

in which

$\#^6$ marks the linkage site with the carbonyl group,

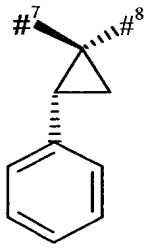
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen,

R^4 is benzyl, 1-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, or $-CH_2-O-R^{11}$,

in which

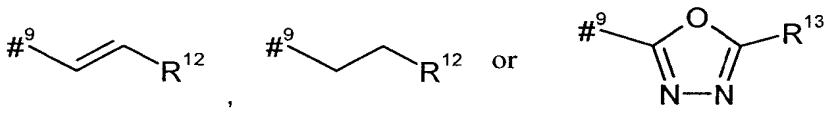
R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

R^5 is hydrogen, methyl or a group of the formula



in which

$\#^9$ marks the linkage site with $-CHCH_2$phenyl,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

5. Binder-drug conjugates of the general formula (Ia) according to any of Claims 1 to 4, in which

n is a number from 1 to 10,

AK is AK_2

where

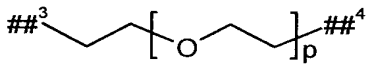
AK_2 is a binder which binds FGFR2 and is bonded via the NH side group of a lysine residue of the binder to the group G,

G is carbonyl,

L^1 is a bond,

B is a bond,

L^2 is linear (C_3-C_6)-alkanediyl or is a group of the formula



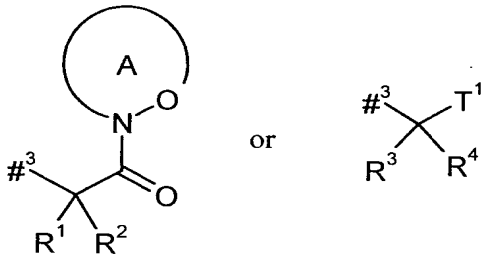
where

p is a number 2 or 3,

##^{3} marks the linkage site with the group B,

##^{4} marks the linkage site with the nitrogen atom,

D is a group of the formula



where

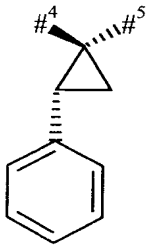
#^{3} marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is benzyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

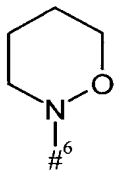


in which

#^{4} marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

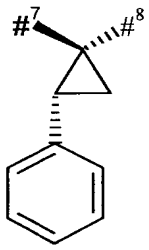
$\#^6$ marks the linkage site with the carbonyl group,

R^3 is hydrogen,

R^4 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$ or $-C(=O)-NR^8R^9$

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen,

R^9 is hydrogen or benzyl,

R^{35} is methyl,

and also their salts, solvates and solvates of the salts.

6. Binder-drug conjugates of the general formula (Ia) according to any of Claims 1 to 5, in which

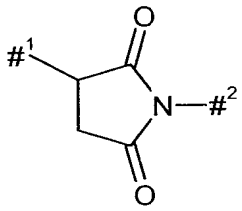
n is a number from 1 to 10,

AK is AK_1

where

AK_1 is a binder which binds FGFR2 and is bonded via the sulphur atom of a cysteine residue of the binder to the group G,

G is a group of the formula

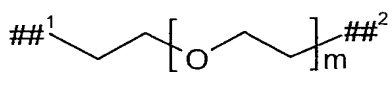


where

$\#^1$ marks the linkage site with the cysteine residue of the binder,

$\#^2$ marks the linkage site with the group L^1,

L^1 is a bond, linear (C_3-C_5)-alkanediyl or a group of the formula



where

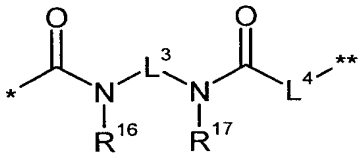
m is a number 2 or 3,

[1] marks the linkage site with the group G,

##[2] marks the linkage site with the group B,

where (C_3-C_5)-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula



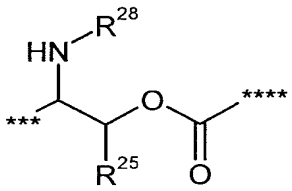
where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a bond or a group of the formula



in which

*** marks the linkage site with the carbonyl group,

**** marks the linkage site with L^2,

R^{25} is methyl,

R^{28} is hydrogen, methylcarbonyl or tert-butyloxycarbonyl,

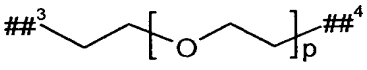
R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a piperazinyl ring,

L^2 is linear (C_3-C_5)-alkanediyl or is a group of the formula



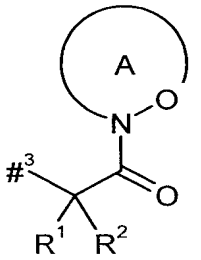
where

p is a number 2 or 3,

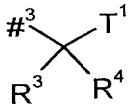
##^{3} marks the linkage site with the group B,

##^{4} marks the linkage site with the nitrogen atom,

D is a group of the formula



or



where

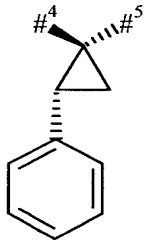
#^{3} marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is benzyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

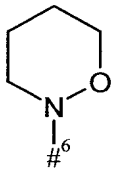


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

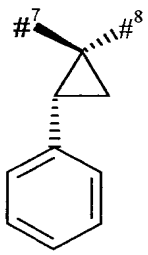
$\#^6$ marks the linkage site with the carbonyl group,

R^3 is hydrogen,

R^4 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$ or $-C(=O)-NR^8R^9$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

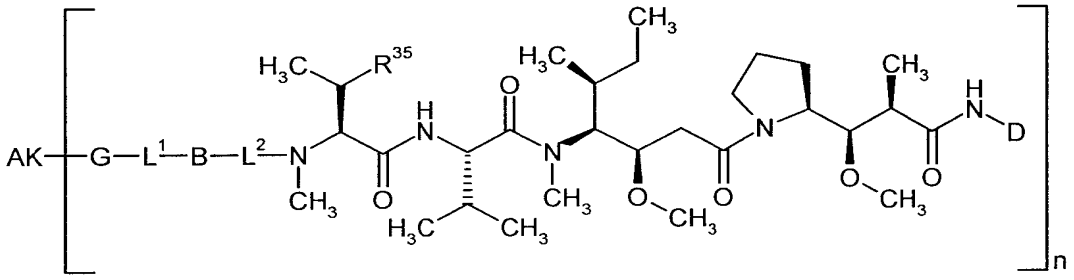
R^8 is hydrogen,

R^9 is hydrogen or benzyl,

R^{35} is methyl,

and also their salts, solvates and solvates of the salts.

7. Binder-drug conjugates of the general formula (Ia)



(Ia),

in which

n is a number from 1 to 50,

AK is a binder which binds to FGFR2,

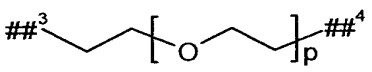
the group §-G-L^1-B-§§ is a linker,

where

§ marks the linkage site with the group AK and

§§ marks the linkage site with the nitrogen atom,

L^2 is linear (C_2-C_{10})-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

^{3} marks the linkage site with the group B,

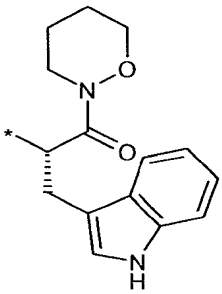
^{4} marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxyl and benzyl,

and

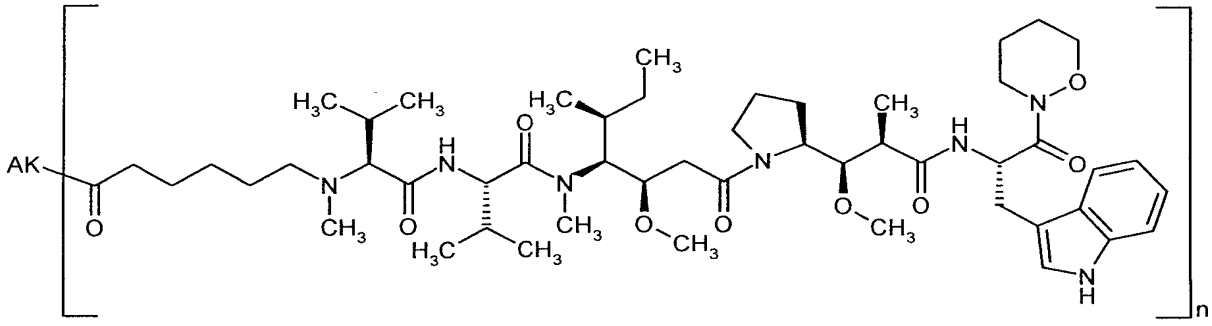
where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

D is a group of the following formula, where * is the linkage site to the nitrogen atom



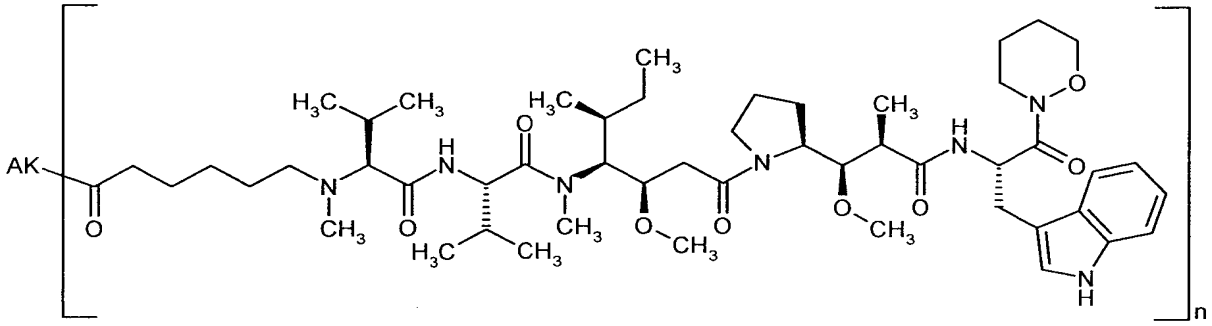
and also their salts, solvates and solvates of the salts.

8. Compounds of the following formula



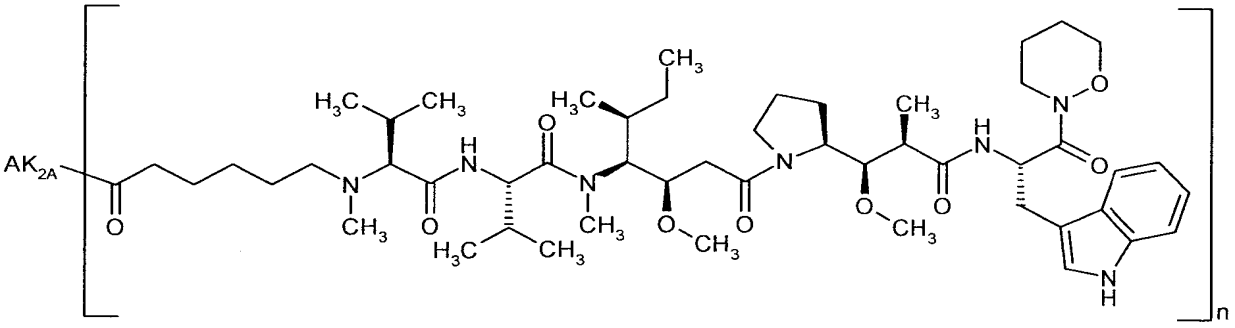
where AK is a binder which binds FGFR2, and n is a number from 1 to 10, and also their salts, solvates and solvates of the salts.

9. Compounds of the following formula



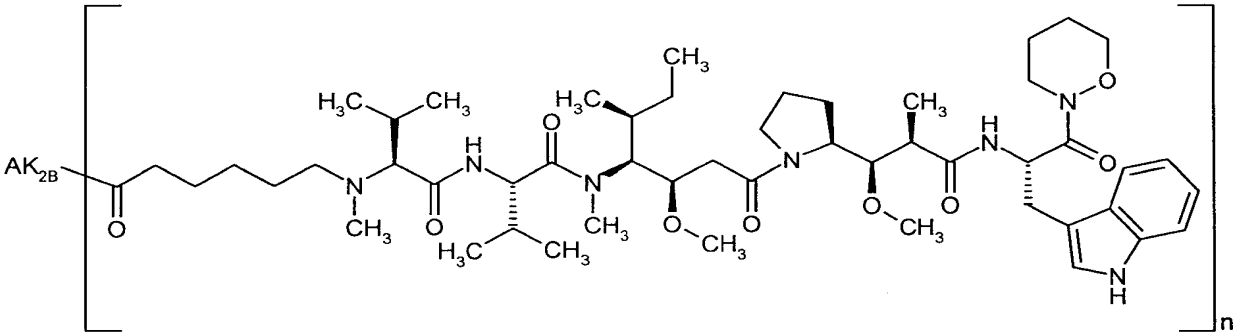
where AK is an antibody or an antibody fragment which binds FGFR2, and n is a number from 1 to 10, and also their salts, solvates and solvates of the salts.

10. Compound of the following formula



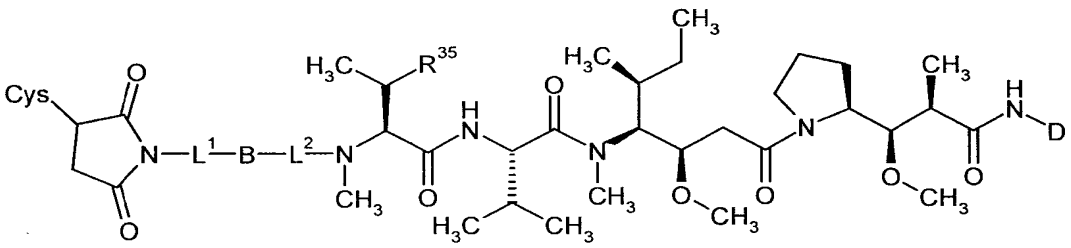
where AK2A is M048-D01-hIgG1 and n is a number from 1 to 10, and also their salts, solvates and solvates of the salts.

11. Compound of the following formula



where AK2B is M048-D01-hIgG1-b and n is a number from 1 to 10, and also their salts, solvates and solvates of the salts.

12. Compounds of the formula (XXXa)

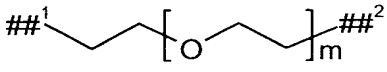
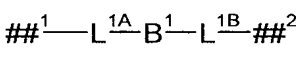


(XXXa),

in which

Cys is a cysteine residue which is bonded via the sulphur atom of the side chain to a carbon atom of the succinimide,

L^1 is a bond, linear (C_1-C_{10})-alkanediyl, a group of the formula

 or 

where

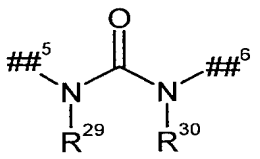
m is a number from 2 to 6,

^{1} marks the linkage site with the group G,

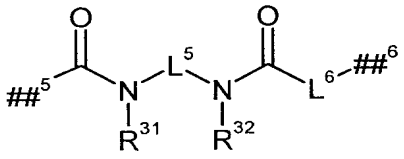
^{2} marks the linkage site with the group B,

L^{1A} is linear (C_2-C_{10})-alkanediyl,

B^1 is a group of the formula



or



in which

[5] marks the linkage site with the group L^{1A},

[6] marks the linkage site with the group L^{1B},

L^5 is a bond or $(C_2$-$C_4)$-alkanediyl,

L^6 is a bond,

R^{29} is hydrogen or $(C_1$-$C_4)$-alkyl,

R^{30} is hydrogen or $(C_1$-$C_4)$-alkyl,

or

R^{29} and R^{30} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{31} is hydrogen or $(C_1$-$C_4)$-alkyl,

R^{32} is hydrogen or $(C_1$-$C_4)$-alkyl,

or

R^{31} and R^{32} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

L^{1B} is linear $(C_2$-$C_{10})$-alkanediyl,

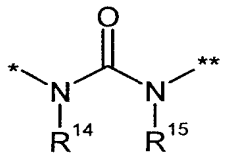
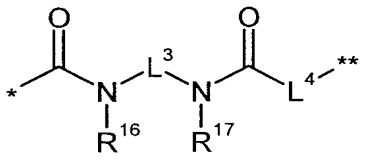
and

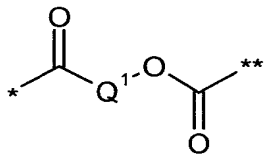
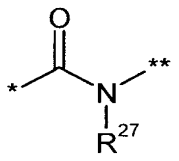
where $(C_1$-$C_{10})$-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

B is a bond or a group of the formula


,

,


or


where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

P is O or NH,

L^3 is a bond or (C_2-C_4)-alkanediyl,

L^4 is a bond,

Q^1 is a 4- to 7-membered heterocycle,

Q^2 is a 3- to 7-membered carbocycle or a 4- to 7-membered heterocycle,

R^{14} is hydrogen or (C_1-C_4)-alkyl,

R^{15} is hydrogen or (C_1-C_4)-alkyl,

or

R^{14} and R^{15} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{16} is hydrogen or (C_1-C_4)-alkyl,

R^{17} is hydrogen or (C_1-C_4)-alkyl,

or

R^{16} and R^{17} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{18} is hydrogen or (C_1-C_4)-alkyl,

R^{19} is hydrogen or the side group of a natural α-amino acid or of its homologues or isomers,

R^{20} is hydrogen or (C_1-C_4)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bonded form a pyrrolidinyl ring,

R^{21} is hydrogen or (C_1-C_4)-alkyl,

R^{22} is hydrogen or (C_1-C_4)-alkyl,

or

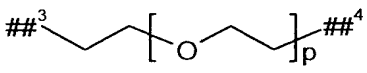
R^{21} and R^{22} together with the atoms to which they are bonded form a 3- to 7-membered carbocycle,

R^{23} is (C_1-C_4)-alkyl,

R^{24} is hydrogen or (C_1-C_4)-alkyl,

R^{27} is hydrogen or (C_1-C_4)-alkyl,

L^2 is linear (C_2-C_{10})-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

[3] marks the linkage site with the group B,

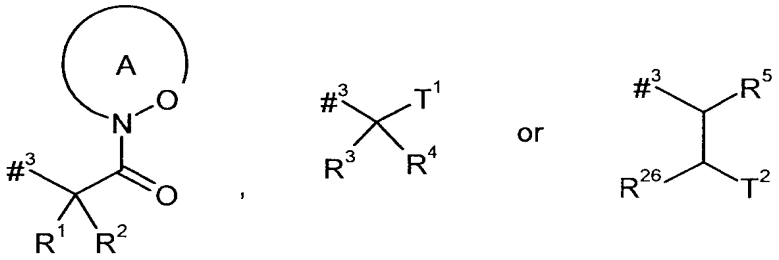
[4] marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2-, 1,3- or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

D is a group of the formula



where

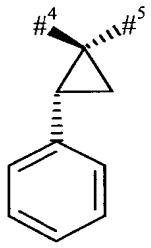
#3 marks the linkage site with the nitrogen atom,

R^1 is hydrogen or methyl,

R^2 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

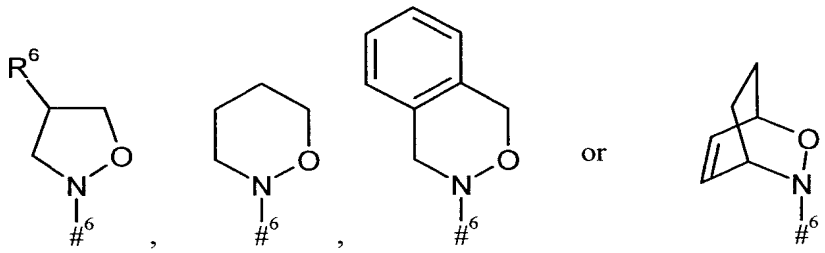


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

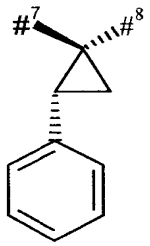
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen or methyl,

R^4 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

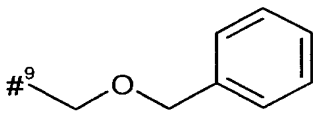
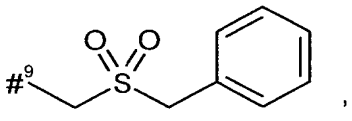
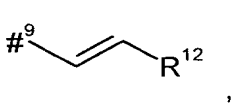
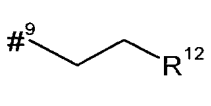
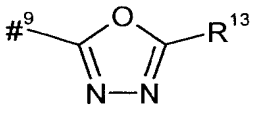
or

R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

R^5 is hydrogen, methyl or a group of the formula

in which

$\#^9$ marks the linkage site with $-CHC(R^{26})-T^2$,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen or hydroxy,

T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

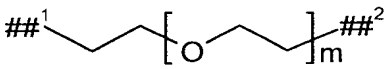
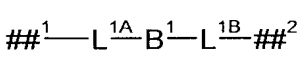
R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

13. Compounds of the formula (XXXa) according to Claim 12, in which

Cys is a cysteine residue which is bonded via the sulphur atom of the side chain via a carbon atom of the succinimide,

L^1 is a bond, linear (C_2-C_6)-alkanediyl, a group of the formula

 or 

where

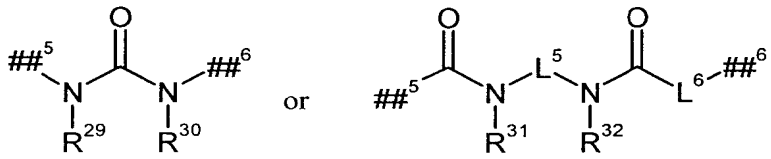
m is a number 2 or 3,

$\#\#^1$ marks the linkage site with the group G,

##^{2} marks the linkage site with the group B,

L^{1A} is linear (C_2-C_6)-alkanediyl,

B^1 is a group of the formula



in which

^{5} marks the linkage site with the group L^{1A},

^{6} marks the linkage site with the group L^{1B},

L^5 is a bond,

L^6 is a bond,

R^{29} is hydrogen,

R^{30} is hydrogen,

R^{31} is hydrogen or methyl,

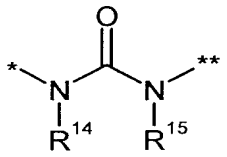
R^{32} is hydrogen or methyl,

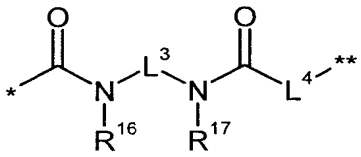
L^{1B} is linear (C_2-C_6)-alkanediyl,

and

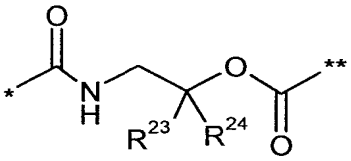
where (C_2-C_6)-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula





or



where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

L^3 is a bond or ethane-1,2-diyl,

L^4 is a bond,

R^{14} is hydrogen,

R^{15} is hydrogen,

R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

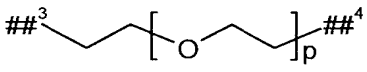
or

R^{16} and R^{17} together with the atoms to which they are bonded form piperazinyl ring,

R^{23} is methyl,

R^{24} is hydrogen or methyl,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



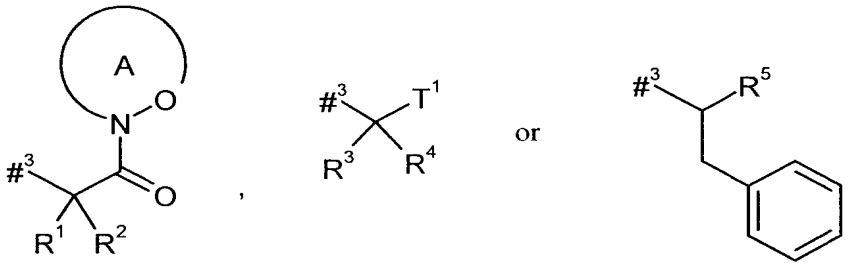
where

p is a number 2 or 3,

[3] marks the linkage site with the group B,

##[4] marks the linkage site with the nitrogen atom,

D is a group of the formula



where

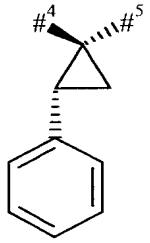
#[3] marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

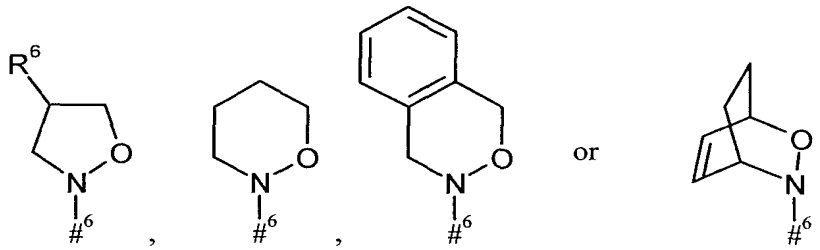


in which

#[4] marks the linkage site with the adjacent nitrogen atom,

#[5] marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

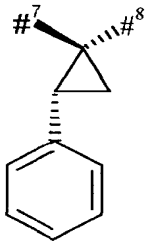
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen,

R^4 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

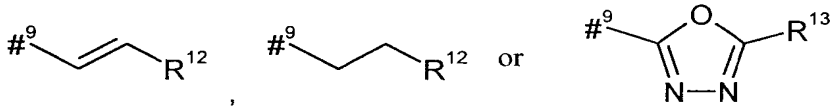
or

R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

R^{11} is benzyl which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

R^5 is hydrogen, methyl or a group of the formula



in which

$\#^9$ marks the linkage site to $-CHCH_2$phenyl,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{35} is methyl or hydroxy,

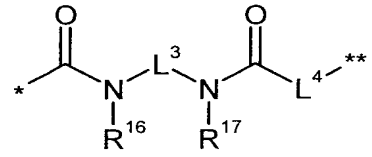
and also their salts, solvates and solvates of the salts.

14. Compounds of the formula (XXXa) according to either of Claims 12 or 13, in which

Cys is a cysteine residue which is bonded via the sulphur atom of the side chain via a carbon atom of the succinimide,

L^1 is a bond or linear (C_2-C_6)-alkanediyl,

B is a bond or a group of the formula



where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

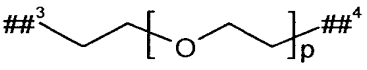
L^3 is a bond,

L^4 is a bond,

R^{16} is hydrogen or methyl,

R^{17} is hydrogen or methyl,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



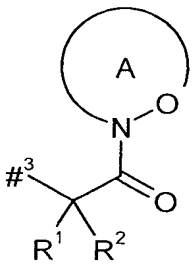
where

p is a number 2 or 3,

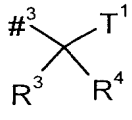
3 marks the linkage site with the group B,

##4 marks the linkage site with the nitrogen atom,

D is a group of the formula



or



where

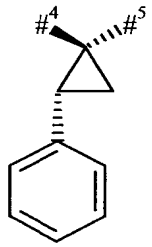
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is benzyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

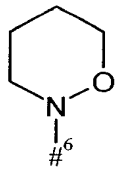


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

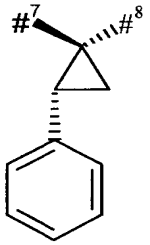
$\#^6$ marks the linkage site with the carbonyl group,

R^3 is hydrogen,

R^4 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$ or $-C(=O)-NR^8R^9$,

in which

R^7 is hydrogen,

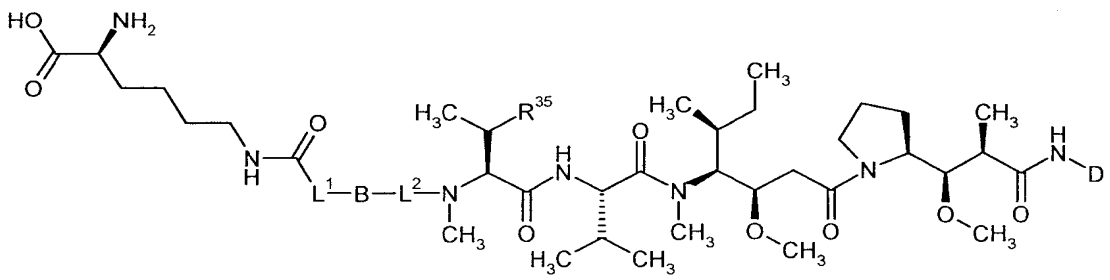
R^8 is hydrogen,

R^9 is hydrogen,

R^{35} is methyl,

and also their salts, solvates and solvates of the salts.

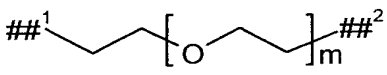
15. Compounds of the formula (XXXI)



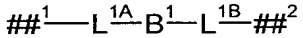
(XXXI),

in which

L^1 is a bond, linear (C_1-C_{10})-alkanediyl, a group of the formula



or



where

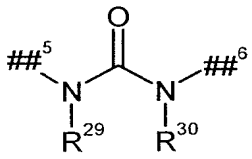
m is a number from 2 to 6,

[1] marks the linkage site with the group G,

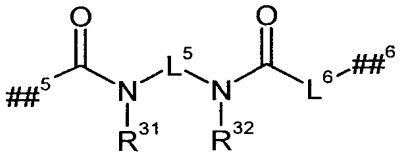
$##^2$ marks the linkage site with the group B,

L^{1A} is linear (C_2-C_{10})-alkanediyl,

B^1 is a group of the formula



or



in which

[5] marks the linkage site with the group L^{1A},

[6] marks the linkage site with the group L^{1B},

L^5 is a bond or (C_2-C_4)-alkanediyl,

L^6 is a bond,

R^{29} is hydrogen or (C_1-C_4)-alkyl,

R^{30} is hydrogen or (C_1-C_4)-alkyl,

or

R^{29} and R^{30} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

R^{31} is hydrogen or (C_1-C_4)-alkyl,

R^{32} is hydrogen or (C_1-C_4)-alkyl,

or

R^{31} and R^{32} together with the atoms to which they are bonded form a 5- or 6-membered heterocycle,

L^{1B} is linear (C_2-C_{10})-alkanediyl,

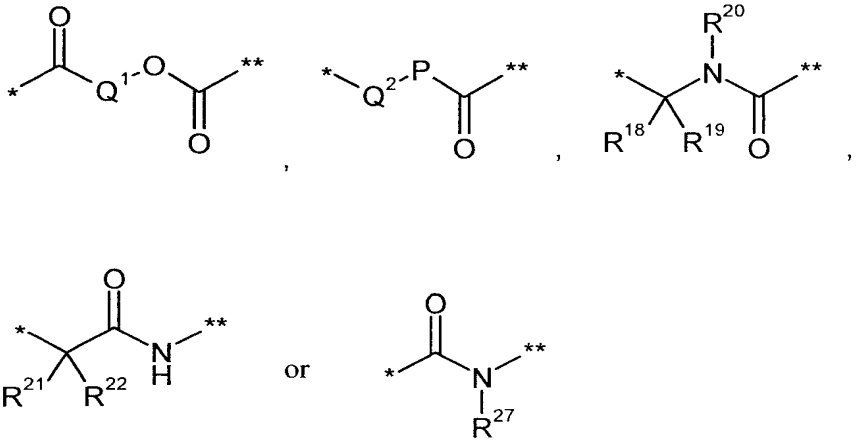
and

where (C_1-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

B is a bond or a group of the formula



where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

P is O or NH,

Q^1 is a 4- to 7-membered heterocycle,

Q^2 is a 3- to 7-membered carbocycle or a 4- to 7-membered heterocycle,

R^{18} is hydrogen or (C_1-C_4)-alkyl,

R^{19} is hydrogen or the side group of a natural α-amino acid or of its homologues or isomers,

R^{20} is hydrogen or (C_1-C_4)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bonded form a pyrrolidinyl ring,

R^{21} is hydrogen or (C_1-C_4)-alkyl,

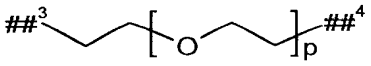
R^{22} is hydrogen or (C_1-C_4)-alkyl,

or

R^{21} and R^{22} together with the atoms to which they are bonded form a 3- to 7-membered carbocycle,

R^{27} is hydrogen or (C_1-C_4)-alkyl,

L^2 is linear (C_2-C_{10})-alkanediyl or is a group of the formula



where

p is a number from 2 to 6,

3 marks the linkage site with the group B,

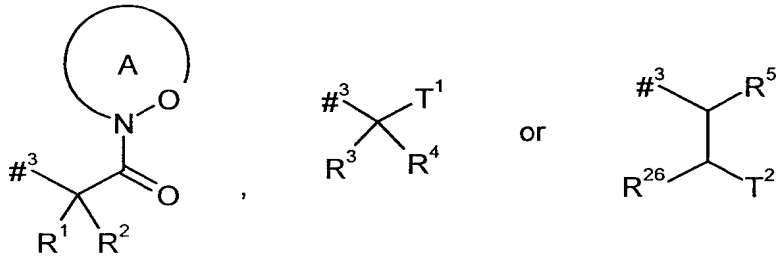
##4 marks the linkage site with the nitrogen atom,

where (C_2-C_{10})-alkanediyl may be substituted by 1 to 4 substituents selected independently of one another from the group consisting of methyl, hydroxy and benzyl,

and

where two carbon atoms of the alkanediyl chain in 1,2, 1,3 or 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a (C_3-C_6)-cycloalkyl ring or a phenyl ring,

D is a group of the formula



in which

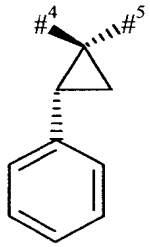
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen or methyl,

R^2 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

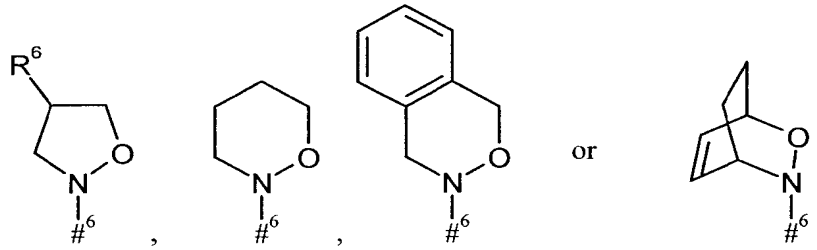


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

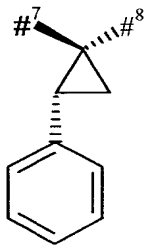
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen or methyl,

R^4 is isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, phenyl, benzyl, 1-hydroxyethyl, 4-hydroxybenzyl, 4-hydroxy-3-nitrobenzyl, 4-hydroxy-3-aminobenzyl, 1-phenylethyl, diphenylmethyl, 1*H*-imidazol-4-ylmethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

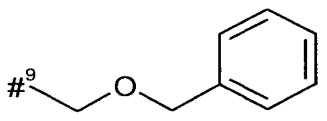
or

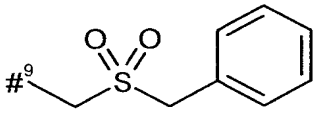
R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

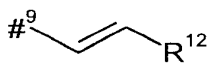
R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

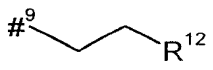
R^5 is hydrogen, methyl or a group of the formula

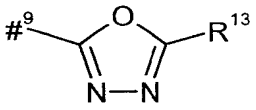


,


,



,


or


#9 marks the linkage site with $-CHC(R^{26})-T^2$,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{26} is hydrogen or hydroxy,

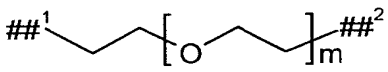
T^2 is phenyl, benzyl, 1*H*-indol-3-yl or 1*H*-indol-3-ylmethyl,

R^{35} is methyl or hydroxy,

and also their salts, solvates and solvates of the salts.

16. Compounds of the formula (XXXI) according to Claim 15, in which

L^1 is a bond, linear (C_2-C_6)-alkanediyl or a group of the formula



where

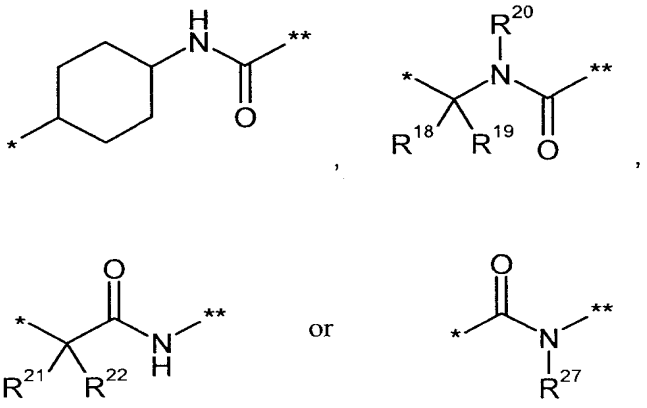
m is a number 2 or 3,

1 marks the linkage site with the group G,

2 marks the linkage site with the group B,

where (C_2-C_6)-alkanediyl may be substituted by 1 or 2 methyl substituents,

B is a bond or a group of the formula



where

* marks the linkage site with L^1,

** marks the linkage site with L^2,

R^{18} is hydrogen,

R^{19} is methyl, propan-2-yl, 2-methylpropan-1-yl or 1-methylpropan-1-yl,

R^{20} is hydrogen or (C_1-C_4)-alkyl,

or

R^{19} and R^{20} together with the atoms to which they are bonded form a pyrrolidinyl ring,

R^{21} is hydrogen or methyl,

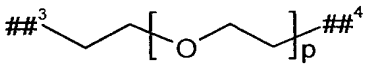
R^{22} is hydrogen or methyl,

or

R^{21} and R^{22} together with the atoms to which they are bonded form a cyclopropyl ring,

R^{27} is hydrogen or methyl,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



where

p is a number 2 or 3,

3 marks the linkage site with the group B,

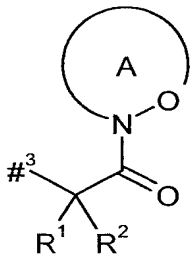
##4 marks the linkage site with the nitrogen atom,

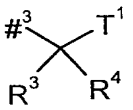
where (C_2-C_{10})-alkanediyl may be substituted by 1 or 2 methyl substituents,

and

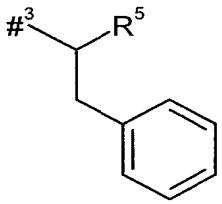
where two carbon atoms of the alkanediyl chain in 1,4-relation to one another, with inclusion of any carbon atoms situated between them, may be bridged to form a phenyl ring,

D is a group of the formula





or



in which

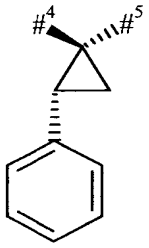
$\#^3$ marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

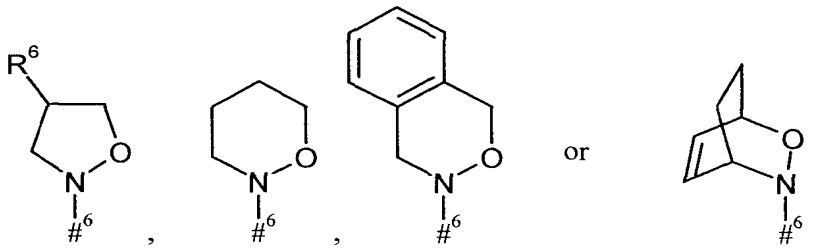


in which

$\#^4$ marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

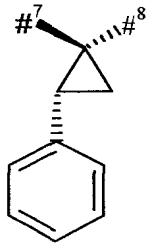
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen,

R^4 is 1-hydroxyethyl, benzyl, 4-hydroxybenzyl, 1-phenylethyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atoms to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula $-C(=O)-OR^7$, $-C(=O)-NR^8R^9$, $-C(=O)-NH-NH-R^{10}$ or $-CH_2-O-R^{11}$,

in which

R^7 is hydrogen, methyl, ethyl, *n*-propyl, *tert*-butyl, benzyl or adamantylmethyl,

R^8 is hydrogen or methyl,

R^9 is hydrogen, methyl, ethyl, *n*-propyl or benzyl,

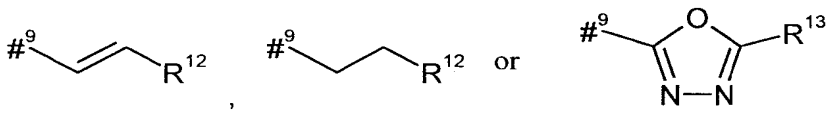
or

R^8 and R^9 together with the nitrogen atom to which they are bonded form a 4- to 7-membered heterocycle,

R^{10} is benzoyl,

R^{11} is benzyl, which may be substituted in the phenyl group by methoxycarbonyl or carboxyl,

R^5 is hydrogen, methyl or a group of the formula



in which

$\#^9$ marks the linkage site with $-CHCH_2$phenyl,

R^{12} is phenyl which may be substituted by methoxycarbonyl, carboxyl or a group of the formula $-S(O)_2OH$,

R^{13} is phenyl which may be substituted by methoxycarbonyl or carboxyl,

R^{35} is methyl or hydroxy,

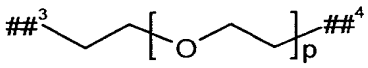
and also their salts, solvates and solvates of the salts.

17. Compounds of the formula (XXXI) according to either of Claims 15 or 16, in which

L^1 is a bond,

B is a bond,

L^2 is linear (C_2-C_6)-alkanediyl or is a group of the formula



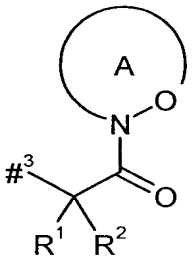
where

p is a number 2 or 3,

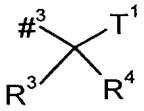
[3] marks the linkage site with the group B,

##[4] marks the linkage site with the nitrogen atom,

D is a group of the formula



or



where

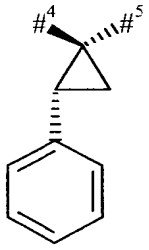
#[3] marks the linkage site with the nitrogen atom,

R^1 is hydrogen,

R^2 is benzyl or 1*H*-indol-3-ylmethyl,

or

R^1 and R^2 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula

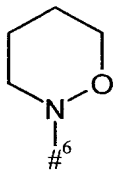


in which

#[4] marks the linkage site with the adjacent nitrogen atom,

$\#^5$ marks the linkage site with the carbonyl group,

the ring A with the N-O moiety present therein is a mono- or bicyclic, optionally substituted heterocycle of the formula



in which

$\#^6$ marks the linkage site with the carbonyl group,

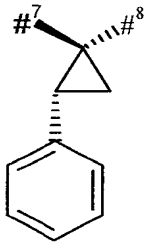
R^6 is hydrogen, hydroxy or benzyloxy,

R^3 is hydrogen,

R^4 is benzyl, 4-hydroxybenzyl or 1*H*-indol-3-ylmethyl,

or

R^3 and R^4 together with the carbon atom to which they are bonded form a (1*S*,2*R*)-2-phenylcyclopropane-1,1-diyl group of the formula



in which

$\#^7$ marks the linkage site with the adjacent nitrogen atom,

$\#^8$ marks the linkage site with the group T^1,

T^1 is a group of the formula -C(=O)-OR^7 or -C(=O)-NR^8R^9,

in which

R^7 is hydrogen,

R^8 is hydrogen,

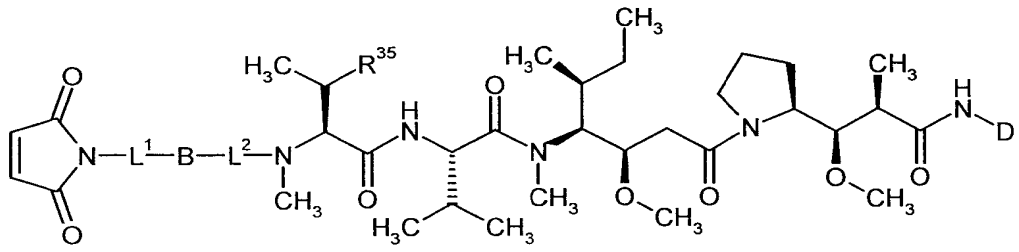
R^9 is hydrogen,

R^{35} is methyl,

and also their salts, solvates and solvates of the salts.

18. Process for preparing the compounds of the invention according to any of Claims 1 to 11, of the general formula (Ia), characterized in that a solution of the binder in a buffer

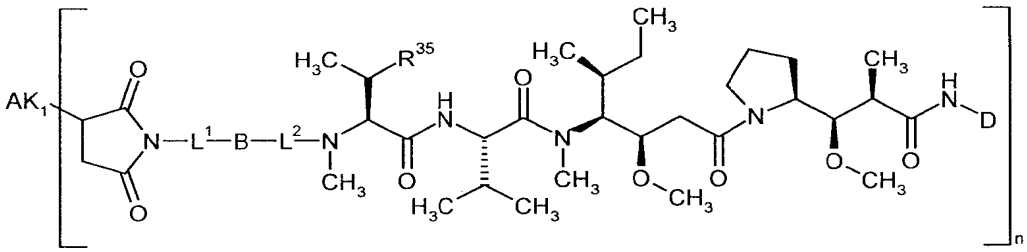
[A] is admixed with a suitable reducing agent, such as, for example, dithiothreitol or tris(2-carboxyethyl)phosphine hydrochloride, and is subsequently reacted with a compound of the formula (II)



(II),

in which D, L^1, B, L^2 and R^{35} each have the definitions indicated in Claims 1 to 11,

to give a compound of the formula (I-A)

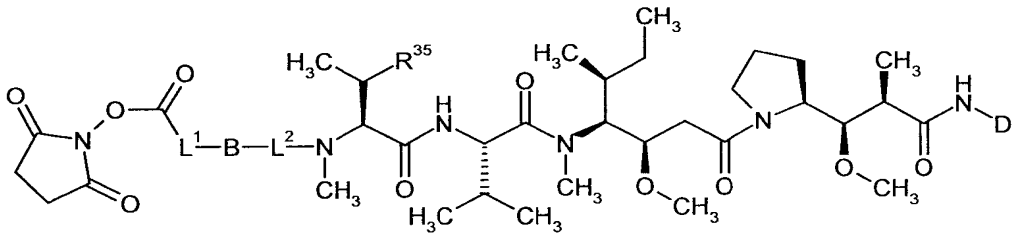


(I-A),

in which n, AK_1, D, L^1, B, L^2 and R^{35} each have the definitions indicated in Claims 1 to 11,

or

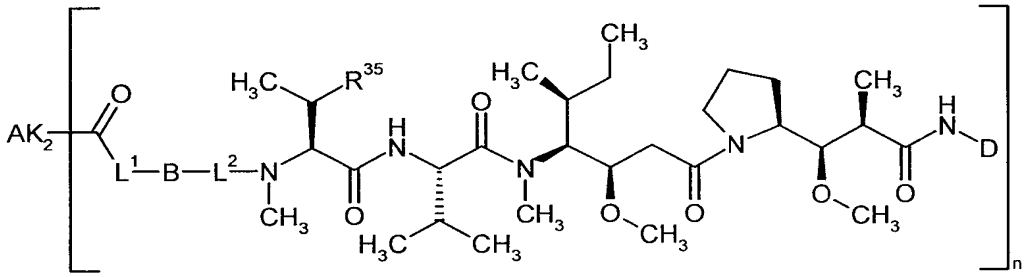
[B] is reacted with a compound of the formula (III)



(III),

in which D, L^1, B, L^2 and R^{35} each have the definitions indicated in Claims 1 to 11,

to give a compound of the formula (I-B)



(I-B),

in which n, AK_2, D, L^1, B, L^2 and R^{35} each have the definitions indicated in Claims 1 to 11.

19. Compounds prepared by the process in Claim 18, where AK_1 and AK_2 is an antibody which comprises the six CDR sequences of the antibody M048-D01-hIgG1 or M048-D01-hIgG1-b, the variable light and variable heavy chain of the antibody M048-D01-hIgG1 or M048-D01-hIgG1-b or the light and heavy chain of the antibody M048-D01-hIgG1 or M048-D01-hIgG1-b,

and also their salts, solvates and solvates of the salts.

20. Binder-drug conjugate according to any of the preceding claims, where the binder binds specifically to FGFR2.

21. Binder-drug conjugate according to any of the preceding claims, where the binder binds to the extracellular domain of FGFR2.

22. Binder-drug conjugate according to any of the preceding claims, where FGFR2 is FGFR2 alpha and/or FGFR2 beta.

23. Binder-drug conjugate according to any of the preceding claims, where the binder binds to the extracellular N-terminal epitope (^{1}RPSFSLVEDTTLEPE^{15}) of FGFR2.

24. Binder-drug conjugate according to any of the preceding claims, where the binder as well as human FGFR2 also binds to murine FGFR2.

25. Binder-drug conjugate according to any of the preceding claims, where the binder, after binding to FGFR2 on the target cell, is internalized by the binding of the target cell.

26. Binder-drug conjugate according to any of the preceding claims, where the binder is a binding protein.

27. Binder-drug conjugate according to any of the preceding claims, where the binder is an antibody or an antigen-binding antibody fragment thereof.

28. Binder-drug conjugate according to any of the preceding claims, where the binder is a monoclonal antibody or an antigen-binding antibody fragment thereof.

29. Binder-drug conjugate according to any of the preceding claims, where the binder is a human, humanized or chimeric antibody or an antigen-binding antibody fragment thereof.

30. Binder-drug conjugate according to any of the preceding claims, where the binder is an IgG antibody or an antigen-binding antibody fragment thereof.

31. Binder-drug conjugate according to any of the preceding claims, where the binder is an IgG1 antibody or an antigen-binding antibody fragment thereof.

32. Binder-drug conjugate according to any of the preceding claims, where the binder competes in binding to the cancer target molecule FGFR2 with the GAL-FR21, GAL-FR22 or M048-D01-hIgG1 antibody.

33. Binder-drug conjugate according to any of the preceding claims, where the binder comprises the amino acid sequence of the CDR sequences of the variable light and heavy chain of the antibody M048-D01-hIgG1 represented in SEQ ID NO:15 (H-CDR1), SEQ ID NO:16 (H-CDR2), SEQ ID NO:17 (H-CDR3), SEQ ID NO:18 (L-CDR1), SEQ ID NO:19 (L-CDR2) and SEQ ID NO:20 (L-CDR3),

the amino acid sequence of the variable light and heavy chains of the antibody M048-D01-hIgG1, represented in SEQ ID NO:12 (Vl) and SEQ ID NO:11 (Vh),

the amino acid sequence of the variable light and heavy chains of the antibody M048-D01-hIgG1-b, represented in SEQ ID NO:14 (Vl) and SEQ ID NO:13 (Vh),

the amino acid sequence of the light and heavy chain of the antibody M048-D01-hIgG1-b represented in SEQ ID NO: 9 (light chain) and SEQ ID NO:10 (heavy chain),

the amino acid sequence of the light and heavy chain of the antibody M048-D01-hIgG1 represented in SEQ ID NO: 7 (light chain) and SEQ ID NO:8 (heavy chain), or

the amino acid sequence of the variable light and heavy chains of the antibody GAL-FR21 or GAL-FR22.

34. Binder-drug conjugate according to any of the preceding claims, where the binder comprises the amino acid sequence of the variable light and heavy chains of the antibody M048-D01-hIgG1-b, represented in SEQ ID NO:14 (Vl) and SEQ ID NO:13 (Vh),

the amino acid sequence of the light and heavy chain of the antibody M048-D01-hIgG1-b represented in SEQ ID NO: 9 (light chain) and SEQ ID NO:10 (heavy chain).

35. Binder-drug conjugate or compounds according to any of Claims 1 to 34 for the treatment and/or prophylaxis of illnesses.

36. Binder-drug conjugate or a compound according to any of Claims 1 to 34 for use in a method for the treatment and/or prophylaxis of hyperproliferative and/or angiogenic diseases.

37. Binder-drug conjugate according to any of Claims 1 to 34 for producing a medicament for the treatment and/or prophylaxis of hyperproliferative and/or angiogenic diseases.

38. Medicament comprising a binder-drug conjugate or a compound according to any of Claims 1 to 34, in combination with an inert, non-toxic, pharmaceutically suitable excipient.

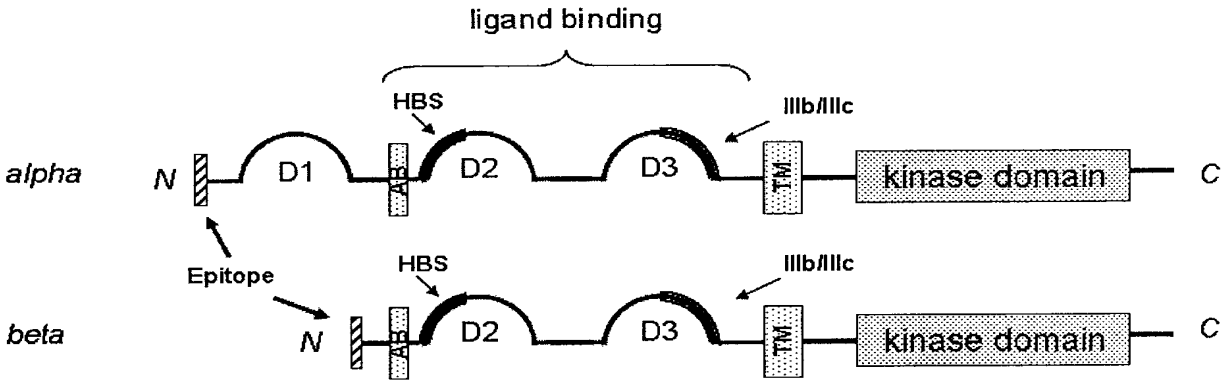
39. Medicament comprising a binder-drug conjugate or a compound according to any of Claims 1 to 34, in combination with one or more anti-hyperproliferative, cytostatic or cytotoxic substances.

40. Medicament according to Claim 38 or 39 for the treatment and/or prophylaxis of hyperproliferative and/or angiogenic diseases.

41. Method for the treatment and/or prophylaxis of hyperproliferative and/or angiogenic diseases in humans and animals, using an effective amount of at least one binder-drug conjugate or a compound according to any of Claims 1 to 34, or of a medicament as defined in either of Claims 39 and 40.

Figures:

Figure 1:



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(54) Titre : COMPOSITIONS ET PROCEDES DE TRAITEMENT DE MALADIES AUTO-IMMUNES ET AUTRES
(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF AUTOIMMUNE AND OTHER DISEASES

(57) Abrégé/Abstract:
Provided are methods relating to the use of CDP-therapeutic agent conjugates for the treatment of autoimmune disease, inflammatory disease, or cancer. Also provided are CDP-therapeutic agent conjugates, particles comprising CDP-therapeutic agent conjugates, and compositions comprising CDP-therapeutic agent conjugates.

ABSTRACT

Provided are methods relating to the use of CDP-therapeutic agent conjugates for the treatment of autoimmune disease, inflammatory disease, or cancer. Also provided are CDP-therapeutic agent conjugates, particles comprising CDP-therapeutic agent conjugates, and compositions comprising CDP-therapeutic agent conjugates.

DEMANDES OU BREVETS VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS COMPREND PLUS D'UN TOME.

CECI EST LE TOME __1___ DE __2 __

NOTE: Pour les tomes additionels, veillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.

THIS IS VOLUME __1___ OF __2___

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COMPOSITIONS AND METHODS FOR TREATMENT OF AUTOIMMUNE AND OTHER DISEASES

This application claims priority to U.S. Serial Number 61/345,641, filed May 18, 2010. The disclosures of the prior application is considered part of (and is incorporated by reference in) the disclosure of this application.

Background of the Invention

Drug delivery and dosing of small molecule therapeutic agents, such as camptothecin, can be problematic due to a number issues including half-life, toxicity, distribution etc.

Summary of the Invention

In one aspect, the invention features a method of treating an autoimmune disease in a subject, e.g., a human subject, comprising administering a CDP-therapeutic agent conjugate, particle or composition to the subject, e.g., a human subject, in an amount effective to treat the disease.

Examples of autoimmune diseases that can be treated according to the methods of the invention include ankylosing spondylitis, arthritis (e.g., rheumatoid arthritis, osteoarthritis, gout), Chagas disease, chronic obstructive pulmonary disease (COPD), dermatomyositis, diabetes mellitus type 1, endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barrè syndrome (GBS), Hashiomoto's disease, Hidradenitis suppurativa, Kawasaki disease, IgA nephropathy, Idiopathic thrombocytopenic purpura, inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitisinterstitial cystitis), lupus (e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus), mixed connective tissue disease, morphea, multiple sclerosis, myasthenia gravis, narcolepsy, neuromyotonia, pemphigus vulgaris, pernicious anemia, psoriasis, psoriatic arthritis, polymyositis, primary biliary

cirrhosis, relapsing polychondritis, schizophrenia, scleroderma, Sjögren's syndrome, Stiff person syndrome, temporal arteritis (also known as giant cell arteritis), vasculitis, vitiligo, Wegener's granulomatosis. In some embodiments, the method includes inhibiting rejection of a transplanted organ, e.g., rejection of a kidney transplant, e.g., rejection of a lung transplant, e.g., rejection of a liver transplant. In an embodiment, the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis.

In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-cytotoxic agent conjugate, particle or composition, e.g., a CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-topoisomerase inhibitor I conjugate (e.g., a CDP-camptothecin conjugate, particle or composition, CDP-irinotecan conjugate, particle or composition, or CDP-SN-38 conjugate, particle or composition, a CDP-topotecan conjugate, particle or composition, a CDP-lamellarin D conjugate, particle or composition, a CDP-lurotecan conjugate, particle or composition, a CDP-exatecan conjugate, particle or composition, a CDP-diflomotecan conjugate, particle or composition, or a CDP-topoisomerase I inhibitor conjugate, particle or composition which includes a derivative of camptothecin, irinotecan, SN-38, lamellarin D, lurotecan, exatecan or diflomotecan).

The topoisomerase inhibitor can be a topoisomerase II inhibitor, thus in an embodiment the conjugate, particle or composition is: a CDP-topoisomerase II inhibitor conjugate, particle or composition (e.g., a CDP-etoposide conjugate, particle or composition, a CDP-tenoposide conjugate, particle or composition, a CDP-amsacrine conjugate, particle or composition, or a CDP-topoisomerase II inhibitor conjugate, particle or composition which includes a derivative of etoposide, tenoposide, and amsacrine).

The therapeutic agent can be an anti-metabolite, thus in an embodiment the conjugate, particle or composition is: a CDP-anti-metabolic agent conjugate, particle or composition (e.g., a CDP-antifolate conjugate, particle or composition (e.g., a CDP-

pemetrexed conjugate, particle or composition, a CDP-floxuridine conjugate, particle or composition, or a CDP-raltitrexed conjugate, particle or composition); or a CDP-pyrimidine analog conjugate, particle or composition (e.g., a CDP-capecitabine conjugate, particle or composition, a CDP-cytarabine conjugate, particle or composition, a CDP-gemcitabine conjugate, particle or composition, or a CDP-5FU conjugate, particle or composition)).

The therapeutic agent can be an alkylating agent, thus in an embodiment the conjugate, particle or composition is: a CDP-alkylating agent conjugate, particle or composition.

The therapeutic agent can be an anthracycline, thus in an embodiment the conjugate, particle or composition is: a CDP-anthracycline conjugate, particle or composition.

The therapeutic agent can be an anti-tumor antibiotic, thus in an embodiment the conjugate, particle or composition is a CDP-anti-tumor antibiotic conjugate, particle or composition (e.g., a CDP-HSP90 inhibitor conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition, a CDP-tanespimycin conjugate, particle or composition or a CDP-alvespimycin conjugate, particle or composition).

The therapeutic agent can be a platinum based agent, thus in an embodiment the conjugate, particle or composition is a CDP-platinum based agent conjugate, particle or composition (e.g., a CDP-cisplatin conjugate, particle or composition, a CDP-carboplatin conjugate, particle or composition, or a CDP-oxaliplatin conjugate, particle or composition).

The therapeutic agent can be a microtubule inhibitor, thus in an embodiment the conjugate, particle or composition is a CDP-microtubule inhibitor conjugate, particle or composition.

The therapeutic agent can be a kinase inhibitor, thus in an embodiment the conjugate, particle or composition is, a CDP-kinase inhibitor conjugate, particle or composition (e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition).

The therapeutic agent can be a proteasome inhibitor, thus in an embodiment the conjugate, particle or composition is a CDP-proteasome inhibitor conjugate, particle or composition, e.g., a CDP-bortezomib inhibitor conjugate, particle or composition.

In an embodiment, the CDP-microtubule inhibitor conjugate, particle or composition comprises a CDP-taxane conjugate, particle or composition or a CDP-epothilone conjugate, particle or composition. In an embodiment, the CDP-proteasome inhibitor conjugate, particle or composition is a CDP-boronic acid containing molecule conjugate, particle or composition, e.g., a CDP-bortezomib conjugate, particle or composition.

The therapeutic agent can be an immunomodulator conjugate, thus in an embodiment the conjugate, particle or composposition is a CDP-immunomodulator conjugate, particle or composition, e.g., a CDP-corticosteroid conjugate, particle or composition. In an embodiment, the CDP-immunomodulator conjugate, particle or composition is a CDP-kinase inhibitor conjugate, particle or composition (e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition).

In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is not (or is other than) methylprednisolone. In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is a Group B corticosteroid, a Group C corticosteroid, or a Group D corticosteroid. In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone, or prednisone. In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is a Group B corticosteroid, a Group C corticosteroid, a Group D corticosteroid, hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone, or prednisone. In an embodiment, the CDP-

therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is a Group B corticosteroid, a Group C corticosteroid, a Group D corticosteroid, hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone, or prednisone. In an embodiment, the CDP-corticosteroid conjugate, e.g., the CDP-methylprednisolone conjugate, includes a linker attaching the corticosteroid to the CDP, wherein the linker is not a glycine. In one embodiment, the linker is one ore more of: alanine, arginine, histidine, lysine, aspartic acid, glutamic acid, serine, threonine, asparganine, glutamine, cysteine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine and valine. In some embodiments, the linker is a linker described herein. In some embodiments, the linker is not an amino acid (e.g., an alpha amino acid). In some embodiments, the linker is alanine glycolate or amino hexanoate. In some embodiments, the loading of the corticosteroid onto the CDP is at least about 13% by weight of the conjugate (e.g., at least about 14%, 15%, 16%, 17%, 18%, 19%, or 20%). In some embodiments, the loading of the corticosteroid onto the CDP is less than about 12% by weight of the conjugate (e.g., less than about 11%, 10%, 9%, 8%, or 7%).

In an embodiment, the CDP-corticosteroid conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition described herein.

In an embodiment, the autoimmune disease is not (or is other than) rheumatoid arthritis. In an embodiment, the autoimmune disease is not (or is other than) rheumatoid arthritis and the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition.

In an embodiment, the autoimmune disease is rheumatoid arthritis and the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is not (or is other than) methylprednisolone. In an embodiment, the autoimmune disease is rheumatoid arthritis and the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is a Group B corticosteroid, a Group C corticosteroid, or a Group D corticosteroid. In an embodiment, the autoimmune disease is rheumatoid arthritis and the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition

wherein the corticosteroid is hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone, or prednisone. In an embodiment, the autoimmune disease is rheumatoid arthritis and the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is a Group B corticosteroid, a Group C corticosteroid, or a Group D corticosteroid, hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone, or prednisone. In an embodiment, the CDP-corticosteroid conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition described herein.

In an embodiment, the autoimmune disease is rheumatoid arthritis, and the CDP-corticosteroid conjugate, particle or composition is a CDP-methylprednisolone conjugate, particle or composition. In an embodiment, the CDP-methylprednisolone conjugate includes a linker attaching the corticosteroid to the CDP, wherein the linker is not a glycine. In one embodiment, the linker is one ore more of: alanine, arginine, histidine, lysine, aspartic acid, glutamic acid, serine, threonine, asparganine, glutamine, cysteine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine and valine. In some embodiments, the linker is a linker described herein. In some embodiments, the linker is not an amino acid (e.g., an alpha amino acid). In some embodiments, the linker is alanine glycolate or amino hexanoate. In some embodiments, the loading of the methylprednisolone onto the CDP is at least about 13% by weight of the conjugate (e.g., at least about 14%, 15%, 16%, 17%, 18%, 19%, or 20%). In some embodiments, the loading of the methylprednisolone onto the CDP is less than about 12% by weight of the conjugate (e.g., less than about 11%, 10%, 9%, 8%, or 7%).

In an embodiment, the autoimmune disease, e.g., rheumatoid arthritis, and the CDP-therapeutic agent conjugate, particle or composition is administered to the subject in combination with a second therapeutic agent. In an embodiment, e.g., wherein the autoimmune disease is rheumatoid arthritis, the second therapeutic agent is one or more of the following agents: an anti-inflammatory agent, a corticosteroid, a disease modifying antirheumatic drug (DMARD), an immunomodulator, a statin, and/or a bisphosphonate.

In an embodiment, e.g., wherein the autoimmune disease is rheumatoid arthritis, the anti-inflammatory agent is one or more of the following agents: aspirin, acetaminophen, and/or a non-steroidal anti-inflammatory drug.

In an embodiment, e.g., wherein the autoimmune disease is rheumatoid arthritis, the corticosteroid is one of more of the corticosteroids described herein.

In an embodiment, e.g., wherein the autoimmune disease is rheumatoid arthritis, the DMARD is one or more of the following agents; azathioprine, cyclosporine A, D-penicillamine, gold salts, hydroxychloroquine, chloroquine (also called anti-malarial agents herein), leflunomide, methotrexate, minocycline, sulfasalazine, and/or cyclophosphamide.

In an embodiment, e.g., wherein the autoimmune disease is rheumatoid arthritis, the immunomodulator includes one or more of the following agents: TNF inhibitors (e.g. etanercept (Enbrel®), infliximab (Remicade®), adalimumab(Humira®), certolixumab pegol (Cimzia®), and golimumab (Simponi®)), IL-1 inhibitors (e.g. anakinra (Kineret®)), antibodies against B cells (rituxamab (Rituxan®)), T cell comstimulation inhibitors (abatacept (Orencia®)), IL-6 inhibitors (tocilizumab (RoActemra®)), and/or other agents, e.g., biologics, that interfere with immune cell function (e.g., antibodies to other immune system targets, e.g., antibodies to IL-15).

In an embodiment, e.g., wherein the autoimmune disease is rheumatoid arthritis,the statin is one or more of the following agents: atorvastatin (Lipitor®), cerivastatin (Baycol®), fluvastatin (Lescol®), lovastatin (Mevacor®), mevastatin, pitavastatin (Livalo®), pravastatin (Pravachol®), rosuvastatin (Crestor®), and/or simvastatin (Zocor®).

In an embodiment, e.g., wherein the autoimmune disease is rheumatoid arthritis,the bisphosphonate is one or more of the following agents: non-N (nitrogen)-containing bisphosphonates (e.g., etidronate (Didronel®), clodronate (Bonefos®), and tiludronate (Skelid®)) and/or N (nitrogen)-containing bisphosphonates (e.g., pamidronate (Aredia®), neridronate, olpadronate, alendronate (Fosamax®), ibandronate (Boniva®), risedronate (Actonel®), and zoledronate (Zometa®)

In an embodiment, the CDP-therapeutic agent conjugate, particle or composition inhibits rejection of a transplanted organ, e.g., rejection of a kidney transplant, rejection

of a lung transplant, rejection of a liver transplant. In an embodiment, the CDP-therapeutic agent conjugate, particle or composition inhibits rejection of a kidney transplant and the CDP-immunomodulator conjugate, particle or composition is a CDP-rapamycin conjugate, particle or composition or a CDP-rapamycin analog conjugate, particle or composition.

In an embodiment, the autoimmune disease is an immune response to a transplanted organ, and the CDP-therapeutic agent conjugate, particle or composition is administered to the subject in combination with a second therapeutic agent. In an embodiment, the second therapeutic agent is one or more of the following agents: an anti-inflammatory agent, a corticosteroid, a disease modifying antirheumatic drug (DMARD), an immunomodulator, a statin, and/or a bisphosphonate, e.g., an anti-inflammatory agent, a corticosteroid, a disease modifying antirheumatic drug (DMARD), an immunomodulator, a statin, and/or a bisphosphonate disclosed herein. In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-rapamycin conjugate, particle or composition or a CDP-rapamycin analog conjugate, particle or composition, and the CDP-rapamycin conjugate, particle or composition or the CDP-rapamycin analog conjugate, particle or composition is administered to inhibit rejection of a transplanted organ, e.g., rejection of a kidney transplant, in combination with cyclosporine.

In another aspect, the invention features a method of treating lupus e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus, in a subject, e.g., a human subject, comprising administering a CDP-therapeutic agent conjugate, particle or composition to the subject in an amount effective to treat the lupus.

In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-topoisomerase I inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition, e.g., CRLX101 and is administered to the subject at 30 mg/m^2 per month or less. In an embodiment, the CDP-topoisomerase I inhibitor conjugate is administered at 30 mg/m^2 per month or less on a dosing schedule described herein (wherein the dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate).

In one aspect, the invention features, a method of treating an autoimmune disease in a subject, e.g., a human subject. The method comprises:

providing an initial administration of a CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition described herein, e.g., CRLX101, to said subject at a dosage of 3 mg/m^2, 4 mg/m^2, 5 mg/m^2, or 6 mg/m^2 (wherein said dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate),

optionally, providing one or more subsequent administrations of said CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition described herein, e.g., CRLX101, at a dosage of 3 mg/m^2, 4 mg/m^2, 5 mg/m^2, or 6 mg/m^2, wherein each subsequent administration is provided, independently, between 5, 6, 7, 8, 9 days after the previous, e.g., the initial, administration, to thereby treat the autoimmune disease (when a range of individual values for parameter is given herein, the invention also includes a range for the parameter, wherein the upper and lower values for the parameter are selected from the individual values given. E.g., when a range of individual values for a dosage is given herein, the invention also includes a range for the dosage, wherein the upper and lower values for the range are selected from the individual values given. By way of example the individual values of 4 and 6 mg/m^2 given above provide a range of 4 and 6 mg/m^2. Similarly, when a range of individual values for a time period is given herein, the invention also includes a range for the time period, wherein the upper and lower values for the range are selected from the individual values given).

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 5-9, e.g., 7, days after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative, a CDP-camptothecin or camptothecin derivative conjugate, particle or composition described herein, e.g., CRLX101, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes. In one embodiment, the CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition, e.g., the CDP-camptothecin or camptothecin derivative conjugate, particle or composition described herein, e.g. CRLX101, is administered at a dosage of 3 mg/m^2, 4 mg/m^2, 5 mg/m^2, or 6 mg/m^2 by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes or 90 minutes, e.g., a period equal to or less than 30 minutes, 45 minutes or 60 minutes.

In an embodiment, the method includes an initial administration of CRLX101 to said subject at a dosage of 3 mg/m^2, 4 mg/m^2, 5 mg/m^2, or 6 mg/m^2 and

one or more subsequent administrations of CRLX101 to said subject, at a dosage of 3 mg/m^2, 4 mg/m^2, 5 mg/m^2, or 6 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is administered, independently, 5-9, e.g., 7, days after the previous, e.g., the initial, administration, and the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis. In an embodiment, the autoimmune disease is lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus.

In one aspect, the invention features, a method of treating an autoimmune disease, e.g., in a subject, e.g., in a human subject. The method comprises:

providing an initial administration of a CDP-topoisomerase inhibitor I conjugate, particle or composition, e.g., a CDP-camptothecin conjugate, particle or composition or camptothecin derivative conjugate, particle or composition, e.g., a CDP-camptothecin

conjugate, particle or composition or camptothecin derivative conjugate, particle or composition described herein, e.g., CRLX101, to the subject at a dosage of 6 mg/m^2, 7 mg/m^2, 8 mg/m^2, 9 mg/m^2, 10 mg/m^2, 11 mg/m^2, 12 mg/m^2, 13 mg/m^2, 14 mg/m^2, 15 mg/m^2, 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2 or 30 mg/m^2 (wherein said dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate) and

optionally, providing one or more subsequent administrations of said CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin conjugate, particle or composition or camptothecin derivative conjugate, particle or composition, e.g., a CDP-camptothecin conjugate, particle or composition or camptothecin derivative conjugate, particle or composition described herein, e.g., CRLX101, at a dosage of 6 mg/m^2, 7 mg/m^2, 8 mg/m^2, 9 mg/m^2, 10 mg/m^2, 11 mg/m^2, 12 mg/m^2, 13 mg/m^2, 14 mg/m^2, 15 mg/m^2, 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2 or 30 mg/m^2, wherein each subsequent administration is provided, independently, between 9, 10, 11, 12, 13, 14, 15 or 16 days after the previous, e.g., the initial, administration, to thereby treat the autoimmune disease.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15 or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 12-16, e.g., 14, days after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-topoisomerase I inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative, a CDP-camptothecin or camptothecin derivative conjugate, particle or composition described herein, e.g., CRLX101, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or

180 minutes. In one embodiment, the CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition, e.g., the CDP-camptothecin or camptothecin derivative conjugate, particle or composition described herein, e.g. CRLX101, is administered at a dosage of 9 mg/m^2, 10 mg/m^2, 11 mg/m^2, 12 mg/m^2, 13 mg/m^2, 14 mg/m^2, 15 mg/m^2, 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2 or 30 mg/m^2 by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes or 90 minutes, e.g., a period equal to or less than 30 minutes, 45 minutes or 60 minutes.

In an embodiment, the method includes an initial administration of CRLX101 to said subject at a dosage of 12 mg/m^2, 13 mg/m^2, 14 mg/m^2 or 15 mg/m^2, and one or more subsequent administrations of CRLX101 to said subject, at a dosage of 12 mg/m^2, 13 mg/m^2, 14 mg/m^2 or 15 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is administered, independently, 12-16, e.g., 14, days after the previous, e.g., the initial, administration, and the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis. In an embodiment, the autoimmune disease is lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus.

In an embodiment, the method includes an initial administration of CRLX101 to said subject at a dosage of 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2 or 30 mg/m^2, and one or more subsequent administrations of CRLX101 to said subject, at a dosage of 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2 or 30 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is administered, independently, 12-16, e.g., 14, days after the previous, e.g., the initial, administration, and the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus,

drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis. In an embodiment, the autoimmune disease is lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus.

In one aspect, the invention features, a method of treating an autoimmune disease, in a subject, e.g., a human subject. The method comprises:

providing an initial administration of a CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition described herein, e.g., CRLX101, to said subject at a dosage of 9 mg/m^2, 10 mg/m^2, 11 mg/m^2,12 mg/m^2, 13 mg/m^2, 14 mg/m^2, 15 mg/m^2, 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2, 30 mg/m^2, 31 mg/m^2, 32 mg/m^2, 33 mg/m^2, 34 mg/m^2, 35 mg/m^2 or 36 mg/m^2 (wherein said dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate) and

optionally, providing one or more subsequent administrations of said CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition described herein, e.g., CRLX101, at a dosage of 9 mg/m^2, 10 mg/m^2, 11 mg/m^2,12 mg/m^2, 13 mg/m^2, 14 mg/m^2, 15 mg/m^2, 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2, 30 mg/m^2, 31 mg/m^2, 32 mg/m^2, 33 mg/m^2, 34 mg/m^2, 35 mg/m^2 or 36 mg/m^2, wherein each subsequent administration is provided, independently, between 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days after the previous, e.g., the initial, administration, to thereby treat the autoimmune disease.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15 or 20 administrations are the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 19-23, e.g., 21, or 25-29, e.g., 27 or 28 days after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative, a CDP-camptothecin or camptothecin derivative conjugate, particle or composition described herein, e.g., CRLX101, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes. In one embodiment, the CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-camptothecin or camptothecin derivative conjugate, particle or composition, e.g., the CDP-camptothecin or camptothecin derivative conjugate, particle or composition described herein, e.g. CRLX101, is administered at a dosage of 9 mg/m^2, 10 mg/m^2, 11 mg/m^2,12 mg/m^2, 13 mg/m^2, 14 mg/m^2, 15 mg/m^2, 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2, 30 mg/m^2, 31 mg/m^2, 32 mg/m^2, 33 mg/m^2, 34 mg/m^2, 35 mg/m^2 or 36 mg/m^2 by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes or 90 minutes, e.g., a period equal to or less than 30 minutes, 45 minutes or 60 minutes.

In an embodiment, the method includes an initial administration of CRLX101 to said subject at a dosage of 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2, 30 mg/m^2, 31 mg/m^2, 32 mg/m^2, 33 mg/m^2, 34 mg/m^2, 35 mg/m^2 or 36 mg/m^2, and one or more subsequent administrations of CRLX101 to said subject, at a dosage of 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2, 30 mg/m^2, 31 mg/m^2, 32 mg/m^2, 33 mg/m^2, 34 mg/m^2, 35 mg/m^2 or 36 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is administered, independently, 19-22, e.g., 21, days after the previous, e.g., the initial, administration, and the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g.,

Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis. In an embodiment, the autoimmune disease is lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus.

In one aspect, the invention features a method of treating lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus in a subject, e.g., a human subject. The method comprises: administering a CDP-therapeutic agent conjugate, particle or composition to the subject in combination with a second therapeutic agent. In one embodiment, the second therapeutic agent is one or more of the following agents: an anti-inflammatory agent, an anti-malarial agent, an immunomodulator, an anti-coagulant, and a hormone.

In one aspect, the invention features, a method of treating an autoimmune disease in a subject, e.g., a human subject. The method comprises:

providing an initial administration of CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein, or, e.g., a CDP-floxuridine conjugate, particle or composition, e.g., a CDP-floxuridine conjugate, particle or composition, described herein, or, e.g., a CDP-raltitrexed conjugate, particle or composition, e.g., a CDP-raltitrexed conjugate, particle or composition, described herein, to said subject, and, optionally, administering one or more subsequent administrations of said CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein, or, e.g., a CDP-floxuridine conjugate, particle or composition, e.g., a CDP-floxuridine conjugate, particle or composition, described herein, or, e.g., a CDP-raltitrexed conjugate, particle or composition, e.g., a CDP-raltitrexed conjugate, particle or composition, described herein, is administered, wherein each subsequent administration is provided, independently, between 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27,

28 days after the previous, e.g., the initial, administration, to thereby treat the autoimmune disease.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 18-24, e.g., 21, days after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein, or, e.g., a CDP-floxuridine conjugate, particle or composition, e.g., a CDP-floxuridine conjugate, particle or composition, described herein, or, e.g., a CDP-raltitrexed conjugate, particle or composition, e.g., a CDP-raltitrexed conjugate, particle or composition, described herein, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes.

In an embodiment, the method includes an initial administration of a CDP-pemetrexed conjugate, particle or composition to said subject at a dosage of 300 mg/m^2, 320 mg/m^2, 350 mg/m^2, 380 mg/m^2, 400 mg/m^2, 420 mg/m^2, 450 mg/m^2, 480 mg/m^2, 500 mg/m^2, 520 mg/m^2, 550 mg/m^2, 580 mg/m^2, 600 mg/m^2, 620 mg/m^2, 650 mg/m^2, 680 mg/m^2, 700 mg/m^2, 720 mg/m^2, or 750 mg/m^2, (wherein the dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate), and one or more subsequent administrations of a CDP-pemetrexed conjugate, particle or composition to said subject, at a dosage of 300 mg/m^2, 320 mg/m^2, 350 mg/m^2, 380 mg/m^2, 400 mg/m^2, 420 mg/m^2, 450 mg/m^2, 480 mg/m^2, 500 mg/m^2, 520 mg/m^2, 550 mg/m^2, 580 mg/m^2, 600 mg/m^2, 620 mg/m^2, 650 mg/m^2, 680 mg/m^2, 700 mg/m^2, 720 mg/m^2, or 750 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is administered, independently, 18-24, e.g., 21 days after the previous, e.g., the initial, administration. In

one embodiment, the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis. In an embodiment, the autoimmune disease is lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus.

In one aspect, the invention features, a method of treating an autoimmune disease in a subject, e.g., a human subject. The method comprises:

providing an initial administration of CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, described herein, or, e.g., a CDP-cytarabine conjugate, particle or composition, e.g., a CDP-cytarabine conjugate, particle or composition, described herein, or, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein, or, e.g., a CDP-5FU conjugate, particle or composition, e.g., a CDP-5FU conjugate, particle or composition, described herein, to said subject, and, optionally, providing one or more subsequent administrations of said CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, described herein, or, e.g., a CDP-cytarabine conjugate, particle or composition, e.g., a CDP-cytarabine conjugate, particle or composition, described herein, or, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein, or, e.g., a CDP-5FU conjugate, particle or composition, e.g., a CDP-5FU conjugate, particle or composition, described herein, wherein each subsequent administration is provided, independently, between 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 days after the previous, e.g., the initial, administration, to thereby treat the autoimmune disease.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 5-14 days, e.g., 7 days after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, described herein, or, e.g., a CDP-cytarabine conjugate, particle or composition, e.g., a CDP-cytarabine conjugate, particle or composition, described herein, or, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein, or, e.g., a CDP-5FU conjugate, particle or composition, e.g., a CDP-5FU conjugate, particle or composition, described herein, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes.

In an embodiment, the method includes an initial administration of a CDP-gemcitabine conjugate, particle or composition at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1330 mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2 (wherein the dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate), and, optionally, one or more subsequent administrations of a CDP-gemcitabine conjugate, particle or composition at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1330

mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is provided, independently, between 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 days after the previous, e.g., the initial, administration. In one embodiment, the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis. In an embodiment, the autoimmune disease is lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus.

In an embodiment, the method includes an initial administration of a CDP-5FU conjugate, particle or composition at a dosage of 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg, or 20 mg/kg (wherein the dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate), and, optionally, one or more subsequent administrations of a CDP-5FU conjugate, particle or composition at a dosage of 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg, or 20 mg/kg, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, day(s) after the previous, e.g., the initial, administration, and the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis. In an embodiment, the autoimmune disease is lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus. In an embodiments,

the CDP-5FU conjugate, particle or composition is administered intravenously once daily for 4 successive days.

In one aspect, the invention features, a method of treating an autoimmune disease in a subject, e.g., a human subject. The method comprises:

providing an initial administration of a CDP-anti-tumor antibiotic conjugate, particle or composition, e.g., a CDP-HSP90 inhibitor conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition described herein, to said subject, and, optionally, providing one or more subsequent administrations of said CDP-anti-tumor antibiotic conjugate, particle or composition, e.g., a CDP-HSP90 inhibitor conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition described herein, wherein each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 days after the previous, e.g., the initial, administration, to thereby treat the autoimmune disease.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 1-15, e.g., 3 or 7, days after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-anti-tumor antibiotic conjugate, particle or composition, e.g., a CDP-HSP90 inhibitor conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition described herein, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes.

In an embodiment, the method includes an initial administration of a CDP-geldanamycin conjugate, particle or composition at a dosage of 20 mg/m^2, 30 mg/m^2, 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 75 mg/m^2, 80 mg/m^2, 85 mg/m^2, 90 mg/m^2, 95 mg/m^2, 100 mg/m^2, 105 mg/m^2, 110 mg/m^2, 115 mg/m^2, 120 mg/m^2, 125 mg/m^2, 130 mg/m^2, 140 mg/m^2, 150 mg/m^2, 160 mg/m^2, or 170 mg/m^2 (wherein the dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate), and, optionally, one or more subsequent administrations of a CDP-geldanamycin conjugate, particle or composition at a dosage of 20 mg/m^2, 30 mg/m^2, 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 75 mg/m^2, 80 mg/m^2, 85 mg/m^2, 90 mg/m^2, 95 mg/m^2, 100 mg/m^2, 105 mg/m^2, 110 mg/m^2, 115 mg/m^2, 120 mg/m^2, 125 mg/m^2, 130 mg/m^2, 140 mg/m^2, 150 mg/m^2, 160 mg/m^2, or 170 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 days after the previous, e.g., the initial, administration. In one embodiment, the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis. In an embodiment, the autoimmune disease is lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus.

In one aspect, the invention features, a method of treating an autoimmune disease in a subject, e.g., a human subject. The method comprises:

providing an initial administration of CDP-platinum based agent conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, described herein, or, e.g., a CDP-carboplatin conjugate, particle or composition, e.g., a CDP-carboplatin conjugate, particle or composition, described herein, or, e.g., a CDP-oxaliplatin conjugate, particle or composition, e.g., a CDP-oxaliplatin conjugate, particle or composition, described herein, and, optionally, providing one or more subsequent administrations of said CDP-platinum based agent conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, described

herein, or, e.g., a CDP-carboplatin conjugate, particle or composition, e.g., a CDP-carboplatin conjugate, particle or composition, described herein, or, e.g., a CDP-oxaliplatin conjugate, particle or composition, e.g., a CDP-oxaliplatin conjugate, particle or composition, described herein wherein each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 39, 30, 31 day(s) after the previous, e.g., the initial, administration, to thereby treat the autoimmune disease.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 17-31 days, e.g., 21 or 28, days after the previous administration. In an embodiment, each subsequent administration is administered 1-5 days, e.g., 1, 3 day(s) after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-platinum based agent conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, described herein, or, e.g., a CDP-carboplatin conjugate, particle or composition, e.g., a CDP-carboplatin conjugate, particle or composition, described herein, or, e.g., a CDP-oxaliplatin conjugate, particle or composition, e.g., a CDP-oxaliplatin conjugate, particle or composition, described herein, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes.

In an embodiment, the method includes an initial administration of a CDP-cisplatin conjugate, particle or composition at a dosage of 10 mg/m^2, 15 mg/m^2, 20 mg/m^2, 25 mg/m^2, 30 mg/m^2, 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 75 mg/m^2, 80 mg/m^2, 85 mg/m^2, 90 mg/m^2, 95 mg/m^2, 100 mg/m^2, 105 mg/m^2, 110 mg/m^2, 115 mg/m^2, 120 mg/m^2, 125 mg/m^2, 130 mg/m^2, 140 mg/m^2, 150 mg/m^2, 160 mg/m^2, or 170 mg/m^2 (wherein the dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate), and, optionally, one or more subsequent administrations of a CDP-cisplatin

conjugate, particle or composition at a dosage of 10 mg/m^2, 15 mg/m^2, 20 mg/m^2, 25 mg/m^2, 30 mg/m^2, 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 75 mg/m^2, 80 mg/m^2, 85 mg/m^2, 90 mg/m^2, 95 mg/m^2, 100 mg/m^2, 105 mg/m^2, 110 mg/m^2, 115 mg/m^2, 120 mg/m^2, 125 mg/m^2, 130 mg/m^2, 140 mg/m^2, 150 mg/m^2, 160 mg/m^2, or 170 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 day(s) after the previous, e.g., the initial, administration. In one embodiment, the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis. In an embodiment, the autoimmune disease is lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus.

In one aspect, the invention features, a method of treating an autoimmune disease in a subject, e.g., a human subject. The method comprises:

providing an initial administration of CDP-kinase inhibitor conjugate, particle or composition, e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, described herein, and, optionally, providing one or more subsequent administrations of said CDP-kinase inhibitor conjugate, particle or composition, e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, described herein, to said subject wherein each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21day(s) after the previous, e.g., the initial, administration, to thereby treat the autoimmune disease.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 1-21 days, e.g., 1, 2, 3, 4 or 5, days after the previous administration. In an embodiment, each subsequent administration is administered 1-5 days, e.g., 1, 3 day(s) after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-kinase inhibitor agent conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, described herein is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes.

In an embodiment, the method includes an initial administration of a CDP-rapamycin conjugate, particle or composition at a dosage of 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 12 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, or 50 mg (wherein the dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate), and, optionally, one or more subsequent administrations of a CDP-rapamycin conjugate, particle or composition at a dosage of 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 12 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, or 50 mg, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21day(s) after the previous, e.g., the initial, administration. In one embodiment, the autoimmune disease is arthritis, e.g., rheumatoid arthritis, osteoarthritis, gout; lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus; inflammatory bowel disease, e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, Behcet's syndrome, infective colitis, indeterminate colitis; psoriasis; or multiple sclerosis. In an embodiment, the autoimmune disease is lupus, e.g., systemic lupus erythematosus, discoid lupus, drug-induced lupus, neonatal lupus.

In an embodiment of the aspects provided above, the CDP-therapeutic agent conjugate, particle or composition (e.g., the CDP-cytotoxic agent conjugate, particle or composition) is administered in combination with an anti-inflammatory agent which is one or more of the following agents: aspirin, acetaminophen, a non-steroidal anti-inflammatory drug, and/or a corticosteroid.

In an embodiment of the aspects provided above, the CDP-therapeutic agent conjugate, particle or composition (e.g., the CDP-cytotoxic agent conjugate, particle or composition) is administered in combination with an anti-malarial agent which is one or more of the following agents: hydroxychloroquine and/or chloroquine.

In an embodiment of the aspects provided above, the CDP-therapeutic agent conjugate, particle or composition (e.g., the CDP-cytotoxic agent conjugate, particle or composition) is administered in combination with an immunomodulator which is one or more of the following agents: an immunomodulator with an intracellular target (e.g., a macrolide), an immunomodulator with a cellular receptor target, an immunomodulator with a serum target, and/or other agents that interfere with immune cell function (e.g., thalidomide, mycophenolate mofetil, tacrolimus, pimecrolimus, cyclosporine (e.g., cyclosporine A), rapamycin and rapamycin analogs-some of these agents may also belong to another class of agents described herein).

In an embodiment of the aspects provided above, the CDP-therapeutic agent conjugate, particle or composition (e.g., the CDP-cytotoxic agent conjugate, particle or composition) is administered in combination with an immunomodulator wherein an intracellular target is an anti-metabolite (e.g., an alkylating agent (e.g., cyclophosphamide (e.g., Cytoxan®), a purine synthesis inhibitor (e.g., azathioprine (Imuran®), a pyrimidine synthesis inhibitor (e.g., leflunomide (Arava®), an antifolate (e.g., methotrexate), an IL-2 inhibitor, an mTOR inhibitor, a TNF inhibitor, or a macrolide.

In an embodiment of the aspects provided above, the CDP-therapeutic agent conjugate, particle or composition (e.g., the CDP-cytotoxic agent conjugate, particle or composition) is administered in combination with an immunomodulator wherein the receptor target is an IL-1 receptor inhibitor or an antibody which inhibits the function of the cellular receptor target. Examples of antibodies which inhibit the function of a cellular receptor target include an anti-CD3 antibody, an anti-CD4 antibody, an anti-CD5

antibody, an anti-CD11a antibody, anti-BLyS antibody, an anti-CD20 antibody, an anti-CD22 antibody, an anti-CD23 antibody, an anti-CD40 antibody, an anti-CD62L antibody, an anti-CD80 antibody, an anti-CD147 antibody, an anti-CD154 antibody, an anti-CAT antibody, an anti-integrin antibody, an CTLA4 antibody, an anti-IL6 receptor antibody, an anti-LFA1 antibody, an anti-IL2 antibody, and an anti-human T cell antibody.

In an embodiment of the aspects provided above, the CDP-therapeutic agent conjugate, particle or composition (e.g., the CDP-cytotoxic agent conjugate, particle or composition) is administered in combination with an immunomodulator wherein the serum target is an antibody which inhibits the function of the serum target. Examples of antibodies which inhibit the function of a serum target include an anti-BLyS antibody, an anti-IL5 antibody, anti-IL6 antibody, and anti-interferon alpha antibody, an anti-IgE antibody, an anti-C5a antibody, an anti-TNF antibody, anti-IL10 antibody, anti-IL12 antibody, and an anti-IL13 antibody. Other immunomodulators can be soluble forms of the cellular receptor targets described herein. A preferred antibody which inhibits the function of a serum target is an anti-BLyS antibody, e.g., belimumab (Benlysta™).

In an embodiment of the aspects provided above, the CDP-therapeutic agent conjugate, particle or composition (e.g., the CDP-cytotoxic agent conjugate, particle or composition) is administered in combination with an anti-coagulant which is one or more of the following agents: aspirin, heparin, and/or warfarin.

In an embodiment of the aspects provided above, the CDP-therapeutic agent conjugate, particle or composition (e.g., the CDP-cytotoxic agent conjugate, particle or composition) is administered in combination with a hormone which selected from the group consisting of an androgen and/or a gonadotropin-hormone releasing agonist.

In one aspect, the invention features, a method of treating an autoimmune disease, e.g., in a subject. The method comprises administering two or more CDP-therapeutic agent conjugates, wherein one CDP is conjugated to a therapeutic agent and the other CDP is conjugated to a second therapeutic agent, or a composition or particle including one or more of the CDP-therapeutic agent conjugates, to the subject to thereby treat the disease.

In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-cytotoxic agent conjugate, particle or composition, e.g., CDP-topoisomerase

inhibitor conjugate, particle or composition, e.g., a CDP-topoisomerase inhibitor I conjugate, particle or composition (e.g., a CDP-camptothecin conjugate, particle or composition, CDP-irinotecan conjugate, particle or composition, CDP-SN-38 conjugate, particle or composition, CDP-topotecan conjugate, particle or composition, CDP-lamellarin D conjugate, particle or composition, a CDP-lurotecan conjugate, particle or composition, a CDP-exatecan conjugate, particle or composition, a CDP-diflomotecan conjugate, particle or composition and CDP-topoisomerase I inhibitor conjugates, particles and compositions which include derivatives of camptothecin, irinotecan, SN-38, lamellarin D, lurotecan, exatecan and diflomotecan), a CDP-topoisomerase II inhibitor conjugate, particle or composition (e.g., a CDP-etoposide conjugate, particle, or composition, CDP-tenoposide conjugate, particle or composition, CDP-amsacrine conjugate, particle or composition and CDP-topoisomerase II inhibitor conjugates, particles and compositions which include derivatives of etoposide, tenoposide, and amsacrine), a CDP-anti-metabolic agent conjugate, particle or composition (e.g., a CDP-antifolate conjugate, particle or composition (e.g., a CDP-pemetrexed conjugate, particle or composition, a CDP-floxuridine conjugate, particle or composition, a CDP-raltitrexed conjugate, particle or composition)) or a CDP-pyrimidine analog conjugate, particle or composition (e.g., a CDP-capecitabine conjugate, particle or composition, a CDP-cytarabine conjugate, particle or composition, a CDP-gemcitabine conjugate, particle or composition, a CDP-5FU conjugate, particle or composition)), a CDP-alkylating agent conjugate, particle or composition, a CDP-anthracycline conjugate, particle or composition, a CDP-anti-tumor antibiotic conjugate, particle or composition (e.g., a CDP-HSP90 inhibitor conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition, a CDP-tanespimycin conjugate, particle or composition or a CDP-alvespimycin conjugate, particle or composition), a CDP-platinum based agent conjugate, particle or composition (e.g., a CDP-cisplatin conjugate, particle or composition, a CDP-carboplatin conjugate, particle or composition, a CDP-oxaliplatin conjugate, particle or composition), a CDP-microtubule inhibitor conjugate, particle or composition, a CDP-kinase inhibitor conjugate, particle or composition (e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate,

particle or composition) or a CDP-proteasome inhibitor conjugate, particle or composition.

In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-immunomodulator conjugate, particle or composition, e.g., a corticosteroid or a rapamycin analog conjugate, particle or composition.

In another aspect, the invention features, a unit dosage of a CDP-therapeutic agent conjugate described herein, and particles and compositions containing a CDP-therapeutic agent conjugate described herein.

In one aspect, the disclosure features a CDP-therapeutic agent conjugate, particle or composition, e.g., a CDP-therapeutic agent conjugate, particle or composition described herein.

In one embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-cytotoxic agent conjugate, particle or composition, e.g.:

a CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-topoisomerase inhibitor I conjugate, particle or composition (e.g., a CDP-camptothecin conjugate, particle or composition, CDP-irinotecan conjugate, particle or composition, CDP-SN-38 conjugate, particle or composition, CDP-topotecan conjugate, particle or composition, CDP-lamellarin D conjugate, particle or composition, a CDP-lurotecan conjugate, particle or composition, a CDP-exatecan conjugate, particle or composition, a CDP-diflomotecan conjugate, particle or composition, and CDP-topoisomerase I inhibitor conjugates, particles and compositions which include derivatives of camptothecin, irinotecan, SN-38, lamellarin D, lurotecan, exatecan, and diflomotecan);

a CDP-topoisomerase II inhibitor conjugate, particle or composition (e.g., a CDP-etoposide conjugate, particle, or composition, CDP-tenoposide conjugate, particle or composition, CDP-amsacrine conjugate, particle or composition and CDP-topoisomerase II inhibitor conjugates, particles and compositions which include derivatives of etoposide, tenoposide, and amsacrine);

a CDP-anti-metabolic agent conjugate, particle or composition (e.g., a CDP-antifolate conjugate, particle or composition (e.g., a CDP-pemetrexed conjugate, particle or composition, a CDP-floxuridine conjugate, particle or composition, a CDP-raltitrexed conjugate, particle or composition) or a CDP-pyrimidine analog conjugate, particle or

composition (e.g., a CDP-capecitabine conjugate, particle or composition, a CDP-cytarabine conjugate, particle or composition, a CDP-gemcitabine conjugate, particle or composition, a CDP-5FU conjugate, particle or composition));

a CDP-alkylating agent conjugate, particle or composition, a CDP-anthracycline conjugate, particle or composition;

a CDP-anti-tumor antibiotic conjugate, particle or composition (e.g., a CDP-HSP90 inhibitor conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition, a CDP-tanespimycin conjugate, particle or composition or a CDP-alvespimycin conjugate, particle or composition);

a CDP-platinum based agent conjugate, particle or composition (e.g., a CDP-cisplatin conjugate, particle or composition, a CDP-carboplatin conjugate, particle or composition, a CDP-oxaliplatin conjugate, particle or composition);

a CDP-microtubule inhibitor conjugate, particle or composition;

a CDP-kinase inhibitor conjugate, particle or composition (e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition);

or a CDP-proteasome inhibitor, e.g., bortezomib, conjugate, particle or composition.

In one embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-immunomodulator conjugate, particle or composition; e.g.,

a CDP-corticosteroid conjugate, particle or composition; or

a CDP-kinase inhibitor conjugate, particle or composition (e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition).

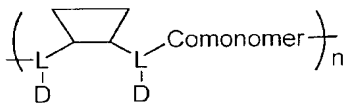
In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is not (or is other than) methylprednisolone. In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is a Group B corticosteroid, a Group C

corticosteroid, or a Group D corticosteroid. In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone, or prednisone. In an embodiment, the CDP-therapeutic agent conjugate, particle or composition is a CDP-corticosteroid conjugate, particle or composition wherein the corticosteroid is a Group B corticosteroid, a Group C corticosteroid, a Group D corticosteroid, hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone, or prednisone. In an embodiment, the CDP-corticosteroid conjugate, e.g., the CDP-methylprednisolone conjugate, includes a linker attaching the corticosteroid to the CDP, wherein the linker is not a glycine. In one embodiment, the linker is one ore more of: alanine, arginine, histidine, lysine, aspartic acid, glutamic acid, serine, threonine, asparganine, glutamine, cysteine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine and valine. In some embodiments, the linker is a linker described herein. In some embodiments, the linker is not an amino acid (e.g., an alpha amino acid). In some embodiments, the linker is alanine glycolate or amino hexanoate. In some embodiments, the loading of the corticosteroid onto the CDP is at least about 13% by weight of the conjugate (e.g., at least about 14%, 15%, 16%, 17%, 18%, 19%, or 20%). In some embodiments, the loading of the corticosteroid onto the CDP is less than about 12% by weight of the conjugate (e.g., less than about 11%, 10%, 9%, 8%, or 7%).

Also included are methods of making the CDP-therapeutic agent conjugates, particles and compositions described herein, e.g., a CDP-cytotoxic agent conjugate, particle or composition, e.g., CDP-topoisomerase inhibitor conjugate, particle or composition, e.g., a CDP-topoisomerase inhibitor I conjugate, particle or composition (e.g., a CDP-camptothecin conjugate, particle or composition, CDP-irinotecan conjugate, particle or composition, CDP-SN-38 conjugate, particle or composition, CDP-topotecan conjugate, particle or composition, CDP-lamellarin D conjugate, particle or composition, a CDP-lurotecan conjugate, particle or composition, a CDP-exatecan conjugate, particle or composition, a CDP-diflomotecan conjugate, particle or composition, and CDP-topoisomerase I inhibitor conjugates, particles and compositions which include

derivatives of camptothecin, irinotecan, SN-38, lamellarin D, lurotecan, exatecan, and diflomotecan), a CDP-topoisomerase II inhibitor conjugate, particle or composition (e.g., a CDP-etoposide conjugate, particle, or composition, CDP-tenoposide conjugate, particle or composition, CDP-amsacrine conjugate, particle or composition and CDP-topoisomerase II inhibitor conjugates, particles and compositions which include derivatives of etoposide, tenoposide, and amsacrine), a CDP-anti-metabolic agent conjugate, particle or composition (e.g., a CDP-antifolate conjugate, particle or composition (e.g., a CDP-pemetrexed conjugate, particle or composition, a CDP-floxuridine conjugate, particle or composition, a CDP-raltitrexed conjugate, particle or composition) or a CDP-pyrimidine analog conjugate, particle or composition (e.g., a CDP-capecitabine conjugate, particle or composition, a CDP-cytarabine conjugate, particle or composition, a CDP-gemcitabine conjugate, particle or composition, a CDP-5FU conjugate, particle or composition)), a CDP-alkylating agent conjugate, particle or composition, a CDP-anthracycline conjugate, particle or composition, a CDP-anti-tumor antibiotic conjugate, particle or composition (e.g., a CDP-HSP90 inhibitor conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition, a CDP-tanespimycin conjugate, particle or composition or a CDP-alvespimycin conjugate, particle or composition), a CDP-platinum based agent conjugate, particle or composition (e.g., a CDP-cisplatin conjugate, particle or composition, a CDP-carboplatin conjugate, particle or composition, a CDP-oxaliplatin conjugate, particle or composition), a CDP-microtubule inhibitor conjugate, particle or composition, a CDP-kinase inhibitor conjugate, particle or composition (e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition) or a CDP-proteasome inhibitor conjugate, particle or composition.

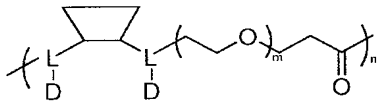
In one embodiment, the CDP-therapeutic agent conjugate has the following formula:



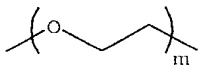
wherein each L is independently a linker, and each D is independently a therapeutic agent, a prodrug derivative thereof, or absent; and each comonomer is independently a comonomer described herein, and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, provided that the polymer comprises at least one therapeutic agent and in some embodiments, at least two therapeutic agents. In some embodiments, the molecular weight of the comonomer is from about 2000 to about 5000 Da (e.g., from about 3000 to about 4000 Da (e.g., about 3400 Da).

In some embodiments, the therapeutic agent is a therapeutic agent described herein (e.g., a cytotoxic agent or an immunomodulator). The therapeutic agent can be attached to the CDP via a functional group such as a hydroxyl group, or where appropriate, an amino group. In some embodiments, one or more of the therapeutic agent in the CDP-therapeutic agent conjugate can be replaced with another therapeutic agent, e.g., another cytotoxic agent or immunomodulator.

In some embodiments, the CDP-therapeutic agent conjugate has the following formula:



wherein each L is independently a linker, and each D is independently a therapeutic agent, a prodrug derivative thereof, or absent, provided that the polymer comprises at least one therapeutic agent and in some embodiments, at least two therapeutic agent moieties; and

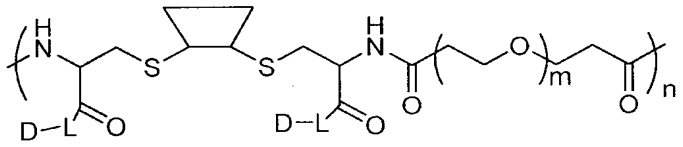
wherein the group 

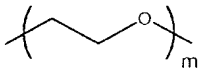
has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20.

In some embodiments, the therapeutic agent is a therapeutic agent described herein (e.g., a cytotoxic agent or an immunomodulator). The therapeutic agent can be attached to the CDP via a functional group such as a hydroxyl group, or where appropriate, an amino group. In some embodiments, one or more of the therapeutic agent in the CDP-therapeutic agent conjugate can be replaced with another therapeutic agent, e.g., another cytotoxic agent or immunomodulator.

In some embodiments, less than all of the L moieties are attached to D moieties, meaning in some embodiments, at least one D is absent. In some embodiments, the loading of the D moieties on the CDP-therapeutic agent conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%). In some embodiments, each L independently comprises an amino acid or a derivative thereof. In some embodiments, each L independently comprises a plurality of amino acids or derivatives thereof. In some embodiments, each L is independently a dipeptide or derivative thereof. In one embodiment, L is one or more of: alanine, arginine, histidine, lysine, aspartic acid, glutamic acid, serine, threonine, asparganine, glutamine, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine and valine.

In one embodiment, the CDP-therapeutic agent conjugate (e.g., the CDP-cytotoxic agent conjugate) has the following formula:



wherein each L is independently a linker or absent and each D is independently a therapeutic agent (e.g., a cytotoxic agent, immunomodulator, a prodrug thereof) or absent, and wherein the group  has a Mw of 5,000 Da or less (e.g., 3,400 Da) and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, provided that the polymer comprises at least one therapeutic agent (e.g., at least one cytotoxic agent immunomodulator, a prodrug thereof). In one embodiment, the cytotoxic agent is a cytotoxic agent described herein. In one embodiment, the immunomodulator is an immunomodulator described herein.

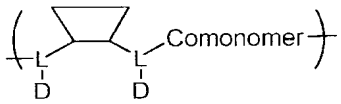
In one embodiment, the CDP is not biodegradable. In one embodiment, the CDP is biodegradable. In one embodiment, the CDP is biocompatible. In one embodiment, the conjugate includes a combination of one or more therapeutic agents.

In one embodiment, each L of the CDP-therapeutic agent conjugate (e.g., the CDP-cytotoxic agent conjugate) is independently an amino acid derivative. In one embodiment, at least a portion of the CDP is covalently attached to the therapeutic agent

(e.g., the cytotoxic agent) through a cysteine moiety. In one embodiment, the linker comprises a moiety formed using "click chemistry" (e.g., as described in WO 2006/115547). In one embodiment, the linker comprises an amide bond, an ester bond, a disulfide bond, or a triazole. In one embodiment, the linker comprises a bond that is cleavable under physiological conditions. In one embodiment, the linker is hydrolysable under physiologic conditions or the linker is enzymatically cleavable under physiological conditions (e.g., the linker comprises a disulfide bond which can be reduced under physiological conditions). In one embodiment, the linker is not cleavable under physiological conditions. In one embodiment, at least a portion of the CDP is covalently attached to the therapeutic agent (e.g., the cytotoxic agent or immunomodulator) through a carboxy terminal of the therapeutic agent.

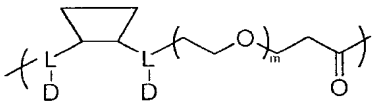
In one embodiment, the therapeutic agents (e.g., the cytotoxic agents or immunomodulators) are from about 1 to about 100 weight % of the conjugate, e.g., from 1 to about 80 weight % of the conjugate, e.g., from 1 to about 70 weight % of the conjugate, e.g., from 1 to about 60 weight % of the conjugate, e.g., from 1 to about 50 weight % of the conjugate, e.g., from 1 to about 40 weight % of the conjugate, e.g., from 1 to about 30 weight % of the conjugate, e.g., from 1 to about 20 weight % of the conjugate, e.g., from 1 to about 10 weight % of the conjugate.

In one embodiment, the CDP-therapeutic agent conjugate (e.g., the CDP-cytotoxic agent conjugate or immunomodulator) comprises a subunit of the following formula:

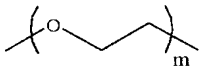


wherein each L is independently a linker, and each D is independently a therapeutic agent, a prodrug derivative thereof, or absent; and each comonomer is independently a comonomer described herein provided that the subunit comprises at least one therapeutic agent.

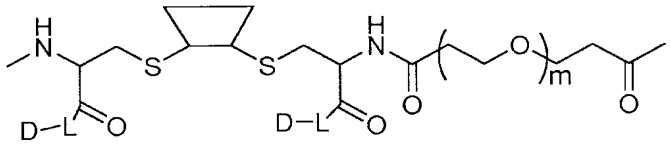
In one embodiment, the CDP-therapeutic agent conjugate (e.g., the CDP-cytotoxic agent conjugate or immunomodulator) comprises a subunit of the following formula:

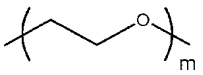


wherein each L is independently a linker, and each D is independently a therapeutic agent, a prodrug derivative thereof, or absent, provided that the subunit comprises at least one therapeutic agent; and

wherein the group  has a Mw of 3400 Da or less.

In one embodiment, the CDP-therapeutic agent conjugate (e.g., the CDP-cytotoxic agent conjugate or immunomodulator) comprises a subunit of the following formula:



wherein each L is independently a linker and each D is independently a therapeutic agent (e.g., the cytotoxic agent or a prodrug thereof) and wherein the group  has a Mw of 5,000 Da or less (e.g., 3,400 Da) , provided that the subunit comprises at least one therapeutic agent. In one embodiment, the cytotoxic agent is a cytotoxic agent described herein. In one embodiment, the immunomodulator is an immunomodulator described herein.

In one embodiment, the CDP is not biodegradable. In one embodiment, the CDP is biodegradable. In one embodiment, the CDP is biocompatible.

In one embodiment, the CDP-therapeutic agent conjugate, e.g., the CDP-cytotoxic agent conjugate or the CDP-immunomodulator conjugate, e.g., a CDP-cytotoxic agent conjugate or CDP-immunomodulator conjugate described herein, forms an inclusion complex between a therapeutic agent attached or conjugated to the CDP, e.g., via a covalent linkage, and another moiety in the CDP (e.g., a cyclodextrin in the CDP) or a moiety (e.g., a cyclodextrin) in another CDP-therapeutic agent conjugate. In one embodiment, the CDP-therapeutic agent conjugate forms a nanoparticle. A plurality of CDP-therapeutic agent conjugates can form a particle (e.g., where the particle is self-

assembled), e.g., through the formation of intramolecular or intermolecular inclusion complexes. In some embodiments, a particle described herein is a nanoparticle. A particle (e.g., a nanoparticle) described herein can include a plurality of CDP-therapeutic agent conjugates (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, or 10). The nanoparticle can range in size from 10 to 300 nm in diameter, e.g., 15 to 280, 30 to 250, 30 to 200, 20 to 150, 30 to 100, 20 to 80, 30 to 70, 30 to 60 or 30 to 50 nm diameter. In one embodiment, the nanoparticle is 15 to 50 nm in diameter. In one embodiment, the nanoparticle is 30 to 60 nm in diameter. In one embodiment, the composition comprises a population or a plurality of nanoparticles with an average diameter from 10 to 300 nm, e.g., 15 to 280, 30 to 250, 30 to 200, 20 to 150, 30 to 100, 20 to 80, 30 to 70, 30 to 60 or 30 to 50 nm. In one embodiment, the nanoparticle is 15 to 50 nm in diameter. In one embodiment, the average nanoparticle diameter is from 30 to 60 nm. In one embodiment, the surface charge of the molecule is neutral, or slightly negative. In some embodiments, the zeta potential of the particle surface is from about -80 mV to about 50 mV, about -20 mV to about 20 mV, about -20 mV to about -10 mV, or about -10 mV to about 0.

In one embodiment, the therapeutic agent (e.g., a cytotoxic agent, e.g., a topoisomerase inhibitor (e.g., a topoisomerase inhibitor I, a topoisomerase II inhibitor), an anti-metabolic agent (e.g., an antifolate, a pyrimidine analog), an alkylating agent, an anthracycline, a platinum based agent, an anti-tumor antibiotic, a microtubule inhibitor (e.g., a taxane or a epothilone), a kinase inhibitor, or a proteasome inhibitor (a boronic acid containing molecule, e.g., a bortezomib); an immunomodulator (e.g., a corticosteroid or a rapamycin analog) conjugated to the CDP is more soluble when conjugated to the CDP than when not conjugated to the CDP.

In one embodiment, the composition comprises a population, mixture or plurality of CDP-therapeutic agent conjugates or particles comprising CDP-therapeutic agent conjugates (e.g., CDP-cytotoxic agent conjugates, e.g., CDP-topoisomerase inhibitor conjugates (e.g., CDP-topoisomerase inhibitor I conjugates, CDP-topoisomerase II inhibitor conjugates), CDP-anti-metabolic agent conjugates (e.g., CDP-antifolate conjugates, CDP-pyrimidine analog conjugates), CDP-alkylating agent conjugates, CDP-anthracycline conjugates, CDP-platinum based agent conjugates, CDP-anti-tumor antibiotic conjugates, CDP-microtubule inhibitor conjugates (e.g., a CDP-taxane

conjugates or CDP-epothilone conjugates), CDP-kinase inhibitor conjugates, CDP-proteasome inhibitor conjugates (CDP-boronic acid containing molecule conjugates, e.g., CDP-bortezomib conjugates); CDP-immunomodulator conjugates (e.g., CDP-corticosteroid conjugates or CDP-rapamycin conjugates). In one embodiment, the population, mixture or plurality of CDP-therapeutic agent conjugates comprises a plurality of different therapeutic agents conjugated to a CDP (e.g., a first therapeutic agent is attached to a first CDP and a different therapeutic agent is attached to a second CDP and both CDP-therapeutic agent conjugates are present in the composition). In one embodiment, the composition comprises a population, mixture or plurality of particles, the particles comprising CDP-therapeutic agent conjugates.

In one aspect, the invention features, a method of treating a proliferative disorder, e.g., cancer, in a subject, e.g., a human subject. The method comprises:

providing an initial administration of a CDP-cytotoxic agent conjugate, particle or composition described herein to said subject, and, optionally, administering one or more subsequent administrations of said CDP-cytotoxic agent conjugate, particle or composition, to said subject.

In one embodiment, the CDP-cytotoxic agent conjugate, particle or composition is administered at a dose and/or dosing schedule described herein.

In one embodiment, the cancer is a bile duct cancer, e.g., a Klatskin tumor.

In one aspect, the invention features, a method of treating cancer in a subject, e.g., a human subject. The method comprises:

providing an initial administration of a CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein, or, e.g., a CDP-floxuridine conjugate, particle or composition, e.g., a CDP-floxuridine conjugate, particle or composition, described herein, or, e.g., a CDP-raltitrexed conjugate, particle or composition, e.g., a CDP-raltitrexed conjugate, particle or composition, described herein, to said subject, and, optionally, administering one or more subsequent administrations of said CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g.,

a CDP-pemetrexed conjugate, particle or composition, described herein, or, e.g., a CDP-floxuridine conjugate, particle or composition, e.g., a CDP-floxuridine conjugate, particle or composition, described herein, or, e.g., a CDP-raltitrexed conjugate, particle or composition, e.g., a CDP-raltitrexed conjugate, particle or composition, described herein, wherein each subsequent administration is provided, independently, between 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 days after the previous, e.g., the initial, administration, to thereby treat the cancer.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 18-24, e.g., 21, days after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein, or, e.g., a CDP-floxuridine conjugate, particle or composition, e.g., a CDP-floxuridine conjugate, particle or composition, described herein, or, e.g., a CDP-raltitrexed conjugate, particle or composition, e.g., a CDP-raltitrexed conjugate, particle or composition, described herein, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes.

In an embodiment, the method includes an initial administration of a CDP-pemetrexed conjugate, particle or composition to said subject at a dosage of 300 mg/m^2, 320 mg/m^2, 350 mg/m^2, 380 mg/m^2, 400 mg/m^2, 420 mg/m^2, 450 mg/m^2, 480 mg/m^2, 500 mg/m^2, 520 mg/m^2, 550 mg/m^2, 580 mg/m^2, 600 mg/m^2, 620 mg/m^2, 650 mg/m^2, 680 mg/m^2, 700 mg/m^2, 720 mg/m^2, or 750 mg/m^2 (wherein the dosage is expressed in mg of drug, as opposed to mg of conjugate), and one or more subsequent administrations of a CDP-pemetrexed conjugate, particle or composition to said subject, at a dosage of 300

mg/m^2, 320 mg/m^2, 350 mg/m^2, 380 mg/m^2, 400 mg/m^2, 420 mg/m^2, 450 mg/m^2, 480 mg/m^2, 500 mg/m^2, 520 mg/m^2, 550 mg/m^2, 580 mg/m^2, 600 mg/m^2, 620 mg/m^2, 650 mg/m^2, 680 mg/m^2, 700 mg/m^2, 720 mg/m^2, or 750 mg/m^2, e.g., at the same dosage as the initial dosage. In one embodiment, each subsequent administration is administered, independently, 18-24, e.g., 21 days after the previous, e.g., the initial, administration.

In an embodiment, the cancer is a cancer described herein. For example, the cancer can be a cancer of the bladder (including accelerated and metastatic bladder cancer), breast (e.g., estrogen receptor positive breast cancer, estrogen receptor negative breast cancer, HER-2 positive breast cancer, HER-2 negative breast cancer, triple negative breast cancer, inflammatory breast cancer), colon (including colorectal cancer), kidney (e.g., renal cell carcinoma), liver, lung (including small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma)), mesothelioma, genitourinary tract, e.g., ovary (including fallopian, endometrial and peritoneal cancers), cervix, prostate and testes, lymphatic system, rectum, larynx, pancreas (including exocrine pancreatic carcinoma), stomach (e.g., gastroesophageal, upper gastric or lower gastric cancer), gastrointestinal cancer (e.g., anal cancer), gall bladder, thyroid, lymphoma (e.g., Burkitt's, Hodgkin's or non-Hodgkin's lymphoma), leukemia (e.g., acute myeloid leukemia), Ewing's sarcoma, nasoesophageal cancer, nasopharyngeal cancer, neural and glial cell cancers (e.g., glioblastoma multiforme), and head and neck. Preferred cancers include breast cancer (e.g., metastatic or locally advanced breast cancer), prostate cancer (e.g., hormone refractory prostate cancer), renal cell carcinoma, lung cancer (e.g., small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma)), mesothelioma, pancreatic cancer, gastric cancer (e.g., gastroesophageal, upper gastric or lower gastric cancer), colorectal cancer, squamous cell cancer of the head and neck, ovarian cancer (e.g., advanced ovarian cancer, platinum-based agent resistant or relapsed ovarian cancer), lymphoma (e.g., Burkitt's, Hodgkin's or non-Hodgkin's lymphoma), leukemia (e.g., acute myeloid leukemia) and gastrointestinal cancer.

In one embodiment, the cancer is lung cancer, e.g., non-small cell lung cancer and/or small cell lung cancer (e.g., squamous cell non-small cell lung cancer, or

nonsquamous cell non-small cell lung cancer, or squamous cell small cell lung cancer). In an embodiment, the cancer is lung cancer, e.g., nonsquamous cell non-small cell lung cancer and the CDP-anti-metabolic agent conjugate, particle or composition is a CDP-pemetrexed conjugate, particle or composition. In one embodiment, the lung cancer is metastatic, recurrent or refractory lung cancer. In one embodiment, the lung cancer is KRAS wild-type lung cancer, e.g., KRAS wild-type non-small cell lung cancer.

In an embodiment, the CDP-anti-metabolic agent conjugate, particle or composition, e.g., the CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, is provided at 300-750 mg/m^2/month, e.g., 300-600 mg/m^2/month or 400-750 mg/m^2/month.

In one embodiment, the CDP-anti-metabolic agent conjugate, particle or composition is administered in combination with one or more additional chemotherapeutic agent, e.g., a chemotherapeutic agent (such as an angiogenesis inhibitor) or combination of chemotherapeutic agents described herein. In one embodiment, the conjugate, particle or composition is administered in combination with one or more of: a platinum based agent (e.g., carboplatin, cisplatin, oxaliplatin), a taxane (e.g., paclitaxel, docetaxel, larotaxel, cabazitaxel), a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), an antimetabolite (e.g., an antifolate (e.g., floxuridine), a pyrimidine analogue (e.g., gemcitabine, 5FU, capecitabine)), an alkylating agent (e.g., cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), a vascular endothelial growth factor (VEGF) pathway inhibitor, a poly ADP-ribose polymerase (PARP) inhibitor and an mTOR inhibitor. In one embodiment, when the CDP-anti-metabolic agent conjugate, particle or composition is administered in combination with an additional chemotherapeutic agent, the dose at which the CDP-anti-metabolic agent conjugate, particle or composition is administered is 1%, 3%, 5%, 10%, 15%, 20%, 25%, 30% less than the doses described herein. In one embodiment, when the CDP-anti-metabolic agent conjugate, particle or composition, e.g., the CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition is provided in combination with one or more additional chemotherapeutic agents, e.g., a chemotherapeutic agent described herein, the CDP-anti-metabolic agent conjugate, particle or composition, e.g., the CDP-antifolate conjugate, particle or

composition, e.g., a CDP-pemetrexed conjugate, particle or composition, is provided at 100-750 mg/m^2/month.

