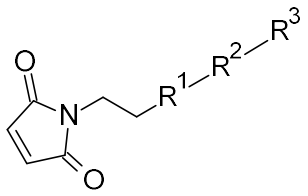
acceptable salts of these novel compounds. Also disclosed herein are methods of preparing an antibody-maleimide conjugate, and methods of making self-hydrolyzing maleimide-containing compounds.

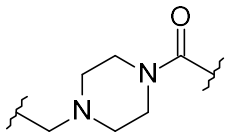
[0010] In an aspect, disclosed herein is a compound of formula:



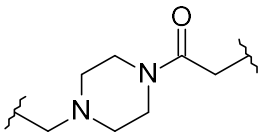
,

or a salt thereof,

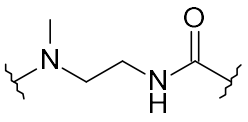
wherein R^1 is



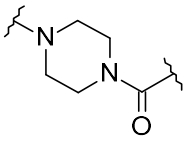
,



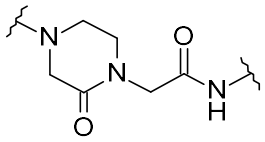
,



,



, or



;

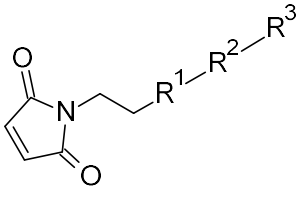
R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;

n is 1 to 50; and

R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl.

[0010a] In another aspect, also disclosed herein is a method of preparing an antibody-maleimide conjugate comprising: combining a monoclonal antibody (mAb) comprising at least one cysteine and a compound, or a salt thereof, as described herein, to form a reaction mixture.

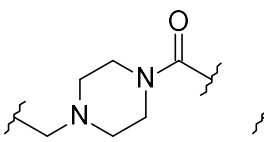
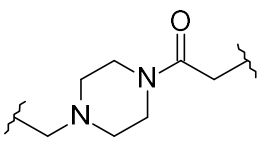
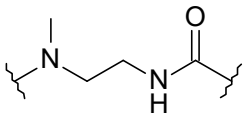
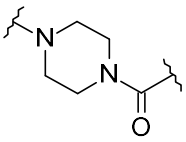
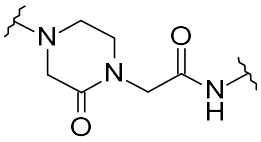
[0010b] In another aspect, also disclosed herein is use of a compound of formula:



,

or a salt thereof, to prepare a pharmaceutical formulation,

wherein R^1 is

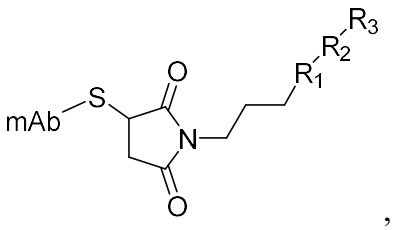
, , , , or ;

R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;

n is 1 to 50; and

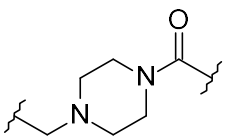
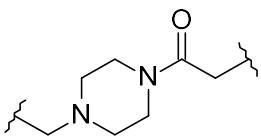
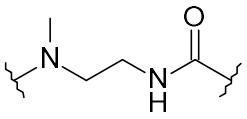
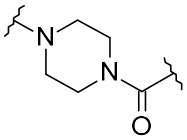
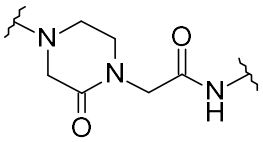
R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl,

[0011] wherein the compound, or a salt thereof, is conjugated to a monoclonal antibody (mAb).In another aspect, also disclosed herein, is a conjugate of the formula:

,

or a salt thereof,

wherein R^1 is


,

,

,

, or

;

R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;

