PROCESSES FOR PREPARING TUBULYSINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 61/371,433 filed on August 6, 2010, the entire disclosure of which is incorporated herein by reference.

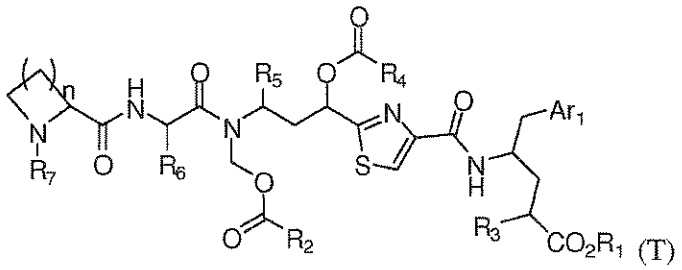
TECHNICAL FIELD

The invention described herein pertains to processes for preparing tubulysins.

BACKGROUND AND SUMMARY ~~OF THE INVENTION~~

The tubulysins are members of a new class of natural products isolated from myxobacterial species (F. Sasse, et al., *J. Antibiot.* **2000**, 53, 879-885). As cytoskeleton interacting agents, the tubulysins are mitotic poisons that inhibit tubulin polymerization and lead to cell cycle arrest and apoptosis (H. Steinmetz, et al., *Chem. Int. Ed.* **2004**, *43*, 4888-4892; M. Khalil, et al., *ChemBioChem.* **2006**, *7*, 678-683;G. Kaur, et al., *Biochem. J.* **2006**, *396*, 235-242). Tubulysins are extremely potent cytotoxic molecules, exceeding the cell growth inhibition of any clinically relevant traditional chemotherapeutic e.g. epothilones, paclitaxel, and vinblastine. Furthermore, they are potent against multidrug resistant cell lines (A. Dömling, et al., *Mol. Diversity* **2005**, *9*, 141-147). These compounds show high cytotoxicity tested against a panel of cancer cell lines with IC_{50} values in the low picomolar range; thus, they are of interest as potential anticancer therapeutics.

Tubulysins are described herein. Structurally, tubulysins often include linear tetrapeptoid backbones, including illustrative compounds having formula T



(T)

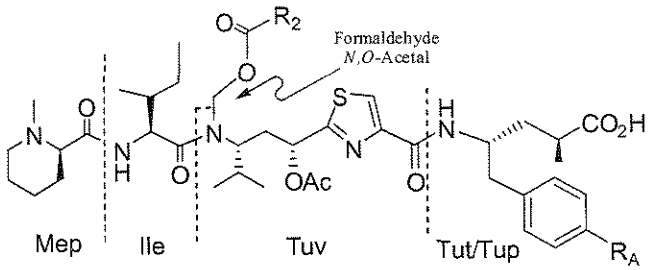
and pharmaceutically acceptable salts thereof;

wherein

Ar_1 is optionally substituted aryl;

R_1 is hydrogen, alkyl, arylalkyl or a pro-drug forming group;

R_2 is selected from the group consisting of optionally substituted alkyl and optionally substituted cycloalkyl;



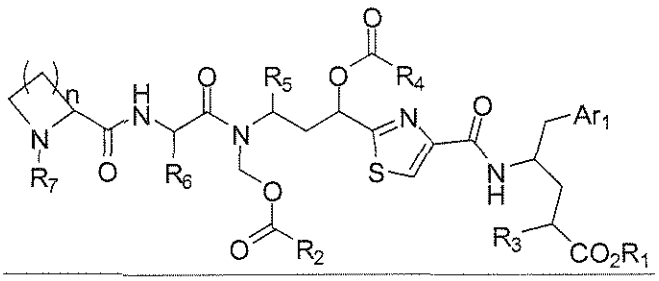
Formula 1, Structures of several natural tubulysins

Tubulysin	R_A	R_2
A	OH	$CH_2CH(CH_3)_2$
B	OH	$CH_2CH_2CH_3$
C	OH	CH_2CH_3
D	H	$CH_2CH(CH_3)_2$
E	H	$CH_2CH_2CH_3$
F	H	CH_2CH_3
G	OH	$CH=C(CH_3)_2$
H	H	CH_3
I	OH	CH_3

A total synthesis of tubulysin D possessing C-terminal tubuphenylalanine (R_A=H) (H. Peltier, et al., J. Am. Chem. Soc. 2006, 128, 16018-16019) has been reported. Recently, a modified synthetic protocol toward the synthesis of tubulysin B (R_A=OH) (O. Pando, et at., Org. Lett. 2009, 11, 5567-5569) has been reported. However, attempts to follow the published procedures to provide larger quantities of tubulysins were unsuccessful, being hampered in part by low yields, difficult to remove impurities, the need for expensive chromatographic steps, and/or the lack of reproducibility of several steps. The interest in using tubulysins for anticancer therapeutics accents the need for reliable and efficient processes for preparing tubulysins, and analogs and derivatives thereof. Described herein are improved processes for making natural tubulysins, or analogs or derivatives thereof, including compounds of formula (T) and formula (1).