In one embodiment, the CDP-anti-metabolic agent conjugate, particle or composition is administered in combination with an angiogenesis inhibitor, e.g., a VEGF pathway inhibitor, e.g., sorafenib or sunitinib. In one embodiment, the angiogenesis inhibitor, e.g., sorafenib, is administered at a dose of about 400 mg per day or less, daily, e.g., 350 mg per day, 300 mg per day, 250 mg per day, 200 mg per day, or 150 mg per day. In one embodiment, the angiogenesis inhibitor, e.g., sunitinib, is administered daily at a dose of about 50 mg per day or less, daily, e.g., 45 mg per day, 40 mg per day, 38 mg per day, 30 mg per day, 25 mg per day, 20 mg per day, or 15 mg per day. In one embodiment, when the CDP-anti-metabolic agent conjugate, particle or composition is administered in combination with an angiogenesis inhibitor, e.g., sorafenib or sunitinib, the dose at which the CDP-anti-metabolic agent conjugate, particle or composition is administered is 1%, 3%, 5%, 10%, 15%, 20%, 25%, or 30% less than a dose described herein.

In one embodiment, the CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein is administered at a dosage of 300 mg/m^2, 320 mg/m^2, 350 mg/m^2, 15 mg/m^2, 380 mg/m^2, 400 mg/m^2, 420 mg/m^2, 450 mg/m^2, 480 mg/m^2, 500 mg/m^2, 520 mg/m^2, 550 mg/m^2, 580 mg/m^2, 600 mg/m^2, 620 mg/m^2, 650 mg/m^2, 680 mg/m^2, 700 mg/m^2, 720 mg/m^2, or 750 mg/m^2 by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes or 90 minutes, e.g., a period equal to or less than 30 minutes, 45 minutes or 60 minutes.

In an embodiment, the method includes an initial administration of the CDP-pemetrexed conjugate, particle or composition to the subject at a dosage of 300 mg/m^2, 320 mg/m^2, 350 mg/m^2, 15 mg/m^2, 380 mg/m^2, 400 mg/m^2, 420 mg/m^2, 450 mg/m^2, 480 mg/m^2, 500 mg/m^2, 520 mg/m^2, 550 mg/m^2, 580 mg/m^2, 600 mg/m^2, 620 mg/m^2, 650 mg/m^2, 680 mg/m^2, 700 mg/m^2, 720 mg/m^2, or 750 mg/m^2, and

one or more subsequent administrations of the CDP-pemetrexed conjugate, particle or composition to the subject, at a dosage of 300 mg/m^2, 320 mg/m^2, 350 mg/m^2,

15 mg/m^2, 380 mg/m^2, 400 mg/m^2, 420 mg/m^2, 450 mg/m^2, 480 mg/m^2, 500 mg/m^2, 520 mg/m^2, 550 mg/m^2, 580 mg/m^2, 600 mg/m^2, 620 mg/m^2, 650 mg/m^2, 680 mg/m^2, 700 mg/m^2, 720 mg/m^2, or 750 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is administered, independently, 18-24, e.g., 21, days after the previous, e.g., the initial, administration, and the cancer is, e.g., lung cancer, e.g., non-small cell lung cancer or small cell lung cancer (e.g., squamous cell non-small cell lung cancer, squamous cell small cell lung cancer, or nonsquamous cell non-small cell lung cancer), or mesothelioma.

In one embodiment, the subject has not been administered a CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein, prior to the initial administration.

In an embodiment, the CDP-anti-metabolic agent conjugate, particle or composition is administered as a first line treatment for the cancer.

In an embodiment, the CDP-anti-metabolic agent conjugate, particle or composition is administered as a second, third or fourth line treatment for the cancer. In an embodiment, the cancer is sensitive to one or more chemotherapeutic agents, e.g., a platinum-based agent, a taxane, an alkylating agent, an antimetabolite and/or a vinca alkaloid. In an embodiment, the cancer is a refractory, relapsed or resistant to one or more chemotherapeutic agents, e.g., a platinum-based agent, a taxane, an alkylating agent, an anthracycline (e.g., doxorubicin (e.g., liposomal doxorubicin)), an antimetabolite and/or a vinca alkaloid. In one embodiment, the cancer is, e.g., lung cancer, and the lung cancer is refractory, relapsed or resistant to a taxane (e.g., paclitaxel, docetaxel, larotaxel, cabazitaxel), a platinum-based agent (e.g., carboplatin, cisplatin, oxaliplatin), a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), a vascular endothelial growth factor (VEGF) pathway inhibitor, and/or an epidermal growth factor (EGF) pathway inhibitor).

In one embodiment, the subject has lung cancer, e.g., nonsquamous non-small cell cancer, or mesothelioma that is refractory, relapsed or resistant to a platinum-based agent, and the subject is administered a CDP-anti-metabolic agent conjugate, particle or

composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein.

In one embodiment, the subject has mesothelioma, and the subject is administered a CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein, in combination with a platinum based agent (e.g., cisplatin, carboplatin, or oxaliplatin). In one embodiment, the platinum based agent (e.g., cisplatin, carboplatin, or oxaliplatin) is administered at a dose of about 20 mg/m^2, about 30 mg/m^2, about 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 80 mg/m^2, every 17, 18, 19, 20, 21, 22, 23 or 24 days, e.g., 21 days. In one embodiment, the CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein is administered at a dose and/or dosing regimen described herein and the platinum-based chemotherapeutic (e.g., cisplatin, carboplatin, or oxaliplatin) is administered at a dose of about 20 mg/m^2, about 30 mg/m^2, about 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 80 mg/m^2, every 17, 18, 19, 20, 21, 22, 23 or 24 days, e.g., 21 days. In one embodiment, when the CDP-anti-metabolic agent conjugate, particle or composition is administered in combination with platinum-based chemotherapeutic (e.g., cisplatin, carboplatin, or oxaliplatin), the dose at which the CDP-anti-metabolic agent conjugate, particle or composition is administered is 1%, 3%, 5%, 10%, 15%, 20%, 25%, 30% less than a dose described herein.

In one embodiment, the subject has radically resected non-small cell lung cancer, and/or advanced non-squamous KRAS wild type non-squamous cell lung cancer and the subject is administered a CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein, in combination with a platinum based agent (e.g., cisplatin, carboplatin, or oxaliplatin). In one embodiment, the platinum based agent (e.g., cisplatin, carboplatin, or oxaliplatin) is administered at a dose of about 20 mg/m^2, about

30 mg/m^2, about 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 80 mg/m^2, every 17, 18, 19, 20, 21, 22, 23 or 24 days, e.g., 21 days. In one embodiment, the CDP-anti-metabolic agent conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, e.g., a CDP-pemetrexed conjugate, particle or composition, described herein is administered at a dose and/or dosing regimen described herein and the platinum-based chemotherapeutic (e.g., cisplatin, carboplatin, or oxaliplatin) is administered at a dose of about 20 mg/m^2, about 30 mg/m^2, about 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 80 mg/m^2, every 17, 18, 19, 20, 21, 22, 23 or 24 days, e.g., 21 days. In one embodiment, when the CDP-anti-metabolic agent conjugate, particle or composition is administered in combination with platinum-based chemotherapeutic (e.g., cisplatin, carboplatin, or oxaliplatin), the dose at which the CDP-anti-metabolic agent conjugate, particle or composition is administered is 1%, 3%, 5%, 10%, 15%, 20%, 25%, 30% less than a dose described herein.

In one aspect, the invention features, a method of treating cancer in a subject, e.g., a human subject. The method comprises:

providing an initial administration of CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, described herein, or, e.g., a CDP-cytarabine conjugate, particle or composition, e.g., a CDP-cytarabine conjugate, particle or composition, described herein, or, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein, or, e.g., a CDP-5FU conjugate, particle or composition, e.g., a CDP-5FU conjugate, particle or composition, described herein, to said subject, and, optionally, providing one or more subsequent administrations of said CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, described herein, or, e.g., a CDP-cytarabine conjugate, particle or composition, e.g., a CDP-cytarabine conjugate, particle or composition, described herein, or, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein, or, e.g., a CDP-5FU conjugate, particle or composition, e.g., a CDP-5FU

conjugate, particle or composition, described herein, wherein each subsequent administration is provided, independently, between 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 days after the previous, e.g., the initial, administration, to thereby treat the cancer.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 20-28, e.g., 24, days after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-antifolate conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, e.g., a CDP-capecitabine conjugate, particle or composition, described herein, or, e.g., a CDP-cytarabine conjugate, particle or composition, e.g., a CDP-cytarabine conjugate, particle or composition, described herein, or, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein, or, e.g., a CDP-5FU conjugate, particle or composition, e.g., a CDP-5FU conjugate, particle or composition, described herein, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes.

In an embodiment, the method includes an initial administration of a CDP-gemcitabine conjugate, particle or composition at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1550 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2 (wherein the dosage is expressed in mg

of drug, as opposed to mg of conjugate), and one or more subsequent administrations of a CDP-gemcitabine conjugate, particle or composition at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1550 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2, e.g., at the same dosage as the initial dosage. In one embodiment, each subsequent administration is provided, independently, between 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 days after the previous, e.g., the initial, administration.

In an embodiment, the cancer is a cancer described herein. For example, the cancer can be a cancer of the bladder (including accelerated and metastatic bladder cancer), breast (e.g., estrogen receptor positive breast cancer, estrogen receptor negative breast cancer, HER-2 positive breast cancer, HER-2 negative breast cancer, triple negative breast cancer, inflammatory breast cancer), colon (including colorectal cancer), kidney (e.g., renal cell carcinoma), liver, lung (including small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma), mesothelioma, genitourinary tract, e.g., ovary (including fallopian, endometrial and peritoneal cancers), cervix, prostate and testes, lymphatic system, rectum, larynx, pancreas (including exocrine pancreatic carcinoma), stomach (e.g., gastroesophageal, upper gastric or lower gastric cancer), gastrointestinal cancer (e.g., anal cancer), gall bladder, thyroid, lymphoma (e.g., Burkitt's, Hodgkin's or non-Hodgkin's lymphoma), leukemia (e.g., acute myeloid leukemia), Ewing's sarcoma, nasoesophageal cancer, nasopharyngeal cancer, neural and glial cell cancers (e.g., glioblastoma multiforme), and head and neck. Preferred cancers include breast cancer (e.g., metastatic or locally advanced breast cancer), prostate cancer (e.g., hormone refractory prostate cancer), renal cell carcinoma, lung cancer (e.g., small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma)), pancreatic cancer (e.g., metastatic or locally advanced pancreatic cancer), gastric cancer (e.g.,

gastroesophageal, upper gastric or lower gastric cancer), colorectal cancer, squamous cell cancer of the head and neck, ovarian cancer (e.g., advanced ovarian cancer, platinum-based agent resistant or relapsed ovarian cancer), lymphoma (e.g., Burkitt's, Hodgkin's or non-Hodgkin's lymphoma), leukemia (e.g., acute myeloid leukemia) and gastrointestinal cancer.

In an embodiment, the CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, is provided at 1200-4950 mg/m^2/month, e.g., 2000-4000 mg/m^2 /month or 3000-3750 mg/m^2/month.

In one embodiment, the CDP-pyrimidine analog conjugate, particle or composition is administered in combination with one or more additional chemotherapeutic agents, e.g., a chemotherapeutic agent (such as an angiogenesis inhibitor) or combination of chemotherapeutic agents described herein. In one embodiment, the conjugate, particle or composition is administered in combination with one or more of: a platinum based agent (e.g., carboplatin, cisplatin, oxaliplatin), a taxane (e.g., paclitaxel, docetaxel, larotaxel, cabazitaxel), a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), an antimetabolite (e.g., an antifolate (e.g., floxuridine, pemetrexed), a pyrimidine analogue (e.g., 5FU, capecitabine)), an alkylating agent (e.g., cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), a vascular endothelial growth factor (VEGF) pathway inhibitor, a poly ADP-ribose polymerase (PARP) inhibitor and an mTOR inhibitor. In one embodiment, when the CDP-pyrimidine analog conjugate, particle or composition is administered in combination with an additional chemotherapeutic agent, the dose at which the CDP-pyrimidine analog conjugate, particle or composition is administered is 1%, 3%, 5%, 10%, 15%, 20%, 25%, 30% less than the doses described herein. In one embodiment, when the CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, is provided in combination with one or more additional chemotherapeutic agents, e.g., a chemotherapeutic agent described herein, the CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, is provided at 1000-4000 mg/m^2/month.

In one embodiment, the CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-

gemcitabine conjugate, particle or composition, described herein is administered at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1550 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2 by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes or 90 minutes, e.g., a period equal to or less than 30 minutes, 45 minutes or 60 minutes.

In an embodiment, the method includes an initial administration of the CDP-gemcitabine conjugate, particle or composition to the subject at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1550 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2, and

one or more subsequent administrations of the CDP-gemcitabine conjugate, particle or composition to the subject, at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1550 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is administered, independently, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 days, e.g., 7 or 14, days after the previous, e.g., the initial, administration, and the cancer is, e.g., lung cancer, e.g., non-small cell lung cancer and/or small cell lung cancer (e.g., squamous cell non-small cell lung cancer, squamous cell small cell lung cancer, or nonsquamous cell non-small cell lung cancer). In one

embodiment, the lung cancer is locally advanced or metastatic lung cancer, e.g., non-small cell lung cancer and/or small cell lung cancer.

In an embodiment, the method includes an initial administration of the CDP-gemcitabine conjugate, particle or composition to the subject at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1550 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2, and

one or more subsequent administrations of the CDP-gemcitabine conjugate, particle or composition to the subject, at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1550 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is administered, independently, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, e.g., 7 or 14, days after the previous, e.g., the initial, administration, and the cancer is, e.g., pancreatic cancer, e.g., unresectable or metastatic pancreatic cancer.

In an embodiment, the method includes an initial administration of the CDP-gemcitabine conjugate, particle or composition to the subject at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1550 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2, and

one or more subsequent administrations of the CDP-gemcitabine conjugate, particle or composition to the subject, at a dosage of 600 mg/m^2, 700 mg/m^2, 730 mg/m^2, 750 mg/m^2, 780 mg/m^2, 800 mg/m^2, 830 mg/m^2, 850 mg/m^2, 880 mg/m^2, 900 mg/m^2, 930 mg/m^2, 950 mg/m^2, 980 mg/m^2, 1000 mg/m^2, 1030 mg/m^2, 1050 mg/m^2, 1080 mg/m^2, 1100 mg/m^2, 1130 mg/m^2, 1150 mg/m^2, 1180 mg/m^2, 1200 mg/m^2, 1230 mg/m^2, 1250 mg/m^2, 1280 mg/m^2, 1300 mg/m^2, 1350 mg/m^2, 1380 mg/m^2, 1400 mg/m^2, 1430 mg/m^2, 1450 mg/m^2, 1480 mg/m^2, 1500 mg/m^2, 1530 mg/m^2, 1550 mg/m^2, 1580 mg/m^2, 1600 mg/m^2, 1630 mg/m^2, or 1650 mg/m^2, e.g., at the same dosage as the initial dosage, wherein each subsequent administration is administered, independently, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 days, e.g., 7 or 14, days after the previous, e.g., the initial, administration, and the cancer is, e.g., breast cancer, e.g., estrogen receptor positive breast cancer, estrogen receptor negative breast cancer, HER-2 positive breast cancer, HER-2 negative breast cancer, triple negative breast cancer or inflammatory breast cancer. In one embodiment, the breast cancer is metastatic breast cancer.

In an embodiment, the method includes an initial administration of a CDP-5FU conjugate, particle or composition at a dosage of 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg, or 20 mg/kg (wherein the dosage is expressed in mg of drug, as opposed to mg of conjugate), and one or more subsequent administrations of a CDP-5FU conjugate, particle or composition at a dosage of 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg, or 20 mg/kg, e.g., at the same dosage as the initial dosage. In some embodiments, each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, day(s) after the previous, e.g., the initial, administration.

In an embodiments, the CDP-5FU conjugate, particle or composition is administered intravenously once daily for 4 successive days.

In an embodiment, the cancer is carcinoma of the colon, rectum, breast, stomach or pancreas, and the CDP-pyrimidine analog conjugate, particle or composition is a CDP-5FU conjugate, particle or composition.

In an embodiment, the cancer is metastatic or refractory colorectal cancer, stage III colorectal cancer, locally advanced squamous cell carcinoma of the head and neck (SCCHN), or gastric adenocarcinoma, and the CDP-pyrimidine analog conjugate, particle or composition is a CDP-5FU conjugate, particle or composition.

In an embodiment, the cancer is superficial basal cell carcinoma or actinic keratosis, and the CDP-pyrimidine analog conjugate, particle or composition is a CDP-5FU conjugate, particle or composition.

In one embodiment, the subject has not been administered a CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein, prior to the initial administration.

In an embodiment, the CDP-pyrimidine analog conjugate, particle or composition is administered as a first line treatment for the cancer.

In an embodiment, the CDP-pyrimidine analog conjugate, particle or composition is administered as a second, third or fourth line treatment for the cancer. In an embodiment, the cancer is sensitive to one or more chemotherapeutic agents, e.g., a platinum-based agent, a taxane, an alkylating agent, an anthracycline, an antimetabolite and/or a vinca alkaloid. In an embodiment, the cancer is a refractory, relapsed or resistant to one or more chemotherapeutic agents, e.g., a platinum-based agent, a taxane, an alkylating agent, an antimetabolite and/or a vinca alkaloid. In one embodiment, the cancer is, e.g., lung cancer, and the cancer is refractory, relapsed or resistant to a taxane (e.g., paclitaxel, docetaxel, larotaxel, cabazitaxel), a platinum-based agent (e.g., carboplatin, cisplatin, oxaliplatin), an anthracycline, a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), a vascular endothelial growth factor (VEGF) pathway inhibitor, an epidermal growth factor (EGF) pathway inhibitor) and/or an antimetabolite (e.g., an antifolate (e.g., pemetrexed, floxuridine, raltitrexed) and a pyrimidine analogue (e.g., capecitabine, cytarabine, 5FU)). In one embodiment, the cancer is, e.g., breast cancer, and the cancer is refractory, relapsed or resistant to a taxane (e.g., paclitaxel, docetaxel, larotaxel, cabazitaxel), a vascular endothelial growth factor (VEGF) pathway inhibitor, an anthracycline (e.g., daunorubicin, doxorubicin (e.g., liposomal doxorubicin), epirubicin, valrubicin, idarubicin), a platinum-based agent (e.g., carboplatin, cisplatin,

oxaliplatin), and/or an antimetabolite (e.g., an antifolate (e.g., pemetrexed, floxuridine, raltitrexed) and a pyrimidine analogue (e.g., capecitabine, cytarabine, 5FU)).

In one embodiment, the subject has breast cancer (e.g., metastatic breast cancer), and the subject is administered a CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein in combination with a taxane. In one embodiment, CDP-pyrimidine analog conjugate, particle or composition is administered in combination with a taxane (e.g., docetaxel, paclitaxel, larotaxel, or cabazitaxel). In one embodiment, the taxane (e.g., docetaxel, paclitaxel, larotaxel, or cabazitaxel) is administered at a dose of about 80 mg/m^2, 100 mg/m^2, 125 mg/m^2, 150 mg/m^2, 175 mg/m^2, or about 200 mg/m^2, every 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 days, e.g., 21 days. In one embodiment, the CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein is administered at a dose and/or dosing regimen described herein and the taxane (e.g., docetaxel, paclitaxel, larotaxel, or cabazitaxel) is administered at a dose of about 80 mg/m^2, 100 mg/m^2, 125 mg/m^2, 150 mg/m^2, 175 mg/m^2, or about 200 mg/m^2, every 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 days, e.g., 21 days. In one embodiment, when the CDP-pyrimidine analog conjugate, particle or composition is administered in combination with the taxane (e.g., docetaxel, paclitaxel, larotaxel, or cabazitaxel), the dose at which the CDP-pyrimidine analog conjugate, particle or composition is administered is 1%, 3%, 5%, 10%, 15%, 20%, 25%, 30% less than a dose described herein.

In one embodiment, the subject has non-small cell lung cancer (e.g., locally advanced or metastatic non-small cell lung cancer), and the subject is administered a CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein. In one embodiment, CDP-pyrimidine analog conjugate, particle or composition is administered in combination with a platinum-based chemotherapeutic (e.g., cisplatin, carboplatin, or oxaliplatin). In one embodiment, the platinum-based chemotherapeutic (e.g., cisplatin, carboplatin, or oxaliplatin) is administered at a dose of about 60 mg/m^2, 80 mg/m^2, 100 mg/m^2, 120 mg/m^2, or 140

mg/m^2, every 21, 24, 25, 26, 27, 28, 29, 30 or 31 days, e.g., 28 days. In one embodiment, the CDP-pyrimidine analog conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, e.g., a CDP-gemcitabine conjugate, particle or composition, described herein is administered at a dose and/or dosing regimen described herein and the platinum-based chemotherapeutic (e.g., cisplatin, carboplatin, or oxaliplatin) is administered at a dose of about 60 mg/m^2, 80 mg/m^2, 100 mg/m^2, 120 mg/m^2, or 140 mg/m^2, every 21, 24, 25, 26, 27, 28, 29, 30 or 31 days, e.g., 28 days. In one embodiment, when the CDP-pyrimidine analog conjugate, particle or composition is administered in combination with platinum-based chemotherapeutic (e.g., cisplatin, carboplatin, or oxaliplatin), the dose at which the CDP-pyrimidine analog conjugate, particle or composition is administered is 1%, 3%, 5%, 10%, 15%, 20%, 25%, 30% less than a dose described herein.

In one embodiment, the subject has non-small cell lung cancer (e.g., locally advanced or metastatic non-small cell lung cancer), and the CDP-pyrimidine analog conjugate, particle or composition is administered in combination with an angiogenesis inhibitor, e.g., a VEGF pathway inhibitor, e.g., soranenib or sunitinib. In one embodiment, the angiogenesis inhibitor, e.g., sorafenib, is administered at a dose of about 400 mg per day or less, daily, e.g., 350 mg per day, 300 mg per day, 250 mg per day, 200 mg per day, or 150 mg per day. In one embodiment, the angiogenesis inhibitor, e.g., sunitinib, is administered daily at a dose of about 50 mg per day or less, daily, e.g., 45 mg per day, 40 mg per day, 38 mg per day, 30 mg per day, 25 mg per day, 20 mg per day, or 15 mg per day. In one embodiment, when the CDP-pyrimidine analog conjugate, particle or composition is administered in combination with an angiogenesis inhibitor, e.g., sorafenib or sunitinib, the dose at which the CDP-pyrimidine analog conjugate, particle or composition is administered is 1%, 3%, 5%, 10%, 15%, 20%, 25%, or 30% less than a dose described herein.

In one aspect, the invention features, a method of treating cancer in a subject, e.g., a human subject. The method comprises:

providing an initial administration of a CDP-anti-tumor antibiotic conjugate, particle or composition, e.g., a CDP-HSP90 inhibitor conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition, e.g., a CDP-geldanamycin

conjugate, particle or composition described herein, to said subject, and, optionally, providing one or more subsequent administrations of said CDP-anti-tumor antibiotic conjugate, particle or composition, e.g., a CDP-HSP90 inhibitor conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition described herein, wherein each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 days, e.g., 3 or 7 days after the previous, e.g., the initial, administration, to thereby treat the cancer.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 1-12, e.g., 3 or 7, days after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-anti-tumor antibiotic conjugate, particle or composition, e.g., a CDP-HSP90 inhibitor conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition, e.g., a CDP-geldanamycin conjugate, particle or composition described herein, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes.

In an embodiment, the method includes an initial administration of a CDP-geldanamycin conjugate, particle or composition at a dosage of 20 mg/m^2, 30 mg/m^2, 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 75 mg/m^2, 80 mg/m^2, 85 mg/m^2, 90 mg/m^2, 95 mg/m^2, 100 mg/m^2, 105 mg/m^2, 110 mg/m^2, 115 mg/m^2, 120 mg/m^2, 125 mg/m^2, 130 mg/m^2, 140 mg/m^2, 150 mg/m^2, 160 mg/m^2, or 170 mg/m^2, and one or more subsequent administrations of a CDP-geldanamycin conjugate, particle or composition at a dosage of 20 mg/m^2, 30 mg/m^2, 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 75 mg/m^2, 80 mg/m^2, 85 mg/m^2, 90 mg/m^2, 95 mg/m^2, 100 mg/m^2, 105 mg/m^2, 110 mg/m^2, 115 mg/m^2, 120 mg/m^2, 125 mg/m^2, 130 mg/m^2, 140 mg/m^2, 150 mg/m^2, 160 mg/m^2, or 170 mg/m^2, e.g.,

at the same dosage as the initial dosage. In one embodiment, each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, days after the previous, e.g., the initial, administration.

In an embodiment, the cancer is a cancer described herein. For example, the cancer can be a cancer of the bladder (including accelerated and metastatic bladder cancer), breast (e.g., estrogen receptor positive breast cancer, estrogen receptor negative breast cancer, HER-2 positive breast cancer, HER-2 negative breast cancer, triple negative breast cancer, inflammatory breast cancer), colon (including colorectal cancer), kidney (e.g., renal cell carcinoma), liver, lung (including small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma), mesothelioma, genitourinary tract, e.g., ovary (including fallopian, endometrial and peritoneal cancers), cervix, prostate and testes, lymphatic system, rectum, larynx, pancreas (including exocrine pancreatic carcinoma), stomach (e.g., gastroesophageal, upper gastric or lower gastric cancer), gastrointestinal cancer (e.g., anal cancer), gall bladder, thyroid, lymphoma (e.g., Burkitt's, Hodgkin's or non-Hodgkin's lymphoma (e.g., mantle cell lymphoma or anaplastic large cell lymphoma)), myeloma, leukemia (e.g., acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, and chronic lymphoblastic leukemia), Ewing's sarcoma, nasoesophageal cancer, nasopharyngeal cancer, neural and glial cell cancers (e.g., glioblastoma multiforme, neuroblastoma), and head and neck. Preferred cancers include breast cancer (e.g., metastatic or locally advanced breast cancer), prostate cancer (e.g., hormone refractory prostate cancer), renal cell carcinoma, lung cancer (e.g., small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma)), pancreatic cancer (e.g., metastatic or locally advanced pancreatic cancer), gastric cancer (e.g., gastroesophageal, upper gastric or lower gastric cancer), bladder cancer, colorectal cancer, squamous cell cancer of the head and neck, ovarian cancer (e.g., advanced ovarian cancer, platinum-based agent resistant or relapsed ovarian cancer), lymphoma (e.g., Burkitt's, Hodgkin's or non-Hodgkin's lymphoma), leukemia (e.g., acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, and chronic lymphoblastic leukemia), myeloma, and gastrointestinal cancer.

In one embodiment, the CDP-geldanamycin conjugate, particle or composition is administered in combination with one or more additional chemotherapeutic agents, e.g., a chemotherapeutic agent (such as an angiogenesis inhibitor) or combination of chemotherapeutic agents described herein. In one embodiment, the conjugate, particle or composition is administered in combination with one or more of: a taxane (e.g., paclitaxel, docetaxel, larotaxel, cabazitaxel), an antimetabolite (e.g., an antifolate (e.g., floxuridine, pemetrexed), a proteasome inhibitor (e.g., a boronic acid containing molecule, e.g., bortezomib), a pyrimidine analogue (e.g., 5FU, cytarabine, capecitabine)), a kinase inhibitor (e.g., imatinib), e.g., a vascular endothelial growth factor (VEGF) pathway inhibitor (e.g., sorafenib), a poly ADP-ribose polymerase (PARP) inhibitor and an mTOR inhibitor. In one embodiment, when the CDP- geldanamycin conjugate, particle or composition is administered in combination with an additional chemotherapeutic agent, the dose at which the CDP- geldanamycin conjugate, particle or composition is administered is 1%, 3%, 5%, 10%, 15%, 20%, 25%, 30% less than the doses described herein. In some embodiments, a CDP-geldanamycin conjugate, particle or composition is administered in combination with bortezomib, gemcitabine, belinostat, cytarabine, paclitaxel, rituximab, sorafenib, imatinib, irinotecan, or docetaxel.

In one aspect, the invention features, a method of treating cancer in a subject, e.g., a human subject. The method comprises:

providing an initial administration of CDP-platinum based agent conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, described herein, or, e.g., a CDP-carboplatin conjugate, particle or composition, e.g., a CDP-carboplatin conjugate, particle or composition, described herein, or, e.g., a CDP-oxaliplatin conjugate, particle or composition, e.g., a CDP-oxaliplatin conjugate, particle or composition, described herein, and, optionally, providing one or more subsequent administrations of said CDP-platinum based agent conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, described herein, or, e.g., a CDP-carboplatin conjugate, particle or composition, e.g., a CDP-carboplatin conjugate, particle or composition, described herein, or, e.g., a CDP-oxaliplatin conjugate, particle or composition, e.g., a CDP-oxaliplatin conjugate, particle

or composition, described herein wherein each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 day(s) after the previous, e.g., the initial, administration, to thereby treat the cancer.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 20-28, e.g., 21 or 28, days after the previous administration. In an embodiment, each subsequent administration is administered 1-5, e.g., 1, 3 day(s) after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-platinum based agent conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, e.g., a CDP-cisplatin conjugate, particle or composition, described herein, or, e.g., a CDP-carboplatin conjugate, particle or composition, e.g., a CDP-carboplatin conjugate, particle or composition, described herein, or, e.g., a CDP-oxaliplatin conjugate, particle or composition, e.g., a CDP-oxaliplatin conjugate, particle or composition, described herein, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes.

In an embodiment, the method includes an initial administration of a CDP-cisplatin conjugate, particle or composition at a dosage of 10 mg/m^2, 15 mg/m^2, 20 mg/m^2, 25 mg/m^2, 30 mg/m^2, 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 75 mg/m^2, 80 mg/m^2, 85 mg/m^2, 90 mg/m^2, 95 mg/m^2, 100 mg/m^2, 105 mg/m^2, 110 mg/m^2, 115 mg/m^2, 120 mg/m^2, 125 mg/m^2, 130 mg/m^2, 140 mg/m^2, 150 mg/m^2, 160 mg/m^2, or 170 mg/m^2, and one or more subsequent administrations of a CDP-cisplatin conjugate, particle or composition at a dosage of 10 mg/m^2, 15 mg/m^2, 20 mg/m^2, 25 mg/m^2, 30 mg/m^2, 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 75 mg/m^2, 80 mg/m^2, 85 mg/m^2, 90 mg/m^2, 95 mg/m^2, 100 mg/m^2, 105 mg/m^2, 110 mg/m^2, 115 mg/m^2, 120 mg/m^2, 125 mg/m^2, 130 mg/m^2, 140 mg/m^2, 150 mg/m^2, 160 mg/m^2, or 170 mg/m^2, e.g., at the same dosage as the

initial dosage. In one embodiment, each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31, day(s) after the previous, e.g., the initial, administration.

In an embodiment, the cancer is a cancer described herein. For example, the cancer can be a cancer of the bladder (including accelerated and metastatic bladder cancer), breast (e.g., estrogen receptor positive breast cancer, estrogen receptor negative breast cancer, HER-2 positive breast cancer, HER-2 negative breast cancer, triple negative breast cancer, inflammatory breast cancer), colon (including colorectal cancer), kidney (e.g., renal cell carcinoma), liver, lung (including small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma), mesothelioma, genitourinary tract, e.g., ovary (including fallopian, endometrial and peritoneal cancers), cervix, prostate and testes (e.g., metastatic testicular cancer), lymphatic system, rectum, larynx, pancreas (including exocrine pancreatic carcinoma), stomach (e.g., gastroesophageal, upper gastric or lower gastric cancer), gastrointestinal cancer (e.g., anal cancer), gall bladder, thyroid, lymphoma (e.g., Burkitt's, Hodgkin's or non-Hodgkin's lymphoma (e.g., mantle cell lymphoma or anaplastic large cell lymphoma)), myeloma, leukemia (e.g., acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, and chronic lymphoblastic leukemia), Ewing's sarcoma, nasoesophageal cancer, nasopharyngeal cancer, neural and glial cell cancers (e.g., glioblastoma multiforme, neuroblastoma), and head and neck. Preferred cancers include breast cancer (e.g., metastatic or locally advanced breast cancer), prostate cancer (e.g., hormone refractory prostate cancer) and testicular cancer (e.g., metastatic testicular cancer), renal cell carcinoma, lung cancer (e.g., small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma)), pancreatic cancer (e.g., metastatic or locally advanced pancreatic cancer), gastric cancer (e.g., gastroesophageal, upper gastric or lower gastric cancer), bladder cancer (e.g., advanced bladder cancer), colorectal cancer, squamous cell cancer of the head and neck, ovarian cancer (e.g., advanced ovarian cancer, resistant or relapsed ovarian cancer), lymphoma (e.g., Burkitt's, Hodgkin's or non-Hodgkin's lymphoma), leukemia (e.g.,

acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, and chronic lymphoblastic leukemia), myeloma, and gastrointestinal cancer.

In one embodiment, the method is a method of treating testicular cancer, e.g., metastatic testicular cancer, in a subject and the method includes an initial administration of a CDP- cisplatin conjugate, particle or composition at a dosage of 10 mg/m^2, 15 mg/m^2, 20 mg/m^2, 25 mg/m^2, 30 mg/m^2, or 40 mg/m^2, and, optionally, one or more subsequent administrations of a CDP-cisplatin conjugate, particle or composition at a dosage of 10 mg/m^2, 15 mg/m^2, 20 mg/m^2, 25 mg/m^2, 30 mg/m^2, or 40 mg/m^2, e.g., at the same dosage as the initial dosage. In one embodiment, each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 day(s) after the previous, e.g., the initial, administration.

In one embodiment, the method is a method of treating ovarian cancer, e.g., metastatic ovarian cancer, in a subject and the method includes an initial administration of a CDP- cisplatin conjugate, particle or composition at a dosage of 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 80 mg/m^2, 90 mg/m^2, 100 mg/m^2, or 110 mg/m^2, 120 mg/m^2, or 130 mg/m^2, and, optionally, one or more subsequent administrations of a CDP-cisplatin conjugate, particle or composition at a dosage of 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 80 mg/m^2, 90 mg/m^2, 100 mg/m^2, or 110 mg/m^2, 120 mg/m^2, or 130 mg/m^2, e.g., at the same dosage as the initial dosage. In one embodiment, each subsequent administration is provided, independently, between 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or 31 day(s) after the previous, e.g., the initial, administration. In some embodiments, the CDP-cisplatin conjugate, particle or composition is administered in combination with a second therapeutic agent, e.g., cyclophosphamide. In some embodiments, the CDP-cisplatin conjugate, particle or composition is administered in combination with surgical intervention or radiation.

In one embodiment, the method is a method of treating bladder cancer, e.g., advanced bladder cancer, in a subject and the method includes an initial administration of a CDP- cisplatin conjugate, particle or composition at a dosage of 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 80 mg/m^2, 90 mg/m^2, 100 mg/m^2, or 110 mg/m^2, 120 mg/m^2, or 130 mg/m^2, and, optionally, one or more subsequent administrations of a CDP-cisplatin conjugate, particle or composition at a dosage of 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70

mg/m^2, 80 mg/m^2, 90 mg/m^2, 100 mg/m^2, or 110 mg/m^2, 120 mg/m^2, or 130 mg/m^2, e.g., at the same dosage as the initial dosage. In one embodiment, each subsequent administration is provided, independently, between 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or 31 day(s) after the previous, e.g., the initial, administration. In some embodiments, the CDP-cisplatin conjugate, particle or composition is administered in combination with surgical intervention or radiation.

In one aspect, the invention features, a method of treating cancer in a subject, e.g., a human subject. The method comprises:

providing an initial administration of CDP-kinase inhibitor conjugate, particle or composition, e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, described herein, to said subject, and, optionally, providing one or more subsequent administrations of said CDP-kinase inhibitor conjugate, particle or composition, e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, described herein, wherein each subsequent administration is provided, independently, between 1, 2, 3, 4, 5, 6, 7, 8, 9 day(s) after the previous, e.g., the initial, administration, to thereby treat the cancer.

In an embodiment, the dosage of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, the time between at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 administrations is the same.

In an embodiment, each subsequent administration is administered 1-9, e.g., 1, 2, 3, 4, days after the previous administration.

In an embodiment, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 50 or 100 administrations are administered to the subject.

In one embodiment, the CDP-kinase inhibitor conjugate, particle or composition, e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin

conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, described herein, is administered by intravenous administration over a period equal to or less than about 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, or 180 minutes.

In one embodiment, the CDP-kinase inhibitor conjugate, particle or composition, e.g., a CDP-seronine/threonine kinase inhibitor conjugate, particle or composition, e.g., a CDP-mTOR inhibitor conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, described herein, is administered at a dosage of 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 12 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, or 50 mg (wherein said dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate).

In an embodiment, the cancer is a cancer described herein. For example, the cancer can be a cancer of the bladder (including accelerated and metastatic bladder cancer), breast (e.g., estrogen receptor positive breast cancer, estrogen receptor negative breast cancer, HER-2 positive breast cancer, HER-2 negative breast cancer, triple negative breast cancer, inflammatory breast cancer), colon (including colorectal cancer), kidney (e.g., renal cell carcinoma), liver, lung (including small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma), mesothelioma, genitourinary tract, e.g., ovary (including fallopian, endometrial and peritoneal cancers), cervix, prostate and testes (e.g., metastatic testicular cancer), lymphatic system, rectum, larynx, pancreas (including exocrine pancreatic carcinoma), stomach (e.g., gastroesophageal, upper gastric or lower gastric cancer), gastrointestinal cancer (e.g., anal cancer), gall bladder, thyroid, lymphoma (e.g., Burkitt's, Hodgkin's or non-Hodgkin's lymphoma (e.g., mantle cell lymphoma or anaplastic large cell lymphoma)), myeloma, leukemia (e.g., acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, and chronic lymphoblastic leukemia), Ewing's sarcoma, nasoesophageal cancer, nasopharyngeal cancer, neural and glial cell cancers (e.g., glioblastoma multiforme, neuroblastoma), and head and neck. Preferred cancers include breast cancer (e.g., metastatic or locally advanced breast cancer), prostate cancer (e.g., hormone refractory prostate cancer) and

testicular cancer (e.g., metastatic testicular cancer), renal cell carcinoma, lung cancer (e.g., small cell lung cancer and non-small cell lung cancer (including adenocarcinoma, squamous cell carcinoma, bronchoalveolar carcinoma and large cell carcinoma)), pancreatic cancer (e.g., metastatic or locally advanced pancreatic cancer), gastric cancer (e.g., gastroesophageal, upper gastric or lower gastric cancer), bladder cancer (e.g., advanced bladder cancer), colorectal cancer, squamous cell cancer of the head and neck, ovarian cancer (e.g., advanced ovarian cancer, resistant or relapsed ovarian cancer), lymphoma (e.g., Burkitt's, Hodgkin's or non-Hodgkin's lymphoma), leukemia (e.g., acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, and chronic lymphoblastic leukemia), myeloma, and gastrointestinal cancer.

In one embodiment, the method is a method of treating AKT-positive lymphomas in a subject and the method comprises administering a CDP-rapamycin conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, described herein, at a dosage of 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 12 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, or 50 mg (wherein said dosage is expressed in mg of therapeutic agent, as opposed to mg of conjugate). In some embodiments, the CDP-rapamycin conjugate, particle or composition, e.g., a CDP-rapamycin conjugate, particle or composition, described herein, is administered in combination with an anthracycline (e.g., doxorubicin (e.g., liposomal doxorubicin)).

In one aspect, the invention features, a method of treating cancer, e.g., in a subject. The method comprises administering two or more CDP-therapeutic agent conjugates, wherein one CDP is conjugated to a therapeutic agent and the other CDP is conjugated to a second therapeutic agent, a composition or particle including one or more of the CDP-therapeutic agent conjugates, to the subject to thereby treat the disease. In an embodiment, the CDP-therapeutic agent conjugate is a CDP-cytotoxic agent conjugate, e.g., CDP-topoisomerase inhibitor conjugate, e.g., a CDP-topoisomerase inhibitor I conjugate (e.g., a CDP-camptothecin conjugate, CDP-irinotecan conjugate, CDP-SN-38 conjugate, CDP-topotecan conjugate, CDP-lamellarin D conjugate, a CDP-lurotecan conjugate, particle or composition, a CDP-exatecan conjugate, particle or composition, a CDP-diflomotecan conjugate, particle or composition, and CDP-topoisomerase I inhibitor conjugates which include derivatives of camptothecin, irinotecan, SN-38, lamellarin D,

lurotecan, exatecan, and diflomotecan), a CDP-topoisomerase II inhibitor conjugate (e.g., a CDP-etoposide conjugate, CDP-tenoposide conjugate, CDP-amsacrine conjugate and CDP-topoisomerase II inhibitor conjugates which include derivatives of etoposide, tenoposide, and amsacrine), a CDP-anti-metabolic agent conjugate (e.g., a CDP-antifolate conjugate (e.g., a CDP-pemetrexed conjugate, a CDP-floxuridine conjugate, a CDP-raltitrexed conjugate) or a CDP-pyrimidine analog conjugate (e.g., a CDP-capecitabine conjugate, a CDP-cytarabine conjugate, a CDP-gemcitabine conjugate, a CDP-5FU conjugate)), a CDP-alkylating agent conjugate, a CDP-anthracycline conjugate, a CDP-anti-tumor antibiotic conjugate (e.g., a CDP-HSP90 inhibitor conjugate, e.g., a CDP-geldanamycin conjugate, a CDP-tanespimycin conjugate or a CDP-alvespimycin conjugate), a CDP-platinum based agent conjugate (e.g., a CDP-cisplatin conjugate, a CDP-carboplatin conjugate, a CDP-oxaliplatin conjugate), a CDP-microtubule inhibitor conjugate, a CDP-kinase inhibitor conjugate (e.g., a CDP-seronine/threonine kinase inhibitor conjugate, e.g., a CDP-mTOR inhibitor conjugate, e.g., a CDP-rapamycin conjugate) or a CDP-proteasome inhibitor conjugate (a CDP-boronic acid containing molecule conjugate, e.g., a CDP-bortezomib conjugate); a CDP-immunomodulator conjugate (e.g., a corticosteroid or a rapamycin analog conjugate).

In any of the above aspects or embodiments, the CDP-therapeutic agent conjugate may be administered in the form of a pharmaceutical composition or a particle, e.g., a nanoparticle, e.g., a nanoparticle with an average diameter from 10 to 300 nm, e.g., 15 to 280, 30 to 250, 30 to 200, 20 to 150, 30 to 100, 20 to 80, 30 to 70, 30 to 60 or 30 to 50 nm. In one embodiment, the nanoparticle is 15 to 50 nm in diameter. In one embodiment, the average nanoparticle diameter is from 30 to 60 nm. In one embodiment, the surface charge of the molecule is neutral, or slightly negative. In some embodiments, the zeta potential of the particle surface is from about -80 mV to about 50 mV, about -20 mV to about 20 mV, about -20 mV to about -10 mV, or about -10 mV to about 0.

The details of one or more embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

Brief Description of the Drawings

FIG. 1 depicts exemplary cyclodextrin-containing polymers (CDPs) which may be used for the delivery of therapeutic agents.

FIG. 2 depicts a schematic representation of (β)-cyclodextrin.

FIG. 3 depicts the structure of an exemplary cyclodextrin-containing polymer that may be used for the delivery of therapeutic agents.

FIG. 4 is a table depicting examples of different CDP-taxane conjugates.

FIG. 5 depicts structures of exemplary epothilones that can be used in the CDP-epothilone conjugates.

FIG. 6 is a table depicting examples of different CDP-epothilone conjugates.

FIG. 7 is a table depicting examples of different CDP-proteasome inhibitor conjugates.

FIG. 8 depicts a general strategy for synthesizing linear, branched, or grafted cyclodextrin-containing polymers (CDPs) for loading therapeutic agents, and, optionally, targeting ligands.

FIG. 9 depicts a general scheme for graft CDPs.

FIG. 10 depicts a general scheme of preparing linear CDPs.

Detailed Description of the Invention

The present invention relates to compositions of therapeutic cyclodextrin-containing polymers (CDP) designed for drug delivery of therapeutic agents described herein. In certain embodiments, these cyclodextrin-containing polymers improve drug stability and/or solubility, and/or reduce toxicity, and/or improve efficacy of the therapeutic agent when used *in vivo*.

Furthermore, by selecting from a variety of linker groups that link or couple CDP to a therapeutic agent described herein, and/or targeting ligands, the rate of drug release from the polymers can be attenuated for controlled delivery. The invention also relates to methods of treating subjects with compositions described herein. The invention further relates to methods for conducting a pharmaceutical business comprising manufacturing,

licensing, or distributing kits containing or relating to the CDP-therapeutic agent conjugates, particles and compositions described herein.

More generally, the present invention provides water-soluble, biocompatible polymer conjugates comprising a water-soluble, biocompatible polymer covalently attached to the topoisomerase inhibitor through attachments that are cleaved under biological conditions to release the therapeutic agent.

Polymeric conjugates featured in the methods described herein may be useful to improve solubility and/or stability of a bioactive/therapeutic agent, reduce drug-drug interactions, reduce interactions with blood elements including plasma proteins, reduce or eliminate immunogenicity, protect the agent from metabolism, modulate drug-release kinetics, improve circulation time, improve drug half-life (e.g., in the serum, or in selected tissues, such as tumors), attenuate toxicity, improve efficacy, normalize drug metabolism across subjects of different species, ethnicities, and/or races, and/or provide for targeted delivery into specific cells or tissues.

Definitions

The term "ambient conditions," as used herein, refers to surrounding conditions at about one atmosphere of pressure, 50% relative humidity and about 25 °C.

The term "attach," as used herein with respect to the relationship of a first moiety to a second moiety, e.g., the attachment of a therapeutic agent to a polymer, refers to the formation of a covalent bond between a first moiety and a second moiety. In the same context, "attachment" refers to the covalent bond. For example, a therapeutic agent attached to a polymer is a therapeutic agent covalently bonded to the polymer (e.g., a hydrophobic polymer described herein). The attachment can be a direct attachment, e.g., through a direct bond of the first moiety to the second moiety, or can be through a linker (e.g., through a covalently linked chain of one or more atoms disposed between the first and second moiety). E.g., where an attachment is through a linker, a first moiety (e.g., a drug) is covalently bonded to a linker, which in turn is covalently bonded to a second moiety (e.g., a hydrophobic polymer described herein).

The term "biodegradable" is art-recognized, and includes polymers, compositions and formulations, such as those described herein, that are intended to degrade during use. Biodegradable polymers typically differ from non-biodegradable polymers in that the

former may be degraded during use. In certain embodiments, such use involves *in vivo* use, such as *in vivo* therapy, and in other certain embodiments, such use involves *in vitro* use. In general, degradation attributable to biodegradability involves the degradation of a biodegradable polymer into its component subunits, or digestion, e.g., by a biochemical process, of the polymer into smaller, non-polymeric subunits. In certain embodiments, two different types of biodegradation may generally be identified. For example, one type of biodegradation may involve cleavage of bonds (whether covalent or otherwise) in the polymer backbone. In such biodegradation, monomers and oligomers typically result, and even more typically, such biodegradation occurs by cleavage of a bond connecting one or more of subunits of a polymer. In contrast, another type of biodegradation may involve cleavage of a bond (whether covalent or otherwise) internal to a side chain or that connects a side chain to the polymer backbone. In certain embodiments, one or the other or both general types of biodegradation may occur during use of a polymer.

The term "biodegradation," as used herein, encompasses both general types of biodegradation. The degradation rate of a biodegradable polymer often depends in part on a variety of factors, including the chemical identity of the linkage responsible for any degradation, the molecular weight, crystallinity, biostability, and degree of cross-linking of such polymer, the physical characteristics (e.g., shape and size) of a polymer, assembly of polymers or particle, and the mode and location of administration. For example, a greater molecular weight, a higher degree of crystallinity, and/or a greater biostability, usually lead to slower biodegradation.

The term "carbohydrate," as used herein refers to and encompasses monosaccharides, disaccharides, oligosaccharides and polysaccharides.

The phrase "cleavable under physiological conditions" refers to a bond having a half life of less than about 100 hours, when subjected to physiological conditions. For example, enzymatic degradation can occur over a period of less than about five years, one year, six months, three months, one month, fifteen days, five days, three days, or one day upon exposure to physiological conditions (e.g., an aqueous solution having a pH from about 4 to about 8, and a temperature from about 25°C to about 37°C).

An "effective amount" or "an amount effective" refers to an amount of the CDP-therapeutic agent conjugate which is effective, upon single or multiple dose

administrations to a subject, in treating a cell, or curing, alleviating, relieving or improving a symptom of a disorder. An effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects.

"Pharmaceutically acceptable carrier or adjuvant," as used herein, refers to a carrier or adjuvant that may be administered to a patient, together with a CDP-therapeutic agent conjugate described herein, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the particle. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose, mannitol and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical compositions.

The term "polymer," as used herein, is given its ordinary meaning as used in the art, i.e., a molecular structure featuring one or more repeat units (monomers), connected by covalent bonds. The repeat units may all be identical, or in some cases, there may be more than one type of repeat unit present within the polymer. In some cases, the polymer is biologically derived, i.e., a biopolymer. Non-limiting examples of biopolymers include peptides or proteins (i.e., polymers of various amino acids), or nucleic acids such as DNA or RNA. In some instances, a polymer may be comprised of subunits, e.g., a subunit described herein, wherein a subunit comprises polymers, e.g., PEG, but the subunit may

be repeated within a conjugate. In some embodiments, a conjugate may comprise only one subunit described herein; however conjugates may comprise more than one identical subunit.

As used herein the term "low aqueous solubility" refers to water insoluble compounds having poor solubility in water, that is <5 mg/ml at physiological pH (6.5-7.4). Preferably, their water solubility is <1 mg/ml, more preferably <0.1 mg/ml. It is desirable that the drug is stable in water as a dispersion; otherwise a lyophilized or spray-dried solid form may be desirable.

A "hydroxy protecting group" as used herein, is well known in the art and includes those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference. Suitable hydroxy protecting groups include, for example, acyl (e.g., acetyl), triethylsilyl (TES), *t*–butyldimethylsilyl (TBDMS), 2,2,2-trichloroethoxycarbonyl (Troc), and carbobenzyloxy (Cbz).

"Inert atmosphere," as used herein, refers to an atmosphere composed primarily of an inert gas, which does not chemically react with the CDP-therapeutic agent conjugates, particles, compositions or mixtures described herein. Examples of inert gases are nitrogen (N_2), helium, and argon.

"Linker," as used herein, is a moiety having at least two functional groups. One functional group is capable of reacting with a functional group on a polymer described herein, and a second functional group is capable of reacting with a functional group on agent described herein. In some embodiments the linker has just two functional groups. A linker may have more than two functional groups (e.g., 3, 4, 5, 6, 7, 8, 9, 10 or more functional groups), which may be used, e.g., to link multiple agents to a polymer. Depending on the context, linker can refer to a linker moiety before attachment to either of a first or second moiety (e.g., agent or polymer), after attachment to one moiety but before attachment to a second moiety, or the residue of the linker present after attachment to both the first and second moiety.

The term "lyoprotectant," as used herein refers to a substance present in a lyophilized preparation. Typically it is present prior to the lyophilization process and persists in the resulting lyophilized preparation. It can be used to protect nanoparticles,

liposomes, and/or micelles during lyophilization, for example to reduce or prevent aggregation, particle collapse and/or other types of damage. In an embodiment the lyoprotectant is a cryoprotectant. In an embodiment the lyoprotectant is a carbohydrate.

As used herein, the term "prevent" or "preventing" as used in the context of the administration of an agent to a subject, refers to subjecting the subject to a regimen, e.g., the administration of a CDP-therapeutic agent conjugate such that the onset of at least one symptom of the disorder is delayed as compared to what would be seen in the absence of the regimen.

As used herein, the term "subject" is intended to include human and non-human animals. Exemplary human subjects include a human patient having a disorder, e.g., a disorder described herein, or a normal subject. The term "non-human animals" includes all vertebrates, e.g., non-mammals (such as chickens, amphibians, reptiles) and mammals, such as non-human primates, domesticated and/or agriculturally useful animals, e.g., sheep, dog, cat, cow, pig, etc.

The term "therapeutic agent," as used herein, refers to a moiety, wherein upon administration of the moiety to a subject, the subject receives a therapeutic effect (e.g., administration of the therapeutic agent treats a cell, or cures, alleviates, relieves or improves a symptom of a disorder).

As used herein, the term "treat" or "treating" a subject having a disorder refers to subjecting the subject to a regimen, e.g., the administration of a CDP-therapeutic agent conjugate such that at least one symptom of the disorder is cured, healed, alleviated, relieved, altered, remedied, ameliorated, or improved. Treating includes administering an amount effective to alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder or the symptoms of the disorder. The treatment may inhibit deterioration or worsening of a symptom of a disorder.

The term "acyl" refers to an alkylcarbonyl, cycloalkylcarbonyl, arylcarbonyl, heterocyclylcarbonyl, or heteroarylcarbonyl substituent, any of which may be further substituted (e.g., by one or more substituents). Exemplary acyl groups include acetyl ($CH_3C(O)$-), benzoyl ($C_6H_5C(O)$-), and acetylamino acids (e.g., acetylglycine, $CH_3C(O)NHCH_2C(O)$-.

The term "alkoxy" refers to an alkyl group, as defined below, having an oxygen radical attached thereto. Representative alkoxy groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl-substituted cycloalkyl groups, and cycloalkyl-substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chains, C_3-C_{30} for branched chains), and more preferably 20 or fewer, and most preferably 10 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure. The term "alkylenyl" refers to a divalent alkyl, e.g., -CH_2-, -CH_2CH_2-, and -$CH_2CH_2CH_2$-.

The term "alkenyl" refers to an aliphatic group containing at least one double bond.

The terms "alkoxyl" or "alkoxy" refers to an alkyl group, as defined below, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl-substituted cycloalkyl groups, and cycloalkyl-substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chains, C_3-C_{30} for branched chains), and more preferably 20 or fewer, and most preferably 10 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

The term "alkynyl" refers to an aliphatic group containing at least one triple bond.

The term "aralkyl" or "arylalkyl" refers to an alkyl group substituted with an aryl group (e.g., a phenyl or naphthyl).

The term "aryl" includes 5-14 membered single-ring or bicyclic aromatic groups, for example, benzene, naphthalene, and the like. The aromatic ring can be substituted at

one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, polycyclyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphate, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, $-CF_3$, -CN, or the like. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls. Each ring can contain, e.g., 5-7 members. The term "arylene" refers to a divalent aryl, as defined herein.

The term "arylalkenyl" refers to an alkenyl group substituted with an aryl group. The term "carboxy" refers to a –C(O)OH or salt thereof.

The term "hydroxy" and "hydroxyl" are used interchangeably and refer to –OH. The term "substituents" refers to a group "substituted" on an alkyl, cycloalkyl, alkenyl, alkynyl, heterocyclyl, heterocycloalkenyl, cycloalkenyl, aryl, or heteroaryl group at any atom of that group. Any atom can be substituted. Suitable substituents include, without limitation, alkyl (e.g., C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12 straight or branched chain alkyl), cycloalkyl, haloalkyl (e.g., perfluoroalkyl such as CF_3), aryl, heteroaryl, aralkyl, heteroaralkyl, heterocyclyl, alkenyl, alkynyl, cycloalkenyl, heterocycloalkenyl, alkoxy, haloalkoxy (e.g., perfluoroalkoxy such as OCF_3), halo, hydroxy, carboxy, carboxylate, cyano, nitro, amino, alkyl amino, SO_3H, sulfate, phosphate, methylenedioxy (-O-CH_2-O- wherein oxygens are attached to vicinal atoms), ethylenedioxy, oxo, thioxo (e.g., C=S), imino (alkyl, aryl, aralkyl), $S(O)_n$alkyl (where n is 0-2), $S(O)_n$ aryl (where n is 0-2), $S(O)_n$ heteroaryl (where n is 0-2), $S(O)_n$ heterocyclyl (where n is 0-2), amine (mono-, di-, alkyl, cycloalkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, and combinations thereof), ester (alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl), amide (mono-, di-, alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, and combinations thereof), sulfonamide (mono-, di-, alkyl, aralkyl, heteroaralkyl, and combinations thereof). In one aspect, the substituents on a group are independently any one single, or any subset of the aforementioned substituents. In another aspect, a substituent may itself be substituted with any one of the above substituents.

The terms "halo" and "halogen" means halogen and includes chloro, fluoro, bromo, and iodo.

The terms "hetaralkyl", "heteroaralkyl" or "heteroarylalkyl" refers to an alkyl group substituted with a heteroaryl group.

The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples of heteroaryl groups include pyridyl, furyl or furanyl, imidazolyl, benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinolinyl, indolyl, thiazolyl, and the like. The term "heteroarylene" refers to a divalent heteroaryl, as defined herein.

The term "heteroarylalkenyl" refers to an alkenyl group substituted with a heteroaryl group.

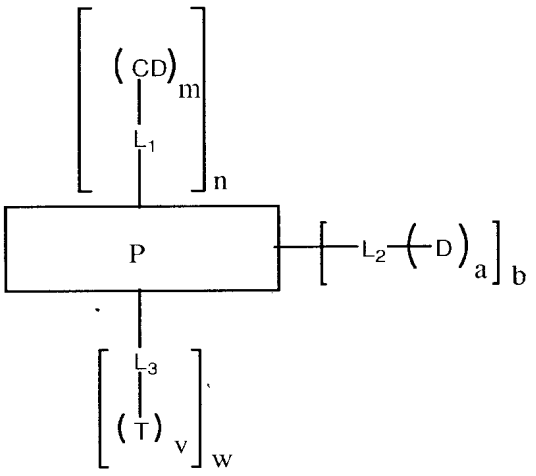
CDP-Therapeutic Agent Conjugates, Particles, and Compositions

Described herein are cyclodextrin containing polymer ("CDP")-therapeutic agent conjugates, wherein one or more therapeutic agents are covalently attached to the CDP (e.g., either directly or through a linker). The CDP-therapeutic agent conjugates include linear or branched cyclodextrin-containing polymers and polymers grafted with cyclodextrin. Exemplary cyclodextrin-containing polymers that may be modified as described herein are taught in U.S. Patent Nos. 7,270,808, 6,509,323, 7,091,192, 6,884,789, U.S. Publication Nos. 20040087024, 20040109888 and 20070025952.

The CDP-therapeutic agent conjugate can include a therapeutic agent such that the CDP-therapeutic agent conjugate can be used to treat an autoimmune disease, inflammatory disease, or cancer. Exemplary therapeutic agents that can be used in a conjugate described herein include the following: a topisomerase inhibitor, an anti-metabolic agent, a pyrimide analog, an alkylating agent, an anthracycline an anti-tumor

antibiotic, a platinum based agent, a microtubule inhibitor, a proteasome inhibitor, and a corticosteroid.

Accordingly, in one embodiment the CDP-therapeutic agent conjugate is represented by Formula I:



(I)

wherein

P represents a linear or branched polymer chain;

CD represents a cyclic moiety such as a cyclodextrin moiety;

L_1, L_2 and L_3, independently for each occurrence, may be absent or represent a linker group;

D, independently for each occurrence, represents a therapeutic agent or a prodrug thereof;

T, independently for each occurrence, represents a targeting ligand or precursor thereof;

a, m, and v, independently for each occurrence, represent integers in the range of 1 to 10 (preferably 1 to 8, 1 to 5, or even 1 to 3);

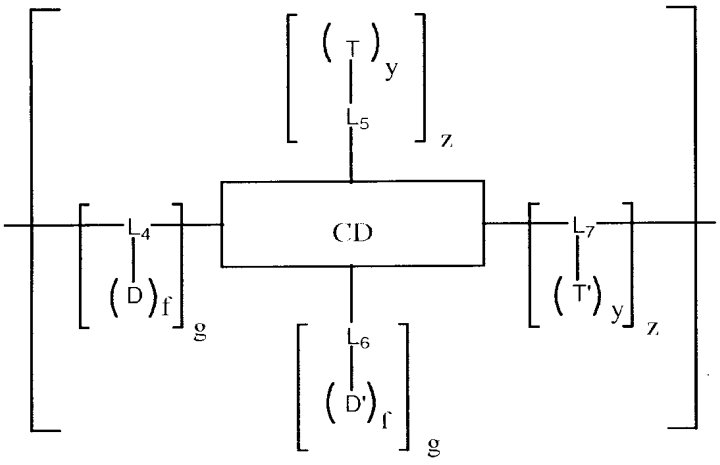
n and w, independently for each occurrence, represent an integer in the range of 0 to about 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5); and

b represents an integer in the range of 1 to about 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5),

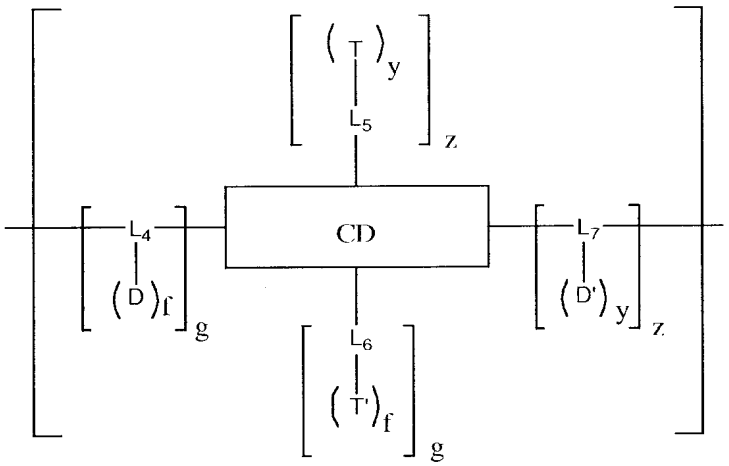
wherein either P comprises cyclodextrin moieties or n is at least 1.

In some embodiments, one or more of one type of therapeutic agent in the CDP-therapeutic agent conjugate can be replaced with another, different type of therapeutic agent, e.g., another cytotoxic agent or immunomodulator. Examples of other cytotoxic agents are described herein. Examples of immunomodulators include a steroid, e.g., prednisone, and a NSAID.

In certain embodiments, P contains a plurality of cyclodextrin moieties within the polymer chain as opposed to the cyclodextrin moieties being grafted on to pendant groups off of the polymeric chain. Thus, in certain embodiments, the polymer chain of formula I further comprises n' units of U, wherein n' represents an integer in the range of 1 to about 30,000, e.g., from 4-100, 4-50, 4-25, 4-15, 6-100, 6-50, 6-25, and 6-15 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <20, <15, <10, or even <5); and U is represented by one of the general formulae below:



or



wherein

CD represents a cyclic moiety, such as a cyclodextrin moiety, or derivative thereof;

L_4, L_5, L_6, and L_7, independently for each occurrence, may be absent or represent a linker group;

D and D', independently for each occurrence, represent the same or different therapeutic agent or prodrug forms thereof;

T and T', independently for each occurrence, represent the same or different targeting ligand or precursor thereof;

f and y, independently for each occurrence, represent an integer in the range of 1 and 10; and

g and z, independently for each occurrence, represent an integer in the range of 0 and 10.

In some embodiments, one g is 0 and one g is 1-10. In some embodiments, one z is 0 and one z is 1-10.

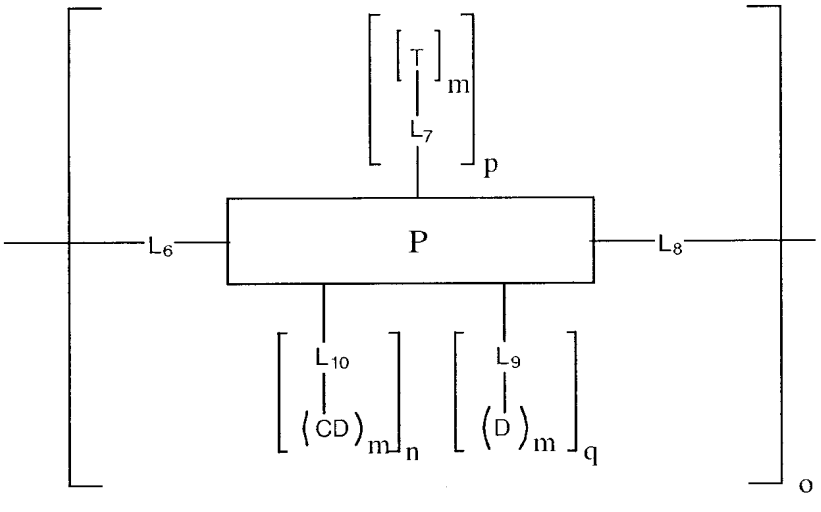
Preferably the polymer has a plurality of D or D' moieties. In some embodiments, at least 50% of the U units have at least one D or D'. In some embodiments, one or more of one type of therapeutic agent in the CDP-therapeutic agent conjugate can be replaced with another, different type of therapeutic agent, e.g., another cytotoxic agent or immunomodulator.

In preferred embodiments, L_4 and L_7 represent linker groups.

The CDP may include a polycation, polyanion, or non-ionic polymer. A polycationic or polyanionic polymer has at least one site that bears a positive or negative charge, respectively. In certain such embodiments, at least one of the linker moiety and the cyclic moiety comprises such a charged site, so that every occurrence of that moiety includes a charged site. In some embodiments, the CDP is biocompatible.

In certain embodiments, the CDP may include polysaccharides, and other non-protein biocompatible polymers, and combinations thereof, that contain at least one terminal hydroxyl group, such as polyvinylpyrrollidone, poly(ethylene glycol) (PEG), polysuccinic anhydride, polysebacic acid, PEG-phosphate, polyglutamate, polyethylenimine, maleic anhydride divinylether (DIVMA), cellulose, pullulans, inulin, polyvinyl alcohol (PVA), N-(2-hydroxypropyl)methacrylamide (HPMA), dextran and hydroxyethyl starch (HES), and have optional pendant groups for grafting therapeutic agents, targeting ligands and/or cyclodextrin moieties. In certain embodiments, the polymer may be biodegradable such as poly(lactic acid), poly(glycolic acid), poly(alkyl 2-cyanoacrylates), polyanhydrides, and polyorthoesters, or bioerodible such as polylactide-glycolide copolymers, and derivatives thereof, non-peptide polyaminoacids, polyiminocarbonates, poly alpha-amino acids, polyalkyl-cyano-acrylate, polyphosphazenes or acyloxymethyl poly aspartate and polyglutamate copolymers and mixtures thereof.

In another embodiment the CDP-therapeutic agent conjugate is represented by Formula II:



(II)

wherein

P represents a monomer unit of a polymer that comprises cyclodextrin moieties;

T, independently for each occurrence, represents a targeting ligand or a precursor thereof;

L_6, L_7, L_8, L_9, and L_{10}, independently for each occurrence, may be absent or represent a linker group;

CD, independently for each occurrence, represents a cyclodextrin moiety or a derivative thereof;

D, independently for each occurrence, represents a therapeutic agent or a prodrug form thereof;

m, independently for each occurrence, represents an integer in the range of 1 to 10 (preferably 1 to 8, 1 to 5, or even 1 to 3);

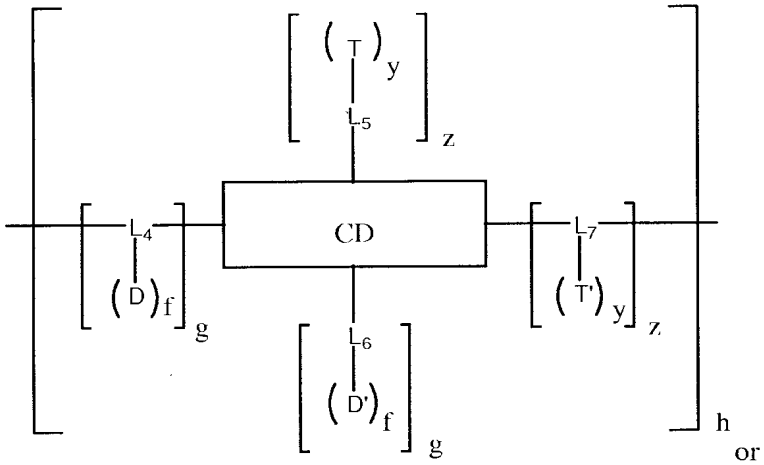
o represents an integer in the range of 1 to about 30,000 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <10, or even <5); and

p, n, and q, independently for each occurrence, represent an integer in the range of 0 to 10 (preferably 0 to 8, 0 to 5, 0 to 3, or even 0 to about 2),

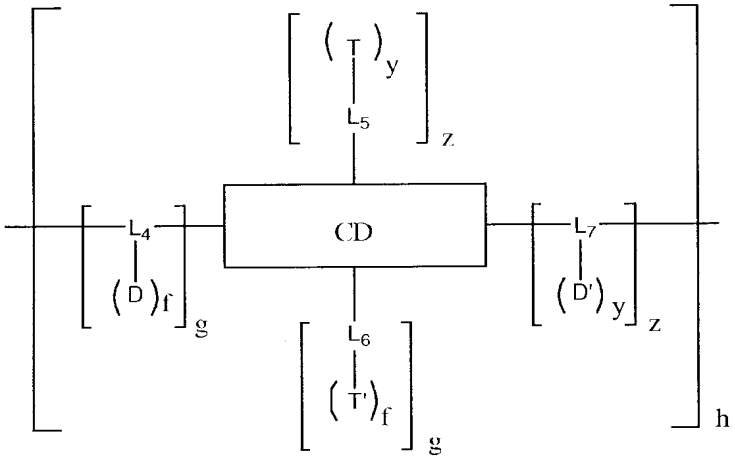
wherein CD and D are preferably each present at least 1 location (preferably at least 5, 10, 25, or even 50 or 100 locations) in the compound.

In some embodiments, one or more of the therapeutic agents in the CDP-therapeutic agent conjugate can be replaced with another, different therapeutic agent, e.g., another cytotoxic agent or immunomodulator. Examples of cytotoxic agents are described herein. Examples of immunomodulators include a steroid, e.g., prednisone, or a NSAID.

In another embodiment the CDP-therapeutic agent conjugate is represented either of the formulae below:



or



wherein

CD represents a cyclic moiety, such as a cyclodextrin moiety, or derivative thereof;

L_4, L_5, L_6, and L_7, independently for each occurrence, may be absent or represent a linker group;

D and D', independently for each occurrence, represent the same or different therapeutic agent;

T and T', independently for each occurrence, represent the same or different targeting ligand or precursor thereof;

f and y, independently for each occurrence, represent an integer in the range of 1 and 10 (preferably 1 to 8, 1 to 5, or even 1 to 3);

g and z, independently for each occurrence, represent an integer in the range of 0 and 10 (preferably 0 to 8, 0 to 5, 0 to 3, or even 0 to about 2); and

h represents an integer in the range of 1 and 30,000, e.g., from 4-100, 4-50, 4-25, 4-15, 6-100, 6-50, 6-25, and 6-15 (preferably <25,000, <20,000, <15,000, <10,000, <5,000, <1,000, <500, <100, <50, <25, <20, <15, <10, or even <5),

wherein at least one occurrence (and preferably at least 5, 10, or even at least 20, 50, or 100 occurrences) of g represents an integer greater than 0.

In some embodiments, one g is 0 and one g is 1-10. In some embodiments, one z is 0 and one z is 1-10.

Preferably the polymer has a plurality of D or D' moieties. In some embodiments, at least 50% of the polymer repeating units have at least one D or D'. In some embodiments, one or more of the therapeutic agent in the CDP-therapeutic agent conjugate can be replaced with another therapeutic agent, e.g., another cytotoxic agent or immunomodulator.

In preferred embodiments, L4 and L7 represent linker groups.

In certain such embodiments, the CDP comprises cyclic moieties alternating with linker moieties that connect the cyclic structures, e.g., into linear or branched polymers, preferably linear polymers. The cyclic moieties may be any suitable cyclic structures, such as cyclodextrins, crown ethers (e.g., 18-crown-6, 15-crown-5, 12-crown-4, etc.), cyclic oligopeptides (e.g., comprising from 5 to 10 amino acid residues), cryptands or cryptates (e.g., cryptand [2.2.2], cryptand-2,1,1, and complexes thereof), calixarenes, or cavitands, or any combination thereof. Preferably, the cyclic structure is (or is modified to be) water-soluble. In certain embodiments, e.g., for the preparation of a linear polymer, the cyclic structure is selected such that under polymerization conditions, exactly two moieties of each cyclic structure are reactive with the linker moieties, such

that the resulting polymer comprises (or consists essentially of) an alternating series of cyclic moieties and linker moieties, such as at least four of each type of moiety. Suitable difunctionalized cyclic moieties include many that are commercially available and/or amenable to preparation using published protocols. In certain embodiments, conjugates are soluble in water to a concentration of at least 0.1 g/mL, preferably at least 0.25 g/mL.

Thus, in certain embodiments, the invention relates to novel compositions of therapeutic cyclodextrin-containing polymeric compounds designed for delivery of a therapeutic agent described herein. In certain embodiments, these CDPs improve drug stability and/or solubility, and/or reduce toxicity, and/or improve efficacy of the therapeutic agent when used *in vivo*. Furthermore, by selecting from a variety of linker groups, and/or targeting ligands, the rate of therapeutic agent release from the CDP can be attenuated for controlled delivery.

Disclosed herein are various types of linear, branched, or grafted CDPs wherein a therapeutic agent is covalently bound to the polymer. In certain embodiments, the therapeutic agent is covalently linked via a biohydrolyzable bond, for example, an ester, amide, carbamates, or carbonate. General strategies for synthesizing linear, branched or grafted cyclodextrin-containing polymers (CDPs) for loading therapeutic agents, and optional targeting ligands are described in U.S. Patent Nos. 7,270,808, 6,509,323, 7,091,192, 6,884,789, U.S. Publication Nos. 20040087024, 20040109888 and 20070025952, all of which are incorporated by reference in their entireties. As shown in FIG. 1, the general strategies can be used to achieve a variety of different cyclodextrin-containing polymers for the delivery of therapeutic agents, e.g., cytotoxic agents, e.g., topoisomerase inhibitors, e.g., a topoisomerase I inhibitor (e.g., camptothecin, irinotecan, SN-38, topotecan, lamellarin D, lurotecan, exatecan, diflomotecan, or derivatives thereof), or a topoisomerase II inhibitor (e.g., an etoposide, a tenoposide, amsacrine, or derivatives thereof), an anti-metabolic agent (e.g., an antifolate (e.g., pemetrexed, floxuridine, or raltitrexed) or a pyrimidine conjugate (e.g., capecitabine, cytarabine, gemcitabine, or 5FU)), an alkylating agent, an anthracycline, an anti-tumor antibiotic (e.g., a HSP90 inhibitor, e.g., geldanamycin), a platinum based agent (e.g., cisplatin, carboplatin, or oxaliplatin), a microtubule inhibitor, a kinase inhibitor (e.g., a seronine/threonine kinase inhibitor, e.g., a mTOR inhibitor, e.g., rapamycin) or a

proteasome inhibitor. The resulting CDPs are shown graphically as polymers (A)-(L) of FIG. 1. Generally, wherein R can be a therapeutic agent or an OH, it is required that at least one R within the polymer be a therapeutic agent, e.g., the loading is not zero. Generally, m, n, and o, if present, are independently from 1 to 1000, e.g., 1 to 500, e.g., 1 to 100, e.g., 1 to 50, e.g., 1 to 25, e.g., 10 to 20, e.g. about 14.

In certain embodiments, the CDP comprises a linear cyclodextrin-containing polymer, e.g., the polymer backbone includes cyclodextrin moieties. For example, the polymer may be a water-soluble, linear cyclodextrin polymer produced by providing at least one cyclodextrin derivative modified to bear one reactive site at each of exactly two positions, and reacting the cyclodextrin derivative with a linker having exactly two reactive moieties capable of forming a covalent bond with the reactive sites under polymerization conditions that promote reaction of the reactive sites with the reactive moieties to form covalent bonds between the linker and the cyclodextrin derivative, whereby a linear polymer comprising alternating units of cyclodextrin derivatives and linkers is produced. Alternatively the polymer may be a water-soluble, linear cyclodextrin polymer having a linear polymer backbone, which polymer comprises a plurality of substituted or unsubstituted cyclodextrin moieties and linker moieties in the linear polymer backbone, wherein each of the cyclodextrin moieties, other than a cyclodextrin moiety at the terminus of a polymer chain, is attached to two of said linker moieties, each linker moiety covalently linking two cyclodextrin moieties. In yet another embodiment, the polymer is a water-soluble, linear cyclodextrin polymer comprising a plurality of cyclodextrin moieties covalently linked together by a plurality of linker moieties, wherein each cyclodextrin moiety, other than a cyclodextrin moiety at the terminus of a polymer chain, is attached to two linker moieties to form a linear cyclodextrin polymer.

In some embodiments, the CDP-therapeutic agent conjugate comprises a water soluble linear polymer conjugate comprising: cyclodextrin moieties; comonomers which do not contain cyclodextrin moieties (comonomers); and a plurality of therapeutic agents; wherein the CDP-therapeutic agent conjugate comprises at least four, five six, seven, eight, etc., cyclodextrin moieties and at least four, five six, seven, eight, etc., comonomers. In some embodiments, the therapeutic agent is a therapeutic gaent

described herein, e.g., the CDP-therapeutic agent conjugate is a CDP-cytotoxic agent conjugate, e.g., CDP-topoisomerase inhibitor conjugate, e.g., a CDP-topoisomerase inhibitor I conjugate (e.g., a CDP-camptothecin conjugate, CDP-irinotecan conjugate, CDP-SN-38 conjugate, CDP-topotecan conjugate, CDP-lamellarin D conjugate, a CDP-lurotecan conjugate, particle or composition, a CDP-exatecan conjugate, particle or composition, a CDP-diflomotecan conjugate, particle or composition, and CDP-topoisomerase I inhibitor conjugates which include derivatives of camptothecin, irinotecan, SN-38, lamellarin D, lurotecan, exatecan, and diflomotecan), a CDP-topoisomerase II inhibitor conjugate (e.g., a CDP-eptoposide conjugate, CDP-tenoposide conjugate, CDP-amsacrine conjugate and CDP-topoisomerase II inhibitor conjugates which include derivatives of etoposide, tenoposide, and amsacrine), a CDP-anti-metabolic agent conjugate (e.g., a CDP-antifolate conjugate (e.g., a CDP-pemetrexed conjugate, a CDP-floxuridine conjugate, a CDP-raltitrexed conjugate) or a CDP-pyrimidine analog conjugate (e.g., a CDP-capecitabine conjugate, a CDP-cytarabine conjugate, a CDP-gemcitabine conjugate, a CDP-5FU conjugate)), a CDP-alkylating agent conjugate, a CDP-anthracycline conjugate, a CDP-anti-tumor antibiotic conjugate (e.g., a CDP-HSP90 inhibitor conjugate, e.g., a CDP-geldanamycin conjugate, a CDP-tanespimycin conjugate or a CDP-alvespimycin conjugate), a CDP-platinum based agent conjugate (e.g., a CDP-cisplatin conjugate, a CDP-carboplatin conjugate, a CDP-oxaliplatin conjugate), a CDP-microtubule inhibitor conjugate, a CDP-kinase inhibitor conjugate (e.g., a CDP-seronine/threonine kinase inhibitor conjugate, e.g., a CDP-mTOR inhibitor conjugate, e.g., a CDP-rapamycin conjugate) or a a CDP-proteasome inhibitor conjugate (e.g., CDP-boronic acid containing molecule conjugate, e.g., a CDP-bortezomib conjugate) or a CDP-immunomodulator conjugate (e.g., a CDP-corticosteroid or a CDP-rapamycin analog conjugate).

The therapeutic agent can be attached to the CDP via a functional group such as a hydroxyl group, or where appropriate, an amino group.

In some embodiments, one or more of one type of therapeutic agent in the CDP-therapeutic agent conjugate can be replaced with another, different type of therapeutic agent, e.g., another anticancer agent or anti-inflammatory agent.

In some embodiments, the least four cyclodextrin moieties and at least four comonomers alternate in the CDP-therapeutic agent conjugate. In some embodiments, the therapeutic agents are cleaved from said CDP-therapeutic agent conjugate under biological conditions to release the therapeutic agent. In some embodiments, the cyclodextrin moieties comprise linkers to which therapeutic agents are linked. In some embodiments, the therapeutic agents are attached via linkers.

In some embodiments, the comonomer comprises residues of at least two functional groups through which reaction and linkage of the cyclodextrin monomers was achieved. In some embodiments, the functional groups, which may be the same or different, terminal or internal, of each comonomer comprise an amino, acid, imidazole, hydroxyl, thio, acyl halide, -HC=CH-, —C≡C— group, or derivative thereof. In some embodiments, the two functional groups are the same and are located at termini of the comonomer precursor. In some embodiments, a comonomer contains one or more pendant groups with at least one functional group through which reaction and thus linkage of a therapeutic agent was achieved. In some embodiments, the functional groups, which may be the same or different, terminal or internal, of each comonomer pendant group comprise an amino, acid, imidazole, hydroxyl, thiol, acyl halide, ethylene, ethyne group, or derivative thereof. In some embodiments, the pendant group is a substituted or unsubstituted branched, cyclic or straight chain C1-C10 alkyl, or arylalkyl optionally containing one or more heteroatoms within the chain or ring. In some embodiments, the cyclodextrin moiety comprises an alpha, beta, or gamma cyclodextrin moiety. In some embodiments, the therapeutic agent is at least 5%, 10%, 15%, 20%, 25%, 30%, or 35% by weight of CDP-therapeutic agent conjugate.

In some embodiments, the comonomer comprises polyethylene glycol of molecular weight 3,400 Da, the cyclodextrin moiety comprises beta-cyclodextrin, the theoretical maximum loading of a therapeutic agent such as a topoisomerase inhibitor on a CDP-therapeutic agent conjugate (e.g., a CDP-topoisomerase inhibitor conjugate) is 25% (e.g., 20%, 15%, 13%, or 10%) by weight, and the therapeutic agent (e.g., a topoisomerase inhibitor) is 4-20% by weight (e.g., 6-10% by weight) of CDP-therapeutic agent conjugate (e.g., CDP-topoisomerase inhibitor conjugate). In some embodiments, the therapeutic agent (e.g., a topoisomerase inhibitor) is poorly soluble in water. In some

embodiments, the solubility of the therapeutic agent (e.g., a topoisomerase inhibitor) is <5 mg/ml at physiological pH. In some embodiments, the therapeutic agent (e.g., a topoisomerase inhibitor) is a hydrophobic compound with a log P>0.4, >0.6, >0.8, >1, >2, >3, >4, or >5.

In some embodiments, the therapeutic agent is attached to the CDP via a second compound (e.g., a linker).

In some embodiments, administration of the CDP-therapeutic agent conjugate to a subject results in release of the therapeutic agent over a period of at least 6 hours. In some embodiments, administration of the CDP-therapeutic agent conjugate to a subject results in release of the thereapeutic agent over a period of 2 hours, 3 hours, 5 hours, 6 hours, 8 hours, 10 hours, 15 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 7 days, 10 days, 14 days, 17 days, 20 days, 24 days, 27 days up to a month. In some embodiments, upon administration of the CDP-therapeutic agent conjugate to a subject, the rate of therapeutic agent release is dependent primarily upon the rate of hydrolysis of the therapeutic agent as opposed to enzymatic cleavage.

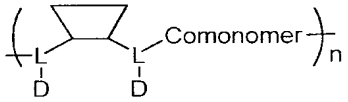
In some embodiments, the CDP-therapeutic agent conjugate has a molecular weight of 10,000-500,000 Da (e.g., 20,000-300,000, 30,000-200,000, or 40,000-200,000, or 50,000-100,000). In some embodiments, the cyclodextrin moieties make up at least about 2%, 5%, 10%, 20%, 30%, 50% or 80% of the CDP-therapeutic agent conjugate by weight.

In some embodiments, the CDP-therapeutic agent conjugate is made by a method comprising providing cyclodextrin moiety precursors modified to bear one reactive site at each of exactly two positions, and reacting the cyclodextrin moiety precursors with comonomer precursors having exactly two reactive moieties capable of forming a covalent bond with the reactive sites under polymerization conditions that promote reaction of the reactive sites with the reactive moieties to form covalent bonds between the comonomers and the cyclodextrin moieties, whereby a CDP comprising alternating units of a cyclodextrin moiety and a comonomer is produced. In some embodiments, the cyclodextrin moiety precursors are in a composition, the composition being substantially free of cyclodextrin moieties having other than two positions modified to bear a reactive

site (e.g., cyclodextrin moieties having 1, 3, 4, 5, 6, or 7 positions modified to bear a reactive site).

In some embodiments, a comonomer of the CDP-therapeutic agent conjugate comprises a moiety selected from the group consisting of: an alkylene chain, polysuccinic anhydride, poly-L-glutamic acid, poly(ethyleneimine), an oligosaccharide, and an amino acid chain. In some embodiments, a CDP-therapeutic agent conjugate comonomer comprises a polyethylene glycol chain. In some embodiments, a comonomer comprises a moiety selected from: polyglycolic acid and polylactic acid chain. In some embodiments, a comonomer comprises a hydrocarbylene group wherein one or more methylene groups is optionally replaced by a group Y (provided that none of the Y groups are adjacent to each other), wherein each Y, independently for each occurrence, is selected from, substituted or unsubstituted aryl, heteroaryl, cycloalkyl, heterocycloalkyl, or -O-, C(=X) (wherein X is NR_1, O or S), -OC(O)-, -C(=O)O, -NR_1-, -NR_1CO-, -C(O)NR_1-, -$S(O)_n$- (wherein n is 0, 1, or 2), -OC(O)-NR_1, -NR_1-C(O)-NR_1-, -$NR_1$1-C(NR_1)-NR_1-, and -B(OR_1)-; and R_1, independently for each occurrence, represents H or a lower alkyl.

In some embodiments, the CDP-therapeutic agent conjugate is a polymer having attached thereto a plurality of D moieties of the following formula:

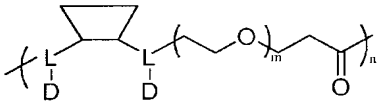


wherein each L is independently a linker, and each D is independently a therapeutic agent, a prodrug derivative thereof, or absent; and each comonomer is independently a comonomer described herein, and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, provided that the polymer comprises at least one therapeutic agent and in some embodiments, at least two therapeutic agent. In some embodiments, the molecular weight of the comonomer is from about 2000 to about 5000 Da (e.g., from about 3000 to about 4000 Da (e.g., about 3400 Da).

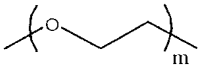
In some embodiments, the therapeutic agent is a therapeutic agent described herein. The therapeutic agent can be attached to the CDP via a functional group such as a hydroxyl group, or where appropriate, an amino group. In some embodiments, one or

more of the therapeutic agent in the CDP-therapeutic agent conjugate can be replaced with another therapeutic agent, e.g., another cytotoxic agent or immunomodulator.

In some embodiments, the CDP-therapeutic agent conjugate is a polymer having attached thereto a plurality of D moieties of the following formula:



wherein each L is independently a linker, and each D is independently a therapeutic agent, a prodrug derivative thereof, or absent, provided that the polymer comprises at least one therapeutic agent and in some embodiments, at least two therapeutic agent; and

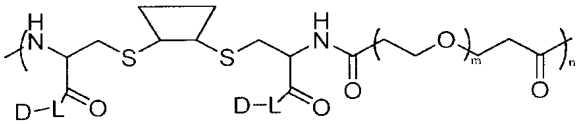
wherein the group 

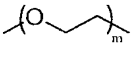
has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20.

In some embodiments, the therapeutic agent is a therapeutic agent described herein. The therapeutic agent can be attached to the CDP via a functional group such as a hydroxyl group, or where appropriate, an amino group. In some embodiments, one or more of the therapeutic agent in the CDP-therapeutic agent conjugate can be replaced with another therapeutic agent, e.g., another cytotoxic agent or immunomodulator.

In some embodiments, less than all of the L moieties are attached to D moieties, meaning in some embodiments, at least one D is absent. In some embodiments, the loading of the D moieties on the CDP-therapeutic agent conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%). In some embodiments, each L independently comprises an amino acid or a derivative thereof. In some embodiments, each L independently comprises a plurality of amino acids or derivatives thereof. In some embodiments, each L is independently a dipeptide or derivative thereof. In one embodiment, L is one ore more of: alanine, arginine, histidine, lysine, aspartic acid, glutamic acid, serine, threonine, asparganine, glutamine, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine and valine.

In some embodiments, the CDP-therapeutic agent conjugate is a polymer having attached thereto a plurality of L-D moieties of the following formula:

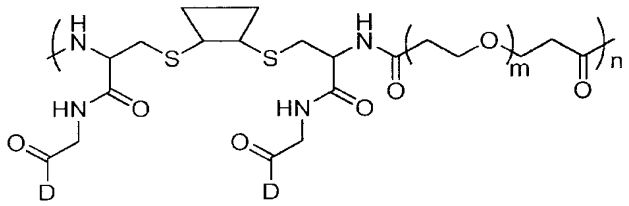


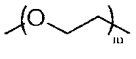
wherein each L is independently a linker or absent and each D is independently a therapeutic agent described herein, a prodrug derivative thereof, or absent and wherein the group  has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, provided that the polymer comprises at least one therapeutic agent and in some embodiments, at least two therapeutic agent.

In some embodiments, less than all of the C(=O) moieties are attached to L-D moieties, meaning in some embodiments, at least one L and/or D is absent. In some embodiments, the loading of the L, D and/or L-D moieties on the CDP-therapeutic agent conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%). In some embodiments, each L is independently an amino acid or derivative thereof. In some embodiments, each L is glycine or a derivative thereof.

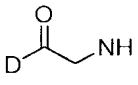
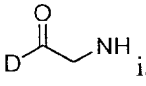
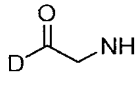
In some embodiments, one or more of the therapeutic agent in the CDP-therapeutic agent conjugate can be replaced with another therapeutic agent, e.g., another cytotoxic agent or immunomodulator.

In some embodiments, the CDP-therapeutic agent conjugate is a polymer having the following formula:



wherein D is independently a therapeutic agent described herein, a prodrug derivative thereof, or absent, the group  has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, provided that the

polymer comprises at least one therapeutic agent and in some embodiments, at least two therapeutic agent.

In some embodiments, less than all of the C(=O) moieties are attached to  moieties, meaning in some embodiments,  is absent, provided that the polymer comprises at least one therapeutic agent and in some embodiments, at least two therapeutic agent. In some embodiments, the loading of the  moieties on the CDP-therapeutic agent conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%).

In some embodiments, one or more of the therapeutic agent in the CDP-therapeutic agent conjugate can be replaced with another therapeutic agent, e.g., another cytotoxic agent or immunomodulator.

In some embodiments, the CDP-therapeutic agent conjugate will contain a therapeutic agent and at least one additional therapeutic agent (e.g., a first and second therapeutic agent where the first and second therapeutic agents are different therapeutic agents). For instance, a therapeutic agent described herein and one more different cancer drugs, an immunosuppressant, an antibiotic or an anti-inflammatory agent may be grafted on to the polymer via optional linkers. By selecting different linkers for different drugs, the release of each drug may be attenuated to achieve maximal dosage and efficacy.

Cyclodextrins

In certain embodiments, the cyclodextrin moieties make up at least about 2%, 5% or 10% by weight, up to 20%, 30%, 50% or even 80% of the CDP by weight. In certain embodiments, the therapeutic agents, or targeting ligands make up at least about 1%, 5%, 10% or 15%, 20%, 25%, 30% or even 35% of the CDP by weight. Number-average molecular weight (M_n) may also vary widely, but generally fall in the range of about 1,000 to about 500,000 daltons, preferably from about 5000 to about 200,000 daltons and, even more preferably, from about 10,000 to about 100,000. Most preferably, M_n varies between about 12,000 and 65,000 daltons. In certain other embodiments, M_n varies

between about 3000 and 150,000 daltons. Within a given sample of a subject polymer, a wide range of molecular weights may be present. For example, molecules within the sample may have molecular weights that differ by a factor of 2, 5, 10, 20, 50, 100, or more, or that differ from the average molecular weight by a factor of 2, 5, 10, 20, 50, 100, or more. Exemplary cyclodextrin moieties include cyclic structures consisting essentially of from 7 to 9 saccharide moieties, such as cyclodextrin and oxidized cyclodextrin. A cyclodextrin moiety optionally comprises a linker moiety that forms a covalent linkage between the cyclic structure and the polymer backbone, preferably having from 1 to 20 atoms in the chain, such as alkyl chains, including dicarboxylic acid derivatives (such as glutaric acid derivatives, succinic acid derivatives, and the like), and heteroalkyl chains, such as oligoethylene glycol chains.

Cyclodextrins are cyclic polysaccharides containing naturally occurring D-(+)-glucopyranose units in an α-(1,4) linkage. The most common cyclodextrins are alpha ((α)-cyclodextrins, beta (β)-cyclodextrins and gamma (γ)-cyclodextrins which contain, respectively six, seven, or eight glucopyranose units. Structurally, the cyclic nature of a cyclodextrin forms a torus or donut-like shape having an inner apolar or hydrophobic cavity, the secondary hydroxyl groups situated on one side of the cyclodextrin torus and the primary hydroxyl groups situated on the other. Thus, using (β)-cyclodextrin as an example, a cyclodextrin is often represented schematically as shown in FIG. 2. Attachment on the trapezoid representing the cyclodextrin depicts only whether the moiety is attached through a primary hydroxyl on the cyclodextrin, i.e., by depicting attachment through the base of the trapezoid, or depicting whether the moiety is attached through a secondary hydroxyl on the cyclodextrin, i.e., by depicting attachment through the top of the trapezoid. For example, a trapezoid with two moieties attached at the right and left bottom of the trapezoid does not indicate anything about the relative position of the moieties around the cyclodextrin ring. The attachment of the moieties can be on any glucopyranose in the cyclodextrin ring. Exemplary relative positions of two moieties on a cyclodextrin ring include the following: moieties positioned such that the derivatization on the cyclodextrin is on the A and D glucopyranose moieties, moieties positioned such that the derivatization on the cyclodextrin is on the A and C glucopyranose moieties, moieties positioned such that the derivatization on the cyclodextrin is on the A and F

glucopyranose moieties, or moieties positioned such that the derivatization on the cyclodextrin is on the A and E glucopyranose moieties.

The side on which the secondary hydroxyl groups are located has a wider diameter than the side on which the primary hydroxyl groups are located. The present invention contemplates covalent linkages to cyclodextrin moieties on the primary and/or secondary hydroxyl groups. The hydrophobic nature of the cyclodextrin inner cavity allows for host-guest inclusion complexes of a variety of compounds, e.g., adamantane. (Comprehensive Supramolecular Chemistry, Volume 3, J.L. Atwood et al., eds., Pergamon Press (1996); T. Cserhati, Analytical Biochemistry, 225:328-332(1995); Husain et al., Applied Spectroscopy, 46:652-658 (1992); FR 2 665 169). Additional methods for modifying polymers are disclosed in Suh, J. and Noh, Y., *Bioorg. Med. Chem. Lett.* 1998, *8*, 1327-1330.

In certain embodiments, the compounds comprise cyclodextrin moieties and wherein at least one or a plurality of the cyclodextrin moieties of the CDP-therapeutic agent conjugate is oxidized. In certain embodiments, the cyclodextrin moieties of P alternate with linker moieties in the polymer chain.

Comonomers

In addition to a cyclodextrin moiety, the CDP can also include a comonomer, for example, a comonomer described herein. In some embodiments, a comonomer of the CDP-topoisomerase inhibitor conjugate comprises a moiety selected from the group consisting of: an alkylene chain, polysuccinic anhydride, poly-L-glutamic acid, poly(ethyleneimine), an oligosaccharide, and an amino acid chain. In some embodiments, a CDP-topoisomerase inhibitor conjugate comonomer comprises a polyethylene glycol chain. In some embodiments, a comonomer comprises a moiety selected from: polyglycolic acid and polylactic acid chain. In some embodiments, a comonomer comprises a hydrocarbylene group wherein one or more methylene groups is optionally replaced by a group Y (provided that none of the Y groups are adjacent to each other), wherein each Y, independently for each occurrence, is selected from, substituted or unsubstituted aryl, heteroaryl, cycloalkyl, heterocycloalkyl, or -O-, C(=X) (wherein X is NR_1, O or S), -OC(O)-, -C(=O)O, -NR_1-, -NR_1CO-, -C(O)NR_1-, -$S(O)_n$- (wherein n is

0, 1, or 2), -OC(O)-NR_1, -NR_1-C(O)-NR_1-, -$NR_1$1-C(NR_1)-NR_1-, and -B(OR_1)-; and R_1, independently for each occurrence, represents H or a lower alkyl.

In some embodiments, a comonomer can be and/or can comprise a linker such as a linker described herein.

Linkers/tethers

The CDPs described herein can include on or more linkers. In some embodiments, a linker can link a therapeutic agent described herein to a CDP. In some embodiments, for example, when referring to a linker that links a therapeutic agent to the CDP, the linker can be referred to as a tether.

In certain embodiments, a plurality of the linker moieties are attached to a therapeutic agent or prodrug thereof and are cleaved under biological conditions.

Described herein are CDP-therapeutic agent conjugates comprising a CDP covalently attached to a therapeutic agent through attachments that are cleaved under biological conditions to release the therapeutic agent. In certain embodiments, a CDP-therapeutic agent conjugate comprises a therapeutic agent covalently attached to a polymer, preferably a biocompatible polymer, through a tether, e.g., a linker, wherein the tether comprises a selectivity-determining moiety and a self-cyclizing moiety which are covalently attached to one another in the tether, e.g., between the polymer and the therapeutic agent.

In some embodiments, such therapeutic agents are covalently attached to CDPs through functional groups comprising one or more heteroatoms, for example, hydroxy, thiol, carboxy, amino, and amide groups. Such groups may be covalently attached to the subject polymers through linker groups as described herein, for example, biocleavable linker groups, and/or through tethers, such as a tether comprising a selectivity-determining moiety and a self-cyclizing moiety which are covalently attached to one another.

In certain embodiments, the CDP-therapeutic agent conjugate comprises a therapeutic agent covalently attached to the CDP through a tether, wherein the tether comprises a self-cyclizing moiety. In some embodiments, the tether further comprises a selectivity-determining moiety. Thus, one aspect of the invention relates to a polymer

conjugate comprising a therapeutic agent covalently attached to a polymer, preferably a biocompatible polymer, through a tether, wherein the tether comprises a selectivity-determining moiety and a self-cyclizing moiety which are covalently attached to one another.

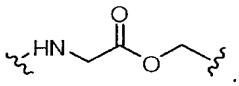
In some embodiments, the selectivity-determining moiety is bonded to the self-cyclizing moiety between the self-cyclizing moiety and the CDP.

In certain embodiments, the selectivity-determining moiety is a moiety that promotes selectivity in the cleavage of the bond between the selectivity-determining moiety and the self-cyclizing moiety. Such a moiety may, for example, promote enzymatic cleavage between the selectivity-determining moiety and the self-cyclizing moiety. Alternatively, such a moiety may promote cleavage between the selectivity-determining moiety and the self-cyclizing moiety under acidic conditions or basic conditions.

In certain embodiments, the invention contemplates any combination of the foregoing. Those skilled in the art will recognize that, for example, any therapeutic agent described herein in combination with any linker (e.g., self-cyclizing moiety, any selectivity-determining moiety, and/or any therapeutic agent described herein) are within the scope of the invention.

In certain embodiments, the selectivity-determining moiety is selected such that the bond is cleaved under acidic conditions.

In certain embodiments, where the selectivity-determining moiety is selected such that the bond is cleaved under basic conditions, the selectivity-determining moiety is an aminoalkylcarbonyloxyalkyl moiety. In certain embodiments, the selectivity-determining moiety has a structure



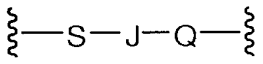
In certain embodiments where the selectivity-determining moiety is selected such that the bond is cleaved enzymatically, it may be selected such that a particular enzyme or class of enzymes cleaves the bond. In certain preferred such embodiments, the

selectivity-determining moiety may be selected such that the bond is cleaved by a cathepsin, preferably cathepsin B.

In certain embodiments the selectivity-determining moiety comprises a peptide, preferably a dipeptide, tripeptide, or tetrapeptide. In certain such embodiments, the peptide is a dipeptide is selected from KF and FK, In certain embodiments, the peptide is a tripeptide is selected from GFA, GLA, AVA, GVA, GIA, GVL, GVF, and AVF. In certain embodiments, the peptide is a tetrapeptide selected from GFYA and GFLG, preferably GFLG.

In certain such embodiments, a peptide, such as GFLG, is selected such that the bond between the selectivity-determining moiety and the self-cyclizing moiety is cleaved by a cathepsin, preferably cathepsin B.

In certain embodiments, the selectivity-determining moiety is represented by Formula A:


(A),

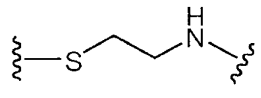
wherein

S a sulfur atom that is part of a disulfide bond;

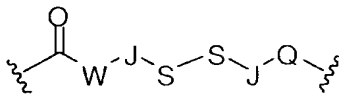
J is optionally substituted hydrocarbyl; and

Q is O or NR^{13}, wherein R^{13} is hydrogen or alkyl.

In certain embodiments, J may be polyethylene glycol, polyethylene, polyester, alkenyl, or alkyl. In certain embodiments, J may represent a hydrocarbylene group comprising one or more methylene groups, wherein one or more methylene groups is optionally replaced by a group Y (provided that none of the Y groups are adjacent to each other), wherein each Y, independently for each occurrence, is selected from, substituted or unsubstituted aryl, heteroaryl, cycloalkyl, heterocycloalkyl, or -O-, C(=X) (wherein X is NR^{30}, O or S), -OC(O)-, -C(=O)O, $-NR^{30}-$, $-NR_1CO-$, $-C(O)NR^{30}-$, $-S(O)_n-$ (wherein n is 0, 1, or 2), $-OC(O)-NR^{30}$, $-NR^{30}-C(O)-NR^{30}-$, $-NR^{30}-C(NR^{30})-NR^{30}-$, and $-B(OR^{30})-$; and R^{30}, independently for each occurrence, represents H or a lower alkyl. In certain embodiments, J may be substituted or unsubstituted lower alkylene, such as ethylene.

For example, the selectivity-determining moiety may be  .

In certain embodiments, the selectivity-determining moiety is represented by Formula B:


(B),

wherein

W is either a direct bond or selected from lower alkyl, NR^{14}, S, O;

S is sulfur;

J, independently and for each occurrence, is hydrocarbyl or polyethylene glycol;

Q is O or NR^{13}, wherein R^{13} is hydrogen or alkyl; and

R^{14} is selected from hydrogen and alkyl.

In certain such embodiments, J may be substituted or unsubstituted lower alkyl, such as methylene. In certain such embodiments, J may be an aryl ring. In certain embodiments, the aryl ring is a benzo ring. In certain embodiments W and S are in a 1,2-relationship on the aryl ring. In certain embodiments, the aryl ring may be optionally substituted with alkyl, alkenyl, alkoxy, aralkyl, aryl, heteroaryl, halogen, -CN, azido, -NR^xR^x, -CO_2OR^x, -C(O)-NR^xR^x, -C(O)-R^x, -NR^x-C(O)-R^x, -$NR^xSO_2R^x$, -SR^x, -S(O)R^x, -SO_2R^x, -$SO_2NR^xR^x$, -$(C(R^x)_2)_n$-OR^x, -$(C(R^x)_2)_n$-NR^xR^x, and -$(C(R^x)_2)_n$-SO_2R^x; wherein R^x is, independently for each occurrence, H or lower alkyl; and n is, independently for each occurrence, an integer from 0 to 2.

In certain embodiments, the aryl ring is optionally substituted with alkyl, alkenyl, alkoxy, aralkyl, aryl, heteroaryl, halogen, -CN, azido, -NR^xR^x, -CO_2OR^x, -C(O)-NR^xR^x, -C(O)-R^x, -NR^x-C(O)-R^x, -$NR^xSO_2R^x$, -SR^x, -S(O)R^x, -SO_2R^x, -$SO_2NR^xR^x$, -$(C(R^x)_2)_n$-OR^x, -$(C(R^x)_2)_n$-NR^xR^x, and -$(C(R^x)_2)_n$-SO_2R^x; wherein R^x is, independently for each occurrence, H or lower alkyl; and n is, independently for each occurrence, an integer from 0 to 2.

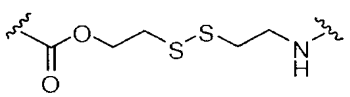
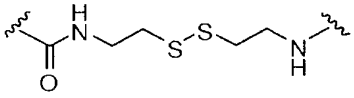
In certain embodiments, J, independently and for each occurrence, is polyethylene glycol, polyethylene, polyester, alkenyl, or alkyl.

In certain embodiments, independently and for each occurrence, the linker comprises a hydrocarbylene group comprising one or more methylene groups, wherein

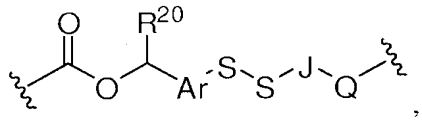
one or more methylene groups is optionally replaced by a group Y (provided that none of the Y groups are adjacent to each other), wherein each Y, independently for each occurrence, is selected from, substituted or unsubstituted aryl, heteroaryl, cycloalkyl, heterocycloalkyl, or -O-, C(=X) (wherein X is NR^{30}, O or S), -OC(O)-, -C(=O)O, -NR^{30}-, -NR_1CO-, -C(O)NR^{30}-, -$S(O)_n$- (wherein n is 0, 1, or 2), -OC(O)-NR^{30}, -NR^{30}-C(O)-NR^{30}-, -NR^{30}-C(NR^{30})-NR^{30}-, and -B(OR^{30})-; and R^{30}, independently for each occurrence, represents H or a lower alkyl.

In certain embodiments, J, independently and for each occurrence, is substituted or unsubstituted lower alkylene. In certain embodiments, J, independently and for each occurrence, is substituted or unsubstituted ethylene.

In certain embodiments, the selectivity-determining moiety is selected from

 and  .

The selectivity-determining moiety may include groups with bonds that are cleavable under certain conditions, such as disulfide groups. In certain embodiments, the selectivity-determining moiety comprises a disulfide-containing moiety, for example, comprising aryl and/or alkyl group(s) bonded to a disulfide group. In certain embodiments, the selectivity-determining moiety has a structure

 ,

wherein

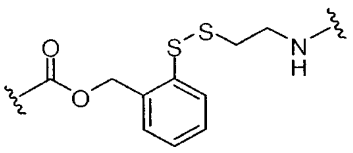
Ar is a substituted or unsubstituted benzo ring;

J is optionally substituted hydrocarbyl; and

Q is O or NR^{13},

wherein R^{13} is hydrogen or alkyl.

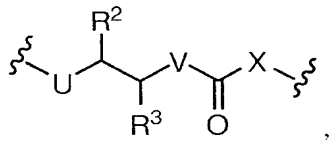
In certain embodiments, Ar is unsubstituted. In certain embodiments, Ar is a 1,2-benzo ring. For example, suitable moieties within Formula B include:



In certain embodiments, the self-cyclizing moiety is selected such that upon cleavage of the bond between the selectivity-determining moiety and the self-cyclizing moiety, cyclization occurs thereby releasing the therapeutic agent. Such a cleavage-cyclization-release cascade may occur sequentially in discrete steps or substantially simultaneously. Thus, in certain embodiments, there may be a temporal and/or spatial difference between the cleavage and the self-cyclization. The rate of the self-cyclization cascade may depend on pH, e.g., a basic pH may increase the rate of self-cyclization after cleavage. Self-cyclization may have a half-life after introduction *in vivo* of 24 hours, 18 hours, 14 hours, 10 hours, 6 hours, 3 hours, 2 hours, 1 hour, 30 minutes, 10 minutes, 5 minutes, or 1 minute.

In certain such embodiments, the self-cyclizing moiety may be selected such that, upon cyclization, a five- or six-membered ring is formed, preferably a five-membered ring. In certain such embodiments, the five- or six-membered ring comprises at least one heteroatom selected from oxygen, nitrogen, or sulfur, preferably at least two, wherein the heteroatoms may be the same or different. In certain such embodiments, the heterocyclic ring contains at least one nitrogen, preferably two. In certain such embodiments, the self-cyclizing moiety cyclizes to form an imidazolidone.

In certain embodiments, the self-cyclizing moiety has a structure



wherein

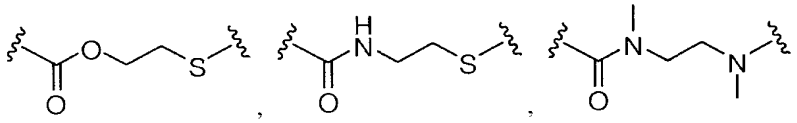
U is selected from NR^1 and S;

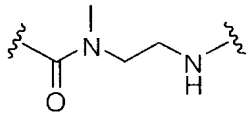
X is selected from O, NR^5, and S, preferably O or S;

V is selected from O, S and NR^4, preferably O or NR^4;
R^2 and R^3 are independently selected from hydrogen, alkyl, and alkoxy; or R^2 and R^3 together with the carbon atoms to which they are attached form a ring; and
R^1, R^4, and R^5 are independently selected from hydrogen and alkyl.

In certain embodiments, U is NR^1 and/or V is NR^4, and R^1 and R^4 are independently selected from methyl, ethyl, propyl, and isopropyl. In certain embodiments, both R^1 and R^4 are methyl. On certain embodiments, both R^2 and R^3 are hydrogen. In certain embodiments R^2 and R^3 are independently alkyl, preferably lower alkyl. In certain embodiments, R^2 and R^3 together are $-(CH_2)_n-$ wherein n is 3 or 4, thereby forming a cyclopentyl or cyclohexyl ring. In certain embodiments, the nature of R^2 and R^3 may affect the rate of cyclization of the self-cyclizing moiety. In certain such embodiments, it would be expected that the rate of cyclization would be greater when R^2 and R^3 together with the carbon atoms to which they are attached form a ring than the rate when R^2 and R^3 are independently selected from hydrogen, alkyl, and alkoxy. In certain embodiments, U is bonded to the self-cyclizing moiety.

In certain embodiments, the self-cyclizing moiety is selected from

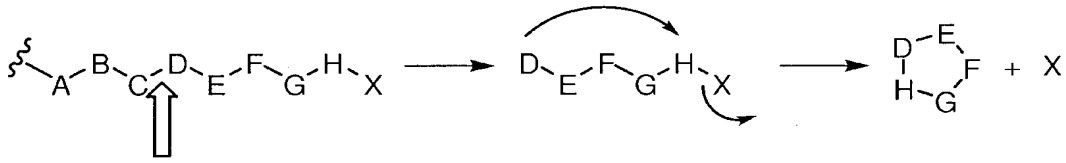

, and


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In certain embodiments, the selectivity-determining moiety may connect to the self-cyclizing moiety through carbonyl-heteroatom bonds, e.g., amide, carbamate, carbonate, ester, thioester, and urea bonds.

In certain embodiments, a therapeutic agent is covalently attached to a polymer through a tether, wherein the tether comprises a selectivity-determining moiety and a self-cyclizing moiety which are covalently attached to one another. In certain embodiments, the self-cyclizing moiety is selected such that after cleavage of the bond between the selectivity-determining moiety and the self-cyclizing moiety, cyclization of the self-cyclizing moiety occurs, thereby releasing the therapeutic agent. As an

illustration, ABC may be a selectivity-determining moiety, and DEFGH maybe be a self-cyclizing moiety, and ABC may be selected such that enzyme Y cleaves between C and D. Once cleavage of the bond between C and D progresses to a certain point, D will cyclize onto H, thereby releasing therapeutic agent X, or a prodrug thereof.



In certain embodiments, the conjugate may further comprise additional intervening components, including, but not limited to another self-cyclizing moiety or a leaving group linker, such as CO_2 or methoxymethyl, that spontaneously dissociates from the remainder of the molecule after cleavage occurs.

In some embodiments, a linker may be and/or comprise an alkylene chain, a polyethylene glycol (PEG) chain, polysuccinic anhydride, poly-L-glutamic acid, poly(ethyleneimine), an oligosaccharide, an amino acid (e.g., glycine or cysteine), an amino acid chain, or any other suitable linkage. In certain embodiments, the linker group itself can be stable under physiological conditions, such as an alkylene chain, or it can be cleavable under physiological conditions, such as by an enzyme (e.g., the linkage contains a peptide sequence that is a substrate for a peptidase), or by hydrolysis (e.g., the linkage contains a hydrolyzable group, such as an ester or thioester). The linker groups can be biologically inactive, such as a PEG, polyglycolic acid, or polylactic acid chain, or can be biologically active, such as an oligo- or polypeptide that, when cleaved from the moieties, binds a receptor, deactivates an enzyme, etc. Various oligomeric linker groups that are biologically compatible and/or bioerodible are known in the art, and the selection of the linkage may influence the ultimate properties of the material, such as whether it is durable when implanted, whether it gradually deforms or shrinks after implantation, or whether it gradually degrades and is absorbed by the body. The linker group may be attached to the moieties by any suitable bond or functional group, including carbon-carbon bonds, esters, ethers, amides, amines, carbonates, carbamates, sulfonamides, etc.

In certain embodiments, the linker group(s) of the present invention represent a hydrocarbylene group wherein one or more methylene groups is optionally replaced by a

group Y (provided that none of the Y groups are adjacent to each other), wherein each Y, independently for each occurrence, is selected from, substituted or unsubstituted aryl, heteroaryl, cycloalkyl, heterocycloalkyl, or -O-, C(=X) (wherein X is NR_1, O or S), -OC(O)-, -C(=O)O, -NR_1-, -NR_1CO-, -C(O)NR_1-, -$S(O)_n$- (wherein n is 0, 1, or 2), -OC(O)-NR_1, -NR_1-C(O)-NR_1-, -NR_1-C(NR_1)-NR_1-, and -B(OR_1)-; and R_1, independently for each occurrence, represents H or a lower alkyl.

In certain embodiments, the linker group represents a derivatized or non-derivatized amino acid (e.g., glycine or cysteine). In certain embodiments, linker groups with one or more terminal carboxyl groups may be conjugated to the polymer. In certain embodiments, one or more of these terminal carboxyl groups may be capped by covalently attaching them to a therapeutic agent, a targeting moiety, or a cyclodextrin moiety via an (thio)ester or amide bond. In still other embodiments, linker groups with one or more terminal hydroxyl, thiol, or amino groups may be incorporated into the polymer. In preferred embodiments, one or more of these terminal hydroxyl groups may be capped by covalently attaching them to a therapeutic agent, a targeting moiety, or a cyclodextrin moiety via an (thio)ester, amide, carbonate, carbamate, thiocarbonate, or thiocarbamate bond. In certain embodiments, these (thio)ester, amide, (thio)carbonate or (thio)carbamates bonds may be biohydrolyzable, i.e., capable of being hydrolyzed under biological conditions.

In certain embodiments, a linker group represents a hydrocarbylene group wherein one or more methylene groups is optionally replaced by a group Y (provided that none of the Y groups are adjacent to each other), wherein each Y, independently for each occurrence, is selected from, substituted or unsubstituted aryl, heteroaryl, cycloalkyl, heterocycloalkyl, or -O-, C(=X) (wherein X is NR_1, O or S), -OC(O)-, -C(=O)O, -NR_1-, -NR_1CO-, -C(O)NR_1-, -$S(O)_n$- (wherein n is 0, 1, or 2), -OC(O)-NR_1, -NR_1-C(O)-NR_1-, -NR_1-C(NR_1)-NR_1-, and -B(OR_1)-; and R_1, independently for each occurrence, represents H or a lower alkyl.

In certain embodiments, a linker group, e.g., between a therapeutic agent described herein and the CDP, comprises a self-cyclizing moiety. In certain embodiments, a linker group, e.g., between a therapeutic agent described herein and the CDP, comprises a selectivity-determining moiety.

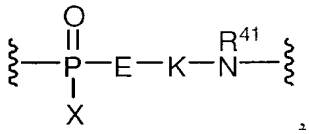
In certain embodiments as disclosed herein, a linker group, e.g., between a therapeutic agent and the CDP, comprises a self-cyclizing moiety and a selectivity-determining moiety.

In certain embodiments as disclosed herein, the therapeutic agent or targeting ligand is covalently bonded to the linker group via a biohydrolyzable bond (e.g., an ester, amide, carbonate, carbamate, or a phosphate).

In certain embodiments as disclosed herein, the CDP comprises cyclodextrin moieties that alternate with linker moieties in the polymer chain.

In certain embodiments, the linker moieties are attached to therapeutic agents or prodrugs thereof that are cleaved under biological conditions.

In certain embodiments, at least one linker that connects the therapeutic agent or prodrug thereof to the polymer comprises a group represented by the formula



wherein

P is phosphorus;

O is oxygen;

E represents oxygen or NR^{40};

K represents hydrocarbyl;

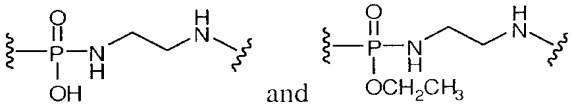
X is selected from OR^{42} or $NR^{43}R^{44}$; and

R^{40}, R^{41}, R^{42}, R^{43}, and R^{44} independently represent hydrogen or optionally substituted alkyl.

In certain embodiments, E is NR^{40} and R^{40} is hydrogen.

In certain embodiments, K is lower alkylene (e.g., ethylene).

In certain embodiments, at least one linker comprises a group selected from



In certain embodiments, X is OR^{42}.

In certain embodiments, the linker group comprises an amino acid or peptide, or derivative thereof (e.g., a glycine or cysteine).

In certain embodiments as disclosed herein, the linker is connected to the therapeutic agent through a hydroxyl group. In certain embodiments as disclosed herein, the linker is connected to the therapeutic agent through an amino group.

In certain embodiments, the linker group that connects to the therapeutic agent may comprise a self-cyclizing moiety, or a selectivity-determining moiety, or both. In certain embodiments, the selectivity-determining moiety is a moiety that promotes selectivity in the cleavage of the bond between the selectivity-determining moiety and the self-cyclizing moiety. Such a moiety may, for example, promote enzymatic cleavage between the selectivity-determining moiety and the self-cyclizing moiety. Alternatively, such a moiety may promote cleavage between the selectivity-determining moiety and the self-cyclizing moiety under acidic conditions or basic conditions.

In certain embodiments, any of the linker groups may comprise a self-cyclizing moiety or a selectivity-determining moiety, or both. In certain embodiments, the selectivity-determining moiety may be bonded to the self-cyclizing moiety between the self-cyclizing moiety and the polymer.

In certain embodiments, any of the linker groups may independently be or include an alkyl chain, a polyethylene glycol (PEG) chain, polysuccinic anhydride, poly-L-glutamic acid, poly(ethyleneimine), an oligosaccharide, an amino acid chain, or any other suitable linkage. In certain embodiments, the linker group itself can be stable under physiological conditions, such as an alkyl chain, or it can be cleavable under physiological conditions, such as by an enzyme (e.g., the linkage contains a peptide sequence that is a substrate for a peptidase), or by hydrolysis (e.g., the linkage contains a hydrolyzable group, such as an ester or thioester). The linker groups can be biologically inactive, such as a PEG, polyglycolic acid, or polylactic acid chain, or can be biologically active, such as an oligo- or polypeptide that, when cleaved from the moieties, binds a receptor, deactivates an enzyme, etc. Various oligomeric linker groups that are biologically compatible and/or bioerodible are known in the art, and the selection of the linkage may influence the ultimate properties of the material, such as whether it is durable when implanted, whether it gradually deforms or shrinks after implantation, or

whether it gradually degrades and is absorbed by the body. The linker group may be attached to the moieties by any suitable bond or functional group, including carbon-carbon bonds, esters, ethers, amides, amines, carbonates, carbamates, sulfonamides, etc.

In certain embodiments, any of the linker groups may independently be an alkyl group wherein one or more methylene groups is optionally replaced by a group Y (provided that none of the Y groups are adjacent to each other), wherein each Y, independently for each occurrence, is selected from aryl, heteroaryl, carbocyclyl, heterocyclyl, or -O-, C(=X) (wherein X is NR^1, O or S), -OC(O)-, -C(=O)O-, -NR^1-, -NR^1CO-, -C(O)NR^1-, -$S(O)_n$- (wherein n is 0, 1, or 2), -OC(O)-NR^1-, -NR^1-C(O)-NR^1-, -NR^1-C(NR^1)-NR^1-, and -B(OR^1)-; and R^1, independently for each occurrence, is H or lower alkyl.

In certain embodiments, the present invention contemplates a CDP, wherein a plurality of therapeutic agents are covalently attached to the polymer through attachments that are cleaved under biological conditions to release the therapeutic agents as discussed above, wherein administration of the polymer to a subject results in release of the therapeutic agent over a period of at least 2, 3, 5, 6, 8, 10, 15, 20, 24, 36, 48 or even 72 hours.

In some embodiments, the conjugation of the therapeutic agent to the CDP improves the aqueous solubility of the therapeutic agent and hence the bioavailability. Accordingly, in one embodiment of the invention, the therapeutic agent has a log P >0.4, >0.6, >0.8, >1, >2, >3, >4, or even >5.

The CDP-therapeutic agent conjugate of the present invention preferably has a molecular weight in the range of 10,000 to 500,000; 30,000 to 200,000; or even 70,000 to 150,000 Da.

In certain embodiments, the present invention contemplates attenuating the rate of release of the therapeutic agent by introducing various tether and/or linking groups between the therapeutic agent and the polymer. Thus, in certain embodiments, the CDP-therapeutic agent conjugates of the present invention are compositions for controlled delivery of the therapeutic agent.

Characteristics of CDP-therapeutic agent conjugates, particles or compositions

In some embodiments, the CDP and/or CDP-therapeutic agent conjugate, particle or composition as described herein have polydispersities less than about 3, or even less than about 2 (e.g., 1.5, 1.25, or less).

