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(54) Title: THIOPEPTIDE COMPOUNDS

(57) Abstract: Formula (I) The present invention relates to compounds of Formula (I) or a pharmaceutically acceptable salt, metabolite, prodrug, or stereoisomer thereof. The present invention also relates to a method for isolating a compound as disclosed herein comprising the steps of: (i) expanding a Nonomuraea jiangxiensis strain under culture conditions that allow expansion of the strain to obtain a seed culture; (ii) using the seed culture to inoculate a fermentation culture; (iii) incubating the fermentation culture; and (iv) isolating the compound from the fermentation culture. The present invention further relates to a method for treating a bacterial, microbial and/or fungal infection, comprising administering a therapeutically effective amount of a compound as defined herein, or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, to a subject in need of treatment.



THIOPEPTIDE COMPOUNDS

Technical Field

The present disclosure generally relates to thiopeptide compounds, and more particularly relates to thiopeptide compounds, their methods of production and uses thereof.

Background Art

Antimicrobial resistance is one of the leading threats to human health globally. High rates of resistance against antibiotics used to treat common infections have been observed worldwide. Recently, it was estimated that 1.2 million people died from antibiotic-resistant bacterial infections, which was more than that caused by HIV/AIDS or malaria. Notably, *Staphylococcus aureus* is one of the leading pathogens (the second after *Escherichia coli*) for fatalities associated with resistance. Strains of *S. aureus* resistant to antistaphylococcal antibiotics, also known as methicillin-resistant *Staphylococcus aureus* (MRSA), have become increasingly common as the cause of infections. MRSA treatments involve second-line antibacterials such as vancomycin, that may come with more serious side effects. The World Health Organization (WHO) estimates MRSA proportions to exceed 20% globally, and that people with MRSA infections are 64% more likely to die than people with drug-sensitive infections.

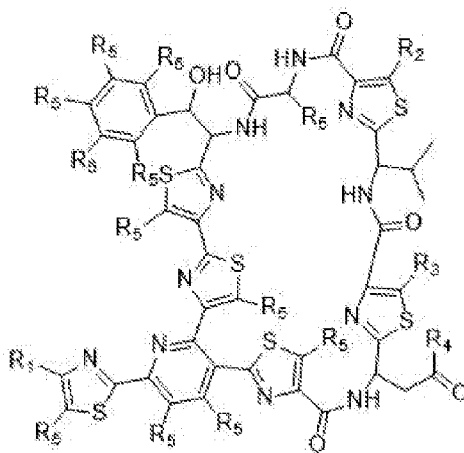
The misuse and overuse of antimicrobial agents in humans, animals, and agriculture are some of the leading causes of the development of resistance. Factors such as incomplete courses of antibiotics, widespread use of antibiotics in livestock and aquaculture, and lack of proper hygiene and infection control practices contribute to the problem.

Addressing antimicrobial resistance requires a comprehensive, multi-sectoral approach that involves both human and animal health sectors, as well as the environment. Strategies include promoting appropriate use of antimicrobials, developing new drugs and treatment regimens, implementing infection prevention and control measures, and raising awareness among healthcare providers, patients, and the general public.

However, there has been a decline in the development of new antimicrobial agents in recent years, partially due to the high cost and low profitability associated with their development. Therefore, there is a pressing need to create new antimicrobials that are effective against resistant strains and have fewer side effects.

Summary

In an aspect of the present disclosure, there is provided a compound of Formula (I) or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof:



Formula (I)

wherein:

R_1 is $-C(O)-R_6$ or $-A-G-J-L$;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or $-C(O)N(R_{7a})-$;

G is $-C(O)-$ or optionally substituted $-alkylene-C(O)-$;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is $-C(O)-R_6$;

R_2 is $-H$, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R_3 is $-H$, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

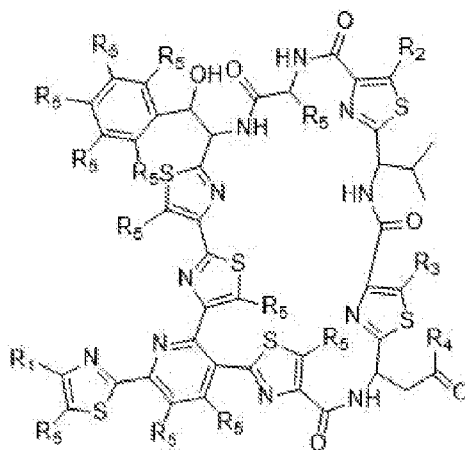
R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl,

wherein when R₂ is optionally substituted alkalkoxy, R₃ is -H, optionally substituted alkenyl, or optionally substituted alkynyl.

In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising a therapeutically effective amount of a compound disclosed herein, or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, and a pharmaceutically acceptable excipient.

In a further aspect of the present disclosure, there is provided a method of treating a bacterial, microbial and/or fungal infection, comprising administering a therapeutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, to a subject in need of treatment:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

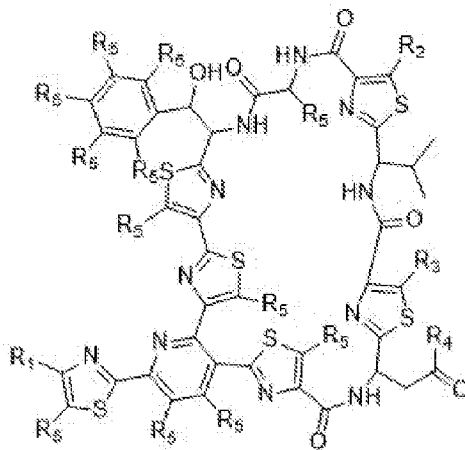
R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

In another aspect of the present disclosure, there is provided a method of treating cancer, comprising administering a therapeutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, to a subject in need of treatment:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

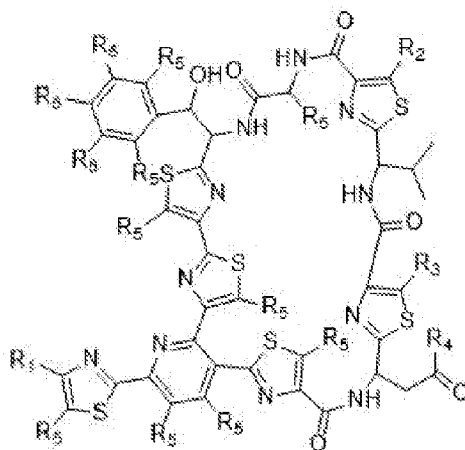
R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

In a further aspect of the present disclosure, there is provided a method of isolating a compound of Formula (I),



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylenc-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl,

the method comprising:

- (i) expanding a *Nonomuraea jiangxiensis* strain under culture conditions that allow expansion of the strain to obtain a seed culture;
- (ii) using the seed culture to inoculate a fermentation culture;
- (iii) incubating the fermentation culture; and
- (iv) isolating the compound from the fermentation culture.

Definitions

Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature used in connection with, and techniques of, chemistry described herein, are those well-known and commonly used in the art.

Unless otherwise indicated, compounds disclosed and/or described herein include all possible enantiomers, diastereomers, meso isomers and other stereoisomeric forms, including racemic mixtures, optically pure forms and intermediate mixtures thereof. Enantiomers, diastereomers, meso isomers and other stereoisomeric forms may be prepared using chiral synthons or reagents, or resolved using conventional techniques. Unless specified otherwise, when the compounds disclosed and/or described herein contain olefinic double bonds or other centers of geometric asymmetry, it is intended that the compounds include both E and Z isomers. When the compounds described herein contain moieties capable of tautomerization, and unless specified otherwise, it is intended that the compounds include all possible tautomers.

As used herein, the term “isomers” refers to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the structural arrangement or configuration of the atoms.

As used here, the term “tautomer” refers to one of two or more structural isomers that exist in equilibrium and which are readily converted from one isomeric form to another. It will be apparent to one skilled in the art that certain compounds of this disclosure may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the disclosure.

Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the disclosure.

As used herein, the term "alkyl" includes within its meaning monovalent (“alkyl”) and divalent (“alkylene”) straight chain or branched chain saturated aliphatic groups having from 1 to 12 carbon atoms, eg, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 carbon atoms. For example, the term alkyl includes, but is not limited to, methyl, ethyl, 1-propyl, isopropyl, 1-butyl, 2-butyl, isobutyl, tert-butyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-

dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, undecyl, dodecyl and the like. Alkyl groups may be optionally substituted.

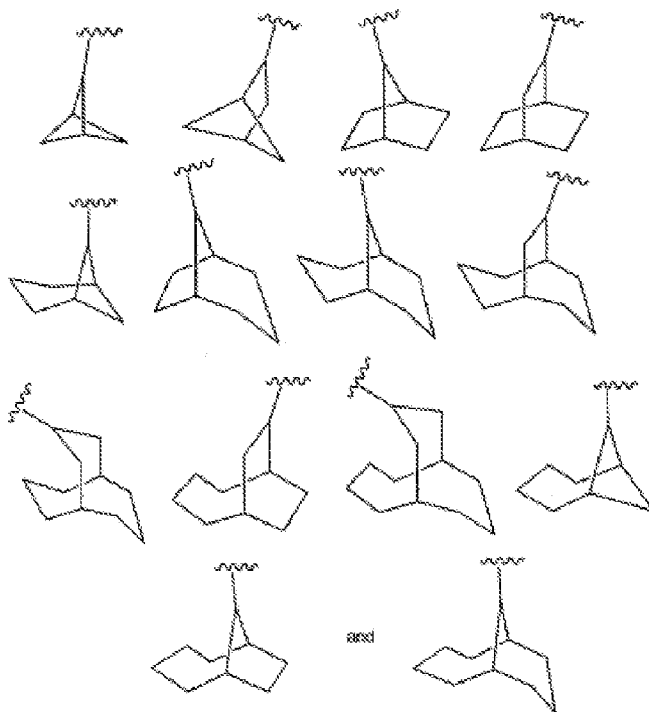
As used herein, the term "alkenyl" includes within its meaning monovalent ("alkenyl") and divalent ("alkenylene") straight chain or branched chain unsaturated aliphatic groups containing at least one carbon-carbon double bond and having from 2 to 12 carbon atoms, eg, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 carbon atoms. For example, the term alkenyl includes, but is not limited to, ethenyl, propenyl, butenyl, 1-butenyl, 2-butenyl, 2-methylpropenyl, 1-pentenyl, 2-pentenyl, 2-methylbut-1-enyl, 3-methylbut-1-enyl, 2-methylbut-2-enyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 2,2-dimethyl-2-butenyl, 2-methyl-2-hexenyl, 3-methyl-1-pentenyl, 1,5-hexadienyl and the like. Alkenyl groups may be optionally substituted.

As used herein, the term "alkynyl" includes within its meaning monovalent ("alkynyl") and divalent ("alkynylene") unsaturated aliphatic groups containing at least one carbon-carbon triple bond and having from 2 to 12 carbon atoms, eg, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 carbon atoms. For example, the term alkynyl includes, but is not limited to, ethynyl, propynyl, 1-butylnyl, 2-butylnyl, 1-pentylnyl, 2-pentylnyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 3-methyl-1-pentylnyl, and the like. Alkynyl groups may be optionally substituted.

The term "carbocyclene" as used herein, refers to a divalent non-aromatic, aromatic, mono- or multi-cyclic ring system comprising at least 3 carbons. Correspondingly, it is understood that the term "heterocarbocyclene" or "heterocyclene" refers to a divalent non-aromatic, aromatic, mono- or multi-cyclic ring system comprising at least 3 carbons and has at least one heteroatom in the ring system. It is also understood that the term can encompass "cycloalkylene", which is a non-aromatic mono- or multi-cyclic ring system, "arylene" which is an aromatic mono- or multi-cyclic ring system, "heterocycloalkylene" which is a non-aromatic mono- or multi-cyclic ring system having at least one heteroatom in the ring system, or "heteroarylene" which is an aromatic mono- or multi-cyclic ring system having at least one heteroatom in the ring system.

The term "aryl", or variants such as "aromatic group" or "arylene" as used herein refers to monovalent ("aryl") and divalent ("arylene") single, polynuclear, conjugated and fused residues of aromatic hydrocarbons having from 6 to 10 carbon atoms. Such groups include, for example, phenyl, biphenyl, naphthyl, phenanthrenyl, and the like. Aryl groups may be optionally substituted.

The term "cycloalkyl" or variants such as "cycloalkylene" as used herein refers to monovalent ("cycloalkyl") and divalent ("cycloalkylene") non-aromatic mono- or multicyclic ring system comprising about 3 to about 10 carbon atoms. The cycloalkyl can be optionally substituted with one or more "ring system substituents" which may be the same or different, and are as defined herein. Non-limiting examples of suitable monocyclic cycloalkyls include cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl and the like. Non-limiting examples of suitable multicyclic cycloalkyls include 1-decalinyl, norbornyl, adamantyl and the like. Further non-limiting examples of cycloalkyl include the following:



As used herein, "heterocycloalkyl" or variants such as "heterocycloalkylene" as used herein refers to a cycloalkyl or cycloalkylene, where one or more of the atoms in the ring system is not a carbon atom, namely a heteroatom that is selected from N, O, P and S. Heterocycloalkyl groups may be optionally substituted.

The term "heteroaryl" or variants such as "heteroarylene" as used herein refers to monovalent ("heteroaryl") and divalent ("heteroarylene") aromatic monocyclic or multicyclic ring system comprising about 5 to about 14 ring atoms, preferably about 5 to about 10 ring atoms, in which one or more of the ring atoms is an element other than carbon, for example nitrogen, oxygen or sulfur, alone or in combination. "Heteroaryl" may also include a heteroaryl as defined above fused to an aryl as defined above. Non-limiting examples of suitable heteroaryls include pyridyl, pyrazinyl, furanyl, thienyl, pyrimidinyl, pyridone (including N-substituted pyridones), isoxazolyl, isothiazolyl, oxazolyl,

thiazolyl, pyrazolyl, furazanyl, pyrrolyl, pyrazolyl, triazolyl, 1,2,4-thiadiazolyl, pyrazinyl, pyridaziny, quinoxaliny, phthalaziny, oxindolyl, imidazo[1,2-a]pyridiny, imidazo[2,1-b]thiazolyl, benzofurazanyl, indolyl, azaindolyl, benzimidazolyl, benzothienyl, quinoliny, imidazolyl, thienopyridyl, quinazolinyl, thienopyrimidyl, pyrrolopyridyl, imidazopyridyl, isoquinoliny, benzoazaindolyl, 1,2,4-triazinyl, benzothiazolyl and the like. The term "heteroaryl" also refers to partially saturated heteroaryl moieties such as, for example, tetrahydroisoquinolyl, tetrahydroquinolyl and the like. Heteroaryl groups may be optionally substituted.

The term "carbocycle", or variants such as "carbocyclic ring" or "carbocyclylene" as used herein refers to monovalent ("carbocycle") and divalent ("carbocyclylene") any stable 3, 4, 5, 6, or 7-membered monocyclic or bicyclic or 7, 8, 9, 10, 11, 12, or 13-membered bicyclic or tricyclic, any of which may be saturated, partially unsaturated, or aromatic. Examples of such carbocycles include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, adamantyl, cyclooctyl, [3.3.0]bicyclooctane, [4.3.0]bicyclononane, [4.4.0]bicyclodecane (decalin), [2.2.2]bicyclooctane, fluorenyl, phenyl, naphthyl, indanyl, adamantyl, or tetrahydronaphthyl (tetralin). Preferred carbocycles, unless otherwise specified, are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl, naphthyl, and indanyl. When the term "carbocycle" is used, it is intended to include "aryl". Carbocycles may be optionally substituted.

As used herein, "heterocarbocyclyl", "heterocyclylene" or "heterocarbocyclylene" refers to a carbocycle or carbocyclylene, where one or more of the atoms in the ring system is not a carbon atom, namely a heteroatom that is selected from N, O, P and S. Heterocarbocyclyl groups may be optionally substituted.

While "alkyl", "alkenyl", "alkynyl", "carbocyclyl", "heterocarbocyclyl", "cycloalkyl", "heterocycloalkyl", "aryl", "heteroaryl" are end-of-chain groups, the corresponding "alkylene", "alkenylene", "alkynylene", "carbocyclylene", "heterocarbocyclylene", "cycloalkylene", "heterocycloalkylene", "arylene", "heteroarylene" are each mid-chain moieties.

When compounded chemical names, e.g. "arylalkyl" and "arylimine" are used herein, they are understood to have a specific connectivity to the core of the chemical structure. The group listed farthest to the right (e.g. alkyl in "arylalkyl"), is the group that is directly connected to the core. Thus, an "arylalkyl" group, for example, is an alkyl group substituted with an aryl group (e.g. phenylmethyl (i.e., benzyl)) and the alkyl group is attached to the core. An "alkylaryl" group is an aryl group substituted with an alkyl group (e.g., p-methylphenyl (i.e., p-tolyl)) and the aryl group is attached to the core.

The term “optionally substituted” as used herein means the group to which this term refers may be unsubstituted, or may be substituted with one or more groups other than hydrogen provided that the indicated atom’s normal valency is not exceeded, and that the substitution results in a stable compound. Such groups may be, for example, halogen, hydroxy, oxo, cyano, nitro, alkyl, alkoxy, haloalkyl, haloalkoxy, aryl-4-alkoxy, alkylthio, hydroxyalkyl, alkoxyalkyl, cycloalkyl, cycloalkylalkoxy, alkanoyl, alkoxycarbonyl, alkylsulfonyl, alkylsulfonyloxy, alkylsulfonylalkyl, arylsulfonyl, arylsulfonyloxy, arylsulfonylalkyl, alkylsulfonamido, alkylamido, alkylsulfonamidoalkyl, alkylamidoalkyl, arylsulfonamido, arylcarboxamido, arylsulfonamidoalkyl, arylcarboxamidoalkyl, aroyl, aroyl-4-alkyl, arylalkanoyl, acyl, aryl, arylalkyl, alkylaminoalkyl, a group R^xR^yN- , $R^xOCO(CH_2)_m$, $R^xCON(R^y)(CH_2)_m$, $R^xR^yNCO(CH_2)_m$, $R^xR^yNSO_2(CH_2)_m$ or $R^xSO_2NR^y(CH_2)_m$ (where each of R^x and R^y is independently selected from hydrogen or alkyl, or where appropriate R^xR^y forms part of carbocyclic or heterocyclic ring and m is 0, 1, 2, 3 or 4), a group $R^xR^yN(CH_2)_p-$ or $R^xR^yN(CH_2)_pO-$ (wherein p is 1, 2, 3 or 4); wherein when the substituent is $R^xR^yN(CH_2)_p-$ or $R^xR^yN(CH_2)_pO$, R^x with at least one CH_2 of the $(CH_2)_p$ portion of the group may also form a carbocyclyl or heterocyclyl group and R^y may be hydrogen, alkyl.

The term “substituted” as used herein means the group to which this term refers is substituted with one or more groups other than hydrogen provided that the indicated atom’s normal valency is not exceeded, and that the substitution results in a stable compound. Such groups may be, for example, halogen, hydroxy, oxo, cyano, nitro, alkyl, alkoxy, haloalkyl, haloalkoxy, arylalkoxy, alkylthio, hydroxyalkyl, alkoxyalkyl, cycloalkyl, cycloalkylalkoxy, alkanoyl, alkoxycarbonyl, alkylsulfonyl, alkylsulfonyloxy, alkylsulfonylalkyl, arylsulfonyl, arylsulfonyloxy, arylsulfonylalkyl, alkylsulfonamido, alkylamido, alkylsulfonamidoalkyl, alkylamidoalkyl, arylsulfonamido, arylcarboxamido, arylsulfonamidoalkyl, arylcarboxamidoalkyl, aroyl, aroyl-4-alkyl, arylalkanoyl, acyl, aryl, arylalkyl, alkylaminoalkyl, a group R^xR^yN- , $R^xOCO(CH_2)_m$, $R^xCON(R^y)(CH_2)_m$, $R^xR^yNCO(CH_2)_m$, $R^xR^yNSO_2(CH_2)_m$ or $R^xSO_2NR^y(CH_2)_m$ (where each of R^x and R^y is independently selected from hydrogen or alkyl, or where appropriate R^xR^y forms part of carbocyclic or heterocyclic ring and m is 0, 1, 2, 3 or 4), a group $R^xR^yN(CH_2)_p-$ or $R^xR^yN(CH_2)_pO-$ (wherein p is 1, 2, 3 or 4); wherein when the substituent is $R^xR^yN(CH_2)_p-$ or $R^xR^yN(CH_2)_pO$, R^x with at least one CH_2 of the $(CH_2)_p$ portion of the group may also form a carbocyclyl or heterocyclyl group and R^y may be hydrogen, alkyl.

Any carbon or heteroatom with unsatisfied valences in the text, schemes, examples, structural formulae, and any Tables herein is assumed to have the hydrogen atom or atoms to satisfy the valences.

The term “pharmaceutically acceptable salt” refers to salts that are within sound medical judgement, that may be suitable for medical applications, or suitable for use to contact human and/or animal tissue without undue toxicity, allergic response, irritation and the like, and have a reasonable

benefit/risk ratio. Such salts are well known in the art and may include, for example, salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid, lactic acid, malic acid or malonic acid. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. alkali metal, alkaline earth metal, ammonium and alkylammonium salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, non-toxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate.

The term "pharmaceutically acceptable carrier" is intended to include solvents, dispersion media, coatings, anti-bacterial and anti-fungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the compound, use thereof in the therapeutic compositions and methods of treatment and prophylaxis is contemplated. Supplementary active compounds may also be incorporated into the compositions according to the present invention. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for the individual to be treated; each unit containing a predetermined quantity of compound(s) is calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The compound(s) may be formulated for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in an acceptable dosage unit. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

As used herein, the term "metabolite" refers to any substance that is produced during metabolism or that takes part in metabolism. Metabolism refers to the biochemical reactions involved in maintaining the living condition of the cells in an organism.

As used herein, the term “prodrug” refers to therapeutic agent precursors that may be intrinsically and/or largely inactive, but can be transformed in vivo into one or more active compounds that exhibit therapeutic efficacy. Suitable prodrugs include esters, phosphonate esters etc, of the active form of the compound.

Brief Description of Drawings

The accompanying drawings illustrate disclosed embodiments and serve to explain the principles of the disclosed embodiments. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

FIG 1

Fig. 1 is a scheme of a phylogenetic tree showing the evolutionary relationship between A7611 and other type species of the genus *Nonomuraea*, with the bar indicating 0.01 substitutions per nucleotide position.

FIG 2

Fig. 2 is an image showing the results of Global Natural Products Social Molecular Networking (GNPS) performed on the extract of A7611 strain, with insets showing the structures attributed to nodes in the thiopeptide GNPS clusters.

FIG 3

Fig. 3 is a diagram showing the chemical structures of compounds 1–9.

FIG 4

Fig. 4 is a series of ultraviolet (UV) spectra for compounds 1–9.

FIG 5

Fig. 5 is a series of (+)-High Resolution Electrospray Ionisation Mass Spectrometry (HRESIMS) spectra for compounds 1–9.

FIG 6

Fig. 6 is a diagram showing selected homonuclear COrrrelation SpectroscopY (COSY) and Heteronuclear Multiple Bond Correlation (HMBC) correlations of compounds 1–9.

FIG. 7A

Fig. 7a is a ^1H NMR spectrum ($\text{DMSO-}d_6$, 400 MHz) of compound 1.

FIG. 7B

Fig. 7b is a ^{13}C NMR spectrum (DMSO- d_6 , 100 MHz) of compound 1.

FIG. 7C

Fig. 7c is a COSY spectrum of compound 1.

FIG. 7D

Fig. 7d is a Heteronuclear Single Quantum Coherence (HSQC) spectrum of compound 1.

FIG. 7E

Fig. 7e is a HMBC spectrum of compound 1.

FIG. 8A

Fig. 8a is a ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of compound 2.

FIG. 8B

Fig. 8b is a ^{13}C NMR spectrum (DMSO- d_6 , 100 MHz) of compound 2.

FIG. 8C

Fig. 8c is a COSY spectrum of compound 2.

FIG. 8D

Fig. 8d is a HSQC spectrum of compound 2.

FIG. 8E

Fig. 8e is a HMBC spectrum of compound 2.

FIG. 9A

Fig. 9a is a ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of compound 3.

FIG. 9B

Fig. 9b is a COSY spectrum of compound 3.

FIG. 9C

Fig. 9c is a HSQC spectrum of compound 3.

FIG. 9D

Fig. 9d is a HMBC spectrum of compound **3**.

FIG. 10A

Fig. 10a is a ^1H NMR spectrum ($\text{DMSO-}d_6$, 400 MHz) of compound **4**.

FIG. 10B

Fig. 10b is a ^{13}C NMR spectrum ($\text{DMSO-}d_6$, 100 MHz) of compound **4**.

FIG. 10C

Fig. 10c is a COSY spectrum of compound **4**.

FIG. 10D

Fig. 10d is a HSQC spectrum of compound **4**.

FIG. 10E

Fig. 10e is a HMBC spectrum of compound **4**.

FIG. 11A

Fig. 11a is a ^1H NMR spectrum ($\text{DMSO-}d_6$, 400 MHz) of compound **5**.

FIG. 11B

Fig. 11b is a ^{13}C NMR spectrum ($\text{DMSO-}d_6$, 100 MHz) of compound **5**.

FIG. 11C

Fig. 11c is a COSY spectrum of compound **5**.

FIG. 11D

Fig. 11d is a HSQC spectrum of compound **5**.

FIG. 11E

Fig. 11e is a HMBC spectrum of compound **5**.

FIG. 12A

Fig. 12a is a ^1H NMR spectrum ($\text{DMSO-}d_6$, 400 MHz) of compound **6**.

FIG. 12B

Fig. 12b is a ^{13}C NMR spectrum (DMSO- d_6 , 100 MHz) of compound **6**.

FIG. 12C

Fig. 12c is a COSY spectrum of compound **6**.

FIG. 12D

Fig. 12d is a HSQC spectrum of compound **6**.

FIG. 12E

Fig. 12e is a HMBC spectrum of compound **6**.

FIG. 13A

Fig. 13a is a ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of compound **7**.

FIG. 13B

Fig. 13b is a ^{13}C NMR spectrum (DMSO- d_6 , 100 MHz) of compound **7**.

FIG. 13C

Fig. 13c is a COSY spectrum of compound **7**.

FIG. 13D

Fig. 13d is a HSQC spectrum of compound **7**.

FIG. 13E

Fig. 13e is a HMBC spectrum of compound **7**.

FIG. 14

Fig. 14 is a ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of compound **8**.

FIG. 15

Fig. 15 is a ^1H NMR spectrum (DMSO- d_6 , 400 MHz) of compound **9**.

FIG. 16

Fig. 16 is a series of images showing (a) the extracted ion chromatogram; (b) the (+)-HRESIMS spectrum; and (c) the MS/MS spectrum of GE2270A (a thiopeptide detected from *Planobispora rosea*) in the extract of the *Nonomuraea jiangxiensis* strain A7611.

FIG. 17

Fig. 17 is a series of inhibitory effect dose response curves of compounds **1–9** against *Staphylococcus aureus* Rosenbach (ATCC® 25923™) with gentamicin as positive control.

FIG. 18A

Fig. 18a is scheme showing the synthetic route for intermediate **I**.

FIG. 18B

Fig. 18b is scheme showing the synthetic route towards intermediate **II**.

FIG. 18C

Fig. 18c is scheme showing the synthetic route towards intermediates **III** and **IV**.

FIG. 18D

Fig. 18d is scheme showing the synthetic route towards compounds **7, 8, and 9**.

FIG. 18E

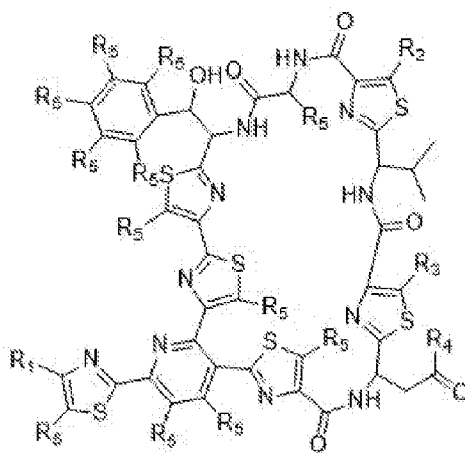
Fig. 18e is scheme showing the synthetic route towards compounds **1, 2, 3, and 4**.

FIG. 18F

Fig. 18f is scheme showing the synthetic route towards compounds **5 and 6**.

Detailed Disclosure of Embodiments

The present invention discloses a compound of Formula (I) or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof:



Formula (I)

wherein:

R_1 is $-C(O)-R_6$ or $-A-G-J-L$;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or $-C(O)N(R_{7a})-$;

G is $-C(O)-$ or optionally substituted $-alkylene-C(O)-$;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is $-C(O)-R_6$;

R_2 is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

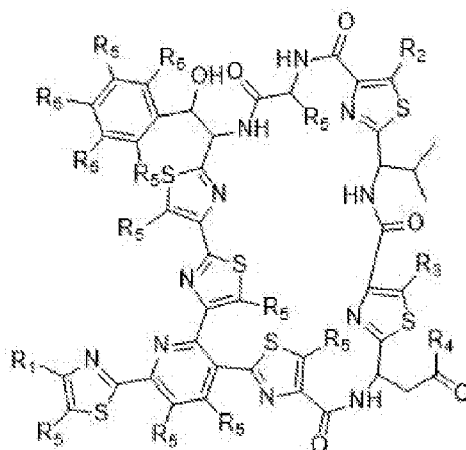
R₄ is H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently H or optionally substituted alkyl.

The present disclose also provides a compound of Formula (I) or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylenc, optionally substituted heteroarylenc, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl,

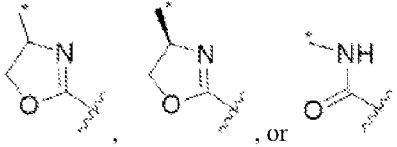
wherein when R₂ is optionally substituted alkalkoxy, R₃ is -H, optionally substituted alkenyl, or optionally substituted alkynyl.

In some embodiments, R₁ is -C(O)-R₆ or -A-G-J-L.

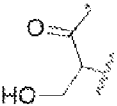
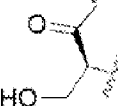
In some embodiments, R₁ is -C(O)-R₆. In other embodiments, R₁ is -COOH. In some other embodiments, R₁ is an acid derivative, e.g., an ester (-COOR), or an amide (-CONR_aR_b), where R, R_a and/or R_b may be optionally substituted alkyl, e.g., optionally substituted C₁₋₁₂ alkyl or optionally substituted C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkyl, optionally substituted alkenyl, e.g.,

optionally substituted C₂₋₁₂ alkenyl or optionally substituted C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkenyl, or optionally substituted alkynyl, e.g., optionally substituted C₂₋₁₂ alkynyl or optionally substituted C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkynyl, or optionally substituted alkalkoxy, e.g., optionally substituted C₁₋₁₂ alkalkoxy or optionally substituted C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkalkoxy, or any other substituents as defined herein. In some embodiments, R₁ is -C(O)-R₆, and R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}). In some embodiments, the alkoxy is MeO-, EtO-, PrO-, iPrO-, n-BuO-, sec-BuO-, or t-BuO-. In some other embodiments, -N(R_{7a})(R_{7b}) is -NH₂, -NHMe, -N(Me)₂, -NHEt, -N(Et)₂, or -NMeEt.

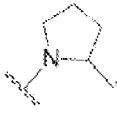
In some embodiments, A is optionally substituted carbocyclylene, e.g., optionally substituted C₃₋₁₂ carbocyclylene or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ carbocyclylene, optionally substituted heterocarbocyclylene, e.g., optionally substituted C₃₋₁₂ heterocarbocyclylene or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ heterocarbocyclylene, optionally substituted cycloalkylene, e.g., optionally substituted C₃₋₁₂ cycloalkylene or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ cycloalkylene, optionally substituted heterocycloalkylene, e.g., optionally substituted C₃₋₁₂ heterocycloalkylene or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ heterocycloalkylene, optionally substituted arylene, e.g., optionally substituted C₆₋₁₂ arylene or optionally substituted C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ arylene, optionally substituted heteroarylene, e.g., optionally substituted C₅₋₁₂ heteroarylene or optionally substituted C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ heteroarylene, or -C(O)N(R_{7a})-. In some embodiments, the heteroatom is N, O, P, S and/or Se. In some preferred embodiments, A is heterocarbocyclylene or -C(O)N(R_{7a})-. In some other embodiments, A is

 , or isomers thereof, wherein  represents a connection to the rest of the compound and * represents a connection to G.

In some embodiments, G is -C(O)- or optionally substituted -alkylene-C(O)-. In some embodiments, the optionally substituted alkylene may be methylene, ethylene, propylene, butylene, pentylene, hexylene, all of which may be optionally substituted or isomers thereof. In some preferred embodiments, the alkylene is optionally substituted methylene. In some other embodiments, G is -C(O)- or -CH(R₈)-C(O)-, wherein R₈ is optionally substituted alkylene, e.g., optionally substituted C₁₋₁₂ alkylene or optionally substituted C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkylene, substituted with -OH. In some preferred embodiments, R₈ is methylene substituted with one -OH, i.e., -CH₂OH. In

some embodiments, G is -C(O)-, , or , or isomers thereof, wherein  represents a connection to A and * represents a connection to J.

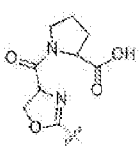
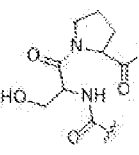
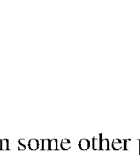
In some embodiments, J is optionally substituted carbocyclylene, e.g., optionally substituted C₃₋₁₂ carbocyclylene or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ carbocyclylene, optionally substituted heterocarbocyclylene, e.g., optionally substituted C₃₋₁₂ heterocarbocyclylene or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ heterocarbocyclylene, optionally substituted cycloalkylene, e.g., optionally substituted C₃₋₁₂ cycloalkylene or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ cycloalkylene, optionally substituted heterocycloalkylene, e.g., optionally substituted C₃₋₁₂ heterocycloalkylene or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ heterocycloalkylene, optionally substituted arylene, e.g., optionally substituted C₆₋₁₂ arylene or optionally substituted C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ arylene, or optionally substituted heteroarylene, e.g., optionally substituted C₅₋₁₂ heteroarylene or optionally substituted C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ heteroarylene. In some embodiments, the heteroatom is N, O, P, S and/or Se. In some preferred

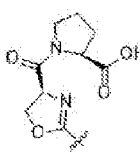
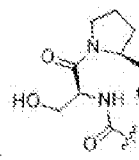
embodiments, J is heterocarbocyclylene. In some preferred embodiments, J is , or , or isomers thereof, wherein  represents a connection to G and * represents a connection to L.

In some embodiments, L is -C(O)-R₆. In other embodiments, L is -COOH. In some other embodiments, L is an acid derivative, e.g., an ester (-COOR), or an amide (-CONR_aR_b), where R, R_a and/or R_b may be optionally substituted alkyl, e.g., optionally substituted C₁₋₁₂ alkyl or optionally substituted C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkyl, optionally substituted alkenyl, e.g., optionally substituted C₂₋₁₂ alkenyl or optionally substituted C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkenyl, or optionally substituted alkynyl, e.g., optionally substituted C₂₋₁₂ alkynyl or optionally substituted C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkynyl, or optionally substituted alkalkoxy, e.g., optionally substituted C₁₋₁₂ alkalkoxy or optionally substituted C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkalkoxy, or any other substituents as defined herein. In some embodiments, R₁ is -C(O)-R₆, and R₆ is -OH, optionally substituted alkoxy, e.g., optionally substituted C₁₋₁₂ alkoxy or optionally substituted C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkoxy, or -N(R_{7a})(R_{7b}). In some embodiments, the optionally substituted alkoxy is MeO-, EtO-, PrO-, iPrO-, n-BuO-, sec-BuO-, or t-BuO-. In some

other embodiments, $-N(R_{7a})(R_{7b})$ is $-NH_2$, $-NHMe$, $-N(Me)_2$, $-NHEt$, $-N(Et)_2$, or $-NMeEt$. In some preferred embodiments, L is $-COOH$ or $-C(O)NH_2$.

In some preferred embodiments, R_1 is selected from the group consisting of $-COOH$, $-$

 $C(O)OCH_3$, , and . In some other preferred embodiments, R_1 is selected

from the group consisting of $-COOH$, $-C(O)OCH_3$, , and .

In some embodiments, R_2 is $-H$, optionally substituted alkyl, e.g., optionally substituted C_{1-12} alkyl or optionally substituted $C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}$ alkyl, optionally substituted alkenyl, e.g., optionally substituted C_{2-12} alkenyl or optionally substituted $C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}$ alkenyl, or optionally substituted alkynyl, e.g., optionally substituted C_{2-12} alkynyl or optionally substituted $C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}$ alkynyl, or optionally substituted alkalkoxy, e.g., optionally substituted C_{1-12} alkalkoxy or optionally substituted $C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}$ alkalkoxy. In some preferred embodiments, R_2 is $-H$ or $-CH_2OCH_3$.

In some embodiments, R_3 is $-H$, optionally substituted alkyl, e.g., optionally substituted C_{1-12} alkyl or optionally substituted $C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}$ alkyl, optionally substituted alkenyl, e.g., optionally substituted C_{2-12} alkenyl or optionally substituted $C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}$ alkenyl, or optionally substituted alkynyl, e.g., optionally substituted C_{2-12} alkynyl or optionally substituted $C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}$ alkynyl. In some preferred embodiments, R_3 is $-H$ or $-CH_3$.

In some embodiments, R_4 is $-H$, $-OH$, optionally substituted alkyl, e.g., optionally substituted C_{1-12} alkyl or optionally substituted $C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}$ alkyl, optionally substituted alkenyl, e.g., optionally substituted C_{2-12} alkenyl or optionally substituted $C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}$ alkenyl, optionally substituted alkynyl, e.g., optionally substituted C_{2-12} alkynyl or optionally substituted $C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}$ alkynyl, or $-N(R_{7a})(R_{7b})$. In some preferred embodiments, R_4 is $-NH(CH_3)$.

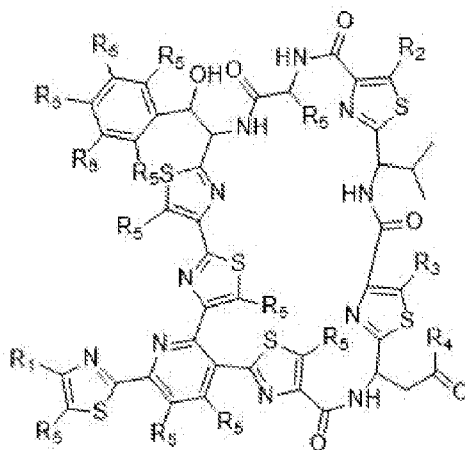
In some embodiments, R_5 is $-H$, halogen, $-N(R_{7a})(R_{7b})$, optionally substituted alkoxy, e.g., optionally substituted C_{1-12} alkoxy or optionally substituted $C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11},$

C₁₂ alkoxy, optionally substituted alkyl, e.g., optionally substituted C₁₋₁₂ alkyl or optionally substituted C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkyl, optionally substituted alkenyl, e.g., optionally substituted C₂₋₁₂ alkenyl or optionally substituted C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkenyl, optionally substituted alkynyl, e.g., optionally substituted C₂₋₁₂ alkynyl or optionally substituted C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkynyl, optionally substituted carbocyclyl, e.g., optionally substituted C₃₋₁₂ carbocyclyl or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ carbocyclyl, optionally substituted heterocarbocyclyl, e.g., optionally substituted C₃₋₁₂ heterocarbocyclyl or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ heterocarbocyclyl, optionally substituted cycloalkyl, e.g., optionally substituted C₃₋₁₂ cycloalkyl or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ cycloalkyl, optionally substituted heterocycloalkyl, e.g., optionally substituted C₃₋₁₂ heterocycloalkyl or optionally substituted C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ heterocycloalkyl, optionally substituted aryl, e.g., optionally substituted C₆₋₁₂ aryl or optionally substituted C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ aryl, or optionally substituted heteroaryl, e.g., optionally substituted C₅₋₁₂ heteroaryl or optionally substituted C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ heteroaryl. In some embodiments, the heteroatom is N, O, P, S and/or Se. In some preferred embodiments, R₅ is -H.

In some embodiments, R₆ is -OH, optionally substituted alkoxy, e.g., optionally substituted C₁₋₁₂ alkoxy or optionally substituted C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkoxy, or -N(R_{7a})(R_{7b}). In some preferred embodiments, R₆ is -OH, -OMe, or -NHMe.

In some embodiments, each of R_{7a} and R_{7b} is independently -H or optionally substituted alkyl, e.g., optionally substituted C₁₋₁₂ alkyl or optionally substituted C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ alkyl. In some preferred embodiments, each of R_{7a} and R_{7b} is independently -H or -Me.

The present invention also discloses a compound of Formula (I) or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

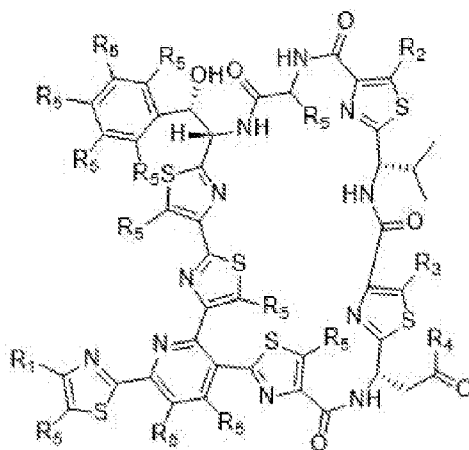
R_5 is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R_6 is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl,

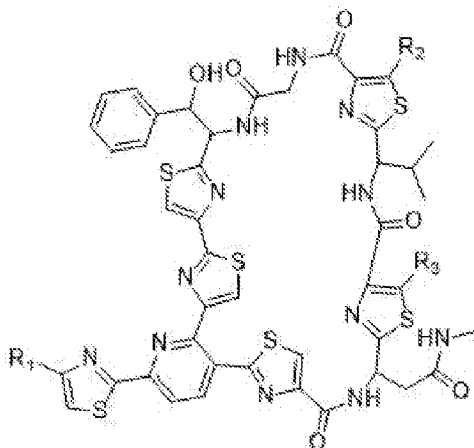
wherein when R_2 is optionally substituted alkalkoxy, R_3 is -H, optionally substituted alkenyl, or optionally substituted alkynyl.

The present invention also discloses a compound having a structure and stereochemical configuration of Formula (II):



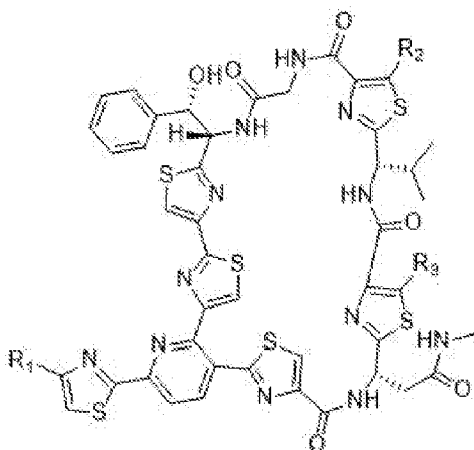
Formula (II)

The present invention also discloses a having a Formula (III):



Formula (III)

The present invention also discloses a compound having a Formula (IV):

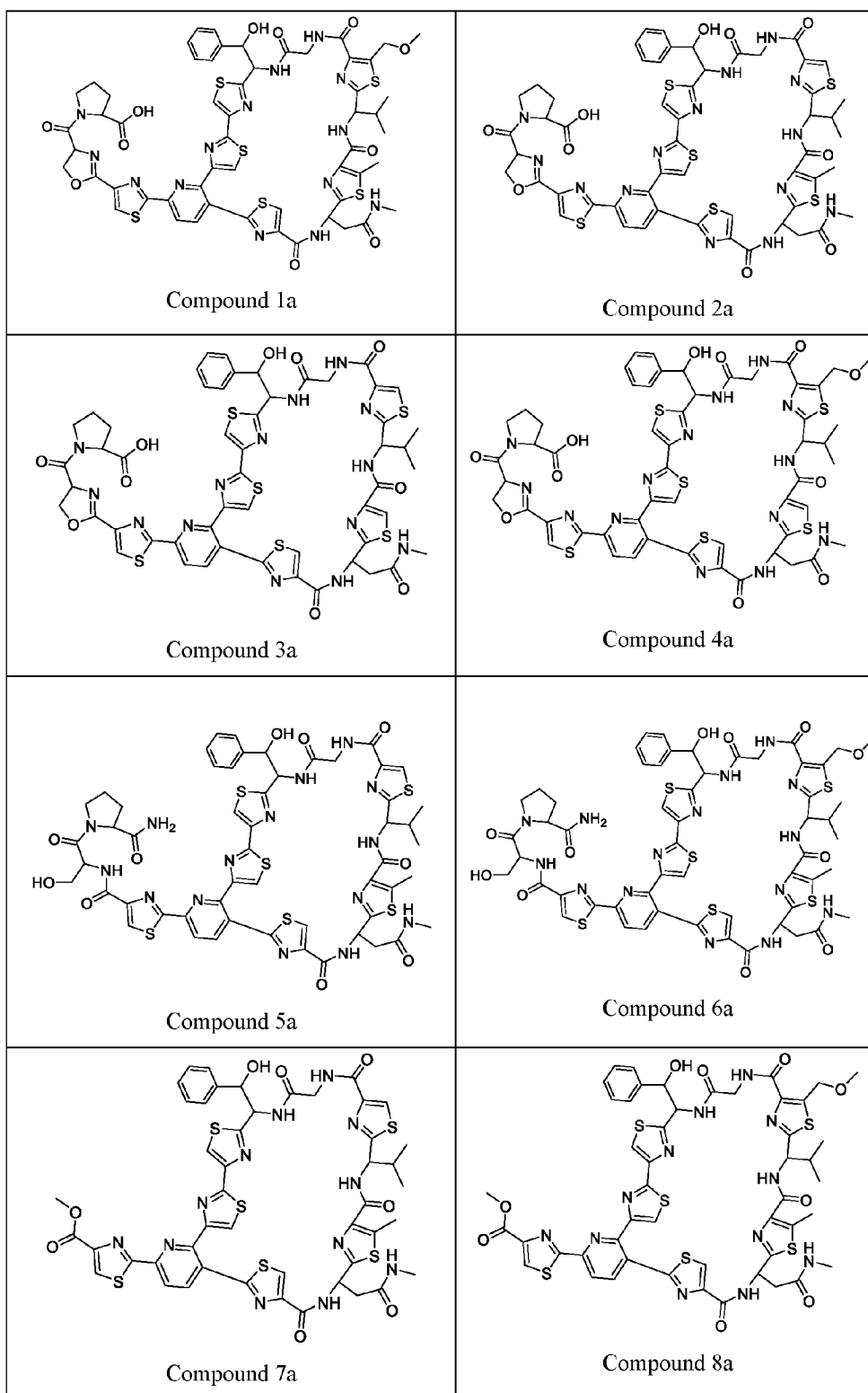


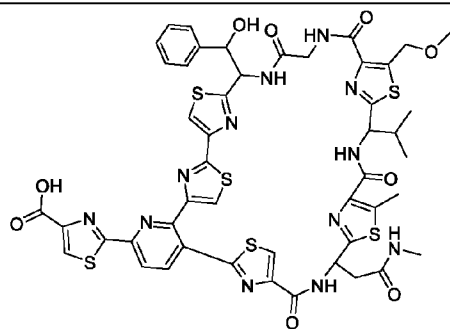
Formula (IV)

In some embodiments, when R₂ is optionally substituted alkalkoxy, R₃ is -H, optionally substituted alkenyl, or optionally substituted alkynyl. In some other embodiments, when R₂ is optionally substituted alkalkoxy, R₃ is not optionally substituted alkyl. Preferably, when R₂ is -CO₂OCH₃, R₃ is -H, optionally substituted alkenyl, or optionally substituted alkynyl. Alternatively, when R₂ is -CO₂OCH₃, R₃ is not -CH₃.

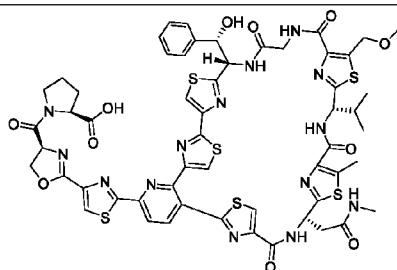
R, R₁, R₂, R₃, R₄, R₅, R₆, R₇, R_{7a}, R_{7b}, R₈, may be as defined anywhere in the specification.

In some embodiments, the compound of Formula (I) or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, may be selected from the following group:

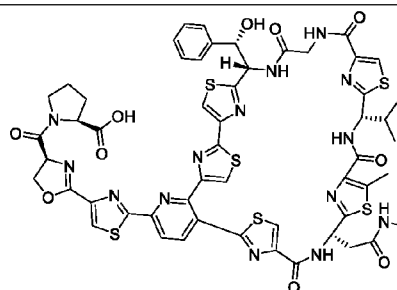




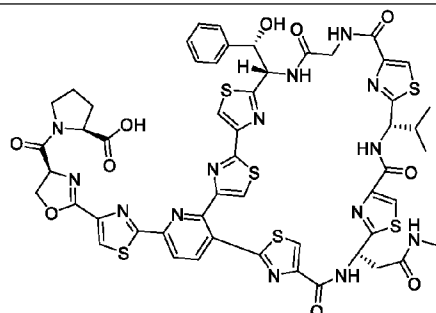
Compound 9a



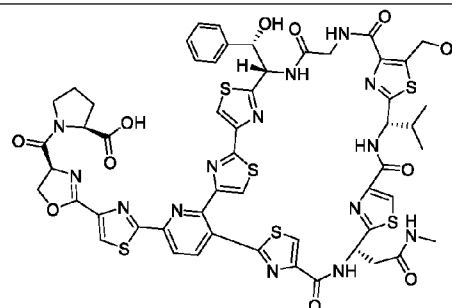
Compound 1



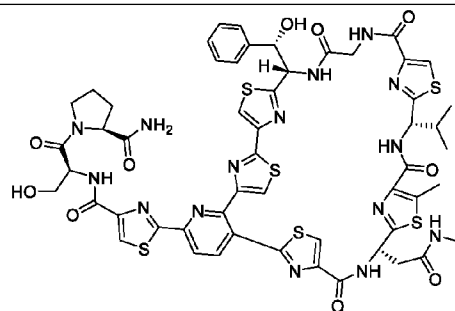
Compound 2



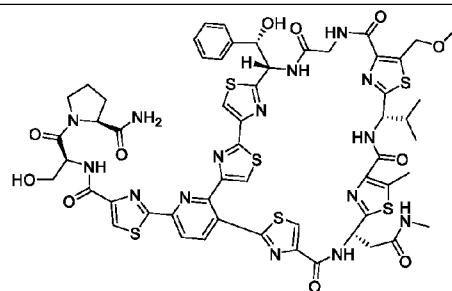
Compound 3



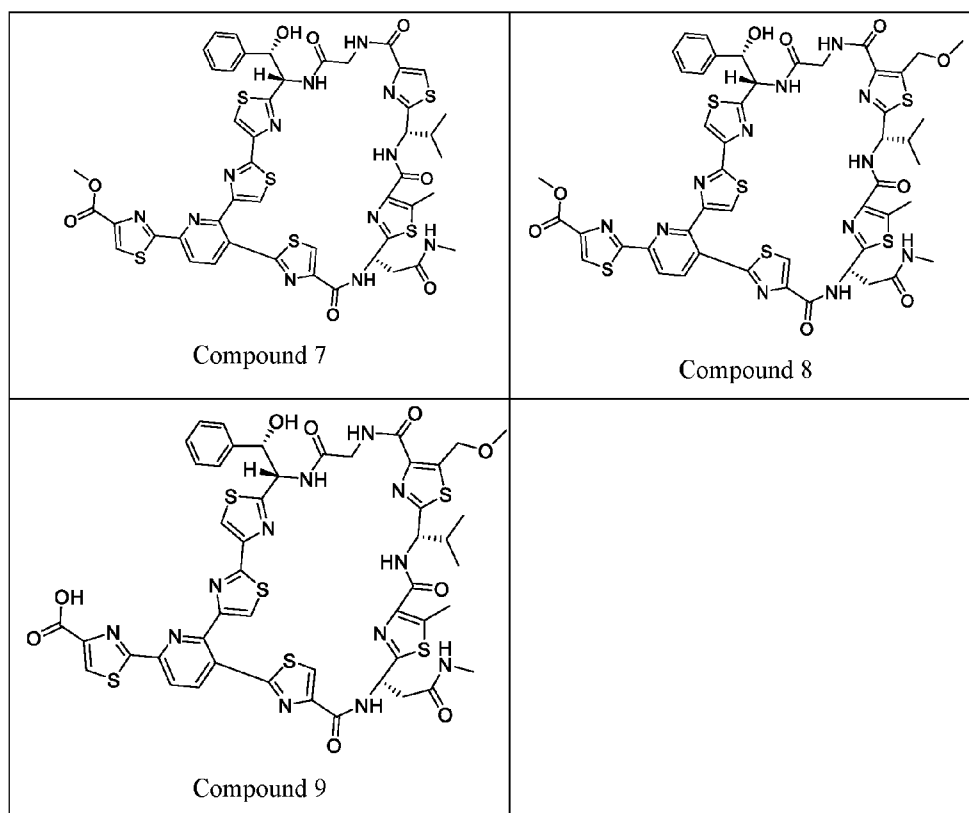
Compound 4



Compound 5



Compound 6



Pharmaceutical Compositions

The present disclosure also provides for a pharmaceutical composition comprising the compound disclosed herein, or its pharmaceutically acceptable salt, metabolite, prodrug, stereoisomer, enantiomer, diastereomer, geometric isomer, and/or isomer thereof, all of which are disclosed herein.