P~~In one illustrative embodiment of the invention, p~~rocesses for preparing natural tubulysins, or analogs or derivatives thereof, including compounds of formula (T) and formula (1) are described herein. The processes include one or more steps described herein.

In one aspect, there is provided a process for preparing a compound of the formula



or a pharmaceutically acceptable salt thereof;

wherein

Ar_1 is 4-hydroxyphenyl;

R_1 is hydrogen, alkyl, arylalkyl or a pro-drug forming group;

R_2 is selected from the group consisting of optionally substituted alkyl and optionally substituted cycloalkyl;

R_3 is optionally substituted alkyl;

R_4 is optionally substituted alkyl or optionally substituted cycloalkyl;

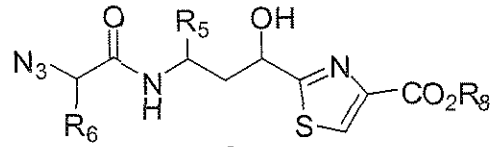
R_5 and R_6 are each independently selected from the group consisting of optionally substituted alkyl and optionally substituted cycloalkyl;

R_7 is optionally substituted alkyl; and

n is 1, 2, 3, or 4;

wherein the process comprises

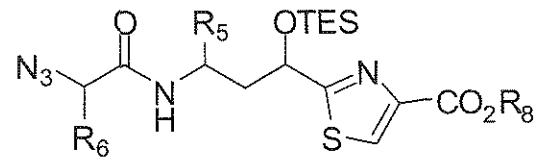
the step of treating a compound of formula A with triethylsilyl chloride and imidazole in an aprotic solvent, where R_8 is C1-C6 unbranched alkyl



A ;

or

the step of treating a compound of formula B with a base and a compound of the formula $ClCH_2OC(O)R_2$ in an aprotic solvent at a temperature from about −78° C. to about 0° C.; wherein the molar ratio of the compound of the formula $ClCH_2OC(O)R_2$ to the compound of formula B from about 1 to about 1.5, where R_8 is C1-C6 unbranched alkyl



B ;

or

the steps of

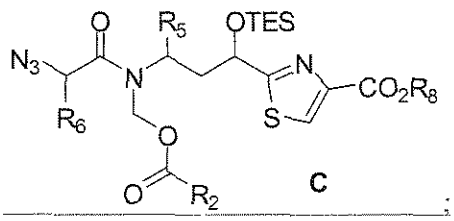
a) preparing a compound of formula (E1) where X_1 is a leaving group from a compound of formula E



E E1

and

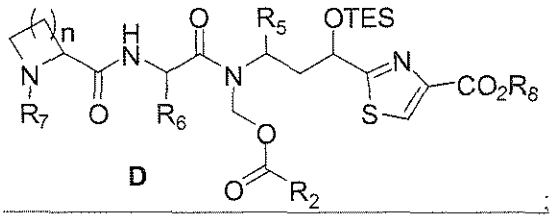
b) treating a compound of formula C under reducing conditions in the presence of the compound of formula E1, where R_8 is C1-C6 unbranched alkyl



C ;

or

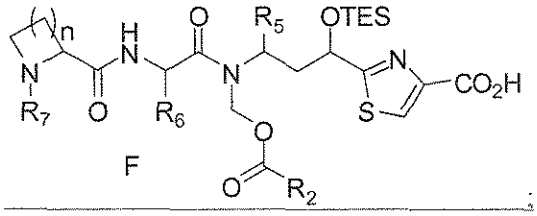
the step of treating compound D with a hydrolase enzyme, where R_8 is C1-C6 unbranched alkyl



D ;

or

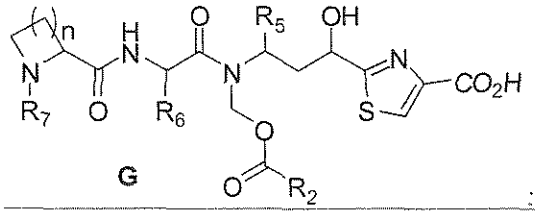
the step of treating the silyl ether of compound F with a non-basic fluoride reagent



F ;

or

the step of treating a compound of formula G with an acylating agent of formula $R_4C(O)X_2$, where X_2 is a leaving group

