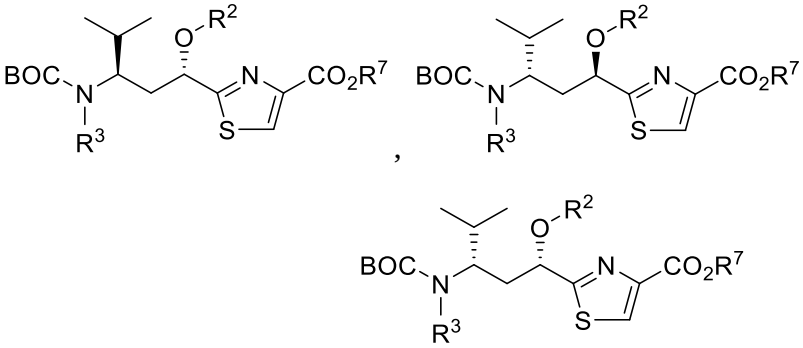
[0007] The methods of synthesis of tubulysin compounds having substitution of the amide nitrogen atom of the tubuvaline component reported in the literature to date involve multiple steps that are not easily scalable. Therefore, a need exists in the art for improved processes in producing N-substituted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



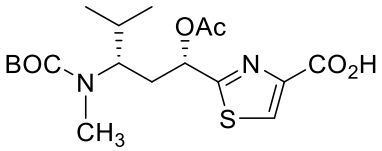
or a salt thereof, wherein

R^2 is H or -CH_2CH_3;

R^3 is–CH_3 or –$CH_2CH_2CH_3$; and

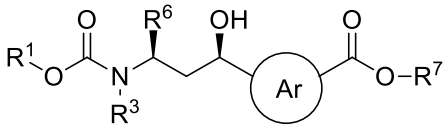
R^7 is –H or -CH_2CH_3.

In yet another aspect, the present disclosure refers to a compound having the structure of:



.

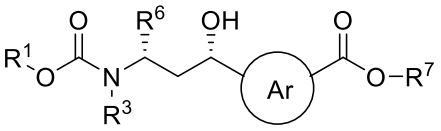
[0008a] In another aspect, the present disclosure refers to a composition comprising a tubuvaline compound of (*R,R*)-Formula **1a**, or a salt thereof, having the structure of



(*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**, or a salt thereof, 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;

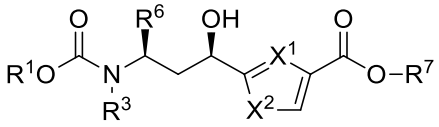
R^1 is phenyl, t-butyl, 9-fluorenyl or allyl, optionally substituted, or other moiety so that R^1-OC(=O)- is a suitable nitrogen protecting group;

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;

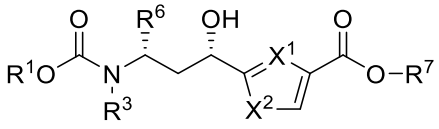
R^6 is optionally substituted saturated C_1-C_8 alkyl or unsaturated C_3-C_8 alkyl; and

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

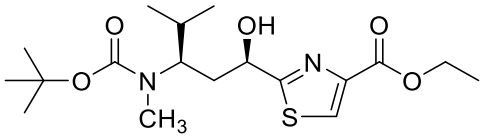
X^1 is =C(R^{X1})-; and

X^2 is NR^{X2},

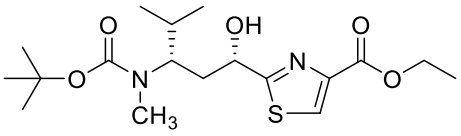
R^{X1} and R^{X2} are independently selected from the group consisting of -H, -CH_3 or –CH_2CH_3; and

wherein the remaining variable group are as previously defined,

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

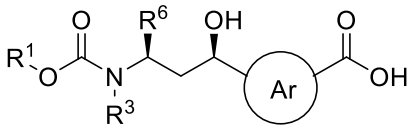


and (*S*,*S*)-Formula **1a** has the structure of:



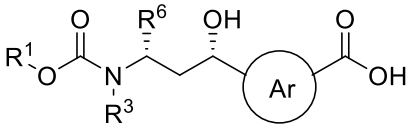
.

[0008b] In yet another aspect, the present disclosure refers to a composition comprising a tubuvaline compound of (*R*,*R*)-Formula **2**, or a salt thereof, 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**, or a salt thereof, is the major optical impurity, which has the structure of:



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

wherein:

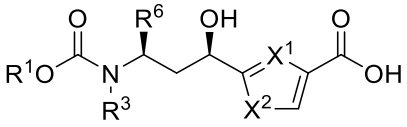
the circled Ar is a 1,3-phenylene or a 5- or 6-membered nitrogen-containing 1,3-heteroarylene, optionally substituted at the remaining positions;

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

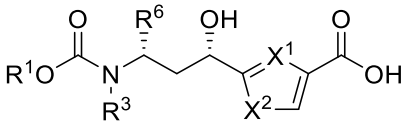
R^3 is optionally substituted saturated C_1-C_8 alkyl, optionally substituted unsaturated C_3-C_8 alkyl or optionally substituted C_3-C_8 heteroalkyl; and

R^6 is optionally substituted saturated C_1-C_8 alkyl or unsaturated C_3-C_8 alkyl,

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

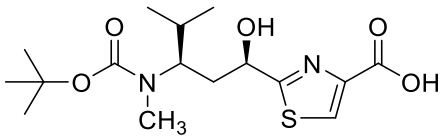
X^1 is =C(R^{X1})-; and

X^2 is NR^{X2},

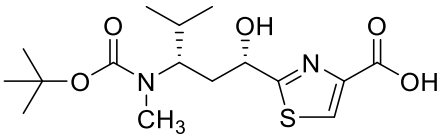
wherein R^{X1} and R^{X2} are independently selected from the group consisting of -H, -CH_3 or –CH_2CH_3;

and the remaining variable groups are as previously defined,

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

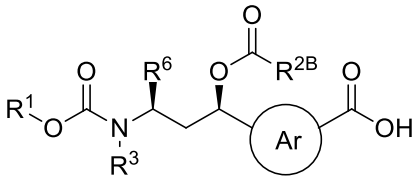


or a salt thereof, and (*S*,*S*)-Formula 2, or a salt thereof, has the structure of:



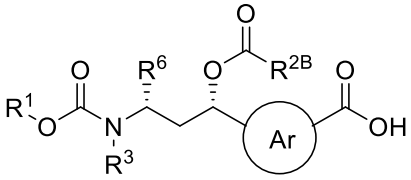
.

[0008c] In yet another aspect, the present disclosure refers to a composition comprising a tubuvaline compound of (*R*,*R*)-Formula **2a**, or a salt thereof, 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**, or a salt thereof, 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;

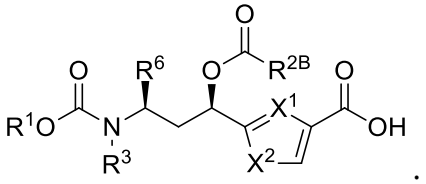
R^1 is phenyl, t-butyl, 9-fluorenyl or allyl, optionally substituted, or other moiety so that R^1-OC(=O)- is a suitable nitrogen protecting group;

wherein R_{2B} is optionally substituted saturated C_1-C_4 alkyl or optionally substituted C_3-C_6 unsaturated alkyl;

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; and

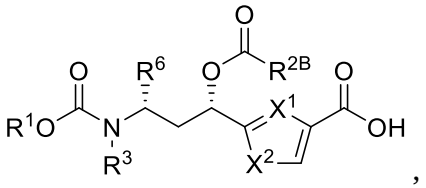
R^6 is optionally substituted saturated C_1-C_8 alkyl or unsaturated C_3-C_8 alkyl,

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

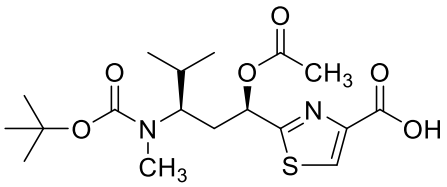
X^2 is S, O, or N(R^{X2})-, or

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

X^2 is NR^{X2},

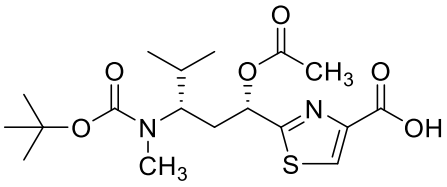
wherein R^{X1} and R^{X2} are independently selected from the group consisting of -H, -CH_3 or –CH_2CH_3,

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



.

