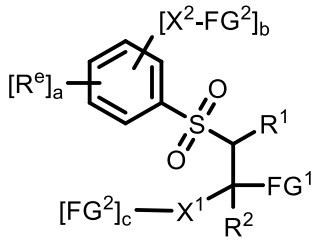
Claims

1. A releasable linker having a structure according to formula (I):



(I)

or stereoisomer, tautomer or mixture thereof, or isotopic variant thereof;

wherein:

X^1 is a first spacer moiety;

X^2 is a second spacer moiety;

R^1 is a hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, or substituted aryl;

R^2 is a hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, or substituted aryl;

R^e is an electron altering group selected from nitro, cyano, halogen, amide, substituted amide, sulfone, substituted sulfone, sulfonamide, substituted sulfonamide, alkoxy, substituted alkoxy, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl;

a is an integer from 0 to 4;

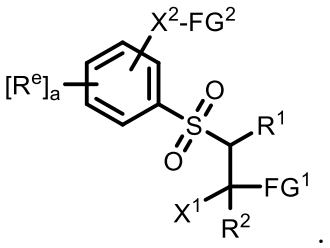
b is 1;

c is an integer from 0 to 1;

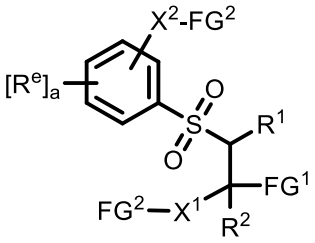
FG^1 is a functional group capable of reacting with an amino group of an active agent to form a releasable linkage; and

FG^2 is a functional group capable of reacting through click chemistry consisting of azide, alkynyl, and cycloalkynyl groups.

2. The releasable linker of claim 1, wherein the releasable linker has a structure according to formula (I-B) or formula (I-C):



(I-B) ; or



(I-C).

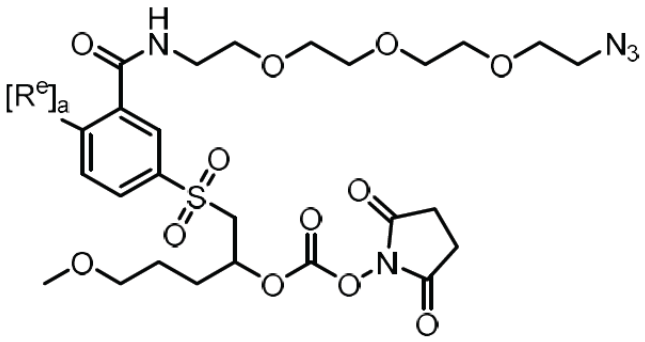
3. The releasable linker of claim 1 or 2, wherein

a is an integer from 0 to 2;

R^1 and R^2 are each independently hydrogen, Me, or Et; and

R^e is nitro, cyano, halogen, $-CF_3$, -CONHMe, $-SO_2NHMe$, -OMe, -NHAc, $-NHSO_2Me$, or $-OCF_3$.

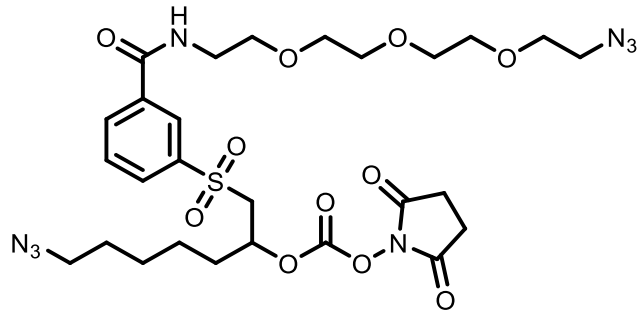
4. The releasable linker of any one of claims 1-3, wherein the linker has the following structure:



wherein:

a is 0 or 1; and

R^e is H, CF_3 or Cl;

or  .

or stereoisomer, tautomer or mixture thereof, or isotopic variant thereof.