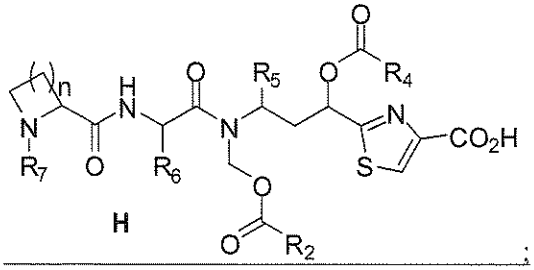


G ;

or

the steps of

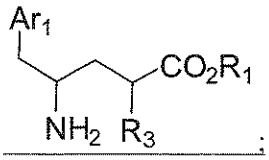
c) forming an active ester intermediate from a compound of formula *H*



;

and

d) reacting the active ester intermediate with a compound of the formula I



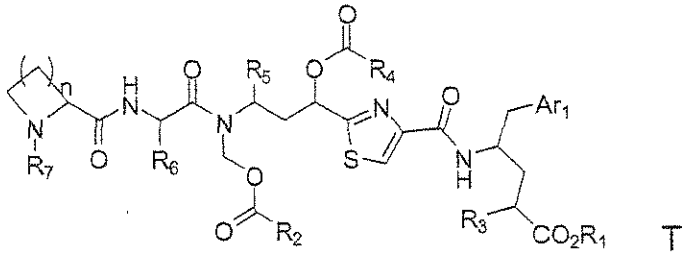
;

or a combination thereof.

In one~~another~~ embodiment, a process is described for preparing a compound of formula B, wherein R_5 and R_6 are as described in the various embodiments herein, such as each being independently selected from optionally substituted alkyl or optionally substituted cycloalkyl; and R_8 is C1-C6 n-alkyl;

~~WHAT IS CLAIMED IS:~~

1. A process for preparing a compound of the formula



T

or a pharmaceutically acceptable salt thereof;

wherein

Ar_1 is aryl optionally substituted by independently replacing one or more hydrogen atoms attached thereto with a group selected from amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, nitro, sulfonate, and carboxylate;

R_1 is hydrogen, alkyl, arylalkyl or a pro-drug forming group selected from 1-indanyl, N-oxysuccinimide, acetoxymethyl, pivaloyloxymethyl, β-acetoxyethyl, β-pivaloyloxyethyl, 1-(cyclohexylcarbonyloxy)prop-1-yl, (1-aminoethyl)carbonyloxymethyl, ethoxycarbonyloxymethyl, α-ethoxycarbonyloxyethyl, β-ethoxycarbonyloxyethyl, dimethylaminomethyl, dimethylaminoethyl, diethylaminomethyl, diethylaminoethyl, 2-(isobutoxycarbonyl)pent-2-enyl, 2-(ethoxycarbonyl)but-2-enyl, phthalidyl, and dimethoxyphthalidyl;

R_2 is selected from the group consisting of alkyl and cycloalkyl, wherein alkyl and cycloalkyl are optionally substituted by independently replacing one or more hydrogen atoms attached thereto with a group selected from amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, nitro, sulfonate, and carboxylate;

R_3 is alkyl optionally substituted by independently replacing one or more hydrogen atoms attached thereto with a group selected from amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, nitro, sulfonate, and carboxylate;

R_4 is alkyl or cycloalkyl, wherein alkyl and cycloalkyl are optionally substituted by independently replacing one or more hydrogen atoms attached thereto with a group selected from amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, nitro, sulfonate, and carboxylate;

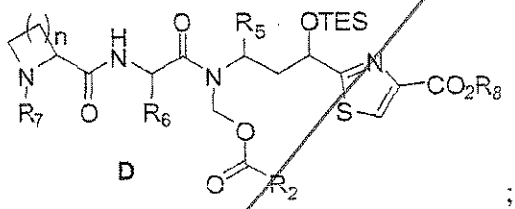
~~R_5 and R_6 are each independently selected from the group consisting of alkyl and~~ cycloalkyl, wherein alkyl and cycloalkyl are optionally substituted by independently replacing one or more hydrogen atoms attached thereto with a group selected from amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, nitro, sulfonate, and carboxylate;

R_7 is alkyl optionally substituted by independently replacing one or more hydrogen atoms attached thereto with a group selected from amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, nitro, sulfonate, and carboxylate; and

n is 1, 2, 3, or 4;

wherein the process comprises

the step of treating compound D with a hydrolase enzyme, where R_8 is C1-C6 unbranched alkyl

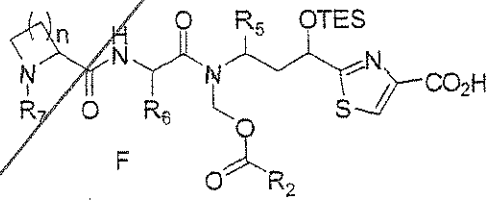


D

;

