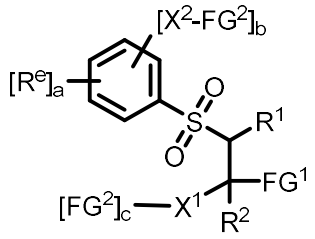
Claims

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;

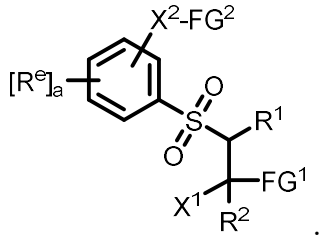
b is an integer from 1 to 3;

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.

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



(I-B)

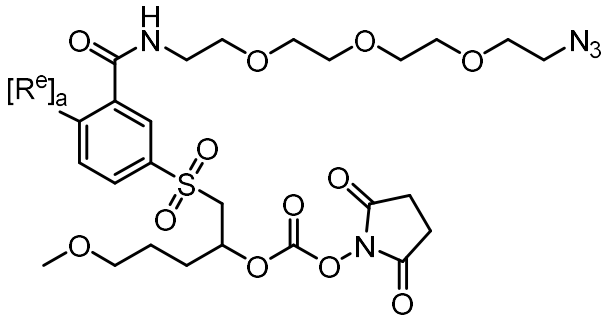
3. The releasable linker of claim 1 or 2, wherein

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, -NHMe, -NHAc, $-NHSO_2Me$, or $-OCF_3$.

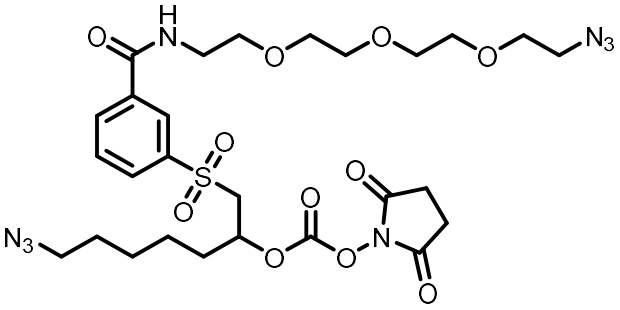
4. A releasable linker having the structure:



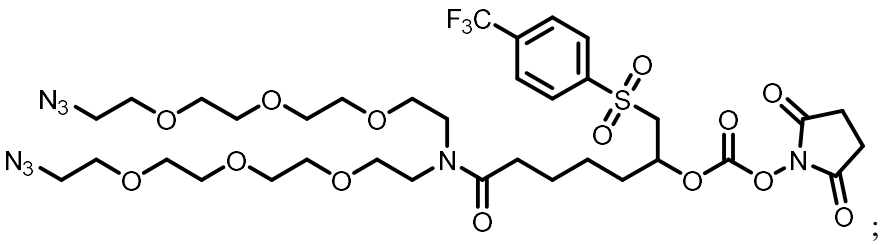
wherein:

a is 0 or 1; and

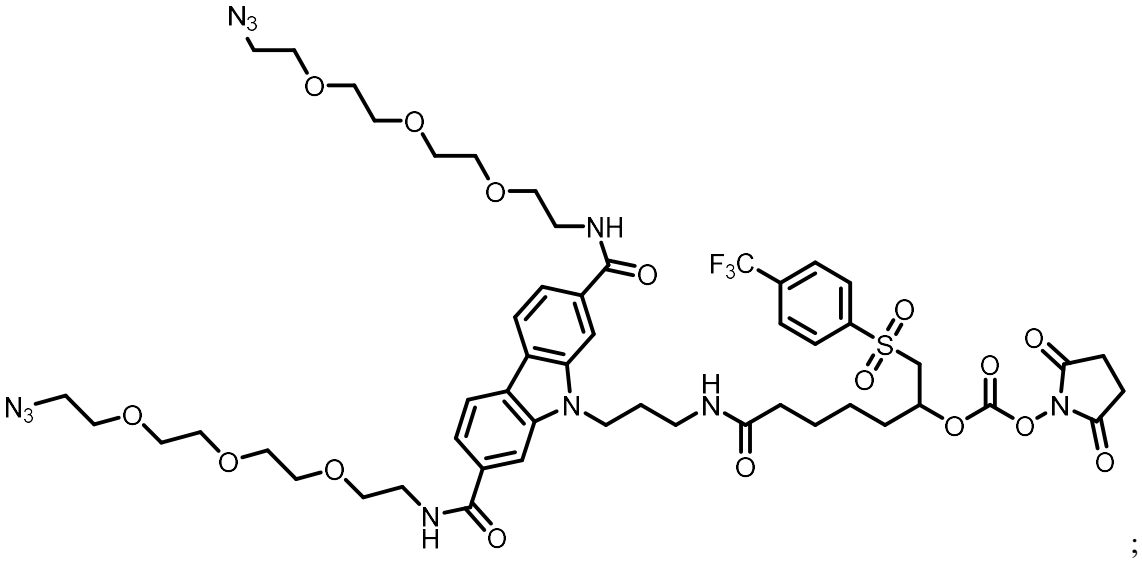
R^e is H, CF_3 or Cl;



