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(71) **Applicant** *(for all designated States except US)*: **ACHAOGEN, INC.** [US/US]; 7000 Shoreline Court, Third Floor, South San Francisco, California 94080 (US).

(72) **Inventors; and**

(75) **Inventors/Applicants** *(for US only)*: **KASAR, Ramesh** [US/US]; 985 Bayview Avenue, Oakland, California 94610 (US). **LINSELL, Martin Sheringham** [GB/US]; 602 East 16th Avenue, San Mateo, California 94402 (US). **AGGEN, James Bradley** [US/US]; 1311 California Drive, Burlingame, California 94010 (US). **LU, Qing (Jane)** [US/US]; 50 Foresail Court, Foster City, California 94404 (US). **WANG, Dan** [CA/US]; 48331 Conifer Street, Fremont, California 94539 (US). **CHURCH, Tim** [US/US]; 3913 Pasadena Drive, San Mateo, California 94403 (US). **MOSER, Heinz E.** [US/US]; 1566 Ascension Drive, San Mateo, California 94402 (US). **PATTEN, Phillip A.** [US/US]; 261 La Cuesta Drive, Portola Valley, California 94028 (US).

(74) **Agents**: **WAGNER, Emily W.** et al.; Cooley LLP, 777 6th Street, NW, Suite 1100, Washington, District of Columbia 20001 (US).

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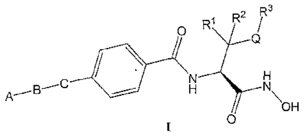

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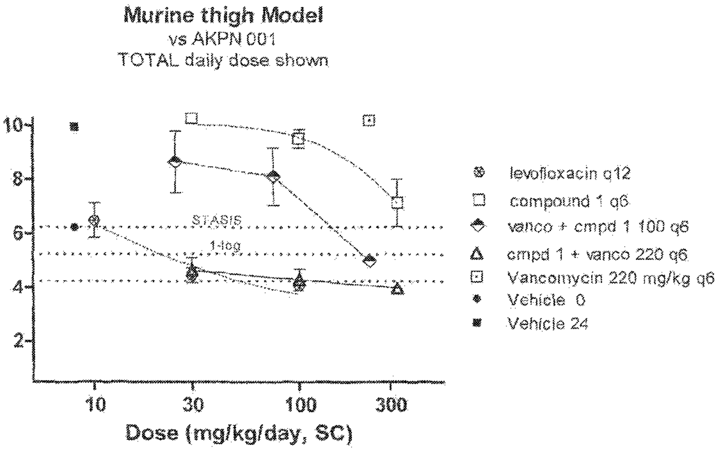
(57) **Abstract**: Antibacterial compounds of Formula I are provided: as well as stereoisomers and pharmaceutically acceptable salts thereof; pharmaceutical compositions comprising such compounds; methods of treating bacterial infections by the administration of such compounds; use of such compounds in the treatment of bacterial infections and processes for the preparation of such compounds.



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HYDROXAMIC ACID DERIVATIVES AND THEIR USE IN THE TREATMENT OF BACTERIAL INFECTIONS

I. CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application No. 61/412,311, filed November 10, 2010, which application is incorporated herein by reference in its entirety.

II. STATEMENT OF GOVERNMENT INTEREST

This invention was made with government support under Contract HDTRA1-07-C-0079 awarded by the United States Department of Defense. The government has certain rights in this invention.

III. BACKGROUND

A. Field

This invention pertains generally to treating infections caused by gram-negative bacteria. More specifically, the invention described herein pertains to treating gram-negative infections by inhibiting activity of UDP-3-O-(R-3-hydroxydecanoyl)-N-acetylglucosamine deacetylase (LpxC). The present invention provides small molecule inhibitors of LpxC, pharmaceutical formulations containing such inhibitors, methods of treating patients with such pharmaceutical formulations, and methods of preparing such pharmaceutical formulations and inhibitors. The invention described herein pertains to treating gram-negative infections by administering compounds capable of inhibiting activity of UDP-3-O-(R-3-hydroxydecanoyl)-N-acetylglucosamine deacetylase (LpxC), either alone or in combination with a second antibacterial agent.

B. Description of the Related Art

Over the past several decades, the frequency of antimicrobial resistance and its association with serious infectious diseases have increased at alarming rates. The problem of antibacterial resistance is compounded by the existence of bacterial strains resistant to multiple antibacterials. Thus there is a need for new antibacterials, particularly antibacterials with novel mechanisms of action. A previously unexploited but highly conserved target, LpxC, provides a new opportunity for developing broad-spectrum antibacterial small molecules that comprise a new class of active bactericidal chemical entities that should encounter little, if any, naturally-occurring, target-related resistance. LpxC (the enzyme uridyldiphospho-3-O-(R-hydroxydecanoyl)-N-acetylglucosamine deacetylase) is present across all Gram-negative bacterial species of interest and is involved in the first committed step in outer membrane biosynthesis. Thus LpxC is essential for survival and presents an ideal target for antibiotic activity in Gram-negative bacterial species.

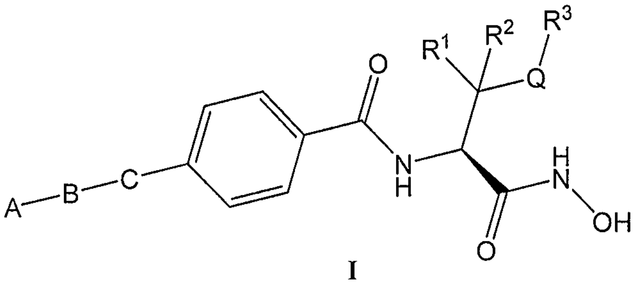
Researchers have identified some compounds with antibacterial activity that target lipid A biosynthesis. For example, Jackman et al. (J. Biol. Chem., 2000, 275(15), 11002-11009); Wyckoff et al. (Trends in Microbiology, 1998, 6(4), 154-159); U.S. Patent Application Publication No. 2001/0053555 (published 20 December 2001, corresponding to International PCT Publication No. WO 98/18754, published 7 May 1998); International PCT Publication No. WO 00/61134 (published 19 October 2000); U.S. Patent Application Publication No. 2004/0229955 (published 18 November 2004); and International PCT Publication No. WO 2008/154642 (published 18 December 2008) all disclose compounds having antibacterial anti-LpxC activity. The commercial development of these LpxC inhibitors has been complicated by toxicity of these compounds in mammalian animals at concentrations at or near those required for antibacterial activity. The compounds presented herein are significantly better tolerated than other closely related compounds having anti-LpxC activity.

Although there have been advances in the field, there remains a need for LpxC inhibitors that have activity as bactericidal agents against gram-negative bacteria and have an acceptable toxicity/tolerance profile. It is, accordingly, an object of this invention to provide compounds and combinations of such compounds for use in the preparation of non-toxic antibacterials and other pharmaceuticals capable of inhibiting gram-negative bacterial infections. An additional object of the present invention is to provide synergistic combinations of antibacterial agents with LpxC inhibitors, which have intrinsic antibacterial properties as well the ability to improve permeability of the outer membrane of gram-negative bacteria to other antibacterial agents. The use of synergistic combinations of drugs could have many advantages over conventional single compound chemotherapy, including lowered side-effects of drugs due to lower doses used or shorter time of treatment, more rapid cure of infection shortening hospital stays, increasing spectrum of pathogens controlled, and decreasing incidence of development of resistance to antibiotics.

IV. BRIEF SUMMARY

The present invention provides novel compounds, pharmaceutical formulations including the compounds, methods of inhibiting UDP-3-O-(R-3-hydroxydecanoyl)-N-acetylglucosamine deacetylase (LpxC), and methods of treating gram-negative bacterial infections.

In one aspect, the invention provides compounds of Formula I:



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and stereoisomers and pharmaceutically acceptable salts thereof, wherein A is a substituted C_3-C_6 cycloalkyl, wherein at least one substituent is a C_1-C_3 primary alcohol; B is absent, -CH=CH-, -C≡C- or an unsubstituted phenyl; C is -CH=CH-, -C≡C- or an unsubstituted phenyl, wherein if B is -CH=CH- then C is not also -CH=CH-; R^1, R^2 and R^3 independently are selected from hydrogen and substituted or unsubstituted C_1-C_3 alkyl, or R^1 and R^2, together with the carbon atom to which they are attached, form an unsubstituted C_3-C_6 cycloalkyl group, or R^2 and R^3, together with the carbon atom and Q to which they are attached, form a substituted or unsubstituted heterocyclic ring, having from 5 to 8 ring atoms, wherein 1-2 ring atoms of the heterocyclic ring are selected from N, O and S; and Q is O or NR, wherein R is hydrogen or an unsubstituted C_1-C_3 alkyl.

In another aspect, the present invention provides a pharmaceutical composition comprising a compound of Formula I, or a stereoisomer or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

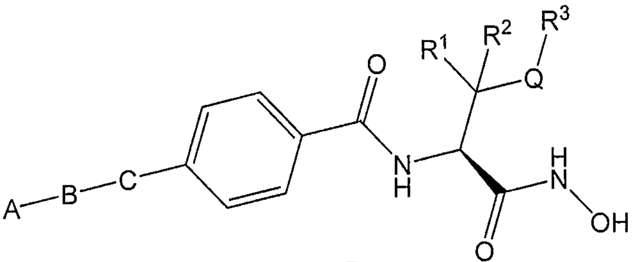
In another aspect, the present invention provides a pharmaceutical composition comprising an effective amount of an antibacterial compound of Formula I, or a stereoisomer or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

In another aspect, the present invention provides a method of inhibiting a deacetylase enzyme in gram-negative bacteria, thereby affecting bacterial growth, comprising administering to a patient in need of such inhibition an LpxC-inhibitory compound of Formula I or a stereoisomer or pharmaceutically acceptable salt thereof.

In another aspect, the present invention provides a method of inhibiting LpxC in gram-negative bacteria, thereby modulating the virulence of a bacterial infection, comprising administering to a patient in need of such inhibition an LpxC-inhibitory compound of Formula I or a stereoisomer or pharmaceutically acceptable salt thereof.

In another aspect, the present invention provides a method for treating a subject having a bacterial infection comprising administering to the subject in need thereof an antibacterially effective amount of a compound of Formula I, or a stereoisomer or pharmaceutically acceptable salt thereof. In a more specific embodiment of the method of treatment, the bacterial infection is a gram-negative bacterial infection. In one such embodiment the bacteria is *Pseudomonas aeruginosa*, *Burkholderia* (e.g., *Burkholderia cepacia*), *Enterobacteriaceae*, *Franciscellaceae* (e.g., *Franciscella tularensis*), *Serratia*, *Proteus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Providencia*, *Yersinia* (e.g., *Yersinia pestis*), *Morganella* or *Escherichia coli*. In one particular embodiment the bacteria is *Pseudomonas aeruginosa*, *Burkholderia, Franciscellaceae*, *Enterobacter*, *Yersinia* or *Escherichia coli*. In one such embodiment the bacteria is *Pseudomonas aeruginosa*. In another such embodiment the bacteria is *Escherichia coli*. In another embodiment the bacteria is *Stenotrophomonas maltophila*, *Alcaligenes xylosoxidans*, *Haemophilus*, *Neisseria species, Cedecea* or *Edwardsiella species*. In a further specific embodiment the subject is a mammal and in certain embodiments, a human.

One aspect of the invention provides pharmaceutical compositions comprising an inhibitor of LpxC and a second antibacterial agent. In one implementation, the second antibacterial agent is selected from the group consisting of vancomycin, linezolid, azithromycin, imipenem, teicoplanin, daptomycin, clindamycin, rifampin, cefotaxime, gentamicin, novobiocin, and telavancin. In one such implementation, the second antibacterial agent is vancomycin or rifampin. In another embodiment, the LpxC inhibitor is a compound of Formula I:



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or a stereoisomer or pharmaceutically acceptable salt thereof, wherein A is a substituted C_3-C_6 cycloalkyl, wherein at least one substituent is a C_1-C_3 primary alcohol; B is absent, -CH=CH-, -C≡C- or an unsubstituted phenyl; C is -CH=CH-, -C≡C- or an unsubstituted phenyl, wherein if B is -CH=CH- then C is not also -CH=CH-; R^1, R^2 and R^3 independently are selected from hydrogen and substituted or unsubstituted C_1-C_3 alkyl, or R^1 and R^2, together with the carbon atom to which they are attached, form an unsubstituted C_3-C_6 cycloalkyl group, or R^2 and R^3, together with the carbon atom and Q to which they are attached, form a substituted or unsubstituted heterocyclic ring, having from 5 to 8 ring atoms, wherein 1-2 ring atoms of the heterocyclic ring are selected from N, O and S; and Q is O or NR, wherein R is hydrogen or an unsubstituted C_1-C_3 alkyl.

Another aspect of the invention provides methods for treating a patient with a gram-negative bacterial infection, comprising co-administering a synergistic amount, for example an *in vivo* synergistic amount, of an antibacterial agent and an LpxC inhibitor of Formula I. In one implementation, the antibacterial agent is selected from the group consisting of vancomycin, linezolid, azithromycin, imipenem, teicoplanin, daptomycin, clindamycin, rifampin, cefotaxime, gentamicin, novobiocin, and telavancin. In one such implementation, the antibacterial agent is vancomycin or rifampin.

These and other aspects of the invention will be evident upon reference to the following detailed description.

V. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates in vivo synergy of compound **I-1** and vancomycin in bacterial strain ATCC 43816.

VI. DETAILED DESCRIPTION

The present invention provides novel compounds, methods for inhibiting LpxC in gram-negative bacteria, and novel methods for treating bacterial infections. The compounds provided herein can be formulated into pharmaceutical formulations and medicaments that are useful in the methods of the invention. The invention also provides for the use of the compounds in preparing medicaments and pharmaceutical formulations, for use of the compounds in inhibiting LpxC, and for use of the compounds in treating bacterial infections in a subject. The invention further provides compositions and methods for treating gram-negative infections by administering compounds capable of inhibiting activity of UDP-3-O-(R-3-hydroxydecanoyl)-N-acetylglucosamine deacetylase (LpxC), either alone or in combination with administering a second antibacterial compound.

A. Definitions

The following abbreviations and definitions are used throughout this application:

"LpxC" is an abbreviation that stands for UDP-3-O-(R-3-hydroxydecanoyl)-N-acetylglucosamine deacetylase.

As used herein, the following definitions shall apply unless otherwise indicated.

"Alkyl" refers to monovalent saturated aliphatic hydrocarbyl groups having from 1 to 10 carbon atoms and preferably 1 to 6 carbon atoms. This term includes, by way of example, linear and branched hydrocarbyl groups such as methyl

(CH_3-), ethyl (CH_3CH_2-), *n*-propyl ($CH_3CH_2CH_2$-), isopropyl (($CH_3)_2CH$-), *n*-butyl ($CH_3CH_2CH_2CH_2$-), isobutyl (($CH_3)_2CHCH_2$-), *sec*-butyl ((CH_3)(CH_3CH_2)CH-), *t*-butyl (($CH_3)_3C$-), *n*-pentyl ($CH_3CH_2CH_2CH_2CH_2$-), and neopentyl (($CH_3)_3CCH_2$-).

"Alkoxy" refers to the group -O-alkyl, wherein alkyl is as defined herein. Alkoxy includes methoxy, ethoxy, *n*-propoxy, isopropoxy, *n*-butoxy, *t*-butoxy, *sec*-butoxy, *n*-pentoxy, and the like.

"Amino" refers to the group -NH_2.

"Primary alcohol" refers to the group -alkyl-OH, wherein the hydroxyl radical is connected to a primary carbon. Examples include -CH_2OH (hydroxymethyl), -CH_2CH_2OH (hydroxymethyl) and -$CH(CH_3)CH_2OH$ (1-hydroxypropan-2-yl).

"Alkenyl" refers to straight chain or branched hydrocarbyl groups having from 2 to 6 carbon atoms and preferably 2 to 4 carbon atoms and having at least 1 and preferably from 1 to 2 sites of vinyl (>C=C<) unsaturation. Such groups are exemplified by vinyl, allyl, and but-3-en-1-yl. Included within this term are the *cis* and *trans* isomers or mixtures of these isomers.

"Alkynyl" refers to straight or branched monovalent hydrocarbyl groups having from 2 to 6 carbon atoms and preferably 2 to 3 carbon atoms and having at least 1 and preferably from 1 to 2 sites of acetylenic -C≡C- unsaturation. Examples of such alkynyl groups include acetylenyl (-C≡CH), and propargyl (-CH_2C≡CH).

"Carboxyl" or "carboxy" refers to -COOH or salts thereof.

"Cyano" or "nitrile" refers to the group -CN.

"Cycloalkyl" refers to cyclic alkyl groups of from 3 to 13 carbon atoms having single. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like.

"Guanidino" refers to the group -NHC(=NH)NH_2.

"Halo" or "halogen" refers to fluoro, chloro, bromo, and iodo and is typically fluoro or chloro.

"Hydroxy" or "hydroxyl" refers to the group -OH.

"Heterocycle," "heterocyclic," and "heterocyclyl" refer to a saturated or unsaturated group having a single ring, and having from 3 to 15 ring atoms, including 1 to 4 hetero atoms. These ring atoms are selected from the group consisting of nitrogen, sulfur, or oxygen. In one implementation, the nitrogen and/or sulfur atom(s) of the heterocyclic group are optionally oxidized to provide for the N-oxide, -S(O)-, or $-SO_2-$ moieties.

"Nitro" refers to the group $-NO_2$.

"Nitroso" refers to the group -NO.

"Oxo" refers to the atom (=O).

"Substituted" refers to a group having one or more hydrogens replaced with substituents selected from the group consisting of alkoxy, acyl, acylamino, acyloxy, amino, aminocarbonyl, aminothiocarbonyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, amidino, carboxyl, carboxyl ester, (carboxyl ester)amino, (carboxyl ester)oxy, cyano, guanidino, halo, hydroxy, nitro, SO_3H, sulfonyl, sulfonyloxy, thioacyl, thiol, and alkylthio, wherein said substituents are as defined herein. In certain substituted cyclic groups, "substituted" also refers to a group having two hydrogens replaced with a single double bonded oxygen atom (an oxo group) or a single double bonded sulfur atom (thioxo). In some implementations, the substituted group has 1 to 3 of the aforementioned substituents. In other implementations, the substituted group has 1 to 2 of the aforementioned substituents

"Sulfonyl" refers to the group $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-alkenyl, $-SO_2$-substituted alkenyl, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, and substituted alkynyl are as defined herein. Sulfonyl includes groups such as methyl-SO_2-.

"Sulfonyloxy" refers to the group $-OSO_2$-alkyl, $-OSO_2$-substituted alkyl, $-OSO_2$-alkenyl, $-OSO_2$-substituted alkenyl, $-OSO_2$-alkynyl, $-OSO_2$-substituted alkynyl, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, and substituted alkynyl are as defined herein.

"Thioacyl" refers to the groups H-C(S)-, alkyl-C(S)-, substituted alkyl-C(S)-, alkenyl-C(S)-, substituted alkenyl-C(S)-, alkynyl-C(S)-, and substituted alkynyl-C(S)-, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, and substituted alkynyl are as defined herein.

"Thiol" refers to the group -SH.

"Thioxo" refers to the atom (=S).

"Alkylthio" refers to the group -S-alkyl, wherein alkyl is as defined herein. In other implementations, sulfur may be oxidized to -S(O)-. The sulfoxide may exist as one or more stereoisomers.

Unless indicated otherwise, the nomenclature of substituents that are not explicitly defined herein are arrived at by naming the terminal portion of the functionality followed by the adjacent functionality toward the point of attachment. For example, the substituent "arylalkyloxycarbonyl" refers to the group (aryl)-(alkyl)-O-C(O)-.

Generally, reference to a certain element such as hydrogen or H is meant to include all isotopes of that element. For example, if a substituent group is defined to include hydrogen or H, it also includes deuterium and tritium.

The subject invention also includes isotopically-labeled compounds of the present invention, that are structurally identical to those disclosed herein, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulfur, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F and ^{36}Cl, respectively. Compounds of the present invention, prodrugs thereof, and pharmaceutically acceptable salts of said compounds and of said prodrugs that contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically labeled compounds of the present invention, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., ^{3}H, and carbon-14, *i.e.*, ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, i.e., ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labeled compounds of this invention and prodrugs thereof can generally be prepared by carrying out known or referenced procedures and by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent.

"Stereoisomer" and "stereoisomers" refer to compounds that have same atomic connectivity but different atomic arrangement in space. Stereoisomers include cis-trans isomers, E and Z isomers, enantiomers, and diastereomers.

"Tautomer" refers to alternate forms of a molecule that differ in the position of a proton, such as enol-keto and imine-enamine tautomers, or the tautomeric forms of heteroaryl groups containing a -N=C(H)-NH- ring atom arrangement, such as pyrazoles, imidazoles, benzimidazoles, triazoles, and tetrazoles. A person of ordinary skill in the art would recognize that other tautomeric ring atom arrangements are possible.

"Patient" refers to human and non-human animals, especially mammals.

"Pharmaceutically acceptable salt" refers to pharmaceutically acceptable salts of a compound, which salts are derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate, phosphate, sulfate and the like.

"Pharmaceutically effective amount" and "therapeutically effective amount" refer to an amount of a compound sufficient to treat a specified disorder or disease or one or more of its symptoms and/or to prevent the occurrence of the disease or disorder.

The term "synergy" or "synergistic" as used herein means the combined effect of the compounds when used in combination is greater than the additive effects of the compounds when used individually. "Synergism" can be defined quantitatively as a fractional inhibitory concentration index (FICI) of ≤0.5, where FICI is defined as the sum of the fractional inhibitory concentrations (FICs) of the individual components in a combination of two compounds, and the FIC is defined as the ratio of the minimal inhibitory concentration (MIC) of the compound in the combination divided by the MIC of the compound alone:

$$FICI = \left(\frac{MIC_{drug\ A\ in\ combo}}{MIC_{drug\ A\ alone}}\right) + \left(\frac{MIC_{drug\ B\ in\ combo}}{MIC_{drug\ B\ alone}}\right)$$

Alternatively, "synergism," more particularly "*in* vivo synergism," can be defined quantitatively as an at least two-fold decrease in the static dose of the agents used in combination as compared to the LpxC inhibitor or second antibacterial agent alone. In certain cases one agent alone may never reach a static dose. In such cases, a combination is synergistic if bacterial growth can be halted (CFU load at 24 hours that is identical to that measured at 0 hours post infection) by combined administration with two compounds that alone cannot achieve stasis.

"Co-administration" can be in the form of a single formulation (combining, for example, a compound of the present invention and a second antibacterial agent with pharmaceutically acceptable excipients, optionally segregating the two active ingredients in different excipient mixtures designed to independently control their respective release rates and durations) or by independent administration of separate formulations containing the active agents. "Co-administration" further includes concurrent administration (administration of a compound of the present invention and a second antibacterial agent at the same time) and time varied administration (administration of a compound of the present invention at a time different from that of the second antibacterial agent), as long as both the compound of the present invention and the second antibacterial agent are present in the body in therapeutically effective concentrations during at least partially overlapping times.

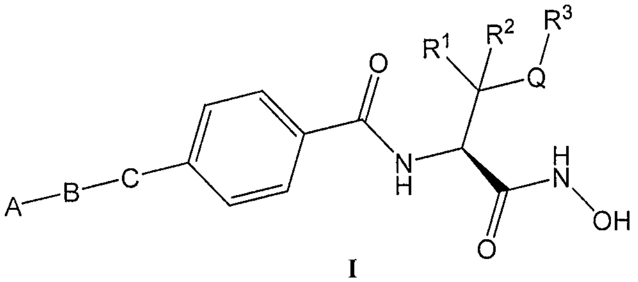
The term "antibacterial agent" refers to agents that have either bactericidal or bacteriostatic activity. The term "inhibiting the growth" indicates that the rate of increase in the numbers of a population of a particular bacterium is reduced. Thus, the term includes situations in which the bacterial population increases but at a reduced rate, as well as situations where the growth of the population is stopped, as well as situations where the numbers of the bacteria in the population are reduced or the population even eliminated. If an enzyme activity assay is used to screen for inhibitors, one can make modifications in uptake/efflux, solubility, half-life, etc. to compounds in order to correlate enzyme inhibition with growth inhibition. The activity of antibacterial agents is not necessarily limited to bacteria but may also encompass activity against parasites, virus, and fungi.

Unless the context requires otherwise, throughout the specification and claims which follow, the word "comprise" and variations thereof, such as, "comprises" and "comprising" are to be construed in an open, inclusive sense, that is as "including, but not limited to".

Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

B. Compounds, Compositions and Use Thereof

In one aspect, the present invention provides compounds of Formula I:



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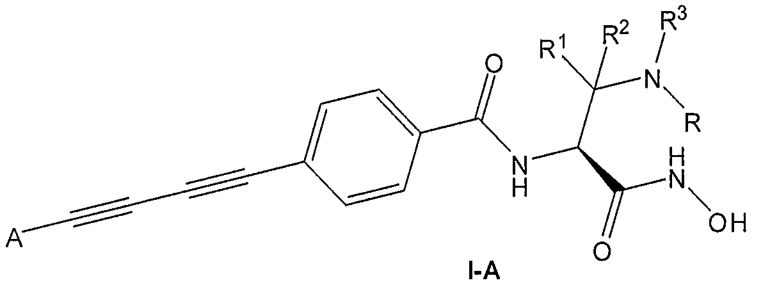
and stereoisomers and pharmaceutically acceptable salts thereof, wherein A is a substituted C_3-C_6 cycloalkyl, wherein at least one substituent is a C_1-C_3 primary alcohol; B is absent, -CH=CH-, -C≡C- or an unsubstituted phenyl; C is -CH=CH-, -C≡C- or an unsubstituted phenyl, wherein if B is -CH=CH- then C is not also -CH=CH-; R^1, R^2 and R^3 independently are selected from hydrogen and substituted or unsubstituted C_1-C_3 alkyl, or R^1 and R^2, together with the carbon atom to which they are attached, form an unsubstituted C_3-C_6 cycloalkyl group, or R^2 and R^3, together with the carbon atom and Q to which they are attached, form a substituted or unsubstituted heterocyclic ring, having from 5 to 8 ring atoms, wherein 1-2 ring atoms of the heterocyclic ring are selected from N, O and S; and Q is O or NR, wherein R is hydrogen or an unsubstituted C_1-C_3 alkyl.

In certain embodiments, Q is NR, and in some such embodiments Q is NH or NCH_3. In certain embodiments, R^1, R^2, and R^3 independently are selected from hydrogen and substituted or unsubstituted C_1-C_3 alkyl, and in some such embodiments are selected from hydrogen and unsubstituted C_1-C_3 alkyl. In certain embodiments, R^1 and R^2 independently are unsubstituted C_1-C_3 alkyl. In certain embodiments, A is a C_3-C_6 cycloalkyl substituted with hydroxymethyl. In other embodiments, both B and C are a -C≡C-.

One aspect of the invention provides compounds of Formula I-A:

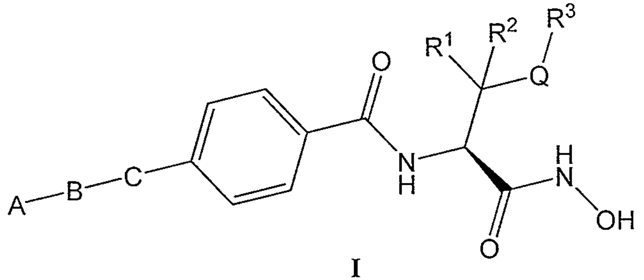


I-A

and stereoisomers and pharmaceutically acceptable salts thereof, wherein A is a substituted C_3-C_6 cycloalkyl, wherein at least one substituent is a C_1-C_3 primary alcohol; R^1, R^2 and R^3 independently are selected from hydrogen and substituted or unsubstituted C_1-C_3 alkyl, or R^1 and R^2, together with the carbon atom to which they are attached, form a C_3-C_6 cycloalkyl group, or R^2 and R^3, together with the carbon atom and nitrogen to which they are attached, form a substituted or unsubstituted heterocyclic ring, having from 5 to 8 ring atoms, wherein 1-2 ring atoms of the heterocyclic ring are selected from N, O and S; and R is hydrogen or an unsubstituted C_1-C_3 alkyl. In certain embodiments, R^1, R^2 and R^3 independently are selected from hydrogen and substituted or unsubstituted C_1-C_3 alkyl, and in some such embodiments are selected from hydrogen and unsubstituted C_1-C_3 alkyl. In one embodiment, A is a C_3-C_6 cycloalkyl, mono-substituted with a C_1-C_3 primary alcohol.

Compounds of the present invention include those listed in Table I. Certain of the compounds illustrated in Table 1 represent mixtures of two diastereomers. In such a case, the notation "*" indicates that the two moieties attached to the cyclic moiety are *trans* to one another. The notation "#" indicates that the two moieties attached to the cyclic moiety are *cis* to one another.

