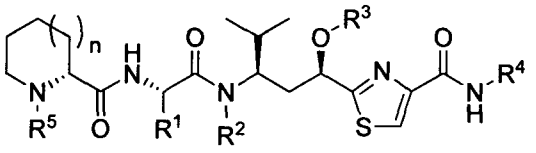
ORIGINAL

ABSTRACT OF THE DISCLOSURE

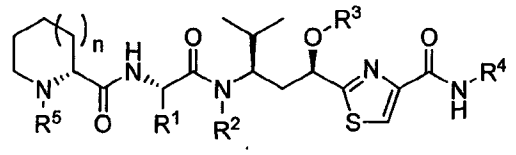
Antiproliferative compounds having a structure represented by formula (II), where n, R^1, R^2, R^3, R^4, and R^5 are as defined herein, can be used to treat tumors, optionally when conjugated to a ligand such as an antibody:



(II)

What is claimed is:

1. A compound having a structure represented by formula (II)



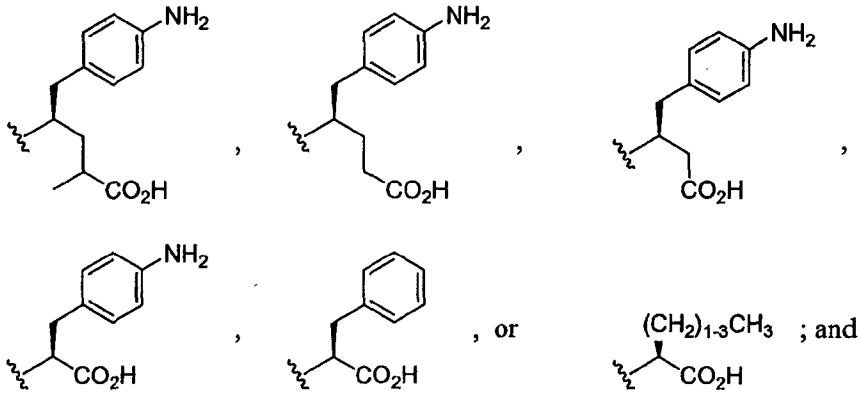
(II)

wherein

n is 0, 1, or 2;

R^1, R^2 and R^3 are independently H, unsubstituted or substituted C_1-C_{10} alkyl, unsubstituted or substituted C_2-C_{10} alkenyl, unsubstituted or substituted C_2-C_{10} alkynyl, unsubstituted or substituted aryl, unsubstituted or substituted heteroaryl, unsubstituted or substituted $(CH_2)_{1-2}O(C_1$-C_{10} alkyl), unsubstituted or substituted $(CH_2)_{1-2}O(C_2$-C_{10} alkenyl), unsubstituted or substituted $(CH_2)_{1-2}O(C_2$-C_{10} alkynyl), $(CH_2)_{1-2}OC(=O)(C_1$-C_{10} alkyl), unsubstituted or substituted $(CH_2)_{1-2}OC(=O)(C_2$-C_{10} alkenyl), unsubstituted or substituted $(CH_2)_{1-2}OC(=O)(C_2$-C_{10} alkynyl), unsubstituted or substituted $C(=O)(C_1$-C_{10} alkyl), unsubstituted or substituted $C(=O)(C_2$-C_{10} alkenyl), unsubstituted or substituted $C(=O)(C_2$-C_{10} alkynyl), unsubstituted or substituted cycloaliphatic, unsubstituted or substituted heterocycloaliphatic, unsubstituted or substituted arylalkyl, or unsubstituted or substituted alkylaryl;

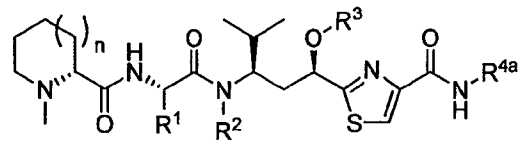
R^4 is



R^5 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, $CO(C_1$-C_5 alkyl), $CO(C_2$-C_5 alkenyl), or $CO(C_2$-C_5 alkynyl);

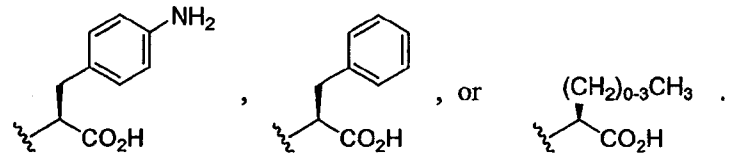
or a pharmaceutically acceptable ester thereof, a pharmaceutically acceptable amide thereof at the carboxyl group of R^4 with the α-amino group of an α-amino acid, or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1, having a structure represented by formula (II-a)

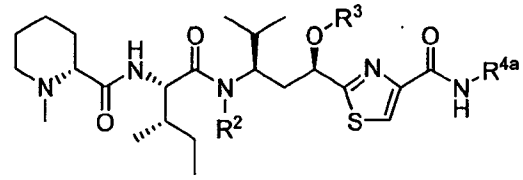


(II-a)

wherein R^{4a} is



3. A compound according to claim 2, having a structure represented by formula (II-a'):



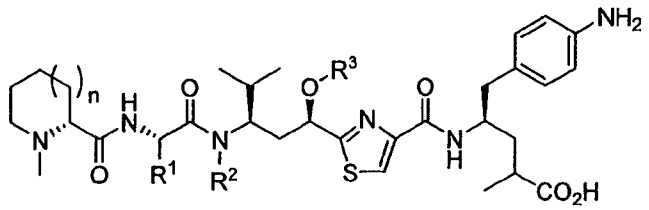
(II-a')

wherein

R^2 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, $CH_2O(C_1$-C_5 alkyl), $CH_2O(C_2$-C_5 alkenyl), $CH_2O(C{=}O)(C_1$-C_5 alkyl), or $CH_2OC(=O)(C_2$-C_5 alkenyl); and

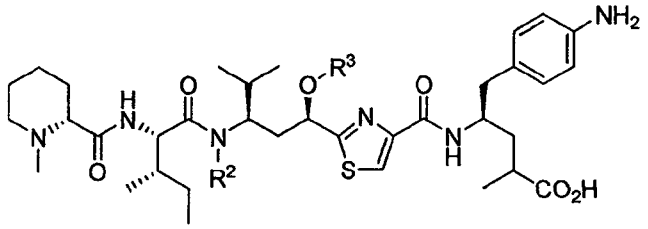
R^3 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, $C(=O)C_1$-C_5 alkyl, or $C(=O)C_2$-C_5 alkenyl.

4. A compound according to claim 1, having a structure represented by formula (II-b):



(II-b)

5. A compound according to claim 4, having a structure represented by formula (II-b'):



(II-b')

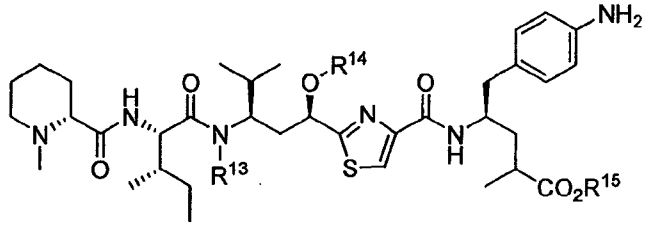
wherein

R^2 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, $CH_2O(C_1$-C_5 alkyl), $CH_2O(C_2$-C_5 alkenyl), $CH_2O(C{=}O)(C_1$-C_5 alkyl), or $CH_2OC(=O)(C_2$-C_5 alkenyl); and

R^3 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, $C(=O)C_1$-C_5 alkyl, or $C(=O)C_2$-C_5 alkenyl.

6. A compound according to claim 1, having a structure according to formula (III-a), III-b), (III-c), (III-d), (III-e), (III-f), (III-g), (III-h), (III-i), (III-j), (III-k), (III-l), (III-m), (III-n), (III-o), (III-p), (III-q), (III-r), (III-s), (III-t), (III-u), (III-v), (III-w), or (III-y).

7. A compound according to claim 1, having a structure represented by formula (II-c)



(II-c)

where R^{13} is Me, n-Pr, CH_2OMe, or $CH_2OC(=O)CH_2CH(Me)_2$; R^{14} is Me or C(=O)Me; and R^{15} is H or C_1-C_5 alkyl.

8. A conjugate comprising a compound according to claim 1 conjugated to an antibody.

9. A conjugate having a structure represented by formula (IV):

$$[D(X^D)_aC(X^Z)_b]_mZ \qquad (IV)$$

wherein

D is a compound according to claim 1;

Z is an antibody;

X^D and X^Z are spacer groups;

C is a group cleavable at the site of intended biological action of D;

each of a and b is independently 0 or 1; and

m is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

10. A composition of matter having a structure represented by formula (V-a)

$$D\text{-}(X^D)_aC(X^Z)_b\text{-}R^{31} \qquad (V\text{-}a)$$

wherein

R^{31} is a functional group suitable for reacting with a functional group on an antibody;

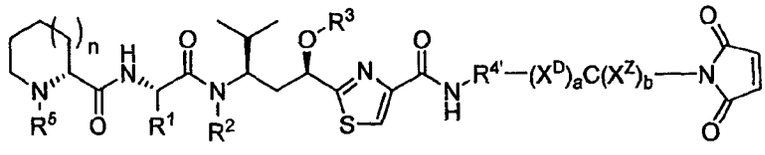
D is a compound according to claim 1;

X^D and X^Z are spacer groups;

C is a group cleavable at the site of intended biological action of D; and

each of a and b is independently 0 or 1.

11. A composition of matter having a structure represented by formula (V-b)



(V-b)

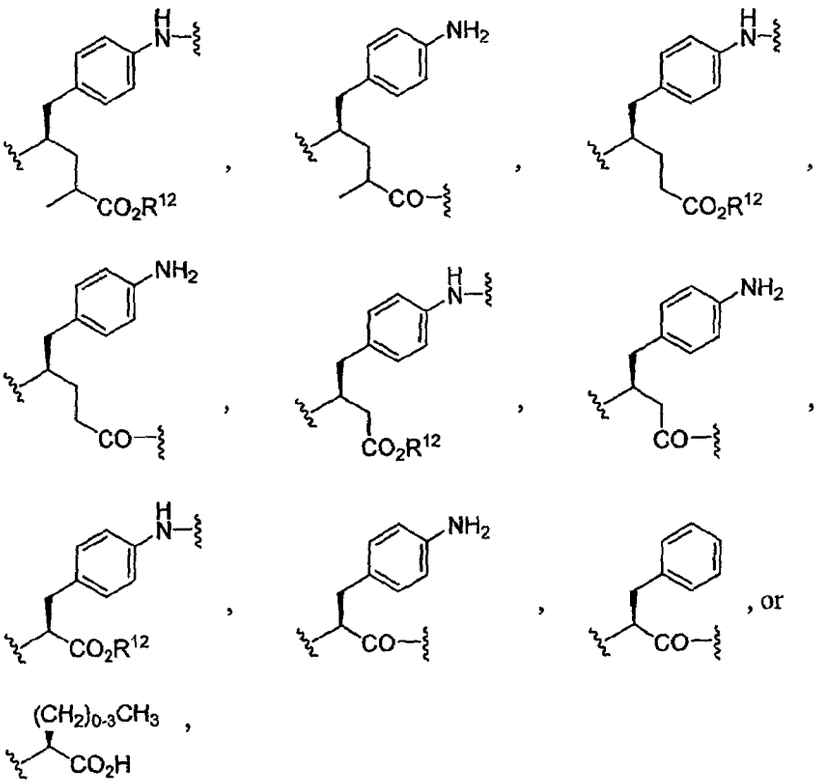
wherein

n is 0, 1, or 2;

R^1, R^2 and R^3 are independently H, unsubstituted or substituted C_1-C_{10} alkyl, unsubstituted or substituted C_2-C_{10} alkenyl, unsubstituted or substituted C_2-C_{10} alkynyl, unsubstituted or substituted aryl, unsubstituted or substituted heteroaryl, unsubstituted or substituted $(CH_2)_{1\text{-}2}O(C_1\text{-}C_{10}$ alkyl), unsubstituted or substituted $(CH_2)_{1\text{-}2}O(C_2\text{-}C_{10}$ alkenyl), unsubstituted or

substituted $(CH_2)_{1-2}O(C_2-C_{10}$ alkynyl), $(CH_2)_{1-2}OC(=O)(C_1-C_{10}$ alkyl), unsubstituted or substituted $(CH_2)_{1-2}OC(=O)(C_2-C_{10}$ alkenyl), unsubstituted or substituted $(CH_2)_{1-2}OC(=O)(C_2-C_{10}$ alkynyl), unsubstituted or substituted $C(=O)(C_1-C_{10}$ alkyl), unsubstituted or substituted $C(=O)(C_2-C_{10}$ alkenyl), unsubstituted or substituted $C(=O)(C_2-C_{10}$ alkynyl), unsubstituted or substituted cycloaliphatic, unsubstituted or substituted heterocycloaliphatic, unsubstituted or substituted arylalkyl, or unsubstituted or substituted alkylaryl;

$R^{4'}$ is



wherein R^{12} is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, or C_2-C_5 alkynyl; and

R^5 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, $CO(C_1$-C_5 alkyl), $CO(C_2$-C_5 alkenyl), or $CO(C_2$-C_5 alkynyl);

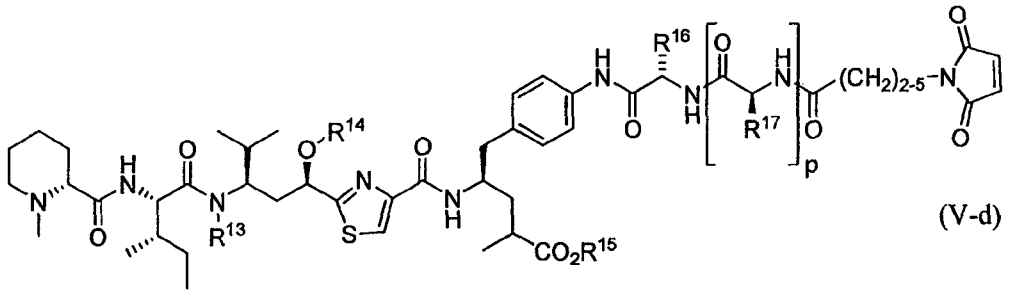
X^D and X^Z are spacer groups;

C is a cleavable group; and

a and b are independently 0 or 1;

wherein group $R^{4'}$ is linked via a carboxyl or amine group therein to either group X^D in the event a is 1 or to group C in the event a is 0.

12. A composition of matter having a structure represented by formula (V-d):

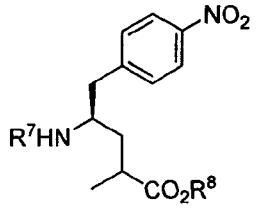


(V-d)

where R^{13} is Me, n-Pr, CH_2OMe, or $CH_2OC(=O)CH_2CH(Me)_2$; R^{14} is Me or C(=O)Me; R^{15} is H or C_1-C_5 alkyl; R^{16} is $(CH_2)_4NH_2$ or $(CH_2)_3NHC(=O)NH_2$; R^{17} is $C(Me)_2$ or Me; and p is 0 or 1.

13. A method for treating a cancer in a subject suffering from such cancer, comprising administering to the subject a therapeutically effective amount of a compound according to claim 1, or a conjugate thereof with a ligand, in particular an antibody.

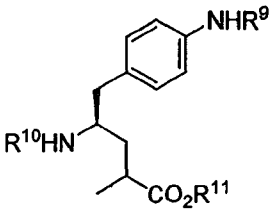
14. A compound having a structure according to formula (VIII-a)



(VIII-a)

wherein R^7 is H or an amine protecting group and R^8 is H, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, aryl, cycloaliphatic, alkylcycloaliphatic, arylalkyl, or alkylaryl.

15. A compound having a structure according to formula (VIII-b)



(VIII-b)

wherein R^9 and R^{10} are independently H or an amine protecting group and R^{11} is H, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, aryl, cycloaliphatic, alkylcycloaliphatic, arylalkyl, or alkylaryl.