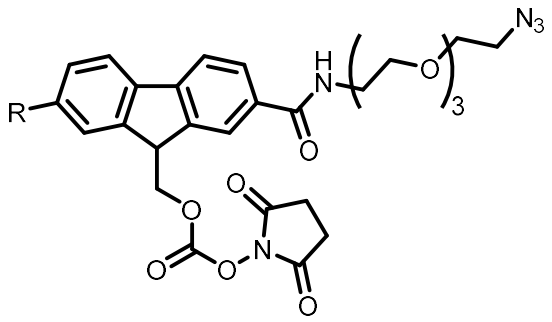
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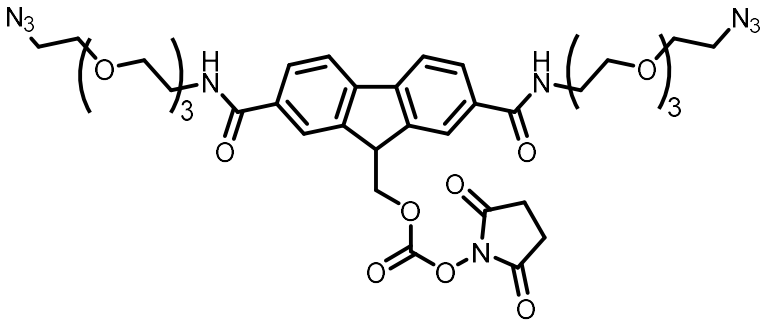
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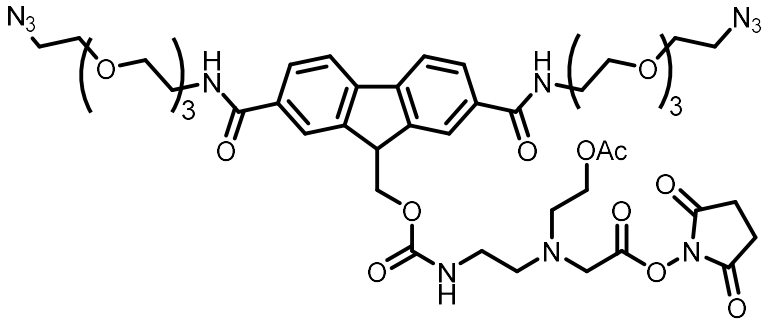
;



;



(II-B); or



(III-A),

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

(I-C)

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

Protein-$(L)_z$

(XIX)

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

wherein:

z is an integer from 1 to 25;

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 a chemokine, a chemokine antagonist, a cytokine, a cytokine antagonist, an antibody, or a therapeutic peptide.