TABLE I



I

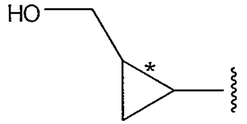
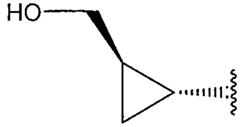
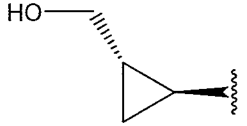
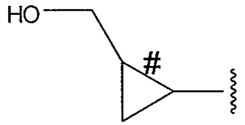
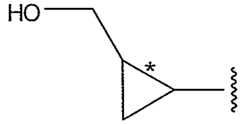
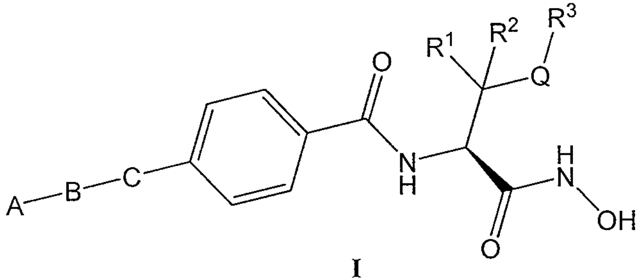
	A	B	C	R^1	R^2	R^3	Q
I-1		≡	≡	CH_3	CH_3	H	NH
I-2		≡	≡	CH_3	CH_3	H	NH
I-3		≡	≡	CH_3	CH_3	H	NH
I-4		≡	≡	CH_3	CH_3	H	NH
I-5		≡	phenyl	CH_3	CH_3	H	NH

TABLE I



I

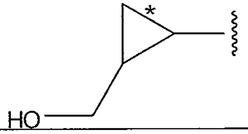
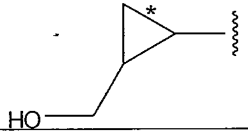
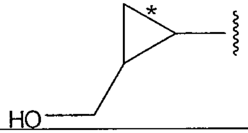
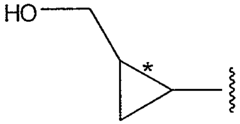
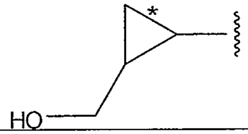
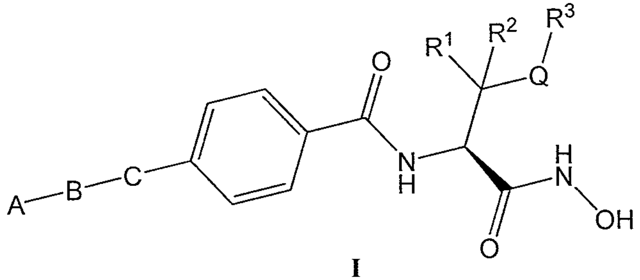
	A	B	C	R^1	R^2	R^3	Q
I-6		≡	≡	CH_3	CH_3	CH_3	NH
I-7		≡	≡	CH_3	CH_3	CH_2CH_3	NH
I-8		≡	≡	CH_3	CH_3	CH_2CH_2OH	NH
I-9		≡	≡	CH_3	CH_3	CH_3	NCH_3
I-10		≡	≡	CH_3	CH_3	H	O

TABLE I



I

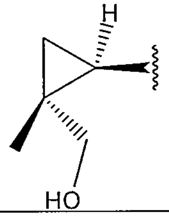
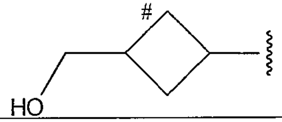
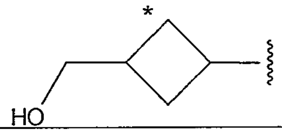
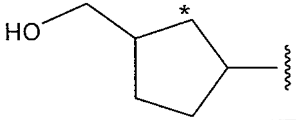
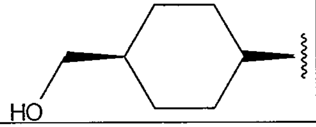
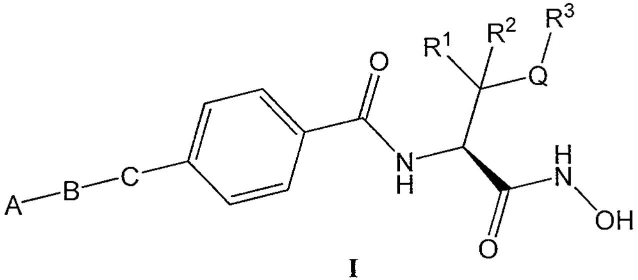
	A	B	C	R^1	R^2	R^3	Q
I-11		≡	≡	CH_3	CH_3	H	NH
I-12		≡	≡	CH_3	CH_3	H	NH
I-13		≡	≡	CH_3	CH_3	H	NH
I-14		≡	≡	CH_3	CH_3	H	NH
I-15		≡	≡	CH_3	CH_3	H	NH

TABLE I



I

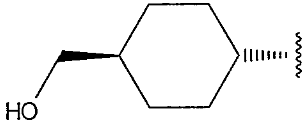
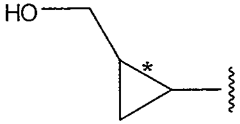
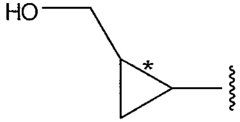
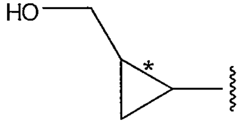
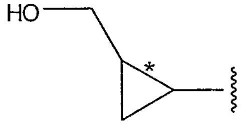
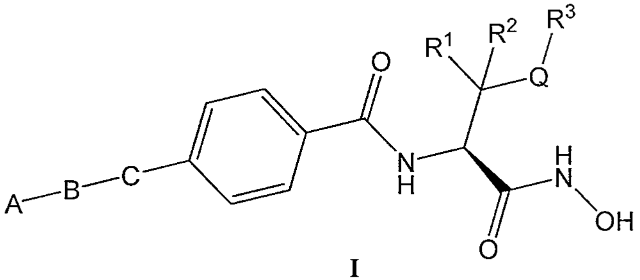
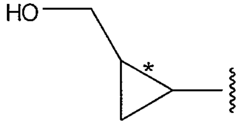
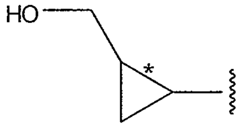
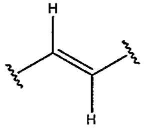
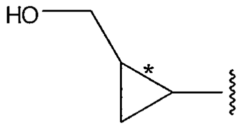
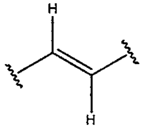
	A	B	C	R^1	R^2	R^3	Q
I-16		≡	≡	CH_3	CH_3	H	NH
I-17		≡	≡	cyclobutyl		H	NH
I-18		≡	≡	cyclobutyl		H	NH
I-19		≡	≡	cyclobutyl		CH_3	NCH_3
I-20		≡	≡	H	morpholino		NH

TABLE I



I

	A	B	C	R^1	R^2	R^3	Q
I-21		≡	≡	H	morpholino		NCH_3
I-22			≡	CH_3	CH_3	H	NH
I-23		≡		CH_3	CH_3	H	NH

* = trans # = cis

Compounds of the present invention can be readily synthesized using the methods described herein, or other methods, that are well known in the art. For example, the synthesis of hxdroxamic acids or similar scaffolds having a wide variety of substituents are comprehensively reviewed in Kline, T., et al., "Potent, novel in vitro inhibitors of the *Pseudomonas aeruginosa* deacetylase LpxC" *J. Med Chem.* **2002,** 45(14), 3112-29; U.S. Patent No. 5,925,659; Pirrung, M. C., et al., "A Convenient Procedure for the Preparation of Amino Acid Hydroxamate from Esters" *J. Org. Chem.* **1995**, 60, 8084-8085; Nhu, K., et al., "A New and Efficient Solid Phase Synthesis of Hydroxamic Acids" *J. Org. Chem.* **1997**, 62, 7088-7089; International PCT Publication No. WO98/18754; Mellor, S. L., et al., "N-Fmoc-aminoxy-2-chlortrityl Polystyrene Resin: A Facile Solid-phase Methodology for the Synthesis of Hydroxamic Acids" *Tetrahedron Lett.* **1997**, 38, 3311-3314; Khan, S. I., et al., "A Facile and Convenient Solid-phase Procedure for Synthesizing Nucleoside Hydroxamic Acids" *Tetrahedron. Lett.* **1998**, 39, 8031-8034; Zhang, Y., et al., "Design, Combinatorial Chemical Synthesis, and in vitro Characterization of Novel Urea Based Gelatinase Inhibitors" *Bioorg. Med. Chem. Lett.* **1999**, 9, 2823-2826; Ito, Y., et al., "Synthetic Reactions by Complex Catalysts. XXXI, A Novel and Versatile Method of Heterocycle Synthesis" *J. Am Chem. Soc.* **1973**, 95, 4447-4448; Ito, Y., et al., "Synthetic Reactions by Complex Catalysts XXXV" *Syn. Commun.* **1974**, 4, 97-103; Witte, H., et al., "Cyclische Imidsaurester aus Nitrilen und Aminoalkoholen" *Liebigs Ann. Chem.* **1974**, 996-1009; Pattenden, G., et al., "Naturally Occurring Linear Fused Thiazoline-Thiazole Containing Metabolites: Total Synthesis of (-) Didehydromirabazole A, a Cytotoxic Alkaloid from Blue-Green Algae" *J. Chem. Soc. Perkin Trans* **1993**, 1, 1629-1636; Boyce, R. J., et al., "Total Synthesis of Thiangazole, A Novel Naturally Occurring HIV-1 Inhibitor from Polyangium sp." *Tetrahedron* **1995**, 51, 7321-7330; Galeotti, N., et al., "Synthesis of Peptidyl Aldehydes from Thiazolines" *Tetrahedron. Lett.* **1997**, 38, 2459-2462; Charette, A. B., et al., "Mild Method for the Synthesis of Thiazolines from Secondary and Tertiary Amides" *J. Org. Chem.* **1998**, 63, 908-909; Bergeron, R. J., et al., "Effects of C-4 Stereochemistry and C-4' Hydroxylation on the Iron Clearing Efficiency and Toxicity of Desferrithiocin Analogues" *J. Med. Chem.* **1999**, 42, 2432-2440; Raman, P., et al., "Titanium (IV)-mediated Tandem Deprotection-cyclodehydration of Protected

Cysteine N-Amides: Biomimetic Synthesis of Thiazoline- and Thiazole-containing Heterocycles" *Org. Lett.* **2000**, 2, 3289-3292; Fernandez, X., et al., "Novel Synthesis of 2-Thioazolines" *Tetrahedron Lett.* **2000**, 41, 3381-3384; and Wipf, P., et al., "C. Thiolysis of Oxazolinenes: A New, Selective Method for the Direct Conversion of Peptide Oxazolines into Thiazolines" *Tetrahedron Lett.* **1995**, 36, 6395-6398, which are incorporated herein by reference.

In another aspect, the present invention provides a pharmaceutical composition comprising a compound of Formula I, or a stereoisomer or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

In another aspect, the invention provides a method of inhibiting a deacetylase enzyme in a gram-negative bacteria, thereby affecting bacterial growth, comprising administering to a patient in need of such inhibition a compound of Formula I or a stereoisomer or pharmaceutically acceptable salt thereof.

In another aspect, the invention provides a method of inhibiting LpxC in a gram-negative bacteria, thereby modulating the virulence of a bacterial infection, comprising administering to a patient in need of such inhibition a compound of Formula I or a stereoisomer or pharmaceutically acceptable salt thereof. In certain embodiments of the method of inhibiting LpxC using a compound of the present invention, the IC_{50} value of the compound is less than or equal to 10 μM with respect to LpxC. In other embodiments, the IC_{50} value is less than or equal to 1 μM, is less than or equal to 0.1 μM, is less than or equal to 0.050 μM, is less than or equal to 0.030 μM, is less than or equal to 0.025 μM, or is less than or equal to 0.010 μM.

In another aspect, the invention provides a method for treating a subject having a gram-negative bacterial infection comprising administering to the subject in need thereof an antibacterially effective amount of a compound of Formula I or a stereoisomer or pharmaceutically acceptable salt thereof. In one such embodiment the bacteria is *Pseudomonas aeruginosa*, *Burkholderia* (e.g., *Burkholderia cepacia*), *Enterobacteriaceae*, *Franciscellaceae* (e.g., *Franciscella tularensis*), *Serratia*, *Proteus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Providencia*, *Yersinia* (e.g., *Yersinia pestis*), *Morganella* or *Escherichia coli*. In one particular embodiment the bacteria is

Pseudomonas aeruginosa, *Burkholderia, Franciscellaceae*, *Enterobacter*, *Yersinia* or *Escherichia coli.* In one such embodiment the bacteria is *Pseudomonas aeruginosa.* In another such embodiment the bacteria is *Escherichia coli.* In another embodiment the bacteria is *Stenotrophomonas maltophila*, *Alcaligenes xylosoxidans*, *Haemophilus*, *Neisseria species, Cedecea* or *Edwardsiella species.*

In certain embodiments, the subject may be a mammal, and in some embodiments, a human.

Bacterial infections susceptible to treatment according to the present invention include primary infections and co-infections caused by a species of bacteria and one or more additional infectious agents such as, for example, bacteria, virus, parasite and fungus.

Compounds of the invention can be used for treating conditions caused by the bacterial production of endotoxin and, in particular, by gram-negative bacteria and bacteria that use LpxC in the biosynthesis of lipopolysaccharide (LPS) or endotoxin.

Compounds of the invention also are useful in treating conditions that are caused or exacerbated by the bacterial production of lipid A and LPS or endotoxin, such as sepsis, septic shock, systemic inflammation, localized inflammation, chronic obstructive pulmonary disease (COPD) and acute exacerbations of chronic bronchitis (AECB). For these conditions, treatment includes the administration of a compound of the invention, or a combination of compounds of the invention, optionally with a second agent wherein the second agent is a second antibacterial agent or a non-antibacterial agent.

For sepsis, septic shock, systemic inflammation, localized inflammation, chronic obstructive pulmonary disease (COPD) and acute exacerbations of chronic bronchitis (AECB), representative non-antibacterial agents include antiendotoxins including endotoxin receptor-binding antibodies, endotoxin-binding antibodies, anti-CD14-binding protein antibodies, anti-lipopolysaccharide-binding protein antibodies and tyrosine kinase inhibitors.

In treatment of serious or chronic respiratory tract infections, compounds of the present invention may also be used with non-antibacterial agents administered via inhalation. Representative non-antibacterial agents used in this treatment include anti-inflammatory steroids, non-steroidal anti-inflammatory agents, bronchodilators, mucolytics, anti-asthma therapeutics and lung fluid surfactants. In particular, the non-antibacterial agent may be albuterol, salbuterol, budesonide, beclomethasone, dexamethasone, nedocromil, beclomethasone, fluticasone, flunisolide, triamcinolone, ibuprofin, rofecoxib, naproxen, celecoxib, nedocromil, ipratropium, metaproterenol, pirbuterol, salmeterol, formoterol, indacaterol, bronchodilators, mucolytics, calfactant, beractant, poractant alfa, surfaxin or pulmozyme (also called domase alfa).

Compounds of the invention can be used alone or in combination with a second antibacterial agent for the treatment of a serious or chronic respiratory tract infection including serious lung and nosocomial infections such as those caused by *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Alcaligenes xylosoxidans*, *Flavobacterium meningosepticum*, *Providencia stuartii* and *Citrobacter freundi*, community lung infections such as those caused by *Haemophilus Influenzae*, *Legionella* species, *Moraxella catarrhalis*, *Branhamella catarrhalis*, *Enterobacter* species, *Klebsiella* species, and *Proteus* species, infections caused by other bacterial species such as *Neisseria* species, *Shigella* species, *Salmonella* species, *Helicobacter pylori*, *Vibrionaceae* and *Bordetella* species, as well as infections caused by a *Brucella* species, *Francisella tularensis* and/or *Yersinia Pestis*.

When used for treating subjects infected with gram-negative bacterial infections, compounds of the present invention can be used to sensitize gram-negative bacteria to the effects of a second antibacterial agent.

The present invention provides novel combinations of compounds including a compound of Formula I, or a stereoisomer or pharmaceutically acceptable salt thereof, as well as methods for treating subjects infected with gram-negative bacteria. The novel combinations provided herein can be formulated into pharmaceutical formulations and medicaments that are useful in the methods of the invention. The invention also provides for the use of the novel combinations in preparing medicaments and pharmaceutical formulations, for use of the combinations in treating bacterial infections in a patient.

One classic method for assessing synergy, referred to as the checkerboard assay, is used to predict the efficacy of antibacterial agents, and is described by Scribner et. al., (1982, Antimicrobial Agents and Chemotherapy 21(6):939-943) and in Goodman & Gilman (1980, The Pharmacological Basis of Therapeutics, Sixth Edition, pp. 1097-1098). The checkerboard assay involves serial two-fold dilutions of the antibiotics individually and in combination in broth, which is then inoculated with the microorganism to be tested. After incubation, the minimum inhibitory concentration (MIC) of each drug used individually and in combination is determined (N.B., the MIC is the lowest concentration of the drug that inhibits growth in the medium). Synergism is indicated by a decrease in the MIC of each drug when used in combination. Antagonism is indicated by an increase in the MIC of either or both drugs when used in combination. Alternate methods of assessing synergy are reviewed in Greco, et al., *Pharmacological Reviews* **47**(2):331-285 (1995), incorporated herein by reference in its entirety.

However, a positive result in a checkerboard assay, *i.e.*, indicating synergy below the MIC, does not necessarily result in synergistic behavior *in vivo*. US Patent Application Publication No. 2004-229955A1 reports strong synergy between erythromycin and an LpxC inhibitor, N-[(1S)-1-(aminomethyl)-2-(hydroxyamino)-2oxoethyl]-4-(4-{4-[({[(3methylphenyl)methyl]amino}acetyl)amino]phenyl}buta-1,3diynyl)benzamide against *E. coli* strain ATCC 25922. International PCT Patent Application No. PCT/US2010/33910 demonstrated that the combination of erythromycin and a variety of LpxC inhibitors show no synergy *in vivo*.

LpxC, an essential gene in gram-negative bacteria, encodes the enzyme uridyldiphospho-3-O-(R-hydroxydecanoyl)-N-acetylglucosamine deacetylase. This enzyme catalyzes an early committed step in the bio-synthesis of lipid A, the lipid moiety of lipopolysaccharide that is an essential component of all gram-negative bacteria. Above the MIC, an LpxC inhibitor is expected to disrupt the outer membrane, thus permitting other antibacterial compounds to penetrate the outer membrane. Once these agents have penetrated the outer membrane, they may affect periplasmic targets as is the case with vancomycin, or they may then diffuse across the inner membrane to interact with an intracellular target such as the ribosome (Erythromycin) or RNA polymerase (Rifampin). In the absence ,of an LpxC inhibitor, the ability of agents such as vancomycin to access their target is greatly diminished by the outer membrane. Thus, without being bound by theory, it is believed that the biochemical mechanism that may underlie the observed synergy is the enhanced permeability of the outer membrane to agents such as vancomycin when combined with LpxC inhibitors.

In one embodiment, the second antibacterial agent used in combination with a compound of Formula I, or stereoisomer or pharmaceutically acceptable salt thereof, is vancomycin, linezolid , azithromycin, imipenem, teicoplanin, daptomycin, clindamycin, rifampin, cefotaxime, gentamicin, novobiocin or telavancin. In one such embodiment, the second antibacterial agent is vancomycin, teicoplanin, rifampin, azithromycin, telavancin or novobiocin. In one such embodiment, the second antibacterial agent is vancomycin or rifampin. In some embodiments of the invention, the second antibacterial agent and/or the compound of Formula I, or stereoisomer or pharmaceutically acceptable salt thereof, is administered at a sub-therapeutic dose, wherein a subtherapeutic dose is a dose that would be insufficient to treat bacterial infections, if administered alone.

Pharmaceutical compositions of the present invention comprise a compound of Formula I, or a stereoisomer or pharmaceutically acceptable salt thereof, formulated together with one or more pharmaceutically acceptable carriers or diluents. As used herein, the term “pharmaceutically acceptable carrier” means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials that can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogenfree water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator. The pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally (as by intravenous, intramuscular or subcutaneous injection), intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, or as an oral or nasal spray, or a liquid aerosol or dry powder formulation for inhalation.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, 1% lidocaine, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form may be accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations may also be prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissues.

Compositions for rectal or vaginal administration are preferably suppositories that can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, acetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The antibacterial compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulations, ear drops, and the like are also contemplated as being within the scope of this invention.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Compositions of the invention may also be formulated for delivery as a liquid aerosol or inhalable dry powder. Liquid aerosol formulations may be nebulized predominantly into particle sizes that can be delivered to the terminal and respiratory bronchioles where bacteria reside in patients with bronchial infections, such as chronic bronchitis and pneumonia. Pathogenic bacteria are commonly present throughout airways down to bronchi, bronchioli and lung parenchema, particularly in terminal and respiratory bronchioles. During exacerbation of infection, bacteria can also be present in alveoli. Liquid aerosol and inhalable dry powder formulations are preferably delivered throughout the endobronchial tree to the terminal bronchioles and eventually to the parenchymal tissue.

Aerosolized formulations of the invention may be delivered using an aerosol forming device, such as a jet, vibrating porous plate or ultrasonic nebulizer, preferably selected to allow the formation of an aerosol particles having with a mass medium average diameter predominantly between 1 to 5 μm. Further, the formulation preferably has balanced osmolarity ionic strength and chloride concentration, and the smallest aerosolizable volume able to deliver effective dose of the compounds of the invention to the site of the infection. Additionally, the aerosolized formulation preferably does not impair negatively the functionality of the airways and does not cause undesirable side effects.

Aerosolization devices suitable for administration of aerosol formulations of the invention include, for example, jet, vibrating porous plate, ultrasonic nebulizers and energized dry powder inhalers, that are able to nebulize the formulation of the invention into aerosol particle size predominantly in the size range from 1-5 pm. Predominantly in this application means that at least 70% but preferably more than 90% of all generated aerosol particles are 1 to 5 μm range. A jet nebulizer works by air pressure to break a liquid solution into aerosol droplets. Vibrating porous plate nebulizers work by using a sonic vacuum produced by a rapidly vibrating porous plate to extrude a solvent droplet through a porous plate. An ultrasonic nebulizer works by a piezoelectric crystal that shears a liquid into small aerosol droplets. A variety of suitable devices are available, including, for example, AeroNeb and AeroDose vibrating porous plate nebulizers (AeroGen, Inc., Sunnyvale, Calif.), Sidestream7 nebulizers (Medic-Aid Ltd., West Sussex, England), Pari LC7 and Pari LC Star7 jet nebulizers (Pari Respiratory Equipment, Inc., Richmond, Va.), and Aerosonic (DeVilbiss Medizinische Produkte (Deutschland) GmbH, Heiden, Germany) and UltraAire7 (Omron Healthcare, Inc., Vernon Hills, Ill.) ultrasonic nebulizers.

Compounds of the invention may also be formulated for use as topical powders and sprays that can contain, in addition to the compounds of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

Transdermal patches have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

According to the methods of treatment of the present invention, bacterial infections are treated or prevented in a patient such as a human or lower mammal by administering to the patient a therapeutically effective amount of a compound of Formula I, or a stereoisomer or pharmaceutically acceptable salt thereof, in such amounts and for such time as is necessary to achieve the desired result. By a "therapeutically effective amount" of a compound of the invention is meant a sufficient amount of the compound to treat bacterial infections, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

The total daily dose of the compounds of this invention administered to a human or other mammal in single or in divided doses can be in amounts, for example, from 0.01 to 200 mg/kg body weight or more usually from 0.1 to 50 mg/kg body weight. In certain embodiments, the total daily dose administered to a human or other mammal is from 1.0 to 100 mg/kg body weight or from 5.0 to 25 mg/kg body weight. Single dose compositions may contain such amounts or submultiples thereof to make up the daily dose. In general, treatment regimens according to the present invention comprise administration to a patient in need of such treatment from about 10 mg to about 15 g of the compound(s) of this invention per day in single or multiple doses, more usually, from 100 mg to 5 g, and even more usually from 250 mg to 1 g per day in single or multiple doses.

Methods of formulation are well known in the art and are disclosed, for example, in Remington: The Science and Practice of Pharmacy, Mack Publishing Company, Easton, Pa., 19th Edition (1995). Pharmaceutical compositions for use in the present invention can be in the form of sterile, non-pyrogenic liquid solutions or suspensions, coated capsules, suppositories, lyophilized powders, transdermal patches or other forms known in the art.

A “kit” as used in the instant application includes a container for containing the pharmaceutical compositions and may also include divided containers such as a divided bottle or a divided foil packet. The container can be in any conventional shape or form as known in the art that is made of a pharmaceutically acceptable material, for example a paper or cardboard box, a glass or plastic bottle or jar, a resealable bag (for example, to hold a “refill” of tablets for placement into a different container), or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. The container employed can depend on the exact dosage form involved, for example a conventional cardboard box would not generally be used to hold a liquid suspension. It is feasible that more than one container can be used together in a single package to market a single dosage form. For example, tablets may be contained in a bottle that is in turn contained within a box.

An example of such a kit is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material covered with a foil of a preferably transparent plastic material. During the packaging process, recesses are formed in the plastic foil. The recesses have the size and shape of individual tablets or capsules to be packed or may have the size and shape to accommodate multiple tablets and/or capsules to be packed. Next, the tablets or capsules are placed in the recesses accordingly and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil that is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are individually sealed or collectively sealed, as desired, in the recesses between the plastic foil and the sheet. Preferably the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

It maybe desirable to provide a written memory aid, where the written memory aid is of the type containing information and/or instructions for the physician, pharmacist or other health care provider, or subject, e.g., in the form of numbers next to the tablets or capsules whereby the numbers correspond with the days of the regimen that the tablets or capsules so specified should be ingested or a card that contains the same type of information. Another example of such a memory aid is a calendar printed on the card e.g., as follows "First Week, Monday, Tuesday,". . . etc . . . "Second Week, Monday, Tuesday, . . ." etc. Other variations of memory aids will be readily apparent. A "daily dose" can be a single tablet or capsule or several tablets or capsules to be taken on a given day. When the kit contains separate compositions, a daily dose of one or more compositions of the kit can consist of one tablet or capsule while a daily dose of another one or more compositions of the kit can consist of several tablets or capsules.

Another specific embodiment of a kit is a dispenser designed to dispense the daily doses one at a time in the order of their intended use. Preferably, the dispenser is equipped with a memory-aid, so as to further facilitate compliance with the regimen. An example of such a memory-aid is a mechanical counter that indicates the number of daily doses that has been dispensed. Another example of such a memory-aid is a battery-powered micro-chip memory coupled with a liquid crystal readout, or audible reminder signal that, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

The kits of the present invention may also include, in addition to a compound of the present invention, one or more additional pharmaceutically active compounds. For example, the additional compound second antibacterial. The additional compounds may be administered in the same dosage form as the compound of the present invention or in a different dosage form. Likewise, the additional compounds can be administered at the same time as the compound of the present invention or at different times.

Compositions of the present compounds may also be used in combination with other known antibacterial agents of similar spectrum to (1) enhance treatment of severe gram-negative infections covered by the spectrum of this compound or (2) add coverage in severe infections in which multiple organisms are suspected in which another agent of a different spectrum may be required in addition to this compound. Potential agents include members of the aminoglycosides, penicillins, cephalosporins, fluoroquinolones, macrolides, glycopeptides, lipopeptides and oxazolidinones. The treatment can involve administering a composition having both a compound of the present invention and a second antibacterial compound or administration of a compound of the present inventive compounds followed by or preceded by administration of a second antibacterial agent.

The foregoing may be better understood by reference to the following examples, that are presented for illustration and not to limit the scope of the inventive concepts.

VII. EXAMPLES

Referring to the examples that follow, compounds of the present invention were characterized by high performance liquid chromatography (HPLC) using a Waters Millenium chromatography system with a 2690 Separation Module (Milford, Mass.). The analytical columns were Alltima C-18 reversed phase, 4.6×250 mm from Alltech (Deerfield, Ill.). A gradient elution was used, typically starting with 5% acetonitrile/95% water and progressing to 100% acetonitrile over a period of 40 minutes. All solvents contained 0.1% trifluoroacetic acid (TFA). Compounds were detected by ultraviolet light (UV) absorption at either 220 or 254 nm. In some instances, purity was assessed by thin layer chromatography (TLC) using glass or plastic backed silica gel plates, such as, for example, Baker-Flex Silica Gel 1 B2-F flexible sheets. TLC results were readily detected visually under ultraviolet light, or by employing well known iodine vapor and other various staining techniques.

Mass spectrometric analysis was performed on one of two LCMS instruments: a Waters System. (Alliance HT HPLC and a Micromass ZQ mass spectrometer; Column: Eclipse XDB-C-18, 2.1×50 mm; solvent system: 5-95% (or 35-95%, or 65-95% or 95-95%) acetonitrile in water with 0.05%TFA; flow rate 0.8 mL/min; molecular weight range 500-1500; cone Voltage 20 V; column temperature 40° C.) or a Hewlett Packard System (Series 1100 HPLC; Column: Eclipse XDB-C18, 2.1×50 mm;

solvent system: 1-95% acetonitrile in water with 0.05% TFA; flow rate 0.4 mL/min; molecular weight range 150-850; cone Voltage 50 V; column temperature 30° C). All masses are reported as those of the protonated parent ions.

GCMS analysis was performed on a Hewlet Packard instrument (HP6890 Series gas chromatograph with a Mass Selective Detector 5973; injector volume: 1 μL; initial column temperature: 50° C.; final column temperature: 250C; ramp time: 20 minutes; gas flow rate: 1 mL/min; column: 5% phenyl methyl siloxane, Model #HP 190915-443, dimensions: 30.0 m×25 m×0.25 m).

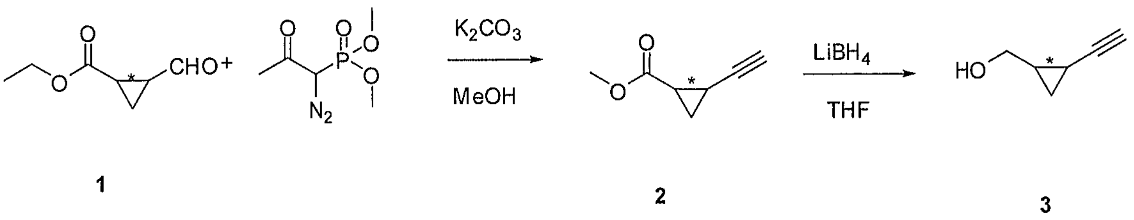
Nuclear magnetic resonance (NMR) analysis was performed with a Varian 300 MHz NMR (Palo Alto, Calif.). The spectral reference was either TMS or the known chemical shift of the solvent. Some compound samples were run at elevated temperatures (e.g. 75° C.) to promote increased sample solubility.

The purity of some of the invention compounds was assessed by elemental analysis (Desert Analytics, Tucson, Ariz.)

Melting points were determined on a Laboratory Devices Mel-Temp apparatus (Holliston, Mass.).

Preparative separations were carried out using a Flash 40 chromatography system and KP-Sil, 60A (Biotage, Charlottesville, Va.), or by flash column chromatography using silica gel (230-400 mesh) packing material, or by HPLC using a C-18 reversed phase column. Typical solvents employed for the Flash 40 Biotage system and flash column chromatography were dichloromethane, methanol, ethyl acetate, hexane, acetone, aqueous hydroxylamine and triethyl amine. Typical solvents employed for the reverse phase HPLC were varying concentrations of acetonitrile and water with 0.1% trifluoroacetic acid.