One embodiment of the present invention provides an improved delivery of certain therapeutic agents by covalently attaching one or more therapeutic agents to a CDP. Such conjugation can improve the aqueous solubility and hence the bioavailability of the therapeutic agent.

In certain embodiments as disclosed herein, the CDP-therapeutic agent conjugate has a number average (M_n) molecular weight between 1,000-500,000 Da, or between 5,000-200,000 Da, or between 10,000-100,000 Da. One method to determine molecular weight is by gel permeation chromatography ("GPC"), e.g., mixed bed columns, CH_2Cl_2 or HFIP (hexafluoroisopropanol) solvent, light scattering detector, and off-line dn/dc. Other methods are known in the art.

In certain embodiments as disclosed herein, the CDP-therapeutic agent conjugate, particle or composition is biodegradable or bioerodable.

In certain embodiments as disclosed herein, the therapeutic agent makes up at least 3% (e.g., at least about 5%) by weight of the CDP-therapeutic agent conjugate or particle. In certain embodiments, the therapeutic agent makes up at least 20% by weight of the CDP-therapeutic agent conjugate. In certain embodiments, the therapeutic agent makees up at least 5%, 10%, 15%, or at least 20% by weight of the CDP-therapeutic agent conjugate or particle.

In one embodiment, the CDP-therapeutic agent conjugate forms a particle, e.g., a nanoparticle. The particle can comprise multiple CDP-therapeutic agent conjugates, e.g., a plurality of CDP-therapeutic agent conjugates, e.g., CDP-therapeutic agent conjugates having the same therapeutic agents or different therapeutic agents. The nanoparticle ranges in size from 10 to 300 nm in diameter, e.g., 15 to 280, 30 to 250, 40 to 200, 20 to 150, 30 to 100, 20 to 80, 30 to 70, 40 to 60 or 40 to 50 nm diameter. In one embodiment, the particle is 50 to 60 nm, 20 to 60 nm, 30 to 60 nm, 35 to 55 nm, 35 to 50 nm or 35 to 45 nm in diameter.

In one embodiment, the CDP-therapeutic agent conjugate forms an inclusion complex. In one embodiment, the CDP-therapeutic agent conjugate containing the inclusion complex forms a particle, e.g., a nanoparticle. The nanoparticle ranges in size from 10 to 300 nm in diameter, e.g., 15 to 280, 30 to 250, 40 to 200, 20 to 150, 30 to 100, 20 to 80, 30 to 70, 40 to 60 or 40 to 50 nm diameter. In one embodiment, the particle is 50 to 60 nm, 20 to 60 nm, 30 to 60 nm, 35 to 55 nm, 35 to 50 nm or 35 to 45 nm in diameter.

In one embodiment, the surface charge of the molecule is neutral, or slightly negative. In some embodiments, the zeta potential of the particle surface is from about -80 mV to about 50 mV, about -20 mV to about 20 mV, about -20 mV to about -10 mV, or about -10 mV to about 0.

CDP-therapeutic agent conjugates, particles and compositions of the present invention may be useful to improve solubility and/or stability of the therapeutic agent, reduce drug-drug interactions, reduce interactions with blood elements including plasma proteins, reduce or eliminate immunogenicity, protect the therapeutic agent from metabolism, modulate drug-release kinetics, improve circulation time, improve therapeutic agent half-life (e.g., in the serum, or in selected tissues, such as tumors), attenuate toxicity, improve efficacy, normalize therapeutic agent metabolism across subjects of different species, ethnicities, and/or races, and/or provide for targeted delivery into specific cells or tissues.

In other embodiments, the CDP-therapeutic agent conjugate, particle or composition may be a flexible or flowable material. When the CDP used is itself flowable, the CDP composition of the invention, even when viscous, need not include a biocompatible solvent to be flowable, although trace or residual amounts of biocompatible solvents may still be present.

While it is possible that the biodegradable polymer or the biologically active agent may be dissolved in a small quantity of a solvent that is non-toxic to more efficiently produce an amorphous, monolithic distribution or a fine dispersion of the biologically active agent in the flexible or flowable composition, it is an advantage of the invention that, in a preferred embodiment, no solvent is needed to form a flowable composition. Moreover, the use of solvents is preferably avoided because, once a

polymer composition containing solvent is placed totally or partially within the body, the solvent dissipates or diffuses away from the polymer and must be processed and eliminated by the body, placing an extra burden on the body's clearance ability at a time when the illness (and/or other treatments for the illness) may have already deleteriously affected it.

However, when a solvent is used to facilitate mixing or to maintain the flowability of the CDP-therapeutic agent conjugate, particle or composition, it should be non-toxic, otherwise biocompatible, and should be used in relatively small amounts. Solvents that are toxic should not be used in any material to be placed even partially within a living body. Such a solvent also must not cause substantial tissue irritation or necrosis at the site of administration.

Examples of suitable biocompatible solvents, when used, include N-methyl-2-pyrrolidone, 2-pyrrolidone, ethanol, propylene glycol, acetone, methyl acetate, ethyl acetate, methyl ethyl ketone, dimethylformamide, dimethylsulfoxide, tetrahydrofuran, caprolactam, oleic acid, or 1-dodecylazacylcoheptanone. Preferred solvents include N-methylpyrrolidone, 2-pyrrolidone, dimethylsulfoxide, and acetone because of their solvating ability and their biocompatibility.

In certain embodiments, the CDP-therapeutic agent conjugates, particles and compositions are soluble in one or more common organic solvents for ease of fabrication and processing. Common organic solvents include such solvents as chloroform, dichloromethane, dichloroethane, 2-butanone, butyl acetate, ethyl butyrate, acetone, ethyl acetate, dimethylacetamide, N-methylpyrrolidone, dimethylformamide, and dimethylsulfoxide.

In certain embodiments, the CDP-therapeutic agent conjugates, particles and compositions described herein, upon contact with body fluids, undergo gradual degradation. The life of a biodegradable polymer in vivo depends upon, among other things, its molecular weight, crystallinity, biostability, and the degree of crosslinking. In general, the greater the molecular weight, the higher the degree of crystallinity, and the greater the biostability, the slower biodegradation will be.

If a subject composition is formulated with a therapeutic agent or other material, release of the therapeutic agent or other material for a sustained or extended period as

compared to the release from an isotonic saline solution generally results. Such release profile may result in prolonged delivery (over, say 1 to about 2,000 hours, or alternatively about 2 to about 800 hours) of effective amounts (e.g., about 0.0001 mg/kg/hour to about 10 mg/kg/hour, e.g., 0.001 mg/kg/hour, 0.01 mg/kg/hour, 0.1 mg/kg/hour, 1.0 mg/kg/hour) of the therapeutic agent or any other material associated with the polymer.

A variety of factors may affect the desired rate of hydrolysis of CDP-therapeutic agent conjugates, particles and compositions, the desired softness and flexibility of the resulting solid matrix, rate and extent of bioactive material release. Some of such factors include the selection/identity of the various subunits, the enantiomeric or diastereomeric purity of the monomeric subunits, homogeneity of subunits found in the polymer, and the length of the polymer. For instance, the present invention contemplates heteropolymers with varying linkages, and/or the inclusion of other monomeric elements in the polymer, in order to control, for example, the rate of biodegradation of the matrix.

To illustrate further, a wide range of degradation rates may be obtained by adjusting the hydrophobicities of the backbones or side chains of the polymers while still maintaining sufficient biodegradability for the use intended for any such polymer. Such a result may be achieved by varying the various functional groups of the polymer. For example, the combination of a hydrophobic backbone and a hydrophilic linkage produces heterogeneous degradation because cleavage is encouraged whereas water penetration is resisted.

One protocol generally accepted in the field that may be used to determine the release rate of a therapeutic agent or other material loaded in the CDP-therapeutic agent conjugates, particles or compositions of the present invention involves degradation of any such matrix in a 0.1 M PBS solution (pH 7.4) at 37 °C, an assay known in the art. For purposes of the present invention, the term "PBS protocol" is used herein to refer to such protocol.

In certain instances, the release rates of different CDP-therapeutic agent conjugates, particles and compositions of the present invention may be compared by subjecting them to such a protocol. In certain instances, it may be necessary to process polymeric systems in the same fashion to allow direct and relatively accurate comparisons of different systems to be made. For example, the present invention teaches

several different methods of formulating the CDP-therapeutic agent conjugates, particles and compositions. Such comparisons may indicate that any one CDP-therapeutic agent conjugate, particle or composition releases incorporated material at a rate from about 2 or less to about 1000 or more times faster than another polymeric system.

Alternatively, a comparison may reveal a rate difference of about 3, 5, 7, 10, 25, 50, 100, 250, 500 or 750 times. Even higher rate differences are contemplated by the present invention and release rate protocols.

In certain embodiments, when formulated in a certain manner, the release rate for CDP-therapeutic agent conjugates, particles and compositions of the present invention may present as mono- or bi-phasic.

Release of any material incorporated into the polymer matrix, which is often provided as a microsphere, may be characterized in certain instances by an initial increased release rate, which may release from about 5 to about 50% or more of any incorporated material, or alternatively about 10, 15, 20, 25, 30 or 40%, followed by a release rate of lesser magnitude.

The release rate of any incorporated material may also be characterized by the amount of such material released per day per mg of polymer matrix. For example, in certain embodiments, the release rate may vary from about 1 ng or less of any incorporated material per day per mg of polymeric system to about 500 or more ng/day/mg. Alternatively, the release rate may be about 0.05, 0.5, 5, 10, 25, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, or 500 ng/day/mg. In still other embodiments, the release rate of any incorporated material may be 10,000 ng/day/mg, or even higher. In certain instances, materials incorporated and characterized by such release rate protocols may include therapeutic agents, fillers, and other substances.

In another aspect, the rate of release of any material from any CDP-therapeutic agent conjugate, particle or composition of the present invention may be presented as the half-life of such material in the matrix.

In addition to the embodiment involving protocols for in vitro determination of release rates, in vivo protocols, whereby in certain instances release rates for polymeric systems may be determined in vivo, are also contemplated by the present invention.

Other assays useful for determining the release of any material from the polymers of the present system are known in the art.

Physical Structures of the CDP-therapeutic agent conjugates, particles and compositions

The CDP-therapeutic agent conjugates, particles and compositions may be formed in a variety of shapes. For example, in certain embodiments, CDP-therapeutic agent conjugates may be presented in the form of microparticles or nanoparticles. Microspheres typically comprise a biodegradable polymer matrix incorporating a drug. Microspheres can be formed by a wide variety of techniques known to those of skill in the art. Examples of microsphere forming techniques include, but are not limited to, (a) phase separation by emulsification and subsequent organic solvent evaporation (including complex emulsion methods such as oil in water emulsions, water in oil emulsions and water-oil-water emulsions); (b) coacervation-phase separation; (c) melt dispersion; (d) interfacial deposition; (e) in situ polymerization; (f) spray drying and spray congealing; (g) air suspension coating; and (h) pan and spray coating. These methods, as well as properties and characteristics of microspheres are disclosed in, for example, U.S. Patent No. 4,438,253; U.S. Patent No. 4,652,441; U.S. Patent No. 5,100,669; U.S. Patent No. 5,330,768; U.S. Patent No. 4,526,938; U.S. Patent No. 5,889,110; U.S. Patent No. 6,034,175; and European Patent 0258780, the entire disclosures of which are incorporated by reference herein in their entireties.

To prepare microspheres, several methods can be employed depending upon the desired application of the delivery vehicles. Suitable methods include, but are not limited to, spray drying, freeze drying, air drying, vacuum drying, fluidized-bed drying, milling, co-precipitation and critical fluid extraction. In the case of spray drying, freeze drying, air drying, vacuum drying, fluidized-bed drying and critical fluid extraction; the components (stabilizing polyol, bioactive material, buffers, etc.) are first dissolved or suspended in aqueous conditions. In the case of milling, the components are mixed in the dried form and milled by any method known in the art. In the case of co-precipitation, the components are mixed in organic conditions and processed as described below. Spray drying can be used to load the stabilizing polyol with the bioactive material. The components are mixed under aqueous conditions and dried using precision nozzles to

produce extremely uniform droplets in a drying chamber. Suitable spray drying machines include, but are not limited to, Buchi, NIRO, APV and Lab-plant spray driers used according to the manufacturer's instructions.

The shape of microparticles and nanoparticles may be determined by scanning electron microscopy. Spherically shaped nanoparticles are used in certain embodiments, for circulation through the bloodstream. If desired, the particles may be fabricated using known techniques into other shapes that are more useful for a specific application.

In addition to intracellular delivery of a therapeutic agent, it also possible that particles of the CDP-therapeutic agent conjugates, such as microparticles or nanoparticles, may undergo endocytosis, thereby obtaining access to the cell. The frequency of such an endocytosis process will likely depend on the size of any particle. In one embodiment, the surface charge of the particle is neutral, or slightly negative. In some embodiments, the zeta potential of the particle surface is from about -80 mV to about 50 mV, e.g., from about -40 mV to about 30 mV, e.g., from about -20 mV to about 30 mV.

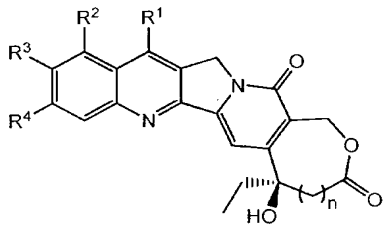
Exemplary CDP-Therapeutic Agent Conjugates

Described herein are cyclodextrin containing polymer ("CDP")-therapeutic agent conjugates, wherein one or more therapeutic agents are covalently attached to the CDP (e.g., either directly or through a linker). These cyclodextrin containing polymer ("CDP")-therapeutic agent conjugates are useful as carriers for delivery of a therapeutic agent and may improve therapeutic agent stability and solubility when used *in vivo*. The CDP-therapeutic agent conjugate can include a therapeutic agent such that the CDP-therapeutic agent conjugate can be used to treat an autoimmune disease or cancer. In an embodiment, the therapeutic agent in the CDP-therapeutic agent conjugate is a cytotoxic agent or immunomodulator. In an embodiment, the CDP-therapeutic agent conjugate is a CDP-cytotoxic agent conjugate, e.g., CDP-topoisomerase inhibitor conjugate, e.g., a CDP-topoisomerase inhibitor I conjugate (e.g., a CDP-camptothecin conjugate, CDP-irinotecan conjugate, CDP-SN-38 conjugate, CDP-topotecan conjugate, CDP-lamellarin D conjugate, a CDP-lurotecan conjugate, particle or composition, a CDP-exatecan conjugate, particle or composition, a CDP-diflomotecan conjugate, particle or

composition, and CDP-topoisomerase I inhibitor conjugates which include derivatives of camptothecin, irinotecan, SN-38, lamellarin D, lurotecan, exatecan, and diflomotecan), a CDP-topoisomerase II inhibitor conjugate (e.g., a CDP-etoposide conjugate, CDP-tenoposide conjugate, CDP-amsacrine conjugate and CDP-topoisomerase II inhibitor conjugates which include derivatives of etoposide, tenoposide, and amsacrine), a CDP-anti-metabolic agent conjugate (e.g., a CDP-antifolate conjugate (e.g., a CDP-pemetrexed conjugate, a CDP-floxuridine conjugate, a CDP-raltitrexed conjugate) or a CDP-pyrimidine analog conjugate (e.g., a CDP-capecitabine conjugate, a CDP-cytarabine conjugate, a CDP-gemcitabine conjugate, a CDP-5FU conjugate)), a CDP-alkylating agent conjugate, a CDP-anthracycline conjugate, a CDP-anti-tumor antibiotic conjugate (e.g., a CDP-HSP90 inhibitor conjugate, e.g., a CDP-geldanamycin conjugate, a CDP-tanespimycin conjugate or a CDP-alvespimycin conjugate), a CDP-platinum based agent conjugate (e.g., a CDP-cisplatin conjugate, a CDP-carboplatin conjugate, a CDP-oxaliplatin conjugate), a CDP-microtubule inhibitor conjugate, a CDP-kinase inhibitor conjugate (e.g., a CDP-seronine/threonine kinase inhibitor conjugate, e.g., a CDP-mTOR inhibitor conjugate, e.g., a CDP-rapamycin conjugate) or a CDP-proteasome inhibitor conjugate.

In one embodiment, the cytotoxic agents include topoisomerase inhibitors, e.g., a topoisomerase I inhibitor (e.g., camptothecin, irinotecan, SN-38, topotecan, lamellarin D, lurotecan, exatecan, diflomotecan, and derivatives thereof), a topoisomerase II inhibitor (e.g., etoposide, tenoposide, amsacrine and derivatives thereof).

In an embodiment, the topoisomerase inhibitor in the CDP-topoisomerase inhibitor conjugate, particle or composition is camptothecin or a camptothecin derivative. For example, camptothecin derivatives can have the following structure:



wherein,

R^1 is H, OH, optionally substituted alkyl (e.g., optionally substituted with NR^a_2 or OR_a, or SiR^a_3), or SiR^a_3; or R^1 and R^2 may be taken together to form an optionally substituted 5- to 8-membered ring (e.g., optionally substituted with NR^a_2 or OR^a);

R^2 is H, OH, NH_2, halo, nitro, optionally substituted alkyl (e.g., optionally substituted with NR^a_2 or OR^a, NR^a_2, $OC(=O)NR^a_2$, or $OC(=O)OR^a$);

R^3 is H, OH, NH_2, halo, nitro, NR^a_2, $OC(=O)NR^a_2$, or $OC(=O)OR^a$;

R^4 is H, OH, NH_2, halo, CN, or NR^a_2; or R^3 and R^4 taken together with the atoms to which they are attached form a 5- or 6-membered ring (e.g. forming a ring including –OCH_2O- or –OCH_2CH_2O-);

each R^a is independently H or alkyl; or two R^as, taken together with the atom to which they are attached, form a 4- to 8-membered ring (e.g., optionally containing an O or NR^b);

R^b is H or optionally substituted alkyl (e.g., optionally substituted with OR^c or NR^c_2);

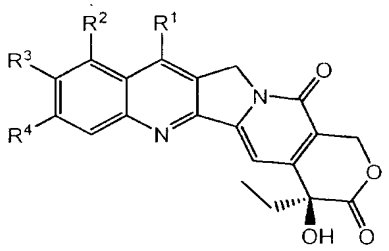
R^c is H or alkyl; or, two R^cs, taken together with the atom to which they are attached, form a 4- to 8-membered ring; and

n = 0 or 1.

In one embodiment, R^1, R^2, R^3 and R^4 of the camptothecin derivative are each H, and n is 0.

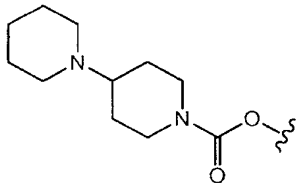
In one embodiment, R^1, R^2, R^3 and R^4 of the camptothecin derivative are each H, and n is 1.

In some embodiments, the camptothecin or camptothecin derivative is the compound as provided below.



In one embodiment, R^1 of the camptothecin derivative is H, R^2 is –$CH_2N(CH_3)_2$, R^3 is –OH, R^4 is H; and n is 0.

In one embodiment, R^1 of the camptothecin derivative is $-CH_2CH_3$, R^2 is H, R^3 is:



, R^4 is H, and n is 0.

In one embodiment, R^1 of the camptothecin derivative is $-CH_2CH_3$, R^2 is H, R^3 is –OH, R^4 is H, and n is 0.

In one embodiment, R^1 of the camptothecin derivative is *tert*-butyldimethylsilyl, R^2 is H, R^3 is –OH and R^4 is H, and n is 0.

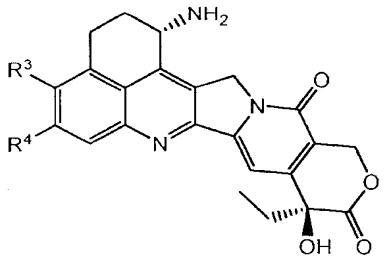
In one embodiment, R^1 of the camptothecin derivative is *tert*-butyldimethylsilyl, R^2 is hydrogen, R^3 is –OH and R^4 is hydrogen, and n is 1.

In one embodiment, R^1 of the camptothecin derivative is *tert*-butyldimethylsilyl, R^2, R^3 and R^4 are each H, and n is 0.

In one embodiment, R^1 of the camptothecin derivative is *tert*-butyldimethylsilyl, R^2, R^3 and R^4 are each H, and n is 1.

In one embodiment, R^1 of the camptothecin derivative is $-CH_2CH_2Si(CH_3)_3$ and R^2, R^3 and R^4 are each H.

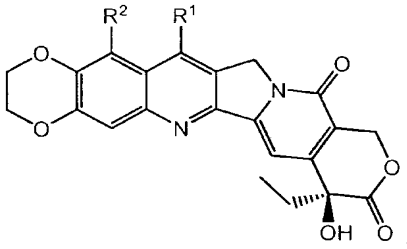
In one embodiment, R^1 and R^2 of the camptothecin derivative are taken together with the carbons to which they are attached to form an optionally substituted ring. In one embodiment, R^1 and R^2 of the camptothecin derivative are taken together with the carbons to which they are attached to form a substituted 6-membered ring. In one embodiment, the camptothecin derivative has the following formula:



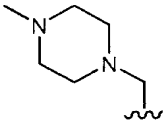
. In one embodiment, R^3 is methyl and R^4 is fluoro.

In one embodiment, R^3 and R^4 are taken together with the carbons to which they are attached to form an optionally substituted ring. In one embodiment, R^3 and R^4 are taken together with the carbons to which they are attached to form a 6-membered

heterocyclic ring. In one embodiment, the camptothecin derivative has the following formula:

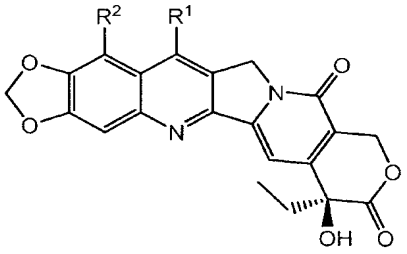


. In one embodiment, R^1 is:

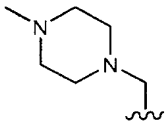


and R^2 is hydrogen.

In one embodiment, the camptothecin derivative has the following formula:

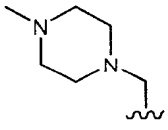


. In one embodiment, R^1 is:



and R^2 is hydrogen.

In one embodiment, R^1 is:



; R^2 is H, R^3 is methyl, R^4 is chloro; and n is 1.

In one embodiment, R^1 is $-CH=NOC(CH_3)_3$, R^2, R^3 and R^4 are each H, and n is 0.

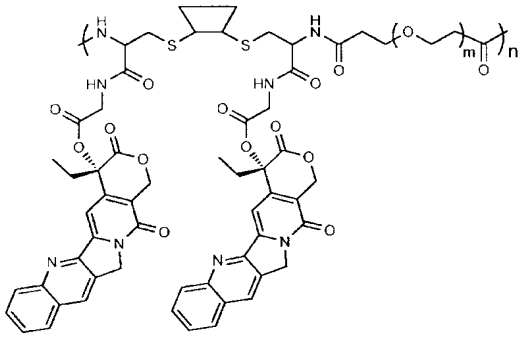
In one embodiment, R^1 is $-CH_2CH_2NHCH(CH_3)_2$, R^2, R^3 and R^4 are each H; and n is 0.

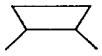
In one embodiment, R^1 and R^2 are H, R^3 and R^4 are fluoro, and n is 1.

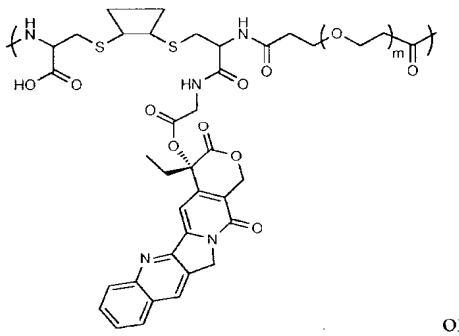
In one embodiment, each of R^1, R^3, and R^4 is H, R^2 is NH_2, and n is 0.

In one embodiment, each of R^1, R^3, and R^4 is H, R^2 is NO_2, and n is 0.

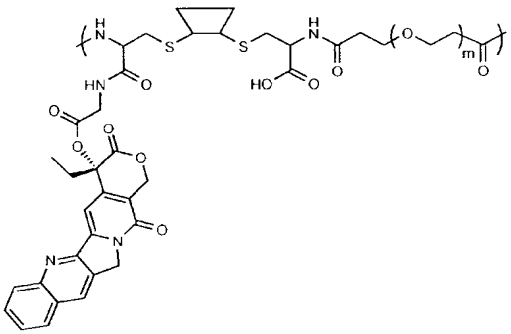
In one embodiment, the CDP-topoisomerase I inhibitor conjugate is a CDP-camptothecin conjugate, e.g., as shown below,

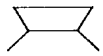


wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-topoisomerase I inhibitor conjugate, e.g., the CDP-camptothecin conjugate, does not have complete loading, e.g., one or more binding sites, e.g., cysteine residues, are not bound to a topoisomerase 1 inhibitor, e.g., a camptothecin moiety, e.g., a glycine-linkage bound camptothecin, e.g., the CDP-camptothecin conjugate comprises one or more subunits having the formulae provided below



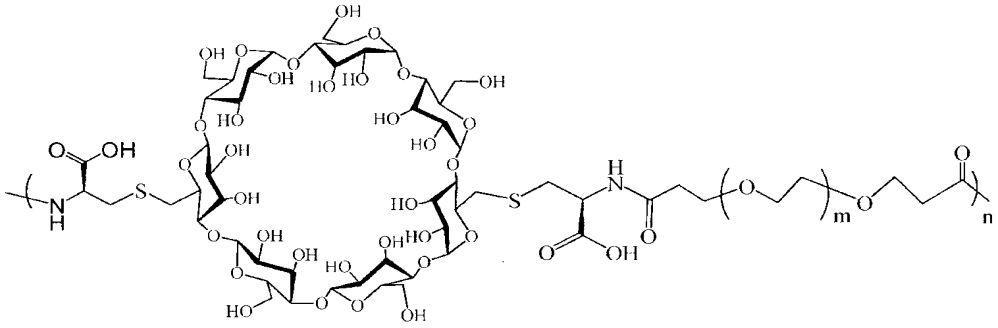
or

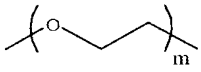


wherein  represents a cyclodextrin; m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-topoisomerase 1 inhibitor

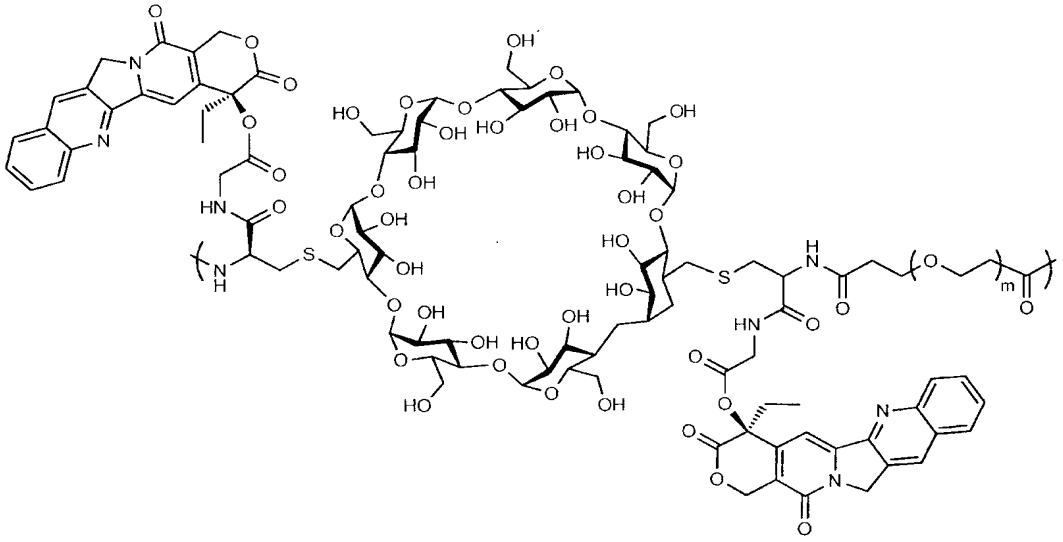
conjugate, particle or composition e.g., the CDP-camptothecin conjugate, particle or composition, comprises a mixture of fully-loaded and partially-loaded CDP-topoisomerase I inhibitor subunits within the conjugates, e.g., CDP-camptothecin conjugates.

In one embodiment, the CDP is the cyclodextrin-containing polymer shown below (as well as in FIG. 3):



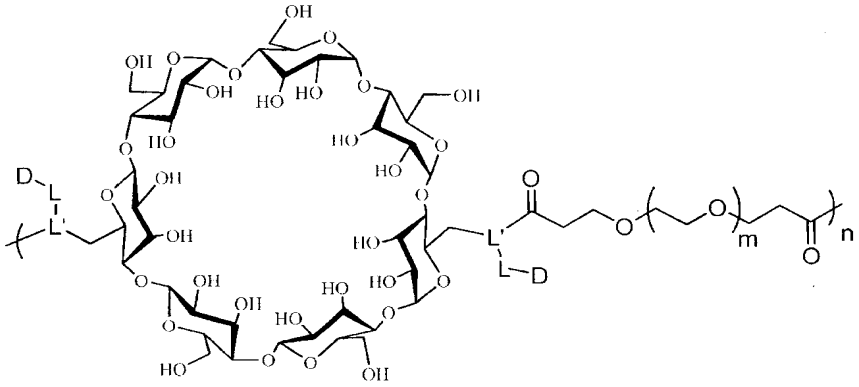
wherein the group  has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. Note that the taxane is conjugated to the CDP through the carboxylic acid moieties of the polymer as provided above. Full loading of the taxane onto the CDP is not required. In some embodiments, at least one, e.g., at least 2, 3, 4, 5, 6 or 7, of the carboxylic acid moieties remains unreacted with the taxane after conjugation (e.g., a plurality of the carboxylic acid moieties remain unreacted).

In one embodiment, the CDP-topoisomerase I inhibitor conjugate comprises a subunit of

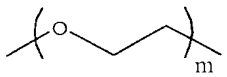


wherein m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40).

In some embodiments, the CDP-topoisomerase inhibitor conjugate is a polymer having the following formula:



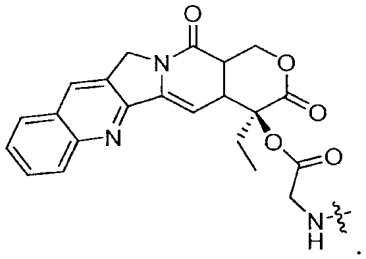
wherein L and L' independently for each occurence, is a linker, a bond, or -OH and D, independently for each occurence, is a topoisomerase inhibitor such as camptothecin ("CPT"), a camptothecin derivative or absent, and

wherein the group  has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, provided that at least one D is CPT

or a camptothecin derivative. In some embodiments, at least 2 D moieties are CPT and/or a camptothecin derivative.

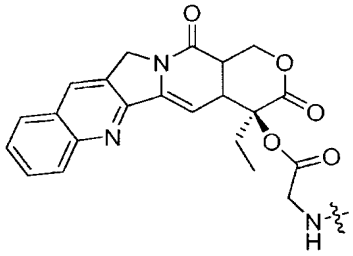
In some embodiments, each L', for each occurence, is a cysteine. In some embodiments, the cysteine is attached to the cyclodextrin via a sulfide bond. In some embodiments, the cysteine is attached to the PEG containing portion of the polymer via an amide bond.

In some embodiments, the L is a linker (e.g., an amino acid such as glycine). In some embodiments, L is absent. In some embodiments, D-L together form

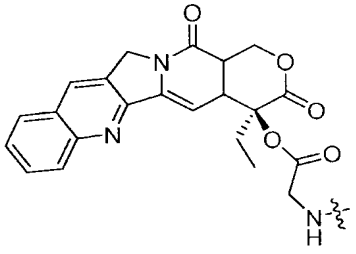


In some embodiments, a plurality of D moieties are absent and at the same position on the polymer, the corresponding L is -OH.

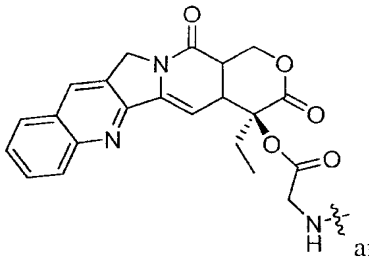
In some embodiments, less than all of the C(=O) moieties of the cysteine residue in the polymer backbone are attached to

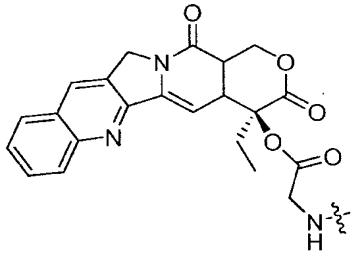


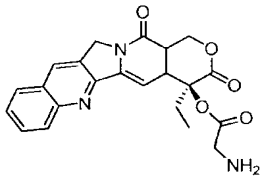
moieties, meaning in some embodiments,

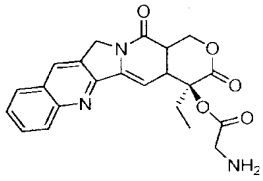


is absent in one or more positions of the polymer backbone, provided that the polymer comprises at least one

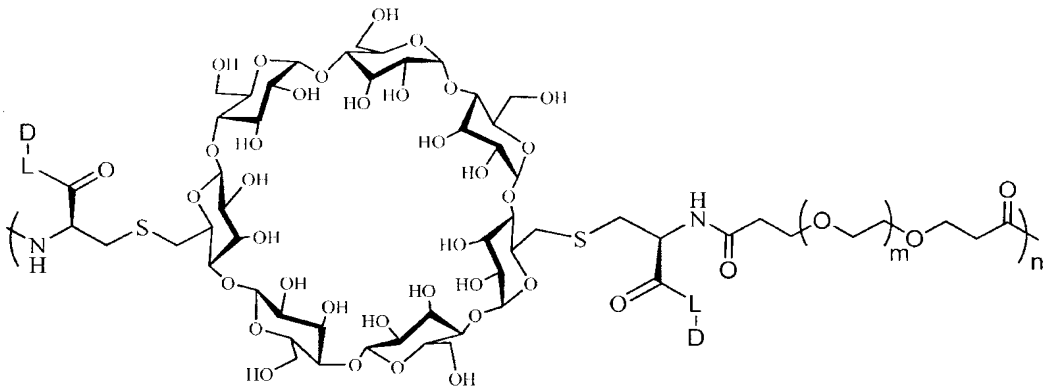

and in some embodiments, at least two


moieties. In some embodiments, the loading of the

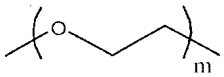

moieties on the CDP-topoisomerase inhibitor conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%, e.g., from about 6 to about 10%). In some embodiments, the loading of


on the CDP is from about 6% to about 10% by weight of the total polymer.

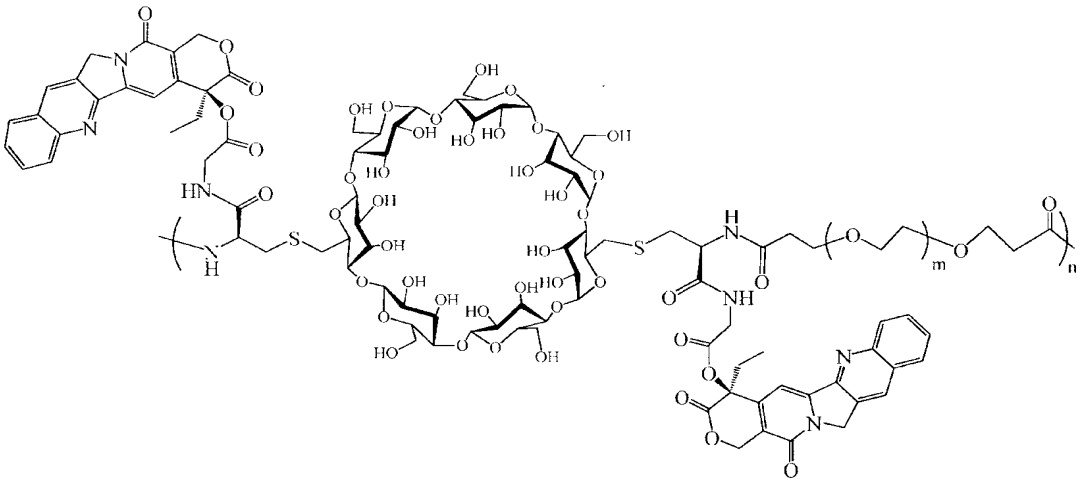
In some embodiments, the CDP-topoisomerase inhibitor conjugate is a polymer having the following formula:



wherein L, independently for each occurrence, is a linker, a bond, or -OH and D, independently for each occurrence, is camptothecin ("CPT"), a camptothecin derivative or absent, and

wherein the group  has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, provided that at least one D is CPT or a camptothecin derivative. In some embodiments, at least 2 D moieties are CPT and/or a camptothecin derivative.

In some embodiments, the CDP-camptothecin conjugate is as shown below, which is referred to herein as "CRLX101." In some embodiments, a CDP-camptothecin conjugate may have one or more binding sites, e.g., a cysteine residue, not bound to the CDP, e.g., as described below:



In the above structure:

m = about 77 or the molecular weight of the PEG moiety is from about 3060 to about 3740 (e.g., about 3400) Da;

n = is from about 10 to about 18 (e.g., about 14);

the molecular weight of the polymer backbone (i.e., the polymer minus the CPT-gly, which results in the cysteine moieties having a free -C(O)OH) is from about 48 to about 8500 Da;

the polydispersity of the polymer backbone is less than about 2.2; and

the loading of the CPT onto the polymer backbone is from about 6 to about 13% by weight, wherein 13% is theoretical maximum, meaning, in some instances, one or more of the cysteine residues has a free -C(O)OH (i.e., it lacks the CPT-gly).

In some embodiments, the polydispersity of the PEG component in the above structure is less than about 1.1.

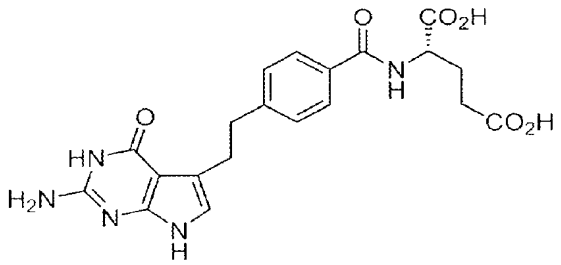
In some embodiments, a CDP-camptothecin conjugate described herein has a terminal amine and/or a terminal carboxylic acid.

In an embodiment, the topoisomerase inhibitor of the CDP-topoisomerase inhibitor conjugate, particle, or composition is a topoisomerase II inhibitor, e.g.,

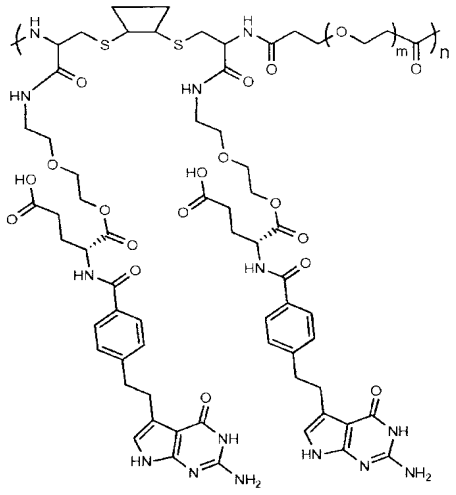
etoposide (Toposar® or VePesid®), teniposide (Vumon®), amsacrine and derivatives thereof.

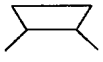
In an embodiment, the therapeutic agent in the CDP-therapeutic agent conjugate is a cytotoxic agent such as an anti-metabolic agent. In some embodiments, the anti-metabolic agent in the CDP-anti-metabolic agent conjugate, particle or composition is an anti-metabolic agent including, without limitation, folic acid antagonists (also referred to herein as antifolates), pyrimidine analogs, purine analogs and adenosine deaminase inhibitors): methotrexate (Rheumatrex®, Trexall®), 5-fluorouracil (Adrucil®, Efudex®, or Fluoroplex®), floxuridine (FUDF®), cytarabine (Cytosar-U® or Tarabine PFS), 6-mercaptopurine (Puri-Nethol®)), 6-thioguanine (Thioguanine Tabloid®), fludarabine phosphate (Fludara®), pentostatin (Nipent®), pemetrexed (Alimta®), raltitrexed (Tomudex®), cladribine (Leustatin®), clofarabine (Clofarex® or Clolar®), mercaptopurine (Puri-Nethol®), capecitabine (Xeloda®), nelarabine (Arranon®), azacitidine (Vidaza®) and gemcitabine (Gemzar®). Preferred anti-metabolites include, e.g., 5-fluorouracil (5FU) (Adrucil®, Efudex®, or Fluoroplex®), floxuridine (FUDF®), capecitabine (Xeloda®), pemetrexed (Alimta®), raltitrexed (Tomudex®) and gemcitabine (Gemzar®).

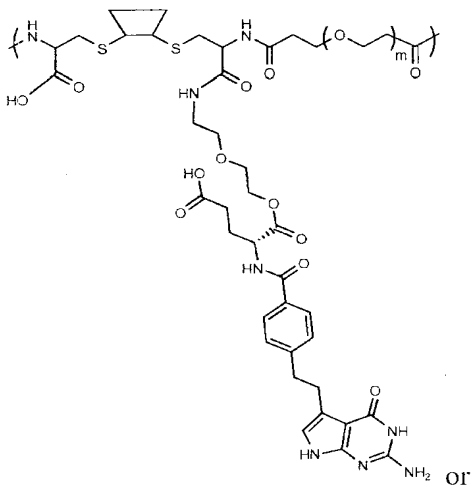
In an embodiment, the anti-metabolic agent in the CDP-anti-metabolic agent conjugate, particle or composition is an antifolate, e.g., a CDP-antifolate conjugate, particle or composition. In preferred embodiments, the antifolate in the CDP-antifolate conjugate, particle or composition is pemetrexed or a pemetrexed derivative. For example, pemetrexed has the following structure:



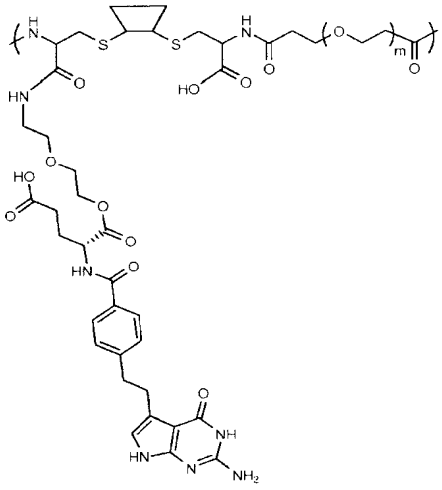
In one embodiment, the CDP-antifolate conjugate is a CDP-pemetrexed conjugate, e.g.,

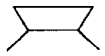


wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-antifolate conjugate, e.g., the CDP-pemetrexed conjugate, does not have complete loading, e.g., one or more binding sites, e.g., cysteine residues, are not bound to an antifolate, e.g., a pemetrexed moiety, e.g., an amine-linkage bound pemetrexed, e.g., the CDP-pemetrexed conjugate comprises one or more subunits having the formulae provided below:

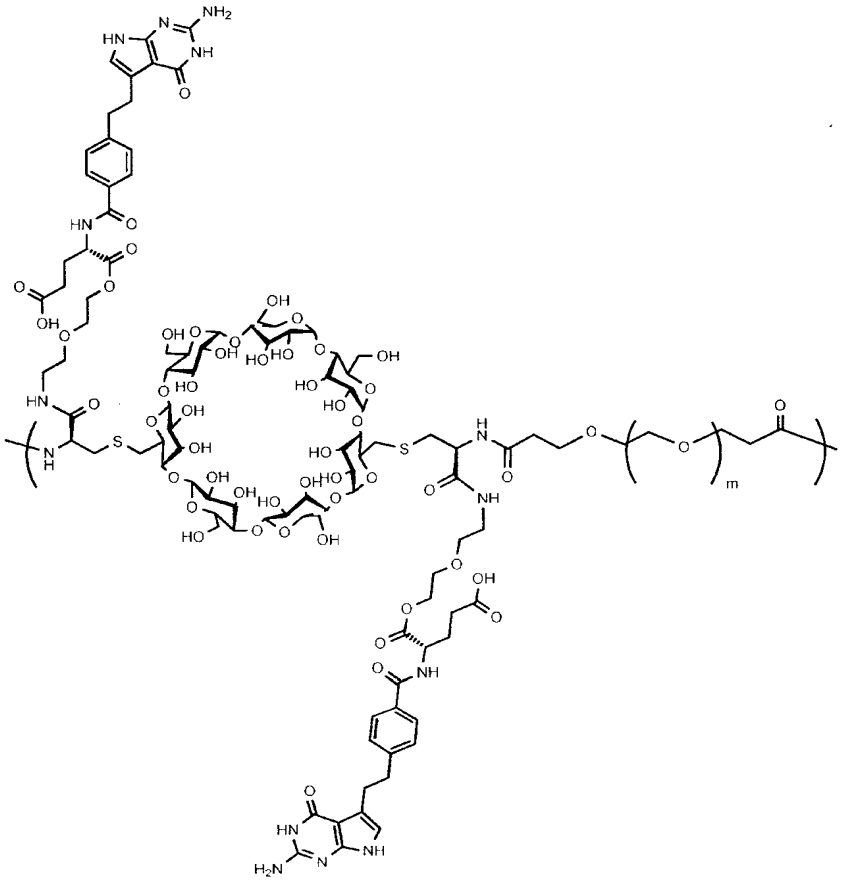


or



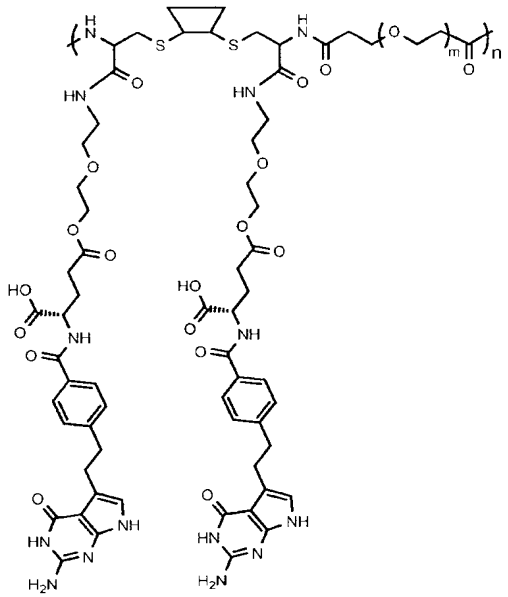
wherein  represents a cyclodextrin and m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-antifolate conjugate, particle or composition e.g., the CDP-pemetrexed conjugate, particle or composition, comprises a mixture of fully-loaded and partially-loaded CDP-antifolate analog conjugates, e.g., CDP-pemetrexed conjugates.

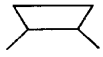
In one embodiment, the CDP-pemetrexed conjugate comprises a subunit of

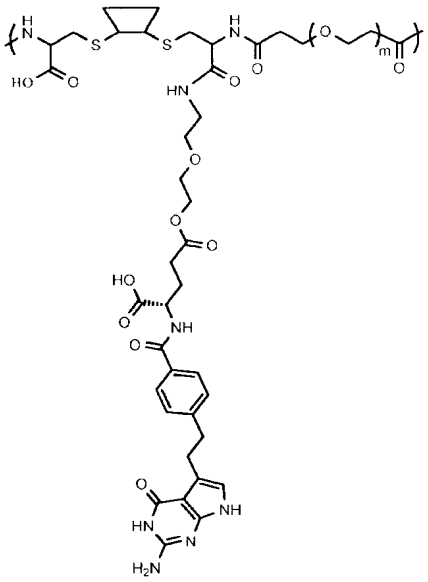


wherein m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40).

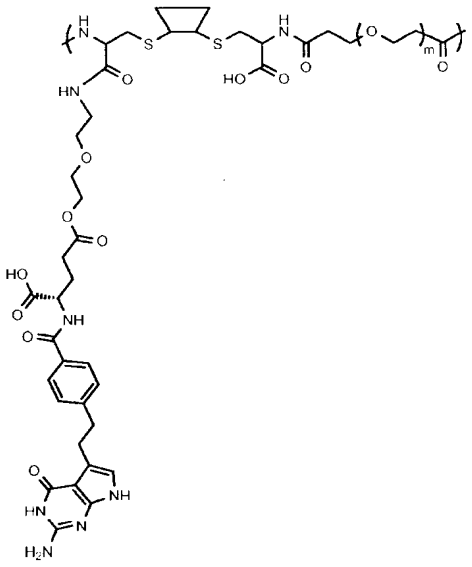
In one embodiment, the CDP-antifolate conjugate is a CDP-pemetrexed conjugate, e.g.,

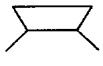


wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-antifolate conjugate, e.g., the CDP-pemetrexed conjugate, does not have complete loading, e.g., one or more binding sites, e.g., cysteine residues, are not bound to an antifolate, e.g., a pemetrexed moiety, e.g., an amine-linkage bound pemetrexed, e.g., the CDP-pemetrexed conjugate comprises one or more subunits having the formulae provided below:

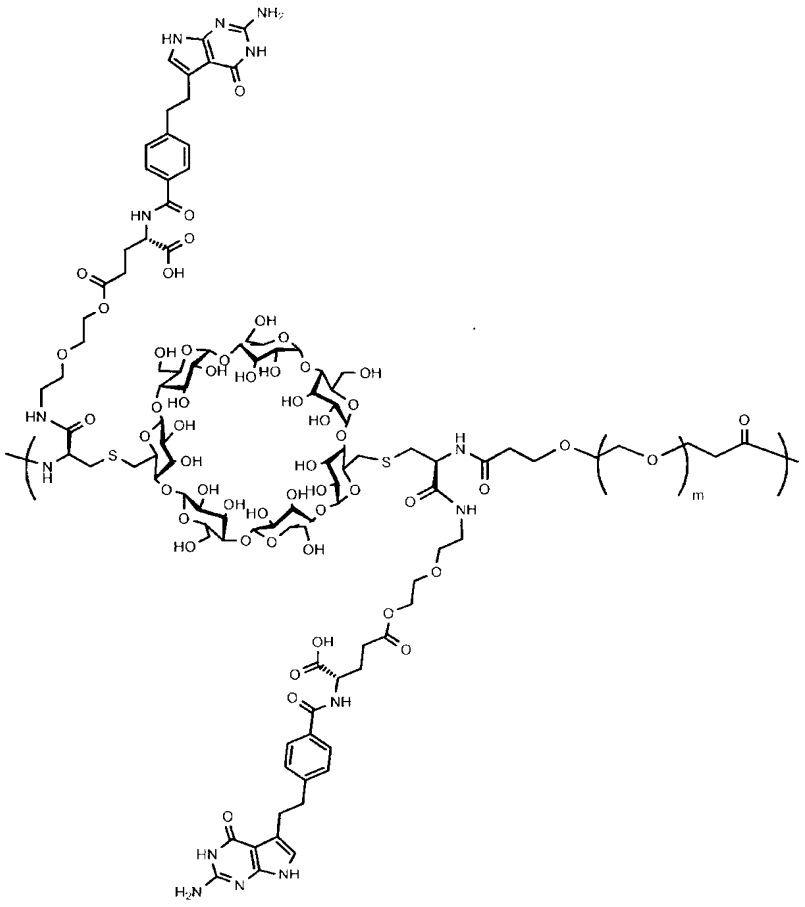


or



wherein  represents a cyclodextrin and m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-antifolate conjugate, particle or composition e.g., the CDP-pemetrexed conjugate, particle or composition, comprises a mixture of fully-loaded and partially-loaded CDP-antifolate analog conjugates, e.g., CDP-pemetrexed conjugates.

In one embodiment, the CDP-pemetrexed conjugate comprises a subunit of



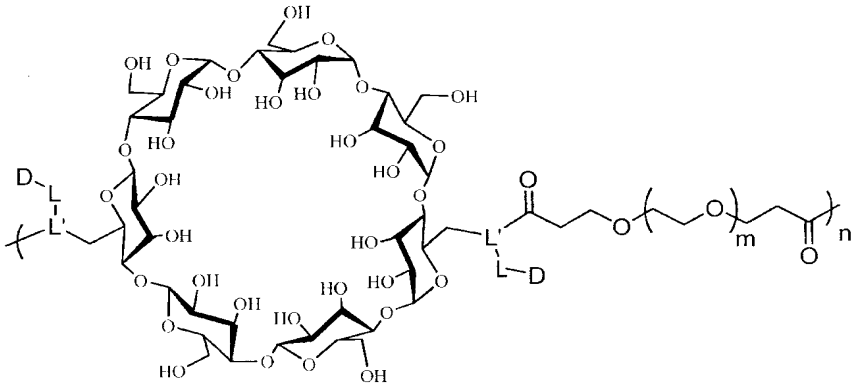
wherein m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40).CDP-pemetrexed conjugates can be made using many different combinations of components described herein. For example, various combinations of cyclodextrins (e.g., beta-cyclodextrin), comonomers (e.g., PEG containing comonomers), linkers linking the cyclodextrins and comonomers, and/or linkers tethering the pemetrexed to the CDP are described herein.

In one embodiment, the CDP-pemetrexed conjugate forms a particle, e.g., a nanoparticle. The compositions described herein comprise a CDP-pemetrexed conjugate or a plurality of CDP-pemetrexed conjugates. The composition can also comprise a particle or a plurality of particles described herein.

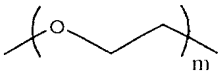
In one embodiment, the CDP-pemetrexed conjugate forms a particle, e.g., a nanoparticle. The nanoparticle ranges in size from 10 to 300 nm in diameter, e.g., 15 to 280, 30 to 250, 40 to 200, 20 to 150, 30 to 100, 20 to 80, 30 to 70, 40 to 60 or 40 to 50 nm diameter. In one embodiment, the particle is 50 to 60 nm, 20 to 60 nm, 30 to 60 nm, 35 to 55 nm, 35 to 50 nm or 35 to 45 nm in diameter.

In one embodiment, the surface charge of the molecule is neutral, or slightly negative. In some embodiments, the zeta potential of the particle surface is from about -80 mV to about 50 mV, about -20 mV to about 20 mV, about -20 mV to about -10 mV, or about -10 mV to about 0.

In some embodiments, the CDP-pemetrexed conjugate is a polymer having the formula:



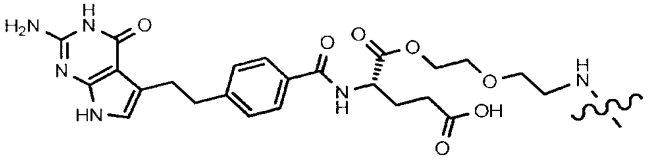
wherein L and L' independently for each occurence, is a linker, a bond, or -OH and D, independently for each occurence, is a pemetrexed, a pemetrexed derivative or absent, and

wherein the group  has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, provided that at least one D is pemetrexed or a pemetrexed derivative. In some embodiments, at least 2 D moieties are pemetrexed and/or a pemetrexed derivative.

In some embodiments, each L', for each occurence, is a cysteine. In some embodiments, the cysteine is attached to the cyclodextrin via a sulfide bond. In some

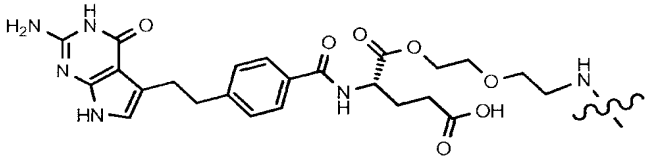
embodiments, the cysteine is attached to the PEG containing portion of the polymer via an amide bond.

In some embodiments, the L is a linker (e.g., an amine linkage). In some embodiments, L is absent. In some embodiments, D-L together form

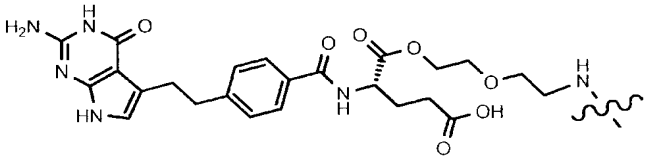


In some embodiments, a plurality of D moieties are absent and at the same position on the polymer, the corresponding L is -OH.

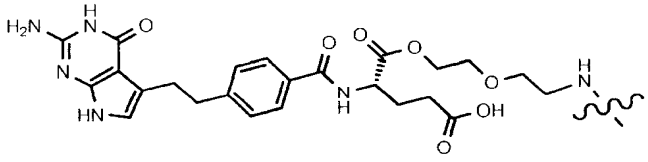
In some embodiments, less than all of the C(=O) moieties of the cysteine residue in the polymer backbone are attached to



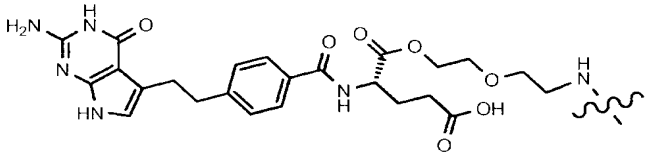
moieties, meaning in some



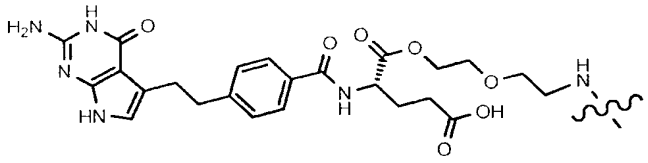
embodiments, is absent in one or more positions of the polymer backbone, provided that the polymer comprises at least



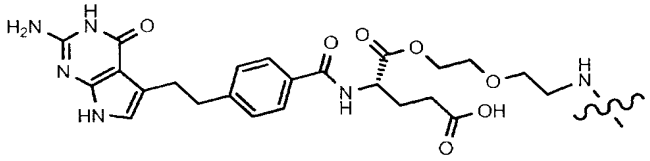
one and in some embodiments, at



least two moieties. In some

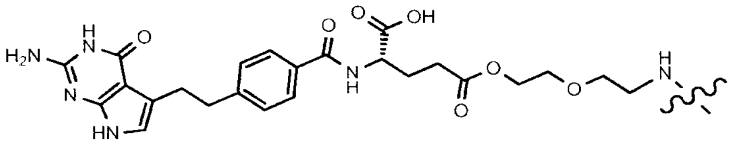


embodiments, the loading of the moieties on the CDP-pemetrexed conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%, e.g., from about 6 to about 10%). In some embodiments, the



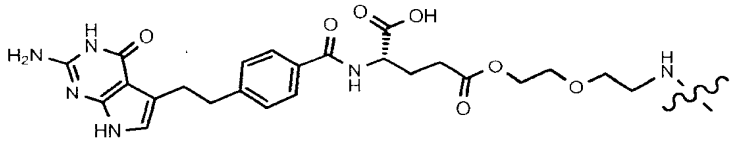
loading of on the CDP is from about 6% to about 10% by weight of the total polymer.

In some embodiments, the L is a linker (e.g., an amine linkage). In some embodiments, L is absent. In some embodiments, D-L together form

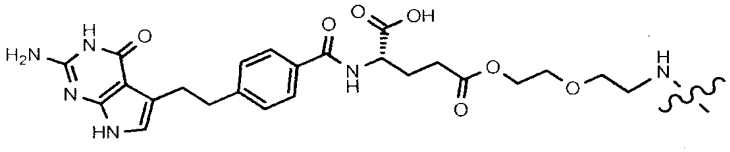


In some embodiments, a plurality of D moieties are absent and at the same position on the polymer, the corresponding L is -OH.

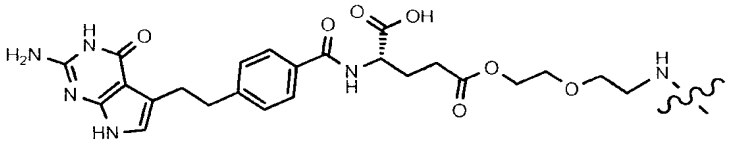
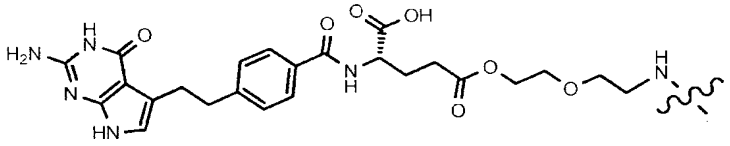
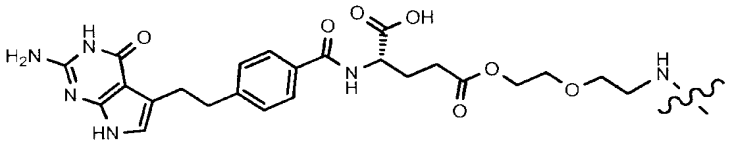
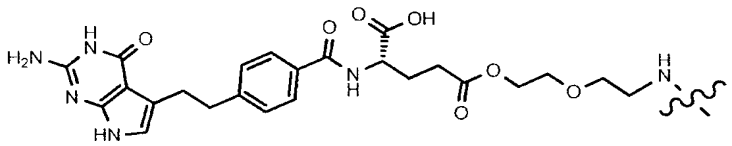
In some embodiments, less than all of the C(=O) moieties of the cysteine residue in the polymer backbone are attached to



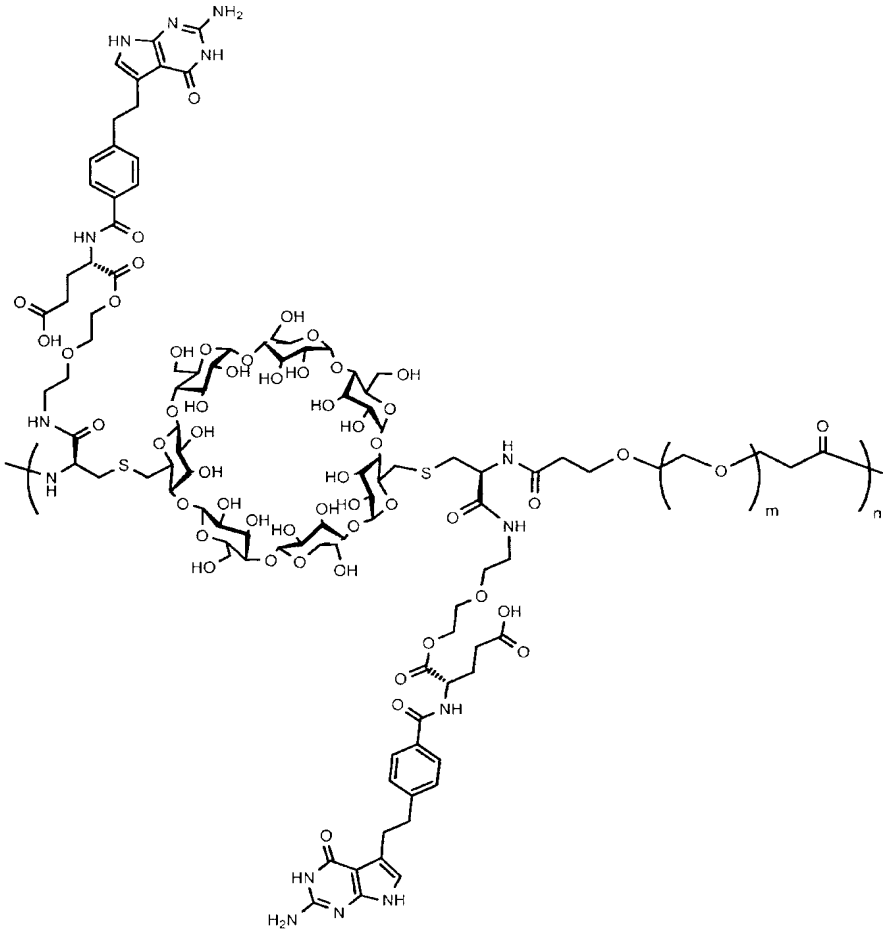
moieties, meaning in some



embodiments, is absent in one or more positions of the polymer backbone, provided that the polymer comprises at

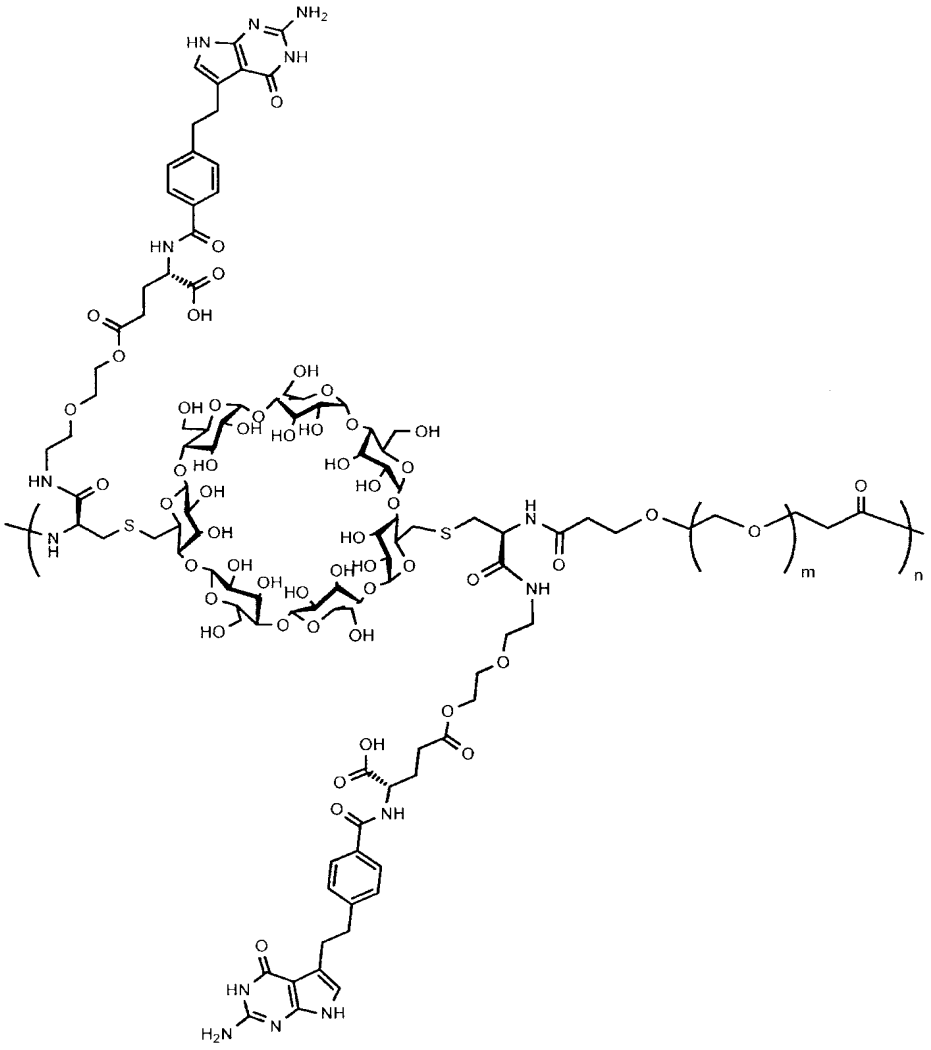
least one  and in some embodiments, at least two  moieties. In some embodiments, the loading of the  moieties on the CDP-pemetrexed conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%, e.g., from about 6 to about 10%). In some embodiments, the loading of  on the CDP is from about 6% to about 10% by weight of the total polymer.

In some embodiments, the CDP-pemetrexed conjugate is a polymer of the formula:



,

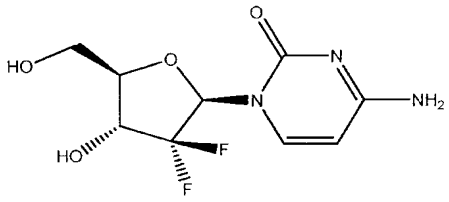
wherein m and n are as defined above, and wherein less than all of the C(=O) sites of the cysteine of the polymer backbone are occupied as indicated above with the pemetrexed-ester, but instead are free acids, meaning, the theoretical loading of the polymer is less than 100%.In some embodiments, the CDP-pemetrexed conjugate is a polymer of the formula:



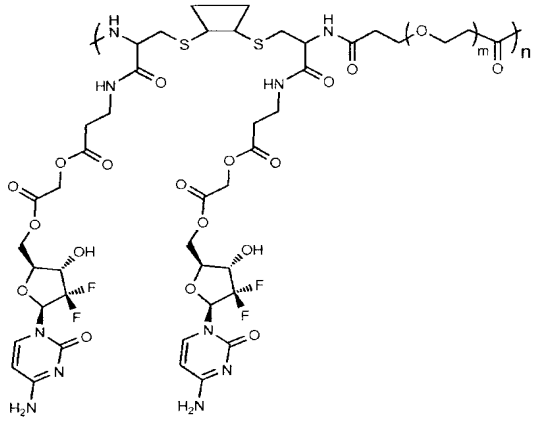
wherein m and n are as defined above, and wherein less than all of the C(=O) sites of the cysteine of the polymer backbone are occupied as indicated above with the pemetrexed-ester, but instead are free acids, meaning, the theoretical loading of the polymer is less than 100%.

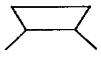
In an embodiment, the anti-metabolic agent in the CDP-anti-metabolic agent conjugate, particle or composition is pyrimidine analog, e.g., a CDP-pyrimidine analog conjugate, particle or composition. In preferred embodiments, the pyrimidine analog agent in the CDP-pyrimidine analog conjugate, particle or composition comprises

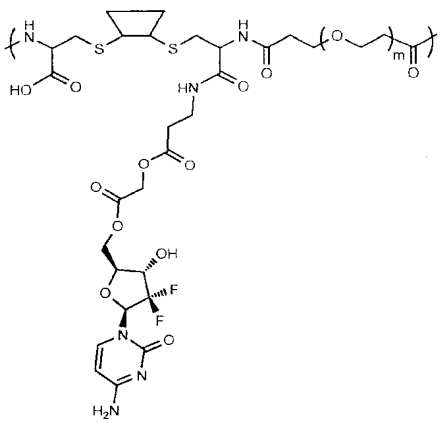
gemcitabine or a gemcitabine derivative. For example, gemcitabine can have the following structure:



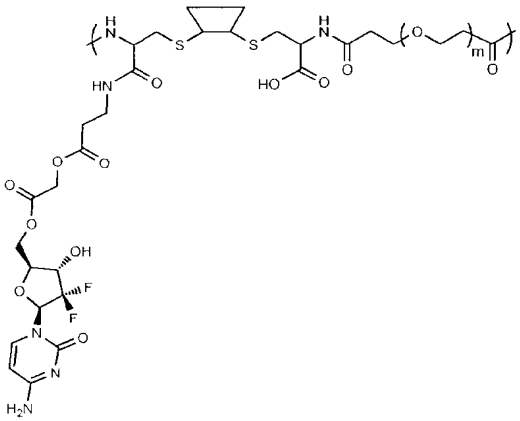
In one embodiment, the CDP-pyrimidine analog conjugate is a CDP-gemcitabine conjugate, e.g.,

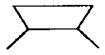


wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-pyrimidine analog conjugate, e.g., the CDP-gemcitabine conjugate, does not have complete loading, e.g., one or more binding sites, e.g., cysteine residues, are not bound to a pyrimidine analog, e.g., a gemcitabine moiety, e.g., an ester-linkage bound gemcitabine, e.g., the CDP-gemcitabine conjugate comprises one or more subunits having the formulae provided below:

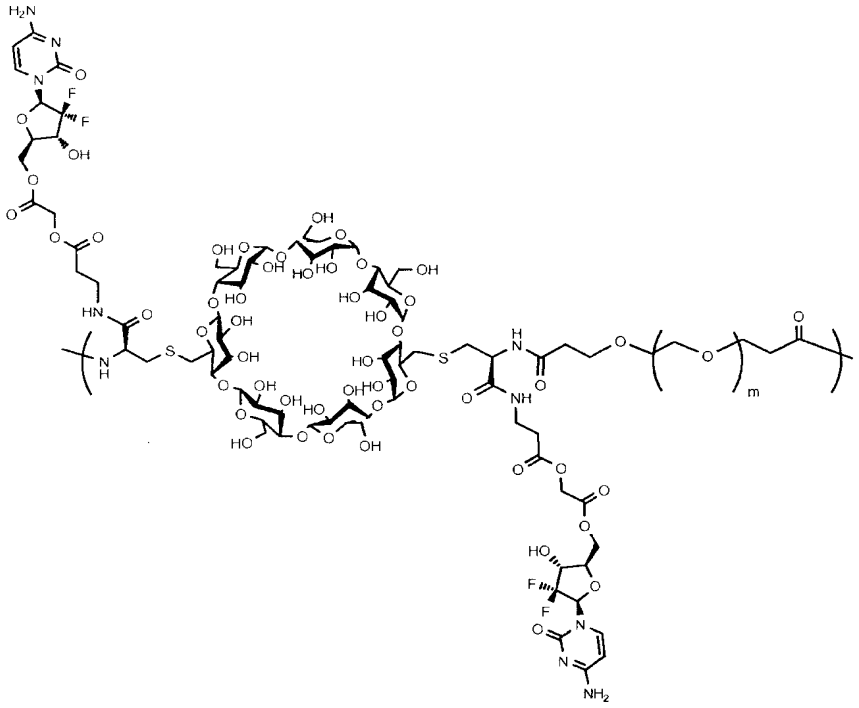


or



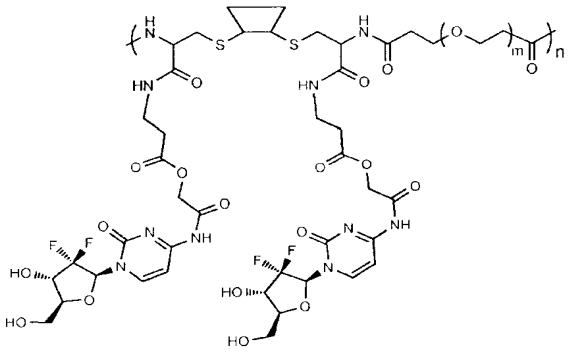
wherein  represents a cyclodextrin and m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-pyrimidine analog conjugate, particle or composition e.g., the CDP-gemcitabine conjugate, particle or composition, comprises a mixture of fully-loaded and partially-loaded CDP-pyrimidine analog conjugates, e.g., CDP-gemcitabine conjugates.

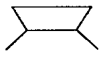
In one embodiment, the CDP-pyrimidine analog conjugate comprises a subunit of



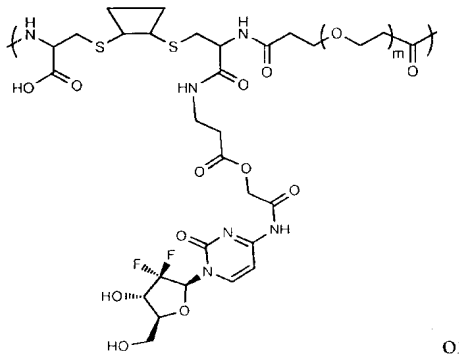
wherein m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40).

In one embodiment, the CDP-pyrimidine analog conjugate is a CDP-gemcitabine conjugate, e.g.,

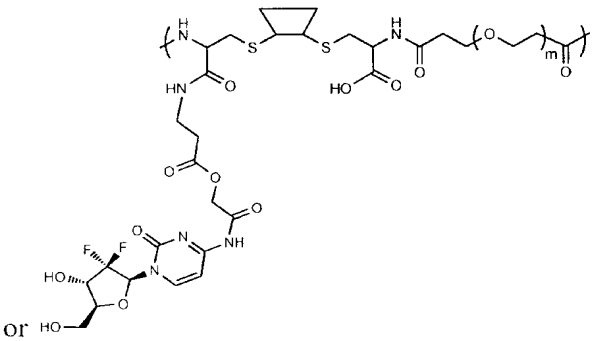


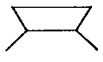
wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to

50, or from 20 to 40). In some embodiments, the CDP-pyrimidine analog conjugate, e.g., the CDP-gemcitabine conjugate, does not have complete loading, e.g., one or more binding sites, e.g., cysteine residues, are not bound to a pyrimidine analog, e.g., a gemcitabine moiety, e.g., an ester-linkage bound gemcitabine, e.g., the CDP-gemcitabine conjugate comprises one or more subunits having the formulae provided below:

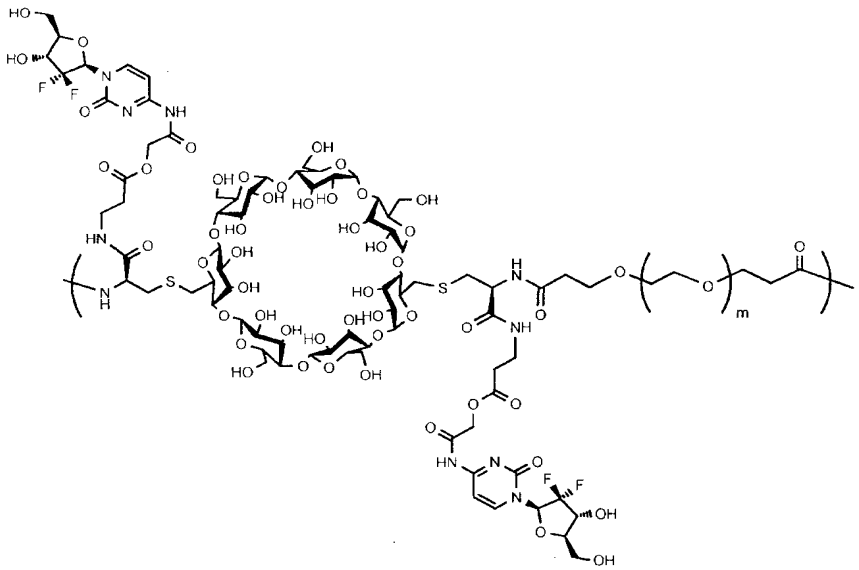


or



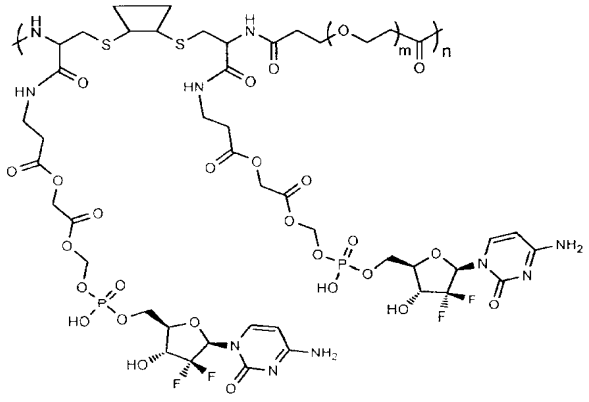
wherein  represents a cyclodextrin and m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-pyrimidine analog conjugate, particle or composition e.g., the CDP-gemcitabine conjugate, particle or composition, comprises a mixture of fully-loaded and partially-loaded CDP-pyrimidine analog conjugates, e.g., CDP-gemcitabine conjugates.

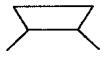
In one embodiment, the CDP-pyrimidine analog conjugate comprises a subunit of



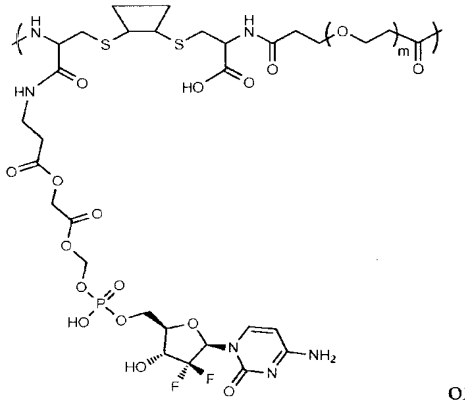
wherein m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40).

In one embodiment, the CDP-pyrimidine analog conjugate is a CDP-gemcitabine derivative conjugate, e.g.,

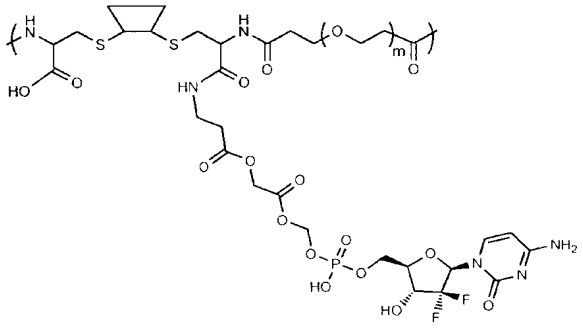


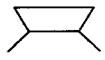
wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-pyrimidine analog conjugate, e.g.,

the CDP-gemcitabine derivative conjugate, does not have complete loading, e.g., one or more binding sites, e.g., cysteine residues, are not bound to a pyrimidine analog, e.g., a gemcitabine derivative, e.g., an ester-linkage bound gemcitabine derivative, e.g., the CDP-gemcitabine derivative conjugate comprises one or more subunits having the formulae provided below:

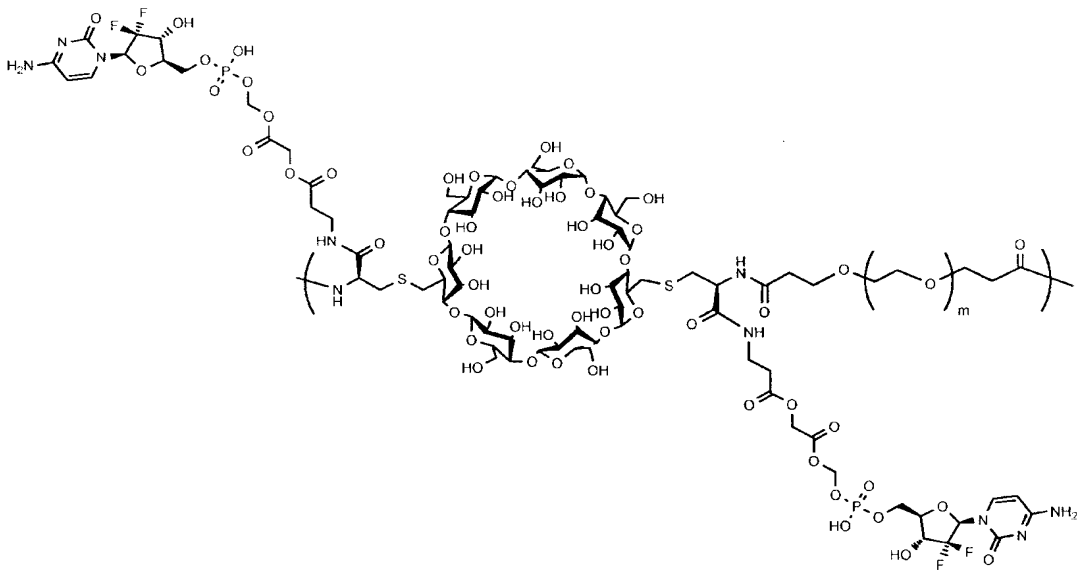


or



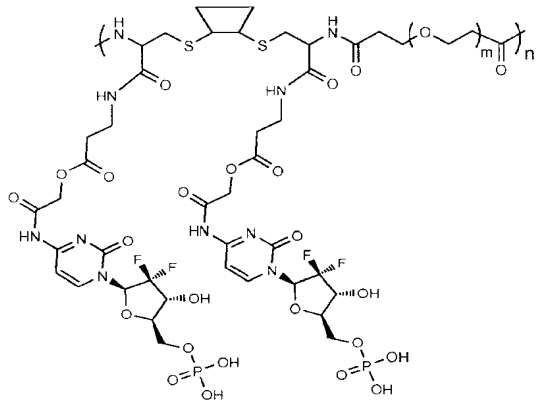
wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-pyrimidine analog conjugate, particle or composition e.g., the CDP-gemcitabine derivative conjugate, particle or composition, comprises a mixture of fully-loaded and partially-loaded CDP-pyrimidine analog conjugates, e.g., CDP-gemcitabine derivative conjugates.

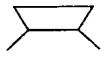
In one embodiment, the CDP-pyrimidine analog conjugate comprises a subunit of



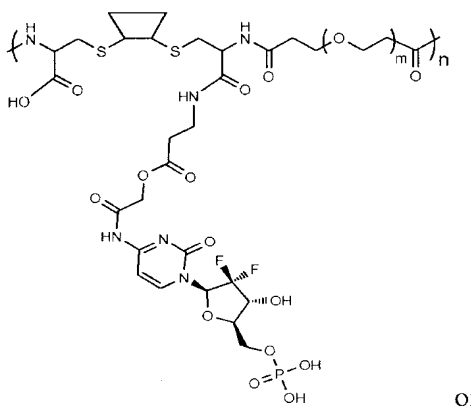
wherein m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40).

In one embodiment, the CDP-pyrimidine analog conjugate is a CDP-gemcitabine derivative conjugate, e.g.,

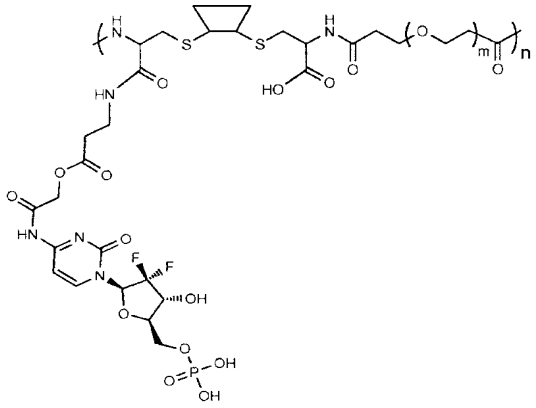


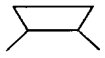
wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-pyrimidine analog conjugate, e.g.,

the CDP-gemcitabine derivative conjugate, does not have complete loading, e.g., one or more binding sites, e.g., cysteine residues, are not bound to a pyrimidine analog, e.g., a gemcitabine derivative, e.g., an ester-linkage bound gemcitabine derivative, e.g., the CDP-gemcitabine derivative conjugate comprises one or more subunits having the formulae provided below:

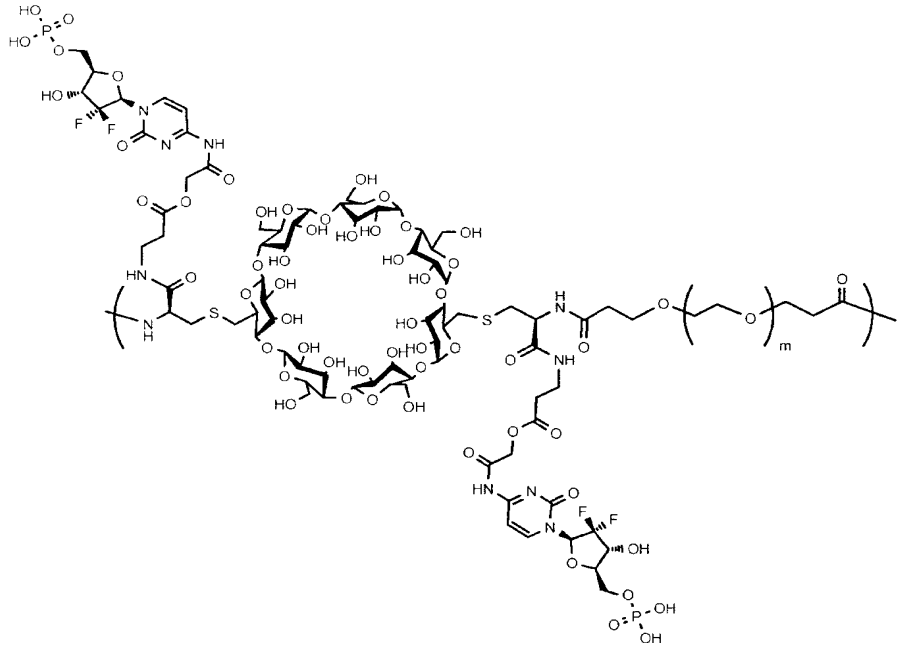


or



wherein  represents a cyclodextrin and m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40). In some embodiments, the CDP-pyrimidine analog conjugate, particle or composition e.g., the CDP-gemcitabine derivative conjugate, particle or composition, comprises a mixture of fully-loaded and partially-loaded CDP-pyrimidine analog conjugates, e.g., CDP-gemcitabine derivative conjugates.

In one embodiment, the CDP-pyrimidine analog conjugate comprises a subunit of



wherein m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40).

CDP-gemcitabine conjugates and CDP-gemcitabine derivative conjugates can be made using many different combinations of components described herein. For example, various combinations of cyclodextrins (e.g., beta-cyclodextrin), comonomers (e.g., PEG containing comonomers), linkers linking the cyclodextrins and comonomers, and/or linkers tethering the gemcitabine to the CDP are described herein.

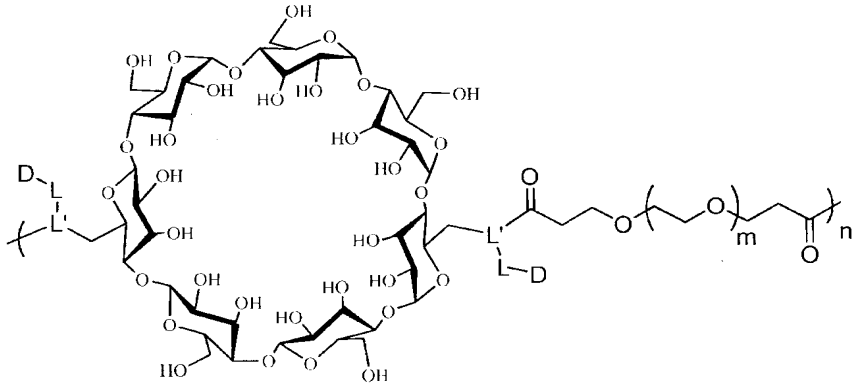
In one embodiment, the CDP-gemcitabine conjugate forms a particle, e.g., a nanoparticle. The particle can comprise a CDP-gemcitabine conjugate, e.g., a plurality of CDP-gemcitabine conjugates, e.g., CDP-gemcitabine conjugates having the same gemcitabine or different gemcitabines. The compositions described herein comprise a CDP-gemcitabine conjugate or a plurality of CDP-gemcitabine conjugates. The composition can also comprise a particle or a plurality of particles described herein.

In one embodiment, the CDP-gemcitabine conjugate containing the inclusion complex forms a particle, e.g., a nanoparticle. The nanoparticle ranges in size from 10 to 300 nm in diameter, e.g., 15 to 280, 30 to 250, 40 to 200, 20 to 150, 30 to 100, 20 to 80,

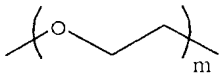
30 to 70, 40 to 60 or 40 to 50 nm diameter. In one embodiment, the particle is 50 to 60 nm, 20 to 60 nm, 30 to 60 nm, 35 to 55 nm, 35 to 50 nm or 35 to 45 nm in diameter.

In one embodiment, the surface charge of the molecule is neutral, or slightly negative. In some embodiments, the zeta potential of the particle surface is from about -80 mV to about 50 mV, about -20 mV to about 20 mV, about -20 mV to about -10 mV, or about -10 mV to about 0.

In some embodiments, the CDP-gemcitabine conjugate or CDP-gemcitabine derivative conjugate is a polymer having a formula:

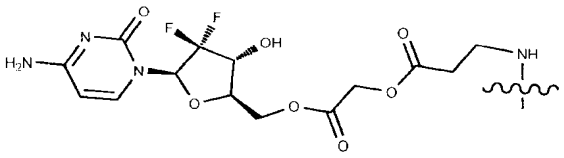


wherein L and L' independently for each occurence, is a linker, a bond, or -OH and D, independently for each occurence, is a gemcitabine, a gemcitabine derivative or absent, and

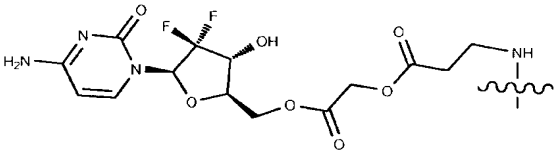
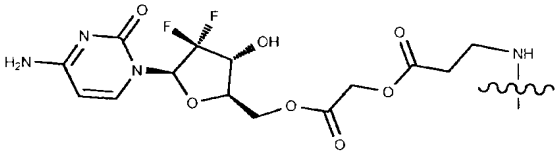
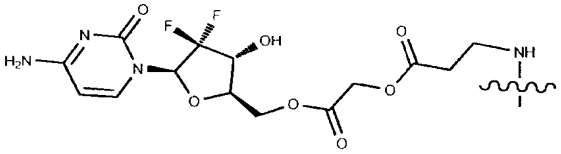
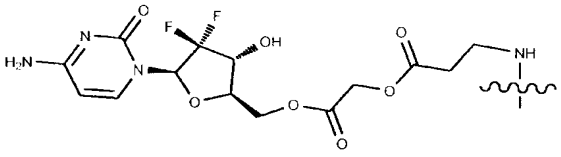
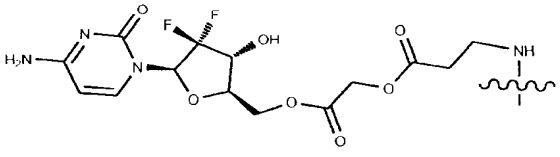
wherein the group  has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, provided that at least one D is gemcitabine or a gemcitabine derivative. In some embodiments, at least 2 D moieties are gemcitabine and/or a gemcitabine derivative.

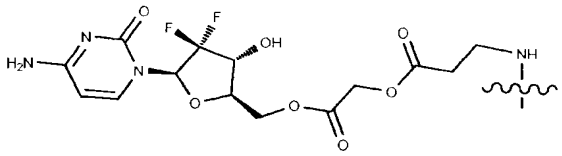
In some embodiments, each L', for each occurence, is a cysteine. In some embodiments, the cysteine is attached to the cyclodextrin via a sulfide bond. In some embodiments, the cysteine is attached to the PEG containing portion of the polymer via an amide bond.

In some embodiments, the L is a linker (e.g., an ester linkage). In some embodiments, L is absent. In some embodiments, D-L together form

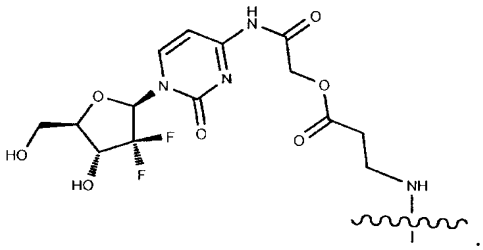


In some embodiments, a plurality of D moieties are absent and at the same position on the polymer, the corresponding L is -OH.

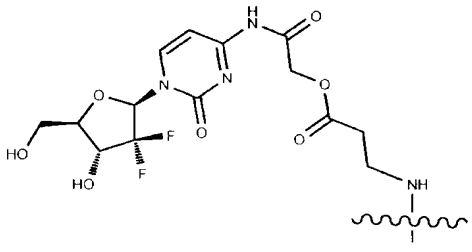
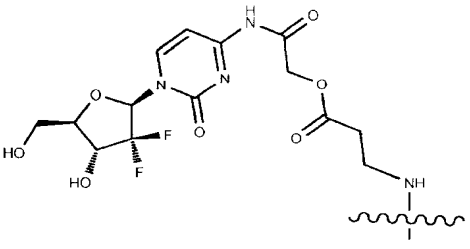
In some embodiments, less than all of the C(=O) moieties of the cysteine residue in the polymer backbone are attached to  moieties, meaning in some embodiments,  is absent in one or more positions of the polymer backbone, provided that the polymer comprises at least one  and in some embodiments, at least two  moieties. In some embodiments, the loading of the  moieties on the CDP-gemcitabine conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%, e.g., from about 6 to about 10%). In some embodiments, the

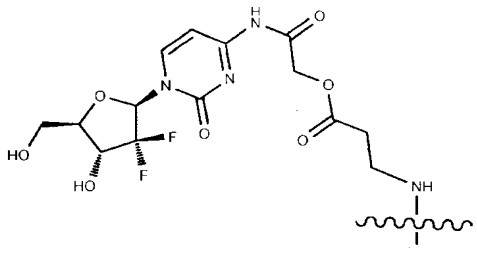
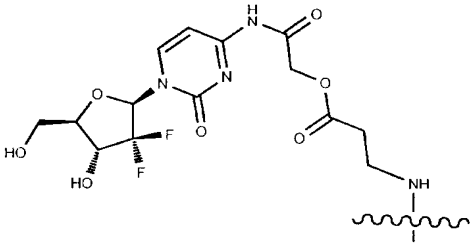
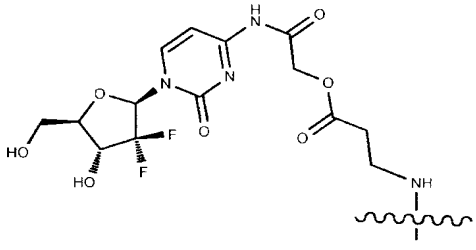
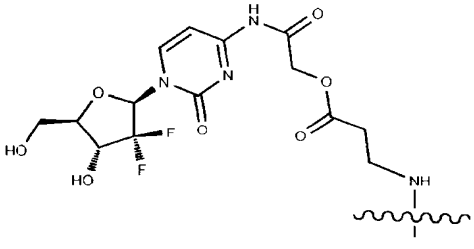
loading of  on the CDP is from about 6% to about 10% by weight of the total polymer.

In some embodiments, the L is a linker (e.g., an ester linkage). In some embodiments, L is absent. In some embodiments, D-L together form

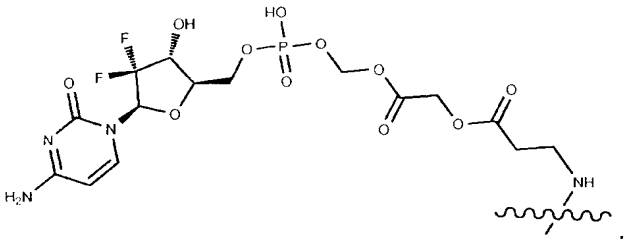


In some embodiments, a plurality of D moieties are absent and at the same position on the polymer, the corresponding L is -OH.

In some embodiments, less than all of the C(=O) moieties of the cysteine residue in the polymer backbone are attached to  moieties, meaning in some embodiments,  is absent in one or more positions of the polymer backbone, provided that the polymer comprises at least

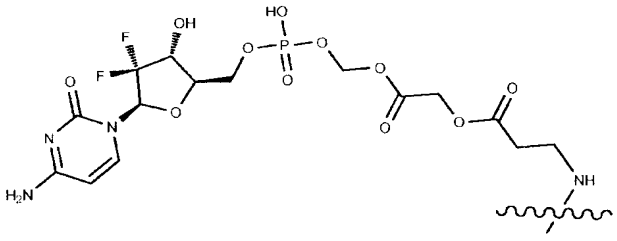
one  and in some embodiments, at least two  moieties. In some embodiments, the loading of the  moieties on the CDP-gemcitabine conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%, e.g., from about 6 to about 10%). In some embodiments, the loading of  on the CDP is from about 6% to about 10% by weight of the total polymer.

In some embodiments, the L is a linker (e.g., an ester linkage). In some embodiments, L is absent. In some embodiments, D-L together form

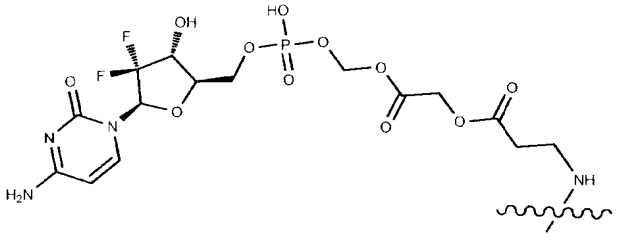


In some embodiments, a plurality of D moieties are absent and at the same position on the polymer, the corresponding L is -OH.

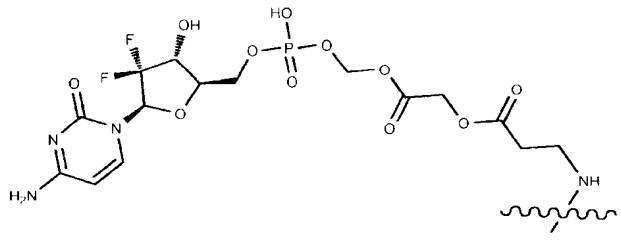
In some embodiments, less than all of the C(=O) moieties of the cysteine residue in the polymer backbone are attached to



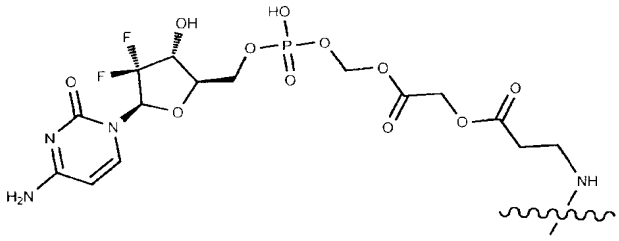
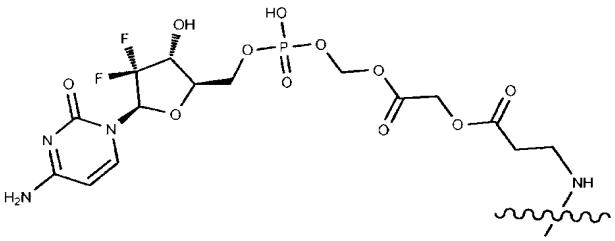
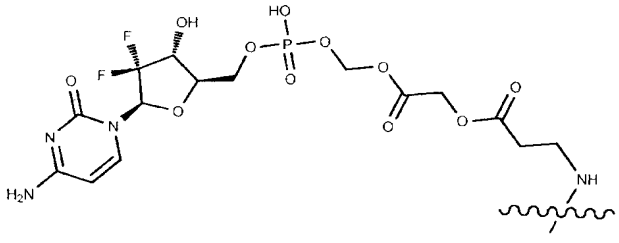
moieties, meaning in some

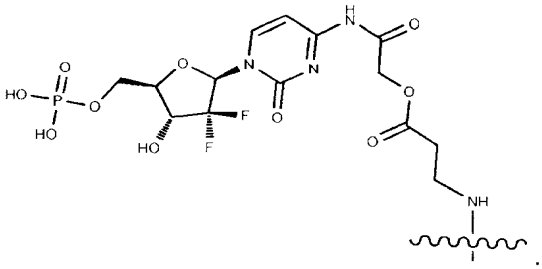


embodiments, is absent in one or more positions of the polymer backbone, provided that the polymer comprises at least



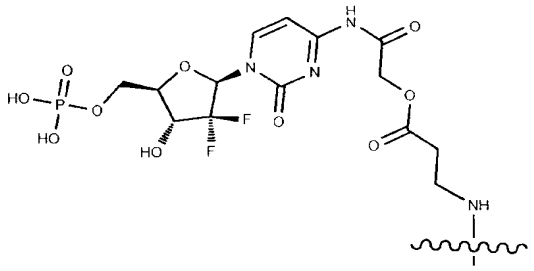
one and in some embodiments, at

least two  moieties. In some embodiments, the loading of the  moieties on the CDP-gemcitabine conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%, e.g., from about 6 to about 10%). In some embodiments, the loading of  on the CDP is from about 6% to about 10% by weight of the total polymer.

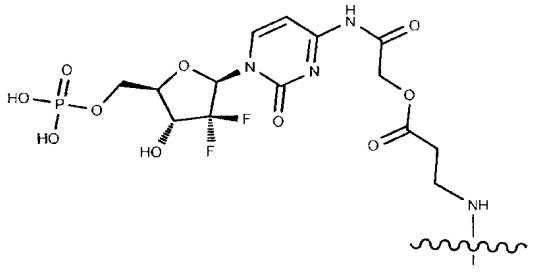
In some embodiments, the L is a linker (e.g., an ester linkage). In some embodiments, L is absent. In some embodiments, D-L together form  .

In some embodiments, a plurality of D moieties are absent and at the same position on the polymer, the corresponding L is -OH.

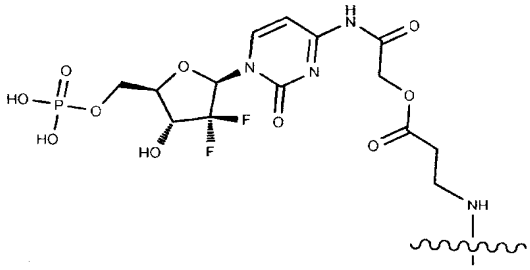
In some embodiments, less than all of the C(=O) moieties of the cysteine residue



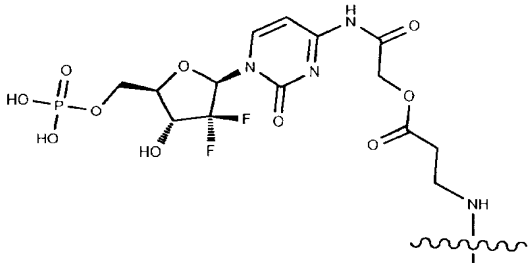
in the polymer backbone are attached to



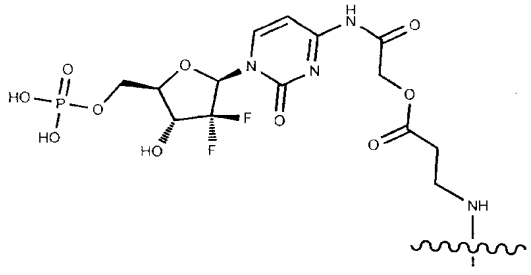
moieties, meaning in some embodiments, is absent in one or more positions of the polymer backbone, provided that the polymer



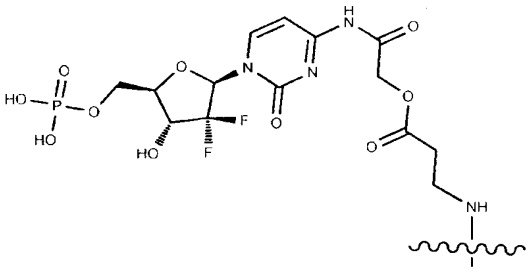
comprises at least one and in some



embodiments, at least two moieties. In some

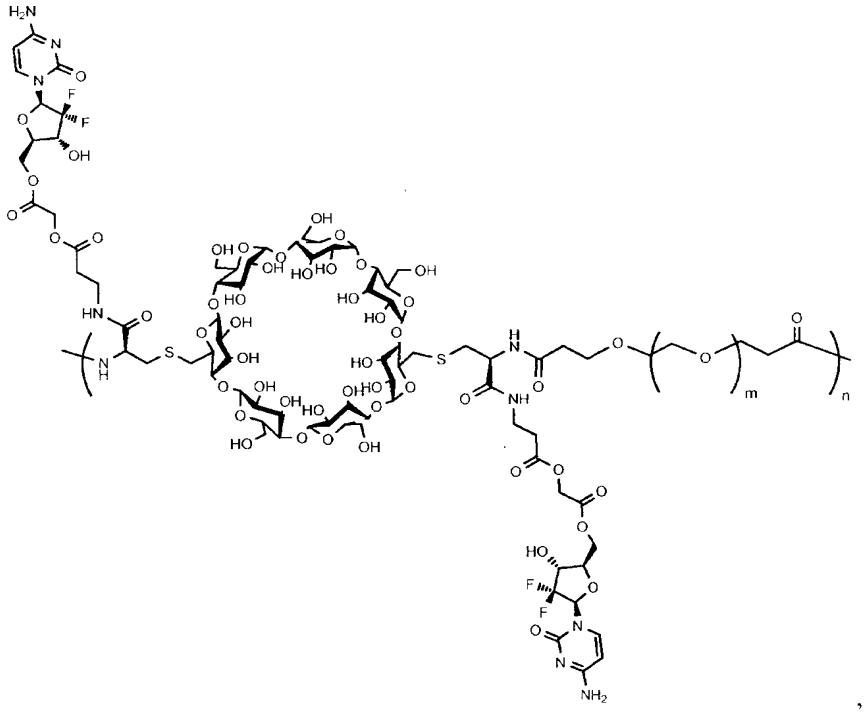


embodiments, the loading of the moieties on the CDP-gemcitabine conjugate is from about 1 to about 50% (e.g., from about 1 to about 40%, from about 1 to about 25%, from about 5 to about 20% or from about 5 to about 15%, e.g., from about 6 to about 10%). In some embodiments, the loading of



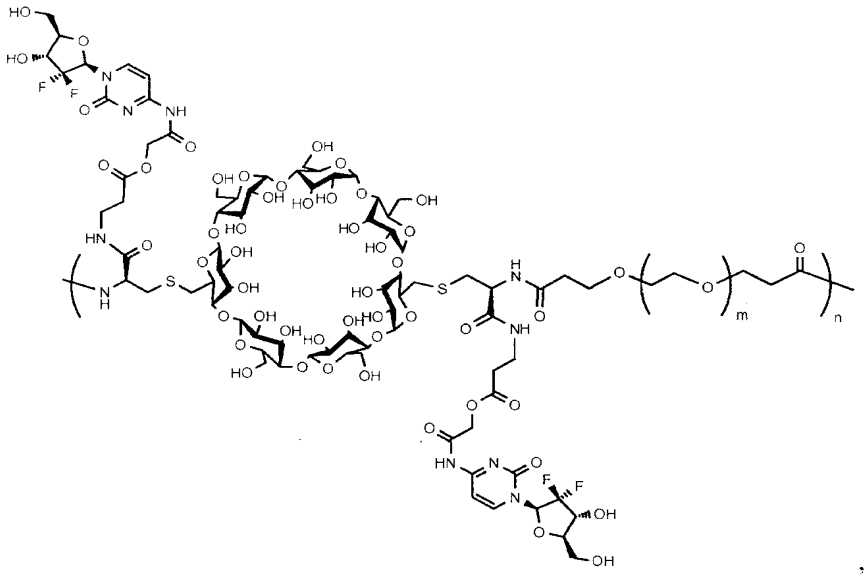
on the CDP is from about 6% to about 10% by weight of the total polymer.

In some embodiments, the CDP-gemcitabine conjugate of formula C is a polymer of formula:



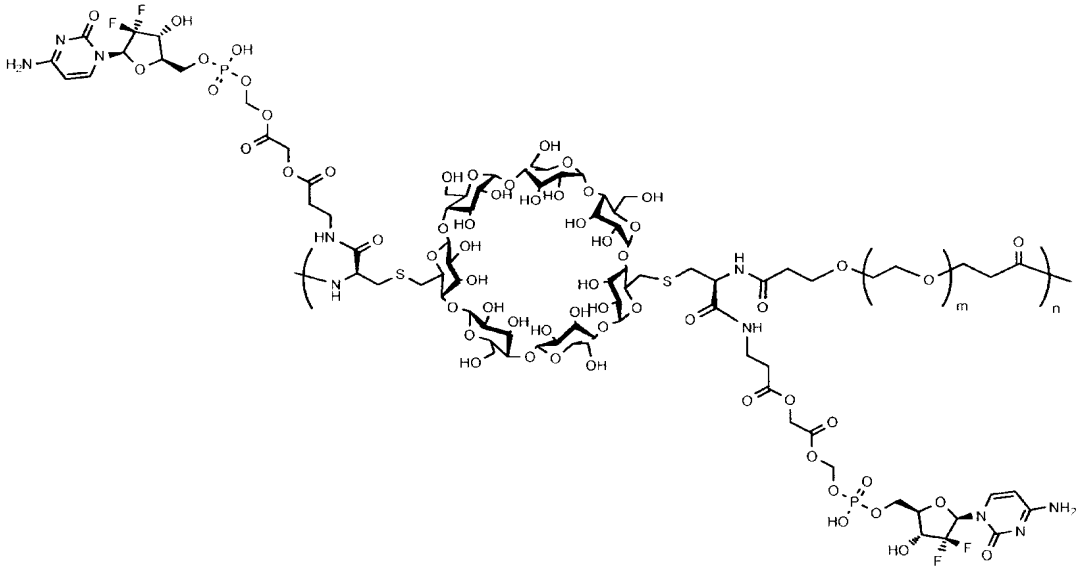
wherein m and n are as defined above, and wherein less than all of the C(=O) sites of the cysteine of the polymer backbone are occupied as indicated above with the gemcitabine-ester, but instead are free acids, meaning, the theoretical loading of the polymer is less than 100%.

In some embodiments, the CDP-gemcitabine conjugate is a polymer of formula:



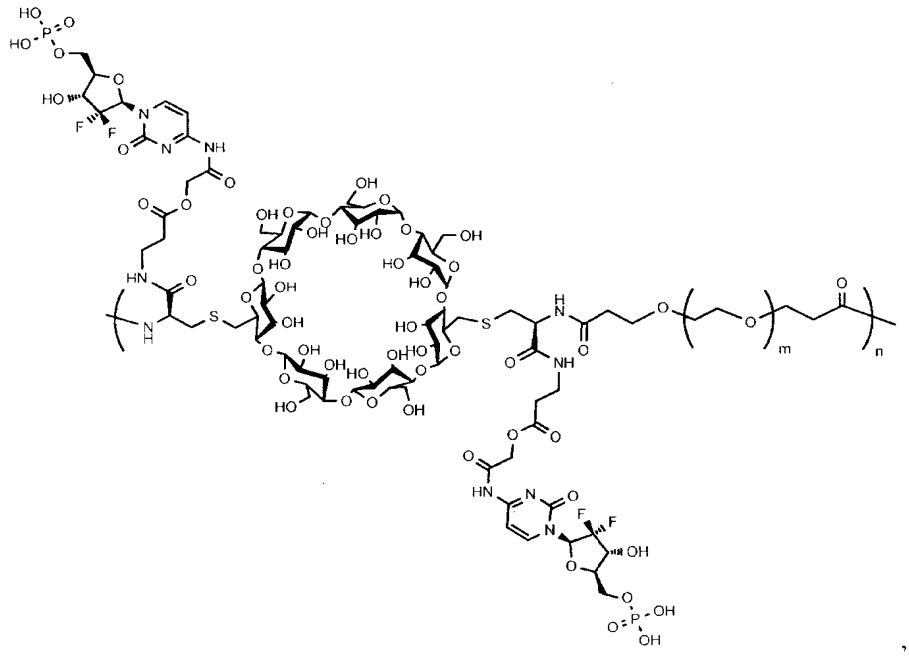
wherein m and n are as defined above, and wherein less than all of the C(=O) sites of the cysteine of the polymer backbone are occupied as indicated above with the gemcitabine-ester, but instead are free acids, meaning, the theoretical loading of the polymer is less than 100%.

In some embodiments, the CDP-gemcitabine conjugate is a polymer of the formula:



wherein m and n are as defined above, and wherein less than all of the C(=O) sites of the cysteine of the polymer backbone are occupied as indicated above with the gemcitabine-ester, but instead are free acids, meaning, the theoretical loading of the polymer is less than 100%.

In some embodiments, the CDP-gemcitabine conjugate is a polymer of the formula:



wherein m and n are as defined above, and wherein less than all of the C(=O) sites of the cysteine of the polymer backbone are occupied as indicated above with the gemcitabine-ester, but instead are free acids, meaning, the theoretical loading of the polymer is less than 100%.

In an embodiment, the therapeutic agent in the CDP-therapeutic agent conjugate is a cytotoxic agent such as an alkylating agent. In some embodiments, the alkylating agent in the CDP-alkylating agent conjugate, particle or composition is an alkylating agent including alkylating agents (including, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen mustard®, Uracillost®, Uracilmostaza®,

UrDastin®, UrDastine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®)

In an embodiment, the therapeutic agent in the CDP-therapeutic agent conjugate is a cytotoxic agent such as an anthracycline agent. In some embodiments, the anthracycline in the CDP-anthracycline conjugate, particle or composition is an anthracycline including, without limitation, daunorubicin (Cerubidine® or Rubidomycin®), doxorubicin (Adriamycin®), epirubicin (Ellence®), idarubicin (Idamycin®), mitoxantrone (Novantrone®), and valrubicin (Valstar®). Preferred anthracyclines include daunorubicin (Cerubidine® or Rubidomycin®) and doxorubicin (Adriamycin®).

In an embodiment, the therapeutic agent in the CDP-therapeutic agent conjugate is a cytotoxic agent such as an anti-tumor-antibiotic agent. In some embodiments, the anti-tumor-antibiotic agent in the CDP-anti-tumor-antibiotic agent conjugate, particle or composition is an anti-tumor-antibiotic agent including, without limitation, a HSP90 inhibitor, e.g., geldanamycin, a CDP-tanespimycin conjugate or a CDP-alvespimycin conjugate.

In an embodiment, the therapeutic agent in the CDP-therapeutic agent conjugate is a cytotoxic agent such as platinum based agent. In some embodiments, the platinum based agent in the CDP-platinum based agent conjugate, particle or composition is a platinum based agent including, without limitation, cisplatin (Platinol® or Platinol-AQ®) carboplatin (Paraplatin® or Paraplatin-AQ®), and oxaliplatin (Eloxatin®).

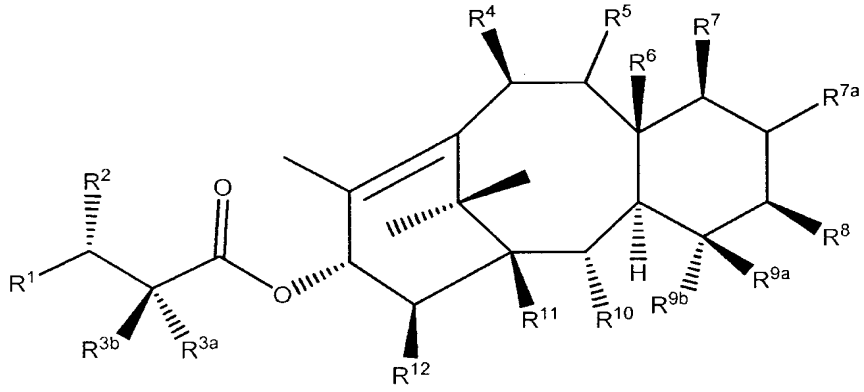
In an embodiment, the therapeutic agent in the CDP-therapeutic agent conjugate is a cytotoxic agent such as microtubule inhibitor. In some embodiments, the microtubule inhibitor in the CDP-microtubule inhibitor conjugate is a taxane. In some embodiments, the taxane in the CDP-taxane conjugate, particle or composition is a taxane

including, without limitation, paclitaxel (Taxol®), docetaxel (Taxotere®), larotaxel, and cabazitaxel.

Taxanes

The term "taxane," as used herein, refers to any naturally occurring, synthetic, or semi-synthetic taxane structure, for example, known in the art. Exemplary taxanes include those compounds shown below, including, for example, formula (X), (XIIa), and (XIIb).

In one embodiment, a taxane is a compound of the following formula (X):



formula (X)

wherein;

R^1 is aryl (e.g., phenyl), heteroaryl (e.g., furanyl, thiophenyl, or pyridyl), alkyl (e.g., butyl such as isobutyl or tert-butyl), cycloalyl (e.g., cyclopropyl), heterocycloalkyl (epoxyl), or R^1, when taken together with one of R^{3b}, R^{9b}, or R^{10} and the carbons to which they are attached, forms a mono- or bi-cyclic ring system; wherein R^1 is optionally substituted with 1-3 R^{1a};

R^2 is $NR^{2a}R^{2b}$ or OR^{2c};

R^{3a} is H, OH, O-polymer, OC(O)alkyl, or OC(O)alkenyl;

R^{3b} is H or OH; or together with R^1 and the carbon to which it is attached, forms a mono- or bi-cyclic ring system;

R^4 is OH, alkoxy (e.g., methoxy), OC(O)alkyl (e.g., Oacyl), OC(O)cycloalkyl, heterocycloalkylalkyl; or R^4 together with R^5 and the carbons to which they are attached,

form an optionally substituted ring; or R^4, together with the carbon to which it is attached, forms a ring (forming a spirocyclic ring) or an oxo;

R^5 is OH, OC(O)alkyl (e.g., Oacyl); or R^5 together with R^4 or R^7 and the carbons to which they are attached, form an optionally substituted ring; or R^5, together with the carbon to which it is attached, forms a ring (forming a spirocyclic ring) or an oxo;

R^6 is alkyl (e.g., methyl); or R^6 together with R^7 and the carbons to which they are attached, form an optionally substituted ring (e.g., a cyclopropyl ring);

R^7 is H, OH, alkoxy (e.g., methoxy), OC(O)Oalkyl, OalkylSalkyl (e.g., OCH_2SMe), or OalkylOalkyl (e.g., OCH_2OMe), thioalkyl, SalkylOalkyl (e.g., SCH_2OMe); or R^7 together with R^5 or R^6 and the carbons to which they are attached, form an optionally substituted ring (e.g., a cyclopropyl ring);

R^{7a} H or OH;

R^8 is OH or a leaving group (e.g., a mesylate, or halo); or R^8 taken together with R^{9a} and the carbons to which they are attached form a ring;

R^{9a} is an activated alkyl (e.g.CH_2I); or R^{9a} taken together with R^8 and the carbons to which they are attached form a ring; or R^{9a}, together with R^{9b} and the carbon to which it is attached, forms a ring (forming a spirocyclic ring);

R^{9b} is OH, OC(O)alkyl (e.g., Oacyl), OC(O)Oalkyl (e.g., OC(O)OMe), or OC(O)cycloalkyl; or R^{9b}, taken together with R^1 and the carbons to which they are attached, form a ring; or R^{9b}, together with R^{9a} and the carbon to which it is attached, forms a ring (forming a spirocyclic ring);

R^{10} is OH, OC(O)aryl (e.g., wherein aryl is optionally substituted for example with halo, alkoxy, or N_3) or OC(O)alkyl; or R^{10} taken together with R^1 or R^{11} and the carbons to which they are attached, forms a ring;

R^{11} H or OH; or R^{11} taken together with R^{10} or R^{12} and the carbons to which they are attached, forms a ring;

R^{12} is H, or OH; or R^{12} taken together with R^{11} and the carbons to which they are attached, forms a ring;

each R^{1a} is independently halo (e.g., fluro), alkyl (e.g., methyl)

each R^{2a} and R^{2b} is independently H, C(O)aryl (e.g, C(O)phenyl), C(O)alkyl (e.g., acyl), C(O)H, C(O)Oalkyl; wherein C(O)aryl (e.g, C(O)phenyl), C(O)alkyl (e.g., acyl), and

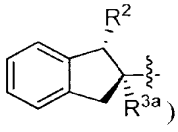
C(O)Oalkyl is each optionally further substituted, for example, with a substituent as descdribed in R^{1a}; and

R^{2c} is H or C(O)NHalkyl.

