EC1456 dosed at 2.0 µmol/kg BIW for two weeks (●) did not decrease TNBC PDX tumor size.

As shown in FIG. 38, **Conjugate 5** dosed at 0.27 µmol/kg BIW for two weeks (■) decreased TNBC PDX tumor size in test mice compared to untreated control (■), whereas erubulin mesylate dosed at 1.0 µmol/kg SIW for two weeks (▲) did not decrease TNBC PDX

tumor size.

EXAMPLE 43: Conjugate 5 *in vivo* activity against ovarian tumors.

Mice were maintained and tumor volumes were measures according to Method 3.

Primary human Ovarian model ST070 fragments (2-4 mm in diameter) were inoculated subcutaneously at the right flank of each mouse. Mice were randomized into experimental groups of 7 mice each and test articles were injected through the lateral tail vein under sterile conditions in a volume of 200 µL of phosphate-buffered saline (PBS).

As shown in FIG. 34, **Conjugate 5** dosed at 0.5 µmol/kg SIW for two weeks (■)decreased ovarian PDX tumor size in test mice compared to untreated control (■), whereas EC1456 dosed at 4.0 µmol/kg SIW for two weeks (▲) and paclitaxel dosed at 15 mg/kg SIW for two weeks (▼) did not decrease ovarian PDX tumor size.

Example 44: Conjugate 5 *in vivo* activity in KB rat tumor model

Female Balb/c *nu/nu* rats were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. KB- tumor cells were inoculated subcutaneously at the right flank of each rat. Rats were dosed through the lateral tail vein under sterile conditions in a volume of 200 µL of phosphate-buffered saline (PBS).

Growth of each s.c. tumor was followed by measuring the tumor two times per week. Tumors were measured in two perpendicular directions using Vernier calipers, and their volumes were calculated as 0.5 x L x W^2, where L = measurement of longest axis in mm and W = measurement of axis perpendicular to L in mm. Results for tumor volume are shown in FIG. 37A. Toxicity was measured as a function of animal weight gain or loss as shown in FIG. 37B.

Example 45: Conjugate 5 *in vivo* activity against endopetrial tumors

Female Balb/c *nu/nu* mice were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. Primary human Endometrial model ST040 fragments (2-4 mm in diameter) were inoculated subcutaneously at the right flank of each mouse. Mice were randomized into experimental groups of 7 mice each and test articles were injected through the lateral tail vein under sterile conditions in a volume of 200 µL of phosphate-buffered saline (PBS). These studies were performed at South Texas Accelerated Research Therapeutics, 4383 Medical Drive, San Antonio, TX 78229.

Growth of each s.c. tumor was followed by measuring the tumor two times per week until a volume of 1200 mm^3 was reached. Tumors were measured in two perpendicular directions using Vernier calipers, and their volumes were calculated as 0.5 x L x W^2, where L =

measurement of longest axis in mm and W = measurement of axis perpendicular to L in mm.

FIG. 39 shows that treatment with paclitaxel at 15 mg/kg SIW for two weeks produced 0% partial response subjects, while **Compound 5** dosed at 0.27 μmol/kg BIW for two weeks produced 43% partial response subjects.

Example 46: Conjugate 17 *in vivo* activity in KB rat tumor model

Female Balb/c *nu/nu* rats were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. KB- tumor cells were inoculated subcutaneously at the right flank of each rat. Rats were dosed through the lateral tail vein under sterile conditions in a volume of 200 μL of phosphate-buffered saline (PBS).

Growth of each s.c. tumor was followed by measuring the tumor two times per week. Tumors were measured in two perpendicular directions using Vernier calipers, and their volumes were calculated as 0.5 x L x W^2, where L = measurement of longest axis in mm and W = measurement of axis perpendicular to L in mm. Results for tumor volume are shown in FIG. 48A. Toxicity was measured as a function of animal weight gain or loss as shown in FIG. 48B.

Example 47: Conjugate 22 *in vivo* activity in KB rat tumor model

Female Balb/c *nu/nu* rats were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. KB- tumor cells were inoculated subcutaneously at the right flank of each rat. Rats were dosed through the lateral tail vein under sterile conditions in a volume of 200 μL of phosphate-buffered saline (PBS).

Growth of each s.c. tumor was followed by measuring the tumor two times per week. Tumors were measured in two perpendicular directions using Vernier calipers, and their volumes were calculated as 0.5 x L x W^2, where L = measurement of longest axis in mm and W = measurement of axis perpendicular to L in mm. Results for tumor volume are shown in FIG. 49A. Toxicity was measured as a function of animal weight gain or loss as shown in FIG. 49B.

Example 48: Conjugate 24 *in vivo* activity in KB rat tumor model

Female Balb/c *nu/nu* rats were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. KB- tumor cells were inoculated subcutaneously at the right flank of each rat. Rats were dosed through the lateral tail vein under sterile conditions in a volume of 200 μL of phosphate-buffered saline (PBS).

Growth of each s.c. tumor was followed by measuring the tumor two times per week. Tumors were measured in two perpendicular directions using Vernier calipers, and their

volumes were calculated as 0.5 x L x W^2, where L = measurement of longest axis in mm and W = measurement of axis perpendicular to L in mm. Results for tumor volume are shown in FIG. 50A. Toxicity was measured as a function of animal weight gain or loss as shown in FIG. 50B.

Example 49: Conjugate 26 *in vivo* activity in KB rat tumor model

Female Balb/c *nu/nu* rats were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. KB- tumor cells were inoculated subcutaneously at the right flank of each rat. Rats were dosed through the lateral tail vein under sterile conditions in a volume of 200 μL of phosphate-buffered saline (PBS).

Growth of each s.c. tumor was followed by measuring the tumor two times per week. Tumors were measured in two perpendicular directions using Vernier calipers, and their volumes were calculated as 0.5 x L x W^2, where L = measurement of longest axis in mm and W = measurement of axis perpendicular to L in mm. Results for tumor volume are shown in FIG. 51A. Toxicity was measured as a function of animal weight gain or loss as shown in FIG. 51B.

Example 50: Conjugate 27 *in vivo* activity in KB rat tumor model

Female Balb/c *nu/nu* rats were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. KB- tumor cells were inoculated subcutaneously at the right flank of each rat. Rats were dosed through the lateral tail vein under sterile conditions in a volume of 200 μL of phosphate-buffered saline (PBS).

Growth of each s.c. tumor was followed by measuring the tumor two times per week. Tumors were measured in two perpendicular directions using Vernier calipers, and their volumes were calculated as 0.5 x L x W^2, where L = measurement of longest axis in mm and W = measurement of axis perpendicular to L in mm. Results for tumor volume are shown in FIG. 52A. Toxicity was measured as a function of animal weight gain or loss as shown in FIG. 52B.

Example 51: Conjugate 28 *in vivo* activity in KB rat tumor model

Female Balb/c *nu/nu* rats were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. KB- tumor cells were inoculated subcutaneously at the right flank of each rat. Rats were dosed through the lateral tail vein under sterile conditions in a volume of 200 μL of phosphate-buffered saline (PBS).

Growth of each s.c. tumor was followed by measuring the tumor two times per week. Tumors were measured in two perpendicular directions using Vernier calipers, and their volumes were calculated as 0.5 x L x W^2, where L = measurement of longest axis in mm and W

= measurement of axis perpendicular to L in mm. Results for tumor volume are shown in FIG. 53A. Toxicity was measured as a function of animal weight gain or loss as shown in FIG. 53B.

Example 52: Conjugate 30 *in vivo* activity in KB rat tumor model

Female Balb/c *nu/nu* rats were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. KB- tumor cells were inoculated subcutaneously at the right flank of each rat. Rats were dosed through the lateral tail vein under sterile conditions in a volume of 200 μL of phosphate-buffered saline (PBS).

Growth of each s.c. tumor was followed by measuring the tumor two times per week. Tumors were measured in two perpendicular directions using Vernier calipers, and their volumes were calculated as 0.5 x L x W^2, where L = measurement of longest axis in mm and W = measurement of axis perpendicular to L in mm. Results for tumor volume are shown in FIG. 54A. Toxicity was measured as a function of animal weight gain or loss as shown in FIG. 54B.

Example 53: Conjugate 32 *in vivo* activity in KB rat tumor model

Female Balb/c *nu/nu* rats were fed *ad libitum* with folate-deficient chow (Harlan diet #TD01013) for the duration of the experiment. KB- tumor cells were inoculated subcutaneously at the right flank of each rat. Rats were dosed through the lateral tail vein under sterile conditions in a volume of 200 μL of phosphate-buffered saline (PBS).

Growth of each s.c. tumor was followed by measuring the tumor two times per week. Tumors were measured in two perpendicular directions using Vernier calipers, and their volumes were calculated as 0.5 x L x W^2, where L = measurement of longest axis in mm and W = measurement of axis perpendicular to L in mm. Results for tumor volume are shown in FIG. 55A. Toxicity was measured as a function of animal weight gain or loss as shown in FIG. 55B.

Example 54: *In vitro* studies of **Conjugate 5** in ovarian cancer cell lines

Reagents

The mouse and human folate binding protein 1 (FBP1, FOLR1) PicoKine™ ELSIA kits were purchased from Boster Biological Technology (Pleasanton, CA). Antibodies used for surface marker staining were purchased from eBioscience: PD-L1 (clone MIH5; cat# 25-5982), F4/80 (clone BM8; cat# 12-4801), CD11b (clone M1/70; cat# 48-0112), CD3ε (clone 145-2C11; cat# 25-0031), CD4 (clone GK1.5; cat# 46-0041), and CD8β (clone H3517.2; cat# 11-0083).

Cell Line

The FR-α expressing cell lines utilized to evaluate **Conjugate 5** activity in in-vitro and ex-vivo studies were (1) ID8-Cl15, an ovarian carcinoma cell line transfected with the murine FR-α, and (2) IGROV1, a human ovarian carcinoma cell line that expresses the human FR-α. The FR-α negative ID8 parent (ID8p) cell line was used as controls in-vivo. ID8p and ID8-Cl15 cells were grown respectively in a folate-replete or folate-free RPMI1640 medium (Gibco BRL) (FFRPMI) containing 10% heat-inactivated fetal calf serum (HIFCS) and antibiotics, and maintained under a 5% CO_2 atmosphere using standard cell culture techniques. IGROV1 cells were grown in the same medium as ID8-Cl15 except that Corning® ultra-low attachment culture flasks (VWR, Cat. #89089-878) were used.

ELISA Analysis

Following manufacturer's instructions, standards and test samples were added to 96-well ELISA plates that were pre-coated with a rat anti-FOLR1 monoclonal antibody. A biotinylated goat anti-FOLR1 polyclonal antibody was added and followed by a buffer wash. The avidin-biotin-peroxidase complex was then added and unbound conjugates were washed away. Subsequently, a horseradish *peroxidase* substrate, 3,3',5,5'-Tetramethylbenzidine was added and catalyzed to produce a blue color product. The absorbance was read at 375 nm in a microplate reader at least two different time points.

Clonogenic Assay

IGROV1 cells seeded in 6-well plates (1000 cells/well) were exposed for 2 h to **Conjugate 5** at 1, 10, and 100 nM and followed by a 9-day chase in drug-free medium. Afterwards, the cells were washed with PBS and fixed for 5 min in a 3:1 methanol:acetic acid solution. The cells were then stained with 0.5% crystal violet/methanol solution for 15 min and washed with tap water. After a drying step, the colonies were photographed and counted using the ImageJ software.

Flow Cytometry

The single-cell suspensions prepared from ascites were blocked in a FACS stain solution on ice for 20 minutes prior to staining for flow cytometry. The FACS stain solution consisted of 1% bovine serum albumin fraction V (Fisher scientific, cat# BP1600), 0.5 mg/mL human immunoglobulin (Equitech-Bio, cat# SLH66) and 0.05% sodium azide in PBS. For surface marker detections (PD-L1, F4/80, CD11b, CD3, CD4, CD8), the tumor cells were

stained in the FACS stain solution containing various fluorophore conjugated antibodies purchased from eBioscience at optimized concentrations (0.4 – 2.5 μg/mL). After 20 minutes on ice, the tumor cells were washed with PBS and re-suspended in PBS containing 3 μM propidium iodide for dead cell exclusion. Data was collected on the Gallios flow cytometer (Beckman Coulter) and analyzed using the Kaluza v1.2 software (Beckman Coulter). Functional folate receptor was measured using a small molecule synthesized in house by coupling folic acid to Alexa Fluor 647.

Results

Conjugate 5 activity against ID8-Cl15 tumor cells was assessed using the XTT cell viability assay. The cells were exposed for 2 h to 10-fold serial dilutions of **Conjugate 5** (up to 1 μM) and followed by a 72-120 h chase in drug-free medium. As determined by the XTT assay, **Conjugate 5** showed a potent dose-dependent inhibition of cell *proliferation* with relative IC_{50} values of ~0.52 (72 h), 0.61 (96 h), and 0.17 (120 h) (FIG. 56). Importantly, the maximal cell kill was observed after 96-120 h chase, supporting the mechanism of action of this class of DNA-crosslinking compound.

Conjugate 5 activity against the slow-growing IGROV tumor cells was assessed using a clonogenic assay. After a 2 h exposure and 9-day chase (FIG. 57), **Conjugate 5** demonstrated a potent activity at all concentrations (1 – 100 nM) tested. More importantly, **Conjugate 5** anti-tumor activity was significantly reduced in the presence of excess amount of folic acid at both 1 and 10 nM concentrations.

Example 55: *In vivoo* studies of **Conjugate 5** in ovarian tumor model

Mice

Female C57BL/6 (ID8p, ID8-Cl15) and nu/nu (IGROV1) mice were purchased from Envigo (Indianapolis, IN) and used when they reached 6-8 weeks of age. The mice were fed a folate-deficient diet (TestDiet, St. Louis, MO) on the day of arrival.

Tumor Implantation

Mouse ascites tumors were generated by intra-peritoneal implantation of cultured cells at 5 x 10^6 in C57BL/6 (ID8p, ID8-Cl15) and nu/nu (IGROV1) mice respectively.

Preparation of Single Cell Suspension from Tumor Bearing Mice

Ascites was collected via an I.P. injection of 5 mL of cold PBS containing 5 mM EDTA then removal of the intra-peritoneal fluid containing ascitic tumor cells. The cells were then

collected by a 5 minute 400 x g centrifugation, followed by an RBC lysis step, then a cold PBS wash and finally a 40 μm nylon filtration to remove tissue and large cellular aggregates.

Preparation of Acellular Ascitic Fluid from Ascites Bearing Mice

Upon euthanasia, total ascitic fluid was collected via an I.P. lavage of the intra-peritoneal fluid containing ascitic tumor cells. The acellular fraction of the ascitic fluid was obtained by a 5-minute 2200 x g centrifugation and stored at -80°C until future use.

Conjugate 5 plus Anti-CTLA-4 Combination Study

To test the effect of **Conjugate 5** alone and in combination with anti-CTLA-4 antibody, ID8-Cl15 tumor cells (5 x 10^6 cells per animal in 1% syngeneic mouse serum/folate-deficient RPMI1640 medium) were inoculated intraperitoneally 13 days post the date of arrival and start of the folate deficient diet. For comparison, EC1456 alone and in combination with the same regimen of anti-CTLA-4 antibody was also evaluated. Starting 7 days after tumor implant, mice were intravenously dosed BIW for a total of 6 doses with **Conjugate 5** at 0.1 μmol/kg or EC1456 at 2 μmol/kg. The anti-CTLA-4 antibody dosing solution was prepared by diluting the stock solution (BioXcell, Clone UC10-4F10-11) to 1.25 mg/mL in PBS, pH 7.4. Anti-CTLA-4 (250 μg/dose) was i.p. administered BIW for a total of 5 doses starting 11 days after the tumor implant. In the **Conjugate 5** plus anti-CTLA-4 and EC1456 plus anti-CTLA-4 combination groups, all compounds were dose- and schedule-matched with the single-agent dosing groups. Mice were weighed 3 times/week and assessed for any clinical sign of swollen bellies indicative of ascites formation and for the evidence of toxicity such as respiratory distress, mobility, weight loss, diarrhea, hunched posture, and failure to eat. Once the animals developed ascites, they were monitored daily and euthanized when ascites became severe (rounded and walking on tip toes). Healthy animals from the same cohort of mice were used as controls for normal weight gain.

Results

Quantification of FBP1 in Mouse Ascitic Fluids

The acellular ascitic fluid samples collected from ID8p, ID8-Cl15 and IGROV1 tumor-bearing mice at the time of euthanasia were assayed for soluble murine (ID8p, ID8-Cl15) and human (IGROV1) FBP1 levels. Murine FBP1 was detected in the ascitic fluid derived from mice intraperitoneally implanted with ID8-Cl15 tumor cells at 0.93 – 4.6 nM (Table 1). Similarly, human FBP1 was detected in the ascitic fluid derived from mice intraperitoneally implanted with IGROV1 tumor cells at 0.70 – 2.8 nM (Table 1). In contract, negligible amount

of the murine FBP1 was found in the ascitic fluid derived from ID8p tumor-bearing mice (Table 1). This suggests that malignant ascites microenvironment renders FOLR1 shedding from cancer cells.

Assessment of Functional FR in Mouse Models of Ovarian Cancer

Functional FR levels were measured on the IGROV1 human ovarian cancer cells (FIG. 58; HLA+ CD45-; label a) grown in the peritoneal cavity of nu/nu mice using a folate-fluorophore conjugate and compared to those on peritoneal macrophages (F480+ CD11b+; label b) and freshly harvested IGROV1 cells from in vitro cultures (label c). There was only a small minority of mouse peritoneal ascites IGROV1 cells (~6%) stained positive for FA-Alexa Fluor, suggesting a loss of FR-α either through shedding or down regulation or a combination of both. Shedding of FR-α by IGROV1 and ID8-Cl15 ascites cells likely occurred as soluble human and mouse FR-α (FBP1, FOLR1) were detected in acellular ascitic fluid by ELISA analysis (Table 1). The ID8p cell line derived ascitic fluid was used as a FRα-negative control and indeed very little soluble murine FR-α was detected by ELISA (Table 1).

Table 1

Tumor models (Intraperitoneal)	Mouse strain (Female)	Ascites fluid ELISA analysis	Results (nM)
IGROV1	Nu/Nu	hFBP1	0.70 – 2.8
ID8-Cl15	C57BL/6	mFBP1	0.93 – 4.6
ID8p (FRα- control)	C57BL/6	mFBP1	0.066 – 0.092

The presence of CD4+ and CD8+ T cells were also quantitated in total peritoneal cells of the immunocompetent C57BL6 mice at 7 day intervals post IP injection of the mouse ovarian cell line, ID8-CL15 (FIG. 59A). The CD45+ CD3e+ CD8+ CD4- T cells (■) slowly increased in number from day 7 to day 42 post implantation. The CD45+ CD3e+ CD4+ CD8- T cells (▲) also increased in number from day 7 to day 35 with a more significant increase from day 35 to day 42 post implantation suggesting an immune response to the ovarian cancer cell had occurred. In addition, CD45- non bone-marrow derived ascites cells from ID8-CL15 implanted mice expressed very little functional FR (see FIG. 59B (■)), whereas ascites macrophages (see FIG. 59B (●) and 59C (insert box)) expressed a significant amount of a functional FR (likely,

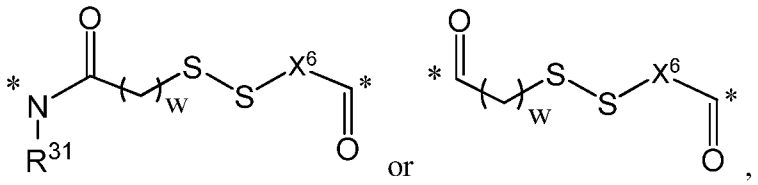
FRβ). These suggest that targeting of FR-β+ ovarian cancer stromal cells such as ascites macrophages could be alterative mechanism of action for compounds such as **Conjugate 5**.

Conjugate 5 *In-Vivo* Activity Alone and in Combination with Anti-CTLA-4

CTLA-4 (CD152) is a protein receptor that functions as an immune checkpoint to downregulate immune responses. CTLA-4 competes with CD28 for binding to B7 on antigen presentation cells in order to shut down T-cell activation. Recent studies showed that CTLA4 antagonists can enhance the activity of chemotherapy in certain tumor types. To examine the antitumor effect of **Conjugate 5** alone and in combination anti-CTLA-4 antibody, we utilized syngeneic intraperitoneal ID8-Cl15 tumor bearing mice (Fig. 60A). For comparison, EC1456 was also tested as single agent or in combination with anti-CTLA-4 antibody. Here, untreated control mice had a median survival time of ~46 days post tumor implant. Both EC1456 alone (i.v. 2 μmol/kg, BIW x 6 doses) and **Conjugate 5** alone (i.v. 0.1 μmol/kg, BIW x 6 doses) produced significant anti-tumor effects in 5 animals each group, with ~67% increase in the median survival time (~77 days post tumor implant, $P = 0.0018$, Log-Rank test). Anti-CTLA-4 antibody alone (i.p. 250 μg/dose, BIW x 5 doses) displayed no significant anti-tumor effect in 5 animals, with ~11% increase in the median survival time (~51 days post tumor implant). EC1456 (i.v. 2 μmol/kg, BIW x 6 doses) plus anti-CTLA-4 antibody (i.p. 250 μg/dose, BIW x 5 doses) displayed no additional benefit in 5 animals with a median survival time of ~81 days post tumor implant. On the other hand, **Conjugate 5** (i.v. 0.1 μmol/kg, BIW x 6 doses) plus anti-CTLA-4 antibody (i.p. 250 μg/dose, BIW x 5 doses), displayed additional therapeutic benefit in 5 animals with a median survival time of ~102 days post tumor implant.

WHAT IS CLAIMED IS:

1. A conjugate, or a pharmaceutically acceptable salt thereof, comprising a binding ligand (B), one or more linkers (L), at least one releasable group, a first drug (D^1) and a second drug (D^2), wherein B is covalently attached to at least one L, at least one L is covalently attached to at least one of the first drug or the second drug, at least one of the first drug or the second drug is a PBD, and the one or more linkers comprises at least one releasable linker (L^r) of the formula



wherein

each R^{31} and $R^{31'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{32}$, $-OC(O)R^{32}$, $-OC(O)NR^{32}R^{32'}$, $-OS(O)R^{32}$, $-OS(O)_2R^{32}$, $-SR^{32}$, $-S(O)R^{32}$, $-S(O)_2R^{32}$, $-S(O)NR^{32}R^{32'}$, $-S(O)_2NR^{32}R^{32'}$, $-OS(O)NR^{32}R^{32'}$, $-OS(O)_2NR^{32}R^{32'}$, $-NR^{32}R^{32'}$, $-NR^{32}C(O)R^{33}$, $-NR^{32}C(O)OR^{33}$, $-NR^{32}C(O)NR^{33}R^{33'}$, $-NR^{32}S(O)R^{33}$, $-NR^{32}S(O)_2R^{33}$, $-NR^{32}S(O)NR^{33}R^{33'}$, $-NR^{32}S(O)_2NR^{33}R^{33'}$, $-C(O)R^{32}$, $-C(O)OR^{32}$ or $-C(O)NR^{32}R^{32'}$;

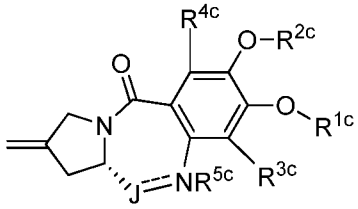
each X^6 is independently selected from the group consisting of $-C_1$-C_6 alkyl-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-, $-C_1$-C_6 alkyl-O-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-O-, $-C_1$-C_6 alkyl-$NR^{31'}$- and $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-$NR^{31'}$-, wherein each hydrogen atom in $-C_1$-C_6 alkyl-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-, $-C_1$-C_6 alkyl-O-, $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-O-, $-C_1$-C_6 alkyl-$NR^{31'}$- or $-C_6$-C_{10} aryl-(C_1-C_6 alkyl)-$NR^{31'}$ is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{34}$, $-OC(O)R^{34}$, $-OC(O)NR^{34}R^{34'}$, $-OS(O)R^{34}$, $-OS(O)_2R^{34}$, $-SR^{34}$, $-S(O)R^{34}$, $-S(O)_2R^{34}$, $-S(O)NR^{34}R^{34'}$, $-S(O)_2NR^{34}R^{34'}$, $-OS(O)NR^{34}R^{34'}$, $-OS(O)_2NR^{34}R^{34'}$, $-NR^{34}R^{34'}$, $-NR^{34}C(O)R^{35}$, $-NR^{34}C(O)OR^{35}$, $-NR^{34}C(O)NR^{35}R^{35'}$, $-NR^{34}S(O)R^{35}$, $-NR^{34}S(O)_2R^{35}$, $-NR^{34}S(O)NR^{35}R^{35'}$, $-NR^{34}S(O)_2NR^{35}R^{35'}$, $-C(O)R^{34}$, $-C(O)OR^{34}$ or $-C(O)NR^{34}R^{34'}$;

each R^{32}, $R^{32'}$, R^{33}, $R^{33'}$, R^{34}, $R^{34'}$, R^{35} and $R^{35'}$ are independently selected from the group consisting of H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, and 5- to 7-membered heteroaryl;

each w is independently an integer from 1 to 4; and

each * represents a covalent bond to the rest of the conjugate.

2. The conjugate of claim 1, wherein at least one of the first drug or the second drug is a PBD of the formula



wherein

J is –C(O)-, $–CR^{13c}=$ or $–(CR^{13c}R^{13c'})$-;

R^{1c}, R^{2c} and R^{5c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-C(O)R^{6c}$, $-C(O)OR^{6c}$ and $-C(O)NR^{6c}R^{6c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{7c}$, $-OC(O)R^{7c}$, $-OC(O)NR^{7c}R^{7c'}$, $-OS(O)R^{7c}$, $-OS(O)_2R^{7c}$, $-SR^{7c}$, $-S(O)R^{7c}$, $-S(O)_2R^{7c}$, $-S(O)_2OR^{7c}$, $-S(O)NR^{7c}R^{7c'}$, $-S(O)_2NR^{7c}R^{7c'}$, $-OS(O)NR^{7c}R^{7c'}$, $-OS(O)_2NR^{7c}R^{7c'}$, $-NR^{7c}R^{7c'}$, $-NR^{7c}C(O)R^{8c}$, $-NR^{7c}C(O)OR^{8c}$, $-NR^{7c}C(O)NR^{8c}R^{8c'}$, $-NR^{7c}S(O)R^{8c}$, $-NR^{7c}S(O)_2R^{8c}$, $-NR^{7c}S(O)NR^{8c}R^{8c'}$, $-NR^{7c}S(O)_2NR^{8c}R^{8c'}$, $-C(O)R^{7c}$, $-C(O)OR^{7c}$ or $-C(O)NR^{7c}R^{7c'}$; or when J is $–CR^{13c}=$, R^{5c} is absent; provided that at least one of R^{1c}, R^{2c} or R^{5c} is a covalent bond to the rest of the conjugate;

R^{3c} and R^{4c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -CN, $-NO_2$, -NCO, $-OR^{9c}$, $-OC(O)R^{9c}$, $-OC(O)NR^{9c}R^{9c'}$, $-OS(O)R^{9c}$, $-OS(O)_2R^{9c}$, $-SR^{9c}$, $-S(O)R^{9c}$, $-S(O)_2R^{9c}$, $-S(O)NR^{9c}R^{9c'}$, $-S(O)_2NR^{9c}R^{9c'}$, $-OS(O)NR^{9c}R^{9c'}$, $-OS(O)_2NR^{9c}R^{9c'}$, $-NR^{9c}R^{9c'}$, $-NR^{9c}C(O)R^{10c}$, $-NR^{9c}C(O)OR^{10c}$, $-NR^{9c}C(O)NR^{10c}R^{10c'}$, $-NR^{9c}S(O)R^{10c}$, $-NR^{9c}S(O)_2R^{10c}$, $-NR^{9c}S(O)NR^{10c}R^{10c'}$, $-NR^{9c}S(O)_2NR^{10c}R^{10c'}$, $-C(O)R^{9c}$, $-C(O)OR^{9c}$ and $-C(O)NR^{9c}R^{9c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered

heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{11c}$, $-OC(O)R^{11c}$, $-OC(O)NR^{11c}R^{11c'}$, $-OS(O)R^{11c}$, $-OS(O)_2R^{11c}$, $-SR^{11c}$, $-S(O)R^{11c}$, $-S(O)_2R^{11c}$, $-S(O)NR^{11c}R^{11c'}$, $-S(O)_2NR^{11c}R^{11c'}$, $-OS(O)NR^{11c}R^{11c'}$, $-OS(O)_2NR^{11c}R^{11c'}$, $-NR^{11c}R^{11c'}$, $-NR^{11c}C(O)R^{12c}$, $-NR^{11c}C(O)OR^{12c}$, $-NR^{11c}C(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)R^{12c}$, $-NR^{11c}S(O)_2R^{12c}$, $-NR^{11c}S(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)_2NR^{12c}R^{12c'}$, $-C(O)R^{11c}$, $-C(O)OR^{11c}$ or $-C(O)NR^{11c}R^{11c}$;

each R^{6c}, $R^{6c'}$, R^{7c}, $R^{7c'}$, R^{8c}, $R^{8c'}$, R^{9c}, $R^{9c'}$, R^{10c}, $R^{10c'}$, R^{11c}, $R^{11c'}$, R^{12c} and $R^{12c'}$ is independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

R^{13c} and $R^{13c'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{11c}$, $-OC(O)R^{11c}$, $-OC(O)NR^{11c}R^{11c'}$, $-OS(O)R^{11c}$, $-OS(O)_2R^{11c}$, $-SR^{11c}$, $-S(O)R^{11c}$, $-S(O)_2R^{11c}$, $-S(O)NR^{11c}R^{11c'}$, $-S(O)_2NR^{11c}R^{11c'}$, $-OS(O)NR^{11c}R^{11c'}$, $-OS(O)_2NR^{11c}R^{11c'}$, $-NR^{11c}R^{11c'}$, $-NR^{11c}C(O)R^{12c}$, $-NR^{11c}C(O)OR^{12c}$, $-NR^{11c}C(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)R^{12c}$, $-NR^{11c}S(O)_2R^{12c}$, $-NR^{11c}S(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)_2NR^{12c}R^{12c'}$, $-C(O)R^{11c}$, $-C(O)OR^{11c}$ and $-C(O)NR^{11c}R^{11c}$.

3. The conjugate of claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein each releasable group comprises at least one cleavable bond.

4. The conjugate of claim 3, or a pharmaceutically acceptable salt thereof, wherein each cleavable bond is broken under physiological conditions.

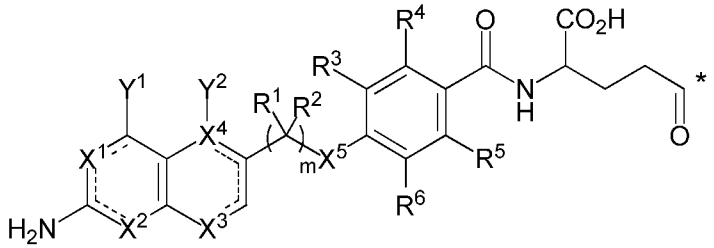
5. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, further comprising a releasable group that is not disulfide bond.

6. The conjugate of claim 5, or a pharmaceutically acceptable salt thereof, wherein the releasable group that is not disulfide bond is a group within the structure of at least one of D^1 or D^2.

7. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein one of D^1 or D^2 is a PBD pro-drug, and the releasable group is a group within the structure of the PBD pro-drug.

8. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein the one or more linkers (L) are independently selected from the group consisting of AA, L^1, L^2, L^3 and L^r, and combinations thereof.

9. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein B is of the formula



wherein

R^1 and R^2 in each instance are independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -OR^7, -SR^7 and -$NR^7R^{7'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl is independently optionally substituted by halogen, –OR^8, -SR^8, -$NR^8R^{8'}$, -$C(O)R^8$, -$C(O)OR^8$ or -$C(O)NR^8R^{8'}$;

R^3, R^4, R^5 and R^6 are each independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -CN, -NO_2, -NCO, -OR^9, -SR^9, –$NR^9R^{9'}$, -$C(O)R^9$, -$C(O)OR^9$ and -$C(O)NR^9R^{9'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl is independently optionally substituted by halogen, –OR^{10}, -SR^{10}, -$NR^{10}R^{10'}$, -$C(O)R^{10}$, -$C(O)OR^{10}$ or -$C(O)NR^{10}R^{10'}$;

each R^7, $R^{7'}$, R^8, $R^{8'}$, R^9, $R^{9'}$, R^{10} and $R^{10'}$ is independently H, C_1-C_6 alkyl, C_2-C_6 alkenyl or C_2-C_6 alkynyl;

X^1 is –NR^{11}-, =N-, -N=, -$C(R^{11})$= or =$C(R^{11})$-;

X^2 is –$NR^{11'}$- or =N-;

X^3 is –$NR^{11''}$-, -N= or -$C(R^{11'})$=;

X^4 is –N= or –C=;

X^5 is NR^{12} or $CR^{12}R^{12'}$;

Y^1 is H,–OR^{13}, –SR^{13} or –$NR^{13}R^{13'}$ when X^1 is -N= or -$C(R^{11})$=, or Y^1 is =O when X^1 is -NR^{11}-, =N- or =$C(R^{11})$-;

Y^2 is H, C_1-C_6 alkyl, C_2-C_6 alkenyl, -$C(O)R^{14}$, -$C(O)OR^{14}$, -$C(O)NR^{14}R^{14'}$ when X^4 is -C=, or Y^2 is absent when X^4 is –N=;

R^{11}, $R^{11'}$, $R^{11''}$, R^{12}, $R^{12'}$, R^{13}, $R^{13'}$, R^{14} and $R^{14'}$ are each independently selected from the group consisting of H, C_1-C_6 alkyl, -C(O)R^{15}, -C(O)OR^{15} and -C(O)N$R^{15}R^{15'}$;

R^{15} and $R^{15'}$ are each independently H or C_1-C_6 alkyl; and

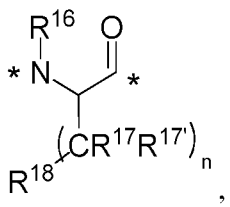
m is 1, 2, 3 or 4;

wherein * represents a covalent bond to the rest of the conjugate.

10. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein the one or more linkers (L) comprises at least one AA selected from the group consisting of L-lysine, L-asparagine, L-threonine, L-serine, L-isoleucine, L-methionine, L-proline, L-histidine, L-glutamine, L-arginine, L-glycine, L-aspartic acid, L-glutamic acid, L-alanine, L-valine, L-phenylalanine, L-leucine, L-tyrosine, L-cysteine, L-tryptophan, L-phosphoserine, L-sulfo-cysteine, L-arginosuccinic acid, L-hydroxyproline, L-phosphoethanolamine, L-sarcosine, L-taurine, L-carnosine, L-citrulline, L-anserine, L-1,3-methyl-histidine, L-alpha-amino-adipic acid, D-lysine, D-asparagine, D-threonine, D-serine, D-isoleucine, D-methionine, D-proline, D-histidine, D-glutamine, D-arginine, D-glycine, D-aspartic acid, D-glutamic acid, D-alanine, D-valine, D-phenylalanine, D-leucine, D-tyrosine, D-cysteine, D-tryptophan, D-citrulline and D-carnosine.

11. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein wherein the one or more linkers (L) comprises at least one AA selected from the group consisting of L-arginine, L-aspartic acid, L-cysteine, D-arginine, D-aspartic acid, and D-cysteine.

12. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein, when the one or more linkers (L) comprises a first spacer linker (L^1), the first spacer linker is of the formula



,

wherein

R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -C(O)R^{19}, -C(O)OR^{19} and -C(O)N$R^{19}R^{19'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl and C_2-C_6 alkynyl is independently optionally substituted by halogen, C_1-C_6

alkyl, C_2-C_6 alkenyl, and C_2-C_6 alkynyl, $-OR^{20}$, $-OC(O)R^{20}$, $-OC(O)NR^{20}R^{20'}$, $-OS(O)R^{20}$, $-OS(O)_2R^{20}$, $-SR^{20}$, $-S(O)R^{20}$, $-S(O)_2R^{20}$, $-S(O)NR^{20}R^{20'}$, $-S(O)_2NR^{20}R^{20'}$, $-OS(O)NR^{20}R^{20'}$, $-OS(O)_2NR^{20}R^{20'}$, $-NR^{20}R^{20'}$, $-NR^{20}C(O)R^{21}$, $-NR^{20}C(O)OR^{21}$, $-NR^{20}C(O)NR^{21}R^{21'}$, $-NR^{20}S(O)R^{21}$, $-NR^{20}S(O)_2R^{21}$, $-NR^{20}S(O)NR^{21}R^{21'}$, $-NR^{20}S(O)_2NR^{21}R^{21'}$, $-C(O)R^{20}$, $-C(O)OR^{20}$ or $-C(O)NR^{20}R^{20'}$;

each R^{17} and $R^{17'}$ is independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{22}$, $-OC(O)R^{22}$, $-OC(O)NR^{22}R^{22'}$, $-OS(O)R^{22}$, $-OS(O)_2R^{22}$, $-SR^{22}$, $-S(O)R^{22}$, $-S(O)_2R^{22}$, $-S(O)NR^{22}R^{22'}$, $-S(O)_2NR^{22}R^{22'}$, $-OS(O)NR^{22}R^{22'}$, $-OS(O)_2NR^{22}R^{22'}$, $-NR^{22}R^{22'}$, $-NR^{22}C(O)R^{23}$, $-NR^{22}C(O)OR^{23}$, $-NR^{22}C(O)NR^{23}R^{23'}$, $-NR^{22}S(O)R^{23}$, $-NR^{22}S(O)_2R^{23}$, $-NR^{22}S(O)NR^{23}R^{23'}$, $-NR^{22}S(O)_2NR^{23}R^{23'}$, $-C(O)R^{22}$, $-C(O)OR^{22}$, and $-C(O)NR^{22}R^{22'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, $-OR^{24}$, $-OC(O)R^{24}$, $-OC(O)NR^{24}R^{24'}$, $-OS(O)R^{24}$, $-OS(O)_2R^{24}$, $-SR^{24}$, $-S(O)R^{24}$, $-S(O)_2R^{24}$, $-S(O)NR^{24}R^{24'}$, $-S(O)_2NR^{24}R^{24'}$, $-OS(O)NR^{24}R^{24'}$, $-OS(O)_2NR^{24}R^{24'}$, $-NR^{24}R^{24'}$, $-NR^{24}C(O)R^{25}$, $-NR^{24}C(O)OR^{25}$, $-NR^{24}C(O)NR^{25}R^{25'}$, $-NR^{24}S(O)R^{25}$, $-NR^{24}S(O)_2R^{25}$, $-NR^{24}S(O)NR^{25}R^{25'}$, $-NR^{24}S(O)_2NR^{25}R^{25'}$, $-C(O)R^{24}$, $-C(O)OR^{24}$ or $-C(O)NR^{24}R^{24'}$; or R^{17} and $R^{17'}$ may combine to form a C_4-C_6 cycloalkyl or a 4- to 6- membered heterocycle, wherein each hydrogen atom in C_4-C_6 cycloalkyl or 4- to 6-membered heterocycle is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{24}$, $-OC(O)R^{24}$, $-OC(O)NR^{24}R^{24'}$, $-OS(O)R^{24}$, $-OS(O)_2R^{24}$, $-SR^{24}$, $-S(O)R^{24}$, $-S(O)_2R^{24}$, $-S(O)NR^{24}R^{24'}$, $-S(O)_2NR^{24}R^{24'}$, $-OS(O)NR^{24}R^{24'}$, $-OS(O)_2NR^{24}R^{24'}$, $-NR^{24}R^{24'}$, $-NR^{24}C(O)R^{25}$, $-NR^{24}C(O)OR^{25}$, $-NR^{24}C(O)NR^{25}R^{25'}$, $-NR^{24}S(O)R^{25}$, $-NR^{24}S(O)_2R^{25}$, $-NR^{24}S(O)NR^{25}R^{25'}$, $-NR^{24}S(O)_2NR^{25}R^{25'}$, $-C(O)R^{24}$, $-C(O)OR^{24}$ or $-C(O)NR^{24}R^{24'}$;

R^{18} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{26}$, $-OC(O)R^{26}$, $-OC(O)NR^{26}R^{26'}$, $-OS(O)R^{26}$, $-OS(O)_2R^{26}$, $-SR^{26}$, $-S(O)R^{26}$, $-S(O)_2R^{26}$, $-S(O)NR^{26}R^{26'}$, $-S(O)_2NR^{26}R^{26'}$, $-OS(O)NR^{26}R^{26'}$, $-OS(O)_2NR^{26}R^{26'}$, $-NR^{26}R^{26'}$, $-NR^{26}C(O)R^{27}$, $-NR^{26}C(O)OR^{27}$, $-NR^{26}C(O)NR^{27}R^{27'}$, $-NR^{26}C(=NR^{26''})NR^{27}R^{27'}$, $-NR^{26}S(O)R^{27}$, $-NR^{26}S(O)_2R^{27}$, $-NR^{26}S(O)NR^{27}R^{27'}$, $-NR^{26}S(O)_2NR^{27}R^{27'}$, $-C(O)R^{26}$, $-C(O)OR^{26}$ and $-C(O)NR^{26}R^{26'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to

7-membered heteroaryl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, $-(CH_2)_pOR^{28}$, $-(CH_2)_p(OCH_2)_qOR^{28}$, $-(CH_2)_p(OCH_2CH_2)_qOR^{28}$, $-OR^{29}$, $-OC(O)R^{29}$, $-OC(O)NR^{29}R^{29'}$, $-OS(O)R^{29}$, $-OS(O)_2R^{29}$, $-(CH_2)_pOS(O)_2OR^{29}$, $-OS(O)_2OR^{29}$, $-SR^{29}$, $-S(O)R^{29}$, $-S(O)_2R^{29}$, $-S(O)NR^{29}R^{29'}$, $-S(O)_2NR^{29}R^{29'}$, $-OS(O)NR^{29}R^{29'}$, $-OS(O)_2NR^{29}R^{29'}$, $-NR^{29}R^{29'}$, $-NR^{29}C(O)R^{30}$, $-NR^{29}C(O)OR^{30}$, $-NR^{29}C(O)NR^{30}R^{30'}$, $-NR^{29}S(O)R^{30}$, $-NR^{29}S(O)_2R^{30}$, $-NR^{29}S(O)NR^{30}R^{30'}$, $-NR^{29}S(O)_2NR^{30}R^{30'}$, $-C(O)R^{29}$, $-C(O)OR^{29}$ or $-C(O)NR^{29}R^{29'}$;

each R^{19}, $R^{19'}$, R^{20}, $R^{20'}$, R^{21}, $R^{21'}$, R^{22}, $R^{22'}$, R^{23}, $R^{23'}$, R^{24}, $R^{24'}$, R^{25}, $R^{25'}$, R^{26}, $R^{26'}$, $R^{26''}$, R^{29}, $R^{29'}$, R^{30} and $R^{30'}$ is independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, or 5- to 7-membered heteroaryl is independently optionally substituted by halogen, -OH, -SH, $-NH_2$ or $-CO_2H$;

R^{27} and $R^{27'}$ are each independently selected from the group consisting of H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_3-C_6 cycloalkyl, $-(CH_2)_p$(sugar), $-(CH_2)_p(OCH_2CH_2)_q$-(sugar) and $-(CH_2)_p(OCH_2CH_2CH_2)_q$(sugar);

R^{28} is a H, D, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl or sugar;

n is 1, 2, 3, 4 or 5;

p is 1, 2, 3, 4 or 5;

q is 1, 2, 3, 4 or 5; and

each * represents a covalent bond to the rest of the conjugate.

13. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein when the one or more linkers (L) comprises at least one second spacer linker (L^2), each second spacer linker is independently selected from the group consisting of C_1-C_6 alkyl, $-OC_1$-C_6 alkyl, $-SC_1$-C_6 alkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-NR^{36}(CR^{36'}R^{36''})_r$-S-(succinimid-1-yl)-, $-(CR^{36'}R^{36''})_rC(O)NR^{36}$-, $-(CR^{39}R^{39'})_rC(O)$-, $-(CR^{39}R^{39'})_rOC(O)$-, $-S(CR^{39}R^{39'})_rOC(O)$-, $-C(O)(CR^{39}R^{39'})_r$-, $-C(O)O(CR^{39}R^{39'})_r$-, $-NR^{39}C(O)(CR^{39'}R^{39''})_r$-, $-NR^{39}C(O)(CR^{39'}R^{39''})_rS$-, $-(CH_2)_rNR^{39}$-, $-NR^{39}(CH_2)_r$-, $-NR^{39}(CH_2)_rS$-, $-NR^{39}(CH_2)_rNR^{39'}$-, $-(OCR^{39}R^{39'}CR^{39}R^{39'})_rC(O)$-, $-(OCR^{39}R^{39'}CR^{39}R^{39'}CR^{39}R^{39'})_rC(O)$-, $-OC(O)(CR^{44}R^{44'})_t$-, $-C(O)(CR^{44}R^{44'})_t$-, $-NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_t$-, $-CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tNR^{42}$-,

-$NR^{42}C_6$-C_{10} aryl(C_1-C_6 alkyl)OC(O)-, -$C(O)CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tNR^{42}$-, -$NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(OCR^{44}R^{44'}CR^{44}R^{44'})_tC(O)$-, and -$NR^{42}CR^{43}R^{43'}CR^{43}R^{43'}(CR^{44}=CR^{44'})_t$-;

wherein

each R^{36}, $R^{36'}$ and $R^{36''}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, -$C(O)R^{37}$, -$C(O)OR^{37}$ and -$C(O)NR^{37}R^{37'}$ wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{37}, -$OC(O)R^{37}$, -$OC(O)NR^{37}R^{37'}$, -$OS(O)R^{37}$, -$OS(O)_2R^{37}$, -SR^{37}, -$S(O)R^{37}$, -$S(O)_2R^{37}$, -$S(O)NR^{37}R^{37'}$, -$S(O)_2NR^{37}R^{37'}$, -$OS(O)NR^{37}R^{37'}$, -$OS(O)_2NR^{37}R^{37'}$, -$NR^{37}R^{37'}$, -$NR^{37}C(O)R^{38}$, -$NR^{37}C(O)OR^{38}$, -$NR^{37}C(O)NR^{38}R^{38'}$, -$NR^{37}S(O)R^{38}$, -$NR^{37}S(O)_2R^{38}$, -$NR^{37}S(O)NR^{38}R^{38'}$, -$NR^{37}S(O)_2NR^{38}R^{38'}$, -$C(O)R^{37}$, -$C(O)OR^{37}$ or -$C(O)NR^{37}R^{37'}$;

R^{37}, $R^{37'}$, R^{38} and $R^{38'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

each R^{39} and $R^{39'}$ is independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{40}, -$OC(O)R^{40}$, -$OC(O)NR^{40}R^{40'}$, -$OS(O)R^{40}$, -$OS(O)_2R^{40}$, -SR^{40}, -$S(O)R^{40}$, -$S(O)_2R^{40}$, -$S(O)NR^{40}R^{40'}$, -$S(O)_2NR^{40}R^{40'}$, -$OS(O)NR^{40}R^{40'}$, -$OS(O)_2NR^{40}R^{40'}$, -$NR^{40}R^{40'}$, -$NR^{40}C(O)R^{41}$, -$NR^{40}C(O)OR^{41}$, -$NR^{40}C(O)NR^{41}R^{41'}$, -$NR^{40}S(O)R^{41}$, -$NR^{40}S(O)_2R^{41}$, -$NR^{40}S(O)NR^{41}R^{41'}$, -$NR^{40}S(O)_2NR^{41}R^{41'}$, -$C(O)R^{40}$, -$C(O)OR^{40}$ and -$C(O)NR^{40}R^{40'}$;

R^{40}, $R^{40'}$, R^{41} and $R^{41'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, and 5- to 7-membered heteroaryl; and

R^{42} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{45}, -$OC(O)R^{45}$, -$OC(O)NR^{45}R^{45'}$, -$OS(O)R^{45}$, -$OS(O)_2R^{45}$, -SR^{45}, -$S(O)R^{45}$, -$S(O)_2R^{45}$, -$S(O)NR^{45}R^{45'}$, -$S(O)_2NR^{45}R^{45'}$, -$OS(O)NR^{45}R^{45'}$, -$OS(O)_2NR^{45}R^{45'}$, -$NR^{45}R^{45'}$, -$NR^{45}C(O)R^{46}$, -$NR^{45}C(O)OR^{46}$, -$NR^{45}C(O)NR^{46}R^{46'}$, -$NR^{45}S(O)R^{46}$, -$NR^{45}S(O)_2R^{46}$, -$NR^{45}S(O)NR^{46}R^{46'}$, -$NR^{45}S(O)_2NR^{46}R^{46'}$, -$C(O)R^{45}$, -$C(O)OR^{45}$ or -$C(O)NR^{45}R^{45'}$,

each R^{43}, $R^{43'}$, R^{44} and $R^{44'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in

C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{47}$, $-OC(O)R^{47}$, $-OC(O)NR^{47}R^{47'}$, $-OS(O)R^{47}$, $-OS(O)_2R^{47}$, $-SR^{47}$, $-S(O)R^{47}$, $-S(O)_2R^{47}$, $-S(O)NR^{47}R^{47'}$, $-S(O)_2NR^{47}R^{47'}$, $-OS(O)NR^{47}R^{47'}$, $-OS(O)_2NR^{47}R^{47'}$, $-NR^{47}R^{47'}$, $-NR^{47}C(O)R^{48}$, $-NR^{47}C(O)OR^{48}$, $-NR^{47}C(O)NR^{48}R^{48'}$, $-NR^{47}S(O)R^{48}$, $-NR^{47}S(O)_2R^{48}$, $-NR^{47}S(O)NR^{48}R^{48'}$, $-NR^{47}S(O)_2NR^{48}R^{48'}$, $-C(O)R^{47}$, $-C(O)OR^{47}$ or $-C(O)NR^{47}R^{47'}$;

R^{45}, $R^{45'}$, R^{46}, $R^{46'}$, R^{47}, $R^{47'}$, R^{48} and $R^{48'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

r in each instance is an integer from 1 to 40; and

t is in each instance is an integer from 1 to 40.

14. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein when the one or more linkers (L) comprises at least one third spacer linker (L^3), each third spacer linker is independently selected from the group consisting of C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, $-(CR^{49}R^{49'})_uC(O)-$, $-CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'})_u-$, $-CH_2CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'}CR^{49}R^{49'})_u-$, $-CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'})_uC(O)-$ and $-CH_2CH_2(OCR^{49}R^{49'}CR^{49}R^{49'}CR^{49}R^{49'})_uC(O)-$,

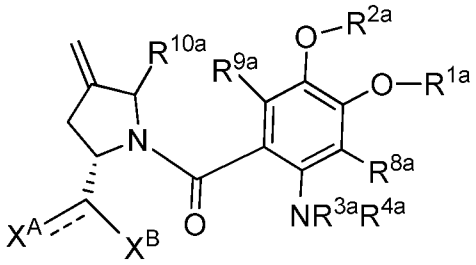
wherein

each R^{49} and $R^{49'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl and C_3-C_6 cycloalkyl is independently optionally substituted by halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{50}$, $-OC(O)R^{50}$, $-OC(O)NR^{50}R^{50'}$, $-OS(O)R^{50}$, $-OS(O)_2R^{50}$, $-SR^{50}$, $-S(O)R^{50}$, $-S(O)_2R^{50}$, $-S(O)NR^{50}R^{50'}$, $-S(O)_2NR^{50}R^{50'}$, $-OS(O)NR^{50}R^{50'}$, $-OS(O)_2NR^{50}R^{50'}$, $-NR^{50}R^{50'}$, $-NR^{50}C(O)R^{51}$, $-NR^{50}C(O)OR^{51}$, $-NR^{50}C(O)NR^{51}R^{51'}$, $-NR^{50}S(O)R^{51}$, $-NR^{50}S(O)_2R^{51}$, $-NR^{50}S(O)NR^{51}R^{51'}$, $-NR^{50}S(O)_2NR^{51}R^{51'}$, $-C(O)R^{50}$, $-C(O)OR^{50}$ or $-C(O)NR^{50}R^{50'}$;

R^{50}, $R^{50'}$, R^{51} and $R^{51'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl; and

u is in each instance 0, 1, 2, 3, 4 or 5.

15. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein the first drug is of the formula



wherein

X^A is $-OR^{6a}$, $=N-OR^{5a}$ or $-NR^{5a}R^{6a}-$, provided that when the hash bond is a pi-bond, X^A is $=NR^{5a}$;

X^B is H or OR^{7a};

R^{1a}, R^{2a}, R^{3a} and R^{4a} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-C(O)R^{11a}$, $-C(O)OR^{11a}$, and $-C(O)NR^{11a}R^{11a'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{11a}$, $-OC(O)R^{11a}$, $-OC(O)NR^{11a}R^{11a'}$, $-OS(O)R^{11a}$, $-OS(O)_2R^{11a}$, $-SR^{11a}$, $-S(O)R^{11a}$, $-S(O)_2R^{11a}$, $-S(O)NR^{11a}R^{11a'}$, $-S(O)_2NR^{11a}R^{11a'}$, $-OS(O)NR^{11a}R^{11a'}$, $-OS(O)_2NR^{11a}R^{11a'}$, $-NR^{11a}R^{11a'}$, $-NR^{11a}C(O)R^{12a}$, $-NR^{11a}C(O)OR^{12a}$, $-NR^{11a}C(O)NR^{12a}R^{12a'}$, $-NR^{11a}S(O)R^{12a}$, $-NR^{11a}S(O)_2R^{12a}$, $-NR^{11a}S(O)NR^{12a}R^{12a'}$, $-NR^{11a}S(O)_2NR^{12a}R^{12a'}$, $-C(O)R^{11a}$, $-C(O)OR^{11a}$ or $-C(O)NR^{11a}R^{11a'}$; or R^{1a} is a bond; or R^{4a} is a bond;

R^{5a}, R^{6a} and R^{7a} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-C(O)R^{13a}$, $-C(O)OR^{13a}$ and $-C(O)NR^{13a}R^{13a'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{14a}$, $-OC(O)R^{14a}$, $-OC(O)NR^{14a}R^{14a'}$, $-OS(O)R^{14a}$, $-OS(O)_2R^{14a}$, $-SR^{14a}$, $-S(O)R^{14a}$, $-S(O)_2R^{14a}$, $-S(O)NR^{14a}R^{14a'}$, $-S(O)_2NR^{14a}R^{14a'}$, $-OS(O)NR^{14a}R^{14a'}$, $-OS(O)_2NR^{14a}R^{14a'}$, $-NR^{14a}R^{14a'}$, $-NR^{14a}C(O)R^{15a}$, $-NR^{14a}C(O)OR^{15a}$, $-NR^{14a}C(O)NR^{15a}R^{15a'}$, $-NR^{14a}S(O)R^{15a}$, $-NR^{14a}S(O)_2R^{15a}$, $-NR^{14a}S(O)NR^{15a}R^{15a'}$, $-NR^{14a}S(O)_2NR^{15a}R^{15a'}$, $-C(O)R^{14a}$, $-C(O)OR^{14a}$ or $-C(O)NR^{14a}R^{14a'}$; wherein R^{6a} and R^{7a} taken together with the atoms to which they are attached optionally

combine to form a 3- to 7-membered heterocycloalkyl or a 3- to 7-membered heterocycloalkyl fused to a 6-membered aryl ring, or R^{5a} and R^{6a} taken together with the atoms to which they are attached optionally combine to form a 3- to 7-membered heterocycloalkyl or 5- to 7-membered heteroaryl, wherein each hydrogen atom in 3- to 7-membered heterocycloalkyl or 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{16a}$, $-OC(O)R^{16a}$, $-OC(O)NR^{16a}R^{16a'}$, $-OS(O)R^{16a}$, $-OS(O)_2R^{16a}$, $-SR^{16a}$, $-S(O)R^{16a}$, $-S(O)_2R^{16a}$, $-S(O)NR^{16a}R^{16a'}$, $-S(O)_2NR^{16a}R^{16a'}$, $-OS(O)NR^{16a}R^{16a'}$, $-OS(O)_2NR^{16a}R^{16a'}$, $-NR^{16a}R^{16a'}$, $-NR^{16a}C(O)R^{17a}$, $-NR^{16a}C(O)CH_2CH_2-$, $-NR^{16a}C(O)OR^{17a}$, $-NR^{16a}C(O)NR^{17a}R^{17a'}$, $-NR^{16a}S(O)R^{17a}$, $-NR^{16a}S(O)_2R^{17a}$, $-NR^{16a}S(O)NR^{17a}R^{17a'}$, $-NR^{16a}S(O)_2NR^{17a}R^{17a'}$, $-C(O)R^{16a}$, $-C(O)OR^{16a}$ or $-C(O)NR^{16a}R^{16a'}$, and wherein when R^{5a} and R^{6a} taken together with the atoms to which they are attached form a 5- to 7-membered heteroaryl, one hydrogen atom in 5- to 7-membered heteroaryl is optionally a bond, or when R^{6a} and R^{7a} taken together with the atoms to which they are attached optionally combine to form a 3- to 7-membered heterocycloalkyl fused to a 6-membered aryl, one hydrogen atom in the 6-membered aryl ring is optionally a bond; or R^{5a} is a bond;

R^{8a} and R^{9a} are each independently selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-CN$, $-NO_2$, $-NCO$, $-OR^{18a}$, $-OC(O)R^{18a}$, $-OC(O)NR^{18a}R^{18a'}$, $-OS(O)R^{18a}$, $-OS(O)_2R^{18a}$, $-SR^{18a}$, $-S(O)R^{18a}$, $-S(O)_2R^{18a}$, $-S(O)NR^{18a}R^{18a'}$, $-S(O)_2NR^{18a}R^{18a'}$, $-OS(O)NR^{18a}R^{18a'}$, $-OS(O)_2NR^{18a}R^{18a'}$, $-NR^{18a}R^{18a'}$, $-NR^{18a}C(O)R^{19a}$, $-NR^{18a}C(O)OR^{19a}$, $-NR^{18a}C(O)NR^{19a}R^{19a'}$, $-NR^{18a}S(O)R^{19a}$, $-NR^{18a}S(O)_2R^{19a}$, $-NR^{18a}S(O)NR^{19a}R^{19a'}$, $-NR^{18a}S(O)_2NR^{19a}R^{19a'}$, $-C(O)R^{18a}$, $-C(O)OR^{18a}$ and $-C(O)NR^{18a}R^{18a'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{20a}$, $-OC(O)R^{20a}$, $-OC(O)NR^{20a}R^{20a'}$, $-OS(O)R^{20a}$, $-OS(O)_2R^{20a}$, $-SR^{20a}$, $-S(O)R^{20a}$, $-S(O)_2R^{20a}$, $-S(O)NR^{20a}R^{20a'}$, $-S(O)_2NR^{20a}R^{20a'}$, $-OS(O)NR^{20a}R^{20a'}$, $-OS(O)_2NR^{20a}R^{20a'}$, $-NR^{20a}R^{20a'}$, $-NR^{20a}C(O)R^{21a}$, $-NR^{20a}C(O)OR^{21a}$, $-NR^{20a}C(O)NR^{21a}R^{21a'}$, $-NR^{20a}S(O)R^{21a}$, $-NR^{20a}S(O)_2R^{21a}$, $-NR^{20a}S(O)NR^{21a}R^{21a'}$, $-NR^{20a}S(O)_2NR^{21a}R^{21a'}$, $-C(O)R^{20a}$, $-C(O)OR^{20a}$ or $-C(O)NR^{20a}R^{20a'}$;

R^{10a} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{22a}$, $-OC(O)R^{22a}$, $-OC(O)NR^{22a}R^{22a'}$, $-OS(O)R^{22a}$, $-OS(O)_2R^{22a}$, $-SR^{22a}$, $-S(O)R^{22a}$, $-S(O)_2R^{22a}$, $-S(O)NR^{22a}R^{22a'}$, $-S(O)_2NR^{22a}R^{22a'}$, $-OS(O)NR^{22a}R^{22a'}$,

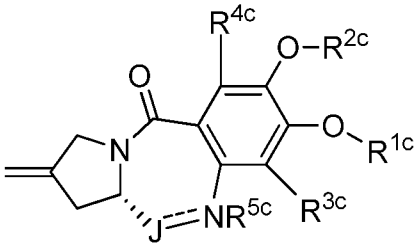
$-OS(O)_2NR^{22a}R^{22a'}$, $-NR^{22a}R^{22a'}$, $-NR^{22a}C(O)R^{23a}$, $-NR^{22a}C(O)OR^{23a}$, $-NR^{22a}C(O)NR^{23a}R^{23a'}$, $-NR^{22a}S(O)R^{23a}$, $-NR^{22a}S(O)_2R^{23a}$, $-NR^{22a}S(O)NR^{23a}R^{23a'}$, $-NR^{22a}S(O)_2NR^{23a}R^{23a}$, $-C(O)R^{22a}$, $-C(O)OR^{23a}$ and $-C(O)NR^{22a}R^{22a'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{24a}$, $-OC(O)R^{24a}$, $-OC(O)NR^{24a}R^{24a'}$, $-OS(O)R^{24a}$, $-OS(O)_2R^{24a}$, $-SR^{24a}$, $-S(O)R^{24a}$, $-S(O)_2R^{24a}$, $-S(O)NR^{24a}R^{24a'}$, $-S(O)_2NR^{24a}R^{24a'}$, $-OS(O)NR^{24a}R^{24a'}$, $-OS(O)_2NR^{24a}R^{24a'}$, $-NR^{24a}R^{24a'}$, $-NR^{24a}C(O)R^{25a}$, $-NR^{24a}C(O)OR^{25a}$, $-NR^{24a}C(O)NR^{25a}R^{25a'}$, $-NR^{24a}S(O)R^{25a}$, $-NR^{24a}S(O)_2R^{25a}$, $-NR^{24a}S(O)NR^{25a}R^{25a'}$, $-NR^{24a}S(O)_2NR^{25a}R^{25a'}$, $-C(O)R^{24a}$, $-C(O)OR^{24a}$ or $-C(O)NR^{24a}R^{24a'}$; and

each R^{11a}, $R^{11a'}$, R^{12a}, $R^{12a'}$, R^{13a}, $R^{13a'}$, R^{14a}, $R^{14a'}$, R^{15a}, $R^{15a'}$, R^{16a}, $R^{16a'}$, R^{17a}, $R^{17a'}$, R^{18a}, $R^{18a'}$, R^{19a}, $R^{19a'}$, R^{20a}, $R^{20a'}$, R^{21a}, $R^{21a'}$, R^{22a}, $R^{22a'}$, R^{23a}, $R^{23a'}$, R^{24a}, $R^{24a'}$, R^{25a} and $R^{25a'}$ is independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_{13} cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, and 5- to 7-membered heteroaryl; and

provided that at least two of R^{1a}, R^{4a}, R^{5a} are a bond; or when R^{5a} and R^{6a} taken together with the atoms to which they are attached optionally combine to form a 5- to 7-membered heteroaryl, one hydrogen atom in 5- to 7-membered heteroaryl is a bond and one of R^{1a} or R^{4a} is a bond.

16. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein the first drug is covalently attached to the second drug by a third spacer linker (L^3).

17. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein the second drug is selected from the group consisting of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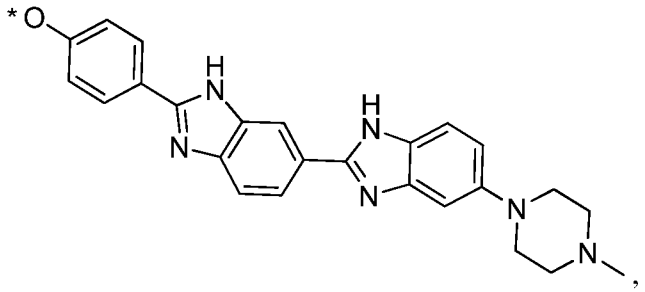
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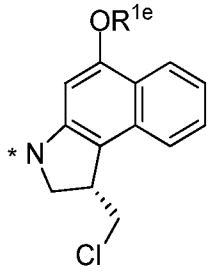
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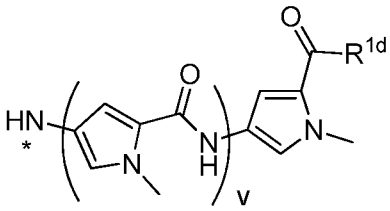
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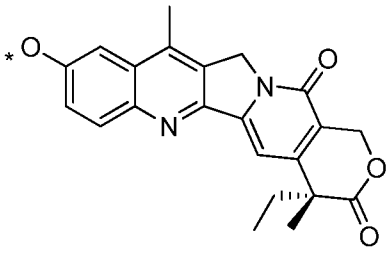
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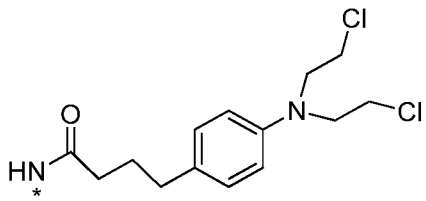


wherein

J is –C(O)-, –CR^{13c}= or –($CR^{13c}R^{13c'}$)-;

R^{1c}, R^{2c} and R^{5c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -C(O)R^{6c}, -C(O)OR^{6c} and -C(O)$NR^{6c}R^{6c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -OR^{7c}, -OC(O)R^{7c}, -OC(O)$NR^{7c}R^{7c'}$, -OS(O)R^{7c}, -$OS(O)_2R^{7c}$, -SR^{7c}, -S(O)R^{7c}, -$S(O)_2R^{7c}$, -$S(O)_2OR^{7c}$, -S(O)$NR^{7c}R^{7c'}$, -$S(O)_2NR^{7c}R^{7c'}$, -OS(O)$NR^{7c}R^{7c'}$, -$OS(O)_2NR^{7c}R^{7c'}$, -$NR^{7c}R^{7c'}$, -NR^{7c}C(O)R^{8c}, -NR^{7c}C(O)OR^{8c}, -NR^{7c}C(O)$NR^{8c}R^{8c'}$, -NR^{7c}S(O)R^{8c}, -$NR^{7c}S(O)_2R^{8c}$, -NR^{7c}S(O)$NR^{8c}R^{8c'}$, -$NR^{7c}S(O)_2NR^{8c}R^{8c'}$, -C(O)$R^{7c}$, -C(O)$OR^{7c}$ or -C(O)$NR^{7c}R^{7c'}$; or when J is –CR^{13c}=, R^{5c} is absent; provided that at least one of R^{1c}, R^{2c} or R^{5c} is a covalent bond to the rest of the conjugate;

R^{3c} and R^{4c} are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, -CN, -NO_2, -NCO, -OR^{9c}, -OC(O)R^{9c}, -OC(O)$NR^{9c}R^{9c'}$, -OS(O)R^{9c}, -$OS(O)_2R^{9c}$, -SR^{9c}, -S(O)R^{9c}, -$S(O)_2R^{9c}$, -S(O)$NR^{9c}R^{9c'}$, -$S(O)_2NR^{9c}R^{9c'}$,

$-OS(O)NR^{9c}R^{9c'}$, $-OS(O)_2NR^{9c}R^{9c'}$, $-NR^{9c}R^{9c'}$, $-NR^{9c}C(O)R^{10c}$, $-NR^{9c}C(O)OR^{10c}$, $-NR^{9c}C(O)NR^{10c}R^{10c'}$, $-NR^{9c}S(O)R^{10c}$, $-NR^{9c}S(O)_2R^{10c}$, $-NR^{9c}S(O)NR^{10c}R^{10c'}$, $-NR^{9c}S(O)_2NR^{10c}R^{10c'}$, $-C(O)R^{9c}$, $-C(O)OR^{9c}$ and $-C(O)NR^{9c}R^{9c'}$, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{11c}$, $-OC(O)R^{11c}$, $-OC(O)NR^{11c}R^{11c'}$, $-OS(O)R^{11c}$, $-OS(O)_2R^{11c}$, $-SR^{11c}$, $-S(O)R^{11c}$, $-S(O)_2R^{11c}$, $-S(O)NR^{11c}R^{11c'}$, $-S(O)_2NR^{11c}R^{11c'}$, $-OS(O)NR^{11c}R^{11c'}$, $-OS(O)_2NR^{11c}R^{11c'}$, $-NR^{11c}R^{11c'}$, $-NR^{11c}C(O)R^{12c}$, $-NR^{11c}C(O)OR^{12c}$, $-NR^{11c}C(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)R^{12c}$, $-NR^{11c}S(O)_2R^{12c}$, $-NR^{11c}S(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)_2NR^{12c}R^{12c'}$, $-C(O)R^{11c}$, $-C(O)OR^{11c}$ or $-C(O)NR^{11c}R^{11c}$;

each R^{6c}, $R^{6c'}$, R^{7c}, $R^{7c'}$, R^{8c}, $R^{8c'}$, R^{9c}, $R^{9c'}$, R^{10c}, $R^{10c'}$, R^{11c}, $R^{11c'}$, R^{12c} and $R^{12c'}$ is independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

R^{13c} and $R^{13c'}$ are each independently selected from the group consisting of H, C_1-C_7 alkyl, C_2-C_7 alkenyl, C_2-C_7 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{11c}$, $-OC(O)R^{11c}$, $-OC(O)NR^{11c}R^{11c'}$, $-OS(O)R^{11c}$, $-OS(O)_2R^{11c}$, $-SR^{11c}$, $-S(O)R^{11c}$, $-S(O)_2R^{11c}$, $-S(O)NR^{11c}R^{11c'}$, $-S(O)_2NR^{11c}R^{11c'}$, $-OS(O)NR^{11c}R^{11c'}$, $-OS(O)_2NR^{11c}R^{11c'}$, $-NR^{11c}R^{11c'}$, $-NR^{11c}C(O)R^{12c}$, $-NR^{11c}C(O)OR^{12c}$, $-NR^{11c}C(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)R^{12c}$, $-NR^{11c}S(O)_2R^{12c}$, $-NR^{11c}S(O)NR^{12c}R^{12c'}$, $-NR^{11c}S(O)_2NR^{12c}R^{12c'}$, $-C(O)R^{11c}$, $-C(O)OR^{11c}$ and $-C(O)NR^{11c}R^{11c}$;

R^{1d} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{2d}$, $-SR^{2d}$ and $-NR^{2d}R^{2d'}$,

R^{2d} and $R^{2d'}$ are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is optionally substituted by $-OR^{3d}$, $-SR^{3d}$, and $-NR^{3d}R^{3d'}$;

R^{3d} and $R^{3d'}$ are each independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

R^{1e} is selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to

7-membered heteroaryl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is independently optionally substituted by C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl, 5- to 7-membered heteroaryl, $-OR^{2e}$, $-OC(O)R^{2e}$, $-OC(O)NR^{2e}R^{2e'}$, $-OS(O)R^{2e}$, $-OS(O)_2R^{2e}$, $-SR^{2e}$, $-S(O)R^{2e}$, $-S(O)_2R^{2e}$, $-S(O)NR^{2e}R^{2e'}$, $-S(O)_2NR^{2e}R^{2e'}$, $-OS(O)NR^{2e}R^{2e'}$, $-OS(O)_2NR^{2e}R^{2e'}$, $-NR^{2e}R^{2e'}$, $-NR^{2e}C(O)R^{3e}$, $-NR^{2e}C(O)OR^{3e}$, $-NR^{2e}C(O)NR^{3e}R^{3e'}$, $-NR^{2e}S(O)R^{3e}$, $-NR^{2e}S(O)_2R^{3e}$, $-NR^{2e}S(O)NR^{2e}R^{2e'}$, $-NR^{2e}S(O)_2NR^{3e}R^{3e'}$, $-C(O)R^{2e}$, $-C(O)OR^{2e}$ or $-C(O)NR^{2e}R^{2e}$;

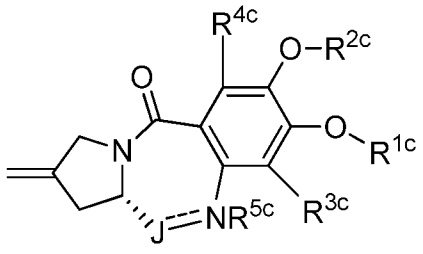
each R^{2e}, $R^{2e'}$, R^{3e} and $R^{3e'}$ is independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl, wherein each hydrogen atom in C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl is optionally substituted by $-OR^{4e}$, $-SR^{4e}$ or $-NR^{4e}R^{4e'}$;

R^{4e} and $R^{4e'}$ are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, 3- to 7-membered heterocycloalkyl, C_6-C_{10} aryl and 5- to 7-membered heteroaryl;

v is 1, 2 or 3; and

each * represents a covalent bond to the rest of the conjugate.

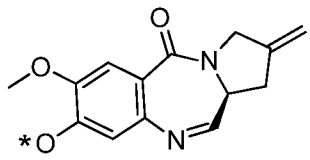
18. The conjugate of any one of the preceding claims, wherein the second drug is of the formula



,

or a pharmaceutically acceptable salt thereof.

19. The conjugate of any one of the preceding claims, wherein the second drug is of the formula



,

wherein * represents a covalent bond to the rest of the conjugate.

20. The conjugate of claim 8, having the formula B-$(L^1)_{z1}$-$(AA)_{z2}$-$(L^1)_{z3}$-$(AA)_{z4}$-$(L^1)_{z5}$-$(AA)_{z6}$-$(L^2)_{z7}$-$(L^r)_{z8}$-$(L^2)_{z9}$-D-L^3-D-$(L^2)_{y9}$-$(L^r)_{y8}$-$(L^2)_{y7}$-$(AA)_{y6}$-$(L^1)_{y5}$-$(AA)_{y4}$-$(L^1)_{y3}$-$(AA)_{y2}$-$(L^1)_{y1}$-X,

wherein

z1 is an integer from 0 to 2, z2 is an integer from 0 to 3, z3 is an integer from 0 to 2, z4 is an integer from 0 to 3, z5 is an integer from 0 to 2, z6 is an integer from 0 to 3, z7 is an integer from 0 to 8, z8 is 0 or 1, z9 is an integer from 0 to 8, y1 is an integer from 0 to 2, y2 is an integer from 0 to 3, y3 is an integer from 0 to 2, y4 is an integer from 0 to 3, y5 is an integer from 0 to 2, y6 is 0 or 1, y7 is an integer from 0 to 8, y8 is 0 or 1; y9 is an integer from 0 to 8;

each D is independently D^1 or D^2;

X is H or B;

each B is independently a binding ligand;

each AA is independently an amino acid;

each L^1 is independently a first spacer linker;

each L^2 is independently a second spacer linker;

each L^3 is independently a third spacer linker; and

each L^r is independently a releasable linker;

or a pharmaceutically acceptable salt thereof.

21. The conjugate of claim 20, or a pharmaceutically acceptable salt thereof, wherein y1 is 0, y2 is 0, y3 is 0, y4 is 0, y5 is 0, y6 is 0, y7 is 0, y8 is 0, y9 is 0 and X is H.

22. The conjugate of claim 20 or 21, or a pharmaceutically acceptable salt thereof, wherein z1 is 0, z2 is 2, z3 is 0, z4 is 1, z5 is 0 and z6 is 1.

23. The conjugate of claim 20 or 21, or a pharmaceutically acceptable salt thereof, wherein z1 is 0, z2 is 2, z3 is 0, z4 is 2, z5 is 0 and z6 is 1.

24. The conjugate of claim 20 or 21, or a pharmaceutically acceptable salt thereof, wherein z1 is 1, z2 is 1, z3 is 1, z4 is 1, z5 is 1 and z6 is 1.

25. The conjugate of claim 20 or 21, or a pharmaceutically acceptable salt thereof, wherein z1 is 1, z2 is 1, z3 is 1, z4 is 1, z5 is 1 and z6 is 0.

26. The conjugate of claim 20, or a pharmaceutically acceptable salt thereof, wherein z1 is 0, z2 is 2, z3 is 0, z4 is 1, z5 is 0, z6 is 1, y1 is 0, y2 is 2, y3 is 0, y4 is 1, y5 is 0 and y6 is 1.

27. The conjugate of claim 20, or a pharmaceutically acceptable salt thereof, wherein z1 is 0, z2 is 2, z3 is 0, z4 is 2, z5 is 0, z6 is 1, y1 is 0, y2 is 2, y3 is 0, y4 is 2, y5 is 0 and y6 is 1.

28. The conjugate of claim 26 or 27, or a pharmaceutically acceptable salt thereof, wherein y7 is 1.

29. The conjugate of claim 28, or a pharmaceutically acceptable salt thereof, wherein y8 is 0.

30. The conjugate of claim 29, or a pharmaceutically acceptable salt thereof, wherein y9 is 0.

31. The conjugate of claim 26 or 27, or a pharmaceutically acceptable salt thereof, wherein y7 is 0.

32. The conjugate of claim 31, or a pharmaceutically acceptable salt thereof, wherein y8 is 1.

33. The conjugate of claim 32, or a pharmaceutically acceptable salt thereof, wherein y9 is 0.

34. The conjugate of claim 26 or 27, or a pharmaceutically acceptable salt thereof, wherein y8 is 0.

35. The conjugate of any one of claims 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 6.

36. The conjugate of any one of claims 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 5.

37. The conjugate of any one of claims 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 4.

38. The conjugate of any one of claims 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 3.

39. The conjugate of any one of claims 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 2.

40. The conjugate of any one of claims 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 1.

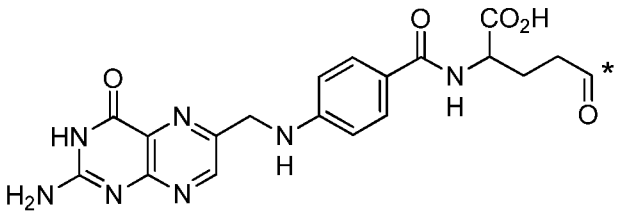
41. The conjugate of any one of claims 20 to 27, or a pharmaceutically acceptable salt thereof, wherein z7 is 0.

42. The conjugate of any one of claims 20 to 41, or a pharmaceutically acceptable salt thereof, wherein z8 is 1.

43. The conjugate of any one of claims 20 to 42, or a pharmaceutically acceptable salt thereof, wherein z8 is 0.

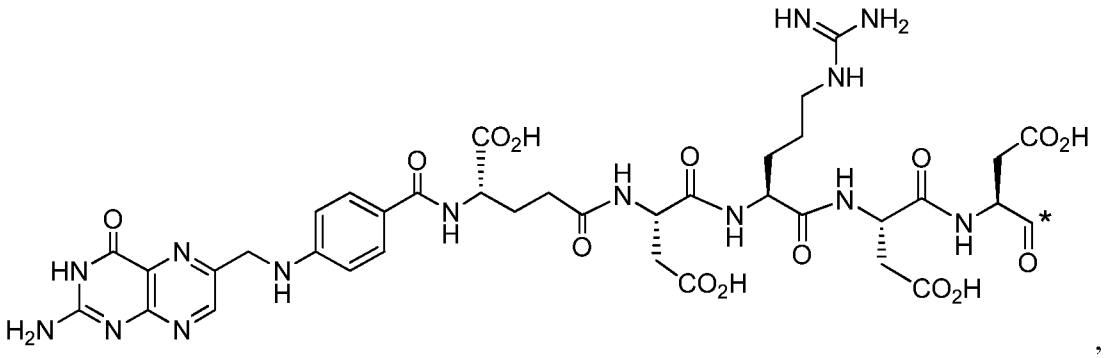
44. The conjugate of any one of claims 20 to 43, or a pharmaceutically acceptable salt thereof, wherein z9 is 0.

45. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, wherein B is of the formula



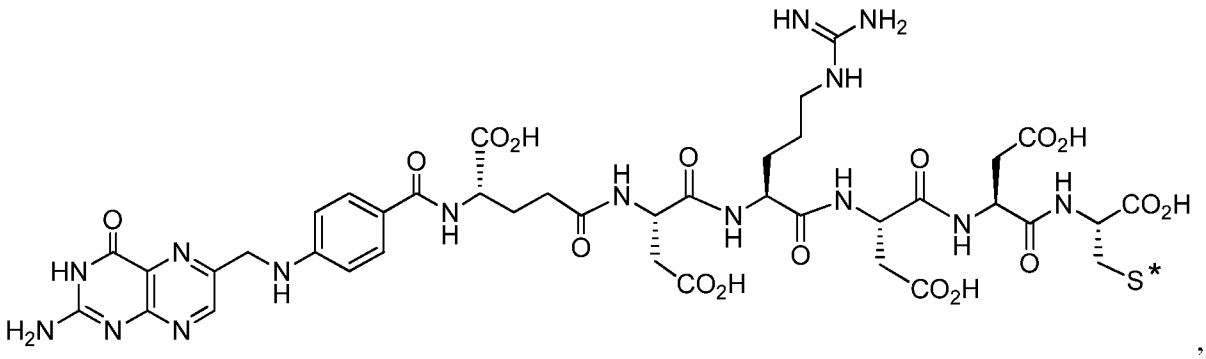
or a pharmaceutically acceptable salt thereof.

46. The conjugate of any one of claims 1 to 22, or a pharmaceutically acceptable salt thereof, comprising the formula



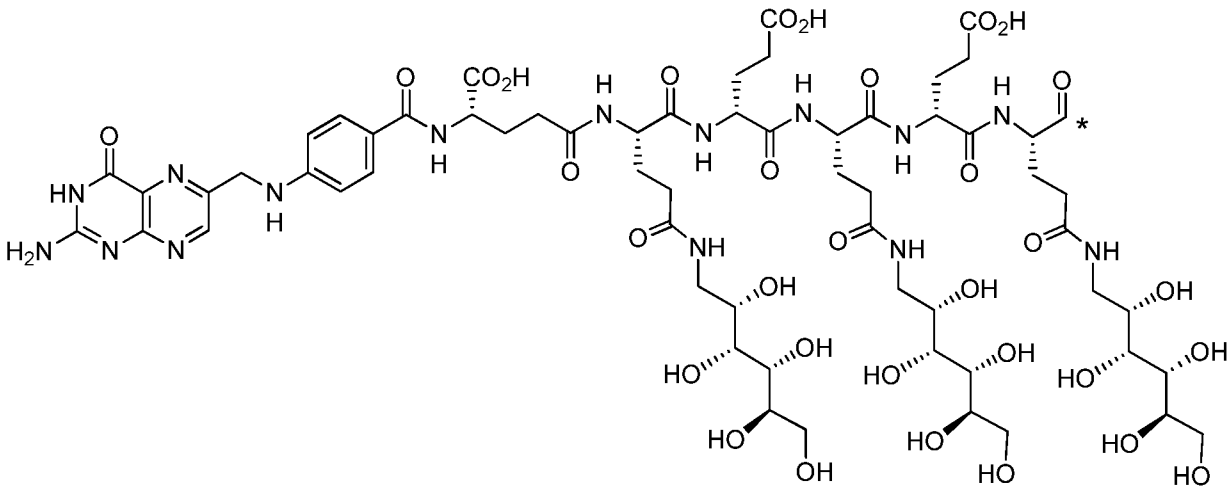
wherein * represents a covalent bond to the rest of the conjugate.

