POLYPEPTIDE CONJUGATION COMPOSITIONS AND METHODS OF USE

CROSS-REFERENCE

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/581,936, filed September 11, 2023, U.S. Provisional Patent Application No. 63/591,072, filed October 17, 2023, U.S. Provisional Patent Application No. 63/607,467, filed December 7, 2023, and U.S. Provisional Patent Application No. 63/631,357, filed April 8, 2024, the disclosures of which are hereby incorporated by reference in their entirety for all purposes.

BACKGROUND

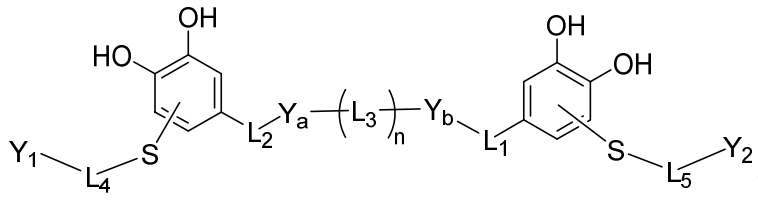
[0002] Coupling biomolecules (e.g., polypeptides such as antibodies) to payloads to generate conjugates, while preserving the function of the biomolecule, has long been a goal of chemical biology and biopharmaceutical research. Examples of conjugates include protein-peptide conjugates for vaccine development, antibody-drug conjugates, and antibody-protein conjugates for immunotherapies.

[0003] While many techniques have been developed to allow for attachment of payloads to polypeptides, it has been challenging to develop methods for attaching payloads in a site-specific manner to any position on a polypeptide's surface. There is a need for improved conjugation procedures that can modify a polypeptide or biomolecule in a simple yet site specific manner.

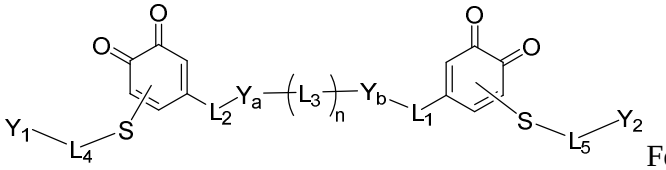
SUMMARY

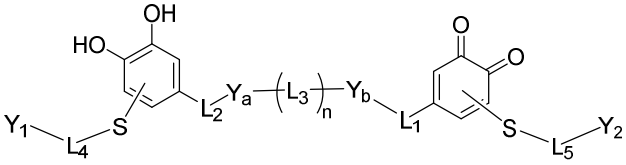
[0004] The present disclosure provides for chemoselective modification of a polypeptide or biomolecule.

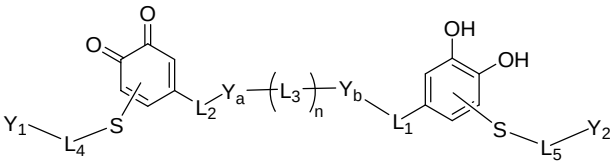
[0005] In one example, disclosed herein is a conjugate of Formula A, Formula B, Formula C, or Formula D:



Formula A,


Formula B,


Formula C, or


Formula D,

wherein:

Y_a is a first polypeptide comprising a first terminal tag comprising $(X_1)_{m1}X_2X_3X_4X_5$, wherein X_1-X_5 is any amino acid provided that at least one amino acid of X_1-X_5 is a tyrosine, and wherein m1 is an integer greater than or equal to 0;

Y_b is a second polypeptide comprising a second terminal tag different from the first terminal tag, the second terminal tag comprising $(X_6)_{m2}X_7X_8X_9X_{10}$, wherein X_6-X_{10} is any amino acid provided that at least one amino acid of X_6-X_{10} is a tyrosine, and wherein m2 is an integer greater than or equal to 0;

n is an integer greater than 0;

L_1 is an optional first linker;

L_2 is an optional second linker;

L_3 is an optional third linker;

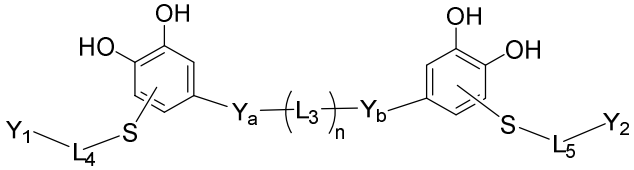
L_4 is an optional fourth linker;

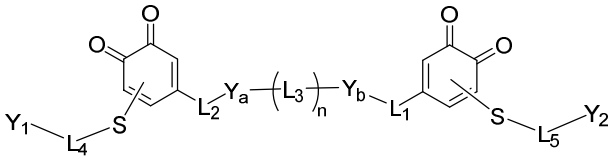
L_5 is an optional fifth linker;

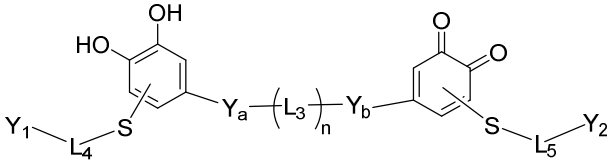
Y_1 is a first payload; and

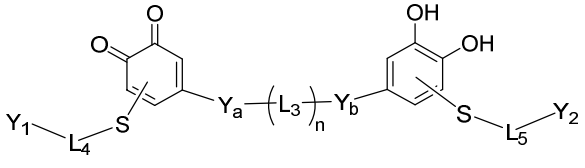
Y_2 is a second payload.

[0005a] In one aspect, the present disclosure refers to a conjugate of (i) Formula A', Formula B', Formula C', or Formula D':