5. A conjugate comprising a protein covalently attached to at least one linker; wherein the conjugate comprises a structure according to formula (XIX):

$$\text{Protein-(L)}_z$$

(XIX)

or a stereoisomer, tautomer or mixture thereof, or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, or hydrate thereof;

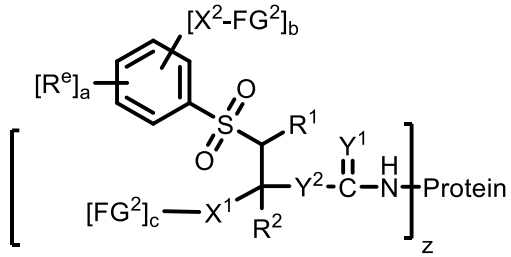
wherein:

z is an integer from 1 to 10;

L is a linker, wherein at least one linker is a releasable linker; or a releasable linker or a non-releasable linker that contains a functional group capable of reacting through click chemistry selected from the group consisting of azide, alkynyl, and cycloalkynyl groups; and

Protein is IL-2;

wherein the conjugate comprises a structure according to formula (VII):



(VII)

wherein:

X^1 is a first spacer moiety;

X^2, when present, is a second spacer moiety;

R^1 is a hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, or substituted aryl;

R^2 is a hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, or substituted aryl;

R^e is an electron altering group selected from nitro, cyano, halogen, amide, substituted amide, sulfone, substituted sulfone, sulfonamide, substituted sulfonamide, alkoxy, substituted alkoxy, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl;

a is an integer from 0 to 5;

b is 1;

c is an integer from 0 to 2;

z is an integer from 1 to 10;

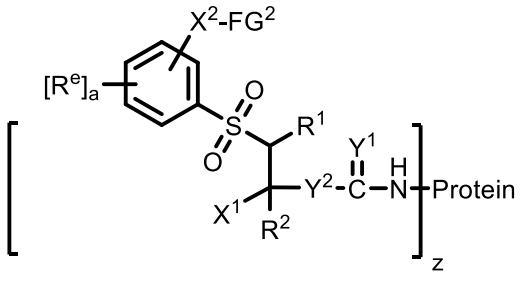
Y^1 is O or S;

Y^2 is O or S;

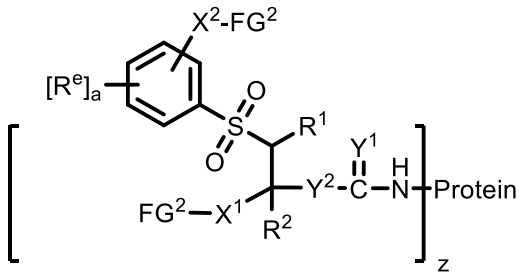
FG^2 is a functional group capable of reacting through click chemistry selected from the group consisting of azide, alkynyl, and cycloalkynyl groups; and

-NH- is an amine group of a residue within the protein.

6. The conjugate of claim 5, wherein the conjugate comprises a structure according to formula (VII-B) or formula (VII-C):



(VII-B) ; or



(VII-C).

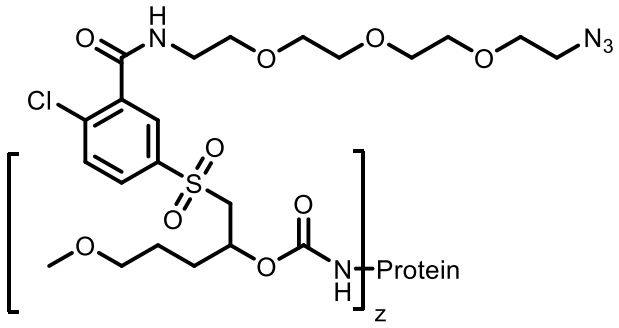
7. The conjugate of claim 5 or 6, wherein

a is an integer from 0 to 2;

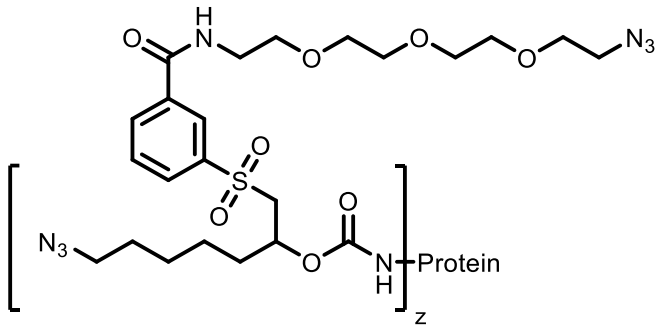
R^1 and R^2 are each independently hydrogen, Me, or Et; and

R^e is nitro, cyano, halogen, $-CF_3$, -CONHMe, $-SO_2NHMe$, -OMe, -NHAc, $-NHSO_2Me$, or $-OCF_3$.