In some embodiments, R^1 is phenyl (e.g., optionally substituted for example with halo such as fluoro). In some embodiments, R^1 is heteroaryl, for example, furanyl, thiophenyl, or pyridyl (e.g., an optionally substituted pyridyl).

In some embodiments, R^1 is alkyl, e.g., butyl such as isobutyl or tert-butyl.

In some embodiments, R^1 is heterocyclcoalkyl (e.g., epoxyl optionally substituted, for example, with one or more alkyl groups such as methyl).

In some embodiments, R^1, taken together with R^{3b} and the carbons to which they are attached form a bicyclic ring system (e.g., 

).

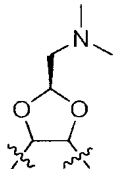
In some embodiments, R^1, taken together with R^{10} and the carbons to which they are attached, form a ring, e.g., a mono- or bi-cyclic ring system).

In some embodiments, R^1, taken together with R^{9b} and the carbons to which they are attached, form a ring, e.g., a mono- or bi-cyclic ring system).

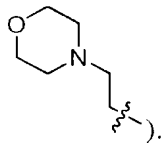
In some embodiments, R^2 is $NR^{2a}R^{2b}$. In some embodiments, at least one of R^{2a} or R^{2b} is H. In some embodiments, R^{2a} is H and R^{2b} is C(O)aryl (e.g, C(O)phenyl), C(O)alkyl (e.g., acyl), C(O)H, or C(O)Oalkyl. In some embodiments, R^2 is NHC(O)aryl or NHC(O)Oalkyl.

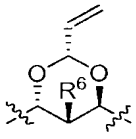
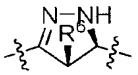
In some embodiments, R^{3a} is OH. In some embodiments, R^{3a} is Opolymer. In some embodiments, polymer is polyglutamic acid. In some embodiments, R^{3a} is $OC(O)C_{21}$alkenyl.

In some embodiments, one of R^{3a} or R^{3b} is H and the other of R^{3a} or R^{3b} is OH.

In some embodiments, R^4 is OAcyl. In some embodiments, R^4 is OH. In some embodiments, R^4 is methoxy. In some embodiments, R^4 together with R^5 and the carbons to which they are attached forms 

. In some embodiments, R^4, together with the

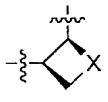
carbon to which it is attached, forms  . In some embodiments, R^4, together with the carbon to which it is attached, forms an oxo. In some embodiments, R^4 is heterocycloalkylalkyl (e.g., ).

In some embodiments, R^5, together with the carbon to which it is attached, forms an oxo. In some embodiments, R^5 together with R^7 and the carbons to which they are attached forms  or  .

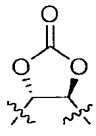
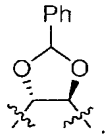
In some embodiments, R^6 is methyl. In some embodiments, R^6 together with R^7 and the carbons to which they are attached form a ring (e.g., cyclopropyl).

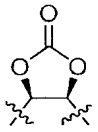
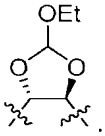
In some embodiments, R^7 is OH. In some embodiments, R^7 is H. In some embodiments, when R^7 is H, R^{7a} is OH.

In some embodiments, R^{7a} is H. In some embodiments, R^{7a} is OH.

In some embobodiments, R^8 together with R^{9a} and the carbons to which they are attached form  , wherein X is O, S, Se, or NR^{8a} (e.g., O), wherein R^{8a} is H, alkyl, arylalkyl (e.g., benzyl), C(O)alkyl, or C(O)H.In some embobodiments, R^8 together with R^{9a} and the carbons to which they are attached form a cyclopropyl ring.

In some embodiments, R^{9b} is OAc.

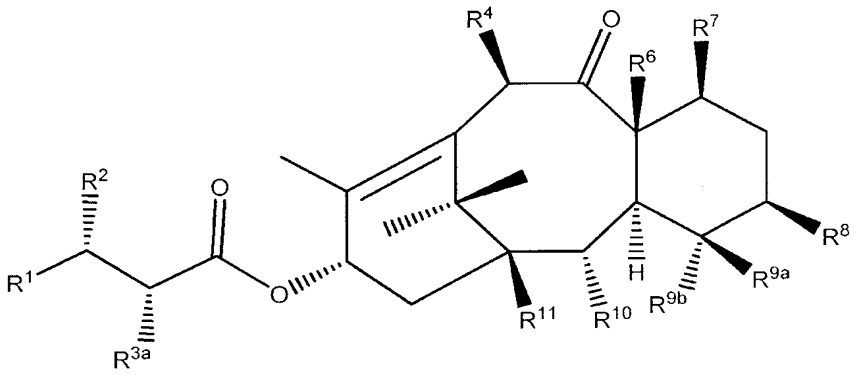
In some embodiments, R^{10} is OC(O)phenyl. In some embodiments, R^{10} taken together with R^{11} and the carbon to which it is attached, forms a ring such as  or  .

In some embodiments, R^{11} is OH. In some embodiments, R^{11} taken together with R^{12} and the carbon to which it is attached, forms a ring such as  or  .

In some embodiments, R^{12} is H.

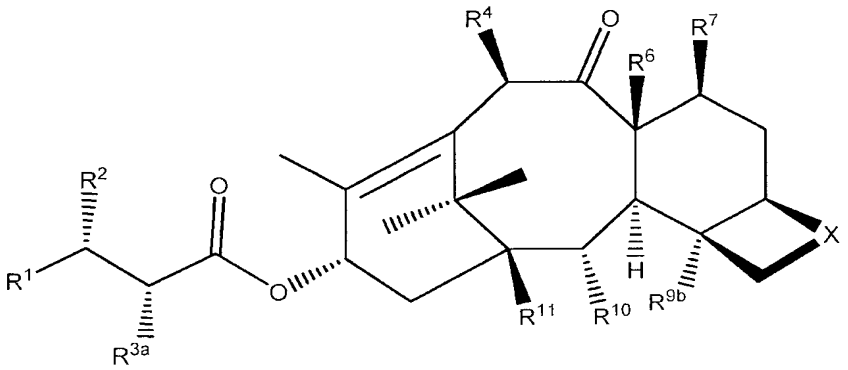
In some embodiments, the variables defined above are chosen so as to form docetaxel, paclitaxel, larotaxel, or cabazitaxel or a structural analogue therof.

In some embodiments, the taxane is a compound of formula (Xa):



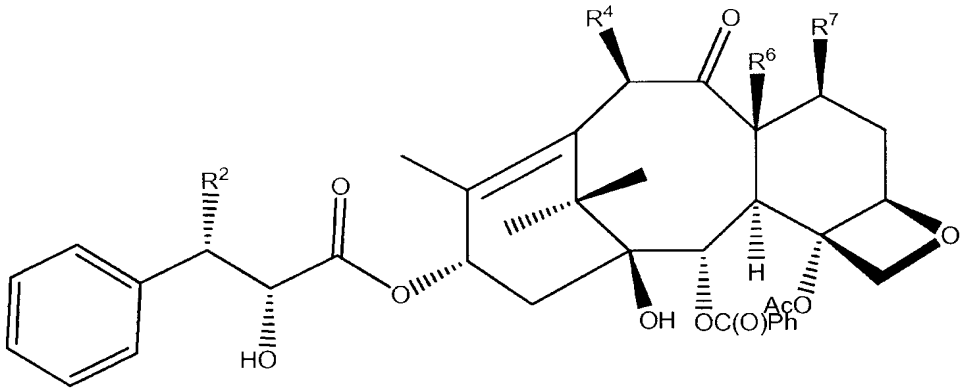
formula (Xa).

In some embodiments, the taxane is a compound of formula (Xb):



formula (Xb).

In some embodiments, the compound is a compound of formula Xc:



(Xc).

In some embodiments, R^2 is NHC(O)aryl or NHC(O)Oalkyl.

In some embodiments, R^4 is OH or OAc.

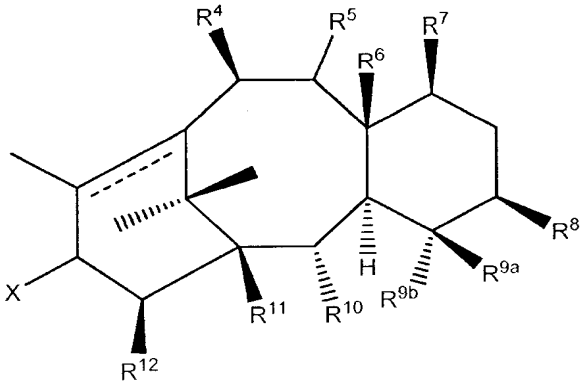
In some embodiments, R^6 is methyl.

In some embodiments, R^7 is OH or OMe.

In some embodiments, R^6 and R^7, together with the carbons to which they are attached, form a ring.

In some embodiments, the variables defined above are chosen so as to form docetaxel, paclitaxel, larotaxel, or cabazitaxel or a structural analogue therof.

In one embodiment, the taxane is a compound of formula (XI):



formula (XI)

wherein,

X is OH, oxo (i.e., when forming a double bond with the carbon to which it is attached), alkoxy, OC(O)alkyl (e.g., Oacyl), or OPg;

R^4 is OH, alkoxy (e.g., methoxy), OC(O)alkyl (e.g., Oacyl), OC(O)cycloalkyl, OPg, heterocycloalkylalkyl; or R^4 together with R^5 and the carbons to which they are attached, form an optionally substituted ring; or R^4, together with the carbon to which it is attached, forms a ring (forming a spirocyclic ring) or an oxo;

R^5 is OH, OC(O)alkyl (e.g., Oacyl), or OPg; or R^5 together with R^4 and the carbons to which they are attached, form an optionally substituted ring; or R^5, together with the carbon to which it is attached, forms an oxo;

R^6 is alkyl (e.g., methyl);

R^7 is H, OH, alkoxy (e.g., methoxy), OC(O)alkyl (e.g., OAc); OPg (e.g., OTES or OTroc), or OC(O)alkenyl (wherein alkenyl is substituted, e.g., with aryl (e.g., napthyl) (e.g., OC(O)CHCHnapthyl), or R^7, together with the carbon to which it is attached, forms an oxo;

R^8 is OH, optionally substituted OC(O)arylalkyl (e.g., OC(O)CHCHphenyl), $OC(O)(CH_2)_{1\text{-}3}$aryl (e.g., $OC(O)CH_2CH_2$phenyl), or a leaving group (e.g., a mesylate, or halo); or R^8 taken together with R^{9a} and the carbons to which they are attached form a ring;

R^{9a} is an activated alkyl (e.g.CH_2I); or R^{9a} taken together with R^8 and the carbons to which they are attached form a ring; or R^{9a}, together with R^{9b} and the carbon to which it is attached, forms a ring (forming a spirocyclic ring)or R^{9a} taken together with R^{9b} and the carbon to which they are attached form an alylenyl;

R^{9b} is OH, alkoxy, OC(O)alkyl (e.g., Oacyl), OC(O)Oalkyl (e.g., OC(O)OMe), OC(O)cycloalkyl, or OPg; or R^{9b}, together with R^{9a} and the carbon to which it is attached, forms a ring (forming a spirocyclic ring); or R^{9b} taken together with R^{9a} and the carbon to which they are attached form an alylenyl;

R^{10} is OH, OC(O)aryl (e.g., wherein aryl is optionally substituted for example with halo, alkoxy, or N_3) or OC(O)alkyl; or R^{10} taken together with R^{11} and the carbons to which they are attached, forms a ring;

R^{11} H, OH; or R^{11} taken together with R^{10} or R^{12} and the carbons to which they are attached, forms a ring;

R^{12} is H, OH, or OC(O)alkyl, wherein alkyl is substituted with 1-4 substituents; or R^{12} taken together with R^{11} and the carbons to which they are attached, forms a ring;

Pg is a protecting group for a heteroatom such as O or N (e.g., Bn, Bz, TES, TMS, DMS, Troc, or Ac); and

 is a single or double bond

In some embodiments, X is OH. In some embodiments, X is oxo. In some embodiments, X is OAc.

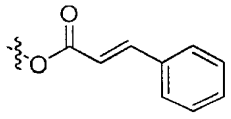
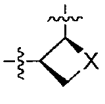
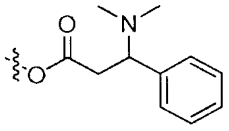
In some embodiments, is a single bond.

In some embodiments, R^4 is OAcyl. In some embodiments, R^4 is OH. In some embodiments, R^4 is methoxy. In some embodiments, R^4 is OPg (e.g., OTroc or OAc). In some embodiments, R^4 together with R^5 and the carbons to which they are attached forms a ring.

In some embodiments, R^5, together with the carbon to which it is attached, forms an oxo. In some embodiments, R^5 is OH or OPg.

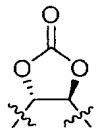
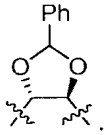
In some embodiments, R^6 is methyl.

In some embodiments, R^7 is H. In some embodiments, R^7 is OH or OPg. In some embodiments, R^7, together with the carbon to which it is attached, forms an oxo.

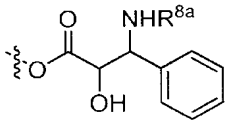
In some embodiments, R^8 is  . In some embodiments, R^8 together with R^{9a} and the carbons to which they are attached form  , wherein X is O, S, Se, or NR^{8a} (e.g., O), wherein R^{8a} is H, alkyl, arylalkyl (e.g., benzyl), C(O)alkyl, Pg, or C(O)H. In some embodiments, R^8 together with R^{9a} and the carbons to which they are attached form a cyclopropyl ring. In some embodiments,  .

In some embodiments, R^{9a} and R^{9b}, together with the carbon to which they are attached form  .

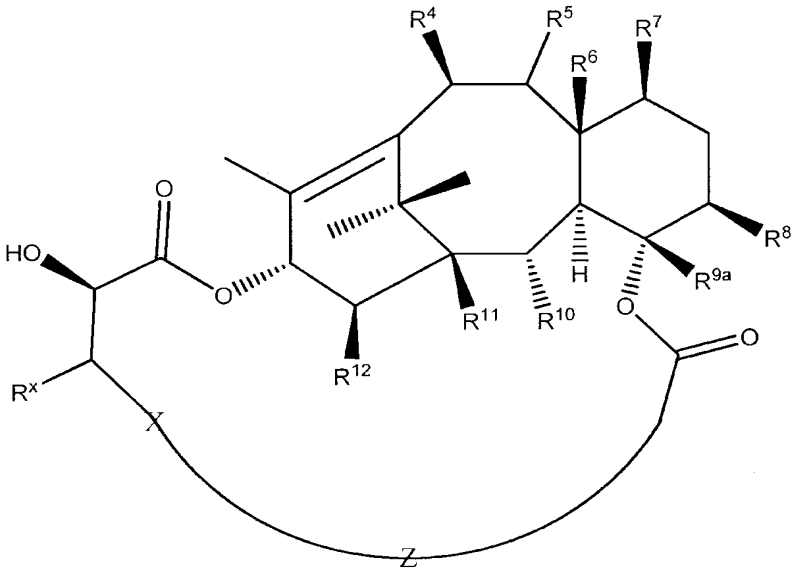
In some embodiments, R^{9b} is OAc.

In some embodiments, R^{10} is OC(O)phenyl. In some embodiments, R^{10} taken together with R^{11} and the carbon to which it is attached, forms a ring such as  or  .

In some embodiments, R^{11} is H. In some embodiments, R^{11} is OH.

In some embodiments, R^{12} is H: In some embodiments, R^{12} is OH. In some embodiments, R^{12} is  .

In one embodiment, the taxane is a compound of formula (XIIa):



formula (XIIa)

wherein,

Z forms a ring by linking O with the atom X attached to $-CHR^x$;

R^4 is OH, alkoxy (e.g., methoxy), OC(O)alkyl (e.g., Oacyl), OC(O)cycloalkyl, heterocycloalkylalkyl; or R^4 together with R^5 and the carbons to which they are attached, form an optionally substituted ring; or R^4, together with the carbon to which it is attached, forms a ring (forming a spirocyclic ring) or an oxo;

R^5 is OH, OC(O)alkyl (e.g., Oacyl); or R^5 together with R^4 or R^7 and the carbons to which they are attached, form an optionally substituted ring; or R^5, together with the carbon to which it is attached, forms a ring (forming a spirocyclic ring) or an oxo;

R^6 is alkyl (e.g., methyl); or R^6 together with R^7 and the carbons to which they are attached, form an optionally substituted ring (e.g., a cyclopropyl ring);

R^7 is H, OH, alkoxy (e.g., methoxy), OC(O)Oalkyl, OalkylSalkyl (e.g., OCH_2SMe), or OalkylOalkyl (e.g., OCH_2OMe), thioalkyl, SalkylOalkyl (e.g., SCH_2OMe); or R^7 together with R^5 or R^6 and the carbons to which they are attached, form an optionally substituted ring (e.g., a cyclopropyl ring);

R^{7a} H or OH;

R^8 is OH or a leaving group (e.g., a mesylate, or halo); or R^8 taken together with R^{9a} and the carbons to which they are attached form a ring;

R^{9a} is an activated alkyl (e.g.CH_2I); or R^{9a} taken together with R^8 and the carbons to which they are attached form a ring;

R^{10} is OH, OC(O)aryl (e.g., wherein aryl is optionally substituted for example with halo, alkoxy, or N_3) or OC(O)alkyl; or R^{10} taken together with R^1 or R^{11} and the carbons to which they are attached, forms a ring;

R^{11} H or OH; or R^{11} taken together with R^{10} or R^{12} and the carbons to which they are attached, forms a ring;

R^{12} is H, or OH; or R^{12} taken together with R^{11} and the carbons to which they are attached, forms a ring;

R^x is NHPg or aryl;

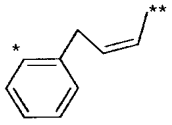
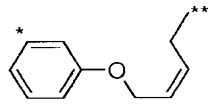
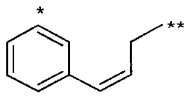
X is C or N; and

Pg is a protecting group for a heteroatom such as O or N (e.g., Bn, Bz, TES, TMS, DMS, Troc, Boc or Ac).

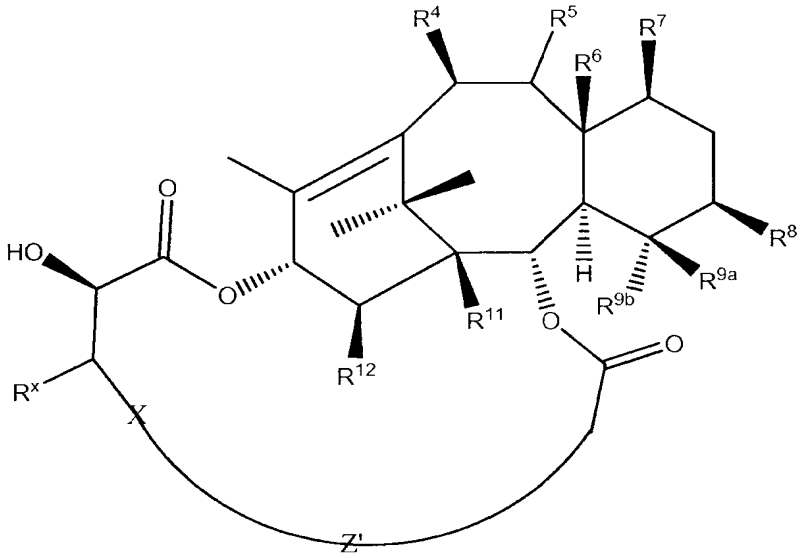
In some embodiments, Z includes one or more phenyl rings.

In some embodiments, Z includes one or more double bonds.

In some embodiments, Z includes one or more heteroatoms.

In some embodiments, Z is  , wherein * indicates the atom X attached to CHR^x and ** indicates the carbon attached to C(O). In some embodiments, Z is  , wherein * indicates the atom X attached to CHR^x and ** indicates the carbon attached to C(O). In some embodiments, Z is  , wherein * indicates the atom X attached to CHR^x and ** indicates the carbon attached to C(O).

In some embodiments, the taxane is a compound of formula (XIIb):



formula(XIIb)

wherein,

Z' forms a ring by linking O with the atom X, which is attached to $-CHR^x$;

R^4 is OH, alkoxy (e.g., methoxy), OC(O)alkyl (e.g., Oacyl), OC(O)cycloalkyl, heterocycloalkylalkyl; or R^4 together with R^5 and the carbons to which they are attached, form an optionally substituted ring; or R^4, together with the carbon to which it is attached, forms a ring (forming a spirocyclic ring) or an oxo;

R^5 is OH, OC(O)alkyl (e.g., Oacyl); or R^5 together with R^4 or R^7 and the carbons to which they are attached, form an optionally substituted ring; or R^5, together with the carbon to which it is attached, forms a ring (forming a spirocyclic ring) or an oxo;

R^6 is alkyl (e.g., methyl); or R^6 together with R^7 and the carbons to which they are attached, form an optionally substituted ring (e.g., a cyclopropyl ring);

R^7 is H, OH, alkoxy (e.g., methoxy), OC(O)Oalkyl, OalkylSalkyl (e.g., OCH_2SMe), or OalkylOalkyl (e.g., OCH_2OMe), thioalkyl, SalkylOalkyl (e.g., SCH_2OMe); or R^7 together with R^5 or R^6 and the carbons to which they are attached, form an optionally substituted ring (e.g., a cyclopropyl ring);

R^{7a} H or OH;

R^8 is OH or a leaving group (e.g., a mesylate, or halo); or R^8 taken together with R^{9a} and the carbons to which they are attached form a ring;

R^{9a} is an activated alkyl (e.g.CH_2I); or R^{9a} taken together with R^8 and the carbons to which they are attached form a ring; or R^{9a}, together with R^{9b} and the carbon to which it is attached, forms a ring (forming a spirocyclic ring);

R^{9b} is OH, OC(O)alkyl (e.g., Oacyl), OC(O)Oalkyl (e.g., OC(O)OMe), or OC(O)cycloalkyl; or R^{9b}, together with R^{9a} and the carbon to which it is attached, forms a ring (forming a spirocyclic ring);

R^{11} H or OH; or R^{11} taken together with R^{10} or R^{12} and the carbons to which they are attached, forms a ring;

R^{12} is H, or OH; or R^{12} taken together with R^{11} and the carbons to which they are attached, forms a ring;

R^x is NHPg or aryl;

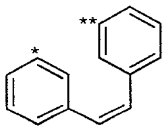
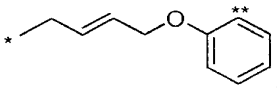
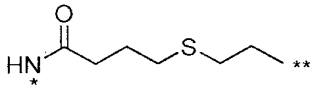
X is C or N; and

Pg is a protecting group for a heteroatom such as O or N (e.g., Bn, Bz, TES, TMS, DMS, Troc, Boc or Ac).

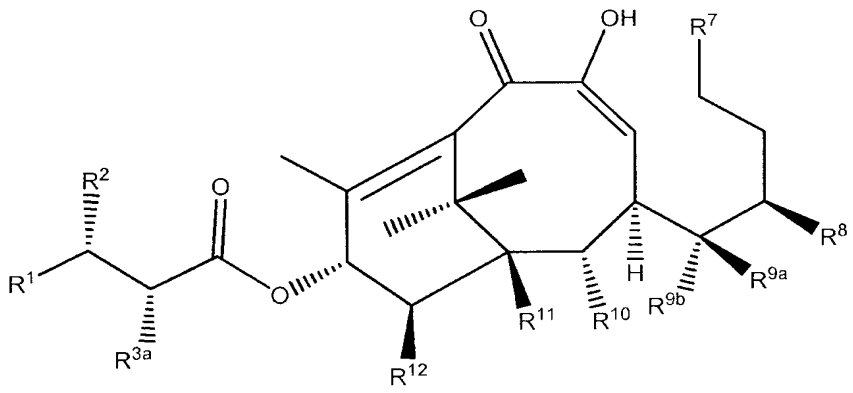
In some embodiments, Z' includes one or more phenyl rings.

In some embodiments, Z' includes one or more double bonds.

In some embodiments, Z' includes one or more heteroatoms.

In some embodiments, Z' is  , wherein * indicates the atom X attached to CHR^x and ** indicates the carbon attached to C(O). In some embodiments, Z' is  , wherein * indicates the atom X attached to CHR^x and ** indicates the carbon attached to C(O). In some embodiments, Z' is  , wherein * indicates the atom X attached to CHR^x and ** indicates the carbon attached to C(O).

In some embodiments, the taxane is a compound of formula (XIII):



formula (XIII)

wherein,

R^1 is aryl (e.g., phenyl), heteroaryl (e.g., furanyl, thiophenyl, or pyridyl), alkyl (e.g., butyl such as isobutyl or tert-butyl), cycloalyl (e.g., cyclopropyl), heterocycloalkyl (epoxyl), or R^1, when taken together with one of R^{3b}, R^{9b}, or R^{10} and the carbons to which they are attached, forms a mono- or bi-cyclic ring system; wherein R^1 is optionally substituted with 1-3 R^{1a};

R^2 is $NR^{2a}R^{2b}$ or OR^{2c};

R^{3a} is H, OH, Opolymer, OC(O)alkyl, or OC(O)alkenyl;

R^7 is OH, alkoxy (e.g., methoxy), OC(O)Oalkyl;

R^8 is OH or a leaving group (e.g., a mesylate, or halo); or R^8 taken together with R^{9a} and the carbons to which they are attached form a ring;

R^{9a} is an activated alkyl (e.g.CH_2I); or R^{9a} taken together with R^8 and the carbons to which they are attached form a ring; or R^{9a}, together with R^{9b} and the carbon to which it is attached, forms a ring (forming a spirocyclic ring)

R^{9b} is OH, OC(O)alkyl (e.g., Oacyl), OC(O)Oalkyl (e.g., OC(O)OMe), or OC(O)cycloalkyl; or R^{9b}, taken together with R^1 and the carbons to which they are attached, form a ring; or R^{9b}, together with R^{9a} and the carbon to which it is attached, forms a ring (forming a spirocyclic ring);

R^{10} is OH, OC(O)aryl (e.g., wherein aryl is optionally substituted for example with halo, alkoxy, or N_3) or OC(O)alkyl; or R^{10} taken together with R^1 or R^{11} and the carbons to which they are attached, forms a ring;

R^{11} H or OH; or R^{11} taken together with R^{10} or R^{12} and the carbons to which they are attached, forms a ring;

R^{12} is H, or OH; or R^{12} taken together with R^{11} and the carbons to which they are attached, forms a ring;

each R^{1a} is independently halo (e.g., fluro), alkyl (e.g., methyl)

each R^{2a} and R^{2b} is independently H, C(O)aryl (e.g, C(O)phenyl), C(O)alkyl (e.g., acyl), C(O)H, C(O)Oalkyl; wherein C(O)aryl (e.g, C(O)phenyl), C(O)alkyl (e.g., acyl), and C(O)Oalkyl is each optionally further substituted, for example, with a substituent as descdribed in R^{1a};

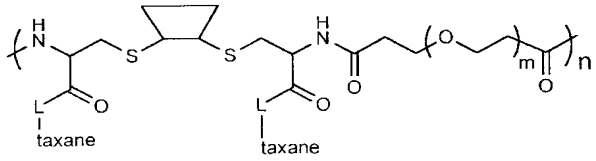
R^{2c} is H or C(O)NHalkyl; and

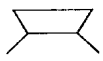
R^{8a} is H, alkyl, arylalkyl (e.g., benzyl), C(O)alkyl, or C(O)H.

In some embodiments, R^7 is OH.

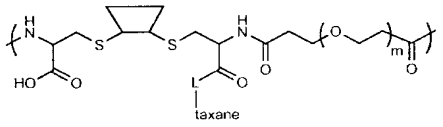
In some preferred embodiments, the taxane is docetaxel, larotaxel, milataxel, TPI-287, TL-310, BMS-275183, BMS-184476, BMS-188797, ortataxel, tesetaxel, or cabazitaxel. Additional taxanes are provided in Fan, Mini-Reviews in Medicinal Chemistry, 2005, 5, 1-12; Gueritte, Current Pharmaceutical Design, 2001, 7, 1229-1249; Kingston, J. Nat. Prod., 2009, 72, 507-515; and Ferlini, Exper Opin. Invest. Drugs, 2008, 17, 3, 335-347; the contents of each of which is incorporated herein by reference in its entirety.

In one embodiment, the CDP-microtubule inhibitor conjugate is a CDP-taxane conjugate, e.g.,

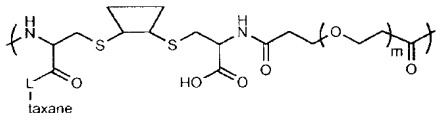


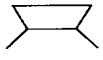
wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an

integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); L is a linker, e.g., a linker described herein; and "taxane" is a taxane, e.g., a taxane described herein, e.g., a taxane shown in FIG. 4. In some embodiments, the CDP-microtubule inhibitor conjugate, e.g., the CDP-taxane conjugate, does not have complete loading, e.g., one or more binding sites, e.g., cysteine residues, are not bound to a microtubule inhibitor, e.g., a taxane moiety, e.g., e.g., a taxane described herein, bound with a linker described herein, e.g., the CDP-taxane conjugate comprises one or more subunits having the formulae provided below:

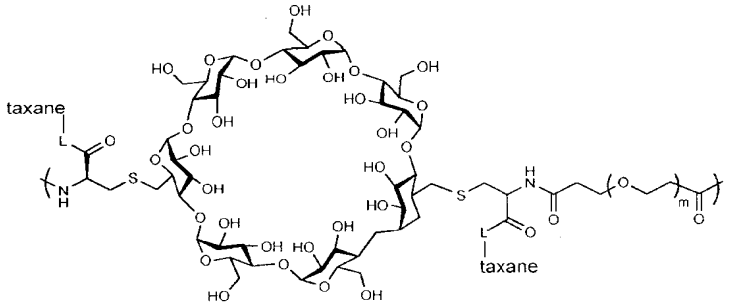


or



wherein  represents a cyclodextrin; m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); L is a linker, e.g., a linker described herein; and "taxane" is a taxane, e.g., a taxane described herein, e.g., a taxane shown in FIG. 4. In some embodiments, the CDP-microtubule inhibitor conjugate, particle or composition e.g., the CDP-taxane conjugate, particle or composition, comprises a mixture of fully-loaded and partially-loaded CDP-microtubule inhibitor conjugates, e.g., CDP-taxane conjugates.

In one embodiment, the CDP-microtubule inhibitor conjugate comprises a subunit of

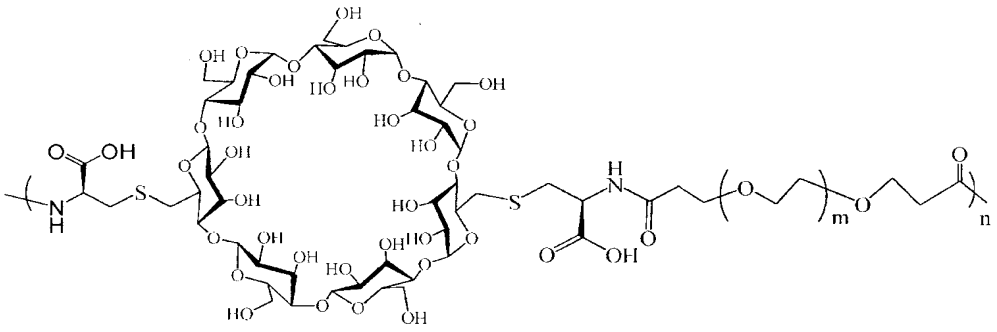


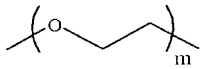
wherein m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); L is a linker, e.g., a linker described herein; and "taxane" is a taxane, e.g., a taxane described herein, e.g., a taxane shown in FIG. 4.

FIG. 4 is a table depicting examples of different CDP-taxane conjugates. The CDP-taxane conjugates in FIG. 4 are represented by the following formula:

CDP-CO-ABX-Taxane

In this formula, CDP is the cyclodextrin-containing polymer shown below (as well as in FIG. 3):



wherein the group  has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. Note that the taxane is conjugated to the CDP through the carboxylic acid moieties of the polymer as provided above. Full loading of the taxane onto the CDP is not required. In some embodiments, at least one, e.g., at least 2, 3, 4, 5, 6 or 7, of the carboxylic acid moieties remains unreacted with the taxane after conjugation (e.g., a plurality of the carboxylic acid moieties remain unreacted).

CO represents the carbonyl group of the cysteine residue of the CDP;

A and B represent the link between the CDP and the taxane. Position A is either a bond between linker B and the cysteine acid carbonyl of CDP (represented as a "-" in FIG. 4), a bond between the taxane and the cysteine acid carbonyl of CDP (represented as a "-"in FIG. 4) or depicts a portion of the linker that is attached via a bond to the cysteine acid carbonyl of the CDP. Position B is either not occupied (represented by "-" in FIG. 4) or represents the linker or the portion of the linker that is attached via a bond to the taxane; and

X represents the heteroatom to which the linker is coupled on the taxane.

As provided in FIG. 4, the column with the heading "Taxane" indicates which taxane is included in the CDP-taxane conjugate.

The three columns on the right of the table in FIG. 4 indicate respectively, what, if any, protecting groups are used to protect the indicated position of the taxane, the process for producing the CDP-taxane conjugate, and the final product of the process for producing the CDP-taxane conjugate.

The processes referred to in FIG. 4 are given a letter representation, e.g., Process A, Process B, etc. as seen in the second column from the right. The steps for each these processes respectively are provided below.

Process A: Couple the protected linker of position B to the taxane, deprotect the linker and couple to CDP via the carboxylic acid group of the CDP to afford the 2'-taxane linked to CDP.

Process B: Couple the activated linker of position B to the 2'-hydroxyl of taxane, and couple to CDP containing linker of position A via the linker of A to afford the 2'-taxane linked to CDP.

Process C: Protect the C2' hydroxy group of the taxane, couple the protected linker of position B to the taxane, deprotect the linker and the C2' hydroxy group, and couple to CDP via the carboxylic acid group of the CDP to afford the 7-taxane linked to CDP.

Process D: Protect the C2' hydroxy group of the taxane, couple the activated linker of position B to the 7-hydroxyl of the taxane, deprotect the C2' hydroxy group and couple to CDP containing linker of position A via the linker of A to afford afford the 7-taxane linked to CDP.

As shown specifically in FIG. 4, the CDP-taxane conjugates can be prepared using a variety of methods known in the art, including those described herein. In some embodiments, the CDP-taxane conjugates can be prepared using no protecting groups on the taxane. For taxanes having hydroxyl groups at both the 2' and the 7-positions, one of skill in the art will understand that the 2'-position is more reactive, and therefore when using no protecting groups, the major product of the reaction(s) will be that which is linked via the 2' position.

One or more protecting groups can be used in the processes described above to make the CDP-taxane conjugates described herein. A protecting group can be used to control the point of attachment of the taxane and/or taxane linker to position A. In some

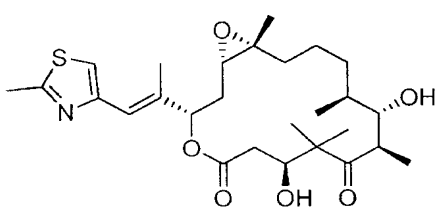
embodiments, the protecting group is removed and, in other embodiments, the protecting group is not removed. If a protecting group is not removed, then it can be selected so that it is removed *in vivo* (e.g., acting as a prodrug). An example is hexanoic acid which has been shown to be removed by lipases *in vivo* if used to protect a hydroxyl group in doxorubicin. Protecting groups are generally selected for both the reactive groups of the taxane and the reactive groups of the linker that are not targeted to be part of the coupling reaction. The protecting group should be removable under conditions which will not degrade the taxane and/or linker material. Examples include t-butyldimethylsilyl ("TBDMS") and TROC (derived from 2,2,2-trichloroethoxy chloroformate). Carboxybenzyl ("CBz") can also be used in place of TROC if there is selectivity seen for removal over olefin reduction. This can be addressed by using a group which is more readily removed by hydrogenation such as –methoxybenzyl OCO-. Other protecting groups may also be acceptable. One of skill in the art can select suitable protecting groups for the products and methods described herein.

In some embodiments, the microtubule inhibitor in the CDP-microtubule inhibitor conjugate is an epothilone. In some embodiments, the epothilone in the CDP-epothilone conjugate, particle or composition is an epothilone including, without limitation, ixabepilone, epothilone B, epothilone D, BMS310705, dehydelone, and ZK-Epothilone (ZK-EPO). Other epothilones described herein can also be included in the CDP-epothilone conjugates.

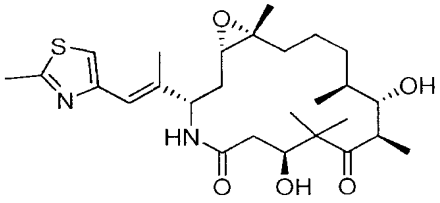
Epothilones

The term "epothilone," as used herein, refers to any naturally occurring, synthetic, or semi-synthetic epothilone structure, for example, known in the art. The term epothilone also includes structures falling within the generic formulae XX, XXI, XXII, XXIII, XXIV, XXV, and XXVI as provided herein.

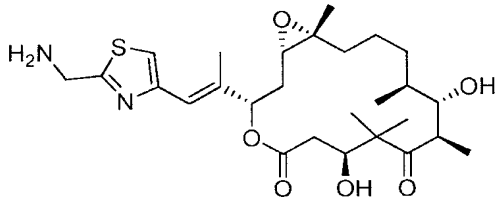
Exemplary epothilones include those described generically and specifically herein. In some embodiments, the epothilone is epothilone B, ixabepilone, BMS310705, epothilone D, dehydelone, or sagopilone. The structures of all of these epothilones are provided below:



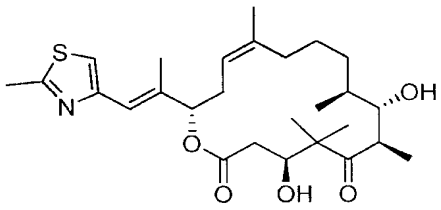
Epothilone B



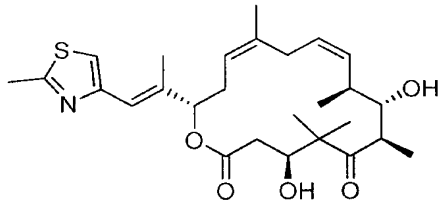
Ixabepilone



BMS-310705

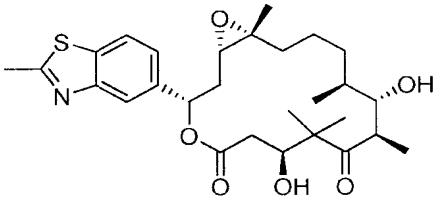


Epothilone D



Dehydelone

and



Sagopilone

Other exemplary epothilones are also provided in FIG. 5 and disclosed in Altmann et al. "Epothilones as Lead Structures for New Anticancer Drugs-Pharmacology, Fermentation, and Structure-activity-relationships;" *Progress in Drug Research* (2008) Vol. 66, page 274-334, which is incorporated herein by reference. Additionally, epothilones may be found, for example, in US 7,317,100; US 6,946,561; US 6,350,878; US 6,302,838; US 7,030,147; US 6,387,927; US 6,346,404; US 2004/0038324; US 2009/0041715; US 2007/0129411; US 2005/0271669; US 2008/0139587; US 2004/0235796; US 2005/0282873; US 2006/0089327; WO 2008/071404; WO 2008/019820; WO 2007/121088; WO 1998/08849; EP 1198225; EP 1420780; EP 1385522; EP 1539768; EP 1485090; and EP 1463504, the contents of these references are incorporated herein in their entireties.

Further epothilones may be found, for example, in US 6,410,301; US 7,091,193; US 7,402,421; US 7,067,286; US 6,489,314; US 6,589,968; US 6,893,859; US 7,176,235; US 7,220,560; US 6,280,999; US 7,070,964; US 2005/0148543; US

2005/0215604; US 2003/0134883; US 2008/0319211; US 2005/0277682; US 2005/0020558; US 2005/0203174; US 20020045609, US 2004/0167097; US 2004/0072882; US 2002/0137152; WO 2009/064800; and WO 2002/012534, the contents of these references are incorporated herein in their entireties.

Further epothilones may be found, for example, in US 6,537,988; US 7,312,237; US 7,022,330; US 6,670,384; US 6,605,599; US 7,125,899; US 6,399,638; US 7,053,069; US 6,936,628; US 7,211,593; US 6,686,380; US 6,727,276; US 6,291,684; US 6,780,620; US 6,719,540; US 2009/0004277; US 2007/0276018; WO 2004/078978; and EP 1157023, the contents of these references are incorporated herein in their entireties.

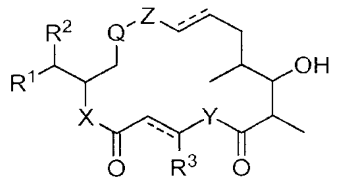
Further epothilones may be found, for example, in US 2008/0146626; US 2009/0076098; WO 2009/003706 and WO 2009/074274, the contents of these references are incorporated herein in their entireties.

Further epothilones may be found, for example, in US 7,169,930; US 6,294,374; US 6,380,394; and US 6,441,186, the contents of these references are incorporated herein in their entireties.

Further epothilones may be found, for example, in US 7,119,071; and German Application Serials Nos. DE 197 13 970.1, DE 100 51 136.8, DE 101 34 172.5, and DE 102 32 094.2, the contents of these references are incorporated herein in their entireties.

In some embodiments, the epothilone is attached to a targeting moiety such as a folate moiety. In some embodiments, the targeting moiety (e.g., a folate) is attached to a functional group on the epothilone such as a hydroxyl group or an amino group where appropriate. In some embodiments, the folate is attached to the epothilone directly. In some embodiments, the folate is attached to the epothilone via a linker. Epofolate (BMS-753493) is an example an epothilone attached to a folate, see, for example, U.S. 7,033,594, which is incorporated herein by reference.

In one embodiment, the epothilone is a compound of formula (XX)



formula (XX)

wherein

R^1 is aryl, heteroaryl, arylalkenyl or heteroarylalkenyl; each of which is optionally substituted with 1-3 R^8;

R^2 is H or alkyl (e.g., a methyl); or

R^1 and R^2, when taken together with the carbon to which they are attached, form an aryl or a heteroaryl moiety optionally substituted with 1-3 R^8;

R^3 is H, OH, NH_2, or CN;

X is O or NR^4;

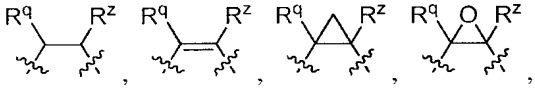
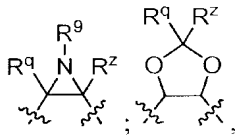
R^4 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)NR^5alkyl, -C(O)NR^5arylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl;

Y is CR^5R^6, O or NR^7;

each of R^5 and R^6 is independently H or alkyl (e.g., methyl);

R^7 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)NR^5alkyl, -C(O) NR^5arylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl;

each R^8, for each occurrence, is independently alkyl, aminoalkyl, hydroxyalkyl, alkylthiol, aryl, arylalkyloxyalkyl or alkoxy;

Q-Z, when taken together, form  ,  ; heteroarylenyl, C(O)NR^4, NR^4C(O), $CR^5R^6NR^4$, or $NR^4CR^5R^6$;

R^q is H, alkyl (e.g., methyl) or hydroxy;

R^z is H, alkyl (e.g., methyl), haloalkyl (e.g., CF_3), heterocyclylalkyl or N_3;

R^9 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)NR^5alkyl, -C(O) NR^5arylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl; and

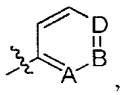
each  , for each occurrence, is independently a single or double bond.

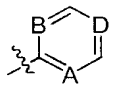
In some embodiments, R^1 is  optionally substituted with 1-3 R^8.

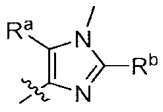
In some embodiments, HET is a five membered ring heteroaryl optionally substituted with 1-3 R^8.

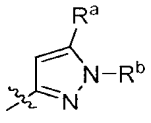
In some embodiments, HET is a thiazolyl optionally substituted with 1-3 R^8. In some embodiments, HET is substituted with alkyl (e.g., methyl), aminoalkyl (e.g., aminomethyl), alkylthiol (e.g., methylthiol), hydroxyalkyl (e.g., hydroxymethyl), alkoxy (e.g., methoxy) or aryl (e.g., phenyl).

In some embodiments, HET is substituted with alkyl (e.g., methyl) or amino alkyl.

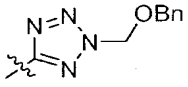
In some embodiments, HET is  , wherein each of A, B and D is independently CH or N. In some embodiments, A is N, B is CH and D is CH. In some embodiments, A is CH, B is N and D is CH. In some embodiments, A is CH, B is CH and D is N.

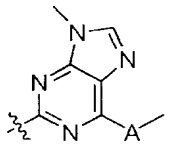
In some embodiments, HET is  , wherein each of A, B and D is independently CH or N. In some embodiments, A is N, B is N and D is CH. In some embodiments, A is N, B is CH and D is N. In some embodiments, A is CH, B is CH and D is CH.

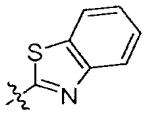
In some embodiments, HET is  , wherein each R^a and R^b is independently H or –SMe.

In some embodiments, HET is  , wherein each R^a is H, alkyl or –Salkyl; and R^b is H, alkyl (e.g., methyl) or aryl (e.g., phenyl).

In some embodiments, HET is  , wherein A is CH or N.

In some embodiments, HET is  .

In some embodiments, HET is  , wherein A is S or O.

In some embodiments, HET is  .

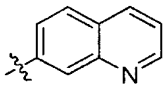
In some embodiments R^2 is H.

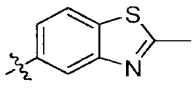
In some embodiments, R^2 is alkyl (e.g., methyl).

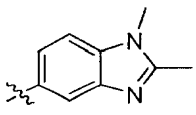
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, form an aryl or a heteroaryl moiety optionally substituted with 1-3 R^8.

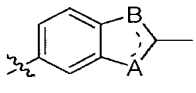
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, form a heteroaryl moiety optionally substituted with 1-3 R^8.

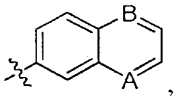
In some embodiments, the heteroaryl moiety is a bicyclic heteroaryl moiety.

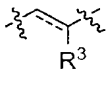
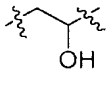
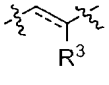
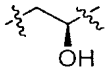
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  .

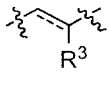
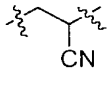
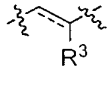
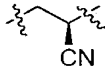
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  .

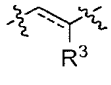
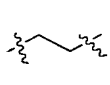
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  .

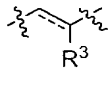
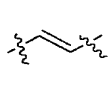
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  , wherein A is N and B is S or wherein A is S and B is N.

In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  , wherein A is N and B is CH or wherein A is CH and B is N.

In some embodiments,  is  . In some embodiments,  is  .

In some embodiments,  is  . In some embodiments,  is  .

In some embodiments,  is  .

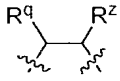
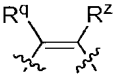
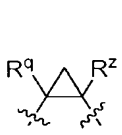
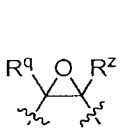
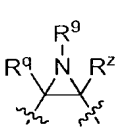
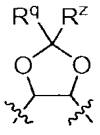
In some embodiments,  is  .

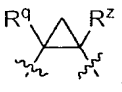
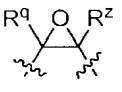
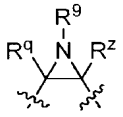
In some embodiments, X is O.

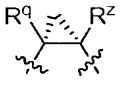
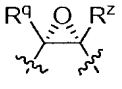
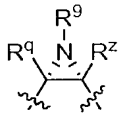
In some embodiments, X is NR^4 (e.g., NH).

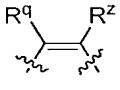
In some embodiments, Y is CR^5R^6. In some embodiments, Y is  . In some embodiments, Y is CH_2.

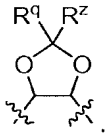
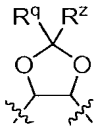
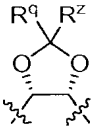
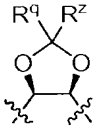
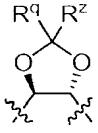
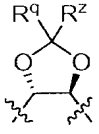
In some embodiments, Y is NR^7 (e.g., NH or NMe).

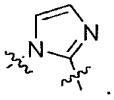
In some embodiments, Q-Z, when taken together, form  ,  ,  ,  ,  ;  or heteroarylenyl.

In some embodiments, Q-Z, when taken together, form  ,  or  .

In some embodiments, Q-Z, when taken together, form  ,  or  .

In some embodiments, Q-Z, when taken together, form  , wherein R^q is H and R^z is H or alkyl (e.g., methyl).

In some embodiments, Q-Z, when taken together, form  . In some embodiments, both R^q and R^z are methyl. In some embodiments,  is selected from  ,  ,  or  . In some embodiments, both R^q and R^z are methyl.

In some embodiments, Q-Z, when taken together, form a heteroarylenyl. In some embodiments, Q-Z, when taken together, form  .

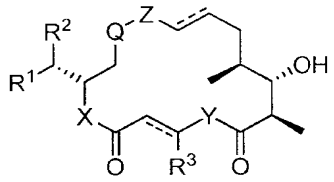
In some embodiments, Q-Z, when taken together, form $C(O)NR^4$. In some embodiments, R^4 is H or alkyl (e.g., methyl or ethyl).

In some embodiments, Q-Z, when taken together, form $NR^4C(O)$. In some embodiments, R^4 is H or alkyl (e.g., methyl or ethyl).

In some embodiments, Q-Z, when taken together, form CH_2NR^4. In some embodiments, R^4 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl. In some embodiments, R^4 is -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl.

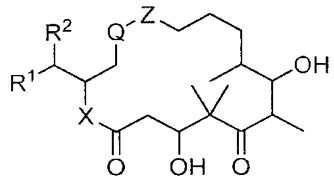
In some embodiments, Q-Z, when taken together, form NR^4CH_2. In some embodiments, R^4 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl. In some embodiments, R^4 is -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl.

In some embodiments, the compound of formula (XX) is a compound of formula (XXa)



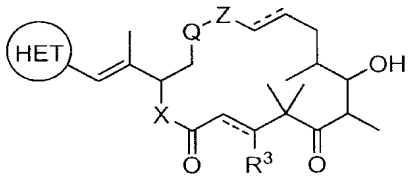
formula (XXa).

In some embodiments, the compound of formula (XX) is a compound of formula (XXb)



formula (XXb).

In some embodiments, the compound of formula (XX) is a compound of formula (XXc)

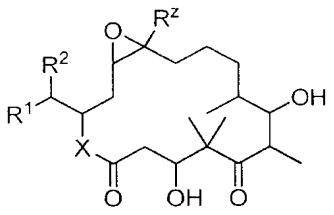


formula (XXc)

wherein HET is an optionally substituted heteroaryl.

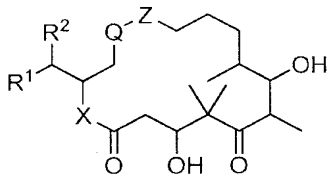
In some embodiments, HET is an optionally substituted 5 membered ring.

In some embodiments, the compound of formula (XX) is a compound of formula (XXd)



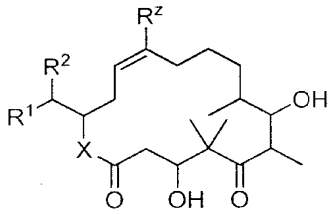
formula (XXd).

In some embodiments, the compound of formula (XX) is a compound of formula (XXe)



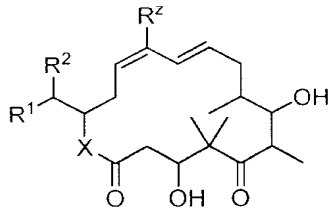
formula (XXe).

In some embodiments, the compound of formula (XX) is a compound of formula (XXf)



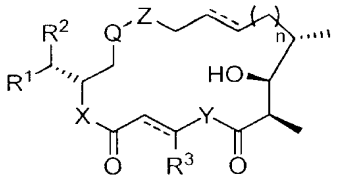
formula (XXf).

In some embodiments, the compound of formula (XX) is a compound of formula (XXg)



formula (XXg).

In one embodiment, the epothilone is a compound of formula (XXI)



formula (XXI)

wherein

R^1 is aryl, heteroaryl, arylalkenyl, or heteroarylalkenyl; each of which is optionally substituted with 1-3 R^8;

R^2 is H or alkyl (e.g., methyl); or

R^1 and R^2, when taken together with the carbon to which they are attached, form an aryl or a heteroaryl moiety optionally substituted with 1-3 R^8;

R^3 is H, OH, NH_2 or CN;

X is O or NR^4;

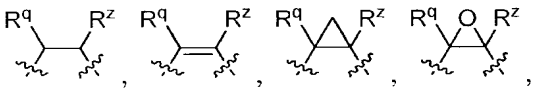
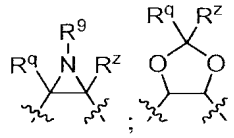
R^4 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)NR^5alkyl, -C(O) NR^5arylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl;

Y is CR^5R^6, O or NR^7;

each of R^5 and R^6 is independently H or alkyl (e.g., methyl);

R^7 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)NR^5alkyl, -C(O) NR^5arylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl;

each R^8, for each occurrence, is independently alkyl, aminoalkyl, hydroxyalkyl, alkylthiol, aryl, arylalkyloxyalkyl or alkoxy;

Q-Z, when taken together, form  ,  , heteroarylenyl, C(O)NR^4, NR^4C(O), $CR^5R^6NR^4$, or $NR^4CR^5R^6NR^4$;

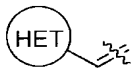
R^q is H, alkyl (e.g., methyl) or hydroxy;

R^z is H, alkyl (e.g., methyl), haloalkyl (e.g., CF_3), heterocyclylalkyl or N_3;

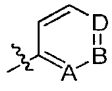
R^9 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)NR^5alkyl, -C(O) NR^5arylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl;

each ⟋⟍, for each occurrence, is independently a single or double bond; and

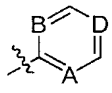
n is 0, 1 or 2.

In some embodiments, R^1 is 

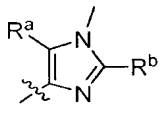
optionally substituted with 1-3 R^8. In some embodiments, HET is a five membered ring heteroaryl optionally substituted with 1-3 R^8. In some embodiments, HET is a thiazolyl optionally substituted with 1-3 R^8. In some embodiments, HET is substituted with alkyl (e.g., a methyl), aminoalkyl (e.g., aminomethyl), alkylthiol (e.g., methylthiol), hydroxyalkyl (e.g., hydroxymethyl), alkoxy (e.g., methoxy) or aryl (e.g., phenyl). In some embodiments, HET is substituted with alkyl (e.g., methyl) or aminoalkyl.

In some embodiments, HET is 

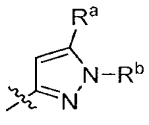
, wherein each of A, B and D is independently CH or N. In some embodiments, A is N, B is CH and D is CH. In some embodiments, A is CH, B is N and D is CH. In some embodiments, A is CH, B is CH and D is N.

In some embodiments, HET is 

, wherein each of A, B and D is independently CH or N. In some embodiments, A is N, B is N and D is CH. In some embodiments, A is N, B is CH and D is N. In some embodiments, A is CH, B is CH and D is CH.

In some embodiments, HET is 

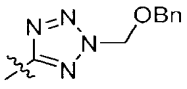
, wherein each R^a and R^b is independently –H or –SMe.

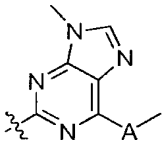
In some embodiments, HET is 

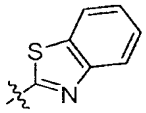
, wherein each R^a is H, alkyl or –Salkyl; and R^b is H, alkyl (e.g., methyl) or aryl (e.g., phenyl).

In some embodiments, HET is 

, wherein A is CH or N.

In some embodiments, HET is  .

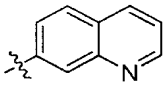
In some embodiments, HET is  , wherein A is S or O.

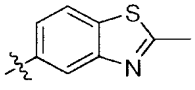
In some embodiments, HET is  .

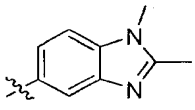
In some embodiments R^2 is H.

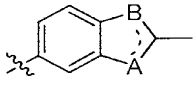
In some embodiments, R^2 is alkyl (e.g., methyl).

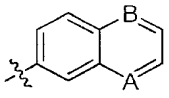
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, form an aryl or a heteroaryl moiety optionally substituted with 1-3 R^8. In some embodiments, the heteroaryl moiety is a bicyclic heteroaryl moiety.

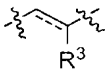
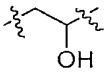
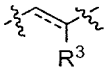
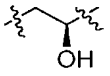
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  .

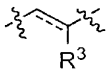
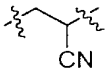
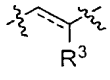
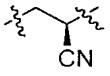
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  .

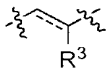
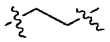
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  .

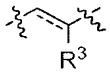
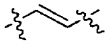
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  , wherein A is N and B is S or wherein A is S and B is N.

In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  , wherein A is N and B is CH or wherein A is CH and B is N.

In some embodiments,  is  . In some embodiments,  is  .

In some embodiments,  is  . In some embodiments,  is  .

In some embodiments,  is  .

In some embodiments,  is  .

In some embodiments, X is O.

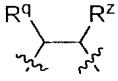
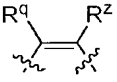
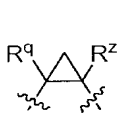
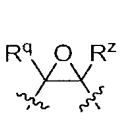
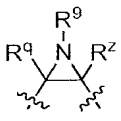
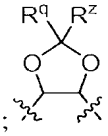
In some embodiments, X is NR^4 (e.g., NH).

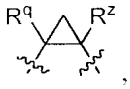
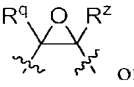
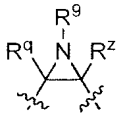
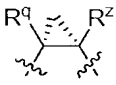
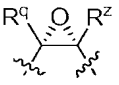
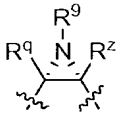
In some embodiments, Y is CR^5R^6.

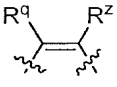
In some embodiments, Y is  .

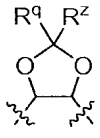
In some embodiments, Y is CH_2.

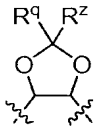
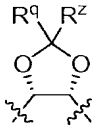
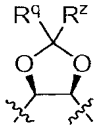
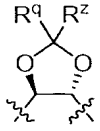
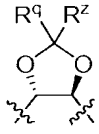
In some embodiments, Y is NR^7 (e.g., NH or NMe).

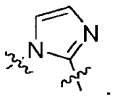
In some embodiments, Q-Z, when taken together, form  ,  ,  ,  ,  ;  or heteroarylenyl.

In some embodiments, Q-Z, when taken together, form  ,  or  . In some embodiments, Q-Z, when taken together, form  ,  or  .

In some embodiments, Q-Z, when taken together, form  , wherein R^q is H and R^z is H or alkyl (e.g., methyl).

In some embodiments, Q-Z, when taken together, form  . In some embodiments, both R^q and R^z are methyl.

In some embodiments,  is selected from  ,  ,  or  . In some embodiments, both R^q and R^z are methyl.

In some embodiments, Q-Z, when taken together, form a heteroarylenyl. In some embodiments, Q-Z, when taken together, form  .

In some embodiments, Q-Z, when taken together, form $C(O)NR^4$. In some embodiments, R^4 is H or alkyl (e.g., methyl or ethyl).

In some embodiments, Q-Z, when taken together, form $NR^4C(O)$. In some embodiments, R^4 is H or alkyl (e.g., methyl or ethyl).

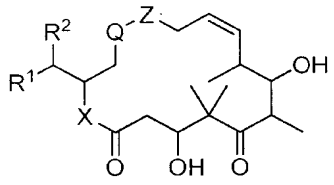
In some embodiments, Q-Z, when taken together, form CH_2NR^4. In some embodiments, R^4 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl. In some embodiments, R^4 is -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl.

In some embodiments, Q-Z, when taken together, form NR^4CH_2. In some embodiments, R^4 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl. In some embodiments, R^4 is -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl.

In some embodiments, n is 0.

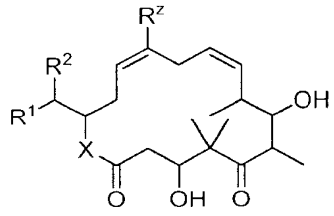
In some embodiments, n is 1.

In some embodiments, the compound of formula (XXI) is a compound of formula (XXIa)



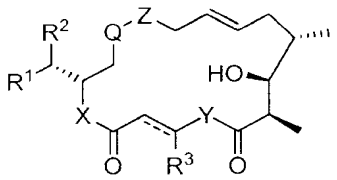
formula (XXIa).

In some embodiments, the compound of formula (XXI) is a compound of formula (XXIb)



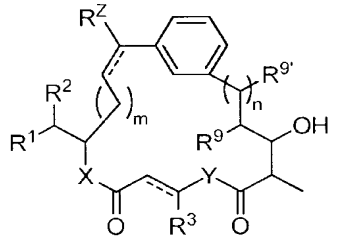
formula (XXIb).

In some embodiments, the compound of formula (XXI) is a compound of formula (XXIc)



formula (XXIc).

In some embodiments, the epothilone is a compound of formula (XXII)



formula (XXII)

wherein,

R^1 is aryl, heteroaryl, arylalkenyl or heteroarylalkenyl; each of which is optionally substituted with 1-3 R^8;

R^2 is H or alkyl (e.g., methyl); or

R^1 and R^2, when taken together with the carbon to which they are attached, form an aryl or a heteroaryl moiety optionally substituted with 1-3 R^8;

R^3 is H, OH, NH_2, or CN;

X is O or NR^4;

R^4 is H,alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)NR^5alkyl, -C(O) NR^5arylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl;

Y is CR^5R^6, O or NR^7;

each of R^5 and R^6 is independently H or alkyl (e.g., methyl);

R^7 is H, alkyl, -C(O)Oalkyl, -C(O)Oarylalkyl, -C(O)NR^5alkyl, -C(O) NR^5arylalkyl, -C(O)alkyl, -C(O)aryl or arylalkyl;

each R^8, for each occurrence, is independently alkyl, aminoalkyl or hydroxyalkyl;

each R^9 and $R^{9'}$ is independently H or alkyl (e.g., methyl);

R^z is H, alkyl (e.g., methyl), haloalkyl (e.g., CF_3), heterocyclylalkyl or N_3;

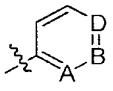
each $\diagdown\!\!\diagup$, for each occurrence, is independently a single or double bond;

m is 0, 1 or 2; and

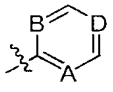
n is 0, 1 or 2.

In some embodiments, R^1 is 

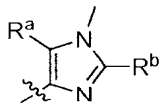
optionally substituted with 1-3 R^8. In some embodiments, HET is a five membered ring heteroaryl optionally substituted with 1-3 R^8. In some embodiments, HET is thiazolyl optionally substituted with 1-3 R^8. In some embodiments, HET is substituted with alkyl (e.g., methyl), aminoalkyl (e.g., aminomethyl), alkylthiol (e.g., methylthiol), hydroxyalkyl (e.g., hydroxymethyl), alkoxy (e.g., methoxy) or aryl (e.g., phenyl). In some embodiments, HET is substituted with alkyl (e.g., methyl) or amino alkyl.

In some embodiments, HET is 

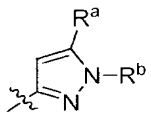
, wherein each of A, B and D is independently CH or N. In some embodiments, A is N, B is CH and D is CH. In some embodiments, A is CH, B is N and D is CH. In some embodiments, A is CH, B is CH and D is N.

In some embodiments, HET is 

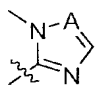
, wherein each of A, B and D is independently CH or N. In some embodiments, A is N, B is N and D is CH. In some embodiments, A is N, B is CH and D is N. In some embodiments, A is CH, B is CH and D is CH.

In some embodiments, HET is 

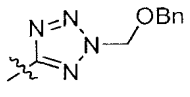
, wherein each R^a and R^b is independently H or –SMe.

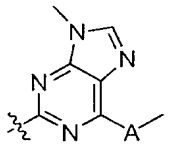
In some embodiments, HET is 

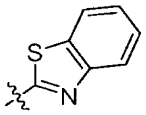
, wherein each R^a is H, an alkyl or –Salkyl; and R^b is H, alkyl (e.g., methyl) or aryl (e.g., phenyl).

In some embodiments, HET is 

, wherein A is CH or N.

In some embodiments, HET is  .

In some embodiments, HET is  , wherein A is S or O.

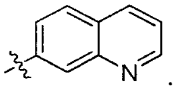
In some embodiments, HET is  .

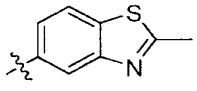
In some embodiments R^2 is H.

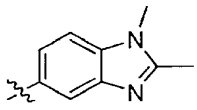
In some embodiments, R^2 is alkyl (e.g., methyl).

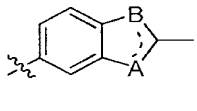
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, form an aryl or a heteroaryl moiety optionally substituted with 1-3 R^8.

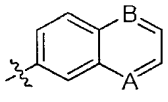
In some embodiments, the heteroaryl moiety is a bicyclic heteroaryl moiety.

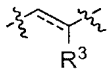
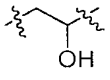
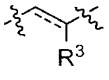
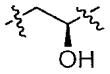
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  .

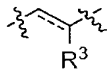
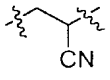
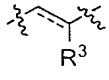
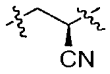
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  .

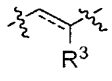
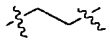
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  .

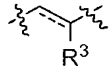
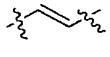
In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  , wherein A is N and B is S or wherein A is S and B is N.

In some embodiments, R^1 and R^2, when taken together with the carbon to which they are attached, are  , wherein A is N and B is CH or wherein A is CH and B is N.

In some embodiments,  is  . In some embodiments,  is  .

In some embodiments,  is  . In some embodiments,  is  .

In some embodiments,  is  .

In some embodiments,  is  .

In some embodiments, X is O.

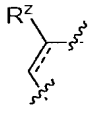
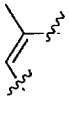
In some embodiments, X is NR^4 (e.g., NH).

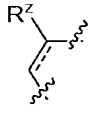
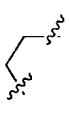
In some embodiments, Y is CR^5R^6. In some embodiments, Y is  . In some embodiments, Y is CH_2.

In some embodiments, Y is NR^7 (e.g., NH or NMe).

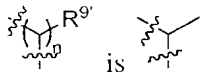
In some embodiments, R^9 is H.

In some embodiments, R^9 is Me.

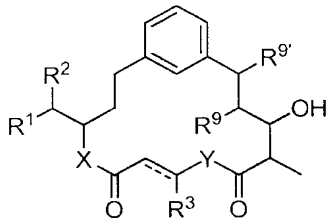
In some embodiments,  is  . In some embodiments, m is 1.

In some embodiments,  is  . In some embodiments, m is 0.

In some embodiments, n is 0.

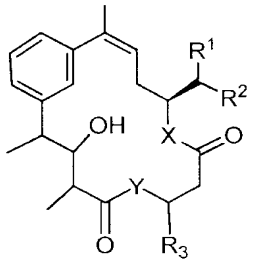
In some embodiments,  is .

In some embodiments, compound of formula (XXII) is a compound of formula (XXIIa)



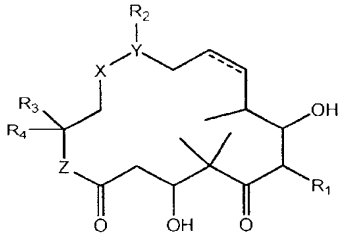
formula (XXIIa).

In some embodiments, compound of formula (XXII) is a compound of formula (XXIIb)



formula (XXIIb).

In some embodiments, the epothilone is a compound of formula (XXIII):



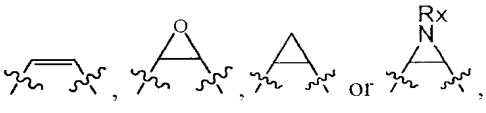
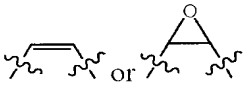
formula (XXIII)

wherein

 represents a single or double bond;

R_1 is $C_{1\text{-}6}$alkyl, $C_{2\text{-}6}$alkynyl or $C_{2\text{-}6}$alkenyl radical;

R_2 is H or $C_{1\text{-}6}$alkyl radical;

X–Y is selected from the following groups:  , preferably  ;

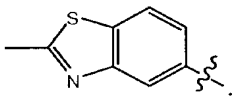
Z is O or NR_X, wherein R_X is hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, alkylcycloalkyl, heteroalkylcycloalkyl, heterocycloalkyl, aralkyl or heteroaralkyl group;

R_3 is halogen atom or $C_{1\text{-}6}$alkyl, $C_{2\text{-}6}$alkenyl or $C_{1\text{-}6}$-heteroalkyl radical;

R_4 is bicycloaryl, bicycloheteroaryl or a group of formula $-C(R_5)=CHR_6$;

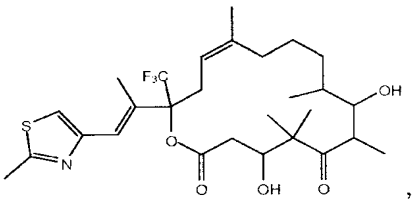
R_5 is H or methyl; and

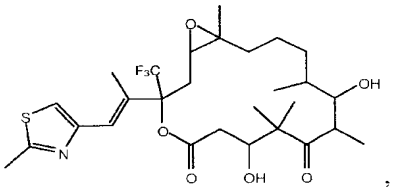
R_6 is an optionally substituted aryl or a heteroaryl group.

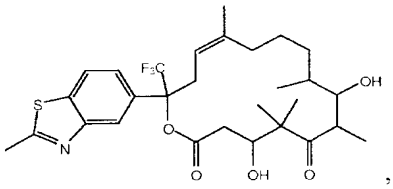
In certain embodiments, R_4 is  .

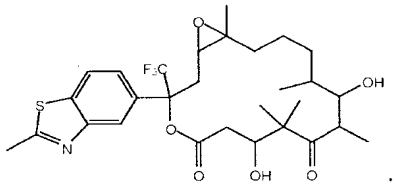
In some embodiments, Z is O. In some embodiments, Z is NH.

In certain embodiments, the compound of formula (XXIII) can be represented by the following structures:

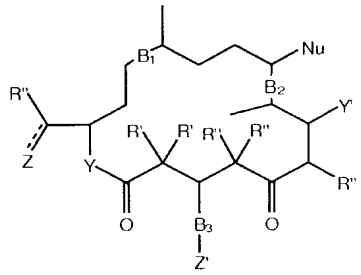
 ,

 ,

 , or



In some embodiments, the epothilone is a compound of formula (XXIV):



formula (XXIV)

wherein

B_1, B_2, B_3 are selected from single bonds; double bonds in the E(trans) form, the Z(cis) form or as an E/Z mixture; epoxide rings in the E(trans) form, the Z(cis) form or an E/Z mixture; aziridine rings in the E(trans) form, the Z(cis) form or an E/Z mixture; cyclopropane rings in the E(trans) form, the Z(cis) form or an E/Z mixture; and/or combinations thereof; and being preferably selected from single and double bonds; and particularly preferably being selected from B_1 as Z double bonds or epoxide and B_2 and B_3 as single bond;

R is selected from H, alkyl, aryl, aralkyl (such as -CH_2-aryl, -C_2H_4-aryl and the like), alkenyl (such as vinyl), cycloalkyl (preferably a 3- to 7-membered cycloalkyl), CH_nF_{3-n} wherein n=0 to 3, oxacycloalkyl (preferably a 3- to 7-membered oxacycloalkyl) and/or combinations thereof. Preferably R is selected from H, methyl, ethyl, phenyl, benzyl and combinations thereof, and more preferably R is selected from H, methyl, ethyl and combinations thereof;

R' is selected from the same group as R, and is preferably H;

R" is selected from the same group as R, and is preferably methyl;

Y is selected from S, NH, N-PG, NR and O; preferably Y is selected from NH, N-PG, NR and O, and more preferably Y is O;

Y' is selected from H, OH, OR, O-PG, NH_2, NR_2, $N(PG)_2$, SR and SH; preferably Y' is O-PG and/or OH;

Nu is selected from R, O-PG, OR, $N(PG)_2$, NR_2, S-PG, SR, SeR, CN, N_3, aryl and heteroaryl; Nu is preferably selected from R, O-PG, OR, $N(PG)_2$ and NR_2, and more preferably Nu is H;

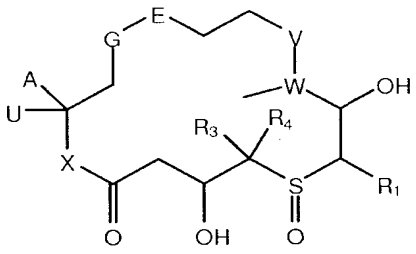
Z is selected from -OH, -O-PG, -OR, =O, =N-Nu, =CH-heteroaryl, =CH-aryl and $=PR_3$, where all previously mentioned double bound groups may be present in the E(trans) form, the Z(cis) form or as an E/Z mixture; preferably Z is =CH-heteroaryl; and more preferably Z is selected from =O, (E)-(2-methylthiazol-4-yl)-CH= and (E)-(2-methyloxazol-4-yl)-CH=;

Z' is selected from O, OH, OR, O-PG, $N(H)_{1-2}$, $N(R)_{1-2}$, $N(PG)_{1-2}$, SR, S-PG and R; preferably Z' is O, O-PG and/or OR;

B_3 is selected from single or double bonds in the E(trans) form, the Z(cis) form or as an E/Z mixture; preferably B_3 is selected from single and double bonds with heteroatoms such as O, S and N; and more preferably B_3 is a single bond to O-PG and/or OH;

PG, as referred to herein, is a protecting group, and is preferably selected from allyl, methyl, t-butyl (preferably with electron withdrawing group), benzyl, silyl, acyl and activated methylene derivative (e.g., methoxymethyl), alkoxyalkyl or 2-oxacycloalkyl. Exemplary protecting groups for alcohol and amines include trimethylsilyl, triethylsilyl, dimethyl-tert-butylsilyl, acetyl, propionyl, benzoyl, or a tetrahydropyranyl protecting group. Protecting groups can also be used to protect two neighboring groups (e.g., -CH(OH)-CH(OH)-), or bivalent groups (PG_2). Such protecting groups can form a ring such as a 5- to 7-membered ring. Exemplary protecting groups include succinyl, phthalyl, methylene, ethylene, propylene, 2,2-dimethylpropa-1,3-diyl, and acetonide. Any combination of protecting groups described herein can be used as determined by one of skill in the art.

In some embodiments, the epothilone is a compound of formula (XXV):

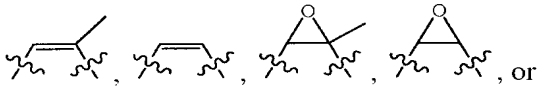


formula (XXV)

wherein

A is heteroalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaryl, heteroaralkenyl or heteroaralkyl group;

U is hydrogen, halogen, alkyl, heteroalkyl, heterocycloalkyl, heteroalkylcycloalkyl, heteroaryl or heteroaralkyl;

G-E is selected from the following groups,  , or is part of an optionally substituted phenyl ring;

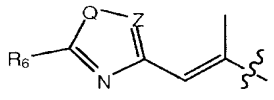
R_1 is C_1-C_4-alkyl, C_2-C_4-alkenyl, C_2-C_4-alkynyl, or C_3-C_4-cycloalkyl group;

V–W is selected from the group consisting of CH_2CH or CH=C;

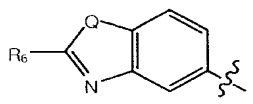
X is oxygen or a group of the formula NR_2, wherein R_2 is hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, alkylcycloalkyl, heteroalkylcycloalkyl, heterocycloalkyl, aralkyl, or heteroaralkyl; and

each of R_3 and R_4, independently from each other, is hydrogen, C_1-C_4-alkyl or R_3 and R_4 together are part of a cycloalkyl group with 3 or 4 ring atoms.

In certain embodiments of formula (XXV), A is a group of Formula (XXVII) or (XXVIII),



(XXVII)



(XXVIII)

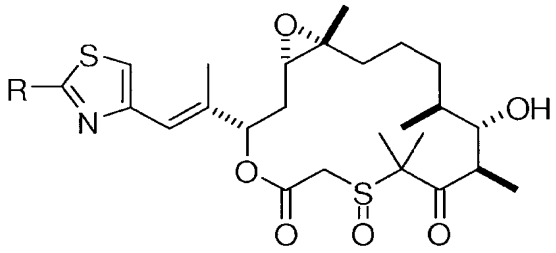
wherein

Q is sulfur, oxygen or NR_7 (preferably oxygen or sulfur), wherein R_7 is hydrogen, C_1-C_4 alkyl or C_1-C_4 heteroalkyl;

Z is nitrogen or CH (preferably CH); and

R_6 is OR_8, NHR_8, C_1-C_4 alkyl, C_1-C_4 alkenyl, C_1-C_4 alkynyl or C_1-C_6 heteroalkyl (preferably methyl, CH_2OR_8 or CH_2NHR_8), wherein R_8 is hydrogen, C_1-C_4 alkyl or C_1-C_4 heteroalkyl (preferably hydrogen).

In some embodiments, the epothilone is a compound of formula (XXVI):



formula (XXVI)

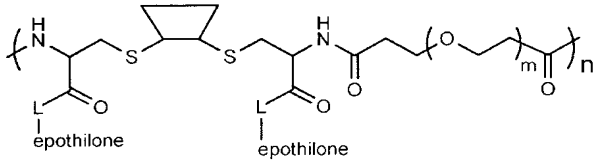
wherein R is selected from OR^1, NHR^1, alkyl, alkenyl, alkynyl and heteroalkyl (e.g., CH_2OR^1 or CH_2NHR^1) and R^1 is selected from hydrogen, C_{1-4} alkyl and C_{1-4} heteroalkyl (preferably hydrogen).

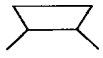
In certain embodiments, R is selected from methyl, CH_2OH and CH_2NH_2. Preparation of naturally occurring and semi-synthetic epothilones and corresponding derivatives is known in the art. Epothilones A & B were first extracted from *Sorangium cellulosum* So ce90 which exists at the German Collection of Microorganisms as DMS 6773 and DSM 11999. It has been reported that DSM 6773 allegedly displays increased production of epothilones A and B over the wild type strain. Representative fermentation conditions for Sorangium are described, for example, in U.S. Patent 6,194,181 and various international PCT publications including WO 98/10121, WO 97/19086, WO 98/22461 and WO 99/42602. Methods of preparing epothilones are also described in WO 93/10121.

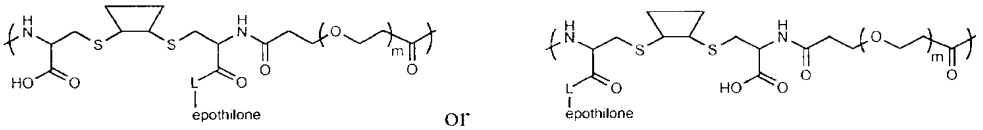
In addition, epothilones can be obtained via de novo synthesis. The total synthesis of epothilones A and B have been reported by a number of research groups including Danishefsky, Schinzer and Nicolaou. These total syntheses are described, for example, in U.S. Patents 6,156,905, 6,043,372, and 5,969,145 and in international PCT

publications WO 98/08849, WO 98/25929, and WO 99/01124. Additional synthetic methods for making epothilone compounds are also described in PCT publications WO 97/19086, WO 98/38192, WO 99/02514, WO 99/07692, WO 99/27890, WO 99/28324, WO 99/43653, WO 99/54318, WO 99/54319, WO 99/54330, WO 99/58534, WO 59985, WO 99/67252, WO 99/67253, WO 00/00485, WO 00/23452, WO 00/37473, WO 00/47584, WO 00/50423, WO 00/57874, WO 00/58254, WO 00/66589, WO 00/71521, WO 01/07439 and WO 01/27308. In preferred embodiments, the microtubule inhibitor in the CDP-microtubule inhibitor conjugate, particle or composition comprises an epothilone, e.g., an epothilone described herein, e.g., an epothilone shown in FIG. 5 or FIG. 6.

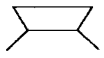
In one embodiment, the CDP-microtubule inhibitor conjugate is a CDP-epothilone conjugate, e.g.,



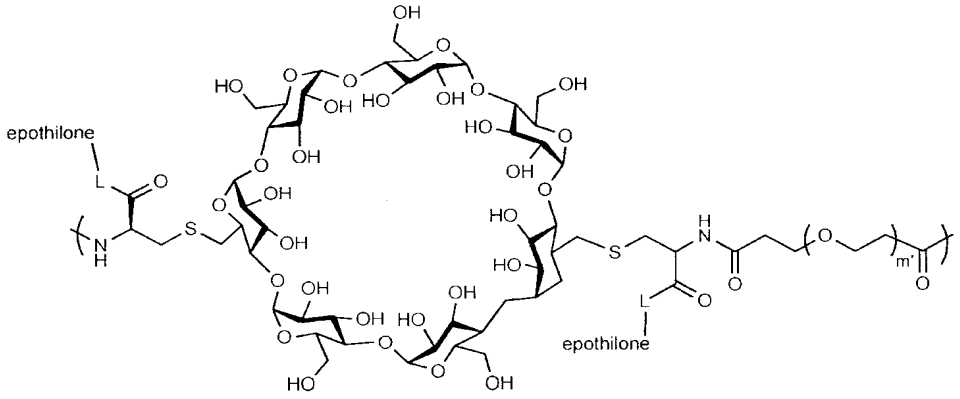
wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); L is a linker, e.g., a linker described herein; and "epothilone" is an epothilone, e.g., an epothilone described herein, e.g., an epothilone shown in FIG. 5 or FIG. 6. In some embodiments, the CDP-microtubule inhibitor conjugate, e.g., the CDP-epothilone conjugate, does not have complete loading, e.g., one or more binding sites, e.g., cysteine residues, are not bound to a microtubule inhibitor, e.g., an epothilone moiety, e.g., e.g., an epothilone described herein, bound with a linker described herein, e.g., the CDP-epothilone conjugate comprises one or more subunits having the formulae provided below:



or

wherein  represents a cyclodextrin; m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); L is a linker, e.g., a linker described herein; and "epothilone" is an epothilone, e.g., an epothilone described herein, e.g., an epothilone shown in FIG. 5 or FIG. 6. In some embodiments, the CDP-microtubule inhibitor conjugate, particle or composition e.g., the CDP-epothilone conjugate, particle or composition, comprises a mixture of fully-loaded and partially-loaded CDP-microtubule inhibitor conjugates, e.g., CDP-epothilone conjugates.

In one embodiment, the CDP-microtubule inhibitor conjugate comprises a subunit of



wherein m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); L is a linker, e.g., a linker described herein; and "epothilone" is an epothilone, e.g., an epothilone described herein, e.g., an epothilone shown in FIG. 5 or FIG. 6.

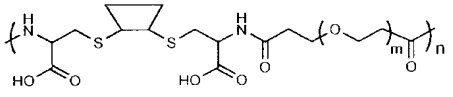
CDP-epothilone conjugates can be made using many different combinations of components described herein. For example, various combinations of cyclodextrins (e.g., beta-cyclodextrin), comonomers (e.g., PEG containing comonomers), linkers linking the cyclodextrins and comonomers, and/or linkers tethering the epothilone to the CDP are described herein.

FIG. 6 is a table depicting examples of different CDP-epothilone conjugates. The CDP-epothilone conjugates in FIG. 6 are represented by the following formula:

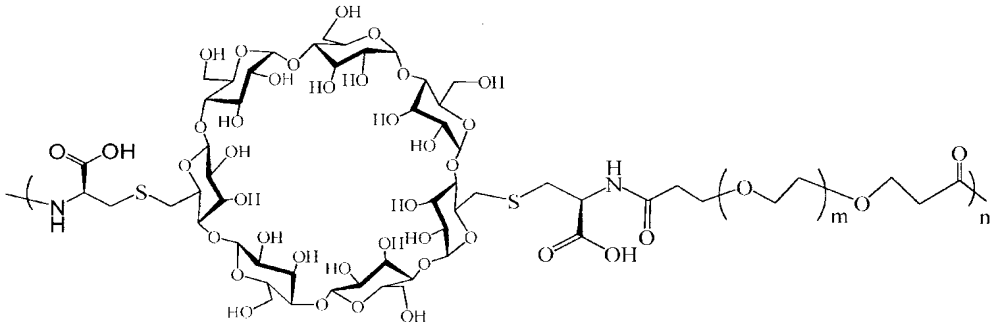
CDP-COABX-Epothilone

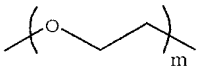
In this formula,

CDP is the cyclodextrin-containing polymer shown below (as well as in FIG. 3):



or



wherein for each example above, the group  has a Mw of 3400 Da or less and n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. Note that the epothilone is conjugated to the CDP through the carboxylic acid moieties of the polymer as provided above. Full loading of the epothilone onto the CDP is not required. In some embodiments, at least one, e.g., at least 2, 3, 4, 5, 6 or 7, of the carboxylic acid moieties remains unreacted with the epothilone after conjugation (e.g., a plurality of the carboxylic acid moieties remain unreacted).

CO represents the carbonyl group of the cysteine residue of the CDP;

A and B represent the link between the CDP and the epothilone. Position A is either a bond between linker B and the cysteine acid carbonyl of CDP (represented as a "-" in FIG. 6), a bond between the epothilone and the cysteine acid carbonyl of CDP (represented as a "-" in FIG. 6) or depicts a portion of the linker that is attached via a bond to the cysteine acid carbonyl of the CDP. Position B is either not occupied

(represented by "-" in FIG. 6) or represents the linker or the portion of the linker that is attached via a bond to the epothilone; and

X represents the heteroatom to which the linker is coupled on the epothilone.

As provided in FIG. 6, the column with the heading "Epothilone" indicates which epothilone is included in the CDP-epothilone conjugate.

The three columns on the right of the table in FIG. 6 indicate respectively, what, if any, protecting groups are used to protect the X on the epothilone, the process for producing the CDP-epothilone conjugate, and the final product of the process for producing the CDP-epothilone conjugate.

The processes referred to in FIG. 6 are given a letter representation, e.g., Process A, Process B, Process C, etc. as seen in the second column from the right. The steps for each these processes respectively are provided below.

Process A: Couple the protected linker of position B to the epothilone, deprotect the linker and couple to CDP via the carboxylic acid group of the CDP to afford a mixture of 3- and 7-linked epothilone to CDP.

Process B: Couple the protected linker of position B to the epothilone, isolate 3-linked epothilone, and deprotect the linker and couple to CDP via the carboxylic acid group of the CDP to afford a 3-linked epothilone to CDP.

Process C: Couple the protected linker of position B to the epothilone, isolate 7-linked epothilone, deprotect the linker and couple to CDP via the carboxylic acid group of the CDP to afford a 7-linked epothilone to CDP.

Process D: Protect the epothilone, couple the protected linker of position B to an unprotected hydroxyl group of the epothilone, deprotect the linker and the epothilone hydroxyl protecting group, and couple to CDP via the carboxylic acid group of the CDP to afford a mixture of 3- and 7-linked epothilone to CDP.

Process E: Protect the epothilone, couple the protected linker of position B to an unprotected hydroxyl group of the epothilone, deprotect the linker protecting group, couple the linker to CDP via the carboxylic acid group of the CDP, and deprotect the hydroxyl protecting group to afford a mixture of 3- and 7-linked epothilone to CDP.

Process F: Protect the epothilone, isolate the 3-protected epothilone, couple the 3-protected epothilone to the protected linker of position B, deprotect linker and hydroxyl

protecting group of the epothilone, and couple to CDP via the carboxylic acid group of the CDP to afford a 7-linked epothilone to CDP.

Process G: Protect the epothilone, isolate the 7-protected epothilone, couple to the protected linker of position B, deprotect linker and hydroxyl protecting group of the epothilone, and couple to CDP via the carboxylic acid group of the CDP to afford 3-linked epothilone to CDP.

Process H: Protect an amino group of the epothilone, couple the protected linker of position B to the epothilone, deprotect linker, couple to CDP via the carboxylic acid group of the CDP to afford a mixture of 3- and 7-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process I: Protect an amino group of the epothilone, couple the protected linker of position B to the epothilone, isolate the 3-linked epothilone, deprotect the linker, couple to CDP via the carboxylic acid group of the CDP to afford 3-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process J: Protect an amino group of the epothilone, couple the protected linker of position B to the epothilone, isolate the 7-linked epothilone, deprotect the linker, couple to CDP via the carboxylic acid group of the CDP to afford 7-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process K: Protect an amino group and a hydroxyl group of the epothilone, couple the protected linker of position B to an unprotected hydroxyl group of the epothilone, deprotect the linker and the hydroxyl group of the epothilone, couple to CDP via the carboxylic acid group of the CDP to afford a mixture of 3- and 7-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process L: Protect epothilone amino group and hydroxyl group, couple the protected linker of position B to unprotected hydroxyl group, deprotect linker protecting group, couple to CDP, deprotect hydroxyl protecting group to afford a mixture of 3- and 7-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process M: Protect an amino group and a hydroxyl group of the epothilone, isolate 3-protected epothilone, couple the epothilone to the linker of position B, deprotect the linker and the hydroxyl group of the epothilone, couple to CDP via the carboxylic

acid group of the CDP to afford 7-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process N: Protect an amino group and a hydroxyl group of the epothilone, isolate 7-protected epothilone, couple the epothilone to the linker of position B, deprotect the linker and the hydroxyl group of the epothilone, couple to CDP via the carboxylic acid group of the CDP to afford 3-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process O: Couple the protected linker of position B to an amino group of epothilone, deprotect the linker, and couple to CDP via the carboxylic acid group to afford NH-linked epothilone to CDP.

Process P: Couple the activated linker of position B to the epothilone, and couple to CDP containing linker of position A via the linker of A to afford a mixture of 3- and 7-linked epothilone to CDP.

Process Q: Couple the activated linker of position B to the epothilone, isolate the 3-linked epothilone, and couple to the CDP containing linker of position A via the linker of A to afford the 3-linked epothilone to CDP.

Process R: Couple the activated linker of position B, isolate the 7-linked epothilone, and couple to the CDP containing linker of position A via the linker of A to afford 7-linked epothilone to CDP.

Process S: Protect the epothilone, couple the activated linker of position B to an unprotected hydroxyl group of the epothilone, deprotect the hydroxyl group of the epothilone, and couple to the CDP containing linker of position A via the linker of A to afford a mixture of 3- and 7-linked epothilone to CDP.

Process T: Protect the epothilone, couple the activated linker of position B to an unprotected hydroxyl group of the epothilone, couple to the CDP containing linker of position A via the linker of A, and deprotect hydroxyl group of the epothilone to afford a mixture of 3- and 7-linked epothilone to CDP.

Process U: Protect the epothilone, isolate the 3-protected epothilone, couple the epothilone to the activated linker of position B, deprotect the hydroxyl protecting group of the epothilone, and couple to the CDP containing linker of position A to afford the 7-linked epothilone to CDP.

Process V: Protect the epothilone, isolate the 7-protected epothilone, couple to the activated linker of position B, deprotect the hydroxyl group of the epothilone, and couple to CDP containing linker of position A via the linker of A to afford the 3-linked epothilone to CDP.

Process W: Couple the epothilone directly to CDP via the free amino group of the epothilone to the carboxylic acid group of the CDP to form NH-linked epothilone to CDP.

Process X: Couple the activated linker of position B to an amino group of epothilone, and couple to CDP containing linker of position A via the linker of A to form NH-linked epothilone to CDP.

Process Y: Protect the epothilone, isolate the 3-protected epothilone, couple the epothilone to the linker of position B, deprotect the linker, and couple to CDP via the carboxylic acid group of CDP to afford the 7-linked epothilone to CDP.

Process Z: Protect the epothilone, isolate the 7-protected epothilone, couple to the protected linker of position B, deprotect linker, and couple to CDP via the carboxylic acid group of CDP to afford the 3-linked epothilone to CDP.

Process AA: Protect the amino and hydroxyl groups of the epothilone, isolate 3-protected epothilone, couple to the protected linker of position B, deprotect the linker, and couple to CDP via the carboxylic acid group of CDP to afford 7-linked epothilone to CDP.

Process BB: Protect the amino and hydroxyl groups of the epothilone, isolate 7-protected epothilone, couple to the protected linker of position B, deprotect the linker, and couple to CDP via the carboxylic acid group of the CDP to afford 3-linked epothilone to CDP.

Process CC: Protect an amino group of the epothilone, couple the activated linker of position B to the epothilone, couple to CDP containing linker of position A via the linker of A to afford a mixture of 3- and 7-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process DD: Protect an amino group of the epothilone, couple the activated linker of position B to the epothilone, isolate the 3-linked epothilone, couple to the CDP

containing linker of position A via the linker of A to afford the 3-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process EE: Protect an amino group of the epothilone, couple the activated linker of position B, isolate the 7-linked epothilone, couple to the CDP containing linker of position A via the linker of A to afford 7-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process FF: Protect an amino group and a hydroxyl group of the epothilone, couple the activated linker of position B to an unprotected hydroxyl group of the epothilone, deprotect the hydroxyl group of the epothilone, couple to the CDP containing linker of position A via the linker of A to afford a mixture of 3- and 7-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process GG: Protect an amino group and a hydroxyl group of the epothilone, couple the activated linker of position B to an unprotected hydroxyl group of the epothilone, couple to the CDP containing linker of position A via the linker of A, deprotect hydroxyl group of the epothilone to afford a mixture of 3- and 7-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process HH: Protect an amino group and a hydroxyl group of the epothilone, isolate the 3-protected epothilone, couple the epothilone to the activated linker of position B, deprotect the hydroxyl protecting group of the epothilone, couple to the CDP containing linker of position A to afford the 7-linked epothilone to CDP, and deprotect the amino group of the epothilone.

Process II: Protect an amino group and a hydroxyl group of the epothilone, isolate the 7-protected epothilone, couple to the activated linker of position B, deprotect the hydroxyl group of the epothilone, couple to CDP containing linker of position A via the linker of A to afford the 3-linked epothilone to CDP, and deprotect the amino group of the epothilone.

As shown specifically in FIG. 6, the CDP-epothilone conjugates can be prepared using a variety of methods known in the art, including those described herein. In some embodiments, the CDP-epothilone conjugates can be prepared using no protecting groups on the epothilone. For example, the CDP-epothilone conjugates can be prepared as a mixture (e.g., where there are two free hydroxyl groups on the epothilone) at the time the

epothilone is coupled to the CDP or the linker. The mixture can be coupled with a linker, e.g., a linker of position A, which is attached to the cysteine acid carbonyl of the CDP. The mixture may also be directly coupled with the CDP, i.e., the cysteine acid carbonyl of the CDP.

In some embodiments, the CDP-epothilone conjugates can be prepared using a protecting group on a hydroxyl group of the epothilone that is not used as a point of attachment to the CDP. When a linker is present, e.g., a linker of position B, the linker can be coupled to the epothilone at an unprotected point of attachment, e.g., at an unprotected hydroxyl group of the epothilone. In one embodiment, the epothilone can be deprotected and a linker of position B can be coupled to CDP via linker of position A. When a linker of position A is present, it can be attached to cysteine acid carbonyl of the CDP. Position A may also be a bond, and therefore, the coupling of the epothilone and/or epothilone linker B may be directly with the CDP, i.e., the cysteine acid carbonyl of the CDP.

In some embodiments, the CDP-epothilone conjugates can be prepared using a prodrug protecting group on a hydroxyl group of the epothilone that is not used as a point of attachment to the CDP. When linker of position B is present, the linker can be coupled to the epothilone without deprotecting the epothilone. For example, the prodrug can be an ester group that remains on a hydroxyl group of the epothilone and a different hydroxyl group of the epothilone can be used as the point of attachment to the CDP (see, e.g., examples 289-400 of FIG. 6). In some embodiments, the protected epothilone can be coupled to the CDP via a linker of position A. When position A includes a linker, the linker at position A is attached to the cysteine acid carbonyl of the CDP. Position A may also be a bond, and therefore, the coupling may be directly with the CDP, i.e., the cysteine acid carbonyl of the CDP.

One or more protecting groups can be used in the processes described above to make the CDP-epothilone conjugates described herein. A protecting group can be used to control the point of attachment of the epothilone and/or epothilone linker to position A. In some embodiments, the protecting group is removed and, in other embodiments, the protecting group is not removed. If a protecting group is not removed, then it can be selected so that it is removed *in vivo* (e.g., acting as a prodrug). An example is hexanoic

acid which has been shown to be removed by lipases *in vivo* if used to protect a hydroxyl group in doxorubicin. Protecting groups are generally selected for both the reactive groups of the epothilone and the reactive groups of the linker that are not targeted to be part of the coupling reaction. The protecting group should be removable under conditions which will not degrade the epothilone and/or linker material. Examples include t-butyldimethylsilyl ("TBDMS") and TROC (derived from 2,2,2-trichloroethoxy chloroformate). Carboxybenzyl ("CBz") can also be used in place of TROC if there is selectivity seen for removal over olefin reduction. This can be addressed by using a group which is more readily removed by hydrogentation such as –methoxybenzyl OCO-. Other protecting groups may also be acceptable. One of skill in the art can select suitable protecting groups for the products and methods described herein.

Although the products in FIG. 6 corresponding to processes E, L, T, and FF result in a mixture of 3- and 7-linked epothilone to CDP. These processes can be readily modified to produce a product having an epothilone linked by a single group, e.g., linked either through the 3- position only or 7- position only. For example, a 3- linked epothilone to CDP can be produced in methods E, L, T, and FF by separating and isoloating a pure isomer of the 7-protected epothilone prior to coupling of the epothilone to the CDP; and a 7- linked epothilone to CDP can be produced in methods E, L, T, and FF by separating and isoloating a pure isomer of the 3-protected epothilone prior to coupling of the epothilone to the CDP.

In some embodiments, microtubule inhibitor in the CDP-microtubule inhibitor conjugate is an a vinca alkaloid, e.g., vinblastine (Velban® or Velsar®), vincristine (Vincasar® or Oncovin®), vindesine (Eldisine®), vinorelbine (Navelbine®).

In some embodiments, the anti-tumor antibiotic in the CDP-anti-tumor antibiotic conjugate, particle or composition is an antibiotic including, without limitation, actinomycin (Cosmegen®), bleomycin (Blenoxane®), hydroxyurea (Droxia® or Hydrea®), mitomycin (Mitozytrex® or Mutamycin®).

In an embodiment, the therapeutic agent in the CDP-therapeutic agent conjugate is a cytotoxic agent such as a kinase inhibitor. In some embodiments, the kinase inhibitor in the CDP-kinase inhibitor conjugate, particle or composition is a kinase inhibitor

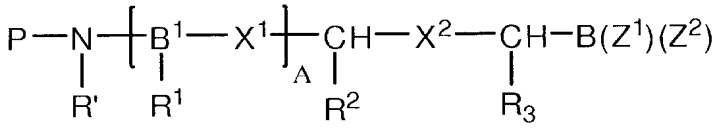
including, without limitation, a seronine/threonine kinase inhibitor conjugate, e.g., an mTOR inhibitor, e.g., rapamycin (RapDane®).

In an embodiment, the therapeutic agent in the CDP-therapeutic agent conjugate is a cytotoxic agent such as a proteasome inhibitor. In some embodiments, the proteasome inhibitor in the CDP-proteasome inhibitor conjugate, particle or composition is a boronic acid containing molecule or a boronic acid derivative, e.g., bortezomib (Velcade®). Other proteasome inhibitors described herein can also be included in the CDP-proteasome inhibitor conjugates.

As used herein, a boronic acid derivative is represented by R-B(Y)$_2$, wherein each Y is a group that is readily displaced by an amine or alcohol group on a liker L to form a covalent bond (e.g., conjugating the therapeutic agent (e.g., a proteasome inhibitor containing a boronic acid or derivative thereof to the CDP)). Examples of boronic acid derivatives include boronic ester (e.g., RB(O-alkyl)$_2$), boronic amides (e.g., RB(N(alkyl)$_2$)$_2$), alkoxyboranamine (e.g., RB(O-alkyl)(N(alkyl)$_2$); and boronic acid anhydride. Mixed boronic acid derivatives are also included, such as RB(O-alkyl)(N(alkyl)$_2$).

A number of CDP-L-boronic acid structures are shown in FIG. 7, wherein the structures for the CDP-proteasome inhibitor are represented by CDP-L-boronic acid, wherein Z^1 and Z^2 each represent bonds to the boron atom of the conjugated drug. For example, the CDP-bortezomib conjugate is represented by CDP-L-B-(*L*)-CH(CH$_2$CH(CH$_3$)$_2$)NH-(*L*)-Phe-CO-pyrazine. In FIG. 7 Z^1 and Z^2 each represents a bond to the boron atom of the boronic acid. Process A comprises: 1) couple linker, optionally protected, to CDP, 2) deprotect linker if protected, 3) conjugate to boronic acid. Process B comprises: 1) conjugate linker, optionally protected, to boronic acid, 2) deprotect linker if protected, 3) couple to CDP.

In one embodiment, for the CDP-proteasome inhibitor conjugates described in any one of 1st to 15th embodiments (below) wherein the proteasome inhibitor contains a boronic acid or derivative thereof, RB(OH)$_2$ or RB(Y)$_2$ is represented by formula (1a) below:



(1a),

or a pharmaceutically acceptable salts thereof, wherein:

P is hydrogen or an amino-group-protecting moiety;

B^1, at each occurrence, is independently one of N or CH;

X^1, at each occurrence, is independently one of -C(O)-NH-, -CH_2-NH-, -CH(OH)-CH_2-, -CH(OH)-CH(OH)-, -CH(OH)-CH_2-NH-, -CH=CH-, -C(O)CH_2-, -SO_2-NH-, -SO_2-CH_2- or -CH(OH)-CH_2-C(O)-NH-, provided that when B^1 is N, then the X^1 attached to said B^1 is -C(O)-NH-;

X^2 is one of -C(O)-NH-, -CH(OH)-CH_2-, -CH(OH)-CH(OH)-, -C(O)-CH_2-, -SO_2–NH-, -SO_2 –CH_2 - or -CH(OH)-CH_2 -C(O)-NH-;

R' is hydrogen or alkyl, or R forms together with the adjacent R^1, or when A is zero, forms together with the adjacent R^2, a nitrogen-containing mono-, bi- or tri-cyclic, saturated or partially saturated ring system having 4-14 ring members, that can be optionally substituted by one or two of keto, hydroxy, alkyl, aryl, aralkyl, alkoxy or aryloxy;

R^1, at each occurrence, is independently one of hydrogen, alkyl, cycloalkyl, aryl, a 5-10 membered saturated, partially unsaturated or aromatic heterocycle or –CH_2 –R^5, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

R^2 is one of hydrogen, alkyl, cycloalkyl, aryl, a 5-10 membered saturated, partially unsaturated or aromatic heterocycle or –CH-R^5, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

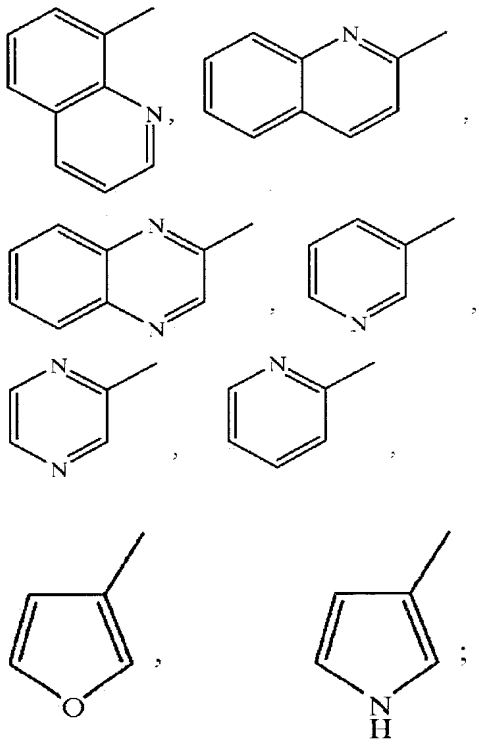
R^3 is one of hydrogen, alkyl, cycloalkyl, aryl, a 5-10 membered saturated, partially unsaturated or aromatic heterocycle or –CH_2-R^5, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

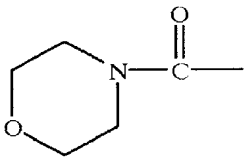
R^5, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, a 5-10 membered saturated, partially unsaturated or aromatic heterocycle or –W-R^6, where W is a chalcogen and R^6 is alkyl, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

Z^1 and Z^2 are independently one of alkyl, hydroxy, alkoxy, or aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O; and A is 0, 1, or 2.

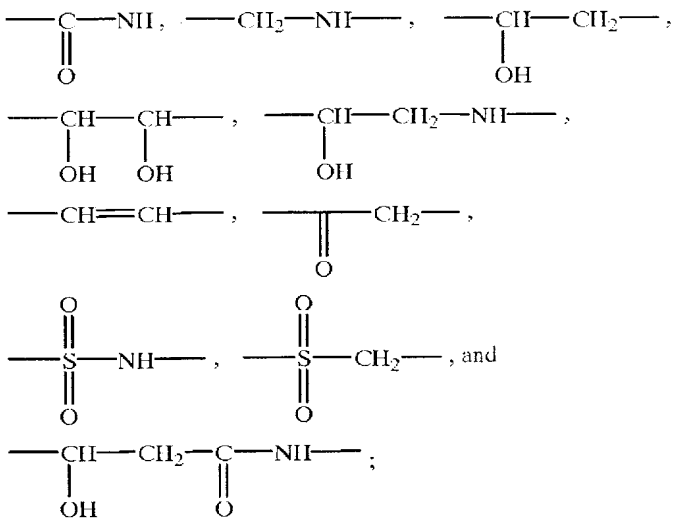
In one embodiment, for formula (1a):

P is R' or R^7-C(=O)- or R^7-SO_2-, wherein R^7 selected from the group consisting of



or P is  ;

X_2 is selected from the group consisting of



R' is hydrogen or alkyl;

R_2 and R_3 are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, heterocycle and $-CH_2-R_5$, where R_5 is aryl, aralkyl, alkaryl, cycloalkyl, heterocycle or $-Y-R_6$,

where Y is a chalcogen, and R_6 is alkyl;

Z_1 and Z_2 are independently alkyl, hydroxy, alkoxy, aryloxy, or together form a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O; and A is 0.

In another embodiment, for structural formula (1a):

P is R_7-C(O)- or R_7-SO_2 -, where R_7 is pyrazinyl;

X_2 is -C(O)-NH-;

R' is hydrogen or alkyl;

R_2 and R_3 are independently hydrogen, alkyl, cycloalkyl, aryl, or $-CH_2-R_5$;

R_5 in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, or -W-R_6, where W is a chalcogen and R_6 is alkyl;

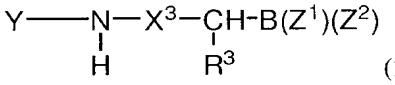
where the ring portion of any of said aryl, aralkyl, or alkaryl in R_2, R_3 and R_5 can be optionally substituted by one or two substituents independently selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{1-6} alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8}

alkynyl, cyano, amino, C_{1-6} alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6} alkoxy, C_{6-10} aryl, C_{6-10} aryl(C_{1-6})alkyl, C_{6-10} aryl(C_{1-6})alkoxy, hydroxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{6-10} arylthio, C_{6-10} arylsulfinyl, C_{6-10} arylsulfonyl, C_{6-10} aryl, C_{1-6} alkyl(C_{6-10}) aryl, and halo(C_{6-10})aryl;

Z_1 and Z_2 are independently one of hydroxy, alkoxy, or aryloxy, or together Z_1 and Z_2 form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O; and

A is zero.

In one embodiment, for CDP-proteasome inhibitor conjugates described in any one of the 1st to 15th embodiments (below) wherein the proteasome inhibitor contains a boronic acid or derivative thereof, $RB(OH)_2$ or its analog is represented by formula 2a below



(2a),

or a pharmaceutically acceptable salts thereof, wherein:

Y is one of R^8 -C(O)-, R^8-SO_2 -, R^8-NH-C(O)- or R^8-O-C(O)-, where R^8 is one of alkyl, aryl, alkaryl, aralkyl, any of which can be optionally substituted, or when Y is R^8 -C(O)-or R^8-SO_2 -, then R^8 can also be an optionally substituted 5-10 membered, saturated, partially unsaturated or aromatic heterocycle;

X^3 is a covalent bond or -C(O)-CH_2-;

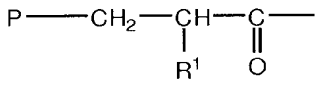
R^3 is one of hydrogen, alkyl, cycloalkyl, aryl, a 5-10 membered saturated, partially unsaturated or aromatic heterocycle or –CH_2 –R^5, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

R^5, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, a 5-10 membered saturated, partially unsaturated or aromatic heterocycle or –W-R^6, where W is a chalcogen and R^6 is alkyl, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted; and

Z^1 and Z^2 are independently alkyl, hydroxy, alkoxy, aryloxy, or together form a

moiety derived from dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O;

provided that when Y is R^8-C(O)-, R^8 is other than phenyl, benzyl or C_1 –C_3 alkyl. Alternatively, the group Y in formula (2a) above, can be as provided in formula 3a below:



(3a)

P is one of R^7-C(O)-, R^7-SO_2-, R^7-NH-C(O)- or R^7-O-C(O)-;

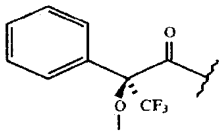
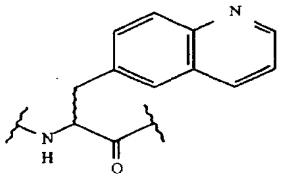
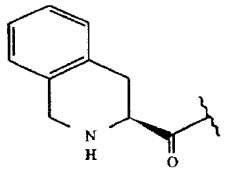
R^7 is one of alkyl, aryl, alkaryl, aralkyl, any of which can be optionally substituted, or when Y is R^7-C(O)- or R^7-SO_2-, R^7 can also be an optionally substituted 5-10 membered saturated, partially unsaturated or aromatic heterocycle; and

R^1 is defined above as for formula (1a).

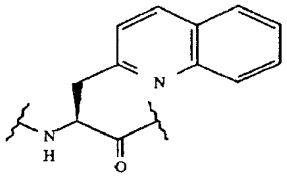
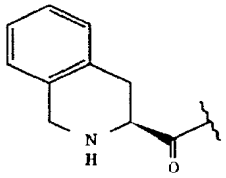
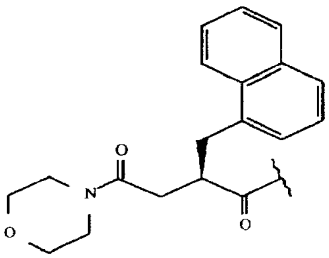
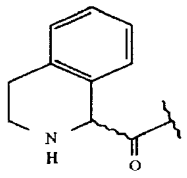
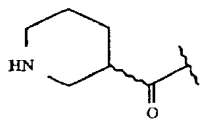
In one embodiments, compounds of formula (1a) or (2a) described above are compounds depicted in Table 1.

Table 1.

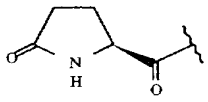
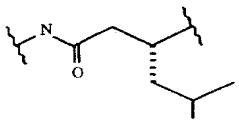
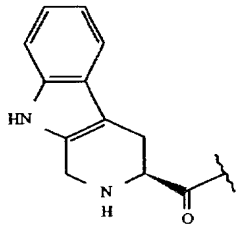
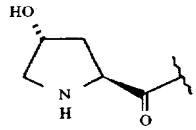
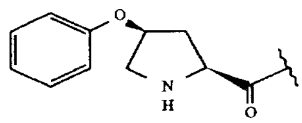
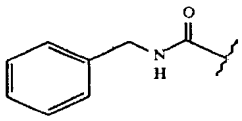
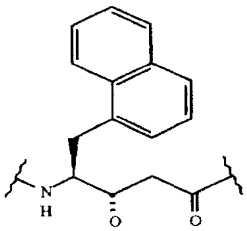
Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
$P-AA^1-AA^2-AA^3-B(Z^1)(Z^2)$

Compound	P[a]	AA^1	AA^{2b}	AA^{3c}	Z^1, Z^2
MG-261	Cbz	L—Leu	L—Leu	L—Leu	pinane diol
MG-262	Cbz	L—Leu	L—Leu	L—Leu	$(OH)_2$
MG-264	Cbz	—	L—Leu	L—Leu	pinane diol
MG-267	Cbz	—	L—Nal	L—Leu	pinane diol
MG-268	Cbz(N—Me)	—	L—Leu	L—Leu	$(OH)_2$
MG-270	Cbz	—	L—Nal	L—Leu	$(OH)_2$
MG-272	Cbz	—	D-(2-Nal)	L—Leu	$(OH)_2$
MG-273	Morph	—	L—Nal	L—Leu	$(OH)_2$
MG-274	Cbz	—	L—Leu	L—Leu	$(OH)_2$
MG-278	Morph	L—Leu	L—Leu	L—Leu	$(OH)_2$
MG-282	Cbz	—	L—His	L—Leu	$(OH)_2$
MG-283	Ac	L—Leu	L—Leu	L—Leu	$(OH)_2$
MG-284		—	—	L—Leu	$(OH)_2$
MG-285	Morph	—	L—Trp	L—Leu	$(OH)_2$
MG-286	Morph	—	L—Nal	L—Leu	diethanol-amine
MG-287	Ac	—	L—Nal	L—Leu	$(OH)_2$
MG-288	Morph	—	L—Nal	D—Leu	$(OH)_2$
MG-289	Ms	—	L-(3-Pal)	L—Leu	$(OH)_2$
MG-290	Ac	—	L-(3-Pal)	L—Leu	$(OH)_2$
MG-291	Ms	—	L—Nal	L—Leu	diethanol-amine
MG-292	Morph	—		L—Leu	$(OH)_2$
MG-293	Morph	—	D—Nal	D—Leu	$(OH)_2$
MG-294	H	—	L-(3-Pal)	L—Leu	$(OH)_2$
MG-295	Ms	—	L—Trp	L—Leu	$(OH)_2$
MG-296	(8-Quin)-SO_2	—	L—Nal	L—Leu	$(OH)_2$
MG-297	Ts	—	L—Nal	L—Leu	$(OH)_2$
MG-298	(2-Quin)-C(O)	—	L—Nal	L—Leu	$(OH)_2$
MG-299	(2-quinoxalinyl)-C(O)	—	L—Nal	L—Leu	$(OH)_2$
MG-300	Morph	—	L-(3-Pal)	L—Leu	$(OH)_2$
MG-301	Ac	—	L—Trp	L—Leu	$(OH)_2$
MG-302	H	—	L—Nal	L—Leu	$(OH)_2$
MG-303	H.HCl	—	L—Nal	L—Leu	$(OH)_2$
MG-304	Ac	L—Leu	L—Nal	L—Leu	$(OH)_2$
MG-305	Morph	—	D—Nal	L—Leu	$(OH)_2$
MG-306	Morph	—	L—Tyr—(O-Benzyl)	L—Leu	$(OH)_2$
MG-307	Morph	—	L—Tyr	L—Leu	$(OH)_2$
MG-308	Morph	—	L-(2-Nal)	L—Leu	$(OH)_2$
MG-309	Morph	—	L—Phe	L—Leu	$(OH)_2$
MG-310	Ac	—		L—Leu	$(OH)_2$
MG-312	Morph	—	L-(2-Pal)	L—Leu	$(OH)_2$
MG-313	Phenethyl-C(O)	—	—	L—Leu	$(OH)_2$

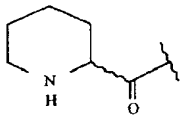
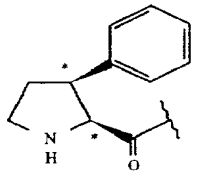
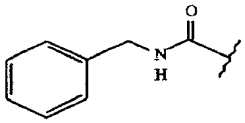
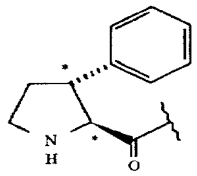
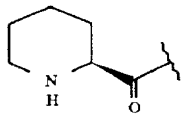
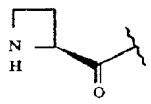
Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
$P-AA^1-AA^2-AA^3-B(Z^1)(Z^2)$

Compound	P[a]	AA^1	AA^{2b}	AA^{3c}	Z^1, Z^2
MG-314	(2-Quin)-C(O)	—	L—Phe	L—Leu	$(OH)_2$
MG-315	Morph	—		L—Leu	$(OH)_2$
MG-316	H.HCl	—		L—Leu	$(OH)_2$
MG-317	Morph	—	L—Nal	L—Leu	$(OH)(CH_3)$
MG-318	Morph	—	L—Nal	L—Leu	$(CH_3)_2$
MG-319	H.HCl	—	L—Pro	L—Leu	$(OH)_2$
MG-321	Morph	—	L—Nal	L—Phe	$(OH)_2$
MG-322	Morph	—	L-homoPhe	L—Leu	$(OH)_2$
MG-323	Ac	—	—	L—Leu	$(OH)_2$
MG-324		—	—	L—Leu	H
MG-325	(2-Quin)-C(O)	—	L-homoPhe	L—Leu	$(OH)_2$
MG-328	Bz	—	L—Nal	L—Leu	$(OH)_2$
MG-329	Cyclohexyl-C(O)	—	L—Nal	L—Leu	$(OH)_2$
MG-332	Cbz(N—Me)	—	L—Nal	L—Leu	$(OH)_2$
MG-333	H.HCl	—	L—Nal	L—Leu	$(OH)_2$
MG-334	H.HCl(N—Me)	—	L—Nal	L—Leu	$(OH)_2$
MG-336	(3-Pyr)—C(O)	—	L—Phe	L—Leu	$(OH)_2$
MG-337	H.HCl	—		L—Leu	$(OH)_2$
MG-338	(2-Quin)-C(O)	—	L-(2-Pal)	L—Leu	$(OH)_2$
MG-339	H.HCl	—		L—Leu	$(OH)_2$

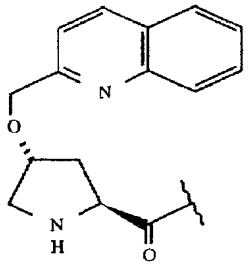
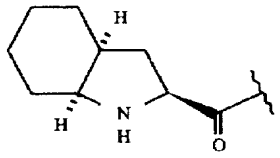
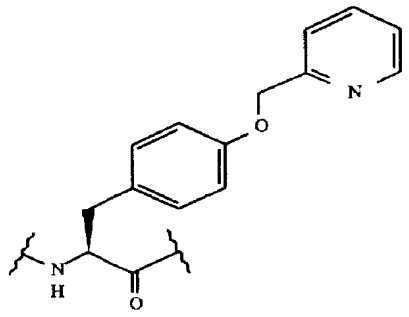
Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
$P-AA^1-AA^2-AA^3-B(Z^1)(Z^2)$

Compound	P[a]	AA^1	AA^{2b}	AA^{3c}	Z^1, Z^2
MG-340	H	—		L−Leu	$(OH)_2$
MG-341	(2-Pyz)−C(O)	—	L−Phe	L−Leu	$(OH)_2$
MG-342	Bn	—		—	$(OH)_2$
MG-343	(2-Pyr)−C(O)	—	L−Phe	L−Leu	$(OH)_2$
MG-344	Ac	—		L−Leu	$(OH)_2$
MG-345	Bz	—	L-(2-Pal)	L−Leu	$(OH)_2$
MG-346	Cyclohexyl-C(O)	—	L-(2-Pal)	L−Leu	$(OH)_2$
MG-347	(8-Quin)-SO_2	—	L-(2-Pal)	L−Leu	$(OH)_2$
MG-348	H.HCl	—		L−Leu	$(OH)_2$
MG-349	H.HCl	—		L−Leu	$(OH)_2$
MG-350		—	L−Phe	L−Leu	$(OH)_2$
MG-351	H.HCl	—	L-(2-Pal)	L−Leu	$(OH)_2$
MG-352	Phenylethyl-C(O)	—	L−Phe	L−Leu	$(OH)_2$
MG-353	Bz	—	L−Phe	L−Leu	$(OH)_2$
MG-354	(8-Quin)-SO_2	—		L−Leu	$(OH)_2$

Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
$P-AA^1-AA^2-AA^3-B(Z^1)(Z^2)$

Compound	P[a]	AA^1	AA^{2b}	AA^{3c}	Z^1, Z^2
MG-356	Cbz	—	L—Phe	L—Leu	$(OH)_2$
MG-357	H.HCl	—		L—Leu	$(OH)_2$
MG-358	(3-Furanyl)-C(O)	—	L—Phe	L—Leu	$(OH)_2$
MG-359	H.HCl	—		L—Leu	$(OH)_2$
MG-361	(3-Pyrrolyl)-C(O)	—	L—Phe	L—Leu	(OH)2
MG-362		—	—	L—Leu	$(OH)_2$
MG-363	H.HCl	—		L—Leu	$(OH)_2$
MG-364	Phenethyl-C(O)	—	—	L—Leu	$(OH)_2$
MG-366	H.HCl	—		L—Leu	$(OH)_2$
MG-368	(2-Pyz)—C(O)	—	L-(2-Pal)	L—Leu	$(OH)_2$
MG-369	H.HCl	—		L—Leu	$(OH)_2$
MG-380	(8-Quin)SO_2	—	L—Phe	L—Leu	$(OH)_2$
MG-382	(2-Pyz)—C(O)	—	L-(4-F)-Phe	L—Leu	$(OH)_2$
MG-383	(2-Pyr)—C(O)	—	L-(4-F)-Phe	L—Leu	$(OH)_2$

Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
$P-AA^1-AA^2-AA^3-B(Z^1)(Z^2)$

Compound	P[a]	AA^1	AA^{2b}	AA^{3c}	Z^1, Z^2
MG-385	H.HCl	—		L—Leu	$(OH)_2$
MG-386	H.HCl	—		L—Leu	$(OH)_2$
MG-387	Morph	—		L—Leu	$(OH)_2$

[a]Cbz = carbobenzyloxy; MS = methylsulfonyl; Morph = 4-morpholinecarbonyl; (8-Quin)-SO_2 = 8-quinolinesulfonyl; (2-Quin)-C(O) = 2-quinolinecarbonyl; Bz = benzoyl; (2-Pyr)—C(O) = 2-pyridinecarbonyl; (3-Pyr)—C(O) = 3-pyridinecarbonyl; (2-Pyz)—C(O) = 2-pyrazinecarbonyl.
[b]Nal = β-(1-naphthyl)alanine; (2-Nal) = β-(2-naphthyl)alanine; (2-Pal) = β-(2-pyridyl)alanine; (3-Pal) = β-(3-pyridyl)alanine; homoPhe = homophenylalanine; (4-F)-Phe = (4-fluorophenyl)alanine.
[c]$B(Z^1)(Z^2)$ takes the place of the carboxyl group of AA^3.

