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(54) **Title:** EFFICIENT PREPARATION OF DOLASTATIN AND AURISTATIN ANALOGS THROUGH A COMMON INTERMEDIATE

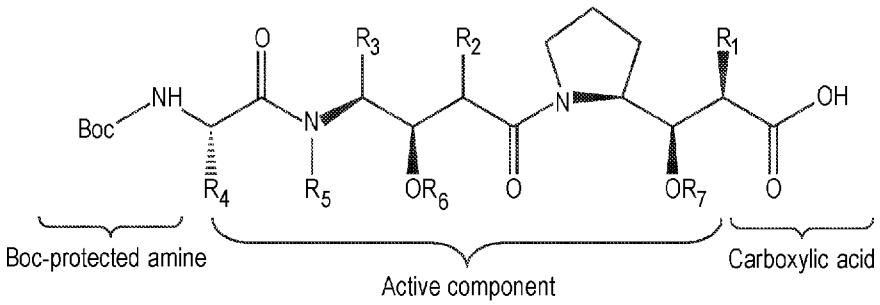

FIG. 1

(57) **Abstract:** Methods for making a dolastatin, auristatin or related compounds comprising the steps of providing a universal dolastatin core of Formula (I) reacting the C-terminal carboxylic acid group with an amine (A) to form an amide bond and reacting the N-terminal amine with a carboxylic acid (CA) to form an amide bond, wherein the steps can be performed in either order. Also provided are an isolated salt of the universal dolastatin core for use in preparation of dolastatins, auristatins and related compounds. Also provided are a number of intermediates and process steps which are useful for the preparation of high purity dolastatin core and high purity dolastatin and auristatin compounds.

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EFFICIENT PREPARATION OF DOLASTATIN AND AURISTATIN ANALOGS THROUGH A COMMON INTERMEDIATE

Cross-reference to related application

[0001] This application claims the benefit of priority of U.S. Provisional Patent Application No. 62/987,150 filed March 9, 2020, the entirety of which is incorporated herein by reference.

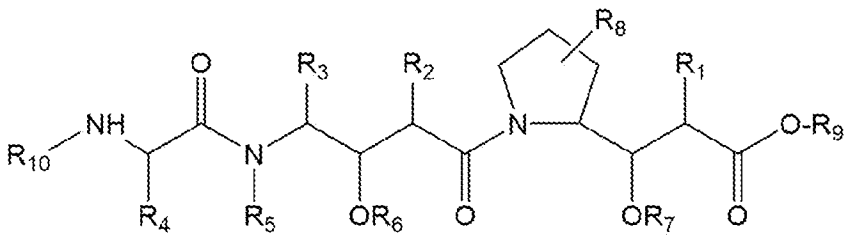
Background

[0002] Dolastatins and related Auristatins are classes of compounds recognized as important antineoplastic agents particularly when coupled to antibodies for delivery directly to cancerous cells. As with many complex natural products and derivatives, the syntheses of these molecules are generally quite intensive requiring numerous chemical steps. Today, scientists must develop each synthesis *de novo* and then require further development to enable cGMP manufacturing of dolastatin and auristatin-containing payloads for conjugates.

[0003] A need exists for new, more efficient methods of synthesis for production of these clinically important compounds and to enable development of new dolastatin, auristatins and related compounds.

Summary

[0004] Provided herein are methods for making dolastatins, auristatins or related compounds by starting with a compound of Formula I



I

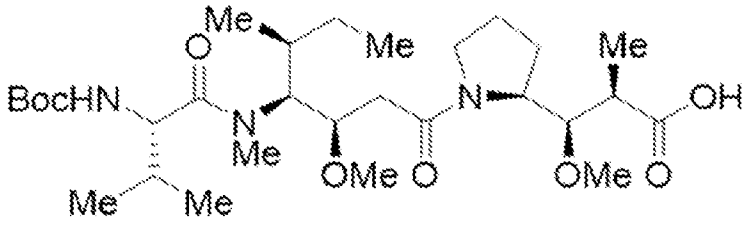
wherein R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo; R_{11} and R_{12} are individually selected from H, C_1-C_6 alkyl; R_6 and R_7 are each individually H or C_1-C_4 alkyl; R_9 is H or an acid protecting group, and R_{10} is H or an amino protecting group, by reacting the C-terminal carboxylic acid group with an amine (A) to form an amide bond and reacting the N-terminal amine with a carboxylic acid (CA) to form an amide bond. The reaction steps, i.e., the reaction of the C-terminal carboxylic acid with an amine

(A), or the reaction of the N-terminal amine with a carboxylic acid (CA), can be done in either order.

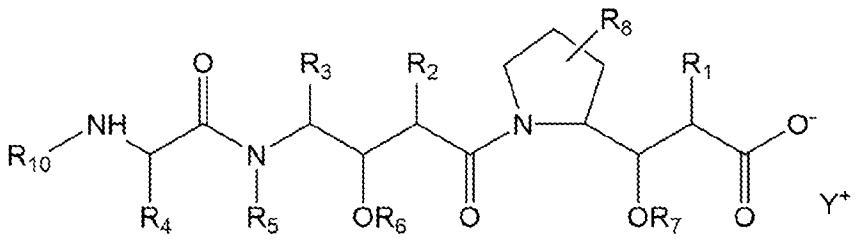
[0005] The amine (A) may be selected from alkylamines, alkanolamines, arylalkanolamines, amino acids, amino acid derivatives, peptides, and peptide derivatives. In various embodiments, the amine (A) may include one or more substituents. In some embodiments, the amine (A) includes a protecting group.

[0006] The carboxylic acid (CA) may be selected from amino acids, amino acid derivatives, peptides and peptide derivatives. In some embodiments, the carboxylic acid (CA) includes one or more substituents. In some embodiments, the carboxylic acid (CA) may have a protecting group.

[0007] In a preferred embodiment, the R groups in the compound of Formula I are selected to give the compound of Formula IA:



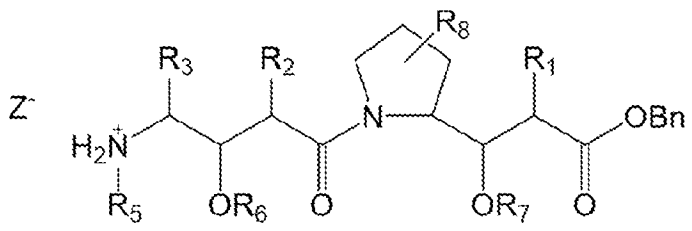
[0008] Also provided is an isolated salt of Formula II:



II

wherein R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo, R_{11} and R_{12} are individually selected from H and C_1-C_6 alkyl, R_6 and R_7 are each individually H or C_1-C_4 alkyl, R_{10} is H or an amino protecting group; and Y^+ is counterion.

[0009] Also provided is a compound of Formula III:



III

wherein R_1, R_2, R_3, R_5 and R_8 are each individually selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo, R_{11} and R_{12} are individually selected from H and C_1-C_6 alkyl, R_6 and R_7 are each individually selected from H or C_1-C_4 alkyl, and Z^- is a counterion.

[0010] Also provided are additional intermediate compounds and process steps which are useful for the preparation of high purity dolastatin core and high purity dolastatin and auristatin compounds.

Brief Description of the Drawings

[0011] FIG 1 shows the structural features of the preferred compound of Formula I.

[0012] FIG 2 shows the synthetic scheme for the preparation of Formula I.

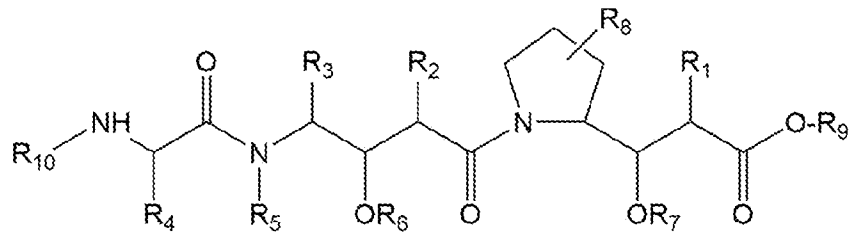
[0013] FIG 3 shows exemplary dolastatin and auristatin payloads that have entered the clinic, including (A) MMAE, (B) MMAF, (C) XMT-1505, XMT-1536, (D) Amberstatin 269, (E) Auristatin W, (F) Dolastatin 10, and (G) Pfizer Auristatin, along with the universal dolastatin core described herein, the dolastatin core is highlighted in each payload.

[0014] FIG 4 shows the synthetic schemes for the preparation of mc-Val-Cit-PAB-N-Me-Val-OH, MMAE and vcMMAE via two routes from Dolastatin Core.

Detailed Description

[0015] To overcome the deficiencies in the conventional methods of preparing auristatins and dolastatins, the inventors have identified and synthesized an advanced intermediate that can be efficiently transformed into numerous dolastatin, auristatin and related compounds. Through this identification and synthesis of a universal dolastatin core, the inventors have been able to develop a new platform that can be tailored to provide highly efficient syntheses of existing dolastatins and auristatins, such as those shown in Figure 3. Additionally, this platform may be used to develop new dolastatins, auristatins and related compounds.

[0016] Provided herein are simplified methods for making dolastatins, auristatins or related compounds using a universal dolastatin core, shown in Formula I:



I.

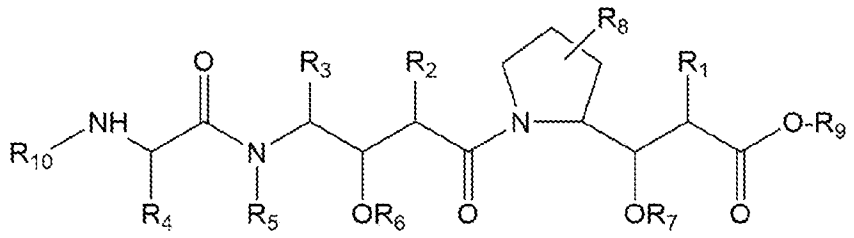
According to the methods provided herein, the C-terminal carboxylic acid is reacted with an amine (A) to form an amide bond, and the N-terminal amine is reacted with a carboxylic acid (CA) to form an amide bond, yielding a dolastatin, an auristatin or a further dolastatin or auristatin intermediate compound that can then be modified further, such as by the addition of one or more of a spacer, a linker, and an attachment group. These steps can be done in either order, that is, in some embodiments, the C-terminal carboxylic acid is first reacted with an amine (A), then the N-terminal amine is reacted with a carboxylic acid (CA). In other embodiments, the N-terminal amine is reacted with a carboxylic acid (CA) first, then C-terminal carboxylic acid is first reacted with an amine (A) to form the a dolastatin, an auristatin or a further dolastatin or auristatin intermediate of interest.

[0017] Suitable acid and amine protecting groups may be used to protect the terminal that is being reacted in a subsequent step. In embodiments in which there is a protecting group on the N-terminal amine, the protecting group can be removed by conventional methods prior to reacting with carboxylic acid (CA). When no protecting group is included, the optional deprotecting step is not necessary. Similarly, in embodiments in which there is a protecting group on the C-terminal carboxylic acid, the protecting group can be removed by conventional methods prior to reacting with amine (A). When no protecting group is included, the optional deprotecting step is not necessary.

[0018] Suitable coupling agents may be used may be used when reacting the C-terminal carboxylic acid with amine (A) and when reacting N-terminal amine with carboxylic acid (CA). Suitable coupling agents used in the methods provided herein include, but are not limited to, carbonyldiimidazole (CDI), propylphosphonic anhydride (T3P) solution and HATU. Other suitable coupling agents are known to those of ordinary skill in the art. In some embodiments, a coupling additive is used. Coupling additives are used in coupling reactions to inhibit side reaction and reduce racemization. Useful coupling additives for the reactions described herein include N-hydroxysuccinimide (HOSu), N-hydroxy-5-norbornene-2,3-dicarboximide (HONB), 1-hydroxybenzotriazole (HOBt), 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HODhbt), its aza derivative (HODhat), and 2-pyridinol 1-oxide (HOPO). Additionally, when reacting N-terminal amine with carboxylic acid (CA), the carboxylic acid may be in the form of a preformed activated ester. Common

preformed activated esters include: N-hydroxysuccinimide (NHS-esters), 4-nitrophenol (PNP-ester), tetra/pentafluorophenol (TFP/PFP-esters) and N-carboxyanhydrides (NCA's). Other suitable preformed activated esters are known to those of ordinary skill in the art.

[0019] With respect to the universal dolastatin core of Formula I



I

R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo; R_{11} and R_{12} are individually selected from H, C_1-C_6 alkyl; R_6 and R_7 are each individually H or C_1-C_4 alkyl; R_9 is H or an acid protecting group, and R_{10} is H or an amino protecting group.

[0020] The amine (A) may be selected from alkylamines, alkanolamines, arylalkanolamines, amino acids, amino acid derivatives, peptides and peptide derivatives. When amine (A) is a peptide or peptide derivative, it is preferably 2-6 amino acid residues in length. In various embodiments, the amine (A) may include one or more substituents, suitable substituents include C_1-C_6 alkyl, hydroxy, C_1-C_6 alkoxy, amino, thiol, C_1-C_6 alkylthio, and halo. In some embodiments, amine (A) includes a protecting group. Suitable amine protecting groups include, but are not limited to, tert-butoxycarbonyl groups (Boc), 9-fluorenylmethoxycarbonyl groups (Fmoc), benzoyloxycarbonyl groups (Cbz, Z), and allyloxycarbonyl (Alloc). Suitable carboxyl protecting groups include, but are not limited to, simple esters, such as methyl ester, ethyl ester, tert-butyl ester, and benzyl ester, as well as esters formed with, e.g., trityl, 2,4-dimethoxylbenyl (Dmb), and 9-fluorenylmethyl (Fm). Other suitable protecting groups are known to those of ordinary skill in the art. In some embodiments, amine (A) may include one or more of a spacer, a linker and an attachment group.

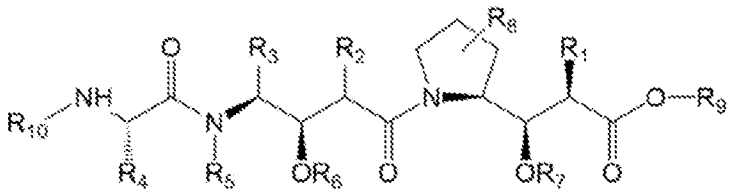
[0021] In various embodiments, the amine (A) is selected from phenylalanine, phenylalanine derivatives, substituted phenylalanine, substituted phenylalanine derivatives, tryptophan, tryptophan derivatives, substituted tryptophan, substituted

tryptophan derivatives, phenylpropanolamine, protected phenylpropanolamine, substituted phenylpropanolamine, protected substituted phenylpropanolamine, dolaphenine and protected dolaphenine, substituted dolaphenine, protected substituted dolaphenine, dolaphenine derivatives, protected dolaphenine derivatives, substituted dolaphenine derivatives, protected dolaphenine derivatives.

[0022] The carboxylic acid (CA) may be selected from amino acids, amino acid derivatives, peptides and peptide derivatives. When carboxylic acid (CA) is a peptide or peptide derivative, it is preferably 2-6 amino acid residues in length. In some embodiments, the carboxylic acid (CA) includes one or more substituents, suitable substituents include C_1-C_6 alkyl, hydroxy, C_1-C_6 alkoxy, amino, thiol, C_1-C_6 alkylthio, and halo. In some embodiments, the carboxylic acid (CA) may have a protecting group. Suitable carboxyl protecting groups include, but are not limited to, simple esters, such as methyl ester, ethyl ester, tert-butyl ester, and benzyl ester, as well as esters formed with, e.g., trityl, 2,4-dimethoxylbenyl (Dmb), and 9-fluorenylmethyl (Fm). Suitable amine protecting groups include, but are not limited to, tert-butoxycarbonyl groups (Boc), 9-fluorenylmethoxycarbonyl groups (Fmoc), benzoyloxycarbonyl groups (Cbz, Z), and allyloxycarbonyl (Alloc). Other suitable protecting groups are known to those of ordinary skill in the art. In some embodiments, carboxylic acid (CA) may include one or more of a spacer, a linker and an attachment group.

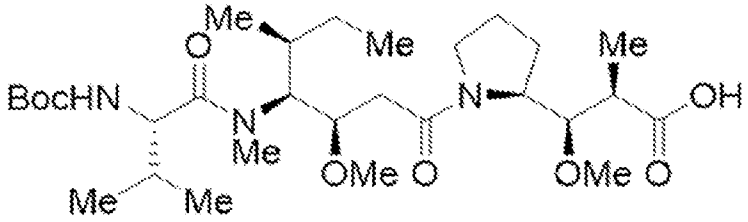
[0023] In various embodiments, carboxylic acid (CA) is selected from valine, protected valine, substituted valine, protected substituted valine, valine derivatives, protected valine derivatives, substituted valine derivatives, protected substituted valine derivatives, alanine, protected alanine, substituted alanine, protected substituted alanine, alanine derivatives, protected alanine derivatives, substituted alanine derivatives and protected substituted alanine derivatives.

[0024] In some preferred embodiments, the compound of Formula I is

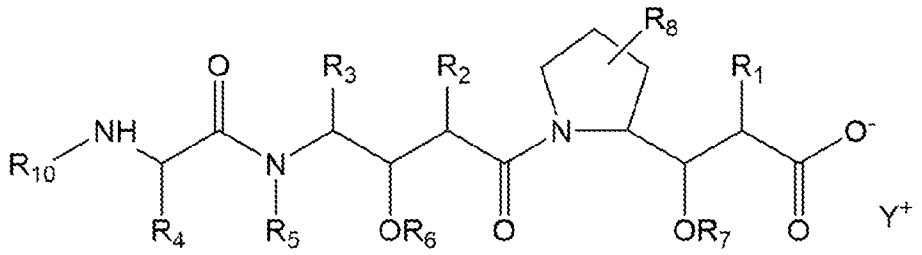


with the R groups as defined above.