The pharmaceutical composition may comprise a therapeutically effective amount of the compound disclosed herein. Further, the pharmaceutical composition may also comprise the compound disclosed herein, or its pharmaceutically acceptable salt, metabolite, prodrug, stereoisomer, enantiomer, diastereomer, geometric isomer, and/or isomer thereof.

In an embodiment of the present disclosure, there is provided a pharmaceutical composition comprising a therapeutically effective amount of a compound disclosed herein, or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, and a pharmaceutically acceptable excipient.

Therapy

The compounds disclosed in the present invention, or their pharmaceutically acceptable salts, metabolites, prodrugs, stereoisomers, enantiomers, diastereomers, geometric isomers, and/or isomers, or pharmaceutical compositions of any of the foregoing, may be used in therapy.

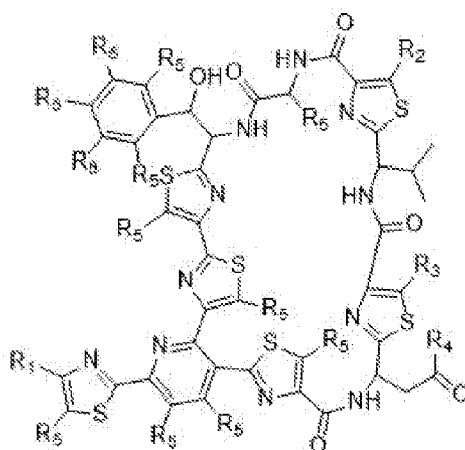
In some embodiments, the present invention discloses methods of treatment using the compounds disclosed herein or their pharmaceutically acceptable salts, metabolites, prodrugs, stereoisomers, enantiomers, diastereomers, geometric isomers, and/or isomers, or pharmaceutical compositions of any of the foregoing, all of which are disclosed herein.

In some embodiments, the present invention discloses the compounds disclosed herein or their pharmaceutically acceptable salts, metabolites, prodrugs, stereoisomers, enantiomers, diastereomers, geometric isomers, and/or isomers, or pharmaceutical compositions of any of the foregoing, all of which are disclosed herein, for use in therapy or in treatment.

In some other embodiments, the present invention also discloses the use of the compounds disclosed herein or their pharmaceutically acceptable salts, metabolites, prodrugs, stereoisomers, enantiomers, diastereomers, geometric isomers, and/or isomers, or pharmaceutical compositions of any of the foregoing, all of which are disclosed herein, in the manufacture of a medicament for therapy, or for treatment of any diseases, infections or indications disclosed herein and throughout the disclosure.

In some embodiments, the compounds disclosed in the present invention, or their pharmaceutically acceptable salts, metabolites, prodrugs, stereoisomers, enantiomers, diastereomers, geometric isomers, and/or isomers, or pharmaceutical compositions of any of the foregoing, may be used to treat various microbial, bacterial, fungal infections, and/or cancer.

In some embodiments, the present invention provides a method of treating a bacterial, microbial and/or fungal infection, comprising administering a therapeutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, to a subject in need of treatment:



Formula (I)

wherein:

R_1 is $-C(O)-R_6$ or $-A-G-J-L$;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or $-C(O)N(R_{7a})-$;

G is $-C(O)-$ or optionally substituted $-alkylene-C(O)-$;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is $-C(O)-R_6$;

R_2 is $-H$, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R_3 is $-H$, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

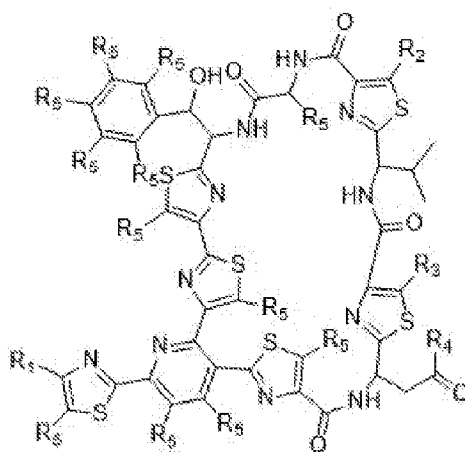
R_4 is $-H$, $-OH$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or $-N(R_{7a})(R_{7b})$;

R_5 is independently selected from the group consisting of -H, halogen, $-N(R_{7a})(R_{7b})$, optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R_6 is -OH, alkoxy, or $-N(R_{7a})(R_{7b})$; and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

In some embodiments, the present invention provides a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, for use in therapy:



Formula (I)

wherein:

R_1 is $-C(O)-R_6$ or $-A-G-J-L$;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or $-C(O)N(R_{7a})-$;

G is $-C(O)-$ or optionally substituted $-alkylene-C(O)-$;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

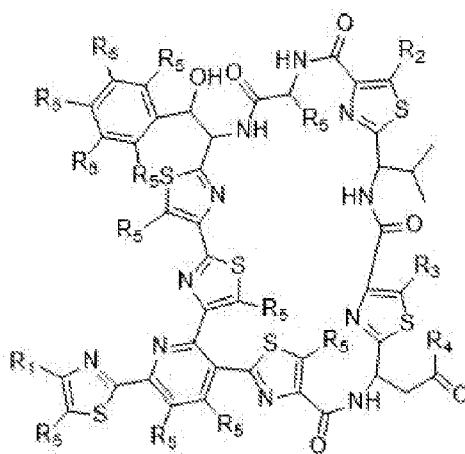
R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

In some embodiments, the present invention provides a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, for use in treating a bacterial, microbial and/or fungal infection:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

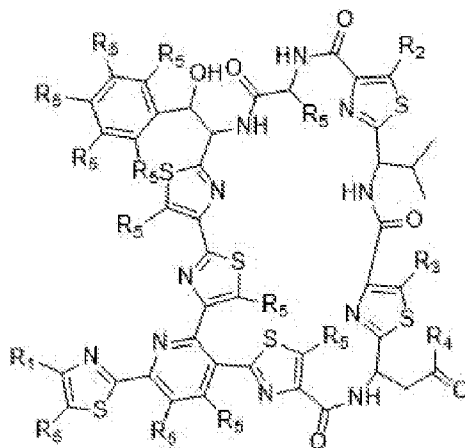
R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

In some embodiments, the present invention provides a use of a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, in the manufacture of a medicament for treating a bacterial, microbial and/or fungal infection:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

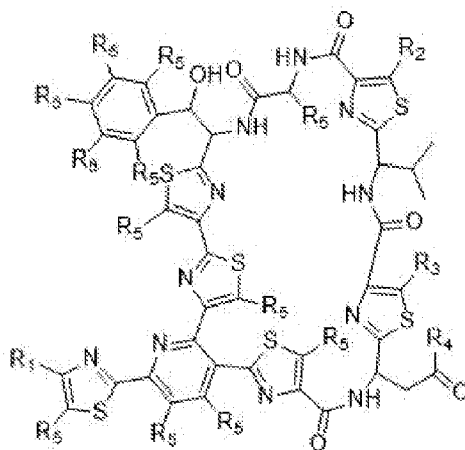
R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

Such treatable infections may be caused by, but are not limited to, *Staphylococcus*, *Bacillus*, *Clostridium*, *Acinetobacter*, *Klebsiella*, *Candida*, *Enterococcus*, *Micrococcus*, or *Pseudomonas* pathogens. In some embodiments, the infection is a *Staphylococcus*, *Bacillus*, *Clostridium*, *Acinetobacter*, *Klebsiella*, *Candida*, *Enterococcus*, *Micrococcus*, or *Pseudomonas* infection.

In some preferred embodiments, the infection is caused by, but not limited to *Staphylococcus aureus*, *Staphylococcus aureus* Rosenbach, gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens*, *Acinetobacter baumannii*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Candida albicans*, *Clostridium difficile*, *Enterococcus faecalis*, *Enterococcus faecium*, or *Micrococcus luteus*.

In some embodiments, the present invention provides a method of treating cancer, comprising administering a therapeutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, to a subject in need of treatment:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

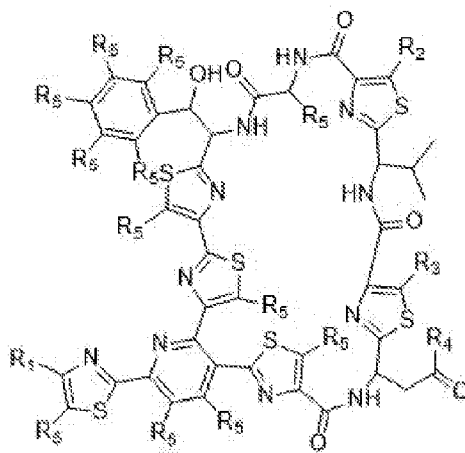
R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R_5 is independently selected from the group consisting of -H, halogen, $-N(R_{7a})(R_{7b})$, optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R_6 is -OH, alkoxy, or $-N(R_{7a})(R_{7b})$; and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

In some embodiments, the present invention provides a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, for use in treating cancer:



Formula (I)

wherein:

R_1 is $-C(O)-R_6$ or $-A-G-J-L$;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or $-C(O)N(R_{7a})-$;

G is $-C(O)-$ or optionally substituted $-alkylene-C(O)-$;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

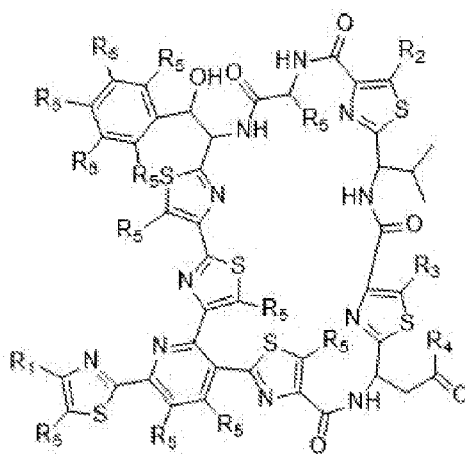
R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

In some embodiments, the present invention provides a use of a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, in the manufacture of a medicament for treating cancer:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

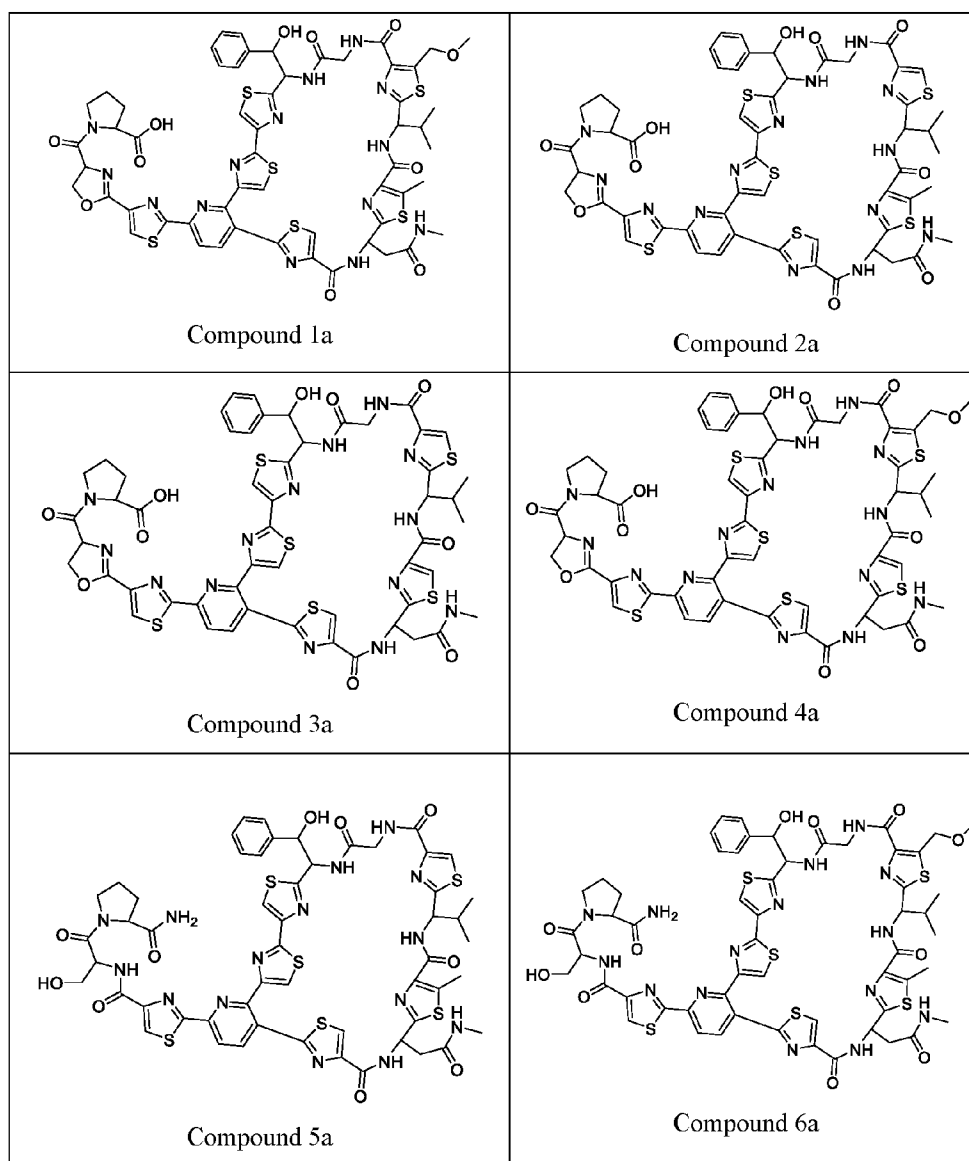
R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

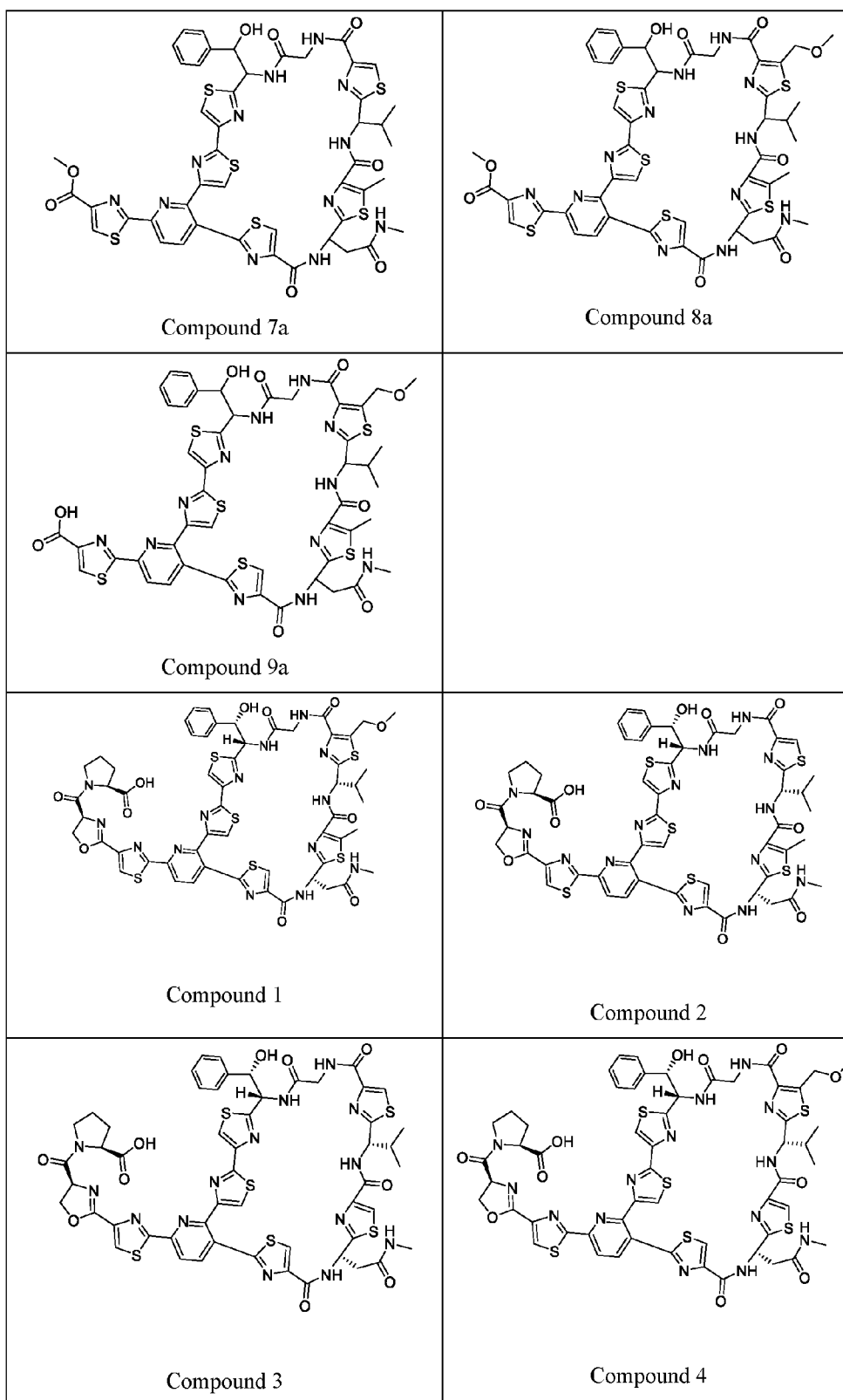
R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

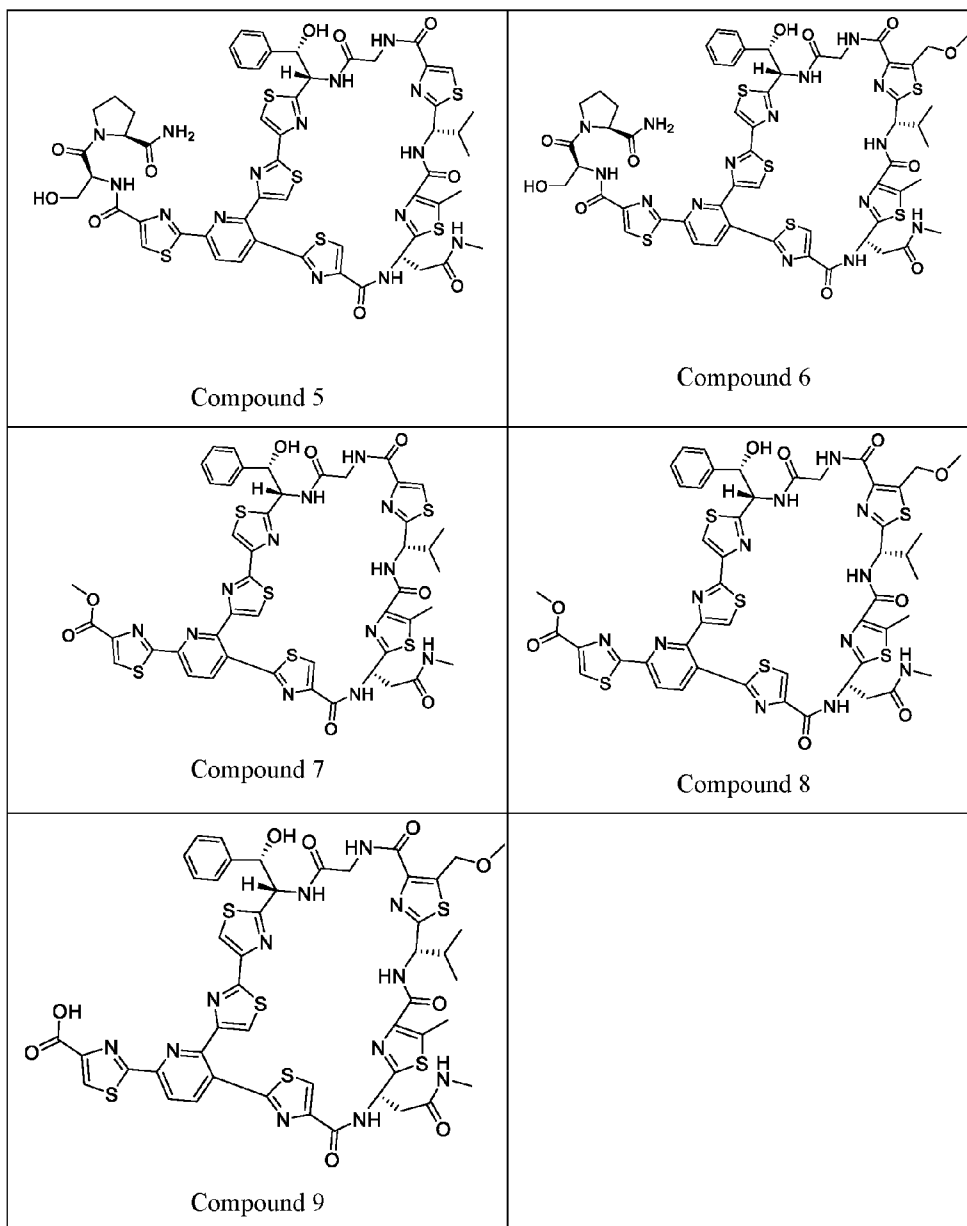
R, R₁, R₂, R₃, R₄, R₅, R₆, R₇, R_{7a}, R_{7b}, R₈, may be as defined anywhere in the specification.

In some embodiments, the compounds for use, or the compound as used in any methods of therapy or manufacture of medicaments for therapy are compounds as disclosed in the present invention, or their pharmaceutically acceptable salts, metabolites, prodrugs, stereoisomers, enantiomers, diastereomers, geometric isomers, and/or isomers, or pharmaceutical compositions of any of the foregoing.

In some other embodiments, the compounds for use, or the compound as used in any methods of therapy or manufacture of medicaments for therapy, or their pharmaceutically acceptable salts, metabolites, prodrugs, stereoisomers, enantiomers, diastereomers, geometric isomers, and/or isomers, or pharmaceutical compositions of any of the foregoing, may be selected from the group consisting of:







In some embodiments, the compounds for use, or the compound as used in any methods of therapy or manufacture of medicaments for therapy, or their pharmaceutically acceptable salts, metabolites, prodrugs, stereoisomers, enantiomers, diastereomers, geometric isomers, and/or isomers, or pharmaceutical compositions of any of the foregoing, may be selected from the group consisting of Compounds 1a-2a, 5a-9a, 1-2 and 5-9.

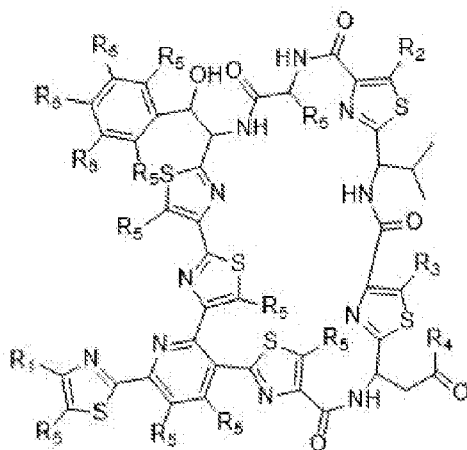
Isolation of Compounds

Special fermentation and/or culture conditions may be required to induce the microbe(s) to produce the compounds of the present invention. In some preferred embodiments, the microbe is a bacteria. In some preferred embodiments, the bacteria is a *Nonomuraea* strain. In further preferred embodiments, the bacteria is *Nonomuraea jiangxiensis*.

The present invention provides a method of isolating compounds disclosed in the present invention, the method comprising:

- (i) expanding a bacteria strain under culture conditions that allow expansion of the strain to obtain a seed culture;
- (ii) using the seed culture to inoculate a fermentation culture;
- (iii) incubating the fermentation culture; and
- (iv) isolating the compound from the fermentation culture.

The present invention also discloses a method of isolating a compound of Formula (I),



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or $-C(O)N(R_{7a})-$;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

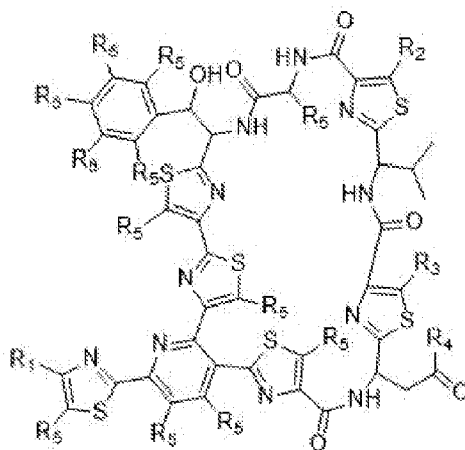
R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl,

the method comprising:

- (i) expanding a bacterial strain under culture conditions that allow expansion of the strain to obtain a seed culture;
- (ii) using the seed culture to inoculate a fermentation culture;
- (iii) incubating the fermentation culture; and
- (iv) isolating the compound from the fermentation culture.

The present invention also discloses a method of isolating a compound of Formula (I),



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl,

the method comprising:

- (i) expanding a *Nonomuraea jiangxiensis* strain under culture conditions that allow expansion of the strain to obtain a seed culture;
- (ii) using the seed culture to inoculate a fermentation culture;
- (iii) incubating the fermentation culture; and
- (iv) isolating the compound from the fermentation culture.

In some embodiments, step (i) comprises expanding the bacteria strain in media, for example an SV2 media. The media may comprise glucose, glycerol, soya peptone, calcium carbonate, and/or combinations thereof.

The pH of the media may be adjusted to be in a range of at least about 5.0, at least about 5.5, at least about 6.0, at least about 6.5, at least about 7.0, at least about 7.5, at least about 8.0; or from about 5.0 to about 8.0, from about 5.0 to about 7.5, from about 5.0 to about 7.0, from about 5.0 to about 6.5, from about 5.0 to about 6.0, from about 5.0 to about 5.5, from about 5.5 to about 8.0, from about 5.5 to about 7.5, from about 5.5 to about 7.0, from about 5.5 to about 6.5, from about 5.5 to about 6.0, from about 6.0 to about 8.0, from about 6.0 to about 7.5, from about 6.0 to about 7.0, from about 6.0 to about 6.5, from about 6.5 to about 8.0, from about 6.5 to about 7.5, from about 6.5 to about 7.0, from about 7.0 to about 8.0, from about 7.0 to about 7.5, from about 7.5 to about 8.0; or at most about 5.0, at most about 5.5, at most about 6.0, at most about 6.5, at most about 7.0, at most about 7.5, at most about 8.0; or about 5.0, about 5.5, about 6.0, about 6.5, about 7.0, about 7.5, about 8.0, or ranges or values therebetween. In some preferred embodiments, the pH of the media may be adjusted to be about 7.0.

In some embodiments, step (i) comprises expanding the bacteria strain in a range of at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days; or from about 1 day to about 5 days, from about 1 day to about 4 days, from about 1 day to about 3 days, from about 1 day to about 2 days, from about 2 days to about 5 days, from about 2 days to about 4 days,

from about 2 days to about 3 days, from about 3 days to about 5 days, from about 3 days to about 4 days, from about 4 days to about 5 days; or at most about 1 day, at most about 2 days, at most about 3 days, at most about 4 days, at most about 5 days; or about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, or any ranges or values therebetween. In some preferred embodiments, step (i) comprises expanding the bacteria strain for 3 days.

In some embodiments, step (i) comprises expanding the bacteria strain at a temperature range of at least about 25 °C, at least about 27 °C, at least about 28 °C, at least about 29 °C, at least about 30 °C, at least about 32 °C, at least about 35 °C; or from about 25 °C to about 35 °C, from about 25 °C to about 32 °C, from about 25 °C to about 30 °C, from about 25 °C to about 29 °C, from about 25 °C to about 28 °C, from about 25 °C to about 27 °C, from about 27 °C to about 35 °C, from about 27 °C to about 32 °C, from about 27 °C to about 30 °C, from about 27 °C to about 29 °C, from about 27 °C to about 28 °C, from about 28 °C to about 35 °C, from about 28 °C to about 32 °C, from about 28 °C to about 30 °C, from about 28 °C to about 29 °C, from about 29 °C to about 35 °C, from about 29 °C to about 32 °C, from about 29 °C to about 30 °C, from about 30 °C to about 35 °C, from about 30 °C to about 32 °C, from about 32 °C to about 35 °C; or at most about 25 °C, at most about 27 °C, at most about 28 °C, at most about 29 °C, at most about 30 °C, at most about 32 °C, at most about 35 °C; or about 25 °C, about 27 °C, about 28 °C, about 29 °C, about 30 °C, about 32 °C, about 35 °C, or ranges or values therebetween. In some preferred embodiments, step (i) comprises expanding the bacteria strain at about 28 °C.

In some embodiments, step (i) comprises expanding the bacteria strain with a shaking condition in a range of at least about 150 rpm, at least about 175 rpm, at least about 200 rpm, at least about 225 rpm, at least about 250 rpm, at least about 300 rpm, at least about 350 rpm; or from about 150 rpm to about 350 rpm, from about 150 rpm to about 300 rpm, from about 150 rpm to about 250 rpm, from about 150 rpm to about 225 rpm, from about 150 rpm to about 200 rpm, from about 150 rpm to about 175 rpm, from about 175 rpm to about 350 rpm, from about 175 rpm to about 300 rpm, from about 175 rpm to about 250 rpm, from about 175 rpm to about 225 rpm, from about 175 rpm to about 200 rpm, from about 200 rpm to about 350 rpm, from about 200 rpm to about 300 rpm, from about 200 rpm to about 250 rpm, from about 200 rpm to about 225 rpm, from about 225 rpm to about 350 rpm, from about 225 rpm to about 300 rpm, from about 225 rpm to about 250 rpm, from about 250 rpm to about 350 rpm, from about 250 rpm to about 300 rpm, from about 300 rpm to about 350 rpm; or at most about 150 rpm, at most about 175 rpm, at most about 200 rpm, at most about 225 rpm, at most about 250 rpm, at most about 300 rpm, at most about 350 rpm; or about 150 rpm, about 175 rpm, about 200 rpm, about 225 rpm, about 250 rpm, about 300 rpm, about 350 rpm, or any ranges or values therebetween. In some preferred embodiments, step (i) comprises expanding the bacteria strain with a shaking condition at 200 rpm.

In some embodiments, the fermentation culture in step (ii) is CA09LB media. In some embodiments, the media may comprise meat extract, yeast extract, glucose, glycerol, and/or combinations thereof.

The pH of the fermentation culture may be adjusted to be in a range of at least about 5.0, at least about 5.5, at least about 6.0, at least about 6.5, at least about 7.0, at least about 7.5, at least about 8.0; or from about 5.0 to about 8.0, from about 5.0 to about 7.5, from about 5.0 to about 7.0, from about 5.0 to about 6.5, from about 5.0 to about 6.0, from about 5.0 to about 5.5, from about 5.5 to about 8.0, from about 5.5 to about 7.5, from about 5.5 to about 7.0, from about 5.5 to about 6.5, from about 5.5 to about 6.0, from about 6.0 to about 8.0, from about 6.0 to about 7.5, from about 6.0 to about 7.0, from about 6.0 to about 6.5, from about 6.5 to about 8.0, from about 6.5 to about 7.5, from about 6.5 to about 7.0, from about 7.0 to about 8.0, from about 7.0 to about 7.5, from about 7.5 to about 8.0; or at most about 5.0, at most about 5.5, at most about 6.0, at most about 6.5, at most about 7.0, at most about 7.5, at most about 8.0; or about 5.0, about 5.5, about 6.0, about 6.5, about 7.0, about 7.5, about 8.0, or ranges or values therebetween. In some preferred embodiments, the pH of the fermentation culture may be adjusted to be about 7.0.

In some embodiments, step (iii) comprises incubating the fermentation culture in a range of at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days; or from about 7 days to about 12 days, from about 7 days to about 11 days, from about 7 days to about 10 days, from about 7 days to about 9 days, from about 7 days to about 8 days, from about 8 days to about 12 days, from about 8 days to about 11 days, from about 8 days to about 10 days, from about 8 days to about 9 days, from about 9 days to about 12 days, from about 9 days to about 11 days, from about 9 days to about 10 days, from about 10 days to about 12 days, from about 10 days to about 11 days, from about 11 days to about 12 days; or at most about 7 days, at most about 8 days, at most about 9 days, at most about 10 days, at most about 11 days, at most about 12 days; or about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, or any ranges or values therebetween. In some preferred embodiments, step (iii) comprises incubating the fermentation culture for about 7 days to about 12 days. In some other preferred embodiments, step (iii) comprises incubating the fermentation culture for about 9 days.

In some embodiments, step (iii) comprises incubating the fermentation culture at a temperature range of at least about 25 °C, at least about 27 °C, at least about 28 °C, at least about 29 °C, at least about 30 °C, at least about 32 °C, at least about 35 °C; or from about 25 °C to about 35 °C, from about 25 °C to about 32 °C, from about 25 °C to about 30 °C, from about 25 °C to about 29 °C, from about 25 °C to about 28 °C, from about 25 °C to about 27 °C, from about 27 °C to about 35 °C, from about 27 °C to

about 32 °C, from about 27 °C to about 30 °C, from about 27 °C to about 29 °C, from about 27 °C to about 28 °C, from about 28 °C to about 35 °C, from about 28 °C to about 32 °C, from about 28 °C to about 30 °C, from about 28 °C to about 29 °C, from about 29 °C to about 35 °C, from about 29 °C to about 32 °C, from about 29 °C to about 30 °C, from about 30 °C to about 35 °C, from about 30 °C to about 32 °C, from about 32 °C to about 35 °C; or at most about 25 °C, at most about 27 °C, at most about 28 °C, at most about 29 °C, at most about 30 °C, at most about 32 °C, at most about 35 °C; or about 25 °C, about 27 °C, about 28 °C, about 29 °C, about 30 °C, about 32 °C, about 35 °C, or ranges or values therebetween. In some preferred embodiments, step (iii) comprises incubating the fermentation culture at about 28 °C.

In some embodiments, step (iii) comprises incubating the fermentation culture with a shaking condition in a range of at least about 150 rpm, at least about 175 rpm, at least about 200 rpm, at least about 225 rpm, at least about 250 rpm, at least about 300 rpm, at least about 350 rpm; or from about 150 rpm to about 350 rpm, from about 150 rpm to about 300 rpm, from about 150 rpm to about 250 rpm, from about 150 rpm to about 225 rpm, from about 150 rpm to about 200 rpm, from about 150 rpm to about 175 rpm, from about 175 rpm to about 350 rpm, from about 175 rpm to about 300 rpm, from about 175 rpm to about 250 rpm, from about 175 rpm to about 225 rpm, from about 175 rpm to about 200 rpm, from about 200 rpm to about 350 rpm, from about 200 rpm to about 300 rpm, from about 200 rpm to about 250 rpm, from about 200 rpm to about 225 rpm, from about 225 rpm to about 350 rpm, from about 225 rpm to about 300 rpm, from about 225 rpm to about 250 rpm, from about 250 rpm to about 350 rpm, from about 250 rpm to about 300 rpm, from about 300 rpm to about 350 rpm; or at most about 150 rpm, at most about 175 rpm, at most about 200 rpm, at most about 225 rpm, at most about 250 rpm, at most about 300 rpm, at most about 350 rpm; or about 150 rpm, about 175 rpm, about 200 rpm, about 225 rpm, about 250 rpm, about 300 rpm, about 350 rpm, or any ranges or values therebetween. In some preferred embodiments, step (iii) comprises incubating the fermentation culture with a shaking condition at 200 rpm.

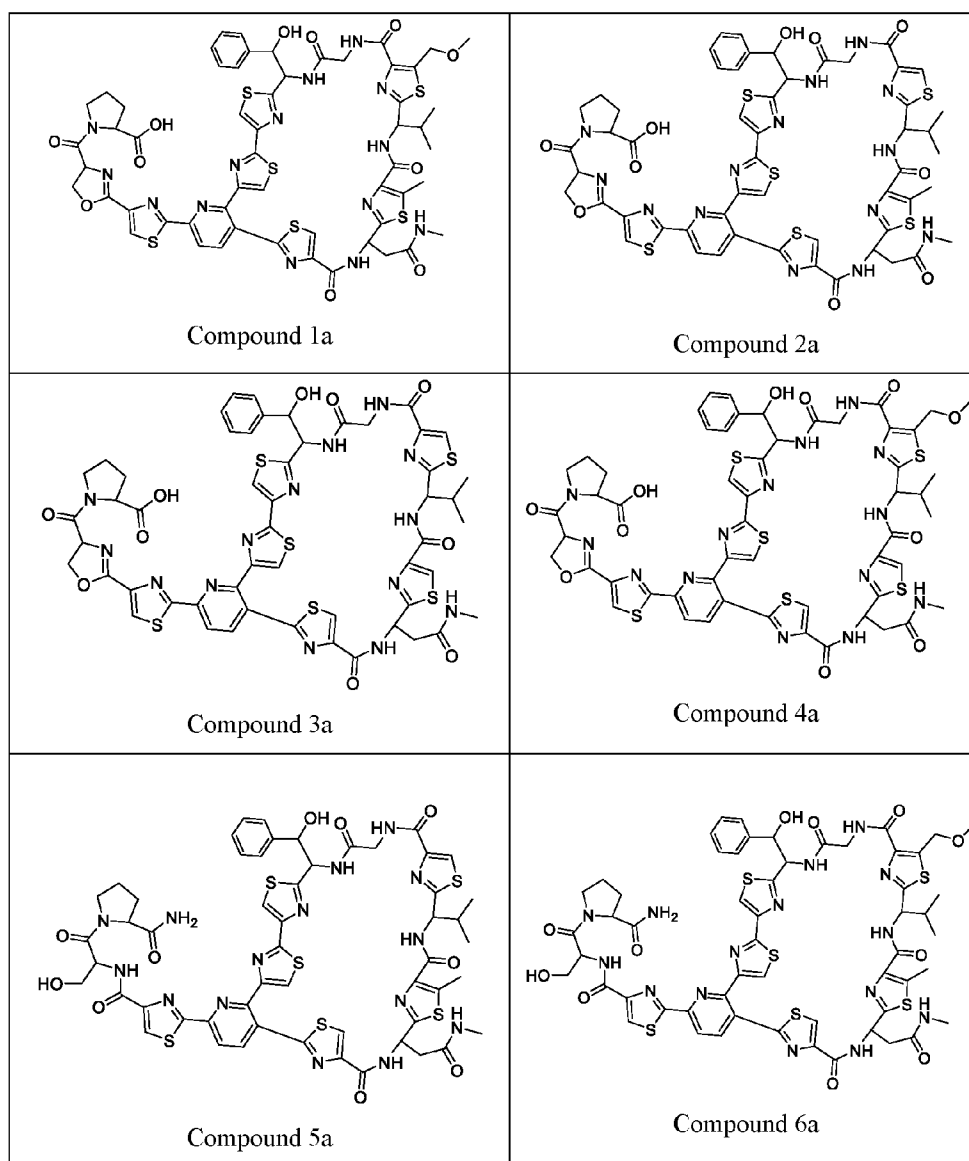
In some embodiments, step (iii) comprises incubating the fermentation culture for 9 days at 28 °C with shaking condition of 200 rpm.

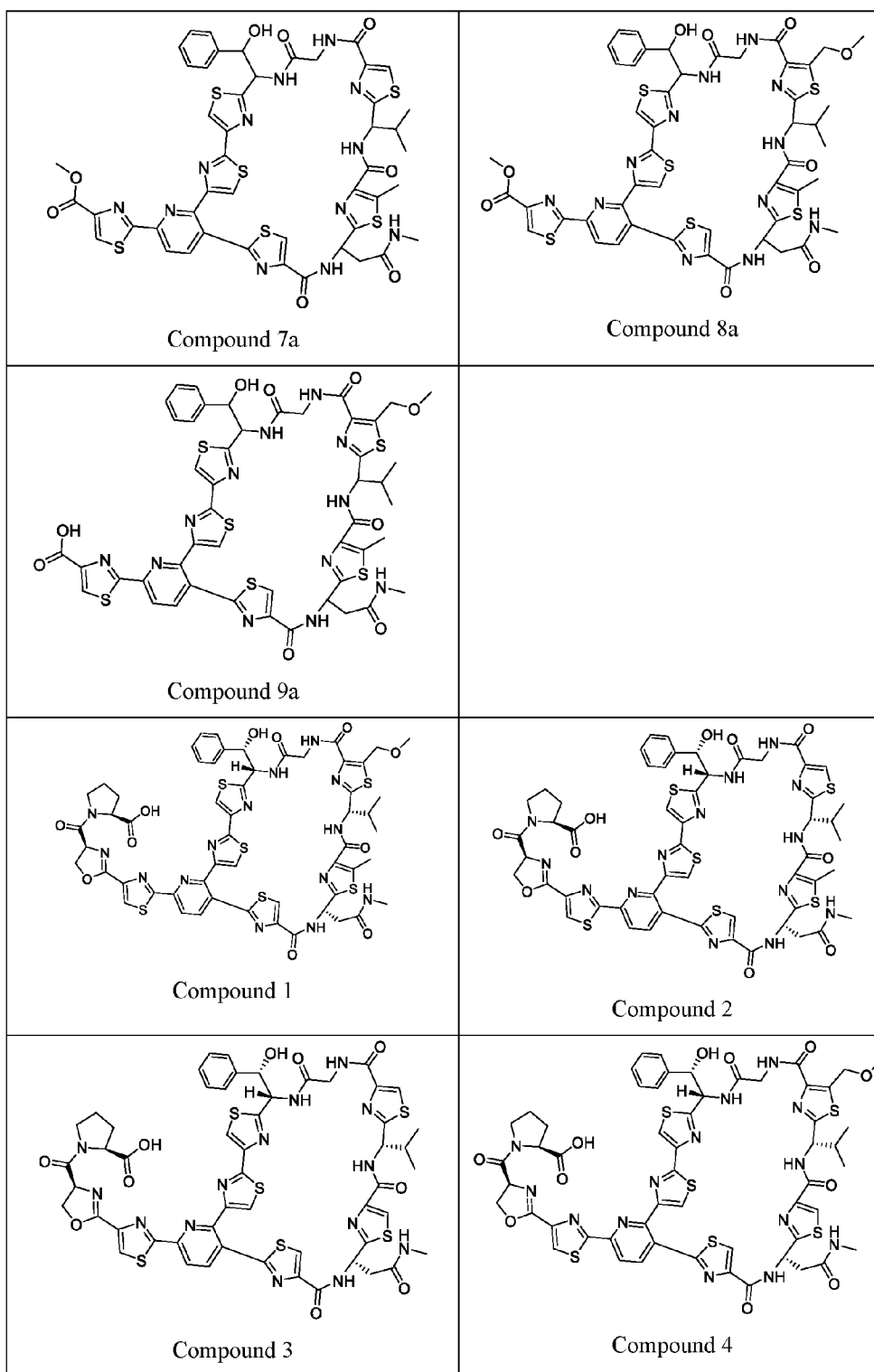
In some embodiments, the compound is isolated using centrifugation, precipitation, filtration, liquid extraction, methanol extraction, chromatography, column chromatography, affinity chromatography, evaporation, lyophilization, and/or combinations thereof.

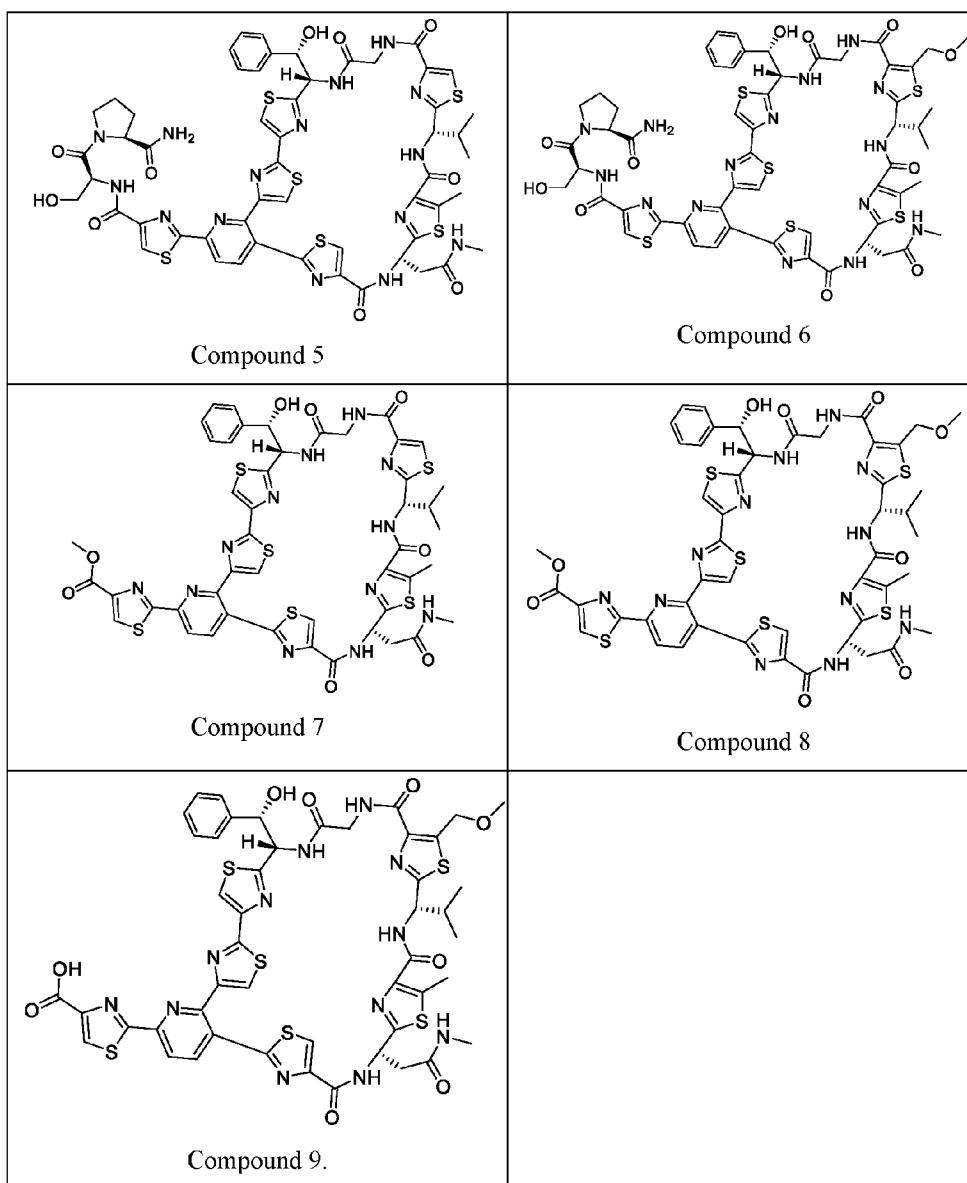
In some embodiments, the compound isolated, or its pharmaceutically acceptable salt, metabolite, prodrug, stereoisomer, enantiomer, diastereomer, geometric isomer, and/or isomer of any of the foregoing, is a compound as disclosed herein.

R, R₁, R₂, R₃, R₄, R₅, R₆, R₇, R_{7a}, R_{7b}, R₈, may be as defined anywhere in the specification.

In some embodiments, the compound isolated, or its pharmaceutically acceptable salt, metabolite, prodrug, stereoisomer, enantiomer, diastereomer, geometric isomer, and/or isomer of any of the foregoing, may be selected from the group consisting of:



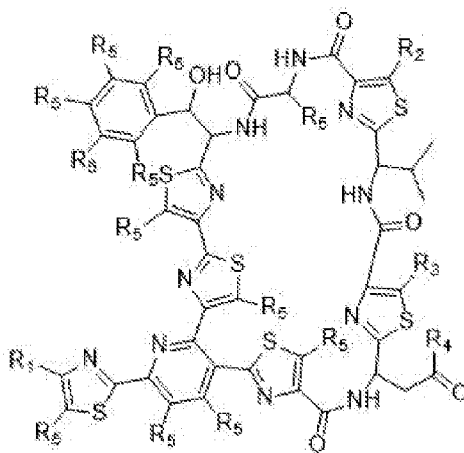




Accordingly, the present invention provides a compound prepared by the method disclosed herein.

List of Embodiments

1. A compound of Formula (I) or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof:



Formula (I)

wherein:

R_1 is $-C(O)-R_6$ or $-A-G-J-L$;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or $-C(O)N(R_{7a})-$;

G is $-C(O)-$ or optionally substituted $-alkylene-C(O)-$;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is $-C(O)-R_6$;

R_2 is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R_3 is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

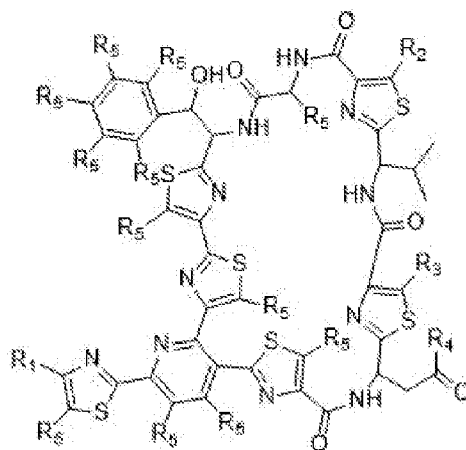
R_4 is H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R_5 is independently selected from the group consisting of H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R_6 is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently H or optionally substituted alkyl.

2. A compound of Formula (I) or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof:



Formula (I)

wherein:

R_1 is -C(O)- R_6 or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylenc-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

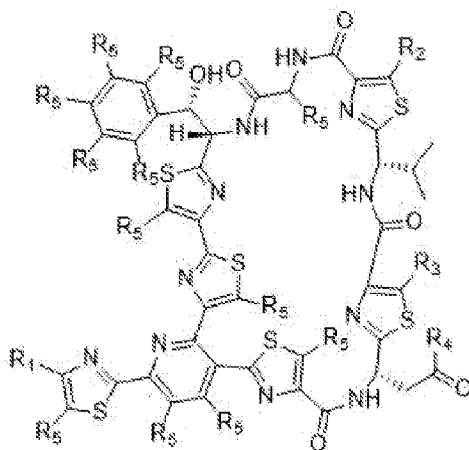
R₅ is independently selected from the group consisting of H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently H or optionally substituted alkyl,

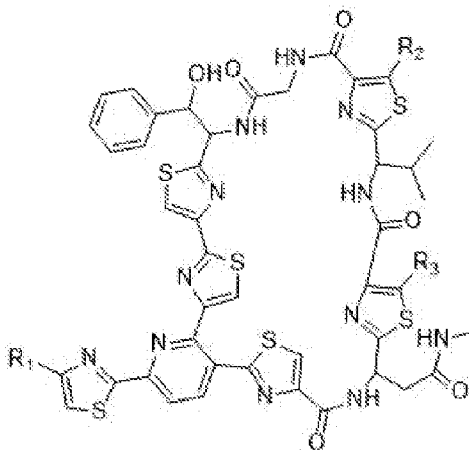
wherein when R₂ is optionally substituted alkalkoxy, R₃ is H, optionally substituted alkenyl, or optionally substituted alkynyl.