A. Synthesis of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-1)

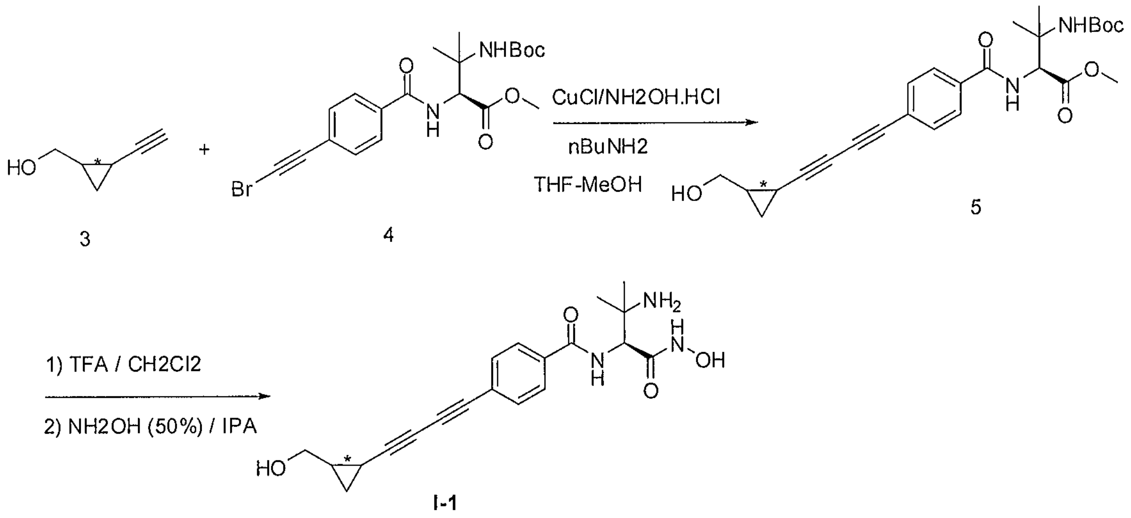


Methyl 2-ethynylcyclopropanecarboxylate(2)

Racemic ethyl 2-formylcyclopropanecarboxylate **1** (10 g, 70.3 mmol) and Bestmann Ohira reagent (16.4g, 85mmol) were dissolved in anhydrous methanol (100ml) under N_2. Potassium carbonate (19.4g, 141 mmol) was then added slowly in portions and the solution was stirred for 18h. The solvents were removed under reduced pressure at 20°C, water (100ml) was added and the product was extracted with dichloromethane (2x 200ml), dried over sodium sulfate, and concentrated slowly to give racemic methyl 2-ethynylcyclopropanecarboxylate (4.16g) which was confirmed by NMR.

(2-Ethynylcyclopropyl)methanol (3)

Lithium borohydride (175mg, 8.06mmol) was then added slowly to racemic methyl 2-ethynylcyclopropanecarboxylate (1g, 20.6mmol) in anhydrous THF (20ml) under nitrogen and stirring was continued for 2h. Reaction mixture was quenched with a few drops of acetic acid and solvent was removed. The crude product was extracted with ethyl acetate (2x50ml), dried over sodium sulfate and concentrated slowly to give racemic (2-trans-ethynylcyclopropyl)methanol, **3**, as yellow liquid (735mg) and used as such for the next step.



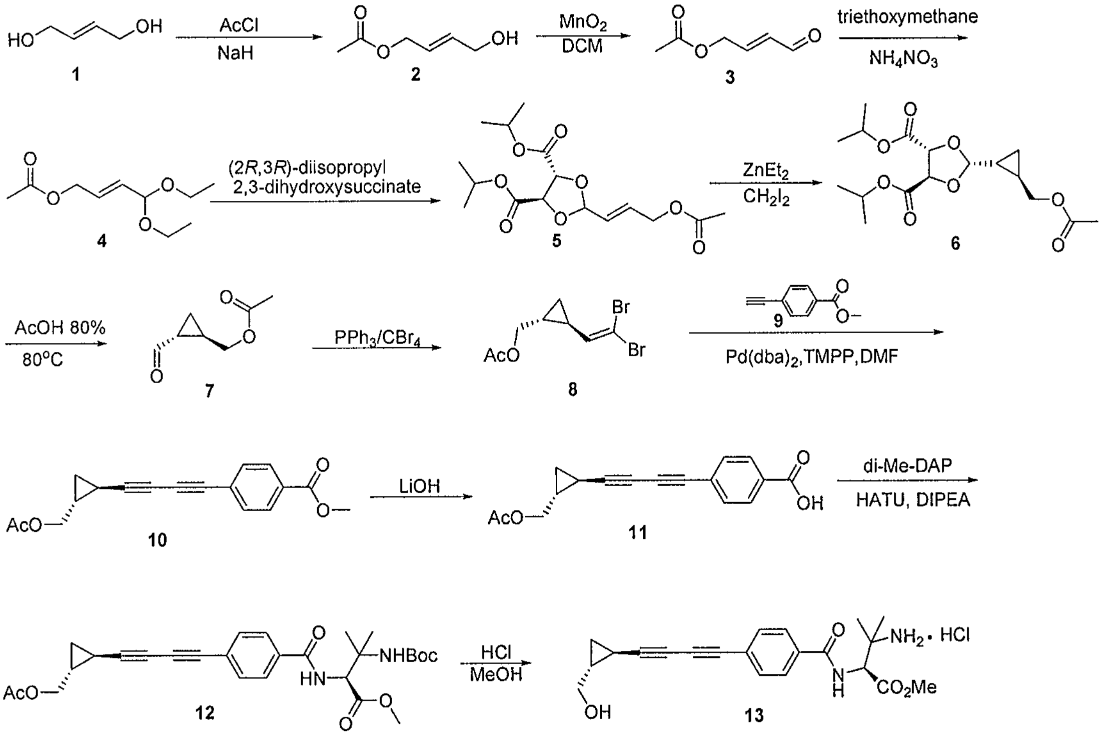
(2S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-((2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (5)

CuCl (42mg, 0.416 mmol) was added slowly to a stirred solution of racemic (2-*trans*-ethynylcyclopropyl)methanol (400mg, 4.16mmol), methyl 2-(S)-(4-(bromoethynyl)benzamido)-3-(tert-butoxycarbonylamino)-3-methylbutanoate (1.9g, 4.16mmol) in a mixture of THF (20ml), methanol (10ml) and butyl amine (10ml) under nitrogen followed by a few crystals of hydroxylamine hydrochloride (30mgs). Stirring was continued for 4h. The solvent was removed under reduced pressure, water (100ml) was added and extracted with ethyl acetate (2x150ml), dried over sodium sulfate, concentrated and purified on ISCO to give 432mg of (2-S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-((2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate.

I-1: N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2 trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide

(2-S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-((2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate 5 (0.370g, 0.814mmol) was taken into dichloromethane (25ml), treated with TFA (2ml) and stirred for 20min. Excess solvent and TFA was removed under reduced pressure to give deprotected material which was re-dissolved in IPA (10mL) and treated with aqueous hydroxylamine (50%, 2mL, excess) and kept in refrigerator for 2 days. Excess solvent was removed under reduced pressure and the crude product was purified by reverse phase HPLC to give 42mgs of I-1 as its trifluoroacetate salt. LC-MS (M+1) 370, Chemical Formula: $C_{20}H_{23}N_3O_4$, Exact Mass: 369.17. ^{1}HNMR (DMSO-d6), TFA salt: δ 0.9 (m, 2H) 1.24(s, 3H), 1.29 (s, 3H), 1.40(m, 1H), 3.24(m, 1H), 3.4(m, 1H), 4.64(d, 1H), 4.69(d, 1H), 7.61(d, 2H), 7.88(d, 2H), 7.90(br.d, 1NH), 8.65(d, 1NH), 9.1(br.S, OH).

B. Synthesis of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1R,2R)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-2)



(E)-4-hydroxybut-2-enyl acetate (2)

Reagent	MW	Eq.	mol	g, mL

Compound **1**	88.05	1	3	264 g
NaH(60%)	24.00	1	3	120.0 g
CH_3COCl	78.50	1	3	235.5 g
THF				1.5 L

To a solution of (E)-but-2-ene-1,4-diol **1** (264g , 3.0 mol) in THF 1.5 L, added sodium hydride (120g, 3.0 mol) in portions under -20°C. Upon the addition, the mixture was kept stirring at -20°C for 30 mins. Then acetyl chloride (235.5g, 3 mol) was added dropwise, the mixture allowed to warm to r.t. and kept stirring at r.t. for another 3 hours. The mixture was filtered and the residue was washed with THF. The combined organic layer were dried and concentrated to give crude **2** which was purified by silica gel column (PE:EA=5:1-2:1) to give **2,** 210 g as a colorless oil. Yield: 54%. 1HNMR:CP-0005065-043 (CDCl3,400 M HZ) δ: 5.85(m ,1H), 5.62(m, 1H), 4.67(t, J=6.2 Hz,2H), 4.26 (t , J=6.0 Hz,2H), 2.10(s,1H), 2.06(s,3H).

(E)-4-oxobut-2-enyl acetate (3)

Reagent	MW	Eq.	mol	g, mL
Compound **2**	130.14	1	1.5	195 g
MnO2(active)	86.94	10	15	1305 g
DCM				3 L

To a suspension of manganese dioxide (active, 1305g, 15mol) in 2.5L dichloromethane, added (E)-4-hydroxybut-2-enyl acetate **2** (195g) in portions. The mixture was kept stirring at r.t. for 48 hours. The mixture was filtered and the residue was washed with dichloromethane. The combined organic layer were dried and concentrated to give crude **3** which was purified by silica gel column (PE:EA=10:1-5:1) to give **3,** 130 g as a colorless oil. Yield: 64%. 1HNMR:CP-0005065-044(CDCl3,400 M HZ) δ: 10.01(d, J=6.4Hz ,1H), 6.52(m, 1H), 6.10(m ,1H), 5.08 (m, 2H), 2.10(s,3H).

(E)-4,4-diethoxybut-2-enyl acetate (4)

Reagent	MW	Eq.	mol	g, mL
Compound **3**	128.13	1	0.75	96 g
Triethoxymethane	148.20	1.2	0.90	133.2 g
NH_4NO_3	79.90	0.05	0.038	3.0 g
EtOH				500 mL

To a solution of (E)-4-oxobut-2-enyl acetate **3** (96.0 g, 0.75 mol) and triethoxymethane (133.2g, 0.9 mol) in 500 ml ethanol, added ammonium nitrate (3.0 g, 0.038mol), the mixture was kept stirring at r.t. for 15 hours. The mixture was diluted with 800 ml EtOAc and washed with saturated sodium bicarbonate. The aqueous layer was back extracted with EtOAc (300ml x 2). The combined organic layers were dried and concentrated to give crude **4** 140 g as a red oil which was used for the next step without for further purification.

(4R,5R,E)-diisopropyl-2-(3-acetoxyprop-1-enyl)-1,3-dioxolane-4,5-dicarboxylate (5)

Reagent	MW	Eq.	mol	g, mL
Compound **4**	202.25	1	0.3	60.6 g
(2R,3R)-diisopropyl 2,3-dihydroxysuccinate	234.25	1.1	0.33	77.2 g
PPTS	251.09	0.05	0.015	3.8 g
benzene				500 ml

To a solution of (E)-4,4-diethoxybut-2-enyl acetate **4** (60.6g, 0.3 mol) and (2R,3R)-diisopropyl 2,3-dihydroxysuccinate (77.2g, 0.33 mol) in 500 ml benzene, added PPTS (3.8 g, 15 mmol), the mixture was heated to 90°C to distill off the ethanol for 15 hours . The mixture was cooled to r.t. and concentrated in vacuum. Purified by silica gel with (PE:EA=50:1-30:1), 38.5 g **5** as a colorless oil was obtained. Yield:37.3%. GCMS:CP-0005065-070-2 (85% pure).

(4R,5R)-diisopropyl-2-((1S,2R)-2-(acetoxymethyl)cyclopropyl)-1,3-dioxolane-4,5-dicarboxylate (6)

Reagent	MW	Eq.	mol	g, mL
Compound **5**	344.36	1	0.1	34.4 g
$ZnEt_2$(1M in hexane)		5	0.5	500 ml
CH_2I_2	267.84	10	1	267.8 g
Hexane				1.5 L

To a solution of (4R,5R,E)-diisopropyl-2-(3-acetoxyprop-1-enyl)-1,3-dioxolane-4,5-dicarboxylate **5** (34.4 g , 0.1 mol) in hexane (1.5L), added diethyl zinc (1M in hexane, 500 mL) in portion under argon at -20°C. Upon the addition, diiodoethane was added dropwise below -20°C with strong stirring. The mixture allowed warm to r.t. and kept stirring for another 8 hours. The reaction mixture was quenched by 800 ml cold aqueous ammonium chloride, then extracted with ether (800 ml x 5). The combined organic layer were washed with aqueous sodium thiosulfate, water, brine, then dried and concentrated to give crude **6** which was purified by silica gel column (PE:EA=30:1-10:1) to give **6,** 16 g as a colorless oil. Yield : 44.7%. 1HNMR:PO5 (CDCl3,400 MHZ) δ: 5.13(m ,2H), 4.95(d, J=5.6 Hz ,1H), 4.67(d, J=3.6 Hz,1H), 4.57 (d, J=4.0 Hz,1H), 4.07(m,1H), 3.88(m,1H), 2.06(s,3H), 1.38(s,12H), 1.23(m,1H), 0.83(m,1H), 0.66(m,1H).

((1R,2R)-2-formylcyclopropyl)methyl acetate (7)

Reagent	MW	Eq.	mmol	g, mL
Compound **6**	358.38	1	40	14.3g
AcOH(80%)				140 mL

A mixture of (4R,5R)-diisopropyl-2-((1S,2R)-2-(acetoxymethyl)cyclopropyl)-1,3-dioxolane-4,5-dicarboxylate **6** (14.3 g, 40 mmol) in 140ml 80% acetic acid was heated to 80°C and kept stirring at this temperature for 2 hours. When TLC showed little **6** remaining, the mixture was added to 300 ml saturated sodium bicarbonate dropwise, then extracted with dichloromethane (200 ml x 3). The combined organic layers were washed with water, brine dried and concentrated to give crude **7** which was purified by silica gel column (PE:EA=10:1-5:1) to give **7**, 3.5 g as a colorless oil. Yield : 62%. 1HNMR:CP-0005065-072(CDCl3,400 M HZ) δ: 9.15(s, 1H), 4.11(m, 1H), 3.91 (m ,1H) , 2.08 (s, 3H), 2.10(s,3H), 1.88(m ,2H), 1.39(m, 1H), 1.12(m ,1H).

((1R,2R)-2-(2,2-dibromovinyl)cyclopropyl)methyl acetate (8)

Reagent	MW	Eq.	mmol	g, mL
Compound **7**	142.15	1	21	3.00 g
CBr_4	331.63	2	42	13.9 g
PPh3	262.29	4	84	22.0 g
DCM				120 mL

To a solution of carbon tetrabromide (13.9 g, 42 mmol) in dichloromethane 30 mL, added a solution of triphenylphosphine (22.0 g, 84 mmol) in 50 mL dichloromethane dropwise at -20°C under argon. The mixture was kept stirring at this temperature for half an hour, then cooled to -78°C. Added a solution of ((1R,2R)-2-formylcyclopropyl)methyl acetate **7** (3.00 g, 21 mmol) in 40 ml dichloromethane dropwise, maintained the temperature for another half an hour. The mixture was allowed warm to r.t. over 30 mins. Removed the solvent and purified by silica gel column (PE:EA=100:1~50:1) to give **8** as a colorless oil 4.3g. Yield: 69%. 1HNMR:CP-0005065-075(CDCl3,400 M HZ) δ: 5.85(d, J=8.8Hz ,1H), 3.98(m, 2H), 2.09 (m ,3H) , 1.61 (m, 1H), 1.35(m,1H), 0.88(m, 2H).

Methyl 4-(((1R,2R)-2-(acetoxymethyl)cyclopropyl)buta-1,3-diynyl)benzoate (10)

Reagent	MW	Eq.	mmol	g, mL
Compound **8**	297.97	1	14.5	4.3 g
Compound **9**	160.17	1.1	16.0	2.56 g
Pd_2dba_2	575.22	0.01	0.15	86.3 mg
TMPP	352.24	0.04	0..58	204.2 mg
NEt3	101.19	3	43.5	4.35 g
DMF				100 mL

To a solution of ((1R,2R)-2-(2,2-dibromovinyl)cyclopropyl)methyl acetate **8** (4.3g, 14.5mmol), Pd_2dba_2 (86.3mg, 0.15mmol), tri(4-methylphenyl)phosphine (204mg, 0.58mmol), triethylamine (4.35g, 43.5mmol) in DMF 100 mL, treated with methyl 4-ethynylbenzoate **9** (2.64g, 11mmol) under argon. The mixture was kept stirring at r.t. for 5 hours. When TLC showed little compound **8** remaining, the reaction was diluted with EtOAc (300 mL) and washed with water (3x100 mL), the organic layer was dried and concentrated to give crude **10** which was purified by silica gel column chromatography PE:EA= 50: 1 ~30:1 to give **10** 2.0 g as a yellow solid. Yield: 46.5 %, LCMS:CP-0005065-085-2 (ESI) m / z =297 (M+1) purity:92.4 % (214nm).

4-(((1R,2R)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzoic acid (11)

Reagent	MW	Eq.	mmol	g, mL
Compound **10**	296.32	1	6.5	1.92 g
NaOH	40.10	10	65	2.60 g
THF				40 mL

Methyl 4-(((1R,2R)-2-(acetoxymethyl)cyclopropyl)buta-1,3-diynyl)benzoate **10** (1.92g, 6.5mmol) was dissolved in THF (40 mL), then added a solution of sodium hydroxide (2.60g, 65 mmol) in 10mL water. The mixture was kept stirring at r.t. for 8 hours. When LCMS showed little compound **10** remaining, the solvent was removed under reduced pressure, the residue was diluted with water (50 mL), adjusted the pH to 4.0, extracted with ethyl acetate (4x50 mL), the organic layer was dried and concentrated to give crude **11** 1.4 g as a yellow solid which was used for next step without further purification. LCMS:CP-0005065-088-3 (ESI) m / z =241 (M+1) purity:89 % (214nm). Yield: 89 %.

(S)-methyl 2-(4-(((1R,2R)-2-(acetoxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-(tert-butoxycarbonylamino)-3-methylbutanoate (12)

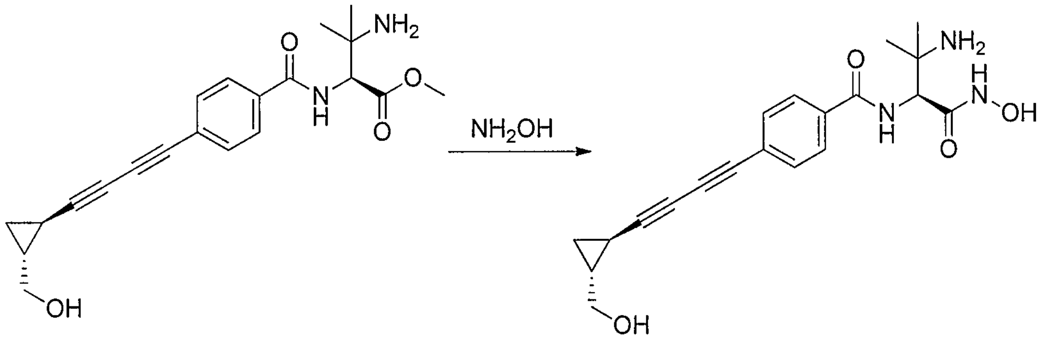
Reagent	MW	Eq.	mmol	g, mL
Compound **11**	240.25	1	5.0	1.20 g
Boc-di-Me-DAP	246.30	1.2	6.0	1.48g
HATU	390.12	1.2	6.0	2.34 g
DIPEA	129.24	4	20	3.58 g
DMF				50 mL

To a solution of 4-(((1R,2R)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzoic acid **11** (1.20g, 5.0mmol), HATU (2.34 g , 6 mmol) in DMF 50mL, treated with (S)-methyl 2-amino-3-(tert-butoxycarbonylamino)-3-methylbutanoate (1.48g, 6.0mmoL) and DIPEA (3.58g, 20mmoL). The mixture was kept stirring at r.t. for 5 hours. When LCMS showed little compound **11** remaining, the reaction was diluted with EtOAc (100 mL), washed with 5% lithium chloride (3x50mL), the organic layer was dried and concentrated to give **12** as a yellow oil. Purification by silica gel column chromatography PE:EA=2:1 gave 2.0g **12** as a colorless oil, yield: 70%, LCMS:CP-0005065-091-3 (ESI) m / z = 469 (M+1) purity:95 % (214nm).

(S)-methyl 3-amino-2-(4-(((1R,2R)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate hydrochloride (13)

Reagent	MW	Eq.	mmol	g, mL
Compound **12**	468.23	1	4.0	1.87 g
HCl(g)	36.5			
CH_3OH				50 mL

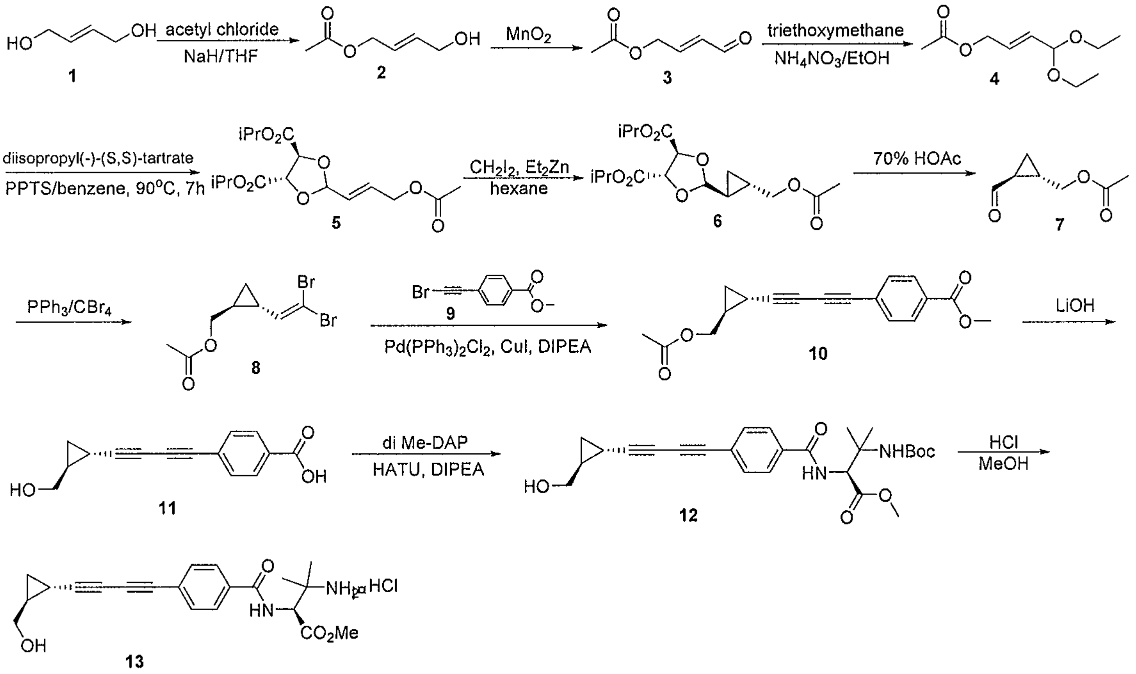
(S)-methyl2-(4-(((1R,2R)-2-(acetoxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-(tert-butoxycarbonylamino)-3-methylbutanoate **12** (1.87g, 4.0mmoL) was dissolved in methanol (50mL), treated with dry HCl_g for 10mins. When LCMS showed little compound **12** remaining, stopped HCl flow. Removed the solvent under reduce pressure, and gave **13,** 1.45g as a yellow solid. Yield: 91%, LCMS: CP-0005065-096-4-LCMSA019 (ESI) m / z = 369 (M+1) purity: 98 % (214nm). 1H NMR:CP-0005065-096-4 (DMSO-d_6, 400 MHz) δ:9.04(d, J=6.8Hz, 1H), 8.36(s ,3H), 7.98(d, J=6.4Hz , 2H), 7.64(d, J=6.8Hz , 2H), 4.89(d, J=6.8Hz, 1H), 3.73(s, 3H), 3.44(m, 1H), 3.22(m, 1H), 1.48(m, 2H), 1.40(s, 6H), 0.94(m, 2H).



(S)-methyl 3-amino-2-(4-(((1R,2R)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate hydrochloride **13** (1.25g, 3.1mmol) was treated with isopropanol (10mL) and 50% aqueous hydroxylamine (4.1mL, 61.7mmol, 20 equivalents) until predominantly complete by LCMS.

The crude material was purified by reverse-phase HPLC (gradient of 0-30% acetonitrile in water, each containing 0.1% TFA, over 120 minutes) and the desired fractions pooled and lyophilized to give N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1R,2R)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide as its trifluoroacetate salt (white solid, 647mg, 1.3mmol, 43%). Mass spec data: expected (M+1): 370.4, observed: 370.2. Proton NMR (400MHz, dmso-d6): 11.20 (s, 1H), 9.43 (br s, 1H), 8.55 (d, 1H, J = 9.6Hz), 8.00 (br s, 3H), 7.89 (dd, 2H, J = 1.8, 6.6Hz), 7.60 (dd, 2H, J = 2.0, 6.8Hz), 8.66 (d, 1H, J = 9.2Hz), 3.39 (dd, 1H, J = 4.8, 11.6Hz), 3.22 (dd, 1H, J = 5.8, 11.4Hz), 1.39 – 1.46 (m, 2H), 1.30 (s, 3H), 1.25 (s, 3H), 0.84 – 0.93 (m, 2H)

C. Synthesis of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1S,2S)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-3)



(4S,5S,E)-diisopropyl-2-(3-acetoxyprop-1-enyl)-1,3-dioxolane-4,5-dicarboxylate (5)

Reagent	MW	Eq.	mol	g, mL

Compound 4	202.25	1	0.37	75 g
diisopropyl(-)-(S,S)-tartrate	234.25	1	0.37	86.8 g
PPTS	251.09	0.05	0.0185	4.66 g
Benzene				800 ml

To a solution of (E)-4,4-diethoxybut-2-enyl acetate **4** (75 g, 0.37 mol) and diisopropyl(-)-(S,S)-tartrate (86.8g, 0.3 mol) in 800 ml benzene, added PPTS (4.66 g, 0.0185 mol), the mixture was heated to 9 °C to distill off the ethanol for 15 hours . The mixture was cooled to r.t. and concentrated in vacuum. Purified with distillation to give Compound **5** (50.0 g, 39%) as a colorless oil. 1H NMR: (CDCl3, 400 MHz) δ:6.04-6.01(m, 1H), 5.81-5.80 (m ,1H), 5.57(d, J = 8Hz , 1H), 5.07-5.01(m, 2H), 4.65(d, J = 4 Hz, 1H), 4.57 (d, J = 4 Hz, 1H), 4.54-4.53 (m, 2H), 1.99(s, 3H), 1.23-1.19(m, 12H).

(4S,5S)-diisopropyl-2-((1R,2S)-2-(acetoxymethyl)cyclopropyl)-1,3-dioxolane-4,5-dicarboxylate (6)

Reagent	MW	Eq.	mol	g, mL
Compound **5**	344.36	1	0.12	40 g
$ZnEt_2$(1M in hexane)		10	1.20	1.16 L
CH_2I_2	267.84	20	2.40	623 g
Hexane				1.0 L

To a solution of (4S,5S,E)-diisopropyl-2-(3-acetoxyprop-1-enyl)-1,3-dioxolane-4,5-dicarboxylate **5** (40g, 0.12mol) in hexane (1.0L), added diethyl zinc (1M in hexane, 1.16L) in portions under argon at -20°C. Upon the addition, diiodomethane (623g, 2.40mol) was added dropwise below -20°C with strong stirring. The mixture allowed warm to r.t. and kept stirring for another 8 hours. The reaction mixture was quenched by 800 ml cold aqueous ammonium chloride, then extracted with ether (800 mL x 5). The combined organic layer was washed with aqueous sodium thiosulfate , water, brine, then dried and concentrated to give crude compound **6** which was purified by silica gel column with ethyl acetate in petroleum ether (3%-10% v/v) to give Compound **6** (20.0 g, 50%) as a colorless oil. ^{1}H NMR: ($CDCl_3$,400 MHz) δ: 5.15-5.08 (m ,2H), 4.95(d, J = 5.6 Hz ,1H), 4.67(d, J = 3.6 Hz,1H), 4.57 (d, J = 4.0 Hz,1H), 4.07-4.04 (m,1H), 3.91-3.86(m,1H), 2.06(s,3H), 1.40-1.37(m,1H), 1.31-1.28 (m,12H), 1.24-1.22(m,1H), 0.85-0.82(m,1H), 0.68-0.63(m,1H).

((1S,2S)-2-formylcyclopropyl)methyl acetate (7)

Reagent	MW	Eq.	mmol	g, mL
Compound **6**	358.38	1	55.80	20.0 g
AcOH (80%)				200 mL

A mixture of (4S,5S)-diisopropyl-2-((1R,2S)-2-(acetoxymethyl)cyclopropyl)-1,3-dioxolane-4,5-dicarboxylate **6** (20.0g, 55.8mmol) in 200ml 80% acetic acid was heated to 80°C and kept stirring at this temperature for 2 hours. When TLC showed little **6** remaining, the mixture was diluted with water (150mL), extracted with ethyl acetate (200ml x 3). The combined organic layer was washed with saturated sodium bicarbonate, water, brine, then dried and concentrated to give crude compound **7** which was purified by silica gel column with ethyl acetate in petroleum ether (8%-20% v/v) to give Compound **7** (3.0, 38%) as a colorless oil . ^{1}H NMR: ($CDCl_3$, 400 MHz) δ: 9.15(d, J = 4.8, 1H), 4.14-4.09(m, 1H), 3.95-3.90 (m ,1H) , 2.08 (s, 3H), 1.92-1.88(m,2H), 1.38-1.36(m ,1H), 1.12-1.09(m ,1H) .

((1S,2S)-2-(2,2-dibromovinyl)cyclopropyl)methyl acetate (8)

Reagent	MW	Eq.	mmol	g, mL
Compound **7**	142.15	1	21.00	3.00 g
CBr_4	331.63	2	42.00	13.90 g
PPh_3	262.29	4	84.00	22.00 g
DCM				120 mL

To a solution of carbon tetrabromide (13.9g, 42.0 mmol) in dichloromethane (30mL), added a solution of triphenylphosphine (22.0g, 84.0mmol) in 50mL dichloromethane dropwise at -20°C under argon. The mixture was kept stirring at this temperature for half an hour, then cooled to -78°C. Added a solution of ((1S,2S)-2-formylcyclopropyl)methyl acetate **7** (3.0g, 21.0mmol) in 40mL dichloromethane dropwise, maintained the temperature for another half an hour. The mixture was allowed warm to r.t. over 30 mins. Removed the solvent under vacuum and the residue was purified by silica gel column with petroleum ether to give Compound **8** (3.20g, 51%) as a colorless oil. Confirmed by GC-MS.

methyl 4-(((1S,2S)-2-(acetoxymethyl)cyclopropyl)buta-1,3-diynyl)benzoate (10)

Reagent	MW	Eq.	mmol	g, mL
Compound **8**	297.97	1	10.85	3.2 g
Compound **9**	160.17	1.1	15.20	2.43 g
Pd_2dba_2	575.22	0.01	0.11	62.4 mg
TMPP	352.24	0.04	0.43	153 mg
NEt3	101.19	3	14.25	1.44 g
DMF				50 mL

To a solution of ((1S,2S)-2-(2,2-dibromovinyl)cyclopropyl)methyl acetate **8** (3.2g, 10.85mmol), Pd_2dba_2 (62.4mg, 0.11mmol), tri(4-methylphenyl) phosphine (153mg, 0.43mmol), triethylamine (1.44g, 14.25mmol) in DMF (50mL), treated with methyl 4-ethynylbenzoate **9** (2.43g, 15.2mmol) under Argon. The mixture was kept stirring at r.t. overnight. The reaction was diluted with EtOAc (300mL), washed with water (3 x 100mL), the combined organic layer was dried and concentrated to give crude Compound **10** which was purified by silica gel column chromatography with ethyl acetate in petroleum ether (3%-10% v/v) to give Compound **10** (1.4g, 46.5%) as a yellow solid. LC-MS:297 $[M+H]^+$.