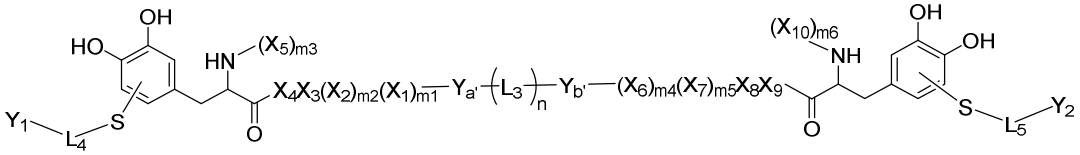

Formula A',


Formula B',

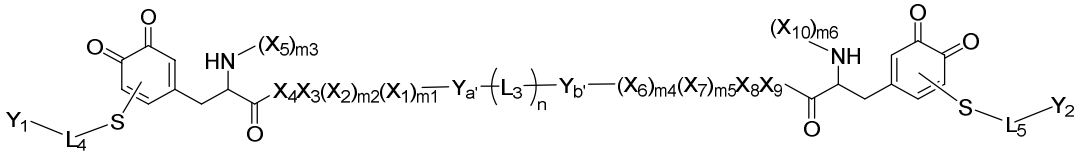

Formula C', or


Formula D',

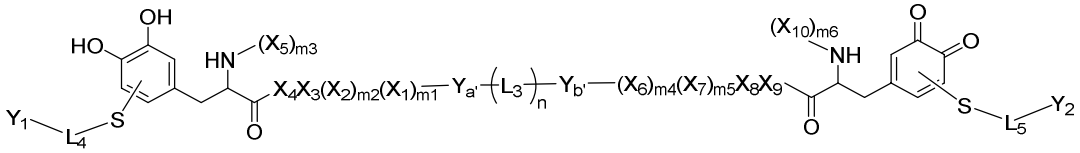
wherein:
Y_a is a first polypeptide comprising a first terminal tag comprising $(X_1)_{m1}X_2X_3X_4X_5$, wherein X_1-X_5 is any amino acid provided that at least one amino acid of X_1-X_5 comprises a tyrosine or a portion thereof, and wherein m1 is an integer greater than or equal to 0, or $X_1X_2X_3$, wherein X_1-X_3 is any amino acid provided that at least one amino acid of X_1-X_3 is a tyrosine or a portion thereof, wherein Y_a is linked to "S" via the tyrosine or a portion thereof ;
Y_b is a second polypeptide comprising a second terminal tag different from the first terminal tag, the second terminal tag comprising $(X_6)_{m2}X_7X_8X_9X_{10}$, wherein X_6-X_{10} is any amino acid provided that at least one amino acid of X_6-X_{10} comprises a tyrosine or a portion thereof, and wherein m2 is an integer greater than or equal to 0, or $X_1X_2X_3$, wherein X_1-X_3 is any amino acid provided that at least one amino acid of X_1-X_3 is a tyrosine or a portion thereof, wherein Y_b is linked to "S" via the tyrosine or a portion thereof;
n is an integer greater than 0;
L_3 is an optional third linker;
L_4 is an optional fourth linker;
L_5 is an optional fifth linker;
Y_1 is a first payload; and
Y_2 is a second payload;
(ii) Formula A-I, Formula B-I, Formula C-I, or Formula D-I:



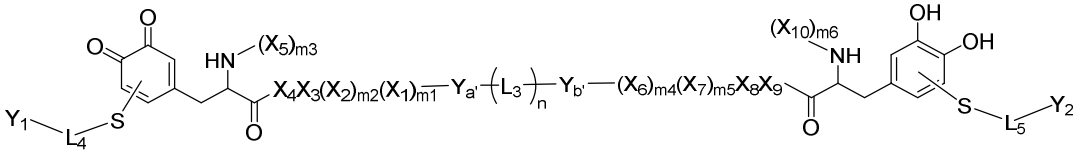
Formula A-I,



Formula B-I,

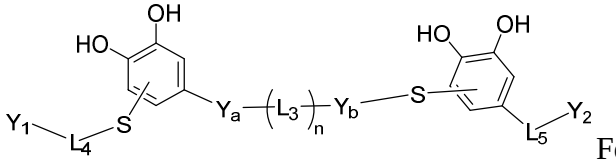


Formula C-I, or

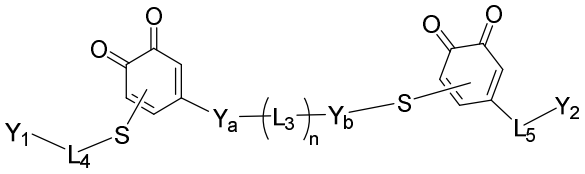


Formula D-I; wherein:
$Y_{a'}$ is a first polypeptide,
each of X_1, X_2, X_3, X_4, and X_5 is independently any amino acid, wherein m1, m2, and m3 are each an integer greater than or equal to 0;
$Y_{b'}$ is a second polypeptide,
each of X_6, X_7, X_8, X_9, and X_{10} is independently any amino acid, wherein m4, m5, and m6 are each an integer greater than or equal to 0, and wherein the $-(X_1)_{m1}(X_2)_{m2}X_3X_4-$ moiety is different from the $-(X_6)_{m1}(X_7)_{m2}X_8\ X_9-$ moiety;
n is an integer greater than 0;
L_3 is an optional third linker;
L_4 is an optional fourth linker;
L_5 is an optional fifth linker;

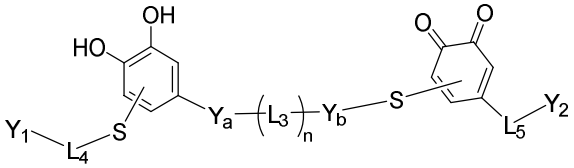
Y_1 is a first payload; and
Y_2 is a second payload;
or
(iii) Formula E', Formula F', Formula G', Formula H', Formula J', or Formula K'



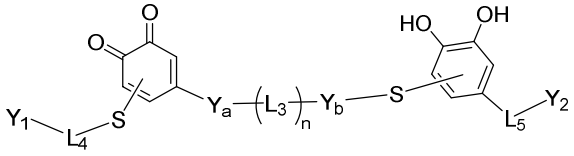
Formula E',



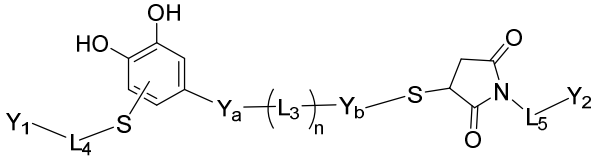
Formula F',



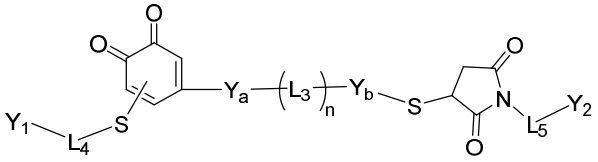
Formula G',



Formula H',



Formula J', or

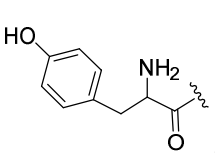
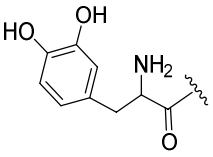
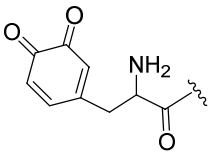
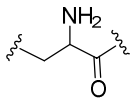


Formula K',

wherein:
Y_a is a first polypeptide comprising a first terminal tag comprising $(X_1)_{m1}X_2X_3X_4X_5Y$, wherein X_1-X_5 is any amino acid provided that no more than two amino acids of X_2, X_3, X_4, and X_5 are aspartate or glutamate and wherein m1 is an integer greater than or equal to 0;
Y_b is a second polypeptide;
n is an integer greater than 0;
L_3 is an optional third linker;
L_4 is an optional fourth linker;
L_5 is an optional fifth linker;
Y_1 is a first payload; and
Y_2 is a second payload.

[0006] In one example, disclosed herein is a conjugate of Formula A', Formula B', Formula C', or Formula D':

Y_2 is a second payload.

[0007] In some examples, the tyrosine or a portion thereof is selected from the group consisting of , , , and .

[0008] In some examples, the first terminal tag comprises at least two amino acids selected from the group consisting of aspartate (D), glutamate (E), arginine (R), and lysine (K).

[0009] In some examples, the second terminal tag comprises at least two amino acids selected from the group consisting of aspartate (D), glutamate (E), arginine (R), and lysine (K).

[0010] In some examples, the first terminal tag comprises at least one of GGGGY, RGGGY, RGRGY, RRRGY, RRRRY, EGGGY, EGEGY, EEEGY, EEEEY, GGGWY, GGWGY, RRRWY, RRWRY, EEEWY, EEWEY, DDDDY, SGGY, SGY, KKKKY, RRKKY, RRRKY, EDEDY, EDDDY, EEDDY, RRKKY, KKGGY, DDGGY, EESY, ESSSY, SSSY, SNNY, SSNY, SEGY, SSSEY, EGGY, SSEEY, SESY, ESSY, ESY, SRY, SKY, SNY, ERY, EKY, EGY, SEYP, SHRKY, SARKY, SPPEY, SSEEY, SEEEY, and SSSSY.

[0011] In some examples, the second terminal tag comprises at least one of GGGGY, RGGGY, RGRGY, RRRGY, RRRRY, EGGGY, EGEGY, EEEGY, EEEEY, GGGWY, GGWGY, RRRWY, RRWRY, EEEWY, EEWEY, DDDDY, SGGY, SGY, KKKKY, RRKKY, RRRKY, EDEDY, EDDDY, EEDDY, RRKKY, KKGGY, DDGGY, EESY, ESSSY, SSSY, SNNY, SSNY, SEGY, SSSEY, EGGY, SSEEY, SESY, ESSY, ESY, SRY, SKY, SNY, ERY, EKY, EGY, SEYP, SHRKY, SARKY, SPPEY, SSEEY, SEEEY, and SSSSY.