6. A composition comprising a mixture of two or more different conjugates of claim 5.

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

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

(XX)

or a stereoisomer, regioisomer, tautomer or mixtures thereof, or an isotopic variant thereof; or a pharmaceutically acceptable salt, solvate, hydrate, or prodrug thereof;

wherein:

z is an integer from 1 to 25;

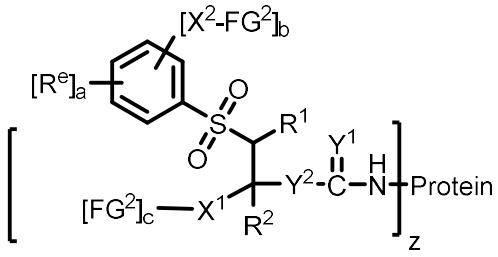
L is a linker comprising functional groups resulting from click chemistry;

Protein is a chemokine, a chemokine antagonist, a cytokine, a cytokine antagonist, an antibody, or a therapeutic peptide; and

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

8. A composition comprising a mixture of two or more different conjugates of claim7.

9. The conjugate of claim 5, 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 an integer from 0 to 3;

c is an integer from 0 to 2;

z is an integer from 1 to 25;

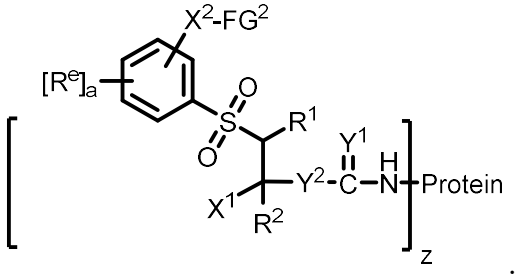
Y^1 is O or S;

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.

10. The conjugate of claim 5 or 9, wherein the conjugate comprises a structure according to formula (VII-B):



(VII-B)

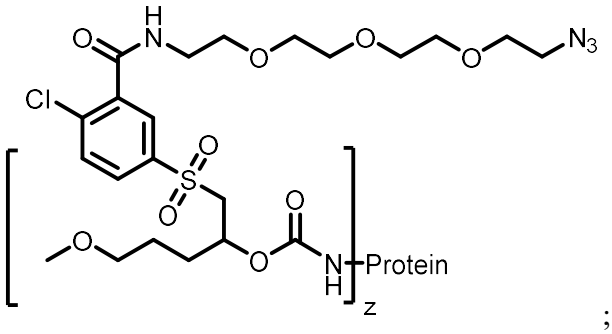
11. The conjugate of claim 9 or 10, wherein

a is an integer from 0 to 2;

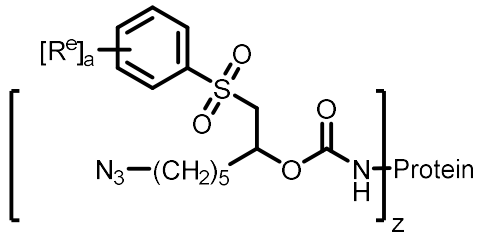
R^1 and R^2 are each independently hydrogen, Me, or Et; and

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

12. The conjugate of any one of claims 5, 9, 10 or 11 wherein the conjugate has following structure:

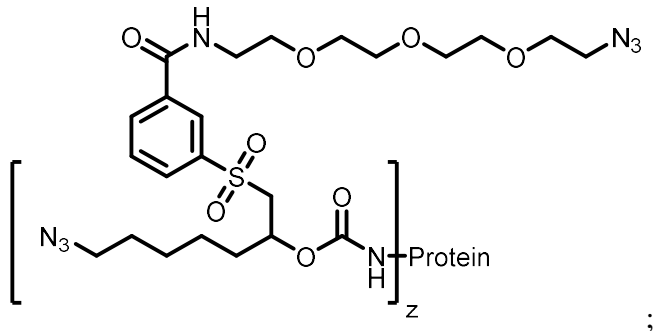


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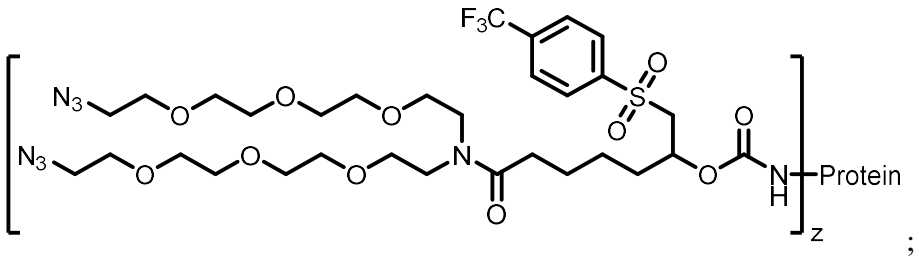