4-(((1S,2S)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzoic acid (11)

Reagent	MW	Eq.	mmol	g, mL
Compound **10**	296.32	1	4.72	1.4 g
NaOH	40.10	4	18.90	756 mg
THF				30 mL

To a solution of methyl 4-(((1S,2S)-2-(acetoxymethyl)cyclopropyl)buta-1,3-diynyl)benzoate **10** (1.4g) in THF (30 mL) was added a solution of sodium hydroxide (75mg, 18.9mmol) in water (10 mL). The mixture was kept stirring at r.t. overnight. After this time, the solvent was removed under reduced pressure, the residue was diluted with water (50mL), adjusted the pH 4.0 with 1M HCl, then extracted with EtOAc (4 x 50mL), the organic layer was dried and concentrated to give Compound **11** (1.05g, 93%) as a yellow solid which used for next step without further purification. LC-MS:241 $[M+H]^+$.

(S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-(((1S,2S)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (12)

Reagent	MW	Eq.	mmol	g, mL
Compound **11**	240.25	1	4.38	1.05 g
Boc-di-Me-DAP	246.30	1	4.38	1.076g
HATU	390.12	1.1	4.81	1.83 g
DIPEA	129.24	3	13.10	1.69 g
DMF				25 mL

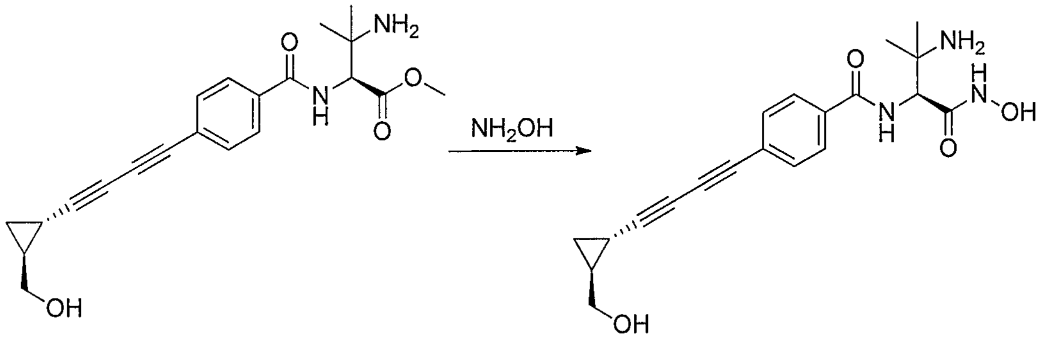
To a solution of 4-(((1S,2S)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzoic acid **11** (1.05g, 4.38mmol), HATU (1.83g, 4.81mmol) in DMF (25mL) was added (S)-methyl 2-amino-3-(tert-butoxycarbonylamino)-3-methylbutanoate

(1.076g, 4.38mmoL) and DIPEA (1.69g,̇ 13.1mmoL). The mixture was kept stirring at r.t. for 5 hours. After this time, the reaction was diluted with water (20mL), then extracted with ethyl acetate (60mL x 3). The combined organic layers were washed with water and brine, then dried and concentrated to give Compound **12** as a yellow oil. (1.35g, 65%) LC-MS:469 $[M+H]^+$.

(S)-methyl 3-amino-2-(4-(((1S,2S)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate hydrochloride (13)

Reagent	MW	Eq.	mmol	g, mL
Compound **12**	468.23	1	2.78	1.3 g
HCl(g)				
CH_3OH				20 mL

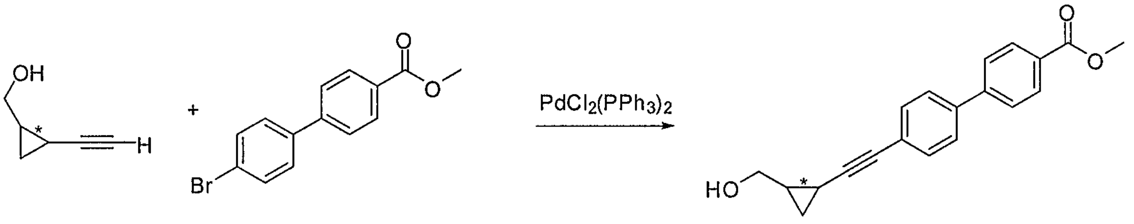
A solution of (S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-(((1S,2S)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate **12** (1.3g, 2.78mmol) in methanol (20mL) was attached to a HCl apparatus. Then the reaction was stirred at room temperature until TLC indicated the total consumption of the starting material. After that, the solution was concentrated under reduced pressure to give **13** as yellow solid (1.1 g, 98%). LC-MS:369 $[M+H]^+$. 1H-NMR: (DMSO-d_6, 400 MHz) δ:9.04(d, J = 6.8Hz, 1H), 8.38(s ,3H), 7.98(d, J = 6.8Hz , 2H), 7.64(d, J = 6.4Hz , 2H), 4.88(d, J = 6.8Hz, 1H), 3.72(s, 3H), 3.45-3.42(m, 1H), 3.28-3.24(m, 1H), 1.49-1.46(m, 2H), 1.40(s, 6H), 0.95-0.90(m, 2H).



(S)-methyl 3-amino-2-(4-(((1S,2S)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate hydrochloride **13** (1.15g, 2.8mmol) was treated with isopropanol (10mL) and 50% aqueous hydroxylamine (3.8mL, 56.8mmol, 20 equivalents) until predominantly complete by LCMS.

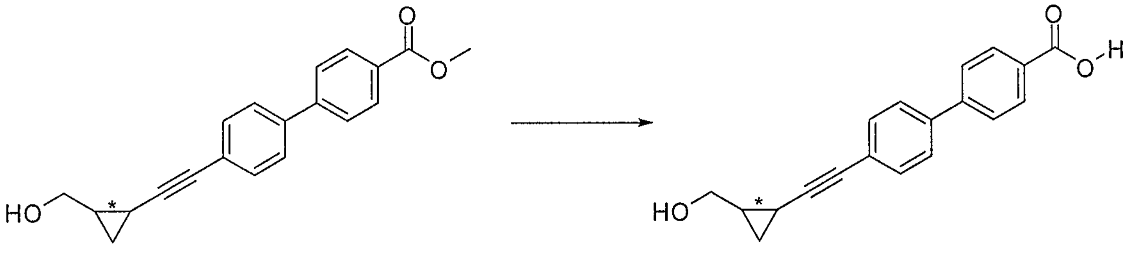
The crude material was purified by reverse-phase HPLC (gradient of 0-30% acetonitrile in water, each containing 0.1% TFA, over 120 minutes) and the desired fractions pooled and lyophilized to give N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1S,2S)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide as its trifluoroacetate salt (white solid, 446mg, 0.92mmol, 33%). Mass spec data: expected (M+1): 370.4, observed: 370.2. Proton NMR (400MHz, dmso-d6): 11.20 (s, 1H), 9.22 (br s, 1H), 8.55 (d, 1H, J = 9.6Hz), 7.99 (br s, 3H), 7.89 (dd, 2H, J = 2.0, 6.8Hz), 7.61 (dd, 2H, J = 1.6, 6.8Hz), 4.66 (d, 1H, J = 9.6Hz), 3.40 (dd, 1H, J = 5.0, 11.4Hz), 3.22 (dd, 1H, J = 5.8, 11.8Hz), 1.38 – 1.46 (m, 2H), 1.30 (s, 3H), 1.25 (s, 1H), 0.84 – 0.93 (m, 2H).

D. Synthesis of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4'-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-carboxamide (I-5)

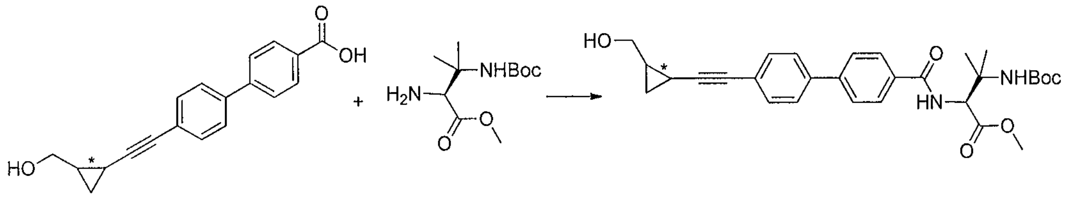


To a round bottom flask was added methyl 4'-bromobiphenyl-4-carboxylate (2.0 g, 6.87 mmol, 1.0 equiv) followed by a solution of racemic 2-*trans*-ethynylcyclopropyl)methanol (0.991 g, 10.30 mmol, 1.5 equiv) dissolved in THF (20 mL). To this was added palladium dichloride bis-triphenylphosphine (241 mg, 0.343 mmol, 0.05 equiv), copper(I) iodide (131 mg, 0.687 mmol, 0.1 equiv), then triethylamine (6.87 mL, 49.3 mmol, 7.18 equiv). The reaction was stirred at 75°C for ~2 h. The reaction was cooled to room temperature, the solids filtered off, rinsing with

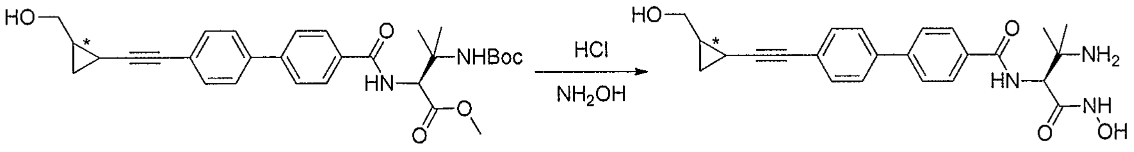
THF. The solution was placed in the freezer for 5 days. Concentrate down to dryness. Add DCM and a precipitate forms. Filter precipitate, rinse with minimal DCM. TLC shows ppt contains mostly product (~1.5g solid). Triturate with 10%EtOAc in hexanes (5 mL), pipet off solvent, TLC shows solid contains product and liquid removed starting material-bromide and some baseline impurities. Additional 10%EtOAc in hexanes (5 mL) was added and stirred for 30 minutes, filtered, rinsed with 10%EtOAc in hexanes (5mL), and dried solid. Yielded 900 mg crude methyl 4'-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-carboxylate. 1H NMR (DMSO-d6): δ 0.82-0.85 (m, 2H), 1.35-1.43 (m, 2H), 3.23-3.29 (m, 1H), 3.40-3.42 (m, 1H), 3.84 (s, 3H), 4.65-4.67 (t, 1H), 7.43-7.45 (d, 2H), 7.67-7.70 (d, 2H), 7.80-7.82 (d, 2H), 7.99-8.01 (d, 2H).



Methyl 4'-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-carboxylate (900 mgs, 2.94 mmol, 1.0 equiv) was dissolved in methanol (5 mL), DMF (2 mL), and THF (5 mL). At room temp was added 1.0M NaOH (4.41 mL, 4.407 mmol, 1.5 equiv). The reaction stirred for 4 days. Reaction concentrated, to remove MeOH and THF, acidify to pH ~3 with 6N HCl (~5mL). Extract with EtOAc (3x50 mL), combine organic layers, wash with sat. NaCl, dried (MgSO4), filtered, concentrated. Yielded 890 mg of 4'-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-carboxylic acid. 1H NMR (DMSO-d6): δ 0.79-0.88 (m, 2H), 1.34-1.44 (m, 2H), 3.22-3.26 (m, 1H), 3.40-3.44 (m, 1H), 7.42-7.44 (d, 2H), 7.66-7.68 (d, 2H), 7.76-7.78 (d, 2H), 7.97-7.99 (d, 2H), 8.10 (s, 1H).



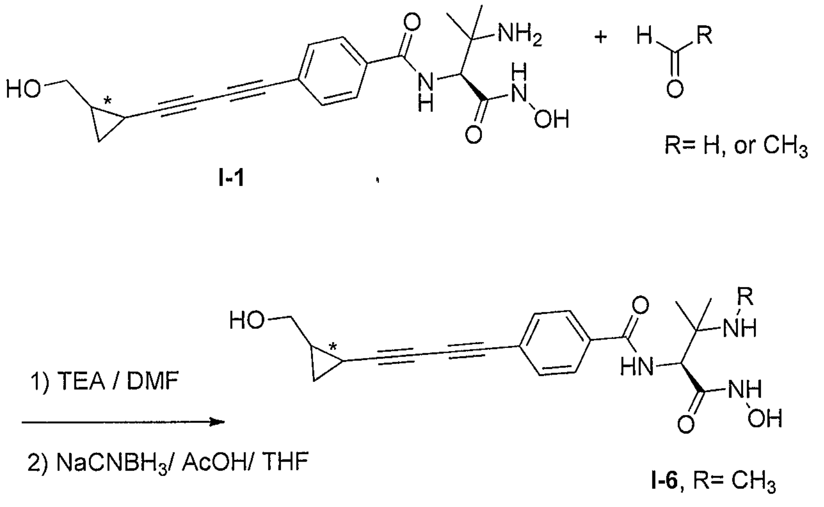
(S)-methyl 2-amino-3-(tert-butoxycarbonylamino)-3-methylbutanoate (150 mg, 0.609 mmol, 1.0 equiv) and 4'-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-carboxylic acid (178 mg, 0.609 mmol, 1.0 equiv) were dissolved in DMF (2 mL). To this was added DIPEA (0.266 mL, 1.523 mmol, 2.5 equiv) then HATU (278 mg, 0.731 mmol, 1.2 equiv). This stirred at room temperature for ~48 h. The reaction was partitioned between 1M citric acid and ethyl acetate. The organics were washed with semi-saturated sodium chloride, saturated sodium bicarbonate then saturated sodium chloride, dried over magnesium sulfate and evaporated to dryness. Yielded 370 mg crude (S)-methyl 3-(tert-butoxycarbonylamino)-2-(4'-(((1S,2S)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-ylcarboxamido)-3-methylbutanoate. LCMS M+1 expected = 521.3, observed = 521.3.



(S)-methyl-3-(tert-butoxycarbonylamino)-2-(4'-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-ylcarboxamido)-3-methylbutanoate (crude) was dissolved in methanol (1 mL). At room temp was added 4.0M HCl in dioxane (1.283 mL, 5.13 mmol, 8.43 equiv). The reaction was complete after 90 minutes (HPLC). The reaction was concentrated on rotovap at 0°C. To this was added IPA (1 mL) then hydroxylamine solution (0.804 mL, 12.18 mmol, 20 equiv). Placed flask at 4°C for ~120h. Reaction concentrated, (keeping reaction at 0°C) to gummy mass. To this was added water (3 mL) and ACN (0.5 mL). Acidified while at 0°C using TFA (3 mL). Additional water (1 mL) and ACN (1 mL) was added. Purified by RP HPLC (1" column, 25mL/min, 0.1% TFA in water/ACN, equil @ 10%B). Loaded onto 1" column (10mL/min, 5%B) using syringe filter (2X6.5 mL). Ramped to

25mL/min over 1 minute. 10%B for 15 minutes, then 10-70%B over 80min, product eluted at 41-48 minutes. Desired fractions were combined, frozen and placed on lyo. Yielded 105 mg of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4'-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-carboxamide (I-5), TFA. LCMS M+1 expected = 422.2, observed = 422.2. 1H NMR (DMSO-d6): δ 0.80-0.88 (m, 2H), 1.27 (s, 3H), 1.31 (s, 3H), 1.36-1.44 (m, 2H), 3.21-3.29 (m, 1H), 3.40-3.45 (m, 1H), 4.65-4.71 (m, 2H), 7.44-7.46 (d, 2H), 7.68-7.71 (d, 2H), 7.79-7.81 (d, 2H), 7.97-7.99 (d, 2H), 8.46-8.48 (d, 1H), 9.22 (s, 1H), 11.21 (br, 1H).

E. Synthesis of N-((S)-1-(hydroxyamino)-3-methyl-3-(methylamino)-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-6) and N-((S)-1-(hydroxyamino)-3-(2-hydroxyethylamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-7)



I-1

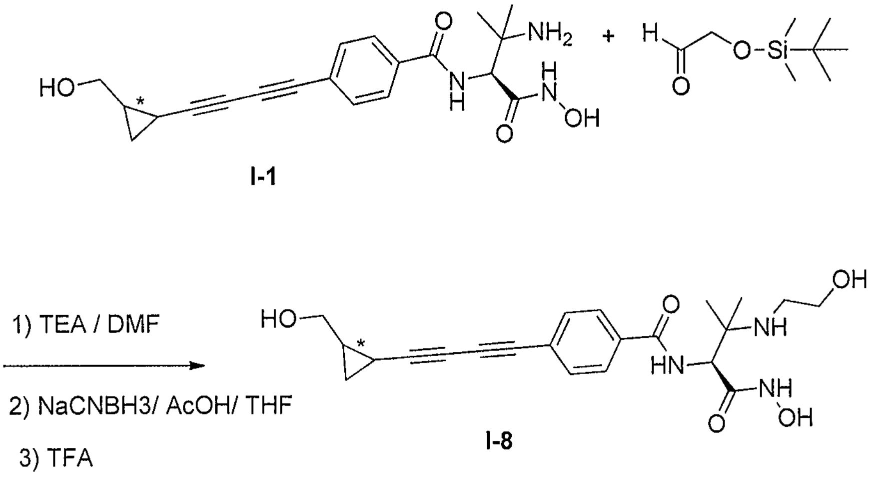
I-6, R= CH_3

I-7, R= CH_2CH_3

In a 250mL round-bottomed flask was N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-1**) (1.2g, 3.25mmol), triethylamine (2 mL, 14.35 mmol), and formaldehyde (0.2 g, 6.66 mmol) in DMF (20 ml) to give a yellow solution. The reaction mixture was stirred for 3h. Excess formaldehyde was quenched with n-butylamine (2ml). Sodium cyanoborohydride (570mg, 14.3mmol) and acetic acid (4ml) were added at room temperature at 0°C. Reaction mixture was checked by LCMS. After removing the solvents, the product was purified by HPLC to give 440mg of N-((S)-1-(hydroxyamino)-3-methyl-3-(methylamino)-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide, **I-6**. LC-MS (M+1) 384; Chemical Formula: $C_{21}H_{25}N_3O_4$, Exact Mass: 383.18. 1HNMR (DMSO-d6), TFA salt: δ 0.88 (m, 2H) 1.30(s, 3H), 1.35 (s, 3H), 1.44(m, 1H), 2.51(s, H), 3.39(m, 1H), 3.41(m, 1H), 4.7(br.d, 1H), 4.84(d, 1H), 7.58(d, 2H), 7.91(d, 2H), 8.51 (br.m, 1NH) 8.64(d, 1NH), 9.23(S, NH) . 11.17(s, OH).

In a 250mL round-bottomed flask was N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-1**) (1g, 2.71mmol), triethylamine (0.377 ml, 2.71 mmol), and acetaldehyde (0.119 g, 2.71 mmol) in DMF (20 ml) to give a yellow solution. Reaction mixture was stirred for 3h. Sodium cyanoborohydride (570mg, 14.3mmol) and acetic acid (2ml) were added at room temperature. Reaction mixture was checked by LCMS. Reaction mixture was concentrated and the product were purified by HPLC to give 642mg of N-((S)-1-(hydroxyamino)-3-(2-hydroxyethylamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide, **I-7**. LC-MS (M+1) 398; Chemical Formula: $C_{22}H_{27}N_3O_4$, Exact Mass: 397.20. 1HNMR (DMSO-d6), TFA salt: δ 0.85 (m, 2H), 1.14(t, 3H), 1.29(s, 3H), 1.39 (s, 3H), 1.44(m, 1H), 2.96(br.m, 2H) 3.24(m, 2H) 3.39(m, 2H), 4.64(br.d, 1H), 4.85(d, 1H), 7.60(d, 2H), 7.92(d, 2H), 8.59 (br.m, 1NH) 8.45(brt, 1NH), 9.23(S, NH), 11.15(s, OH).

F. Synthesis of N-((S)-1-(hydroxyamino)-3-(2-hydroxyethylamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-8)

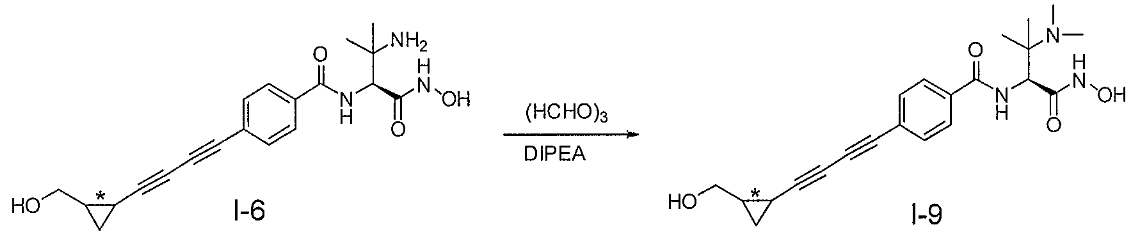


I-1

I-8

In a 250mL round-bottomed flask (t=g) was N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (1g, 2.71mmol), and triethylamine (0.377ml, 2.71mmol) in DMF (20 ml) to give a yellow solution. 2-(tert-butyldimethylsilyloxy)acetaldehyde (0.494g, 2.83mmol) was added. The reaction mixture was stirred for 18h, concentrated to dryness. The residue was dissolved in THF (15ml) and acetic acid (2ml) and sodium cyanoborohydride (600mg, 15mmol) were added at room temperature. Reaction mixture was checked by LCMS. After stirring at room temperature for 2h, TFA (5ml) was added and stirred for additional 3h. The completion of reaction was confirmed by LCMS and the reaction was concentrated, and the product was purified on HPLC to give 370mg of N-((S)-1-(hydroxyamino)-3-(2-hydroxyethylamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide, **I-8**. LC-MS (M+1) 414; Chemical Formula: $C_{22}H_{27}N_3O_5$, Exact Mass: 413.20 ; 1HNMR (DMSO-d6), TFA salt: δ 0.88 (m, 2H) 1.30(s, 3H), 1.35 (s, 3H), 1.44(m, 1H), 3.22(m, 2H), 3.39(m, 2H), 4.64(br.d, 1H), 4.90(d, 1H), 5.25(br. S, 1H) 7.60(d, 2H), 7.91(d, 2H), 8.59 (br.m, 1NH) 8.71(d, 1NH), 9.26(S, NH) . 11.19(s, OH).

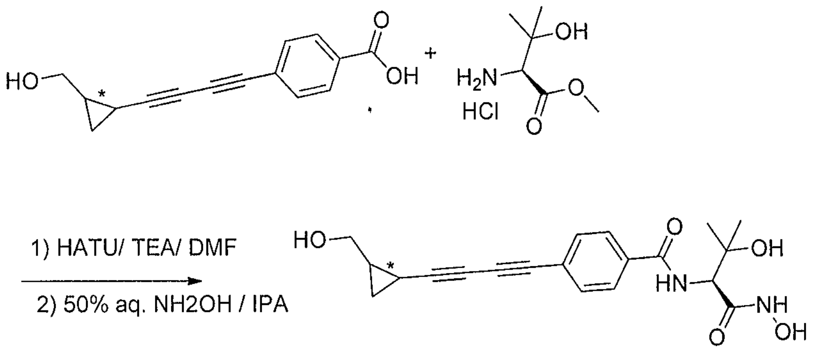
G. Synthesis of N-((S)-3-(dimethylamino)-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-9)



N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-((2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (300mg, 0.81mmol) was dissolved in *N,N*-dimethylformamide (10mL) and treated with paraformaldehyde (732mg, 8.1mmol, 10eq) and *N,N*-diisopropylethylamine (0.56mL, 3.3mmol, 4eq) at room temperature for 16 hours. Trifluoroacetic acid (1.3mL, 16.2mmol, 20eq) and sodium cyanoborohydride (101mg, 1.6mmol, 2 eq) were introduced.

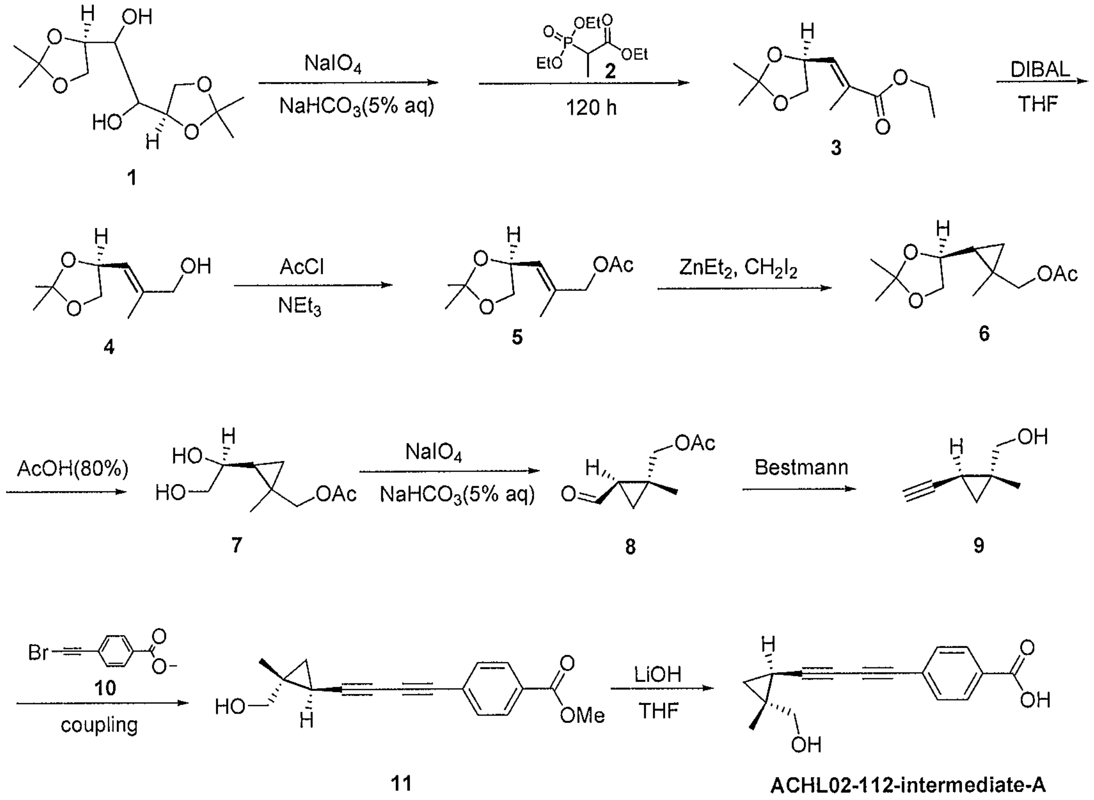
The crude material was purified by reverse-phase HPLC and the desired fractions pooled and lyophilized to give N-((S)-3-(dimethylamino)-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide as its trifluoroacetate salt (white solid, 2.9mg, 5.3µmol, 0.7%). Mass spec data: expected (M+1): 398.5, observed: 398.2. Proton NMR (400MHz, dmso-d6): 11.16 (s, 1H), 9.25 (br s, 1H), 9.05 (br s, 1H), 8.68 (d, 1H, J = 10.0Hz), 7.94 (dd, 2H, J = 1.6, 8.0HZ), 7.59 (dd, 2H, J = 1.8, 8.2Hz), 5.06 (d, 1H, J = 9.6Hz), 3.4 (peak obscured by water), 3.21 (dd, 1H, J = 5.2, 11.2Hz), 2.75 (d, 3H, J = 5.6Hz), 2.73 (d, 3H, J = 5.2Hz), 1.48 (s, 3H), 1.39 – 1.45 (m, 2H), 1.28 (s, 3H), 0.85 – 0.91 (m, 2H).

H. Synthesis of N-((S)-3-hydroxy-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-10)



To a stirred solution of 4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzoic acid (750 mg, 3.12mmol), HATU (570mg, 1.5 mmol) and DIEA (3.4ml excess) in DMF (30 ml) at room temperature, (S)-methyl 2-amino-3-hydroxy-3-methylbutanoate hydrochloride (402 mg, 1.5 mmol) was added. Reaction mixture was stirred at ambient temperature 1h followed by the dilution with water (50 ml). Solution was extracted with ethyl acetate (100 ml x 3) and brine (20 ml). Organic layer was dried over $MgSO_4$ and evaporated. The resulting product (1.3g) was dissolved in IPA (20mL) and treated with NH_2OH (10mL, excess) and stirred at room temperature for 3 days. Excess solvent was removed and crude product was purified on a reverse phase HPLC to give 178 mg of N-((S)-3-hydroxy-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide **I-10**; LC-MS (M+1) 371; Chemical Formula: $C_{20}H_{22}N_2O_5$ Exact Mass: 370.15. 1HNMR (DMSO-d6), : δ 0.88 (m, 2H) 1.11(s, 3H), 1.16 (s, 3H), 1.44(m, 1H), 3.20(m, 1H), 3.40(m, 2H), 4.34(d, 1H), 4.84(d, 1H), 7.58(d, 2H), 7.84(d, 2H), 7.6(d, 1NH), 8.88(br.s, NH) . 10.57(s, OH).

I. Synthesis of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1S,2R)-2-(hydroxymethyl)-2-methylcyclopropyl)buta-1,3-diynyl)benzamide (I-11)



(S,E)-ethyl 3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-methylacrylate (3)

Reagent	MW	Eq.	mmol	g, mL
Compound 1	262.30	1	49.6	13.0g
$NaIO_4$	213.89	1.2	59.5	12.7 g
Compound 2	238.22	2	99.2	23.0 g
K_2CO_3	138.15	2.1	103.6	14.3 g
$NaHCO_3$(5% aq)				60 mL

To a stirred solution of D-mannitol diacetonide **1** (13.0 g , 49.6 mmol) in sodium bicarbonate (5% aq, 60 ml), added a saturated solution of sodium periodate (12.74 g, 59.5 mmol) drop wise at r.t. Upon the addition, the mixture was kept stirring at r.t. for 2 hours. Then ethyl 2-(diethoxyphosphoryl)propanoate **2** (23.0 g, 99.2 mmol) was added followed by potassium carbonate (14.3 g, 103.6 mmol), the mixture was kept stirring at r.t. for another 120 hours. The mixture was added to 500 ml water, extracted with EtOAc (300mLx3). The combined organic layer was dried over anhydrous sodium sulfate, concentrated and then chromatographed with PE:EA(30/1) to give the desired compound **3** 5.5 g as a colorless oil, yield:51.8%.

