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Claims EN 1, 8, 25

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(22) Date of filing: **25.10.2016**

(54) **ANTIBODY CONJUGATES COMPRISING TOLL-LIKE RECEPTOR AGONIST**

ANTIKÖRPERKONJUGATE MIT TOLL-LIKE-REZEPTORAGONIST

CONJUGUÉS D'ANTICORPS COMPRENANT UN AGONISTE DU RÉCEPTEUR DE TYPE TOLL

(84) Designated Contracting States:
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GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO
PL PT RO RS SE SI SK SM TR
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(56) References cited:
WO-A1-2014/056953 WO-A1-2015/168279
US-A1- 2012 231 023

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Description

[0001] This application claims the benefit of U.S. Provisional Application No. 62/247896, filed 29 October 2015.

5 **SEQUENCE LISTING**

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format. Said ASCII copy, created on October 5, 2016, is named PAT057064-WO-PCT_SL.txt and is 55,525 bytes in size.

10 **FIELD OF THE INVENTION**

[0003] The invention provides antibody conjugates comprising toll-like receptor agonists and the use of such conjugates for the treatment of cancer.

15 **BACKGROUND OF THE INVENTION**

[0004] Innate immunity is a rapid nonspecific immune response that fights against environmental insults including pathogens such as bacteria or viruses. Adaptive immunity is a slower but more specific immune response, which confers long-lasting or protective immunity to the host and involves differentiation and activation of naive T lymphocytes into CD4+ T helper cells and/or CD8+ cytotoxic T cells, to promote cellular and humoral immunity. Antigen presentation cells of the innate immune system, such as dendritic cells or macrophages, serve as a critical link between the innate and adaptive immune systems by phagocytosing and processing the foreign antigens and presenting them on the cell surface to the T cells, thereby activating T cell response.

[0005] Toll-like receptors (TLRs) are pattern recognition receptors (PRR) that are expressed predominantly on dendritic cells, macrophages, monocytes, natural killer cells, and T lymphocytes. TLRs bind to pathogen-associated molecular patterns (PAMPS) from bacteria, fungi, protozoa and viruses, and act as a first line of defense against invading pathogens. TLR activation leads to increased antigen uptake, maturation, and T-cell stimulatory capacity of the dendritic cells. TLRs comprise an extracellular N-terminal leucine-rich repeat (LRR) domain, followed by a cysteine-rich region, a transmembrane domain, and an intracellular (cytoplasmic) tail that contains a conserved region named the Toll/IL-1 receptor (TIR) domain. The LRR domain is important for ligand binding and associated signaling and is a common feature of PRRs. The TIR domain is important in protein-protein interactions and is associated with innate immunity. TLRs are essential to induce expression of genes involved in inflammatory responses, and play critical roles in the development of antigen-specific acquired immunity.

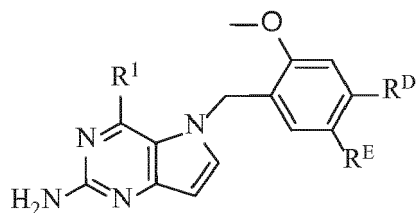
[0006] Whilst TLR agonists have been described previously in US2012/231023 A1, which discloses an adjuvant composition comprising an anti-dendritic cell (DC)-specific antibody conjugated to a TLR agonist, the TLR agonists differ structurally to those disclosed herein at several points. Furthermore, US2012/231023 A1 does not disclose a conjugate wherein the antibody binds specifically to human Her2.

[0007] There remains a need for new immunotherapies for the treatment of diseases, in particular cancer.

40 **SUMMARY OF THE INVENTION**

[0008] The invention provides antibody conjugates comprising toll-like receptor agonists, pharmaceutically acceptable salts thereof, pharmaceutical compositions thereof and combinations thereof, which are useful for the treatment of diseases, in particular, cancer. The invention further provides an effective amount of an antibody conjugate of the invention for use in treating, preventing, or ameliorating cancer in a subject in need thereof. The invention also provides compounds comprising TLR7 agonists and a linker which are useful to conjugate to an anti-HER2 antibody and thereby make the immunostimulatory conjugates of the invention. Various embodiments of the invention are described herein.

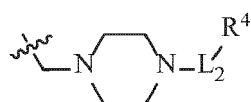
[0009] In one aspect of the invention are compounds having the structure of Formula (I), and the pharmaceutically acceptable salts thereof, which are TLR7 agonists:



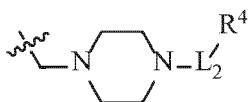
Formula (I)

wherein:

R^D is



and R^E is H; or R^E is



and R^D is H;

R¹ is -NHR² or -NHCHR²R³;

R² is -C₃-C₆alkyl or -C₄-C₆alkyl;

R³ is L₁OH;

L₁ is -(CH₂)_m-;

L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_n-,

-(CH₂)_nNHC(=O)(CH₂)_nC(=O)NH(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)(CH₂)_n-,

-C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,

-C(=O)((CH₂)_nO)_t(CH₂)_nC(=O)NH(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,

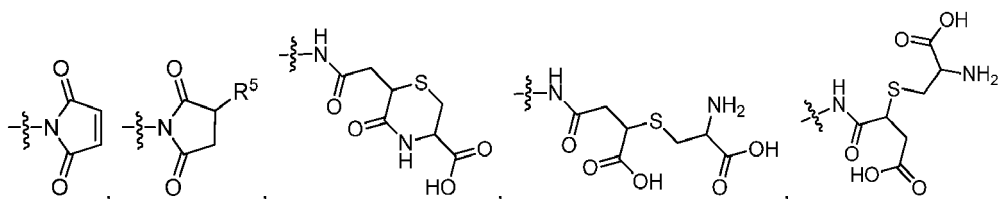
-C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)X₂X₃C(=O)(CH₂)_n-,

-C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)X₂C(=O)(CH₂)_nNHC(=O)((CH₂)_nO)_t(CH₂)_n-,

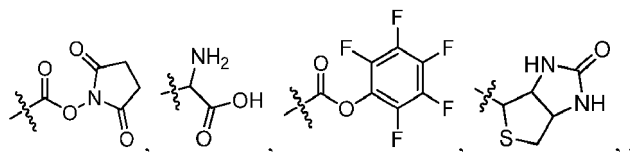
-C(=O)(CH₂)_nC(R₇)₂-, -C(=O)(CH₂)_nC(R₇)₂SS(CH₂)_nNHC(=O)(CH₂)_n-,

-(CH₂)_nX₂C(=O)(CH₂)_nNHC(=O)((CH₂)_nO)_t(CH₂)_n- or -C(=O)(CH₂)_nC(=O)NH(CH₂)_n;

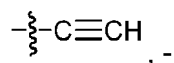
R⁴ is



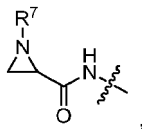
-ONH₂, -NH₂,



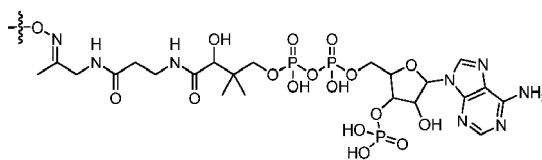
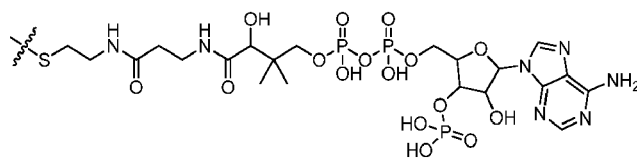
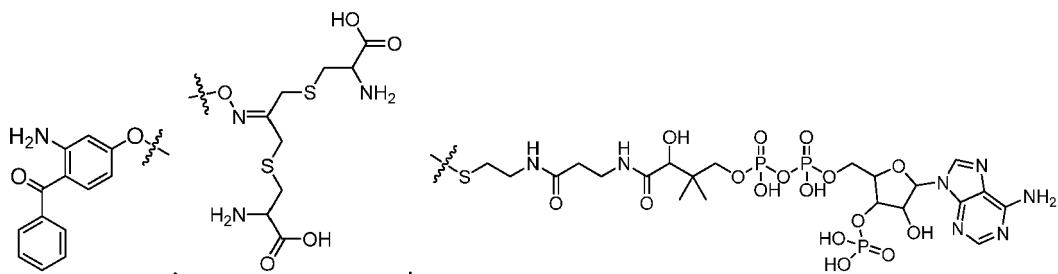
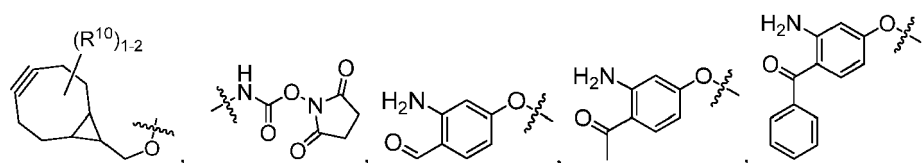
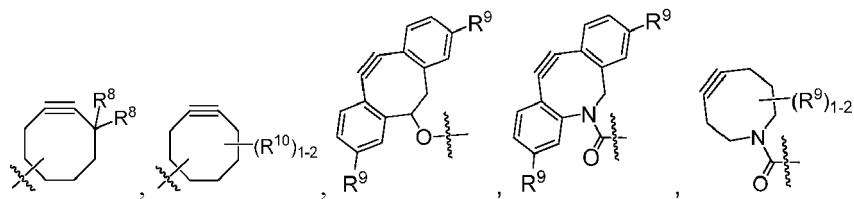
-N₃,



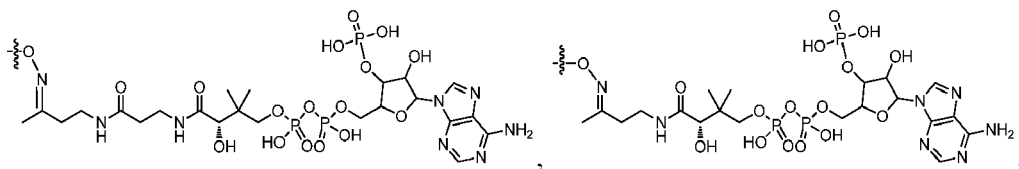
NHC(=O)CH=CH₂, -SH, -SR⁷, -OH, -SSR⁶, -S(=O)₂(CH=CH₂), -(CH₂)₂S(=O)₂(CH=CH₂), -NHS(=O)₂(CH=CH₂),
-NHC(=O)CH₂Br, -NHC(=O)CH₂I, -C(O)NHNH₂,



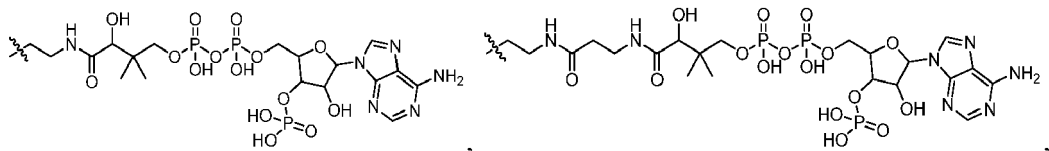
-CO₂H, -C(O)NHNH₂,



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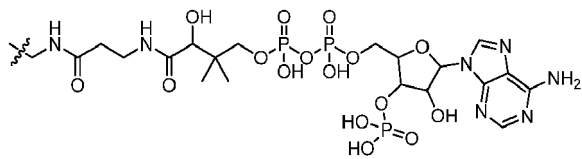
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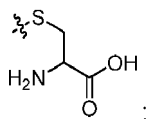
or

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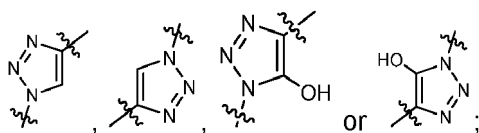
25

R⁵ is



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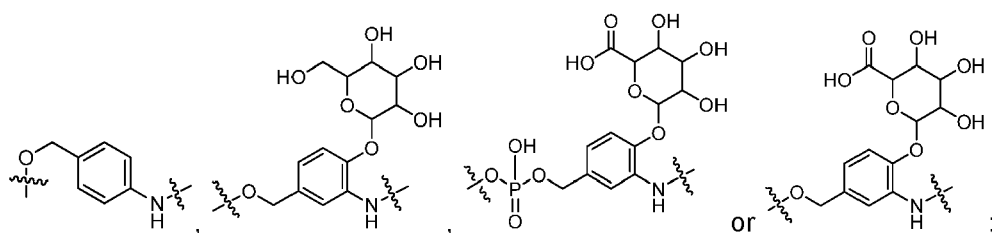
X₁ is



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X₂ is

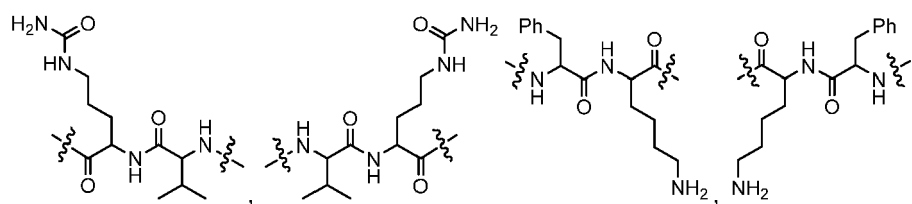
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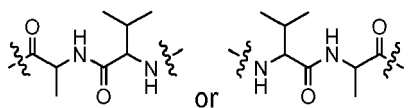
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X₃ is



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R^6 is 2-pyridyl or 4-pyridyl;

each R^7 is independently selected from H and C_1 - C_6 alkyl;

each R^8 is independently selected from H, C_1 - C_6 alkyl, F, Cl, and -OH;

each R^9 is independently selected from H, C_1 - C_6 alkyl, F, Cl, -NH₂, -OCH₃, -OCH₂CH₃, -N(CH₃)₂, -CN, -NO₂ and -OH;

each R^{10} is independently selected from H, C_1 - C_6 alkyl, fluoro, benzyloxy substituted with -C(=O)OH, benzyl substituted with -C(=O)OH, C_{1-4} alkoxy substituted with -C(=O)OH and C_{1-4} alkyl substituted with -C(=O)OH;

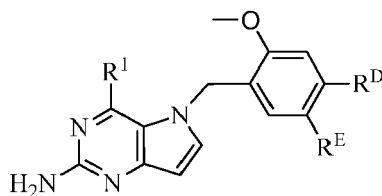
each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

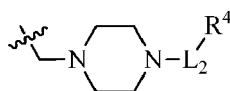
[0010] In one aspect of the invention are compounds having the structure of Formula (I), and the pharmaceutically acceptable salts thereof, which are TLR7 agonists:



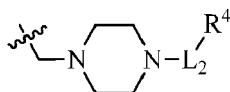
Formula (I)

wherein:

R^D is



and R^E is H; or R^E is



and R^D is H;

R^1 is -NHR² or -NHCHR²R³;

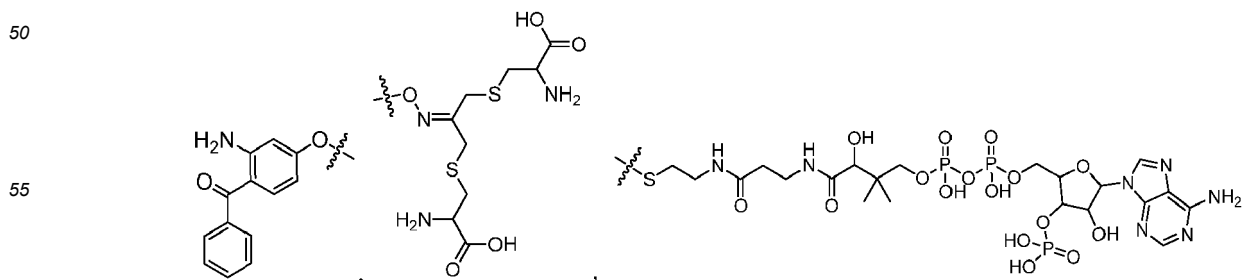
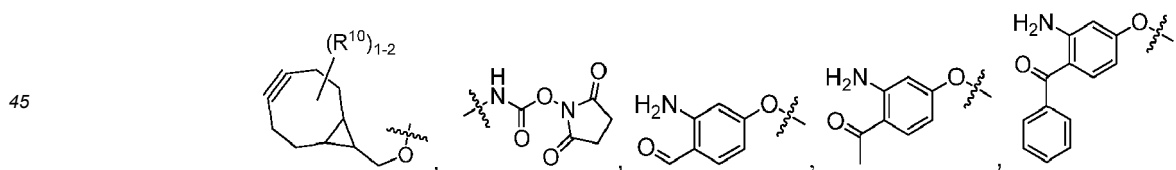
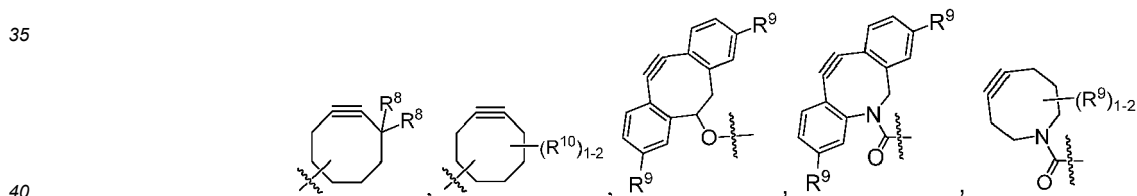
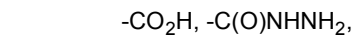
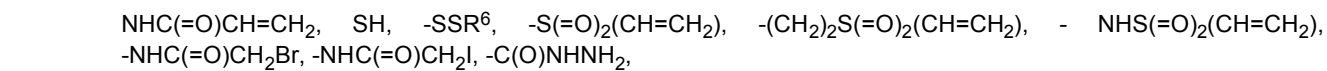
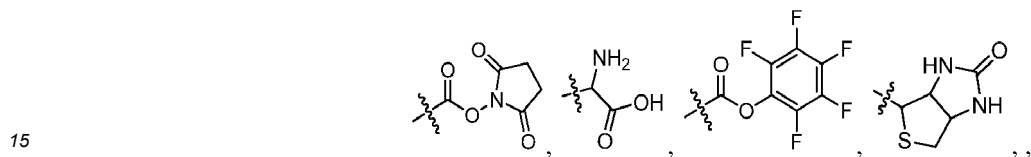
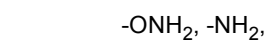
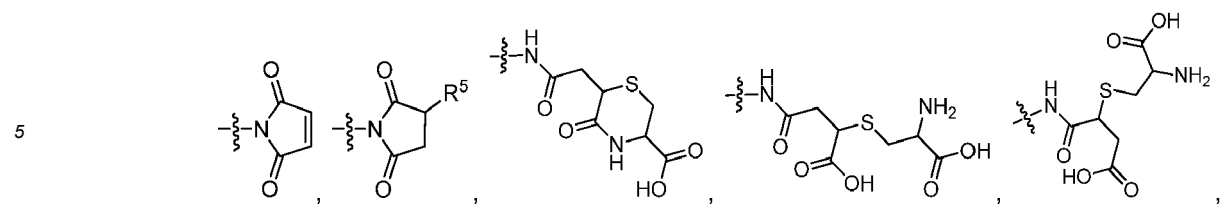
R^2 is -C₃-C₆alkyl or -C₄-C₆alkyl;

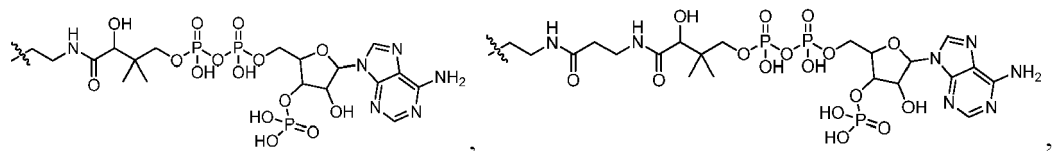
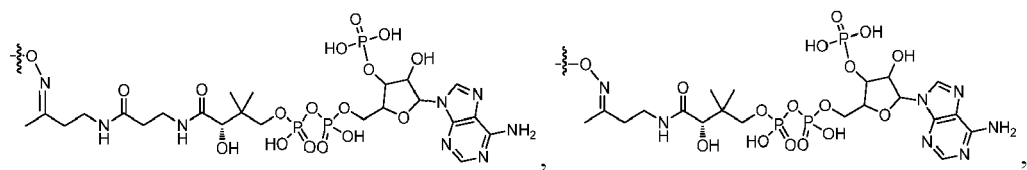
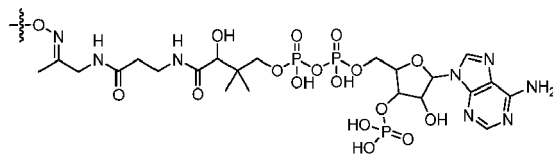
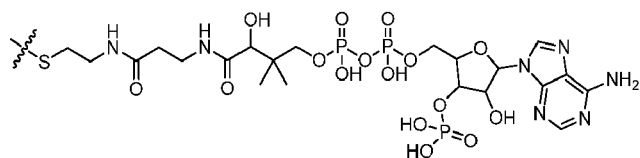
R^3 is L₁OH;

L₁ is -(CH₂)_m-;

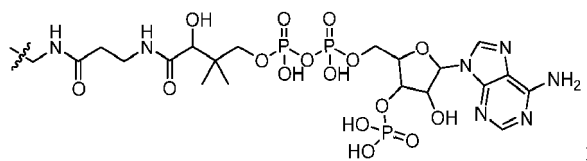
L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_n-,
 -(CH₂)_nNHC(=O)(CH₂)_nC(=O)NH(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)(CH₂)_n-,
 -C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,
 -C(=O)((CH₂)_nO)_t(CH₂)_nC(=O)NH(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,
 -C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-, or -C(=O)(CH₂)_nC(=O)NH(CH₂)_n;

R^4 is

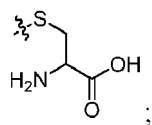




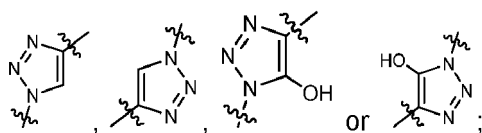
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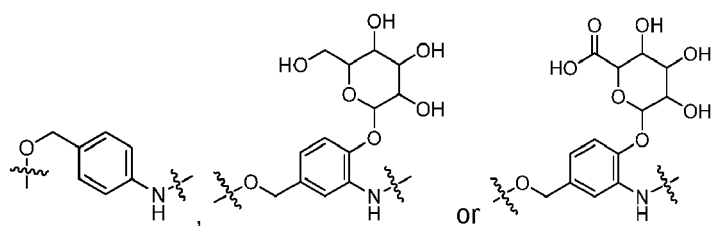
R⁵ is



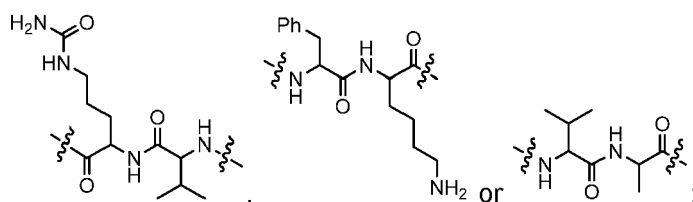
X₁ is



X₂ is



X_3 is



R^6 is 2-pyridyl or 4-pyridyl;

each R^7 is independently selected from H and C_1 - C_6 alkyl;

each R^8 is independently selected from H, C_1 - C_6 alkyl, F, Cl, and -OH;

each R^9 is independently selected from H, C_1 - C_6 alkyl, F, Cl, - NH_2 , - OCH_3 , - OCH_2CH_3 , - $N(CH_3)_2$, -CN, - NO_2 and -OH;

each R^{10} is independently selected from H, C_1 - C_6 alkyl, fluoro, benzyloxy substituted with -C(=O)OH, benzyl substituted

with -C(=O)OH, C_{1-4} alkoxy substituted with -C(=O)OH and C_{1-4} alkyl substituted with -C(=O)OH;

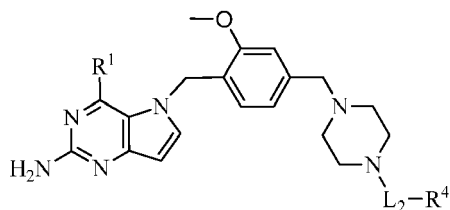
each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

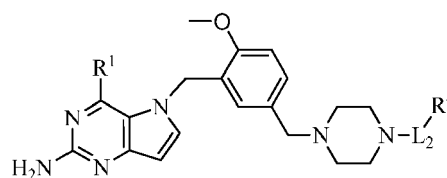
and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

[0011] In one aspect of the invention are compounds of Formula (I) having the structure of Formula (Ia) or Formula (Ib), and the pharmaceutically acceptable salts thereof:



Formula (Ia)



Formula (Ib)

wherein:

R^1 is -NHR² or -NHCHR²R³;

R^2 is - C_3 - C_6 alkyl or - C_4 - C_6 alkyl;

R^3 is L_1OH ;

L_1 is $-(CH_2)_m$;

L_2 is $-(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_n$, $-(CH_2)_nX_1(CH_2)_n$, $-(CH_2)_nNHC(=O)(CH_2)_n$,

$-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$, $-C(=O)(CH_2)_n$,

$-C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$,

$-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$,

$-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)X_2X_3C(=O)(CH_2)_n$,

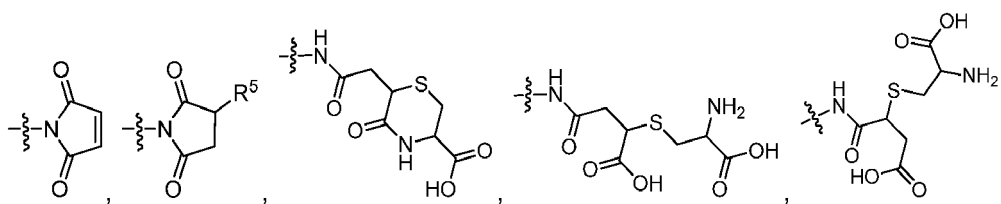
$-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n$,

$-C(=O)(CH_2)_nC(R_7)_2$, $-C(=O)(CH_2)_nC(R_7)_2SS(CH_2)_nNHC(=O)(CH_2)_n$,

$-(CH_2)_nX_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n$ or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

R^4 is

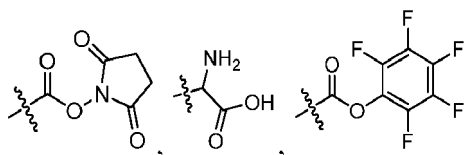
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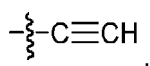
-ONH₂, -NH₂,

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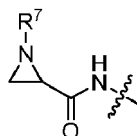
-NHC(=O)CH=CH₂, -N₃,



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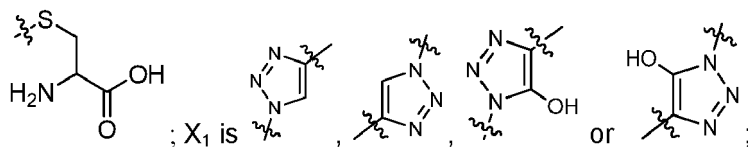
SH, -SR⁷, -OH, -SSR⁶, -S(=O)₂(CH=CH₂), -(CH₂)₂S(=O)₂(CH=CH₂), -NHS(=O)₂(CH=CH₂), -NHC(=O)CH₂Br, -NHC(=O)CH₂I, -C(O)NHNH₂,

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or -CO₂H;
R⁵ is

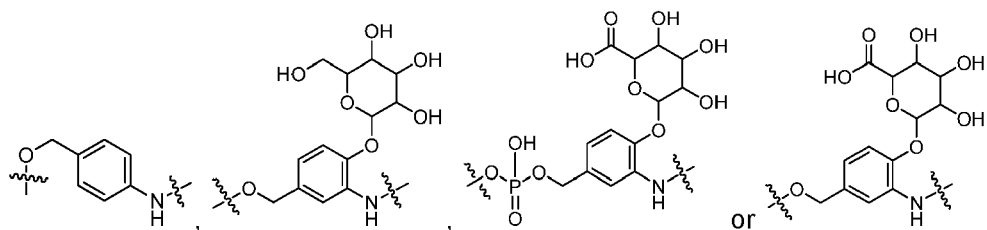
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X₂ is

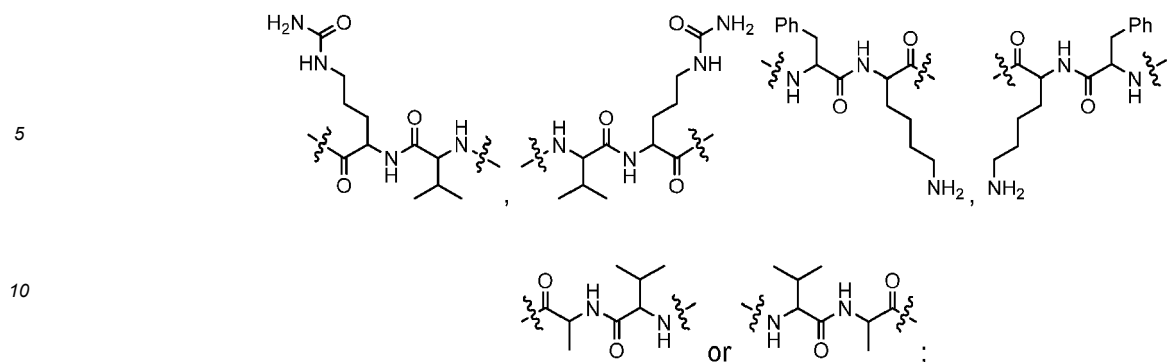
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X₃ is

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R^6 is 2-pyridyl or 4-pyridyl;

each R^7 is independently selected from H and C_1 - C_6 alkyl;

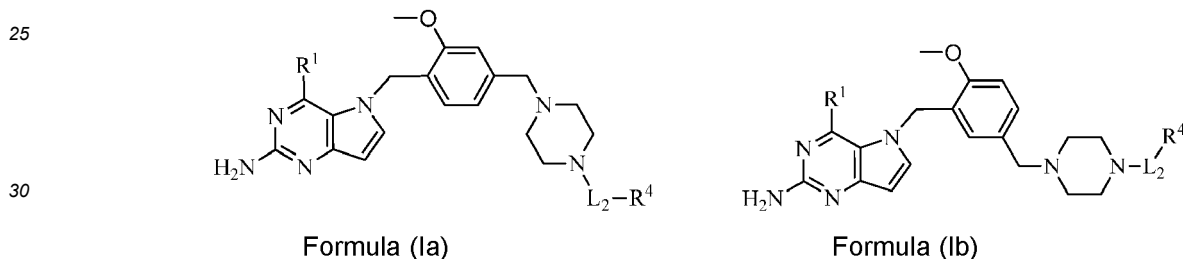
each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

[0012] In one aspect of the invention are compounds of Formula (I) having the structure of Formula (Ia) or Formula (Ib), and the pharmaceutically acceptable salts thereof:



wherein:

R^1 is $-NHR^2$ or $-NHCHR^2R^3$;

R^2 is $-C_3$ - C_6 alkyl or $-C_4$ - C_6 alkyl;

R^3 is L_1OH ;

L_1 is $-(CH_2)_m$;

L_2 is $-(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_n$, $-(CH_2)_nX_1(CH_2)_n$, $-(CH_2)_nNHC(=O)(CH_2)_n$,

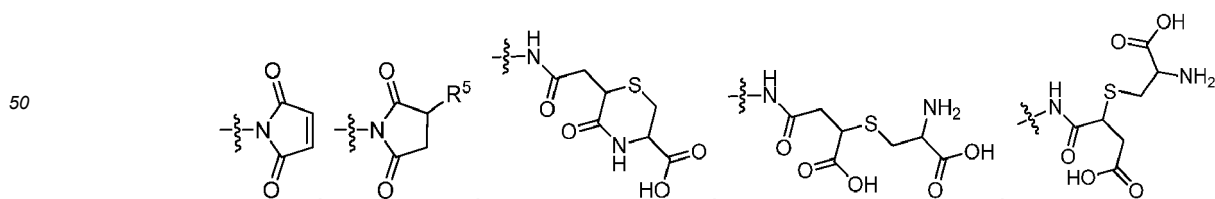
$-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$, $-C(=O)(CH_2)_n$,

$-C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$,

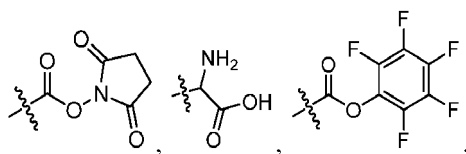
$-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$,

$-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n$, or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

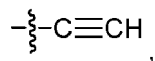
R^4 is



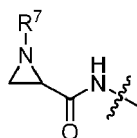
$-ONH_2$, $-NH_2$,



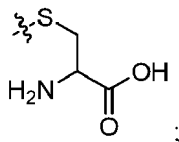
-NHC(=O)CH=CH₂, -N₃,



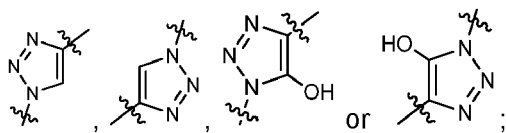
SH, -SSR⁶, -S(=O)₂(CH=CH₂), -(CH₂)₂S(=O)₂(CH=CH₂), -NHS(=O)₂(CH=CH₂), -NHC(=O)CH₂Br, -NHC(=O)CH₂I, -C(O)NHNH₂,



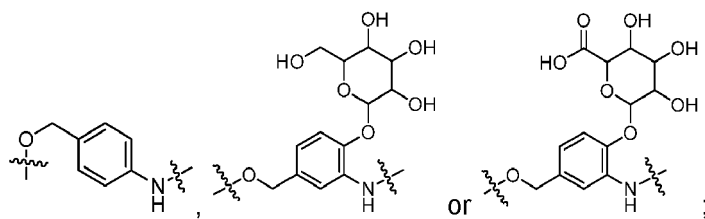
or -CO₂H;
R⁵ is



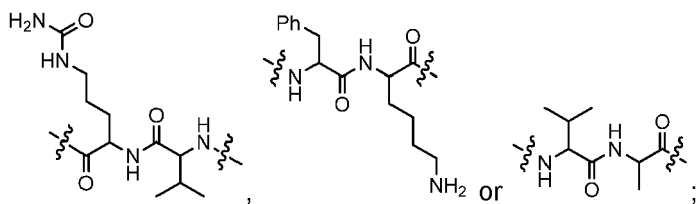
X₁ is



X₂ is



X₃ is



R^6 is 2-pyridyl or 4-pyridyl;

each R^7 is independently selected from H and C_1 - C_6 alkyl;

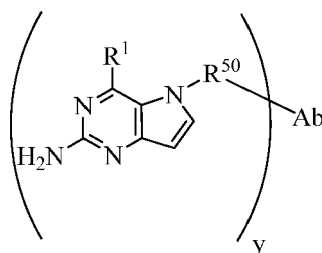
each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

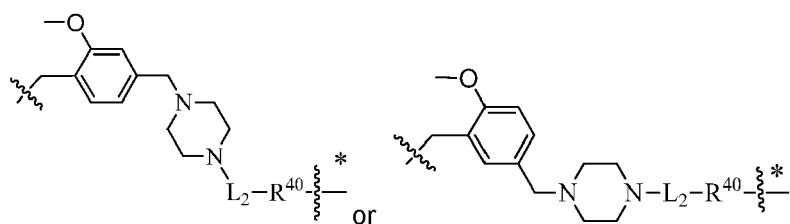
[0013] Another aspect of the invention are antibody conjugates having the structure of Formula (II), and the pharmaceutically acceptable salts thereof:



Formula (II)

wherein:

R^{50} is



where the * indicates the point of attachment to Ab;

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R^1 is $-NHR^2$ or $-NHCHR^2R^3$;

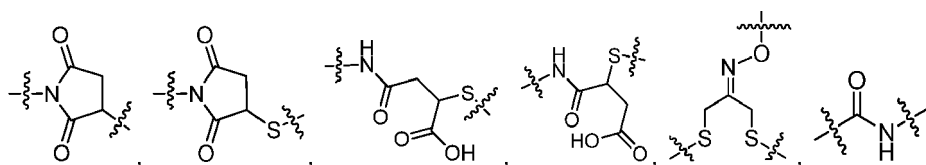
R^2 is $-C_3$ - C_6 alkyl or $-C_4$ - C_6 alkyl;

R^3 is L_1OH ;

L_1 is $-(CH_2)_m$;

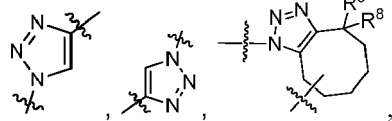
L_2 is $-(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_n$, $-(CH_2)_nX_1(CH_2)_n$, $-(CH_2)_nNHC(=O)(CH_2)_n$, $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$, $-C(=O)(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)X_2X_3C(=O)(CH_2)_n$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)(CH_2)_nC(R_7)_2$, $-C(=O)(CH_2)_nC(R_7)_2SS(CH_2)_nNHC(=O)(CH_2)_n$, $-(CH_2)_nX_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n$ or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

R^{40} is



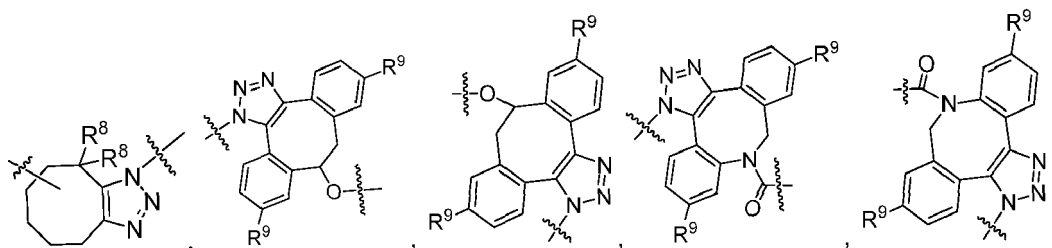
-S-, -NHC(=O)CH₂-, -S(=O)₂CH₂CH₂-, -(CH₂)₂S(=O)₂CH₂CH₂-, -NHS(=O)₂CH₂CH₂-, -NHC(=O)CH₂CH₂-,
-CH₂NHCH₂CH₂-, -NHCH₂CH₂-,

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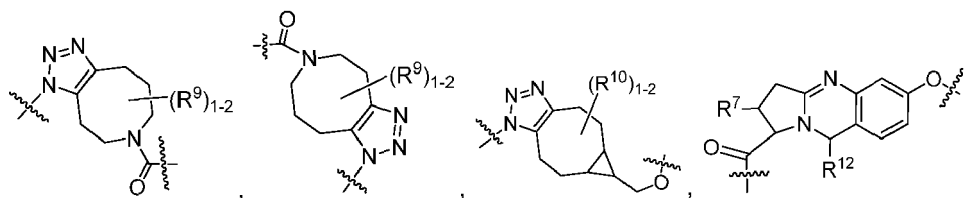
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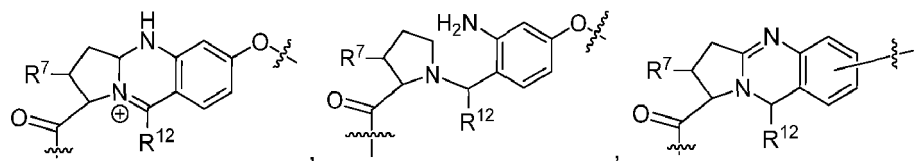


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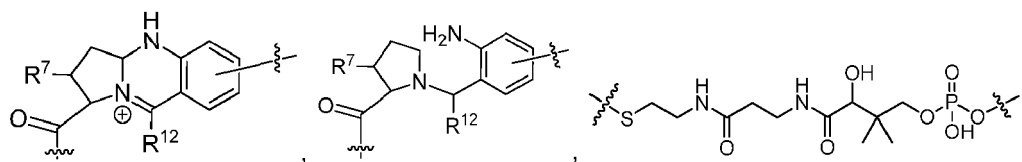


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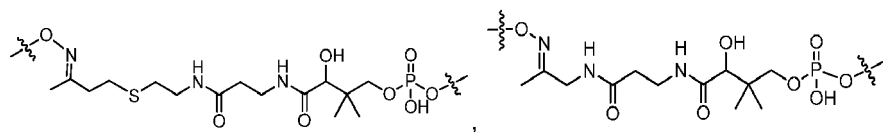


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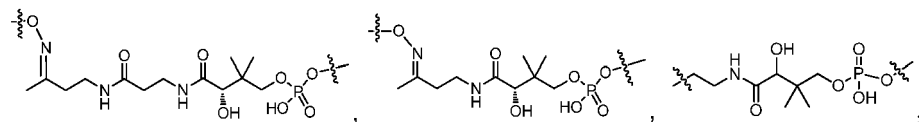
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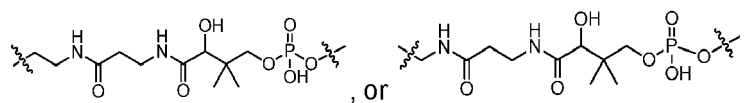
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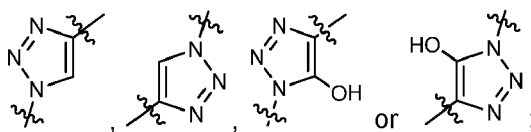
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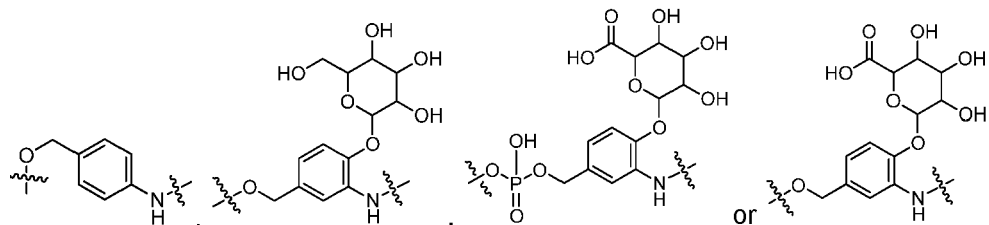
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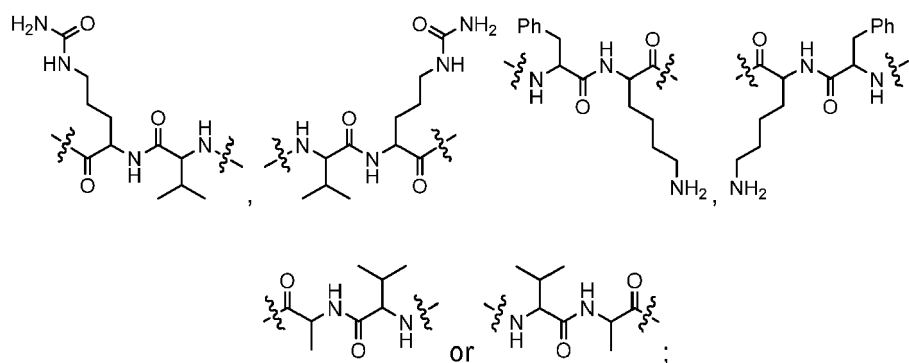
X₁ is



X₂ is



X₃ is



each R⁷ is independently selected from H and C₁-C₆alkyl;

each R⁸ is independently selected from H, C₁-C₆alkyl, F, Cl, and -OH;

each R⁹ is independently selected from H, C₁-C₆alkyl, F, Cl, -NH₂, -OCH₃, -OCH₂CH₃, -N(CH₃)₂, -CN, -NO₂ and -OH;

each R¹⁰ is independently selected from H, C₁₋₆alkyl, fluoro, benzyloxy substituted with -C(=O)OH, benzyl substituted with -C(=O)OH, C₁₋₄alkoxy substituted with -C(=O)OH and C₁₋₄alkyl substituted with -C(=O)OH;

R¹² is H, methyl or phenyl;

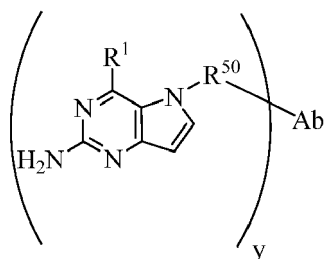
each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and

y is an integer from 1 to 16.

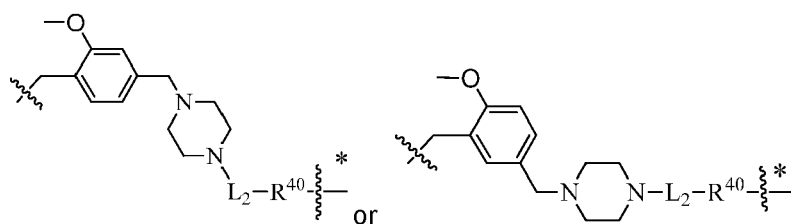
[0014] Another aspect of the invention are antibody conjugates having the structure of Formula (II), and the pharmaceutically acceptable salts thereof:



Formula (II)

wherein:

R^{50} is



where the * indicates the point of attachment to Ab;

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

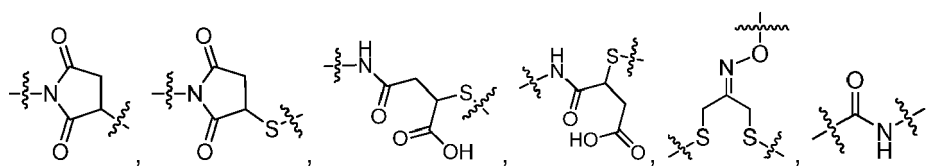
R^1 is $-NHR^2$ or $-NHCHR^2R^3$;

R^2 is $-C_3-C_6$ alkyl or $-C_4-C_6$ alkyl;

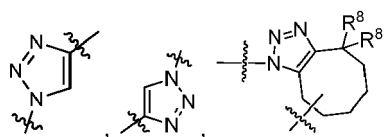
R^3 is L_1OH ;

L_1 is $-(CH_2)_m-$;

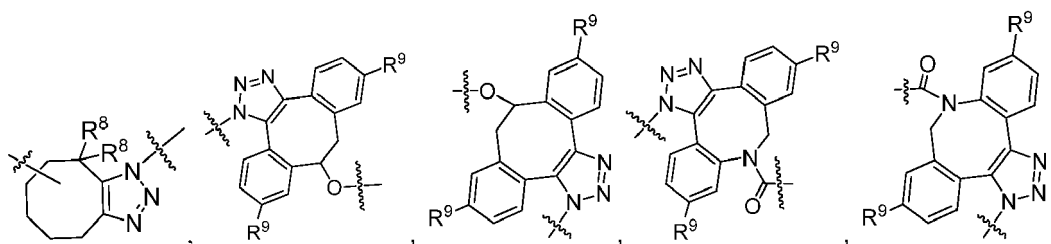
L_2 is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$,
 $-(CH_2)_nNHC(=O)(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$,
 $-C(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_n-$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$,
 $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;
 R^{40} is



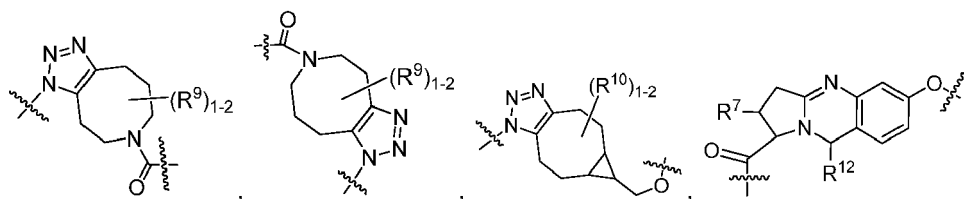
$-S-$, $-NHC(=O)CH_2-$, $-S(=O)_2CH_2CH_2-$, $-(CH_2)_2S(=O)_2CH_2CH_2-$, $-NHS(=O)_2CH_2CH_2-$, $-NHC(=O)CH_2CH_2-$,
 $-CH_2NHCH_2CH_2-$, $-NHCH_2CH_2-$,



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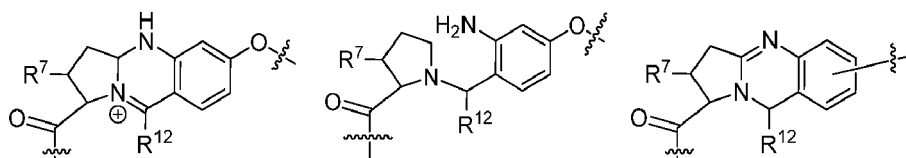


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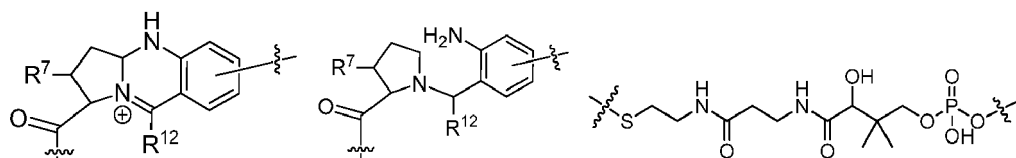


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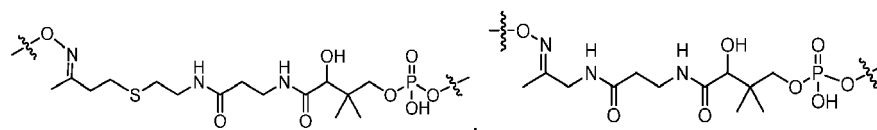
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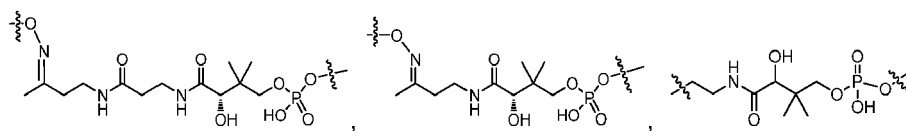
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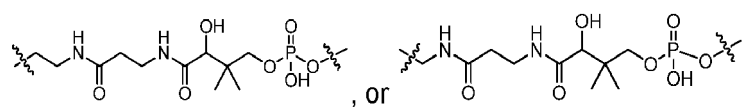
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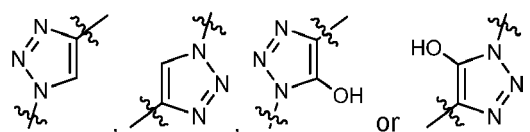
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X₁ is

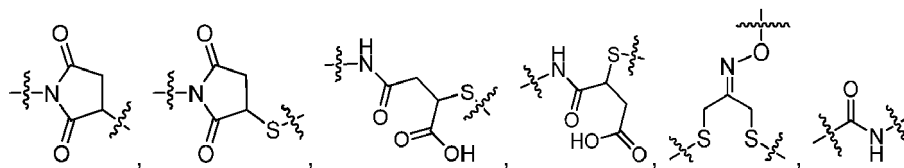
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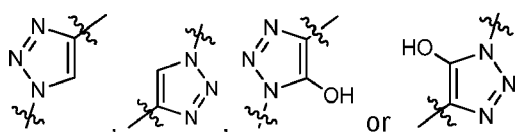
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X₂ is

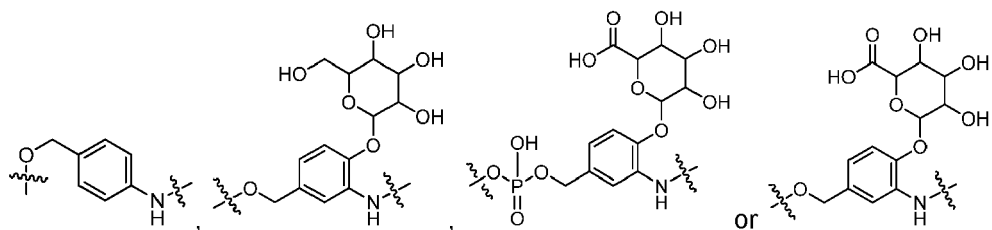
$-\text{C}(=\text{O})(\text{CH}_2)_n\text{C}(\text{R}_7)_2-\text{C}(=\text{O})(\text{CH}_2)_n\text{C}(\text{R}_7)_2\text{SS}(\text{CH}_2)_n\text{NHC}(=\text{O})(\text{CH}_2)_n-$,
 $-(\text{CH}_2)_n\text{X}_2\text{C}(=\text{O})(\text{CH}_2)_n\text{NHC}(=\text{O})((\text{CH}_2)_n\text{O})_t(\text{CH}_2)_n-$ or $-\text{C}(=\text{O})(\text{CH}_2)_n\text{C}(=\text{O})\text{NH}(\text{CH}_2)_n-$;
 R^{40} is



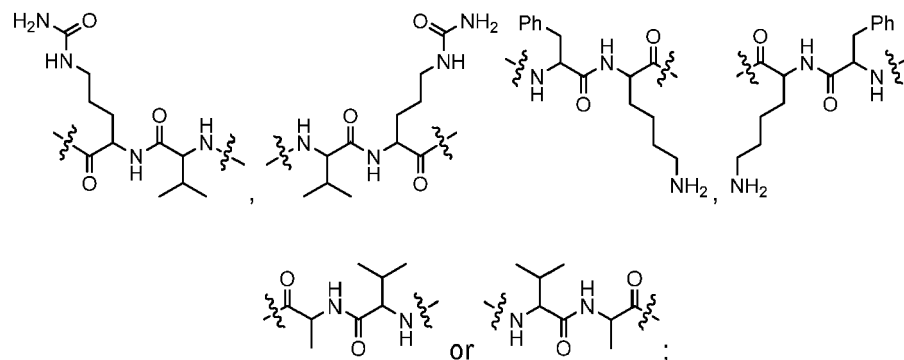
or $-\text{S}-$;
 X_1 is



X_2 is



X_3 is



each R^7 is independently selected from H and $\text{C}_1\text{-C}_6$ alkyl;

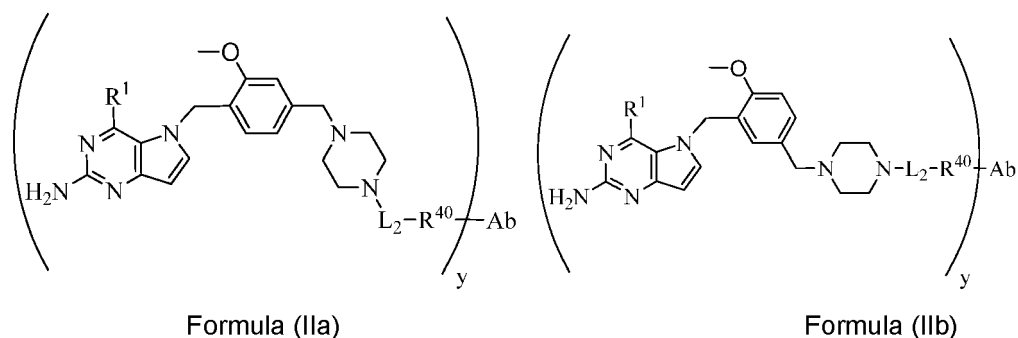
each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and

y is an integer from 1 to 16.

[0016] Another aspect of the invention are antibody conjugates of Formula (II) having the structure of Formula (IIa) or Formula (IIb), and the pharmaceutically acceptable salts thereof:



wherein:

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R¹ is -NHR² or -NHCHR²R³;

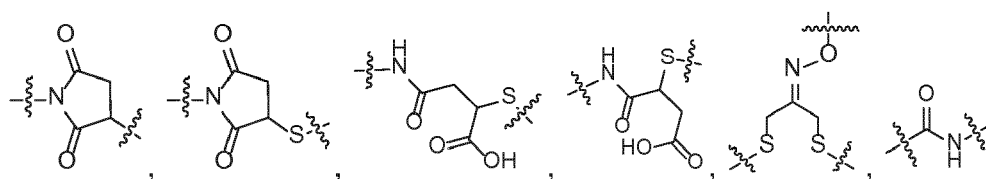
R² is -C₃-C₆alkyl or -C₄-C₆alkyl;

R³ is L₁OH;

L₁ is -(CH₂)_m-;

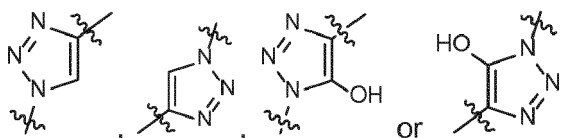
L₂ is -(CH₂)_n-, -((CH₂)_nO)(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_n-,
 -(CH₂)_nNHC(=O)(CH₂)_nC(=O)NH(CH₂)_n-, -((CH₂)_nO)(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)(CH₂)_n-,
 -C(=O)((CH₂)_nO)(CH₂)_n-, -C(=O)((CH₂)_nO)(CH₂)_nX₁(CH₂)_n-, -C(=O)((CH₂)_nO)(CH₂)_nNHC(=O)(CH₂)_n-,
 -C(=O)((CH₂)_nO)(CH₂)_nC(=O)NH(CH₂)_n-, -C(=O)NH((CH₂)_nO)(CH₂)_nX₁(CH₂)_n-,
 -C(=O)X₂X₃C(=O)((CH₂)_nO)(CH₂)_n-, -C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-, or -C(=O)(CH₂)_nC(=O)NH(CH₂)_n;

R⁴⁰ is

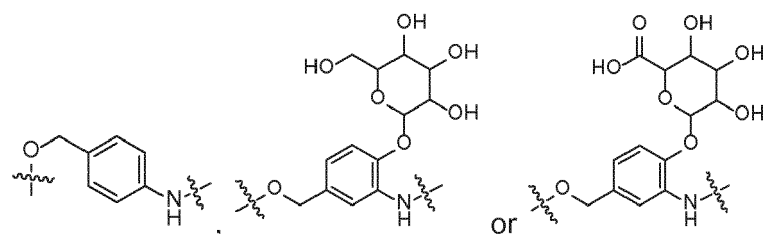


or -S-;

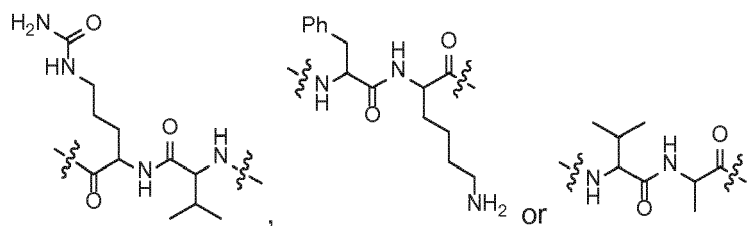
X₁ is



X₂ is



X₃ is



each R^7 is independently selected from H and C_1 - C_6 alkyl;
 each m is independently selected from 1, 2, 3, and 4;
 each n is independently selected from 1, 2, 3, and 4;
 each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and
 y is an integer from 1 to 16.

[0017] Another aspect of the invention is a pharmaceutical composition that includes a therapeutically effective amount of an antibody conjugate of Formula (II), Formula (IIa) or Formula (Iib), or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[0018] Another aspect of the invention is use of an effective amount of antibody conjugate of Formula (II), Formula (IIa) or Formula (Iib), or pharmaceutically acceptable salt thereof for treating a HER2-positive cancer, in a subject in need of such treatment. A HER2-positive cancer can be any of gastric cancer, esophageal cancer, gastroesophageal junction adenocarcinoma, colon cancer, rectal cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, endometrial cancer, bladder cancer, urinary tract cancer, pancreatic cancer, lung cancer, prostate cancer, osteosarcoma, neuroblastoma, glioblastoma, and head and neck cancer. A HER2-positive cancer can have high HER2 expression (e.g., having 3+ IHC score), or low HER2 expression (e.g., having 2+ IHC score).

[0019] Another aspect of the invention is the use of an antibody conjugate of Formula (II), Formula (IIa) or Formula (Iib), or pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treating a HER2-positive cancer.

[0020] Another aspect of the invention is an antibody conjugate for use in a method of medical treatment, wherein the method of medical treatment is for treating a HER2-positive cancer, and wherein the antibody conjugate is an antibody conjugate of Formula (II), Formula (IIa) or Formula (Iib), or pharmaceutically acceptable salt thereof. In addition, a further aspect of the invention is an antibody conjugate for use in a method of suppressing a HER2-positive cancer for a sustained period and/or reducing recurrence of a HER2-positive cancer, when compared to an anti-HER2 antibody alone.

[0021] The antibody conjugates described herein can be used to treat not only high HER2-expressing tumors (e.g., having 3+ IHC scores), but also lower HER2-expressing tumors (e.g., having 2+ IHC scores).

BRIEF DESCRIPTION OF THE DRAWINGS

[0022]

FIG. 1 depicts results following a single treatment of anti-HER2-mAb2-(C-1) conjugate in the N87 xenograft tumor model. Regression of tumor was observed for all doses tested, including 1 mg/kg (filled diamond), 2.5 mg/kg (filled triangle), 5 mg/kg (filled circle), and 10 mg/kg (filled square) when compared to untreated animals (open circle). Regression of N87 gastric tumors was not observed in the N87 xenograft mice treated with 10 mg/kg of unconjugated anti-HER2-mAb2 alone (open triangle), or an isotype control antibody-(C-1) conjugate (open diamond) when compared to untreated animals (open circle). Data represent mean tumor volumes (mean $\pm$ SEM) over time (post-dose).

FIG. 2 depicts results following treatment of human N87 xenograft tumors with a single dose of anti-HER2-mAb1-(C-1) or anti-HER2-mAb1-(C-5). Regression of human N87 xenograft tumors was observed after treatment with 1 mg/kg of anti-HER2-mAb1-(C-1) (filled square) or 1 mg/kg of anti-HER2-mAb1-(C-5) (filled triangle), while treatment with 0.3 mg/kg of anti-HER2-mAb1-(C-1) (filled circle) or 0.3 mg/kg of anti-HER2-mAb1-(C-5) (filled diamond) resulted in tumor stasis, when compared to untreated animals (open circle). Regression of N87 gastric tumors was not observed in the N87 xenograft mice treated with an isotype control antibody-(C-5) conjugate (open diamond) when compared to untreated animals (open circle). Data represent mean tumor volumes (mean $\pm$ SEM) over time (post-dose).

FIG. 3 depicts results following treatment of human N87 xenograft tumors with a single dose of anti-HER2-mAb1-(C-5). Regression of human N87 xenograft tumors was observed after treatment with 5 mg/kg of anti-HER2-mAb1-(C-5) (filled square) or 3 mg/kg of anti-HER2-mAb1-(C-5) (filled circle), while treatment with 1 mg/kg of anti-HER2-

mAb1-(C-5) (filled triangle) resulted in tumor stasis, when compared to untreated animals (open circle). Data represent mean tumor volumes (mean $\pm$ SEM) over time (post-dose).

FIG. 4 depicts results following treatment of human N87 xenograft tumors with a single dose of anti-HER2-mAb1 conjugated with different compounds. Initial regression, followed by stasis of human N87 xenograft tumors was observed after treatment with 1 mg/kg of anti-HER2-mAb1-(C-5) (filled triangles), anti-HER2-mAb1-(C-60) (open triangles), anti-HER2-mAb1-(C-59) (filled square), anti-HER2-mAb1-(C-61) (open square), anti-HER2-mAb1-(C-35) (filled hexagon), anti-HER2-mAb1-(C-37) (open hexagon), anti-HER2-mAb1-(C-64) (filled diamond) or anti-HER2-mAb1-(C-62) (open diamond), when compared to untreated animals (open circle). Data represent mean tumor volumes (mean $\pm$ SEM) over time (post-dose).

FIGs. 5A and 5B depict the results of treatment of MMC mouse breast tumors (ratHER2-positive) with a single dose of anti-ratHER2-(C-46) conjugate. Results demonstrate complete tumor regression was observed in seven out of eight mice treated with anti-ratHER2-(C-46) conjugate (FIG. 5A), but only in three out of eight mice treated with the naked anti-ratHER2 antibody (FIG. 5B). Treatment was initiated when tumors reached an average size of 200 mm³ in MMC breast cancer syngeneic model. Data represent mean tumor volumes (mean $\pm$ SEM) over time (post-dose).

FIG. 6 depicts results following treatment of human HCC1954 breast xenograft tumors with a single dose of anti-HER2-mAb1-(C-5). Regression of human HCC1954 xenograft tumors was observed after treatment with 10 mg/kg of anti-HER2-mAb1-(C-5) (filled square) or 3 mg/kg of anti-HER2-mAb1-(C-5) (filled circle), while treatment with 1 mg/kg of anti-HER2-mAb1-(C-5) (filled triangle) resulted in tumor stasis, when compared to untreated animals (open circle). Regression of tumors was not observed in the HCC1954 xenograft mice treated with 10 mg/kg of an isotype control antibody-(C-5) conjugate (open diamond) or unconjugated anti-HER2-mAb1 alone (open triangle) when compared to untreated animals (open circle). Data represent mean tumor volumes (mean $\pm$ SEM) over time (post-dose).

FIG. 7 depicts results following treatment of human SKOV3 ovarian xenograft tumors with a single dose of anti-HER2-mAb1-(C-5). Regression of human SKOV3 xenograft tumors was observed after treatment with 10 mg/kg of anti-HER2-mAb1-(C-5) (filled square), while treatment with 3 mg/kg of anti-HER2-mAb1-(C-5) (filled circle) resulted in initial tumor regression followed by tumor regrowth, when compared to untreated animals (open circle). Regression of tumors was not observed in the SKOV3 xenograft mice treated with 10 mg/kg of an isotype control antibody-(C-5) conjugate (open diamond) or unconjugated anti-HER2-mAb1 alone (open triangle) when compared to untreated animals (open circle). Data represent mean tumor volumes (mean $\pm$ SEM) over time (post-dose).

FIGs. 8A-8C depict representative ImmunoHistoChemistry (IHC) images showing HER2 expression on N87 (FIG. 8A), HCC1954 (FIG. 8B) and SKOV3 (FIG. 8C) xenografts tumors. Tumors were scored based on their HER2 expression level as 3+ (N87 and HCC1954) and 2+ (SKOV3).

DETAILED DESCRIPTION OF THE INVENTION

[0023] Various enumerated embodiments of the invention are described herein. It will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present invention.

[0024] Throughout the text of this application, should there be a discrepancy between the text of the specification (e.g., Table 1) and the sequence listing, the text of the specification shall prevail.

Definitions

[0025] The term "C₄-C₆alkyl", as used herein, refers to a fully saturated branched or straight chain hydrocarbon containing 4 to 6 carbon atoms. Non-limiting examples of "C₄-C₆alkyl" groups include n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl and hexyl.

[0026] As used herein, "HER2" (also known as ERBB2; NEU; NGL; TKR1; CD340; p185; MLN19; HER-2/neu) refers to a transmembrane tyrosine kinase receptor of the epidermal growth factor (EGF) receptor family. HER2 comprises an extracellular binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. HER2 does not have a ligand binding domain of its own and therefore cannot bind growth factors, however, HER2 binds tightly to other ligand-bound EGF receptor family members such as HER1 or HER3, to form a heterodimer, stabilizing ligand binding and enhancing kinase-mediated activation of downstream signalling pathways. The human HER2/NEU gene is mapped to chromosomal location 17q12, and the genomic sequence of HER2/NEU gene can be found in GenBank at

NG_007503.1. In human, there are five HER2 isoforms: A, B, C, D, and E; the term "HER2" is used herein to refer collectively to all HER2 isoforms. As used herein, a human HER2 protein also encompasses proteins that have over its full length at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with HER2 isoforms: A, B, C, D, and E, wherein such proteins still have at least one of the functions of HER2. The mRNA and protein sequences for human HER2 isoform A, the longest isoform, are:

Homo sapiens erb-b2 receptor tyrosine kinase 2 (ERBB2), transcript variant 1, mRNA [NM_004448.3]

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1 gcttgctccc aatcacagga gaaggaggag gtggaggagg agggctgctt gaggaagtat
61 aagaatgaag ttgtgaagct gagattcccc tccattggga ccggagaaac caggggagcc
121 ccccgggcag ccgcgcgccc ctcccaagg ggccctttac tgcgccgcgc gcccgggccc
181 caccctcgc agcaccgccg gcccgcgccc ctccagcgc ggtccagccg gagccatggg
241 gccggagccg cagtgaacac catggagctg gcggccttgt gccgctgggg gtcctcctc

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301 gccctcttgc ccccgaggc cgcgagcacc caagtgtgca ccggcacaga catgaagctg
 361 cggctccctg ccagtcgga gaccacctg gacatgctcc gccaccta ccagggctgc
 421 cagggtgtgc agggaaacct ggaactcacc tacctgcca ccaatgccag cctgtccttc
 5 481 ctgcaggata tccaggaggt gcagggctac gtgctcatcg ctcaacca agtgaggcag
 541 gtccactgc agaggctgc gattgtgca ggcaccagc tcttgagga caactatgcc
 601 ctggccgtgc tagacaatgg agaccgctg aacaatacca cccctgtcac aggggctcc
 661 ccaggaggcc tgcgggagct gcagcttga agcctcacag agatctgaa aggaggggtc
 721 ttgatccagc ggaaccccca gctgtctac caggacacga tttgtggaa ggacatcttc
 781 cacaagaaca accagctggc tctcacactg atagacacca accgctctcg ggctgccac
 10 841 cctgttctc cgatgtgaa gggctccgc tctgtgggag agagtctga ggattgtcag
 901 agcctgacgc gactgtctg tgcgggtggc tgtgccgct gcaaggggc actgccact
 961 gactgtgcc atgagcagtg tctgccggc tgcagggcc ccaagcactg tactgcctg
 1021 gcctgcctcc actcaacca cagtggcatc tgtagctgc actgccagc cctgttcacc
 1081 tacaacacag acacgttga gtccatgcc aatcccgagg gccggtatac attggcgcc
 1141 agctgtgtga ctgcctgtcc ctacaactac ctttctacgg acgtgggac ctgcacccctc
 1201 gtctgcccc tgcacaacca agaggtaga gcagaggatg gaacacagcg gtgtgagaag
 1261 tgcagcaagc cctgtgccc agtgtgtat ggtctgggca tggagcactt gcgagagggtg
 1321 agggcagtta ccagtgcga tatccaggag ttgtctggct gcaagaagat cttgggagc
 1381 ctggcatttc tgcgggagag cttgatggg gaccagcct ccaacactgc cccgtccag
 20 1441 ccagagcagc tcaagtgtt tgagactctg gaagagatca caggttacct atactctca
 1501 gcatggccgg acagcctgcc tgacctcagc gtctccaga acctgcaagt aatccgggga
 1561 cgaattctgc acaatgggc ctactcgtg acctgcaag ggctgggcat cagctggctg
 1621 gggctgcgt cactgaggga actgggagc ggactggccc tcatccacca taacaccac
 1681 ctctgtctg tgcacaggt gccctgggac cagctcttc ggaacccgca ccaagctctg
 25 1741 ctccactc ccaaccggc agaggacag tgtgtggcg agggcctggc ctgccaccag
 1801 ctgtgcgcc gagggactg ctgggtcca gggccaccc agtgtgtca ctgcagccag
 1861 ttctctggg gccaggagtg cgtggaggaa tgcagagac tgcaggggt cccaggagag
 1921 tatgtaatg ccaggcactg ttgcccgtc caccctgagt gtcagccca gaatggctca
 1981 gtgacctgt ttggaccgga ggctgaccag tgtgtggct gtgcccacta taaggacct
 30 2041 ccttctgctg tggcccgctg cccagcgggt gtgaaacctg acctctcta catgccatc
 2101 tggagtttc cagttagga gggcgcatg cagcctgcc ccatcaactg caccactcc
 2161 tgtgtggacc tggatgaca gggctgccc gccgagcaga gagccagccc tctgacgtcc
 2221 atcatctctg cgtgtgttg cattctctg gtctgtgtct tgggggtgtt cttgggac
 2281 ctcatcaagc gacggcagca gaagatccg aagtacacga tgcggagact gctgcaggaa
 35 2341 acggagctgg tggagccgt gacacctagc ggagcgatg ccaaccaggc gcagatggc
 2401 atctgaaag agacggagct gaggaagggt aagggtctg gatctggcg tttggcaca
 2461 gtctacaagg gcatctggat cctgatggg gagaatgtga aaattccagt ggccatcaa
 2521 gtgttaggg aaaacacatc cccaaagcc acaaagaaa tcttagacga agcatatctg
 2581 atggctgtg tgggtcccc atatgtctc cgccttctg gcatctgcct gacatccag
 40 2641 gtgcagctg tgcacagct tatgccctat ggctgcctt tagaccatgt ccgggaaaac
 2701 cgcggacgc tgggtccca ggacctgtg aactggtga tgcagattgc caaggggatg
 2761 agctacctg aggatgtgc gctgtacac agggacttg ccgctcgga cgtgtgtg
 2821 aagagtcca accatgtcaa aattacagac ttcgggctg ctcggctgt ggacattgac
 2881 gagacagag accatgcaga tgggggcaag gtgccatca agtgatggc gctggagtcc
 45 2941 attctccgc ggcgttcac ccaccagag gatgtgtga gttatggtg gactgtgtg
 3001 gagctgatga ctttggggc caaacctac gatgggatcc cagcccgga gatccctgac
 3061 ctgttgaaa agggggagcg gctgcccag ccccatct gacattga tgtctacatg
 3121 atcatggtca aatgttgat gattgactt gaatgtggc caagattccg ggagtgtg
 3181 tctgaattct cccgatggc cagggaccc cagcgttg tggcatcca gaatgaggac
 50 3241 ttggggccag ccagtcctt ggacagcacc ttctaccgt cactgtgga ggacgatgac
 3301 atgggggacc tgggtgatg tgaggagat ctggtaccc agcagggct cttctgtcca
 3361 gacctgccc cggcgctgg ggcaggtg caccacagg accgcagtc atctaccag
 3421 agtggcggtg gggacctgac actagggct gagccctct aagaggagg cccaggtct
 3481 ccactggcac cctccgaagg ggctggctc gatgtattg atggtgacct ggaatggg
 55 3541 gcagccaagg ggctgcaaag cctcccaca catgaccca gcccttaca gcggtacatg
 3601 gaggaccca cagtacctt gccctctgag actgatggct acgtgcccc cctgacctg

3661 agccccagc ctgaatatgt gaaccagcca gatgttcggc cccagcccc ttcgccccga
 3721 gagggccctc tgcctgctgc cgcacctgct ggtgccactc tggaaaggcc caagactctc
 3781 tccccaggga agaattgggt cgtcaaagac gttttgcct tgggggtgc cgtggagaac
 3841 cccgagtact tgacacccca gggaggagct gccctcagc cccaccctcc tctgccttc
 3901 agccagcctc tcgacaacct ctattactgg gaccaggacc caccagagcg gggggctcca
 3961 cccagcacct tcaaaggac acctacggca gagaaccag agtacctggg tctggacgtg
 4021 ccagtgtgaa ccagaaggcc aagtcgcag aagccctgat gtgtcctcag ggagcagga
 4081 aggcctgact tctgtggca tcaagagggt ggagggccct cgcaccactt ccagggaac
 4141 ctgccatgcc aggaacctgt cctaaggaa cctccttct gcttgagttc ccagatggct
 4201 ggaaggggtc cagcctcgtt ggaagaggaa cagcactggg gactctttgt ggattctgag
 4261 gccctgcca atgagactct aggtccagt ggatgccaca gccagcttg gcccttctt
 4321 tccagatcct gggtagtcaa agccttaggg aagctggcct gagaggggaa gggccctaa
 4381 gggagtgtct aagaacaaaa gcgaccatt cagagactgt cctgaaacc tagtactgcc
 4441 ccccatgagg aaggaacagc aatgggtca gtatccaggc ttgtacaga gtgctttct
 4501 gtttagttt tactttttt gtttgttt ttaaagatg aaataaagac ccagggggag
 4561 aatgggtgtt gtatggggag gcaagtgtg ggggtcctt tccacacca cttgtccat
 4621 ttgcaatat attttgaaa acagctaaa aaaaaaaaaa aaaa (SEQ ID NO: 25)

Receptor tyrosine-protein kinase erbB-2 isoform a precursor [Homo sapiens] [NP_004439.2]

MELAALCRWG LLLALLPPGA ASTQVCTGTD MKLRLPASPE THLDMLRHLY
 QGCQVVQGNL ELTYLPTNAS LSFLQDIQEV QGYVLIAHNQ VRQVPLQLRL
 IVRGTQLFED NYALAVLDNG DPLNNTTPVT GASPGGLREL QLRSLTEILK
 GGVLIQRNPQ LCYQDTILWK DIFHKNNQLA LTLIDTNRSR ACHPCSPMCK
 GSRCWGESSE DCQSLTRTVC AGGCARCKGP LPTDCCHEQC AAGCTGPKHS
 DCLACLHFNH SGICELHCPA LVTYNTDTFE SMPNPEGRYT FGASCVTACP
 YNYLSTDVGS CTLVCPLHNQ EVTAEDGTQR CEKCSKPCAR VCYGLGMEHL
 REVRAVTSAN IQEFAGCKKI FGSLAFLPES FDGDPAANTA PLQPEQLQVF
 ETLEEITGYL YISAWPDSLPL DLSVFQNLQV IRGRILHNGA YSLTLQGLGI
 SWLGLRSLRE LGSGLALHH NTHLCFVHTV PWDQLFRNPH QALLHTANRP
 EDECVGEGLA CHQLCARGHC WGPPTQCVN CSQFLRGQEC VEECRVLQGL
 PREYVNARHC LPCHPECQPQ NGSVTCFGPE ADQCVACAHY KDPPFCVARC
 PSGVKPDLSY MPIWKFPDEE GACQPCPINC THSCVDLDDK GCPAEQRASP
 LTSIISAVVG ILLVVVLGVV FGILIKRRQQ KIRKYTMRR LQETELVEPL
 TPSGAMPNQA QMRILKETEL RKVKVLGSGA FGTVYKGIW PDGENVKIPV
 AIKVLRENTS PKANKEILDE AYVMAGVGSP YVSRLGICL TSTVQLVTQL
 MPYGCLLDHV RENRGRGSG DLLNWCMQIA KGMSYLEDVR LVHRDLAARN
 VLVKSPNHVK ITDFGLARLL DIDETEYHAD GGVPIKWMA LESILRRRFT
 HQSDVWSYGV TVWELMTFGA KPYDGIPARE IPDLLEKGER LPQPPICTID
 VYMIMVKCWM IDSECRPRFR ELVSEFSRMA RDPQRFVVIQ NEDLGPASPL
 DSTFYRSLLE DDDMGDLVDA EEYLVPQQGF FCPDPAPGAG GMVHHRHRSS
 STRSGGGDLT LGLEPSEEEA PRSPLAPSEG AGSDVFDGDL GMGAAGKLQS
 LPTHDPSPPL RYSEDPTVPL PSETDGYVAP LTCSPQPEYV NQPDVRPQPP
 SPREGPLPAA RPAGATLERP KTLSPGKNGV VKDVFAFGGA VENPEYLTPQ
 GGAAPQPHPP PAFSPAFDNL YYWDQDPPER GAPPSTFKGT PTAENPEYLG LDVPV
 (SEQ ID NO: 26)

[0027] The mRNA and protein sequences of the other human HER2 isoforms can be found in GeneBank with the following Accession Nos:

HER2 isoform B: NM_001005862.2 (mRNA)----> NP_001005862.1 (protein);
 HER2 isoform C: NM_001289936.1 (mRNA)----> NP_001276865.1 (protein);
 HER2 isoform D: NM_001289937.1 (mRNA)----> NP_001276866.1 (protein);
 HER2 isoform E: NM_001289938.1 (mRNA)----> NP_001276867.1 (protein).

[0028] The term "antibody," as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin

molecule that specifically binds to an antigen. Antibodies can be polyclonal or monoclonal, multiple or single chain, or intact immunoglobulins, and may be derived from natural sources or from recombinant sources. A naturally occurring "antibody" is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. An antibody can be a monoclonal antibody, human antibody, humanized antibody, camelised antibody, or chimeric antibody. The antibodies can be of any isotype (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass.

[0029] The term "antibody fragment" or "antigen-binding fragment" refers to at least one portion of an antibody, that retains the ability to specifically interact with (e.g., by binding, steric hinderance, stabilizing/destabilizing, spatial distribution) an epitope of an antigen. Examples of antibody fragments include Fab, Fab', F(ab')₂, Fv fragments, scFv antibody fragments, disulfide-linked Fvs (sdFv), a Fd fragment consisting of the VH and CH1 domains, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, multi-specific antibodies formed from antibody fragments such as a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, and an isolated CDR or other epitope binding fragments of an antibody. An antigen binding fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, *Nature Biotechnology* 23:1126-1136, 2005). Antigen binding fragments can also be grafted into scaffolds based on polypeptides such as a fibronectin type III (Fn3) (see U.S. Patent No.: 6,703,199, which describes fibronectin polypeptide minibodies). The term "scFv" refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked, e.g., via a synthetic linker, e.g., a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the VL and VH variable regions in either order, e.g., with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

[0030] The terms "complementarity determining region" or "CDR," as used herein, refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (e.g., HCDR1, HCDR2, and HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, and LCDR3). The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani et al., (1997) *JMB* 273,927-948 ("Chothia" numbering scheme), or a combination thereof. In a combined Kabat and Chothia numbering scheme for a given CDR region (for example, HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2 or LC CDR3), in some embodiments, the CDRs correspond to the amino acid residues that are defined as part of the Kabat CDR, together with the amino acid residues that are defined as part of the Chothia CDR.

[0031] The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or otherwise interacting with a molecule. Epitopic determinants generally consist of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and can have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope may be "linear" or "conformational." Conformational and linear epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0032] The phrases "monoclonal antibody" or "monoclonal antibody composition" as used herein refers to polypeptides, including antibodies, bispecific antibodies, etc., that have substantially identical amino acid sequence or are derived from the same genetic source. This term also includes preparations of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[0033] The phrase "human antibody," as used herein, includes antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region is also derived from such human sequences, e.g., human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from

human framework sequences analysis, for example, as described in Knappik, et al. (2000. J Mol Biol 296, 57-86). The structures and locations of immunoglobulin variable domains, e.g., CDRs, may be defined using well known numbering schemes, e.g., the Kabat numbering scheme, the Chothia numbering scheme, or a combination of Kabat and Chothia (see, e.g., Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services (1991), eds. Kabat et al.; Al Lazikani et al., (1997) J. Mol. Bio. 273:927-948); Kabat et al., (1991) Sequences of Proteins of Immunological Interest, 5th edit., NIH Publication no. 91-3242 U.S. Department of Health and Human Services; Chothia et al., (1987) J. Mol. Biol. 196:901-917; Chothia et al., (1989) Nature 342:877-883; and Al-Lazikani et al., (1997) J. Mol. Biol. 273:927-948.

[0034] The human antibodies of the invention may include amino acid residues not encoded by human sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*, or a conservative substitution to promote stability or manufacturing). However, the term "human antibody" as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0035] The phrase "recombinant human antibody" as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the human antibody, e.g., from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of all or a portion of a human immunoglobulin gene, sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0036] The term "Fc region" as used herein refers to a polypeptide comprising the CH3, CH2 and at least a portion of the hinge region of a constant domain of an antibody. Optionally, an Fc region may include a CH4 domain, present in some antibody classes. An Fc region may comprise the entire hinge region of a constant domain of an antibody. In one embodiment, the invention comprises an Fc region and a CH1 region of an antibody. In one embodiment, the invention comprises an Fc region CH3 region of an antibody. In another embodiment, the invention comprises an Fc region, a CH1 region and a Ckappa/lambda region from the constant domain of an antibody. In one embodiment, a binding molecule of the invention comprises a constant region, e.g., a heavy chain constant region. In one embodiment, such a constant region is modified compared to a wild-type constant region. That is, the polypeptides of the invention disclosed herein may comprise alterations or modifications to one or more of the three heavy chain constant domains (CH1, CH2 or CH3) and/or to the light chain constant region domain (CL). Example modifications include additions, deletions or substitutions of one or more amino acids in one or more domains. Such changes may be included to optimize effector function, half-life, etc.

[0037] The term "binding specificity" as used herein refers to the ability of an individual antibody combining site to react with one antigenic determinant and not with a different antigenic determinant. The combining site of the antibody is located in the Fab portion of the molecule and is constructed from the hypervariable regions of the heavy and light chains. Binding affinity of an antibody is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody. It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody.

[0038] The term "affinity" as used herein refers to the strength of interaction between antibody and antigen at single antigenic sites. Within each antigenic site, the variable region of the antibody "arm" interacts through weak non-covalent forces with antigen at numerous sites; the more interactions, the stronger the affinity.

[0039] The term "conservative sequence modifications" refers to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody or antibody fragment containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody or antibody fragment of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within an antibody can be replaced with other amino acid residues from the same side chain family and the altered antibody can

be tested using the functional assays described herein.

[0040] The term "homologous" or "identity" refers to the subunit sequence identity between two polymeric molecules, e.g., between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (e.g., 9 of 10), are matched or homologous, the two sequences are 90% homologous. Percentage of "sequence identity" can be determined by comparing two optimally aligned sequences over a comparison window, where the fragment of the amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage can be calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity. The output is the percent identity of the subject sequence with respect to the query sequence.

[0041] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, carcinoma, lymphoma, blastoma (including medulloblastoma and retinoblastoma), sarcoma (including liposarcoma and synovial cell sarcoma), neuroendocrine tumors (including carcinoid tumors, gastrinoma, and islet cell cancer), mesothelioma, schwannoma (including acoustic neuroma), meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, neuroblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, urinary tract cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophageal cancer, tumors of the biliary tract, as well as head and neck cancer.

[0042] A "HER2-positive cancer" or "HER2-expressing cancer" is a cancer comprising cells that have HER2 protein present at their cell surface. Many methods are known in the art for detecting or determining the presence of HER2 on a cancer cell. For example, in some embodiments, the presence of HER2 on the cell surface may be determined by immunohistochemistry (IHC), flow cytometry, Western blotting, immunofluorescent assay, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), homogeneous time resolved fluorescence (HTRF), or positron emission tomography (PET).

[0043] The terms "combination" or "pharmaceutical combination," as used herein mean a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, by way of example, a compound of the invention and one or more additional therapeutic agent, are administered to a subject simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, by way of example, a compound of the invention and one or more additional therapeutic agent, are administered to a subject as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the active ingredients in the body of the subject. The latter also applies to cocktail therapy, e.g. the administration of 3 or more active ingredients.

[0044] The terms "composition" or "pharmaceutical composition," as used herein, refers to a mixture of a compound of the invention with at least one and optionally more than one other pharmaceutically acceptable chemical components, such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, and/or excipients.

[0045] The term "an optical isomer" or "a stereoisomer", as used herein, refers to any of the various stereo isomeric configurations which may exist for a given compound of the present invention and includes geometric isomers. It is understood that a substituent may be attached at a chiral center of a carbon atom. The term "chiral" refers to molecules which have the property of non-superimposability on their mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner. Therefore, the invention includes enantiomers, diastereomers or racemates of the compound. "Enantiomers" are a pair of stereoisomers that are non- superimposable mirror images of each other. A 1:1 mixture of a pair of enantiomers is a "racemic" mixture. The term is used to designate a racemic mixture where appropriate. "Diastereoisomers" are stereoisomers that have at least two asymmetric atoms, but which are not mirror-images of each other. The absolute stereochemistry is specified according to the Cahn-Ingold- Prelog R-S system. When a compound is a pure enantiomer the stereochemistry at each chiral carbon may be specified by either R or S. Resolved compounds whose absolute configuration is unknown can be designated (+) or (-) depending on the direction (dextro- or levorotatory) which they rotate plane polarized light at the wavelength of the sodium D line. Certain

compounds described herein contain one or more asymmetric centers or axes and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)-.

[0046] The term "pharmaceutically acceptable carrier", as used herein, includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drug stabilizers, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, and combinations thereof, as would be known to those skilled in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289- 1329). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

[0047] The term "pharmaceutically acceptable salt," as used herein, refers to a salt which does not abrogate the biological activity and properties of the compounds of the invention, and does not cause significant irritation to a subject to which it is administered.

[0048] The term "subject", as used herein, encompasses mammals and non-mammals. Examples of mammals include humans, chimpanzees, apes, monkeys, cattle, horses, sheep, goats, swine; rabbits, dogs, cats, rats, mice, guinea pigs. Examples of non-mammals include birds, fish. Frequently the subject is a human.

[0049] The term "a subject in need of such treatment", refers to a subject which would benefit biologically, medically or in quality of life from such treatment.

[0050] The term "therapeutically effective amount," as used herein, refers to an amount of an antibody conjugate of the invention that will elicit the biological or medical response of a subject, for example, reduction or inhibition of an enzyme or a protein activity, or ameliorate symptoms, alleviate conditions, slow or delay disease progression, or prevent a disease, etc. In one non-limiting embodiment, the term "a therapeutically effective amount" refers to the amount of an antibody conjugate of the invention that, when administered to a subject, is effective to at least partially alleviate, inhibit, prevent and/or ameliorate a condition, or a disorder or a disease.

[0051] The term "TLR7 agonist", as used herein, refers to a compound or antibody conjugate capable of activating Toll-like Receptor 7 (TLR7).

[0052] The terms "treat," "treating" or "treatment," as used herein, refers to methods of alleviating, abating or ameliorating a disease or condition symptoms, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms, inhibiting the disease or condition, arresting the development of the disease or condition, relieving the disease or condition, causing regression of the disease or condition, relieving a condition caused by the disease or condition, or stopping the symptoms of the disease or condition either prophylactically and/or therapeutically.

[0053] The compound names provided herein were obtained using ChemDraw Ultra version 12.0 (CambridgeSoft®) or JChem version 5.3.1 (ChemAxon).

[0054] The compound names provided herein were obtained using ChemDraw Ultra version 12.0 (CambridgeSoft®) or JChem version 5.3.1 (ChemAxon).

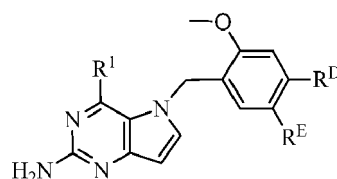
[0055] Unless specified otherwise, the term "compounds of the present invention", "compounds of the invention" or "compounds provided herein" refers to compounds of Formula (I) and subformulae thereof (i.e. compounds of Formula (Ia) and Formula (Ib)), and pharmaceutically acceptable salts, stereoisomers (including diastereoisomers and enantiomers), tautomers and isotopically labeled compounds (including deuterium substitutions) thereof.

[0056] Unless specified otherwise, the term "antibody conjugate of the invention", refers to antibody conjugates of Formula (II) and subformulae thereof (i.e. compounds of Formula (IIa) and Formula (IIb)), and pharmaceutically acceptable salts, stereoisomers (including diastereoisomers and enantiomers), tautomers and isotopically labeled compounds (including deuterium substitutions) thereof.

[0057] As used herein, the term "a," "an," "the" and similar terms used in the context of the present invention (especially in the context of the claims) are to be construed to cover both the singular and plural unless otherwise indicated herein or clearly contradicted by the context.

Immunostimulatory Compounds of the Invention

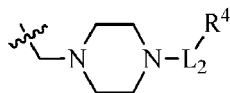
[0058] The immunostimulatory compounds of the invention are TLR7 agonists having the structure of Formula (I):



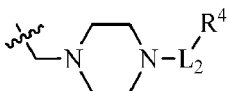
Formula (I)

wherein:

R^D is



and R^E is H; or R^E is



and R^D is H;

R^1 is $-NHR^2$ or $-NHCHR^2R^3$;

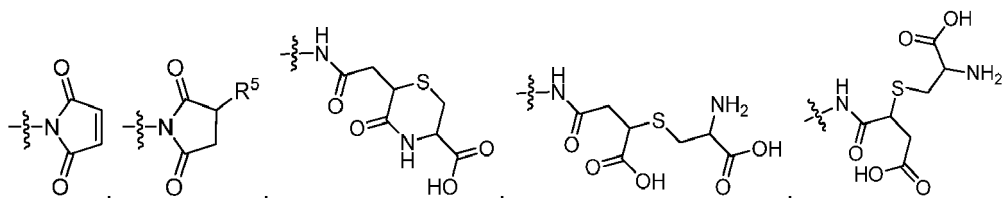
R^2 is $-C_3-C_6$ alkyl or $-C_4-C_6$ alkyl;

R^3 is L_1OH ;

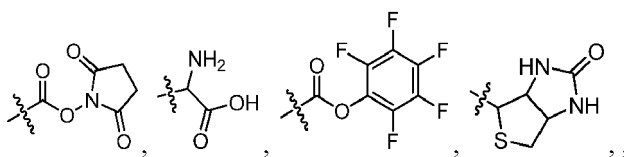
L_1 is $-(CH_2)_m-$;

L_2 is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_n-$,
 $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)(CH_2)_n-$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$,
 $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)X_2X_3C(=O)(CH_2)_n-$,
 $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n-$,
 $-C(=O)(CH_2)_nC(R_7)_2-$, $-C(=O)(CH_2)_nC(R_7)_2SS(CH_2)_nNHC(=O)(CH_2)_n-$,
 $-(CH_2)_nX_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n-$ or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

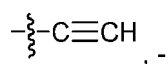
R^4 is



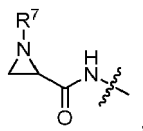
$-ONH_2$, $-NH_2$,



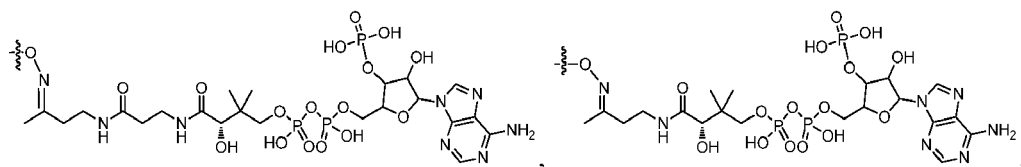
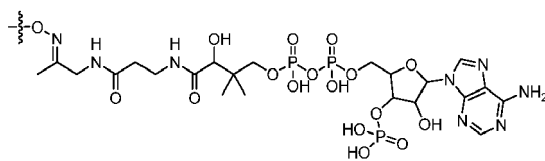
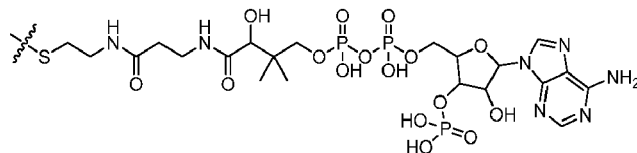
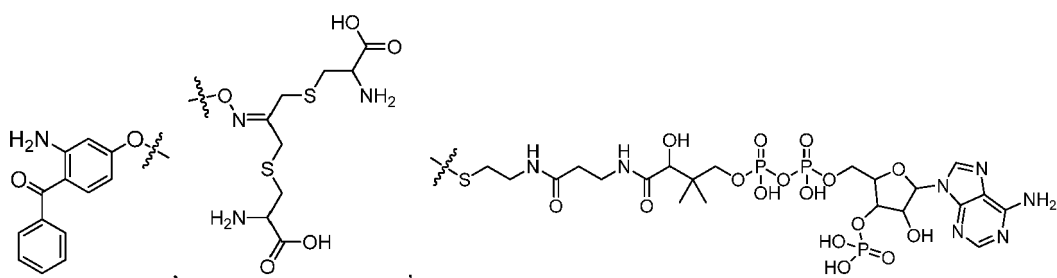
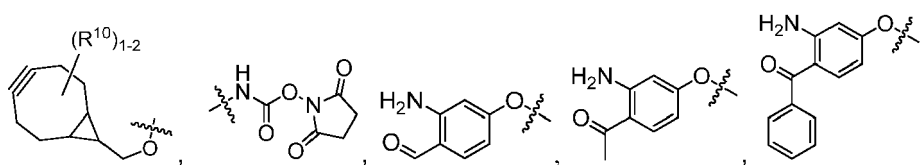
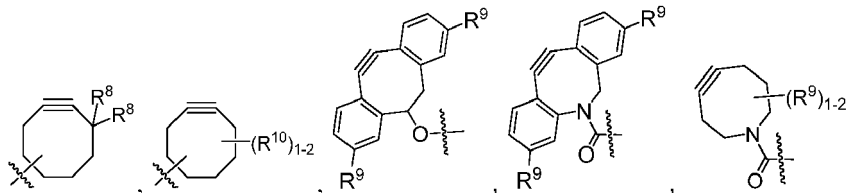
$-N_3$,

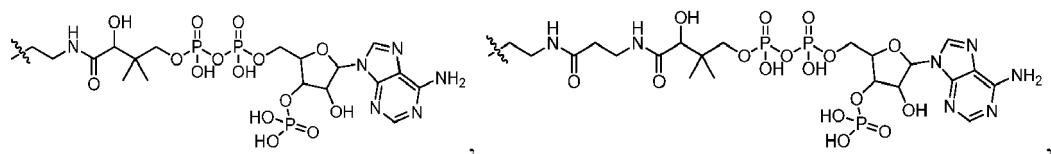


$NHC(=O)CH=CH_2$, $-SH$, $-SR^7$, $-OH$, $-SSR^6$, $-S(=O)_2(CH=CH_2)$, $-(CH_2)_2S(=O)_2(CH=CH_2)$, $-NHS(=O)_2(CH=CH_2)$,
 $-NHC(=O)CH_2Br$, $-NHC(=O)CH_2I$, $-C(O)NHNH_2$,

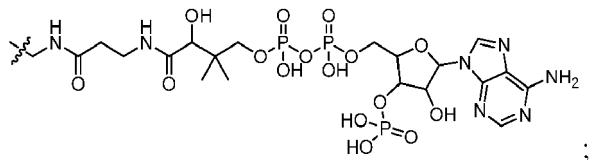


$-\text{CO}_2\text{H}$, $-\text{C}(\text{O})\text{NHNH}_2$,

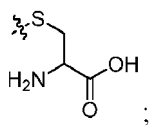




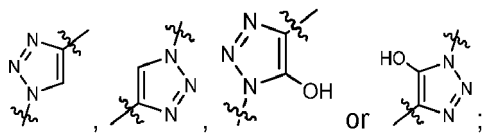
or



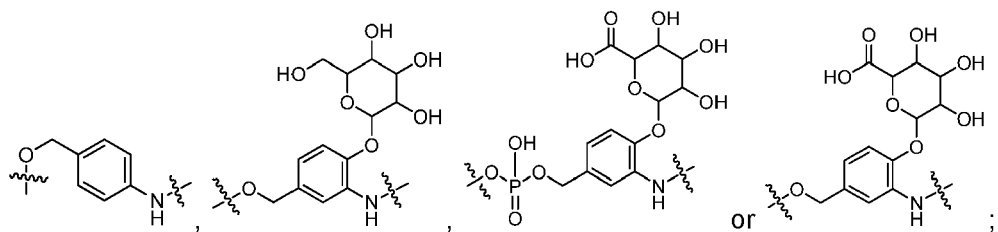
R⁵ is



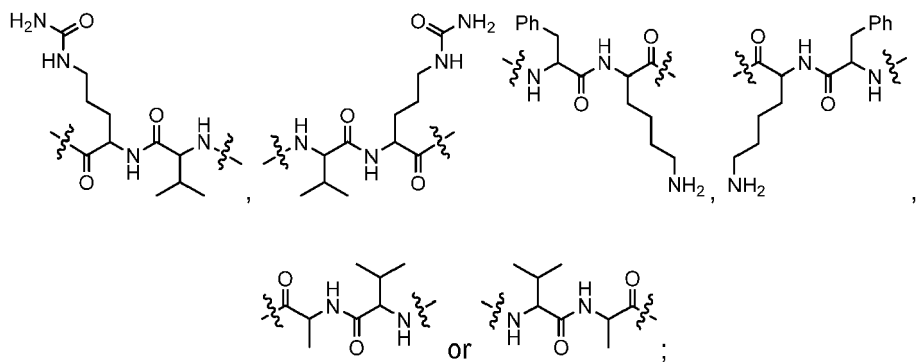
X₁ is



X₂ is



X₃ is



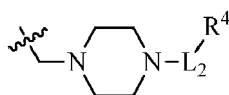
R⁶ is 2-pyridyl or 4-pyridyl;

each R^7 is independently selected from H and C_1 - C_6 alkyl;
 each R^8 is independently selected from H, C_1 - C_6 alkyl, F, Cl, and -OH;
 each R^9 is independently selected from H, C_1 - C_6 alkyl, F, Cl, -NH₂, -OCH₃, -OCH₂CH₃, -N(CH₃)₂, -CN, -NO₂ and -OH;
 each R^{10} is independently selected from H, C_1 - C_6 alkyl, fluoro, benzyloxy substituted with -C(=O)OH, benzyl substituted
 5 with -C(=O)OH, C_{1-4} alkoxy substituted with -C(=O)OH and C_{1-4} alkyl substituted with -C(=O)OH;
 each m is independently selected from 1, 2, 3, and 4;
 each n is independently selected from 1, 2, 3, and 4;
 and
 each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

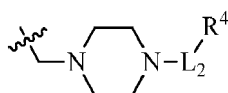
[0059] Certain aspects and examples of the compounds of the invention are provided in the following listing of additional, enumerated embodiments. It will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present invention.