In another embodiment, compounds of formula (1a) or (2a) described above are selected from the following compounds as well as pharmaceutically acceptable salts and boronate esters thereof:

N-(4-morpholine)carbonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid,

N-(8-quinoline)sulfonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid,

N-(2-pyrazine)carbonyl-L-phenylalanine-L-leucine boronic acid,

L-proline-L-leucine boronic acid,

N-(2-quinoline)carbonyl-L-homophenylalanine-L-leucine boronic acid,

N-(3-pyridine)carbonyl-L-phenylalanine-L-leucine boronic acid,

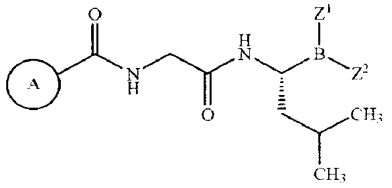
N-(3-phenylpropionyl)-L-phenylalanine-L-leucine boronic acid,

N-(4-morpholine)carbonyl-L-phenylalanine-L-leucine boronic acid,

N-(4-morpholine)carbonyl-(O-benzyl)-L-tyrosine-L-leucine boronic acid, N-(4-morpholine)carbonyl-L-tyrosine-L-leucine boronic acid, and

N-(4-morpholine)carbonyl-[O-(2-pyridylmethyl)]-L-tyrosine-L-leucine boronic acid.

In one embodiment, for the CDP-proteasome inhibitor conjugates described in any one of 1st to 15th embodiments wherein the proteasome inhibitor contains a boronic acid or derivative thereof, $RB(OH)_2$ or $RB(Y)_2$ is represented by the formula (3b):

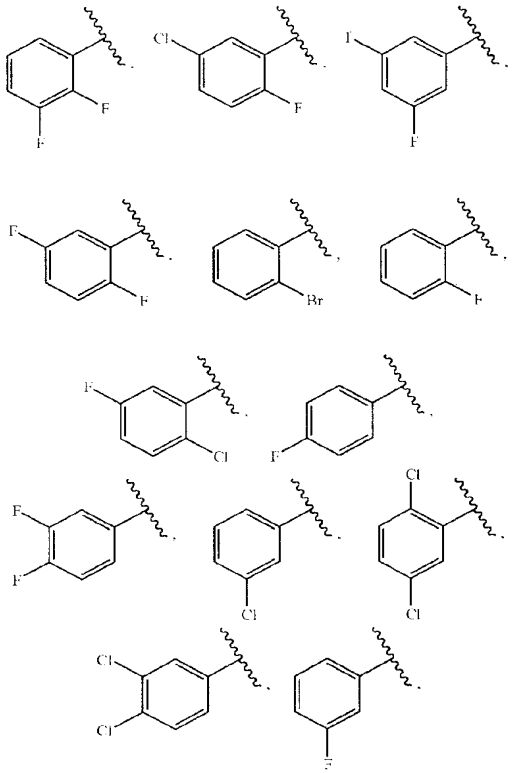


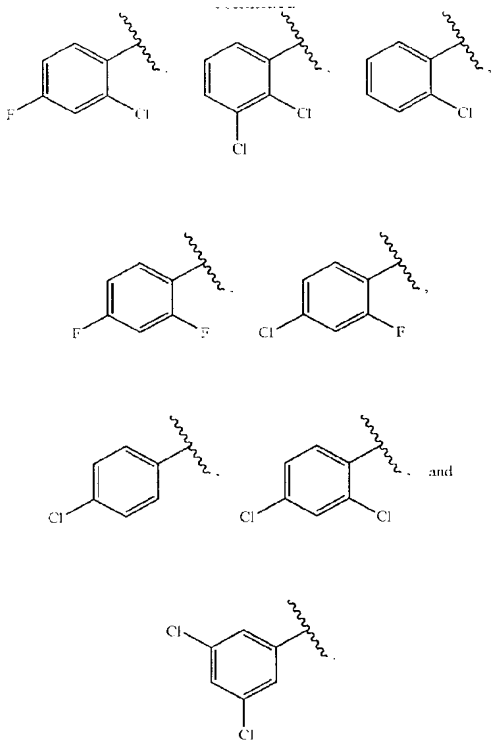
(3b)

or a pharmaceutically acceptable salt or boronic acid anhydride thereof, wherein:

Z^1 and Z^2 are each independently hydroxy, alkoxy, aryloxy, or aralkoxy; or Z^1 and Z^2 together form a moiety derived from a boronic acid completing agent; and

Ring A is selected from the group consisting of:





More specifically, compounds of formula (3b) are referred to by the following chemical names:

I-1 [(1R)-1-({[(2,3-difluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-2 [(1R)-1-({[(5-chloro-2-fluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-3 [(1R)-1-({[(3,5-difluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-4 [(1R)-1-({[(2,5-difluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-5 [(1R)-1-({[(2-bromobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-6 [(1R)-1-({[(2-fluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-7 [(1R)-1-({[(2-chloro-5-fluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-8 [(1R)-1-({[(4-fluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-9 [(1R)-1-({[(3,4-difluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-10 [(1R)-1-({[(3-chlorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-11 [(1R)-1-({[(2,5-dichlorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-12 [(1R)-1-({[(3,4-dichlorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-13 [(1R)-1-({[(3-fluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-14 [(1R)-1-({[(2-chloro-4-fluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-15 [(1R)-1-({[(2,3-dichlorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-16 [(1R)-1-({[(2-chlorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-17 [(1R)-1-({[(2,4-difluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

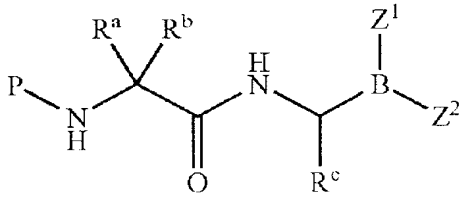
I-18 [(1R)-1-({[(4-chloro-2-fluorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-19 [(1R)-1-({[(4-chlorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-20 [(1R)-1-({[(2,4-dichlorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid

I-21 [(1R)-1-({[(3,5-dichlorobenzoyl)amino]acetyl}amino)-3-methylbutyl]boronic acid.

In another embodiment, for the CDP-proteasome inhibitor conjugates described in any one of the 1st to 15th embodiments (below) wherein the proteasome inhibitor contains a boronic acid or derivative thereof, $RB(OH)_2$ or $RB(Y)_2$ is represented by formula (4a):



(4a)

or a pharmaceutically acceptable salt or boronic acid anhydride thereof, wherein:

P is hydrogen or an amino-group-blocking moiety;

R^a is a C_{1-4} aliphatic or C_{1-4} fluoroaliphatic group that is substituted with 0-1 R^A; or R^a and R^b taken together with the carbon atom to which they are attached, form a substituted or unsubstituted 3- to 6-membered cycloaliphatic group;

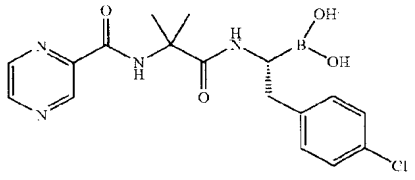
R^A is a substituted or unsubstituted aromatic or cycloaliphatic ring;

R^b is a C_{1-4} aliphatic or C_{1-4} fluoroaliphatic group; or R^a and R^b, taken together with the carbon atom to which they are attached, form a substituted or unsubstituted 3- to 6-membered cycloaliphatic group;

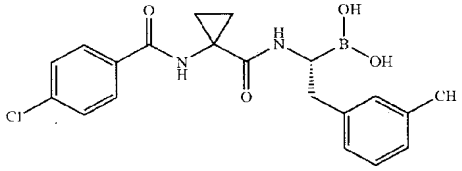
R^c is a C_{1-4} aliphatic or C_{1-4} fluoroaliphatic group that is substituted with 0-1 R^C;

R^C is a substituted or unsubstituted aromatic or cycloaliphatic ring; and Z^1 and Z^2 are each independently hydroxy, alkoxy, aryloxy, or aralkoxy; or Z^1 and Z^2 together form a moiety derived from a boronic acid complexing agent.

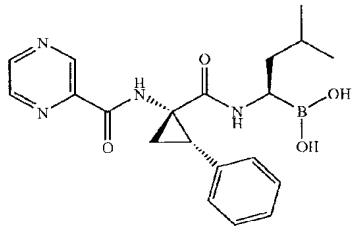
Representative examples of compounds of formula (4a), wherein Z^1 and Z^2 are each –OH are shown as the following:



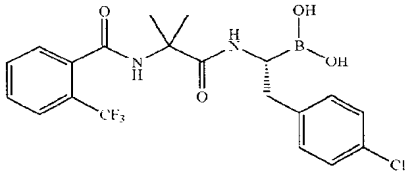
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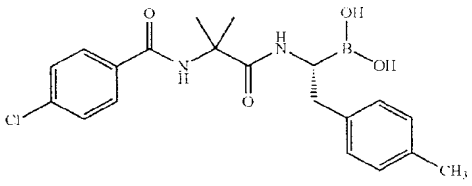
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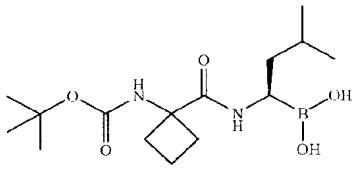
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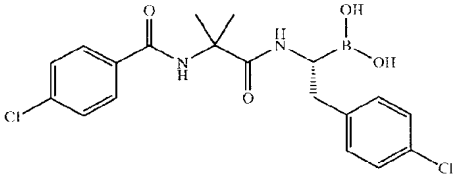
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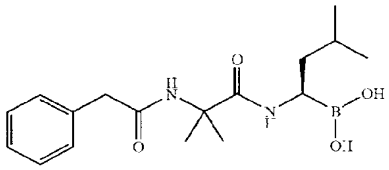
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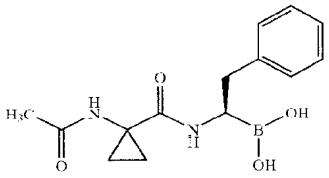
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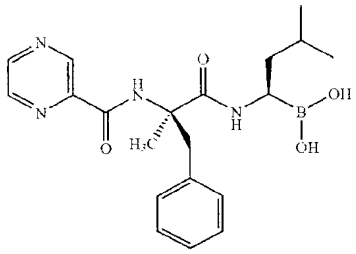
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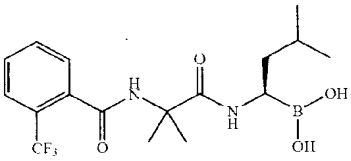
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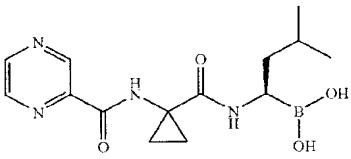
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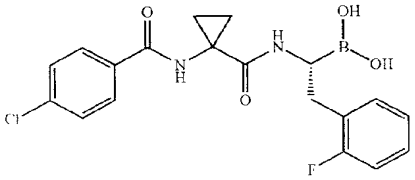
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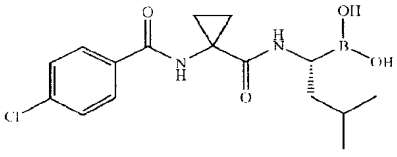
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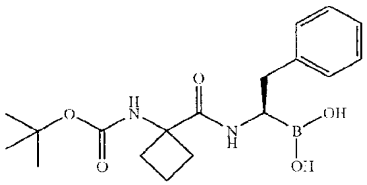
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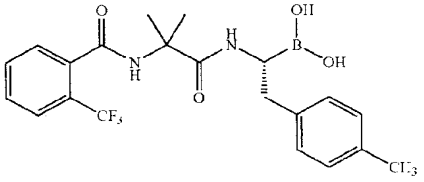
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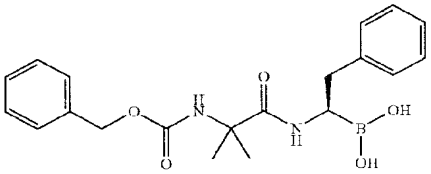
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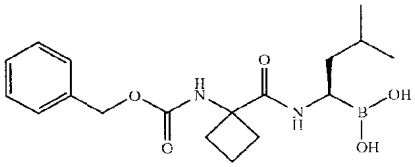
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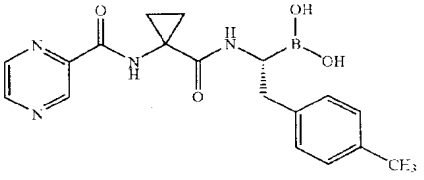
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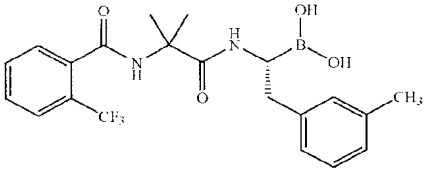
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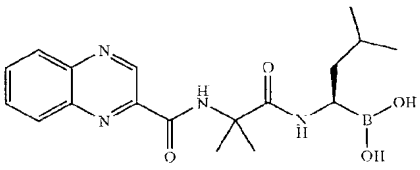
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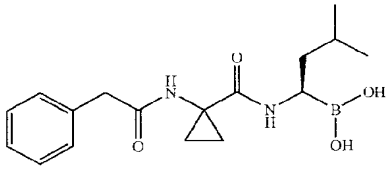
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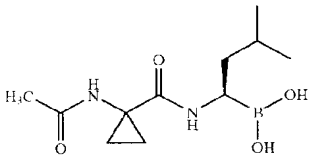
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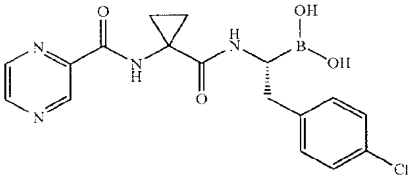
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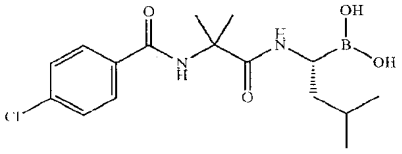
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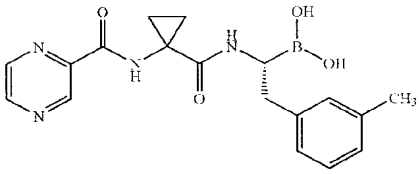
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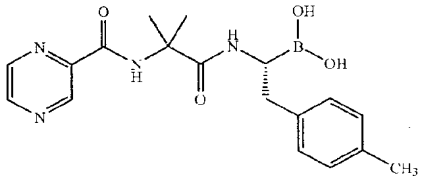
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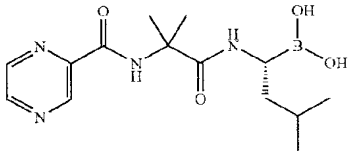
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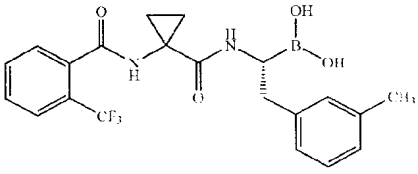
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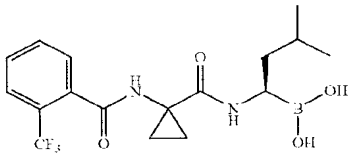
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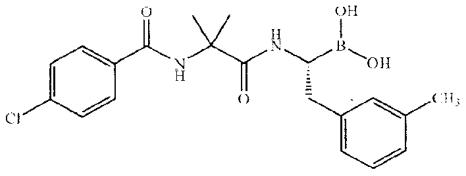
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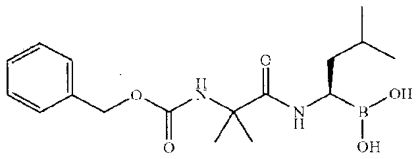
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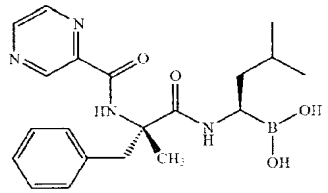
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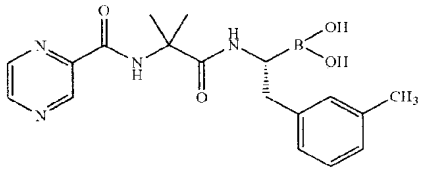
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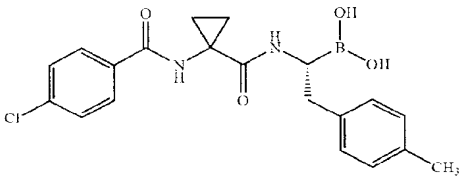
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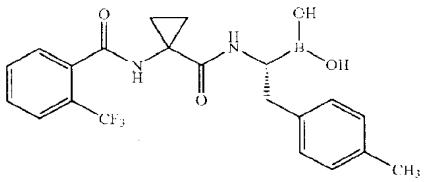
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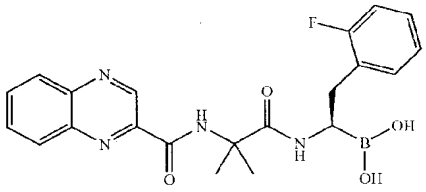
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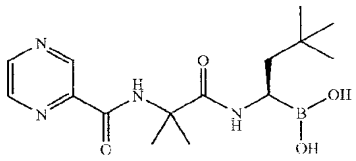
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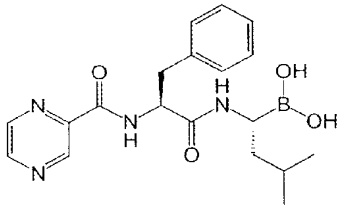


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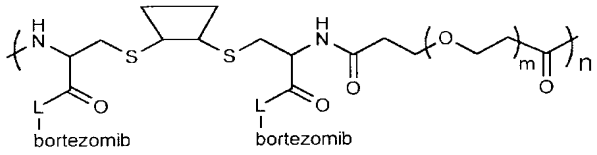


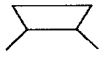
38

In preferred embodiments, the proteasome inhibitor in the CDP-proteasome inhibitor conjugate, particle or composition comprises a boronic acid containing molecule, e.g., a boronic acid containing molecule described herein, e.g., bortezomib;

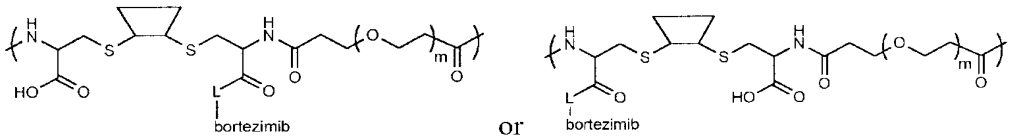


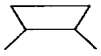
In one embodiment, the CDP-proteasome inhibitor conjugate is a CDP-bortezomib conjugate, e.g.,



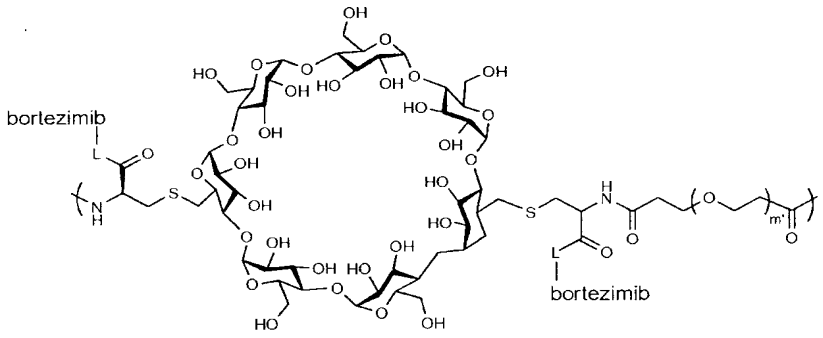
wherein  represents a cyclodextrin; n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an

integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); and "L-bortezemib" is a bortezemib-linker moiety, e.g., a bortezemib-linker moiety described herein, e.g., a bortezemib-linker moiety described in FIG. 7. In some embodiments, the CDP-proteasome inhibitor conjugate, e.g., the CDP-bortezomib conjugate, does not have complete loading, e.g., one or more binding sites, e.g., cysteine residues, are not bound to a proteasome inhibitor, e.g., a bortezomib moiety, bound with a linker described herein, e.g., the CDP-bortezemib conjugate comprises one or more subunits having the formulae provided below:



wherein  represents a cyclodextrin; m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); and "L-bortezemib" is a bortezemib-linker moiety, e.g., a bortezemib-linker moiety described herein, e.g., a bortezemib-linker moiety described in FIG. 7. In some embodiments, the CDP-proteasome inhibitor conjugate, particle or composition e.g., the CDP-bortezomib conjugate, particle or composition, comprises a mixture of fully-loaded and partially-loaded CDP-proteasome inhibitor conjugates, e.g., CDP-bortezomib conjugates.

In one embodiment, the CDP-proteasome inhibitor conjugate comprises a subunit of

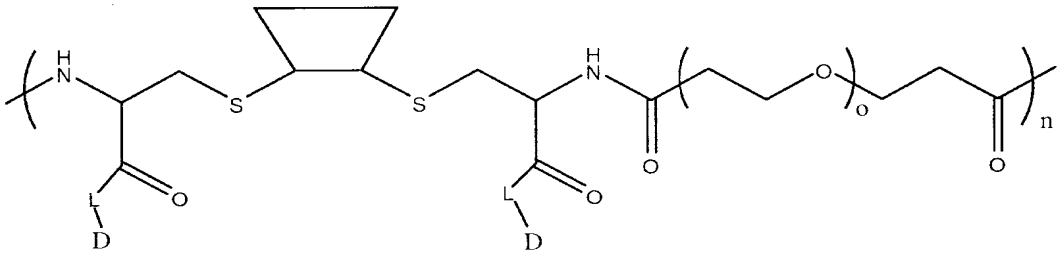


wherein m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); and "L-

bortezemib" is a bortezemib-linker moiety, e.g., a bortezemib-linker moiety described herein, e.g., a bortezemib-linker moiety described in FIG. 7.

The CDP-proteasome inhibitor conjugate (such as a boronic acid containing proteasome inhibitor) of the invention comprises a proteasome inhibitor (such as a boronic acid containing proteasome inhibitor, e.g., bortezomib) covalently linked to a CDP described herein. In one embodiment, the proteasome inhibitor is a pharmaceutically active agent, preferably comprises a boronic acid moiety or a boronic acid derivative described herein.

In the 1st embodiment, the CDP-proteasome inhibitor conjugate is formula (K) below:



wherein:

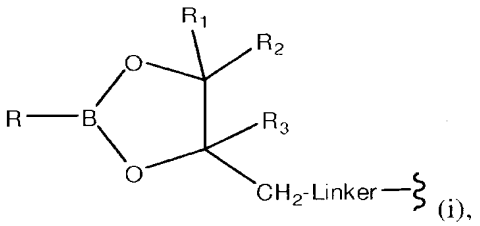
n is an integer from 1 to 100;

o is an integer from 1 to 1000;

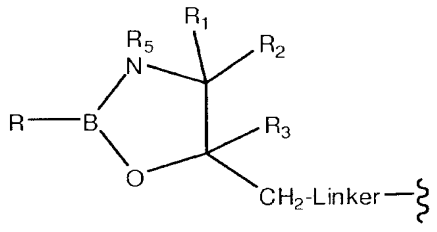
L is a linker described in Formulas (I)-(VIII); and

D is –B-R, wherein R is as described in $RB(OH)_2$ or $RB(Y)_2$ described above.

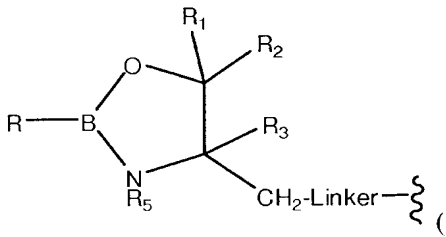
In another embodiment, the L-D moiety in formula (K) is represented by the following formula:



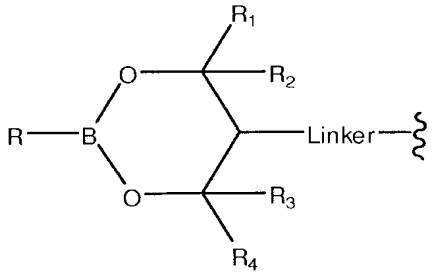
(i),


R1
R5
R2
N
R
B
R3
O
CH2-Linker

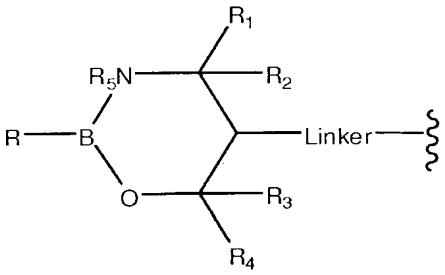
(ii),


R1
R2
O
R
B
R3
N
R5
CH2-Linker

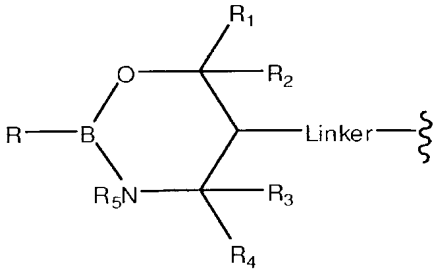
(iii),


R1
O
R2
R
B
Linker
O
R3
R4

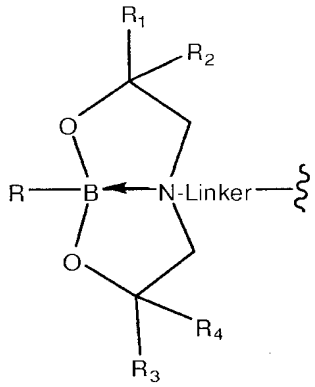
(iv),


R1
R5N
R2
R
B
Linker
O
R3
R4

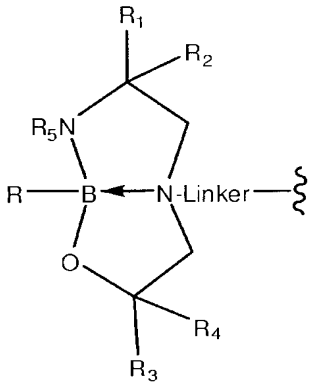
(v),


R1
O
R2
R
B
Linker
R5N
R3
R4

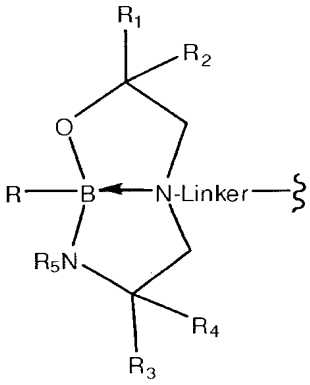
(vi),



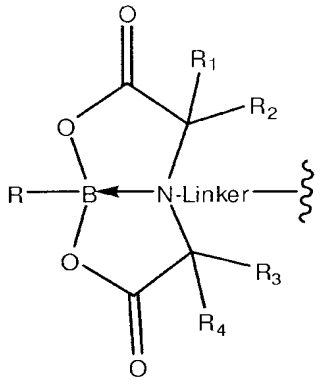
(vii),



(viii),



(ix), or



(x),

wherein:

R is the non-boronic acid moiety in R-$B(OH)_2$ or R is as described in a boronic acid derivative $RB(Y)_2$ described herein;

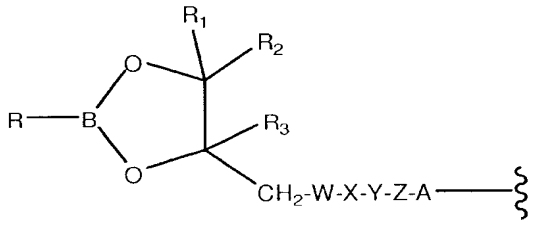
$RB(OH)_2$ is a pharmaceutically active agent, preferably a proteasome inhibitor comprising a boronic acid moiety, such as bortezomib;

$RB(Y)_2$ is a pharmaceutically active agent, preferably a proteasome inhibitor such as a proteosome inhibitor comprising a boronic acid derivative;

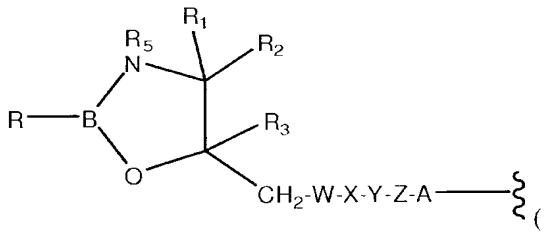
R_1, R_2, R_3, R_4 and R_5 are each independently –H or a (C_1-C_5)alkyl;

Linker is a linker group comprising an amino terminal group.

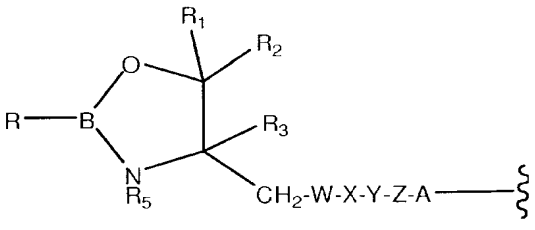
In a 2nd embodiment, for CDP-proteasome inhibitor conjugate represented by formulas (K), the L-D moiety is represented by a formula selected from:



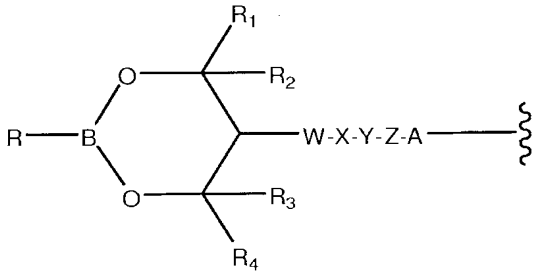
(ia),



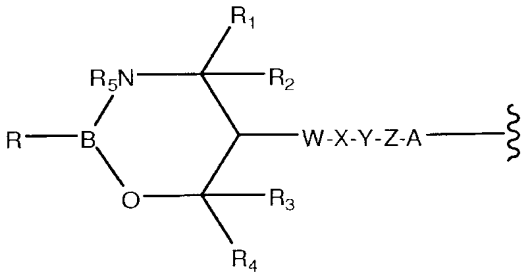
(iia),



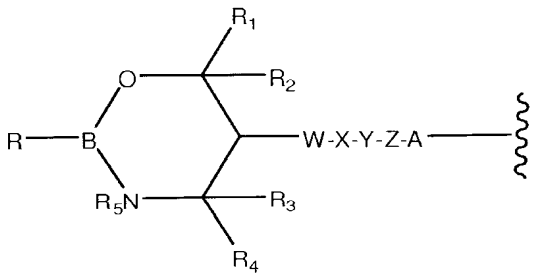
(iiia),



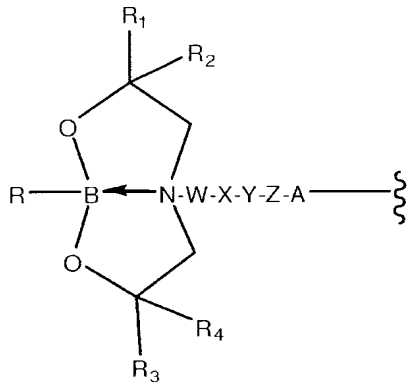
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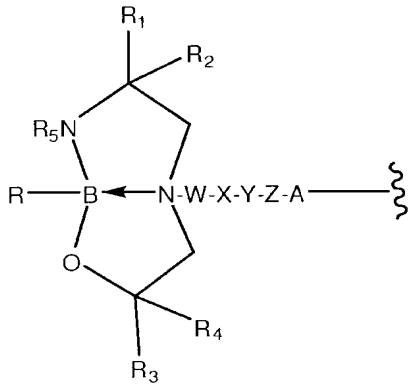
(va),



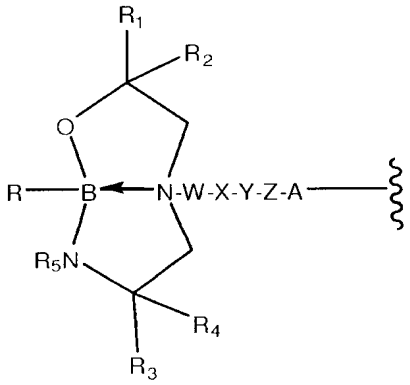
(via),



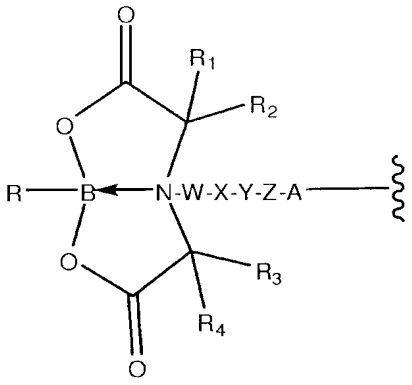
(viia),



(viiia),



(ixa), and



(xa),

wherein:

R_1, R_2, R_3, R_4 and R_5 are each independently –H or a (C_1-C_5)alkyl;

R is as described in $RB(OH)_2$ or $RB(Y)_2$ described above;

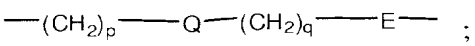
W is -$(CH_2)_m$-, -O- or –$N(R_5')$-, when the polymer-agent conjugate is represented by structural formulas (ia)-(via); or

W is -$(CH_2)_m$-, when the polymer-agent conjugate is represented by structural formulas (viia)-(xa);

X is a bond when W is –$(CH_2)_m$- and X is –C(=O)- when W is -O-, or –$N(R_5')$;

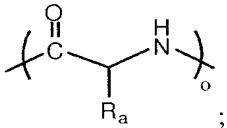
Y is a bond, -O-, or –$N(R_5')$-;

Z is represented by the following structural formula:



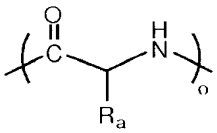
;

E is a bond, aryl (e.g., phenyl) or heteroaryl (e.g., pyridyl, furyl or furanyl, imidazolyl, benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinolinyl, indolyl and thiazolyl);

Q is a bond, -O-, –$N(R_5')$-, -$N(R_5')$-C(=O)-O-, -O-C(=O)-$N(R_5')$-, -OC(=O)-, -C(=O)-O-, -S-S-, -$(O\text{-}CH_2\text{-}CH_2)_n$- or 

;

R_a is a side chain of a naturally occurring amino acid or an analog thereof;



A is –N(R_5')-, or A is a bond when Q is and q is 0;

R_5' is –H or (C_1-C_6)alkyl;

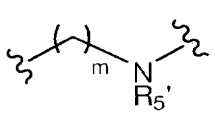
m, p, q are each an integer from 0 to 10;

n is an integer from 1 to 10; and

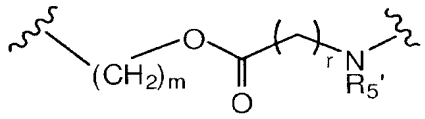
o is an integer from 1 to 10, provided when Y is -O- or –N(R_5')- and Q is -O-, -N(R_5')-, -(O-CH_2-CH_2)$_n$-, -N(R_5')-C(=O)-O-, -O-C(=O)-N(R_5')-, -OC(=O)- or -S-S-, then p is an integer from 2 to 10; when Q is -O-, –N(R_5')-, -N(R_5')-C(=O)-O-, -O-C(=O)-N(R_5')-, -OC(=O)-, -C(=O)-O-, or -S-S- and E is a bond, then q is an integer from 2 to 10; when Y is -O- or –N(R_5')-, Q and E are both a bond, then p+q ≥ 2; when W is –O- or -N(R_5')-, Y, Q and E are all bond, then p+q ≥ 1; and when W is –O- or -N(R_5')-, Y is a bond, and Q is -N(R_5')-C(=O)-O-, -O-C(=O)-N(R_5')-, -OC(=O)-, -C(=O)-O-, -S-S- or -(O-CH_2-CH_2)$_n$-, then p is an integer from 2 to 10.

In one embodiment, Z is a bond or –(CH_2)$_r$-, wherein r is an integer from 1 to 10.

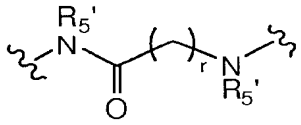
In a 3rd embodiment, for CDP-proteasome inhibitor conjugate described in the 2nd embodiment, the linker (i.e. -W-X-Y-Z-A) is represented by any one of the following formula:



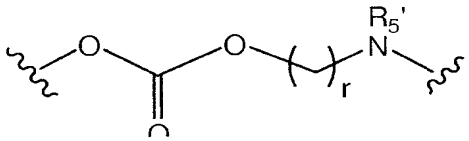
(a);



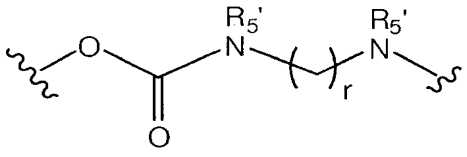
(b);



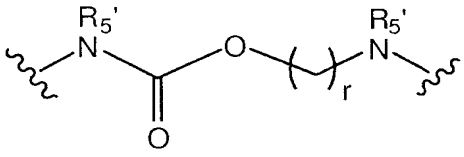
(c);



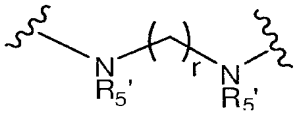
(d);



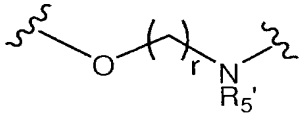
(e);



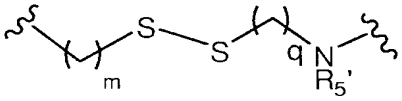
(f);



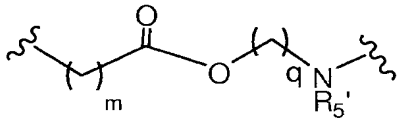
(g);



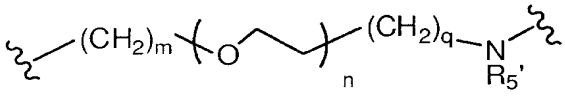
(h);



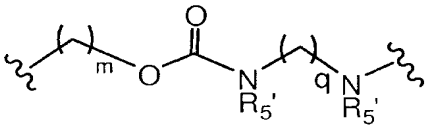
(i);



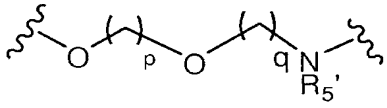
(j);



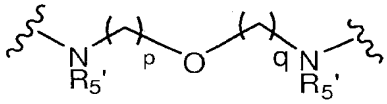
(k);



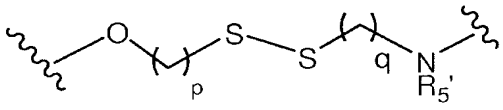
(l);



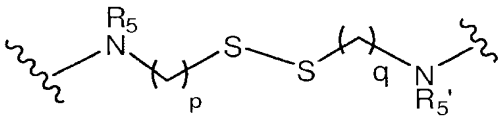
(m);



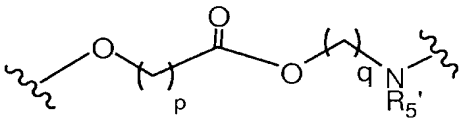
(n);



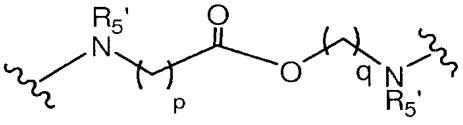
(o);



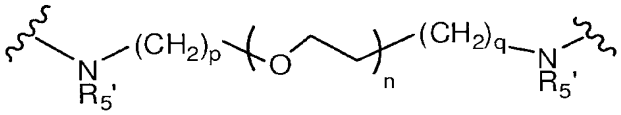
(p);



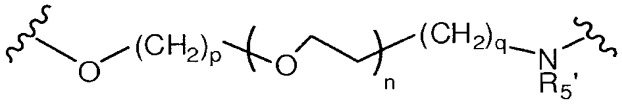
(q);



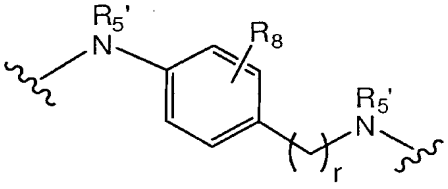
(r);



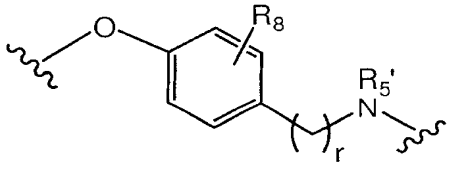
(s);



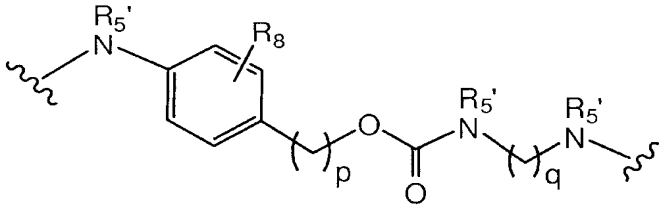
(t);



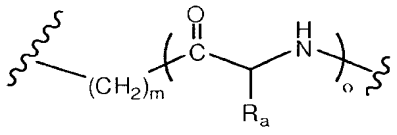
(u);



(v);



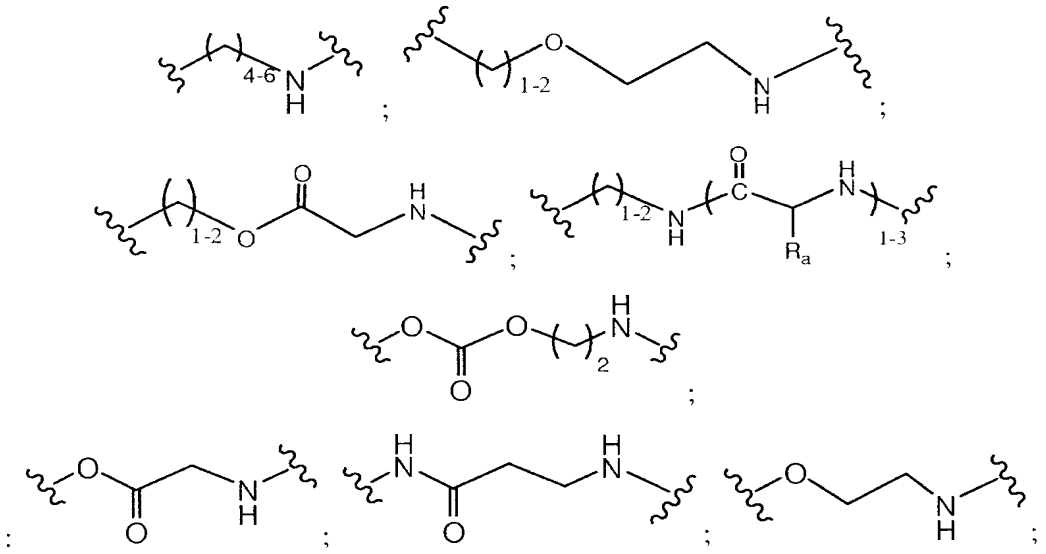
(w);

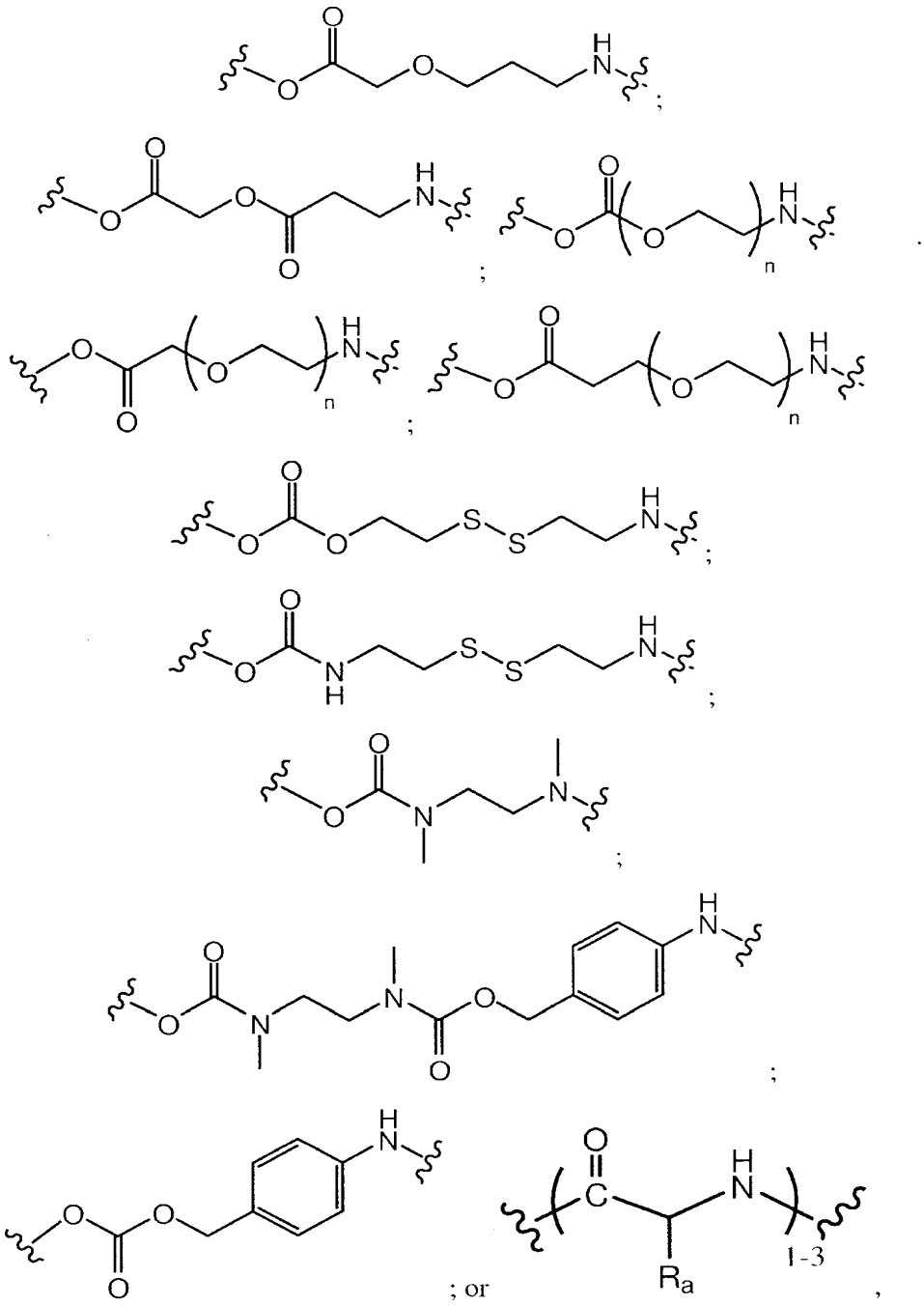


(x),

wherein R_5' is –H or (C_1-C_6)alkyl; R_a is a side chain of a naturally occurring amino acid or an analog thereof; R_8 is a substituent; n is an integer from 1 to 10; r is an integer from 1 to 10; m, p and q are each an integer from 0 to 10; and o is an integer from 1 to 10. For formulas (d)-(h), r is an integer from 2 to 10. For formulas (i), (j) and (l), q is an integer from 2 to 10. For formulas (m)-(p), p and q are each an integer from 2 to 10. For formulas (q) and (r), p is an integer from 1 to 10 and q is an integer from 2 to 10. For formulas (s) and (t), p is an integer from 2 to 10. For formula (w), q is an integer from 2 to 10. More specifically, R_8 is selected from H, halo, -CN, -NO_2, -OH, (C_1-C_6)alkyl, halo(C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, (C_1-C_6)alkoxy, halo(C_1-C_6)alkoxy, (C_1-C_3)alkoxy(C_1-C_3)alkyl and –NR_9R_{10}; wherein R_9 and R_{10} are each independently H, (C_1-C_6)alkyl, halo(C_1-C_6)alkyl, (C_1-C_6)alkoxy, halo(C_1-C_6)alkoxy, (C_1-C_3)alkoxy(C_1-C_3)alkyl.

In a 4th embodiment, for CDP-proteasome inhibitor conjugate described in the 3rd embodiment, the linker (i.e., -W-X-Y-Z-A) is represented by any one of the following formulas:





wherein n is an integer from 2 to 5; and R_a is a side chain of a naturally occurring amino acid or an analog thereof.

In a 5th embodiment, for the CDP-proteasome inhibitor conjugate described in the

1st embodiment, the linker is represented by formulas (AA1), (BB1) or (CC1):

$$-(CH_2)_m-O-CH_2-O-(CH_2)_q-N(R_5)- \quad (AA1),$$

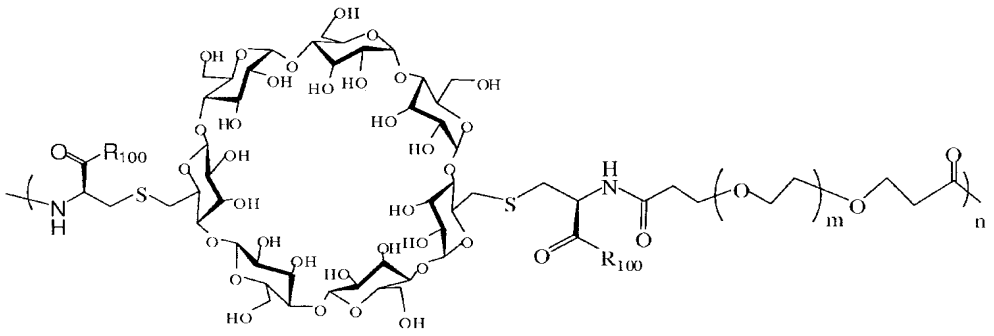
$$-(CH_2)_m-O-(CH_2)_p-O-CH_2-N(R_5)- \quad (BB1)$$

$$-(CH_2)_m-(CH_2)_p- O-CH_2-N(R_5)- \quad (CC1)$$

wherein m is an integer from 0 to 10; q is an integer from 2 to 10; p is an integer from 0 to 10 for structural formula (CC1) and p is an integer from 2 to 10 for structural formula (BB1).

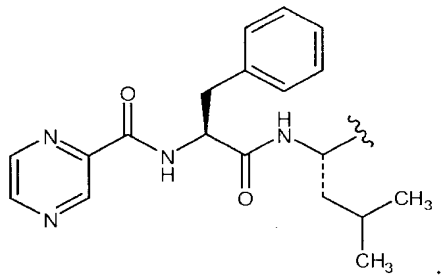
In a 6th embodiment, for CDP-proteasome inhibitor conjugate of formula (K) described in the 1st embodiment, the L-D moiety is as described in FIG. 7.

In a 7th embodiment, the CDP-proteasome inhibitor conjugate is represented by the following formula:

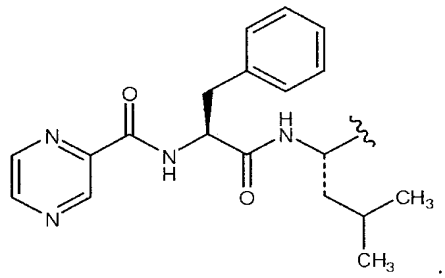


(M),

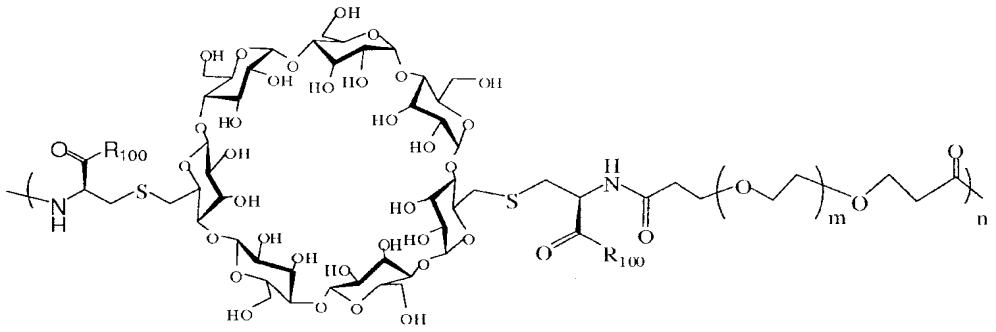
wherein n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); and R_{100} is –OH or a group comprising a –B-R moiety, wherein R is as described in $RB(OH)_2$ or $RB(Y)_2$ described above. At least one R_{100} in the conjugate is a group comprising a –B-R moiety. Alternatively, the conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by a group comprising a –B-R moiety per repeat unit. In one embodiment, at least one R_{100} in the conjugate is a group comprising a –B-R moiety and R is represented by the following structural formula:



Alternatively, the conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by a group comprising a –B-R moiety per repeat unit and R is represented by the following structural formula:



In a 8th embodiment, the CDP-proteasome inhibitor conjugate is represented by formula (M):

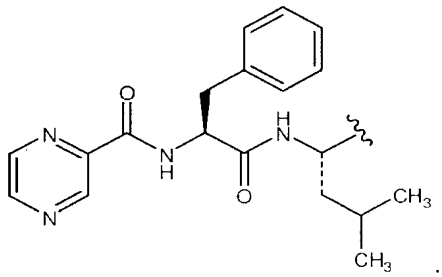


(M),

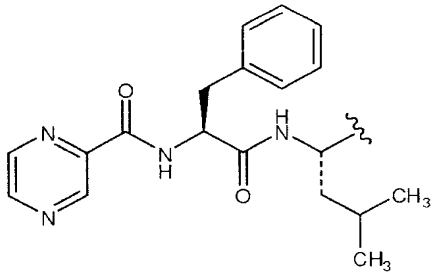
wherein n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); R_{100} is –OH or a group represented by a formula selected from formulas (i)-(x). At least one R_{100} group

in the conjugate is a group represented by a formula selected from formulas (i)-(x). Alternatively, the conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by a formula selected from formulas (i)-(x) per repeat unit.

In a 9th embodiment, for the CDP-proteasome inhibitor conjugate represented by formula (M), n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); R_{100} is –OH or a group represented by a formula selected from formulas (i)-(x). At least one R_{100} group in the conjugate is a group represented by a formula selected from formulas (i)-(x); and R in formulas (i)-(x) is as described in $RB(OH)_2$ or $RB(Y)_2$ described above. More specifically, at least one R_{100} group in the conjugate is a group represented by a formula selected from formulas (i)-(x); and R in formulas (i)-(x) is represented by the following structural formula:

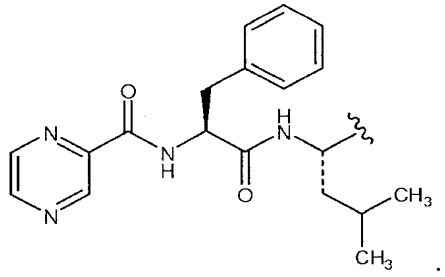


Alternatively, the CDP-proteasome inhibitor conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by a formula selected from formulas (i)-(x) per repeat unit; and R in formulas (i)-(x) is as described in $RB(OH)_2$ or $RB(Y)_2$ described above. More specifically, the CDP-proteasome inhibitor conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by a formula selected from formulas (i)-(x) per repeat unit; and R in formulas (i)-(x) is represented by the following structural formula:

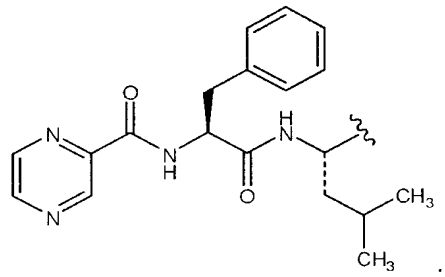


In a 10th embodiment, for the CDP-proteasome inhibitor conjugate represented by formula (M), n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); R_{100} is –OH or a group represented by a formula selected from formulas (ia)-(xa). At least one R_{100} group in the conjugate is a group represented by a formula selected from formulas (ia)-(xa). Alternatively, the conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by a formula selected from formulas (ia)-(xa) per repeat unit.

In a 11th embodiment, for the CDP-proteasome inhibitor conjugate represented by formula (M), n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); R_{100} is –OH or a group represented by a formula selected from formulas (ia)-(xa). At least one R_{100} group in the conjugate is a group represented by a formula selected from formulas (ia)-(xa); and R in formulas (ia)-(xa) is as described in $RB(OH)_2$ or $RB(Y)_2$ described above. More specifically, at least one R_{100} group in the conjugate is a group represented by a formula selected from formulas (ia)-(xa); and R in formulas (i)-(x) is represented by the following structural formula:



Alternatively, the CDP-proteasome inhibitor conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by a formula selected from formulas (ia)-(xa) per repeat unit; and R in formulas (ia)-(xa) is as described in $RB(OH)_2$ or $RB(Y)_2$ described above. More specifically, the CDP-proteasome inhibitor conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by a formula selected from formulas (ia)-(xa) per repeat unit; and R in formulas (ia)-(xa) is represented by the following structural formula:



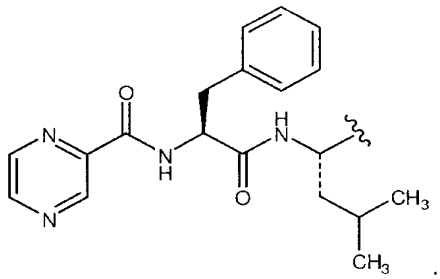
In a 12th embodiment, for the CDP-proteasome inhibitor conjugate represented by formula (M), n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); R_{100} is –OH or a group represented by formula (ia). At least one R_{100} group in the conjugate is a group represented by formula (1a) and the group -W-X-Y-Z-A in R_{100} represented by formula (ia) is represented by a formula selected from formulas (a)-(x) described in the 3rd embodiment and formulas (AA1), (BB1) and (CC1) described in the 5th embodiment.

Alternatively, the CDP-proteasome inhibitor conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by formula (ia) per repeat unit; and the group -W-X-Y-Z-A in R_{100} represented by formula (ia) is represented by a formula selected from formulas (a)-(x) described in the 3rd embodiment and formulas (AA1), (BB1) and (CC1) described in the 5th embodiment.

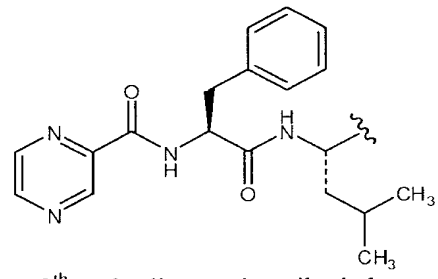
Alternatively, in the 12th embodiment described above, R_{100} is represented by formula (iia) instead of formula (ia). Alternatively, in the 12th embodiment described above, R_{100} is represented by formula (iiia) instead of formula (ia). Alternatively, in the 12th embodiment above, R_{100} is represented by formula (iva) instead of formula (ia). Alternatively, in the 12th embodiment described above, R_{100} is represented by formula (va) instead of formula (ia). Alternatively, in the 12th embodiment described above, R_{100} is represented by formula (via) instead of formula (ia). Alternatively, in the 12th embodiment described above, R_{100} is represented by formula (viia) instead of formula (ia). Alternatively, in the 12th embodiment described above, R_{100} is represented by formula (viiia) instead of formula (ia). Alternatively, in the 12th embodiment described above, R_{100} is represented by formula (ixa) instead of formula (ia). Alternatively, in the 12th embodiment described above, R_{100} is represented by formula (xa) instead of formula (ia).

In a 13th embodiment, for the CDP-proteasome inhibitor conjugate represented by formula (M), n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); R_{100} is -OH or a group represented by (ia). At least one R_{100} group in the conjugate is a group represented by (ia); the group -W-X-Y-Z-A in formula (ia) is represented by a formula selected from formulas (a)-(x) described in the 3rd embodiment and formulas (AA1), (BB1) and (CC1) described in the 5th embodiment; and R in R_{100} represented by formula (ia) is as describe in $RB(OH)_2$ or $RB(Y)_2$ described above. More specifically, at least one R_{100} group in the conjugate is a group represented by formula (ia); the group -W-X-Y-Z-A in R_{100} represented by formula (ia) is represented by a formula selected

from formulas (a)-(x) described in the 3rd embodiment and formulas (AA1), (BB1) and (CC1) described in the 5th embodiment; and R in R_{100} represented by formula (ia) is represented by the following structural formula:



Alternatively, the CDP-proteasome inhibitor conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by formula (ia) per repeat unit; the group -W-X-Y-Z-A in R_{100} represented by formula (ia) is represented by a formula selected from formulas (a)-(x) described in the 3rd embodiment and formulas (AA1), (BB1) and (CC1) described in the 5th embodiment; and R in R_{100} represented by formula (ia) is as described in $RB(OH)_2$ or $RB(Y)_2$ described above. More specifically, the CDP-proteasome inhibitor conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by formula (ia) per repeat unit; the group -W-X-Y-Z-A in R_{100} represented by formula (ia) is represented by a formula selected from formulas (a)-(x) described in the 3rd embodiment and formulas (AA1), (BB1) and (CC1) described in the 5th embodiment; and R in R_{100} represented by formula (ia) is represented by the following structural formula:



Alternatively, in the 13th embodiment described above, R_{100} is represented by formula (iia) instead of formula (ia). Alternatively, in the 13th embodiment described above, R_{100} is represented by formula (iiia) instead of formula (ia). Alternatively, in the

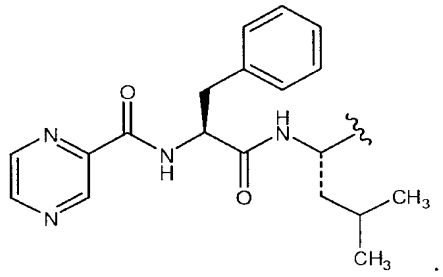
13th embodiment above, R_{100} is represented by formula (iva) instead of formula (ia). Alternatively, in the 13th embodiment described above, R_{100} is represented by formula (va) instead of formula (ia). Alternatively, in the 13th embodiment described above, R_{100} is represented by formula (via) instead of formula (ia). Alternatively, in the 13th embodiment described above, R_{100} is represented by formula (viia) instead of formula (ia). Alternatively, in the 13th embodiment described above, R_{100} is represented by formula (viiia) instead of formula (ia). Alternatively, in the 13th embodiment described above, R_{100} is represented by formula (ixa) instead of formula (ia). Alternatively, in the 13th embodiment described above, R_{100} is represented by formula (xa) instead of formula (ia).

In a 14th embodiment, for the CDP-proteasome inhibitor conjugate represented by formula (M), n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); R_{100} is –OH or a group represented by formula (ia). At least one R_{100} group in the conjugate is a group represented by formula (ia) and the group -W-X-Y-Z-A in R_{100} represented by formula (ia) is represented by a formula selected from the formulas described in the 4th embodiment. Alternatively, the CDP-proteasome inhibitor conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by formula (ia) per repeat unit; and the group -W-X-Y-Z-A in R_{100} represented by formula (ia) is represented by a formula selected from the formulas described in the 4th embodiment.

Alternatively, in the 14th embodiment described above, R_{100} is represented by formula (iia) instead of formula (ia). Alternatively, in the 14th embodiment described above, R_{100} is represented by formula (iiia) instead of formula (ia). Alternatively, in the 14th embodiment above, R_{100} is represented by formula (iva) instead of formula (ia). Alternatively, in the 14th embodiment described above, R_{100} is represented by formula (va) instead of formula (ia). Alternatively, in the 14th embodiment described above, R_{100} is represented by formula (via) instead of formula (ia). Alternatively, in the 14th embodiment described above, R_{100} is represented by formula (viia) instead of formula

(ia). Alternatively, in the 14th embodiment described above, R_{100} is represented by formula (viiia) instead of formula (ia). Alternatively, in the 14th embodiment described above, R_{100} is represented by formula (ixa) instead of formula (ia). Alternatively, in the 14th embodiment described above, R_{100} is represented by formula (xa) instead of formula (ia).

In a 15th embodiment, for the CDP-proteasome inhibitor conjugate represented by formula (M), n is an integer from 1 to 100 (e.g., n is an integer from 4 to 80, from 4 to 50, from 4 to 30 or from 4 to 20, or n is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20); m is an integer from 1 to 1000 (e.g., m is an integer from 1 to 200, from 1 to 100, from 1 to 80, from 2 to 80, from 5 to 70, from 10 to 50, or from 20 to 40); R_{100} is –OH or a group represented by formula (ia). At least one R_{100} group in the conjugate is a group represented by formula (ia); the group -W-X-Y-Z-A in R_{100} represented by formula (ia) is represented by a formula selected from the formulas described in the 4th embodiment; and R in R_{100} represented by formula (ia) is as described in $RB(OH)_2$ or $RB(Y)_2$ described above. More specifically, at least one R_{100} group in the conjugate is a group represented by formula (ia); the group -W-X-Y-Z-A in R_{100} represented by formula (ia) is represented by a formula selected from the formulas described in the 4th embodiment; and R in R_{100} represented by formulas (ia) is represented by the following structural formula:



Alternatively, the CDP-proteasome inhibitor conjugate represented by formula (M) comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2.0 R_{100} groups represented by formula (ia) per repeat unit; the group -W-X-Y-Z-A in R_{100} represented by formula (ia) is represented by a formula selected from the formulas described in the 4th embodiment; and R in R_{100} represented by formula (ia) is as described in $RB(OH)_2$ or $RB(Y)_2$ described above. More specifically, the CDP-proteasome inhibitor conjugate represented by formula (M) comprises at least 0.1, 0.2,

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JUMBO APPLICATIONS / PATENTS

THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.

THIS IS VOLUME __1___ OF __2___

NOTE: For additional volumes please contact the Canadian Patent Office.

CLAIMS:

1. A cyclodextrin-containing polymer (CDP)-therapeutic agent conjugate for use in a method of treating a disease or disorder associated with an immune response in a subject, wherein the CDP-therapeutic agent conjugate is a CDP-cytotoxic agent conjugate.

2. The CDP-therapeutic agent conjugate of claim 1, wherein the disease or disorder associated with an immune response is an autoimmune disease.

3. The CDP-therapeutic agent conjugate of claim 1, wherein the disease or disorder associated with an immune response is lupus.

4. The CDP-therapeutic agent conjugate of claim 1, wherein the disease or disorder associated with an immune response is an inflammatory disease.

5. The CDP-therapeutic agent conjugate of any one of claims 1 to 4, wherein the CDP-cytotoxic agent conjugate is selected from the group consisting of a CDP-topoisomerase II inhibitor conjugate, a CDP-anti-metabolic agent conjugate, a CDP-anti-folate agent conjugate, a CDP-alkylating agent conjugate, a CDP-anthracycline conjugate, a CDP-anti-tumor antibiotic conjugate, and a CDP-microtubule inhibitor conjugate.

6. The CDP-therapeutic agent conjugate of claim 5 wherein the CDP-microtubule inhibitor conjugate is a CDP-taxane conjugate.

7. The CDP-therapeutic agent conjugate of claim 5, wherein the CDP-microtubule inhibitor conjugate is a CDP-epothilone conjugate.

8. The CDP-therapeutic agent conjugate of any one of claims 1 to 7, wherein the CDP-therapeutic agent conjugate is formulated for administration to the subject in combination with a second therapeutic agent.

9. The CDP-therapeutic agent conjugate of claim 3, wherein the CDP-therapeutic agent conjugate is formulated for administration to the subject in combination with a second therapeutic agent, wherein the second therapeutic agent is one or more of the following agents:

(a) an anti-inflammatory agent;

(b) an anti-malarial agent;

(c) an immunomodulator;

(d) an anti-coagulant; and

(e) a hormone.

10. The CDP-therapeutic agent conjugate of claim 9, wherein the anti-inflammatory agent is: aspirin, acetaminophen, a non-steroidal anti-inflammatory drug, a corticosteroid, or any combination thereof.

11. The CDP-therapeutic agent conjugate of claim 9, wherein the anti-malarial agent is hydroxychloroquine, chloroquine, or any combination thereof.

12. The CDP-therapeutic agent conjugate of claim 9, wherein the immunomodulator is an immunomodulator with an intracellular target.

13. The CDP-therapeutic agent conjugate of claim 12, wherein the immunomodulator with an intracellular target is: an anti-metabolite, an IL-2 inhibitor, an mTor inhibitor, a TNF inhibitor, a macrolide, or any combination thereof.

14. The CDP-therapeutic agent conjugate of claim 13, wherein the anti-metabolite is a purine synthesis inhibitor, a pyrimidine synthesis inhibitor, or an anti-folate.

15. The CDP-therapeutic agent conjugate of claim 9, wherein the immunomodulator is an immunomodulator with a cellular receptor target.

16. The CDP-therapeutic agent conjugate of claim 15, wherein the immunomodulator with a cellular receptor target is: an IL-1 receptor inhibitor; an antibody which inhibits the function of the cellular receptor target, or any combination thereof.

17. The CDP-therapeutic agent conjugate of claim 16, wherein the antibody which inhibits the function of a cellular receptor target is an anti-CD3 antibody, an anti-CD4 antibody, an anti-CD5 antibody, an anti-CD11a antibody, anti-BLyS antibody, an anti-CD20 antibody, an anti-CD22 antibody, an anti-CD23 antibody, an anti-CD40 antibody, an anti-CD62L antibody, an anti-CD80 antibody, an anti-CD147 antibody, an anti-CD154 antibody, an anti-CAT antibody, an anti-integrin antibody, a CTLA4 antibody, an anti-IL6 receptor antibody, an anti-LFA1 antibody, an anti-IL2 antibody, or an anti-human T cell antibody.

18. The CDP-therapeutic agent conjugate of claim 9, wherein the immunomodulator is an immunomodulator with a serum target.

19. The CDP-therapeutic agent conjugate of claim 18, wherein the immunomodulator with a serum target is an antibody which inhibits the function of the serum target.

20. The CDP-therapeutic agent conjugate of claim 19, wherein the antibody which inhibits the function of the serum target is an anti-BLyS antibody, an anti-IL5 antibody, anti-IL6 antibody, and anti-interferon alpha antibody, an anti-IgE antibody, an anti-C5a antibody, an anti-TNF antibody, anti-IL10 antibody, anti-IL12 antibody, or an anti-IL13 antibody.

21. The CDP-therapeutic agent conjugate of claim 20, wherein the anti-BLyS antibody is belimumab.

22. The CDP-therapeutic agent conjugate of claim 9, wherein the immunomodulator is a biologic that interferes with immune cell function.

23. The CDP-therapeutic agent conjugate of claim 9, wherein the immunomodulator is a macrolide.

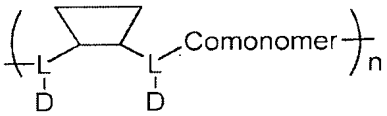
24. The CDP-therapeutic agent conjugate of claim 9, wherein the anti-coagulant is aspirin, heparin, or warfarin.

25. The CDP-therapeutic agent conjugate of claim 9, wherein the hormone is an androgen, a gonadotropin-hormone releasing agonist, or any combination thereof.

26. The CDP-therapeutic agent conjugate of any one of claims 1 to 25, wherein the CDP-therapeutic agent conjugate is in the form of a nanoparticle.

27. A cyclodextrin-containing polymer (CDP)-therapeutic agent conjugate, wherein said therapeutic agent is an anti-metabolic agent.

28. The CDP-therapeutic agent conjugate of claim 27, wherein the CDP-therapeutic agent conjugate is formed of repeating units of the following formula:



wherein

each L is independently a linker;

each D is independently an anti-metabolic agent or is absent;

each Comonomer is independently a comonomer; and

n is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20;

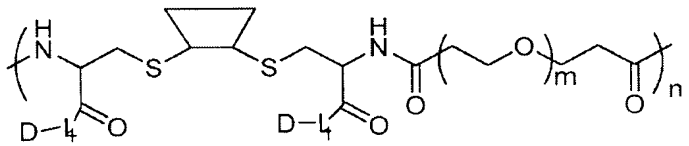
provided that the polymer comprises at least one anti-metabolic agent.

29. The CDP-therapeutic agent conjugate of claim 28, wherein each Comonomer is independently a comonomer comprising a polyethylene glycol chain.

30. The CDP-therapeutic agent conjugate of claim 28, wherein the molecular weight of each Comonomer is from about 2000 to about 5000 Da.

31. The CDP-therapeutic agent conjugate of claim 30, wherein the molecular weight of each Comonomer is from about 3000 to about 4000 Da.

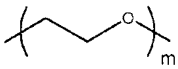
32. The CDP-therapeutic agent conjugate of claim 28, wherein said repeating units are of the following formula:



wherein

D and n are as defined in claim 33;

each L_1 is independently a linker, absent or, when D is absent, -OH; and

the group  has a Mw of 5,000 Da or less.

33. The CDP-therapeutic conjugate of any one of claims 27 to 32, wherein said anti-metabolic agent is an anti-folate agent.

34. The CDP-therapeutic conjugate of claim 33, wherein said anti-folate agent is pemetrexed, floxuridine or raltitrexed.

35. The CDP-therapeutic agent conjugate of claim 34, wherein said anti-folate agent is pemetrexed.

36. The CDP-therapeutic conjugate of any one of claims 27 to 32, wherein said anti-metabolic agent is a pyrimidine analog.

37. The CDP-therapeutic conjugate of claim 36, wherein said pyrimidine analog is gemcitabine, fluorouracil (5FU), capecitabine or cytarabine.

38. The CDP-therapeutic conjugate of claim 37, wherein said pyrimidine analog is gemcitabine.

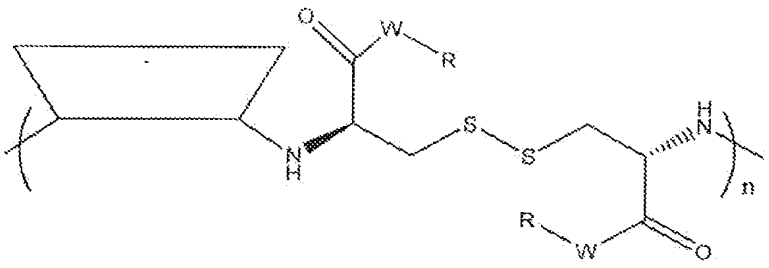
39. The CDP-therapeutic conjugate of claim 37, wherein said pyrimidine analog is fluorouracil (5FU).

40. A pharmaceutical composition comprising a CDP-therapeutic agent conjugate of any one of claims 27 to 39 and a pharmaceutically acceptable diluent or excipient.

41. A CDP-therapeutic agent conjugate of any one of claims 27 to 39, for use in a method of treating a proliferative disorder.

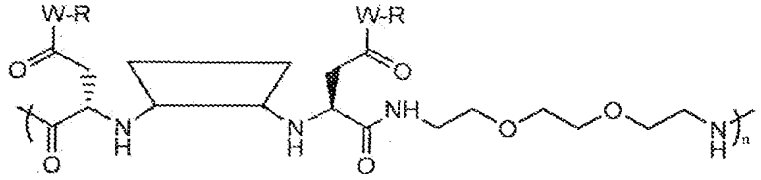
42. The CDP-therapeutic agent conjugate for use of claim 41, wherein the proliferative disorder is a cancer.

FIG. 1



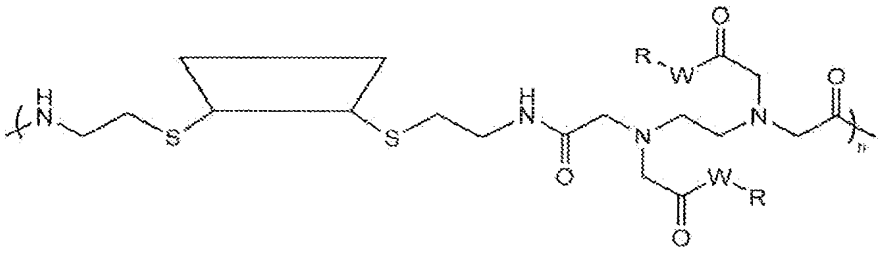
(A)

wherein n = 1 to 1000; R = therapeutic agent or OH; W = absent or a linker



(B)

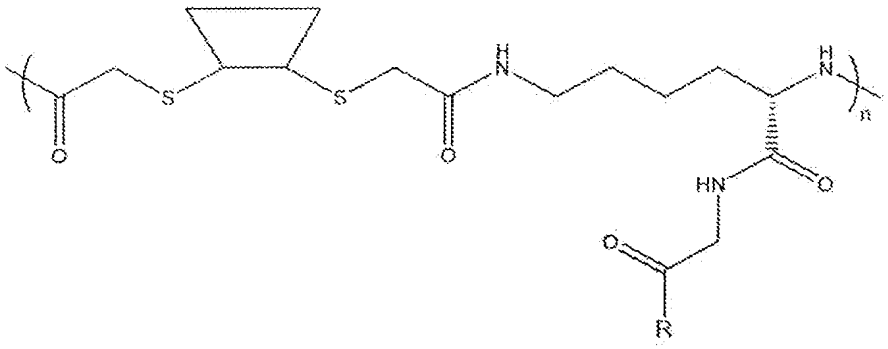
wherein n = 1 to 1000; R = therapeutic agent or OH; W = absent or a linker



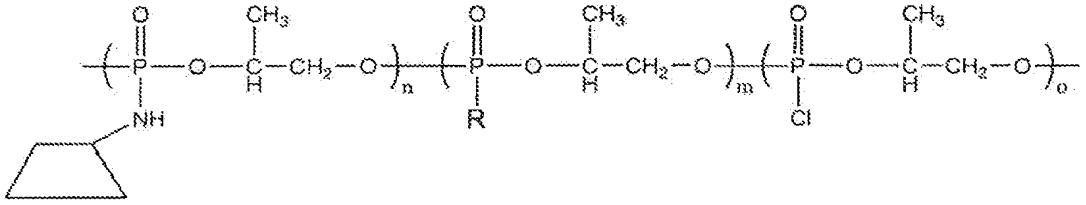
(C)

wherein n = 1 to 1000; R = therapeutic agent or OH; W = absent or a linker

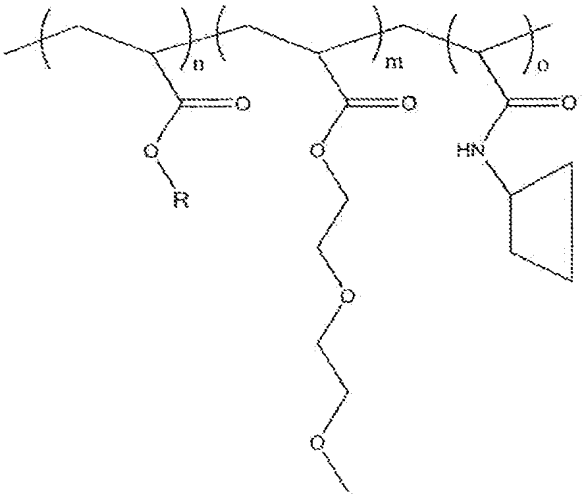
FIG. 1 (Cont'd)



(F) wherein n = 1 to 1000; R = therapeutic agent or OH

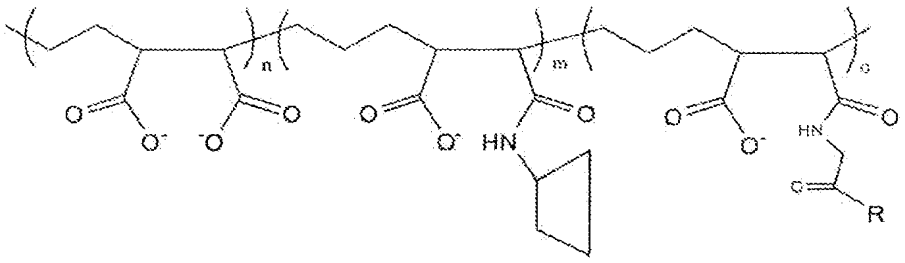


(G) wherein m, n, and o, independently, = 1 to 1000; R = therapeutic agent or OH

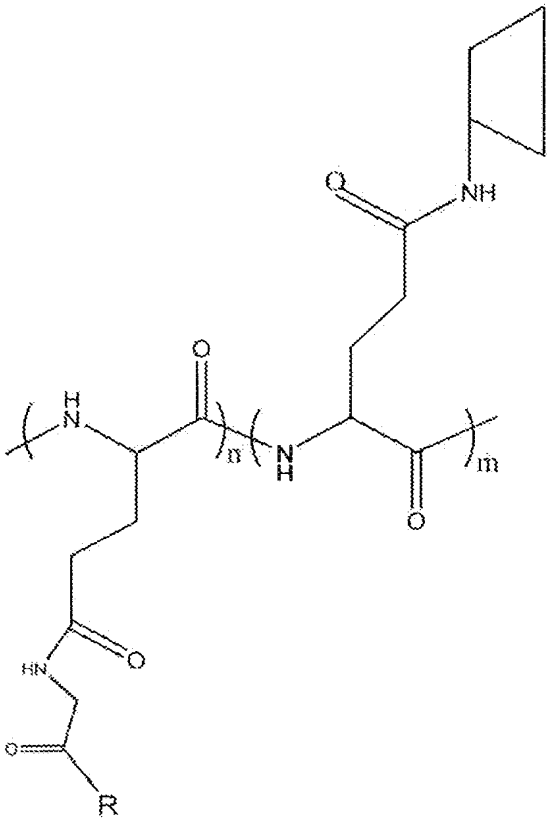


(H) wherein m, n, and o, independently, = 1 to 1000; R = therapeutic agent or OH

FIG. 1 (Cont'd)

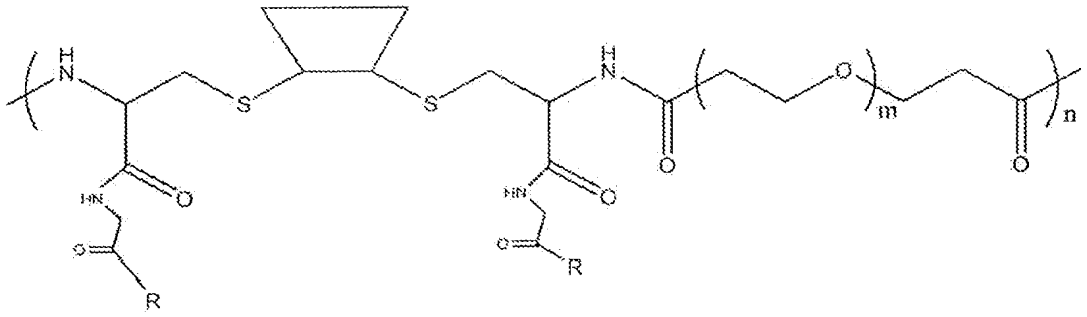


(I) wherein m, n, and o, independently, = 1 to 1000; R = therapeutic agent or OH

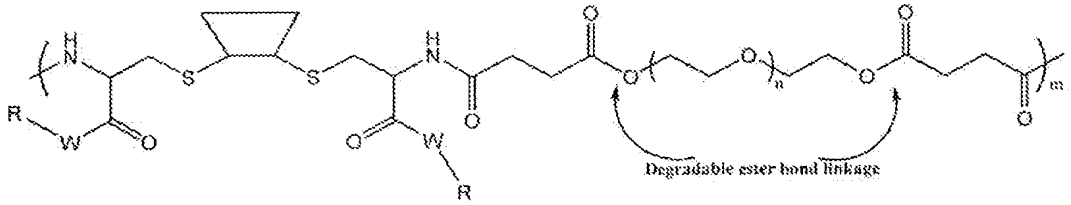


(J) wherein m and n, independently, = 1 to 1000; R = therapeutic agent or OH

FIG. 1 (Cont'd)



(K) wherein m and n, independently, = 1 to 1000; R = therapeutic agent or OH



(L) wherein m and n, independently, = 1 to 1000; R = therapeutic agent or OH; W = absent or a linker

FIG. 2

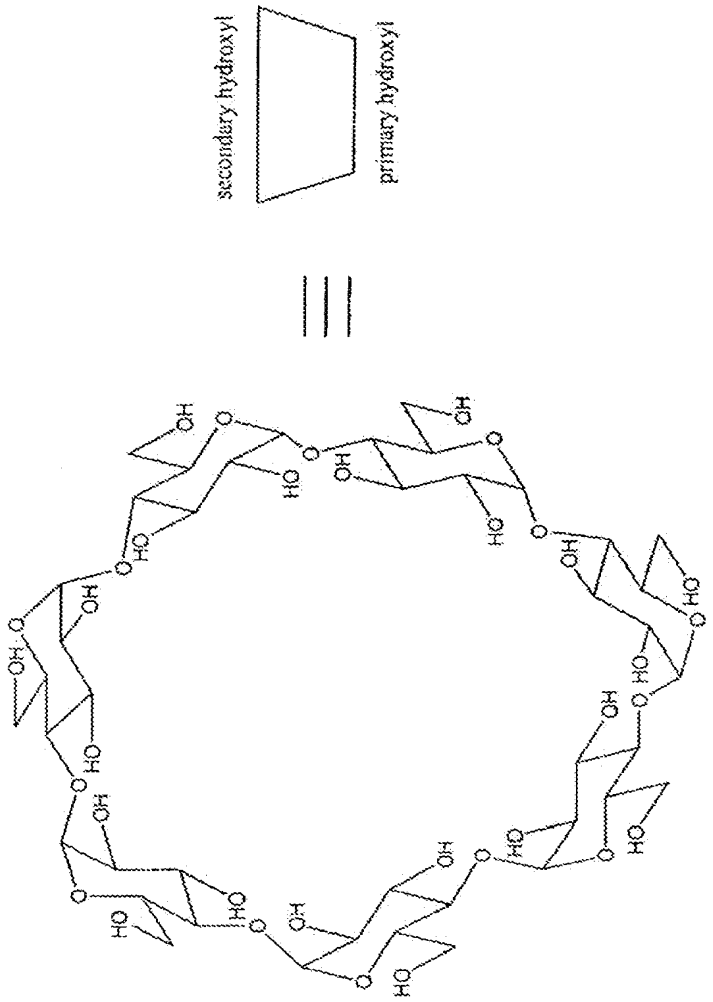
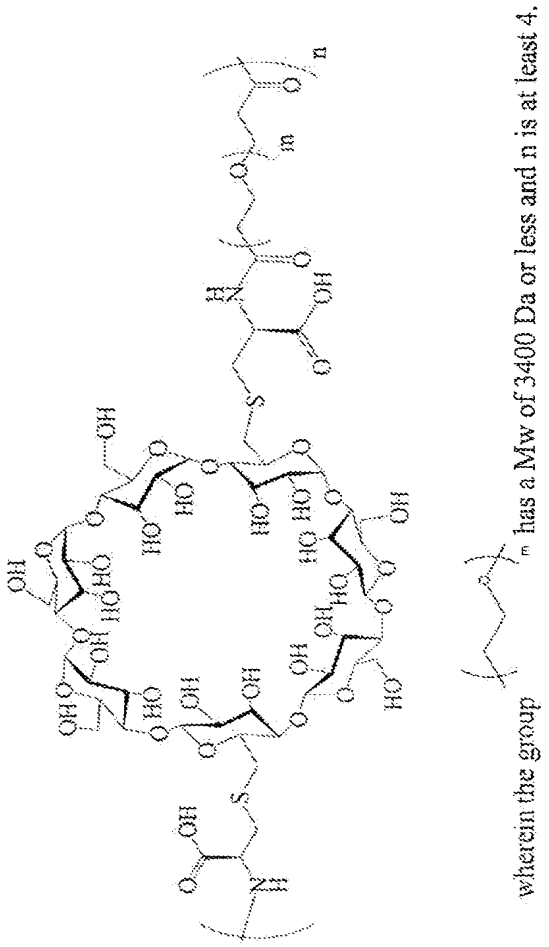


FIG 3



wherein the group has a Mw of 3400 Da or less and n is at least 4.

FIG. 4

Example	A	B	X	Taxane	Hydroxy Protecting Groups	Process for Preparation of taxane linker CDP molecules	Final Product
1.	-	-NH(CH$_2$)$_5$CO-	2'-OH	docetaxel	None	Process A	2'-docetaxel linked to CDP
2.	-	-NH(CH$_2$)$_5$CO-	7-OH	docetaxel	2' TBDMS or TROC	Process C	7-docetaxel linked to CDP
3.	-	-NH(CH$_2$)$_5$CO-	2'-OH	larotaxel	None	Process A	2'-larotaxel linked to CDP
4.	-	-NH(CH$_2$)$_5$CO-	2'-OH	cabazitaxel	None	Process A	2'-cabazitaxel linked to CDP
5.	-	-NH(CH$_2$)$_5$CO-	2'-OH	paclitaxel	None	Process A	2'-paclitaxel linked to CDP
6.	-	-NH(CH$_2$)$_5$CO-	7-OH	paclitaxel	2' TBDMS or TROC	Process C	7-paclitaxel linked to CDP
7.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	2'-OH	docetaxel	None	Process A	2'-docetaxel linked to CDP
8.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	7-OH	docetaxel	2' TBDMS or TROC	Process C	7-docetaxel linked to CDP
9.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	2'-OH	larotaxel	None	Process A	2'-larotaxel linked to CDP
10.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	2'-OH	cabazitaxel	None	Process A	2'-cabazitaxel linked to CDP
11.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	2'-OH	paclitaxel	None	Process A	2'-paclitaxel linked to CDP
12.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	7-OH	paclitaxel	2' TBDMS or TROC	Process C	7-paclitaxel linked to CDP
13.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	2'-OH	docetaxel	None	Process A	2'-docetaxel linked to CDP

FIG. 4 (cont'd)

Example	A	B	X	Taxane	Hydroxy Protecting Groups	Process for Preparation of taxane linker CDP molecules	Final Product
14.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	7-OH	docetaxel	2' TBDMS or TROC	Process C	7-docetaxel linked to CDP
15.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	2'-OH	larotaxel	None	Process A	2'-larotaxel linked to CDP
16.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	2'-OH	cabazitaxel	None	Process A	2'-cabazitaxel linked to CDP
17.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	2'-OH	paclitaxel	None	Process A	2'-paclitaxel linked to CDP
18.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	7-OH	paclitaxel	2' TBDMS or TROC	Process C	7-paclitaxel linked to CDP
19.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	2'-OH	docetaxel	None	Process A	2'-docetaxel linked to CDP
20.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	7-OH	docetaxel	2' TBDMS or TROC	Process C	7-docetaxel linked to CDP
21.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	2'-OH	larotaxel	None	Process A	2'-larotaxel linked to CDP
22.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	2'-OH	cabazitaxel	None	Process A	2'-cabazitaxel linked to CDP
23.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	2'-OH	paclitaxel	None	Process A	2'-paclitaxel linked to CDP
24.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	7-OH	paclitaxel	2' TBDMS or TROC	Process C	7-paclitaxel linked to CDP
25.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	2'-OH	docetaxel	None	Process B	2'-docetaxel linked to CDP
26.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	docetaxel	2' TBDMS or TROC	Process D	7-docetaxel linked to CDP
27.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	2'-OH	larotaxel	None	Process B	2'-larotaxel linked to CDP

FIG. 4 (cont'd)

Example	A	B	X	Taxane	Hydroxy Protecting Groups	Process for Preparation of taxane linker CDP molecules	Final Product
28.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	2'-OH	cabazitaxel	None	Process B	2'-cabazitaxel linked to CDP
29.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	2'-OH	paclitaxel	None	Process B	2'-paclitaxel linked to CDP
30.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	paclitaxel	2' TBDMS or TROC	Process D	7-paclitaxel linked to CDP
31.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	2'-OH	docetaxel	None	Process A	2'-docetaxel linked to CDP
32.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	7-OH	docetaxel	2' TBDMS or TROC	Process C	7-docetaxel linked to CDP
33.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	2'-OH	larotaxel	None	Process A	2'-larotaxel linked to CDP
34.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	2'-OH	cabazitaxel	None	Process A	2'-cabazitaxel linked to CDP
35.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	2'-OH	paclitaxel	None	Process A	2'-paclitaxel linked to CDP
36.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	7-OH	paclitaxel	2' TBDMS or TROC	Process C	7-paclitaxel linked to CDP
37.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	2'-OH	docetaxel	None	Process B	2'-docetaxel linked to CDP
38.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	docetaxel	2' TBDMS or TROC	Process D	7-docetaxel linked to CDP
39.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	2'-OH	larotaxel	None	Process B	2'-larotaxel linked to CDP
40.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	2'-OH	cabazitaxel	None	Process B	2'-cabazitaxel linked to CDP
41.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	2'-OH	paclitaxel	None	Process B	2'-paclitaxel linked to CDP

FIG. 4 (cont'd)

Example	A	B	X	Taxane	Hydroxy Protecting Groups	Process for Preparation of taxane linker CDP molecules	Final Product
42.	$-NH(CH_2)_nS-$	$-S(CH_2)_2OCO-$	7-OH	paclitaxel	2' TBDMS or TROC	Process D	7-paclitaxel linked to CDP
43.	-	$-NH(CH_2)_nCO-$ n is 1, 2, or 3	2'-OH	docetaxel	None	Process A	2'-docetaxel linked to CDP
44.	-	$-NH(CH_2)_nCO-$ n is 1, 2, or 3	7-OH	docetaxel	2' TBDMS or TROC	Process C	7-docetaxel linked to CDP
45.	-	$-NH(CH_2)_nCO-$ n is 1, 2, or 3	2'-OH	larotaxel	None	Process A	2'-larotaxel linked to CDP
46.	-	$-NH(CH_2)_nCO-$ n is 1, 2, or 3	2'-OH	cabazitaxel	None	Process A	2'-cabazitaxel linked to CDP
47.	-	$-NH(CH_2)_nCO-$ n is 1, 2, or 3	2'-OH	paclitaxel	None	Process A	2'-paclitaxel linked to CDP
48.	-	$-NH(CH_2)_nCO-$ n is 1, 2, or 3	7-OH	paclitaxel	2' TBDMS or TROC	Process C	7-paclitaxel linked to CDP
49.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	2'-OH	docetaxel	None	Process A	2'-docetaxel linked to CDP
50.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	docetaxel	2' TBDMS or TROC	Process C	7-docetaxel linked to CDP

FIG. 4 (cont'd)

Example	A	B	X	Taxane	Hydroxy Protecting Groups	Process for Preparation of taxane linker CDP molecules	Final Product
51.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	2'-OH	larotaxel	None	Process A	2'-larotaxel linked to CDP
52.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	2'-OH	cabazitaxel	None	Process A	2'-cabazitaxel linked to CDP
53.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	2'-OH	paclitaxel	None	Process A	2'-paclitaxel linked to CDP
54.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	paclitaxel	2' TBDMS or TROC	Process C	7-paclitaxel linked to CDP

FIG. 5

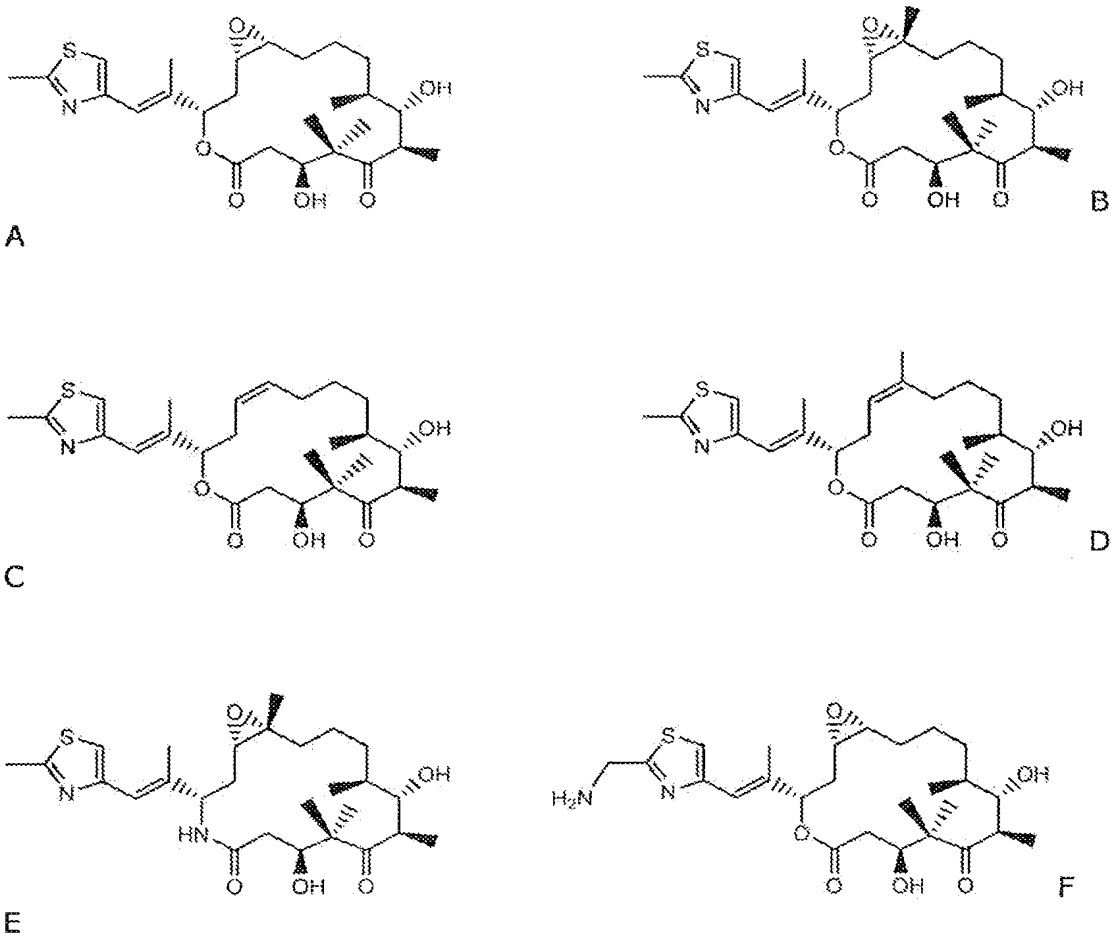
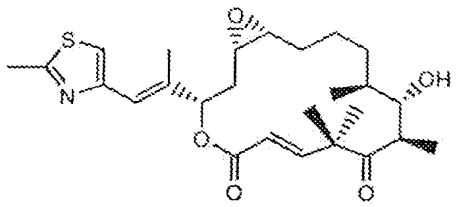
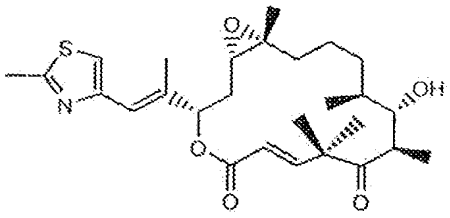


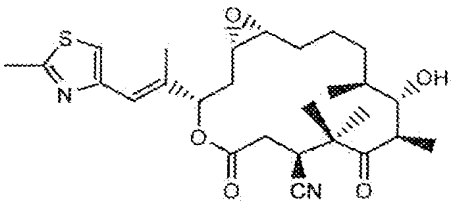
FIG. 5 (cont'd)



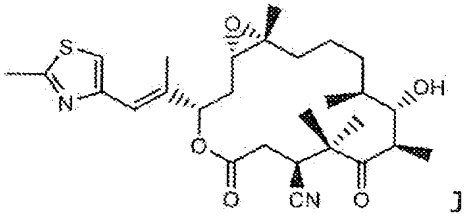
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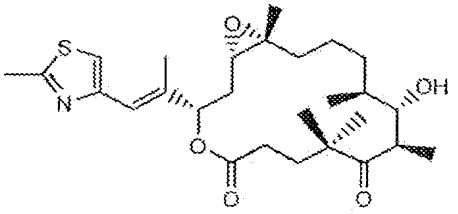
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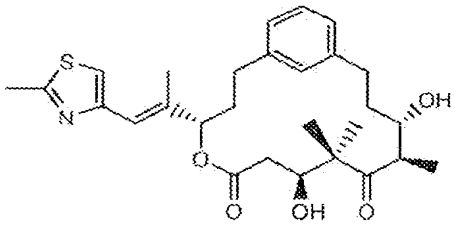
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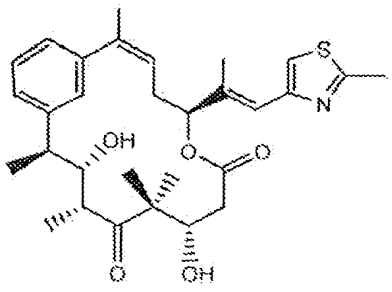
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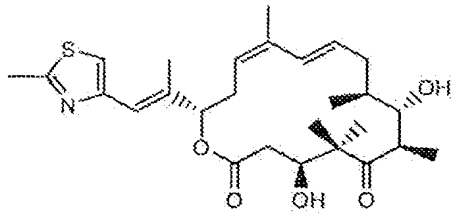


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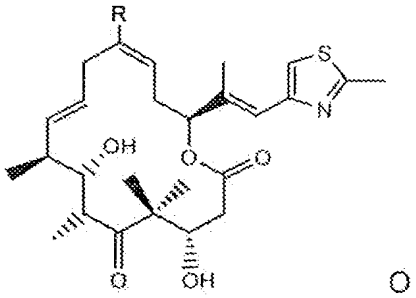


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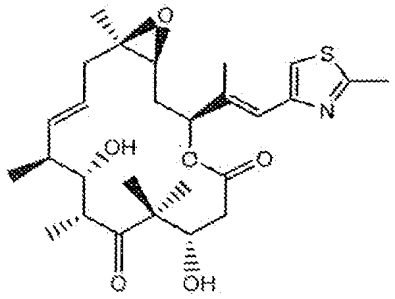
FIG. 5 (cont'd)



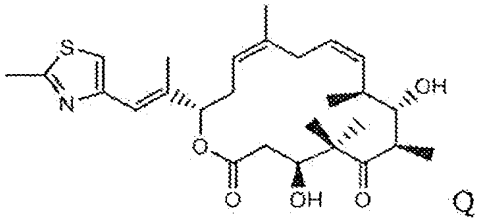
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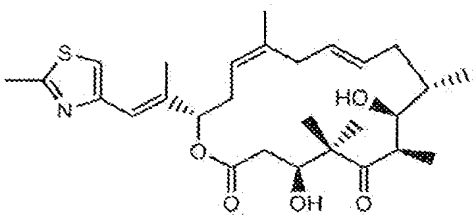
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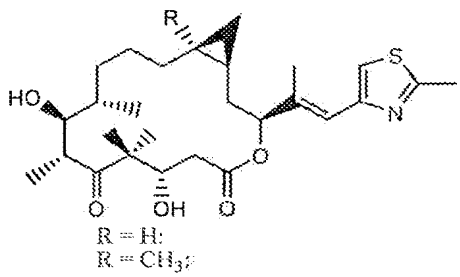


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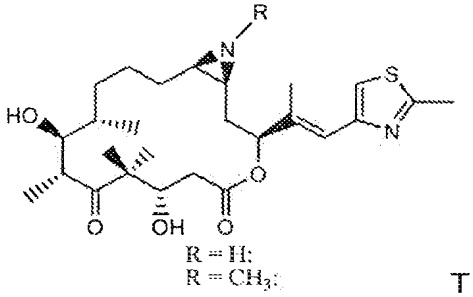


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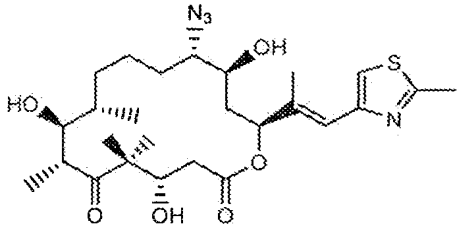
FIG. 5 (cont'd)



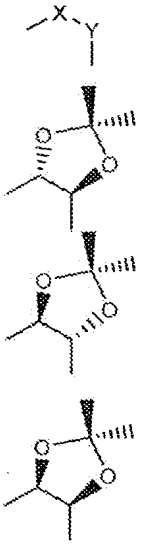
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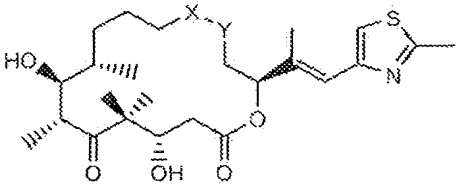


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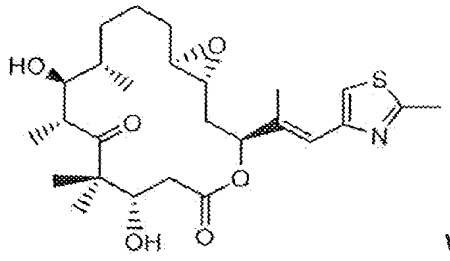
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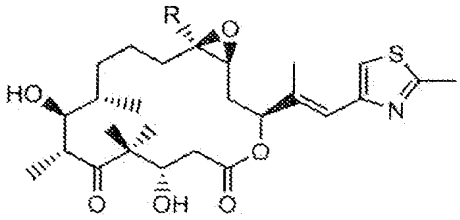


V

FIG. 5 (cont'd)

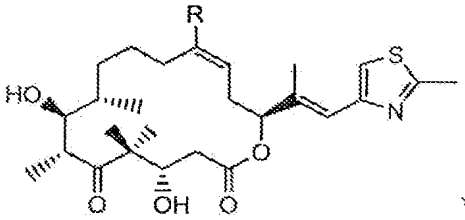


W



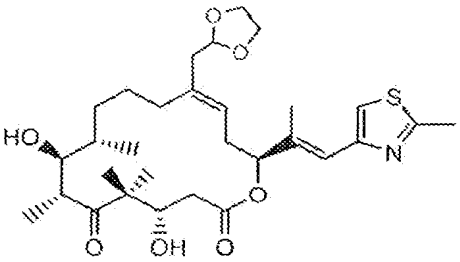
R = CH_2F, CH_2Cl, C_2H_5, n-C_3H_7

X

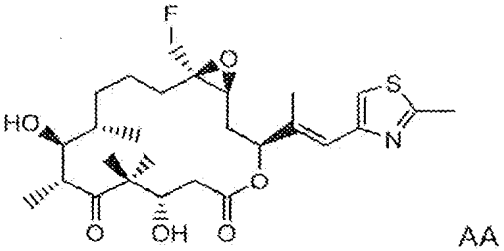


R = CH_2F, CH_2Cl, C_2H_5, n-C_3H_7

Y

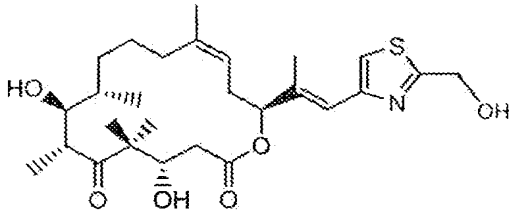


Z

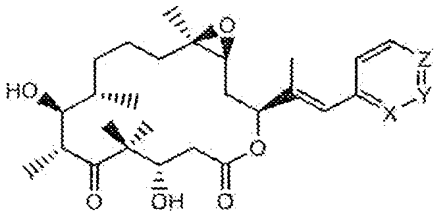


AA

FIG. 5 (cont'd)

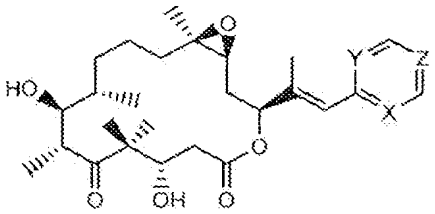


BB



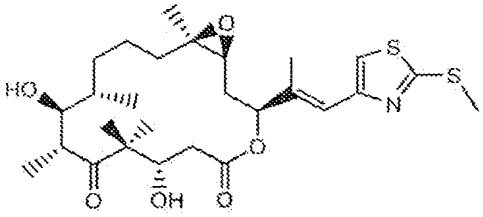
X,Y,Z = N, CH, CH;
X,Y,Z = CH, N, CH;
X,Y,Z = CH, CH, N;

CC

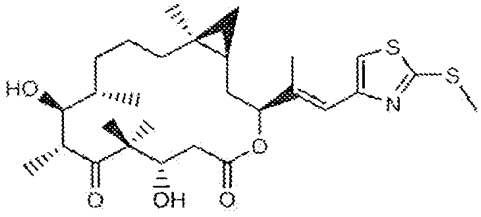


X,Y,Z = N, N, CH;
X,Y,Z = N, CH, N;
X,Y,Z = CH, CH, CH;

DD

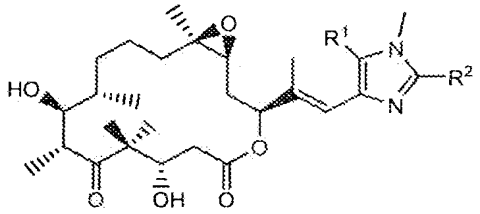


EE



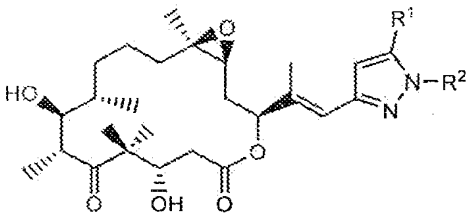
FF

FIG. 5 (cont'd)



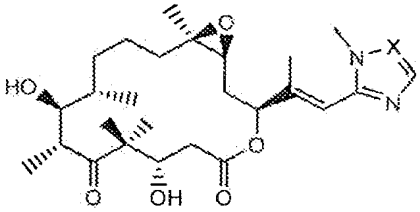
$R^1 = R^2 = H$:
$R^1 = R^2 = SMe$:

GG



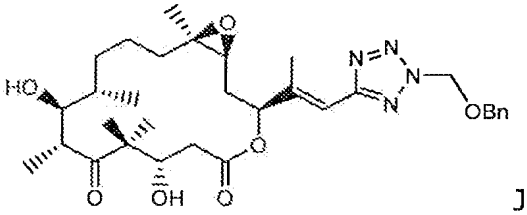
$R^1 = H, R^2 = Me$:
$R^1 = H, R^2 = Ph$:
$R^1 = SMe, R^2 = Me$:

HH

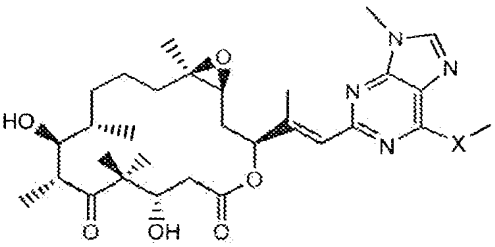


X = CH:
X = N:

II

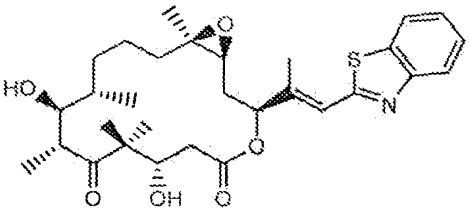


JJ



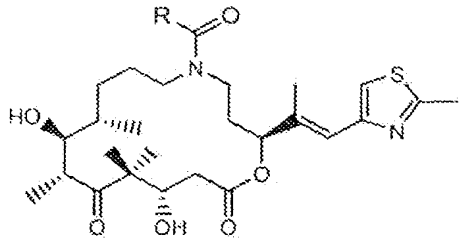
X = S:
X = O:

KK



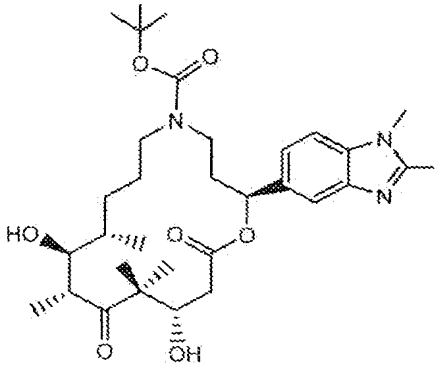
LL

FIG. 5 (cont'd)

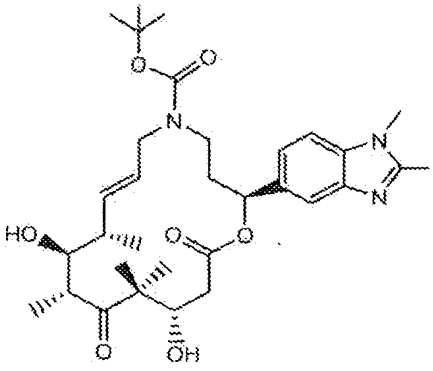


R = O-tert.-C_4H_9;
R = OCH_2Ph;
R = OC_2H_5;
R = O-iso-C_3H_7;
R = CH_3;
R = C_2H_5;
R = tert.-C_4H_9;
R = C_6H_5;