(S,E)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-methylprop-2-en-1-ol (4)

Reagent	MW	Eq.	mmol	g, mL
Compound 3	214.26	1	25	5.35 g
DIBALH	1M in toluene	2	50	50 mL
THF				150 mL

To a solution of (S,E)-ethyl 3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-methylacrylate **3** (5.35 g, 5 mmol) in THF 150 ml , added a solution of DIBAL-H (50 mL, 50 mmol) drop wise under argon at 0 °C. Upon the addition, the mixture was kept stirring at 0°C for 3 hours. When TLC showed little compound 3 remained, the reaction was quenched with 30 ml aqueous ammonia and stirred with 300 ml dichloromethane for another 1 hour. The mixture was filtered, and the residue was washed with THF, the combined organic layer was dried and concentrated to give crude **4** which was purified by silica gel column with PE:EA=10:1-5:1, 3.0 g compound **4** as a colorless oil was obtained, Yield: 70%.

(S,E)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-methylallyl acetate (5)

Reagent	MW	Eq.	mmol	g, mL
Compound 4	172.22	1	17	2.92 g
NEt_3	101.19	2.5	42.5	4.3 g
AcCl	78.5	2	34	2.7 g
THF				100 mL

To a solution of (S,E)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-methylprop-2-en-1-ol **4** (2.92 g, 17 mmol) in THF 100 ml, added triethylamine (4.3 g, 42.5 mmol). Then acetyl chloride (2.7 g , 34 mmol) was added dropwise under argon at 0 °C. Upon the addition, the mixture was kept stirring at 0°C for 3 hours. When TLC showed little compound **4** remaining, the reaction was poured to 150 ml water. Extracted with EtOAc (50mLx3). The combined organic layer was dried over anhydrous sodium sulfate, concentrated and then chromatographed with PE:EA(10/1) to give the desired compound **5,** 3.4 g as a colorless oil, yield: 89%.

((trans)-2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-methylcyclopropyl)methyl acetate (6)

Reagent	MW	Eq.	mol	g, mL
Compound 5	214.26	1	15	3.2 g
Et_2Zn	IM in hexane	5	75	75 mL
CH_2I_2	267.84	10	150	40 g
Hexane				200 mL

To a solution of (S,E)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-methylallyl acetate **5** (3.2 g, 15 mmol) in hexane 200 mL, added diethyl zinc (1M in hexane, 75 mL) in portions under argon at -20°C. After the addition, diiodomethane (40 g, 150 mmol) was added dropwise below -20°C with strong stirring. The mixture was kept stirring below -20°C for another 8 hours and allowed warm to r.t. and kept stirring for another 8 hours. The reaction mixture was quenched by 100 ml cold aqueous NH_4Cl, then extracted with ether (100 ml x 5). The combined organic layer were washed with aqueous sodium thiosulfate, water, brine then dried and concentrated to give crude **6** which was purified by silica gel column (PE:EA=30:1-20:1) to give **6,** 3.0 g as a colorless oil. Yield: 88%.

((trans)-2-((S)-1,2-dihydroxyethyl)-1-methylcyclopropyl)methyl acetate (7)

Reagent	MW	Eq.	mol	g, mL
Compound 6	228.28	1	13	2.97 g
AcOH(80%)				30 mL

A mixture of ((trans)-2-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1-methylcyclopropyl)methyl acetate **6** (2.97 g, 13 mmol) in 30 ml 80% acetic acid was kept stirring at room temperature for 15 hours. When TLC showed little **6** remaining, the mixture was added to 300 ml saturated aqueous sodium bicarbonate dropwise. Then extracted with dichloromethane (200 ml x 3). The combined organic layer was washed with water, brine dried and concentrated to give crude **7** which was purified by silica gel column (PE:EA=3:1-1:1) to give Compound **7,** 1.5 g as a colorless oil. Yield : 62%.

((trans)-2-formyl-1-methylcyclopropyl)methyl acetate (8)

Reagent	MW	Eq.	mmol	g, mL
Compound 7	188.22	1	7.5	1.41 g
$NaIO_4$	213.89	1.2	9	1.93 g
$NaHCO_3$(5% aq)				20 mL

To a stirred solution of ((trans)-2-((S)-1,2-dihydroxyethyl)-1-methylcyclopropyl)methyl acetate **7** (1.41 g , 7.5 mmol) in sodium bicarbonate (5% aq) 20 ml, added a saturated solution of sodium periodate (1.93 g, 9 mmol) drop wise at rt. Upon the addition, the mixture was kept stirring at r.t. for 2 hours. When TLC showed little **7** remained, the mixture was added to 50 ml water, extracted with EtOAc (100mLx3). The combined organic layer was dried over anhydrous sodium sulfate, concentrated and then chromatographed with PE:EA(20/1) to give the desired compound **8,** 1.0 g as a colorless oil, yield:85%.

((trans)-2-ethynyl-1-methylcyclopropyl)methanol (9)

Reagent	MW	Eq.	mmol	g, mL
Compound 8	156.08	1	5	780 mg
Bestmann reagent	192.11	1.5	7.5	1.44 g
K_2CO_3	138.46	3	15	2.07 g
CH_3OH				15 mL

To a solution of ((trans)-2-formyl-1-methylcyclopropyl)methyl acetate **8** (780 mg, 5 mmol) in methanol 15 mL, added Bestmann reagent (1.44 g, 7.5 mmol) and potassium carbonate (2.07 g, 15 mmol), the mixture was kept stirring at r.t. for 5 hours. When TLC showed little **8** remained, the reaction mixture was diluted with water (20 mL). Extracted with ether (20mLx3), the combined organic layer was dried and concentrated to give crude **9**, which was purified by silica gel column (PE:Et_2O=10:1~5:1) to give **9** as a colorless oil 400 mg . Yield: 73%.

Methyl 4-(((trans)-2-(hydroxymethyl)-2-methylcyclopropyl)buta-1,3-diynyl) benzoate (11)

Reagent	MW	Eq.	mmol	g, mL
Compound 9	110.17	1	3.6	400 mg
Compound 10	239.07	1	3.6	870 mg
$Pd(PPh_3)_2Cl_2$	700.13	0.05	0. 18	126 mg
CuI	190.23	0.1	0.36	68.4 mg
i-Pr_2NH	101.19	3	10.8	1.09 g
THF				20 mL

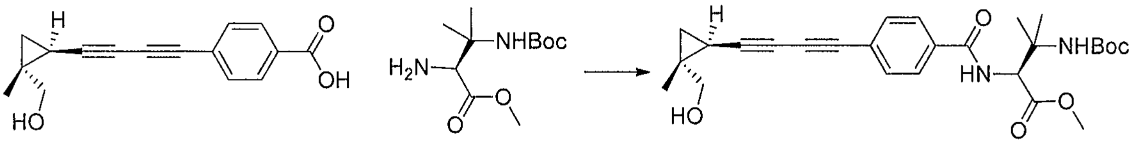
To a solution of ((trans)-2-ethynyl-1-methylcyclopropyl)methanol **9** (400 mg, 3.6 mmol), $Pd(PPh_3)_2Cl_2$ (126 mg, 0.18 mmol), CuI (68.4 mg ,0.36 mmol), DIPEA

(1.09g, 10.8 mmol) in THF 20 mL, treated with methyl 4-(bromoethynyl)benzoate **10** (870 mg, 3.6 mmol) under Argon. The mixture was kept stirring at r.t. for 5 hours. When TLC showed little compound **9** remained, the solvent was removed under reduced pressure, the residue was diluted with water (50 mL), extracted with dichloromethane (3x50 mL), the organic layer was dried and concentrated to give crude **11** as a red oil which was purified by silica gel column chromatography PE:EA= 20: 1 ~10:1 to give **11** 430 mg as a yellow solid. Yield: 40 %.

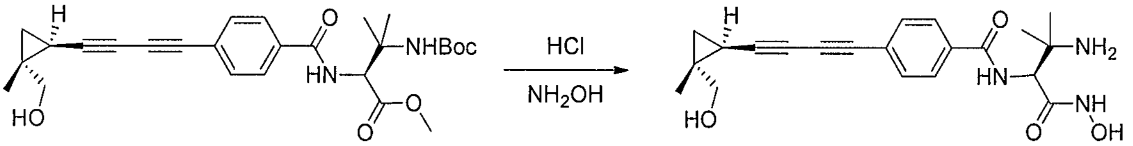
4-(((1S,2R)-2-(hydroxymethyl)-2-methylcyclopropyl)buta-1,3-diynyl)benzoic acid (ACHL02-112-intermediate-A)

Reagent	MW	Eq.	Mmol	g, mL
Compound 11	268.31	1	1.5	400 mg
LiOH H_2O	40.10	4	6	240 mg
THF/ H_2O(V/V=4:1)				20 mL

To a stirred solution of methyl 4-(((trans)-2-(hydroxymethyl)-2-methylcyclopropyl)buta-1,3-diynyl) benzoate **11** (400 mg, 1.5 mmol) in THF 16 ml, added a solution of lithium hydroxide mono-hydrate (240 mg, 6 mmol) in 4 mL water. Upon the addition, the mixture was kept stirring at r.t. for 15 hours. When LCMS showed little Compound **11** remaining, the mixture was added to 50 ml water. Adjusted the pH to 3.0 with 1M HCl at 0 °C, extracted with EtOAc (100mLx3). The combined organic layer was dried over anhydrous sodium sulfate, concentrated to give the desired compound 370 mg as a yellow powder, yield:92%. LCMS: CP-0004344-006-3-03665-LCMSA035(ESI)m/z=255(M+1). 1HNMR: CP-0004344-006-3 (DMSO-*d*$_6$, 400 MHz) δ:13.1(s, 1H), 7.91(d, J=8.4 Hz , 2H), 7.62(d, J=8.4Hz , 2H), 4.75(s, 1H), 3.22(m, 2H), 1.59(m, 1H), 1.18(s, 3H), 1.08(m, 1H), 0.66(m, 1H).

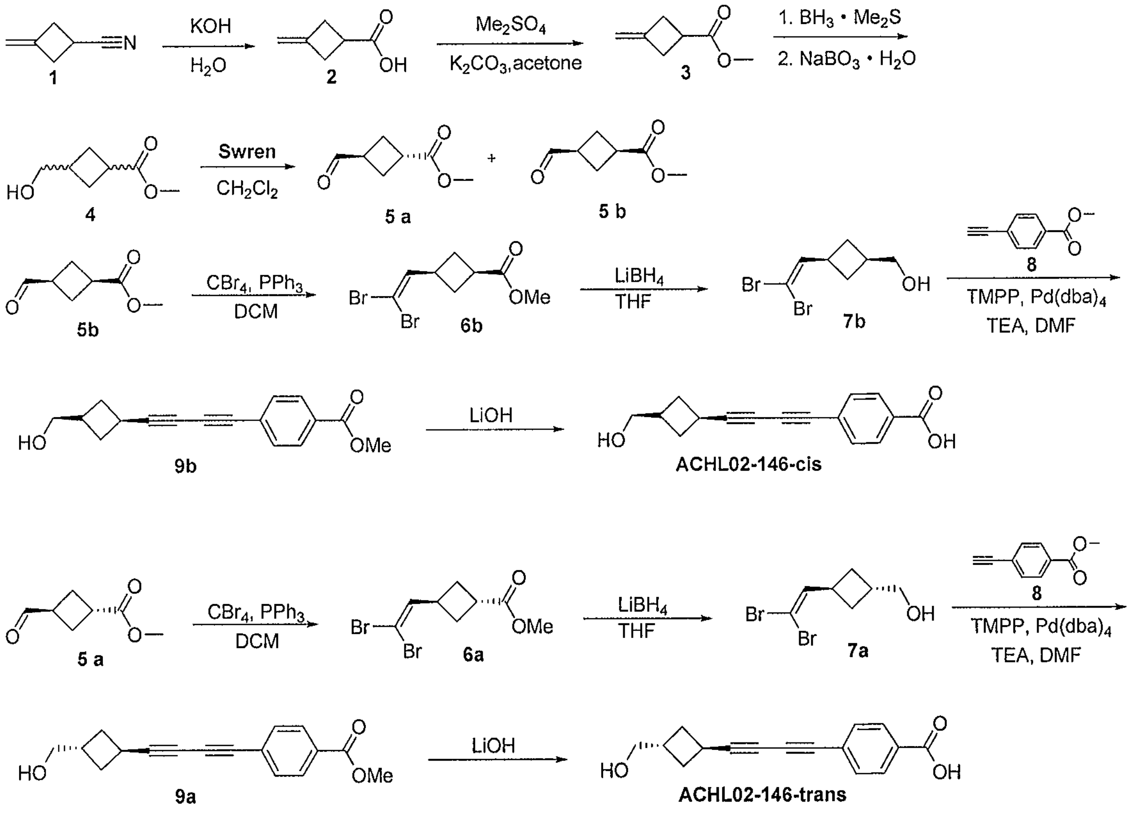


(S)-methyl 2-amino-3-(tert-butoxycarbonylamino)-3-methylbutanoate (347 mg, 1.408 mmol, 1.0 equiv) and 4-(((1S,2R)-2-(hydroxymethyl)-2-methylcyclopropyl)buta-1,3-diynyl)benzoic acid (358 mg, 1.408 mmol, 1.0 equiv) were dissolved in DMF (3 mL). To this was added DIPEA (0.615 mL, 3.52 mmol, 2.5 equiv) then HATU (642 mg, 1.689 mmol, 1.2 equiv). This stirred at room temperature for ~16 h. The reaction was partitioned between 1M citric acid and ethyl acetate. The organics were washed with semi-saturated sodium chloride, saturated sodium bicarbonate then saturated sodium chloride, dried over magnesium sulfate and evaporated to dryness. Yielded 912 mg crude (S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-(((1S,2R)-2-(hydroxymethyl)-2-methylcyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate. LCMS M+1 expected = 483.2, observed = 483.2.



(S)-methyl-3-(tert-butoxycarbonylamino)-2-(4-(((1S,2R)-2-(hydroxymethyl)-2-methylcyclopropyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (crude) was dissolved in methanol (1 mL). At room temp was added 4.0M HCl in dioxane (2.97 mL, 11.86 mmol, 8.43 equiv). The reaction complete after 60 minutes (HPLC). The reaction was concentrated on rotovap at 0°C. To this was added IPA (2 mL) then hydroxylamine solution (1.859 mL, 28.1 mmol, 20 equiv). Placed flask at 4°C for ~40h. Reaction concentrated, (keeping reaction at 0°C) to gummy mass. To this was added water (3 mL) and ACN (0.5 mL). Acidified while at 0°C using TFA (3 mL). Additional water (1 mL) and ACN (1 mL) was added. Purified by RP HPLC (2" column, 50mL/min, 0.1% TFA in water/ACN, equil @ 5%B). Loaded onto 2" column (10mL/min, 5%B) using syringe filter (2X6.5 mL). Ramped to 50mL/min over 1 minute. 5-30%B over 66min, prod elutes at 25%B. Desired fractions were combined, frozen and placed on lyo. Yielded 410 mg of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1S,2R)-2-(hydroxymethyl)-2-methylcyclopropyl)buta-1,3-diynyl)benzamide (**I-11**), TFA. LCMS M+1 expected = 384.2, observed = 384.2. 1H NMR (DMSO-d6): δ 0.61-0.64 (t, 1H), 1.02-1.06 (m, 1H), 1.16 (s, 3H), 1.25 (s, 3H), 1.30 (s, 3H), 1.55-1.60 (m, 1H), 3.17-3.29 (m, 2H), 4.65-4.67 (d, 1H), 7.61-7.63 (d, 2H), 7.88-7.90 (d, 2H), 7.98 (s, 2H), 8.53-8.56 (d, 1H), 9.21 (br, 1H), 11.19 (s, 1H).

J. Synthesis of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-*cis*)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamide (I-12) and N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-*trans*)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamide (I-13)



3-methylenecyclobutanecarboxylic acid (2)

Reagent	MW	Eq.	mol	g, mL
Compound **1**	93	1.0	0.3	27.9 g
KOH	56	5.0	1.5	84 g
EtOH				200mL
H_2O				200mL

The solution of 3-methylenecyclobutanecarbonitrile **1** (27.9g, 0.3mol) in ethanol (200mL) and water (200mL) was treated with potassium hydroxide (84g, 1.5mol). The resulting mixture was stirred and heated to 105°C for 4h. The ethanol was removed under reduced pressure. The residue was cooled to 0°C, and the pH was adjusted to 1~2, then extracted with ethyl acetate, dried over sodium sulfate$_4$, filtered and the solvent was removed. (30.2g, yield= 90%).

methyl 3-methylenecyclobutanecarboxylate (3)

Reagent	MW	Eq.	mol	g, mL
Compound **2**	112	1.0	0.260	29.12 g
Me_2SO_4	126	1.2	0.312	39.312g
K_2CO_3	138	2.0	0.520	70.2g
Acetone				500mL

The mixture of 3-methylenecyclobutanecarboxylic acid **2** (29.12g, 0.26mol), potassium carbonate (70.2g, 0.52mol), acetone (500mL) and dimethyl sulfate (39.312g, 0.312mol) was heated to reflux for 2h. The reaction mixture was cooled to room temperature and filtered. The solvent was removed under reduced pressure. The residue was purified with silica gel chromatography to afford the desired product as colorless oil. (27.5g, yield=84%).

methyl 3-(hydroxymethyl)cyclobutanecarboxylate (4)

Reagent	MW	Eq.	mol	g, mL
Compound **3**	126	1.0	0.210	26.46g
BH_3 THF (1M in THF)		0.3	0.070	70.0mL
THF				130mL
NaOH(3M)	40			30mL
H_2O_2 (30%)	34	1.0	0.210	34.0g

A dry three-neck flask was charged with methyl 3-methylenecyclobutanecarboxylate **3** (24.46g, 0.21mol) and dry THF (130ml) and cooled to -10°C, then borane-THF complex (70.0mL) was added via a syringe dropwise. The resulting mixture was stirred for 4h at r.t and was cooled to -20°C~-10°C; methanol was added, stirred for 15min. Sodium hydroxide (3M 30mL) and hydrogen peroxide (34.0g, 0.210mol) were added in sequence. The mixture was stirred for 2h and saturated sodium sulfite solution (100mL) was added. The reaction mixture was diluted with water, then extracted with ethyl acetate, washed with water and brine, dried over sodium sulfate, filtered, removed the solvent and the residue was purified with silica gel chromatography. (28.73g, 95%)

(trans)-methyl 3-formylcyclobutanecarboxylate (5a) and (cis)-methyl 3-formylcyclobutanecarboxylate (5b)

Reagent	MW	Eq.	mmol	g, mL
Compound **4**	144	1.0	69.4	10g
oxalyl dichloride	128	2.0	139	17.8g
DMSO	78	4.0	278	21.7g
DCM				650mL
TEA	101	10.0	700	71g

To a solution of oxalyl chloride (17.8g, 139mmol, 2.0eq) in CH_2Cl_2 (450mL) was added slowly DMSO (21.7g, 278mmol, 4.0eq) in dichloromethane (50mL) at –78°C. After 30 min, methyl 3-(hydroxymethyl)cyclobutanecarboxylate **4** (10g, 69.4mmol, 1.0eq) in dichloromethane (150mL) was added dropwise at -78°C. The mixture was stirred for additional 2h at –78°C, and then triethylamine (70g, 700mmol, 10.0eq) was added at –78°C. After 20 min, the mixture was warmed to room temperature, and saturated aqueous NH_4Cl was added. The layers were separated, and the aqueous layer was extracted with dichloromethane (3 X 200mL). The combined organic layers were washed with brine, dried (sodium sulfate), filtered and concentrated to give the crude product as a mixture of cis and trans isomers. Purification to get **5a** (trans, 3.1 g) and **5b** (cis, 2.9 g) by silica column.

(cis)-methyl 3-(2,2-dibromovinyl)cyclobutanecarboxylate (6b)

Reagent	MW	Eq.	mmol	g, mL
Compound **5a**	142	1.0	20.40	2.9g
PPh_3	262	4.0	81.80	21.41g
CBr_4	332	2.0	40.90	13.56g
DCM				200mL

To a solution of carbon tetrabromide (13.56g, 40.89mmol, 2.0eq) in 40mL dichloromethane, added a solution of triphenylphosphine (21.41g, 81.80mmol, 4.0eq) in 80mL dichloromethane dropwise below -20°C under argon. The mixture was kept stirring at -20°C for 30min, then cooled to -78°C. (cis)-methyl 3-formylcyclobutanecarboxylate **5b** (2.9g, 20.40mmol, 1.0eq) in 80mL dichloromethane was added dropwise, and kept stirring at -78°C for another 1h. The mixture was allowed warm to r.t. The reaction mixture was added dropwise to stirring 300mL PE. The mixture was filtered and the residue was washed with PE. The combined organic layer was dried over sodium sulfate and concentrated to give the target compound which was purified by silica gel column. (2.3g, yield=37%)

((cis)-3-(2,2-dibromovinyl)cyclobutyl)methanol (7b)

Reagent	MW	Eq.	mmol	g, mL
Compound **6b**	298	1.0	8.4	2.5g
LiBH4	22	1.1	9.2	203mg
THF				10mL

Compound **6b** (2.5g, 8.4mmol, 1.0eq) dissolved in THF (10mL) was added lithium borohydride (203mg) at 0°C. The reaction mixture was kept stirring for 3h at r.t. water (5mL) was added and extracted with ethyl acetate. The combined ethyl acetate layer was dried and concentrated under reduced pressure. The crude was purified by silica gel column with PE: EA=5:1. (1.88g yield=83%).

methyl 4-(((cis)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzoate (9b)

Reagent	MW	Eq.	mmol	g, mL
Compound **7b**	270	1.0	6.9	1.88g
Compound **8**	160	1.4	9.7	1.56g
TMPP	352	0.04	0.28	98mg
Pd(dba)$_4$	916	0.01	0.069	64mg
DMF				30mL
TEA	101	3.0	20.9	2.11g

A mixture of ((cis)-3-(2,2-dibromovinyl)cyclobutyl)methanol **7b** (1.88g, 6.9mmol, 1.0eq), methyl 4-ethynylbenzoate **8** (1.56g, 9.7mmol, 1.4eq), tri(4-methylphenyl)phosphine (98mg, 0.28mmol, 0.04eq), Pd(dba)$_4$ (64mg, 0.069mmol, 0.01eq) in dry DMF (30mL) was added triethylamine (2.11g, 20.9mmol, 3.0eq) under nitrogen atmosphere. The reaction mixture was kept stirring at 80°C for 15h. LCMS monitored the reaction. The reaction mixture was diluted with NH_4Cl_{aq} solution and extracted with ethyl acetate. The combined ethyl acetate layer was washed with water, brine and dried. The solvent was evaporated under reduced pressure to get the crude which was purified by silica gel column with PE: EA=5:1. (450mg, yield=24%).

4-(((cis)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzoic acid (ACHL02-146-cis)

Reagent	MW	Eq.	mmol	g, mL
Compound **9a**	268	1.0	1.68	450mg
LiOH H_2O	42	4.0	6.72	282mg
THF				20mL

To a solution of methyl 4-(((cis)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzoate **9b** (450mg, 1.0 equiv) in THF (200mL) was added lithium hydroxide mono-hydrate (282mg, 4.0 equiv), then the mixture reacted at 25°C for 5h. Then the reaction solvent was removed and neutralized with 3 N HCl to PH 5-6, extracted with ethyl acetate. The combined ethyl acetate layer was washed with brine and dried. The solvent was evaporated to obtain the target compound. (400mg, yield=94%).

(trans)-methyl 3-(2,2-dibromovinyl)cyclobutanecarboxylate (6a)

Reagent	MW	Eq.	Mmol	g, mL
Compound **5a**	142	1.0	16.90	2.4g
PPh_3	262	4.0	67.60	17.7g
CBr_4	332	2.0	33.80	11.2g
DCM				200mL

To a solution of carbon tetrabromide (11.2g, 33.80mmol, 2.0eq) in 40mL dichloromethane, added a solution of triphenylphosphine (17.7g, 67.60mmol, 4.0eq) in 80mL dichloromethane dropwise below -20°C under argon. The mixture was kept stirring at -20°C for 30min, then cooled to -78°C. (trans)-methyl 3-

formylcyclobutanecarboxylate **5a** (2.4g, 16.90mmol, 1.0eq) in 80mL dichloromethane was added dropwise, and kept stirring at -78°C for another 1h. The mixture was allowed warm to r.t. the reaction mixture was added dropwise to a stirring 300mL PE. Filtered and the residue was washed with PE. The combined organic layer was dried over sodium sulfate and concentrated to give the target compound which was purified by silica gel column. (2.5g, yield=50%).

((trans)-3-(2,2-dibromovinyl)cyclobutyl)methanol (7a)

Reagent	MW	Eq.	mmol	g, mL
Compound **6a**	298	1.0	8.4	2.5g
LiBH4	22	1.1	9.2	203mg
THF				10mL

(trans)-methyl 3-(2,2-dibromovinyl)cyclobutanecarboxylate **6a** (2.5g, 8.4mmol, 1.0eq) dissolved in THF (10mL) was added lithium borohydride (203mg) at 0°C. The reaction mixture was kept stirring for 3h at r.t. water (5mL) was added and extracted with ethyl acetate. The combined ethyl acetate layer was dried and concentrated under reduced pressure. The crude was purified by silica gel column with PE: EA=5:1. (1.70g, yield=75%).

methyl 4-(((trans)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzoate (9a)

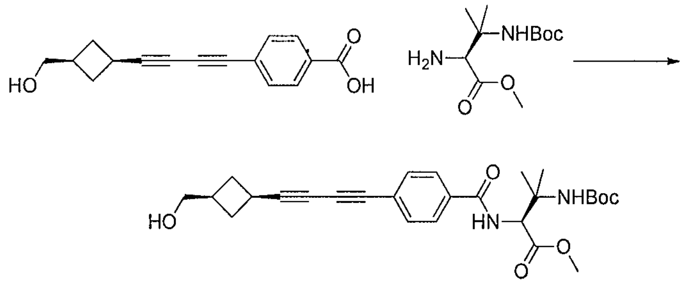
Reagent	MW	Eq.	mmol	g, mL
Compound **7a**	270	1.0	6.3	1.70g
Compound **8**	160	1.4	8.8	1.41g
TMPP	352	0.04	0.25	89mg
Pd(dba)$_4$	916	0.01	0.063	58mg
DMF				30mL
TEA	101	3.0	18.9	1.91g

A mixture of ((trans)-3-(2,2-dibromovinyl)cyclobutyl)methanol **7a** (1.70g, 6.3mmol, 1.0eq), Compound **8** (1.41g, 8.8mmol, 1.4eq), tri(4-methylphenyl)phosphine (89mg, 0.25mmol, 0.04eq), Pd(dba)$_4$ (58mg, 0.063mmol, 0.01eq) in dried DMF (30mL) was added triethylamine (1.91g, 18.9mmol, 3.0eq) under nitrogen. The reaction mixture was kept stirring at 80°C for 15h. LCMS monitored the reaction. The reaction mixture was diluted with NH_4Cl_{aq} solution and extracted with ethyl acetate. The combined ethyl acetate layer was washed with water, brine and dried. The solvent was evaporated under reduced pressure to get the crude which was purified by silica gel column with PE: EA=5:1. (550mg, yield=33%).

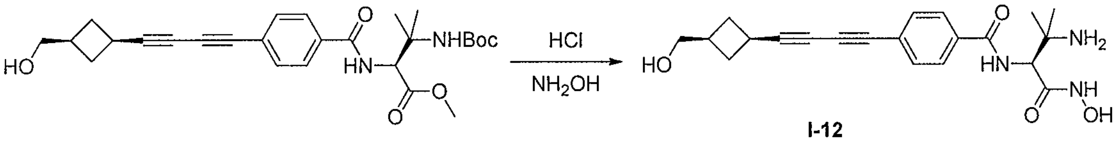
4-(((trans)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzoic acid (ACHL02-146-trans)

Reagent	MW	Eq.	mmol	g, mL
Compound **9a**	268	1.0	2.05	550mg
LiOH H_2O	42	4.0	8.21	345mg
THF				20mL

To a solution of methyl 4-(((trans)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzoate **9a** (550mg, 1.0 equiv) in THF (20mL) was added lithium hydroxide mono-hydrate (345mg, 4.0 equiv), then the mixture reacted at 25°C for 5h. Then the reaction solvents was removed and neutralized with 3 N HCl to pH 5-6, extracted with ethyl acetate. The combined ethyl acetate layer was washed with brine and dried. The solvent was evaporated to obtain the target compound. (340mg, yield=65%).

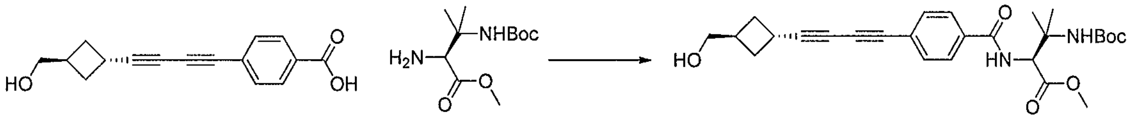


(S)-methyl 2-amino-3-(tert-butoxycarbonylamino)-3-methylbutanoate (387 mg, 1.573 mmol, 1.0 equiv) and 4-(((1,3-*cis*)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzoic acid (400 mg, 1.573 mmol, 1.0 equiv) were dissolved in DMF (3 mL). To this was added DIPEA (0.687 mL, 3.93 mmol, 2.5 equiv) then HATU (718 mg, 1.888 mmol, 1.2 equiv). This stirred at room temperature for ~16 h. The reaction was partitioned between 1M citric acid and ethyl acetate. The organics were washed with semi-saturated sodium chloride, saturated sodium bicarbonate then saturated sodium chloride, dried over magnesium sulfate and evaporated to dryness. Yielded 1.035 g crude (S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-(((1,3-*cis*)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate. LCMS M+1 expected = 483.2, observed = 483.2.