Embodiment 1. The compound of Formula (I), and the pharmaceutically acceptable salts thereof, wherein:

R^D is



and R^E is H; or R^E is



and R^D is H;

R^1 is -NHR² or -NHCHR²R³;

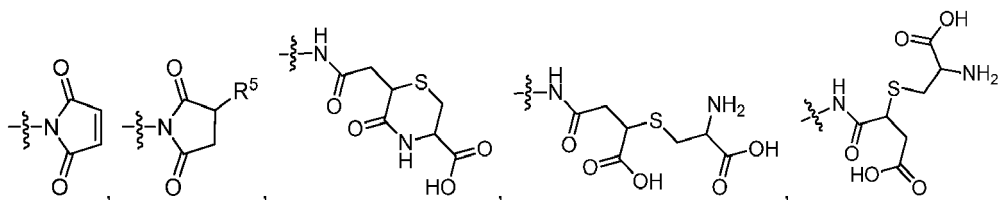
R^2 is -C₃-C₆alkyl or -C₄-C₆alkyl;

R^3 is L₁OH;

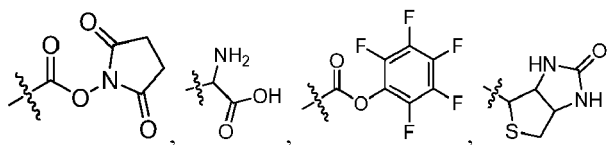
L₁ is -(CH₂)_m-;

L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_n-,
 -(CH₂)_nNHC(=O)(CH₂)_nC(=O)NH(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)(CH₂)_n-,
 -C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,
 -C(=O)((CH₂)_nO)_t(CH₂)_nC(=O)NH(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,
 -C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-, or
 -C(=O)(CH₂)_nC(=O)NH(CH₂)_n;

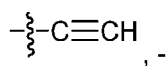
R^4 is



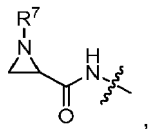
-ONH₂, -NH₂,



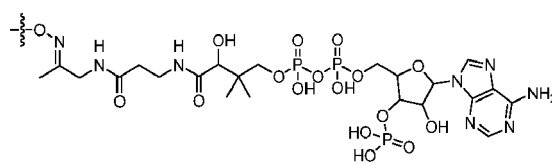
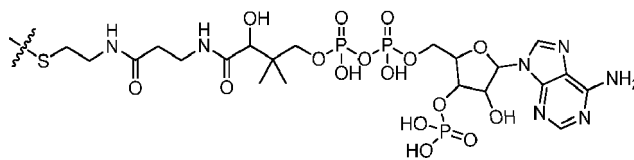
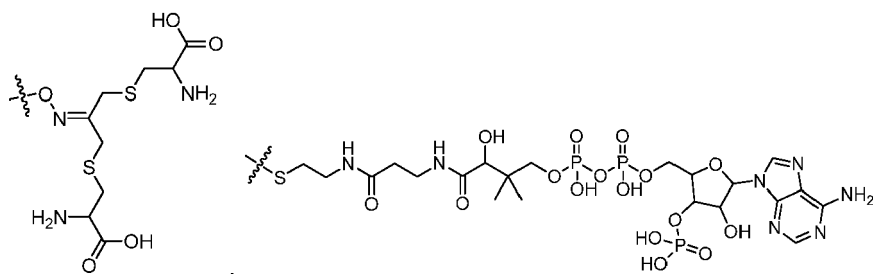
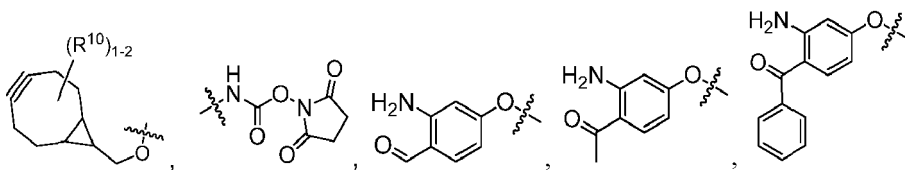
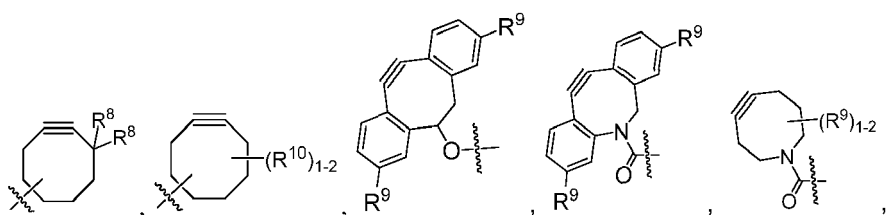
-N₃,



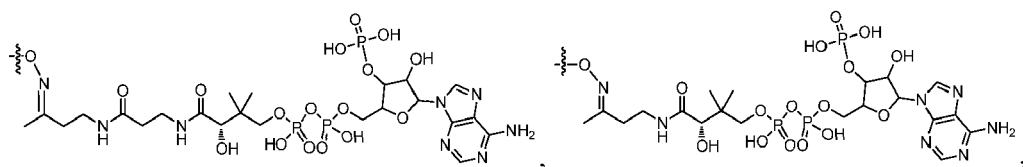
NHC(=O)CH=CH₂, SH, -SSR⁶, -S(=O)₂(CH=CH₂), -(CH₂)₂S(=O)₂(CH=CH₂), -NHS(=O)₂(CH=CH₂),
-NHC(=O)CH₂Br, -NHC(=O)CH₂I, -C(O)NHNH₂,



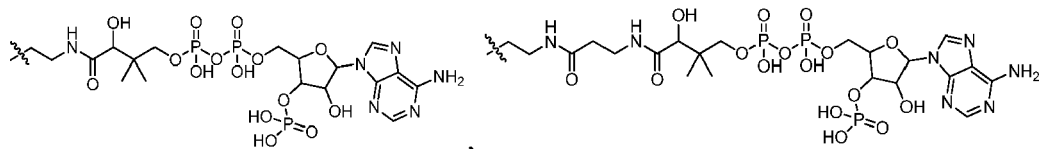
-CO₂H, -C(O)NHNH₂,



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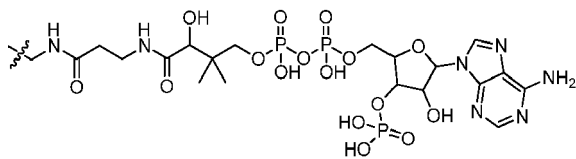


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or

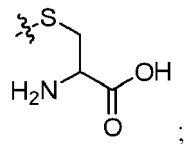
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R⁵ is

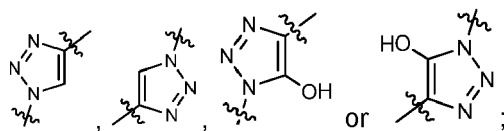
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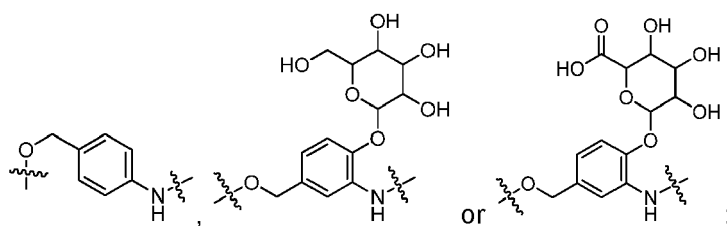
X₁ is

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X₂ is

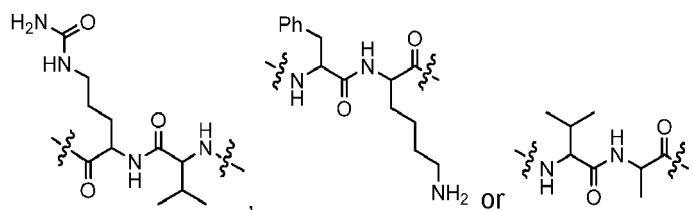
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X₃ is

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R^6 is 2-pyridyl or 4-pyridyl;

each R^7 is independently selected from H and C_1 - C_6 alkyl;

each R^8 is independently selected from H, C_1 - C_6 alkyl, F, Cl, and -OH;

each R^9 is independently selected from H, C_1 - C_6 alkyl, F, Cl, -NH₂, -OCH₃, -OCH₂CH₃, -N(CH₃)₂, -CN, -NO₂ and -OH;

each R^{10} is independently selected from H, C_1 - C_6 alkyl, fluoro, benzyloxy substituted with -C(=O)OH, benzyl substituted with -C(=O)OH, C_{1-4} alkoxy substituted with -C(=O)OH and C_{1-4} alkyl substituted with -C(=O)OH;

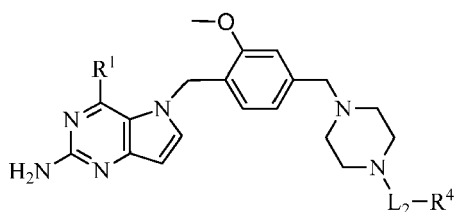
each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

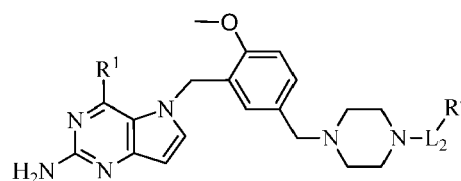
and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

Embodiment 2. The compound of Formula (I) having the structure of Formula (Ia) or Formula (Ib), and the pharmaceutically acceptable salts thereof:



Formula (Ia)



Formula (Ib)

wherein:

R^1 is -NHR² or -NHCHR²R³;

R^2 is -C₃-C₆alkyl or -C₄-C₆alkyl;

R^3 is L₁OH;

L₁ is -(CH₂)_m-;

L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_n-,

-(CH₂)_nNHC(=O)(CH₂)_nC(=O)NH(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)(CH₂)_n-,

-C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,

-C(=O)((CH₂)_nO)_t(CH₂)_nC(=O)NH(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,

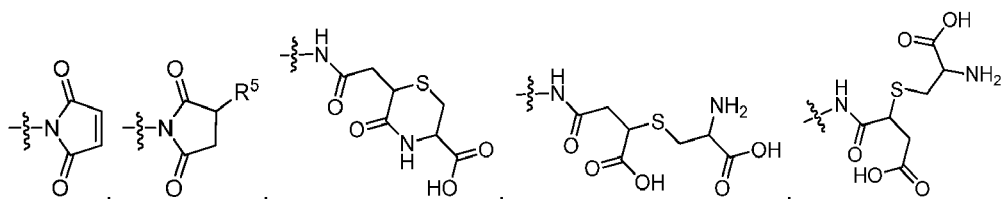
-C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)X₂X₃C(=O)(CH₂)_n-,

-C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)X₂C(=O)(CH₂)_nNHC(=O)((CH₂)_nO)_t(CH₂)_n-,

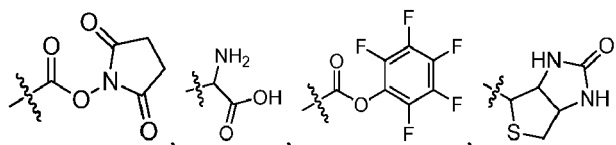
-C(=O)(CH₂)_nC(R₇)₂-, -C(=O)(CH₂)_nC(R₇)₂SS(CH₂)_nNHC(=O)(CH₂)_n-,

-(CH₂)_nX₂C(=O)(CH₂)_nNHC(=O)((CH₂)_nO)_t(CH₂)_n- or -C(=O)(CH₂)_nC(=O)NH(CH₂)_n;

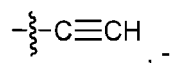
R^4 is



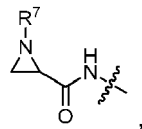
-ONH₂, -NH₂,



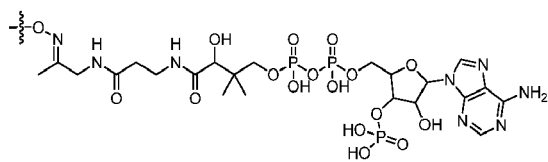
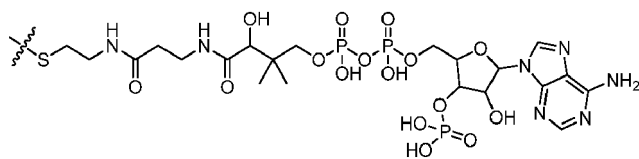
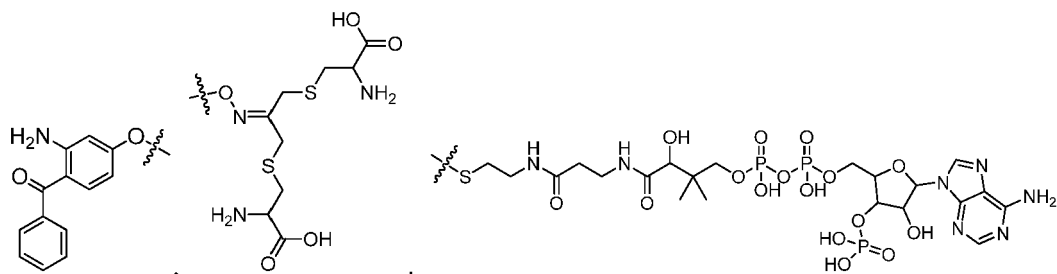
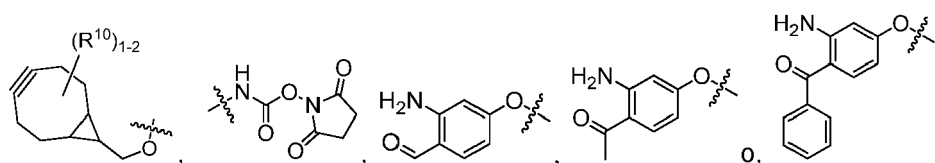
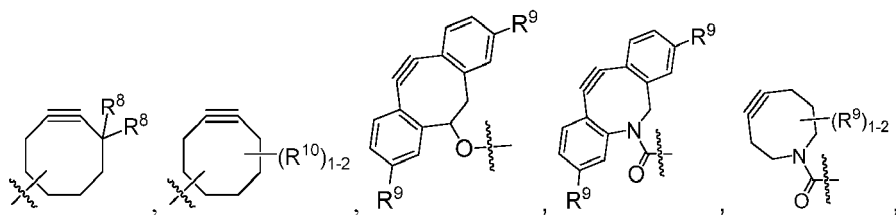
-N₃,



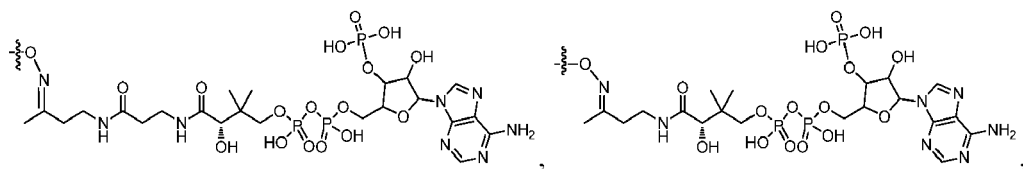
NHC(=O)CH=CH₂, SH, -SR⁷, -OH, -SSR⁶, -S(=O)₂(CH=CH₂), -(CH₂)₂S(=O)₂(CH=CH₂), -NHS(=O)₂(CH=CH₂),
-NHC(=O)CH₂Br, -NHC(=O)CH₂I, -C(O)NHNH₂,



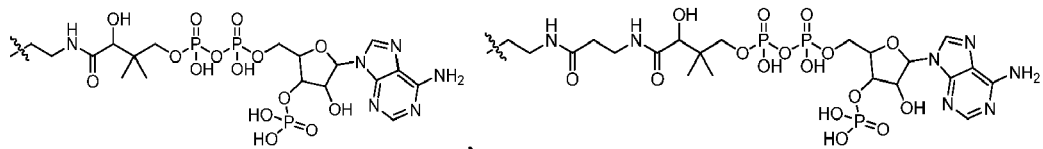
-CO₂H, -C(O)NHNH₂,



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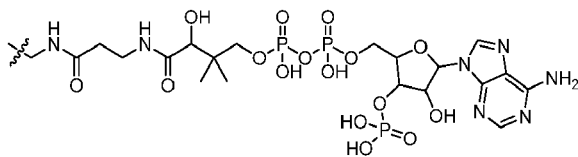


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or

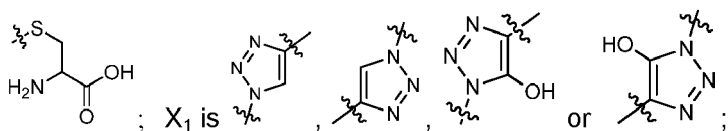
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R⁵ is

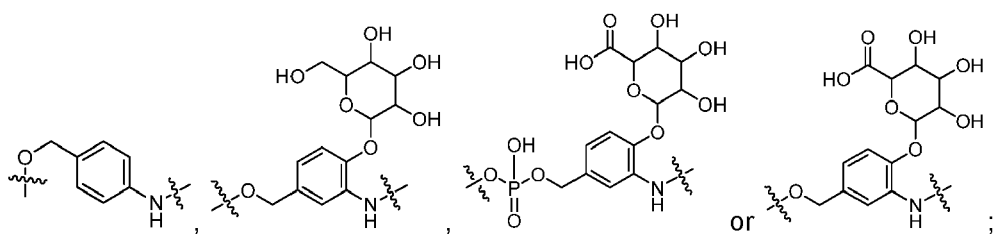
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X₂ is

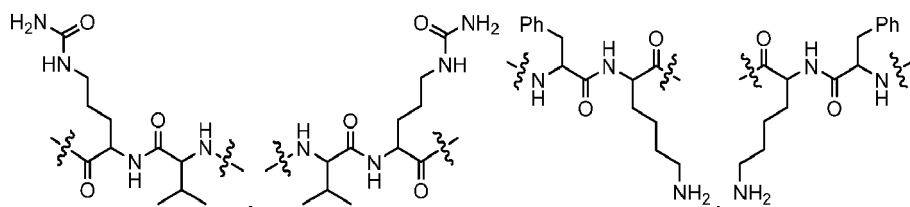
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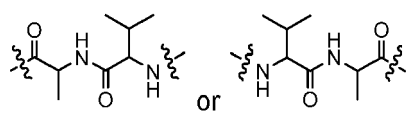
X₃ is

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R⁶ is 2-pyridyl or 4-pyridyl;

each R⁷ is independently selected from H and C₁-C₆alkyl;

each R^8 is independently selected from H, C_1 - C_6 alkyl, F, Cl, and -OH;

each R^9 is independently selected from H, C_1 - C_6 alkyl, F, Cl, $-NH_2$, $-OCH_3$, $-OCH_2CH_3$, $-N(CH_3)_2$, $-CN$, $-NO_2$ and -OH;

each R^{10} is independently selected from H, C_{1-6} alkyl, fluoro, benzyloxy substituted with $-C(=O)OH$, benzyl substituted with $-C(=O)OH$, C_{1-4} alkoxy substituted with $-C(=O)OH$ and C_{1-4} alkyl substituted with $-C(=O)OH$;

each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

Embodiment 3. The compound of Formula (Ia) or Formula (Ib), and the pharmaceutically acceptable salts thereof, wherein:

R^1 is $-NHR^2$ or $-NHCHR^2R^3$;

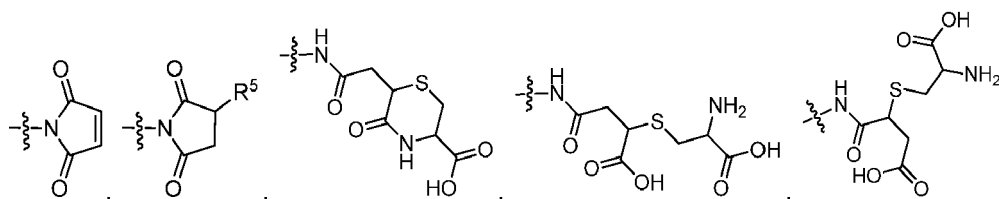
R^2 is $-C_3$ - C_6 alkyl or $-C_4$ - C_6 alkyl;

R^3 is L_1OH ;

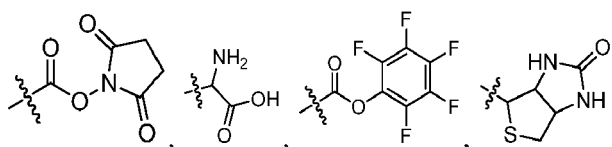
L_1 is $-(CH_2)_m-$;

L_2 is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

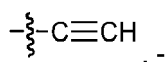
R^4 is



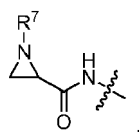
$-ONH_2$, $-NH_2$,



$-N_3$,

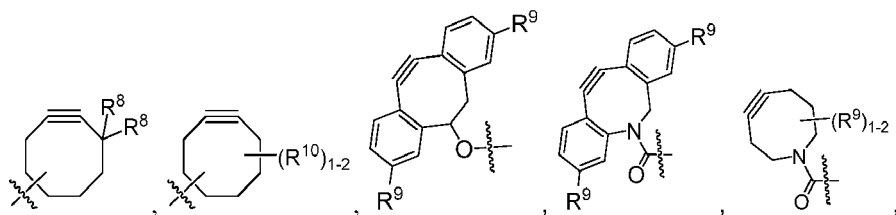


$NHC(=O)CH=CH_2$, SH , $-SSR^6$, $-S(=O)_2(CH=CH_2)$, $-(CH_2)_2S(=O)_2(CH=CH_2)$, $-NHS(=O)_2(CH=CH_2)$, $-NHC(=O)CH_2Br$, $-NHC(=O)CH_2I$, $-C(O)NHNH_2$,

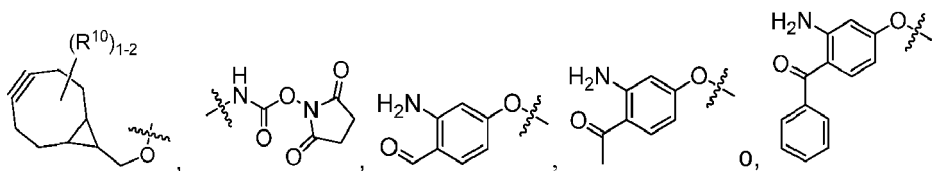


$-CO_2H$, $-C(O)NHNH_2$,

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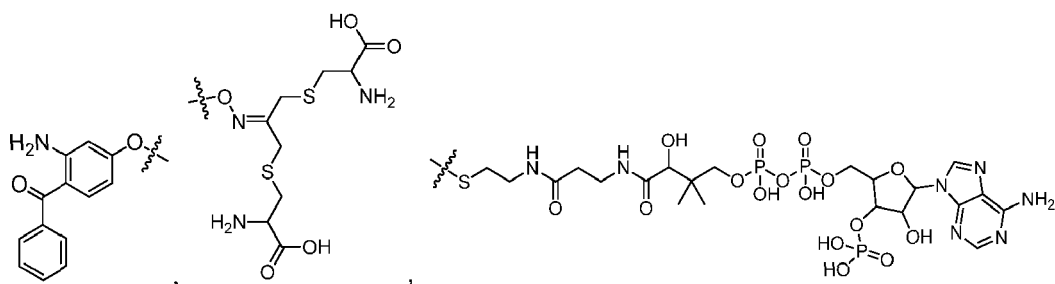


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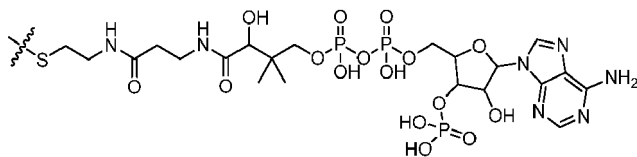
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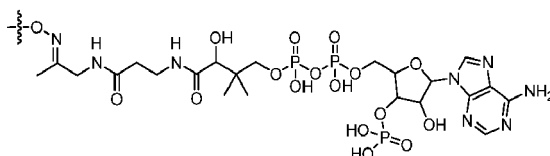


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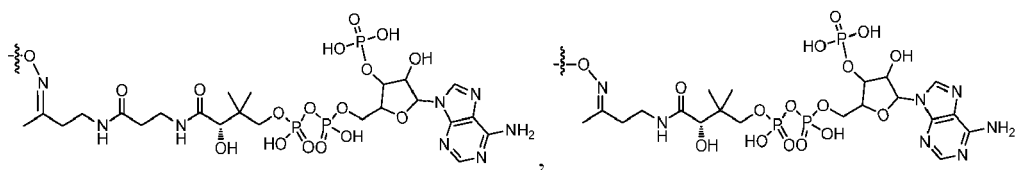


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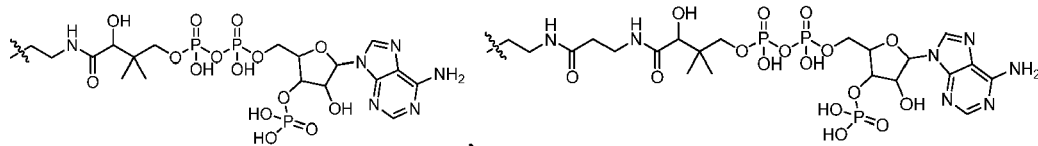


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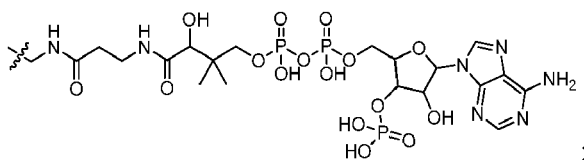


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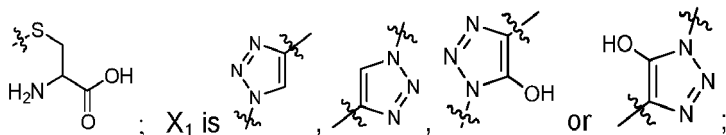


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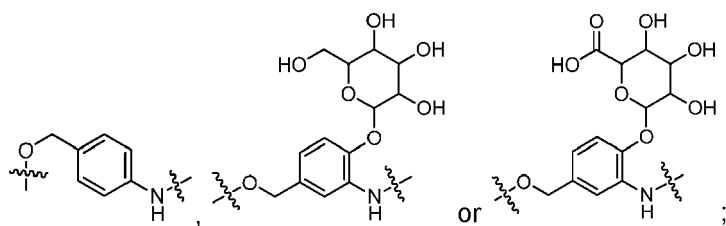
or



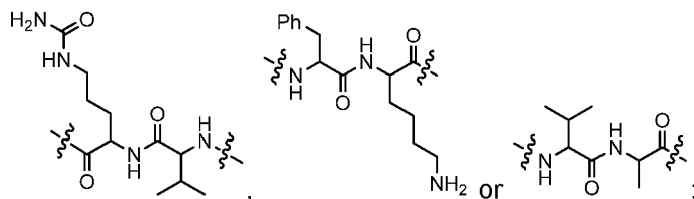
R⁵ is



X₂ is



X₃ is



R⁶ is 2-pyridyl or 4-pyridyl;

each R⁷ is independently selected from H and C₁-C₆alkyl;

each R⁸ is independently selected from H, C₁-C₆alkyl, F, Cl, and -OH;

each R⁹ is independently selected from H, C₁-C₆alkyl, F, Cl, -NH₂, -OCH₃, -OCH₂CH₃, -N(CH₃)₂, -CN, -NO₂ and -OH;

each R¹⁰ is independently selected from H, C₁₋₆alkyl, fluoro, benzyloxy substituted with -C(=O)OH, benzyl substituted with -C(=O)OH, C₁₋₄alkoxy substituted with -C(=O)OH and C₁₋₄alkyl substituted with -C(=O)OH;

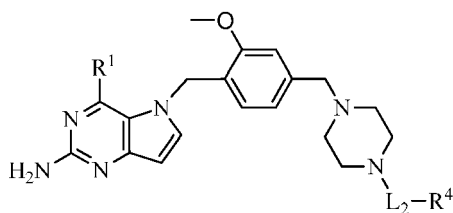
each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

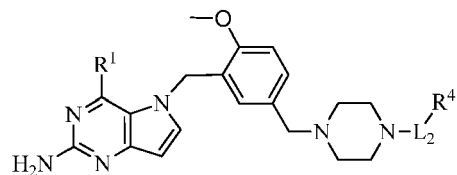
and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

Embodiment 4. The compound of Formula (I) having the structure of Formula (Ia) or Formula (Ib), and the pharmaceutically acceptable salts thereof:



Formula (Ia)



Formula (Ib)

wherein:

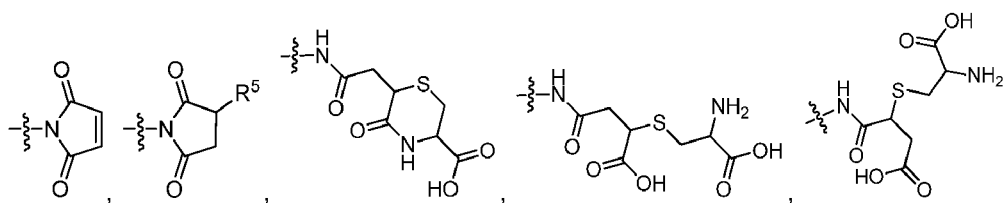
R¹ is -NHR² or -NHCHR²R³;

R² is -C₃-C₆alkyl or -C₄-C₆alkyl;

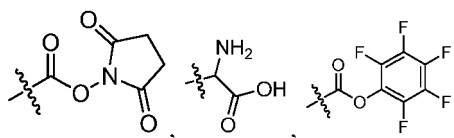
R³ is L₁OH;

L₁ is -(CH₂)ₘ-;

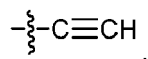
L₂ is -(CH₂)ₙ-, -((CH₂)ₙO)ₜ(CH₂)ₙ-, -(CH₂)ₙX₁(CH₂)ₙ-, -(CH₂)ₙNHC(=O)(CH₂)ₙ-,
 -(CH₂)ₙNHC(=O)(CH₂)ₙC(=O)NH(CH₂)ₙ-, -((CH₂)ₙO)ₜ(CH₂)ₙNHC(=O)(CH₂)ₙ-, -C(=O)(CH₂)ₙ-,
 -C(=O)((CH₂)ₙO)ₜ(CH₂)ₙ-, -C(=O)((CH₂)ₙO)ₜ(CH₂)ₙX₁(CH₂)ₙ-, -C(=O)((CH₂)ₙO)ₜ(CH₂)ₙNHC(=O)(CH₂)ₙ-,
 -C(=O)((CH₂)ₙO)ₜ(CH₂)ₙC(=O)NH(CH₂)ₙ-, -C(=O)NH((CH₂)ₙO)ₜ(CH₂)ₙX₁(CH₂)ₙ-,
 -C(=O)X₂X₃C(=O)((CH₂)ₙO)ₜ(CH₂)ₙ-, -C(=O)X₂C(=O)(CH₂)ₙNHC(=O)(CH₂)ₙ-, or
 -C(=O)(CH₂)ₙC(=O)NH(CH₂)ₙ-;
 R⁴ is



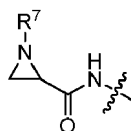
-ONH₂, -NH₂,



-NHC(=O)CH=CH₂, -N₃,

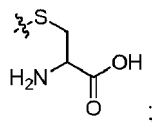


SH, -SSR⁶, -S(=O)₂(CH=CH₂), -(CH₂)₂S(=O)₂(CH=CH₂), -NHS(=O)₂(CH=CH₂), -NHC(=O)CH₂Br,
 -NHC(=O)CH₂I, -C(O)NHNH₂,

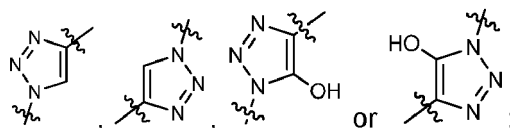


or -CO₂H;

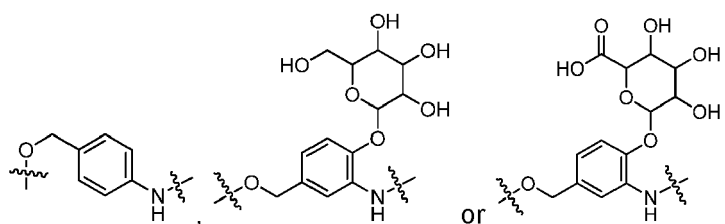
R⁵ is



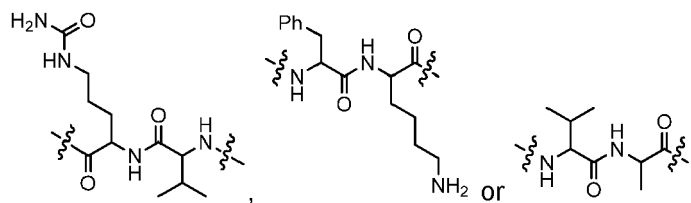
X_1 is



X_2 is



X_3 is



R^6 is 2-pyridyl or 4-pyridyl;

each R^7 is independently selected from H and C_1 - C_6 alkyl;

each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

Embodiment 5. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

R^1 is $-NHR^2$ or $-NHCHR^2R^3$;

R^2 is $-C_4$ - C_6 alkyl;

R^3 is L_1OH ;

L_1 is $-(CH_2)_m$;

L_2 is $-(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_n$, $-(CH_2)_nX_1(CH_2)_n$,

$-(CH_2)_nNHC(=O)(CH_2)_n$, $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$,

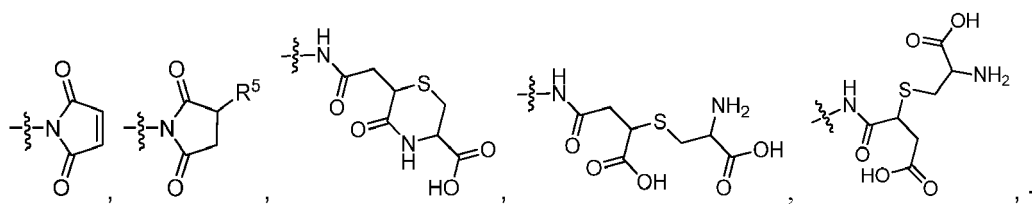
$-C(=O)(CH_2)_n$, $-C(=O)(CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$, $-C(=O)(CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$,

$-C(=O)(CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$,

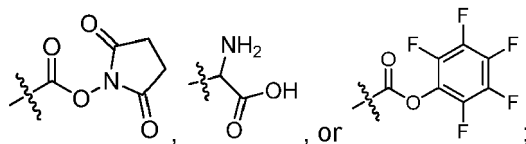
$-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n$, or

$-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

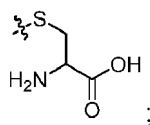
R^4 is



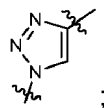
ONH₂, -NH₂,



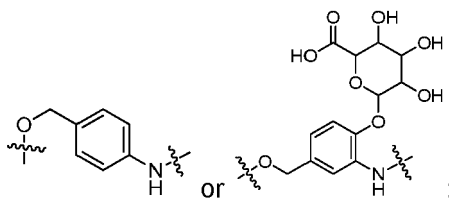
R⁵ is



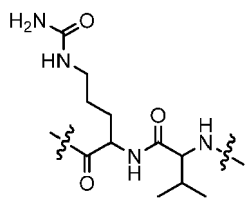
X₁ is



X₂ is



X₃ is



each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

and

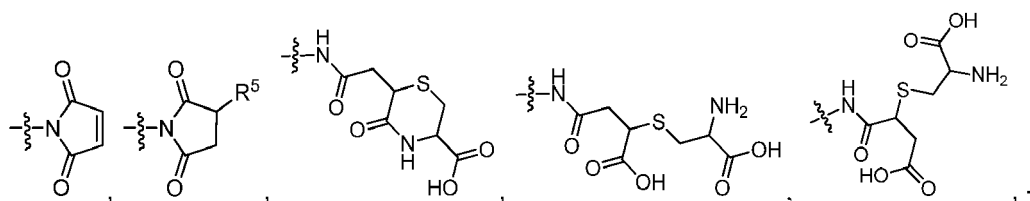
each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

Embodiment 6. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein::

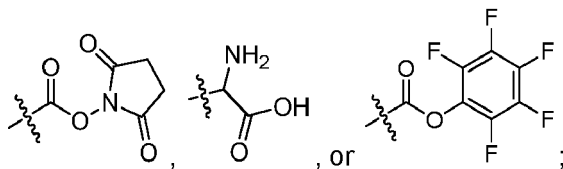
R¹ is -NHR²; R² is -C₄-C₆alkyl;

L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-,
 -(CH₂)_nNHC(=O)(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_nC(=O)NH(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,
 -C(=O)(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,
 -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-,
 -C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-, or -C(=O)(CH₂)_nC(=O)NH(CH₂)_n-;

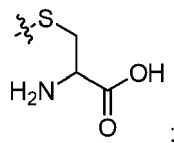
R⁴ is



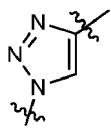
ONH₂, -NH₂,



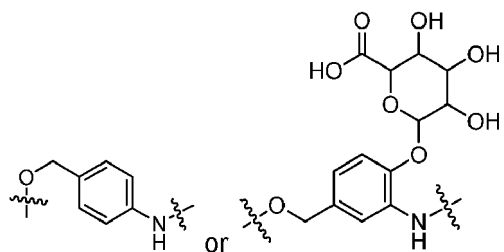
R⁵ is



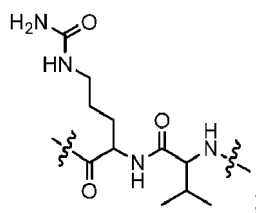
X₁ is



X₂ is



X₃ is



each n is independently selected from 1, 2, 3, and 4,

and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

Embodiment 7. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

R^1 is $-NHR^2$;

R^2 is $-C_4-C_6$ alkyl;

L_2 is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-C(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_n-$,

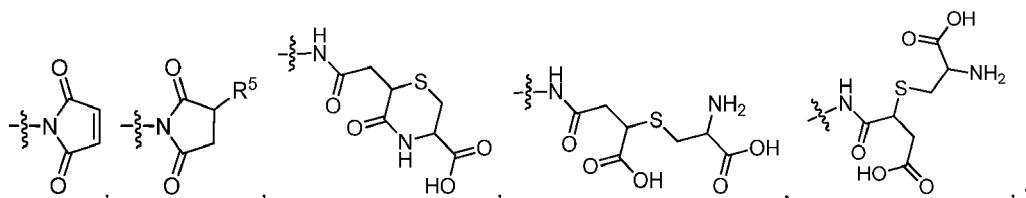
$-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$,

$-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$

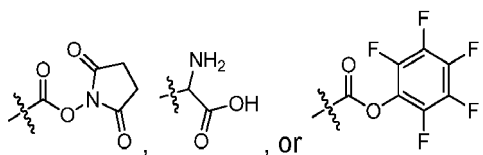
or

$-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$;

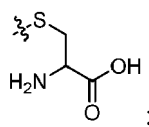
R^4 is



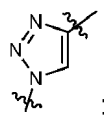
ONH_2 , $-NH_2$,



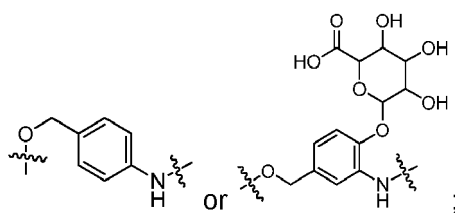
R^5 is



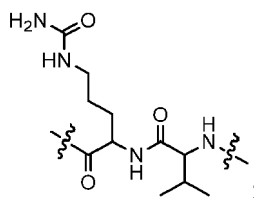
X_1 is



X_2 is



X_3 is



each n is independently selected from 1, 2, 3, and 4,

and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

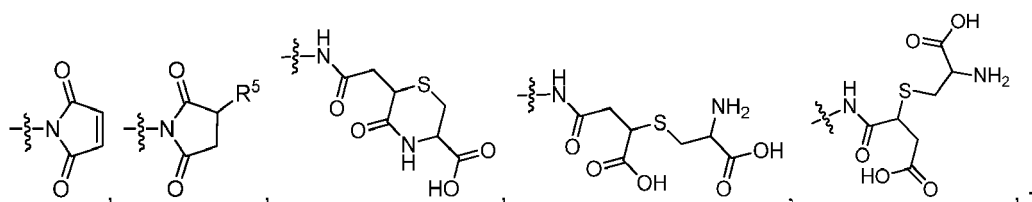
Embodiment 8. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

R^1 is $-NHR^2$;

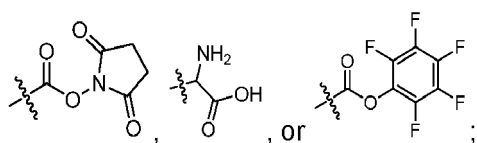
R^2 is $-C_4-C_6$ alkyl;

L_2 is $-(CH_2)_n-$ or $-C(=O)(CH_2)_n-$;

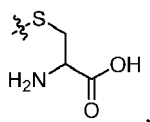
R^4 is



ONH_2 , $-NH_2$,



R^5 is

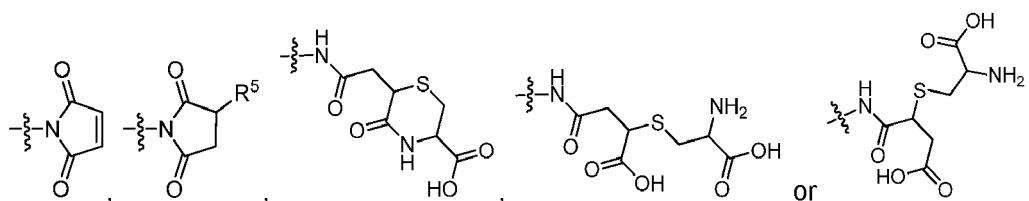


and

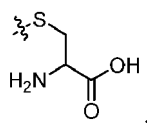
each n is independently selected from 1, 2, 3, and 4.

Embodiment 9. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

R^1 is $-NHR^2$;
 R^2 is $-C_4-C_6$ alkyl;
 L_2 is $-(CH_2)_n-$ or $-C(=O)(CH_2)_n-$;
 R^4 is



R^5 is



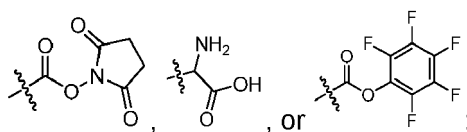
and
 each n is independently selected from 1, 2, 3, and 4.

Embodiment 10. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

R^1 is $-NHR^2$;
 R^2 is $-C_4-C_6$ alkyl;
 L_2 is $-(CH_2)_n-$ or $-C(=O)(CH_2)_n-$;
 R^4 is $-ONH_2$ or $-NH_2$;
 and
 each n is independently selected from 1, 2, 3, and 4.

Embodiment 11. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

R^1 is $-NHR^2$;
 R^2 is $-C_4-C_6$ alkyl;
 L_2 is $-(CH_2)_n-$ or $-C(=O)(CH_2)_n-$;
 R^4 is



and
 each n is independently selected from 1, 2, 3, and 4.

Embodiment 12. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: R^1 is $-NHR^2$.

Embodiment 13. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: R^1 is $-NHCHR^2R^3$.

Embodiment 14. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: R^2 is $-C_4$ alkyl.

Embodiment 15. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: R^2 is $-C_5$ alkyl.

Embodiment 16. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: R^2 is $-C_6$ alkyl.

Embodiment 17. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: R^3 is L_1OH .

Embodiment 18. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: L_1 is $-(CH_2)-$.

Embodiment 19. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: L_1 is $-(CH_2CH_2)-$.

Embodiment 20. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

L_2 is $-(CH_2)_n^*$, $-((CH_2)_nO)_t(CH_2)_n^*$, $-(CH_2)_nX_1(CH_2)_n^*$, $-(CH_2)_nNHC(=O)(CH_2)_n^*$, $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n^*$, or $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n^*$, where the * denotes attachment point to R^4 .

Embodiment 21. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

L_2 is $-C(=O)(CH_2)_n^*$, $-C(=O)((CH_2)_nO)_t(CH_2)_n^*$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n^*$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n^*$, $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n^*$, $C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n^*$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n^*$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n^*$, or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n^*$, where the * denotes attachment point to R^4 .

Embodiment 22. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

L_2 is $-(CH_2)_n^*$ or $-C(=O)(CH_2)_n^*$, where the * denotes attachment point to R^4 .

Embodiment 23. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

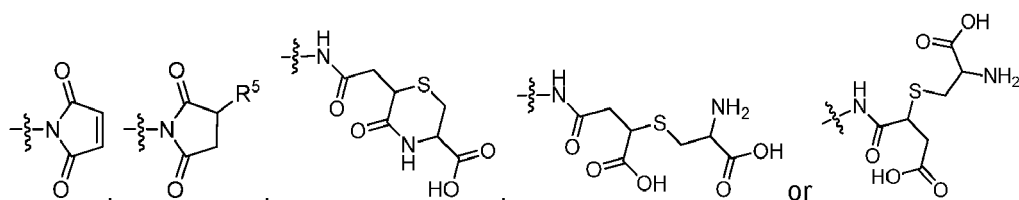
L_2 is $-(CH_2CH_2)^*$ or $-C(=O)(CH_2CH_2)^*$, where the * denotes attachment point to R^4 .

Embodiment 24. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

L_2 is $-C(=O)X_2X_3C(=O)(CH_2)_n^*$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n^*$, $-C(=O)(CH_2)_nC(R_7)_2^*$, $-C(=O)(CH_2)_nC(R_7)_2SS(CH_2)_nNHC(=O)(CH_2)_n^*$, or $-(CH_2)_nX_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n^*$, where the * denotes attachment point to R^4 .

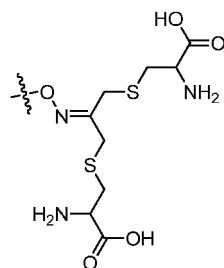
Embodiment 25. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

R^4 is



Embodiment 26. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

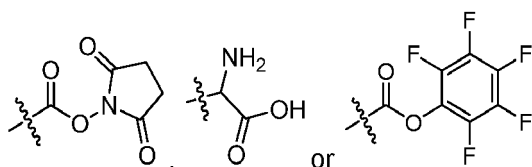
R^4 is $-ONH_2$,



or $-NH_2$.

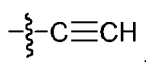
Embodiment 27. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

R^4 is

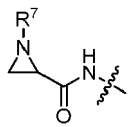


Embodiment 28. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:

R^4 is $-NHC(=O)CH=CH_2$, $-N_3$,

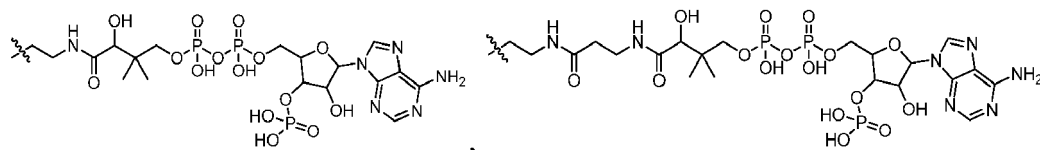
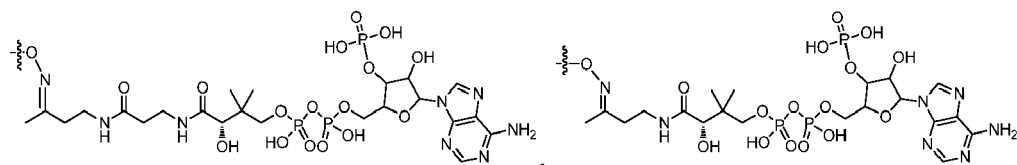
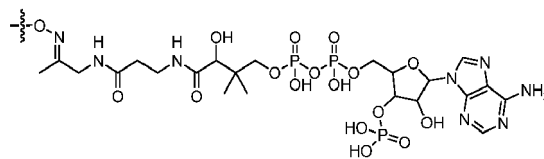
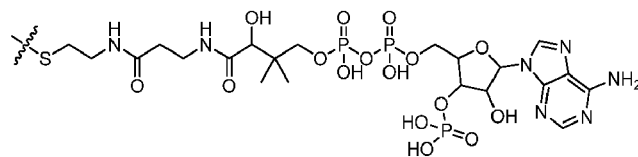
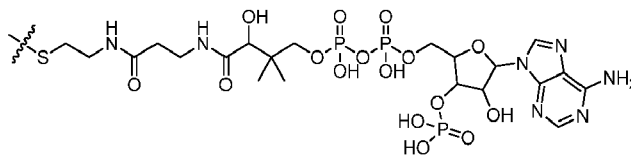


SH, -SSR⁶, -S(=O)₂(CH=CH₂), -(CH₂)₂S(=O)₂(CH=CH₂), -NHS(=O)₂(CH=CH₂), -NHC(=O)CH₂Br, -NHC(=O)CH₂I, -C(O)NHNH₂,

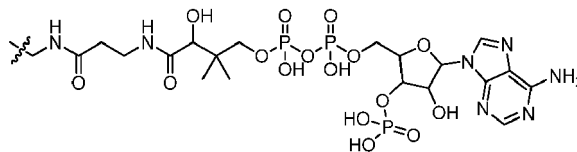


-CO₂H, -NHCH(=O) or -NHCH(=S).

Embodiment 29. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein:
R⁴ is

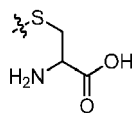


or

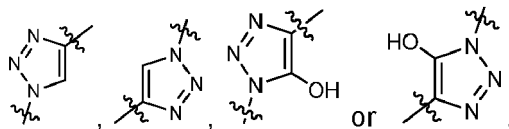


Embodiment 30. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: R⁴ is -SR⁷ or -OH.

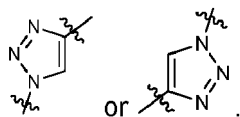
Embodiment 31. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein R⁵ is



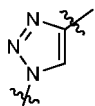
Embodiment 32. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_1 is



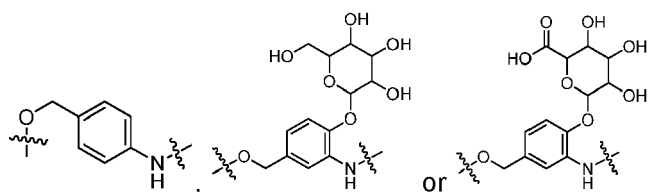
Embodiment 33. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_1 is



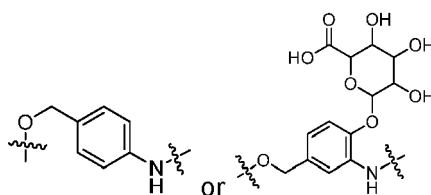
Embodiment 34. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_1 is



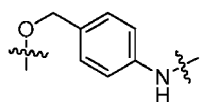
Embodiment 35. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_2 is



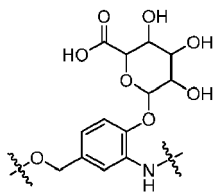
Embodiment 36. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_2 is



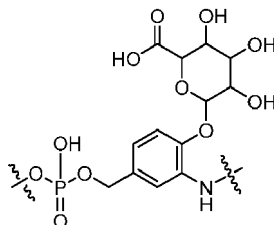
Embodiment 37. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_2 is



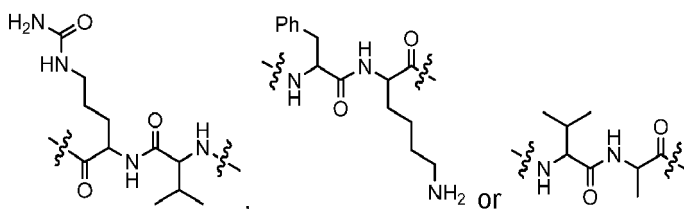
Embodiment 38. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_2 is



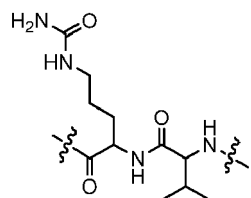
Embodiment 39. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_2 is



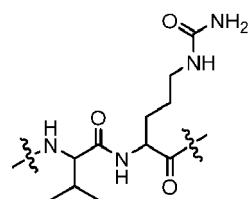
Embodiment 40. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_3 is



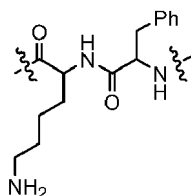
Embodiment 41. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_3 is



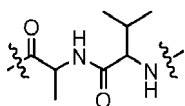
Embodiment 42. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_3 is



Embodiment 43. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_3 is



Embodiment 44. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: X_3 is



Embodiment 45. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: R^6 is 2-pyridyl or 4-pyridyl.

Embodiment 46. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: each R^7 is independently selected from H and C_1 - C_6 alkyl.

Embodiment 47. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: each R^7 is H.

Embodiment 48. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: each R^7 is C_1 - C_6 alkyl.

Embodiment 49. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: each m is independently selected from 1, 2, 3, and 4.

Embodiment 50. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: each m is 1 or 2.

Embodiment 51. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: each n is independently selected from 1, 2, 3, and 4.

Embodiment 52. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: each n is 2 or 3.

Embodiment 53. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

Embodiment 54. The compound of Formula (I), Formula (Ia) or Formula (Ib), wherein: each t is independently selected from 1, 2, 3, 4, 5 and 6.

Embodiment 55. The compound of Formula (I), Formula (Ia) or Formula (Ib) selected from:

1-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1 H-pyrrole-2,5-dione;
 (2R)-2-amino-3-((1-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)thio)propanoic acid;
 (6R)-6-(2-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-2-oxoethyl)-5-oxothiophosphorine-3-carboxylic acid;
 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 (S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 (R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 (R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 (S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1 H-pyrrole-2,5-dione;
 (2S)-2-amino-3-((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-2,5-dioxopyrrolidin-3-yl)thio)propanoic acid;
 (6R)-6-(2-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-2-oxoethyl)-5-oxothiophosphorine-3-carboxylic acid;
 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid;
 (S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid;
 (R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid;
 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid;
 (R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid;

(S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid;
 1-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)-1H-pyrrole-2,5-dione;
 5 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid;
 (S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid;
 10 (R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid;
 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid;
 (R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid;
 15 (S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid;
 1-(2-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-pyrrole-2,5-dione;
 (2R)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,19-dioxo-10,13,16-trioxa-4-thia-7-azanonadecan-1-oic acid;
 20 (2R,5S)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,19-dioxo-10,13,16-trioxa-4-thia-7-azanonadecan-1-oic acid;
 (2R,5R)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,19-dioxo-10,13,16-trioxa-4-thia-7-azanonadecan-1-oic acid;
 25 (19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,14-dioxo-4,7,10-trioxa-17-thia-13-azaicosan-20-oic acid;
 (16R,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,14-dioxo-4,7,10-trioxa-17-thia-13-azaicosan-20-oic acid;
 (16S,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,14-dioxo-4,7,10-trioxa-17-thia-13-azaicosan-20-oic acid;
 30 1-(21-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-21-oxo-3,6,9,12,15,18-hexaoxahenicosyl)-1H-pyrrole-2,5-dione;
 (2R)-2-amino-28-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,28-dioxo-10,13,16,19,22,25-hexaoxa-4-thia-7-azaotacosan-1-oic acid;
 35 (2R,5S)-2-amino-28-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,28-dioxo-10,13,16,19,22,25-hexaoxa-4-thia-7-azaotacosan-1-oic acid;
 (2R,5R)-2-amino-28-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,28-dioxo-10,13,16,19,22,25-hexaoxa-4-thia-7-azaotacosan-1-oic acid;
 40 (28R)-28-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-25-carboxy-1,23-dioxo-4,7,10,13,16,19-hexaoxa-26-thia-22-azanonacosan-29-oic acid;
 (25R,28R)-28-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-25-carboxy-1,23-dioxo-4,7,10,13,16,19-hexaoxa-26-thia-22-azanonacosan-29-oic acid;
 45 (25S,28R)-28-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-25-carboxy-1,23-dioxo-4,7,10,13,16,19-hexaoxa-26-thia-22-azanonacosan-29-oic acid;
 1-(((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrole-2,5-dione;
 50 (2R)-2-amino-3-((2-(((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-2-oxoethyl)thio)pentanedioic acid;
 N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide;
 55 (19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-(carboxymethyl)-1,11,15-trioxo-4,7-dioxo-17-thia-10,14-diazaicosan-20-oic acid;
 (16S,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-(carboxymethyl)-1,11,15-trioxo-4,7-dioxo-17-thia-10,14-diazaicosan-20-oic acid;

(16R,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-(carboxymethyl)-1,11,15-trioxo-4,7-dioxo-17-thia-10,14-diazaicosan-20-oic acid;
 (20R)-20-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-17-carboxy-1,11,15-trioxo-4,7-dioxo-18-thia-10,14-diazaheptacosan-21-oic acid;
 (17R,20R)-20-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-17-carboxy-1,11,15-trioxo-4,7-dioxo-18-thia-10,14-diazaheptacosan-21-oic acid;
 (17S,20R)-20-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-17-carboxy-1,11,15-trioxo-4,7-dioxo-18-thia-10,14-diazaheptacosan-21-oic acid;
 5-(4-((4-(3-aminopropyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine;
 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-(2-aminoethoxy)ethoxy)propan-1-one;
 N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide;
 (2R)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,9,19-trioxo-13,16-dioxo-4-thia-7,10-diazanonadecan-1-oic acid;
 (2R,5S)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,9,19-trioxo-13,16-dioxo-4-thia-7,10-diazanonadecan-1-oic acid;
 (2R,5R)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,9,19-trioxo-13,16-dioxo-4-thia-7,10-diazanonadecan-1-oic acid;
 (19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,11,14-trioxo-4,7-dioxo-17-thia-10,13-diazaicosan-20-oic acid;
 (16R,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,11,14-trioxo-4,7-dioxo-17-thia-10,13-diazaicosan-20-oic acid;
 (16S,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,11,14-trioxo-4,7-dioxo-17-thia-10,13-diazaicosan-20-oic acid;
 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)-N-(2-(2-(2-(4-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethyl)piperazine-1-carboxamide;
 3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 (S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 (R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 (R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 (S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 1-(2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)-1H-pyrrole-2,5-dione;
 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid;
 (S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid;
 (R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid;
 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid;
 (R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-

5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid;
 (S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid;
 1-((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrole-2,5-dione;
 3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 (S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 (R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 (R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 (S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid;
 N-(21-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-21-oxo-3,6,9,12,15,18-hexaoxahenicosyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide;
 4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate;
 (2R,3R,4R,5S)-6-4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid;
 (S)-1-(3-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1 H-pyrrole-2,5-dione;
 1-(3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1 H-pyrrole-2,5-dione;
 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 (S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 (R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 (R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 (S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid;
 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-(aminooxy)ethanone;
 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-aminoethoxy)propan-1-one;
 N-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)-2-(aminooxy)acetamide;
 (S)-1-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-2-(aminooxy)ethanone;
 (S)-1-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-(2-(2-aminoethoxy)ethoxy)propan-1-one;
 (S)-N-(2-(2-(3-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(aminooxy)acetamide;
 N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(aminooxy)acetamide;
 5-(4-((4-(2-(2-(aminooxy)ethoxy)ethyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine;
 N-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-

yl)propyl)-2-(aminooxy)acetamide;
 5-(4-((4-(2-(2-(2-aminoethoxy)ethoxy)ethyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidin-2,4-diamine;
 N-(2-(2-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethoxyethyl)-2-(aminooxy)acetamide;
 2,5-dioxopyrrolidin-1-yl 5-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate;
 (S)-2,5-dioxopyrrolidin-1-yl 5-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate;
 (S)-2-amino-6-(5-(4-(3-((2-amino-4-((S)-1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)hexanoic acid;
 (S)-2-amino-6-(5-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)hexanoic acid;
 2,5-dioxopyrrolidin-1-yl 5-((3-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)amino)-5-oxopentanoate;
 (S)-2-amino-6-(5-((3-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)amino)-5-oxopentanamido)hexanoic acid;
 2,5-dioxopyrrolidin-1-yl 5-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate;
 (S)-2-amino-6-(5-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)hexanoic acid;
 perfluorophenyl 5-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate;
 perfluorophenyl 3-(3-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)propanoate;
 perfluorophenyl 3-(2-(3-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)propanoate;
 (S)-2-amino-6-(3-(3-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)propanamido)hexanoic acid, and
 N-(15-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-15-oxo-3,6,9,12-tetraoxapentadecyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide.

Embodiment 56. The compound of Formula (I), Formula (Ia) or Formula (Ib) selected from:

1-(3-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1 H-pyrrole-2,5-dione;
 1-(2-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-pyrrole-2,5-dione;
 1-(2-(3-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)-1H-pyrrole-2,5-dione, and
 1-(2-(2-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)-1H-pyrrole-2,5-dione.

Embodiment 57. The compound of Formula (I), Formula (Ia) or Formula (Ib) selected from:

(2R,3R,4R,5S)-6-(4-(((4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid;
 4-((R)-6-amino-2-((S)-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-3-phenylpropanamido)hexanamido)benzyl 4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate;
 4-((S)-2-((S)-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)propanamido)benzyl 4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate;
 (2S,3S,4S,5R,6S)-6-(4-(((4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid;

(2S,3S,4S,5R,6S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyloxy)methyl)-2-(3-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid;
 N-(2-((5-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-methyl-5-oxopentan-2-yl)disulfanyl)ethyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide;
 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-4-methyl-4-(methylthio)pentan-1-one;
 (2S,3S,4S,5R,6S)-6-(4-(((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)(hydroxy)phosphoryloxy)methyl)-2-(3-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid;
 (2R,2'R)-3,3'-((2-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-oxoethoxy)imino)propane-1,3-diyl)bis(sulfanediyl))bis(2-aminopropanoic acid);
 (R)-2-amino-6-(((R)-2-amino-2-carboxyethyl)thio)methyl)-17-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-10,17-dioxo-8,14-dioxo-4-thia-7,11-diazaheptadec-6-enoic acid, and
 2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethan-1-ol.

[0060] Further, substitution with heavier isotopes, particularly deuterium (i.e., ^2H or D) may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements or an improvement in therapeutic index. It is understood that deuterium in this context is regarded as a substituent of a compound of the formula (I). The concentration of such a heavier isotope, specifically deuterium, may be defined by the isotopic enrichment factor. The term "isotopic enrichment factor" as used herein means the ratio between the isotopic abundance and the natural abundance of a specified isotope. If a substituent in a compound of this invention is denoted deuterium, such compound has an isotopic enrichment factor for each designated deuterium atom of at least 3500 (52.5% deuterium incorporation at each designated deuterium atom), at least 4000 (60% deuterium incorporation), at least 4500 (67.5% deuterium incorporation), at least 5000 (75% deuterium incorporation), at least 5500 (82.5% deuterium incorporation), at least 6000 (90% deuterium incorporation), at least 6333.3 (95% deuterium incorporation), at least 6466.7 (97% deuterium incorporation), at least 6600 (99% deuterium incorporation), or at least 6633.3 (99.5% deuterium incorporation).

[0061] Pharmaceutically acceptable solvates in accordance with the invention include those wherein the solvent of crystallization may be isotopically substituted, e.g. D_2O , d_6 -acetone, d_6 -DMSO.

Processes for Making Compounds of Formula (I) and subformulae thereof

[0062] General procedures for preparing compounds of Formula (I), and sub-Formulae thereof, are described herein. In the reactions described, reactive functional groups, for example hydroxy, amino, imino, thiol or carboxy groups, where these are desired in the final product, may be protected to avoid their unwanted participation in the reactions. Within the scope of this text, only a readily removable group that is not a constituent of the particular desired end product of the compounds of the present invention is designated a "protecting group", unless the context indicates otherwise. The protection of functional groups by such protecting groups, the protecting groups themselves, and their cleavage reactions are described for example in standard reference works, such as J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London and New York 1973, in T. W. Greene and P. G. M. Wuts, "Protective Groups in Organic Synthesis", Third edition, Wiley, New York 1999, in "The Peptides"; Volume 3 (editors: E. Gross and J. Meienhofer), Academic Press, London and New York 1981, in "Methoden der organischen Chemie" (Methods of Organic Chemistry), Houben Weyl, 4th edition, Volume 15/I, Georg Thieme Verlag, Stuttgart 1974, in H.-D. Jakubke and H. Jeschkeit, "Aminosäuren, Peptide, Proteine" (Amino acids, Peptides, Proteins), Verlag Chemie, Weinheim, Deerfield Beach, and Basel 1982, and in Jochen Lehmann, "Chemie der Kohlenhydrate: Monosaccharide und Derivate" (Chemistry of Carbohydrates: Monosaccharides and Derivatives), Georg Thieme Verlag, Stuttgart 1974. A characteristic of protecting groups is that they can be removed readily (i.e. without the occurrence of undesired secondary reactions) for example by solvolysis, reduction, photolysis or alternatively under physiological conditions (e.g. by enzymatic cleavage).

[0063] In certain embodiments, compounds of Formula (I) and subformulae thereof, provided herein are prepared as a pharmaceutically acceptable acid addition salt by reacting the free base form of a compound of Formula (I) and subformulae thereof, with a stoichiometric amount of an appropriate pharmaceutically acceptable organic acid or inorganic acid or a suitable anion exchange reagent.

[0064] Such reactions are typically carried out in water or in an organic solvent, or in a mixture of the two. Generally, use of non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile is desirable, where practicable.

[0065] Alternatively, the salt forms of compounds of Formula (I) and subformulae thereof, are prepared using salts of

the starting materials or intermediates.

[0066] Salts of compounds of the present invention having at least one salt-forming group may be prepared in a manner known to those skilled in the art. For example, salts of compounds of the present invention having acid groups may be formed, for example, by treating the compounds with metal compounds, such as alkali metal salts of suitable organic carboxylic acids, e.g. the sodium salt of 2-ethylhexanoic acid, with organic alkali metal or alkaline earth metal compounds, such as the corresponding hydroxides, carbonates or hydrogen carbonates, such as sodium or potassium hydroxide, carbonate or hydrogen carbonate, with corresponding calcium compounds or with ammonia or a suitable organic amine, stoichiometric amounts or only a small excess of the salt-forming agent preferably being used. Acid addition salts of compounds of the present invention are obtained in customary manner, e.g. by treating the compounds with an acid or a suitable anion exchange reagent. Internal salts of compounds of the present invention containing acid and basic salt-forming groups, e.g. a free carboxy group and a free amino group, may be formed, e.g. by the neutralisation of salts, such as acid addition salts, to the isoelectric point, e.g. with weak bases, or by treatment with ion exchangers.

[0067] Salts can be converted into the free compounds in accordance with methods known to those skilled in the art. Metal and ammonium salts can be converted, for example, by treatment with suitable acids, and acid addition salts, for example, by treatment with a suitable basic agent.

[0068] All the above-mentioned process steps can be carried out under reaction conditions that are known to those skilled in the art, including those mentioned specifically, in the absence or, customarily, in the presence of solvents or diluents, including, for example, solvents or diluents that are inert towards the reagents used and dissolve them, in the absence or presence of catalysts, condensation or neutralizing agents, for example ion exchangers, such as cation exchangers, e.g. in the H^+ form, depending on the nature of the reaction and/or of the reactants at reduced, normal or elevated temperature, for example in a temperature range of from about $-100\text{ }^{\circ}\text{C}$ to about $190\text{ }^{\circ}\text{C}$, including, for example, from approximately $-80\text{ }^{\circ}\text{C}$ to approximately $150\text{ }^{\circ}\text{C}$, for example at from -80 to $-60\text{ }^{\circ}\text{C}$, at room temperature, at from -20 to $40\text{ }^{\circ}\text{C}$ or at reflux temperature, under atmospheric pressure or in a closed vessel, where appropriate under pressure, and/or in an inert atmosphere, for example under an argon or nitrogen atmosphere.

[0069] Pharmaceutically acceptable acid addition salts of compounds of Formula (I) and subformulae thereof, include acetate, adipate, ascorbate, aspartate, benzoate, besylate, benzenesulfonate, bicarbonate/carbonate, bisulfate/sulfate, bromide/hydrobromide, camphor sulfonate, camsylate, caprate, chloride/hydrochloride, chlorotheophyllinate, citrate, edisylate, ethanedisulfonate, fumarate, gluceptate, glucoheptonate, gluconate, glucuronate, glutamate, glutarate, glycolate, hippurate, hydroiodide/iodide, isethionate, lactate, lactobionate, laurylsulphate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, naphthoate, napsylate, 2-napsylate, naphthalenesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, octadecanoate, oleate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, polygalacturonate, propionate, sebacate, stearate, succinate, sulfosalicylate, sulfate, tartrate, tosylate, p-toluenesulfonate, trifluoroacetate, trifenatate, triphenylacetate and xinafoate salt forms.

[0070] The organic acid or inorganic acids used to form certain pharmaceutically acceptable acid addition salts of compounds of Formula (I) and subformulae thereof, include acetic acid, adipic acid, ascorbic acid, aspartic acid, benzoic acid, benzenesulfonic acid, carbonic acid, camphor sulfonic acid, capric acid, chlorotheophyllinate, citric acid, ethanedisulfonic acid, fumaric acid, D-glycero-D-gulo-Heptonicacid, galactaric acid, galactaric acid/mucic acid, gluceptic ethanedisulfonic acid, fumaric acid, D-glycero-D-gulo-Heptonicacid, galactaric acid, galactaric acid/mucic acid, gluceptic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, hydroiodic acid, isethionic acid, lactic acid, lactobionic acid, lauryl sulfuric acid, malic acid, maleic acid, malonic acid, mandelic acid, mesylic acid, methanesulfonic acid, mucic acid, naphthoic acid, 1-hydroxy-2-naphthoic acid, naphthalenesulfonic acid, 2-naphthalenesulfonic acid, nicotinic acid, nitric acid, octadecanoic acid, oleic acid, oxalic acid, palmitic acid, pamoic acid, phosphoric acid, polygalacturonic acid, propionic acid, sebacic acid, stearic acid, succinic acid, sulfosalicylic acid, sulfuric acid, tartaric acid, p-toluenesulfonic acid, trifluoroacetic acid and triphenylacetic acid.

[0071] In one embodiment, the present invention provides 3-(3-fluoro-4-(3-(piperidin-4-yl)propoxy)phenyl)-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine in an acetate, adipate, ascorbate, aspartate, benzoate, besylate, benzenesulfonate, bicarbonate/carbonate, bisulfate/sulfate, bromide/hydrobromide, camphor sulfonate, camsylate, caprate, chloride/hydrochloride, chlorotheophyllonate, citrate, edisylate, ethanedisulfonate, fumarate, gluceptate, glucoheptonate, gluconate, glucuronate, glutamate, glutarate, glycolate, hippurate, hydroiodide/iodide, isethionate, lactate, lactobionate, laurylsulphate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, naphthoate, napsylate, 2-napsylate, naphthalenesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, octadecanoate, oleate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, polygalacturonate, propionate, sebacate, stearate, succinate, sulfosalicylate, sulfate, tartrate, tosylate, p-toluenesulfonate, trifluoroacetate, trifenatate, triphenylacetate or xinafoate salt form.

[0072] In one embodiment, the present invention provides 3-(4-(((1r,4r)-4-aminocyclohexyl)methoxy)-3-fluorophenyl)-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine in an acetate, adipate, ascorbate, aspartate, benzoate, besylate, benzenesulfonate, bicarbonate/carbonate, bisulfate/sulfate, bromide/hydrobromide, camphor sulfonate, camsylate, caprate,

chloride/hydrochloride, chlorthephyllonate, citrate, edisylate, ethanedisulfonate, fumarate, gluceptate, glucoheptonate, gluconate, glucuronate, glutamate, glutarate, glycolate, hippurate, hydroiodide/iodide, isethionate, lactate, lactobionate, laurylsulphate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, naphthoate, napsylate, 2-napsylate, naphthalenesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, octadecanoate, oleate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, polygalacturonate, propionate, sebacate, stearate, succinate, sulfosalicylate, sulfate, tartrate, tosylate, p-toluenesulfonate, trifluoroacetate, trifenatate, triphenylacetate or xinafoate salt form.

[0073] In one embodiment, the present invention provides 3-(4-((4-aminobicyclo[2.2.2]octan-1-yl)methoxy)-3-fluorophenyl)-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine in an acetate, adipate, ascorbate, aspartate, benzoate, besylate, benzenesulfonate, bicarbonate/carbonate, bisulfate/sulfate, bromide/hydrobromide, camphor sulfonate, camsylate, caprate, chloride/hydrochloride, chlorthephyllonate, citrate, edisylate, ethanedisulfonate, fumarate, gluceptate, glucoheptonate, gluconate, glucuronate, glutamate, glutarate, glycolate, hippurate, hydroiodide/iodide, isethionate, lactate, lactobionate, laurylsulphate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, naphthoate, napsylate, 2-napsylate, naphthalenesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, octadecanoate, oleate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, polygalacturonate, propionate, sebacate, stearate, succinate, sulfosalicylate, sulfate, tartrate, tosylate, p-toluenesulfonate, trifluoroacetate, trifenatate, triphenylacetate or xinafoate salt form.

[0074] In one embodiment, the present invention provides 3-(4-((4-aminobicyclo[2.2.2]octan-1-yl)methoxy)-3-chlorophenyl)-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine in an acetate, adipate, ascorbate, aspartate, benzoate, besylate, benzenesulfonate, bicarbonate/carbonate, bisulfate/sulfate, bromide/hydrobromide, camphor sulfonate, camsylate, caprate, chloride/hydrochloride, chlorthephyllonate, citrate, edisylate, ethanedisulfonate, fumarate, gluceptate, glucoheptonate, gluconate, glucuronate, glutamate, glutarate, glycolate, hippurate, hydroiodide/iodide, isethionate, lactate, lactobionate, laurylsulphate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, naphthoate, napsylate, 2-napsylate, naphthalenesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, octadecanoate, oleate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, polygalacturonate, propionate, sebacate, stearate, succinate, sulfosalicylate, sulfate, tartrate, tosylate, p-toluenesulfonate, trifluoroacetate, trifenatate, triphenylacetate or xinafoate salt form.

[0075] In one embodiment, the present invention provides 4-((2-chloro-4-(6-methoxy-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenoxy)methyl)bicyclo[2.2.2]octan-1-amine in an acetate, adipate, ascorbate, aspartate, benzoate, besylate, benzenesulfonate, bicarbonate/carbonate, bisulfate/sulfate, bromide/hydrobromide, camphor sulfonate, camsylate, caprate, chloride/hydrochloride, chlorthephyllonate, citrate, edisylate, ethanedisulfonate, fumarate, gluceptate, glucoheptonate, gluconate, glucuronate, glutamate, glutarate, glycolate, hippurate, hydroiodide/iodide, isethionate, lactate, lactobionate, laurylsulphate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, naphthoate, napsylate, 2-napsylate, naphthalenesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, octadecanoate, oleate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, polygalacturonate, propionate, sebacate, stearate, succinate, sulfosalicylate, sulfate, tartrate, tosylate, p-toluenesulfonate, trifluoroacetate, trifenatate, triphenylacetate or xinafoate salt form.

[0076] Lists of additional suitable acid addition salts can be found, e.g., in "Remington's Pharmaceutical Sciences", 20th ed., Mack Publishing Company, Easton, Pa., (1985); and in "Handbook of Pharmaceutical Salts: Properties, Selection, and Use" by Stahl and Wermuth mentioned specifically or, for example, water, esters, such as lower alkyl-lower alkanooates, for example ethyl acetate, ethers, such as aliphatic ethers, for example diethyl ether, or cyclic ethers, for example tetrahydrofuran or dioxane, liquid aromatic hydrocarbons, such as benzene or toluene, alcohols, such as methanol, ethanol or 1- or 2-propanol, nitriles, such as acetonitrile, halogenated hydrocarbons, such as methylene chloride or chloroform, acid amides, such as dimethylformamide or dimethyl acetamide, bases, such as heterocyclic nitrogen bases, for example pyridine or N-methylpyrrolidin-2-one, carboxylic acid anhydrides, such as lower alkanic acid anhydrides, for example acetic anhydride, cyclic, linear or branched hydrocarbons, such as cyclohexane, hexane or isopentane, methycyclohexane, or mixtures of those solvents, for example aqueous solutions, unless otherwise indicated in the description of the processes. Such solvent mixtures may also be used in working up, for example by chromatography or partitioning.

[0077] In certain embodiments, compounds of Formula (I) and subformulae thereof, are prepared or formed, as solvates (e.g., hydrates). In certain embodiments, hydrates of compounds of Formula (I) and subformulae thereof, are prepared by recrystallization from an aqueous/organic solvent mixture, using organic solvents such as dioxin, tetrahydrofuran or methanol. Furthermore, the compounds of the present invention, including their salts, can also be obtained in the form of their hydrates, or include other solvents used for their crystallization.

[0078] The compounds of the present invention may inherently or by design form solvates with pharmaceutically acceptable solvents (including water); therefore, it is intended that the invention embrace both solvated and unsolvated forms. The term "solvate" refers to a molecular complex of a compound of the present invention (including pharmaceutically acceptable salts thereof) with one or more solvent molecules. Such solvent molecules are those commonly used

in the pharmaceutical art, which are known to be innocuous to the recipient, e.g., water, ethanol. The term "hydrate" refers to the complex where the solvent molecule is water.

[0079] Any asymmetric atom (e.g., carbon or the like) of the compound(s) of the present invention can be present in racemic or enantiomerically enriched, for example the (*R*)-, (*S*)- or (*R,S*)- configuration. In certain embodiments, each asymmetric atom has at least 50 % enantiomeric excess, at least 60 % enantiomeric excess, at least 70 % enantiomeric excess, at least 80 % enantiomeric excess, at least 90 % enantiomeric excess, at least 95 % enantiomeric excess, or at least 99 % enantiomeric excess in the (*R*)- or (*S*)- configuration. Substituents at atoms with unsaturated double bonds may, if possible, be present in *cis*- (*Z*)- or *trans*- (*E*)- form.

[0080] Accordingly, as used herein a compound of the present invention can be in the form of one of the possible isomers, rotamers, atropisomers, tautomers or mixtures thereof, for example, as substantially pure geometric (*cis* or *trans*) isomers, diastereomers, optical isomers (antipodes), racemates or mixtures thereof.

[0081] Any resulting mixtures of isomers can be separated on the basis of the physicochemical (antipodes), racemates or mixtures thereof.

[0082] Any resulting mixtures of isomers can be separated on the basis of the physicochemical differences of the constituents, into the pure or substantially pure geometric or optical isomers, diastereomers, racemates, for example, by chromatography and/or fractional crystallization.

[0083] Any resulting racemates of final products or intermediates can be resolved into the optical antipodes by known methods, e.g., by separation of the diastereomeric salts thereof, obtained with an optically active acid or base, and liberating the optically active acidic or basic compound. In particular, a basic moiety may thus be employed to resolve the compounds of the present invention into their optical antipodes, e.g., by fractional crystallization of a salt formed with an optically active acid, e.g., tartaric acid, dibenzoyl tartaric acid, diacetyl tartaric acid, di-*O,O'*-*p*-toluoyl tartaric acid, mandelic acid, malic acid or camphor-10-sulfonic acid. Racemic products can also be resolved by chiral chromatography, e.g., high pressure liquid chromatography (HPLC) using a chiral adsorbent.

[0084] In certain embodiments, compounds of Formula (I), or subformulae thereof, are prepared as their individual stereoisomers. In other embodiments, the compounds of Formula (I), or subformulae thereof, are prepared as their individual stereoisomers by reacting a racemic mixture of the compound with an optically active resolving agent to form a pair of diastereoisomeric compounds, separating the diastereomers and recovering the optically pure enantiomers. In certain embodiments, resolution of enantiomers is carried out using covalent diastereomeric derivatives of the compounds of Formula (I), or subformulae thereof, or by using dissociable complexes (e.g., crystalline diastereomeric salts). Diastereomers have distinct physical properties (e.g., melting points, boiling points, solubility, reactivity, etc.) and are readily separated by taking advantage of these dissimilarities. In certain embodiments, the diastereomers are separated by chromatography, or by separation/resolution techniques based upon differences in solubility. The optically pure enantiomer is then recovered, along with the resolving agent, by any practical means that would not result in racemization. A more detailed description of the techniques applicable to the resolution of stereoisomers of compounds from their racemic mixture can be found in Jean Jacques, Andre Collet, Samuel H. Wilen, "Enantiomers, Racemates and Resolutions," John Wiley And Sons, Inc., 1981.

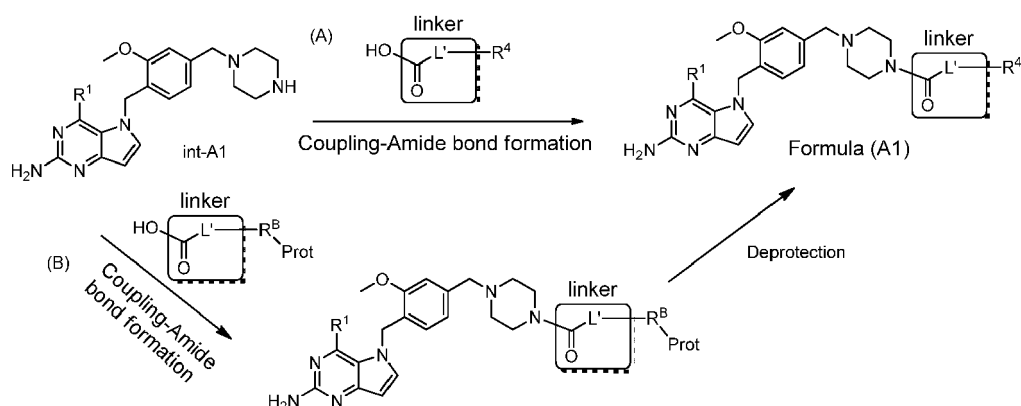
[0085] Mixtures of isomers obtainable according to the invention can be separated in a manner known to those skilled in the art into the individual isomers; diastereoisomers can be separated, for example, by partitioning between polyphasic solvent mixtures, recrystallisation and/or chromatographic separation, for example over silica gel or by e.g. medium pressure liquid chromatography over a reversed phase column, and racemates can be separated, for example, by the formation of salts with optically pure salt-forming reagents and separation of the mixture of diastereoisomers so obtainable, for example by means of fractional crystallisation, or by chromatography over optically active column materials. or as mixtures thereof, for example as pure optical isomers, or as isomer mixtures, such as racemates and diastereoisomer mixtures, depending on the number of asymmetric carbon atoms. The present invention is meant to include all such possible isomers, including racemic mixtures, diastereomeric mixtures and optically pure forms. Optically active (*R*)- and (*S*)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. If the compound contains a double bond, the substituent may be *E* or *Z* configuration. If the compound contains a disubstituted cycloalkyl, the cycloalkyl substituent may have a *cis*- or *trans*-configuration. All tautomeric forms are also intended to be included.

[0086] Intermediates and final products can be worked up and/or purified according to standard methods, e.g. using chromatographic methods, distribution methods, (re-) crystallization. The invention relates also to those forms of the process in which a compound obtainable as an intermediate at any stage of the process is used as starting material and the remaining process steps are carried out, or in which a starting material is formed under the reaction conditions or is used in the form of a derivative, for example in a protected form or in the form of a salt, or a compound obtainable by the process according to the invention is produced under the process conditions and processed further *in situ*. All starting materials, building blocks, reagents, acids, bases, dehydrating agents, solvents and catalysts utilized to synthesize the compounds of the present invention are either commercially available or can be produced by organic synthesis methods known to one of ordinary skill in the art.

[0087] Compounds of Formula (I) and subformulae thereof (Formula (Ia) and Formula (Ib)) are made by processes described in the general schemes herein and as illustrated in the Examples.

[0088] Scheme 1A illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (A1) where the -linker- R^4 moiety is attached to intermediate (int-A1) by an amide bond. In Scheme 1A the linker is any linker (L') having a terminal carbonyl moiety (i.e. $-L'-C(=O)-$). Also in Scheme 1A, R^1 is as described herein and R^4 is a reactive moiety which can react with a thiol, a disulfide, an amine, a ketone, a diketone, an azide or an alkyne. Scheme 1B illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (A1) where the -linker- R^4 moiety is attached to intermediate (int-A1) by an amide bond. In Scheme 1B the linker is any linker (L') having a terminal carbonyl moiety (i.e. $-L'-C(=O)-$). Also in Scheme 1B, R^1 is as described herein and R^4 moiety having an amino group (such as a hydroxyl amine or an amine) and R^B is moiety having a protected amino group, where Prot is a protecting group such as Boc, Fmoc and Cbz.

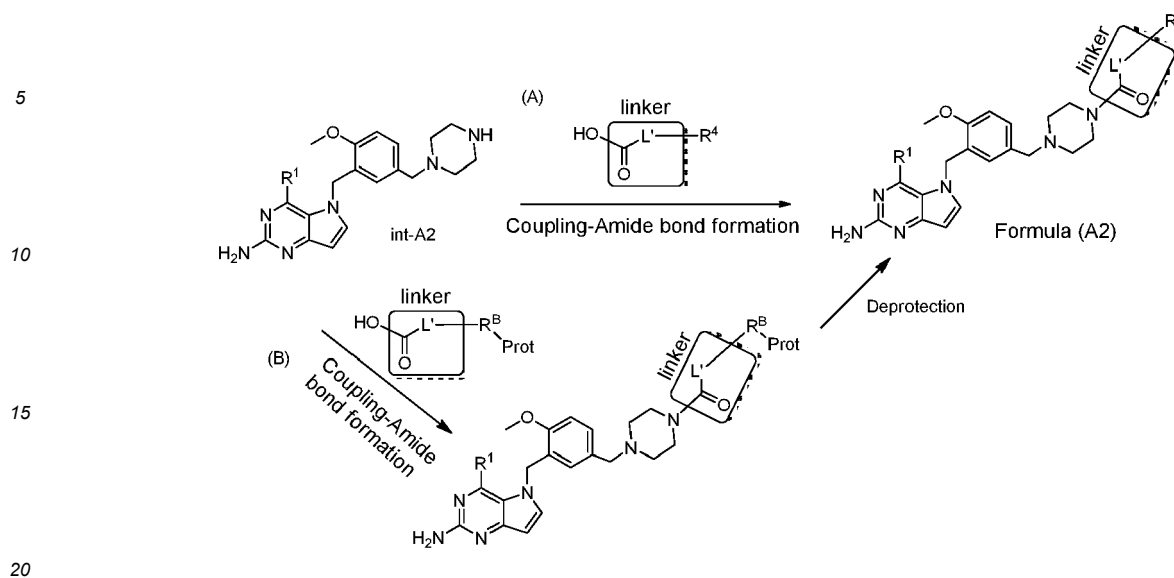
Scheme 1



Such amide bond formation can be accomplished using heat, EDCI coupling, HATU coupling, HBTU coupling, TBTU coupling or T3P coupling.

[0089] Scheme 2A illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (A2) where the -linker- R^4 moiety is attached to intermediate (int-A2) by an amide bond. In Scheme 2A the linker is any linker (L') having a terminal carbonyl moiety (i.e. $-L'-C(=O)-$). Also in Scheme 2A, R^1 is as described herein and R^4 is a reactive moiety which can react with a thiol, a disulfide, an amine, a ketone, a diketone, an azide or an alkyne. Scheme 2B illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (A2) where the -linker- R^4 moiety is attached to intermediate (int-A2) by an amide bond. In Scheme 2B the linker is any linker (L') having a terminal carbonyl moiety (i.e. $-L'-C(=O)-$). Also in Scheme 2B, R^1 is as described herein and R^4 moiety having an amino group (such as a hydroxyl amine or an amine) and R^B is moiety having a protected amino group, where Prot is a protecting group such as Boc, Fmoc and Cbz.

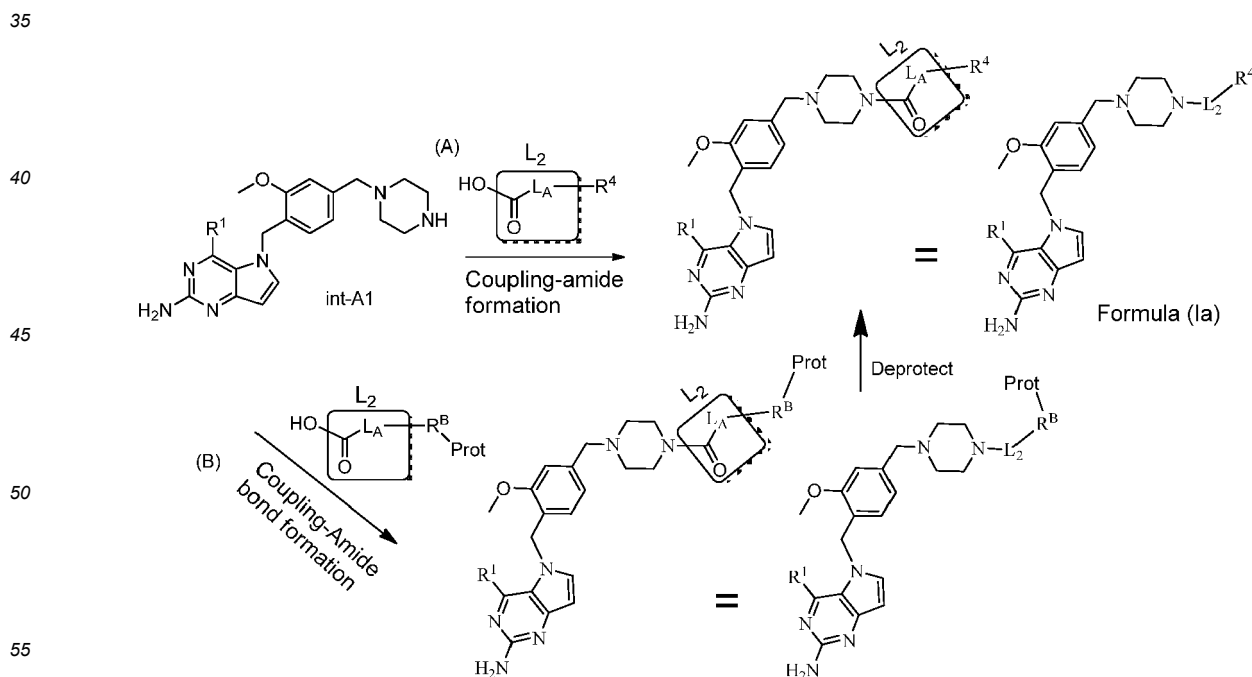
Scheme 2



Such amide bond formation can be accomplished using heat, EDCI coupling, HATU coupling, HBTU coupling, TBTU coupling or T3P coupling.

[0090] Scheme 3A illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (Ia) wherein the -L₂-R⁴ moiety is attached to intermediate (int-A1) by an amide bond. Such amide bond formation can be accomplished using heat, EDCI coupling, HATU coupling, HBTU coupling, TBTU coupling or T3P coupling. In Scheme 3A the linker (L₂) comprises a linker moiety (L_A) having a terminal carbonyl moiety (i.e. -L_A-C(=O)). Scheme 3B illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (I) wherein the -L₂-R⁴ moiety is attached to intermediate (int-A1) by an amide bond. Such amide bond formation can be accomplished using heat, EDCI coupling, HATU coupling, HBTU coupling, TBTU coupling or T3P coupling. In Scheme 3B the linker (L₂) comprises a linker moiety (L_A) having a terminal carbonyl moiety (i.e. -L_A-C(=O)), and R^B is moiety having a protected amino group, where Prot is a protecting group such as Boc, Fmoc and Cbz.

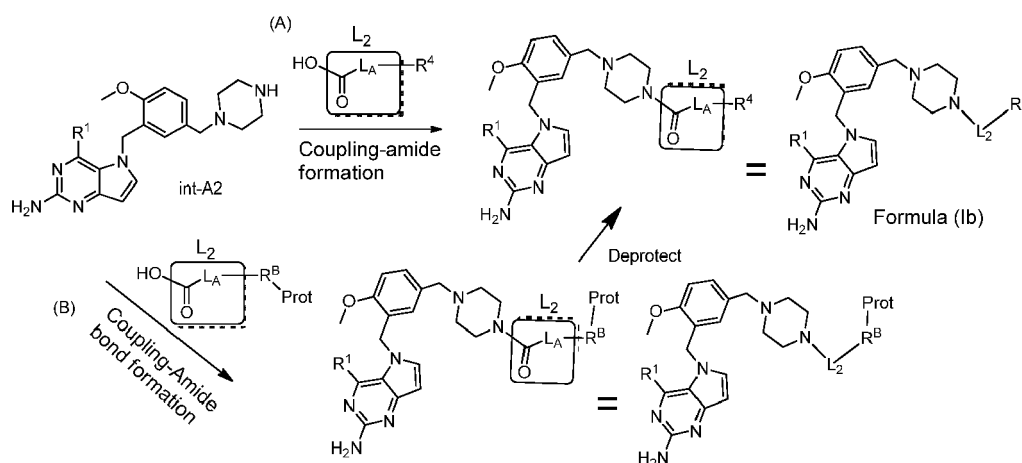
Scheme 3



Scheme 4A illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (Ib) wherein the

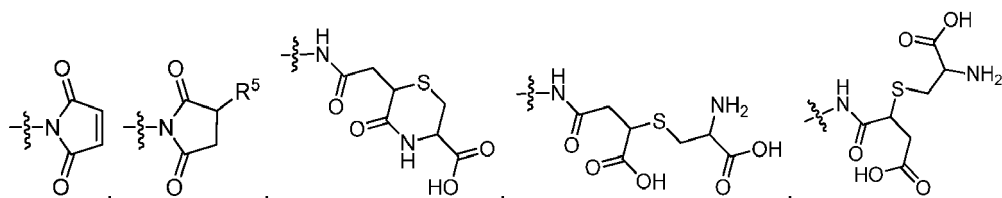
$-L_2-R^4$ moiety is attached to intermediate (int-A2) by an amide bond. Such amide bond formation can be accomplished using heat, EDCI coupling, HATU coupling, HBTU coupling, TBTU coupling or T3P coupling. In Scheme 4A the linker (L_2) comprises a linker moiety (L_A) having a terminal carbonyl moiety (i.e. $-L_A-C(=O)-$). Scheme 4B illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (Ib) wherein the $-L_2-R^4$ moiety is attached to intermediate (int-A2) by an amide bond. Such amide bond formation can be accomplished using heat, EDCI coupling, HATU coupling, HBTU coupling, TBTU coupling or T3P coupling. In Scheme 4B the linker (L_2) comprises a linker moiety (L_A) having a terminal carbonyl moiety (i.e. $-L_A-C(=O)-$), and R^B is moiety having a protected amino group, where Prot is a protecting group such as Boc, Fmoc and Cbz.

Scheme 4

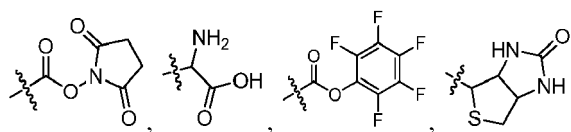


[0091] In Schemes 3 and 4,

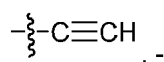
R^4 is



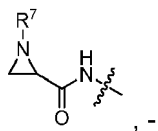
$-ONH_2$, $-NH_2$,



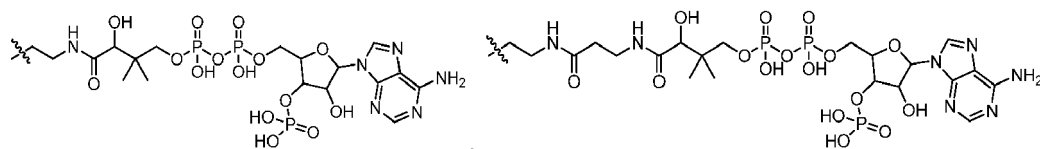
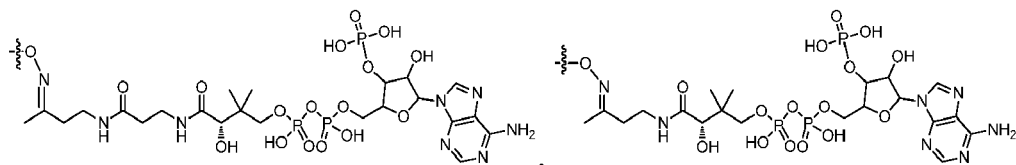
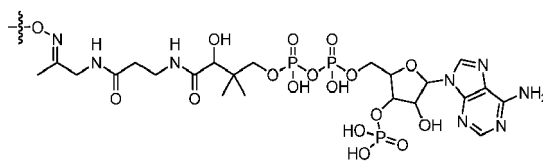
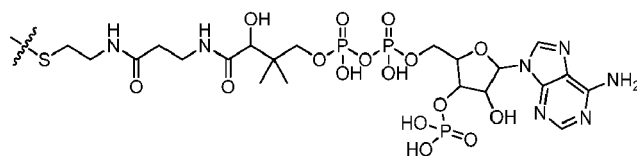
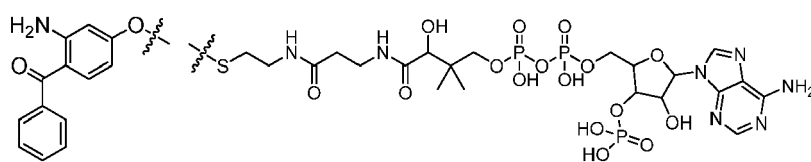
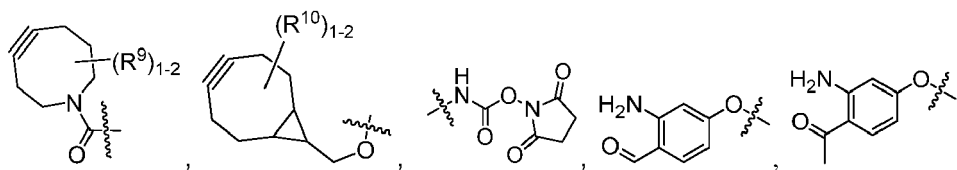
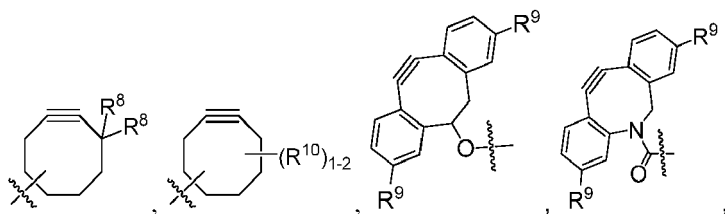
$-N_3$,



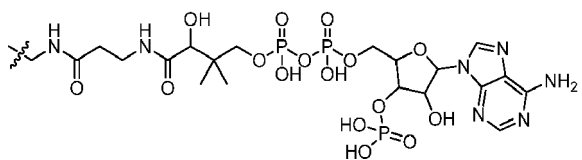
$NHC(=O)CH=CH_2$, SH , $-SSR^6$, $-S(=O)_2(CH=CH_2)$, $-(CH_2)_2S(=O)_2(CH=CH_2)$, $-NHS(=O)_2(CH=CH_2)$,
 $-NHC(=O)CH_2Br$, $-NHC(=O)CH_2I$, $-C(O)NHNH_2$,



CO_2H , $-\text{C}(\text{O})\text{NHNH}_2$,



or

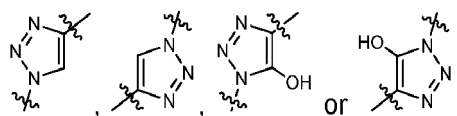


R^B is $-ONH-$;

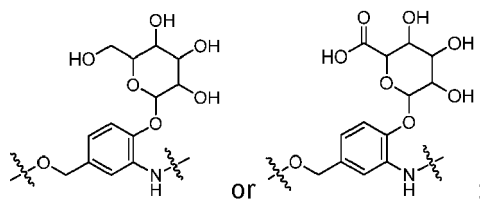
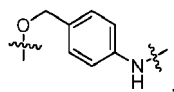
L_A is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n-$, $-NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, or $-(CH_2)_nC(=O)NH(CH_2)_n$;

L_2 is $-C(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

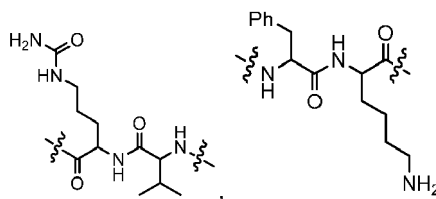
where X_1 is



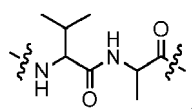
X_2 is



and X_3 is



or



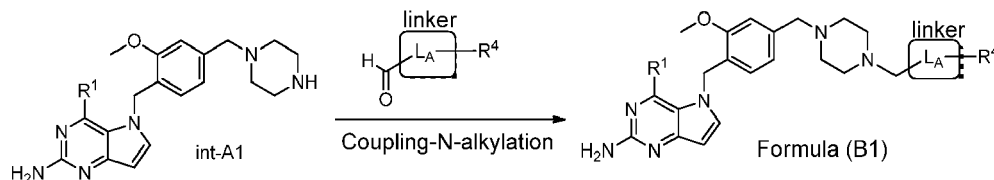
and

R^1 , R^7 , R^8 , R^9 and R^{10} , are as defined herein.

[0092] Scheme 5 illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (B1) where

the -linker-R⁴ moiety is attached to intermediate (int-A1) by alkylation of the secondary amine of intermediate (int-A1). In Scheme 5 the linker (L_A) is initially functionalized with a terminal aldehyde (i.e. -L_A-C(=O)H) and then reacted with the secondary amine of intermediate (int-A1). Also in Scheme 5, R¹ is as described herein and R⁴ is a reactive moiety which can react with a thiol, a disulfide, an amine, a ketone, a diketone, an azide or an alkyne.

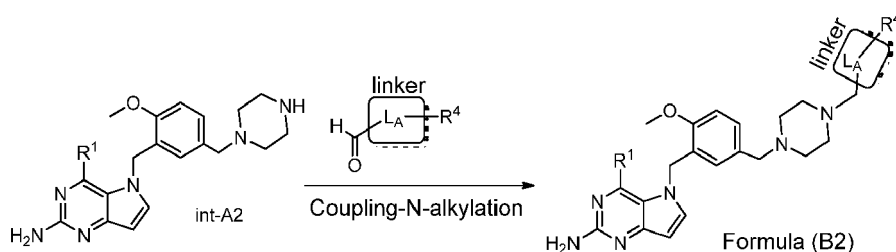
Scheme 5



Such N-alkylation can be accomplished using a reducing agent such as NaCNBH₃, NaBH₄ or NaBH(OAC)₃.

[0093] Scheme 6 illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (B2) where the -linker-R⁴ moiety is attached to intermediate (int-A2) by alkylation of the secondary amine of intermediate (int-A2). In Scheme 6 the linker (L_A) is initially functionalized with a terminal aldehyde (i.e. -L_A-C(=O)H) and then reacted with the secondary amine of intermediate (int-A2). Also in Scheme 6, R¹ is as described herein and R⁴ is a reactive moiety which can react with a thiol, a disulfide, an amine, a ketone, a diketone, an azide or an alkyne.

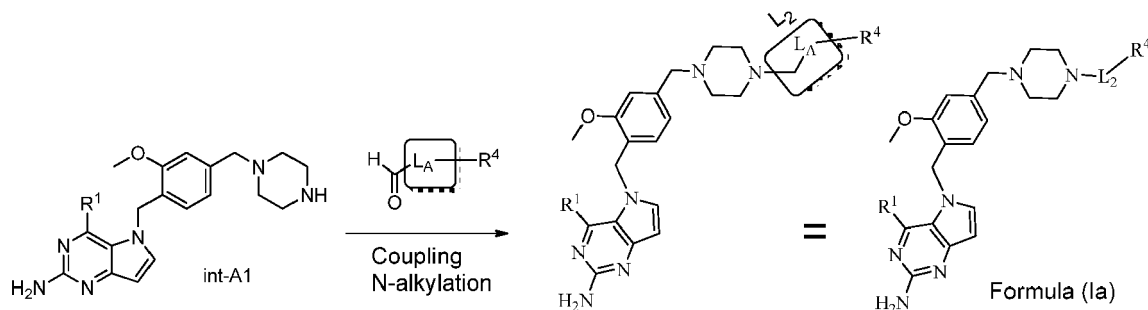
Scheme 6



Such N-alkylation can be accomplished using a reducing agent such as NaCNBH₃, NaBH₄ or NaBH(OAC)₃.

[0094] Scheme 7 illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (Ia) wherein the -L₂-R⁴ moiety is attached to intermediate (int-A1) by alkylation of the secondary amine of intermediate (int-A1). In Scheme 7 the linker moiety, L_A, initially functionalized with a terminal aldehyde (i.e. -L'₁-C(=O)H) is then reacted with the secondary amine of intermediate (int-A1), thereby forming the linker, L₂, which comprises the linker moiety L_A with a terminal -CH₂- group. Such N-alkylation can be accomplished using a reducing agent such as NaCNBH₃, NaBH₄ or NaBH(OAC)₃.

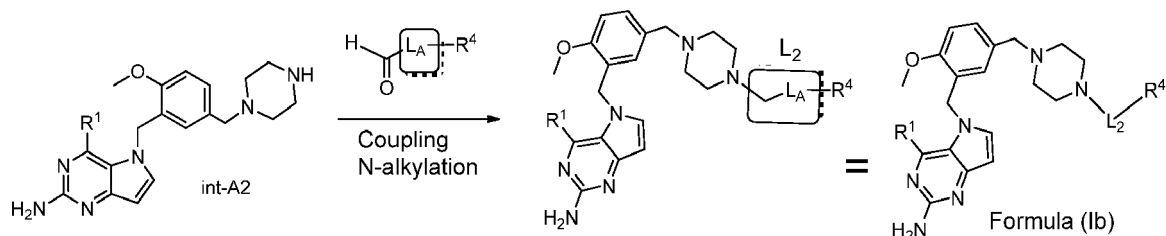
Scheme 7



Scheme 8 illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (Ib) wherein the -L₂-R⁴ moiety is attached to intermediate (int-A2) by alkylation of the secondary amine of intermediate (int-A2). In Scheme 8 the linker moiety (L_A) initially functionalized with a terminal aldehyde (i.e. -L'₁-C(=O)H) which is then reacted with the

secondary amine of intermediate (int-A2), thereby forming the linker, L_2 , which comprises the linker moiety L_A with a terminal $-CH_2-$ group. Such N-alkylation can be accomplished using a reducing agent such as $NaCNBH_3$, $NaBH_4$ or $NaBH(OAc)_3$.

Scheme 8

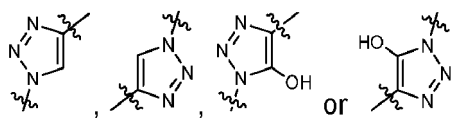


[0095] In Schemes 7 and 8,

R^4 is as defined for Schemes 3 and 4;

L_A is $-(CH_2)_{(n-1)-}$, $-((CH_2)_{(n-1)}O)((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_{(n-1)}X_1(CH_2)_n-$, $-(CH_2)_{(n-1)}NHC(=O)(CH_2)_n-$, $-(CH_2)_{(n-1)}NHC(=O)(CH_2)_nC(=O)NH(CH_2)_n-$ or $-((CH_2)_{(n-1)}O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$;

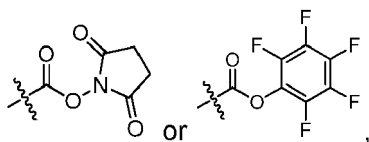
L_2 is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n-$ or $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$; where X_1 is



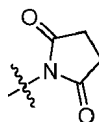
and

R^1 and R^7 are as defined herein.

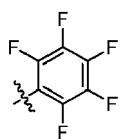
[0096] Scheme 9 illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (A1) where the -linker- R^4 moiety is attached to intermediate (int-A1) by an amide bond. In Scheme 9 the linker is any linker (L') having a terminal carbonyl moiety (i.e. $-L'-C(=O)-$). Also in Scheme 9, R^1 is as described herein, R^4 is



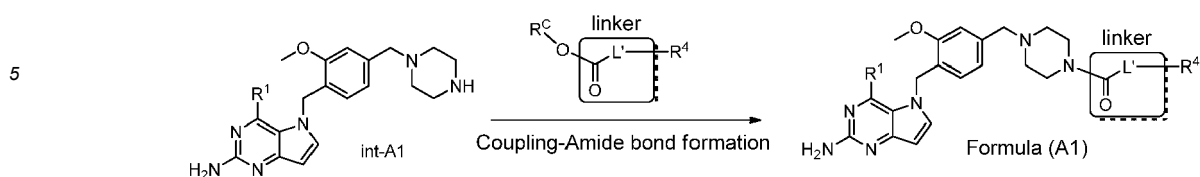
and R^C is



or



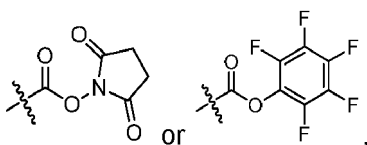
Scheme 9



10 Such amide bond formation can be accomplished using heat, EDCI coupling, HATU coupling, HBTU coupling, TBTU coupling or T3P coupling.

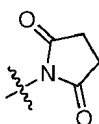
[0097] Scheme 10 illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (A2) where the -linker-R⁴ moiety is attached to intermediate (int-A2) by an amide bond. In Scheme 10 the linker is any linker (L') having a terminal carbonyl moiety (i.e. -L'-C(=O)). Also in Scheme 10, R¹ is as described herein, R⁴ is

15

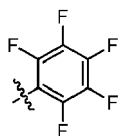


and R^C is

25

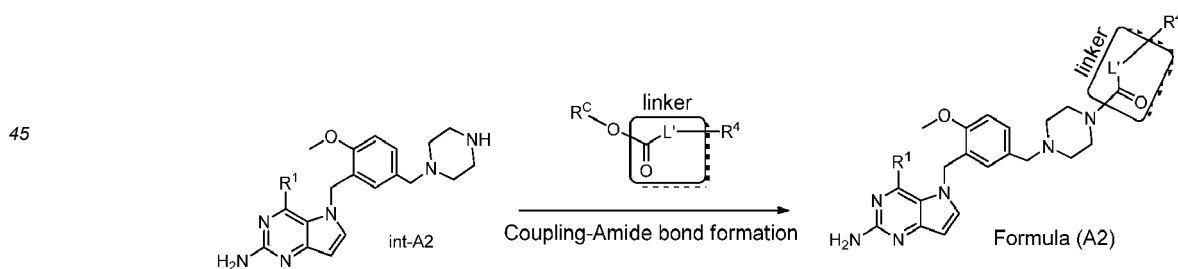


30 or



Scheme 10

40



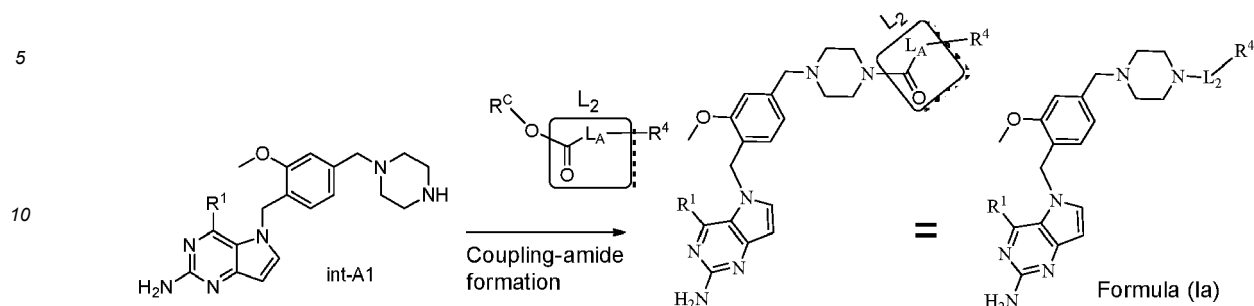
50

Such amide bond formation can be accomplished using heat, EDCI coupling, HATU coupling, HBTU coupling, TBTU coupling or T3P coupling.

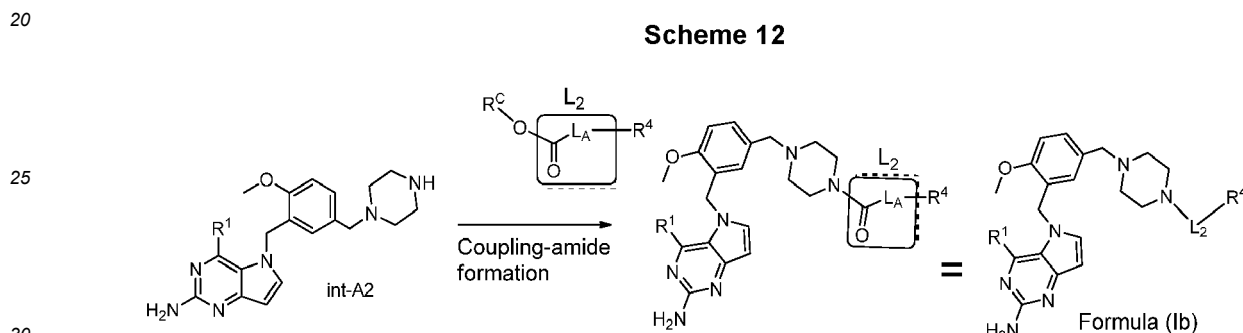
[0098] Scheme 11 illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (1a) wherein the -L₂-R⁴ moiety is attached to intermediate (int-A1) by an amide bond. In Scheme 11 the linker (L₂) comprises a linker moiety (L_A) having a terminal carbonyl moiety (i.e. -L_A-C(=O)). Such amide bond formation can be accomplished using heat, EDCI coupling, HATU coupling, HBTU coupling, TBTU coupling or T3P coupling.

55

Scheme 11



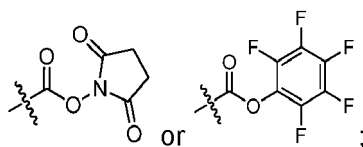
15 **[0099]** Scheme 12 illustrates a non-limiting synthetic scheme used to make certain compounds of Formula (Ib) wherein the $-L_2-R^4$ moiety is attached to intermediate (int-A2) by an amide bond. In Scheme 12 the linker (L_2) comprises a linker moiety (L_A) having a terminal carbonyl moiety (i.e. $-L_A-C(=O)-$). Such amide bond formation can be accomplished using heat, EDCI coupling, HATU coupling, HBTU coupling, TBTU coupling or T3P coupling.



[0100] In Schemes 11 and 12,

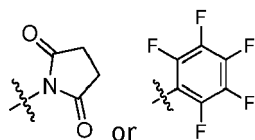
R^4 is

35

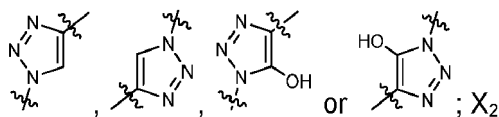


R^C is

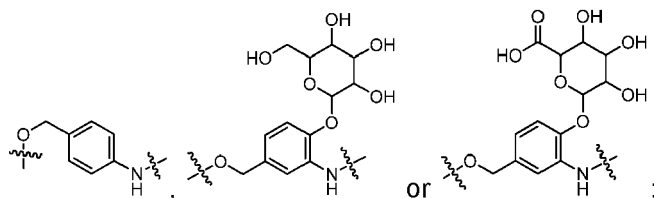
45



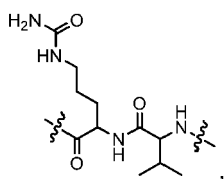
55 L_A is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, or $-(CH_2)_nC(=O)NH(CH_2)_n-$;
 L_2 is $-C(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n-$;
 where X_1 is



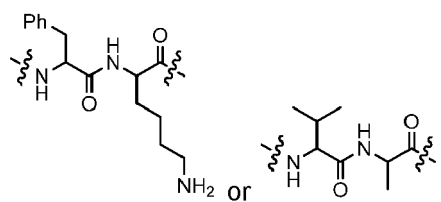
is



and X_3 is



and



R^1 and R^7 are as defined herein.

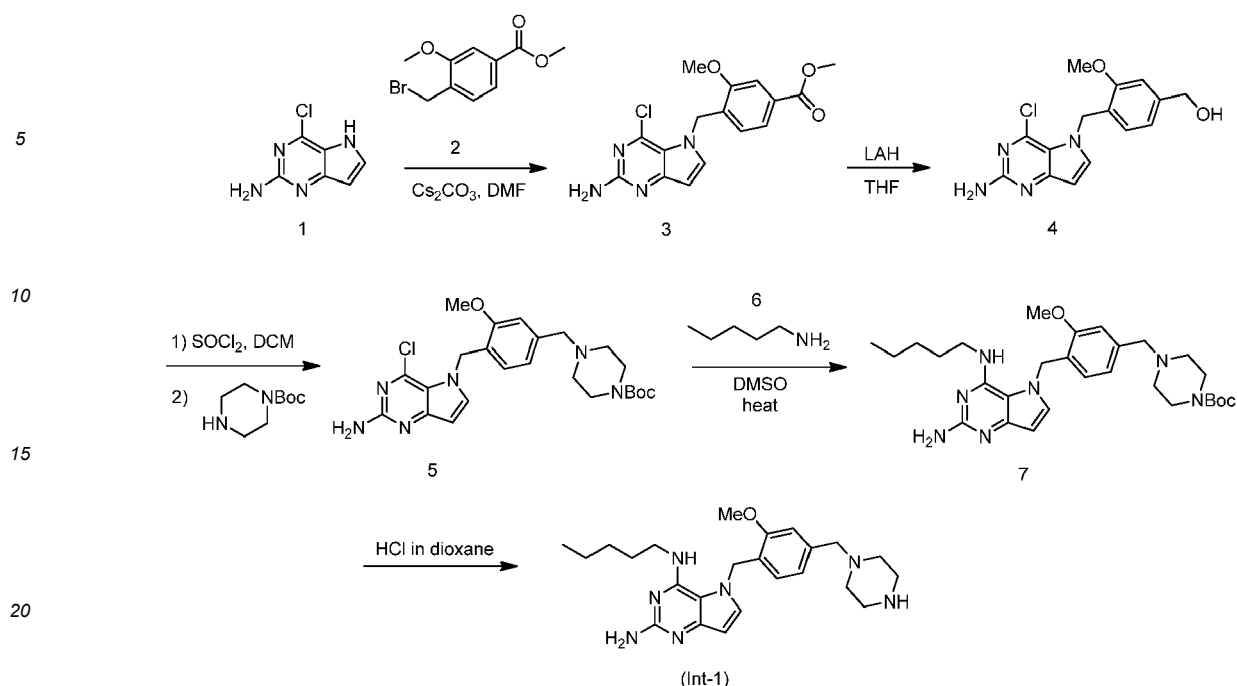
Intermediates

[0101] The synthesis of the intermediates used to make the compounds of Formula (I) and subformulae thereof (i.e. compounds of Formula (Ia) and Formula (Ib)) of the invention are given below.

Intermediate 1

Synthesis of 5-(2-methoxy-4-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1)

[0102]



Step 1: Preparation of methyl 4-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzoate (3)

[0103] A round bottom flask was charged with 4-chloro-5H-pyrrolo[3,2-d]pyrimidin-2-amine (1, commercially available, 1.0 equiv.), methyl 4-(bromomethyl)-3-methoxybenzoate (2, commercially available, 1.0 equiv.), caesium carbonate (1.0 equiv.) and DMF (1.0 M). The reaction mixture was stirred at room temperature for 18 hours and the solvent was then removed in vacuo. To the resulting mixture was added EtOAc and the solvent was removed in vacuo. To this mixture was added DCM and the solvent removed in vacuo. The crude reaction mixture was then purified by ISCO chromatography (0 - 10% MeOH:DCM, gradient) to afford methyl 4-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzoate (3) as a solid.

Step 2: 4-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxyphenyl)methanol (4)

[0104] A slurry of lithium aluminum hydride (LAH) (1.0 equiv., powder) in THF (0.3 M) was prepared in a round bottom flask, cooled to 0 °C and vigorously stirred for 15 minutes. To this mixture was added methyl 4-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzoate (3, 1.0 equiv. from previous step) in portions. The ice bath was removed and the reaction mixture was stirred at room temperature for 4 hours, with additional LAH being added until the reaction was complete. Et_2O was added to the reaction mixture and the mixture then transferred to an Erlenmeyer flask and cooled to 0 °C under vigorous stirring. The reaction was then quenched by the slow addition of a saturated sodium sulfate solution. A white precipitate was obtained and the mixture was filtered through a frit containing Celite and washed with THF and Et_2O . The volatiles were then removed in vacuo and the material used in the next step without further purification.

Step 3: tert-butyl 4-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (5)

[0105] Thionyl chloride (10.0 equiv.) was added to a round bottom flask containing 4-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxyphenyl)methanol (4, 1.0 equiv. from step 2) in DCM (0.1 M) at 0 °C. The ice bath was then removed and the reaction mixture was stirred at room temperature for 4 hours. The reaction mixture was then cooled back to 0 °C and slowly quenched by the addition of NaOH (1.0 M, 40.0 equiv.) and saturated NaHCO_3 (aq.). The material was transferred to a separatory funnel and washed with DCM 3x. The combined organic layers were dried with sodium sulfate, filtered and volatiles removed in vacuo. The resulting crude product was then dissolved in DMF (0.1 M) in a round bottom flask and used without further purification. To this material was added tert-butyl piperazine-1-carboxylate (1.0 equiv.) and Huenig's base (1.2 equiv.) and stirred at room temperature for 18 hours. The reaction mixture was then diluted with EtOAc, transferred to a separatory funnel and washed with saturated NaCl (aq.) 2x and water 2x. The combined organic layers were dried with sodium sulfate, filtered and volatiles removed in vacuo. The

crude reaction mixture was purified by ISCO chromatography (0 - 10% MeOH:DCM, gradient) to afford tert-butyl 4-(4-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (5) as a solid.

Step 4: tert-butyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (7)

[0106] A round bottom flask was charged with tert-butyl 4-(4-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (5, 1.0 equiv. from step 3), commercially available pentylamine (6, 3.0 equiv.), Huenig's base (5.0 equiv.) and DMSO (0.5 M). The reaction mixture was heated to 120 °C and stirred for 18 hours. The reaction mixture was then cooled to room temperature and water added. This mixture was then frozen and the majority of volatiles removed by lyophilization. The crude reaction mixture was purified by ISCO chromatography (0 - 10% MeOH (the MeOH contained 0.7 N NH₃):DCM, gradient) to afford tert-butyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (7) as a solid.

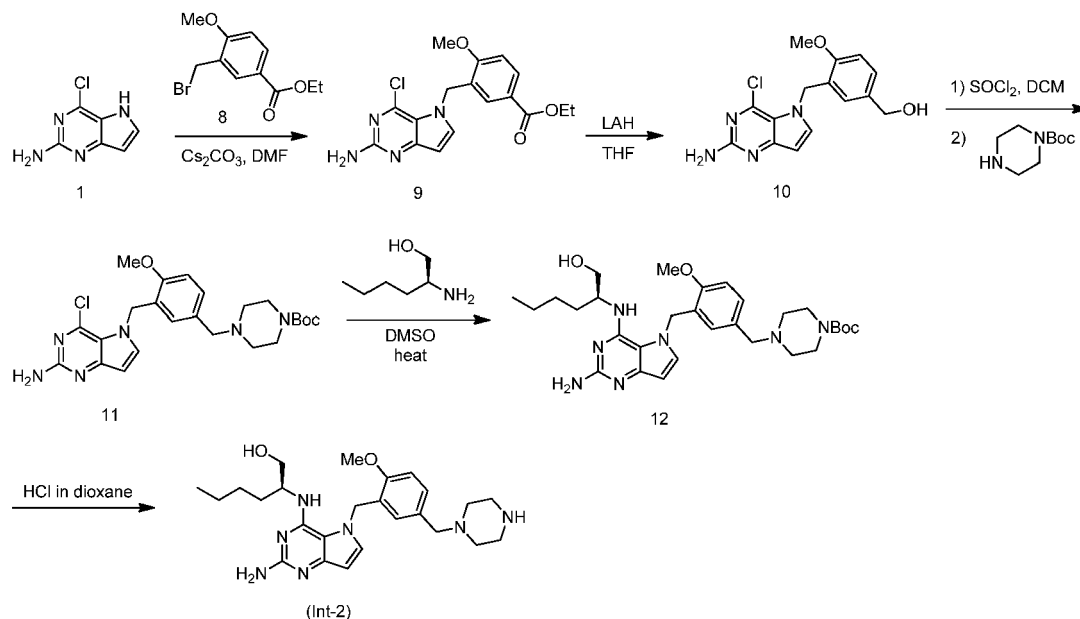
Step 5: 5-(2-methoxy-4-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1)

[0107] HCl in dioxane (4.0 M, 20.0 equiv.) was added to a solution of tert-butyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (6, 1.0 equiv. from step 4) in DCM (0.1 M) in a round bottom flask at 0 °C. The ice-bath was then removed and the reaction mixture was stirred at room temperature for 3 hours. NH₃ in MeOH (0.7 N) was then added to the reaction mixture and the volatiles removed in vacuo. The addition of NH₃ in MeOH (0.7 N) and removal of volatiles in vacuo was repeated two more times. The crude reaction mixture was then purified by ISCO chromatography (0 - 20% MeOH (the MeOH contained 0.7 N NH₃):DCM, gradient) to provide 5-(2-methoxy-4-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1) as a solid: ¹H NMR (CD₃OD): δ 7.37 (d, 1H), 7.10 (s, 1H), 6.91 (d, 1H), 6.74 (d, 1H), 6.22 (d, 1H), 5.52 (s, 2H), 3.92 (s, 3H), 3.61 (s, 2H), 3.54 (t, 2H), 3.35 (s, 3H), 3.22 (m, 4H), 2.69 (m, 4H), 1.51 (m, 2H), 1.30 (m, 2H), 1.18 (m, 2H), 0.89 (s, 3H). LRMS [M+H] = 438.3.