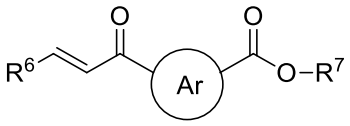
or salt thereof, and (*S*,*S*)-Formula **2a**, or a salt thereof, has the structure of:



.

[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:**



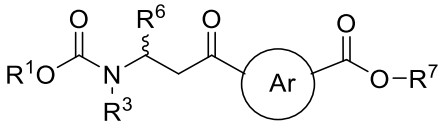
(**A**),

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 moiety so that R^7-O- provides for a suitable carboxylic acid protecting group,

with a compound of Formula **B:**

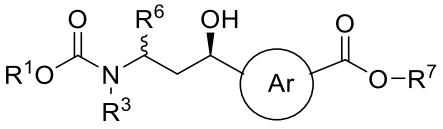
$$R^3NHC(O)OR^1 \text{ (}\mathbf{B}\text{)},$$

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



(**AB)**,

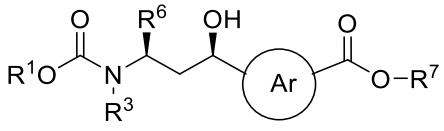
(b) contacting the enantiomeric mixture with a suitable chiral reducing agent so as to form a composition comprising an equimolar mixture of diastereomers, wherein the diastereomeric mixture is represented by Formula *R*-**1a**,



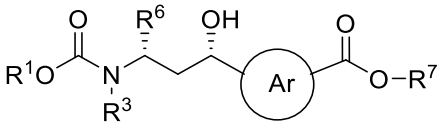
(*R*-**1a**)

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

(b') separating the diastereomers from the composition of the Formula *R*-**1a** diastereomeric mixture so that the composition, which comprises (*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, or a salt of either or both of the foregoing, have the structures of:



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

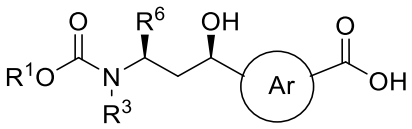


(*S,S*-**1a**)

respectively.

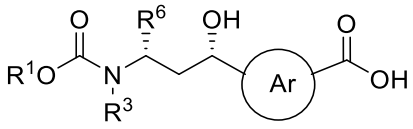
[0008e] In yet another aspect, the present discosure refers to a method for preparing a composition as described herein, the method comprising the steps of:

(c) contacting the composition obtained from steps (a), (b) and step (b') of the method described herein with a suitable hydrolysis agent, wherein the predominate optical isomer of the composition so obtained is (*R,R*)-Formula **2**, or a salt thereofm, having the structure of:



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

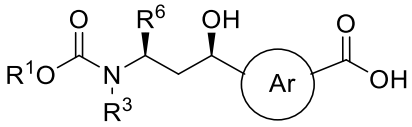
and the major optical impurity is (*S*,*S*)-Formula **2**, or a salt thereof, having the structure of:



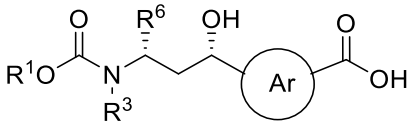
(*S*,*S*-**2**).

[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 the method described hereinwith an suitable hydrolysis agent to obtain a composition comprising (*R*,*R*)-Formula **2** as the predominate optical isomer, or a salt thereof, which has the structure of:

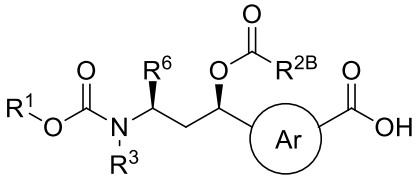


and further comprising its enantiomer, or a salt thereof, as the major optical impurity, which has the structure of:

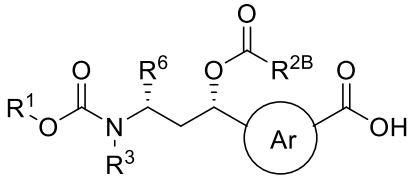


and

step (d): contacting the composition so obtained with a suitable acylating agent to obtain a composition comprising (*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 major optical impurity, or a salt thereof, have the structures of:



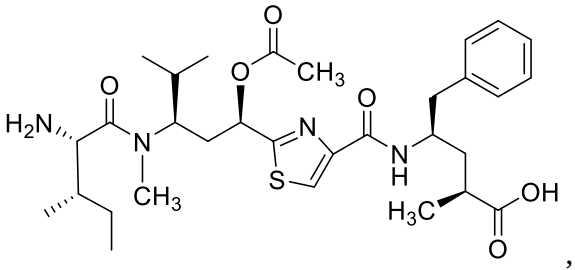
(*R*,*R*-**2a**),



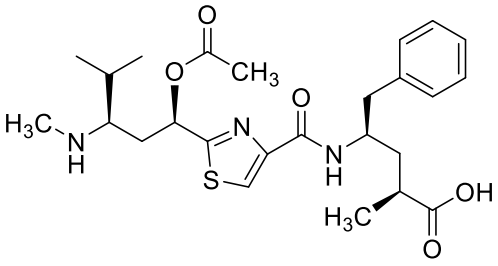
(*S,S*-**2a**)

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.

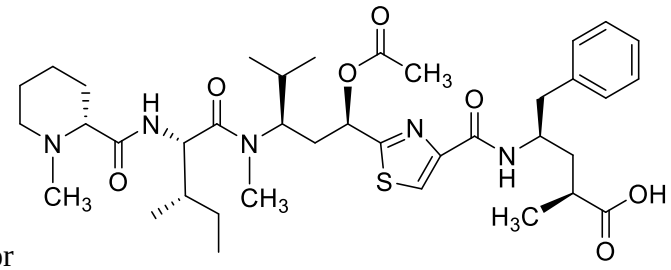
[0008g] In still another aspect, the present disclosure refers to a composition comprising a compound, or a salt thereof, having the structure of:



,

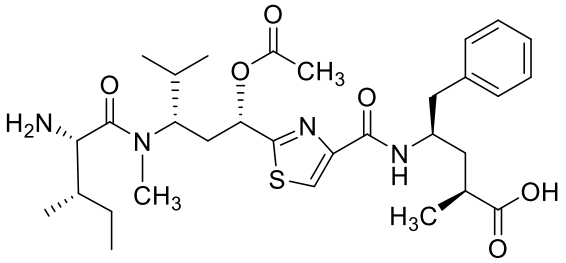


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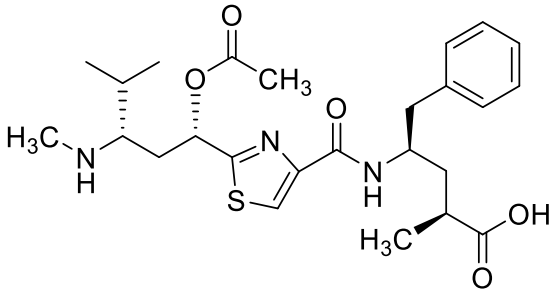


or

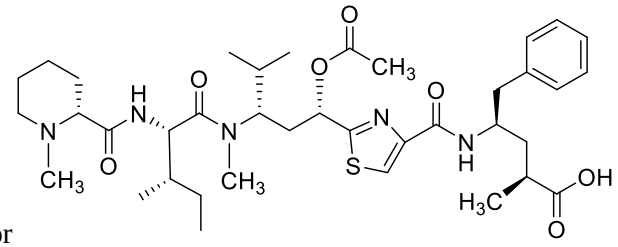
as the predominate optical isomer, and a corresponding optical impurity, or a salt thereof, having the structure of:



,

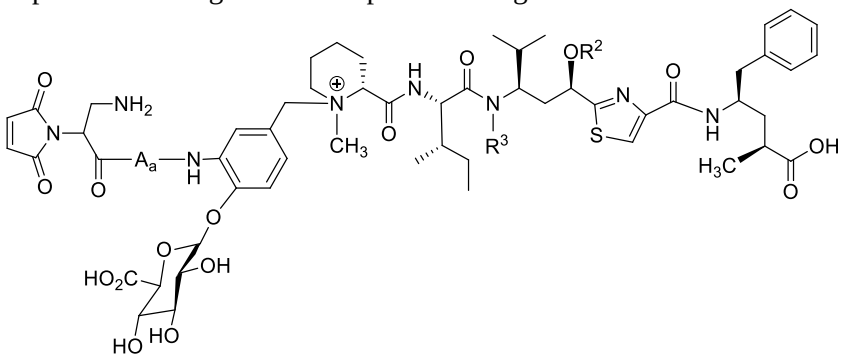


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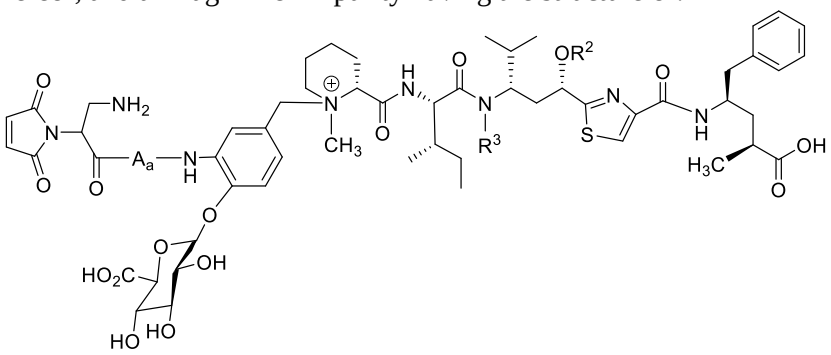


or .

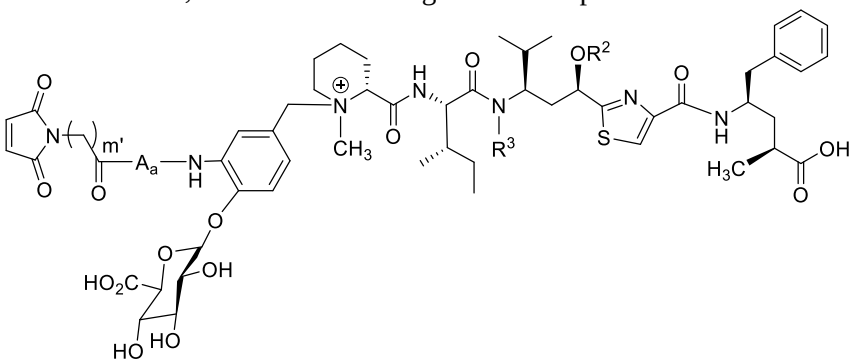
[0008h]In yet another aspect, the present disclosure refers to a Drug Linker composition, wherein the composition is comprised of a Drug Linker compound having the structure of:



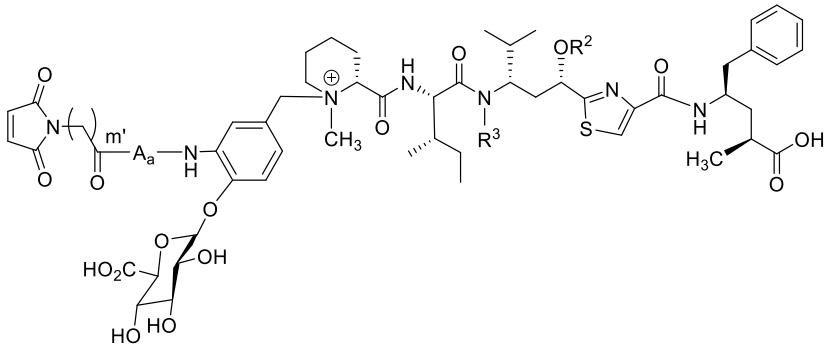
or a salt thereof, and a Drug linker impurity having the structure of:



or a salt thereof, or wherein the Drug Linker compound has the structure of:



or a salt thereof, and the Drug linker impurity has the structure of:



or a salt thereof, wherein the Drug Linker impurity is no more than 10% w/w of the composition relative to the Drug Linker compound, wherein A is a Stretcher Unit, 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 $–CH_2CH_2CH_3$.

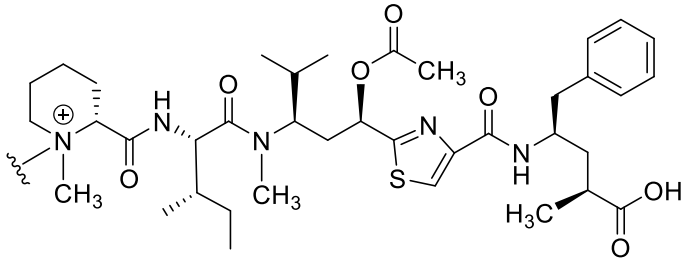
[0008i] In still another aspect, the present disclosure refers to a Ligand Drug Conjugate composition having the structure of Formula **1**:

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

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,

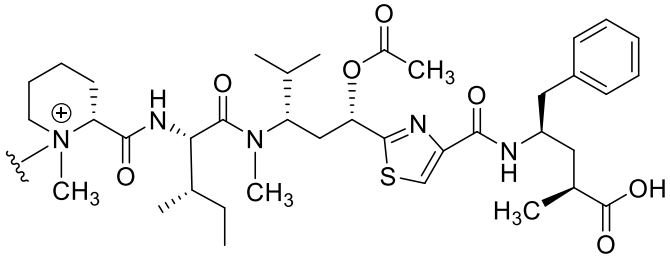
wherein a plurality of Ligand Drug Conjugate compounds of the composition have identical quaternized tubulysin Drug Units having the structure of (*R*,*R*)-Formula D^+:



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

or a salt thereof, wherein the wavy line indicate the site of covalent attachment to LU.

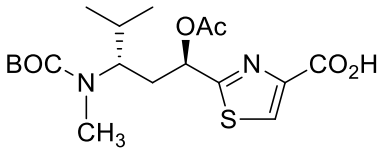
and wherein at least one compound of the composition has at least one quaternized tubulysin Drug Unit having the structure of (*S*,*S*)-Formula D^+:



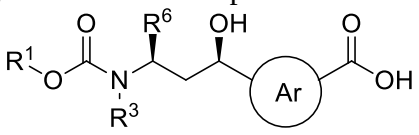
(S,S-D^+)

with the remainder having identical (R,R)-Formula D^+ structures, wherein no more than 10% w/w of the tubulysin drug units of the composition have the structure of (S,S)-Formula D^+.

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



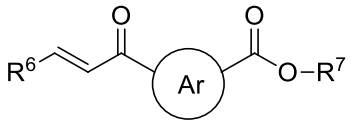
.A principal example of the disclosure provides for a method for preparing a tubuvaline compound of Formula **2**:



(**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,

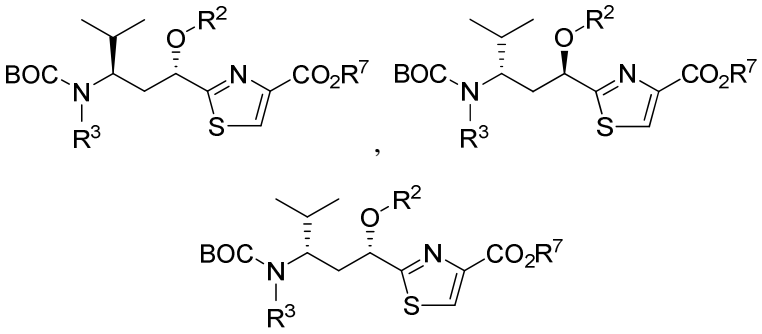
[0010] the method comprising the steps of: (a) contacting a tubuvaline intermediate of Formula **A:**



(**A**),

WHAT IS CLAIMED IS:

1. A compound having the structure of:



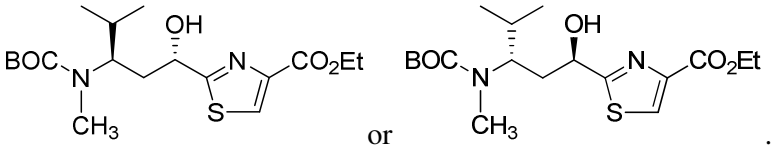
or a salt thereof, wherein

R^2 is H or -CH_2CH_3;

R^3 is–CH_3 or –$CH_2CH_2CH_3$; and

R^7 is –H or -CH_2CH_3.