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Dated this the 31st day of January, 2012

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Fig. 1a

Scheme 1 (part 1 of 2)

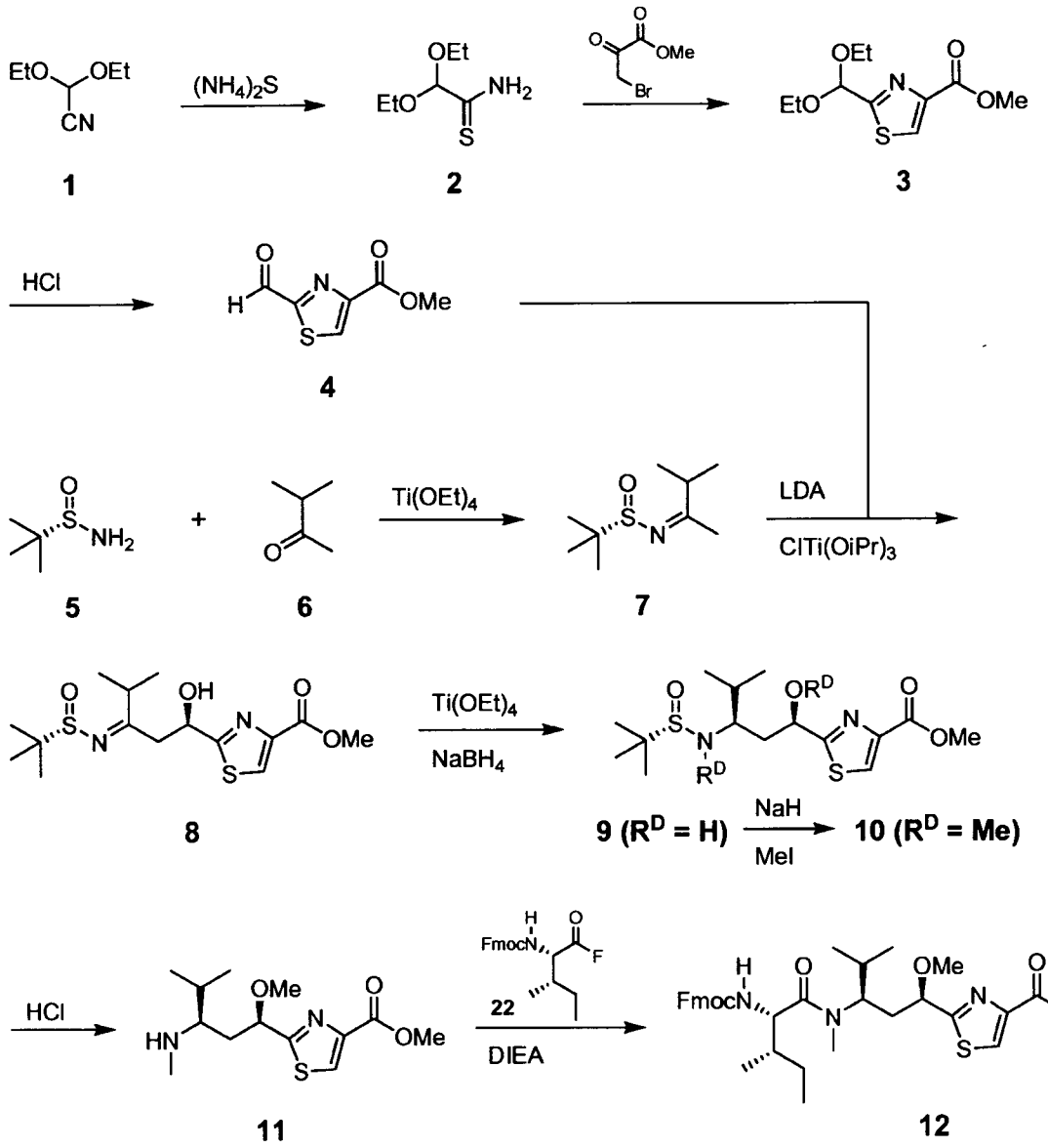

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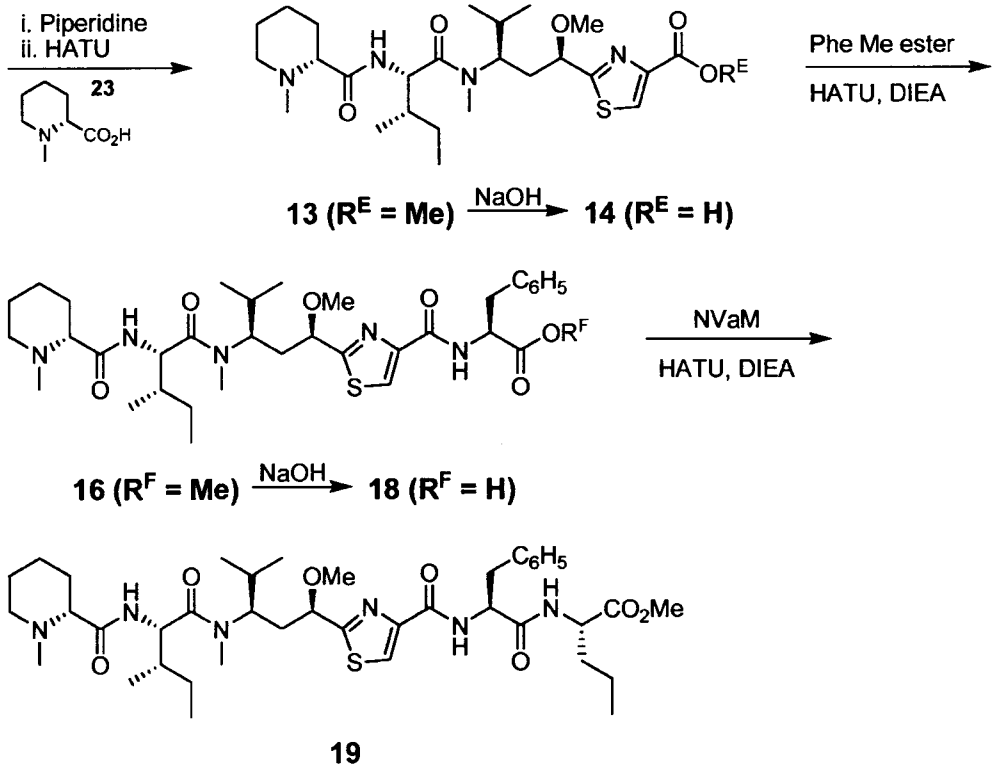
Fig. 1b

Scheme 1 (part 2 of 2)



Archana Shanker

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Fig. 2

Scheme 2

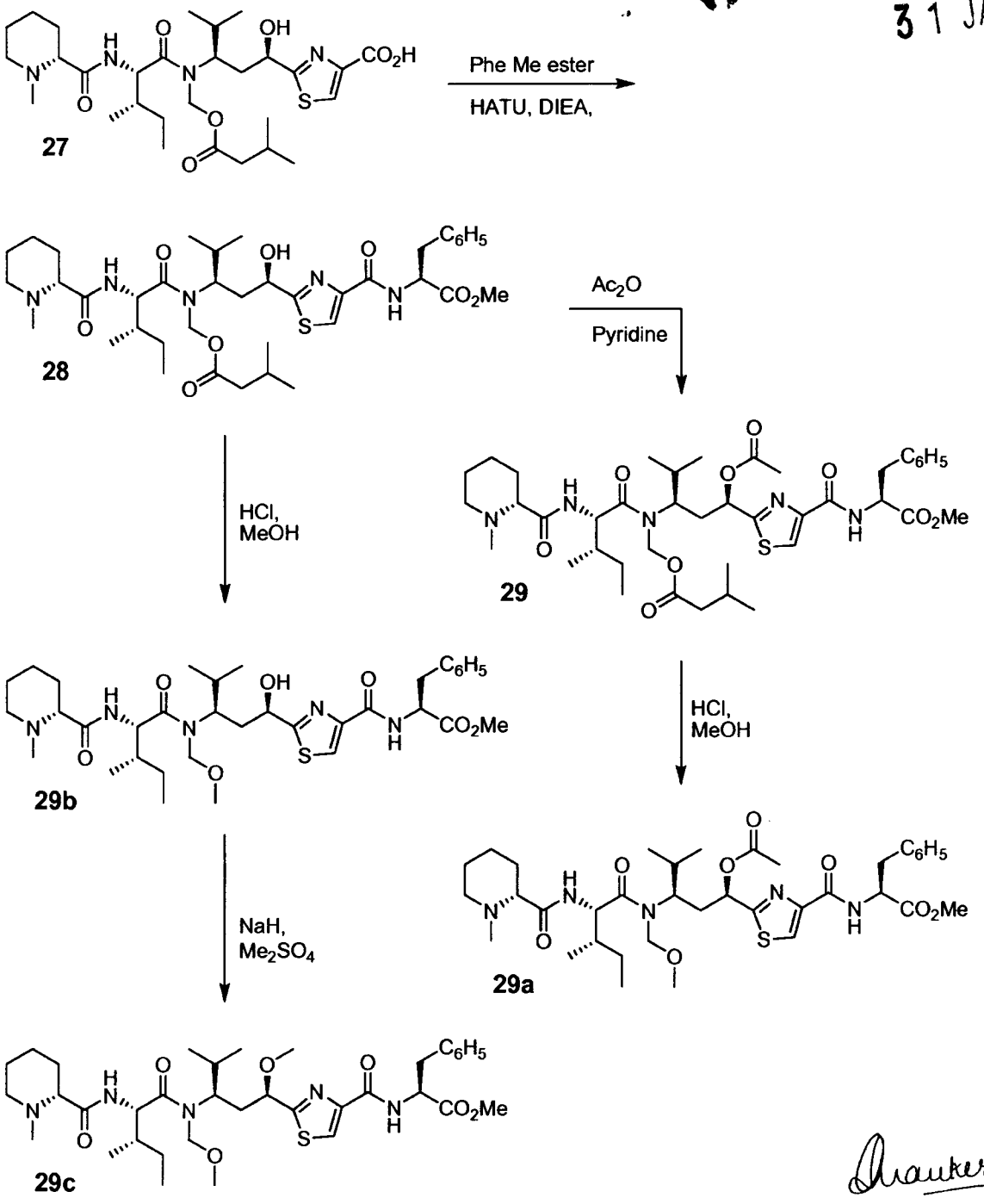

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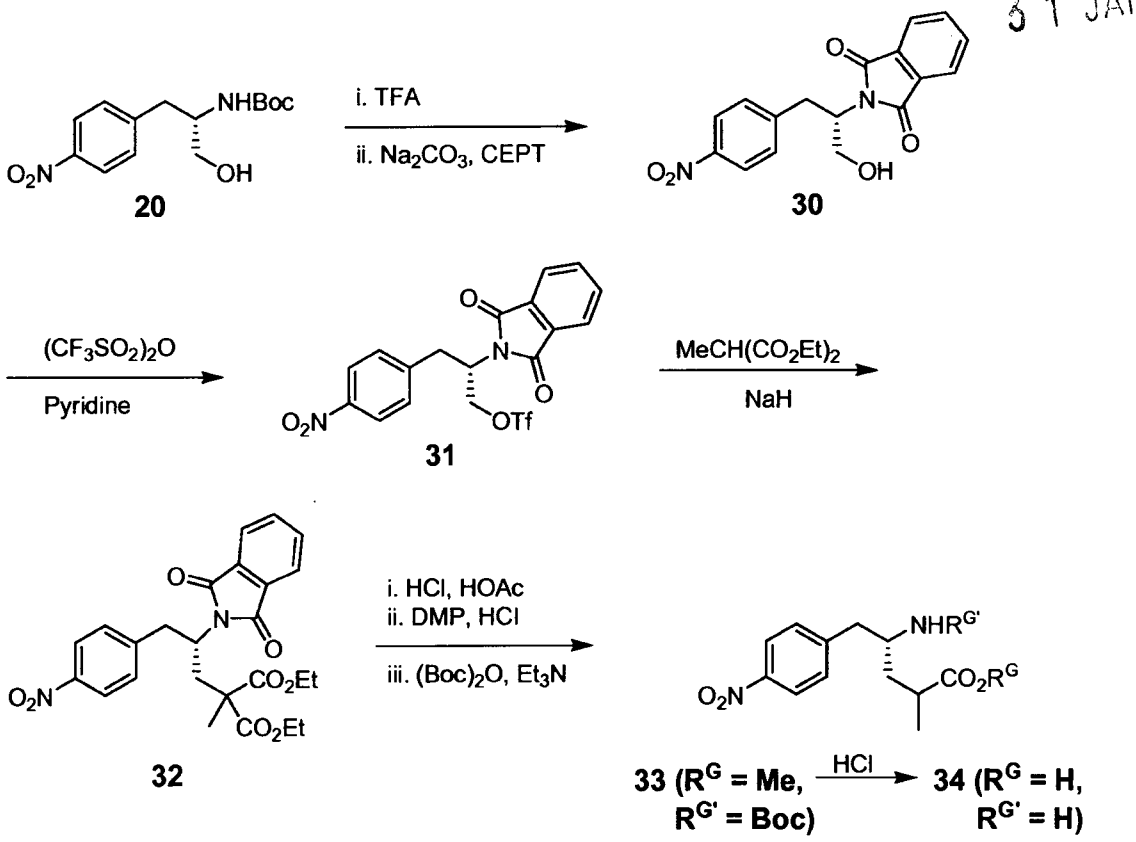
Fig. 3