(VII-A1),

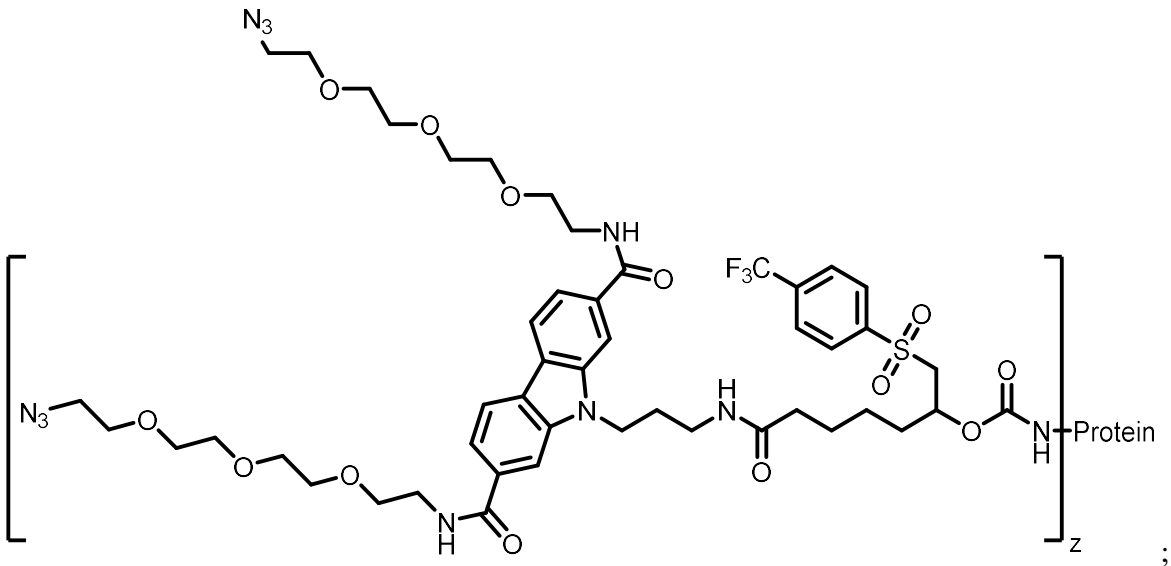
wherein a is an integer from 1 to 2; and R^e is 4-F, 4-Cl, 4-CF_3, 2,4-difluoro, or 2-CF_3-4-F;



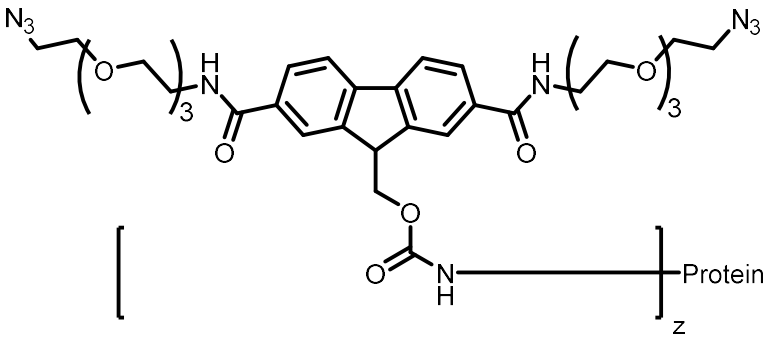
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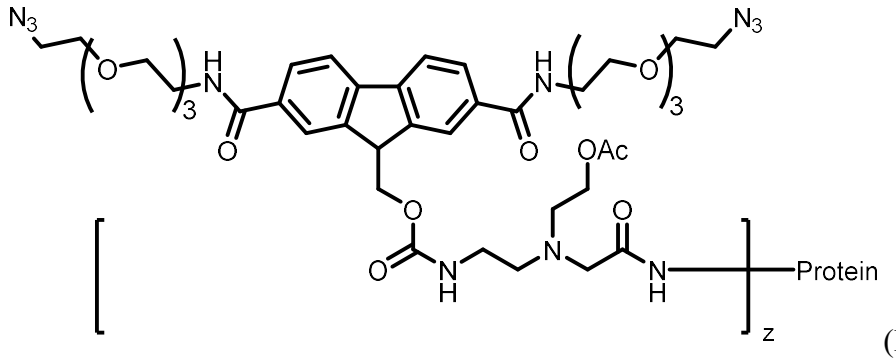
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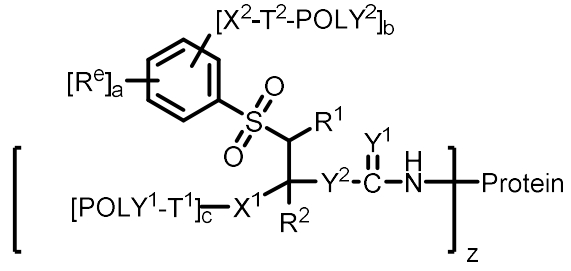