47. The conjugate of any one of claims 1 to 21 or 23, or a pharmaceutically acceptable salt thereof, comprising the formula



wherein * represents a covalent bond to the rest of the conjugate.

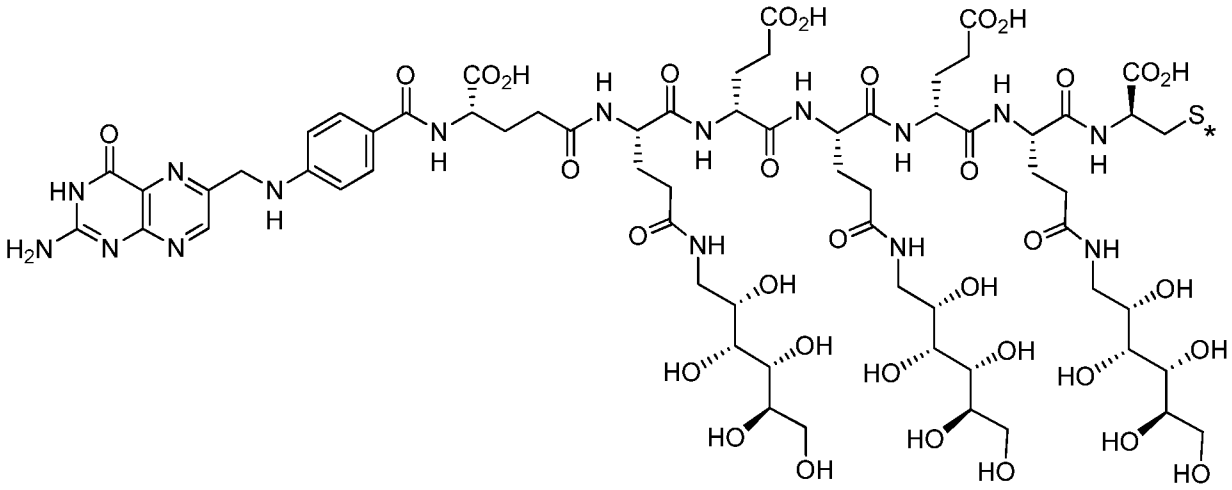
48. The conjugate of any one of claims 1 to 21 or 25, or a pharmaceutically acceptable salt thereof, comprising the formula



,

wherein * represents a covalent bond to the rest of the conjugate.

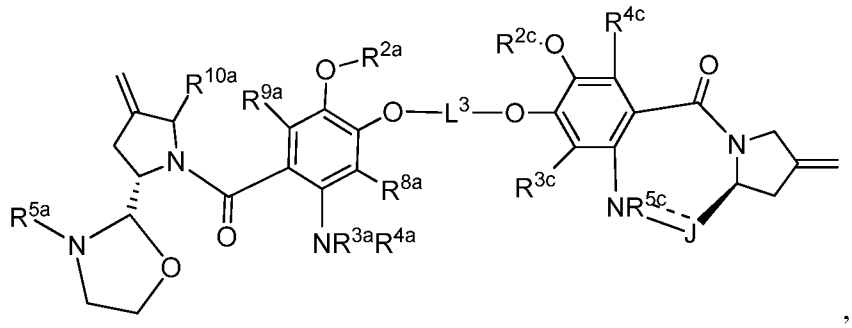
48. The conjugate of any one of claims 1 to 21 or 24, or a pharmaceutically acceptable salt thereof, comprising the formula



,

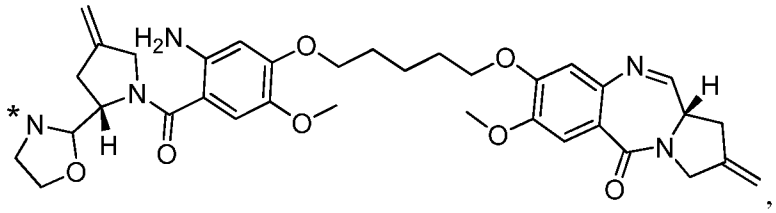
wherein * represents a covalent bond to the rest of the conjugate.

49. The conjugate of any one of the preceding claims, or a pharmaceutically acceptable salt thereof, comprising the formula



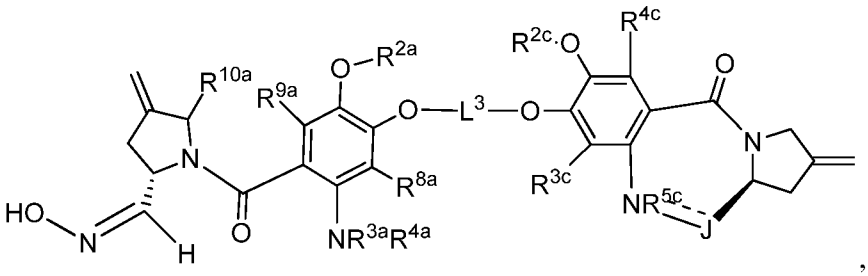
wherein R^{5a} is a covalent bond to the rest of the conjugate.

50. The conjugate of claim 49, any one of the preceding claims, or a pharmaceutically acceptable salt thereof, comprising the formula



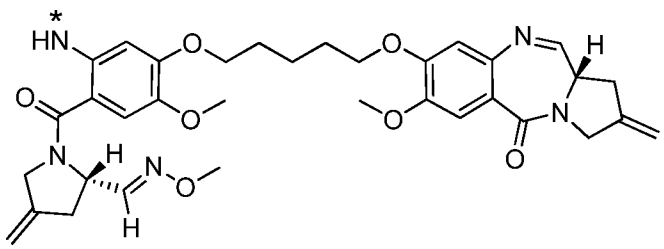
wherein * represents a covalent bond to the rest of the conjugate.

51. The conjugate of any one of claims 1 to 48, or a pharmaceutically acceptable salt thereof, comprising the formula



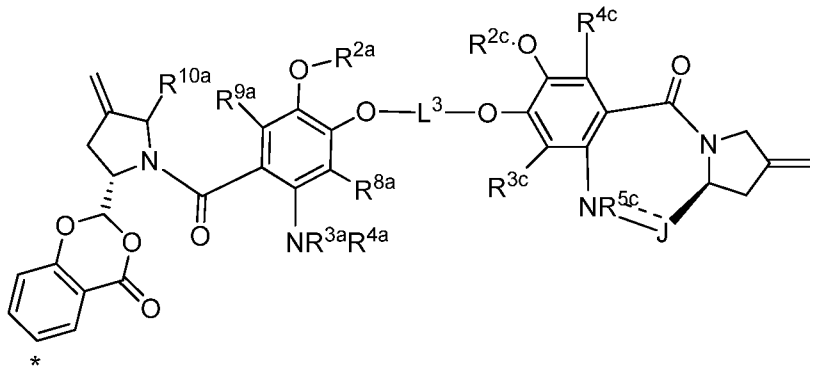
wherein R^{4a} is a covalent bond to the rest of the conjugate.

52. The conjugate of claim 51, or a pharmaceutically acceptable salt thereof, comprising the formula



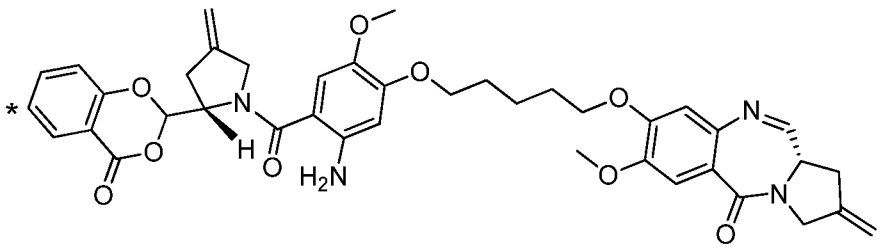
wherein * represents a covalent bond to the rest of the conjugate.

53. The conjugate of any one of claims 1 to 48, or a pharmaceutically acceptable salt thereof, comprising the formula



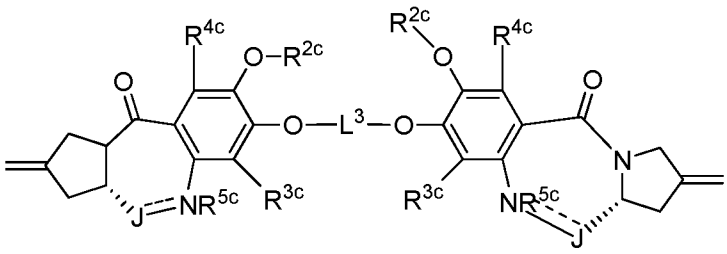
wherein * represents a covalent bond to the rest of the conjugate.

54. The conjugate of claim 53, or a pharmaceutically acceptable salt thereof, comprising the formula



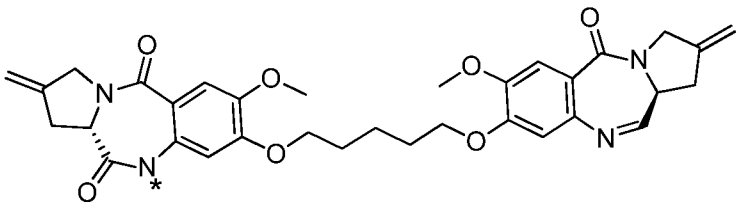
wherein * represents a covalent bond to the rest of the conjugate.

55. The conjugate of any one of claims 1 to 48, or a pharmaceutically acceptable salt thereof, comprising the formula

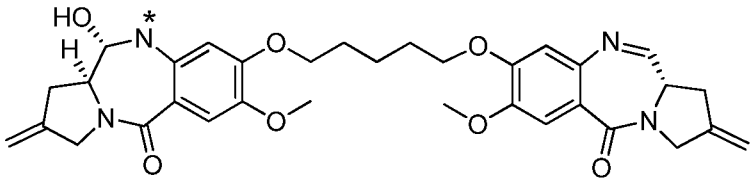


wherein at least one R^{5c} is a covalent bond to the rest of the conjugate.

56. The conjugate of claim 55, or a pharmaceutically acceptable salt thereof, comprising the formula



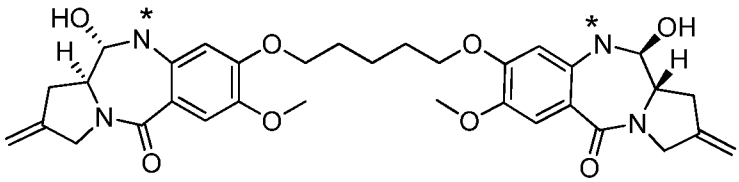
or



,

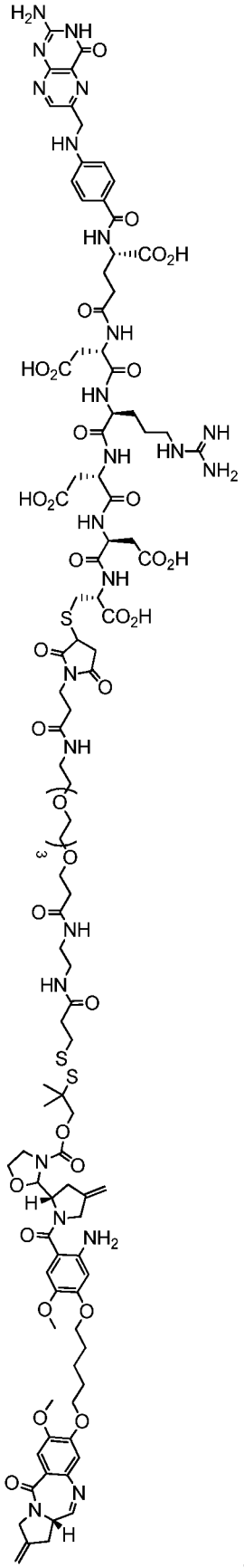
wherein * represents a covalent bond to the rest of the conjugate.

57. The conjugate of claim 55, or a pharmaceutically acceptable salt thereof, comprising the formula



wherein * represents a covalent bond to the rest of the conjugate.

58. A conjugate selected from the group consisting of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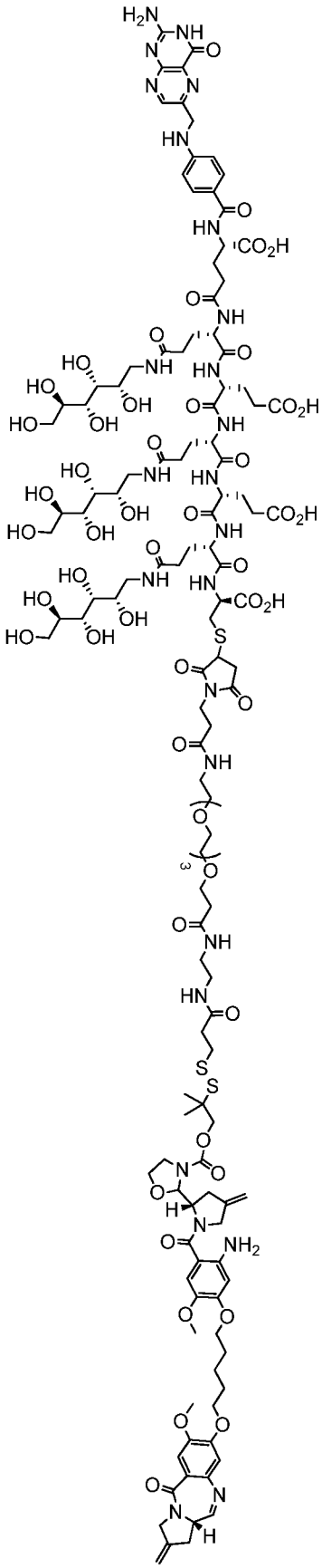
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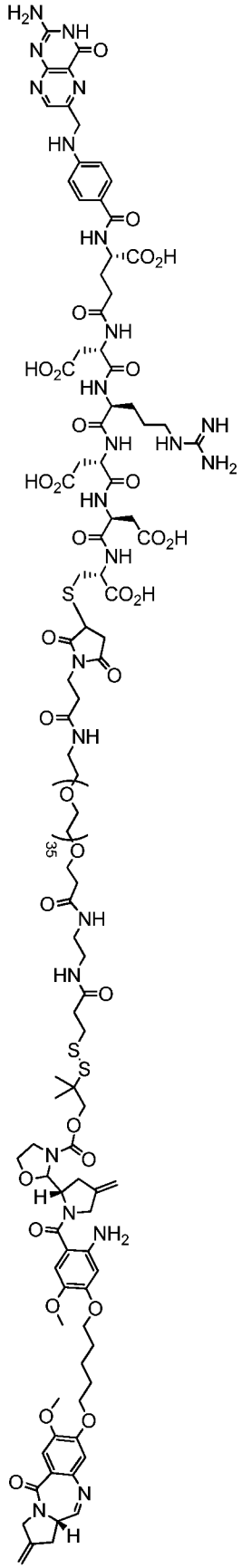
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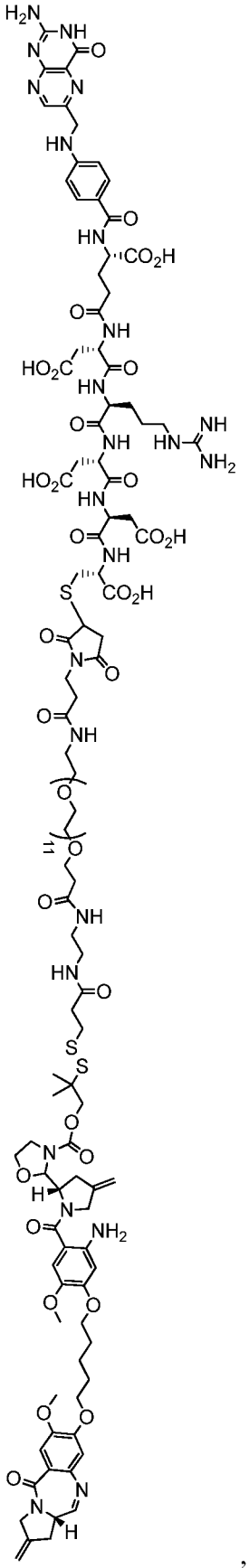
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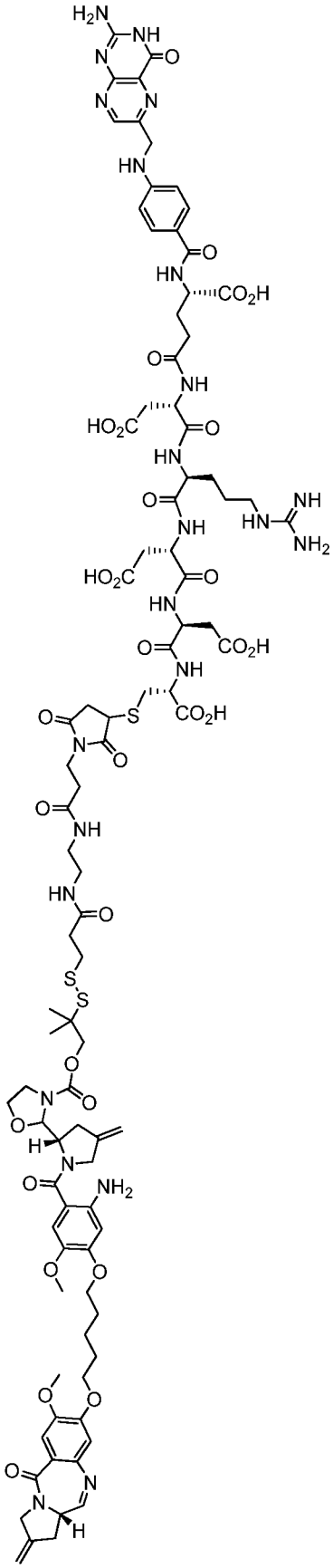

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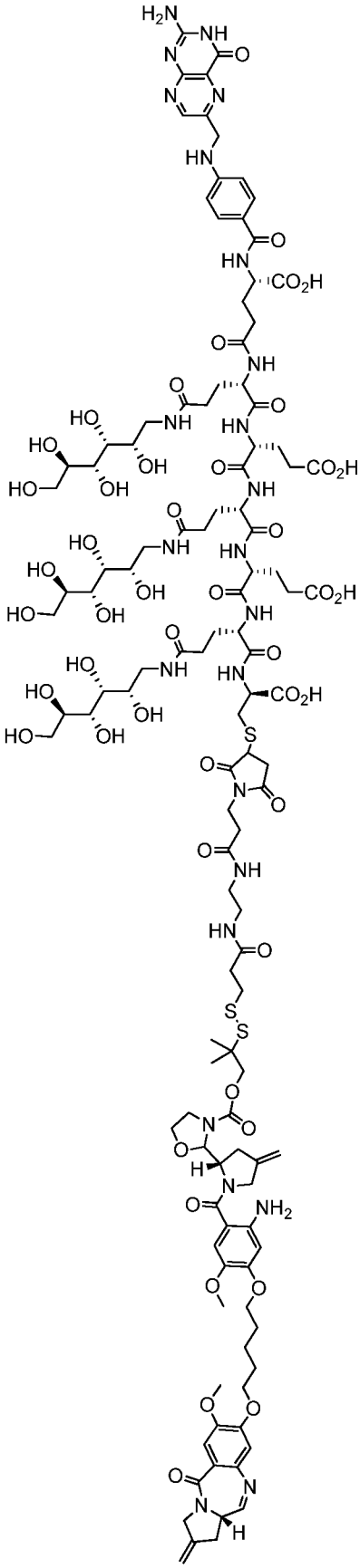

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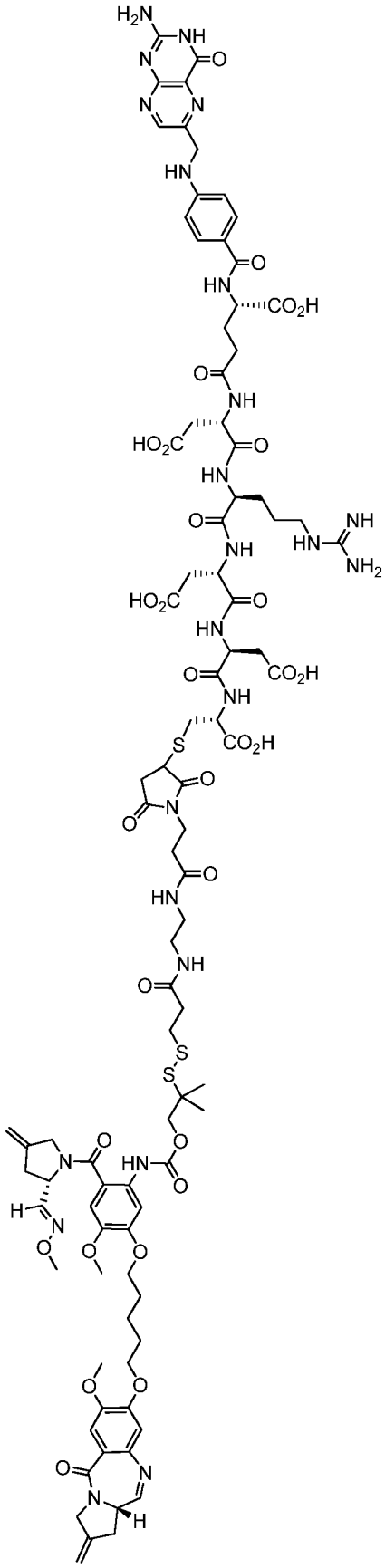

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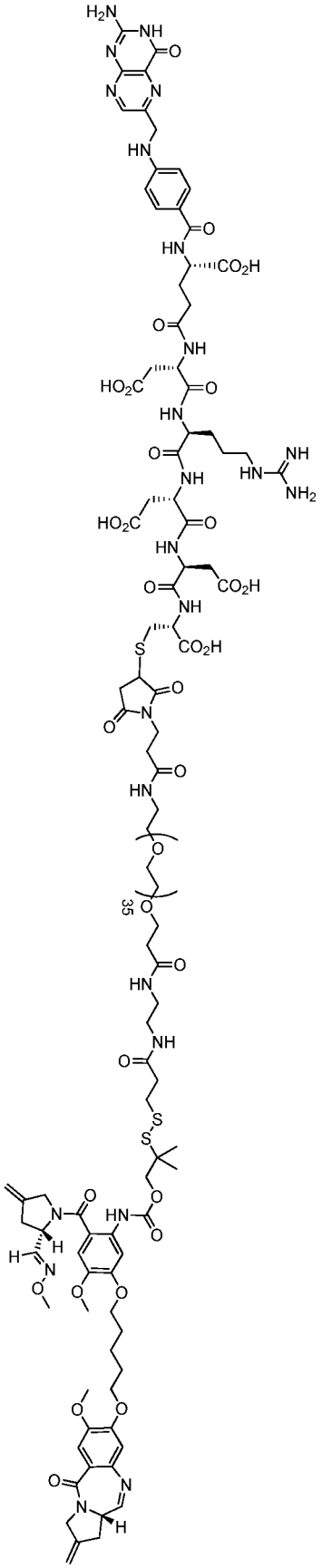

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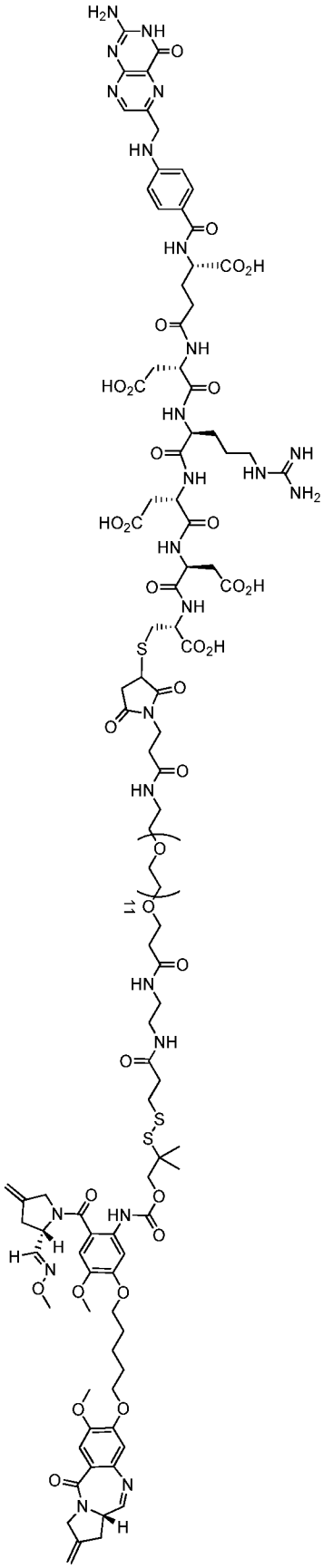

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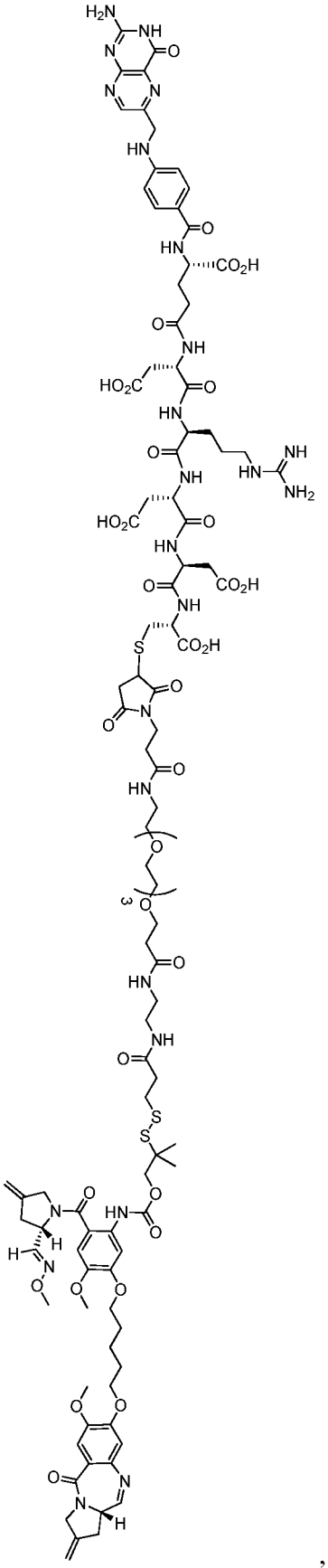

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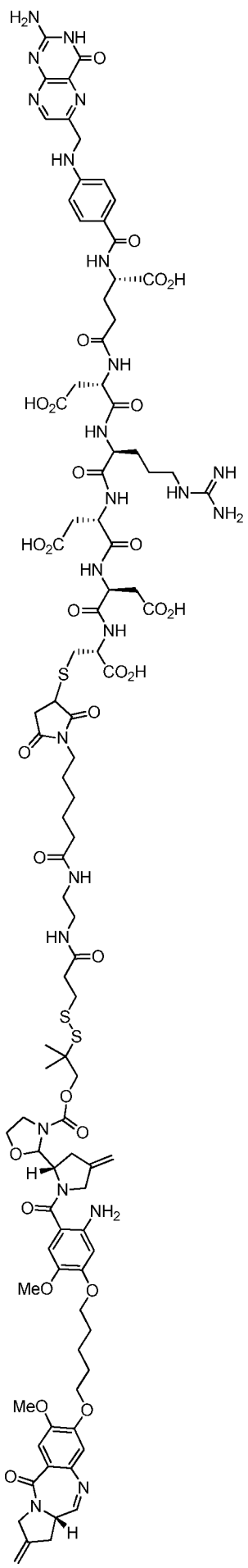

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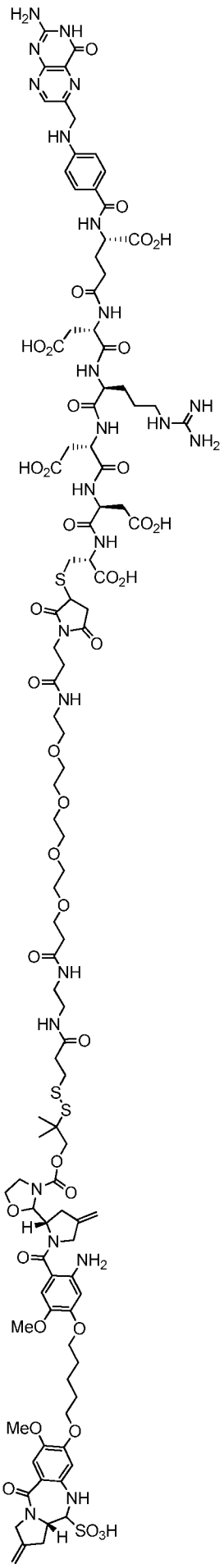

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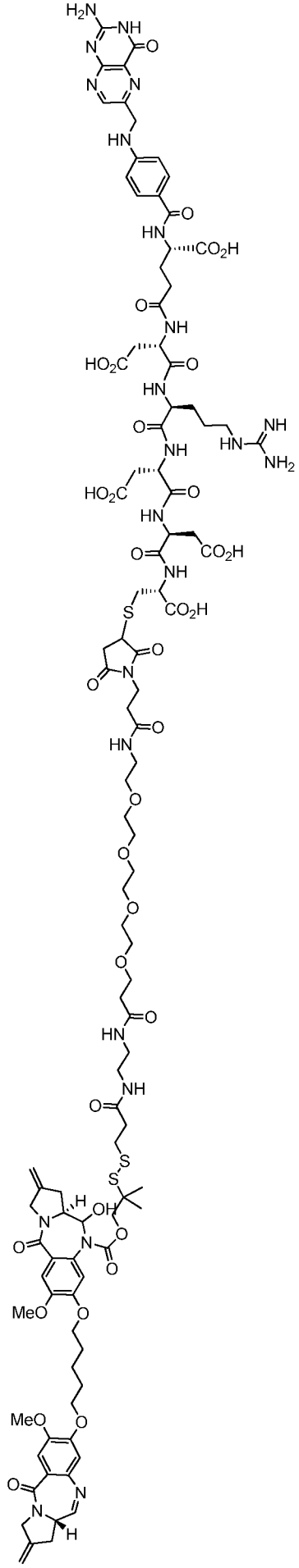

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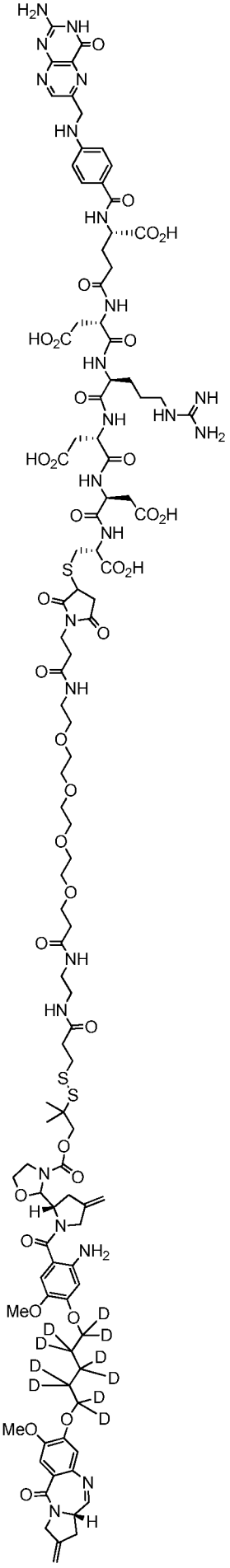

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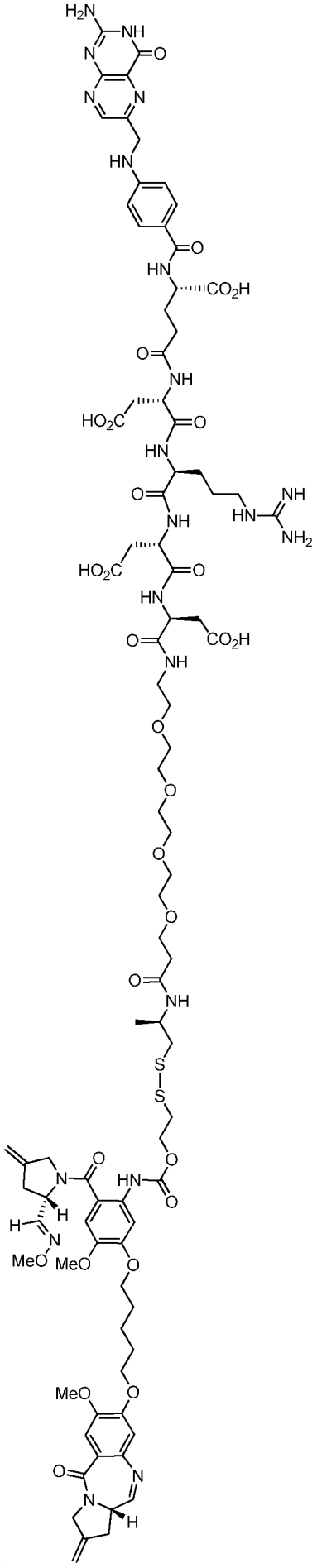
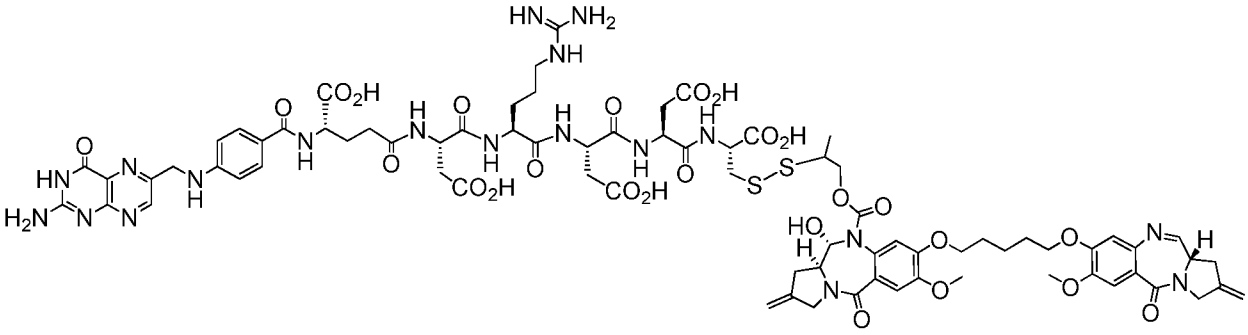


,

or a pharmaceutically acceptable salt thereof.

59. A conjugate selected from the group consisting of



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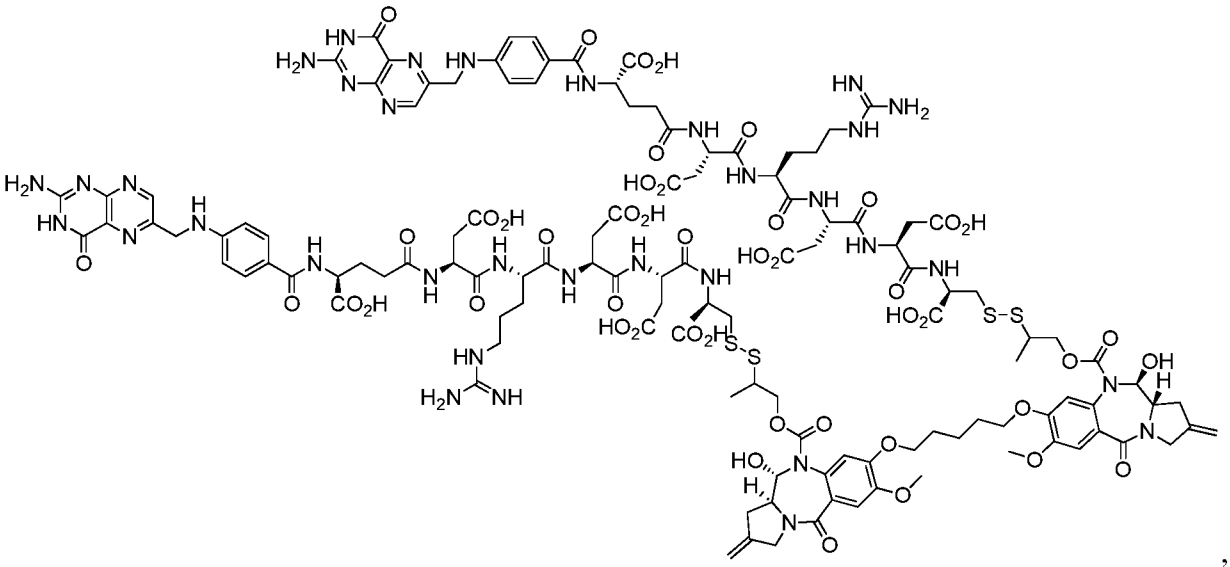

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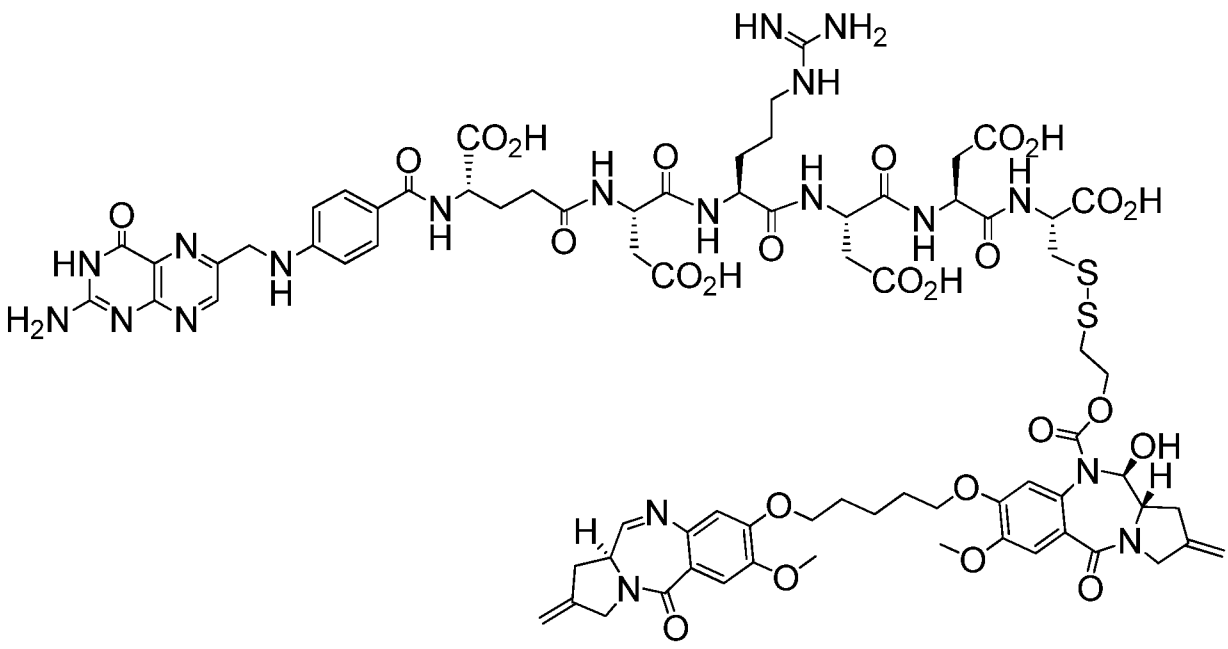

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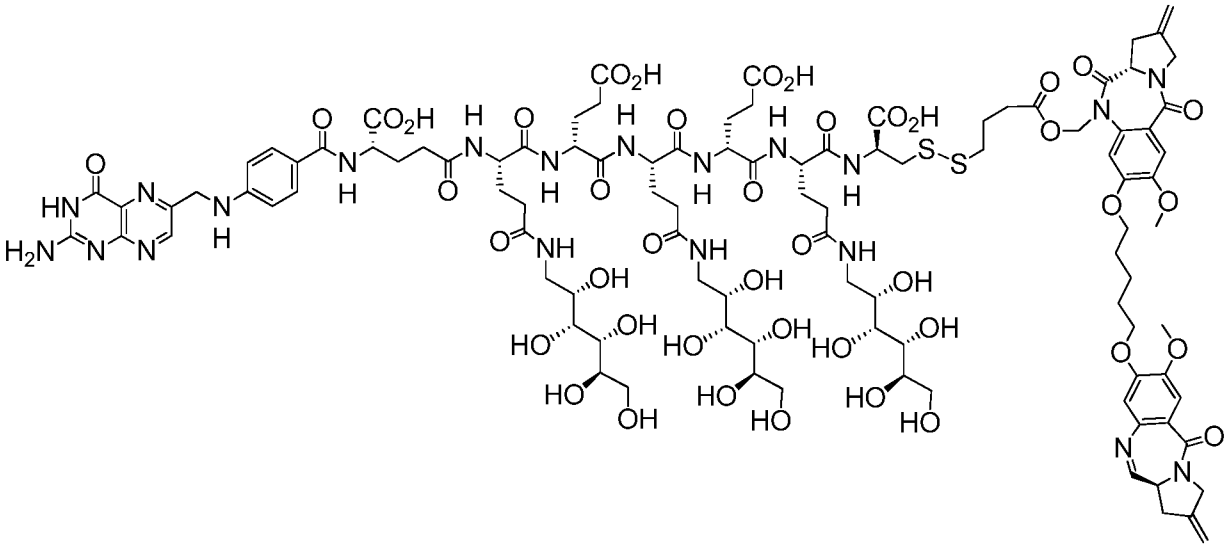

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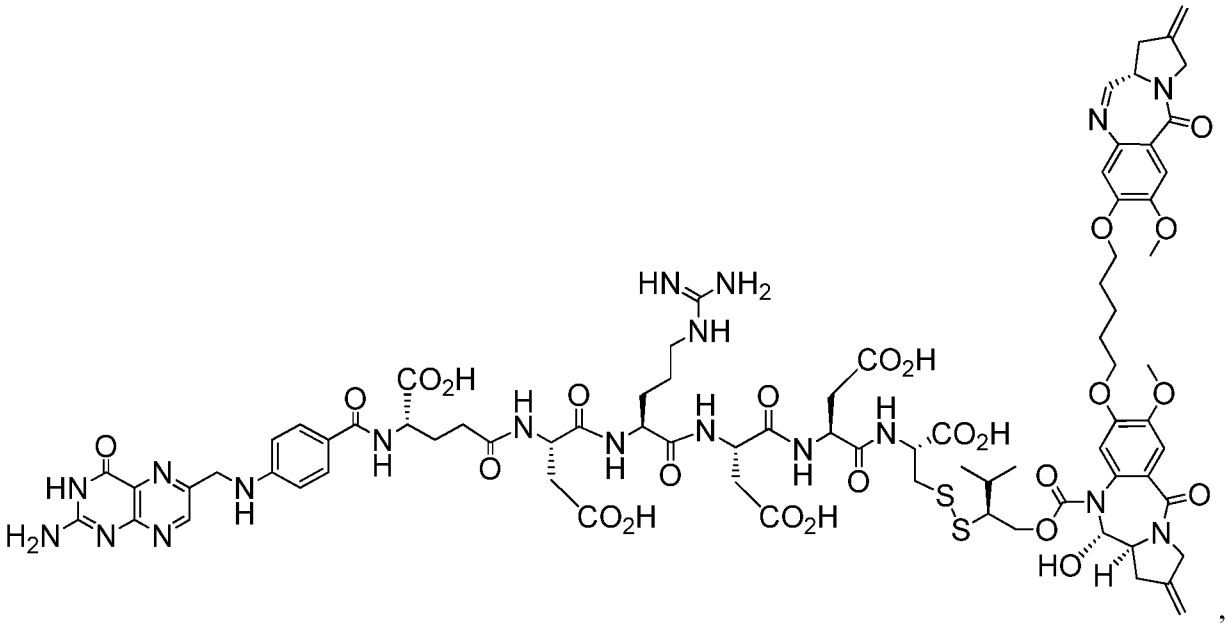
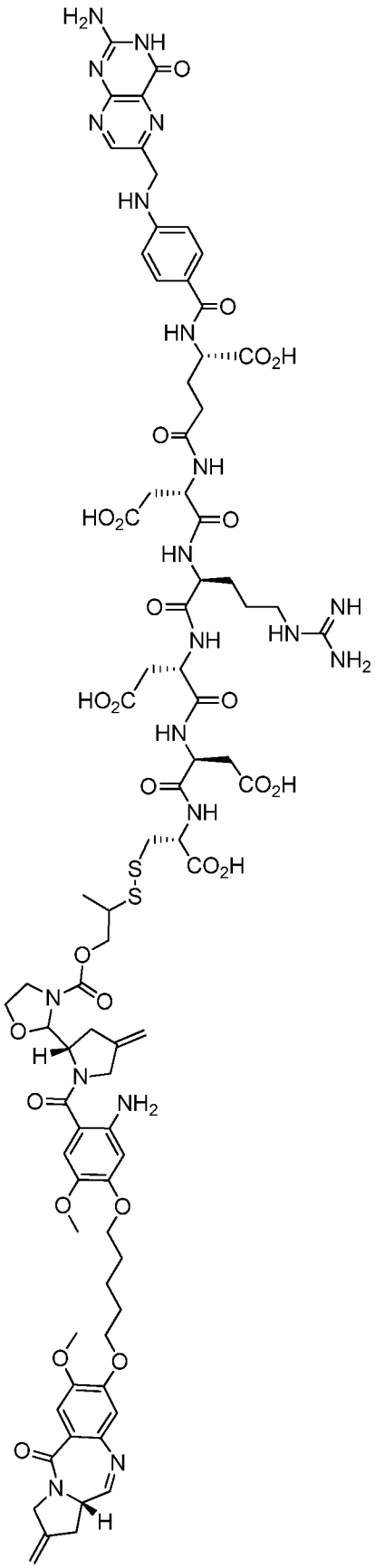
and



,

or a pharmaceutically acceptable salt thereof.