Scheme 3



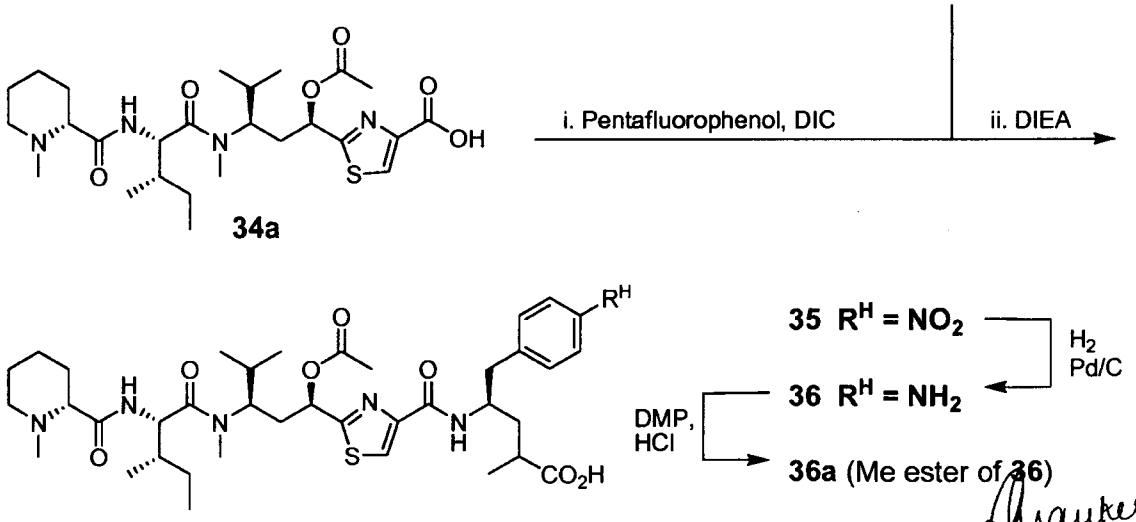


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Fig. 4

Scheme 4

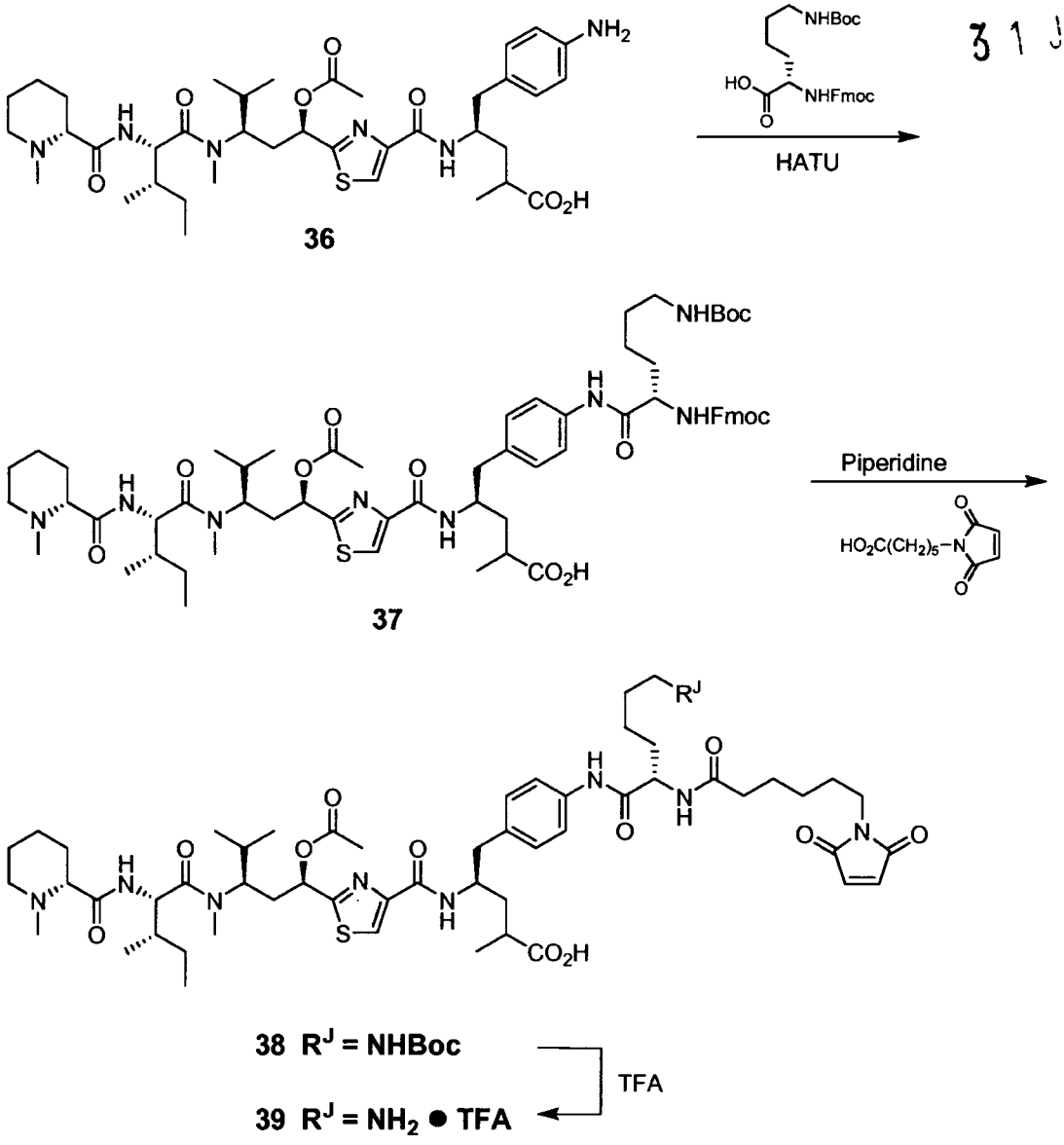

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Fig. 5

Scheme 5

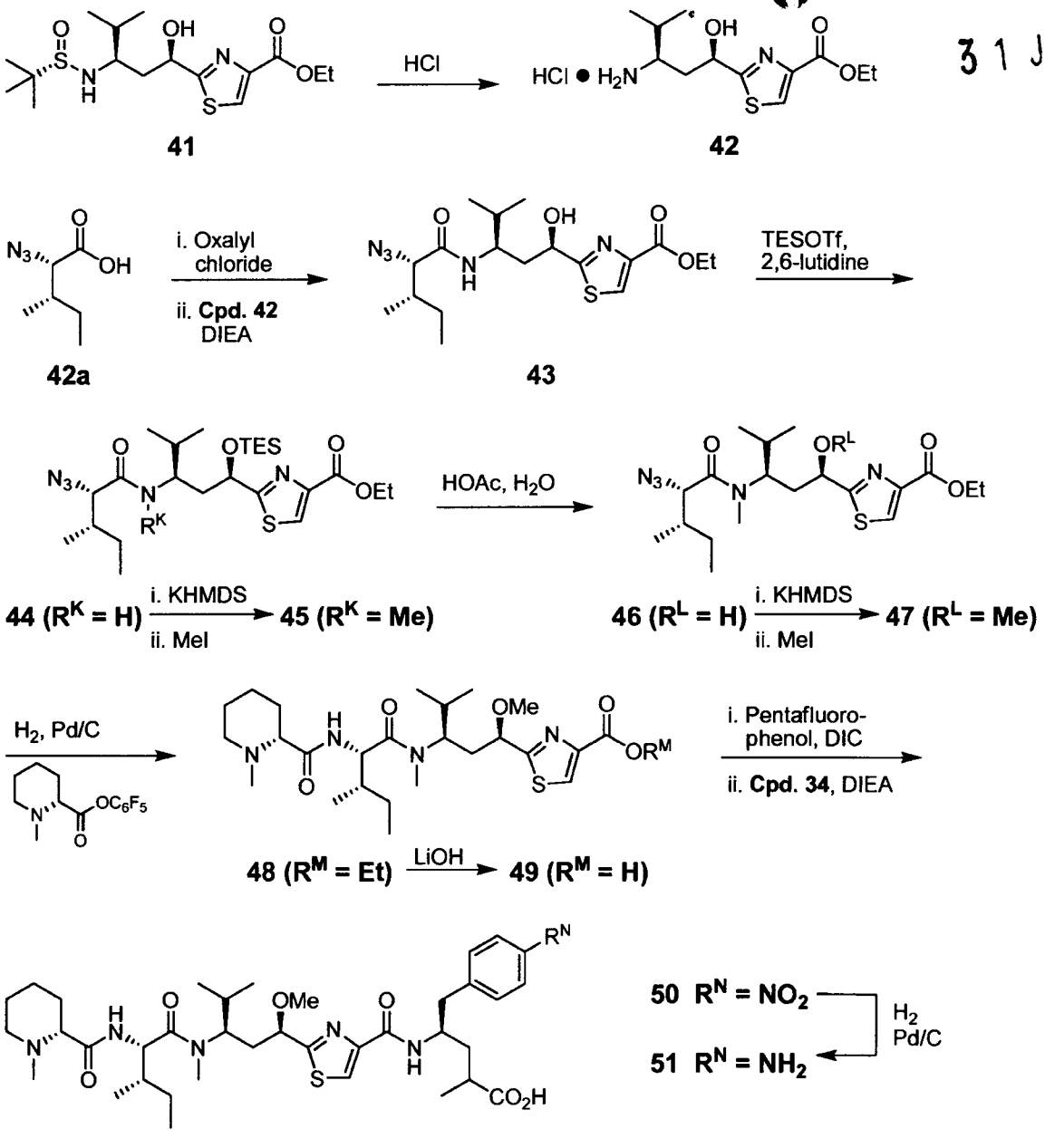

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Fig. 6

Scheme 6

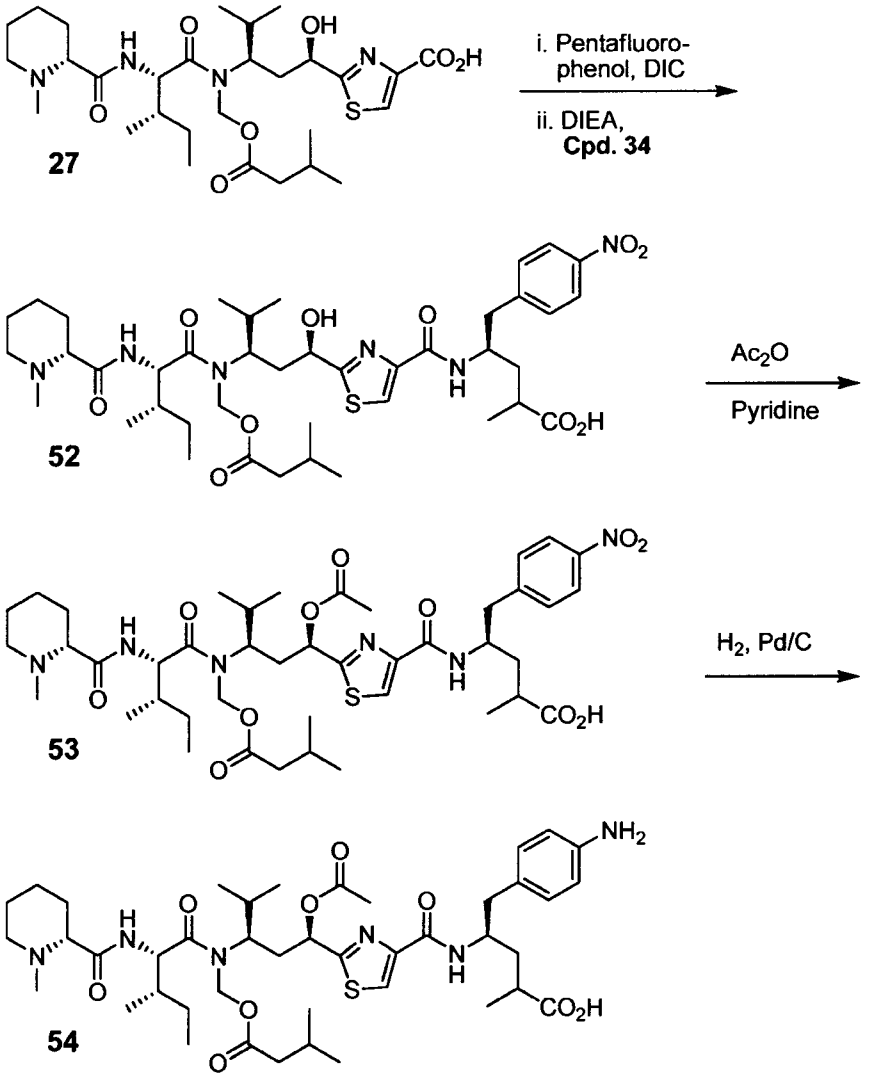

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Fig. 7

Scheme 7

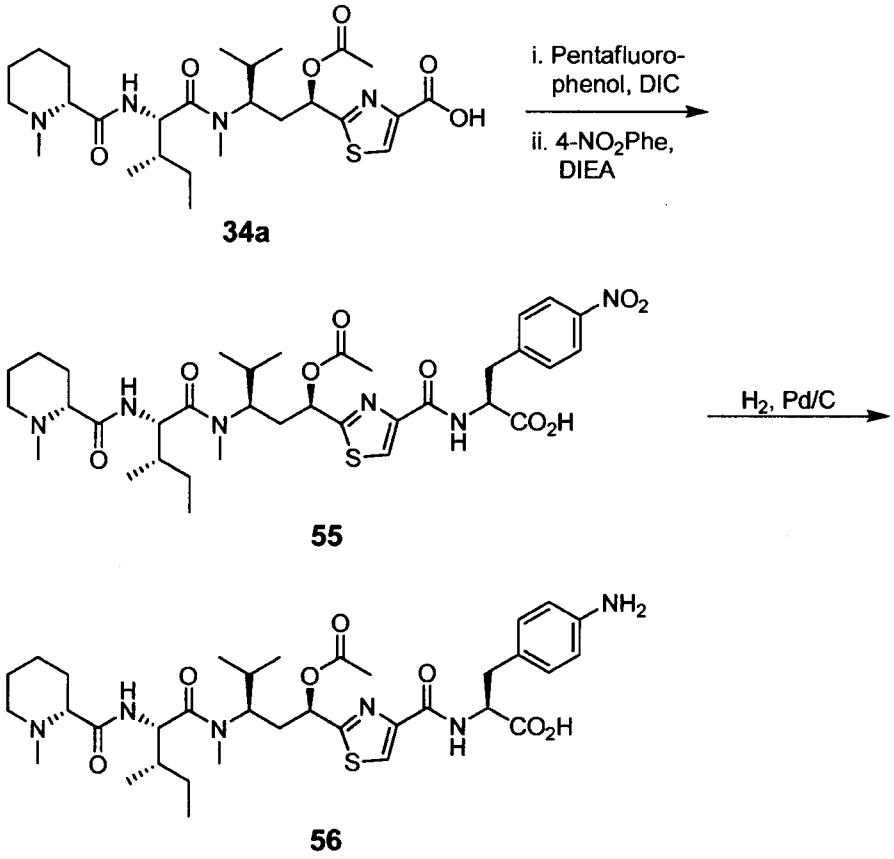

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Fig. 8a

Scheme 8

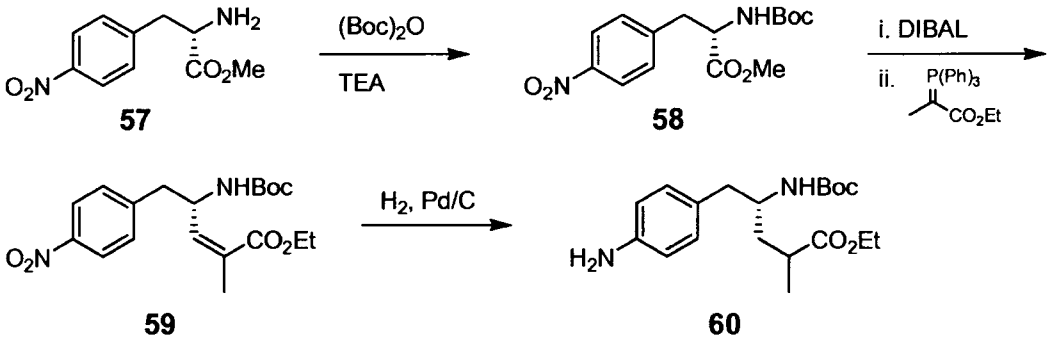

Fig. 8b

Scheme 9

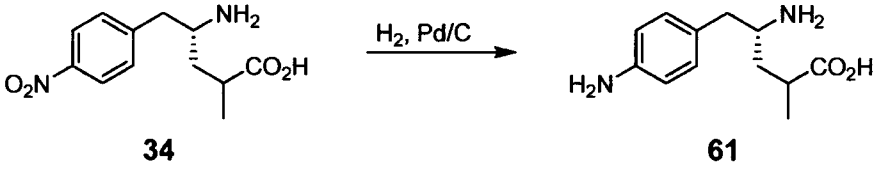

Fig. 8c

Scheme 10

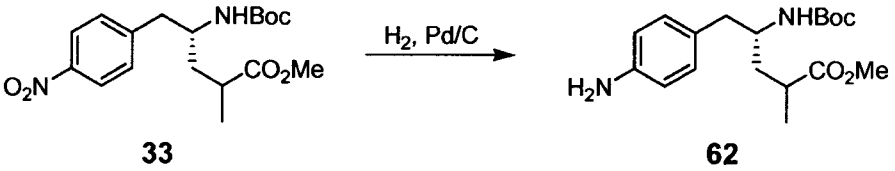

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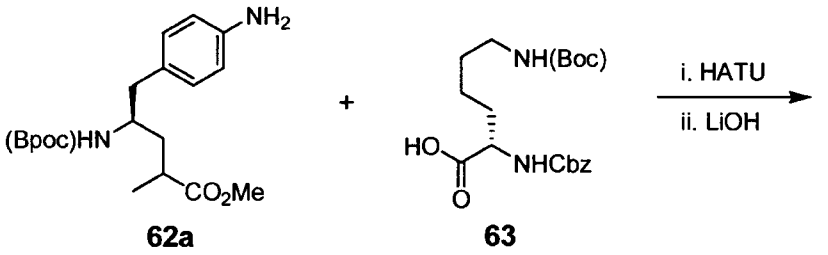
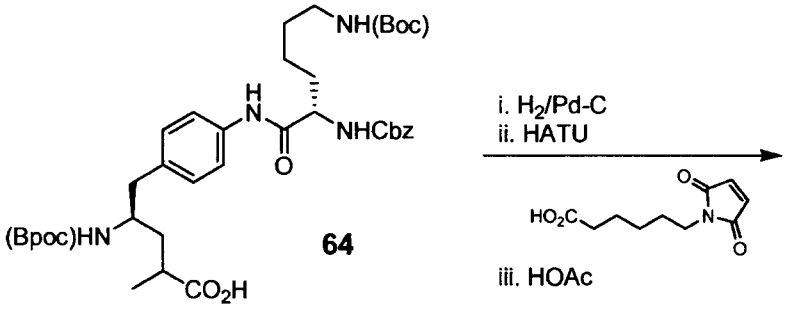
Fig. 9