3. The compound of embodiments 1 or 2, wherein the compound has the structure and stereochemical configuration of Formula (II):



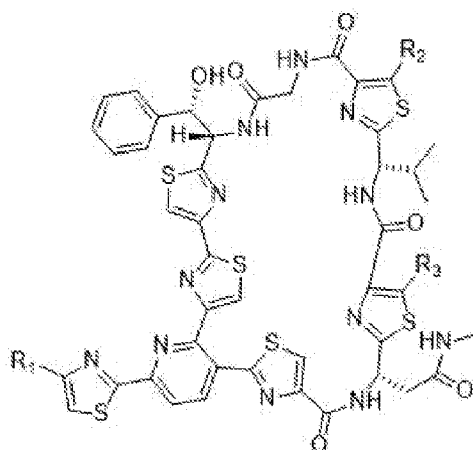
Formula (II)

4. The compound of any one of embodiments 1 to 3, wherein the compound is of Formula (III):



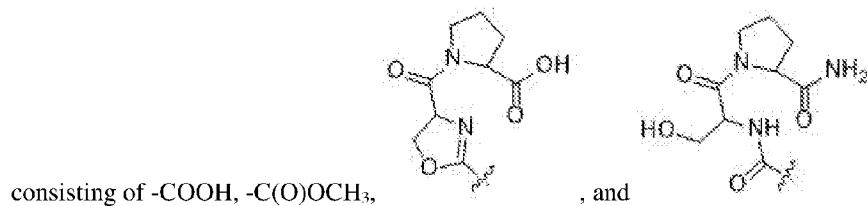
Formula (III)

5. The compound of any one of embodiments 1 to 4, wherein the compound is of Formula (IV):

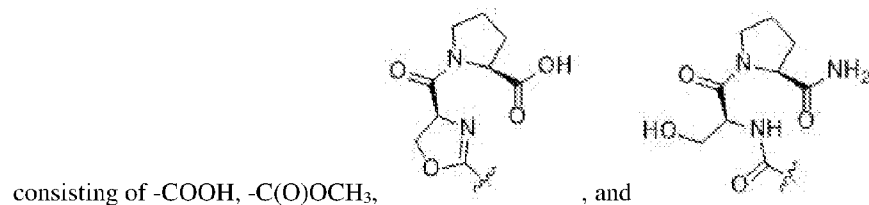


Formula (IV)

6. The compound of any one of embodiments 1 to 5, wherein A is heterocarbocyclylene or $-\text{C}(\text{O})\text{N}(\text{R}_{7a})-$.
7. The compound of any one of embodiments 1 to 6, wherein G is $-\text{C}(\text{O})-$ or optionally substituted $-\text{alkylene}-\text{C}(\text{O})-$.
8. The compound of any one of embodiments 1 to 7, wherein G is $-\text{C}(\text{O})-$ or $-\text{CH}(\text{R}_8)-\text{C}(\text{O})-$, wherein R_8 is alkylene substituted with $-\text{OH}$.
9. The compound of embodiment 8, wherein R_8 is $-\text{CH}_2-\text{OH}$.
10. The compound of any one of embodiments 1 to 9, wherein J is heterocarbocyclylene.
11. The compound of any one of embodiments 1 to 10, wherein L is $-\text{COOH}$ or $-\text{C}(\text{O})\text{NH}_2$.
12. The compound of any one of embodiments 1 to 11, wherein R_1 is selected from the group



13. The compound of any one of embodiments 1 to 12, wherein R_1 is selected from the group

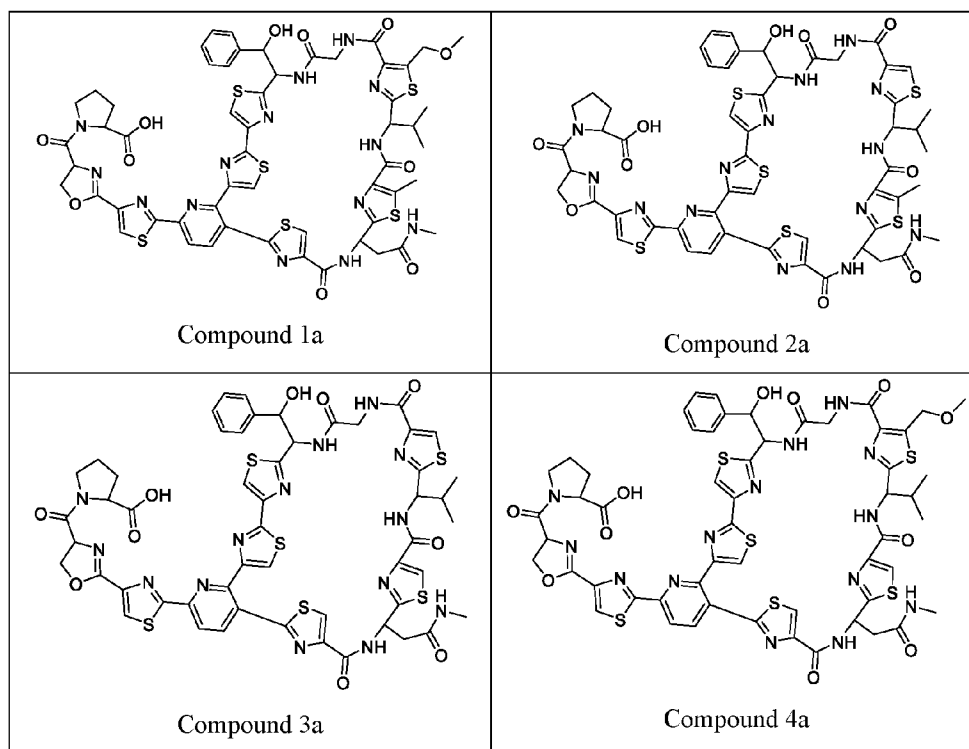


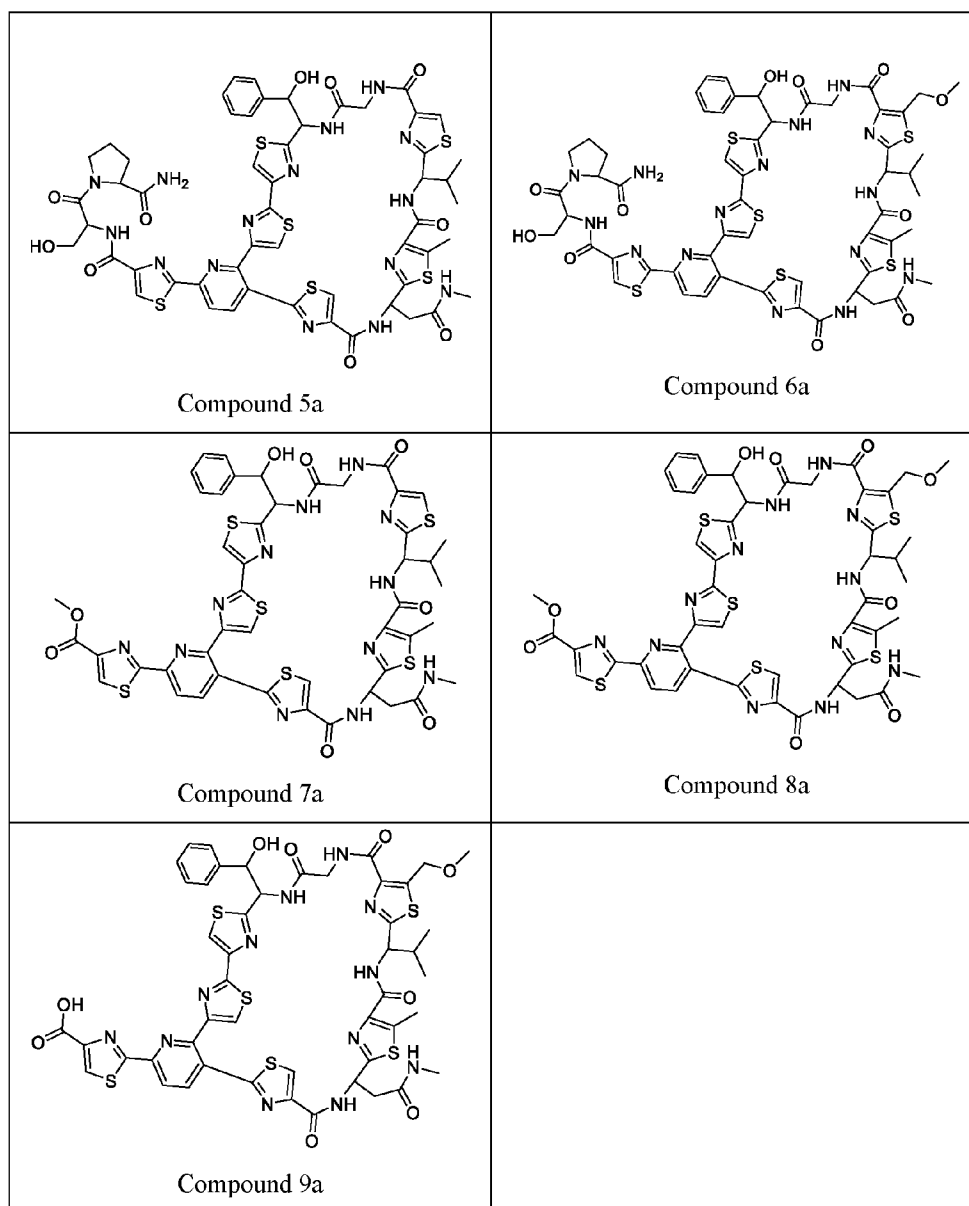
14. The compound of any one of embodiments 1 to 13, wherein R_2 is H or $-\text{CH}_2\text{OCH}_3$.

15. The compound of any one of embodiments 1 to 14, wherein R_3 is H or $-\text{CH}_3$.

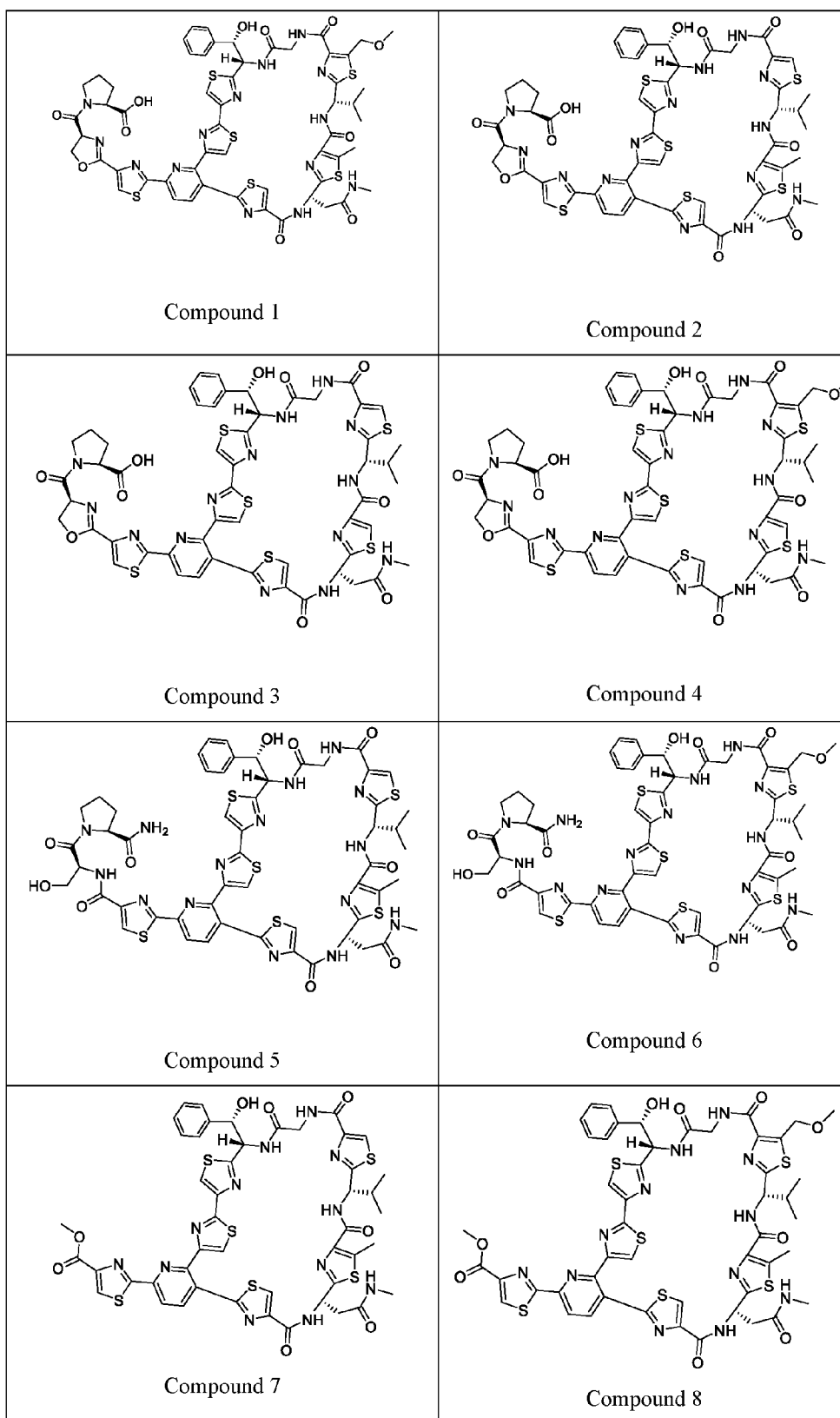
16. The compound of any one of embodiments 1 to 15, wherein R_4 is $-\text{NH}(\text{CH}_3)$.

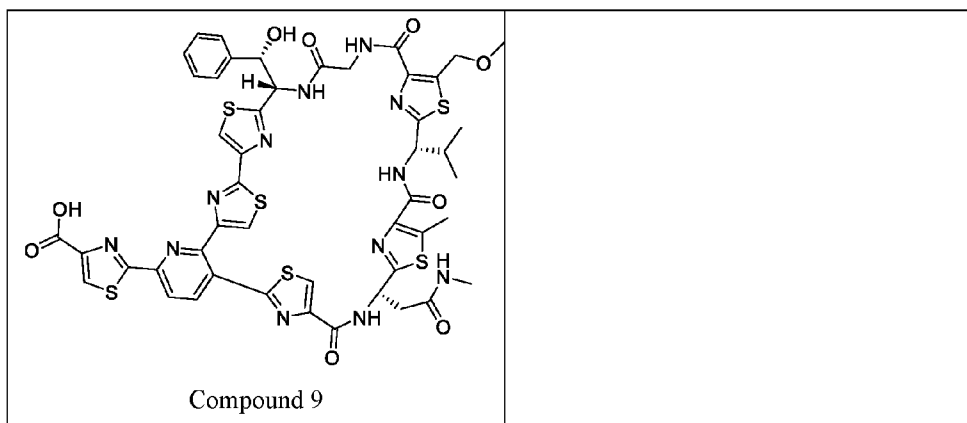
17. The compound of any one of embodiments 1 to 16, wherein the compound is selected from the group consisting of:





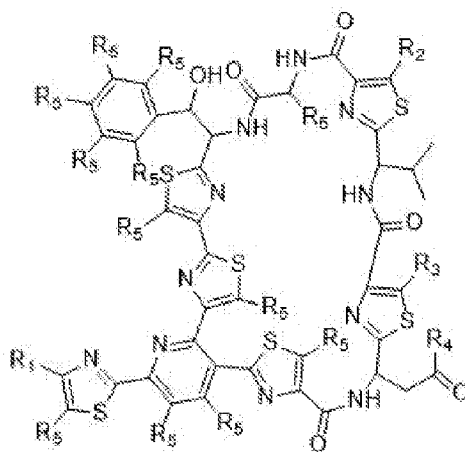
18. The compound of any one of embodiments 1 to 17, wherein the compound is selected from the group consisting of:





19. A pharmaceutical composition comprising a therapeutically effective amount of a compound of any one of embodiments 1 to 18, or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, and a pharmaceutically acceptable excipient.
20. A compound of any one of embodiments 1 to 18, or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, for use in therapy.
21. The compound or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof of any one of embodiments 1 to 18, for use in treating an infection caused by *Staphylococcus aureus*, *Staphylococcus aureus* Rosenbach, *gram-positive methicillin-resistant Staphylococcus aureus (MRSA)*, *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens*, *Acinetobacter baumannii*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Candida albicans*, *Clostridium difficile*, *Enterococcus faecalis*, *Enterococcus faecium*, or *Micrococcus luteus*.
22. The compound or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof of any one of embodiments 1 to 18, for use in treating cancer.
23. Use of a compound of any one of embodiments 1 to 18, or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, in the manufacture of a medicament for treating bacterial, microbial and/or fungal infections.
24. The use of embodiment 23, wherein the infection is caused by *Staphylococcus aureus*, *Staphylococcus aureus* Rosenbach, *gram-positive methicillin-resistant Staphylococcus aureus (MRSA)*, *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens*, *Acinetobacter baumannii*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Candida albicans*, *Clostridium difficile*, *Enterococcus faecalis*, *Enterococcus faecium*, or *Micrococcus luteus*.

25. Use of a compound of any one of embodiments 1 to 24, or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, in the manufacture of a medicament for treating cancer.
26. A method of treating a bacterial, microbial and/or fungal infection, comprising administering a therapeutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, to a subject in need of treatment



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

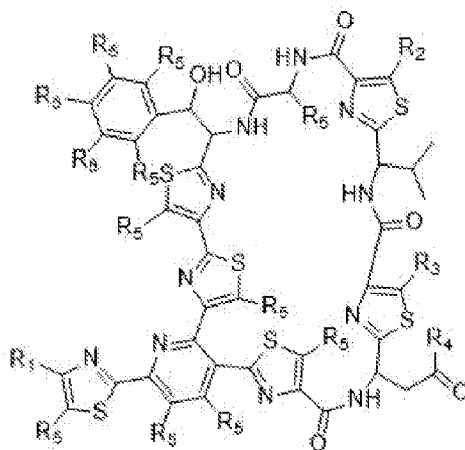
R₄ is H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently H or optionally substituted alkyl.

27. The method of any one of embodiments 21-26, wherein the infection is a *Staphylococcus*, *Bacillus*, *Clostridium*, *Acinetobacter*, *Klebsiella*, *Pseudomonas*, *Candida*, *Enterococcus*, or *Micrococcus* infection.
28. The method of any one of embodiments 21-27, wherein the infection is caused by *Staphylococcus aureus*, *Staphylococcus aureus* Rosenbach, gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens*, *Acinetobacter baumannii*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Candida albicans*, *Clostridium difficile*, *Enterococcus faecalis*, *Enterococcus faecium*, or *Micrococcus luteus*.
29. A method of treating cancer, comprising administering a therapeutically effective amount of a compound selected from the following, or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, to a subject in need of treatment:



Formula (I)

wherein:

R_1 is $-C(O)-R_6$ or $-A-G-J-L$;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or $-C(O)N(R_{7a})-$;

G is $-C(O)-$ or optionally substituted $-alkylene-C(O)-$;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is $-C(O)-R_6$;

R_2 is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R_3 is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R_4 is H, $-OH$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or $-N(R_{7a})(R_{7b})$;

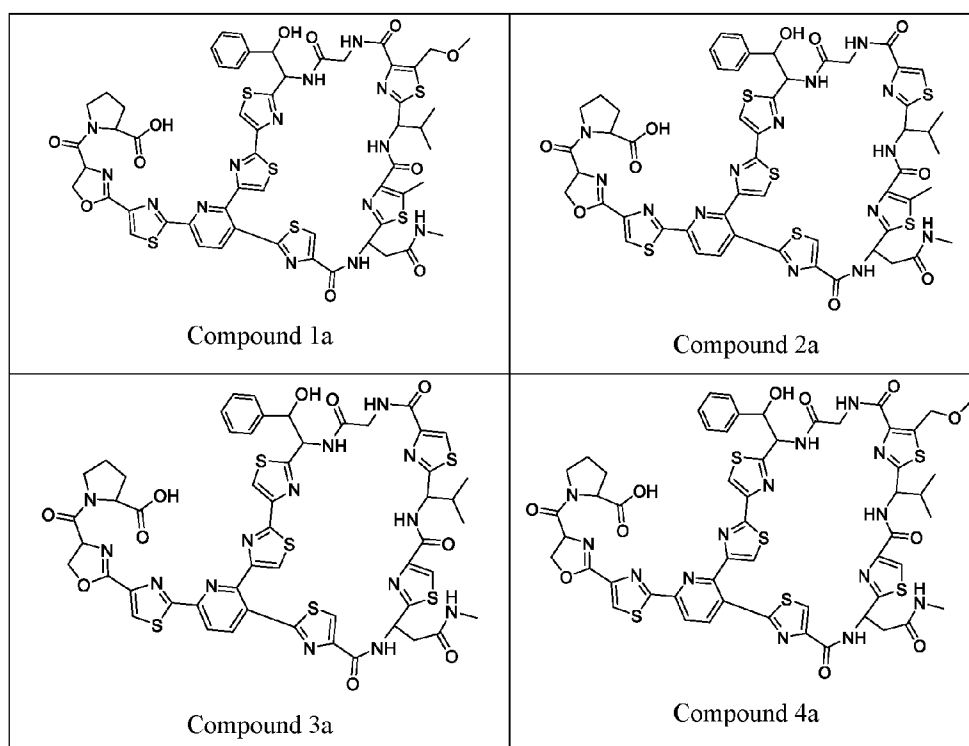
R₅ is independently selected from the group consisting of H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

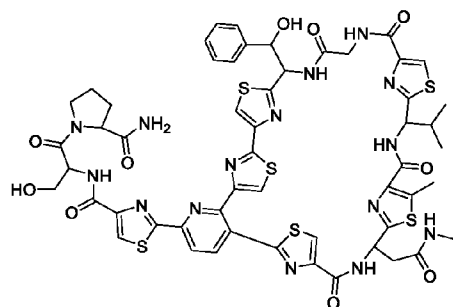
R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently H or optionally substituted alkyl.

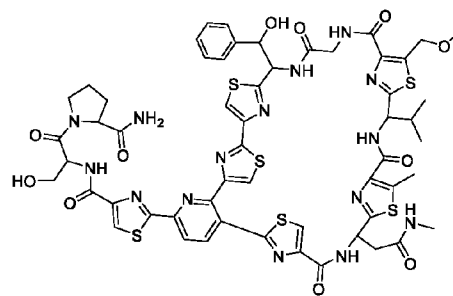
30. The method of any one of embodiments 21-29, wherein the compound is a compound of any one of embodiments 1-18.

31. The method of any one of embodiments 21-30, wherein the compound is selected from:

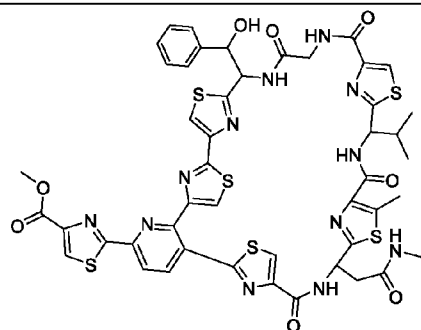




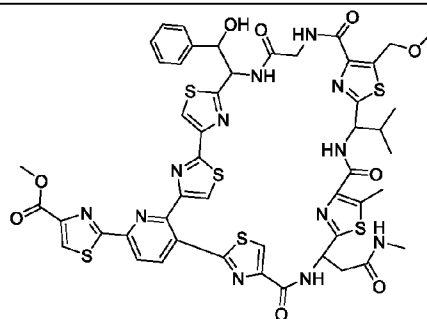
Compound 5a



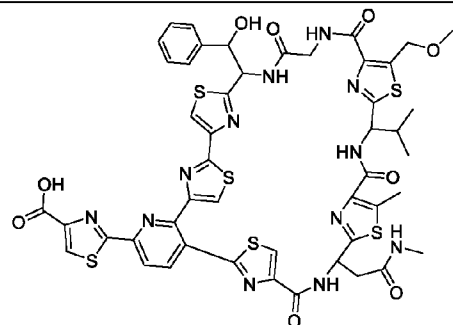
Compound 6a



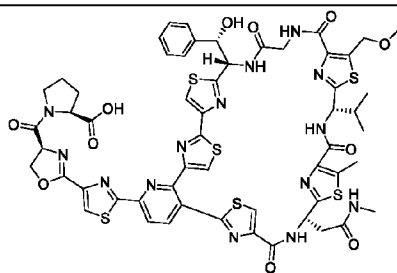
Compound 7a



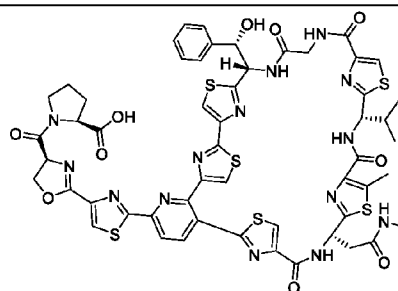
Compound 8a



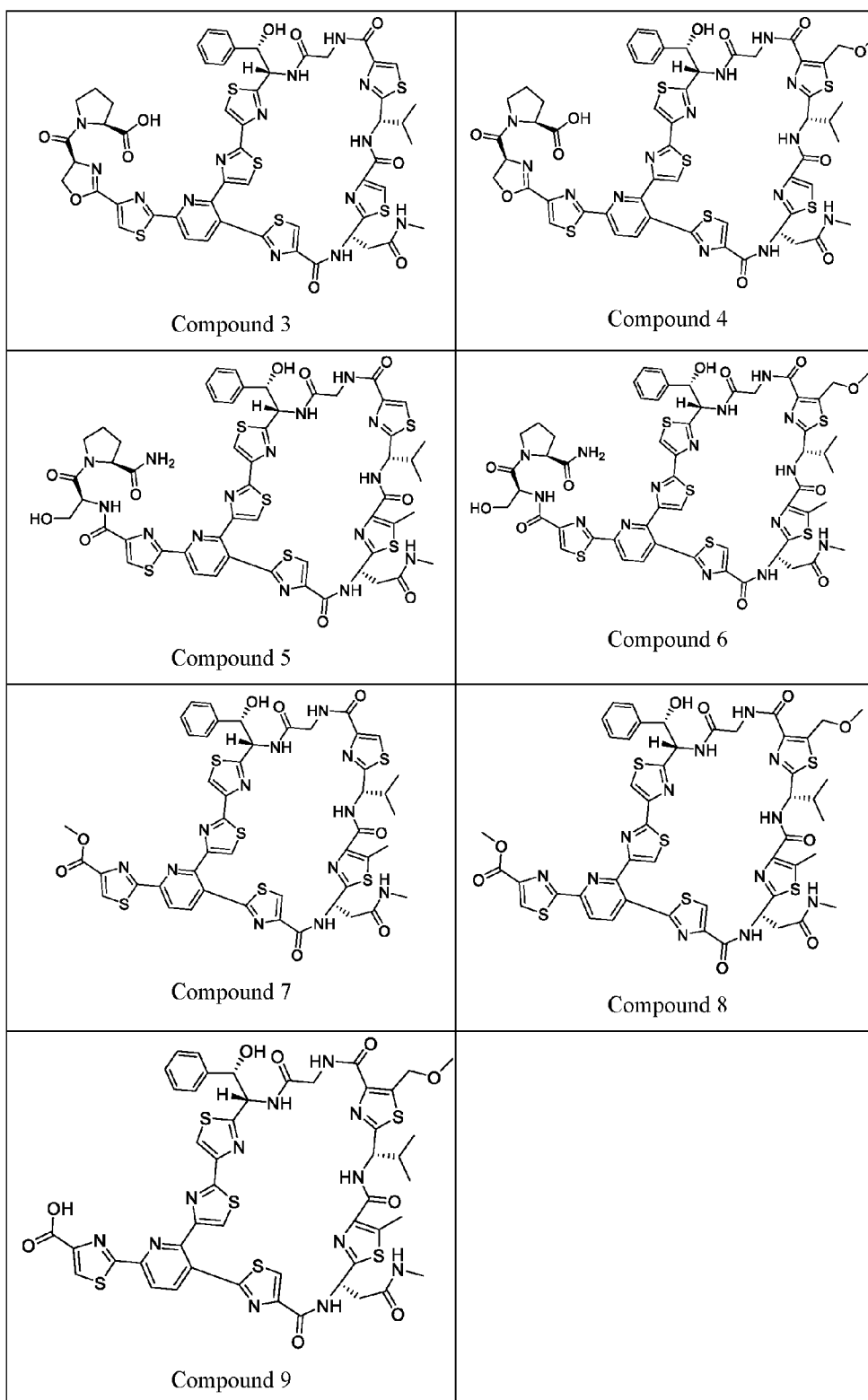
Compound 9a



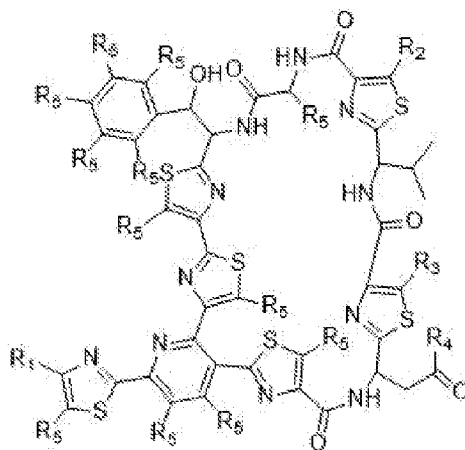
Compound 1



Compound 2



32. A method of isolating a compound of Formula (I),



Formula (I)

wherein:

R_1 is $-C(O)-R_6$ or $-A-G-J-L$;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or $-C(O)N(R_{7a})-$;

G is $-C(O)-$ or optionally substituted $-alkylene-C(O)-$;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is $-C(O)-R_6$;

R_2 is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R_3 is H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R_4 is H, $-OH$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or $-N(R_{7a})(R_{7b})$;

R₅ is independently selected from the group consisting of H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

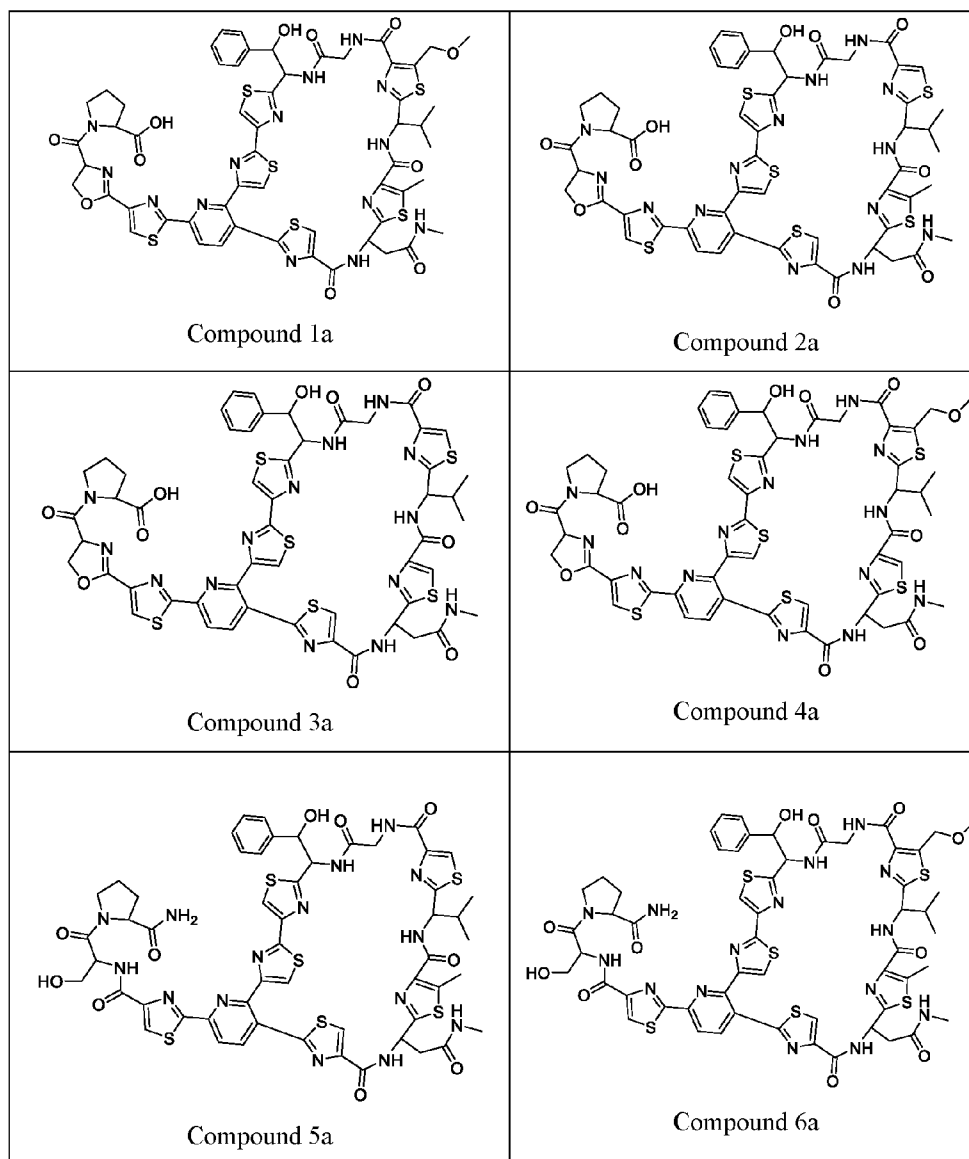
R_{7a} and R_{7b} are independently H or optionally substituted alkyl.

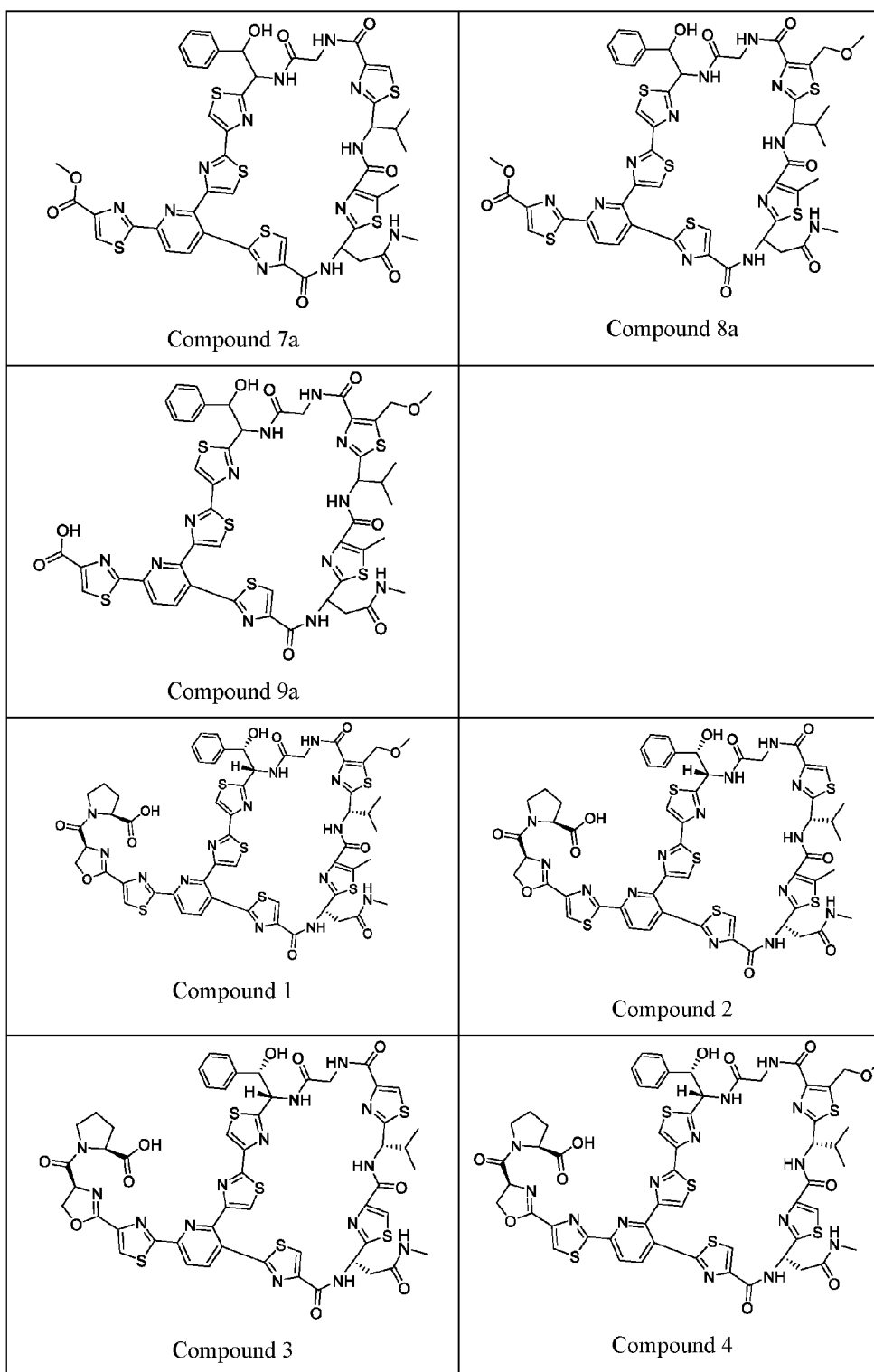
the method comprising:

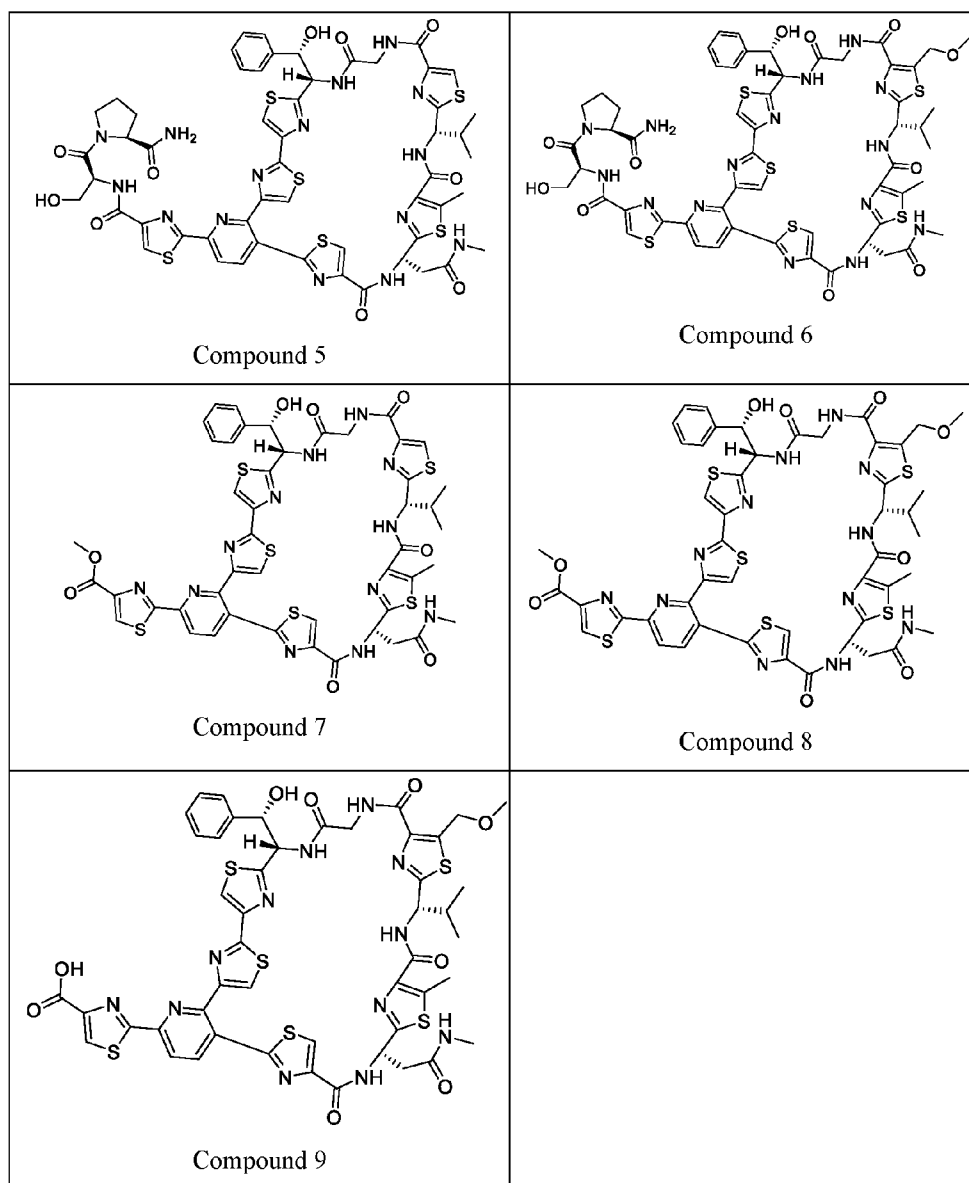
- (i) expanding a *Nonomuraea jiangxiensis* strain under culture conditions that allow expansion of the strain to obtain a seed culture;
- (ii) using the seed culture to inoculate a fermentation culture;
- (iii) incubating the fermentation culture; and
- (iv) isolating the compound from the fermentation culture.

- 33. The method of embodiment 32, wherein step (iii) comprises incubating the fermentation culture for about 7 to about 12 days.
- 34. The method of embodiment 32 or 33, wherein step (iii) comprises incubating the fermentation culture at a temperature of about 25 °C to 35 °C.
- 35. The method of any one of embodiments 32 to 34, wherein step (iii) comprises incubating the fermentation culture with a shaking condition of about 150 to 350 rpm.
- 36. The method of any one of embodiments 32 to 35, wherein step (iii) comprises incubating the fermentation culture for 9 days at 28 °C with shaking condition of 200 rpm.
- 37. The method of any one of embodiments 32 to 36, wherein the compound is isolated using a method selected from the group consisting of centrifugation, precipitation, filtration, liquid extraction, methanol extraction, chromatography, column chromatography, affinity chromatography, evaporation, lyophilization, and combinations thereof.
- 38. The method of any one of embodiments 32 to 37, wherein the compound is a compound of any one of embodiments 1 to 18.

39. The method of any one of embodiments 32 to 38, wherein the compound is a compound selected from the group consisting of:







40. A compound prepared by the method of any one of embodiments 32 to 39.

Examples

Non-limiting examples of the invention and comparative examples will be further described in greater detail by reference to specific examples, which should not be construed as in any way limiting the scope of the invention.

Material and Methods

The bacterial strain A7611 was isolated from terrestrial soil in Singapore.

Example 1: Molecular Identification of Bacteria Isolate A7611

The isolated bacterial strain A7611 was grown on Bennett Agar for 5 days to 7 days at 28 °C.

The DNA of the strain was extracted from the plate using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol where the cells underwent a beat-beating step for cell disruption using an automated tissue homogenizer and cell lyser 1600 MiniG (SPEX SamplePrep, Metuchen, New Jersey, US) at 1500 rpm for 3 minutes. The NanoDrop2000 spectroscopy system (ThermoFisher Scientific, Waltham, Massachusetts, US) was used to measure the DNA purity and yield extracted.

Bacterial 16S rRNA genes were amplified from the DNA extracted from the isolated actinobacteria with universal 16S primers 27F (5' – AGA GTT TGA TCC TGG CTC AG – 3') and 1492R (5' – TAC GGY TAC CTT GTT ACG ACT T – 3') [12,13]. The PCR amplification reactions were performed using Applied Biosystems ProFlex Thermocycler (ThermoFisher Scientific, Waltham, Massachusetts, US) with a total reaction of 20 µl that comprised 2.0 µl of 10× PCR buffer with 20 mM MgCl₂, 2.0 µl of 2 mM dNTPs, 1 unit of Taq polymerase (ThermoFisher Scientific, Waltham, Massachusetts, US), 1.0 µl of 10 µM of each primer and 1.0 µl of purified DNA templates. A non-template and negative control using sterile resuspension buffer were included in the run. The reactions were subjected to the following temperature cycling profile of initial denaturation at 95 °C for 5 minutes; 30 cycles each of 30 seconds at 95 °C for denaturation, 50 seconds at 60 °C for annealing and 1 minute at 72 °C for extension, with a final extension of 5 minutes at 72 °C.

Commercial service of forward and reverse Sanger Sequencing were done on the PCR amplified DNA fragment (1st BASE, Singapore). The sequences obtained were aligned using

Benchling and further analyzed using Basic Local Alignment Search Tool (BLAST) [National Center for Biotechnology Information (NCBI)].

Example 2: Phylogenetic Analysis of Bacteria Isolate A7611

The 16S rRNA region sequence of the isolated strain A7611 was aligned with related actinobacteria strains retrieved from the GenBank databases using ClustalW. A neighbor-joining tree algorithm method was used to determine the genetic relationship between the strains. The phylogenetic tree was constructed with a bootstrapped database containing 1000 replicates in MEGA 11.0 software (Mega, US). The DNA sequence for sample A7611 reported in the present disclosure were deposited with GenBank database of NCBI under the accession numbers OM967343.

A nucleotide BLAST search of the 16S rRNA gene sequence of A7611 performed against the NCBI 16S ribosomal RNA database revealed that the isolate shared 99.93% sequence identity (E-value = 0.0) to the 16S rRNA of *Nonomuraea jiangxiensis* having an accession number NR_116645.1. The phylogenetic relatedness using the neighbor-joining analysis method of isolated strain and its closely related species obtained from the GenBank database is shown in Fig. 1. The neighbor-joining phylogenetic tree was constructed based on 16S rRNA gene sequence showing the relationship between isolated strain A7611 and representatives or related actinobacteria strains retrieved from the GenBank with their respective accession numbers. Bootstrap values greater than 50% are shown at the number on the branches nodes which were analyzed based on 1,000 replicates. Bar, 0.01 substitutions per nucleotide position.

Example 3: Fermentation and extraction of bacterial crude extract

The *Nonomuraea jiangxiensis* strain A7611 was cultured in 5 mL SV2 media, (for 1 L, add 15 g glucose (1st BASE, Singapore), 15 g glycerol (VWR, Radnor, Pennsylvania, US), 15 g soya peptone (Oxoid, Basingstoke, Hampshire, UK), and 1 g calcium carbonate (Sigma-Aldrich, St. Louis, Missouri, US), pH adjusted to 7.0) for 3 days at 28 °C with shaking at 200 rpm.

Saturated seed cultures were diluted in a 1:20 volume ratio into fresh fermentation CA09LB media (For 1 L, add 10 g meat extract (Sigma-Aldrich, St. Louis, Missouri, US), 4 g yeast extract (BD Biosciences, Franklin Lakes, New Jersey, US), 20 g glucose (1st BASE, Singapore), and glycerol 3 g (VWR, Radnor, Pennsylvania, US), pH adjusted to 7.0) and fermented with 200 rpm shaking at 28 °C in the dark for 9 days.

The cultures were pelleted after 9 days followed by lyophilization of the separated biomass and supernatant. The dried samples were extracted with MeOH and filtered through filter paper (Whatman Grade 4, Maidstone, Kent, UK). MeOH was removed under reduced pressure to give a crude extract of a combined weight of 15.70 g. The crude extract consists of broth extract 84.4% (13.25 g) and biomass extract 15.6% (2.45 g).

Example 4: Isolation and Purification of Compounds

The dried extracts obtained were combined and partitioned with CH₂Cl₂/MeOH/H₂O in a ratio of 2:1:1. The CH₂Cl₂ was removed under reduced pressure and the CH₂Cl₂ crude extract (692 mg) was redissolved in CH₂Cl₂ and subjected to a silica gel column chromatography (Merck, Silica gel 60, 0.040-0.063 mm). The column was eluted with a stepwise gradient of 0%, 2%, 4%, 8%, 10% and 12% MeOH in CH₂Cl₂ followed by 100% MeOH. The 12% MeOH in CH₂Cl₂ and 100% MeOH fractions were combined to obtain an enriched fraction of thiopeptide analogues (350 mg).

Compounds 1, 2 and 7-9

The enriched fraction was then redissolved in CH₂Cl₂:MeOH in a ratio of 1:1. Further separation was performed using a Sephadex LH-20 column (mobile phase: CH₂Cl₂:MeOH = 1:1) to obtain a subfraction of thiopeptide analogues (53 mg). The dried mixtures were dissolved in MeOH and separated with C₁₈ RP-HPLC (solvent A: H₂O + 0.1% HCOOH, solvent B: acetonitrile + 0.1% HCOOH; flow rate: 24 mL/min, gradient conditions: 70:30 isocratic for 5 minutes; 30% to 60% of solvent B over 55 minutes, 60% to 100% of solvent B over 2 minutes, and finally isocratic at 100% of solvent B for 10 minutes. The HPLC gave 6.9 mg of compound **1** (RT = 37 min), 5.1 mg of compound **2** (RT = 30.5 min), 1.1 mg of compound **7** (RT = 45 min), 0.2 mg compound **8** (RT = 51 min), and 0.5 mg of compound **9** (RT = 39 min).

Compounds 3 and 5

Fractions collected between retention time 23.5 min and 27 min were combined. The dried combined fraction was separated by preparative normal phase TLC (Merck, TLC Silica gel 60 F₂₅₄, 20 x 20 cm, 6% MeOH/CH₂Cl₂ for the first TLC run followed by 10% MeOH/CH₂Cl₂ for the second elution) to obtain Compound **3** (1.0 mg) and Compound **5** (2.0 mg).

Compounds 4 and 6

In addition, fractions collected between retention time 31.5 min and 33.75 min were combined. The combined fraction was further separated by preparatory TLC using the same conditions used for the separation of 5 and 3, to obtain Compound **4** (1.7 mg) and Compound **6** (2.7 mg) and.

Example 5: Structure Elucidation of Compounds

Example 5a: General Characterisation Procedures

JASCO P-2000 digital polarimeter was utilized to measure specific rotations of the compounds.

Preparative HPLC experiment was performed using Agilent 1260 Infinity Preparative-scale LC/MS Purification System coupled to Agilent 6130B single quadrupole mass spectrometer with XTerra Prep MS C₁₈ column (19 x 300 mm, 10 μ m). The detection wavelength used in the preparative HPLC was 254 nm.

The HPLC-MS was performed using an Agilent UHPLC 1290 Infinity coupled to Agilent 1290 DAD detector (for UV measurement) and Agilent 6540 accurate-mass quadrupole time-of-flight (QTOF) mass spectrometer equipped with an ESI source and a splitter. The analyses were conducted with an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 μ m), at a flow rate of 0.5 mL/min and under standard gradient condition of 2% MeCN (0.1% formic acid) to 100% MeCN (0.1% formic acid) over 8.6 minutes.

A Bruker DRX-400 NMR spectrometer was utilized to obtain NMR spectra of the compounds. Specifications of the NMR spectrometer include a Cryoprobe, and a 5-mm BBI (1H, G-COSY, multiplicity-edited G-HSQC, and G-HMBC spectra) or BBO (13C spectra) probe heads equipped with z-gradients. Residual solvent peaks for DMSO-*d*₆ were set at δ_H 2.50 and δ_C 39.5 ppm as reference signals in the ¹H and ¹³C NMR spectra, respectively.

The LC-MS/MS data file (.d) created from the Agilent QTOF mass was converted to .mgf file format with Agilent Qualitative 10.0 and uploaded to the GNPS Web platform (<http://gnps.ucsd.edu>) for classical molecular networking generation. MS-Cluster (0.1 Da tolerance) and a 0.02 Da tolerance for fragment ions were applied to create consensus parent mass spectra. A network was generated where there were more than six matched fragment ions and the edges were filtered to have a minimal cosine score of 0.7. A maximum size of a molecular family was also set to 100. The output molecular networking was visualized and analyzed using Cytoscape

3.9.0. The GNPS data may be found at <https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=e9f7b2ac30bd4ddabcc847f44b089a4b>.

Example 5b: GNPS Analysis

HPLC-MS analysis of the extract of *Nonomuraea jiangxiensis* strain A7611 found several masses of sulfur and nitrogen containing compounds, indicating the presence of thiopeptide. To visualize the overall chemical space in the extract, HPLC-MS/MS experiments were performed and the MS/MS data used to generate a consolidated GNPS molecular network as shown in Fig. 2. In this molecular network, each node represents one molecular feature (m/z). The nodes are connected to each other based on their MS/MS fragmentations pattern, forming a network, and suggesting their structural similarity. From the analysis of the network, specific clusters containing potentially new thiopeptides were found.

To identify the thiopeptides, large scale fermentation was performed, and the CH₂Cl₂ extract was subjected to RP-HPLC and PTLC to obtain **1–9** (Fig. 3).

Example 5c: Characterisation of Compounds

The characterisation data for the isolated compounds are summarised in Table 1.