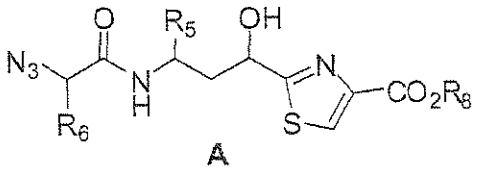
followed by

the step of treating the silyl ether of compound F with a non-basic fluoride reagent



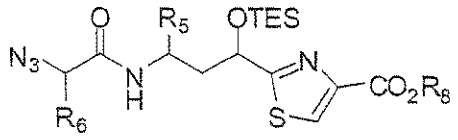
F

2. The process of claim 1 further comprising the step of treating a compound of formula A with triethylsilyl chloride and imidazole in an aprotic solvent, where R_8 is C1-C6 unbranched alkyl



A

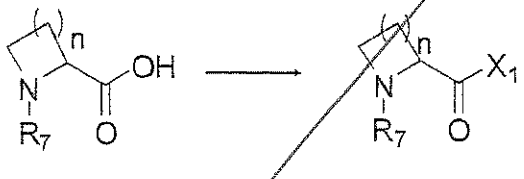
~~3. The process of claim 1 further comprising the step of treating a compound of formula B~~ with a base and a compound of the formula $ClCH_2OC(O)R_2$ in an aprotic solvent at a temperature from about -78°C to about 0°C; wherein the molar ratio of the compound of the formula $ClCH_2OC(O)R_2$ to the compound of formula B from about 1 to about 1.5, where R_8 is C1-C6 unbranched alkyl



B

4. The process of claim 1 further comprising the steps of

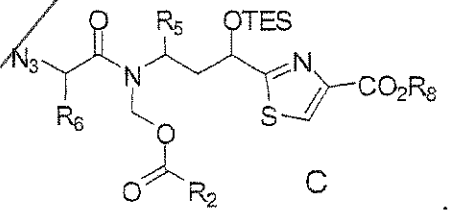
a) preparing a compound of formula (E1) where X_1 is a leaving group from a compound of formula E



E E1

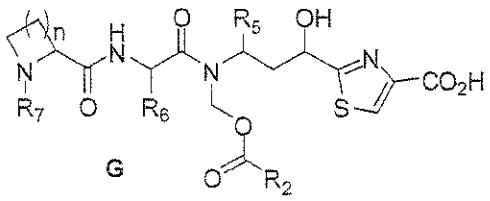
and

b) treating a compound of formula C under reducing conditions in the presence of the compound of formula E1, where R_8 is C1-C6 unbranched alkyl



C

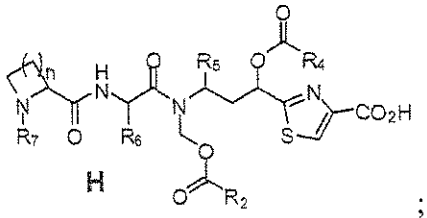
5. The process of claim 1 further comprising the step of treating a compound of formula G with an acylating agent of formula $R_4C(O)X_2$, where X_2 is a leaving group



G

6. The process of claim 1 further comprising the steps of

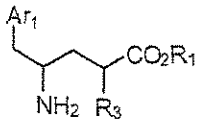
c) forming an active ester intermediate from a compound of formula II



;

and

d) reacting the active ester intermediate with a compound of the formula I



7. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_1 is hydrogen, benzyl, or C1-C4 alkyl.

8. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_1 is hydrogen.

9. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_2 is C1-C8 alkyl or C3-C8 cylcoalkyl.

10. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_2 is n-propyl.

11. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_3 is C1-C4 alkyl.

12. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_3 is methyl.

13. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein Ar_1 is phenyl or hydroxyphenyl.

14. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein Ar_1 is 4-hydroxyphenyl.

15. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_4 is C1-C8 alkyl or C3-C8 cylcoalkyl.

16. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_4 is methyl.

17. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_5 is branched C3-C6 or C3-C8 cycloalkyl.

18. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_5 is iso-propyl.

19. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_6 is branched C3-C6 or C3-C8 cycloalkyl.

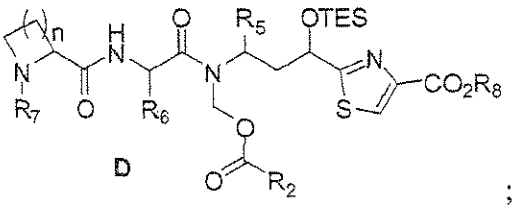
20. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_6 is sec-butyl.

21. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_7 is C1-C6 alkyl.