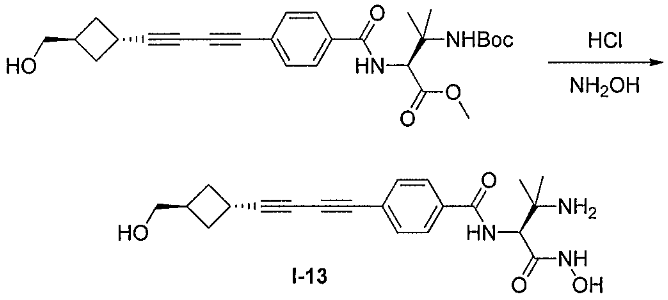


I-12

(S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-(((1,3-*cis*)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (crude) was dissolved in methanol (1 mL). At room temp was added 4.0M HCl in dioxane (3.3 mL, 13.26 mmol, 8.43 equiv). The reaction complete after 60 minutes (HPLC). The reaction was concentrated on rotovap at 0°C. To this was added IPA (2 mL) then hydroxylamine solution (2 mL, 31.5 mmol, 20 equiv). Placed flask at 4°C for 24h. Reaction concentrated, (keeping reaction at 0°C) to gummy mass. To this was added water (3 mL) and ACN (0.5 mL). Acidify while at 0°C using TFA (3 mL). Additional water (1 mL) and ACN (1 mL) was added. Purify by RP HPLC (2" column, 50mL/min, 0.1% TFA in water/ACN, equil @ 5%B). Load onto 2" column (10mL/min, 5%B) using syringe filter (2X6 mL). Ramp to 50mL/min over 1 minute. 5-35%B over 55min, product eluted at 24.5-26%B. Desired fractions were combined, frozen and placed on lyo. Yielded 460 mg of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-*cis*)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamide, TFA. LCMS M+1 expected = 384.2, observed = 384.2. 1H NMR (DMSO-d6): δ 1.25 (s, 3H), 1.30 (s, 3H), 1.81-1.88 (m, 2H), 2.23-2.34 (m, 3H), 3.08-3.15 (m, 1H), 3.30-3.31 (d, 1H), 4.54 (br, 1H), 4.65-4.67 (d, 1H), 7.61-7.64 (d, 2H), 7.89-7.91 (d, 2H), 7.99 (s, 2H), 8.54-8.56 (d, 1H), 9.22 (br, 1H), 11.20 (s, 1H).



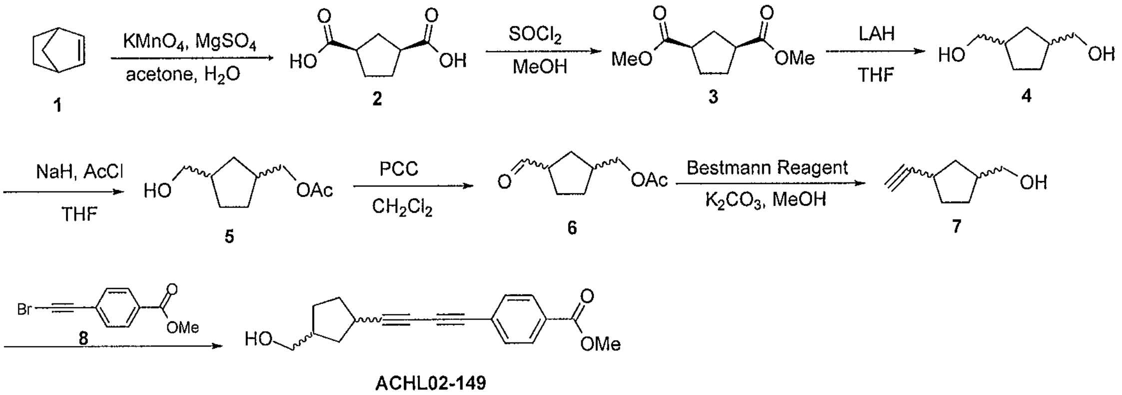
(S)-methyl 2-amino-3-(tert-butoxycarbonylamino)-3-methylbutanoate (329 mg, 1.377 mmol, 1.0 equiv) and 4-(((1,3-*trans*)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzoic acid (340 mg, 1.377 mmol, 1.0 equiv) were dissolved in DMF (3 mL). To this was added DIPEA (0.584 mL, 3.34 mmol, 2.5 equiv) then HATU (610 mg, 1.605 mmol, 1.2 equiv). This stirred at room temperature for ~16 h. The reaction was partitioned between 1M citric acid and ethyl acetate. The organics were washed with semi-saturated sodium chloride, saturated sodium bicarbonate then saturated sodium chloride, dried over magnesium sulfate and evaporated to dryness. Yielded 990 mg crude (S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-(((1,3-*trans*)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate. LCMS M+1 expected = 483.2, observed = 483.2.



I-13

(S)-methyl-3-(tert-butoxycarbonylamino)-2-(4-(((1,3-*trans*)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (crude) was dissolved in methanol (1 mL). At room temp was added 4.0M HCl in dioxane (2.82 mL, 11.27 mmol, 8.43 equiv). The reaction was complete after 60 minutes (HPLC). The reaction was concentrated on rotovap at 0°C. To this was added IPA (2 mL) then hydroxylamine solution (1.77 mL, 26.7 mmol, 20 equiv). The flask was placed at 4°C for 96h. Then the reaction was concentrated (keeping reaction at 0°C) to a gummy mass. To this was added water (3 mL) and ACN (0.5 mL). The solution was acidified at 0°C using TFA (3 mL). Additional water (1 mL) and ACN (1 mL) was added. The compound was purified by RP HPLC (2" column, 50mL/min, 0.1% TFA in water/ACN, equil @ 5%B); loaded onto 2" column (10mL/min, 5%B) using syringe filter (2X7 mL); ramped to 50mL/min over 1 minute and 5-35%B over 55min. The product eluted at 22.9-25.5%B. Desired fractions were combined, frozen and placed on lyo. The process yielded 410 mg of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-*trans*)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamide, TFA. LCMS M+1 expected = 384.2, observed = 384.2. 1H NMR (DMSO-d6): δ 1.25 (s, 3H), 1.30 (s, 3H), 2.07-2.11 (t, 4H), 2.40-2.43 (m, 1H), 3.22-3.26 (m, 1H), 3.37-3.38 (d, 1H), 4.57 (br, 1H), 4.65-4.67 (d, 1H), 7.62-7.64 (d, 2H), 7.89-7.91 (d, 2H), 7.97 (s, 2H), 8.54-8.56 (d, 1H), 9.21 (br, 1H), 11.19 (s, 1H).

K. Synthesis of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-*trans*)-3-(hydroxymethyl)cyclopentyl)buta-1,3-diynyl)benzamide (I-14)



(cis)-cyclopentane-1,3-dicarboxylic acid (2)

Reagent	MW	Eq.	mmol	g, mL

Compound **1**	94	1.0	106	10 g
KMnO4	158	2.8	297	47 g
MgSO4	120	0.33	35.4	4.3 g
H2O				500mL
Acetone				15 mL

To a stirred suspension of potassium permanganate (47 g, 0.297 mol) and magnesium sulfate (4.3 g, 35.4 mmol) in water (500 mL) cooled to 5 °C was added a solution of bicyclo[2.2.1]hept-2-ene **1** (10 g, 0.106 mol) in acetone (15 mL) in one portion (5 °C —40 °C). The ice bath was removed and the reaction was stirred for further 2 h . After this time, the mixture was filtered and the filtrate was treated with sodium hydrogensulfite and acidified to pH=2 with 1N HCl. Then extracted with ethyl acetate (800 mL x 3) and the combined organic layer was washed with water (1 L), brine (1 L), dried, concentrated to give target compound **2** (7.5 g, 46 %) as a white solid without purification for next step.

(cis)-dimethyl cyclopentane-1,3-dicarboxylate *(3)*

Reagent	MW	Eq.	mmol	g, mL
Compound **2**	158	1.0	47.5	7.5 g
$SOCl_2$	119	4.0	190	22.6 g
MeOH				200mL

To a stirred solution of (cis)-cyclopentane-1,3-dicarboxylic acid **2** (7.5 g, 47.5 mmol) in methanol (200 mL) was added thionyl chloride (22.6 g, 0.19 mol) at ice bath. Then the reaction mixture was heated to reflux overnight. After this time, the mixture was concentrated to give Compound **3** (8.3 g, 94%) as a yellow oil without purification for next step. Confirmed by GC-MS.

cyclopentane-1,3-diyldimethanol (4)

Reagent	MW	Eq.	mmol	g, mL
Compound **2**	186	1	44.6	8.3 g
$LiAlH_4$	38	2.0	89.2	3.4 g
THF				150 ml

To a stirred solution of LAH (3.4 g, 89.2 mmol) in THF (100 mL) was added a solution of (cis)-dimethyl cyclopentane-1,3-dicarboxylate 3 (8.3 g, 44.6 mmol) in THF (50 mL) at ice bath. Then the reaction mixture was stirred at room temperature overnight. After this time, the mixture was diluted with water (6.5 mL), then filtered, and the filtrate was concentrated, the residue was purified with silica-gel chromatography (PE:EA=2:1) to give Compound 4 (4.0 g, 69 %) as a yellow oil. HNMR (400 MHz, DMSO-d6) 0.53-0.50 (m, 1H), 0.96-1.00 (m, 2H), 1.29-1.35 (m, 2H), 1.47-1.54 (m, 1H), 1.66-1.73 (m, 2H), 2.98-3.01 (m, 4H), 4.29 (s, 1H)

3-(hydroxymethyl)cyclopentyl)methyl acetate (5)

Reagent	MW	Eq.	mmol	g, mL
Compound **4**	130	1.0	31	4.0 g
AcCl	78	1.0	34	2.7 g
NaH (60%)	24	0.9	28	1.1 g
THF				60 mL

To a stirred solution of cyclopentane-1,3-diyldimethanol **4** (4.0 g, 31 mmol) in THF (60 mL) was added NaH (60 %) (1.1 g, 28 mol) at ice bath. Then the reaction mixture was stirred at ice bath for 0.5 h, then acetyl chloride (2.7 g, 34 mmol) was added to the reaction mixture. And the mixture was stirred at room temperature overnight. After this time, the mixture was diluted with water (1.0 mL), then filtered, and the filtrate was concentrated, the residue was purification with silica-gel chromatography to give Compound **5** (1.7 g, 25 %) as a yellow oil. HNMR (400 MHz, DMSO-d6) 0.84-0.87 (m, 1H), 1.28-1.31 (m, 2H), 1.62-1.65 (m, 2H), 1.80-1.83 (m, 1H), 1.99 (s, 1H), 2.10-2.18 (m, 1H), 3.26-3.29 (m, 2H), 3.89 (d, *J* = 8, 2H), 4.43 (s, 1H).

3-formylcyclopentyl)methyl acetate (6)

Reagent	MW	Eq.	mmol	g, mL
Compound **5**	172	1	9.9	1.7 g
PCC	215	3.0	29.7	6.4 g
CH2Cl2				50 ml

To a stirred solution of 3-(hydroxymethyl)cyclopentyl)methyl acetate **5** (1.7 g, 9.9 mmol) in CH_2Cl_2 (50 mL) was added PCC (6.4 g, 29.7 mmol) in portions. Then the reaction mixture was stirred at room temperature overnight. After this time, the mixture was concentrated, the residue was purified with silica-gel chromatography (PE:EA=6:1) to give Compound **6** (0.7 g, 44 %) as a yellow oil.

3-ethynylcyclopentyl)methanol (7)

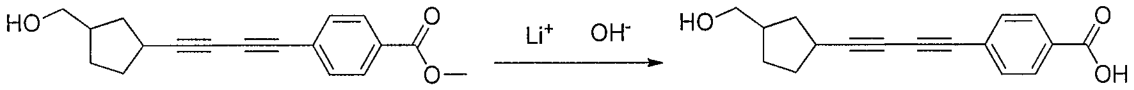
Reagent	MW	Eq.	mmol	g, mL
Compound **6**	170	1.0	4.1	0.7 g
Bestmann reagent (80%)	192	1.5	6.15	1.18 g
K_2CO_3	138	1.5	6.15	0.85 g
MeOH				50 mL

To a stirred solution of 3-formylcyclopentyl)methyl acetate **6** (0.7 g, 4.1 mmol) in MeOH (50 mL) was added potassium carbonate (0.85 g, 6.15 mmol) and Bestmann Reagent (1.18 g, 6.15 mmol). The reaction mixture was stirred at room temperature overnight. After this time, the mixture was diluted with water (50 mL), extracted with ether (80 mL x 3) and the combined organic layer was dried, concentrated the residue was purified with silica-gel chromatography (PE:EA=2:1) to give Compound **7** (0.31 g, 60 %) as a yellow oil.

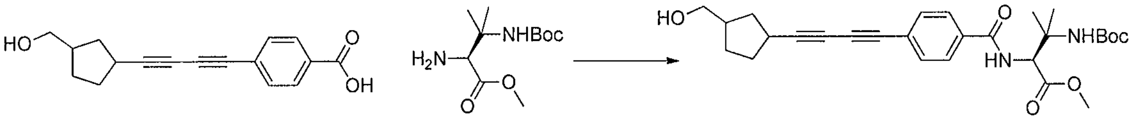
Methyl 4-((3-(hydroxymethyl)cyclopentyl)buta-1,3-diynyl)benzoate (ACHL02-149)

Reagent	MW	Eq.	mmol	g, mL
Compound **7**	124	1.0	2.42	0.3 g
Compound **8**	240	1.2	2.9	0.7 g
$PdCl_2(PPh_3)_2$	701	0.03	0.1	70 mg
CuI	191	0.1	0.242	46 mg
DIPEA	129	3.0	7.26	936 g
THF				60 mL

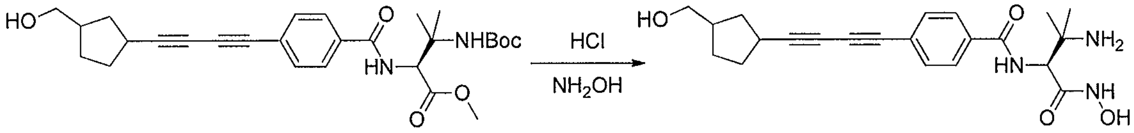
Under nitrogen, DIPEA (936 mg, 7.26 mmol) was added dropwise to a mixture of 3-ethynylcyclopentyl)methanol 7 (0.3 g, 2.42 mmol), methyl 4-(bromoethynyl)benzoate 8 (0.7 g, 2.9 mmol), $PdCl_2(PPh_3)_2$ (70mg, 0.1 mmol) and CuI (46mg, 0.242 mmol) in THF (60 mL) at RT. The reaction mixture was stirring at RT overnight. LCMS monitored the reaction. The reaction mixture was diluted with water (60 mL) and extracted with ethyl acetate (80mL x 3). The ethyl acetate layer was washed with saturated NH4Claq solution, water and brine. After dried, the ethyl acetate solution was concentrated under reduced pressure. The residue was purified with silica-gel column (PE: EA = 5:1) to obtain compound ACHL02-149 (as a mixture of cis and trans isomers) (140 mg, 25%). H NMR (400MHz, DMSO-d6): 7.95 (d, J = 8.4Hz, 2H), 7.51 (d, J = 8Hz, 2H), 3.91 (s, 1H), 3.61 (d, J = 4Hz,1H), 3.53 (d, J = 8Hz, 1H), 2.89-2.87 (m, 1H), 2.43-2.40 (m, 1H), 2.21-2.17 (m, 1H), 2.02-1.99 (m, 1H), 1.82-1.76 (m, 1H), 1.54-1.45 (m,1H), 1.36-1.31 (m,1H).



Methyl 4-((3-(hydroxymethyl)cyclopentyl)buta-1,3-diynyl)benzoate (115 mg, 0.407 mmol, 1.0 equiv) was dissolved in MeOH (5mL), followed by the addition of lithium hydroxide (29.3 mg, 1.222 mmol, 3.0 equiv) and water (5 mL). This stirred at room temperature for ~72 h. The reaction was concentrated to remove MeOH, acidify to pH ~3 with 6N HCl (~5mL). Extract with EtOAc (3x50 mL), combine organic layers, wash with sat. NaCl, dried (MgSO4), filtered, concentrated. Yielded 0.14 g crude 4-((3-(hydroxymethyl)cyclopentyl)buta-1,3-diynyl)benzoic acid. LCMS M+1 expected = 269.1, observed = 269.1.



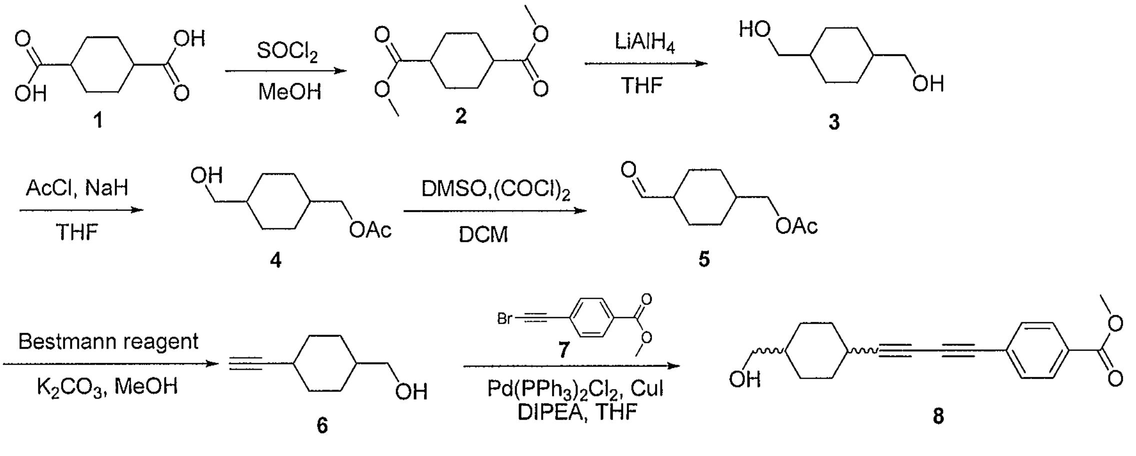
(S)-methyl 2-amino-3-(tert-butoxycarbonylamino)-3-methylbutanoate (100 mg, 0.406 mmol, 1.0 equiv) and 4-((3-(hydroxymethyl)cyclopentyl)buta-1,3-diynyl)benzoic acid (crude) (theoretical 109 mg, 0.406 mmol, 1.0 equiv) were dissolved in DMF (1 mL). To this was added DIPEA (0.177 mL, 1.016 mmol, 2.5 equiv) then HATU (185 mg, 0.488 mmol, 1.2 equiv). This stirred at room temperature for ~16 h. The reaction was partitioned between 1M citric acid and ethyl acetate. The organics were washed with semi-saturated sodium chloride, saturated sodium bicarbonate then saturated sodium chloride, dried over magnesium sulfate and evaporated to dryness. Yielded 500 mg crude (S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-((3-(hydroxymethyl)cyclopentyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate. LCMS M+1 expected = 497.3, observed = 497.2.



(2S)-methyl-3-(tert-butoxycarbonylamino)-2-(4-((3-(hydroxymethyl)cyclopentyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (crude) was dissolved in methanol (1 mL). At room temp was added 4.0M HCl in dioxane

(0.857 mL, 3.43 mmol, 8.43 equiv). The reaction complete after 3 hours (HPLC). The reaction was concentrated on rotovap at 0°C. To this was added IPA (1 mL) then hydroxylamine solution (0.537 mL, 8.14 mmol, 20 equiv). Place flask at 4°C for 168h. Reaction concentrated, (keeping reaction at 0°C) to gummy mass. To this was added water (3 mL) and ACN (0.5 mL). Acidified while at 0°C using TFA (3 mL). Additional water (1 mL) and ACN (1 mL) was added. Purified by RP HPLC (1" column, 20mL/min, 0.1% TFA in water/ACN, equil @ 5%B). Loaded onto 1" column (10mL/min, 5%B) using syringe filter (2X8 mL). Ramped to 20mL/min over 1 minute. 5-19%B over 80min, prod elutes at 19%B. Desired fractions were combined, frozen and placed on lyo. Yielded 145 mg of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-((3-(hydroxymethyl)cyclopentyl)buta-1,3-diynyl)benzamide, **I-14**, TFA. LCMS M+1 expected = 398.2, observed = 398.2. 1H NMR (DMSO-d6): δ 1.25 (s, 3H), 1.29 (s, 3H), 1.38-1.45 (m, 1H), 1.53-1.77 (m, 2H), 1.88-2.17 (m, 3H), 2.83-2.91 (m, 1H), 3.21-3.38 (m, 4H), 4.52 (br, 1H), 4.65-4.67 (d, 1H), 7.61-7.63 (d, 2H), 7.88-7.90 (d, 2H), 7.97 (s, 2H), 8.53-8.56 (d, 1H), 9.21 (br, 1H), 11.19 (s, 1H).

L. Synthesis of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,4-*cis*)-4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamide (I-15) and N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,4-*trans*)-4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamide (I-16)



<u>Step 1:</u> dimethyl cyclohexane-1,4-dicarboxylate (2)

Reagent	MW	Eq.	mmol	g, mL
Compound **1**	172	1.0	290.7	50g
$SOCl_2$	119	4.0	1162.8	138g
MeOH				500mL

Methanol (500 mL) was cooled in an ice-salt bath and thionyl chloride (138 g, 4.0eq) was added dropwise. To the resultant solution of HCl in methanol, cyclohexane-1,4-dicarboxylic acid **1** (50g, 1.0 equiv) was added and the reaction mixture was refluxed for 1h. TLC monitored the reaction. The solvent was evaporated and the residue was diluted with water, extracted with ethyl acetate. The ethyl acetate layer was washed with $NaHCO_3$ solution, brine and dried over Na_2SO_4.The solvent was evaporated to obtain the target compound (56.4g, 97%).

Step 2: cyclohexane-1,4-diyldimethanol (3)

Reagent	MW	Eq.	mmol	g, mL
Compound **2**	200	1	150.0	30g
$LiAlH_4$	38	3.0	450.0	17.1g
THF				200ml

An ice-cooled suspension of $LiAlH_4$ in THF was added dropwise a solution of dimethyl cyclohexane-1,4-dicarboxylate **2** at 0°C. The reaction mixture was kept stirring at r.t.for 30min. TLC monitored the reaction. 12eq Water (32.4mL) was added to quench the reaction. The reaction mixture was filtered, and the filtrate was concentrated. The residue was diluted with Et_2O, dried over Na_2SO_4. Et_2O was removed to collect the target compound which was used for next step without further purification (19.9g, 92%).

Step 3: *(4-(hydroxymethyl)cyclohexyl)methyl acetate (4)*

Reagent	MW	Eq.	mmol	g, mL
Compound **3**	144	1.0	138.2	19.9g
AcCl	78	1.0	138.2	10.78g
NaH (60%)	24	1.0	138.2	5.53g
THF				150mL

An ice-cooled suspension of NaH and cyclohexane-1,4-diyldimethanol **3** in THF was added dropwise AcCl at 0°C. The reaction was kept stirring for 3h at 15°C. TLC monitored the reaction. 1 eq H_2O (2.5mL) was added to quench the reaction. The reaction mixture was filtered, and the filtrate was diluted with ethyl acetate. The ethyl acetate layer was washed with brine (5mL), dried over Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified with silica-gel column (DCM: MeOH=50:1 to 10:1) to obtain the target compound (8.6g, 33%).

Step 4: *(4-formylcyclohexyl)methyl acetate (5)*

Reagent	MW	Eq.	mmol	g, mL
Compound **4**	186	1.0	46.24	8.6g
$(COCl)_2$	128	2.0	92.47	11.8g
DMSO	78	4.0	184.95	14.4g
DCM				200mL
TEA	101	8.0	369.92	37.36g

To a solution of oxalyl chloride in DCM (60mL) was added dropwise DMSO in DCM (60mL) at -78°C. 30min later, (4-(hydroxymethyl)cyclohexyl)methyl acetate **4** in DCM (80mL) was added dropwise at -78°C. The reaction mixture was stirring for additional 2h at -78°C, then TEA was added dropwise at -78 °C to quench the reaction. The reaction mixture was allowed warm to rt. The reaction mixture was diluted with DCM, and washed with saturated aqueous ammonium chloride and brine. The DCM layer was dried and concentrated. The residue was purified with silica-gel column (DCM) to obtain the target compound (9.0g, still wet with dichloromethane).

Step 5: (4-ethynylcyclohexyl)methanol (6)

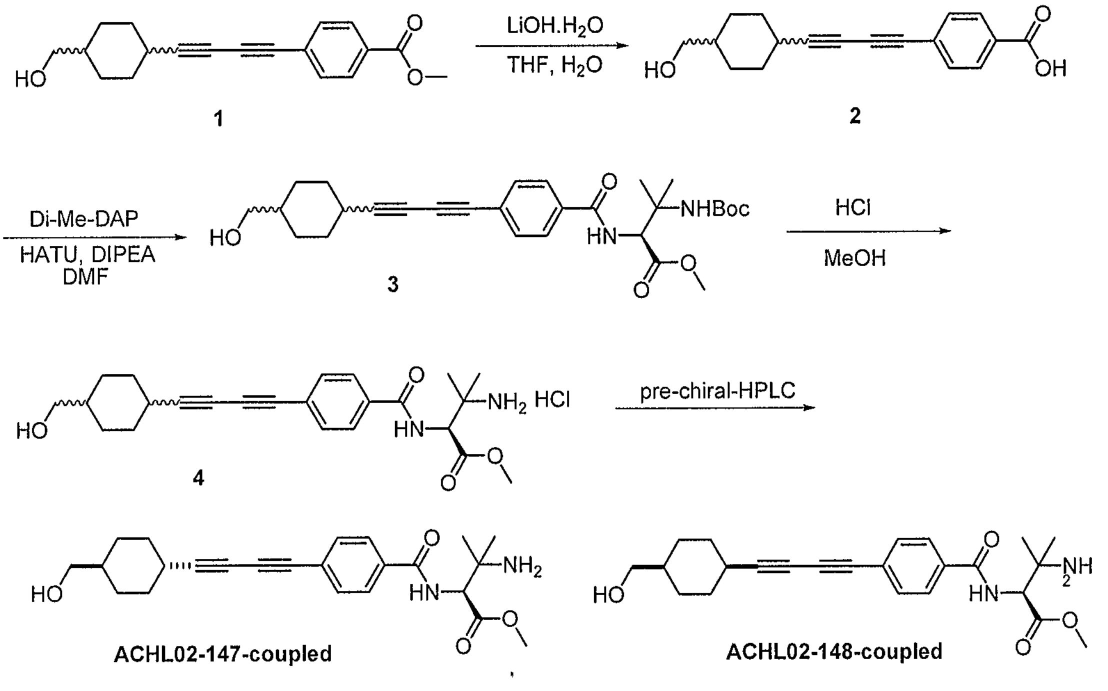
Reagent	MW	Eq.	mmol	g, mL
Compound **5**	184	1.0	46.2	8.5g
Bestmann reagent (80%)	192	1.3	60.0	11.56g
K_2CO_3	138	3.0	138.6	19.04g
MeOH				100mL

A mixture of (4-formylcyclohexyl)methyl acetate **5** (8.5g, 1.0eq), K_2CO_3 (19.04g, 3.0eq) in CH_3OH (100mL) was added Bestmann Reagent (11.56g, 1.3eq, 80%) at 0°C. The mixture was stirred at room temperature for 15h. TLC monitored the reaction. The mixture was diluted with water, extracted with ethyl acetate, washed with brine, dried Na_2SO_4, concentrated under reduced pressure. The crude was purified with silica-gel column (PE: EA=3:1) to obtain the target compound (2.6g, 40%).

Step 6: methyl 4-((4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzoate (8)

Reagent	MW	Eq.	mmol	g, mL
Compound **6**	138	1.0	10.14	1.4g
Compound **7**	240	1.2	12.17	2.9g
$PdCl_2(PPh_3)_2$	701	0.03	0.304	213mg
CuI	191	0.1	1.014	194mg
DIPEA	129	3.0	30.43	3.92g
THF				150mL

Under nitrogen, DIPEA (3.92g, 3.0 equiv) was added dropwise to a mixture of (4-ethynylcyclohexyl)methanol **6** (1.4g, 1.0 equiv), methyl 4-(bromoethynyl)benzoate **7** (2.9g, 1.2 equiv), $PdCl_2(PPh_3)_2$ (213mg, 0.03 equiv) and CuI (194mg, 0.1 equiv) in THF (150mL) at RT. The reaction mixture was stirring at RT overnight. LCMS monitored the reaction. The reaction mixture was diluted with water (100mL) and extracted with ethyl acetate (300mL). The ethyl acetate layer was washed with saturated NH_4Cl aq solution, water and brine. After being dried, the ethyl acetate solution was concentrated under reduced pressure. The residue was purified with silica-gel column (PE: EA=5:1 to 3:1) to obtain the target compound (as a mixture of cis and trans isomers, 1.2 g, 40%). ^1H NMR (400MHz, DMSO-d_6): 7.959-7.937(d, J=8.8Hz, 2H), 7.682-7.660(d, J=8.8Hz, 2H), 4.421-4.395(t, J=5.2Hz, 1H), 3.860(s.3H), 3.207-3.179(t, J=5.6Hz, 2H), 2.085(s, 1H), 1.980-1.941 (m, 2H), 1.747-1.708(m, 2H), 1.366-1.328(m, 3H), 0.935-0.897(m, 2H).



4-((4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzoic acid (2)

Reagent	MW	Eq.	Mmol	g, mL

Compound **1**	296	1	4.1	1.2 g
$LiOH.H_2O$	42	3	12.3	517 mg
THF				20 mL
H_2O				5 mL

To a solution of methyl 4-((4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzoate **1** (1.2g, 4.1mmol) in THF (20mL) was added a solution of lithium hydroxide mono-hydrate (517mg, 12.3mmol) in water (5mL). The mixture was kept stirring at r.t. overnight. After this time, the solvent was removed under reduced pressure, the residue was diluted with water (40mL), the pH was adjusted to 4.0 with 1M HCl, then extracted with ethyl acetate (3 x 60mL). The organic layer was dried and concentrated to give 4-((4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzoic acid **2** (1.0 g, 93%) as a yellow solid which was used for next step without further purification. LC-MS:283 $[M+H]^+$.