[0012] In some examples, the first terminal tag comprises SGGY, SGY, SGGGY, ESY, EESY, ESSY, ESSSY, SESY, SGGGY, EESY, SSSY, SNNY, SSNY, EGGY, SESY, or SEGY.

[0013] In some examples, the second terminal tag comprises EEEY, EEEEY, SSSEY, SSEEY, SSSSY, or SSNNY.

[0014] In some examples, Y_a, Y_b, or both Y_a and Y_b are glycosylated.

[0015] In some examples, Y_a, Y_b, or both Y_a and Y_b comprise a non-terminal tyrosine.

[0016] In some examples, at least one of L_3, L_4, or L_5 is a cleavable linker.

[0017] In some examples, the cleavable linker is an electrophilically cleavable linker, a nucleophilically cleavable linker, a photocleavable linker, a metal cleavable linker, an electrolytically-cleavable linker, an acid cleavable linker, or a proteolytically cleavable linker.

[0018] In some examples, the cleavable linker further include a pegylated group, a sugar group, or a modification that increases hydrophilicity.

[0019] In some examples, the cleavable linker is cleavable under reductive and/or oxidative conditions.

[0020] In some examples, the cleavable linker is cleavable under acidic conditions.

[0021] In some examples, the cleavable linker comprises a disulfide bond.

[0022] In some examples, the cleavable linker is a proteolytically cleavable linker and comprises a protease recognition sequence.

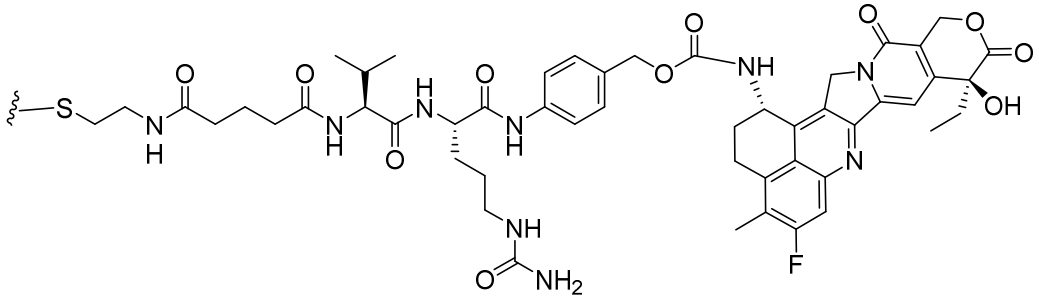
[0023] In some examples, the protease recognition sequence is recognized by a protease selected from the group comprising a metalloprotease, cathepsin B, and tobacco etch virus (TEV).

[0024] In some examples, the cleavable linker comprises a dipeptide, tripeptide or tetrapeptide. In some examples, the dipeptide is a valine-citrulline (Val-Cit) dipeptide, a valine-lysine dipeptide, a valine-alanine dipeptide. In some examples, the tetrapeptide is a glycine-glycine-phenylalanine-glycine (GGFG) tetrapeptide.

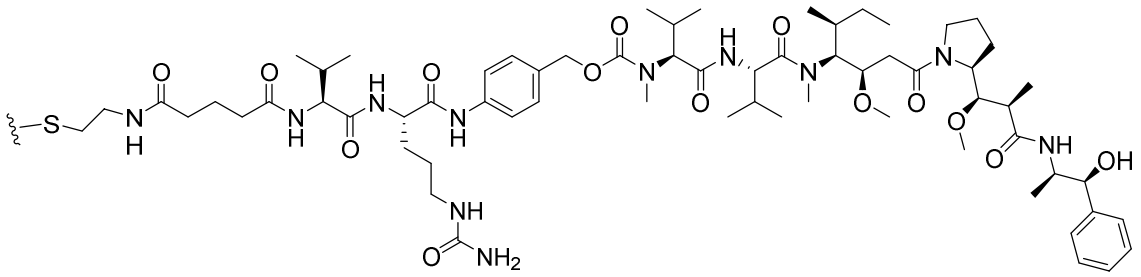
[0025] In some examples, the cleavable linker is selected from the group comprising PABC (p-aminobenzyl alcohol), glucuronide, and MABC (m-aminobenzyl alcohol).

[0026] In some examples, the cleavable linker is Val-Cit-PABC.

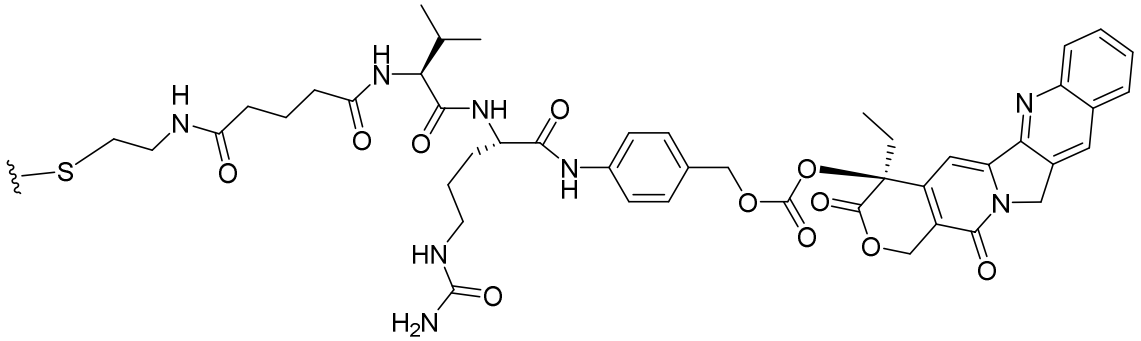
[0027] In some examples, wherein “Y1-L4-S-“ and/or “-S-L5-Y2” is selected from the group consisting of:



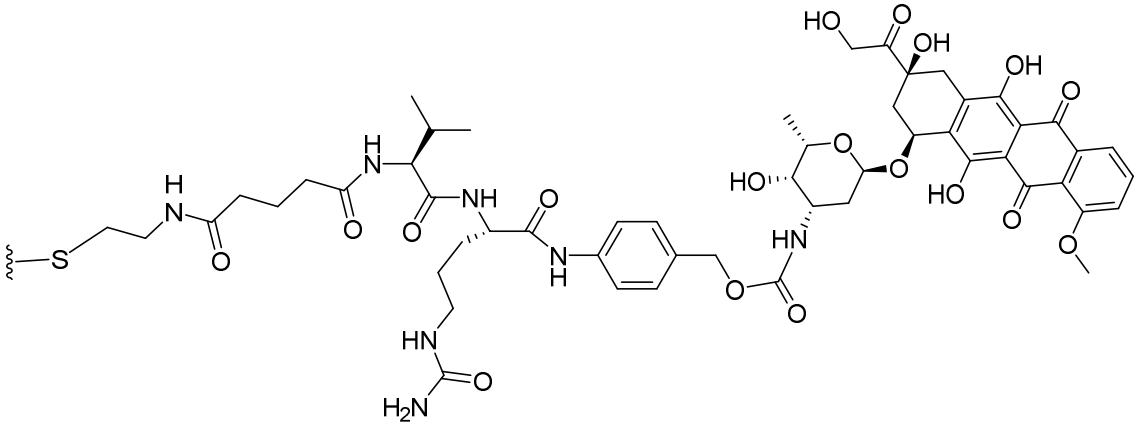
S-VC-PAB-DXD,



S-VC-PAB-MMAE,



S-VC-PAB-Camptothecin, and



S-VC-PAB-Doxorubicin.