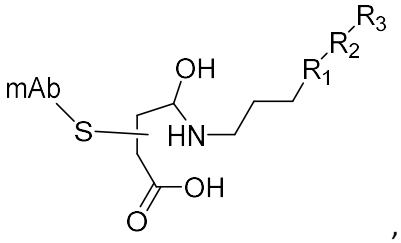
n is 1 to 50;

R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl;

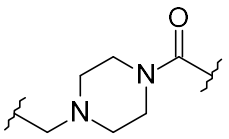
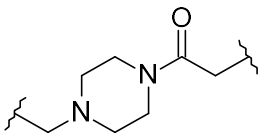
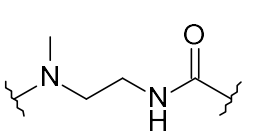
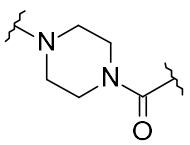
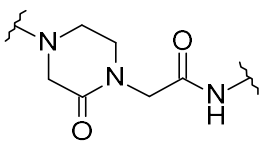
mAb is a monoclonal antibody; and

S is a sulfur atom from a cysteine residue on the monoclonal antibody.

[0012] In another aspect, also disclosed herein is conjugate of the formula:

[0013] 
,

or a salt thereof, wherein R^1 is

[0014] 
,

,

,

, or

;

R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;

n is 1 to 50;

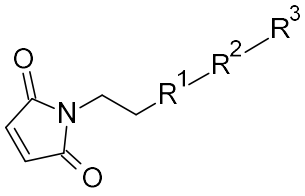
R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl;

mAb is a monoclonal antibody; and

S is a sulfur atom from a cysteine residue on the monoclonal antibody.

[0015] In an example, disclosed herein are compounds of Formula I, or salts thereof, methods of making compounds of Formula I, or salts thereof, and methods of using compounds of Formula I or salts thereof, are disclosed.

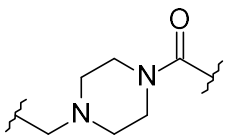
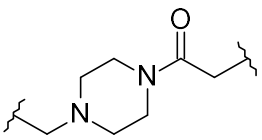
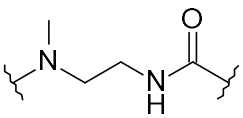
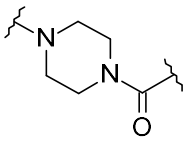
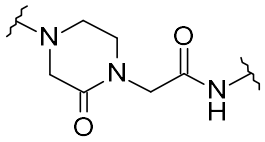
In an example, disclosed herein is a compound of Formula I:



Formula I,

or a salt thereof,

wherein R^1 is

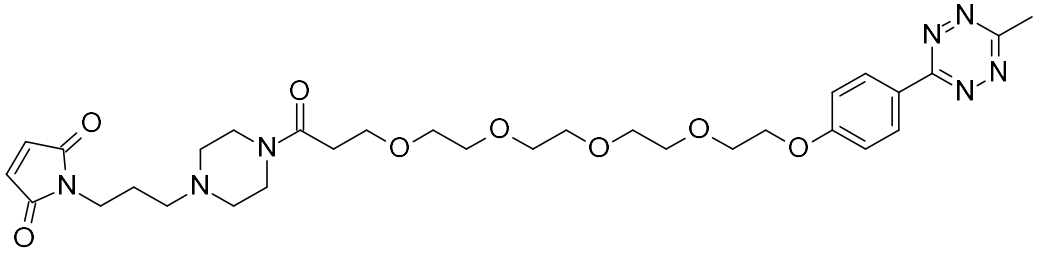
, , , , or ;

R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;

n is 1 to 50;

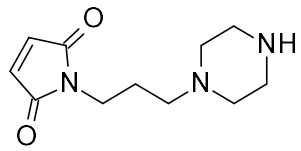
R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl.