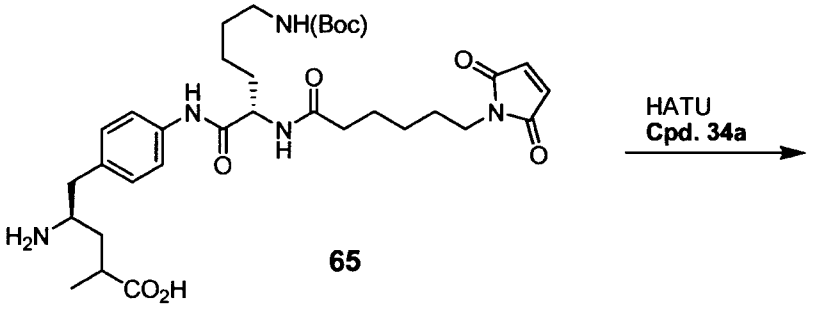
Scheme 11



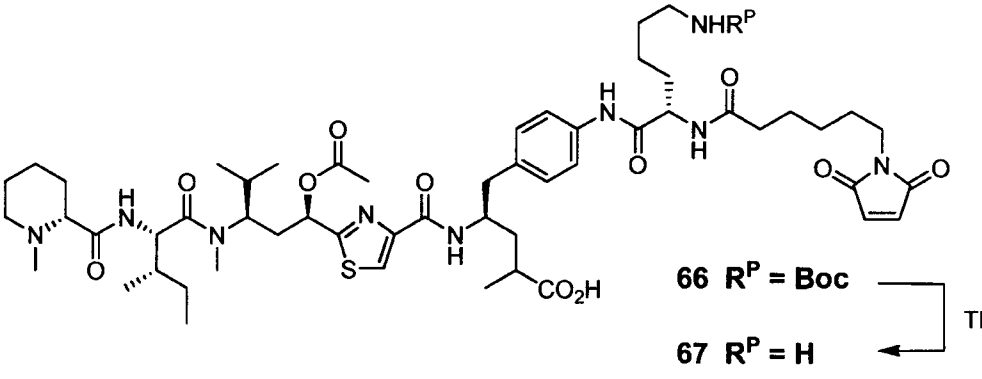
62a **63**



64



65



66 R^P = Boc

67 R^P = H

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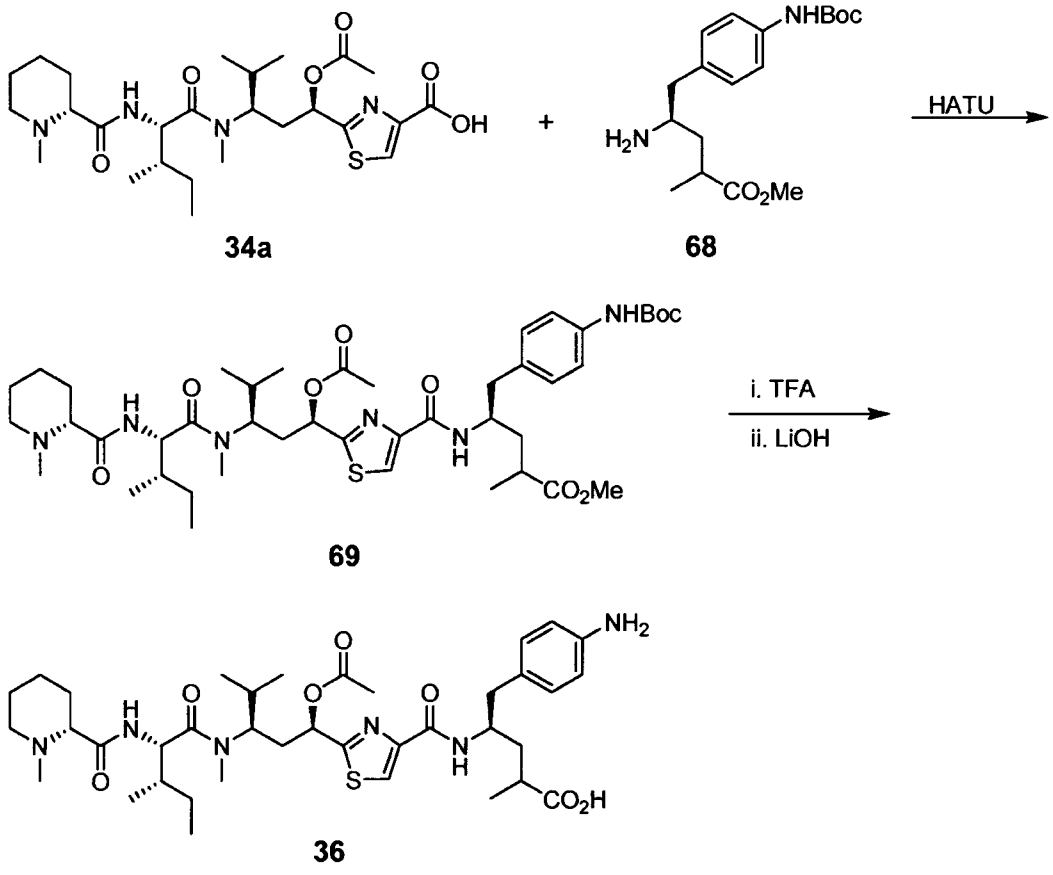
Fig. 10

