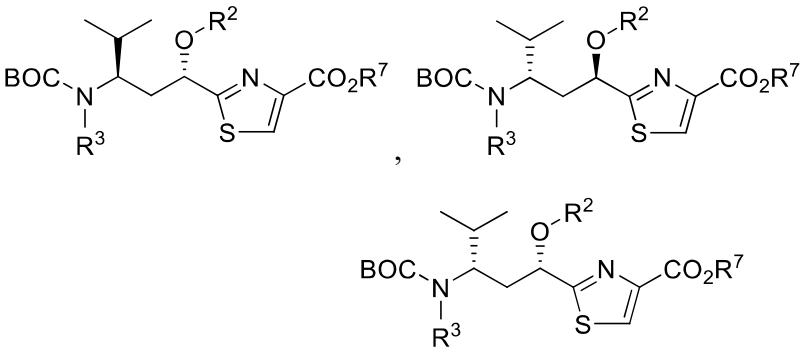
[0007] The methods of synthesis of tubulysin compounds having substitution of the amide nitrogen atom of the tubuvaline component reported in the literature to date involve multiple steps that are not easily scalable. Therefore, a need exists in the art for improved processes in producing N-substituted tubuvaline for the preparation of the tubulysin compounds. The methods described herein address that unmet need.

SUMMARY

[0008] In one aspect, the present disclosure refers to a compound having the structure of:



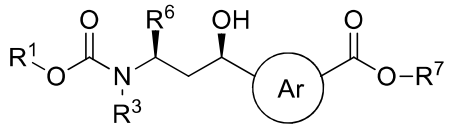
optionally in salt form, wherein

R^2 is selected from the group consisting of –H, C_1-C_4 alkyl and R^{2A}, wherein R^{2A} is –C(=O)R^{2B}, wherein R^{2B} is optionally substituted saturated C_1-C_4 alkyl or optionally substituted C_3-C_6 unsaturated alkyl;

R^3 is–CH_3, -CH_2CH_3 or –$CH_2CH_2CH_3$

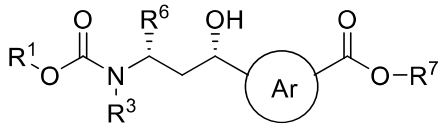
R^7 is –H or C_1-C_4 saturated alkyl.

[0008a] In another aspect, the present disclosure refers to a composition comprised of a tubuvaline compound of (*R,R*)-Formula **1a**, optionally in salt form, having the structure of



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

and one or more optical isomers thereof as optical impurities, wherein the (*R,R*)-Formula **1a** tubuvaline compound, or salt thereof, is the predominate optical isomer, and wherein its corresponding enantiomer, (*S,S*)-Formula **1a**, optionally in salt form, is the major optical impurity, which has the structure of:



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

wherein:

the circled Ar is a 1,3-phenylene or a 5- or 6-membered nitrogen-containing 1,3-heteroarylene, optionally substituted at the remaining positions;

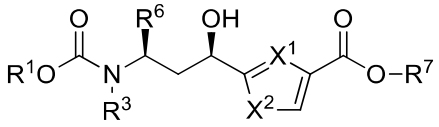
R^1 is phenyl, t-butyl, 9-fluorenyl or allyl, optionally substituted, or other moiety so that R^1-OC(=O)- is a suitable nitrogen protecting group;

R^3 is optionally substituted saturated C_1-C_8 alkyl, optionally substituted unsaturated C_3-C_8 alkyl or optionally substituted C_3-C_8 heteroalkyl;

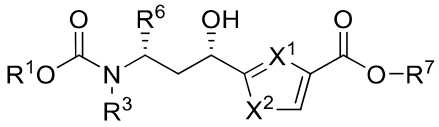
R^6 is optionally substituted C_1-C_8 alkyl; and

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 suitable carboxylic acid protecting group,

in particular, (*R,R*)-Formula **1a** has the structure of:



and (*S,S*)-Formula **1a** has the structure of:



wherein:

X^1 is =N-; and

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

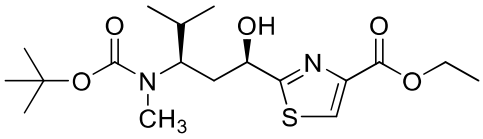
X^1 is =C(R^{X1})-; and

X^2 is NR^{X2},

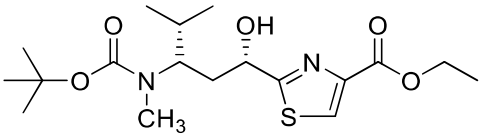
R^{X1} and R^{X2} are independently selected from the group consisting of -H, -CH_3 or –CH_2CH_3; and

wherein the remaining variable group are as previously defined,

more particularly, (*R,R*)-Formula **1a** has the structure of:

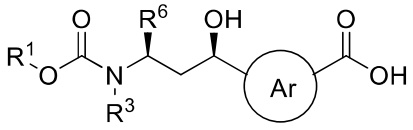


and (*S,S*)-Formula **1a** has the structure of:



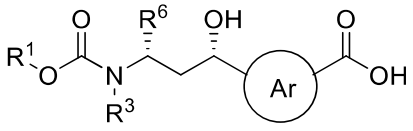
.

[0008b] In yet another aspect, the present disclosure refers to a composition comprised of a tubuvaline compound of (*R,R*)-Formula **2**, optionally in salt form, having the structure of:



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

and one or more optical isomers thereof as optical impurities, wherein the (*R,R*)-Formula **2** tubuvaline compound, or salt thereof, is the predominate optical isomer, and wherein its corresponding enantiomer, (*S,S*)-Formula **2**, optionally in salt form, is the major optical impurity, which has the structure of:



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

wherein:

the circled Ar is a 1,3-phenylene or a 5- or 6-membered nitrogen-containing 1,3-heteroarylene, optionally substituted at the remaining positions;

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

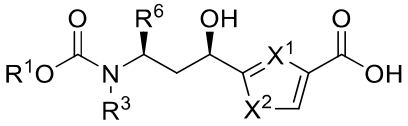
R^3 is optionally substituted saturated C_1-C_8 alkyl, optionally substituted unsaturated C_3-C_8 alkyl or optionally substituted C_3-C_8 heteroalkyl;

R^6 is optionally substituted C_1-C_8 alkyl; and

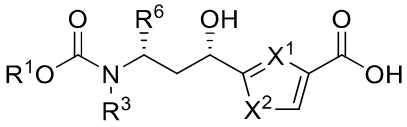
R^7 is optionally substituted saturated C_1-C_{20} alkyl, optionally substituted unsaturated C_3-C_{20} alkyl, optionally substituted C_3-C_{20} heteroalkyl, optionally substituted C_2-C_{20} alkenyl, optionally substituted C_3-C_{20} heteroalkenyl, optionally substituted C_2-C_{20} alkynyl,

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 suitable carboxylic acid protecting group,

in particular, wherein (*R,R*)-Formula **2** has the structure of:



or a salt thereof, and (*S,S*)-Formula **2** has the structure of:



or a salt thereof, wherein:

X^1 is =N-; and

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

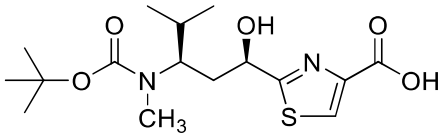
X^1 is =C(R^{X1})-; and

X^2 is NR^{X2},

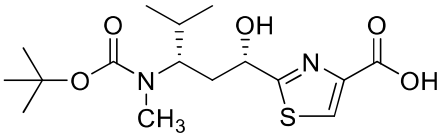
wherein R^{X1} and R^{X2} are independently selected from the group consisting of -H, -CH_3 or –CH_2CH_3;

and the remaining variable groups are as previously defined,

more particularly, (*R,R*)-Formula **2** has the structure of:

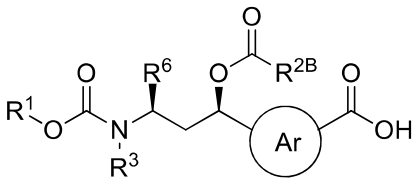


or a salt thereof, and (*S,S*)-Formula 2, optionally in salt form, has the structure of:



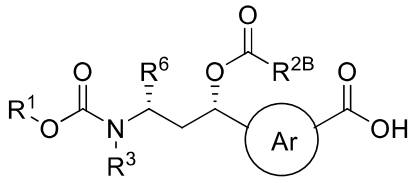
.