In an example, disclosed herein is a compound,



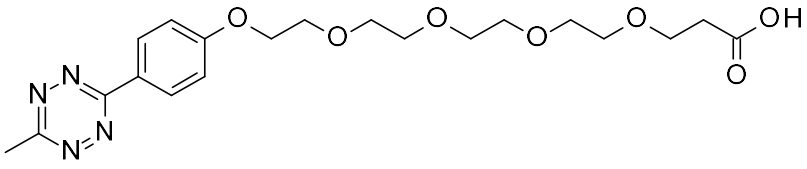
,

1-[3-[4-[3-[2-[2-[2-[2-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoyl]piperazin-1-yl]propyl]pyrrole-2,5-dione ("Compound A") or a salt thereof, obtained by combining 1-(3-piperazin-1-ylpropyl)pyrrole-2,5-dione,



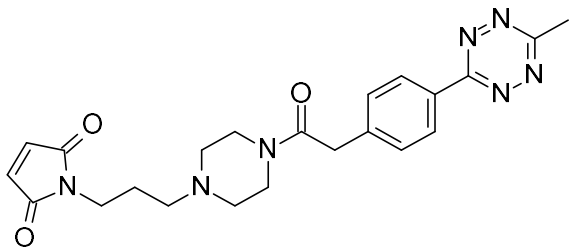
,

with 3-[2-[2-[2-[2-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid ("methyltetrazine-PEG4-acid"),



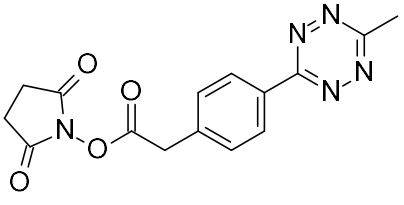
.

In an example, disclosed herein is 1-[3-[4-[2-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl]acetyl]piperazin-1-yl]propyl]pyrrole-2,5-dione ("Compound B"), which has the formula:



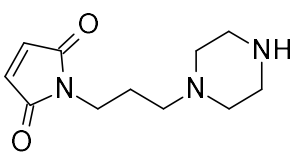
,

or a salt thereof, obtained by combining (2,5-dioxopyrrolidin-1-yl) 2-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl]acetate,



,

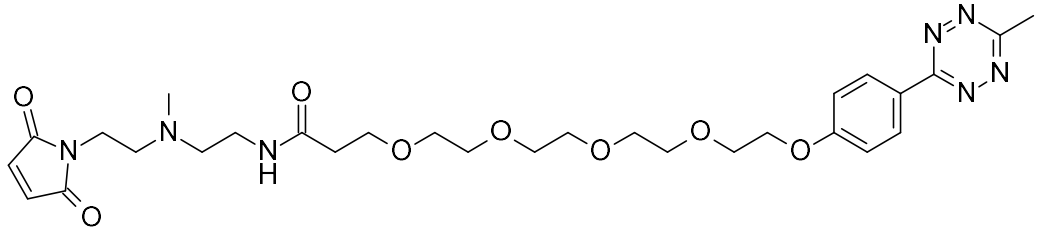
1-(3-piperazin-1-ylpropyl)pyrrole-2,5-dione,



,

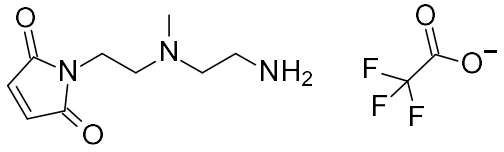
and a base, in the presence of a solvent and to give Compound B.

In an example, disclosed herein is N-[2-[2-(2,5-dioxopyrrol-1-yl)ethyl-methyl-amino]ethyl]-3-[2-[2-[2-[2-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanamide ("Compound C"), which has the following formula:



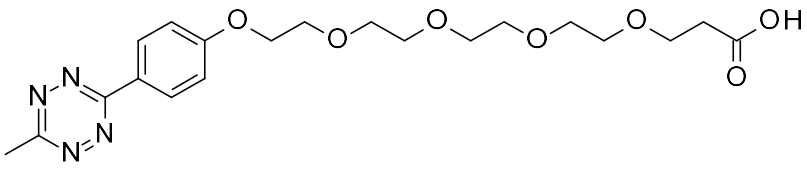
,

or a salt thereof, obtained by combining 1-[2-[2-aminoethyl(methyl)amino]ethyl]pyrrole-2,5-dione trifluoroacetate salt,