60. A conjugate selected from the group consisting of



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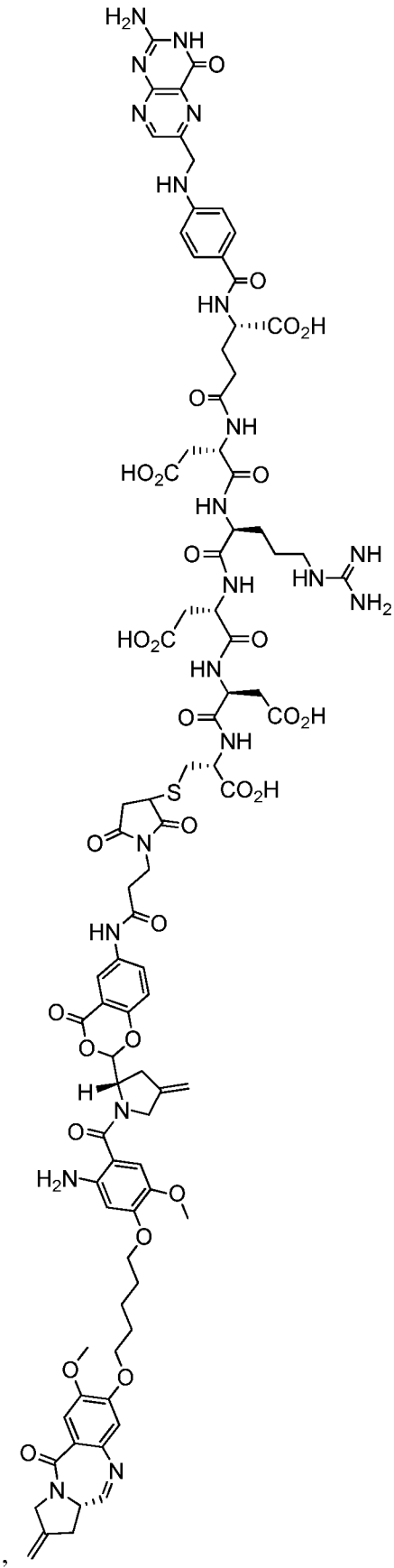

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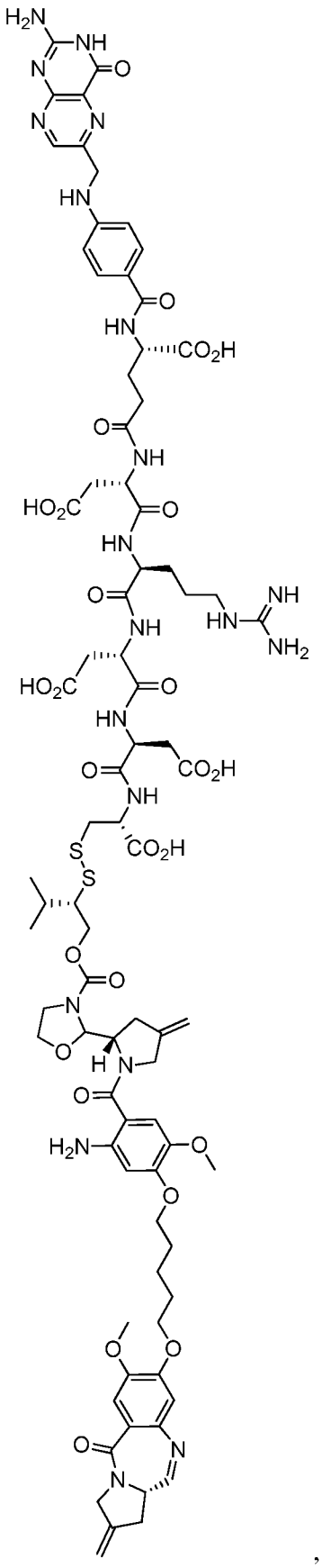

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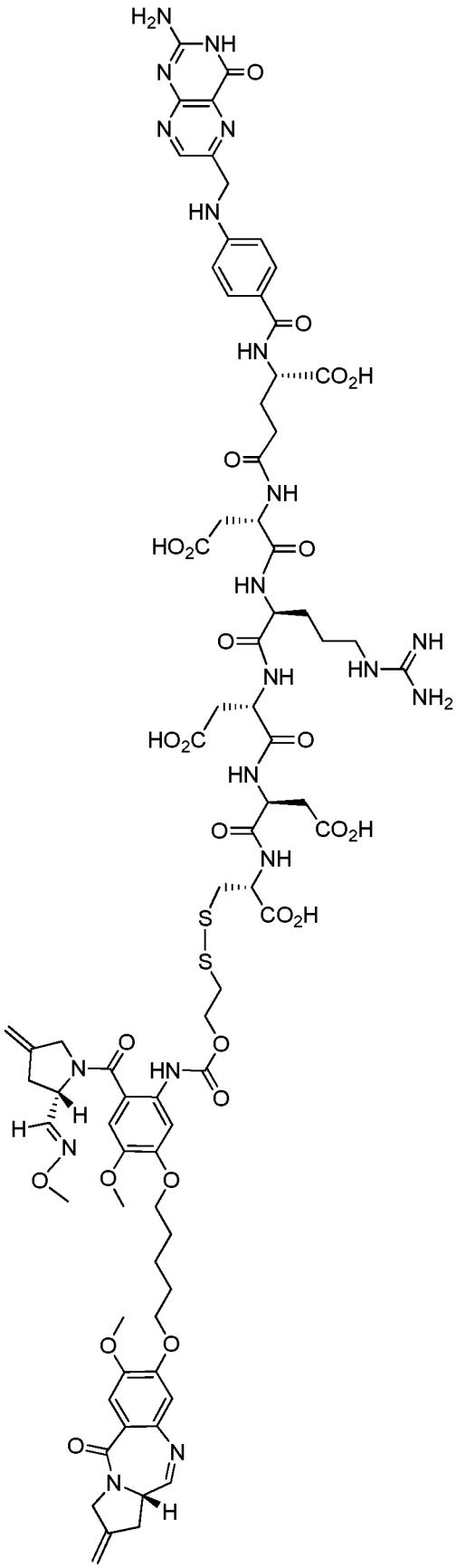

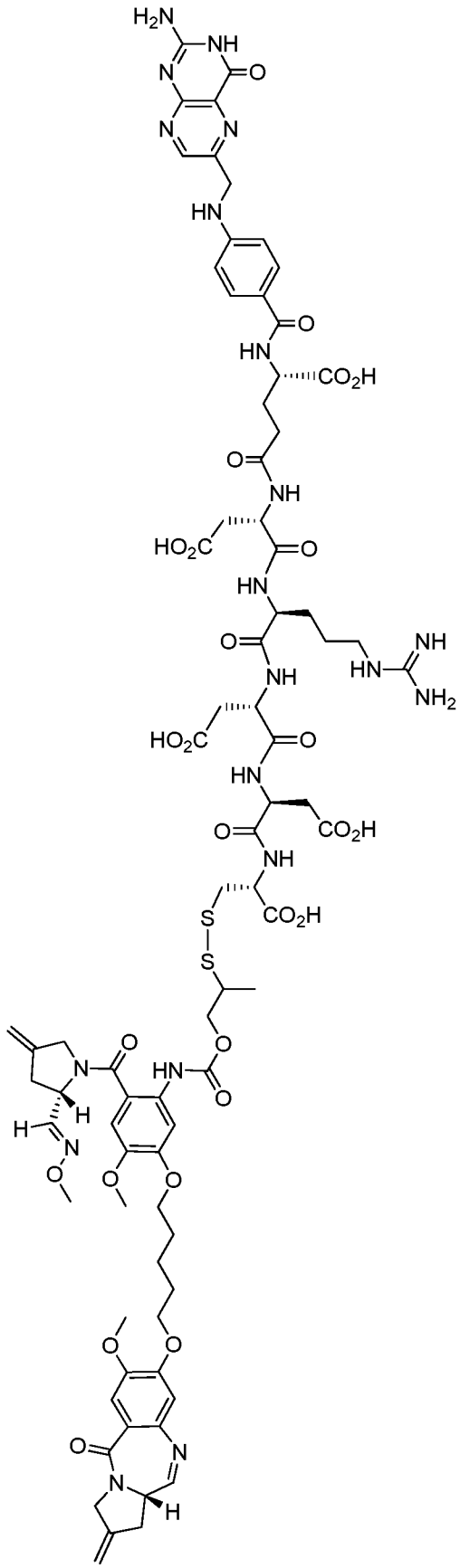

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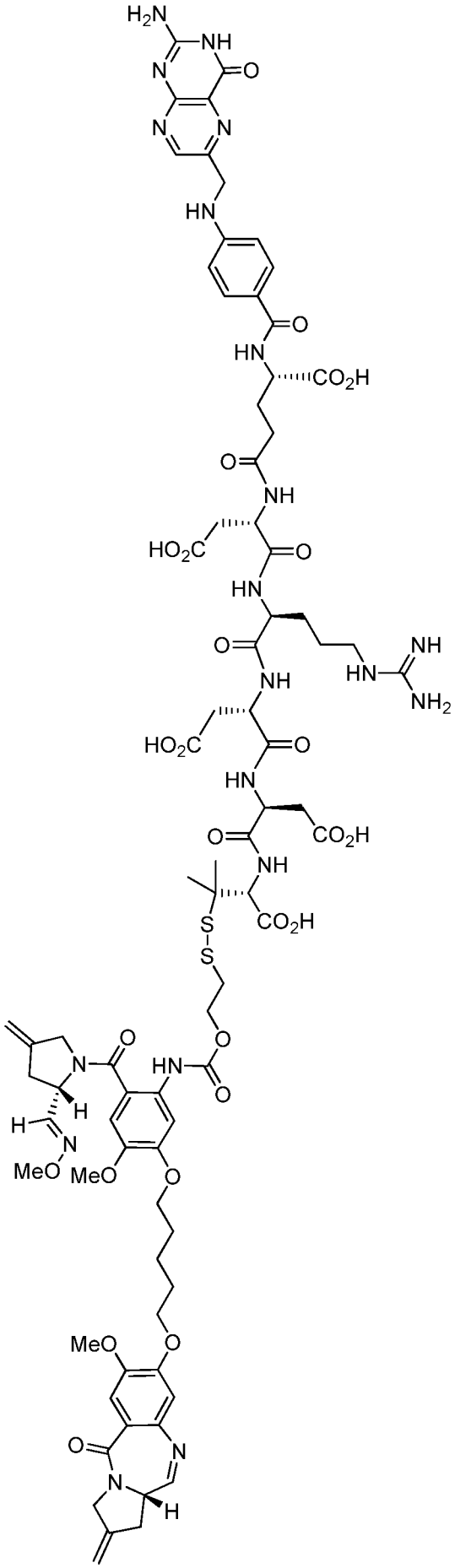

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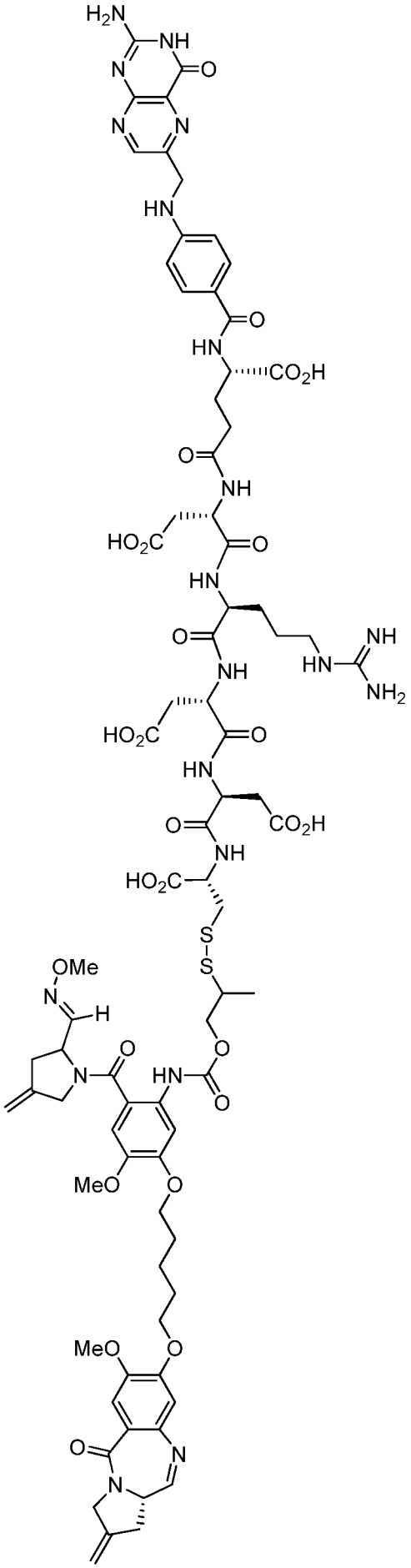

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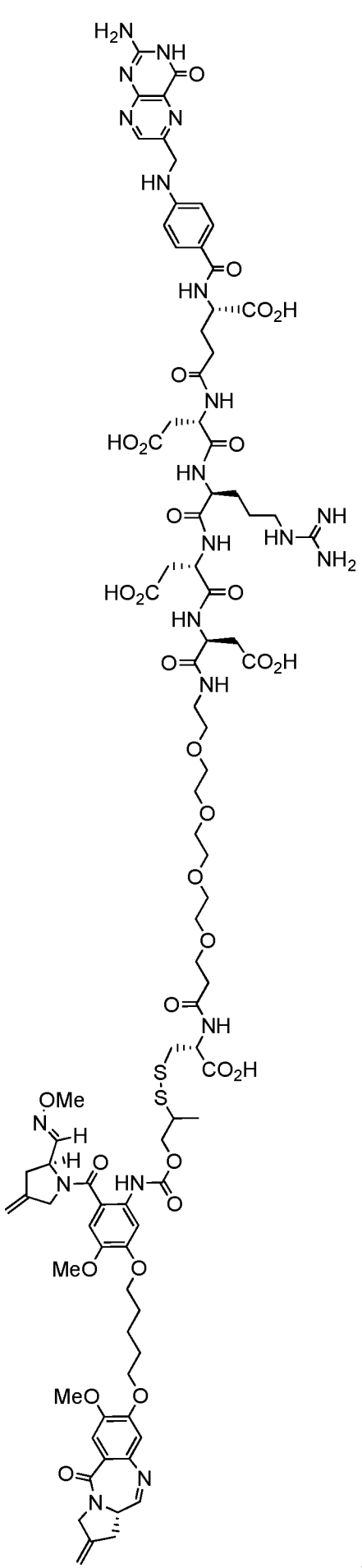

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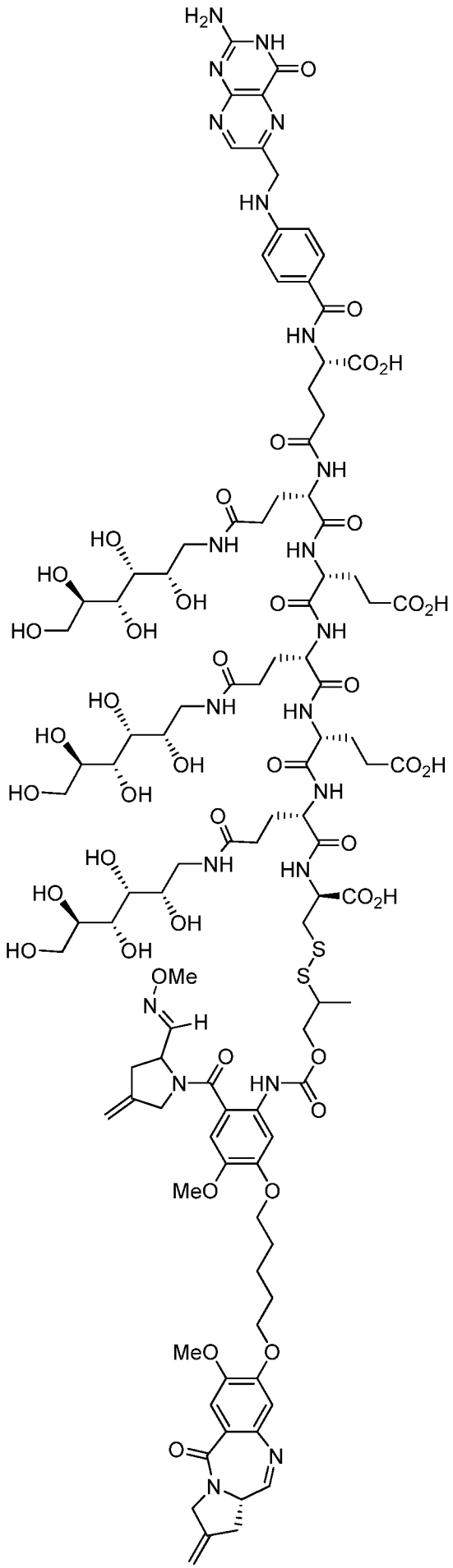
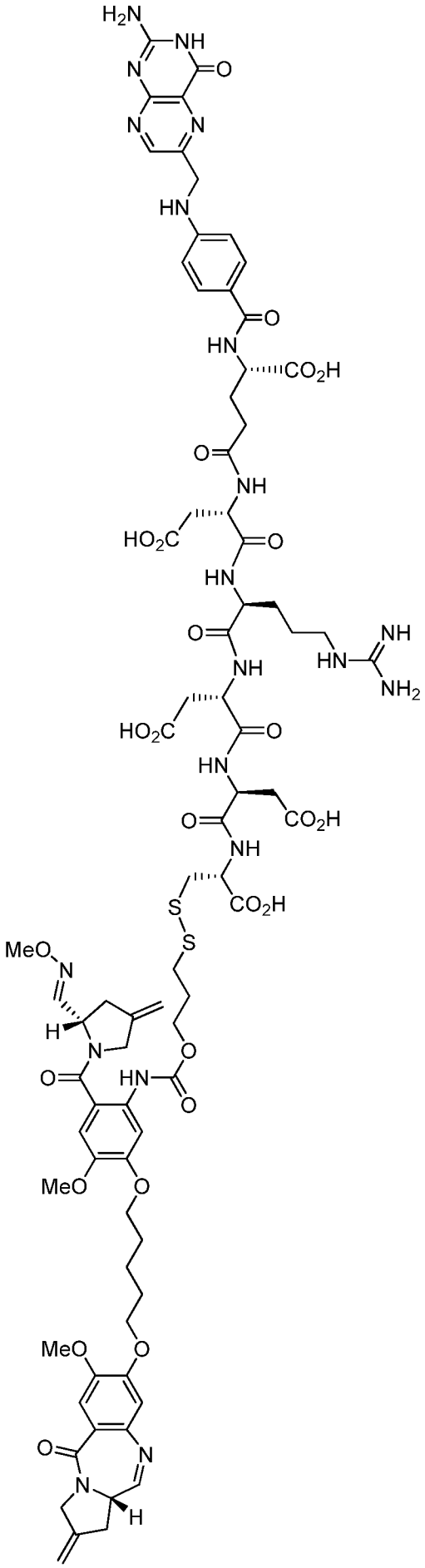


and



,

or a pharmaceutically acceptable salt thereof.

61. A pharmaceutical composition comprising a therapeutically effective amount of a conjugate according to any one of the preceding claims, or a pharmaceutically acceptable salt thereof, and optionally at least one pharmaceutically acceptable excipient.

62. A method of treating abnormal cell growth in a patient, comprising

a. administering to the patient a therapeutically effective amount of a conjugate, or a pharmaceutically acceptable salt thereof, or pharmaceutical composition, of any one of the preceding claims.

63. The method of claim 62, wherein the abnormal cell growth is cancer

64. The method of claim 63. wherein the cancer is selected from the group consisting of lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, triple negative breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma and pituitary adenoma.

65. Use of a conjugate according to any one of claims 1 to 60 in the preparation of a medicament for the treatment of cancer.

66. A conjugate according to any one of claims 1 to 60 for use in a method of treating cancer in a patient.

67. The conjugate of claim 66, where the method comprises administering to the patient a therapeutically effective amount of a conjugate, or a pharmaceutically acceptable salt thereof.

68. The conjugate of claim 67, wherein the cancer is selected from the group consisting of lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, triple negative breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma and pituitary adenoma.

FIG. 1

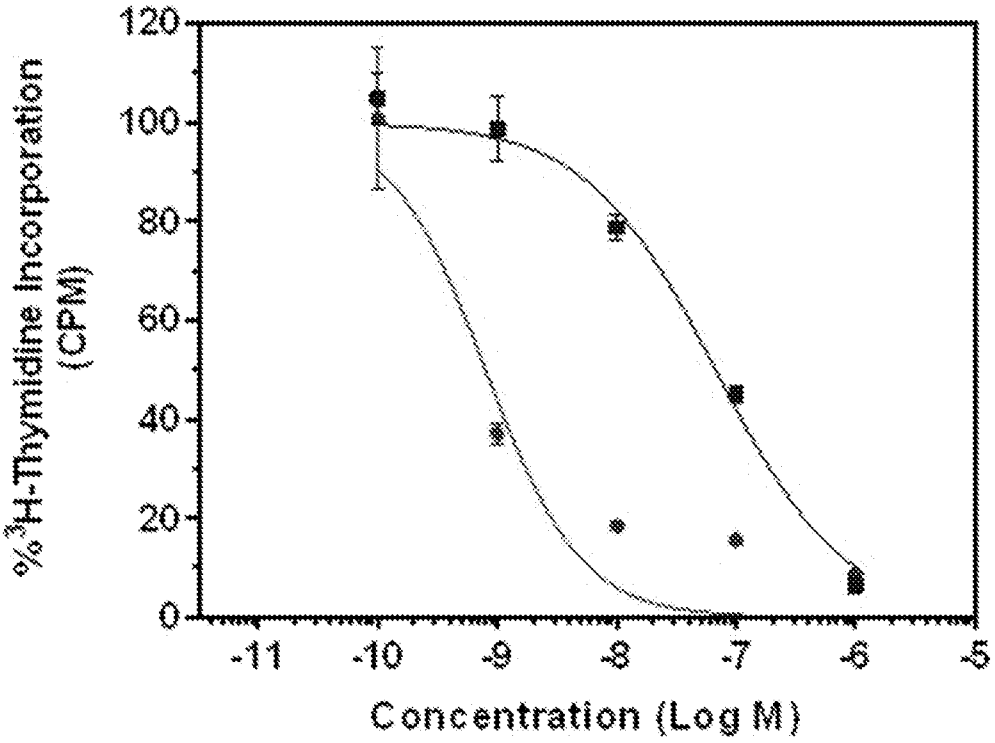

120
100
80
60
40
20
0
% ^{3}H-Thymidine Incorporation (CPM)
-11
-10
-9
-8
-7
-6
-5
Concentration (Log M)

FIG 2A

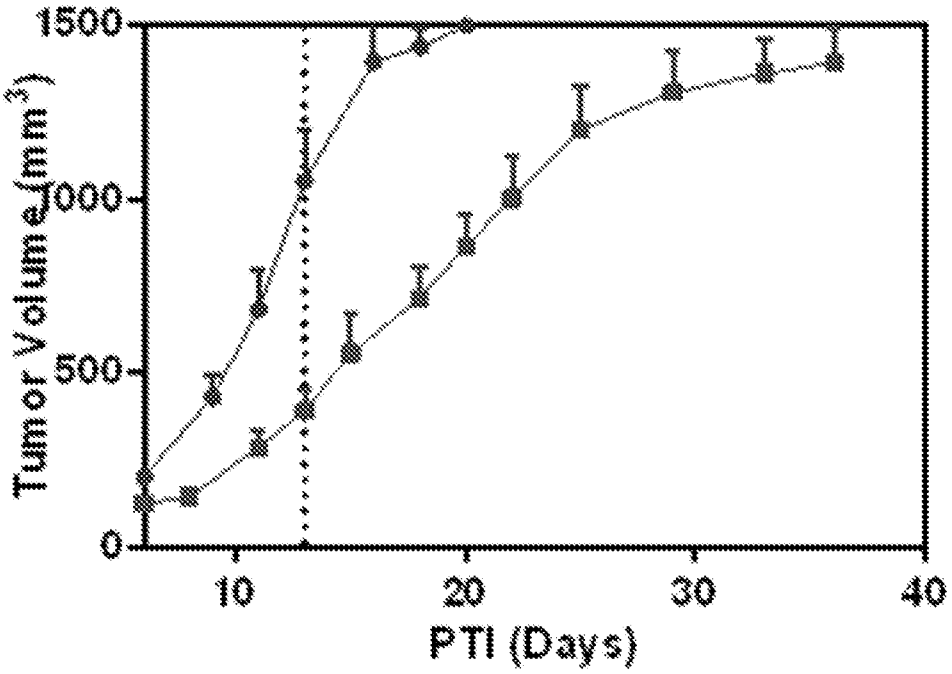

1500
1000
500
0
Tumor Volume (mm^3)
10
20
30
40
PTI (Days)

FIG. 2B

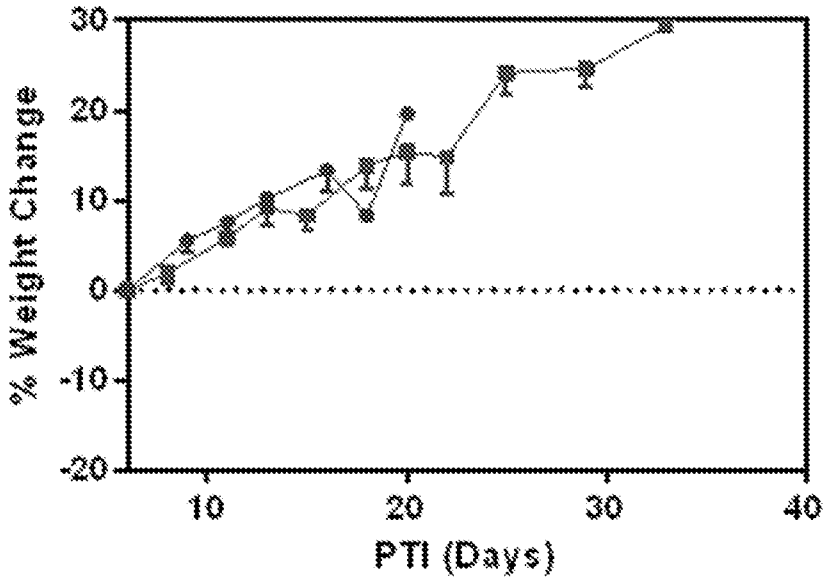

% Weight Change
30
20
10
0
-10
-20
10
20
30
40
PTI (Days)

FIG. 3

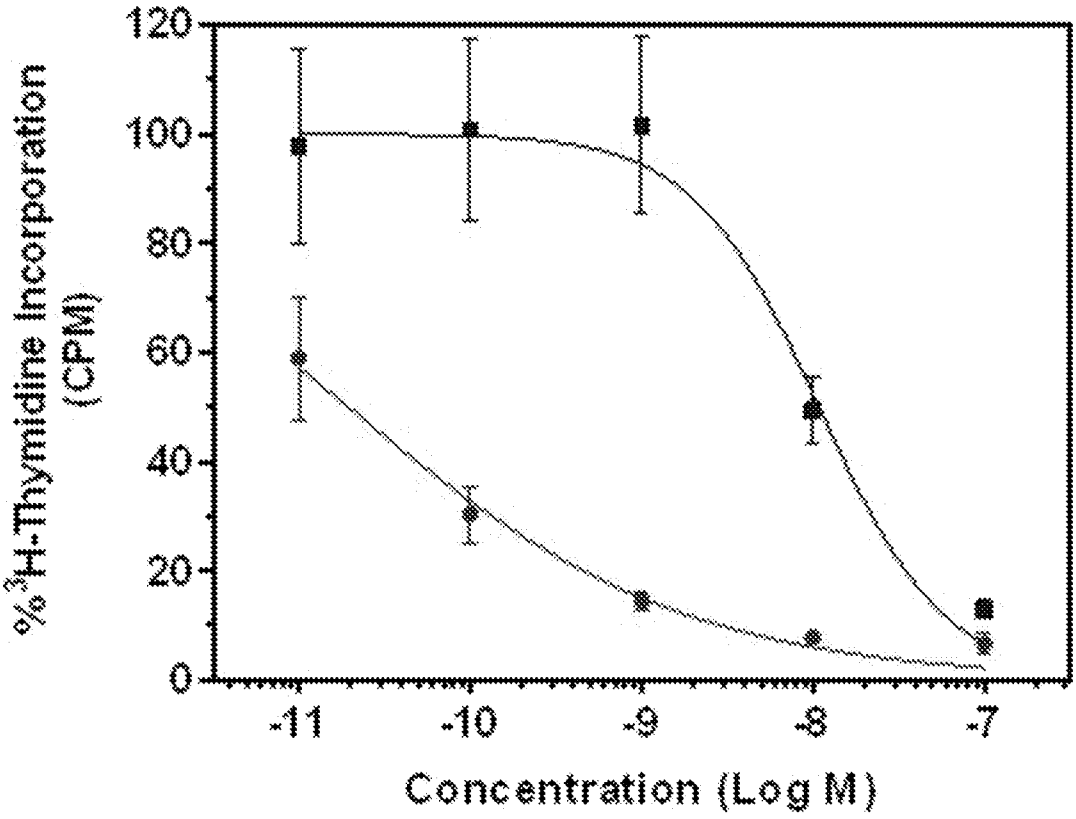

% ^{3}H-Thymidine Incorporation (CPM)
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
Concentration (Log M)

FIG. 4A

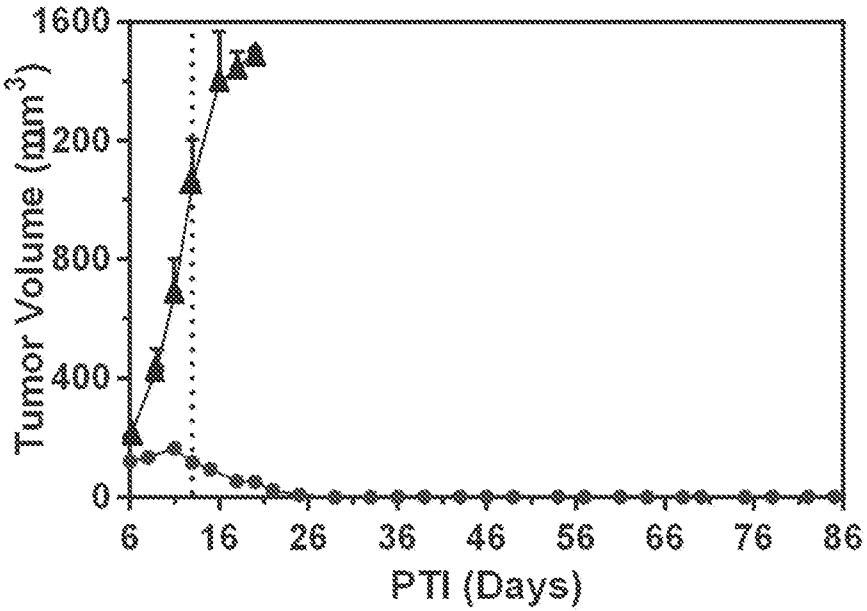

Tumor Volume (mm3)
1600
1200
800
400
0
6
16
26
36
46
56
66
76
86
PTI (Days)

FIG. 4B

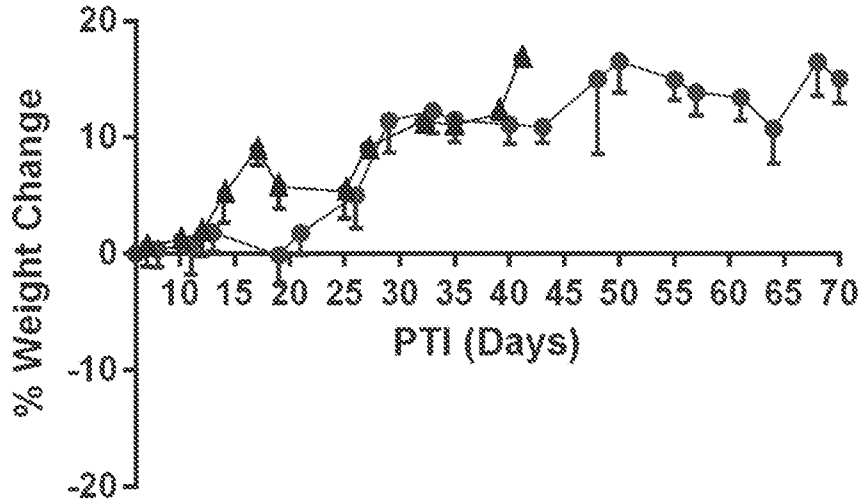

% Weight Change
20
10
0
-10
-20
10
15
20
25
30
35
40
45
50
55
60
65
70
PTI (Days)

FIG. 5

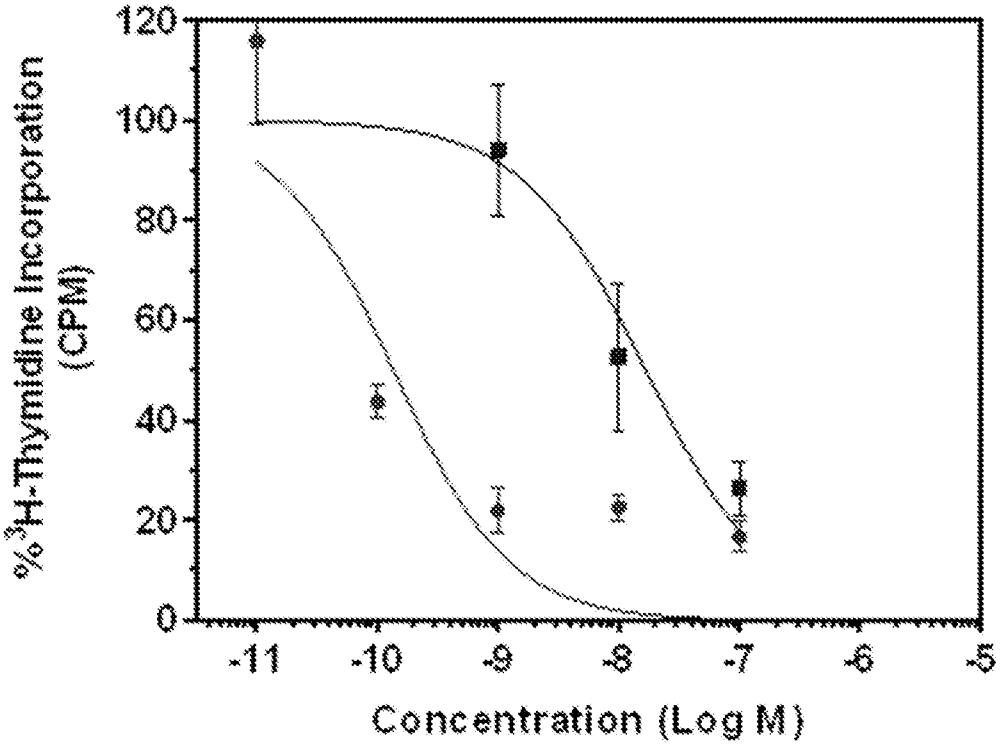

% ^{3}H-Thymidine Incorporation (CPM)
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
-5
Concentration (Log M)

FIG. 6A

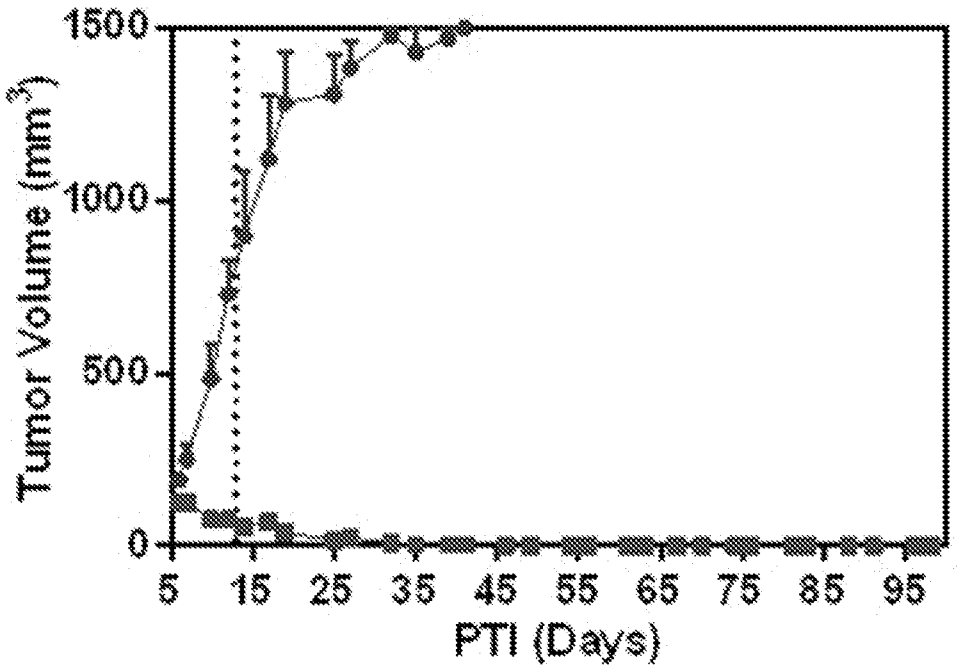

Tumor Volume (mm^3)
1500
1000
500
0
5
15
25
35
45
55
65
75
85
95
PTI (Days)