Table 1: Characterisation data of Compounds 1-11 and GE2270A

Compounds	1	2	3	4	5	6
Labels	GE2270F ₁	GE2270F ₂	GE2270F ₃	GE2270F ₄	-	-
Physical Feature	White amorphous powders	White amorphous powders	White amorphous powders	White amorphous powders	White amorphous powders	White amorphous powders
$[\alpha]_D^{23}$	+78 (c 1.2, CH ₂ Cl ₂ :MeOH = 1:1)	+111 (c 0.9, CH ₂ Cl ₂ :MeOH = 1:1)	+58 (c 0.2, CH ₂ Cl ₂ :MeOH = 1:1)	+88 (c 0.3, CH ₂ Cl ₂ :MeOH = 1:1)	+102 (c 0.3, CH ₂ Cl ₂ :MeOH = 1:1)	+79 (c 0.2, CH ₂ Cl ₂ :MeOH = 1:1)
UV (MeCN/H ₂ O) λ_{max} (%)	221 (100%), 308 (33%) nm (Fig 4)	221 (100%), 308 (33%) nm (Fig 4)	220 (100%), 310 (33%) nm (Fig 4)	220 (100%), 310 (33%) nm (Fig 4)	221 (100%), 310 (33%) nm (Fig 4)	220 (100%), 310 (33%) nm (Fig 4)
(+)-HRESIMS m/z ; Δ ppm	1291.2544 [M+H] ⁺ (calcd for C ₅₆ H ₅₅ N ₁₄ O ₁₁ S ₆ , 1291.2499); 3.91 (Fig 5)	1247.2240 [M+H] ⁺ (calcd for C ₅₄ H ₅₁ N ₁₄ O ₁₀ S ₆ , 1247.2237); 0.69 (Fig 5)	1233.2096 [M+H] ⁺ (calcd for C ₅₃ H ₄₉ N ₁₄ O ₁₀ S ₆ , 1233.2080); 1.72 (Fig 5)	1277.2336 [M+H] ⁺ (calcd for C ₅₅ H ₅₃ N ₁₄ O ₁₁ S ₆ , 1277.2337); -0.08 (Fig 5)	1264.2495* [M+H] ⁺ (calcd for C ₅₄ H ₅₄ N ₁₅ O ₁₀ S ₆ , 1264.2502); -0.15 (Fig 5)	1308.2770* [M+H] ⁺ (calcd for C ₅₆ H ₅₈ N ₁₅ O ₁₁ S ₆ , 1308.2764); 0.84 (Fig 5)
¹ H NMR	Table 1, Fig 7a	Table 1, Fig 8a	Table S1, Fig 9a	Table S1, Fig 10a	Table S2, Fig 11a	Table S2, Fig 12a
¹³ C NMR	Table 1, Fig 7b	Table 1, Fig 8b	Table S1	Table S1, Fig 10b	Table S2, Fig 11b	Table S2, Fig 12b
COSY	Fig 7c	Fig 8c	Fig 9b	Fig 10c	Fig 11c	Fig 12c
HSQC	Fig 7d	Fig 8d	Fig 9c	Fig 10d	Fig 11d	Fig 12d
HMBC	Fig 7e	Fig 8e	Fig 9d	Fig 10e	Fig 11e	Fig 12e
COSY and HMBC Correlations	Fig 6	Fig 6	Fig 6	Fig 6	Fig 6	Fig 6

Compounds	7	8	9	10	11	
Labels	-	-	-	-	-	GE2270A
Physical Feature	White amorphous powders	White amorphous powders	White amorphous powders	-	-	-
$[\alpha]_D^{23}$	+72 (c 0.2, CH ₂ Cl ₂ :MeOH = 1:1)	+36 (c 0.2, CH ₂ Cl ₂ :MeOH = 1:1)	+24 (c 0.2, CH ₂ Cl ₂ :MeOH = 1:1)	-	-	-
UV (MeCN/H ₂ O) λ_{max} (%)	222 (100%), 308 (33%) nm (Fig 4)	221 (100%), 307 (33%) nm (Fig 4)	221 (100%), 308 (33%) nm (Fig 4)	-	-	-
(+)-HRESIMS m/z ; Δ ppm	1095.1655 [M+H] ⁺ (calcd for C ₄₇ H ₄₃ N ₁₂ O ₈ S ₆ , 1095.1651); 0.86 (Fig 5)	1139.1898 [M+H] ⁺ (calcd for C ₄₉ H ₄₇ N ₁₂ O ₉ S ₆ , 1139.1913); -0.85 (Fig 5)	1125.1740 [M+H] ⁺ (calcd for C ₄₈ H ₄₅ N ₁₂ O ₉ S ₆ , 1125.1757); -1.00 (Fig 5)	1081.1486* [M+H] ⁺ (calcd for C ₄₈ H ₄₃ N ₉ O ₉ S ₆ , 1081.1502); -1.53	1219.1926* [M+H] ⁺ (calcd for C ₅₆ H ₅₆ N ₁₅ O ₁₀ S ₆ , 1219.1918); 0.63	1290.2663 [M+H] ⁺ (calcd for C ₅₆ H ₅₆ N ₁₅ O ₁₀ S ₆ , 1290.2653); 0.75 (Fig. 16)
¹ H NMR	Table S3, Fig 13a	Table S3, Fig 14	Table S3, Fig 15	-	-	-
¹³ C NMR	Table S3, Fig 13b	-	-	-	-	-
COSY	Fig 13c	-	-	-	-	-
HSQC	Fig 13d	-	-	-	-	-
HMBC	Fig 13e	-	-	-	-	-
COSY and HMBC Correlations	Fig 6	-	-	-	-	-

*Unconnected singleton nodes in molecular network, #Uncertain structures

Table 2. NMR spectral data of thiopeptides 1 and 2.

Residue	Pos.	1		2	
		¹³ C	¹ H, mult. (J = Hz)	¹³ C	¹ H, mult. (J = Hz)
Proline (Pro)	α	58.5, CH	4.25, t (8.2)	58.5, CH	4.24, m
	β	27.9, CH ₂	1.92, m, 2.19, m	27.9, CH ₂	1.91, m, 2.19, m
	γ	22.1, CH ₂	1.84, m, 1.91, m	22.1, CH ₂	1.84, m, 1.91, m
	δ	44.8, CH ₂	3.41, m, 3.50, m	44.8, CH ₂	3.39, m, 3.50, m
	CO	169.3, C	-	169.3	-
Oxazoline (Oxa)	α	53.9, CH	4.55, m	53.9, CH	4.55, m
	β	63.2, CH ₂	4.53; 4.68, dd (14.9, 6.7)	63.2, CH ₂	4.53; 4.68, dd (14.9, 6.7)
	CN	160.1, C	-	160.1, C	-
	CO	163.2, C	-	163.2, C	-
Thiazole A	2	167.9, C	-	168.0, C	-
	4	147.2, C	-	147.2, C	-
	5	132.7, CH	8.76, s	132.7, CH	8.75, s
Pyridine (Py)	2	150.1, C	-	150.1, C	-
	3	127.6, C	-	127.70, C	-
	4	141.3, CH	8.44, d (8.2)	141.3, CH	8.43, d (8.1)
	5	118.4, CH	8.27, d (8.1)	118.3, CH	8.27, d (8.1)
	6	150.0, C	-	150.0, C	-
Thiazole B	2	160.3, C	-	160.4, C	-
	4	153.1, C	-	153.1, C	-
	5	123.0, CH	8.29, s	123.0, CH	8.29, s
Thiazole C	2	170.8	-	170.8, C	-
	4	146.6	-	146.6, C	-
	5	116.2, CH	7.34, s	116.3, CH	7.34, s
Phenylserine (PheSer)	α	58.2, CH	5.24, dd (14.0, 6.8)	58.2, CH	5.24, m
	β	73.3, CH	5.03, m	73.3, CH	5.04, m
	1	141.6	-	141.6, C	-
	2,6	126.5, CH	7.29, m	126.5, CH	7.28, m
	3,5	127.7, CH	7.28, m	127.75, CH	7.26, m
	4	127.4, CH	7.28, m	127.4, CH	7.27, m
	NH	-	9.29, d (7.7)	-	9.22, d (7.9)
Glycine (Gly)	OH	6.43, br s	-	-	6.35, br s
	α	41.0, CH ₂	3.81, dd (17.1, 3.9); 4.32, t (17.1, 8.7)	41.3, CH ₂	3.88, dd (16.9, 4.3); 4.24, t (7.6)
	CO	169.4, C	-	169.3, C	-
Thiazole D	NH	-	8.45, m	-	8.55, m
	2	165.4, C	-	168.3,	-
	4	143.5, C	-	148.4, C	-
	5	140.8, C	-	124.5, CH	8.28, s
	5-CH ₂	67.2, C	4.99, s	-	-
	5-CH ₂ OMe	58.4, C	3.39, s	-	-
	CO	161.2, C	-	160.3, C	-
Valine (Val)	α	55.2, CH	5.20, dd (4.7, 8.0)	55.3, CH	5.25, m
	β	33.8, CH	2.18, m	34.0, CH	2.19, m
	γ	18.3, CH ₃	0.86, d (6.9)	18.4, CH ₃	0.86, d (6.8)
	γ'	17.8, CH ₃	0.89, d (6.9)	17.8, CH ₃	0.89, d (6.8)
	NH	-	8.69, d (7.7)	-	8.75, m
Thiazole E	2	168.3, C	-	167.9, C	-
	4	141.9, C	-	141.7, C	-
	5	139.3, C	-	139.5, C	-
	5-Me	11.8, CH ₃	2.59, s	11.8, CH ₃	2.60, s
	CO	161.0, C	-	161.0, C	-
Asparagine (Asn)	α	47.9, CH	5.29, dt (4.1, 8.7)	47.9, CH	5.35, m
	β	37.4, CH ₂	1.31, m, 2.74, dd (4.1, 16.8)	37.6, CH ₂	1.47, m, 2.74, dd (3.8, 17.1)
	CONHMe	169.6, C	-	169.6, C	-
	NHMe	-	7.41, q (4.5)	-	7.48, m
	Me	25.6, CH ₃	2.47, d (4.5)	25.6, CH ₃	2.46, d (4.5)
	NH	-	8.69, d (7.7)	-	8.74, m
Thiazole F	2	164.5, C	-	164.6, C	-
	4	149.2, C	-	149.3, C	-
	5	126.8, CH	8.61, s	126.8, CH	8.60, s
	CO	160.2, C	-	160.2, C	-

¹H (400 MHz) and ¹³C (100 MHz) in DMSO-*d*₆. Assignments based on COSY, HSQC and HMBC and comparison with literature compounds. Chemical shifts (δ) in ppm. s: singlet; br s: broad singlet; d: doublet; br d: broad doublet; t: triplet; m: multiplet.

Table 3. NMR spectral data of **3** and **4**.

Residuc	Position	3		4	
		¹³ C	¹ H, mult. (<i>J</i> = Hz)	¹³ C	¹ H, mult. (<i>J</i> = Hz)
Proline (Pro)	α	58.6, CH	4.25, m	58.5, CH	4.24, m
	β	27.9, CH ₂	1.92, m; 2.19, m	27.9, CH ₂	1.90, m; 2.19, m
	γ	22.1, CH ₂	1.88, m; 1.91, m	22.1, CH ₂	1.84, m; 1.91, m
	δ	44.6, CH ₂	3.40, m; 3.51, m	44.8, CH ₂	3.41, m; 3.51, m
	CO	169.4, C	-	169.3, C	-
Oxazoline (Oxa)	α	53.9, CH	4.55, m	53.9, CH	4.55, m
	β	63.2, CH ₂	4.54, m; 4.68, m	63.2, CH ₂	4.54, m; 4.68, dd (16.0, 8.0)
	CN	160.5, C	-	160.1, C	-
	CO	163.2, C	-	163.2, C	-
Thiazole A	2	167.9, C	-	167.9, C	-
	4	147.2, C	-	147.2, C	-
	5	132.5, CH	8.76, s	132.8, CH	8.75, s
Pyridine (Py)	2	150.1, C	-	150.1, C	-
	3	127.77, C	-	127.6, C	-
	4	141.2, CH	8.44, d (8.2)	141.3, CH	8.45, d (8.2)
	5	118.3, CH	8.27, d (8.2)	118.4, CH	8.28, d (8.2)
	6	150.0, C	-	150.0, C	-
Thiazole B	2	160.5, C	-	160.3, C	-
	4	153.2, C	-	153.2, C	-
	5	122.8, CH	8.31, s	123.1, CH	8.31, s
Thiazole C	2	170.7, C	-	171.0, C	-
	4	146.4, C	-	146.7, C	-
	5	116.4, CH	7.37, s	116.3, CH	7.37, s
Phenylserine (PheSer)	α	58.1, CH	5.24, m	58.0, CH	5.24, m
	β	73.3, CH	5.03, m	73.6, CH	5.02, m
	1	141.1, C	-	141.6, C	-
	2,6	126.5, CH	7.29, (2H, m)	126.6, CH	7.31, (2H, m)
	3,5	127.80, CH	7.29, (2H, m)	127.8, CH	7.31, (2H, m)
	4	127.4, CH	7.24, m	127.5, CH	7.25, m
	NH	-	9.00, d (7.7)	-	9.01, d (8.0)
	OH	-	6.03, d (4.3)	-	6.03, d (4.5)
Glycine (Gly)	α	41.0, CH ₂	3.88, dd (16.6, 4.0); 4.25, m	41.0, CH ₂	3.81, dd (17.2, 4.0); 4.30 dd (17.2, 8.6)
	CO	169.6, C	-	169.4, C	-
	NH	-	8.56, m	-	8.46, m
Thiazole D	2	168.2, C	-	165.4, C	-
	4	148.0, C	-	143.7, C	-
	5	124.4, CH	8.29, s	140.6, C	-
	5-CH ₂	-	-	67.2, CH ₂	4.98, s
	5-CH ₂ OMe	-	-	58.4, CH ₃	3.39, s
	CO	160.4, C	-	161.2, C	-
Valine (Val)	α	55.4, CH	5.29, dd (8.0, 4.9)	55.5, CH	5.26, s
	β	33.8, CH	2.22, m	33.9, CH	2.21, s
	γ	18.5, CH ₃	0.87, d (6.9)	18.4, CH ₃	0.86, d (6.6)
	γ'	17.7, CH ₃	0.89, d (7.0)	17.7, CH ₃	0.89, d (6.8)
	NH	-	8.76, d (8.0)	-	8.70, d (8.4)
Thiazole E	2	173.0, C	-	173.0, C	-
	4	148.4, C	-	148.6, C	-
	5	123.4, CH	8.10, s	123.3, CH	8.08, s
	CO	159.0, C	-	159.6, C	-
Asparagine (Asn)	α	48.0, CH	5.42, m	48.2, CH	5.37, m
	β	37.5, CH ₂	1.50, m; 2.76, m	37.7, CH ₂	1.35, m; 2.74, dd (16.5, 3.7)
	CONHMe	169.3, C	-	169.5, C	-
	NHMe	-	7.48, m	-	7.40, m
	Me	25.3, CH ₃	2.46, d (4.6)	25.6, CH ₃	2.46, d (4.7)
	NH	-	8.81, d (9.0)	-	8.74, d (8.0)
Thiazole F	2	164.5, C	-	164.6, C	-
	4	149.3, C	-	149.2, C	-
	5	126.8, CH	8.61, s	126.9, CH	8.62, s
	CO	160.0, C	-	160.2, C	-

¹H (400 MHz) and ¹³C (100 MHz) in DMSO-*d*₆. Assignments based on COSY, HSQC and HMBC and comparison with literature compounds. Chemical shifts (δ) in ppm. s: singlet; br s: broad singlet; d: doublet; br d: broad doublet; t: triplet, m: multiplet. One proton unless otherwise stated.

Table 4. ¹H and ¹³C NMR data of **5** and **6**.

Residue	Position	5		6	
		¹³ C	¹ H, mult. (<i>J</i> = Hz)	¹³ C	¹ H, mult. (<i>J</i> = Hz)
Proline (Pro)	α	59.8, CH	4.33, dd (8.6, 3.8)	59.8, CH	4.33, dd (8.8, 3.6)
	β	29.1, CH ₂	2.08, m; 1.86, m	29.3, CH ₂	1.87, m; 2.09, m
	γ	24.4, CH ₂	1.92, (2H, m)	24.4, CH ₂	1.92, (2H, m)
	δ	47.0, CH ₂	3.77, (2H, m)	47.1, CH ₂	3.77, (2H, m)
	CO	173.5, C	-	173.6, C	-
Serine (Ser)	α	53.0, CH	4.90, dd (12.0, 4.0)	53.0, CH	4.90, dd (12.0, 4.0)
	β	61.7, CH ₂	3.76, m; 3.82, m	61.8, CH ₂	3.80, m; 3.84, m
	CO	168.9	-	168.9, C	-
	NH	-	8.47, m	-	8.47, m
	OH	-	5.28, br t (6.0)	-	5.28, m
Thiazole A	2	167.3, C	-	167.3, C	-
	4	150.8, C	-	150.7, C	-
	5	127.6, CH	8.55, s	127.5, CH	8.55, s
	CO	160.1, C	-	160.1, C	-
Pyridine (Py)	2	150.3, C	-	150.2, C	-
	3	127.7, C	-	127.6, C	-
	4	141.3, CH	8.44, d (7.0)	141.2, CH	8.46, d (6.8)
	5	118.5, CH	8.40, d (7.0)	118.6, CH	8.41, d (6.8)
	6	150.1, C	-	150.0, C	-
Thiazole B	2	160.4, C	-	160.3, C	-
	4	153.1, C	-	153.2, C	-
	5	123.0, CH	8.30, s	123.0, CH	8.30, s
Thiazole C	2	170.9, C	-	171.0, C	-
	4	146.7, C	-	146.7, C	-
	5	116.3, CH	7.36, s	116.3, CH	7.36, s
Phenylserine (PheSer)	α	58.0, CH	5.24, m	58.1, CH	5.24, m
	β	73.5, CH	5.03, br t (4.0)	73.7, CH	5.01, dd (6.2, 5.0)
	1	141.6, CH	-	141.7, C	-
	2,6	126.6, CH	7.30, (2H, m)	126.6, CH	7.31, m
	3,5	127.8, CH	7.27, (2H, m)	127.8, CH	7.30, m
	4	127.8, CH	7.25, m	127.7, CH	7.25, m
	NH	-	9.01, d (8.3)	-	9.01, d (7.8)
	OH	-	6.02, d (4.5)	-	6.03, d (4.6)
Glycine (Gly)	α	41.4, CH ₂	3.87, m; 4.21, dd (16.6, 7.9)	41.1, CH ₂	3.79, m; 4.26, dd (17.5, 8.6)
	CO	169.3, C	-	169.3, C	-
	NH	-	8.55, m	-	8.44, m
Thiazole D	2	168.4, C	-	165.5, C	-
	4	148.3, C	-	143.6, C	-
	5	124.5, CH	8.28, s	140.8, C	-
	5-CH ₂	-	-	67.3, CH ₂	4.98, s
	5-CH ₂ OMe	-	-	58.5, CH ₃	3.38, s
	CO	161.0, C	-	161.2, C	-
Valine (Val)	α	55.4, CH	5.24, m	55.2, CH	5.20, m
	β	34.0, CH	2.20, m	34.0, CH	2.18, m
	γ	18.4, CH ₃	0.86, d (8.0)	18.3, CH ₃	0.85, d (6.8)
	γ'	17.7, CH ₃	0.88, d (8.0)	17.8, CH ₃	0.88, d (6.8)
	NH	-	8.73, d (7.8)	-	8.67, d (8.2)
Thiazole E	2	167.9, C	-	168.3, C	-
	4	141.7, C	-	141.9, C	-
	5	139.6, C	-	139.3, C	-
	5-Me	11.8, CH ₃	2.60, s	11.8, CH ₃	2.59, s
	CO	160.3, C	-	159.8, C	-
Asparagine (Asn)	α	47.9, CH	5.37, m	47.9, CH	5.31, m
	β	37.6, CH ₂	1.50, m; 2.73, dd (16.4, 3.7)	37.5, CH ₂	1.37, m; 2.72, dd (16.4, 3.2)
	CONHMe	169.6, C	-	169.6, C	-
	NHMe	-	7.48, m	-	7.38, m
	Me	25.6, CH ₃	2.46, d (4.6)	25.7, CH ₃	2.47, d (4.5)
	NH	-	8.77, d (9.0)	-	8.70, d (8.7)
Thiazole F	2	164.7, C	-	164.7, C	-
	4	149.3, C	-	149.3, C	-
	5	126.8, CH	8.60, s	126.9, CH	8.61, s
	CO	159.8, C	-	161.1, C	-

¹H (400 MHz) and ¹³C (100 MHz) in DMSO-*d*₆. Assignments based on COSY, HSQC and HMBC and comparison with literature compounds. Chemical shifts (δ) in ppm. s: singlet; br s: broad singlet; d: doublet; br d: broad doublet; t: triplet, m: multiplet. One proton unless otherwise stated.

Table 5. ¹H and ¹³C NMR data of **7** and ¹H NMR data of **8** and **9**.

Residue	Position				
		¹³ C	7 ¹ H, mult. (<i>J</i> = Hz)	8 ¹ H, mult. (<i>J</i> = Hz)	9 ¹ H, mult. (<i>J</i> = Hz)
Thiazole A	2	168.1, C	-	-	-
	4	147.3, C	-	-	-
	5	132.3, CH	8.78, s	8.79, s	8.79, s
	CO	161.1, C	-	-	-
	COOMe	52.2 CH ₃	3.91, s	3.91, s	-
Pyridine (Py)	2	150.1, C	-	-	-
	3	127.6, C	-	-	-
	4	141.3, CH	8.41, d (8.2)	8.43, d (8.1)	8.43, d (8.2)
	5	118.5, CH	8.30, d (8.2)	8.30, d (8.1)	8.30, d (8.2)
	6	150.0, C	-	-	-
Thiazole B	2	160.4, C	-	-	-
	4	153.1, C	-	-	-
	5	123.0, CH	8.29, s	8.29, s	8.29, s
Thiazole C	2	170.8, C	-	-	-
	4	146.6, C	-	-	-
	5	116.3, CH	7.32, s	7.33, s	7.34, s
Phenylserine (PheSer)	α	58.3, CH	5.23, m	5.20, m	5.20, m
	β	73.2, CH	5.06, m	5.04, m	5.03, m
	1	141.6, C	-	-	-
	2,6	126.5, CH	7.26 (2H, m)	7.28 (2H, m)	7.29, m
	3,5	127.7, CH	7.25 (2H, m)	7.27 (2H, m)	7.28, m
	4	127.5, CH	7.21, m	7.22, m	7.22, m
	NH	-	9.46, d (7.6)	9.45, d (6.2)	9.29, d (6.6)
	OH	-	6.70, br s	6.65, br s	6.44, br s
Glycine (Gly)	α	41.3, CH ₂	3.89, dd (17.2, 4.0); 4.29, dd (17.2, 8.9)	3.81, m; 4.34, m	3.80, dd (16.7, 3.6); 4.31, dd (16.7, 8.6)
	CO	169.4, C	-	-	8.45, m
	NH	-	8.57, m	8.46, m	-
Thiazole D	2	168.3, C	-	-	-
	4	148.4, C	-	-	-
	5	124.5, CH	8.28, s	-	-
	5-CH ₂	-	-	4.99, s	4.98, s
	5-CH ₂ OMe	-	-	3.39	3.39, s
	CO	161.1, C	-	-	-
Valine (Val)	α	55.4, CH	5.24, m	5.23, m	5.24, m
	β	34.0, CH	2.19, m	2.18, m	2.18, m
	γ	18.4, CH ₃	0.86, d (6.8)	0.89, d (6.8)	0.88, d (6.6)
	γ'	17.8, CH ₃	0.89, d (6.8)	0.85, d (6.8)	0.85, d (6.8)
	NH	-	8.76, d (8.2)	8.70, d (8.3)	8.69, br s
Thiazole E	2	167.9, C	-	-	-
	4	141.7, C	-	-	-
	5	139.6, C	-	-	-
	5-Me	11.8, CH ₃	2.60, s	2.59, s	2.59, s
	CO	160.5, C	-	-	-
Asparagine (Asn)	α	47.9, CH	5.34, m	5.29, m	5.30, dd (8.6, 4.6)
	β	37.6, CH ₂	1.43, m; 2.75, m	1.29, m; 2.72, m	1.34, m; 2.72, dd (16.0, 3.4)
	CONHMe	169.6, C	-	-	-
	NHMe	-	7.50, m	7.42, m	7.41, m
	Me	25.7, CH ₃	2.45, d (4.5)	2.46, d (4.6)	2.47, d (4.6)
Thiazole F	NH	-	8.73, d (9.4)	8.68, d (8.8)	8.67, br s
	2	164.6, C	-	-	-
	4	149.3, C	-	-	-
	5	126.9, CH	8.61, s	8.61, s	8.61, br s
	CO	160.2	-	-	-

¹H (400 MHz) and ¹³C (100 MHz) in DMSO-*d*₆. Assignments based on COSY, HSQC and HMBC and comparison with literature compounds. Chemical shifts (δ) in ppm. s: singlet; br s: broad singlet; d: doublet; br d: broad doublet; t: triplet, m: multiplet. One proton unless otherwise stated.

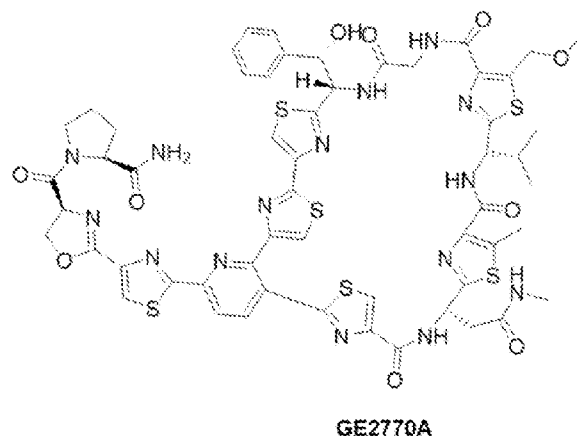
Example 5c: Compound 1

Compound **1** (**Fig. 3**) was isolated as a white amorphous powder and its molecular formula was established as $C_{56}H_{54}N_{14}O_{11}S_6$ based on HR-ESIMS measurement.

The NMR data of compound **1** may be found in Figs. **1a-1e**, with peak assignments found in Table 2.

The 1H NMR data (**Table 2, Fig. 7a**) revealed features of a peptide-derived compound, including five amide 1H signals (δ_H 9.29, 8.69, 8.69, 8.45, 7.41). The ^{13}C NMR data (**Table 2, Fig. 7b**) was also consistent with a peptide-derived compound, comprising of oxazoline and thiazole units including five amide carbonyls and one carboxylic acid carbonyl signals (δ_C 169.4, 169.3, 163.2, 161.2, 161.0, 160.2), 6 thiazole (δ_C 170.8, 168.3, 167.9, 165.4, 164.5, 160.3) and oxazoline (δ_C 160.1) moieties. The presence of thiazole rings A, B, C, D, E and F, pyridine (Py), phenylserine (PheSer), valine (Val), glycine (Gly), asparagine (Asn), oxazoline (Oxa) and proline (Pro) residues in **1** was supported by the observed COSY and HMBC correlations (**Figs. 6, 7c and 7e**).

Further analysis of 2D-NMR data (**Figs. 7d and 7e**) revealed that the structure of **1** was similar to that of GE2270A, a thiopeptide detected from *Planobispora rosea*. NMR data comparison between **1** and those of GE2270A shown a high degree of similarity, implying their structural analogy. The 1D NMR resonances of the aromatic protons of Py and of the thiazole rings A, B, C, D, E and F for GE2270A and **1** also coincided. This showed that the building blocks of the thiazolyl peptide backbone and the sequence was conserved. A detailed inspection of the ^{13}C NMR data of **1** (**Table 2, Fig. 7b**) together with comparison with reported NMR data of GE2270A revealed a missing signal of δ_C 173.5 that corresponded to the C-terminal amide/carbonyl of proline (Pro) residue in GE2270A, which was replaced by a carboxylic acid group (δ_C 169.3) in **1**. Based on these data, the structure of **1** was established to be an analog of GE2270A possessing a C-terminal carboxylic acid. The structure of GE2270A is depicted below:



Example 5d: Compounds 2, 3, 4

Compounds **2**, **3**, and **4** (**Fig. 3**) were isolated as white amorphous powders. HR-ESIMS measurement determined the molecular formula of **2** as $C_{54}H_{50}N_{14}O_{10}S_6$, **3** as $C_{53}H_{48}N_{14}O_{10}S_6$, and **4** as $C_{55}H_{52}N_{14}O_{11}S_6$, respectively.

The NMR data for compounds **2** to **4** may be found in Figs. **8a-8e**, **9a-9d**, and **10a-10e** respectively.

1H NMR data of **2-4** (**Table 2** and **3**) were consistent with that of **1** with minor differences. For instance, the methyl singlet (δ_H 2.59) on thiazole ring E in **1** was not observed in the 1H NMR spectra of **3** (**Fig. 9a**) and **4** (**Fig. 10a**). This implied that C-5 of thiazole ring E in **3** and **4** was unsubstituted, which was further supported by an additional singlet at around δ_H 8.10 in the 1H NMR spectra of **3** and **4**. On the other hand, the singlets at δ_H 3.39 and 4.99 of the methoxymethyl substituent at C-5 position of thiazole ring D were not detected in **2** and **3**. This observation suggested that thiazole ring D was unsubstituted at C-5 position supported by an additional singlet at δ_H 8.29 in the 1H NMR spectra of **2** (**Fig. 8a**) and **3**. The remaining structural moieties in compounds **2-4** were established by COSY and HMBC correlations (**Figs. 4, 6, 8c-8e, 9b-9d, and 10c-10e**). Compounds **1-4** shared the same thiazolyl peptide backbone and C-terminal carboxylic acid group while possessing altered decorations at thiazole rings D and E, they are grouped together and named GE2270F₁, GE2270F₂, GE2270F₃ and GE2270F₄, respectively.

Example 5e: Compounds 5, 6

Compounds **5** and **6** (**Fig. 3**) were isolated as white amorphous powders and their molecular formulae were established as $C_{54}H_{54}N_{15}O_{10}S_6$ and $C_{56}H_{58}N_{15}O_{11}S_6$, respectively based on HR-ESIMS measurement.

NMR data for compounds **5-6** may be found in Figs. **11a-11e** and **12a-12e** respectively.

Interpretation of the COSY, HSQC and HMBC spectra of **5** (**Figs. 11c-11e**) and **6** (**Figs. 12c-12e**) together with NMR data comparison (**Figs. 11a, 11b, 12a, and 12b**) with those of GE2270A revealed the replacement of Oxa group with a serine (Ser) moiety which was indicated by an amide carbonyl at δ_C 173.5 (**Fig. 6** and **Table 4**). The presence of Ser residue was evident from the COSY correlations between NH (δ_H 8.47)/H- α (δ_H 4.90), H- α (δ_H 4.90)/H₂- β (δ_H 3.76, 3.82), and H₂- β (δ_H 3.76, 3.82)/OH (δ_H 5.28), as well as HMBC correlation from H- α to a carbonyl carbon at δ_C 168.9 (**Fig. 6**). Compounds **5** and **6** may be intermediates or precursors in the biosynthesis of GE2270C₁ and GE2270A respectively.

Example 5f: Compounds 7, 8, 9

Compounds **7**, **8** and **9** (**Fig. 3**) were isolated as white amorphous powder and their molecular formulae were determined as $C_{47}H_{42}N_{12}O_8S_6$, $C_{49}H_{46}N_{12}O_9S_6$ and $C_{48}H_{44}N_{12}O_9S_6$, respectively.

NMR data for compounds **7-9** are found in Figs. 13a-13e, 14 and 15 respectively.

Compounds **7-9** possessed the same thiazolyl peptide backbone as in **1** with the loss of Pro and Oxa groups as indicated by the missing proton and carbon signals that correspond to Pro and Oxa groups (**Fig. 3** and **Table 5**). Thiopeptides **7** and **8** were analogues of one another with or without substitution at C-5 position of thiazole ring D and both possessed terminal methyl-ester functionality which is indicated by an additional singlet at δ_H 3.91 (**Table 5**). On the other hand, **9** was the free carboxylic acid form of **8**. These compounds were likely to be intermediates or precursors in the biosynthesis of GE2270A. Compounds **8** and **9** were previously reported and synthesized as intermediates in the total synthesis of thiopeptide GE2270 analogues. This was the first report of the isolation of thiopeptides **8** and **9** from nature. Notably, one close structural analog of compounds **8** and **9**, which possessed a terminal amide group in place of ester or carboxylic acid group was also synthesized and tested for antibacterial activity and was inactive against gram-positive *S. aureus*.

Example 5g: Compounds 10 and 11

Although the thiopeptide GNPS molecular clusters showed several related thiopeptides, it was not possible to isolate and acquire NMR spectroscopic data for two minor compounds (m/z 1081.15 for **10**, and m/z 1219.19 for **11**) due to their minute amount in the extract. Nevertheless, their molecular formulae were determined by exact mass calculation (**Table 1**).

Due to the small amount of samples, absolute configurations could not be assigned for Compounds **1-12** using Marfey's reagent. However, the structures of thiopeptides **1-9** are closely related to a series of known thiopeptides, GE2270. Further manual observation of the HPLC-HRESIMS/MS of the crude extract indeed revealed the presence of GE2270A as shown by a molecular ion m/z 1290.2663 $[M+H]^+$ consistent with that of GE2270A (**Fig. 16**). Thus, **1-9** were most likely to be biosynthetically related to GE2270A, a thiopeptide initially isolated from *Planobispora rosea*. Although the structures of compounds **1-9** and GE2270A contain several typical characteristics of a nonribosomal peptide (NRP), GE2270A is a ribosomally synthesized, post-translationally modified peptide (RiPP), as what have also been observed in other known thiopeptides. Therefore, **1-9** was presumed to occur in the configuration as shown in **Fig. 3**, and determined to be new members of GE2270 thiopeptides. This was further supported by comparison of the specific rotation and 1H and ^{13}C NMR data of **1-9** with those of GE2270A, whose configuration was confirmed by total synthesis.

Example 6: Biological assaysExample 6a: General experimental procedures

Isolated compounds of interest were tested against 5 microbial strains for antimicrobial testing which are *Acinetobacter baumannii* (ATCC® 19606™), *Klebsiella aerogenes* (ATCC® 13048™), *Pseudomonas aeruginosa* (ATCC® 9027™), *Staphylococcus aureus* Rosenbach (ATCC® 25923™) and *Aspergillus fumigatus* (ATCC® 46645™).

Minimum inhibition/bactericidal/fungicidal concentration (MIC/MBC/MFC)

The minimum inhibition concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were carried out using the microbroth dilution method according to the Clinical Laboratory Standards Institute (CLSI) guidelines, with some modifications.

To establish the MIC values, the bacterial cells were seeded at a concentration of 5.5×10^5 cells/mL and fungal spores at a concentration of 2.5×10^4 spores/mL. The tested compounds were then incubated together with bacterial cells at 37 °C for 24 hrs and with fungal spores at 25 °C for 72 hrs respectively. OD₆₀₀ measurement was subsequently carried out to evaluate the inhibitory effect of the compounds. To further determine the bactericidal and fungicidal effects of the compounds, 5 µL of the treated culture was transferred onto new media microplates. The plates were incubated under the same condition, followed by OD₆₀₀ measurement. The cytotoxicity effect of the isolated compounds was also tested on A549 human lung carcinoma cells (ATCC® CCL-185™), where cells were seeded at 3.3×10^4 cells/mL. The cells were then treated with the compounds for 72 hrs at 37 °C in the presence of 5% CO₂.

Cytotoxic effect was detected with PrestoBlue™ cell viability reagent (ThermoFisher Scientific, Waltham, Massachusetts, US). The cells were read with fluorescence reading at excitation of 560 nm and emission 590 nm. Standard inhibitors gentamicin (Gibco, Waltham, Massachusetts, US), amphotericin (Sigma-Aldrich, St. Louis, Missouri, US) and puromycin (Sigma-Aldrich, St. Louis, Missouri, US) were used as the assay controls respectively for the antibacterial, antifungal and cytotoxicity assay. All compounds were tested in triplicates to ensure reproducibility of the results. GraphPad Prism 8 software (GraphPad, San Diego, California, US) was used for analysis of bioactivity to determine the respective IC₉₀ and IC₅₀ values.

Example 6b: Biological activities against *Staphylococcus aureus* Rosenbach (ATCC® 25923™)

Table 6. Biological activities of compounds 1–9 and positive control gentamicin against *Staphylococcus aureus* Rosenbach (ATCC® 25923™)

Thiopeptide Compounds	MIC ₉₀ [(μM) mean ± SD]	MBC ₉₀ [(μM) mean ± SD]
1	2.63 ± 0.12	18.48 ± 1.34
2	6.94 ± 0.83	67.93 ± 1.55
3	>70	>70
4	>70	>70
5	>70	>70
6	7.07 ± 0.55	31.80 ± 1.38
7	4.71 ± 0.37	12.44 ± 1.06
8	3.17 ± 0.28	16.22 ± 1.48
9	10.27 ± 0.75	51.99 ± 1.41
Gentamicin	0.04 ± 0.01	0.10 ± 0.01

Values are expressed as mean ± SD in triplicates.

Notably, it has been well documented that thiopeptides exhibited a wide range of biological properties; and are strong antibiotics against Gram-positive bacteria, including contemporary strains of methicillin-resistant *Staphylococcus aureus* (MRSA).

while **1**, **2**, **6**, **7**, **8** and **9** displayed activities against *S. aureus* Rosenbach (ATCC® 25923™), the only Gram-positive bacterial strain tested (**Table 6** and **Fig. 17**). Interestingly, **3**, **4** and **5** were found to be inactive against *S. aureus*.

Compounds **1-4** possessed the same R₁ group on thiazole ring A, but various substitutions, i.e. R₂ and R₃ groups on thiazole rings D and E respectively. As shown in **Table 6**, **1** was three-fold more active (i.e. minimal inhibitory concentration (MIC₉₀) of 2.63 μM and minimal bactericidal concentration (MBC₉₀) of 18.48 μM) against *S. aureus* Rosenbach (ATCC® 25923™) than **2** (i.e. MIC₉₀ of 6.94 μM and MBC₉₀ of 67.93 μM). This was likely attributed to the presence of both R₂ (i.e. methylene-oxy-methyl) and R₃ (i.e. methyl) groups in **1**, resulting in a significant increase in antibacterial activity. In addition, the presence of R₃ group and the absence of R₂ group in **2** resulted in a weaker antibacterial activity. The absence of both R₂ and R₃ groups in **3**, and the absence of the R₃ group in **4** resulted in no bioactivity. Compounds **5** and **6** shared similar chemical structures, each possessing a terminal serine-proline group (R₁) on thiazole ring A. Compound **6** possessing both R₂ and R₃ groups demonstrated antibacterial activity against *S. aureus* Rosenbach (ATCC® 25923™), while **5** possessing only the R₃ group displayed no activity against *S. aureus* Rosenbach (ATCC® 25923™). This indicated that the presence of both R₂ and R₃ groups was important for bioactivity in this series of thiopeptides.

Both compounds **7** and **8** possessed a terminal ester group (R_1) on thiazole ring A, with **7** possessing only the R_3 group, whilst **8** possessed both R_2 and R_3 groups. A slight increase in antibacterial activity (i.e. MIC_{90} of $3.17\ \mu M$) was observed in **8** as compared to **7** (i.e. MIC_{90} of $4.71\ \mu M$) when both R_2 and R_3 groups are present. **9** is the only compound with a terminal carboxylic acid group (R_1) on thiazole ring A. Interestingly, at least three-fold reduction in antibacterial activity (i.e. MIC_{90} of $10.27\ \mu M$) was observed in **9** as compared to **8** when the ester group was changed to a carboxylic acid. This could be due to the poor cell membrane permeability of the carboxylic acid group, thus resulted in poorer antibacterial activity. The mechanism of action of thiopeptides has been previously studied, and it was well established that thiopeptides exert their antibacterial function in the bacterial cell via the inhibition of ribosomal protein synthesis.

Example 7: Synthesis of compounds 1-9

Example 7a: Synthesis of key intermediate I

Intermediate **I** may be synthesized via the following procedure and as shown in Fig. 18a. Commercially available L-serine may be used to form Garner's aldehyde according to Koskinen's procedure, after which the Garner's aldehyde may then undergo a subsequent Hantzsch thiazole synthesis to obtain intermediate **Ia**. To form intermediate **Ib**, a second thiazole may first be formed by subjecting cysteine methyl ester to Hantzsch thiazole synthesis, following which **Ia** and the second thiazole may be condensed under $KHCO_3$ to form intermediate **Ib** through a cyclodehydration reaction. Dimerization of intermediate **Ib** in the presence of Ag_2CO_3 , DBU and benzylamine affords intermediate **Ic**. Intermediate **Ic** may be heated with DBU to give intermediate **Id**, following which reaction of **Id** with an L-erythro phenylserine derivative would provide intermediate **Ie**. To form intermediate **I**, intermediate **Ie** may be protected at the -OH group, following which treatment with Lawesson's reagent would provide intermediate **I**.

Example 7b: Synthesis of building block II

Intermediate **II** may be synthesized via the following procedure (Fig. 18b). Intermediate **I** may be deprotected, following which treatment with DAST would form Intermediate **IIb** with the 2,3-dihydrothiazolidine ring via dehydration of the corresponding free alcohol. Intermediate **IIb** may be oxidised in the presence of $BrCCl_3$ to form intermediate **IIc**, following which deprotection and amide condensation with Boc-glycine would furnish intermediate **IIf**. Further deprotection of the -NHBoc group would provide intermediate **II**.

Example 7c: Synthesis of building blocks III and IV

Compound **III** may be synthesized via the following procedure (Fig. 18c). Boc-L-valinamide may be reacted with the corresponding ethyl 2-diazo-3-oxopropanoate, in the presence of Rh(OAc)₂ to form intermediate **IIIa**, following which cyclodehydration with Lawesson's reagent to form the thiazole and treatment with LiOH to hydrolyse the ester would form intermediate **III**.

To form intermediate **IV**, 5-methyl-2-[(1S)-3-(methylamino)-1-[(2-methylpropan-2-yl)oxy-oxomethyl]amino]-3-oxopropyl]-4-thiazolecarboxylic acid methyl ester (CAS no. 348155-30-6) may cross-esterified to the corresponding O-allyl ester before being deprotected to release the amine.

7d: Synthesis of Compounds 7, 8 and 9

Compounds **7** and **8** may be synthesized via the following procedure (Fig. 18d). Condensation of intermediates **II** and **III** would furnish compound **Va**, after which treatment with Me₃SnOH would provide intermediate **Vb**. Further condensation with intermediate **IV** would provide intermediate **Vc**. Subsequent deprotection of the -Oallyl and -NHTBS groups, and subsequent macrolactamisation with FDPP would provide intermediate **Vd**. Compounds **7** and **8** may be synthesized by simply removing the -OTBS group using TBAF.

To form compound **9**, the -COOMe of intermediate **Vd** may first be removed, following which deprotection of the -OTBs group would afford compound **9**.

7e: Synthesis of Compounds 1 to 4

Compounds **1** and **4** may be synthesized via the following procedure (Fig. 18e).

Intermediate **Ve** may first be coupled with L-serine methyl ester to furnish intermediate **VIa**, following which cyclisation using DAST would form intermediate **VIIb**. Deprotection of the -COOMe followed by condensation with proline methyl ester would furnish intermediate **VIc**. Deprotection of both the -COOMe and -OTBS groups would then provide compounds **1**, **2**, **3** and **4**.

7f: Synthesis of Compounds 5 and 6

Compounds **5** and **6** may be synthesized via the following procedure (Fig. 18f).

Intermediate **VIa** may first be treated with Me₃SnOH, following which condensation with proline amide would furnish intermediate **VIIc**. The -OTBs group of intermediate **VIIc** may be simply removed to afford compounds **5** and **6**.

Industrial Applicability

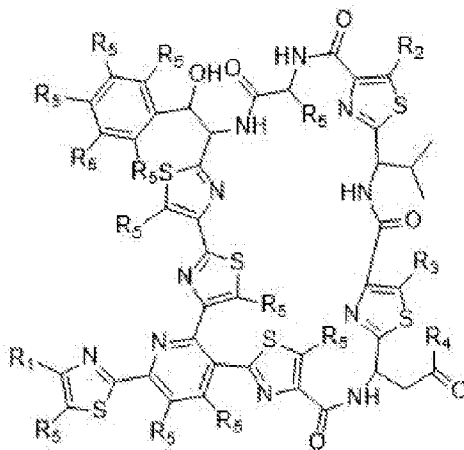
The present invention relates to thiopeptide compounds, and more particularly relates to thiopeptide compounds, their methods of production and uses thereof. The thiopeptide compounds of the present disclosure possess bacterial activity against Gram-positive bacteria, in particular, *S. aureus*.

The present invention also refers to a method isolating the disclosed compounds. The disclosed method may be easily scaled up for industrial-scale production. Current industrial infrastructure may also be used to produce the disclosed compounds in large quantities. Thus, this invention is capable of industrial applicability.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Claims

1. A compound of Formula (I) or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclene, optionally substituted heterocarbocyclene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclene, optionally substituted heterocarbocyclene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

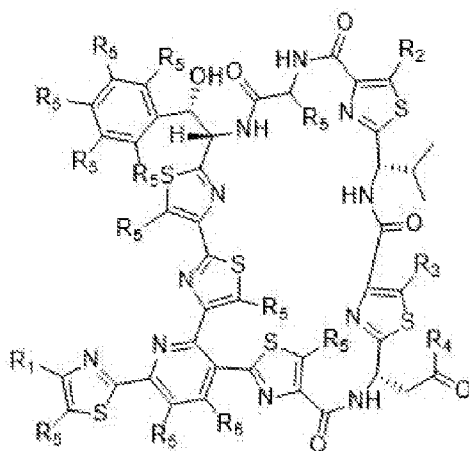
R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl,

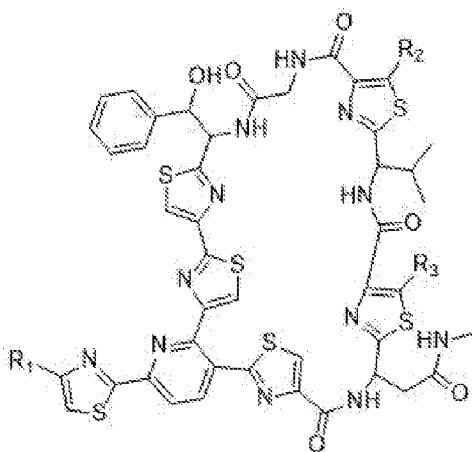
wherein when R₂ is optionally substituted alkalkoxy, R₃ is -H, optionally substituted alkenyl, or optionally substituted alkynyl.

2. The compound of claim 1, wherein the compound has the structure and stereochemical configuration of Formula (II):



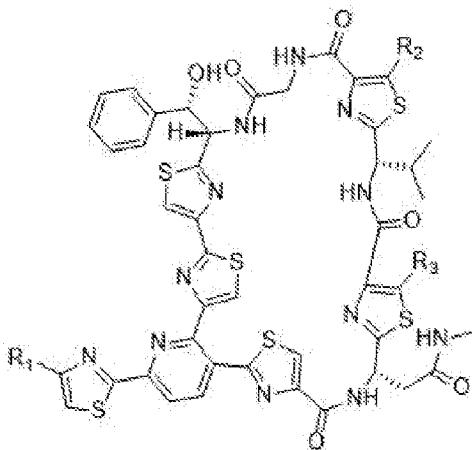
Formula (II)

3. The compound of any one of claims 1 to 2, wherein the compound is of Formula (III):



Formula (III)

4. The compound of any one of claims 1 to 3, wherein the compound is of Formula (IV):

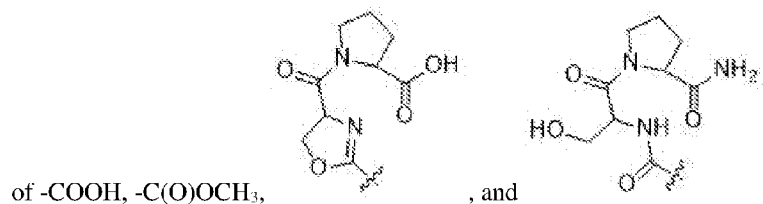


Formula (IV)

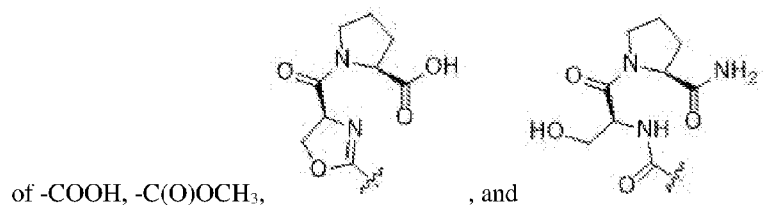
5. The compound of any one of claims 1 to 4, wherein A is heterocarbocyclylene or -C(O)N(R_{7a})-.
6. The compound of any one of claims 1 to 5, wherein G is -C(O)- or optionally substituted -alkylene-C(O)-.
7. The compound of any one of claims 1 to 6, wherein G is -C(O)- or -CH(R₈)-C(O)-, wherein R₈ is alkylene substituted with -OH.
8. The compound of claim 7, wherein R₈ is -CH₂-OH.
9. The compound of any one of claims 1 to 8, wherein J is heterocarbocyclylene.

10. The compound of any one of claims 1 to 9, wherein L is -COOH or -C(O)NH₂.

11. The compound of any one of claims 1 to 10, wherein R₁ is selected from the group consisting



12. The compound of any one of claims 1 to 11, wherein R₁ is selected from the group consisting

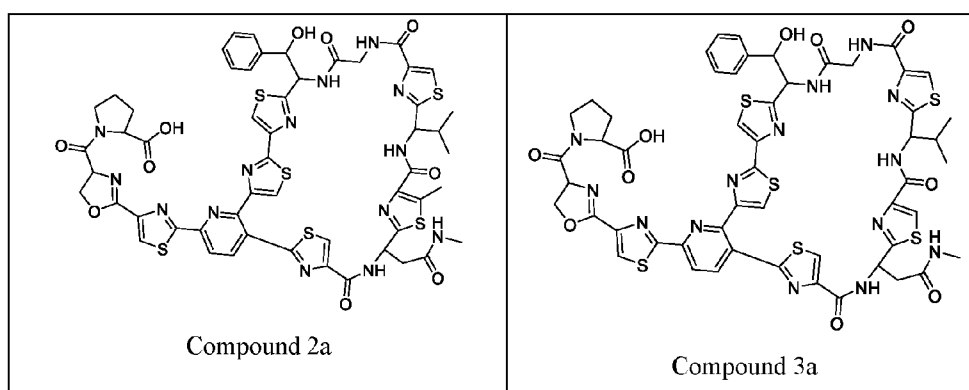


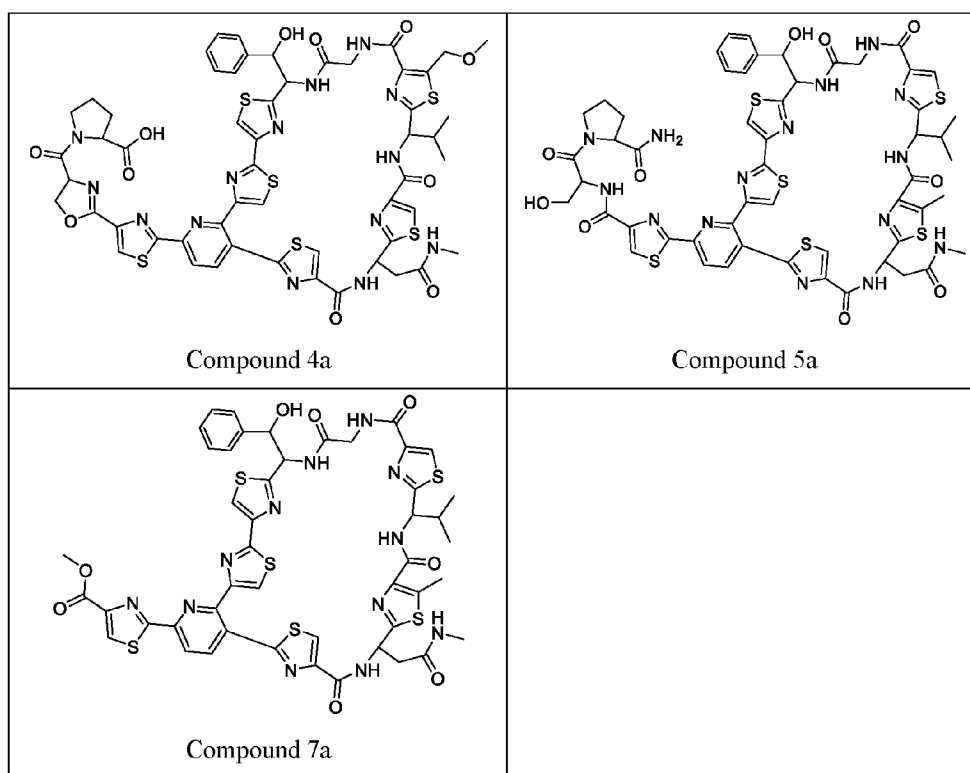
13. The compound of any one of claims 1 to 12, wherein R₂ is -H or -CH₂OCH₃.

14. The compound of any one of claims 1 to 13, wherein R₃ is -H or -CH₃.

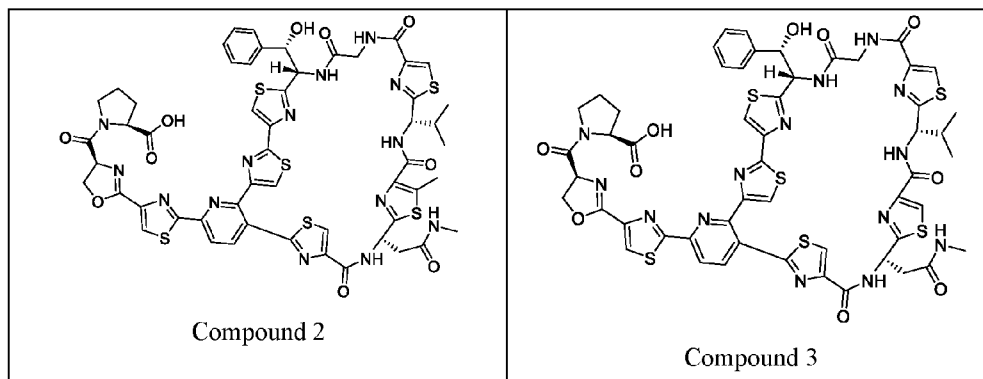
15. The compound of any one of claims 1 to 14, wherein R₄ is -NH(CH₃).