,

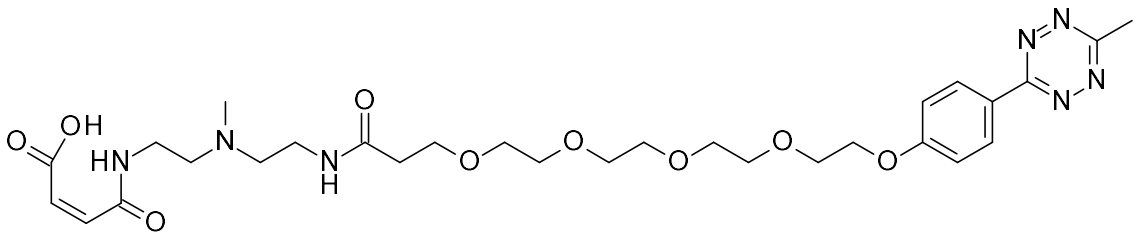
methyltetrazine-PEG4-acid,



,

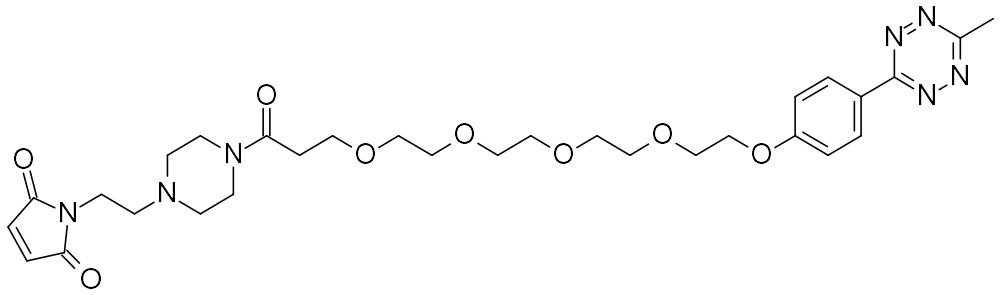
and an amide coupling reagent, and a base in a solvent.

In an alternate example, Compound C or a salt thereof, is obtained by combining (Z)-4-[2-[methyl-[2-[3-[2-[2-[2-[2-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]ethyl]amino]ethylamino]-4-oxo-but-2-enoic acid,



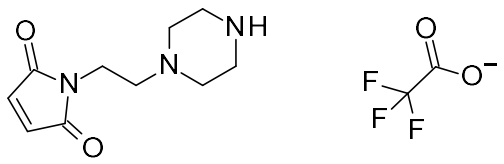
, acetate salt, and acetic anhydride.

In an example, disclosed herein is -[3-[2-[2-[2-[2-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy]ethoxy]ethoxy]ethoxy]propanoyl]piperazin-1-yl]ethyl]pyrrole-2,5-dione ("Compound D"):



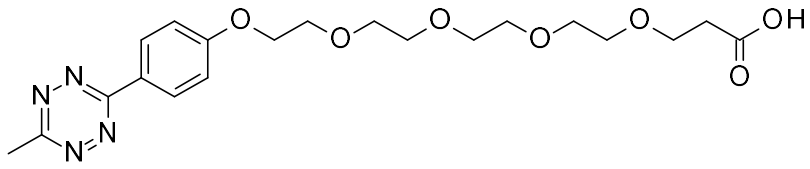
,

1-[2-[4 or a salt thereof, obtained by combining 1-(2-piperazin-1-ylethyl)pyrrole-2,5-dione trifluoroacetate,