[0025] In some preferred embodiments, the R groups in the compound of Formula I are selected to give the compound of Formula IA:



[0026] Also provided is an isolated salt of Formula II:



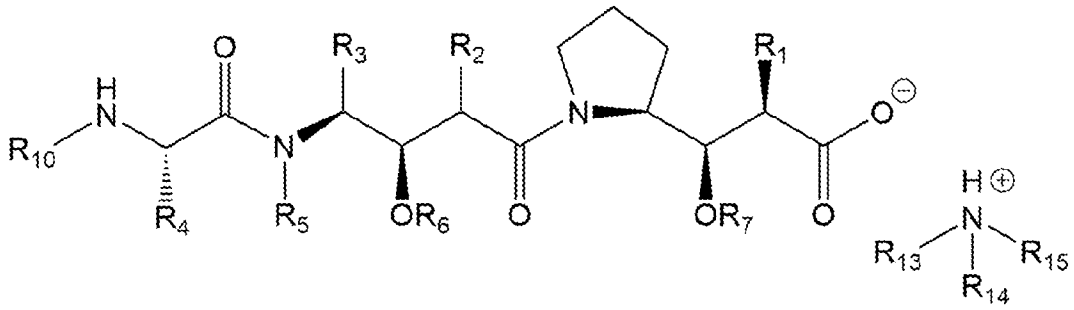
II

wherein R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo, R_{11} and R_{12} are individually selected from H and C_1-C_6 alkyl, R_6 and R_7 are each individually H or C_1-C_4 alkyl, R_{10} is H or an amino protecting group; and Y^+ is counterion.

[0027] In some embodiments of the isolated salt of Formula II, R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected from H, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, and iso-butyl; R_6 and R_7 are each individually H or methyl, R_{10} is H or tert-butoxycarbonyl (Boc), and Y^+ is an ammonium ion of the formula $N^+HR_{13}R_{14}R_{15}$ wherein R_{13} is selected from optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; R_{14} and R_{15} are independently selected from H, optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; wherein each optional substituent, if present, is selected from alkyl and aryl.

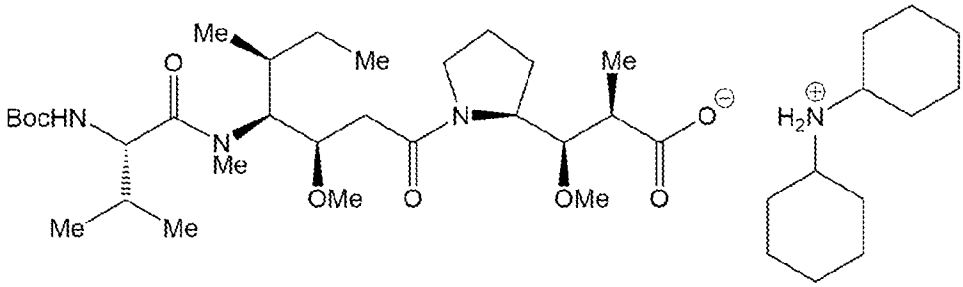
[0028] The isolated salt according either of claims 10 or 11 wherein Y^+ is selected from the group consisting diethylammonium ion, dibutylammonium ion, dicyclohexylammonium ion, methylcyclohexylammonium ion and methylbenzylammonium ion.

[0029] In some embodiments, the isolated salt of Formula II has the structure



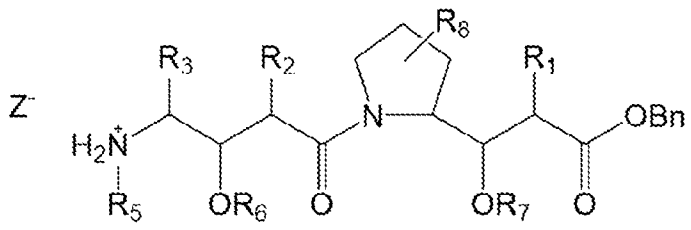
wherein the R groups are defined as above.

[0030] In a preferred embodiment, the isolated salt of Formula II has the structure



[0031] Also provided are additional compounds useful both in the preparation of the universal dolastatin core described herein as well as in alternate methods of preparing dolastatins, auristatins and related compounds. These compounds are intermediates in the preparation of the universal dolastatin core described herein. The use of these intermediates yields high purity dolastatin core as well as high purity dolastatin and auristatin compounds. It has also been found that the usefulness of these compounds in producing high purity compounds extends beyond the synthesis of the universal dolastatin core; they are also suitable in other methods of producing high purity dolastatin and auristatin compounds through other pathways.

[0032] Provided herein is a compound of Formula III:



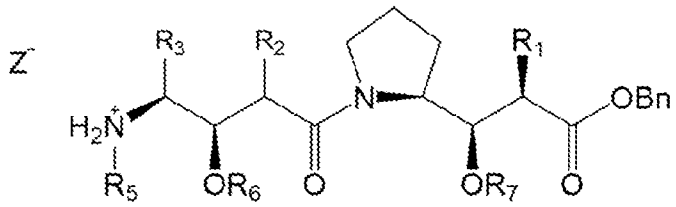
III

wherein R_1, R_2, R_3, R_5 and R_8 are each individually selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo, R_{11} and R_{12} are individually

selected from H and C_1-C_6 alkyl, R_6 and R_7 are each individually selected from H or C_1-C_4 alkyl, and Z^- is a counterion. In some embodiments, the compound of Formula III is in solution. In other embodiments, the compound of Formula III may be isolated. The salt form enables facile isolation and purification at scale. The crystallization enables impurity purge beyond the capabilities of column chromatography. The salt is a high purity, bench stable solid, whereas the free base is an oil. Using this salt will enable significantly larger scale production compared with methods requiring column chromatography.

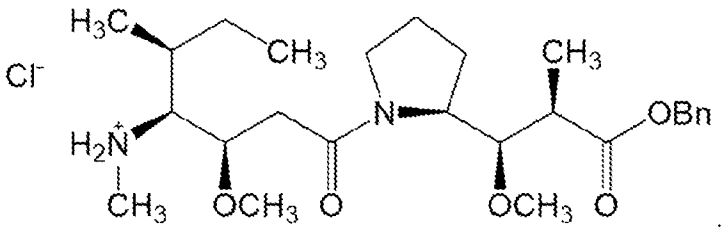
[0033] In some embodiments of Formula III, R_1, R_2, R_3, R_5 and R_8 are each individually selected from H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl; R_6 and R_7 are each individually selected from H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl; and Z^- is selected from halide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, mesylate, tosylate, benzene sulfonate, ethylsulfonate, nitrate, formate, acetate, trifluoroacetate, oxalate, and citrate.

[0034] In some embodiments, the compound of Formula III is

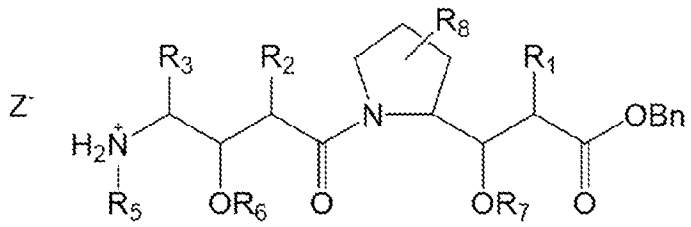


wherein the R groups and Z^- are defined as above.

[0035] In a preferred embodiment, the compound is of Formula III is:

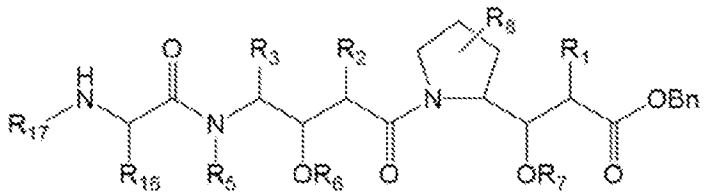


[0036] Also provided is a method of coupling an amino acid to a compound of Formula III



III

wherein R_1, R_2, R_3, R_5 and R_8 are each individually selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo; R_{11} and R_{12} are individually selected from H and C_1-C_6 alkyl; R_6 and R_7 are each individually selected from H or C_1-C_4 alkyl, and Z^- is a counterion; by first contacting the compound of Formula III with an aqueous base to remove the counterion, and then contacting the compound of Formula III with an N-protected amino acid N-carboxyanhydride to yield a compound of Formula IV:



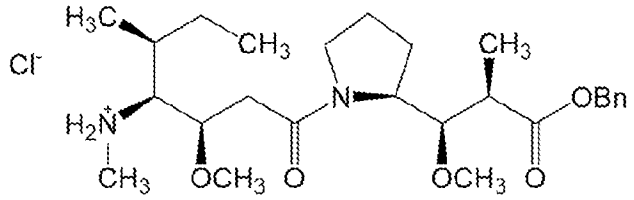
IV

wherein R_1, R_2, R_3, R_5, R_6, R_7 and R_8 are defined as above; R_{16} is an amino acid side chain, and R_{17} is a protecting group. In various embodiments, the aqueous base is selected from Na_2CO_3, $NaHCO_3$, NaOH, Na_2HPO_4, and Na_3PO_4. The Boc-NCA coupling is operationally convenient and facilitates isolation of compounds of Formula IV, the reaction goes to completion and side products are easily purged by aqueous workup. Less than 0.5% epimerization is observed using Boc-NCA compared to 5-10% for HATU-mediated coupling. This is particularly noteworthy as HATU is a preferred coupling agent to minimize epimerization. This is particularly important because diastereomeric impurities are difficult to remove.

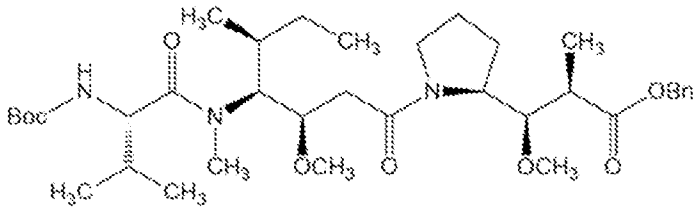
[0037] In some embodiments, of this method, the starting compound III, and final compound, IV, have R_1, R_2, R_3, R_5 and R_8 each individually selected from H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl; R_6 and R_7 are each individually selected from H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl, and Z^- is selected from halide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, mesylate, tosylate,

benzene sulfonate, ethylsulfonate, nitrate, formate, acetate, trifluoroacetate, oxalate, and citrate, and the N-protected amino acid N-carboxyanhydride is a Boc- protected amino acid N-carboxyanhydride, and the the aqueous base is selected from Na_2CO_3, $NaHCO_3$, NaOH, Na_2HPO_4, and Na_3PO_4 and is preferably Na_2CO_3.

[0038] In still other embodiments, the compound of Formula III is

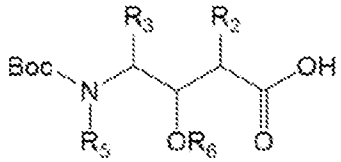


the aqueous base is Na_2CO_3, the N-protected amino acid N-carboxyanhydride is Boc-Val-NCA, and the compound of Formula IV is:



IV.

[0039] Also provided is a method of preparing a crystalline compound of Formula V without using column chromatography, the method involving the steps of providing a crude hydroxy acid of Formula V

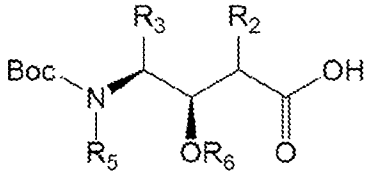


V

wherein R_2, R_3, and R_5 are each individually selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo; R_{11} and R_{12} are individually selected from H, C_1-C_6 alkyl, and R_6 is selected from H and C_1-C_4 alkyl; dissolving crude hydroxy acid in a soluble solvent, adding an insoluble solvent, initiating crystallization, and allowing the crystallization to complete yielding a purified hydroxy acid of Formula V. The "soluble solvent" is a solvent in which the hydroxy acid is soluble in; in some embodiments, the soluble solvent is heated when dissolving the hydroxy acid. The "insoluble solvent" is a solvent in which the hydroxy acid is insoluble. In a preferred embodiment, the soluble solvent is tert-butyl methyl ether (MTBE) and the insoluble solvent is heptane. In some embodiments, crystallization is

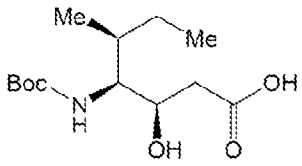
initiated by seeding the hydroxy acid. In other embodiments, crystallization is initiated by heat cycling. In still other embodiments, crystallization may be initiated by a combination of seeding and heat cycling. The resulting purified hydroxy acid may be isolated as a crystalline solid and is ready to use without additional purification—that is, no column chromatography is necessary when using this process. Advantageously, crystallization allows complete control of diastereoselectivity improving the quality and purity of downstream materials.

[0040] In some embodiments, the hydroxy acid of Formula V is



wherien R_2, R_3 and R_5 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl; R_6 is selected from the group consisting of H and methyl. In a preferred embodiment, the soluble solvent is hot MTBE and the insoluble solvent is heptane. In various embodiments, crystallization may be initiated by seeding, by heat cycling or a combination of seeding and heat cycling.

[0041] In a preferred embodiment, the hydroxy acid of Formula V is



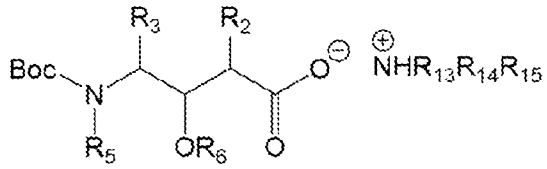
[0042] In some embodiments, the method further includes a step of isolating the purified hydroxy acid of Formula V. The resulting purified hydroxy acid of formula V is a crystalline solid.

[0043] In some embodiments, the method includes the synthesis of the hydroxy acid of Formula V without the need for column chromatography at any step. In an exemplary embodiment, the Ile-hydroxy acid shown above is synthesized starting with N-Boc isoleucine. The method involves condensing the N-Boc-isoleucine with mono-ethyl malonate to provide an Ile-keto-ester, reducing the Ile-keto-ester to provide an Ile-hydroxy-ester, and saponifying the Ile-hydroxy-ester to form a Ile-hydroxy-acid. The resulting Ile-hydroxy-acid is then purified by dissolving the crude Ile-hydroxy acid in a soluble solvent, adding an insoluble solvent, seeding the

hydroxy acid to initiate crystallization, and allowing the crystallization to complete, yielding purified Ile-hydroxy-acid. The purified Ile-hydroxy acid is optionally filtered and dried to yield a crystalline solid.

[0044] Advantageously, this method provides a highly scalable method of preparing a compound of Formula V in high diastereoselectivity without the need for column chromatography. The crystallization step eliminates the need for column chromatography and provides the material in a crystalline form that can be stored for subsequent use.

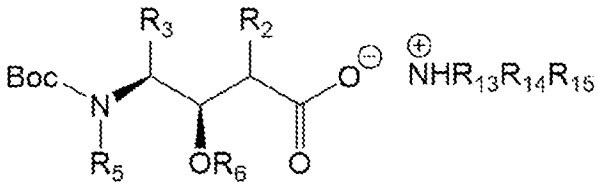
[0045] Also provided is compound of Formula VI:



VI

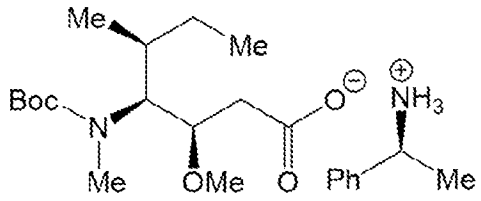
wherein R_2, R_3, and R_5 are each individually selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo; R_{11} and R_{12} are individually selected from H, C_1-C_6 alkyl, and R_6 is selected from H and C_1-C_4 alkyl; and R_{13} is selected from optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; R_{14} and R_{15} are independently selected from H, optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; wherein each optional substituent, if present, is selected alkyl and aryl. The salt form enables facile isolation and purification at scale. The crystallization enables impurity purge beyond the capability of column chromatography. The salt is a high purity, bench stable solid, whereas the free acid is an oil.

[0046] In some embodiment, the compound Formula VI is



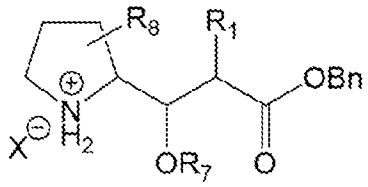
wherien R_2, R_3 and R_5 are each individually selected from H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl, R_6 is selected from H and methyl; and $NHR_{13}R_{14}R_{15}$ is selected from diethylammonium ion, dibutylammonium ion, dicyclohexylammonium ion, methylcyclohexylammonium ion and methylbenzylammonium ion.

[0047] In still other embodiments, the compound of Formula VI is:



[0048] Advantageously, the compound of formula VI may be isolated as a solid. In some embodiments, the compound of Formula VI is a crystalline solid.