[0028] In some examples, Y_a is a heavy chain variable region (VH), a light chain variable region (VL), a heavy chain, a light chain, a constant chain of an antibody or antibody fragment, a peptide, or a cyclic peptide.

[0029] In some examples, the peptide is a binding peptide.

[0030] In some examples, Y_b is a heavy chain variable region (VH), a light chain variable region (VL), a heavy chain, a light chain, a constant chain of an antibody, antibody fragment, a peptide, or a cyclic peptide. In some examples, the peptide is a binding peptide.

[0031] In some examples, Y_a and Y_b are attached via L_3. In some examples, L_3 comprises a peptide sequence, a dimerization and docking domain, a leucine zipper, or knobs-into-holes. In some examples, L_3 comprises a peptide bond, a disulfide bond, a

maleimide bond, thioether bond, an azide-alkyne cycloaddition, a cystinyl-dopa, or a hydrogen bond. In some examples, L_3 is a linker.

[0032] In some examples, the linker comprises a sequence selected from the group consisting of $(GS)_{n3}$, $(G2S)_{n3}$, $(G3S)_{n3}$, $(G4S)_{n3}$, $(G)_{n3}$, $(GGSGGD)_{n3}$, $(GGSGGE)_{n3}$, $(GGGSGSGGGGS)_{n3}$, and $(GGGGGPGGGGP)_{n3}$ and wherein n3 is an integer from 2 to 20.

[0033] In some examples, L_3 comprises a terminal tyrosine or a portion thereof.

[0034] In some examples, n is 1, 2, 3, 4, or 5. In some examples, n is 1.

[0035] In some examples, Y_1 and Y_2 are the same.

[0036] In some examples, Y_1 and Y_2 are different.

[0037] In some examples, Y_1, Y_2, or both Y_1 and Y_2 is a small molecule.

[0038] In some examples, the small molecule is selected from the group consisting of deruxtecan, exatecan, FL118, irinotecan, topotecan, SN-38, rubitecan, belotecan, lurototecan, gimatecan, diflomotecan, karenitecan, silatecan, namitecan, elomotecan, DRF-1042, delimotecan, NSC606985, chimmitecan, ZBH-1205, auristatin (MMAE, MMAF, MMAG, MMAH) calicheamicin, doxorubicin, taxol and taxol derivatives, maytansinoids (DM1-4), pyrolodiazepines (PBDs), tubulysins, eribulin, anthramycin, duocarmycin, anthracycline, and camptothecin (CPT), including the lactone and carboxylate forms of CPT.

[0039] In some examples, the small molecule is selected from the group of a topoisomerase inhibitor and a tubulin inhibitor.

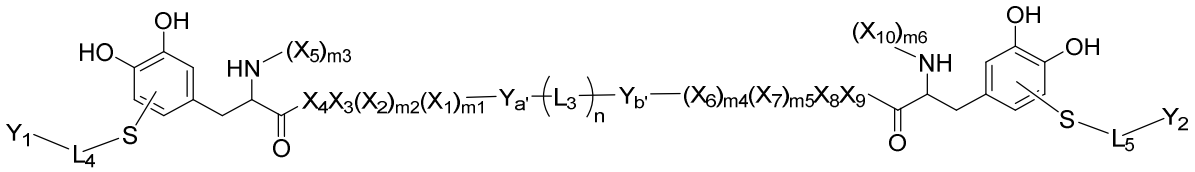
[0040] In some examples, Y_1, Y_2, or both Y_1 and Y_2 comprise a nucleic acid, an immune agonist, a peptide, a cytokine, or a binding domain. In some examples, Y_1, Y_2, or both Y_1 and Y_2 comprise a nucleic acid.

[0041] In some examples, Y_1, Y_2, or both Y_1 and Y_2 comprise an oligonucleotide.

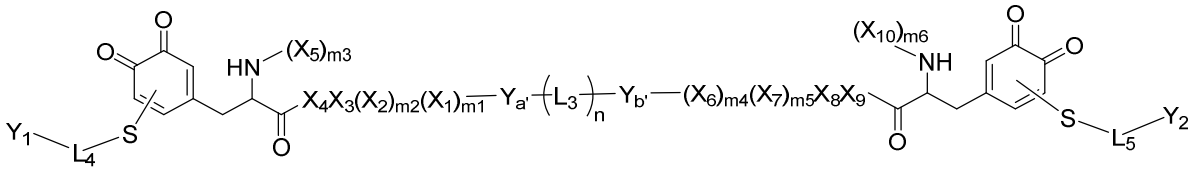
[0042] In some examples, Y_1, Y_2, or both Y_1 and Y_2 comprises a peptide.

[0043] In some examples, Y_1, Y_2, or both Y_1 and Y_2 comprises a binding peptide.

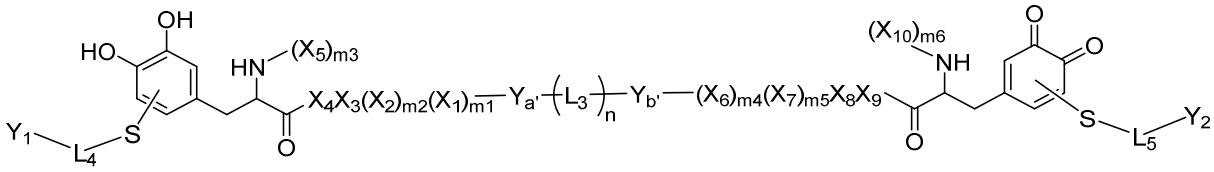
[0044] In another example, provided herein is a conjugate of Formula A-I, Formula B-I, Formula C-I, or Formula D-I:



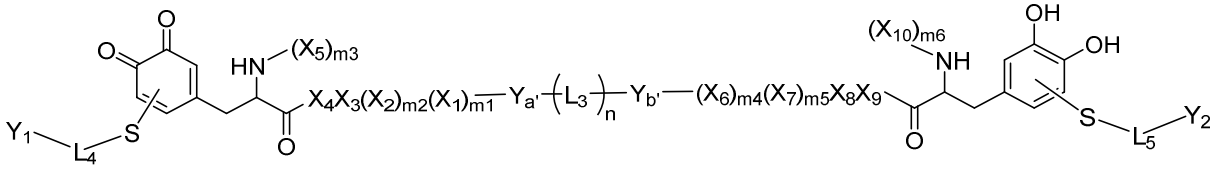
Formula A-I,



Formula B-I,



Formula C-I, or



Formula D-I; wherein:

$Y_{a'}$ is a first polypeptide,

each of X_1, X_2, X_3, X_4, and X_5 is independently any amino acid, wherein m1, m2, and m3 are each an integer greater than or equal to 0;

$Y_{b'}$ is a second polypeptide,

each of X_6, X_7, X_8, X_9, and X_{10} is independently any amino acid, wherein m4, m5, and m6 are each an integer greater than or equal to 0, and wherein the $-(X_1)_{m1}(X_2)_{m2}X_3X_4-$ moiety is different from the $-(X_6)_{m1}(X_7)_{m2}X_8\ X_9-$ moiety;

n is an integer greater than 0;

L_3 is an optional third linker;

L_4 is an optional fourth linker;

L_5 is an optional fifth linker;

Y_1 is a first payload; and

Y_2 is a second payload.

[0045] In some examples, the $-(X_1)_{m1}(X_2)_{m2}X_3X_4-$ moiety comprises at least two amino acids selected from the group consisting of aspartate (D), glutamate (E), arginine (R), and lysine (K).