Scheme 12



34a **68**

69

36

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Fig. 11a

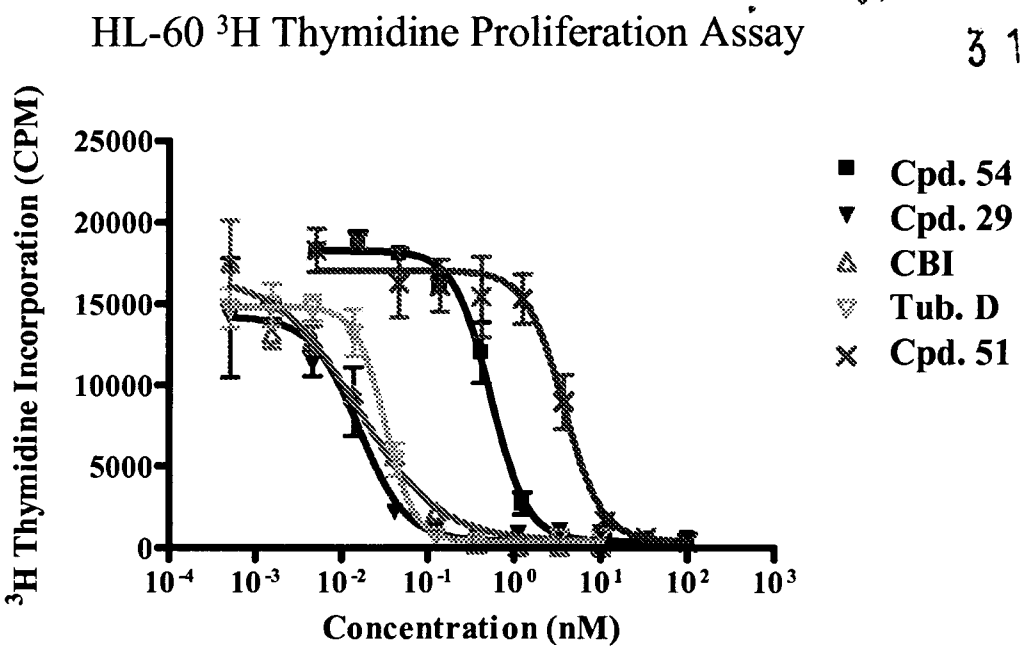

Fig. 11b

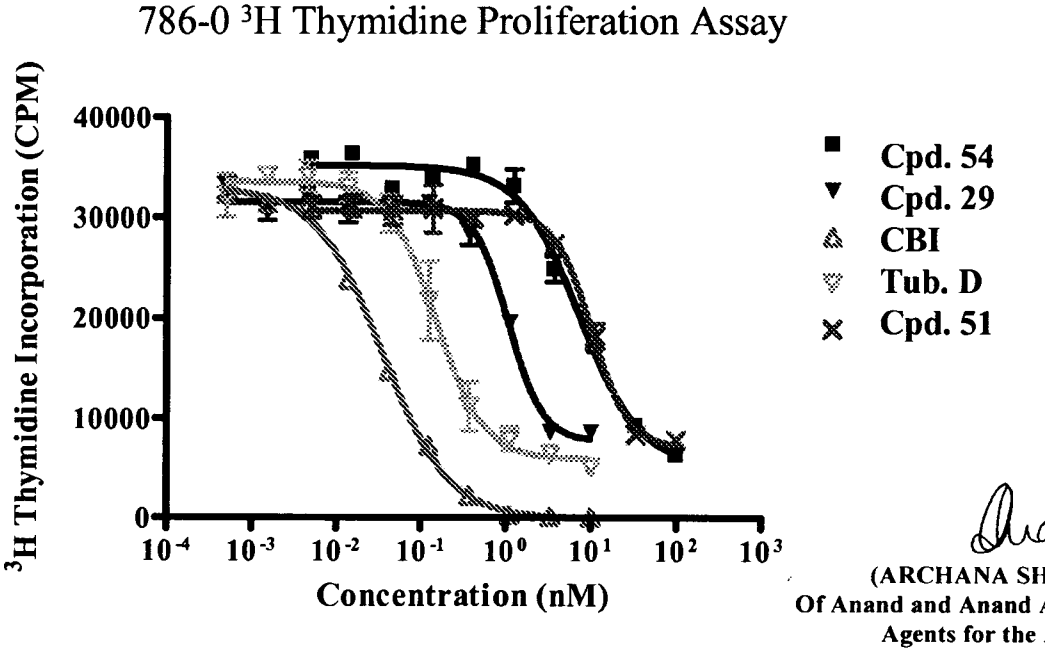

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Fig. 12a

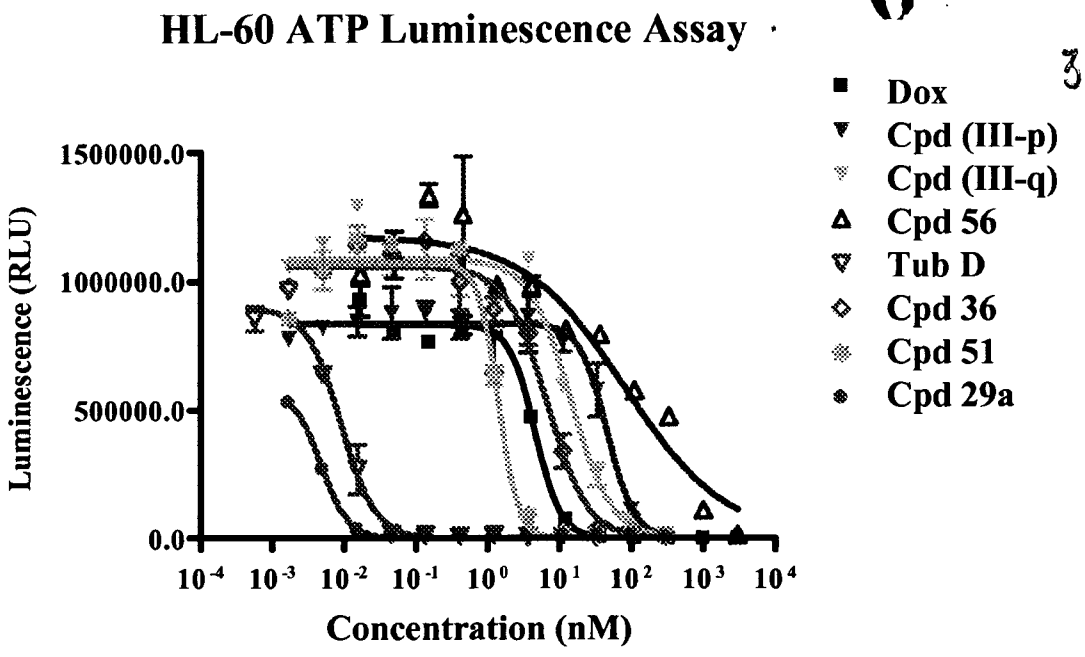

Fig. 12b

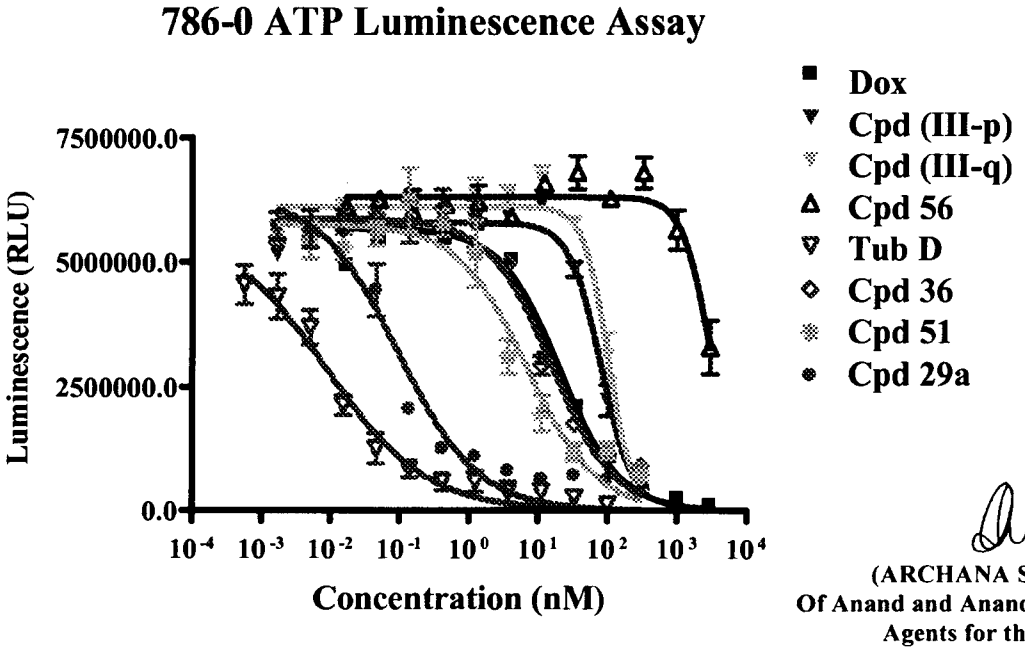

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Fig. 12c

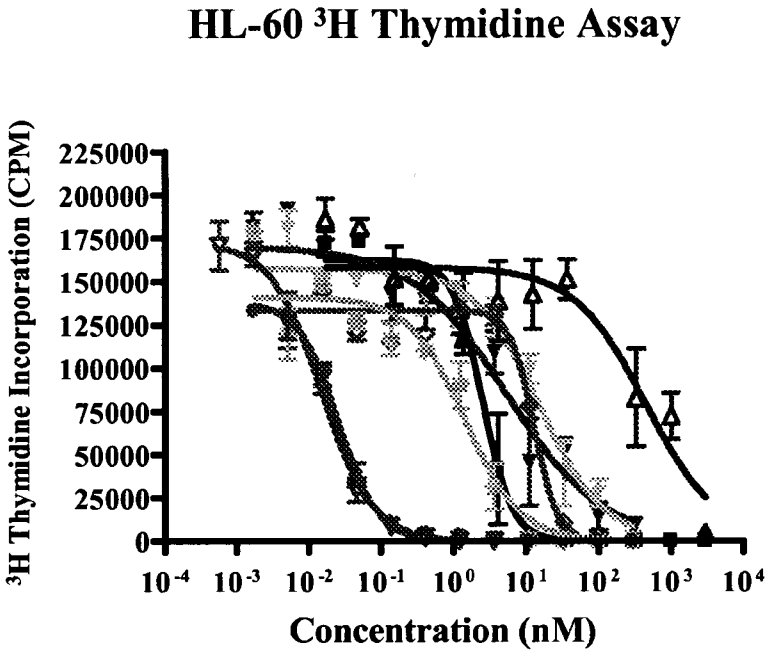

Fig. 12d

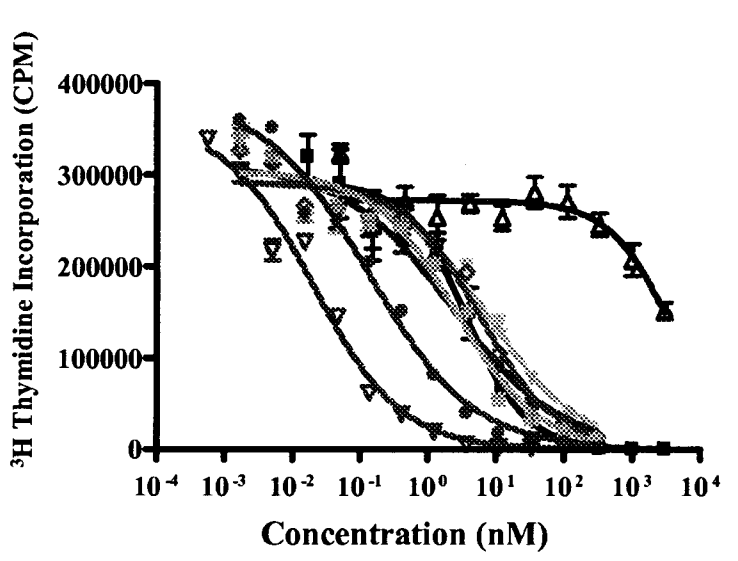

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Fig. 13

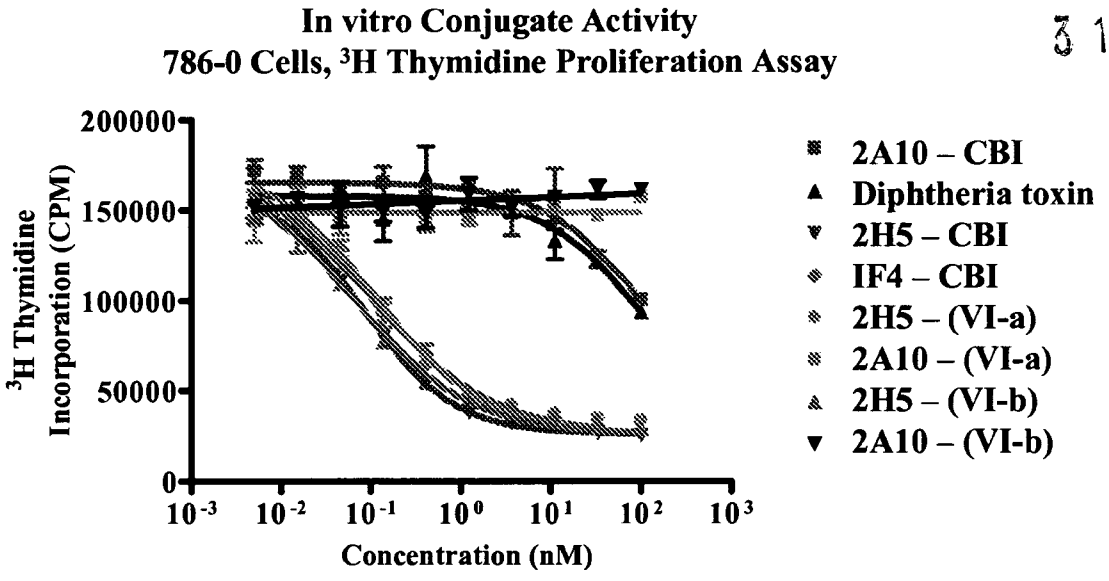

Fig. 14

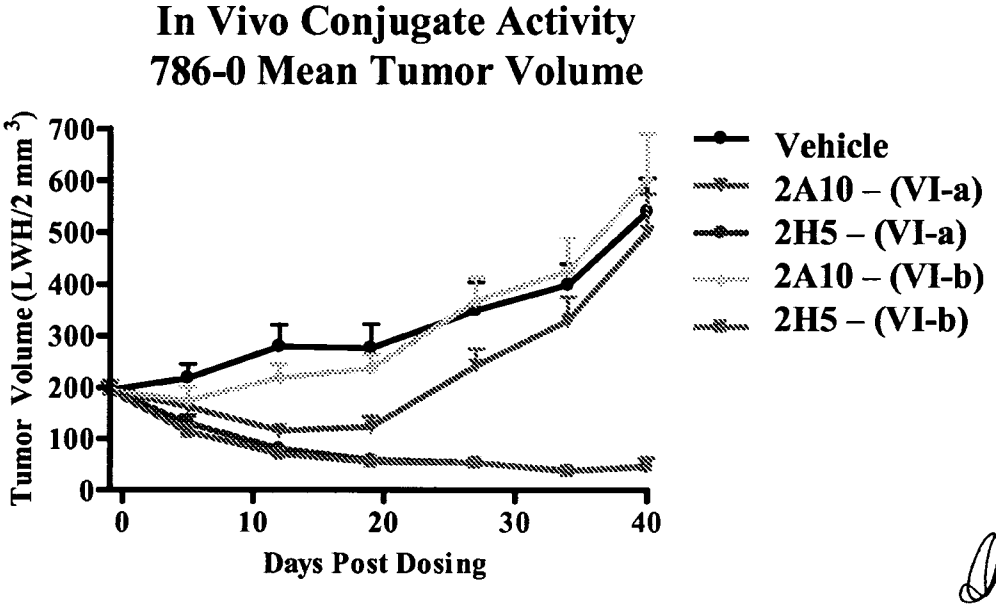

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Fig. 15

Scheme 13

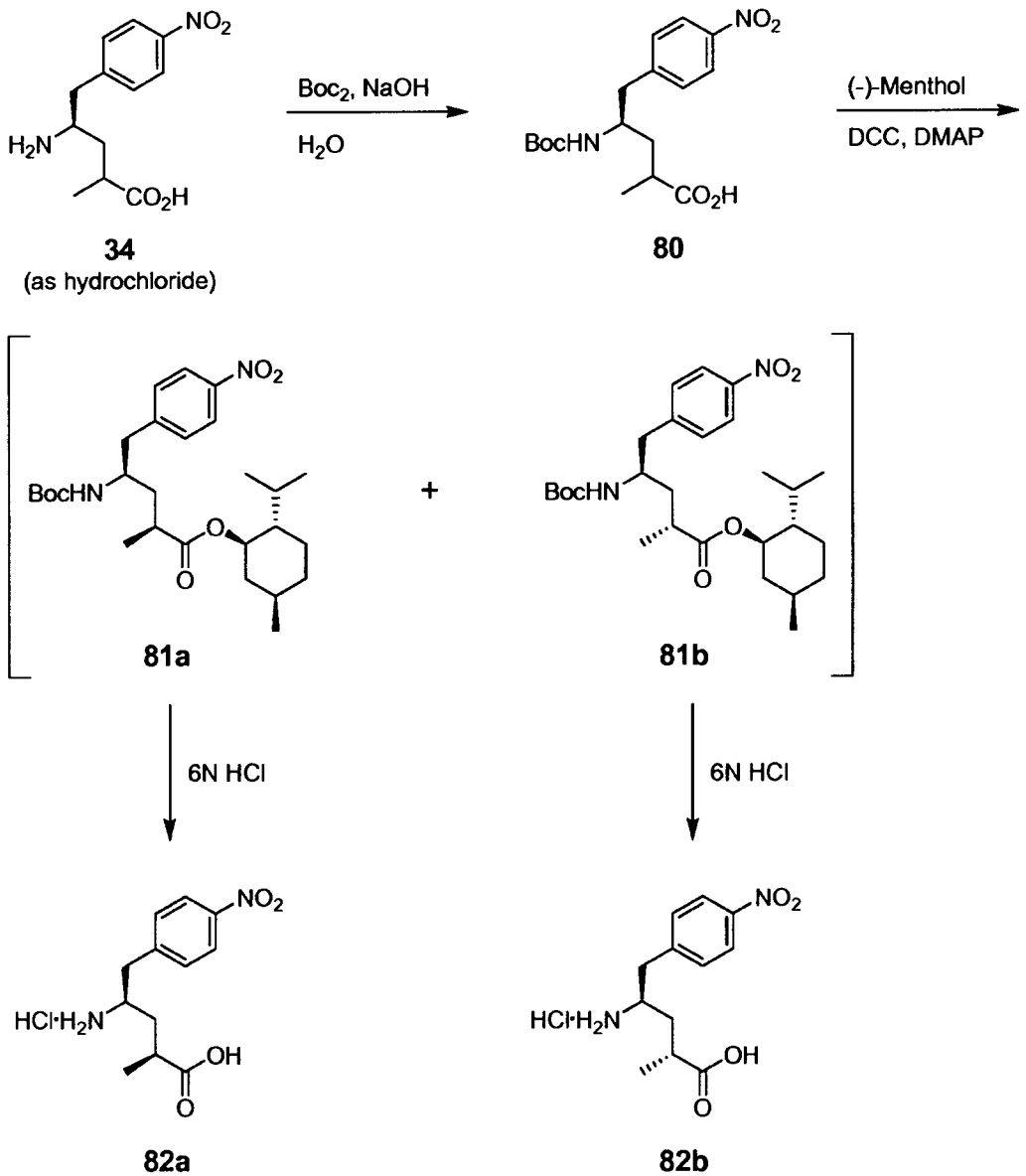

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Fig. 16

Scheme 14

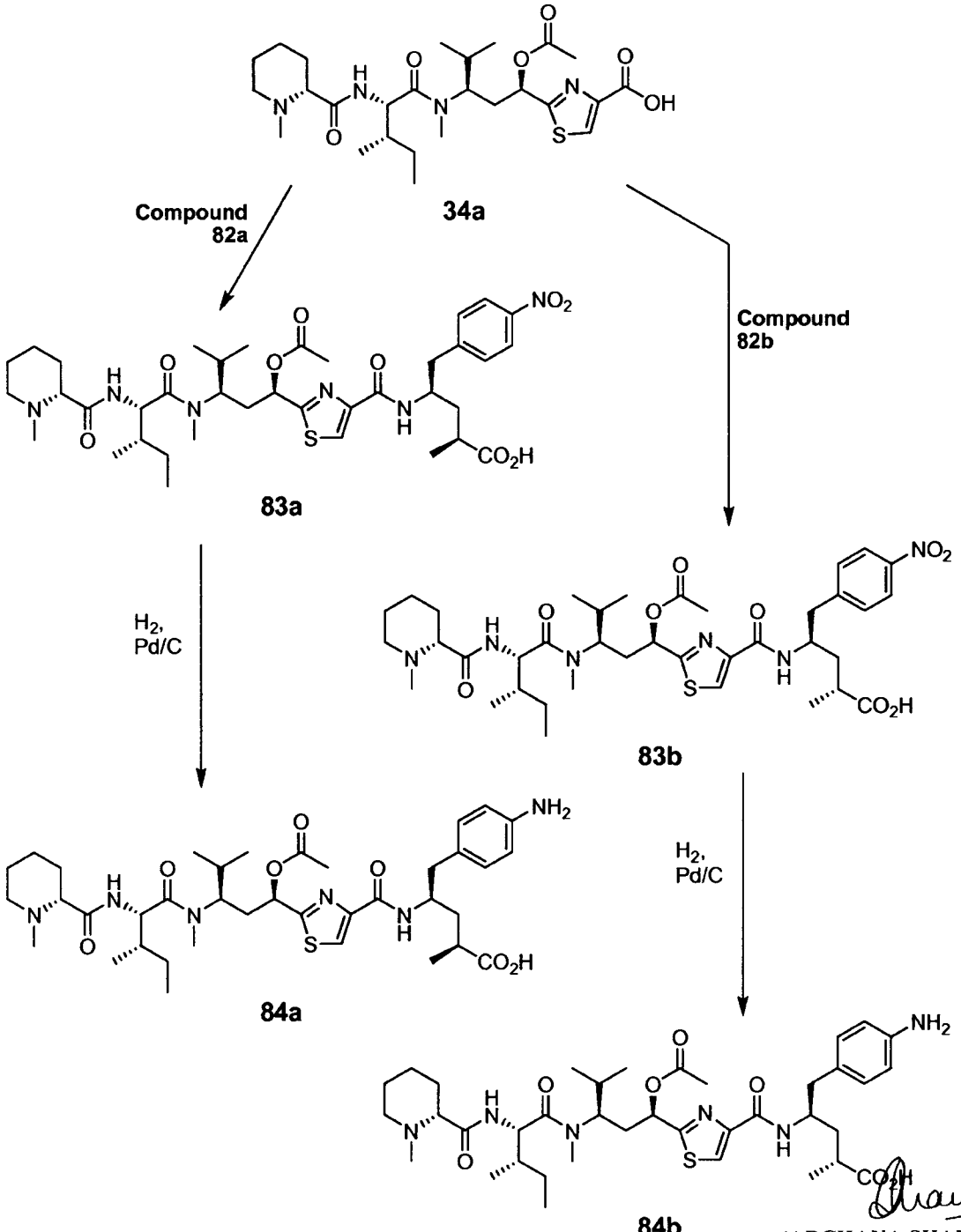

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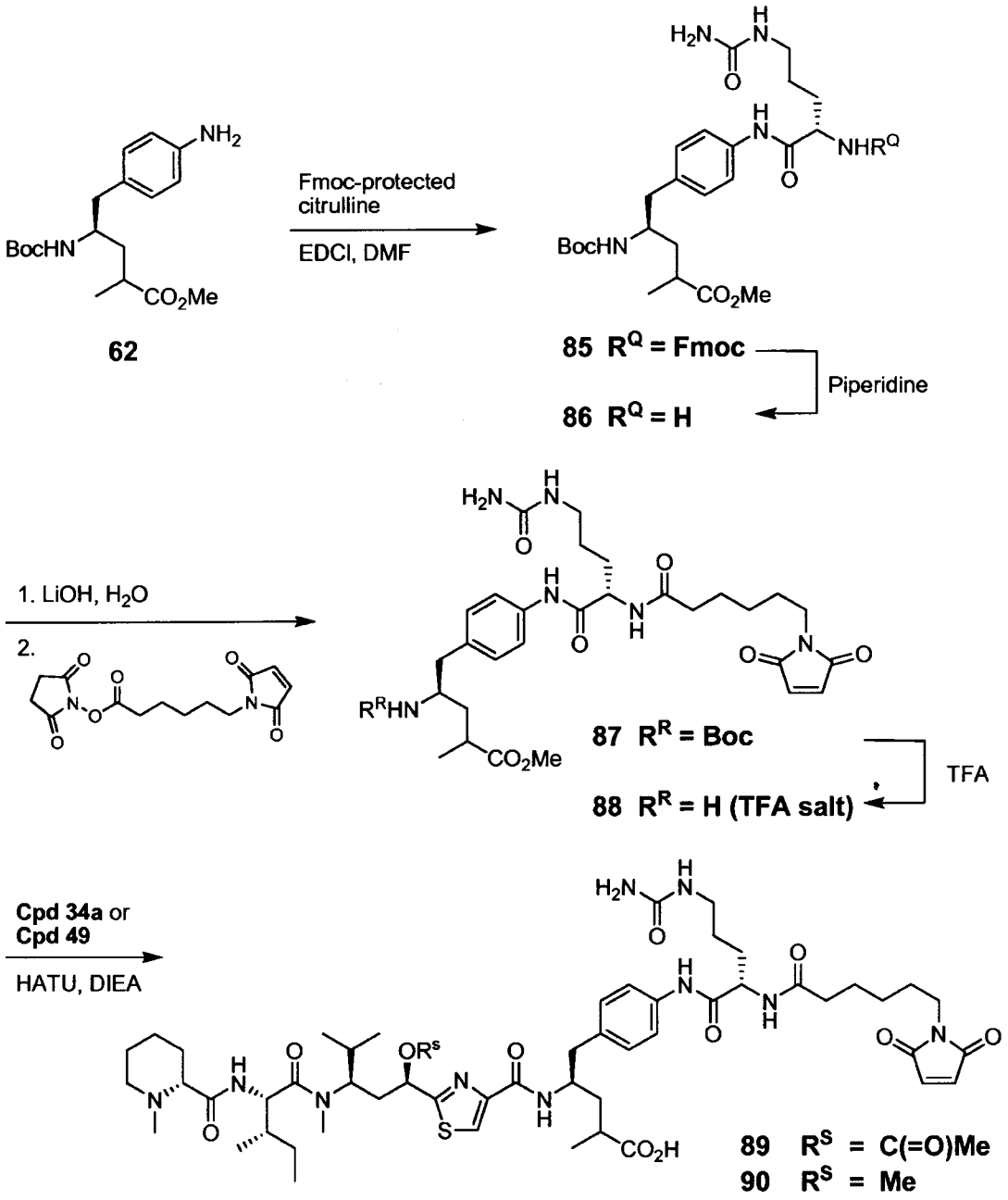
Fig. 17