Intermediate 2

Synthesis of (S)-2-((2-amino-5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-5H-pyrrolo[3,2-d]pyrimidin-4-yl)amino)hexan-1-ol (Int-2)

[0108]



Step 1: Preparation of ethyl 3-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzoate (9)

[0109] A round bottom flask was charged with 4-chloro-5H-pyrrolo[3,2-d]pyrimidin-2-amine (1, commercially available,

1.0 equiv.), ethyl 3-(bromomethyl)-4-methoxybenzoate (8, commercially available, 1.0 equiv.), caesium carbonate (1.0 equiv.) and DMF (1.0 M). The reaction mixture was stirred at room temperature for 18 hours. The solvent was then removed in vacuo. To the resulting mixture was added EtOAc and the solvent was removed in vacuo. To this mixture was added DCM and the solvent removed in vacuo. The crude reaction mixture was then purified by ISCO chromatography (0 - 10% MeOH:DCM, gradient) to afford ethyl 3-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzoate (9) as a solid.

Step 2: (3-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxyphenyl)methanol (10)

[0110] A slurry of LAH (1.0 equiv., powder) in THF (0.3 M) was prepared in a round bottom flask, cooled to 0 °C and vigorously stirred for 15 minutes. To this mixture was added ethyl 3-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzoate (9, 1.0 equiv. from step 1) in portions. The ice-bath was then removed and the reaction mixture was stirred at room temperature for 4 hours (if the reaction was not complete by this time additional LAH was added and stirring continued until the reaction was complete). The reaction mixture was then transferred to an Erlenmeyer flask using Et₂O. The mixture was cooled to 0 °C and vigorously stirred. The reaction was then quenched by the slow addition of a saturated sodium sulfate solution. A white precipitate was obtained and the mixture was filtered through a frit containing Celite and washed with THF and Et₂O. The volatiles were then removed in vacuo and the material used in the next step without further purification.

Step 3: tert-butyl 4-(3-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazine-1-carboxylate (11)

[0111] Thionyl chloride (10.0 equiv.) was added to a round bottom flask containing (3-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxyphenyl)methanol (10, 1.0 equiv. from step 2) in DCM (0.1 M) at 0 °C. The ice-bath was then removed and the reaction mixture stirred at room temperature for 4 hours. The reaction mixture was then cooled to 0 °C and slowly quenched by the addition of NaOH (1.0 M, 40.0 equiv.) and saturated NaHCO₃ (aq.). The material was transferred to a separatory funnel and washed with DCM 3x. The combined organic layers were dried with sodium sulfate, filtered and volatiles removed in vacuo. The resulting crude product was then dissolved in DMF (0.1 M) in a round bottom flask and used without further purification. To this material was added tert-butyl piperazine-1-carboxylate (1.0 equiv.) and Huenig's base (1.2 equiv.) and stirred at room temperature for 18 hours. The reaction mixture was then diluted with EtOAc, transferred to a separatory funnel and washed with saturated NaCl (aq.) 2x and water 2x. The combined organic layers were dried with sodium sulfate, filtered and volatiles removed in vacuo. The crude reaction mixture was purified by ISCO chromatography (0 - 10% MeOH:DCM, gradient) to afford tert-butyl 4-(3-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazine-1-carboxylate (11) as a solid.

Step 4: (S)-tert-butyl 4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazine-1-carboxylate (12)

[0112] A round bottom flask was charged with tert-butyl 4-(3-((2-amino-4-chloro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazine-1-carboxylate (11, 1.0 equiv. from step 3), commercially available (S)-2-aminohexan-1-ol (3.0 equiv.), Huenig's base (5.0 equiv.) and DMSO (0.5 M). The reaction mixture was heated to 120 °C and stirred for 18 hours. The reaction mixture was then cooled to room temperature and water added. This mixture was then frozen and the majority of volatiles removed by lyophilization. The crude reaction mixture was purified by ISCO chromatography (0 - 10% MeOH (the MeOH contained 0.7 N NH₃):DCM, gradient) to afford (S)-tert-butyl 4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazine-1-carboxylate (12) as a solid.

Step 5: Example 1- (S)-2-((2-amino-5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-5H-pyrrolo[3,2-d]pyrimidin-4-yl)amino)hexan-1-ol (Int-2)

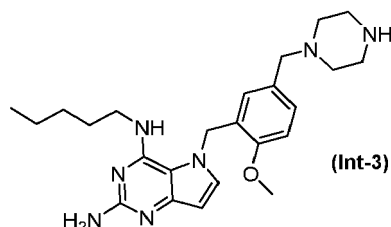
[0113] HCl in dioxane (4.0 M, 20.0 equiv.) was added to a solution of (S)-tert-butyl 4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazine-1-carboxylate (12, 1.0 equiv. from step 4) in DCM (0.1 M) in a round bottom flask at 0 °C. The ice-bath was then removed and the reaction mixture was stirred at room temperature for 3 hours. NH₃ in MeOH (0.7 N) was then added to the reaction mixture and the volatiles removed in vacuo. The addition of NH₃ in MeOH (0.7 N) and removal of volatiles in vacuo was repeated two more times. The crude reaction mixture was then purified by ISCO chromatography (0 - 20% MeOH (the MeOH contained 0.7 N NH₃):DCM, gradient) to provide (S)-2-((2-amino-5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-5H-pyrrolo[3,2-d]pyrimidin-4-yl)amino)hexan-1-ol (Int-2) as a solid: ¹H (CD₃OD): δ 7.50 (d, 1H), 7.29 (d, 1H), 7.09 (d, 1H), 6.63 (s, 1H), 6.29 (d, 1H), 5.69 (d, 1H), 5.40 (d, 1H), 4.34 (m, 1H), 3.95 (s, 3H), 3.51 (m, 2H), 3.42 (s, 2H), 3.12 (m, 4H), 2.56 (m, 2H), 1.48 (m, 1H),

1.21 (m, 3H), 0.96 (m, 2H), 0.83 (t, 3H). LRMS [M+H] = 468.3.

Intermediate 3

Synthesis of 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-3)

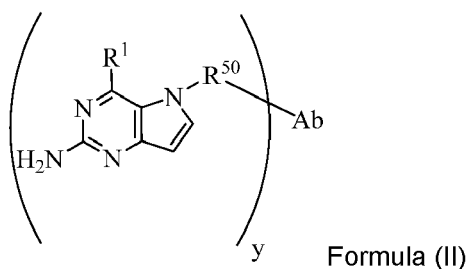
[0114]



[0115] 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-3) was prepared according to the synthesis of (S)-2-((2-amino-5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-5H-pyrrolo[3,2-d]pyrimidin-4-yl)amino)hexan-1-ol (Int-2), except commercially available N-pentylamine was used in place of (S)-2-amino-5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-5H-pyrrolo[3,2-d]pyrimidine-4-yl in Step 4. ¹H NMR (CD₃OD): δ 7.42 (d, 1H), 7.32 (d, 1H), 7.09 (d, 1H), 6.70 (s, 1H), 6.25 (d, 1H), 5.54 (d, 2H), 3.92 (s, 3H), 3.52 (t, 2H), 3.46 (s, 2H), 3.14 (m, 4H), 2.60 (m, 4H), 1.48 (m, 2H), 1.30 (m, 2H), 1.13 (m, 2H), 0.88 (t, 3H). LRMS [M+H] = 438.3.

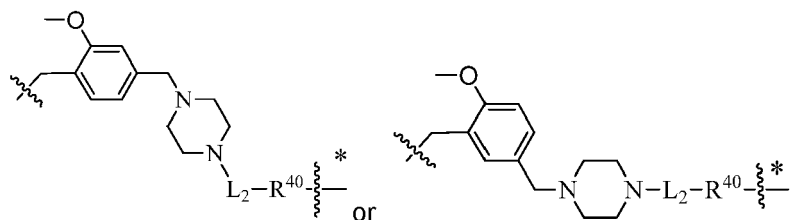
Antibody conjugates of the Invention

[0116] The antibody conjugates of the invention comprise a TLR7 agonist and have the structure of Formula (II):



wherein:

R⁵⁰ is



where the * indicates the point of attachment to Ab;

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R¹ is -NHR² or -NHCHR²R³;

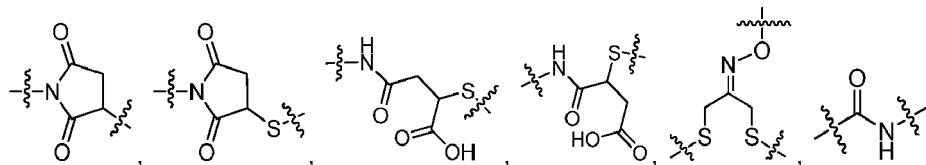
R² is -C₃-C₆alkyl or -C₄-C₆alkyl;

R³ is L₁OH;

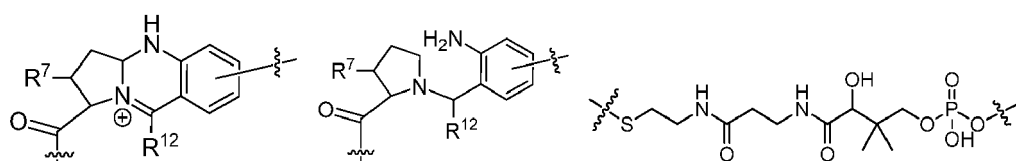
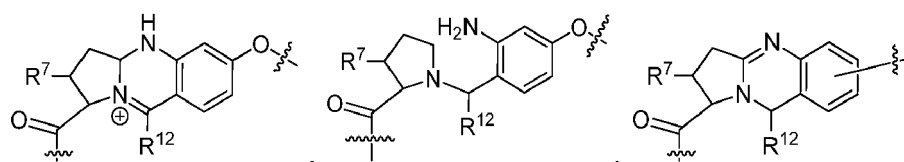
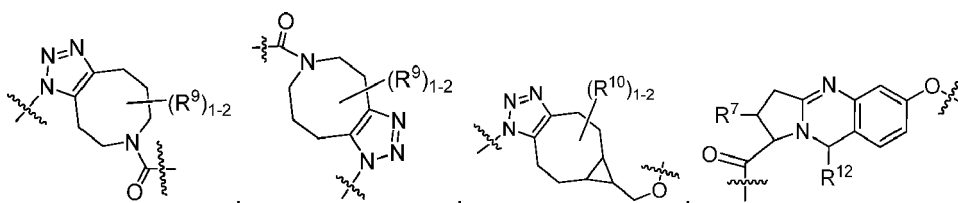
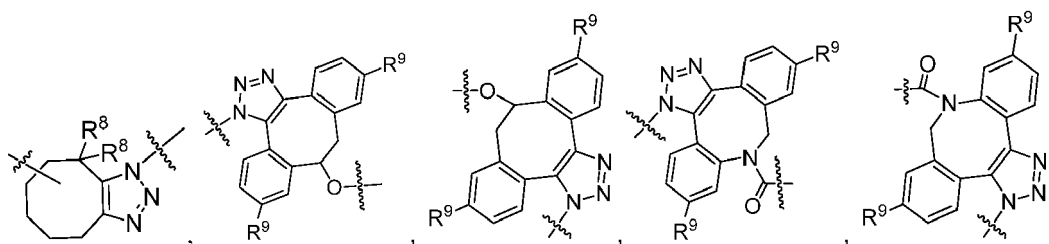
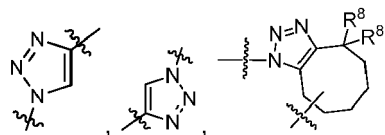
L₁ is -(CH₂)_m;

L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, or -(CH₂)_nX₁(CH₂)_n-;

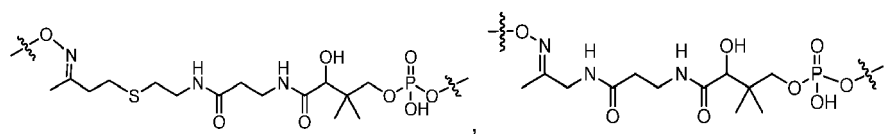
$-(\text{CH}_2)_n\text{NHC(=O)(CH}_2)_n-$, $-(\text{CH}_2)_n\text{NHC(=O)(CH}_2)_n\text{C(=O)NH(CH}_2)_n-$, $-((\text{CH}_2)_n\text{O})_t(\text{CH}_2)_n\text{NHC(=O)(CH}_2)_n-$,
 $-\text{C(=O)(CH}_2)_n-$, $-\text{C(=O)((CH}_2)_n\text{O})_t(\text{CH}_2)_n-$,
 $-\text{C(=O)((CH}_2)_n\text{O})_t(\text{CH}_2)_n\text{X}_1(\text{CH}_2)_n-$, $-\text{C(=O)((CH}_2)_n\text{O})_t(\text{CH}_2)_n\text{NHC(=O)(CH}_2)_n-$,
 $-\text{C(=O)((CH}_2)_n\text{O})_t(\text{CH}_2)_n\text{C(=O)NH(CH}_2)_n-$, $-\text{C(=O)NH((CH}_2)_n\text{O})_t(\text{CH}_2)_n\text{X}_1(\text{CH}_2)_n-$,
 $-\text{C(=O)X}_2\text{X}_3\text{C(=O)((CH}_2)_n\text{O})_t(\text{CH}_2)_n-$, $-\text{C(=O)X}_2\text{X}_3\text{C(=O)(CH}_2)_n-$,
 $-\text{C(=O)X}_2\text{C(=O)(CH}_2)_n\text{NHC(=O)(CH}_2)_n-$, $-\text{C(=O)X}_2\text{C(=O)(CH}_2)_n\text{NHC(=O)((CH}_2)_n\text{O})_t(\text{CH}_2)_n-$,
 $-\text{C(=O)(CH}_2)_n\text{C(R}_7)_2-$, $-\text{C(=O)(CH}_2)_n\text{C(R}_7)_2\text{SS(CH}_2)_n\text{NHC(=O)(CH}_2)_n-$,
 $-(\text{CH}_2)_n\text{X}_2\text{C(=O)(CH}_2)_n\text{NHC(=O)((CH}_2)_n\text{O})_t(\text{CH}_2)_n-$ or $-\text{C(=O)(CH}_2)_n\text{C(=O)NH(CH}_2)_n-$;
 R^{40} is



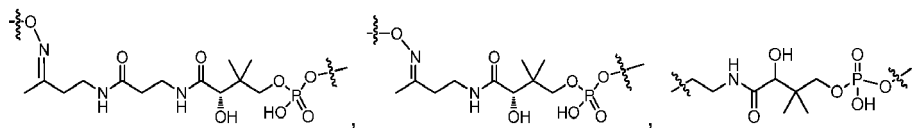
$-\text{S}-$, $-\text{NHC(=O)CH}_2-$, $-\text{S(=O)}_2\text{CH}_2\text{CH}_2-$, $-(\text{CH}_2)_2\text{S(=O)}_2\text{CH}_2\text{CH}_2-$, $-\text{NHS(=O)}_2\text{CH}_2\text{CH}_2-$, $-\text{NHC(=O)CH}_2\text{CH}_2-$,
 $-\text{CH}_2\text{NHCH}_2\text{CH}_2-$, $-\text{NHCH}_2\text{CH}_2-$,



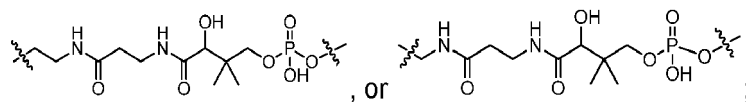
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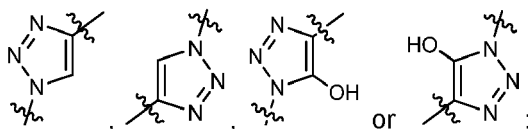
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 X_1 is

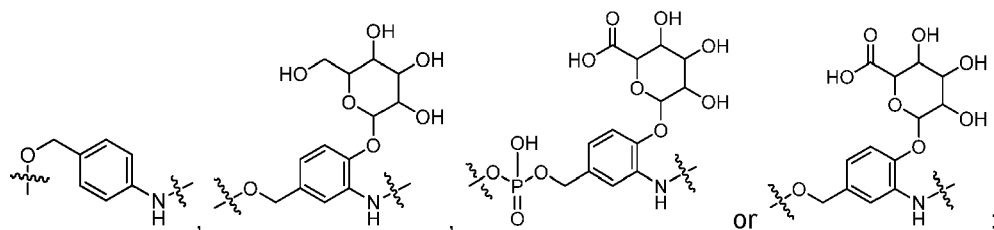
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 X_2 is

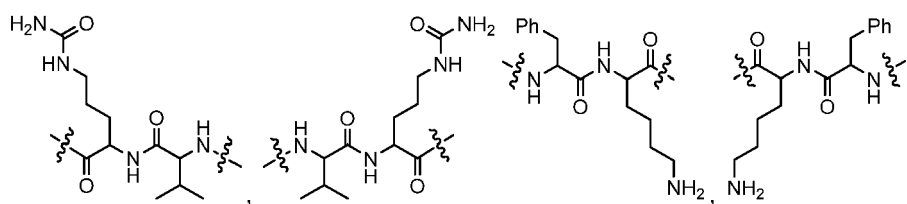
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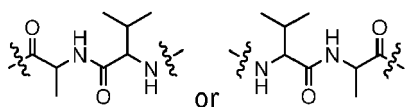
 X_3 is

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each R^7 is independently selected from H and C_1 - C_6 alkyl;each R^8 is independently selected from H, C_1 - C_6 alkyl, F, Cl, and -OH;each R^9 is independently selected from H, C_1 - C_6 alkyl, F, Cl, - NH_2 , - OCH_3 , - OCH_2CH_3 , - $N(CH_3)_2$, -CN, - NO_2 and -OH;

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each R^{10} is independently selected from H, C_1 - C_6 alkyl, fluoro, benzyloxy substituted with -C(=O)OH, benzyl substituted with -C(=O)OH, C_{1-4} alkoxy substituted with -C(=O)OH and C_{1-4} alkyl substituted with -C(=O)OH; R^{12} is H, methyl or phenyl;

each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

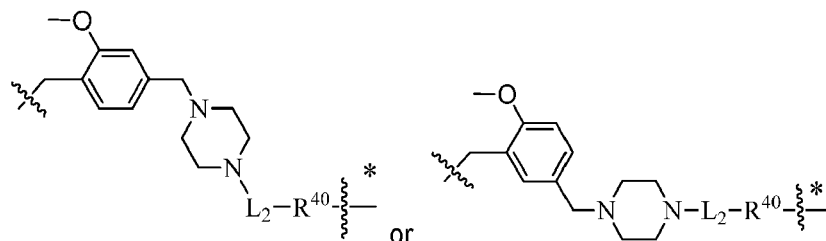
each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and

y is an integer from 1 to 16.

[0117] Certain aspects and examples of the compounds of the invention are provided in the following listing of additional, enumerated embodiments. It will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present invention.

Embodiment 58. The antibody conjugates of Formula (II), wherein:

R⁵⁰ is



where the * indicates the point of attachment to Ab;

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R¹ is -NHR² or -NHCHR²R³;

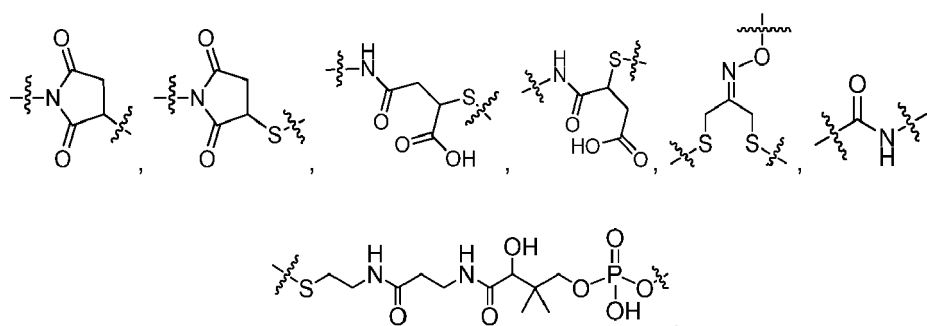
R² is -C₃-C₆alkyl or -C₄-C₆alkyl;

R³ is L₁OH;

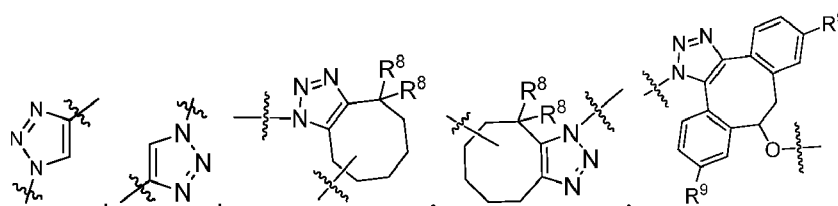
L₁ is -(CH₂)_m;

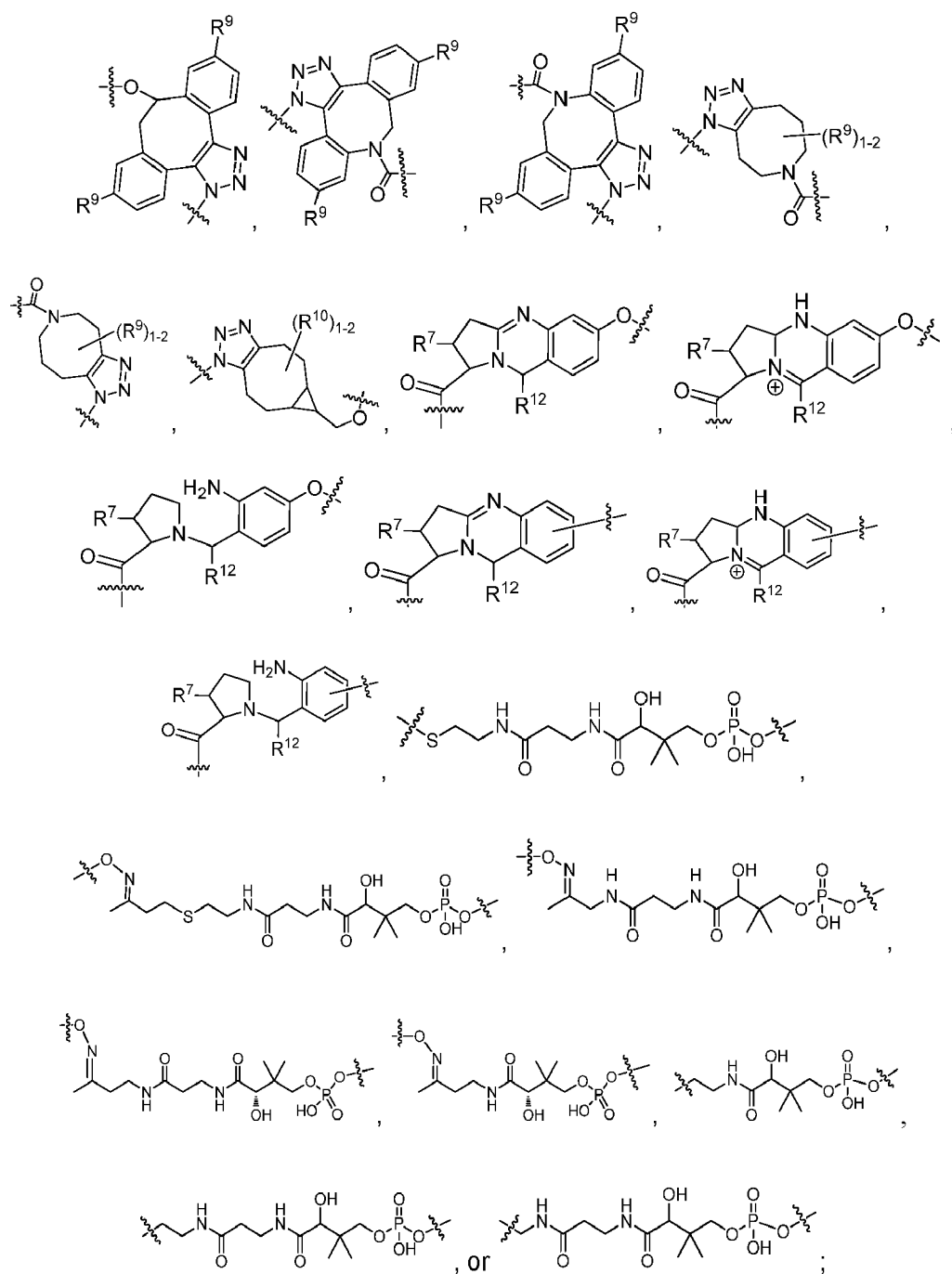
L₂ is $-(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_n$, $-(CH_2)_nX_1(CH_2)_n$, $-(CH_2)_nNHC(=O)(CH_2)_n$,
 $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$, $-C(=O)(CH_2)_n$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$,
 $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n$, or
 $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

R⁴⁰ is

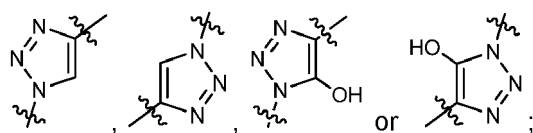


-S-, -NHC(=O)CH₂-, -S(=O)₂CH₂CH₂-, -(CH₂)₂S(=O)₂CH₂CH₂-, -NHS(=O)₂CH₂CH₂-, -NHC(=O)CH₂CH₂-,
 -CH₂NHCH₂CH₂-, -NHCH₂CH₂-;

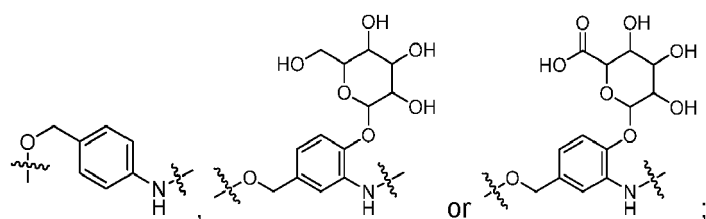




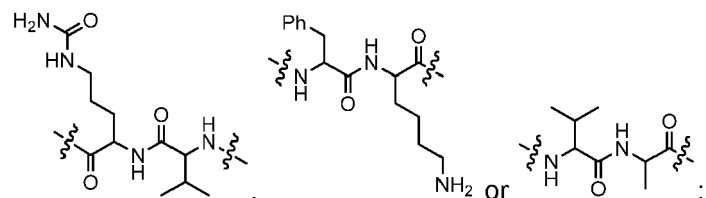
X_1 is



X_2 is



X_3 is



each R^7 is independently selected from H and C_1 - C_6 alkyl;

each R^8 is independently selected from H, C_1 - C_6 alkyl, F, Cl, and -OH;

each R^9 is independently selected from H, C_1 - C_6 alkyl, F, Cl, - NH_2 , -OCH₃, -OCH₂CH₃, -N(CH₃)₂, -CN, -NO₂ and -OH;

each R^{10} is independently selected from H, C_{1-6} alkyl, fluoro, benzyloxy substituted with -C(=O)OH, benzyl substituted with -C(=O)OH, C_{1-4} alkoxy substituted with -C(=O)OH and C_{1-4} alkyl substituted with -C(=O)OH;

R^{12} is H, methyl or phenyl;

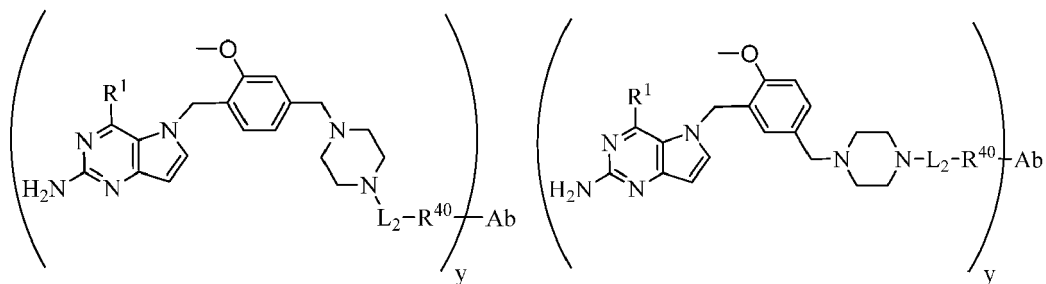
each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and

y is an integer from 1 to 16.

Embodiment 59. The antibody conjugate of Formula (II) having the structure of Formula (IIa) or Formula (IIb), and the pharmaceutically acceptable salts thereof:



Formula (IIa)

Formula (IIb)

wherein:

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R^1 is -NHR² or -NHCHR²R³;

R^2 is -C₃-C₆alkyl or -C₄-C₆alkyl;

R^3 is L₁OH;

L₁ is -(CH₂)_m;

L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-,

-(CH₂)_nNHC(=O)(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_nC(=O)NH(CH₂)_n-,

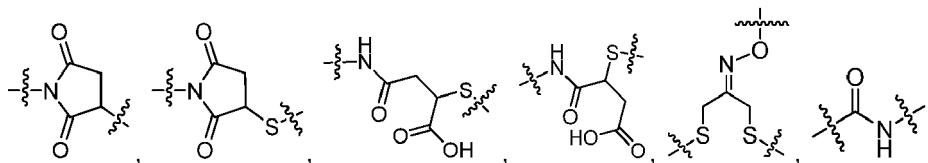
-(CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)(CH₂)_n-,

-C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,

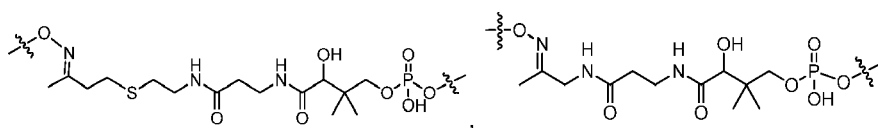
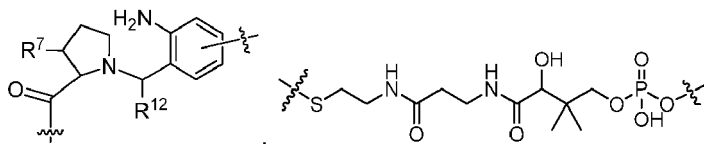
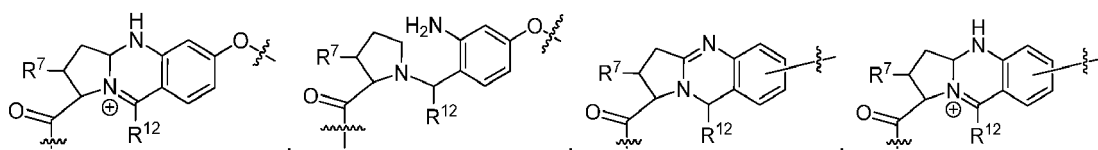
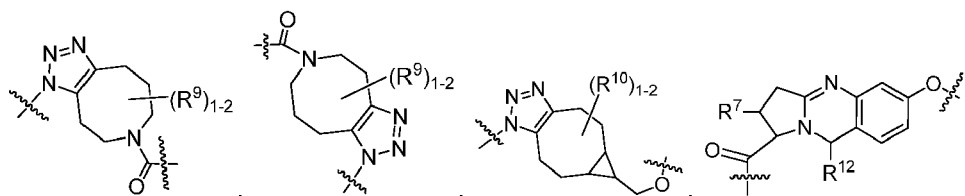
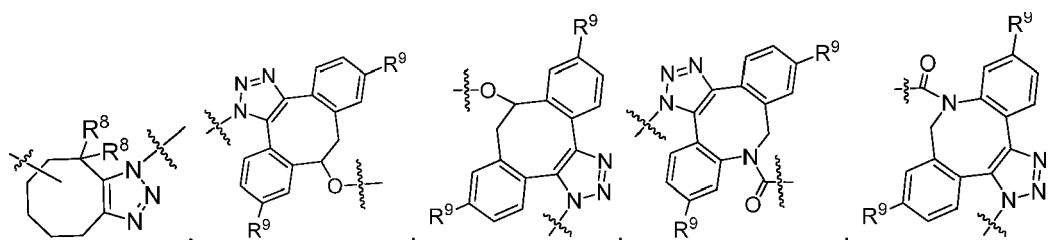
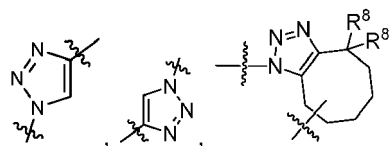
-C(=O)((CH₂)_nO)_t(CH₂)_nC(=O)NH(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,

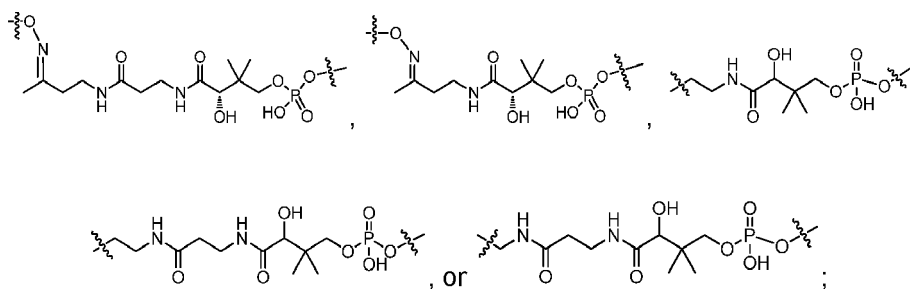
-C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)X₂X₃C(=O)(CH₂)_n-,

$-\text{C}(=\text{O})\text{X}_2\text{C}(=\text{O})(\text{CH}_2)_n\text{NHC}(=\text{O})(\text{CH}_2)_n-$, $-\text{C}(=\text{O})\text{X}_2\text{C}(=\text{O})(\text{CH}_2)_n\text{NHC}(=\text{O})((\text{CH}_2)_n\text{O})_t(\text{CH}_2)_n-$,
 $-\text{C}(=\text{O})(\text{CH}_2)_n\text{C}(\text{R}_7)_2-$, $-\text{C}(=\text{O})(\text{CH}_2)_n\text{C}(\text{R}_7)_2\text{SS}(\text{CH}_2)_n\text{NHC}(=\text{O})(\text{CH}_2)_n-$,
 $-(\text{CH}_2)_n\text{X}_2\text{C}(=\text{O})(\text{CH}_2)_n\text{NHC}(=\text{O})((\text{CH}_2)_n\text{O})_t(\text{CH}_2)_n-$ or $-\text{C}(=\text{O})(\text{CH}_2)_n\text{C}(=\text{O})\text{NH}(\text{CH}_2)_n-$;
 R^{40} is

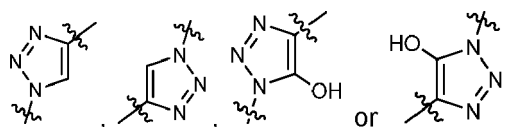


$-\text{S}-$, $-\text{NHC}(=\text{O})\text{CH}_2-$, $-\text{S}(=\text{O})_2\text{CH}_2\text{CH}_2-$, $-(\text{CH}_2)_2\text{S}(=\text{O})_2\text{CH}_2\text{CH}_2-$, $-\text{NHS}(=\text{O})_2\text{CH}_2\text{CH}_2-$, $-\text{NHC}(=\text{O})\text{CH}_2\text{CH}_2-$,
 $-\text{CH}_2\text{NHCH}_2\text{CH}_2-$, $-\text{NHCH}_2\text{CH}_2-$,

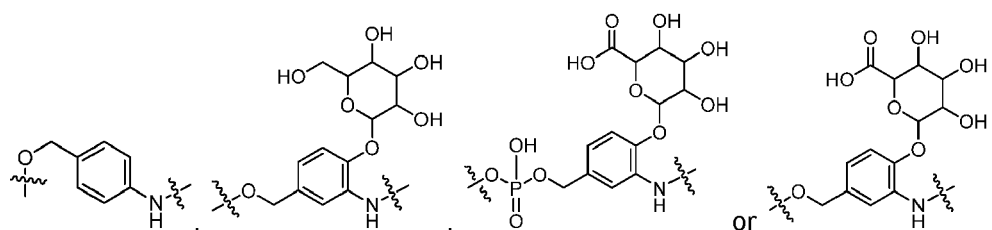




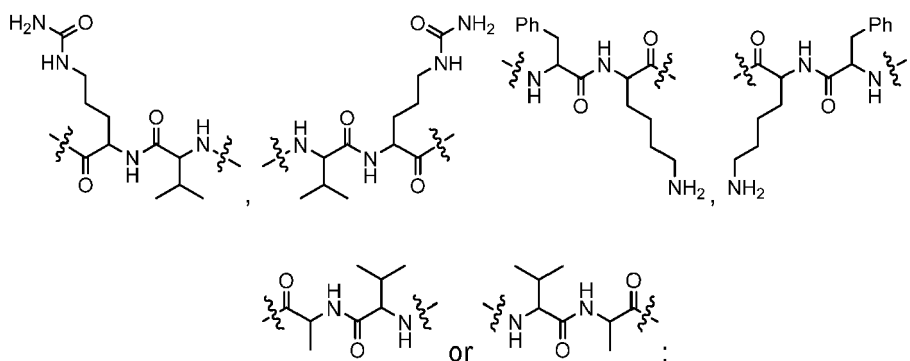
X_1 is



X_2 is



X_3 is



each R^7 is independently selected from H and C_1 - C_6 alkyl;

each R^8 is independently selected from H, C_1 - C_6 alkyl, F, Cl, and -OH;

each R^9 is independently selected from H, C_1 - C_6 alkyl, F, Cl, - NH_2 , - OCH_3 , - OCH_2CH_3 , - $N(CH_3)_2$, -CN, - NO_2 and -OH;

each R^{10} is independently selected from H, C_1 - C_6 alkyl, fluoro, benzyloxy substituted with - $C(=O)OH$, benzyl substituted with - $C(=O)OH$, C_{1-4} alkoxy substituted with - $C(=O)OH$ and C_{1-4} alkyl substituted with - $C(=O)OH$;

R^{12} is H, methyl or phenyl;

each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and

y is an integer from 1 to 16.

Embodiment 60. The antibody conjugate of Formula (IIa) or Formula (IIb), and the pharmaceutically acceptable

salts thereof, wherein:

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R¹ is -NHR² or -NHCHR²R³;

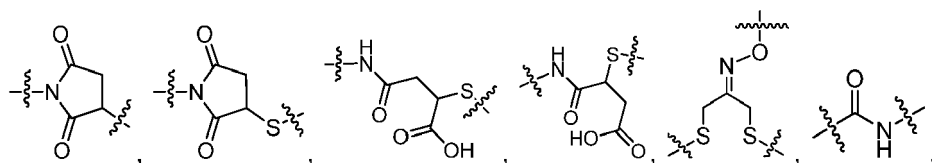
R² is -C₃-C₆alkyl or -C₄-C₆alkyl;

R³ is L₁OH;

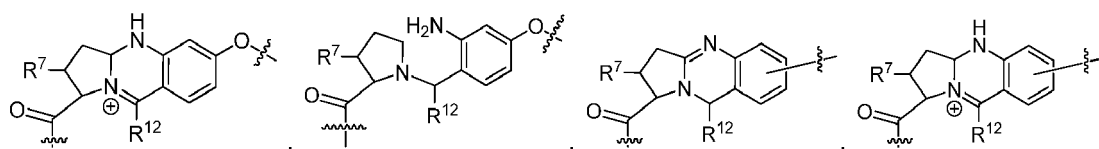
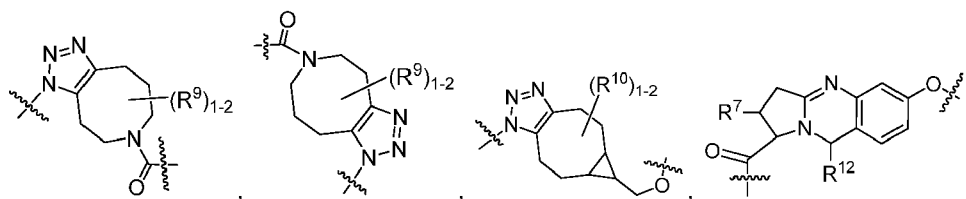
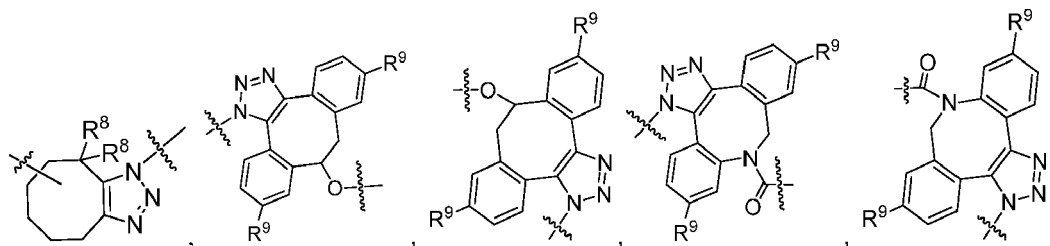
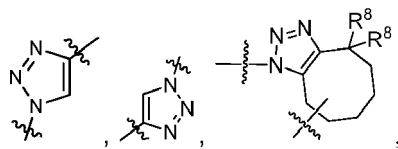
L₁ is -(CH₂)_m-;

L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_n-,
 -(CH₂)_nNHC(=O)(CH₂)_nC(=O)NH(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)(CH₂)_n-,
 -C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,
 -C(=O)((CH₂)_nO)_t(CH₂)_nC(=O)NH(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,
 -C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-, or
 -C(=O)(CH₂)_nC(=O)NH(CH₂)_n;

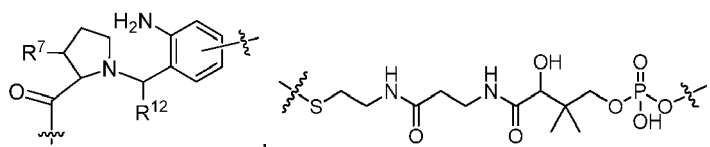
R⁴⁰ is



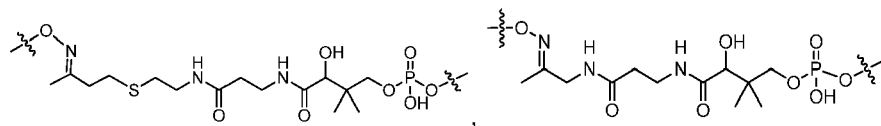
-S-, -NHC(=O)CH₂-, -S(=O)₂CH₂CH₂-, -(CH₂)₂S(=O)₂CH₂CH₂-, -NHS(=O)₂CH₂CH₂-, -NHC(=O)CH₂CH₂-,
 -CH₂NHCH₂CH₂-, -NHCH₂CH₂-;



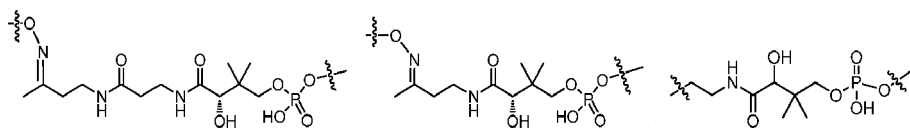
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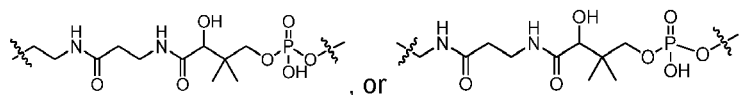
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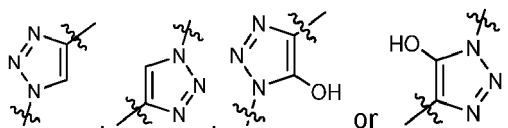
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X₁ is

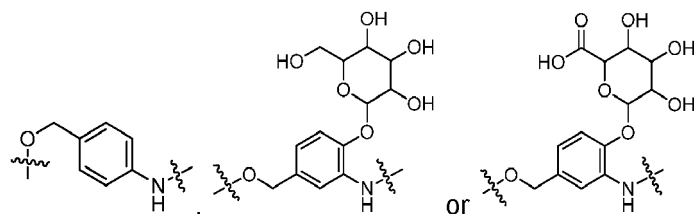
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X₂ is

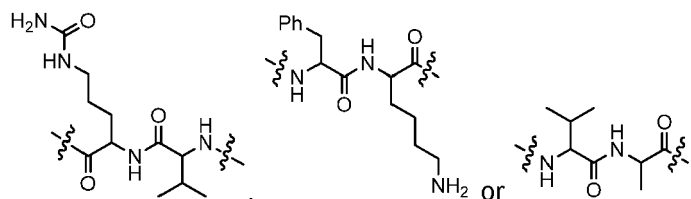
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X₃ is

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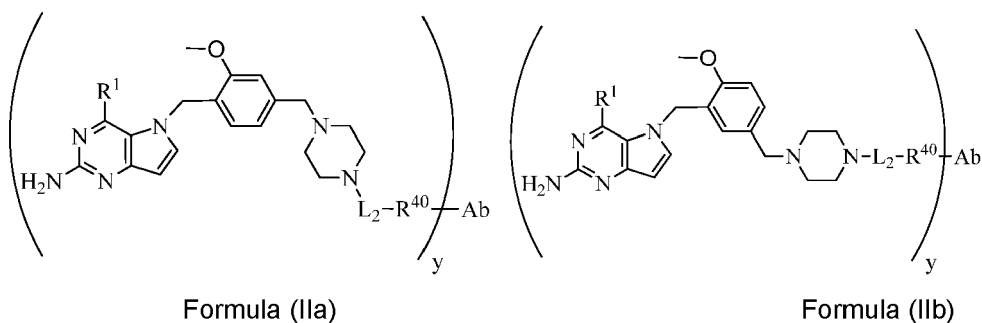
each R⁷ is independently selected from H and C₁-C₆alkyl;each R⁸ is independently selected from H, C₁-C₆alkyl, F, Cl, and -OH;each R⁹ is independently selected from H, C₁-C₆alkyl, F, Cl, -NH₂, -OCH₃, -OCH₂CH₃, -N(CH₃)₂, -CN, -NO₂ and -OH;

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each R¹⁰ is independently selected from H, C₁₋₆alkyl, fluoro, benzyloxy substituted with -C(=O)OH, benzyl substituted with -C(=O)OH, C₁₋₄alkoxy substituted with -C(=O)OH and C₁₋₄alkyl substituted with -C(=O)OH;R¹² is H, methyl or phenyl;

each m is independently selected from 1, 2, 3, and 4;
 each n is independently selected from 1, 2, 3, and 4;
 each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and
 y is an integer from 1 to 16.

Embodiment 61. The antibody conjugate of Formula (II) having the structure of Formula (IIa) or Formula (IIb), and the pharmaceutically acceptable salts thereof:



wherein:

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

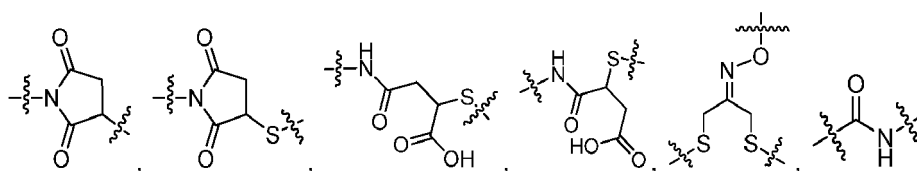
R¹ is -NHR² or -NHCHR²R³;

R² is -C₃-C₆alkyl or -C₄-C₆alkyl;

R³ is L₁OH;

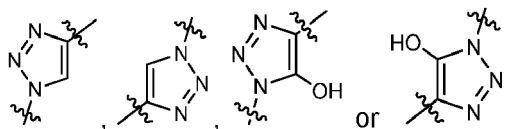
L₁ is -(CH₂)_m;

L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_n-,
 -(CH₂)_nNHC(=O)(CH₂)_nC(=O)NH(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)(CH₂)_n-,
 -C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,
 -C(=O)((CH₂)_nO)_t(CH₂)_nC(=O)NH(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,
 -C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-, or
 -C(=O)(CH₂)_nC(=O)NH(CH₂)_n;
 R⁴⁰ is

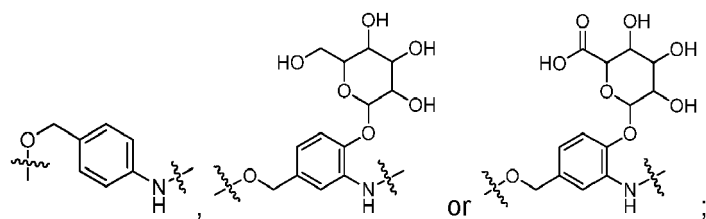


or -S-;

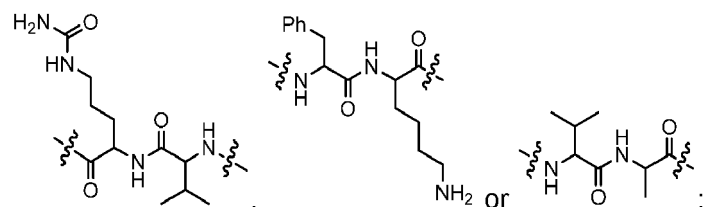
X₁ is



X₂ is



X_3 is



each R^7 is independently selected from H and C_1 - C_6 alkyl;

each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and y is an integer from 1 to 16.

Embodiment 62. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein:

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R^1 is $-NHR^2$ or $-NHCHR^2R^3$;

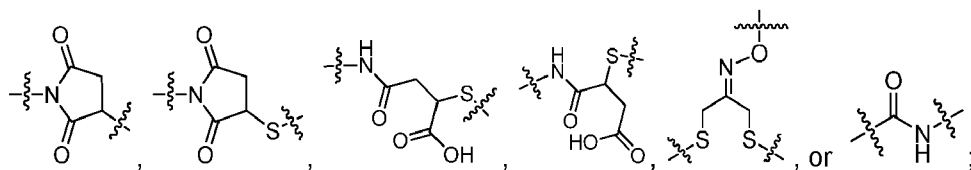
R^2 is $-C_4$ - C_6 alkyl;

R^3 is L_1OH ;

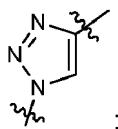
L_1 is $-(CH_2)_m$;

L_2 is $-(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_n$, $-(CH_2)_nX_1(CH_2)_n$, $-(CH_2)_nNHC(=O)(CH_2)_n$, $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$, $-C(=O)(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n$, $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n$, or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

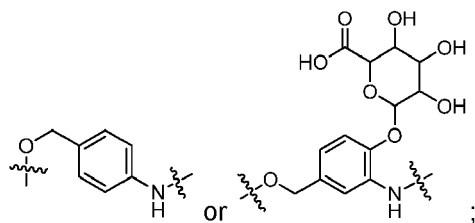
R^{40} is



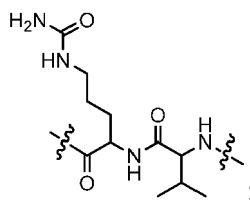
X_1 is



X_2 is



X_3 is



each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and y is an integer from 1 to 16.

Embodiment 63. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein:

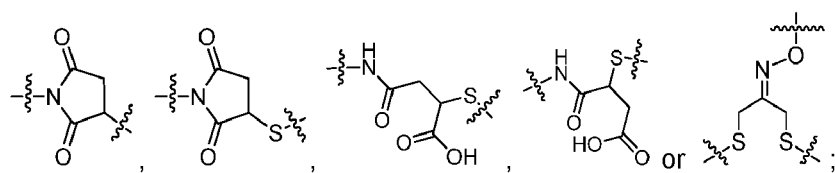
Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R^1 is $-NHR^2$;

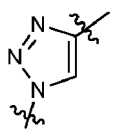
R^2 is $-C_4-C_6$ alkyl;

L_2 is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_n-$,
 $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)(CH_2)_n-$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$,
 $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, or
 $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

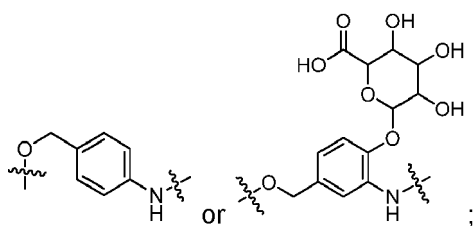
R^{40} is



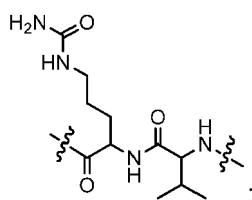
X_1 is



X_2 is



X_3 is



each n is independently selected from 1, 2, 3, and 4;
each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and
 y is an integer from 1 to 16.

Embodiment 64. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein:

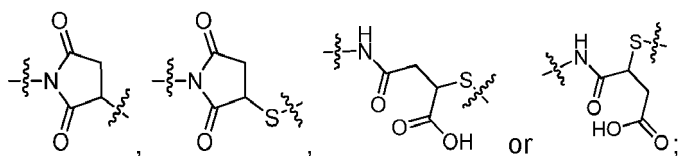
Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R^1 is $-NHR^2$;

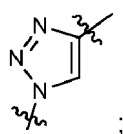
R^2 is $-C_4-C_6$ alkyl;

L_2 is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-C(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_n-$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$,
 $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$ or $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$;

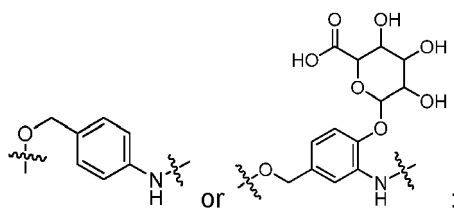
R^{40} is



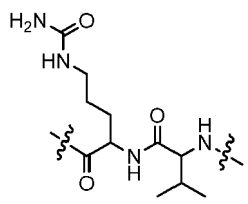
X_1 is



X_2 is



X_3 is



each n is independently selected from 1, 2, 3, and 4;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and y is an integer from 1 to 16.

Embodiment 65. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein:

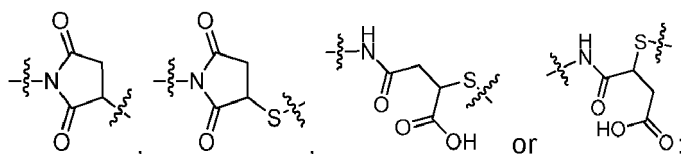
Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R^1 is $-NHR^2$;

R^2 is $-C_4-C_6$ alkyl;

L_2 is $-(CH_2)_n-$ or $-C(=O)(CH_2)_n-$;

R^{40} is



and

each n is independently selected from 1, 2, 3, and 4, and

y is an integer from 1 to 16.

Embodiment 66. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein:

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

R^1 is $-NHR^2$;

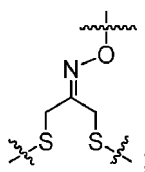
R^2 is $-C_4-C_6$ alkyl;

L_2 is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-C(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_n-$,

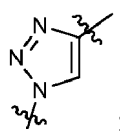
$-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$,

$-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$ or $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$;

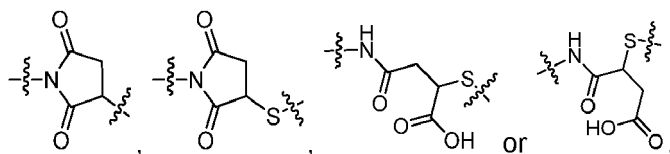
R^{40} is



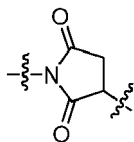
X_1 is



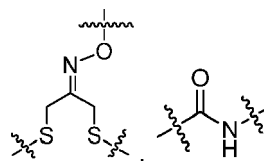
X_2 is



Embodiment 81. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: R⁴⁰ is

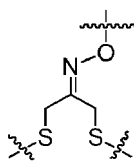


Embodiment 82. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: R⁴⁰ is

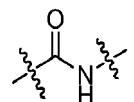


or -S-.

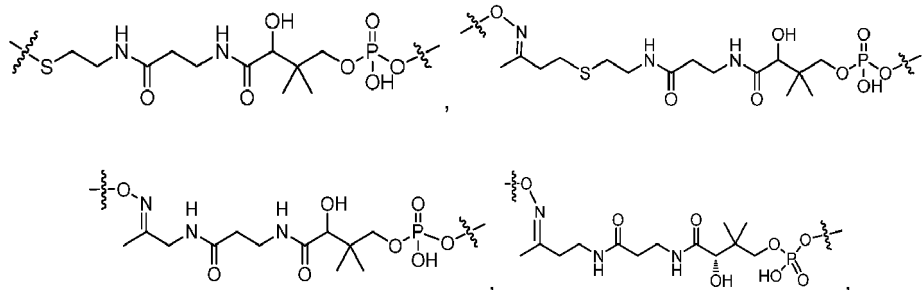
Embodiment 83. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: R⁴⁰ is

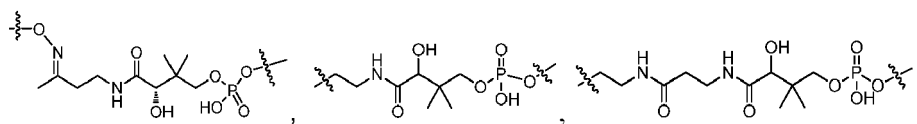


Embodiment 84. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: R⁴⁰ is

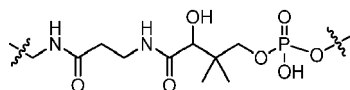


Embodiment 85. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: R⁴⁰ is

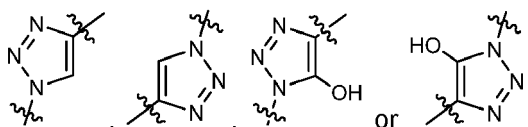




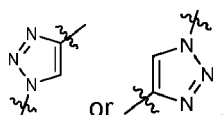
or



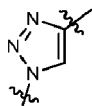
Embodiment 86. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: X_1 is



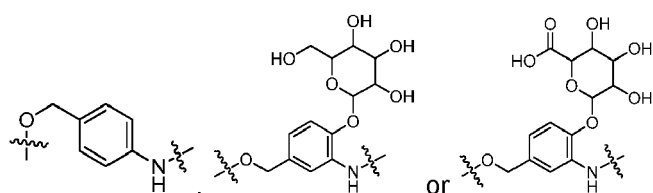
Embodiment 87. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: X_1 is



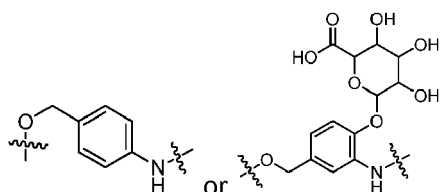
Embodiment 88. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: X_1 is



Embodiment 89. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: X_2 is

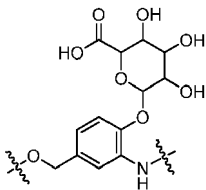


Embodiment 90. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: X_2 is

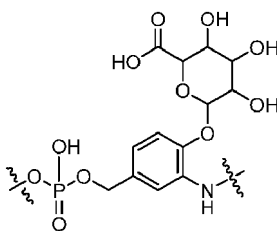


Embodiment 91. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: X_2 is

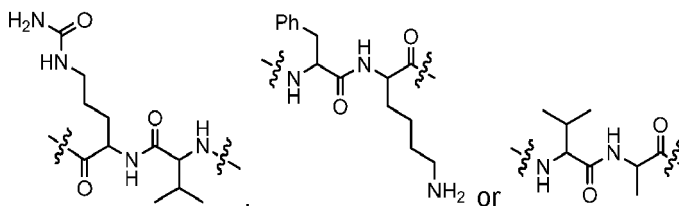
5



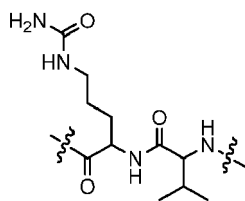
10



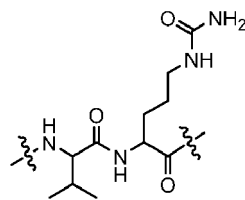
15



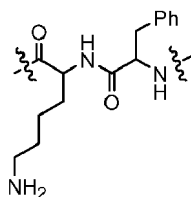
20



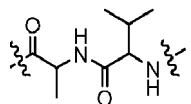
35



40



Embodiment 98. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: X_3 is



Embodiment 99. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: each m is independently selected from 1, 2, 3, and 4.

Embodiment 100. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: each m is 1 or 2.

Embodiment 101. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: each n is independently selected from 1, 2, 3, and 4.

Embodiment 102. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: each n is 2 or 3.

Embodiment 103. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

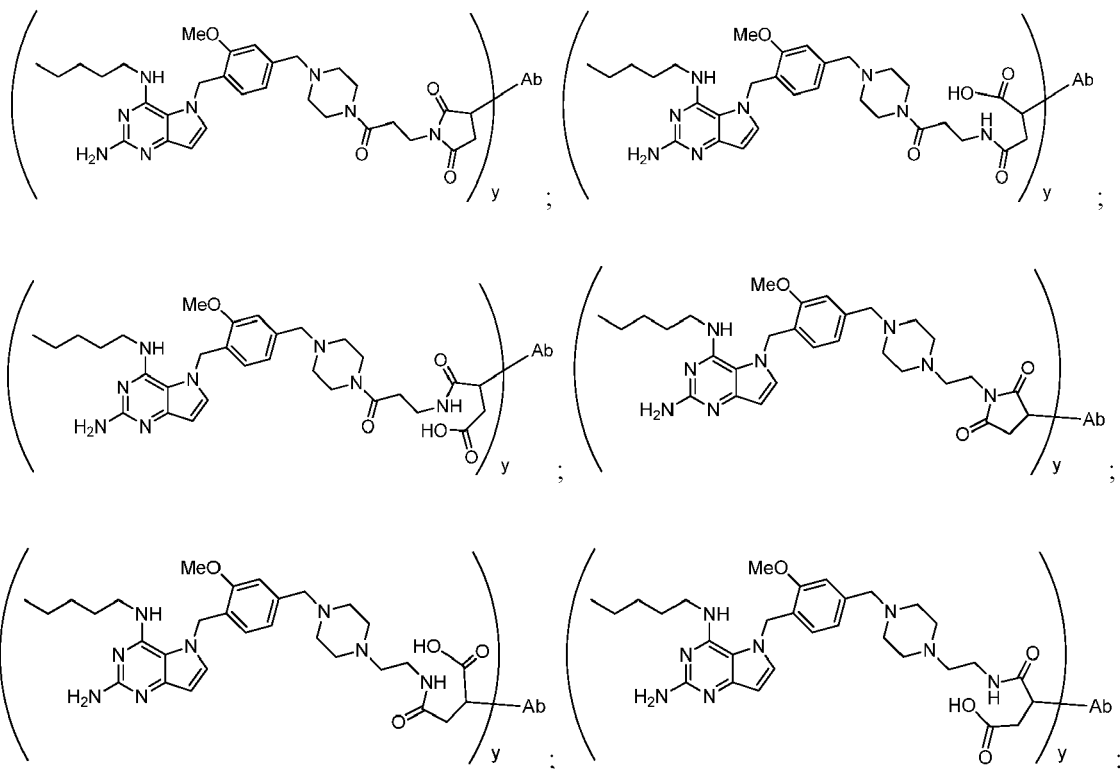
Embodiment 104. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein: each t is independently selected from 1, 2, 3, 4, 5 and 6.

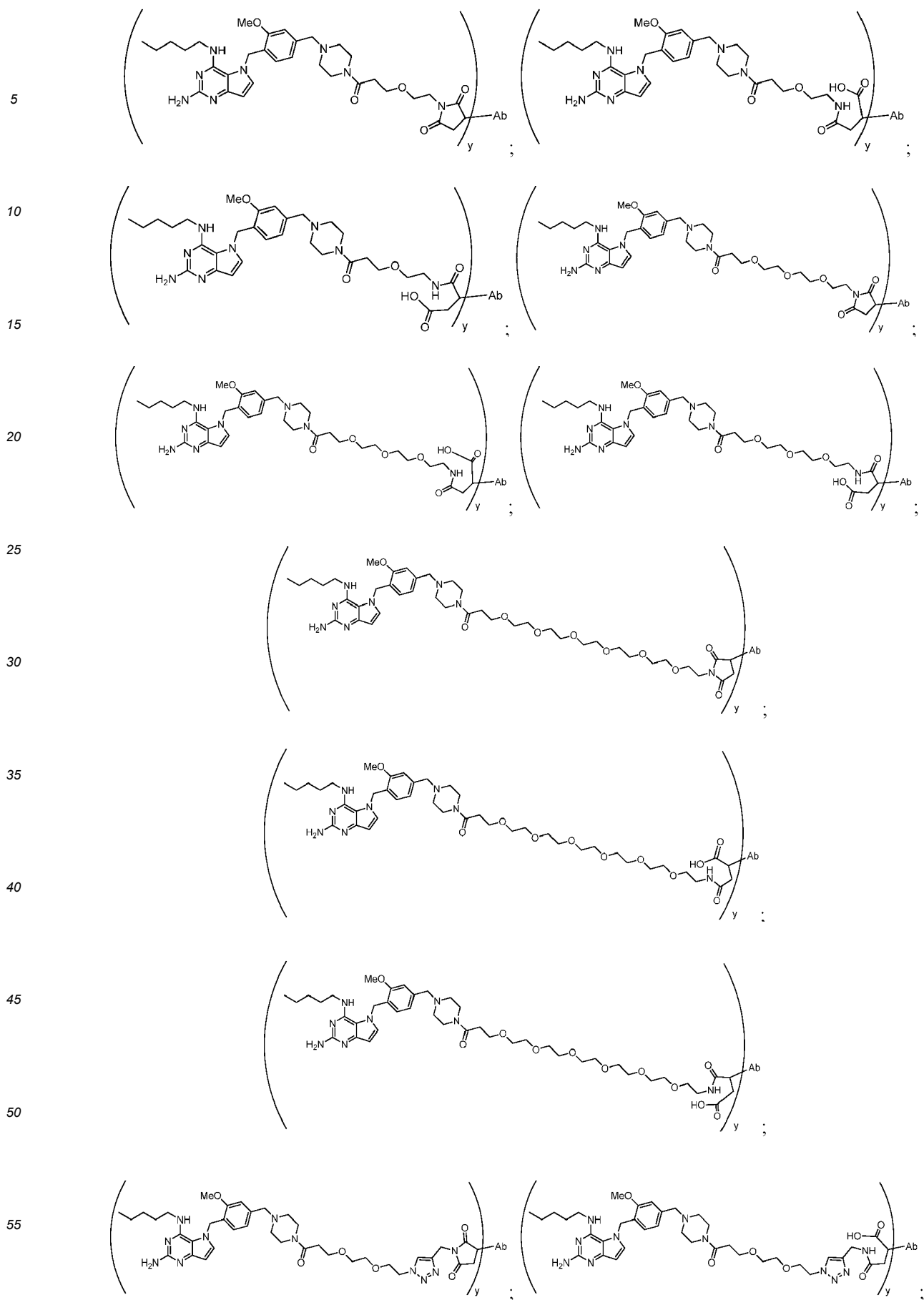
Embodiment 105. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein y is an integer from 1 to 16.

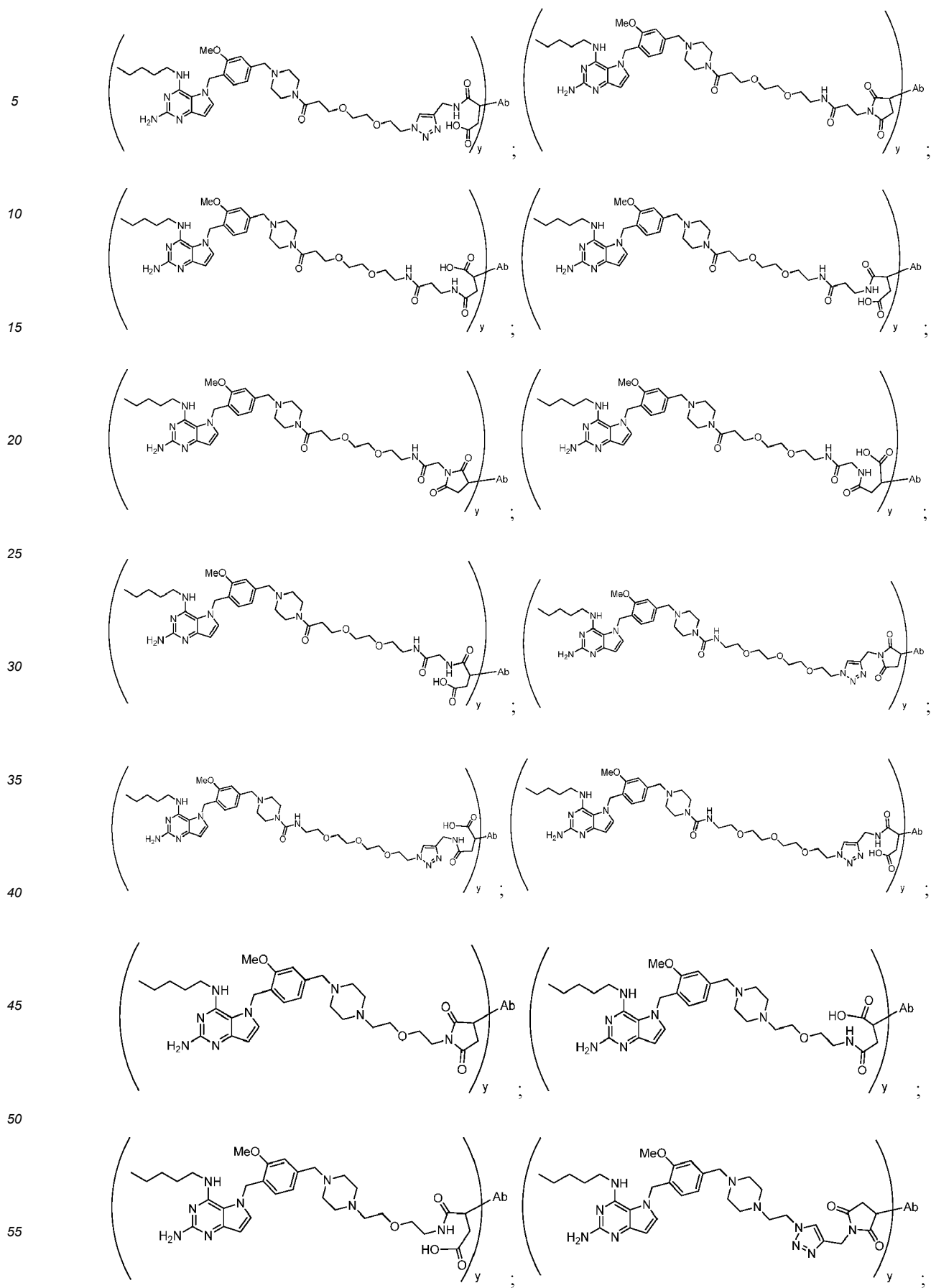
Embodiment 106. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein y is an integer from 1 to 8.

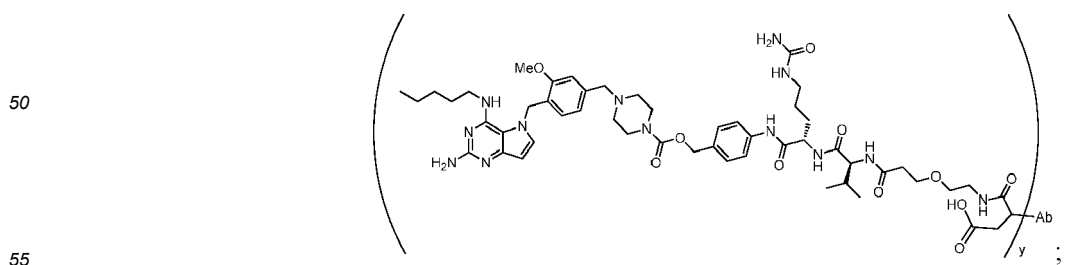
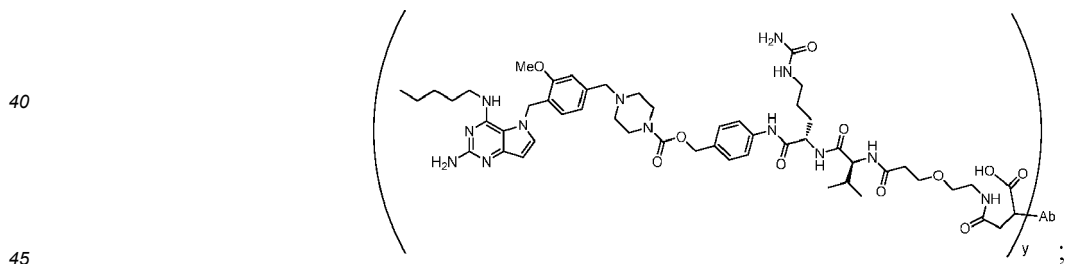
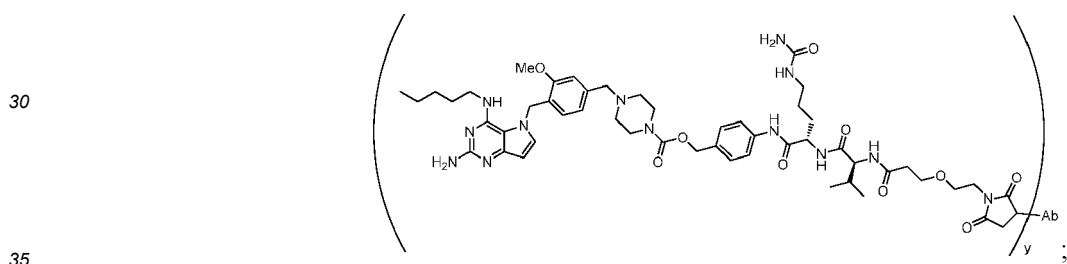
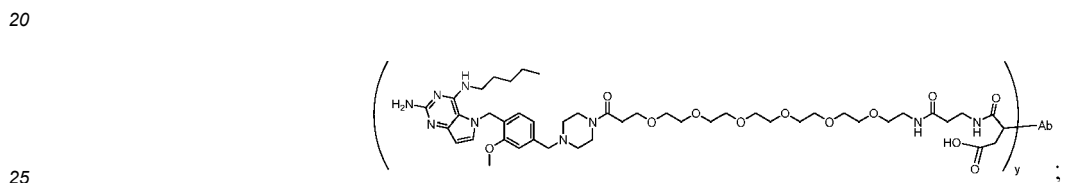
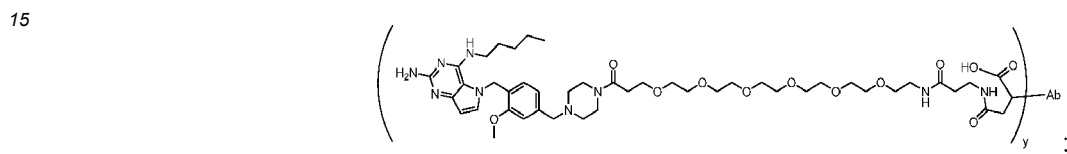
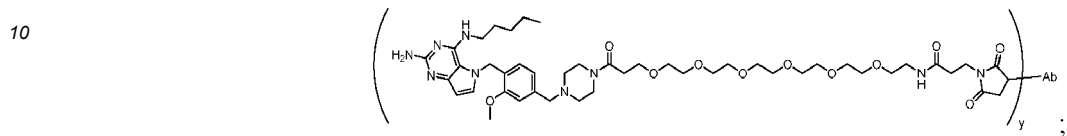
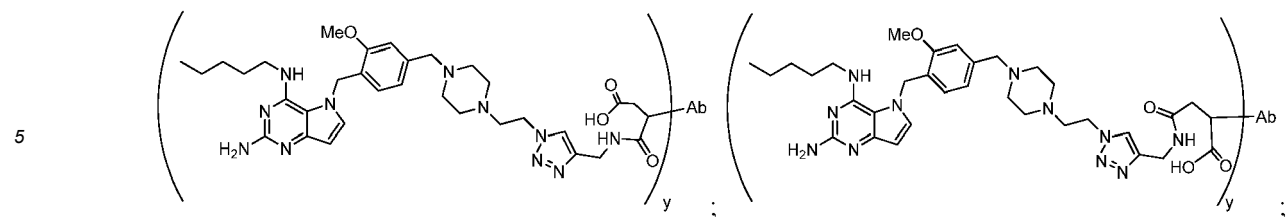
Embodiment 107. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb), wherein y is an integer from 1 to 4.

Embodiment 108. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb) selected from:

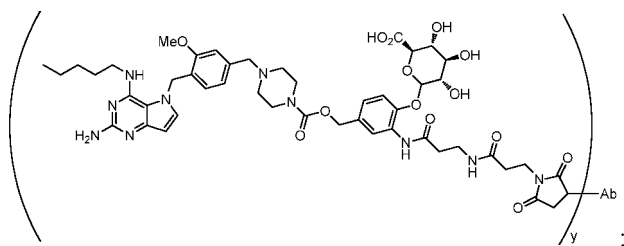




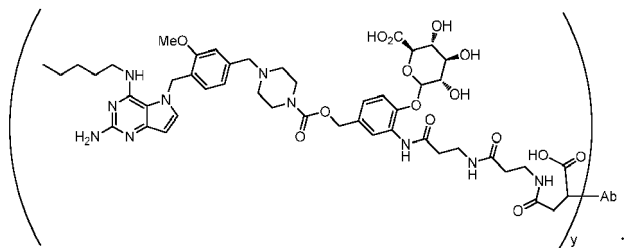




5

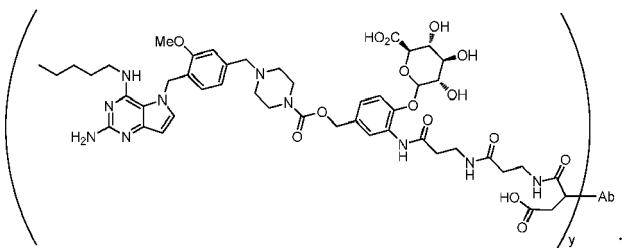


10



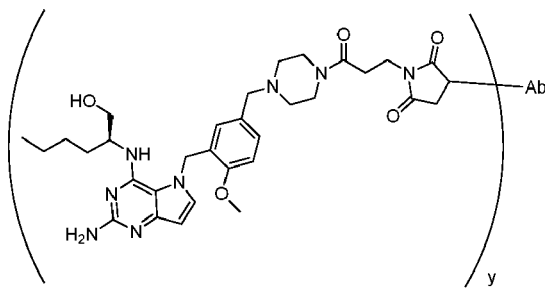
15

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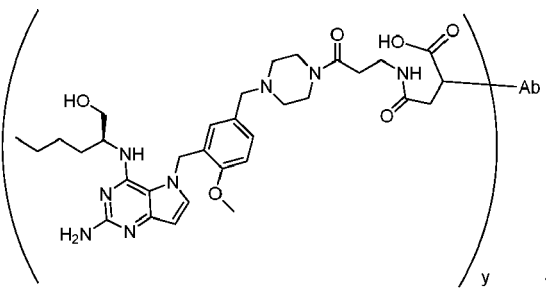


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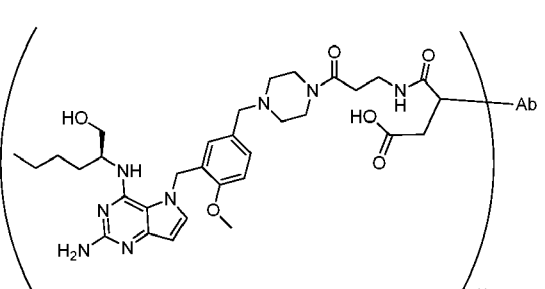


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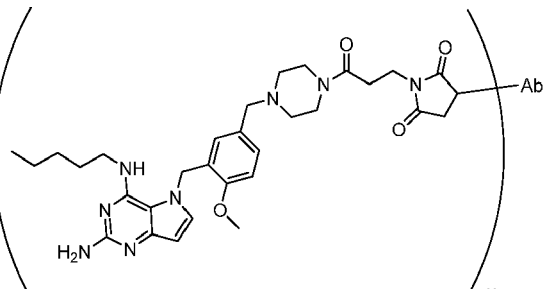


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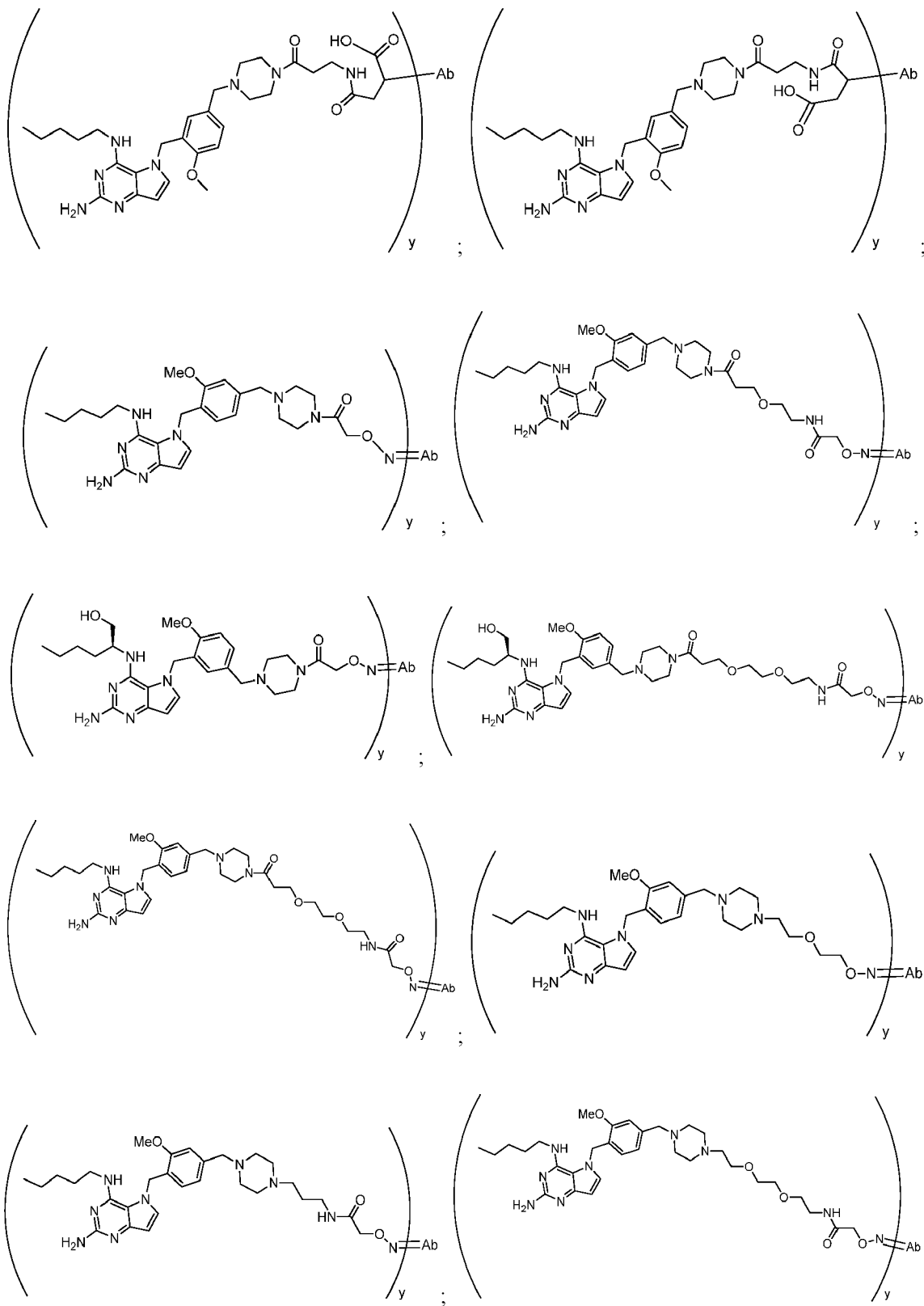
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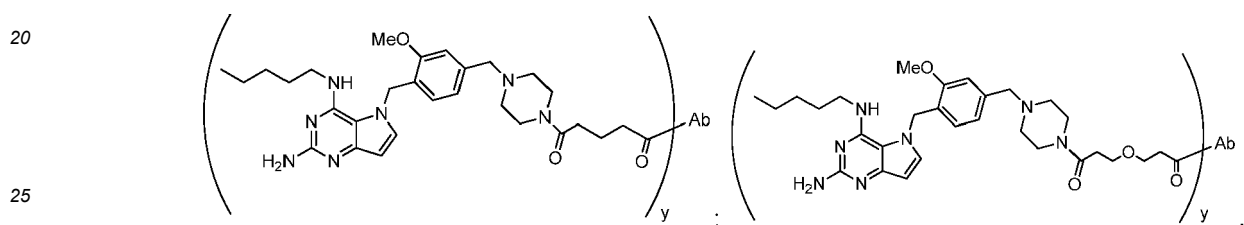
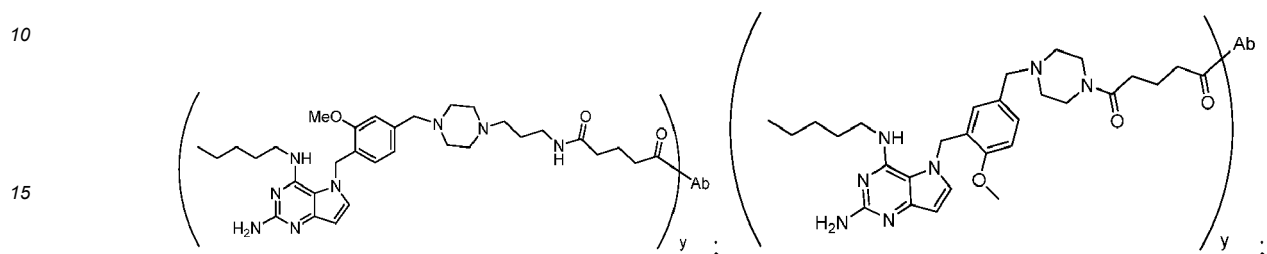
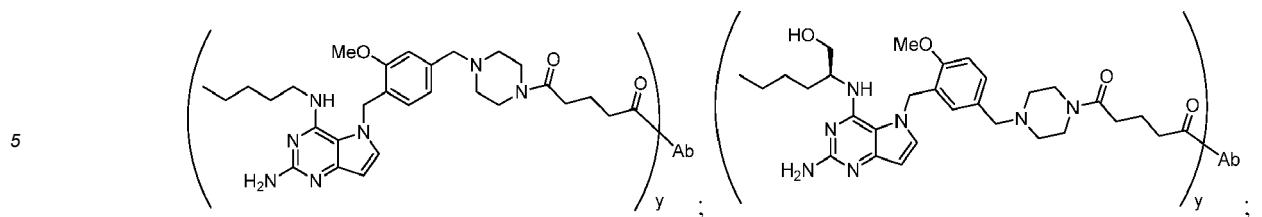
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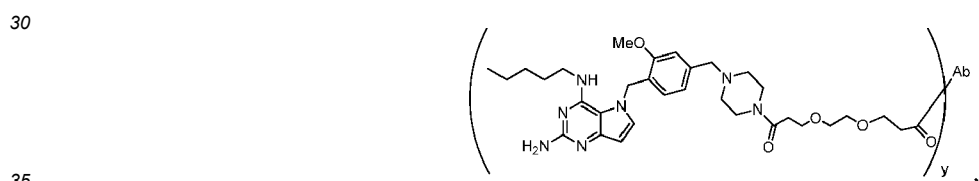
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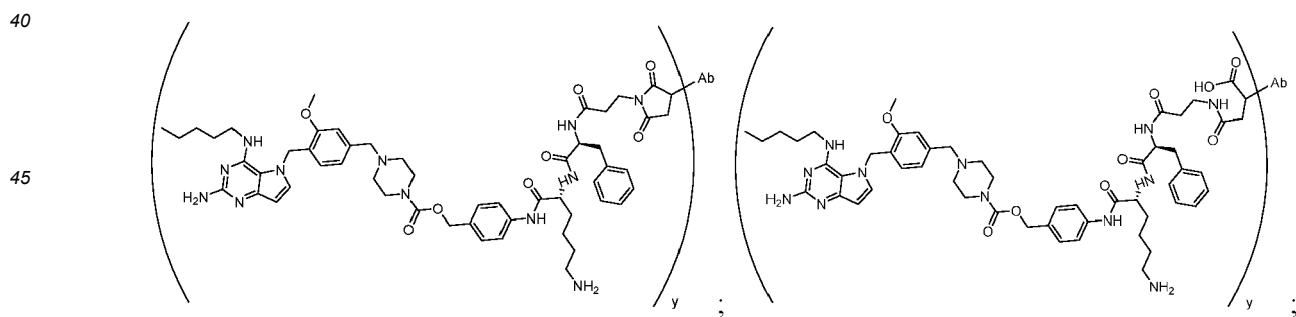




and



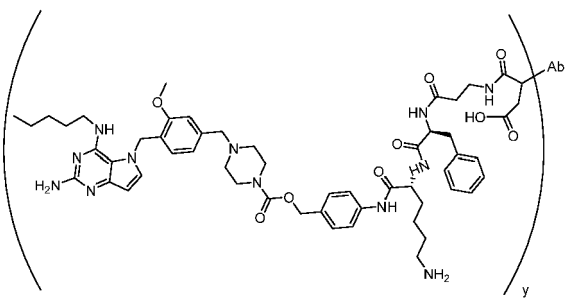
wherein y is an integer from 1 to 4 and Ab is an anti-HER2 antibody or antigen binding fragment thereof.
Embodiment 109. The antibody conjugate of Formula (II), Formula (IIa) or Formula (IIb) selected from:



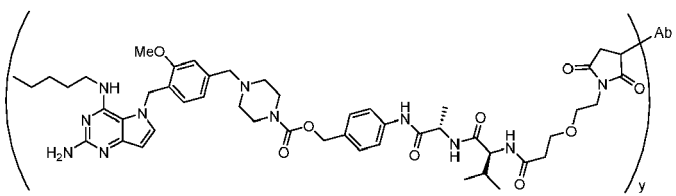
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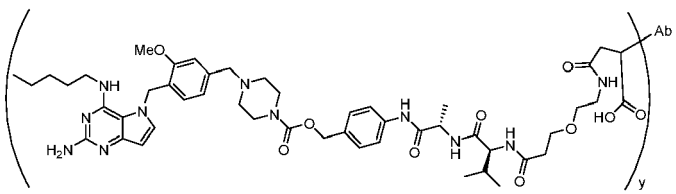
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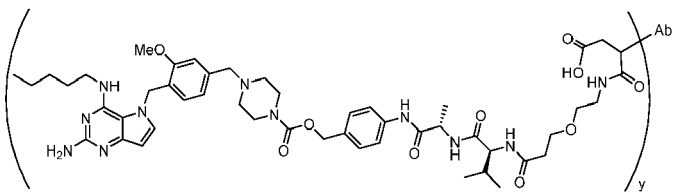
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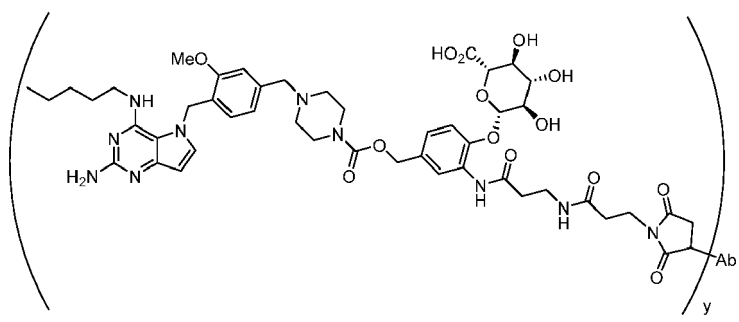


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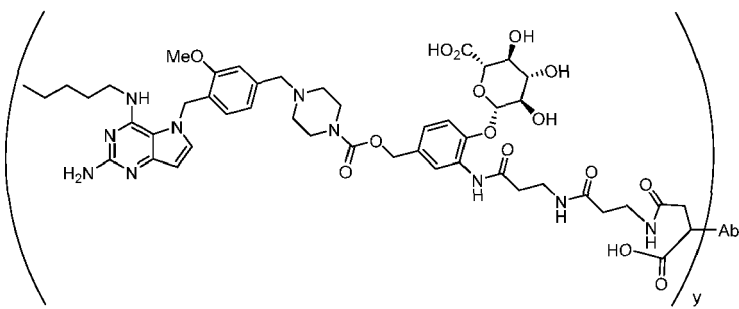
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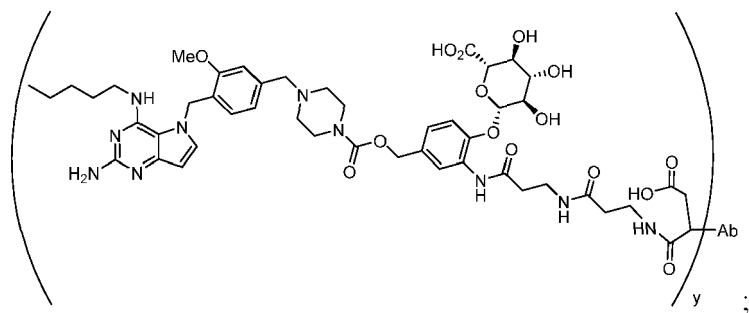


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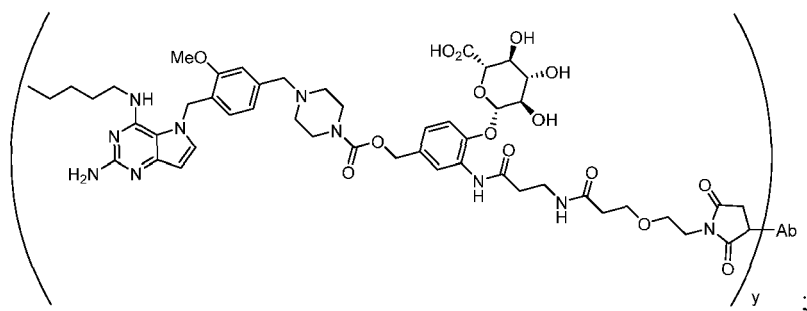
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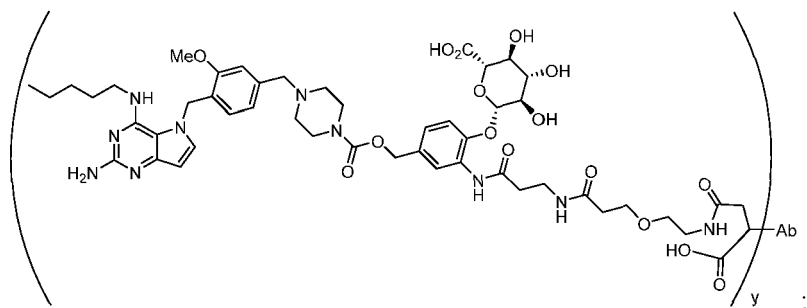
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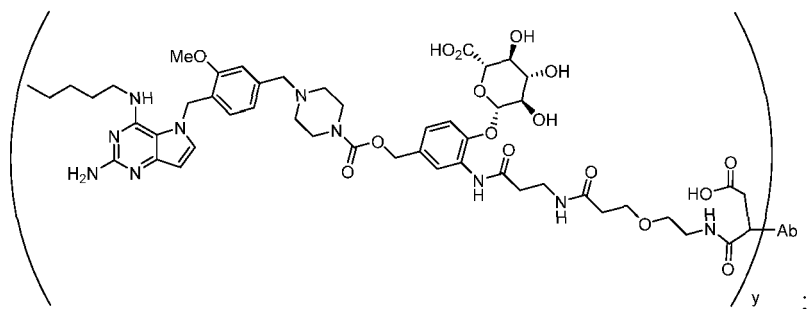
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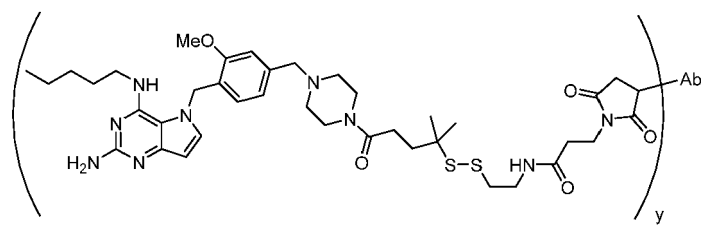
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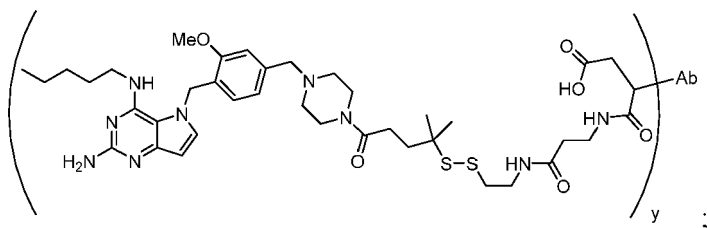


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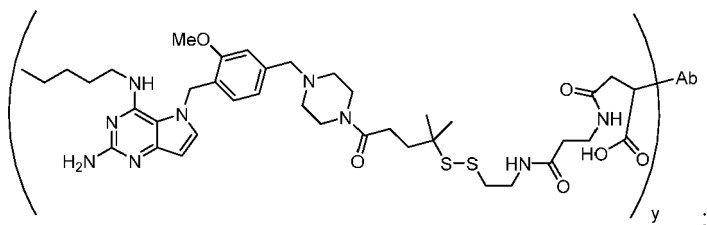
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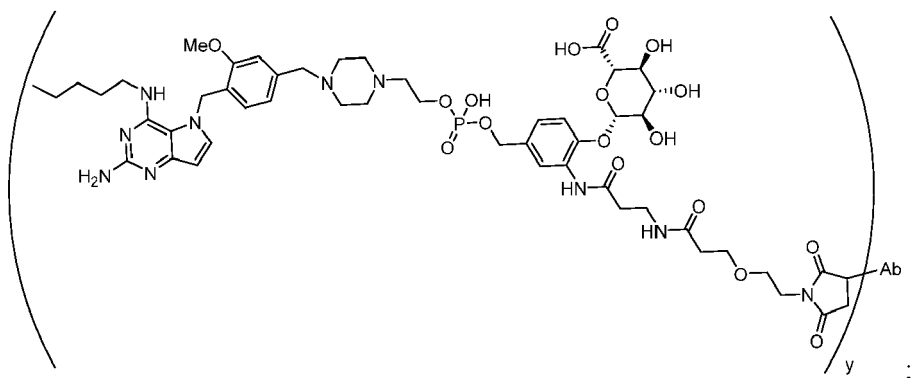


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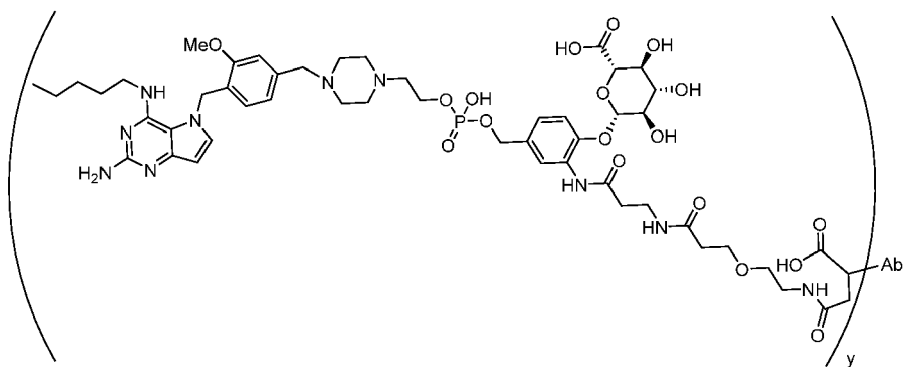
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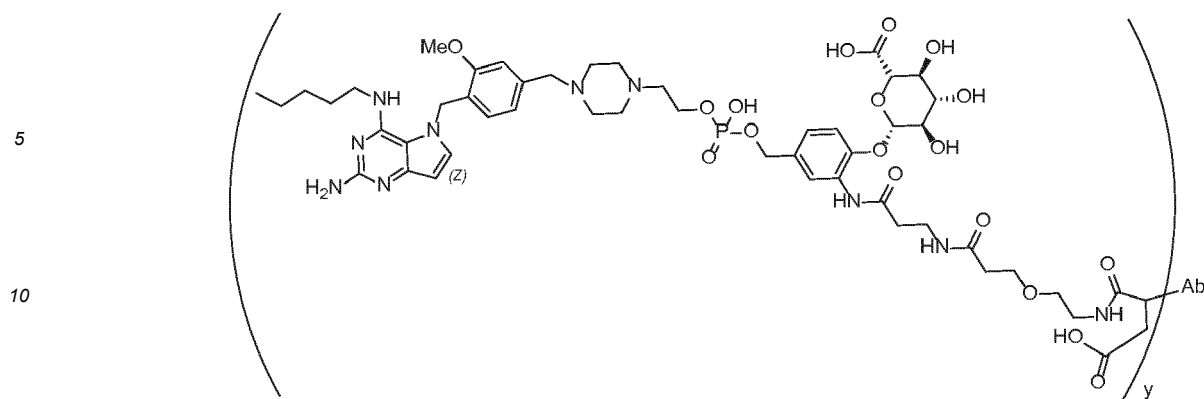
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[0118] Provided are also protocols for some aspects of analytical methodology for evaluating antibody conjugates of the invention. Such analytical methodology and results can demonstrate that the conjugates have favorable properties, for example properties that would make them easier to manufacture, easier to administer to patients, more efficacious, and/or potentially safer for patients. One example is the determination of molecular size by size exclusion chromatography (SEC) wherein the amount of desired antibody species in a sample is determined relative to the amount of high molecular weight contaminants (e.g., dimer, multimer, or aggregated antibody) or low molecular weight contaminants (e.g., antibody fragments, degradation products, or individual antibody chains) present in the sample. In general, it is desirable to have higher amounts of monomer and lower amounts of, for example, aggregated antibody due to the impact of, for example, aggregates on other properties of the antibody sample such as clearance rate, immunogenicity, and toxicity. A further example is the determination of the hydrophobicity by hydrophobic interaction chromatography (HIC) wherein the hydrophobicity of a sample is assessed relative to a set of standard antibodies of known properties. In general, it is desirable to have low hydrophobicity due to the impact of hydrophobicity on other properties of the antibody sample such as aggregation, aggregation over time, adherence to surfaces, hepatotoxicity, clearance rates, and pharmacokinetic exposure. See Damle, N.K., *Nat Biotechnol.* 2008; 26(8):884-885; Singh, S.K., *Pharm Res.* 2015; 32(11):3541-71. When measured by hydrophobic interaction chromatography, higher hydrophobicity index scores (i.e. elution from HIC column faster) reflect lower hydrophobicity of the conjugates. As shown in Example 70 and Table 3, a majority of the tested antibody conjugates showed a hydrophobicity index of greater than 0.8. In some embodiments, provided are antibody conjugates having a hydrophobicity index of 0.8 or greater, as determined by hydrophobic interaction chromatography.

Anti-HER2 Antibody

[0119] Antibody conjugates provided herein include an antibody or antibody fragment thereof (e.g., antigen binding fragment) that specifically binds to human HER2 (anti-HER2 antibody). HER2 overexpression is observed in many types of cancers, such as gastric cancer, esophageal cancer, colon cancer, rectal cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, endometrial cancer, bladder cancer, pancreatic cancer, lung cancer, prostate cancer, osteosarcoma, neuroblastoma, or head and neck cancer. Antibody conjugates comprising an anti-HER2 antibody can be specifically targeted to HER2-positive cancers or tumors.

[0120] In some embodiments, antibody conjugates provided herein include a monoclonal antibody or antibody fragment thereof that specifically binds to human HER2, e.g., a human or humanized anti-HER2 monoclonal antibody. In some embodiments, the antibody or antibody fragment thereof that specifically binds to human HER2 can be selected from trastuzumab, pertuzumab, margetuximab, or HT-19, or an antibody fragment thereof or a site-specific cysteine mutant thereof.

[0121] Trastuzumab (trade name Herceptin or Herclon) is a humanized monoclonal antibody that binds to the juxtamembrane portion of the extracellular domain of the HER2 receptor (Hudis CA, *N Engl J Med.* 2007; 357(1):39-51). The amino acid sequences of trastuzumab heavy chain and light chain variable regions were described in U.S. Patent No. 5,821,337. Trastuzumab interacts with three loop regions formed by residues 557-561, 570-573, and 593-603 of human HER2 (Cho et al., *Nature* 421: 756-760, 2003). Trastuzumab interferes with HER2 signaling possibly by prevention of HER2-receptor dimerization, facilitation of endocytotic destruction of the HER2 receptor, inhibition of shedding of the extracellular domain (Hudis CA, *N Engl J Med.* 2007; 357(1):39-51). Another important mechanism of action of an anti-HER2 antibody is the mediation of Antibody Dependent Cellular Cytotoxicity (ADCC). In ADCC, the anti-HER2 antibody binds to tumor cells and then recruits immune cells, such as macrophages, through Fc γ receptor (Fc γ R) interactions. Trastuzumab has a conserved human IgG Fc region, and is capable of recruiting immune effector cells that are responsible for antibody-dependent cytotoxicity (Hudis CA, *N Engl J Med.* 2007; 357(1):39-51). Trastuzumab gained U.S. FDA approval in September 1998 for the treatment of metastatic breast cancer in patients whose tumors overexpress HER2

and who received one or more chemotherapy regimens for their metastatic disease.

[0122] Pertuzumab (also called 2C4, Omnitarg, Perjeta) is a humanized monoclonal antibody that binds to the extracellular domain of the HER2 receptor and inhibits dimerization of HER2 with other HER receptors. The amino acid sequences of pertuzumab heavy chain and light chain were described in U.S. Patent No. 7,560,111. Pertuzumab mainly interact with residues within region 245-333 of human HER2, particularly residues His 245, Val 286, Ser 288, Leu 295, His 296, or Lys 311 (Franklin et al., Cancer Cell 5: 317-328, 2004). Pertuzumab was shown to be more effective than trastuzumab in disrupting the formation of HER1-HER2 and HER3-HER2 complexes in breast and prostate cancer cell lines (Agus et al., J Clin Oncol. 2005; 23(11):2534-43. Epub Feb 7, 2005). Pertuzumab does not require antibody-dependent cellular cytotoxicity for efficacy because an intact Fc region is not required for its activity (Agus et al., J Clin Oncol. 2005; 23(11):2534-43. Epub Feb 7, 2005). Pertuzumab received U.S. FDA approval for use in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive metastatic breast cancer who have not received anti-HER2 therapy or chemotherapy for metastatic disease in June 2012.

[0123] Margetuximab (also called MGAH22) is another anti-HER2 monoclonal antibody (See <http://www.macrogenics.com/products-margetuximab.html>). The Fc region of margetuximab was optimized so that it has increased binding to the activating FcγRs but decreased binding to the inhibitory FcγRs on immune effector cells. Margetuximab is currently under clinical trial for treating patients with relapsed or refractory advanced breast cancer whose tumors express HER2 at the 2+ Level by immunohistochemistry and lack evidence of HER2 gene amplification by FISH.

[0124] HT-19 is another anti-HER2 monoclonal antibody that binds to an epitope in human HER2 distinct from the epitope of trastuzumab or pertuzumab and was shown to inhibit HER2 signaling comparable to trastuzumab and enhance HER2 degradation in combination with trastuzumab and pertuzumab (Bergstrom D. A. et al., Cancer Res. 2015; 75:LB-231).

[0125] Other suitable anti-HER2 monoclonal antibodies include the anti-HER2 antibodies described in US Patent Nos.: 9,096,877; 9,017,671; 8,975,382; 8,974,785; 8,968,730; 8,937,159; 8,840,896; 8,802,093; 8,753,829; 8,741,586; 8,722,362; 8,697,071; 8,652,474; 8,652,466; 8,609,095; 8,512,967; 8,349,585; 8,241,630; 8,217,147; 8,192,737; 7,879,325; 7,850,966; 7,560,111; 7,435,797; 7,306,801; 6,399,063; 6,387,371; 6,165,464; 5,772,997; 5,770,195; 5,725,856; 5,720,954; 5,677,171.

[0126] In some embodiments, the anti-HER2 antibody or antibody fragment (e.g., an antigen binding fragment) comprises a VH domain having an amino acid sequence of any VH domain described in Table 1. Other suitable anti-HER2 antibodies or antibody fragments (e.g., antigen binding fragments) can include amino acids that have been mutated, yet have at least 80, 85, 90, 95, 96, 97, 98, or 99 percent identity in the VH domain with the VH regions depicted in the sequences described in Table 1. The present disclosure in certain embodiments also provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind to HER2, wherein the antibodies or antibody fragments (e.g., antigen binding fragments) comprise a VH CDR having an amino acid sequence of any one of the VH CDRs listed in Table 1. In particular embodiments, the invention provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind to HER2, comprising (or alternatively, consist of) one, two, three, four, five or more VH CDRs having an amino acid sequence of any of the VH CDRs listed in Table 1.

[0127] In some embodiments, the anti-HER2 antibody or antibody fragment (e.g., antigen binding fragments) comprises a VL domain having an amino acid sequence of any VL domain described in Table 1. Other suitable anti-HER2 antibodies or antibody fragments (e.g., antigen binding fragments) can include amino acids that have been mutated, yet have at least 80, 85, 90, 95, 96, 97, 98, or 99 percent identity in the VL domain with the VL regions depicted in the sequences described in Table 1. The present disclosure also provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind to HER2, the antibodies or antibody fragments (e.g., antigen binding fragments) comprise a VL CDR having an amino acid sequence of any one of the VL CDRs listed in Table 1. In particular, the invention provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind to HER2, which comprise (or alternatively, consist of) one, two, three or more VL CDRs having an amino acid sequence of any of the VL CDRs listed in Table 1.