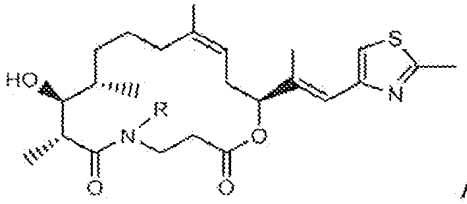
XX



YY



ZZ



AAA

FIG. 6

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
1.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	ixabepilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
2.	-	-NH(CH$_2$)$_5$CO-	3-OH	ixabepilone	None	Process B	3-epothilone linked to CDP
3.	-	-NH(CH$_2$)$_5$CO-	7-OH	ixabepilone	None	Process C	7-epothilone linked to CDP
4.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	ixabepilone	t-butyldimethylsilyl (TBDMS) or 2,2,2-trichloroethoxycarbonyl (TROC)	Process D	Mixture of 3, 7-epothilone linked to CDP
5.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
6.	-	-NH(CH$_2$)$_5$CO-	7-OH	ixabepilone	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
7.	-	-NH(CH$_2$)$_5$CO-	3-OH	ixabepilone	TBDMS or TROC	Process G	3-epothilone linked to CDP
8.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	epothilone B	None	Process A	Mixture of 3, 7-epothilone linked to CDP
9.	-	-NH(CH$_2$)$_5$CO-	3-OH	epothilone B	None	Process B	3-epothilone linked to CDP
10.	-	-NH(CH$_2$)$_5$CO-	7-OH	epothilone B	None	Process C	7-epothilone linked to CDP
11.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
12.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
13.	-	-NH(CH$_2$)$_5$CO-	7-OH	epothilone B	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
14.	-	-NH(CH$_2$)$_5$CO-	3-OH	epothilone B	TBDMS or TROC	Process G	3-epothilone linked to CDP
15.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	dehydelone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
16.	-	-NH(CH$_2$)$_5$CO-	3-OH	dehydelone	None	Process B	3-epothilone linked to CDP
17.	-	-NH(CH$_2$)$_5$CO-	7-OH	dehydelone	None	Process C	7-epothilone linked to CDP
18.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
19.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
20.	-	-NH(CH$_2$)$_5$CO-	7-OH	dehydelone	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
21.	-	-NH(CH$_2$)$_5$CO-	3-OH	dehydelone	TBDMS or TROC	Process G	3-epothilone linked to CDP
22.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	epothilone D	None	Process A	Mixture of 3, 7-epothilone linked to CDP
23.	-	-NH(CH$_2$)$_5$CO-	3-OH,	epothilone D	None	Process B	3-epothilone linked to CDP
24.	-	-NH(CH$_2$)$_5$CO-	7-OH	epothilone D	None	Process C	7-epothilone linked to CDP
25.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
26.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
27.	-	-NH(CH$_2$)$_5$CO-	7-OH	epothilone D	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
28.	-	-NH(CH$_2$)$_5$CO-	3-OH	epothilone D	TBDMS or TROC	Process G	3-epothilone linked to CDP
29.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	sagopilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
30.	-	-NH(CH$_2$)$_5$CO-	3-OH,	sagopilone	None	Process B	3-epothilone linked to CDP
31.	-	-NH(CH$_2$)$_5$CO-	7-OH	sagopilone	None	Process C	7-epothilone linked to CDP
32.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
33.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
34.	-	-NH(CH$_2$)$_5$CO-	7-OH	sagopilone	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
35.	-	-NH(CH$_2$)$_5$CO-	3-OH	sagopilone	TBDMS or TROC	Process G	3-epothilone linked to CDP
36.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	BMS-310705	None	Process H	Mixture of 3, 7-epothilone linked to CDP
37.	-	-NH(CH$_2$)$_5$CO-	3-OH	BMS-310705	None	Process I	3-epothilone linked to CDP
38.	-	-NH(CH$_2$)$_5$CO-	7-OH	BMS-310705	None	Process J	7-epothilone linked to CDP
39.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process K	Mixture of 3, 7-epothilone linked to CDP
40.	-	-NH(CH$_2$)$_5$CO-	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process L	Mixture of 3, 7-epothilone linked to CDP
41.	-	-NH(CH$_2$)$_5$CO-	7-OH	BMS-310705	TBDMS or TROC	Process M	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
42.	~	-NH(CH$_2$)$_5$CO-	3-OH	BMS-310705	TBDMS or TROC	Process N	3-epothilone linked to CDP
43.	~	-NH(CH$_2$)$_5$CO-	-NH2	BMS-310705	None	Process O	Amino linked epothilone to CDP
44.	~	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH, 7-OH	ixabepilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
45.	~	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH	ixabepilone	None	Process B	3-epothilone linked to CDP
46.	~	-NH(CH$_2$)$_3$OCH$_2$CO-	7-OH	ixabepilone	None	Process C	7-epothilone linked to CDP
47.	~	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
48.	~	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
49.	-	$-NH(CH_2)_3OCH_2CO-$	7-OH	ixabepilone	TBDMS or TROC	Process F	7-epothilone linked to CDP
50.	-	$-NH(CH_2)_3OCH_2CO-$	3-OH	ixabepilone	TBDMS or TROC	Process G	3-epothilone linked to CDP
51.	-	$-NH(CH_2)_3OCH_2CO-$	3-OH, 7-OH	epothilone B	None	Process A	Mixture of 3, 7-epothilone linked to CDP
52.	-	$-NH(CH_2)_3OCH_2CO-$	3-OH	epothilone B	None	Process B	3-epothilone linked to CDP
53.	-	$-NH(CH_2)_3OCH_2CO-$	7-OH	epothilone B	None	Process C	7-epothilone linked to CDP
54.	-	$-NH(CH_2)_3OCH_2CO-$	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
55.	-	$-NH(CH_2)_3OCH_2CO-$	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
56.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	7-OH	epothilone B	TBDMS or TROC	Process F	7-epothilone linked to CDP
57.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH	epothilone B	TBDMS or TROC	Process G	3-epothilone linked to CDP
58.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH, 7-OH	dehydelone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
59.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH	dehydelone	None	Process B	3-epothilone linked to CDP
60.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	7-OH	dehydelone	None	Process C	7-epothilone linked to CDP
61.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
62.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
63.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	7-OH	dehydelone	TBDMS or TROC	Process F	7-epothilone linked to CDP
64.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH	dehydelone	TBDMS or TROC	Process G	3-epothilone linked to CDP
65.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH, 7-OH	epothilone D	None	Process A	Mixture of 3, 7-epothilone linked to CDP
66.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH	epothilone D	None	Process B	3-epothilone linked to CDP
67.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	7-OH	epothilone D	None	Process C	7-epothilone linked to CDP
68.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
69.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
70.	-	-NH(CH_2)$_3$OCH_2CO-	7-OH	epothilone D	TBDMS or TROC	Process F	7-epothilone linked to CDP
71.	-	-NH(CH_2)$_3$OCH_2CO-	3-OH	epothilone D	TBDMS or TROC	Process G	3-epothilone linked to CDP
72.	-	-NH(CH_2)$_3$OCH_2CO-	3-OH, 7-OH	sagopilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
73.	-	-NH(CH_2)$_3$OCH_2CO-	3-OH	sagopilone	None	Process B	3-epothilone linked to CDP
74.	-	-NH(CH_2)$_3$OCH_2CO-	7-OH	sagopilone	None	Process C	7-epothilone linked to CDP
75.	-	-NH(CH_2)$_3$OCH_2CO-	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
76.	-	-NH(CH_2)$_3$OCH_2CO-	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
77.	-	$-NH(CH_2)_3OCH_2CO-$	7-OH	sagopilone	TBDMS or TROC	Process F	7-epothilone linked to CDP
78.	-	$-NH(CH_2)_3OCH_2CO-$	3-OH	sagopilone	TBDMS or TROC	Process G	3-epothilone linked to CDP
79.	-	$-NH(CH_2)_3OCH_2CO-$	3-OH, 7-OH	BMS-310705	None	Process H	Mixture of 3, 7-epothilone linked to CDP
80.	-	$-NH(CH_2)_3OCH_2CO-$	3-OH	BMS-310705	None	Process I	3-epothilone linked to CDP
81.	-	$-NH(CH_2)_3OCH_2CO-$	7-OH	BMS-310705	None	Process J	7-epothilone linked to CDP
82.	-	$-NH(CH_2)_3OCH_2CO-$	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process K	Mixture of 3, 7-epothilone linked to CDP
83.	-	$-NH(CH_2)_3OCH_2CO-$	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process L	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
84.	-	-NH$(CH_2)_3OCH_2CO$-	7-OH	BMS-310705	TBDMS or TROC	Process M	7-epothilone linked to CDP
85.	-	-NH$(CH_2)_3OCH_2CO$-	3-OH	BMS-310705	TBDMS or TROC	Process N	3-epothilone linked to CDP
86.	-	-NH$(CH_2)_3OCH_2CO$-	-NH2	BMS-310705	None	Process O	Amino linked epothilone to CDP
87.	-	-NH$CH_2CH_2COOCH_2CO$-	3-OH, 7-OH	ixabepilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
88.	-	-NH$CH_2CH_2COOCH_2CO$-	3-OH,	ixabepilone	None	Process B	3-epothilone linked to CDP
89.	-	-NH$CH_2CH_2COOCH_2CO$-	7-OH	ixabepilone	None	Process C	7-epothilone linked to CDP
90.	-	-NH$CH_2CH_2COOCH_2CO$-	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
91.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
92.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	7-OH	ixabepilone	TBDMS or TROC	Process F	7-epothilone linked to CDP
93.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH	ixabepilone	TBDMS or TROC	Process G	3-epothilone linked to CDP
94.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH, 7-OH	epothilone B	None	Process A	Mixture of 3, 7-epothilone linked to CDP
95.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH,	epothilone B	None	Process B	3-epothilone linked to CDP
96.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	7-OH	epothilone B	None	Process C	7-epothilone linked to CDP
97.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
98.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
99.	-	-$NHCH_2CH_2COOCH_2CO$-	7-OH	epothilone B	TBDMS or TROC	Process F	7-epothilone linked to CDP
100.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH	epothilone B	TBDMS or TROC	Process G	3-epothilone linked to CDP
101.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH, 7-OH	dehydelone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
102.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH,	dehydelone	None	Process B	3-epothilone linked to CDP
103.	-	-$NHCH_2CH_2COOCH_2CO$-	7-OH	dehydelone	None	Process C	7-epothilone linked to CDP
104.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
105.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
106.	-	-$NHCH_2CH_2COOCH_2CO$-	7-OH	dehydelone	TBDMS or TROC	Process F	7-epothilone linked to CDP
107.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH	dehydelone	TBDMS or TROC	Process G	3-epothilone linked to CDP
108.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH, 7-OH	epothilone D	None	Process A	Mixture of 3, 7-epothilone linked to CDP
109.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH,	epothilone D	None	Process B	3-epothilone linked to CDP
110.	-	-$NHCH_2CH_2COOCH_2CO$-	7-OH	epothilone D	None	Process C	7-epothilone linked to CDP
111.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
112.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
113.	-	-$NHCH_2CH_2COOCH_2CO$-	7-OH	epothilone D	TBDMS or TROC	Process F	7-epothilone linked to CDP
114.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH	epothilone D	TBDMS or TROC	Process G	3-epothilone linked to CDP
115.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH, 7-OH	sagopilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
116.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH,	sagopilone	None	Process B	3-epothilone linked to CDP
117.	-	-$NHCH_2CH_2COOCH_2CO$-	7-OH	sagopilone	None	Process C	7-epothilone linked to CDP
118.	-	-$NHCH_2CH_2COOCH_2CO$-	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
119.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
120.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	7-OH	sagopilone	TBDMS or TROC	Process F	7-epothilone linked to CDP
121.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH	sagopilone	TBDMS or TROC	Process G	3-epothilone linked to CDP
122.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH, 7-OH	BMS-310705	None	Process H	Mixture of 3, 7-epothilone linked to CDP
123.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH,	BMS-310705	None	Process I	3-epothilone linked to CDP
124.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	7-OH	BMS-310705	None	Process J	7-epothilone linked to CDP
125.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process K	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
126.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process L	Mixture of 3, 7-epothilone linked to CDP
127.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	7-OH	BMS-310705	TBDMS or TROC	Process M	7-epothilone linked to CDP
128.	-	-NHCH$_2$CH$_2$COOCH$_2$CO-	3-OH	BMS-310705	TBDMS or TROC	Process N	3-epothilone linked to CDP
129.	-	-NH(CH$_2$)$_3$OCH$_2$CO-	-NH2	BMS-310705	None	Process O	Amino linked epothilone to CDP
130.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	ixabepilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
131.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH	ixabepilone	None	Process B	3-epothilone linked to CDP
132.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	7-OH	ixabepilone	None	Process C	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
133.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	ixabepilone	TBDMS	Process D	Mixture of 3, 7-epothilone linked to CDP
134.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	ixabepilone	TBDMS	Process E	Mixture of 3, 7-epothilone linked to CDP
135.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	7-OH	ixabepilone	TBDMS	Process F	7-epothilone linked to CDP
136.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH	ixabepilone	TBDMS	Process G	3-epothilone linked to CDP
137.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	epothilone B	None	Process A	Mixture of 3, 7-epothilone linked to CDP
138.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH	epothilone B	None	Process B	3-epothilone linked to CDP
139.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	7-OH	epothilone B	None	Process C	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
140.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	epothilone B	TBDMS	Process D	Mixture of 3, 7-epothilone linked to CDP
141.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	epothilone B	TBDMS	Process E	Mixture of 3, 7-epothilone linked to CDP
142.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	7-OH	epothilone B	TBDMS	Process F	7-epothilone linked to CDP
143.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH	epothilone B	TBDMS	Process G	3-epothilone linked to CDP
144.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	dehydelone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
145.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH	dehydelone	None	Process B	3-epothilone linked to CDP
146.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	7-OH	dehydelone	None	Process C	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
147.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	dehydelone	TBDMS	Process D	Mixture of 3, 7-epothilone linked to CDP
148.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	dehydelone	TBDMS	Process E	Mixture of 3, 7-epothilone linked to CDP
149.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	7-OH	dehydelone	TBDMS	Process F	7-epothilone linked to CDP
150.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH	dehydelone	TBDMS	Process G	3-epothilone linked to CDP
151.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	epothilone D	None	Process A	Mixture of 3, 7-epothilone linked to CDP
152.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH	epothilone D	None	Process B	3-epothilone linked to CDP
153.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	7-OH	epothilone D	None	Process C	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
154.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	3-OH, 7-OH	epothilone D	TBDMS	Process D	Mixture of 3, 7-epothilone linked to CDP
155.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	3-OH, 7-OH	epothilone D	TBDMS	Process E	Mixture of 3, 7-epothilone linked to CDP
156.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	7-OH	epothilone D	TBDMS	Process F	7-epothilone linked to CDP
157.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	3-OH	epothilone D	TBDMS	Process G	3-epothilone linked to CDP
158.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	3-OH, 7-OH	sagopilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
159.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	3-OH	sagopilone	None	Process B	3-epothilone linked to CDP
160.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	7-OH	sagopilone	None	Process C	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
161.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	3-OH, 7-OH	sagopilone	TBDMS	Process D	Mixture of 3, 7-epothilone linked to CDP
162.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	3-OH, 7-OH	sagopilone	TBDMS	Process E	Mixture of 3, 7-epothilone linked to CDP
163.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	7-OH	sagopilone	TBDMS	Process F	7-epothilone linked to CDP
164.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	3-OH	sagopilone	TBDMS	Process G	3-epothilone linked to CDP
165.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	3-OH, 7-OH	BMS-310705	None	Process H	Mixture of 3, 7-epothilone linked to CDP
166.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	3-OH	BMS-310705	None	Process I	3-epothilone linked to CDP
167.	-	-$NHCH_2CH_2SSCH_2CH_2QCO$- Q is O or NH	7-OH	BMS-310705	None	Process J	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
168.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	BMS-310705	TBDMS	Process K	Mixture of 3, 7-epothilone linked to CDP
169.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH, 7-OH	BMS-310705	TBDMS	Process L	Mixture of 3, 7-epothilone linked to CDP
170.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	7-OH	BMS-310705	TBDMS	Process M	7-epothilone linked to CDP
171.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	3-OH	BMS-310705	TBDMS	Process N	3-epothilone linked to CDP
172.	-	-NHCH$_2$CH$_2$SSCH$_2$CH$_2$QCO- Q is O or NH	-NH2	BMS-310705	None	Process O	Amino linked epothilone to CDP
173.	- NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	ixabepilone	None	Process P	Mixture of 3, 7-epothilone linked to CDP
174.	- NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	ixabepilone	None	Process Q	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
175.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	ixabepilone	None	Process R	7-epothilone linked to CDP
176.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process S	Mixture of 3, 7-epothilone linked to CDP
177.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	ixabepilone	TBDMS	Process T	Mixture of 3, 7-epothilone linked to CDP
178.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	ixabepilone	TBDMS or TROC	Process U	7-epothilone linked to CDP
179.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	ixabepilone	TBDMS or TROC	Process V	3-epothilone linked to CDP
180.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone B	None	Process P	Mixture of 3, 7-epothilone linked to CDP
181.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	epothilone B	None	Process Q	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
182.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	epothilone B	None	Process R	7-epothilone linked to CDP
183.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process S	Mixture of 3, 7-epothilone linked to CDP
184.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone B	TBDMS	Process T	Mixture of 3, 7-epothilone linked to CDP
185.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	epothilone B	TBDMS or TROC	Process U	7-epothilone linked to CDP
186.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	epothilone B	TBDMS or TROC	Process V	3-epothilone linked to CDP
187.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	dehydelone	None	Process P	Mixture of 3, 7-epothilone linked to CDP
188.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	dehydelone	None	Process Q	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
189.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	dehydelone	None	Process R	7-epothilone linked to CDP
190.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process S	Mixture of 3, 7-epothilone linked to CDP
191.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	dehydelone	TBDMS	Process T	Mixture of 3, 7-epothilone linked to CDP
192.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	dehydelone	TBDMS or TROC	Process U	7-epothilone linked to CDP
193.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	dehydelone	TBDMS or TROC	Process V	3-epothilone linked to CDP
194.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone D	None	Process P	Mixture of 3, 7-epothilone linked to CDP
195.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	epothilone D	None	Process Q	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
196.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	epothilone D	None	Process R	7-epothilone linked to CDP
197.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process S	Mixture of 3, 7-epothilone linked to CDP
198.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone D	TBDMS	Process T	Mixture of 3, 7-epothilone linked to CDP
199.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	epothilone D	TBDMS or TROC	Process U	7-epothilone linked to CDP
200.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	epothilone D	TBDMS or TROC	Process V	3-epothilone linked to CDP
201.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	sagopilone	None	Process P	Mixture of 3, 7-epothilone linked to CDP
202.	~ NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	sagopilone	None	Process Q	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
203.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	sagopilone	None	Process R	7-epothilone linked to CDP
204.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process S	Mixture of 3, 7-epothilone linked to CDP
205.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	sagopilone	TBDMS	Process T	Mixture of 3, 7-epothilone linked to CDP
206.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	sagopilone	TBDMS or TROC	Process U	7-epothilone linked to CDP
207.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	sagopilone	TBDMS or TROC	Process V	3-epothilone linked to CDP
208.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	BMS-310705	None	Process CC	Mixture of 3, 7-epothilone linked to CDP
209.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	BMS-310705	None	Process DD	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
210.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	BMS-310705	None	Process EE	7-epothilone linked to CDP
211.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process FF	Mixture of 3, 7-epothilone linked to CDP
212.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH, 7-OH	BMS-310705	TBDMS	Process GG	Mixture of 3, 7-epothilone linked to CDP
213.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	7-OH	BMS-310705	TBDMS or TROC	Process HH	7-epothilone linked to CDP
214.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	3-OH	BMS-310705	TBDMS or TROC	Process II	3-epothilone linked to CDP
215.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$NHCO-	-NH2	BMS-310705	None	Process X	Amino linked epothilone to CDP
216.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	ixabepilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
217.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH	ixabepilone	None	Process B	3-epothilone linked to CDP
218.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	7-OH	ixabepilone	None	Process C	7-epothilone linked to CDP
219.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	ixabepilone	TBDMS	Process D	Mixture of 3, 7-epothilone linked to CDP
220.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	ixabepilone	TBDMS	Process E	Mixture of 3, 7-epothilone linked to CDP
221.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	7-OH	ixabepilone	TBDMS	Process F	7-epothilone linked to CDP
222.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH	ixabepilone	TBDMS	Process G	3-epothilone linked to CDP
223.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone B	None	Process A	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
224.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH	epothilone B	None	Process B	3-epothilone linked to CDP
225.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	7-OH	epothilone B	None	Process C	7-epothilone linked to CDP
226.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone B	TBDMS	Process D	Mixture of 3, 7-epothilone linked to CDP
227.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone B	TBDMS	Process E	Mixture of 3, 7-epothilone linked to CDP
228.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	7-OH	epothilone B	TBDMS	Process F	7-epothilone linked to CDP
229.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH	epothilone B	TBDMS	Process G	3-epothilone linked to CDP
230.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	dehydelone	None	Process A	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
231.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH	dehydelone	None	Process B	3-epothilone linked to CDP
232.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	7-OH	dehydelone	None	Process C	7-epothilone linked to CDP
233.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	dehydelone	TBDMS	Process D	Mixture of 3, 7-epothilone linked to CDP
234.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	dehydelone	TBDMS	Process E	Mixture of 3, 7-epothilone linked to CDP
235.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	7-OH	dehydelone	TBDMS	Process F	7-epothilone linked to CDP
236.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH	dehydelone	TBDMS	Process G	3-epothilone linked to CDP
237.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone D	None	Process A	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
238.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH	epothilone D	None	Process B	3-epothilone linked to CDP
239.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	7-OH	epothilone D	None	Process C	7-epothilone linked to CDP
240.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone D	TBDMS	Process D	Mixture of 3, 7-epothilone linked to CDP
241.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	epothilone D	TBDMS	Process E	Mixture of 3, 7-epothilone linked to CDP
242.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	7-OH	epothilone D	TBDMS	Process F	7-epothilone linked to CDP
243.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH	epothilone D	TBDMS	Process G	3-epothilone linked to CDP
244.	-	-NH(CH$_2$)$_2$SS(CH$_2$)$_2$NHCO-	3-OH, 7-OH	sagopilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
245.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	3-OH	sagopilone	None	Process B	3-epothilone linked to CDP
246.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	7-OH	sagopilone	None	Process C	7-epothilone linked to CDP
247.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	3-OH, 7-OH	sagopilone	TBDMS	Process D	Mixture of 3, 7-epothilone linked to CDP
248.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	3-OH, 7-OH	sagopilone	TBDMS	Process E	Mixture of 3, 7-epothilone linked to CDP
249.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	7-OH	sagopilone	TBDMS	Process F	7-epothilone linked to CDP
250.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	3-OH	sagopilone	TBDMS	Process G	3-epothilone linked to CDP
251.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	3-OH, 7-OH	BMS-310705	None	Process H	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
252.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	3-OH	BMS-310705	None	Process I	3-epothilone linked to CDP
253.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	7-OH	BMS-310705	None	Process J	7-epothilone linked to CDP
254.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	3-OH, 7-OH	BMS-310705	TBDMS	Process K	Mixture of 3, 7-epothilone linked to CDP
255.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	3-OH, 7-OH	BMS-310705	TBDMS	Process L	Mixture of 3, 7-epothilone linked to CDP
256.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	7-OH	BMS-310705	TBDMS	Process M	7-epothilone linked to CDP
257.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	3-OH	BMS-310705	TBDMS	Process N	3-epothilone linked to CDP
258.	-	-NH(CH_2)$_2$SS(CH_2)$_2$NHCO-	-NH_2	BMS-310705	None	Process O	Amino linked epothilone to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
259.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	ixabepilone	None	Process P	Mixture of 3, 7-epothilone linked to CDP
260.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH	ixabepilone	None	Process Q	3-epothilone linked to CDP
261.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	ixabepilone	None	Process R	7-epothilone linked to CDP
262.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process S	Mixture of 3, 7-epothilone linked to CDP
263.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	ixabepilone	TBDMS	Process T	Mixture of 3, 7-epothilone linked to CDP
264.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	ixabepilone	TBDMS or TROC	Process U	7-epothilone linked to CDP
265.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH	ixabepilone	TBDMS or TROC	Process V	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
266.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	epothilone B	None	Process P	Mixture of 3, 7-epothilone linked to CDP
267.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH	epothilone B	None	Process Q	3-epothilone linked to CDP
268.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	epothilone B	None	Process R	7-epothilone linked to CDP
269.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process S	Mixture of 3, 7-epothilone linked to CDP
270.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	epothilone B	TBDMS	Process T	Mixture of 3, 7-epothilone linked to CDP
271.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	epothilone B	TBDMS or TROC	Process U	7-epothilone linked to CDP
272.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH	epothilone B	TBDMS or TROC	Process V	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
273.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	dehydelone	None	Process P	Mixture of 3, 7-epothilone linked to CDP
274.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH	dehydelone	None	Process Q	3-epothilone linked to CDP
275.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	dehydelone	None	Process R	7-epothilone linked to CDP
276.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process S	Mixture of 3, 7-epothilone linked to CDP
277.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	dehydelone	TBDMS	Process T	Mixture of 3, 7-epothilone linked to CDP
278.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	dehydelone	TBDMS or TROC	Process U	7-epothilone linked to CDP
279.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH	dehydelone	TBDMS or TROC	Process V	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
280.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	epothilone D	None	Process P	Mixture of 3, 7-epothilone linked to CDP
281.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH	epothilone D	None	Process Q	3-epothilone linked to CDP
282.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	epothilone D	None	Process R	7-epothilone linked to CDP
283.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process S	Mixture of 3, 7-epothilone linked to CDP
284.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	epothilone D	TBDMS	Process T	Mixture of 3, 7-epothilone linked to CDP
285.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	epothilone D	TBDMS or TROC	Process U	7-epothilone linked to CDP
286.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH	epothilone D	TBDMS or TROC	Process V	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
287.	- $NH(CH_2)_2S$-	-$S(CH_2)_2OCO$-	3-OH, 7-OH	sagopilone	None	Process P	Mixture of 3, 7-epothilone linked to CDP
288.	- $NH(CH_2)_2S$-	-$S(CH_2)_2OCO$-	3-OH	sagopilone	None	Process Q	3-epothilone linked to CDP
289.	- $NH(CH_2)_2S$-	-$S(CH_2)_2OCO$-	7-OH	sagopilone	None	Process R	7-epothilone linked to CDP
290.	- $NH(CH_2)_2S$-	-$S(CH_2)_2OCO$-	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process S	Mixture of 3, 7-epothilone linked to CDP
291.	- $NH(CH_2)_2S$-	-$S(CH_2)_2OCO$-	3-OH, 7-OH	sagopilone	TBDMS	Process T	Mixture of 3, 7-epothilone linked to CDP
292.	- $NH(CH_2)_2S$-	-$S(CH_2)_2OCO$-	7-OH	sagopilone	TBDMS or TROC	Process U	7-epothilone linked to CDP
293.	- $NH(CH_2)_2S$-	-$S(CH_2)_2OCO$-	3-OH	sagopilone	TBDMS or TROC	Process V	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
294.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	BMS-310705	None	Process CC	Mixture of 3, 7-epothilone linked to CDP
295.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH	BMS-310705	None	Process DD	3-epothilone linked to CDP
296.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	BMS-310705	None	Process EE	7-epothilone linked to CDP
297.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process FF	Mixture of 3, 7-epothilone linked to CDP
298.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH, 7-OH	BMS-310705	TBDMS	Process GG	Mixture of 3, 7-epothilone linked to CDP
299.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	7-OH	BMS-310705	TBDMS or TROC	Process HH	7-epothilone linked to CDP
300.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	3-OH	BMS-310705	TBDMS or TROC	Process II	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
301.	-NH(CH$_2$)$_2$S-	-S(CH$_2$)$_2$OCO-	-NH$_2$	BMS-310705	None	Process X	Amino linked epothilone to CDP
302.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	ixabepilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
303.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH	ixabepilone	None	Process B	3-epothilone linked to CDP
304.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	7-OH	ixabepilone	None	Process C	7-epothilone linked to CDP
305.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
306.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
307.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	7-OH	ixabepilone	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
308.	~	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH	ixabepilone	TBDMS or TROC	Process G	3-epothilone linked to CDP
309.	~	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	epothilone B	None	Process A	Mixture of 3, 7-epothilone linked to CDP
310.	~	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH	epothilone B	None	Process B	3-epothilone linked to CDP
311.	~	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	7-OH	epothilone B	None	Process C	7-epothilone linked to CDP
312.	~	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
313.	~	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
314.	~	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	7-OH	epothilone B	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
315.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH	epothilone B	TBDMS or TROC	Process G	3-epothilone linked to CDP
316.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	dehydelone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
317.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH	dehydelone	None	Process B	3-epothilone linked to CDP
318.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	7-OH	dehydelone	None	Process C	7-epothilone linked to CDP
319.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
320.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
321.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	7-OH	dehydelone	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
322.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH	dehydelone	TBDMS or TROC	Process G	3-epothilone linked to CDP
323.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	epothilone D	None	Process A	Mixture of 3, 7-epothilone linked to CDP
324.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH	epothilone D	None	Process B	3-epothilone linked to CDP
325.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	7-OH	epothilone D	None	Process C	7-epothilone linked to CDP
326.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
327.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
328.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	7-OH	epothilone D	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
329.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH	epothilone D	TBDMS or TROC	Process G	3-epothilone linked to CDP
330.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	sagopilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
331.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH	sagopilone	None	Process B	3-epothilone linked to CDP
332.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	7-OH	sagopilone	None	Process C	7-epothilone linked to CDP
333.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
334.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
335.	-	-NH(CH$_2$)$_n$CO- n is 1, 2, or 3	7-OH	sagopilone	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
336.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	3-OH	sagopilone	TBDMS or TROC	Process G	3-epothilone linked to CDP
337.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	BMS-310705	None	Process H	Mixture of 3, 7-epothilone linked to CDP
338.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	3-OH	BMS-310705	None	Process I	3-epothilone linked to CDP
339.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	7-OH	BMS-310705	None	Process J	7-epothilone linked to CDP
340.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process K	Mixture of 3, 7-epothilone linked to CDP
341.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process L	Mixture of 3, 7-epothilone linked to CDP
342.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	7-OH	BMS-310705	TBDMS or TROC	Process M	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
343.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	3-OH	BMS-310705	TBDMS or TROC	Process N	3-epothilone linked to CDP
344.	-	-NH(CH_2)$_n$CO- n is 1, 2, or 3	-NH_2	BMS-310705	None	Process O	Amino linked epothilone to CDP
345.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	ixabepilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
346.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	ixabepilone	None	Process B	3-epothilone linked to CDP
347.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	ixabepilone	None	Process C	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
348.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
349.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	ixabepilone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
350.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	ixabepilone	TBDMS or TROC	Process F	7-epothilone linked to CDP
351.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	ixabepilone	TBDMS or TROC	Process G	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
352.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	epothilone B	None	Process A	Mixture of 3, 7-epothilone linked to CDP
353.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	epothilone B	None	Process B	3-epothilone linked to CDP
354.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	epothilone B	None	Process C	7-epothilone linked to CDP
355.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
356.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	epothilone B	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
357.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	epothilone B	TBDMS or TROC	Process F	7-epothilone linked to CDP
358.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	epothilone B	TBDMS or TROC	Process G	3-epothilone linked to CDP
359.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	dehydelone	None	Process A	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
360.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	dehydelone	None	Process B	3-epothilone linked to CDP
361.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	dehydelone	None	Process C	7-epothilone linked to CDP
362.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
363.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	dehydelone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
364.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	dehydelone	TBDMS or TROC	Process F	7-epothilone linked to CDP
365.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	dehydelone	TBDMS or TROC	Process G	3-epothilone linked to CDP
366.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	epothilone D	None	Process A	Mixture of 3, 7-epothilone linked to CDP
367.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	epothilone D	None	Process B	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
368.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	epothilone D	None	Process C	7-epothilone linked to CDP
369.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
370.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	epothilone D	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
371.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	epothilone D	TBDMS or TROC	Process F	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
372.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	epothilone D	TBDMS or TROC	Process G	3-epothilone linked to CDP
373.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	sagopilone	None	Process A	Mixture of 3, 7-epothilone linked to CDP
374.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	sagopilone	None	Process B	3-epothilone linked to CDP
375.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	sagopilone	None	Process C	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
376.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process D	Mixture of 3, 7-epothilone linked to CDP
377.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	sagopilone	TBDMS or TROC	Process E	Mixture of 3, 7-epothilone linked to CDP
378.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	sagopilone	TBDMS or TROC	Process F	7-epothilone linked to CDP
379.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	sagopilone	TBDMS or TROC	Process G	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
380.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	BMS-310705	None	Process H	Mixture of 3, 7-epothilone linked to CDP
381.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	BMS-310705	None	Process I	3-epothilone linked to CDP
382.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	BMS-310705	None	Process J	7-epothilone linked to CDP
383.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process K	Mixture of 3, 7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
384.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH, 7-OH	BMS-310705	TBDMS or TROC	Process L	Mixture of 3, 7-epothilone linked to CDP
385.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	7-OH	BMS-310705	TBDMS or TROC	Process M	7-epothilone linked to CDP
386.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	3-OH	BMS-310705	TBDMS or TROC	Process N	3-epothilone linked to CDP
387.	-	-NHZCO- Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	$-NH_2$	BMS-310705	None	Process O	Amino linked epothilone to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
388.	-	-	$-NH_2$	BMS-310705	None	Process W	Epothilone linked directly to CDP via an amide bond
389.	-	-Linker-	7-OH where 3-O-hexanoyl remains functionalized	ixabepilone	3-O-hexanoyl	Process Y	7-epothilone linked to CDP
390.	-	-Linker-	7-OH where 3-O-hexanoyl remains functionalized	epothilone B	3-O-hexanoyl	Process Y	7-epothilone linked to CDP
391.	-	-Linker-	7-OH where 3-O-hexanoyl remains functionalized	dehydelone	3-O-hexanoyl	Process Y	7-epothilone linked to CDP
392.	-	-Linker-	7-OH where 3-O-hexanoyl remains functionalized	epothilone D	3-O-hexanoyl	Process Y	7-epothilone linked to CDP
393.	-	-Linker-	7-OH where 3-O-hexanoyl remains functionalized	sagopilone	3-O-hexanoyl	Process Y	7-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
394.	-	-Linker-	7-OH where 3-O-hexanoyl remains functionalized	BMS-310705	3-O-hexanoyl	Process AA	7-epothilone linked to CDP
395.	-	-Linker-	3-OH where 7-O-hexanoyl remains functionalized	ixabepilone	7-O-hexanoyl	Process Z	3-epothilone linked to CDP
396.	-	-Linker-	3-OH where 7-O-hexanoyl remains functionalized	epothilone B	7-O-hexanoyl	Process Z	3-epothilone linked to CDP
397.	-	-Linker-	3-OH where 7-O-hexanoyl remains functionalized	dehydelone	7-O-hexanoyl	Process Z	3-epothilone linked to CDP
398.	-	-Linker-	3-OH where 7-O-hexanoyl remains functionalized	epothilone D	7-O-hexanoyl	Process Z	3-epothilone linked to CDP
399.	-	-Linker-	3-OH where 7-O-hexanoyl remains functionalized	sagopilone	7-O-hexanoyl	Process Z	3-epothilone linked to CDP

FIG. 6 (cont'd)

Example	A	B	X	Epothilone	Hydroxy Protecting Groups	Process for Prep. of epothilone linker CDP molecules	Final Product
400.	-	-Linker-	3-OH where 7-O-hexanoyl remains functionalized	BMS-310705	7-O-hexanoyl	Process BB	3-epothilone linked to CDP

FIG. 7

Example	L	Boronic Acid	Process for Preparation of boronic acid linker CDP molecules	Final Product
1.	-NH(CH$_2$)$_6$C(CH$_3$)(OZ')C(CH$_3$)$_2$OZ'	bortezomib	Process A	bortezomib linked to CDP
2.	-NH(CH$_2$)$_3$CH(CH$_2$OZ')CH$_2$OZ'	bortezomib	Process A	bortezomib linked to CDP
3.	-NH(CH$_2$)$_6$C(CH$_3$)(OZ')CH$_2$NH(CH$_3$)Z'	bortezomib	Process A	bortezomib linked to CDP
4.	-NH(CH$_2$)$_6$N(CH$_2$CH$_2$OZ')CH$_2$CH$_2$OZ'	bortezomib	Process A	bortezomib linked to CDP
5.	-NH(CH$_2$)$_6$N(CH$_2$CO$_2$Z')CH$_2$CO$_2$Z'	bortezomib	Process A	bortezomib linked to CDP
6.	-NH(CH$_2$)$_6$N((CH$_2$)$_2$OZ')(CH$_2$)$_2$N(CH$_3$)Z'	bortezomib	Process A	bortezomib linked to CDP
7.	-NH(CH$_2$)$_6$C(CH$_3$)(OZ')C(CH$_3$)$_2$OZ'	bortezomib	Process B	bortezomib linked to CDP
8.	-NH(CH$_2$)$_4$CH(CH$_2$OZ')CH$_2$OZ'	bortezomib	Process B	bortezomib linked to CDP
9.	-NH(CH$_2$)$_6$C(CH$_3$)(OZ')CH$_2$NH(CH$_3$)Z'	bortezomib	Process B	bortezomib linked to CDP
10.	-NH(CH$_2$)$_6$N(CH$_2$CH$_2$OZ')CH$_2$CH$_2$OZ'	bortezomib	Process B	bortezomib linked to CDP
11.	-NH(CH$_2$)$_6$N(CH$_2$CO$_2$Z')CH$_2$CO$_2$Z'	bortezomib	Process B	bortezomib linked to CDP
12.	-NH(CH$_2$)$_6$N((CH$_2$)$_2$OZ')(CH$_2$)$_2$N(CH$_3$)Z'	bortezomib	Process B	bortezomib linked to CDP
13.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$C(CH$_3$)(OZ')C(CH$_3$)$_2$OZ'	bortezomib	Process A	bortezomib linked to CDP

FIG. 7 (cont'd)

Example	L	Boronic Acid	Process for Preparation of boronic acid linker CDP molecules	Final Product
14.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$CH(CH$_2$OZ')CH$_2$OZ'	bortezomib	Process A	bortezomib linked to CDP
15.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$C(CH$_3$)(OZ')CH$_2$NH(CH$_2$)Z'	bortezomib	Process A	bortezomib linked to CDP
16.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$N(CH$_2$CH$_2$OZ')CH$_2$CH$_2$OZ'	bortezomib	Process A	bortezomib linked to CDP
17.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$N(CH$_2$CO$_2$Z')CH$_2$CO$_2$Z'	bortezomib	Process A	bortezomib linked to CDP
18.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$N((CH$_2$)$_2$OZ')(CH$_2$)$_2$N(CH$_3$)Z'	bortezomib	Process A	bortezomib linked to CDP
19.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$C(CH$_3$)(OZ')C(CH$_3$)$_2$OZ'	bortezomib	Process B	bortezomib linked to CDP
20.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$CH(CH$_2$OZ')CH$_2$OZ'	bortezomib	Process B	bortezomib linked to CDP
21.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$C(CH$_3$)(OZ')CH$_2$NH(CH$_2$)Z'	bortezomib	Process B	bortezomib linked to CDP
22.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$N(CH$_2$CH$_2$OZ')CH$_2$CH$_2$OZ'	bortezomib	Process B	bortezomib linked to CDP
23.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$N(CH$_2$CO$_2$Z')CH$_2$CO$_2$Z'	bortezomib	Process B	bortezomib linked to CDP
24.	-NHCH$_2$CH$_2$OCH$_2$CH$_2$N((CH$_2$)$_2$OZ')(CH$_2$)$_2$N(CH$_3$)Z'	bortezomib	Process B	bortezomib linked to CDP
25.	-NHCH$_2$COOCH$_2$CH$_2$C(CH$_3$)(OZ')C(CH$_3$)$_2$OZ'	bortezomib	Process A	bortezomib linked to CDP
26.	-NHCH$_2$COOCH$_2$CH$_2$CH(CH$_2$OZ')CH$_2$OZ'	bortezomib	Process A	bortezomib linked to CDP

FIG. 7 (cont'd)

Example	L	Boronic Acid	Process for Preparation of boronic acid linker CDP molecules	Final Product
27.	-NHCH$_2$COOCH$_2$CH$_2$C(CH$_3$)(OZ')CH$_2$NH(CH$_3$)Z'	bortezomib	Process A	bortezomib linked to CDP
28.	-NHCH$_2$COOCH$_2$CH$_2$N(CH$_2$CH$_2$OZ')CH$_2$CH$_2$OZ'	bortezomib	Process A	bortezomib linked to CDP
29.	-NHCH$_2$COOCH$_2$CH$_2$N(CH$_2$CO$_2$Z')CH$_2$CO$_2$Z'	bortezomib	Process A	bortezomib linked to CDP
30.	-NHCH$_2$COOCH$_2$CH$_2$N((CH$_2$)$_2$OZ')(CH$_2$)$_2$N(CH$_3$)Z'	bortezomib	Process A	bortezomib linked to CDP
31.	-NHCH$_2$COOCH$_2$CH$_2$C(CH$_3$)(OZ')C(CH$_3$)$_2$OZ'	bortezomib	Process B	bortezomib linked to CDP
32.	-NHCH$_2$COOCH$_2$CH$_2$CH(CH$_2$OZ')CH$_2$OZ'	bortezomib	Process B	bortezomib linked to CDP
33.	-NHCH$_2$COOCH$_2$CH$_2$C(CH$_3$)(OZ')CH$_2$NH(CH$_3$)Z'	bortezomib	Process B	bortezomib linked to CDP
34.	-NHCH$_2$COOCH$_2$CH$_2$N(CH$_2$CH$_2$OZ')CH$_2$CH$_2$OZ'	bortezomib	Process B	bortezomib linked to CDP
35.	-NHCH$_2$COOCH$_2$CH$_2$N(CH$_2$CO$_2$Z')CH$_2$CO$_2$Z'	bortezomib	Process B	bortezomib linked to CDP
36.	-NHCH$_2$COOCH$_2$CH$_2$N((CH$_2$)$_2$OZ')(CH$_2$)$_2$N(CH$_3$)Z'	bortezomib	Process B	bortezomib linked to CDP
37.	-NHZCONHCH$_2$CH$_2$C(CH$_3$)(OZ')C(CH$_3$)$_2$OZ' Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process A	bortezomib linked to CDP

FIG. 7 (cont'd)

Example	L	Boronic Acid	Process for Preparation of boronic acid linker CDP molecules	Final Product
38.	-NHZCONHCH$_2$CH$_2$CH(CH$_2$OZ^1)CH$_2$OZ^2 Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process A	bortezomib linked to CDP
39.	-NHZCONHCH$_2$CH$_2$C(CH$_3$)(OZ^1)CH$_2$NH(CH$_3$)Z^2 Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process A	bortezomib linked to CDP
40.	-NHZCONHCH$_2$CH$_2$N(CH$_2$CH$_2$OZ^1)CH$_2$CH$_2$OZ^2 Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process A	bortezomib linked to CDP
41.	-NHZCONHCH$_2$CH$_2$N(CH$_2$CO$_2$Z^1)CH$_2$CO$_2$Z^2 Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process A	bortezomib linked to CDP
42.	-NHZCONHCH$_2$CH$_2$N((CH$_2$)$_2$OZ^1)(CH$_2$)$_2$N(CH$_3$)Z^2 Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process A	bortezomib linked to CDP
43.	-NHZCONHCH$_2$CH$_2$C(CH$_3$)(OZ^1)C(CH$_3$)$_2$OZ^2 Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process B	bortezomib linked to CDP

FIG. 7 (cont'd)

Example	L	Boronic Acid	Process for Preparation of boronic acid linker CDP molecules	Final Product
44.	-NHZCONHCH$_2$CH$_2$CH(CH$_2$OZ')CH$_2$OZ' Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process B	bortezomib linked to CDP
45.	-NHZCONHCH$_2$CH$_2$C(CH$_3$)(OZ')CH$_2$NH(CH$_3$)Z' Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process B	bortezomib linked to CDP
46.	-NHZCONHCH$_2$CH$_2$N(CH$_2$CH$_2$OZ')CH$_2$CH$_2$OZ' Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process B	bortezomib linked to CDP
47.	-NHZCONHCH$_2$CH$_2$N(CH$_2$CO$_2$Z')CH$_2$CO$_2$Z' Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process B	bortezomib linked to CDP
48.	-NHZCONHCH$_2$CH$_2$N((CH$_2$)$_2$OZ')(CH$_2$)$_2$N(CH$_3$)Z' Z is a mono, di, or tripeptide or other peptide or derivative thereof where NH and CO represent the amino and acid terminus of the amino acid or peptide	bortezomib	Process B	bortezomib linked to CDP

FIG. 8

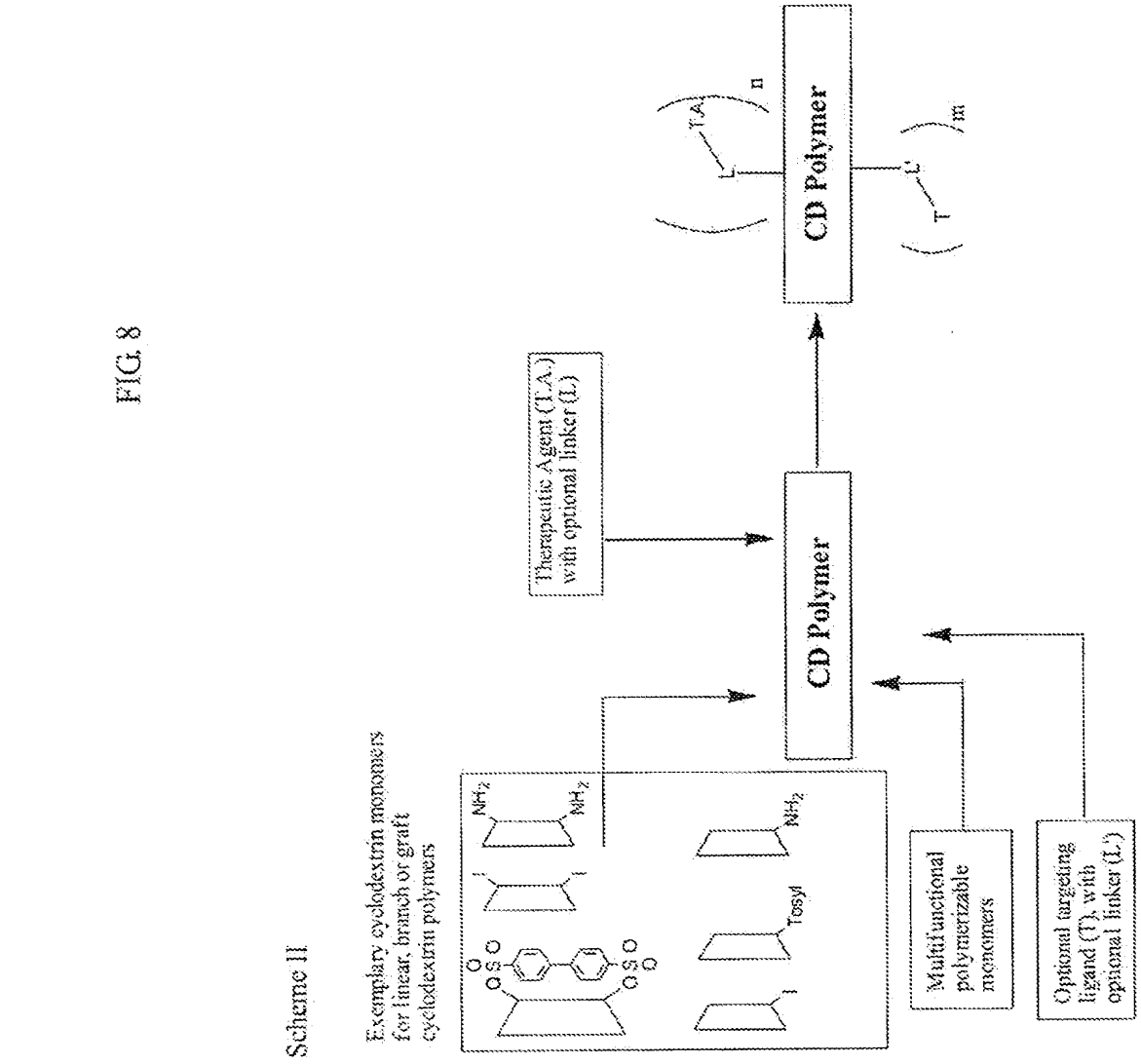

Scheme II
Exemplary cyclodextrin monomers for linear, branch or graft cyclodextrin polymers
Therapeutic Agent (T.A.) with optional linker (L)
CD Polymer
CD Polymer
Multifunctional polymerizable monomers
Optional targeting ligand (T), with optional linker (L')
Tosyl
NH2
T.A
L
n
L'
T
m

FIG. 9

Scheme IIa: General scheme for graft cyclodextrin-containing polymers.

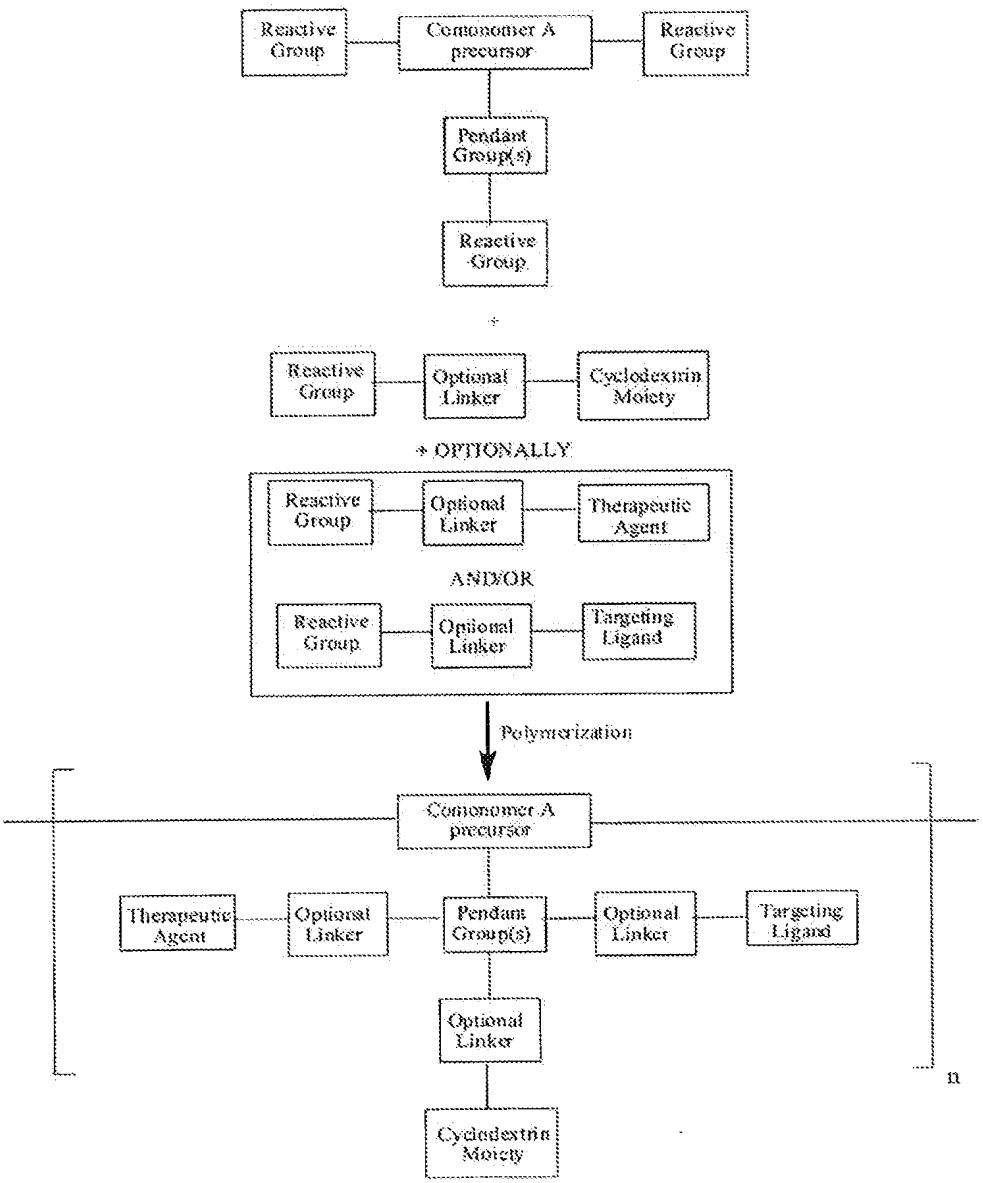
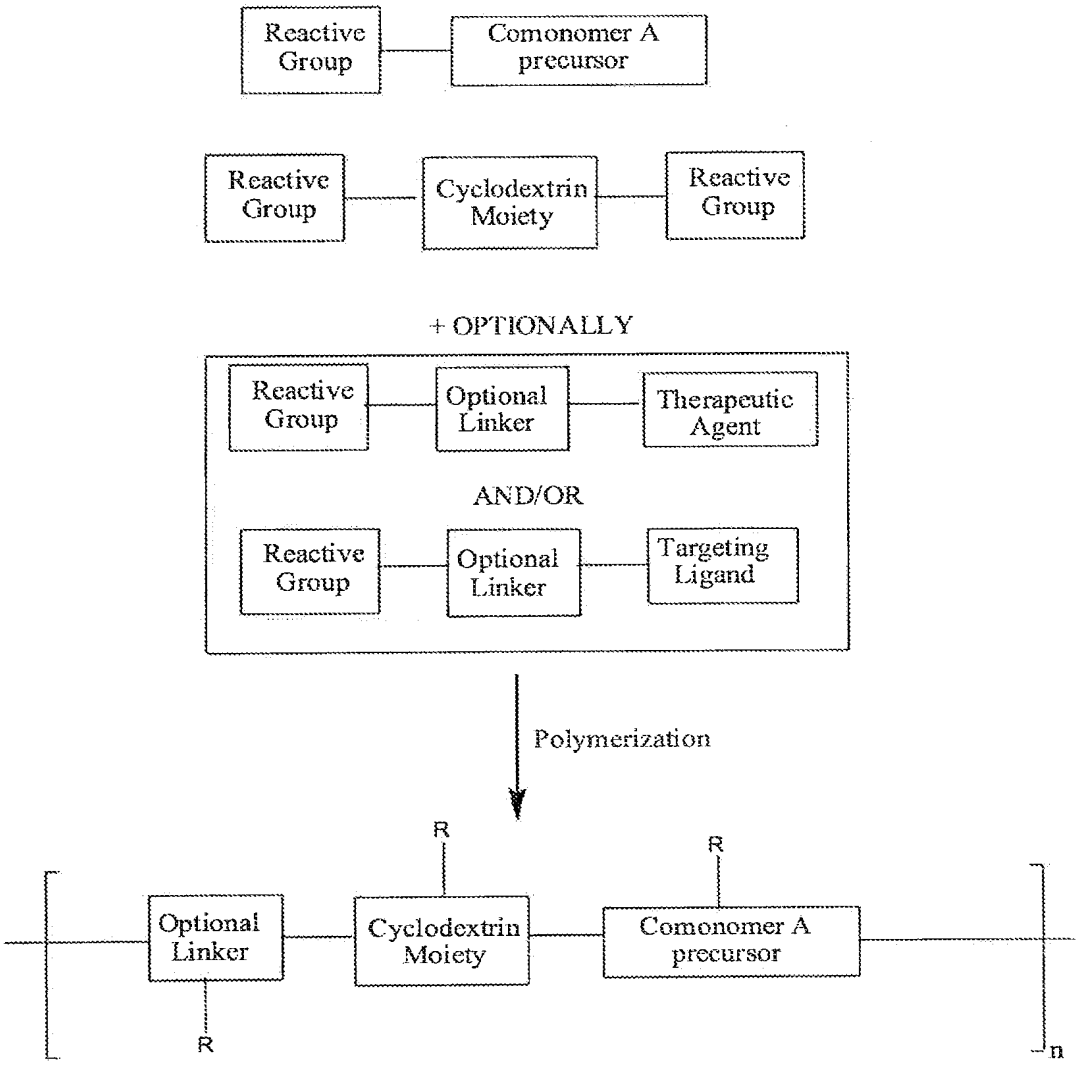


FIG. 10

Scheme IIb: General scheme of preparing linear CDPs.



Wherein R is a therapeutic agent and/or targeting ligand, either of which may be absent or present.

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权利要求书 2 页 说明书 70 页 附图 23 页

[54] 发明名称

HER2 抗体组合物

[57] 摘要

本发明公开了包含结合 HER2 结构域 II 的主要种类 HER2 抗体及其包含氨基末端前导延伸的氨基酸序列变体的组合物。还公开了包含所述组合物的药用配制剂以及所述组合物的治疗用途。

1. 包含结合 HER2 结构域 II 的主要种类 HER2 抗体及其包含氨基末端前导延伸的氨基酸序列变体的组合物。

2. 权利要求 1 的组合物，其中所述氨基末端前导延伸位于所述抗体变体的轻链上。

3. 权利要求 2 的组合物，其中所述氨基末端前导延伸位于所述抗体变体的一条或两条轻链上。

4. 前述权利要求任一项的组合物，其中所述主要种类 HER2 抗体和抗体变体都是完整抗体。

5. 前述权利要求任一项的组合物，其中所述主要种类 HER2 抗体包含分别在 SEQ ID No. 3 和 4 中的轻链和重链可变区氨基酸序列。

6. 权利要求 5 的组合物，其中所述主要种类 HER2 抗体包含分别在 SEQ ID No. 15 和 16 中的轻链和重链氨基酸序列。

7. 前述权利要求任一项的组合物，其中所述组合物中约 20%或更少的抗体分子包含氨基末端前导延伸。

8. 权利要求 7 的组合物，其中所述组合物中约 1%至约 15%的抗体分子包含氨基末端前导延伸。

9. 权利要求 8 的组合物，其中根据阳离子交换分析的量化，所述组合物中约 5%至约 15%的抗体分子包含氨基末端前导延伸。

10. 前述权利要求任一项的方法，其中所述氨基末端前导延伸包含 VHS-。

11. 权利要求 10 的方法，其中所述氨基末端前导延伸由 VHS-组成。

12. 前述权利要求任一项的组合物，它还包含主要种类 HER2 抗体的第二氨基酸序列变体。

13. 权利要求 12 的组合物，其中所述第二抗体变体选自在其一条或所有两条重链上包含 C-末端赖氨酸残基的抗体，脱酰胺抗体变体、和具有一个或多个氧化后甲硫氨酸残基的抗体。

14. 前述权利要求任一项的组合物，它还包含所述主要种类抗体的糖基化变体。

15. 权利要求 14 的组合物，其中所述糖基化变体选自包含附着于其 Fc

区的 G1 或 G2 寡糖结构的抗体，包含附着于其轻链的碳水化合物模块的抗体、包含未糖基化重链的抗体、和有唾液酸化寡糖结构附着于其 Fc 区的抗体。

16. 前述权利要求任一项的组合物，其中所述主要种类抗体结合 HER2 结构域 I、II 和 III 之间的连接处。

17. 在药学可接受载体中包含权利要求 1 的组合物的药用配制剂。

18. 权利要求 17 的药用配制剂，它是无菌的。

19. 包含主要种类 HER2 抗体及其氨基酸序列变体的组合物，所述主要种类 HER2 抗体包含分别在 SEQ ID No. 3 和 4 中的轻链和重链可变区序列，所述氨基酸序列变体包含附着于其一个或两个轻链可变区的 VHS-氨基末端前导延伸，其中所述组合物中约 1%至约 20%的抗体分子包含 VHS-氨基末端前导延伸。

20. 包含 SEQ ID No. 23 中的氨基酸序列的多肽或其脱酰胺和/或氧化变体。

21. 包含轻链和重链的抗体，(a) 所述轻链包含权利要求 20 的多肽，且 (b) 所述重链包含选自下组的氨基酸序列：SEQ ID No. 16、SEQ ID No. 24、和 SEQ ID No. 16 或 SEQ ID No. 24 的脱酰胺和/或氧化变体。

22. 在药学可接受载体中包含权利要求 21 的抗体的药用配制剂。

23. 在患者中治疗表达 HER2 的癌症的方法，包括以有效治疗癌症的量对患者施用权利要求 17 的药用配制剂。

HER2 抗体组合物

发明领域

本发明关注包含结合 HER2 结构域 II 的主要种类 HER2 抗体及其包含氨基末端前导延伸的氨基酸序列变体的组合物。本发明还涉及包含所述组合物的药用配制剂以及所述组合物的治疗用途。

发明背景

HER2 抗体

受体酪氨酸激酶的 HER 家族是细胞生长、分化和存活的重要介质。该受体家族包括四个独特的成员：表皮生长因子受体（EGFR、ErbB1 或 HER1）、HER2（ErbB2 或 $p185^{neu}$）、HER3（ErbB3）和 HER4（ErbB4 或 tyro2）。

由 *erb*B1 基因编码的 EGFR 已被认为是人类恶性肿瘤的病因。具体而言，在乳癌、膀胱癌、肺癌、头癌、颈癌和胃癌以及成胶质细胞瘤中已经观察到 EGFR 表达的升高。EGFR 受体表达的升高常常与 EGFR 配体，转化生长因子 α (TGF-α)的产量增加有关，这样的肿瘤细胞通过自分泌刺激途径导致受体活化。Baselga and Mendelsohn, *Pharmac. Ther.* 64: 127-154 (1994)。针对 EGFR 或其配体 TGF-α 或 EGF 的单克隆抗体已经作为治疗此类恶性肿瘤的治疗剂进行了评估。参见例如 Baselga and Mendelsohn, 见上文; Masui *et al.*, *Cancer Research* 44: 1002-1007 (1984); 及 Wu *et al.*, *J. Clin. Invest.* 95: 1897-1905 (1995)。

HER 家族的第二个成员 $p185^{neu}$ 最初被鉴定成转化基因的产物，来自经化学处理的大鼠的成神经细胞瘤。*neu* 原癌基因的活化形式产生自所编码蛋白质跨膜区中的点突变（缬氨酸变成谷氨酸）。在乳癌和卵巢癌中观察到 *neu* 的人同系物的扩增，而且这与预后不良有关（Slamon *et al.*, *Science* 235: 177-182 (1987); Slamon *et al.*, *Science* 244: 707-712 (1989); 及美国专利 4,968,603）。迄今为止，对于人类肿瘤尚无 *neu* 原癌基因中与之类似的点突变的报道。在其它癌瘤中也已观察到 HER2 的过表达（频繁但不均一，原因在于基因扩增），包括胃、子宫内膜、唾液腺、肺、肾、结肠、甲状腺、胰

和膀胱的癌瘤。参见 King *et al.*, *Science* 229: 974 (1985); Yokota *et al.*, *Lancet* 1: 765-767 (1986); Fukushige *et al.*, *Mol. Cell Biol.* 6: 955-958 (1986); Guerin *et al.*, *Oncogene Res.* 3: 21-31 (1988); Cohen *et al.*, *Oncogene* 4: 81-88 (1989); Yonemura *et al.*, *Cancer Res.* 51: 1034 (1991); Borst *et al.*, *Gynecol. Oncol.* 38: 364 (1990); Weinere *et al.*, *Cancer Res.* 50: 421-425 (1990); Kern *et al.*, *Cancer Res.* 50: 5184 (1990); Park *et al.*, *Cancer Res.* 49: 6605 (1989); Zhau *et al.*, *Mol. Carcinog.* 3: 254-257 (1990); Aasland *et al.*, *Br. J. Cancer* 57: 358-363 (1988); Williams *et al.*, *Pathobiology* 59: 46-52 (1991); 及 McCann *et al.*, *Cancer* 65: 88-92 (1990)等。HER2 可在前列腺癌中过表达（Gu *et al.*, *Cancer Lett.* 99: 185-9 (1996); Ross *et al.*, *Hum. Pathol.* 28: 827-33 (1997); Ross *et al.*, *Cancer* 79: 2162-70 (1997); 及 Sadasivan *et al.*, *J. Urol.* 150: 126-31 (1993)）。

已经描述了针对大鼠 $p185^{neu}$ 和人 HER2 蛋白质产物的抗体。Drebin 及其同事制备了针对大鼠 *neu* 基因产物，$p185^{neu}$ 的抗体。参见例如 Drebin *et al.*, *Cell* 41: 695-706 (1985); Myers *et al.*, *Meth. Enzym.* 198: 277-290 (1991); 及 WO94/22478。Drebin *et al.*, *Oncogene* 2: 273-277 (1988)报道了与 $p185^{neu}$ 的两个独特区域有反应性的抗体混合物对植入裸鼠的 *neu* 转化 NIH-3T3 细胞产生协同的抗肿瘤作用。还可参见 1998 年 10 月 20 日发布的美国专利 5,824,311。

Hudziak *et al.*, *Mol. Cell. Biol.* 9(3): 1165-1172 (1989)描述了一组 HER2 抗体的生成，并利用人乳房肿瘤细胞系 SK-BR-3 鉴定。通过 72 小时后单层细胞的结晶紫染色测定了暴露于抗体后 SK-BR-3 细胞的相对细胞增殖。利用此测定法，用称作 4D5 的抗体获得了最大抑制，它抑制细胞增殖达 56%。该组其它抗体在此测定法中以较低程度降低细胞增殖。还发现抗体 4D5 使过表达 HER2 的乳房肿瘤细胞系对 TNF-α 的细胞毒性作用变得敏感。还可参见 1997 年 10 月 14 日发布的美国专利 5,677,171。Hudziak 等人的文章中讨论的抗体还在以下文献中进行了鉴定：Fendly *et al.*, *Cancer Research* 50: 1550-1558 (1990); Kotts *et al.*, *In Vitro* 26(3): 59A (1990); Sarup *et al.*, *Growth Regulation* 1: 72-82 (1991); Shepard *et al.*, *J. Clin. Immunol.* 11(3): 117-127 (1991); Kumar *et al.*, *Mol. Cell. Biol.* 11(2): 979-986 (1991); Lewis *et al.*, *Cancer Immunol. Immunother.* 37: 255-263 (1993); Pietras *et al.*, *Oncogene* 9: 1829-1838 (1994); Vitetta *et al.*, *Cancer Research* 54: 5301-5309 (1994); Sliwkowski *et al.*, *J. Biol. Chem.* 269(20): 14661-14665 (1994); Scott *et al.*, *J. Biol. Chem.* 266:

14300-5 (1991); D'souza *et al.*, *Proc. Natl. Acad. Sci.* 91: 7202-7206 (1994); Lewis *et al.*, *Cancer Research* 56: 1457-1465 (1996); 及 Schaefer *et al.*, *Oncogene* 15: 1385-1394 (1997)。

鼠 HER2 抗体 4D5 的重组人源化型式（huMAb4D5-8、rhuMAb HER2、Trastuzumab 或 HERCEPTIN®; 美国专利 5,821,337）在临床上对患有 HER2 过表达的转移性乳癌并已接受广泛的早期抗癌疗法的患者起作用（Baselga *et al.*, *J. Clin. Oncol.* 14: 737-744 (1996)）。Trastuzumab 在 1998 年 9 月 25 日得到了食品和药品管理局的销售许可，可用于治疗所患肿瘤过表达 HER2 蛋白质的转移性乳癌患者。

以下文献中描述了具有各种特性的其它 HER2 抗体: Tagliabue *et al.*, *Int. J. Cancer* 47: 933-937 (1991); McKenzie *et al.*, *Oncogene* 4: 543-548 (1989); Maier *et al.*, *Cancer Res.* 51: 5361-5369 (1991); Bacus *et al.*, *Molecular Carcinogenesis* 3: 350-362 (1990); Stancovski *et al.*, *PNAS (USA)* 88: 8691-8695 (1991); Bacus *et al.*, *Cancer Research* 52: 2580-2589 (1992); Xu *et al.*, *Int. J. Cancer* 53: 401-408 (1993); WO94/00136; Kasprzyk *et al.*, *Cancer Research* 52: 2771-2776 (1992); Hancock *et al.*, *Cancer Res.* 51: 4575-4580 (1991); Shawver *et al.*, *Cancer Res.* 54: 1367-1373 (1994); Arteaga *et al.*, *Cancer Res.* 54: 3758-3765 (1994); Harwerth *et al.*, *J. Biol. Chem.* 267: 15160-15167 (1992); 美国专利 5,783,186; 及 Klapper *et al.*, *Oncogene* 14: 2099-2109 (1997)。

同源性筛选使得 HER 受体家族的两个其它成员得以鉴定: HER3（美国专利 5,183,884 和 5,480,968 以及 Kraus *et al.*, *PNAS (USA)* 86: 9193-9197 (1989)）和 HER4（欧洲专利申请 599,274; Plowman *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 1746-1750 (1993); 及 Plowman *et al.*, *Nature* 366: 473-475 (1993)）。这两种受体均在至少有些乳癌细胞系中展示出表达升高。

通常发现 HER 受体在细胞中有多种组合，而且认为它的二聚化增加了对各种 HER 配体的细胞应答的多样性（Earp *et al.*, *Breast Cancer Research and Treatment* 35: 115-132 (1995)）。EGFR 受到 6 种不同配体的结合: 表皮生长因子(EGF)、转化生长因子 α (TGF-α)、双调蛋白、肝素结合表皮生长因子(HB-EGF)、betacellulin 和 epiregulin（Groenen *et al.*, *Growth Factors* 11: 235-257 (1994)）。由单一基因的可变剪接产生的一个调蛋白蛋白质家族是 HER3 和 HER4 的配体。调蛋白家族包括 α、β 和 γ 调蛋白（Holmes *et al.*,

Science 256: 1205-1210 (1992); 美国专利 5,641,869; 及 Schaefer *et al.*, *Oncogene* 15: 1385-1394 (1997)); neu 分化因子(NDF); 神经胶质生长因子(GGF); 乙酰胆碱受体诱导活性(ARIA); 及感觉和运动神经元衍生因子(SMDF)。有关综述参见 Groenen *et al.*, *Growth Factors* 11: 235-257 (1994); Lemke, G, *Molec. & Cell. Neurosci.* 7: 247-262 (1996); 及 Lee *et al.*, *Pharm. Rev.* 47: 51-85 (1995)。最近还鉴定了另外三种 HER 配体: 神经调节蛋白-2 (NRG-2), 据报道它结合 HER3 或 HER4 (Chang *et al.*, *Nature* 387: 509-512 (1997); 及 Carraway *et al.*, *Nature* 387: 512-516 (1997)); 神经调节蛋白-3, 它结合 HER4 (Zhang *et al.*, *PNAS (USA)* 94(18): 9562-7 (1997)); 和神经调节蛋白-4, 它结合 HER4 (Harari *et al.*, *Oncogene* 18: 2681-89 (1999))。HB-EGF、betacellulin 和 epiregulin 也结合 HER4。

尽管 EGF 和 TGFα 不结合 HER2, 但是 EGF 刺激 EGFR 和 HER2 形成异二聚体, 它活化 EGFR 并导致异二聚体中 HER2 的转磷酸作用。二聚化作用和/或转磷酸作用看来活化 HER2 酪氨酸激酶。参见 Earp *et al.*, 见上文。同样, 当 HER3 与 HER2 共表达时, 形成有活性的信号复合物, 而针对 HER2 的抗体能破坏此复合物 (Sliwkowski *et al.*, *J. Biol. Chem.* 269(20): 14661-14665 (1994))。此外, 在与 HER2 共表达时, HER3 对调蛋白(HRG)的亲和力提高到了较高的亲和力状态。关于 HER2-HER3 蛋白质复合物还可参见 Levi *et al.*, *Journal of Neuroscience* 15: 1329-1340 (1995); Morrissey *et al.*, *Proc. Natl. Acad. Sci. USA* 92: 1431-1435 (1995); 及 Lewis *et al.*, *Cancer Res.* 56: 1457-1465 (1996)。HER4, 与 HER3 类似、与 HER2 形成有活性的信号复合物 (Carraway and Cantley, *Cell* 78: 5-8 (1994))。

为了靶向 HER 信号途径, 将 rhuMAb 2C4 (Pertuzumab, OMNITARG™) 开发成抑制 HER2 与其它 HER 受体二聚化的人源化抗体, 从而抑制配体驱动的磷酸化作用和活化作用, 及 RAS 和 AKT 途径的下游激活。在 Pertuzumab 作为治疗实体瘤的单一药剂的 I 期试验中, 用 Pertuzumab 治疗患有晚期卵巢癌的 3 名受试者。一人具有持久的部分响应, 而另一名受试者疾病稳定了 15 周。Agus *et al. Proc Am Soc Clin Oncol* 22: 192, Abstract 771 (2003)。

抗体变体组合物

美国专利 6,339,142 描述了包含抗 HER2 抗体及其一种或多种酸性变体

的混合物的 HER2 抗体组合物，其中所述酸性变体的量少于约 25%。Trastuzumab 是例示性的 HER2 抗体。

Reid 等人在充分表征的生物技术药物会议(Well Characterized Biotech Pharmaceuticals Conference)（2003 年 1 月）上呈现的海报 "Effects of Cell Culture Process Changes on Humanized Antibody Characteristics"描述了一种未命名的人源化 IgG1 抗体组合物，它由于其重链上 VHS 信号肽、N-末端谷氨酰胺和焦谷氨酸的组合而具有 N-末端异质性。

Reed 等人在 IBC 抗体生成会议(IBC Antibody Production Conference)（2002 年 2 月）上呈现的海报 "The Ideal Chromatographic Antibody Characterization Method" 报告了人源化抗 IgE 抗体 E25 的重链上的 VHS 延伸。

Rouse 等人在 WCBP（2004 年 1 月 6-9 日）上呈现的海报 " 'Top Down' Glycoprotein Characterization by High Resolution Mass Spectrometry and Its Application to Biopharmaceutical Development" 描述了一种单克隆抗体组合物，它由于其轻链上的$^{-3}$AHS 或$^{-2}$HS 信号肽残基而具有 N-末端异质性。

Jill Porter 在 IBC 会议（2000 年 9 月）上呈现的海报 "Strategic Use of Comparability Studies and Assays for Well Characterized Biologicals" 讨论了 ZENAPAX™的晚期洗脱形式，它在其重链上具有三个额外的氨基酸残基。

发明概述

本发明关注包含结合 HER2 结构域 II 的主要种类 HER2 抗体及其包含氨基末端前导延伸的氨基酸序列变体的组合物。

另外，本发明提供了包含主要种类 HER2 抗体及其氨基酸序列变体的混合物的组合物，所述主要种类抗体包含分别在 SEQ ID No. 3 和 4 中的轻链和重链可变区序列，而所述氨基酸序列变体包含附着于其一个或两个轻链可变区的 VHS-氨基末端前导延伸，其中组合物中约 1%至约 20%的抗体分子包含 VHS-氨基末端前导延伸。

本发明还关注包含 SEQ ID No. 23 中的氨基酸序列的多肽或其脱酰胺和/或氧化变体，以及包含轻链和重链的抗体，(a) 所述轻链包含该多肽，而(b) 所述重链包含选自下组的氨基酸序列：SEQ ID No. 16、SEQ ID No. 24、和 SEQ ID No. 16 或 SEQ ID No. 24 的脱酰胺和/或氧化变体。

本发明还关注在药学可接受载体中包含所述组合物的药用配制剂，以及在患者中治疗表达HER2的癌症的方法，包括以有效治疗癌症的量对患者施用所述药用配制剂。

附图简述

图1提供了HER2蛋白质结构的示意图，及其胞外域的结构域I-IV的氨基酸序列（分别是SEQ ID No. 19-22）。

图2A和2B描绘了鼠源单克隆抗体2C4的轻链可变区(V_L)（图2A）和重链可变区(V_H)（图2B）（分别是SEQ ID No. 1和2）；人源化2C4型式574的V_L和V_H结构域（分别是SEQ ID No. 3和4），及人V_L和V_H共有框架（hum κ1，轻链κ亚组I；hum III，重链亚组III）（分别是SEQ ID No. 5和6）的氨基酸序列对比。星号标示人源化2C4型式574和鼠源单克隆抗体2C4之间或人源化2C4型式574和人框架之间的差异。互补决定区(CDR)包在括号中。

图3A和3B显示了Pertuzumab轻链（SEQ ID No. 15）和重链（SEQ ID No. 16）的氨基酸序列。CDR以粗体显示。碳水化合物模块(moiety)附着于重链的Asn299。

图4A和4B显示了Pertuzumab轻链（SEQ ID No. 17）和重链的氨基酸序列，各自包含完整的氨基末端信号肽序列（SEQ ID No. 18）。

图5以图描述了2C4在HER2的异二聚体结合位点处的结合，由此阻止与活化的EGFR或HER3的异二聚化。

图6描绘了HER2/HER3与MAPK和Akt途径的偶联。

图7比较了Trastuzumab和Pertuzumab的活性。

图8A和8B显示了还原的Pertuzumab轻链（图8A）和重链（图8B）的重建质谱。

图9A和9B描绘了天然Pertuzumab（图9A）和经CPB消化的Pertuzumab（图9B）的阳离子交换层析分析。

图10显示了Pertuzumab的大小排阻层析分析。

图11A和11B显示了还原的Pertuzumab（图11A）和完整Pertuzumab（图11B）的CE-SDS-LIF分析。

图12A和12B描绘了Pertuzumab的胰蛋白酶消化肽图（图12A）和Pertuzumab的LYS-C消化肽图（图12B）。

图 13 显示了从 Pertuzumab 释放的 N-连接寡糖的 CE 分析。

图 14 显示了常常在 IgG 抗体中观察到的寡糖结构。

图 15 描绘了从 Pertuzumab 释放的中性寡糖的主动模式(positive mode) MALDI-TOF 质谱。

图 16A 和 16B 显示了 Trastuzumab 轻链（SEQ ID No. 13）和重链（SEQ ID No. 14）的氨基酸序列。

图 17A 和 17B 描绘了一种变体 Pertuzumab 轻链序列（SEQ ID No. 23）和一种变体 Pertuzumab 重链序列（SEQ ID No. 24）。

优选实施方案的详述

I. 定义

术语“主要种类抗体”在本文中指组合物中这样的抗体氨基酸序列结构，它是该组合物中在数量上最主要的抗体分子。优选的是，主要种类抗体是 HER2 抗体，诸如结合 HER2 结构域 II 的抗体、比 Trastuzumab 更有效的抑制 HER 二聚化的抗体、和/或结合 HER2 的异二聚体结合位点的抗体。本文中主要种类抗体的优选实施方案是包含 SEQ ID No. 3 和 4 中的轻链和重链可变区氨基酸序列的抗体，且最优选包含 SEQ ID No. 15 和 16 中的轻链和重链氨基酸序列（Pertuzumab）。

“氨基酸序列变体”抗体在本文中指具有与主要种类抗体不同的氨基酸序列的抗体。通常，氨基酸序列变体将与主要种类抗体具有至少约 70%的同源性，而且优选的是，它们将是与主要种类抗体至少约 80%，且更优选至少约 90%同源。氨基酸序列变体在主要种类抗体氨基酸序列内部或邻近的某些位置具有替代、删除和/或添加。本文中氨基酸序列变体的例子包括酸性变体（如脱酰胺抗体变体）、碱性变体、在其一条或两条轻链上具有氨基末端前导延伸（如 VHS-）的抗体、在其一条或两条重链上具有 C-末端赖氨酸残基的抗体、具有一个或多个氧化后甲硫氨酸残基的抗体等，且包括重链和/或轻链氨基酸序列变异的组合。本文中特别感兴趣的抗体变体是在其一条或两条轻链上包含氨基末端前导延伸的抗体，任选相对于主要种类抗体还包含其它氨基酸序列和/或糖基化差异。

“糖基化变体”抗体在本文中指有一个或多个碳水化合物模块附着于它的抗体、所述碳水化合物与附着于主要种类抗体的一个或多个碳水化合物模

块不同。本文中糖基化变体的例子包括有 G1 或 G2 寡糖结构代替 G0 寡糖结构附着于其 Fc 区的抗体、有一个或多个碳水化合物模块附着于其一条或两条轻链的抗体、没有碳水化合物模块附着于其一条或两条重链的抗体等，以及这些糖基化改变的组合。

若抗体具有 Fc 区，则本文中诸如图 14 所示的寡糖结构可附着于抗体的一条或两条重链，如位于残基 299。对于 Pertuzumab，G0 是最主要的寡糖结构，而在 Pertuzumab 组合物中发现其它寡糖结构，诸如 G0-F、G-1、Man5、Man6、G1-1、G1(1-6)、G1(1-3)和 G2 的量较少。

除非另有说明，“G1 寡糖结构”在本文中包括 G1(1-6)和 G1(1-3)结构。

“氨基末端前导延伸”在本文中指在抗体的任何一条或多条重链或轻链的氨基末端存在的氨基末端前导序列的一个或多个氨基酸残基。例示性的氨基末端前导延伸包含三个氨基酸残基、VHS 或由其组成，它们存在于抗体变体的一条或所有两条轻链上。

“脱酰胺”抗体指其中一个或多个天冬酰胺残基衍生成例如天冬氨酸、琥珀酰亚胺或异天冬氨酸的抗体。

“同源性”定义为在必要时对比序列和引入缺口以获取最大百分比同一性后，氨基酸序列变体中相同残基的百分比。用于对比的方法和计算机程序在本领域是众所周知的。一种这样的计算机程序是“Align 2”，由 Genentech, Inc.创作，已经在 1991 年 12 月 10 日与用户文档(user documentation)一起提交给美国版权局（Washington, DC 20559）。

为了本文的目的，“阳离子交换分析”指根据电荷差异利用阳离子交换剂将包含两种或多种化合物的组合物分开的任何方法。阳离子交换剂通常包含共价结合的，带负电荷的基团。优选的是，本文中的阳离子交换剂是弱阳离子交换剂和/或包含羧酸盐/酯官能团，诸如 Dionex 出售的 PROPAC WCX-10™阳离子交换柱。

“HER 受体”是属于 HER 受体家族的受体蛋白质酪氨酸激酶，包括 EGFR、HER2、HER3 和 HER4 受体及未来将鉴定的此家族其它成员。HER 受体通常将包含胞外结构域，它可结合 HER 配体；亲脂性跨膜结构域；保守的胞内酪氨酸激酶结构域；和含有几个可被磷酸化的酪氨酸残基的羧基末端信号结构域。优选的是，HER 受体是天然序列人 HER 受体。

HER2 的胞外域包含 4 个结构域：结构域 I（大约第 1-195 位氨基酸残基）、

结构域 II（大约第 196-319 位氨基酸残基）、结构域 III（大约第 320-488 位氨基酸残基）和结构域 IV（大约第 489-630 位氨基酸残基）（残基编号不包括信号肽）。参见 Garrett *et al.*, *Mol. Cell.* 11: 495-505 (2003); Cho *et al.*, *Nature* 421: 756-760 (2003); Franklin *et al.*, *Cancer Cell* 5: 317-328 (2004); 或 Plowman *et al.*, *Proc. Natl. Acad. Sci.* 90: 1746-1750 (1993)。还可参见本文图 1。

术语 "ErbB1"、"HER1"、"表皮生长因子受体" 和 "EGFR" 在本文中可互换使用，指例如 Carpenter *et al.*, *Ann. Rev. Biochem.* 56: 881-914 (1987)中公开的 EGFR，包括其天然存在的突变体形式（如 Humphrey *et al.*, *PNAS (USA)* 87: 4207-4211 (1990)中的删除突变体 EGFR）。*erb*B1 指编码 EGFR 蛋白质产物的基因。

表述"ErbB2"和"HER2"在本文中可互换使用，指例如 Semba *et al.*, *PNAS (USA)* 82: 6497-6501 (1985)和 Yamamoto *et al.*, *Nature* 319: 230-234 (1986)中描述的人 HER2 蛋白（Genebank 编号 X03363）。术语"*erb*B2"指编码人 ErbB2 的基因，而 "*neu*" 指编码大鼠 p185^{neu} 的基因。优选的 HER2 是天然序列人 HER2。

"ErbB3" 和 "HER3" 指例如美国专利 5,183,884 和 5,480,968 及 Kraus *et al.*, *PNAS (USA)* 86: 9193-9197 (1989)中公开的受体多肽。

术语"ErbB4"和"HER4"在本文中指例如欧洲专利申请 599,274; Plowman *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 1746-1750 (1993); 及 Plowman *et al.*, *Nature* 366: 473-475 (1993)中公开的受体多肽，包括其同种型(isoform)，例如 1999 年 4 月 22 日出版的 WO99/19488 中所公开的。

"HER 配体" 指结合和/或活化 HER 受体的多肽。本文中特别感兴趣的 HER 配体是天然序列人 HER 配体，诸如表皮生长因子(EGF)（Savage *et al.*, *J. Biol. Chem.* 247: 7612-7621 (1972)）；转化生长因子 α (TGF-α)（Marquardt *et al.*, *Science* 223: 1079-1082 (1984)）；双调蛋白，又称为施旺细胞瘤(schwannoma)或角质形成细胞自分泌生长因子（Shoyab *et al.*, *Science* 243: 1074-1076 (1989); Kimura *et al.*, *Nature* 348: 257-260 (1990); 及 Cook *et al.*, *Mol. Cell. Biol.* 11: 2547-2557 (1991)）；betacellulin（Shing *et al.*, *Science* 259: 1604-1607 (1993); 及 Sasada *et al.*, *Biochem. Biophys. Res. Commun.* 190: 1173 (1993)）；肝素结合表皮生长因子(HB-EGF)（Higashiyama *et al.*, *Science* 251: 936-939

(1991)); epiregulin (Toyoda *et al.*, *J. Biol. Chem.* 270: 7495-7500 (1995); 及 Komurasaki *et al.*, *Oncogene* 15: 2841-2848 (1997)); α调蛋白 (见下文); 神经调节蛋白-2 (NRG-2) (Carraway *et al.*, *Nature* 387: 512-516 (1997)); 神经调节蛋白-3 (NRG-3) (Zhang *et al.*, *Proc. Natl. Acad. Sci.* 94: 9562-9567 (1997)); 神经调节蛋白-4 (NRG-4) (Harari *et al.*, *Oncogene* 18: 2681-89 (1999)); 或 cripto (CR-1) (Kannan *et al.*, *J. Biol. Chem.* 272(6): 3330-3335 (1997))。结合 EGFR 的 HER 配体包括 EGF、TGF-α、双调蛋白、betacellulin、HB-EGF 和 epiregulin。结合 HER3 的 HER 配体包括调蛋白。能够结合 HER4 的 HER 配体包括 betacellulin、epiregulin、HB-EGF、NRG-2、NRG-3、NRG-4 和调蛋白。

"调蛋白" (HRG)在用于本文时指由调蛋白基因编码的多肽，如美国专利 5,641,869 或 Marchionni *et al.*, *Nature* 362: 312-318 (1993)中所公开的。调蛋白的例子包括调蛋白-α、调蛋白-β1、调蛋白-β2 和调蛋白-β3 (Holmes *et al.*, *Science* 256: 1205-1210 (1992); 及美国专利 5,641,869); *neu* 分化因子 (NDF) (Peles *et al.*, *Cell* 69: 205-216 (1992)); 乙酰胆碱受体诱导活性(ARIA) (Falls *et al.*, *Cell* 72: 801-815 (1993)); 神经胶质生长因子(GGF) (Marchionni *et al.*, *Nature* 362: 312-318 (1993)); 感觉和运动神经元衍生因子(SMDF) (Ho *et al.*, *J. Biol. Chem.* 270: 14523-14532 (1995)); γ-调蛋白 (Schaefer *et al.*, *Oncogene* 15: 1385-1394 (1997))。此术语包括天然序列 HRG 多肽的生物学活性片段和/或氨基酸序列变体，诸如其 EGF 样结构域片段 (如 $HRG\beta1_{177-244}$)。

"HER 二聚体" 在本文中指包含至少两个不同 HER 受体的非共价结合二聚体。当表达两种或多种 HER 受体的细胞暴露于 HER 配体时可能形成此类复合物，可通过免疫沉淀进行分离并通过 SDS-PAGE 进行分析，如例如 Sliwkowski *et al.*, *J. Biol. Chem.* 269(20): 14661-14665 (1994)中所述。此类 HER 二聚体的例子包括 EGFR-HER2、HER2-HER3 和 HER3-HER4 异二聚体。此外，HER 二聚体可包含与诸如 HER3、HER4 或 EGFR 等不同 HER 受体组合的两个或多个 HER2 受体。其它蛋白质，诸如细胞因子受体亚基(如 gp130) 可与所述二聚体结合。

HER2 上的 "异二聚体结合位点" 指 HER2 胞外域中在与 EGFR、HER3 或 HER4 形成二聚体时，接触 EGFR、HER3 或 HER4 胞外域中某区域或与 EGFR、HER3 或 HER4 胞外域中某区域形成介面的区域。已发现所述区域

在 HER2 的结构域 II 中。Franklin *et al.*, *Cancer Cell* 5: 317-328 (2004)。

"HER 活化"或"HER2 活化"指任一种或多种 HER 受体或 HER2 受体的活化或磷酸化作用。一般而言，HER 活化导致信号转导（例如由 HER 受体胞内激酶结构域引起的，磷酸化 HER 受体或底物多肽中的酪氨酸残基）。HER 活化可由结合包含目的 HER 受体的 HER 二聚体的 HER 配体介导。结合 HER 二聚体的 HER 配体可活化二聚体中一种或多种 HER 受体的激酶结构域，从而导致一种或多种 HER 受体中酪氨酸残基的磷酸化作用和/或其它底物多肽中酪氨酸残基的磷酸化作用，诸如 Akt 或 MAPK 胞内激酶。

术语"抗体"在本文中使用最广的含义，具体覆盖完整的单克隆抗体、多克隆抗体、由至少两种完整抗体形成的多特异性抗体（如双特异性抗体）、及抗体片段，只要它们展现所需生物学活性。

术语"单克隆抗体"在用于本文时指由一群基本上同质的抗体获得的抗体，即构成群体的各个抗体相同和/或结合相同表位，除了生产单克隆抗体的过程中可能产生的可能变体外，诸如本文描述的那些抗体。与典型的包含针对不同决定簇（表位）的不同抗体的多克隆抗体制品不同，每种单克隆抗体针对抗原上的同一决定簇。除了它们的特异性以外，单克隆抗体的优越性体现在它们未受到其它免疫球蛋白的污染。修饰语"单克隆"指示由基本上同质的抗体群获得的抗体的特征，并不解释为需要通过任何特定方法来生产抗体。例如，依照本发明使用的单克隆抗体可通过最初由 Kohler *et al.*, *Nature* 256: 495 (1975)描述的杂交瘤方法来制备，或者可通过重组 DNA 方法来制备（参见例如美国专利 4,816,567）。"单克隆抗体"还可使用例如 Clackson *et al.*, *Nature* 352: 624-628 (1991)和 Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1991)中描述的技术从噬菌体抗体库分离。

单克隆抗体在本文中具体包括"嵌合"抗体，其中重链和/或轻链的一部分与衍生自特定物种或属于特定抗体类别或亚类的抗体中的相应序列相同或同源，而链的剩余部分与衍生自另一物种或属于另一抗体类别或亚类的抗体中的相应序列相同或同源，以及此类抗体的片段，只要它们展现所需生物学活性（美国专利 4,816,567; 及 Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81: 6851-6855 (1984)）。本文中感兴趣的嵌合抗体包括包含衍生自非人灵长类动物（如旧大陆猴类(Old World Monkey)、猿等）的可变区抗原结合序列和人恒定区序列的"灵长类化(primatized)"抗体。

“抗体片段”包含完整抗体的一部分，优选包含其抗原结合区或可变区。抗体片段的例子包括 Fab、Fab'、$F(ab')_2$ 和 Fv 片段；双抗体；线性抗体；单链抗体分子；及由抗体片段形成的多特异性抗体。

“完整抗体”指包含抗原结合可变区以及轻链恒定区（C_L）和重链恒定区 C_H1、C_H2 和 C_H3 的抗体。恒定区可以是天然序列恒定区（如人天然序列恒定区）或其氨基酸序列变体。优选的是，完整抗体具有一项或多项效应物功能，且包含附着于其一条或两条重链的寡糖结构。

抗体“效应物功能”指那些可归于抗体 Fc 区（天然序列 Fc 区或氨基酸序列变体 Fc 区）的生物学活性。抗体效应物功能的例子包括 C1q 结合；补体依赖性细胞毒性；Fc 受体结合；抗体依赖性细胞介导的细胞毒性(ADCC)；吞噬作用；细胞表面受体（如 B 细胞受体；BCR）的下调等。

根据完整抗体的重链恒定区的氨基酸序列，可将其归入不同的“类”(class)。完整抗体有五种主要的类别：IgA、IgD、IgE、IgG 和 IgM，其中有些可进一步分为“亚类”(subclass)（同种型），如 IgG1、IgG2、IgG3、IgG4、IgA1 和 IgA2。将与不同抗体类别对应的重链恒定区分别称作 α、δ、ε、γ 和 μ。不同类别免疫球蛋白的亚基结构和三维构造是众所周知的。

“抗体依赖性细胞介导的细胞毒性”和“ADCC”指由细胞介导的反应，其中表达 Fc 受体(FcR)的非特异性细胞毒性细胞（如天然杀伤(NK)细胞、嗜中性粒细胞和巨噬细胞）识别靶细胞上结合的抗体，随后引起靶细胞溶解。介导 ADCC 的主要细胞，NK 细胞，只表达 FcγRIII，而单核细胞表达 FcγRI、FcγRII 和 FcγRIII。Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991)中第 464 页表 3 总结了造血细胞上的 FcR 表达。为了评估目的分子的 ADCC 活性，可进行体外 ADCC 测定法，诸如美国专利 5,500,362 或 5,821,337 中所描述的。可用于此类测定法的效应细胞包括外周血单核细胞(PBMC)和天然杀伤(NK)细胞。或者/另外，可在体内评估目的分子的 ADCC 活性，例如在动物模型中，诸如 Clynes *et al.*, *PNAS(USA)* 95: 652-656 (1998)中所公开的。

“人效应细胞”指表达一种或多种 FcR 并行使效应物功能的白细胞。优选的是，该细胞至少表达 FcγRIII 并执行 ADCC 效应物功能。介导 ADCC 的人白细胞的例子包括外周血单核细胞(PBMC)、天然杀伤(NK)细胞、单核细胞、细胞毒性 T 细胞和嗜中性粒细胞，优选 PBMC 和 NK 细胞。效应细胞可以从其天然来源中分离，例如血液或 PBMC，正如本文所述。

术语“Fc 受体”和“FcR”用于描述与抗体 Fc 区结合的受体。优选的 FcR 是天然序列人 FcR。此外，优选的 FcR 是与 IgG 抗体结合的 FcR（γ 受体），包括 FcγRI、FcγRII 和 FcγRIII 亚类的受体，包括这些受体的等位基因变体和可变剪接形式。FcγRII 受体包括 FcγRIIA（“活化受体”）和 FcγRIIB（“抑制受体”），它们具有相似的氨基酸序列，区别主要在于其胞质域。活化受体 FcγRIIA 在其胞质域中包含免疫受体基于酪氨酸的活化基序(ITAM)。抑制受体 FcγRIIB 在其胞质域中包含免疫受体基于酪氨酸的抑制基序(ITIM)（参见综述 Daëron, *Annu. Rev. Immunol.* 15: 203-234 (1997)）。FcR 的综述参见 Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-492 (1991); Capel *et al.*, *Immunomethods* 4: 25-34 (1994); 及 de Haas *et al.*, *J. Lab. Clin. Med.* 126: 330-341 (1995)）。术语“FcR”在本文中涵盖其它 FcR，包括未来将会鉴定的 FcR。该术语还包括新生儿受体，FcRn，它负责将母体的 IgG 转移给胎儿（Guyer *et al.*, *J. Immunol.* 117: 587 (1976)和 Kim *et al.*, *J. Immunol.* 24: 249 (1994)）。

“补体依赖性细胞毒性”或“CDC”指在存在补体时分子溶解靶物的能力。补体激活途径是由补体系统第一组分(C1q)结合与关联抗原复合的分子（如抗体）起始的。为了评估补体激活，可进行 CDC 测定法，例如 Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202: 163 (1996)中所描述的。

“天然抗体”通常是由两条相同的轻链(L)和两条相同的重链(H)构成的约 150,000 道尔顿的异四聚体糖蛋白。每条轻链通过一个共价二硫键与重链连接，而二硫键的数目在不同免疫球蛋白同种型的重链中有所变化。每条重链和轻链还具有间隔规律的链内二硫桥。每条重链在一端具有一个可变区(V_H)，接着是多个恒定区。每条轻链在一端具有一个可变区(V_L)，而另一端是一个恒定区。轻链的恒定区与重链的第一恒定区排列在一起，而轻链的可变区与重链的可变区排列在一起。认为特定的氨基酸残基在轻链和重链可变区之间形成界面。

术语“可变的”指可变区中的某些部分在抗体序列中差异广泛且用于每种特定抗体针对其特定抗原的结合和特异性的实情。然而，变异性并非均匀分布于抗体的整个可变区。它集中于轻链和重链可变区中称作高变区的三个区段。可变区中更加高度保守的部分称作框架区(FR)。天然重链和轻链的可变区各自包含四个 FR，它们大多采取 β-折叠构象，通过形成环状连接且在

有些情况中形成β-折叠结构一部分的三个高变区连接。每条链中的高变区通过 FR 非常接近的保持在一起，并与另一条链的高变区一起促成抗体的抗原结合位点的形成（参见 Kabat 等人，《*Sequences of Proteins of Immunological Interest*》，第 5 版，Public Health Service，National Institutes of Health，Bethesda，MD，1991）。恒定区不直接涉及抗体与抗原的结合，但展现多种效应物功能，诸如抗体依赖性细胞细胞毒性(ADCC)中抗体的参与。

术语“高变区”在用于本文时指抗体中负责抗原结合的氨基酸残基。高变区通常包含来自“互补决定区”或“CDR”的氨基酸残基（例如轻链可变区中的残基 24-34（L1）、50-56（L2）和 89-97（L3）及重链可变区中的 31-35（H1）、50-65（H2）和 95-102（H3）；Kabat 等人，《*Sequences of Proteins of Immunological Interest*》，第 5 版，Public Health Service，National Institutes of Health，Bethesda，MD，1991）和/或那些来自“高变环”的残基（例如轻链可变区中的残基 26-32（L1）、50-52（L2）和 91-96（L3）及重链可变区中的 26-32（H1）、53-55（H2）和 96-101（H3）；Chothia and Lesk, *J. Mol. Biol.* 196: 901-917 (1987)）。“框架区”或“FR”残基指本文定义的高变区残基以外的可变区残基。

用木瓜蛋白酶消化抗体产生两个相同的抗原结合片段，称作“Fab”片段，各自具有一个抗原结合位点，和一个残余“Fc”片段，其名称反映了它易于结晶的能力。胃蛋白酶处理产生一个 $F(ab')_2$ 片段，它具有两个抗原结合位点，并且仍能够交联抗原。

“Fv”是包含完整抗原识别和抗原结合位点的最小抗体片段。该区域由紧密、非共价结合的一个重链可变区和一个轻链可变区的二聚体组成。正是在这种构造中，各个可变区的三个高变区相互作用而在 V_H-V_L 二聚体表面确定了一个抗原结合位点。六个高变区共同赋予抗体以抗原结合特异性。然而，即使是单个可变区（或只包含对抗原特异的三个高变区的半个 Fv）也具有识别和结合抗原的能力，尽管亲和力低于完整结合位点。

Fab 片段还包含轻链的恒定区和重链的第一恒定区(CH1)。Fab’片段因在重链 CH1 结构域的羧基末端增加了少数残基而与 Fab 片段有所不同，包括来自抗体铰链区的一个或多个半胱氨酸。Fab’-SH 是本文中对其中恒定区的半胱氨酸残基携带至少一个游离硫醇基的 Fab’的称谓。$F(ab')_2$ 抗体片段最初是作为成对 Fab’片段生成的，在 Fab’片段之间具有铰链半胱氨酸。还知道抗

体片段的其它化学偶联。

来自任何脊椎动物物种的抗体的“轻链”，根据其恒定区的氨基酸序列，可归入两种截然不同类型中的一种，称作卡帕(κ)和拉姆达(λ)。

“单链 Fv”或“scFv”抗体片段包含抗体的 V_H 和 V_L 结构域，其中这些结构域存在于一条多肽链上。优选的是，该 Fv 多肽在 V_H 和 V_L 结构域之间还包含多肽接头，使得 scFv 形成抗原结合所需的结构。关于 scFv 的综述参见 Plückthun，在《*The Pharmacology of Monoclonal Antibodies*》中，第 113 卷，Rosenburg 和 Moore 编，Springer-Verlag，New York，269-315，1994。HER2 抗体 scFv 片段描述于 WO93/16185；美国专利 5,571,894；和美国专利 5,587,458。

术语“双抗体”指具有两个抗原结合位点的小型抗体片段，该片段在同一条多肽链（V_H-V_L）中包含相连的重链可变区(V_H)和轻链可变区(V_L)。通过使用过短的接头使得同一条链上的两个结构域之间不能配对，迫使结构域与另一条链的互补结构域配对，并产生两个抗原结合位点。双抗体更完整的描述于例如 EP 404,097；WO 93/11161；和 Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993)。

非人（如啮齿类）抗体的“人源化”形式指最低限度包含衍生自非人免疫球蛋白的序列的嵌合抗体。在极大程度上，人源化抗体指人免疫球蛋白（受体抗体）中的高变区残基用具有所需特异性、亲和力和能力的非人物种（供体抗体）诸如小鼠、大鼠、兔或非人灵长类的高变区残基替换的免疫球蛋白。在有些情况中，将人免疫球蛋白的框架区(FR)残基用相应的非人残基替换。此外，人源化抗体可包含在受体抗体或供体抗体中没有发现的残基。进行这些修饰是为了进一步改进抗体的性能。通常，人源化抗体将包含基本上不少于至少一个、通常两个如下可变区，其中所有或基本上所有的高变环对应于非人免疫球蛋白的高变环，且所有或基本上所有的 FR 是人免疫球蛋白序列的 FR。任选的是，人源化抗体还将包含至少部分的免疫球蛋白恒定区(Fc)，通常是人免疫球蛋白的恒定区。更多细节参见 Jones *et al.*, *Nature* 321: 522-525 (1986); Riechmann *et al.*, *Nature* 332: 323-329 (1988)；及 Presta, *Curr. Op. Struct. Biol.* 2: 593-596 (1992)。

人源化 HER2 抗体包括 huMAb4D5-1、huMAb4D5-2、huMAb4D5-3、huMAb4D5-4、huMAb4D5-5、huMAb4D5-6、huMAb4D5-7 和 huMAb4D5-8

或 Trastuzumab（HERCEPTIN®），如美国专利 5,821,337 的表 3 中所述，明确收入本文作为参考；人源化 520C9（WO93/21319）和人源化 2C4 抗体，如本文所述。

为了本文的目的，“Trastuzumab”、“HERCEPTIN®”和“huMAb4D5-8”指包含分别在 SEQ ID No. 13 和 14 中的轻链和重链氨基酸序列的抗体。

在本文中，“Pertuzumab”、“OMNITARG™”和“rhuMAb 2C4”指包含分别在 SEQ ID No. 3 和 4 中的轻链和重链可变区氨基酸序列的抗体。若 Pertuzumab 是完整抗体，则它优选包含分别在 SEQ ID No. 15 和 16 中的轻链和重链氨基酸序列。

“裸抗体”指未偶联异源分子，诸如细胞毒性模块或放射性标记物的抗体（如本文所定义的）。

“分离的”抗体指已经由其天然环境的一种成分鉴别和分离和/或回收的抗体。其天然环境的污染成分指将会干扰抗体的诊断或治疗用途的物质，可包括酶、激素、和其它蛋白质性质或非蛋白质性质的溶质。在优选的实施方案中，将抗体纯化至(1) 根据 Lowry 法的测定，抗体重量超过 95%，最优选重量超过 99%，(2) 足以通过使用转杯式测序仪获得至少 15 个残基的 N-末端或内部氨基酸序列的程度，或(3) 通过使用考马斯蓝或优选银染的还原或非还原条件下的 SDS-PAGE 达到同质。既然抗体的天然环境的至少一种成分不会存在，那么分离的抗体包括重组细胞内的原位抗体。然而，分离的抗体通常将通过至少一个纯化步骤来制备。

“比 Trastuzumab 更有效的抑制 HER 二聚化的”HER2 抗体指比 Trastuzumab 更有效（例如有效性提高至少约 2 倍）的减少或消除 HER 二聚体的抗体。优选的是，这样的抗体至少大约像选自下组的抗体那样有效的抑制 HER 二聚化：鼠单克隆抗体 2C4、鼠单克隆抗体 2C4 的 Fab 片段、完整的 Pertuzumab、和 Pertuzumab 的 Fab 片段。可直接通过研究 HER 二聚体，或者通过评估由 HER 二聚化引起的 HER 活化或下游信号传导，和/或通过评估抗体-HER2 结合位点等来评估对 HER 二聚化的抑制。用于筛选有能力比 Trastuzumab 更有效的抑制 HER 二聚化的抗体的测定法见 Agus *et al.*, *Cancer Cell* 2: 127-137 (2002)和 WO01/00245（Adams *et al.*）。只是作为例示，可通过评估例如下列各项来分析对 HER 二聚化的抑制：对 HER 二聚体形成的抑制（参见例如 Agus *et al.*, *Cancer Cell* 2: 127-137 (2002)的附图 1A-B 和

WO01/00245）；表达 HER 二聚体的细胞中 HER 配体活化的降低（例如 WO01/00245 和 Agus *et al.*, *Cancer Cell* 2: 127-137 (2002)的附图 2A-B）；对 HER 配体结合表达 HER 二聚体的细胞的阻断（例如 WO01/00245 和 Agus *et al.*, *Cancer Cell* 2: 127-137 (2002)的附图 2E）；在存在（或缺乏）HER 配体时对表达 HER 二聚体的癌细胞（如 MCF7、MDA-MD-134、ZR-75-1、MD-MB-175、T-47D 细胞）的细胞生长的抑制（例如 WO01/00245 和 Agus *et al.*, *Cancer Cell* 2: 127-137 (2002)的附图 3A-D）；对下游信号传导的抑制（例如对 HRG 依赖性 AKT 磷酸化的抑制或对 HRG 或 TGFα 依赖性 MAPK 磷酸化的抑制）（参见例如 WO01/00245 和 Agus *et al.*, *Cancer Cell* 2: 127-137 (2002)的附图 2C-D）。还可通过研究抗体-HER2 结合位点来评估抗体是否抑制 HER 二聚化，例如通过评估与 HER2 结合的抗体的结构或模型，诸如晶体结构（参见例如 Franklin *et al.*, *Cancer Cell* 5: 317-328 (2004)）。

HER2 抗体可比 Trastuzumab 更有效（例如有效性提高至少 2 倍）的“抑制 HRG 依赖性 AKT 磷酸化”和/或抑制“HRG 或 TGFα 依赖性 MAPK 磷酸化”（参见例如 Agus *et al.*, *Cancer Cell* 2: 127-137 (2002)和 WO01/00245）。

HER2 抗体可以是“不抑制 HER2 胞外域切割”的抗体（Molina *et al.*, *Cancer Res.* 61: 4744-4749 (2001)）。

“结合 HER2 的异二聚体结合位点的”HER2 抗体结合结构域 II 中的残基（且任选还结合 HER2 的胞外域以外的其它结构域，诸如结构域 I 和 III 中的残基），且至少在一定程度上能在空间上阻碍 HER2-EGFR、HER2-HER3 或 HER2-HER4 异二聚体的形成。Franklin *et al.*, *Cancer Cell* 5: 317-328 (2004)表征了存放在 RCSB 蛋白质数据库（ID Code IS78）的 HER2-Pertuzumab 晶体结构，举例说明了结合 HER2 的异二聚体结合位点的例示性抗体。

“结合 HER2 结构域 II 的”抗体结合结构域 II 中的残基和任选 HER2 的其它结构域，诸如结构域 I 和 III 中的残基。优选的是，结合结构域 II 的抗体结合 HER2 结构域 I、II 和 III 之间的连接处。

“生长抑制剂”在用于本文时指在体外或在体内抑制细胞，尤其是表达 HER 的癌细胞生长的化合物或组合物。因此，生长抑制剂可以是显著降低 S 期的表达 HER 细胞百分比的药剂。生长抑制剂的例子包括阻断细胞周期行进（处于 S 期以外的位置）的药剂，诸如诱导 G1 停滞和 M 期停滞的药剂。经典的 M 期阻断剂包括长春花生物碱类（长春新碱和长春碱）、紫杉醇、和

拓扑异构酶 II 抑制剂诸如多柔比星、表柔比星、柔红霉素、依托泊苷和博来霉素。那些阻滞 G1 的药剂也溢出进入 S 期停滞，例如 DNA 烷化剂诸如他莫昔芬、泼尼松、达卡巴嗪、双氯乙基甲胺、顺铂、甲氨喋呤、5-氟尿嘧啶和 ara-C。其它信息可参见在《*The Molecular Basis of Cancer*》中，Mendelsohn 和 Israel 编，第 1 章，题为 "Cell cycle regulation, oncogenes, and antieioplastic drugs"，Murakaini 等人，WB Saunders，Philadelphia，1995，尤其是第 13 页。

"生长抑制性" 抗体的例子是那些结合 HER2 并抑制过表达 HER2 的癌细胞生长的抗体。优选的生长抑制性 HER2 抗体在约 0.5-30μg/ml 的抗体浓度对细胞培养中 SK-BR-3 乳房肿瘤细胞的生长抑制大于 20%，优选大于 50%（例如从约 50%至约 100%），其中生长抑制是在 SK-BR-3 细胞暴露于抗体后 6 天测定的（参见 1997 年 10 月 14 日发布的美国专利 5,677,171）。该专利及下文中更详细的描述了 SK-BR-3 细胞生长抑制测定法。优选的生长抑制性抗体是鼠单克隆抗体 4D5 的人源化变体，例如 Trastuzumab。

"诱导凋亡" 的抗体指那些根据膜联蛋白 V 结合、DNA 断裂、细胞收缩、内质网膨胀、细胞破裂和/或膜囊形成（称作凋亡小体）的测定，诱导程序性细胞死亡的抗体。所述细胞通常是过表达 HER2 受体的细胞。优选的是，所述细胞是肿瘤细胞，例如乳房、卵巢、胃、子宫内膜、唾液腺、肺、肾、结肠、甲状腺、胰或膀胱细胞。在体外，所述细胞可以是 SK-BR-3、BT474、Calu3 细胞、MDA-MB-453、MDA-MB-361 或 SKOV3 细胞。有多种方法可用于评估与凋亡有关的细胞事件。例如，可通过膜联蛋白结合来测量磷脂酰丝氨酸(PS)易位；可通过 DNA 梯化(laddering)来评估 DNA 断裂；而可通过亚二倍体细胞的任何增加来评估伴随着 DNA 断裂的核/染色质浓缩。优选的是，诱导凋亡的抗体是在使用 BT474 细胞的膜联蛋白结合测定法（见下文）中导致对膜联蛋白结合的诱导相对于未处理细胞提高约 2 至 50 倍，优选约 5 至 50 倍，最优选约 10 至 50 倍的抗体。诱导凋亡的 HER2 抗体的例子是 7C2 和 7F3。

"表位 2C4" 指 HER2 胞外域中抗体 2C4 所结合的区域。为了筛选结合 2C4 表位的抗体，可进行常规的交叉阻断测定法，诸如《*Antibodies, A Laboratory Manual*》，Cold Spring Harbor Laboratory，Ed Harlow 和 David Lane，1988 中所描述的。或者，可使用本领域知道的方法进行表位作图以评估抗体是否结合 HER2 的 2C4 表位，和/或可研究抗体-HER2 结构（Franklin

et al., *Cancer Cell* 5: 317-328 (2004)）以了解抗体结合 HER2 的哪个/些结构域。表位 2C4 包含来自 HER2 胞外域中结构域 II 的残基。2C4 和 Pertuzumab 在结构域 I、II 和 III 的连接处结合 HER2 的胞外域。Franklin *et al.*, *Cancer Cell* 5: 317-328 (2004)。

“表位 4D5”指 HER2 胞外域中抗体 4D5（ATCC CRL 10463）和 Trastuzumab 所结合的区域。此表位接近 HER2 的跨膜域，且在 HER2 的结构域 IV 之内。为了筛选结合 4D5 表位的抗体，可进行常规的交叉阻断测定法，诸如《*Antibodies, A Laboratory Manual*》，Cold Spring Harbor Laboratory，Ed Harlow 和 David Lane，1988 中所描述的。或者，可进行表位作图以评估抗体是否结合 HER2 的 4D5 表位（例如大约第 529 位残基至大约第 625 位残基区域内的任何一个或多个残基，含；见图 1）。

“表位 7C2/7F3”指 HER2 胞外域的结构域 I 内 N 末端处 7C2 和/或 7F3 抗体（各自保藏于 ATCC，见下文）所结合的区域。为了筛选结合 7C2/7F3 表位的抗体，可进行常规的交叉阻断测定法，诸如《*Antibodies, A Laboratory Manual*》，Cold Spring Harbor Laboratory，Ed Harlow 和 David Lane，1988 中所描述的。或者，可进行表位作图以确定抗体是否结合 HER2 上的 7C2/7F3 表位（例如图 1 中 HER2 的大约第 22 位残基至大约第 53 位残基区域内的任何一个或多个残基）。

“治疗”指治疗性处理和预防性措施二者。需要治疗的受试者包括早就患有疾病的受试者以及要预防疾病的受试者。因此，本文中待治疗的患者可能已经诊断为患有疾病或者可能有患上疾病的倾向性或易感性。

术语“癌症”和“癌的”指的是或描述哺乳动物中特征通常为细胞生长不受调节的生理状况。癌症的例子包括但不限于癌、淋巴瘤、母细胞瘤（包括髓母细胞瘤和视网膜母细胞瘤）、肉瘤（包括脂肪肉瘤和滑膜细胞肉瘤）、神经内分泌肿瘤（包括类癌瘤、胃泌素瘤和胰岛细胞癌）、间皮瘤、施旺细胞瘤（包括听神经瘤）、脑膜瘤、腺癌、黑素瘤、和白血病或淋巴样恶性肿瘤。这些癌症的更具体例子包括鳞状细胞癌（如上皮鳞状细胞癌）、肺癌包括小细胞肺癌、非小细胞肺癌、肺的腺癌和肺的鳞癌、腹膜的癌症、肝细胞癌症、胃癌包括胃肠癌、胰腺癌、成胶质细胞瘤、宫颈癌、卵巢癌、肝癌、膀胱癌、肝瘤(hepatoma)、乳癌、结肠癌、直肠癌、结肠直肠癌、子宫内膜或子宫癌、唾液腺癌、肾癌、前列腺癌、外阴癌、甲状腺癌、肝的癌、肛门

癌、阴茎癌、睾丸癌、食道癌、胆管肿瘤、及头和颈癌。

术语“有效量”指在患者中有效治疗疾病的药物量。若疾病是癌症，则药物的有效量可减少癌细胞的数目；缩小肿瘤的尺寸；抑制（即减缓到一定程度和优选阻止）癌细胞浸润到周围器官中；抑制（即减缓到一定程度和优选阻止）肿瘤转移；在某种程度上抑制肿瘤生长；和/或在一定程度上减轻一种或多种与癌症有关的症状。在药物可阻止生长和/或杀死现有癌细胞的程度上，它可以是抑制细胞的和/或细胞毒性的。有效量可延长无进展存活，导致客观响应（包括部分响应，PR 或完全响应，CR），增加总体存活时间，和/或改善癌症的一种或多种症状。

“表达 HER2 的癌症”是包含在其细胞表面存在 HER2 蛋白质的细胞的癌症。

“过表达”HER 受体的癌症是与同一组织类型的非癌细胞相比，在其细胞表面具有显著更高水平的 HER 受体诸如 HER2 的癌症。这样的过表达可以是由基因扩增或者转录或翻译增加引起的。可在诊断或预后测定法中通过评估细胞表面上存在的 HER 蛋白质水平的增加（例如通过免疫组化测定法，IHC）来确定 HER 受体的过表达。或者/另外，可测量细胞中编码 HER 的核酸的水平，例如通过荧光原位杂交（FISH；参见 1998 年 10 月公布的 WO98/45479）、Southern 印迹或聚合酶链式反应(PCR)技术，诸如实时定量 PCR (RT-PCR)。还可通过测量生物学流体诸如血清中的脱落抗原（例如 HER 胞外域）来研究 HER 受体过表达（参见例如 1990 年 6 月 12 日发布的美国专利 4,933,294；1991 年 4 月 18 日发布的 WO91/05264；1995 年 3 月 28 日发布的美国专利 5,401,638；及 Sias *et al., J. Immunol. Methods* 132: 73-80 (1990)）。除了上述测定法之外，熟练技术人员还可利用多种体内测定法。例如，可将患者体内细胞暴露于任选用可检测标记物例如放射性同位素标记的抗体，并且可评估抗体与患者体内细胞的结合，例如通过外部扫描放射性或通过分析取自事先已暴露于所述抗体的患者的活组织检查切片。

相反，“不过表达 HER2 受体”的癌症是与同一组织类型的非癌细胞相比，不表达高于正常水平的 HER2 受体的癌症。

“过表达”HER 配体的癌症是与同一组织类型的非癌细胞相比，产生显著更高水平的该配体的癌症。这样的过表达可以是由基因扩增或者转录或翻译增加引起的。可通过评估患者体内，例如肿瘤活组织检查切片中所述配体

（或编码它的核酸）的水平，或者通过各种诊断测定法诸如 IHC、FISH、Southern 印迹、PCR 或上文所述体内测定法在诊断上确定 HER 配体的过表达。

术语“胞毒剂”在用于本文时指抑制或阻止细胞功能和/或引起细胞破坏的物质。该术语意欲包括放射性同位素（如 At^{211}、I^{131}、I^{125}、Y^{90}、Re^{186}、Re^{188}、Sm^{153}、Bi^{212}、P^{32}和 Lu 的放射性同位素）、化疗剂、和毒素诸如小分子毒素或细菌、真菌、植物或动物起源的酶活毒素，包括其片段和/或变体。

“化疗剂”指可用于治疗癌症的化学化合物。化疗剂的例子包括烷化剂类，诸如噻替哌(thiotepa)和环磷酰胺(cyclophosphamide)（CYTOXAN®）；烷基磺酸酯类，诸如白消安(busulfan)、英丙舒凡(improsulfan)和哌泊舒凡(piposulfan)；氮丙啶类，诸如苯佐替哌(benzodopa)、卡波醌(carboquone)、美妥替哌(meturedopa)和乌瑞替哌(uredopa)；乙撑亚胺类(ethylenimine)和甲基蜜胺类(methylamelamine)，包括六甲蜜胺(altretamine)、三乙撑蜜胺(triethylenemelamine)、三乙撑磷酰胺(trietylenephosphoramide)、三乙撑硫代磷酰胺(triethylenethiophosphoramide)和三羟甲蜜胺(trimethylolomelamine)；番荔枝内酯类(acetogenin)（尤其是布拉他辛(bullatacin)和布拉他辛酮(bullatacinone)）；δ-9-四氢大麻酚（曲大麻酚(dronabinol)，MARINOL®）；β-拉帕醌(lapachone)；拉帕醇(lapachol)；秋水仙素类(colchicine)；白桦脂酸(betulinic acid)；喜树碱(camptothecin)（包括合成类似物拓扑替康(topotecan)（HYCAMTIN®）、CPT-11（伊立替康(irinotecan)，CAMPTOSAR®）、乙酰喜树碱、东莨菪素(scopolectin)和 9-氨基喜树碱）；苔藓抑素(bryostatin)；callystatin；CC-1065（包括其阿多来新(adozelesin)、卡折来新(carzelesin)和比折来新(bizelesin)合成类似物）；鬼臼毒素(podophyllotoxin)；鬼臼酸(podophyllinic acid)；替尼泊苷(teniposide)；隐藻素类(cryptophycin)（特别是隐藻素 1 和隐藻素 8）；多拉司他丁(dolastatin)；duocarmycin（包括合成类似物 KW-2189 和 CB1-TM1）；艾榴塞洛素(eleutherobin)；pancratistatin；sarcodictyin；海绵抑素(spongistatin)；氮芥类，诸如氯丁酸氮芥(chlorambucil)、萘氮芥(chlornaphazine)、胆磷酰胺(cholophosphamide)、雌氮芥(estramustine)、异磷酰胺(ifosfamide)、双氯乙基甲胺(mechlorethamine)、盐酸氧氮芥(mechlorethamine oxide hydrochloride)、美法仑(melphalan)、新氮芥(novembichin)、苯芥胆甾醇(phenesterine)、松龙苯芥(prednimustine)、曲磷胺

(trofosfamide)、尿嘧啶氮芥(uracil mustard)；硝基脲类，诸如卡莫司汀(carmustine)、氯脲菌素(chlorozotocin)、福莫司汀(fotemustine)、洛莫司汀(lomustine)、尼莫司汀(nimustine)和雷莫司汀(ranimustine)；抗生素类，诸如烯二炔类(enediyne)抗生素（如加利车霉素(calicheamicin)，尤其是加利车霉素γ1I和加利车霉素ω1I（参见例如 Agnew, *Chem. Intl. Ed. Engl.* 33: 183-186 (1994)）；蒽环类(dynemicin)抗生素，包括 dynemicin A；埃斯波霉素(esperamicin)；以及新抑癌菌素(neocarzinostatin)发色团和相关色蛋白烯二炔类抗生素发色团)、阿克拉霉素(aclacinomysin)、放线菌素(actinomycin)、氨茴霉素(authramycin)、氮丝氨酸(azaserine)、博来霉素(bleomycin)、放线菌素C (cactinomycin)、carabicin、洋红霉素(carminomycin)、嗜癌霉素(carzinophilin)、色霉素(chromomycin)、放线菌素D (dactinomycin)、柔红霉素(daunorubicin)、地托比星(detorubicin)、6-二氮-5-氧-L-正亮氨酸、多柔比星(doxorubicin)（包括ADRIAMYCIN®、吗啉代多柔比星、氰基吗啉代多柔比星、2-吡咯代多柔比星、盐酸多柔比星脂质体注射剂（DOXIL®）、脂质体多柔比星TLC D-99（MYOCET®）、PEG化脂质体多柔比星（CAELYX®）和脱氧多柔比星)、表柔比星(epirubicin)、依索比星(esorubicin)、依达比星(idarubicin)、麻西罗霉素(marcellomycin)、丝裂霉素(mitomycin)诸如丝裂霉素C、霉酚酸(mycophenolic acid)、诺加霉素(nogalamycin)、橄榄霉素(olivomycin)、培来霉素(peplomycin)、potfiromycin、嘌呤霉素(puromycin)、三铁阿霉素(quelamycin)、罗多比星(rodorubicin)、链黑菌素(streptonigrin)、链脲霉素(streptozocin)、杀结核菌素(tubercidin)、乌苯美司(ubenimex)、净司他丁(zinostatin)、佐柔比星(zorubicin)；抗代谢物类，诸如甲氨喋呤、吉西他滨(gemcitabine)（GEMZAR®）、替加氟(tegafur)（UFTORAL®）、卡培他滨(capecitabine)（XELODA®）、epothilone和5-氟尿嘧啶(5-FU)；叶酸类似物，诸如二甲叶酸(denopterin)、甲氨喋呤、蝶酰三谷氨酸(pteropterin)、三甲曲沙(trimetrexate)；嘌呤类似物，诸如氟达拉滨(fludarabine)、6-巯基嘌呤、硫咪嘌呤、硫鸟嘌呤；嘧啶类似物，诸如安西他滨(ancitabine)、阿扎胞苷(azacitidine)、6-氮尿苷、卡莫氟(carmofur)、阿糖胞苷(cytarabine)、双脱氧尿苷(dideoxyuridine)、多西氟尿啶(doxifluridine)、依诺他滨(enocitabine)、氟尿苷(floxuridine)；抗肾上腺类，诸如氨鲁米特(aminoglutethimide)、曼托坦(mitotane)、曲洛司坦(trilostane)；叶酸补充剂，诸如亚叶酸；醋葡内酯

(aceglatone)；醛磷酰胺糖苷(aldophosphamide glycoside)；氨基酮戊酸(aminolevulinic acid)；恩尿嘧啶(eniluracil)；氨苯吖啶(amsacrine)；bestrabucil；比生群(bisantrene)；依达曲沙(edatraxate)；defofamine；地美可辛(demecolcine)；地吖醌(diaziquone)；依洛尼塞(elformithine)；醋酸羟吡咔唑(elliptinium acetate)；依托格鲁(etoglucid)；硝酸镓；羟脲；香菇多糖(lentinan)；氯尼达明(lonidamine)；美登木素生物碱类(maytansinoids)，诸如美登素(maytansine)和美登醇(maytansinol)；安丝菌素(ansamitocin)；米托胍腙(mitoguazone)；米托蒽醌(mitoxantrone)；莫哌达醇(mopidamol)；二胺硝吖啶(nitracrine)；喷司他丁(pentostatin)；蛋氨氮芥(phenamet)；吡柔比星(pirarubicin)；洛索蒽醌(losoxantrone)；2-乙基酰肼；丙卡巴肼(procarbazine)；PSK®多糖复合物（JHS Natural Products，Eugene，OR）；雷佐生(razoxane)；根霉素(rhizoxin)；西索菲兰(sizofiran)；锗螺胺(spirogermanium)；细交链孢菌酮酸(tenuazonic acid)；三亚胺醌(triaziquone)；2,2',2"-三氯三乙胺；单端孢菌素类(trichothecene)（尤其是 T-2 毒素、verracurin A、杆孢菌素 A (roridin A)和蛇形菌素(anguidine)）；乌拉坦(urethan)；达卡巴嗪(dacarbazine)；甘露醇氮芥(mannomustine)；二溴甘露醇(mitobronitol)；二溴卫矛醇(mitolactol)；哌泊溴烷(pipobroman)；gacytosine；阿糖胞苷(arabinoside)（“Ara-C”）；塞替哌；类紫杉醇(taxoid)，例如紫杉醇(paclitaxel)（TAXOL®）、紫杉醇的清蛋白改造纳米颗粒剂型（ABRAXANE™）和多西他塞(doxetaxel)（TAXOTERE®）；苯丁酸氮芥(chloranbucil)；6-硫鸟嘌呤；巯基嘌呤；甲氨喋呤；铂剂，诸如顺铂、奥沙利铂(oxaliplatin)和卡铂；阻止微管蛋白聚合形成微管的长春花生物碱类(vincas)，包括长春碱(vinblastine)（VELBAN®）、长春新碱(vincristine)（ONCOVIN®）、长春地辛(vindesine)（ELDISINE®、FILDESIN®）和长春瑞宾(vinorelbine)（NAVELBINE®）；依托泊苷(etoposide)（VP-16）；异磷酰胺(ifosfamide)；米托蒽醌(mitoxantrone)；亚叶酸(leucovovin)；诺安托(novantrone)；依达曲沙(edatrexate)；柔红霉素(daunomycin)；氨基蝶呤；伊拜膦酸盐(ibandronate)；拓扑异构酶抑制剂 RFS 2000；二氟甲基鸟氨酸(DMFO)；类维 A 酸，诸如视黄酸，包括贝沙罗汀(bexarotene)（TARGRETIN®）；二碳磷酸盐类(bisphosphonate)，诸如氯膦酸盐(clodronate)（例如 BONEFOS®或 OSTAC®）、etidronate（DIDROCAL®）、NE-58095、唑来膦酸(zoledronic acid)/唑来膦酸盐/酯(zoledronate)（ZOMETA®）、阿仑膦