22. The process of any one of claims 1 to 6 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_7 is methyl.

23. The process of claim 1, wherein the process comprises

the step of treating compound D with a hydrolase enzyme, where R_8 is C1-C6 unbranched alkyl

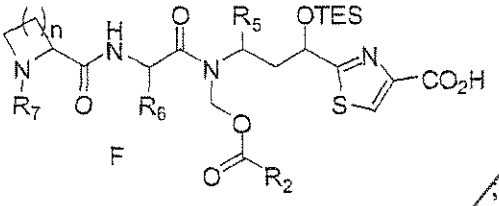


D

;

followed by

the step of treating the silyl ether of compound F with a non-basic fluoride reagent

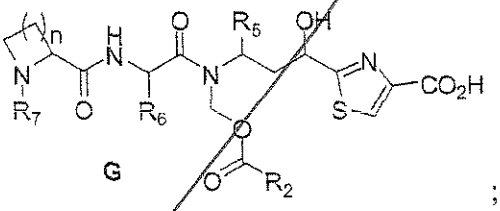


F

;

followed by

the step of treating a compound of formula G with an acylating agent of formula $R_4C(O)X_2$, where X_2 is a leaving group



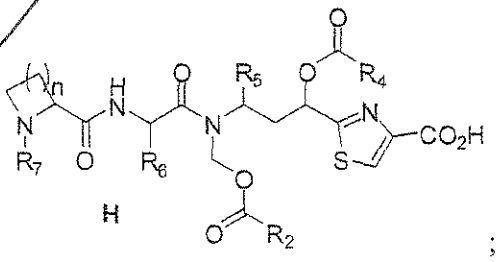
G

;

followed by

the steps of

c) forming an active ester intermediate from a compound of formula H

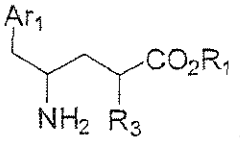


H

;

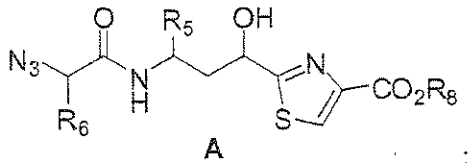
and

d) reacting the active ester intermediate with a compound of the formula I



~~24. The process of claim 1, wherein the process comprises~~

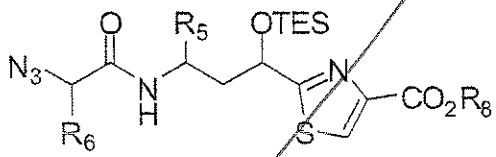
the step of treating a compound of formula A with triethylsilyl chloride and imidazole in an aprotic solvent, where R_8 is C1-C6 unbranched alkyl



A ;

followed by

the step of treating a compound of formula B with a base and a compound of the formula $ClCH_2OC(O)R_2$ in an aprotic solvent at a temperature from about -78°C to about 0°C; wherein the molar ratio of the compound of the formula $ClCH_2OC(O)R_2$ to the compound of formula B from about 1 to about 1.5, where R_8 is C1-C6 unbranched alkyl



B ;

followed by

the steps of

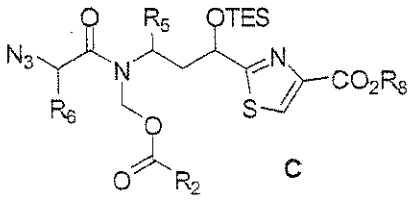
a) preparing a compound of formula (E1) where X_1 is a leaving group from a compound of formula E



E E1

and

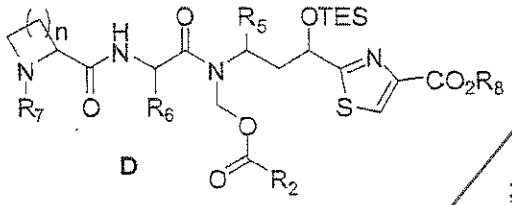
b) treating a compound of formula C under reducing conditions in the presence of the compound of formula E1., where R_8 is C1-C6 unbranched alkyl



C ;

followed by

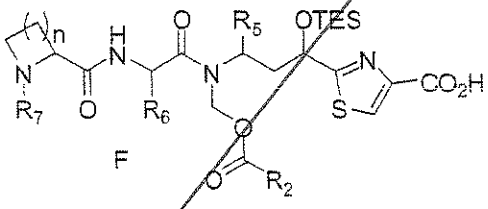
the step of treating compound D with a hydrolase enzyme, where R_8 is C1-C6 unbranched alkyl



D ;

followed by

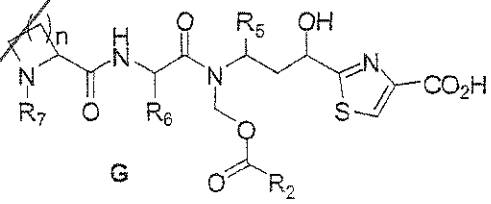
the step of treating the silyl ether of compound F with a non-basic fluoride reagent