[0046] In some examples, the $-(X_6)_{m1}(X_7)_{m2}X_8\ X_9-$ moiety comprises at least two amino acids selected from the group consisting of aspartate (D), glutamate (E), arginine (R), and lysine (K).

[0047] In some examples, the -$(X_1)_{m1}(X_2)_{m2}X_3X_4$- moiety is selected from the group consisting of GGGG, RGGG, RGRG, RRRG, RRRR, EGGG, EGEG, EEEG, EEEE, GGGW, GGWG, RRRW, RRWR, EEEW, EEWE, DDDD, SGG, SG, KKKK, RRKK, RRRK, EDED, EDDD, EEDD, RRKK, KKGG, DDGG EES, ESSS, SSS, SNN, SSN, SEG, SSSE, EGG, SSEE, SES, ESS, ES, SR, SK, SN, ER, EK, EG, SE, SHRK, SARK, SPPE, SSEE, SEEE, and SSSS.

[0048] In some examples, the -$(X_6)_{m1}(X_7)_{m2}X_8$ X_9- moiety is selected from the group consisting of GGGG, RGGG, RGRG, RRRG, RRRR, EGGG, EGEG, EEEG, EEEE, GGGW, GGWG, RRRW, RRWR, EEEW, EEWE, DDDD, SGG, SG, KKKK, RRKK, RRRK, EDED, EDDD, EEDD, RRKK, KKGG, DDGG, EES, ESSS, SSS, SNN, SSN, SEG, SSSE, EGG, SSEE, SES, ESS, ES, SR, SK, SN, ER, EK, EG, SE, SHRK, SARK, SPPE, SSEE, SEEE, and SSSS.

[0049] In some examples, the -$(X_1)_{m1}(X_2)_{m2}X_3X_4$- moiety is SGG, SG, SGGG, ES, EES, ESS, ESSS, SES, SGGG, EES, SSS, SNN, SSN, EGG, SES, or SEG.

[0050] In some examples, the -$(X_6)_{m1}(X_7)_{m2}X_8$ X_9- - moiety is EEE, EEEE, SSSE, SSEE, SSSS, or SSNN.

[0051] In some examples, $Y_{a'}$, $Y_{b'}$, or both $Y_{a'}$ and $Y_{b'}$ are glycosylated.

[0052] In some examples, at least one of L_3, L_4, or L_5 is a cleavable linker. In some examples, the cleavable linker is an electrophilically cleavable linker, a nucleophilically cleavable linker, a photocleavable linker, a metal cleavable linker, an electrolytically-cleavable linker, an acid cleavable linker, or a proteolytically cleavable linker.

[0053] In some examples, the cleavable linker further include a pegylated group, a sugar group, or a modification that increases hydrophilicity. In some examples, the cleavable linker is cleavable under reductive and/or oxidative conditions. In some examples, the cleavable linker is cleavable under acidic conditions. In some examples, the cleavable linker comprises a disulfide bond.

[0054] In some examples, the cleavable linker is a proteolytically cleavable linker and comprises a protease recognition sequence. In some examples, the protease recognition sequence is recognized by a protease selected from the group comprising a metalloprotease, cathepsin B, and tobacco etch virus (TEV).

[0055] In some examples, the cleavable linker comprises a dipeptide, tripeptide or tetrapeptide. In some examples, the dipeptide is a valine-citrulline (Val-Cit) dipeptide, a valine-lysine dipeptide, a valine-alanine dieptide. In some examples, the tetrapeptide is a glycine-glycine-phenylalanine-glycine (GGFG) tetrapeptide.

[0056] In some examples, the cleavable linker is selected from the group comprising PABC (p-aminobenzyl alcohol), glucuronide, and MABC (m-aminobenzyl alcohol),

[0057] In some examples, $Y_{a'}$ is a heavy chain variable region (VH), a light chain variable region (VL), a heavy chain, a light chain, a constant chain of an antibody or antibody fragment, a peptide, or a cyclic peptide. In some examples, the peptide is a binding peptide.

[0058] In some examples, $Y_{b'}$ is a heavy chain variable region (VH), a light chain variable region (VL), a heavy chain, a light chain, a constant chain of an antibody, antibody fragment, a peptide, or a cyclic peptide. In some examples, the peptide is a binding peptide.

[0059] In some examples, $Y_{a'}$ and $Y_{b'}$ are attached via L3.

[0060] In some examples, L_3 comprises a peptide sequence, a dimerization and docking domain, a leucine zipper, or knobs-into-holes.

[0061] In some examples, L_3 comprises a peptide bond, a disulfide bond, a maleimide bond, thioether bond, an azide-alkyne cycloaddition, a cystinyl-dopa, or a hydrogen bond.

[0062] In some examples, L_3 is a linker. In some examples, the linker comprises a sequence selected from the group consisting of $(GS)_{n3}$, $(G2S)_{n3}$, $(G3S)_{n3}$, $(G4S)_{n3}$, $(G)_{n3}$, $(GGSGGD)_{n3}$, $(GGSGGE)_{n3}$, $(GGGSGSGGGGS)_{n3}$, and $(GGGGGPGGGGP)_{n3}$ and wherein n3 is an integer from 2 to 20. In some examples, n is 1, 2, 3, 4, or 5. In some examples, n is 1.

[0063] In some examples, Y_1 and Y_2 are the same.

[0064] In some examples, Y_1 and Y_2 are different.

[0065] In some examples, Y_1, Y_2, or both Y_1 and Y_2 is a small molecule.

[0066] In some examples, the small molecule is selected from the group consisting of deruxtecan, exatecan, FL118, irinotecan, topotecan, SN-38, rubitecan, belotecan, lurototecan, gimatecan, diflomotecan, karenitecan, silatecan, namitecan, elomotecan, DRF-1042, delimotecan, NSC606985, chimmitecan, ZBH-1205, auristatin (MMAE, MMAF, MMAG, MMAH) calicheamicin, doxorubicin, taxol and taxol derivatives, maytansinoids (DM1-4), pyrolodiazepines (PBDs), tubulysins, eribulin, anthramycin, duocarmycin, anthracycline, and camptothecin (CPT), including the lactone and carboxylate forms of CPT.

[0067] In some examples, the small molecule is selected from the group of a topoisomerase inhibitor and a tubulin inhibitor.

[0068] In some examples, Y_1 and Y_2, or both Y_1 and Y_2 comprises a nucleic acid, an immune agonist, a peptide, a cytokine, or a binding domain. In some examples, Y_1 and

Y_2, or both Y_1 and Y_2 comprises a nucleic acid. In some examples, Y_1 and Y_2, or both Y_1 and Y_2 comprises an oligonucleotide.

[0069] In some examples, Y_1 and Y_2, or both Y_1 and Y_2 comprises a peptide. In some examples, Y_1 and Y_2, or both Y_1 and Y_2 comprises a binding peptide.

[0070] In another example, provided herein is a polypeptide comprising a first terminal tag comprising $(X_1)_{m1}X_2X_3X_4X_5$, wherein X_1-X_5 is any amino acid provided that at least one amino acid of X_1-X_5 is a tyrosine or a portion thereof, and wherein m1 is an integer greater than or equal to 0, or $X_1X_2X_3$, wherein X_1-X_3 is any amino acid provided that at least one amino acid of X_1-X_3 is a tyrosine or a portion thereof; and a second terminal tag different from the first terminal tag, the second terminal tag comprising $(X_6)_{m2}X_7X_8X_9X_{10}$,, wherein X_6-X_{10} is any amino acid provided that at least one amino acid of X_6-X_{10} is a tyrosine or a portion thereof, and wherein m2 is an integer greater than or equal to 0, or $X_1X_2X_3$, wherein X_1-X_3 is any amino acid provided that at least one amino acid of X_1-X_3 is a tyrosine or a portion thereof.