(VIII-A); or



(IX-A)..

13. The conjugate of claim 7, 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 an integer from 0 to 3;

c is an integer from 0 to 2;

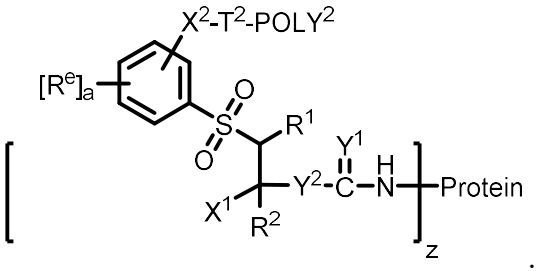
z is an integer from 1 to 25;

Y^1 is O or S;

Y^2 is O or S; and

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

14. The conjugate of claim 7 or 13, wherein the conjugate comprises a structure according to formula (XIII-B):



(XIII-B)

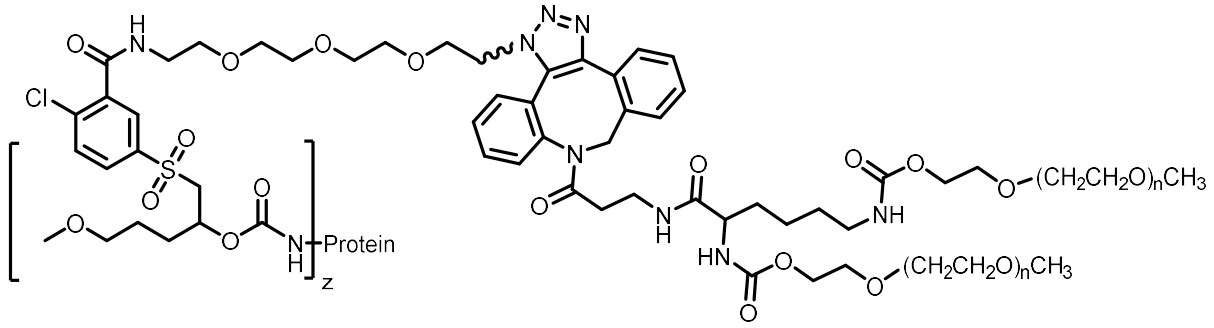
15. The conjugate of claim 13 or 14, wherein

a is an integer from 0 to 2;