Table 1. Sequences of exemplary anti-HER2 monoclonal antibodies

anti-HER2 mAb1		
SEQ ID NO: 1	HCDR1 (Kabat)	DTYIH
SEQ ID NO: 2	HCDR2 (Kabat)	RIYPTNGYTRYADSVKG
SEQ ID NO: 3	HCDR3 (Kabat)	WGGDGFYAMDY
SEQ ID NO: 4	HCDR1 (Chothia)	GFNIKDT
SEQ ID NO: 5	HCDR2 (Chothia)	YPTNGY

(continued)

	anti-HER2 mAb1		
5	SEQ ID NO: 3	HCDR3 (Chothia)	WGGDGFYAMDY
	SEQ ID NO: 6	HCDR1 (Combined)	GFNIKDTYIH
	SEQ ID NO: 2	HCDR2 (Combined)	RIYPTNGYTRYADSVKG
10	SEQ ID NO: 3	HCDR3 (Combined)	WGGDGFYAMDY
	SEQ ID NO: 7	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYI HWRQAPGKGLEWWARIYPTNGYTRYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCSRWG GDGFYAMDYWGQGTLLTVSS
15	SEQ ID NO: 8	VH DNA	GAGGTTCAGCTGGTGGAGTCTGGCGGTGGCCT GGTGCAGCCAGGGGGCTCACTCCGTTTGTCT GTGCAGCTTCTGGCTTCAACATTAAAGACACCT ATATACACTGGGTGCGTCAGGCCCCGGGTAAG GGCCTGGAATGGGTTGCAAGGATTTATCCTAC GAATGGTTATACTAGATATGCCGATAGCGTCAA GGGCCGTTTCACTATAAGCGCAGACACATCCA AAAACACAGCCTACCTGCAGATGAACAGCCTG CGTGCTGAGGACACTGCCGTCTATTATTGTTCT AGATGGGGAGGGGACGGCTTCTATGCTATGGA CTACTGGGGTCAAGGAACCCTGGTCACCGTCT CCTCG
20	SEQ ID NO: 9	Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYI HWRQAPGKGLEWWARIYPTNGYTRYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCSRWG GDGFYAMDYWGQGTLLTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPCPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPCDIAVEWESNGQPENNYKTTTPVLDS GSFFLYSKLTVDKSRWQQGNVFSVMSHEALHN HYTQKSLSLSPGK

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(continued)

	anti-HER2 mAb1		
5	SEQ ID NO: 14	LCDR1 (Chothia)	SQDVNTA
	SEQ ID NO: 15	LCDR2 (Chothia)	SAS
	SEQ ID NO: 16	LCDR3 (Chothia)	HYTTPP
	SEQ ID NO: 11	LCDR1 (Combined)	RASQDVNTAVA
10	SEQ ID NO: 12	LCDR2 (Combined)	SASFL YS
	SEQ ID NO: 13	LCDR3 (Combined)	QQHYTTPPT
15	SEQ ID NO: 17	VL	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSG TDFTLTISLQPEDFATYYCQQHYTTPPTFGQGT KVEIK
20	SEQ ID NO: 18	VL DNA	GATATCCAGATGACCCAGTCCCCGAGCTCCCT GTCCGCCTCTGTGGGCGATAGGGTCACCATCA CCTGCCGTGCCAGTCAGGATGTGAATACTGCT GTAGCCTGGTATCAACAGAAACCAGGAAAAGC TCCGAAACTACTGATTTACTCGGCATCCTTCCT CTACTCTGGAGTCCCTTCTCGCTTCTCTGGATC CAGATCTGGGACGGATTTCACTCTGACCATCA GCAGTCTGCAGCCGGAAGACTTCGCAACTTAT TACTGTCAGCAACATTATACTACTCCTCCACG TTCGGACAGGGTACCAAGGTGGAGATCAAA
30	SEQ ID NO: 19	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSG TDFTLTISLQPEDFATYYCQQHYTTPPTFGQGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
40	SEQ ID NO: 20	Light Chain DNA	GATATCCAGATGACCCAGTCCCCGAGCTCCCT GTCCGCCTCTGTGGGCGATAGGGTCACCATCA CCTGCCGTGCCAGTCAGGATGTGAATACTGCT GTAGCCTGGTATCAACAGAAACCAGGAAAAGC TCCGAAACTACTGATTTACTCGGCATCCTTCCT CTACTCTGGAGTCCCTTCTCGCTTCTCTGGATC CAGATCTGGGACGGATTTCACTCTGACCATCA GCAGTCTGCAGCCGGAAGACTTCGCAACTTAT TACTGTCAGCAACATTATACTACTCCTCCACG TTCGGACAGGGTACCAAGGTGGAGATCAAACG TACGGTGGCCGCTCCCAGCGTGTTTCATCTTCC CCCCCAGCGACGAGCAGCTGAAGAGTGGCAC CGCCAGCGTGGTGTGCCTGCTGAACAACCTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGT GGACAACGCCCTGCAGAGCGGCAACAGCCAG GAGAGCGTCACCGAGCAGGACAGCAAGGACT CCACCTACAGCCTGAGCAGCACCCCTGACCCCTG AGCAAGGCCGACTACGAGAAGCATAAGGTGTA CGCCTGCGAGGTGACCCACCAGGGCCTGTCC AGCCCCGTGACCAAGAGCTTCAACAGGGGCGA GTGC

(continued)

	anti-HER2 mAb2		
5	SEQ ID NO: 1	HCDR1 (Kabat)	DTYIH
	SEQ ID NO: 2	HCDR2 (Kabat)	RIYPTNGYTRYADSVKG
	SEQ ID NO: 3	HCDR3 (Kabat)	WGGDGFYAMDY
	SEQ ID NO: 4	HCDR1 (Chothia)	GFNIKDT
10	SEQ ID NO: 5	HCDR2 (Chothia)	YPTNGY
	SEQ ID NO: 3	HCDR3 (Chothia)	WGGDGFYAMDY
	SEQ ID NO: 6	HCDR1 (Combined)	GFNIKDTYIH
15	SEQ ID NO: 2	HCDR2 (Combined)	RIYPTNGYTRYADSVKG
	SEQ ID NO: 3	HCDR3 (Combined)	WGGDGFYAMDY
20	SEQ ID NO: 7	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYI HWVRQAPGKGLEWWARIYPTNGYTRYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCSRWG GDGFYAMDYWGQGTLVTVSS
25	SEQ ID NO: 8	VH DNA	GAGGTTTCAGCTGGTGGAGTCTGGCGGTGGCCT GGTGCAGCCAGGGGGCTCACTCCGTTTGTCT GTGCAGCTTCTGGCTTCAACATTAAAGACACCT ATATACACTGGGTGCGTCAGGCCCCGGGTAAG GGCCTGGAATGGGTTGCAAGGATTTATCCTAC GAATGGTTATACTAGATATGCCGATAGCGTCAA GGGCCGTTTCACTATAAGCGCAGACACATCCA AAAACACAGCCTACCTGCAGATGAACAGCCTG CGTGCTGAGGACACTGCCGTCTATTATTGTTCT AGATGGGGAGGGGACGGCTTCTATGCTATGGA CTACTGGGGTCAAGGAACCCTGGTCACCGTCT CCTCG
35	SEQ ID NO: 21	Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYI HWVRQAPGKGLEWWARIYPTNGYTRYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCSRWG GDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPCPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPCDIAVEWESNGQPENNYKTTTPVLDS GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK

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anti-HER2 mAb2		
SEQ ID NO: 22	Heavy Chain DNA	GAGGTTTCAGCTGGTGGAGTCTGGCGGTGGCCT GGTGCAGCCAGGGGGCTCACTCCGTTTGTCT GTGCAGCTTCTGGCTTCAACATTAAGACACCT ATATACTGGGTGCGTCAGGCCCCGGGTAAG GGCCTGGAATGGGTTGCAAGGATTTATCCTAC GAATGGTTATACTAGATATGCCGATAGCGTCAA GGGCCGTTTCACTATAAGCGCAGACACATCCA AAAACACAGCCTACCTGCAGATGAACAGCCTG CGTGCTGAGGACACTGCCGTCTATTATTGTTCT AGATGGGGAGGGGACGGCTTCTATGCTATGGA CTA CTGGGGTCAAGGAACCTGGTCACCGTCT CCTCGGCTAGCACCAAGGGCCCCAGCGTGTTT CCCCTGGCCCCCAGCAGCAAGAGCACCAGCG GCGGCACAGCCGCCCTGGGCTGCCTGGTGAA GGACTACTTCCCTTGTCCCGTGACCGTGTCT GGAACAGCGGAGCCCTGACCTCCGGCGTGCA CACCTTCCCCGCCGTGCTGCAGAGCAGCGGC CTGTACAGCCTGTCCAGCGTGGTGACAGTGCC CAGCAGCAGCCTGGGCACCCAGACCTACATCT GCAACGTGAACCACAAGCCCAGCAACACCAAG GTGGACAAGAAAGTGGAGCCCAAGAGCTGCGA CAAGACCCACACCTGCCCCCCTGCCAGCCC CAGAGCTGCTGGGCGGACCCTCCGTGTTCTCTG TTCCCCCCCCAAGCCCAAGGACACCCTGATGAT CAGCAGGACCCCCGAGGTGACCTGCGTGGTG GTGGACGTGAGCCACGAGGACCCAGAGGTGA AGTTCAACTGGTACGTGGACGGCGTGAGAGTG CACAACGCCAAGACCAAGCCCAGAGAGGAGCA GTACAACAGCACCTACAGGGTGGTGTCCTGTC TGACCGTGCTGCACCAGGACTGGCTGAACGGC AAGGAATACAAGTGCAAGGTCTCCAACAAGGC CCTGCCAGCCCCCATCGAAAAGACCATCAGCA AGGCCAAGGGCCAGCCACGGGAGCCCCAGGT GTACACCCTGCCCCCCTCCCGGGAGGAGATGA CCAAGAACCAGGTGTCCCTGACCTGTCTGGTG AAGGGCTTCTACCCCTGCGACATCGCCGTGGA GTGGGAGAGCAACGGCCAGCCCGAGAACAAC TACAAGACCACACCTCCAGTGCTGGACAGCGA CGGCAGCTTCTTCTGTACAGCAAGCTGACCG TGGACAAGTCCAGGTGGCAGCAGGGCAACGT GTTCAGCTGCAGCGTGATGCACGAGGCCCTGC ACAACCACTACACCCAGAAGAGCCTGAGCCTG TCCCCCGGCAAG
SEQ ID NO: 11	LCDR1 (Kabat)	RASQDVNTAVA
SEQ ID NO: 12	LCDR2 (Kabat)	SASFL YS
SEQ ID NO: 13	LCDR3 (Kabat)	QQHYTPPT

(continued)

	anti-HER2 mAb2		
5	SEQ ID NO: 14	LCDR1 (Chothia)	SQDVNTA
	SEQ ID NO: 15	LCDR2 (Chothia)	SAS
	SEQ ID NO: 16	LCDR3 (Chothia)	HYTTPP
	SEQ ID NO: 11	LCDR1 (Combined)	RASQDVNTAVA
10	SEQ ID NO: 12	LCDR2 (Combined)	SASFL YS
	SEQ ID NO: 13	LCDR3 (Combined)	QQHYTTPPT
15	SEQ ID NO: 17	VL	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSG TDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGT KVEIK
20	SEQ ID NO: 18	VL DNA	GATATCCAGATGACCCAGTCCCCGAGCTCCCT GTCCGCCTCTGTGGGCGATAGGGTCACCATCA CCTGCCGTGCCAGTCAGGATGTGAATACTGCT GTAGCCTGGTATCAACAGAAACCAGGAAAAGC TCCGAACTACTGATTTACTCGGCATCCTTCCT CTACTCTGGAGTCCCTTCTCGCTTCTCTGGATC CAGATCTGGGACGGATTTCACTCTGACCATCA GCAGTCTGCAGCCGGAAGACTTCGCAACTTAT TACTGTCAGCAACATTATACTACTCCTCCACG TTCGGACAGGGTACCAAGGTGGAGATCAAA
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30	SEQ ID NO: 19	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSG TDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
35			
40	SEQ ID NO: 34	Light Chain DNA	GATATCCAGATGACCCAGTCCCCGAGCTCCCT GTCCGCCTCTGTGGGCGATAGGGTCACCATCA CCTGCCGTGCCAGTCAGGATGTGAATACTGCT GTAGCCTGGTATCAACAGAAACCAGGAAAAGC TCCGAACTACTGATTTACTCGGCATCCTTCCT CTACTCTGGAGTCCCTTCTCGCTTCTCTGGATC CAGATCTGGGACGGATTTCACTCTGACCATCA GCAGTCTGCAGCCGGAAGACTTCGCAACTTAT TACTGTCAGCAACATTATACTACTCCTCCACG TTCGGACAGGGTACCAAGGTGGAGATCAAACG AACGGTGGCCGCTCCCAGCGTGTTTCATCTTCC CCCCCAGCGACGAGCAGCTGAAGAGCGGCAC CGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGT GGACAACGCCCTGCAGAGCGGCAACAGCCAG GAGAGCGTCACCGAGCAGGACAGCAAGGACT CCACCTACAGCCTGAGCAGCACCCCTGACCCCTG AGCAAGGCCGACTACGAGAAGCATAAGGTGTA CGCCTGCGAGGTGACCCACCAGGGCCTGTCC AGCCCCGTGACCAAGAGCTTCAACAGGGGCGA GTGC
45			
50			
55			

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	anti-HER2 mAb3		
5	SEQ ID NO: 1	HCDR1 (Kabat)	DTYIH
	SEQ ID NO: 2	HCDR2 (Kabat)	RIYPTNGYTRYADSVKG
	SEQ ID NO: 3	HCDR3 (Kabat)	WGGDGFYAMDY
	SEQ ID NO: 4	HCDR1 (Chothia)	GFNIKDT
10	SEQ ID NO: 5	HCDR2 (Chothia)	YPTNGY
	SEQ ID NO: 3	HCDR3 (Chothia)	WGGDGFYAMDY
	SEQ ID NO: 6	HCDR1 (Combined)	GFNIKDTYIH
15	SEQ ID NO: 2	HCDR2 (Combined)	RIYPTNGYTRYADSVKG
	SEQ ID NO: 3	HCDR3 (Combined)	WGGDGFYAMDY
20	SEQ ID NO: 7	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYI HWRQAPGKGLEWWARIYPTNGYTRYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCSRWG GDGFYAMDYWGQGTLLTVSS
25	SEQ ID NO: 8	VH DNA	GAGGTTTCAGCTGGTGGAGTCTGGCGGTGGCCT GGTGCAGCCAGGGGGCTCACTCCGTTTGTCT GTGCAGCTTCTGGCTTCAACATTAAAGACACCT ATATACTGGGTGCGTCAGGCCCCGGGTAAG GGCCTGGAATGGGTTGCAAGGATTTATCCTAC GAATGGTTATACTAGATATGCCGATAGCGTCAA GGGCCGTTTCACTATAAGCGCAGACACATCCA AAAACACAGCCTACCTGCAGATGAACAGCCTG CGTGCTGAGGACACTGCCGTCTATTATTGTTCT AGATGGGGAGGGGACGGCTTCTATGCTATGGA CTACTGGGGTCAAGGAACCCTGGTCACCGTCT CCTCG
35	SEQ ID NO: 23	Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYI HWRQAPGKGLEWWARIYPTNGYTRYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCSRWG GDGFYAMDYWGQGTLLTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK

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	anti-HER2 mAb3		
5	SEQ ID NO: 14	LCDR1 (Chothia)	SQDVNTA
	SEQ ID NO: 15	LCDR2 (Chothia)	SAS
	SEQ ID NO: 16	LCDR3 (Chothia)	HYTTPP
	SEQ ID NO: 11	LCDR1 (Combined)	RASQDVNTAVA
10	SEQ ID NO: 12	LCDR2 (Combined)	SASFL YS
	SEQ ID NO: 13	LCDR3 (Combined)	QQHYTTPPT
15	SEQ ID NO: 17	VL	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSG TDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGT KVEIK
20	SEQ ID NO: 18	VL DNA	GATATCCAGATGACCCAGTCCCCGAGCTCCCT GTCCGCCTCTGTGGGCGATAGGGTCACCATCA CCTGCCGTGCCAGTCAGGATGTGAATACTGCT GTAGCCTGGTATCAACAGAAACCAGGAAAAGC TCCGAAACTACTGATTTACTCGGCATCCTTCT CTACTCTGGAGTCCCTTCTCGCTTCTCTGGATC CAGATCTGGGACGGATTTCACTCTGACCATCA GCAGTCTGCAGCCGGAAGACTTCGCAACTTAT TACTGTCAGCAACATTATACTACTCCTCCACG TTCGGACAGGGTACCAAGGTGGAGATCAAA
30	SEQ ID NO: 19	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSG TDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
35	SEQ ID NO: 34	Light Chain DNA	GATATCCAGATGACCCAGTCCCCGAGCTCCCT GTCCGCCTCTGTGGGCGATAGGGTCACCATCA CCTGCCGTGCCAGTCAGGATGTGAATACTGCT GTAGCCTGGTATCAACAGAAACCAGGAAAAGC TCCGAAACTACTGATTTACTCGGCATCCTTCT CTACTCTGGAGTCCCTTCTCGCTTCTCTGGATC CAGATCTGGGACGGATTTCACTCTGACCATCA GCAGTCTGCAGCCGGAAGACTTCGCAACTTAT TACTGTCAGCAACATTATACTACTCCTCCACG TTCGGACAGGGTACCAAGGTGGAGATCAAACG AACGGTGGCCGCTCCCAGCGTGTTTCATCTTCC CCCCAGCGACGAGCAGCTGAAGAGCGGCAC CGCCAGCGTGGTGTGCCTGCTGAACAACTTCT ACCCCCGGGAGGCCAAGGTGCAGTGGAAGGT GGACAACGCCCTGCAGAGCGGCAACAGCCAG GAGAGCGTCACCGAGCAGGACAGCAAGGACT CCACCTACAGCCTGAGCAGCACCCCTGACCCTG AGCAAGGCCGACTACGAGAAGCATAAGGTGTA CGCCTGCGAGGTGACCCACCAGGGCCTGTCC AGCCCCGTGACCAAGAGCTTCAACAGGGGCGA GTGC

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anti-HER2 mAb4		
SEQ ID NO: 30	Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYI HWRQAPGKGLEWWARIYPTNGYTRYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCSRWG GDGFYAMDYWGQGTLLTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPCPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK
SEQ ID NO: 19	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSG TDFTLTISLQPEDFATYYCQQHYTTPPTFGQGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
anti-HER2 mAb5		
SEQ ID NO: 32	Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYI HWRQAPGKGLEWWARIYPTNGYTRYADSVKGR FTISADTSKNTAYLQMNSLRAEDTAVYYCSRWG GDGFYAMDYWGQGTLLTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPEGDSLDMLEWSLM NNYKTTTPVLDSGSFFLYSKLTVDKSRWQQGN VFSCFSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 19	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSG TDFTLTISLQPEDFATYYCQQHYTTPPTFGQGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC

[0128] Other anti-HER2 antibodies or antibody fragments (e.g., antigen binding fragments) disclosed herein include amino acids that have been mutated, yet have at least 80, 85, 90, 95, 96, 97, 98, or 99 percent identity in the CDR regions with the CDR regions depicted in the sequences described in Table 1. In some embodiments, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the CDR regions when compared with the CDR regions depicted in the sequence described in Table 1.

[0129] Also provided herein are nucleic acid sequences that encode VH, VL, full length heavy chain, and full length light chain of antibodies and antigen binding fragments thereof that specifically bind to HER2, e.g., the nucleic acid sequences in Table 1. Such nucleic acid sequences can be optimized for expression in mammalian cells.

[0130] Other anti-HER2 antibodies disclosed herein include those where the amino acids or nucleic acids encoding the amino acids have been mutated, yet have at least 80, 85, 90, 95, 96, 97, 98, or 99 percent identity to the sequences described in Table 1. In some embodiments, antibodies or antigen binding fragments thereof include mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the variable regions when compared with the variable regions depicted in the sequence described in Table 1, while retaining substantially the same therapeutic activity.

[0131] Since each provided antibody binds to HER2, the VH, VL, full length light chain, and full length heavy chain sequences (amino acid sequences and the nucleotide sequences encoding the amino acid sequences) can be "mixed and matched" to create other HER2-binding antibodies disclosed herein. Such "mixed and matched" HER2-binding antibodies can be tested using binding assays known in the art (e.g., ELISAs, assays described in the Exemplification). When chains are mixed and matched, a VH sequence from a particular VH/VL pairing should be replaced with a structurally similar VH sequence. A full length heavy chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length heavy chain sequence. A VL sequence from a particular VH/VL pairing should be replaced with a structurally similar VL sequence. A full length light chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length light chain sequence.

[0132] Accordingly, in one embodiment, the invention provides an isolated monoclonal antibody or antigen binding region thereof having: a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 7; and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 17; wherein the antibody specifically binds to HER2. In another embodiment, the invention provides (i) an isolated monoclonal antibody having: a full length heavy chain comprising an amino acid sequence of any of SEQ ID NOs: 9, 21, 23, 30 or 32; and a full length light chain comprising an amino acid sequence of SEQ ID NO: 19; or (ii) a functional protein comprising an antigen binding portion thereof.

[0133] In another embodiment, the present disclosure provides HER2-binding antibodies that comprise the heavy chain CDR1, CDR2 and CDR3 and light chain CDR1, CDR2 and CDR3 as described in Table 1, or combinations thereof. The amino acid sequences of the VH CDR1s of the antibodies are shown in SEQ ID NOs: 1, 4, and 6. The amino acid sequences of the VH CDR2s of the antibodies are shown in SEQ ID NOs: 2 and 5. The amino acid sequences of the VH CDR3s of the antibodies are shown in SEQ ID NO: 3. The amino acid sequences of the VL CDR1s of the antibodies are shown in SEQ ID NOs: 11 and 14. The amino acid sequences of the VL CDR2s of the antibodies are shown in SEQ ID NOs 12 and 15. The amino acid sequences of the VL CDR3s of the antibodies are shown in SEQ ID NOs: 13 and 16.

[0134] Given that each of the antibodies binds HER2 and that antigen-binding specificity is provided primarily by the CDR1, CDR2 and CDR3 regions, the VH CDR1, CDR2 and CDR3 sequences and VL CDR1, CDR2 and CDR3 sequences can be "mixed and matched" (i.e., CDRs from different antibodies can be mixed and match, although each antibody must contain a VH CDR1, CDR2 and CDR3 and a VL CDR1, CDR2 and CDR3 to create other HER2-binding molecules disclosed herein. Such "mixed and matched" HER2-binding antibodies can be tested using the binding assays known in the art and those described in the Examples (e.g., ELISAs). When VH CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VH sequence should be replaced with a structurally similar CDR sequence(s). Likewise, when VL CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VL sequence should be replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel VH and VL sequences can be created by substituting one or more VH and/or VL CDR region sequences with structurally similar sequences from CDR sequences shown herein for monoclonal antibodies of the present disclosure.

[0135] Accordingly, the present disclosure provides an isolated monoclonal antibody or antigen binding region thereof comprising a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 4, and 6; a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 5; a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 3; a light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 11 and 14; a light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 12 and 15; and a light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 13 and 16; wherein the antibody specifically binds HER2.

[0136] In certain embodiments, an antibody that specifically binds to HER2 is an antibody or antibody fragment (e.g., antigen binding fragment) that is described in Table 1.

[0137] In some embodiments, the antibody that specifically binds to human HER2 comprises a heavy chain complementary determining region 1 (HCDR1) comprising the amino acid sequence of SEQ ID NO: 1; a heavy chain complementary determining region 2 (HCDR2) comprising the amino acid sequence of SEQ ID NO: 2; a heavy chain complementary determining region 3 (HCDR3) comprising the amino acid sequence of SEQ ID NO: 3; a light chain complementary determining region 1 (LCDR1) comprising the amino acid sequence of SEQ ID NO: 11; a light chain complementary determining region 2 (LCDR2) comprising the amino acid sequence of SEQ ID NO: 12; a light chain complementary determining region 3 (LCDR3) comprising the amino acid sequence of SEQ ID NO: 13; and a light chain complementary determining region 4 (LCDR4) comprising the amino acid sequence of SEQ ID NO: 14.

mentary determining region 2 (LCDR2) comprising the amino acid sequence of SEQ ID NO: 12; and a light chain complementary determining region 3 (LCDR3) comprising the amino acid sequence of SEQ ID NO: 13.

[0138] In some embodiments, the antibody that specifically binds to human HER2 comprises a HCDR1 comprising the amino acid sequence of SEQ ID NO: 4; a HCDR2 comprising the amino acid sequence of SEQ ID NO: 5; a HCDR3 comprising the amino acid sequence of SEQ ID NO: 3; a LCDR1 comprising the amino acid sequence of SEQ ID NO: 14; a LCDR2 comprising the amino acid sequence of SEQ ID NO: 15; and a LCDR3 comprising the amino acid sequence of SEQ ID NO: 16.

[0139] In some embodiments, the antibody that specifically binds to human HER2 comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 17.

[0140] In some embodiments, the antibody that specifically binds to human HER2 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9, and a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0141] In some embodiments, the antibody that specifically binds to human HER2 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 21, and a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0142] In some embodiments, the antibody that specifically binds to human HER2 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23, and a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0143] In some embodiments, the antibody that specifically binds to human HER2 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 30, and a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0144] In some embodiments, the antibody that specifically binds to human HER2 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 32, and a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0145] In some embodiments, the present disclosure provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind an epitope in human HER2. In some embodiments, the present disclosure provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind to an epitope in human HER2, wherein the epitope comprises one or more of the residues 557-561, 570-573, and 593-603 of SEQ ID NO: 26. In some embodiments, the present disclosure provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind to an epitope in human HER2, wherein the epitope comprises one or more of the residues 245-333 of SEQ ID NO: 26. In some embodiments, the present disclosure provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind to an epitope in human HER2, wherein the epitope comprises one or more of the following residues: His 245, Val 286, Ser 288, Leu 295, His 296, or Lys 311 of SEQ ID NO: 26.

[0146] Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., using the techniques described in the present invention. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct cross-competition studies to find antibodies that competitively bind with one another, e.g., the antibodies compete for binding to the antigen. A high throughput process for "binning" antibodies based upon their cross-competition is described in International Patent Application No. WO 2003/48731. As will be appreciated by one of skill in the art, practically anything to which an antibody can specifically bind could be an epitope. An epitope can comprises those residues to which the antibody binds.

Modification of Framework or Fc Region

[0147] Antibodies and antibody conjugates disclosed herein may comprise modified antibodies or antigen binding fragments thereof that comprise modifications to framework residues within VH and/or VL, e.g. to improve the properties of the antibody/antibody conjugate.

[0148] In some embodiments, framework modifications are made to decrease immunogenicity of an antibody. For example, one approach is to "back-mutate" one or more framework residues to a corresponding germline sequence. Such residues can be identified by comparing antibody framework sequences to germline sequences from which the antibody is derived. To "match" framework region sequences to desired germline configuration, residues can be "back-mutated" to a corresponding germline sequence by, for example, site-directed mutagenesis. Such "back-mutated" antibodies are also intended to be encompassed by the invention.

[0149] Another type of framework modification involves mutating one or more residues within a framework region, or even within one or more CDR regions, to remove T-cell epitopes to thereby reduce potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication No. 20030153043 by Carr et al.

[0150] In addition or alternative to modifications made within a framework or CDR regions, antibodies disclosed herein may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity.

[0151] Furthermore, an antibody disclosed herein may be chemically modified (e.g., one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Each of these embodiments is described in further detail below.

[0152] In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, e.g., increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer et al. The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

[0153] In some embodiments antibodies or antibody fragments (e.g., antigen binding fragment) useful in antibody conjugates disclosed herein include modified or engineered antibodies, such as an antibody modified to introduce one or more cysteine residues as sites for conjugation to a drug moiety (Junutula JR, et al.: Nat Biotechnol 2008, 26:925-932). In one embodiment, the invention provides a modified antibody or antibody fragment thereof comprising a substitution of one or more amino acids with cysteine at the positions described herein. Sites for cysteine substitution are in the constant regions of the antibody and are thus applicable to a variety of antibodies, and the sites are selected to provide stable and homogeneous conjugates. A modified antibody or fragment can have two or more cysteine substitutions, and these substitutions can be used in combination with other antibody modification and conjugation methods as described herein. Methods for inserting cysteine at specific locations of an antibody are known in the art, see, e.g., Lyons et al, (1990) Protein Eng., 3:703-708, WO 2011/005481, WO2014/124316, WO 2015/138615. In certain embodiments a modified antibody or antibody fragment comprises a substitution of one or more amino acids with cysteine on its constant region selected from positions 117, 119, 121, 124, 139, 152, 153, 155, 157, 164, 169, 171, 174, 189, 205, 207, 246, 258, 269, 274, 286, 288, 290, 292, 293, 320, 322, 326, 333, 334, 335, 337, 344, 355, 360, 375, 382, 390, 392, 398, 400 and 422 of a heavy chain of the antibody or antibody fragment, and wherein the positions are numbered according to the EU system. In some embodiments a modified antibody or antibody fragment comprises a substitution of one or more amino acids with cysteine on its constant region selected from positions 107, 108, 109, 114, 129, 142, 143, 145, 152, 154, 156, 159, 161, 165, 168, 169, 170, 182, 183, 197, 199, and 203 of a light chain of the antibody or antibody fragment, wherein the positions are numbered according to the EU system, and wherein the light chain is a human kappa light chain. In certain embodiments a modified antibody or antibody fragment thereof comprises a combination of substitution of two or more amino acids with cysteine on its constant regions wherein the combinations comprise substitutions at positions 375 of an antibody heavy chain, position 152 of an antibody heavy chain, position 360 of an antibody heavy chain, or position 107 of an antibody light chain and wherein the positions are numbered according to the EU system. In certain embodiments a modified antibody or antibody fragment thereof comprises a substitution of one amino acid with cysteine on its constant regions wherein the substitution is position 375 of an antibody heavy chain, position 152 of an antibody heavy chain, position 360 of an antibody heavy chain, position 107 of an antibody light chain, position 165 of an antibody light chain or position 159 of an antibody light chain and wherein the positions are numbered according to the EU system, and wherein the light chain is a kappa chain.

[0154] In particular embodiments a modified antibody or antibody fragment thereof comprises a combination of substitution of two amino acids with cysteine on its constant regions, wherein the modified antibody or antibody fragment thereof comprises cysteines at positions 152 and 375 of an antibody heavy chain, wherein the positions are numbered according to the EU system.

[0155] In other particular embodiments a modified antibody or antibody fragment thereof comprises a substitution of one amino acid with cysteine at position 360 of an antibody heavy chain and wherein the positions are numbered according to the EU system.

[0156] In other particular embodiments a modified antibody or antibody fragment thereof comprises a substitution of one amino acid with cysteine at position 107 of an antibody light chain and wherein the positions are numbered according to the EU system, and wherein the light chain is a kappa chain.

[0157] In additional embodiments antibodies or antibody fragments (e.g., antigen binding fragment) useful in antibody conjugates disclosed herein include modified or engineered antibodies, such as an antibody modified to introduce one or more other reactive amino acid (other than cysteine), including Pcl, pyrrolysine, peptide tags (such as S6, A1 and ybbR tags), and non-natural amino acids, in place of at least one amino acid of the native sequence, thus providing a reactive site on the antibody or antigen binding fragment for conjugation to a drug moiety of Formula (I) or subformulae thereof. For example, the antibodies or antibody fragments can be modified to incorporate Pcl or pyrrolysine (W. Ou et al. (2011) PNAS 108 (26), 10437-10442; WO2014124258) or unnatural amino acids (J.Y. Axup, et al. Proc Natl Acad Sci U S A, 109 (2012), pp. 16101-16106; for review, see C.C. Liu and P.G. Schultz (2010) Annu Rev Biochem 79, 413-444; C.H. Kim, et al., (2013) Curr Opin Chem Biol. 17, 412-419) as sites for conjugation to a drug. Similarly, peptide tags for enzymatic conjugation methods can be introduced into an antibody (Strop P. et al. Chem Biol. 2013, 20(2):161-7; Rabuka D., Curr Opin Chem Biol. 2010 Dec;14(6):790-6; Rabuka D, et al., Nat Protoc. 2012, 7(6):1052-67). One other example is the use of 4'-phosphopantetheinyl transferases (PPTase) for the conjugation of Coenzyme A analogs (WO2013184514). Methods for conjugating such modified or engineered antibodies with payloads or linker-payload combinations are known in the art.

[0158] In another embodiment, an Fc hinge region of an antibody is mutated to decrease the biological half-life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcal Protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward et al.

[0159] In yet other embodiments, an Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in, e.g., U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter et al.

[0160] In another embodiment, one or more amino acids selected from amino acid residues can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in, e.g., U.S. Patent Nos. 6,194,551 by Idusogie et al.

[0161] In another embodiment, one or more amino acid residues are altered to thereby alter the ability of the antibody to fix complement. This approach is described in, e.g., the PCT Publication WO 94/29351 by Bodmer et al. Allotypic amino acid residues include constant region of a heavy chain of the IgG1, IgG2, and IgG3 subclasses as well as constant region of a light chain of the kappa isotype as described by Jefferis et al., MAbs. 1:332-338 (2009).

[0162] In yet another embodiment, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or antibody dependent cellular phagocytosis (ADCP), for example, by modifying one or more amino acid residues to increase the affinity of the antibody for an activating Fc γ receptor, or to decrease the affinity of the antibody for an inhibitory Fc γ receptor. Human activating Fc γ receptors include Fc γ RIa, Fc γ RIIa, Fc γ RIIIa, and Fc γ RIIIb, and human inhibitory Fc γ receptor includes Fc γ RIIb. This approach is described in, e.g., the PCT Publication WO 00/42072 by Presta. Moreover, binding sites on human IgG1 for Fc γ RI, Fc γ RII, Fc γ RIII and FcRn have been mapped and variants with improved binding have been described (see Shields et al., J. Biol. Chem. 276:6591-6604, 2001). Optimization of Fc-mediated effector functions of monoclonal antibodies such as increased ADCC/ADCP function has been described (see Strohl, W.R., Current Opinion in Biotechnology 2009; 20:685-691.) In some embodiments, an antibody conjugate comprises an immunoglobulin heavy chain comprising a mutation or combination of mutations conferring enhanced ADCC/ADCP function, e.g., one or more mutations selected from G236A, S239D, F243L, P247I, D280H, K290S, R292P, S298A, S298D, S298V, Y300L, V305I, A330L, I332E, E333A, K334A, A339D, A339Q, A339T, P396L (all positions by EU numbering).

[0163] In another embodiment, the Fc region is modified to increase the ability of the antibody to mediate ADCC and/or ADCP, for example, by modifying one or more amino acids to increase the affinity of the antibody for an activating receptor that would typically not recognize the parent antibody, such as Fc α RI. This approach is described in, e.g., Borrok et al., mAbs. 7(4):743-751. In particular embodiments, an antibody conjugate comprises an immunoglobulin heavy chain comprising a mutation or a fusion of one or more antibody sequences conferring enhanced ADCC and/or ADCP function.

[0164] In still another embodiment, glycosylation of an antibody is modified. For example, an aglycosylated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for "antigen." Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in, e.g., U.S. Patent Nos. 5,714,350 and 6,350,861 by Co et al.

[0165] Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hang et al. describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation. PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields et al., (2002) J. Biol. Chem. 277:26733-26740). PCT Publication WO 99/54342 by Umana et al. describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (e.g., beta(1,4)-N acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNAc structures which results in increased ADCC activity of the antibodies (see also Umana et al., Nat. Biotech. 17:176-180, 1999).

[0166] In another embodiment, the antibody is modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described

in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half-life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta et al.

5 **Production of anti-HER2 Antibodies**

[0167] Anti-HER2 antibodies and antibody fragments (*e.g.*, antigen binding fragments) thereof can be produced by any means known in the art, including recombinant expression, chemical synthesis, and enzymatic digestion of antibody tetramers, whereas full length monoclonal antibodies can be obtained by, *e.g.*, hybridoma or recombinant production. Recombinant expression can be from any appropriate host cells known in the art, for example, mammalian host cells, bacterial host cells, yeast host cells, insect host cells, etc.

[0168] Also provided herein are polynucleotides encoding antibodies described herein, *e.g.*, polynucleotides encoding heavy or light chain variable regions or segments comprising complementarity determining regions as described herein. In some embodiments, a polynucleotide encoding the heavy chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 8. In some embodiments, a polynucleotide encoding the light chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 18.

[0169] In some embodiments, a polynucleotide encoding the heavy chain has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of any of SEQ ID NOs: 10, 22, or 24. In some embodiments, a polynucleotide encoding the light chain has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 20 or 34.

[0170] Some polynucleotides disclosed herein encode a variable region of an anti-HER2 antibody. Some polynucleotides disclosed herein encode both a variable region and a constant region of an anti-HER2 antibody. Some polynucleotide sequences encode a polypeptide that comprises variable regions of both a heavy chain and a light chain of an anti-HER2 antibody. Some polynucleotides encode two polypeptide segments that respectively are substantially identical to the variable regions of a heavy chain and a light chain of any anti-HER2 antibodies disclosed herein.

[0171] Polynucleotide sequences can be produced by *de novo* solid-phase DNA synthesis or by PCR mutagenesis of an existing sequence (*e.g.*, sequences as described in the Examples below) encoding an anti-HER2 antibody or its binding fragment. Direct chemical synthesis of nucleic acids can be accomplished by methods known in the art, such as the phosphotriester method of Narang et al., *Meth. Enzymol.* 68:90, 1979; the phosphodiester method of Brown et al., *Meth. Enzymol.* 68:109, 1979; the diethylphosphoramidite method of Beaucage et al., *Tetra. Lett.*, 22:1859, 1981; and the solid support method of U.S. Patent No. 4,458,066. Introducing mutations to a polynucleotide sequence by PCR can be performed as described in, *e.g.*, PCR Technology: Principles and Applications for DNA Amplification, H.A. Erlich (Ed.), Freeman Press, NY, NY, 1992; PCR Protocols: A Guide to Methods and Applications, Innis et al. (Ed.), Academic Press, San Diego, CA, 1990; Mattila et al., *Nucleic Acids Res.* 19:967, 1991; and Eckert et al., *PCR Methods and Applications* 1:17, 1991.

[0172] Also provided are expression vectors and host cells for producing anti-HER2 antibodies described above. Various expression vectors can be employed to express polynucleotides encoding anti-HER2 antibody chains or binding fragments. Both viral-based and nonviral expression vectors can be used to produce antibodies in a mammalian host cell.

[0173] Nonviral vectors and systems include plasmids, episomal vectors, typically with an expression cassette for expressing a protein or RNA, and human artificial chromosomes (see, *e.g.*, Harrington et al., *Nat Genet* 15:345, 1997). For example, nonviral vectors useful for expression of anti-HER2 polynucleotides and polypeptides in mammalian (*e.g.*, human) cells include pThioHis A, B & C, pCDNATM3.1/His, pEBVHis A, B & C (Invitrogen, San Diego, CA), MPSV vectors, and numerous other vectors known in the art for expressing other proteins. Useful viral vectors include vectors based on retroviruses, adenoviruses, adenoassociated viruses, herpes viruses, vectors based on SV40, papilloma virus, HBP Epstein Barr virus, vaccinia virus vectors and Semliki Forest virus (SFV). See, Brent *et al.*, *supra*; Smith, *Annu. Rev. Microbiol.* 49:807, 1995; and Rosenfeld et al., *Cell* 68:143, 1992.

[0174] Choice of expression vector depends on the intended host cells in which a vector is to be expressed. Typically, expression vectors contain a promoter and other regulatory sequences (*e.g.*, enhancers) that are operably linked to polynucleotides encoding an anti-HER2 antibody chain or fragment. In some embodiments, an inducible promoter is employed to prevent expression of inserted sequences except under inducing conditions. Inducible promoters include, *e.g.*, arabinose, lacZ, metallothionein promoter or a heat shock promoter. Cultures of transformed organisms can be expanded under noninducing conditions without biasing the population for coding sequences whose expression products are better tolerated by host cells. In addition to promoters, other regulatory elements may also be required or desired for efficient expression of an anti-HER2 antibody chain or fragment. Elements typically include an ATG initiation codon and adjacent ribosome binding site or other sequences. In addition, efficiency of expression may be enhanced by the

inclusion of enhancers appropriate to the cell system in use (see, e.g., Scharf et al., *Results Probl. Cell Differ.* 20:125, 1994; and Bittner et al., *Meth. Enzymol.*, 153:516, 1987). For example, an SV40 enhancer or CMV enhancer may be used to increase expression in mammalian host cells.

[0175] Expression vectors may also provide a secretion signal sequence position to form a fusion protein with polypeptides encoded by inserted anti-HER2 antibody sequences. More often, inserted anti-HER2 antibody sequences are linked to a signal sequence before inclusion in the vector. Vectors to be used to receive sequences encoding anti-HER2 antibody light and heavy chain variable domains sometimes also encode constant regions or parts thereof. Such vectors allow expression of variable regions as fusion proteins with constant regions, thereby leading to production of intact antibodies or fragments thereof. Typically, such constant regions are human.

[0176] Host cells for harboring and expressing anti-HER2 antibody chains can be either prokaryotic or eukaryotic. *E. coli* is one prokaryotic host useful for cloning and expressing polynucleotides of the present disclosure. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as a lactose promoter system, a tryptophan (*trp*) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters typically control expression, optionally with an operator sequence, and have ribosome binding site sequences, for initiating and completing transcription and translation. Other microbes, such as yeast, can also be employed to express anti-HER2 polypeptides disclosed herein. Insect cells in combination with baculovirus vectors can also be used.

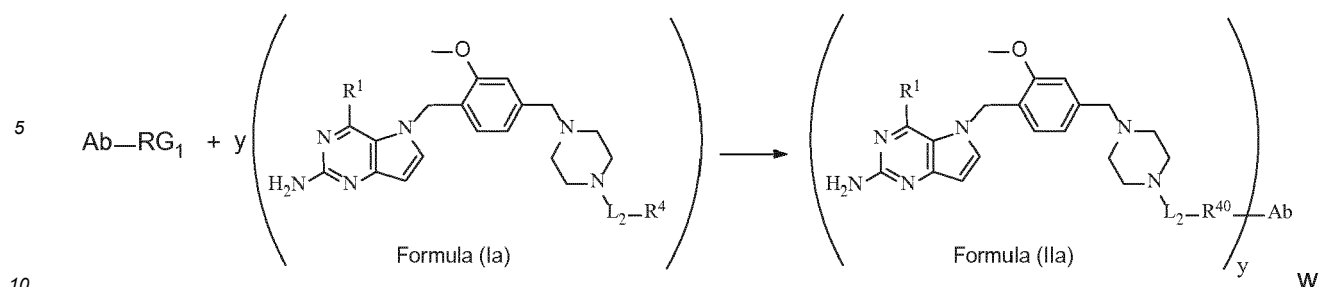
[0177] In some particular embodiments, mammalian host cells are used to express and produce anti-HER2 polypeptides of the present disclosure. For example, they can be either a hybridoma cell line expressing endogenous immunoglobulin genes (e.g., myeloma hybridoma clones) or a mammalian cell line harboring an exogenous expression vector (e.g., the SP2/0 myeloma cells). These include any normal mortal or normal or abnormal immortal animal or human cell. For example, a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed, including various CHO cell lines, Cos cell lines, HeLa cells, myeloma cell lines, transformed B-cells and hybridomas. Use of mammalian tissue cell culture to express polypeptides is discussed generally in, e.g., Winnacker, *From Genes to Clones*, VCH Publishers, N.Y., N.Y., 1987. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (see, e.g., Queen et al., *Immunol. Rev.* 89:49-68, 1986), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Expression vectors usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters may be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, a metallothionein promoter, a constitutive adenovirus major late promoter, a dexamethasone-inducible MMTV promoter, a SV40 promoter, a MRP polIII promoter, a constitutive MPSV promoter, a tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), a constitutive CMV promoter, and promoter-enhancer combinations known in the art.

[0178] Methods for introducing expression vectors containing polynucleotide sequences of interest vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts (see generally Sambrook *et al.*, *supra*). Other methods include, e.g., electroporation, calcium phosphate treatment, liposome-mediated transformation, injection and microinjection, ballistic methods, virosomes, immunoliposomes, polycation:nucleic acid conjugates, naked DNA, artificial virions, fusion to the herpes virus structural protein VP22 (Elliot and O'Hare, *Cell* 88:223, 1997), agent-enhanced uptake of DNA, and *ex vivo* transduction. For long-term, high-yield production of recombinant proteins, stable expression will often be desired. For example, cell lines which stably express anti-HER2 antibody chains or binding fragments can be prepared using expression vectors disclosed herein which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth of cells which successfully express the introduced sequences in selective media. Resistant, stably transfected cells can be proliferated using tissue culture techniques appropriate to the cell type.

Processes for Making Antibody conjugate of Formula (IIa) and Formula (IIb)

[0179] A general reaction scheme for the formation of immunostimulatory conjugates of Formula (II) is shown in Scheme 13 below:

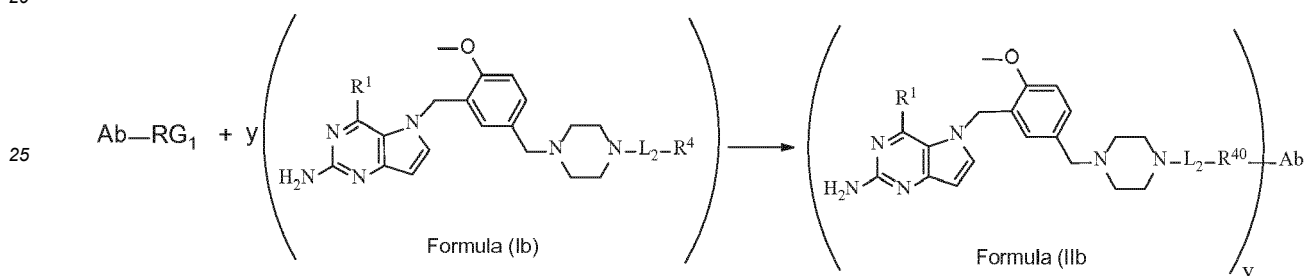
Scheme 13



here: RG_1 is a reactive group which reacts with a compatible R^4 group of a compound of Formula (Ia) to form a corresponding R^{40} group, such as maleimide reacting with a thiol to give a succinimide ring, or a hydroxylamine reacting with a ketone to give an oxime; R^1 , R^4 , L_2 , Ab and R^{40} are as defined herein.

15 **[0180]** A general reaction scheme for the formation of immunostimulatory conjugates of Formula (IIb) is shown in Scheme 14 below:

Scheme 14



30 where: RG_1 is a reactive group which reacts with a compatible R^4 group of a compound of Formula (Ib) to form a corresponding R^{40} group, such as maleimide reacting with a thiol to give a succinimide ring, or a hydroxylamine reacting with a ketone to give an oxime; R^1 , R^4 , L_2 , Ab and R^{40} are as defined herein.

35 Therapeutic Uses

[0181] Provided antibody conjugates are useful in a variety of applications including treatment of cancer, such as HER2 positive cancer. In certain embodiments, antibody conjugates provided herein are useful for inhibiting tumor growth, reducing tumor volume, inducing differentiation, and/or reducing the tumorigenicity of a tumor, e.g., a HER2 solid tumor. The methods of use can be *in vitro*, *ex vivo*, or *in vivo* methods.

40 **[0182]** In some embodiments, provided herein is use of the antibody conjugates of the invention to treat or prevent disease in a subject, e.g., a human patient. Additionally provided is use of antibody conjugates in treatment or prevention of disease in a subject. In some embodiments provided are antibody conjugates for use in manufacture of a medicament for treatment or prevention of disease in a subject. In certain embodiments, the disease treated with antibody conjugates is a cancer, e.g., a HER2-positive cancer. Various cancers that can be treated with the antibody conjugates are listed in the definitions section above. The HER2-positive cancer can be any cancer comprising cells that have HER2 protein present at their cell surface. For example, a HER2-positive cancer can be either primary tumor or metastasis of any of gastric cancer, esophageal cancer, gastroesophageal junction adenocarcinoma, colon cancer, rectal cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, endometrial cancer, bladder cancer, urinary tract cancer, pancreatic cancer, lung cancer, prostate cancer, osteosarcoma, neuroblastoma, glioblastoma, neuroendocrine tumors, and head and neck cancer. In certain embodiments, the cancer is characterized by HER2 expressing cells to which the antibodies, antibody fragments (e.g., antigen binding fragments) of the antibody conjugates bind. In certain embodiments, the cancer is characterized by concurrent expression of multiple human epidermal growth factor receptors in addition to HER2 expression. In some embodiments, the HER2-positive cancer can have high HER2 expression, e.g., having an immunohistochemistry (IHC) score of 3+, which is defined as uniform intense membrane staining of >30% of invasive tumor cells as determined by the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) IHC score (see English et al., Mol Diagn Ther. 2013 Apr; 17(2): 85-99). In some embodiments, the HER2-positive cancer can have relatively low HER2 expression, e.g., having an IHC score of 2+, which is defined as complete

membrane staining that is either non-uniform or weak in intensity but with obvious circumferential distribution in at least 10% cells or very rarely tumors that show complete membranes staining of 30% or fewer tumor cells by the ASCO/CAP IHC score (see English et al., Mol Diagn Ther. 2013 Apr; 17(2): 85-99).

[0183] In some embodiments, provided is a therapeutically effective amount of any of the antibody conjugates described herein for treating a HER2-positive cancer in a subject in need thereof. The HER2-positive cancer can be any cancer comprising cells that have HER2 protein present at their cell surface. In some embodiments, the antibody conjugate used is capable of suppressing the HER2-positive cancer for a sustained period and/or reducing recurrence of the HER2-positive cancer, when compared to an anti-HER2 antibody alone.

[0184] It is also contemplated that the antibody conjugates described herein may be used to treat various non-malignant diseases or disorders, such as inflammatory bowel disease (IBD), gastrointestinal ulcers, Menetrier's disease, hepatitis B, hepatitis C, secreting adenomas or protein loss syndrome, renal disorders, angiogenic disorders, ocular disease such as age related macular degeneration, presumed ocular histoplasmosis syndrome, or age related macular degeneration, bone associated pathologies such as osteoarthritis, rickets and osteoporosis, hyperviscosity syndrome systemic, Osler Weber-Rendu disease, chronic occlusive pulmonary disease, or edema following burns, trauma, radiation, stroke, hypoxia or ischemia, diabetic nephropathy, Paget's disease, photoaging (e.g., caused by UV radiation of human skin), benign prostatic hypertrophy, certain microbial infections including microbial pathogens selected from adenovirus, hantaviruses, *Borrelia burgdorferi*, *Yersinia* spp., and *Bordetella pertussis*, thrombus caused by platelet aggregation, reproductive conditions such as endometriosis, ovarian hyperstimulation syndrome, preeclampsia, dysfunctional uterine bleeding, or menometrorrhagia, acute and chronic nephropathies (including proliferative glomerulonephritis), hypertrophic scar formation, endotoxin shock and fungal infection, familial adenomatosis polyposis, myelodysplastic syndromes, aplastic anemia, ischemic injury, fibrosis of the lung, kidney or liver, infantile hypertrophic pyloric stenosis, urinary obstructive syndrome, psoriatic arthritis.

[0185] Method of administration of such antibody conjugates include, parenteral (e.g., intravenous) administration, e.g., injection as a bolus or continuous infusion over a period of time, oral administration, intramuscular administration, intratumoral administration, intramuscular administration, intraperitoneal administration, intracerebrospinal administration, subcutaneous administration, intra-articular administration, intrasynovial administration, injection to lymph nodes, or intrathecal administration.

[0186] For treatment of disease, appropriate dosage of antibody conjugates of the present invention depends on various factors, such as the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, previous therapy, patient's clinical history, and so on. Antibody conjugates can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved (e.g., reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of a particular antibody conjugate. In some embodiments, dosage is from 0.01 mg to 20 mg (e.g., 0.01 mg, 0.02 mg, 0.03 mg, 0.04 mg, 0.05 mg, 0.06 mg, 0.07 mg, 0.08 mg, 0.09 mg, 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, or 20 mg) per kg of body weight, and can be given once or more daily, weekly, monthly or yearly. In certain embodiments, the antibody conjugate of the present invention is given once every two weeks or once every three weeks. In certain embodiments, the antibody conjugate of the present invention is given only once. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues.

Combination Therapy

[0187] In certain instances, an antibody conjugate of the present invention can be combined with other therapeutic agents, such as other anti-cancer agents, anti-allergic agents, anti-nausea agents (or anti-emetics), pain relievers, cytoprotective agents, and combinations thereof.

[0188] General chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegen), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycytidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-

DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), vinorelbine (Navelbine®), epirubicin (Ellence®), oxaliplatin (Eloxatin®), exemestane (Aromasin®), letrozole (Femara®), and fulvestrant (Faslodex®).

[0189] The term "pharmaceutical combination" as used herein refers to either a fixed combination in one dosage unit form, or non-fixed combination or a kit of parts for the combined administration where two or more therapeutic agents may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, e.g. synergistic effect.

[0190] The term "combination therapy" refers to the administration of two or more therapeutic agents to treat a therapeutic condition or disorder described in the present disclosure. Such administration encompasses co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single capsule having a fixed ratio of active ingredients. Alternatively, such administration encompasses co-administration in multiple, or in separate containers (e.g., capsules, powders, and liquids) for each active ingredient. Powders and/or liquids may be reconstituted or diluted to a desired dose prior to administration. In addition, such administration also encompasses use of each type of therapeutic agent in a sequential manner, either at approximately the same time or at different times. In either case, the treatment regimen will provide beneficial effects of the drug combination in treating the conditions or disorders described herein.

[0191] The combination therapy can provide "synergy" and prove "synergistic", i.e., the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect can be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect can be attained when the compounds are administered or delivered sequentially, e.g., by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e., serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

[0192] In one embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more other anti-HER2 antibodies, e.g., trastuzumab, pertuzumab, margetuximab, or HT-19 described above, or with other anti-HER2 conjugates, e.g., ado-trastuzumab emtansine (also known as Kadcyla®, or T-DM1) for use in treating cancer in a subject in need thereof.

[0193] In one embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more tyrosine kinase inhibitors, including EGFR inhibitors, Her3 inhibitors, IGFR inhibitors, and Met inhibitors for use in treating cancer in a subject in need thereof.

[0194] For example, tyrosine kinase inhibitors include Erlotinib hydrochloride (Tarceva®); Linifanib (N-[4-(3-amino-1H-indazol-4-yl)phenyl]-N'-(2-fluoro-5-methylphenyl)urea, also known as ABT 869, available from Genentech); Sunitinib malate (Sutent®); Bosutinib (4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile, also known as SKI-606, and described in US Patent No. 6,780,996); Dasatinib (Sprycel®); Pazopanib (Votrient®); Sorafenib (Nexavar®); Zactima (ZD6474); and Imatinib or Imatinib mesylate (Gleevec® and Gleevec®).

[0195] Epidermal growth factor receptor (EGFR) inhibitors include Erlotinib hydrochloride (Tarceva®), Gefitinib (Iressa®); N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[[3"S"]-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butenamide, Tovok®; Vandetanib (Caprelsa®); Lapatinib (Tykerb®); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); Canertinib dihydrochloride (CI-1033); 6-[4-[(4-Ethyl-1-piperazinyl)methyl]phenyl]-N-[(1R)-1-phenylethyl]-7H-Pyrrolo[2,3-d]pyrimidin-4-amine (AEE788, CAS 497839-62-0); Mubritinib (TAK165); Pelitinib (EKB569); Afatinib (Gilotrif®); Neratinib (HKI-272); N-[4-[[1-[(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamic acid, (3S)-3-morpholinylmethyl ester (BMS599626); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[[3α,5β,6α]-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); and 4-[4-[(1R)-1-Phenylethyl]amino]-7H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol (PKI166, CAS187724-61-4).

[0196] EGFR antibodies include Cetuximab (Erbix®); Panitumumab (Vectibix®); Matuzumab (EMD-72000); Nimotuzumab (hR3); Zalutumumab; TheraCIM h-R3; MDX0447 (CAS 339151-96-1); and ch806 (mAb-806, CAS 946414-09-1).

[0197] Other HER2 inhibitors include Neratinib (HKI-272, (2E)-N-[4-[[3-chloro-4-[(pyridin-2-yl)methoxy]phenyl]amino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide, and described PCT Publication No. WO 05/028443); Lapatinib or Lapatinib ditosylate (Tykerb®); (3R,4R)-4-amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); (2E)-N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[[3S]-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4-(dimethylamino)-2-butenamide (BIBW-2992, CAS 850140-72-6); N-[4-[[1-[(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamic acid, (3S)-3-morpholinylmethyl ester (BMS 599626, CAS 714971-09-2); Canertinib dihydrochloride (PD183805 or CI-1033); and N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[[3α,5β,6α]-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS

781613-23-8).

[0198] HER3 inhibitors include LJM716, MM-121, AMG-888, RG7116, REGN-1400, AV-203, MP-RM-1, MM-111, and MEHD-7945A.

[0199] MET inhibitors include Cabozantinib (XL184, CAS 849217-68-1); Foretinib (GSK1363089, formerly XL880, CAS 849217-64-7); Tivantinib (ARQ197, CAS 1000873-98-2); 1-(2-Hydroxy-2-methylpropyl)-N-(5-(7-methoxyquinolin-4-yloxy)pyridin-2-yl)-5-methyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carboxamide (AMG 458); Cryzotinib (Xalkori®, PF-02341066); (3Z)-5-(2,3-Dihydro-1H-indol-1-ylsulfonyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1H-pyrrol-2-yl)methylene)-1,3-dihydro-2H-indol-2-one (SU11271); (3Z)-N-(3-Chlorophenyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1H-pyrrol-2-yl)methylene)-N-methyl-2-oxoindoline-5-sulfonamide (SU11274); (3Z)-N-(3-Chlorophenyl)-3-[[3,5-dimethyl-4-(3-morpholin-4-ylpropyl)-1H-pyrrol-2-yl]methylene]-N-methyl-2-oxoindoline-5-sulfonamide (SU11606); 6-[Difluoro[6-(1-methyl-1H-pyrazol-4-yl)-1,2,4-triazolo[4,3-b]pyridazin-3-yl]methyl]-quinoline (JNJ38877605, CAS 943540-75-8); 2-[4-[1-(Quinolin-6-ylmethyl)-1H-[1,2,3]triazolo[4,5-b]pyrazin-6-yl]-1H-pyrazol-1-yl]ethanol (PF04217903, CAS 956905-27-4); N-((2R)-1,4-Dioxan-2-ylmethyl)-N-methyl-N'-[3-(1-methyl-1H-pyrazol-4-yl)-5-oxo-5H-benzo[4,5]cyclohepta[1,2-b]pyridin-7-yl]sulfamide (MK2461, CAS 917879-39-1); 6-[[6-(1-Methyl-1H-pyrazol-4-yl)-1,2,4-triazolo[4,3-b]pyridazin-3-yl]thio]-quinoline (SGX523, CAS 1022150-57-7); and (3Z)-5-[[[2,6-Dichlorophenyl]methyl]sulfonyl]-3-[[3,5-dimethyl-4-[(2R)-2-(1-pyrrolidinylmethyl)-1-pyrrolidinyl]carbonyl]-1H-pyrrol-2-yl]methylene]-1,3-dihydro-2H-indol-2-one (PHA665752, CAS 477575-56-7).

[0200] IGF1R inhibitors include BMS-754807, XL-228, OSI-906, GSK0904529A, A-928605, AXL1717, KW-2450, MK0646, AMG479, IMCA12, MEDI-573, and BI836845. See e.g., Yee, JNCI, 104; 975 (2012) for review.

[0201] In another embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more proliferation signaling pathway inhibitors, including MEK inhibitors, BRAF inhibitors, PI3K/Akt inhibitors, SHP2 inhibitors, and also mTOR inhibitors, and CDK inhibitors for use in treating cancer in a subject in need thereof.

[0202] For example, mitogen-activated protein kinase (MEK) inhibitors include XL-518 (also known as GDC-0973, Cas No. 1029872-29-4, available from ACC Corp.); 2-[(2-Chloro-4-iodophenyl)amino]-N-(cyclopropylmethoxy)-3,4-difluoro-benzamide (also known as CI-1040 or PD184352 and described in PCT Publication No. WO2000035436); N-[(2R)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide (also known as PD0325901 and described in PCT Publication No. WO2002006213); 2,3-Bis[amino[(2-aminophenyl)thio]methylene]-butanedinitrile (also known as U0126 and described in US Patent No. 2,779,780); N-[3,4-Difluoro-2-[(2-fluoro-4-iodophenyl)amino]-6-methoxyphenyl]-1-[(2R)-2,3-dihydroxypropyl]-cyclopropanesulfonamide (also known as RDEA119 or BAY869766 and described in PCT Publication No. WO2007014011); (3S,4R,5Z,8S,9S,11E)-14-(Ethylamino)-8,9,16-trihydroxy-3,4-dimethyl-3,4,9,19-tetrahydro-1H-2-benzoxacyclotetradecine-1,7(8H)-dione (also known as E6201 and described in PCT Publication No. WO2003076424); 2'-Amino-3'-methoxyflavone (also known as PD98059 available from BIAFFIN GmbH & Co., KG, Germany); Vemurafenib (PLX-4032, CAS 918504-65-1); (R)-3-(2,3-Dihydroxypropyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido[2,3-d]pyrimidine-4,7(3H,8H)-dione (TAK-733, CAS 1035555-63-5); Pimasertib (AS-703026, CAS 1204531-26-9); and Trametinib dimethyl sulfoxide (GSK-1120212, CAS 1204531-25-80).

[0203] BRAF inhibitors include Vemurafenib (or Zelboraf®), GDC-0879, PLX-4720 (available from Symansis), Dabrafenib (or GSK2118436), LGX 818, CEP-32496, UI-152, RAF 265, Regorafenib (BAY 73-4506), CCT239065, or Sorafenib (or Sorafenib Tosylate, or Nexavar®), or Ipilimumab (or MDX-010, MDX-101, or Yervoy).

[0204] Phosphoinositide 3-kinase (PI3K) inhibitors include 4-[2-(1H-Indazol-4-yl)-6-[[4-(methylsulfonyl)piperazin-1-yl]methyl]thieno[3,2-d]pyrimidin-4-yl]morpholine (also known as GDC0941, RG7321, GNE0941, Pictrelisib, or Pictilisib; and described in PCT Publication Nos. WO 09/036082 and WO 09/055730); 2-Methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in PCT Publication No. WO 06/122806); 4-(trifluoromethyl)-5-(2,6-dimorpholinopyrimidin-4-yl)pyridin-2-amine (also known as BKM120 or NVP-BKM120, and described in PCT Publication No. WO2007/084786); Tozasertib (VX680 or MK-0457, CAS 639089-54-6); (5Z)-5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidinedione (GSK1059615, CAS 958852-01-2); (1E,4S,4aR,5R,6aS,9aR)-5-(Acetyloxy)-1-[(di-2-propenylamino)methylene]-4,4a,5,6,6a,8,9,9a-octahydro-11-hydroxy-4-(methoxymethyl)-4a,6a-dimethylcyclopenta[5,6]naphtho[1,2-c]pyran-2,7,10(1H)-trione (PX866, CAS 502632-66-8); 8-Phenyl-2-(morpholin-4-yl)-chromen-4-one (LY294002, CAS 154447-36-6); (S)-N1-(4-methyl-5-(2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl)thiazol-2-yl)pyrrolidine-1,2-dicarboxamide (also known as BYL719 or Alpelisib); 2-(4-(2-(1-isopropyl-3-methyl-1H-1,2,4-triazol-5-yl)-5,6-dihydrobenzo[f]imidazo[1,2-d][1,4]oxazepin-9-yl)-1H-pyrazol-1-yl)-2-methylpropanamide (also known as GDC0032, RG7604, or Taselisib).

[0205] mTOR inhibitors include Temsirolimus (Torisel®); Ridaforolimus (formally known as deferolimus, (1R,2R,4S)-4-[(2R)-2-[(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28Z,30S,32S,35R)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-2,3,10,14,20-penta-11,36-dioxo-4-azatricyclo[30.3.1.0^{4,9}]hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); Everolimus (Afinitor® or RAD001); Rapamycin (AY22989, Sirolimus®); Simapimod (CAS 164301-51-3); (5-{2,4-Bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl)-2-

methoxyphenyl)methanol (AZD8055); 2-Amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF04691502, CAS 1013101-36-4); and *N*²-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4*H*-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L- α -aspartyl-L-serine-, inner salt (SF1126, CAS 936487-67-1).

[0206] CDK inhibitors include Palbociclib (also known as PD-0332991, Ibrance®, 6-Acetyl-8-cyclopentyl-5-methyl-2-[[5-(1-piperazinyl)-2-pyridinyl]amino]pyrido[2,3-*d*]pyrimidin-7(8*H*)-one).

[0207] In yet another embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more pro-apoptotics, including IAP inhibitors, BCL2 inhibitors, MCI1 inhibitors, TRAIL agents, CHK inhibitors for use in treating cancer in a subject in need thereof.

[0208] For examples, IAP inhibitors include LCL161, GDC-0917, AEG-35156, AT406, and TL32711. Other examples of IAP inhibitors include those disclosed in WO04/005284, WO 04/007529, WO05/097791, WO 05/069894, WO 05/069888, WO 05/094818, US2006/0014700, US2006/0025347, WO 06/069063, WO 06/010118, WO 06/017295, and WO08/134679.

[0209] BCL-2 inhibitors include 4-[4-[[2-(4-Chlorophenyl)-5,5-dimethyl-1-cyclohexen-1-yl]methyl]-1-piperazinyl]-N-[[4-[[[(1*R*)-3-(4-morpholinyl)-1-[(phenylthio)methyl]propyl]amino]-3-[(trifluoromethyl)sulfonyl]phenyl]sulfonyl]benzamide (also known as ABT-263 and described in PCT Publication No. WO 09/155386); Tetrocarcin A; Antimycin; Gossypol ((-)-JBL-193); Obatoclax; Ethyl-2-amino-6-cyclopentyl-4-(1-cyano-2-ethoxy-2-oxoethyl)-4Hchromone-3-carboxylate (HA14 -1); Oblimersen (G3139, Genasense®); Bak BH3 peptide; (-)-Gossypol acetic acid (AT-101); 4-[4-[(4'-Chloro[1,1'-biphenyl]-2-yl)methyl]-1-piperazinyl]-N-[[4-[[[(1*R*)-3-(dimethylamino)-1-[(phenylthio)methyl]propyl]amino]-3-nitrophenyl]sulfonyl]benzamide (ABT-737, CAS 852808-04-9); and Navitoclax (ABT-263, CAS 923564-51-6).

[0210] Proapoptotic receptor agonists (PARAs) including DR4 (TRAILR1) and DR5 (TRAILR2), including Dulanermin (AMG-951, RhApo2L/TRAIL); Mapatumumab (HRS-ETR1, CAS 658052-09-6); Lexatumumab (HGS-ETR2, CAS 845816-02-6); Apomab (Apomab®); Conatumumab (AMG655, CAS 896731-82-1); and Tigatuzumab (CS1008, CAS 946415-34-5, available from Daiichi Sankyo).

[0211] Checkpoint Kinase (CHK) inhibitors include 7-Hydroxystaurosporine (UCN-01); 6-Bromo-3-(1-methyl-1*H*-pyrazol-4-yl)-5-(3*R*)-3-piperidinylpyrazolo[1,5-*a*]pyrimidin-7-amine (SCH900776, CAS 891494-63-6); 5-(3-Fluorophenyl)-3-ureidothiophene-2-carboxylic acid N-[(S)-piperidin-3-yl]amide (AZD7762, CAS 860352-01-8); 4-[[[(3*S*)-1-Azabicyclo[2.2.2]oct-3-yl]amino]-3-(1*H*-benzimidazol-2-yl)-6-chloroquinolin-2(1*H*)-one (CHIR 124, CAS 405168-58-3); 7-Aminodactinomycin (7-AAD), Isogranulatimide, debromohymenialdisine; N-[5-Bromo-4-methyl-2-[(2*S*)-2-morpholinylmethoxy]-phenyl]-N'-(5-methyl-2-pyrazinyl)urea (LY2603618, CAS 911222-45-2); Sulforaphane (CAS 4478-93-7, 4-Methylsulfanylbutyl isothiocyanate); 9,10,11,12-Tetrahydro-9,12-epoxy-1*H*-diindolo[1,2,3-*fg*:3',2',1'-*k'*]pyrrolo[3,4-*l*][1,6]benzodiazocine-1,3(2*H*)-dione (SB-218078, CAS 135897-06-2); and TAT-S216A (YGRKKRRQRRRLYRSPAMPENL (SEQ ID NO: 33)), and CBP501 ((d-Bpa)sws(d-Phe-F5)(d-Cha)rrrrrr).

[0212] In a further embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more immunomodulators (e.g., one or more of an activator of a costimulatory molecule or an inhibitor of an immune checkpoint molecule) for use in treating cancer in a subject in need thereof.

[0213] In certain embodiments, the immunomodulator is an activator of a costimulatory molecule. In one embodiment, the agonist of the costimulatory molecule is chosen from an agonist (e.g., an agonistic antibody or antigen-binding fragment thereof, or a soluble fusion) of OX40, CD2, CD27, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3 or CD83 ligand.

[0214] In certain embodiments, the immunomodulator is an inhibitor of an immune checkpoint molecule. In one embodiment, the immunomodulator is an inhibitor of PD-1, PD-L1, PD-L2, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and/or TGFRbeta. In one embodiment, the inhibitor of an immune checkpoint molecule inhibits PD-1, PD-L1, LAG-3, TIM-3 or CTLA4, or any combination thereof. The term "inhibition" or "inhibitor" includes a reduction in a certain parameter, e.g., an activity, of a given molecule, e.g., an immune checkpoint inhibitor. For example, inhibition of an activity, e.g., a PD-1 or PD-L1 activity, of at least 5%, 10%, 20%, 30%, 40%, 50% or more is included by this term. Thus, inhibition need not be 100%.

[0215] Inhibition of an inhibitory molecule can be performed at the DNA, RNA or protein level. In some embodiments, an inhibitory nucleic acid (e.g., a dsRNA, siRNA or shRNA), can be used to inhibit expression of an inhibitory molecule. In other embodiments, the inhibitor of an inhibitory signal is a polypeptide e.g., a soluble ligand (e.g., PD-1-Ig or CTLA-4 Ig), or an antibody or antigen-binding fragment thereof, that binds to the inhibitory molecule; e.g., an antibody or fragment thereof (also referred to herein as "an antibody molecule") that binds to PD-1, PD-L1, PD-L2, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and/or TGFR beta, or a combination thereof.

[0216] In one embodiment, the antibody molecule is a full antibody or fragment thereof (e.g., a Fab, F(ab')₂, Fv, or a single chain Fv fragment (scFv)). In yet other embodiments, the antibody molecule has a heavy chain constant region (Fc) chosen from, e.g., the heavy chain constant regions of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE; particularly, chosen from, e.g., the heavy chain constant regions of IgG1, IgG2, IgG3, and IgG4, more particularly, the heavy chain constant region of IgG1 or IgG4 (e.g., human IgG1 or IgG4). In one embodiment, the heavy chain constant

region is human IgG1 or human IgG4. In one embodiment, the constant region is altered, e.g., mutated, to modify the properties of the antibody molecule (e.g., to increase or decrease one or more of Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function).

[0217] In certain embodiments, the antibody molecule is in the form of a bispecific or multispecific antibody molecule.

In one embodiment, the bispecific antibody molecule has a first binding specificity to PD-1 or PD-L1 and a second binding specificity, e.g., a second binding specificity to TIM-3, LAG-3, or PD-L2. In one embodiment, the bispecific antibody molecule binds to PD-1 or PD-L1 and TIM-3. In another embodiment, the bispecific antibody molecule binds to PD-1 or PD-L1 and LAG-3. In another embodiment, the bispecific antibody molecule binds to PD-1 and PD-L1. In yet another embodiment, the bispecific antibody molecule binds to PD-1 and PD-L2. In another embodiment, the bispecific antibody molecule binds to TIM-3 and LAG-3. Any combination of the aforesaid molecules can be made in a multispecific antibody molecule, e.g., a trispecific antibody that includes a first binding specificity to PD-1 or PD-1, and a second and third binding specificities to two or more of: TIM-3, LAG-3, or PD-L2.

[0218] In certain embodiments, the immunomodulator is an inhibitor of PD-1, e.g., human PD-1. In another embodiment, the immunomodulator is an inhibitor of PD-L1, e.g., human PD-L1. In one embodiment, the inhibitor of PD-1 or PD-L1 is an antibody molecule to PD-1 or PD-L1. The PD-1 or PD-L1 inhibitor can be administered alone, or in combination with other immunomodulators, e.g., in combination with an inhibitor of LAG-3, TIM-3 or CTLA4. In an exemplary embodiment, the inhibitor of PD-1 or PD-L1, e.g., the anti-PD-1 or PD-L1 antibody molecule, is administered in combination with a LAG-3 inhibitor, e.g., an anti-LAG-3 antibody molecule. In another embodiment, the inhibitor of PD-1 or PD-L1, e.g., the anti-PD-1 or PD-L1 antibody molecule, is administered in combination with a TIM-3 inhibitor, e.g., an anti-TIM-3 antibody molecule. In yet other embodiments, the inhibitor of PD-1 or PD-L1, e.g., the anti-PD-1 antibody molecule, is administered in combination with a LAG-3 inhibitor, e.g., an anti-LAG-3 antibody molecule, and a TIM-3 inhibitor, e.g., an anti-TIM-3 antibody molecule.

[0219] Other combinations of immunomodulators with a PD-1 inhibitor (e.g., one or more of PD-L2, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and/or TGFR) are also within the present invention. Any of the antibody molecules known in the art or disclosed herein can be used in the aforesaid combinations of inhibitors of checkpoint molecule.

[0220] In one embodiment, the PD-1 inhibitor is an anti-PD-1 antibody chosen from Nivolumab, Pembrolizumab or Pidilizumab. In some embodiments, the anti-PD-1 antibody is Nivolumab. Alternative names for Nivolumab include MDX-1106, MDX-1106-04, ONO-4538, or BMS-936558. In some embodiments, the anti-PD-1 antibody is Nivolumab (CAS Registry Number: 946414-94-4). Nivolumab is a fully human IgG4 monoclonal antibody which specifically blocks PD1. Nivolumab (clone 5C4) and other human monoclonal antibodies that specifically bind to PD1 are disclosed in US Pat No. 8,008,449 and PCT Publication No. WO2006/121168.

[0221] In other embodiments, the anti-PD-1 antibody is Pembrolizumab. Pembrolizumab (Trade name KEYTRUDA formerly Lambrolizumab, also known as Merck 3745, MK-3475 or SCH-900475) is a humanized IgG4 monoclonal antibody that binds to PD1. Pembrolizumab is disclosed, e.g., in Hamid, O. et al. (2013) New England Journal of Medicine 369 (2): 134-44, PCT Publication No. WO2009/114335, and US Patent No. 8,354,509.

[0222] In some embodiments, the anti-PD-1 antibody is Pidilizumab. Pidilizumab (CT-011; Cure Tech) is a humanized IgG1k monoclonal antibody that binds to PD1. Pidilizumab and other humanized anti-PD-1 monoclonal antibodies are disclosed in PCT Publication No. WO2009/101611. Other anti-PD1 antibodies are disclosed in US Patent No. 8,609,089, US Publication No. 2010028330, and/or US Publication No. 20120114649. Other anti-PD1 antibodies include AMP 514 (Amplimmune).

[0223] In some embodiments, the PD-1 inhibitor is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 inhibitor is AMP-224.

[0224] In some embodiments, the PD-L1 inhibitor is anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 inhibitor is chosen from YW243.55.S70, MPDL3280A, MEDI-4736, or MDX 1105MSB-0010718C (also referred to as A09-246-2) disclosed in, e.g., WO 2013/0179174, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[0225] In one embodiment, the PD-L1 inhibitor is MDX-1105. MDX-1105, also known as BMS 936559, is an anti-PD-L1 antibody described in PCT Publication No. WO 2007/005874.

[0226] In one embodiment, the PD-L1 inhibitor is YW243.55.S70. The YW243.55.S70 antibody is an anti-PD-L1 described in PCT Publication No. WO 2010/077634.

[0227] In one embodiment, the PD-L1 inhibitor is MDPL3280A (Genentech / Roche). MDPL3280A is a human Fc optimized IgG1 monoclonal antibody that binds to PD-L1. MDPL3280A and other human monoclonal antibodies to PD-L1 are disclosed in U.S. Patent No.: 7,943,743 and U.S. Publication No.: 20120039906.

[0228] In other embodiments, the PD-L2 inhibitor is AMP-224. AMP-224 is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD1 and B7-H1 (B7-DCIg; Amplimmune; e.g., disclosed in PCT Publication Nos. WO2010/027827 and WO2011/066342).

[0229] In one embodiment, the LAG-3 inhibitor is an anti-LAG-3 antibody molecule. In one embodiment, the LAG-3 inhibitor is BMS-986016.

[0230] In yet another embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more cytokines, including interferon, IL2, IL15, IL7, or IL21 for use in treating cancer in a subject in need thereof.

[0231] In yet another embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more agonists of STING receptor (Stimulator of Interferon Genes), e.g., the compounds described in WO 2014/189805 for use in treating cancer in a subject in need thereof.

[0232] In another embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more angiogenesis inhibitors, e.g., Bevacizumab (Avastin®), axitinib (Inlyta®); Brivanib alaninate (BMS-582664, (S)-((R)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate); Sorafenib (Nexavar®); Pazopanib (Votrient®); Sunitinib malate (Sutent®); Cediranib (AZD2171, CAS 288383-20-1); Vargatef (BIBF1120, CAS 928326-83-4); Foretinib (GSK1363089); Telatinib (BAY57-9352, CAS 332012-40-5); Apatinib (YN968D1, CAS 811803-05-1); Imatinib (Gleevec®); Ponatinib (AP24534, CAS 943319-70-8); Tivozanib (AV951, CAS 475108-18-0); Regorafenib (BAY73-4506, CAS 755037-03-7); Vatalanib dihydrochloride (PTK787, CAS 212141-51-0); Brivanib (BMS-540215, CAS 649735-46-6); Vandetanib (Caprelsa® or AZD6474); Motesanib diphosphate (AMG706, CAS 857876-30-3, N-(2,3-dihydro-3,3-dimethyl-1H-indol-6-yl)-2-[(4-pyridinylmethyl)amino]-3-pyridinecarboxamide, described in PCT Publication No. WO 02/066470); Dovitinib dilactate (TKI258, CAS 852433-84-2); Linfanib (ABT869, CAS 796967-16-3); Cabozantinib (XL184, CAS 849217-68-1); Lestaurtinib (CAS 111358-88-4); N-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (BMS38703, CAS 345627-80-7); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[(3 α ,5 β ,6 α)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); 4-Methyl-3-[[1-methyl-6-(3-pyridinyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl]amino]-N-[3-(trifluoromethyl)phenyl]-benzamide (BHG712, CAS 940310-85-0); or Aflibercept (Eylea®) for use in treating cancer in a subject in need thereof.

[0233] In another embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more heat shock protein inhibitors, e.g., Tanespimycin (17-allylamino-17-demethoxygeldanamycin, also known as KOS-953 and 17-AAG, available from SIGMA, and described in US Patent No. 4,261,989); Retaspimycin (IPI504), Ganetespib (STA-9090); [6-Chloro-9-(4-methoxy-3,5-dimethylpyridin-2-ylmethyl)-9H-purin-2-yl]amine (BIIB021 or CNF2024, CAS 848695-25-0); *trans*-4-[[2-(Aminocarbonyl)-5-[4,5,6,7-tetrahydro-6,6-dimethyl-4-oxo-3-(trifluoromethyl)-1H-indazol-1-yl]phenyl]amino]cyclohexyl glycine ester (SNX5422 or PF04929113, CAS 908115-27-5); 5-[2,4-Dihydroxy-5-(1-methylethyl)phenyl]-N-ethyl-4-[4-(morpholinylmethyl)phenyl]-3-isoxazolecarboxamide (AU922, CAS 747412-49-3); or 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) for use in treating cancer in a subject in need thereof.

[0234] In another embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more HDAC inhibitors or other epigenetic modifiers. Exemplary HDAC inhibitors include Voninostat (Zolinza®); Romidepsin (Istodax®); Treichostatin A (TSA); Oxamflatin; Vorinostat (Zolinza®, Suberoylanilide hydroxamic acid); Pyroxamide (syberoyl-3-aminopyridineamide hydroxamic acid); Trapoxin A (RF-1023A); Trapoxin B (RF-10238); Cyclo[(α S,2S)- α -amino- η -oxo-2-oxiraneoctanoyl-O-methyl-D-tyrosyl-L-isoleucyl-L-prolyl] (Cyl-1); Cyclo[(α S,2S)- α -amino- η -oxo-2-oxiraneoctanoyl-O-methyl-D-tyrosyl-L-isoleucyl-(2S)-2-piperidinecarbonyl] (Cyl-2); Cyclic[L-alanyl-D-alanyl-(2S)- η -oxo-L- α -aminooxiraneoctanoyl-D-prolyl] (HC-toxin); Cyclo[(α S,2S)- α -amino- η -oxo-2-oxiraneoctanoyl-D-phenylalanyl-L-leucyl-(2S)-2-piperidinecarbonyl] (WF-3161); Chlamydocin ((S)-Cyclic(2-methylalanyl-L-phenylalanyl-D-prolyl- η -oxo-L- α -aminooxiraneoctanoyl); Apicidin (Cyclo(8-oxo-L-2-aminodecanoyl-1-methoxy-L-tryptophyl-L-isoleucyl-D-2-piperidinecarbonyl); Romidepsin (Istodax®, FR-901228); 4-Phenylbutyrate; Spiruchostatin A; Mylproin (Valproic acid); Entinostat (MS-275, N-(2-Aminophenyl)-4-[N-(pyridine-3-yl-methoxycarbonyl)-amino-methyl]-benzamide); Depudecin (4,5:8,9-dianhydro-1,2,6,7,11-pentadeoxy- D-*threo*-D-*ido*-Undeca-1,6-dienitol); 4-(Acetylamino)-N-(2-aminophenyl)-benzamide (also known as CI-994); N1-(2-Aminophenyl)-N8-phenyl-octanediamide (also known as BML-210); 4-(Dimethylamino)-N-(7-(hydroxyamino)-7-oxoheptyl)benzamide (also known as M344); (E)-3-(4-(((2-(1H-indol-3-yl)ethyl)(2-hydroxyethyl)amino)-methyl)phenyl)-N-hydroxyacrylamide; Panobinostat (Farydak®); Mocetinostat, and Belinostat (also known as PXD101, Beleodaq®, or (2E)-N-Hydroxy-3-[3-(phenylsulfamoyl)phenyl]prop-2-enamide), or chidamide (also known as CS055 or HBI-8000, (E)-N-(2-amino-5-fluorophenyl)-4-((3-(pyridin-3-yl)acrylamido)methyl)benzamide). Other epigenetic modifiers include inhibitors of EZH2 (enhancer of zeste homolog 2), EED (embryonic ectoderm development), or LSD1 (lysine-specific histone demethylase 1A or KDM1A) for use in treating cancer in a subject in need thereof.

[0235] In yet another embodiment, the present invention provides an antibody conjugate of the present invention in combination with one or more inhibitors of indoleamine-pyrrole 2,3-dioxygenase (IDO), for example, Indoximod (also known as NLG-8189), α -Cyclohexyl-5H-imidazo[5,1-a]isoindole-5-ethanol (also known as NLG919), or (4E)-4-[(3-Chloro-4-fluoroanilino)-nitrosomethylidene]-1,2,5-oxadiazol-3-amine (also known as INCB024360) for use in treating cancer

in a subject in need thereof.

[0236] In some embodiments, the present invention provides an antibody conjugate of the present invention in combination with two or more of any of the above described inhibitors, activators, immunomodulators, agonists, or modifiers for use in treating cancer in a subject in need thereof. For example, the antibody conjugate of the present invention can be used in combination with one or more checkpoint inhibitors and/or one or more immune activators.

[0237] In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy.

Pharmaceutical Compositions

[0238] To prepare pharmaceutical or sterile compositions including one or more antibody conjugates described herein, provided antibody conjugate can be mixed with a pharmaceutically acceptable carrier or excipient.

[0239] Formulations of therapeutic and diagnostic agents can be prepared by mixing with physiologically acceptable carriers, excipients, or stabilizers in the form of, e.g., lyophilized powders, slurries, aqueous solutions, lotions, or suspensions (see, e.g., Hardman et al., Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y., 2001; Gennaro, Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y., 2000; Avis, et al. (eds.), Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY, 1993; Lieberman, et al. (eds.), Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY, 1990; Lieberman, et al. (eds.) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY, 1990; Weiner and Kotkoskie, Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y., 2000).

[0240] In some embodiments, the pharmaceutical composition comprising the antibody conjugate of the present invention is a lyophilisate preparation. In certain embodiments a pharmaceutical composition comprising the antibody conjugate is a lyophilisate in a vial containing an antibody conjugate, histidine, sucrose, and polysorbate 20. In certain embodiments the pharmaceutical composition comprising the antibody conjugate is a lyophilisate in a vial containing an antibody conjugate, sodium succinate, and polysorbate 20. In certain embodiments the pharmaceutical composition comprising the antibody conjugate is a lyophilisate in a vial containing an antibody conjugate, trehalose, citrate, and polysorbate 8. The lyophilisate can be reconstituted, e.g., with water, saline, for injection. In a specific embodiment, the solution comprises the antibody conjugate, histidine, sucrose, and polysorbate 20 at a pH of about 5.0. In another specific embodiment the solution comprises the antibody conjugate, sodium succinate, and polysorbate 20. In another specific embodiment, the solution comprises the antibody conjugate, trehalose dehydrate, citrate dehydrate, citric acid, and polysorbate 8 at a pH of about 6.6. For intravenous administration, the obtained solution will usually be further diluted into a carrier solution.

[0241] Selecting an administration regimen for a therapeutic depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix. In certain embodiments, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available (see, e.g., Wawrzynczak, Antibody Therapy, Bios Scientific Pub. Ltd, Oxfordshire, UK, 1996; Kresina (ed.), Monoclonal Antibodies, Cytokines and Arthritis, Marcel Dekker, New York, N.Y., 1991; Bach (ed.), Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases, Marcel Dekker, New York, N.Y., 1993; Baert et al., New Engl. J. Med. 348:601-608, 2003; Milgrom et al., New Engl. J. Med. 341:1966-1973, 1999; Slamon et al., New Engl. J. Med. 344:783-792, 2001; Beniaminovitz et al., New Engl. J. Med. 342:613-619, 2000; Ghosh et al., New Engl. J. Med. 348:24-32, 2003; Lipsky et al., New Engl. J. Med. 343:1594-1602, 2000).

[0242] Determination of the appropriate dose is made by the clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, e.g., the inflammation or level of inflammatory cytokines produced.

[0243] Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors known in the medical arts.

[0244] Compositions comprising the antibody conjugate of the invention can be provided by continuous infusion, or

by doses at intervals of, e.g., one day, one week, or 1-7 times per week, once every other week, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, or once every eight weeks. Doses may be provided intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, or by inhalation. A specific dose protocol is one involving the maximal dose or dose frequency that avoids

significant undesirable side effects.

[0245] For the antibody conjugates of the invention, the dosage administered to a patient may be 0.0001 mg/kg to 100 mg/kg of the patient's body weight. The dosage may be between 0.001 mg/kg and 50 mg/kg, 0.005 mg/kg and 20 mg/kg, 0.01 mg/kg and 20 mg/kg, 0.02 mg/kg and 10 mg/kg, 0.05 and 5 mg/kg, 0.1 mg/kg and 10 mg/kg, 0.1 mg/kg and 8 mg/kg, 0.1 mg/kg and 5 mg/kg, 0.1 mg/kg and 2 mg/kg, 0.1 mg/kg and 1 mg/kg of the patient's body weight. The dosage of the antibody conjugate may be calculated using the patient's weight in kilograms (kg) multiplied by the dose to be administered in mg/kg.

[0246] Doses of the antibody conjugates the invention may be repeated and the administrations may be separated by less than 1 day, at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, 4 months, 5 months, or at least 6 months. In some embodiments, an antibody conjugate of the invention is administered twice weekly, once weekly, once every two weeks, once every three weeks, once every four weeks, or less frequently. In a specific embodiment, doses of the antibody conjugates of the invention are repeated every 2 weeks.

[0247] An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the method, route and dose of administration and the severity of side effects (see, e.g., Maynard et al., A Handbook of SOPs for Good Clinical Practice, Interpharm Press, Boca Raton, Fla., 1996; Dent, Good Laboratory and Good Clinical Practice, Urch Publ., London, UK, 2001).

[0248] The route of administration may be by, e.g., topical or cutaneous application, injection or infusion by subcutaneous, intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intracerebrospinal, intralesional administration, or by sustained release systems or an implant (see, e.g., Sidman et al., Biopolymers 22:547-556, 1983; Langer et al., J. Biomed. Mater. Res. 15:167-277, 1981; Langer, Chem. Tech. 12:98-105, 1982; Epstein et al., Proc. Natl. Acad. Sci. USA 82:3688-3692, 1985; Hwang et al., Proc. Natl. Acad. Sci. USA 77:4030-4034, 1980; U.S. Pat. Nos. 6,350,466 and 6,316,024). Where necessary, the composition may also include a solubilizing agent or a local anesthetic such as lidocaine to ease pain at the site of the injection, or both. In addition, pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903.

[0249] Examples of such additional ingredients are well-known in the art.

[0250] Methods for co-administration or treatment with a second therapeutic agent, e.g., a cytokine, steroid, chemotherapeutic agent, antibiotic, or radiation, are known in the art (see, e.g., Hardman et al., (eds.) (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10^{sup}.th ed., McGraw-Hill, New York, N.Y.; Poole and Peterson (eds.) (2001) Pharmacotherapeutics for Advanced Practice: A Practical Approach, Lippincott, Williams & Wilkins, Phila., Pa.; Chabner and Longo (eds.) (2001) Cancer Chemotherapy and Biotherapy, Lippincott, Williams & Wilkins, Phila., Pa.). An effective amount of therapeutic may decrease the symptoms by at least 10%; by at least 20%; at least about 30%; at least 40%, or at least 50%.

[0251] Additional therapies (e.g., prophylactic or therapeutic agents), which can be administered in combination with the antibody conjugates of the invention may be administered less than 5 minutes apart, less than 30 minutes apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, at about 12 hours to 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours apart from the antibody conjugates of the invention. The two or more therapies may be administered within one same patient visit.

[0252] In certain embodiments, the antibody conjugates of the invention can be formulated to ensure proper distribution *in vivo*. Exemplary targeting moieties include folate or biotin (see, e.g., U.S. Pat. No. 5,416,016 to Low et al.); mannosides (Umezawa et al., (1988) Biochem. Biophys. Res. Commun. 153:1038); antibodies (Bloeman et al., (1995) FEBS Lett. 357:140; Owais et al., (1995) Antimicrob. Agents Chemother. 39:180); surfactant Protein A receptor (Briscoe et al., (1995) Am. J. Physiol. 1233:134); p 120 (Schreier et al., (1994) J. Biol. Chem. 269:9090); see also K. Keinänen; M. L. Laukkanen (1994) FEBS Lett. 346:123; J. J. Killian; I. J. Fidler (1994) Immunomethods 4:273.

[0253] The invention provides protocols for the administration of pharmaceutical composition comprising antibody conjugates of the invention alone or in combination with other therapies to a subject in need thereof. The therapies (e.g., prophylactic or therapeutic agents) of the combination therapies of the present invention can be administered concomitantly or sequentially to a subject. The therapy (e.g., prophylactic or therapeutic agents) of the combination therapies

of the present invention can also be cyclically administered. Cycling therapy involves the administration of a first therapy (e.g., a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy (e.g., a second prophylactic or therapeutic agent) for a period of time and repeating this sequential administration, i.e., the cycle, in order to reduce the development of resistance to one of the therapies (e.g., agents) to avoid or reduce the side effects of one of the therapies (e.g., agents), and/or to improve, the efficacy of the therapies.

[0254] The therapies (e.g., prophylactic or therapeutic agents) of the combination therapies of the invention can be administered to a subject concurrently.

[0255] The term "concurrently" is not limited to the administration of therapies (e.g., prophylactic or therapeutic agents) at exactly the same time, but rather it is meant that a pharmaceutical composition comprising antibodies or fragments thereof of the invention are administered to a subject in a sequence and within a time interval such that the antibodies or antibody conjugates of the invention can act together with the other therapy(ies) to provide an increased benefit than if they were administered otherwise. For example, each therapy may be administered to a subject at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapy can be administered to a subject separately, in any appropriate form and by any suitable route. In various embodiments, the therapies (e.g., prophylactic or therapeutic agents) are administered to a subject less than 5 minutes apart, less than 15 minutes apart, less than 30 minutes apart, less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, 24 hours apart, 48 hours apart, 72 hours apart, or 1 week apart. In other embodiments, two or more therapies (e.g. prophylactic or therapeutic agents) are administered within the same patient visit.

[0256] Prophylactic or therapeutic agents of the combination therapies can be administered to a subject in the same pharmaceutical composition. Alternatively, the prophylactic or therapeutic agents of the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions. The prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

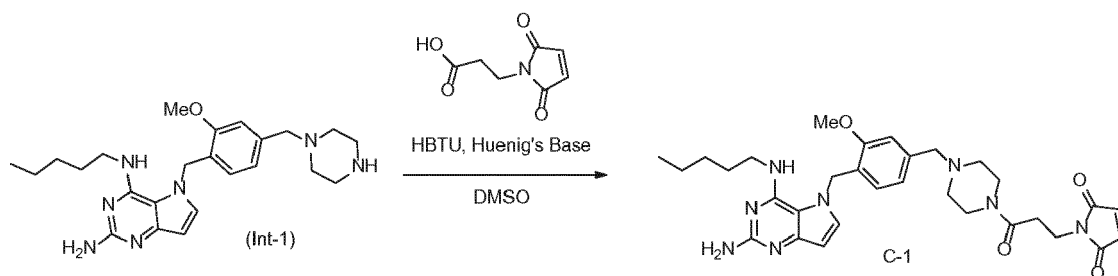
EXAMPLES

[0257] The invention is further described in the following examples.

Example 1

Synthesis of 1-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1H-pyrrole-2,5-dione (C-1)

[0258]

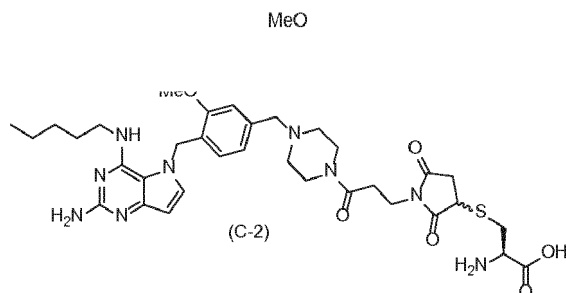


[0259] A round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), HBTU (1.2 equiv.), Huenig's base (3.0 equiv.), 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid (1.2 equiv.) and DMSO (0.1 M). The reaction mixture was stirred at room temperature for 3 hours and then the crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 1-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1H-pyrrole-2,5-dione (C-1) as a solid as the TFA salt: ¹H NMR (CDCl₃): δ 7.35 (d, 1H), 7.12 (d, 1H), 6.86 (d, 1H), 6.72 (s, 2H), 6.69 (d, 1H), 6.40 (s, 1H), 5.46 (t, 1H), 5.33 (s, 2H), 4.16 (s, 2H), 3.95 (s, 3H), 3.82 (m, 6H), 3.40 (m, 4H), 3.21 (m, 2H), 2.67 (m, 4H), 1.39 (m, 2H), 1.26 (m, 2H), 1.14 (m, 2H), 0.86 (t, 3H). LRMS [M+H] = 589.3.

Example 2

Synthesis of (2R)-2-amino-3-((1-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)thio)propanoic acid (C-2)

[0260]

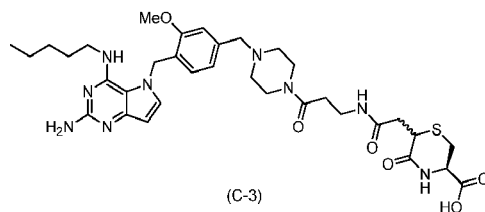


[0261] A round bottom flask was charged with 1-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1H-pyrrole-2,5-dione (C-1, 1.0 equiv.) and dissolved in ACN-PBS buffer (1:2, 0.02 M). To this mixture was added L-cysteine (2.0 equiv.) dissolved in DPBS buffer (0.07 M). The reaction mixture was stirred at room temperature for 1 hour. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford (2R)-2-amino-3-((1-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)thio)propanoic acid (C-2) as a solid as the TFA salt of a mixture of diastereomers: ¹H NMR (CD₃OD): δ 7.36 (d, 1H), 7.28 (d, 1H), 7.05 (d, 1H), 6.81 (d, 1H), 6.24 (d, 1H), 5.57 (s, 2H), 4.26 (m, 2H), 4.02 (m, 1H), 3.95 (s, 3H), 3.78 (m, 6H), 3.55 (m, 2H), 3.44 (m, 1H), 3.23 (m, 3H), 3.12 (m, 2H), 2.76 (m, 2H), 2.53 (m, 1H), 1.53 (m, 2H), 1.30 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LCMS [M+H] = 710.3.

Example 3

Synthesis of (6R)-6-(2-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-2-oxoethyl)-5-oxothiomorpholine-3-carboxylic acid (C-3)

[0262]

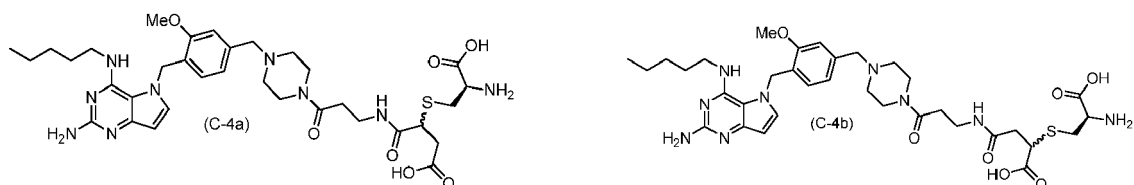


[0263] A round bottom flask was charged with (2R)-2-amino-3-((1-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-2,5-dioxopyrrolidin-3-yl)thio)propanoic acid (C-1) and dissolved in PBS buffer (pH 7.5, 100 mM phosphate with 5 nM EDTA) and acetonitrile (1:1, 0.012 M). The reaction mixture was then stirred at 40 °C for 6 hours. At this point the crude reaction mixture was allowed to cool to room temperature and purified by RP-HPLC (0.5M NH₄OAc in ACN:10mM NH₄OAc in H₂O, C18 column) to afford (6R)-6-(2-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-2-oxoethyl)-5-oxothiomorpholine-3-carboxylic acid (C-3) as a solid as a mixture of regio- and diastereomers. ¹H NMR (CD₃OD): δ 7.38 (d, 1H), 7.13 (s, 1H), 6.94 (d, 1H), 6.74 (d, 1H), 6.22 (d, 1H), 5.52 (s, 2H), 4.24 (m, 1H), 3.93 (s, 3H), 3.82 (m, 1H), 3.67 (s, 2H), 3.60 (m, 4H), 3.54 (t, 2H), 3.43 (m, 2H), 3.18 (m, 1H), 3.01 (m, 1H), 2.87 (m, 1H), 2.58 (m, 7H), 1.50 (m, 2H), 1.29 (m, 2H), 1.17 (m, 2H), 0.88 (t, 3H). LCMS [M+H] = 710.4.

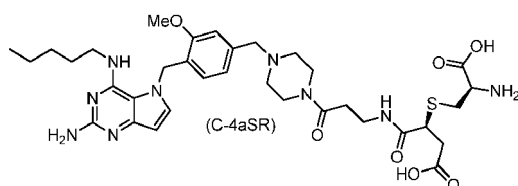
Example 4

Synthesis of 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-4a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-4b)

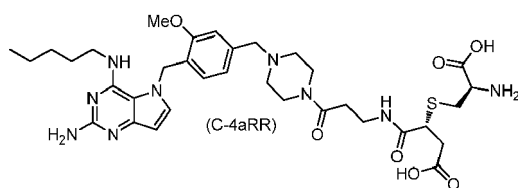
[0264]



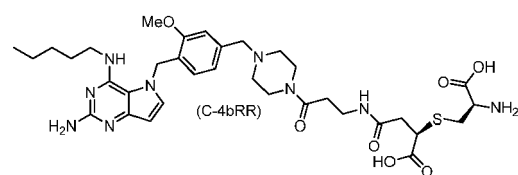
[0265] A round bottom flask was charged with 1-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1H-pyrrole-2,5-dione (C-1, 1.0 equiv.), L-cysteine (1.0 equiv.), and PBS:MeCN (2:1, 0.008 M). The reaction mixture was stirred at room temperature for 1 hour and then 1M NaOH (20.0 equiv.) was added to the reaction mixture. The reaction was then stirred an additional 3 hours, then the crude reaction mixture was purified by RP-HPLC (0.5 mM NH₄OAc in MeCN:10 mM NH₄OAc in H₂O, C18 column) to afford a mixture of 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-4a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-4b), as their respective diastereomers (Compounds (C-4aSR), C-4aRR), (C-4bRR) and (C-4bRR) below) as a solid: ¹H NMR (DMSO): δ 7.88 (s, 1), 7.26 (s, 1H), 6.98 (s, 1H), 6.77 (d, 1H), 6.64 (s, 1H), 6.46 (s, 1H), 6.01 (s, 1H), 5.40 (s, 2H), 3.85 (s, 3H), 3.36 (m, 17H), 2.29 (m, 8H), 1.90 (s, 2H), 1.39 (m, 2H), 1.21 (m, 2H), 1.09 (m, 2H), 0.81 (t, 3H). LRMS [M+H] = 728.4.



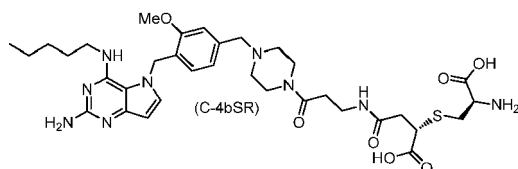
(S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-4aSR);



(R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-4aRR)



(R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-4bRR)

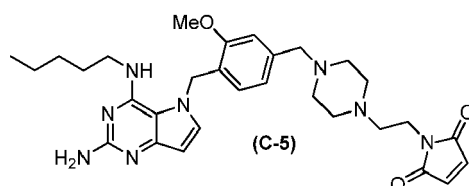


(S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-4bSR).

Example 5

Synthesis of 1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-pyrrole-2,5-dione (C-5)

[0266]



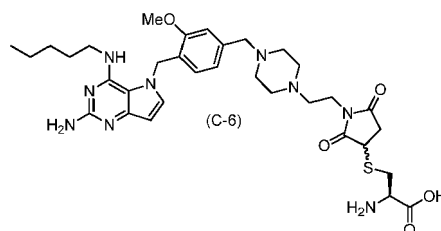
[0267] A round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetaldehyde (4.0 equiv.), sodium cyanoborohydride (13.0 equiv.), and MeOH (0.04 M). The reaction mixture was stirred at room temperature for 1 hour and the crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-pyrrole-2,5-dione (C-5) as a solid as the TFA salt: ¹H NMR (CDCl₃): δ 7.32 (d, 1H), 7.12 (d, 1H), 6.87 (d, 1H), 6.72 (s, 2H), 6.70 (d, 1H), 6.41 (d, 1H), 5.45 (t, 1H), 5.31 (s, 2H), 4.07 (s, 2H), 3.95 (s, 3H), 3.73 (t, 2H), 3.40 (m, 4H), 3.17 (m, 6H), 2.89 (m, 4H), 1.39 (m, 2H), 1.26 (m, 2H), 1.14 (m, 2H), 0.86 (t, 3H). LRMS [M+H] = 561.3.

[0268] Note: 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetaldehyde was prepared by adding 1-(2-hydroxyethyl)-1H-pyrrole-2,5-dione (1.0 equiv.), Dess-Martin periodinane (1.5 equiv.) and DCM (0.1 M) to a round bottom flask and stirring at room temperature for 2 hours. The reaction mixture was then filtered, the volatiles removed in vacuo and the product used without further purification.

Example 6

Synthesis of (2S)-2-amino-3-((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-2,5-dioxopyrrolidin-3-yl)thio)propanoic acid (C-6)

[0269]



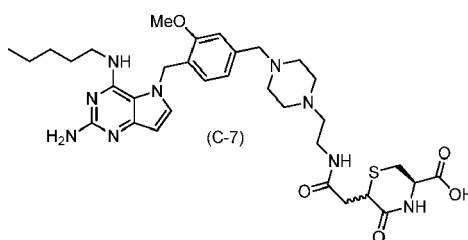
[0270] (2S)-2-amino-3-((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-2,5-dioxopyrrolidin-3-yl)thio)propanoic acid (C-6) was prepared following a procedure similar to Example 2, except Compound (C-5) was used in place of Compound (C-1), to afford (2S)-2-amino-3-((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-2,5-dioxopyrrolidin-3-yl)thio)propanoic acid (C-6) as a solid as the TFA salt of a mixture of diastereomers: ¹H NMR (CD₃OD): δ 7.36 (d,

1H), 7.21 (m, 1H), 7.02 (m, 1H), 6.78 (m, 1H), 6.23 (d, 1H), 5.56 (m, 2H), 4.21 (m, 1H), 4.09 (s, 1H), 4.03 (m, 1H), 3.95 (m, 3H), 3.75 (m, 2H), 3.54 (t, 2H), 3.43 (m, 1H), 3.34 (m, 1H), 3.22 (m, 2H), 3.03 (m, 6H), 2.84 (m, 2H), 2.63 (m, 1H), 1.52 (m, 2H), 1.30 (m, 2H), 1.18 (m, 2H), 0.88 (t, 3H). LCMS [M+H] = 682.4.

5 Example 7

Synthesis of (6R)-6-(2-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-2-oxoethyl)-5-oxothiomorpholine-3-carboxylic acid (C-7)

10 [0271]

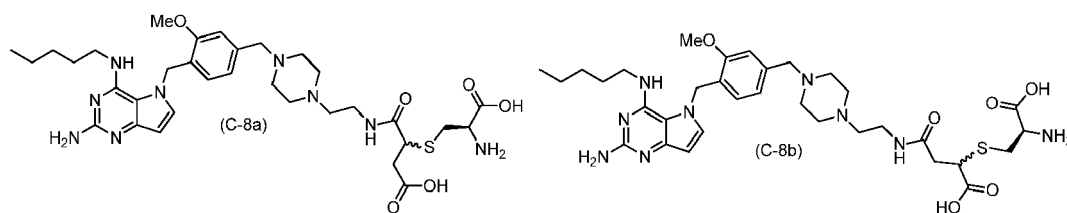


20 [0272] (6R)-6-(2-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-2-oxoethyl)-5-oxothiomorpholine-3-carboxylic acid (C-7) was prepared following a procedure similar to Example 3, except Compound (C-5) was used in place of Compound (C-1), to afford (6R)-6-(2-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-2-oxoethyl)-5-oxothiomorpholine-3-carboxylic acid (C-7) as a solid as a mixture of regio- and diastereomers: ¹H NMR (CD₃OD): δ 7.37 (d, 1H), 7.10 (s, 1H), 6.91 (d, 1H), 6.72 (d, 1H), 6.22 (d, 1H), 5.51 (s, 2H), 4.13 (m, 1H), 3.92 (s, 3H), 3.88 (m, 1H), 3.58 (s, 2H), 3.52 (t, 2H), 3.40 (m, 2H), 3.16 (m, 1H), 2.99 (m, 1H), 2.86 (m, 1H), 2.67 (m, 10H), 1.49 (m, 2H), 1.29 (m, 2H), 1.17 (m, 2H), 0.88 (t, 3H). LCMS [M+H] = 682.3.

30 Example 8

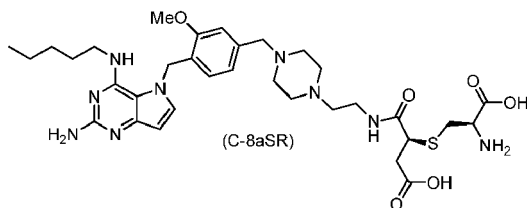
Synthesis of 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid (C-8a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid (C-8b)

35 [0273]



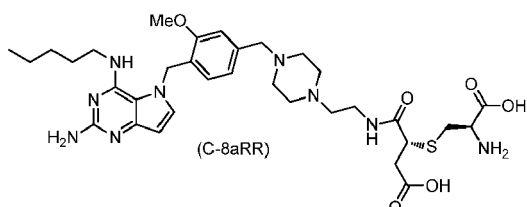
40 [0274] 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid (C-8a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid (C-8b) were prepared following a procedure similar to Example 4, except Compound (C-5) was used in place of Compound (C-1), to give a mixture of Compounds (C-8a) and (C-8b), as their respective diastereomers (Compounds (C-8aSR), C-8aRR), (C-8bRR) and (C-8bRR) below), as a solid: ¹H NMR (DMSO): δ 7.81 (s, 1), 7.33 (s, 1H), 6.96 (s, 1H), 6.76 (d, 1H), 6.69 (s, 1H), 6.48 (s, 1H), 6.10 (s, 1H), 5.45 (s, 2H), 3.82 (s, 3H), 3.37 (m, 17H), 2.35 (m, 8H), 1.90 (s, 2H), 1.41 (m, 2H), 1.20 (m, 2H), 1.08 (m, 2H), 0.80 (t, 3H). LRMS [M+H] = 700.4.

5



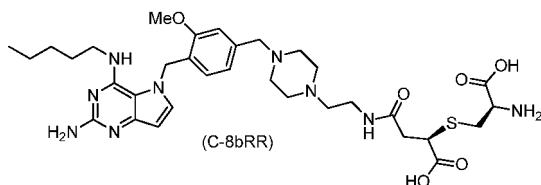
10 (S)-3-(((R)-2-amino-2-carboxyethylthio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid (C-8aSR);

15



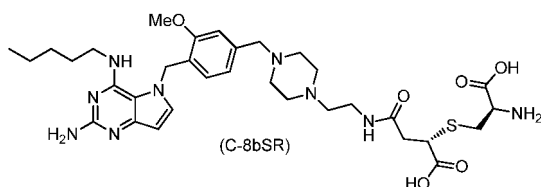
20 (R)-3-(((R)-2-amino-2-carboxyethylthio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid (C-8aRR);

25



30 (R)-2-(((R)-2-amino-2-carboxyethylthio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid (C-8bRR);

35



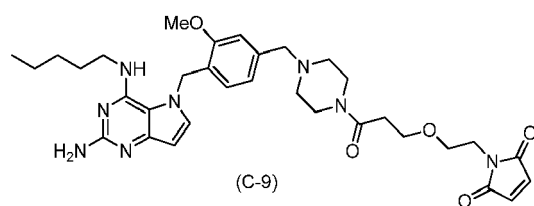
40 (S)-2-(((R)-2-amino-2-carboxyethylthio)-4-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)amino)-4-oxobutanoic acid (C-8bSR).

Example 9

45 Synthesis of 1-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)-1 H-pyrrole-2,5-dione (C-9)

[0275]

50



55

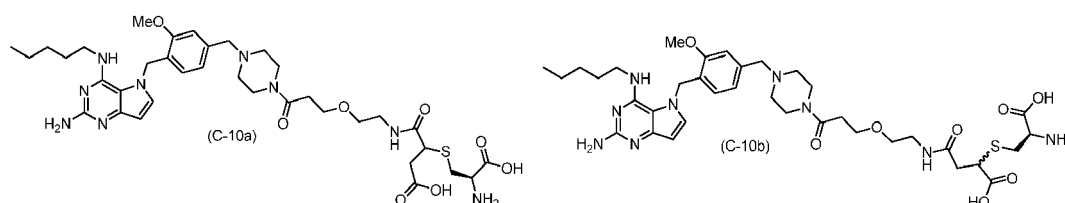
[0276] 1-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-

1-yl)-3-oxopropoxy)ethyl)-1H-pyrrole-2,5-dione (C-9) was prepared following a procedure similar to Example 1, except 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoic acid was used in place of 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid, to afford 1-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)-1H-pyrrole-2,5-dione (C-9) as a solid as the TFA salt: ^1H NMR (CD_3OD): δ 7.37 (d, 1H), 7.27 (d, 1H), 7.06 (d, 1H), 6.82 (s, 2H), 6.81 (d, 1H), 6.24 (d, 1H), 5.58 (s, 2H), 4.38 (s, 2H), 3.96 (s, 3H), 3.86 (m, 4H), 3.67 (m, 4H), 3.56 (m, 4H), 3.24 (m, 4H), 2.61 (t, 2H), 1.53 (m, 2H), 1.31 (m, 2H), 1.20 (m, 2H), 0.88 (t, 3H). LCMS $[\text{M}+\text{H}] = 633.3$.