FIG. 6B

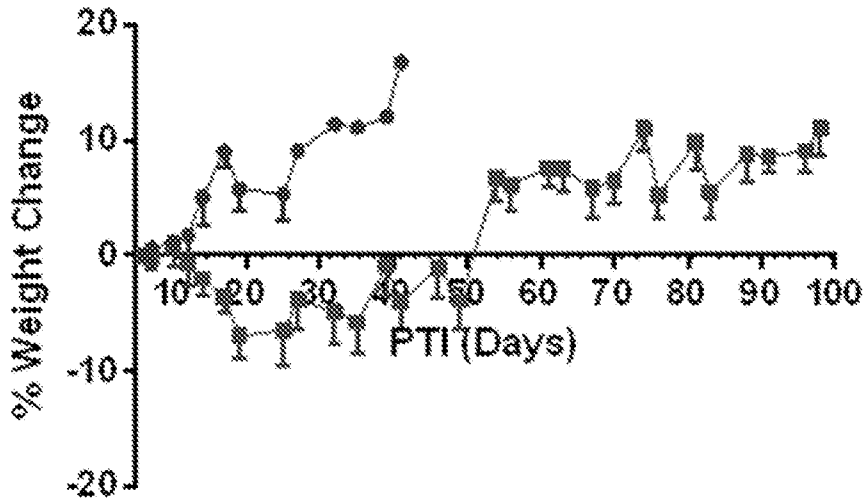

% Weight Change
20
10
0
-10
-20
10
20
30
40
50
60
70
80
90
100
PTI (Days)

FIG. 7

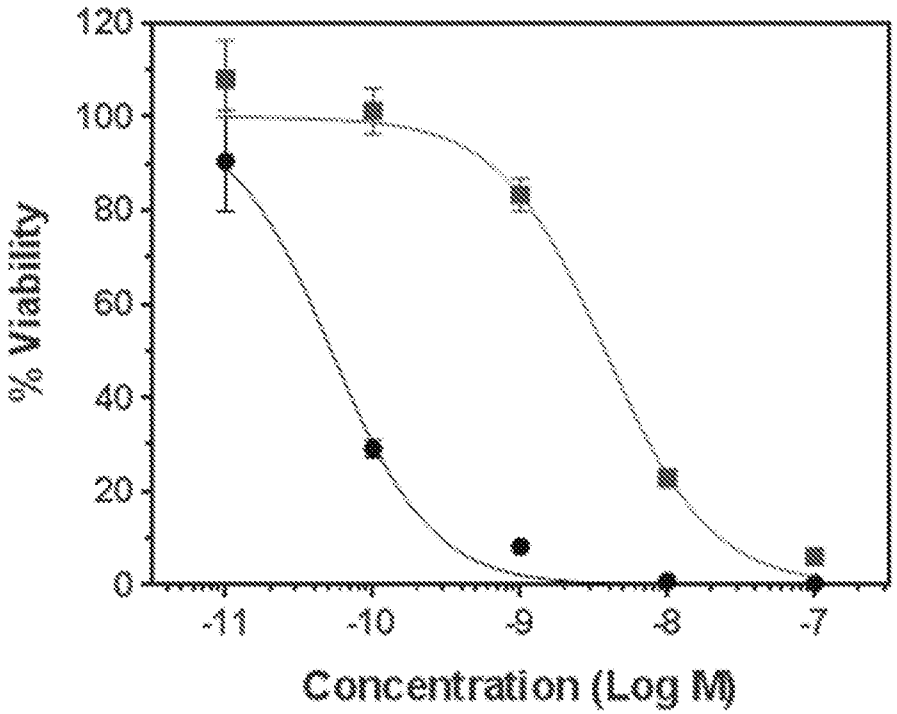

% Viability
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
Concentration (Log M)

FIG. 8A

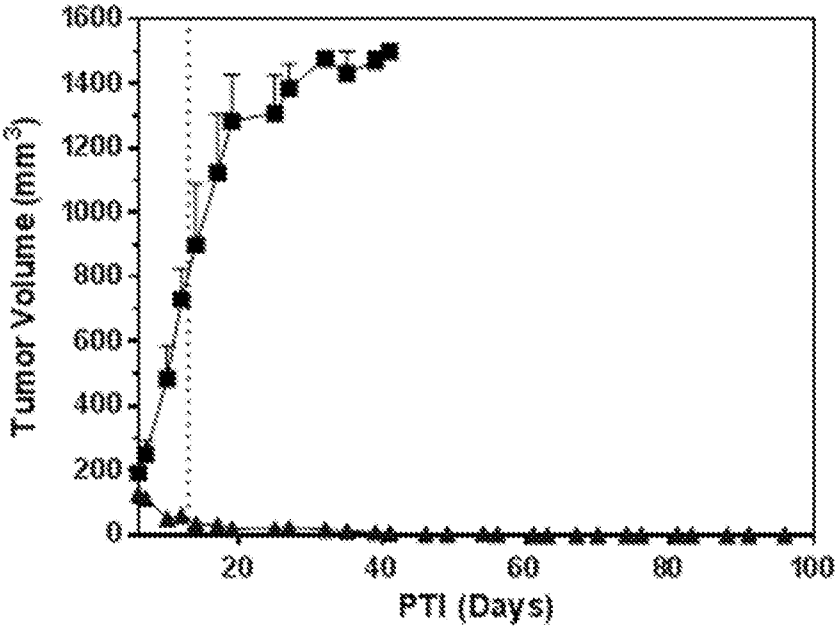

Tumor Volume (mm3)
1600
1400
1200
1000
800
600
400
200
0
20
40
60
80
100
PTI (Days)

FIG. 8B

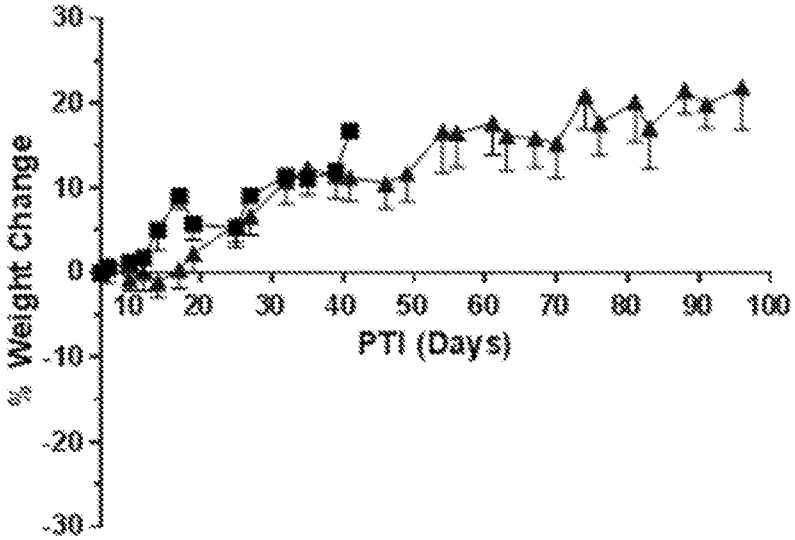

% Weight Change
30
20
10
0
-10
-20
-30
10
20
30
40
50
60
70
80
90
100
PTI (Days)

FIG. 9

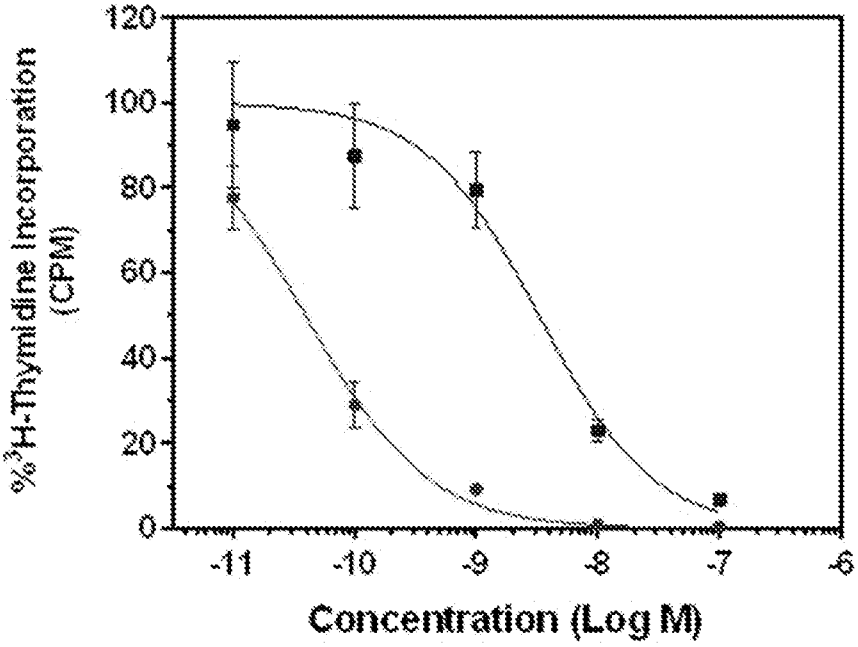

120
100
80
60
40
20
0
%^{3}H-Thymidine Incorporation (CPM)
-11
-10
-9
-8
-7
-6
Concentration (Log M)

FIG. 10A

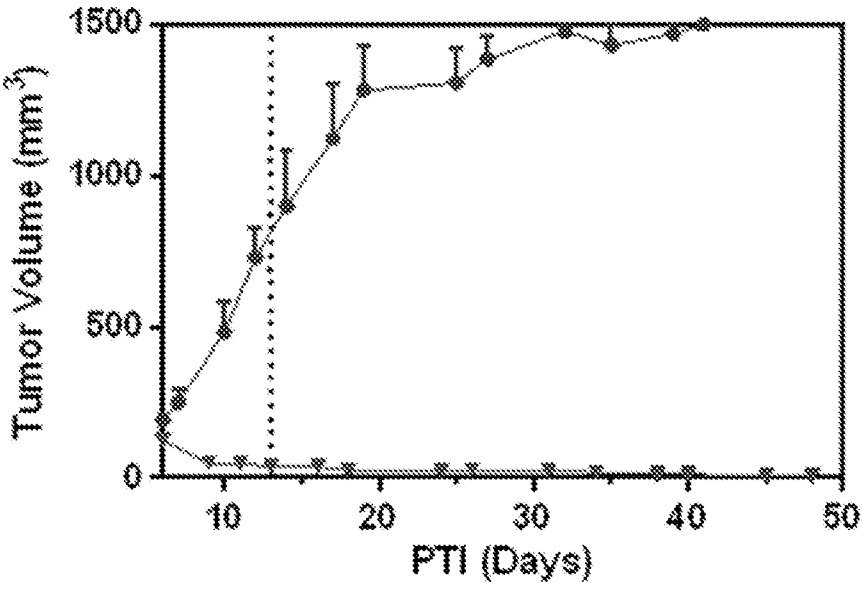

1500
1000
500
0
Tumor Volume (mm^3)
10
20
30
40
50
PTI (Days)

FIG. 10B

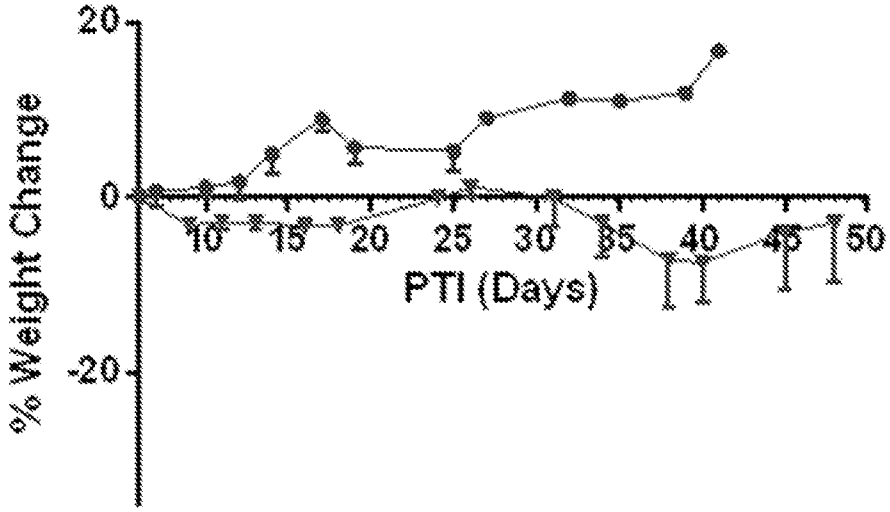


FIG. 11

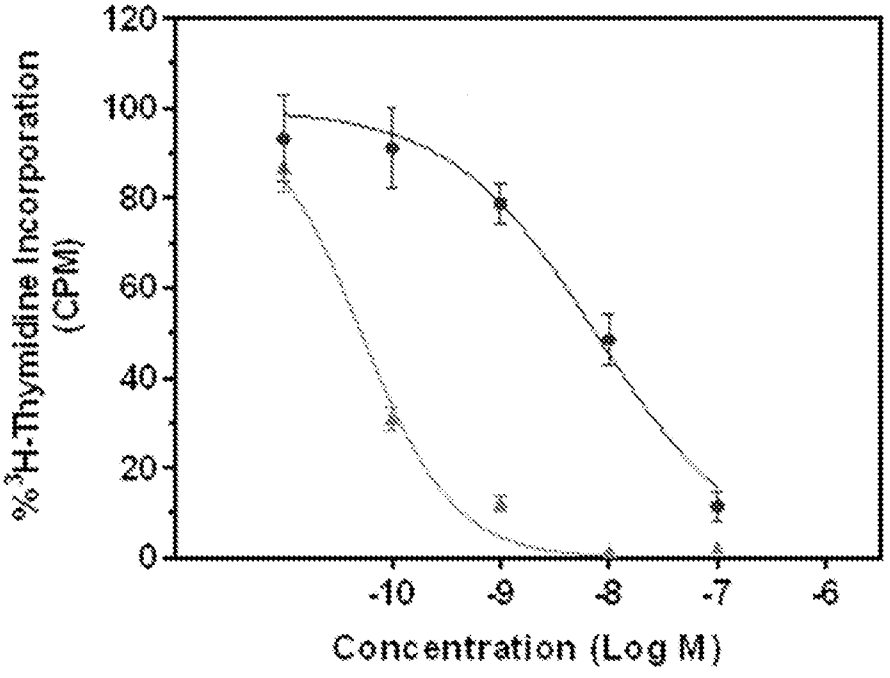


FIG. 12

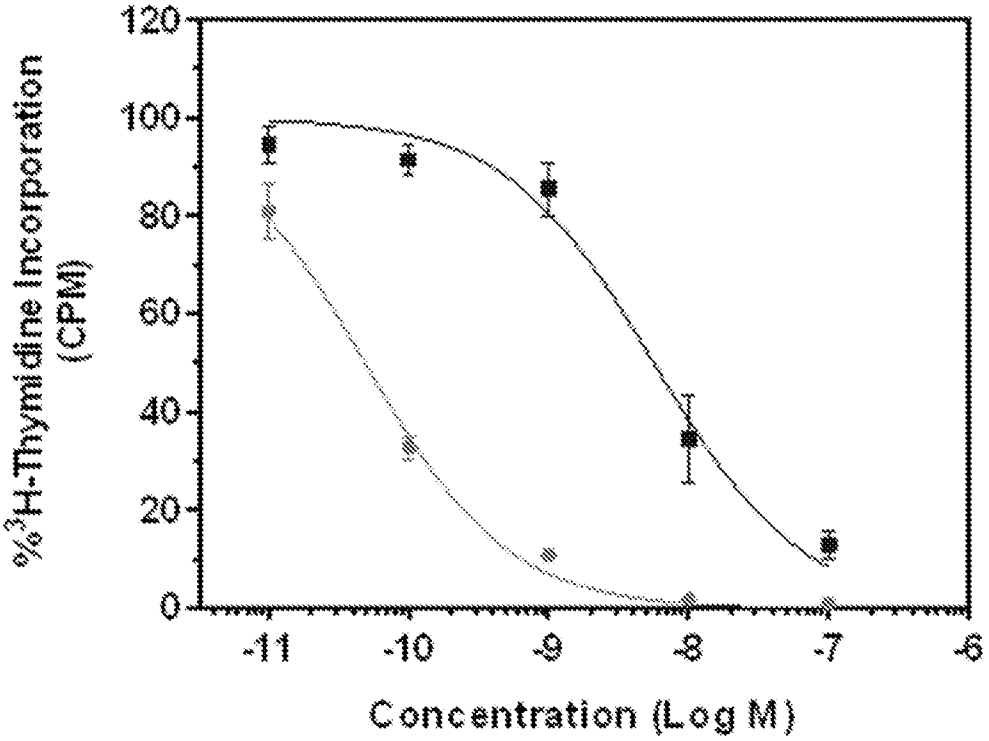


FIG. 13A

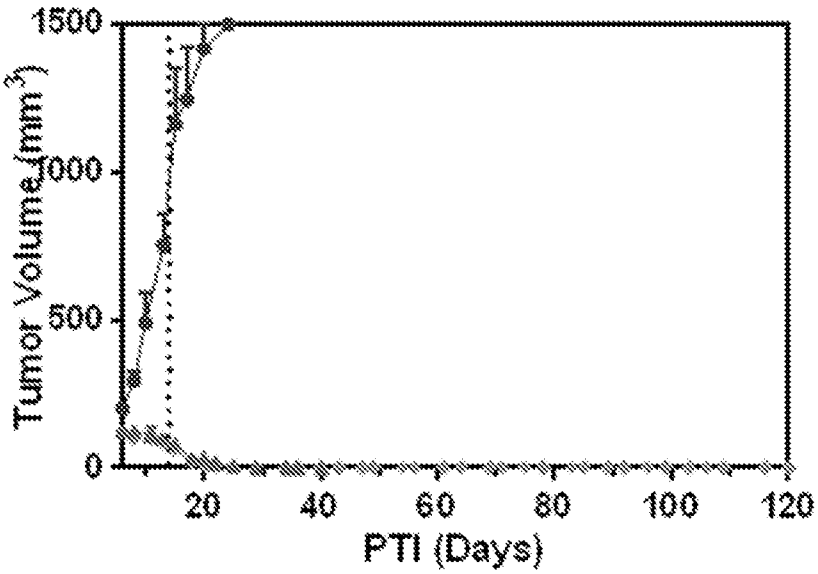


FIG. 13B

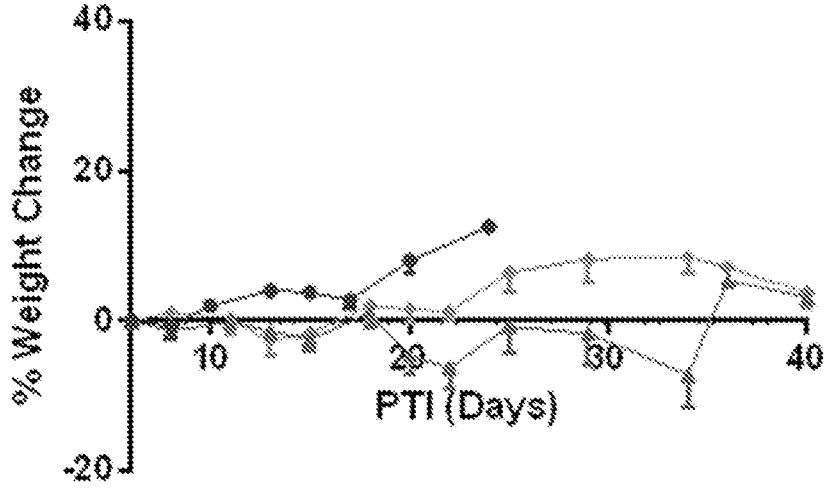


FIG. 14

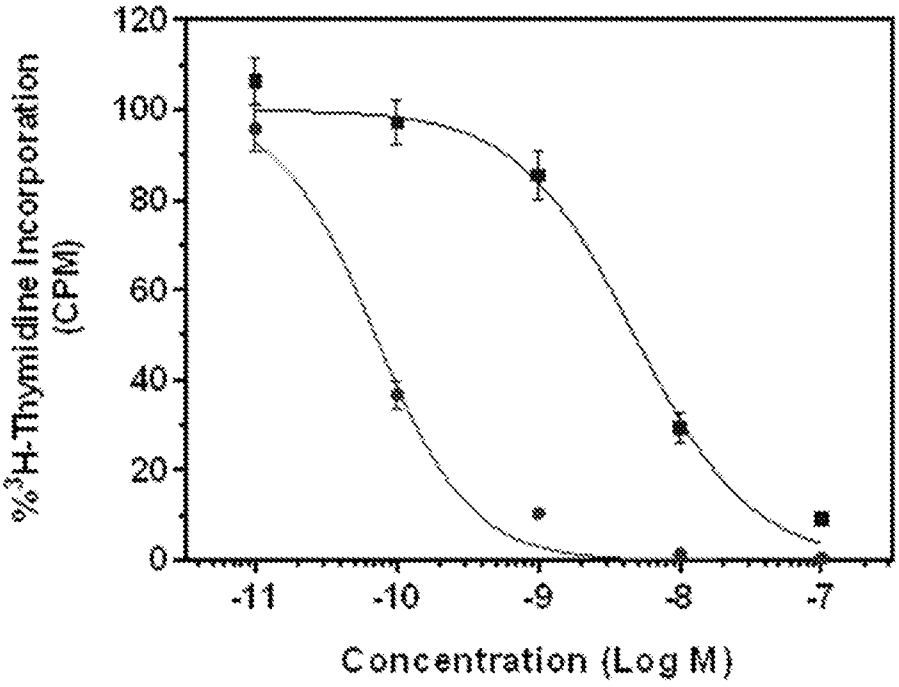


FIG. 15A

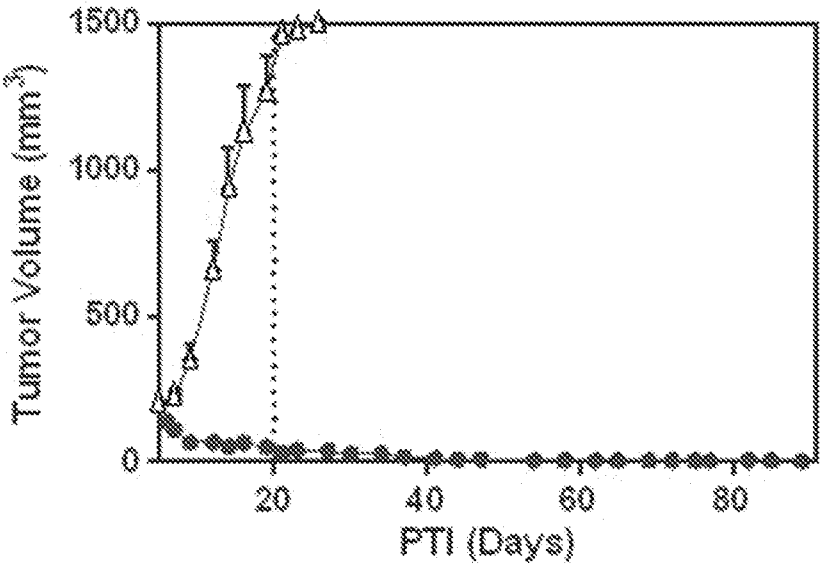


FIG. 15B

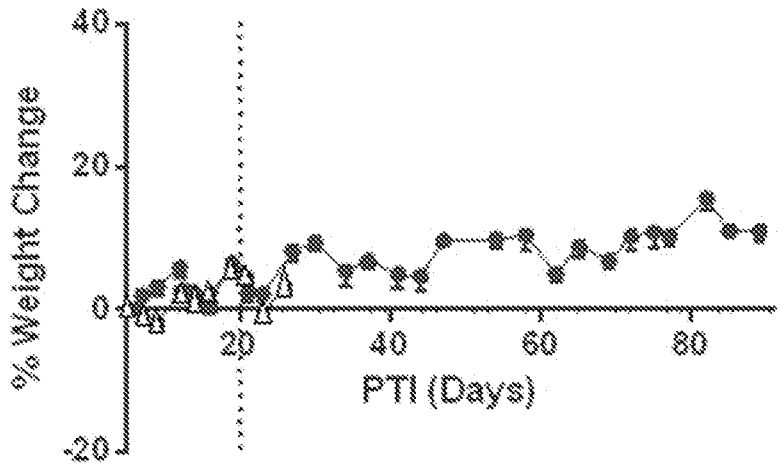


FIG. 16

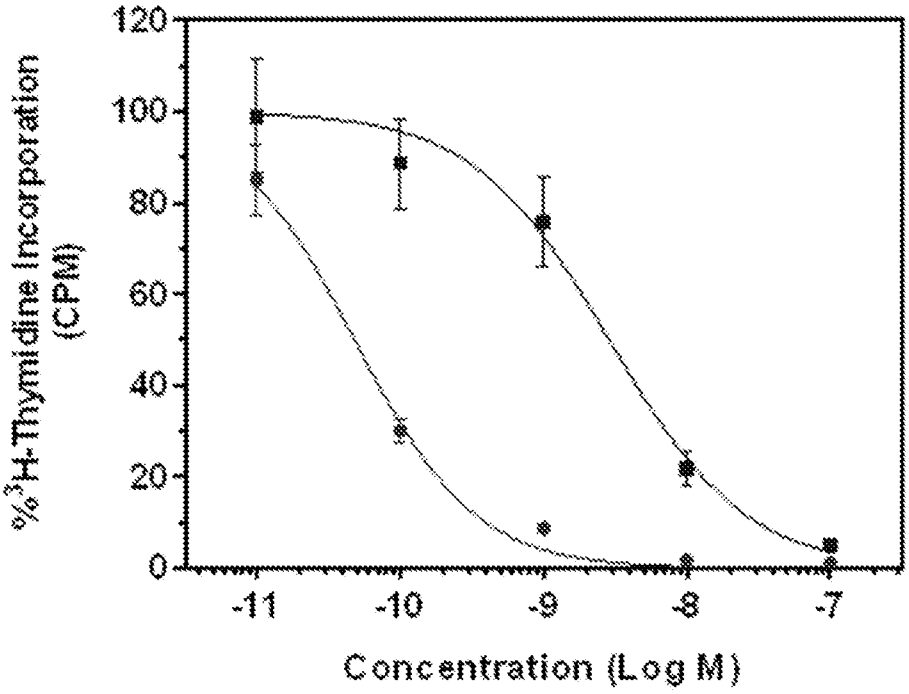


FIG. 17A

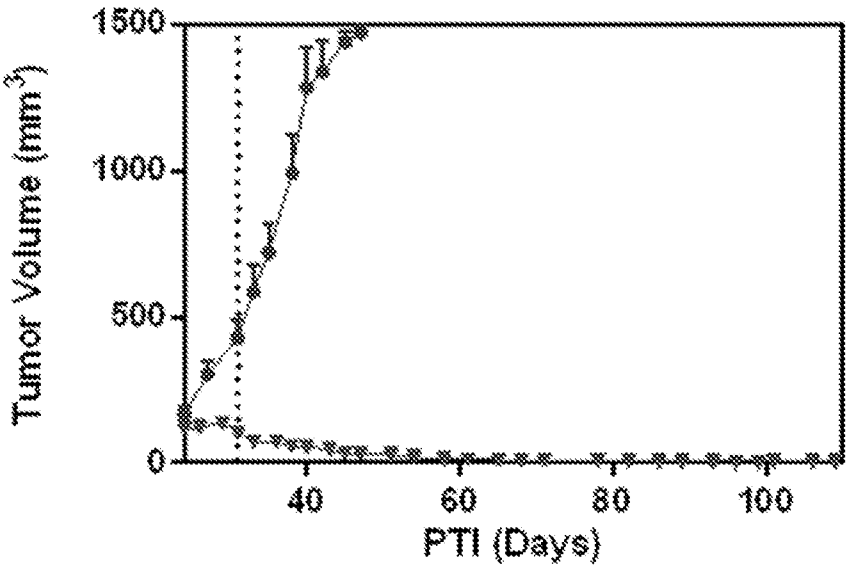


FIG. 17B

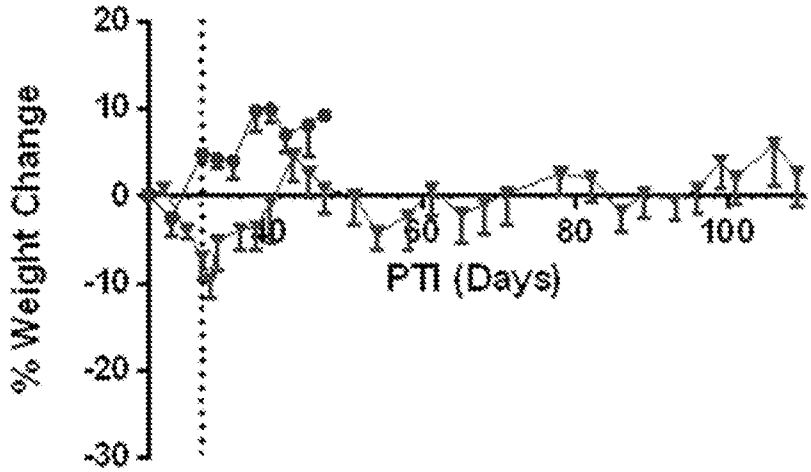


FIG. 18

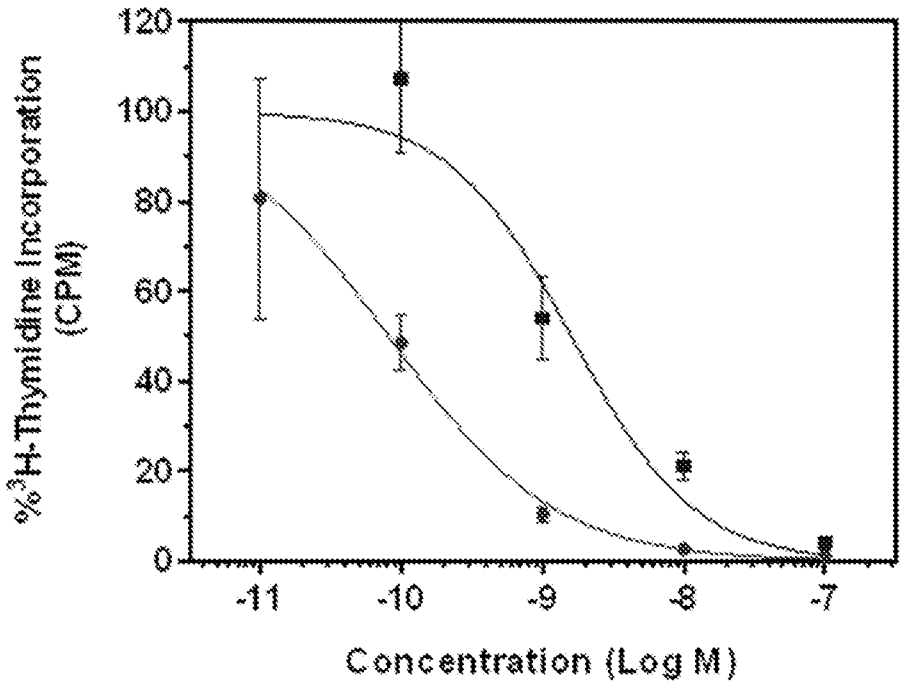


FIG. 19A

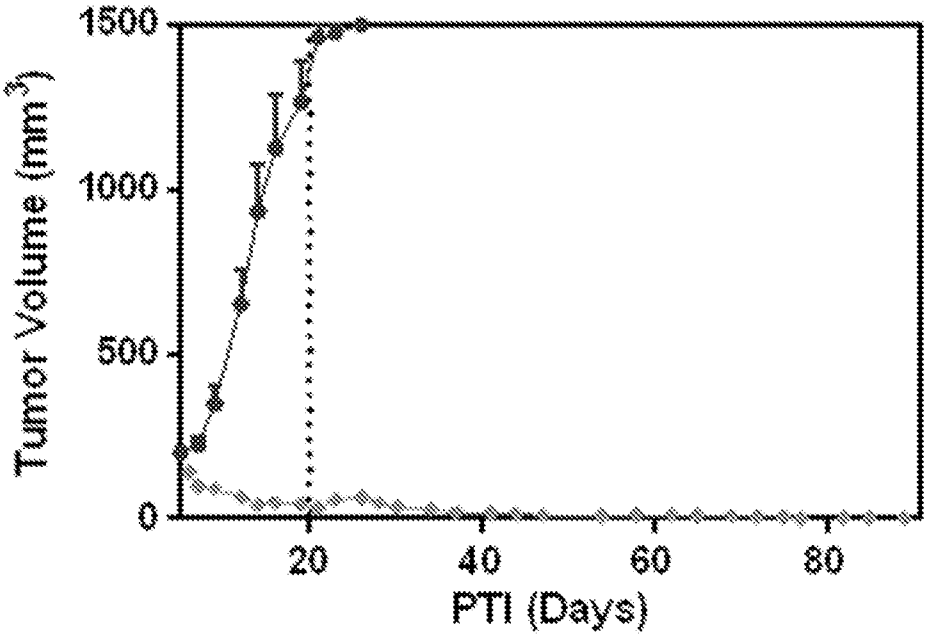


FIG. 19B

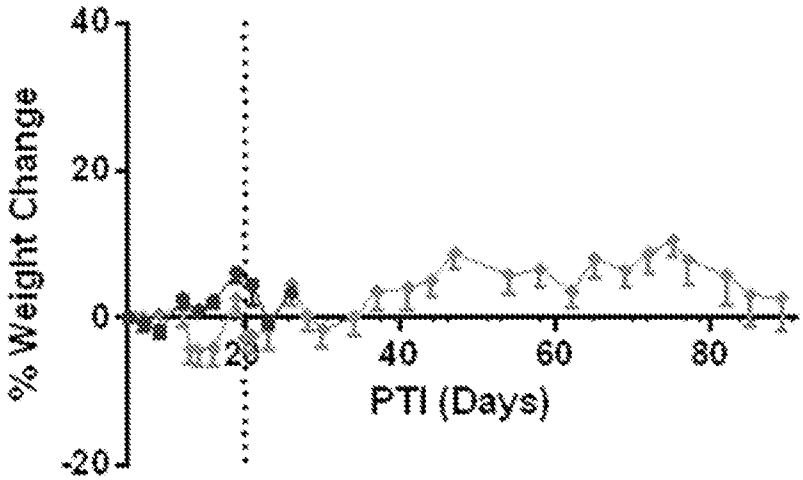


FIG. 20

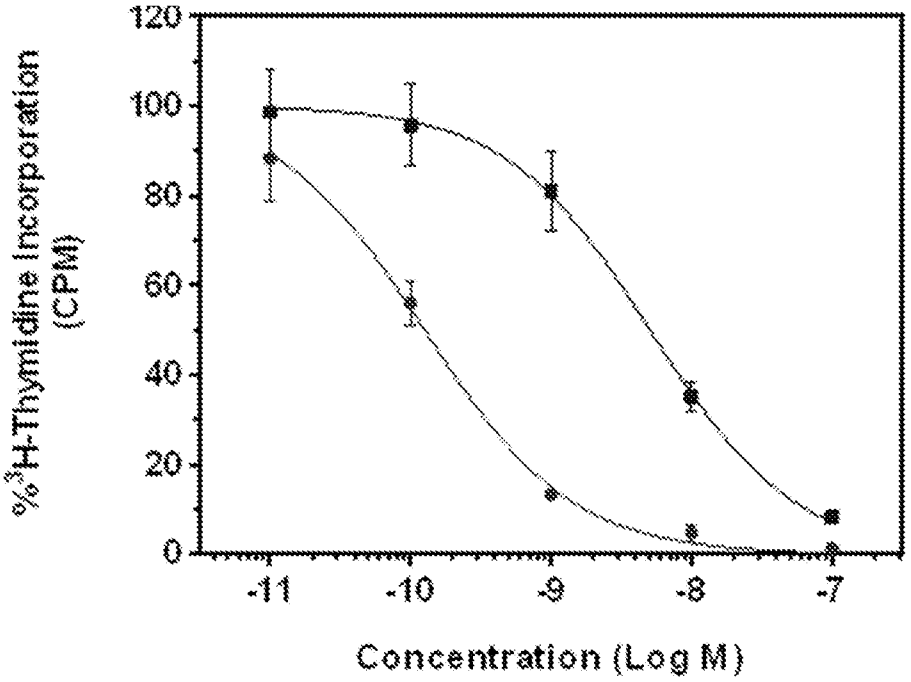


FIG. 21A

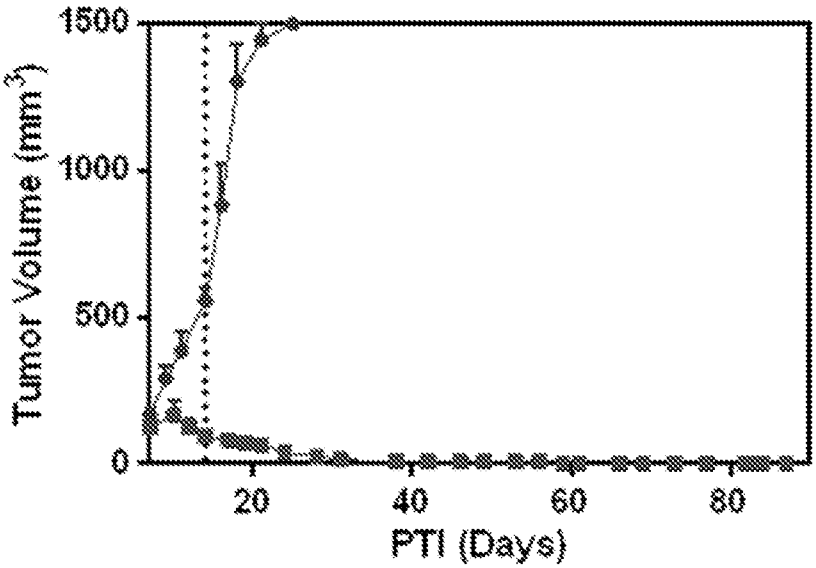


FIG. 21B

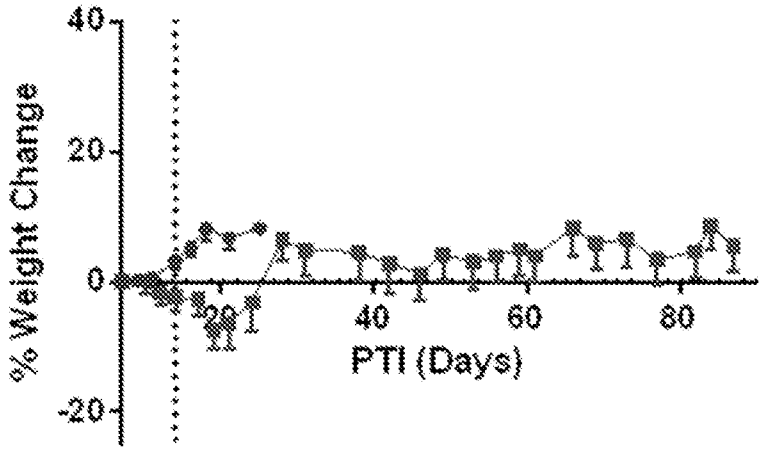


FIG. 22

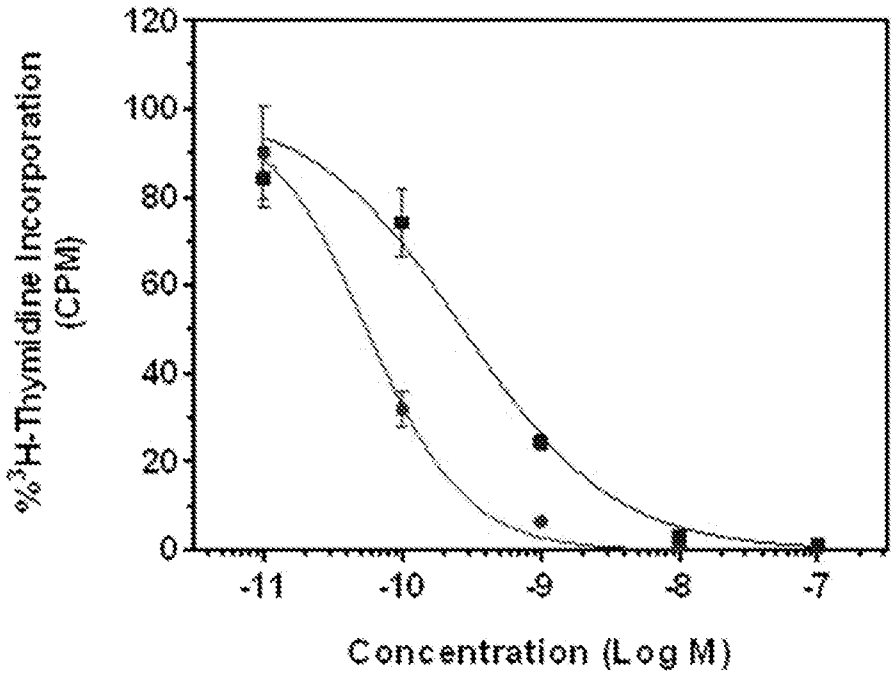


FIG. 23A

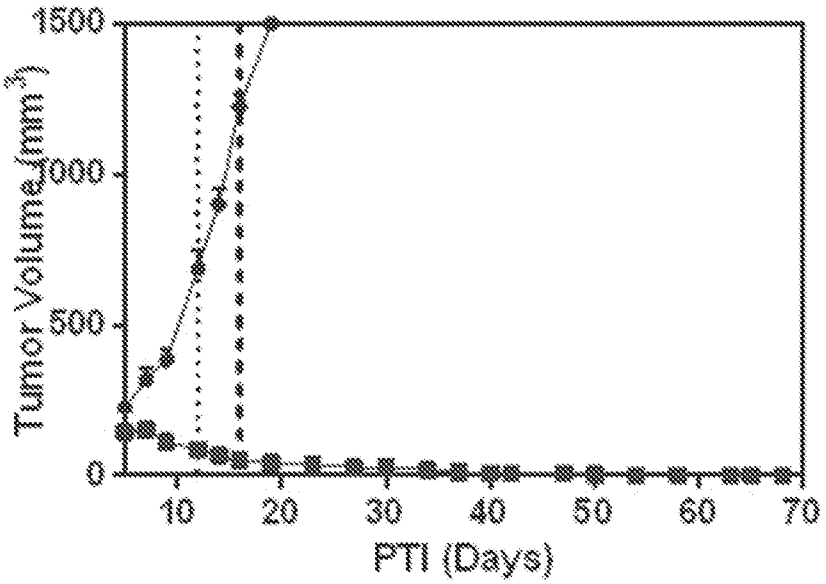


FIG. 23B

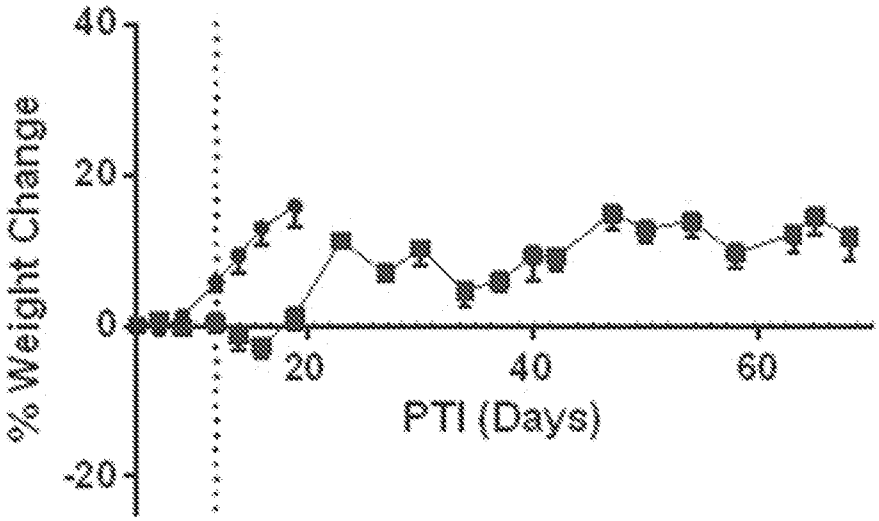


FIG. 24

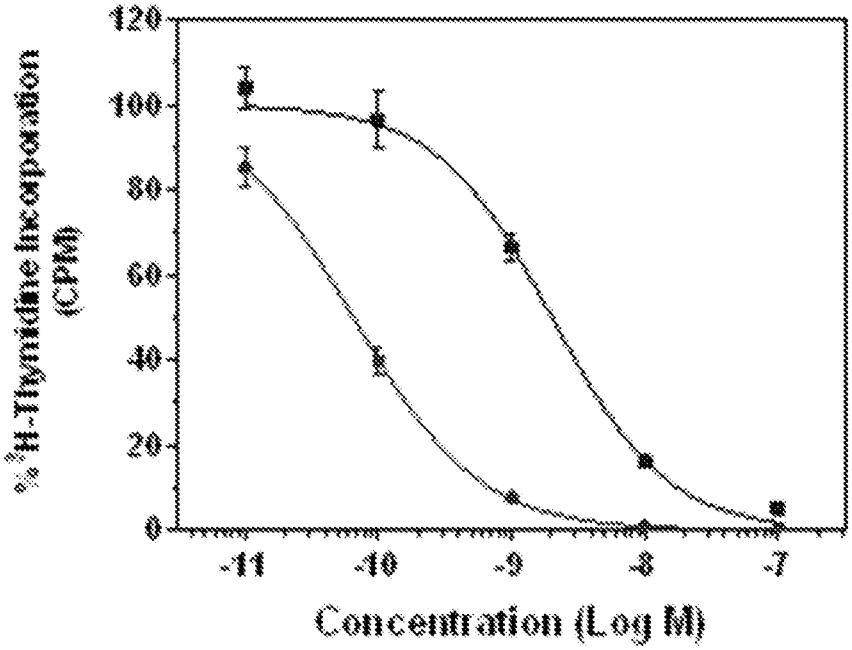

120
100
80
60
40
20
0
% ³H-Thymidine Incorporation (CPM)
-11
-10
-9
-8
-7
Concentration (Log M)

