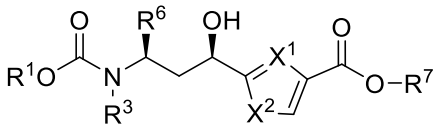
Drug Conjugate. A synthetic method that does not require the use of a heavy metal catalyst or has fewer steps requiring cryogenic temperatures (below -70 °C) would be particularly useful in the manufacturing of a medicant. Eliminating the use of heavy metal catalysts reduces the potential for heavy metal contamination in the therapeutic Conjugate. Those impurities must be carefully controlled and cannot exceed a threshold level for it to be suitable for human use, which adds to the cost of manufacturing. Cryogenic temperatures can also be problematic beyond benchtop scale due to cost, so a reaction sequence having fewer such steps would be beneficial. Therefore, the methods described herein address the unmet need for scalable methods at reduced cost for preparing the tubuvaline component of a tubulysin compound and therapeutic Conjugates related thereto.

SUMMARY

[006a] In an aspect of the present disclosure, there is provided a method for preparing a composition comprising a compound of Formula *R,R*-1a:



(*R,R*-1a)

or a salt thereof, wherein

X^1 is =N-; and

X^2 is S, O, or N(R^{X2})-, or

X^1 is =C(R^{X1})-; and

X^2 is NR^{X2},

wherein R^{X1} and R^{X2} are independently selected from the group consisting of -H, -CH_3, and -CH_2CH_3;

R^1 is phenyl, t-butyl, 9-fluorenyl, or allyl, optionally substituted, or other moiety so that R^1-OC(=O)- is a nitrogen protecting group;

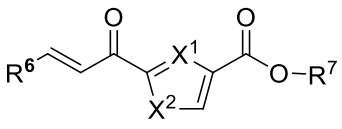
R^3 is optionally substituted saturated C_1-C_6 alkyl, optionally substituted unsaturated C_3-C_6 alkyl, or optionally substituted C_3-C_6 heteroalkyl;

R^6 is optionally substituted C_1-C_6 alkyl; and

R^7 is optionally substituted saturated C_1-C_{20} alkyl, optionally substituted unsaturated C_3-C_{20} alkyl, optionally substituted C_3-C_{20} heteroalkyl, optionally substituted C_2-C_{20} alkenyl, optionally substituted C_3-C_{20} heteroalkenyl, optionally substituted C_2-C_{20} alkynyl, optionally substituted C_3-C_{20} heteroalkynyl, optionally substituted C_6-C_{24} aryl, optionally substituted C_5-C_{24} heteroaryl,

optionally substituted C_3-C_{20} heterocyclyl, or other moiety so that R^7-O- provides for a carboxylic acid protecting group, the method comprising the steps of:

(a) contacting a compound of Formula A:



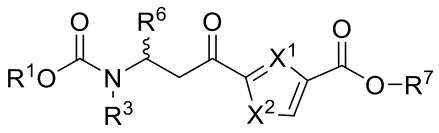
(A),

with a carbamate anion of a compound of Formula B:

$R^3NHC(O)OR^1$ (B),

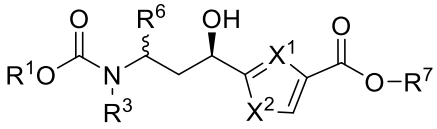
in a polar, aprotic solvent, wherein said contacting is effective for aza-Michael conjugate addition of the Formula B compound anion to the Formula A compound: and

(b) quenching the reaction mixture from said conjugate addition with a Brønstead acid, so as to form an optical isomer mixture of tubuvaline intermediates, or salts thereof, or a composition comprising or consisting of that mixture, wherein the optical isomer mixture is represented by Formula AB:



(AB),

(c) contacting the optical isomer mixture with a chiral reducing agent so as to form a composition comprising an equimolar mixture of diastereomers, wherein the diastereomeric mixture is represented by Formula *R*-1a:

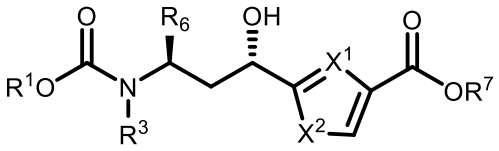


(*R*-1a)

wherein the composition further comprises an equimolar mixture of optical impurities that are enantiomers of the diastereomers, and

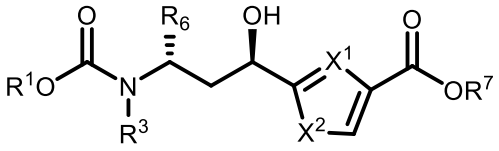
wherein the chiral reducing agent is a chiral oxazaborolidine prepared from contacting BH_3-DMS in THF with a chiral ligand;

(c') separating the compound of Formula *S*,*R*-1a



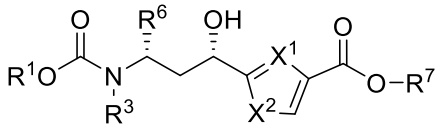
(*S*,*R*-1a)

and the compound of formula *R*,*S*-1a



(*R*,*S*-1a)

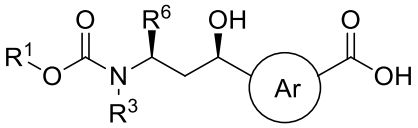
from the composition of the Formula *R*-1a diastereomeric mixture to obtain the composition comprising the compound of Formula *R,R*-1a as the predominate optical isomer and a compound of Formula *S,S*-1a:



(*S,S*-1a)

or a salt thereof, as the major optical impurity, wherein the variable groups of A, B, AB, Formula *R*-1a, Formula *S,R*-1a, Formula *R,S*-1a and Formula *S,*S-1a retain the previous meanings from the compound of Formula *R,R*-1a.

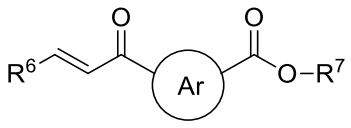
[0007] A principal example of the disclosure provides for a method for preparing a tubuvaline compound of (*R,R*)-Formula **2**:



(**2**),

[0008] or a composition comprised or consisting essentially of that compound, optionally in salt form, wherein: the circled Ar is a 1,3-phenylene or a nitrogen-containing 5- or 6-membered 1,3-heteroarylene, optionally substituted at the remaining positions; R^1 is t-butyl, 9-fluorenyl, allyl, optionally substituted phenyl or other moeity so that R^1-OC(=O)- is a suitable nitrogen protecting group; R^3 is optionally substituted saturated C_1-C_8 alkyl, optionally substituted unsaturated C_3-C_8, or optionally substituted C_3-C_8 heteroalkyl; and R^6 is optionally substituted C_1-C_8 alkyl,

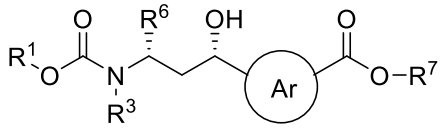
[0009] the method comprising the steps of: (a) contacting a tubuvaline intermediate of Formula **A:**



(**A**),

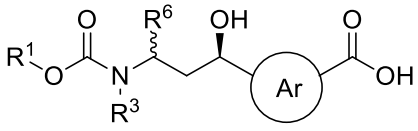
[0010] optionally in salt for, wherein R^7 is optionally substituted saturated C_1-C_{20} alkyl, optionally substituted unsaturated C_3-C_{20} alkyl, optionally substituted C_3-C_{20} heteroalkyl, optionally substituted C_2-C_{20} alkenyl, optionally substituted C_3-C_{20} heteroalkenyl, optionally substituted C_2-C_{20} alkynyl, optionally substituted C_3-C_{20}

isomer; and having (*S*,*S*)-Formula **1a** as the major optical impurity, which has the structure of:



(S,S-**2a**)

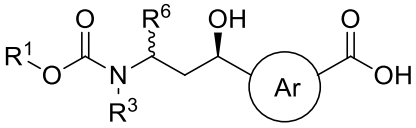
[0018] (d) contacting the diastereomeric mixture of Formula *R*-**1a**, or the composition comprising or consisting essentially of that mixture from step (b) with a suitable hydrolysis agent so as to form a mixture of two tubuvaline diastereomeric compounds, each optionally in salt form, represented by Formula *R*-**2** having the structure of:



(*R*-**2**),

[0019] or a composition comprised or consisting essentially of these diastereomers, or salts thereof, wherein the variable groups of Formulae **A, B** and **AB** are as defined for Formula **2**, or

[0020] (d') contacting the purified tubuvaline composition from step (b') to form a composition comprised or consisting essentially of the (*R*,*R*)-Formula **2** compound as the major optical isomer and having a (*S*,*S*)-Formula **2** compound as the predominate optical impurity, each optionally in salt form, wherein the (*S*,*S*)-Formula **2** compound has the structure of:



(*S*,*S*)-Formula **2**.

[0021] Other principle examples provides for methods of preparing from tubuvaline compounds of Formula *R*-**1a** or Formula *R*-**2** tubuvaline compounds of related structure in which another O-linked substituent replaces the hydroxyl group. Those examples include replacement of the hydroxyl group in Formula *R*-**1a** by an ether group of formula $-OR^2$, wherein R^2 is optionally substituted saturated C_1-C_8 alkyl, optionally substituted unsaturated C_3-C_8 alkyl, optionally substituted C_2-C_8 alkenyl or optionally substituted C_2-C_8 alkynyl, or wherein R^2 is R^{2A}, wherein R^{2A} is $–CH_2R^{2C}$, wherein R^{2C} is optionally substituted saturated C_1-C_8 ether, optionally substituted unsaturated C_3-C_8 ether, optionally substituted C_2-C_8 alkenyl or optionally substituted C_2-C_8 alkynyl, followed by hydrolysis of the carboxylic acid protecting group, which is provided by $–OR^1$, and further include examples in which the hydroxyl group in

Formula **2** is replaced by an ester substituent of formula $-OR^{2A}$ wherein R^{2A} is $R^{2B}C(=O)-$, wherein R^{2B} is optionally substituted saturated C_1-C_8 alkyl, optionally substituted unsaturated C_3-C_8 alkyl, optionally substituted C_2-C_8 alkenyl or optionally substituted C_2-C_8 alkynyl.

[0022] Still other principle examples provide for Drug Linker compositions having a quaternized tubulysin Drug Unit prepared from the tubuvaline compositions and further provide for Ligand Drug Conjugates derived therefrom.

[0023] Those and other examples of the disclosure are described in more detail in the following “Detailed Description of the Invention” and “Claims”.

DETAILED DESCRIPTION OF THE INVENTION

[0024] General

[0025] The present invention is based, in part, on the discovery that tubuvaline analogs can be prepared using a significantly simplified sequence of synthetic steps from commercially available starting materials that significantly shortens the route and which does not require the use of a heavy metal. Specifically, the present invention provides tubuvaline derivatives that are readily generated using a Michael addition by a carbamate anion that introduces a suitable protected secondary amine into a tubuvaline precursor without the need for difficult to control reaction conditions, particularly exceedingly low temperatures typically associated with generation of reactive anions thus increasing yields and shortening overall reaction times. Thus, the present invention further provides improved processes for preparing certain tubulysin compounds and related Drug Linker compounds and Ligand Drug Conjugates.

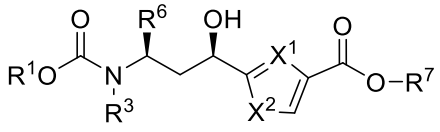
[0026] 1. Definitions

[0027] Unless otherwise stated or implied by context, terms that are used herein have the meanings defined below. Unless otherwise contraindicated or implied, e.g., by including mutually exclusive elements or options, in those definitions and throughout this specification, the terms "a" and "an" mean one or more and the term "or" means and/or where permitted by context. Thus, as presented in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0028] At various locations in the present disclosure, e.g., in any disclosed embodiments or in the claims, reference is made to compounds, compositions, or methods

WHAT IS CLAIMED IS:

1. A method for preparing a composition comprising a compound of Formula *R,R*-1a:



(*R,R*-1a)

or a salt thereof, wherein

X^1 is =N-; and

X^2 is S, O, or N(R^{X2})-, or

X^1 is =C(R^{X1})-; and

X^2 is NR^{X2},

wherein R^{X1} and R^{X2} are independently selected from the group consisting of -H, -CH_3, and -CH_2CH_3;

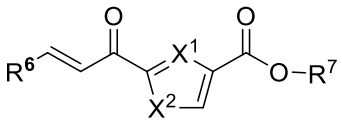
R^1 is phenyl, t-butyl, 9-fluorenyl, or allyl, optionally substituted, or other moiety so that R^1-OC(=O)- is a nitrogen protecting group;

R^3 is optionally substituted saturated C_1-C_6 alkyl, optionally substituted unsaturated C_3-C_6 alkyl, or optionally substituted C_3-C_6 heteroalkyl;

R^6 is optionally substituted C_1-C_6 alkyl; and

R^7 is optionally substituted saturated C_1-C_{20} alkyl, optionally substituted unsaturated C_3-C_{20} alkyl, optionally substituted C_3-C_{20} heteroalkyl, optionally substituted C_2-C_{20} alkenyl, optionally substituted C_3-C_{20} heteroalkenyl, optionally substituted C_2-C_{20} alkynyl, optionally substituted C_3-C_{20} heteroalkynyl, optionally substituted C_6-C_{24} aryl, optionally substituted C_5-C_{24} heteroaryl, optionally substituted C_3-C_{20} heterocyclyl, or other moiety so that R^7-O- provides for a carboxylic acid protecting group, the method comprising the steps of:

(a) contacting a compound of Formula A:



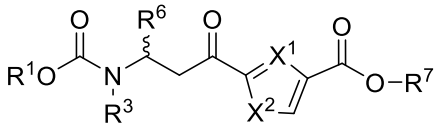
(A),

with a carbamate anion of a compound of Formula B:

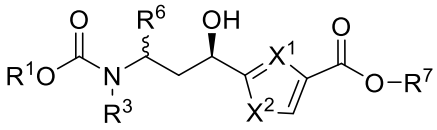
$R^3NHC(O)OR^1$ (B),

in a polar, aprotic solvent, wherein said contacting is effective for aza-Michael conjugate addition of the Formula B compound anion to the Formula A compound: and

(b) quenching the reaction mixture from said conjugate addition with a Brønstead acid, so as to form an optical isomer mixture of tubuvaline intermediates, or salts thereof, or a composition comprising or consisting of that mixture, wherein the optical isomer mixture is represented by Formula AB:


(AB),

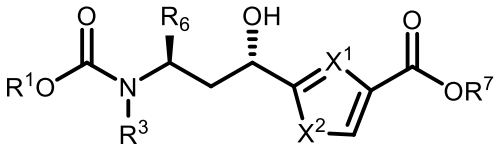
(c) contacting the optical isomer mixture with a chiral reducing agent so as to form a composition comprising an equimolar mixture of diastereomers, wherein the diastereomeric mixture is represented by Formula *R*-1a:


(*R*-1a)

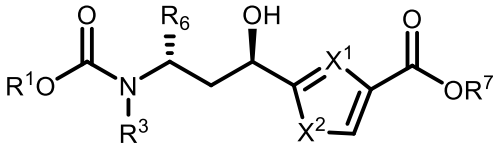
wherein the composition further comprises an equimolar mixture of optical impurities that are enantiomers of the diastereomers, and

wherein the chiral reducing agent is a chiral oxazaborolidine prepared from contacting BH_3-DMS in THF with a chiral ligand;

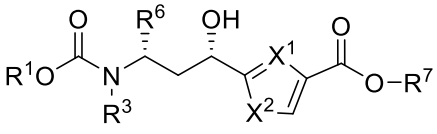
(c') separating the compound of Formula *S*,*R*-1a


(*S*,*R*-1a)

and the compound of formula *R*,*S*-1a


(*R*,*S*-1a)

from the composition of the Formula *R*-1a diastereomeric mixture to obtain the composition comprising the compound of Formula *R*,*R*-1a as the predominate optical isomer and a compound of Formula *S*,*S*-1a:

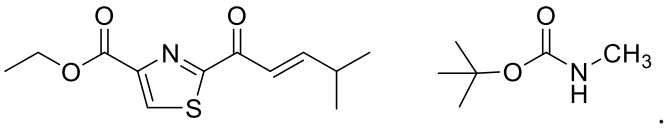

(*S*,*S*-1a)

or a salt thereof, as the major optical impurity, wherein the variable groups of A, B, AB, Formula *R*-1a, Formula *S*,*R*-1a, Formula *R*,*S*-1a and Formula *S*,S-1a retain the previous meanings from the compound of Formula *R*,*R*-1a.

2. The method of claim 1, wherein the optical purity of the composition from step (c') is retained by the composition obtained from step (c).

3. The method of claim 1, wherein said step (c') separation is by silica gel flash chromatography.

4. The method of claim 1, wherein compound A and compound B have the structures of:



.

5. The method of any one of claims 1-4, wherein the carbamate anion is prepared by contacting the Formula B compound in a polar, aprotic solvent at about -20 °C to about -40 °C, with a hindered base which deprotonates the carbamate functional group of the Formula B compound, wherein "about" indicate that the numeric value or range of values can vary by 10% of the recited value.

6. The method of claim 5, wherein the hindered base is potassium bis(trimethylsilyl)amide (KHMDS) in THF.

7. The method of claim 5, wherein the polar, aprotic solvent for preparing the carbamate anion is the same used for conducting the aza Michael conjugate addition of step (a).

8. The method of any one of claims 1-4, wherein step (a) is conducted by addition of a solution of the compound of Formula A to the Formula B compound anion solution while maintaining a reaction temperature of about -20 °C to about -40 °C, wherein both solutions are in the same polar, aprotic solvent, wherein "about" indicate that the numeric value or range of values can vary by 10% of the recited value.

9. The method of claim 8, wherein the polar, aprotic solvent is diethyl ether, THF, or dioxane, or a mixture of two or three of these solvents.

10. The method of any one of claims 1-4, wherein step (b) quenching is by addition of 50% AcOH/water to the reaction mixture of step (a).

11. The method of any one of claims 1-4, wherein the chiral reducing agent is (S)-(-)-CBS.

12. The method of any one of claims 1-4, wherein step (c) is conducted in the polar, aprotic solvent by admixing a solution of BH_3-SMe_2 with solution of (*S*)-(-)-CBS ligand at a temperature between about -10 °C to about 4 °C, followed by stirring for about 5 min. to about 30 min. so as to

form the chiral reducing agent then cooling the chiral reducing agent to between about -20 °C to about -50 °C, whereupon a solution of the Formula AB tubuvaline intermediate mixture is added while maintaining the original temperature of the chiral reducing agent, followed by stirring of the resulting reaction mixture until consumption of the Formula AB tubuvaline intermediates is complete, wherein "about" indicate that the numeric value or range of values can vary by 10% of the recited value.

13. The method of any one of claims 1-4, wherein step (c) is conducted in THF by admixing a solution of BH_3-SMe_2 with solution of (*S*)-(-)-CBS ligand in a molar excess of about 5% to about 10% at a temperature about -4 °C or about 0 °C, followed by about 15 min. or about 10 min. of stirring so as to form the chiral reducing agent then cooling the chiral reducing agent to about – 40 °C, whereupon a solution of the Formula AB tubuvaline intermediate mixture is added while maintaining the original temperature of the chiral reducing agent, followed by stirring of the resulting reaction mixture until consumption of the Formula AB tubuvaline intermediates is complete, wherein "about" indicate that the numeric value or range of values can vary by 10% of the recited value.

14. The method of claim 1, wherein R^3 is $–CH_3$ or $–CH_2CH_2CH_3$.

15. The method of claim 1, wherein R^7 is $–CH_3$ or $–CH_2CH_3$.

16. The method of claim 9, wherein the polar, aprotic solvent is THF.