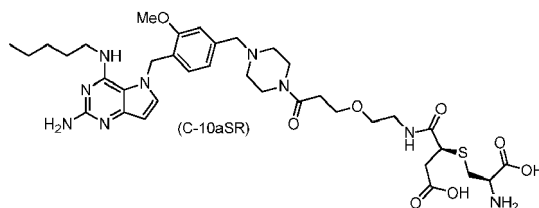
Example 10

Synthesis of 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid (C-10a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid (C-10b)

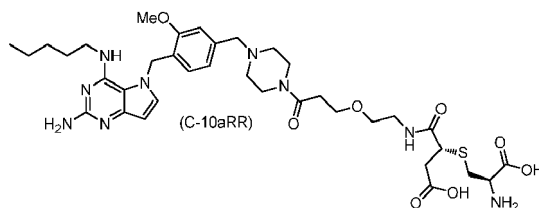
[0277]



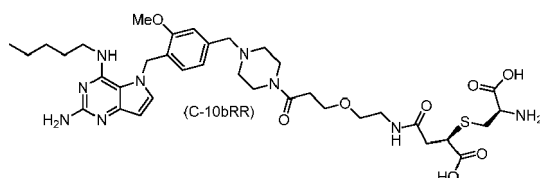
[0278] 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid (C-10a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid (C-10b) were prepared following a procedure similar to Example 4, except Compound (C-9) was used in place of Compound (C-1), to afford a mixture of Compounds (C-10a) and (C-10b), as their respective diastereomers (Compounds (C-10aSR), C-10aRR), (C-10bRR) and (C-10bRR) below), as a solid as the TFA salt. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN :0.05% TFA in H_2O , C18 column): ^1H NMR (CD_3OD): δ 7.35 (d, 1H), 7.29 (d, 1H), 7.05 (d, 1H), 6.77 (m, 1H), 6.23 (s, 1H), 5.56 (s, 2H), 4.32 (m, 2H), 3.94 (s, 3H), 3.86 (m, 3H), 3.72 (m, 3H), 3.54 (m, 10H), 3.21 (m, 4H), 2.67 (m, 4H), 1.52 (m, 2H), 1.30 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LCMS $[\text{M}+\text{H}] = 772.4$.



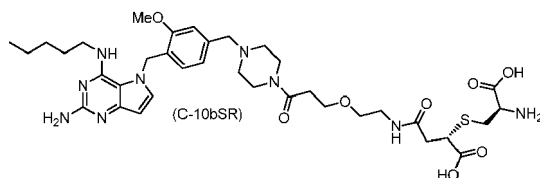
(S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid (C-10aSR);



(R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid (C-10aRR);



(R)-2-(((R)-2-amino-2-carboxyethylthio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid (C-10bRR);

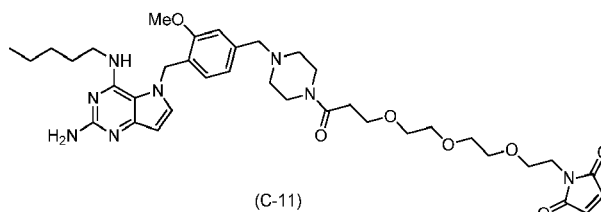


(S)-2-(((R)-2-amino-2-carboxyethylthio)-4-((2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)amino)-4-oxobutanoic acid (C-10bSR).

Example 11

Synthesis of 1-(2-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethoxy)ethyl)-1H-pyrrole-2,5-dione (C-11)

[0279]

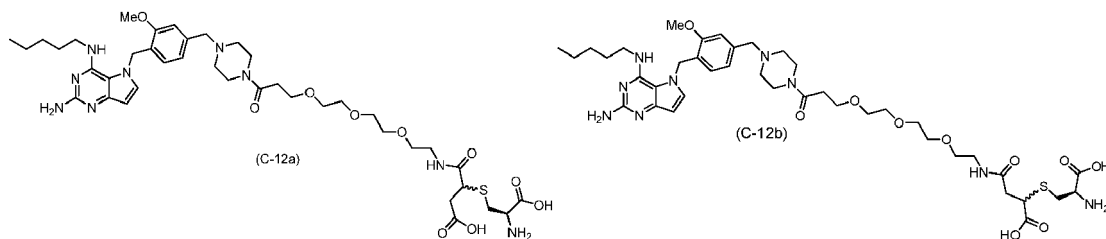


[0280] 1-(2-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethoxy)ethyl)-1H-pyrrole-2,5-dione (C-11) was prepared following a procedure similar to Example 1, except 3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanoic acid was used in place of 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid, to afford 1-(2-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethoxy)ethyl)-1H-pyrrole-2,5-dione (C-11) as a solid as the TFA salt: ^1H NMR (CD_3OD): δ 7.37 (d, 1H), 7.26 (d, 1H), 7.05 (d, 1H), 6.82 (d, 1H), 6.80 (s, 2H), 6.24 (d, 1H), 5.58 (s, 2H), 4.32 (s, 2H), 3.96 (s, 3H), 3.74 (t, 2H), 3.64 (m, 2H), 3.58 (m, 12H), 3.64 (m, 4H), 3.20 (m, 4H), 2.68 (m, 2H), 1.53 (m, 2H), 1.32 (m, 2H), 1.20 (m, 2H), 0.88 (t, 3H). LCMS $[\text{M}+\text{H}] = 721.4$.

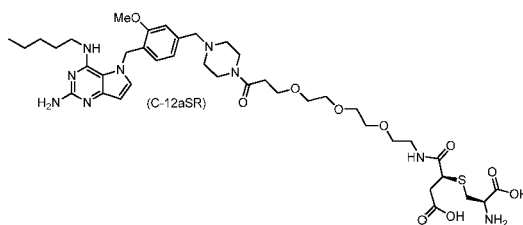
Example 12

Synthesis of (2R)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,19-dioxo-10,13,16-trioxa-4-thia-7-azanonadecan-1-oic acid (C-12a) and (19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,14-dioxo-4,7,10-trioxa-17-thia-13-azaicosan-20-oic acid (C-12b)

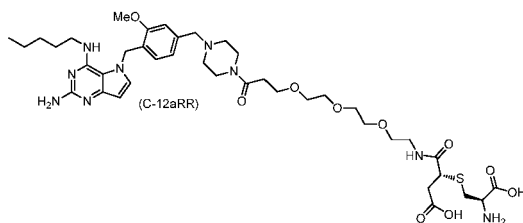
[0281]



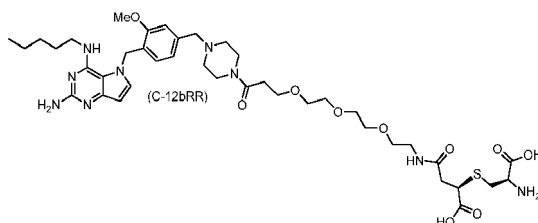
[0282] (2R)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,19-dioxo-10,13,16-trioxa-4-thia-7-azanonadecan-1-oic acid (C-12) and (19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,14-dioxo-4,7,10-trioxa-17-thia-13-azaicosan-20-oic acid (C-12b) were prepared following a procedure similar to Example 4, except Compound (C-11) was used in place of Compound (C-1), to afford a mixture of Compounds (C-12a) and (C-12b), as their respective diastereomers (Compounds (C-12aSR), (C-12aRR), (C-12bRR) and (C-12bRR) below), as a solid as the TFA salt. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column): ¹H NMR (CD₃OD): δ 7.36 (d, 1H), 7.31 (s, 1H), 7.06 (d, 1H), 6.79 (d, 1H), 6.24 (d, 1H), 5.57 (s, 2H), 4.34 (s, 2H), 4.23 (m, 1H), 3.96 (s, 3H), 3.86 (m, 4H), 3.76 (m, 4H), 3.58 (m, 14H), 3.27 (m, 4H), 3.22 (m, 2H), 2.84 (m, 1H), 2.71 (m, 2H), 1.53 (m, 2H), 1.31 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LCMS [M+H]⁺ = 860.4.



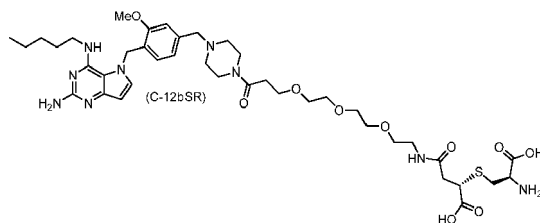
(2R,5S)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,19-dioxo-10,13,16-trioxa-4-thia-7-azanonadecan-1-oic acid (C-12aSR);



(2R,5R)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,19-dioxo-10,13,16-trioxa-4-thia-7-azanonadecan-1-oic acid (C-12aRR);



(16R,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,14-dioxo-4,7,10-trioxa-17-thia-13-azaicosan-20-oic acid (C-12bRR);

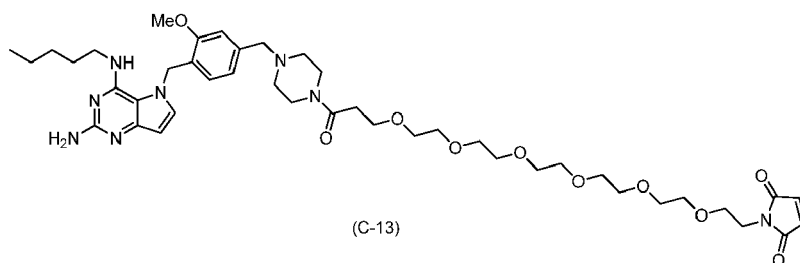


(16S,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,14-dioxo-4,7,10-trioxa-17-thia-13-azaicosan-20-oic acid (C-12bSR).

Example 13

Synthesis of 1-(21-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-21-oxo-3,6,9,12,15,18-hexaoxahenicosyl)-1H-pyrrole-2,5-dione (C-13)

[0283]

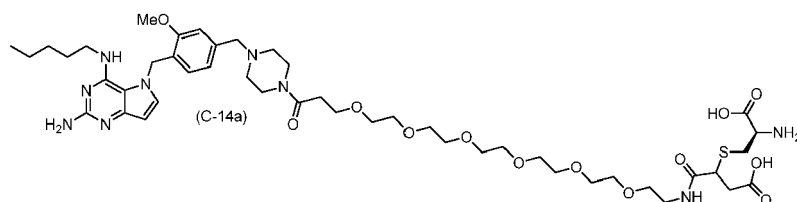


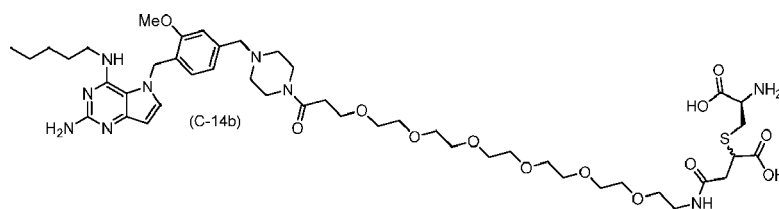
[0284] 1-(21-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-21-oxo-3,6,9,12,15,18-hexaoxahenicosyl)-1H-pyrrole-2,5-dione (C-13) was prepared following a procedure similar to example 1, except 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,6,9,12,15,18-hexaoxahenicosan-21-oic acid was used in place of 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid, to afford 1-(21-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-21-oxo-3,6,9,12,15,18-hexaoxahenicosyl)-1H-pyrrole-2,5-dione (C-13) as a solid as the TFA salt: ^1H NMR (CD_3OD): δ 7.38 (d, 1H), 7.27 (d, 1H), 7.07 (d, 1H), 6.84 (d, 1H), 6.82 (s, 2H), 6.25 (d, 1H), 5.59 (s, 2H), 4.36 (s, 2H), 3.97 (s, 3H), 3.65 (m, 32H), 3.20 (m, 4H), 2.71 (m, 2H), 1.55 (m, 2H), 1.32 (m, 2H), 1.21 (m, 2H), 0.89 (t, 3H). LCMS $[\text{M}+\text{H}] = 853.5$.

Example 14

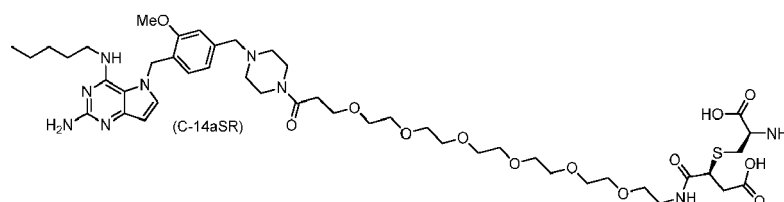
Synthesis of (2R)-2-amino-28-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,28-dioxo-10,13,16,19,22,25-hexaoxa-4-thia-7-azaoctacosan-1-oic acid (C-14a) and (28R)-28-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-25-carboxy-1,23-dioxo-4,7,10,13,16,19-hexaoxa-26-thia-22-azanonacosan-29-oic acid (C-14b)

[0285]

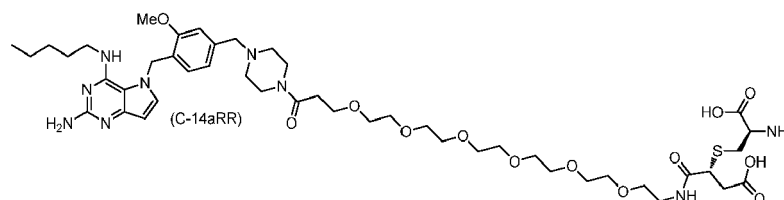




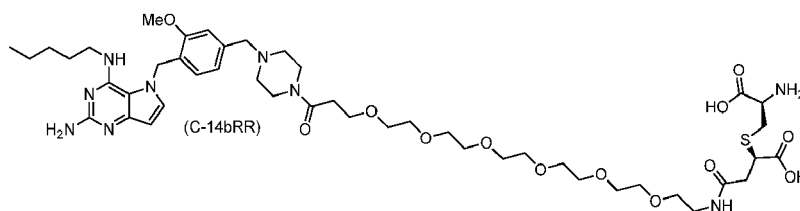
[0286] (2R)-2-amino-28-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,28-dioxo-10,13,16,19,22,25-hexaoxa-4-thia-7-aza-octacosan-1-oic acid (C-14a) and (28R)-28-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-25-carboxy-1,23-dioxo-4,7,10,13,16,19-hexaoxa-26-thia-22-azanonacosan-29-oic acid (C-14b) were prepared following a procedure similar to Example 4, except Compound (C-13) was used in place of Compound (C-1), to provide a mixture of Compounds (C-14a) and (C-14b), as their respective diastereomers (Compounds (C-14aSR), C-14aRR), (C-14bRR) and (C-14bRR) below), as a solid as the HCl salt (After RP-HPLC purification the product was dissolved in acetonitrile, treated with excess 2N HCl, and then lyophilized): ¹H NMR (CD₃OD): δ 7.47 (s, 1H), 7.39 (d, 1H), 7.13 (d, 1H), 6.82 (d, 1H), 6.25 (d, 1H), 5.58 (s, 2H), 4.38 (s, 2H), 4.32 (m, 1H), 4.00 (s, 3H), 3.77 (m, 4H), 3.76 (m, 4H), 3.64 (m, 28H), 3.55 (m, 5H), 3.31 (m, 4H), 3.12 (m, 1H), 2.86 (m, 1H), 2.72 (s, 2H), 2.62 (m, 1H), 1.54 (m, 2H), 1.31 (m, 2H), 1.20 (m, 2H), 0.89 (t, 3H). LCMS [M+H] = 992.4.



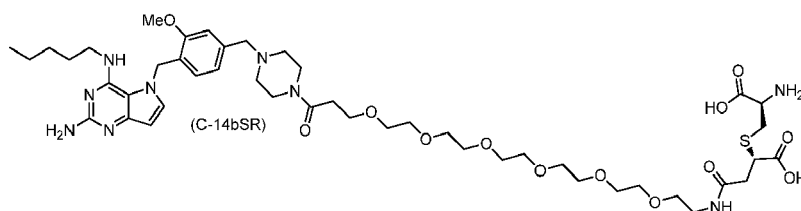
(2R,5S)-2-amino-28-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,28-dioxo-10,13,16,19,22,25-hexaoxa-4-thia-7-aza-octacosan-1-oic acid (C-14aSR);



(2R,5R)-2-amino-28-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,28-dioxo-10,13,16,19,22,25-hexaoxa-4-thia-7-aza-octacosan-1-oic acid (C-14aRR);



(25R,28R)-28-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-25-carboxy-1,23-dioxo-4,7,10,13,16,19-hexaoxa-26-thia-22-azanonacosan-29-oic acid (C-14bRR);

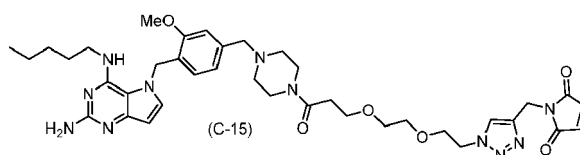


(25S,28R)-28-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-25-carboxy-1,23-dioxo-4,7,10,13,16,19-hexaoxa-26-thia-22-azanonacosan-29-oic acid (C-14bSR).

Example 15

Synthesis of 1-((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrole-2,5-dione (C-15)

[0287]



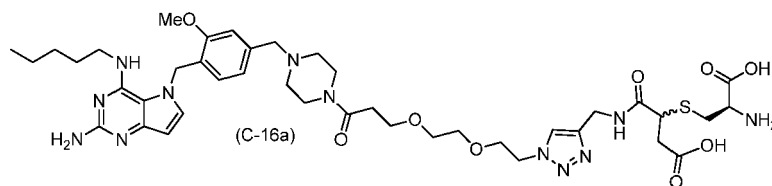
[0288] Step 1: 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-(2-azidoethoxy)ethoxy)propan-1-one was prepared following the procedure similar to Example 1, except 3-(2-(2-azidoethoxy)ethoxy)propanoic acid was used in place of 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid.

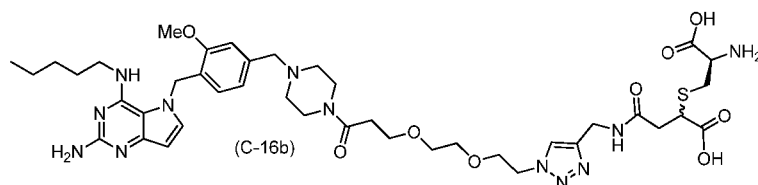
[0289] Step 2: A round bottom flask was charged with 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-(2-azidoethoxy)ethoxy)propan-1-one (1.0 equiv.), CuSO₄ (0.25 equiv.), L-Ascorbic acid sodium salt (1.1 equiv.), 1-(prop-2-yn-1-yl)-1H-pyrrole-2,5-dione (2.2 equiv.), and a mixture of t-BuOH/water (1:1, v/v, 0.012 M). The reaction mixture was placed under vacuum and subsequently flushed with N₂ (this was repeated four more times). The reaction mixture was then stirred at room temperature for 2 hours and the crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 1-((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrole-2,5-dione (C-15) as a solid as the TFA salt: ¹H NMR (CD₃OD): δ 7.94 (s, 1H), 7.37 (d, 1H), 7.29 (s, 1H), 7.05 (d, 1H), 6.85 (s, 2H), 6.81 (d, 1H), 6.24 (d, 1H), 5.57 (s, 2H), 4.73 (s, 2H), 4.52 (t, 2H), 4.36 (s, 2H), 3.95 (s, 3H), 3.85 (t, 2H), 3.84 (m, 4H), 3.66 (t, 2H), 3.54 (m, 6H), 3.27 (m, 4H), 2.63 (t, 2H), 1.53 (m, 2H), 1.30 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LCMS [M+H] = 758.4.

Example 16

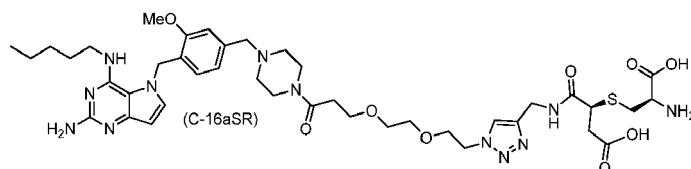
Synthesis of 3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-16a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-16b)

[0290]

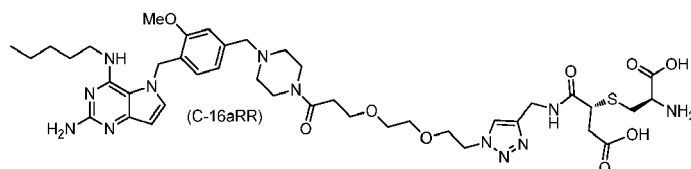




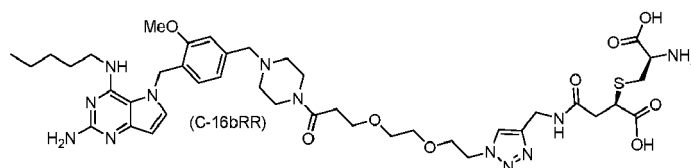
[0291] 3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-16a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-16b) were prepared following a procedure similar to Example 4, except Compound (C-15) was used in place of Compound (C-1), to afford a mixture of Compounds (C-16a) and (C-16b), as their respective diastereomers (Compounds (C-16aSR), (C-16aRR), (C-16bRR) and (C-16bRR) below), as a solid as the TFA salt. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column): ¹H NMR (CD₃OD): δ 7.91 (s, 1H), 7.36 (d, 1H), 7.30 (s, 1H), 7.06 (d, 1H), 6.80 (d, 1H), 6.24 (d, 1H), 5.57 (s, 2H), 4.54 (s, 2H), 4.44 (m, 2H), 4.34 (s, 2H), 4.25 (m, 1H), 3.95 (s, 3H), 4.83 (m, 6H), 3.68 (t, 2H), 3.55 (m, 6H), 3.25 (m, 2H), 2.86 (m, 1H), 2.64 (m, 2H), 1.53 (m, 2H), 1.30 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LCMS [M+H]⁺ = 897.4



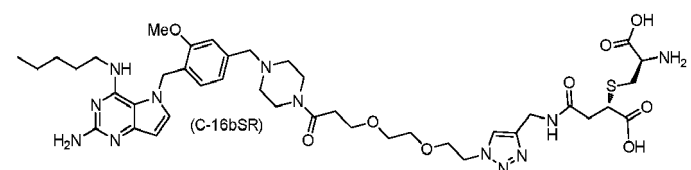
(S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-16aSR);



(R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-16aRR);



(R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-16bRR);

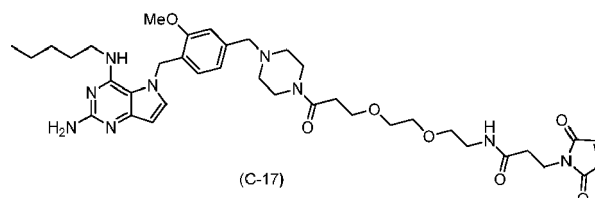


(S)-2-(((R)-2-amino-2-carboxyethylthio)-4-(((1-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-16bSR).

Example 17

Synthesis of N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide (C-17)

[0292]

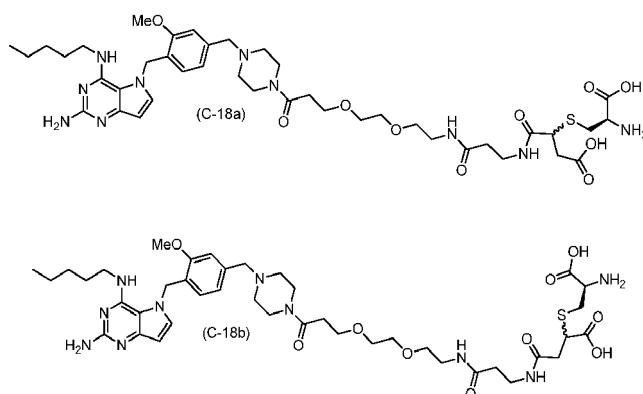


[0293] N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide (C-17) was prepared following a procedure similar to Example 1, except 3-(2-(2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)ethoxy)ethoxy)propanoic acid was used in place of 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid, to afford N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide (C-17) as a solid as the TFA salt: ¹H NMR (CD₃OD): δ 7.37 (d, 1H), 7.28 (d, 1H), 7.06 (d, 1H), 6.82 (d, 1H), 6.80 (s, 2H), 6.24 (d, 1H), 5.58 (s, 2H), 4.37 (s, 2H), 3.96 (s, 3H), 3.84 (m, 4H), 3.40 (m, 4H), 3.56 (m, 6H), 3.48 (t, 2H), 3.20 (m, 6H), 2.69 (t, 2H), 2.45 (t, 2H), 1.53 (m, 2H), 1.31 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LCMS [M+H] = 748.4.

Example 18

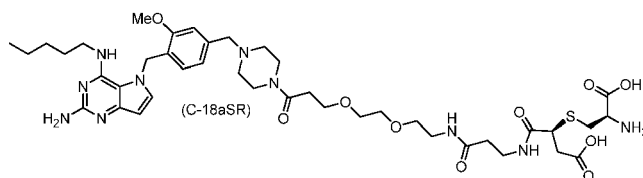
Synthesis of (19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-(carboxymethyl)-1,11,15-trioxo-4,7-dioxo-17-thia-10,14-diazaisicosan-20-oic acid (C-18a) and (20R)-20-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-17-carboxy-1,11,15-trioxo-4,7-dioxo-18-thia-10,14-diazahenicosan-21-oic acid (C-18b)

[0294]

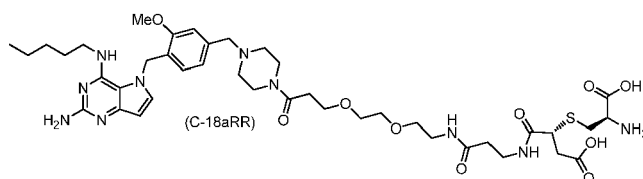


[0295] (19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-(carboxymethyl)-1,11,15-trioxo-4,7-dioxo-17-thia-10,14-diazaisicosan-20-oic acid (C-18a) and (20R)-20-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-17-carboxy-1,11,15-trioxo-4,7-dioxo-18-thia-10,14-diazahenicosan-21-oic acid (C-18b) were prepared following a procedure similar to Example 4, except Compound (C-17) was used in place of Compound (C-1), to afford a mixture of Compounds (C-18a) and (C-18b), as their respective diastereomers (Compounds (C-18aSR), (C-18aRR), (C-18bRR)

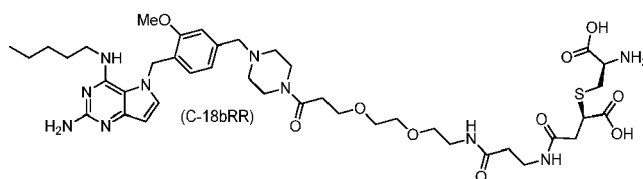
and (C-18bRR) below), as a solid as the TFA salt. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column): ¹H NMR (CD₃OD): δ 7.37 (d, 1H), 7.30 (s, 1H), 7.07 (d, 1H), 6.80 (d, 1H), 6.25 (d, 1H), 5.57 (s, 2H), 4.35 (s, 2H), 4.19 (m, 1H), 3.95 (s, 3H), 3.89 (s, 3H), 3.76 (m, 3H), 3.60 (s, 4H), 3.53 (m, 4H), 3.41 (m, 1H), 3.36 (m, 2H), 3.22 (s, 2H), 2.70 (t, 2H), 2.42 (2H), 1.53 (m, 2H), 1.30 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LCMS [M+H]⁺ = 887.4.



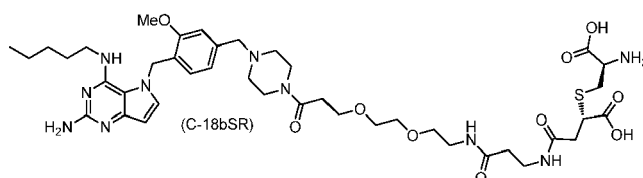
(16S,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-(carboxymethyl)-1,11,15-trioxo-4,7-dioxo-17-thia-10,14-diazaisicosan-20-oic acid (C-18aSR);



(16R,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-(carboxymethyl)-1,11,15-trioxo-4,7-dioxo-17-thia-10,14-diazaisicosan-20-oic acid (C-18aRR);



(17R,20R)-20-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-17-carboxy-1,11,15-trioxo-4,7-dioxo-18-thia-10,14-diazahenicosan-21-oic acid (C-18bRR);

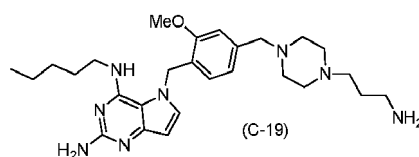


(17S,20R)-20-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-17-carboxy-1,11,15-trioxo-4,7-dioxo-18-thia-10,14-diazahenicosan-21-oic acid (C-18bSR).

Example 19

Synthesis of 5-(4-((4-(3-aminopropyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (C-19)

[0296]

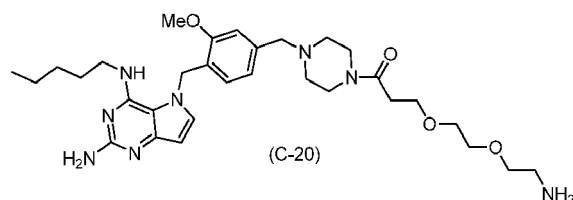


[0297] 5-(4-((4-(3-aminopropyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (C-19) was prepared by a two step sequence. In the first step a round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), tert-butyl (3-bromopropyl)carbamate (1.2 equiv.), Huenig's base (2.4 equiv.), and DMF (0.2 M). The reaction mixture was heated to 60 °C and then stirred for 18 hours. The crude reaction mixture was then cooled to room temperature and purified by ISCO chromatography (0 - 20% MeOH:DCM) to provide the intermediate tert-butyl (3-(4-(4-(2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)carbamate. In the second step a procedure similar to the last step in the synthesis of (Int-1) was used to obtain 5-(4-((4-(3-aminopropyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (C-19) as a solid: ¹H NMR (CD₃OD): δ 7.24 (d, 1H), 7.10 (d, 1H), 6.85 (d, 1H), 6.57 (d, 1H), 6.11 (s, 1H), 5.42 (s, 2H), 3.95 (s, 3H), 3.52 (s, 2H), 3.35 (m, 2H), 2.80 (t, 2H), 2.51 (m, 4H), 2.45 (m, 4H), 1.72 (m, 2H), 1.40 (m, 2H), 1.28 (m, 4H), 1.15 (m, 2H), 0.88 (t, 3H). LRMS [M+H] = 495.3.

Example 20

Synthesis of 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-(2-aminoethoxy)ethoxy)propan-1-one (C-20)

[0298]

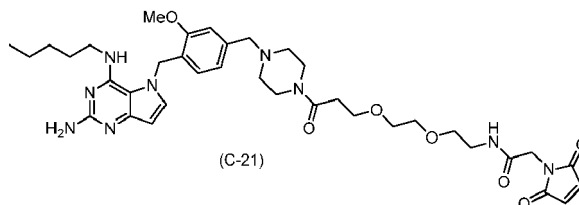


[0299] 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-(2-aminoethoxy)ethoxy)propan-1-one (C-20) was prepared following a procedure of Example 19, except 2,2-dimethyl-4-oxo-3,8,11-trioxo-5-azatetradecan-14-oic acid was used in place of tert-butyl (3-bromopropyl)carbamate, to afford 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-(2-aminoethoxy)ethoxy)propan-1-one (C-20) as a solid: ¹H NMR (CD₃OD): δ 7.24 (d, 1H), 7.11 (s, 1H), 6.86 (d, 1H), 6.57 (d, 1H), 6.12 (d, 1H), 5.42 (s, 2H), 3.96 (s, 3H), 3.76 (t, 2H), 3.59 (m, 12H), 3.37 (t, 2H), 2.76 (t, 2H), 2.66 (t, 2H), 2.45 (m, 4H), 1.41 (m, 2H), 1.28 (m, 2H), 1.16 (m, 2H), 0.89 (t, 3H). LRMS [M+H] = 597.4.

Example 21

Synthesis of N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (C-21)

[0300]



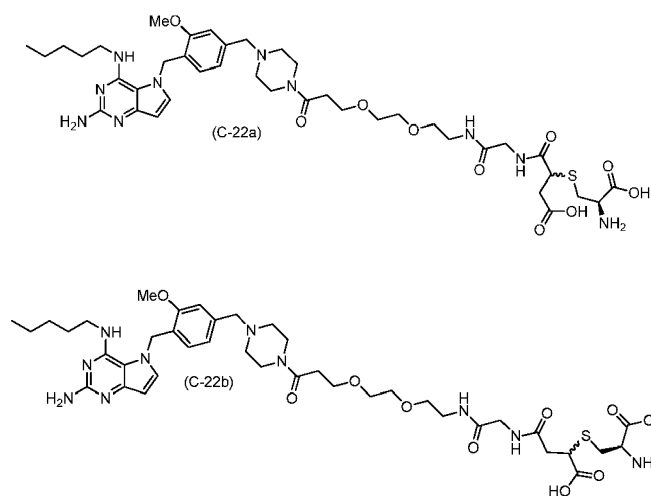
[0301] A round bottom flask was charged with 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-(2-aminoethoxy)ethoxy)propan-1-one (C-20) (1.0 equiv.), DIEA (10.0 equiv.) and DMF (0.004 M) and the mixture was stirred at room temperature for 15 minutes. A separate flask was then charged with 2,5-dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate (1.5 equiv.), DIEA (10.0 equiv.) and DMF (0.006 M). This mixture was also stirred for 15 minutes at room temperature and then the two solutions were mixed and the reaction mixture stirred at room temperature for 1 hour. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrro-

lo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (C-21) as a solid as the TFA salt: ^1H NMR (CD_3CN): δ 7.30 (d, 1H), 7.05 (s, 1H), 6.98 (s, 1H), 6.86 (d, 1H), 6.82 (s, 2H), 6.74 (s, 1H), 6.68 (d, 1H), 6.21 (d, 1H), 6.08 (t, 1H), 5.38 (s, 2H), 4.08 (s, 2H), 3.89 (s, 3H), 3.70 (t, 2H), 3.41 (m, 14H), 3.29 (m, 2H), 2.55 (t, 2H), 2.38 (m, 4H), 1.41 (m, 2H), 1.26 (m, 2H), 1.13 (m, 2H), 0.85 (t, 3H). LCMS $[\text{M}+\text{H}] = 734.4$.

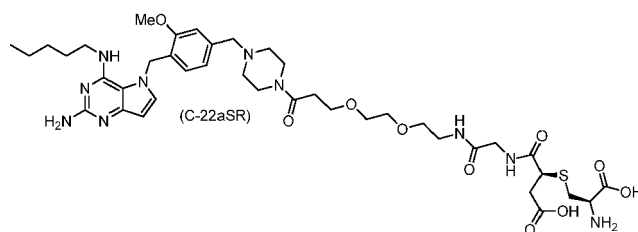
Example 22

Synthesis of (2R)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,9,19-trioxo-13,16-dioxo-4-thia-7,10-diazanonadecan-1-oic acid (C-22a) and (19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,11,14-trioxo-4,7-dioxo-17-thia-10,13-diazaicosan-20-oic acid (C-22b)

[0302]

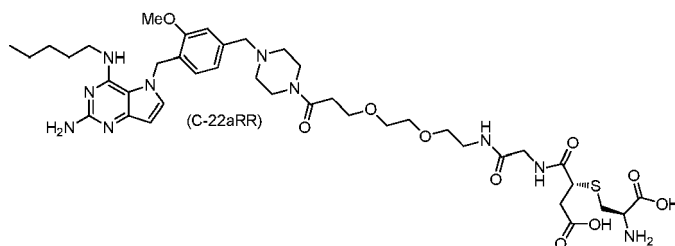


[0303] (2R)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,9,19-trioxo-13,16-dioxo-4-thia-7,10-diazanonadecan-1-oic acid (C-22a) and (19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,11,14-trioxo-4,7-dioxo-17-thia-10,13-diazaicosan-20-oic acid (C-22b) were prepared following a procedure similar to Example 4, except Compound (C-21) was used in place of Compound (C-1), to afford a mixture of Compounds (C-22a) and (C-22b), as their respective diastereomers (Compounds (C-22aSR), C-22aRR), (C-22bRR) and (C-22bRR) below), as a solid as the TFA salt. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H_2O , C18 column): ^1H NMR (CD_3OD): δ 7.37 (d, 1H), 7.32 (s, 1H), 7.08 (d, 1H), 6.81 (d, 1H), 6.24 (d, 1H), 5.57 (s, 2H), 4.34 (s, 2H), 4.20 (m, 1H), 3.96 (s, 3H), 3.82 (m, 9H), 3.56 (m, 9H), 3.38 (m, 3H), 3.21 (m, 2H), 2.70 (t, 2H), 1.54 (m, 2H), 1.32 (m, 2H), 1.19 (m, 2H), 0.89 (t, 3H). LCMS $[\text{M}+\text{H}] = 873.4$.



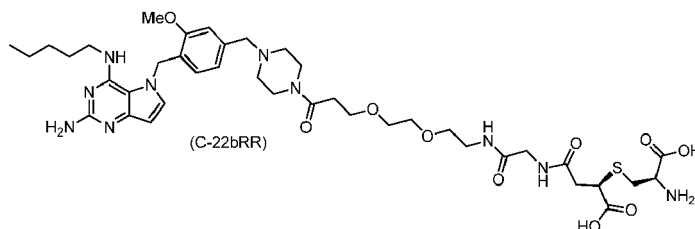
(2R,5S)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,9,19-trioxo-13,16-dioxo-4-thia-7,10-diazanonadecan-1-oic acid (C-22aSR);

5



10 (2R,5R)-2-amino-19-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-(carboxymethyl)-6,9,19-trioxo-13,16-dioxo-4-thia-7,10-diazanonadecan-1-oic acid (C-22aRR);

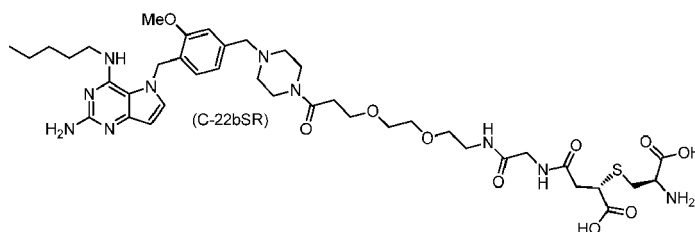
15



20

(16R,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,11,14-trioxo-4,7-dioxo-17-thia-10,13-diazaicosan-20-oic acid (C-22bRR);

25



30

(16S,19R)-19-amino-1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-16-carboxy-1,11,14-trioxo-4,7-dioxo-17-thia-10,13-diazaicosan-20-oic acid (C-22bSR).

35

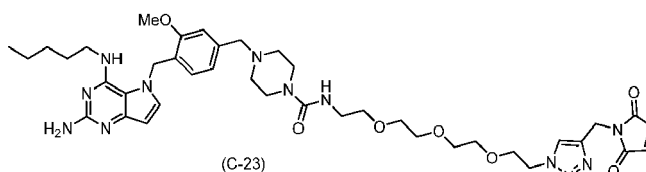
Example 23

Synthesis of 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)-N-(2-(2-(2-(2-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)piperazine-1-carboxamide (C-23)

40

[0304]

45



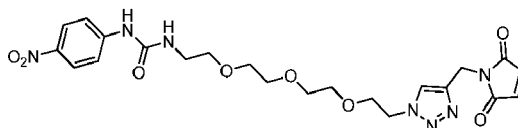
50

[0305] A round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1 equiv.), 4-nitrophenyl (2-(2-(2-(2-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)carbamate (0.9 equiv.), triethylamine (3.0 equiv.) and DMSO (0.01 M). The reaction mixture was stirred at room temperature for 2 hours and the crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)-N-(2-(2-(2-(2-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)piperazine-1-carboxamide (C-23) as a solid as the TFA salt: ¹H NMR (CD₃OD):

55

δ 7.96 (s, 1H), 7.36 (d, 1H), 7.26 (d, 1H), 7.05 (d, 1H), 6.85 (s, 2H), 6.79 (d, 1H), 6.24 (d, 1H), 5.57 (s, 2H), 4.74 (s, 2H), 4.53 (t, 2H), 4.35 (s, 2H), 3.95 (s, 3H), 3.86 (t, 2H), 3.85 (m, 4H), 3.54 (m, 12H), 3.22 (m, 6H), 1.53 (m, 2H), 1.30 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LCMS [M+H] = 817.4.

Note: 4-nitrophenyl (2-(2-(2-(2-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)carbamate



was prepared using the following procedure:

Step 1: Triethylamine (2.5 equiv.) and di-tert-butyl dicarbonate (1.1 equiv.) were added to a solution of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanamine (1.0 equiv.) in CH_2Cl_2 (0.05 M) and the reaction mixture was stirred at room temperature for 30 minutes. The reaction mixture was then concentrated in vacuo and the residue was purified using RP-C18 ISCO and then lyophilized to give tert-butyl (2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)carbamate.

Step 2: A solution of tert-butyl (2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)carbamate (1 equiv.) and 1-(prop-2-yn-1-yl)-1H-pyrrole-2,5-dione (2.0 equiv.) in *t*-BuOH (0.08 M) was flushed with N_2 gas five times and then L-ascorbic acid sodium salt (1.0 equiv. 0.16 M in H_2O) and CuSO_4 (0.2 equiv. 0.03 M in H_2O) were added. The reaction mixture was again flushed with N_2 gas five times and then stirred at room temperature for 4 h. The reaction mixture was then purified by ISCO RP-C18 and lyophilized to afford tert-butyl (2-(2-(2-(2-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl) carbamate.

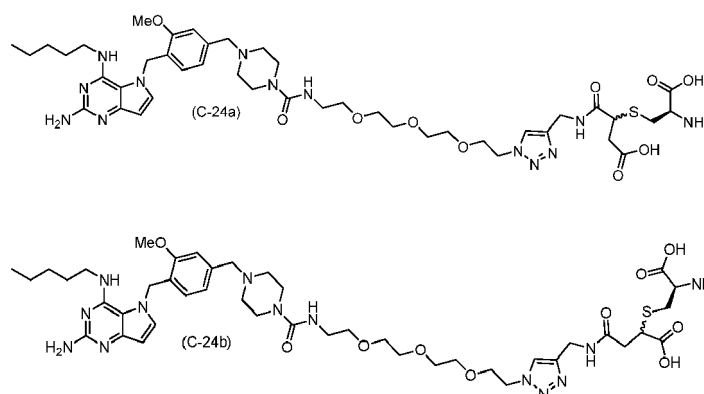
Step 3: A solution of tert-butyl (2-(2-(2-(2-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl) carbamate in TFA (0.02 M) was concentrated in vacuo to afford 1-((1-(23-amino-3,6,9,12,15,18,21-heptaaoxatricosyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrole-2,5-dione. LCMS [M+H] = 354.2.

Step 4: 4-Nitrophenyl carbonochloridate (1.10 equiv.) and triethylamine (2.50 equiv.) were added to a solution of 1-((1-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrole-2,5-dione (1 equiv.) in CH_2Cl_2 (0.01 M) and the reaction mixture was stirred at room temperature for 10 minutes. The reaction mixture was then concentrated in vacuo, purified by RP-C18 ISCO and then lyophilized to afford 4-nitrophenyl (2-(2-(2-(2-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)carbamate LCMS [M+H] = 519.2.

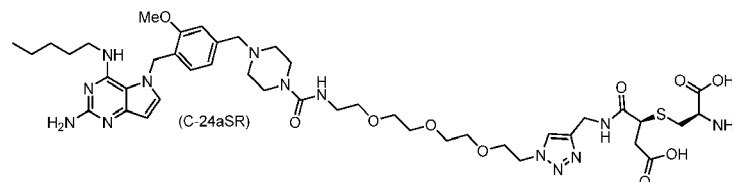
Example 24

Synthesis of 3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-24a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-24b)

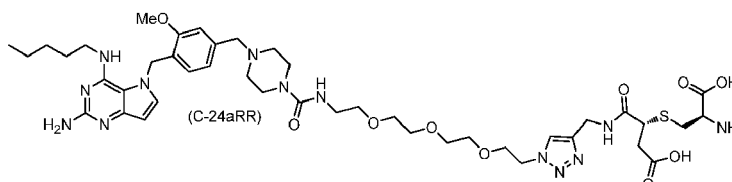
[0306]



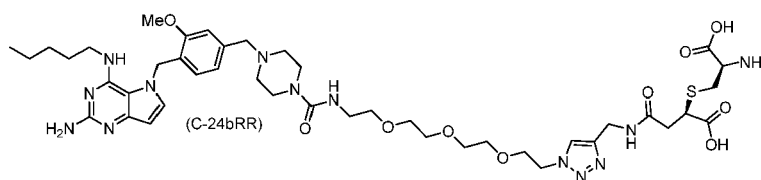
[0307] 3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-24a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-24b) were prepared following a procedure similar to Example 4, except Compound (C-23) was used in place of Compound (C-1), to provide a mixture of Compounds (C-24a) and (C-24b), as their respective diastereomers (Compounds (C-24aSR), C-24aRR), (C-24bRR) and (C-24bSR) below), as a solid as the TFA salt. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column): LCMS [M+H]⁺ = 956.4.



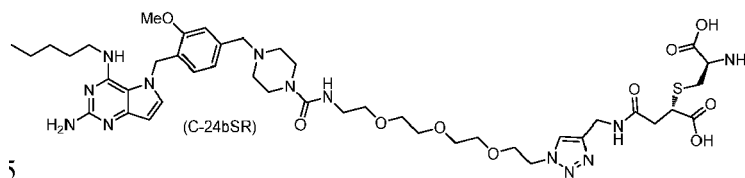
(S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-24aSR);



(R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-24aRR);



(R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-24bRR);

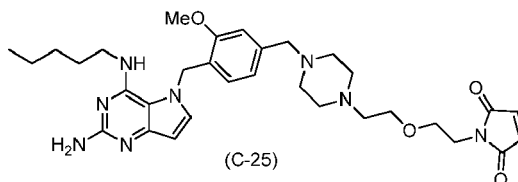


(S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-24bSR).

Example 25

Synthesis of 1-(2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)-1H-pyrrole-2,5-dione (C-25)

[0308]



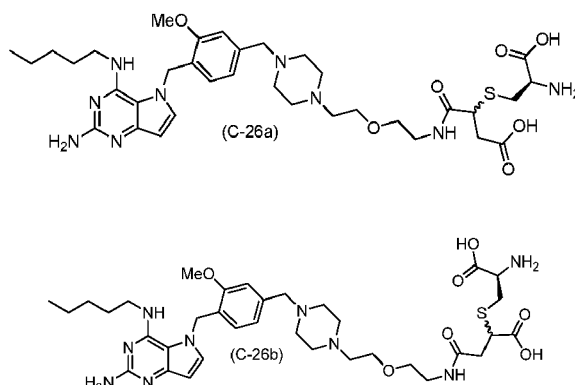
[0309] A round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), 2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)acetaldehyde (4.0 equiv.), sodium cyanoborohydride (13.0 equiv.), and MeOH (0.04 M). The reaction mixture was stirred at room temperature for 1 hour. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 1-(2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)-1H-pyrrole-2,5-dione (C-25) as a solid as the TFA salt: ¹H NMR (CD₃OD): δ 7.36 (d, 1H), 7.16 (d, 1H), 6.96 (d, 1H), 6.83 (s, 2H), 6.76 (d, 1H), 6.23 (d, 1H), 5.53 (s, 2H), 3.93 (s, 3H), 3.84 (s, 2H), 3.78 (m, 2H), 3.71 (m, 2H), 3.64 (m, 2H), 3.54 (m, 2H), 3.35 (m, 4H), 3.27 (t, 2H), 2.95 (m, 4H), 1.52 (m, 2H), 1.30 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LCMS [M+H]⁺ = 605.4.

[0310] Note: 2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)acetaldehyde was prepared by adding 1-(2-(2-hydroxyethoxy)ethyl)-1H-pyrrole-2,5-dione (1.0 equiv.), Dess-Martin periodinane (1.5 equiv.) and DCM (0.1 M) to a round bottom flask and stirring the reaction mixture at room temperature for 2 hours. The reaction mixture was then filtered, the volatiles removed in vacuo and the product used without further purification.

Example 26

Synthesis of 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid (C-26a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid (C-26b)

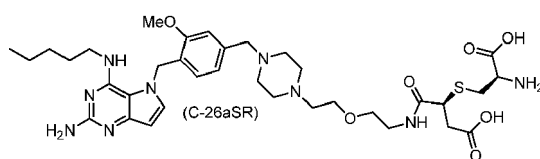
[0311]



[0312] 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid (C-26a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid (C-26b) were prepared following a procedure similar to Example 4, except Compound (C-25) was used in place of Compound (C-1), to afford a mixture of Compounds (C-26a) and (C-26b), as their respective diastereomers (Compounds (C-26aSR), (C-26aRR), (C-26bRR) and (C-26bRR) below), as a solid as the TFA salt. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O,

C18 column): LCMS [M+H] = 744.4

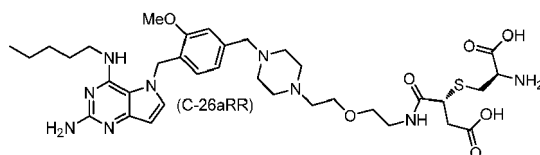
5



10

(S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid (C-26aSR);

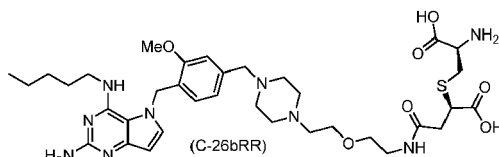
15



20

(R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid (C-26aRR);

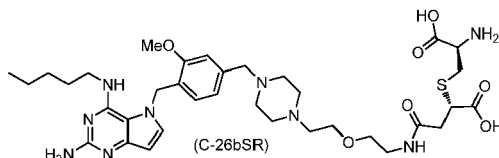
25



30

(R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid (C-26bRR);

35



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(S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethyl)amino)-4-oxobutanoic acid (C-26bSR).

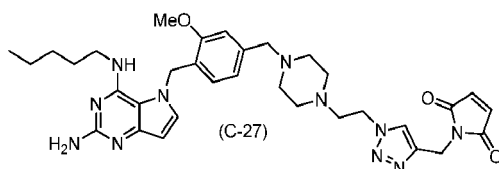
Example 27

Synthesis of 1-((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1 H-1,2,3-triazol-4-yl)methyl)-1 H-pyrrole-2,5-dione (C-27)

45

[0313]

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[0314] Step 1: A round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), 2-azidoacetaldehyde (4.0 equiv.), sodium cyanoborohydride (32.0 equiv.), and MeOH (0.02 M). The reaction mixture was stirred at room temperature for 2 hours. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 5-(4-((4-(2-

azidoethyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine as a solid: LC-MS [M+H] = 507.3.

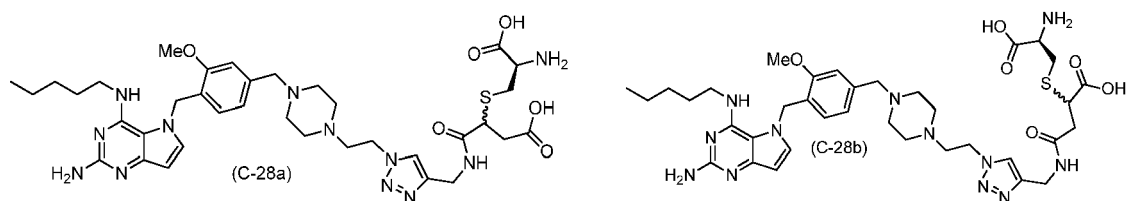
[0315] Step 2: A round bottom flask was charged with 5-((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrole-2,5-dione (2.3 equiv.) and a mixture of *t*-BuOH and water (2:1, v/v, 0.008 M). The reaction mixture was degassed under vacuum and flushed with N₂ five times to remove O₂. L-ascorbic acid sodium salt (1.1 equiv in 0.5 ml H₂O, degassed under and flushed with N₂ five times to remove O₂) was added using a syringe to the reaction mixture, then and CuSO₄ (0.2 equiv. in 0.5 ml water, degassed under vacuum and flushed with N₂ five times to remove O₂) was added using a syringe. The reaction mixture was then stirred at room temperature for 2 hours. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 1-((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrole-2,5-dione (C-27) as a solid as the TFA salt: ¹H NMR (CD₃OD): δ 7.95 (s, 1H), 7.36 (d, 1H), 7.22 (d, 1H), 7.02 (d, 1H), 6.86 (s, 2H), 6.79 (d, 1H), 6.23 (d, 1H), 5.57 (s, 2H), 4.76 (s, 2H), 4.52 (t, 2H), 4.26 (s, 2H), 3.95 (s, 3H), 3.54 (t, 2H), 2.85 (m, 8H), 2.94 (t, 2H), 1.53 (m, 2H), 1.31 (m, 2H), 1.18 (m, 2H), 0.88 (t, 3H). LCMS [M+H] = 642.4.

[0316] Note: 2-azidoacetaldehyde was prepared by adding 2-azidoethanol (1.0 equiv.), Dess-Martin periodinane (1.5 equiv.) and DCM (0.20 M) to a round bottom flask and then stirring the reaction mixture at room temperature for 2 hours. The reaction mixture was then filtered, the volatiles removed in vacuo and the product used without further purification.

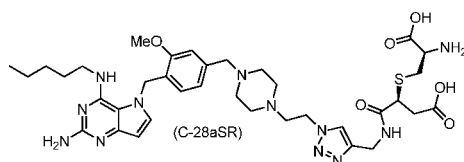
Example 28

Synthesis of 3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-28a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-28b)

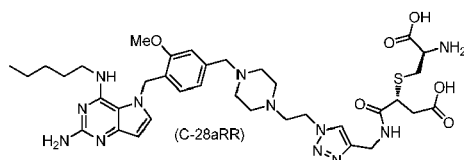
[0317]



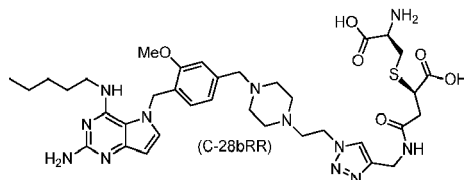
[0318] 3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-28a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-28b) were prepared following a procedure similar to Example 4, except Compound (C-27) was used in place of Compound (C-1), to afford a mixture of Compounds (C-28a) and (C-28b), as their respective diastereomers (Compounds (C-28aSR), C-28aRR), (C-28bRR) and (C-28bRR) below), as a solid as the TFA salt. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column): LCMS [M+H] = 781.4



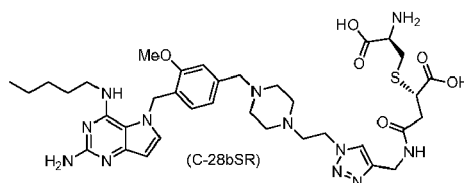
(S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-28aSR);



(R)-3-(((R)-2-amino-2-carboxyethylthio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-28aRR);



(R)-2-(((R)-2-amino-2-carboxyethylthio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-28bRR);

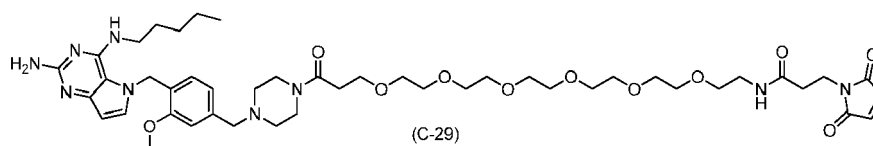


(S)-2-(((R)-2-amino-2-carboxyethylthio)-4-(((1-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4-oxobutanoic acid (C-28bSR).

Example 29

Synthesis of N-(21-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-21-oxo-3,6,9,12,15,18-hexaoxahenicosyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide (C-29)

[0319]

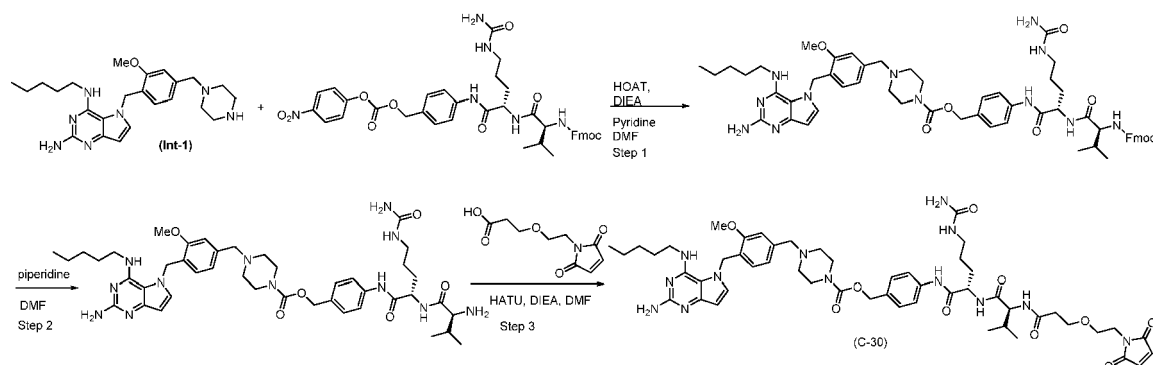


[0320] N-(21-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-21-oxo-3,6,9,12,15,18-hexaoxahenicosyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide (C-29) was prepared following a procedure similar to Example 1, except 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16,19,22-hexaoxa-4-azapentacosan-25-oic acid was used in place of 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid, to afford N-(21-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-21-oxo-3,6,9,12,15,18-hexaoxahenicosyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide (C-29) as a solid as the TFA salt: ^1H NMR (DMSO): δ 8.00 (t, 1H), 7.42 (d, 1H), 7.38 (s, 3H), 7.20 (s, 1H), 7.00 (s, 2H), 6.95 (s, 1H), 6.57 (s, 1H), 6.23 (d, 1H), 5.57 (s, 2H), 4.30 (s, 2H), 3.87 (s, 3H), 3.59 (m, 4H), 3.49 (m, 28H), 3.35 (t, 2H), 3.14 (m, 2H), 2.32 (m, 2H), 1.45 (m, 2H), 1.21 (m, 2H), 1.09 (m, 2H), 0.81 (t, 3H). LRMS $[\text{M}+\text{H}] = 924.4$.

Example 30

Synthesis of 4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (C-30)

[0321]



[0322] Step 1: A round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), HOAT (2.0 equiv.), Huenig's base (14.0 equiv.), (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-(((S)-1-(4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate (1.2 equiv.), and pyridine:DMF (1:4, 0.02 M). The reaction mixture was stirred at room temperature for 4 hours, and the crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 4-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate as a solid: LCMS [M+H] = 1065.5.

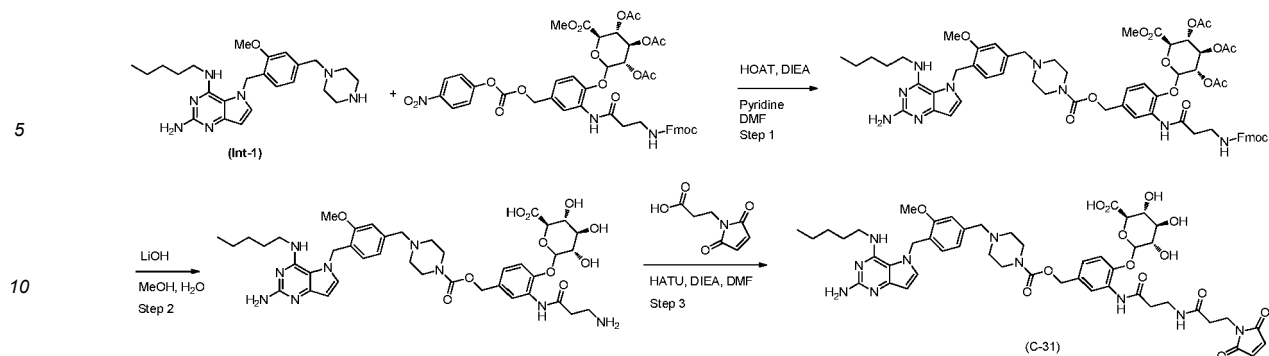
[0323] Step 2: 4-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate was dissolved in DMF (0.007 M) and piperidine (100.0 equiv.) was added. The reaction was stirred at room temperature for 30 minutes. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate as a solid: LCMS [M+H] = 843.5.

[0324] Step 3: A round bottom flask was charged with 4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (1.0 equiv.), 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoic acid (1.1 equiv.), Huenig's base (5.0 equiv.), HATU (1.05 equiv.) and DMF (0.004 M). The reaction mixture was stirred at room temperature for 2 hours. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (C-30) as a solid as the TFA salt: LCMS [M+H] = 1038.5.

Example 31

Synthesis of (2R,3R,4R,5S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (C-31)

[0325]



[0326] Step 1: A round bottom flask was charged with 5-(2-methoxy-4-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), HOAT (2.0 equiv.), Huenig's base (14.0 equiv.), (3S,4R,5R,6R)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (1.2 equiv.), and pyridine:DMF (1:4, 0.015 M). The reaction mixture was stirred at room temperature for 4 hours. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford (3S,4R,5R,6R)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate as a solid: LCMS [M+H] = 1212.4.

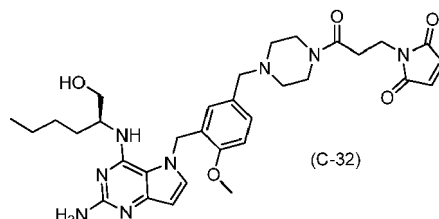
[0327] Step 2: (3S,4R,5R,6R)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (1.0 equiv.) was dissolved in MeOH, THF and water (2:1:0.4) (0.005 M). LiOH (8.0 equiv.) was then added and the reaction was stirred at room temperature for 2 hours. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford (2R,3R,4R,5S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-aminopropanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid as a solid: LCMS [M+H] = 850.4.

[0328] Step 3: A round bottom flask was charged with (2R,3R,4R,5S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-aminopropanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (1.0 equiv.), 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid (2.0 equiv.), Huenig's base (6.0 equiv.), HBTU (1.8 equiv.) and DMF (0.003 M). The reaction was kept stirring at room temperature for 15 minutes. The reaction mixture was stirred at room temperature for 2 hours. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford (2R,3R,4R,5S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (C-31) as a solid as the TFA salt: LCMS [M+H] = 1001.3.

Example 32

Synthesis of (S)-1-(3-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1H-pyrrole-2,5-dione (C-32)

[0329]



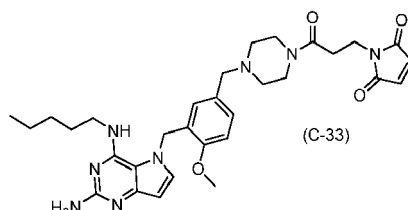
[0330] (S)-1-(3-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1H-pyrrole-2,5-dione (C-32) was prepared following a procedure similar to Example

1, except Compound (Int-2) was used in place of Compound (Int-1), to afford (S)-1-(3-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1H-pyrrole-2,5-dione (C-32) as a solid as the TFA salt: ^1H NMR (CD_3OD): δ 7.49 (d, 2H), 7.21 (d, 1H), 6.82 (s, 2H), 6.77 (d, 1), 6.28 (d, 1H), 5.67 (d, 1H), 5.51 (d, 1H), 4.36 (m, 1H), 4.18 (s, 2H), 3.98 (s, 3H), 3.76 (t, 2H), 3.54 (dd, 1H), 3.46 (dd, 1H), 3.16 (m, 4H), 3.05 (m, 4H), 2.71 (t, 2H), 1.48 (m, 1H), 1.26 (m, 3H), 1.05 (m, 1H), 0.84 (t, 3H). LRMS $[\text{M}+\text{H}] = 619.4$.

Example 33

Synthesis of 1-(3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1H-pyrrole-2,5-dione (C-33)

[0331]

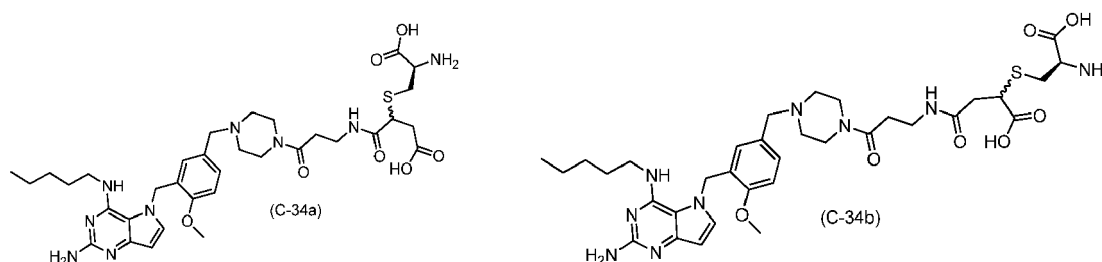


[0332] 1-(3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1H-pyrrole-2,5-dione (C-33) was prepared following a procedure similar to Example 1, except Compound (Int-3) was used in place of Compound (Int-1), to afford 1-(3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)-1H-pyrrole-2,5-dione (C-33) as a solid as the TFA salt. LRMS $[\text{M}+\text{H}] = 589.3$.

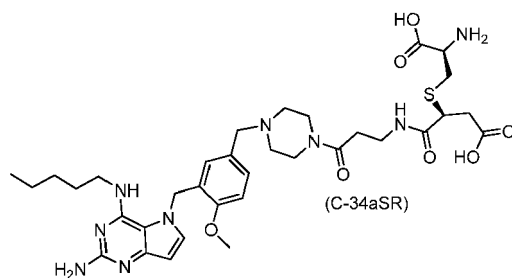
Example 34

Synthesis of 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-34a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-34b)

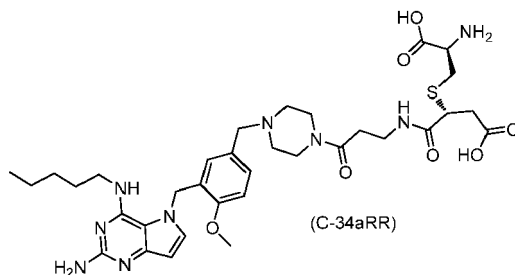
[0333]



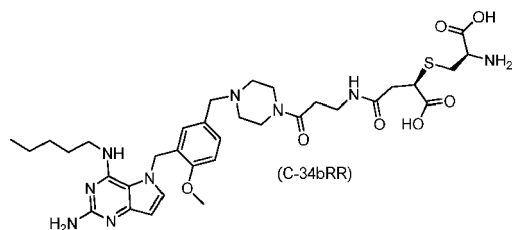
[0334] 3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-34a) and 2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-34b) were prepared following a procedure similar to Example 4, except Compound (C-33) was used in place of Compound (C-1), to afford a mixture of Compounds (C-34a) and (C-34b), as their respective diastereomers (Compounds (C-34aSR), (C-34aRR), (C-34bRR) and (C-34bRR) below), as a solid as the TFA salt. The crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN :0.05% TFA in H_2O , C18 column): ^1H NMR (DMSO): δ 7.51 (s, 2H), 7.39 (m, 2H), 7.27 (d, 1H), 7.15 (d, 1H), 6.59 (s, 1H), 6.22 (t, 1H), 5.56 (s, 2H), 3.86 (s, 4H), 3.66 (m, 3H), 3.42 (m, 8H), 3.25 (m, 4H), 3.08 (m, 2H), 2.81 (m, 3H), 2.65 (m, 1H), 1.43 (m, 2H), 1.22 (m, 3H), 1.07 (m, 2H), 0.83 (t, 3H). LCMS $[\text{M}+\text{H}] = 728.3$



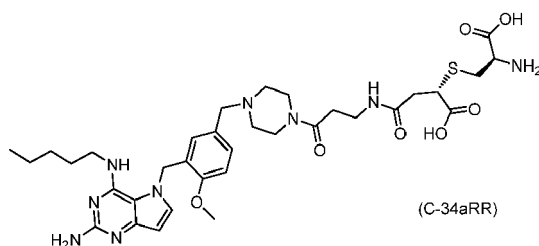
(S)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-34aSR);



(R)-3-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-34aRR);



(R)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-34bRR);

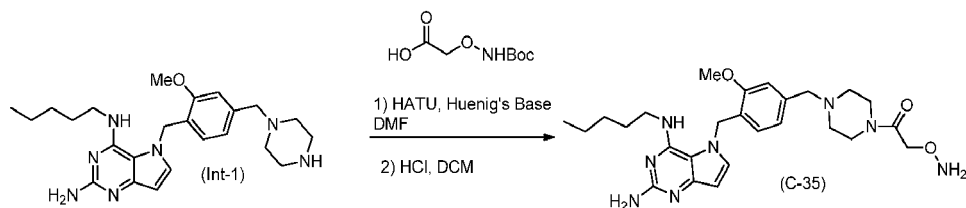


(S)-2-(((R)-2-amino-2-carboxyethyl)thio)-4-((3-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropyl)amino)-4-oxobutanoic acid (C-34bSR).

Example 35

Synthesis of 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-(aminoxy)ethanone (C-35)

[0335]



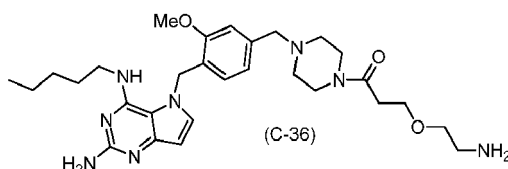
[0336] Step 1: A round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), 2-(((tert-butoxycarbonyl)amino)oxy)acetic acid (1.1 equiv.), HATU (1.05 equiv.), Huenig's base (5.0 equiv.), and DMF (0.2 M). The reaction mixture was stirred at room temperature for 18 hours and the crude reaction mixture was then purified by ISCO chromatography (0 - 20% MeOH:DCM) to provide tert-butyl 2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-oxoethoxycarbamate.

[0337] Step 2: HCl (20.0 equiv., 4M in dioxane) was added to a round bottom flask charged with tert-butyl 2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-oxoethoxycarbamate (1.0 equiv.) and DCM (0.1 M) at 0°C. The ice bath was removed and reaction mixture stirred at room temperature for 3 hours. The volatiles were removed in vacuo. MeOH (with 8% NH₄OH) was added to the resulting residue and the volatiles removed in vacuo. This was repeated 2 more times. The crude reaction mixture was then purified by ISCO chromatography (0 - 10% MeOH (8% NH₄OH):DCM) to deliver 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-(aminooxy)ethanone (C-35) as a solid: ¹H NMR (CDCl₃): δ 7.12 (d, 1H), 7.00 (s, 1H), 6.90 (s, 1H), 6.69 (d, 1H), 6.38 (d, 1H), 5.52 (t, 1H), 5.30 (s, 2H), 4.35 (s, 2H), 3.94 (s, 3H), 3.64 (s, 2H), 3.52 (m, 2H), 3.38 (m, 4H), 2.44 (m, 4H), 1.62 (s, 2H), 1.45 (m, 2H), 1.38 (m, 2H), 1.25 (m, 2H), 1.12 (m, 2H), 0.87 (t, 3H). LRMS [M+H] = 511.4.

Example 36

Synthesis of 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-aminoethoxy)propan-1-one (C-36)

[0338]

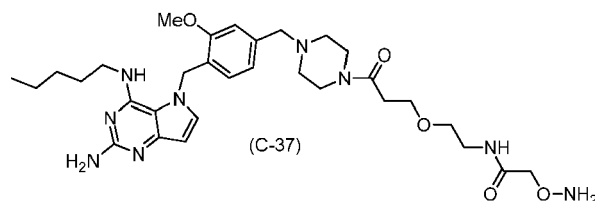


[0339] 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-aminoethoxy)propan-1-one (C-36) was prepared following a procedure similar to Example 35, except 3-(2-(((tert-butoxycarbonyl)amino)ethoxy)propanoic acid was used in place of 2-(((tert-butoxycarbonyl)amino)oxy)acetic acid, to afford 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-aminoethoxy)propan-1-one (C-36) as a solid: ¹H NMR (CD₃OD): δ 7.26 (d, 1H), 7.09 (d, 1H), 6.86 (d, 1H), 6.59 (d, 1H), 6.13 (d, 1H), 5.43 (s, 2H), 4.57 (s, 2H), 3.94 (s, 3H), 3.73 (t, 2H), 3.58 (m, 4H), 3.54 (m, 2H), 3.37 (m, 2H), 2.93 (t, 2H), 2.66 (m, 2H), 2.44 (m, 4H), 1.41 (m, 2H), 1.27 (m, 2H), 1.15 (m, 2H), 0.87 (t, 3H). LRMS [M+H] = 553.4.

Example 37

Synthesis of N-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)-2-(aminooxy)acetamide (C-37)

[0340]

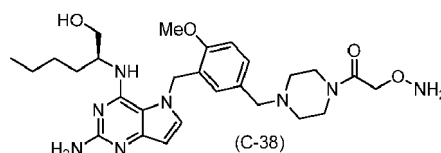


[0341] N-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)-2-(aminooxy)acetamide (C-37) was prepared following a procedure similar to Example 35, except 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-(2-aminoethoxy)propan-1-one (C-36) was used in place of Int-1, to afford N-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)-2-(aminooxy)acetamide (C-37) as a solid: ^1H NMR (CD_3OD): δ 7.27 (d, 1H), 7.09 (d, 1H), 6.86 (d, 1H), 6.59 (d, 1H), 6.13 (d, 1H), 5.44 (s, 2H), 4.08 (s, 2H), 3.93 (s, 3H), 3.72 (t, 2H), 3.56 (m, 8H), 3.40 (m, 4H), 2.64 (t, 2H), 2.44 (m, 4H), 1.43 (m, 2H), 1.27 (m, 2H), 1.14 (m, 2H), 0.87 (t, 3H). LRMS $[\text{M}+\text{H}] = 626.4$.

Example 38

Synthesis of (S)-1-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-2-(aminooxy)ethanone (C-38)

[0342]

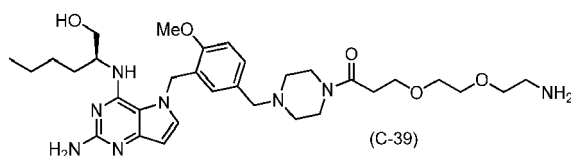


[0343] (S)-1-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-2-(aminooxy)ethanone (C-38) was prepared following a procedure similar to Example 35, except Compound (Int-2) was used in place of Compound (Int-1), to afford (S)-1-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-2-(aminooxy)ethanone (C-38) as a solid: ^1H NMR (CD_3OD): δ 7.54 (d, 1), 7.40 (d, 1H), 7.13 (d, 1H), 6.68 (s, 1H), 6.29 (d, 1H), 5.69 (d, 1H), 5.48 (d, 1H), 4.36 (m, 3H), 3.96 (s, 3H), 3.74 (m, 2H), 3.51 (m, 4H), 2.66 (m, 4H), 1.49 (m, 1H), 1.38 (m, 3H), 1.24 (m, 2H), 0.96 (m, 2H), 0.84 (t, 3H). LRMS $[\text{M}+\text{H}] = 541.3$.

Example 39

(S)-1-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-(2-(2-aminoethoxy)ethoxy)propan-1-one (C-39)

[0344]



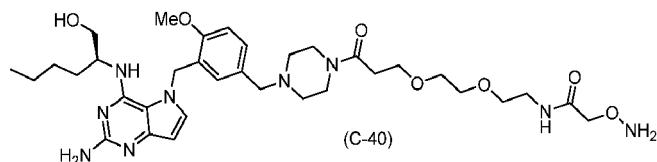
[0345] (S)-1-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-(2-(2-aminoethoxy)ethoxy)propan-1-one (C-39) was prepared following a procedure similar to Example 35, except Compound (Int-2) was used in place of Compound (Int-1) and 2,2-dimethyl-4-oxo-3,8,11-trioxo-5-azatetradecan-14-oic acid was used in place of 2-(((tert-butoxycarbonyl)amino)oxy)acetic acid, to afford (S)-1-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-(2-(2-aminoethoxy)ethoxy)propan-1-one (C-39) as a solid: ^1H NMR (CD_3OD): δ 7.56 (d, 1H), 7.44 (d, 1H), 7.16 (d, 1H), 6.77 (s, 1H), 6.31 (d, 1H), 5.71 (d, 1H), 5.50 (d, 1H), 4.38 (m, 1H), 3.98 (s, 3H), 3.78 (m, 4H), 3.72 (m, 2H), 3.67

(m, 6H), 3.53 (m, 4H), 3.14 (m, 2H), 2.77 (m, 2H), 2.69 (m, 4H), 1.51 (m, 1H), 1.26 (m, 3H), 1.02 (m, 2H), 0.86 (t, 3H). LRMS [M+H] = 627.5.

Example 40

Synthesis of (S)-N-(2-(2-(3-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(aminooxy)acetamide (C-40)

[0346]

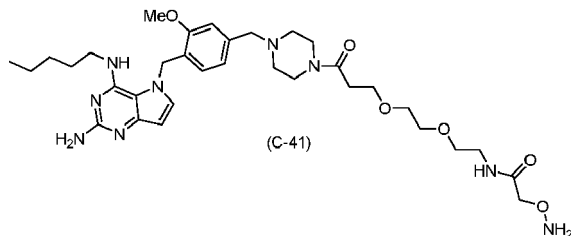


[0347] (S)-N-(2-(2-(3-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(aminooxy)acetamide (C-40) was prepared following a procedure similar to Example 35, except Compound (C-39) was used in place of Compound (Int-1), to afford (S)-N-(2-(2-(3-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(aminooxy)acetamide (C-40) as a solid: ¹H NMR (CD₃OD): δ 7.54 (d, 1H), 7.47 (d, 1H), 7.17 (d, 1H), 6.78 (s, 1H), 6.30 (d, 1H), 5.68 (d, 1H), 5.50 (d, 1H), 4.36 (m, 1H), 4.09 (s, 2H), 3.97 (s, 3H), 3.73 (m, 8H), 3.56 (m, 4H), 3.43 (t, 2H), 3.23 (m, 2H), 2.88 (m, 4H), 2.66 (t, 2H), 1.49 (m, 1H), 1.26 (m, 3H), 1.04 (m, 2H), 0.84 (t, 3H). LRMS [M+H] = 700.4.

Example 41

Synthesis of N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(aminooxy)acetamide (C-41)

[0348]



[0349] N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(aminooxy)acetamide (C-41) was prepared following a procedure similar to Example 35, except Compound (C-20) was used in place of Compound (Int-1), to afford N-(2-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)ethyl)-2-(aminooxy)acetamide (C-41) as a solid: ¹H NMR (CD₃OD): δ 7.25 (d, 1H), 7.11 (s, 1H), 6.86 (d, 1H), 6.58 (d, 1H), 6.12 (d, 1H), 5.43 (s, 2H), 4.10 (s, 2H), 3.96 (s, 3H), 3.76 (t, 2H), 3.60 (m, 12H), 3.44 (t, 2H), 3.36 (t, 2H), 2.66 (t, 2H), 2.46 (m, 4H), 1.40 (m, 2H), 1.30 (m, 2H), 1.15 (m, 2H), 0.89 (t, 3H). LRMS [M+H] = 670.4.

Example 42

Synthesis of 5-(4-((4-(2-(2-(aminooxy)ethoxy)ethyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (C-42)

[0350]



15 and purified by reverse phase HPLC, using C18 column (eluted with 10-50% acetonitrile-H₂O containing 0.05% TFA) to deliver 2-(2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethoxy)isoindoline-1,3-dione. LCMS [M+H]⁺= 671.40.

(10.0 equiv.), MeOH (0.02 M) and water (0.2 M). The mixture was stirred for 4 hours at room temperature. The reaction mixture was purified by reverse phase HPLC, using C18 column (eluted with 10-50% acetonitrile-H₂O containing 0.05% TFA). The fractions containing desired product were pooled and concentrated under reduced pressure, the residue was then dissolved in MeOH and loaded to a preconditioned Sphere PL-HCO₃ MP-resin column and eluted with MeOH, the eluent was concentrated to afford 5-(4-((4-(2-(2-(aminooxy)ethoxy)ethyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (C-42) as a solid: ¹H NMR (CD₃OD): δ 7.22 (d, 1H), 7.08 (d, 1H), 6.83 (d, 1H), 6.56 (d, 1H), 6.10 (d, 1H), 5.40 (s, 2H), 3.94 (s, 3H), 3.76 (m, 2H), 3.60 (m, 4H), 3.50 (s, 2H), 3.34 (d, 3H), 2.59 (m, 4H), 2.49 (s, 4H), 1.38 (m, 2H), 1.26 (m, 2H), 1.12 (m, 2H), 0.87 (t, 3H). LCMS [M+H]⁺ = 541.40.

[0353] Step 1: To a solution of N-hydroxyphthalimide (1.0 equiv.), diethylene glycol (1.0 equiv.) and triphenylphosphine (1.3 equiv.) in THF (0.2 M) was added DEAD (2.2 M solution in toluene, 1.3 equiv.) at 0°C. The resulting solution was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo. The residue was purified by silica gel chromatography (eluted with 20-70% EtOA/Hexanes). The product still contained some Ph₃PO after this chromatography, it was then repurified by reverse phase chromatography (C18 column, eluted with 20-40-100% CH₃CN/water) to afford 2-(2-(2-hydroxyethoxy)ethoxy)isoindoline-1,3-dione LCMS [M+H] = 252.10.

40 and brine, the organic layer was separated and dried over MgSO₄ and evaporated in vacuo. The crude mixture was purified by silica gel chromatography (eluted with 30-70% EtAcOc/Hexanes), to deliver 2-(2-((1,3-dioxoisindolin-2-yl)oxy)ethoxy)acetaldehyde. LCMS [M+H]⁺ = 250.10.

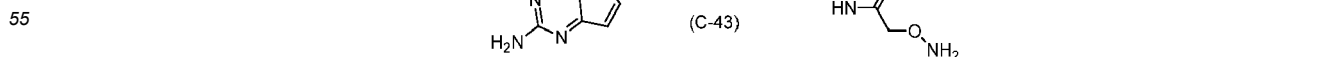
45 Synthesis of N-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)-2-(aminooxy)acetamide (C-43)

50

55

(C-43)

Chemical structure of compound (C-43) is shown, which is a substituted purine derivative. The structure includes a 7-aminopurine-2,6-dione core. The substituents are: a butylamino group at position 9, a (4-methoxyphenyl)methyl group at position 2, and a (3-((3-aminooxypropyl)carbamoyl)propyl)piperidin-1-ylmethyl group at position 6.



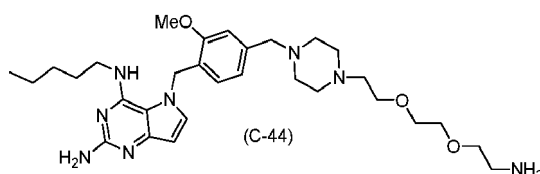
[0356] N-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-

yl)propyl)-2-(aminooxy)acetamide (C-43) was prepared following a procedure similar to Example 35, except Compound (C-19) was used in place of Compound (Int-1), to afford N-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)-2-(aminooxy)acetamide (C-43) as a solid: ^1H NMR (CD_3OD): δ 7.12 (d, 1H), 6.98 (d, 1H), 6.73 (d, 1H), 6.45 (d, 1H), 6.00 (d, 1H), 5.30 (s, 2H), 3.97 (s, 2H), 3.84 (s, 3H), 3.41 (s, 2H), 3.25 (s, 2H), 2.40 (s, 6H), 2.27 (m, 3H), 1.63 (m, 2H), 1.28 (m, 2H), 1.17 (m, 3H), 1.02 (m, 2H), 0.77 (t, 3H). LCMS $[\text{M}+\text{H}] = 568.40$.

Example 44

Synthesis of 5-(4-((4-(2-(2-(2-aminoethoxy)ethoxy)ethyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (C-44)

[0357]

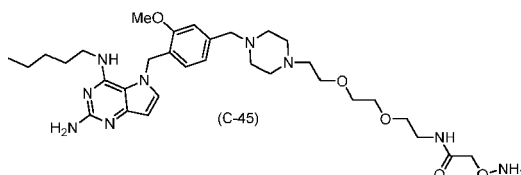


[0358] 5-(4-((4-(2-(2-(2-aminoethoxy)ethoxy)ethyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (C-44) was prepared following a procedure similar to Example 19, except tert-butyl (2-(2-(2-bromoethoxy)ethoxy)ethyl)carbamate was used in place of tert-butyl (3-bromopropyl)carbamate, to afford 5-(4-((4-(2-(2-(2-aminoethoxy)ethoxy)ethyl)piperazin-1-yl)methyl)-2-methoxybenzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (C-44) as a solid: ^1H NMR (CD_3OD): δ 7.36 (d, 1H), 7.13 (d, 1H), 6.92 (d, 1H), 6.73 (d, 1H), 6.21 (s, 1H), 5.51 (s, 2H), 3.92 (s, 3H), 3.69 (m, 12H), 3.53 (t, 2H), 3.12 (m, 2H), 2.84 (m, 8H), 1.50 (m, 2H), 1.28 (m, 2H), 1.17 (m, 2H), 0.87 (t, 3H). LRMS $[\text{M}+\text{H}] = 569.3$.

Example 45

Synthesis of N-(2-(2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethoxy)ethyl)-2-(aminooxy)acetamide (C-45)

[0359]

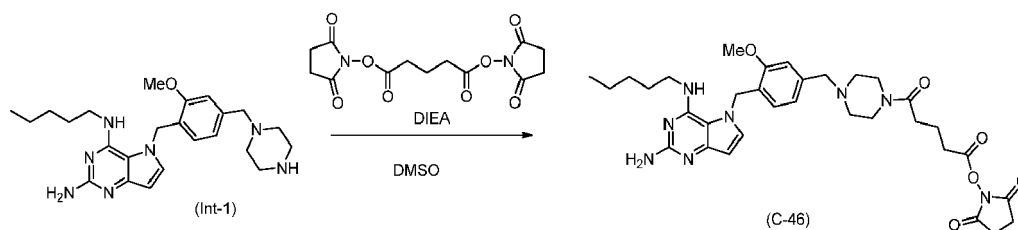


[0360] N-(2-(2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethoxy)ethyl)-2-(aminooxy)acetamide (C-43) was prepared following a procedure similar to Example 35, except Compound (C-44) was used in place of Compound (Int-1), to afford N-(2-(2-(2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)ethoxy)ethyl)-2-(aminooxy)acetamide (C-45) as a solid: ^1H NMR (CDCl_3): δ 7.20 (s, 1H), 6.97 (d, 1H), 6.90 (s, 1H), 6.87 (s, 1H), 6.76 (d, 1H), 6.56 (d, 1H), 6.17 (d, 1H), 5.84 (s, 2H), 5.21 (s, 2H), 4.69 (m, 2H), 4.07 (s, 2H), 3.85 (s, 3H), 3.53 (m, 8H), 3.45 (m, 2H), 3.39 (s, 2H), 3.24 (m, 2H), 2.52 (t, 2H), 2.40 (m, 8H), 1.22 (m, 2H), 1.16 (m, 2H), 1.02 (m, 2H), 0.78 (t, 3H). LRMS $[\text{M}+\text{H}] = 642.4$.

Example 46

Synthesis of 2,5-dioxopyrrolidin-1-yl 5-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-46)

[0361]

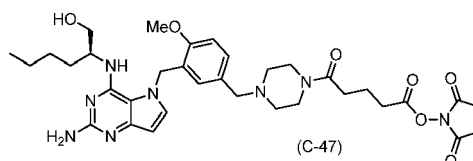


[0362] A round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), diisopropyl amine (1.3 equiv.), disuccinimidyl glutarate (1.3 equiv.), and DMSO (0.1 M). The reaction mixture was stirred room temperature for 3 hours. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford 2,5-dioxopyrrolidin-1-yl 5-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-46) as a solid as the TFA salt: ¹H NMR (DMSO): δ 7.41 (s, 1H), 7.37 (s, 3H), 7.19 (s, 1H), 6.94 (s, 1H), 6.57 (s, 1H), 6.22 (d, 1H), 5.56 (s, 2H), 4.30 (s, 2H), 3.86 (s, 3H), 3.44 (m, 4H), 3.35 (m, 2H), 2.92 (m, 2H), 2.80 (m, 8H), 2.71 (m, 2H), 1.83 (m, 2H), 1.44 (m, 2H), 1.20 (m, 2H), 1.09 (m, 2H), 0.80 (t, 3H). LRMS [M+H]⁺ = 649.3.

Example 47

Synthesis of (S)-2,5-dioxopyrrolidin-1-yl 5-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-47)

[0363]

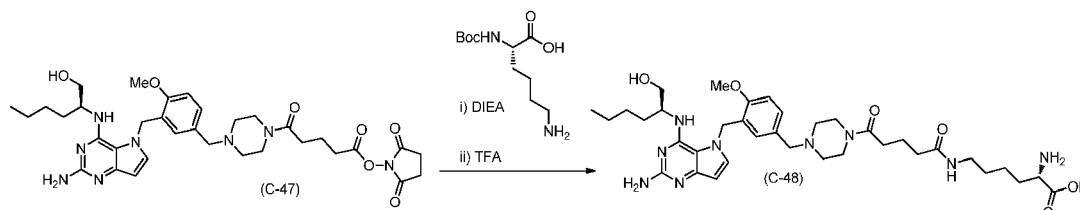


[0364] (S)-2,5-dioxopyrrolidin-1-yl 5-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-47) was prepared following a procedure similar to Example 46, except Compound (Int-2) was used in place of Compound (Int-1), to afford (S)-2,5-dioxopyrrolidin-1-yl 5-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-47) as a solid as the TFA salt: ¹H NMR (DMSO): δ 7.54 (s, 1H), 7.43 (s, 3H), 7.22 (s, 1H), 6.61 (s, 1H), 6.28 (d, 1H), 6.24 (d, 1H), 5.67 (d, 1H), 5.50 (d, 1H), 4.82 (s, 1H), 4.39 (s, 1H), 4.22 (m, 2H), 3.89 (s, 3H), 3.36 (m, 4H), 3.28 (m, 2H), 2.92 (m, 2H), 2.82 (m, 8H), 2.72 (m, 2H), 1.84 (m, 2H), 1.34 (m, 2H), 1.15 (m, 2H), 0.86 (m, 2H), 0.77 (t, 3H). LRMS [M+H]⁺ = 679.3.

Example 48

Synthesis of (S)-2-amino-6-(5-(4-(3-((2-amino-4-((S)-1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanamide)hexanoic acid (C-48)

[0365]



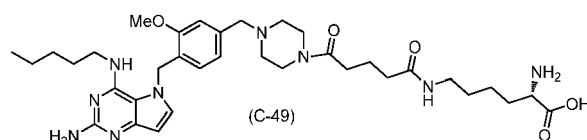
[0366] A round bottom flask was charged with (S)-2,5-dioxopyrrolidin-1-yl 5-(4-(3-((2-amino-4-((1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-47 (1.0 eq),

Boc-Lys-OH (2.0 eq), DIEA (5.0 eq) and DMF (30 mM). The reaction was stirred at room temperature for 16 hours and the volatiles were removed in vacuo. The crude reaction mixture was purified using RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to obtain (S)-6-(5-(4-(3-((2-amino-4-(((S)-1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)-2-((tert-butoxycarbonyl)amino)hexanoic acid LCMS [M+1] = 810.5. (S)-6-(5-(4-(3-((2-amino-4-(((S)-1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)-2-((tert-butoxycarbonyl)amino)hexanoic acid was treated with 30% TFA by volume in 0.1 M DCM and the volatiles removed in vacuo to obtain (S)-2-amino-6-(5-(4-(3-((2-amino-4-(((S)-1-hydroxyhexan-2-yl)amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)hexanoic acid (C-48) as a solid as the TFA salt: ¹H NMR (CD₃OD): δ 7.49 (m, 2H), 7.21 (d, 1H), 6.77 (s, 1H), 6.29 (d, 1H), 5.68 (d, 1H), 5.50 (d, 1H), 4.36 (m, 1H), 4.20 (m, 2H), 3.99 (s, 3H), 3.93 (m, 1H), 3.76 (m, 2H), 3.50 (m, 2H), 3.19 (m, 4H), 2.44 (t, 2H), 2.24 (t, 2H), 2.16 (m, 4H), 1.88 (m, 4H), 1.51 (m, 2H), 1.25 (m, 6H), 1.03 (m, 2H), 0.84 (t, 3H). LRMS [M+H] = 710.3.

Example 49

Synthesis of (S)-2-amino-6-(5-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)hexanoic acid (C-49)

[0367]

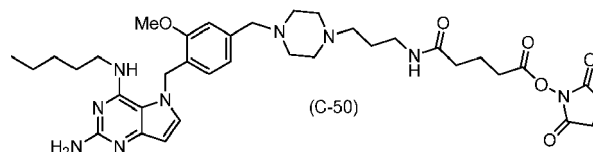


(S)-2-amino-6-(5-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)hexanoic acid (C-49) was prepared following a procedure similar to Example 48, except Compound (C-46) was used in place of Compound (C-47), to afford (S)-2-amino-6-(5-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)hexanoic acid (C-49) as a solid as the TFA salt: ¹H NMR (CD₃OD): δ 7.37 (d, 1H), 7.22 (d, 1H), 7.01 (d, 1H), 6.78 (d, 1H), 6.23 (s, 1H), 5.56 (s, 2H), 4.07 (m, 2H), 3.95 (s, 3H), 3.79 (m, 1H), 3.73 (m, 2H), 3.55 (m, 2H), 2.98 (m, 4H), 2.43 (t, 2H), 2.23 (t, 2H), 2.04 (m, 4H), 1.89 (m, 4H), 1.54 (m, 6H), 1.30 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LRMS [M+H] = 680.4.

Example 50

Synthesis of 2,5-dioxopyrrolidin-1-yl 5-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)amino)-5-oxopentanoate (C-50)

[0369]

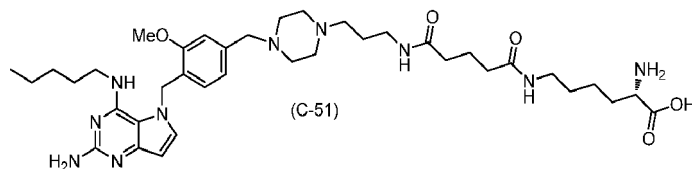


2,5-dioxopyrrolidin-1-yl 5-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)amino)-5-oxopentanoate (C-50) was prepared following a procedure similar to Example 46, except Compound (C-19) was used in place of Compound (Int-1), to afford 2,5-dioxopyrrolidin-1-yl 5-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)amino)-5-oxopentanoate (C-50) as a solid as the TFA salt: ¹H NMR (DMSO): δ 8.00 (s, 1H), 7.40 (m, 4H), 7.02 (s, 1H), 6.82 (s, 1H), 6.55 (d, 1H), 6.21 (d, 1H), 5.53 (s, 2H), 3.83 (m, 5H), 3.00 (m, 8H), 2.81 (m, 4H), 2.69 (m, 2H), 2.19 (m, 2H), 1.84 (m, 2H), 1.75 (m, 4H), 1.45 (m, 2H), 1.22 (m, 4H), 1.09 (m, 4H), 0.80 (t, 3H). LRMS [M+H] = 706.4.

Example 51

Synthesis of (S)-2-amino-6-(5-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)amino)-5-oxopentanamido)hexanoic acid (C-51)

[0371]

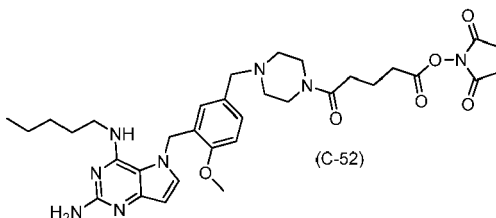


[0372] (S)-2-amino-6-(5-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)amino)-5-oxopentanamido)hexanoic acid (C-51) was prepared following a procedure similar to Example 48, except Compound (C-50) was used in place of Compound (C-47), to afford (S)-2-amino-6-(5-((3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)propyl)amino)-5-oxopentanamido)hexanoic acid (C-51) as a solid as the TFA salt: ^1H NMR (CD_3OD): δ 7.35 (d, 1H), 7.12 (s, 1H), 6.94 (d, 1H), 6.75 (d, 1H), 6.22 (s, 1H), 5.52 (s, 2H), 3.92 (s, 3H), 3.86 (t, 1H), 3.71 (s, 2H), 3.54 (, m, 2H), 3.22 (m, 8H), 3.05 (m, 2H), 2.82 (m, 2H), 2.21 (m, 4H), 1.89 (m, 4H), 1.53 (m, 6H), 1.30 (m, 4H), 1.18 (m, 2H), 0.88 (t, 3H). LRMS $[\text{M}+\text{H}] = 737.4$.

Example 52

Synthesis of 2,5-dioxopyrrolidin-1-yl 5-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-52)

[0373]

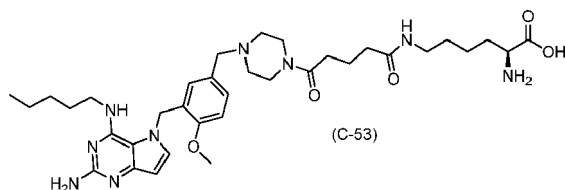


[0374] 2,5-dioxopyrrolidin-1-yl 5-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-52) was prepared following a procedure similar to Example 46, except Compound (Int-3) was used in place of Compound (Int-1), to afford 2,5-dioxopyrrolidin-1-yl 5-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-52) as a solid as the TFA salt: LRMS $[\text{M}+\text{H}] = 649.4$.

Example 53

Synthesis of (S)-2-amino-6-(5-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)hexanoic acid (C-53)

[0375]

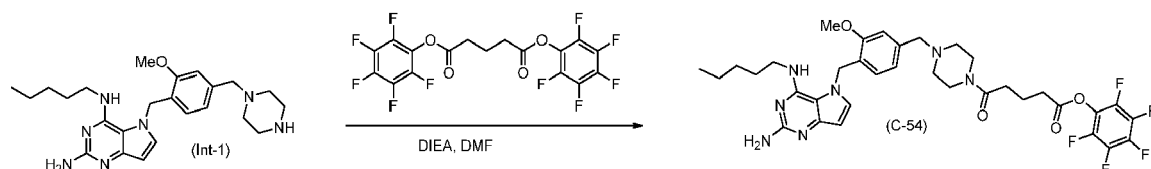


[0376] (S)-2-amino-6-(5-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)hexanoic acid (C-53) was prepared following a procedure similar to Example 48, except Compound (C-52) was used in place of Compound (C-47), to afford S)-2-amino-6-(5-(4-(3-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-4-methoxybenzyl)piperazin-1-yl)-5-oxopentanamido)hexanoic acid (C-53) as a solid as the TFA salt: ^1H NMR (DMSO): δ 8.22 (s, 3H), 7.79 (t, 1H), 7.51 (s, 2H), 7.42 (m, 2H), 7.27 (t, 1H), 7.17 (d, 1H), 6.61 (s, 1H), 6.23 (d, 1H), 5.57 (s, 2H), 4.05 (m, 2H), 3.87 (s, 5H), 3.42 (m, 3H), 3.02 (m, 3H), 2.89 (m, 2H), 2.31 (t, 2H), 2.09 (t, 2H), 1.72 (m, 4H), 1.41 (m, 5H), 1.22 (m, 2H), 1.07 (m, 2H), 0.83 (t, 3H). LRMS $[\text{M}+\text{H}] = 680.4$.

Example 54

Synthesis of perfluorophenyl 5-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-54)

[0377]



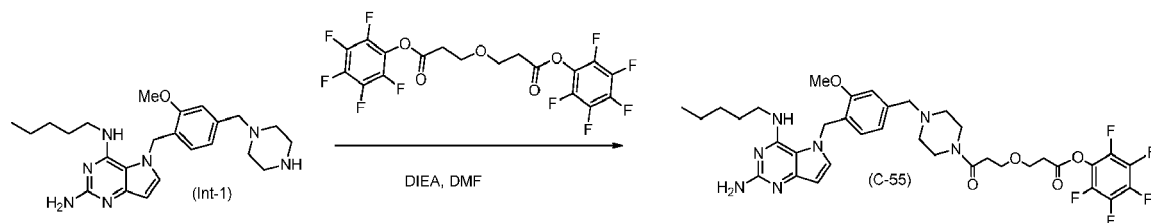
[0378] A round-bottom flask was charged with 5-(2-methoxy-4-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), DIEA (3.0 equiv.), bis(perfluorophenyl) glutarate (2.0 equiv.), and DMF (0.01 M). The reaction was stirred at room temperature for 2 hours and then the crude reaction mixture was purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H_2O , C18 column) yielding perfluorophenyl 5-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-5-oxopentanoate (C-54) as a solid as the TFA salt. LCMS $[\text{M}+1] = 718.4$.

[0379] Note: Bis(perfluorophenyl) glutarate was prepared by glutamyl dichloride (1.0 equiv.), THF (0.15 M) and triethylamine (2.2 equiv.) to a round bottom flask and cooling the reaction mixture to 0°C . A solution of 2,3,4,5,6-pentafluorophenol (2.1 equiv.) in THF (1.2 M) was then added slowly. The reaction mixture was stirred for 2 hours at room temperature. The mixture was filtered through silica gel and then concentrated in vacuo. The residue was purified by silica gel column eluted with hexane-ethyl acetate (9:1) and concentrated to give bis(perfluorophenyl) glutarate as solid. LCMS $[\text{M}+23] = 487.2$.

Example 55

Synthesis of perfluorophenyl 3-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)propanoate (C-55)

[0380]

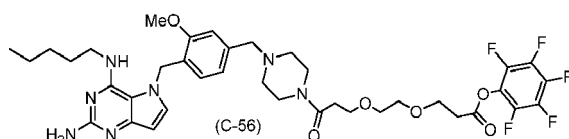


[0381] Perfluorophenyl 3-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)propanoate (C-55) was prepared as a solid as the TFA salt following a procedure similar to Example 54, except bis(perfluorophenyl) 3,3'-oxydipropoate was used in place of bis(perfluorophenyl) glutarate. ¹H NMR (Acetonitrile-*d*₃) δ 7.33 (d, 1H), 7.30 (d, 1H), 6.95 (d, 1H), 6.73 (d, 1H), 6.22 (d, 1H), 6.06 (m, 1H), 5.43 (s, 2H), 4.18 (s, 2H), 3.92 (s, 3H), 3.81 (t, 2H), 3.74 (t, 2H), 3.47 (m, 2H), 2.95 (t, 2H), 2.60 (t, 2H), 2.14 (d, 2H), 1.45 (m, 2H), 1.28 (m, 2H), 1.15 (m, 2H), 0.87 (t, 3H). LRMS [M+H]⁺ = 748.4. ¹⁹F NMR (471 MHz, Acetonitrile-*d*₃) δ -154.71 (d, 2F), -160.40 (d, 1F), -164.57 (dd, 2F).

Example 56

Synthesis of perfluorophenyl 3-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)propanoate (C-56)

[0382]

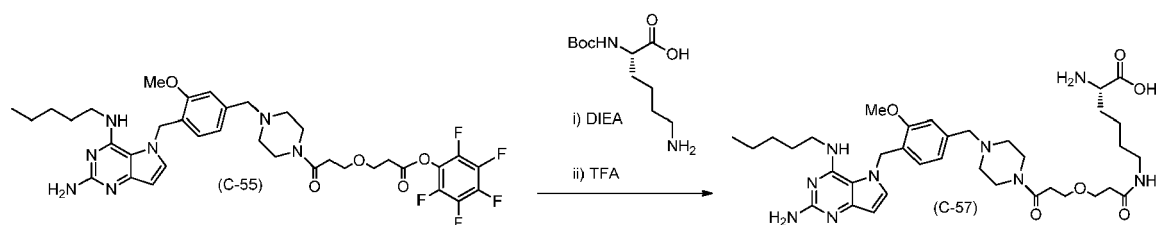


[0383] 3-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)propanoate (C-56) was prepared following a procedure similar to Example 54, except bis(perfluorophenyl) 3,3'-(ethane-1,2-diylbis(oxy))dipropoate was used in place of bis(perfluorophenyl) glutarate to obtain 3-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethoxy)propanoate (C-54). LRMS [M+H]⁺ = 792.4.

Example 57

Synthesis of (S)-2-amino-6-(3-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)propanamido)hexanoic acid (C-57)

[0384]

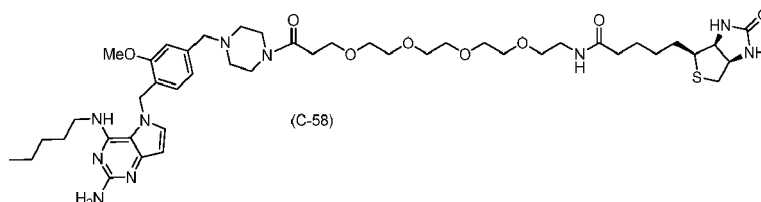


[0385] A round bottom flask was charged with perfluorophenyl 3-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)propanoate (C-55, 1.0 equiv.), Boc-Lys-OH (2.0 equiv.), DIEA (5.0 equiv.) and DMF (30 mM). The reaction was stirred at room temperature for 16 hours and the volatiles were removed in vacuo. The crude reaction mixture was purified using RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to obtain (S)-6-(3-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)propanamido)-2-((tert-butoxycarbonyl)amino)hexanoic acid. LCMS [M+1]⁺ = 810.5. The boc protected compound was treated with 30% TFA by volume in 0.1M DCM and then the volatiles removed in vacuo to obtain (S)-2-amino-6-(3-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)propanamido)hexanoic acid (C-57) as a solid as the TFA salt: ¹H NMR (DMSO): δ 8.18 (m, 3H), 7.80 (s, 1H), 7.41 (m, 4H), 7.18 (s, 1H), 6.94 (d, 1H), 6.59 (d, 1H), 6.22 (d, 1H), 5.56 (s, 2H), 4.24 (m, 1H), 3.86 (m, 7H), 3.56 (m, 4H), 3.44 (m, 4H), 3.01 (m, 4H), 2.60 (m, 2H), 2.28 (m, 2H), 1.74 (m, 2H), 1.45 (m, 2H), 1.38 (m, 3H), 1.21 (m, 3H), 1.09 (m, 2H), 0.80 (t, 3H). LCMS [M+1]⁺ = 710.5.

Example 58

Synthesis of N-(15-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-15-oxo-3,6,9,12-tetraoxapentadecyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (C-58)

[0386]

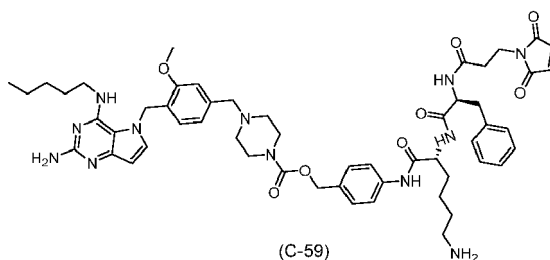


[0387] N-(15-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-15-oxo-3,6,9,12-tetraoxapentadecyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (C-58) was prepared following a procedure similar to Example 46, except 2,5-dioxopyrrolidin-1-yl 17-oxo-21-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-4,7,10,13-tetraoxa-16-azahenicosan-1-oate was used in place of disuccinimidyl glutarate, to afford N-(15-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-15-oxo-3,6,9,12-tetraoxapentadecyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (C-58) as a solid as the TFA salt: ^1H NMR (DMSO): δ 7.84 (m, 2H), 7.42 (m, 4H), 7.22 (m, 1H), 6.94 (d, 1H), 6.56 (d, 1H), 6.42 (s, 1H), 6.37 (s, 1H), 6.22 (s, 1H), 5.57 (s, 2H), 4.29 (m, 2H), 4.11 (m, 2H), 3.86 (s, 3H), 3.60 (m, 4H), 3.48 (m, 16H), 3.37 (m, 4H), 3.16 (m, 4H), 3.08 (m, 2H), 2.80 (m, 1H), 2.56 (m, 2H), 2.05 (m, 2H), 1.58 (m, 1H), 1.45 (m, 5H), 1.23 (m, 4H), 1.07 (m, 2H), 0.80 (t, 3H). LRMS $[\text{M}+\text{H}] = 911.6$.

Example 59

Synthesis of 4-((R)-6-amino-2-((S)-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-3-phenylpropanamido)hexanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (C-59)

[0388]

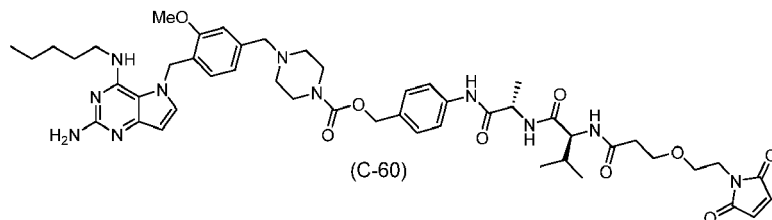


[0389] 4-((R)-6-amino-2-((S)-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-3-phenylpropanamido)hexanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (C-59) was prepared as a solid as the TFA salt according to the scheme shown for Example (C-30), except (9H-fluoren-9-yl)methyl ((S)-1-(((R)-6-amino-1-((4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxohexan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate was used in place of (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-(((S)-1-((4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate in the first step: ^1H NMR (CD_3OD): δ 8.26 (d, 1H), 7.91 (t, 1H), 7.61 (d, 2H), 7.35 (m, 3H), 7.25 (m, 3H), 7.19 (m, 3H), 7.03 (d, 1H), 6.79 (d, 1H), 6.76 (s, 2H), 6.24 (d, 1H), 5.57 (s, 2H), 5.11 (s, 2H), 4.41 (m, 1H), 4.33 (s, 2H), 3.98 (t, 1H), 3.95 (s, 3H), 3.70 (m, 3H), 3.54 (t, 2H), 3.24 (m, 4H), 3.10 (m, 1H), 3.02 (m, 1H), 2.83 (m, 1H), 2.47 (t, 2H), 1.92 (m, 2H), 1.52 (m, 4H), 1.42 (m, 2H), 1.30 (m, 3H), 1.18 (m, 2H), 0.88 (t, 3H). LRMS $[\text{M}+\text{H}] = 1013.5$.

Example 60

Synthesis of 4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)propanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (C-60)

[0390]

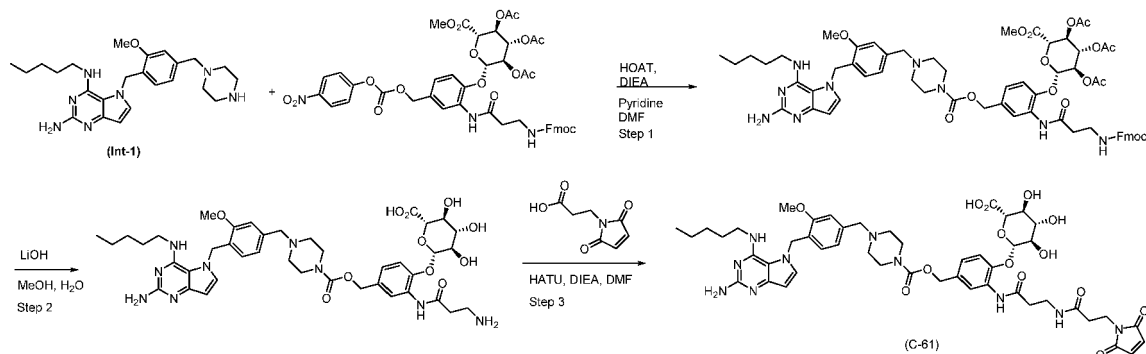


[0391] 4-((S)-2-((S)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)-3-methylbutanamido)propanamido)benzyl 4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxylate (C-60) was prepared as a solid as the TFA salt according to the scheme shown for Example (C-30), except (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-((S)-1-((4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxobutan-2-yl)carbamate was used in place of (9H-fluoren-9-yl)methyl ((S)-3-methyl-1-((S)-1-((4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate in the first step: ^1H NMR (CD_3OD): δ 9.65 (s, 1H), 8.20 (d, 1H), 7.97 (d, 1H), 7.60 (m, 2H), 7.34 (m, 2H), 7.31 (s, 1H), 7.22 (d, 1H), 7.03 (d, 1H), 6.80 (m, 2H), 6.77 (s, 2H), 6.23 (d, 1H), 5.57 (s, 2H), 5.11 (s, 2H), 4.48 (t, 1H), 4.31 (s, 3H), 4.15 (t, 1H), 3.95 (m, 4H), 3.68 (m, 4H), 3.62 (m, 2H), 3.53 (m, 8H), 2.49 (t, 2H), 2.11 (m, 1H), 1.52 (m, 2H), 1.44 (d, 3H), 1.28 (m, 2H), 1.18 (m, 2H), 0.98 (m, 6H), 0.87 (t, 3H). LRMS $[M+H] = 952.6$.