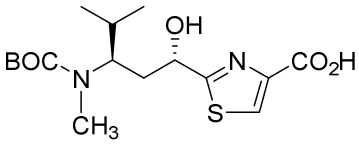
2. The compound of claim **1**, wherein the compound has the structure of:



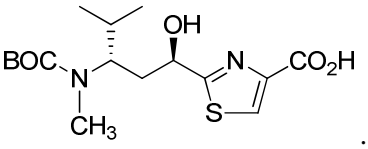
or

.

3. The compound of claim **1**, wherein the compound has the structure of:

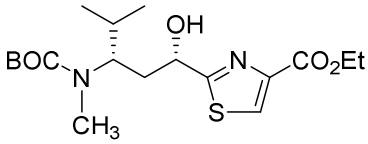


or

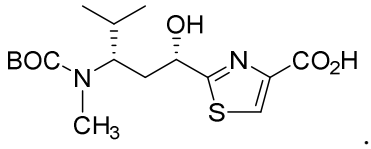


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4. The compound of claim **1**, wherein the compound has the structure of:

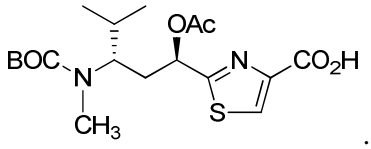


or



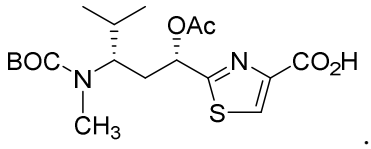
.

5. A compound having the structure of:



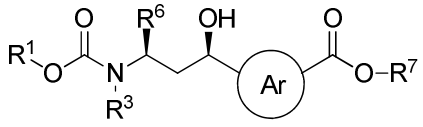
.

6. A compound having the structure of:



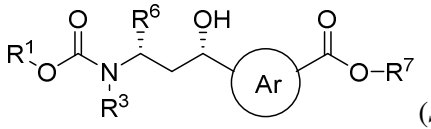
.

7. A composition comprising a tubuvaline compound of (*R,R*)-Formula **1a**, or a salt thereof, 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**, or a salt thereof, 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;

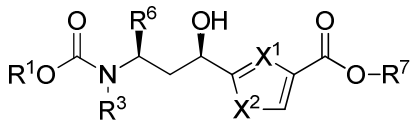
R^3 is optionally substituted saturated C_1-C_8 alkyl, optionally substituted unsaturated C_3-C_8 alkyl or optionally substituted C_3-C_8 heteroalkyl;

R^6 is optionally substituted saturated C_1-C_8 alkyl or unsaturated C_3-C_8 alkyl; and

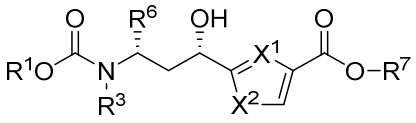
R^7 is optionally substituted saturated C_1-C_{20} alkyl, optionally substituted unsaturated C_3-C_{20} alkyl, optionally substituted C_3-C_{20} heteroalkyl, optionally substituted C_2-C_{20} alkenyl, optionally substituted C_3-C_{20} heteroalkenyl, optionally substituted C_2-C_{20} alkynyl, optionally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

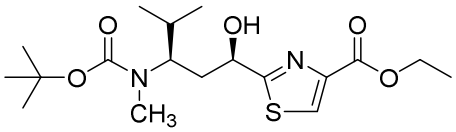
X^1 is $=C(R^{X1})$-; and

X^2 is NR^{X2},

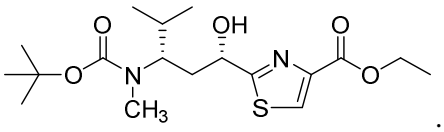
R^{X1} and R^{X2} are independently selected from the group consisting of -H, $-CH_3$ or $–CH_2CH_3$; and

wherein the remaining variable group are as previously defined,

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



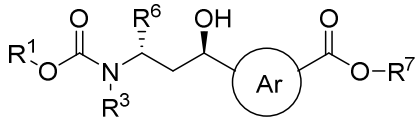
and (*S,S*)-Formula **1a** has the structure of:



.

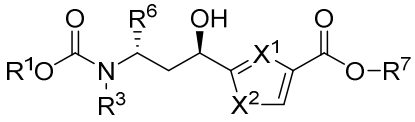
8. The composition of claim **7**, wherein the tubuvaline composition comprising the (*R,R*)-Formula **1a** tubuvaline compound, or salt thereof, as the predominate optical isomer has no more than a 5% w/w of the diastereomeric tubuvaline compound, (*R,S*)-Formula **1a**, relative to the total amount of optical isomers present in the composition, in particular, no more than 1.5 % w/w, more particularly, or no more than 1.0 % w/w, as

determined by chiral HPLC, wherein the diastereomeric tubuvaline compound, or a salt thereof, has the structure of:



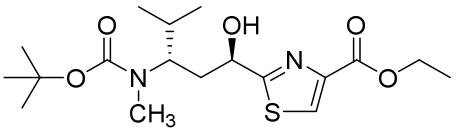
(*R,S*-**1a**)

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



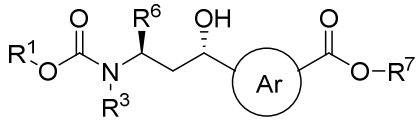
wherein the variable groups remain as previously defined for (*R,R*)-Formula **1a** and (*S,S*)-Formula **1a**,

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



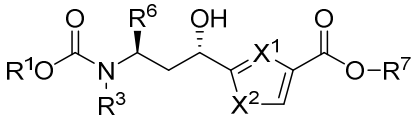
.

9. The composition of claim **8**, wherein the composition has no more than 1.0% combined w/w of the diastereomer (*R,S*)-Formula **1a** and its corresponding enantiomer, (*S,R*)-Formula **1a**, as determined by chiral HPLC, wherein (*S,R*)-Formula **1a** has the structure of:



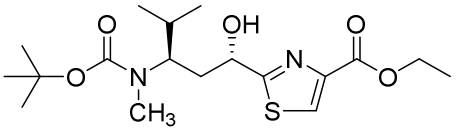
(*S,R*-**1a**)

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



wherein the variable groups remain as previously defined for (*R,R*)-Formula **1a**, (*S,S*)-Formula **1a** and (*R,S*)-Formula **1a**,

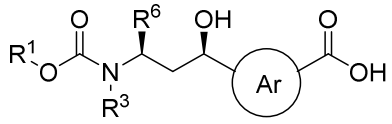
more particularly, (*S,R*)-Formula **1a** has the structure of:



.

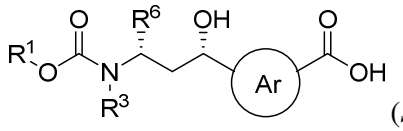
10. The composition of any one of claims **7** to **9**, wherein the composition contains no more than 3% w/w, in particular no more than 2% w/w or no more than 1.5% w/w of the optical impurity of (*S,S*)-Formula **1a**, as determined by chiral HPLC, relative to the total amount of optical isomers present in the composition.