[0071] In some examples, the first terminal is on a N-terminus of the polypeptide and the second terminal tag is on the C-terminus of the polypeptide.

[0072] In some examples, the polypeptide comprises a non-terminal tyrosine.

[0073] In some examples, the polypeptide is modified to expose the non-terminal tyrosine to be accessible to an enzyme.

[0074] In another example, provided herein is a composition of a first polypeptide and a second polypeptide, wherein the first polypeptide comprises a first terminal tag comprising $(X_1)_{m1}X_2X_3X_4X_5$, wherein X_1-X_5 is any amino acid provided that at least one amino acid of X_1-X_5 is a tyrosine or a portion thereof, or $X_1X_2X_3$, wherein X_1-X_3 is any amino acid provided that at least one amino acid of X_1-X_3 is a tyrosine or a portion thereof; and wherein m1 is an integer greater than or equal to 0, and a second polypeptide comprising a second terminal tag different from the first terminal tag, the second terminal tag comprising $(X_6)_{m2}X_7X_8X_9X_{10}$, wherein X_6-X_{10} is any amino acid provided that at least one amino acid of X_6-X_{10} is a tyrosine or a portion thereof, and wherein m2 is an integer greater than or equal to 0.

[0075] In some examples, the first polypeptide and/or the second polypeptide comprise a non-terminal tyrosine.

[0076] In some examples, the first polypeptide and/or the second polypeptide is modified to expose the non-terminal tyrosine to be accessible to an enzyme.

[0077] In some examples, the first terminal tag comprises at least one of GGGGY, RGGGY, RGRGY, RRRGY, RRRRY, EGGGY, EGEGY, EEEGY, EEEEY, GGGWY, GGWGY, RRRWY, RRWRY, EEEWY, EEWEY, DDDDY, SGGY, SGY, KKKKY, RRKKY, RRRKY, EDEDY, EDDDY, EEDDY, RRKKY, KKGGY, DDGGY, EESY, ESSSY, SSSY, SNNY, SSNY, SEGY, SSSEY, EGGY, SSEEY, SESY, ESSY, ESY, SRY, SKY, SNY, ERY, EKY, EGY, SEYP, SHRKY, SARKY, SPPEY, SSEEY, SEEEY, and SSSSY.

[0078] In some examples, the second terminal tag comprises at least one of GGGGY, RGGGY, RGRGY, RRRGY, RRRRY, EGGGY, EGEGY, EEEGY, EEEEY, GGGWY, GGWGY, RRRWY, RRWRY, EEEWY, EEWEY, DDDDY, SGGY, SGY, KKKKY, RRKKY, RRRKY, EDEDY, EDDDY, EEDDY, RRKKY, KKGGY, DDGGY, EESY, ESSSY, SSSY, SNNY, SSNY, SEGY, SSSEY, EGGY, SSEEY, SESY, ESSY, ESY, SRY, SKY, SNY, ERY, EKY, EGY, SEYP, SHRKY, SARKY, SPPEY, SSEEY, SEEEY, and SSSSY.

[0079] In some examples, the first terminal tag comprises SGGY, SGY, SGGGY, ESY, EESY, ESSY, ESSSY, SESY, SGGGY, EESY, SSSY, SNNY, SSNY, EGGY, SESY, or SEGY.

[0080] In some examples, the second terminal tag comprises EEEY, EEEEY, SSEEY, SSSEY, SSSSY, or SSNNY.

[0081] In another example, provided herein is a method of covalently linking at least two polypeptides to at least two payloads, the method comprising: a) contacting a first polypeptide of the at least two polypeptides and a first payload of the at least two payloads using a first tyrosinase, wherein the first polypeptide comprises a first terminal tag comprising $(X_1)_{m1}X_2X_3X_4X_5$, wherein X_1-X_5 is any amino acid provided that at least one amino acid of X_1-X_5 is a tyrosine or a portion thereof, and wherein m1 is an integer greater than or equal to 0, or $X_1X_2X_3$, wherein X_1-X_3 is any amino acid provided that at least one amino acid of X_1-X_3 is a tyrosine or a portion thereof; and b) contacting a second polypeptide of the at least two polypeptides and a second payload of the at least two payloads using a second tyrosinase, wherein the second polypeptide comprises a second terminal tag comprising $(X_6)_{m2}X_7X_8X_9X_{10}$, wherein X_6-X_{10} is any amino acid provided that at least one amino acid of X_6-X_{10} is a tyrosine or a portion thereof, and wherein m2 is an integer greater than or equal to 0, or $X_1X_2X_3$, wherein X_1-X_3 is any amino acid provided that at least one amino acid of X_1-X_3 is a tyrosine or a portion thereof.

[0082] In some examples, the first tyrosinase, the second tyrosinase, or both the first tyrosinase and the second tyrosinase is Agricus bisporus tyrosinase (abTYR).

[0083] In some examples, the first tyrosinase, the second tyrosinase, or both comprise a sequence at least 80% identity to any one of SEQ ID NOs: 1-6, 52, or 53.

[0084] In some examples, the first tyrosinase, the second tyrosinase, or both the first tyrosinase and the second tyrosinase is a Catenase.

[0085] In some examples, the first tyrosinase and the second tyrosinase is a Catenase.

[0086] In some examples, the first tyrosinase, the second tyrosinase, or both comprises a sequence at least 90% identity any one of SEQ ID NOs: 2-6.

[0087] In some examples, the first tyrosinase, the second tyrosinase, or both comprises a sequence at least 90% identity to SEQ ID NO: 2.

[0088] In some examples, the first tyrosinase is *Agricus bisporus* tyrosinase (abTYR) and the second tyrosinase is Catenase.

[0089] In some examples, the first tyrosinase and the second tyrosinase are provided at the same time.

[0090] In some examples, the first tyrosinase is provided first followed by the second tyrosinase.

[0091] In another example, provided herein is a method of covalently linking at least two polypeptides to at least two payloads, the method comprising: a) contacting a first polypeptide of the at least two polypeptides and a first payload of the at least two payloads in the presence of a tyrosinase, wherein the first polypeptide comprises a first terminal tag comprising $(X_1)_{m1}X_2X_3X_4X_5$, wherein X_1-X_5 is any amino acid provided that at least one amino acid of X_1-X_5 is a tyrosine or a portion thereof, and wherein m1 is an integer greater than or equal to 0, or $X_1X_2X_3$, wherein X_1-X_3 is any amino acid provided that at least one amino acid of X_1-X_3 is a tyrosine or a portion thereof; and b) contacting a second polypeptide of the at least two polypeptides and a second payload of the at least two payloads in the presence of the tyrosinase, wherein the second polypeptide comprises a second terminal tag comprising $(X_6)_{m2}X_7X_8X_9X_{10}$, wherein X_6-X_{10} is any amino acid provided that at least one amino acid of X_6-X_{10} is a tyrosine or a portion thereof, and wherein m2 is an integer greater than or equal to 0, or $X_1X_2X_3$, wherein X_1-X_3 is any amino acid provided that at least one amino acid of X_1-X_3 is a tyrosine or a portion thereof.

[0092] In some examples, the first polypeptide is contacted with the tyrosinase at a temperature between 0-15℃. In some examples, the first polypeptide is contacted with the tyrosinase at a temperature of 4 ℃.