Scheme 15



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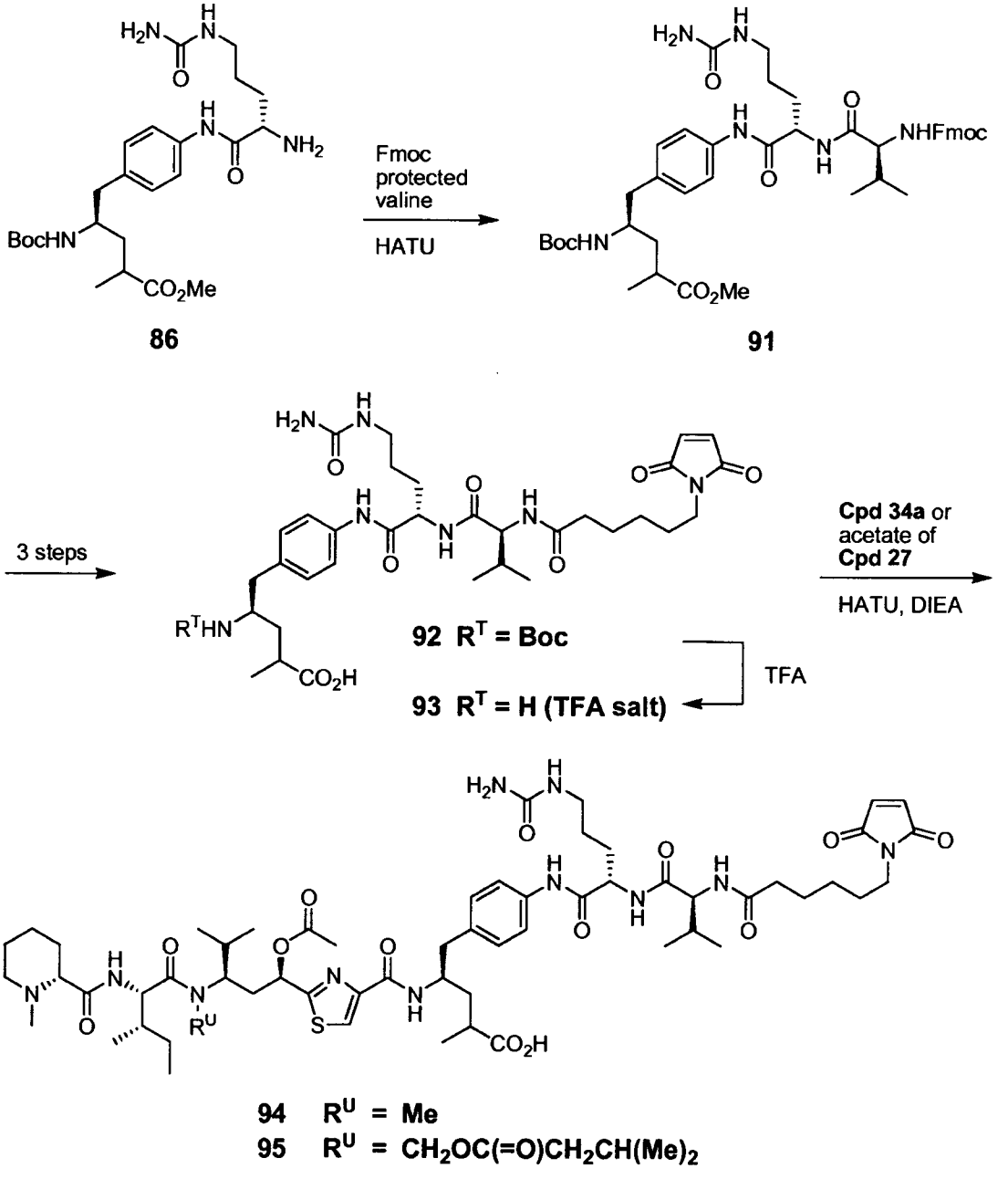
0860/MUM/12

31 JAN 2012

Fig. 18

Scheme 16



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31 JAN 2012

Fig. 19

Scheme 17

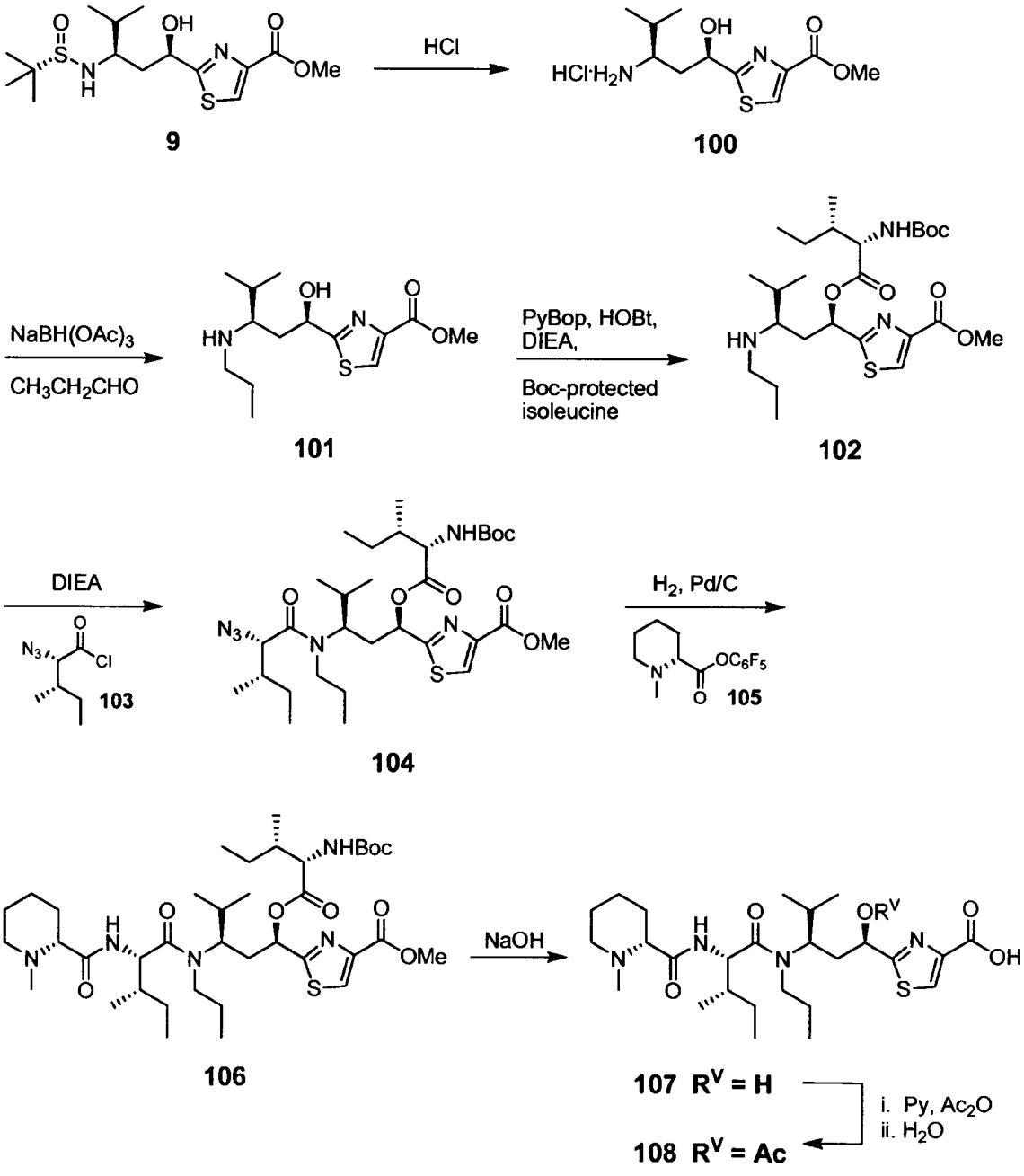

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Fig. 20a

Scheme 18 (part 1 of 2)

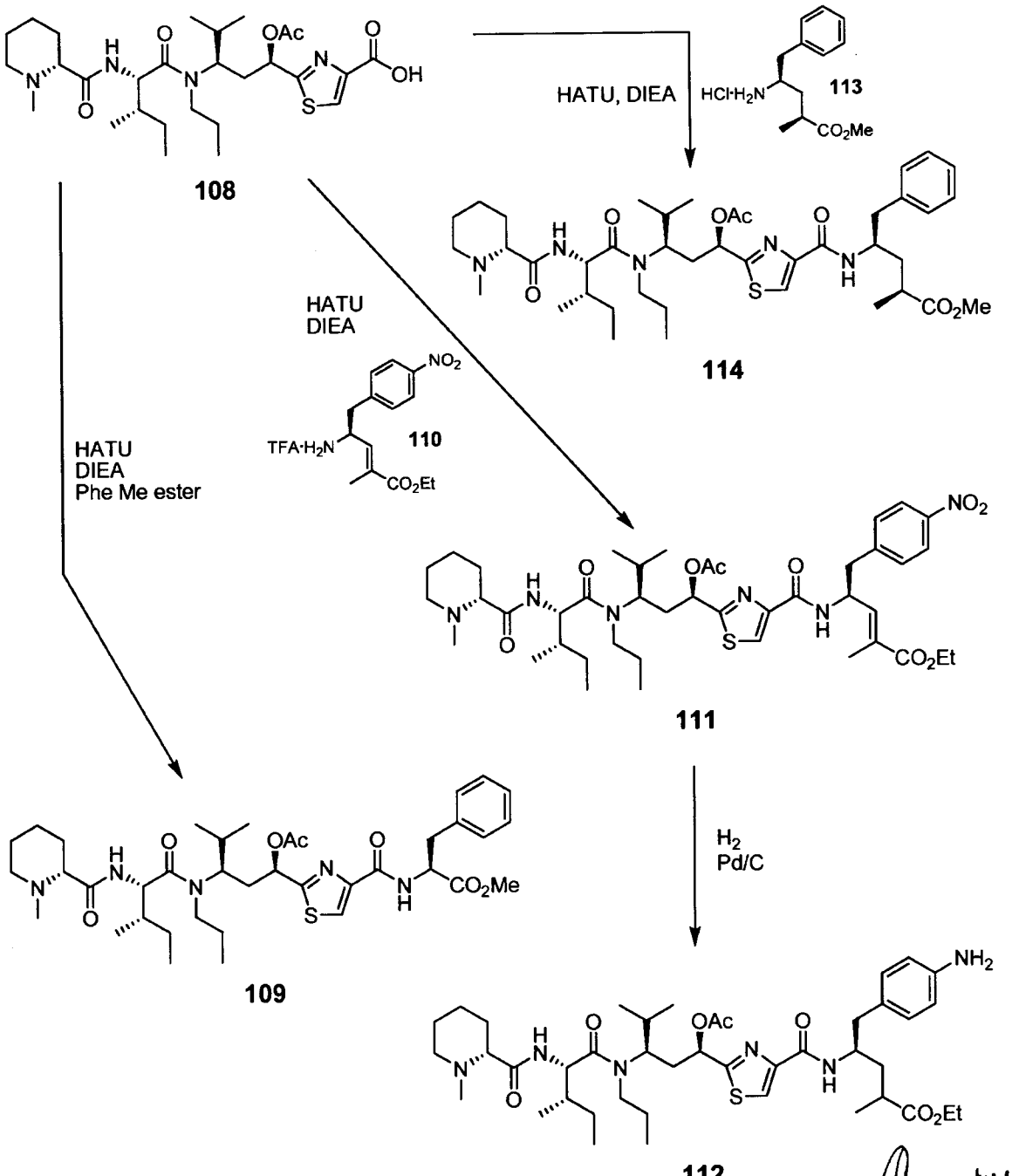

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Fig. 20b

Scheme 18 (part 2 of 2)