(S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-((4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (3)

Reagent	MW	Eq.	mmol	g, mL
Compound **2**	282	1	3.72	1.05 g
Boc-Di-Me-DAP	246	1	3.72	915 mg
HATU	380	1.1	4.1	1.55 g
DIPEA	129	3	11.2	1.44 g
DMF				25 mL

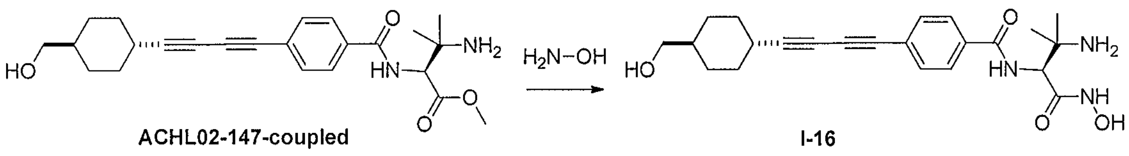
To a solution of 4-((4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzoic acid **2** (1.05g, 3.72mmol), HATU (1.55g, 4.1mmol) in DMF (25mL) was added (S)-methyl 2-amino-3-(tert-butoxycarbonylamino)-3-methylbutanoate (915 mg, 3.72mmoL) and DIPEA (1.44 g, 11.2mmoL). The mixture was kept stirring at r.t. for 5 hours. After this time, the reaction was diluted with water (20mL), then extracted with ethyl acetate

(60mL x 3). The combined organic layers were washed with water then brine, dried and concentrated, the residue was purified with silica-gel chromatography (PE:EA 2:1) to give (S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-((4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate **3** as a yellow solid. (1.6 g, 85%). LC-MS:511 $[M+H]^+$.455 [M-56]

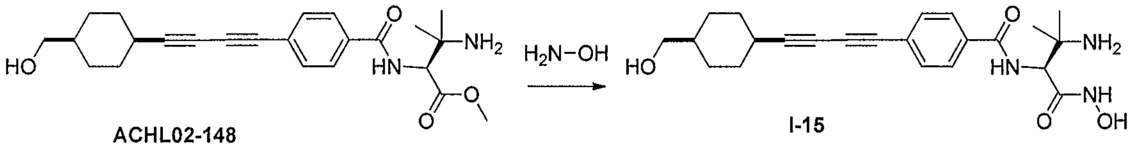
(S)-methyl 3-amino-2-(4-((4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate hydrochloride (4)

Reagent	MW	Eq.	mmol	g, mL
Compound **3**	510	1	3.14	1.6g
HCl(g)				
CH_3OH				20 mL

A solution of (S)-methyl 3-(tert-butoxycarbonylamino)-2-(4-((4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate **3** (1.6g, 3.14mmol) in methanol (20mL) was attached to a HCl apparatus. Then the reaction was stirred at room temperature until TLC indicated the total consumption of the starting material. After that, the solution was concentrated under reduced pressure to give (S)-methyl 3-amino-2-(4-((4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate hydrochloride **4** as yellow solid (1.4 g, 99%). LC-MS:411 $[M+H]^+$.The cis and trans isomers were separated by chiral HPLC to give (S)-methyl 3-amino-2-(4-(((1,4-*cis*)-4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (ACHL02-148-coupled, 90 mg) as peak 1 and (S)-methyl 3-amino-2-(4-(((1,4-*trans*)-4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (ACHL02-147-coupled, 750 mg) as peak 2.



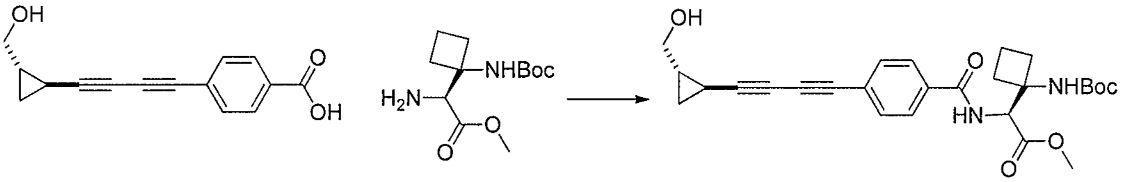
(S)-Methyl-3-amino-2-(4-(((1,4-*trans*)-4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (750 mg, 1.827 mmol, 1.0 equiv) was added to a round bottom flask. To this was added IPA (6 mL), the suspension was sonicated for 5 minutes, then THF (2 mL) was added to form a solution. Hydroxylamine solution (2.4 mL, 36.5 mmol, 20 equiv) was added and the flask was placed at 4°C for ~96h. Reaction concentrated, (keeping reaction at 0°C) to remove IPA and THF. Acidified while at 0°C using TFA (4 mL). Additional ACN (2mL) was added. Purified by RP HPLC (2" column, 50mL/min, 0.1% TFA in water/ACN, equil @ 10%B). Loaded onto 2" column (10mL/min, 10%B) using syringe filter (3X8 mL). Ramped to 50mL/min over 1 minute. 10-40%B over 60min, prod elutes at 30.5-35%B. Desired fractions were combined, frozen and placed on lyo. Yielded 706 mg of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,4-*trans*)-4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamide, **I-16**, TFA. LCMS M+1 expected = 412.2, observed = 412.2. 1H NMR (DMSO-d6): δ 0.84-0.94 (m, 2H), 1.25 (s, 3H), 1.30 (s, 3H), 1.32-1.36 (m, 1H), 1.68-1.72 (m, 2H), 1.90-1.95 (m, 2H), 2.39-2.42 (m, 1H), 3.15-3.18 (m, 2H), 4.38-4.40 (t, 1H), 4.65-4.67 (d, 1H), 7.62-7.64 (d, 2H), 7.89-7.91 (d, 2H), 7.98 (br, 2H), 8.54-8.56 (d, 1H), 9.22 (br, 1H), 11.21 (br, 1H).



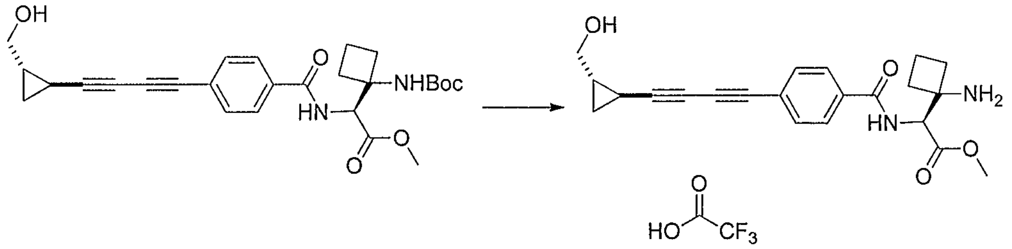
(S)-methyl-3-amino-2-(4-(((1,4-*cis*)-4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamido)-3-methylbutanoate (90 mg, 0.219 mmol, 1.0 equiv) was added to a round bottom flask. To this was added IPA (0.5 mL), the solution was vortexed for 2 minutes, then hydroxylamine solution (0.29 mL, 4.38 mmol, 20 equiv) was added and the flask was placed at 4°C for ~72h. Reaction concentrated, (keeping reaction at 0°C) to remove IPA. Acidified while at 0°C using TFA (3 mL). Acetonitrile (0.5 mL) was added. Purified by RP HPLC (1" column, 25mL/min, 0.1% TFA in water/ACN, equil @ 10%B). Loaded onto 1" column (10mL/min, 10%B) using syringe filter (7 mL). Ramped to 25mL/min over 1 minute. 10-40%B over 90min, prod elutes at 25.5-28%B.

Desired fractions were combined, frozen and placed on lyo. Yielded 53 mg of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,4-*cis*)-4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamide, **I-15**, TFA. LCMS M+1 expected = 412.2, observed = 412.2. 1H NMR (DMSO-d6): δ 1.20-1.35 (m, 2H), 1.26 (s, 3H), 1.30 (s, 3H), 1.47-1.59 (m, 4H), 1.71-1.75 (m, 2H), 2.97-2.99 (m, 1H), 3.21-3.22 (d, 2H), 4.30 (br, 1H), 4.65-4.68 (d, 1H), 7.64-7.66 (d, 2H), 7.89-7.91 (d, 2H), 7.98 (br, 2H), 8.54-8.56 (d, 1H), 9.22 (br, 1H), 11.20 (s, 1H).

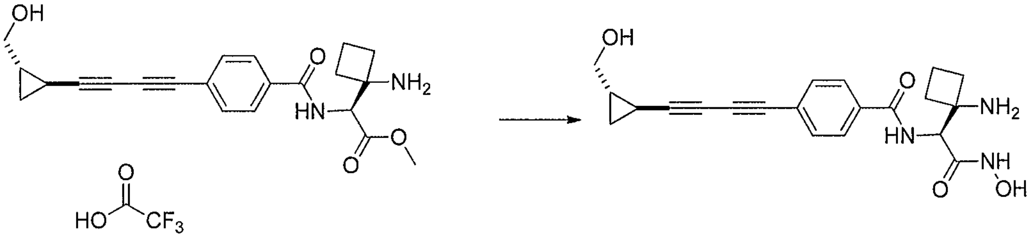
M. Synthesis of N-((S)-1-(1-aminocyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide trifluoroacetate (I-17) and N-((S)-1-(1-aminocyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((*cis*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide trifluoroacetate (I-18)



4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzoic acid (205 mg, 0.85 mmol, 1 eq) and (S)-methyl-2-amino-2-(1-(tert-butoxycarbonylamino)cyclobutyl)acetate (220 mg, 0.85 mmol, 1 eq) were dissolved in *N,N*-dimethylformamide (3mL). *N,N*-diisopropylethylamine (0.45mL, 2.6mmol, 3eq) was added, followed by HATU (356mg, 0.94mmol, 1.1eq). The mixture was kept at room temperature for 2.5 hours, then partitioned between 1M citric acid and ethyl acetate. The organics were washed with 1M citric acid, saturated sodium bicarbonate then saturated sodium chloride, dried over magnesium sulfate and evaporated to dryness to give (S)-methyl 2-(1-(tert-butoxycarbonylamino)cyclobutyl)-2-(4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)acetate (550mg crude, 134%, used without further purification).



(S)-methyl 2-(1-(tert-butoxycarbonylamino)cyclobutyl)-2-(4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)acetate (550mg crude, 1.1mmol) was dissolved in dichloromethane (6mL) and treated with 6mL trifluoroacetic acid for 10 minutes at room temperature. The volatiles were removed and the residue re-evaporated from dichloromethane to give (S)-methyl 2-(1-aminocyclobutyl)-2-(4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)acetate as its trifluoroacetate salt, which was used without further purification.



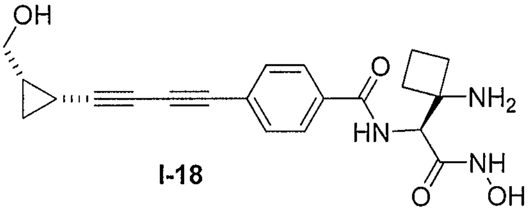
The crude (S)-methyl 2-(1-aminocyclobutyl)-2-(4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)acetate trifluoroacetate obtained in the above reaction was dissolved in isopropanol (2mL) and treated at 4°C for 20 hours with 50% aqueous hydroxylamine (2mL). The volatiles were removed and the residue dissolved in water/acetonitrile on acidification with trifluoroacetic acid. The material was purified by reverse-phase HPLC (2" column, 50mL/min, 0.1% TFA in water/ACN, equil @ 2%) using the following gradient:

2% 10min

2-15 5min

15-45 300min

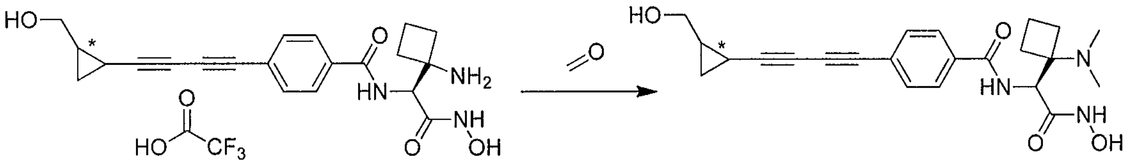
The product eluted between 58 and 78 minutes, and the desired fractions were lyophilized to obtain N-((S)-1-(1-aminocyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide as its trifluoroacetate salt (white solid, 120mg, 0.24mmol, 28% from 4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzoic acid). Mass spec data: expected (M+1): 382.4, observed: 382.1. Proton NMR (400MHz, dmso-d6): 11.3 (br s, 1H), 9.24 (s, 1H), 8.62 (d, 1H, J = 8.8Hz), 8.17 (br s, 3H), 7.86 (d, 2H, J = 8.4Hz), 7.63 (d, 2H, J = 8.4Hz), 4.92 (d, 1H, J = 9.2Hz), 4.69 (t, 1H, J = 5.8Hz), 3.40 (m, 1H), 3.26 (m, 1H), 2.12 – 2.21 (m, 4H), 1.89 (m, 1H), 1.80 (m, 1H), 1.39 – 1.46 (m, 2H), 0.84 – 0.93 (m, 2H). An additional 80mg was obtained upon re-purification of impure fractions.



I-18

Fractions containing mostly N-((S)-1-(1-aminocyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((cis)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (an impurity in the major isomer) were lyophilized separately and repurified under the same gradient. Desired fractions were pooled and lyophilized to give N-((S)-1-(1-aminocyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((cis)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide as its trifluoroacetate salt (3.2mg, 8.4umol, 1% from 4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzoic acid). Mass spec data: expected (M+1): 382.4, observed: 382.1. Proton NMR (400MHz, dmso-d6): 11.30 (bs s, 1H), 9.24 (s, 1H), 8.62 (d, 1H, J = 9.2Hz), 8.16 (br s, 3H), 7.86 (d, 2H, J = 7.6Hz), 7.65 (d, 2H, J = 7.6Hz), 4.92 (d, 1H, J = 9.2Hz), 4.70 (t, 1H, J = 5.2Hz), 3.49 (m, 1H), 3.40 (m, 1H), 2.12 – 2.22 (m, 4H), 1.82-1.95 (m, 1H), 1.70 – 1.80 (m, 2H), 1.36 (m, 1H), 1.08 (m, 1H), 0.58 (m, 1H)

N. Synthesis of N-((S)-1-(1-(dimethylamino)cyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-19)

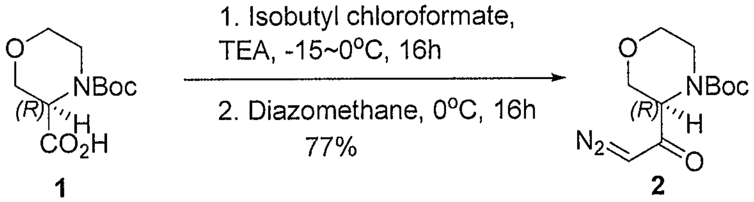


N-((S)-1-(1-aminocyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide trifluoroacetate **I-17** (80mg, 0.16mmol, 1eq) was dissolved in *N,N*-dimethylformamide (800μL) and treated with paraformaldehyde (48.5mg, 1.1mmol, 10eq) and *N,N*-diisopropylethylamine (56μL, 0.32mmol, 2eq) at room temperature for 21 hours. Sodium cyanoborohydride (30mg, 0.48mmol, 3eq) was added followed by methanol (800μL) and acetic acid (28μL, 0.48mmol, 3eq). The mixture was kept at room temperature for 3 days, then more sodium cyanoborohydride and acetic acid (28μL) was added. After a further day at room temperature another 28μL of acetic acid was added. After one more day at room temperature the material was purified by reverse-phase HPLC (1" column, 20mL/min, 0.1%TFA in water/acetonitrile, equilibrated at 2%) using the following gradient:

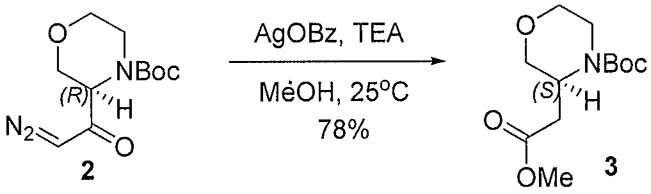
2% 5min
2-18% 5min
18-98% 80min

Desired fraction were pooled and lyophilized to give N-((S)-1-(1-(dimethylamino)cyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide as its trifluoroacetate salt (63mg, 0.12mmol, 74%). Mass spec data: expected (M+1): 410.5, observed: 410.2. Proton NMR (400MHz, dmso-d6): 11.29 (s, 1H), 9.72 (br s, 1H), 9.30 (s, 1H), 8.95 (d, 1H, J = 9.2Hz), 7.88 (d, 2H, J = 8.4Hz), 7.61 (d, 2H, J = 8.4Hz), 5.05 (d, 1H, J = 9.2Hz), 4.7 (br s, 1H), 3.40 (dd, 1H, J = 5.2, 11.6Hz), 3.22 (dd, 1H, J = 6.4, 11.2Hz), 2.76 (d, 3H, J = 4.4Hz), 2.68 (d, 3H, J = 4.4Hz), 2.33 – 2.42 (m, 3H), 1.62 – 1.78 (m, 2H), 1.37 – 1.46 (m, 2H), 0.84 – 0.92 (m, 2H).

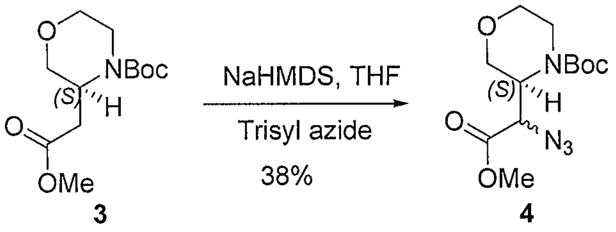
O. Synthesis of N-((S)-2-(hydroxyamino)-1-((S)-morpholin-3-yl)-2-oxoethyl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-20)



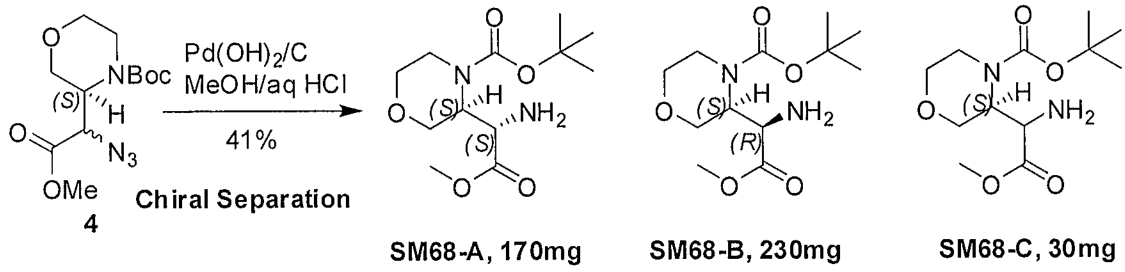
To a precooled suspension of (R)-4-(tert-butoxycarbonyl)morpholine-3-carboxylic acid **1** (5 g, 0.02162 mol) in dry ether (160 mL) was added triethylamine (3.91 mL, 0.0281 mol) dropwise at -16°C and after 5min, the resulting mixture was treated with isobutyl chloroformate (3.37 mL, 0.02600 mol) slowly at -15°C for a 30min duration, then the reaction mixture was stirred at -15°C ~6°C for 16h (overnight), LCMS monitored the reaction process, filtered, washed the solid with ether (30mL x 2), the filtrate was transferred to the next step. Treated the previous filtrate with a solution of diazomethane (0.24 mol) in ether (480 mL) at 0~7°C for 16h (overnight), then quenched the reaction with glacial acetic acid (5 mL), water (50 mL), and the organic layer was washed with sodium bicarbonate (50 mL x 2), brine (50 mL), the aqueous layer was re-extracted with ether (200 mL x 2), dried the combined organic layers over anhydrous sodium sulfate, filtered, concentrated to give the crude residue which was purified by silica-gel chromatography (20% ethyl acetate in petroleum as eluent), afforded (R)-tert-butyl-3-(2-diazoacetyl)morpholine-4-carboxylate **2** as a yellow solid, 4.56g (87% purity, LCMS, 254nm), yield: 77%, 2 steps.



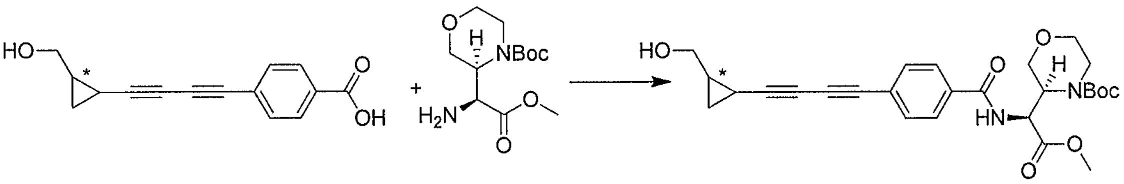
To a precooled suspension of (R)-tert-butyl-3-(2-diazoacetyl)morpholine-4-carboxylate **2** (4.55 g, 17.824 mmol) and silver benzoate (25 mg, 0.11 mmol) in methanol (30 mL) was added triethylamine (250 μL, 1.78 mmol) dropwise and the suspension was stirred at 25^{0}C for 16h in the dark (overnight, oil bath), then filtered, the filtrate was evaporated to dryness and the residue was purified by silica-gel chromatography (10% ethyl acetate in petroleum as eluent), affording (S)-tert-butyl-3-(2-methoxy-2-oxoethyl)morpholine-4-carboxylate **3** as a colorless solid, 3.34 g, yield: 78%.



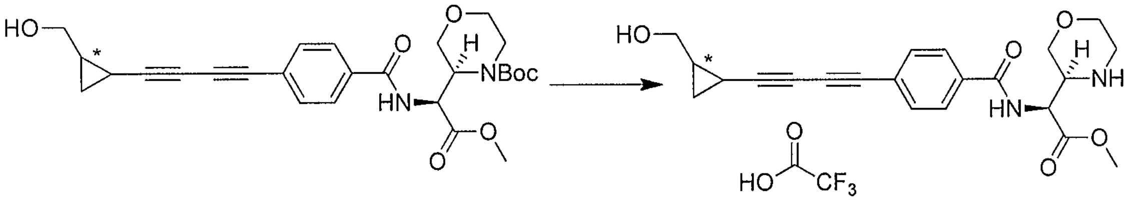
To a precooled solution of (S)-tert-butyl-3-(2-methoxy-2-oxoethyl)morpholine-4-carboxylate **3** (2.737 g, 0.0106 mol) in THF (100 mL) was added NaHMDS (8.15 mL, 0.0159 mol) slowly at -100^{0}C and the resulting suspension was stirred at -100^{0}C for 1h, then a solution of trisilylazide (5.99 g, 0.0170 mol) in THF (10 mL) was added slowly (kept the inner temperature below -100^{0}C), and additional 1h stirring at -100^{0}C was continued. Glacial acetic acid (3.18 g, 0.0530 mol) was added rapidly to quench the reaction, and the reaction mixture was allowed to warm to r.t., stirred for 3h, finally diluted with DCM (200mL x 2) and washed with saturated $NaHCO_3$ (100mL), brine (100mL) and water (100mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, concentrated to give the crude residue which was purified by reverse phase chromatography (Gradient: 10% acetonitrile in water for 5min, increase to 30% acetonitrile in water within 3min, 30% acetonitrile in water for 3min, 30-45% acetonitrile in water, 3min, 45% acetonitrile in water for 5min; Flow rate: 30mL/min), to afford (S)-tert-butyl-3-(1-azido-2-methoxy-2-oxoethyl)morpholine-4-carboxylate **4** (mixture of two diasteroisomers) as a white solid, 1.22 g, yield: 38%



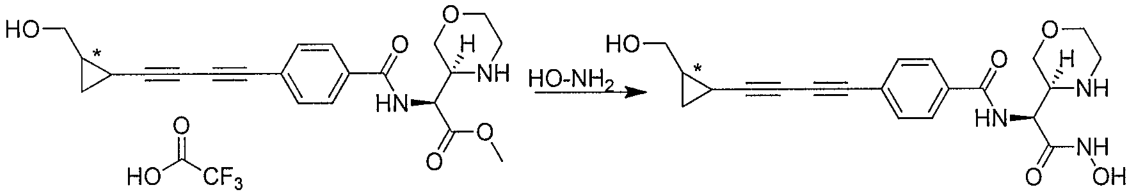
A mixture of (S)-tert-butyl-3-(1-azido-2-methoxy-2-oxoethyl)morpholine-4-carboxylate 4 (1.2 g, 4 mmol), Pd(OH)2/C (200 mg, 15%wt), 2M HCl (2 mL, 4 mmol) in EtOAc/MeOH (20mL/3mL) was degassed and treated with hydrogen gas at 30^{0}C under 1 atm. for 16h. The mixture was filtered, and the solid was washed with EtOAc (20mL). The combined organics were washed with water (20mL), saturated NaHCO3 (20mL, to adjust pH ≥ 8), then dried over anhydrous sodium sulfate, filtered, concentrated to give the crude residue which was purified by reverse phase chromatography (Gradient: 10% acetonitrile in water for 5min, increase to 30% acetonitrile in water within 3 min, 30% acetonitrile in water for 3 min, 30-45% acetonitrile in water, 3min, 45% acetonitrile in water for 5min; Flow rate: 30mL/min). The purified residue was then submitted to chiral separation, affording (S)-tert-butyl-3-((S)-1-amino-2-methoxy-2-oxoethyl)morpholine-4-carboxylate (SM68-A, 170 mg, yellow oil); (S)-tert-butyl-3-((R)-1-amino-2-methoxy-2-oxoethyl)morpholine-4-carboxylate (SM68-B, 230 mg, yellow solid); and SM68-C, 30 mg, yellow oil, total yield: 41%.



4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzoic acid (140 mg, 0.58 mmol) and (S)-tert-butyl-3-((S)-1-amino-2-methoxy-2-oxoethyl)morpholine-4-carboxylate (160 mg, 0.58 mmol) were dissolved in DMF (2 mL). *N,N*-Diisopropylethylamine (255 µL, 1.46 mmol) was added followed by HATU (266 mg, 0.70 mmol). The reaction was kept at r.t. for 30 minutes, then partitioned between 1M citric acid and ethyl acetate. The organics were washed with 1M citric acid, saturated sodium bicarbonate then saturated sodium chloride, dried over magnesium sulfate and evaporated to dryness. 380mg crude (S)-tert-butyl-3-((S)-1-(4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-2-methoxy-2-oxoethyl)morpholine-4-carboxylate was obtained.



(S)-tert-butyl-3-((S)-1-(4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-2-methoxy-2-oxoethyl)morpholine-4-carboxylate (crude, 380 mg) was dissolved in dichloromethane (4 mL), trifluoroacetic acid (4 mL) was added. After 5 minutes at r.t. the mixture was diluted with 10 mL dichloromethane and evaporated. The (S)-methyl-2-(4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-2-((S)-morpholin-3-yl)acetate 2,2,2-trifluoroacetate thus obtained was used crude in the next step.



(S)-methyl-2-(4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamido)-2-((S)-morpholin-3-yl)acetate 2,2,2-trifluoroacetate (crude from above reaction) was dissolved in isopropanol (1 mL), and aqueous hydroxylamine (50%, 1 mL) was added. The solution was kept at 4°C for 18 hours then evaporated. Water was added and the mixture acidified with TFA. The crude material was purified by RP HPLC (2" column, 50mL/min, 0.1% TFA in water/ACN, equil @ 2%):

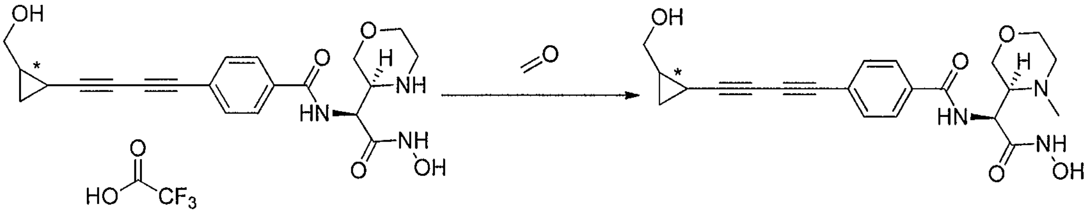
2% 10min

2-11% 5min

11-41% 300min

The desired compound eluted at 88-99 minutes. Desired fractions were lyophilized down to give N-((S)-2-(hydroxyamino)-1-((S)-morpholin-3-yl)-2-oxoethyl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide **I-20** as its trifluoroacetate salt (white solid, 118 mg, 0.30 mmol, 52% from 4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzoic acid). Mass spec data: expected (M+1): 398.2, observed: 398.1. Proton NMR (400MHz, dmso-d6): 11.17 (s, 1H), 9.14 (s, 1H), 9.08 (br s, 1H), 8.93 (br s, 1H), 8.83 (d, 1H, J = 8.4Hz), 7.89 (d, 2H, J = 8.0Hz), 7.62 (d, 2H, J = 8.0Hz), 4.69 (br s, 1H), 4.63 (t, 1H, J = 8.8HZ), 3.83 – 3.86 (m, 2H), 3.60 – 3.66 (m, 2H), 3.50 (t, 1H, J = 11.0Hz), 3.39 (m, 1H), 3.20 – 3.23 (m, 2H), 3.05 (m, 1H), 1.39 – 1.45 (m, 2H), 0.87 – 0.92 (m, 2H).

P. Synthesis of N-((S)-2-(hydroxyamino)-1-((S)-4-methylmorpholin-3-yl)-2-oxoethyl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (I-21)



N-((S)-2-(hydroxyamino)-1-((S)-morpholin-3-yl)-2-oxoethyl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide trifluoroacetate **I-20** (10 mg, 20 µmol) was dissolved in DMF (100 µL). Paraformaldehyde (5.9 mg, 200 µmol) was added followed by *N,N*-diisopropylethylamine (6.8 µL, 39 µmol). The mixture was stirred at r.t. for 19 hours. Sodium cyanoborohydride (3.7 mg, 59 µmol), methanol (100 µL) and acetic acid (4.5 µL, 78 µmol) were added and the mixture stirred at r.t. for a further 3 days. The mixture was purified by RP HPLC (2" column, 50mL/min, 0.1% TFA in water/ACN, equil @ 2%):

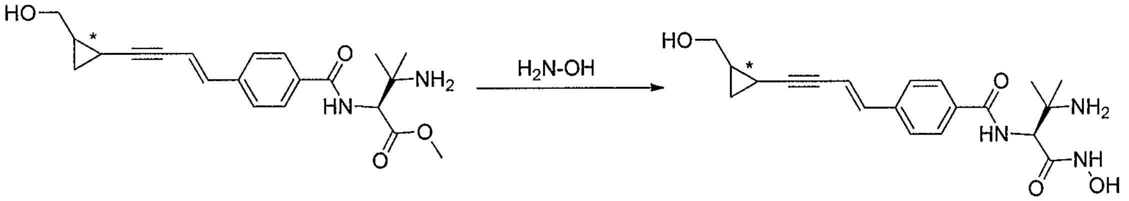
2% 10min

2-15% 5min

15-95% 80min

Product eluted between 23 and 24 minutes. Desired fractions were lyophilized to give N-((S)-2-(hydroxyamino)-1-((S)-4-methylmorpholin-3-yl)-2-oxoethyl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide as its trifluoroacetate salt (7.0 mg, 13 μmol, 68%). Mass spec data: expected (M+1): 411.2, observed: 412.2.