F ;

followed by

the step of treating a compound of formula G with an acylating agent of formula $R_4C(O)X_2$, where X_2 is a leaving group

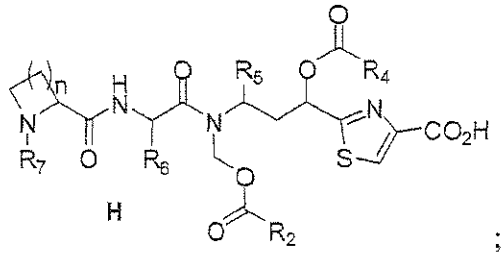


G ;

followed by

the steps of

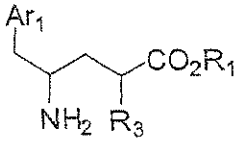
c) forming an active ester intermediate from a compound of formula H



H ;

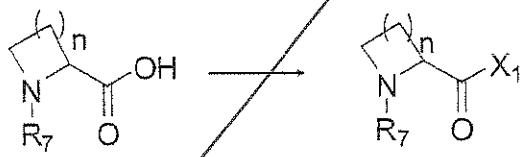
and

d) reacting the active ester intermediate with a compound of the formula I



25. The process of claim 1, wherein the process comprises

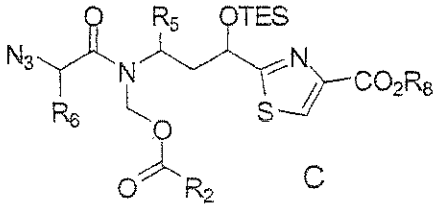
a) preparing a compound of formula (E1) where X_1 is a leaving group from a compound of formula E by reacting the compound of formula E with pentafluorophenol in the presence of diisopropylcarbodiimide for about 1 hour



E E1

and

b) treating a compound of formula C in the presence of the compound of formula E1 with dry 10%Pd/C, where R_8 is C1-C6 unbranched alkyl



C

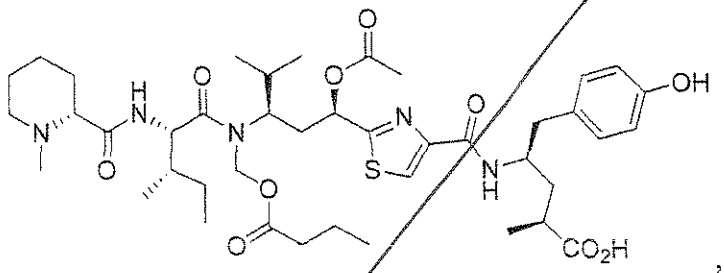
26. The process of any one of claims 23 to 25 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein Ar_1 is 4-hydroxyphenyl.

27. The process of any one of claims 1 to 6 or 23-25 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_1 is hydrogen, R_2 is C1-C8

alkyl or C3-C8 cylcoalkyl, R_3 is C1-C4 alkyl, R_4 is C1-C8 alkyl or C3-C8 cylcoalkyl, R_5 is branched C3-C6 or C3-C8 cycloalkyl, R_6 is branched C3-C6 or C3-C8 cycloalkyl, R_7 is C1-C6 alkyl, and Ar_1 is phenyl or hydroxyphenyl.

28. The process of claim 26 for preparing a compound of the formula T, or a pharmaceutically acceptable salt thereof, wherein R_1 is hydrogen, R_2 is C1-C8 alkyl or C3-C8 cylcoalkyl, R_3 is C1-C4 alkyl, R_4 is C1-C8 alkyl or C3-C8 cylcoalkyl, R_5 is branched C3-C6 or C3-C8 cycloalkyl, R_6 is branched C3-C6 or C3-C8 cycloalkyl, and R_7 is C1-C6 alkyl.

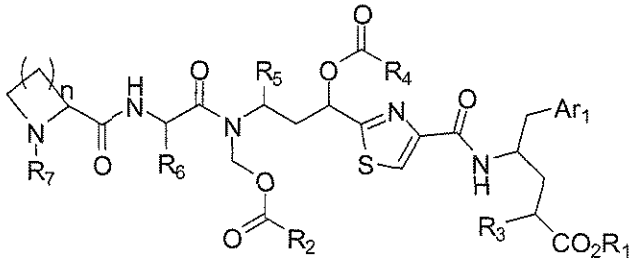
29. The process of any one of claims 1 to 6 or 23-25, wherein the compound of the formula T is of the formula



or a pharmaceutically acceptable salt thereof.