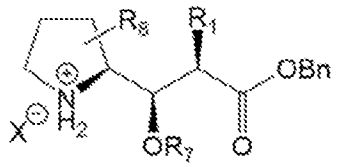
[0049] Further provided is a compound of Formula VII



VII

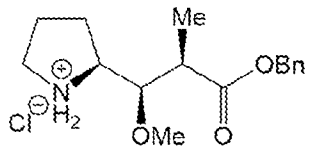
wherein R_1 and R_8 are individually selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo; R_{11} and R_{12} are individually selected from H, C_1-C_6 alkyl; R_7 is selected from H or C_1-C_4 alkyl; and X^- is selected from halide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, mesylate, tosylate, benzene sulfonate, ethylsulfonate, nitrate, formate, acetate, oxalate, and citrate. Salt formation is advantageous as this material is unstable as the free base. The physical characteristics of the salt depends on the counterion. In some salt forms the material is a high purity, bench stable solid that when crystallized efficiently purges impurities. Other salt forms generate a viscous oil.

[0050] In some embodiments, the compound of Formula VII is



wherein R_1 and R_8 are individually selected from H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl, R_7 is selected from H and methyl; and X^- is a halide, preferably chloride.

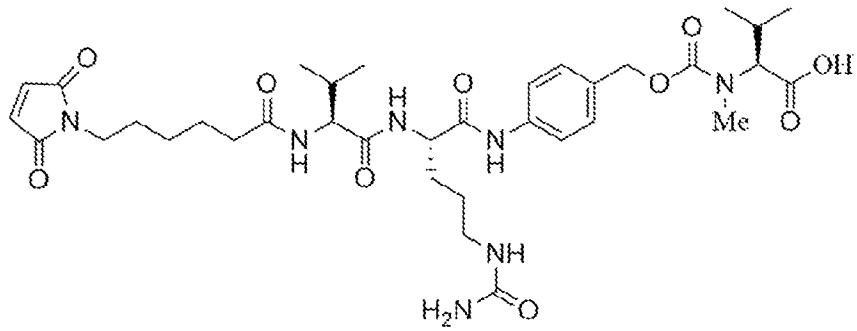
[0051] In still other embodiments, the Formula VII is



which is a high purity, bench stable solid that when crystallized efficiently purges impurities.

[0052] Advantageously, the compound of formula VII may be isolated as a solid. In some embodiments, the compound of Formula VII is a crystalline solid.

[0053] Also provided is a compound of Formula VIII:

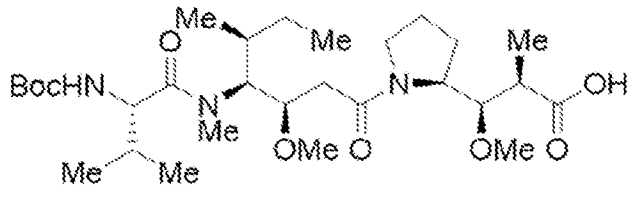


VIII.

[0054] This compound can advantageously be used in a simplified preparation of vcMMAE as well as other auristatins and dolastatins.

[0055] Also provided are methods of preparing specific auristatins of interest.

[0056] Also provided is a method of preparing monomethyl auristatin E from a core compound of Formula IA:

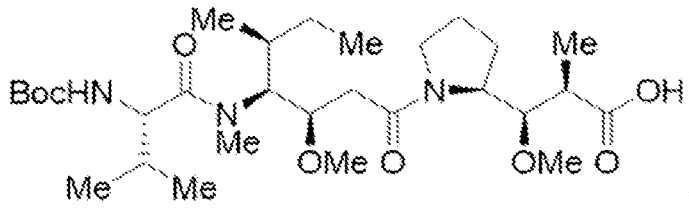


IA

by contacting the core compound with norephedrine in the presence of a coupling agent to form a core compound-norephedrine intermediate, deprotecting the core compound-norephedrine intermediate to form a deprotected core compound-norephedrine intermediate, contacting the deprotected core compound-norephedrine intermediate with N-Boc-N-Me-Val-OH in the presence of a coupling agent to form N-Boc-MMAE, and deprotecting the N-Boc-MMAE to yield MMAE.

[0057] MMAE may then be used to prepare vcMMAE. For example, MMAE is contacted with mc-Val-Cit-PABC-PNP in the presence of a coupling additive. The reaction is allowed to proceed to completion and the product is purified by rp-HPLC.

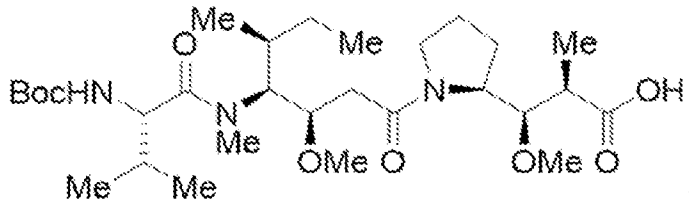
[0058] Further provided is a method of preparing vc-MMAE from a core compound of Formula IA:



IA

by contacting the core compound with norephedrine in the presence of a coupling agent to form a core compound-norephedrine intermediate, deprotecting the core compound-norephedrine intermediate to form a deprotected core compound-norephedrine intermediate, contacting the deprotected core compound-norephedrine intermediate with mc-Val-Cit-PAB-N-Me-Val-OH in the presence of a coupling agent to yield vcMMAE.

[0059] Also provided is a method of preparing monomethyl auristatin F from a core compound of Formula IA:



IA

by contacting the core compound with L-phenylalanine methyl ester hydrochloride in the presence of a coupling agent to form N-Boc-Val-Dil-Dap-Phe-OMe, deprotecting the N-Boc-Val-Dil-Dap-Phe-OMe to yield Val-Dil-Dap-Phe-OMe, contacting Val-Dil-Dap-Phe-OMe with N-Boc-Me-Val-OH in the presence of a coupling agent to form N-Boc-N-Me-Val-Val-Dil-Dap-Phe-OMe, and deprotecting the N-Boc-N-Me-Val-Val-Dil-Dap-Phe-OMe to yield MMAF.

[0060] Other auristatins, dolastatins and related compounds may also be made using methods analogous to those described herein.

[0061] As used herein, the term "alkyl" refers to a straight chain or branched, saturated hydrocarbon. Representative alkyl groups include, but are not limited to, -methyl, -ethyl, -n-propyl,-n-butyl, -n-pentyl, -n-hexyl and so forth; exemplary

branched alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl and 2-methylbutyl. An alkyl group may be attached at any available point to produce a stable compound. The term alkyl is also meant to encompass a fully substituted carbon.

[0062] The term “amino” refers to $-NH_2$, as well as "disubstituted amino" wherein one of the hydrogen atoms is replaced by a non-hydrogen substituent; and “trisubstituted amino” wherein both of the hydrogen atoms are replaced by non-hydrogen substituents, which may be identical or different.

[0063] The term “amine (A)” refers specifically to the amine-containing compound that is reacted with the C-terminal carboxylic acid of the universal dolastatin core described herein. The amine (A) may be selected from alkylamines, alkanolamines, arylalkanolamines, amino acids, amino acid derivatives, and peptides. In various embodiments, the amine (A) may include one or more substituents, suitable substituents include C_1-C_6 alkyl, hydroxy, C_1-C_6 alkoxy, amino, thiol, C_1-C_6 alkylthio, and halo. In some embodiments, amine (A) includes a protecting group. The protecting group may be an amine protecting group, a carboxyl protecting group, or a protecting group on the side chain or other protectable location. Suitable amine protecting groups include, but are not limited to, tert-butoxycarbonyl groups (Boc). 9-fluorenylmethoxycarbonyl groups (Fmoc), benzoyloxycarbonyl groups (Cbz, Z), and Allyloxycarbonyl (Alloc). Suitable carboxyl protecting groups include, but are not limited to, simple esters, such as methyl ester, ethyl ester, tert-butyl ester, and benzyl ester, as well as esters formed with, e.g., trityl, 2,4-dimethoxylbenyl (Dmb), and 9-fluorenylmethyl (Fm). Other suitable protecting groups are known to those of ordinary skill in the art. In some embodiments, amine (A) is selected from phenylalanine, phenylalanine derivatives, substituted phenylalanine, substituted phenylalanine derivatives, tryptophan, tryptophan derivatives, substituted tryptophan, substituted tryptophan derivatives, phenylpropanolamine, protected phenylpropanolamine, substituted phenylpropanolamine, protected substituted phenylpropanolamine, dolaphenine and protected dolaphenine, substituted dolaphenine, protected substituted dolaphenine, dolaphenine derivatives, protected dolaphenine derivatives, substituted dolaphenine derivatives, protected dolaphenine derivatives. In still other embodiments, the amine may be a peptide, preferably having 2 – 6 amino acids residues, the amino acid residues may include combinations of

naturally occurring amino acids, non-standard amino acids, substituted amino acids, and amino acid derivatives, and may include a protecting group.

[0064] The term “amino acid” refers to both naturally occurring amino acids, i.e., standard and non-standard amino acids, as well as chemically synthesized amino acids, and includes both L- and D-isomers. Substituted amino acids are amino acids that include one or more substituents, typically on the side chain. Amino acid derivatives are amino acids which the α-amino group or acyl group have been chemically modified. Such modifications may include, for example, the addition of protecting groups, spacers, linkers, or other functional groups that are useful for further modification of the amino acid. Protected amino acids are amino acid derivatives that have a protecting group on the α-amino group, the acyl group or both the α-amino group and the acyl group. Suitable amine protecting groups include, but are not limited to, tert-butoxycarbonyl groups (Boc). 9-fluorenylmethoxycarbonyl groups (Fmoc), benzoyloxycarbonyl groups (Cbz, Z), and Allyloxycarbonyl (Alloc). Suitable carboxyl protecting groups include, but are not limited to, simple esters, such as methyl ester, ethyl ester, tert-butyl ester, and benzyl ester, as well as esters formed with, e.g., trityl, 2,4-dimethoxylbenyl (Dmb), and 9-fluorenylmethyl (Fm).

[0065] The term "aryl" refers to a carbocyclic aromatic group. Examples of aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. In various embodiments, carboxylic acid (CA) is selected from amino acids, amino acid derivatives, peptides and peptide derivatives.

[0066] The term “carboxylic acid (CA)” as used herein refers specifically to the carboxylic-acid containing compound that is reacted with the N-terminal of the universal dolastatin core in the methods described herein. The carboxylic acid (CA) may be selected from amino acids, amino acid derivatives, peptides and peptide derivatives. When carboxylic acid (CA) is a peptide or peptide derivative, it is preferably 2-6 amino acid residues in length. In some embodiments, the carboxylic acid (CA) includes one or more substituents, suitable substituents include C_1-C_6 alkyl, hydroxy, C_1-C_6 alkoxy, amino, thiol, C_1-C_6 alkylthio, and halo. In some embodiments, the carboxylic acid (CA) may have a protecting group. The protecting group may a carboxyl protecting group, be an amine protecting group, or a protecting group on the side chain or other protectable location. Suitable carboxyl protecting groups include, but are not limited to, simple esters, such as methyl ester, ethyl ester, tert-butyl ester, and benzyl ester, as well as esters formed with, e.g., trityl, 2,4-dimethoxylbenyl

(Dmb), and 9-fluorenylmethyl (Fm). Suitable amine protecting groups include, but are not limited to, tert-butoxycarbonyl groups (Boc). 9-fluorenylmethoxycarbonyl groups (Fmoc), benzoyloxycarbonyl groups (Cbz, Z), and Allyloxycarbonyl (Alloc). Other suitable protecting groups are known to those of ordinary skill in the art. In some embodiments, carboxylic acid (CA) may include one or more of a spacer, a linker and an attachment group.

[0067] The term "coupling agent" refers to peptide coupling reagents used to activate a carboxyl moiety of a carboxylic acid to facilitate reaction with an amino group, such as the α-amino group of an amino acid, or the N-terminal amine of the universal dolastatin core. Suitable coupling agents used in the methods provided herein include, but are not limited to, cabonyldiimidazole (CDI), propylphosphonic anhydride (T3P) solution and HATU. Other suitable coupling agents are known to those of ordinary skill in the art. Coupling agents are generally used in the presence of a base, such as diisopropylethylamine (DIPEA) and N-methylmorpholine (NMM).

[0068] The term "coupling additive" refers to peptide coupling reagents that, in addition to facilitating the formation of a peptide bond, also inhibit side reactions and reduce racemization. Useful coupling additives for the reactions described herein include N-hydroxysuccinimide (HOSu), N-hydroxy-5-norbornene-2,3-dicarboximide (HONB), 1-hydroxybenzotriazole (HOBt), 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HODhbt), its aza derivative (HODhat), and 2-pyridinol 1-oxide (HOPO). Coupling additives are generally used in the presence of a base, such as 2,6-lutidine, DIPEA and NMM.

[0069] The term "halo" refers to elements in VIIa of the periodic table, such as fluorine, chlorine, bromine and iodine. The term "halide" refers to the ion of a halogen.

[0070] The term "heterocyclic" refers to any alkyl or aryl ring containing at least one non-carbon atom in the ring, i.e., heteroalkyl and heteroaryl, respectively. Exemplary non-carbon atoms include, but are not limited to, oxygen, nitrogen and sulfur; heterocyclic rings may include two or more non-carbon atoms in the ring; in such instances, the two or more non-carbon atoms may be the same or different.

[0071] The term "linker" refers to a chemical entity modified to attach to an antibody or small molecule targeting group at one end and a cytotoxic agent, such as a dolastatin or auristatin at the other end. Conventional linkers include cleavable linkers

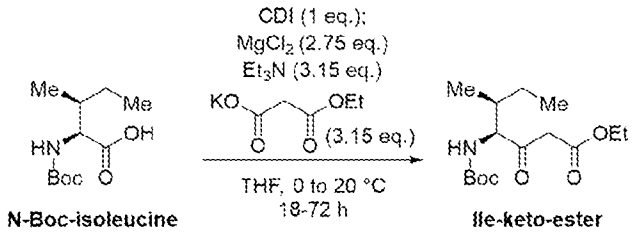
and non-cleavable linkers. The linkers may include an attachment group, for attachment to an antibody, antibody fragment or other targeting entity, and a spacer, which allows for interaction at, e.g., a cleavable site.

[0072] The term "peptide" as used herein, are 2 or more amino acids, substituted amino acids, amino acid derivatives, or substituted amino acid derivatives, including standard, non-standard, and chemically synthesized amino acids, and including for L- and D-isomers, that are linked together in an amide linkage. Peptides may include protecting groups at the N-terminal or the C-terminal. Suitable amine protecting groups include, but are not limited to, tert-butoxycarbonyl groups (Boc), 9-fluorenylmethoxycarbonyl groups (Fmoc), benzoyloxycarbonyl groups (Cbz, Z), and Allyloxycarbonyl (Alloc). Suitable carboxyl protecting groups include, but are not limited to, simple esters, such as methyl ester, ethyl ester, tert-butyl ester, and benzyl ester, as well as esters formed with, e.g., trityl, 2,4-dimethoxylbenyl (Dmb), and 9-fluorenylmethyl (Fm). When peptides, substituted peptides, peptide derivatives, or substituted peptide derivatives are used for amine (A) or carboxylic acid (CA), they are preferably 2-6 amino acid residues in length.

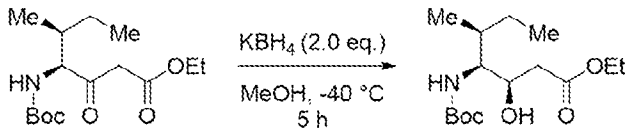
[0073] The term "substituent" refers to any group that replaces a hydrogen atom of, e.g., an alkyl, cycloalkyl, aryl, heteroaryl, amine, and so forth. Substituents may include, but are not limited to, such groups as alkyls, substituted alkyls, aryls, heteroaryls, ethers, amines, amides, thiols, sulfides, disulfides, halo and protecting groups. Suitable substituents for the amine (A) and the carboxylic acid (CA) used in the methods described herein include C_1-C_6 alkyl, e.g., methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, tert-butyl, pentyl, and so forth, hydroxy, C_1-C_6 alkoxy, e.g., methoxy, ethoxy, and so forth, amino, thiol, C_1-C_6 alkylthio, and halo.

[0074] Examples. The following examples are illustrative of the synthesis of the universal dolastatin core and useful intermediates.

[0075] Example 1. Preparation of N-Boc-Dolaisoleuine methylbenzylamine salt.

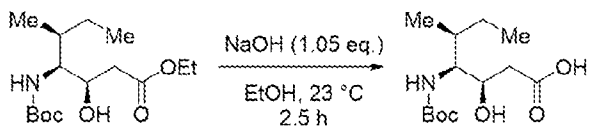


[0076] To a cooled solution (7 °C) of N-Boc-isoleucine (100 g, 1 equiv.) in THF (5 vol.) was charged CDI (1 equiv.), portion-wise. The reaction was warmed to 20 °C and stirred for 4 h, then cooled to 0 °C. To a suspension of potassium mono-ethyl malonate (2.2 equiv.) in THF (15 vol.) at 7 °C was charged anhydrous $MgCl_2$ (2.75 equiv.), portion-wise, then warmed to 20 °C and stirred for 1 h. This suspension was cooled to 0 °C, charged with Et_3N (3.15 equiv.), and stirred for 2 h at 0 °C. The 0 °C imidazolide solution was charged slowly to the malonate suspension maintaining the temperature ≤ 3 °C. The combined suspension was warmed to 20 °C and stirred for 18-72 h. The reaction was quenched with 10% (w/w) aqueous citric acid (20 vol.), maintaining an internal temperature of ≤ 23 °C, and then concentrated under reduced pressure. The acidic aqueous layer was extracted with MTBE (3 x 5 vol.). The combined organic extracts were washed with 20% (w/w) aqueous Na_2CO_3 (2 x 5 vol.), dried over Na_2SO_4 and concentrated under reduced pressure to give the desired Ile-keto-ester (95% yield) without further purification.