,

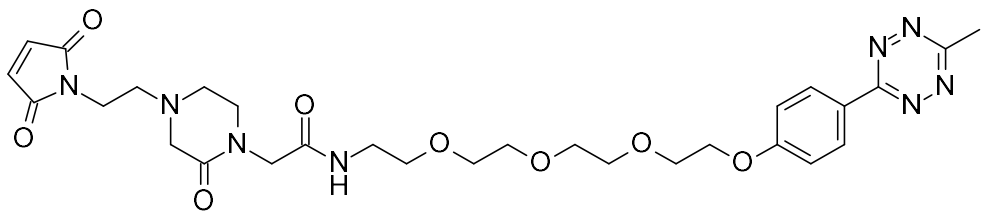
methyltetrazine-PEG4-acid,



,

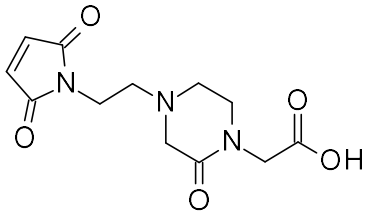
an amide coupling reagent, and a base, to a solvent.

In an example, disclosed herein is 2-[4-[2-(2,5-dioxopyrrol-1-yl)ethyl]-2-oxopiperazin-1-yl]-N-[2-[2-[2-[2-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy]ethoxy]ethoxy]ethoxy]ethyl]acetamide ("Compound E") which has the following formula:



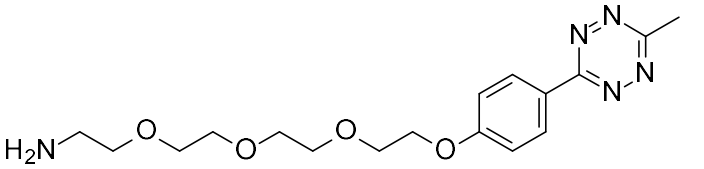
,

or a salt thereof, obtained by combining 2-[4-[2-(2,5-dioxopyrrol-1-yl)ethyl]-2-oxo-piperazin-1-yl]acetic acid,



,

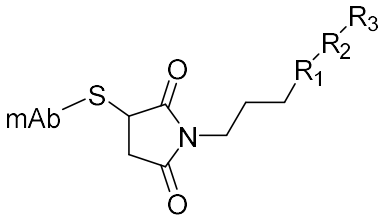
and 2-[2-[2-[2-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy]ethoxy]ethoxy]ethoxy]ethanamine ("methyltetrazine-PEG4-amine"),



,

an amide coupling reagent, an amide coupling solvent, and a base.

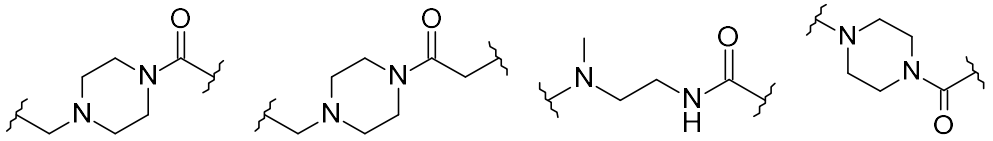
In an example, disclosed herein is a conjugate of the formula:



,

or a salt thereof,

wherein R^1 is



;

R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;

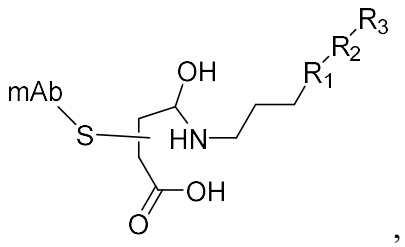
n is 1 to 50;

R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl;

mAb is a monoclonal antibody; and

S is a sulfur atom from a cysteine residue on the monoclonal antibody.

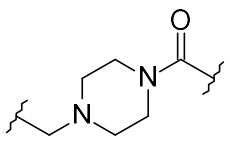
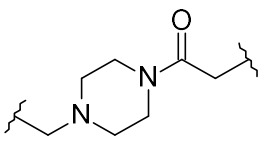
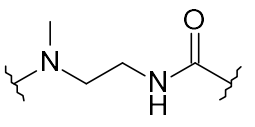
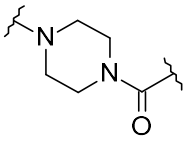
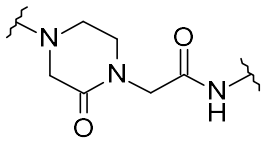
In an example, disclosed herein is a conjugate of the formula:



,

or a salt thereof,

wherein R^1 is


,

,

,

, or

;

R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;

n is 1 to 50;

R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl;

mAb is a monoclonal antibody; and

S is a sulfur atom from a cysteine residue on the monoclonal antibody.