Example 61

Synthesis of (2S,3S,4S,5R,6S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxyl)oxy)methyl)-2-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (C-61)

[0392]



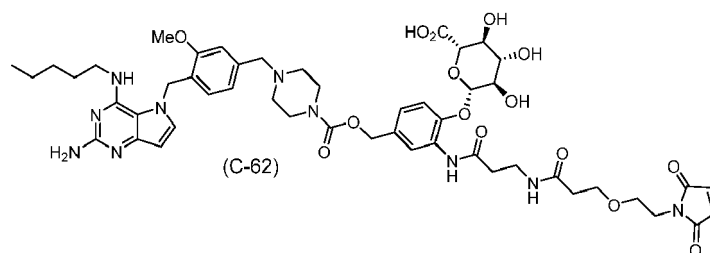
[0393] Step 1: A round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), HOAT (2.0 equiv.), Huenig's base (14.0 equiv.), (3S,4R,5R,6R)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (1.2 equiv.), and pyridine:DMF (1:4, 0.015 M). The reaction mixture was stirred at room temperature for 4 hours. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H_2O , C18 column) to afford (3S,4R,5R,6R)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carboxyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate as a solid: LCMS $[M+H] = 1212.4$.

[0394] Step 2: (3S,4R,5R,6R)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (1.0 equiv.) was dissolved in MeOH, THF and water (2:1:0.4) (0.005 M). LiOH (8.0 equiv.) was then added and the reaction was stirred at room temperature for 2 hours. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford (2R,3R,4R,5S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-aminopropanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid as a solid: LCMS [M+H] = 850.4.

[0395] Step 3: A round bottom flask was charged with (2R,3R,4R,5S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-aminopropanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (1.0 equiv.), 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid (2.0 equiv.), Huenig's base (6.0 equiv.), HBTU (1.8 equiv.) and DMF (0.003 M). The reaction was kept stirring at room temperature for 15 minutes. The reaction mixture was stirred at room temperature for 2 hours. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford (2S,3S,4S,5R,6S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (C-61) as a solid as the TFA salt: LCMS [M+H] = 1001.3.

Example 62

Synthesis of (2S,3S,4S,5R,6S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (C-62)

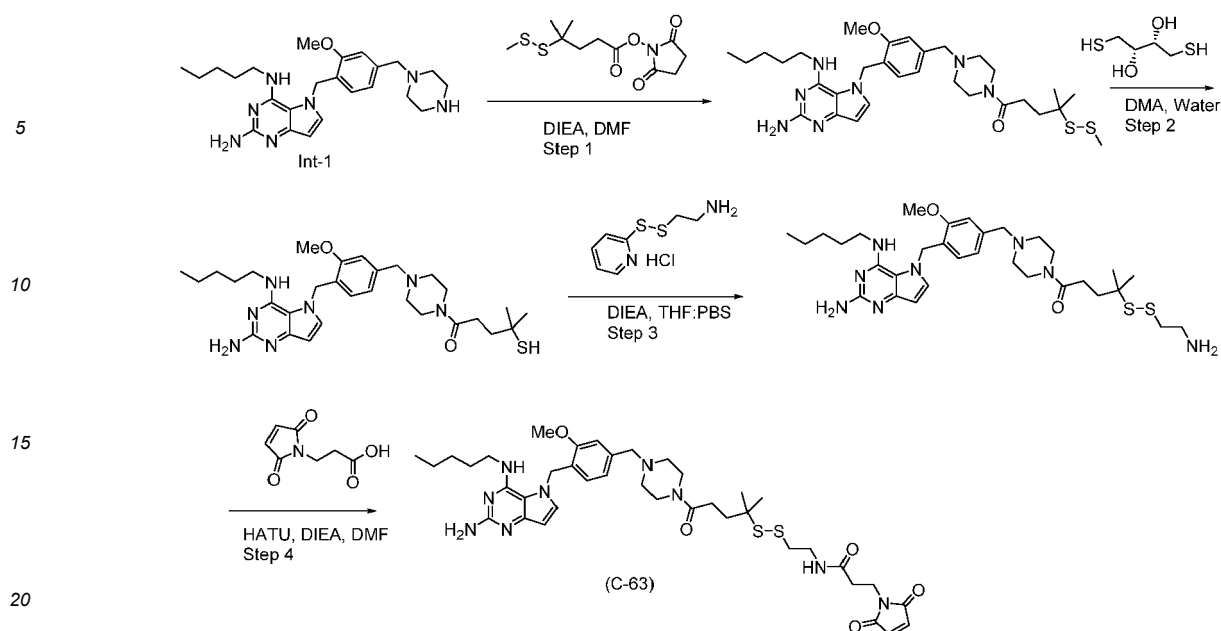


[0397] (2S,3S,4S,5R,6S)-6-(4-(((4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-carbonyl)oxy)methyl)-2-(3-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (C-62) was prepared as a solid as the TFA salt according to the scheme shown for Example (C-61), except 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoic acid was used in place of 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid in the last step: ¹H NMR (CD₃OD): δ 8.20 (d, 1H), 7.37 (d, 1H), 7.21 (m, 2H), 7.05 (m, 1H), 6.99 (d, 1H), 6.78 (m, 3H), 6.23 (d, 1H), 5.55 (s, 2H), 5.09 (s, 2H), 3.92 (m, 4H), 4.81 (d, 1H), 4.00 (s, 2H), 3.94 (s, 3H), 3.89 (d, 1H), 3.62 (m, 9H), 3.53 (m, 8H), 2.90 (m, 3H), 2.66 (t, 2H), 2.37 (t, 2H), 1.51 (m, 2H), 1.29 (m, 2H), 1.17 (m, 2H), 0.87 (t, 3H). LRMS [M+H] = 1045.4.

Example 63

Synthesis of N-(2-(((5-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazine-1-yl)-2-methyl-5-oxopentan-2-yl)disulfany)ethyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide (C-63)

[0398]



[0399] Step 1: A round bottom flask was charged with 5-(2-methoxy-5-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), 2,5-dioxopyrrolidin-1-yl 4-methyl-4-(methylthio)pentanoate (1.3 equiv.), Huenig's base (20.0 equiv.), and DMF (0.03 M). The reaction mixture was stirred at room temperature for 2 hours. The crude reaction mixture was then purified using RP-C18 ISCO (ACN:H₂O, with TFA as modifier) and then lyophilized to give 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-4-methyl-4-(methylthio)pentan-1-one as a solid as the TFA salt: LCMS [M+H] = 614.3.

[0400] Step 2: A round bottom flask was charged with 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-4-methyl-4-(methylthio)pentan-1-one (1.0 equiv.), (2S,3S)-1,4-dimercaptobutane-2,3-diol (1.0 equiv.), and dimethyl acetamide:H₂O (1:1, 0.03 M). The reaction mixture was stirred at room temperature for 2 hours. The crude reaction mixture was then purified using RP-C18 ISCO (ACN:H₂O, with TFA as modifier) and then lyophilized to give 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-4-mercapto-4-methylpentan-1-one as a solid as the TFA salt: LCMS [M+H] = 568.3.

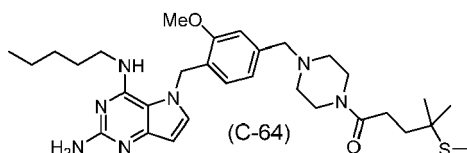
[0401] Step 3: A round bottom flask was charged with 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-4-mercapto-4-methylpentan-1-one (1.0 equiv.), 2-(pyridin-2-yl)disulfanylethan-1-amine HCl salt (2.0 equiv.), Huenig's base (10.0 equiv.), and THF:PBS (1:1, 0.03 M). The reaction mixture was stirred at room temperature for 15 minutes. The crude reaction mixture was then purified using RP-C18 ISCO (ACN:H₂O, with TFA as modifier) and then lyophilized to give 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-4-((2-aminoethyl)disulfanyl)-4-methylpentan-1-one as a solid as the TFA salt: LCMS [M+H] = 643.4.

[0402] Step 4: A round bottom flask was charged with 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-4-((2-aminoethyl)disulfanyl)-4-methylpentan-1-one (1.0 equiv.), 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid (1.0 equiv.), Huenig's base (5.0 equiv.), HATU (1.0 equiv.) and DMF (0.02 M). The reaction mixture was stirred at room temperature for 2 hours. The crude reaction mixture was then purified using RP-C18 ISCO (ACN:H₂O, with TFA as modifier) and then lyophilized to give N-(2-((5-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-methyl-5-oxopentan-2-yl)disulfanyl)ethyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide (C-63) as a solid as the TFA salt: ¹H NMR (CD₃OD): δ 7.37 (d, 1H), 7.26 (d, 1H), 7.08 (m, 1H), 6.83 (d, 1H), 6.81 (s, 2H), 6.24 (d, 1H), 5.58 (s, 2H), 4.37 (s, 2H), 4.20 (br, 4H), 3.97 (s, 3H), 3.75 (t, 2H), 3.55 (t, 2H), 3.38 (m, 2H), 3.38 (br, 4H), 2.72 (t, 2H), 2.55 (m, 2H), 2.45 (t, 2H), 1.89 (m, 2H), 1.54 (m, 2H), 1.31 (m, 8H), 1.19 (m, 2H), 0.88 (t, 3H). LRMS [M+H] = 794.4.

Example 64

Synthesis of 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-4-methyl-4-(methylthio)pentan-1-one (C-64)

[0403]

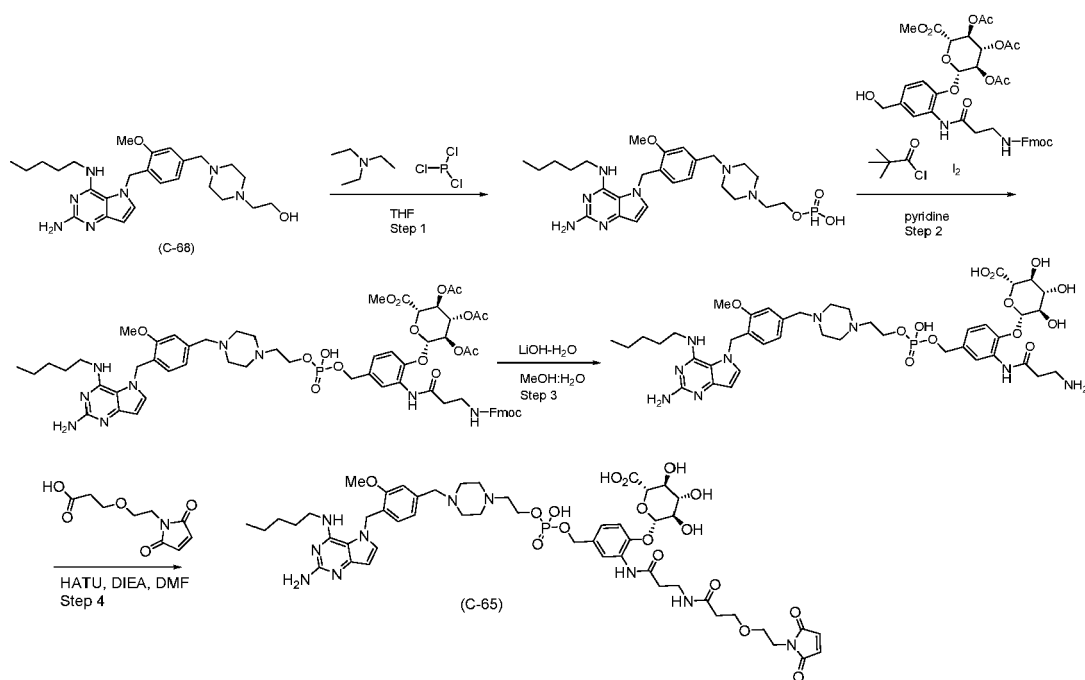


[0404] 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-4-methyl-4-(methylthio)pentan-1-one (C-63) was prepared following the procedure described for intermediate Int-1, except using 4-methyl-4-(methylthio)-1-(piperazin-1-yl)pentan-1-one in place of tert-butyl piperazine-1-carboxylate in step 3. The crude reaction mixture was purified using RP-C18 ISCO (ACN:H₂O, with TFA as modifier) and then lyophilized to give 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-4-methyl-4-(methylthio)pentan-1-one (C-64) as a solid as the TFA salt: ¹H NMR (CD₃OD): δ 7.36 (d, 1H), 7.25 (d, 1H), 7.05 (m, 1H), 6.81 (d, 1H), 6.24 (d, 1H), 5.58 (s, 2H), 4.34 (s, 2H), 3.90 (br, 4H), 3.96 (s, 3H), 3.55 (t, 2H), 3.28 (br, 4H), 2.55 (m, 2H), 1.95 (s, 3H), 1.80 (m, 2H), 1.54 (m, 2H), 1.31 (m, 2H), 1.27 (s, 6H), 1.19 (m, 2H), 0.88 (t, 3H). LRMS [M+H] = 582.4.

Example 65

Synthesis of (2S,3S,4S,5R,6S)-6-(4-((((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)(hydroxy)phosphoryl)oxy)methyl)-2-(3-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (C-65)

[0405]



[0406] Step 1: A round bottom flask was charged with 2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethan-1-ol (C-68) (1.0 equiv.), trichlorophosphine (3.0 equiv.), triethylamine (9.0 equiv.), and THF (0.2 M) at 0°C and allowed to stir for 1 h. The reaction was then quenched by the slow addition of ice-water and washed with EtOAc 3x. The aqueous layer containing the desired product was then lyophilized. 2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl hydrogen phosphonate was isolated and used in the next step without further purification: LCMS [M+H] = 546.3.

[0407] Step 2: A round bottom flask was charged with (2S,3R,4S,5S,6S)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (1.0 equiv.), 2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethyl hydrogen phosphonate (2.0 equiv.), pivaloyl chloride (42.0 equiv.), and pyridine (0.03 M). The reaction mixture was stirred at room temperature for 2 hours. At this point diiodide (1.06 equiv.) in pyridine:H₂O (1:0.1, 0.14 M) was added

and the mixture stirred for 10 min. The crude reaction mixture was then purified using RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to obtain (2S,3R,4S,5S,6S)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)(hydroxy)phosphoryl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate as a solid as the TFA salt: LCMS [M+H] = 1292.5.

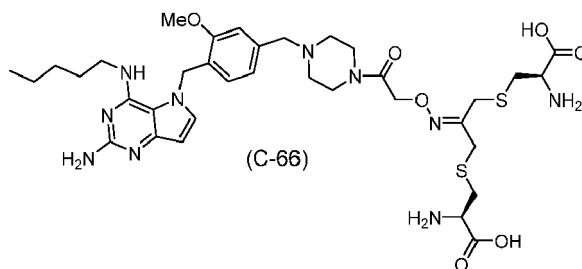
[0408] Step 3: A round bottom flask was charged with (2S,3R,4S,5S,6S)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)(hydroxy)phosphoryl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (1.0 equiv.), lithium hydroxide-H₂O (10.0 equiv.) and MeOH:H₂O (3:1.5, 0.007 M). The reaction mixture was stirred at room temperature for 2 hours. The crude reaction mixture was then purified using RP-C18 ISCO (ACN:H₂O, with TFA as modifier) and then lyophilized to give (2S,3S,4S,5R,6S)-6-(4-(((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)(hydroxy)phosphoryl)oxy)methyl)-2-(3-aminopropanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid as a solid as the TFA salt: LC-MS [M+H] = 930.4.

[0409] Step 4: A round bottom flask was charged with (2S,3S,4S,5R,6S)-6-(4-(((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)(hydroxy)phosphoryl)oxy)methyl)-2-(3-aminopropanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (1.0 equiv.), 3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanoic acid (1.0 equiv.), Huenig's base (6.0 equiv.), HATU (1.0 equiv.) and DMF (0.005 M). The reaction was kept stirring at room temperature for 15 minutes. The crude reaction mixture was then purified by RP-HPLC (0.035% TFA in ACN:0.05% TFA in H₂O, C18 column) to afford (2S,3S,4S,5R,6S)-6-(4-(((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethoxy)(hydroxy)phosphoryl)oxy)methyl)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)propanamido)propanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (C-65) as a solid as the TFA salt: ¹H NMR (CD₃OD): δ 8.19 (s, 1H), 7.37 (d, 1H), 7.14 (m, 3H), 6.79 (s, 2H), 6.77 (d, 1H), 6.22 (d, 1H), 5.53 (s, 2H), 4.86 (s, 2H), 4.84 (d, 1H), 4.08 (s, 2H), 3.95 (d, 1H), 3.92 (s, 3H), 4.00 (br, 4H), 3.76 (s, 2H), 3.62 (m, 5H), 3.53 (m, 10H), 3.27 (m, 2H), 2.85 (m, 4H), 2.63 (m, 2H), 2.37 (t, 2H), 1.52 (m, 2H), 1.31 (m, 2H), 1.17 (m, 2H), 0.88 (t, 3H). LRMS [M+H/2Z] = 563.4.

Example 66

Synthesis of (2R,2'R)-3,3'-((2-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-oxoethoxy)imino)propane-1,3-diyl)bis(sulfanediy))bis(2-aminopropanoic acid) (C-66)

[0410]

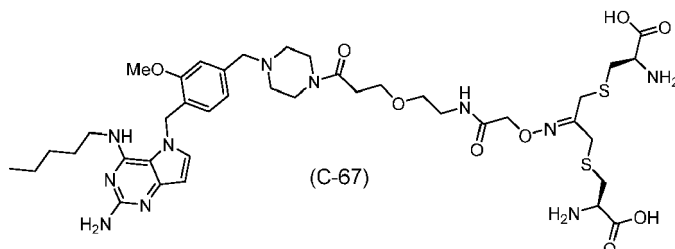


[0411] A round bottom flask was charged with 1-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-(aminooxy)ethan-1-one (C-35) (2.4 equiv.), (2R,2'R)-3,3'-((2-oxopropane-1,3-diyl)bis(sulfanediy))bis(2-aminopropanoic acid) (1.0 equiv.), and ethanol (0.02 M). The reaction mixture was stirred at room temperature for 30 min. The crude reaction mixture was purified using RP-C18 ISCO (ACN:H₂O, with TFA as modifier) and then lyophilized to give (2R,2'R)-3,3'-((2-((2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-2-oxoethoxy)imino)propane-1,3-diyl)bis(sulfanediy))bis(2-aminopropanoic acid) (C-66) as a solid: ¹H NMR (CD₃OD): δ 7.35 (d, 1H), 7.28 (d, 1H), 7.05 (m, 1H), 6.80 (d, 1H), 6.23 (d, 1H), 5.57 (s, 2H), 4.32 (s, 2H), 4.20 (m, 1H), 4.05 (m, 1H), 3.94 (s, 3H), 3.81 (m, 4H), 3.55 (m, 2H), 3.44 (m, 2H), 3.20 (m, 4H), 2.96 (m, 1H), 2.88 (m, 1H), 1.53 (m, 2H), 1.31 (m, 2H), 1.18 (m, 2H), 0.88 (t, 3H). LRMS [M+H] = 789.3.

Example 67

Synthesis of (R)-2-amino-6-(((R)-2-amino-2-carboxyethylthio)methyl)-17-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-10,17-dioxo-8,14-dioxo-4-thia-7,11-diazaheptadec-6-enoic acid (C-67)

[0412]

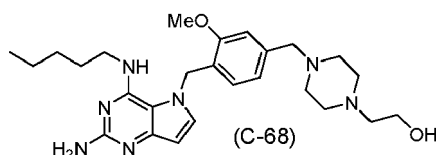


[0413] A round bottom flask was charged with N-(2-(3-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-3-oxopropoxy)ethyl)-2-(aminooxy)acetamide (C-37) (2.4 equiv.), (2R,2'R)-3,3'-((2-oxopropane-1,3-diyl)bis(sulfanediyl))bis(2-aminopropanoic acid) (1.0 equiv.), and ethanol (0.02 M). The reaction mixture was stirred at room temperature for 30 min. The crude reaction mixture was purified using RP-C18 ISCO (ACN:H₂O, with TFA as modifier) and then lyophilized to give (R)-2-amino-6-(((R)-2-amino-2-carboxyethylthio)methyl)-17-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)-10,17-dioxo-8,14-dioxo-4-thia-7,11-diazaheptadec-6-enoic acid (C-67) as a solid: ¹H NMR (CD₃OD): δ 7.36 (d, 1H), 7.29 (d, 1H), 7.07 (m, 1H), 6.80 (d, 1H), 6.24 (d, 1H), 5.57 (s, 2H), 4.57 (s, 2H), 4.31 (m, 2H), 4.11 (m, 1H), 4.03 (m, 1H), 3.95 (s, 3H), 3.86 (br, 4H), 3.73 (t, 2H), 3.54 (m, 6H), 3.40 (m, 2H), 3.20 (m, 8H), 2.96 (m, 2H), 2.67 (t, 2H), 1.52 (m, 2H), 1.30 (m, 2H), 1.19 (m, 2H), 0.88 (t, 3H). LRMS [M+H] = 904.4.

Example 68

Synthesis of 2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethan-1-ol (C-68)

[0414]



[0415] A round bottom flask was charged with 5-(2-methoxy-4-(piperazin-1-ylmethyl)benzyl)-N4-pentyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (Int-1, 1.0 equiv.), 2-bromoethan-1-ol (1.3 equiv.), triethylamine (20.0 equiv.), and acetonitrile (0.03 M). The reaction mixture was stirred at room temperature for 2 hours. The crude reaction mixture was then purified by ISCO chromatography (0 - 10% MeOH:DCM, gradient) to afford 2-(4-(4-((2-amino-4-(pentylamino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl)methyl)-3-methoxybenzyl)piperazin-1-yl)ethan-1-ol (C-68) as a solid: ¹H NMR (CD₃OD): δ 7.22 (d, 1H), 7.08 (d, 1H), 6.83 (d, 1H), 6.55 (d, 1H), 6.10 (d, 1H), 5.39 (s, 2H), 3.93 (s, 3H), 3.66 (t, 2H), 3.50 (s, 2H), 3.32 (m, 2H), 3.20 (s, 1H), 2.51 (m, 10H), 1.37 (m, 2H), 1.27 (m, 2H), 1.25 (s, 1H), 1.12 (m, 2H), 0.86 (t, 3H). LRMS [M+H] = 482.4.

Example 69

[0416] Compounds of Formula (I) were assayed to measure their activity as toll-like receptor 7 agonists.

Reporter gene assay

[0417] Human embryonic kidney 293 (HEK293) cells were stably transfected with human TLR7 and an NF-κB-driven luciferase reporter vector (pNifty-Luciferase). As a control assay, normal HEK293 transfected with pNifty-Luc were used.

Cells were cultured in DMEM supplemented with 2 mM L-glutamine, 10% heat inactivated FBS, 1% penicillin and streptomycin, 2 μ g/ml puromycin (InvivoGen #ant-pr-5) and 5 μ g/ml of blasticidin (Invitrogen #46-1120). Bright-Glo™ Luciferase assay buffer and substrate were supplied by Promega #E263B and #E264B (assay substrate and buffer respectively). 384 well clear-bottom plates were supplied by Greiner bio-one (#789163-G) and were custom bar-coded plates.

[0418] Cells were plated at 25,000 cells/well in 384-well plates in a final volume of 50 μ l of media. Cells were allowed to adhere to the plates after overnight (18 hours) culture at 37°C and 5% CO₂. Serially diluted experimental and positive control compounds were then dispensed to each well and incubated for 7 hours at 37°C and 5% CO₂. Cells stimulated with DMSO alone also serve as negative controls. After the incubation, 30 μ l of the pre-mix assay buffer and substrate buffer were added to each well according to manufacturer's instructions. The luminescence signal was read on a CLIPR machine with an integration time of 20 seconds per plate.

[0419] Dose response curves are generated for each compound and EC₅₀ values were determined as the concentration that gives 50% of the maximal signal.

Selected Assay Results

[0420] Various compounds of Formula (I), in free form or in pharmaceutically acceptable salt form, exhibit pharmacological properties, for example, as indicated by the *in vitro* tests described in this application. The EC₅₀ value in those experiments is given as that concentration of the test compound in question that provokes a response halfway between the baseline and maximum responses. In other examples, compounds of Formula (I) have EC₅₀ values in the range from 1 nM to 2 μ M. In other examples, compounds of Formula (I) have EC₅₀ values in the range from 1 nM to 1 μ M. In other examples, compounds of Formula (I) have EC₅₀ values in the range from 1 nM to 500 nM. In other examples, compounds of Formula (I) have EC₅₀ values in the range from 1 nM to 250 nM. In other examples, compounds of Formula (I) have EC₅₀ values in the range from 1 nM to 100 nM. In other examples, compounds of Formula (I) have EC₅₀ values in the range from 1 nM to 50 nM. In other examples, compounds of Formula (I) have EC₅₀ values in the range from 1 nM to 25 nM. In other examples, compounds of Formula (I) have EC₅₀ values in the range from 1 nM to 10 nM.

[0421] To illustrate the in-vitro activity of the compounds of the invention, the EC₅₀ values for TLR7 stimulation by certain compounds of Formula (I) are listed in Table 2. Cysteine adduct are thought to be putative catabolites that arise from degradation within the lysosome (Bioconjugate Chem. 2006, 17, 114-124). Certain compounds of Table 2 are the result of derivatization of the corresponding parent compound with cysteine.

Table 2

Compound Number	Human TLR7 EC ₅₀ (nM) HEK293	Compound Number	Human TLR7 EC ₅₀ (nM) HEK293
C-2	10	C-36	4
C-3	96	C-37	57
C-4	35	C-38	278
C-6	16	C-39	192
C-7	77	C-40	2101
C-8	32	C-41	52
C-10	157	C-42	1
C-12	144	C-43	6
C-14	8	C-44	2
C-16	289	C-45	11
C-18	518	C-48	1900
C-19	2	C-49	264
C-20	11	C-51	80
C-22	598	C-53	753
C-24	277	C-57	16
C-26	134	C-64	3
C-28	230	C-66	2

(continued)

Compound Number	Human TLR7 EC ₅₀ (nM) HEK293	Compound Number	Human TLR7 EC ₅₀ (nM) HEK293
C-34	585	C-67	30
C-35	5	C-68	<1

Example 70**Generation of anti-HER2-TLR7 agonist conjugates by conjugation of TLR7 agonists to specific cysteine residues of anti-HER2 antibody mutants**Preparation of anti-HER2 antibody with specific Cysteine (Cys) mutations

[0422] Preparation of anti-HER2 antibodies, e.g., trastuzumab, with site-specific cysteine mutations has been described previously in WO 2014/124316 and WO 2015/138615. Briefly, DNA encoding variable regions of the heavy and light chains of an anti-HER2 antibody, e.g., trastuzumab, were chemically synthesized and cloned into two mammalian expression vectors, pOG-HC and pOG-LC, that contain constant regions of human IgG1 and human kappa light chain. Vectors contain a CMV promoter and a signal sequence: MKTFILLWVLLWVIFLLPGATA (SEQ ID NO: 27). Oligonucleotide directed mutagenesis was employed to prepare Cys mutant constructs of the anti-HER2 antibody, and the sequences of Cys mutant constructs were confirmed by DNA sequencing.

[0423] For example, cysteine can be introduced at one or more of the following positions (all positions by EU numbering) in an anti-HER2 antibody: (a) positions 152, 360 and/or 375 of the antibody heavy chain, and (b) positions 107, 159, and/or 165 of the antibody light chain. For example, cysteine can be introduced at position 152 of the heavy chain resulting in anti-HER2 mAb4, which has a light chain sequence of SEQ ID NO: 19 and a heavy chain sequence of SEQ ID NO: 30.

[0424] Cys mutants of the anti-HER2 antibody were expressed in 293 Freestyle™ cells by co-transfecting heavy chain and light chain plasmids using transient transfection methods as described previously (Meissner, et al., Biotechnol Bioeng. 75:197-203 (2001)). The expressed antibodies were purified from the cell supernatants by standard Protein A affinity chromatography.

[0425] Similar methods were used to clone the variable regions of the heavy chain and light chain of trastuzumab into two vectors for expression in CHO cells. The heavy chain vector encodes the constant region of the human IgG1 antibody, includes a signal peptide (MPLLLLLLPLLWAGALA) (SEQ ID NO: 28), a CMV promoter to drive expression of the heavy chain, and appropriate signal and selection sequences for stable transfection into CHO cells. The light chain vector encodes the constant region of the human kappa light chain, includes a signal peptide (MSVLTQVLALLLWLTGTRC) (SEQ ID NO: 29), a CMV promoter to drive expression of the light chain, and appropriate signal and selection sequences for stable transfection into CHO cells. To produce antibodies, a heavy chain vector and a light chain vector were co-transfected into a CHO cell line. Cells underwent selection, and stably transfected cells were then cultured under conditions optimized for antibody production. Antibodies were purified from the cell supernatants by standard Protein A affinity chromatography.

[0426] Additional mutations to the constant region of the antibody vectors were made using standard mutagenesis methods.

Reduction, re-oxidation and conjugation of Cys mutant anti-HER2 antibodies to TLR7 agonists

[0427] Compounds of Formula (I) of the invention comprising a linker were conjugated to Cys residues engineered into an antibody using methods described in Junutula JR, et al., Nature Biotechnology 26:925-932 (2008).

[0428] Because engineered Cys residues in antibodies expressed in mammalian cells are modified by adducts (disulfides) such as glutathione (GSH) and/or cysteine during biosynthesis (Chen *et al.* 2009), the modified Cys as initially expressed is unreactive to thiol reactive reagents such as maleimido or bromo- acetamide or iodo-acetamide groups. To conjugate engineered Cys residues, glutathione or cysteine adducts need to be removed by reducing disulfides, which generally entails reducing all disulfides in the expressed antibody. This can be accomplished by first exposing antibody to a reducing agent such as dithiothreitol (DTT) followed by re-oxidation of all native disulfide bonds of the antibody to restore and/or stabilize the functional antibody structure. Accordingly, in order to reduce native disulfide bonds and disulfide bond between the cysteine or GSH adducts of engineered Cys residue(s), freshly prepared DTT was added to previously purified Cys mutants of trastuzumab, to a final concentration of 10 mM or 20 mM. After antibody incubation with DTT at 37°C for 1 hour, mixtures were dialyzed against PBS for three days with daily buffer exchange

to remove DTT and re-oxidize native disulfide bonds. The re-oxidation process was monitored by reverse-phase HPLC, which is able to separate antibody tetramer from individual heavy and light chain molecules. Reactions were analyzed on a PRLP-S 4000A column (50 mm x 2.1 mm, Agilent) heated to 80°C and column elution was carried out by a linear gradient of 30-60% acetonitrile in water containing 0.1% TFA at a flow rate of 1.5 ml/min. The elution of proteins from the column was monitored at 280 nm. Dialysis was allowed to continue until reoxidation was complete. Reoxidation restores intra-chain and interchain disulfides, while dialysis allows cysteines and glutathiones connected to the newly-introduced Cys residue(s) to dialyze away.

[0429] After re-oxidation, maleimide-containing compounds were added to re-oxidized antibodies in PBS buffer (pH 7.2) at ratios of typically 1.5:1, 2:1, or 5:1 to engineered Cys, and incubations were carried out for 1 hour. Typically, excess free compound was removed by purification over Protein A resin by standard methods followed by buffer exchange into PBS.

[0430] Cys mutants of anti-HER2 antibody, e.g., trastuzumab, were alternatively reduced and re-oxidized using an on-resin method. Protein A Sepharose beads (1 ml per 10 mg antibody) were equilibrated in PBS (no calcium or magnesium salts) and then added to an antibody sample in batch mode. A stock of 0.5 M cysteine was prepared by dissolving 850 mg of cysteine HCl in 10 ml of a solution prepared by adding 3.4 g of NaOH to 250 ml of 0.5 M sodium phosphate pH 8.0 and then 20 mM cysteine was added to the antibody/bead slurry, and mixed gently at room temperature for 30-60 minutes. Beads were loaded to a gravity column and washed with 50 bed volumes of PBS in less than 30 minutes, then the column was capped with beads resuspended in one bed volume of PBS. To modulate the rate of re-oxidation, 50 nM to 1 µM copper chloride was optionally added. The re-oxidation progress was monitored by removing a small test sample of the resin, eluting in IgG Elution buffer (Thermo), and analyzing by RP-HPLC as described above. Once re-oxidation progressed to desired completeness, conjugation could be initiated immediately by addition of 2-3 molar excess of compound over engineered cysteines, and allowing the mixture to react for 5-10 minutes at room temperature before the column was washed with at least 20 column volumes of PBS. Antibody conjugates were eluted with IgG elution buffer and neutralized with 0.1 volumes 0.5 M sodium phosphate pH 8.0 and buffer exchanged to PBS. Alternatively, instead of initiating conjugation with antibody on the resin, the column was washed with at least 20 column volumes of PBS, and antibody was eluted with IgG elution buffer and neutralized with buffer pH 8.0. Antibodies were then either used for conjugation reactions or flash frozen for future use.

Properties of the anti-HER2-TLR7 agonist conjugates

[0431] Antibody-TLR7 agonist conjugates were analyzed to determine extent of conjugation. A compound-to-antibody ratio was extrapolated from LC-MS data for reduced and deglycosylated samples. LC/MS allows quantitation of the average number of molecules of linker-payload (compound) attached to an antibody in a conjugate sample. HPLC separates antibody into light and heavy chains, and separates heavy chain (HC) and light chain (LC) according to the number of linker-payload groups per chain. Mass spectral data enables identification of the component species in the mixture, e.g., LC, LC+1, LC+2, HC, HC+1, HC+2, etc. From the average loading on the LC and HC chains, the average compound to antibody ratio can be calculated for an antibody conjugate. A compound-to-antibody ratio for a given conjugate sample represents the average number of compound (linker-payload) molecules attached to a tetrameric antibody containing two light chains and two heavy chains.

[0432] Conjugates were profiled using analytical size-exclusion chromatography (AnSEC) on Superdex 200 10/300 GL (GE Healthcare) and/or Protein KW-803 5 µm 300 x 8 mm (Shodex) columns; aggregation was analyzed based on analytical size exclusion chromatography. Conjugates were also profiled by analytical hydrophobic interaction chromatography (AnHIC) on a Tosoh Bioscience (King of Prussia, PA, USA) TSKgel Butyl-NPR column (100 mm x 4.6 mm, 2.5 µm) installed on an Agilent 1260 LC system (Santa Clara, CA, USA) using a binary gradient of buffer A (20 mM His-HCl, 1.5 M ammonium sulfate, pH 6.0) and buffer B (20 mM His-HCl, 15% isopropanol, pH 6.0) with samples prepared by diluting approximately 20 µg of antibody (initially in PBS) with 0.5 volume of 3 M ammonium sulfate. The hydrophobicity index is calculated against a linear regression of four standard samples of known hydrophobicity. The hydrophobicity of the largest peak by area is reported.

[0433] Most conjugates achieved high compound-to-antibody ratio, were mainly monomeric and showed low hydrophobicity (high hydrophobicity index corresponding to early elution from the HIC column). Conjugation through this method results in conjugation efficiencies of greater than 95% for most compounds (Table 3). The majority of the conjugates contain less than 4% dimeric and oligomeric material (Table 3). A hydrophobicity index (HI) of 0.80 or greater is considered a favorable characteristic. A majority of the conjugates showed HI values of greater than 0.8 (Table 3). This suggests that conjugates can be made efficiently and have favorable characteristics.

Table 3. Properties of anti-HER2-TLR7 agonist conjugates

Conjugate ^a	Conjugation efficiency (by LCMS)	Compound-to- antibody ratio ^b	Aggregation (%) ^c	Hydrophobicity Index (HI) ^d
anti-HER2 mAb2-(C-9)	98	3.9	3.2	0.90
anti-HER2 mAb2-(C-11)	98	3.9	3.4	0.88
anti-HER2 mAb2-(C-13)	98	3.9	3.1	0.84
anti-HER2 mAb2-(C-23)	98	3.9	2.9	0.87
anti-HER2 mAb2-(C-15)	98	3.9	3.2	0.89
anti-HER2 mAb2-(C-17)	98	3.9	3.6	0.87
anti-HER2 mAb2-(C-5)	95	3.8	3.8	0.91
anti-HER2 mAb2-(C-25)	98	3.9	3.0	0.90
anti-HER2 mAb2-(C-21)	95	3.8	3.1	0.87
anti-HER2 mAb2-(C-1)	98	3.9	3.2	0.88
anti-HER2 mAb2-(C-27)	95	3.8	0.5	0.89
anti-HER2 mAb2-(C-31)	88	3.5	1.1	0.87
anti-HER2 mAb2-(C-30)	88	3.5	0.6	0.75
anti-HER2 mAb3-(C-46)	n/a	1.9	0.7 ^e	0.81
anti-HER2 mAb1-(C-5)	95	3.8	3.1	0.90
anti-HER2 mAb1-(C-1)	95	3.8	2.3	0.87
anti-HER2 mAb4-(C-29)	>95	2.0	0.6	Not determined
anti-HER2 mAb3-(C-35)	90	3.6	1.1	0.90
anti-HER2 mAb3-(C-37)	88	3.5	1.9	0.87
anti-HER2 mAb3-(C-1)	n/a	7.0	0.3	0.65
anti-HER2 mAb5-(C-69)-(C-35)	>95	2.0	0.7	0.70
anti-HER2 mAb5-(C-69)-(C-37)	>95	2.0	1	0.70
anti-ratHER2-(C-47)	n/a	2.6	BLQ ^e	Not determined
anti-ratHER2-(C-50)	n/a	1.3	BLQ ^e	Not determined
anti-ratHER2-(C-46)	n/a	2.8	BLQ ^e	Not determined
anti-Her2-HC-E152C-S375C-(C-61)	>95	4	4	Not determined
anti-Her2-HC-E152C-S375C-(C-59)	95	3.8	0	Not determined
anti-Her2-HC-E152C-S375C-(C-60)	>95	4	4	Not determined
anti-Her2-HC-E152C-S375C-(C-64)	90	3.6	3	Not determined

(continued)

Conjugate ^a	Conjugation efficiency (by LCMS)	Compound-to- antibody ratio ^b	Aggregation (%) ^c	Hydrophobicity Index (HI) ^d
anti-Her2-HC-E152C- S375C-(C-62)	>95	4	0	Not determined
^a The anti-HER2 antibodies in the conjugates are: the anti-HER2 mAb1 has a LC of SEQ ID NO: 19; a HC of SEQ ID NO: 9. The anti-HER2 mAb2 has a LC of SEQ ID NO: 19; a HC of SEQ ID NO: 21. The anti-hHER2 mAb3 has a LC of SEQ ID NO: 19; a HC of SEQ ID NO: 23. The anti-HER2 mAb4 has a LC of SEQ ID NO: 19; a HC of SEQ ID NO: 30. The anti-HER2 mAb5 has a LC of SEQ ID NO: 19; a HC of SEQ ID NO: 32. ^b Compound-to-antibody ratio according to LCMS. ^c Aggregation measured by analytical size exclusion chromatography; includes dimeric and oligomeric species. BLQ = below limit of quantitation. ^d Hydrophobic Interaction Chromatography (HIC) measurements: Retention time of the peak maximum was used to calculate the hydrophobicity index. ^e Although aggregation was not observed or observed at a low level by AnSEC, late elution from the column suggests an invalid result.				

Example 71**Generation of anti-HER2-TLR7 agonist conjugates through partial reduction of native disulfide bonds of non-engineered anti-HER2 antibodies**

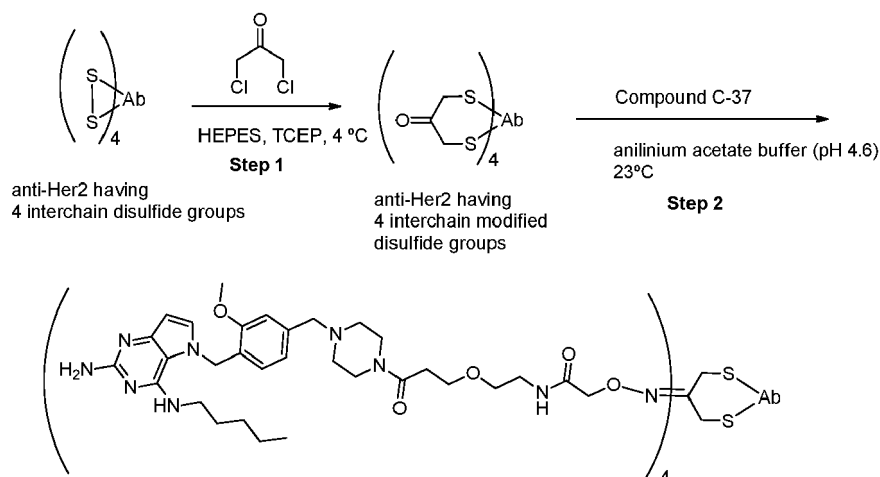
[0434] Some compounds of the invention can also be conjugated to native cysteine residues of non-engineered antibodies using a procedure that involves partial reduction of the antibodies (Doronina, S. O. et al., Nat. Biotechnol. 21, 778-784, 2003). Inter- and intra-chain disulfides bonds of anti-HER2 antibody (at a concentration of 5 to 10 mg/ml) were first partially reduced in PBS containing 2 mM EDTA by adding TCEP to a final concentration of 10 mM and incubating the mixture at 37°C for 1 hour. After desalting and addition of 1% w/v PS-20 detergent, the partially reduced antibodies (1-2 mg/ml) were reacted overnight at 4°C with 0.5 to 1 mg TLR7 agonist compound per 10 mg antibody. Resulting conjugates were purified by Protein A chromatography by standard methods and buffer exchanged to PBS, and profiled by MS, AnSEC, and AnHIC as described above. Measured compound-to-antibody ratio, aggregation behavior, and hydrophobicity data are summarized in Table 3 for one conjugate example made by reduction of anti-HER2 mAb3 followed by conjugation with Compound C-1.

Example 72**Generation of anti-HER2-TLR7 agonist conjugates using 1,3-dichloropropan-2-one to reconnect native inter-chain disulfide bonds of non-engineered anti-HER2 antibodies**

[0435] In an alternative method (United States Patent Application 20150150998), interchain disulfides bonds of a non-engineered, recombinant anti-HER2 antibody can be modified and conjugated to an agonist compound of the invention using the following two steps.

Scheme 15

[0436] Two step conjugation to native cysteine residues using 1,3 dichloropropan-2-one bridging followed by addition to the introduced ketones.



[0437] Step 1: Reduction of interchain disulfide bridges and re-bridging using 1,3-dichloropropan-2-one: TCEP·HCl (1.63 mM) was added to a solution of anti-HER2 antibody mAb3 (136 μ M) and 1,3-dichloropropan-2-one (33 mM) in 0.1 M HEPES buffer (pH 8.0) at 4°C. The resulting mixture was gently agitated at 4°C for 16 h. The reaction mixture was then buffer-exchanged into PBS using a PD-10 desalting column (GE Healthcare). The resulting solution was concentrated using a 50K Amicon filter to give the modified anti-HER2 antibody. The modification was confirmed by ESI-MS (Eluent A: water + 0.1% Formic acid; Eluent B: Acetonitrile + 0.04% Formic acid; Gradient: from 3 to 80% B in 2 minutes - Flow 1.0 ml/min. Column: Proswift Monolith 4.6*50mm 40°C); 145398 Da (after deglycosylation by PNGase F).

[0438] Step 2: Conjugation of the agonists Compound (C-37): The modified anti-HER2 antibody (30 mg/ml) was reacted with 3.0 mM Compound (C-37) comprising a linked amino-oxy moiety in 0.1 M anilinium acetate buffer (pH 4.6) at a final concentration of 15% (v/v) DMSO. The reaction mixture was incubated for approximately 16 hours at 23°C. The reaction mixture was then buffer-exchanged into PBS (pH 7.4) using a 50K Amicon filters, giving rise to the modified anti-HER2-compound conjugate.

[0439] Similar conjugates were obtained using Compound (C-35) to conjugate to the modified anti-HER2 antibody.

[0440] Conjugates were profiled by MS, AnSEC, and AnHIC as described above. The measured compound-to-antibody ratio, aggregation behavior, and hydrophobicity data are summarized in Table 3. The two example conjugates achieved high compound-to-antibody ratio, were mainly monomeric and showed low hydrophobicity (high hydrophobicity index corresponding to early elution from the HIC column). Conjugation through this method results in conjugation efficiencies of greater than 85% (Table 3). The conjugates contain less than 2% dimeric and oligomeric material (Table 3). The conjugates showed HI values of greater than 0.85 (Table 3). This suggests that conjugates can be made efficiently and have favorable characteristics.

Example 73

Generation of anti-HER2-TLR7 agonist conjugates by conjugation to native lysine residues of anti-HER2 antibody

[0441] Native antibodies can be functionalized with certain compounds of the invention through established methods. For example, anti-ratHER2 antibody (7.16.4; purchased from Bio X Cell; West Lebanon, NH) in PBS pH 7.2 at 4 mg/ml was mixed with 760 μ M of Compound C-47) with a final DMSO concentration of 20% (v/v). The reaction was incubated at room temperature overnight, and then quenched with 50 mM Tris pH 8.. Similar methods were used to make conjugates with anti-HER2 mAb3 or with agonist Compounds C-46 and C-50. The resulting antibody conjugates were purified by Protein A chromatography by standard methods and buffer exchanged to PBS.

[0442] Antibody conjugates were profiled by MS, AnSEC, and AnHIC as described above. Measured compound-to-antibody ratio, aggregation behavior, and hydrophobicity data are summarized in Table 3. Several lysine-reacted antibody conjugates show late elution and/or tailing of peaks on the AnSEC columns used, suggesting column interaction, which made detection of aggregate difficult.

Example 74

Generation of anti-HER2-TLR7 agonist conjugates using two-step conjugation of an A1-tagged anti-HER2 mutant antibody with agonist compounds containing an amino-oxy reactive group

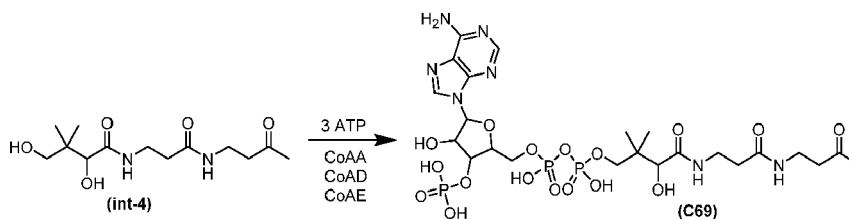
[0443] Post-translational 4'-phosphopantetheinylation is a versatile method for the site-specific labeling of recombinant proteins with structurally diverse small molecules (Yin J, et al., Proc. Natl. Acad. Sci. U.S.A. 102:15815-15820, 2005; Zhou Z, et al., ACS Chem. Biol. 2:337-346, 2007). This enzymatic approach, which is based on the catalytic action of promiscuous 4'-phosphopantetheinyl transferases (PPTases), was adopted for the preparation of highly homogeneous antibody conjugates (see WO2013184514). Enzymatic labeling is accomplished by incorporating 11 or 12-mer S6, ybbR, and A1 peptide sequences at various sites of the constant region of an antibody. For example, an A1 tag of sequence GDSLDMLEWSLM (SEQ ID NO: 31) can be incorporated after residue E388 (EU numbering) in the heavy chain of anti-HER2 mAb2 to produce anti-HER2 mAb5, which has a light chain sequence of SEQ ID NO: 19 and a heavy chain sequence of SEQ ID NO: 32. One strategy is a two-step method to prepare site-specific antibody-compound conjugates by post-translational 4'-phosphopantetheinylation (see WO2013184514). The first step of this approach is based on the PPTase-catalyzed labeling of a peptide-tagged antibody with a CoA analogue containing a bioorthogonal group, such as an azido, alkene, alkyne, ketone, or aldehyde moiety. Following affinity purification of the bioorthogonally labeled antibody, the second step of the two-step method involves the conjugation of a compound comprising a moiety reactive with the bioorthogonal group. As way of example, the following section describes the two-step method for anti-HER2 mutant antibodies containing an A1 tag insertion at a specific site within the constant region of the heavy chain. In addition, although the two-step method is exemplified for oxime ligation chemistry, this strategy can be extended to other bioorthogonal chemistries, such as click chemistry, including copper-free click chemistry, Staudinger ligation, isonitrile-based click chemistry, and tetrazine ligation.

[0444] Oxime ligation chemistry have been used by several research groups as an efficient, bioorthogonal method for the preparation of site-specific protein conjugates (Axup JY, et al., Proc Natl Acad Sci U S A. 109:16101-16106, 2012; Rabuka D, et al., Nat Protoc. 7:1052-1067, 2012). In order to combine post-translational 4'-phosphopantetheinylation with oxime ligation, a ketone-modified CoA analog was prepared chemoenzymatically from the corresponding pantothenate precursor molecule (Compound int-4) using the CoA biosynthetic enzymes CoAA, CoAD, and CoAE (Worthington AS, Burkart MD (2006) Org Biomol Chem. 4:44-46) (Kosa NM, Haushalter RW, Smith AR, Burkart MD (2012) Nat Methods 9:981-984). Next, PPTase catalysis was used to enzymatically conjugate the bioorthogonal ketone group site-specifically onto the embedded A1 tag of an anti-HER2 antibody. Specifically, 2.5 μ M of anti-HER2 mAb5 was conjugated with 30 μ M of ketone-CoA analogue (Compound C-69) (12 molar equivalents relative to the antibody) in the presence of about 0.5 μ M of AcpS PPTase from *Escherichia coli* for 2 days at 37°C in 75 mM Tris-HCl buffer (pH 8.0) supplemented with 12.5 mM MgCl₂ and 20 mM NaCl. To drive the conjugation reaction to completion, the reaction mixture was supplemented with approximately 1 μ M *B. subtilis* Sfp PPTase, while the concentration of Compound C-69 was increased to about 60 μ M. The reaction was incubated for another 4 days at room temperature. Labeling of the anti-HER2 mAb5 antibody with the ketone-CoA analogue (Compound C-69) was verified by obtaining deconvoluted ESI-MS spectra of the reduced and deglycosylated sample. The observed masses were in agreement with the calculated molecular weights of the corresponding ketone-functionalized heavy chains. After removing PPTase enzymes and excess ketone-CoA analogue by Protein A affinity chromatography (MabSelect SuRe, GE Healthcare Life Sciences), the ketone-activated antibody, anti-HER2-mAb5-(C-69) was eluted with Pierce™ IgG Elution Buffer (Thermo Fisher Scientific) followed by immediate neutralization with 1 M Tris-HCl buffer (pH 8.0). The neutralized antibody solution was buffer-exchanged into PBS and concentrated using a 50K Amicon filter.

[0445] Site-specific attachment of a ketone group enabled subsequent oxime ligation of an agonist compound to ketone-activated anti-HER2 mAb5-(C-69) as the second step of the two-step method. 48 μ M of ketone-functionalized antibody was reacted with 30-fold molar excess (1.4 mM) of the aminooxy-agonists C-35 and C-37 in 100 mM anilinium acetate buffer (pH 4.6) containing 7% (v/v) DMSO. After 17 hours of incubation at room temperature, excess aminooxy reagent was removed by ultrafiltration with a 50K Amicon filter and repeated washing with PBS. Antibody conjugates were profiled by MS, AnSEC, and AnHIC as described above. Measured compound-to-antibody ratio, aggregation behavior, and hydrophobicity data are summarized in Table 3. As shown in Table 3, the two-step method afforded near quantitative labeling of ketone-activated anti-HER2 mAb5-(C-69) with the aminooxy-agonists C-35 and C-37.

Ketone-Coenzyme A Analogue (Compound C-69)

[0446]



[0447] Compound (int-4) was converted into the ketone-functionalized CoA analog (C69) by reacting 5 mM of compound (int-4) with 25 mM of ATP in the presence of 10 μM *Staphylococcus aureus* CoAA, 25 μM *Escherichia coli* CoAD, and 20 μM *Escherichia coli* CoAE for about 16 h at 37°C in 50 mM HEPES buffer (pH 8.0) containing 20 mM MgCl_2 . After centrifugation of the reaction mixture at 20,817 x g for 2 min, soluble enzyme was separated by ultrafiltration through an Amicon Ultra centrifugal filter with 10 kDa cutoff. Enzymatic conversion of compound (i-4) into the ketone-functionalized CoA analog (C59) was verified by formation of anti-HER2 mAb5-(C-69)-(C-35) and anti-HER2 mAb5-(C-69)-(C-37) (see Table 3).

Example 75

In vitro stability testing of anti-HER2-TLR7 agonist conjugates

[0448] The stability of the bond formed between maleimide containing payloads and Cys residues of the antibody is enhanced by the hydrolysis of the succinimide ring formed in this reaction. The effects of succinimide ring hydrolysis on the stability of antibody conjugates prepared with agonist compounds of the invention were studied after *in vitro* incubation in mouse serum. Mass changes resulting from payload deconjugation and the hydrolysis of the succinimide ring of maleimide payloads conjugated to antibodies were monitored by LC-MS. The hydrolysis of the succinimide ring has been reported to be stimulated by certain conditions such as high pH, high temperature, or high salts (J. Am. Chem. Soc. 1955, 77: 3922; Biochemistry 1976, 15: 2836; Biochem. J. 1979, 179: 191-197; J Pharm Sci. 1984, 73:1767-1771; Bioorg. Med. Chem. Lett. 17: 6286-6289, 2007). To probe the *in vitro* stability of conjugates, anti-HER2 antibody mAb2 conjugates were incubated at 37°C in 50-70% mouse serum. Fifty microgram samples of conjugate were taken at each timepoint (typically 0, 8, 24, 48, and 72 hours) and flash frozen immediately. The samples were later thawed for processing and analysis. Briefly, antibodies were treated with PNGaseF to remove N-linked glycans and a proteolytic enzyme that cuts near the hinge region of the heavy chain in order to separate the Fab from the Fc before reduction with DTT to break the disulfide bonds. The light chain, heavy chain Fab, and heavy chain Fc fragments were then analyzed by ESI-MS. The relative populations of deconjugated antibody, conjugates with attached payload with hydrolyzed succinimide ring, and conjugates with attached payload with intact succinimide ring, were calculated from the relative MS intensities of the corresponding conjugate species. The extent of deconjugation and the extent of succinimide hydrolysis are shown in Tables 4 and 5 for a subset of conjugates. In general, the conjugates lose less than 13% of compound loaded during a 72 hour *in vitro* incubation and generally the succinimide ring hydrolysis is above 85% complete by 48 hours. Certain compounds of the invention, exemplified by Compound (C-5) and Compound (C-21) exhibit improved conjugate stability due to lower susceptibility to deconjugation through the reverse maleimide reaction and further stabilization through succinimide ring hydrolysis.

Table 4. Succinimide ring hydrolysis of anti-HER2-TLR7 agonist conjugates as a function of *in vitro* incubation time in mouse serum.

Conjugate ^a	% Ring opening (MS)									
	S375C Adduct					E152C Adduct				
	0	8 hr	24 hr	48 hr	72 hr	0	8 hr	24 hr	48 hr	72 hr
anti-HER2 mAb2-(C-9)	0	45	64	86	94	19	58	73	86	88
anti-HER2 mAb2-(C-11)	0	52	71	91	95	0	51	66	84	87
anti-HER2 mAb2-(C-13)	0	50	69	90	96	0	58	75	87	89

(continued)

	% Ring opening (MS)									
	S375C Adduct					E152C Adduct				
Conjugate ^a	0	8 hr	24 hr	48 hr	72 hr	0	8 hr	24 hr	48 hr	72 hr
anti-HER2 mAb2-(C-23)	20	82	93	96	96	0	60	74	86	88
anti-HER2 mAb2-(C-15)	0	81	91	95	96	0	58	71	85	87
anti-HER2 mAb2-(C-17)	17	64	81	94	96	0	59	75	88	90
anti-HER2 mAb2-(C-5)	79	93	93	93	94	51	88	87	90	88
anti-HER2 mAb2-(C-25)	18	55	73	91	95	0	61	76	87	87
anti-HER2 mAb2-(C-21)	49	97	96	96	96	26	87	91	92	92
anti-HER2 mAb2-(C-1)	0	58	76	92	96	0	82	86	86	89
anti-HER2 mAb2-(C-27)	35	90	97	98	100	27	82	90	90	90
anti-HER2 mAb2-(C-31)	23	64	90	95	97	23	48	74	85	88
anti-HER2 mAb2-(C-30)	24	59	86	97	100	25	49	76	89	90
^a The anti-HER2 mAb2 has a LC of SEQ ID NO: 19; a HC of SEQ ID NO: 21.										

Table 5. Compound-to-antibody ratio of anti-HER2-TLR7 agonist conjugates as a function of *in vitro* incubation time in mouse serum

	Compound-to-antibody ratio									
	S375C Adduct					E152C Adduct				
Conjugate ^a	0	8 hr	24 hr	48 hr	72 hr	0	8 hr	24 hr	48 hr	72 hr
anti-HER2 mAb2-(C-9)	0.93	0.88	0.84	0.82	0.80	1.00	0.92	0.91	0.87	0.87
anti-HER2 mAb2-(C-11)	0.94	0.90	0.86	0.84	0.84	1.00	0.93	0.90	0.89	0.87
anti-HER2 mAb2-(C-13)	0.93	0.88	0.85	0.82	0.80	1.00	0.94	0.91	0.88	0.86
anti-HER2 mAb2-(C-23)	0.93	0.93	0.86	0.86	0.86	1.00	0.95	0.93	0.92	0.92
anti-HER2 mAb2-(C-15)	0.93	0.88	0.87	0.86	0.85	1.00	0.95	0.93	0.90	0.90

(continued)

Compound-to-antibody ratio										
	S375C Adduct					E152C Adduct				
Conjugate ^a	0	8 hr	24 hr	48 hr	72 hr	0	8 hr	24 hr	48 hr	72 hr
anti-HER2 mAb2-(C-17)	0.94	0.85	0.86	0.84	0.84	1.00	0.92	0.91	0.87	0.88
anti-HER2 mAb2-(C-5)	0.95	0.95	0.94	0.94	0.94	1.00	0.98	0.98	0.90	0.96
anti-HER2 mAb2-(C-25)	0.92	0.89	0.87	0.85	0.85	1.00	0.96	0.95	0.93	0.92
anti-HER2 mAb2-(C-21)	0.94	0.91	0.91	0.91	0.90	1.00	0.96	0.95	0.95	0.94
anti-HER2 mAb2-(C-1)	0.93	0.86	0.83	0.81	0.79	1.00	0.97	0.96	0.96	0.96
anti-HER2 mAb2-(C-27)	0.95	0.87	0.85	0.85	0.85	0.95	0.91	0.90	0.90	0.90
anti-HER2 mAb2-(C-31)	0.94	0.88	0.84	0.81	0.80	0.85	0.93	0.88	0.87	0.85
anti-HER2 mAb2-(C-30)	0.94	0.86	0.80	0.80	0.79	0.95	0.88	0.81	0.80	0.78
^a The anti-HER2 mAb2 has a LC of SEQ ID NO: 19; a HC of SEQ ID NO: 21.										

Example 76***In vivo* testing of anti-HER2-TLR7 agonist conjugates in a N87 gastric tumor xenograft model**Materials and Methods

[0449] For N87 gastric carcinoma xenograft mouse model, female SCID-beige mice at 6-8 weeks of age (purchased from Harlan Laboratories) were used for implantation. N87 cells (obtained from ATCC, Catalog#CRL-5822, Vendor lot#7686255) were grown in sterile conditions in a 37°C incubator with 5% CO₂ for two weeks. Cells were grown in RPMI medium with 10% fetal bovine serum. Cells were passaged every 3-4 days with 0.05% Trypsin/EDTA. On the day of implantation, N87 cells were lifted (passage x4) and re-suspended in RPMI1640 serum-free media at a concentration of 1 x 10⁶ cells and 50% matrigel/100 µl. Cells were Raddi tested to assure that they are free of mycoplasma and murine viruses.

[0450] N87 cells were implanted with a subcutaneous injection into the lower flank using a 28g needle (100 µl injection volume). After implant, tumors were measured by caliper and mice weighed two times per week once tumors were palpable. Tumors then were measured twice a week in two dimensions. Caliper measurements were calculated using $(L \times W^2)/2$. Mice were fed with normal diet and housed in SPF animal facility in accordance with the Guide for Care and Use of Laboratory Animals and regulations of the Institutional Animal Care and Use Committee.

[0451] When xenograft tumors reached about 200 mm³, mice were administered by intravenous route 0.3-10 mg/kg of anti-HER2 antibody or anti-HER2-TLR7 agonist conjugate. Isotype control antibody was generated by expressing an antibody against a target not found in rodents and conjugating through similar methods described for anti-HER2 antibodies. Tumors were measured twice a week. Average tumor volumes were plotted using Prism 5 (GraphPad) software. An endpoint for efficacy studies was achieved when tumor size reached a volume of 2000 mm³. Following injection,

mice were also closely monitored for signs of clinical deterioration. If for any reason mice showed any signs of morbidity, including respiratory distress, hunched posture, decreased activity, hind leg paralysis, tachypnea as a sign for pleural effusions, weight loss approaching 20% or 15% plus other signs, or if their ability to carry on normal activities (feeding, mobility), was impaired, mice were euthanized.

Results

[0452] N87 gastric tumor xenograft mice were treated intravenously with a single dose of anti-HER2-mAb2-(C-1) conjugate, where Compound (C-1) is conjugated to Cys 152 and Cys 375 of the anti-HER2-mAb2 heavy chain, at 1 mg/kg, 2.5 mg/kg, 5 mg/kg, or 10 mg/kg. Complete regression of N87 xenograft tumors was observed in mice treated with anti-HER2-mAb2-(C-1) conjugate at all doses tested, including the lowest dose tested-1 mg/kg (FIG. 1). Tumor regression was not observed in the N87 xenograft mice treated with 10 mg/kg of unconjugated anti-HER2-mAb2 alone, or an isotype control antibody-(C-1) conjugate, when compared to untreated animals (FIG. 1).

[0453] N87 gastric tumor xenograft mice were treated with a single dose of anti-HER2-mAb1-(C-1) or anti-HER2-mAb1-(C-5), at either 0.3 mg/kg or 1 mg/kg (10 mice per group). While treatment with a single dose of 1 mg/kg anti-HER2-mAb1-(C-1) led to complete regression of human N87 xenograft tumors, 0.3 mg/kg anti-HER2-mAb1-(C-1) resulted in tumor stasis (FIG. 2). Similarly, while treatment with a single dose of 1 mg/kg anti-HER2-mAb1-(C-5) led to complete regression of human N87 xenograft tumors, 0.3 mg/kg anti-HER2-mAb1-(C-5) resulted in tumor stasis (FIG. 2). Regression of N87 gastric tumors was not observed in the N87 xenograft mice treated with an isotype control antibody-(C-5) conjugate when compared to untreated animals. These data showed that tumor regression can be achieved by a single treatment of an anti-HER2-TLR7 agonist conjugate (e.g., anti-HER2-mAb1-(C-1) or anti-HER2-mAb1-(C-5), anti-HER2-mAb2-(C-1)) at a low dose, e.g., 1mg/kg.

[0454] In a separate study, N87 gastric tumor xenograft mice were treated with a single dose of anti-HER2-mAb1-(C-5), at 1 mg/kg, 3 mg/kg or 5 mg/kg (8 mice per group). While treatment with a single dose of either 3 mg/kg or 5 mg/kg anti-HER2-mAb1-(C-5) led to complete regression of human N87 xenograft tumors, 1 mg/kg anti-HER2-mAb1-(C-5) resulted in tumor stasis (FIG. 3) in this study.

[0455] In addition, N87 gastric tumor xenograft mice were treated with a single dose of either anti-HER2-mAb1-(C-5), anti-HER2-mAb1-(C-35), anti-HER2-mAb1-(C-37), anti-HER2-mAb1-(C-59), anti-HER2-mAb1-(C-60), anti-HER2-mAb1-(C-61), anti-HER2-mAb1-(C-62) or anti-HER2-mAb1-(C-64) at 1 mg/kg (6 mice per group). Treatment with a single dose of 1 mg/kg anti-HER2-mAb1 conjugated with different compounds resulted in tumor stasis (FIG. 4), similar to what was observed after a single dose treatment of 1 mg/kg anti-HER2-mAb1-(C-5).

Example 77

In vivo testing of an anti-ratHER2-TLR7 agonist conjugate in MMC (ratHER2⁺) breast cancer syngeneic model

Materials and Methods

[0456] For the MMC (ratHER2⁺) breast cancer syngeneic model, 6-10 week old female FVB/N transgenic mice expressing the activated rat Erbb2 (c-neu) oncogene containing the Val⁶⁶⁴ to Glu⁶⁶⁴ mutation (FVB-Tg(MMTV-ErbB2)NK1Mul/J; originally purchased from Jackson Laboratories, breed in house) were used for implantation. MMC cells (derived from tumors obtained from FVB/N transgenic mice, obtained from Professor Nora Disis, University of Washington) were grown in sterile conditions in a 37°C incubator with 5% CO₂ for two weeks. Cells were grown in DMEM medium with 20% fetal bovine serum and Penicillin/Strep. Cells were passaged every 3-4 days with 0.05% Trypsin/EDTA. On the day of implantation, cells were lifted (passage x4) and re-suspended in RPMI1640 serum-free media at a concentration of 2.5 x 10⁵ cells and 10% matrigel/100 µl. Cells were Radii tested to assure that they are free of mycoplasma and murine viruses.

[0457] MMC cells were implanted with a subcutaneous injection into the lower flank using a 28 gauge needle (100 µl injection volume). After implant, tumors were measured by caliper and mice weighed 2 times per week once tumors were palpable. Tumors then were measured twice a week in two dimensions. Caliper measurements were calculated using $(L \times W^2)/2$. Mice were fed with normal diet and housed in SPF animal facility in accordance with the Guide for Care and Use of Laboratory Animals and regulations of the Institutional Animal Care and Use Committee.

[0458] When tumors reached about 200 mm³, groups of eight mice were administered by intravenous route with 1 mg/kg of anti-ratHER2 antibody (7.16.4, purchased from Bio X Cell; West Lebanon, NH) or 1 mg/kg of anti-ratHER2-TLR7 agonist conjugate (anti-ratHER2-(C-46)). Tumors were measured twice a week. Average tumor volumes were plotted using Prism 5 (GraphPad) software. An endpoint for efficacy studies was achieved when tumor size reached a volume of 2000 mm³. Following injection, mice were also closely monitored for signs of clinical deterioration. If for any reason mice showed any signs of morbidity, including respiratory distress, hunched posture, decreased activity, hind

leg paralysis, tachypnea as a sign for pleural effusions, weight loss approaching 20% or 15% plus other signs, or if their ability to carry on normal activities (feeding, mobility), was impaired, mice were euthanized.

Results

[0459] To test the efficacy of anti-ratHER2-(C-46) conjugates in MMC ratHER2+ breast cancer syngeneic model, mice bearing subcutaneous MMC breast tumors were treated intravenously with 1 mg/kg of anti-ratHER2-(C-46) conjugate, or unconjugated anti-ratHER2 (8 mice per group). As shown in FIGs. 5A and 5B, complete regression of MMC mouse breast tumors (ratHER2+) was observed in seven out of eight mice treated with a single dose of anti-ratHER2-(C-46) conjugates (FIG. 5A), but only in three out of eight mice treated with the naked anti-ratHER2 antibody (FIG. 5B).

[0460] These data suggest that the anti-ratHER2-(C-46) conjugate is therapeutically more effective against ratHER2-positive syngeneic breast cancer than the unconjugated anti-ratHER2 antibody alone.

Example 78

In vivo testing of anti-HER2-TLR7 agonist conjugates in a HCC1954 breast tumor xenograft model

Materials and Methods

[0461] For HCC1954 breast xenograft mouse model, female SCID-beige mice at 6-8 weeks of age (purchased from Harlan Laboratories) were used for implantation. HCC1954 cells (obtained from ATCC, Catalog # CRL-2338, Vendor lot # 5107643) were grown in sterile conditions in a 37°C incubator with 5% CO₂ for two weeks. Cells were grown in RPMI medium with 10% fetal bovine serum. Cells were passaged every 3-4 days with 0.05% Trypsin/EDTA. On the day of implantation, HCC1954 cells were (harvested) lifted (passage x17) and re-suspended in RPMI1640 serum-free media at a concentration of 1 x 10⁶ cells and 50% matrigel/100 µl. Cells were Rapii tested to assure that they are free of mycoplasma and murine viruses.

[0462] HCC1954 cells were implanted with a subcutaneous injection into the right mammary fat pad using a 27G needle (100 µl injection volume). After implant, tumors were measured by caliper and mice weighed two times per week once tumors were palpable. Tumors then were measured twice a week in two dimensions. Caliper measurements were calculated using $(L \times W^2)/2$. Mice were fed with normal diet and housed in SPF animal facility in accordance with the Guide for Care and Use of Laboratory Animals and regulations of the Institutional Animal Care and Use Committee.

[0463] When xenograft tumors reached about 200 mm³, mice were administered by intravenous route 1-10 mg/kg of anti-HER2 antibody or anti-HER2-TLR7 agonist conjugate. Isotype control antibody was generated by expressing an antibody against a target not found in rodents and conjugating through similar methods described for anti-HER2 antibodies. Tumors were measured twice a week. Average tumor volumes were plotted using Prism 5 (GraphPad) software. An endpoint for efficacy studies was achieved when tumor size reached a volume of 2000 mm³. Following injection, mice were also closely monitored for signs of clinical deterioration. If for any reason mice showed any signs of morbidity, including respiratory distress, hunched posture, decreased activity, hind leg paralysis, tachypnea as a sign for pleural effusions, weight loss approaching 20% or 15% plus other signs, or if their ability to carry on normal activities (feeding, mobility), was impaired, mice were euthanized.

Results

[0464] HCC1954 breast tumor xenograft mice were treated intravenously with a single dose of anti-HER2-mAb1-(C-5) conjugate, where Compound (C-5) is conjugated to Cys 152 and Cys 375 of the anti-HER2-mAb1 heavy chain, at 1 mg/kg, 3 mg/kg or 10 mg/kg (8 mice per group). While treatment with a single dose of 10 mg/kg or 3 mg/kg anti-HER2-mAb1-(C-5) led to complete regression of human HCC1954 xenograft tumors, 1 mg/kg anti-HER2-mAb1-(C-5) resulted in initial tumor regression followed by tumor stasis (FIG. 6). Tumor regression was not observed in the HCC1954 xenograft mice treated with 10 mg/kg of unconjugated anti-HER2-mAb2 alone (FIG. 6).

[0465] These data show that tumor regression can be achieved in the high HER2 expressing HCC1954 breast tumor xenograft by a single treatment of an anti-HER2-TLR7 agonist conjugate (anti-HER2-mAb1-(C-5)) at 3 mg/kg.

Example 79***In vivo* testing of anti-HER2-TLR7 agonist conjugates in a SKOV3 ovarian tumor xenograft model****Materials and Methods**

[0466] For SKOV3 ovarian xenograft mouse model, female SCID-beige mice at 6-8 weeks of age (purchased from Harlan Laboratories) were used for implantation. SKOV3 cells (obtained from ATCC, Catalog # HTB-77, Vendor lot # 7349765) were grown in sterile conditions in a 37°C incubator with 5% CO₂ for two weeks. Cells were grown in McCoy's5A medium with 10% fetal bovine serum. Cells were passaged every 3-4 days with 0.05% Trypsin/EDTA. On the day of implantation, SKOV3 cells were (harvested) lifted (passage x11) and re-suspended in McCoy's5A serum-free media at a concentration of 5 x 10⁶ cells and 50% matrigel/100 µl. Cells were Radii tested to assure that they are free of mycoplasma and murine viruses.

[0467] SKOV3 cells were implanted with a subcutaneous injection into the lower flank using a 28 ½ G (100 µl injection volume). After implant, tumors were measured by caliper and mice weighed two times per week once tumors were palpable. Tumors then were measured twice a week in two dimensions. Caliper measurements were calculated using (L x W²)/2. Mice were fed with normal diet and housed in SPF animal facility in accordance with the Guide for Care and Use of Laboratory Animals and regulations of the Institutional Animal Care and Use Committee.

[0468] When xenograft tumors reached about 200 mm³, mice were administered by intravenous route 3-10 mg/kg of anti-HER2 antibody or anti-HER2-TLR7 agonist conjugate. Isotype control antibody was generated by expressing an antibody against a target not found in rodents and conjugating through similar methods described for anti-HER2 antibodies. Tumors were measured twice a week. Average tumor volumes were plotted using Prism 5 (GraphPad) software. An endpoint for efficacy studies was achieved when tumor size reached a volume of 2000 mm³. Following injection, mice were also closely monitored for signs of clinical deterioration. If for any reason mice showed any signs of morbidity, including respiratory distress, hunched posture, decreased activity, hind leg paralysis, tachypnea as a sign for pleural effusions, weight loss approaching 20% or 15% plus other signs, or if their ability to carry on normal activities (feeding, mobility), was impaired, mice were euthanized.

[0469] For HER2 ImmunoHistoChemistry (IHC), standardized guidelines and protocols for HER2 staining and xenograft HER2 scoring were used (see e.g., English et al., Mol Diagn Ther. 2013 Apr; 17(2): 85-99).

Results

[0470] SKOV3 ovarian tumor xenograft mice were treated intravenously with a single dose of anti-HER2-mAb1-(C-5) conjugate, where Compound (C-5) is conjugated to Cys 152 and Cys 375 of the anti-HER2-mAb1 heavy chain, at 3 mg/kg or 10 mg/kg. While treatment with a single dose of 10 mg/kg anti-HER2-mAb1-(C-5) led to complete regression of human SKOV3 xenograft tumors in 7 out of 8 mice, 3 mg/kg anti-HER2-mAb1-(C-5) resulted in initial tumor regression followed by tumor regrowth (FIG. 7). Tumor regression was not observed in the SKOV3 xenograft mice treated with 10 mg/kg of unconjugated anti-HER2-mAb1 alone, or an isotype control antibody-(C-5) conjugate, when compared to untreated animals (FIG. 7).

[0471] These data show that tumor regression can be achieved by a single treatment of an anti-HER2-TLR7 agonist conjugate (e.g., anti-HER2-mAb1-(C-1) or anti-HER2-mAb1-(C-5)) at 10 mg/kg in a xenograft model in which Her2 is expressed at lower levels compared to N87 and HCC xenograft models (FIG. 8C as compared to FIGs. 8A and 8B). Based on HER2 expression level, N87 and HCC1954 have 3+ IHC score, and SKOV3 has 2+ IHC score. Therefore, the anti-HER2-TLR7 agonist conjugates described herein can suppress tumor growth not only in high HER2-expressing tumors (e.g., having 3+ IHC scores), but also in low HER2-expressing tumors (e.g., having 2+ IHC scores).

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 <220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

55 <400> 15

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Ser Ala Ser
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5 <210> 16
<211> 6
<212> PRT
<213> Artificial Sequence

10 <220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 16

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1 5

20 <210> 17
<211> 107
<212> PRT
<213> Artificial Sequence

25 <220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 17

30 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

35 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

40 Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

45 Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

50 Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

55 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 18
<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 18

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 atcacctgcc gtgccagtca ggatgtgaat actgctgtag cctggatatca acagaaacca 120
 15 ggaaaagctc cgaaactact gatttactcg gcataccttc tctactctgg agtcccttct 180
 cgcttctctg gatccagatc tgggacggat ttactctga ccatcagcag tctgcagccg 240
 gaagacttcg caacttatta ctgtcagcaa cattatacta ctcctccac gttcggacag 300
 20 ggtaccaagg tggagatcaa a 321

<210> 19

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 19

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	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	
	1				5					10					15		
5	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Asp	Val	Asn	Thr	Ala	
				20					25					30			
	Val	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	
10			35					40					45				
	Tyr	Ser	Ala	Ser	Phe	Leu	Tyr	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	
		50					55					60					
15																	
	Ser	Arg	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	
	65					70					75					80	
20	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	His	Tyr	Thr	Thr	Pro	Pro	
					85					90					95		
	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	
25				100					105					110			
	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	
			115					120					125				
30																	
	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	
		130				135						140					
35	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	
	145					150					155					160	
	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	
40					165					170					175		
	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	
				180					185					190			
45																	
	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	
			195					200					205				
50	Phe	Asn	Arg	Gly	Glu	Cys											
		210															

<210> 20

<211> 642

55 <212> DNA

<213> Artificial Sequence

<220>

EP 3 368 092 B9

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 20

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 10 ggaaaagctc cgaaactact gatttactcg gcacccctcc tctactctgg agtcccttct 180
 cgcttctctg gatccagatc tgggacggat ttactctga ccatcagcag tctgcagccg 240
 gaagacttctg caacttatta ctgtcagcaa cattatacta ctccctccac gttcggacag 300
 15 ggtaccaagg tggagatcaa acgtacggtg gccgctccca gcgtgttcat cttccccccc 360
 agcgacgagc agctgaagag tggcaccgcc agcgtggtgt gcctgctgaa caacttctac 420
 ccccgggagg ccaaggtgca gtggaaggtg gacaacgccc tgcagagcgg caacagccag 480
 20 gagagcgtca ccgagcagga cagcaaggac tccacctaca gcctgagcag caccctgacc 540
 ctgagcaagg ccgactacga gaagcataag gtgtacgcct gcgaggtgac ccaccagggc 600
 25 ctgtccagcc ccgtgaccaa gagcttcaac aggggagagt gc 642

<210> 21

<211> 450

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 21

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

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5	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Asn	Ile	Lys	Asp	Thr
				20					25					30		
	Tyr	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
10			35					40					45			
	Ala	Arg	Ile	Tyr	Pro	Thr	Asn	Gly	Tyr	Thr	Arg	Tyr	Ala	Asp	Ser	Val
		50					55					60				
15	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Ala	Asp	Thr	Ser	Lys	Asn	Thr	Ala	Tyr
	65					70					75				80	
	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
20				85						90					95	
	Ser	Arg	Trp	Gly	Gly	Asp	Gly	Phe	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln
				100					105					110		
25	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val
			115					120					125			
30	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala
		130					135					140				
	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Cys	Pro	Val	Thr	Val	Ser
35	145					150					155				160	
	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val
				165						170					175	
40	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro
				180					185					190		
	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys
45			195					200					205			
	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp
50		210					215					220				
	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly
	225					230					235				240	
55	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile
				245						250					255	

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	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu
				260					265					270		
5	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His
			275					280					285			
10	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg
		290					295					300				
15	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys
	305					310					315					320
20	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu
					325					330					335	
25	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr
				340					345					350		
30	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu
			355					360					365			
35	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Cys	Asp	Ile	Ala	Val	Glu	Trp
	370						375					380				
40	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val
	385					390					395					400
45	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp
					405					410					415	
50	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His
				420					425					430		
55	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro
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60	Gly	Lys														
		450														

<210> 22

<211> 1350

<212> DNA

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 22

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	tcctgtgcag	cttctggctt	caacattaaa	gacacctata	tacactgggt	gcgtcaggcc	120
5	ccgggtaagg	gcctggaatg	ggttgcaagg	atttatccta	cgaatgggta	tactagatat	180
	gccgatagcg	tcaagggccg	tttcaactata	agcgcagaca	catccaaaaa	cacagcctac	240
10	ctgcagatga	acagcctgcg	tgctgaggac	actgccgtct	attattgttc	tagatgggga	300
	ggggacggct	tctatgctat	ggactactgg	ggtcaaggaa	ccctggtcac	cgtctcctcg	360
	gctagcacca	aggggccccag	cgtgttcccc	ctggccccca	gcagcaagag	caccagcggc	420
15	ggcacagccg	ccctgggctg	cctgggtgaag	gactacttcc	cttgtcccgt	gaccgtgtcc	480
	tggaacagcg	gagccctgac	ctccggcgtg	cacaccttcc	ccgccgtgct	gcagagcagc	540
	ggcctgtaca	gcctgtccag	cgtgggtgaca	gtgcccagca	gcagcctggg	caccagacc	600
20	tacatctgca	acgtgaacca	caagcccagc	aacaccaagg	tggacaagaa	agtggagccc	660
	aagagctgcg	acaagacca	cacctgcccc	ccctgcccag	ccccagagct	gctgggcgga	720
25	ccctccgtgt	tcctgttccc	ccccaaagcc	aaggacaccc	tgatgatcag	caggaccccc	780
	gaggtgacct	gcgtgggtgg	ggacgtgagc	cacgaggacc	cagaggtgaa	gttcaactgg	840
	tacgtggacg	gcgtggaggt	gcacaacgcc	aagaccaagc	ccagagagga	gcagtacaac	900
30	agcacctaca	gggtgggtgtc	cgtgctgacc	gtgctgcacc	aggactggct	gaacggcaag	960
	gaatacaagt	gcaaggtctc	caacaaggcc	ctgccagccc	ccatcgaaaa	gaccatcagc	1020
	aaggccaagg	gccagccacg	ggagccccag	gtgtacaccc	tgccccctc	ccgggaggag	1080
35	atgaccaaga	accaggtgtc	cctgacctgt	ctggtgaagg	gcttctaccc	ctgcgacatc	1140
	gccgtggagt	gggagagcaa	cggccagccc	gagaacaact	acaagaccac	acctccagtg	1200
40	ctggacagcg	acggcagctt	cttcctgtac	agcaagctga	ccgtggacaa	gtccaggtgg	1260
	cagcagggca	acgtgttcag	ctgcagcgtg	atgcacgagg	ccctgcacaa	ccactacacc	1320
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45	<210> 23						
	<211> 450						
	<212> PRT						
	<213> Artificial Sequence						
50	<220>						
	<221> source						
	<223> /note="Description of Artificial Sequence: Synthetic polypeptide"						
55	<400> 23						

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

5

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr

10

15

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	20	25	30
5	Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45		
10	Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val 50 55 60		
15	Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr 65 70 75 80		
20	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95		
25	Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln 100 105 110		
30	Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val 115 120 125		
35	Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala 130 135 140		
40	Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser 145 150 155 160		
45	Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val 165 170 175		
50	Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro 180 185 190		
55	Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys 195 200 205		
	Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp 210 215 220		
	Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly 225 230 235 240		
	Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile 245 250 255		
	Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu 260 265 270		

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	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	
			275					280					285				
5	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	
		290					295					300					
10	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	
	305					310					315					320	
	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	
					325					330					335		
15	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	
				340					345					350			
20	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	
			355					360					365				
25	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	
	370						375					380					
	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	
	385					390					395					400	
30	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	
					405					410					415		
35	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	
				420					425					430			
40	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	
			435					440					445				
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		450															
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	<210> 24																
	<211> 1350																
	<212> DNA																
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	<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"																
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	ccgggtaagg	gcctggaatg	ggttgcaagg	atttatccta	cgaatggtta	tactagatat	180
	gccgatagcg	tcaagggccg	tttcactata	agcgcagaca	catccaaaaa	cacagcctac	240
10	ctgcagatga	acagcctgcg	tgctgaggac	actgccgtct	attattgttc	tagatgggga	300
	ggggacggct	tctatgctat	ggactactgg	ggtcaaggaa	ccctggtcac	cgtctcctcg	360
	gctagcacca	agggccccag	cgtgttcccc	ctggccccca	gcagcaagag	caccagcggc	420
15	ggcacagccg	ccctgggctg	cctggtgaag	gactacttcc	ccgagcccgt	gaccgtgtcc	480
	tggaacagcg	gagccctgac	ctccggcgtg	cacaccttcc	ccgccgtgct	gcagagcagc	540
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	tacatctgca	acgtgaacca	caagcccagc	aacaccaagg	tggacaagaa	agtggagccc	660
	aagagctgcg	acaagacca	cacctgcccc	ccctgcccag	ccccagagct	gctgggcgga	720
25	ccctccgtgt	tcctgttccc	ccccagccc	aaggacaccc	tgatgatcag	caggaccccc	780
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	tacgtggacg	gcgtggaggt	gcacaacgcc	aagaccaagc	ccagagagga	gcagtacaac	900
30	agcacctaca	gggtggtgtc	cgtgctgacc	gtgctgcacc	aggactggct	gaacggcaag	960
	gaatacaagt	gcaaggtctc	caacaaggcc	ctgccagccc	ccatcgaaaa	gaccatcagc	1020
35	aaggccaagg	gccagccacg	ggagccccag	gtgtacaccc	tgccccctc	ccgggaggag	1080
	atgaccaaga	accaggtgtc	cctgacctgt	ctggtgaagg	gcttctaccc	cagcgacatc	1140
	gccgtggagt	gggagagcaa	cggccagccc	gagaacaact	acaagaccac	acctccagtg	1200
40	ctggacagcg	acggcagctt	cttcctgtac	agcaagctga	ccgtggacaa	gtccaggtgg	1260
	cagcagggca	acgtgttcag	ctgcagcgtg	atgcacgagg	ccctgcacaa	ccactacacc	1320
45	cagaagagcc	tgagcctgtc	ccccggcaag				1350
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	<212> DNA						
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50	<400> 25						

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5	ccccgggcag ccgcgcgccc ctccccacgg ggccctttac tgcgccgcgc gcccggtccc	180
	caccctcgc agcaccctgc gccccgcgcc ctcccagccg ggtccagccg gagccatggg	240
10	gccggagccg cagtgagcac catggagctg gcggccttgt gccgctgggg gtcctcctc	300
	gccctcttgc cccccggagc cgcgagcacc caagtgtgca ccggcacaga catgaagctg	360
	cggctccctg ccagtcccga gaccacctg gacatgctcc gccaccta ccagggctgc	420
15		
20		
25		
30		
35		
40		
45		
50		
55		

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	caggtggtgc agggaaacct ggaactcacc tacctgccca ccaatgccag cctgtccttc	480
	ctgcaggata tccaggaggt gcagggctac gtgctcatcg ctcaacaacca agtgaggcag	540
5	gtcccactgc agaggctgcg gattgtgcga ggcaccagc tctttgagga caactatgcc	600
	ctggccgtgc tagacaatgg agaccgctg aacaatacca cccctgtcac aggggcctcc	660
	ccaggaggcc tgcgggagct gcagcttcga agcctcacag agatcttgaa aggaggggtc	720
10	ttgatccagc ggaacccccca gctctgctac caggacacga ttttgtggaa ggacatcttc	780
	cacaagaaca accagctggc tctcacactg atagacacca accgctctcg ggcctgccac	840
	ccctgttctc cgatgtgtaa gggctccgc tgcctgggag agagttctga ggattgtcag	900
15	agcctgacgc gactgtctg tgcgggtggc tgtgccgct gcaaggggcc actgccact	960
	gactgctgcc atgagcagtg tgcctgggc tgcacgggc ccaagcactc tgactgcctg	1020
	gcctgcctcc acttcaacca cagtggcatc tgtgagctgc actgccagc cctggtcacc	1080
20	tacaacacag acacgtttga gtccatgcc aatcccgagg gccggtatac attcggcgcc	1140
	agctgtgtga ctgcctgtcc ctacaactac ctttctacgg acgtgggac ctgcaccctc	1200
	gtctgcccc tgcacaacca agaggtgaca gcagaggatg gaacacagcg gtgtgagaag	1260
25	tgcagcaagc cctgtgccc agtgtgctat ggtctgggca tggagcactt gcgagaggtg	1320
	agggcagtta ccagtgccaa tatccaggag tttgctggct gcaagaagat ctttgggagc	1380
	ctggcatttc tgccggagag ctttgatggg gaccagcct ccaacactgc cccgctccag	1440
30	ccagagcagc tccaagtgtt tgagactctg gaagagatca caggttacct atacatctca	1500
	gcatggccgg acagcctgcc tgacctcagc gtcttccaga acctgcaagt aatccgggga	1560
	cgaattctgc acaatggcgc ctactcgctg accctgcaag ggctgggcat cagctggctg	1620
35	gggctgcgct cactgaggga actgggcagt ggactggccc tcatccacca taacaccac	1680
	ctctgcttcg tgcacacggt gccctgggac cagctcttcc ggaaccgca ccaagctctg	1740
40	ctccacactg ccaaccggcc agaggacgag tgtgtggcg agggcctggc ctgccaccag	1800
	ctgtgcgccc gagggcactg ctggggtcca gggccaccc agtgtgtcaa ctgcagccag	1860
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<212> PRT

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15	Leu	Tyr	Gln	Gly	Cys	Gln	Val	Val	Gln	Gly	Asn	Leu	Glu	Leu	Thr	Tyr	
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25	Gln	Gly	Tyr	Val	Leu	Ile	Ala	His	Asn	Gln	Val	Arg	Gln	Val	Pro	Leu	
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30	Gln	Arg	Leu	Arg	Ile	Val	Arg	Gly	Thr	Gln	Leu	Phe	Glu	Asp	Asn	Tyr	
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35	Ala	Leu	Ala	Val	Leu	Asp	Asn	Gly	Asp	Pro	Leu	Asn	Asn	Thr	Thr	Pro	
			115					120					125				
40	Val	Thr	Gly	Ala	Ser	Pro	Gly	Gly	Leu	Arg	Glu	Leu	Gln	Leu	Arg	Ser	
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45	Leu	Thr	Glu	Ile	Leu	Lys	Gly	Gly	Val	Leu	Ile	Gln	Arg	Asn	Pro	Gln	
		145				150					155					160	

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	Leu	Cys	Tyr	Gln	Asp	Thr	Ile	Leu	Trp	Lys	Asp	Ile	Phe	His	Lys	Asn	
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5	Asn	Gln	Leu	Ala	Leu	Thr	Leu	Ile	Asp	Thr	Asn	Arg	Ser	Arg	Ala	Cys	
				180					185					190			
10	His	Pro	Cys	Ser	Pro	Met	Cys	Lys	Gly	Ser	Arg	Cys	Trp	Gly	Glu	Ser	
			195					200					205				
15	Ser	Glu	Asp	Cys	Gln	Ser	Leu	Thr	Arg	Thr	Val	Cys	Ala	Gly	Gly	Cys	
		210					215					220					
20	Ala	Arg	Cys	Lys	Gly	Pro	Leu	Pro	Thr	Asp	Cys	Cys	His	Glu	Gln	Cys	
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25	Ala	Ala	Gly	Cys	Thr	Gly	Pro	Lys	His	Ser	Asp	Cys	Leu	Ala	Cys	Leu	
					245					250					255		
30	His	Phe	Asn	His	Ser	Gly	Ile	Cys	Glu	Leu	His	Cys	Pro	Ala	Leu	Val	
				260					265					270			
35	Thr	Tyr	Asn	Thr	Asp	Thr	Phe	Glu	Ser	Met	Pro	Asn	Pro	Glu	Gly	Arg	
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40	Tyr	Thr	Phe	Gly	Ala	Ser	Cys	Val	Thr	Ala	Cys	Pro	Tyr	Asn	Tyr	Leu	
		290					295					300					
45	Ser	Thr	Asp	Val	Gly	Ser	Cys	Thr	Leu	Val	Cys	Pro	Leu	His	Asn	Gln	
	305					310					315					320	
50	Glu	Val	Thr	Ala	Glu	Asp	Gly	Thr	Gln	Arg	Cys	Glu	Lys	Cys	Ser	Lys	
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55	Pro	Cys	Ala	Arg	Val	Cys	Tyr	Gly	Leu	Gly	Met	Glu	His	Leu	Arg	Glu	
				340					345					350			
60	Val	Arg	Ala	Val	Thr	Ser	Ala	Asn	Ile	Gln	Glu	Phe	Ala	Gly	Cys	Lys	
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65	Lys	Ile	Phe	Gly	Ser	Leu	Ala	Phe	Leu	Pro	Glu	Ser	Phe	Asp	Gly	Asp	
		370					375					380					
70	Pro	Ala	Ser	Asn	Thr	Ala	Pro	Leu	Gln	Pro	Glu	Gln	Leu	Gln	Val	Phe	
	385					390					395					400	
75	Glu	Thr	Leu	Glu	Glu	Ile	Thr	Gly	Tyr	Leu	Tyr	Ile	Ser	Ala	Trp	Pro	
				405						410					415		

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	Asp	Ser	Leu	Pro	Asp	Leu	Ser	Val	Phe	Gln	Asn	Leu	Gln	Val	Ile	Arg	
				420					425					430			
5	Gly	Arg	Ile	Leu	His	Asn	Gly	Ala	Tyr	Ser	Leu	Thr	Leu	Gln	Gly	Leu	
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	Gly	Ile	Ser	Trp	Leu	Gly	Leu	Arg	Ser	Leu	Arg	Glu	Leu	Gly	Ser	Gly	
10		450					455					460					
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	465					470					475					480	
15	Pro	Trp	Asp	Gln	Leu	Phe	Arg	Asn	Pro	His	Gln	Ala	Leu	Leu	His	Thr	
					485					490					495		
	Ala	Asn	Arg	Pro	Glu	Asp	Glu	Cys	Val	Gly	Glu	Gly	Leu	Ala	Cys	His	
20				500					505					510			
	Gln	Leu	Cys	Ala	Arg	Gly	His	Cys	Trp	Gly	Pro	Gly	Pro	Thr	Gln	Cys	
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30	Arg	Val	Leu	Gln	Gly	Leu	Pro	Arg	Glu	Tyr	Val	Asn	Ala	Arg	His	Cys	
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	Leu	Pro	Cys	His	Pro	Glu	Cys	Gln	Pro	Gln	Asn	Gly	Ser	Val	Thr	Cys	
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40	Pro	Pro	Phe	Cys	Val	Ala	Arg	Cys	Pro	Ser	Gly	Val	Lys	Pro	Asp	Leu	
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	Ser	Tyr	Met	Pro	Ile	Trp	Lys	Phe	Pro	Asp	Glu	Glu	Gly	Ala	Cys	Gln	
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	Gly	Cys	Pro	Ala	Glu	Gln	Arg	Ala	Ser	Pro	Leu	Thr	Ser	Ile	Ile	Ser	
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55	Ala	Val	Val	Gly	Ile	Leu	Leu	Val	Val	Val	Leu	Gly	Val	Val	Phe	Gly	
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	Ile	Leu	Ile	Lys	Arg	Arg	Gln	Gln	Lys	Ile	Arg	Lys	Tyr	Thr	Met	Arg	
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10	Ala	Met	Pro	Asn	Gln	Ala	Gln	Met	Arg	Ile	Leu	Lys	Glu	Thr	Glu	Leu	
	705					710					715					720	
15	Arg	Lys	Val	Lys	Val	Leu	Gly	Ser	Gly	Ala	Phe	Gly	Thr	Val	Tyr	Lys	
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20	Gly	Ile	Trp	Ile	Pro	Asp	Gly	Glu	Asn	Val	Lys	Ile	Pro	Val	Ala	Ile	
				740					745					750			
25	Lys	Val	Leu	Arg	Glu	Asn	Thr	Ser	Pro	Lys	Ala	Asn	Lys	Glu	Ile	Leu	
			755					760					765				
30	Asp	Glu	Ala	Tyr	Val	Met	Ala	Gly	Val	Gly	Ser	Pro	Tyr	Val	Ser	Arg	
	770						775					780					
35	Leu	Leu	Gly	Ile	Cys	Leu	Thr	Ser	Thr	Val	Gln	Leu	Val	Thr	Gln	Leu	
	785					790					795					800	
40	Met	Pro	Tyr	Gly	Cys	Leu	Leu	Asp	His	Val	Arg	Glu	Asn	Arg	Gly	Arg	
					805					810					815		
45	Leu	Gly	Ser	Gln	Asp	Leu	Leu	Asn	Trp	Cys	Met	Gln	Ile	Ala	Lys	Gly	
				820					825					830			
50	Met	Ser	Tyr	Leu	Glu	Asp	Val	Arg	Leu	Val	His	Arg	Asp	Leu	Ala	Ala	
			835					840					845				
55	Arg	Asn	Val	Leu	Val	Lys	Ser	Pro	Asn	His	Val	Lys	Ile	Thr	Asp	Phe	
		850					855					860					
60	Gly	Leu	Ala	Arg	Leu	Leu	Asp	Ile	Asp	Glu	Thr	Glu	Tyr	His	Ala	Asp	
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65	Gly	Gly	Lys	Val	Pro	Ile	Lys	Trp	Met	Ala	Leu	Glu	Ser	Ile	Leu	Arg	
				885						890					895		
70	Arg	Arg	Phe	Thr	His	Gln	Ser	Asp	Val	Trp	Ser	Tyr	Gly	Val	Thr	Val	
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75	Trp	Glu	Leu	Met	Thr	Phe	Gly	Ala	Lys	Pro	Tyr	Asp	Gly	Ile	Pro	Ala	

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5	Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro				
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10	Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met				
	945		950		955 960
15	Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe				
		965		970	975
20	Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu				
		980		985	990
25	Asp Leu Gly Pro Ala Ser Pro Leu Asp Ser Thr Phe Tyr Arg Ser Leu				
		995		1000	1005
30	Leu Glu Asp Asp Asp Met Gly Asp Leu Val Asp Ala Glu Glu Tyr				
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35	Leu Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly				
	1025		1030		1035
40	Ala Gly Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg				
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45	Ser Gly Gly Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu				
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50	Glu Ala Pro Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser				
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55	Asp Val Phe Asp Gly Asp Leu Gly Met Gly Ala Ala Lys Gly Leu				
	1085		1090		1095
60	Gln Ser Leu Pro Thr His Asp Pro Ser Pro Leu Gln Arg Tyr Ser				
	1100		1105		1110
65	Glu Asp Pro Thr Val Pro Leu Pro Ser Glu Thr Asp Gly Tyr Val				
	1115		1120		1125
70	Ala Pro Leu Thr Cys Ser Pro Gln Pro Glu Tyr Val Asn Gln Pro				
	1130		1135		1140
75	Asp Val Arg Pro Gln Pro Pro Ser Pro Arg Glu Gly Pro Leu Pro				
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5	Ser	Pro	Gly	Lys	Asn	Gly	Val	Val	Lys	Asp	Val	Phe	Ala	Phe	Gly	
	1175						1180					1185				
10	Gly	Ala	Val	Glu	Asn	Pro	Glu	Tyr	Leu	Thr	Pro	Gln	Gly	Gly	Ala	
	1190						1195					1200				
15	Ala	Pro	Gln	Pro	His	Pro	Pro	Pro	Ala	Phe	Ser	Pro	Ala	Phe	Asp	
	1205						1210					1215				
20	Asn	Leu	Tyr	Tyr	Trp	Asp	Gln	Asp	Pro	Pro	Glu	Arg	Gly	Ala	Pro	
	1220						1225					1230				
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Gly	Thr	Arg	Cys
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5	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Asn	Ile	Lys	Asp	Thr	
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10	Tyr	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
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15	Ala	Arg	Ile	Tyr	Pro	Thr	Asn	Gly	Tyr	Thr	Arg	Tyr	Ala	Asp	Ser	Val	
	50						55					60					
20	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Ala	Asp	Thr	Ser	Lys	Asn	Thr	Ala	Tyr	
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25	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	
				85						90				95			
30	Ser	Arg	Trp	Gly	Gly	Asp	Gly	Phe	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln	
				100					105					110			
35	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	
			115					120					125				
40	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	
	130						135					140					

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	Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Cys Pro Val Thr Val Ser	145	150	155	160
5	Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val	165	170	175	
10	Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro	180	185	190	
15	Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys	195	200	205	
20	Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp	210	215	220	
25	Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly	225	230	235	240
30	Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile	245	250	255	
35	Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu	260	265	270	
40	Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His	275	280	285	
45	Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg	290	295	300	
50	Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys	305	310	315	320
55	Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu	325	330	335	
	Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr	340	345	350	
	Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu	355	360	365	
	Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp	370	375	380	
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5	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Asn	Ile	Lys	Asp	Thr
				20					25					30		
10	Tyr	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
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15	Ala	Arg	Ile	Tyr	Pro	Thr	Asn	Gly	Tyr	Thr	Arg	Tyr	Ala	Asp	Ser	Val
		50					55					60				
20	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Ala	Asp	Thr	Ser	Lys	Asn	Thr	Ala	Tyr
	65					70					75				80	
25																
30																
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	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
					85					90					95	
5	Ser	Arg	Trp	Gly	Gly	Asp	Gly	Phe	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln
				100					105					110		
10	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val
			115					120					125			
15	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala
	130						135					140				
20	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser
	145					150					155					160
25	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val
				165						170					175	
30	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro
				180					185					190		
35	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys
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40	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp
	210						215					220				
45	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly
	225					230					235					240
50	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile
				245						250					255	
55	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu
				260					265					270		
60	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His
			275					280					285			
65	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg
	290						295					300				
70	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys
	305					310					315					320
75	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu

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	325	330	335
5	Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr 340 345 350		
10	Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu 355 360 365		
15	Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp 370 375 380		
20	Glu Ser Asn Gly Gln Pro Glu Gly Asp Ser Leu Asp Met Leu Glu Trp 385 390 395 400		
25	Ser Leu Met Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp 405 410 415		
30	Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp 420 425 430		
35	Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His 435 440 445		
40	Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys 450 455 460		
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50	<220> <221> source <223> /note="Description of Artificial Sequence: Synthetic peptide"		
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	Ala Met Pro Glu Asn Leu 20		
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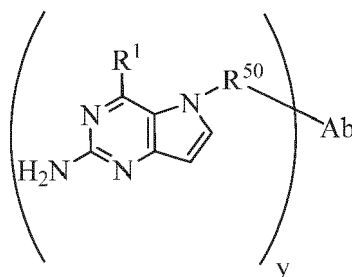
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	ggaaaagctc cgaaactact gatttactcg gcacccctcc tctactctgg agtcccttct	180
10	cgcttctctg gatccagatc tgggacggat ttcactctga ccatcagcag tctgcagccg	240
	gaagacttcg caacttatta ctgtcagcaa cattatacta ctccctccac gttcggacag	300
15	ggtaccaagg tggagatcaa acgaacggtg gccgctccca gcgtgttcat cttccccccc	360
	agcgacgagc agctgaagag cggcaccgcc agcgtggtgt gcctgctgaa caacttctac	420
	ccccgggagg ccaaggtgca gtggaaggtg gacaacgccc tgcagagcgg caacagccag	480
20	gagagcgtca ccgagcagga cagcaaggac tccacctaca gcctgagcag caccctgacc	540
	ctgagcaagg ccgactacga gaagcataag gtgtacgcct gcgagggtgac ccaccagggc	600
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Claims

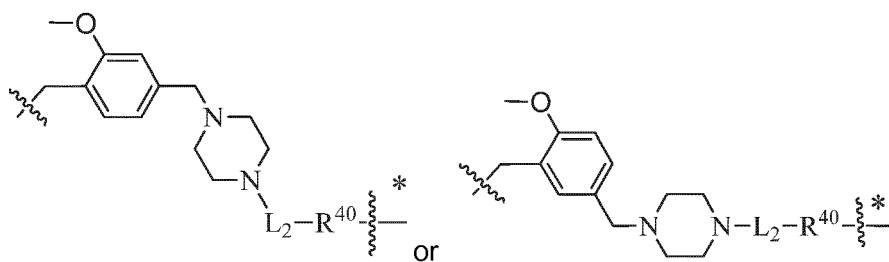
1. A conjugate of Formula (II), or pharmaceutically acceptable salt thereof:



Formula (II)

wherein:

R⁵⁰ is



where the * indicates the point of attachment to Ab;

Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2;

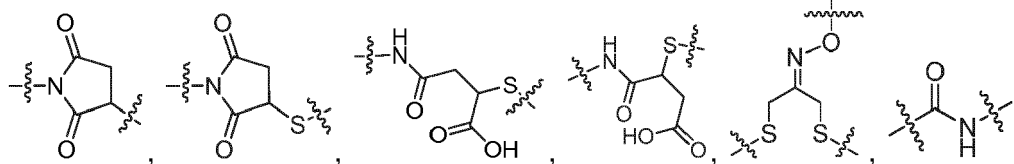
R^1 is $-NHR^2$ or $-NHCHR^2R^3$;

R^2 is $-C_3-C_6$ alkyl or $-C_4-C_6$ alkyl;

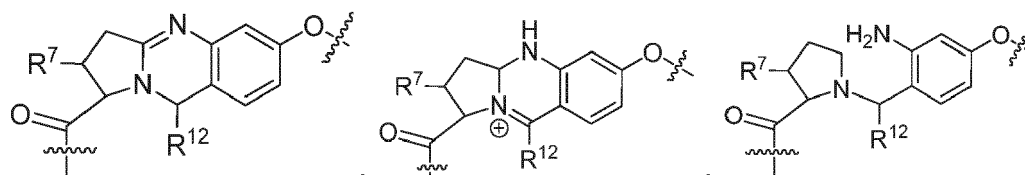
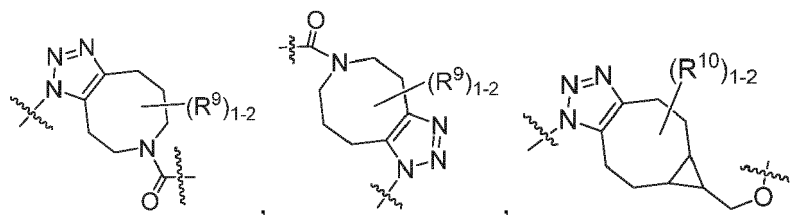
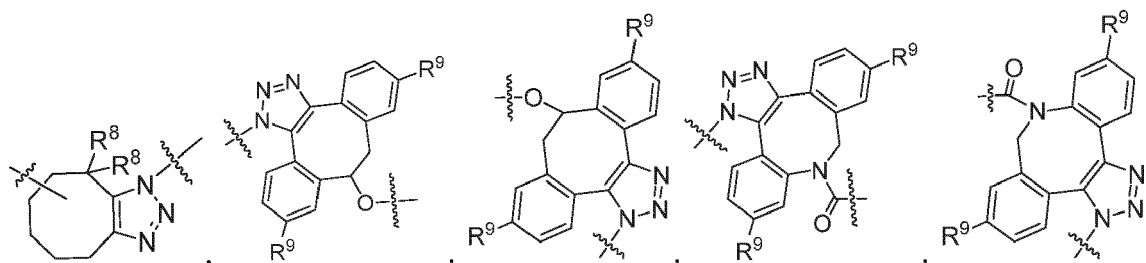
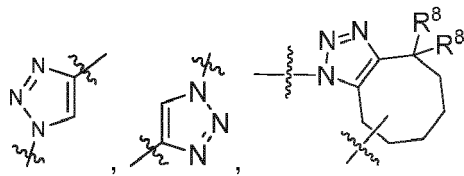
R^3 is L_1OH ;

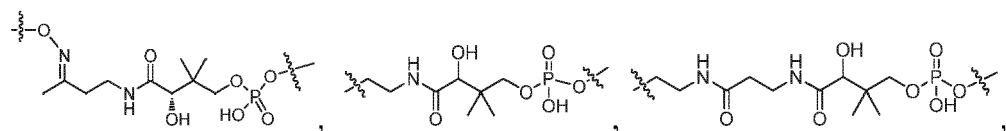
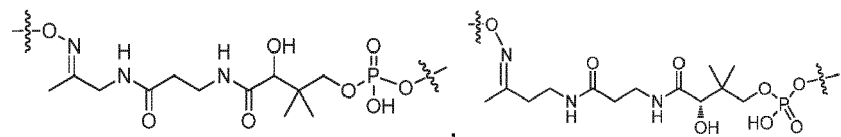
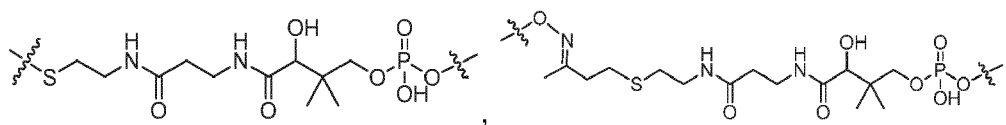
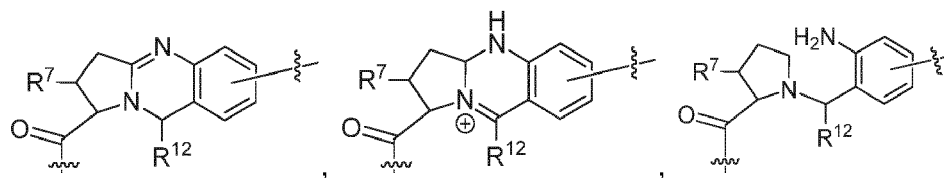
L_1 is $-(CH_2)_m-$;

L_2 is $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_n-$,
 $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)(CH_2)_n-$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$,
 $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$,
 $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)X_2X_3C(=O)(CH_2)_n-$,
 $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n-$,
 $-C(=O)(CH_2)_nC(R_7)_2-$, $-C(=O)(CH_2)_nC(R_7)_2SS(CH_2)_nNHC(=O)(CH_2)_n-$,
 $-(CH_2)_nX_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n-$ or $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;
 R^{40} is

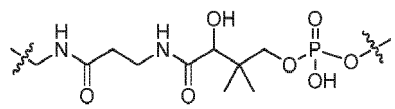


$-S-$, $-NHC(=O)CH_2-$, $-S(=O)_2CH_2CH_2-$, $-(CH_2)_2S(=O)_2CH_2CH_2-$, $-NHS(=O)_2CH_2CH_2-$, $-NHC(=O)CH_2CH_2-$,
 $-CH_2NHCH_2CH_2-$, $-NHCH_2CH_2-$,

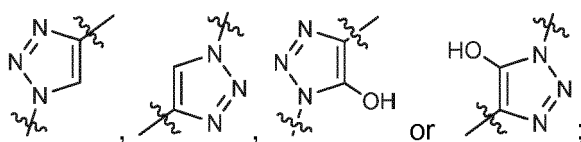




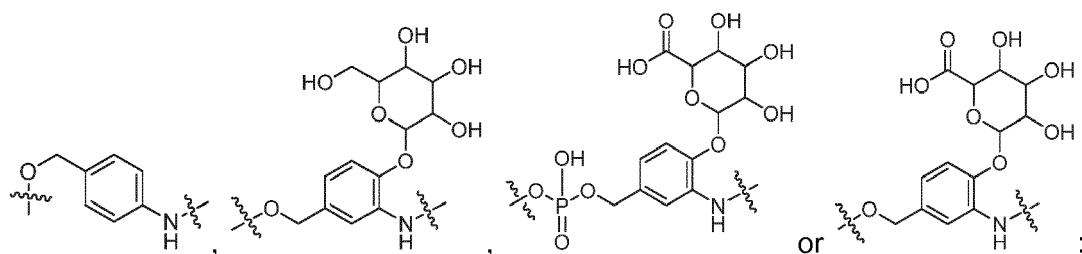
or



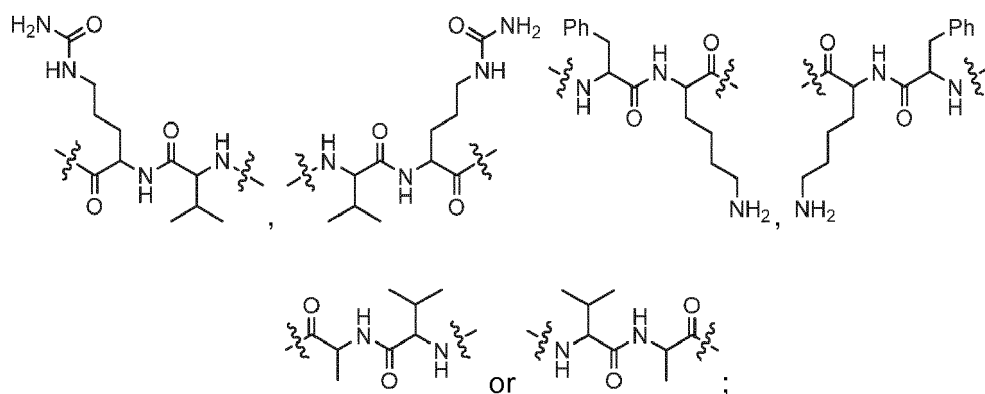
X_1 is



X_2 is



X_3 is



each R^7 is independently selected from H and C_1 - C_6 alkyl;
 each R^8 is independently selected from H, C_1 - C_6 alkyl, F, Cl, and -OH;
 each R^9 is independently selected from H, C_1 - C_6 alkyl, F, Cl, - NH_2 , - OCH_3 , - OCH_2CH_3 , - $N(CH_3)_2$, -CN, - NO_2 and -OH;
 each R^{10} is independently selected from H, C_1 - C_6 alkyl, fluoro, benzyloxy substituted with -C(=O)OH, benzyl substituted with -C(=O)OH, C_{1-4} alkoxy substituted with -C(=O)OH and C_{1-4} alkyl substituted with -C(=O)OH;
 R^{12} is H, methyl or phenyl;
 each m is independently selected from 1, 2, 3, and 4;
 each n is independently selected from 1, 2, 3, and 4;
 each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and
 y is an integer from 1 to 16.