8. The conjugate of any one of claims 5-7, wherein the conjugate has following structure:



; or



.

9. A composition comprising the two or more different conjugates of any one of claims 5-8.

10. A conjugate comprising a structure according to formula (XX):

$$\text{Protein-(L-Macromolecule)}_z$$

(XX)

or a stereoisomer, tautomer or mixtures thereof, or an isotopic variant thereof; or a

pharmaceutically acceptable salt, solvate, or hydrate thereof;

wherein:

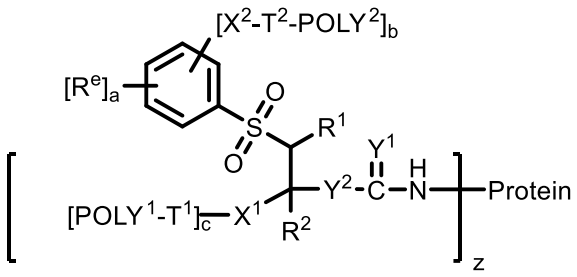
z is an integer from 1 to 10;

L is a linker comprising functional groups resulting from click chemistry;

Protein is IL-2; and

Macromolecule is a water-soluble polymer, a lipid, a protein or a polypeptide;

wherein the conjugate comprises a structure according to formula (XIII):



(XIII)

wherein:

$POLY^1$ is a first straight or branched water-soluble polymer;

$POLY^2$ is a second straight or branched water-soluble polymer;

X^1 is a first spacer moiety; or -X-FG^2;

X^2, when present, is a second spacer moiety;

T^1 is a first triazole functional group;

T^2 is a second triazole functional group;

R^1 is a hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, or substituted aryl;

R^2 is a hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, or substituted aryl;

R^e is an electron altering group selected from nitro, cyano, halogen, amide, substituted amide, sulfone, substituted sulfone, sulfonamide, substituted sulfonamide, alkoxy, substituted alkoxy, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, and substituted

heteroaryl; and -X-FG^2;

wherein:

X is a spacer moiety; and

FG^2 is a functional group capable of reacting through click chemistry selecting from the group consisting of azide, alkynyl, and cycloalkynyl groups.

a is an integer from 0 to 5;

b is 1;

c is an integer from 0 to 2;

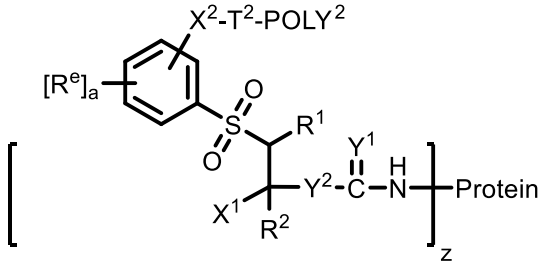
z is an integer from 1 to 10;

Y^1 is O or S;

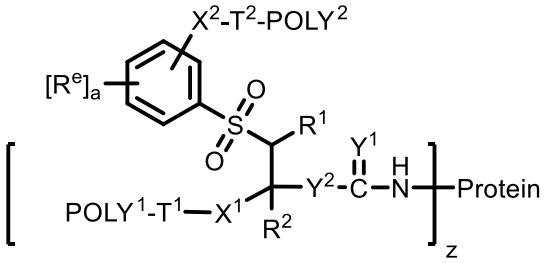
Y^2 is O or S; and

-NH- is an amine group of a residue within the protein.

11. The conjugate of claim 10, wherein the conjugate comprises a structure according to formula (XIII-B) or formula (XIII-C):



(XIII-B) ; or



(XIII-C).