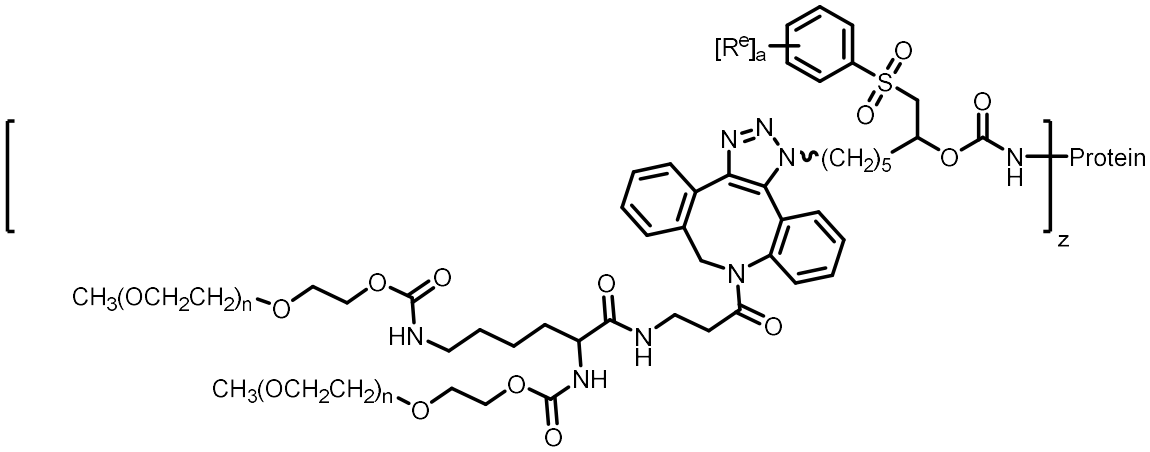
R^1 and R^2 are each independently hydrogen, Me, or Et; and

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

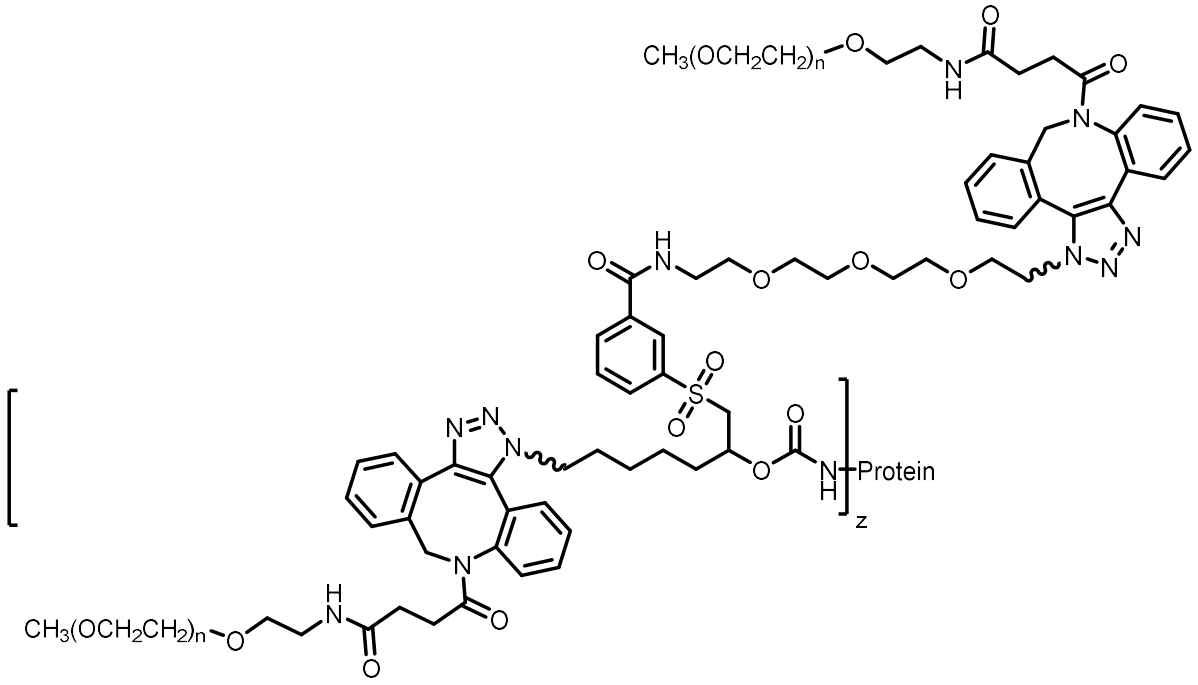
16. The conjugate of any one of claims 7, 13, 14 and 15, wherein the conjugate comprises the structure:



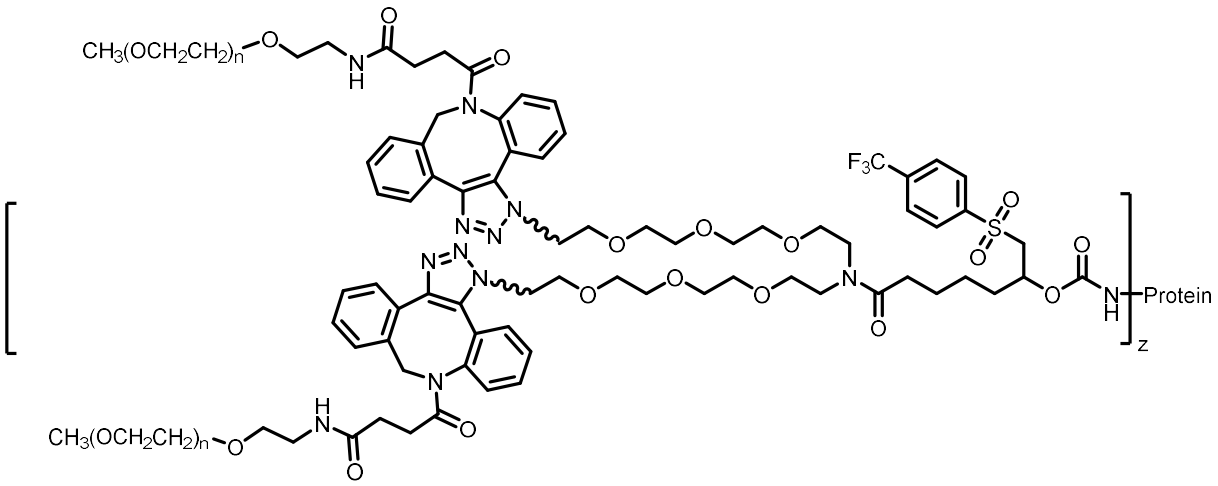
(XIII-B1);



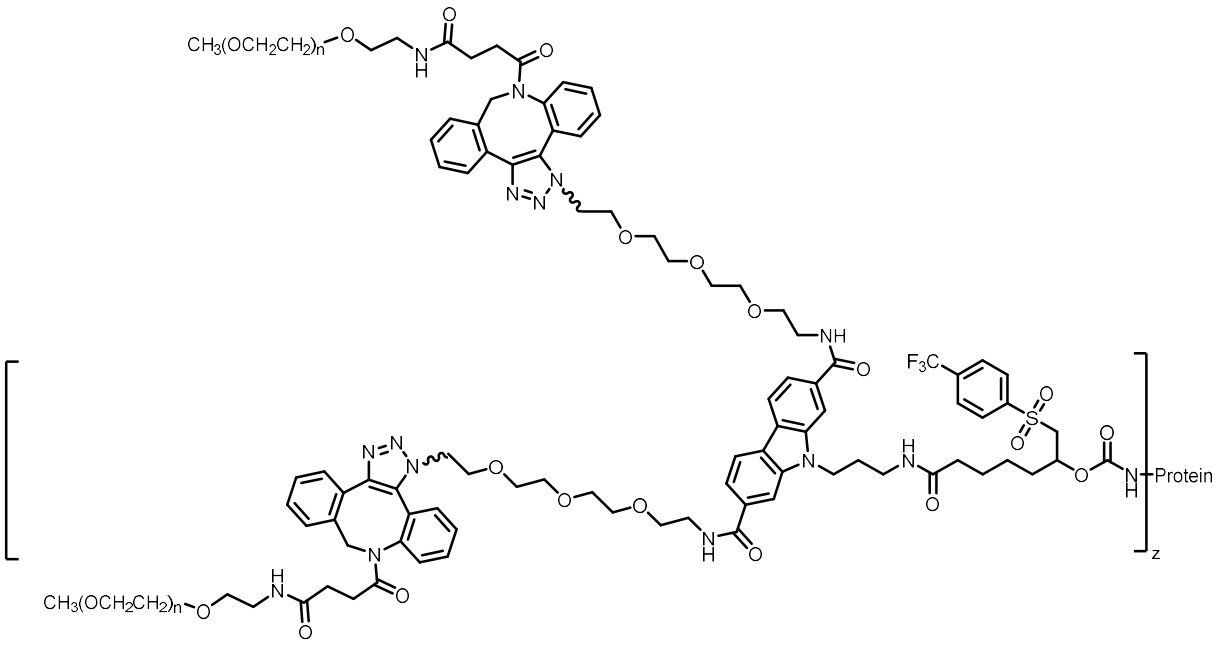
(XIII-A1);