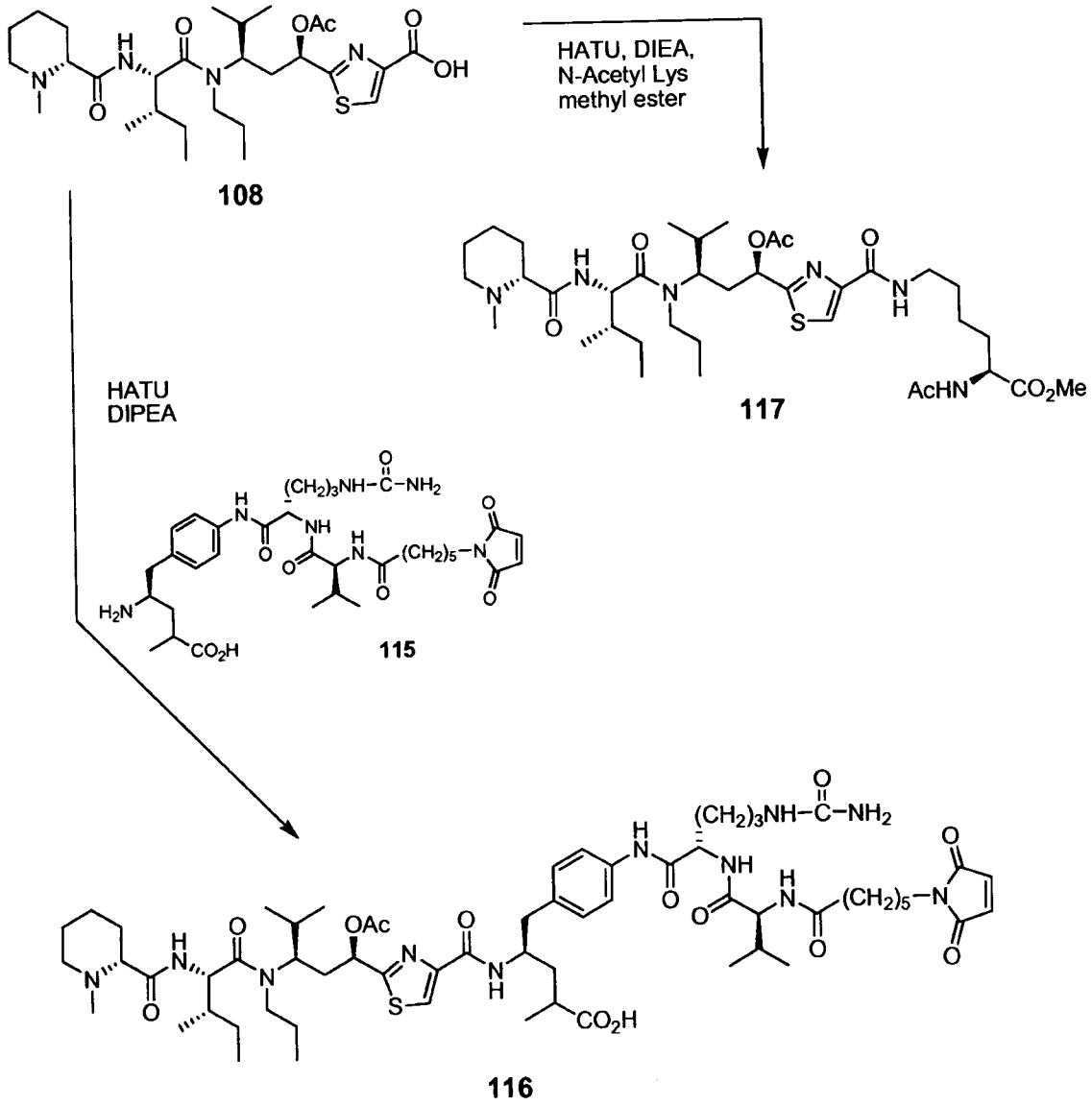

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Fig. 21

Scheme 19

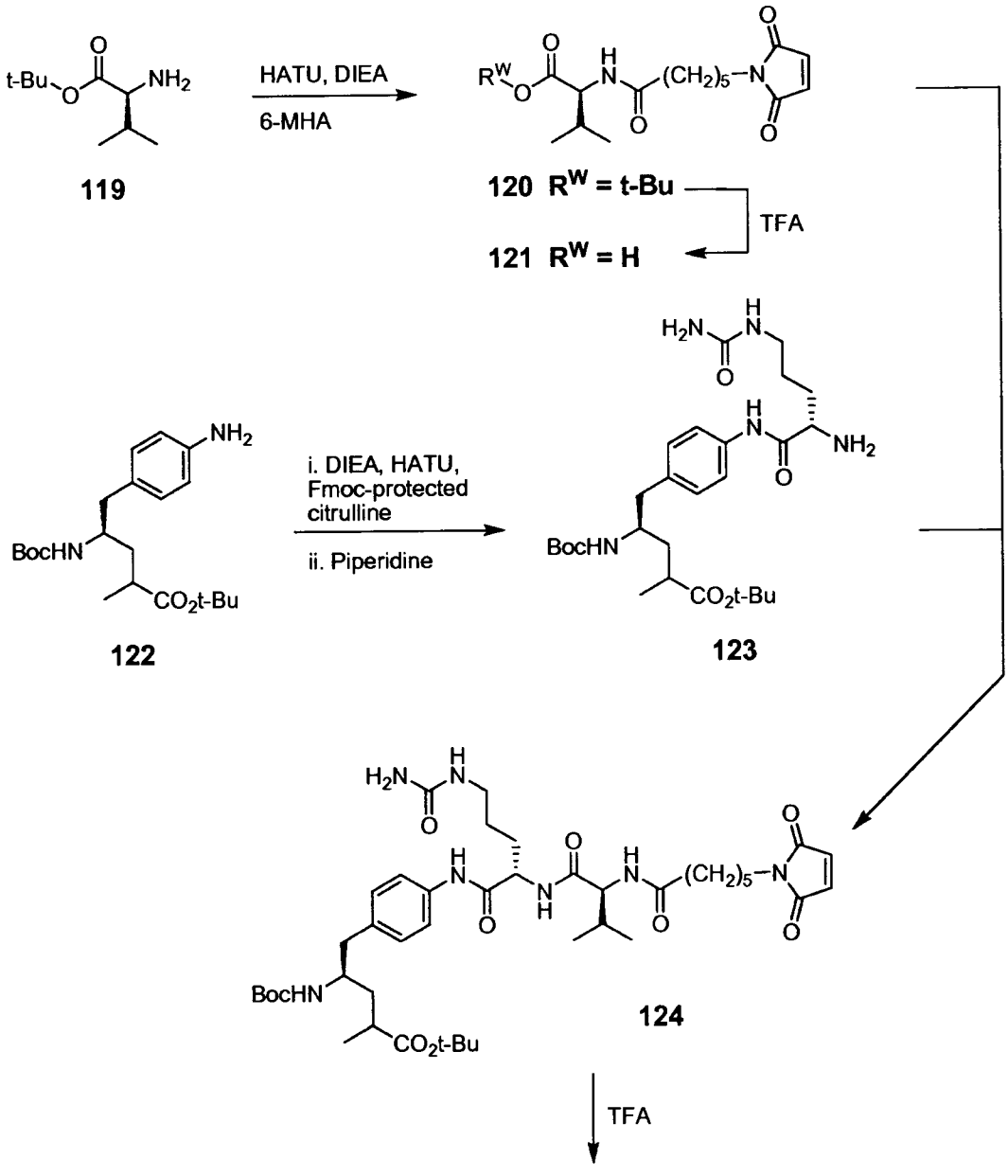

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Fig. 22

Scheme 20

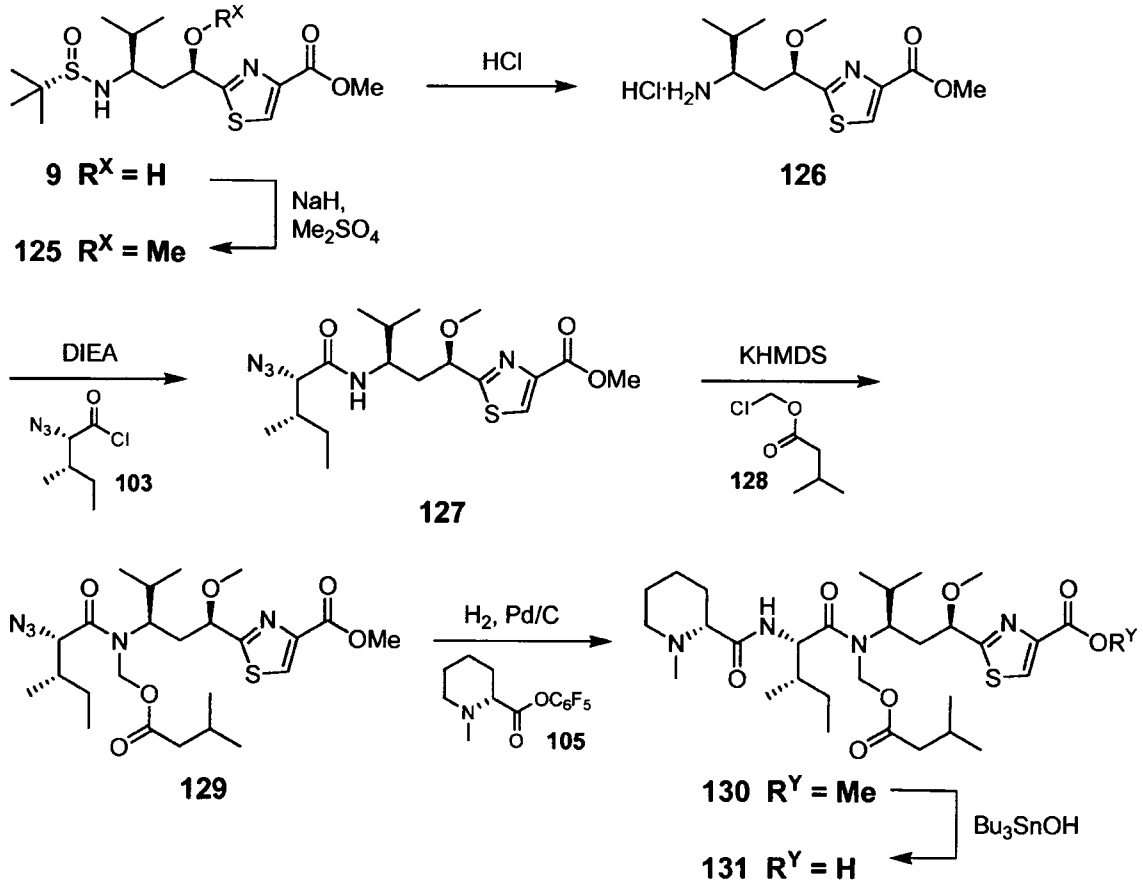

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Fig. 23

Scheme 21

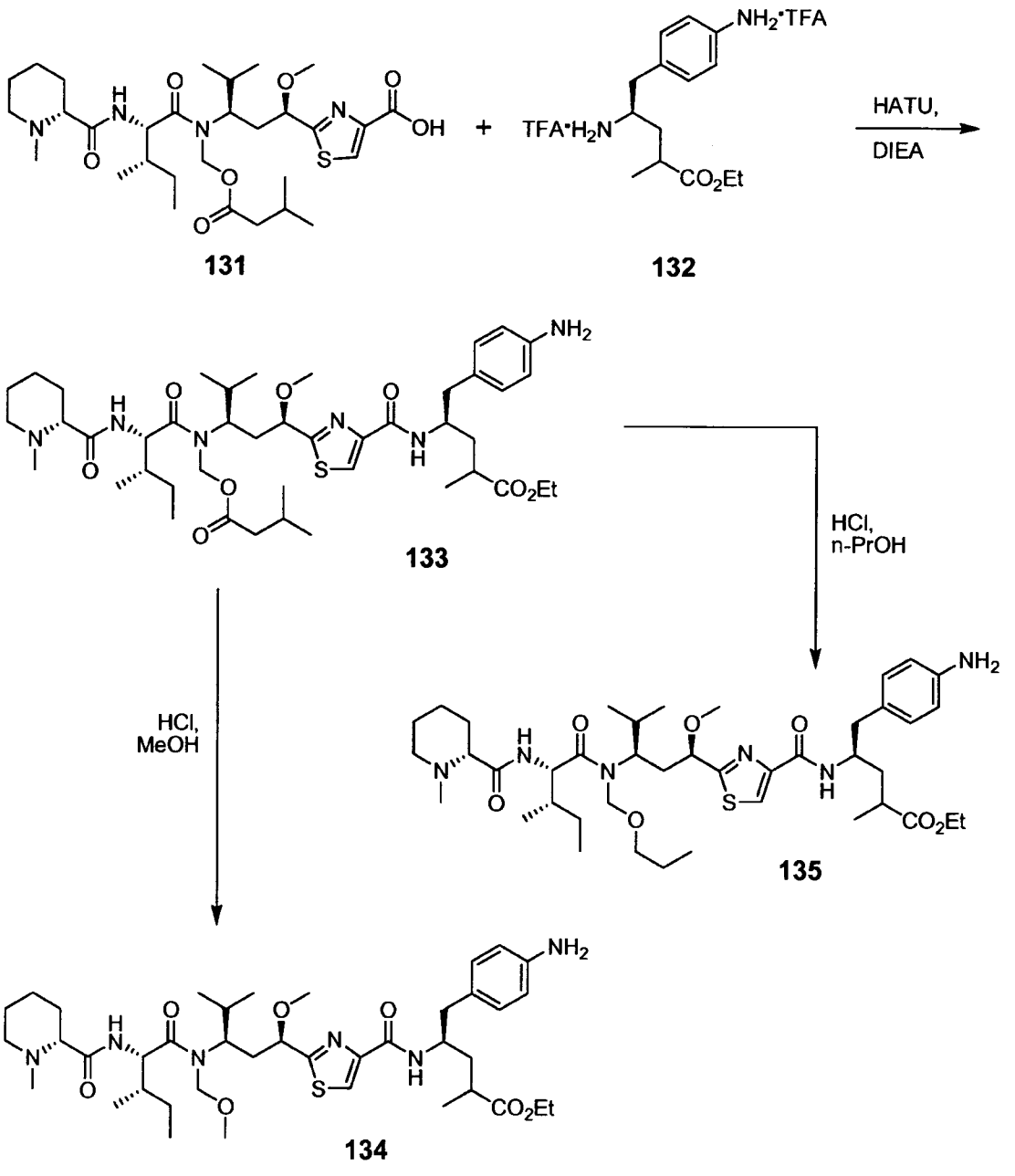

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Fig. 24

Scheme 22

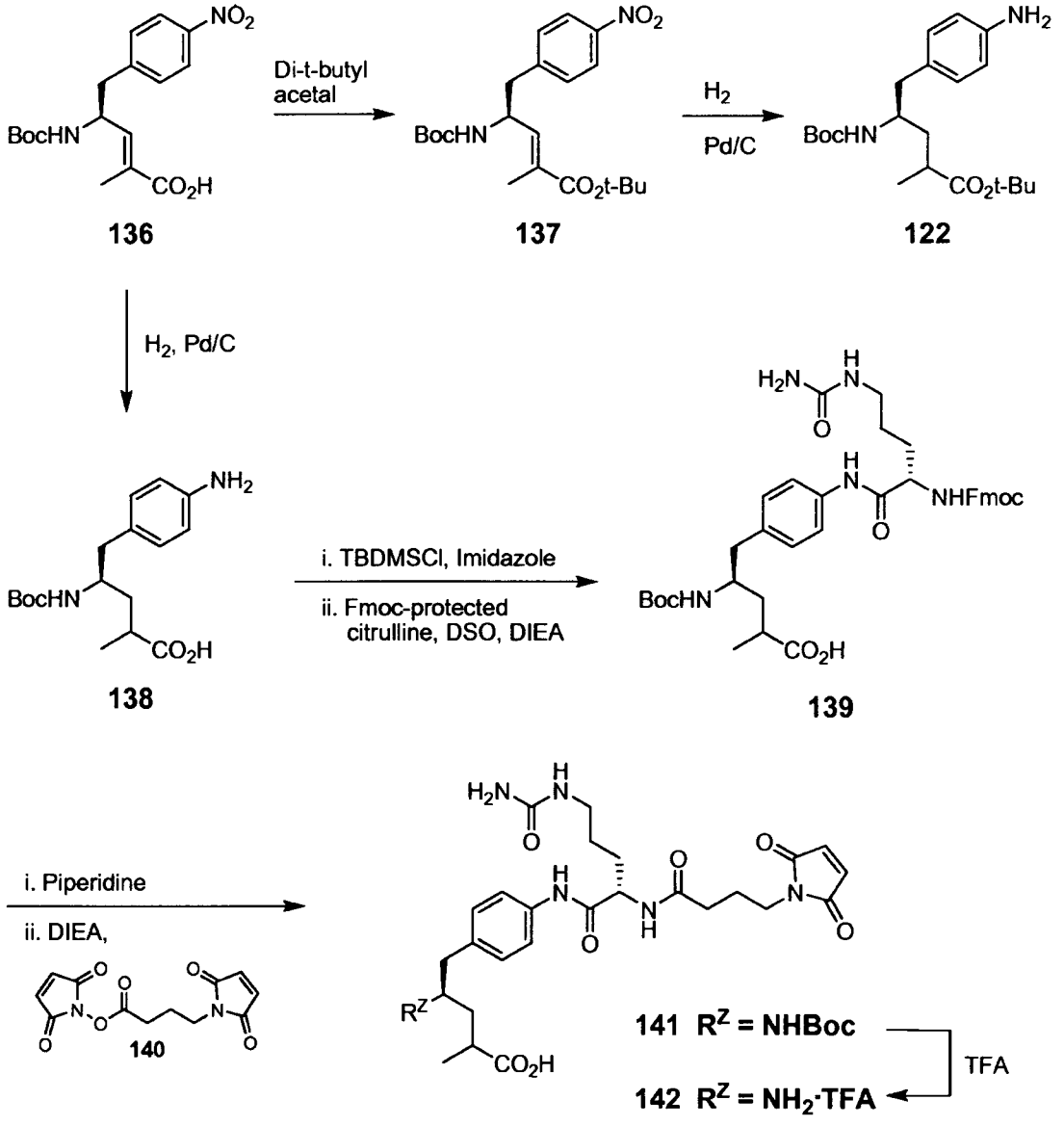

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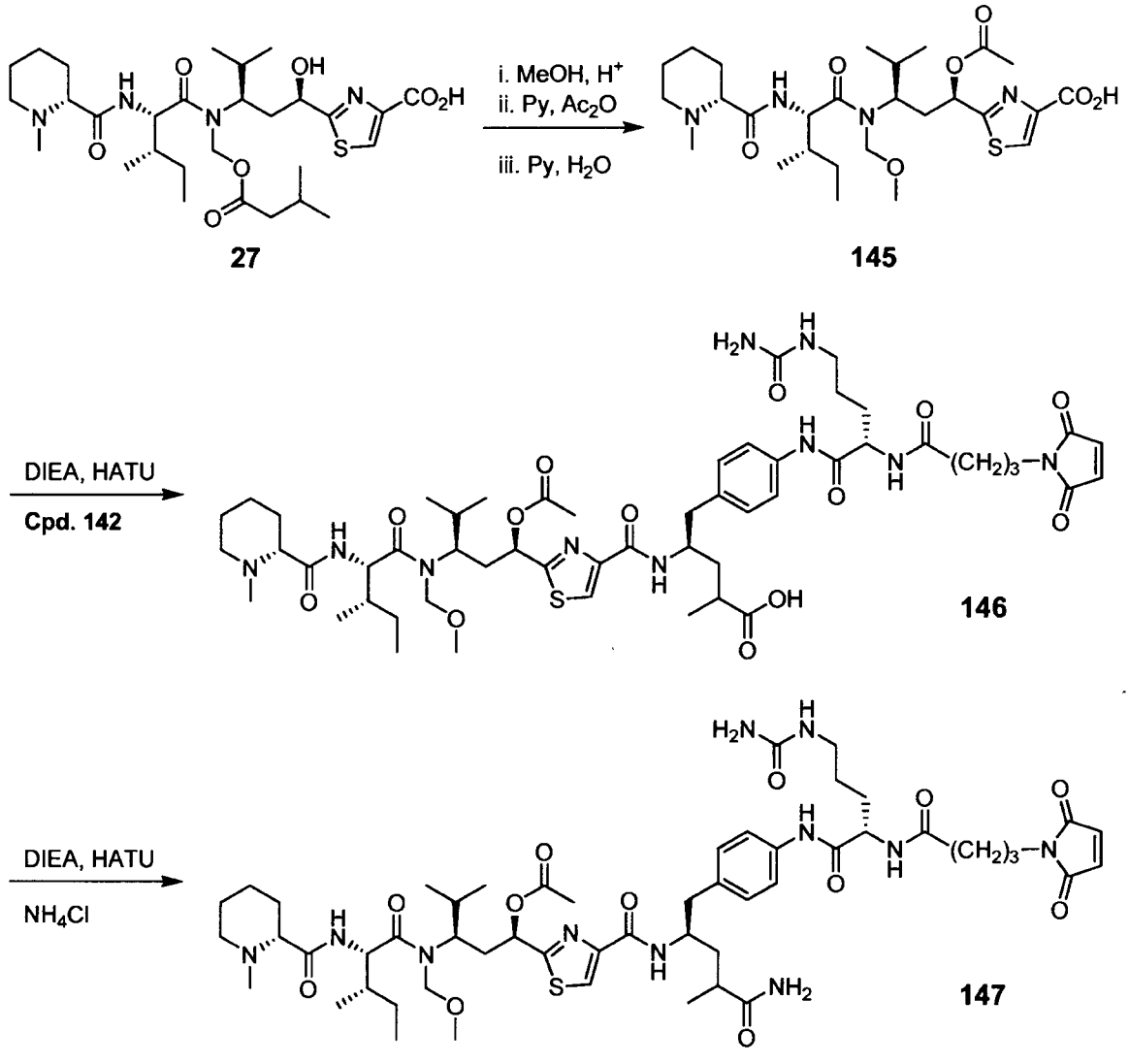
Fig. 25

Scheme 23



Archana Shanker

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ANTIPROLIFERATIVE COMPOUNDS, CONJUGATES THEREOF, METHODS THEREFOR, AND USES THEREOF

BACKGROUND OF THE INVENTION

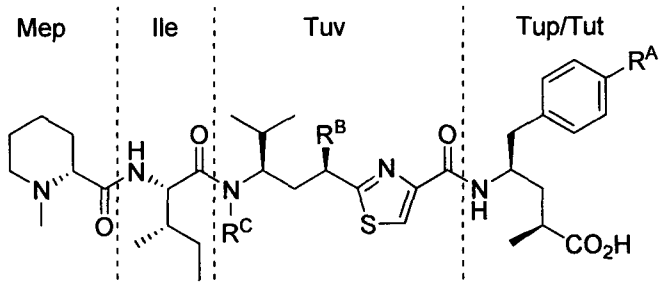
[0001] This invention relates to compounds structurally related to the tubulysins, conjugates thereof with a ligand, methods for making and using such compounds and conjugates, and compositions comprising such compounds and conjugates.

[0002] The tubulysins are cytotoxins originally isolated from cultures of the myxobacteria *Archangium gephyra* or *Angiococcus disciformis*, with each organism producing a different mixture of tubulysins (Sasse et al. 2000; Reichenbach et al. 1998). Their crystal structure and biosynthetic pathway have been elucidated (Steinmetz et al. 2004) and their biosynthesis genes have been sequenced (Hoefle et al. 2006b). Pretubulysin, a biosynthetic precursor of the tubulysins, also has been shown to possess significant activity in its own right (Ullrich et al. 2009). (Full citations for the documents cited herein by first author or inventor and year are listed at the end of this specification.)

[0003] The tubulysins belong to a group of naturally occurring antimitotic polypeptides and depsipeptides that includes the phomopsins, the dolastatins, and the cryptophycins (Hamel 2002). Antimitotic agents other than polypeptides or depsipeptides also exist, for example paclitaxel, the maytansines, and the epothilones. During mitosis, a cell's microtubules reorganize to form the mitotic spindle, a process requiring the rapid assembly and disassembly of the microtubule constituent proteins α- and β-tubulin. Antimitotic agents block this process and prevent a cell from undergoing mitosis, although at the molecular level the exact blockage mechanism may differ from one agent to another. The tubulysins prevent the assembly of the tubulins into microtubules, causing the affected cells to accumulate in the G_2/M phase and undergo apoptosis (Khalil et al. 2006). Conversely, paclitaxel effects the same end result by binding to microtubules and preventing their disassembly.

[0004] The tubulysins have a tetrapeptidyl scaffold constructed from one proteinogenic and three non-proteinogenic amino acid subunits: N-methylpipecolinic acid (Mep), isoleucine (Ile), tubuvaline (Tuv), and either tubuphenylalanine (Tup, R^A equals H in formula (I) below) or tubutyrosine (Tut, R^A equals OH). About a dozen naturally

occurring tubulysins (named A, B, etc.) are known, the sites of structural variation among them being at residues R^A, R^B and R^C as shown in Formula (I) and Table 1:



(I)

Table 1 – Naturally Occurring Tubulysins

Tubulysin	R^A	R^B	R^C
A	OH	OC(=O)Me	$CH_2OC(=O)i$-Bu
B	OH	OC(=O)Me	$CH_2OC(=O)n$-Pr
C	OH	OC(=O)Me	$CH_2OC(=O)Et$
D	H	OC(=O)Me	$CH_2OC(=O)i$-Bu
E	H	OC(=O)Me	$CH_2OC(=O)n$-Pr
F	H	OC(=O)Me	$CH_2OC(=O)Et$
G	OH	OC(=O)Me	$CH_2OC(=O)CH=CH_2$
H	H	OC(=O)Me	$CH_2OC(=O)Me$
I	OH	OC(=O)Me	$CH_2OC(=O)Me$
U	H	OC(=O)Me	H
V	H	OH	H
Z	OH	OH	H
Pretubulysin	H	H	Me

[0005] Kaur et al. 2006 studied the antiproliferative properties of tubulysin A and found that it was more potent than other antimitotic agents such as paclitaxel and vinblastine and was active in xenograft assays against a variety of cancer cell lines. Further, tubulysin A induced apoptosis in cancer cells but not normal cells and showed significant potential antiangiogenic properties in *in vitro* assays. The antimitotic properties of other tubulysins have also been evaluated and generally have been found to compare favorably against those of non-tubulysin antimitotic agents (see, e.g., Balasubramanian et al. 2009;

Steinmetz et al. 2004; Wipf et al. 2004). For these reasons, there is considerable interest in the tubulysins as anti-cancer agents (see, e.g., Domling et al. 2005c; Hamel 2002).