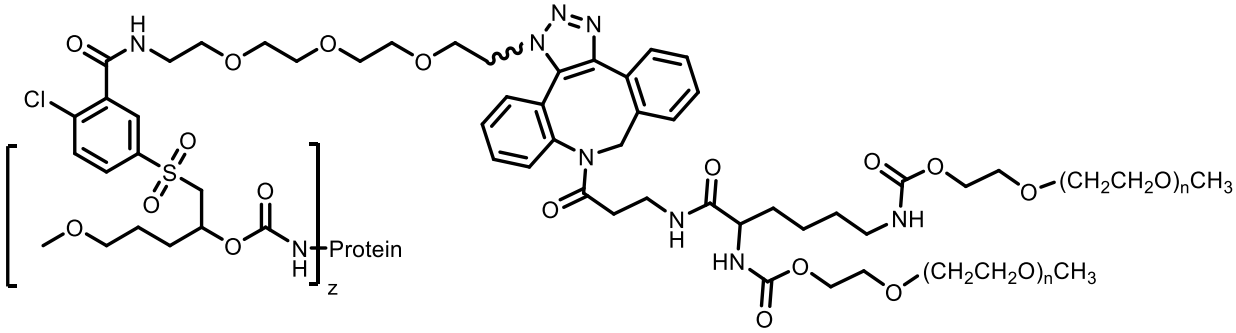
12. The conjugate of claim 10 or 11, wherein

a is an integer from 0 to 2;

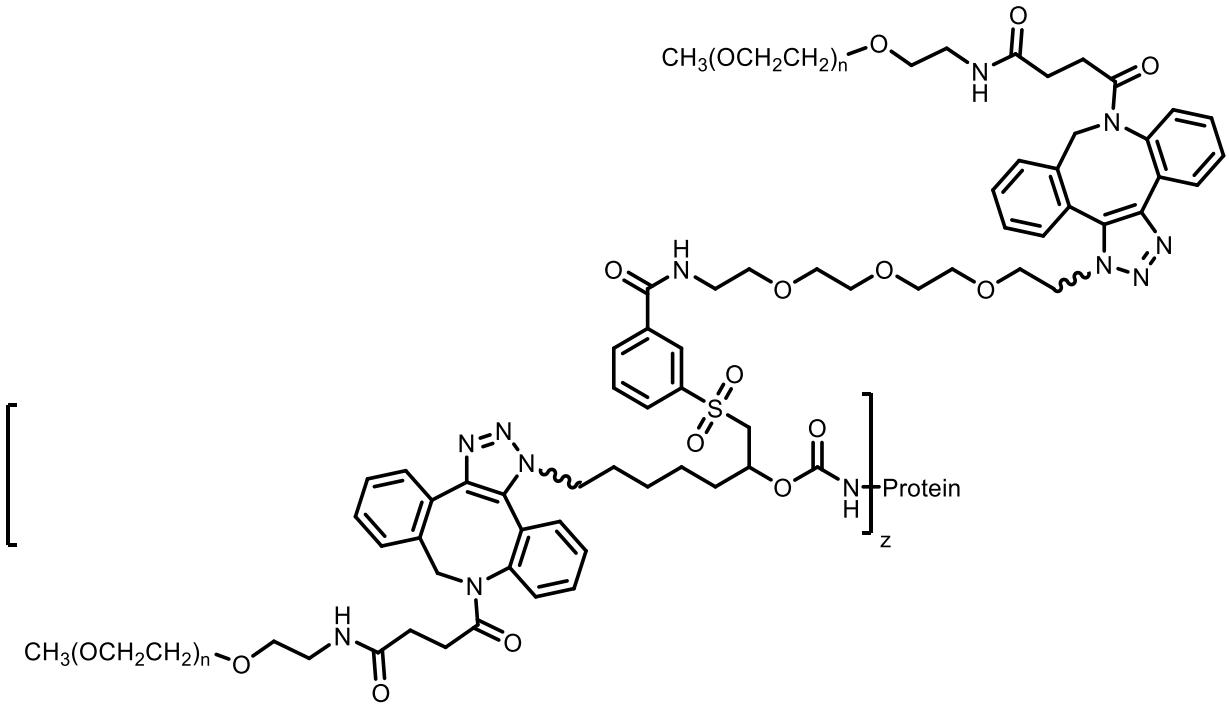
R^1 and R^2 are each independently hydrogen, Me, or Et; and

R^e is nitro, cyano, halogen, $-CF_3$, -CONHMe, $-SO_2NHMe$, -OMe, -NHAc, $-NHSO_2Me$, or $-OCF_3$.