Ile-keto-ester Ile-hydroxy-ester

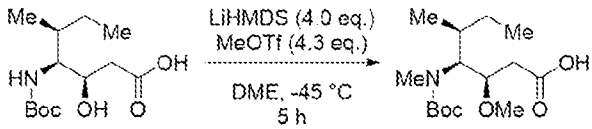
[0077] MeOH (5 vol.) was cooled to ≤ -40 °C and KBH_4 (2.0 equiv.) was charged and stirred at -40 °C for 30 min. A solution of Ile-keto-ester (130 g, 1 equiv.) in MeOH (5 vol.) and charged slowly to the KBH_4 slurry, maintaining the internal temperature ≤ -40 °C. The reaction was stirred for 5 h at -40 °C. The reaction was quenched by charging to a stirred solution of 10% (w/w) aqueous citric acid (20 vol.), with internal temperature ≤ 8 °C and a resulting pH of 3-5. The MeOH was removed under reduced pressure, and the remaining aqueous phase extracted with MTBE (3 x 5 vol.). The combined organic extracts were washed with 20% (w/w) aqueous Na_2CO_3 (2 x 5 vol.), dried over Na_2SO_4, and concentrated under reduced pressure to give the desired Ile-hydroxy-ester (89% yield, dr ≥ 13:1) without further purification.



Ile-hydroxy-ester Ile-hydroxy-acid

[0078] To a RT solution of Ile-hydroxy-ester (118 g, 1 equiv.) in EtOH (5 vol.) was charged 2.5 M aqueous NaOH (1.05 equiv.). The reaction was stirred with an internal temperature ≤ 23 °C for 2.5 h. EtOH was removed under reduced pressure and the basic aqueous phase extracted with MTBE (2 x 5 vol.). The combined organic phases were extracted with 2.5 M aqueous NaOH (2 x 0.5 vol.). The aqueous phases were combined and acidified with H_3PO_4 (85 wt%, 1.5 equiv.) to a pH of 4. The acidified aqueous phase was extracted with MTBE (3 x 5 vol.). The combined organic extractions were dried over Na_2SO_4 and concentrated under reduced pressure to a constant weight of crude Ile-hydroxy-acid (99% yield). The resultant weight of the crude was used in the subsequent crystallization.

[0079] The crude Ile-hydroxy-acid was dissolved in MTBE (2 vol.) and heptane (2 vol.), and then warmed to 55 °C. Heptane (4 vol.) was charged, maintaining the temperature at 55 °C. The mixture was cooled to 45 °C and seeded (0.5 wt%). After initiation of crystallization was observed, the mixture was held at 45 °C for 2 h, cooled to RT over 1 h, stirred vigorously for another 12 h, and then the solid products were isolated by filtration. The filter cake was washed with heptane (2 vol.) and dried under vacuum to give the desired Ile-hydroxy-acid (75% yield, dr > 99:1). The crystallization step eliminates the need for purification by column chromatography, providing both a stable, solid product and scalability.

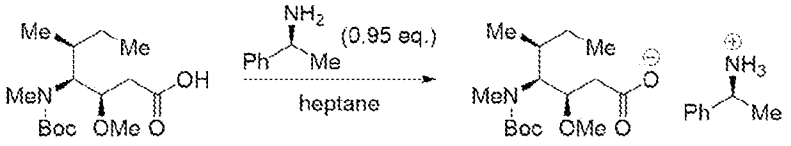


Ile-hydroxy-acid **N-Boc-Dil**

[0080] To a chilled (-45 °C) solution of Ile-hydroxy-acid (5 g, 1 equiv.) in anhydrous dimethoxyethane (20 vol.) was charged LiHMDS (1.0 M in hexanes, 4.0 equiv.), maintaining the internal temperature ≤ -45 °C. MeOTf (4.3 equiv.) was then charged, maintaining the internal temperature at ≤ -45 °C. The reaction was stirred at ≤ -45 °C for 5 h until judged complete. The reaction was quenched by the addition of methanol (5 vol.) and 10% (w/w) aqueous NaOH (4.0 equiv.), maintaining an internal temperature of ≤ -45 °C, then warmed to 5 °C and stirred until judged complete. The reaction mixture was concentrated under reduced pressure to remove organic solvents and then diluted with heptane (20 vol.), which was extracted with 10% (w/w) aqueous NaOH (2 x 5 vol.) The combined basic aqueous phases were adjusted to pH 4 by the

addition of H_3PO_4 and then extracted with 1:1 heptane/MTBE (3 x 5 vol.). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to crude N-Boc-Dil. The resultant weight of the isolated crude was used in the subsequent chromatographic step.

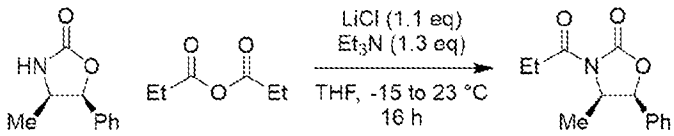
[0081] The crude N-Boc-Dil was loaded onto silica (not less than 20 g silica per 1 g of crude; silica equilibrated with eluent) using a minimal amount of 1:5 MTBE/heptane and eluted with 20% MTBE/heptane/0.1% acetic acid. Positive fractions were combined and reduced under pressure, and the resultant weight was used for the subsequent recrystallization.



N-Boc-Dil **N-Boc-Dil.methylbenzylamine**

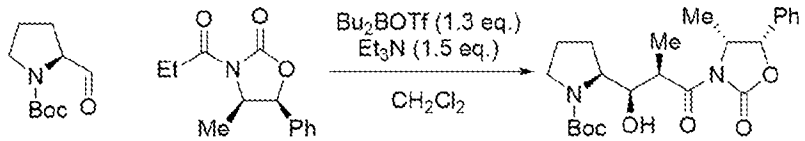
[0082] N-Boc-Dil (1 equiv.) was dissolved in heptane (10 vol.) and heated to 50 °C for 30 min. S-α-methylbenzylamine (0.95 equiv.) was charged, the reaction was cooled to 37 °C, and then seeded (0.5 wt%). The reaction was cooled to 20 °C over 1 h and stirred for an additional 6 h, and then solid products were isolated by filtration. The filter cake was washed with heptane (2 vol.) and dried under vacuum to give the final N-Boc-Dil.methylbenzylamine.

[0083] Example 2. Preparation of O-benzyl ester Dolaproine hydrochloride salt.



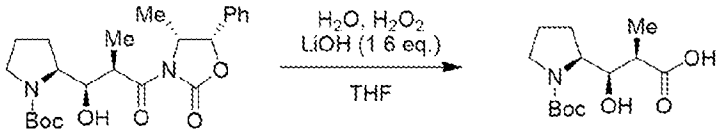
[0084] A solution of (4R,5S)-(+)-4-methyl-5-phenyl-2-oxazolidinone (100.0 g, 1 equiv.) in THF (30 vol.) was cooled to -20 °C. To the precooled solution was added lithium chloride (1.1 equiv.) followed by Et_3N (1.3 equiv.) while maintaining an internal temperature < -15 °C. Propionic anhydride (1.2 equiv.) was added over 30 min. while maintaining an internal temperature < -15 °C. The mixture was warmed to 23 °C and stirred for 16 h. The reaction was concentrated under reduced pressure and partitioned between EtOAc (5 vol.) and 0.2 M aqueous HCl (5 vol.). The organic layer was washed with 1 M aqueous $NaHCO_3$ (2 x 2 vol.) and brine (2 x 1 vol.). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced

pressure to afford the desired Evans-type oxazolidinone (130.1 g, 99%) as a viscous, light yellow oil which was used without further purification.



N-Boc-L-prolinal (1.1 eq) Evans aldol adduct

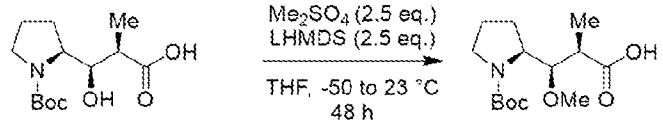
[0085] A solution of (4R,5S)-4-methyl-5-phenyl-3-propionyloxazolidin-2-one (25.75 g, 1.1 equiv.) in CH_2Cl_2 (10 vol.) was cooled to 0 °C. Triethylamine (1.5 equiv.) was charged to this cold reaction mixture followed by the addition of dibutyl boron triflate (1 M in CH_2Cl_2, 1.3 equiv.) while maintaining the reaction temperature < 4 °C. The reaction was stirred for 1 h at 0 °C then cooled to -70 °C. A solution of N-Boc-L-prolinal (20.0 g, 1.0 equiv.) in CH_2Cl_2 (6 vol.) was charged, maintaining the temperature < -60 °C. The reaction was stirred for 2 h at -70 °C, 1 h at -0 °C, then 15 min at room temperature. The reaction was quenched with 0.1 M aqueous sodium phosphate buffer (pH=7, 8 vol.) followed by the slow addition of 30% aqueous H_2O_2/MeOH (1:2, 30 vol.) maintaining the temperature < 10 °C and stirred for 1 h. The mixture was diluted with DI water (15 vol.) and concentrated under reduced pressure to complete removal of organic solvent. DI water (15 vol.) was added to the residue. The mixture was extracted with EtOAc (3 x 15 vol.). The combined organics were washed with 1 M $KHSO_4$ (15 vol.), DI water (15 vol.), saturated aqueous $NaHCO_3$ (15 vol.) and brine (15 vol.). Charcoal (20 wt%) was charged and removed by filtration. The filtrate was concentrated under reduced pressure to obtain Evans aldol adduct as a white foam that was utilized without further purification (45 g).



Evans aldol adduct N-Boc-Dap-hydroxy-acid

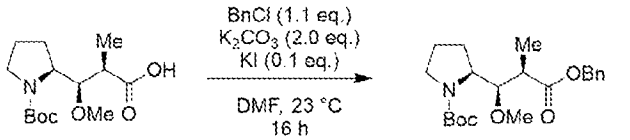
[0086] To a solution of Evans aldol adduct (50 g, 1.0 equiv.) in anhydrous THF (13 vol.) was charged water (3.3 vol.) and cooled to 5 °C. 30% aqueous H_2O_2 (1 vol.) was added, followed by the addition of LiOH (1.6 equiv.) in DI water (2 vol.). The reaction mass was stirred for 5 h at 5 °C under nitrogen atmosphere. The reaction was quenched by the addition of $NaHSO_3$ (4 equiv.) in DI water (5 vol.) and stirred for 16

h. The resulting mixture was adjusted to pH 9 with saturated aqueous $NaHCO_3$ and washed with CH_2Cl_2 (2 x 10 vol.). The aqueous layer was cooled and adjusted to pH 2 by addition of 1 M aqueous $KHSO_4$ and extracted with EtOAc (2 x 10 vol.). The combined EtOAc extracts were washed with brine (10 vol.) then dried over Na_2SO_4 and concentrated under reduced pressure to obtain N-Boc-Dap-hydroxy-acid as a yellow oil that was used without further purification (24 g).



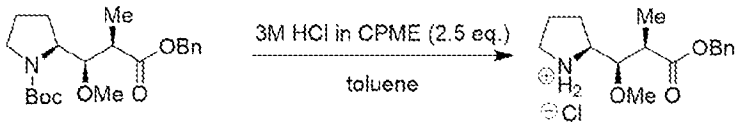
N-Boc-Dap-hydroxy-acid N-Boc-Dap

[0087] To a -50 °C solution of N-Boc-Dap-hydroxy-acid (6.0 g, 1.0 equiv.) in anhydrous THF (20 vol.) was added Me_2SO_4 (2.5 equiv.) followed by LiHMDS (1 M in THF, 2.5 equiv.) maintaining the temperature < -50 °C. The reaction was warmed to room temperature and stirred for 48 h under nitrogen atmosphere. The reaction was quenched by the addition of 10% aqueous NaOH and stirred for 12 h. The mixture was concentrated under reduced pressure to remove THF and adjusted to pH 4 by the addition of 1 M aqueous H_3PO_4. The acidified aqueous layer was extracted with MTBE (3 x 10 vol.) and concentrated under reduced pressure to obtain N-Boc-Dap as a yellow oil which was used without further purification (5.5 g).



N-Boc-Dap N-Boc-Dap benzyl ester

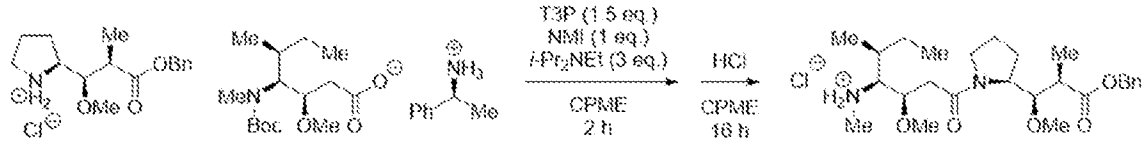
[0088] To a solution of N-Boc-Dap (2.45 g, 1 equiv.) in DMF (8 vol.) was added K_2CO_3 (2.0 equiv.), KI (0.1 equiv.) and benzyl chloride (1.1 equiv.). The reaction was stirred for 16 h at RT and then quenched by the addition of toluene (5 vol.) and DI water (5 vol.). The organic layer was collected and the aqueous layer extracted with toluene (5 vol.). The combined organic layers were washed with DI water (5 vol.) and concentrated under reduced pressure. The residue was purified via silica gel column chromatography (Sfär 50 g duo) eluting with a heptane to 60:40 heptane/EtOAc gradient to obtain N-Boc-Dap benzyl ester as a colorless oil (2.84 g, 88%).



N-Boc-Dap benzyl ester O-Bn-Dap.HCl

[0089] To a stirred solution of N-Boc-Dap benzyl ester (5.7 g, 1 equiv.) in toluene (5 vol.) was added 3 M HCl in CPME (2.5 equiv.). After stirring for 16 h, the reaction was concentrated under reduced pressure to removed CPME and HCl. The residue was dissolved in toluene (5 vol.) and heated to 80 °C. After dissolution, the reaction was cooled to RT and the solid product isolated by filtration. The solid was washed with toluene (2 vol.) and dried to afford O-Bn-Dap.HCl (2.9 g, 61%) as a white crystalline solid.

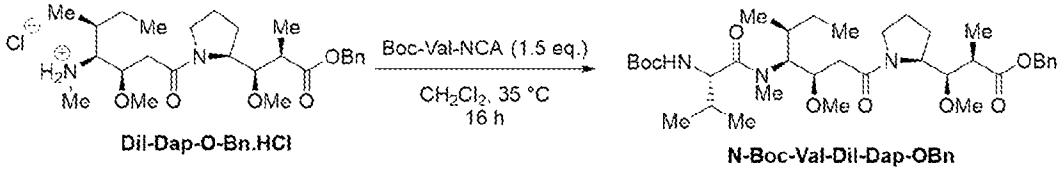
[0090] Example 3. Preparation of Dil-Dap-O-Bn.HCl



O-Bn-Dap.HCl N-Boc-Dil.methylbenzylamine Dil-Dap-O-Bn.HCl

A stirred mixture of N-Boc-Dil.methylbenzylamine (10 g, 1 equiv.) in CPME (4 vol.) was washed with 4 M aqueous HCl (3 x 1.5 vol.) and DI water (3 vol.). The combined aqueous washes were extracted with CPME (3 vol.) and the combined organic layers dried by constant volume distillation (10 vol.). To the stirred solution was added OBn-Dap.HCl (1 equiv.), NMI (1 equiv.), T3P (50% solution in EtOAc, 1.5 equiv.) and DIPEA (3 equiv.). After stirring for 2 h the reaction was quenched by the addition of 4 M aqueous HCl (3 vol.) and the layers separated. The organic layer was washed with 4 M aqueous HCl (3 vol.) then DI water (3 vol.). The combined aqueous layers were extracted with CPME (3 vol.) then the combined organic layers dried by constant volume distillation (10 vol.). To the stirred solution was added 3 M HCl in CPME (5 equiv.). After stirring for 16 h the reaction was concentrated. The residue was crystallized from MTBE/CPME to give Dil-Dap-OBn.HCl as a white solid (8.6 g, 68%, 99.6 A%).

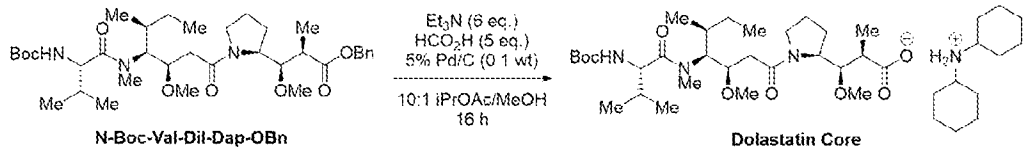
[0091] Example 4. Preparation of isolated universal dolastatin core N-Boc-Val-Dil-Dap-OH.DCHA from Dil-Dap-OBn.HCl



Dil-Dap-O-Bn.HCl

N-Boc-Val-Dil-Dap-OBn

[0092] A solution of Dil-Dap-OBn.HCl in CH_2Cl_2 (4 vol.) was washed with 20% aqueous Na_2CO_3 (3 x 2 vol.) and the aqueous layer extracted with CH_2Cl_2 (2 vol.). The combined organic layers were washed with DI water (3 vol.) and dried by constant volume distillation (10 vol.). N-Boc-Val-NCA (1.5 equiv.) was added to the stirred solution and the reaction stirred at 35 °C for 16 h. The solvent was swapped to iPrOAc and the reaction quenched by the addition of 1 M aqueous $NaHCO_3$ (5 vol.) and glycine (5 equiv.). The organic layer was washed with 1 M $NaHCO_3$ (5 vol.) and DI water (5 vol.). The N-Boc-Val-Dil-Dap-OBn product was utilized in the subsequent step as a solution in iPrOAc.