2. The conjugate of claim 1, wherein Ab is selected from trastuzumab, pertuzumab, margetuximab, or HT-19, or a site-specific cysteine mutant thereof, wherein the site-specific cysteine mutant comprises cysteine at one or more of the following positions (all positions by EU numbering):

- (a) positions 152, 360 and 375 of the antibody heavy chain, and
 (b) positions 107, 159, and 165 of the antibody light chain.

3. The conjugate of claim 1, wherein Ab is selected from any of the following:

- (a) an antibody that comprises:

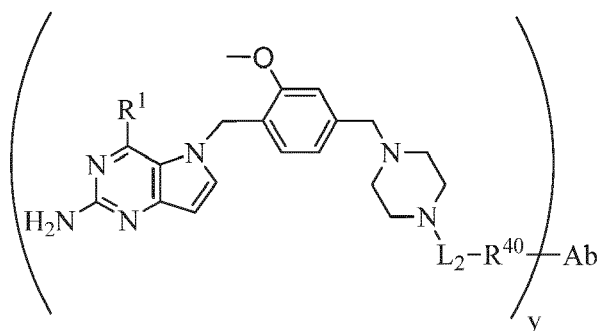
a heavy chain complementary determining region 1 (HCDR1) comprising the amino acid sequence of SEQ ID NO: 1;
 a heavy chain complementary determining region 2 (HCDR2) comprising the amino acid sequence of SEQ ID NO: 2;
 a heavy chain complementary determining region 3 (HCDR3) comprising the amino acid sequence of SEQ ID NO: 3;
 a light chain complementary determining region 1 (LCDR1) comprising the amino acid sequence of SEQ ID NO: 11;
 a light chain complementary determining region 2 (LCDR2) comprising the amino acid sequence of SEQ ID NO: 12; and
 a light chain complementary determining region 3 (LCDR3) comprising the amino acid sequence of SEQ ID NO: 13;

- (b) an antibody that comprises:

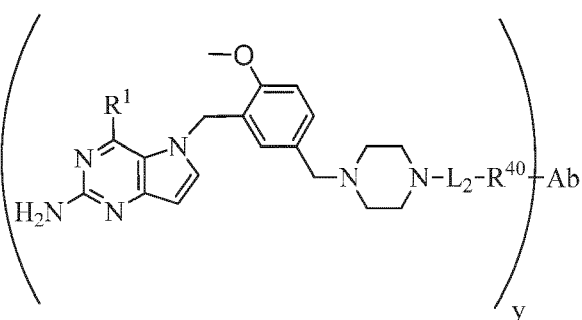
a HCDR1 comprising the amino acid sequence of SEQ ID NO: 4;
 a HCDR2 comprising the amino acid sequence of SEQ ID NO: 5;
 a HCDR3 comprising the amino acid sequence of SEQ ID NO: 3;
 a LCDR1 comprising the amino acid sequence of SEQ ID NO: 14;
 a LCDR2 comprising the amino acid sequence of SEQ ID NO: 15; and
 a LCDR3 comprising the amino acid sequence of SEQ ID NO: 16;

- (c) an antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 17;
 (d) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9, and a light chain comprising the amino acid sequence of SEQ ID NO: 19;
 (e) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 21, and a light chain comprising the amino acid sequence of SEQ ID NO: 19;
 (f) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23, and a light chain comprising the amino acid sequence of SEQ ID NO: 19; or
 (g) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 32, and a light chain comprising the amino acid sequence of SEQ ID NO: 19.

4. The conjugate of any of claims 1-3, wherein Ab is a human or humanized anti-HER2 antibody.
 5. The conjugate of any of claims 1-4, wherein Ab comprises a modified Fc region.
 6. The conjugate of any of claims 1-5, wherein Ab comprises cysteine at one or more of the following positions (all positions by EU numbering):
 (a) positions 152, 360 and 375 of the antibody heavy chain, and
 (b) positions 107, 159, and 165 of the antibody light chain.
 7. The conjugate of any of claims 1-6, wherein Ab comprises cysteines at positions 152 and 375 of the antibody heavy chains (all positions by EU numbering).
 8. The conjugate of any of claims 1-7, wherein the antibody conjugate of Formula (II) comprises the structure of Formula (IIa) or Formula (IIb):



Formula (IIa)



Formula (IIb)

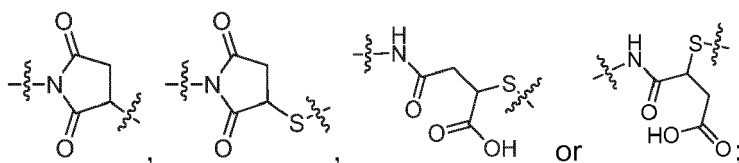
wherein:

R¹ is -NHR²;

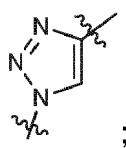
R² is -C₄-C₆alkyl;

L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -C(=O)(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_n-,
 -C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,
 -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-,
 -C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-; or

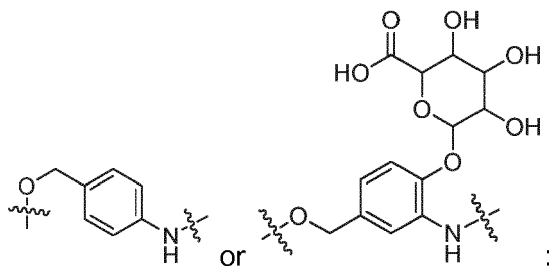
R⁴⁰ is



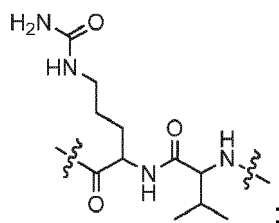
X₄ is



X₂ is



X₃ is



each n is independently selected from 1, 2, 3, and 4;

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18, and
y is an integer from 1 to 16.

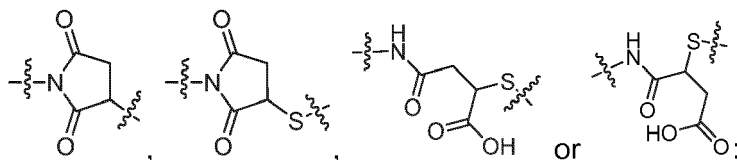
9. The conjugate of any of claims 1-7, wherein

R¹ is -NHR²;

R² is -C₄-C₆alkyl;

L₂ is -(CH₂)_n- or -C(=O)(CH₂)_n;

R⁴⁰ is

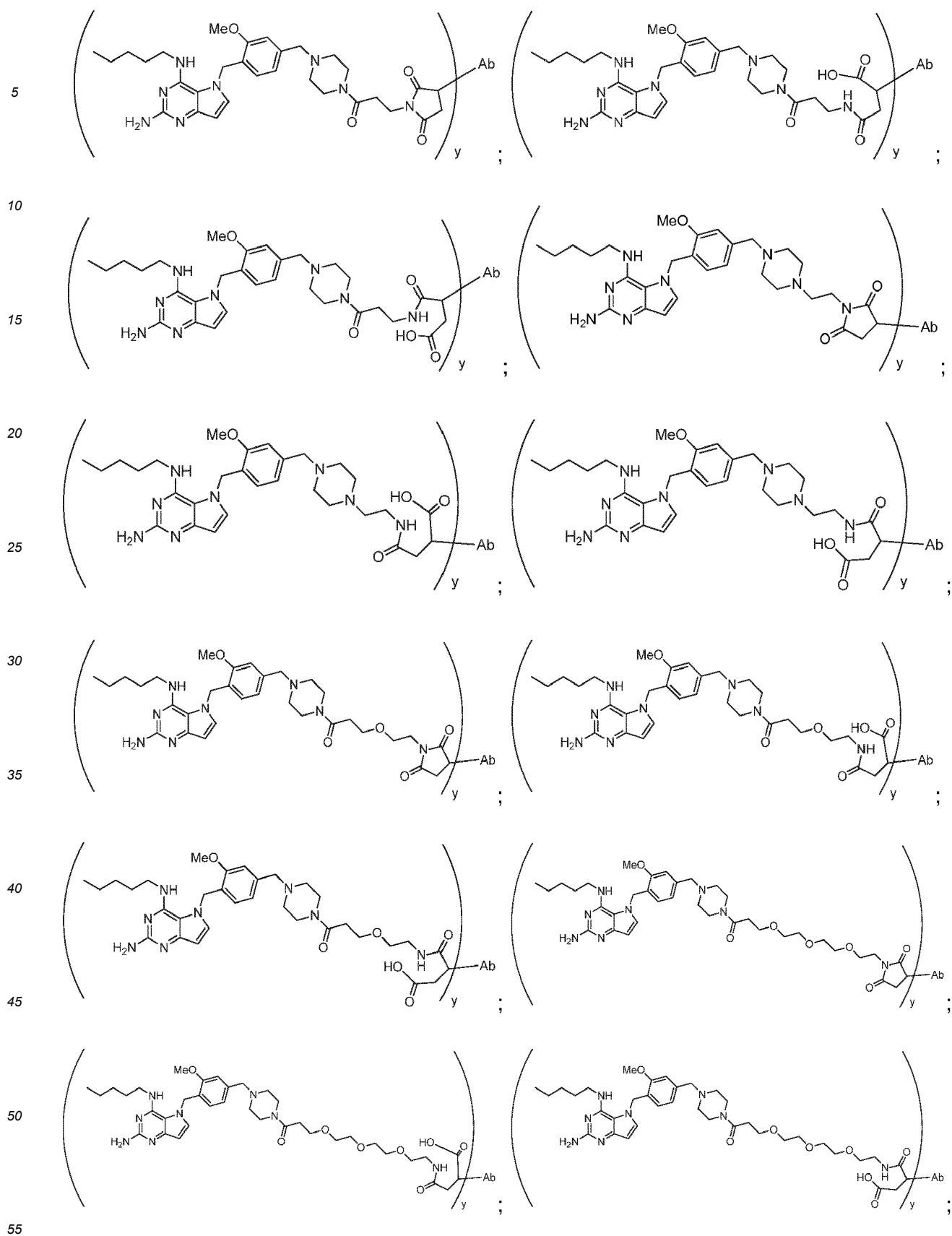


and

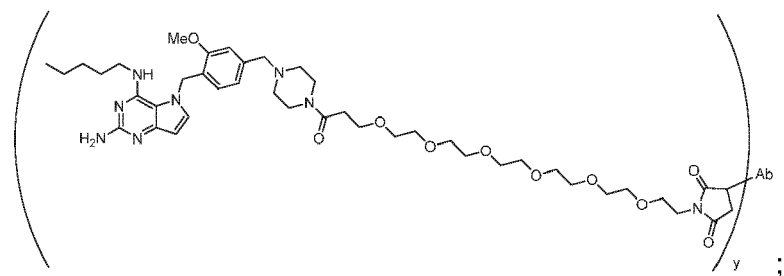
each n is independently selected from 1, 2, 3, and 4, and
y is an integer from 1 to 16.

10. The conjugate of any of claims 1-9, wherein the conjugate has a hydrophobicity index of 0.8 or greater, as determined
by hydrophobic interaction chromatography.

11. A conjugate comprising any of the following formulas:

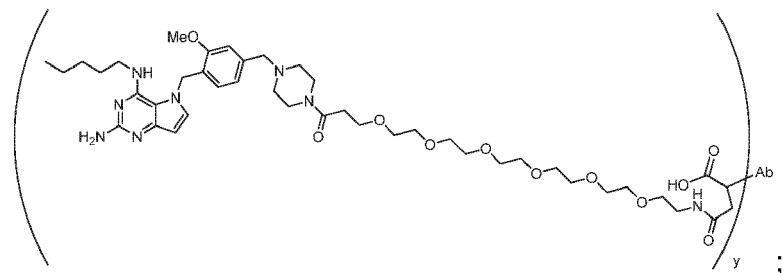


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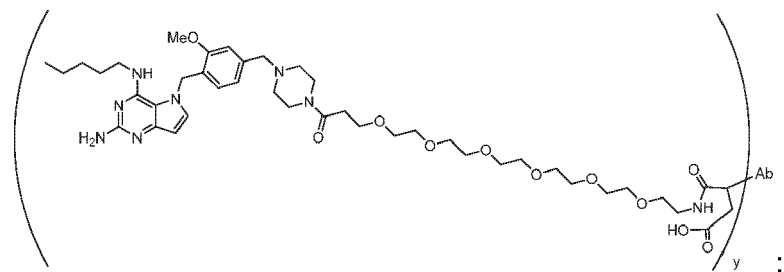
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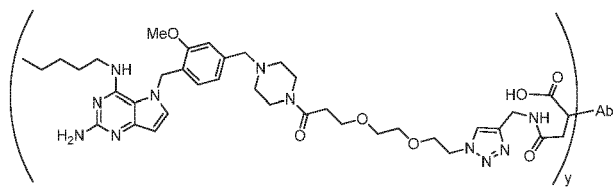
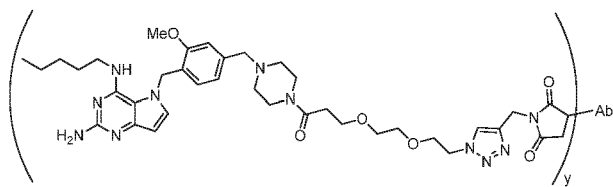
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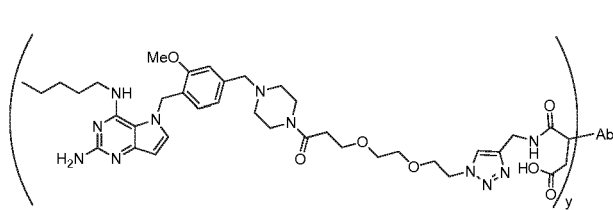


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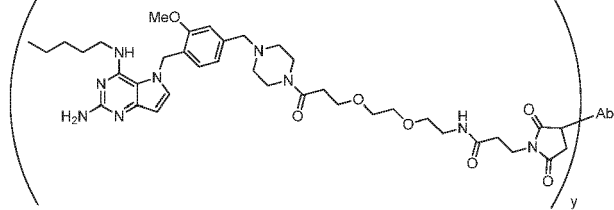
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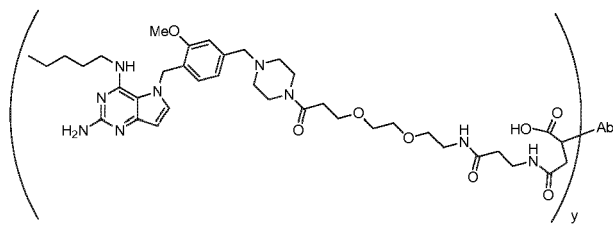
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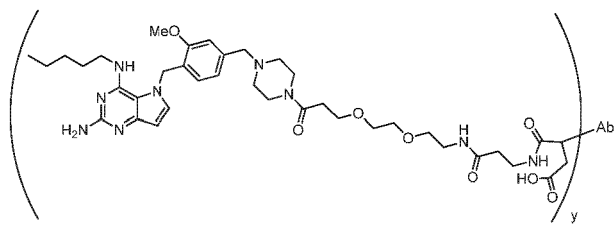
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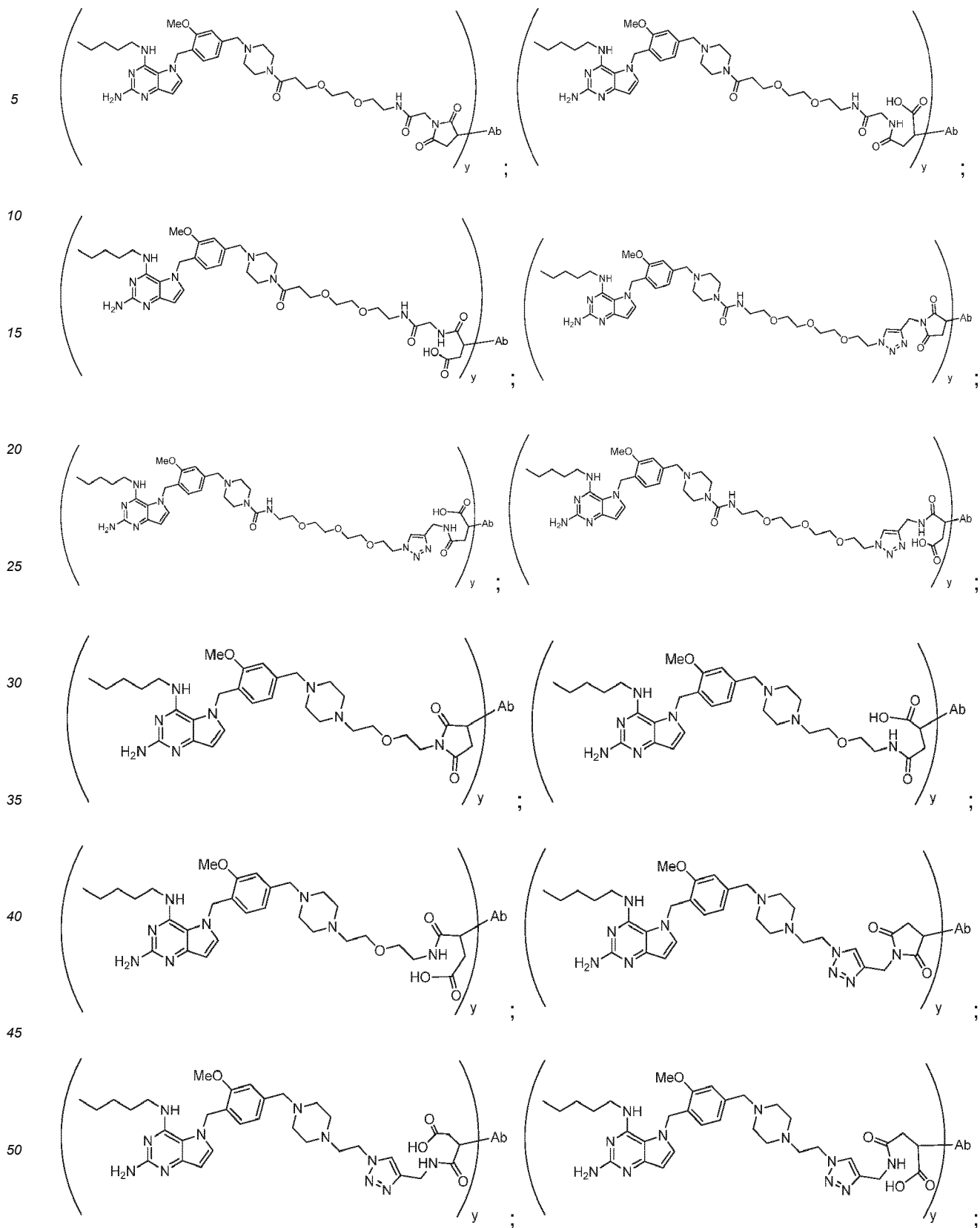


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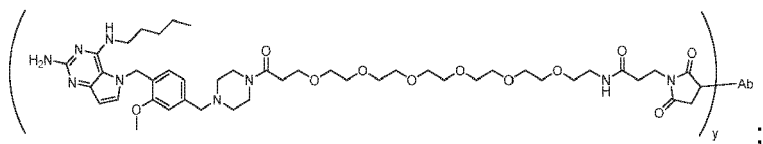


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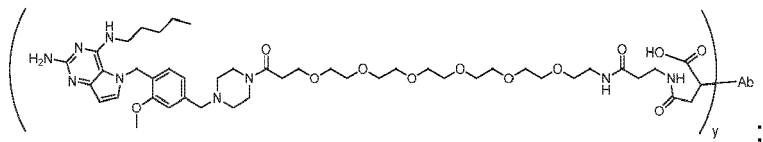




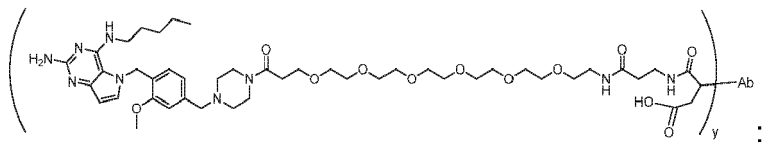
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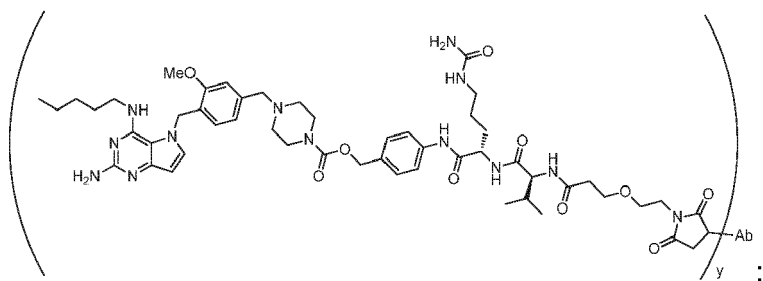
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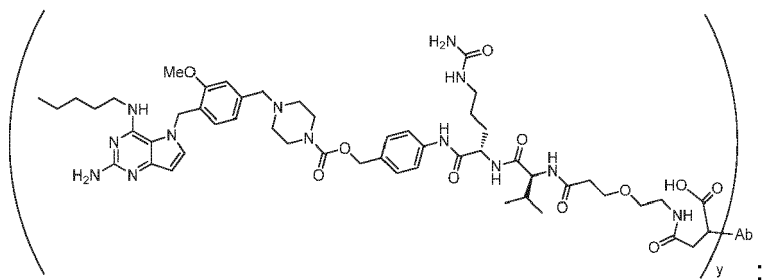


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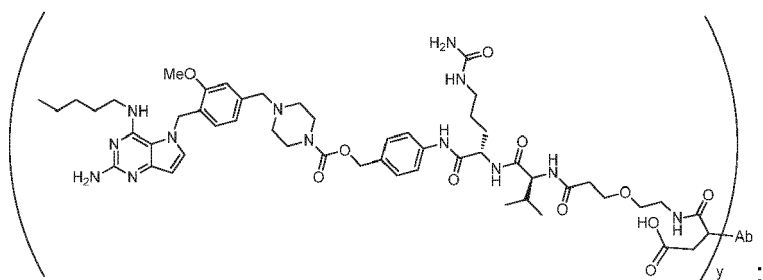
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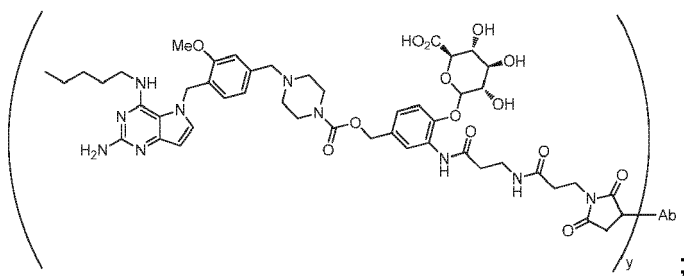


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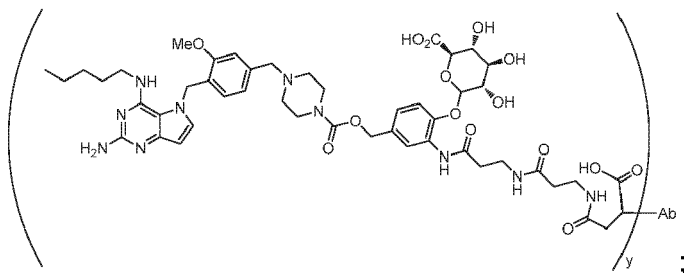
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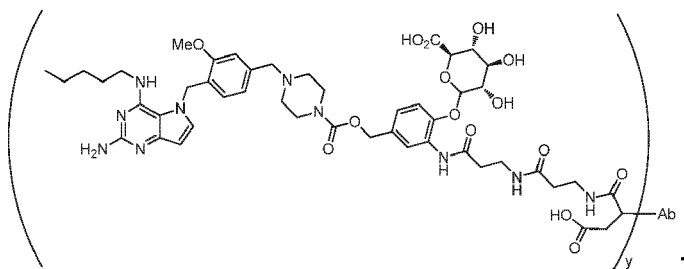


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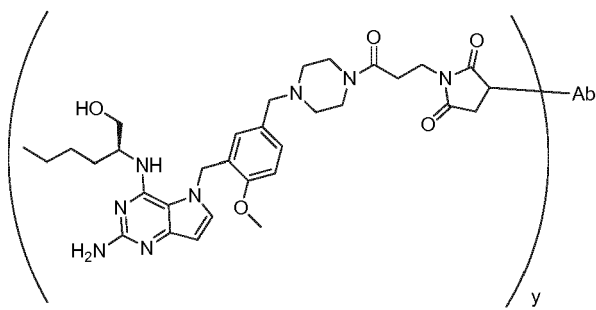
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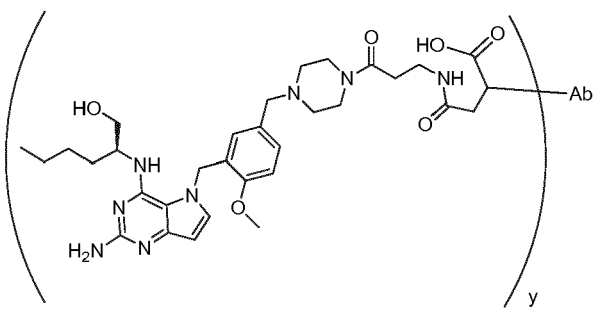
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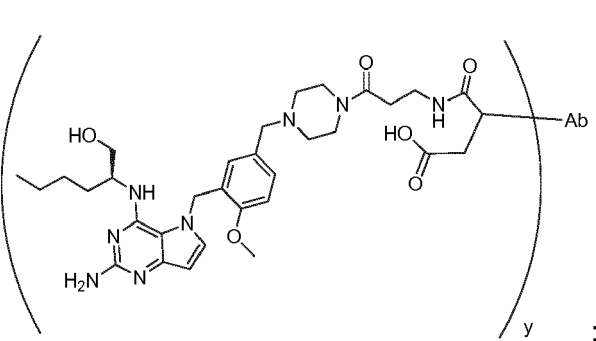
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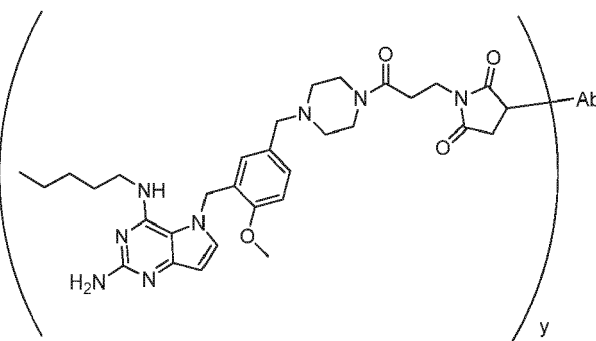


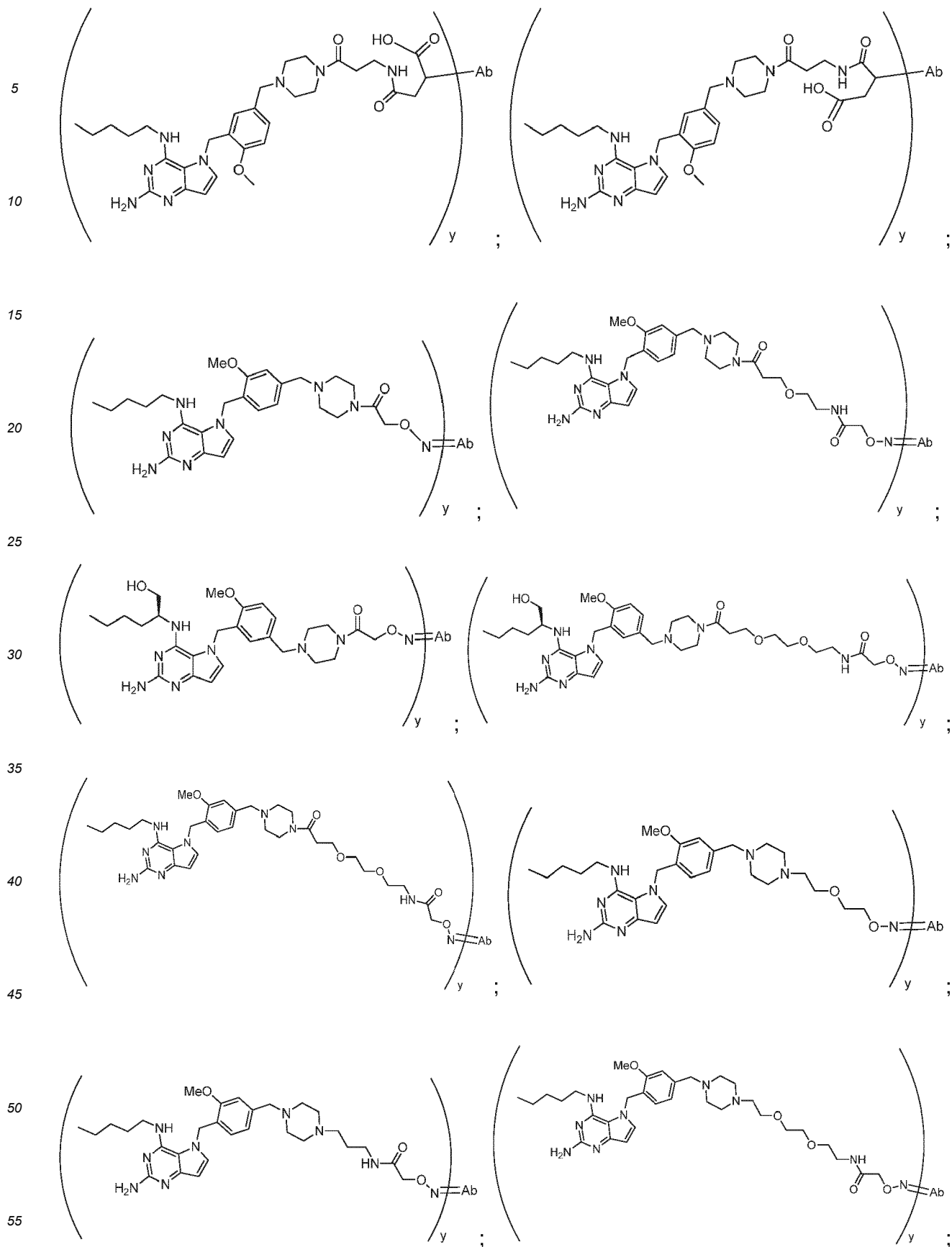
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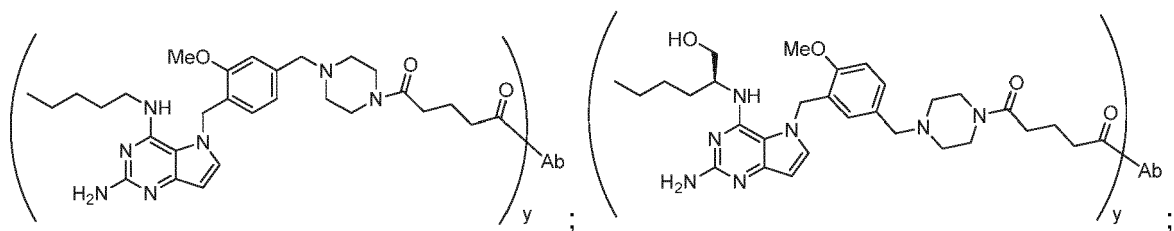


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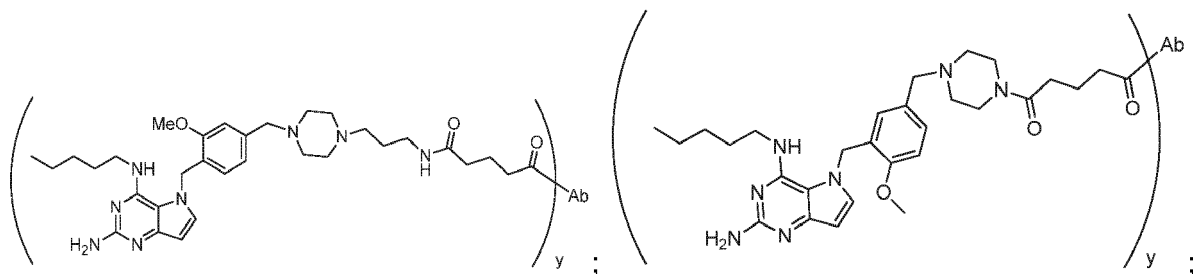




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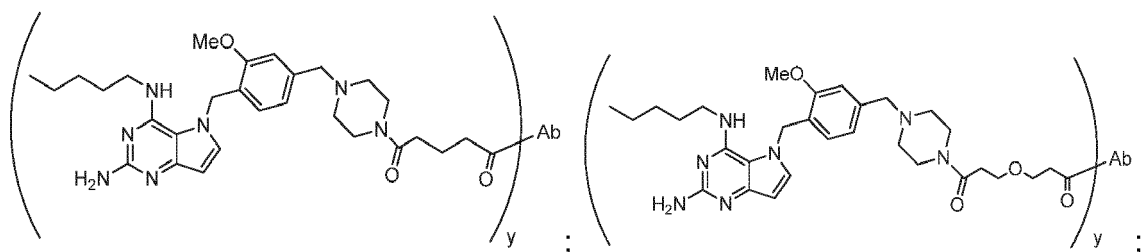
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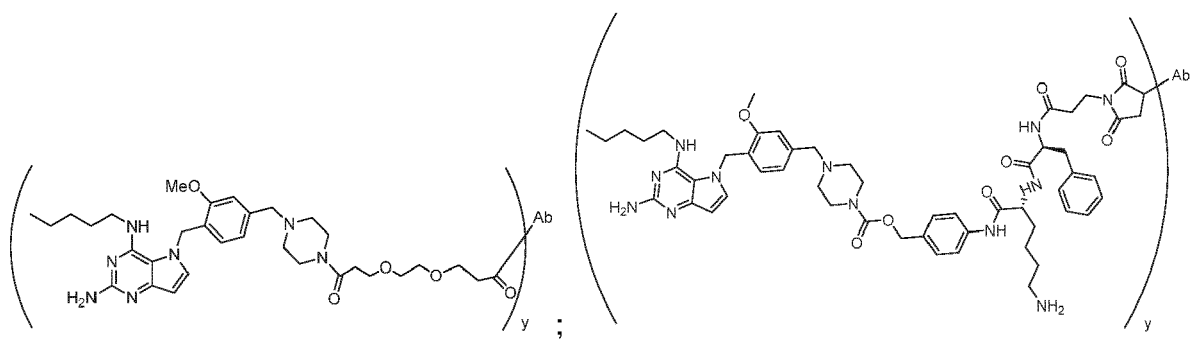
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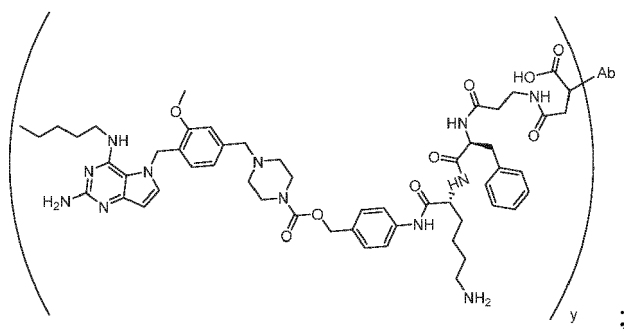
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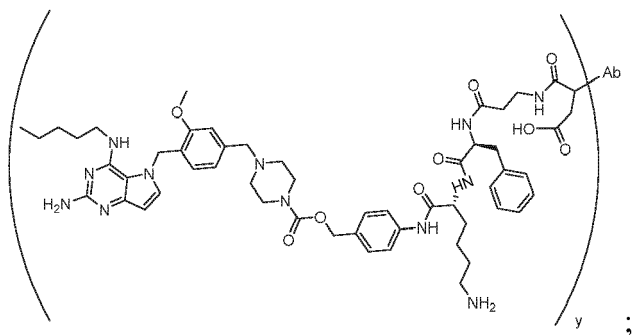
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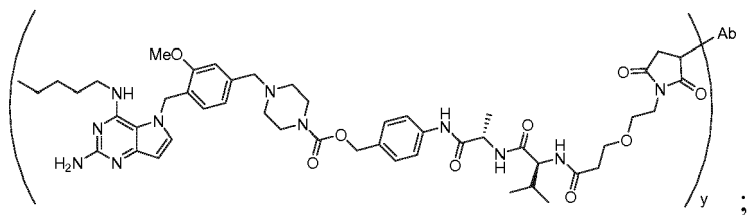
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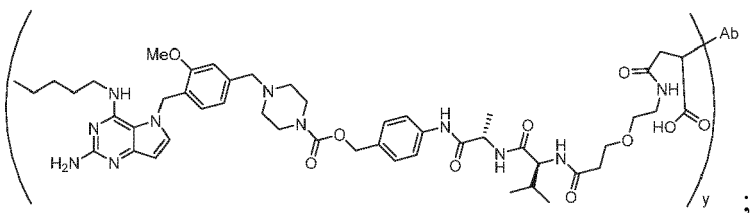


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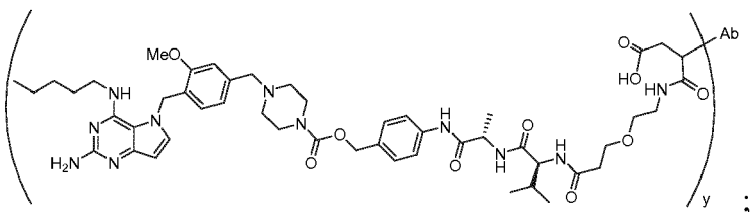


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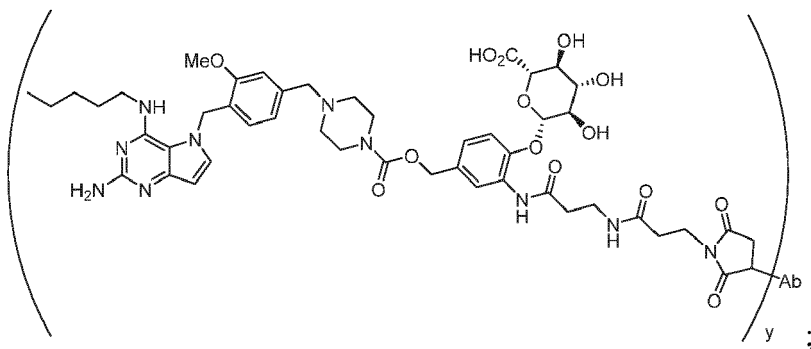
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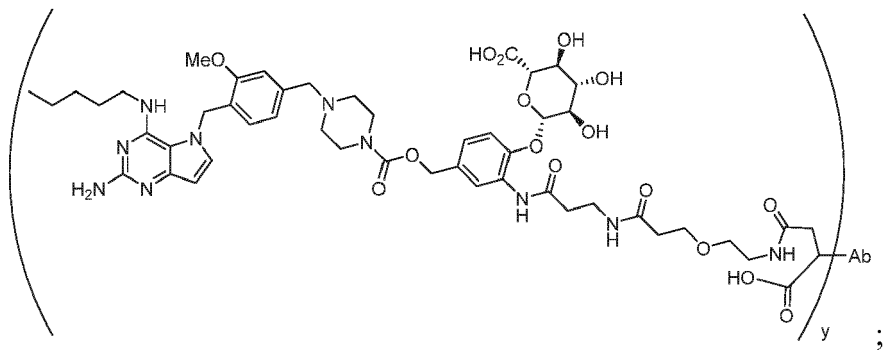
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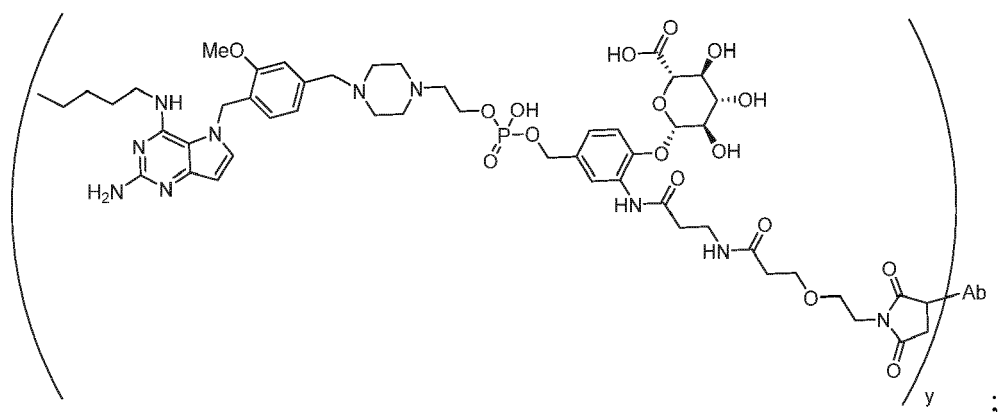
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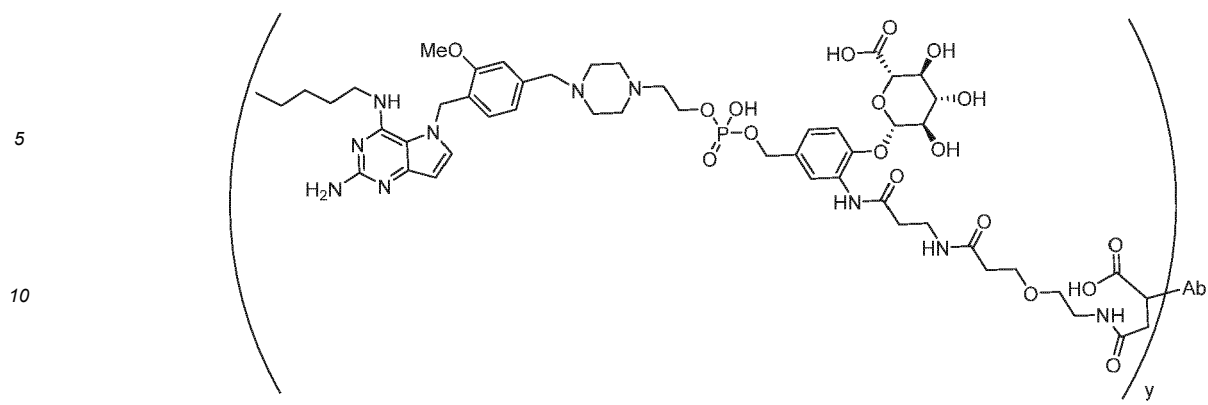


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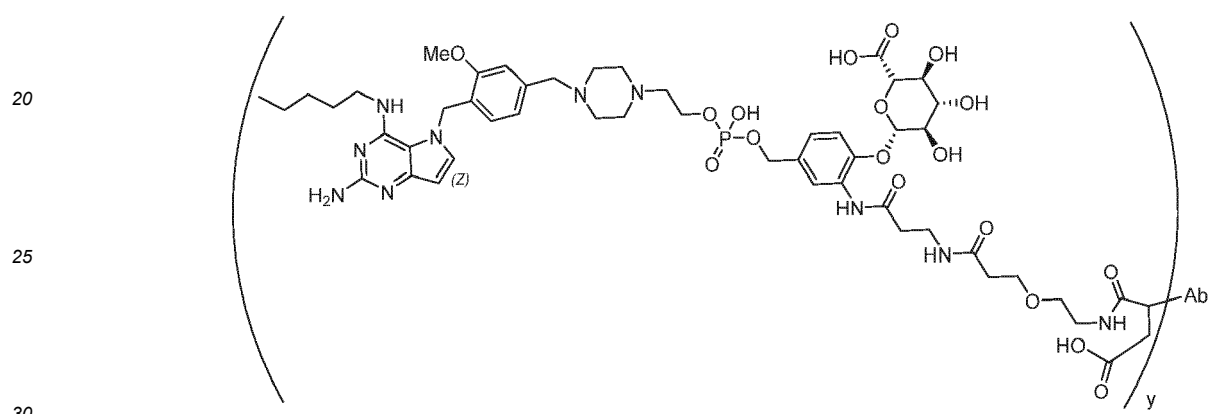
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and



wherein Ab is an antibody or antigen binding fragment thereof that specifically binds to human HER2, and y is an integer from 1 to 4.

12. The conjugate of claim 11, wherein Ab is selected from trastuzumab, pertuzumab, margetuximab, or HT-19, or a site-specific cysteine mutant thereof, wherein the site-specific cysteine mutant comprises cysteine at one or more of the following positions (all positions by EU numbering):

- (a) positions 152, 360 and 375 of the antibody heavy chain, and
(b) positions 107, 159, and 165 of the antibody light chain.

13. The conjugate of claim 11, wherein Ab is selected from any of the following:

- (a) an antibody that comprises:

- a heavy chain complementary determining region 1 (HCDR1) comprising the amino acid sequence of SEQ ID NO: 1;
a heavy chain complementary determining region 2 (HCDR2) comprising the amino acid sequence of SEQ ID NO: 2;
a heavy chain complementary determining region 3 (HCDR3) comprising the amino acid sequence of SEQ ID NO: 3;
a light chain complementary determining region 1 (LCDR1) comprising the amino acid sequence of SEQ ID NO: 11;
a light chain complementary determining region 2 (LCDR2) comprising the amino acid sequence of SEQ ID NO: 12; and
a light chain complementary determining region 3 (LCDR3) comprising the amino acid sequence of SEQ ID NO: 13;

(b) an antibody that comprises:

- a HCDR1 comprising the amino acid sequence of SEQ ID NO: 4;
- a HCDR2 comprising the amino acid sequence of SEQ ID NO: 5;
- a HCDR3 comprising the amino acid sequence of SEQ ID NO: 3;
- a LCDR1 comprising the amino acid sequence of SEQ ID NO: 14;
- a LCDR2 comprising the amino acid sequence of SEQ ID NO: 15; and
- a LCDR3 comprising the amino acid sequence of SEQ ID NO: 16;

(c) an antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 17;

(d) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9, and a light chain comprising the amino acid sequence of SEQ ID NO: 19;

(e) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 21, and a light chain comprising the amino acid sequence of SEQ ID NO: 19;

(f) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23, and a light chain comprising the amino acid sequence of SEQ ID NO: 19; or

(g) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 32, and a light chain comprising the amino acid sequence of SEQ ID NO: 19.

14. The conjugate of claim 11, wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9, and a light chain comprising the amino acid sequence of SEQ ID NO: 19.

15. The conjugate of claim 14, wherein the compound is attached to cysteines at positions 152 and 375 of the antibody heavy chain (all positions by EU numbering).

16. The conjugate of claim 11, wherein y is about 3 to 4.

17. The conjugate of any of claims 11-16, wherein the conjugate has a hydrophobicity index of 0.8 or greater, as determined by hydrophobic interaction chromatography.

18. A pharmaceutical composition comprising one or more conjugates of any of claims 1-17 and a pharmaceutically acceptable carrier.

19. The conjugate of any of claims 1-17 or the pharmaceutical composition of claim 18 for use in treating a HER2-positive cancer in a subject in need thereof.

20. The conjugate for use according to claim 19, wherein the conjugate is capable of suppressing the HER2-positive cancer for a sustained period and/or reducing recurrence of the HER2-positive cancer, when compared to an anti-HER2 antibody alone.

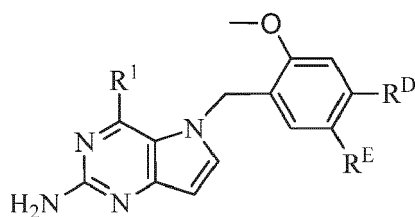
21. The conjugate for use according to claim 19, wherein the HER2-positive cancer is selected from gastric cancer, esophageal cancer, gastroesophageal junction adenocarcinoma, colon cancer, rectal cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, endometrial cancer, bladder cancer, urinary tract cancer, pancreatic cancer, lung cancer, prostate cancer, osteosarcoma, neuroblastoma, glioblastoma, and head and neck cancer.

22. The conjugate for use according to claim 19, wherein the conjugate is administered to the subject intravenously, intratumorally, or subcutaneously.

23. The conjugate for use according to claim 19, wherein the conjugate is administered at a dose of about 0.01-20 mg per kg of body weight.

24. The conjugate for use according to claim 19 further comprising administering a second agent to the subject.

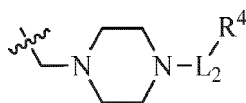
25. A compound having the structure of Formula (I), or the pharmaceutically acceptable salts thereof:



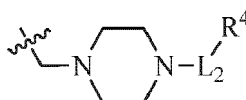
Formula (I)

wherein:

R^D is



and R^E is H; or R^E is



and R^D is H;

R¹ is -NHR² or -NHCHR²R³;

R² is -C₃-C₆alkyl or -C₄-C₆alkyl;

R³ is L₁OH;

L₁ is -(CH₂)_n-;

L₂ is -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_n-,

-(CH₂)_nNHC(=O)(CH₂)_nC(=O)NH(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)(CH₂)_n-,

-C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,

-C(=O)((CH₂)_nO)_t(CH₂)_nC(=O)NH(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,

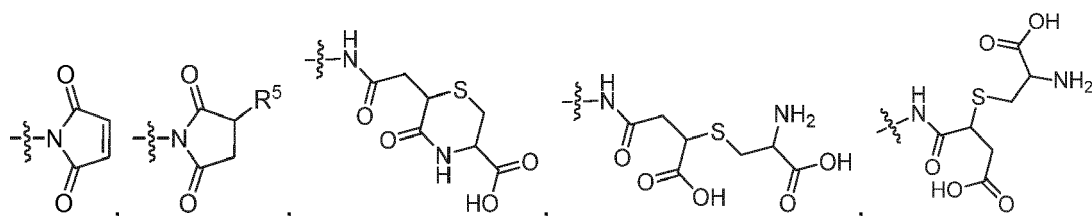
-C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)X₂X₃C(=O)(CH₂)_n-,

-C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)X₂C(=O)(CH₂)_nNHC(=O)((CH₂)_nO)_t(CH₂)_n-,

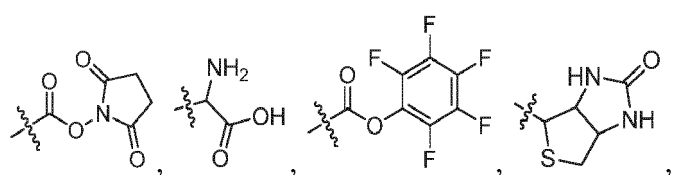
-C(=O)(CH₂)_nC(R₇)₂-, -C(=O)(CH₂)_nC(R₇)₂SS(CH₂)_nNHC(=O)(CH₂)_n-,

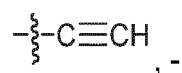
-(CH₂)_nX₂C(=O)(CH₂)_nNHC(=O)((CH₂)_nO)_t(CH₂)_n- or -C(=O)(CH₂)_nC(=O)NH(CH₂)_n;

R⁴ is

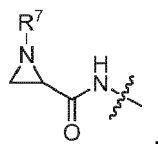
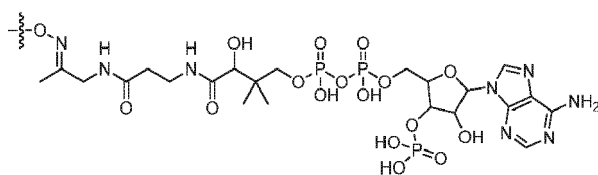
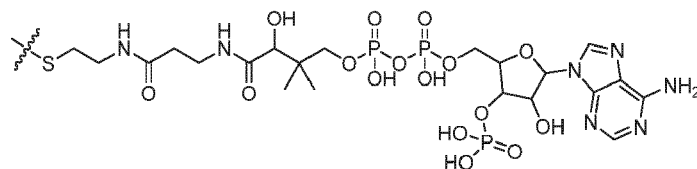
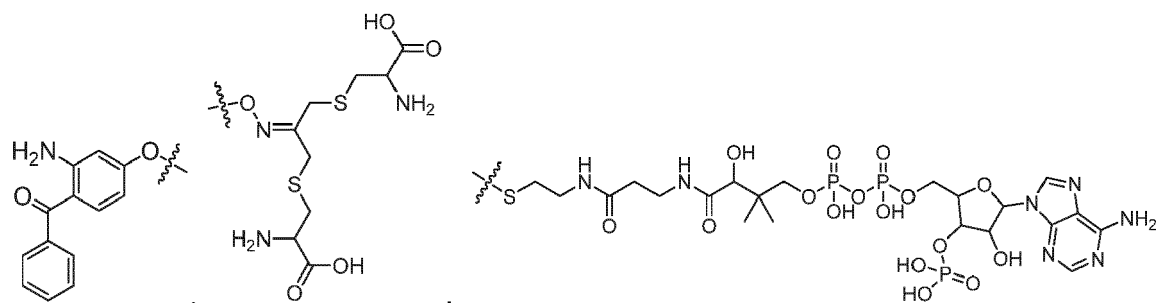
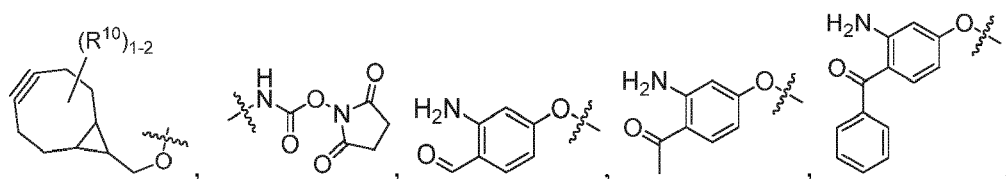
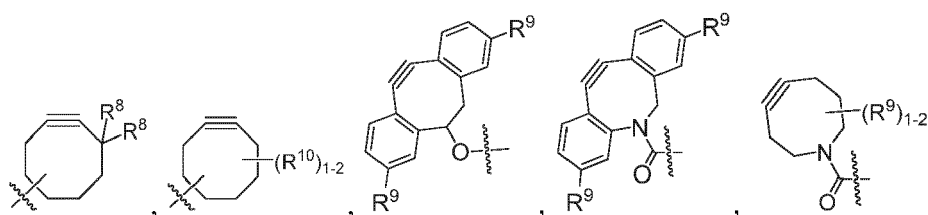


-ONH₂, -NH₂,

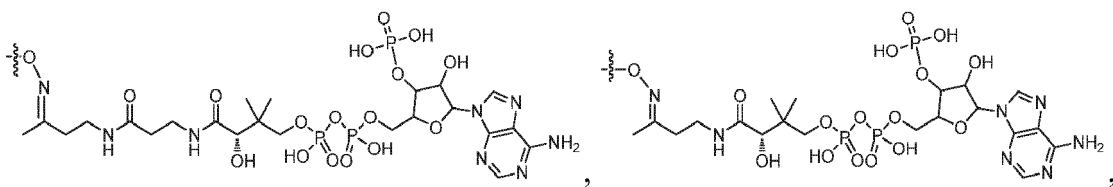


-N₃,

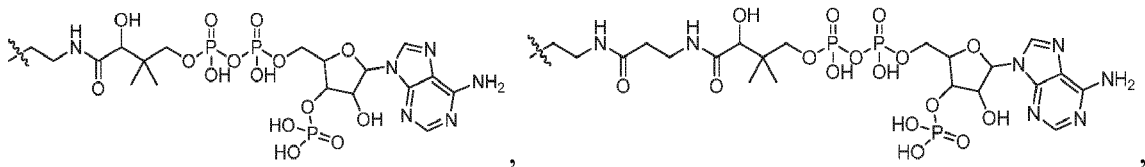
NHC(=O)CH=CH₂, -SH, -SR⁷, -OH -SSR⁶, -S(=O)₂(CH=CH₂), -(CH₂)₂S(=O)₂(CH=CH₂), -NHS(=O)₂(CH=CH₂),
-NHC(=O)CH₂Br, -NHC(=O)CH₂I, -C(O)NHNH₂,

-CO₂H, -C(O)NHNH₂,

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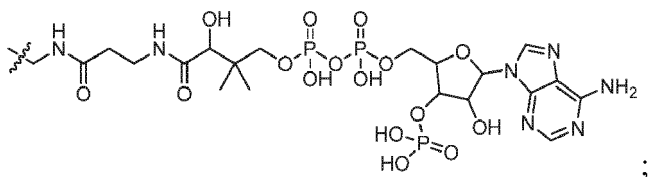
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or

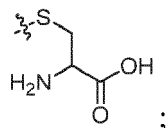
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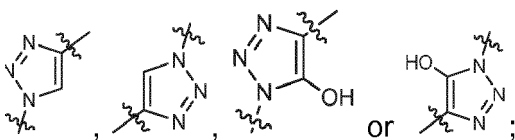
R⁵ is

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X₁ is

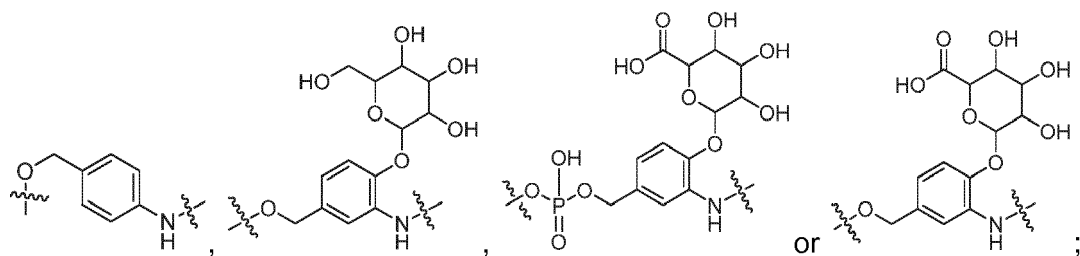
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X₂ is

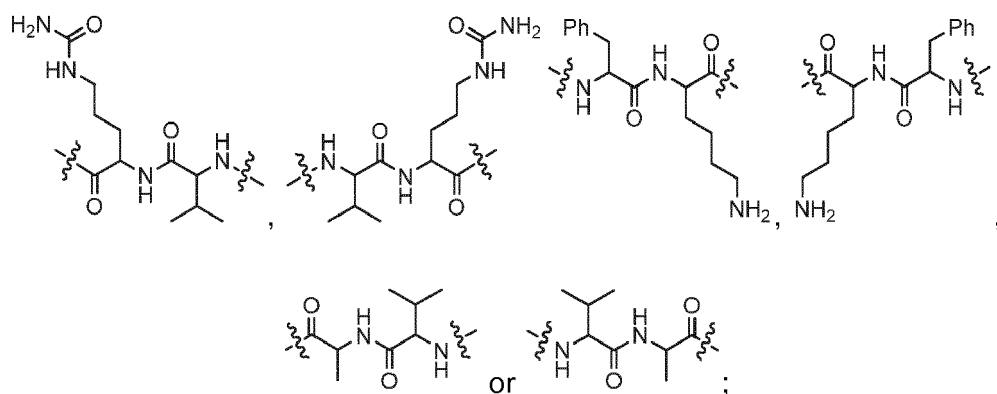
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X₃ is

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R^6 is 2-pyridyl or 4-pyridyl;

each R^7 is independently selected from H and C_1 - C_6 alkyl;

each R^8 is independently selected from H, C_1 - C_6 alkyl, F, Cl, and -OH;

each R^9 is independently selected from H, C_1 - C_6 alkyl, F, Cl, - NH_2 , - OCH_3 , - OCH_2CH_3 , - $N(CH_3)_2$, -CN, - NO_2 and -OH;

each R^{10} is independently selected from H, C_1 - C_6 alkyl, fluoro, benzyloxy substituted with - $C(=O)OH$, benzyl substituted with - $C(=O)OH$, C_{1-4} alkoxy substituted with - $C(=O)OH$ and C_{1-4} alkyl substituted with - $C(=O)OH$;

each m is independently selected from 1, 2, 3, and 4;

each n is independently selected from 1, 2, 3, and 4;

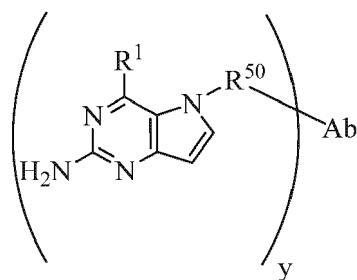
and

each t is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

26. A conjugate comprising the compound of claim 25 attached to an antibody or antigen binding fragment thereof.

Patentansprüche

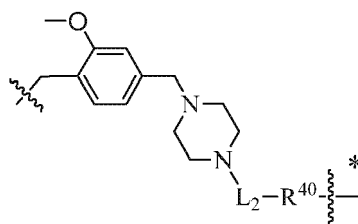
1. Konjugat der Formel (II) oder ein pharmazeutisch unbedenkliches Salz davon:



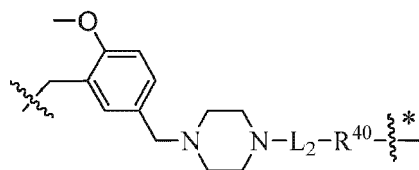
Formel (II),

wobei:

R^{50} für

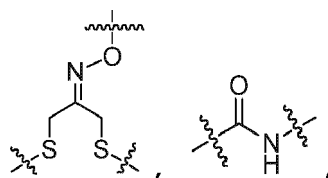
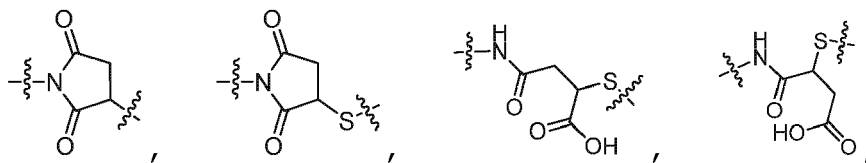
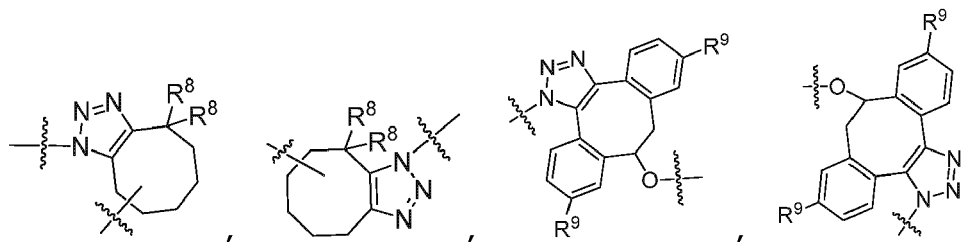
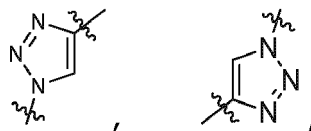


oder

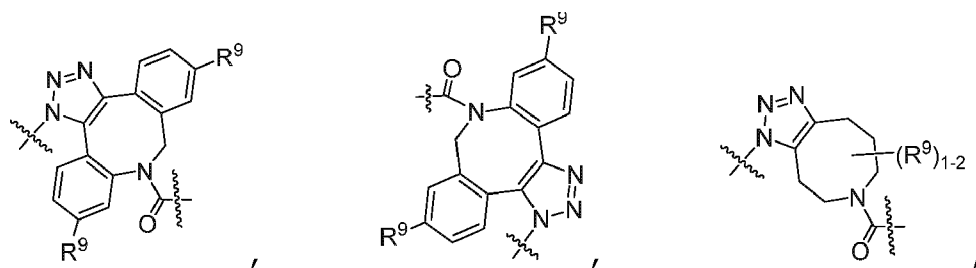


steht, wobei das * den Anbindungspunkt an Ab kennzeichnet;

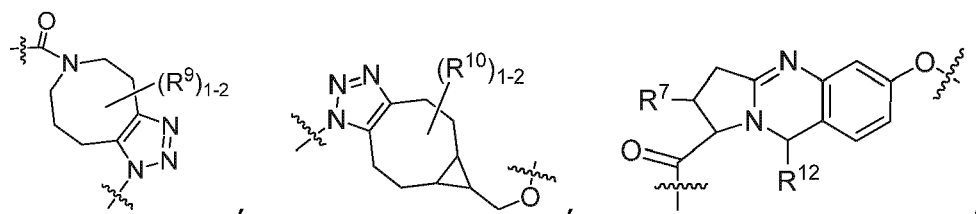
Ab für einen Antikörper oder ein antigenbindendes Fragment davon, der/das spezifisch an humanes HER2 bindet, steht,

R¹ für -NHR² oder -NHCHR²R³ steht,R² für -C₃-C₆-Alkyl oder -C₄-C₆-Alkyl steht,R³ für L₁OH steht,L₁ für -(CH₂)_m- steht,L₂ für -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -(CH₂)_nNHC(=O)(CH₂)_n-, -(CH₂)_nN-HC(=O)(CH₂)_nC(=O)NH(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-, -C(=O)(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_n-,-C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)(CH₂)_nO)_t(CH₂)_nNHC(=O)(CH₂)_n-,-C(=O)((CH₂)_nO)_t(CH₂)_nC(=O)NH(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,-C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)X₂X₃C(=O)(CH₂)_n-, -C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n-,-C(=O)X₂C(=O)(CH₂)_nNHC(=O)((CH₂)_nO)_t(CH₂)_n-, -C(=O)(CH₂)_nC(R₇)₂-, -C(=O)(CH₂)_nC(R₇)₂SS(CH₂)_nN-HC(=O)(CH₂)_n-, -(CH₂)_nX₂C(=O)(CH₂)_nNHC(=O)((CH₂)_nO)_t(CH₂)_n oder -C(=O)(CH₂)_nC(=O)NH(CH₂)_n steht,R⁴⁰ für-S-, -NHC(=O)CH₂-, -S(=O)₂CH₂CH₂-, -(CH₂)₂S(=O)₂CH₂CH₂-, -NHS(=O)₂CH₂CH₂-, -NHC(=O)CH₂CH₂-,
-CH₂NHCH₂CH₂-, -NHCH₂CH₂-,

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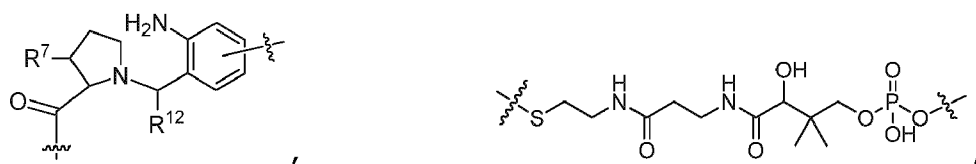
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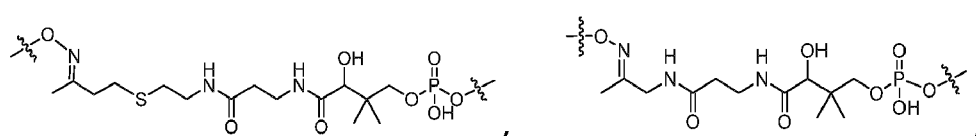
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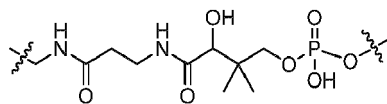
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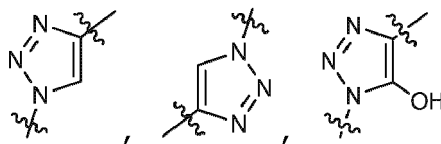
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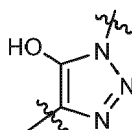
oder



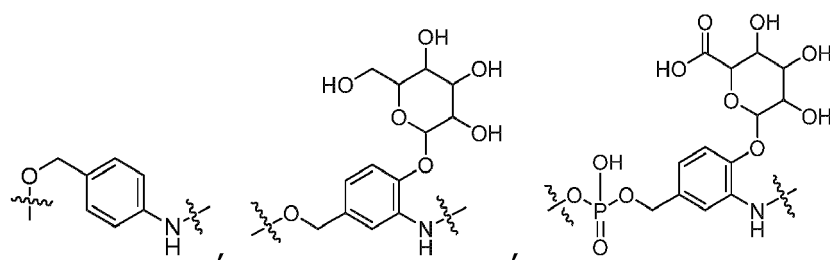
steht,
X₁ für



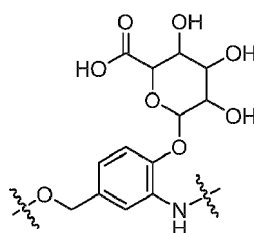
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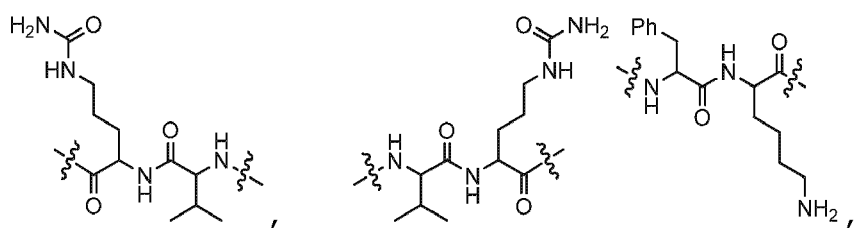
steht,
X₂ für

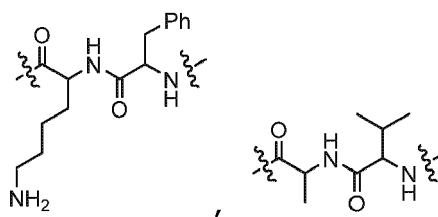


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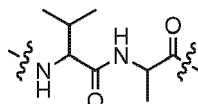


steht,
X₃ für





oder



steht,

R⁷ jeweils unabhängig aus H und C₁-C₆-Alkyl ausgewählt ist,

R⁸ jeweils unabhängig aus H, C₁-C₆-Alkyl, F, Cl und -OH ausgewählt ist,

R⁹ jeweils unabhängig aus H, C₁₋₆-Alkyl, F, Cl, -NH₂, -OCH₃, -OCH₂CH₃, -N(CH₃)₂, -CN, -NO₂ und -OH ausgewählt ist,

R¹⁰ jeweils unabhängig aus H, C₁₋₆-Alkyl, Fluor, durch -C(=O)OH substituiertem Benzyloxy, durch -C(=O)OH substituiertem Benzyl, durch -C(=O)OH substituiertem C₁₋₄-Alkoxy und durch -C(=O)OH substituiertem C₁₋₄-Alkyl ausgewählt ist,

R¹² für H, Methyl oder Phenyl steht,

m jeweils unabhängig aus 1, 2, 3 und 4 ausgewählt ist,

n jeweils unabhängig aus 1, 2, 3 und 4 ausgewählt ist,

t jeweils unabhängig aus 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 und 18 ausgewählt ist und

y für eine ganze Zahl von 1 bis 16 steht.

2. Konjugat nach Anspruch 1, wobei Ab aus Trastuzumab, Pertuzumab, Margetuximab oder HT-19 oder einer ortsspezifischen Cysteinmutante davon ausgewählt ist, wobei die ortsspezifische Cysteinmutante in einer oder mehreren der folgenden Positionen (alle Positionen gemäß EU-Nummerierung) Cystein umfasst:

(a) Positionen 152, 360 und 375 der schweren Kette des Antikörpers und

(b) Positionen 107, 159 und 165 der leichten Kette des Antikörpers.

3. Konjugat nach Anspruch 1, wobei Ab aus den Folgenden ausgewählt ist:

(a) einem Antikörper, der Folgendes umfasst:

eine Complementary Determining Region 1 der schweren Kette (HCDR1), die die Aminosäuresequenz von SEQ ID NO: 1 umfasst,

eine Complementary Determining Region 2 der schweren Kette (HCDR2), die die Aminosäuresequenz von SEQ ID NO: 2 umfasst,

eine Complementary Determining Region 3 der schweren Kette (HCDR3), die die Aminosäuresequenz von SEQ ID NO: 3 umfasst,

eine Complementary Determining Region 1 der leichten Kette (LCDR1), die die Aminosäuresequenz von SEQ ID NO: 11 umfasst,

eine Complementary Determining Region 2 der leichten Kette (LCDR2), die die Aminosäuresequenz von SEQ ID NO: 12 umfasst, und

eine Complementary Determining Region 3 der leichten Kette (LCDR3), die die Aminosäuresequenz von SEQ ID NO: 13 umfasst,

(b) einem Antikörper, der Folgendes umfasst:

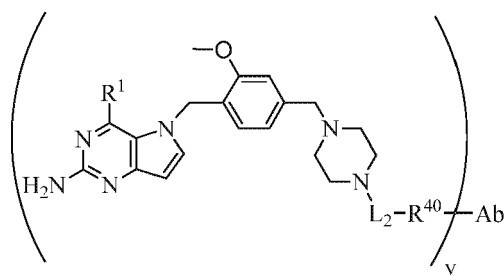
eine HCDR1, die die Aminosäuresequenz von SEQ ID NO: 4 umfasst,

eine HCDR2, die die Aminosäuresequenz von SEQ ID NO: 5 umfasst,

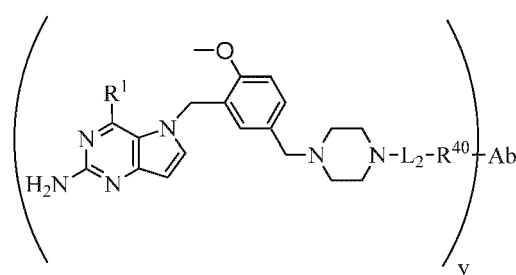
eine HCDR3, die die Aminosäuresequenz von SEQ ID NO: 3 umfasst,

eine LCDR1, die die Aminosäuresequenz von SEQ ID NO: 14 umfasst,
 eine LCDR2, die die Aminosäuresequenz von SEQ ID NO: 15 umfasst, und
 eine LCDR3, die die Aminosäuresequenz von SEQ ID NO: 16 umfasst,

- (c) einem Antikörper, der eine variable Region der schweren Kette, die die Aminosäuresequenz von SEQ ID NO: 7 umfasst, und eine variable Region der leichten Kette, die die Aminosäuresequenz von SEQ ID NO: 17 umfasst, umfasst,
- (d) einem Antikörper, der eine schwere Kette, die die Aminosäuresequenz von SEQ ID NO: 9 umfasst, und eine leichte Kette, die die Aminosäuresequenz von SEQ ID NO: 19 umfasst, umfasst,
- (e) einem Antikörper, der eine schwere Kette, die die Aminosäuresequenz von SEQ ID NO: 21 umfasst, und eine leichte Kette, die die Aminosäuresequenz von SEQ ID NO: 19 umfasst, umfasst,
- (f) einem Antikörper, der eine schwere Kette, die die Aminosäuresequenz von SEQ ID NO: 23 umfasst, und eine leichte Kette, die die Aminosäuresequenz von SEQ ID NO: 19 umfasst, umfasst, oder
- (g) einem Antikörper, der eine schwere Kette, die die Aminosäuresequenz von SEQ ID NO: 32 umfasst, und eine leichte Kette, die die Aminosäuresequenz von SEQ ID NO: 19 umfasst, umfasst.
4. Konjugat nach einem der Ansprüche 1-3, wobei es sich bei Ab um einen humanen oder humanisierten Anti-HER2-Antikörper handelt.
5. Konjugat nach einem der Ansprüche 1-4, wobei Ab eine modifizierte Fc-Region umfasst.
6. Konjugat nach einem der Ansprüche 1-5, wobei Ab in einer oder mehreren der folgenden Positionen (alle Positionen gemäß EU-Nummerierung) Cystein umfasst:
- (a) Positionen 152, 360 und 375 der schweren Kette des Antikörpers und
 (b) Positionen 107, 159 und 165 der leichten Kette des Antikörpers.
7. Konjugat nach einem der Ansprüche 1-6, wobei Ab in den Positionen 152 und 375 der schweren Ketten des Antikörpers (alle Positionen gemäß EU-Nummerierung) Cysteinreste umfasst.
8. Konjugat nach einem der Ansprüche 1-7, wobei das Antikörperkonjugat der Formel (II) die Struktur der Formel (IIa) oder der Formel (IIb) umfasst:



Formel (IIa)



Formel (IIb),

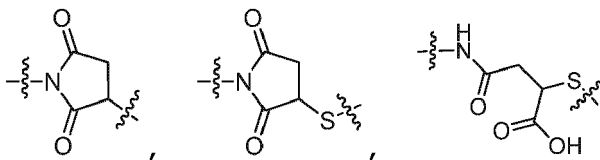
wobei:

R¹ für -NHR² steht,

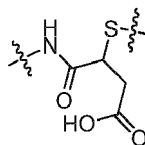
R² für -C₄-C₆-Alkyl steht,

L₂ für -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -C(=O)(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_n-,
 -C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,
 -C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n- oder -C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n- steht,

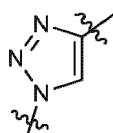
R⁴⁰ für



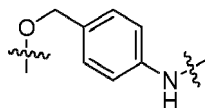
oder



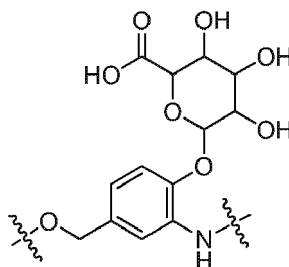
steht,
X₁ für



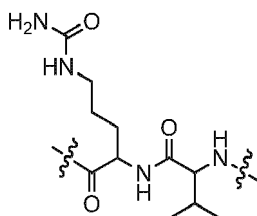
steht, X₂ für



oder



steht, X₃ für



steht,

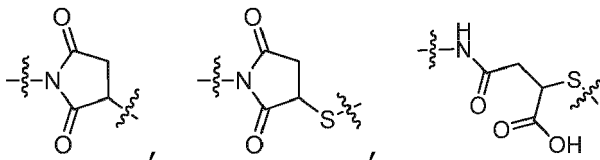
n jeweils unabhängig aus 1, 2, 3 und 4 ausgewählt ist,
t jeweils unabhängig aus 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 und 18 ausgewählt ist und
y für eine ganze Zahl von 1 bis 16 steht.

9. Konjugat nach einem der Ansprüche 1-7, wobei

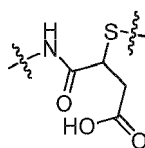
R^1 für $-NHR^2$ steht,

R² für -C₄-C₆-Alkyl steht,

L_2 für $-(CH_2)_n-$ oder $-C(=O)(CH_2)_n$ steht,

R⁴⁰ für

oder



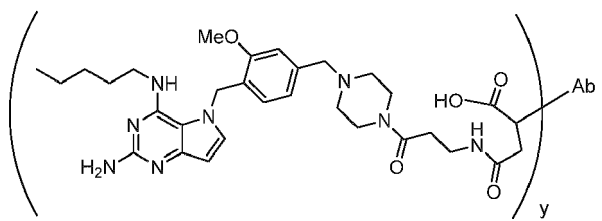
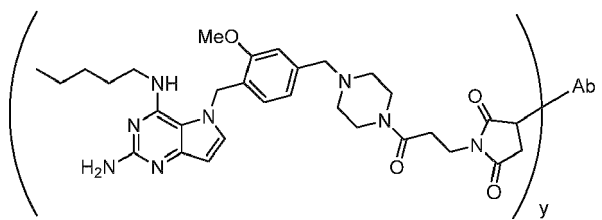
steht,

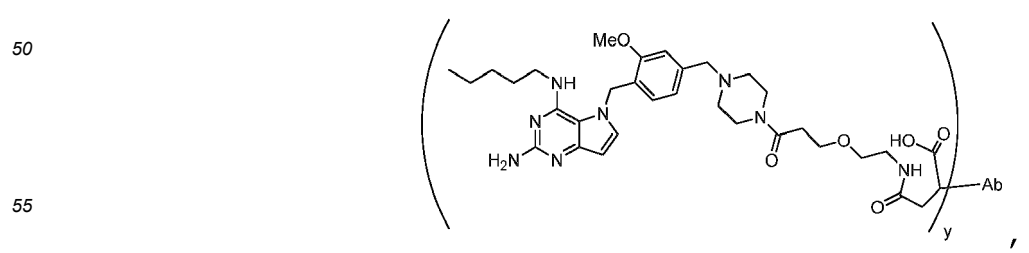
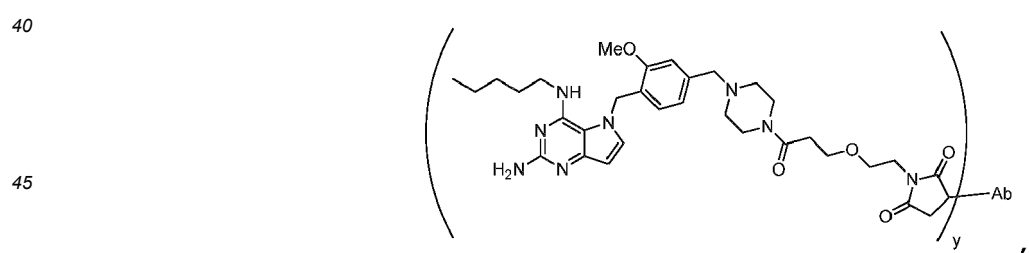
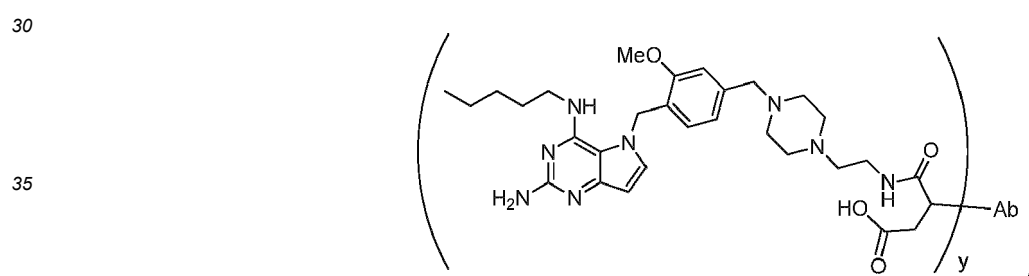
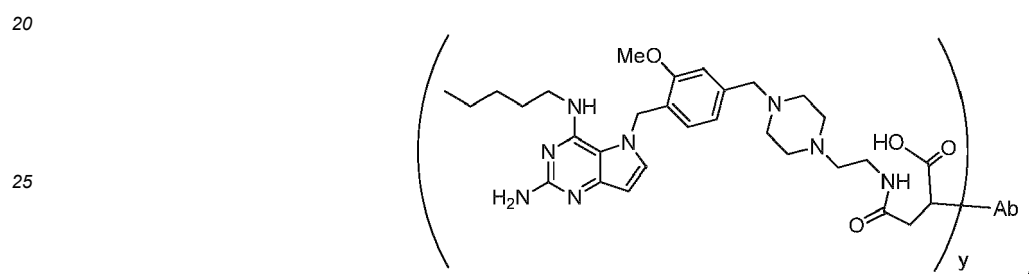
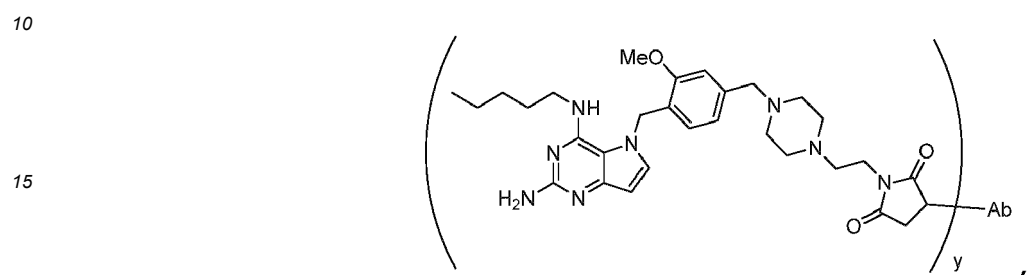
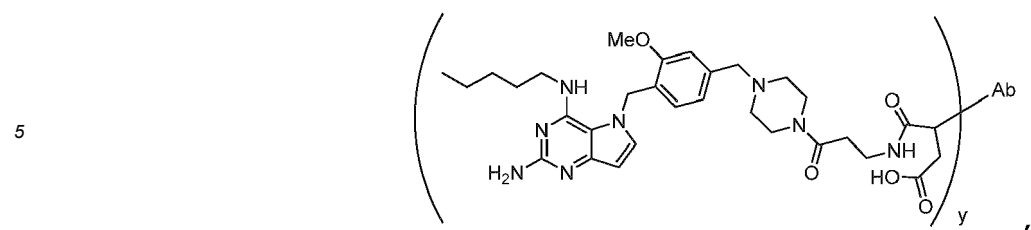
und

n jeweils unabhängig aus 1, 2, 3 und 4 ausgewählt ist und y für eine ganze Zahl von 1 bis 16 steht.

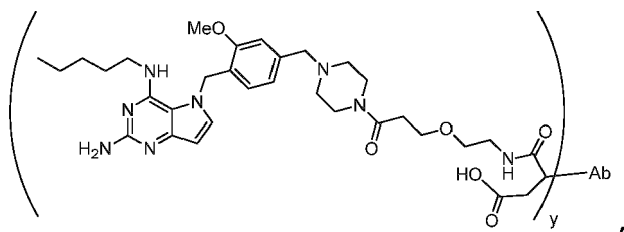
10. Konjugat nach einem der Ansprüche 1-9, wobei das Konjugat einen Hydrophobizitätsindex von 0,8 oder mehr, bestimmt durch Hydrophobe-Wechselwirkungs-Chromatografie, aufweist.

11. Konjugat, umfassend eine der folgenden Formeln:

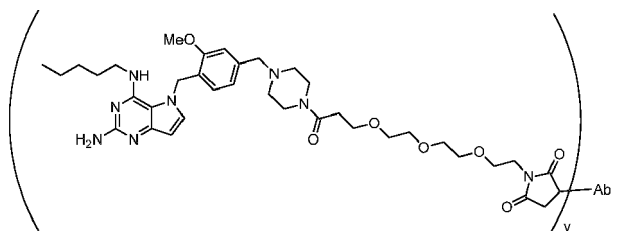




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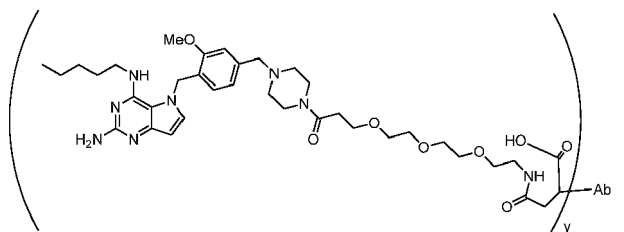


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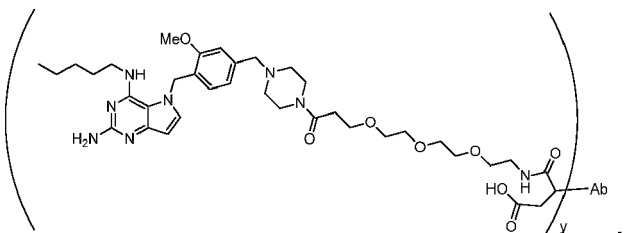
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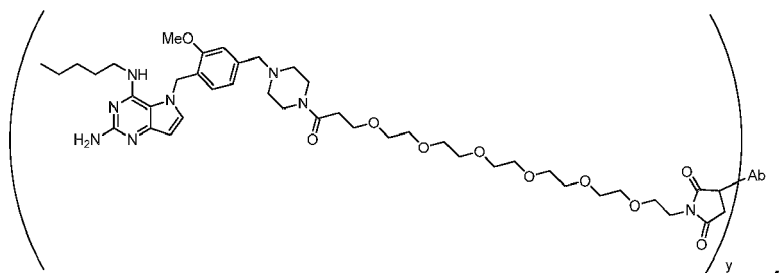
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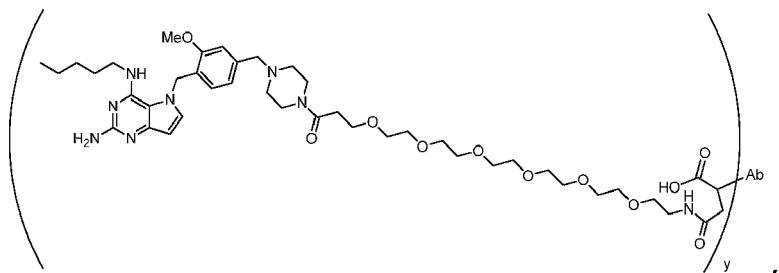
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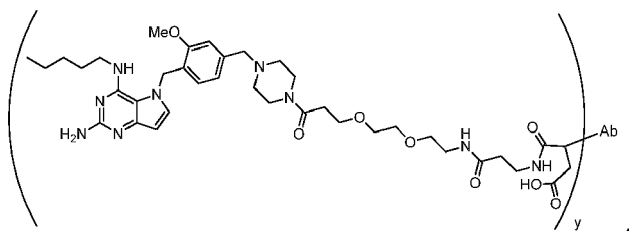
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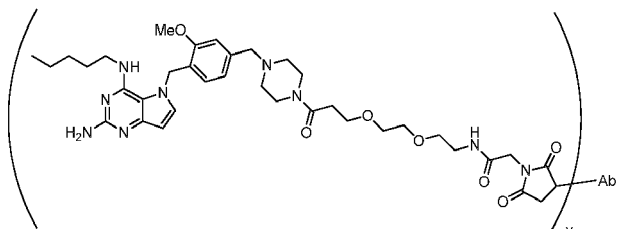


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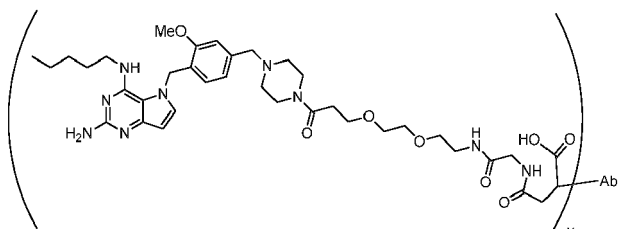


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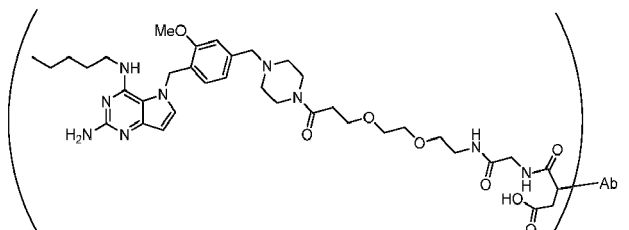
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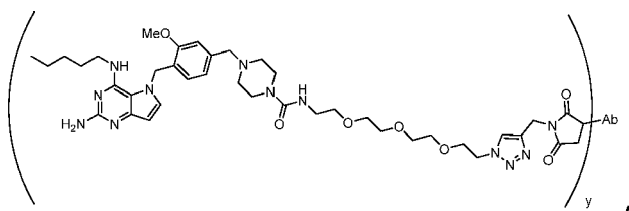
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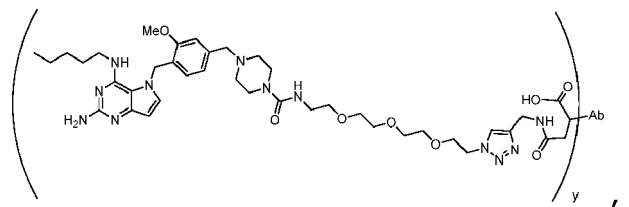
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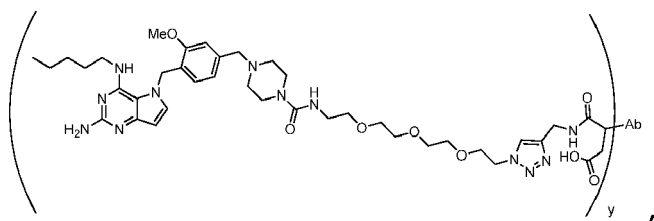
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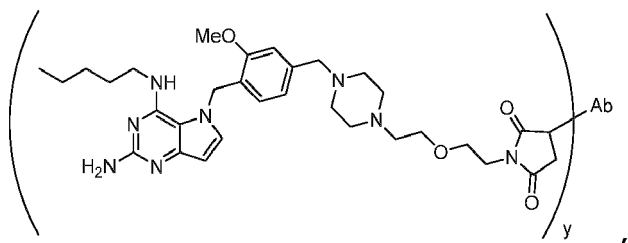


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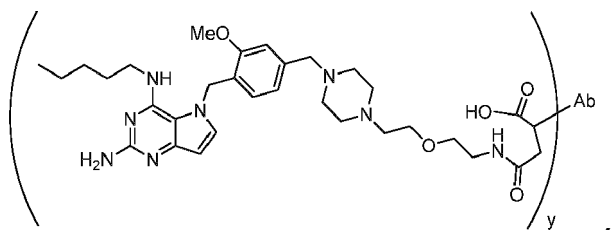


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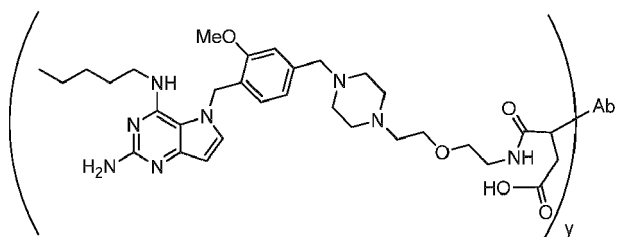
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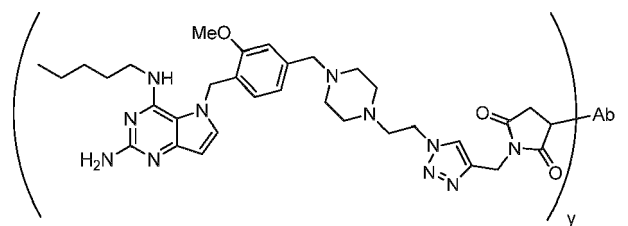
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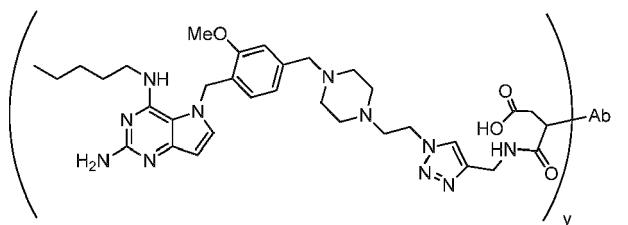
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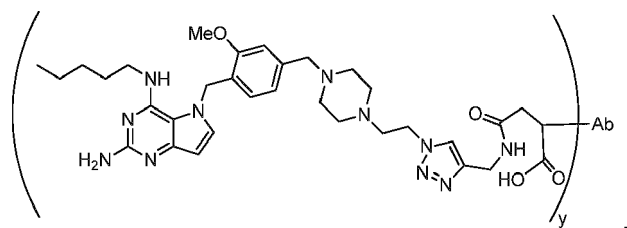
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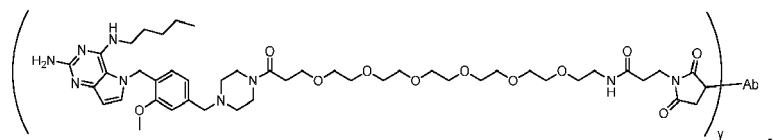


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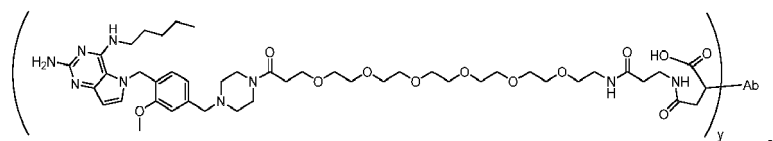
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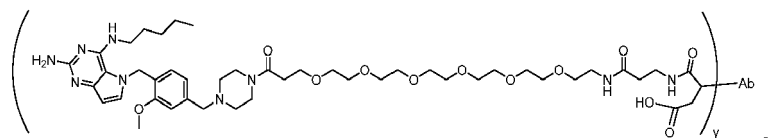
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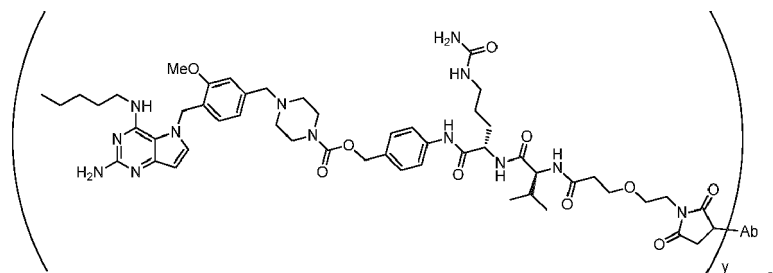
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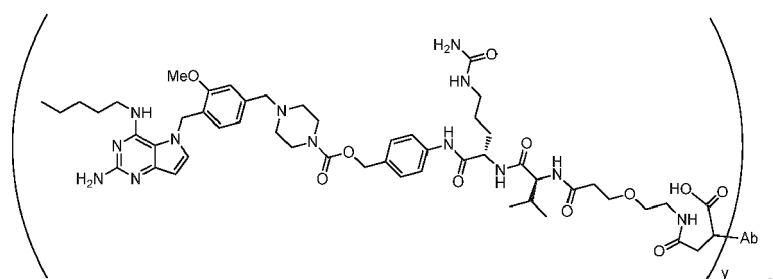
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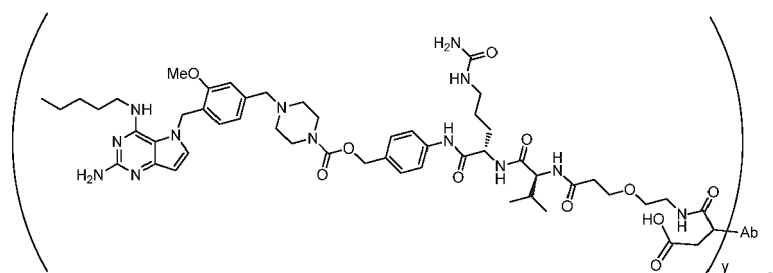
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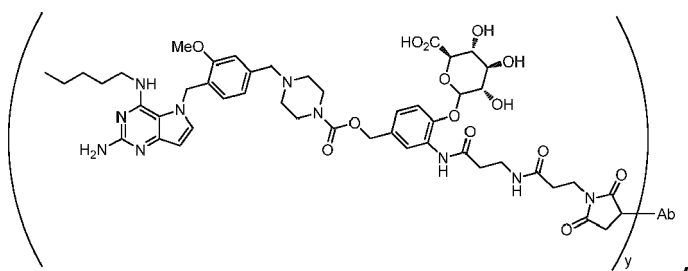


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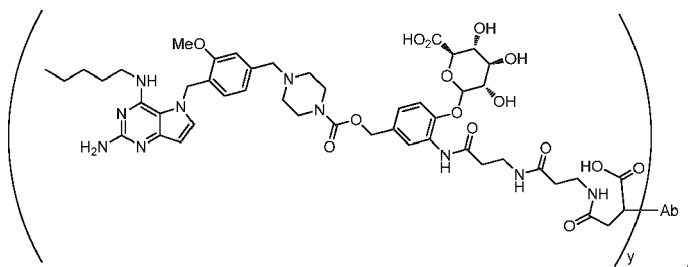
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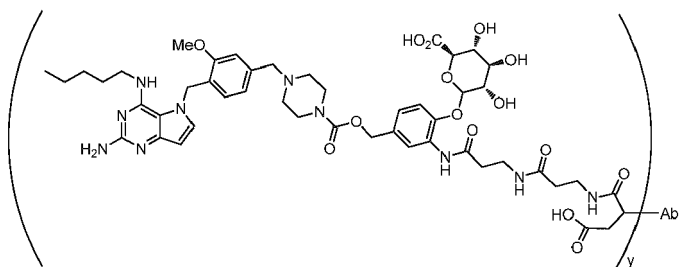
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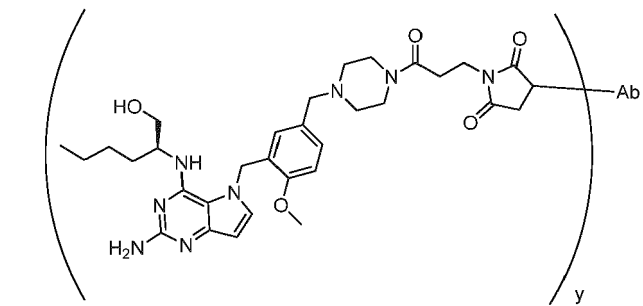
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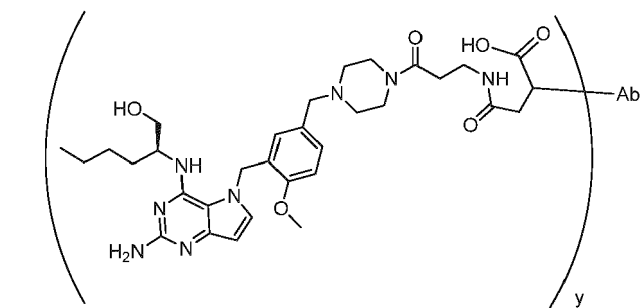
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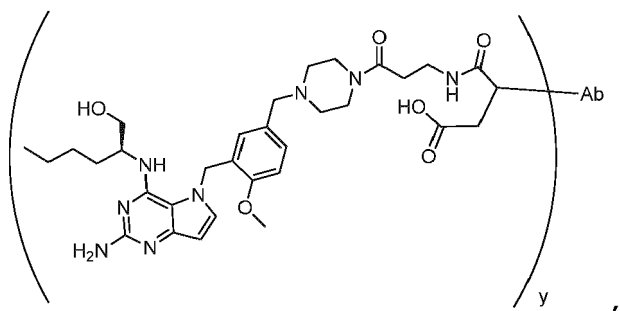


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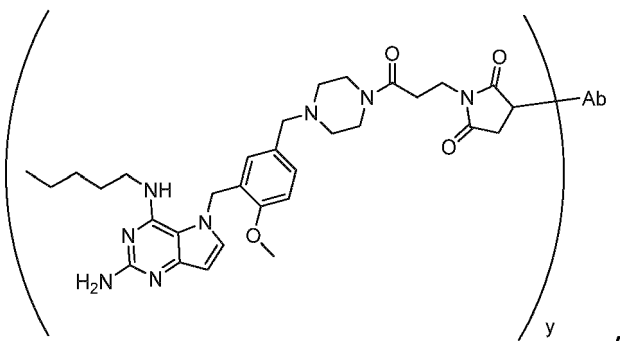
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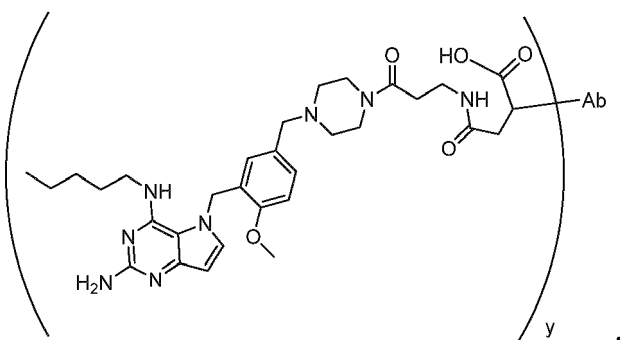
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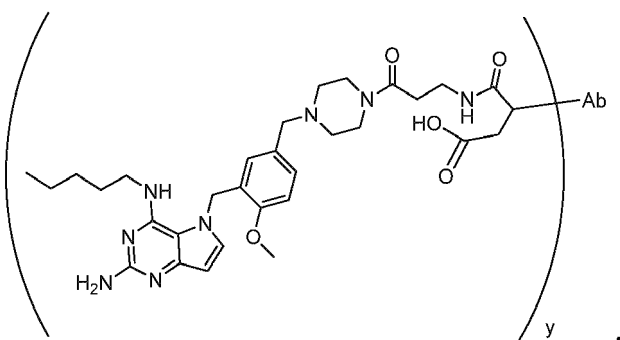
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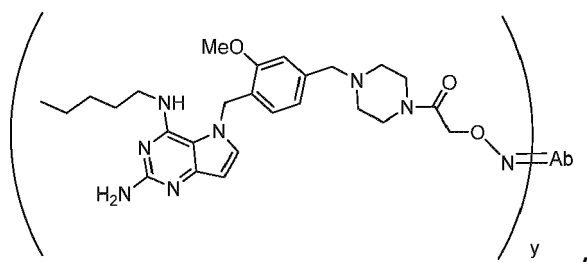
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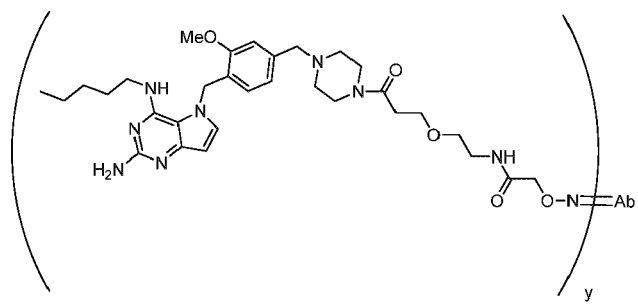
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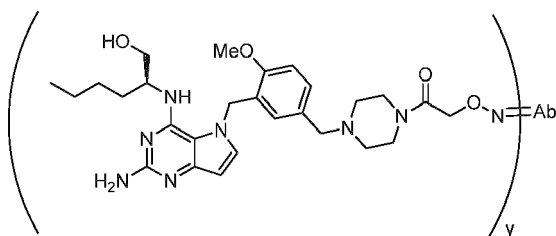
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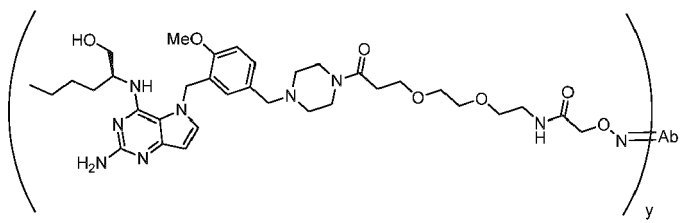
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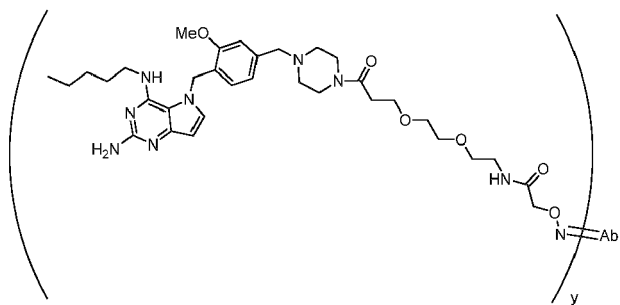
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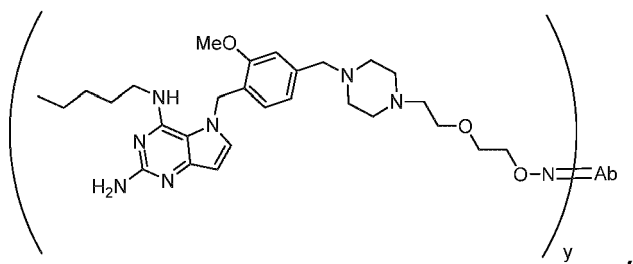
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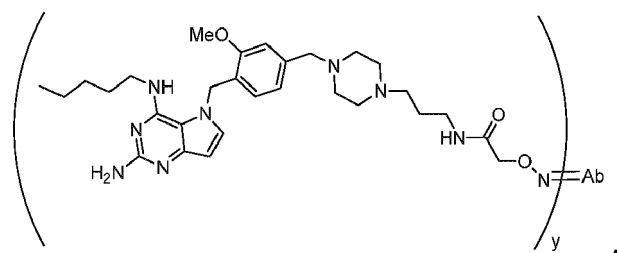
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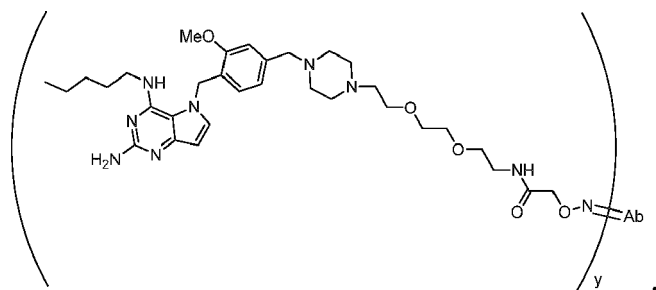
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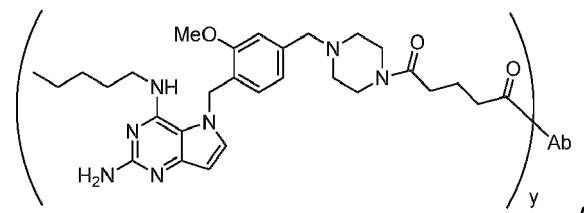
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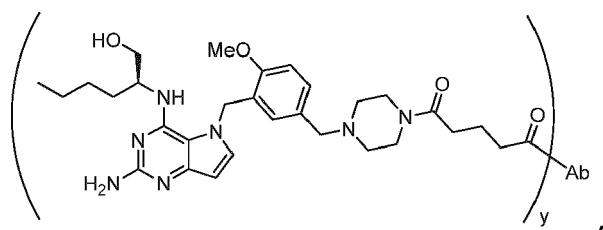
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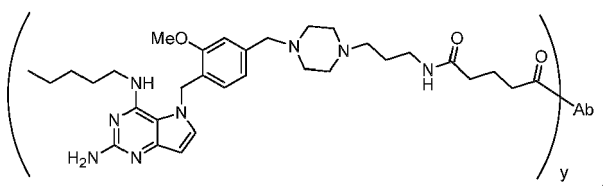


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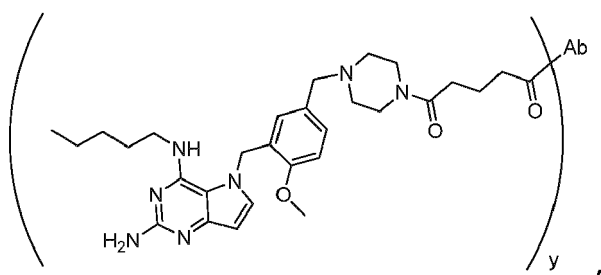


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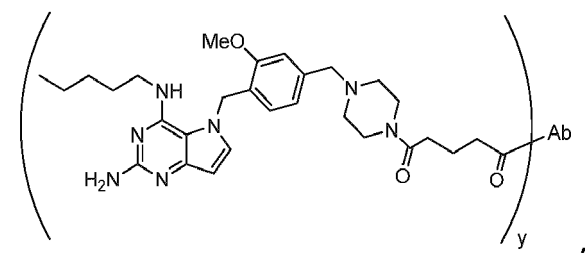
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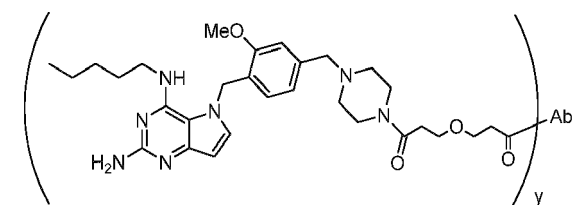
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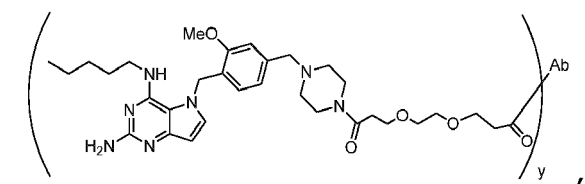
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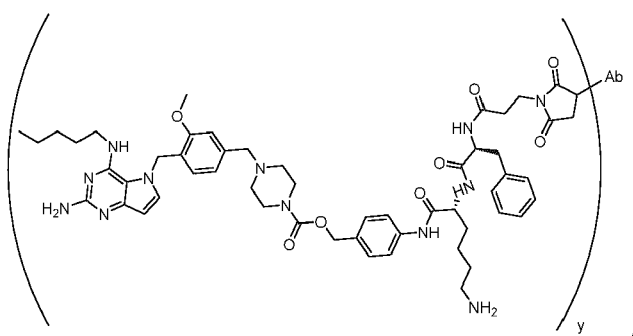
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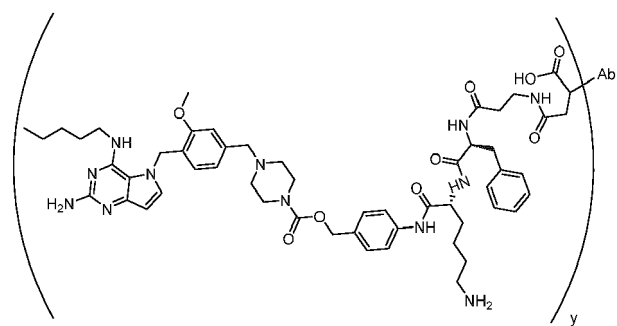
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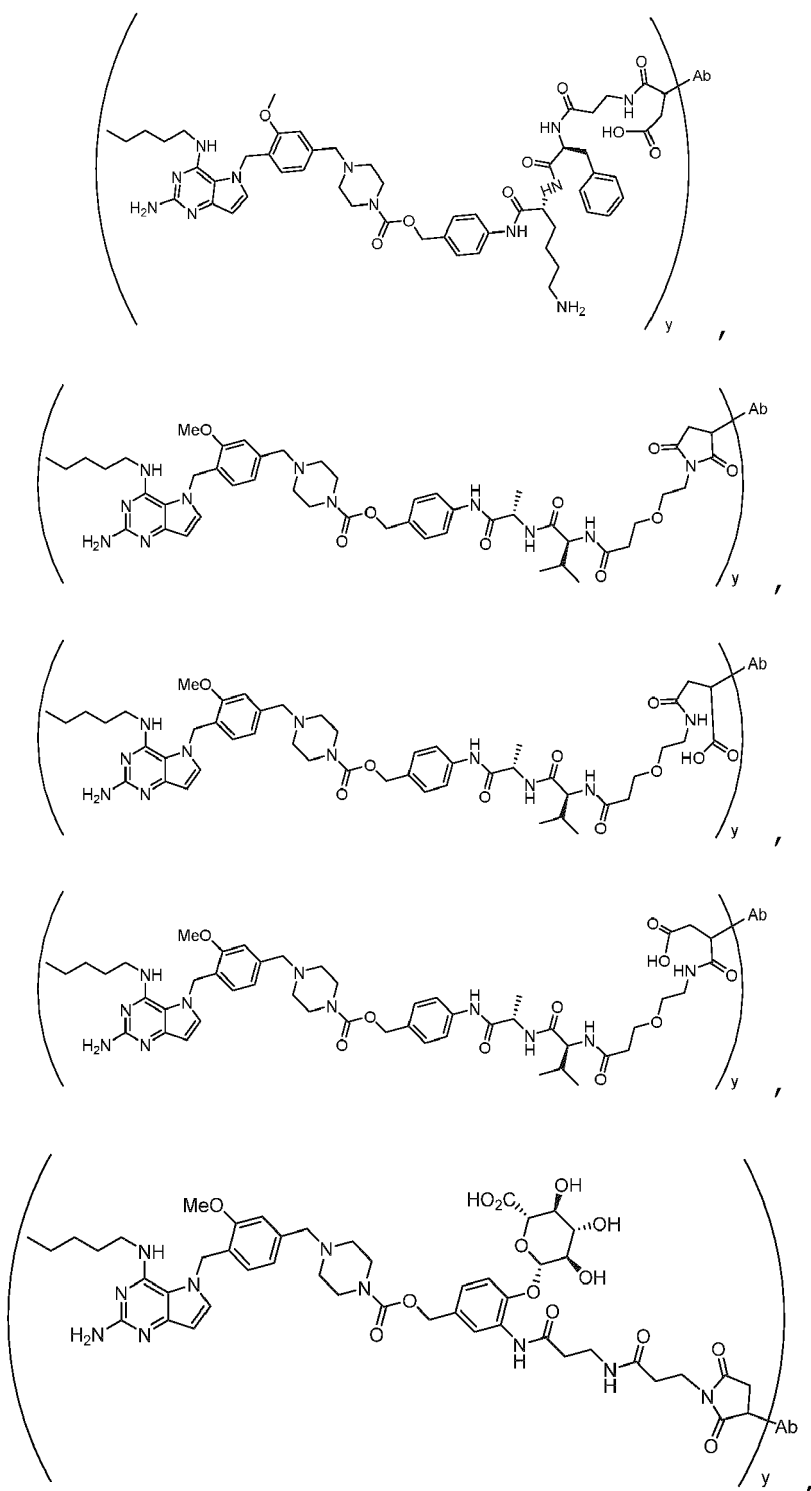
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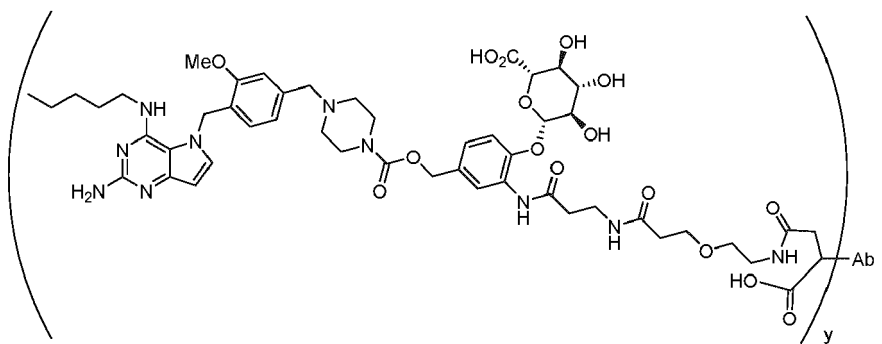
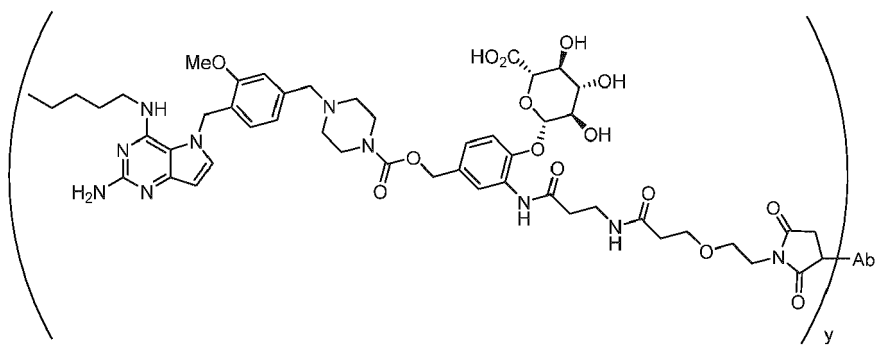
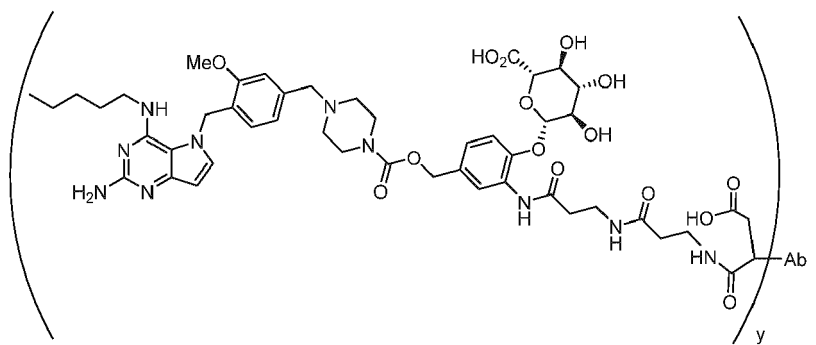
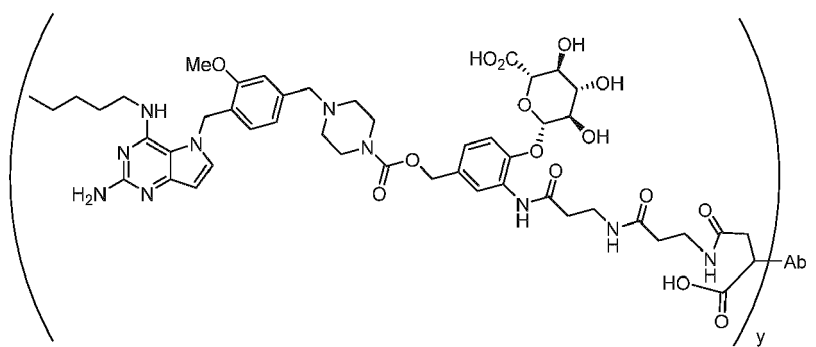
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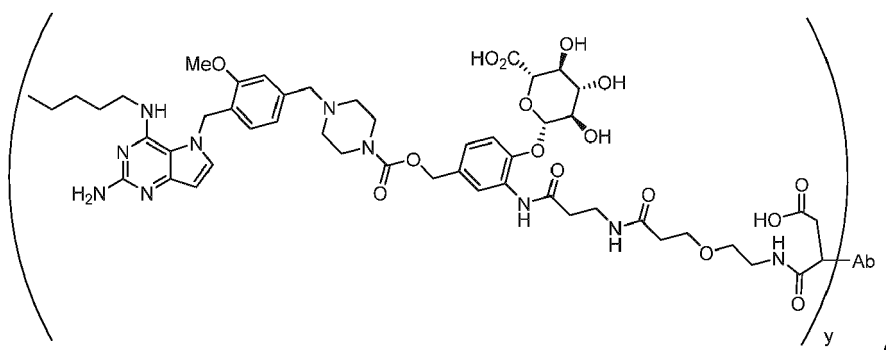
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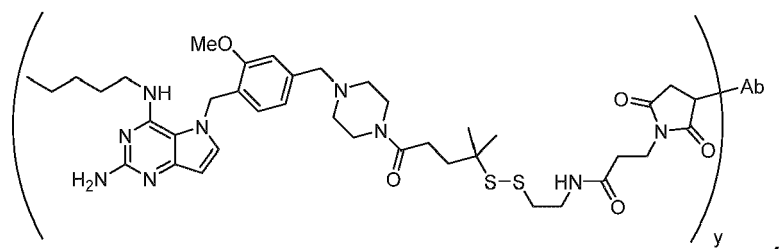
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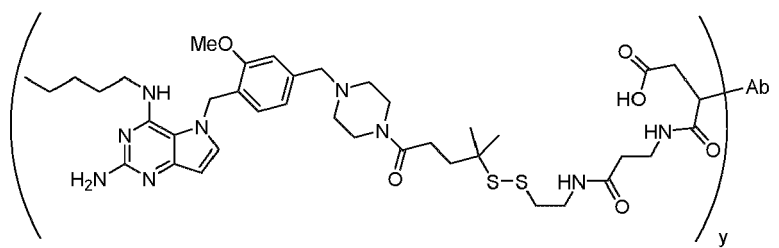
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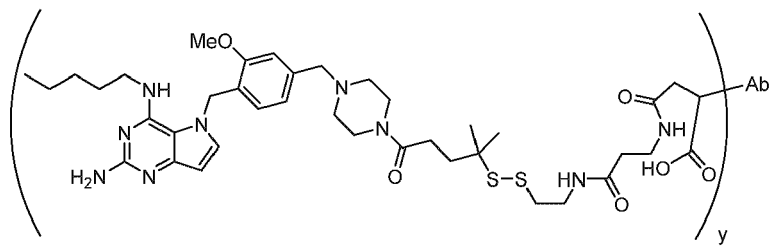
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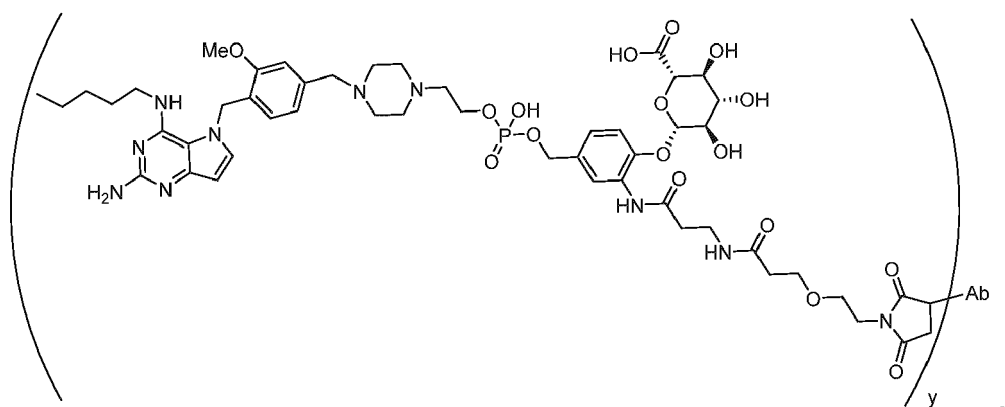
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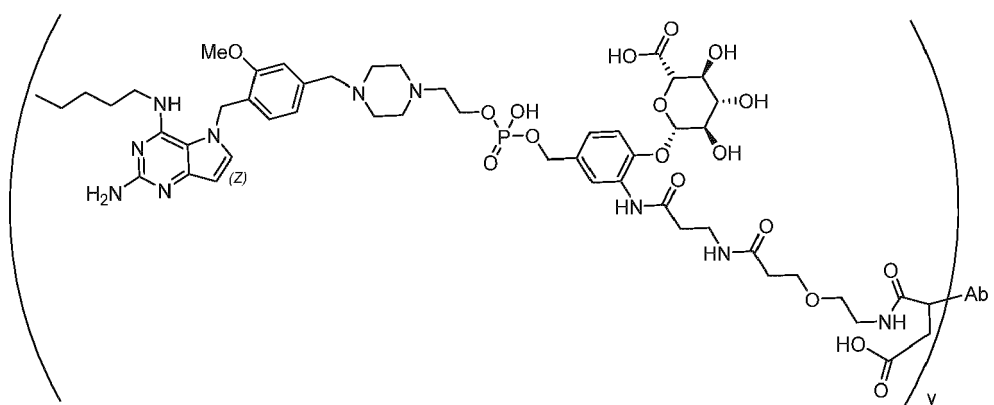
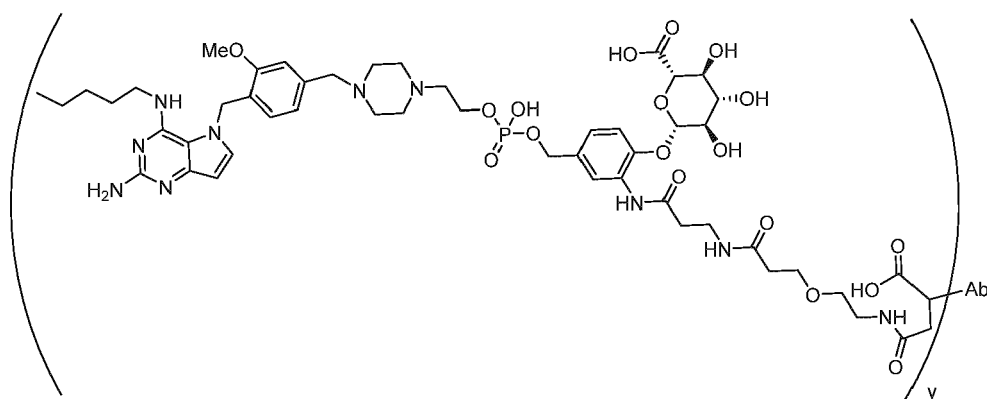
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und

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wobei Ab für einen Antikörper oder ein antigenbindendes Fragment davon, der/das spezifisch an humanes HER2 bindet, steht und y für eine ganze Zahl von 1 bis 4 steht.