Q. Synthesis of N-((S)-3-amino-1-hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-((E)-4-(1,2-trans)-2-(hydroxymethyl)cyclopropyl)but-1-ene-3-ynyl)benzamide (I-22)



(S)-methyl 3-amino-2-(4-(E)-4-(1S,2S)-2-(hydroxymethyl)cyclopropyl)but-1-en-3-ynyl)benzamido)-3-methylbutanoate (400 mg, 1.08 mmol, 1.0 equiv) was added to a round bottom flask. To this was added isopropyl alcohol (2.0 mL), the solution was vortexed for 2 minutes, the flask was cooled in an ice bath, then a hydroxylamine solution (1.4 mL, 21.60 mmol, 20 equiv) was added and the flask was placed at 4°C for approximately 48h. The reaction was concentrated, (at 0°C) to remove the isopropyl alcohol, and then was acidified at 0°C using TFA (3 mL). Additional water (15 mL) and ACN (3 mL) were added. The product was purified by reverse phase HPLC (2" column,50mL/min, 0.1% TFA in water/ACN, equil @ 2%B). The column was loaded at 10mL/min, 2%B using syringe filter (24 mL) and then ramped to 50mL/min over 1 minute. A gradient from 2%B to 95%B was run over 73 min. Desired fractions were combined, frozen and placed on a lyophilizer. The reaction yielded 177 mg of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-((E)-4-((1,2-trans)2-(hydroxymethyl)cyclopropyl)but-1-en-3-ynyl)benzamide, **I-22**, TFA. LCMS M+1 expected = 372.2, observed = 372.2. 1H NMR (DMSO-d6): δ 0.77-0.81 (m, 2H), 1.25 (s, 3H), 1.30 (s,3H), 1.34-1.37 (m, 1H), 3.20-3.24 (dd, 1H), 3.37-3.41 (dd, 1H), 4.65-4.67 (d, 1H), 6.43-6.47 (d, 1H), 6.87-6.91 (d, 1H), 7.55-7.57 (d, 2H), 7.84-7.86 (d, 2H), 7.99 (br, 2H), 8.38-8.41 (d,1H), 9.22 (br, 1H), 11.20 (s, 1H).

R. Antimicrobial Activity

Bacterial Screens and Cultures

Bacterial isolates were cultivated from -70° C. frozen stocks by overnight passages at 35° C in ambient air on Mueller-Hinton agar (Beckton Dickinson, Franklin Lakes, NJ). Clinical isolates tested were obtained from various geographically diverse hospitals in the US and abroad (Focus Diagnostics, Herndon, VA and JMI, North Liberty, IA). Quality control strains were from the American Type Culture Collection (ATCC; Rockville, Md.).

Susceptibility Testing

Minimum Inhibitory Concentrations (MICs) were determined by the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. In brief, organism suspensions were adjusted to a 0.5 McFarland standard to yield a final inoculum between 3×10^5 and 7×10^5 colony-forming units (CFU)/mL. Drug dilutions and inocula were made in sterile, cation adjusted Mueller-Hinton Broth (Beckton Dickinson). An inoculum volume of 100 μL was added to wells containing 100 μL of broth with 2-fold serial dilutions of drug. All inoculated microdilution trays were incubated in ambient air at 35° C for 18-24 h. Following incubation, the lowest concentration of the drug that prevented visible growth (OD600 nm < 0.05) was recorded as the MIC. Performance of the assay was monitored by the use of laboratory quality-control strains and levofloxacin, a compound with a defined MIC spectrum, in accordance with CLSI guidelines. Typically, compounds of the present invention have MIC values of 0.03 – 16 μg/mL. To this end, data for certain representative compounds is shown in Table II below.

Table II

Minimum Inhibitory Concentrations (MICs)

Cmp#	APAE001	AECO001	APAE002	AKPN001
I-1	A	A	A	A
I-2	A	A	A	A
I-3	A	A	A	A
I-4	A	A	A	A
I-5	A	A	A	A
I-6	A	A	A	A
I-7	A	A	A	A
I-8	A	A	A	A
I-9	A	A	A	A
I-10	A	A	A	A
I-11	A	A	A	A
I-12	A	A	A	A
I-13	A	A	A	A
I-14	A	A	A	A
I-15	A	A	A	A
I-16	A	A	A	A
I-17	A	A	A	A
I-18	A	A	A	A
I-19	A	A	A	A
I-20	A	A	A	A
I-21	B	A	A	B
I-22	A	A	A	A
I-23	A	A	A	A

MIC Key:

A = MIC's of 2.0 µg/mL or less

B = MIC's of greater than 2.0 µg/mL to 16.0 µg/mL

C = MIC's of greater than 16.0 µg/mL

*AECO001 is *E. coli* ATCC25922; APAE001 is *Pseudomonas aeruginosa* ATCC27853; AKPN001 is *Klebsiella pneumoniae* ATCC43816; APAE002 is a clinical isolate of *Pseudomonas aeruginosa* expressing a normal level of efflux activity.

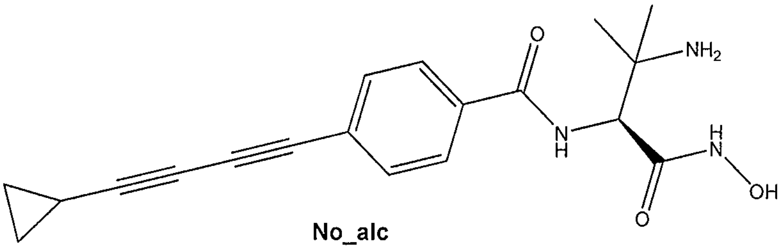
S. *In Vivo* Tolerability

Each compound was administered in mice via subcutaneous injection. The mice were group-housed (five per cage) at 18-28^{0}C and ~50% humidity, and fed with standard rodent chow. Water and food were given *ad libitum*. Mice were given subcutaneous doses of no more than 20 mL per kg of body weight.

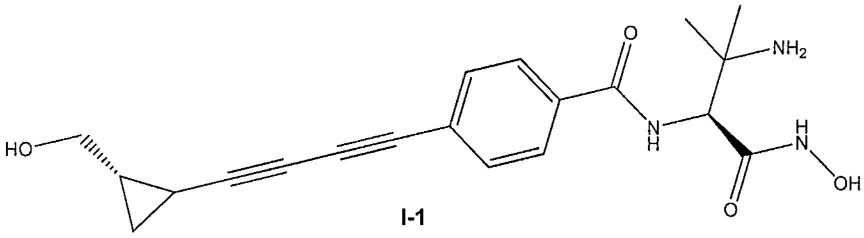
Groups of three mice were dosed at 50, 100, 200, 400 or 600 mg/kg/day as a single dose of the test compound by subcutaneous injection in a formulation consisting of 15% Captisol in 20 mM acetate buffer, pH 5. Subcutaneous injections were done on the back in the interscapular area. Volumes of 10-20 mL/kg were injected into this area. The needle was inserted parallel to the surface of the skin to such a depth that the point of the needle lay within the subcutaneous pocket. A gentle but firm pressure on the plunger was used to expel the contents of the syringe.

Observations were taken at a variety of time points post-dose: 30sec - 1min, 5min, 15min, 30min, 45min, 1hr, 75min, 90min, 105min, 2hr, and every hour afterwards until animals showed recovery signs to near baseline, or up to 4 hr post-dose, whichever came first. The minimum time range to monitor the mice was 30 minutes post-dose if the animals looked alert, normal, and responsive. If animals showed toxicity effects, they were monitored closely until they showed recovery signs to near baseline, or up to 4 hr post-dose, whichever came first. Animals were kept for 72 hours after dosing for clinical observation including monitoring of survival and activity level. Observations considered symptoms of central nervous system distress (such as seizures, lethargy, recumbency, motionlessness, hyperactivity), neuromuscular abnormalities (such as ataxia, twitching, convulsions, splayed limbs, jumping or kicking), autonomic symptoms (such as salivation, lacrimation, urination, defecation, piloerection or squinting), respiratory distress (such as labored or rapid breathing, depression, panting or gasping), stereotypic behaviors (such as repetitive chewing, circling, pacing, grooming, sniffing, head movements or hunched stance), and abnormal behaviors such as escape behavior or wet dog shake.

A known compound, **No_alc** (the synthesis and activity of which is disclosed in International PCT Publication No. 2008/06676; compound 91-12), has shown a maximum tolerated dose in mice of less than 50 mg/kg when subcutaneously injected in mice, while a dose of 30 mg/kg by subcutaneous injection is required as a static dose against *K. pneumonia* in mice. Compound **I-1** of the present invention, which differs from this known compound, simply by a hydroxymethyl substitution of the cyclopropyl group, has antimicrobial activity comparable to that of **No_alc** but is substantially better tolerated in mammals (maximum tolerated dose of approximately 200 mg/kg when subcutaneously injected into mice).



No_alc



I-1

Table III provides the results of observations of the subcutaneously dosed mice. Tolerability was characterized by clinical observation. Class A was assigned to animals with no to little toxicity symptoms such as occasional short pausing when moving, slight labored breathing, or slight lethargy with a quick recovery (e.g., within 10 min). Class B was assigned to animals showing some toxicity symptoms such as longer pausing when moving, slight lethargy with a longer recovery time (up to 1 hour) during which animals are still able to move around. Class C was assigned to animals with moderate to severe toxicity symptoms such as lethargy, recumbence, lethargy with squinting and labored breathing, severe twitching (jumping, kicking), or escape behavior. Finally, Class D was assigned when any lethal effects (including moribund state requiring euthanasia) occurred within the clinical observation time (up to 72 hour post-dose).

Table III

***In Vivo* Tolerability**

Cmpd	**50 mg/kg**	**100 mg/kg**	**200 mg/kg**	**400 mg/kg**	**600 mg/kg**
No_alc	B	B (n=2) C (n=1)	C	C	
I-1			A	B	C
I-5		A	A (n=1) B (n=2)	C	
I-6			A	C	C
I-7			A	B (n=1) C (n=2)	C
I-8			A	B (n=1) C (n=2)	C
I-9			A	C	
I-12		A	A (n=1) B (n=2)	C	C
I-13		A	B	C	C (n=1) D (n=2)
I-14		A	B (n=2)	C (n=2)	

Cmpd	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
			C (n=1)		

T. Synergy with a Second Antibacterial Agent

Bacterial isolates were cultivated from -70°C frozen stocks of *K. pneumoniae* (ATCC 43816) by overnight passages at 35° C. in ambient air on Mueller-Hinton agar (Beckton Dickinson, Franklin Lakes, NJ). Minimum Inhibitory Concentrations (MICs) were determined by the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. In brief, organism suspensions were adjusted to a 0.5 McFarland standard to yield a final inoculum between 3×10^5 and 7×10^5 colony-forming units (CFU)/mL. Drug dilutions and inocula were made in sterile, cation adjusted Mueller-Hinton Broth (Beckton Dickinson). An inoculum volume of 100 μL was added to wells containing 100 μL of broth with 2-fold serial dilutions of drug. All inoculated microdilution trays were incubated in ambient air at 35° C. for 18-24 hours. Following incubation, the lowest concentration of the drug that prevented visible growth (OD_{600} nm < 0.05) was recorded as the MIC.

Standard checkerboard assays were performed with a combination of the indicated agents and N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-1**). Table IV provides the FICI calculated according to standard techniques. Compound **I-1** was synergistic *in vitro* with vancomycin, teicoplanin, erythromycin, azithromycin, rifampin and novobiocin.

Table IV

FICI

Antibiotic	FICI (cmpd I-1)
Vancomycin	≤ 0.4
Teicoplanin	≤ 0.5

Antibiotic	FICI (cmpd I-1)
Erythromycin	0.3
Azithromycin	0.2
Rifampin	0.1
Novobiocin	0.3
Telavancin	> 2.0
Daptomycin	> 2.0
Linezolid	> 1.5
Clindamycin	0.6
Levofloxacin	1.0
Ceftobiprole	1.0
Cefotaxime ,	1.0
Gentamycin	0.5

In vivo synergy of N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-1**) with vancomycin was examined in the neutropenic thigh *in vivo* efficacy model. The model was run essentially as described by Craig and others (see Gudmundsson et al., "Murine Thigh Infection Model," Handbook of Animal Models of Infection, M. A. Sande and O. Zak, Eds.; London: Academic Press, 1999, pp 137-144). Briefly, mice were rendered neutropenic prior to infection with 2 doses of cyclophosphamide, and then infected intramuscularly in the thigh with inocula of 10^3 – 10^5 CFU of *K. pneumo.* (ATCC 43816). Antibiotics or vehicle alone as a negative control were administered twice at 2 hrs and 14 hrs post-infection. The animals were kept neutropenic for the duration of the experiment in order to minimize the effect of white blood cells on the infection such that the microbiological readout measures the *in vivo* interaction of drugs and bacteria. At 24 hrs post-infection, thighs were harvested, homogenized, and plated to measure the number of CFUs surviving per thigh. Thighs from a subset of animals were also harvested 2 hrs post-infection to record the CFUs present just prior to the first antibiotic treatment (pre-treatment). The static dose, defined as the dose required to result in a CFU load at 24 hours that is identical to that measured at 0 hours post infection, was calculated by standard methods in Prizm (GraphPad Software) from a dose response curve.

The purpose of these studies was to quantitatively assess whether the combination of I-1 with a candidate synergizing agent gave a greater reduction in counts in this *in vivo* efficacy model than does the sum of each agent alone.

Vancomycin exhibits significant *in vivo* synergy with LpxC inhibitors for treatment of infections with *K. pneumo.* ATCC43816. As indicated in Figure 1, treatment of infected mice with vancomycin alone at 220 mg/kg/day results in no significant reduction in CFU. However, when co-dosed with compound I-1 the static doses of the LpxC inhibitors is reduced significantly (Figure 1 & Table V).

TABLE V

Drug	**Dose Regimen**	**Dose (mg/kg)**	**Mean Log_{10} CFU[a]**		**Mean Log_{10} CFU reduction vs.**	
			2 hr	**26 hr**	**2 hr**	**26 hr**
Vehicle	q8hr	-	n.d.	9.95	-3.71	0.00
Levofloxacin	q12hr	10	n.d.	6.48	-0.25	3.46
		30	n.d.	4.44	1.79	5.50
		100	n.d.	4.07	2.17	5.88
I-1	q6hr	30	n.d.	10.26	-4.02	-0.31
		100	n.d.	9.51	-3.27	0.44
		300	n.d.	7.13	-0.89	2.82
I-1+ Vancomycin	q6hr	30+220	n.d.	4.63	1.61	5.32
		100+220	n.d.	4.28	1.95	5.67
		300+220	n.d.	3.98	2.26	5.97
Vancomycin+ I-1	q6hr	25+100	n.d.	8.65	-2.41	1.30
		75+100	n.d.	8.11	-1.87	1.84
		220+100	n.d.	4.99	1.25	4.96

Drug	Dose Regimen	Dose (mg/kg)	Mean Log_{10} CFU[a]		Mean Log_{10} CFU reduction vs.	
			2 hr	**26 hr**	**2 hr**	**26 hr**
Vancomycin	q6hr	220	n.d.	10.21	-3.97	-0.26
2 Hr Control	n/a	-	6.24	n.d.	0.00	3.71

Taken together, these data show that vancomycin demonstrates surprising *in vivo* synergy with N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-*trans*)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-1**).

It should be understood that the organic compounds according to the invention may exhibit the phenomenon of tautomerism. As the chemical structures within this specification can only represent one of the possible tautomeric forms, it should be understood that the invention encompasses any tautomeric form of the drawn structure.

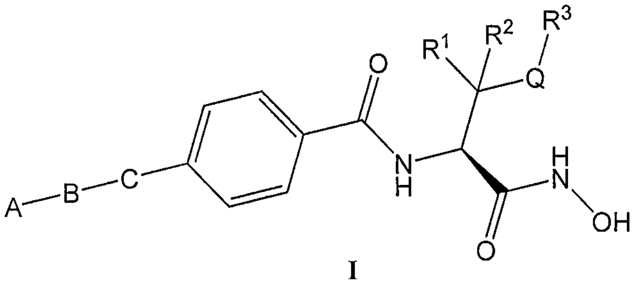
Furthermore, while particular embodiments of the present invention have been shown and described herein for purposes of illustration, it will be understood, of course, that the invention is not limited thereto since modifications may be made by persons skilled in the art, particularly in light of the foregoing teachings, without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification are incorporated herein by reference, in their entirety to the extent not inconsistent with the present description.

CLAIMS

What is claimed is:

1. A compound of Formula I:



I

or a stereoisomer or pharmaceutically acceptable salt thereof, wherein

A is a substituted C_3-C_6 cycloalkyl, wherein at least one substituent is a C_1-C_3 primary alcohol;

B is absent, -CH=CH-, -C≡C- or an unsubstituted phenyl;

C is -CH=CH-, -C≡C- or an unsubstituted phenyl, wherein if B is -CH=CH- then C is not also -CH=CH-;

R^1, R^2 and R^3 independently are selected from hydrogen and substituted or unsubstituted C_1-C_3 alkyl, or

R^1 and R^2, together with the carbon atom to which they are attached, form an unsubstituted C_3-C_6 cycloalkyl group, or

R^2 and R^3, together with the carbon atom and Q to which they are attached, form a substituted or unsubstituted heterocyclic ring, having from 5 to 8 ring atoms, wherein 1-2 ring atoms of the heterocyclic ring are selected from N, O and S; and

Q is O or NR, wherein R is hydrogen or an unsubstituted C_1-C_3 alkyl.

2. A compound of claim 1, wherein Q is NR.

3. A compound of claim 1 or 2, wherein R^1, R^2, and R^3 independently are selected from hydrogen and substituted or unsubstituted C_1-C_3 alkyl.

4. A compound of any one of claims 1-3, wherein R^1, R^2, and R^3 independently are selected from hydrogen and unsubstituted C_1-C_3 alkyl.

5. A compound of any one of claims 1-4, wherein R^1 and R^2 independently are unsubstituted C_1-C_3 alkyl.

6. A compound of any one of claims 1-5, wherein A is a C_3-C_6 cycloalkyl substituted with hydroxymethyl.

7. A compound of any one of claims 1-6, wherein B is -C≡C- and C is -C≡C-.

8. A compound of claim 1, wherein said compound is:

N-((*S*)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-1**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-y1)-4-(((1R,2R)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-2**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1S,2S)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-3**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-cis)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-4**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4'-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-carboxamide (**I-5**);

N-((S)-1-(hydroxyamino)-3-methyl-3-(methylamino)-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-6**);

N-((S)-1-(hydroxyamino)-3-(2-hydroxyethylamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-7**);

N-((S)-1-(hydroxyamino)-3-(2-hydroxyethylamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-8**);

N-((S)-3-(dimethylamino)-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-9**);

N-((S)-3-hydroxy-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-10**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1S,2R)-2-(hydroxymethyl)-2-methylcyclopropyl)buta-1,3-diynyl)benzamide (**I-11**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-cis)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamide (**I-12**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-trans)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamide (**I-13**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-trans)-3-(hydroxymethyl)cyclopentyl)buta-1,3-diynyl)benzamide (**I-14**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,4-cis)-4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamide (**I-15**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,4-trans)-4-(hydroxymethyl)cyclohexyl)buta-1,3-diynyl)benzamide (**I-16**);

N-((S)-1-(1-aminocyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((trans)-2-(hydroxymethyl)cyclopropyl)buta-1 ,3-diynyl)benzamide trifluoroacetate (**I-17**);

N-((S)-1-(1-aminocyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((cis)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide trifluoroacetate (**I-18**);

N-((S)-1-(1-(dimethylamino)cyclobutyl)-2-(hydroxyamino)-2-oxoethyl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-19**);

N-((S)-2-(hydroxyamino)-1-((S)-morpholin-3-yl)-2-oxoethyl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-20**);

N-((S)-2-(hydroxyamino)-1-((S)-4-methylmorpholin-3-yl)-2-oxoethyl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-21**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-((E)-4-((1,2-trans)-2-(hydroxymethyl)cyclopropyl)but-1-en-3-ynyl)benzamide (**I-22**); or

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-((E)-4-((1,2-trans)-2-(hydroxymethyl)cyclopropyl)but-1-en-3-ynyl)benzamide (**I-23**).

9. A compound of claim 8, wherein said compound is:

N-((*S*)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-1**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4'-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-carboxamide (**I-5**);

N-((S)-1-(hydroxyamino)-3-methyl-3-(methylamino)-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-6**);

N-((S)-1-(hydroxyamino)-3-(2-hydroxyethylamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-7**);

N-((S)-1-(hydroxyamino)-3-(2-hydroxyethylamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-8**);

N-((S)-3-(dimethylamino)-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-9**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-cis)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamide (**I-12**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-trans)-3-(hydroxymethyl)cyclobutyl)buta-1,3-diynyl)benzamide (**I-13**); or

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,3-trans)-3-(hydroxymethyl)cyclopentyl)buta-1,3-diynyl)benzamide (**I-14**).

10. A compound of claim 9, wherein said compound is:

N-((*S*)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-1**);

N-((S)-3-amino-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4'-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)ethynyl)biphenyl-4-carboxamide (**I-5**);

N-((S)-1-(hydroxyamino)-3-methyl-3-(methylamino)-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-6**);

N-((S)-1-(hydroxyamino)-3-(2-hydroxyethylamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-7**);

N-((S)-1-(hydroxyamino)-3-(2-hydroxyethylamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-8**); or

N-((S)-3-(dimethylamino)-1-(hydroxyamino)-3-methyl-1-oxobutan-2-yl)-4-(((1,2-trans)-2-(hydroxymethyl)cyclopropyl)buta-1,3-diynyl)benzamide (**I-9**).

11. A pharmaceutical composition comprising a compound of any one of claims 1-10, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

12. A method for treating a subject having a bacterial infection comprising administering to a subject in need thereof a therapeutically effective amount of a compound according to any one of claims 1-10 or a pharmaceutical composition of claim 11.

13. A method according to claim 11, wherein said bacterial infection is a gram-negative bacterial infection.

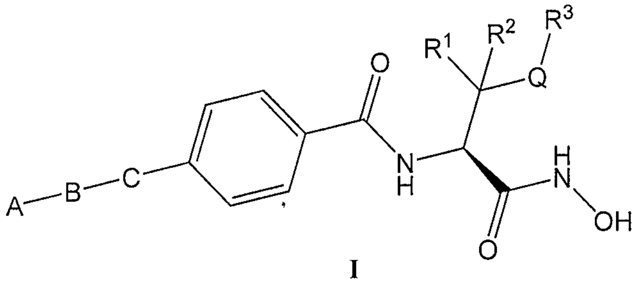
14. A method according to claim 13, wherein said gram-negative bacterial infection is *Pseudomonas aeruginosa*, *Burkholderia, Enterobacteriaceae*, *Franciscellaceae*, *Serratia*, *Proteus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Providencia*, *Yersinia*, *Morganella* or *Escherichia coli.*

15. A method according to claim 14, wherein said gram-negative bacterial infection is *Pseudomonas aeruginosa*, *Burkholderia, Franciscellaceae*, *Enterobacter*, *Yersinia* or *Escherichia coli.*

16. A method according to claim 15, wherein said gram-negative bacterial infection is *Pseudomonas aeruginosa*.

17. A method according to claim 15, wherein said gram-negative bacterial infection is *Escherichia coli*.

18. A pharmaceutical composition comprising a second antibacterial agent and a compound of Formula I:



I

or a stereoisomer or pharmaceutically acceptable salt thereof, wherein

A is a substituted C_3-C_6 cycloalkyl, wherein at least one substituent is a C_1-C_3 primary alcohol;

B is absent, -CH=CH-, -C≡C- or an unsubstituted phenyl;

C is -CH=CH-, -C≡C- or an unsubstituted phenyl, wherein if B is -CH=CH- then C is not also -CH=CH-;

R^1, R^2 and R^3 independently are selected from hydrogen and substituted or unsubstituted C_1-C_3 alkyl, or

R^1 and R^2, together with the carbon atom to which they are attached, form an unsubstituted C_3-C_6 cycloalkyl group, or

R^2 and R^3, together with the carbon atom and Q to which they are attached, form a substituted or unsubstituted heterocyclic ring, having from 5 to 8 ring atoms, wherein 1-2 ring atoms of the heterocyclic ring are selected from N, O and S; and

Q is O or NR, wherein R is hydrogen or an unsubstituted C_1-C_3 alkyl.

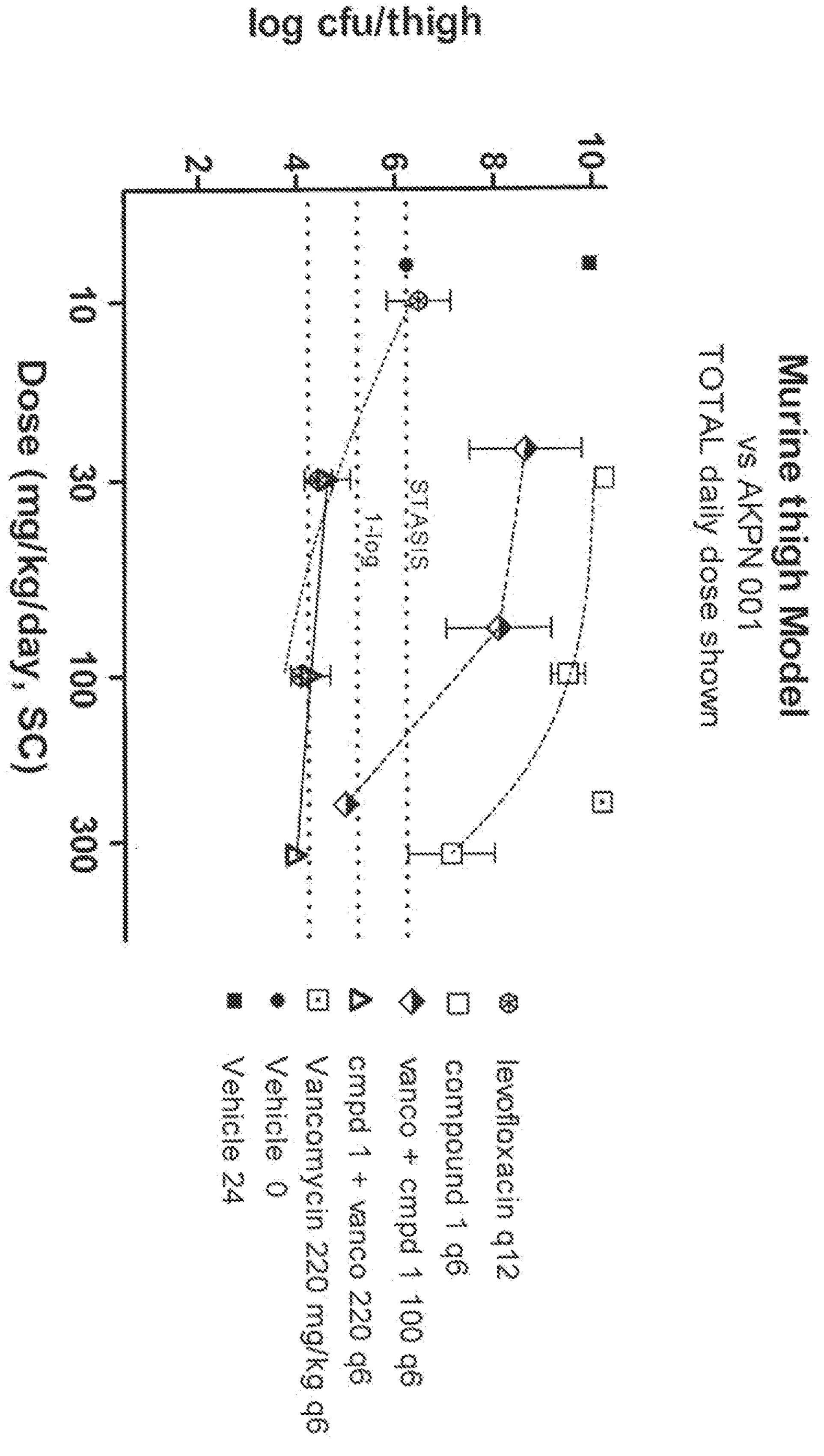
19. A pharmaceutical composition of claim 18, wherein the antibacterial agent is vancomycin or rifampin.

20. A pharmaceutical composition of claim 18, wherein the combination demonstrates *in vivo* synergy.

21. A method of inhibiting a deacetylase enzyme in a gram-negative bacteria comprising administering to a patient in need of such inhibition a compound according to any one of claims 1-10 or a pharmaceutical composition of claim 11.

22. A method of claim 21, wherein said gram-negative bacteria is *Pseudomonas aeruginosa*, *Burkholderia, Enterobacteriaceae*, *Franciscellaceae*, *Serratia*, *Proteus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Providencia*, *Yersinia*, *Morganella* or *Escherichia coli.*

23. A method of inhibiting LpxC in a gram-negative bacteria comprising administering to a patient in need of such inhibition a compound according to any one of claims 1-10 or a pharmaceutical composition of claim 11.


Murine thigh Model
vs AKPN 001
TOTAL daily dose shown
log cfu/thigh
10
8
6
4
2
STASIS
1-log
10
30
100
300
Dose (mg/kg/day, SC)
levofloxacin q12
compound 1 q6
vanco + cmpd 1 100 q6
cmpd 1 + vanco 220 q6
Vancomycin 220 mg/kg q6
Vehicle 0
Vehicle 24

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/059280

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07C259/06 C07D265/30 A61K31/166 A61K31/5375 A61P31/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/190766 A1 (MOSER HEINZ E [US] ET AL) 29 July 2010 (2010-07-29) cited in the application page 1, paragraph [0004] pages 157-160; compounds 91-2 to 91-6, 91-10 to 91-12, 91-14, 91-16, 91-17 page 174; compounds 95-1, 95-2 pages 190,191; compounds 101-1 to 101-6 pages 218,219; compounds 112-1 to 112-5 page 225; compounds 114-15, 114-17, 114-18, 114-20 page 265; compounds 113-4 page 273; compounds 137-5 pages 275-278; compounds 137-21, 137-22, 137-25, 138-9, 138-10 ----- -/--	1-23

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

☒ See patent family annex.

* Special categories of cited documents :

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

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

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"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
27 August 2012

Date of mailing of the international search report
05/09/2012

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

Authorized officer
Kiernan, Andrea

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2004/062601 A2 (CHIRON CORPORATION) 29 July 2004 (2004-07-29) cited in the application page 1, paragraph 2 examples 202, 424,; table 1 -----	1-23
Y,P	WO 2011/005355 A1 (ACHAOGEN, INC.) 13 January 2011 (2011-01-13) cited in the application abstract; examples; tables page 1, lines 15-21 page 3, lines 14-31; compounds LpxCi-4, LpxCi-5, LpxCi-6 -----	1-23

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/059280

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