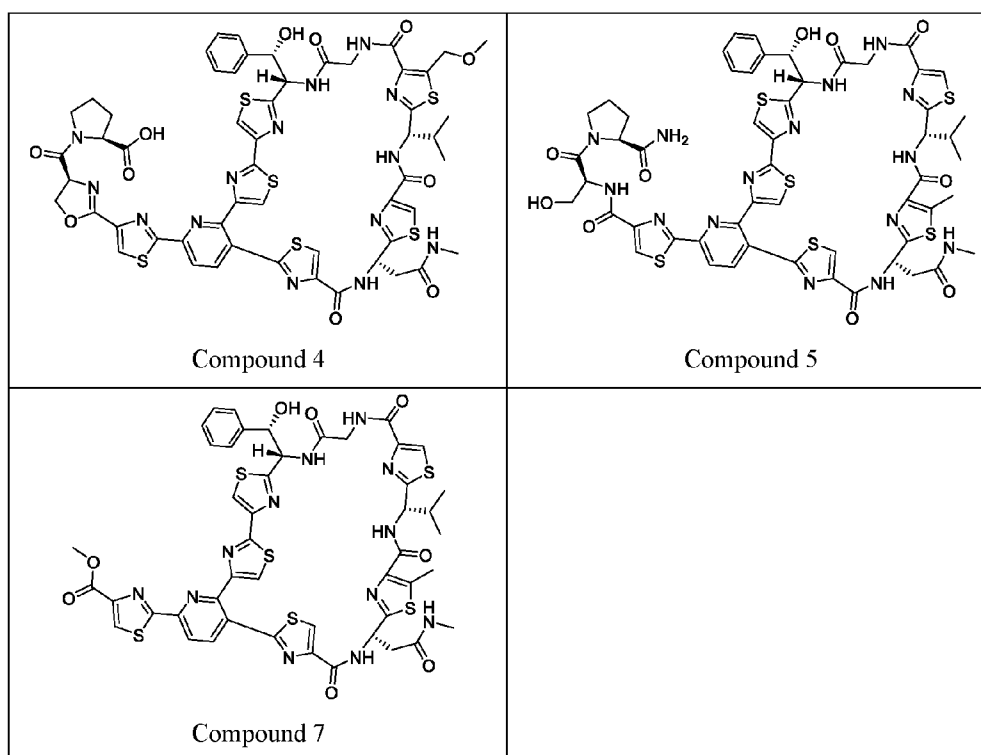
16. The compound of any one of claims 1 to 15, wherein the compound is selected from the group consisting of:



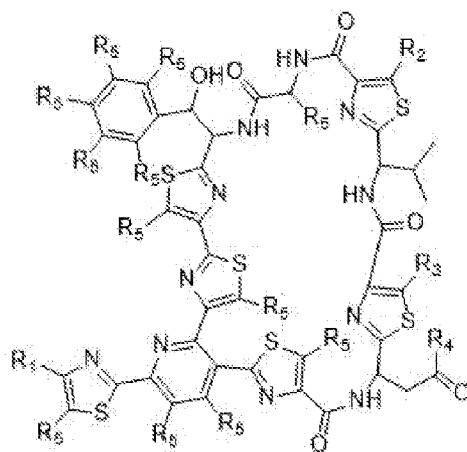


17. The compound of any one of claims 1 to 16, wherein the compound is selected from the group consisting of:





18. A pharmaceutical composition comprising a therapeutically effective amount of a compound of any one of claims 1 to 17, or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, and a pharmaceutically acceptable excipient.
19. A method of treating a bacterial, microbial and/or fungal infection, comprising administering a therapeutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, to a subject in need of treatment:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

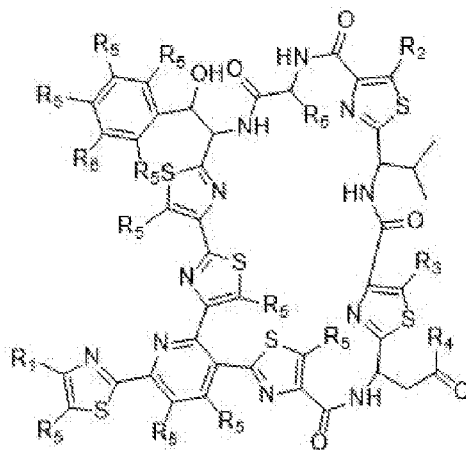
R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

20. The method of claim 19, wherein the infection is a *Staphylococcus*, *Bacillus*, *Clostridium*, *Acinetobacter*, *Klebsiella*, *Pseudomonas*, *Candida*, *Enterococcus*, or *Micrococcus* infection.

21. The method of claim 19 or 20, wherein the infection is caused by *Staphylococcus aureus*, *Staphylococcus aureus* Rosenbach, gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens*, *Acinetobacter baumannii*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Candida albicans*, *Clostridium difficile*, *Enterococcus faecalis*, *Enterococcus faecium*, or *Micrococcus luteus*.
22. A method of treating cancer, comprising administering a therapeutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, metabolite, prodrug or stereoisomer thereof, to a subject in need of treatment:



Formula (I)

wherein:

R₁ is -C(O)-R₆ or -A-G-J-L;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or -C(O)N(R_{7a})-;

G is -C(O)- or optionally substituted -alkylene-C(O)-;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

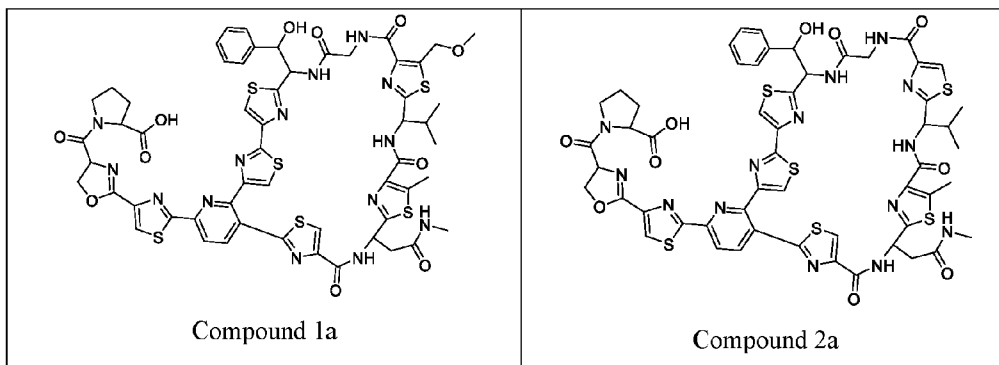
R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

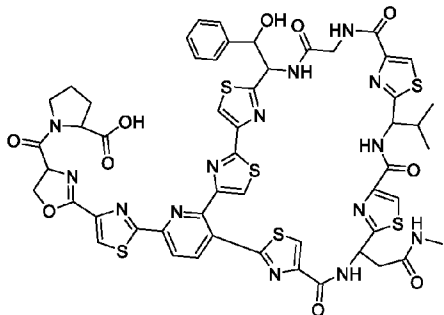
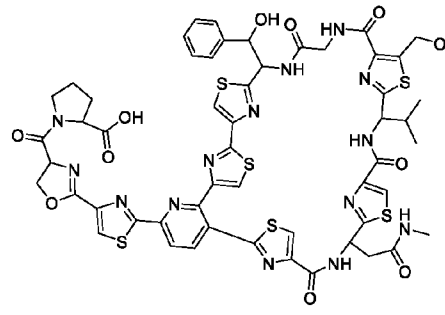
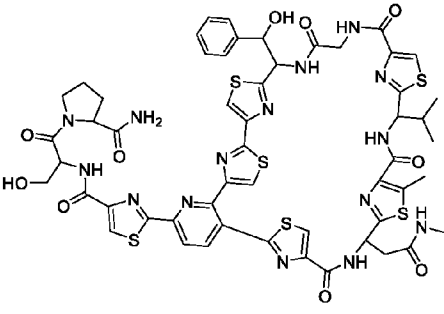
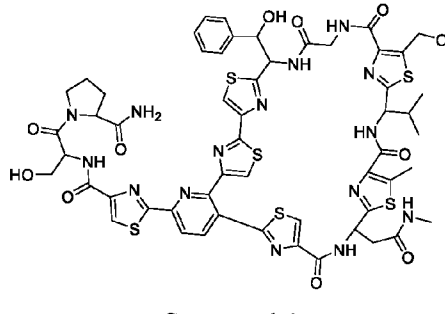
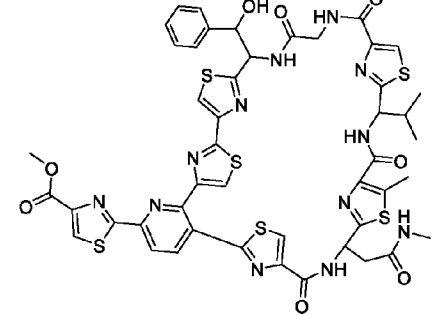
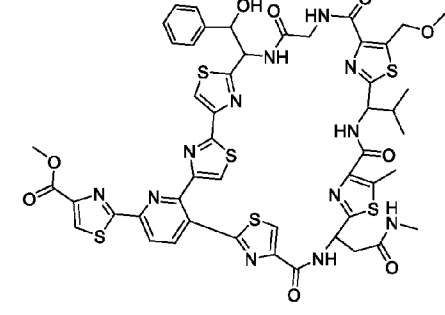
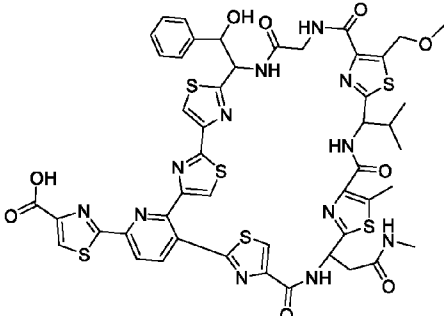
R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

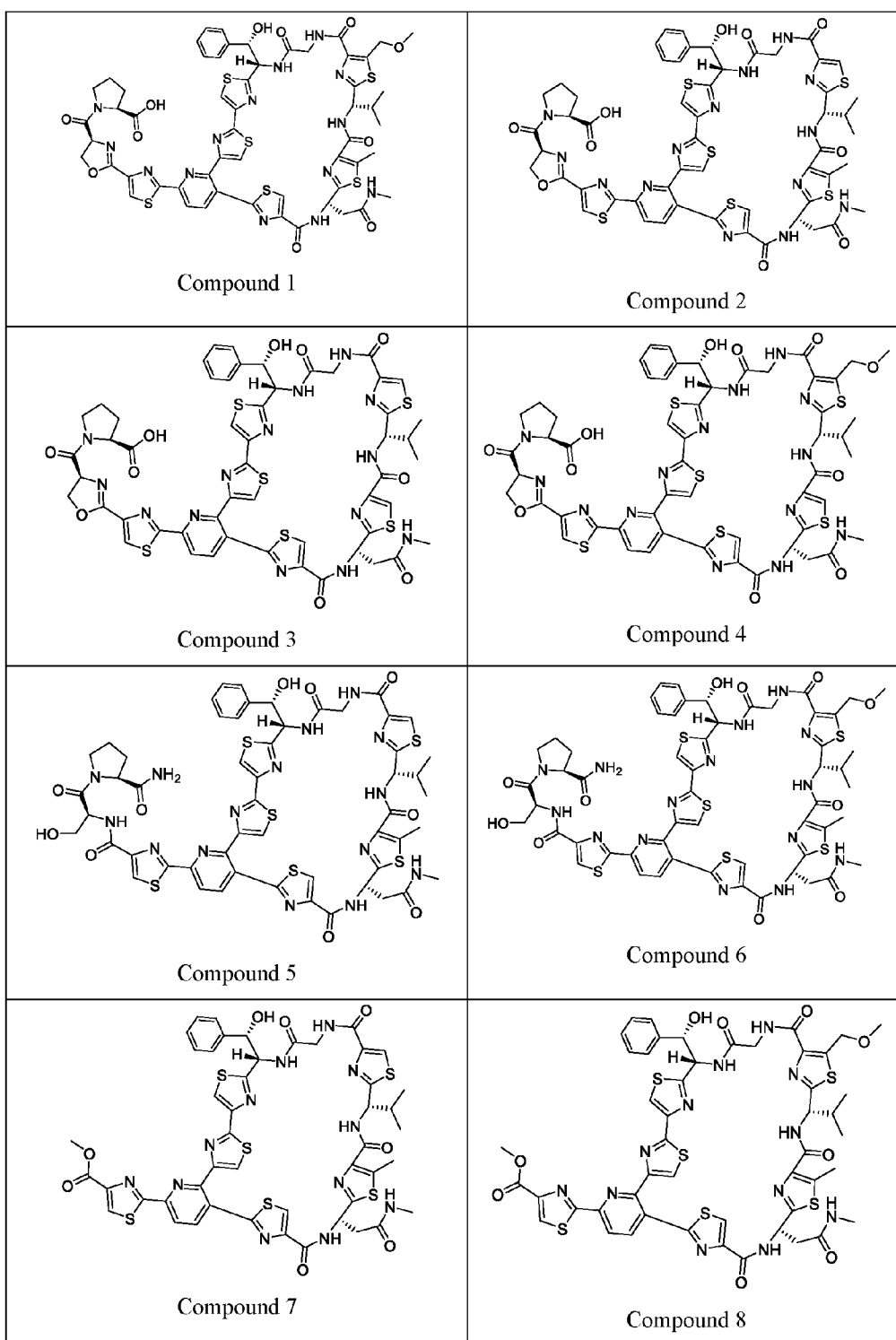
R_{7a} and R_{7b} are independently -H or optionally substituted alkyl.

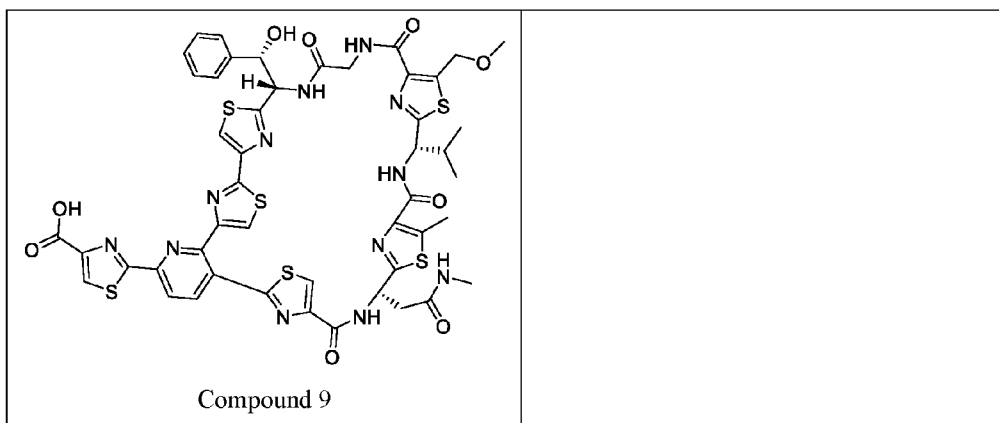
23. The method of any one of claims 19-22, wherein the compound is a compound of any one of claims 1-17.

24. The method of any one of claims 19-22, wherein the compound is selected from:

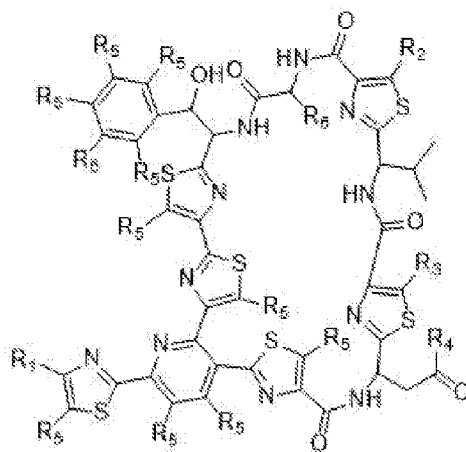


 Compound 3a	 Compound 4a
 Compound 5a	 Compound 6a
 Compound 7a	 Compound 8a
 Compound 9a	





25. A method of isolating a compound of Formula (I),



Formula (I)

wherein:

R_1 is $-C(O)-R_6$ or $-A-G-J-L$;

A is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, optionally substituted heteroarylene, or $-C(O)N(R_{7a})-$;

G is $-C(O)-$ or optionally substituted $-alkylene-C(O)-$;

J is optionally substituted carbocyclylene, optionally substituted heterocarbocyclylene, optionally substituted cycloalkylene, optionally substituted heterocycloalkylene, optionally substituted arylene, or optionally substituted heteroarylene;

L is -C(O)-R₆;

R₂ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl, or optionally substituted alkalkoxy;

R₃ is -H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl;

R₄ is -H, -OH, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or -N(R_{7a})(R_{7b});

R₅ is independently selected from the group consisting of -H, halogen, -N(R_{7a})(R_{7b}), optionally substituted alkoxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocarbocyclyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, or optionally substituted heteroaryl;

R₆ is -OH, alkoxy, or -N(R_{7a})(R_{7b}); and

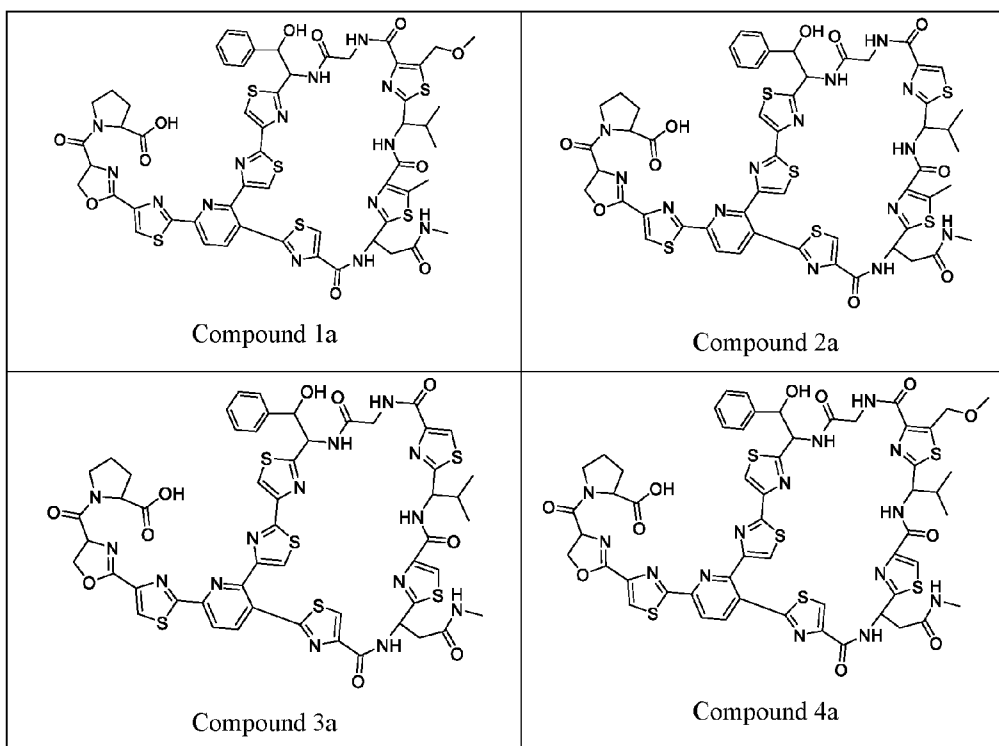
R_{7a} and R_{7b} are independently -H or optionally substituted alkyl,

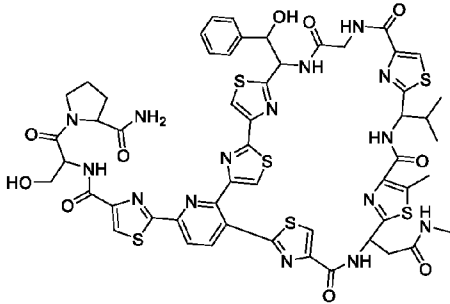
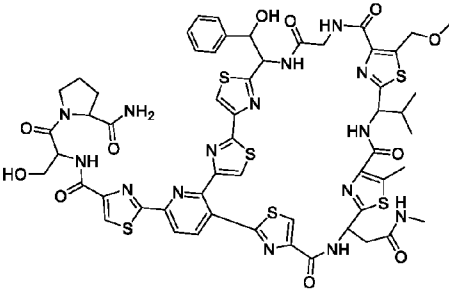
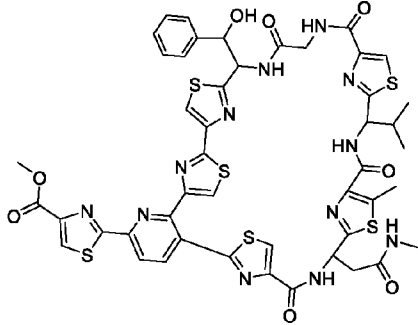
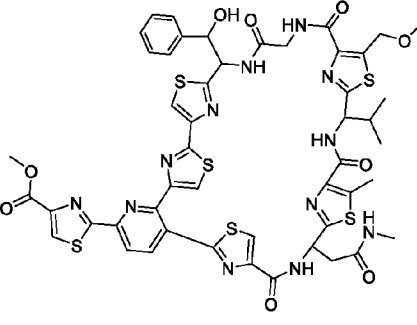
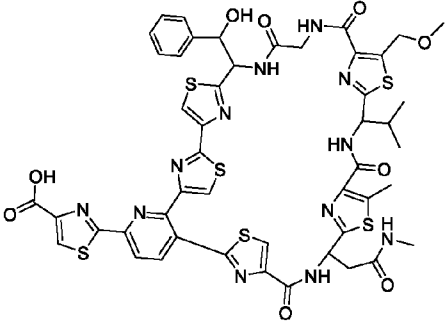
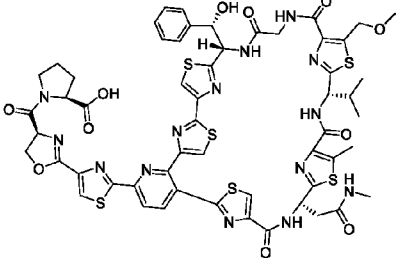
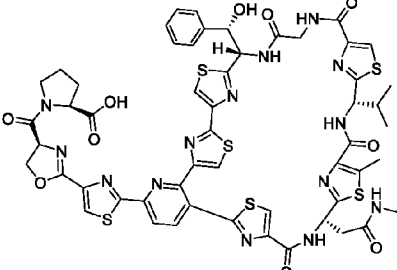
the method comprising:

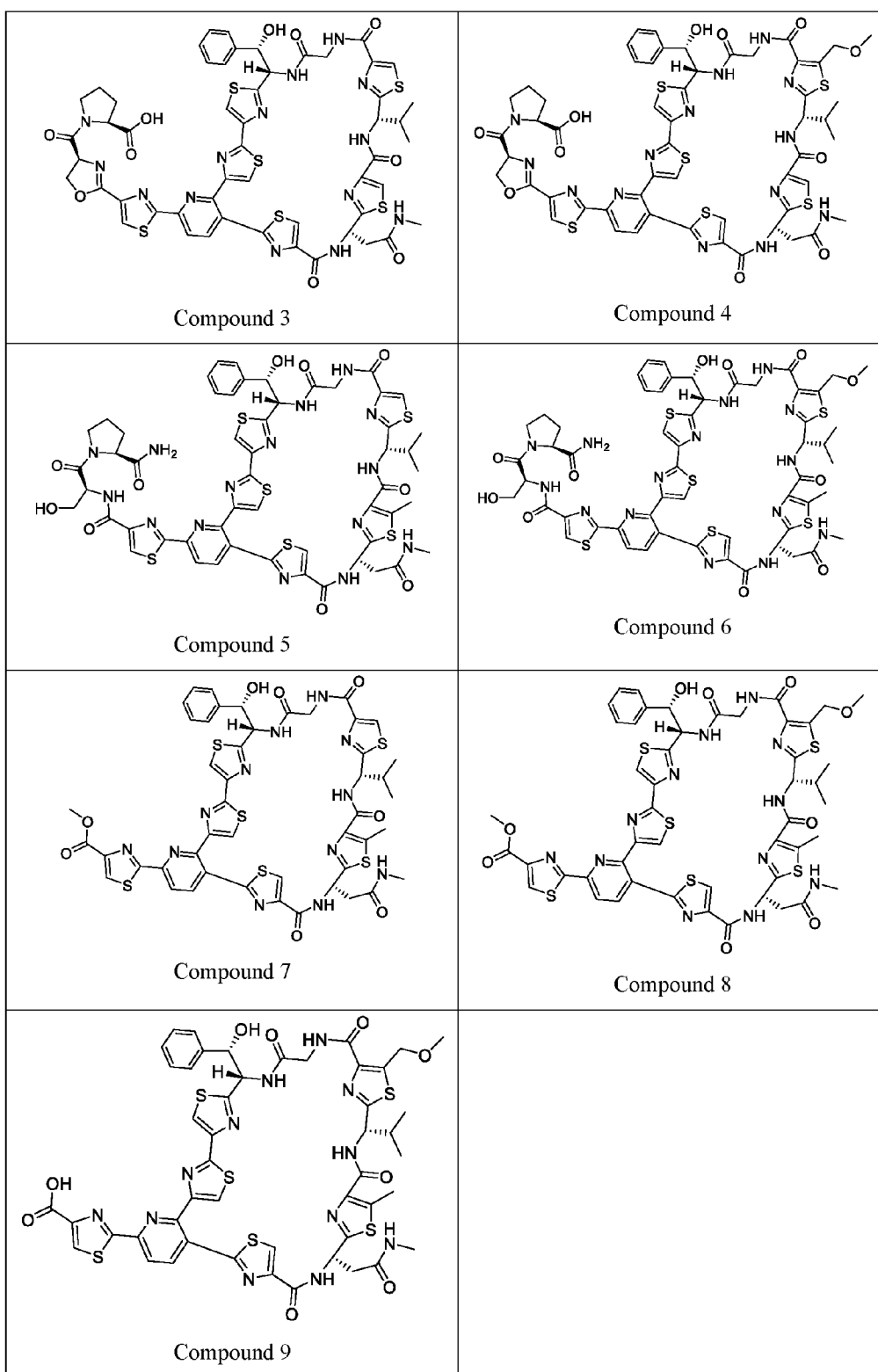
- (i) expanding a *Nonomuraea jiangxiensis* strain under culture conditions that allow expansion of the strain to obtain a seed culture;
- (ii) using the seed culture to inoculate a fermentation culture;
- (iii) incubating the fermentation culture; and
- (iv) isolating the compound from the fermentation culture.

- 26. The method of claim 25, wherein step (iii) comprises incubating the fermentation culture for about 7 to about 12 days.
- 27. The method of claim 25 or 26, wherein step (iii) comprises incubating the fermentation culture at a temperature of about 25 °C to 35 °C.
- 28. The method of any one of claims 25 to 27, wherein step (iii) comprises incubating the fermentation culture with a shaking condition of about 150 to 350 rpm.

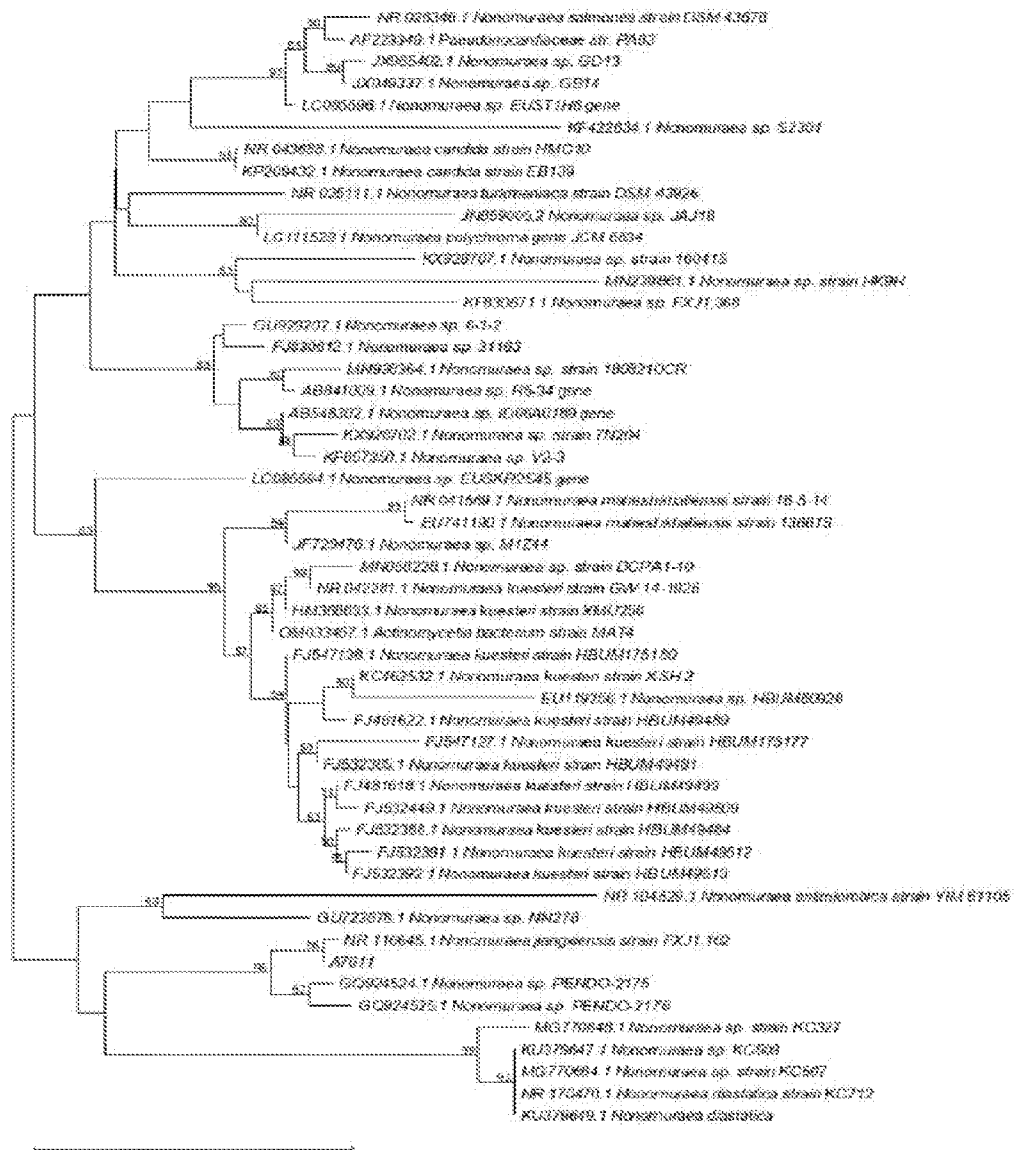
29. The method of any one of claims 25 to 28, wherein step (iii) comprises incubating the fermentation culture for 9 days at 28 °C with shaking condition of 200 rpm.
30. The method of any one of claims 25 to 29, wherein the compound is isolated using a method selected from the group consisting of centrifugation, precipitation, filtration, liquid extraction, methanol extraction, chromatography, column chromatography, affinity chromatography, evaporation, lyophilization, and combinations thereof.
31. The method of any one of claims 25 to 30, wherein the compound is a compound of any one of claims 1 to 17.
32. The method of any one of claims 25 to 30, wherein the compound is a compound selected from the group consisting of



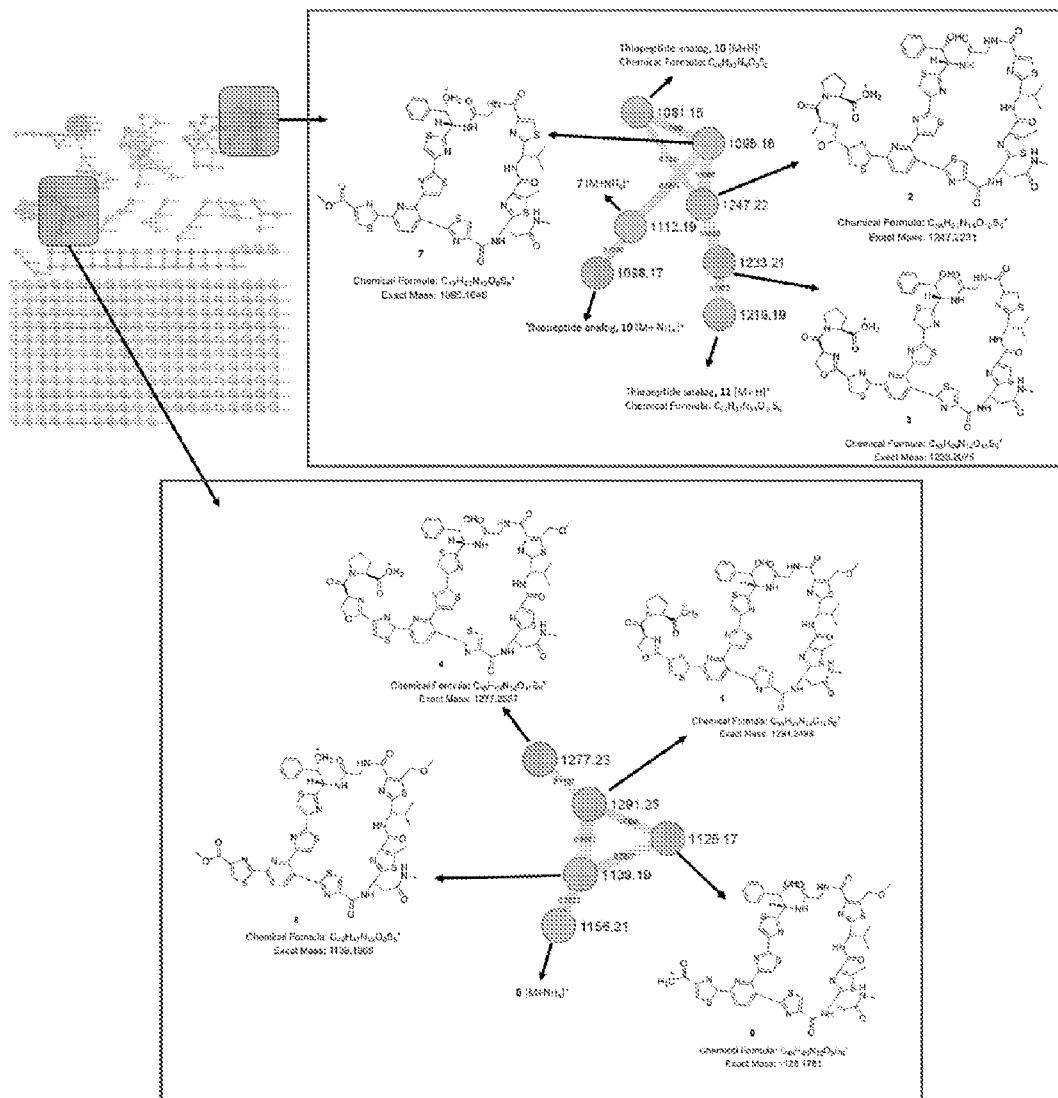
	
Compound 5a	Compound 6a
	
Compound 7a	Compound 8a
	
Compound 9a	
	
Compound 1	Compound 2



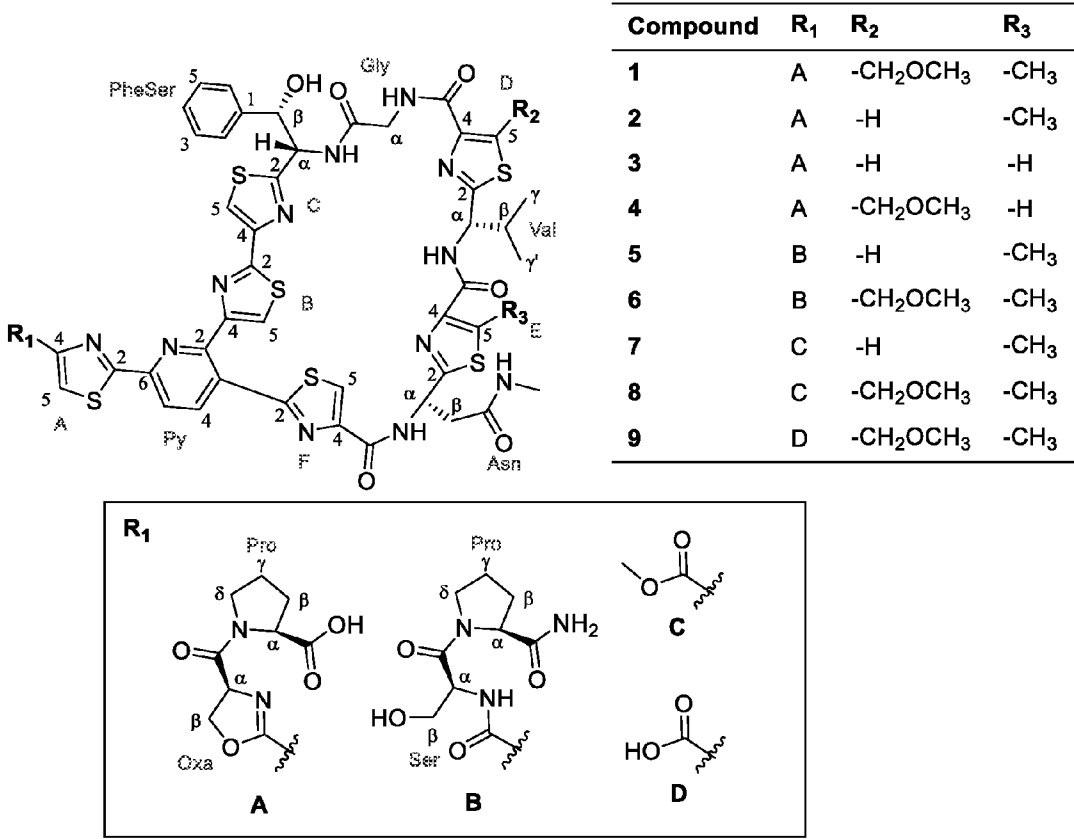
[FIG. 1]



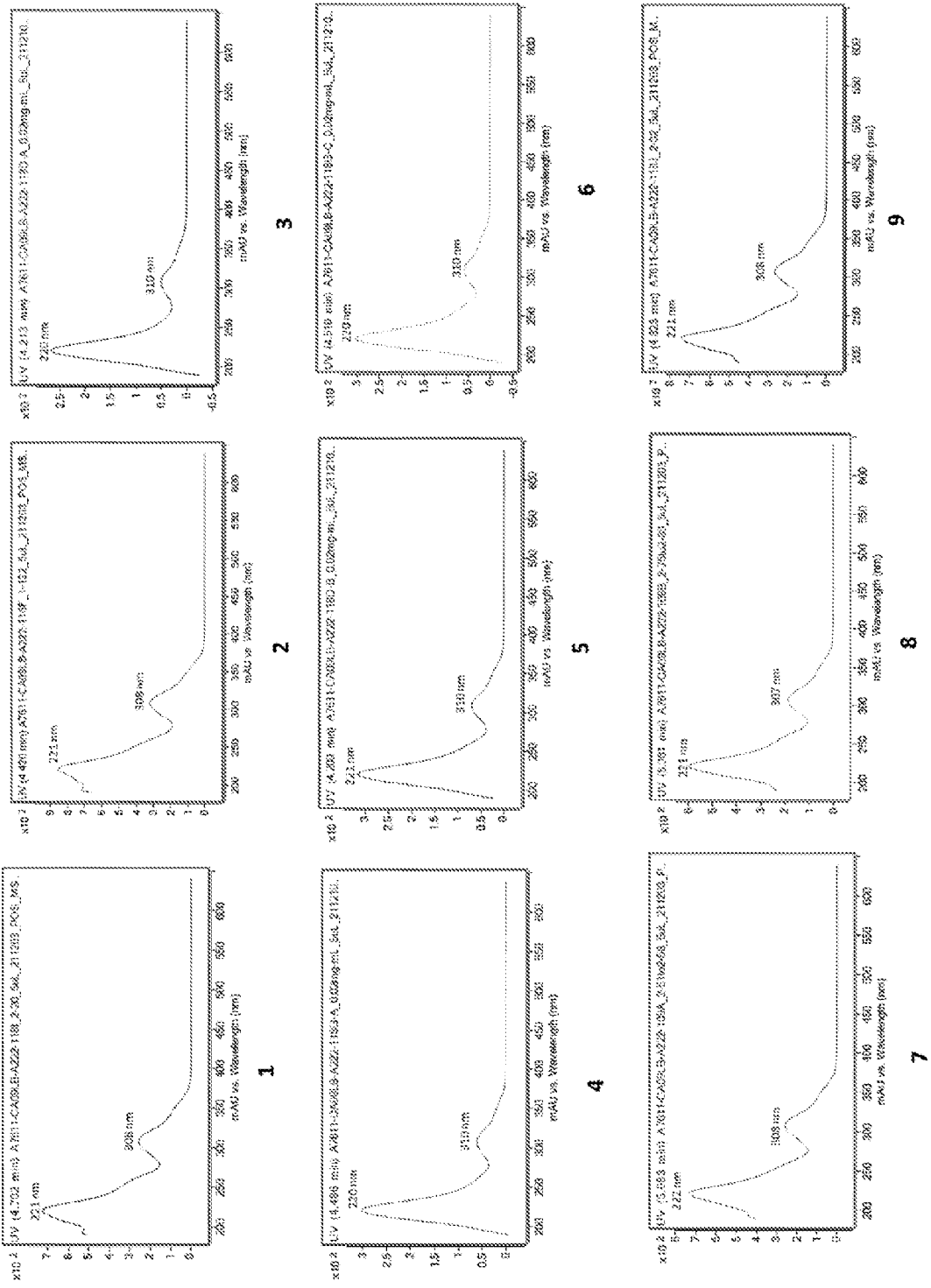
[FIG. 2]



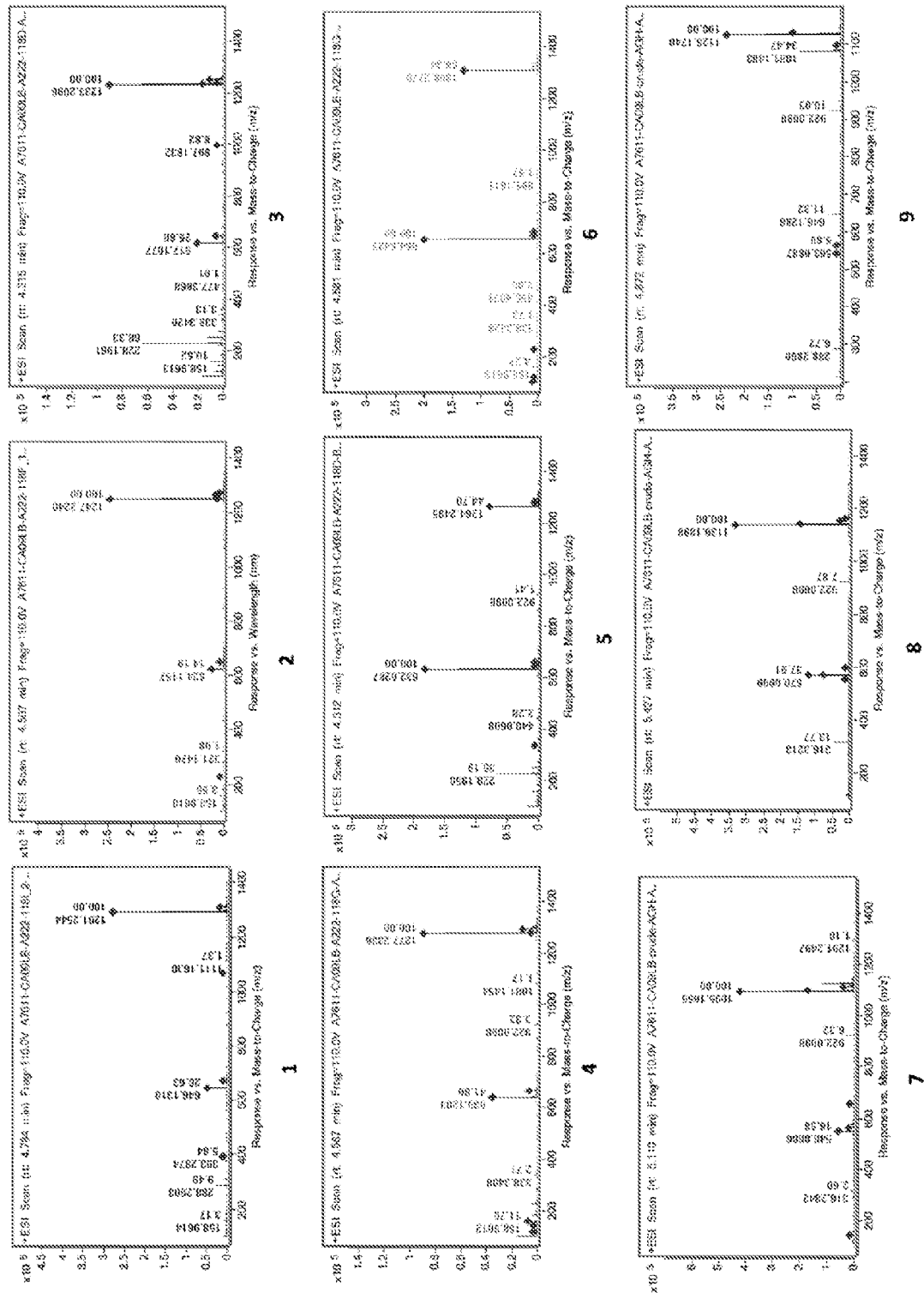
[FIG. 3]



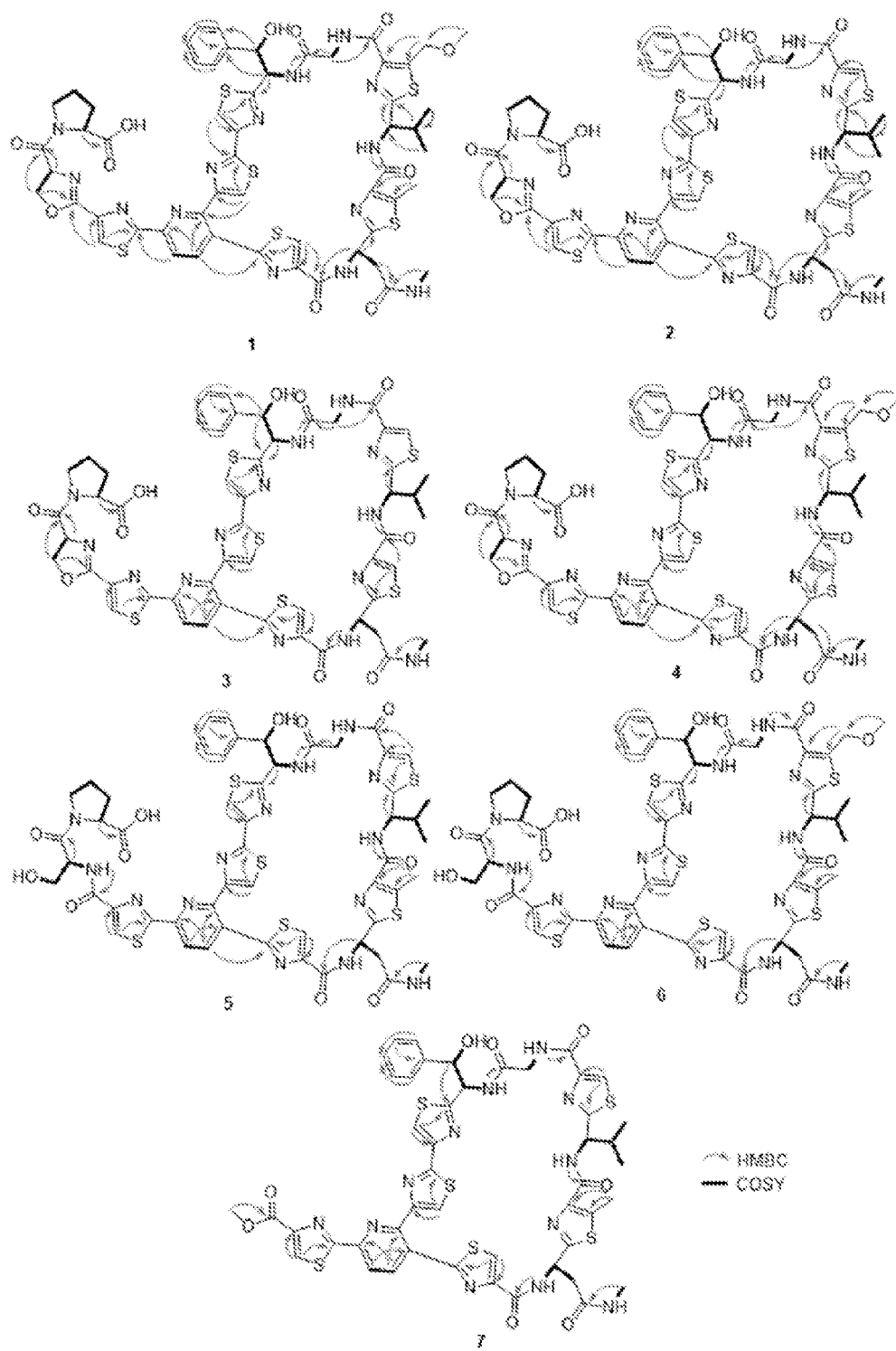
[FIG. 4]



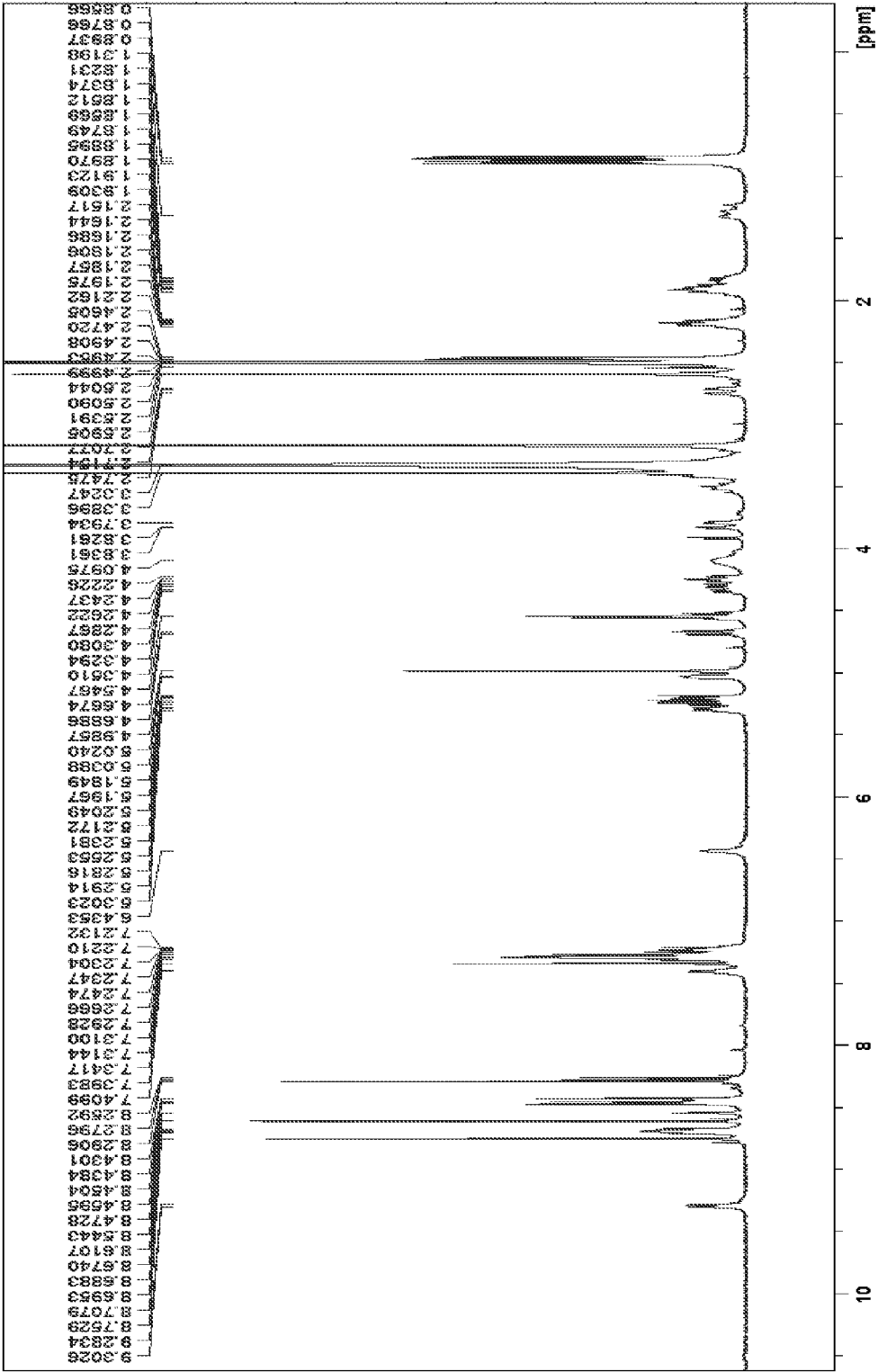
[FIG. 5]