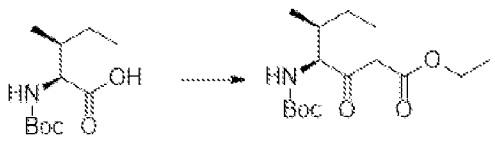


N-Boc-Val-Dil-Dap-OBn

Dolastatin Core

[0093] To a stirred solution N-Boc-Val-Dil-Dap-OBn (2 g, 1 equiv.) in iPrOAc (10 vol.) was added MeOH (1 vol.), 5% Pd/C (0.1 wt.), TEA (6 equiv.) and formic acid (5 equiv.). After 16 h the solution was filtered through Celite and filtrate washed with 4 M aqueous HCl (3 x 5 vol.) and DI water (5 vol.). The solvent was swapped to 80:20 heptane/iPrOAc and DCHA (1 equiv.) was charged. Dolastatin Core, N-Boc-Val-Dil-Dap-OH.DCHA, (75%, 99 A%) was isolated as a white crystalline solid by filtration.

[0094] Example 5. Full preparation of the universal dolastatin core

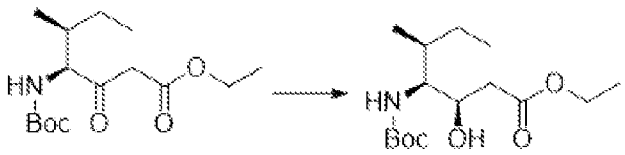


N-Boc-Isoleucine

Ile-Keto-Ester

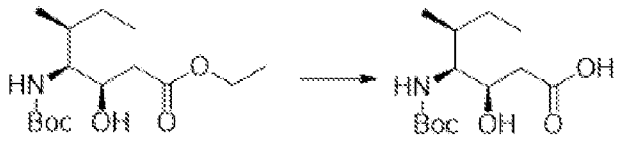
[0095] To a cooled solution (0 °C) of N-Boc-isoleucine (1 eq.) in THF (5 vol.) was charged CDI (1 eq.) portion wise. The reaction was allowed to warm to RT and stirred for 3 h. To a suspension of potassium monoethyl malonate (2.2 eq.) in THF (15 vol.) at 0 °C was added Et_3N (3.15 eq.) and anhydrous $MgCl_2$ (2.75 eq.). The suspension was allowed to warm to RT, stirred for 3 h then cooled to 0 °C. The RT imidazolide

solution was charged slowly to the malonate suspension maintaining the temperature at NMT 5 °C. The combined suspension was allowed to warm to RT and stirred for 72 h. The reaction was quenched with 10% aqueous citric acid (20 vol.) and concentrated under reduced pressure to remove THF. The acidic aqueous layer was extracted with MTBE (3 x 5 vol.). The combined organic extracts were washed with sat. aq. $NaHCO_3$ (5 vol.), dried over Na_2SO_4 and concentrated under reduced pressure to give the desired product (95% yield) without further purification.



Ile-Keto-Ester Ile-hydroxy-Ester

[0096] Methanol (5 vol.) was cooled to NMT -40 °C and KBH_4 (2 eq.) was charged and stirred at NMT -40 °C for 30 min. Keto-Ester (1 eq.) was dissolved in Methanol (5 vol.) and charged slowly to the KBH_4 slurry maintaining the internal temperature NMT -40 °C. The reaction was stirred for 4 h at that temperature then quenched by charging to a stirred solution of 10% aqueous citric acid (20 vol.) The pH of the resulting solution was 3-5. The MeOH was removed under reduced pressure then the aqueous layer extracted with MTBE (3 x 5 vol.). The combined organic extracts were washed with sat. aq. $NaHCO_3$ (5 vol.), dried over Na_2SO_4 and concentrated under reduced pressure to give the desired product (89% yield) without further purification (diastereoselectivity 13:1).



Ile-hydroxy-Ester Ile-hydroxy-acid

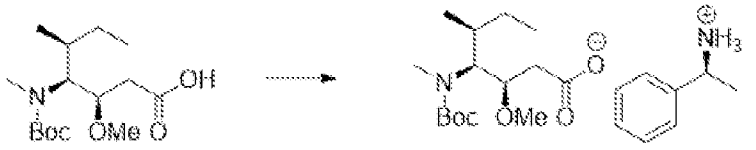
[0097] To a RT solution of Ile-hydroxy-ester (1 eq.) in EtOH (5 vol.) was charged 10% aqueous NaOH (1.05 eq.) and diluted with water (4 vol.). The reaction was stirred at RT for 2.5 h after which time HPLC indicated the reaction was complete. The EtOH was removed under reduced pressure and the basic aqueous phase extracted with MTBE (2 x 5 vol.). The combined organic phases were extracted with 10% aqueous NaOH (0.5 vol.) and the aqueous extract combined with the product containing basic aqueous phase. The combined aqueous phase was acidified with

H_3PO_4 (85 wt%, 1.5 eq.) to adjust the pH to 4. The acidified aqueous phase was extracted with MTBE (3 x 5 vol.). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to a constant weight (99% yield).

[0098] The weight of the isolated product was used in the subsequent crystallization. The crude Ile-hydroxy-acid was dissolved in MTBE (2 vol.) and Heptane (2 vol.) and the solution warmed to NMT 55 °C. Heptane (4 vol.) was charged maintaining the temperature NLT 50 °C. After the addition the mixture was cooled to 45 °C. Upon cooling to 45 °C the crystallization was initiated, (either spontaneous, or addition of 0.5 wt% of seed). After crystallization initiation was observed the mixture was held at 45 °C for 2 h then cooled to RT. After cooling to RT the slurry was stirred vigorously for 12 h then isolated by filtration. The filter cake was washed with heptane (2 vol.) and dried under vacuum. The product was isolated in 75% yield with a dr > 99:1.

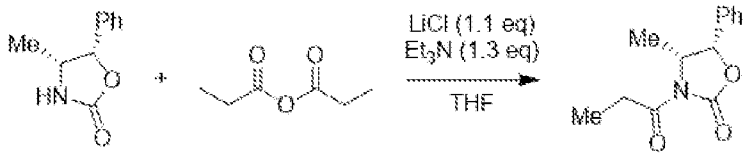
Ile-hydroxy-acid N-Boc-Dil

[0099] A solution of Ile-hydroxy-acid (1 eq.) in anhydrous THF (20 vol.) was cooled to NMT -50°C and Me_2SO_4 (2.05 eq.) was charged maintaining the temperature NMT -50 °C. LiHMDS (1.0 M in THF, 3.3 eq.) was charged maintaining the temperature NMT -50 °C. The reaction was allowed to warm to RT and stirred overnight. The reaction was quenched by the addition of 10% aqueous NaOH (10 eq.) and stirred for 12 h. The mixture was concentrated under reduced pressure to remove THF then the basic aqueous phase was extracted with MTBE (2 x 5 vol.). The aqueous phase was adjusted to pH 4 by the addition of H_3PO_4 and extracted with MTBE (3 x 5 vol.). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure, the crude material was isolated in good yield. N-Boc-Dil was further purified by crystallization in the subsequent step.

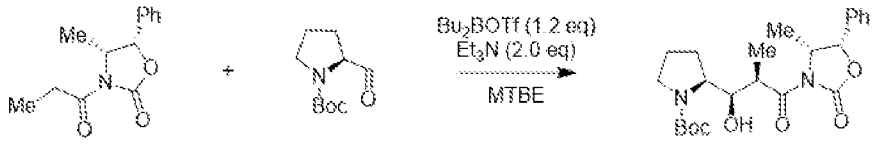


N-Boc-Dil N-Boc-Dil-Methylbenzylamine

[0100] N-Boc-Dil (1 eq.) was dissolved in heptane (10 vol.) and heated to 50 °C. S-α-methylbenzylamine (1 eq.) was charged and the reaction cooled to 35 °C. After stirring at 35 °C for 1 h the reaction was cooled to RT and stirred vigorously for 12 h. The solid was isolated by filtration and washed with heptane (2 vol.) to give the salt in high yield (70%).

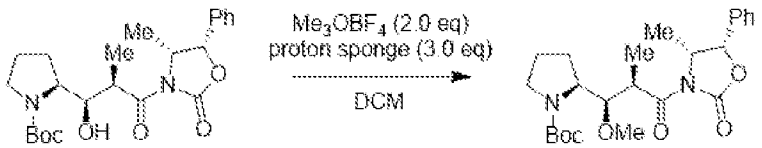


[0101] (4R,5S)-4-methyl-5-phenyl-3-propionyloxazolidine. A solution of (4R,5S)-(+)-4-methyl-5-phenyl-2-oxazolidinone (100.0 g, 0.564 mol, 1 equiv) in 30 vol. of tetrahydrofuran was cooled to -20 °C in a 70% water-MeOH/dry ice bath. To the precooled solution was added lithium chloride (26.32 g, 0.621 mol, 1.1 equiv) followed by triethylamine (102.2 mL, 0.734 mol, 1.3 equiv) at such a rate to keep the temperature below -15 °C. Propionic anhydride (86.82 mL, 0.677 mol, 1.2 equiv) was then added over 30 min to keep the temperature below -15 °C. The mixture was then removed from the bath and stirred at room temperature overnight (~15 h). Once the reaction was complete (confirmed by LCMS or HPLC), the mixture was concentrated under reduced pressure. The mixture was then partitioned between 500 mL of ethyl acetate and 500 mL of 0.2 M hydrochloric acid. The organic layer was then collected and washed with 2 x 100 mL of 1 M sodium bicarbonate and 2 x 100 mL brine. The organic layer was collected and dried over sodium sulfate. Concentration under reduced pressure afforded the final product (130.1 g, 0.558 mol, 99%) as a viscous, slightly yellow oil. No purification was required.



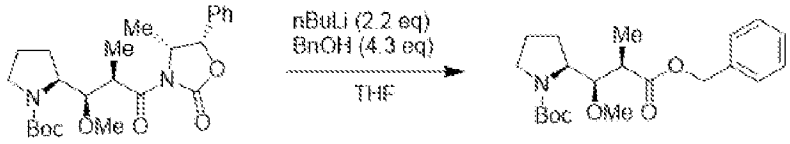
[0102] N-Boc-Pro-Xc. A solution of (4R,5S)-4-methyl-5-phenyl-3-propionyloxazolidine (35.9 g, 0.164 mol, 1.3 equiv) in 4 vol of MTBE was cooled to 0 °C in an ice bath. The cooled solution was charged with triethylamine (34.8 mL, 0.201 mol, 2.0 equiv) followed by careful addition of 1.0 M dibutylboryl trifluoromethanesulfonate solution in dichloromethane (150.0 mL, 0.150 mol, 1.20 equiv) at such a rate to keep the temperature below 5 °C. The mixture was allowed to

stir for 5 hrs. After 5 hrs, the solution was cooled to -78 °C in an acetone/dry ice bath. Next a solution of Boc-L-prolinal (25.0 g, 0.125 mol, 1.0 equiv) in 25 mL of MTBE was prepared and cooled to -78 °C. The cooled solution was then cannulated to the reaction mixture at such a rate to keep the temperature below -60°C. The reaction was allowed to slowly warm to room temperature for 24 hrs. The reaction was then quenched with phosphate buffer (pH=7, 50 mL), followed by the addition of 75 mL of methanol, and then cooled to 0 °C. To the cooled solution was added a solution of 30% aqueous hydrogen peroxide and methanol (1:2, 100 mL) at such a rate to keep the internal temperature below 10 °C. The reaction was then stirred at 0 °C for 1 hr. The reaction was quenched with 100 mL of water, then concentrated under reduced pressure. The aqueous mixture was then extracted with MTBE (2 x 100 mL) and the organic layers are combined. The organic layer was then washed with 1 M potassium bisulfate (2 x 30 mL), saturated sodium bicarbonate (2 x 30 mL), and brine (2 x 30 mL). The organic layer was then collected and dried over sodium sulfate then concentrated under reduced pressure. The crude product was then purified via Si column chromatography using 10% to 30% ethyl acetate in heptane to afford the syn-adduct (11.62 g, 0.027 mol, 22%) as a white foam.

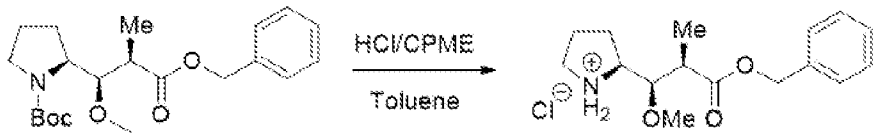


[0103] N-Boc-Dap-Xc. A solution of N-Boc-Dap-Xc (19.0 g, 0.044 mol, 1.0 equiv) in 5 vol of dichloromethane was cooled to 0 °C in an ice bath. To the cooled solution was added proton-sponge (28.3 g, 0.132 mol, 3.0 equiv) followed by trimethyloxonium tetrafluoroborate (12.85 g, 0.087 mol, 2.0 eq). The reaction mixture was allowed to warm to room temperature overnight (~18 h). The reaction was cooled to 0 °C and quenched by the addition of 30 mL of water that has been cooled to 0 °C. The reaction was then filtered through a pad of celite, and the filtrate was evaporated under reduced pressure and partitioned between 250 mL of MTBE and 200 mL of water. The organic layer was separated, and the aqueous layer was extracted with 3 x 20 mL of MTBE. The organic layers were combined and washed with 1 M aqueous potassium bisulfate solution (2 x 20 mL), saturated sodium bicarbonate solution (2 x 20 mL), and brine (2 x 20 mL). The organic layer was then dried over sodium sulfate and concentrated under reduced pressure to afford the crude product as an oil. The product was then purified

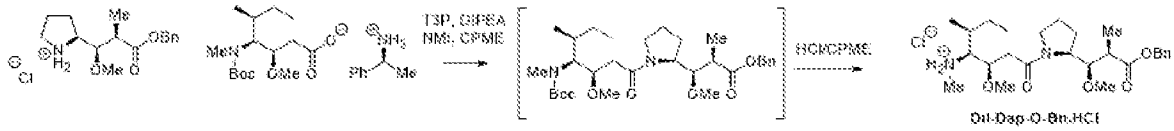
via Si column chromatography (0% to 15% ethyl acetate in heptane). N-Boc-Dap-Xc (11.54 g, 0.026 mol, 59%) was isolated as a clear oil.



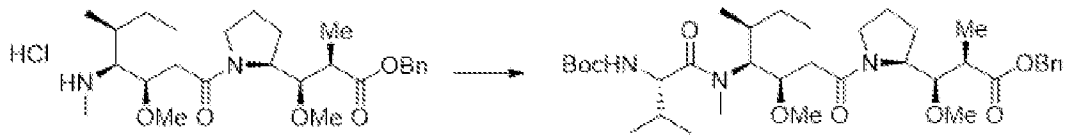
[0104] N-Boc-Dap-OBn. A solution of n-BuLi (2.5M in hexanes, 4.93 mL, 0.012 mol, 2.2 equiv) in THF (9.2 mL) was cooled to 0 °C. Benzyl alcohol (2.55 mL, 0.025 mol, 4.3 eq) was added dropwise to the solution. The reaction was allowed to stir at 0 °C for 1 hr. Meanwhile, a 0.2 M solution of N-Boc-Dap-Xc (2.56 g, 5.73 mmol, 1.0 equiv) in THF was prepared and cooled to 0 °C. The BnOLi-BnOH was cannulated into the N-Boc-Dap-Xc solution at such a rate to maintain the reaction temperature below 5 °C. The reaction was allowed to stir for 1-3 h, until complete by HPLC. The reaction mixture was diluted with 15 mL of ethyl acetate and quenched via the slow addition of 15 mL of sodium bicarbonate. The organic layer was separated and washed with an additional 15 mL of sodium bicarbonate followed by 2 x 15 mL of water and 2 x 15 mL of brine. The organic layer was then collected and dried over sodium sulfate. The solution was concentrated under rotary evaporation to afford the crude product. The crude product was purified via Si column chromatography using a 0-50% EtOAc/Heptane gradient. The product (1.78 g, 4.72 mmol, 82%) was isolated as a clear oil.



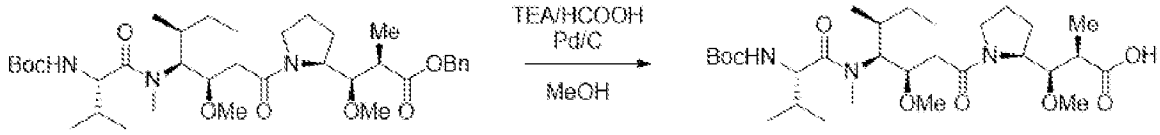
[0105] OBn-Dap-HCl Salt. N-Boc-Dap-OBn (5.7 g, 15 mmol, 1.0 equiv) was dissolved in 5 vol of toluene. To the solution was added 3 M hydrogen chloride solution in CPME (12.6 mL, 2.5 equiv). The reaction mixture was allowed to stir overnight. Upon completion, the solution was concentrated under rotary evaporation. The crude material was taken up in 5 vol of toluene, and heated to 80 °C then allowed to cool to RT. After stirring for 12 h at RT the product was isolated by filtration. The filter cake was washed with 2 vol. of toluene to give the product as a white solid (2.9 g, >99% purity, 62%).