13. The conjugate of any one of claims 10-12, wherein the conjugate comprises the structure:



(XIII-B1); or



(XIII-C1);

wherein:

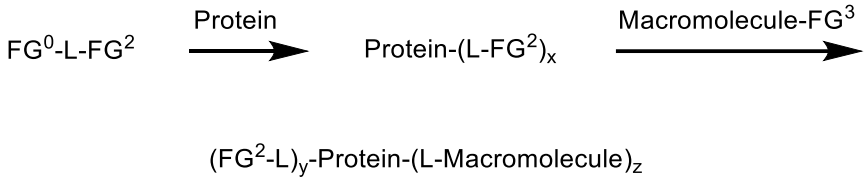
n is independently an integer from 4 to 1500;

z is an integer from 1 to 10; and

-NH- is an amine group of a residue within the protein.

14. A composition comprising a mixture of two or more different conjugates of any one of claims 10-13.

15. A method for preparing Protein-Macromolecule conjugates according to scheme (I):



(Scheme I)

wherein

x is an integer from 1 to 10;

y is an integer from 0 to 9;

z is an integer from 1 to 10, wherein x = y + z;

L is the releasable linker of any one of claims 1-4;

FG^0 is a functional group capable of reacting with a nucleophilic group of an active protein agent to form a linkage, including a carbamate linkage and the like;

FG^2 is a functional group capable of reacting with FG^3 through click chemistry selected from the group consisting of azide, alkynyl, and cycloalkynyl groups;

FG^3 is a functional group capable of reacting with FG^2 through click chemistry selected from the group consisting of azide, alkynyl, and cycloalkynyl groups;

protein is IL-2;

macromolecule is a water-soluble polymer, a lipid, a protein or a polypeptide. It can include any of the following: a fatty acid comprises from about 6 to about 26 carbon atoms, one of the polymers selected from the group consisting of 2-methacryloyl-oxyethyl phosphoyl cholins, poly(acrylic acids), poly(acrylates), poly(acrylamides), poly(N-acryloylmorpholine), poly(alkyloxy) polymers, poly(amides), poly(amidoamines), poly(amino acids), poly(anhydrides), poly(aspartamides), poly(butyric acids), poly(glycolic acids), polybutylene terephthalates, poly(caprolactones), poly(carbonates), poly(cyanoacrylates), poly(dimethylacrylamides), poly(esters), poly(ethylenes), poly(ethylene glycols), poly(ethylene oxides), poly(ethyl phosphates), poly(ethyloxazolines), poly(glycolic

acids), poly(α-hydroxy acid), poly(hydroxyethyl acrylates), poly(hydroxyethyloxazolines), poly(hydroxymethacrylates), poly(hydroxyalkylmethacrylamides), poly(hydroxyalkylmethacrylates), poly(hydroxypropyloxazolines), poly(iminocarbonates), poly(lactic acids), poly(lactic-co-glycolic acids), poly(methacrylamides), poly(methacrylates), poly(methyloxazolines), poly(organophosphazenes), poly(ortho esters), poly(oxazolines), poly(oxyethylated polyol), poly(olefinic alcohol), polyphosphazene, poly(propylene glycols), poly(saccharide), poly(siloxanes), poly(urethanes), poly(vinyl alcohols), poly(vinyl amines), poly(vinylmethylethers), poly(vinylpyrrolidones), silicones, amylose, celluloses, carbomethyl celluloses, hydroxypropyl methylcelluloses, chitins, chitosans, dextrans, dextrins, gelatins, hyaluronic acids (HA) and derivatives, functionalized hyaluronic acids, mannans, pectins, heparin, heparan sulfate (HS), rhamnogalacturonans, starches, hydroxyalkyl starches, hydroxyethyl starches (HES), polysialic acid (PSA) and other carbohydrate-based polymers, xylans, and copolymers, of albumin, transferrin, transthyretin, immunoglobulin, a XTEN peptide, a glycine-rich homoamino acid polymer (HAP), a PAS polypeptide, an elastin-like polypeptide (ELP), a CTP peptide, or a gelatin-like protein (GLK) polymer.

16. A pharmaceutical composition comprising the conjugate of any one of claims 5-14 and one or more pharmaceutically acceptable excipients.

17. Use of the pharmaceutical composition of claim 16 in the manufacture of a medicament for treating cancer, an infection, or an autoimmune disease in a subject.

18. The use of claim 17, wherein the medicament further comprises other suitable therapeutic agents for treating cancer, an infection, or an autoimmune disease in a subject.