DETAILED DESCRIPTION

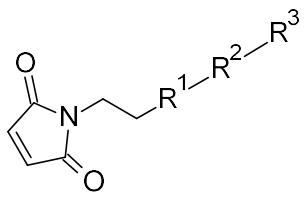
Definitions

The term “salt” as used herein refers to a salt of a compound. The term “pharmaceutically acceptable salt” as used herein refers to a salt of a compound considered to be acceptable for clinical and/or veterinary use. Examples of pharmaceutically acceptable salts and common methodology for preparing them can be found in “Handbook of Pharmaceutical Salts: Properties, Selection and Use” P. Stahl, *et*

CLAIMS

WE (I) CLAIM:

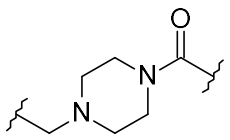
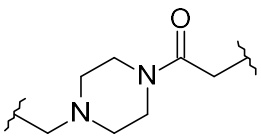
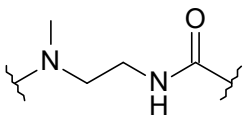
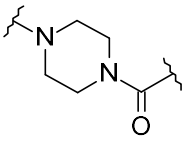
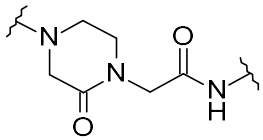
1. A compound of formula:



,

or a salt thereof,

wherein R^1 is

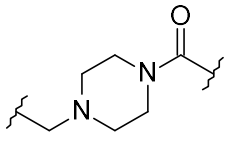

,

,

,

, or

;

R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;

n is 1 to 50; and

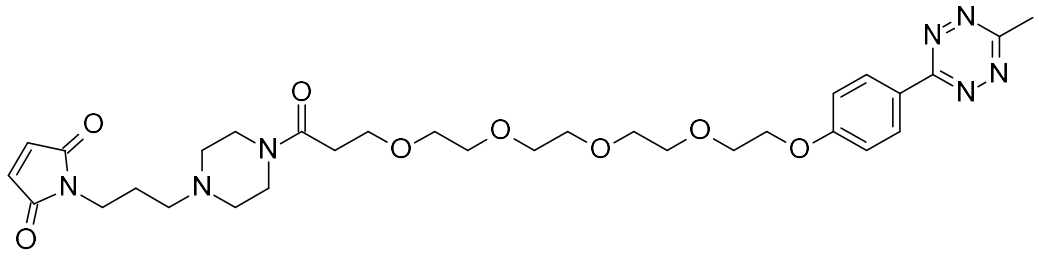
R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl.

2. The compound or a salt thereof according to claim 1 wherein R^1 is


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3. The compound or a salt thereof according to claim 1 or 2, wherein R^2 is PEG_n, and wherein n is 2 to 6.

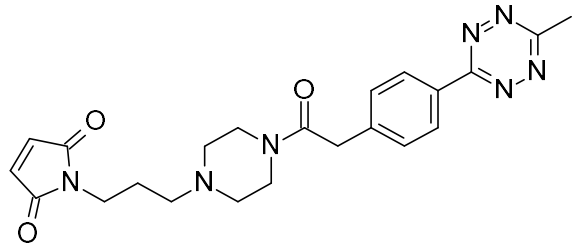
4. The compound or a salt thereof according to any one of claims 1 to 3, wherein the compound is



.

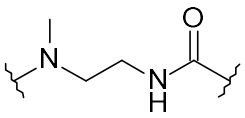
5. The compound or a salt thereof according to claim 1, wherein R^2 is a bond.

6. The compound or a salt thereof according to claim 1 or 5, wherein the compound is



.