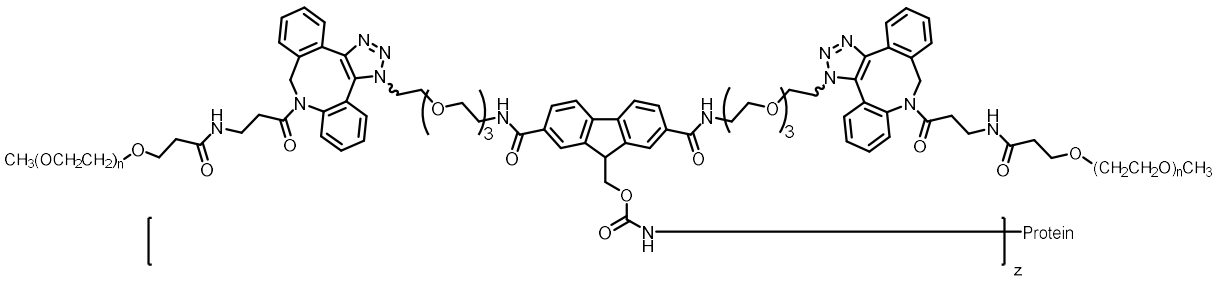
(XIII-C1);



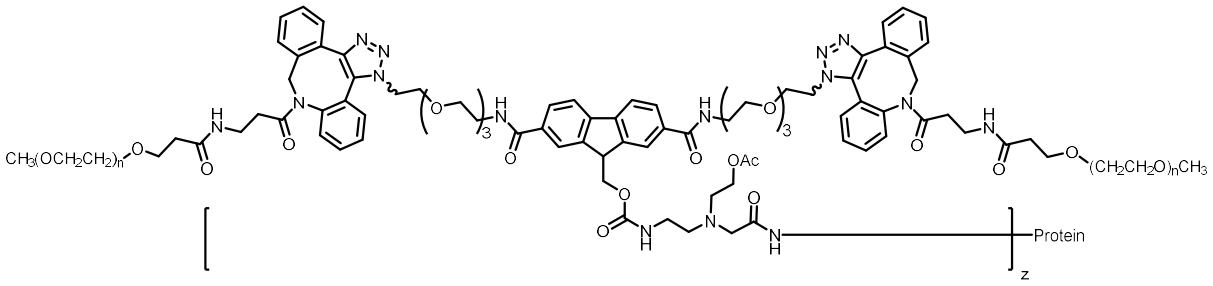
(XIII-D1);



(XIII-D2);



(XIV-A); or



(XV-A);

wherein:

a is 1 or 2;

R^e is 4-F, 4-Cl, 4-CF_3, 2,4-difluoro, or 2-CF_3-4-F;

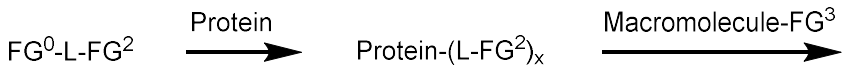
n is independently an integer from 4 to 1500;

z is an integer from 1 to 25; and

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

17. The conjugate of any one of claims 5, 7 and 9-16, wherein the protein is IL-2.

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



$(FG^2\text{-}L)_y\text{-Protein-(L-Macromolecule)}_z$

(Scheme I)

wherein x is an integer from 1 to 25;

y is an integer from 0 to 24;

z is an integer from 1 to 25, wherein x = y + z;

L is a linker;

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, a thiol bridge 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 a chemokine, a chemokine antagonist, a cytokine, a cytokine antagonist, an antibody, or a therapeutic peptide;

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.

19. A pharmaceutical composition comprising the conjugate of any one of claims 7 and 13-16 and one or more pharmaceutically acceptable excipients.

20. Use of the pharmaceutical composition of claim 19 in the manufacture of a medicament for treating cancer, an infection, or an autoimmune disease in a subject.