[0106] N-Boc-Dil.amine salt (1 eq.) was dissolved in CPME (10 vol.) and washed three times with 2.0 M HCl (5 vol.) and once with H_2O, the combined aqueous washes were extracted with CPME (10 vol.) and the combined organic layers concentrated to 10 vol then constant volume vacuum distilled until water content was <0.1%. OBn-Dap.HCl (1 eq.) was charged followed by DIPEA (3.0 eq), 1-methylimidazole (1.0 eq), and T3P (1.5 eq.). The reaction was stirred at RT O/N at which time HPLC analysis indicated the desired intermediate was formed. The reaction was washed twice with 2.0 M HCl (5 vol.) and twice with water (5 vol.) then constant volume vacuum distilled until water content was <0.1%. 3.0 M HCl in CPME (7.5 eq.) was charged and the reaction stirred at RT O/N at which time HPLC analysis indicated the desired product was formed. Constant volume vacuum distillation to remove HCl and swap the solvent to MTBE was performed and the product recrystallized from MTBE. The product was isolated by filtration as a white solid (70% yield).



[0107] Dil-Dap-OBn.HCl was dissolved in DMF (15 vol.). To this solution, was charged N-Boc-Val (1.5 eq.), DIPEA (3 eq.) and COMU (1.9 eq.). The reaction was stirred at RT O/N. After this time the reaction was quenched by the addition of 2 M aqueous HCl (10 vol.) and extracted with EtOAc (2 x 10 vol.). The combined organics were washed with 1 M aqueous $NaHCO_3$ (10 vol.), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by column chromatography (25 g/g loading) with an EtOAc in Heptane gradient. N-Boc-Val-Dil-Dap-OBn was isolated as a clear oil (72%).



N-Boc-Val-Dil-Dap-OBn N-Boc-Val-Dil-Dap-OH

[0108] N-Boc-Val-Dil-Dap-OBn was dissolved MeOH (10 vol.) and 10% Palladium on activated carbon paste type 487-10R487 (0.1 eq.) was charged. Triethylamine (10 eq.) and formic acid (9 eq.) were added and the reaction stirred at RT for 48 h. Celite (1 wt.) was added and the reaction filtered through a celite pad rinsing with MeOH. The filtrates were concentrated and purified by column chromatography (25 g/g loading) with an EtOAc in Heptane gradient with 0.1% AcOH additive. N-Boc-Val-Dil-Dap-OH was isolated in 95% yield.

[0109] Example 6. Use of universal dolastatin core in the preparation of monomethylauristatin E (MMAE)

[0110] A. Preparation of Val-Dil-Dap-(1S,2R)-(+)-norephedrine

[0111] A solution of N-Boc-Val-Dil-Dap-OH.DCHA (Example 4) (1 g, 1 equiv.) in iPrOAc (10 vol.) was washed with 4 M aqueous H_3PO_4 (2 x 2 vol.) then DI Water (2 vol.) and the organic layer dried with Na_2SO_4, filtered and concentrated under reduced pressure. To the residue was added iPrOAc (10 vol.), (1S,2R)-(+)-norephedrine (1.2 equiv.), NMI (1 equiv.), DIPEA (1.5 equiv.) and T3P (50% in EtOAc, 2 equiv.). After stirring at RT for 2 h the reaction was quenched by the addition of 2 M aqueous HCl (10 vol.) and the layers partitioned. The organic layer was washed with 2 M aqueous HCl (10 vol.) then 20% aqueous Na_2CO_3 (10 vol.), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in toluene (10 vol.) and 3 M HCl in CPME (5 equiv.). After stirring at RT for 72 h the reaction was quenched by the addition of 1 M aqueous $NaHCO_3$ to pH 9 and the layers partitioned. The aqueous layer was extracted with iPrOAc (3 x 10 vol.) and the combined organic layers dried over Na_2SO_4, filtered and concentrated to afford Val-Dil-Dap-(1S,2R)-(+)-norephedrine (650 mg, 80%) which can be utilized in subsequent steps without further purification.

[0112] B. Preparation of N-Boc-MMAE

[0113] To a stirred solution of Val-Dil-Dap-(1S,2R)-(+)-norephedrine (100 mg, 1 equiv.) in DMF (10 vol.) was charged HATU (1.5 equiv.), N-Boc-N-Me-Val-OH (1.5 equiv.), and DIPEA (2.5 equiv.). After stirring at RT for 16 h the reaction was quenched

by the addition of 2 M aqueous HCl (10 vol.). EtOAc (10 vol.) was charged and the layers partitioned. The aqueous layer was extracted with EtOAc (2 x 10 vol.) and the combined organic layers washed with 1 M aqueous $NaHCO_3$ (10 vol.). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Sfär HC 10 g) eluting with 80:20 EtOAc/Heptane to 80:20 EtOAc/EtOH gradient. Product containing fractions were combined and concentrated under reduced pressure to afford N-Boc-MMAE (130 mg, 96%) as a colorless oil.

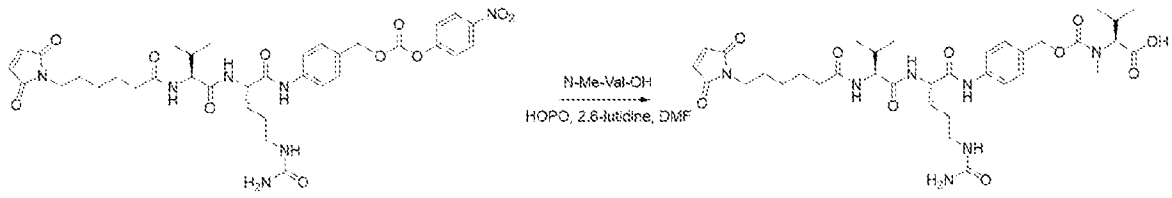
[0114] C. Preparation of MMAE

[0115] To a stirred solution of Boc-MMAE (130 mg, 1 equiv.) in toluene (20 vol.) was added 4 M HCl in dioxane (5 equiv.). After stirring at RT for 16 h the reaction was quenched by the addition of 1 M aqueous $NaHCO_3$ to pH 9 and the layers partitioned. The aqueous layer was extracted with EtOAc (3 x 10 vol.) and the combined organic layers dried over Na_2SO_4, filtered and concentrated to afford crude MMAE. The residue was purified by silica gel column chromatography (Sfär HC 10 g) eluting with 80:20 EtOAc/Heptane to 60/40 EtOAc/EtOH gradient. Product containing fractions were combined and concentrated under reduced pressure to afford MMAE (100 mg, 80%) as a colorless oil. A portion of the purified material was isolated by preparative rp-HPLC (0.05% formic acid in water and acetonitrile, YMC PackPro C18, 250 x 20 mm, 10 µm). The structure was verified by ^1H NMR (spectrum consistent with literature) and high-resolution mass spectrometry (ESI, m/z = 718.5136; calc. $[M+H]^+$ 718.5113).

[0116] Example 7. Preparation of vcMMAE from MMAE

[0117] To a stirred solution of MMAE (36 mg, 1 equiv.) in DMF (10 vol.) was added mc-Val-Cit-PABC-PNP (1.1 equiv.), HOPO (1.1 equiv.) and 2,6-lutidine (2 vol.). After stirring overnight at RT the material was purified by preparative rp-HPLC (0.05% formic acid in water and acetonitrile, YMC PackPro C18, 250 x 20 mm, 10 µm). The positive fractions were lyophilized to isolate vcMMAE (65 mg, 98%) and the structure was verified by ^1H NMR (spectrum consistent with literature) and high-resolution mass spectrometry (ESI, m/z = 1316.7820; calc. $[M+H]^+$ 1316.7864)

[0118] Example 8. Preparation of vcMeVal-OH



[0119] To a scintillation vial were added N-Methyl-L-valine (6.2 equiv.) and mc-Val-Cit-PABC-PNP (500 mg, 1.0 equiv.). The vial was purged with nitrogen (x 3), and the solids were suspended in 2,6-lutidine (4.0 vol.) and DMF (4.0 vol.). Solid HOPO (1.2 equiv.) was added in one portion, the vessel was sealed, and the reaction was stirred vigorously for 48 h. The reaction was poured into MTBE (200 vol.), and the resulting mixture was vacuum filtered (washing with MTBE) to deliver a grey solid. The solid was solubilized in minimal AcOH (4.0 vol.), and the resulting solution was chromatographically purified on silica gel (5% MeOH in CH_2Cl_2 to 20%) to provide mc-Val-Cit-PAB-N-Me-Val-OH as a pale-yellow residue (200 mg).

[0120] Example 9. Preparation of vcMMAE from Val-Dil-Dap-(1S,2R)-(+)-norephedrine

[0121] To a stirred solution of Val-Dil-Dap-(1S,2R)-(+)-norephedrine (50 mg, 1 equiv.) in DMF (10 vol.) was added mc-Val-Cit-PAB-N-Me-Val-OH (1.5 equiv.), HATU (1.5 equiv.) and 2,6-lutidine (10 vol.). The reaction was stirred O/N and purified by preparative rp-HPLC (0.05% formic acid in water and acetonitrile, YMC PackPro C18, 250 x 20 mm, 10 µm). The compound was isolated (20 mg, 20%) and the structure was verified by HPLC (retention time matched previous vcMMAE material) and high-resolution mass spectrometry (ESI, m/z = 1316.7766; calc. $[M+H]^+$ 1316.7864).

[0122] Example 10. Preparation of Val-Dil-Dap-Phe-OMe from universal dolastatin core

[0123] A solution of N-Boc-Val-Dil-Dap-OH.DCHA (Example 4) (0.525 g, 1 equiv.) in iPrOAc (10 vol.) was washed with 4 M aqueous H_3PO_4 (2 x 2 vol.) then DI Water (2 vol.) and the organic layer dried with Na_2SO_4, filtered and concentrated under reduced pressure. To the residue was added iPrOAc (10 vol.), L-phenylalanine methyl ester hydrochloride (1.1 equiv.), NMI (1 equiv.), DIPEA (2.5 equiv.) and T3P (50% in EtOAc, 2 equiv.). After stirring at RT for O/N the reaction was quenched by the addition of 2 M aqueous HCl (10 vol.) and the layers partitioned. The organic layer was washed with 2 M aqueous HCl (10 vol.) then 20% aqueous Na_2CO_3 (10 vol.) dried over Na_2SO_4,

filtered and concentrated under reduced pressure. The residue was dissolved in toluene (5 vol.), 1,4-dioxane (5 vol.) and 3 M HCl in CPME (5 equiv.). After stirring at RT for 16 h the reaction was quenched by the addition of 1 M aqueous $NaHCO_3$ to pH 9 and the layers partitioned. The aqueous layer was extracted with iPrOAc (3 x 10 vol.) and the combined organic layers dried over Na_2SO_4, filtered and concentrated to afford Val-Dil-Dap-Phe-OMe (350 mg, 80%) which can be utilized without further purification.

[0124] Example 11. Preparation of monomethylauristatin F (MMAF)

[0125] A. Preparation of Boc-MMAF-OMe

[0126] To a stirred solution of Val-Dil-Dap-Phe-OMe (100 mg, 1 equiv.) in DMF (10 vol.) was charged HATU (1.1 equiv.), N-Me-Val-OH (1.1 equiv.) and 2,6-lutidine (10 vol.) After stirring at RT for 2 h the reaction was quenched by the addition of 2 M aqueous HCl (10 vol.). EtOAc (10 vol.) was added and the layers partitioned. The aqueous layer was extracted with EtOAc (2 x 10 vol.) and the combined organic layers dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Sfär HC 10 g) eluting with 80:20 EtOAc/Heptane to 80:20 EtOAc/EtOH gradient. Concentration of positive fractions gave Boc-MMAF-OMe (124 mg, 93%) as a colorless residue.

[0127] B. Preparation of MMAF

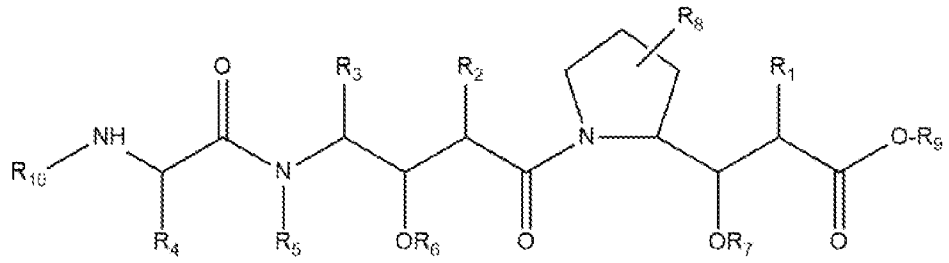
[0128] To a stirred solution of Boc-MMAF-OMe (124 mg, 1 equiv.) in toluene (8 vol.) and 1,4-dioxane (8 vol.) was added 3 M HCl in CPME (16 vol.). The reaction was stirred at RT for 16 h and quenched by the addition of 20% aqueous Na_2CO_3 to pH 11. The mixture was extracted with iPrOAc (3 x 10 vol.), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in 4 M aqueous HCl (20 vol.) and AcOH (20 vol.) and stirred at RT for 24 h. The reaction was purified by preparative rp-HPLC (0.05% formic acid in water and acetonitrile, Phenomenex Kinetex F5, 150 x 21.2 mm, 5 µm). The highest purity fractions were combined and lyophilized to yield MMAF (7 mg, 5%). The structure was verified by ^1H NMR (spectrum consistent with literature) and high-resolution mass spectrometry (ESI, m/z = 732.4901; calc. $[M+H]^+$ 732.4906).

[0129] All examples provided herein are exemplary in nature and are not meant to limit the scope of the invention as defined by the claims.

The invention claimed is:

1. A method for making a dolastatin, auristatin or related compounds comprising the steps of:

providing a compound of Formula I, or salt thereof,



I

wherein

R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo,

R_{11} and R_{12} are individually selected from the group consisting of H, C_1-C_6 alkyl,

R_6 and R_7 are each individually H or C_1-C_4 alkyl,

R_9 is H or an acid protecting group, and

R_{10} is H or an amino protecting group;

if R_9 is an acid protecting group, deprotecting the C-terminal carboxylic acid group, reacting the C-terminal carboxylic acid group with an amine (A) to form an amide bond;

if R_{10} is an amino protecting group, deprotecting the N-terminal amine, reacting the N-terminal amine with a carboxylic acid (CA) to form an amide bond.

2. The method of claim 1 wherein the steps of

if R_9 is an acid protecting group, deprotecting the C-terminal carboxylic acid group, then

reacting the C-terminal carboxylic acid group with an amine (A) to form an amide bond;

are performed before the steps of

if R_{10} is an amino protecting group, deprotecting the N-terminal amine, then

reacting the N-terminal amine with a carboxylic acid (CA) to form an amide bond.

3. The method of claim 1 wherein the steps of

if R_{10} is an amino protecting group, deprotecting the N-terminal amine, then

reacting the N-terminal amine with a carboxylic acid (CA) to form an amide bond

are performed before the steps of

if R_9 is an acid protecting group, deprotecting the C-terminal carboxylic acid group, then

reacting the C-terminal carboxylic acid group with an amine (A) to form an amide bond.

4. The method according to any of claims 1 to 3 wherein the amine (A) is selected from the group consisting of alkylamines, alkanolamines, arylalkanolamines, amino acids, amino acid derivatives, peptides and peptide derivatives,

wherein the amine (A) may have one or more substituents, and

wherein the amine (A) may have a protecting group.

5. The method according to any of claims 1 to 4 wherein the amine (A) is selected from the group consisting of phenylalanine, phenylalanine derivatives, substituted phenylalanine, substituted phenylalanine derivatives, tryptophan, tryptophan derivatives, substituted tryptophan, substituted tryptophan derivatives, phenylpropanolamine, protected phenylpropanolamine, substituted phenylpropanolamine, protected substituted phenylpropanolamine, dolaphenine and protected dolaphenine, substituted dolaphenine, protected substituted dolaphenine, dolaphenine derivatives, protected dolaphenine derivatives, substituted dolaphenine derivatives, protected dolaphenine derivatives.

6. The method according to any of claims 1 to 5 wherein the carboxylic acid (CA) is selected from the group consisting of amino acids, amino acid derivatives, peptides and peptide derivatives,

wherein the carboxylic acid (CA) may have one or more substituents, and

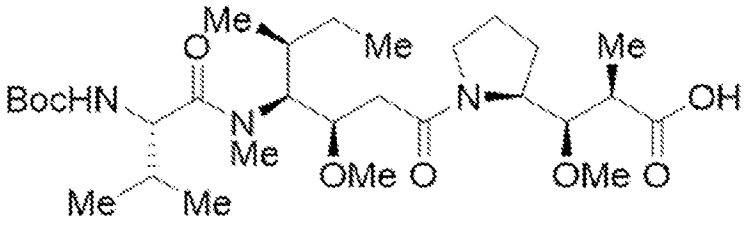
wherein the carboxylic acid (CA) may have a protecting group.