[0006] Numerous publications describe efforts directed at the synthesis of tubulysins, including: Balasubramanian et al. 2009; Domling et al. 2006; Hoefle et al. 2003; Neri et al. 2006; Peltier et al. 2006; Sani et al. 2007; Sasse et al. 2007; Shankar et al. 2009; Shibue et al. 2009; and Wipf et al. 2004. Other publications describe structure-activity relationship (SAR) studies, via the preparation and evaluation of tubulysin analogs or derivatives: Balasubramanian et al. 2008 and 2009; Domling 2006; Domling et al. 2005a; Ellman et al. 2009; Hoefle et al. 2001 & 2006a; Patterson et al. 2007 & 2008; Richter 2008; Vlahov et al. 2009; Wang et al. 2007; and Wipf et al. 2007 and 2010. The SAR studies mainly explored structural variations in the Mep ring, residues R^B and R^C of the Tuv subunit, and the aromatic ring or aliphatic carbon chain of the Tup/Tut subunit.

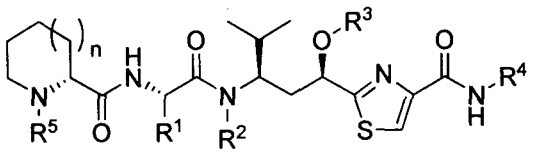
[0007] Domling et al. 2005 disclose conjugates of tubulysins with a partner molecule generically described as a polymer or a biomolecule, but with actual examples limited to polyethylene glycol (PEG) as the partner molecule. Other documents disclosing conjugates of tubulysins are Boyd et al. 2008 and 2010; Vlahov et al. 2008a, 2008b and 2010; Leamon et al. 2008 and 2009; Reddy et al. 2009; and Low et al. 2009. Leung et al. 2002 disclose polyanionic polypeptides that can be conjugated to drugs (including tubulysins) to improve their bioactivity and water solubility.

[0008] Davis et al. 2008 and Schluep et al. 2009 disclose cyclodextrin based formulations in which tubulysins are covalently attached to a cyclodextrin via a hydrazide-disulfide linker moiety bonded to the Tup/Tut carboxyl group.

BRIEF SUMMARY OF THE INVENTION

[0009] The present invention discloses novel antiproliferative compounds that are structurally related to the tubulysins, are cytotoxic or cytostatic against many cancer cells, and are believed to act by an antimitotic mechanism. These compounds can be conjugated to ligands such as antibodies for targeted delivery against cancer cells.

[0010] In one embodiment, this invention provides a compound having a structure represented by formula (II)



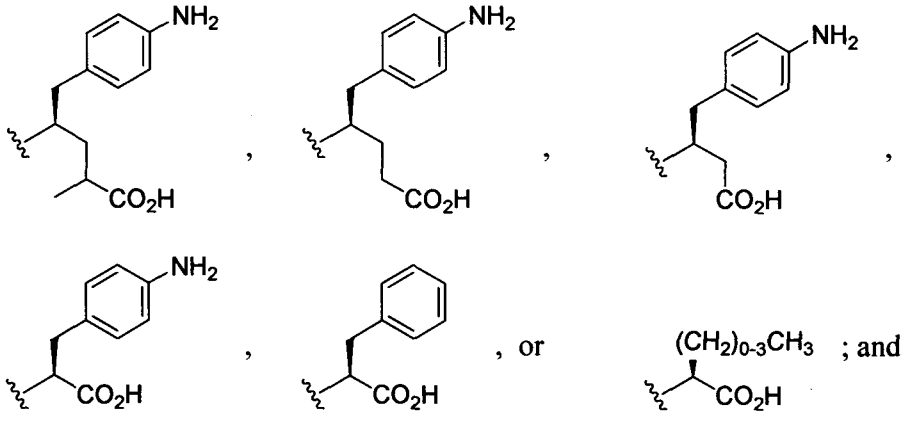
(II)

wherein

n is 0, 1, or 2;

R^1, R^2 and R^3 are independently H, unsubstituted or substituted C_1-C_{10} alkyl, unsubstituted or substituted C_2-C_{10} alkenyl, unsubstituted or substituted C_2-C_{10} alkynyl, unsubstituted or substituted aryl, unsubstituted or substituted heteroaryl, unsubstituted or substituted $(CH_2)_{1\text{-}2}O(C_1\text{-}C_{10}$ alkyl), unsubstituted or substituted $(CH_2)_{1\text{-}2}O(C_2\text{-}C_{10}$ alkenyl), unsubstituted or substituted $(CH_2)_{1\text{-}2}O(C_2\text{-}C_{10}$ alkynyl), $(CH_2)_{1\text{-}2}OC(=O)(C_1\text{-}C_{10}$ alkyl), unsubstituted or substituted $(CH_2)_{1\text{-}2}OC(=O)(C_2\text{-}C_{10}$ alkenyl), unsubstituted or substituted $(CH_2)_{1\text{-}2}OC(=O)(C_2\text{-}C_{10}$ alkynyl), unsubstituted or substituted $C(=O)(C_1\text{-}C_{10}$ alkyl), unsubstituted or substituted $C(=O)(C_2\text{-}C_{10}$ alkenyl), unsubstituted or substituted $C(=O)(C_2\text{-}C_{10}$ alkynyl), unsubstituted or substituted cycloaliphatic, unsubstituted or substituted heterocycloaliphatic, unsubstituted or substituted arylalkyl, or unsubstituted or substituted alkylaryl;

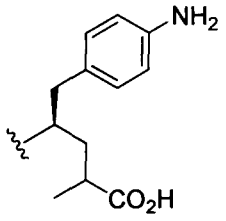
R^4 is



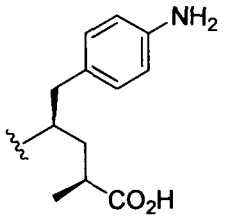
R^5 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, $CO(C_1\text{-}C_5$ alkyl), $CO(C_2\text{-}C_5$ alkenyl), or $CO(C_2\text{-}C_5$ alkynyl);

or a pharmaceutically acceptable ester thereof, a pharmaceutically acceptable amide thereof at the carboxyl group of R^4 with the α-amino group of an α-amino acid, or a pharmaceutically acceptable salt thereof.

[0011] A preferred R^4 is



with the stereochemistry at the methyl group alpha to the carboxyl being more preferably that corresponding to the natural tubulysins, that is:



[0012] This invention also provides novel intermediates useful for synthesizing compounds according to formula (II).

[0013] In another embodiment, this invention provides a compound of this invention conjugated via a linker moiety to a ligand (preferably an antibody, more preferably a monoclonal antibody, and most preferably a human monoclonal antibody) for its selective delivery to a target cell such as a cancer cell.

[0014] In another embodiment, there is provided a composition of matter comprising a compound of this invention and a linker moiety, suitable for conjugation to a ligand.

[0015] In another embodiment, this invention provides a method for inhibiting the proliferation of cancer cells in a subject suffering from cancer, comprising administering to the subject a therapeutically effective amount of a compound of this invention or a conjugate thereof with a ligand (particularly an antibody). In another embodiment, there is provided a method for inhibiting the proliferation of cancer cells, comprising contacting such cells with a compound of this invention or a conjugate thereof with a ligand (particularly an antibody), under conditions sufficient to inhibit the growth of such cancer cells. The cancer cells can be colorectal cancer, liver cancer, prostate cancer, breast cancer,