FIG. 25

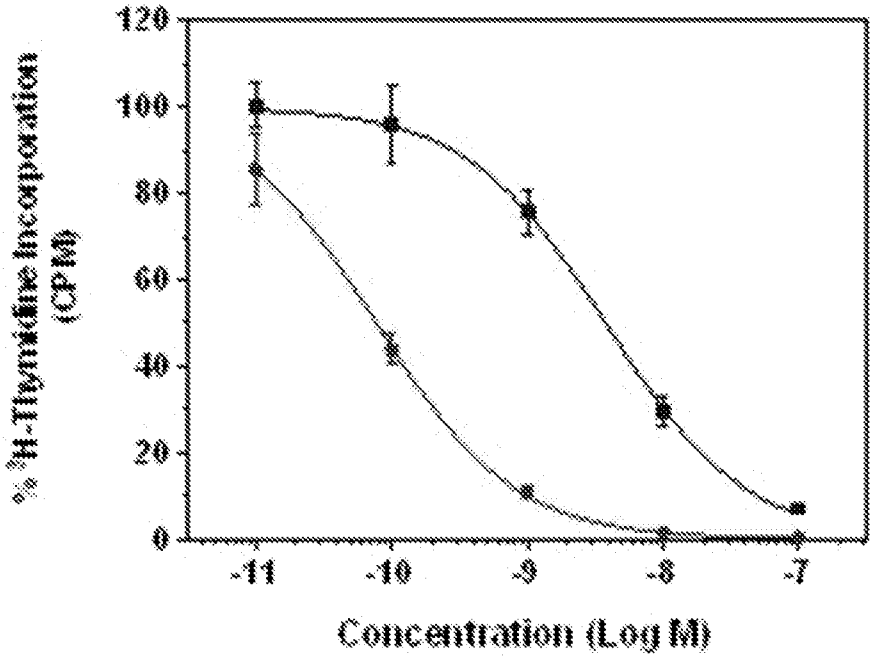

120
100
80
60
40
20
0
% ³H-Thymidine Incorporation (CPM)
-11
-10
-9
-8
-7
Concentration (Log M)

FIG. 26

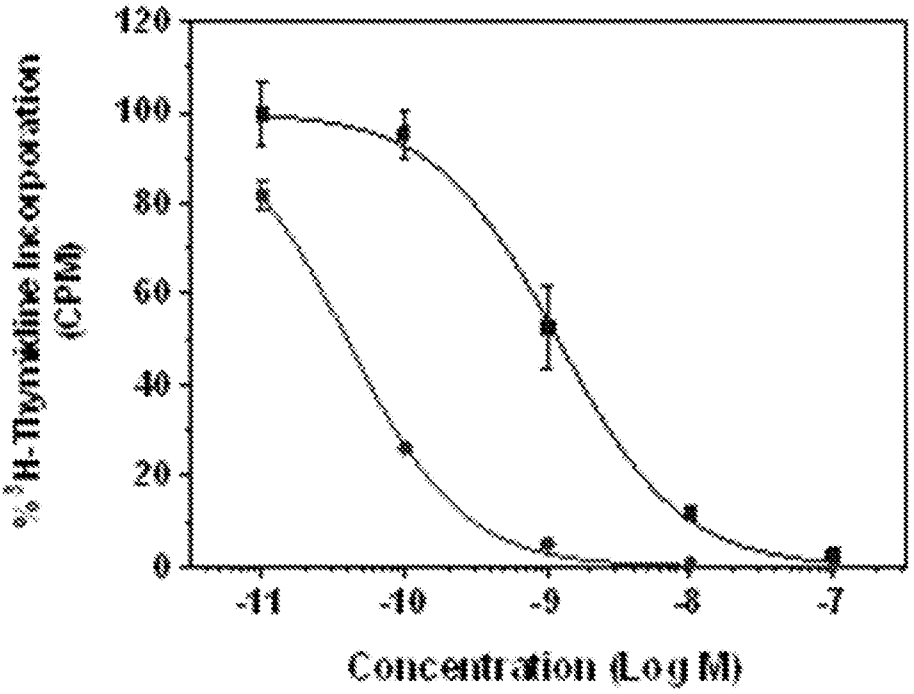


FIG. 27A

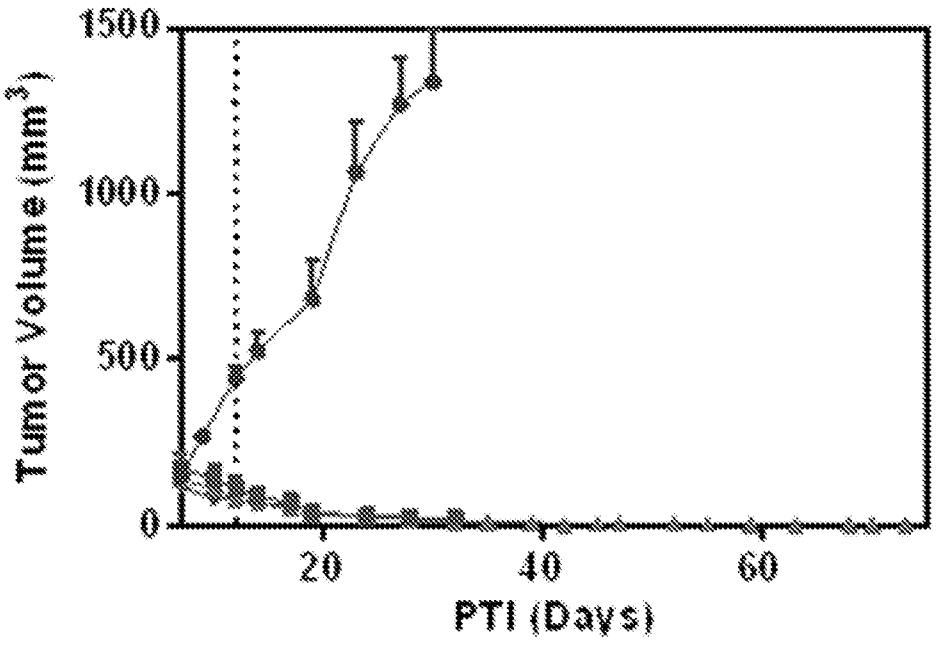


FIG. 27B

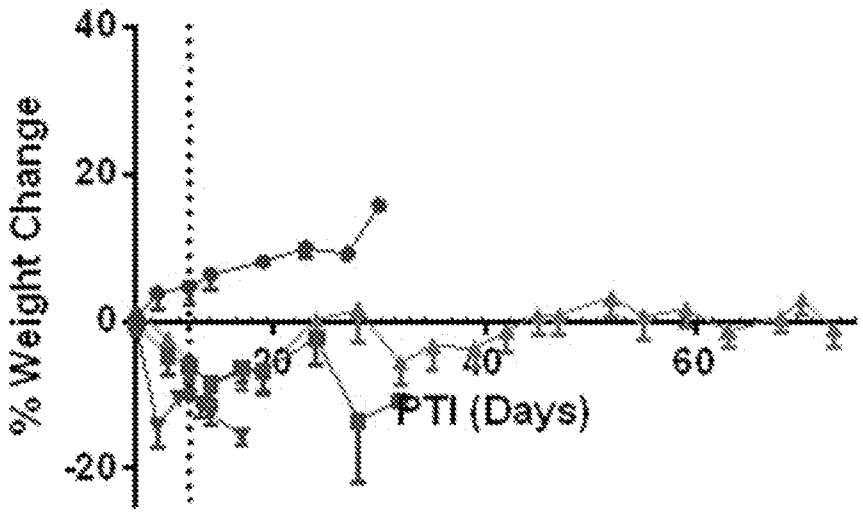


FIG. 28

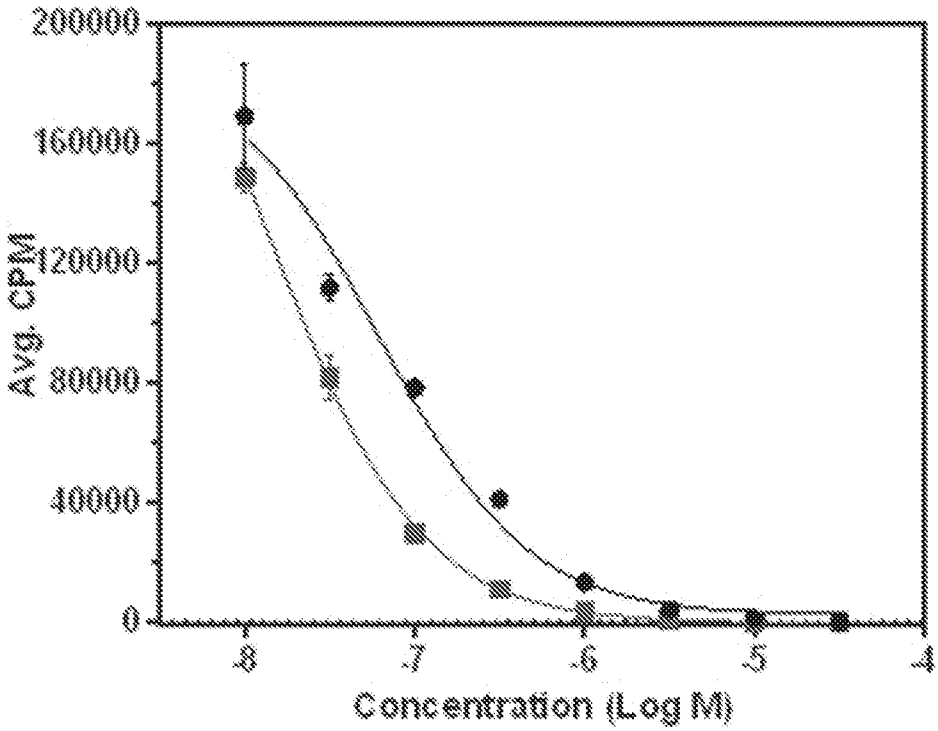


FIG. 29

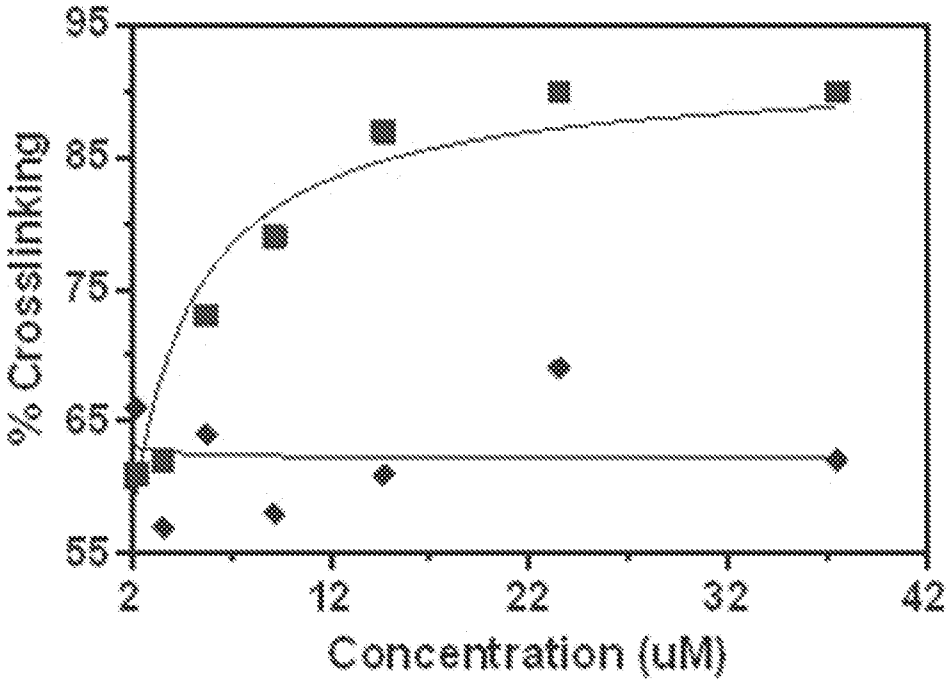

95
85
75
65
55
% Crosslinking
2
12
22
32
42
Concentration (uM)

FIG. 30

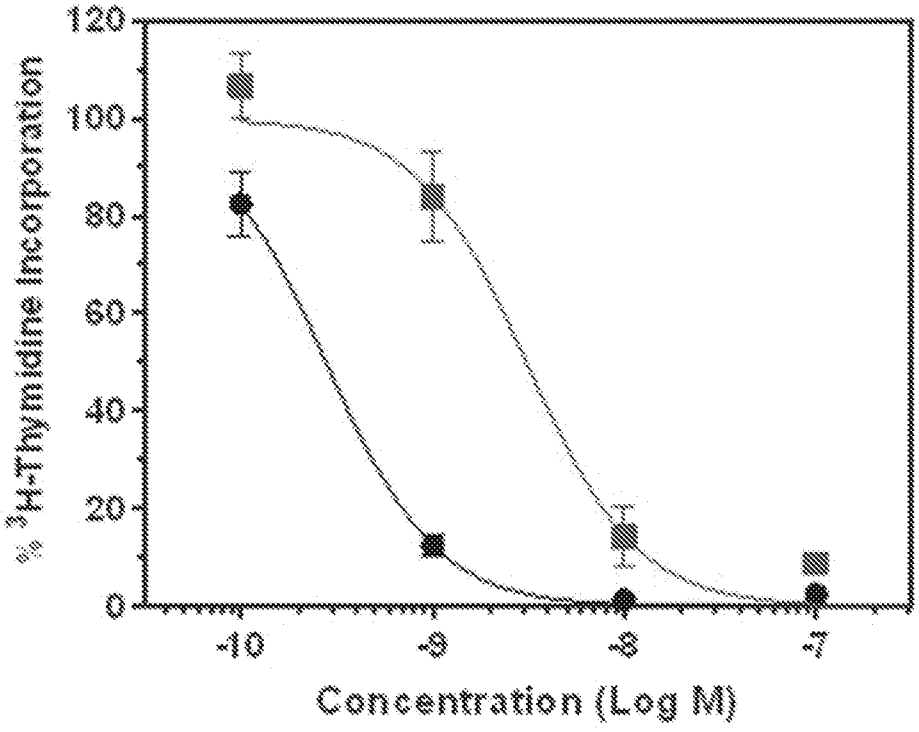

120
100
80
60
40
20
0
% ³H-Thymidine Incorporation
-10
-9
-8
-7
Concentration (Log M)

FIG. 31

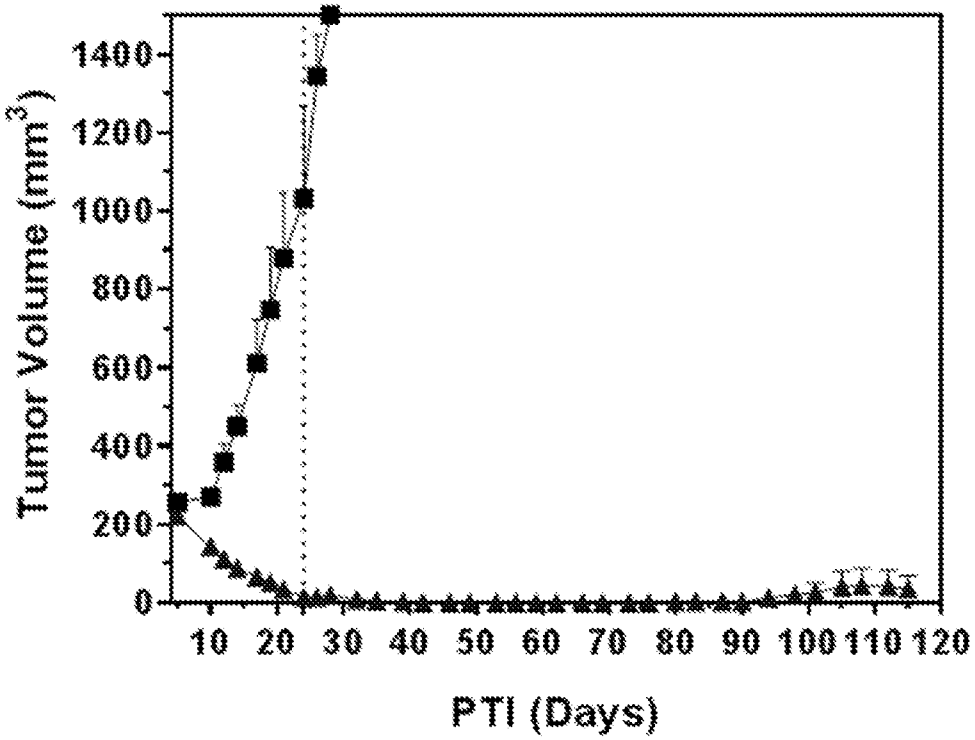

Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
10 20 30 40 50 60 70 80 90 100 110 120
PTI (Days)

FIG. 32

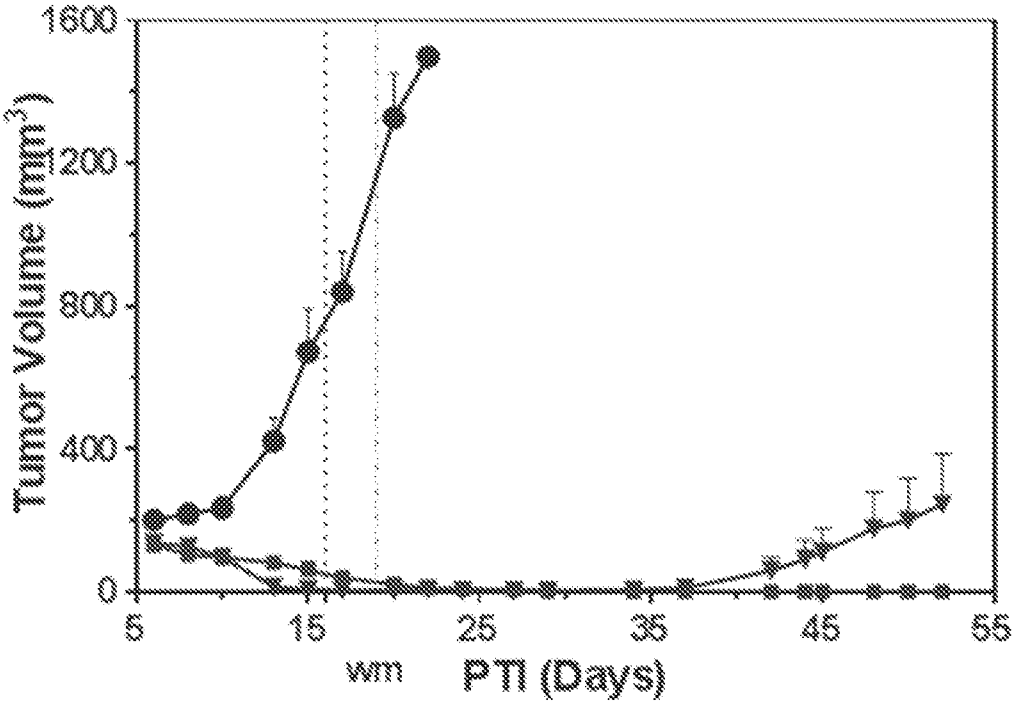

Tumor Volume (mm3)
1600
1200
800
400
0
5 15 25 35 45 55
wm
PTI (Days)

FIG. 33

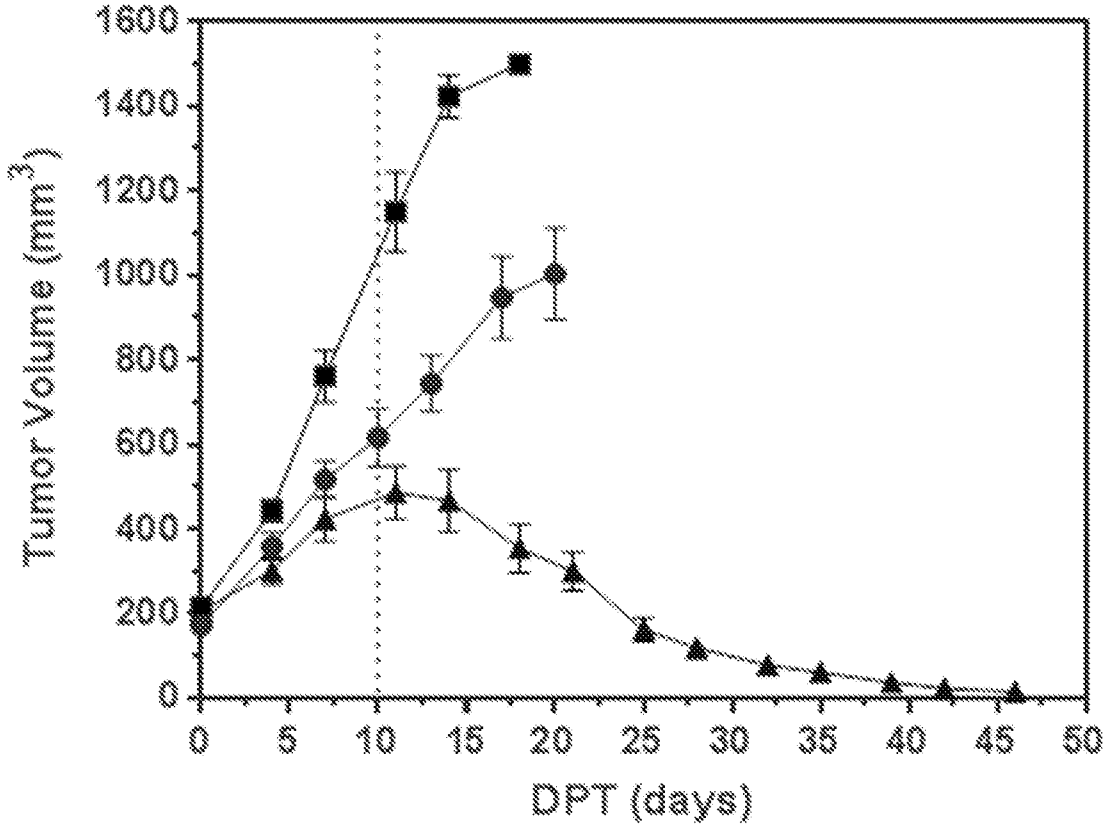

Tumor Volume (mm3)
1600
1400
1200
1000
800
600
400
200
0
0 5 10 15 20 25 30 35 40 45 50
DPT (days)

FIG. 34

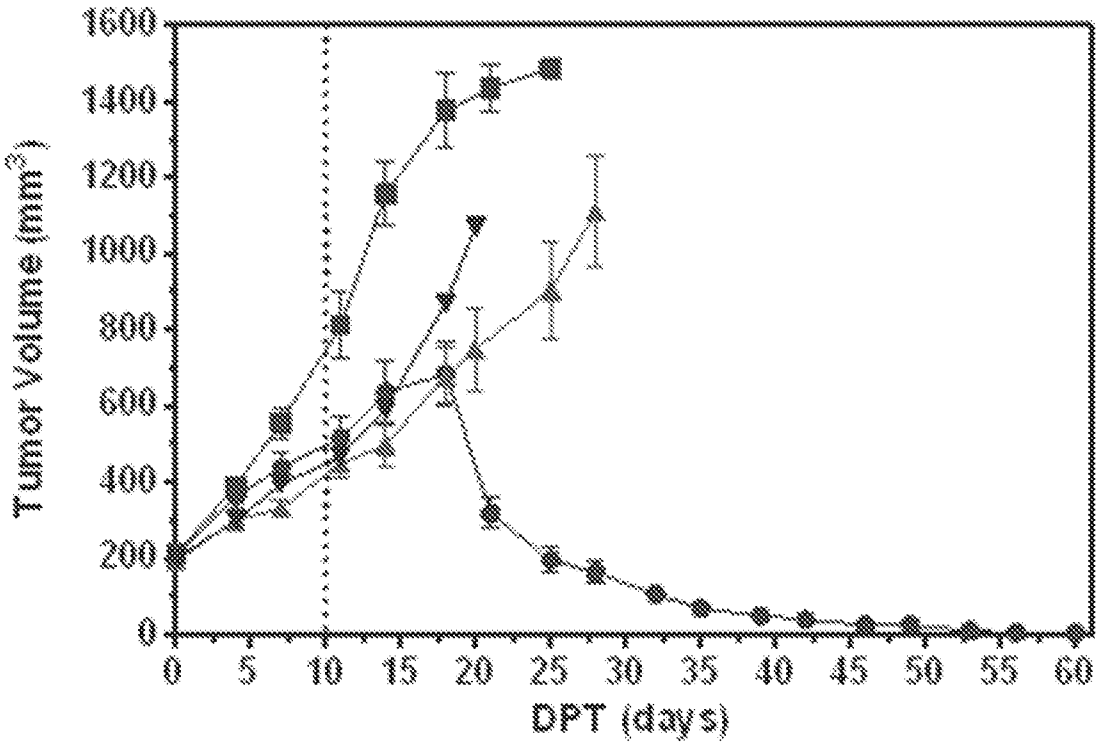

Tumor Volume (mm3)
1600
1400
1200
1000
800
600
400
200
0
0 5 10 15 20 25 30 35 40 45 50 55 60
DPT (days)

FIG. 35

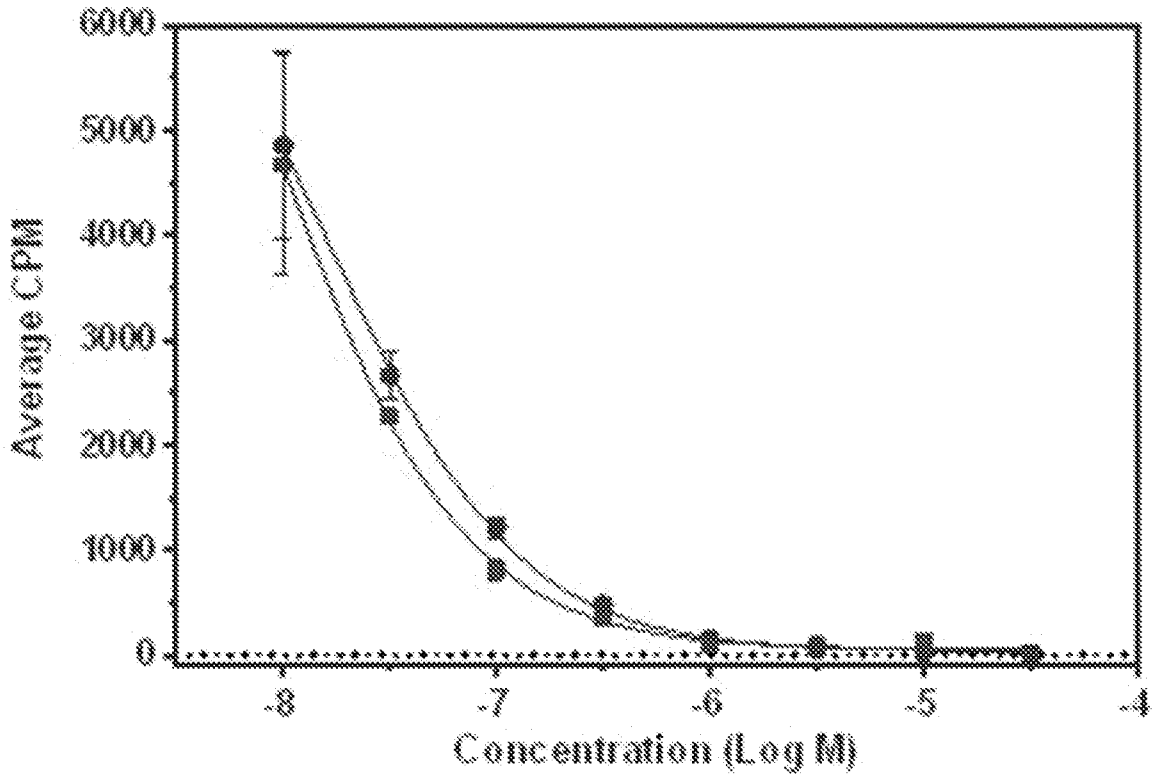

6000
5000
4000
3000
2000
1000
0
Average CPM
-8
-7
-6
-5
-4
Concentration (Log M)

FIG. 36

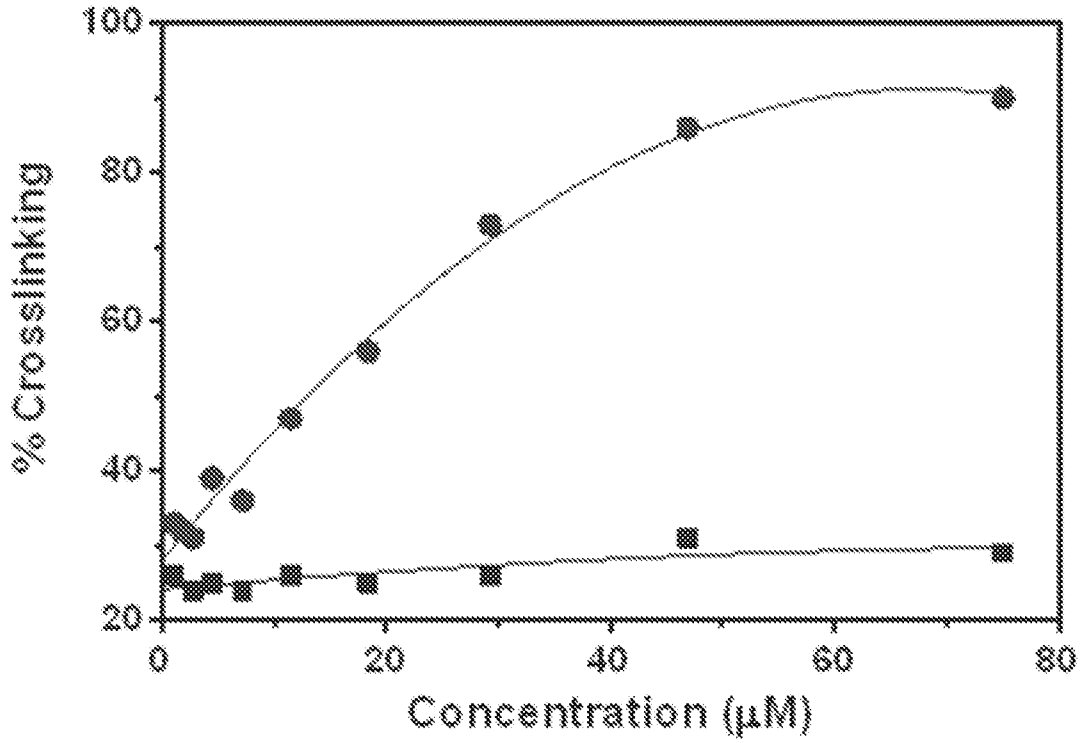

100
80
60
40
20
% Crosslinking
0
20
40
60
80
Concentration (µM)

FIG. 37A

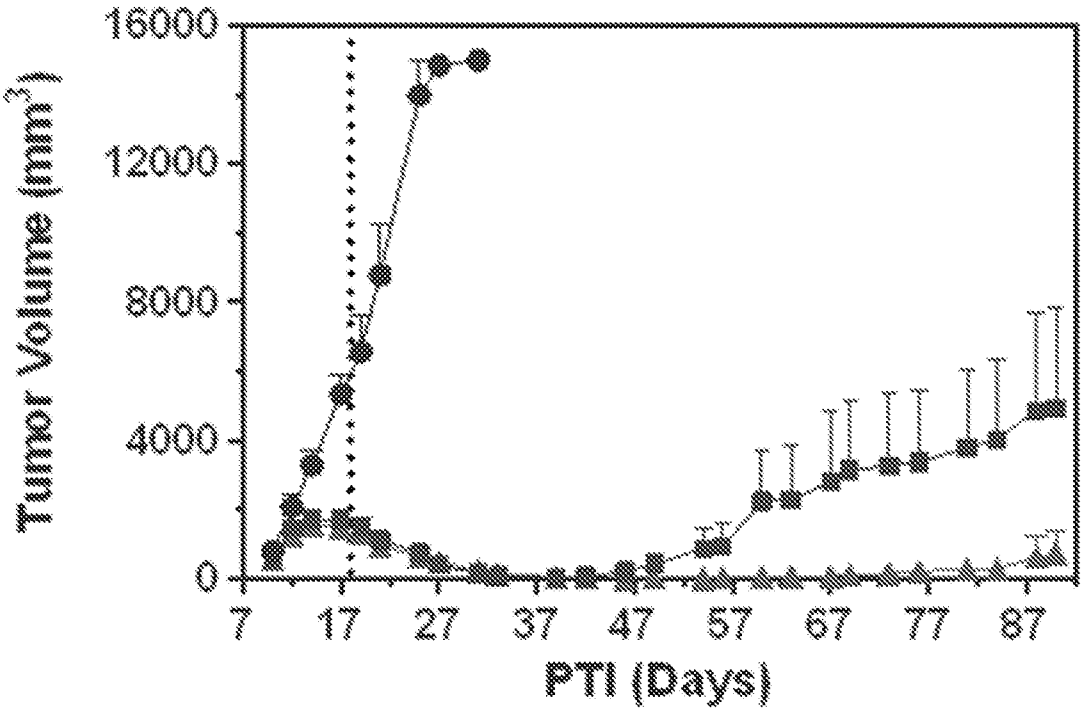


FIG. 37B

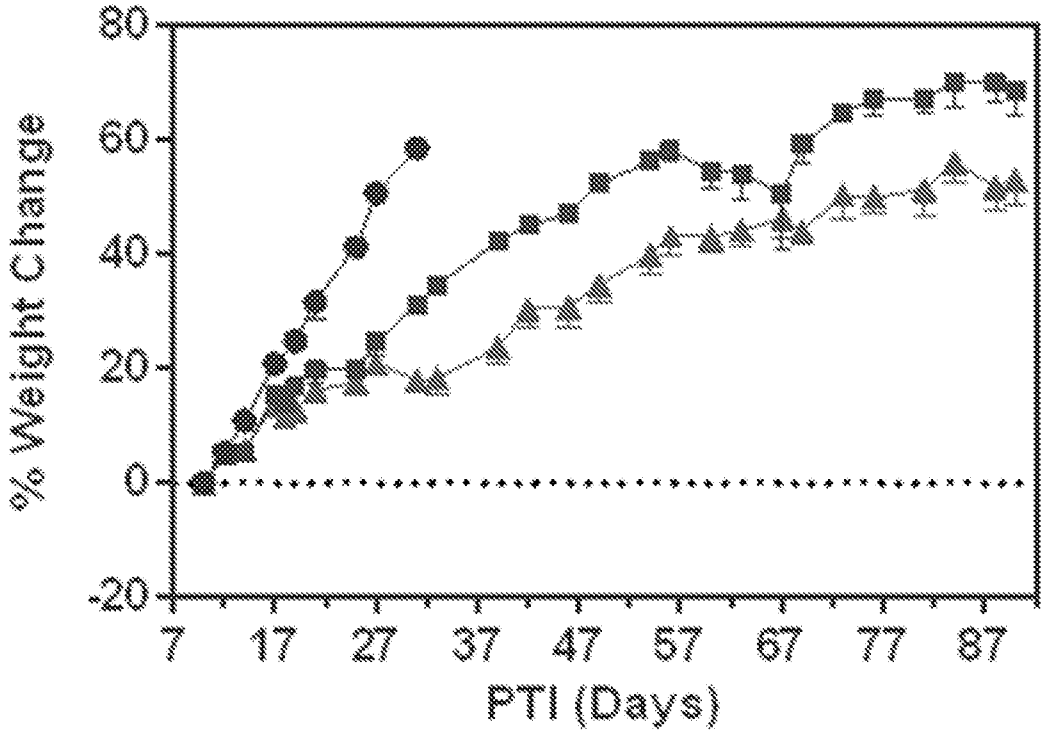


FIG. 38

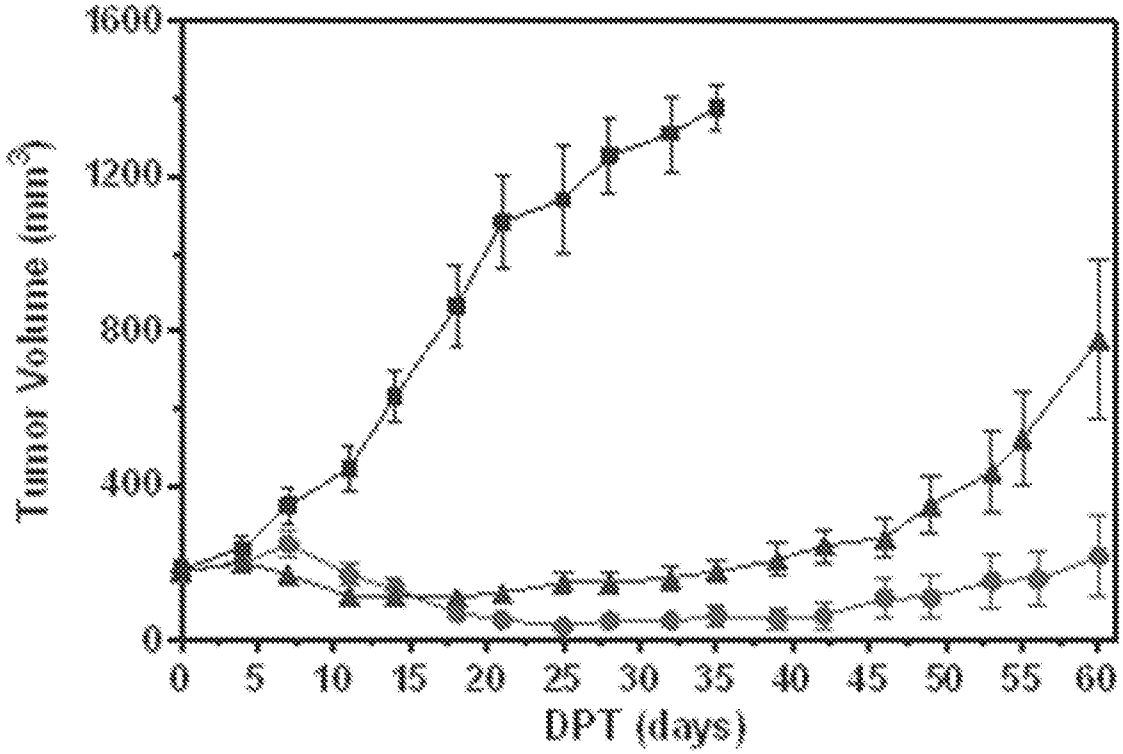

Tumor Volume (mm3)
1600
1200
800
400
0
0 5 10 15 20 25 30 35 40 45 50 55 60
DPT (days)