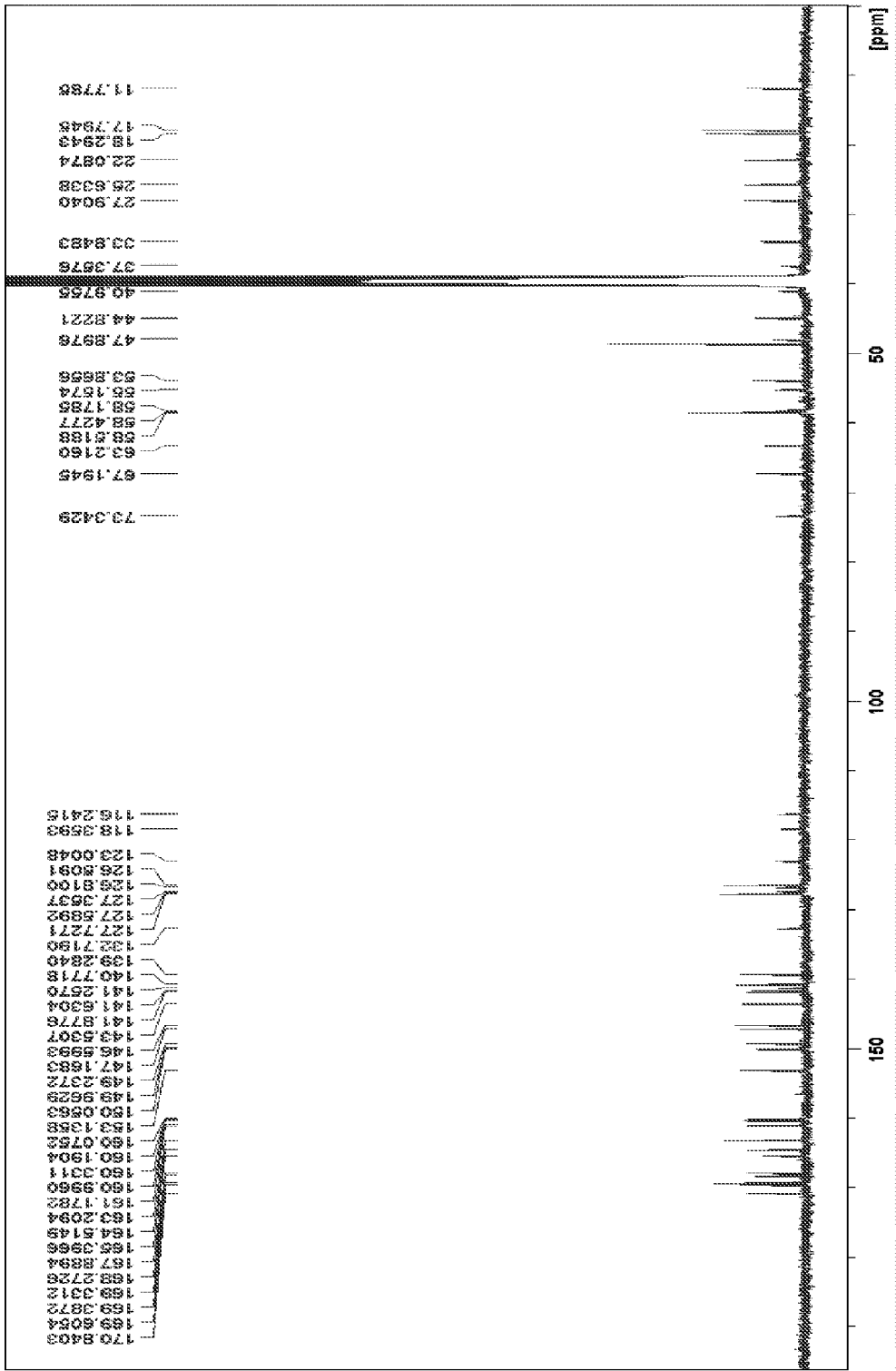
[FIG. 6]



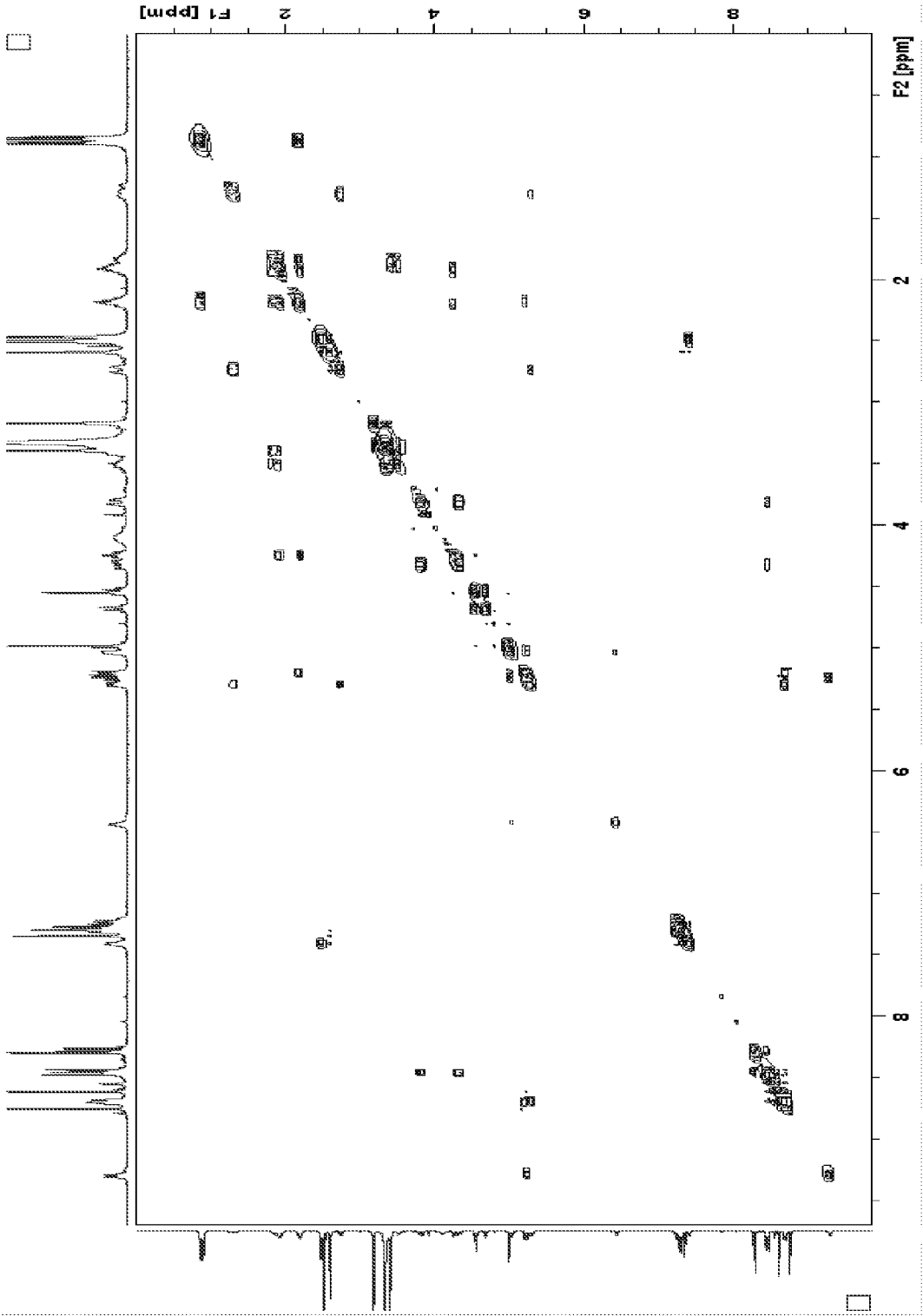
[FIG. 7A]



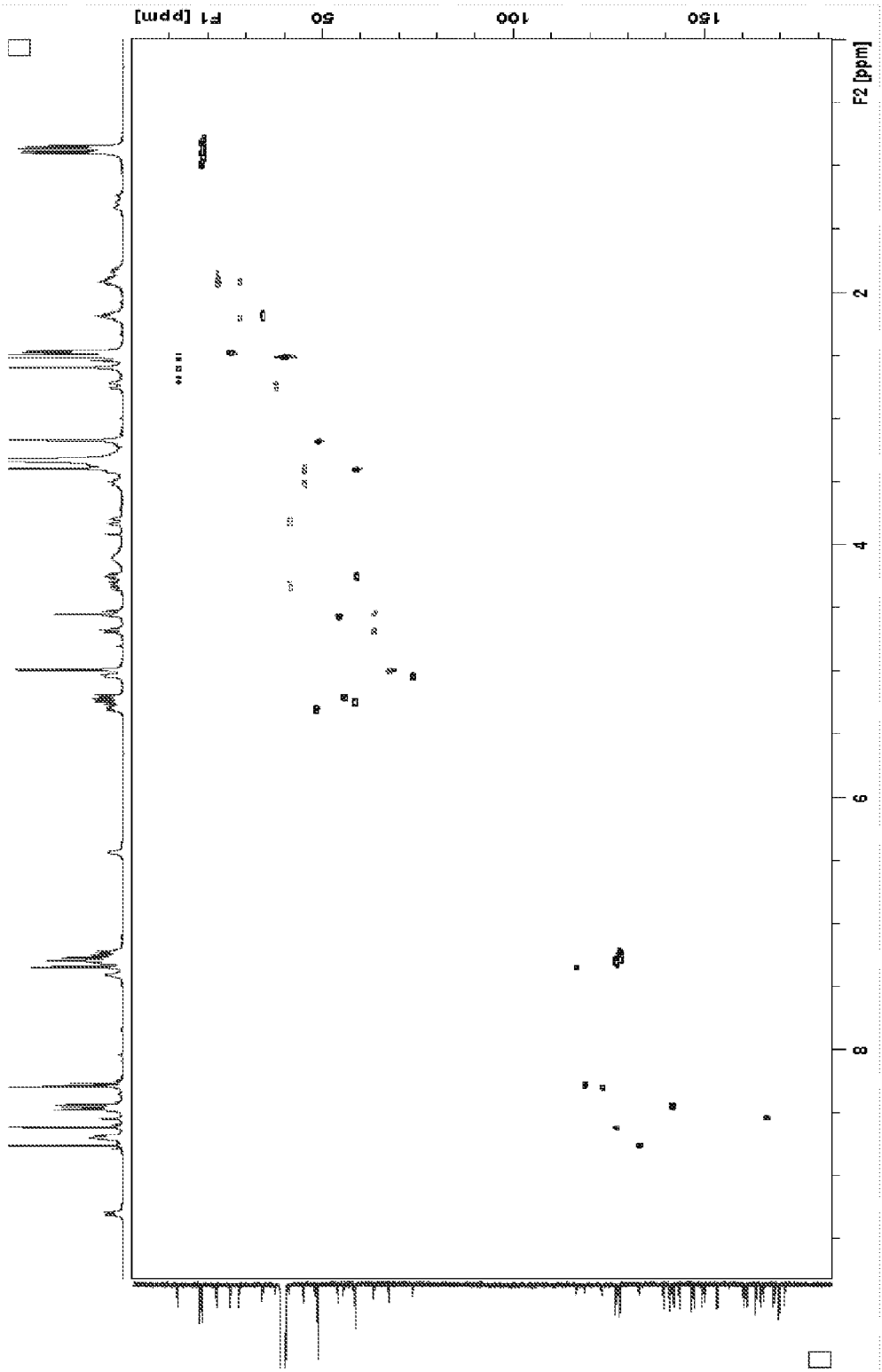
[FIG. 7B]



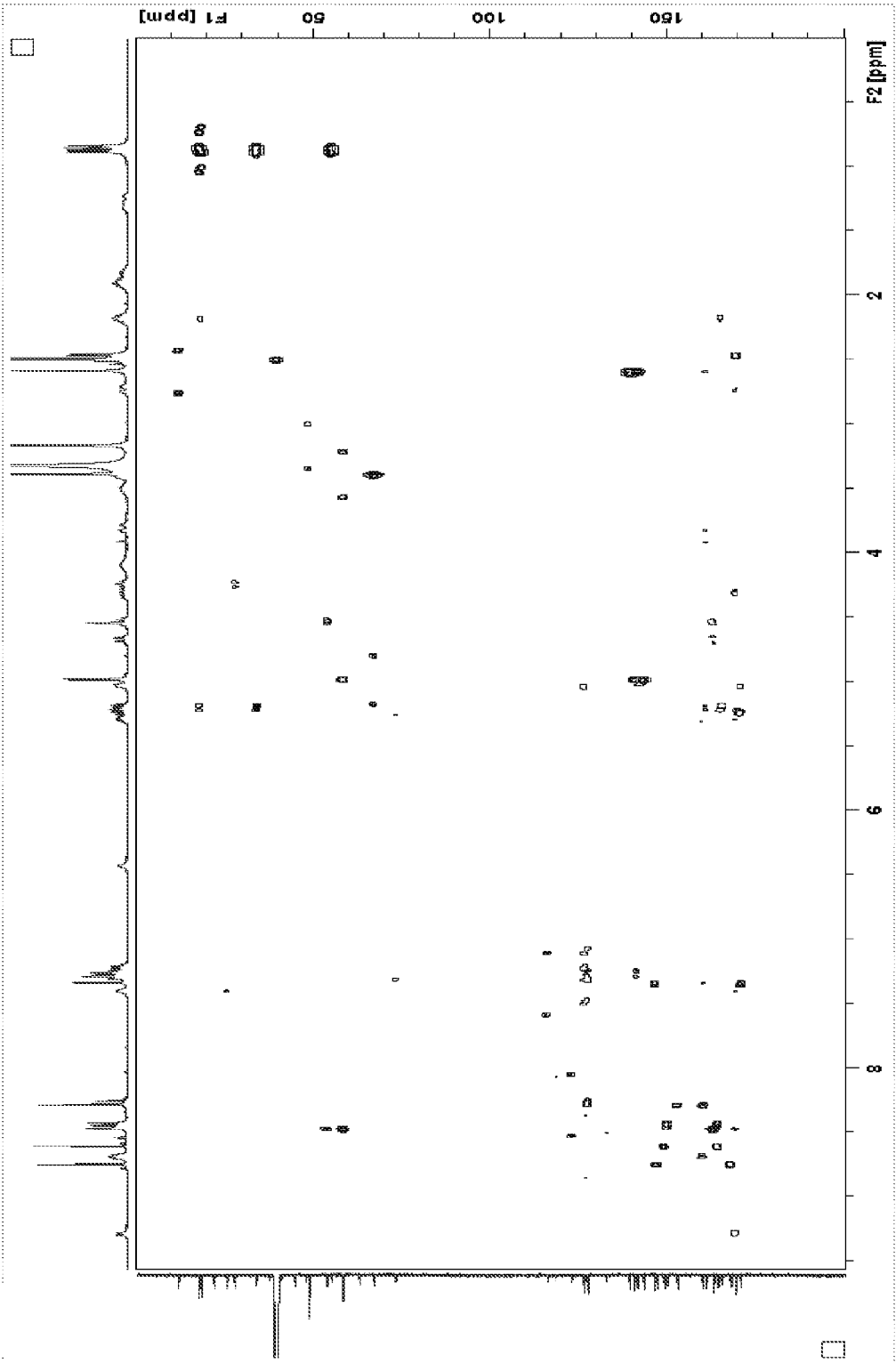
[FIG. 7C]



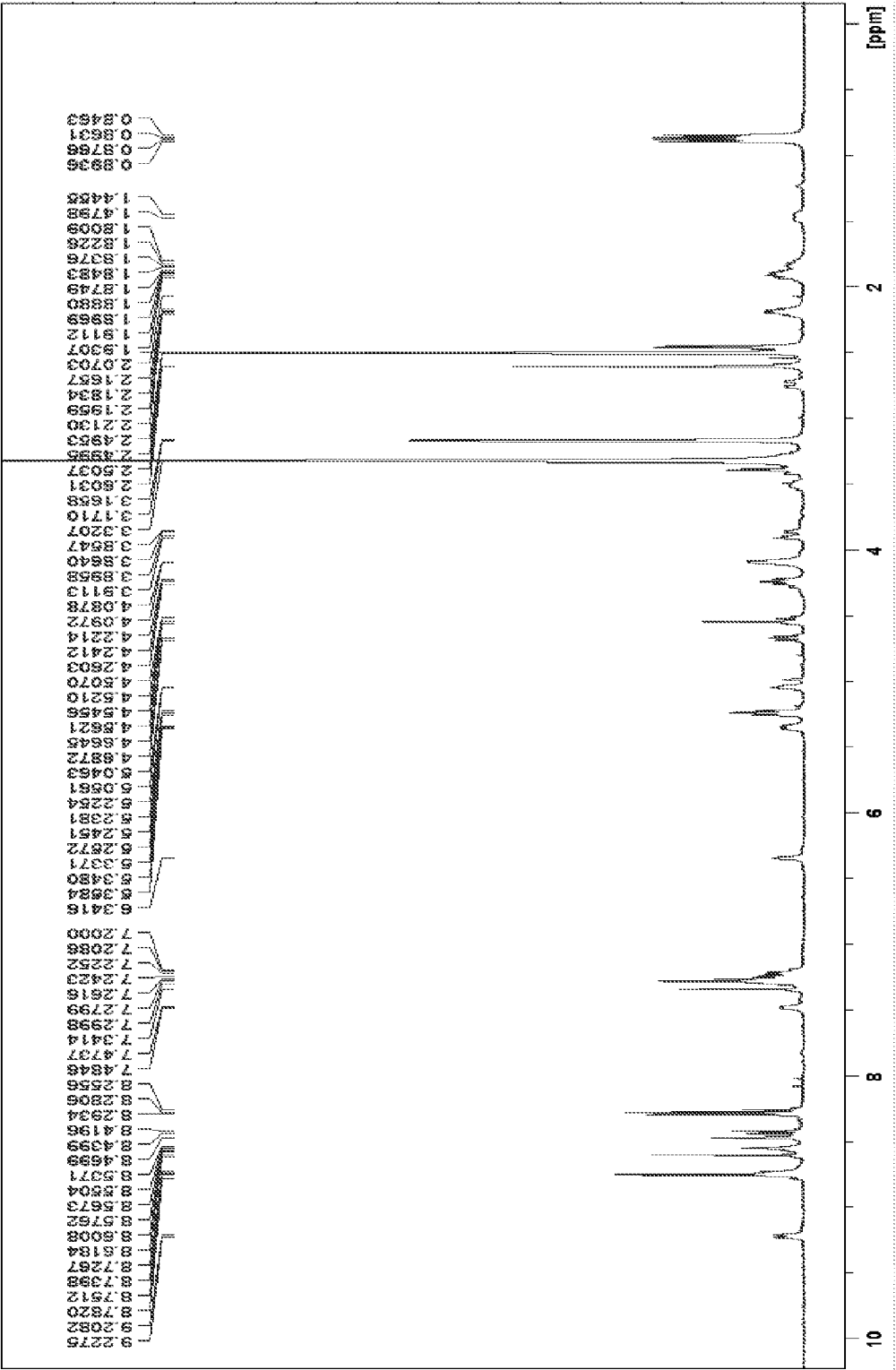
[FIG. 7D]



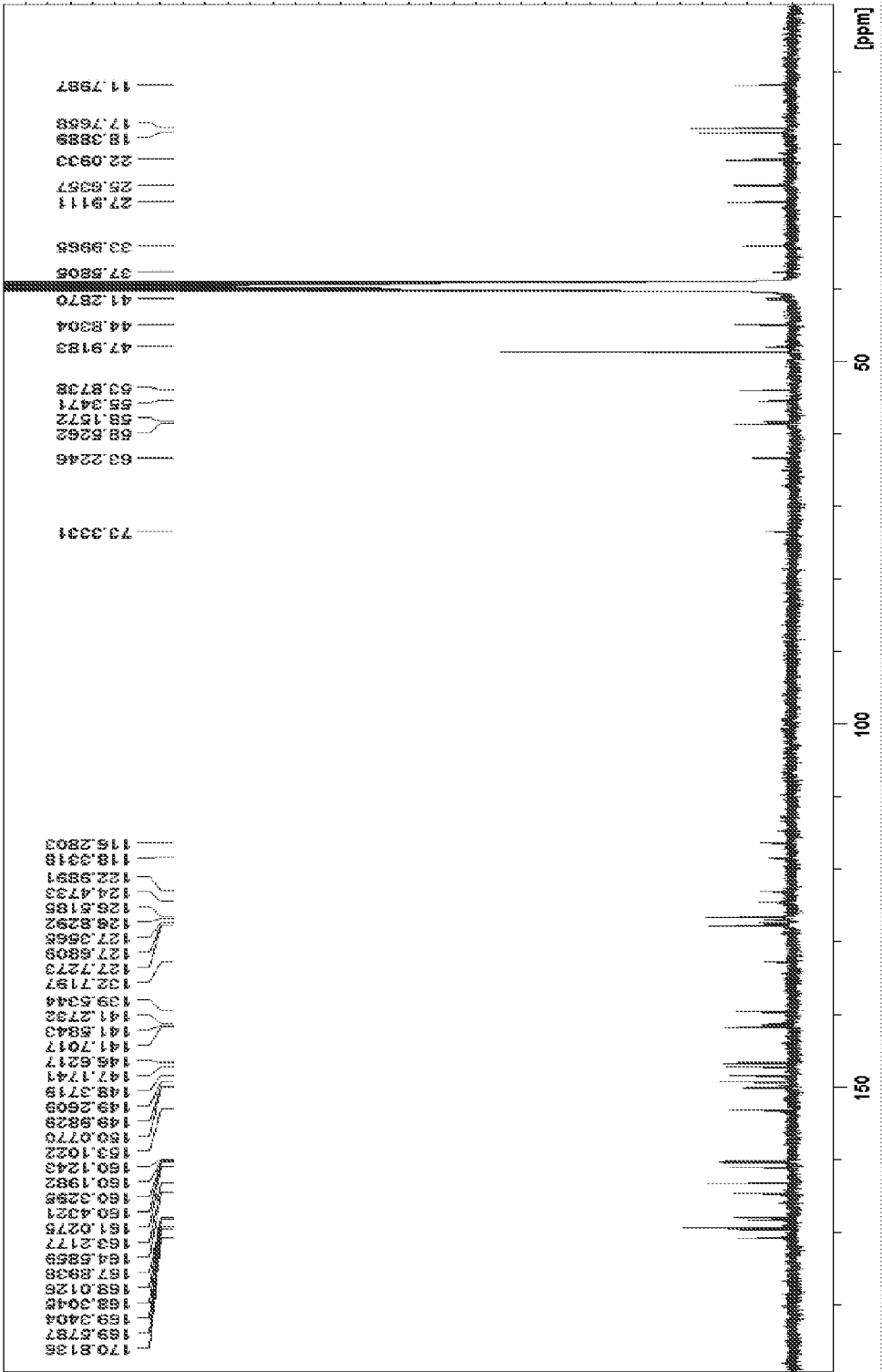
[FIG. 7E]



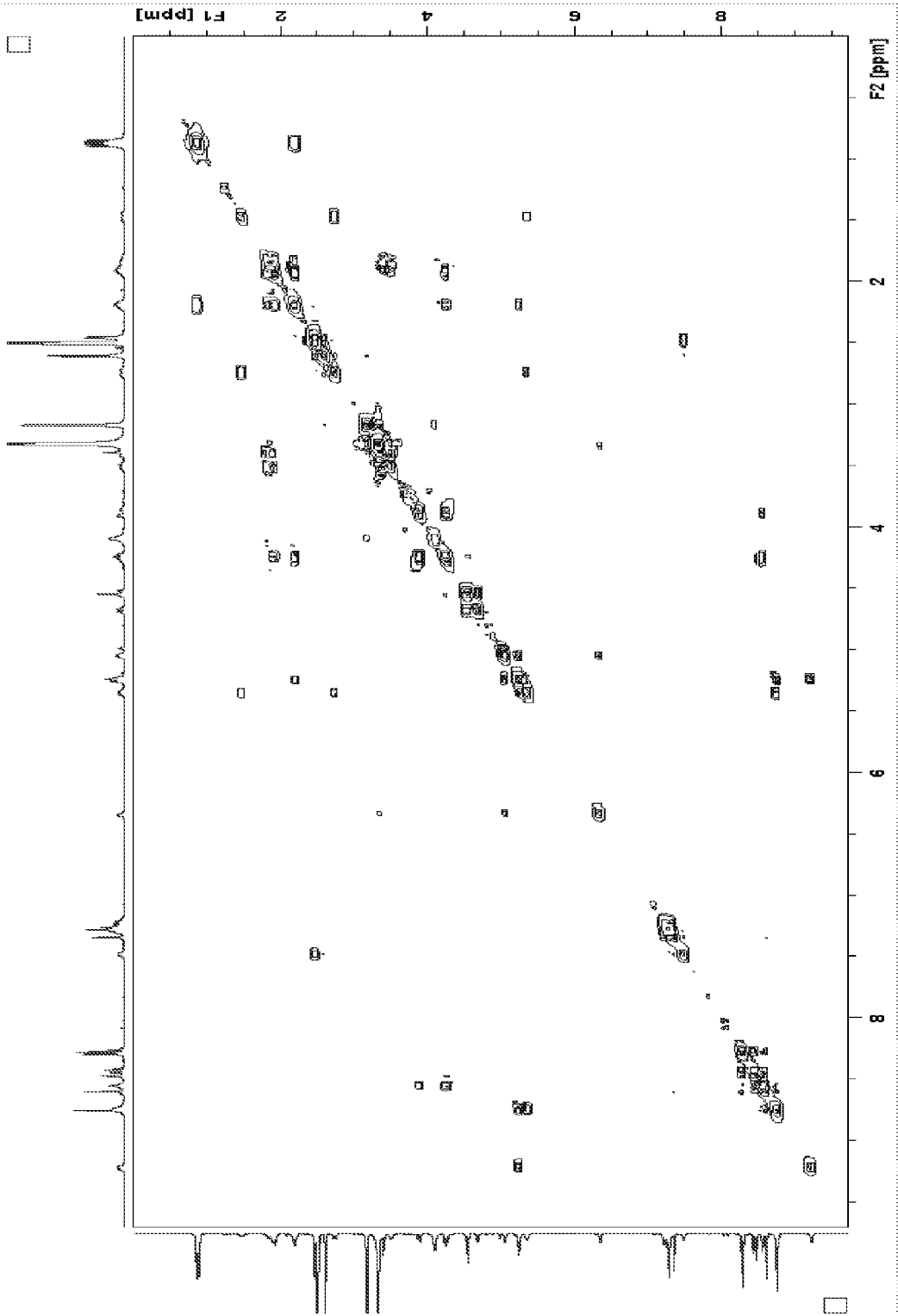
[FIG. 8A]



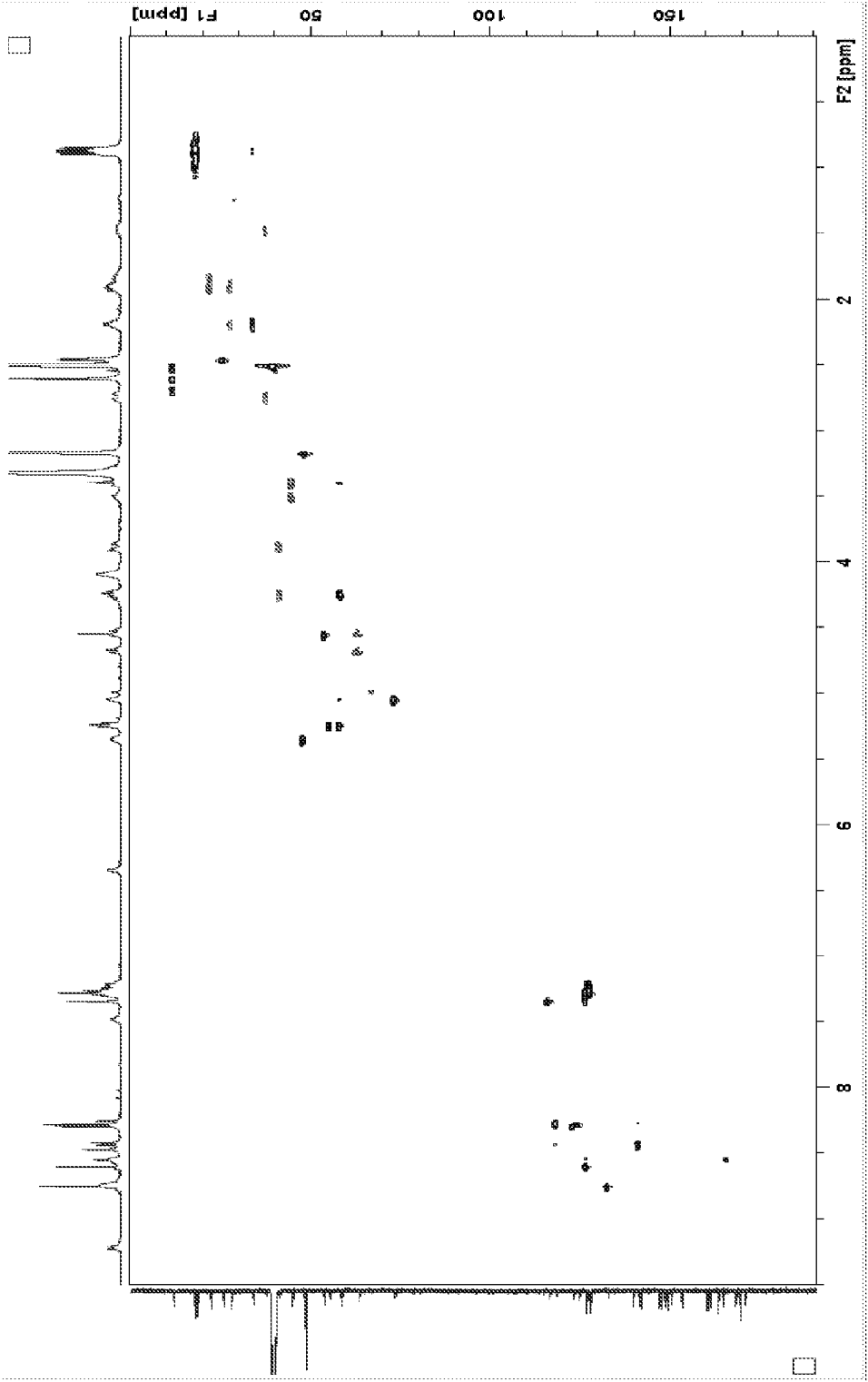
[FIG. 8B]



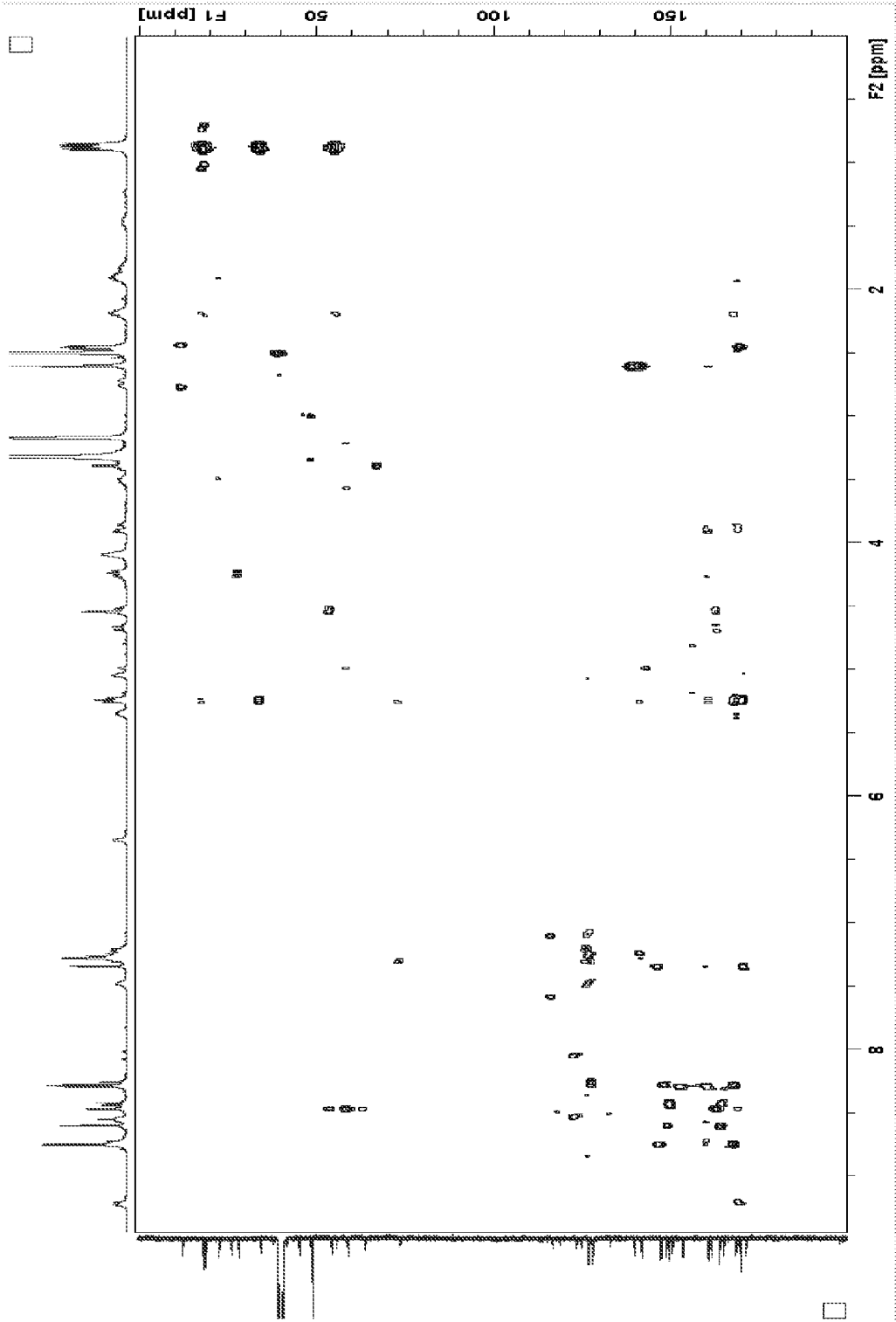
[FIG. 8C]



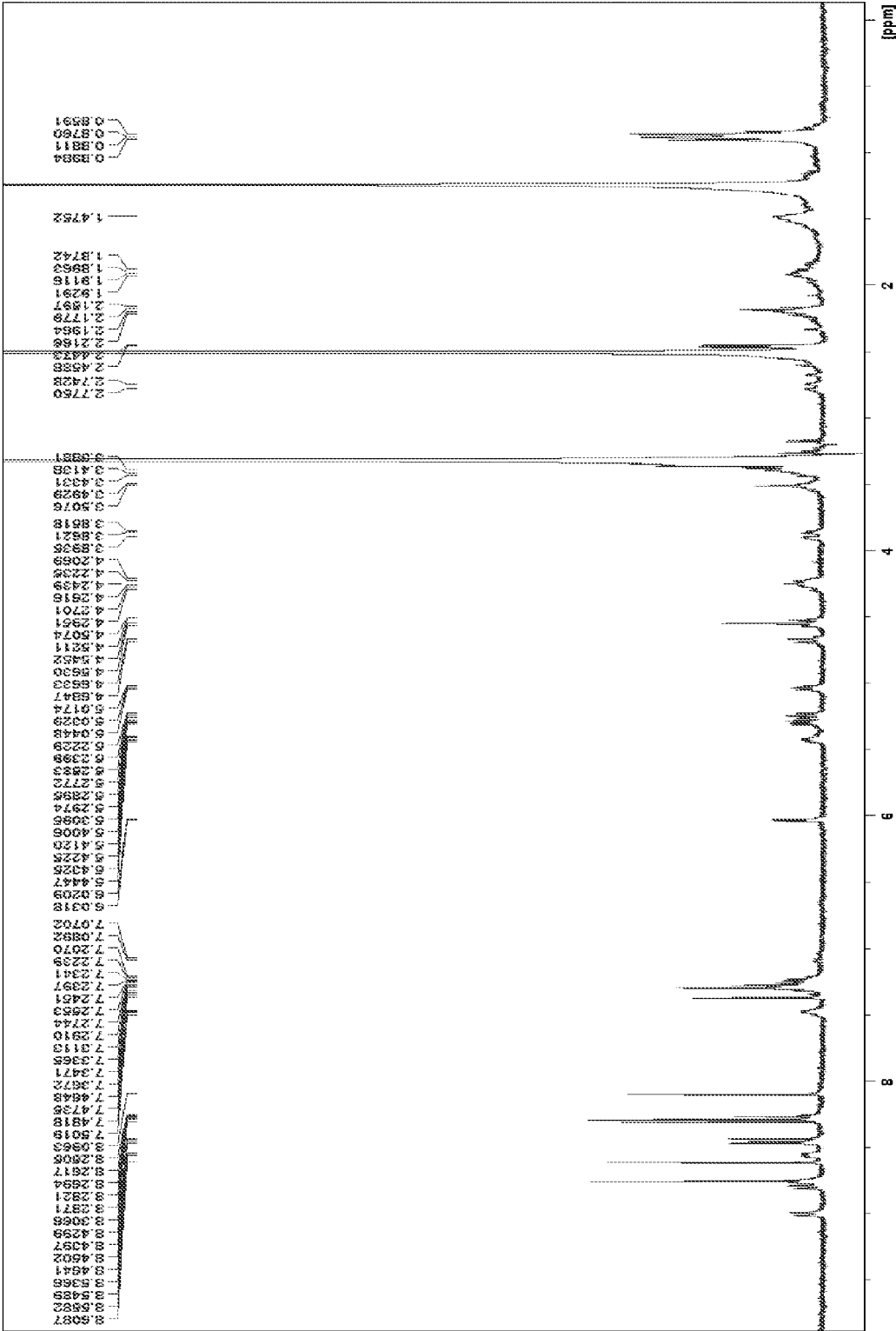
[FIG. 8D]



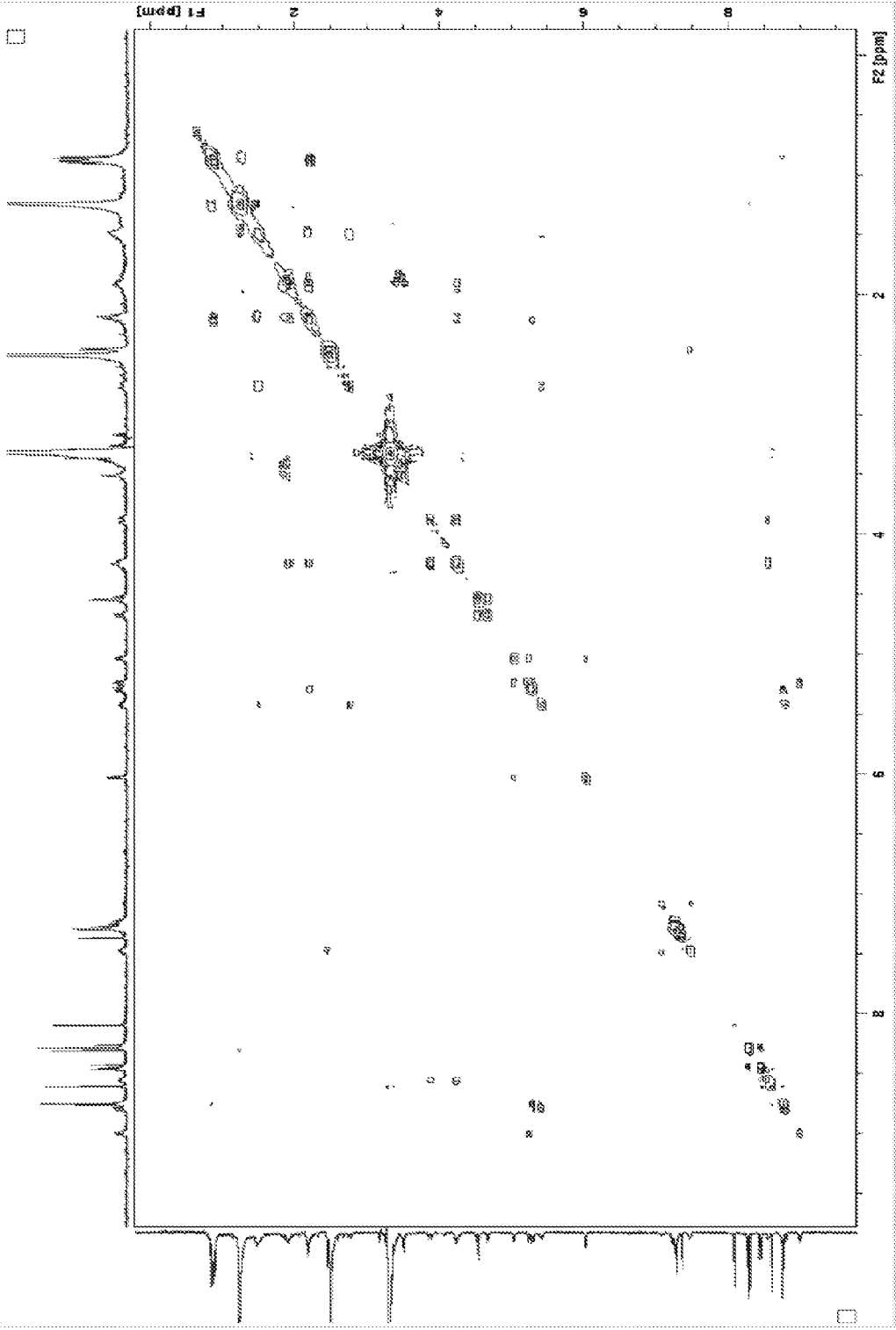
[FIG. 8E]



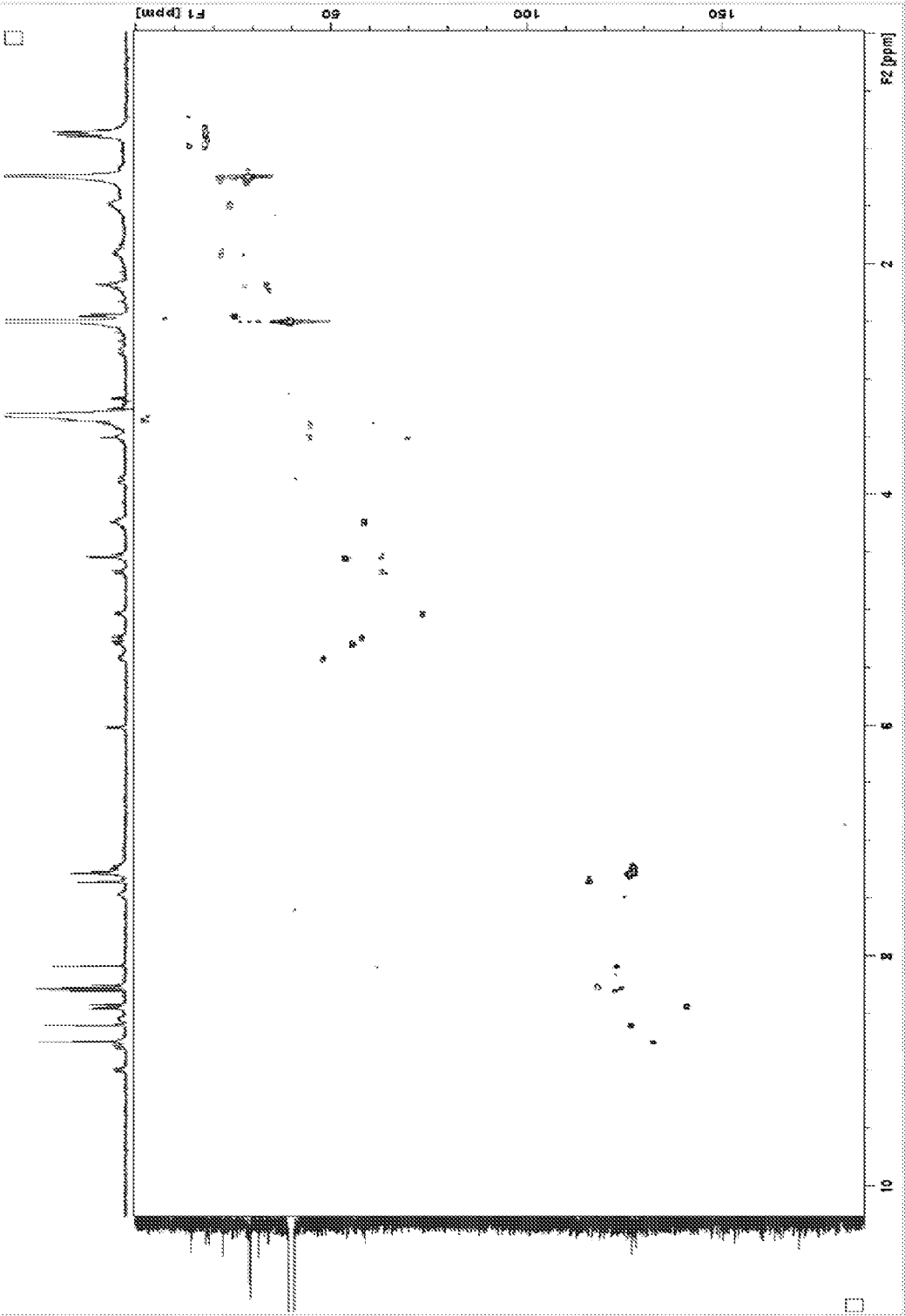
[FIG. 9A]



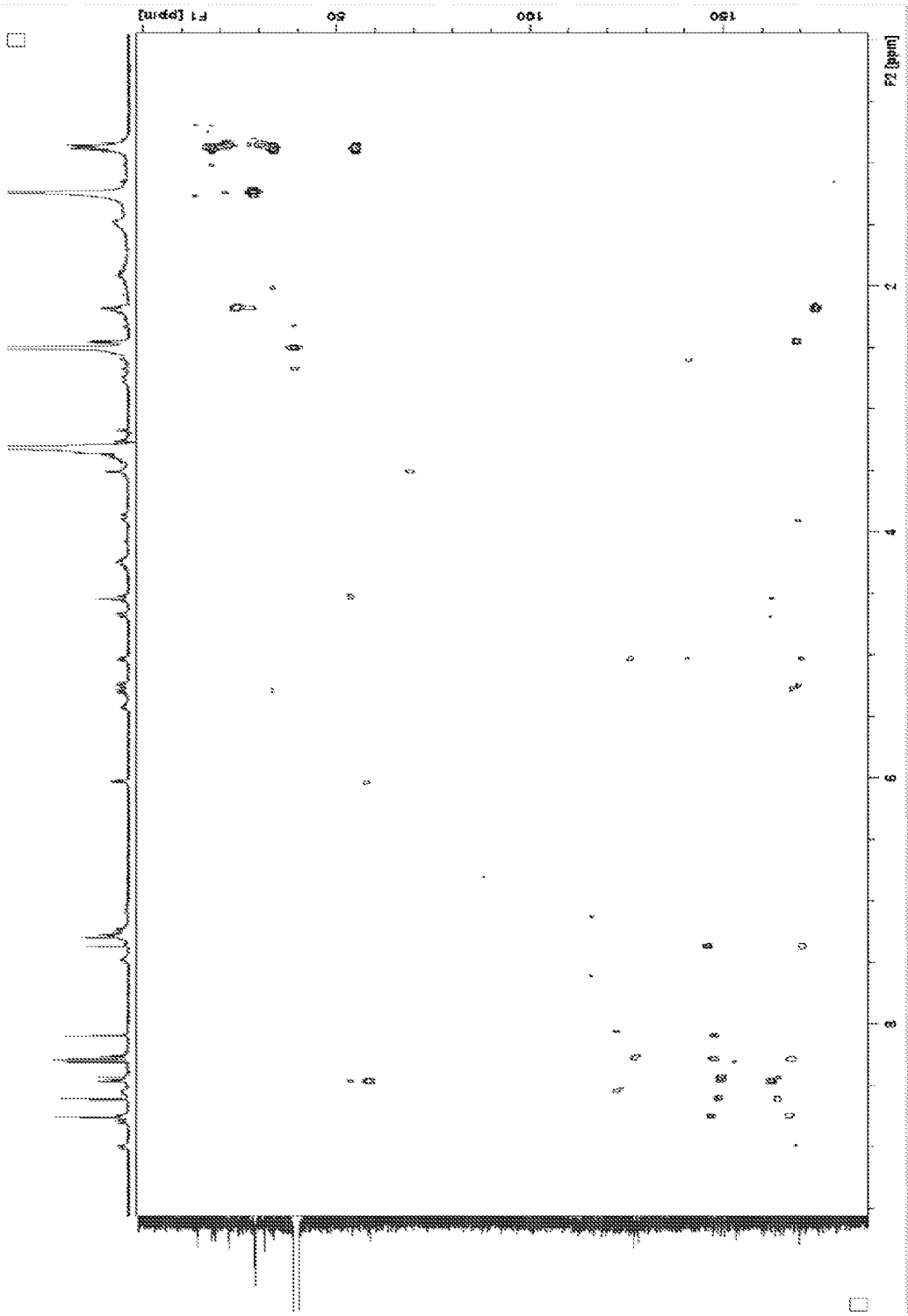
[FIG. 9B]



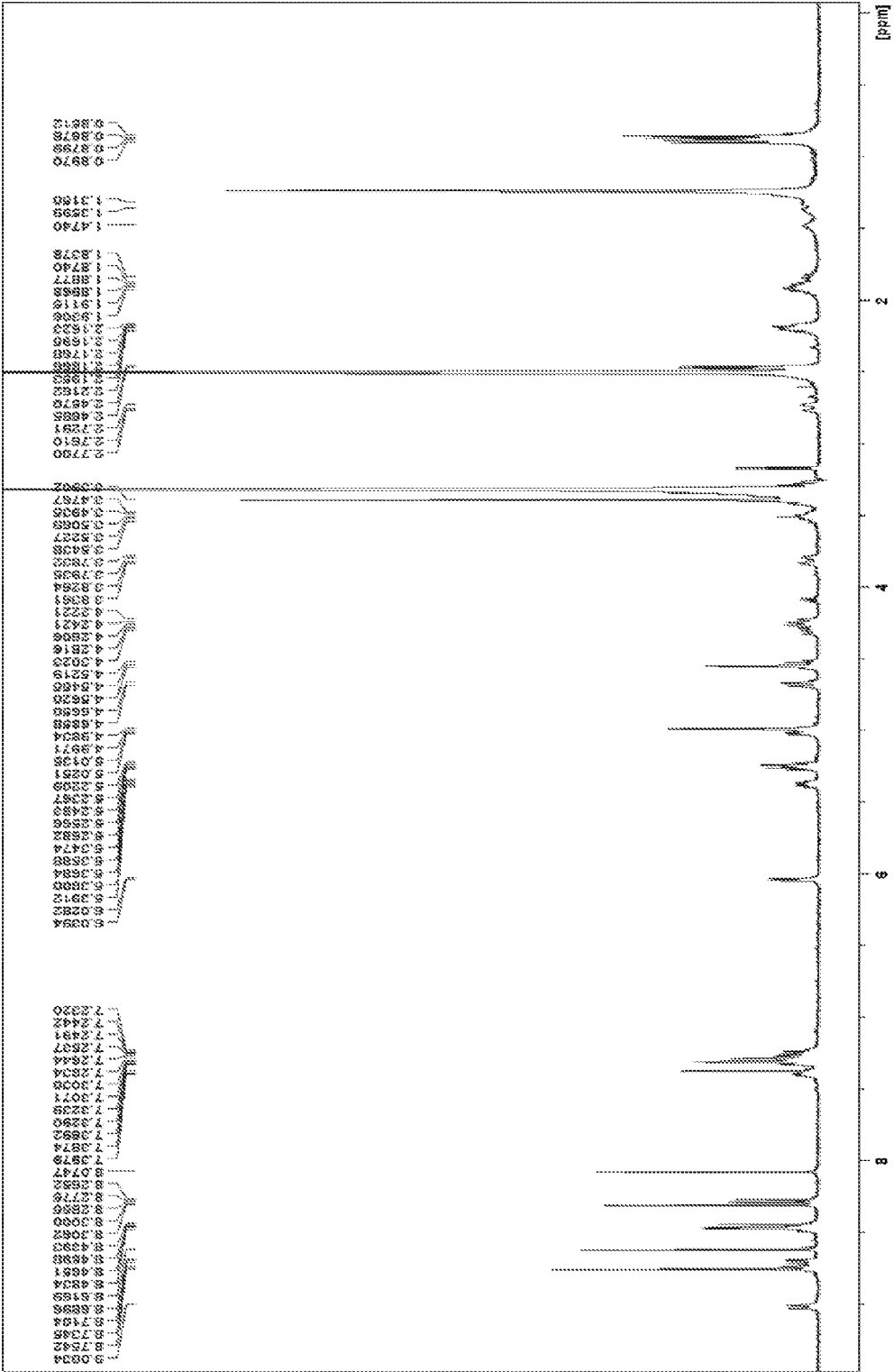
[FIG. 9C]