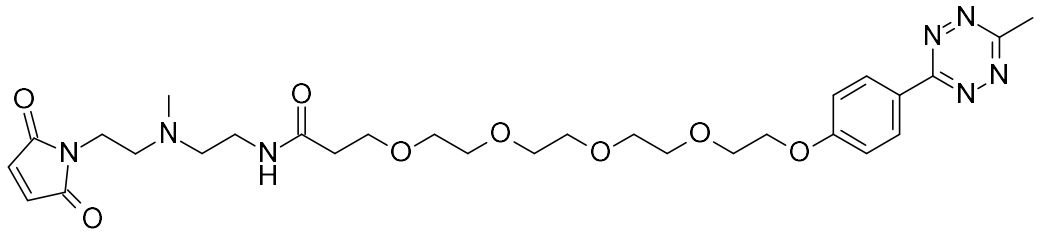
7. The compound or a salt thereof according to claim 1, wherein R^1 is



.

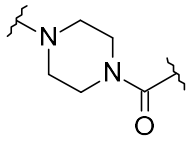
8. The compound or a salt thereof according to claim 7, wherein R^2 is PEG_n, and wherein n is 2 to 6.

9. The compound or a salt thereof according to any one of claims 1, 7, or 8, wherein the compound is



.

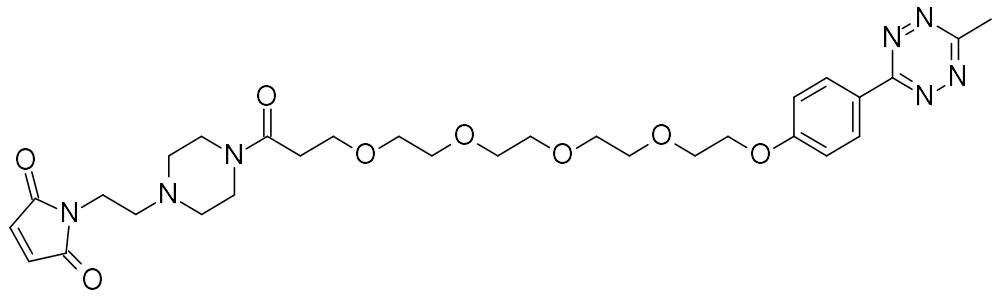
10. The compound or a salt thereof according to claim 1 wherein R^1 is



.

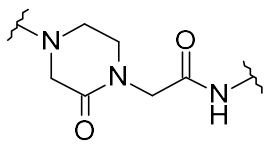
11. The compound or a salt thereof according to claim 10, wherein R^2 is PEG_n, and wherein n is 2 to 6.

12. The compound or a salt thereof according to any one of claims 1, 10, or 11, wherein the compound is



.

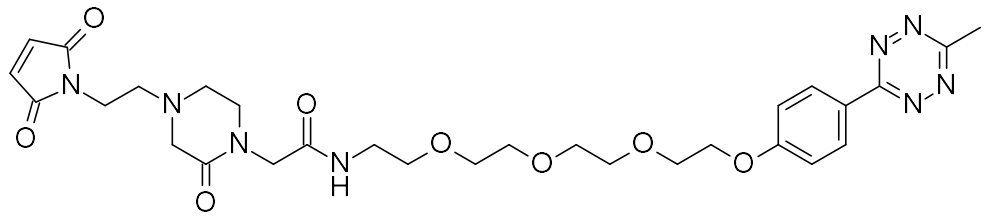
13. The compound or a salt thereof according to claim 1, wherein R^1 is



.

14. The compound or a salt thereof according to claim 13, wherein R^2 is PEG_n, and wherein n is 2 to 6.

15. The compound or a salt thereof according to any one of claims 1, 13, or 14, wherein the compound is

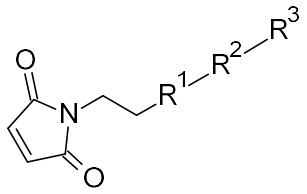


.

16. A method of preparing an antibody-maleimide conjugate comprising: combining a monoclonal antibody (mAb) comprising at least one cysteine and a compound, or a salt thereof, according to any one of claims 1 to 15, to form a reaction mixture.