FIG. 39

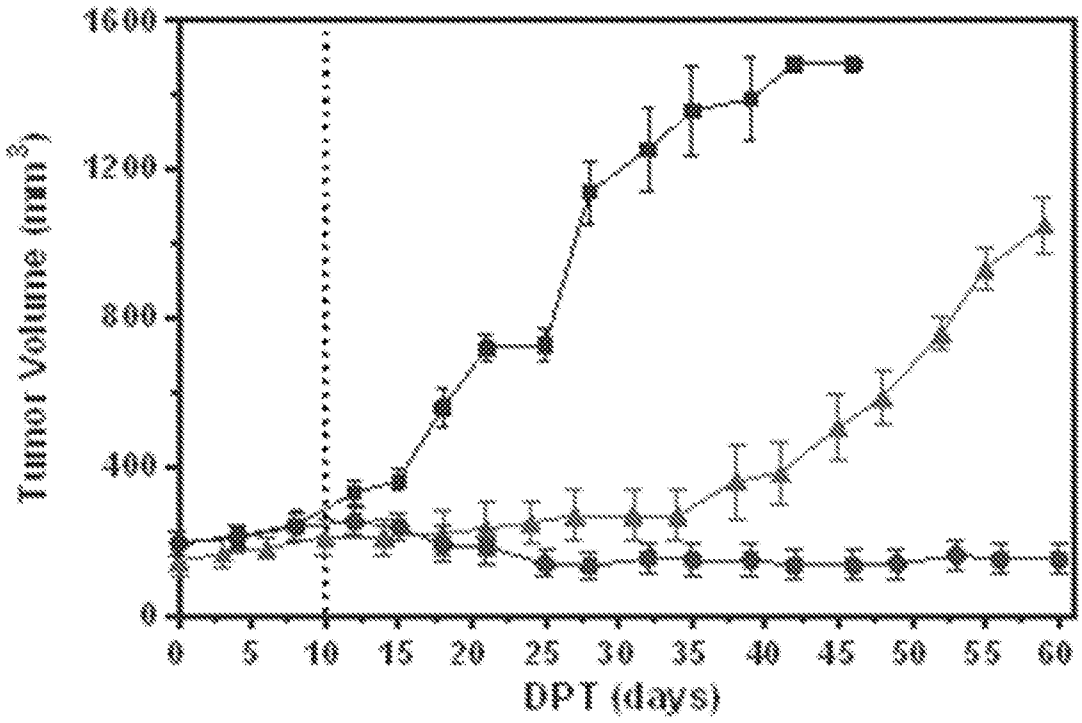

Tumor Volume (mm3)
1600
1200
800
400
0
0 5 10 15 20 25 30 35 40 45 50 55 60
DPT (days)

FIG. 40

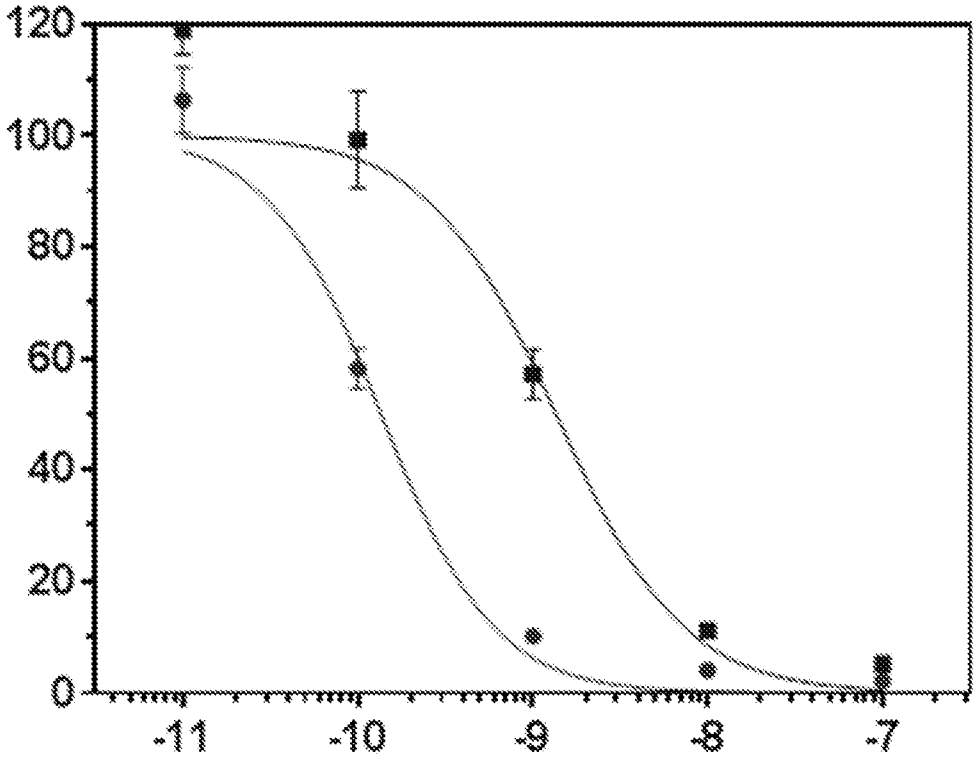

120
100
80
60
40
20
0
-11
-10
-9
-8
-7

FIG. 41

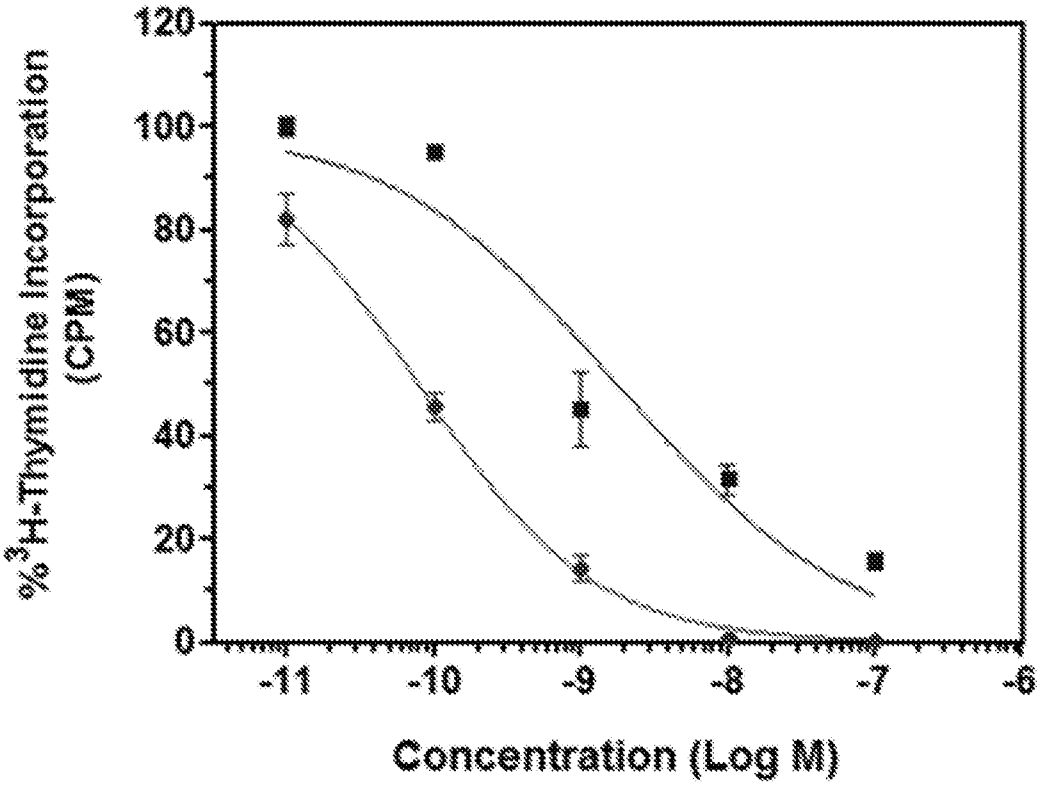

%^{3}H-Thymidine Incorporation (CPM)
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
Concentration (Log M)

FIG. 42

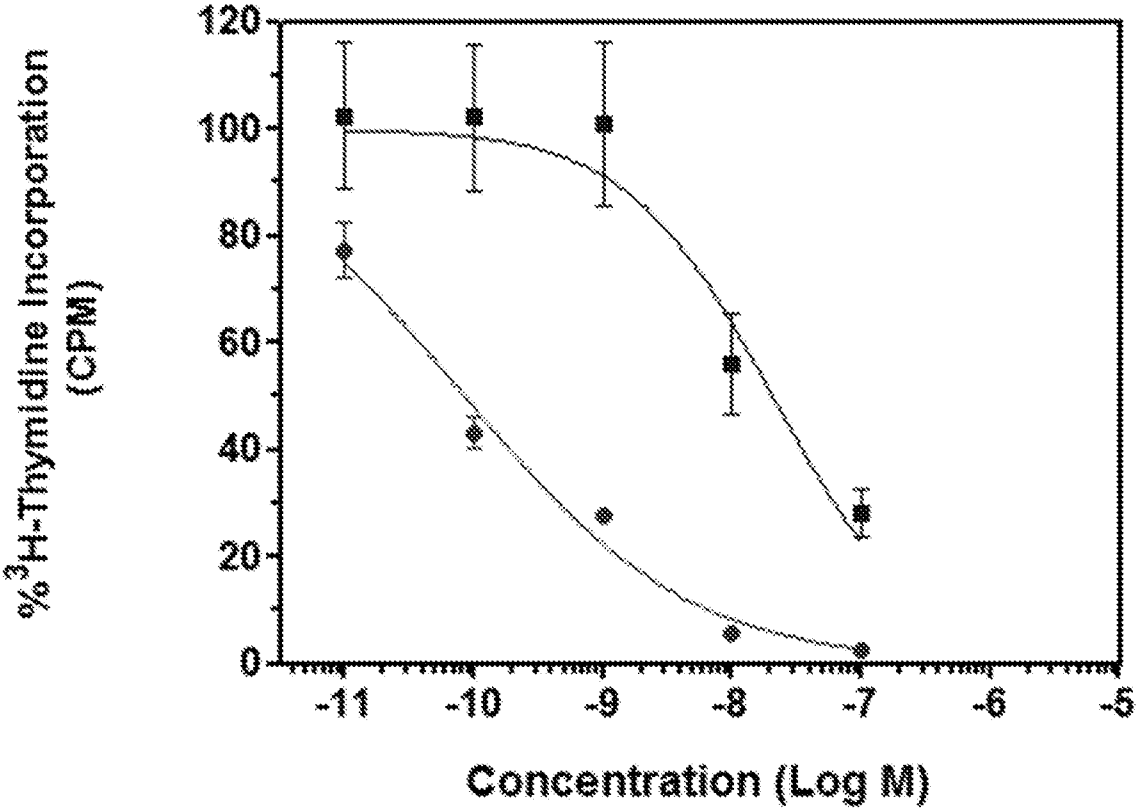

% ^{3}H-Thymidine Incorporation (CPM)
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
-5
Concentration (Log M)

FIG. 43

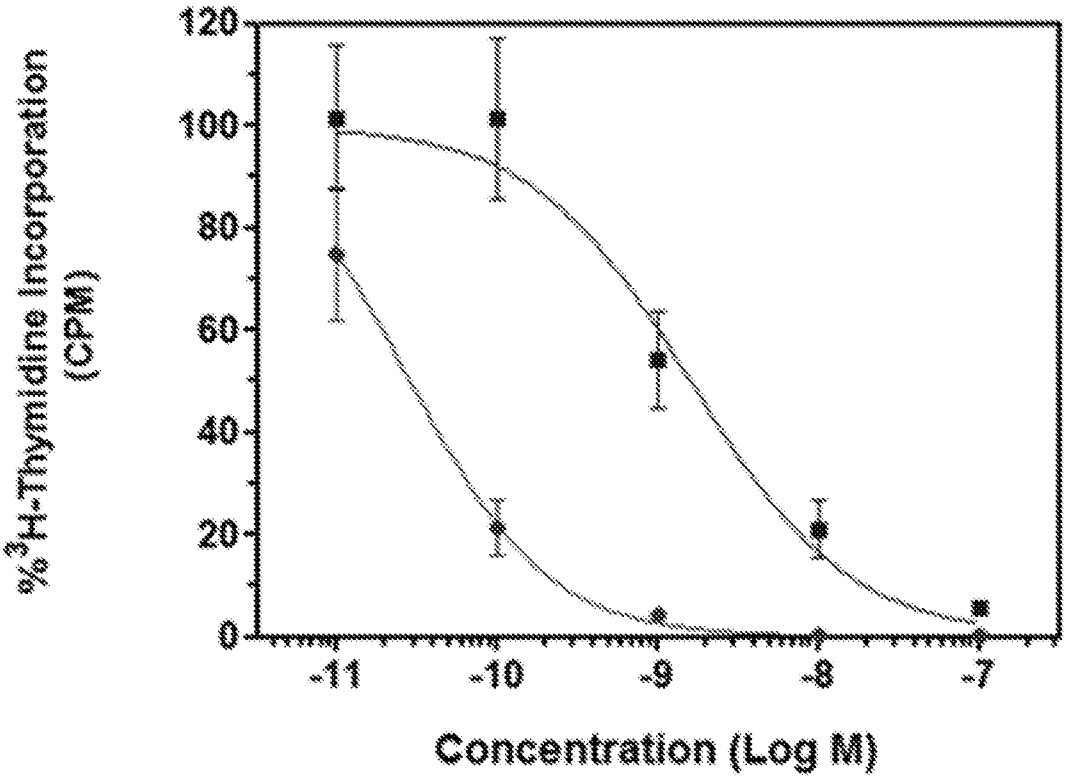

% ^{3}H-Thymidine Incorporation (CPM)
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
Concentration (Log M)

FIG. 44

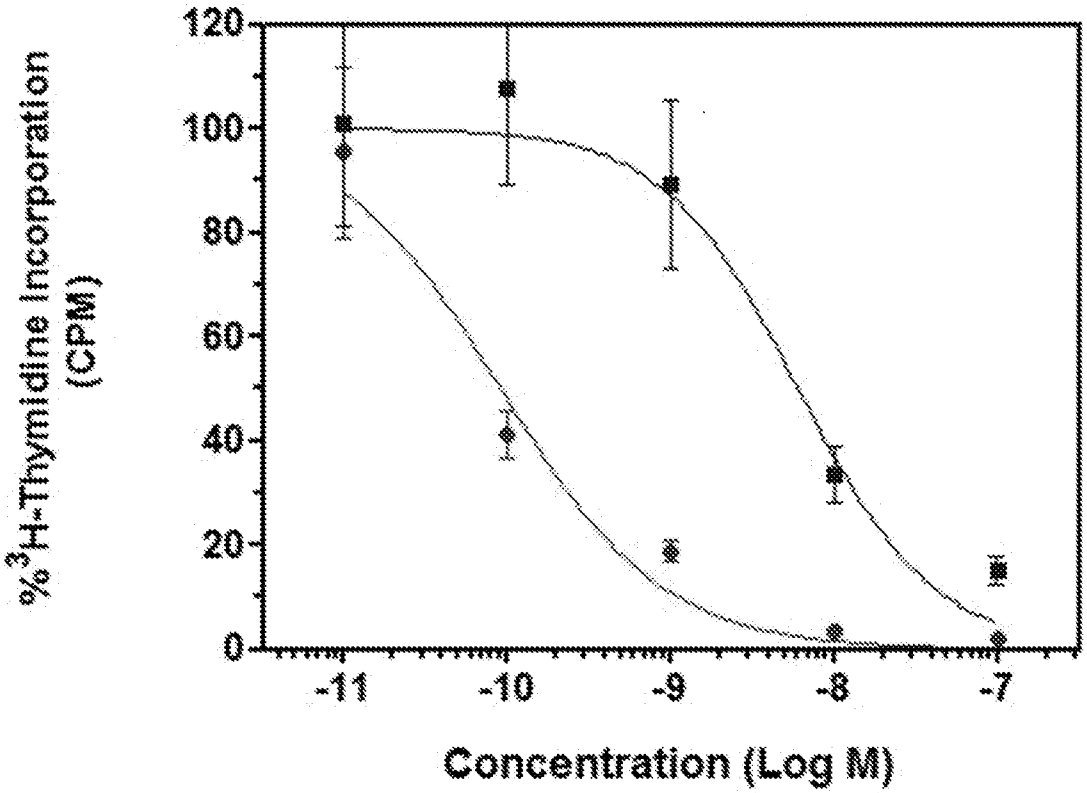

120
100
80
60
40
20
0
-11
-10
-9
-8
-7
% ^{3}H-Thymidine Incorporation (CPM)
Concentration (Log M)

FIG. 45

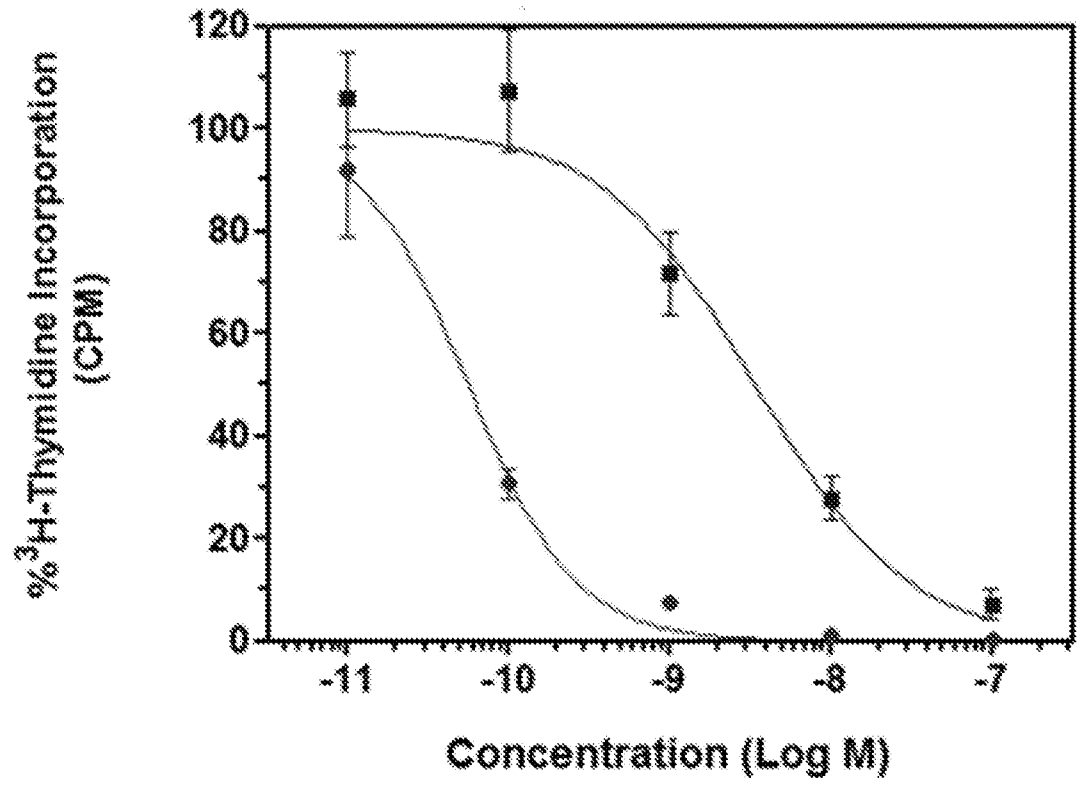

120
100
80
60
40
20
0
-11
-10
-9
-8
-7
% ^{3}H-Thymidine Incorporation (CPM)
Concentration (Log M)

FIG. 46

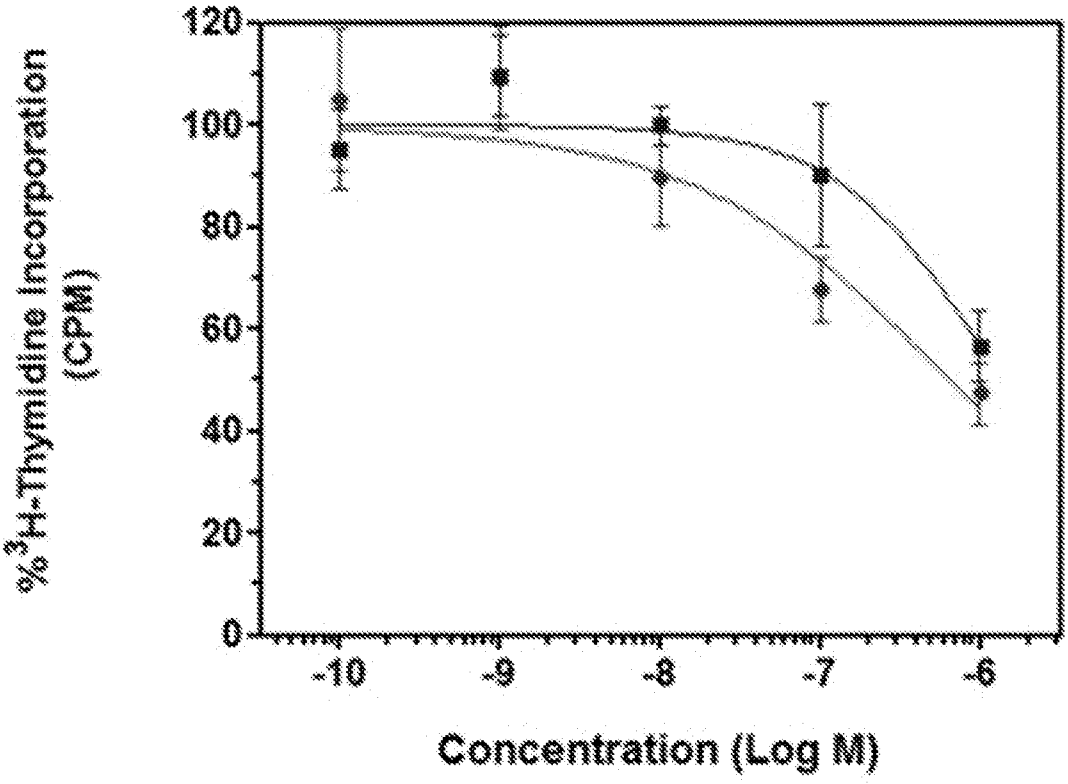

% ^{3}H-Thymidine Incorporation (CPM)
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
Concentration (Log M)

FIG. 47

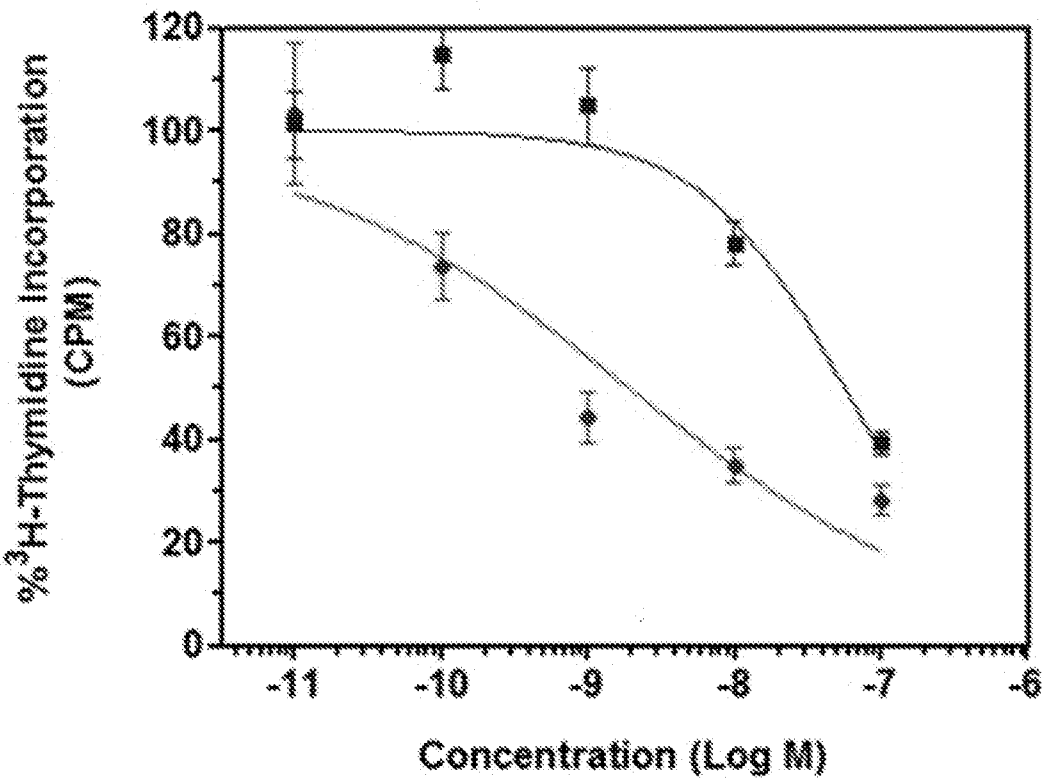

% ^{3}H-Thymidine Incorporation (CPM)
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
Concentration (Log M)