7. The method according to any of claims 1 to 6 wherein the carboxylic acid (CA) is selected from the group consisting of valine, protected valine, substituted valine, protected substituted valine, valine derivatives, protected valine derivatives, substituted valine derivatives, protected substituted valine derivatives, alanine,

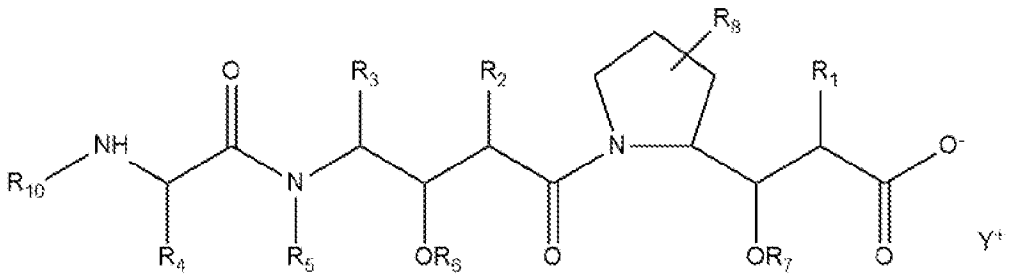
protected alanine, substituted alanine, protected substituted alanine, alanine derivatives, protected alanine derivatives, substituted alanine derivatives and protected substituted alanine derivatives.

8. The method according to any of claims 1 to 7 wherein the carboxylic acid (CA) comprises one or more of a spacer, a linker and an attachment group.

9. The method according to any of claims 1, 2 or 4 to 8 wherein the compound of Formula I is



10. An isolated salt of Formula II:



II

wherein

R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo,

R_{11} and R_{12} are individually selected from the group consisting of H and C_1-C_6 alkyl,

R_6 and R_7 are each individually H or C_1-C_4 alkyl,

R_{10} is H or an amino protecting group; and

Y^+ is counterion.

11. The isolated salt according to claim 10, wherein

R_1, R_2, R_3, R_4 R_5 and R_8 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, and iso-butyl;

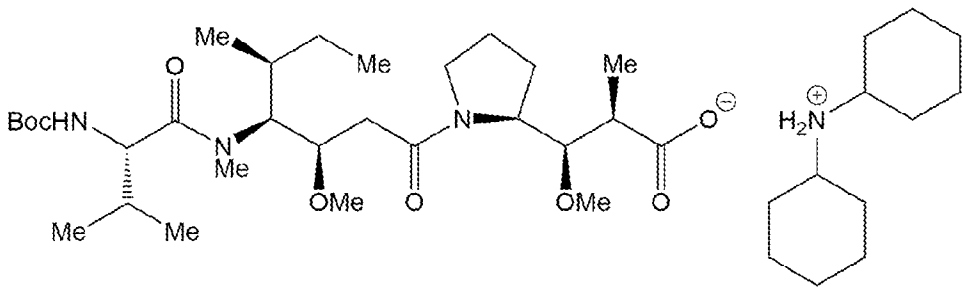
R_6 and R_7 are each individually H or methyl,

R_{10} is H or tert-butoxycarbonyl (Boc), and

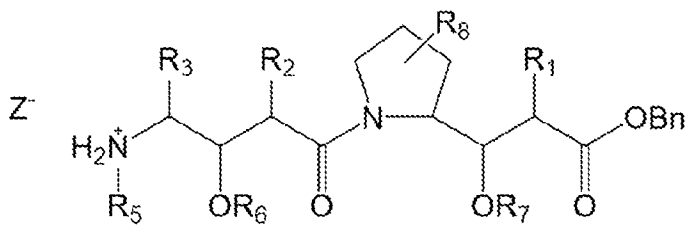
Y^+ is an ammonium ion of the formula $N^+HR_{13}R_{14}R_{15}$ wherein R_{13} is selected from the group consisting of optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; R_{14} and R_{15} are independently selected from the group consisting of H, optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; wherein each optional substituent, if present, is selected from the group consisting of alkyl and aryl.

12. The isolated salt according to either of claims 10 or 11 wherein Y^+ is selected from the group consisting diethylammonium ion, dibutylammonium ion, dicyclohexylammonium ion, methylcyclohexylammonium ion and methylbenzylammonium ion.

13. The salt according to any of claims 10 to 12, wherein the salt is



14. A compound of Formula III:



III

wherein

R_1, R_2, R_3, R_5 and R_8 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo,

R_{11} and R_{12} are individually selected from the group consisting of H and C_1-C_6 alkyl,

R_6 and R_7 are each individually selected from the group consisting of H or C_1-C_4 alkyl, and

Z^- is a counterion.

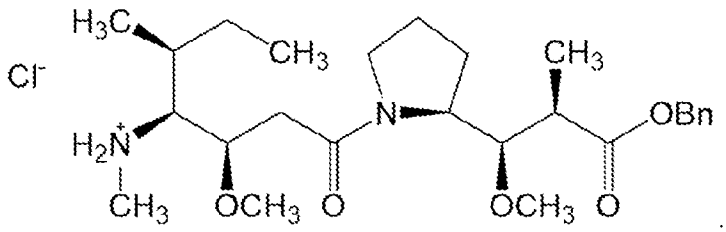
15. The compound of Formula III according to claim 14, wherein

R_1, R_2, R_3, R_5 and R_8 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl,

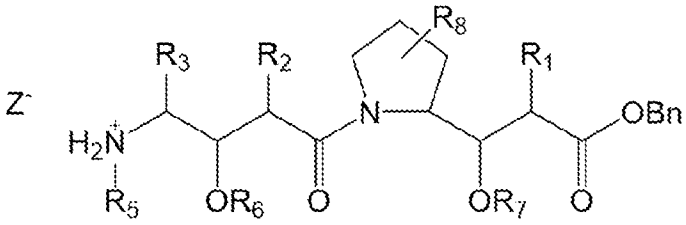
R_6 and R_7 are each individually selected from the group consisting of H and methyl, and

Z^- is selected from the group consisting of halide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, mesylate, tosylate, benzene sulfonate, ethylsulfonate, nitrate, formate, acetate, trifluoroacetate, oxalate, and citrate.

16. The compound of Formula III according to either of claims 14 or 15, wherein the compound is:



17. A method of coupling an amino acid to a compound of Formula III



III

wherein

R_1, R_2, R_3, R_5 and R_8 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo,

R_{11} and R_{12} are individually selected from the group consisting of H and C_1-C_6 alkyl,

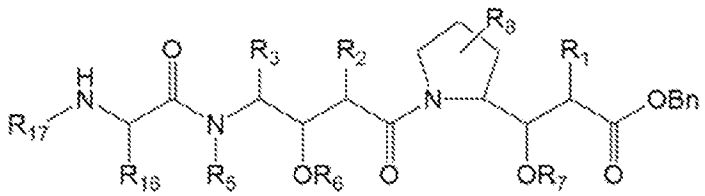
R_6 and R_7 are each individually selected from the group consisting of H or C_1-C_4 alkyl, and

Z^- is a counterion

comprising the steps of

contacting the compound of Formula III with an aqueous base to remove the counterion, and

contacting the compound of Formula III with an N-protected amino acid N-carboxyanhydride to yield a compound of Formula IV:



IV

wherein

R_1, R_2, R_3, R_5, R_6, R_7 and R_8 are defined as above,

R_{16} is an amino acid side chain, and

R_{17} is a protecting group.

18. The method according to claim 17 wherein

R_1, R_2, R_3, R_5 and R_8 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl,

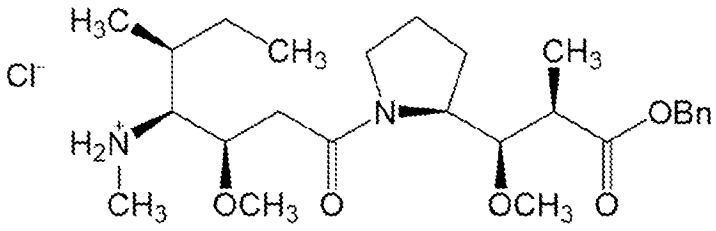
R_6 and R_7 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl,

Z^- is selected from the group consisting of halide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, mesylate, tosylate, benzene sulfonate, ethylsulfonate, nitrate, formate, acetate, trifluoroacetate, oxalate, and citrate;

the aqueous base is selected from the group consisting of Na_2CO_3, $NaHCO_3$, NaOH, Na_2HPO_4, and Na_3PO_4,

the N-protected amino acid N-carboxyanhydride is a Boc- protected amino acid N-carboxyanhydride.

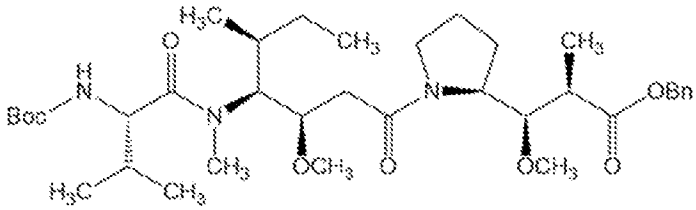
19. The method according to either of claims 17 or 18 wherein the compound of Formula III is



the aqueous base is Na_2CO_3,

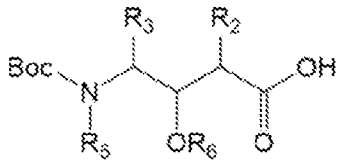
the N-protected amino acid N-carboxyanhydride is Boc-Val-NCA, and

the compound of Formula IV is:



20. A method for purifying a crude hydroxy acid of Formula V without using column chromatography, the method comprising the steps of

providing a crude hydroxy acid of Formula V



V

wherein

R_2, R_3, and R_5 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo; R_{11} and R_{12} are individually selected from the group consisting of H, C_1-C_6 alkyl, and

R_6 is selected from the group consisting of H and C_1-C_4 alkyl;

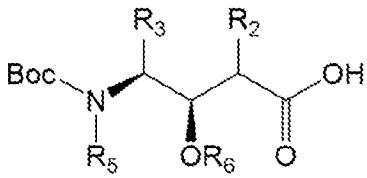
dissolving crude hydroxy acid in a soluble solvent,

adding an insoluble solvent,

initiating crystallization, and

allowing the crystallization to complete, yielding a purified hydroxy acid of Formula V.

21. The method according to claim 20, wherein the hydroxy acid of Formula V is



wherien

R_2, R_3 and R_5 are each individually selected from the group consisting of H, methyl,ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl,

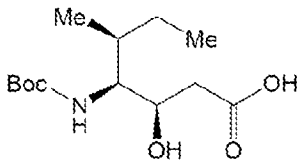
R_6 is selected from the group consisting of H and methyl,

the soluble solvent comprises tert-butyl methyl ether (MTBE),

the insoluble solvent comprises heptane, and

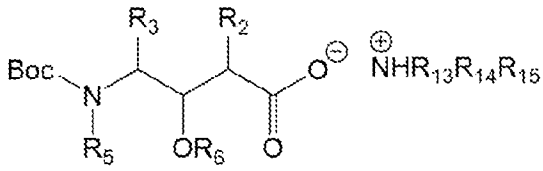
crystallization is initiated by seeding, by heat cycling or by a combination thereof.

22. The method according to either of claims 20 or 21 wherein the hydroxy acid of Formula V is



23. The method according to any of claims 20-22, further comprising the step of isolating the purified hydroxy acid of Formula V.

24. A compound of Formula VI:



VI

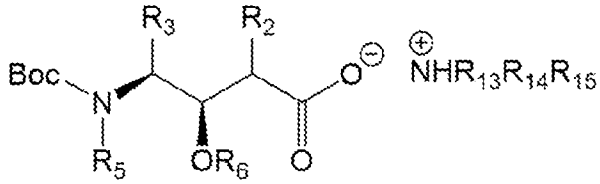
wherein

R_2, R_3, and R_5 are each individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo; R_{11} and R_{12} are individually selected from the group consisting of H, C_1-C_6 alkyl, and

R_6 is selected from the group consisting of H and C_1-C_4 alkyl; and

R_{13} is selected from the group consisting of optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; R_{14} and R_{15} are independently selected from the group consisting of H, optionally substituted C_1-C_8 alkyl and optionally substituted C_3-C_8 cycloalkyl; wherein each optional substituent, if present, is selected from the group consisting of alkyl and aryl.

25. The compound according to claim 24, wherein the compound of Formula VI is



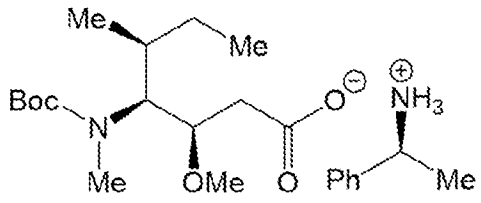
wherien

R_2, R_3 and R_5 are each individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl, and

R_6 is selected from the group consisting of H and methyl; and

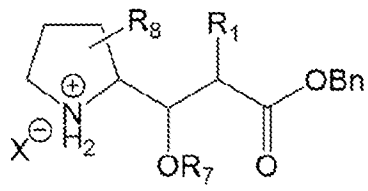
$NHR_{13}R_{14}R_{15}$ is selected from the group consisting of selected from the group consisting diethylammonium ion, dibutylammonium ion, dicyclohexylammonium ion, methylcyclohexylammonium ion and methylbenzylammonium ion.

26. The compound according to either of claims 24 or 25, wherein the compound of Formula VI is:



27. The compound according to any of claims 24-26 wherein the compound of Formula VI is a solid.

28. A compound of Formula VII



VII

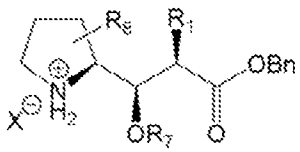
wherein

R_1 and R_8 are individually selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, -OR_{11}, -$NR_{11}R_{12}$, -SR_{11} and halo; R_{11} and R_{12} are individually selected from the group consisting of H, C_1-C_6 alkyl;

R_7 is selected from the group consisting of H or C_1-C_4 alkyl; and

X^- is selected from the group consisting of halide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, mesylate, tosylate, benzene sulfonate, ethylsulfonate, nitrate, formate, acetate, oxalate, and citrate.

29. The compound according to claim 28 wherein the compound of Formula VII is



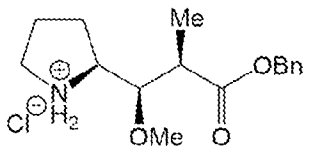
wherein

R_1 and R_8 are individually selected from the group consisting of H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, and tert-butyl,

R_7 is selected from H and methyl; and

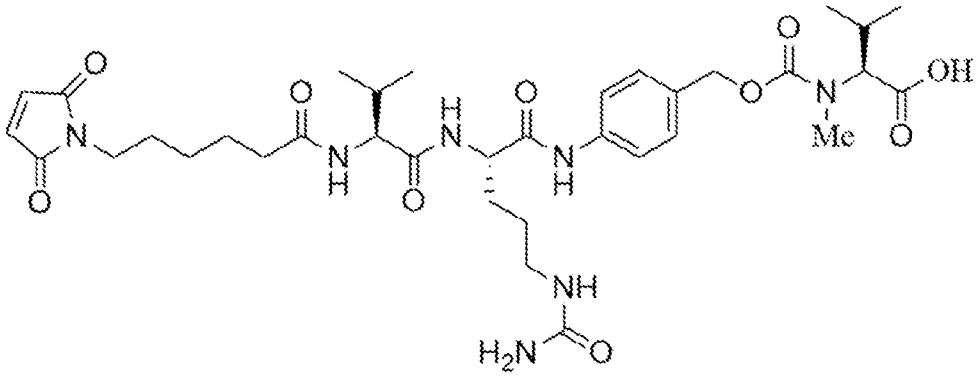
X^- is a halide.

30. The compound according to either of claims 28 or 29 wherein the compound of Formula VII is



31. The compound according to any of claims 28-30 wherein the compound of Formula VII is a solid.

32. A compound of Formula VIII:



VIII.