What is claimed is:

1. A process for preparing a compound of the formula



or a pharmaceutically acceptable salt thereof;

wherein

Ar_1 is 4-hydroxyphenyl;

R_1 is hydrogen, alkyl, arylalkyl or a pro-drug forming group;

R_2 is selected from the group consisting of optionally substituted alkyl and optionally substituted cycloalkyl;

R_3 is optionally substituted alkyl;

R_4 is optionally substituted alkyl or optionally substituted cycloalkyl;

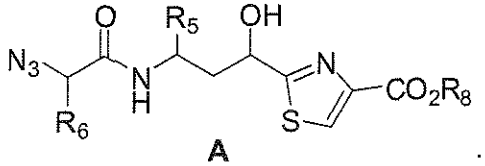
R_5 and R_6 are each independently selected from the group consisting of optionally substituted alkyl and optionally substituted cycloalkyl;

R_7 is optionally substituted alkyl; and

n is 1, 2, 3, or 4;

wherein the process comprises

the step of treating a compound of formula A with triethylsilyl chloride and imidazole in an aprotic solvent, where R_8 is C1-C6 unbranched alkyl

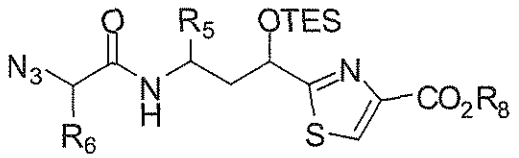


A

;

or

the step of treating a compound of formula B with a base and a compound of the formula $ClCH_2OC(O)R_2$ in an aprotic solvent at a temperature from about −78° C. to about 0° C.; wherein the molar ratio of the compound of the formula $ClCH_2OC(O)R_2$ to the compound of formula B from about 1 to about 1.5, where R_8 is C1-C6 unbranched alkyl



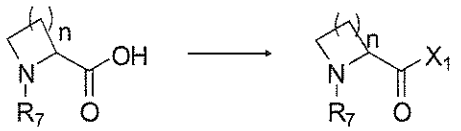
B

;

or

the steps of

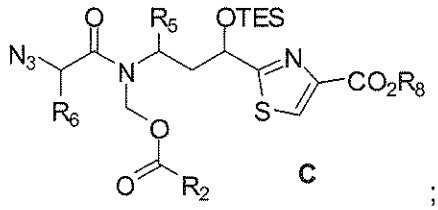
a) preparing a compound of formula (E1) where X_1 is a leaving group from a compound of formula E



E E1

and

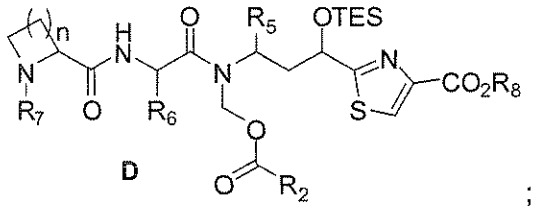
b) treating a compound of formula C under reducing conditions in the presence of the compound of formula E1, where R_8 is C1-C6 unbranched alkyl



C ;

or

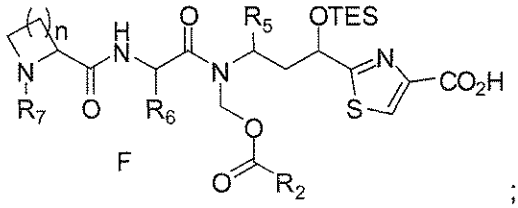
the step of treating compound D with a hydrolase enzyme, where R_8 is C1-C6 unbranched alkyl



D ;

or

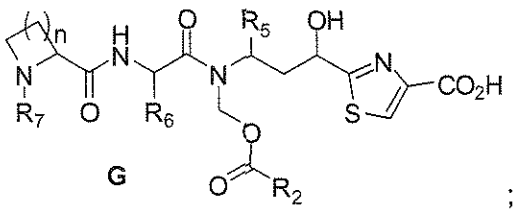
the step of treating the silyl ether of compound F with a non-basic fluoride reagent



F ;

or

the step of treating a compound of formula G with an acylating agent of formula $R_4C(O)X_2$, where X_2 is a leaving group

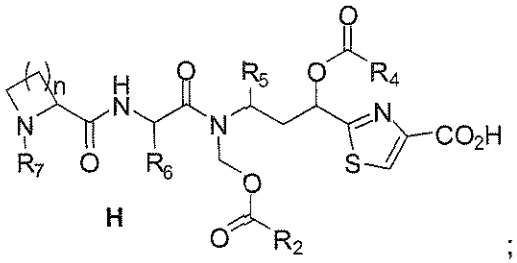


G ;

or

the steps of

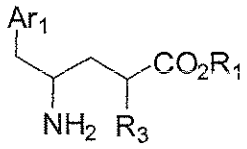
c) forming an active ester intermediate from a compound of formula H