11. A composition comprising a tubuvaline compound of (*R,R*)-Formula **2**, or a salt thereof, 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**, or a salt thereof, is the major optical impurity, which has the structure of:



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

wherein:

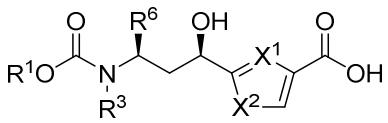
the circled Ar is a 1,3-phenylene or a 5- or 6-membered nitrogen-containing 1,3-heteroarylene, optionally substituted at the remaining positions;

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

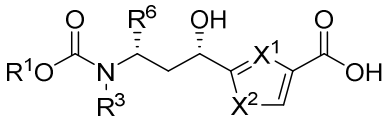
R^3 is optionally substituted saturated C_1-C_8 alkyl, optionally substituted unsaturated C_3-C_8 alkyl or optionally substituted C_3-C_8 heteroalkyl; and

R^6 is optionally substituted saturated C_1-C_8 alkyl or unsaturated C_3-C_8 alkyl,

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

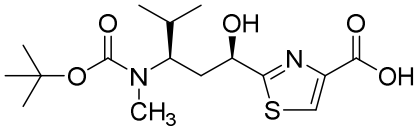
X^1 is =C(R^{X1})-; and

X^2 is NR^{X2},

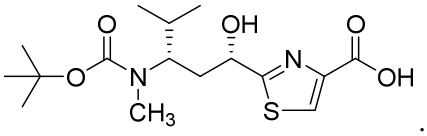
wherein R^{X1} and R^{X2} are independently selected from the group consisting of -H, -CH_3 or –CH_2CH_3;

and the remaining variable groups are as previously defined,

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

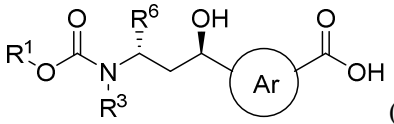


or a salt thereof, and (*S,S*)-Formula 2, or salt thereof, has the structure of:



.

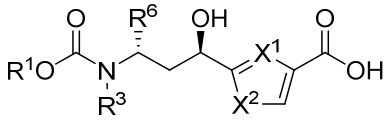
12. The composition of claim **11**, wherein the tubuvaline composition comprising the (*R,R*)-Formula **2** tubuvaline compound, or salt thereof, as the predominate optical isomer has no more than 5% w/w of the diastereomeric tubuvaline compound, (*R,S*)-Formula **2**, relative to the total amount of optical isomers present in the composition, in particular, no more than 1.5% w/w, more particularly, or no more than 1.0% w/w, as determined by chiral HPLC, wherein the diastereomeric tubuvaline compound, or salt thereof, has the structure of (*R,S*)-Formula **2**:



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

wherein the variable groups remain as previously defined,

in particular, wherein (*R,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

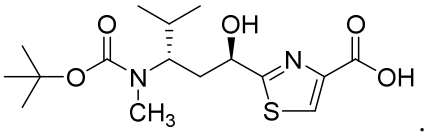
X^1 is $=C(R^{X1})$-; and

X^2 is NR^{X2},

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

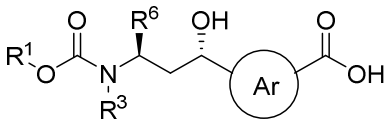
wherein the remaining variable groups are as previously defined,

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



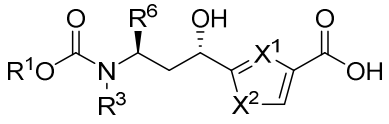
.

13. The composition of claim **12**, wherein the composition has no more than 1.0% combined w/w of the diastereomer, (*R,S*)-Formula **2**, or salt thereof, and its corresponding enantiomer, (*S,R*)-Formula **2**, as determined by chiral HPLC, wherein (*S*,*R*)-Formula **2** has the structure of:



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

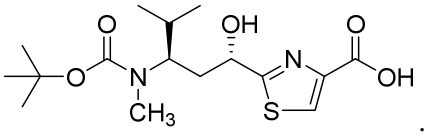
or salt thereof, in particular, wherein (*S,R*)-Formula **2** has the structure of:



;

or salt thereof, wherein the variable groups are as previously defined for (*R,R*)-Formula **1a**, (*R,S*)-Formula **1a**, and (*S,S*)-Formula **1a**,

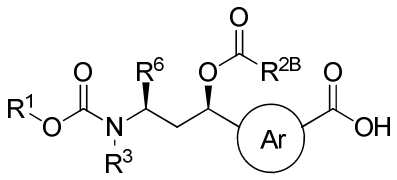
more particularly, wherein (*S,R*)-Formula **2**, or a salt thereof, has the structure of:



.

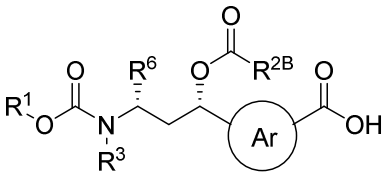
14. The composition of any one of claims **11** to **13**, wherein the composition contains no more than about 3% w/w, in particular no more than about 2% w/w or no more than about 1.5% w/w of the optical impurity of (*S,S*)-Formula **2**, as determined by chiral HPLC, relative to the total amount of optical isomers present in the composition.

15. A composition comprising a tubuvaline compound of (*R,R*)-Formula **2a**, or a salt thereof, 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**, or a salt thereof, 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;

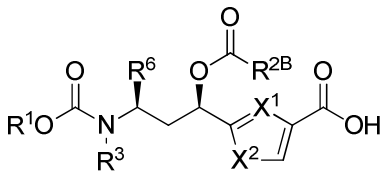
R^1 is phenyl, t-butyl, 9-fluorenyl or allyl, optionally substituted, or other moiety so that R^1-OC(=O)- is a suitable nitrogen protecting group;

wherein R^{2B} is optionally substituted saturated C_1-C_4 alkyl or optionally substituted C_3-C_6 unsaturated alkyl;

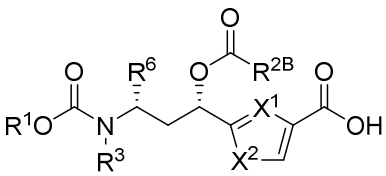
R^3 is optionally substituted saturated C_1-C_8 alkyl, optionally substituted unsaturated C_3-C_8 alkyl or optionally substituted C_3-C_8 heteroalkyl; and

R^6 is optionally substituted saturated C_1-C_8 alkyl or unsaturated C_3-C_8 alkyl,

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

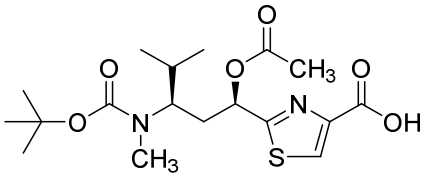
X^2 is S, O, or N(R^{X2})-, or

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

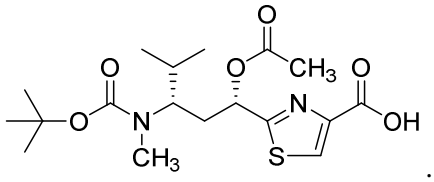
X^2 is NR^{X2},

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

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

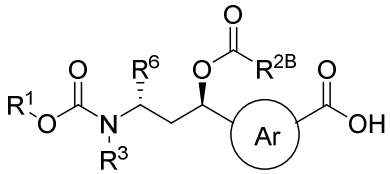


or salt thereof, and (*S,S*)-Formula **2a**, or a salt thereof, has the structure of:



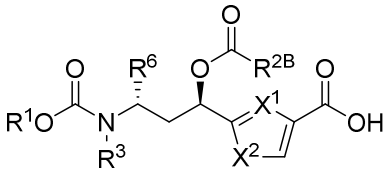
.