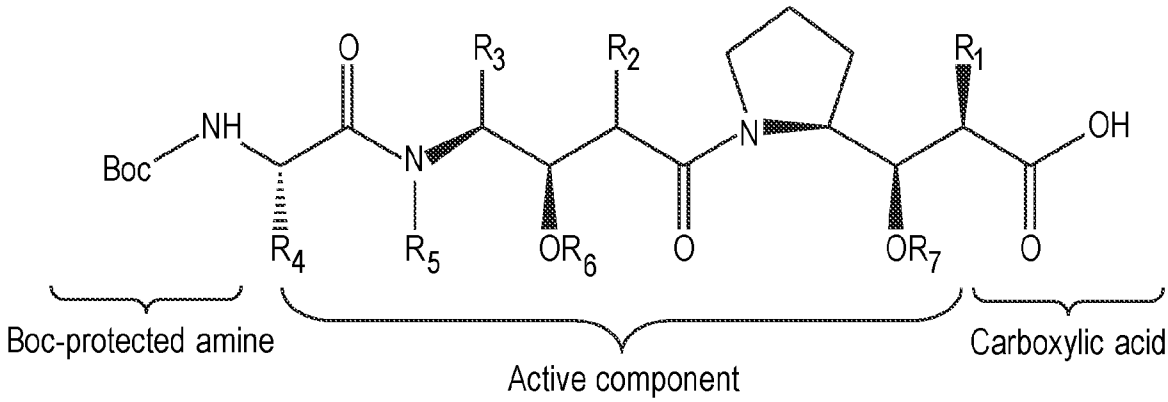

Boc
NH
O
R3
R2
R1
OH
N
N
R4
R5
OR6
O
OR7
O
Boc-protected amine
Active component
Carboxylic acid

FIG. 1

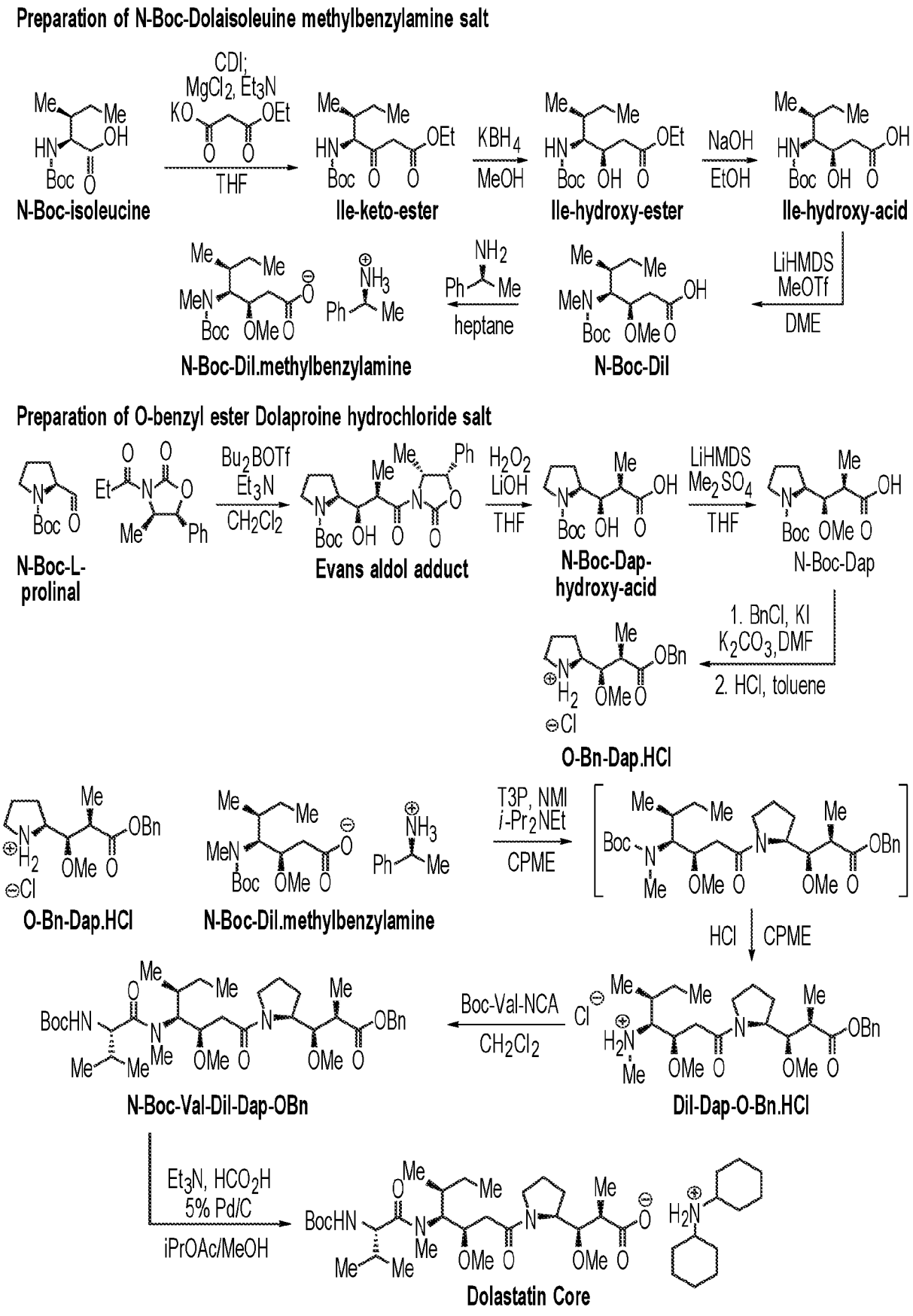
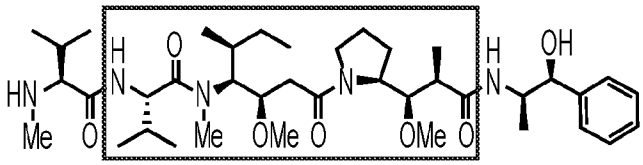
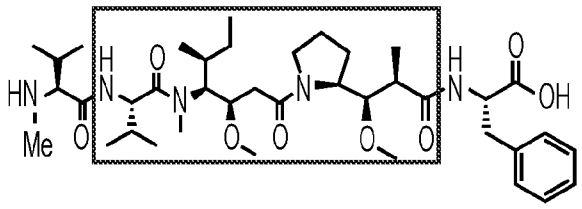

Preparation of N-Boc-Dolaisoleuine methylbenzylamine salt
CDI;
MgCl2, Et3N
THF
KBH4
MeOH
NaOH
EtOH
N-Boc-isoleucine
Ile-keto-ester
Ile-hydroxy-ester
Ile-hydroxy-acid
LiHMDS
MeOTf
DME
heptane
N-Boc-Dil.methylbenzylamine
N-Boc-Dil
Preparation of O-benzyl ester Dolaproine hydrochloride salt
Bu2BOTf
Et3N
CH2Cl2
H2O2
LiOH
THF
LiHMDS
Me2SO4
THF
N-Boc-L-prolinal
Evans aldol adduct
N-Boc-Dap-hydroxy-acid
N-Boc-Dap
1. BnCl, KI
K2CO3, DMF
2. HCl, toluene
O-Bn-Dap.HCl
T3P, NMI
i-Pr2NEt
CPME
HCl
CPME
Boc-Val-NCA
CH2Cl2
N-Boc-Val-Dil-Dap-OBn
Dil-Dap-O-Bn.HCl
Et3N, HCO2H
5% Pd/C
iPrOAc/MeOH
Dolastatin Core

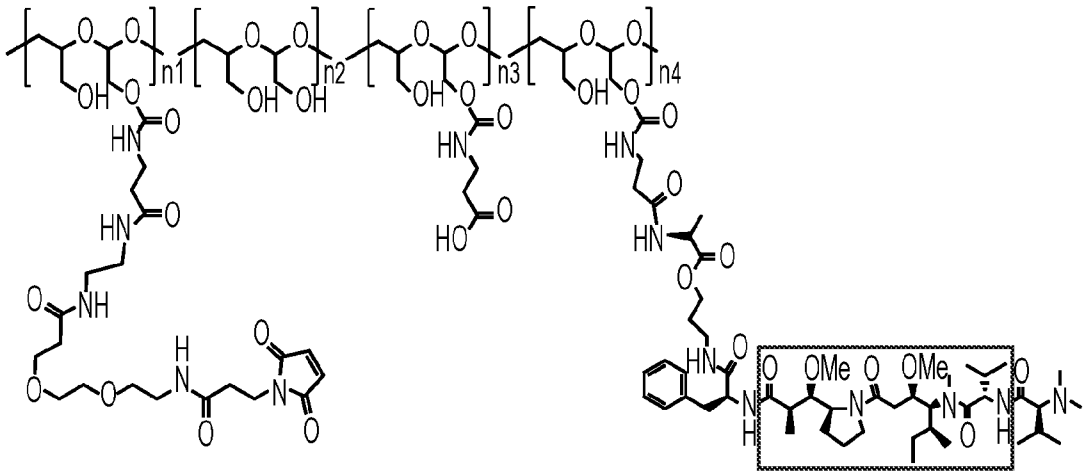
FIG. 2

(A)

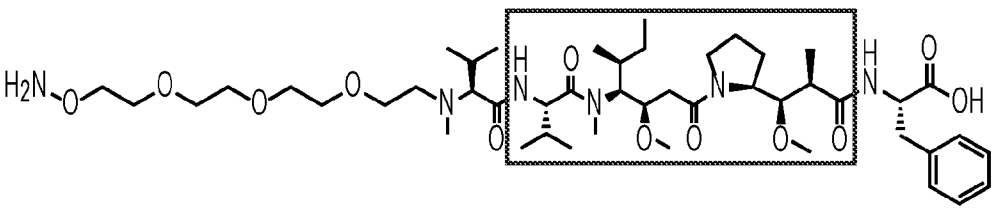




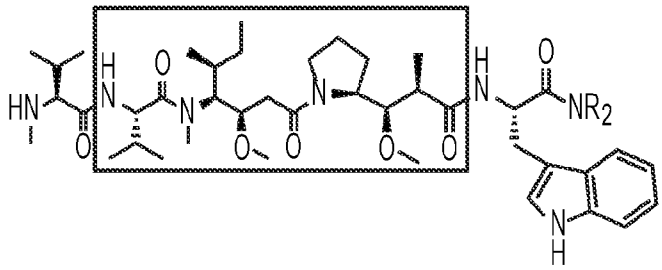
(B)



(C)

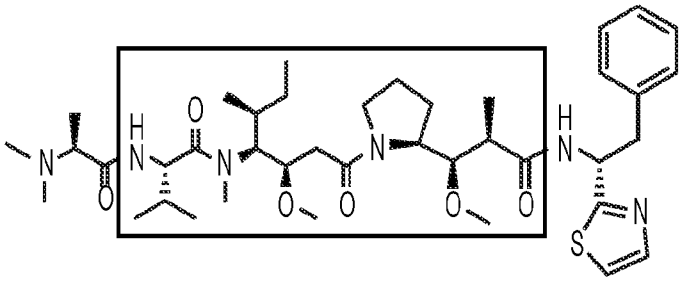


(D)

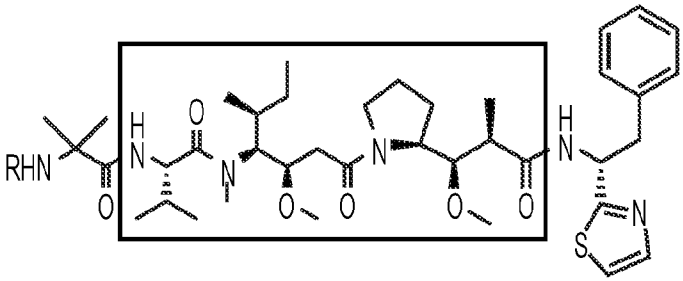


(E)

FIG. 3



(F)



(G)

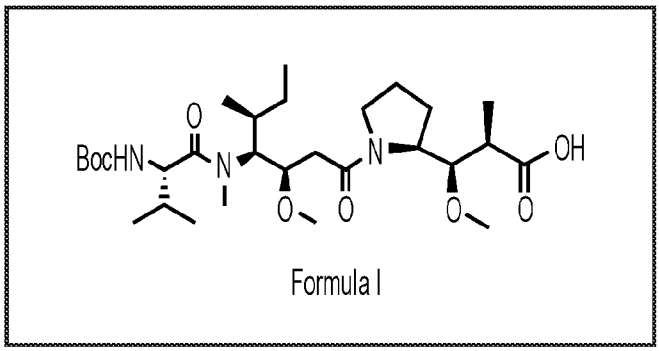


FIG. 3 (cont.)

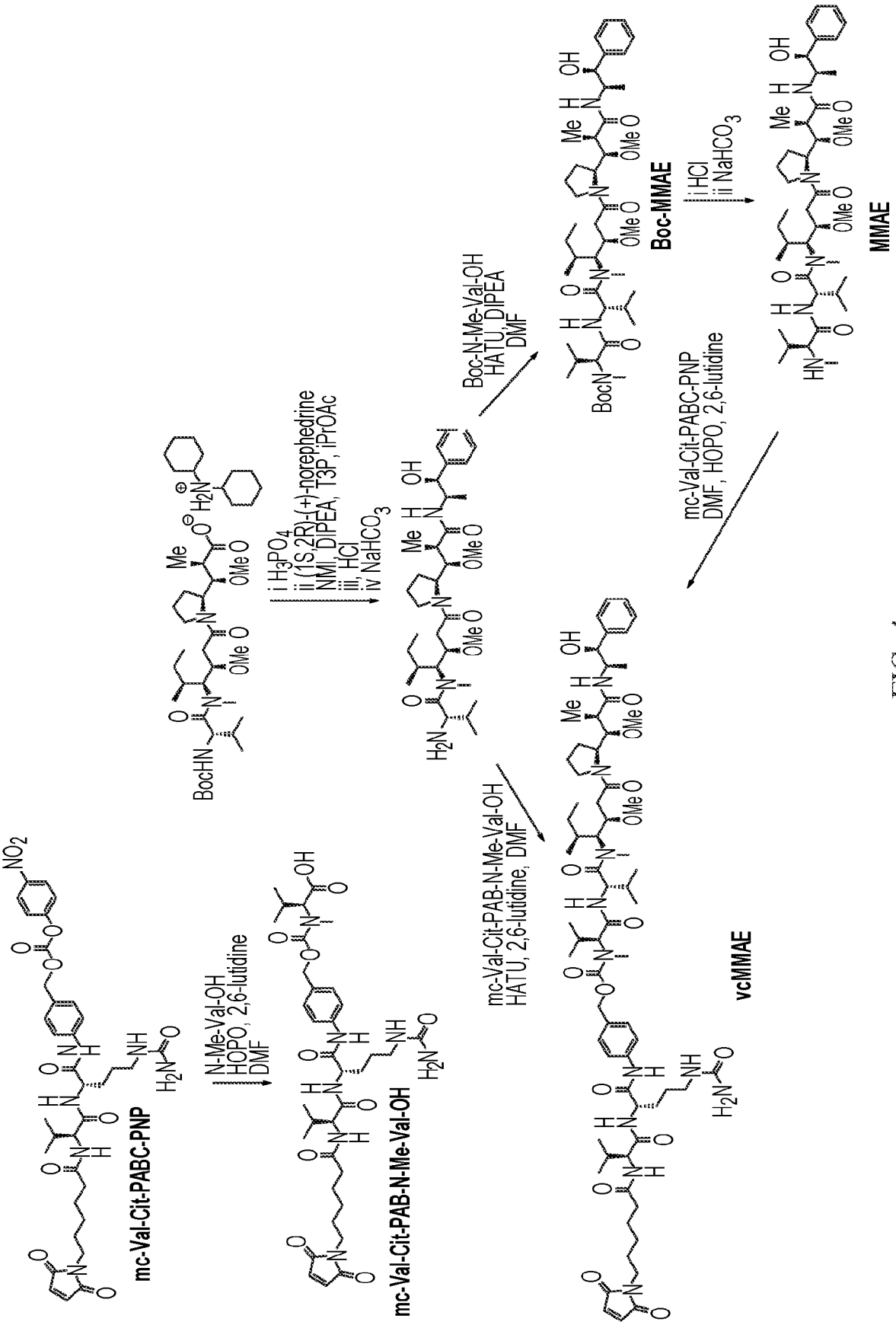

mc-Val-Cit-PABC-PNP
N-Me-Val-OH
HOPO, 2,6-lutidine
DMF
mc-Val-Cit-PAB-N-Me-Val-OH
i H3PO4
ii (1S,2R)-(+)-norephedrine
NMI, DIPEA, T3P, iPrOAc
iii, HCl
iv NaHCO3
Boc-N-Me-Val-OH
HATU, DIPEA
DMF
Boc-MMAE
i HCl
ii NaHCO3
MMAE
mc-Val-Cit-PAB-N-Me-Val-OH
HATU, 2,6-lutidine, DMF
mc-Val-Cit-PABC-PNP
DMF, HOPO, 2,6-lutidine
vcMMAE

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/021547

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K5/02 C07K5/062 C07C211/35 C07C211/63 C07C271/22
C07D207/08
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C07D C07B C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/166559 A1 (AMBRX INC [US]; MIAO ZHENWEI [US] ET AL.) 6 December 2012 (2012-12-06)	1,2,4-12
A	e.g. examples 4, 6	13
A	WO 2013/173391 A1 (CONCORTIS BIOSYSTEMS CORP [US]) 21 November 2013 (2013-11-21) example 1	1,2,4-13

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 June 2021	13/08/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schleifenbaum, A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/021547

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

2, 9-13(completely); 1, 4-8(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 2, 9-13(completely); 1, 4-8(partially)

   Method of making a dolastatin (or related compound) by reacting the C-terminal group before the N-terminal group

   ---

2. claims: 3, 14-19(completely); 1, 4-8(partially)

   Method of making a dolastatin (or related compound) by reacting the N-terminal group before the C-terminal group

   ---

3. claims: 20-23

   Method of purifying compound V

   ---

4. claims: 24-31

   Compound VI and (VII)

   ---

5. claim: 32

   Compound of formula (VIII)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/021547

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012166559 A1	06-12-2012	AU 2012262559 A1	09-05-2013
		AU 2016204081 A1	07-07-2016
		AU 2017216489 A1	31-08-2017
		BR 112013030362 A2	06-09-2016
		CA 2837167 A1	06-12-2012
		CN 103702996 A	02-04-2014
		CN 110078789 A	02-08-2019
		DK 2714685 T3	09-01-2017
		EP 2714685 A1	09-04-2014
		EP 3170821 A1	24-05-2017
		ES 2611359 T3	08-05-2017
		IL 229353 A	31-03-2019
		JP 6215197 B2	18-10-2017
		JP 2014516987 A	17-07-2014
		KR 20140037105 A	26-03-2014
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		MX 371526 B	31-01-2020
		NZ 618261 A	29-04-2016
		SG 195183 A1	30-12-2013
		SG 10201605293P A	30-08-2016
		US 2015080559 A1	19-03-2015
		WO 2012166559 A1	06-12-2012
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		CN 104640572 A	20-05-2015
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		EP 2849790 A1	25-03-2015
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		EP 2850059 A1	25-03-2015
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		EP 3590541 A1	08-01-2020
		ES 2734259 T3	05-12-2019
		HK 1208163 A1	26-02-2016
		HK 1208165 A1	01-04-2016
		HK 1208216 A1	26-02-2016
		JP 6239597 B2	29-11-2017
		JP 6239598 B2	29-11-2017
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