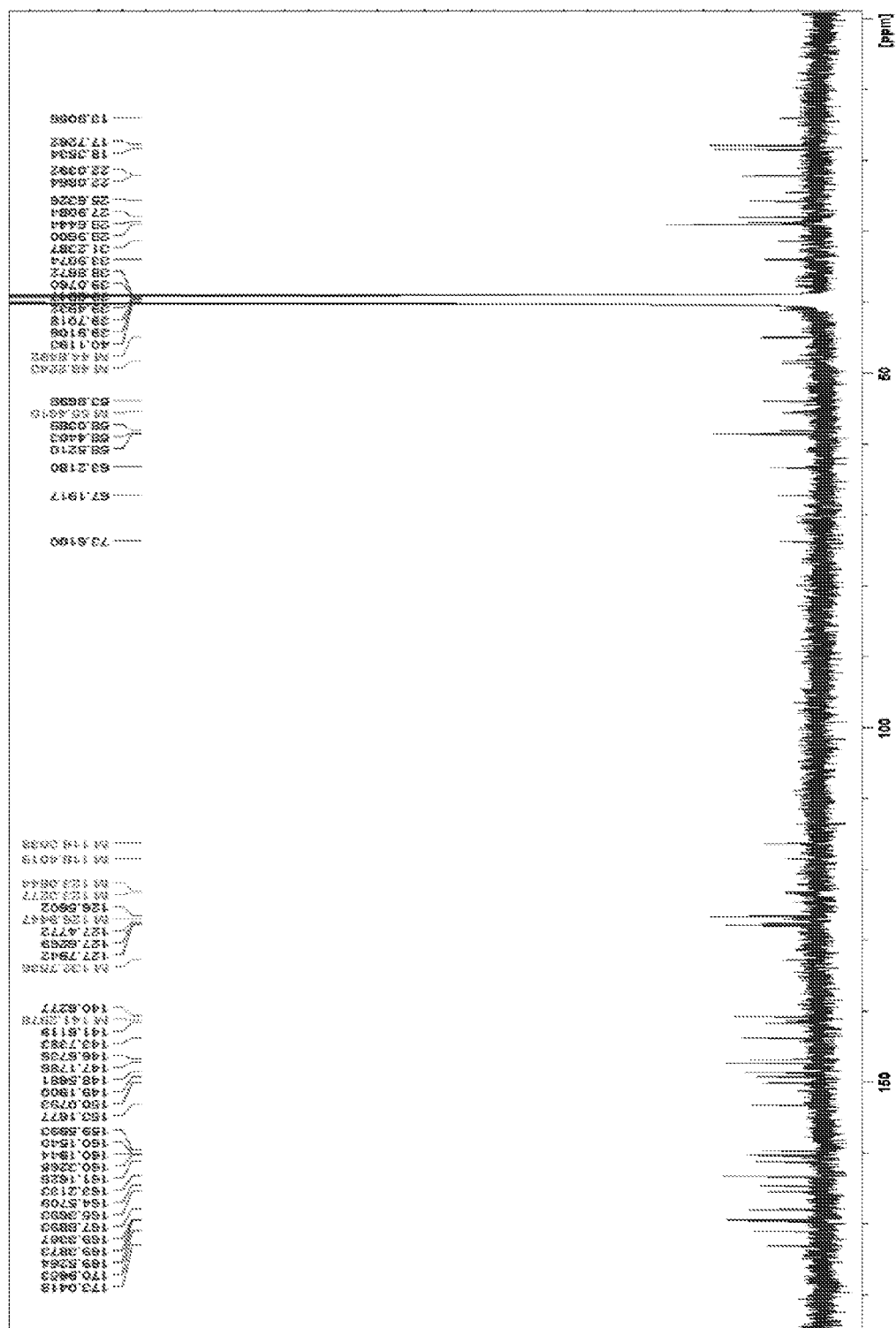
[FIG. 9D]



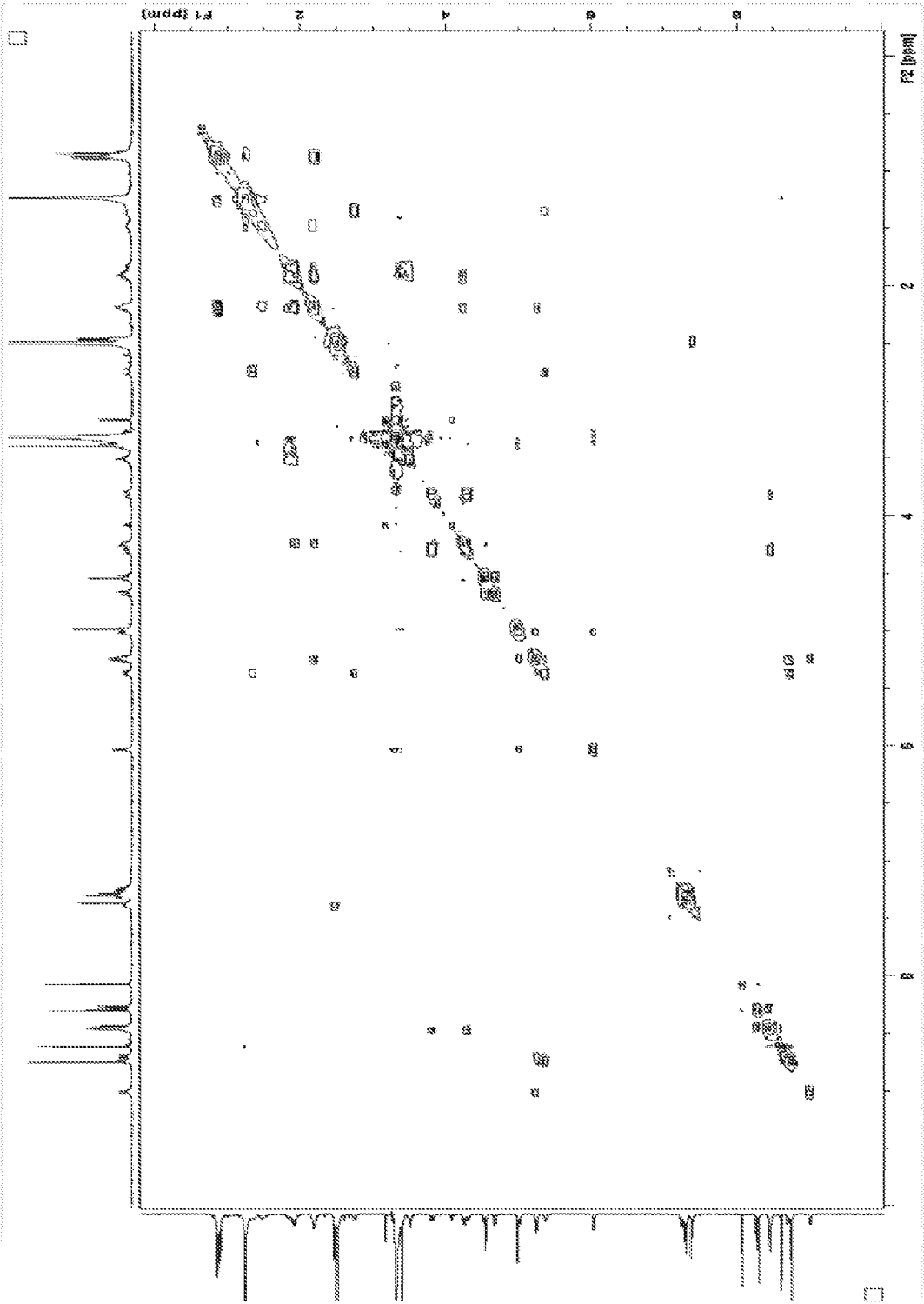
[FIG. 10A]



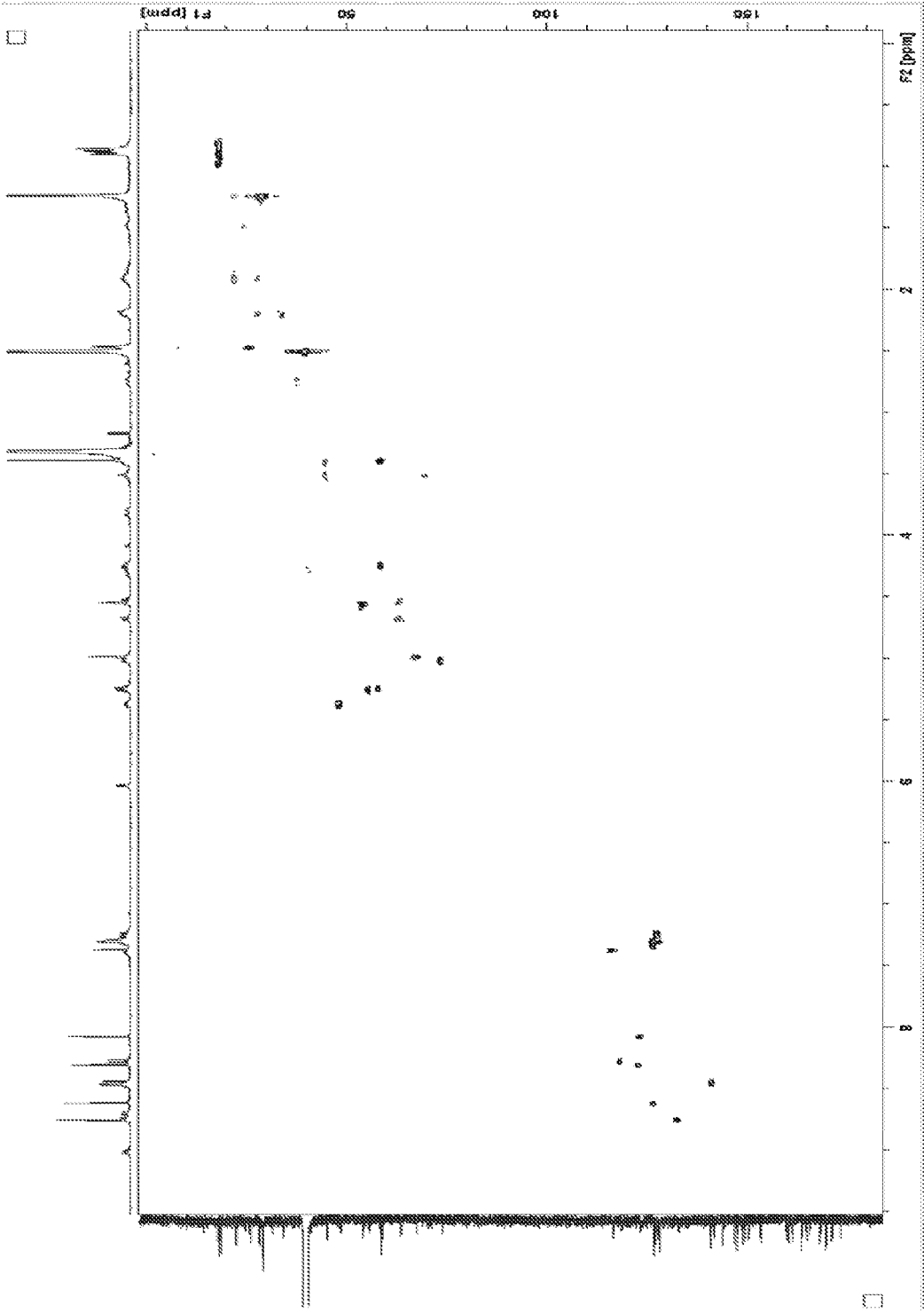
[FIG. 10B]



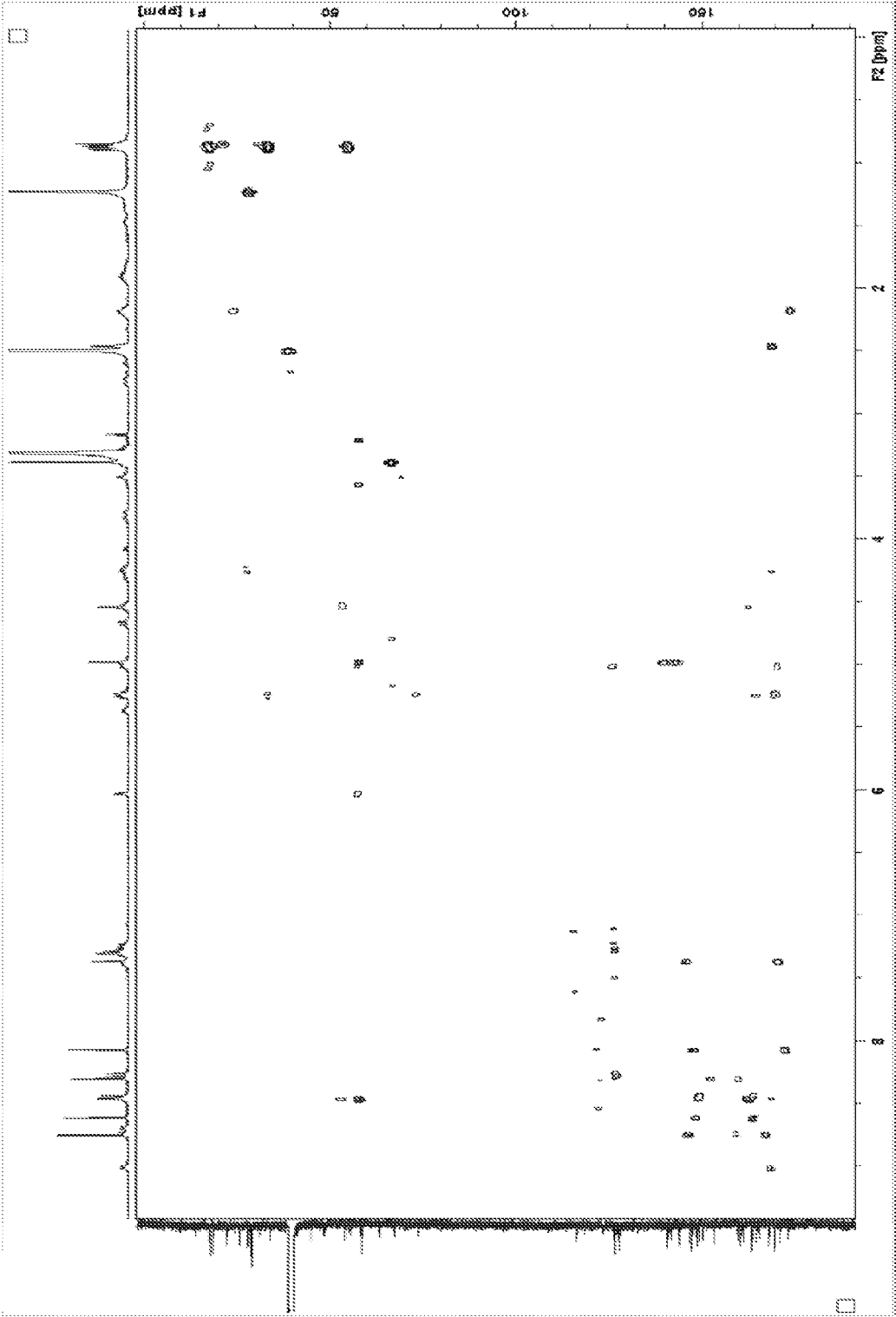
[FIG. 10C]



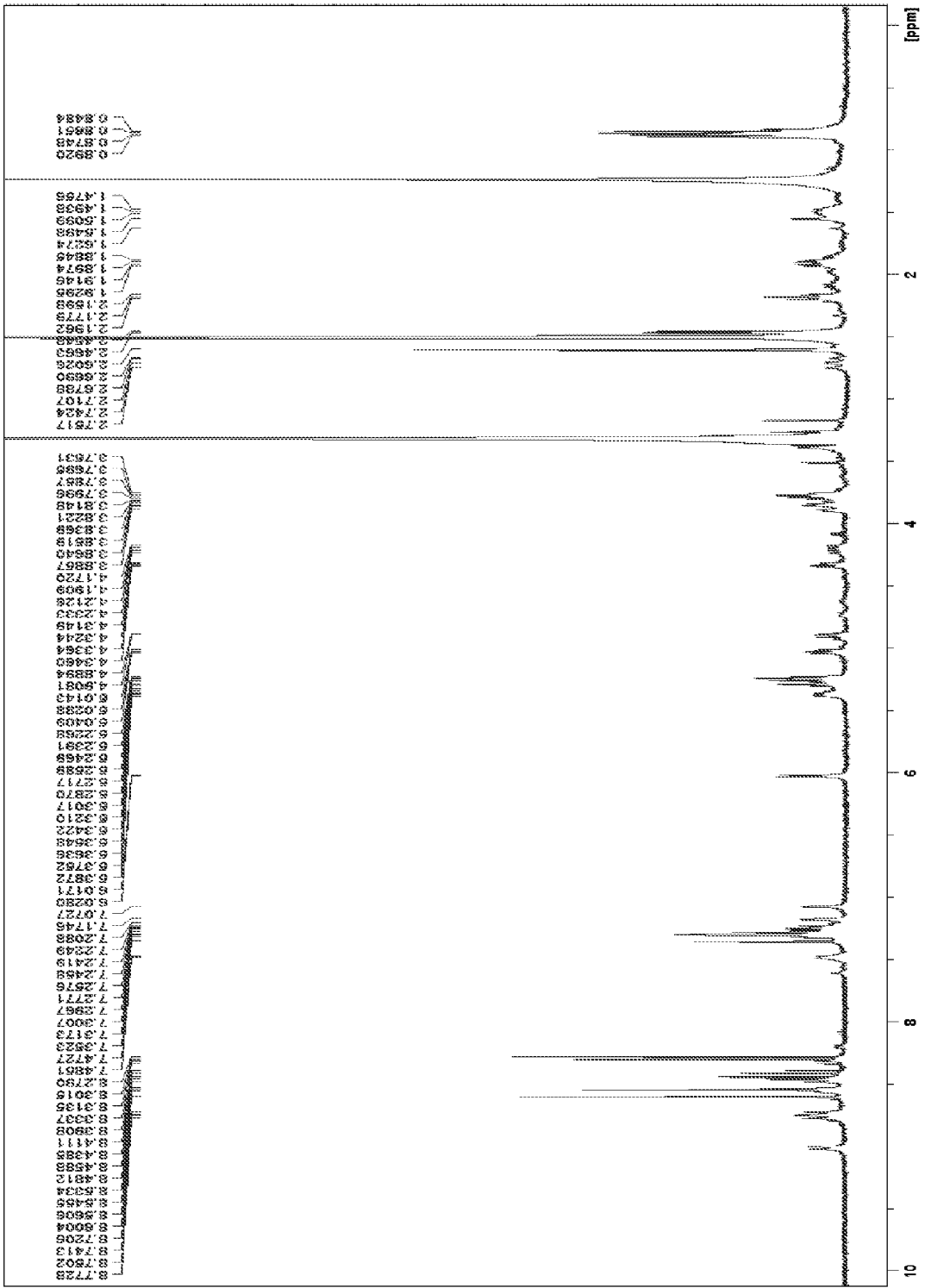
[FIG. 10D]



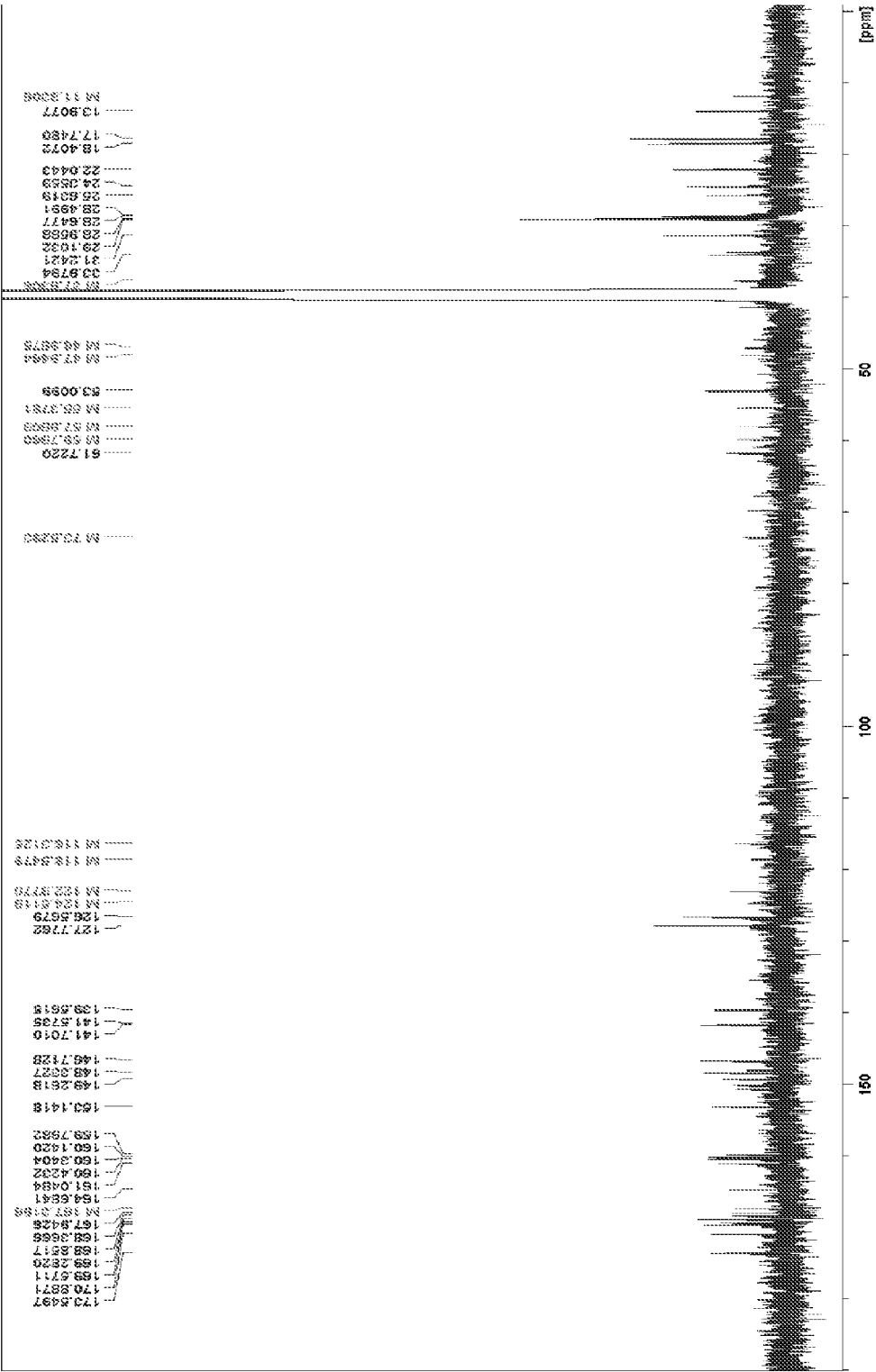
[FIG. 10E]



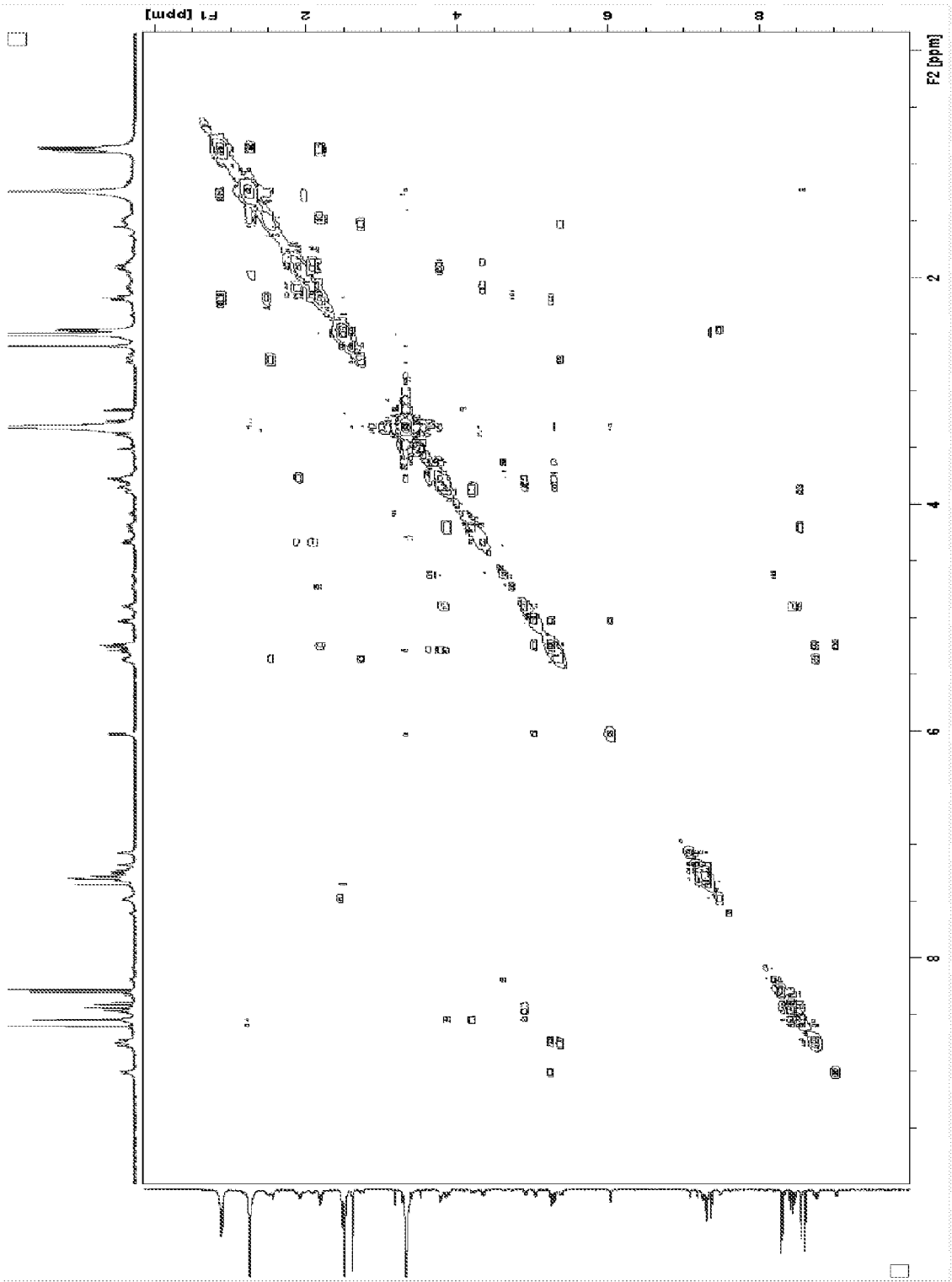
[FIG. 11A]



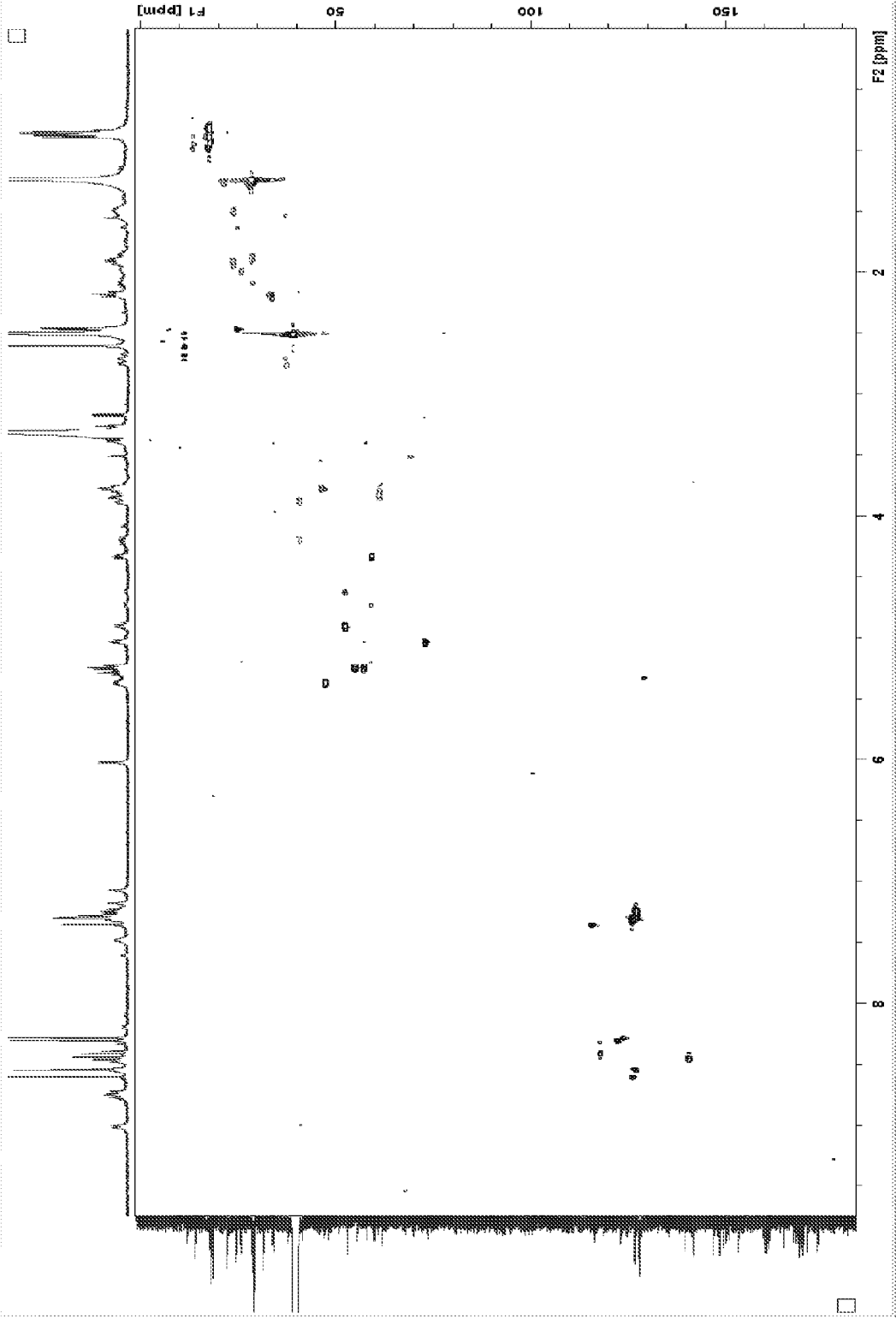
[FIG. 11B]



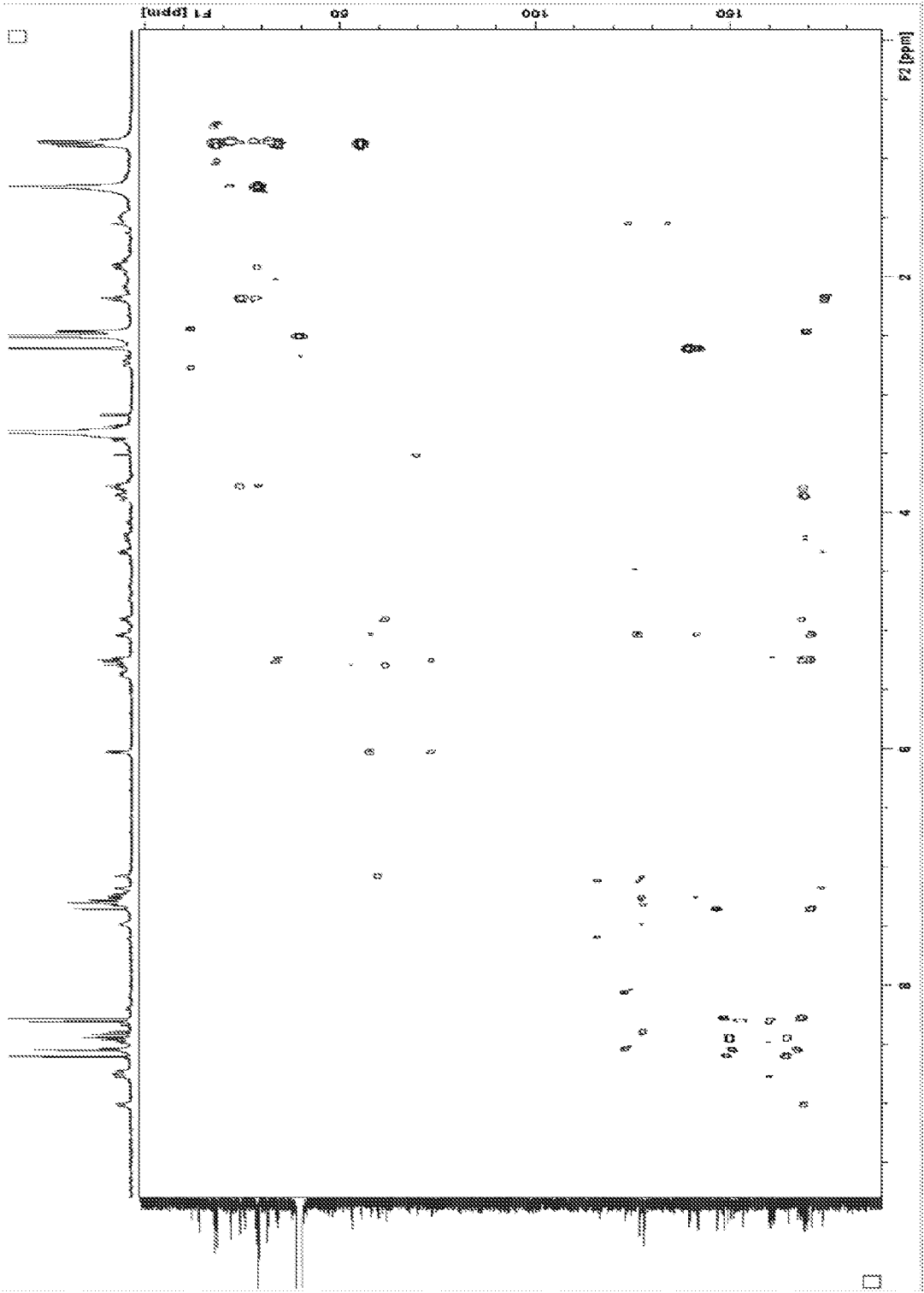
[FIG. 11C]



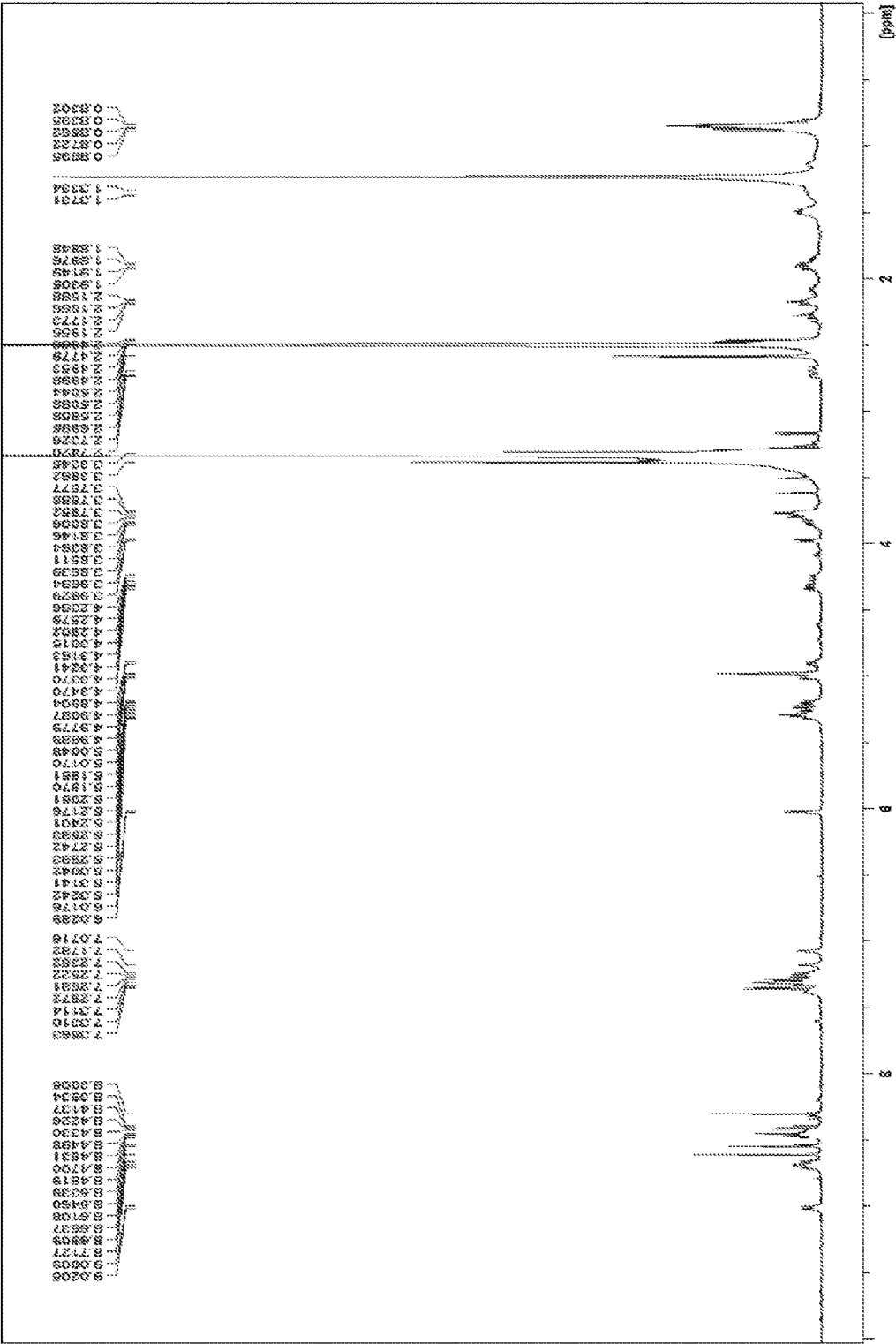
[FIG. 11D]



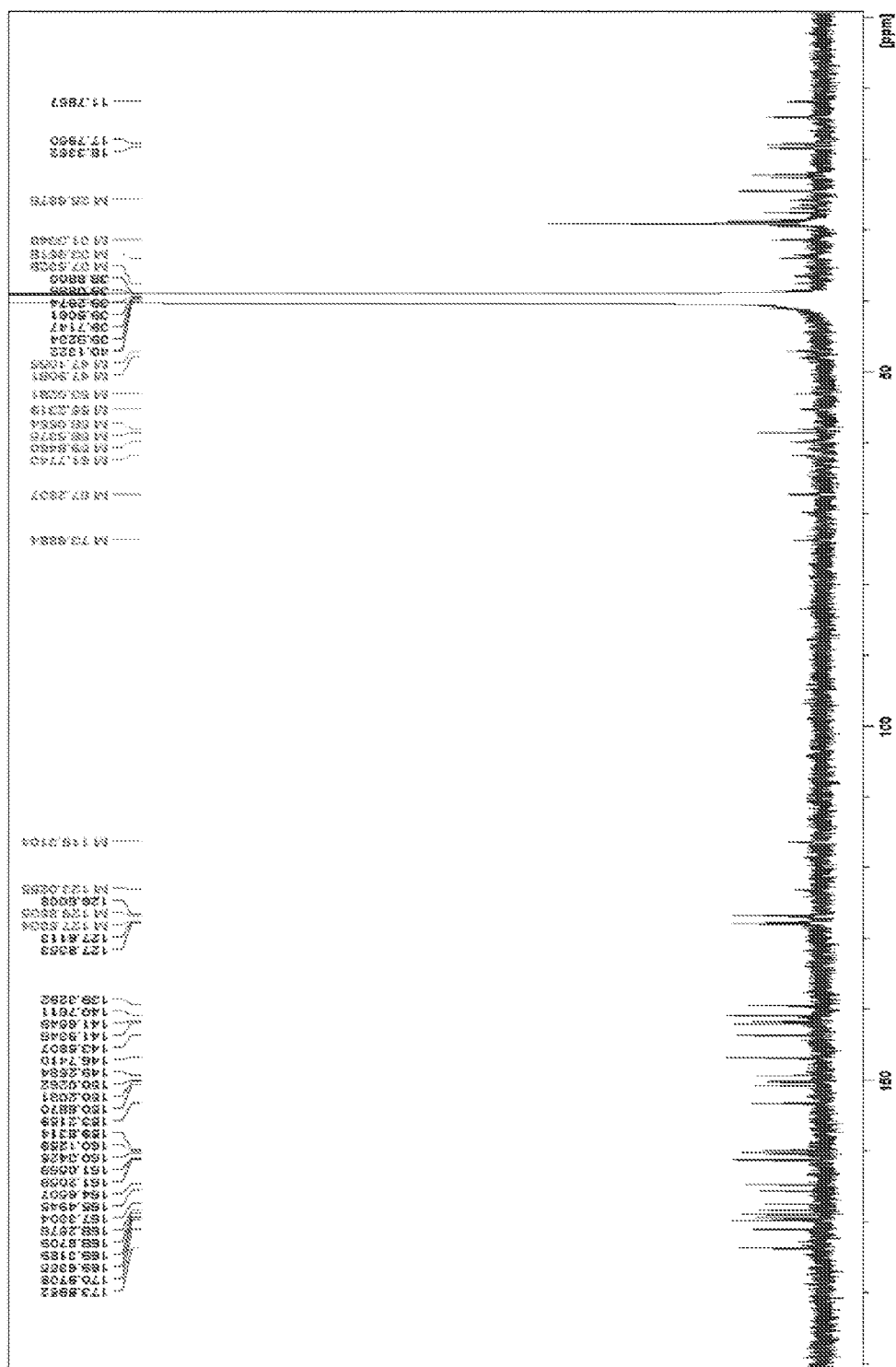
[FIG. 11E]



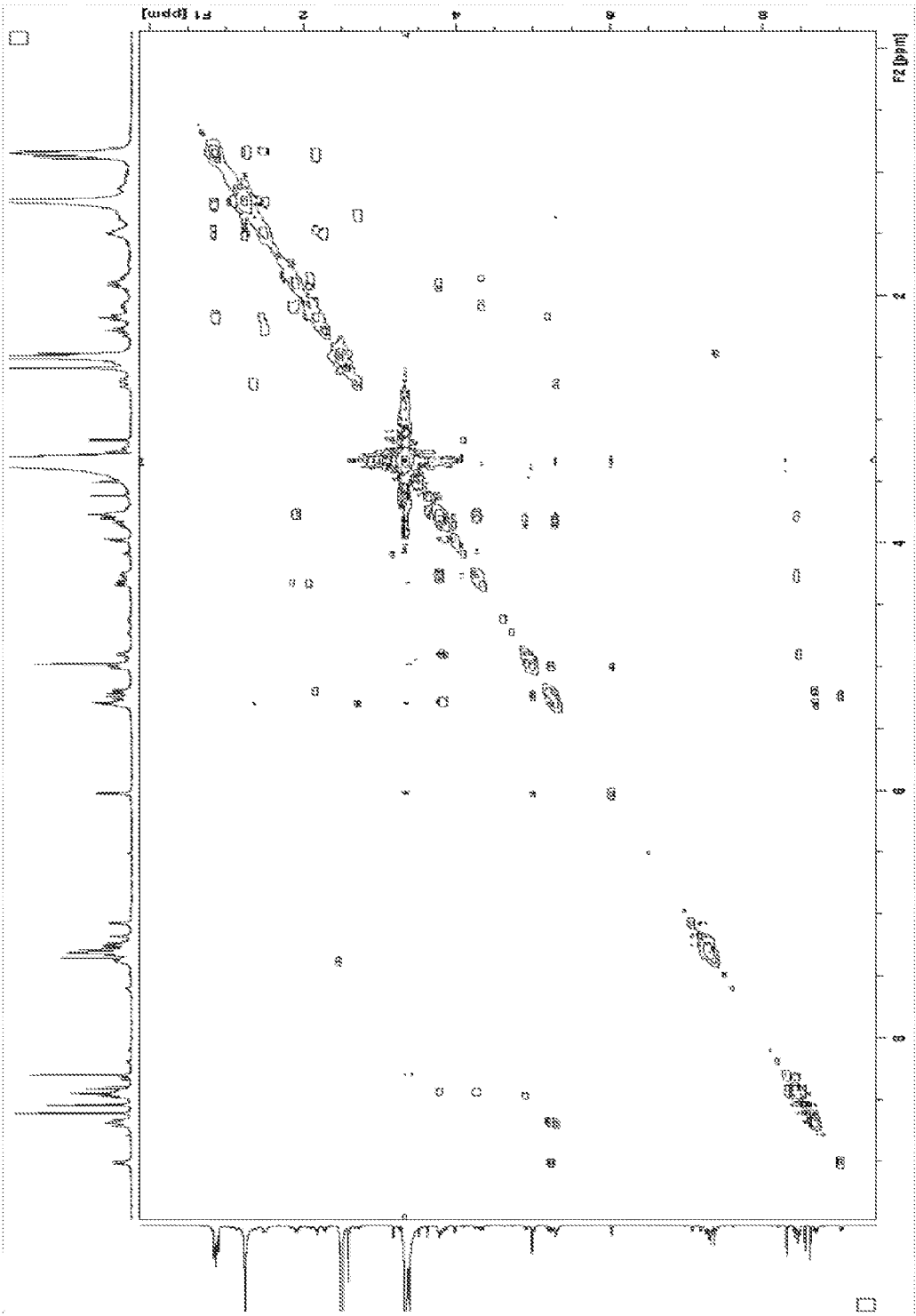
[FIG. 12A]



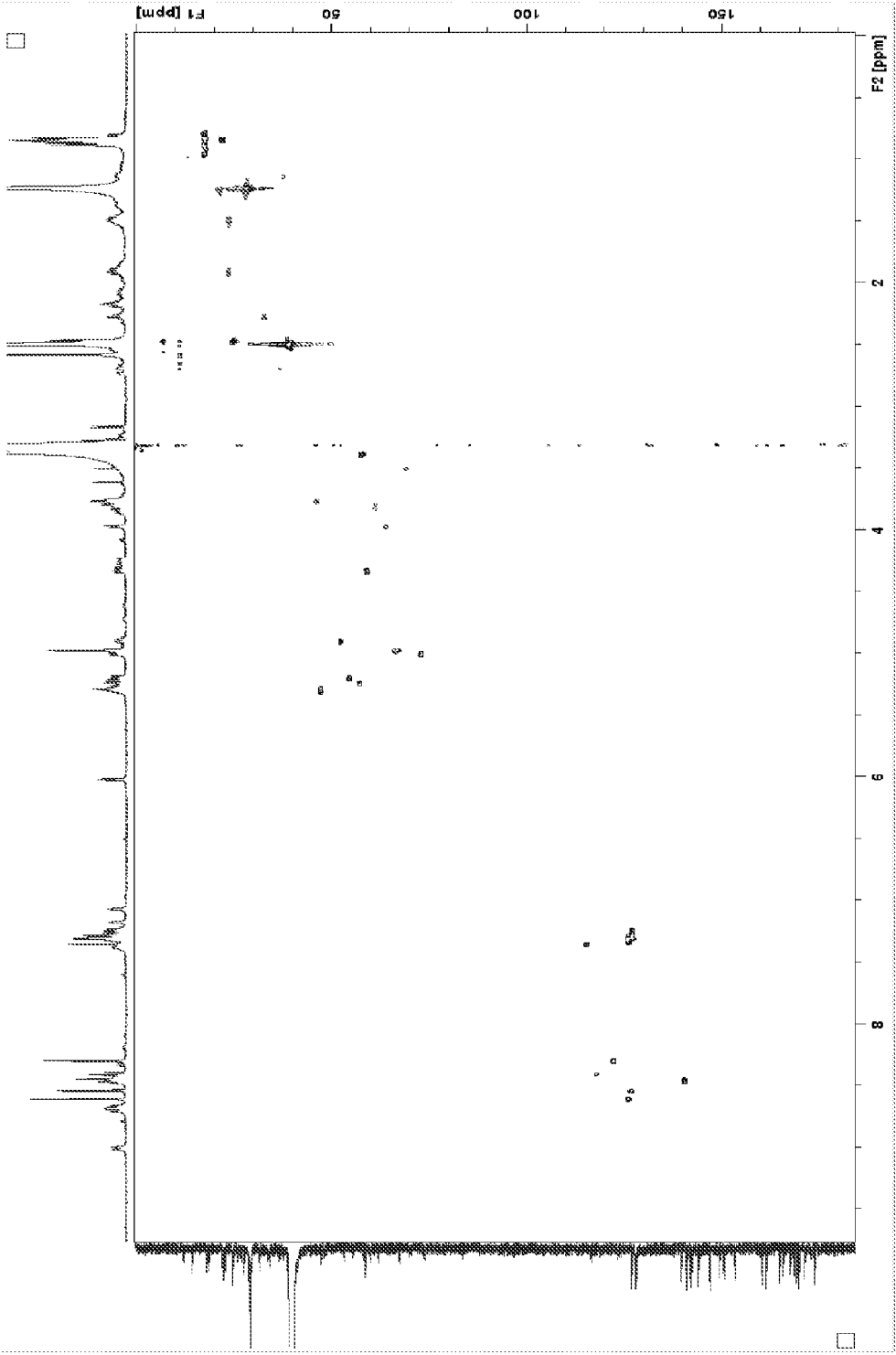
[FIG. 12B]



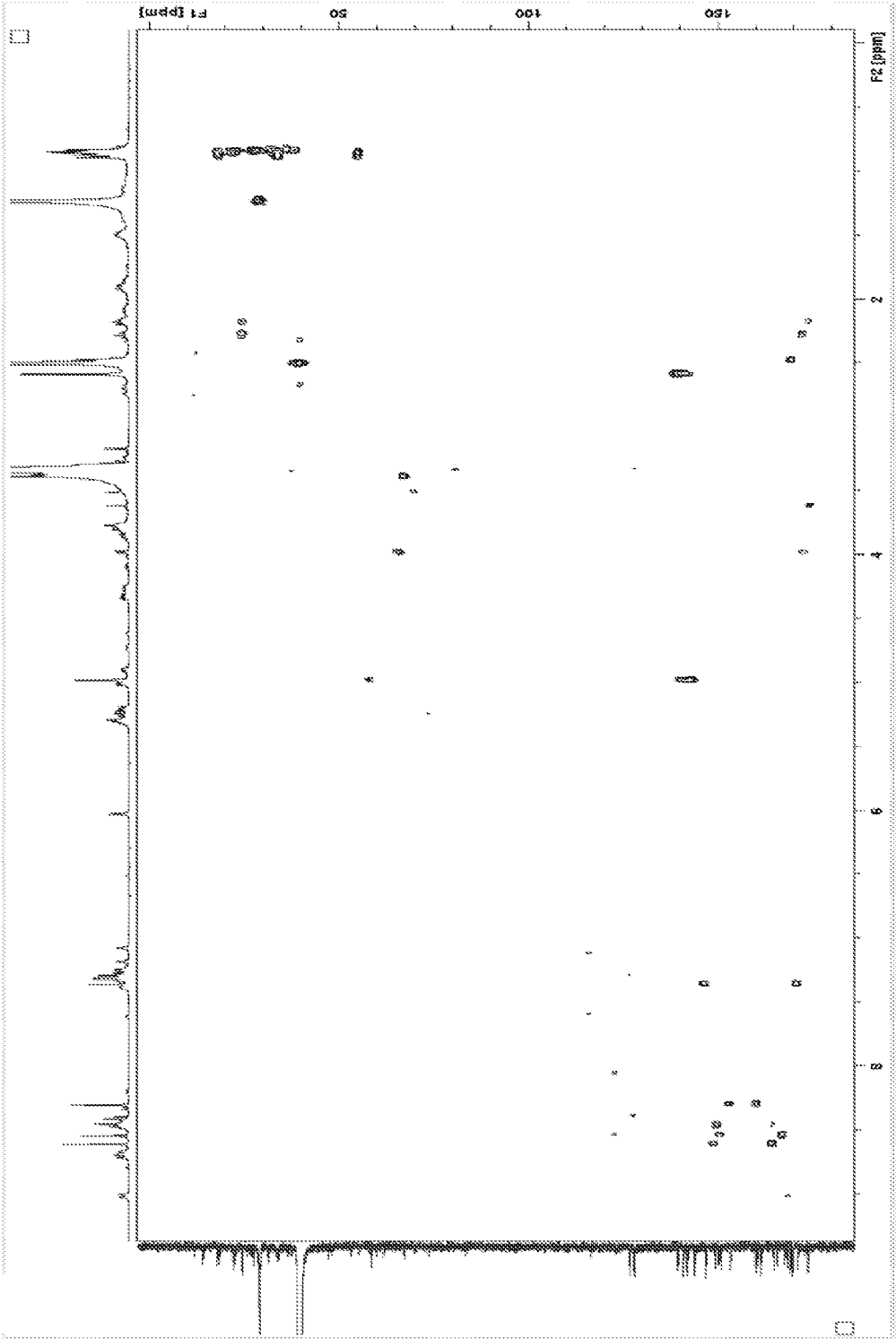
[FIG. 12C]