[0008c] In yet another aspect, the present disclosure refers to a composition comprised of a tubuvaline compound of (*R,R*)-Formula **2a**, optionally in salt form, having the structure of:



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

and one or more optical isomers thereof as optical impurities, wherein the (*R,R*)-Formula **2a** tubuvaline compound, or salt thereof, is the predominate optical isomer, and wherein its corresponding enantiomer, (*S,S*)-Formula **2a**, optionally in salt form, is the major optical impurity, which has the structure of:



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

wherein:

the circled Ar is a 1,3-phenylene or a 5- or 6-membered nitrogen-containing 1,3-heteroarylene, optionally substituted at the remaining positions;

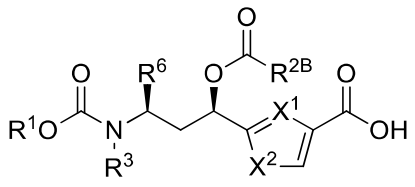
R^1 is phenyl, t-butyl, 9-fluorenyl or allyl, optionally substituted, or other moeity so that R^1-OC(=O)- is a suitable nitrogen protecting group;

R^3 is optionally substituted saturated C_1-C_8 alkyl, optionally substituted unsaturated C_3-C_8 alkyl or optionally substituted C_3-C_8 heteroalkyl;

R^6 is optionally substituted C_1-C_8 alkyl; and

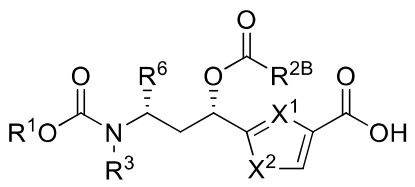
R^7 is optionally substituted saturated C_1-C_{20} alkyl, optionally substituted unsaturated C_3-C_{20} alkyl, optionally substituted C_3-C_{20} heteroalkyl, optionally substituted C_2-C_{20} alkenyl, optionally substituted C_3-C_{20} heteroalkenyl, optionally substituted C_2-C_{20} alkynyl, optionally 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 moeity so that R^7-O- provides for a suitable carboxylic acid protecting group,

in particular, wherein (*R,R*)-Formula **2a** has the structure of:



.

or salt thereof, and (*S,S*)-Formula **2a** has the structure of:



,

or salt thereof, wherein:

X^1 is =N-; and

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

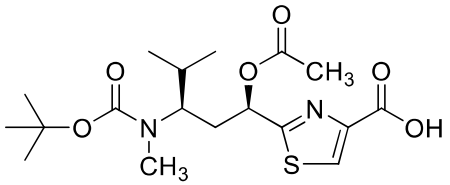
X^1 is =C(R^{X1})-; and

X^2 is NR^{X2},

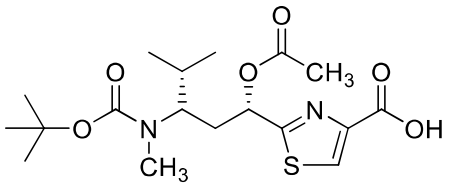
wherein R^{X1} and R^{X2} are independently selected from the group consisting of -H, -CH_3 or –CH_2CH_3; and

wherein the remaining variable groups are as previously defined,

more particularly, wherein (*R,R*)-Formula **2a** has the structure of:

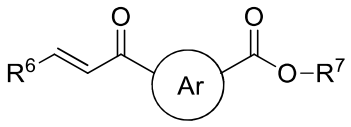


or salt thereof, and (*S,S*)-Formula **2a**, optionally in salt form, has the structure of:



[0008d] In yet another aspect, the present disclosure refers to a method for preparing a composition as described herein, the method comprising the steps of:

(a) contacting a compound of Formula **A:**



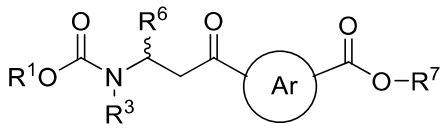
(A),

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} heteroalkynyl, optionally substituted C_6-C_{24} aryl, optionally substituted C_5-C_{24} heteroaryl, optionally substituted C_3-C_{20} heterocyclyl, or other moeity so that R^7-O- provides for a suitable carboxylic acid protecting group,

with a compound of Formula **B:**

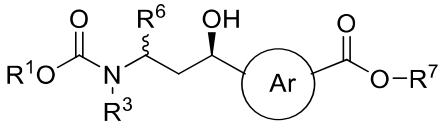
$R^3NHC(O)OR^1$ (**B**),

in a suitable polar, aprotic solvent in the presence of a suitable transition metal (II) or transition metal (III) catalyst, in particular, one comprising a transition metal selected from the group consisting of Cu(II), Zn(II) and Yb(III), so as to form a composition comprised of an enantiomeric mixture of tubuvaline intermediates represented by Formula **AB:**