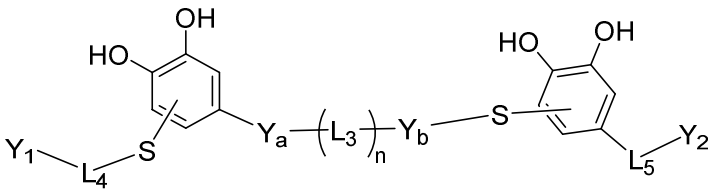
[0093] In some examples, the second polypeptide is contacted with the tyrosinase at a temperature between 25-50℃. In some examples, the second polypeptide is contacted with the tyrosinase at a temperature of 30℃.

[0094] In some examples, the first polypeptide is contacted with the tyrosinase for 5-70 min. In some examples, the first polypeptide is contacted with the tyrosinase for 45 min. In some examples, the first polypeptide is contacted with the tyrosinase for 60 min.

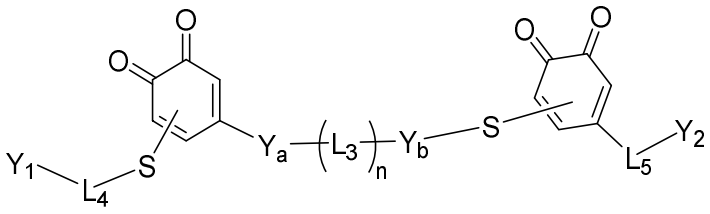
[0095] In some examples, the second polypeptide is contacted with the tyrosinase for more than 15 min. In some examples, the second polypeptide is contacted with the tyrosinase for 60-160 min. In some examples, the second polypeptide is contacted with the tyrosinase for 90 min. In some examples, the second polypeptide is contacted with the tyrosinase for 120 min.

[0096] In some examples, the tyrosinase comprises at least 80% identity to any one of SEQ ID NOs: 1-6, 52, or 53.

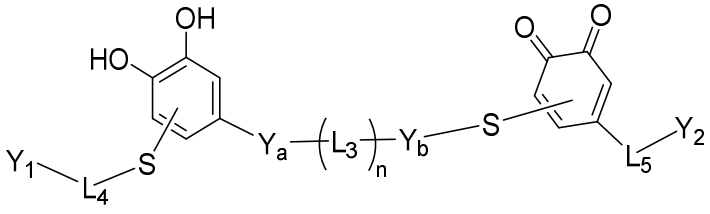
[0097] In another example, provided herein is a conjugate of Formula E', Formula F', Formula G', Formula H', Formula J', or Formula K':



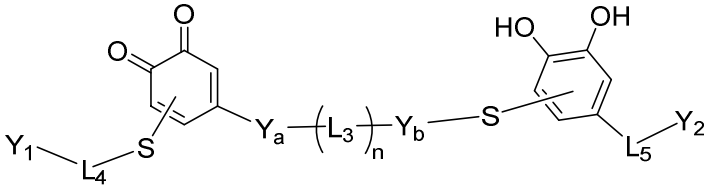
Formula E',



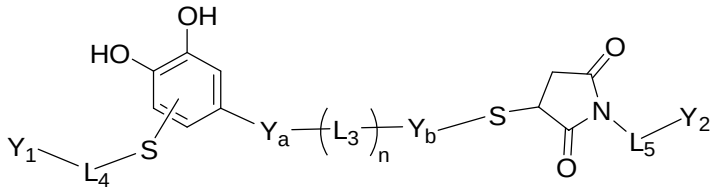
Formula F',



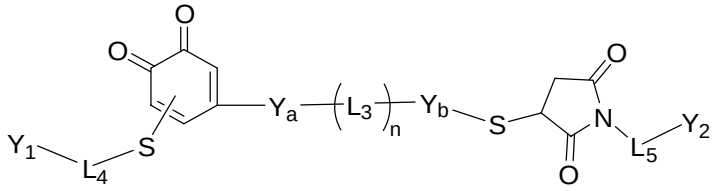
Formula G',



Formula H',



Formula J', or



Formula K',

wherein:

Y_a is a first polypeptide comprising a first terminal tag comprising $(X_1)_{m1}X_2X_3X_4X_5Y$, wherein X_1-X_5 is any amino acid provided that no more than two amino acids of X_2, X_3, X_4, and X_5 are aspartate or glutamate and wherein m1 is an integer greater than or equal to 0;

Y_b is a second polypeptide;

n is an integer greater than 0;

L_3 is an optional third linker;

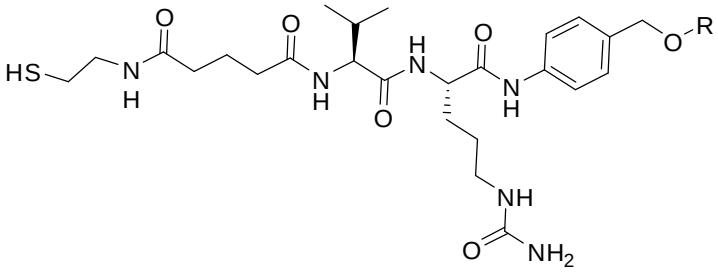
L_4 is an optional fourth linker;

L_5 is an optional fifth linker;

Y_1 is a first payload; and

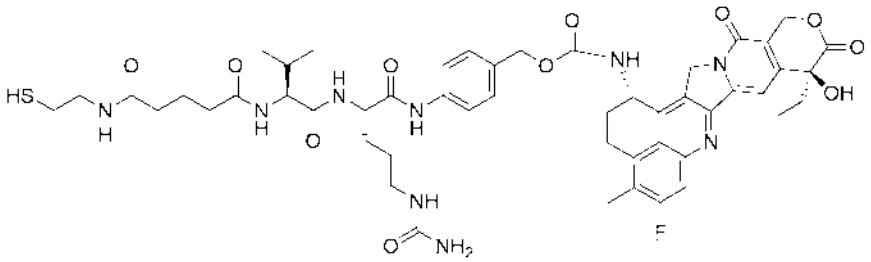
Y_2 is a second payload.

[0098] In another example, provided herein is a compound of formula L-I:

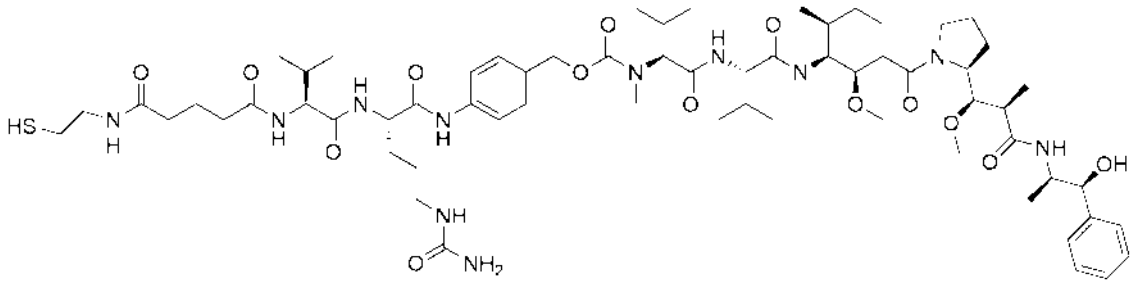


Formula L-I, wherein R is a payload.