FIG. 48A

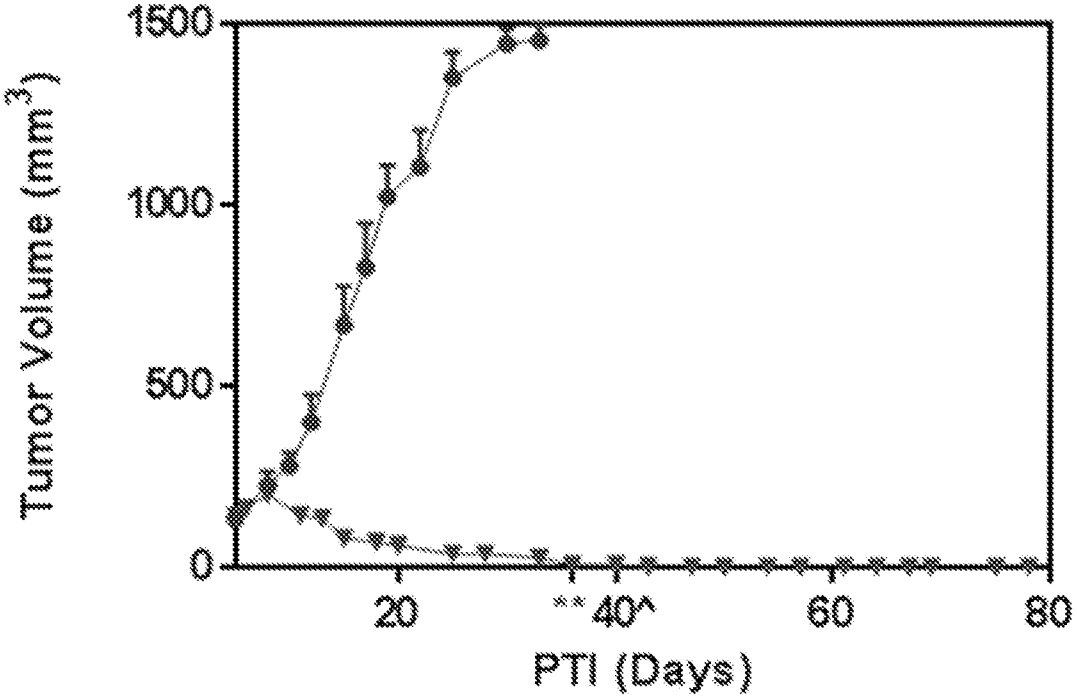


FIG. 48B

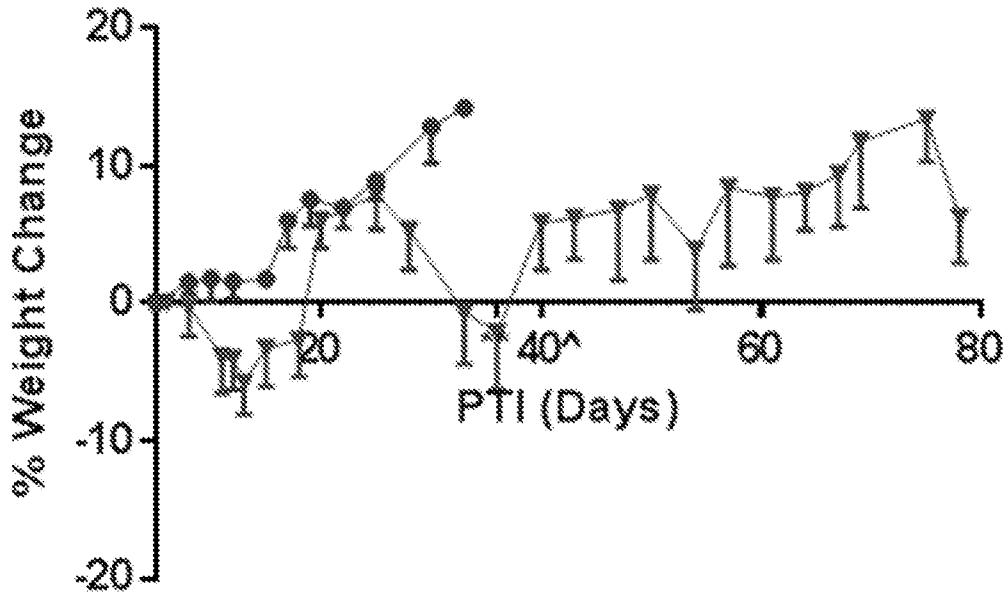


FIG. 49A

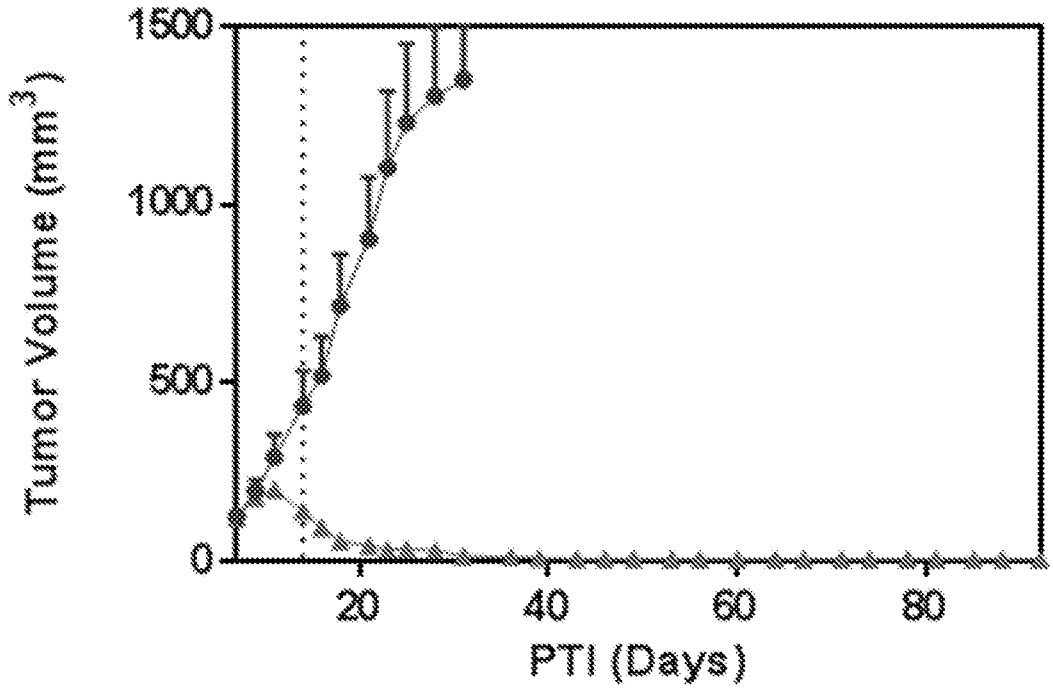


FIG. 49B

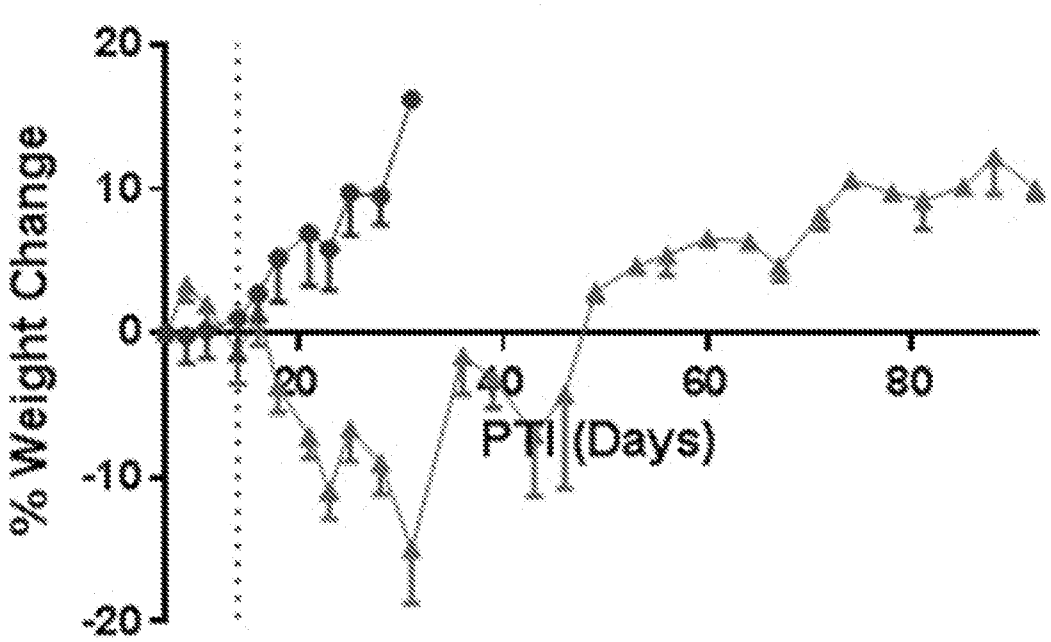


FIG. 50A

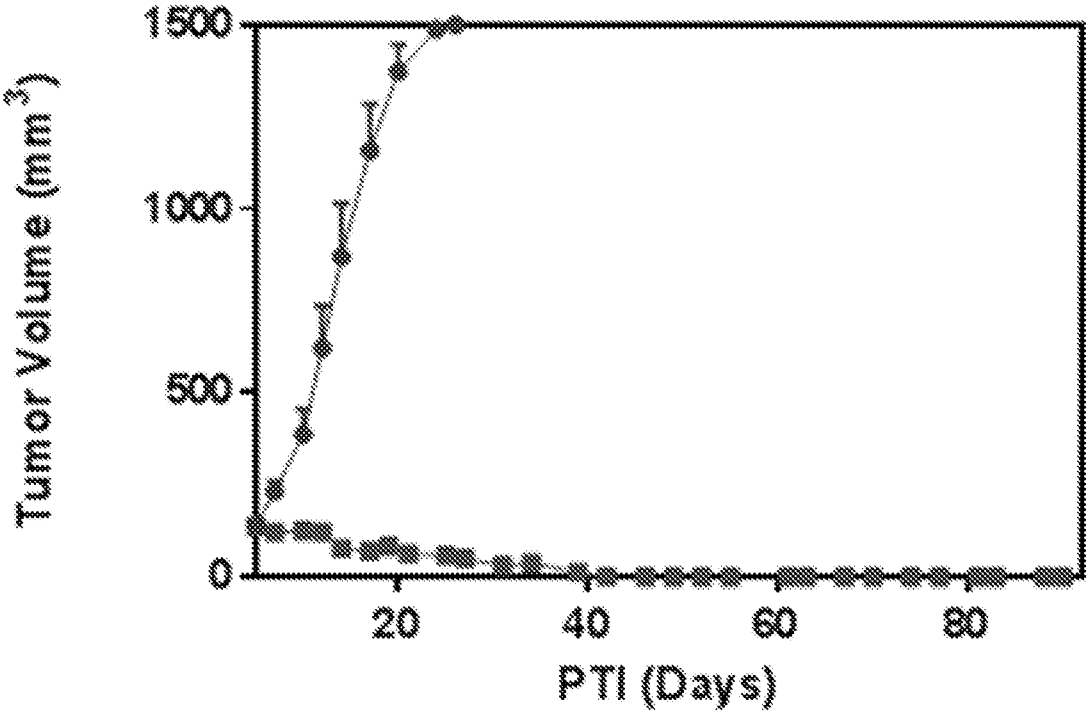


FIG. 50B

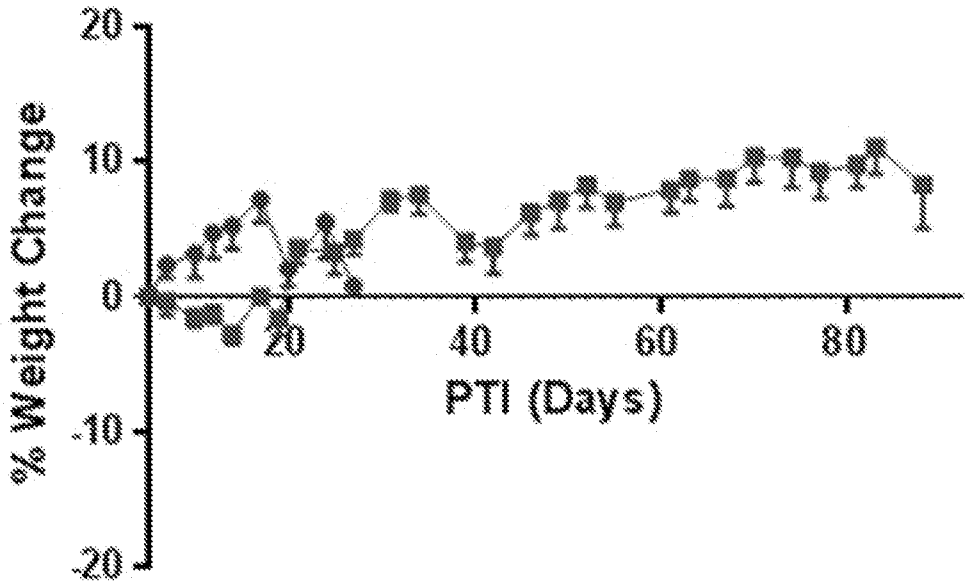


FIG. 51A

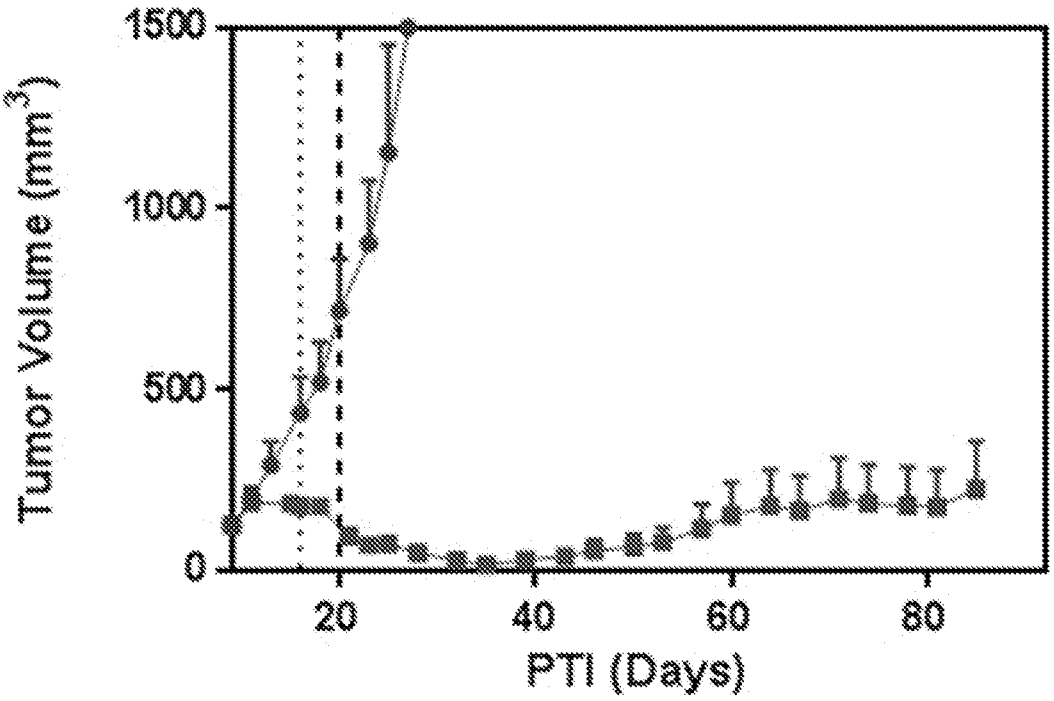


FIG. 51B

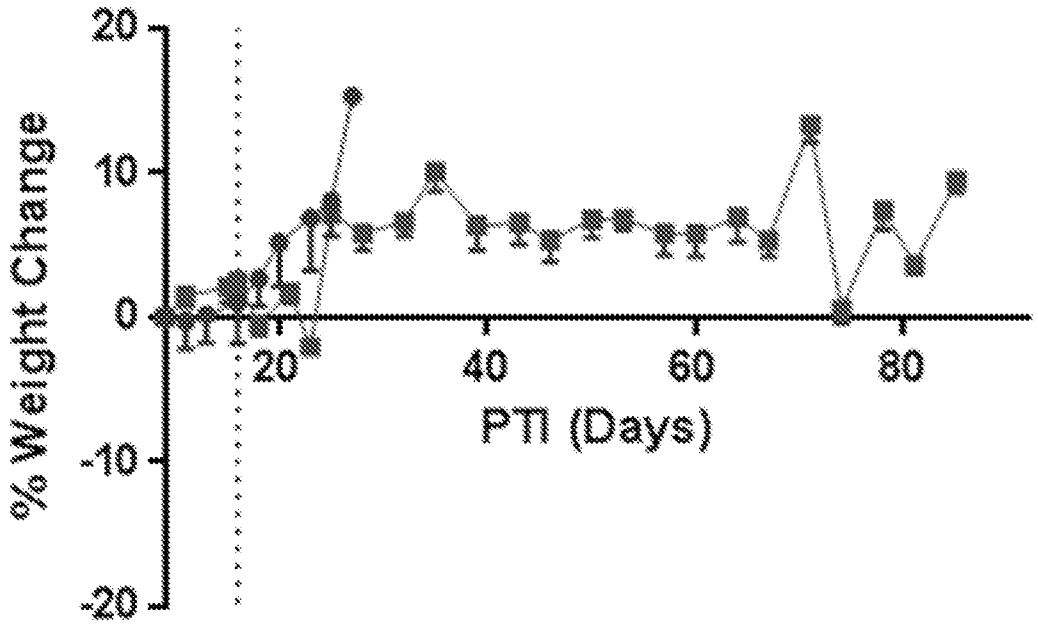


FIG. 52A

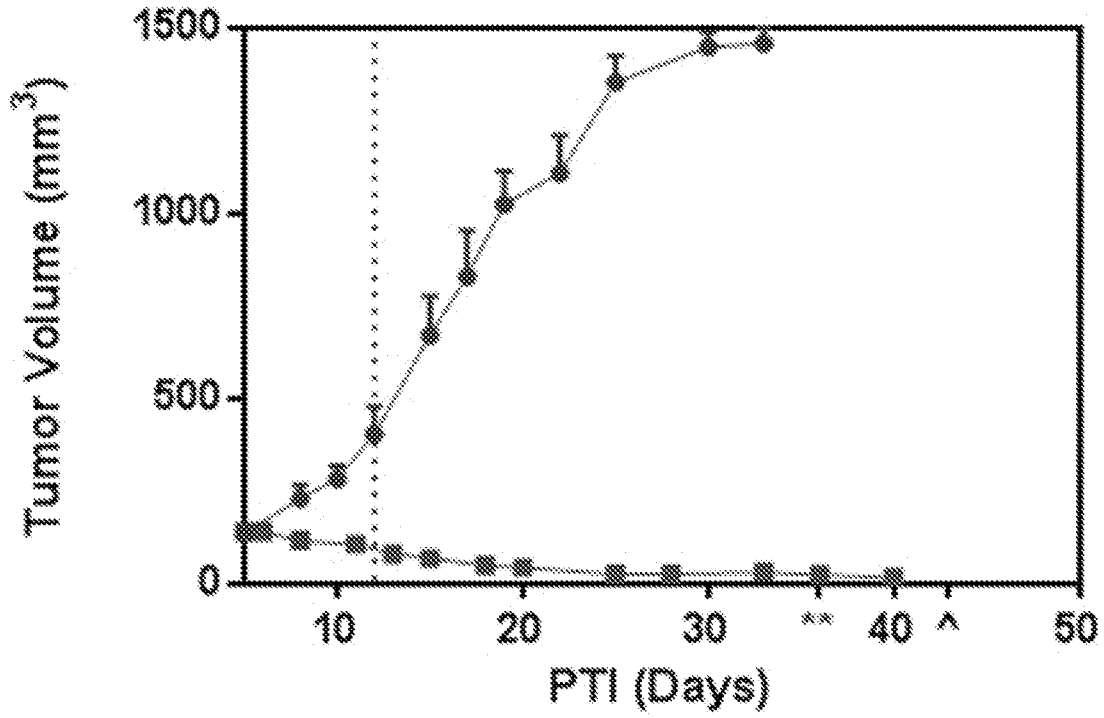


FIG. 52B

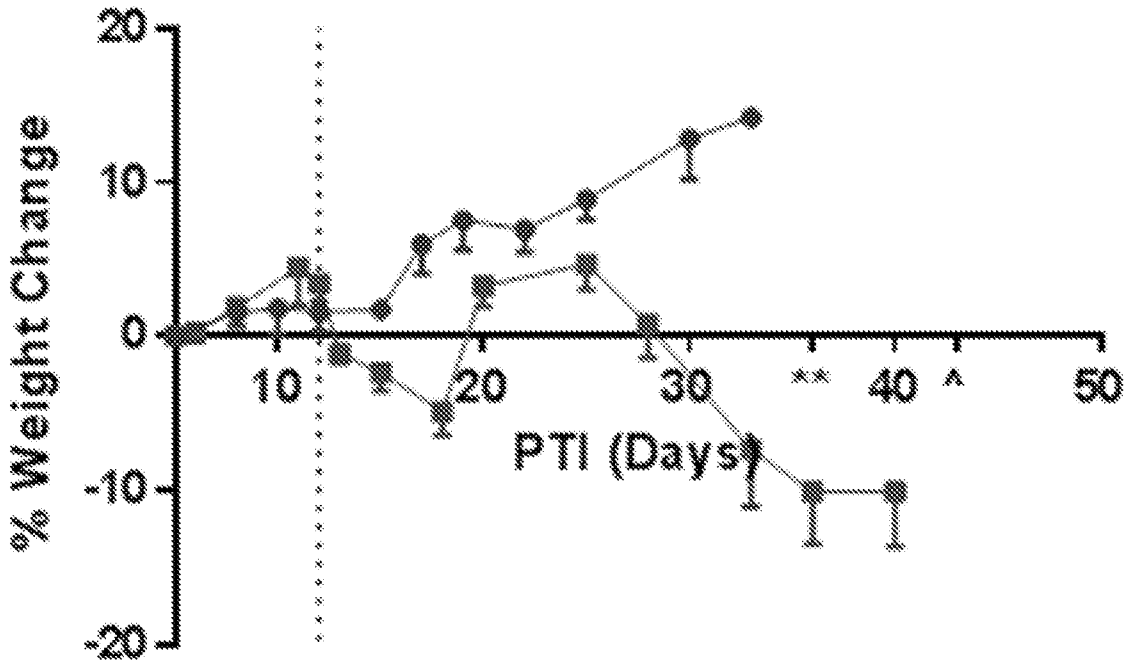


FIG. 53A

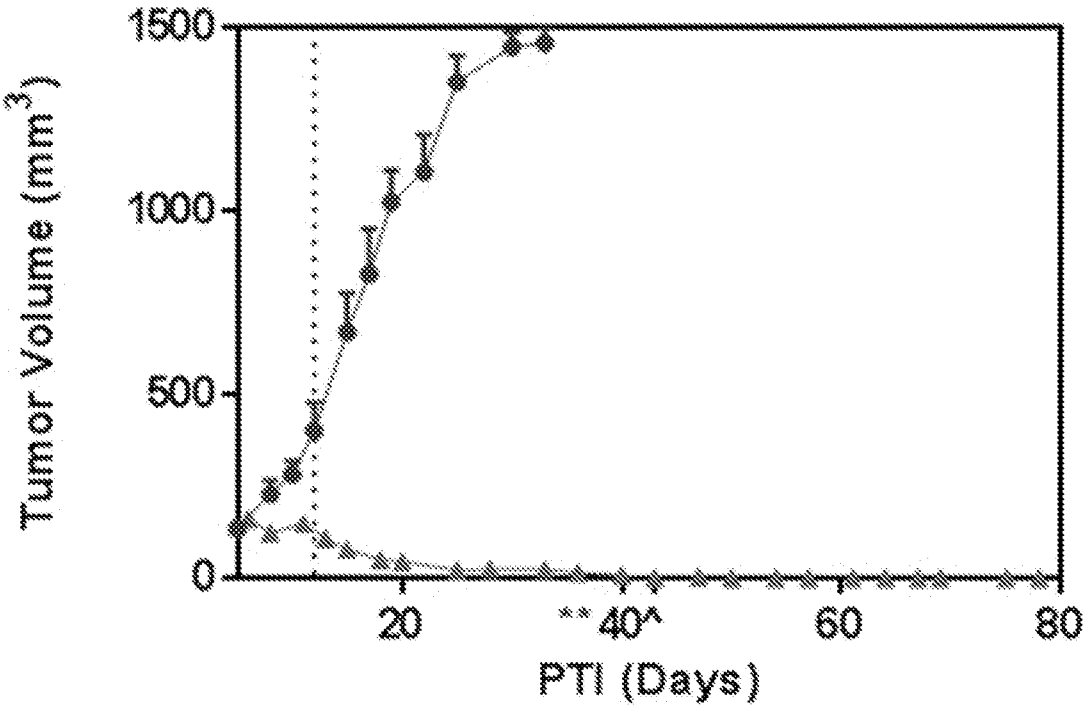


FIG. 53B

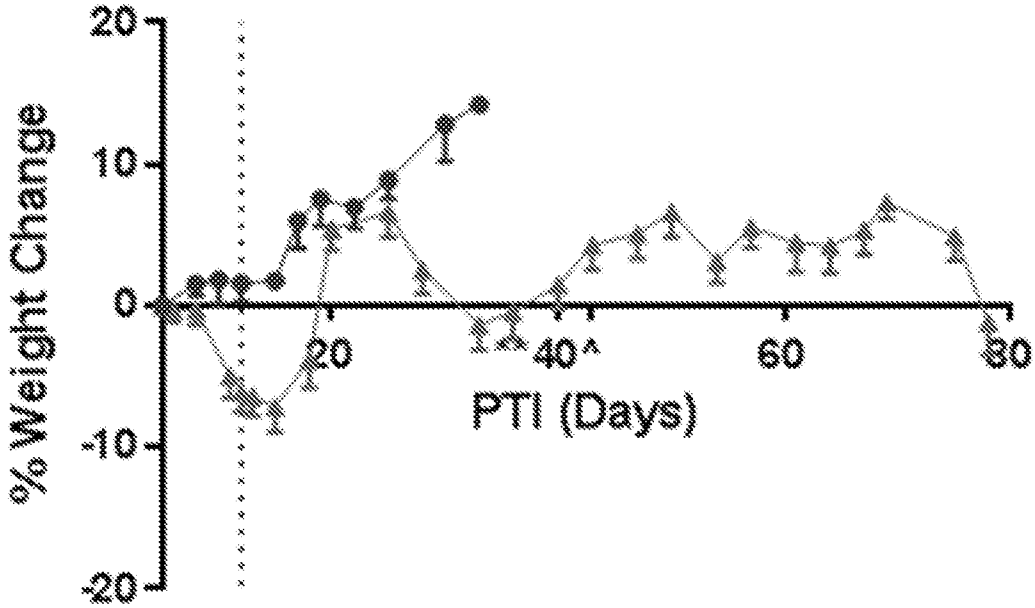


FIG. 54A

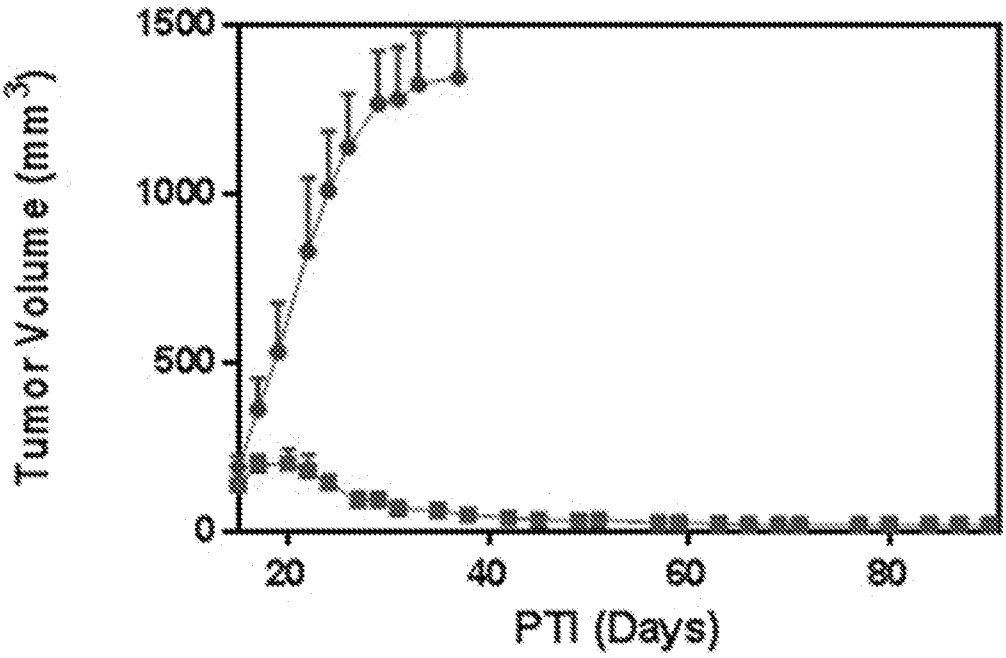


FIG. 54B

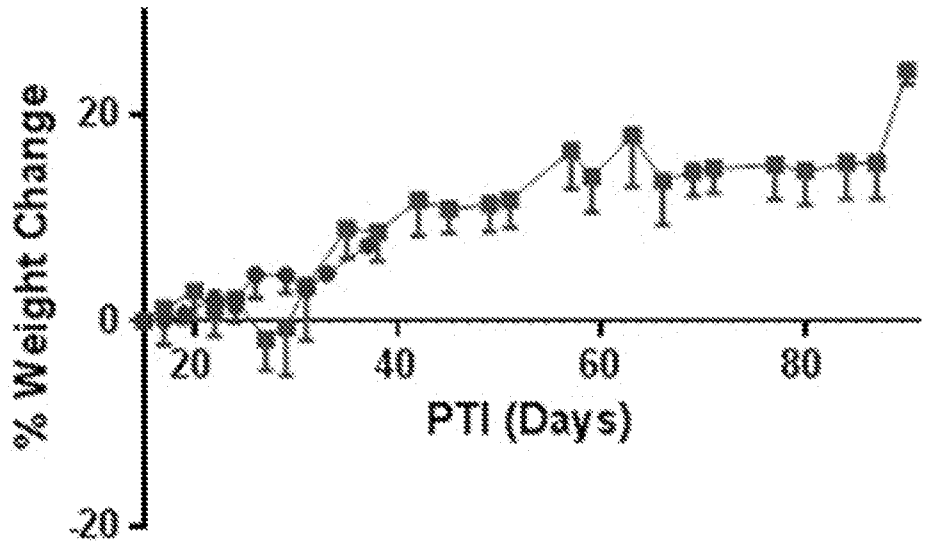


FIG. 55A

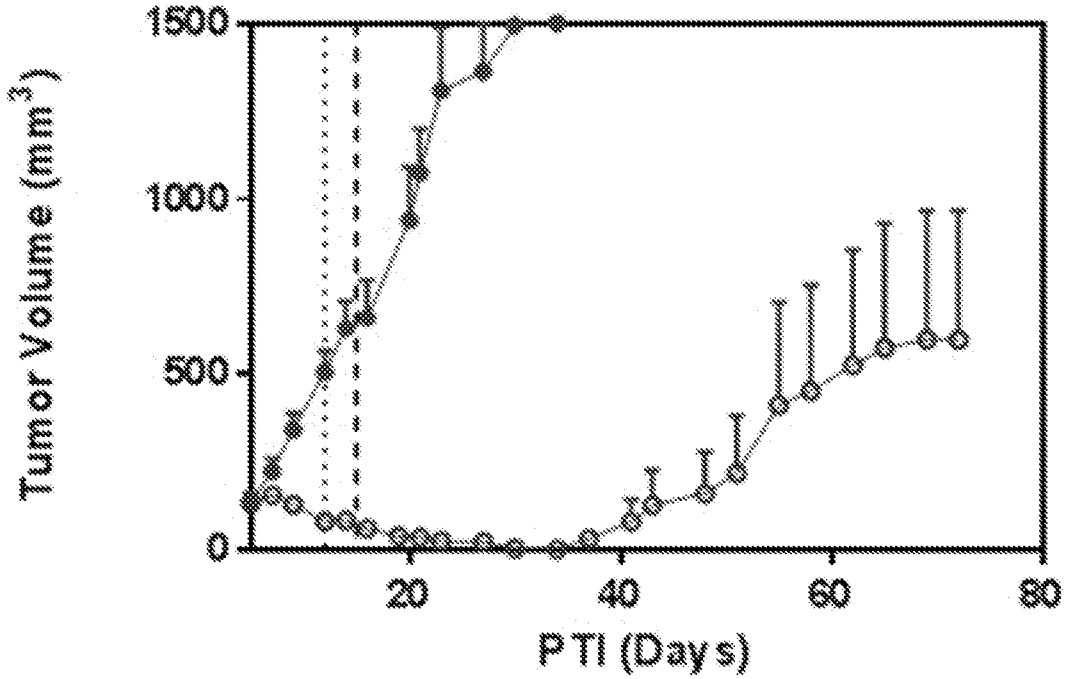


FIG. 55B

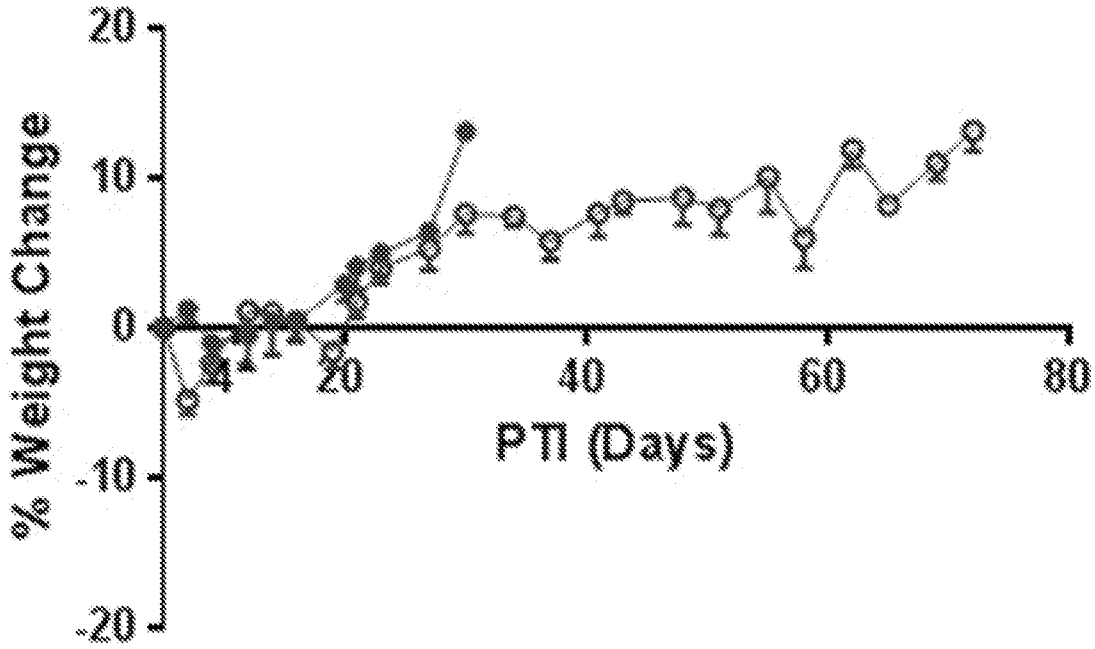


FIG. 56

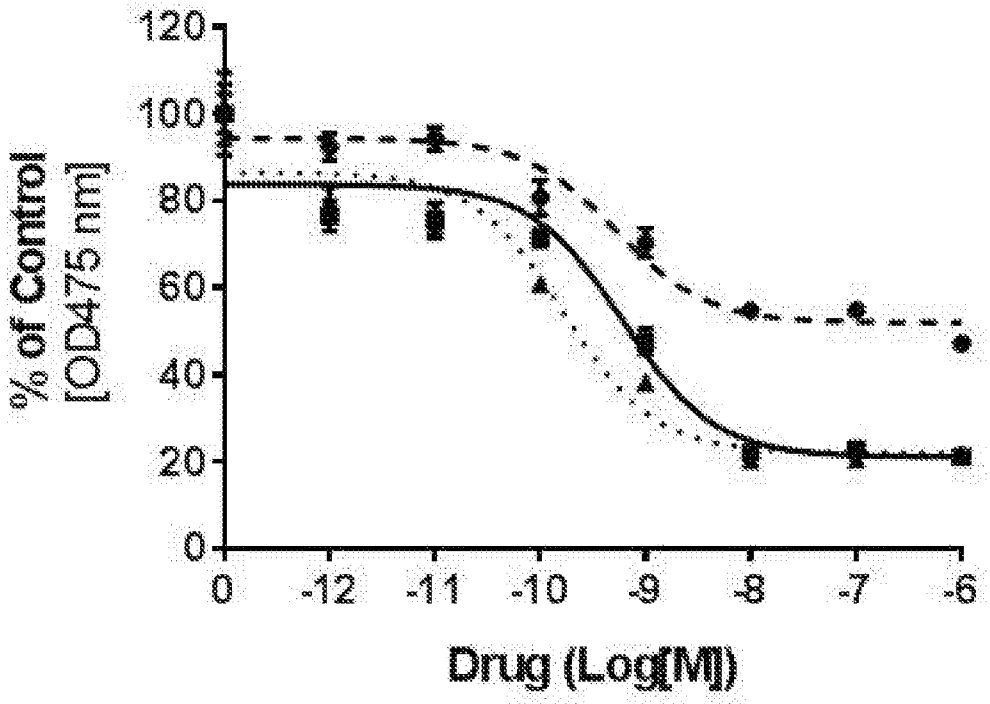

120
100
80
60
40
20
0
% of Control
[OD475 nm]
0
-12
-11
-10
-9
-8
-7
-6
Drug (Log[M])

FIG. 57

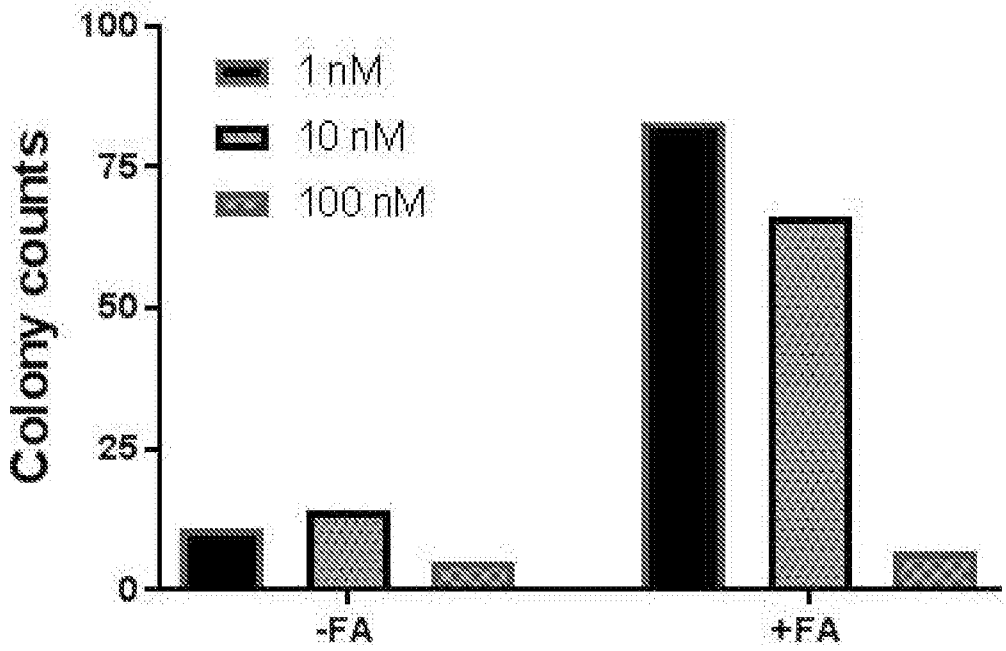

100
75
50
25
0
Colony counts
1 nM
10 nM
100 nM
-FA
+FA

FIG. 58

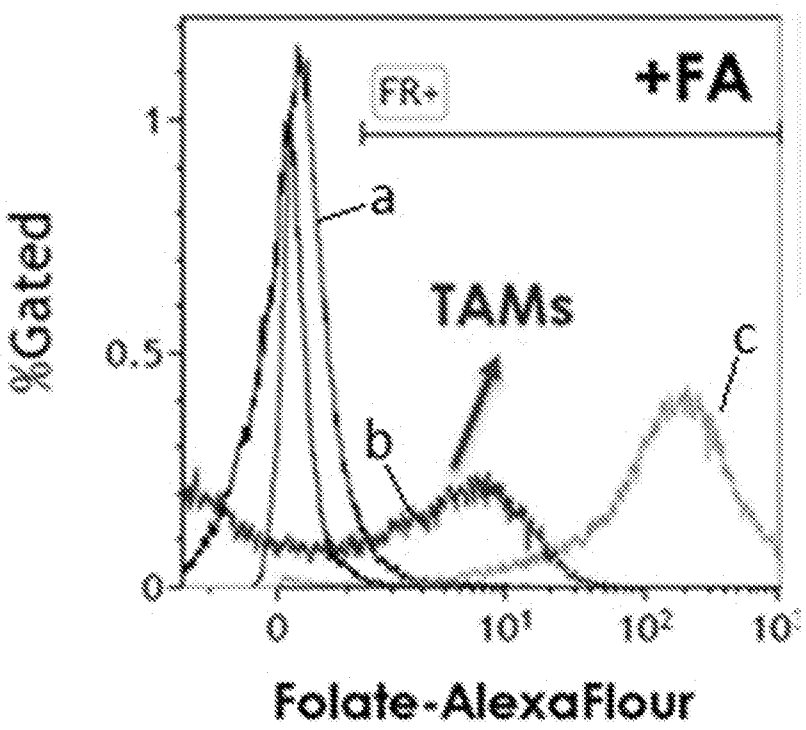


FIG. 59A

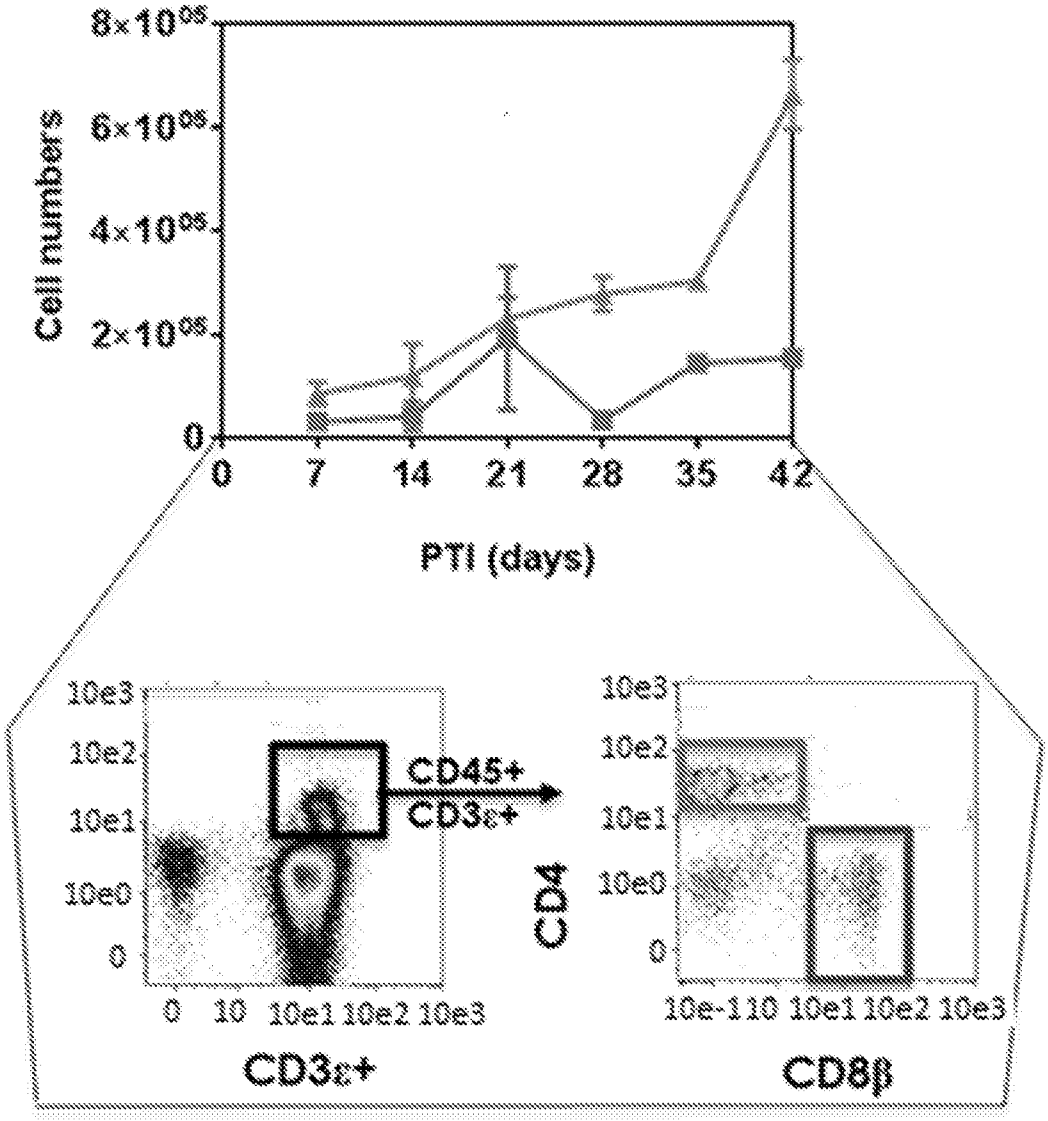

8×10^08
6×10^08
4×10^08
2×10^08
0
Cell numbers
0 7 14 21 28 35 42
PTI (days)
10e3
10e2
10e1
10e0
0
CD45+
CD3ε+
0 10 10e1 10e2 10e3
CD3ε+
CD4
10e-1 10 10e1 10e2 10e3
CD8β

FIG. 59B

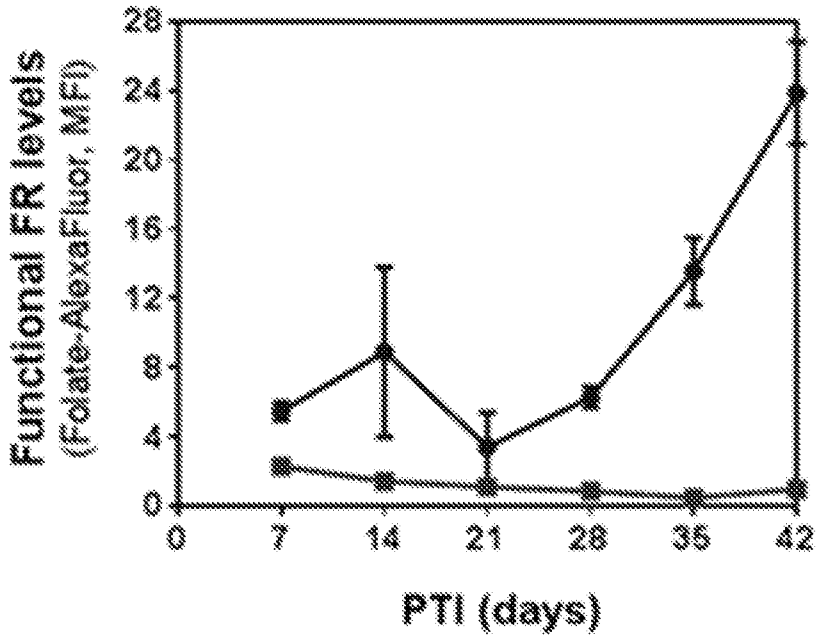


FIG. 59C

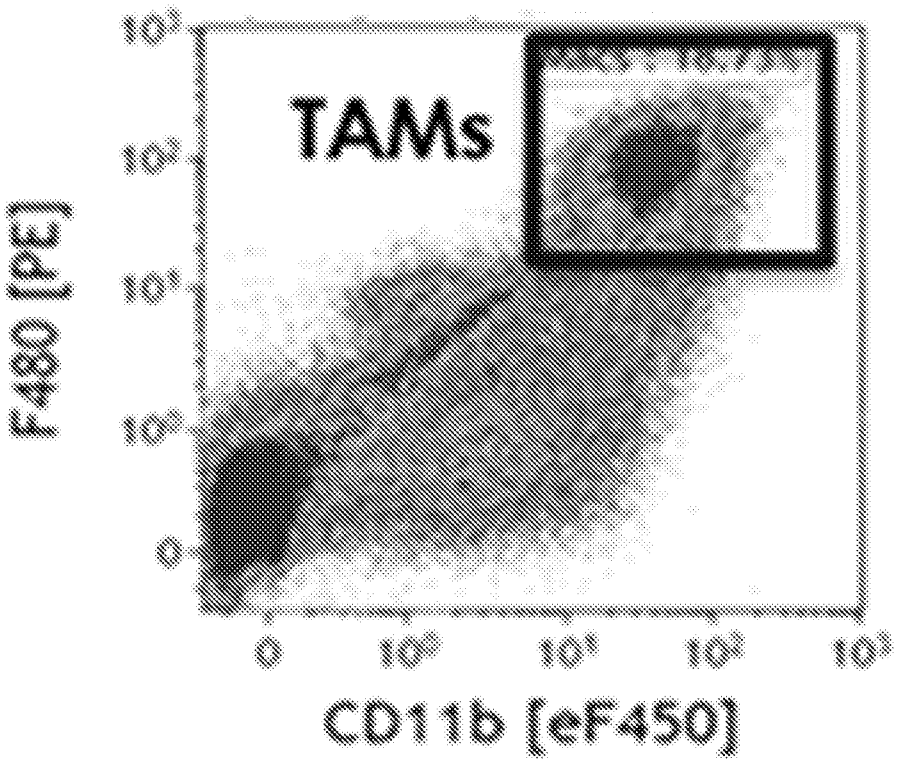


FIG. 60A

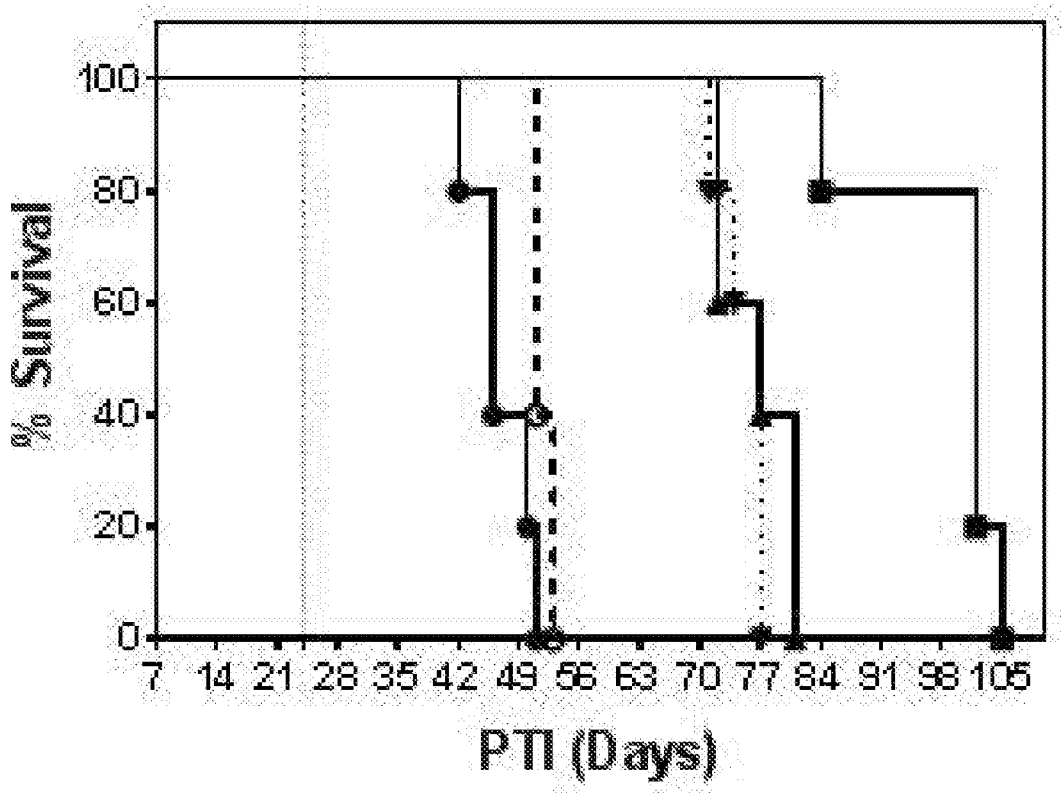
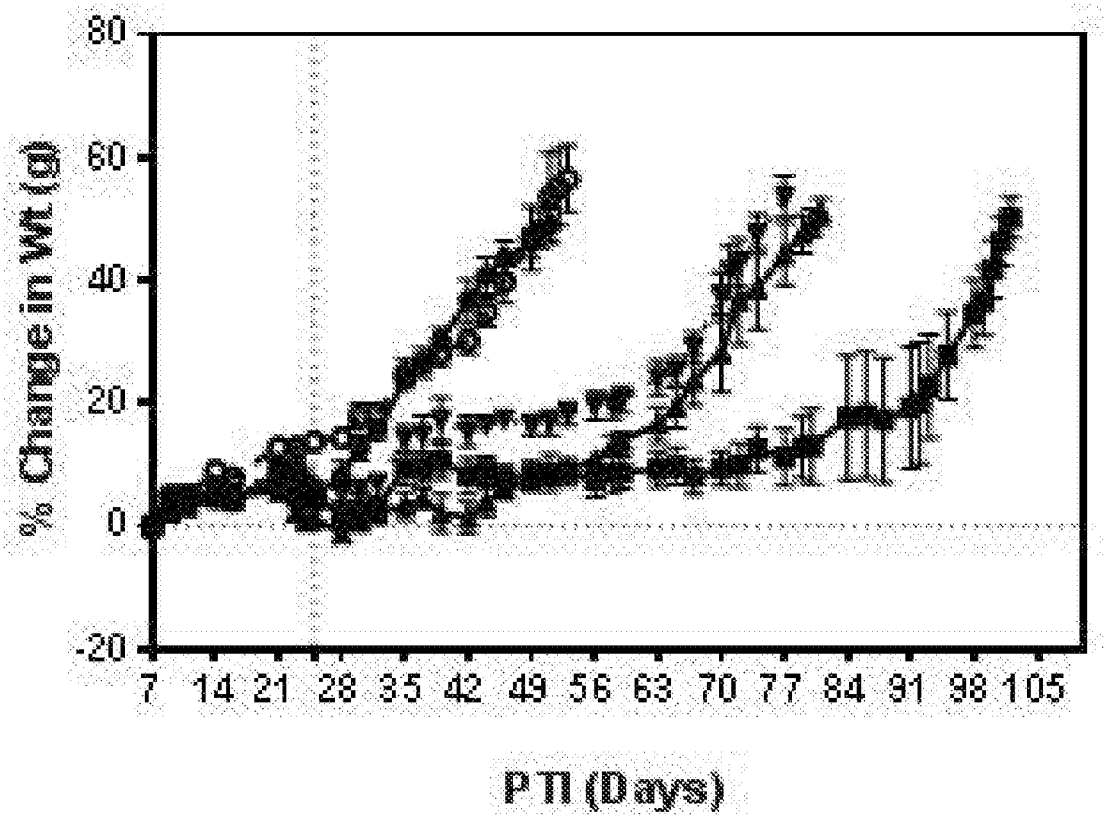


FIG. 60B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/24770

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/5517, A61K 45/06, A61K 47/48 (2017.01)
CPC - A61K 31/5517, A61K 47/48384, A61K 45/06, A61K 47/48561

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y -- A	WO 2016/040723 A1 (GENENTECH, INC.) 17 March 2016 (17.03.2016); para [0016], [0021], [00287], [00373], [00406], [00484]	1-4 ----- 59 ----- 58, 60
Y -- A	WO 2014/062697 A2 (ENDOCYTE, INC.) 24 April 2014 (24.04.2014); pg. 120, ln 3-5, pg. 126, ln 1-5	59 ----- 58, 60
A	US 2014/0088089 A1 (Chari) 27 March 2014 (27.03.2014); Fig. 36	1-4, 58-60
A	US 2015/315196 A1 (Howard) 05 November 2015 (05.11.2015); para [1788], [1795]	1-4, 58-60

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
02 June 2017	22 JUN 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/24770

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

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

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

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

3. ☒ Claims Nos.: 5-57, 61-68
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.