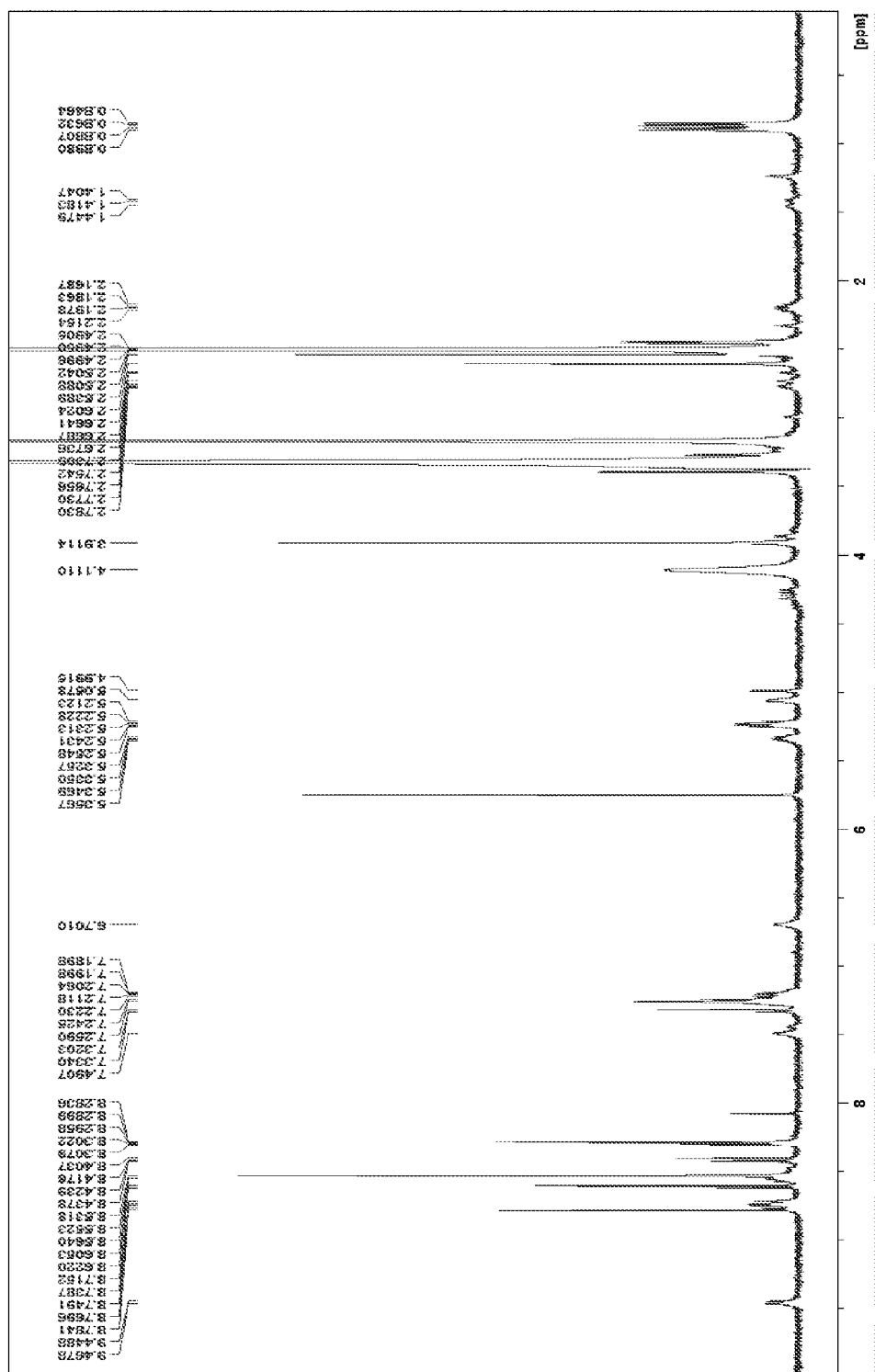
[FIG. 12D]



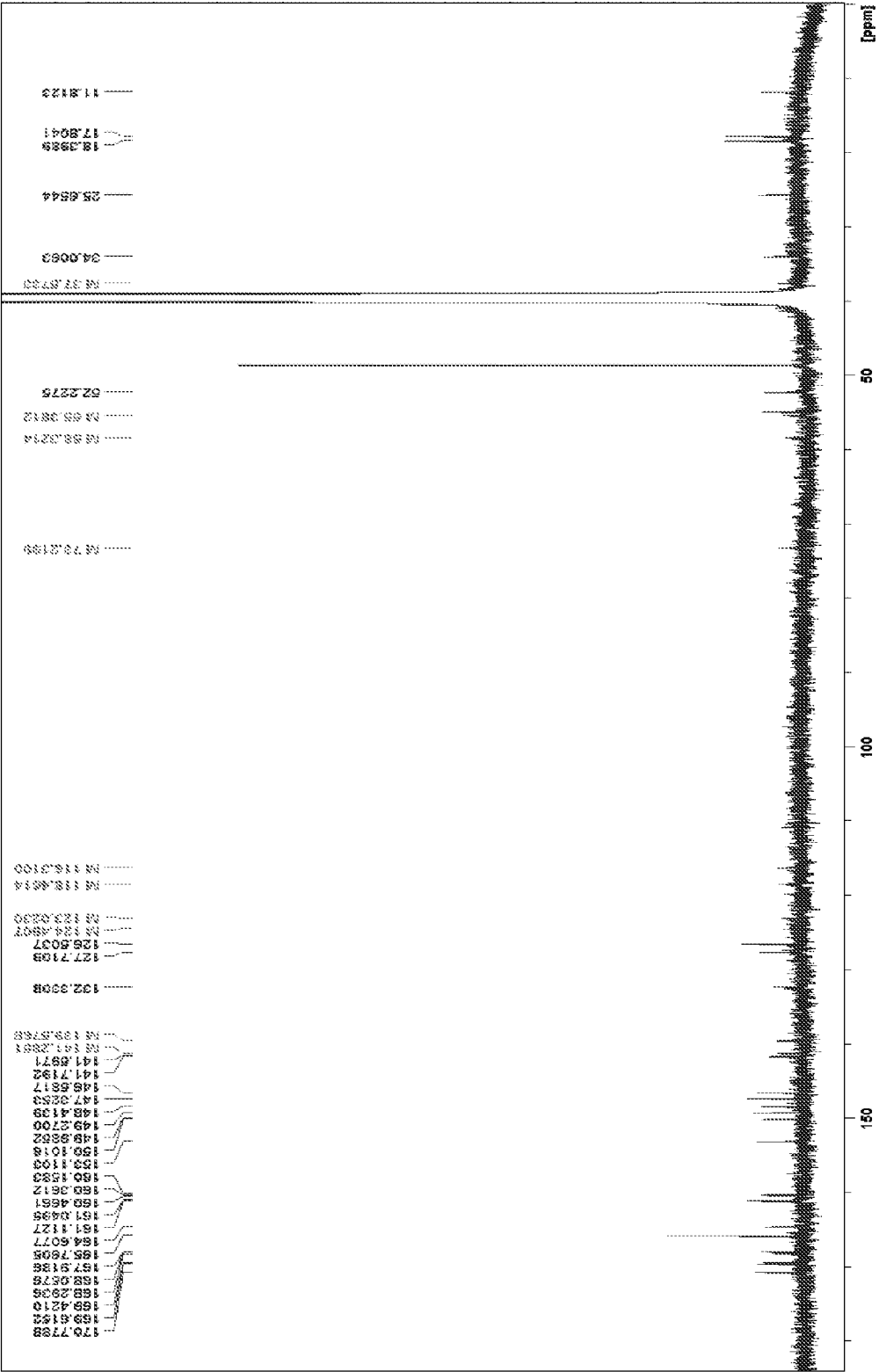
[FIG. 12E]



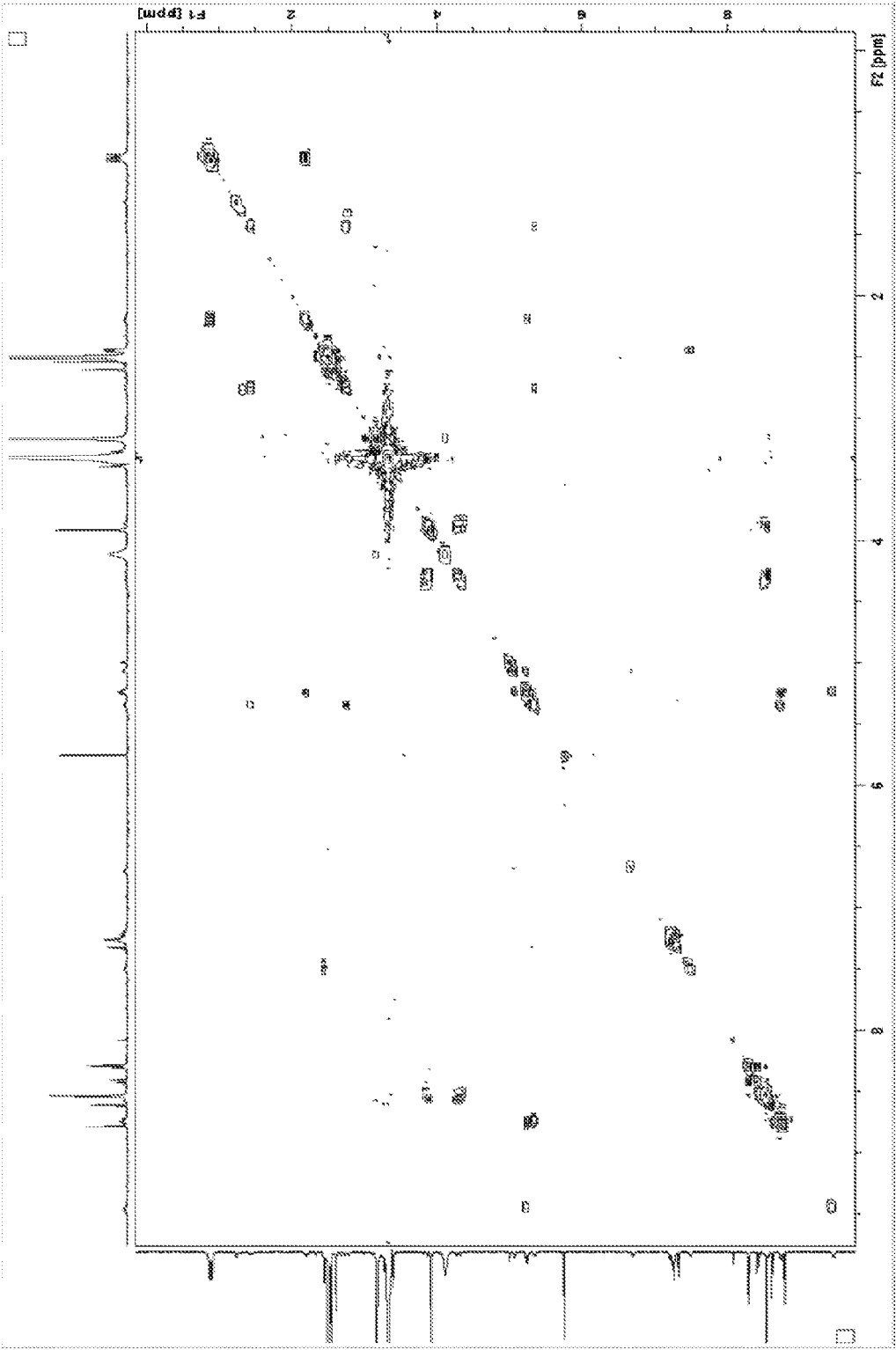
[FIG. 13A]



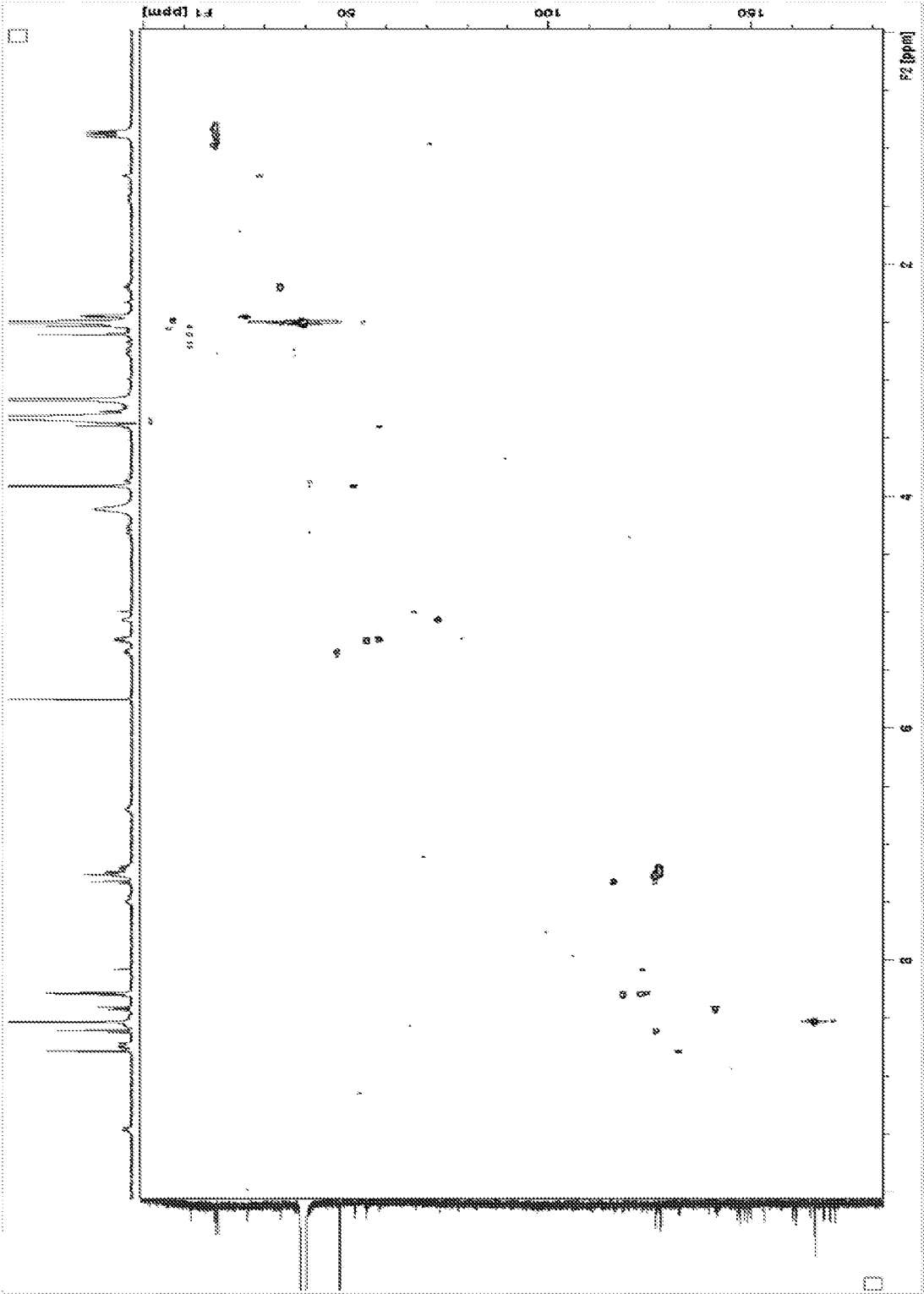
[FIG. 13B]



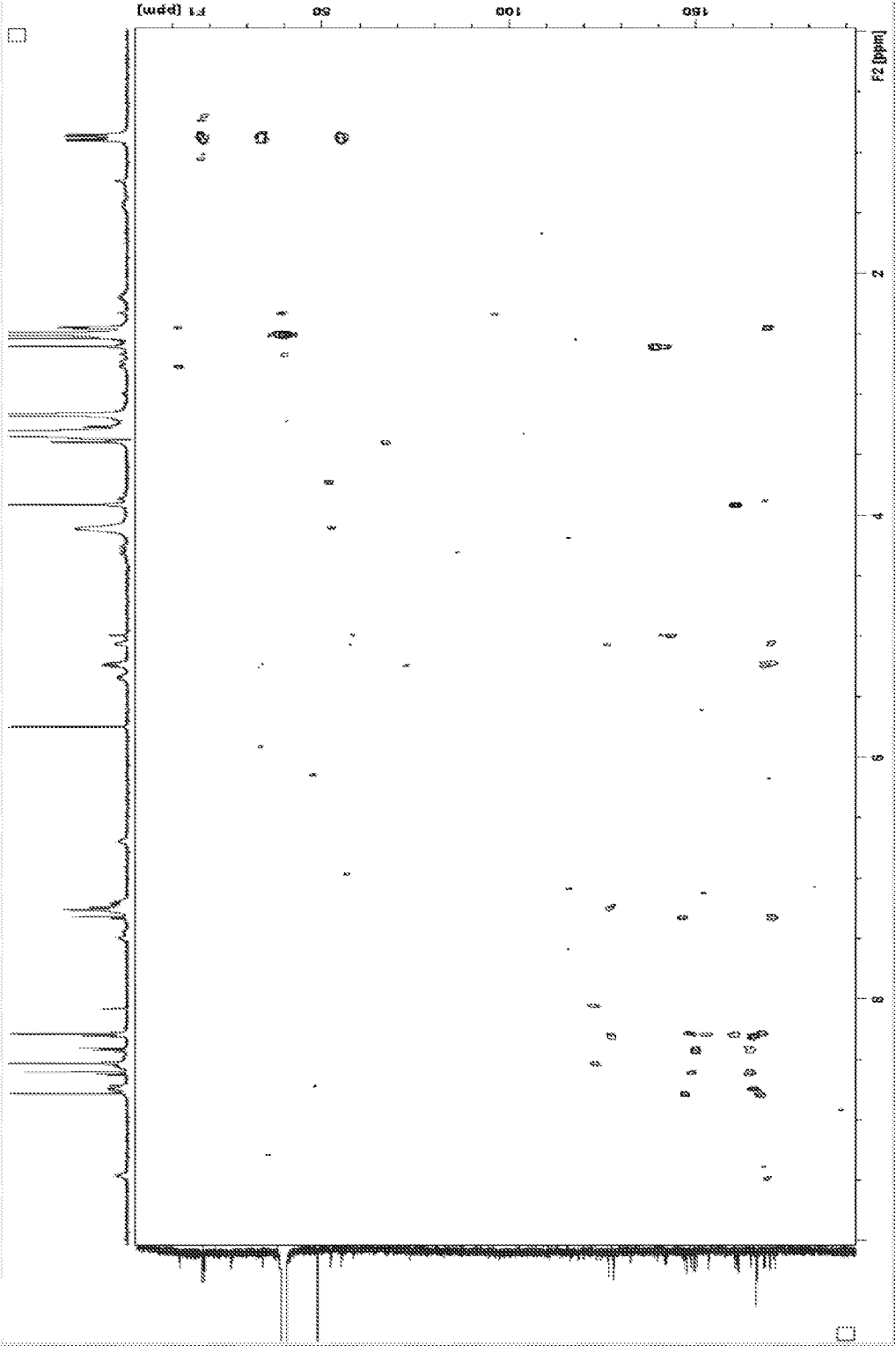
[FIG. 13C]



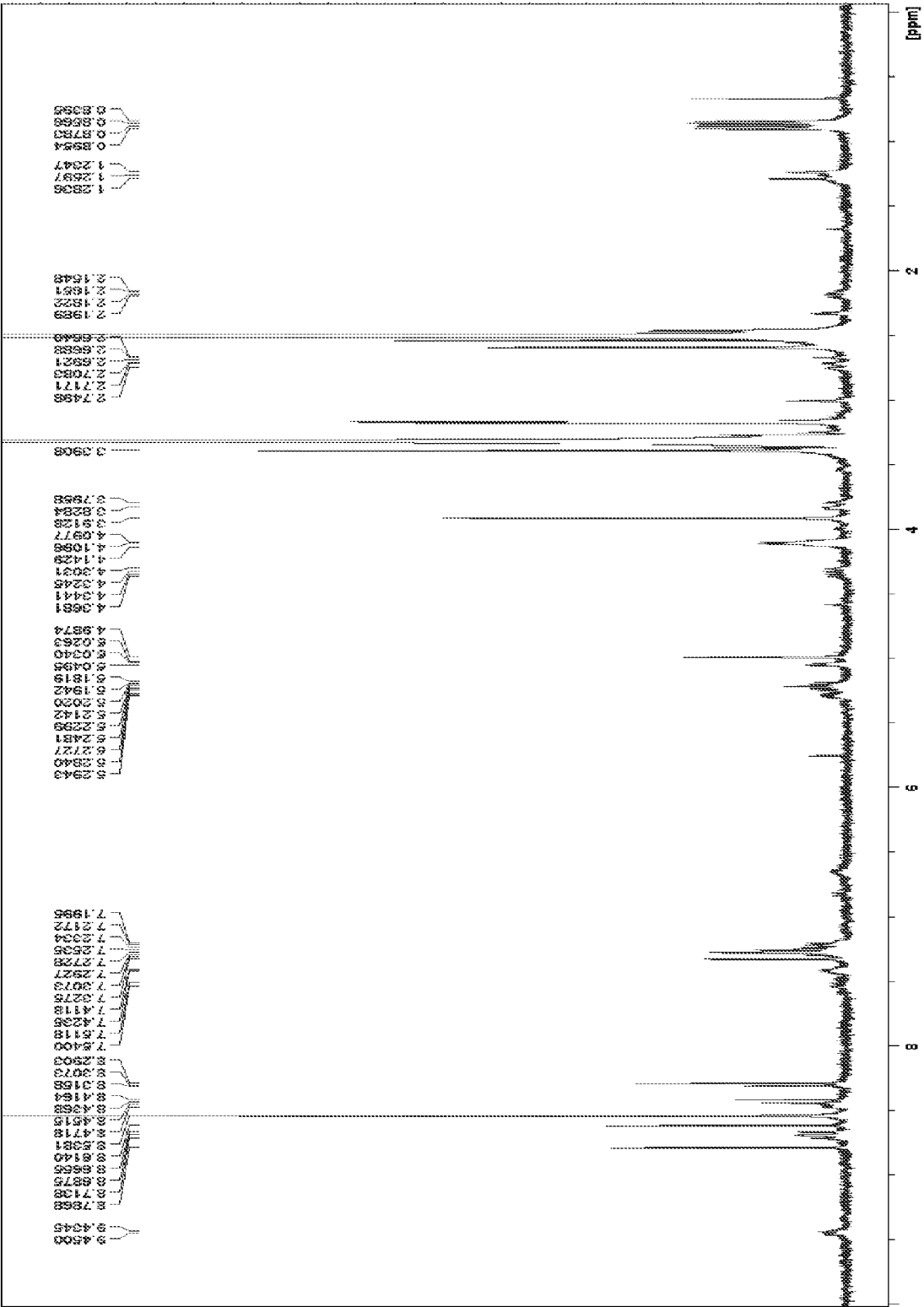
[FIG. 13D]



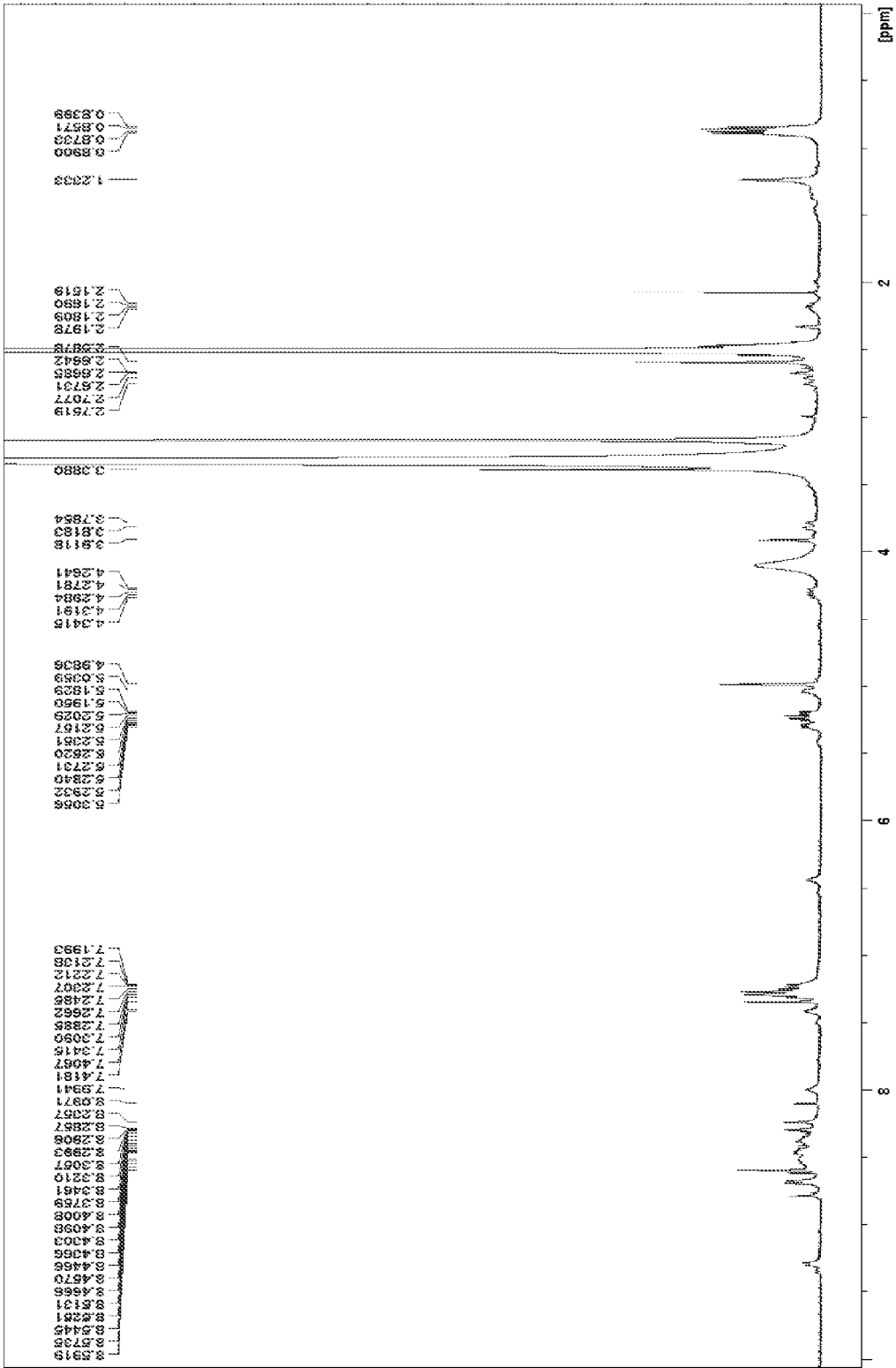
[FIG. 13E]



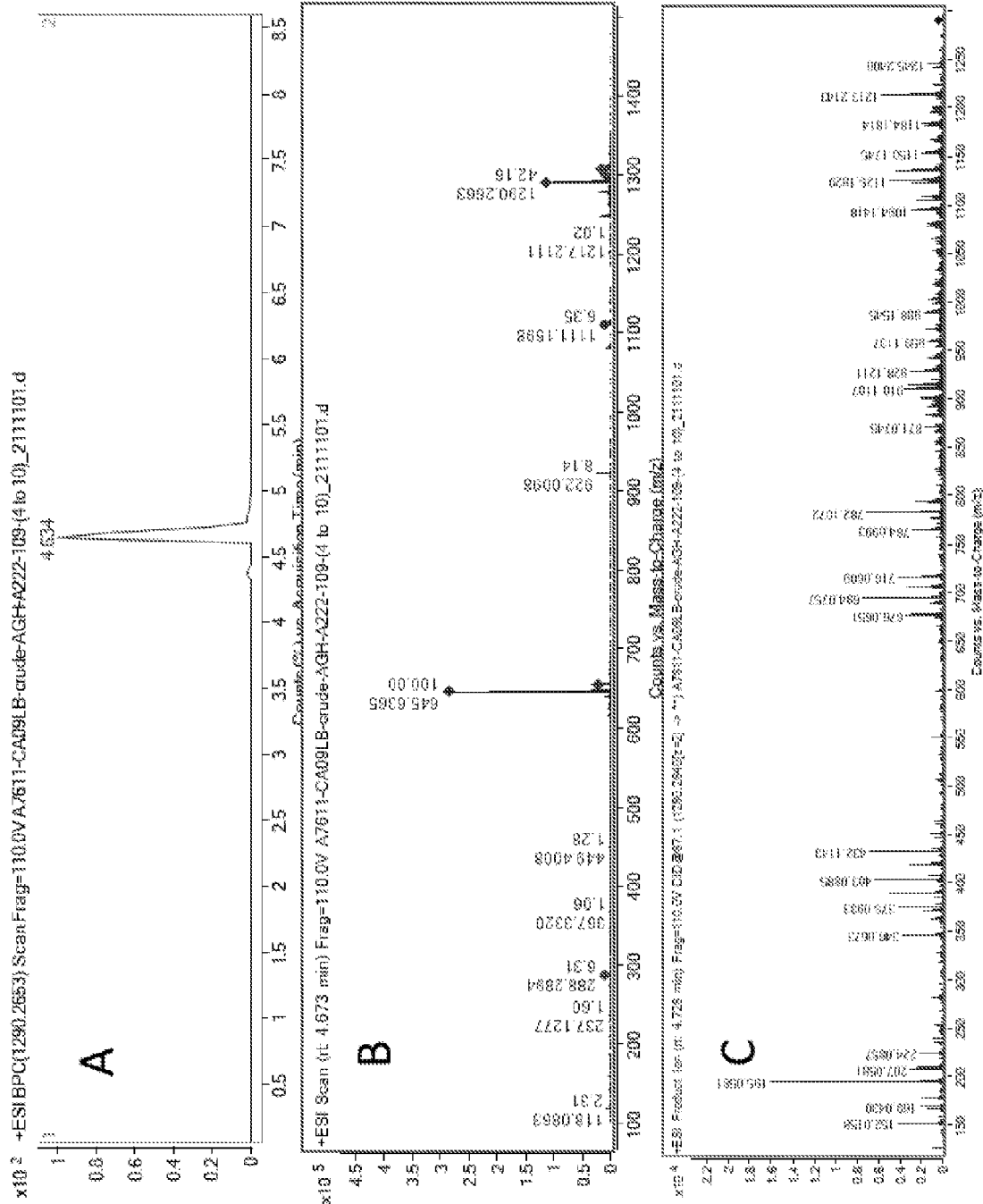
[FIG. 14]



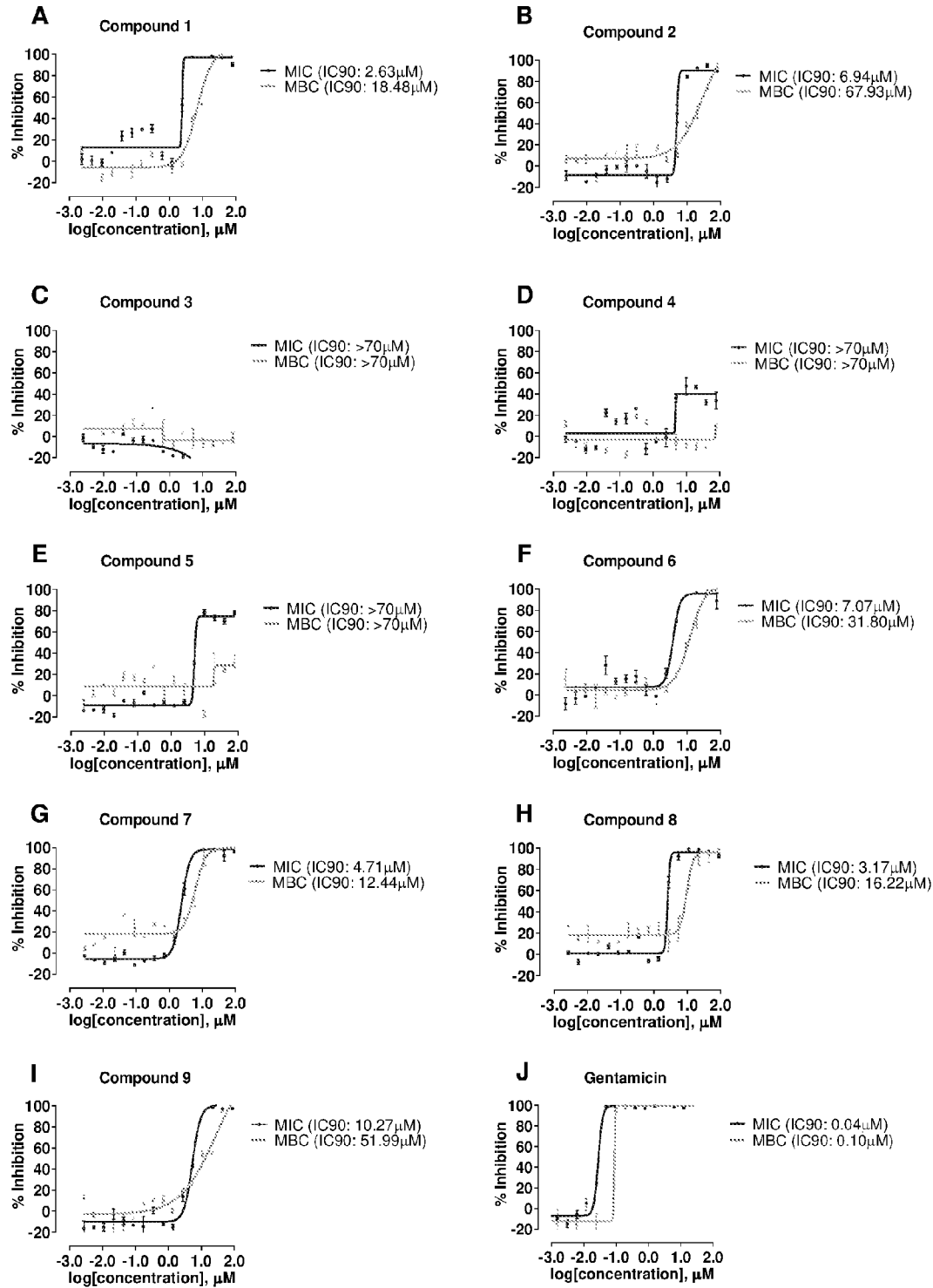
[FIG. 15]



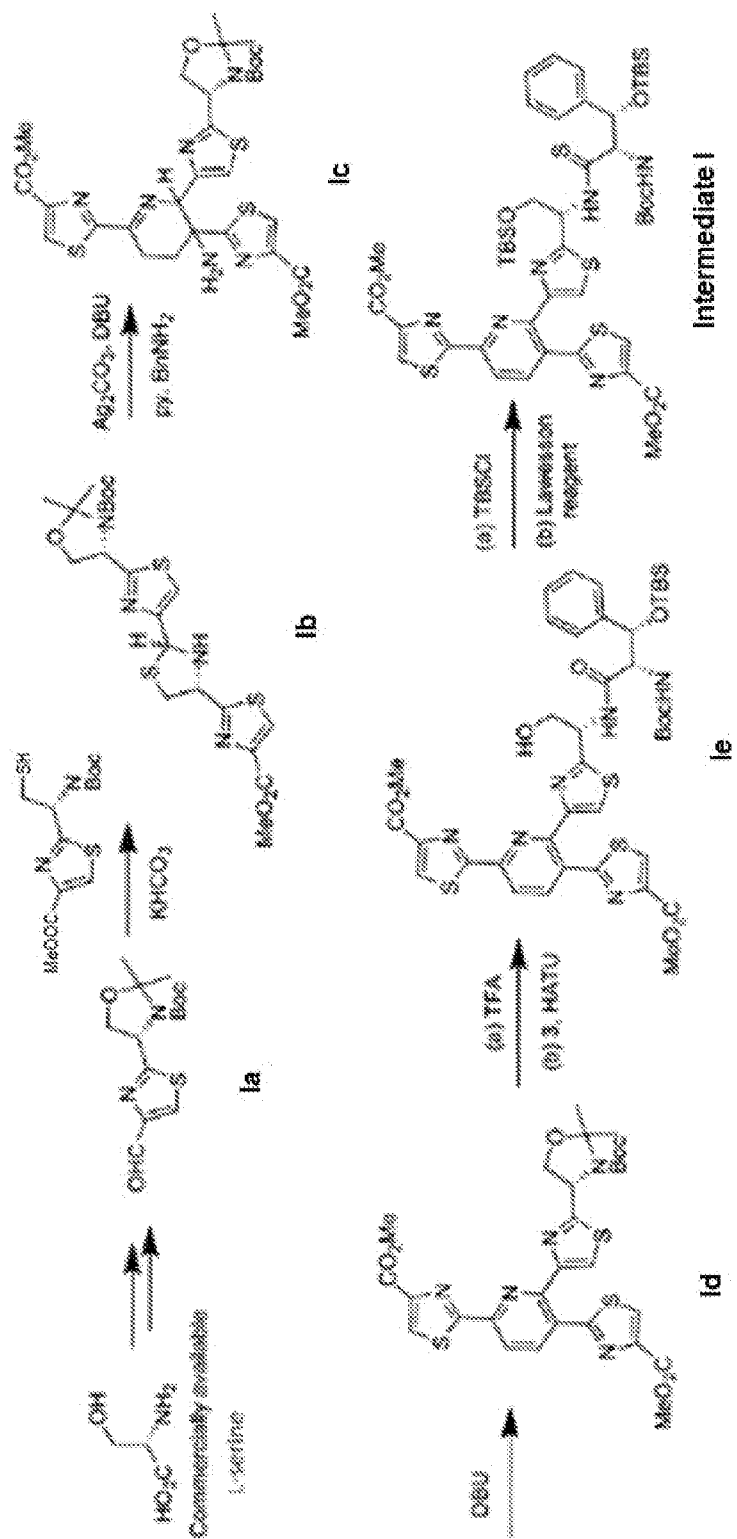
[FIG. 16]



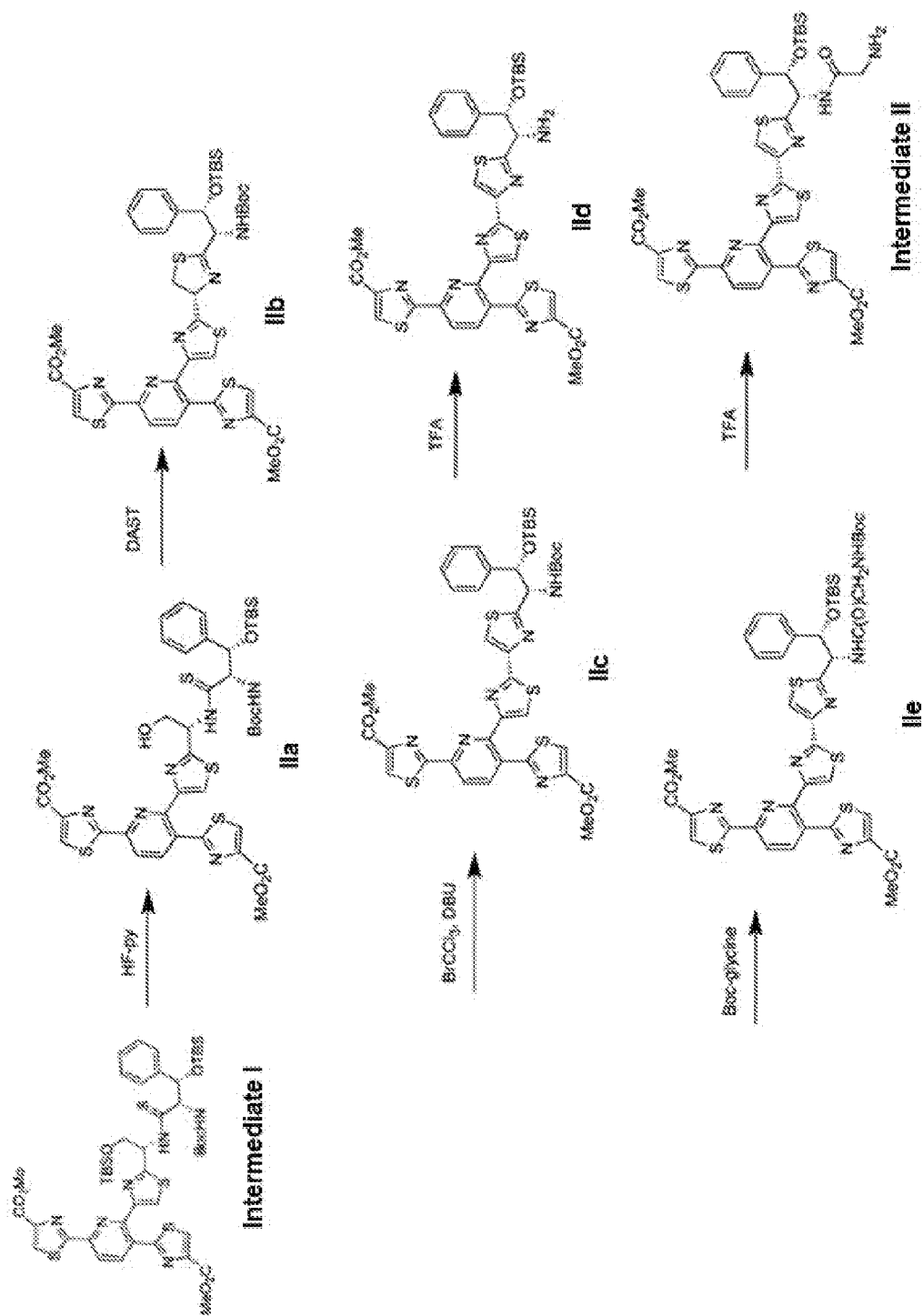
[FIG. 17]



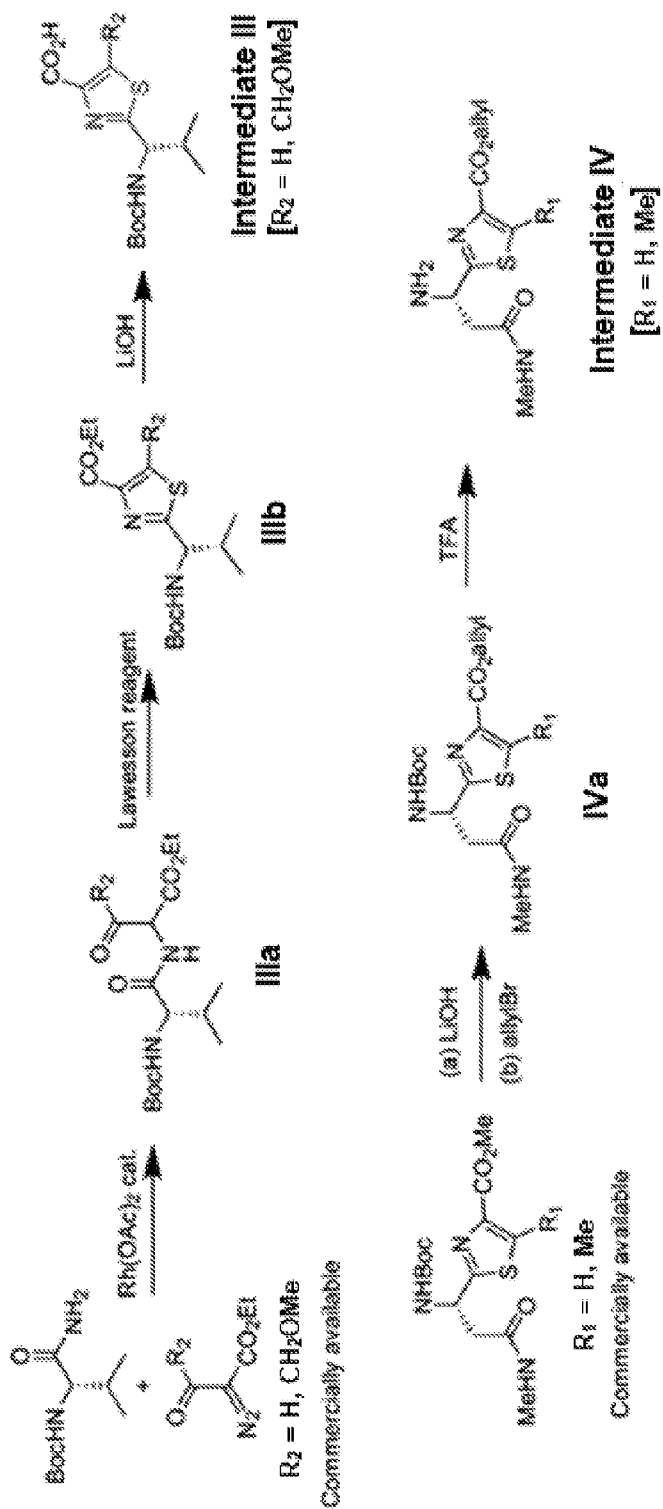
[FIG. 18A]



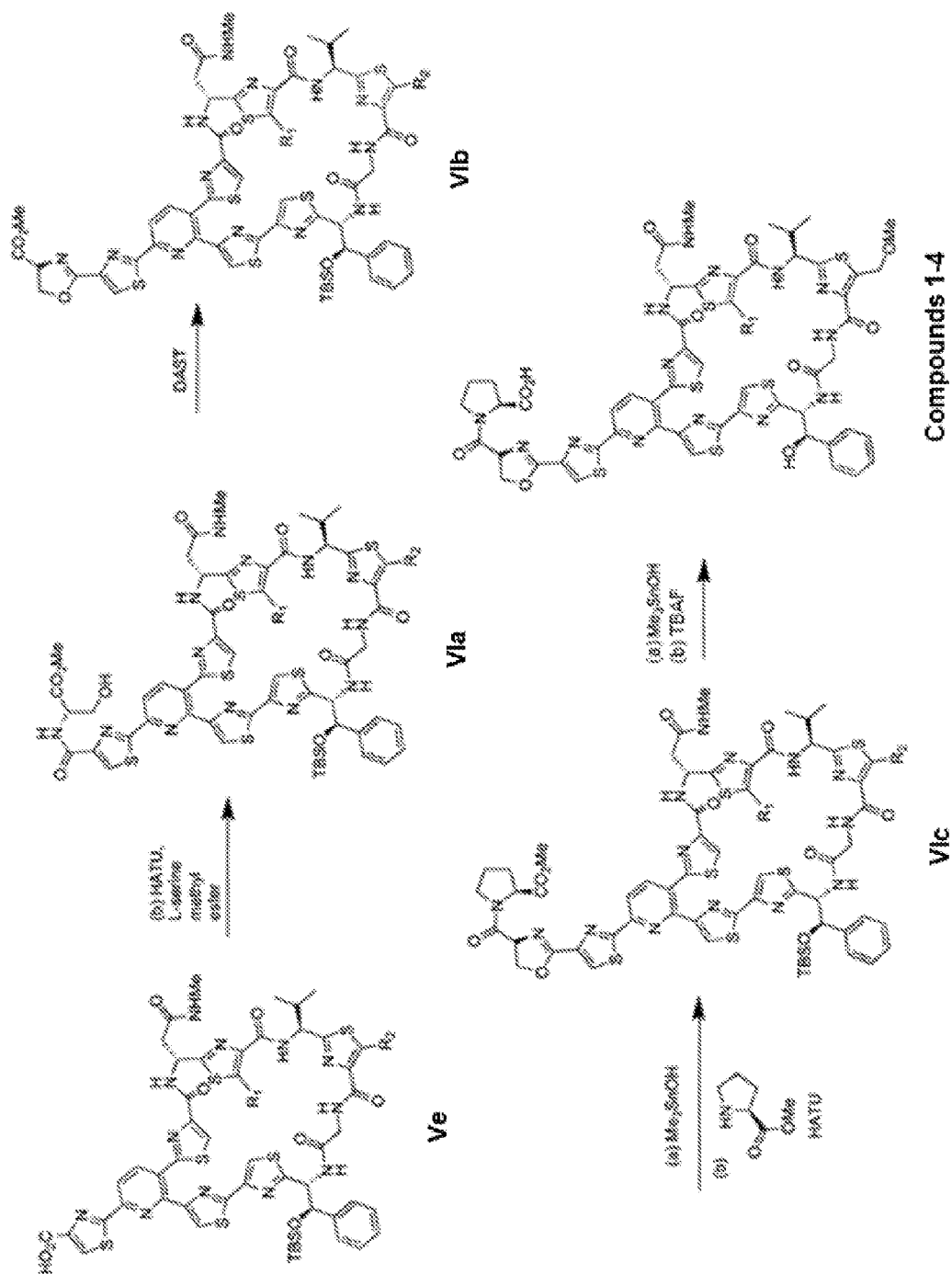
[FIG. 18B]



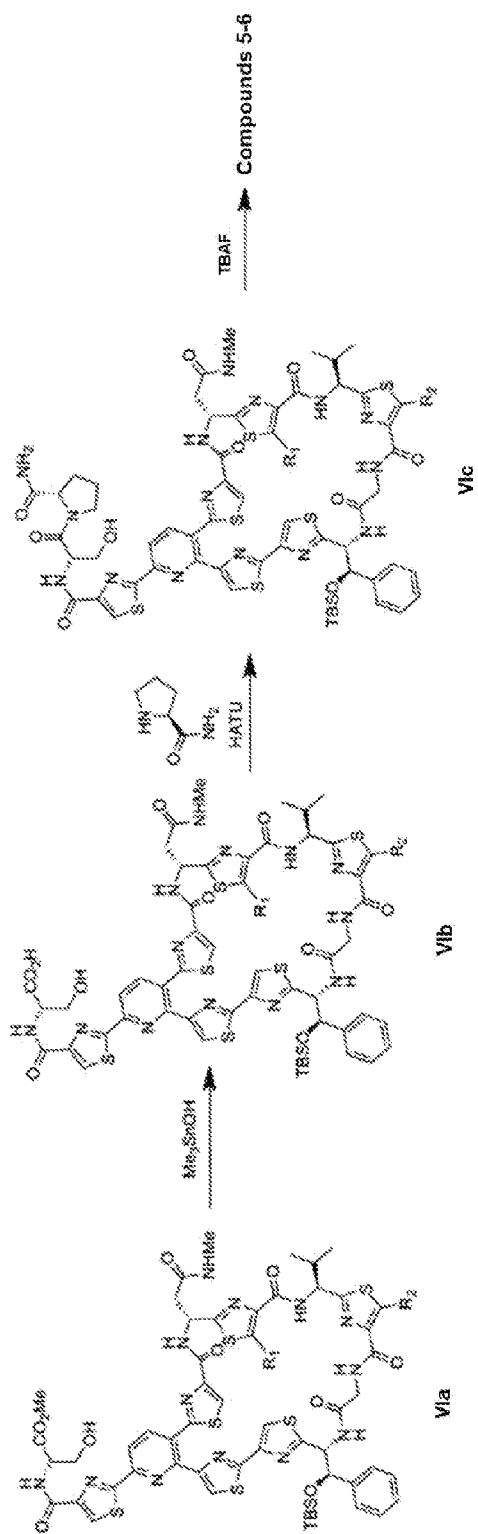
[FIG. 18C]



[FIG. 18E]



[FIG. 18F]



INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2023/050853

A. CLASSIFICATION OF SUBJECT MATTER

See Supplemental Box

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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)

CAS Registry, CAlplus: search based on the formula (I)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CHING, K.C. ET AL., Antibacterial Thiopeptide GE2270-Congeners from <i>Nonomuraea jiangxiensis</i> . <i>Molecules</i> , 23 December 2022, Vol. 28, No. 1, pages 101 [Retrieved on 2024-03-18] <DOI: 10.3390/MOLECULES28010101> Whole document, especially Fig. 4 and Table 3	1-32
X	DATABASE REGISTRY, Compounds having the structure of formula (I). 30 August 2009	1, 3, 5-10, 14-15
X	DATABASE REGISTRY, Compounds having the structure of formula (I). 24 February 2008	1, 3, 5-10, 14-15

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

*Special categories of cited documents:

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"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

18/03/2024

(day/month/year)

Date of mailing of the international search report

01/04/2024

(day/month/year)

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2023/050853

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 2014/167371 A1 (NAICONS S C A R L) 16 October 2014 Pages 3-4; Table 4; compound 1c, 1a, 2-7, 2b, 5a; Examples 3-11	1-24 25-32
X A	WO 02066046 A1 (KIM JONGHEE) 29 August 2002 Formula 3-5, 7 and 13-14; Tables 1-2	1-24 25-32
X A	WO 97/30078 A1 (BIOSEARCH ITALIA SPA ET AL.) 21 August 1997 page 6 lines 24-27; formula III, IIIa; page 31 line 10; formula IXb; compounds 1-4 on page 42; page 45 para 3; Table 1	1-24 25-32
X A	NICOLAOU, K. ET AL., Total Synthesis of Thiopeptide Antibiotics GE2270A, GE2270T, and GE2270C1. <i>Chemistry – An Asian Journal</i> , 29 January 2008, Vol. 3, No. 2, pages 413-429 [Retrieved on 2024-03-18] <DOI: 10.1002/ASIA.200700361> Introduction, Scheme 2	1-24 25-32
A	LI, X. ET AL., <i>Nonomurea jiangxiensis</i> sp. nov., isolated from acidic soil. <i>International Journal of Systematic and Evolutionary Microbiology</i> , 1 June 2012, Vol. 62, No. Pt_6, pages 1409-1413 [Retrieved on 2024-03-18] <DOI: 10.1099/IJS.0.034520-0> Whole document	--

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2023/050853

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014/167371 A1	16/10/2014	NONE	
WO 02066046 A1	29/08/2002	NONE	
WO 97/30078 A1	21/08/1997	HU P9901007 A2 IL 125107 A AU 1768797 A KR 19990082541 A US 6008225 A DK 0880541 T3 EP 0880541 A1 AT E231163 T1 DE 69718442 T2 JP 2000505438 A ES 2186866 T3 CA 2242067 A1	28/07/1999 01/12/2002 02/09/1997 25/11/1999 28/12/1999 05/05/2003 02/12/1998 15/02/2003 30/04/2003 09/05/2000 16/05/2003 21/08/1997

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2023/050853

Supplemental Box

(Classification of Subject Matter)

Int. Cl.

C07K 5/02 (2006.01)

A61K 38/07 (2006.01)

A61P 31/04 (2006.01)

A61P 35/00 (2006.01)

C12P 21/04 (2006.01)