16. The composition of claim **15**, wherein the tubuvaline composition comprising the (*R,R*)-Formula **2a** tubuvaline compound, or salt thereof, as the predominate optical isomer has no more than 5% w/w of the diastereomeric tubuvaline compound, (*R,S*)-Formula **2a**, relative to the total amount of optical isomers present in the composition, in particular, no more than 1.5% w/w, more particularly, or no more than 1.0% w/w, as determined by chiral HPLC, wherein the diastereomeric tubuvaline compound, or a salt thereof, has the structure of (*R,S*)-Formula **2a**:



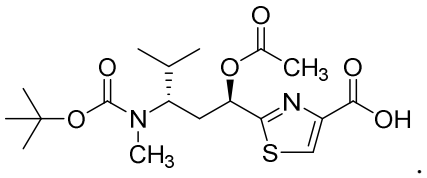
(*R,S*-**2a**),

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



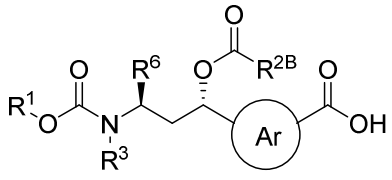
or salt thereof, wherein the variable groups remain as previously defined for (*R,R*)-Formula **2a** and (*S,S*)-Formula **2a**,

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



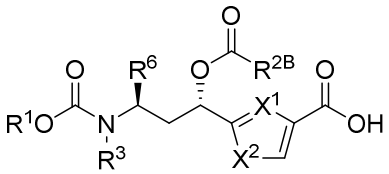
.

17. The composition of claim **16**, wherein the composition has no more than 1.0% combined w/w of the diastereomer, (*R,S*)-Formula **2a**, and its corresponding enantiomer, (*S,R*)-Formula **2a**, as determined by chiral HPLC, wherein (*S,R*)-Formula **2a** has the structure of:



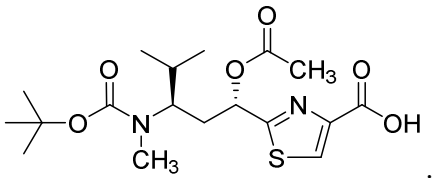
(*S,R*-**2a**)

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



or salt thereof, wherein the variable groups remain as previously defined for (*R,R*)-Formula **2a**, (*S,S*)-Formula **2a** and (*R,S*)-Formula **2a**,

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

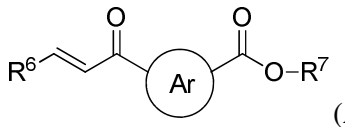


.

18. The composition of any one of claims **15** to **17**, wherein the composition contains no more than 3% w/w, in particular no more than 2% w/w or no more than 1.5% w/w of the optical impurity of (*S,S*)-Formula **2a**, as determined by chiral HPLC, relative to the total amount of optical isomers present in the composition.

19. A method for preparing a composition of any one of claims **7** to **18**, the method comprising the steps of:

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



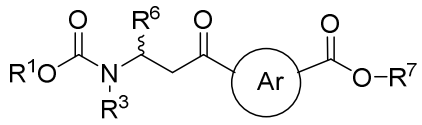
(**A**)

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 moiety so that R^7-O- provides for a suitable carboxylic acid protecting group,

with a compound of Formula **B**:

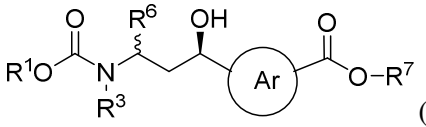
$R^3NHC(O)OR^1$ (**B**)

in a suitable polar, aprotic solvent in the presence of a suitable transition metal (II) or transition metal (III) catalyst comprising a transition metal selected from the group consisting of Cu(II), Zn(II) and Yb(III), so as to form a composition comprising 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 comprising an equimolar mixture of diastereomers, wherein the diastereomeric mixture is represented by Formula *R*-**1a**,

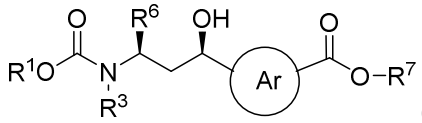


(*R*-**1a**)

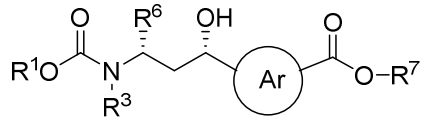
wherein the composition further comprises an equimolar mixture of optical impurities that are enantiomers of the diastereomers, and

(b') separating the diastereomers from the composition of the Formula *R*-**1a** diastereomeric mixture so that the composition, which comprises (*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, or a salt of either or both of the foregoing, have the structures of:



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



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

respectively.

20. The method of claim **19**, wherein the suitable polar, aprotic solvent is dichloromethane.

21. The method of claim **20**, wherein the transition metal (II) catalyst or the transition metal (III) catalyst is $Cu(OTf)_2$, $Zn(OTf)_2$, or $Yb(OTf)_3$.

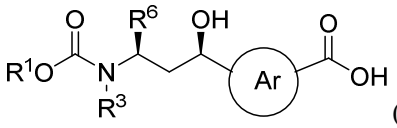
22. The method of claim **19**, wherein the transition metal (II) catalyst comprises Cu(II), in particular $Cu(OTf)_2$, $Cu(SbF_6)_2$, or $CuCl_2$, more particularly $Cu(OTf)_2$.

23. The method of claim **19** or **22**, wherein the suitable polar, aprotic solvent is acetonitrile, dichloromethane, THF, dioxane, or a mixture of two or three of these solvents, in particular, dichloromethane.

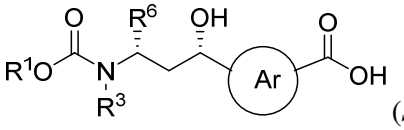
24. The method of any one of claims **19** to **23**, wherein the chiral reducing agent is a chiral oxazaborolidine prepared from contacting BH_3-DMS in THF with a suitable chiral ligand, in particular (S)-(-)-CBS.

25. A method for preparing a composition of any one of claims **11** to **14**, the method comprising the steps of:

(c) contacting the composition obtained from steps (a), (b) and step (b') of any one of claims **19** to **24** with a suitable hydrolysis agent, wherein the predominate optical isomer of the composition so obtained is (*R,R*)-Formula **2**, or a salt thereof, having the structure of:

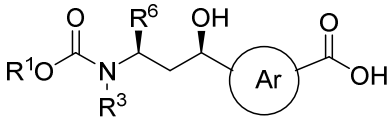

(*R,R*-**2**)

and the major optical impurity is (*S,S*)-Formula **2**, or a salt thereof, having the structure of:

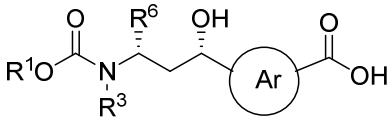

(*S,S*-**2**).

26. A method for preparing a composition of any one of claims **15** to **18**, the method comprising the steps of:

(c) contacting the composition obtained from steps (a), (b) and step (b') of any one of claims **19** to **24** with an suitable hydrolysis agent to obtain a composition comprising (*R,R*)-Formula **2** as the predominate optical isomer, or a salt thereof, which has the structure of:

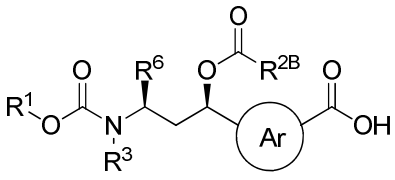


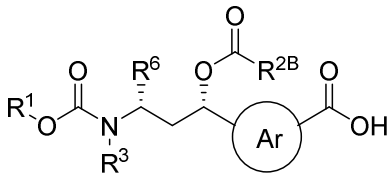
and further comprising its enantiomer, or a salt thereof, as the major optical impurity, which has the structure of:



and

step (d): contacting the composition so obtained with a suitable acylating agent to obtain a composition comprising (*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 major optical impurity, or a salt thereof, have the structures of:


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



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

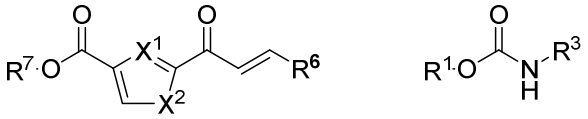
wherein R^{2B} is saturated C_1-C_6 alkyl, unsaturated C_3-C_8 alkyl, C_2-C_8 alkenyl or C_2-C_4 alkynyl, optionally substituted, in particular -CH_3, -CH_2CH_3, -$CH_2CH_2CH_3$, -$CH_2CH{=}CH_2$, -$CH_2CH(CH_3)_2$, -$CH_2C(CH_3)_3$, -$CH_2C(CH_3){=}CH_2$, -$CH{=}CH_2$ or -CHC≡CH, more particularly, –CH_3.

27. The method of any one of claims **19** to **26**, wherein the optical purity of the composition from step (b') is substantially or essentially retained by the composition obtained from step (c) and/or step (d).