H ;

and

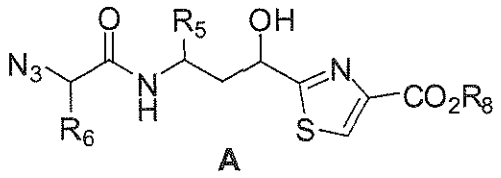
d) reacting the active ester intermediate with a compound of the formula I



;

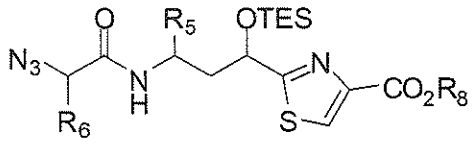
or a combination thereof.

2. The process of claim 1 comprising the step of treating a compound of formula A with triethylsilyl chloride and imidazole in an aprotic solvent, where R_8 is C1-C6 unbranched alkyl



A .

3. The process of claim 1 comprising the step of treating a compound of formula B with a base and a compound of the formula $ClCH_2OC(O)R_2$ in an aprotic solvent at a temperature from about −78° C. to about 0° C.; wherein the molar ratio of the compound of the formula $ClCH_2OC(O)R_2$ to the compound of formula B from about 1 to about 1.5, where R_8 is C1-C6 unbranched alkyl



B

4. The process of claim 1 comprising the steps of

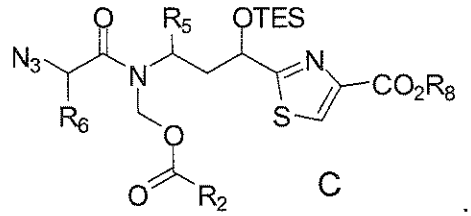
a) preparing a compound of formula (E1) where X_1 is a leaving group from a compound of formula E



E E1

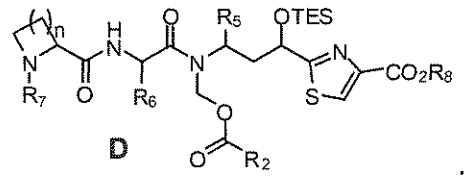
and

b) treating a compound of formula C under reducing conditions in the presence of the compound of formula E1, where R_8 is C1-C6 unbranched alkyl



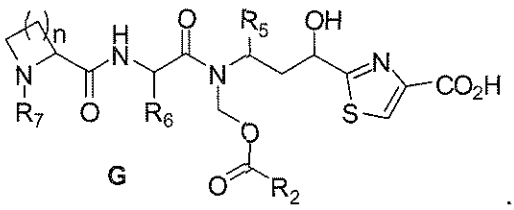
C

5. The process of claim 1 comprising the step of treating compound D with a hydrolase enzyme, where R_8 is C1-C6 unbranched alkyl



D

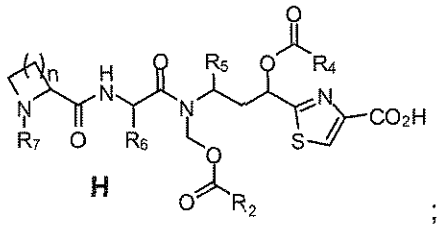
6. The process of claim 1 comprising the step of treating a compound of formula G with an acylating agent of formula $R_4C(O)X_2$, where X_2 is a leaving group



G

7. The process of claim 1 comprising the steps of

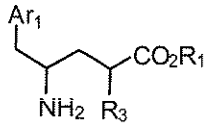
c) forming an active ester intermediate from a compound of formula H



H ;

and

d) reacting the active ester intermediate with a compound of the formula I



8. The process of claim 1 wherein R_1 is hydrogen, benzyl, or C1-C4 alkyl.

9. The process of claim 1 wherein R_1 is hydrogen.

10. The process of claim 1 wherein R_2 is C1-C8 alkyl or C3-C8 cycloalkyl.

11. The process of claim 1 wherein R_2 is n-butyl.

12. The process of claim 1 wherein R_3 is C1-C4 alkyl.

13. The process of claim 1 wherein R_3 is methyl.

14. The process of claim 1 wherein R_4 is C1-C8 alkyl or C3-C8 cycloalkyl.

15. The process of claim 1 wherein R_4 is methyl.

16. The process of claim 1 wherein R_5 is branched C3-C6 alkyl or C3-C8 cycloalkyl.

17. The process of claim 1 wherein R_5 is iso-propyl.

18. The process of claim 1 wherein R_6 is branched C3-C6 alkyl or C3-C8 cycloalkyl.

19. The process of claim 1 wherein R_5 is sec-butyl.

20. The process of claim 1 wherein R_7 is C1-C6 alkyl.

21. The process of claim 1 wherein R_7 is methyl.