17. The method according to claim 16, wherein the cysteine is an engineered cysteine.

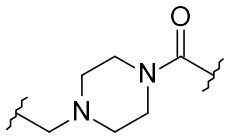
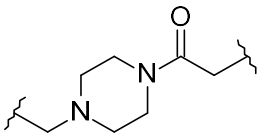
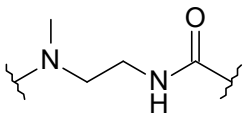
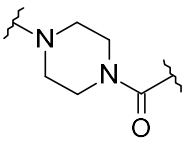
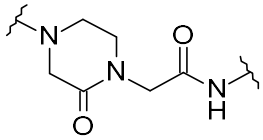
18. Use of a compound of formula:



,

or a salt thereof, to prepare a pharmaceutical formulation,

wherein R^1 is


,

,

,

, or

;

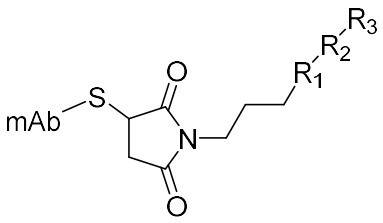
R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;

n is 1 to 50; and

R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl,
wherein the compound, or a salt thereof, is conjugated to a monoclonal antibody (mAb).

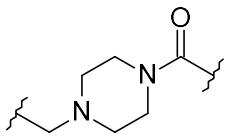
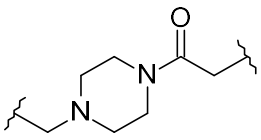
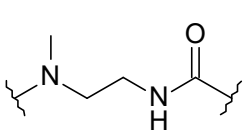
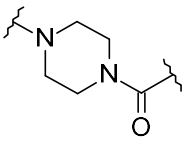
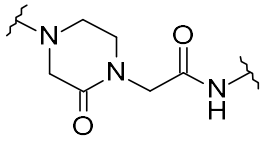
19. The use according to claim 18, wherein the cysteine is an engineered cysteine.

20. A conjugate of the formula:



,

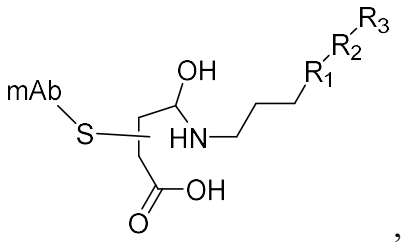
or a salt thereof,
wherein R^1 is


,

,

,

, or

;

R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;
n is 1 to 50;
R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl;
mAb is a monoclonal antibody; and
S is a sulfur atom from a cysteine residue on the monoclonal antibody.

21. The conjugate of claim 20, wherein the cysteine is an engineered cysteine, and wherein R^3 comprises a drug payload.

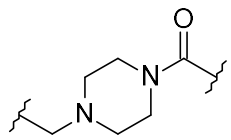
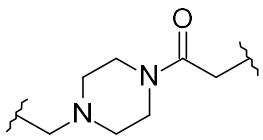
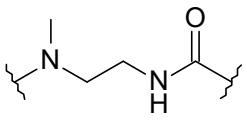
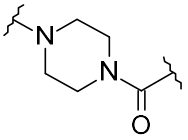
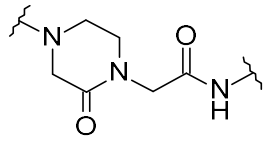
22. A conjugate of the formula:



,

or a salt thereof,

wherein R^1 is


,

,

,

, or

;

R^2 is selected from the group consisting of PEG_n, a bond, and a peptide, or a combination thereof;

n is 1 to 50;

R^3 is a -phenyl-tetrazine group, or a ligation group, wherein the tetrazine is optionally substituted with methyl;

mAb is a monoclonal antibody; and

S is a sulfur atom from a cysteine residue on the monoclonal antibody.

23. The conjugate or a salt thereof of claim 22, wherein the cysteine is an engineered cysteine, and wherein R^3 comprises a drug payload.