28. The method of any one of claims **19** to **27**, wherein said step (b') separation is by silica gel flash chromatography.

29. The method of any one of claims **19** to **28**, wherein the circled Ar is a 5-membered nitrogen-containing 1,3-heteroarylene, optionally substituted at the remaining positions.

30. The method of any one of claims **19** to **29**, wherein compound **A** and compound **B** of step (a) have the structures of:



respectively, 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;

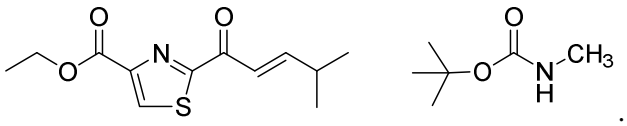
R^1 is phenyl, t-butyl, 9-fluorenyl or allyl, optionally substituted, or other moiety so that R^1-OC(=O)- is a suitable nitrogen protecting group, in particular t-butyl;

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, in particular $–CH_3$ or $–CH_2CH_2CH_3$;

R^6 is saturated C_1-C_6 alkyl, in particular $–CH(CH_3)_2$, or unsaturated C_3-C_8 alkyl; and

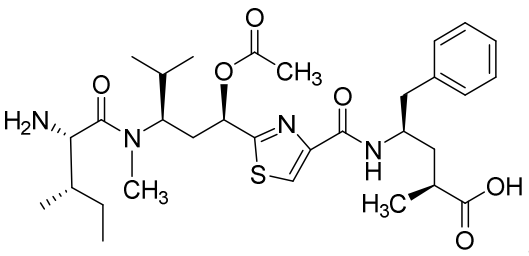
R^7 is optionally substituted saturated C_1-C_{20} alkyl, optionally substituted unsaturated C_3-C_{20} alkyl, optionally substituted C_3-C_{20} heteroalkyl, optionally substituted C_2-C_{20} alkenyl, optionally substituted C_3-C_{20} heteroalkenyl, optionally substituted C_2-C_{20} alkynyl, optionally substituted C_3-C_{20} heteroalkynyl, optionally substituted C_6-C_{24} aryl, optionally substituted C_5-C_{24} heteroaryl or optionally substituted C_3-C_{20} heterocyclyl, or other moiety so that R^7-O- provides a suitable carboxylic acid protecting group, in particular R^7 is $–CH_3$ or $–CH_2CH_3$.

in particular, compound **A** and compound **B** have the structures of:

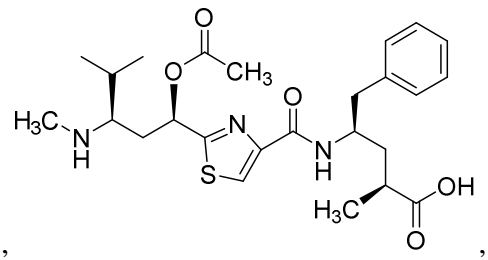


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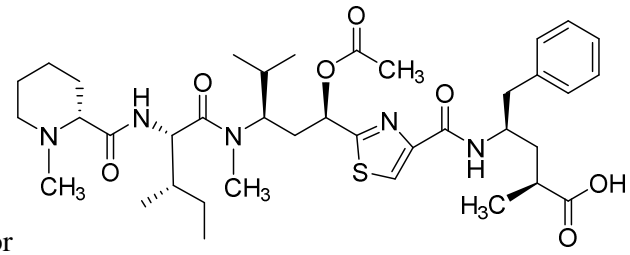
31. A composition comprising a compound, or a salt thereof, having the structure of:



,

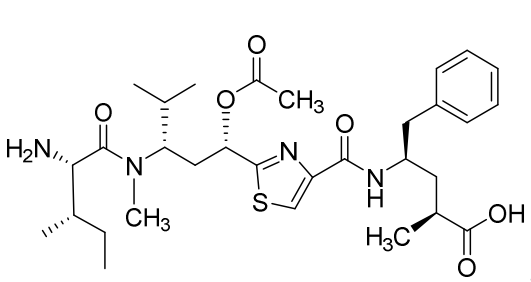
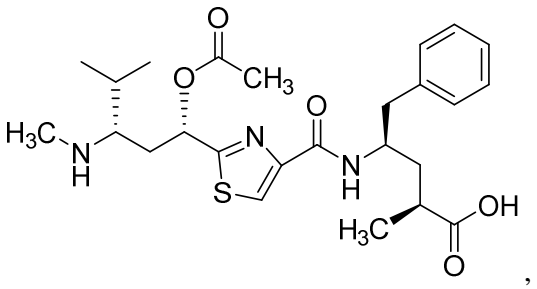


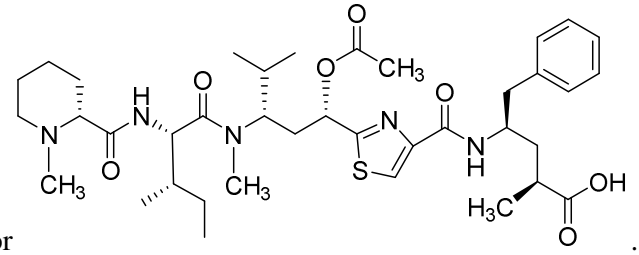
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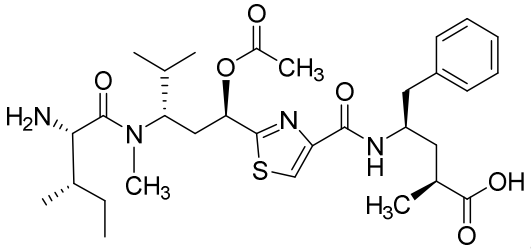
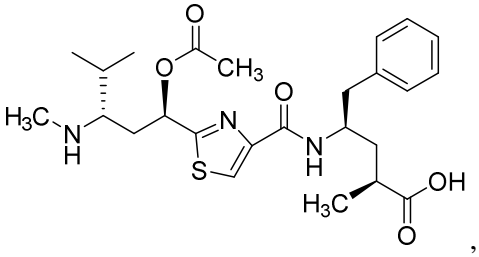
or

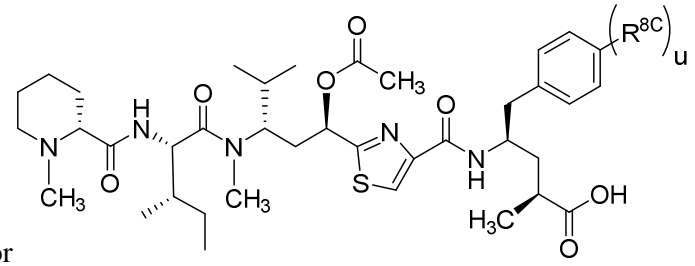
as the predominate optical isomer, and a corresponding optical impurity, or a salt thereof, having the structure of:


,

,

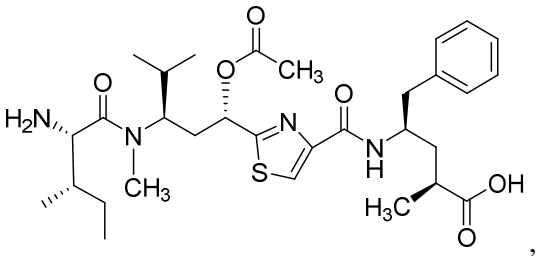
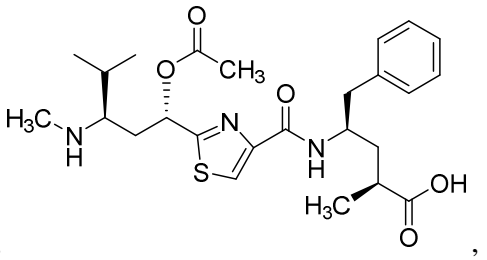

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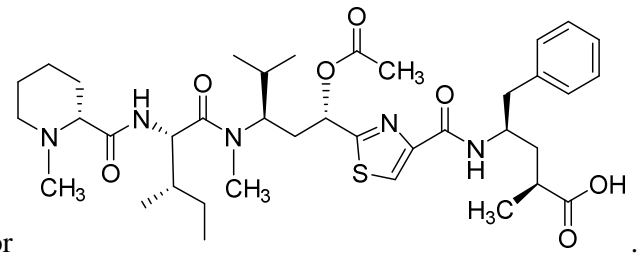
32. The composition of claim **31**, wherein the composition is free of the corresponding optical impurity of:


,

,


or

and is free of the corresponding optical impurity having the structure of:

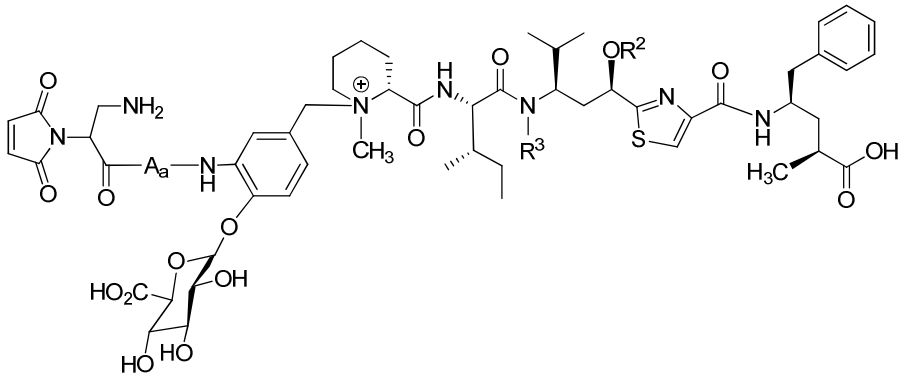

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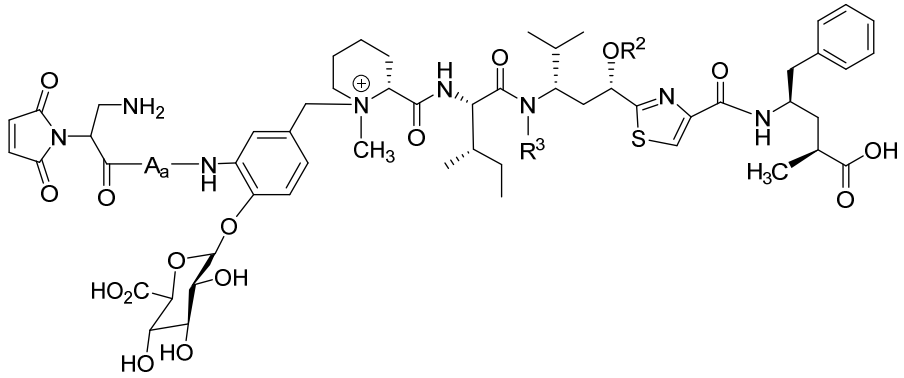
33. The composition of claim **32**, wherein the composition is prepared from a composition of claim **17**,

wherein the composition of claim **17** contains no more than 3% w/w, in particular no more than 2% w/w or no more than 1.5% w/w of the optical impurity of (*S,S*)-Formula **2a**, as determined by chiral HPLC, relative to the total amount of optical isomers present in the composition.

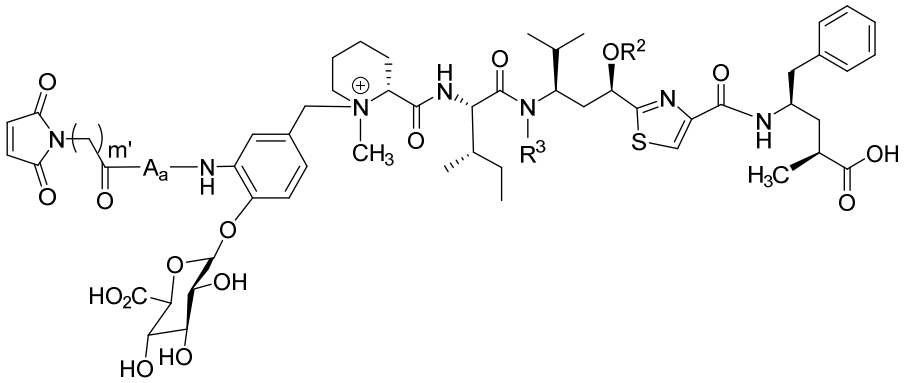
34. A Drug Linker composition, wherein the composition comprises a Drug Linker compound having the structure of:



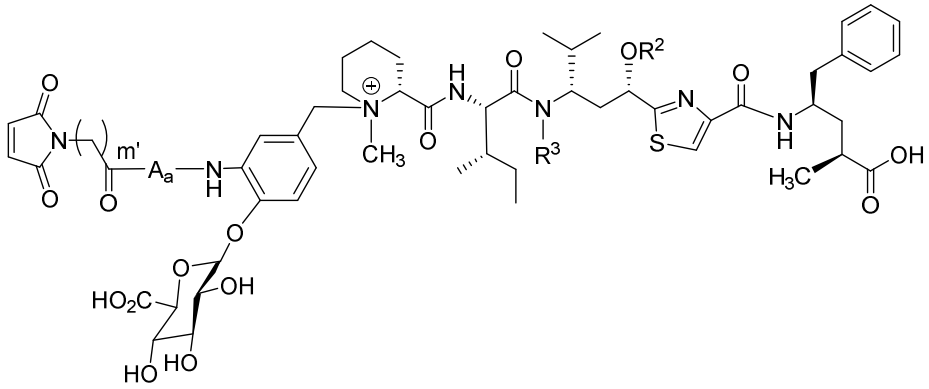
or a salt thereof, and a Drug linker impurity having the structure of:



or a salt thereof, or wherein the Drug Linker compound has the structure of:



or a salt thereof, and the Drug linker impurity has the structure of:



or a salt thereof, wherein the Drug Linker impurity is no more than 10% w/w of the composition relative to the Drug Linker compound, wherein A is a Stretcher Unit, 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 $–CH_2CH_2CH_3$.

35. The Drug Linker composition of claim **34**, wherein R^2 is R^{2A}, wherein R^{2A} is $–C(=O)CH_3$ and R^3 is $–CH_3$, and wherein the composition is prepared from a composition of any one of claims **31** to **33**.

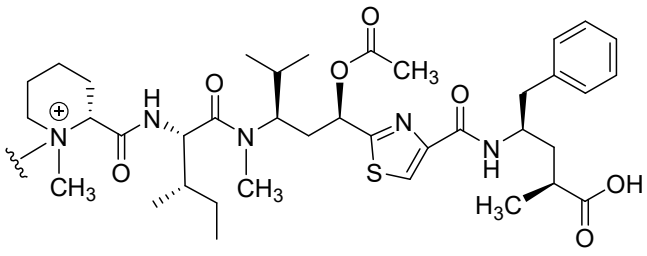
36. A Ligand Drug Conjugate composition having the structure of Formula **1**:

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

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,

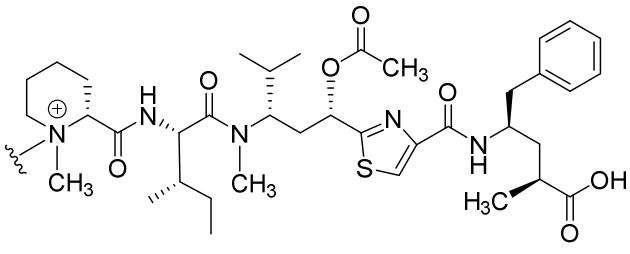
wherein a plurality of Ligand Drug Conjugate compounds of the composition have identical quaternized tubulysin Drug Units having the structure of (*R,R*)-Formula D^+:



(*R,R*-D^+)

or a salt thereof, 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, wherein no more than 10% w/w of the tubulysin drug units of the composition have the structure of (*S*,*S*)-Formula D^+.

37. The Ligand Drug Conjugate composition of claim **36**, wherein the Ligand Drug Conjugate composition is prepared from a composition of any one of claims **31** to **33**.

38. The Ligand Drug Conjugate composition of claim **36** or **37**, wherein the Ligand Unit is an antibody Ligand Unit directed to a cancer cell antigen, in particular CD30, CD33 or CD70.