(**AB**),

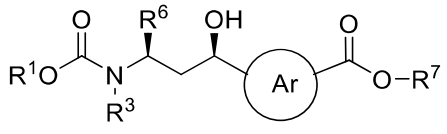
(b) contacting the enantiomeric mixture with a suitable chiral reducing agent so as to form a composition comprised of essentially an equimolar mixture of diastereomers, wherein the diastereomeric mixture is represented by Formula *R*-**1a**,



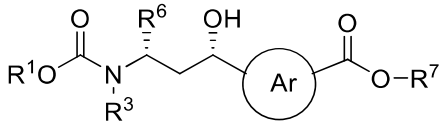
(*R*-**1a**)

wherein the composition is further comprised of essentially an equimolar mixture of optical impurities that are enantiomers of the diastereomers.

(b') separating the diastereomers from the composition of the Formula *R*-**1a** diastereomeric mixture so that the composition, which is comprised of (*R,R*)-Formula **1a** as the predominate optical isomer and having (*S,S*)-Formula **1a** as the major optical impurity, is obtained, wherein the predominate optical isomer and the major optical impurity, each optionally in salt form, have the structures of:



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

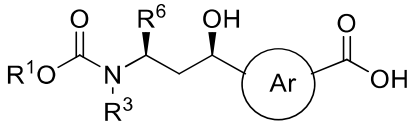


(*S,S*-**1a**)

respectively, wherein the variable groups of **AB**, Formula *R*-**1a**, *R,R*-Formula **1a** and (*S,S*)-Formula **1a** retain the previous meanings from the Formula **A** and Formula **B** compounds.

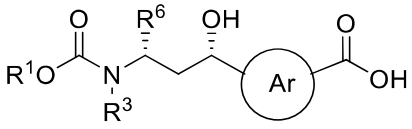
[0008e] In yet another aspect, the present discosure refers to a method for preparing a composition as described herein, the method comprising the steps of:

(c) contacting the composition obtained from steps (a), (b) and step (b') of any one of claims **23-26** with an suitable hydrolysis agent, wherein the predominate optical isomer of the composition so obtained is (*R,R*)-Formula **2**, optionally in salt form, having the structure of:



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

and the major optical impurity is (*S,S*)-Formula **2**, optionally in salt form, having the structure of:

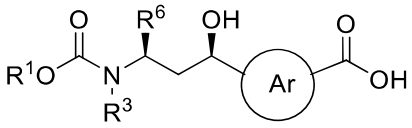


(*S,S*-**2**),

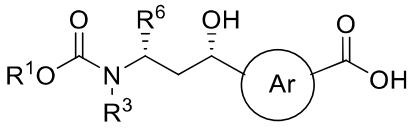
wherein the variable groups of *R,R*-Formula **2** and *S,S*-Formula **2** retain the previous meanings from the Formula **A** and Formula **B** compounds.

[0008f] In yet another aspect, the present disclosure refers to a method for preparing a composition as described herein, the method comprising the steps of:

(c) contacting the composition obtained from steps (a), (b) and step (b') of any one of claims **23-26** with an suitable hydrolysis agent to obtain a composition comprised of (*R,R*)-Formula **2** as the predominate optical isomer, optionally in salt form, which has the structure of:

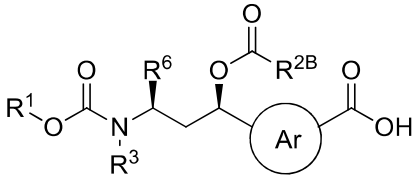


and further comprised of its enantiomer, optionally in salt form, as the major optical impurity, which has the structure of:

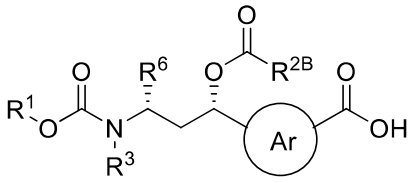


and

step (d): contacting the composition so obtained with a suitable acylating agent to obtain a composition comprised (*R,R*)-Formula **2a** as the predominate optical isomer and (*S,S*)-Formula **2a** as the major optical impurity, wherein the predominate optical isomer and the predominate optical isomer, each optionally in salt form, have the structures of:



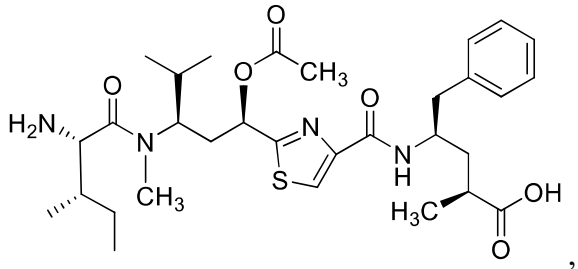
(*R,R*-**2a**),



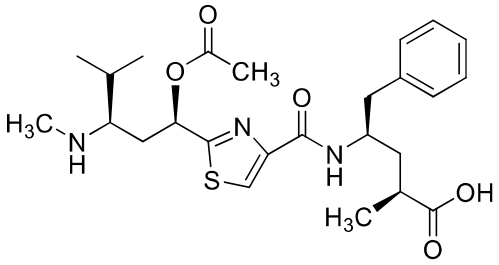
(*S,S*-**2a**)

wherein R^{2B} is saturated C_1-C_6 alkyl, unsaturated C_3-C_8 alkyl, C_2-C_8 alkenyl or C_2-C_4 alkynyl, optionally substituted, in particular $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-CH_2CH{=}CH_2$, $-CH_2CH(CH_3)_2$, $-CH_2C(CH_3)_3$, $-CH_2C(CH_3){=}CH_2$, $-CH{=}CH_2$ or $-CHC{\equiv}CH$, more particularly, $–CH_3$, and the remaining variable groups retain the previous meanings of the Formula **A** and Formula **B** compounds.

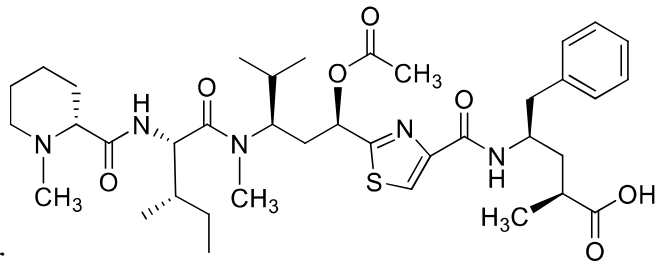
[0008g] In still another aspect, the present disclosure refers to a composition comprised of a compound, optionally in salt form, having the structure of:



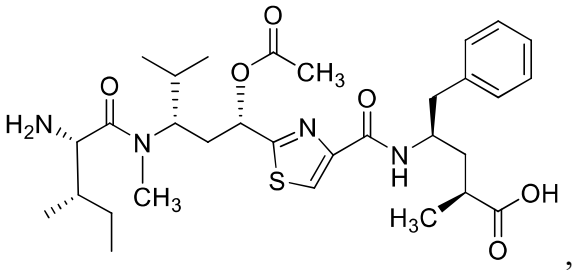
,



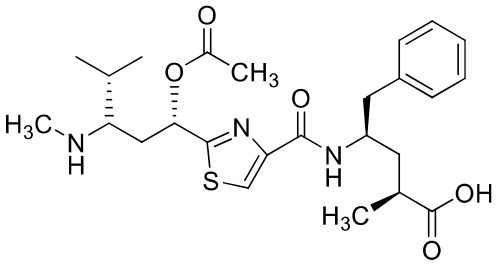
or

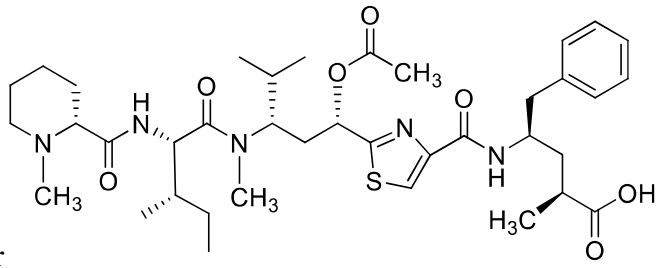


as the predominate optical isomer, and a corresponding optical impurity, optionally in salt form, having the structure of:



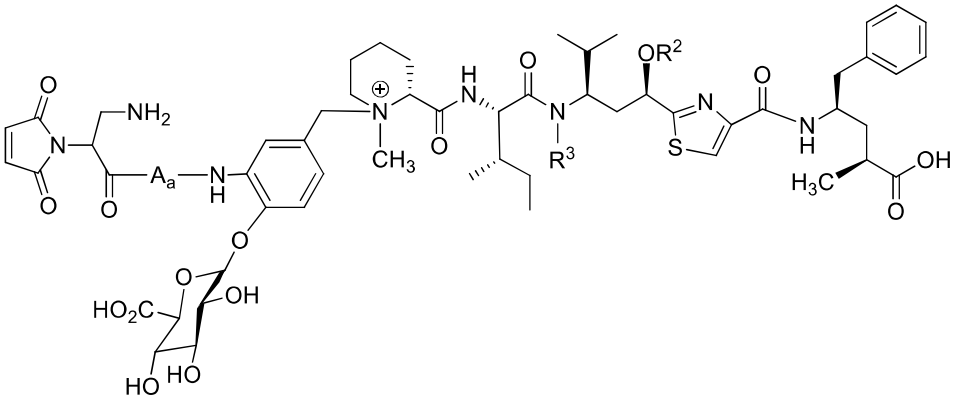
,



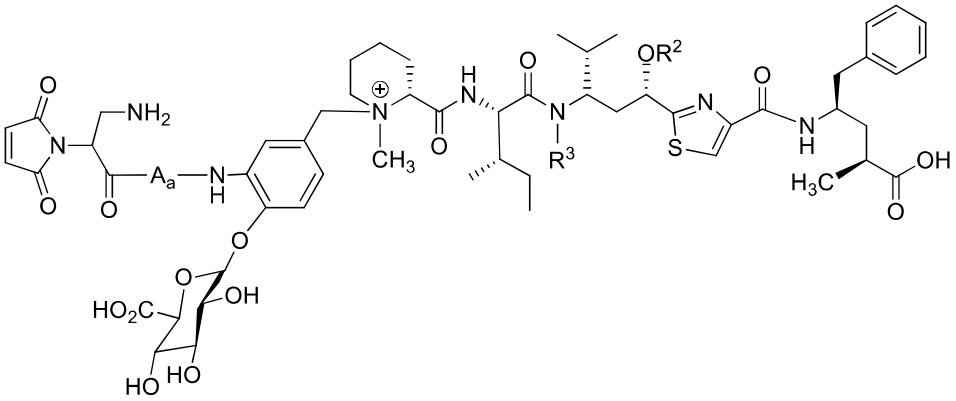


or .

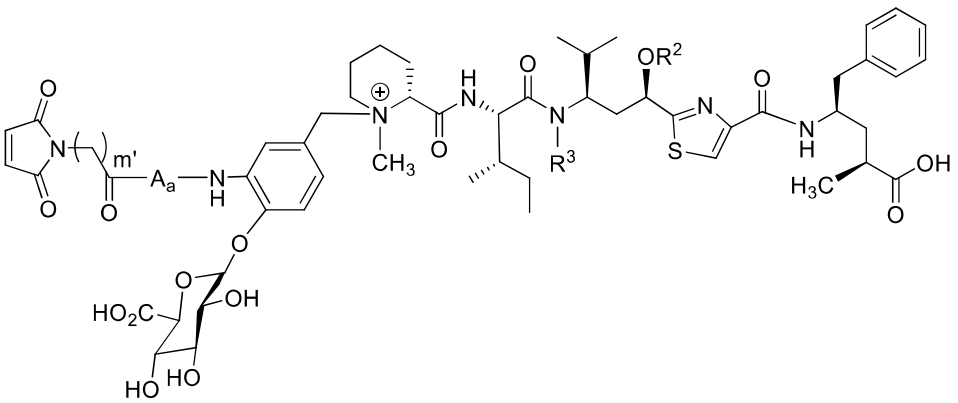
[0008h] In yet another aspect, the present disclosure refers to a Drug Linker composition, wherein the composition is comprised of a Drug Linker compound having the structure of:



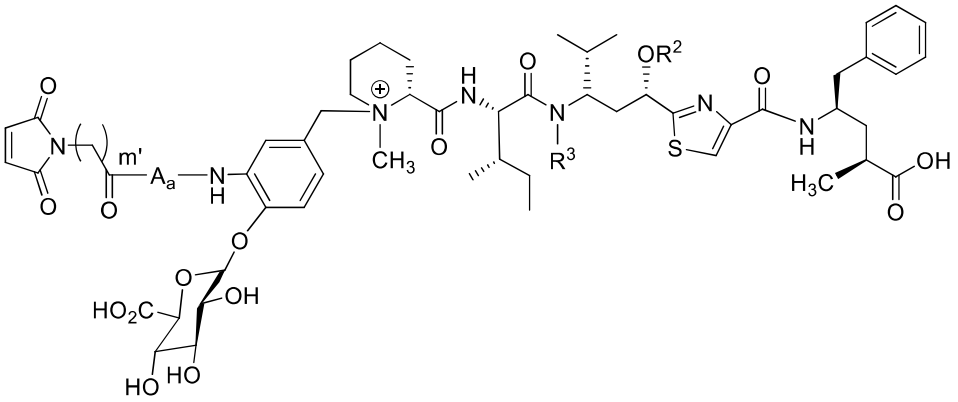
in suitable salt form, and a Drug linker impurity having the structure of:



also in salt form, or wherein the Drug Linker compound has the structure of:



in suitable salt form, and the Drug linker impurity has the structure of:



also in salt form, wherein subscript a is 1 so that A is present, preferably as an α-amino acid or β-amino acid residue; subscript m' ranges from 1 to 5; R^2 is saturated C_1-C_6 alkyl, or R^2 is R^{2A}, wherein R^{2A} is $–C(=O)R^{2B}$, wherein R^{2B} is saturated C_1-C_6 alkyl or unsaturated C_3-C_6 alkyl, in particular, R^2 is $–CH_3$, $-CH_2CH_3$ or $–C(=O)CH_3$; and R^3 is $–CH_3$ or $–CH_2CH_2CH_3$.

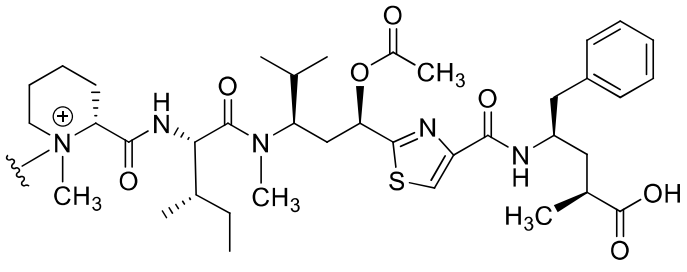
[0008i] In still another aspect, the present disclosure refers to a Ligand Drug Conjugate composition having the structure of Formula **1**:

$$L\text{-}(LU\text{-}D^+)_p \qquad (\mathbf{1})$$

wherein L is a Ligand Unit, LU is a Linker Unit and D^+ is a quaternized tubulysin Drug Unit,

and subscript p ranges from 2 to 12,

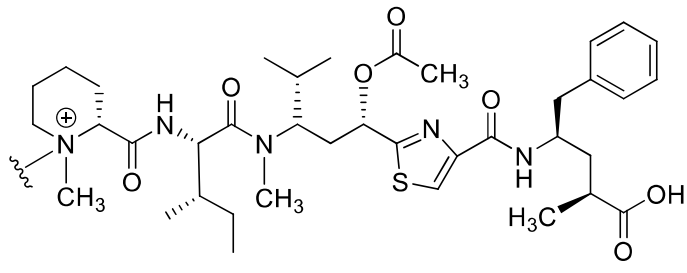
wherein a plurality of Ligand Drug Conjugate compounds of the composition have identical quaternized tubulysin Drug Units having the structure of (*R,R*)-Formula D^+:



(*R,R*-D$^+$)

in pharmaceutically acceptable salt form, wherein the wavy line indicate the site of covalent attachment to LU.

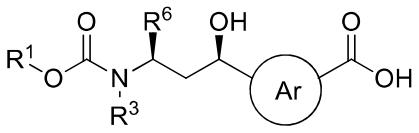
and wherein at least one compound of the composition has at least one quaternized tubulysin Drug Unit having the structure of (*S,S*)-Formula D$^+$:



(*S,S*-D$^+$)

with the remainder having identical (*R,R*)-Formula D$^+$ structures.

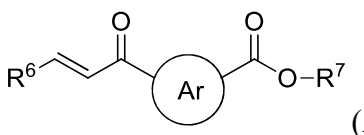
A principal example of the disclosure provides for a method for preparing a tubuvaline compound of Formula **2**:



(**2**),

[0009] 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,

[0010] the method comprising the steps of: (a) contacting a tubuvaline intermediate of Formula **A:**



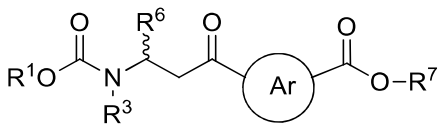
(**A**),

[0011] 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} heteroalkynyl, optionally substituted C_6-C_{24} aryl, optionally substituted C_5-C_{24} heteroaryl, optionally substituted C_3-C_{20} heterocyclyl, or other moeity so that R^7-O- provides a suitable carboxylic acid protecting group,