酸盐/酯(alendronate)（FOSAMAX®）、pamidronate（AREDIA®）、替鲁膦酸盐/酯(tiludronate)（SKELID®）或利塞膦酸盐/酯(risedronate)（ACTONEL®）；曲沙他滨(troxacitabine)（1,3-二氧戊环核苷胞嘧啶类似物）；反义寡核苷酸，特别是那些抑制涉及粘着性细胞增殖的信号途经中的基因表达的反义寡核苷酸，诸如例如PKC-α、Ralf、H-Ras和表皮生长因子受体(EGF-R)；疫苗，诸如THERATOPE®疫苗和基因疗法疫苗，例如ALLOVECTIN®疫苗、LEUVECTIN®疫苗和VAXID®疫苗；拓扑异构酶1抑制剂（如LURTOTECAN®）；rmRH（ABARELIX®）；BAY439006（sorafenib; Bayer）；SU-11248（Pfizer）；哌立福辛(perifosine)，COX-2抑制剂（如塞来考昔(celecoxib)或艾托考昔(etoricoxib)），蛋白体抑制剂（如PS341）；bortezomib（VELCADE®）；CCI-779；tipifarnib（R11577）；orafenib，ABT510；Bcl-2抑制剂，诸如oblimersen sodium（GENASENSE®）；pixantrone；EGFR抑制剂（见下文定义）；酪氨酸激酶抑制剂（见下文定义）；及上述任何物质的药学可接受的盐、酸或衍生物；以及上述两种或多种的组合，诸如CHOP（环磷酰胺、多柔比星、长春新碱和泼尼松龙联合疗法的缩写）和FOLFOX（奥沙利铂(ELOXATIN™)联合5-FU和亚叶酸(leucovovin)的治疗方案的缩写）。

该定义还包括作用于调节或抑制激素对肿瘤的作用的抗激素剂，诸如具有混合的激动剂/拮抗剂特性(profile)的抗雌激素类，包括他莫昔芬(tamoxifen)（NOLVADEX®）、4-羟基他莫昔芬、托瑞米芬(toremifene)（FARESTON®）、艾多昔芬(idoxifene)、屈洛昔芬(droloxifene)、雷洛昔芬(raloxifene)（EVISTA®）、曲沃昔芬(trioxifene)、keoxifene，及选择性雌激素受体调节剂(SERM)，诸如SERM3；没有激动剂特性的纯粹的抗雌激素，诸如氟维司群(fulvestrant)（FASLODEX®）和EM800（此类药剂可阻断雌激素受体(ER)二聚化，抑制DNA结合，提高ER周转，和/或遏制ER水平）；芳香酶抑制剂，包括类固醇芳香酶抑制剂，诸如福美坦(formestane)和依西美坦(exemestane)（AROMASIN®），及非类固醇芳香酶抑制剂，诸如阿那曲唑(anastrozole)（ARIMIDEX®）、来曲唑(letrozole)（FEMARA®）和氨鲁米特(aminoglutethimide)，及其它芳香酶抑制剂，包括伏罗唑(vorozole)（RIVISOR®）、醋酸甲地孕酮(megestrol acetate)（MEGASE®）、法倔唑(fadrozole)、咪唑(imidazole)；lutenizing hormone释放激素激动剂，包括亮丙瑞林(leuprolide)（LUPRON®和ELIGARD®）、戈舍瑞林(goserelin)、布舍瑞

林(buserelin)和曲普瑞林(triptorelin); 性类固醇类，包括孕激素，诸如醋酸甲地孕酮(megestrol acetate)和醋酸甲羟孕酮(medroxyprogesterone acetate)，雌激素，诸如乙烯雌酚(diethylstilbestrol)和倍美力(premarin)，及雄激素/类维 A 酸，诸如氟甲睾酮(fluoxymesterone)、所有反式维 A 酸(transretionic acid)和芬维 A 胺(fenretinide); 奥那司酮(onapristone); 抗孕酮类; 雌激素受体下调调节剂(ERD); 抗雄激素类，诸如氟他米特(flutamide)、尼鲁米特(nilutamide)和比卡米特(bicalutamide); 睾内酯(testolactone); 及上述任何物质的药学可接受的盐、酸或衍生物; 以及两种或多种上述物质的组合。

在用于本文时，术语“EGFR 靶向药物”指结合 EGFR 并任选抑制 EGFR 活化的治疗剂。此类药剂的例子包括结合 EGFR 的抗体和小分子。结合 EGFR 的抗体的例子包括 MAb 579（ATCC CRL HB 8506）、MAb 455（ATCC CRL HB 8507）、MAb 225（ATCC CRL 8508）、MAb 528（ATCC CRL 8509）（参见 Mendelsohn 等人的美国专利 4,943,533 及其变体，诸如嵌合化 225（C225 或 Cetuximab; ERBUTIX®）和重构人 225（H225）（参见 Imclone Systems Inc. 的 WO 96/40210）; IMC-11F8，完全人的 EGFR 靶向抗体（Imclone）; 结合 II 型突变体 EGFR 的抗体（美国专利 5,212,290）; 结合 EGFR 的人源化和嵌合抗体，如美国专利 5,891,996 中所述; 及结合 EGFR 的人抗体，诸如 ABX-EGF 或 Panitumumab（参见 Abgenix/Amgen 的 WO 98/50433）; EMD 55900（Stragliotto *et al.*, *Eur. J. Cancer* 32A: 636-640 (1996)）; EMD7200（matuzumab），针对 EGFR 且与 EGF 和 TGF-α 竞争 EGFR 结合的人源化 EGFR 抗体（EMD/Merck）; 人 EGFR 抗体，HuMax-EGFR（GenMab）; 称为 E1.1、E2.4、E2.5、E6.2、E6.4、E2.11、E6.3 和 E7.6.3 和 US 6,235,883 中描述的完全人的抗体; MDX-447（Medarex Inc.）; 及 mAb 806 或人源化 mAb 806（Jones *et al.*, *J. Biol. Chem.* 279(29): 30375-30384 (2004)）。抗 EGFR 抗体可与胞毒剂偶联，由此产生免疫偶联物（参见例如 EP 659,439 A2，Merck Patent GmbH）。EGFR 拮抗剂包括小分子，诸如美国专利 5,616,582、5,457,105、5,475,001、5,654,307、5,679,683、6,084,095、6,265,410、6,455,534、6,521,620、6,596,726、6,713,484、5,770,599、6,140,332、5,866,572、6,399,602、6,344,459、6,602,863、6,391,874、6,344,455、5,760,041、6,002,008 和 5,747,498 以及 PCT 出版物 WO98/14451、WO98/50038、WO99/09016 和 WO99/24037 中描述的化合物。具体的小分子 EGFR 拮抗剂包括 OSI-774（CP-358774, erlotinib,

TARCEVA®, Genentech/OSI Pharmaceuticals）; PD 183805（CI 1033, 2-propenamide, N-[4-[(3-氯-4-氟苯基)氨基]-7-[3-(4-吗啉基)丙氧基]-6-喹唑啉基]-, 二盐酸化物, Pfizer Inc.）; ZD1839, gefitinib（IRESSA™, 4-(3'-氯-4'-氟苯胺基)-7-甲氧基-6-(3-吗啉代丙氧基)喹唑啉, AstraZeneca）; ZM 105180（(6-氨基-4-(3-甲基苯基-氨基)-喹唑啉, Zeneca）; BIBX-1382（N8-(3-氯-4-氟-苯基)-N2-(1-甲基-哌啶-4-基)-嘧啶并[5,4-d]嘧啶-2,8-二胺, Boehringer Ingelheim）; PKI-166（(R)-4-[4-[(1-苯基乙基)氨基]-1H-吡咯并[2,3-d]嘧啶-6-基]-酚）; (R)-6-(4-羟基苯基)-4-[(1-苯基乙基)氨基]-7H-吡咯并[2,3-d]嘧啶）; CL-387785（N-[4-[(3-溴苯基)氨基]-6-喹唑啉基]-2-丁炔酰胺(butynamide)）; EKB-569（N-[4-[(3-氯-4-氟苯基)氨基]-3-氰基-7-乙氧基-6-喹啉基]-4-(二甲基氨基)-2-丁烯酰胺(butenamide), Wyeth）; AG1478（Sugen）; AG1571（SU 5271; Sugen）; 双重 EGFR/HER2 酪氨酸激酶抑制剂, 诸如 lapatinib（GW 572016 或 N-[3-氯-4-[(3-氟苯基)甲氧基]苯基]6[5[[[2-甲基磺酰基]乙基]氨基]甲基]-2-呋喃基]-4-喹唑啉胺(quinazolinamine); Glaxo-SmithKline）或氰基胍喹唑啉(cyanoguanidine quinazoline)和氰基脒喹唑胺(cyanoamidine quinazolamine)衍生物。

"酪氨酸激酶抑制剂"指抑制酪氨酸激酶诸如 HER 受体的酪氨酸激酶活性的分子。这样的抑制剂的例子包括上一段中提到的 EGFR 靶向药物; 小分子 HER2 酪氨酸激酶抑制剂, 诸如可从 Takeda 购买的 TAK165; CP-724,714, 一种口服 ErbB2 受体酪氨酸激酶选择性抑制剂（Pfizer and OSI）; 优先结合 EGFR 但抑制 HER2 和 EGFR 过表达细胞的双重 HER 抑制剂, 诸如 EKB-569（可从 Wyeth 购买）; lapatinib(GW572016; 可从 Glaxo-SmithKline 购买), 一种口服 HER2 和 EGFR 酪氨酸激酶抑制剂; PKI-166（可从 Novartis 购买）; 泛 HER (pan-HER)抑制剂, 诸如 canertinib（CI-1033; Pharmacia）; Raf-1 抑制剂, 诸如可从 ISIS Pharmaceuticals 购买的, 抑制 Raf-1 信号传导的反义剂 ISIS-5132; 非 HER 靶向 TK 抑制剂, 诸如可从 Glaxo 购买的 Imatinib mesylate（GLEEVAC™）; MAPK 胞外调控激酶 I 抑制剂 CI-1040（可从 Pharmacia 购买）; 喹唑啉类, 诸如 PD 153035, 4-(3-氯苯胺基)喹唑啉; 吡啶并嘧啶类; 嘧啶并嘧啶类; 吡咯并嘧啶类, 诸如 CGP 59326、CGP 60261 和 CGP 62706; 吡唑并嘧啶类, 4-(苯氨基)-7H-吡咯[2,3-d]嘧啶; 姜黄素（二阿魏酰甲烷, 4,5-双(4-氟苯胺基)-酞亚胺）; 含硝基噻吩模块的 tyrphostines; PD-0183805

（Warner-Lambert）；反义分子（例如那些结合 HER 编码核酸的反义分子）；喹喔啉类（美国专利 5,804,396）；tryphostins（美国专利 5,804,396）；ZD6474（Astra Zeneca）；PTK-787（Novartis/Schering AG）；泛 HER 抑制剂，诸如 CI-1033（Pfizer）；Affinitac（ISIS 3521；Isis/Lilly）；Imatinib mesylate（Gleevec；Novartis）；PKI 166（Novartis）；GW2016（Glaxo SmithKline）；CI-1033（Pfizer）；EKB-569（Wyeth）；Semaxinib（Sugen）；ZD6474（AstraZeneca）；PTK-787（Novartis/Schering AG）；INC-1C11（Imclone）；氰基脒喹唑啉和氰基脒喹唑胺衍生物；或任何如下专利出版物中所记载的：美国专利 5,804,396；WO 99/09016（American Cyanimid）；WO 98/43960（American Cyanamid）；WO 97/38983（Warner Lambert）；WO 99/06378（Warner Lambert）；WO 99/06396（Warner Lambert）；WO 96/30347（Pfizer, Inc.）；WO 96/33978（Zeneca）；WO 96/3397（Zeneca）；WO 96/33980（Zeneca）；及 US 2005/0101617。

“抗血管生成剂”指阻断或在某种程度上干扰血管发育的化合物。抗血管生成因子可以是例如结合涉及促进血管生成的生长因子或生长因子受体的小分子或抗体。在本文中优选的抗血管生成因子是结合血管内皮生长因子(VEGF)的抗体，诸如 Bevacizumab（AVASTIN®）。

术语“细胞因子”指由一种细胞群释放的、作为细胞间介质作用于另一细胞的蛋白质的通称。这样的细胞因子的例子是淋巴因子、单核因子和传统的多肽激素。细胞因子包括生长激素，诸如人生长激素、N-甲硫氨酰人生长激素、和牛生长激素；甲状旁腺素；甲状腺素；胰岛素；胰岛素原；松弛素；松弛素原；糖蛋白激素类，诸如促卵泡激素(FSH)、促甲状腺激素(TSH)和促黄体激素(LH)；肝生长因子；成纤维细胞生长因子；促乳素；胎盘催乳激素；肿瘤坏死因子-α 和-β；穆勒氏(Mullerian)抑制性物质；小鼠促性腺激素相关肽；抑制素；激活素(activin)；血管内皮生长因子；整联蛋白；血小板生成素(TPO)；神经生长因子，诸如 NGF-β；血小板衍生生长因子；转化生长因子(TGF)，诸如 TGF-α 和 TGF-β；胰岛素样生长因子-I 和-II；促红细胞生成素(EPO)；骨诱导因子(osteoinductive factor)；干扰素，诸如干扰素-α、-β 和-γ；集落刺激因子(CSF)，诸如巨噬细胞 CSF (M-CSF)、粒细胞-巨噬细胞 CSF (GM-CSF)和粒细胞 CSF (G-CSF)；白介素(IL)，诸如 IL-1、IL-1α、IL-2、IL-3、IL-4、IL-5、IL-6、IL-7、IL-8、IL-9、IL-10、IL-11、IL-12；肿瘤坏死因子，诸如 TNF-α 或 TNF-β；及其它多肽因子，包括 LIF 和 kit 配体(KL)。在用于

本文时，术语细胞因子包括来自天然来源或来自重组细胞培养物的蛋白质和天然序列细胞因子的生物学活性等效物。

II. HER2 抗体变体组合物

本发明至少部分关注某些 HER2 抗体组合物。优选的是，所述 HER2 抗体（主要种类 HER2 抗体及其抗体变体其中任一或二者兼之）是结合 HER2 结构域 II，比 Trastuzumab 更有效的抑制 HER 二聚化，和/或结合 HER2 的异二聚体结合位点的抗体。本文中主要种类抗体的优选实施方案是包含 SEQ ID No. 3 和 4 中的轻链和重链可变区氨基酸序列，且最优选包含 SEQ ID No. 15 和 16 中的轻链和重链氨基酸序列（Pertuzumab）的抗体。

本文中的组合物包含主要种类 HER2 抗体及其包含氨基末端前导延伸的氨基酸序列变体的混合物。优选的是，所述氨基末端前导延伸位于抗体变体的轻链上（例如位于抗体变体的一条或两条轻链上）。所述主要种类 HER2 抗体或抗体变体可以是完整抗体或抗体片段（如 Fab 或 $F(ab')_2$ 片段），但优选二者都是完整抗体。

本文中的抗体变体在其任何一条或多条重链或轻链上包含氨基末端前导延伸。优选的是，所述氨基末端前导延伸位于抗体的一条或两条轻链上。所述氨基末端前导延伸优选包含 VHS-或由其组成。

组合物中氨基末端前导延伸的存在可通过多种分析技术来检测，包括但不限于 N-末端序列分析、电荷异质性的测定法（例如阳离子交换层析或毛细管区带电泳）、质谱等。组合物中抗体变体的量通常在如下范围内，从构成用于检测变体的任何测定法（优选阳离子交换分析）的检测下限的量至少于主要种类抗体量的量。通常，组合物中约 20%或更少（例如从约 1%至约 15%，例如从 5%至约 15%，且优选从约 8%至约 12%）的抗体分子包含氨基末端前导延伸。这样的百分比量优选通过阳离子交换分析来测定。

除了氨基末端前导延伸变体以外，还设想了主要种类抗体和/或变体的氨基酸序列改变，包括但不限于在其一条或所有两条重链上包含 C-末端赖氨酸残基的抗体（这样的抗体变体可以以约 1%至约 20%的量存在）、脱酰胺抗体变体（例如其中 Pertuzumab 一条或两条重链上的 Asn386 和/或 Asn391 脱酰胺）、具有一个或多个氧化后甲硫氨酸残基的抗体（例如包含氧化后 met-254 的 Pertuzumab）等。

此外，主要种类抗体或变体还可包含糖基化变异，其非限制性例子包括包含附着于其 Fc 区的 G1 或 G2 寡糖结构的抗体、包含附着于其轻链的碳水化合物模块的抗体（例如一个或多个碳水化合物模块，诸如葡萄糖或半乳糖附着于抗体的一条或两条轻链，例如附着于一个或多个赖氨酸残基）、包含一条或两条非糖基化重链的抗体、或包含附着于其一条或两条重链的唾液酸化寡糖的抗体等。

本发明还关注包含 SEQ ID No. 23 中的氨基酸序列的多肽或其脱酰胺和/或氧化变体。另外，本发明提供了包含一条或两条轻链的抗体，其中两条轻链其中任一或二者兼之包含 SEQ ID No. 23 中的氨基酸序列。所述抗体还包含一条或两条重链，其中两条重链其中任一或二者兼之包含 SEQ ID No. 16 或 SEQ ID No. 24 中的氨基酸序列（或其脱酰胺和/或氧化变体）。

组合物可从表达 HER2 抗体的基因工程细胞系回收，例如中国仓鼠卵巢(CHO)细胞系，或者可通过肽合成来制备。

III. HER2 抗体的生成

下文描述了用于生成依照本发明使用的抗体的例示性技术。用于生成抗体的 HER2 抗原可以是例如可溶形式的 HER2 胞外域或其含有所需表位的部分。或者，在其细胞表面表达 HER2 的细胞（例如经转化而过表达 HER2 的 NIH-3T3 细胞；或癌细胞系，诸如 SK-BR-3 细胞，参见 Stancovski *et al.*, *PNAS (USA)* 88: 8691-8695 (1991)）可用于生成抗体。可用于生成抗体的其它形式 HER2 对于本领域技术人员是显而易见的。

(i) 多克隆抗体

多克隆抗体优选通过在动物中多次皮下(sc)或腹膜内(ip)注射相关抗原和佐剂来生成。使用双功能或衍生化试剂，例如马来酰亚胺苯甲酰硫代琥珀酰亚胺酯（通过半胱氨酸残基偶联），N-羟基琥珀酰亚胺（通过赖氨酸残基）、戊二醛、琥珀酸酐、$SOCl_2$ 或 $R^1N=C=NR$，其中 R 和 R^1 是不同的烃基，将相关抗原与在待免疫的物种中有免疫原性的蛋白质偶联可能是有用的，例如匙孔血蓝蛋白、血清清蛋白、牛甲状腺球蛋白或大豆胰蛋白酶抑制剂。

通过将例如 100μg 或 5μg 蛋白质或偶联物（分别用于兔或小鼠）与 3 倍体积的弗氏完全佐剂混和，并将该溶液皮内注射于多个部位，由此将动物针对抗原、免疫原性偶联物或衍生物进行免疫。一个月后，通过多个部位的皮

下注射，用弗氏完全佐剂中肽或偶联物初始量的 1/5-1/10 对动物进行强化。7-14 天后，采集动物的血液，并测定血清的抗体滴度。对动物进行强化直到滴度达到稳定。优选的是，将动物用相同抗原但与不同蛋白质和/或通过不同交联剂偶联得到的偶联物进行强化。偶联物还可在重组细胞培养物中作为蛋白质融合物来制备。同样，适当使用凝聚剂诸如明矾来增强免疫应答。

(ii) 单克隆抗体

单克隆抗体是从一群基本上同质的抗体获得的，即构成群体的各个抗体相同和/或结合相同表位，除了单克隆抗体生产过程中可能产生的可能突变外，诸如本文所述那些变体。因此，修饰语“单克隆”表示抗体的特征，即不是分立抗体的混合物。

例如，单克隆抗体可通过最初由 Kohler *et al.*, *Nature* 256: 495 (1975)描述的杂交瘤方法来制备，或者可通过重组 DNA 方法来制备（美国专利 4,816,567）。

在杂交瘤方法中，如上所述免疫小鼠或其它合适的宿主动物，诸如仓鼠，以引发生成或能够生成如下抗体的淋巴细胞，所述抗体将特异性结合用于免疫的蛋白质。或者，可以在体外免疫淋巴细胞。然后，使用合适的融合剂诸如聚乙二醇将淋巴细胞与骨髓瘤细胞融合，以形成杂交瘤细胞（Goding，《*Monoclonal Antibodies: Principles and Practice*》，59-103，Academic Press，1986）。

将如此制备的杂交瘤细胞在合适的培养基中接种和培养，所述培养基优选含有抑制未融合的亲本骨髓瘤细胞生长或存活的一种或多种物质。例如，如果亲本骨髓瘤细胞缺乏酶次黄嘌呤鸟嘌呤磷酸核糖转移酶(HGPRT 或 HPRT)，则用于杂交瘤的培养基通常将含有次黄嘌呤、氨基喋呤和胸苷（HAT 培养基），这些物质阻止 HGPRT 缺陷细胞生长。

优选的骨髓瘤细胞是那些高效融合、支持所选抗体生成细胞稳定的高水平生成抗体、并对诸如 HAT 培养基等培养基敏感的骨髓瘤细胞。在这些细胞中，优选的骨髓瘤细胞系是鼠源骨髓瘤系，诸如那些可从 Salk Institute Cell Distribution Center（San Diege，California，USA）获得的 MOPC-21 和 MPC-11 小鼠肿瘤的衍生系，及可从 American Type Culture Collection（Rockville，Maryland，USA）获得的 SP-2 或 X63-Ag8-653 细胞。用于生成人单克隆抗体的人骨髓瘤和小鼠-人异源骨髓瘤细胞系也已有描述（Kozbor, *J. Immunol.*

133: 3001 (1984); 及 Brodeur 等人，《*Monoclonal Antibody Production Techniques and Applications*》，51-63，Marcel Dekker, Inc.，New York，1987）。

可对杂交瘤细胞正在其中生长的培养基测定针对抗原的单克隆抗体的生成。优选的是，通过免疫沉淀或通过体外结合测定法，诸如放射性免疫测定法(RIA)或酶联免疫吸附测定法(ELISA)，测定由杂交瘤细胞生成的单克隆抗体的结合特异性。

单克隆抗体的结合亲和力可通过例如 Munson *et al.*, *Anal. Biochem.* 107: 220 (1980)的 Scatchard 分析来测定。

在鉴定得到生成具有所需特异性、亲和力和/或活性的抗体的杂交瘤细胞后，所述克隆可通过有限稀释流程进行亚克隆，并通过标准方法进行培养（Goding，《*Monoclonal Antibodies: Principles and Practice*》，59-103，Academic Press，1986）。适于这一目的的培养基包括例如 D-MEM 或 RPMI-1640 培养基。另外，杂交瘤细胞可在动物中作为腹水瘤进行体内培养。

可通过常规抗体纯化流程，诸如例如蛋白 A-Sepharose、羟磷灰石层析、凝胶电泳、透析或亲和层析，将亚克隆分泌的单克隆抗体与培养基、腹水或血清适当分开。

编码单克隆抗体的 DNA 易于通过常规流程分离并测序（例如使用能够特异性结合编码鼠源抗体重链和轻链的基因的寡核苷酸探针）。以杂交瘤细胞作为所述 DNA 的优选来源。一旦分离，可将 DNA 置于表达载体中，然后将该表达载体转染到宿主细胞中，诸如不另外产生抗体蛋白质的大肠杆菌细胞、猿猴 COS 细胞、中国仓鼠卵巢(CHO)细胞或骨髓瘤细胞，以在重组宿主细胞中获得单克隆抗体的合成。关于编码抗体的 DNA 在细菌中的重组表达的综述性论文包括 Skerra *et al.*, *Curr. Opinion in Immunol.* 5: 256-262 (1993)和 Plückthun, *Immunol. Revs.* 130: 151-188 (1992)。

在另一个实施方案中，可从使用 McCafferty *et al.*, *Nature* 348: 552-554 (1990)所述技术构建的噬菌体抗体库中分离单克隆抗体或抗体片段。Clackson *et al.*, *Nature* 352: 624-628 (1991)和 Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1991)分别描述了使用噬菌体文库分离鼠源和人源抗体。后续出版物描述了通过链改组（Marks *et al.*, *Bio/Technology* 10: 779-783 (1992)），以及组合感染和体内重组作为构建非常大的噬菌体文库的策略（Waterhouse *et al.*, *Nuc. Acids Res.* 21: 2265-2266 (1993)），生成高亲和力（nM 范围）的人源抗

体。因此，这些技术是用于分离单克隆抗体的传统单克隆抗体杂交瘤技术的可行替代方法。

还可以修饰 DNA，例如通过替代即用人重链和轻链恒定区的编码序列代替同源鼠源序列（美国专利 4,816,567；及 Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81: 6851 (1984)），或通过共价接合免疫球蛋白编码序列和非免疫球蛋白多肽的整个或部分编码序列。

通常，用此类非免疫球蛋白多肽替代抗体的恒定区，或者用它们替代抗体的一个抗原结合位点的可变区，以产生嵌合二价抗体，它包含对一种抗原具有特异性的一个抗原结合位点及对不同抗原具有特异性的另一个抗原结合位点。

(iii) 人源化抗体

本领域已经描述了用于将非人抗体人源化的方法。优选的是，人源化抗体具有一个或多个从非人来源引入的氨基酸残基。这些非人氨基酸残基常常称作“输入”残基，它们通常取自“输入”可变区。人源化可基本上依照 Winter 及其同事的方法进行（Jones *et al.*, *Nature* 321: 522-525 (1986); Riechmann *et al.*, *Nature* 332: 323-327 (1988); Verhoeyen *et al.*, *Science* 239: 1534-1536 (1988)），通过用高变区序列替代相应的人抗体序列。因此，此类“人源化”抗体是嵌合抗体（美国专利 4,816,567），其中本质上少于整个人可变区用来自非人物种的相应序列替代。在实践中，人源化抗体通常是其中一些高变区残基和可能的一些 FR 残基用来自啮齿类抗体中类似位点的残基替代的人抗体。

用于制备人源化抗体的人可变区的选择，包括轻链和重链，对于降低抗原性非常重要。依照所谓的“最适(best-fit)”方法，用啮齿类抗体可变区序列对已知的人可变区序列的整个文库进行筛选。然后选择与啮齿类最接近的人序列作为人源化抗体的人框架区(FR)（Sims *et al.*, *J. Immunol.* 151: 2296 (1993); Chothia *et al.*, *J. Mol. Biol.* 196: 901 (1987)）。另一种方法使用由特定轻链或重链亚组的所有人抗体的共有序列衍生的特定框架区。同一框架可用于数种不同的人源化抗体（Carter *et al.*, *Proc. Natl. Acad. Sci. USA* 89: 4285 (1992); Presta *et al.*, *J. Immunol.* 151: 2623 (1993)）。

更为重要的是，抗体在人源化后保持对抗原的高亲和力及其它有利的生物学特性。为了实现这一目的，依照一种优选的方法，通过使用亲本和人源

化序列的三维模型分析亲本序列和各种概念性人源化产物的方法来制备人源化抗体。三维免疫球蛋白模型通常是可获得的，且为本领域熟练技术人员所熟悉。还可获得图解和显示所选候选免疫球蛋白序列的可能三维构象结构的计算机程序。检查这些显示图像能够分析残基在候选免疫球蛋白序列行使功能中的可能作用，即分析影响候选免疫球蛋白结合其抗原的能力的残基。这样，可从受体和输入序列中选出 FR 残基并进行组合，从而获得所需抗体特征，诸如对靶抗原的亲和力提高。通常，高变区残基直接且最实质的涉及对抗原结合的影响。

WO01/00245 描述了结合 HER2 并阻断 HER 受体的配体活化的例示性人源化 HER2 抗体的生成。本文中特别感兴趣的人源化抗体基本上像完整鼠单克隆抗体 2C4（或其 Fab 片段）一样有效的阻断 EGF、TGF-α 和/或 HRG 介导的 MAPK 激活，和/或基本上像完整鼠单克隆抗体 2C4（或其 Fab 片段）一样有效的结合 HER2。本文中的人源化抗体可例如包含掺入人重链可变区的非人高变区残基，而且还可在选自 69H、71H 和 73H 的位置包含框架区(FR)替代，采用的是 Kabat 等人，《*Sequences of Proteins of Immunological Interest*》，第 5 版，Public Health Service，National Institutes of Health，Bethesda，MD，1991 中给出的可变区编号系统。在一个实施方案中，人源化抗体在 69H、71H 和 73H 的两个或所有位置包含 FR 替代。

本文中的例示性目的人源化抗体包含重链可变区互补决定残基 GFTFTDYTMX，其中 X 优选是 D 或 S（SEQ ID NO: 7）；DVNPNSGGSIYNQRFKG（SEQ ID NO: 8）；和/或 NLGPSFYFDY（SEQ ID NO: 9），任选包含那些 CDR 残基的氨基酸修饰，例如其中修饰基本上保持或改善抗体的亲和力。例如，目的抗体变体可在上述重链可变区 CDR 序列中具有约 1 个至约 7 个或者约 5 个氨基酸替代。这样的抗体变体可通过亲和力成熟来制备，例如如下文所述。最优选的人源化抗体包含 SEQ ID NO: 4 中的重链可变区氨基酸序列。

例如，除了前一段落中的那些重链可变区 CDR 残基之外，人源化抗体还可包含轻链可变区互补决定残基 KASQDVSIGVA（SEQ ID NO: 10）；SASYX^{1}X^{2}X^3，其中 X^1 优选是 R 或 L，X^2 优选是 Y 或 E，而 X^3 优选是 T 或 S（SEQ ID NO: 11）；和/或 QQYYIYPYT（SEQ ID NO: 12）。这样的人源化抗体任选包含上述 CDR 残基的氨基酸修饰，例如其中修饰基本上保持或改

善抗体的亲和力。例如，目的抗体变体可在上述轻链可变区 CDR 序列中具有约 1 个至约 7 个或者约 5 个氨基酸替代。这样的抗体变体可通过亲和力成熟来制备，例如如下文所述。最优选的人源化抗体包含 SEQ ID NO: 3 中的轻链可变区氨基酸序列。

本申请还设想了亲和力成熟的抗体，它结合 HER2 并阻断 HER 受体的配体活化。亲本抗体可以是人抗体或人源化抗体，例如包含轻链和/或重链可变区序列分别为 SEQ ID NO: 3 和 4 的抗体（即变体 574）。亲和力成熟的抗体优选以高于完整鼠 2C4 或完整变体 574 的亲和力结合 HER2 受体（例如根据采用 HER2 胞外域(ECD) ELISA 的评估，例如亲和力提高约 2 倍或约 4 倍至约 100 倍或约 1000 倍）。例示性的用于替代的重链可变区 CDR 残基包括 H28、H30、H34、H35、H64、H96、H99，或者两个或多个的组合（例如这些残基中的 2、3、4、5、6 或 7 个）。用于改变的轻链可变区 CDR 残基的例子包括 L28、L50、L53、L56、L91、L92、L93、L94、L96、L97，或者两个或多个的组合（例如这些残基中的 2 至 3、4、5 或直到约 10 个）。

还设想了各种形式的人源化抗体或亲和力成熟的抗体。例如，人源化抗体或亲和力成熟的抗体可以是抗体片段，诸如 Fab，它任选与一种或多种胞毒剂偶联以产生免疫偶联物。或者，人源化抗体或亲和力成熟的抗体可以是完整抗体，诸如完整的 IgG1 抗体。

(iv) 人抗体

作为人源化的替代方法，可生成人抗体。例如，现在有可能生成在缺乏内源免疫球蛋白生成的情况下能够在免疫后生成人抗体完整全集的转基因动物（如小鼠）。例如，已经描述了嵌合和种系突变小鼠中抗体重链连接区(J_H)基因的纯合删除导致内源抗体生成的完全抑制。在此类种系突变小鼠中转移大量人种系免疫球蛋白基因将导致在抗原攻击后生成人抗体。参见例如 Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggermann *et al.*, *Year in Immuno.* 7: 33 (1993); 及美国专利 5,591,669、5,589,369 和 5,545,807。

或者，噬菌体展示技术（McCafferty *et al.*, *Nature* 348: 552-553 (1990)）可用于在体外从来自未免疫供体的免疫球蛋白可变(V)区基因全集生成人抗体和抗体片段。依照这种技术，将抗体 V 区基因克隆到丝状噬菌体诸如 M13 或 fd 的主要或次要外壳蛋白基因的读码框中，并在噬菌体颗粒表面上展示

为功能性抗体片段。因为丝状颗粒包含噬菌体基因组的单链 DNA 拷贝，以抗体的功能特性为基础进行的选择也导致编码展示那些特性的抗体的基因的选择。因此，噬菌体模拟 B 细胞的一些特性。噬菌体展示可以多种形式进行；有关综述参见例如 Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3: 564-571 (1993)。V 基因区段的数种来源可用于噬菌体展示。Clackson *et al.*, *Nature* 352: 624-628 (1991)从衍生自经免疫小鼠脾的小型 V 基因随机组合文库分离得到大量不同的抗噁唑酮抗体。可本质上依照 Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1991)或 Griffith *et al.*, *EMBO J.* 12: 725-734 (1993)所述技术，由未免疫人供体构建 V 基因全集，并分离针对大量不同抗原（包括自身抗原）的抗体。还可参见美国专利 5,565,332 和 5,573,905。

还可通过体外激活 B 细胞（参见美国专利 5,567,610 和 5,229,275）来生成人抗体。

1998 年 6 月 30 日发布的美国专利 5,772,997 和 1997 年 1 月 3 日出版的 WO 97/00271 中描述了人 HER2 抗体。

(v) 抗体片段

已经开发了用于生成抗体片段的多种技术。传统上，通过蛋白水解消化完整抗体来衍生这些片段（参见例如 Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24: 107-117 (1992)及 Brennan *et al.*, *Science* 229: 81 (1985)）。然而，现在可直接由重组宿主细胞生成这些片段。例如，可从上文讨论的噬菌体抗体库分离抗体片段。或者，可直接从大肠杆菌回收 Fab'-SH 片段，并通过化学方法偶联形成 $F(ab')_2$ 片段（Carter *et al.*, *Bio/Technology* 10: 163-167 (1992)）。依照另一种方法，可直接从重组宿主细胞培养物分离 $F(ab')_2$ 片段。用于生成抗体片段的其它技术对熟练从业人员是显而易见的。在其它实施方案中，选择的抗体是单链 Fv 片段(scFv)。参见 WO 93/16185；美国专利 5,571,894；和美国专利 5,587,458。抗体片段还可以是“线性抗体”，例如如美国专利 5,641,870 中所述。所述线性抗体片段可以是单特异性的或双特异性的。

(vi) 双特异性抗体

双特异性抗体指对至少两种不同表位具有结合特异性的抗体。例示性的双特异性抗体可结合 HER2 蛋白质的两种不同表位。其它此类抗体可将

HER2 结合位点与 EGFR、HER3 和/或 HER4 的结合位点进行组合。或者，HER2 臂可与结合白细胞上触发分子诸如 T 细胞受体分子（如 CD2 或 CD3）或 IgG 的 Fc 受体(FcγR)，诸如 FcγRI（CD64）、FcγRII（CD32）和 FcγRIII（CD16）的臂组合，使得细胞防御机制聚焦于 HER2 表达细胞。双特异性抗体还可用于将胞毒剂定位于表达 HER2 的细胞。这些抗体拥有 HER2 结合臂及结合胞毒剂（如肥皂草毒蛋白、抗干扰素-α、长春花生物碱、蓖麻毒蛋白 A 链、甲氨蝶呤或放射性同位素半抗原）的臂。可将双特异性抗体制备成全长抗体或抗体片段（如 $F(ab')_2$ 双特异性抗体）。

WO 96/16673 描述了一种双特异性 HER2/FcγRIII 抗体，而美国专利 5,837,234 公开了一种双特异性 HER2/FcγRI 抗体 IDM1（Osidem）。WO 98/02463 显示了一种双特异性 HER2/Fcα 抗体。美国专利 5,821,337 教导了一种双特异性 HER2/CD3 抗体。MDX-210 是一种双特异性 HER2-FcγRIII 抗体。

用于制备双特异性抗体的方法是本领域已知的。全长双特异性抗体的传统生成基于两种免疫球蛋白重链-轻链对的共表达，其中两种链具有不同的特异性（Millstein *et al.*, *Nature* 305: 537-539 (1983)）。由于免疫球蛋白重链和轻链的随机分配，这些杂交瘤（四源杂交瘤(quadroma)）生成 10 种不同抗体分子的潜在混合物，其中只有一种具有正确的双特异性结构。通常通过亲和层析步骤进行的正确分子的纯化相当麻烦，且产物产量低。WO 93/08829 及 Traunecker *et al.*, *EMBO J.* 10: 3655-3659 (1991)中公开了类似的流程。

依照一种不同的方法，将具有所需结合特异性（抗体-抗原结合位点）的抗体可变区与免疫球蛋白恒定区序列融合。融合优选使用包含至少部分铰链、CH2 和 CH3 区的免疫球蛋白重链恒定区。优选的是，在至少一种融合物中具有包含轻链结合所必需的位点的第一重链恒定区(CH1)。将编码免疫球蛋白重链融合物以及，如果需要，免疫球蛋白轻链的 DNA 插入分开的表达载体，并共转染到合适的宿主生物体中。在用于构建的三种多肽链比例不等时提供最佳产量的实施方案中，这为调整三种多肽片段的相互比例提供了很大的灵活性。然而，在至少两种多肽链以相同比率表达导致高产量时或在该比率没有特别意义时，有可能将两种或所有三种多肽链的编码序列插入同一个载体。

在该方法的一个优选实施方案中，双特异性抗体由一个臂上具有第一结

合特异性的杂合免疫球蛋白重链，和另一个臂上的杂合免疫球蛋白重链-轻链对（提供第二结合特异性）构成。由于免疫球蛋白轻链仅在半个双特异性分子中的存在提供了分离的便利途径，因此发现这种不对称结构便于将所需双特异性复合物与不想要的免疫球蛋白链组合分开。该方法公开于 WO 94/04690。关于生成双特异性抗体的其它细节参见例如 Suresh *et al.*, *Methods in Enzymology* 121: 210 (1986)。

依照美国专利 5,731,168 中描述的另一种方法，可改造一对抗体分子之间的界面，将从重组细胞培养物中回收的异二聚体的百分比最大化。优选的界面包含至少部分 C_H3 抗体恒定区。在该方法中，将第一抗体分子界面的一个或多个小氨基酸侧链用较大侧链（如酪氨酸或色氨酸）替换。通过用较小氨基酸侧链（如丙氨酸或苏氨酸）替换大氨基酸侧链，在第二抗体分子的界面上产生针对大侧链的相同或相似大小的补偿性“空腔”。这提供了较之其它不想要的终产物诸如同二聚体提高异二聚体产量的机制。

双特异性抗体包括交联或“异源偶联”抗体。例如，异源偶联物中的一种抗体可与亲合素偶联，另一种抗体与生物素偶联。例如，此类抗体建议用于将免疫系统细胞靶向不想要的细胞(美国专利 4,676,980)，和用于治疗 HIV 感染（WO 91/00360、WO 92/200373 和 EP 03089）。可使用任何便利的交联方法来制备异源偶联抗体。合适的交联剂是本领域众所周知的，并且连同许多交联技术一起公开于美国专利 4,676,980。

文献中还描述了由抗体片段生成双特异性抗体的技术。例如，可使用化学连接来制备双特异性抗体。Brennan *et al.*, *Science* 229: 81 (1985)描述了通过蛋白水解切割完整抗体以生成 $F(ab')_2$ 片段的方法。将这些片段在存在二硫醇络合剂亚砷酸钠的情况下还原，以稳定邻近的二硫醇并防止分子间二硫键的形成。然后将产生的 Fab' 片段转变为硫代硝基苯甲酸酯(TNB)衍生物。然后将 Fab'-TNB 衍生物之一通过巯基乙胺的还原重新恢复成 Fab'-硫醇，并与等摩尔量的另一种 Fab'-TNB 衍生物混合，以形成双特异性抗体。产生的双特异性抗体可用作酶的选择性固定化试剂。

最近的进展使得从大肠杆菌中直接回收 Fab'-SH 片段变得更加容易，这些片段可化学偶联以形成双特异性抗体。Shalaby *et al.*, *J. Exp. Med.* 175: 217-225 (1992)描述了生成完全人源化的双特异性抗体 $F(ab')_2$ 分子。每个 Fab' 片段由大肠杆菌分开分泌，并在体外进行定向化学偶联以形成双特异性抗

体。如此形成的双特异性抗体能够结合过表达 HER2 受体的细胞和正常人 T 细胞，以及触发人细胞毒性淋巴细胞针对人乳房肿瘤靶的溶解活性。

还描述了从重组细胞培养物直接制备和分离双特异性抗体片段的多种技术。例如，已使用亮氨酸拉链生成双特异性抗体。Kostelny *et al.*, *J. Immunol.* 148(5): 1547-1553 (1992)。将来自 Fos 和 Jun 蛋白的亮氨酸拉链肽通过基因融合与两种不同抗体的 Fab'部分连接。抗体同二聚体在铰链区还原而形成单体，然后重新氧化而形成抗体异二聚体。这种方法也可用于生成抗体同二聚体。由 Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993)描述的“双抗体”技术提供了制备双特异性抗体片段的替代机制。该片段包含通过接头相连的轻链可变区(V_L)和重链可变区(V_H)，所述接头太短使得同一条链上的两个结构域之间不能配对。因此，迫使一个片段上的 V_H 和 V_L 结构域与另一个片段上的互补 V_L 和 V_H 结构域配对，由此形成两个抗原结合位点。还报道了通过使用单链 Fv (sFv)二聚体制备双特异性抗体片段的另一种策略。参见 Gruber *et al.*, *J. Immunol.* 152: 5368 (1994)。

设想了具有超过两个效价的抗体。例如，可制备三特异性抗体。Tutt *et al.*, *J. Immunol.* 147: 60 (1991)。

(vii) 其它氨基酸序列修饰

设想了本文所述 HER2 抗体的氨基酸序列修饰。例如，可能希望改进抗体的结合亲和力和/或其它生物学特性。通过将适当的核苷酸改变引入 HER2 抗体核酸或者通过肽合成来制备 HER2 抗体的氨基酸序列变体。此类修饰包括例如 HER2 抗体氨基酸序列中的残基删除和/或插入和/或替代。只要最终的构建物具有所需特性，可进行删除、插入和替代的任何组合以获得最终的构建物。氨基酸变化还可改变 HER2 抗体的翻译后加工，诸如改变糖基化位点的数目或位置。

可用于鉴定 HER2 抗体中作为优选诱变位置的某些残基或区域的一种方法称作“丙氨酸扫描突变”，如 Cunningham and Wells, *Science* 244: 1081-1085 (1989)所述。这里，鉴定了一个或一组靶残基（例如带电荷的残基，诸如精氨酸、天冬氨酸、组氨酸、赖氨酸和谷氨酸），并用中性或带负电荷的氨基酸（最优选丙氨酸或聚丙氨酸）替代，以影响氨基酸与 HER2 抗原的相互作用。然后通过在或对替代位点引入更多或其它变体，推敲那些对替代显示功能敏感性的氨基酸位置。因此，虽然引入氨基酸序列变异的位点是预先决定

的，但是突变本身的性质不需要预先决定。例如，为了分析在指定位点处的突变的后果，在靶密码子或区域进行丙氨酸扫描诱变或随机突变，并对所表达的 HER2 抗体变体筛选所需活性。

氨基酸序列插入包括氨基-和/或羧基-末端的融合，长度范围从一个残基至包含上百或更多残基的多肽，以及单个或多个氨基酸残基的序列内插入。末端插入的例子包括具有 N-末端甲硫氨酰残基的 HER2 抗体或与细胞毒性多肽融合的抗体。HER2 抗体分子的其它插入变体包括在 HER2 抗体的 N-或 C-末端融合酶（例如用于 ADEPT）或提高抗体血清半衰期的多肽。

另一类变体是氨基酸替代变体。这些变体在 HER2 抗体分子中有至少一个氨基酸残基用不同残基替换。最感兴趣进行替代诱变的位点包括高变区或 CDR，但也考虑了 FR 或 Fc 区改变。保守替代在表 1 中显示在标题“优选替代”下。如果此类替代导致生物学活性的改变，那么可以引入表 1 中称为“例示替代”的更实质性改变，或如下文中关于氨基酸种类的进一步描述，并筛选产物。

表 1

原始残基	例示替代	优选替代
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp; Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; 正亮氨酸	Leu
Leu (L)	正亮氨酸; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; 正亮氨酸	Leu

对抗体生物学特性的实质性修饰通过选择在保持以下方面的效果上显著不同的替代来完成：(a) 替代区域的多肽主链的结构，例如作为折叠片或

螺旋构象，(b) 靶位点处分子的电荷或疏水性，或(c) 侧链的体积。根据其侧链特性的相似性，可将氨基酸如下分组（A. L. Lehninger，在《*Biochemistry*》中，第 2 版，73-75，Worth Publishers，New York，1975）：

(1) 非极性的：Ala (A)、Val (V)、Leu (L)、Ile (I)、Pro (P)、Phe (F)、Trp (W)、Met (M)

(2) 不带电荷的、极性的：Gly (G)、Ser (S)、Thr (T)、Cys (C)、Tyr (Y)、Asn (N)、Gln (Q)

(3) 酸性的：Asp (D)、Glu (E)

(4) 碱性的：Lys (K)、Arg (R)、His(H)

或者，根据共同的侧链特性，可将天然存在残基如下分组：

(1) 疏水性的：正亮氨酸、Met、Ala、Val、Leu、Ile;

(2) 中性、亲水性的：Cys、Ser、Thr、Asn、Gln;

(3) 酸性的：Asp、Glu;

(4) 碱性的：His、Lys、Arg;

(5) 影响链取向的残基：Gly、Pro;

(6) 芳香族的：Trp、Tyr、Phe。

非保守替代将需要用这些类别之一中的一个成员交换另一个类别的。

任何不涉及保持 HER2 抗体正确构象的半胱氨酸残基也可替代，通常用丝氨酸，以改进分子的氧化稳定性和防止异常交联。相反，可向抗体中添加半胱氨酸键以改善其稳定性（特别是当抗体是抗体片段诸如 Fv 片段时）。

特别优选的一类替代变体牵涉替代亲本抗体（如人源化或人抗体）的一个或多个高变区残基。通常，选择用于进一步开发的所得变体相对于产生它们的亲本抗体将具有改进的生物学特性。产生此类替代变体的一种便利方法牵涉使用噬菌体展示的亲和力成熟。简而言之，将数个高变区位点（例如 6-7 个位点）突变，在各个位点产生所有可能的氨基酸替代。如此产生的抗体变体以单价形式展示在丝状噬菌体颗粒上，作为与各个颗粒内包装的 M13 基因 III 产物的融合物。然后如本文所公开的对噬菌体展示的变体筛选其生物学活性（例如结合亲和力）。为了鉴定用于修饰的候选高变区位点，可进行丙氨酸扫描诱变以鉴定对抗原结合具有重要贡献的高变区残基。或者/另外，分析抗原-抗体复合物的晶体结构，以鉴定抗体和人 HER2 之间的接触点可能是有益的。所述接触残基及邻近残基是依照本文详述的技术进行替代的候

选位点。一旦产生这样的变体，如本文所述对该组变体进行筛选，可选择在一种或多种相关测定法中具有优良特性的抗体用于进一步的开发。

抗体的另一类氨基酸变体改变了抗体的原始糖基化样式。改变意味着删除在抗体中发现的一个或多个碳水化合物模块，和/或添加抗体中不存在的一个或多个糖基化位点。

抗体的糖基化通常或是 N-连接的或是 O-连接的。N-连接指碳水化合物模块附着于天冬酰胺残基的侧链。三肽序列天冬酰胺-X-丝氨酸和天冬酰胺-X-苏氨酸（其中 X 是除脯氨酸外的任何氨基酸）是将碳水化合物模块酶促附着于天冬酰胺侧链的识别序列。因此，多肽中这些三肽序列其中任一的存在产生了潜在的糖基化位点。O-连接的糖基化指将糖类 N-乙酰半乳糖胺、半乳糖或木糖之一附着于羟基氨基酸，最常见的是丝氨酸或苏氨酸，但也可使用 5-羟脯氨酸或 5-羟赖氨酸。

向抗体中添加糖基化位点可通过改变氨基酸序列使其包含一个或多个上述三肽序列而便利的完成（用于 N-连接的糖基化位点）。所述改变还可通过向原始抗体的序列中添加或替代一个或多个丝氨酸或苏氨酸残基来进行（用于 O-连接的糖基化位点）。

若抗体包含 Fc 区，则可改变附着其上的任何寡糖结构。例如，美国专利申请 US 2003/0157108 A1（Presta,L.）中描述了有缺乏岩藻糖的成熟碳水化合物结构附着于抗体 Fc 区的抗体。还可参见 US 2004/0093621 A1（Kyowa Hakko Kogyo Co., Ltd.）。WO 03/011878（Jean-Mairet 等人）和美国专利 6,602,684（Umana 等人）中提到了在附着于抗体 Fc 区的寡糖结构中有等分 N-乙酰葡糖胺(GlcNAc)的抗体。WO 97/30087（Patel 等人）中报告了在附着于抗体 Fc 区的寡糖结构中有至少一个半乳糖残基的抗体。关于有更改碳水化合物附着于其 Fc 区的抗体还可参见 WO 98/58964（Raju,S.）和 WO 99/22764（Raju,S.）。本文还设想了包含有此类碳水化合物结构附着于 Fc 区一条或两条重链上的主要种类抗体的抗体组合物

编码HER2抗体氨基酸序列变体的核酸分子可通过本领域已知的多种方法制备。这些方法包括但不限于从天然来源分离（在天然存在氨基酸序列变体的情况中），或者通过对早期制备的变体或非变体型式的 HER2 抗体进行寡核苷酸介导的（或定点）诱变、PCR 诱变和盒式诱变来制备。

(viii) 筛选具有期望特性的抗体

上文已经描述了用于生成抗体的技术。如果需要，还可以选择具有某些生物学特征的抗体。

为了鉴定阻断 HER 受体的配体活化的抗体，可测定抗体阻断 HER 配体结合表达 HER 受体（例如偶联另一种 HER 受体，该 HER 受体与目的 HER 受体形成 HER 异寡聚体）的细胞的能力。例如，可将天然表达或者经转染而表达 HER 异寡聚体的 HER 受体的细胞与抗体一起温育，然后暴露于经标记的 HER 配体。然后可评估 HER2 抗体阻断配体结合 HER 异寡聚体中的 HER 受体的能力。

例如，可以基本上如 WO01/00245 中所述，以 24 孔板的形式在冰上使用单层 MCF7 培养物进行 HER2 抗体对 HRG 结合 MCF7 乳房肿瘤细胞系的抑制。可将 HER2 单克隆抗体添加到各个孔中并温育 30 分钟。然后可加入 ^{125}I 标记的 rHRGβ1$_{177-224}$（25pm），并可继续温育 4-16 小时。可绘制剂量-应答曲线，并可计算目的抗体的 IC_{50} 值。在一个实施方案中，阻断 HER 受体的配体活化的抗体在此测定法中抑制 HRG 结合 MCF7 细胞的 IC_{50} 为约 50nM 或更低，更优选 10nM 或更低。当抗体是抗体片段诸如 Fab 片段时，在此测定法中抑制 HRG 结合 MCF7 细胞的 IC_{50} 可以是例如约 100nM 或更低，更优选 50nM 或更低。

或者/另外，可评估 HER2 抗体阻断 HER 异寡聚体中存在的 HER 受体的 HER 配体刺激的酪氨酸磷酸化的能力。例如，可将内源表达或者经转染而表达 HER 受体的细胞与抗体一起温育，然后使用抗磷酸酪氨酸单克隆（任选偶联有可检测标记物）测定 HER 配体依赖性酪氨酸磷酸化活性。美国专利 5,766,863 中描述的激酶受体活化测定法也可用于测定 HER 受体活化和抗体对该活性的阻断。

在一个实施方案中，可以基本上如 WO01/00245 中所述，筛选在 MCF7 细胞中抑制 HRG 刺激 p180 酪氨酸磷酸化的抗体。例如，可将 MCF7 细胞铺到 24 孔板中，并将 HER2 的单克隆抗体加入每个孔中，于室温温育 30 分钟；然后可向每个孔中加入 rHRGβ1$_{177-244}$ 至终浓度 0.2nM，并可继续温育 8 分钟。从每个孔中吸走培养基，通过加入 100μl SDS 样品缓冲液（5% SDS, 25mM DTT, 25mM Tris-HCl, pH 6.8）来终止反应。可在 4-12%梯度凝胶（Novex）上对每一份样品（25μl）进行电泳，然后通过电泳转移到聚偏二氟乙烯膜上。可对抗磷酸酪氨酸（以 1μg/ml）免疫印迹显影，并通过反射密度法(reflectance

densitometry)量化 Mr 约 180,000 的主要反应性条带的强度。在此测定法中，所选抗体优选将 HRG 刺激 p180 酪氨酸磷酸化显著抑制至对照的约 0-35%。可绘制通过反射密度法测定的对 HRG 刺激 p180 酪氨酸磷酸化的抑制作用的剂量-应答曲线，并可计算目的抗体的 IC_{50}。在一个实施方案中，阻断 HER 受体的配体活化的抗体在此测定法中抑制 p180 酪氨酸磷酸化的 HRG 刺激的 IC_{50} 为约 50nM 或更低，更优选 10nM 或更低。当抗体是抗体片段诸如 Fab 片段时，在此测定法中抑制 HRG 刺激 p180 酪氨酸磷酸化的 IC_{50} 可以是例如约 100nM 或更低，更优选 50nM 或更低。

还可评估抗体对 MDA-MB-175 细胞的生长抑制作用，例如基本上如 Schaefer *et al.*, *Oncogene* 15: 1385-1394 (1997)中所述。依照此测定法，可将 MDA-MB-175 细胞用 HER2 单克隆抗体（10μg/mL）处理 4 天，并用结晶紫染色。与 HER2 抗体的温育对此细胞系显示的生长抑制作用可能与使用单克隆抗体 2C4 相似。在还有一个实施方案中，外源 HRG 不会显著逆转这种抑制作用。优选的是，无论是否存在外源 HRG，该抗体将能够以大于单克隆抗体 4D5 的程度（并且任选以大于单克隆抗体 7F3 的程度）抑制 MDA-MB-175 细胞的细胞增殖。

在一个实施方案中，根据免疫共沉淀实验的测定，诸如 WO01/00245 中所述，目的 HER2 抗体可在 MCF7 和 SK-BR-3 细胞中阻断调蛋白依赖性的 HER2 与 HER3 结合，实质上比单克隆抗体 4D5 更有效，且优选实质上比单克隆抗体 7F3 更有效。

为了鉴定生长抑制性 HER2 抗体，可筛选抑制过表达 HER2 的癌细胞生长的抗体。在一个实施方案中，在约 0.5-30μg/ml 的抗体浓度，所选生长抑制性抗体能够将细胞培养中的 SK-BR-3 细胞生长抑制约 20-100%，优选约 50-100%。为了鉴定这样的抗体，可进行美国专利 5,677,171 中描述的 SK-BR-3 测定法。依照此测定法，在添加了 10%胎牛血清、谷氨酰胺和青霉素链霉素的，F12 和 DMEM 培养基的 1:1 混合液中培养 SK-BR-3 细胞。以 20,000 个细胞将 SK-BR-3 细胞铺入 35mm 细胞培养皿（2ml/35mm 培养皿）。每个培养皿加入 0.5-30μg/ml HER2 抗体。6 天后，使用电子 COULTER™细胞计数器对细胞数计数，与未处理细胞比较。可选择那些将 SK-BR-3 细胞生长抑制约 20-100%或约 50-100%的抗体作为生长抑制性抗体。关于用于筛选生长抑制性抗体，诸如 4D5 和 3E8 的测定法，参见美国专利 5,677,171。

为了选择诱导凋亡的抗体，可利用使用 BT474 细胞的膜联蛋白结合测定法。如前一段落所讨论的，在培养皿中培养和接种 BT474 细胞。然后除去培养基，只用新鲜培养基或者含有 10μg/ml 单克隆抗体的培养基替换。在 3 天温育期后，用 PBS 洗涤细胞单层，通过胰蛋白酶消化脱离。然后如上文关于细胞死亡测定法所讨论的，离心细胞，重悬于 Ca^{2+}结合缓冲液中，并等分到试管中。然后往试管中加入经标记的膜联蛋白（如膜联蛋白 V-FTIC）（1μg/ml）。可使用 FACSCAN™流式细胞计数仪和 FACSCONVERT™ CellQuest 软件（Becton Dickinson）来分析样品。选择那些相对于对照诱导统计学显著水平的膜联蛋白结合的抗体作为诱导凋亡的抗体。除了膜联蛋白结合测定法，还可利用使用 BT474 细胞的 DNA 染色测定法。为了进行此测定法，将已经如前面两段所述用目的抗体处理过的 BT474 细胞与 9μg/ml HOECHST 33342™于 37℃温育 2 小时，然后使用 MODFIT LT™软件（Verity Software House）在 EPICS ELITE™流式细胞计数仪（Coulter Corporation）上进行分析。可选择在此测定法中诱导的凋亡细胞百分比变化比未处理细胞高 2 倍或更高（优选 3 倍或更高）（直到 100%凋亡细胞）的抗体作为促凋亡(pro-apoptotic)抗体。用于筛选诱导凋亡抗体诸如 7C2 和 7F3 的测定法参见 WO98/17797。

为了筛选结合 HER2 上目的抗体所结合的表位的抗体，可进行常规交叉阻断测定法，诸如《*Antibodies, A Laboratory Manual*》，Cold Spring Harbor Laboratory，Ed Harlow 和 David Lane，1988 中所述，以评估所述抗体是否交叉阻断诸如 2C4 或 Pertuzumab 等抗体与 HER2 的结合。或者/另外，可通过本领域已知的方法进行表位作图，且/或可研究抗体-HER2 结构（Franklin *et al.*, *Cancer Cell* 5: 317-328 (2004)）以了解抗体结合 HER2 的哪个/哪些结构域。

(ix) 免疫偶联物

本发明还涉及包含偶联有胞毒剂的抗体的免疫偶联物，所述胞毒剂诸如化疗剂、毒素（如小分子毒素或细菌、真菌、植物或动物起源的酶活毒素，包括其片段和/或变体）或放射性同位素（即放射偶联物）。

上文已经描述了可用于生成此类免疫偶联物的化疗剂。本文还设想了抗体与一种或多种小分子毒素的偶联物，诸如加利车霉素、美登素（美国专利 5,208,020）、单端孢菌毒素和 CC1065。

在本发明的一个优选实施方案中，将抗体与一个或多个美登素分子偶联（例如每个抗体分子偶联约 1 个至约 10 个美登素分子）。例如可将美登素转变为 May-SS-Me，后者可还原为 May-SH3 并与经修饰抗体反应（Chari *et al.*, *Cancer Research* 52: 127-131 (1992)）以产生美登木素生物碱-抗体免疫偶联物。

另一种目的免疫偶联物包含偶联有一个或多个加利车霉素分子的 HER2 抗体。加利车霉素抗生素家族能够在亚皮摩尔浓度产生双链 DNA 断裂。可使用的加利车霉素的结构类似物包括但不限于 γ_1^I、α_2^I、α_3^I、N-乙酰基-γ_1^I、PSAG 和 θ^I_1（Hinman *et al.*, *Cancer Research* 53: 3336-3342 (1993)和 Lode *et al.*, *Cancer Research* 58: 2925-2928 (1998)）。还可参见美国专利 5,714,586; 5,712,374; 5,264,586; 和 5,773,001，明确收入本文作为参考。

可使用的酶活毒素及其片段包括白喉毒素 A 链、白喉毒素的非结合活性片段、外毒素 A 链（来自铜绿假单孢菌(*Pseudomonas aeruginosa*)）、蓖麻毒蛋白 A 链、相思豆毒蛋白 A 链、蒴莲根毒素(modeccin) A 链、α-帚曲毒素(sarcin)、油桐(*Aleurites fordii*)毒蛋白、香石竹(dianthin)毒蛋白、美洲商陆(*Phytolaca Americana*)毒蛋白（PAPI、PAPII 和 PAP-S）、苦瓜(momordica charantia)抑制物、麻疯树毒蛋白(curcin)、巴豆毒蛋白(crotin)、肥皂草(sapaonaria officinalis)抑制物、白树毒素(gelonin)、丝林霉素(mitogellin)、局限曲菌素(restrictocin)、酚霉素(phenomycin)、依诺霉素(enomycin)和单端孢菌毒素(tricothecenes)。参见例如 1993 年 10 月 28 日出版的 WO 93/21232。

本发明还设想了抗体与具有核酸水解活性的化合物（如核糖核酸酶或 DNA 内切核酸酶诸如脱氧核糖核酸酶; DNA 酶）之间形成的免疫偶联物。

有多种放射性同位素可用于生成放射偶联 HER2 抗体。例子包括 At^{211}、I^{131}、I^{125}、Y^{90}、Re^{186}、Re^{188}、Sm^{153}、Bi^{212}、P^{32} 和 Lu 的放射性同位素。

可使用多种双功能蛋白质偶联剂来制备抗体和胞毒剂的偶联物，诸如 3-(2-吡啶基二巯基)丙酸 N-琥珀酰亚胺酯(SPDP)、4-(N-马来酰亚胺甲基）环己胺-1-羧酸琥珀酰亚胺酯、亚氨基硫烷(IT)、亚氨酸酯（诸如盐酸己二酰亚胺二甲酯）、活性酯类（诸如辛二酸二琥珀酰亚胺酯）、醛类（诸如戊二醛）、双叠氮化合物（诸如双(对叠氮苯甲酰基)己二胺）、双重氮衍生物（诸如双(对重氮苯甲酰基)己二胺）、二异氰酸酯（诸如甲苯 2,6-二异氰酸酯）、和双活性氟化合物（诸如 1,5-二氟-2,4-二硝基苯）的双功能衍生物。例如，可如 Vitetta

et al., *Science* 238: 1098 (1987)中所述制备蓖麻毒蛋白免疫毒素。碳-14 标记的 1-异硫氰酸苯甲基-3-甲基二亚乙基三胺五乙酸(MX-DTPA)是用于将放射性核苷酸与抗体偶联的例示性螯合剂。参见 WO 94/11026。接头可以是便于在细胞中释放细胞毒性药物的“可切割接头”。例如，可使用酸不稳定接头、肽酶敏感接头、二甲基接头或含二硫化物接头（Chari *et al.*, *Cancer Research* 52: 127-131 (1992)）。

或者，可例如通过重组技术或肽合成来制备包含 HER2 抗体和胞毒剂的融合蛋白。

在另一个实施方案中，可将抗体与“受体”（诸如链霉亲合素）偶联，用于肿瘤的预定位，其中对患者施用抗体-受体偶联物，随后使用清除剂从循环中除去未结合的偶联物，然后施用与胞毒剂（如放射性核苷酸）偶联的“配体”（例如亲合素）。

(xi) 其它抗体修饰

本文还设想了抗体的其它修饰。例如，可将抗体与多种非蛋白质性质聚合物中的一种连接，例如聚乙二醇(PEG)、聚丙二醇、聚氧化烯，或聚乙二醇和聚丙二醇的共聚物。还可将抗体包载于例如通过凝聚技术或通过界面聚合制备的微胶囊中（例如分别是羟甲基纤维素或明胶微胶囊和聚(甲基丙烯酸甲酯)微胶囊）、在胶状药物传递系统中（例如脂质体、清蛋白微球体、微乳剂、纳米颗粒和纳米胶囊）、或在粗滴乳状液中。此类技术公开于《*Remington's Pharmaceutical Sciences*》，第 16 版，Osol,A.编，1980。

可能希望在效应物功能方面修饰本发明的抗体，例如增强抗体的抗体依赖性细胞介导的细胞毒性(ADCC)和/或补体依赖性细胞毒性(CDC)。这可通过在抗体 Fc 区中引入一个或多个氨基酸替代来实现。或者/另外，可在 Fc 区中引入半胱氨酸残基，从而使得在该区中形成链间二硫键。如此产生的同二聚体抗体可具有改善的内在化能力和/或提高的补体介导的细胞杀伤和抗体依赖性细胞细胞毒性(ADCC)。参见 Caron *et al.*, *J. Exp. Med.* 176: 1191-1195 (1992)和 Shopes,B., *J. Immunol.* 148: 2918-2922 (1992)。具有增强的抗肿瘤活性的同二聚体抗体还可使用如 Wolff *et al.*, *Cancer Research* 53: 2560-2565 (1993)中描述的异双功能交联剂来制备。或者，抗体可改造成具有双重 Fc 区，由此可具有增强的补体溶解和 ADCC 能力。参见 Stevenson *et al.*, *Anti-Cancer Drug Design* 3: 219-230 (1989)。

WO 00/42072（Presta,L.）描述了在存在人效应细胞时具有改进的 ADCC 功能的抗体，其中所述抗体在其 Fc 区中包含氨基酸替代。优选的是，具有改进的 ADCC 的抗体在 Fc 区的位置 298、333 和/或 334 包含替代。优选的是，改变的 Fc 区是在一个、两个或三个这些位置处包含替代或由其组成的人 IgG1 Fc 区。

WO 99/51642、美国专利 6,194,551 B1、美国专利 6,242,195 B1、美国专利 6,528,624 B1 和美国专利 6,538,124（Idusogie 等人）中描述了具有改变的 C1q 结合和/和补体依赖性细胞毒性(CDC)的抗体。这些抗体在其 Fc 区的氨基酸位置 270、322、326、327、329、313、333 和/或 334 其中一个或多个处包含氨基酸替代。

为了提高抗体的血清半衰期，可如例如美国专利 5,739,277 中所述将补救受体结合表位掺入抗体（尤其是抗体片段）。在用于本文时，术语“补救受体结合表位”指 IgG 分子（如 IgG_1、IgG_2、IgG_3 或 IgG_4）Fc 区中负责提高 IgG 分子体内血清半衰期的表位。WO00/42072（Presta,L.）中还描述了在其 Fc 区中具有替代且血清半衰期延长的抗体。

还设想了具有三个或更多（优选四个）功能性抗原结合位点的改造抗体（美国专利申请 US 2002/0004587 A1，Miller 等人）。

本文公开的 HER2 抗体还可配制成脂质体。可通过本领域已知的方法来制备包含抗体的脂质体，诸如 Epstein *et al.*, *Proc. Natl. Acad. Sci. USA* 82: 3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA* 77: 4030 (1980); 美国专利 4,485,045 和 4,544,545; 及 1997 年 10 月 23 日出版的 WO 97/38731 中所述。美国专利 5,013,556 中公开了循环时间延长的脂质体。

特别有用的脂质体可用包含磷脂酰胆碱、胆固醇和 PEG 衍生化磷脂酰乙醇胺(PEG-PE)的脂质组合物通过反相蒸发法生成。将脂质体挤过具有设定孔径的滤器，以产生具有所需直径的脂质体。可如 Martin *et al.*, *J. Biol. Chem.* 257: 286-288 (1982)中所述，将本发明抗体的 Fab'片段经二硫化物交换反应与脂质体偶联。任选在脂质体中包含化疗剂。参见 Gabizon *et al.*, *J. National Cancer Inst.* 81(19): 1484 (1989)。

IV. 药用配制剂

制备本发明组合物的治疗用配制剂，即将组合物与任选的药学可接受的

载体、赋形剂或稳定剂混合（《*Remington's Pharmaceutical Sciences*》，第 16 版，Osol,A.编，1980），以冻干配制剂或水溶液的形式贮存。可接受的载体、赋形剂或稳定剂在所采用的剂量和浓度对接受者是无毒的，还包括缓冲剂，诸如磷酸盐、柠檬酸盐和其它有机酸；抗氧化剂，包括抗坏血酸和甲硫氨酸；防腐剂（诸如氯化十八烷基二甲基苄基铵；氯化己烷双胺；苯扎氯铵、苯索氯铵；酚、丁醇或苯甲醇；对羟基苯甲酸烷基酯，诸如对羟基苯甲酸甲酯或丙酯；邻苯二酚；间苯二酚；环己醇；3-戊醇；和间甲酚）；低分子量（少于约 10 个残基）多肽；蛋白质，诸如血清清蛋白、明胶或免疫球蛋白；亲水性聚合物，诸如聚乙烯吡咯烷酮；氨基酸，诸如甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、精氨酸或赖氨酸；单糖、二糖和其它碳水化合物，包括葡萄糖，甘露糖或糊精；螯合剂，诸如 EDTA；糖类，诸如蔗糖、甘露醇、海藻糖或山梨醇；成盐相反离子，诸如钠；金属复合物（如 Zn-蛋白质复合物）；和/或非离子表面活性剂，诸如 TWEEN™、PLURONICS™或聚乙二醇(PEG)。WO 97/04801 中描述了冻干 HER2 抗体配制剂。

本文中的配制剂还可含有所治疗具体适应症所必需的超过一种活性化合物，优选那些活性互补且彼此没有不利影响的化合物。例如，可能还希望在配制剂中提供结合 EGFR、HER2（例如结合 HER2 上不同表位的抗体）、HER3、HER4 或血管内皮生长因子(VEGF)的抗体。或者/另外，配制剂中还可包含化疗剂、胞毒剂、细胞因子、生长抑制剂、抗激素剂、EGFR 靶向药物、抗血管生成剂、酪氨酸激酶抑制剂和/或心脏保护剂。此类分子适于以有效用于所需目的的量而联合存在。

活性成分还可包载于例如通过凝聚技术或通过界面聚合制备的微胶囊中，例如分别是羟甲基纤维素或明胶微胶囊和聚(甲基丙烯酸甲酯)微胶囊、在胶状药物传递系统中（例如脂质体、清蛋白微球体、微乳剂、纳米颗粒和纳米胶囊）、或在粗滴乳状液中。此类技术公开于《*Remington's Pharmaceutical Sciences*》，第 16 版，Osol,A.编，1980。

可制备持续释放制剂。持续释放制剂的合适例子包括含有抗体的固体疏水性聚合物半透性基质，该基质以定型产品的形式存在，如薄膜或微胶囊。持续释放基质的例子包括聚酯、水凝胶（例如聚(2-羟乙基-甲基丙烯酸酯)或聚(乙烯醇)）、聚交酯（美国专利 3,773,919）、L-谷氨酸和 L-谷氨酸 γ-乙酯的共聚物、不可降解的乙烯-乙酸乙烯、可降解的乳酸-乙醇酸共聚物诸如

LUPRON DEPOT™（由乳酸-乙醇酸共聚物和醋酸亮丙瑞林构成的可注射微球体）及聚-D-(-)-3-羟基丁酸。

用于体内施用的配制剂必须是无菌的。这可容易的通过使用无菌滤膜过滤来实现。

V. 筛选接受治疗的患者

依照本发明的一个优选实施方案，为治疗选择的患者具有展示 HER（且优选 HER2）活化的肿瘤或其它细胞或组织。在一个实施方案中，癌细胞或其它测试细胞中 HER（或 HER2）活化的程度显著超过同一组织类型的非癌性或正常细胞中该受体的活化水平。这样的过度活化可能是由于癌细胞中 HER 受体过表达和/或可用于活化 HER 受体的 HER 配体高于正常水平造成的。这样的过度活化可能引起癌细胞的恶性状态和/或是由癌细胞的恶性状态引起的。在有些实施方案中，将对癌症进行诊断或预后测定法以确定是否存在导致所述 HER 受体过度活化的 HER 受体扩增和/或过表达。或者/另外，可对癌症进行诊断或预后测定法以确定癌中是否存在造成所述受体过度活化的 HER 配体扩增和/或过表达。在此类癌症的子集中，所述受体的过度活化可能是由自分泌刺激途径造成的。下文将更为详细的描述用于测定 HER 活化的多种例示性测定法。

(i) HER 二聚体

可对样品评估 HER 二聚体的存在，如指示 HER 或 HER2 活化。可使用本领域已知的任何方法来检测 HER2 二聚体，诸如 EGFR-HER2、HER2-HER3。下文描述了数种优选方法。这些方法检测非共价蛋白质-蛋白质相互作用或者另外指示目的蛋白质之间的接近性。

可使用基于免疫亲和的方法，诸如免疫沉淀或 ELISA 来检测 HER 二聚体。在一个实施方案中，用 HER2 抗体免疫沉淀来自肿瘤细胞的包含 HER2 的复合物，然后通过免疫印迹对所得免疫沉淀物探查 EGFR 或 HER3 的存在。在另一个实施方案中，可将 EGFR 或 HER3 抗体用于免疫沉淀步骤，然后用 HER2 抗体探查免疫沉淀物。在又一个实施方案中，可用对 EGFR、HER3、EGFR/HER2 复合物或 HER2/HER3 复合物特异的 HER 配体来沉淀复合物，然后探查 HER2 的存在。例如，可将配体与亲合素偶联，并用生物素柱纯化复合物。

在其它实施方案中，诸如 ELISA 或抗体“夹心(sandwich)”型测定法中，将 HER2 的抗体固定在固相支持物上，接触肿瘤细胞或肿瘤细胞裂解物，洗涤，然后暴露于针对 EGFR 或 HER3 的抗体。后一种抗体的结合，可直接检测或者通过偶联有可检测标记物的二抗来检测，指示异二聚体的存在。在某些实施方案中，固定 EGFR 或 HER3 抗体，将 HER2 抗体用于检测步骤。在其它实施方案中，可使用 HER 配体，替换或联合 HER 抗体。

化学或紫外交联也可用于共价连接活细胞表面的二聚体。Hunter *et al.*, *Biochem. J.* 320: 847-53。化学交联剂的例子包括二硫代双(琥珀酰亚胺基)丙酸酯(DSP)和 3,3'-二硫代双(磺基琥珀酰亚胺基)丙酸酯(DTSSP)。在一个实施方案中，通过 SDS-PAGE 分析来自化学交联肿瘤细胞的细胞提取物，并用 EGFR 和/或 HER3 的抗体进行免疫印迹。适当分子量的超位移带(supershifted band)最有可能代表 EGFR-HER2 或 HER2-HER3 二聚体，因为 HER2 是 EGFR 和 HER3 的优选二聚化配偶。此结果可通过后续使用 HER2 抗体的免疫印迹得以证实。

荧光共振能量转移(FRET)也可用于检测 EGFR-HER2 或 HER2-HER3 二聚体。根据能量从供体荧光团向受体荧光团的转移，FRET 检测体内和体外的蛋白质构象改变和蛋白质-蛋白质相互作用。Selvin, *Nat. Struct. Biol.* 7: 730-34 (2000)。只有在供体荧光团足够接近受体荧光团时才发生能量转移。在典型的 FRET 实验中，用不同荧光探针标记两个蛋白质或单一蛋白质上的两个位点。用指定波长的入射光将探针之一，供体探针激发至较高的能量状态。然后供体探针将其能量传递给第二种探针，受体探针，导致供体的荧光强度下降，而受体的荧光发射增加。为了测量能量转移的程度，将用供体和受体探针标记的样品中的供体强度与只用供体探针标记的样品中它的强度进行比较。任选的是，比较供体/受体以及只有受体的样品中受体的强度。合适的探针是本领域已知的，包括例如膜渗入染料，诸如荧光素和罗丹明；有机染料，诸如花青染料；以及镧系元素原子。Selvin，见上文。检测和测量能量转移的方法和仪器也是本领域已知的。Selvin，见上文。

适于检测和测量个体细胞中蛋白质-蛋白质相互作用的基于 FRET 的技术也是本领域已知的。例如，可使用供体光漂白荧光共振能力转移(pbFRET)显微术和荧光寿命成像显微术(FLIM)来检测细胞表面受体的二聚化。Selvin, 见上文; Gadella & Jovin, *J. Cell Biol.* 129: 1543-58 (1995)。在一个实施方案

中，将 pbFRET 用于“悬浮液中”或“原位”细胞以检测和测量 EGFR-HER2 或 HER2-HER3 二聚体的形成，如 Nagy *et al.*, *Cytometry* 32: 120-131 (1998) 中所述。这些技术测量由于能量转移而造成的供体荧光寿命的缩短。在一个具体的实施方案中，可使用流式细胞术 Foerster 型 FRET 技术(FCET)来研究 EGFR-HER2 和 HER2-HER3 二聚化，如 Nagy *et al.*, 见上文; 及 Brockhoff *et al.*, *Cytometry* 44: 338-48 (2001)中所述。

优选将 FRET 与标准免疫组化标记技术联合使用。Kenworthy, *Methods* 24: 289-96 (2001)。例如，可将偶联有合适荧光染料的抗体作为探针用于标记两种不同的蛋白质。如果蛋白质彼此接近，则所述荧光染料起到 FRET 的供体和受体的作用。通过标准方法检测能量转移。可通过流式细胞术手段或者通过数字显微镜检术系统诸如与电荷耦合器件(CCD)相机偶联的共聚焦显微镜检术或宽视野荧光显微镜检术来检测能量转移。

在本发明的一个实施方案中，用两种不同荧光团直接标记 HER2 抗体及或是 EGFR 或是 HER3 抗体，例如如 Nagy *et al.*, 见上文中所述。使肿瘤细胞或肿瘤细胞裂解物接触差异标记的抗体，它们在存在 EGFR-HER2 或 HER2-HER3 二聚体时起到 FRET 的供体和受体的作用。或者，将未标记的针对 HER2 及或是 EGFR 或是 HER3 的抗体连同差异标记的二抗一起用作供体和受体。参见例如 Brockhoff *et al.*, 见上文。检测能量转移并确定二聚体的存在，如果在极接近处发现标记物的话。

在其它实施方案中，荧光标记对 HER2 及或是 EGFR 或是 HER3 特异的 HER 受体配体并用于 FRET 研究。

在本发明的其它实施方案中，通过使用标准的直接或间接的免疫荧光技术和共聚焦激光扫描显微术得出的 HER2 与或是 EGFR 或是 HER3 的共定位，证明了肿瘤细胞表面上二聚体的存在。或者，用激光扫描成像(LSI)以高通量的形式诸如微孔板检测抗体结合以及 HER2 与或是 EGFR 或是 HER3 的共定位，如 Zuck *et al.*, *Proc. Natl. Acad. Sci. USA* 96: 11122-27 (1999)中所述。

在其它实施方案中，通过鉴定依赖于二聚体组分接近的酶活性来确定 EGFR-HER2 和/或 HER2-HER3 二聚体的存在。将 HER2 抗体与一种酶偶联，而将 EGFR 或 HER3 抗体与第二种酶偶联。加入第一种酶的第一种底物，且该反应产生第二种酶的第二种底物。这导致与另一种分子的反应，从而产生可检测的化合物，诸如染料。另一种化学药品的存在破坏第二种底物，从而

阻止与第二种酶的反应，除非第一种和第二种酶以及因此两种抗体极其接近。在一个具体的实施方案中，使肿瘤细胞或细胞裂解物接触偶联有葡萄糖氧化酶的 HER2 抗体以及偶联有辣根过氧化物酶的 HER3 或 HER1 抗体。向反应中加入葡萄糖以及染料前体，诸如 DAB，以及过氧化氢酶。通过 DAB 染色后的显色来确定二聚体的存在。

还可使用基于 eTag™测定体系（Aclara Bio Sciences, Mountain View, CA）的方法来检测二聚体，如例如 2001 年 12 月 6 日公开的美国专利申请 2001/0049105 中所述，将其全文明确收入本文作为参考。eTag™或“电泳标签”包含可检测报导物模块，诸如荧光基团。它还可包含“迁移率修饰物”，基本上由具有独特电泳迁移率的模块组成。这些模块容许在规定的电泳条件诸如毛细管电泳(CE)下从复杂混合物中分离并检测 eTag™。将 eTag™中含有报导物模块和任选的迁移率修饰物的部分通过可切割连接基团与第一靶结合模块连接以产生第一结合化合物。第一靶结合模块特异性识别特定的第一靶物，诸如核酸或蛋白质。第一靶结合模块不受任何形式的限制，它可以是例如多核苷酸或多肽。优选的是，第一靶结合模块是抗体或抗体片段。或者，第一靶结合模块可以是 HER 受体配体或其有结合能力的片段。

连接基团优选包含可切割模块，诸如酶底物，或者可在规定条件下断裂的任何化学键。当第一靶结合模块与其靶物结合时，引入和/或活化裂解剂，连接基团遭到切断，由此释放 eTag™中含有报导物模块和迁移率修饰物的部分。因此，“游离”eTag™的出现指示靶结合模块与其靶物的结合。

优选的是，第二结合化合物包含裂解剂和特异性识别第二靶物的第二靶结合模块。第二靶结合模块也不受任何方式的限制，它可以是例如抗体或抗体片段或 HER 受体配体或有结合能力的配体片段。裂解剂是这样的，它在第一结合化合物和第二结合化合物极其接近的情况下将只切割连接基团。

在本发明的一个实施方案中，第一结合化合物包含 eTag™，其中 HER2 的抗体担当第一靶结合模块。第二结合化合物包含与能切割 eTag™连接基团的裂解剂连接的 EGFR 或 HER3 抗体。优选的是，所述裂解剂必须活化才能切割连接基团。使肿瘤细胞或肿瘤细胞裂解物接触结合 HER2 的 eTag™以及结合细胞表面上的 EGFR 或 HER3 的经修饰 EGFR 或 HER3 抗体。如果需要，优选除去未结合的结合化合物并活化裂解剂。如果存在 EGFR-HER2 或 HER2-HER3 二聚体，由于裂解剂接近连接基团，裂解剂将切割连接基团并

释放 eTag™。然后可通过本领域已知的任何方法来检测游离 eTag™，诸如毛细管电泳。

在一个实施方案中，所述裂解剂是作用于连接基团的可活化化学药品。例如，所述裂解剂可通过使样品暴露于光照而活化。

在另一个实施方案中，使用 EGFR 或 HER3 抗体作为第一靶结合模块来构建 eTag™，而第二结合化合物则从 HER2 抗体来构建。

在又一个实施方案中，使用与其对二聚体中任一 HER 受体的结合相比，特异或优先结合二聚体的抗体或其它试剂来检测 HER 二聚体。

(ii) HER2 磷酸化

如前一节所论述的，用 EGFR、HER2 或 HER3 抗体免疫沉淀后，可任选进行二聚体的功能性测定法，作为免疫印迹的替换或补充。在一个实施方案中，用 HER3 抗体免疫沉淀后测定免疫沉淀物中的受体酪氨酸激酶活性。因为 HER3 不具有内在的酪氨酸激酶活性，所以免疫沉淀物中存在酪氨酸激酶活性指示 HER3 最有可能与 HER2 相结合。Graus-Porta *et al.*, *EMBO J.* 16: 1647-55 (1997); Klapper *et al.*, *Proc. Natl. Acad. Sci. USA* 96: 4995-5000 (1999)。此结果可通过用 HER2 抗体进行免疫印迹得到证实。在另一个实施方案中，用 HER2 抗体免疫沉淀后测定 EGFR 受体酪氨酸激酶活性。在此测定法中，使免疫沉淀物接触放射性 ATP 和模拟 EGFR 对 HER2 的转磷酸作用的体内位点的肽底物。所述肽的磷酸化指示共沉淀及由此 EGFR 与 HER2 的二聚化。受体酪氨酸激酶活性测定法是本领域众所周知的，包括检测靶底物磷酸化的测定法，例如用磷酸酪氨酸抗体，以及检测相关信号转导途径诸如 MAPK 途径激活的测定法。

可通过一种或多种 HER 受体诸如 HER (HER2)受体的免疫沉淀和 Western 印迹分析来评估 HER 受体的磷酸化。例如，使用抗磷酸酪氨酸抗体来检测免疫沉淀的 HER 受体中磷酸化酪氨酸残基，通过凝胶上存在磷酸-HER2 带确定为阳性。抗磷酸酪氨酸抗体可购自 Pan Vera（Madison, WI）、Invitrogen 的子公司、Chemicon International Inc.（Temecula, CA）或 Upstate Biotechnology（Lake Placid, NY）。通过缺乏该条带而确定为阴性。

在另一个实施方案中，使用磷酸特异性 HER2 抗体（克隆 PN2A; Thor *et al.*, *J. Clin. Oncol.* 18(18): 3230-3239 (2000)）通过免疫组化来评估 HER (HER2)受体的磷酸化。

用于检测 HER 受体磷酸化的其它方法包括但不限于 KIRA ELISA(美国专利 5,766,863; 5,891,650; 5,914,237; 6,025,145; 和 6,287,784)、质谱(比较磷酸化和未磷酸化 HER2 的大小)、以及使用 HER(例如 HER2)抗体和磷酸特异性或磷酸酪氨酸特异性抗体二者的 e 标签接近性测定法(例如使用购自 Aclara BioSciences(Mountain View, CA)的 eTag™测定试剂盒)。有关 eTag 测定法的细节见上文。

还可在细胞阵列中使用磷酸特异性抗体来检测细胞样品中信号转导蛋白的磷酸化状态(US 2003/0190689)。

(iii) HER2 配体

可依照已知流程来测定肿瘤中或与肿瘤有关的 HER 配体诸如 TGF-α 的水平。这样的测定法可检测待测样品中的蛋白质和/或编码它的核酸。在一个实施方案中,可使用免疫组化(IHC)来测定肿瘤中的 HER 配体水平;参阅例如 Scher *et al.*, *Clin. Cancer Research* 1: 545-550 (1995)。或者/另外,可评估待测样品中 HER 配体编码核酸的水平;例如通过 FISH、Southern 印迹或 PCR 技术。

(iv) 非 HER2 过表达癌症

尽管癌症的特征可以是 HER2 受体的过表达,但本申请还提供了用于治疗不认为是 HER2 过表达的癌症的方法。

为了测定癌症中的 HER2 表达,可利用多种诊断/预后测定法。在一个实施方案中,可通过 IHC 来分析 HER2 过表达,例如使用 HERCEPTEST® (Dako)。可将来自肿瘤活组织检查的石蜡包埋组织切片进行 IHC 测定法并遵循如下 HER2 蛋白质染色强度标准:

得分 0:未观察到染色或者在少于 10%的肿瘤细胞中观察到膜染色。

得分 1+:在超过 10%的肿瘤细胞中检测到微弱的/刚刚可察觉的膜染色。所述细胞只在其部分膜上有染色。

得分 2+:在超过 10%的肿瘤细胞中观察到微弱至中等的完全膜染色。

得分 3+:在超过 10%的肿瘤细胞中观察到中等至强烈的完全膜染色。

那些 HER2 过表达评估得分 0 或 1+的肿瘤可鉴定为未过表达 HER2,而那些得分 2+或 3+的肿瘤可鉴定为过表达 HER2。

过表达 HER2 的肿瘤可根据对应于每个细胞表达的 HER2 分子拷贝数的免疫组化得分进行定级,并且可通过生化方法测定:

0 = 0-10,000 个拷贝/细胞，

1+ = 至少约 200,000 个拷贝/细胞，

2+ = 至少约 500,000 个拷贝/细胞，

3+ = 至少约 2,000,000 个拷贝/细胞。

导致酪氨酸激酶的配体依赖性活化的3+水平的HER2过表达(Hudziak *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 7159-7163 (1987))发生于约30%的乳癌中，而且在这些患者中，无复发存活和总体存活减少（Slamon *et al.*, *Science* 244: 707-712 (1989); Slamon *et al.*, *Science* 235: 177-182 (1987))。或者/另外，可对福尔马林固定、石蜡包埋的肿瘤组织进行FISH测定法诸如INFORM™（由Ventana, Arizona销售)或PATHVISION™(Vysis, Illinois)以测定肿瘤中HER2过表达的程度（如果有的话)。

在一个实施方案中，所述癌症将是表达（且可以是过表达）EGFR的癌症，可对所述表达进行评估，作为用于评估如上所述HER2表达的方法。

还可利用体内诊断测定法来评估HER受体或HER配体过表达或扩增，例如通过施用结合待检测分子且标记有可检测标记物（如放射性同位素）的分子（诸如抗体)，然后对患者进行外部扫描以定位所述标记物。

VI. 使用 HER2 抗体的治疗

依照本发明，设想了HER2抗体可用于治疗癌症。所述癌症通常将包含HER2表达细胞，使得本文的HER2抗体能够结合所述癌细胞。上文定义部分列出了可用本发明组合物治疗的癌症。

还设想了HER2抗体可用于治疗各种非恶性疾病或紊乱，诸如自身免疫性疾病（如银屑病)；子宫内膜异位；硬皮病；再狭窄；息肉，诸如结肠息肉、鼻息肉或胃肠息肉；纤维腺瘤；呼吸疾病；胆囊炎；神经纤维瘤病；多囊性肾病；炎性疾病；皮肤紊乱，包括银屑病和皮炎；脉管疾病；牵涉脉管上皮细胞异常增殖的状况；胃肠溃疡；门内特里埃氏(Menetrier)病，分泌型腺瘤或蛋白质流失综合征(protein loss syndrome)；肾紊乱；血管生成性紊乱；眼病，诸如年龄相关黄斑变性、假定的眼组织胞浆菌病综合症(presumed ocular histoplasmosis syndrome)、来自增殖性糖尿病性视网膜病、视网膜血管形成、糖尿病性视网膜病或年龄相关的黄斑变性的视网膜新血管形成；骨相关病理学，诸如骨关节炎、佝偻病和骨质疏松；脑缺血事件后的损伤；纤维

变性或水肿(edema)疾病，诸如肝硬化、肺纤维化、carcoidosis、throiditis、系统性高粘滞综合症、奥斯勒-韦伯-朗迪(Osler-Weber-Rendu)病、慢性阻塞性肺病，或者烧伤、外伤、辐射、中风、组织缺氧或缺血后的水肿；皮肤的超敏反应；糖尿病性视网膜病变和糖尿病性肾病；格-巴二氏(Guillain-Barre)综合征；移植物抗宿主病或移植排斥；佩吉特氏(Paget)病；骨或关节发炎；光老化（例如由 UV 照射人皮肤引起的）；良性前列腺肥大；某些微生物感染，包括选自腺病毒、汉坦病毒(hantaviruses)、布氏疏螺旋体（*Borrelia burgdorferi*）、耶尔森氏菌属（*Yersinia spp.*）和百日咳博德特氏菌（*Bordetella pertussis*）的微生物病原体；由血小板聚集引起的血栓；生殖疾患，诸如子宫内膜异位、卵巢过刺激综合征、先兆子痫、功能障碍性子宫出血或月经频多；滑膜炎；粥样斑；急性和慢性肾病（包括增生性肾小球肾炎和糖尿病诱发的肾病）；湿疹；肥厚性瘢痕形成；内毒素性休克和真菌感染；家族性腺瘤息肉病；神经变性疾病（如阿尔茨海默氏(Alzheimer)病、AIDS 相关痴呆、帕金森氏(Parkinson)病、肌萎缩侧索硬化、视网膜色素变性、脊髓性肌萎缩和小脑变性）；骨髓发育异常综合症(myelodysplastic syndrome)；再生障碍性贫血；缺血性损伤；肺、肾或肝的纤维化；T 细胞介导的超敏性疾病；婴儿肥厚性幽门狭窄；泌尿阻塞综合症；银屑病关节炎；和桥本氏(Hasimoto)甲状腺炎。本文疗法优选的非恶性适应症包括银屑病、子宫内膜异位、硬皮病、脉管疾病（如再狭窄、动脉粥样硬化、冠状动脉病或高血压）、结肠息肉、纤维腺瘤或呼吸疾病（如哮喘、慢性支气管炎、支气管扩张或囊性纤维化）。

使用 HER2 抗体的治疗将导致疾病的体征或症状改善。例如，若所治疗疾病是癌症，则此类疗法可导致存活（总体存活和/或无进展存活）有所提高和/或可导致客观临床响应（部分的或完全的）。

优选的是，所施用组合物中的 HER2 抗体是裸抗体。然而，所施用的 HER2 抗体可偶联胞毒剂。优选的是，免疫偶联物和/或其所结合的 HER2 蛋白质受到细胞的内在化，导致免疫偶联物杀伤其所结合的癌细胞的治疗功效提高。在一个优选的实施方案中，胞毒剂靶向或干扰癌细胞中的核酸。此类胞毒剂的例子包括美登木素生物碱、加利车霉素、核糖核酸酶和 DNA 内切核酸酶。

依照已知方法对人类患者施用 HER2 抗体，诸如静脉内施用，例如推注或一段时间的连续输注，通过肌肉内、腹膜内、脑脊髓内、皮下、关节内、

滑膜内、鞘内、口服、局部或吸入路径。优选静脉内施用抗体组合物。

对于疾病的预防或治疗，HER2 抗体的适当剂量将取决于如上定义的待治疗疾病的类型、疾病的严重程度和进程、施用 HER2 抗体是为了预防还是治疗目的、先前的疗法、患者的临床史和对 HER2 抗体的响应、及主治医师的判断。适当的在某一时间或者通过一系列治疗对患者施用 HER2 抗体。根据疾病的类型和严重程度，对患者施用的初始候选剂量是约 1μg/kg 至 50mg/kg（如 0.1-20mg/kg）HER2 抗体，无论是通过例如一次或多次分开的施用，或者通过连续输注。在一个实施方案中，HER2 抗体的初始输注时间可长于后续输注时间，例如初始输注为约 90 分钟，而后续输注为约 30 分钟（如果初始输注耐受良好的话）。优选的 HER2 抗体剂量将在约 0.05mg/kg 至约 10mg/kg 的范围内。因此，可对患者施用约 0.5mg/kg、2.0mg/kg、4.0mg/kg 或 10mg/kg（或其任意组合）的一剂或多剂。这样的剂量可间歇施用，例如每周或每三周（例如使得患者接受约 2 剂至约 20 剂，例如约 6 剂 HER2 抗体）。可首先施用较高的加载剂量，后续较低的一剂或多剂。在一个实施方案中，首先以约 840mg 的加载剂量施用 HER2 抗体，然后大约每三周施用约 420mg。在另一个实施方案中，大约每 3 周以 1050mg 的一剂施用 HER2 抗体。

其它治疗剂可与 HER2 抗体联合。这样的联合施用包括使用分开的配制剂或单一的药用配制剂的共施用或同时施用，以及任一次序的连续施用，其中优选有一段时间所有两种（或多种）活性剂同时发挥其生物学活性。因此，可在施用 HER2 抗体之前或之后施用其它治疗剂。在此实施方案中，至少一次其它治疗剂的施用和至少一次 HER2 抗体的施用之间的计时优选为约 1 个月或更短，且最优选约 2 周或更短。或者，以单一的配制剂或分开的配制剂同时对患者施用其它治疗剂和 HER2 抗体。

可与 HER2 抗体联合的其它治疗剂的例子包括以下任一种或多种：化疗剂，诸如抗代谢物，如吉西他滨；第二种不同的 HER2 抗体（例如生长抑制性 HER2 抗体诸如 Trastuzumab 或诱导 HER2 过表达细胞凋亡的 HER2 抗体诸如 7C2、7F3 或其人源化变体）；靶向另一种肿瘤相关抗原诸如 EGFR、HER3、HER4 的第二种抗体；抗激素化合物，例如抗雌激素化合物诸如他莫昔芬，或芳香酶抑制剂；心脏保护剂（以预防或减轻与治疗有关的任何心肌功能障碍）；细胞因子；EGFR 靶向药物（诸如 TARCEVA®、IRESSA®或

Cetuximab)；抗血管生成剂（尤其是 Genentech 销售的 Bevacizumab，商标为 AVASTIN™）；酪氨酸激酶抑制剂；COX 抑制剂（例如 COX-1 或 COX-2 抑制剂）；非类固醇抗炎药，Celecoxib（CELEBREX®）；法呢基转移酶抑制剂（例如可从 Johnson and Johnson 购买的 Tipifarnib/ZARNESTRA® R115777 或可从 Schering-Plough 购买的 Lonafarnib SCH66336）；结合癌胚蛋白 CA 125 的抗体，诸如 Oregovomab（MoAb B43.13）；HER2 疫苗（诸如 Pharmexia 的 HER2 Auto Vac 疫苗，或 Dendreon 的 APC8024 蛋白质疫苗，或 GSK/Corixa 的 HER2 肽疫苗）；其它 HER 靶向疗法（如 trastuzumab、cetuximab、gefitinib、erlotinib、CI1033、GW2016 等）；Raf 和/或 ras 抑制剂（参见例如 WO 2003/86467）；Doxil；托泊替康(Topetecan)；紫杉烷；GW572016；TLK286；EMD-7200；治疗恶心的药物，诸如血清素拮抗剂、类固醇或苯并二氮卓类(benzodiazepien)；预防或治疗皮疹的药物或标准痤疮疗法，包括局部或口服抗生素；降体温药物，诸如醋氨酚(acetaminophen)、苯海拉明(diphenhydramine)或哌替啶(meperidine)；造血生长因子，等等。

任何上述共施用药剂的合适剂量就是那些目前所使用的剂量，并且可由于该药剂与 HER2 抗体的联合作用（协同作用）而降低。联合 HER2 抗体组合物和其它治疗剂的治疗可对患者产生协同的、或者大于累加的治疗效益。

如果施用化疗剂，通常以对此已知的剂量施用，或者任选由于所述药物的联合作用或可归于施用化疗剂的不良副作用而降低。所述化疗剂的制备和给药方案可依照制造商的说明书进行或由熟练从业人员根据经验确定。所述化疗的制备和给药方案还可参阅《Chemotherapy Service》，Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992)。

除了上述治疗方案，还可对患者进行手术去除癌细胞和/或放疗。

VI. 材料保藏

以下杂交瘤细胞系已保藏于美国典型培养物保藏中心（American Type Culture Collection, ATCC, 10801 University Boulevard, Manassas, VA 20110-2209, USA）：

抗体名称	ATCC 编号	保藏日期
7C2	ATCC HB-12215	1996 年 10 月 17 日
7F3	ATCC HB-12216	1996 年 10 月 17 日
4D5	ATCC CRL 10463	1990 年 5 月 24 日

2C4	ATCC HB-12697	1999 年 4 月 8 日

下面的非限制性实施例例示了本发明的更多细节。说明书中所有引用文献的内容均明确收入本文作为参考。

实施例

PERTUZUMAB 组合物的鉴定

Pertuzumab 是在人 IgG1( )框架基础上产生的重组人源化单克隆抗体。它包含两条重链（448 个残基）和两条轻链（214 个残基）。两条重链由两个链间二硫键连接，且各轻链通过一个链间二硫键附着于重链。在 Pertuzumab 的 Fc 区中在两条重链的 Asn-299 处有一个 N-连接糖基化位点。Pertuzumab 与 HERCEPTIN® （Trastuzumab）的不同在于轻链（12 个氨基酸差异）和重链（30 个氨基酸差异）的表位结合区。由于这些差异，Pertuzumab 结合 HER2 受体上完全不同的表位。Pertuzumab 与人上皮细胞上 HER2 受体的结合阻止了它与 HER 受体家族的其它成员形成复合物（Agus *et al.*, *Cancer Cell* 2: 127-137 (2002)）。通过阻断复合物的形成，Pertuzumab 阻止了所述复合物的配体（例如 EGF 和 heregulin）的生长刺激作用。体外实验证明了 Pertuzumab 和 Pertuzumab-Fab 都抑制 heregulin (HRG)与 MCF7 细胞的结合，并且 HRG 刺激的 HER2-HER3 复合物的磷酸化作用可被 Pertuzumab 和 Pertuzumab-Fab 二者所抑制（Agus *et al.*, *Cancer Cell* 2: 127-137 (2002)）。此外，发现 Pertuzumab 和 Pertuzumab 的聚乙二醇衍生化 Fab 在体内对肿瘤生长的抑制作用在鼠前列腺癌异种移植模型中是相当的（Agus *et al.*, *Cancer Cell* 2: 127-137 (2002)）。这些资料表明所述抗体的 Fc 区不是抑制肿瘤生长所必需的，而且二价性和 Fc 介导的效应物功能也不是体内或体外生物学活性所必需的。

分析了以下由重组改造的中国仓鼠卵巢(CHO)细胞表达的样品：

样品	制备过程	规模
参照材料	I 期	400 L
Lot S9802A	II 期	2000 L
工艺开发材料（第 1、2、3、5 和 6 轮）	包括 III 期在内的临床开发程序	400 L

注：由于第 2 天 100 L 接种体培养发生污染，所以未能获得 400 L 第 4 轮的数据。

N-末端序列分析

采用标准 Edman 降解法进行 N-末端序列分析，参照材料、Lot S9802A 和 400 升规模第 1 轮工艺开发材料的结果分别显示于表 2A、表 2B 和表 2C。在所有样品中都观察到预期的轻链和重链 N-末端序列（图 3A 和图 3B）。在 5 种样品中还观察到对应与在预期 N-末端序列之前有三个附加氨基酸 Val-His-Ser (VHS)的轻链对应的附加小序列。VHS 序列是分泌时从蛋白质上去除的信号肽的一部分。信号肽的可变切割导致 Pertuzumab N-末端的 VHS 延伸。在先前为 I/II 期临床研究生产的材料中，约 2%-4%的 Pertuzumab 分子两条轻链有一条含有 N-末端 VHS 序列。不过，这些材料中有 N-末端 VHS 序列的轻链水平（各轻链的 1%-2%）太低了，以至于不能在 N-末端序列分析中检测到。在 5 份工艺开发样品中，此轻链种类的水平稍微高于约 4%-5%的 N-末端分析的检测极限。这 5 份工艺开发样品的 N-末端测序数据与阳离子交换层析结果一致，它显示了这 5 份工艺开发样品中约 9%的 Pertuzumab 分子有一条轻链含有 VHS 延伸（参阅下文的阳离子交换层析(CEC)）。

没有检测到其它序列（检测极限估计是 3%），表明缺乏内部切割位点。

质谱分析

用二硫苏糖醇还原 Pertuzumab 样品，并使用 PE SCIEX API 3000™质谱仪通过电喷射-离子化质谱(ESI-MS)进行分析以证实重链和轻链的质量与它们的预期序列一致。图 8A 和图 8B 中比较了参照材料、Lot S9802A 和 400 升规模第 1 轮工艺开发材料的重建质谱。对于所有三种材料而言，观察到的轻链质量（23,524 Da）都与从其序列预测的值一致。在 23,685 Da 和 23,847 Da（分别比轻链质量高 161 和 323 Da）观察到另外两个较小的峰。第一个峰（23,685 Da）可能源自轻链的糖化(glycation)。第二个峰（23,847 Da）在 400 升规模工艺开发材料中可更清楚的观察到。此峰大概来自具有 Val-His-Ser 延伸的轻链（参见上文的 N-末端序列分析及阳离子交换层析分析）或具有两个糖化位点的轻链。

观察到几个相应于不同形式重链的质量。主要的重链种类具有 50,532 Da 的质量，源自由残基 1-448 组成且具有 G0 寡糖结构的重链。观察到的其它形式包括含有残基 1-448 且具有 G1 或 G2 寡糖结构的重链（图 8B）。

通过阳离子交换层析和毛细管区带电泳评估的电荷异质性

采用阳离子交换层析(CEC)来评估 Pertuzumab 中的电荷异质性。使用 DIONEX™阳离子交换柱（PROPAC WCX-10™, 4 mm x 250 mm）以 pH 6.0, 20mM MES 缓冲液和浅 NaCl 梯度分析用羧肽酶 B (CPB)处理之前和之后的样品。层析图谱的比较显示于图 9A（CPB 处理之前）和图 9B（CPB 处理之后）。在所有三个批次中观察到多个峰。为了达到鉴定目的，将层析图谱分成 6 个区（在图 9A 和 9B 中标以 A 至 F）。6 个区的相对峰面积列于表 3A 和表 3B。Lot S9802A 和 400 升规模工艺开发材料中酸性变体的量（区域 A 中）高于参照材料中此变体的量。与参照材料相比，Lot S9802A 和 400 升规模工艺开发材料中区域 C 和 D 中的碱性变体有所减少，所述碱性变体已显示出含有在一条（C 区）或所有两条（D 区）重链上有 C-末端赖氨酸残基的 Pertuzumab。CPB 处理后，具有一个或两个重链 C-末端赖氨酸的 Pertuzumab 分子转变成 B 区中的主要种类，而在 C 区和 D 区中不再检测到。CPB 处理后 C 区中只剩下一个小峰，它的同一性尚未测定。E 区中的碱性变体，据显示源自一条轻链含有 VHS 延伸的 Pertuzumab 分子，它在 400 升规模工艺开发材料中的含量（9%-10%）高于参照材料和 Lot S9802A 中的含量（4%）。F 区中的碱性变体显示出是在一条轻链上含有 N-末端 VHS 延伸且在一条重链上含有 C-末端赖氨酸的 Pertuzumab。由于酸性和碱性变体的水平较高，B 区中主要种类在 400 升规模工艺开发材料中的含量比在参照材料或 Lot S9802A 中的含量低。

除了阳离子交换层析之外，采用毛细管区带电泳(CZE)来检查 Pertuzumab 中的电荷异质性。通过用 CZE 分析个别收集的 CEC 级分，鉴定了 CZE 峰并与 CEC 中观察到的那些峰联系起来。对于分析的所有材料，通过 CZE 和 CEC 测定的电荷变体的相对量是相当的。

根据基于细胞的抗增殖测定法，不同 CEC 级分中 Pertuzumab 电荷变体的生物学活性看起来是相同的。因此，Pertuzumab 中的电荷异质性预计不会影响它的功效。400 升规模工艺开发材料中的生物学活性与参照材料和 Lot S9802A 中的生物学活性是相当的（参见生物学活性和表 4）。

分子排阻层析

采用分子排阻层析(SEC)来测定 Pertuzumab 的聚集程度。在 TOSOHAAS

TSK G3000SWXL™柱（7.8 mm x 300 mm）上分析样品。使用等度洗脱（100mM 磷酸钾, pH 6.8, 0.5 mL/分钟）并在 280nm 监测紫外(UV)吸光度。参照材料、Lot S9802A 和 400 升规模第 1 轮工艺开发材料的 SEC 资料显示于图 10。在分析的所有材料中，根据峰面积，单体一贯高于 99.6%，而聚集体则低于 0.3%。

CE-SDS-LIF 分析

还可采用具有激光诱导荧光的毛细管电泳-十二烷基硫酸钠分析(CE-SDS-LIF)来评估 Pertuzumab 样品的纯度。对于还原(reduction)和未还原的参照材料、Lot S9802A 和 400 升规模第 1 轮工艺开发材料，观察到相似的电泳图谱（图 11A 和 11B）。从还原材料的电泳图谱测定的非糖基化重链水平在 2.6%和 4.3%之间（就总的重链而言）。通过 CE-SDS-LIF 分析测定的这些样品（未还原的）中聚集体的量与 SEC 的结果一致。在这些样品的 CE-SDS-LIF 分析中没有发现重要产物片段或其它杂质的迹象。CE-SDS-LIF 数据表明 400 升规模工艺开发材料的杂质图谱与参照材料和 Lot S9802A 的杂质图谱相似。

SDS-PAGE 分析

进行 SDS-聚丙烯酰胺凝胶电泳(SDS-PAGE)分析（4%-20% T 凝胶（Daiichi Pure Chemicals Co., 东京, 日本）及 SYPRORUBY™染色）来比较参照材料、Lot S9802A 和 400 升规模工艺开发材料的还原和完整样品。在 400 升规模工艺开发材料中没有观察到新的条带，再次表明 400 升规模工艺开发材料中的总杂质图谱与参照材料和 Lot S9802A 中的相似。

胰蛋白酶和 LYS-C 消化肽图分析

进行胰蛋白酶和 Lys-C 消化肽图分析来检查和比较参照材料、Lot S9802A 和 400 升规模工艺开发材料中 Pertuzumab 的一级结构。用胰蛋白酶消化还原的和 S-羧甲基化的 Pertuzumab 的等分试样，用内切蛋白酶 Lys-C 消化还原的和亚硫酸解(sulfitolyzed)的 Pertuzumab 等分试样。使用 VYDAC C-18™柱（4.6 mm x 250 mm）以 0%-60%的乙腈梯度通过反相层析分离胰蛋白酶消化产物。监测 214nm 的吸光度，并使用 THERMO FINNIGAN LCQ™

通过 ESI-MS 测定肽质量。使用 ZORBAX C-8™柱（4.6 mm x 150 mm）以 0%-100%异丙醇(IPA)梯度通过反相层析分离 Lys-C 消化产物。监测 214nm 的吸光度，并使用 THERMO FINNIGAN LCQ™通过 ESI-MS 测定肽质量。

图 12A 和图 12B 中分别比较了参照材料、Lot S9802A 和 400 升规模第 1 轮工艺开发材料的胰蛋白酶和 Lys-C 消化肽图。所有三种材料的胰蛋白酶和 Lys-C 消化图谱都是基本上相同的。通过 LC-MS 鉴定了大多数肽并与预期肽质量相符。观察到的胰蛋白酶消化肽鉴定为重链残基的 97.1%（436/449）和轻链残基的 95.8%（205/214）。在 Lys-C 图中，重链和轻链的序列覆盖率分别为 98.4%（442/449）和 70.6%（151/214）。通过 LC-MS 发现在胰蛋白酶消化肽图中具有 VHS 延伸的 N-末端肽与不含 VHS 延伸的 N-末端肽共洗脱。在肽图中没有检测到显著量的脱酰胺或氧化肽。

生物学活性

Pertuzumab 的生物学活性是通过测量其抑制人乳癌细胞系 MDA-MB-175-VII 增殖的能力而测定的。对 5 份 400 升规模工艺开发样品获得的百分比比活（表 4）在 90%-96%的范围内，与参照材料（定义为 100%）和 Lot S9802A（在分析证书中报告的是 98%）的活性相似。正如所预期的，电荷异质性不影响 Pertuzumab 的功效。所有材料都具有相当的抗增殖活性。

N-连接寡糖分析

重链的质量异质性部分源自 Asn-299 处的糖基化。为了评估寡糖的异质性，用 PNG 酶 F 将 Pertuzumab 样品消化过夜以释放 N-连接聚糖。然后将释放的聚糖用荧光团 9-氨基-1,4,6-三磺酸盐(APTS)加以衍生。通过毛细管电泳(CE)（Beckman P/ACE 5500 CE，配备 Beckman 的涂层毛细管）分离各种聚糖形式并通过荧光检测进行量化。

从参照材料、Lot S9802A 和 400 升规模第 1 轮工艺开发材料释放的中性寡糖的 CE 分析电泳图谱显示于图 13。作为参考，图 14 中包括了通常在人 IgG1 抗体中找到的寡糖结构以及所使用命名法的概要。表 5 总结了所有材料中寡糖的相对量。

具有 G0 和 G1 结构的寡糖是主要的聚糖。在电泳图谱中还观察到源自其它寡糖结构的峰。这些结构包括 G2、G0-F、G-1、Man5 和 G1-1（或 Man6）

糖型(glycoform)。另外，还解析了异构体(isoform)。所有材料中观察到的聚糖分布是相似的(表 5)。不过，与参照材料和 400 升规模工艺开发材料相比，II 期材料(Lot S9802A)具有较小量的 G0 结构聚糖而具有更多的 G1 结构聚糖。尽管聚糖分布有差异，但所有材料都具有相似的生物学活性。另外，聚糖异质性的变化对于 Pertuzumab 对 FcRn(表 6)或 Fcγ 受体(表 7)的结合亲和力没有显著影响(参阅 FcRn 受体和 Fcγ 受体结合测定法)。

还通过 MALDI-TOF 质谱(MALDI-TOF/MS)分析了释放的中性寡糖。图 15 比较了从参照材料、Lot S9802A 和 400 升规模第 1 轮工艺开发材料释放的中性寡糖的 MALDI-TOF 谱。由 MALDI-TOF/MS 分析获得的聚糖结构和分布与 CE 结果一致。

毛细管等电聚焦分析

采用毛细管等电聚焦(cIEF)来测定参照材料、Lot S9802A 和 400 升规模第 1 轮工艺开发材料中 Pertuzumab 的 pI(等电点)。尽管在 CEC 分析中观察到这些材料中不同带电荷种类的相对量稍有差异，但发现在所有样品中主要种类的 pI 都是 8.7。

游离巯基分析

采用 Ellman 氏分析法对参照材料、Lot S9802A 和 400 升规模工艺开发材料测试游离硫醇(未配对的半胱氨酸残基)。在所有测试材料中游离硫醇水平都低于检测限度(约 0.05 摩尔游离硫醇/每摩尔蛋白质)。

FcRn 受体结合测定法

采用与 Shields *et al.*, J. Biol. Chem. 276: 6591-6604 (2001)中所述相似的 ELISA 测定法比较来自参照材料、Lot S9802A 和 400 升规模工艺开发材料的 Pertuzumab 的 FcRn 受体结合亲和力。使用参照材料作为此测定法中的标准品。

首先用 NEUTRAVADIN™(Pierce, Rockford, IL)将 MAXISORP™ 96 孔微孔板(Nunc, Roskilde, Denmark)在 4℃包被过夜。然后将生物素化的人 FcRn 以 2μg/mL 加到板中并保温 1 小时。将 11 份两倍连续稀释的 Pertuzumab 样品(3.1-3200 ng/mL)加到板中并保温 2 小时。通过添加过氧化物酶标记

的山羊 F(ab')$_2$ 抗人 IgG F(ab')$_2$（Jackson ImmunoResearch, West Grove, PA）并使用 3,3',5,5'-四甲基联苯胺(TMB)（Kirkegaard & Perry Laboratories, Gaithersburg, MD）作为底物来检测结合的 Pertuzumab。在 TITERTEK MULTISKAN™（MCC/340）读数仪（ICN, Costa Mesa, CA）上读取 450nm 的吸光度。还以第二种 ELISA 形式评估 Pertuzumab 的 FcRn 结合亲和力，其中将 HER2-ECD 包被到板上。向板中加入连续稀释的 Pertuzumab 样品并保温 2 小时。洗板并加入 2mg/mL 生物素化人 FcRn。用链霉亲合素-HRP 并以 TMB 作为底物来检测结合的 FcRn。

测定标准品（即参照材料）在滴定曲线中点的吸光度(mid-OD)及标准品和样品在此 mid-OD 处的对应浓度。通过用标准品的 mid-OD 浓度除样品的 mid-OD 浓度来计算相对结合亲和力。

表 6 列出了由这两种 ELISA 形式获得的相对 FcRn 结合亲和力。来自这两种不同 ELISA 形式的数据是相当的。尽管不同 Pertuzumab 材料中的聚糖分布是不同的，但这些材料的 FcRn 结合亲和力是相当的，表明 Pertuzumab 中的聚糖异质性对它的 FcRn 结合亲和力没有明显影响。

Fcγ 受体结合测定法

通过依照 Shields *et al.*, *J. Biol. Chem.* 276: 6591-6604 (2001)并作修改的 ELISA 测定法来评估 Pertuzumab 对人 Fcγ 受体(Fc(R)的结合。

单体 IgG 可结合高亲和力的 FcγRIa (CD64)；不过，低亲和力的受体（FcγRIIa (CD32A)、FcγRIIb (CD32B)和 FcγRIIIa (CD16)）需要多聚体 IgG 才能有显著结合。因此，对于低亲和力受体结合测定法，在测定前通过将各样品（200mg/mL）与山羊抗人 κ 链（400mg/mL；ICN Biomedical, Irvine, CA）混合来形成 Pertuzumab 的多聚体。人 FcγR 表达具成 Gly/His6/GST (甘氨酸/6 组氨酸/谷胱甘肽-s-转移酶)的受体 α 链胞外域的重组融合蛋白。用抗 GST 包被的、牛血清清蛋白(BSA)封闭的测定板捕捉 FcγR。向板中加入受体（100mL, 0.25mg/mL）并保温 1 小时。加入连续稀释的 Pertuzumab 样品（100mL），FcγRIa 用单体而低亲和力 FcγR 用多聚体，并将板保温 2 小时。通过添加辣根过氧化物酶缀合的山羊抗人 F(ab')$_2$（Jackson ImmunoResearch Laboratories, West Grove, PA）并使用 TMB 作为底物来检测结合的 Pertuzumab。通过非线性回归分析以四参数模型（KaleidaGraph, Synergy

Software, Reading, PA)测定Pertuzumab结合FcγR的EC_{50}值。使用RITUXAN®（Lot C2B81298-2）作为对照抗体。

表7总结了Pertuzumab结合FcγR的EC_{50}值。结果显示参照材料、Lot S9802A和400升规模工艺开发材料对FcγR具有相当的结合亲和力。这些结果表明Pertuzumab中的聚糖异质性对它的FcγR结合亲和力没有显著影响。

表 2A

Pertuzumab 参照材料的 N-末端序列分析

（在各个循环中观察到的残基 nmol 数）

	循环											
残基	1	2	3	4	5	6	7	8	9	10	11	12
ALA	0.01	0.01	0.02	0.04	0.04	0.05	0.06	0.07	0.09	0.09	0.10	0.11
ARG	0.01	0.01	0.01	0.01	0.03	0.03	0.04	0.04	0.05	0.06	0.05	0.05
ASN	0.00	0.00	0.01	0.01	0.01	0.02	0.03	0.04	0.05	0.06	0.06	0.07
ASP	0.48	0.08	0.03	0.04	0.04	0.04	0.05	0.07	0.07	0.06	0.09	0.10
CYS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GLN	0.00	0.01	**0.92**	0.13	0.04	0.46	0.13	0.07	0.08	0.08	0.08	0.09
GLU	**0.49**	0.05	0.13	0.03	0.03	**0.51**	0.12	0.07	0.07	0.07	0.07	0.08
GLY	0.01	0.01	0.03	0.03	0.05	0.05	0.06	**0.30**	**0.35**	**0.36**	0.13	0.09
HIS	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
ILE	0.00	0.48	0.05	0.02	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04
LEU	0.00	0.03	0.04	**0.43**	0.11	0.08	0.09	0.11	0.12	0.13	**0.62**	0.26
LYS	0.00	0.00	0.03	0.00	0.05	0.06	0.07	0.08	0.06	0.09	0.00	0.12
MET	0.00	0.00	0.00	0.53	0.07	0.02	0.02	0.02	0.02	0.02	0.02	0.02
PHE	0.00	0.01	0.02	0.02	0.03	0.04	0.05	0.05	0.06	0.07	0.07	0.08
PRO	0.00	0.01	0.02	0.03	0.04	0.04	0.05	0.32	0.13	0.09	0.09	0.10
SER	0.01	0.01	0.03	0.04	0.05	0.07	**0.56**	0.16	0.30	0.33	0.18	0.39
THR	0.00	0.01	0.00	0.04	0.33	0.12	0.07	0.07	0.08	0.09	0.10	0.10
TRP	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.02	0.02
TYR	0.01	0.01	0.03	0.04	0.05	0.06	0.08	0.08	0.09	0.10	0.11	0.13
VAL	0.01	**0.51**	0.08	0.10	**0.53**	0.15	0.11	0.12	0.14	0.15	0.16	**0.61**
轻链	ASP	ILE	GLN	MET	THR	GLN	SER	PRO	SER	SER	LEU	SER
重链	GLU	VAL	GLN	LEU	VAL	GLU	SER	GLY	GLY	GLY	LEU	VAL

注：给出了各个循环中观察到的苯基乙内酰硫脲(phenylthiohydantoin)氨基酸残基的纳摩尔数。来自重链的残基以粗体标示，来自轻链的残基以下划线标示。上样量为约 0.5nmol 蛋白质，相当于轻链和重链各 1.0nmol。在序列分析中没有观察到半胱氨酸(CYS)。

表 2B

Pertuzumab Lot S9802A 的 N-末端序列分析

（在各个循环中观察到的残基 nmol 数）

	循环											
残基	1	2	3	4	5	6	7	8	9	10	11	12
ALA	0.01	0.01	0.03	0.04	0.06	0.06	0.08	0.09	0.11	0.12	0.14	0.14
ARG	0.01	0.01	0.02	0.02	0.03	0.03	0.05	0.05	0.06	0.07	0.07	0.05
ASN	0.00	0.00	0.01	0.02	0.02	0.02	0.04	0.05	0.06	0.07	0.08	0.09
ASP	0.61	0.12	0.04	0.05	0.05	0.06	0.07	0.09	0.09	0.10	0.11	0.13
CYS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GLN	0.00	0.01	**1.11**	0.22	0.06	0.55	0.19	0.10	0.11	0.11	0.11	0.12
GLU	**0.61**	0.09	0.15	0.05	0.04	**0.61**	0.19	0.10	0.09	0.10	0.10	0.11
GLY	0.01	0.01	0.04	0.04	0.06	0.06	0.07	**0.37**	**0.44**	**0.46**	0.19	0.13
HIS	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02
ILE	0.00	0.56	0.10	0.02	0.04	0.04	0.03	0.04	0.05	0.05	0.05	0.06
LEU	0.00	0.04	0.05	**0.51**	0.17	0.11	0.12	0.14	0.15	0.17	**0.78**	0.37
LYS	0.00	0.00	0.04	0.00	0.06	0.08	0.09	0.10	0.11	0.12	0.00	0.16
MET	0.00	0.00	0.01	0.61	0.12	0.03	0.03	0.03	0.02	0.02	0.02	0.03
PHE	0.00	0.01	0.03	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.09	0.10
PRO	0.00	0.01	0.02	0.03	0.05	0.06	0.06	0.37	0.18	0.12	0.12	0.13
SER	0.01	0.02	0.04	0.05	0.06	0.08	**0.63**	0.24	0.36	0.41	0.24	0.35
THR	0.00	0.01	0.00	0.05	0.39	0.17	0.10	0.10	0.11	0.12	0.13	0.14
TRP	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02
TYR	0.16	0.07	0.05	0.06	0.07	0.08	0.10	0.10	0.12	0.13	0.14	0.16
VAL	0.02	**0.58**	0.13	0.12	**0.62**	0.22	0.15	0.16	0.18	0.20	0.21	**0.62**
轻链	ASP	ILE	GLN	MET	THR	GLN	SER	PRO	SER	SER	LEU	SER
重链	GLU	VAL	GLN	LEU	VAL	GLU	SER	GLY	GLY	GLY	LEU	VAL