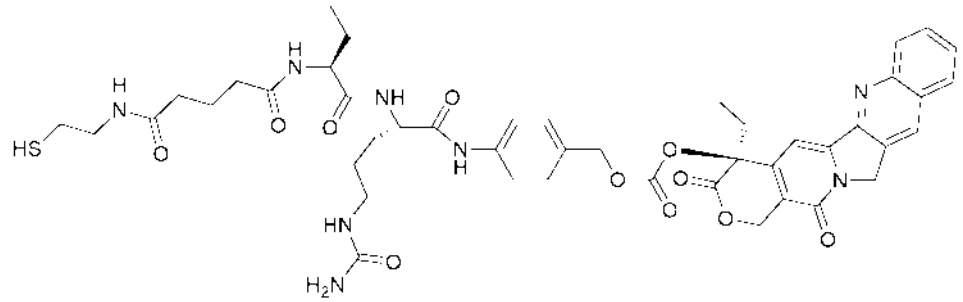
[0099] In some examples, the compound is selected from the group consisting of:



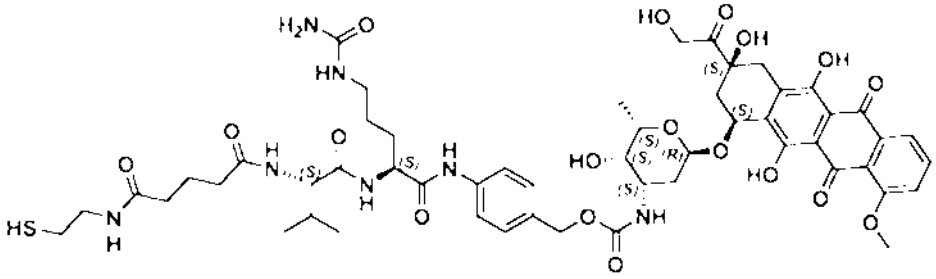
SH-VC-PAB-DXD,



SH-VC-PAB-MMAE,

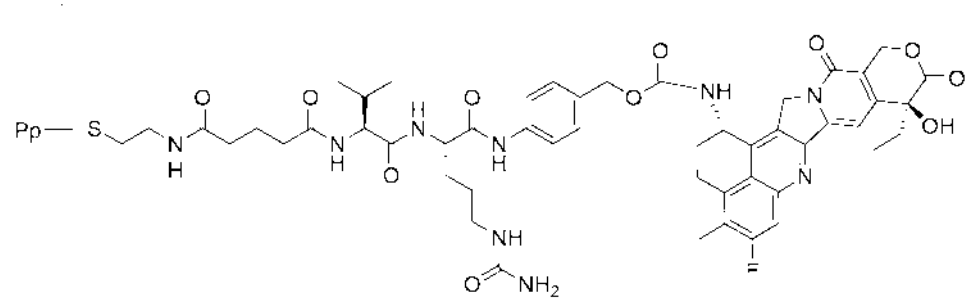


SH-VC-PAB-Camptothecin, and

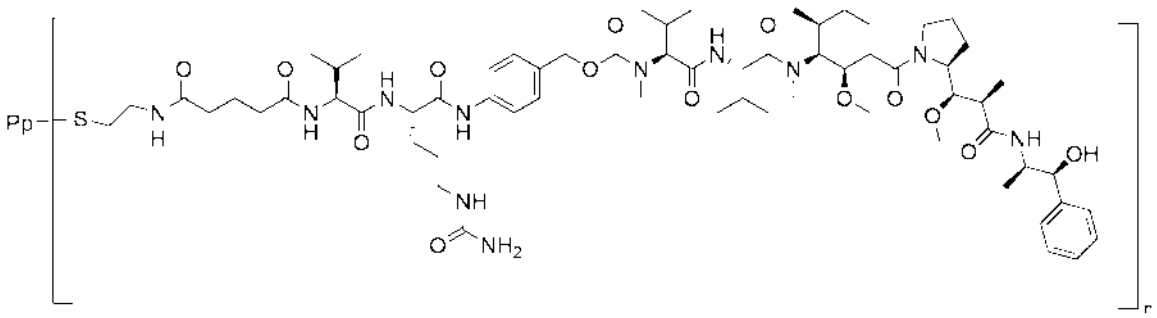


SH-VC-PAB-Doxorubicin.

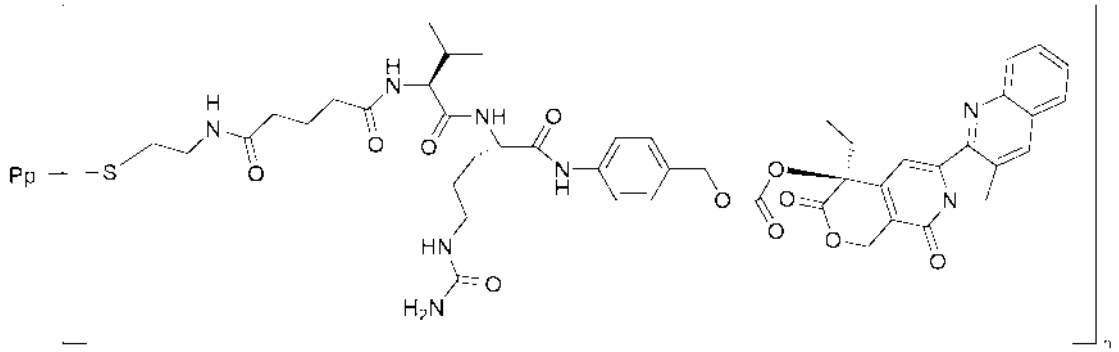
[00100] In another example, provided herein is a polypeptide-conjugate represented by Formula S1, Formula S2, Formula S3, or Formula S4,



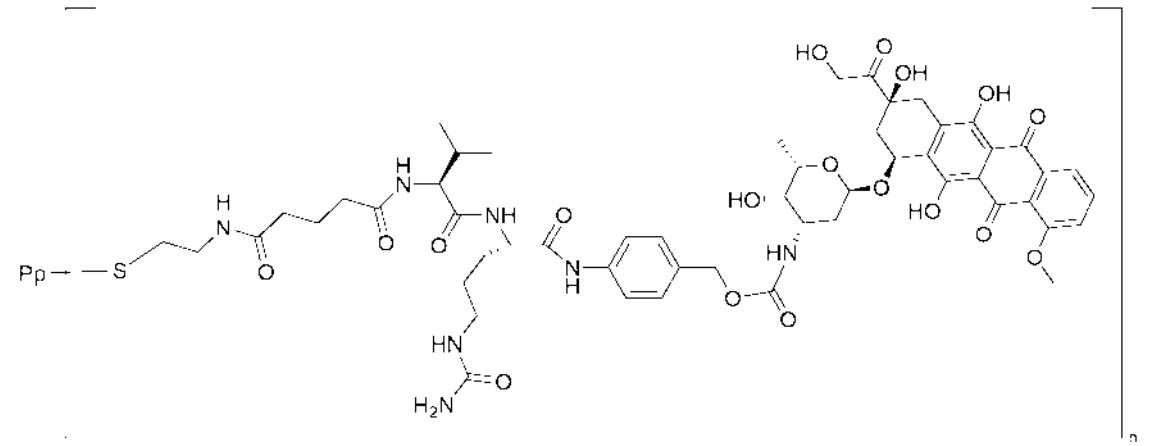
Formula S1,



Formula S2,



Formula S3, or



Formula S4,

wherein: Pp is a polypeptide and contains an oxidized tyrosine; n is 1, 2, or 3; and wherein the “-S-” of the compound is conjugated to the oxidized tyrosine on the polypeptide.

[00101] In some examples, the polypeptide is an enzyme, an antibody or a portion thereof, a structural polypeptide, a ligand for a receptor, or a receptor.

[00102] In some examples, the polypeptide is an antibody or a portion thereof.

[00103] In some examples, the polypeptide is a heavy chain variable region (VH), a light chain variable region (VL), a heavy chain, a light chain, a constant chain of an antibody or antibody fragment, a peptide, or a cyclic peptide.