[0012] with a compound of Formula **B**:

$$R^3NHC(O)OR^1 \text{ (}\mathbf{B}\text{)},$$

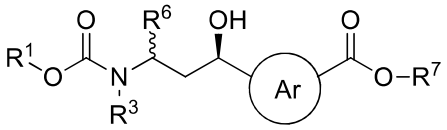
[0013] in a suitable polar, aprotic solvent in the presence of a suitable transition metal (II) or transition metal (III) catalyst, in particular, one comprising a transition metal selected from the group consisting of Cu(II), Zn(II) and Yb(III), so as to form a mixture of two enantiomeric tubuvaline intermediates represented by Formula **AB**:



(AB),

[0014] or a composition comprised or consisting essentially of these enantiomeric intermediates, each optionally in salt form; and

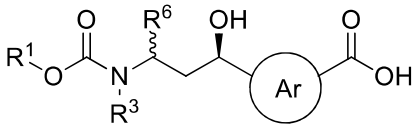
[0015] (b) contacting the enantiomeric Formula **AB** tubuvaline intermediates, or composition comprised or consisting essentially of these intermediates, each optionally in salt form, with a suitable reducing agent, in particular, a chiral reducing agent, so as to form a mixture of two diastereomeric tubuvaline compounds, each optionally in salt form, represented by the structure of Formula *R*-**1a**:



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

[0016] or a composition comprised or consisting essentially of these diastereomers or salts thereof; and

[0017] (c) contacting the diastereomeric mixture of Formula *R*-**1a**, or the composition comprising or consisting essentially of that mixture 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**),

[0018] or 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**.

[0019] Other principle examples provide methods for preparing a tubuvaline composition comprised or consisting essentially of a diastereomer indicated as Formula *R*-**1a** or Formula *R*-**2**, either optionally in salt form, as the predominate optical isomer in which R^6 is in the *R*-configuration, which are described herein by (*R,R*)-Formula **1a** or (*R,R*)-Formula **2**, respectively, wherein the composition contains a diastereomeric impurity or salt thereof of no more than about 10% w/w, preferably no more than about 5% w/w, more preferably no more than about 1% w/w or about 0.5% w/w, relative to the total amount of the Formula **1a** or Formula **2** optical isomers present in the composition, which is attributable to these diastereomers and their respective enantiomers, or is essentially free of that diastereomer in which R^6 of Formula *R*-**1a** or Formula *R*-**2** is in the *S*-configuration, which is described herein as (*R,S*)-Formula **1a** or (*R,S*)-Formula **2**, respectively, and is essentially or substantially free of the corresponding enantiomer, which is indicated as (*S,R*)-Formula **1a** or (*S,R*)-Formula **2**, each optionally in salt form, and optionally containing as the major optical impurity the enantiomer or salt thereof of the predominate optical isomer, which is described as (*S,S*)-Formula **1a** or (*S,S*)-Formula **2**, respectively. In related examples provided are those Formula **1a** and Formula **2** optical isomers each in purified form useful as analytical standards in the development of other methods for stereoselective preparation of tubuvaline compounds of (*R,R*)-Formula **1a** or (*R,R*)-Formula **2** tubuvaline compounds obtained therefrom by hydrolysis.

[0020] 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.

[0021] Still other principle examples provide for Drug Linker compositions, wherein the compounds of the composition have a quaternized tubulysin Drug Unit, that are prepared from the tubuvaline compositions and further provide for Ligand Drug Conjugate compositions derived therefrom.

[0022] 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

[0023] General

[0024] 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. Specifically, the present invention provides tubuvaline derivatives that are readily generated using a transition metal (II)-catalyzed Michael addition that introduces a suitably protected secondary amine into a tubuvaline precursor without the need for an inert atmosphere and difficult to control reaction conditions, 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.

[0025] 1. Definitions

[0026] 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.

[0027] 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 that “comprise” one or more specified components, elements or steps. Invention embodiments also specifically include those compounds, compositions, compositions or methods that are, or that consist of, or that consist essentially of those specified components, elements or steps. For example, disclosed compositions, devices, articles of manufacture or methods that "comprise" a component or step are open and they include or read on those compositions or methods plus an additional component(s) or step(s). However, those terms do not encompass unrecited elements that would destroy the functionality of the disclosed compositions, devices, articles of manufacture or methods