注：给出了各个循环中观察到的苯基乙内酰硫脲(phenylthiohydantoin)氨基酸残基的纳摩尔数。来自重链的残基以粗体标示，来自轻链的残基以下划线标示。上样量为约 0.5nmol 蛋白质，相当于轻链和重链各 1.0nmol。在序列分析中没有观察到半胱氨酸(CYS)。

表 2C

Pertuzumab 400 升规模第 1 轮的 N-末端序列分析（在各个循环中观察到的残基 nmol 数）

残基	循环 1	2	3	4	5	6	7	8	9	10	11	12
ALA	0.01	0.02	0.03	0.05	0.06	0.06	0.08	0.10	0.11	0.13	0.14	0.14
ARG	0.03	0.05	0.07	0.09	0.17	0.15	0.20	0.23	0.24	0.29	0.30	0.33
ASN	0.00	0.00	0.01	0.02	0.02	0.03	0.04	0.06	0.07	0.08	0.09	0.09
ASP	0.89	0.08	0.05	0.11	0.08	0.09	0.11	0.15	0.14	0.16	0.18	0.19
CYS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
GLN	0.00	0.01	**1.17**	0.11	0.05	0.60	0.12	0.09	0.14	0.12	0.11	0.12
GLU	**0.68**	0.05	0.19	0.04	0.04	**0.62**	0.15	0.10	0.10	0.11	0.11	0.12
GLY	0.01	0.01	0.05	0.04	0.08	0.07	0.07	**0.37**	**0.42**	**0.44**	0.16	0.13
HIS	0.00	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.03
ILE	0.00	0.56	0.03	0.02	0.07	0.04	0.03	0.04	0.05	0.06	0.05	0.06
LEU	0.00	0.04	0.05	**0.61**	0.15	0.12	0.13	0.15	0.16	0.18	**0.79**	0.31
LYS	0.00	0.00	0.04	0.05	0.08	0.09	0.10	0.12	0.14	0.15	0.17	0.19
MET	0.00	0.00	0.01	0.55	0.04	0.02	0.05	0.03	0.02	0.02	0.03	0.03
PHE	0.00	0.01	0.03	0.03	0.05	0.05	0.07	0.08	0.08	0.09	0.10	0.11
PRO	0.00	0.01	0.02	0.04	0.05	0.06	0.07	0.41	0.14	0.11	0.13	0.14
SER	0.01	0.02	0.07	0.06	0.08	0.10	**0.76**	0.21	0.43	**0.48**	0.23	0.42
THR	0.00	0.01	0.00	0.06	0.57	0.18	0.12	0.16	0.18	0.17	0.18	0.19
TRP	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.02	0.02	0.02	0.03	0.03
TYR	0.00	0.02	0.04	0.06	0.07	0.08	0.10	0.11	0.13	0.14	0.15	0.17
VAL	0.04	**0.56**	0.10	0.13	**0.62**	0.19	0.15	0.17	0.19	0.21	0.22	**0.62**
轻链	ASP	ILE	GLN	MET	THR	GLN	SER	PRO	SER	SER	LEU	SER
重链	**GLU**	**VAL**	**GLN**	**LEU**	**VAL**	**GLU**	**SER**	**GLY**	**GLY**	**GLY**	**LEU**	**VAL**
VHS-轻链	*VAL*	*HIS*	*SER*	*ASP*	*ILE*	*GLN*	*MET*	*THR*	*GLN*	*SER*	*PRO*	*SER*

注：给出了各个循环中观察到的苯基乙内酰硫脲(phenylthiohydantoin)氨基酸残基的纳摩尔数。来自重链的残基以粗体标示，来自轻链的残基以下划线标示。来自额外 VHS-轻链序列的残基以斜体标示。上样量为约 0.5nmol 蛋白质，相当于轻链和重链各 1.0nmol。在序列分析中没有观察到半胱氨酸(CYS)。

表 3A

天然 Pertuzumab 的阳离子交换层析分析（%峰面积）

样品	离子交换变体峰 A	B	C	D	E	F
参照材料	10	73	10	3	3	0.4
S9802A	20	68	5	2	4	0.2
400升规模第1轮	16	65	5	2	10	0.9
400升规模第2轮	15	67	5	2	9	0.6
400升规模第3轮	15	67	6	2	10	0.6
400升规模第5轮	15	67	5	2	9	0.5
400升规模第6轮	13	63	9	3	9	0.6

注：由于第 2 天 100L 接种体培养发生污染，所以未能获得 400L 第 4 轮的数据。

表 3B

CPB 消化的 Pertuzumab 的阳离子交换层析分析（%峰面积）

样品	离子交换变体峰					
	A	B	C	D	E	F
参照材料	11	78	4	ND	4	ND
S9802A	20	71	3	ND	4	ND
400 升规模第 1 轮	17	68	3	ND	10	ND
400 升规模第 2 轮	15	71	3	ND	9	ND
400 升规模第 3 轮	15	71	3	ND	10	ND
400 升规模第 5 轮	17	70	3	ND	9	ND
400 升规模第 6 轮	16	71	3	ND	9	ND

注：级分 A 至 F 的定义见图 9B。所有数值四舍五入成两个有效数字。由于四舍五入，任一行相加的总数可能不是 100%。由于第 2 天 100L 接种体培养发生污染，所以未能获得 400L 第 4 轮的数据。
ND=未检测或者不可积分。

表 4

通过基于细胞的抗增殖测定法评估的 Pertuzumab 比活

材料	比活（%）	%CV
参照材料	100[a]	—
S9802A	98[b]	10
400 升规模第 1 轮	96[c]	18
400 升规模第 2 轮	90[c]	11
400 升规模第 3 轮	86[c]	17
400 升规模第 5 轮	96[c]	3
400 升规模第 6 轮	95[c]	3

注：由于第 2 天 100L 接种体培养发生污染，所以未能获得 400L 第 4 轮的数据。
[a]根据定义，参照材料的比活是 100%。
[b]Lot S9802A 的报告值。
[c]三次测定的平均值。

表 5

通过 CE 测定的 Pertuzumab 中的寡糖结构分布

样品	%G0-F	%G-1	%Man5	%G0	%Man6 +%G1-1	%G1[a]	%G2
参照材料	1	4	1	71	2	19	2
S9802A	1	4	1	62	2	27	3
400 升规模第 1 轮	2	6	1	73	3	15	2
400 升规模第 2 轮	1	6	1	77	2	13	1
400 升规模第 3 轮	2	6	1	74	4	14	1
400 升规模第 5 轮	1	5	1	71	2	18	1
400 升规模第 6 轮	1	5	1	71	3	18	1

注：由于第 2 天 100L 接种体培养发生污染，所以未获得 400L 第 4 轮的数据。
[a]两种 G1 异构体的总和。

表 6

Pertuzumab 对 FcRn 的相对结合亲和力

样品	相对结合亲和力 NeutrAvidin 包被形式	相对结合亲和力 Her2ECD 包被形式
参照材料(标准品)	1.00	1.00
S9802A	1.34	1.39
400升规模第1轮	1.54	1.22
400升规模第2轮	1.09	1.31
400升规模第3轮	1.14	1.42
400升规模第5轮	1.22	1.38
400升规模第6轮	1.06	1.29

注:由于第2天100L接种体培养发生污染,所以未获得400L第4轮的数据。

[a] 测定了标准品(即参照材料)在滴定曲线中点的吸光度(mid-OD)及标准品和样品在此 mid-OD 处的对应浓度。用标准品的 mid-OD 浓度除样品的 mid-OD浓度得到相对结合亲和力。

表 7

Pertuzumab 结合 Fcγ 受体的 EC_{50} 值

样品	EC_{50} (μg/mL) FcγR Ia 平均值	FcγR Ia SD	FcγRIIa 平均值	FcγRIIa SD	FcγRIIb 平均值	FcγRIIb SD	FcγRIIIa (F158) 平均值	FcγRIIIa (F158) SD	FcγRIIIa (V158) 平均值	FcγRIIIa (V158) SD
参照材料	0.0061	0.0014	5.6	1.8	57	17	14.0	1.4	1.8	0.1
S9802A	0.0075	0.0020	5.4	1.9	45	17	7.8	0.3	1.1	0.1
400升规模第1轮	0.0079	0.0013	5.4	1.5	70	25	8.4	0.9	1.2	0.1
400升规模第2轮	0.0074	0.0016	5.8	1.6	54	18	10.4	0.7	1.4	0.2
400升规模第3轮	0.0075	0.0015	5.5	1.1	54	10	8.2	0.9	1.2	0.1
400升规模第5轮	0.0075	0.0011	6.1	2.7	50	12	10.9	1.5	1.4	0.2
400升规模第6轮	0.0080	0.0005	6.2	1.9	44	1	10.3	0.9	1.4	0.2

注:平均值和标准偏差(SD)获自多轮测定(N=4)。

FcγRIIIa 受体有两种型式:F158 和 V158。

由于第2天100L接种体培养发生污染,所以未获得400L第4轮的数据。

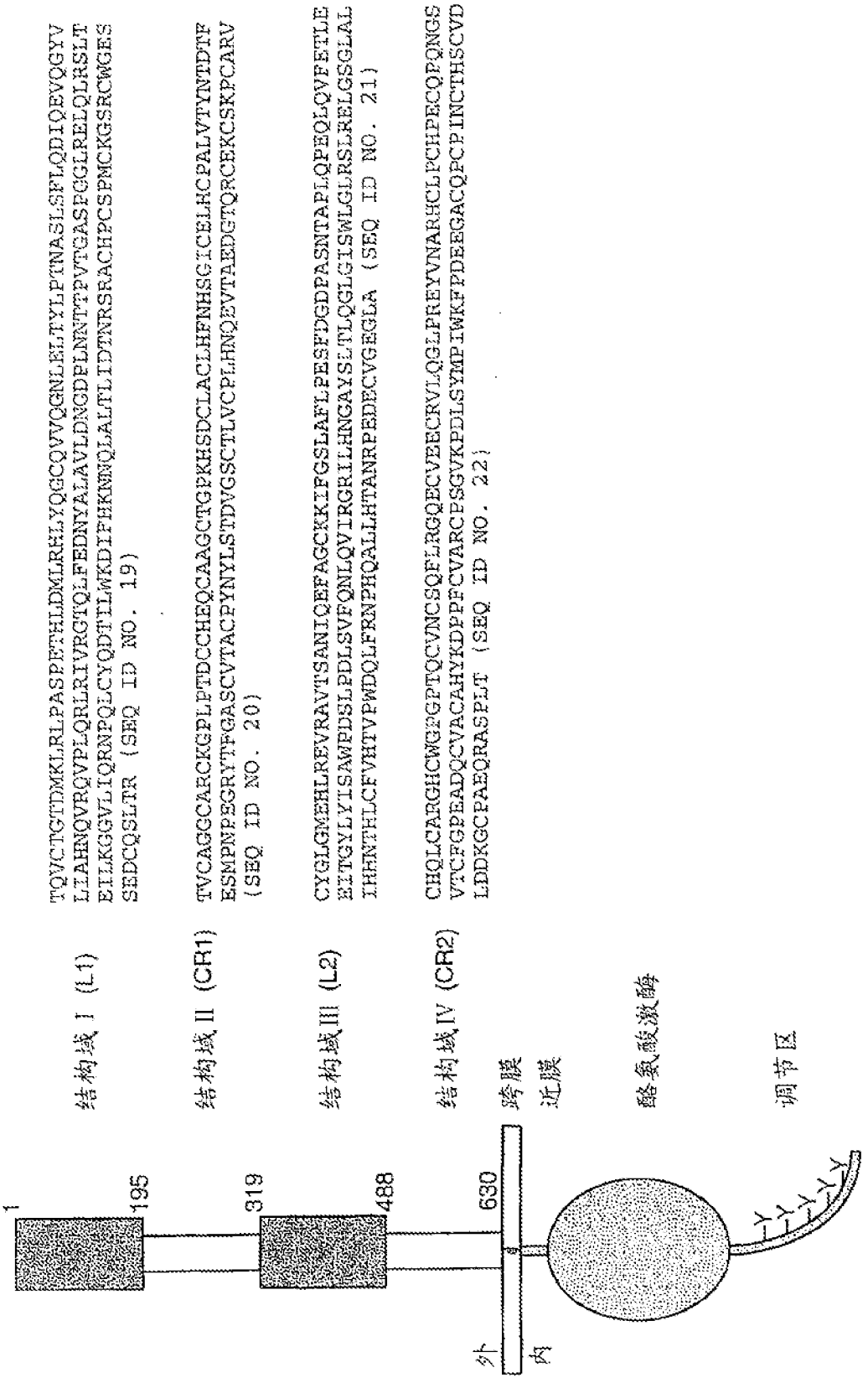

结构域 I (L1)
TQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFLQDIQEVQGYV
LIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLT
EILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGES
SEDCQSLTR (SEQ ID NO. 19)
结构域 II (CR1)
TVCAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTF
ESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLVCPLHNQEVTAEDGTQRCEKCSKPCARV
(SEQ ID NO. 20)
结构域 III (L2)
CYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLE
EITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLAL
IHHNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDECVGEGLA (SEQ ID NO. 21)
结构域 IV (CR2)
CHQLCARGHCWGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHPECQPQNGS
VTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVD
LDDKGCPAEQRASPLT (SEQ ID NO. 22)
跨膜
近膜
酪氨酸激酶
调节区
外
内
1
195
319
488
630
Y
Y
Y
Y
Y

图 1

轻链可变区

```
                  10          20          30          40
2C4       DTVMTQSHKIMSTSVGDRVSITC [KASQDVSIGVA] WYQQRP
           **    **** *       *                     *
574       DIQMTQSPSSLSASVGDRVTITC [KASQDVSIGVA] WYQQKP
                                   *   ** ***
hum κI    DIQMTQSPSSLSASVGDRVTITC [RASQSISNYLA] WYQQKP

                       50          60          70          80
2C4       GQSPKLLIY [SASYRYT] GVPDRFTGSGSGTDFTFTISSVQA
           **                    *  *           *    * *
574       GKAPKLLIY [SASYRYT] GVPSRFSGSGSGTDFTLTISSLQP
                     * *****
hum κI    GKAPKLLIY [AASSLES] GVPSRFSGSGSGTDFTLTISSLQP

                     90          100
2C4       EDLAVYYC [QQYYIYPYT] FGGGTKLEIK (SEQ ID NO:1)
            * *                  *   *
574       EDFATYYC [QQYYIYPYT] FGQGTKVEIK (SEQ ID NO:3)
                       *** *
hum κI    EDFATYYC [QQYNSLPWT] FGQGTKVEIK (SEQ ID NO:5)
```

图 2A

重链可变区

```
                  10          20          30          40
2C4       EVQLQQSGPELVKPGTSVKISCKAS [GFTFTDYTMD] WVKQS
             **  **  *  * ***  *                    * *
574       EVQLVESGGGLVQPGGSLRLSCAAS [GFTFTDYTMD] WVRQA
                                       ** * *
hum III   EVQLVESGGGLVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                       50  a       60          70          80
2C4       HGKSLEWIG [DVNPNSGGSIYNQRFKG] KASLTVDRSSRIVYM
           *  *   **                      *** *    **** *
574       PGKGLEWVA [DVNPNSGGSIYNQRFKG] RFTLSVDRSKNTLYL
                     ****** *** ****       * * *
hum III   PGKGLEWVA [VISGDGGSTYYADSVKG] RFTISRDNSKNTLYL

                    abc     90          100ab       110
2C4       ELRSLTFEDTAVYYCAR [NLGPSFYFDY] WGQGTTLTVSS (SEQ ID NO:2)
          ***  **                                   **
574       QMNSLRAEDTAVYYCAR [NLGPSFYFDY] WGQGTLVTVSS (SEQ ID NO:4)
                             ********
hum III   QMNSLRAEDTAVYYCAR [GRVGYSLYDY] WGQGTLVTVSS (SEQ ID NO:6)
```

图 2B

Pertuzumab的轻链氨基酸序列

```
1         10        20        30        40        50        60
|         |         |         |         |         |         |
DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPS

          70        80        90        100       110       120
          |         |         |         |         |         |
RFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPP

                  130       140       150       160       170       180
                  |         |         |         |         |         |
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT

                  190       200       210
                  |         |         |
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
```

图 3A

Pertuzumab的重链氨基酸序列

```
1         10        20        30        40        50        60
|         |         |         |         |         |         |
EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIY

          70        80        90        100       110       120
          |         |         |         |         |         |
NQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSA

                  130       140       150       160       170       180
                  |         |         |         |         |         |
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG

                  190       200       210       220       230       240
                  |         |         |         |         |         |
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP

                  250       260       270       280       290       300
                  |         |         |         |         |         |*
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

                  310       320       330       340       350       360
                  |         |         |         |         |         |
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM

                  370       380       390       400       410       420
                  |         |         |         |         |         |
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ

                  430       440       448
                  |         |         |
QGNVFSCSVMHEALHNHYTQKSLSLSPG
```

图 3B

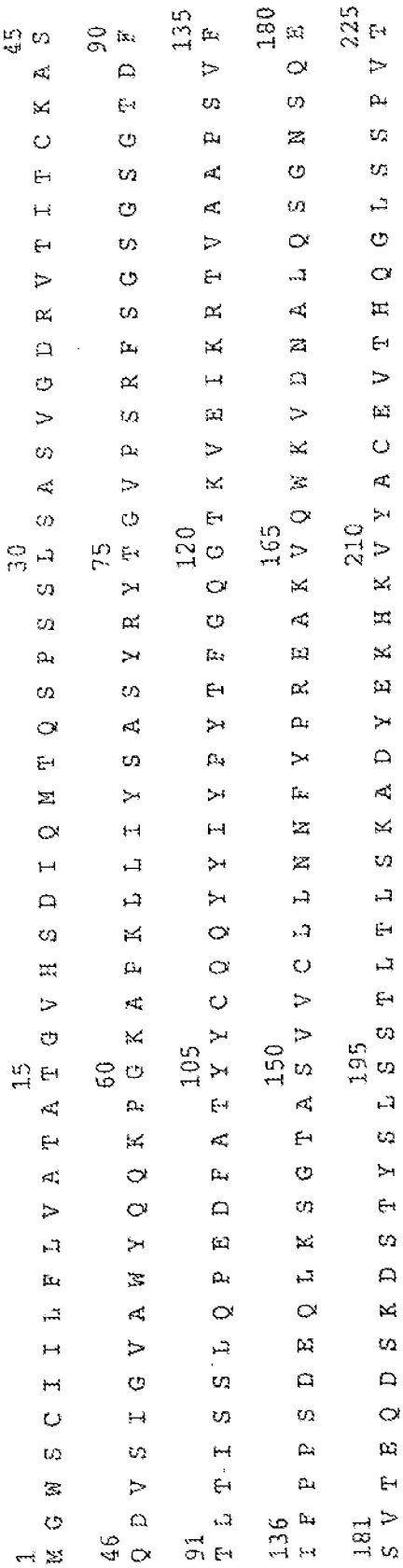

1 M G W S C I I L F L V A T A T G V H S D I Q M T Q S P S S L S A S V G D R V T I T C K A S
46 Q D V S I G V A W Y Q Q K P G K A P K L L I Y S A S Y R Y T G V P S R F S G S G S G T D F
91 T L T I S S L Q P E D F A T Y Y C Q Q Y Y I Y P Y T F G Q G T K V E I K R T V A A P S V F
136 I F P P S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E
181 S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T
226 K S F N R G E C (SEQ ID NO. 17)
15
30
45
60
75
90
105
120
135
150
165
180
195
210
225
233

图 4A

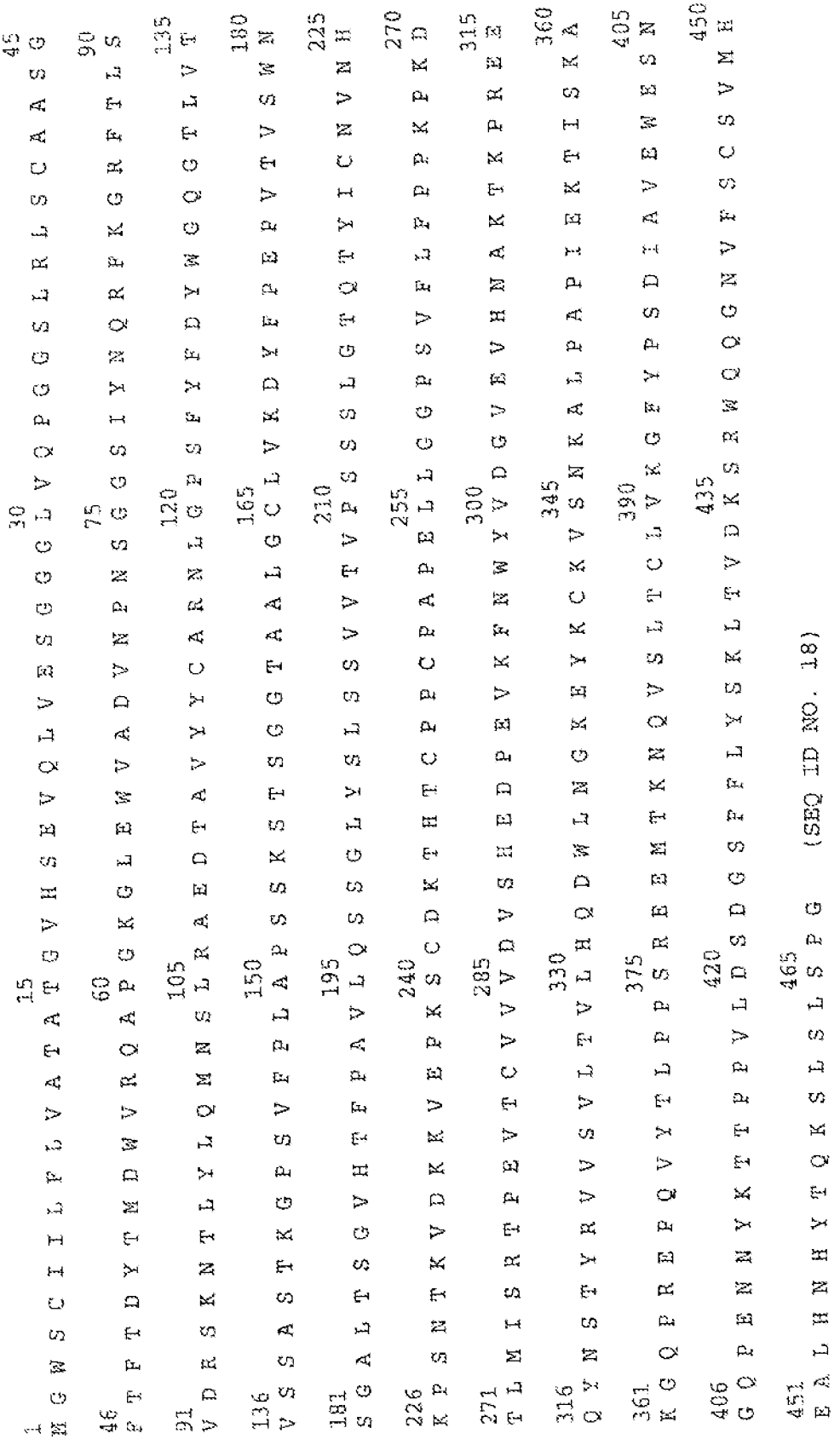

1 M G W S C I I L F L V A T A T G V H S E V Q L V E S G G G L V Q P G G S L R L S C A A S G
46 P T F T D Y T M D W V R Q A P G K G L E W V A D V N P N S G G S I Y N Q R F K G R F T L S
91 V D R S K N T L Y L Q M N S L R A E D T A V Y Y C A R N L G P S F Y F D Y W G Q G T L V T
136 V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S W N
181 S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H
226 K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V F L F P P K P K D
271 T L M I S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E
316 Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A
361 K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N
406 G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H
451 E A L H N H Y T Q K S L S L S P G (SEQ ID NO. 18)
15
30
45
60
75
90
105
120
135
150
165
180
195
210
225
240
255
270
285
300
315
330
345
360
375
390
405
420
435
450
465

图 4B

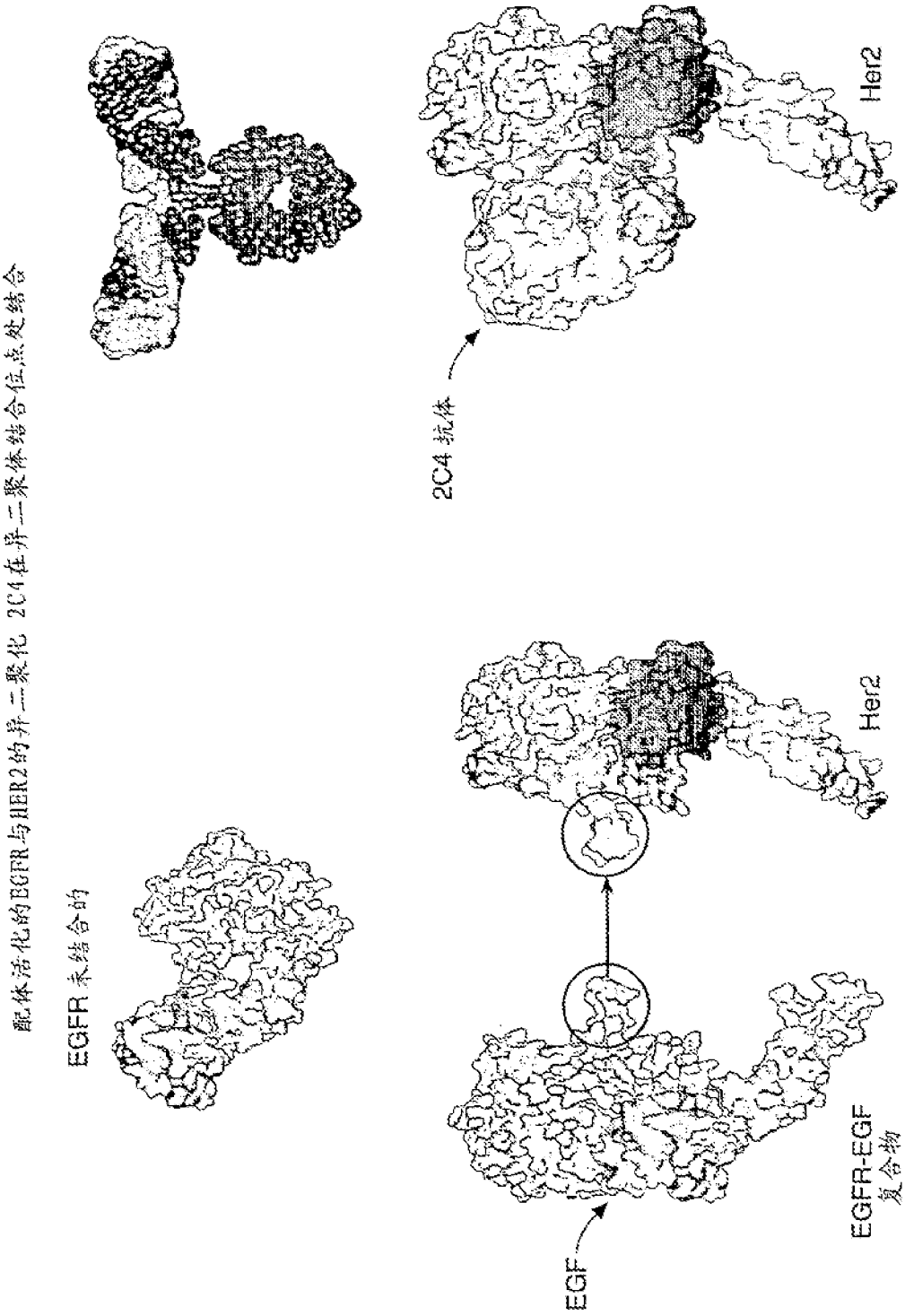

配体活化的EGFR与HER2的异二聚化 2C4在异二聚体结合位点处结合
EGFR 未结合的
2C4 抗体
Her2
EGF
EGFR-EGF
复合物
Her2

图 5

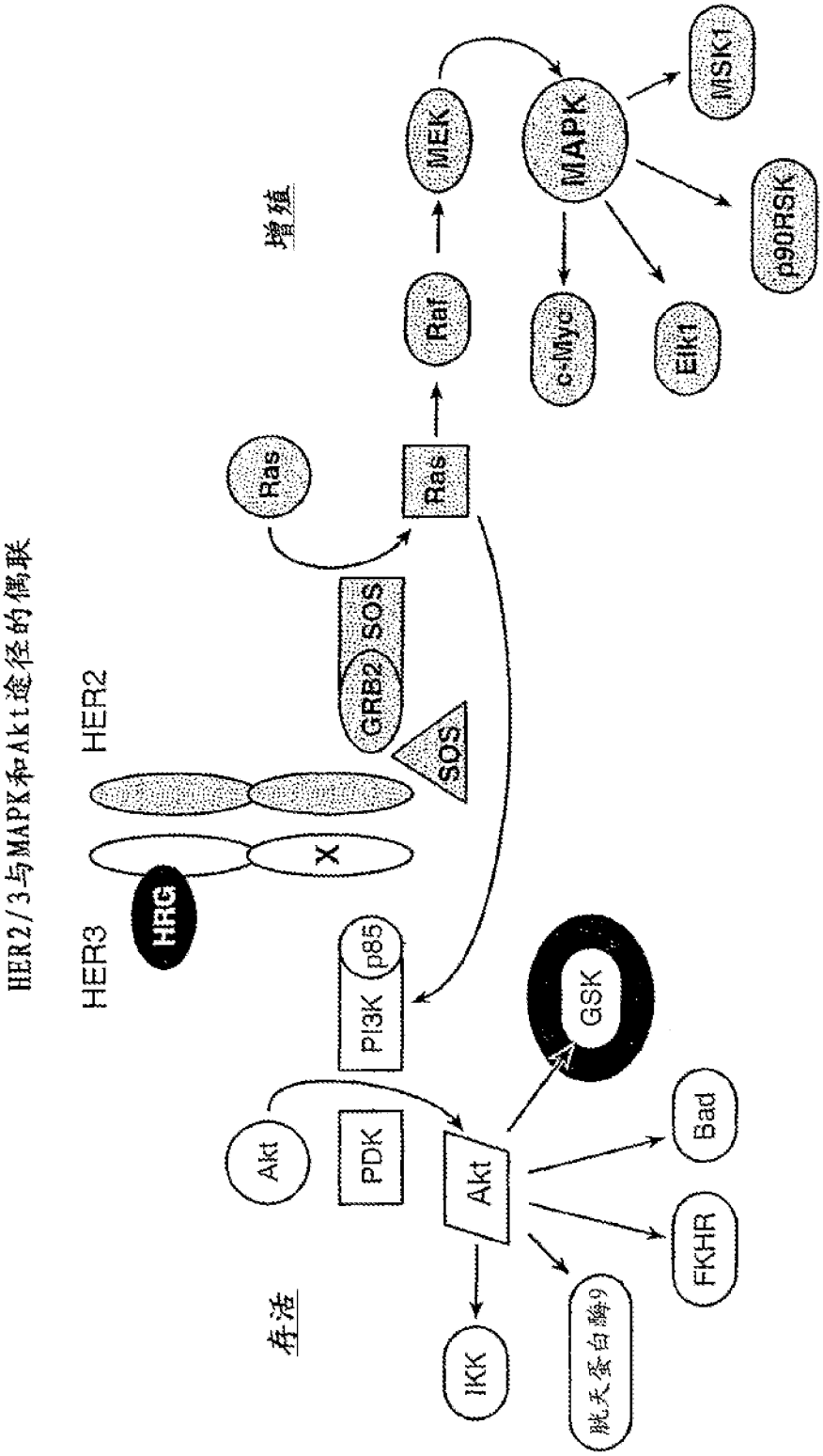
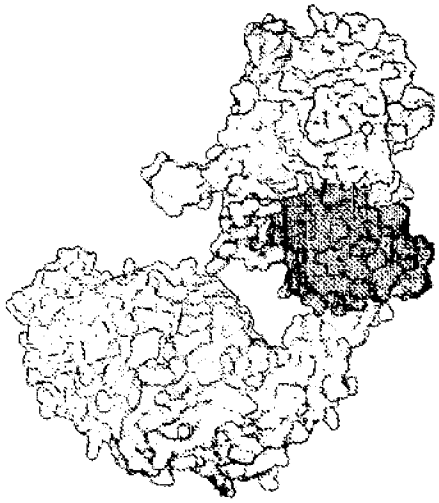

HER2/3与MAPK和Akt途径的偶联
HER3
HER2
HRG
X
SOS
GRB2
SOS
Ras
Ras
Raf
MEK
MAPK
MSK1
p90RSK
Elk1
c-Myc
增殖
PI3K
p85
GSK
Akt
PDK
Akt
Bad
FKHR
IKK
胱天蛋白酶9
存活

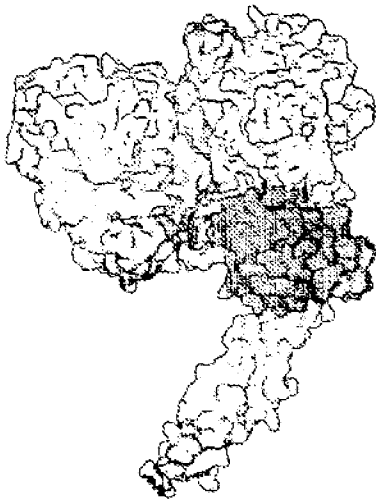
图 6

Trastuzumab
Herceptin



- 在IV中JM附近结合
- 防止受体脱落
- 适度影响受体下调
- 对HER2作为共同受体的角色有轻微影响

Pertuzumab
Omnitarg



- 在II中二聚化界面处结合
- 不能防止受体脱落
- 适度影响受体下调
- 对HER2作为共同受体的角色有较大影响

图 7

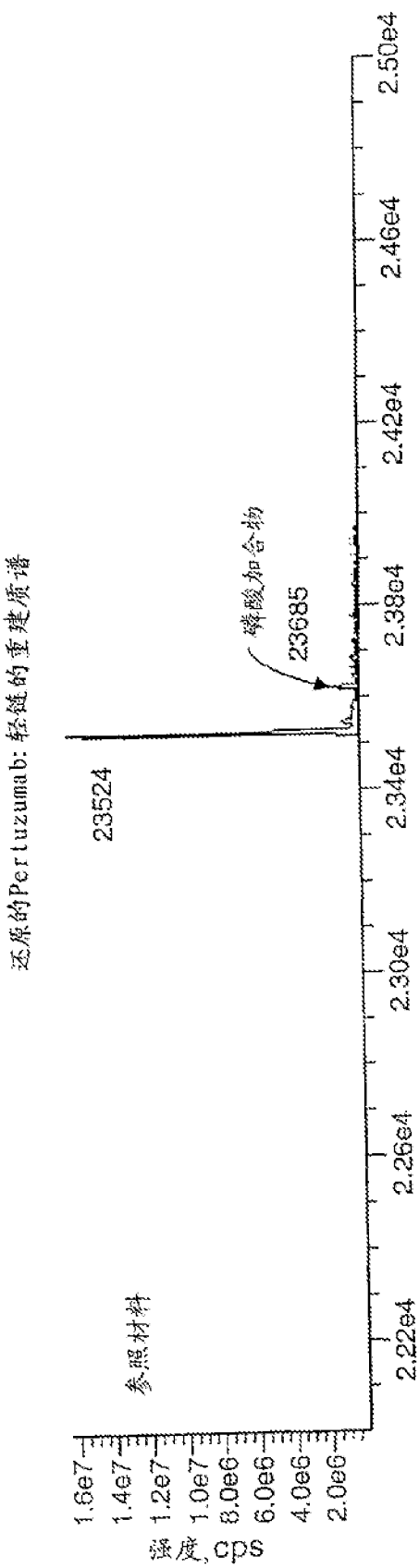

还原的Pertuzumab: 轻链的重建质谱
参照材料
23524
磷酸加合物
23685
强度, cps
质量, amu
1.6e7
1.4e7
1.2e7
1.0e7
8.0e6
6.0e6
4.0e6
2.0e6
2.22e4
2.26e4
2.30e4
2.34e4
2.38e4
2.42e4
2.46e4
2.50e4

图 8A-1

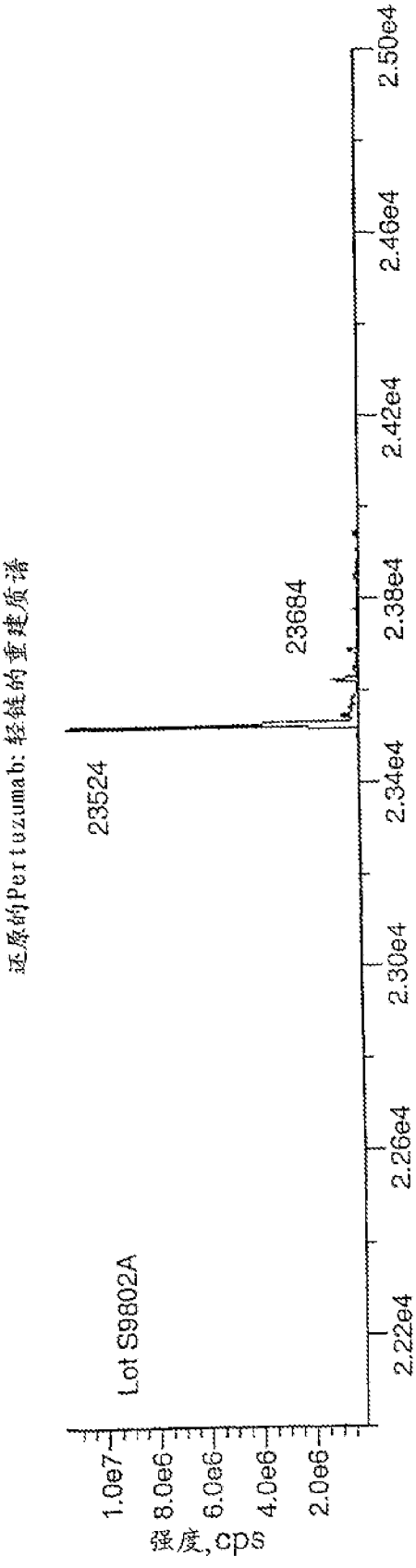

还原的Pertuzumab: 轻链的重建质谱
Lot S9802A
23524
23684
强度, cps
质量, amu
1.0e7
8.0e6
6.0e6
4.0e6
2.0e6
2.22e4
2.26e4
2.30e4
2.34e4
2.38e4
2.42e4
2.46e4
2.50e4

图 8A-2

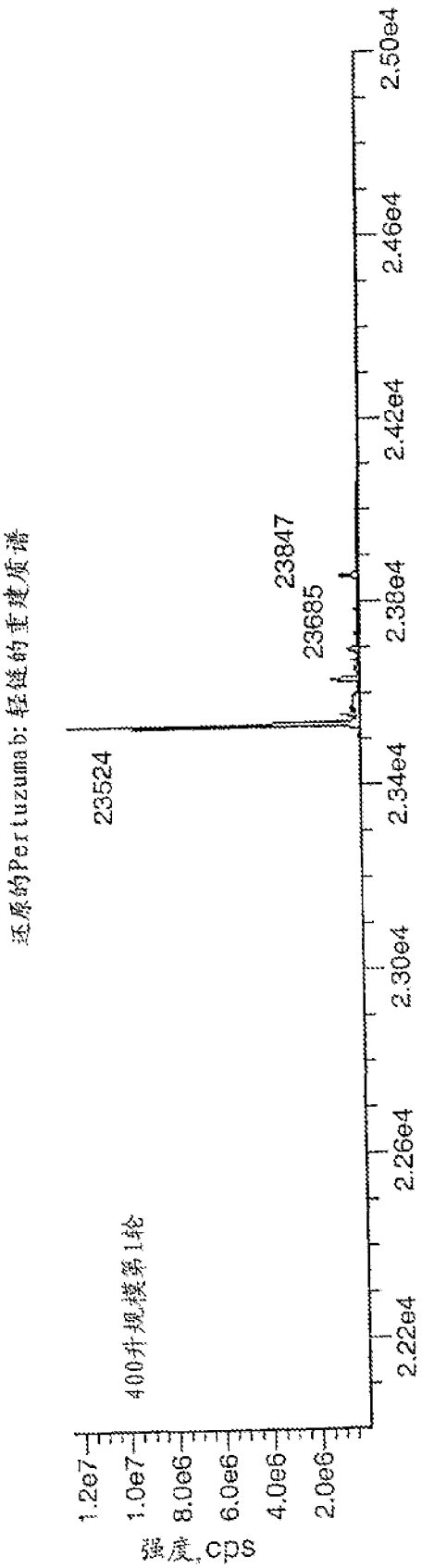

还原的Pertuzumab: 轻链的重建质谱
400升规模第1轮
强度, cps
质量, amu
23524
23685
23847

图 8A-3

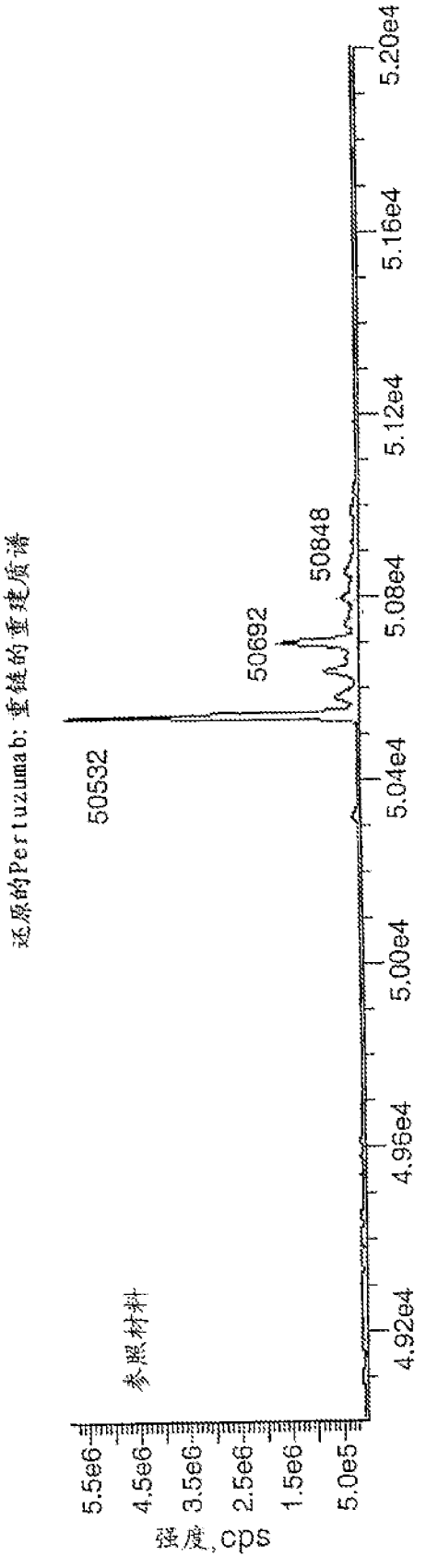

还原的Pertuzumab: 重链的重建质谱
参照材料
强度, cps
质量, amu
50532
50692
50848

图 8B-1

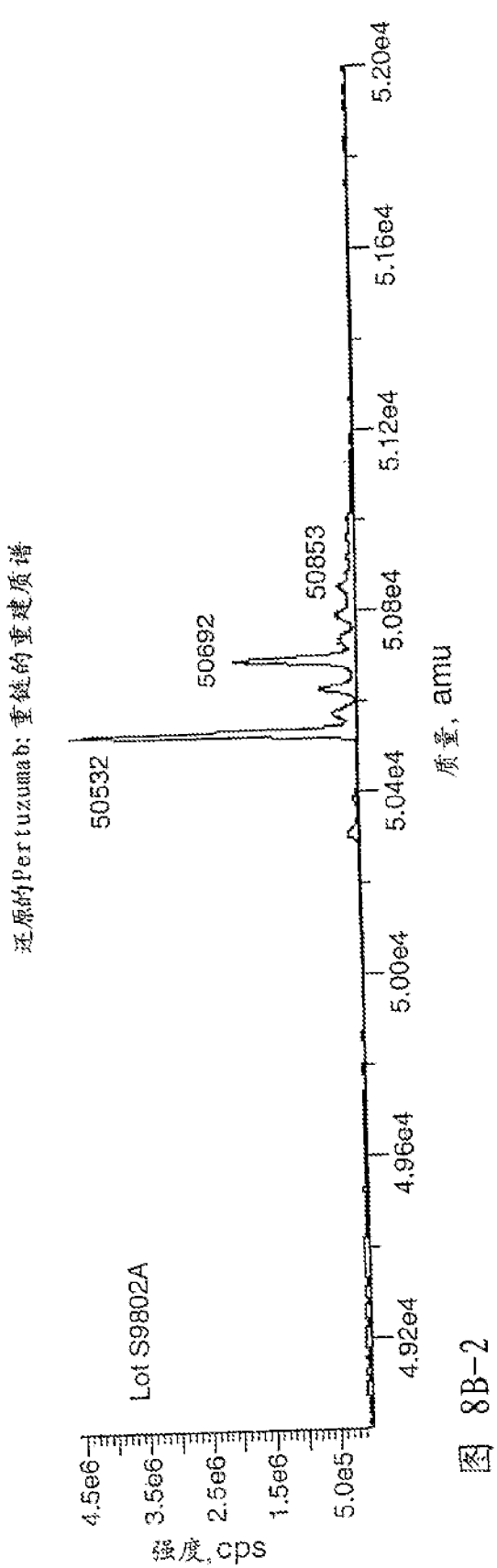

还原的Pertuzumab: 重链的重建质谱
Lot S9802A
50532
50692
50853
4.92e4
4.96e4
5.00e4
5.04e4
5.08e4
5.12e4
5.16e4
5.20e4
质量, amu
4.5e6
3.5e6
2.5e6
1.5e6
5.0e5
强度, cps

图 8B-2

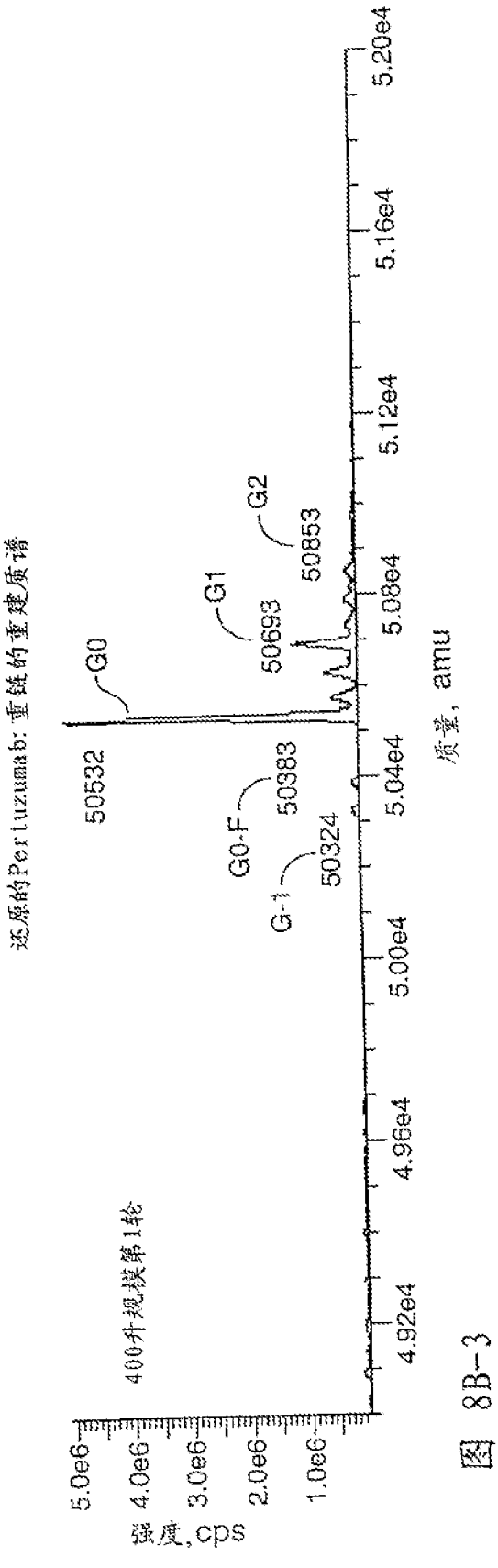

还原的Pertuzumab: 重链的重建质谱
400升规模第1轮
50532
G0
G1
G2
50693
50853
G0-F
50383
G-1
50324
4.92e4
4.96e4
5.00e4
5.04e4
5.08e4
5.12e4
5.16e4
5.20e4
质量, amu
5.0e6
4.0e6
3.0e6
2.0e6
1.0e6
强度, cps

图 8B-3

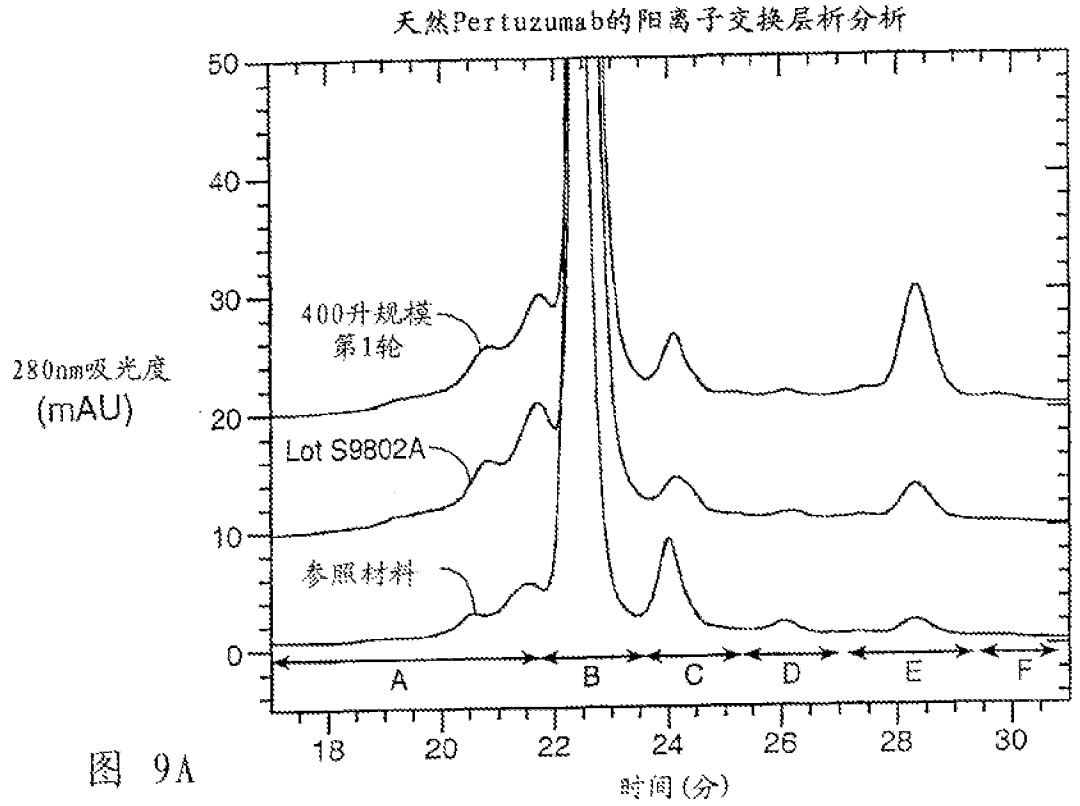

天然Pertuzumab的阳离子交换层析分析
280nm吸光度
(mAU)
400升规模
第1轮
Lot S9802A
参照材料
A
B
C
D
E
F
时间(分)

图 9A

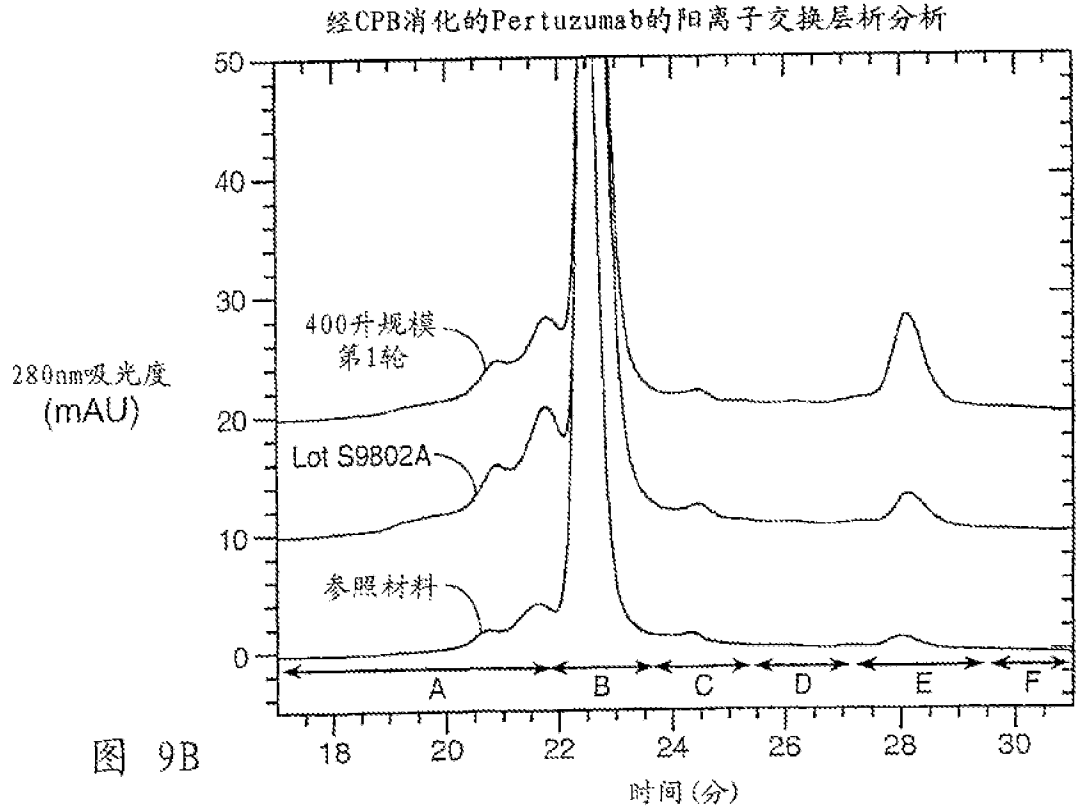

经CPB消化的Pertuzumab的阳离子交换层析分析
280nm吸光度
(mAU)
400升规模
第1轮
Lot S9802A
参照材料
A
B
C
D
E
F
时间(分)

图 9B

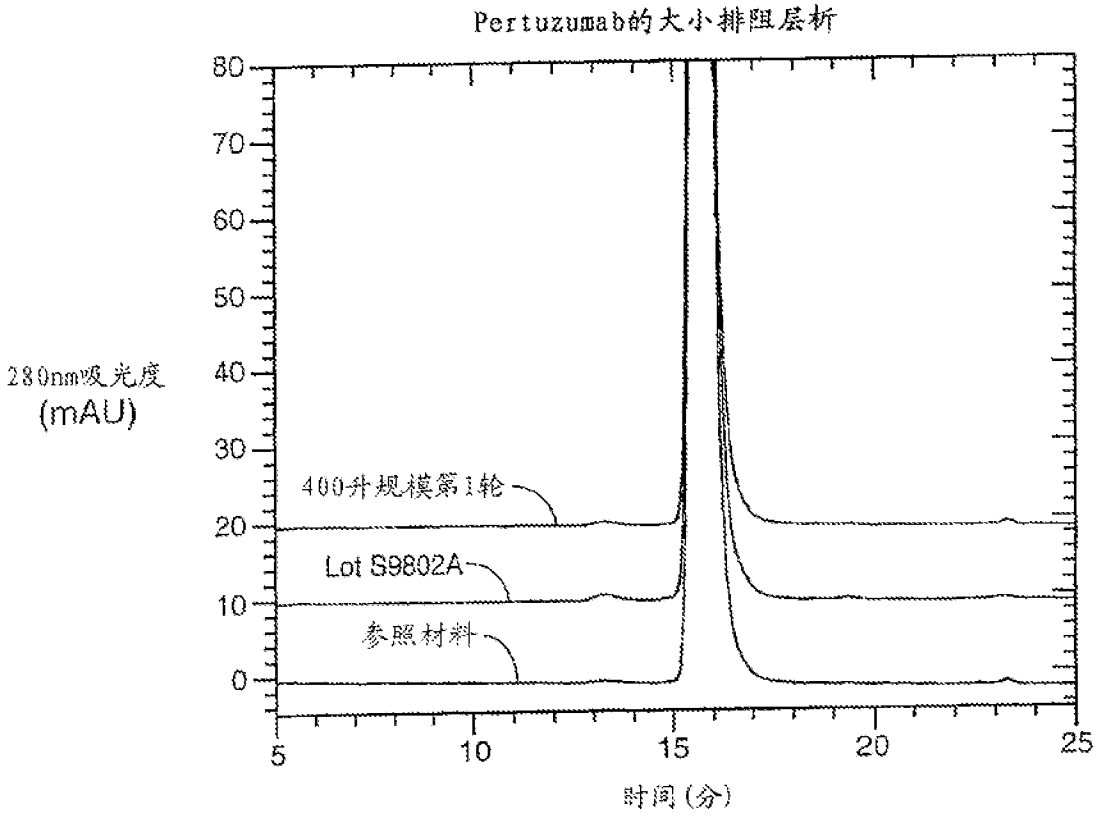

Pertuzumab的大小排阻层析
280nm吸光度
(mAU)
80
70
60
50
40
30
20
10
0
400升规模第1轮
Lot S9802A
参照材料
5
10
15
20
25
时间(分)

图 10

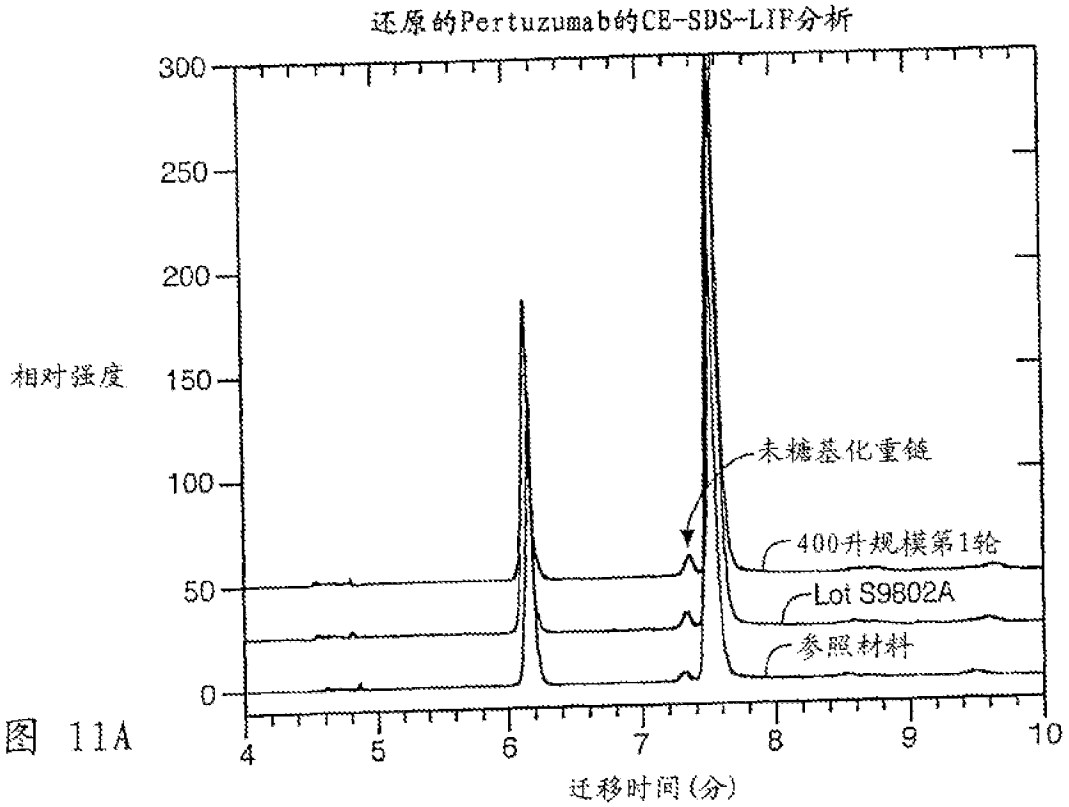

还原的Pertuzumab的CE-SDS-LIF分析
相对强度
300
250
200
150
100
50
0
4
5
6
7
8
9
10
迁移时间(分)
未糖基化重链
400升规模第1轮
Lot S9602A
参照材料

图 11A

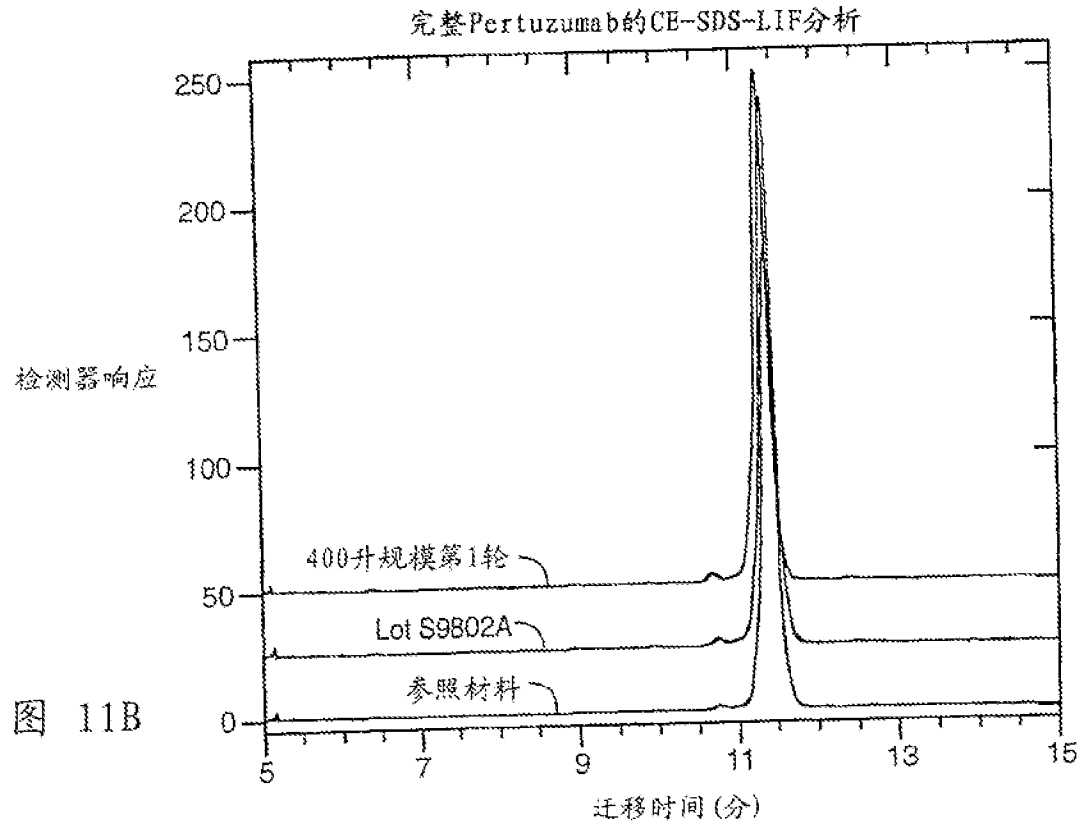

完整Pertuzumab的CE-SDS-LIF分析
检测器响应
250
200
150
100
50
0
5
7
9
11
13
15
迁移时间(分)
400升规模第1轮
Lot S9802A
参照材料

图 11B

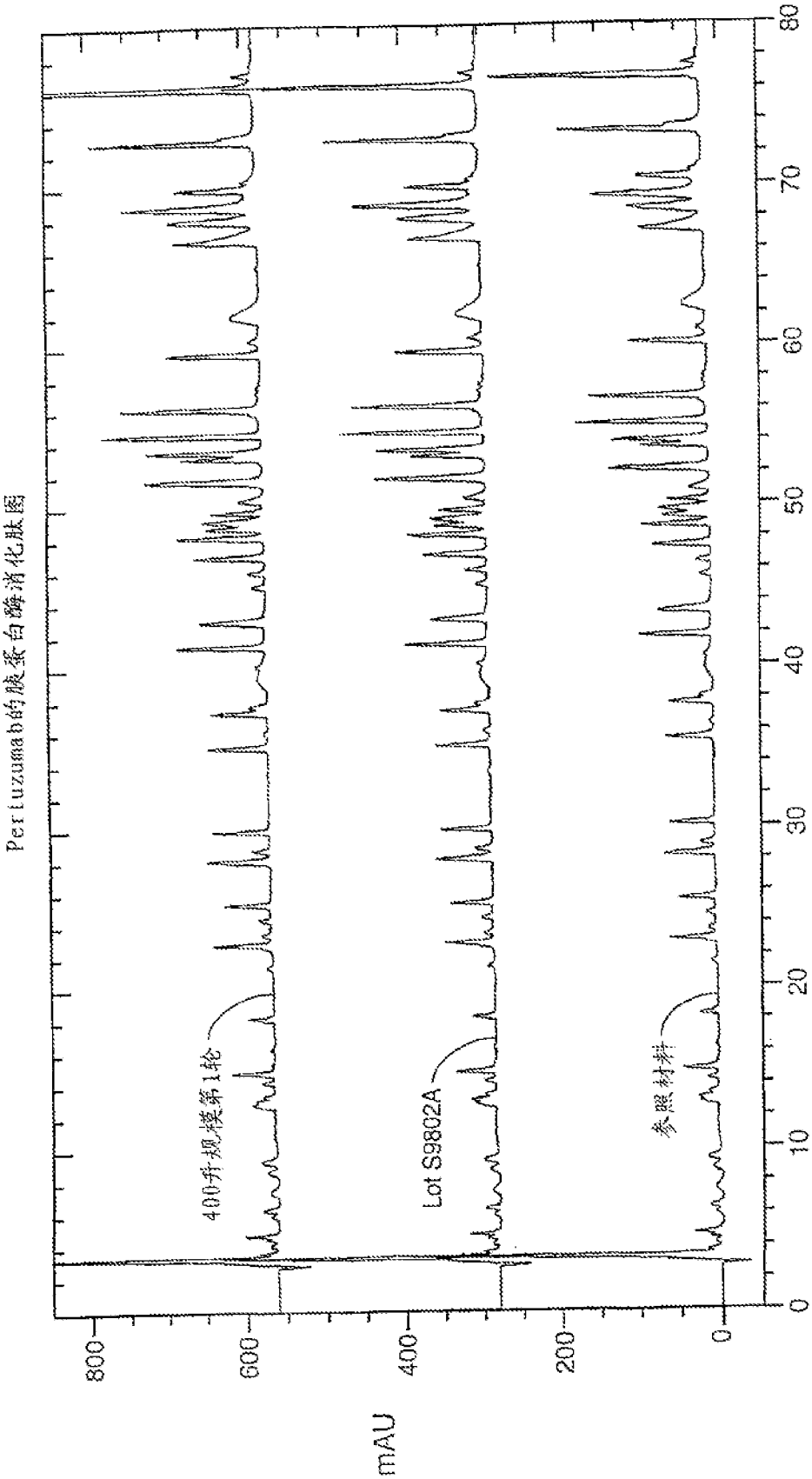

Pertuzumab的胰蛋白酶消化肽图
400升规模第1轮
Lot S9802A
参照材料
mAU
800
600
400
200
0
0
10
20
30
40
50
60
70
80
时间(分)

图 12A

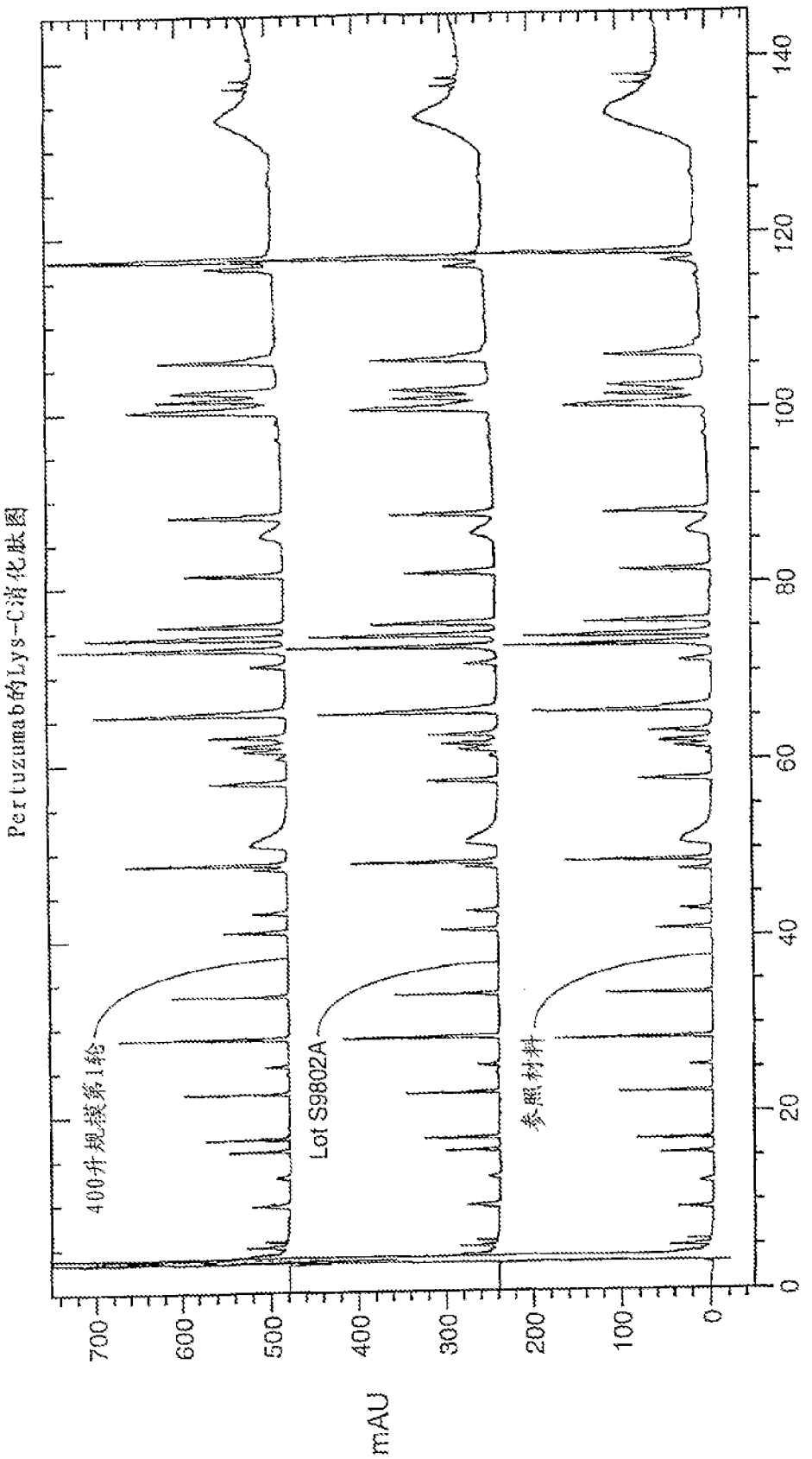

Pertuzumab的Lys-C消化肽图
400升规模第1轮
Lot S9802A
参照材料
mAU
700
600
500
400
300
200
100
0
0
20
40
60
80
100
120
140
时间(分)

图 12B

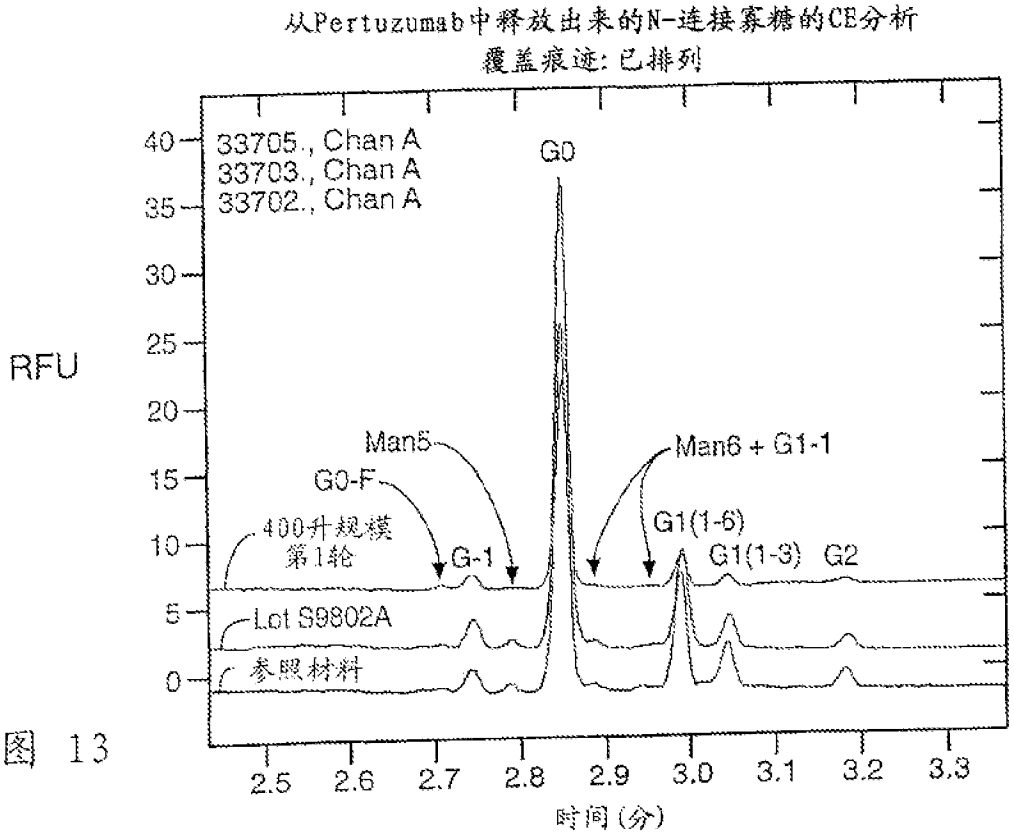

从Pertuzumab中释放出来的N-连接寡糖的CE分析
覆盖痕迹: 已排列
RFU
33705., Chan A
33703., Chan A
33702., Chan A
G0
Man5
G0-F
400升规模
第1轮
G-1
Man6 + G1-1
G1(1-6)
G1(1-3)
G2
Lot S9802A
参照材料
40
35
30
25
20
15
10
5
0
2.5
2.6
2.7
2.8
2.9
3.0
3.1
3.2
3.3
时间(分)

图 13

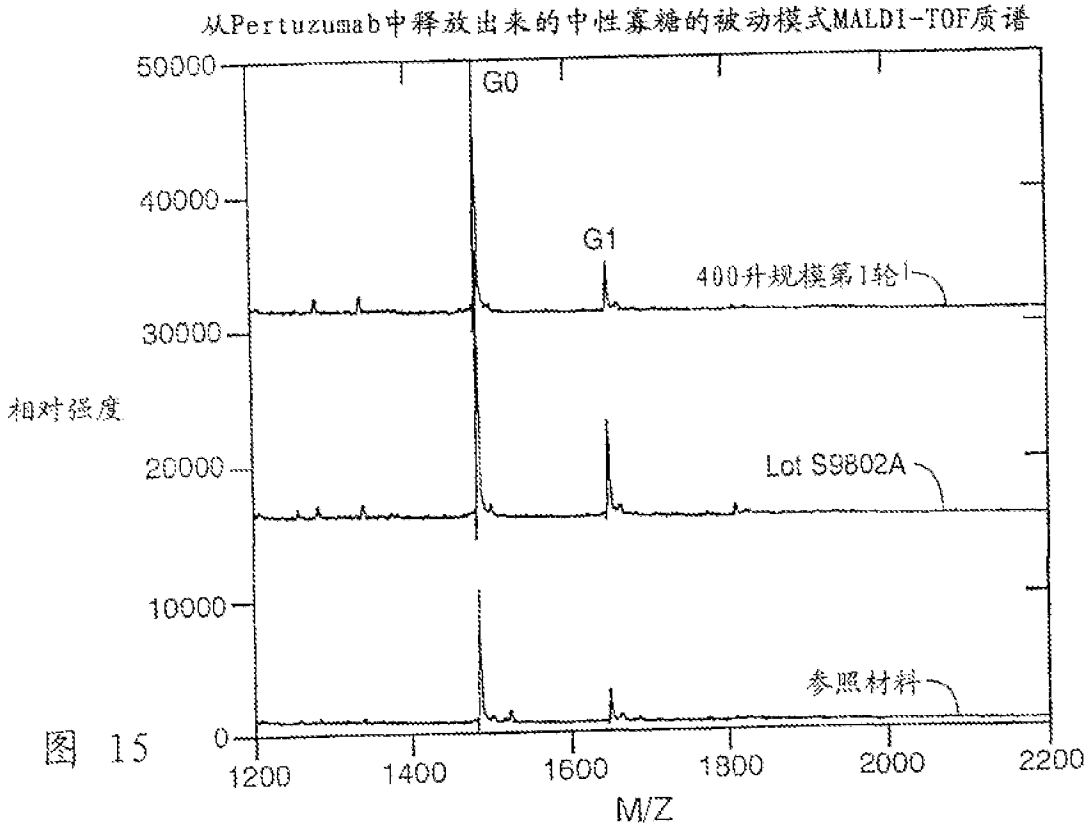

从Pertuzumab中释放出来的中性寡糖的被动模式MALDI-TOF质谱
相对强度
G0
G1
400升规模第1轮
Lot S9802A
参照材料
50000
40000
30000
20000
10000
0
1200
1400
1600
1800
2000
2200
M/Z

图 15

常常在IgG抗体中观察到的寡糖结构

结构	缩写	质量
	Man5	1235
	G-1	1260
	G0-F	1317
	Man6	1398

图 14A

常常在IgG抗体中观察到的寡糖结构

结构	缩写	质量
Fucα(1-->6) | Galβ(1-->4)GlcNAcβ(1-->2){Manα(1-->6)>Manβ(1-->4)GlcNAcβ(1-->4)GlcNAc- Manα(1-->3)}	G1-1	1423
Fucα(1-->6) | GlcNAcβ(1-->2)Manα(1-->6)>Manβ(1-->4)GlcNAcβ(1-->4)GlcNAc- GlcNAcβ(1-->2)Manα(1-->3)	G0	1463
Fucα(1-->6) | Galβ(1-->4)GlcNAcβ(1-->2)Manα(1-->6)>Manβ(1-->4)GlcNAcβ(1-->4)GlcNAc- GlcNAcβ(1-->2)Manα(1-->3)	G1 (1-6)	1626
Fucα(1-->6) | GlcNAcβ(1-->2)Manα(1-->6)>Manβ(1-->4)GlcNAcβ(1-->4)GlcNAc- Galβ(1-->4)GlcNAcβ(1-->2)Manα(1-->3)	G1 (1-3)	1626
Fucα(1-->6) | Galβ(1-->4)GlcNAcβ(1-->2)Manα(1-->6)>Manβ(1-->4)GlcNAcβ(1-->4)GlcNAc- Galβ(1-->4)GlcNAcβ(1-->2)Manα(1-->3)	G2	1788

此图中显示的分子量对应于 $(M+Na)^+$ 值

图 14B

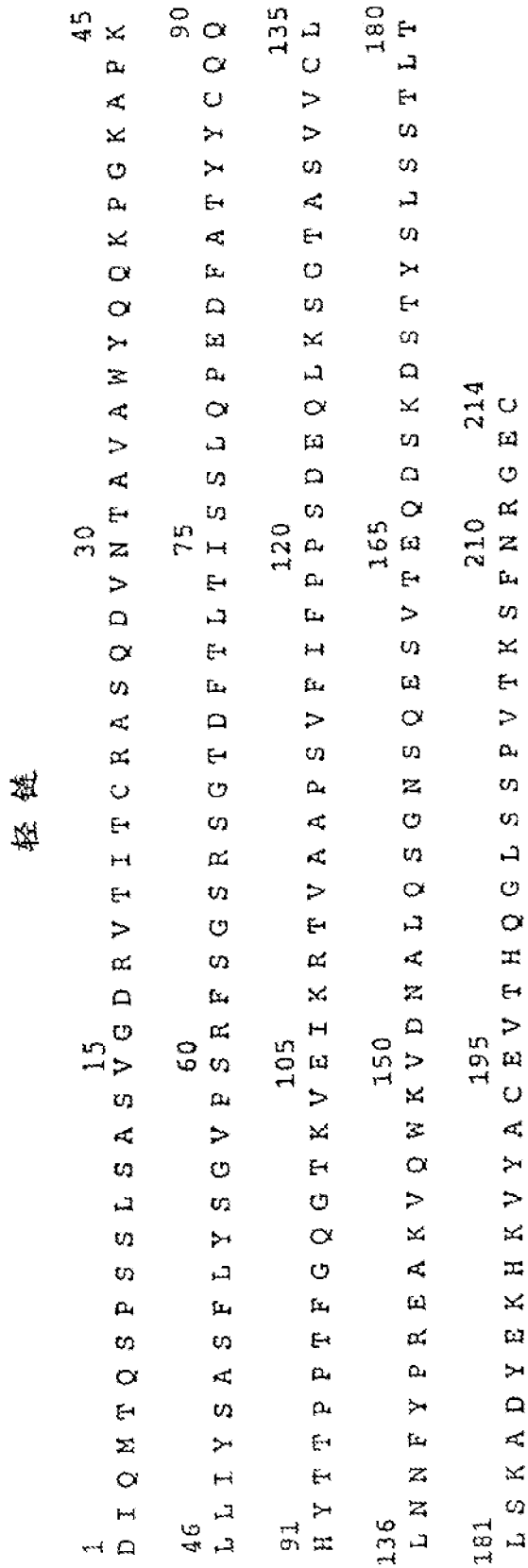

轻链
1
D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V N T A V A W Y Q Q K P G K A P K
15
30
45
46
L L I Y S A S F L Y S G V P S R F S G S R S G T D F T L T I S S L Q P E D F A T Y Y C Q Q
60
75
90
91
H Y T T P P T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L
105
120
135
136
L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T
150
165
180
181
L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C
195
210
214

图 16A

重链

```
1   E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K D T Y I H W V R Q A P G K G L
                15                  30                  45
46  E W V A R I Y P T N G Y T R Y A D S V K G R F T I S A D T S K N T A Y L Q M N S L R A E D
                60                  75                  90
91  T A V Y Y C S R W G G D G F Y A M D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S
                105                 120                 135
136 K S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S
                150                 165                 180
181 G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K
                195                 210                 225
226 T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S
                240                 255                 270
271 H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D
                285                 300                 315
316 W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E
                330                 345                 360
361 M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G
                375                 390                 405
406 S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G
                420                 435                 449
```

图 16B

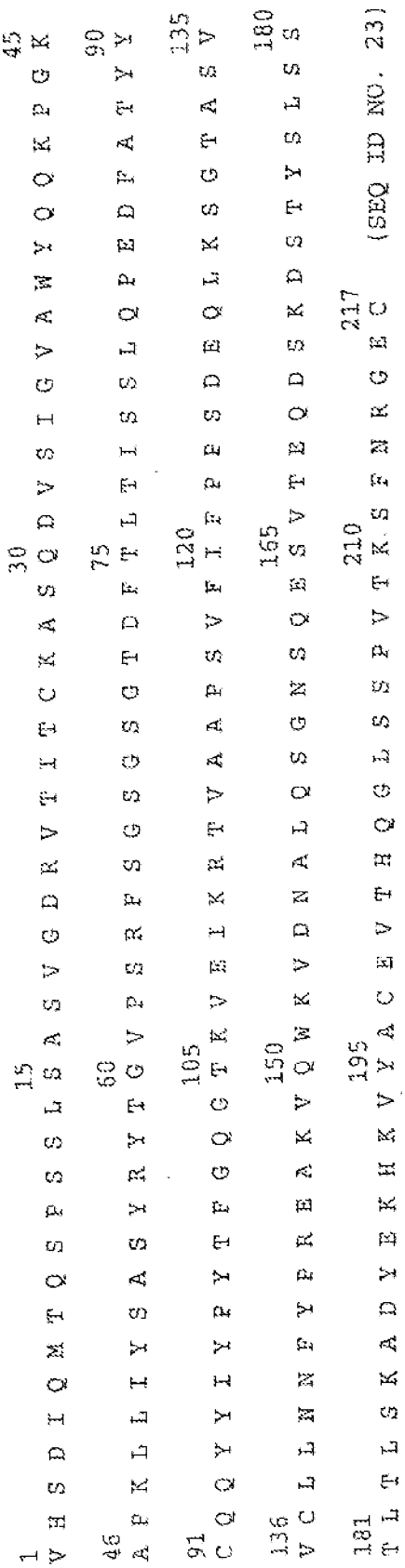
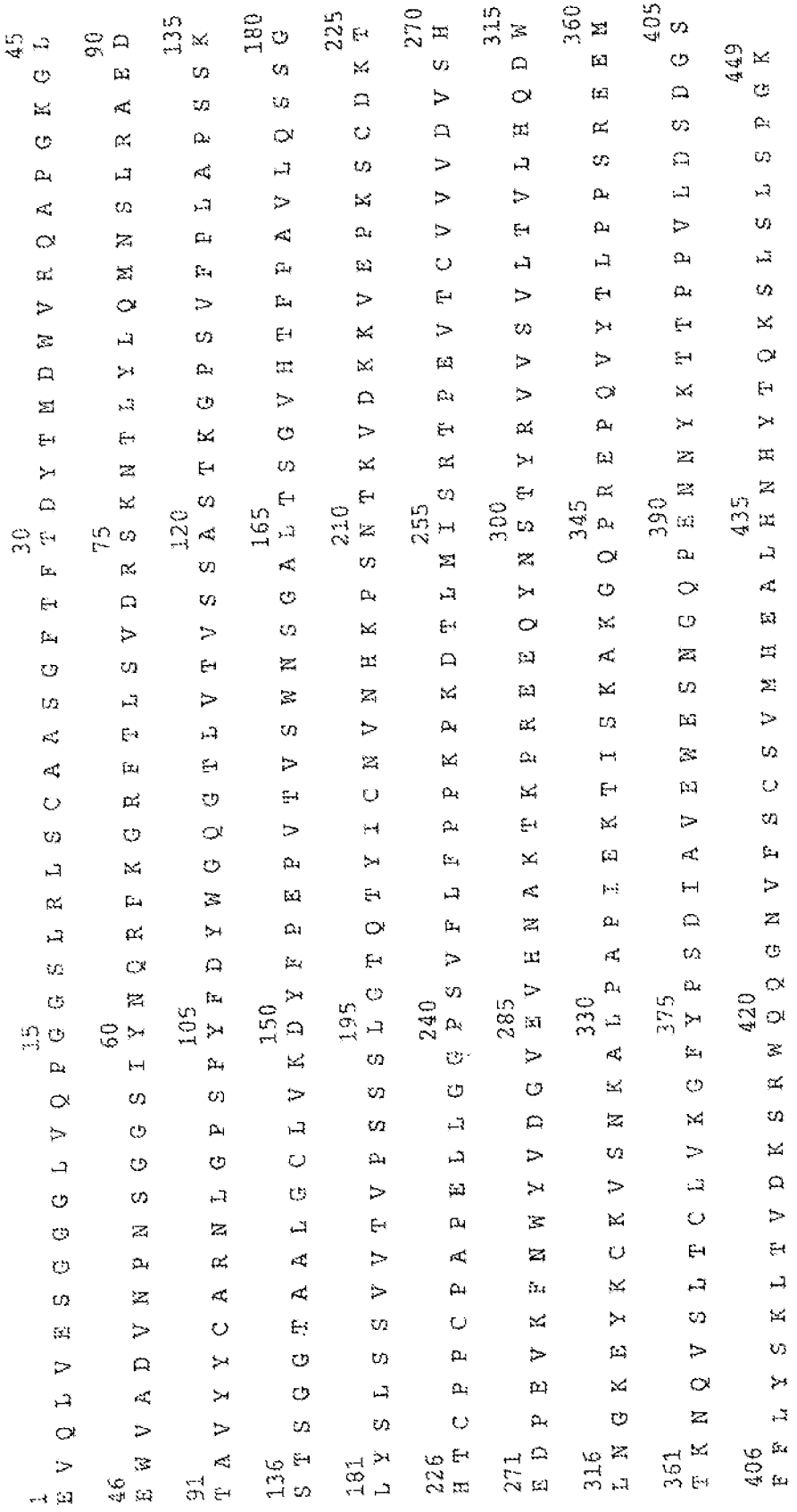

1 V H S D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q D V S I G V A W Y Q Q K P G K
46 A P K L L I Y S A S Y R Y T G V P S R F S G S G S G T D F T L T I S S L Q P E D F A T Y Y
91 C Q Q Y Y I Y P Y T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V
136 V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S
181 T L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C (SEQ ID NO. 23)

图 17A



(SEQ ID NO. 24)

图 17B

OnCusp
Ex. 1038

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A61K 47/48 (2006.01)

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[32] 2007.3.29 [33] US [31] 60/908,941
[32] 2007.4.13 [33] US [31] 60/911,829
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权利要求书 19 页 说明书 208 页 序列表 38 页
附图 31 页

[54] 发明名称

抗体和免疫偶联物及其用途

[57] 摘要

提供了抗 CD22 抗体及其免疫偶联物。 提供了使用抗 CD22 抗体及其免疫偶联物的方法。

1. 一种结合CD22的抗体，其中该抗体包含：(a) HVR-L1，其包含选自SEQ ID NO:9、10、19-23、32和33的氨基酸序列，和(b) 至少一种、两种、三种、四种或五种HVR，其选自

(1) HVR-H1，其包含SEQ ID NO:2的氨基酸序列；

(2) HVR-H2，其包含SEQ ID NO:4的氨基酸序列；

(3) HVR-H3，其包含SEQ ID NO:6的氨基酸序列；

(4) HVR-L2，其包含SEQ ID NO:12的氨基酸序列；和

(5) HVR-L3，其包含SEQ ID NO:14的氨基酸序列。

2. 权利要求1的抗体，该抗体包含HVR-L1，其包含符合共有序列SEQ ID NO:10的氨基酸序列。

3. 权利要求2的抗体，该抗体进一步包含：HVR-H1，其包含SEQ ID NO:2的氨基酸序列；HVR-H2，其包含SEQ ID NO:4的氨基酸序列；和HVR-H3，其包含SEQ ID NO:6的氨基酸序列。

4. 权利要求1的抗体，其中所述HVR-L1包含SEQ ID NO:9，且该抗体进一步包含：HVR-H1，其包含SEQ ID NO:2的氨基酸序列；HVR-H2，其包含SEQ ID NO:4的氨基酸序列；和HVR-H3，其包含SEQ ID NO:6的氨基酸序列。

5. 权利要求1的抗体，其中所述HVR-L1包含SEQ ID NO:19，且该抗体进一步包含：HVR-H1，其包含SEQ ID NO:2的氨基酸序列；HVR-H2，其包含SEQ ID NO:4的氨基酸序列；和HVR-H3，其包含SEQ ID NO:6的氨基酸序列。

6. 权利要求1的抗体，其中所述HVR-L1包含SEQ ID NO:20，且该抗体进一步包含：HVR-H1，其包含SEQ ID NO:2的氨基酸序列；HVR-H2，其包含SEQ ID NO:4的氨基酸序列；和HVR-H3，其包含SEQ ID NO:6的氨基酸序列。

7. 权利要求1的抗体，其中所述HVR-L1包含SEQ ID NO:21，且该抗体进一步包含：HVR-H1，其包含SEQ ID NO:2的氨基酸序列；HVR-H2，其包含SEQ ID NO:4的氨基酸序列；和HVR-H3，其包含SEQ ID NO:6的氨基酸序列。

8. 权利要求1的抗体，其中所述HVR-L1包含SEQ ID NO:22，且该抗体进一步包含：HVR-H1，其包含SEQ ID NO:2的氨基酸序列；HVR-H2，其包含SEQ ID NO:4的氨基酸序列；和HVR-H3，其包含SEQ ID NO:6的氨基酸序列。
9. 权利要求1的抗体，其中所述HVR-L1包含SEQ ID NO:23，且该抗体进一步包含：HVR-H1，其包含SEQ ID NO:2的氨基酸序列；HVR-H2，其包含SEQ ID NO:4的氨基酸序列；和HVR-H3，其包含SEQ ID NO:6的氨基酸序列。
10. 权利要求1的抗体，其中所述HVR-L1包含SEQ ID NO:32，且该抗体进一步包含：HVR-H1，其包含SEQ ID NO:2的氨基酸序列；HVR-H2，其包含SEQ ID NO:4的氨基酸序列；和HVR-H3，其包含SEQ ID NO:6的氨基酸序列。
11. 权利要求1的抗体，其中所述HVR-L1包含SEQ ID NO:33，且该抗体进一步包含：HVR-H1，其包含SEQ ID NO:2的氨基酸序列；HVR-H2，其包含SEQ ID NO:4的氨基酸序列；和HVR-H3，其包含SEQ ID NO:6的氨基酸序列。
12. 权利要求1的抗体，该抗体进一步包含至少一种框架，其选自VH亚组III共有框架和VL亚组I共有框架。
13. 权利要求1的抗体，其中该抗体包含重链可变域，其与选自SEQ ID NO:16的氨基酸序列具有至少90%的序列同一性。
14. 权利要求1的抗体，其中该抗体包含轻链可变域，其与选自SEQ ID NO:17的氨基酸序列具有至少90%的序列同一性。
15. 权利要求1的抗体，其中该抗体包含轻链可变域，其与选自SEQ ID NO:18的氨基酸序列具有至少90%的序列同一性。
16. 权利要求1的抗体，其中该抗体包含重链可变域，其包含一种、两种、三种或四种选自SEQ ID NO:1、3、5和7的框架氨基酸序列。
17. 权利要求1的抗体，其中该抗体包含轻链可变域，其包含一种、两种、三种或四种选自SEQ ID NO:8、11、13和15的框架氨基酸序列。
18. 权利要求13的抗体，该抗体进一步包含轻链可变域，其与选自SEQ ID NO:17的氨基酸序列具有至少90%的序列同一性。
19. 权利要求13的抗体，该抗体进一步包含轻链可变域，其与选自SEQ ID

NO:18的氨基酸序列具有至少90%的序列同一性。

20. 权利要求1的抗体，其中该抗体包含重链，其与选自SEQ ID NO:88的氨基酸序列具有至少90%的序列同一性。
21. 权利要求1的抗体，其中该抗体包含轻链，其与选自SEQ ID NO:87的氨基酸序列具有至少90%的序列同一性。
22. 权利要求1的抗体，其中该抗体包含具有SEQ ID NO:88的氨基酸序列的重链和具有SEQ ID NO:87的氨基酸序列的轻链。
23. 一种结合CD22的抗体，其中该抗体包含重链可变域，其与SEQ ID NO:16的氨基酸序列具有至少90%的序列同一性。
24. 权利要求23的抗体，该抗体进一步包含轻链可变域，其与SEQ ID NO:17的氨基酸序列具有至少90%的序列同一性。
25. 权利要求23的抗体，该抗体进一步包含轻链可变域，其与SEQ ID NO:18的氨基酸序列具有至少90%的序列同一性。
26. 权利要求1的抗体，其中该抗体包含一种、两种、三种、四种、五种或六种来自杂交瘤ATCC编号PTA-7621 (10F4.4.1)所产生的抗体的HVR。
27. 一种抗体，该抗体包含一种、两种、三种、四种、五种或六种来自杂交瘤ATCC编号PTA-7620 (5E8.1.8)所产生的抗体的HVR。
28. 权利要求1的抗体，其中该抗体是人源化的。
29. 权利要求23的抗体，其中该抗体是人源化的。
30. 权利要求1的抗体，其中所述CD22是哺乳动物CD22。
31. 权利要求30的抗体，其中所述CD22选自啮齿类CD22和灵长类CD22。
32. 权利要求31的抗体，其中所述CD22是人CD22。
33. 权利要求23的抗体，其中所述CD22是哺乳动物CD22。
34. 权利要求33的抗体，其中所述CD22选自啮齿类CD22和灵长类CD22。
35. 权利要求34的抗体，其中所述CD22是人CD22。
36. 一种编码权利要求1的抗体的多核苷酸。
37. 一种编码权利要求23的抗体的多核苷酸。
38. 一种包含权利要求36的多核苷酸的载体。
39. 一种包含权利要求37的多核苷酸的载体。
40. 一种包含权利要求38的载体的宿主细胞。
41. 一种包含权利要求39的载体的宿主细胞。

42. 权利要求40的宿主细胞，其中该宿主细胞是真核的。
43. 权利要求42的宿主细胞，其中该宿主细胞是CHO细胞。
44. 权利要求41的宿主细胞，其中该宿主细胞是真核的。
45. 权利要求44的宿主细胞，其中该宿主细胞是CHO细胞。
46. 一种制备抗CD22抗体的方法，其中该方法包括：a) 在适于表达编码该抗体的多核苷酸的条件下培养权利要求38的宿主细胞，和b) 分离该抗体。
47. 一种制备抗CD22抗体的方法，其中该方法包括：a) 在适于表达编码该抗体的多核苷酸的条件下培养权利要求39的宿主细胞，和b) 分离该抗体。
48. 权利要求28的抗体，其中所述CD22是在细胞表面上表达的。
49. 权利要求48的抗体，其中所述细胞是B细胞。
50. 权利要求29的抗体，其中所述CD22是在细胞表面上表达的。
51. 权利要求50的抗体，其中所述细胞是B细胞。
52. 权利要求1的抗体，其中该抗体结合CD22中SEQ ID NO:27的第22-240位氨基酸的区域内的表位。
53. 权利要求23的抗体，其中该抗体结合CD22中SEQ ID NO:27的第22-240位氨基酸的区域内的表位。
54. 权利要求49的抗体，其中所述B细胞与B细胞增殖性病症有关。
55. 权利要求54的抗体，其中所述B细胞增殖性病症是癌症。
56. 权利要求54的抗体，其中所述B细胞增殖性病症选自：淋巴瘤、非何杰金氏淋巴瘤(NHL)、攻击性NHL、复发性攻击性NHL、复发性无痛性NHL、顽固性NHL、顽固性无痛性NHL、慢性淋巴细胞性白血病(CLL)、小淋巴细胞淋巴瘤、白血病、毛细胞白血病(HCL)、急性淋巴细胞性白血病(ALL)和套细胞淋巴瘤。
57. 权利要求51的抗体，其中所述B细胞与B细胞增殖性病症有关。
58. 权利要求57的抗体，其中所述B细胞增殖性疾病是癌症。
59. 权利要求57的抗体，其中所述B细胞增殖性病症选自：淋巴瘤、非何杰金氏淋巴瘤(NHL)、攻击性NHL、复发性攻击性NHL、复发性无痛性NHL、顽固性NHL、顽固性无痛性NHL、慢性淋巴细胞性白血病(CLL)、小淋巴细胞淋巴瘤、白血病、毛细胞白血病(HCL)、急性淋巴细胞性白

血病(ALL)和套细胞淋巴瘤。

60. 权利要求1的抗体，其中该抗体是单克隆抗体。

61. 权利要求60的抗体，其中该抗体是抗体片段，其选自Fab、Fab'-SH、Fv、scFv或$(Fab')_2$片段。

62. 权利要求60的抗体，其中该抗体是人源化的。

63. 权利要求60的抗体，其中该抗体是人的。

64. 权利要求23的抗体，其中该抗体是单克隆抗体。

65. 权利要求64的抗体，其中该抗体是抗体片段，其选自Fab、Fab'-SH、Fv、scFv或$(Fab')_2$片段。

66. 权利要求64的抗体，其中该抗体是人源化的。

67. 权利要求64的抗体，其中该抗体是人的。

68. 权利要求1的抗体，其中该抗体与选自下组的抗体结合相同的表位：ATCC PTA-7621 (10F4.4.1)；ATCC PTA-7620 (5E8.1.8)；及包含SEQ ID NO:88的重链序列和SEQ ID NO:87的轻链序列的抗体。

69. 权利要求23的抗体，其中该抗体与选自下组的抗体结合相同的表位：ATCC PTA-7621 (10F4.4.1)；ATCC PTA-7620 (5E8.1.8)；及包含SEQ ID NO:88的重链序列和SEQ ID NO:87的轻链序列的抗体。

70. 一种检测CD22在生物学样品中存在的方法，该方法包括：使该生物学样品与权利要求1的抗体在容许该抗体结合至CD22的条件下接触，和检测该抗体和CD22之间是否形成复合物。

71. 权利要求70的方法，其中所述生物学样品来自怀疑患有B细胞增殖性病症的患者。

72. 权利要求71的方法，其中所述B细胞增殖性病症选自：淋巴瘤、非何杰金氏淋巴瘤(NHL)、攻击性NHL、复发性攻击性NHL、复发性无痛性NHL、顽固性NHL、顽固性无痛性NHL、慢性淋巴细胞性白血病(CLL)、小淋巴细胞淋巴瘤、白血病、毛细胞白血病(HCL)、急性淋巴细胞性白血病(ALL)和套细胞淋巴瘤。

73. 一种检测CD22在生物学样品中存在的方法，该方法包括：使该生物学样品与权利要求23的抗体在容许该抗体结合至CD22的条件下接触，和检测该抗体和CD22之间是否形成复合物。

74. 权利要求73的方法，其中所述生物学样品来自怀疑患有B细胞增殖性病

症的患者。

75. 权利要求74的方法，其中所述B细胞增殖性病症选自：淋巴瘤、非何杰金氏淋巴瘤(NHL)、攻击性NHL、复发性攻击性NHL、复发性无痛性NHL、顽固性NHL、顽固性无痛性NHL、慢性淋巴细胞性白血病(CLL)、小淋巴细胞淋巴瘤、白血病、毛细胞白血病(HCL)、急性淋巴细胞性白血病(ALL)和套细胞淋巴瘤。

76. 一种免疫偶联物，其包含共价附着至细胞毒剂的权利要求1的抗体。

77. 一种免疫偶联物，其包含共价附着至细胞毒剂的权利要求23的抗体。

78. 权利要求76的免疫偶联物，其中所述细胞毒剂选自毒素、化疗剂、药物模块、抗生素、放射性同位素和核溶酶。

79. 权利要求78的免疫偶联物，该免疫偶联物具有通式Ab-(L-D)p，其中：

(a) Ab是权利要求1的抗体；

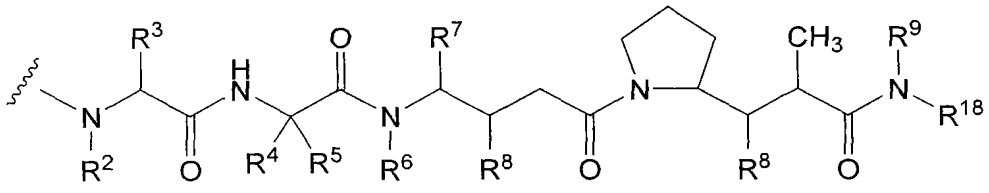
(b) L是接头；

(c) D是药物模块。

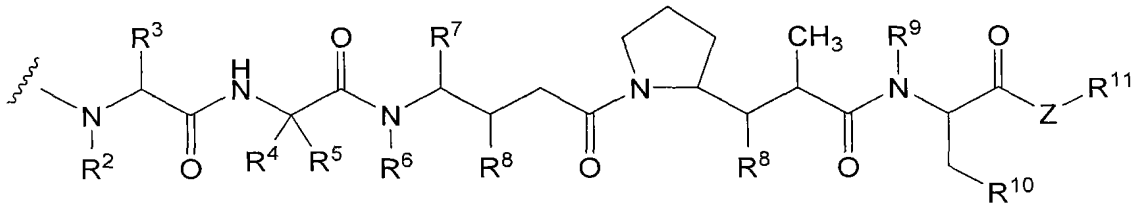
80. 权利要求79的免疫偶联物，其中L选自：6-马来酰亚氨基己酰基(MC)、马来酰亚氨基丙酰基(MP)、缬氨酸-瓜氨酸(val-cit)、丙氨酸-苯丙氨酸(ala-phe)、对氨基苄氧羰基(PAB)、N-琥珀酰亚氨基4-(2-吡啶基硫代)戊酸酯(SPP)、N-琥珀酰亚氨基4-(N-马来酰亚氨基甲基)-环己烷-1-羧酸酯(SMCC)、和N-琥珀酰亚氨基(4-碘-乙酰基)氨基苯甲酸酯(SIAB)。

81. 权利要求79的免疫偶联物，其中D选自auristatin和多拉司他汀。

82. 权利要求81的免疫偶联物，其中D是通式D_E或D_F的药物模块：



D_E



D_F

且其中R^2和R^6各自是甲基；R^3和R^4各自是异丙基；R^7是仲丁基；各个R^8独立地选自CH_3、O-CH_3、OH和H；R^9是H；R^{10}是芳基；Z是–O–

或–NH–; R^{11}是H、C_1-C_8烃基或–$(CH_2)_2$–O–$(CH_2)_2$–O–$(CH_2)_2$–O–CH_3; 和R^{18}是–$C(R^8)_2$–$C(R^8)_2$–芳基; 和

(d) p的范围为大约1至8。

83. 权利要求76的免疫偶联物，其具有体外或体内细胞杀伤活性。

84. 权利要求79的免疫偶联物，其中所述接头是经所述抗体上的硫醇基团附着至该抗体的。

85. 权利要求79的免疫偶联物，其中所述接头是蛋白酶可切割的。

86. 权利要求80的免疫偶联物，其中所述接头包含val-cit二肽。

87. 权利要求79的免疫偶联物，其中所述接头包含对氨基苄基单元。

88. 权利要求80的免疫偶联物，其中所述接头包含6-马来酰亚氨基己酰基。

89. 权利要求82的免疫偶联物，其中所述药物选自MMAE和MMAF。

90. 权利要求89的免疫偶联物，其中所述药物是MMAE。

91. 权利要求89的免疫偶联物，其中所述药物是MMAF。

92. 权利要求77的免疫偶联物，其中所述细胞毒剂选自毒素、化疗剂、药物模块、抗生素、放射性同位素和核溶酶。

93. 权利要求92的免疫偶联物，该免疫偶联物具有通式Ab-(L-D)p，其中:

(a) Ab是权利要求23的抗体;

(b) L是接头;

(c) D是药物模块。

94. 权利要求93的免疫偶联物，其中L选自: 6-马来酰亚氨基己酰基(MC)、马来酰亚氨基丙酰基(MP)、缬氨酸-瓜氨酸(val-cit)、丙氨酸-苯丙氨酸(ala-phe)、对氨基苄氧羰基(PAB)、N-琥珀酰亚氨基4-(2-吡啶基硫代)戊酸酯(SPP)、N-琥珀酰亚氨基4-(N-马来酰亚氨基甲基)-环己烷-1-羧酸酯(SMCC)、和N-琥珀酰亚氨基(4-碘-乙酰基)氨基苯甲酸酯(SIAB)。

95. 权利要求93的免疫偶联物，其中所述接头L是蛋白酶可切割的。

96. 权利要求94的免疫偶联物，其中L包含val-cit二肽。

97. 权利要求93的免疫偶联物，其中L包含对氨基苄基单元。

98. 权利要求97的免疫偶联物，其中该对氨基苄基单元是对氨基苄氧羰基(PAB)。

99. 权利要求94的免疫偶联物，其中L包含6-马来酰亚氨基己酰基(MC)。

100. 权利要求94的免疫偶联物，其中所述接头包含6-马来酰亚氨基己酰基和

对氨基苄氧羰基。

101. 权利要求79的免疫偶联物，其中该免疫偶联物具有通式Ab-(L-MMAE)p，其中L是接头，且p的范围为2至5。

102. 权利要求101的免疫偶联物，其中L包含val-cit。

103. 权利要求101的免疫偶联物，其中L包含MC。

104. 权利要求101的免疫偶联物，其中L包含PAB。

105. 权利要求101的免疫偶联物，其中L包含MC-PAB。

106. 权利要求93的免疫偶联物，其中该免疫偶联物具有通式Ab-(L-MMAE)p，其中L是接头，且p的范围为2至5。

107. 权利要求106的免疫偶联物，其中L包含val-cit。

108. 权利要求106的免疫偶联物，其中L包含MC。

109. 权利要求106的免疫偶联物，其中L包含PAB。

110. 权利要求106的免疫偶联物，其中L包含MC-PAB。

111. 权利要求79的免疫偶联物，其中该免疫偶联物具有通式Ab-(L-MMAF)p，其中L是接头，且p的范围为2至5。

112. 权利要求111的免疫偶联物，其中L包含val-cit。

113. 权利要求111的免疫偶联物，其中L包含MC。

114. 权利要求111的免疫偶联物，其中L包含PAB。

115. 权利要求111的免疫偶联物，其中L包含MC-PAB。

116. 权利要求93的免疫偶联物，其中该免疫偶联物具有通式Ab-(L-MMAF)p，其中L是接头，且p的范围为2至5。

117. 权利要求116的免疫偶联物，其中L包含val-cit。

118. 权利要求116的免疫偶联物，其中L包含MC。

119. 权利要求116的免疫偶联物，其中L包含PAB。

120. 权利要求116的免疫偶联物，其中L包含MC-PAB。

121. 权利要求79的免疫偶联物，其中D是美登木素生物碱。

122. 权利要求121的免疫偶联物，其中D选自DM1、DM3和DM4。

123. 权利要求121的免疫偶联物，其具有体外或体内细胞杀伤活性。

124. 权利要求121的免疫偶联物，其中所述接头是经所述抗体上的硫醇基团附着至该抗体的。

125. 权利要求121的免疫偶联物，其中所述接头L选自N-琥珀酰亚氨基4-(2-

吡啶基硫代)戊酸酯(SPP)、N-琥珀酰亚氨基4-(N-马来酰亚氨基甲基)-环己烷-1-羧酸酯(SMCC)、和N-琥珀酰亚氨基(4-碘-乙酰基)氨基苯甲酸酯(SIAB)。

126. 权利要求122的免疫偶联物，其中所述药物是DM1。

127. 权利要求122的免疫偶联物，其中L包含SPP。

128. 权利要求122的免疫偶联物，其中L包含SMCC。

129. 权利要求122的免疫偶联物，其中p为2-4。

130. 权利要求122的免疫偶联物，其中p为3-4。

131. 权利要求93的免疫偶联物，其中D是美登木素生物碱。

132. 权利要求131的免疫偶联物，其中D选自DM1、DM3和DM4。

133. 权利要求131的免疫偶联物，其具有体外或体内细胞杀伤活性。

134. 权利要求131的免疫偶联物，其中所述接头是经所述抗体上的硫醇基团附着至该抗体的。

135. 权利要求131的免疫偶联物，其中所述接头L选自N-琥珀酰亚氨基4-(2-吡啶基硫代)戊酸酯(SPP)、N-琥珀酰亚氨基4-(N-马来酰亚氨基甲基)-环己烷-1-羧酸酯(SMCC)、和N-琥珀酰亚氨基(4-碘-乙酰基)氨基苯甲酸酯(SIAB)。

136. 权利要求132的免疫偶联物，其中所述药物是DM1。

137. 权利要求136的免疫偶联物，其中L包含SPP。

138. 权利要求136的免疫偶联物，其中L包含SMCC。

139. 权利要求136的免疫偶联物，其中p为2-4。

140. 权利要求136的免疫偶联物，其中p为3-4。

141. 一种药物组合物，其包含权利要求79的免疫偶联物和药学可接受载体。

142. 一种治疗B细胞增殖性病症的方法，包括给个体施用有效量的权利要求141的药物组合物。

143. 权利要求142的方法，其中所述B细胞增殖性病症选自：淋巴瘤、非何杰金氏淋巴瘤(NHL)、攻击性NHL、复发性攻击性NHL、复发性无痛性NHL、顽固性NHL、顽固性无痛性NHL、慢性淋巴细胞性白血病(CLL)、小淋巴细胞淋巴瘤、白血病、毛细胞白血病(HCL)、急性淋巴细胞性白血病(ALL)和套细胞淋巴瘤。

144. 一种药物组合物，其包含权利要求93的免疫偶联物和药学可接受载体。

145. 一种治疗B细胞增殖性病症的方法，包括给个体施用有效量的权利要求144的药物组合物。

146. 权利要求145的方法，其中所述B细胞增殖性病症选自：淋巴瘤、非何杰金氏淋巴瘤(NHL)、攻击性NHL、复发性攻击性NHL、复发性无痛性NHL、顽固性NHL、顽固性无痛性NHL、慢性淋巴细胞性白血病(CLL)、小淋巴细胞淋巴瘤、白血病、毛细胞白血病(HCL)、急性淋巴细胞性白血病(ALL)和套细胞淋巴瘤。

147. 一种药物组合物，其包含权利要求76、77、78或92的免疫偶联物和药学可接受载体。

148. 一种治疗B细胞增殖性病症的方法，包括给个体施用有效量的权利要求147的药物组合物。

149. 权利要求148的方法，其中所述B细胞增殖性病症选自：淋巴瘤、非何杰金氏淋巴瘤(NHL)、攻击性NHL、复发性攻击性NHL、复发性无痛性NHL、顽固性NHL、顽固性无痛性NHL、慢性淋巴细胞性白血病(CLL)、小淋巴细胞淋巴瘤、白血病、毛细胞白血病(HCL)、急性淋巴细胞性白血病(ALL)和套细胞淋巴瘤。

150. 一种抑制B细胞增殖的方法，包括使细胞暴露于权利要求79的免疫偶联物，该暴露在容许该免疫偶联物结合至CD22的条件下进行。

151. 权利要求150的方法，其中所述B细胞增殖与选自下组的病症有关：淋巴瘤、非何杰金氏淋巴瘤(NHL)、攻击性NHL、复发性攻击性NHL、复发性无痛性NHL、顽固性NHL、顽固性无痛性NHL、慢性淋巴细胞性白血病(CLL)、小淋巴细胞淋巴瘤、白血病、毛细胞白血病(HCL)、急性淋巴细胞性白血病(ALL)和套细胞淋巴瘤。

152. 权利要求150的方法，其中所述B细胞是异种移植物。

153. 权利要求150的方法，其中所述暴露在体外发生。

154. 权利要求150的方法，其中所述暴露在体内发生。

155. 权利要求1的抗体，其中该抗体是包含一个或多个具有在0.6至1.0范围内的硫醇反应性值的游离半胱氨酸氨基酸的半胱氨酸改造抗体，其中该半胱氨酸改造抗体是通过包括用半胱氨酸替代亲本抗体的一个或多个氨基酸残基的方法制备的。

156. 权利要求155的半胱氨酸改造抗体，其中该半胱氨酸改造抗体比亲本抗

体更能与硫醇反应性试剂起反应。

157. 权利要求155的半胱氨酸改造抗体，其中所述方法进一步包括通过使半胱氨酸改造抗体与硫醇反应性试剂起反应来测定该半胱氨酸改造抗体的硫醇反应性；其中该半胱氨酸改造抗体比亲本抗体更能与硫醇反应性试剂起反应。

158. 权利要求155的半胱氨酸改造抗体，其中所述一个或多个游离半胱氨酸氨基酸残基是位于轻链中的。

159. 权利要求155的半胱氨酸改造抗体，其中该抗体是免疫偶联物，其包含共价附着至细胞毒剂的半胱氨酸改造抗体。

160. 权利要求159的半胱氨酸改造抗体，其中所述细胞毒剂选自毒素、化疗剂、药物模块、抗生素、放射性同位素和核溶酶。

161. 权利要求155的半胱氨酸改造抗体，其中该抗体是共价附着至捕获标记物、检测标记物或固体支持物的。

162. 权利要求161的半胱氨酸改造抗体，其中该抗体是共价附着至生物素捕获标记物的。

163. 权利要求161的半胱氨酸改造抗体，其中该抗体是共价附着至荧光染料检测标记物的。

164. 权利要求163的半胱氨酸改造抗体，其中所述荧光染料选自荧光素类、罗丹明类、丹酰、丽丝胺、花青、藻红蛋白、德克萨斯红和它们的类似物。

165. 权利要求161的半胱氨酸改造抗体，其中该抗体是共价附着至放射性核素检测标记物的，所述放射性核素检测标记物选自^{3}H、^{11}C、^{14}C、^{18}F、^{32}P、^{35}S、^{64}Cu、^{68}Ga、^{86}Y、^{99}Tc、^{111}In、^{123}I、^{124}I、^{125}I、^{131}I、^{133}Xe、^{177}Lu、^{211}At和^{213}Bi。

166. 权利要求161的半胱氨酸改造抗体，其中该抗体是通过螯合配体共价附着至检测标记物的。

167. 权利要求166的半胱氨酸改造抗体，其中所述螯合配体选自DOTA、DOTP、DOTMA、DTPA和TETA。

168. 权利要求23的抗体，其中该抗体是包含一个或多个具有在0.6至1.0范围内的硫醇反应性值的游离半胱氨酸氨基酸的半胱氨酸改造抗体，其中该半胱氨酸改造抗体是通过包括用半胱氨酸替代亲本抗体的一个或多个

氨基酸残基的方法制备的。

169. 权利要求168的半胱氨酸改造抗体，其中该半胱氨酸改造抗体比亲本抗体更能与硫醇反应性试剂起反应。

170. 权利要求168的半胱氨酸改造抗体，其中所述方法进一步包括通过使半胱氨酸改造抗体与硫醇反应性试剂起反应来测定该半胱氨酸改造抗体的硫醇反应性；其中该半胱氨酸改造抗体比亲本抗体更能与硫醇反应性试剂起反应。

171. 权利要求168的半胱氨酸改造抗体，其中所述一个或多个游离半胱氨酸氨基酸残基是位于轻链中的。

172. 权利要求168的半胱氨酸改造抗体，其中该抗体是免疫偶联物，其包含共价附着至细胞毒剂的该半胱氨酸改造抗体。

173. 权利要求172的半胱氨酸改造抗体，其中所述细胞毒剂选自毒素、化疗剂、药物模块、抗生素、放射性同位素和核溶酶。

174. 权利要求168的半胱氨酸改造抗体，其中该抗体是共价附着至捕获标记物、检测标记物或固体支持物的。

175. 权利要求174的半胱氨酸改造抗体，其中该抗体是共价附着至生物素捕获标记物的。

176. 权利要求174的半胱氨酸改造抗体，其中该抗体是共价附着至荧光染料检测标记物的。

177. 权利要求176的半胱氨酸改造抗体，其中所述荧光染料选自荧光素类、罗丹明类、丹酰、丽丝胺、花青、藻红蛋白、德克萨斯红和它们的类似物。

178. 权利要求174的半胱氨酸改造抗体，其中该抗体是共价附着至放射性核素检测标记物的，所述放射性核素检测标记物选自^{3}H、^{11}C、^{14}C、^{18}F、^{32}P、^{35}S、^{64}Cu、^{68}Ga、^{86}Y、^{99}Tc、^{111}In、^{123}I、^{124}I、^{125}I、^{131}I、^{133}Xe、^{177}Lu、^{211}At和^{213}Bi。

179. 权利要求178的半胱氨酸改造抗体，其中该抗体是通过螯合配体共价附着至检测标记物的。

180. 权利要求179的半胱氨酸改造抗体，其中所述螯合配体选自DOTA、DOTP、DOTMA、DTPA和TETA。

181. 权利要求93的免疫偶联物，其中所述接头是经所述抗体上的硫醇基团附

着至该抗体的。

182. 权利要求1的抗体，其包含清蛋白结合肽。

183. 权利要求182的抗体，其中所述清蛋白结合肽选自SEQ ID NO:42-46。

184. 权利要求23的抗体，其包含清蛋白结合肽。

185. 权利要求184的抗体，其中所述清蛋白结合肽选自SEQ ID NO:42-46。

186. 权利要求1的抗体，其中该抗体进一步包含在一个或多个选自下组的位置上的半胱氨酸：依照Kabat编号规则的轻链第15位、第43位、第110位、第144位、第168位和第205位和依照EU编号规则的重链第41位、第88位、第115位、第118位、第120位、第171位、第172位、第282位、第375位和第400位。

187. 权利要求186的抗体，其中半胱氨酸位于轻链第205位。

188. 权利要求186的抗体，其中半胱氨酸位于重链第118位。

189. 权利要求186的抗体，其中半胱氨酸位于重链第400位。

190. 权利要求186的抗体，其中该抗体选自单克隆抗体、双特异性抗体、嵌合抗体、人抗体和人源化抗体。

191. 权利要求186的抗体，其是抗体片段。

192. 权利要求191的抗体，其中所述抗体片段是Fab片段。

193. 权利要求186的抗体，其选自嵌合抗体、人抗体或人源化抗体。

194. 权利要求186的抗体，其是在细菌中产生的。

195. 权利要求186的抗体，其是在CHO细胞中产生的。

196. 一种测定CD22蛋白在怀疑含所述蛋白质的样品中存在的方法，所述方法包括使所述样品暴露于权利要求186的抗体，和测定所述抗体对所述样品中的所述CD22蛋白的结合，其中所述抗体对所述蛋白质的结合表明所述样品中存在所述蛋白质。

197. 权利要求196的方法，其中所述样品包含怀疑表达所述CD22蛋白的细胞。

198. 权利要求196的方法，其中所述细胞是B细胞。

199. 权利要求196的方法，其中所述抗体是共价附着至标记物的，所述标记物选自荧光染料、放射性同位素、生物素或金属螯合配体。

200. 一种药物配制剂，其包含权利要求186的抗CD22抗体，和药学可接受的稀释剂、载体或赋形剂。