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- 12.** Konjugat nach Anspruch 11, wobei Ab aus Trastuzumab, Pertuzumab, Margetuximab und HT-19 bzw. einer ortsspezifischen Cysteinmutante davon ausgewählt ist, wobei die ortsspezifische Cysteinmutante in einer oder mehreren der folgenden Positionen (alle Positionen gemäß EU-Nummerierung) Cystein umfasst:

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- (a) Positionen 152, 360 und 375 der schweren Kette des Antikörpers und
- (b) Positionen 107, 159 und 165 der leichten Kette des Antikörpers.

- 13.** Konjugat nach Anspruch 11, wobei Ab aus den Folgenden ausgewählt ist:

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- (a) einem Antikörper, der Folgendes umfasst:

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eine Complementary Determining Region 1 der schweren Kette (HCDR1), die die Aminosäuresequenz von SEQ ID NO: 1 umfasst,
 eine Complementary Determining Region 2 der schweren Kette (HCDR2), die die Aminosäuresequenz von SEQ ID NO: 2 umfasst,
 eine Complementary Determining Region 3 der schweren Kette (HCDR3), die die Aminosäuresequenz von SEQ ID NO: 3 umfasst,
 eine Complementary Determining Region 1 der leichten Kette (LCDR1), die die Aminosäuresequenz von SEQ ID NO: 11 umfasst,
 eine Complementary Determining Region 2 der leichten Kette (LCDR2), die die Aminosäuresequenz von SEQ ID NO: 12 umfasst, und
 eine Complementary Determining Region 3 der leichten Kette (LCDR3), die die Aminosäuresequenz von SEQ ID NO: 13 umfasst,

(b) einem Antikörper, der Folgendes umfasst:

eine HCDR1, die die Aminosäuresequenz von SEQ ID NO: 4 umfasst,
eine HCDR2, die die Aminosäuresequenz von SEQ ID NO: 5 umfasst,
eine HCDR3, die die Aminosäuresequenz von SEQ ID NO: 3 umfasst,
eine LCDR1, die die Aminosäuresequenz von SEQ ID NO: 14 umfasst,
eine LCDR2, die die Aminosäuresequenz von SEQ ID NO: 15 umfasst, und
eine LCDR3, die die Aminosäuresequenz von SEQ ID NO: 16 umfasst,

(c) einem Antikörper, der eine variable Region der schweren Kette, die die Aminosäuresequenz von SEQ ID NO: 7 umfasst, und eine variable Region der leichten Kette, die die Aminosäuresequenz von SEQ ID NO: 17 umfasst, umfasst,

(d) einem Antikörper, der eine schwere Kette, die die Aminosäuresequenz von SEQ ID NO: 9 umfasst, und eine leichte Kette, die die Aminosäuresequenz von SEQ ID NO: 19 umfasst, umfasst,

(e) einem Antikörper, der eine schwere Kette, die die Aminosäuresequenz von SEQ ID NO: 21 umfasst, und eine leichte Kette, die die Aminosäuresequenz von SEQ ID NO: 19 umfasst, umfasst,

(f) einem Antikörper, der eine schwere Kette, die die Aminosäuresequenz von SEQ ID NO: 23 umfasst, und eine leichte Kette, die die Aminosäuresequenz von SEQ ID NO: 19 umfasst, umfasst, oder

(g) einem Antikörper, der eine schwere Kette, die die Aminosäuresequenz von SEQ ID NO: 32 umfasst, und eine leichte Kette, die die Aminosäuresequenz von SEQ ID NO: 19 umfasst, umfasst.

14. Konjugat nach Anspruch 11, wobei Ab eine schwere Kette, die die Aminosäuresequenz von SEQ ID NO: 9 umfasst, und eine leichte Kette, die die Aminosäuresequenz von SEQ ID NO: 19 umfasst, umfasst.

15. Konjugat nach Anspruch 14, wobei die Verbindung an Cysteinreste in den Positionen 152 und 375 der schweren Kette des Antikörpers (alle Positionen gemäß EU-Nummerierung) gebunden ist.

16. Konjugat nach Anspruch 11, wobei y für etwa 3 bis 4 steht.

17. Konjugat nach einem der Ansprüche 11-16, wobei das Konjugat einen Hydrophobizitätsindex von 0,8 oder mehr, bestimmt durch Hydrophobe-Wechselwirkungs-Chromatografie, aufweist.

18. Pharmazeutische Zusammensetzung, umfassend ein oder mehrere Konjugate nach einem der Ansprüche 1-17 und einen pharmazeutisch unbedenklichen Träger.

19. Konjugat nach einem der Ansprüche 1-17 oder pharmazeutische Zusammensetzung nach Anspruch 18 zur Verwendung bei der Behandlung einer HER2-positiven Krebserkrankung in einem dessen bedürftigen Subjekt.

20. Konjugat zur Verwendung nach Anspruch 19, wobei das Konjugat dazu fähig ist, die HER2-positive Krebserkrankung über einen anhaltenden Zeitraum zu unterdrücken und/oder das Wiederauftreten der HER2-positiven Krebserkrankung zu reduzieren, im Vergleich zu einem Anti-HER2-Antikörper allein.

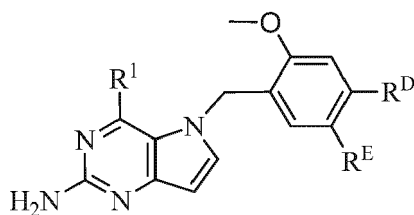
21. Konjugat zur Verwendung nach Anspruch 19, wobei die HER2-positive Krebserkrankung aus Magenkrebs, Speiseröhrenkrebs, Adenokarzinom des ösophagogastralen Übergangs, Dickdarmkrebs, Rektalkrebs, Brustkrebs, Eierstockkrebs, Gebärmutterhalskrebs, Gebärmutterkrebs, Endometriumkrebs, Blasenkrebs, Krebs des Harntrakts, Bauchspeicheldrüsenkrebs, Lungenkrebs, Prostatakrebs, Osteosarkom, Neuroblastom, Glioblastom und Kopf- und Halskrebs ausgewählt ist.

22. Konjugat zur Verwendung nach Anspruch 19, wobei das Konjugat dem Subjekt intravenös, intratumoral oder subkutan verabreicht wird.

23. Konjugat zur Verwendung nach Anspruch 19, wobei das Konjugat in einer Dosis von etwa 0,01-20 mg pro kg Körpergewicht verabreicht wird.

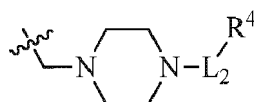
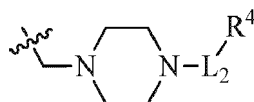
24. Konjugat zur Verwendung nach Anspruch 19, weiterhin umfassend die Verabreichung eines zweiten Mittels an das Subjekt.

25. Verbindung mit der Struktur der Formel (I) oder pharmazeutisch unbedenkliches Salz davon:

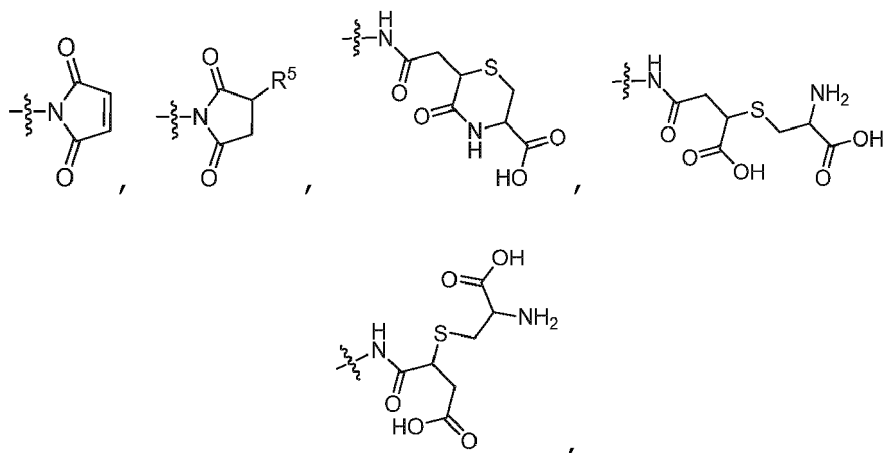


Formel (I),

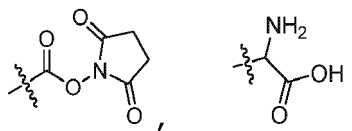
wobei

 R^D fürsteht und R^E für H steht oder R^E fürsteht und R^D für H steht, R^1 für $-NHR^2$ oder $-NHCHR^2R^3$ steht, R^2 für $-C_3-C_6$ -Alkyl oder $-C_4-C_6$ -Alkyl steht, R^3 für L_1OH steht, L_1 für $-(CH_2)_m-$ steht,

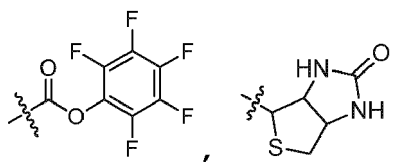
L_2 für $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)X_2X_3C(=O)(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)(CH_2)_nC(R_7)_2-$, $-C(=O)(CH_2)_nC(R_7)_2SS(CH_2)_nNHC(=O)(CH_2)_n-$, $-(CH_2)_nX_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n-$ oder $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$ steht, R^4 für

 $-ONH_2$, $-NH_2$,

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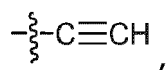


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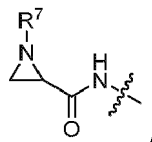
-N₃,



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-NHC(=O)CH=CH₂, -SH, -SR⁷, -OH -SSR⁶, -S(=O)₂(CH=CH₂), -(CH₂)₂S(=O)₂(CH=CH₂),
-NHS(=O)₂(CH=CH₂), -NHC(=O)CH₂Br, -NHC(=O)CH₂I, -C(O)NHNH₂,

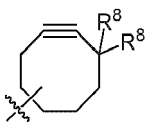
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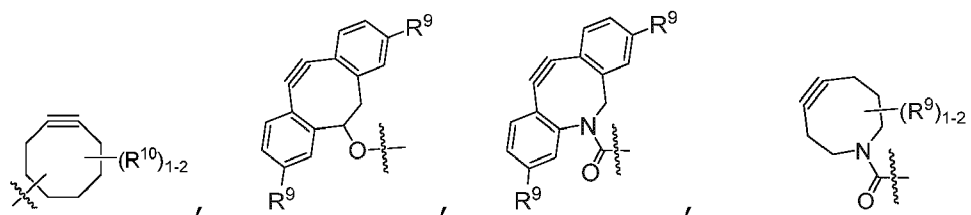
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-CO₂H, -C(O)NHNH₂,

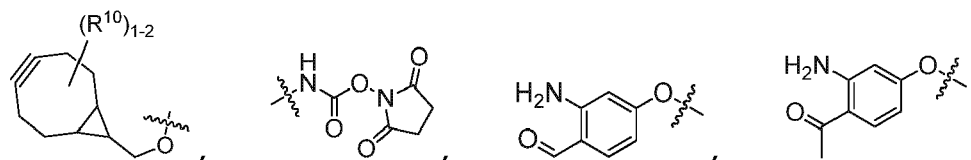
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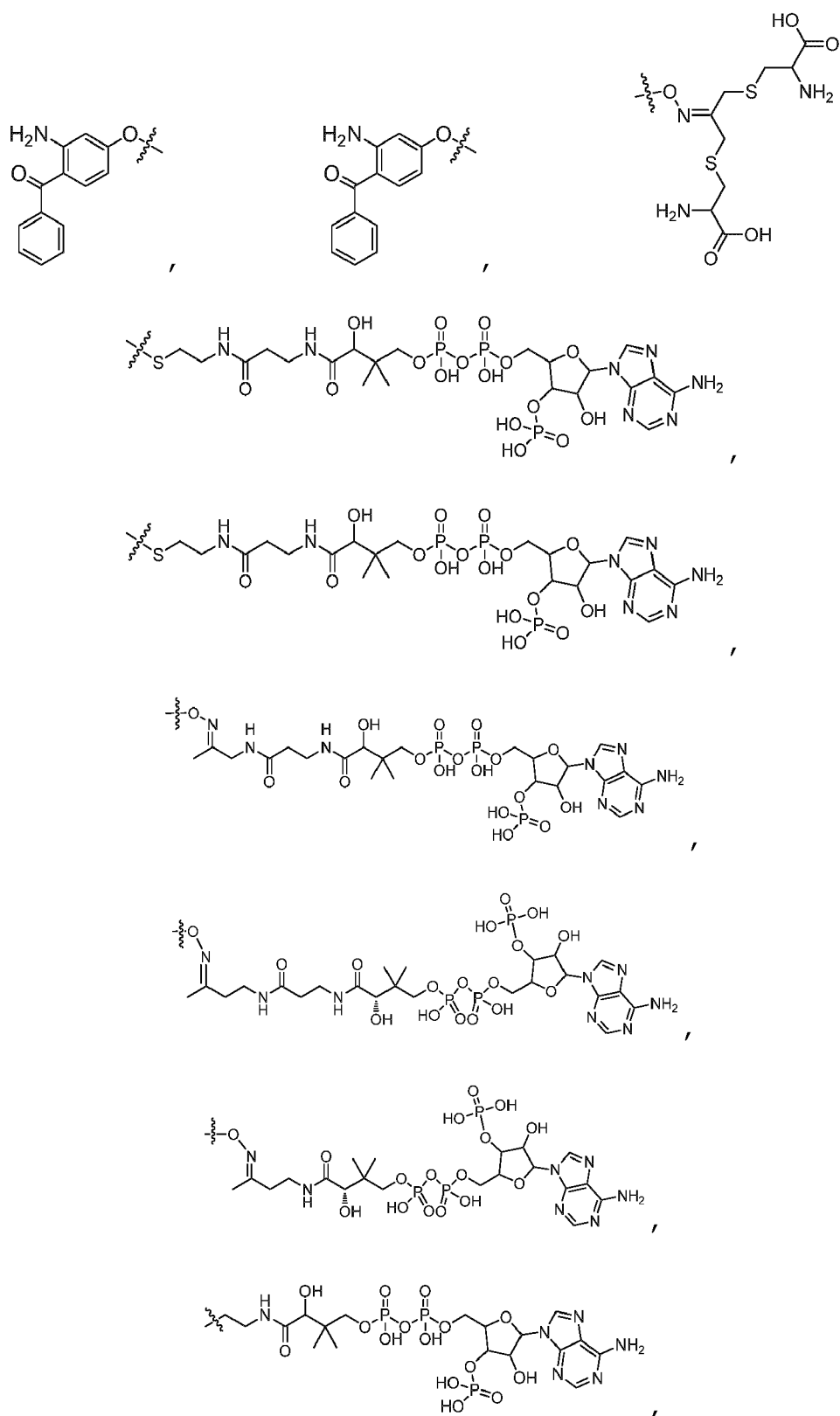
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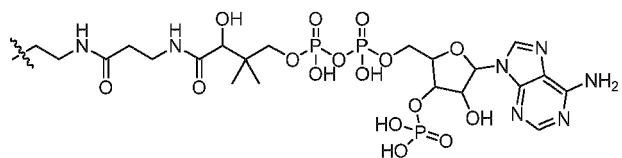


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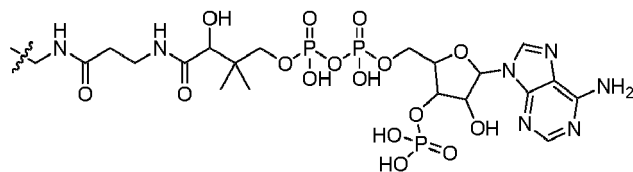
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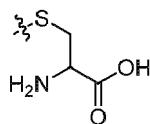




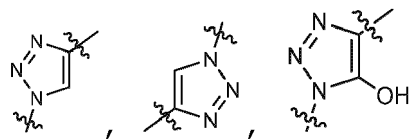
oder



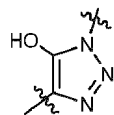
steht,
R⁵ für



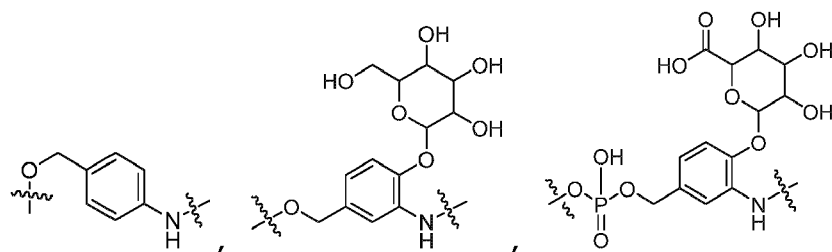
steht,
X₁ für



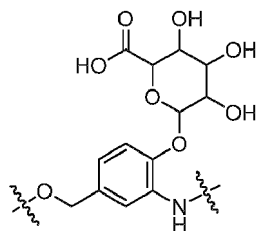
oder



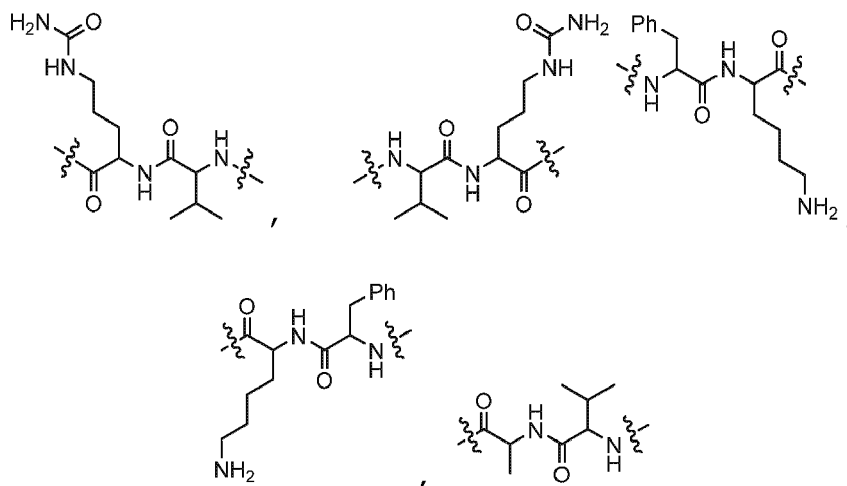
steht,
X₂ für



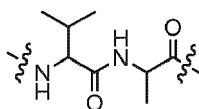
oder



steht,
X₃ für



oder



steht,

R⁶ für 2-Pyridyl oder 4-Pyridyl steht,

R⁷ jeweils unabhängig aus H und C₁-C₆-Alkyl ausgewählt ist,

R⁸ jeweils unabhängig aus H, C₁-C₆-Alkyl, F, Cl und -OH ausgewählt ist,

R⁹ jeweils unabhängig aus H, C₁₋₆-Alkyl, F, Cl, -NH₂, -OCH₃, -OCH₂CH₃, -N(CH₃)₂, -CN, -NO₂ und -OH ausgewählt ist,

R¹⁰ jeweils unabhängig aus H, C₁₋₆-Alkyl, Fluor, durch -C(=O)OH substituiertem Benzyloxy, durch -C(=O)OH substituiertem Benzyl, durch -C(=O)OH substituiertem C₁₋₄-Alkoxy und durch -C(=O)OH substituiertem C₁₋₄-Alkyl ausgewählt ist,

m jeweils unabhängig aus 1, 2, 3 und 4 ausgewählt ist,

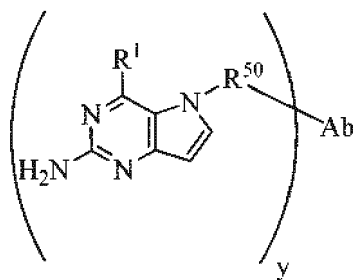
n jeweils unabhängig aus 1, 2, 3 und 4 ausgewählt ist und

t jeweils unabhängig aus 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 und 18 ausgewählt ist.

26. Konjugat, umfassend die Verbindung nach Anspruch 25 gebunden an einen Antikörper oder ein antigenbindendes Fragment davon.

Revendications

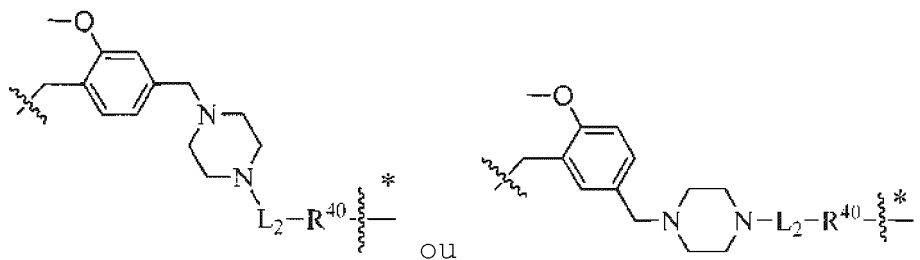
1. Conjugué de formule (II), ou sel pharmaceutiquement acceptable de celui-ci :



Formule (II)

dans lequel :

R^{50} est



où le * indique le point de liaison à Ab ;

Ab est un anticorps ou un fragment de liaison d'antigène de celui-ci qui se lie spécifiquement à HER2 humain ;

R^1 est $-NHR^2$ ou $-NHCHR^2R^3$;

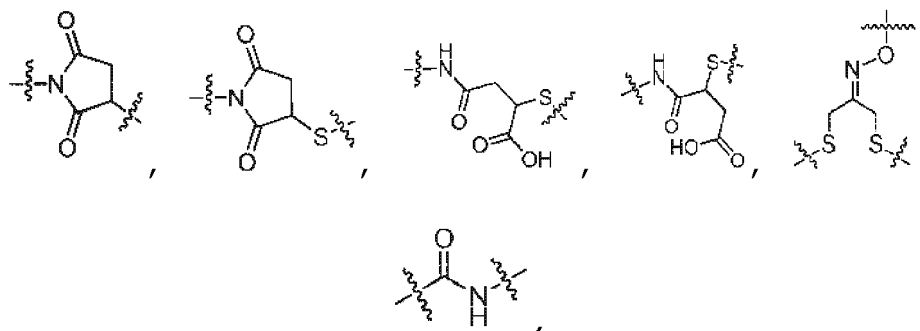
R^2 est $-(alkyle \text{ en } C_3-C_6)$ ou $-(alkyle \text{ en } C_4-C_6)$;

R^3 est L_1OH ;

L_1 est $-(CH_2)_m-$;

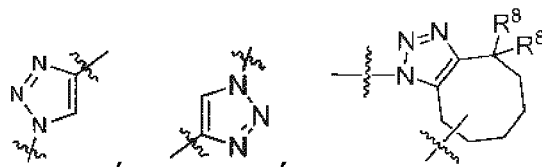
L_2 est $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)X_2X_3C(=O)(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)(CH_2)_nC(R_7)_2-$, $-C(=O)(CH_2)_nC(R_7)_2SS(CH_2)_nNHC(=O)(CH_2)_n-$, $-(CH_2)_nX_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n-$ ou $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

R^{40} est

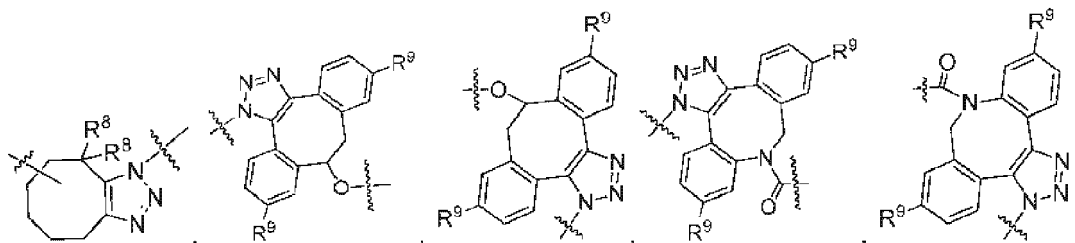


$-S-$, $-NHC(=O)CH_2-$, $-S(=O)_2CH_2CH_2-$, $-(CH_2)_2S(=O)_2CH_2CH_2-$, $-NHS(=O)_2CH_2CH_2-$, $-NHC(=O)CH_2CH_2-$, $-CH_2NHCH_2CH_2-$, $-NHCH_2CH_2-$,

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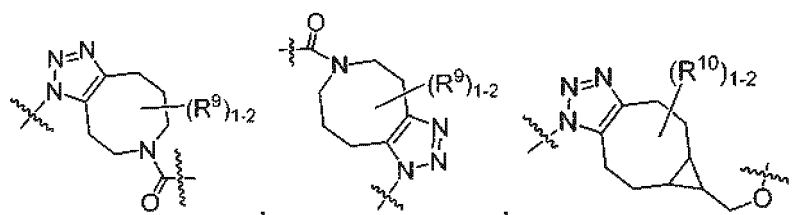


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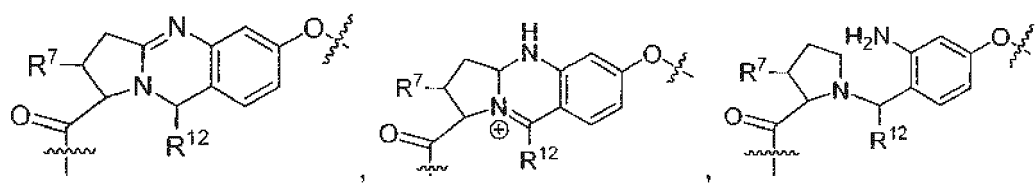


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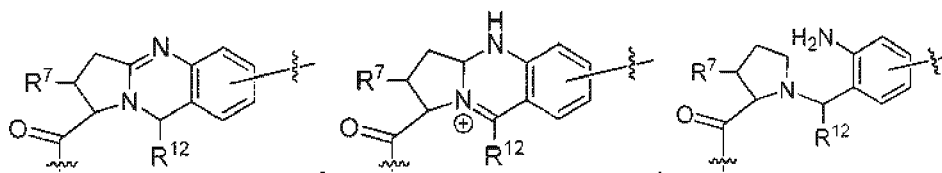


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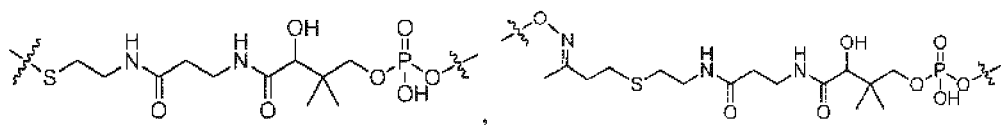


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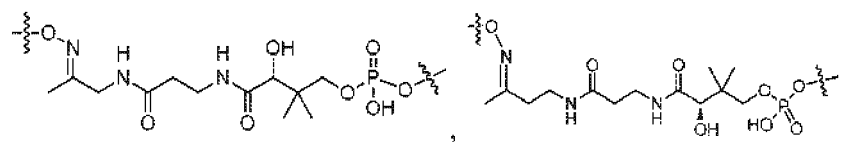
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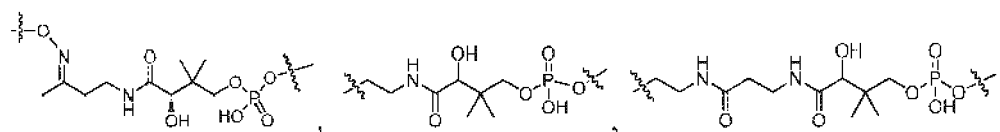


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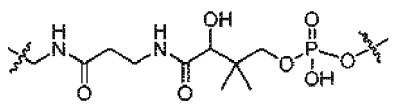


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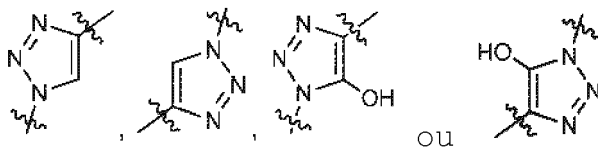
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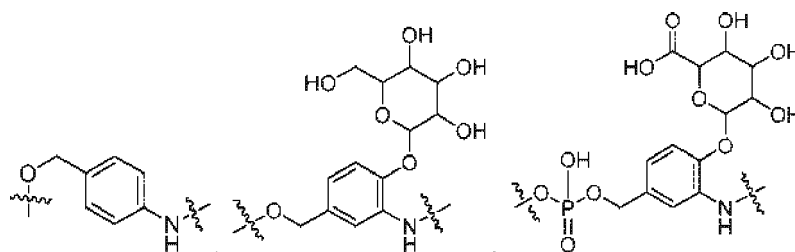
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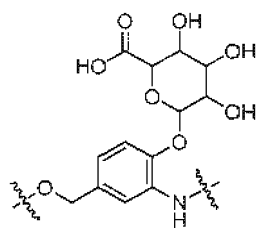
X_1 est



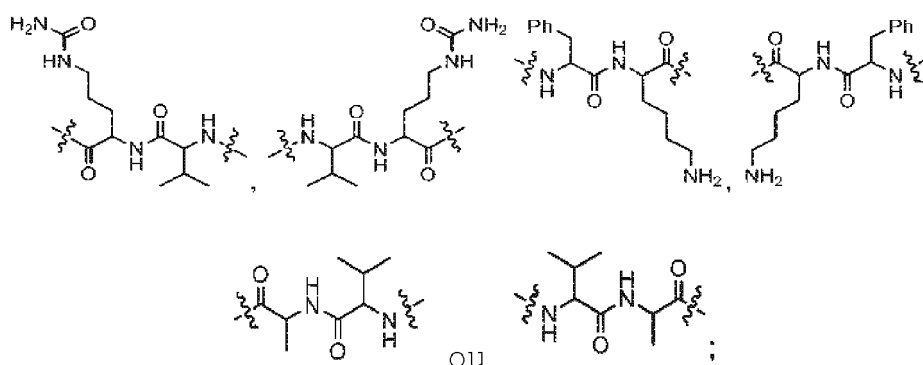
X_2 est



ou



X_3 est



chaque R^7 est indépendamment choisi parmi H et alkyle en C_1 - C_6 ;

chaque R^8 est indépendamment choisi parmi H, alkyle en C_1 - C_6 , F, Cl et -OH ;

chaque R^9 est indépendamment choisi parmi H, alkyle en C_1 - C_6 , F, Cl, - NH_2 , - OCH_3 , - OCH_2CH_3 , - $N(CH_3)_2$, -CN, - NO_2 et -OH ;

chaque R^{10} est indépendamment choisi parmi H, alkyle en C_1 - C_6 , fluoro, benzyloxy substitué par -C(=O)OH, benzyle substitué par -C(=O)OH, alcoxy en C_1 - C_4 substitué par -C(=O)OH et alkyle en C_1 - C_4 substitué par -C(=O)OH ;

R¹² est H, méthyle ou phényle ;
chaque m est indépendamment choisi parmi 1, 2, 3 et 4 ;
chaque n est indépendamment choisi parmi 1, 2, 3 et 4 ;
chaque t est indépendamment choisi parmi 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 et 18, et
y est un entier de 1 à 16.

2. Conjugué selon la revendication 1, dans lequel Ab est choisi parmi le trastuzumab, le pertuzumab, le margétuximab ou HT-19, ou un mutant de cystéine spécifique à un site de ceux-ci, dans lequel le mutant de cystéine spécifique à un site comprend de la cystéine à une ou plusieurs des positions suivantes (toutes les positions étant selon la numérotation EU) :

- (a) positions 152, 360 et 375 de la chaîne lourde d'anticorps, et
(b) positions 107, 159 et 165 de la chaîne légère d'anticorps.

3. Conjugué selon la revendication 1, dans lequel Ab est choisi parmi l'un quelconque des suivants :

- (a) un anticorps qui comprend :

une région déterminant la complémentarité de chaîne lourde 1 (HCDR1) comprenant la séquence d'acides aminés de SEQ ID NO : 1 ;
une région déterminant la complémentarité de chaîne lourde 2 (HCDR2) comprenant la séquence d'acides aminés de SEQ ID NO : 2 ;
une région déterminant la complémentarité de chaîne lourde 3 (HCDR3) comprenant la séquence d'acides aminés de SEQ ID NO : 3 ;
une région déterminant la complémentarité de chaîne légère 1 (LCDR1) comprenant la séquence d'acides aminés de SEQ ID NO : 11 ;
une région déterminant la complémentarité de chaîne légère 2 (LCDR2) comprenant la séquence d'acides aminés de SEQ ID NO : 12 ; et
une région déterminant la complémentarité de chaîne légère 3 (LCDR3) comprenant la séquence d'acides aminés de SEQ ID NO : 13 ;

- (b) un anticorps qui comprend :

une HCDR1 comprenant la séquence d'acides aminés de SEQ ID NO : 4 ;
une HCDR2 comprenant la séquence d'acides aminés de SEQ ID NO : 5 ;
une HCDR3 comprenant la séquence d'acides aminés de SEQ ID NO : 3 ;
une LCDR1 comprenant la séquence d'acides aminés de SEQ ID NO : 14 ;
une LCDR2 comprenant la séquence d'acides aminés de SEQ ID NO : 15 ; et
une LCDR3 comprenant la séquence d'acides aminés de SEQ ID NO : 16 ;

(c) un anticorps qui comprend une région variable de chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 7, et une région variable de chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 17 ;

(d) un anticorps qui comprend une chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 9, et une chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 19 ;

(e) un anticorps qui comprend une chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 21, et une chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 19 ;

(f) un anticorps qui comprend une chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 23, et une chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 19 ; ou

(g) un anticorps qui comprend une chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 32, et une chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 19.

4. Conjugué selon l'une quelconque des revendications 1 à 3, dans lequel Ab est un anticorps anti-HER2 humain ou humanisé.

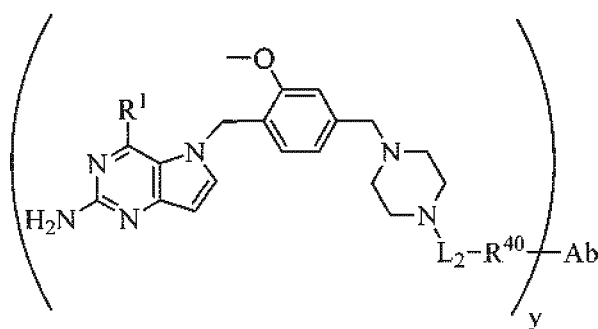
5. Conjugué selon l'une quelconque des revendications 1 à 4, dans lequel Ab comprend une région Fc modifiée.

6. Conjugué selon l'une quelconque des revendications 1 à 5, dans lequel Ab comprend de la cystéine à une ou

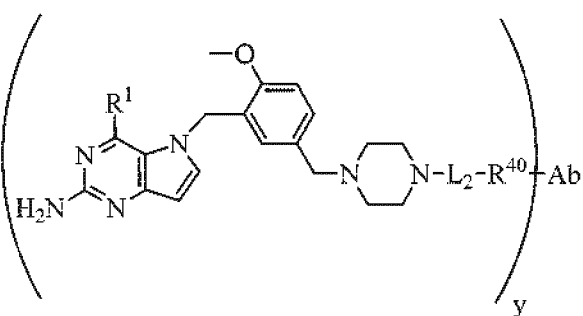
plusieurs des positions suivantes (toutes les positions étant selon la numérotation EU) :

- (a) positions 152, 360 et 375 de la chaîne lourde d'anticorps, et
(b) positions 107, 159 et 165 de la chaîne légère d'anticorps.

7. Conjugué selon l'une quelconque des revendications 1 à 6, dans lequel Ab comprend des cystéines aux positions 152 et 375 des chaînes lourdes d'anticorps (toutes les positions étant selon la numérotation EU).
8. Conjugué selon l'une quelconque des revendications 1 à 7, le conjugué d'anticorps de formule (II) comprend la structure de formule (IIa) ou de formule (IIb) :



Formule (IIa)



Formule (IIb)

dans lequel :

R¹ est -NHR² ;

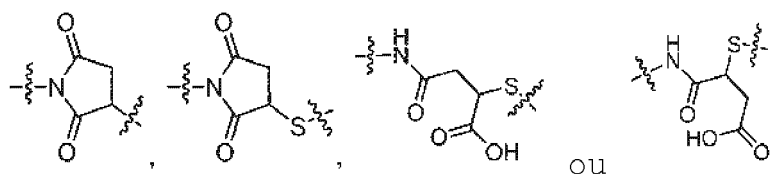
R² est - (alkyle en C₄-C₆) ;

L₂ est -(CH₂)_n-, -((CH₂)_nO)_t(CH₂)_n-, -(CH₂)_nX₁(CH₂)_n-, -C(=O)(CH₂)_n-, -C(=O)((CH₂)_nO)_t(CH₂)_n-,
-C(=O)((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-, -C(=O)NH((CH₂)_nO)_t(CH₂)_nX₁(CH₂)_n-,

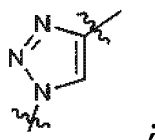
-C(=O)X₂X₃C(=O)((CH₂)_nO)_t(CH₂)_n-

ou -C(=O)X₂C(=O)(CH₂)_nNHC(=O)(CH₂)_n- ;

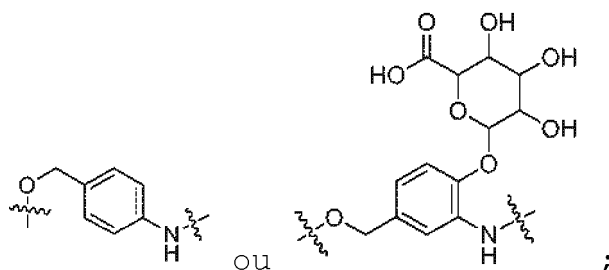
R⁴⁰ est



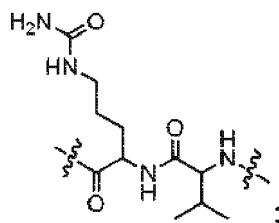
X₁ est



X₂ est



X_3 est



chaque n est indépendamment choisi parmi 1, 2, 3 et 4 :

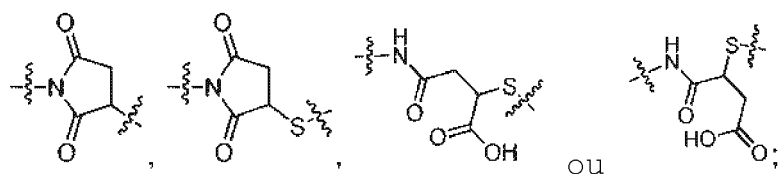
chaque t est indépendamment choisi parmi 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 et 18, et y est un entier de 1 à 16.

9. Conjugué selon l'une quelconque des revendications 1 à 7, dans lequel

R^1 est $-NHR^2$;

R² est -(alkyle en C₄-C₆) ;

L_2 est $-(CH_2)_n-$ ou $-C(=O)(CH_2)_n-$;

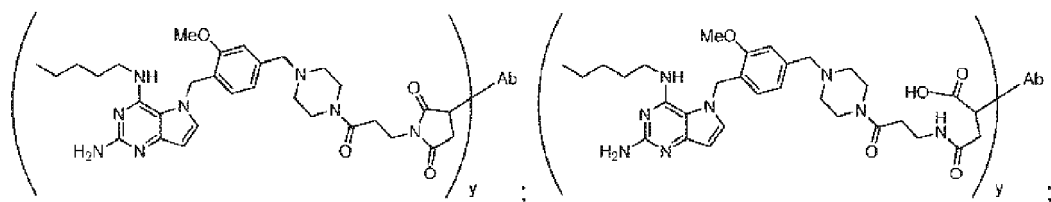
 R^{40} est

et

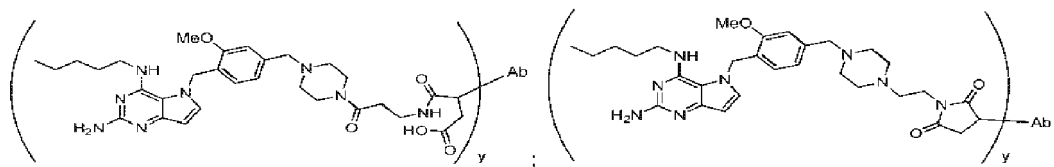
chaque n est indépendamment choisi parmi 1, 2, 3 et 4, et y est un entier de 1 à 16.

10. Conjugué selon l'une quelconque des revendications 1 à 9, le conjugué présentant un indice d'hydrophobicité de 0,8 ou plus, comme déterminé par chromatographie d'interaction hydrophobe.

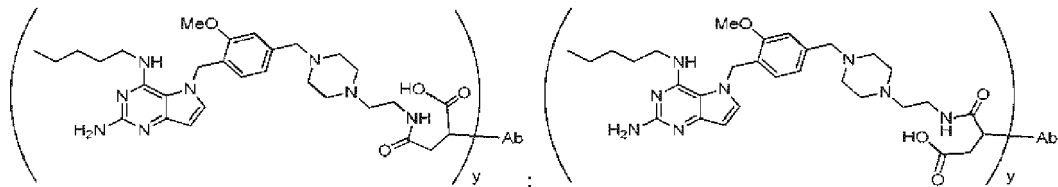
11. Conjugué comprenant l'une quelconque des formules suivantes :



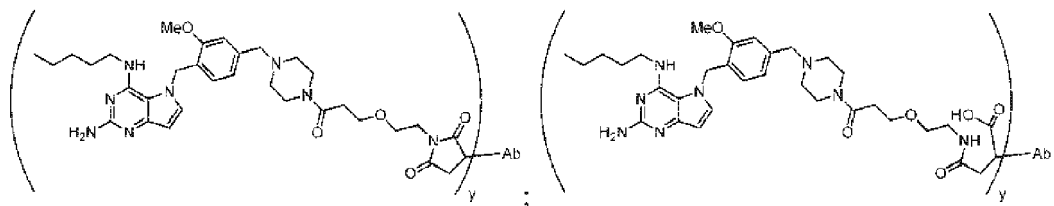
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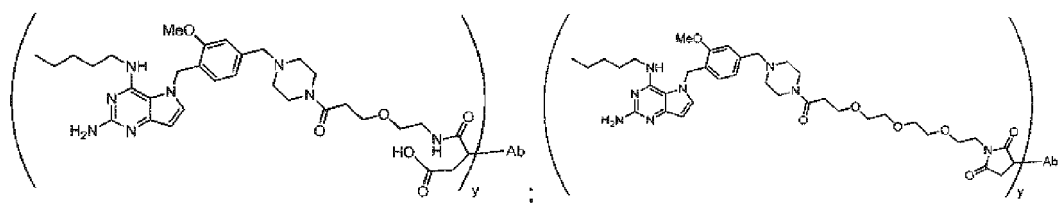
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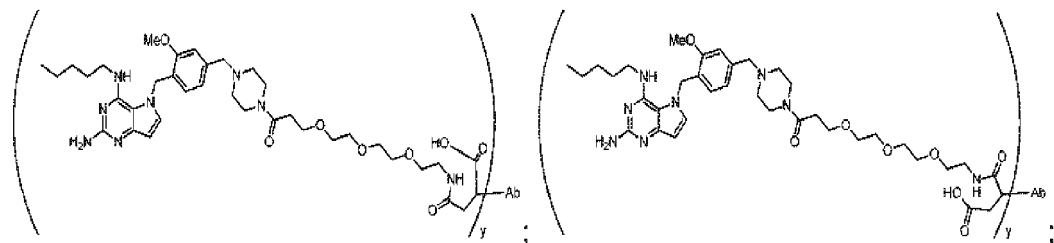
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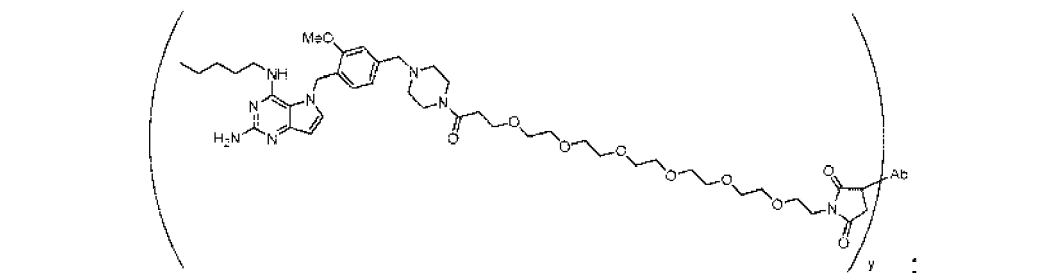
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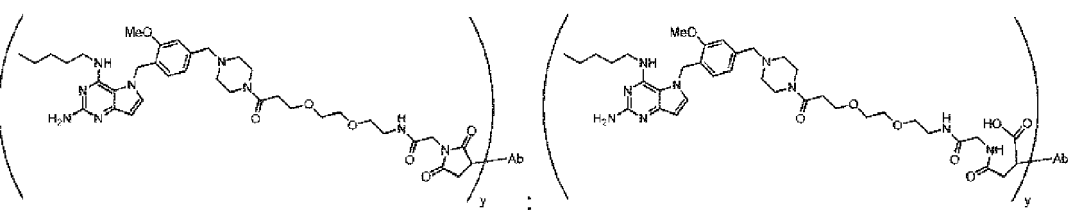
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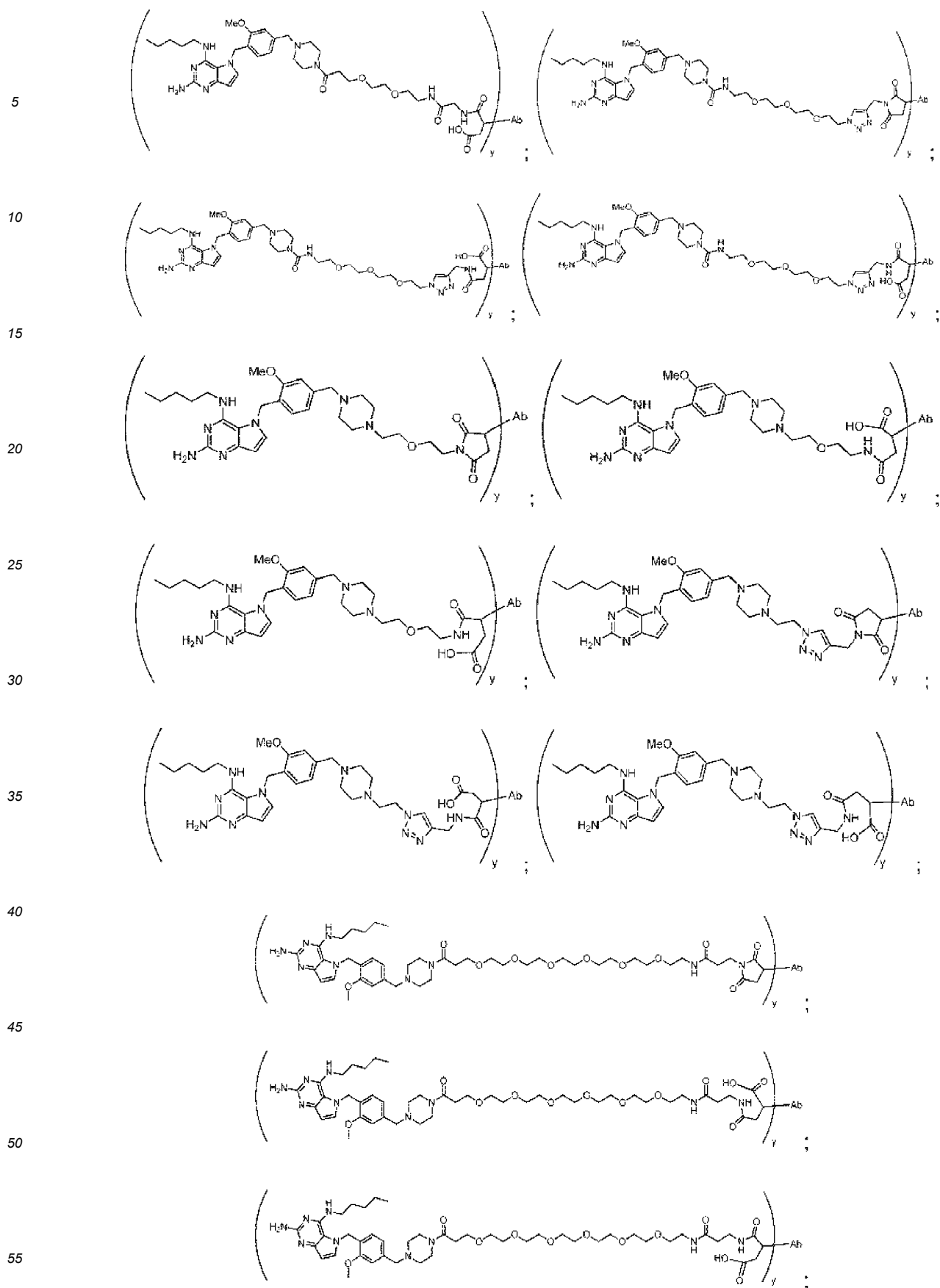
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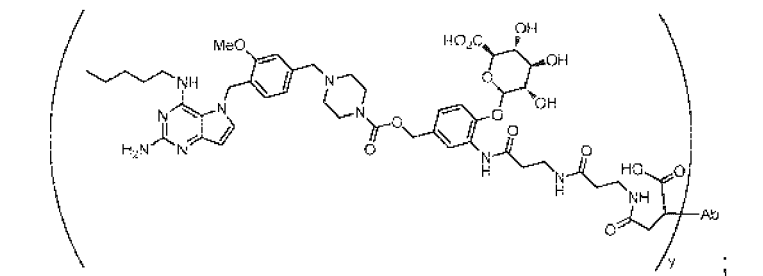
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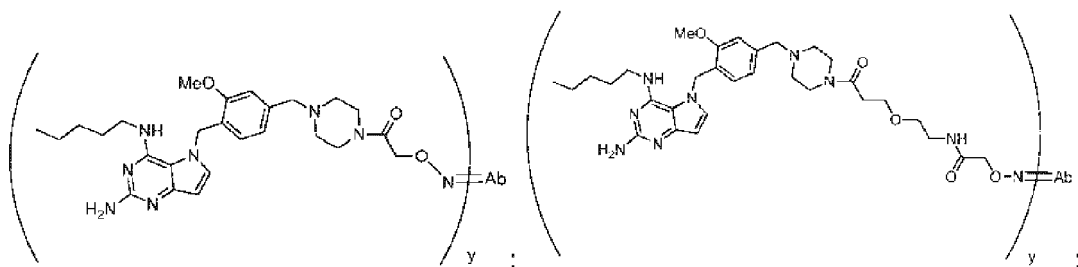
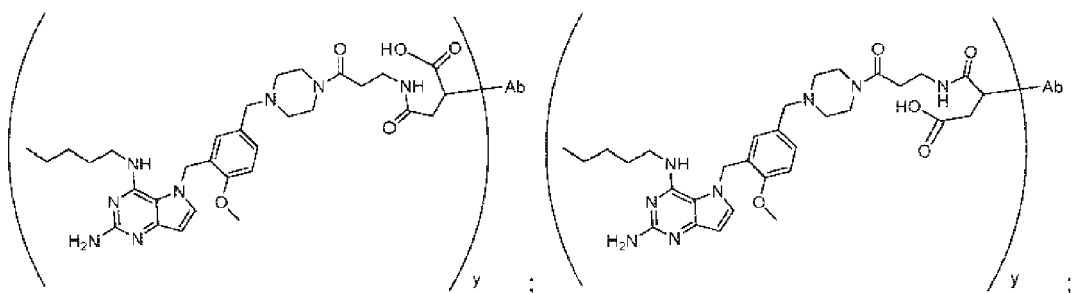
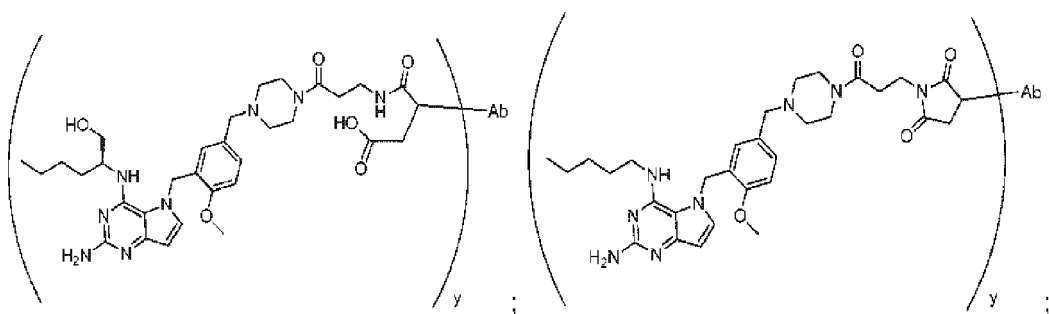
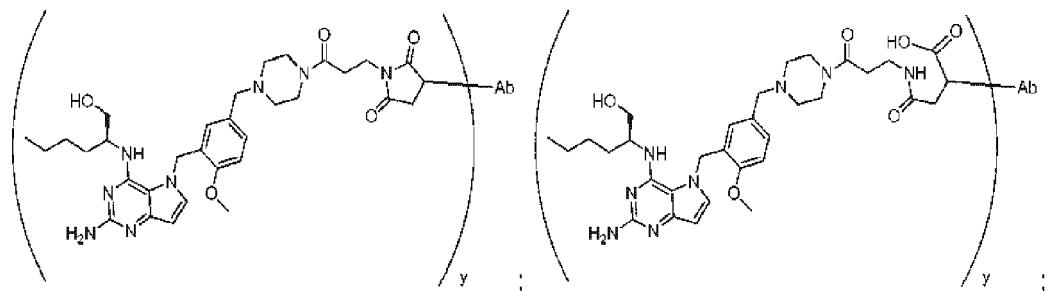
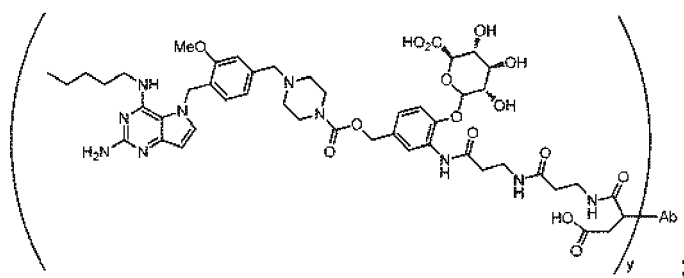
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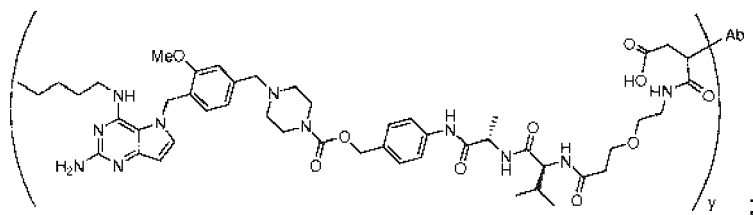
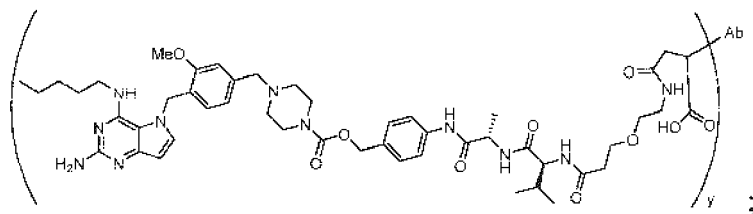
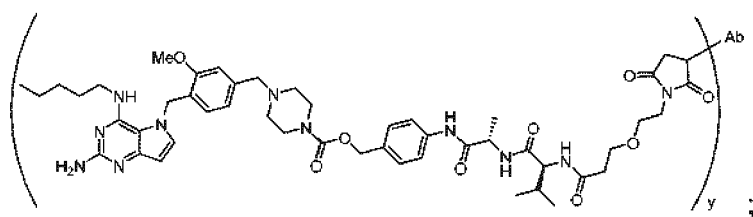
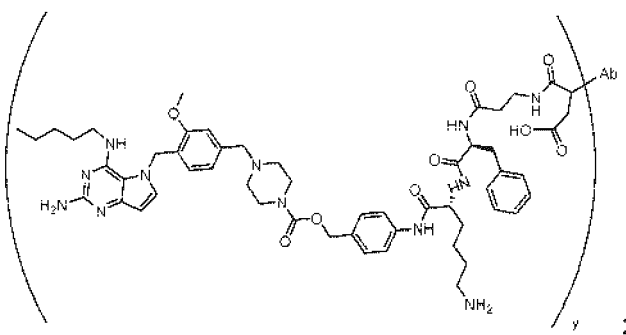
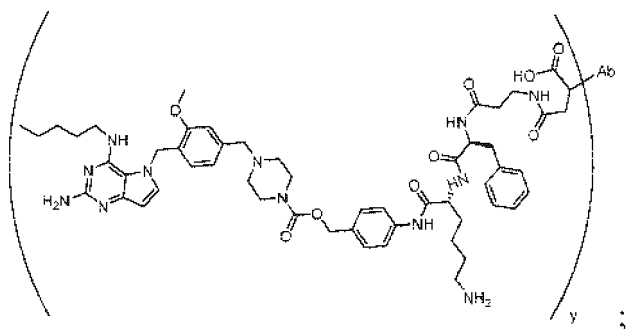
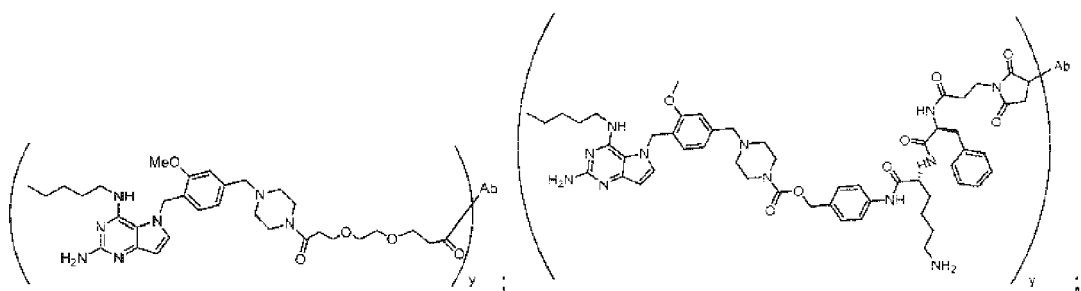












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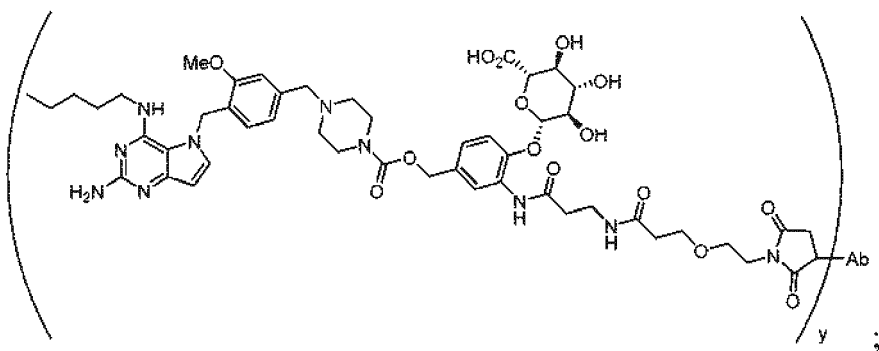
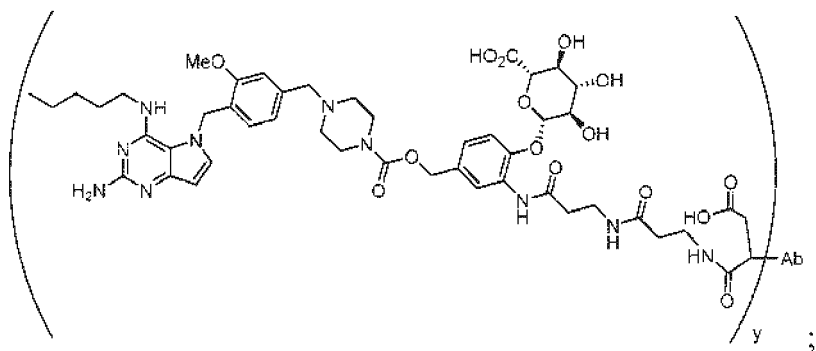
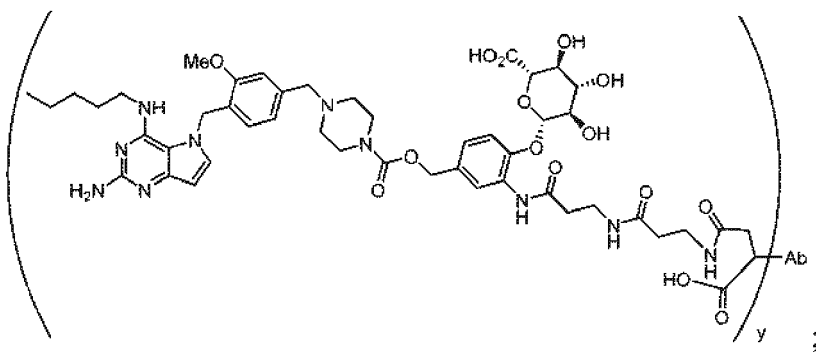
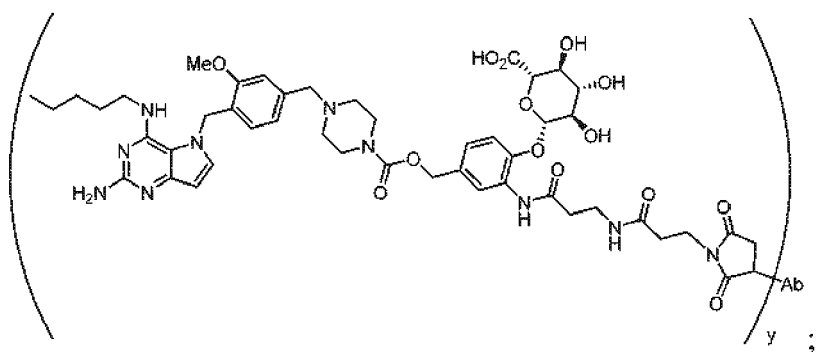
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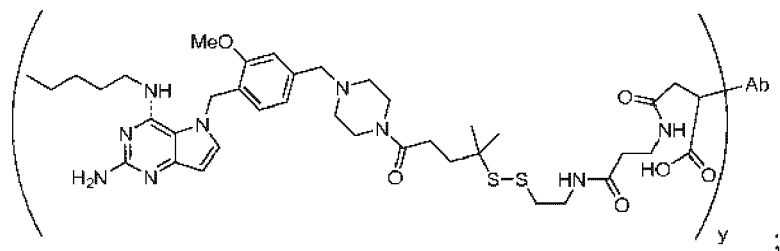
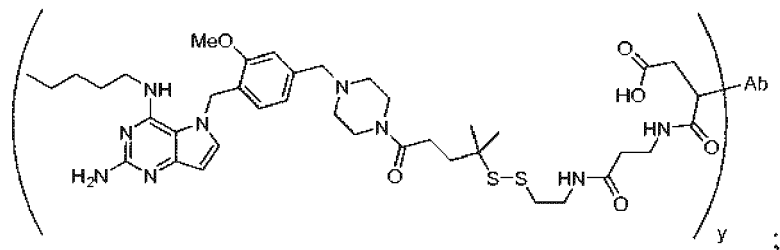
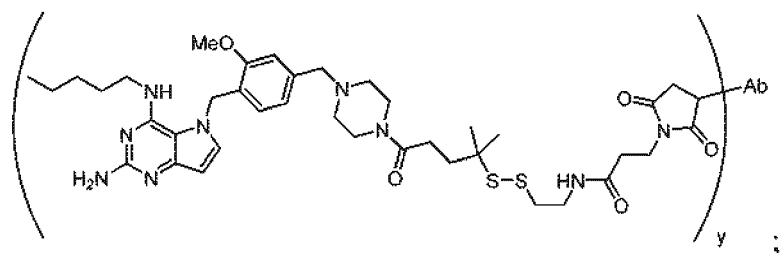
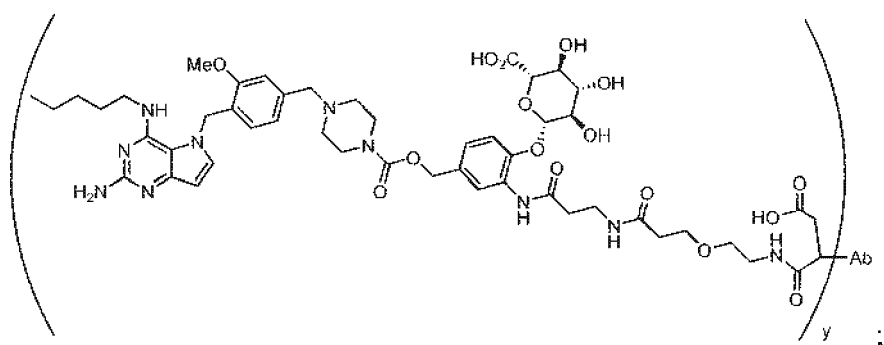
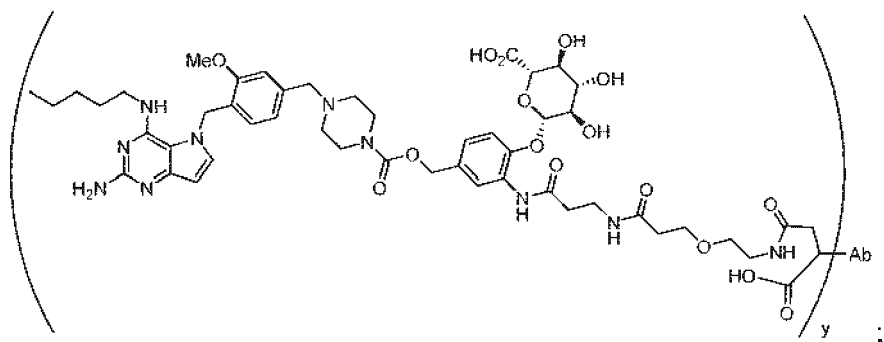
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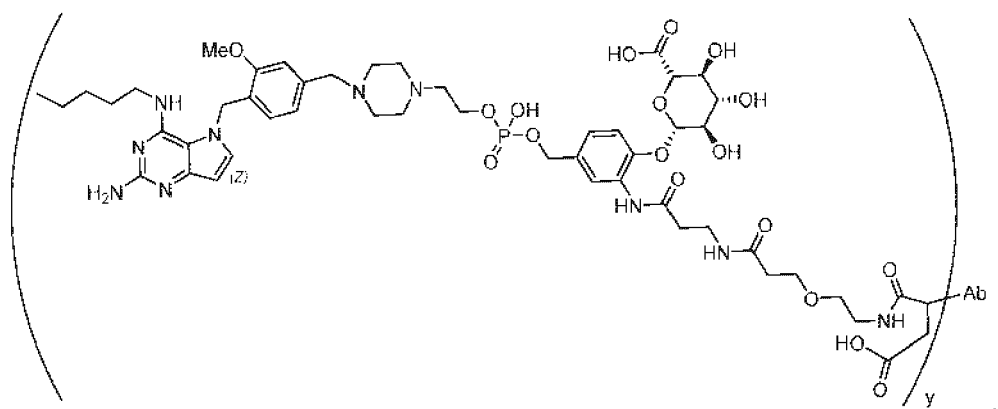
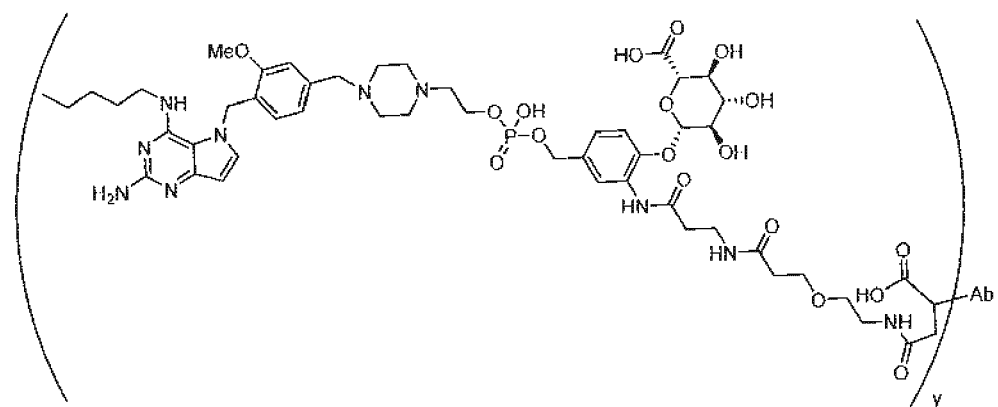
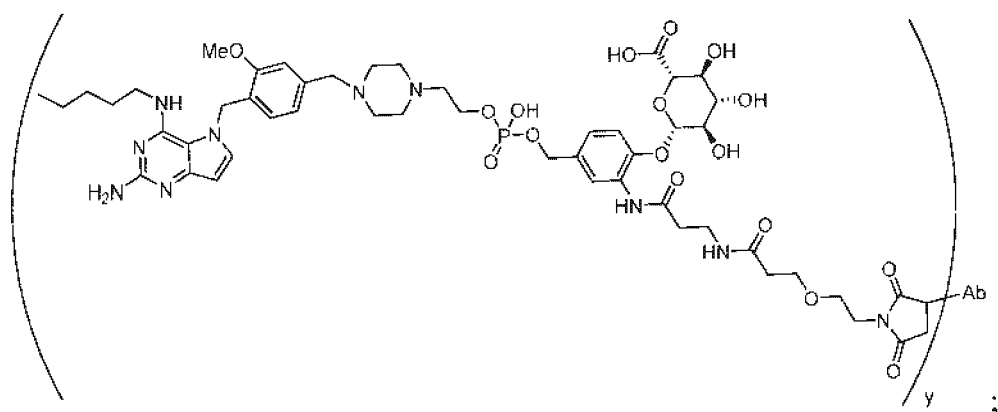
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et

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dans lequel Ab est un anticorps ou un fragment de liaison d'antigène de celui-ci qui se lie spécifiquement à HER2 humain, et y est un entier de 1 à 4.

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12. Conjugué selon la revendication 11, dans lequel Ab est choisi parmi le trastuzumab, le pertuzumab, le margétuximab ou HT-19, ou un mutant de cystéine spécifique à un site de ceux-ci, dans lequel le mutant de cystéine spécifique à un site comprend de la cystéine à une ou plusieurs des positions suivantes (toutes les positions étant selon la numérotation EU) :

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- (a) positions 152, 360 et 375 de la chaîne lourde d'anticorps, et
- (b) positions 107, 159 et 165 de la chaîne légère d'anticorps.

13. Conjugué selon la revendication 11, dans lequel Ab est choisi parmi l'un quelconque des suivants :

(a) un anticorps qui comprend :

une région déterminant la complémentarité de chaîne lourde 1 (HCDR1) comprenant la séquence d'acides aminés de SEQ ID NO : 1 ;
 une région déterminant la complémentarité de chaîne lourde 2 (HCDR2) comprenant la séquence d'acides aminés de SEQ ID NO : 2 ;
 une région déterminant la complémentarité de chaîne lourde 3 (HCDR3) comprenant la séquence d'acides aminés de SEQ ID NO : 3 ;
 une région déterminant la complémentarité de chaîne légère 1 (LCDR1) comprenant la séquence d'acides aminés de SEQ ID NO : 11 ;
 une région déterminant la complémentarité de chaîne légère 2 (LCDR2) comprenant la séquence d'acides aminés de SEQ ID NO : 12 ; et
 une région déterminant la complémentarité de chaîne légère 3 (LCDR3) comprenant la séquence d'acides aminés de SEQ ID NO : 13 ;

(b) un anticorps qui comprend :

une HCDR1 comprenant la séquence d'acides aminés de SEQ ID NO : 4 ;
 une HCDR2 comprenant la séquence d'acides aminés de SEQ ID NO : 5 ;
 une HCDR3 comprenant la séquence d'acides aminés de SEQ ID NO : 3 ;
 une LCDR1 comprenant la séquence d'acides aminés de SEQ ID NO : 14 ;
 une LCDR2 comprenant la séquence d'acides aminés de SEQ ID NO : 15 ; et
 une LCDR3 comprenant la séquence d'acides aminés de SEQ ID NO : 16 ;

(c) un anticorps qui comprend une région variable de chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 7, et une région variable de chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 17 ;

(d) un anticorps qui comprend une chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 9, et une chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 19 ;

(e) un anticorps qui comprend une chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 21, et une chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 19 ;

(f) un anticorps qui comprend une chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 23, et une chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 19 ; ou

(g) un anticorps qui comprend une chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 32, et une chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 19.

14. Conjugué selon la revendication 11, dans lequel Ab comprend une chaîne lourde comprenant la séquence d'acides aminés de SEQ ID NO : 9, et une chaîne légère comprenant la séquence d'acides aminés de SEQ ID NO : 19.

15. Conjugué selon la revendication 14, dans lequel le composé est lié aux cystéines aux positions 152 et 375 de la chaîne lourde d'anticorps (toutes les positions étant selon la numérotation EU).

16. Conjugué selon la revendication 11, dans lequel y est environ 3 à 4.

17. Conjugué selon l'une quelconque des revendications 11 à 16, le conjugué présentant un indice d'hydrophobicité de 0,8 ou plus, comme déterminé par chromatographie d'interaction hydrophobe.

18. Composition pharmaceutique comprenant un ou plusieurs conjugués selon l'une quelconque des revendications 1 à 17 et un véhicule pharmaceutiquement acceptable.

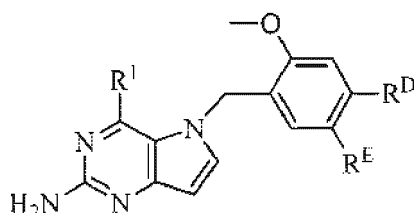
19. Conjugué selon l'une quelconque des revendications 1 à 17 ou composition pharmaceutique selon la revendication 18 pour utilisation dans le traitement d'un cancer positif pour HER2 chez un sujet en ayant besoin.

20. Conjugué pour utilisation selon la revendication 19, le conjugué étant capable de supprimer le cancer positif pour HER2 pendant une durée prolongée et/ou réduire la récurrence du cancer positif pour HER2, par rapport à un anticorps anti-HER2 seul.

21. Conjugué pour utilisation selon la revendication 19, le cancer positif pour HER2 étant choisi parmi un cancer de

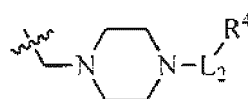
l'estomac, un cancer de l'œsophage, un adénocarcinome de la jonction gastro-œsophagienne, un cancer du côlon, un cancer rectal, un cancer du sein, un cancer de l'ovaire, un cancer du col utérin, un cancer de l'utérus, un cancer de l'endomètre, un cancer de la vessie, un cancer des voies urinaires, un cancer du pancréas, un cancer du poumon, un cancer de la prostate, un ostéosarcome, un neuroblastome, un glioblastome et un cancer de la tête et du cou.

22. Conjugué pour utilisation selon la revendication 19, le conjugué étant administré au sujet par voie intraveineuse, par voie intratumorale ou par voie sous-cutanée.
23. Conjugué pour utilisation selon la revendication 19, le conjugué étant administré à une dose d'environ 0,01 à 20 mg par kg de poids corporel.
24. Conjugué pour utilisation selon la revendication 19 comprenant en outre l'administration d'un deuxième agent au sujet.
25. Composé ayant la structure de formule (I), ou sels pharmaceutiquement acceptables de celui-ci :

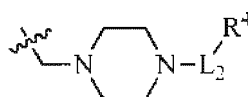


dans lequel :

R^D est



et R^E est H ; ou R^E est



et R^D est H ;

R^1 est $-NHR^2$ ou $-NHCHR^2R^3$;

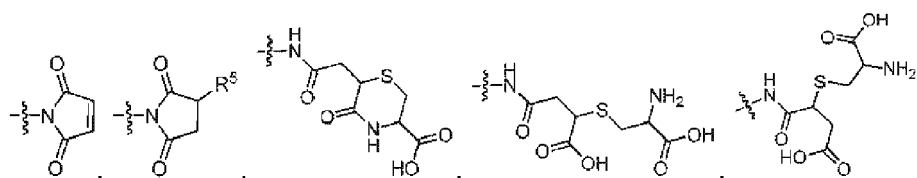
R^2 est $-(alkyle \text{ en } C_3-C_6)$ ou $-(alkyle \text{ en } C_4-C_6)$;

R^3 est L_1OH ;

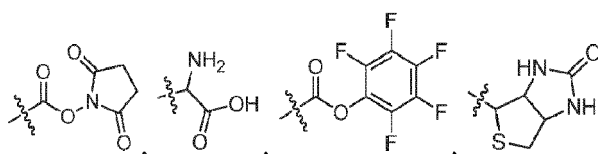
L_1 est $-(CH_2)_m-$;

L_2 est $-(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_n-$, $-(CH_2)_nX_1(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_n-$, $-(CH_2)_nNHC(=O)(CH_2)_nC(=O)NH(CH_2)_n-$, $-((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)((CH_2)_nO)_t(CH_2)_nC(=O)NH(CH_2)_n-$, $-C(=O)NH((CH_2)_nO)_t(CH_2)_nX_1(CH_2)_n-$, $-C(=O)X_2X_3C(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)X_2X_3C(=O)(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)(CH_2)_n-$, $-C(=O)X_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n-$, $-C(=O)(CH_2)_nC(R_7)_2-$, $-C(=O)(CH_2)_nC(R_7)_2SS(CH_2)_nNHC(=O)(CH_2)_n-$, $-(CH_2)_nX_2C(=O)(CH_2)_nNHC(=O)((CH_2)_nO)_t(CH_2)_n-$ ou $-C(=O)(CH_2)_nC(=O)NH(CH_2)_n$;

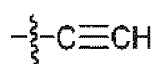
R^4 est



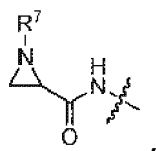
-ONH₂, -NH₂,



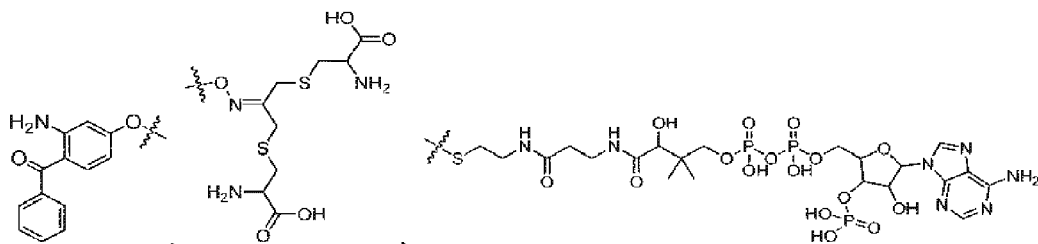
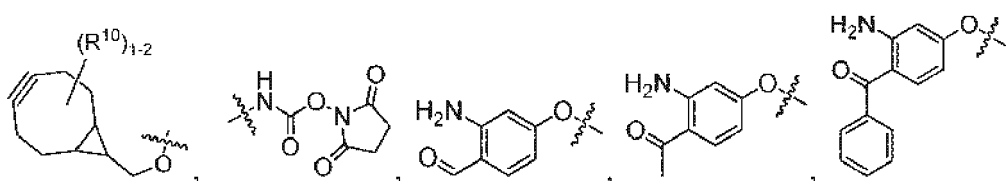
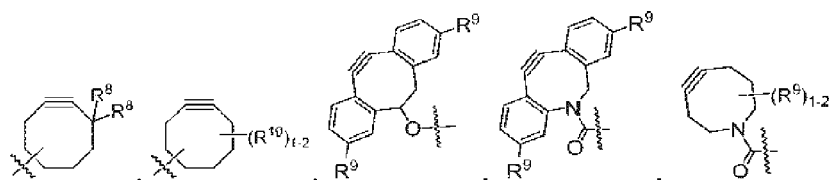
-N₃,

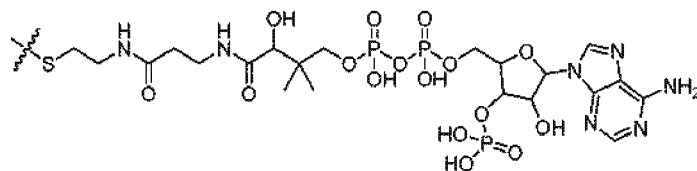


, -NHC(=O)CH=CH₂, -SH, -SR⁷, -OH, -SSR⁶, -S(=O)₂(CH=CH₂), -(CH₂)₂S(=O)₂(CH=CH₂),
-NHS(=O)₂(CH=CH₂), -NHC(=O)CH₂Br, -NHC(=O)CH₂I, -C(O)NHNH₂,

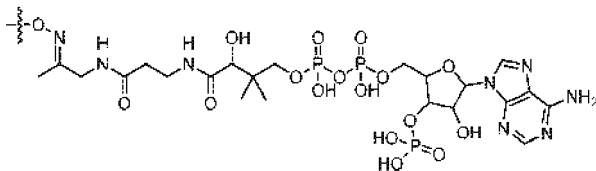


-CO₂H, -C(O)NHNH₂,

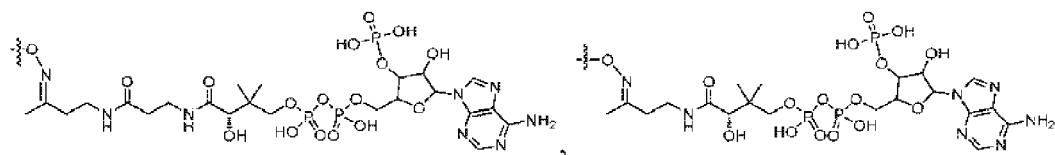




5

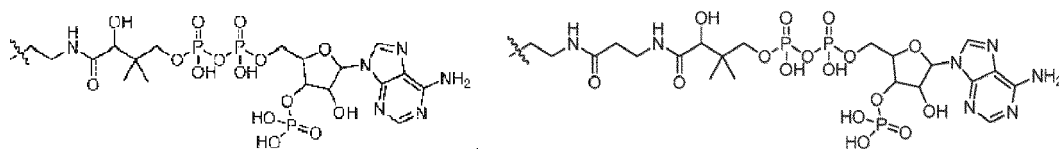


10



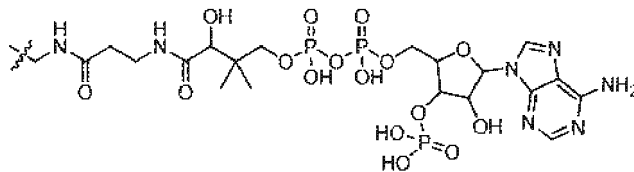
15

20



25

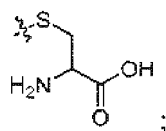
ou



30

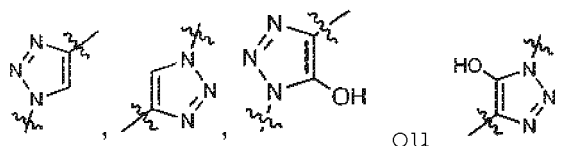
35

R⁵ est



40

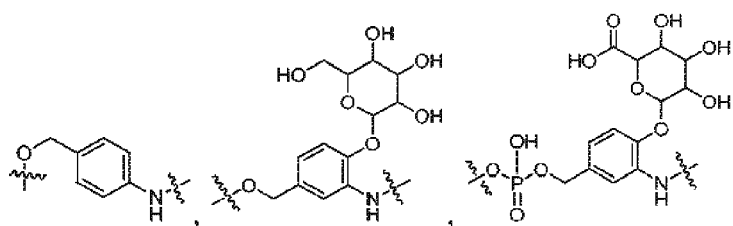
X₁ est



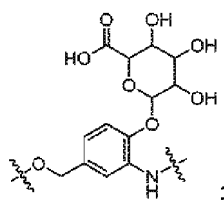
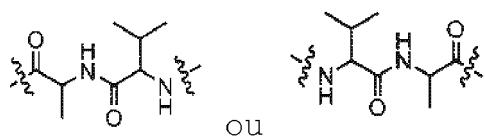
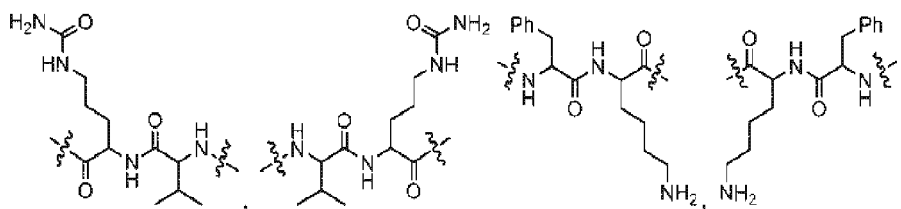
50

X₂ est

55



ou

 X_3 est R^6 est 2-pyridyle ou 4-pyridyle ;chaque R^7 est indépendamment choisi parmi H et alkyle en C_1-C_6 ;chaque R^8 est indépendamment choisi parmi H, alkyle en C_1-C_6 , F, Cl et -OH ;chaque R^9 est indépendamment choisi parmi H, alkyle en C_1-C_6 , F, Cl, $-NH_2$, $-OCH_3$, $-OCH_2CH_3$, $-N(CH_3)_2$, $-CN$, $-NO_2$ et -OH ;chaque R^{10} est indépendamment choisi parmi H, alkyle en C_{1-6} , fluoro, benzyloxy substitué par $-C(=O)OH$, benzyle substitué par $-C(=O)OH$, alcoxy en C_{1-4} substitué par $-C(=O)OH$ et alkyle en C_{1-4} substitué par $-C(=O)OH$;

chaque m est indépendamment choisi parmi 1, 2, 3 et 4 ;

chaque n est indépendamment choisi parmi 1, 2, 3 et 4 ; et

chaque t est indépendamment choisi parmi 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 et 18.

26. Conjugué comprenant le composé selon la revendication 25 lié à un anticorps ou un fragment de liaison d'antigène de celui-ci.

Fig. 1

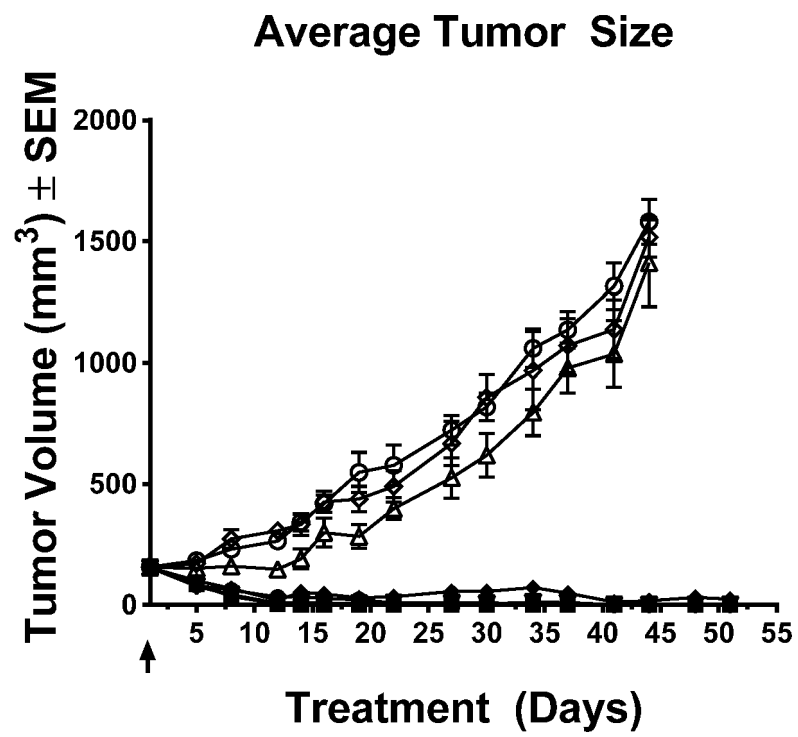


Fig. 2

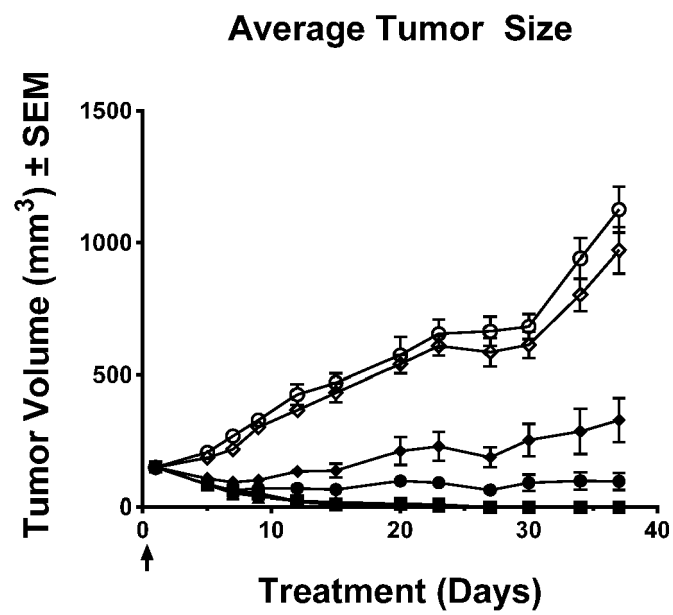


Fig. 3

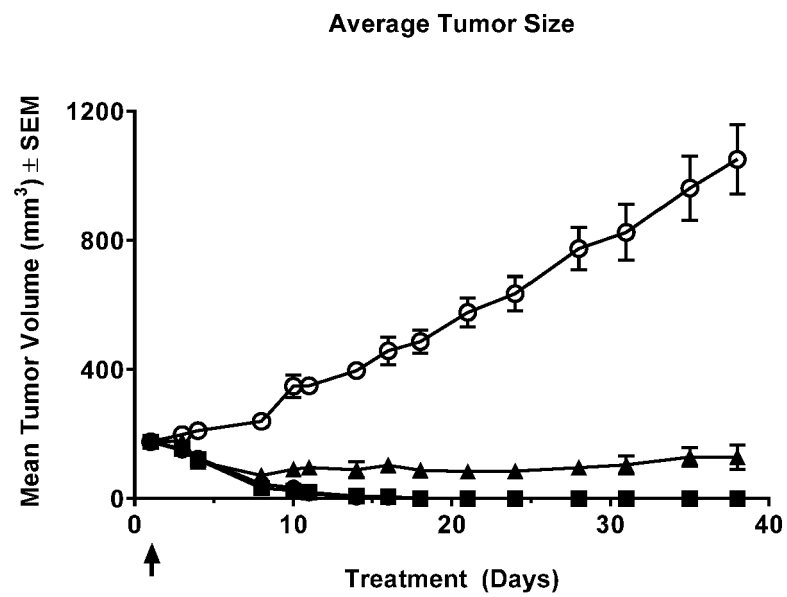


Fig. 4

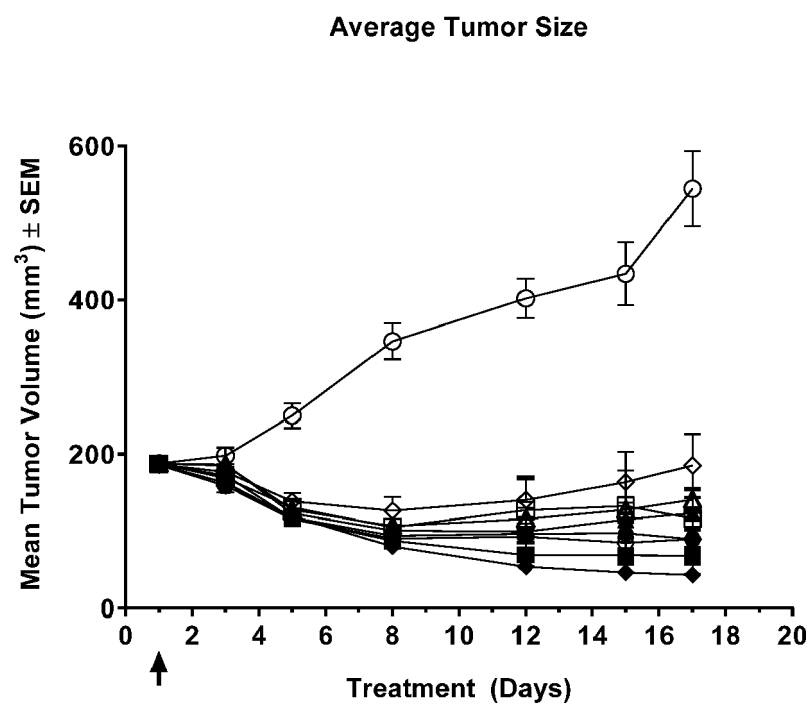


Fig. 5A

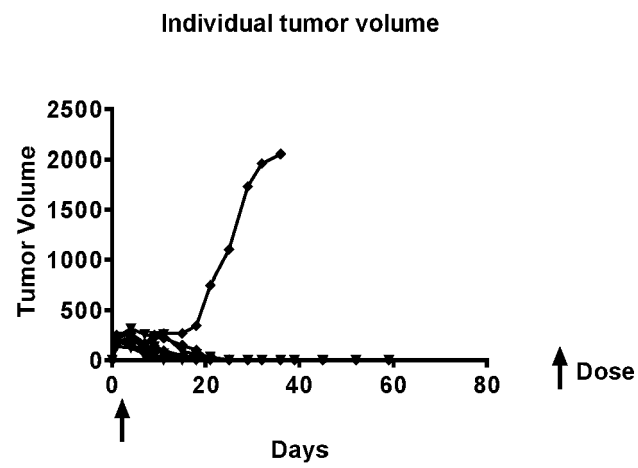


Fig. 5B

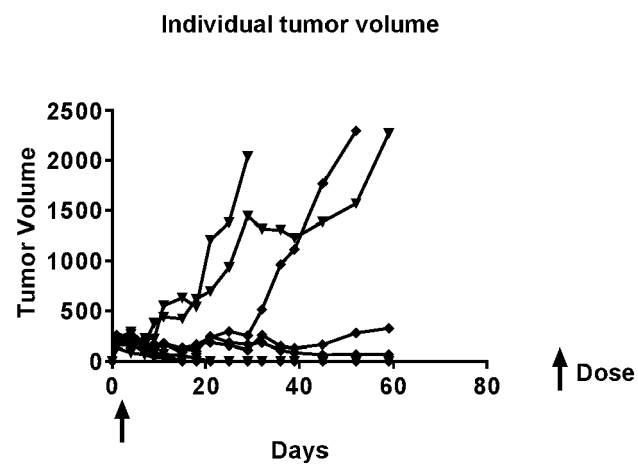


Fig. 6

Average Tumor Size

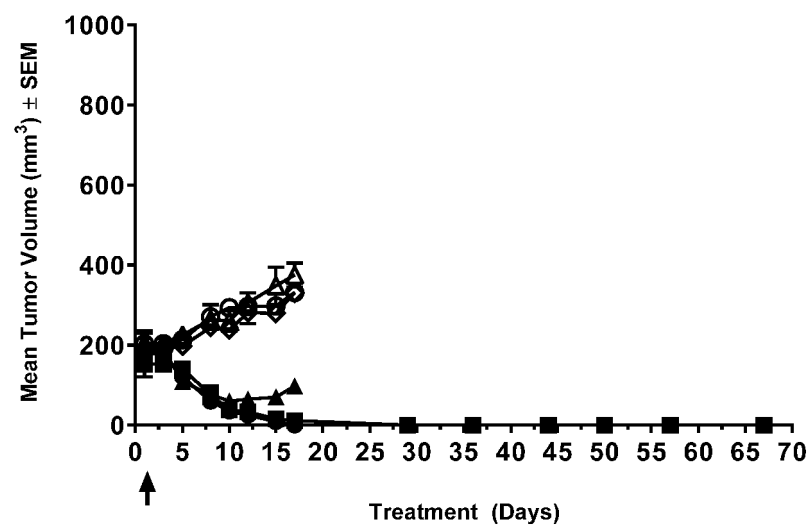


Fig. 7

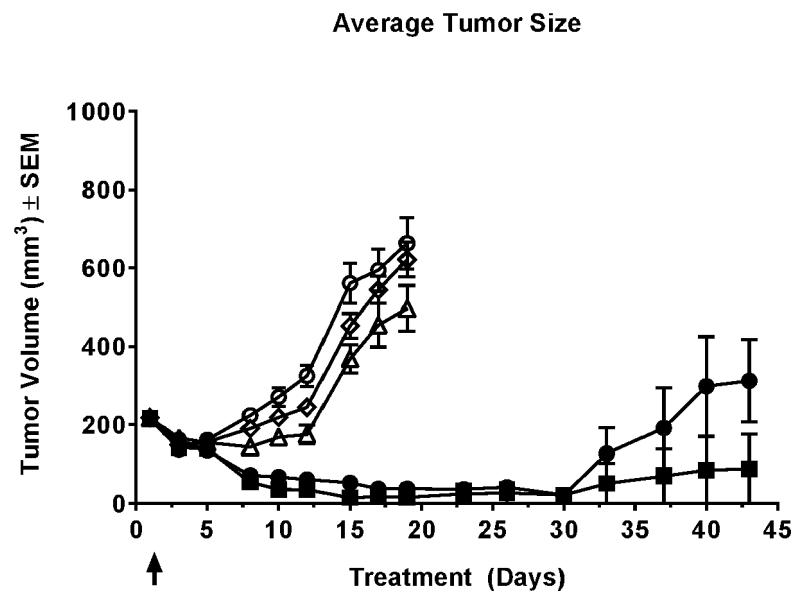


Fig. 8A

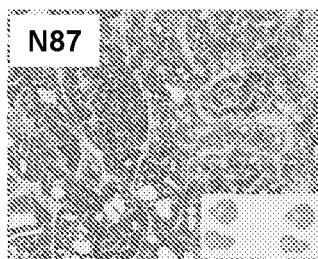


Fig. 8B

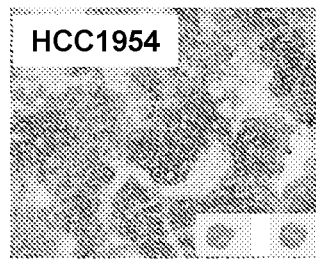
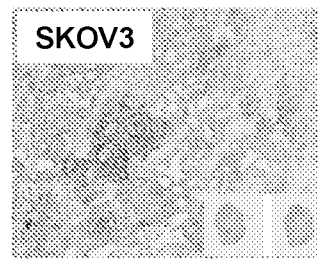


Fig. 8C



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