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(54) Title: COMPOUNDS AND METHODS FOR THE TREATMENT OF PARASITIC DISEASES

(57) Abstract: Provided herein are compounds useful for the treatment of various parasitic diseases. These compounds, as well as pharmaceutically acceptable salts thereof may be formulated in pharmaceutical compositions, veterinary compositions and may be used in methods of treatment and/or prophylaxis of diseases spread by parasites, including malaria and cryptosporidiosis.

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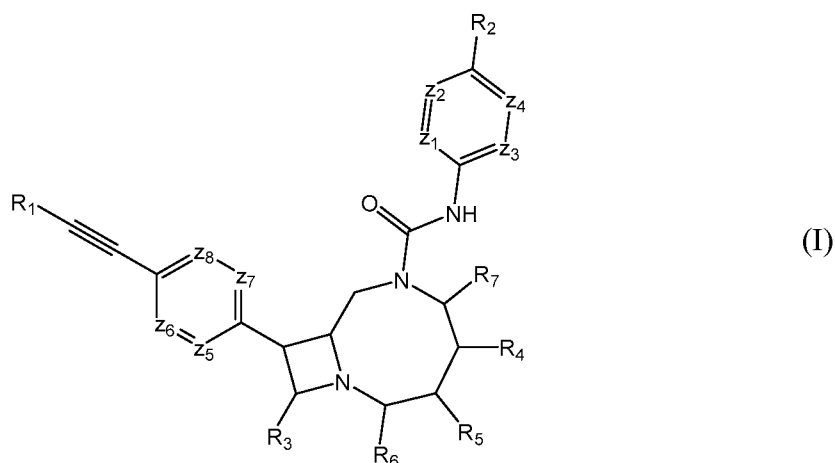
- 5 current standard of care for cryptosporidiosis, but the drug only exhibits partial efficacy in children and is no more effective than placebo in patients with AIDS.

### SUMMARY

- Disclosed herein are compounds, pharmaceutical compositions, and methods of treating or preventing parasitic diseases including malaria and cryptosporidium including/using a compound as described above or elsewhere herein.

In some embodiments, these pharmaceutical compositions are formulated as veterinary compositions for use with subjects other than human.

The compounds may have the structure of formula (I):



wherein the “dashed” bond may be a single or double bond;

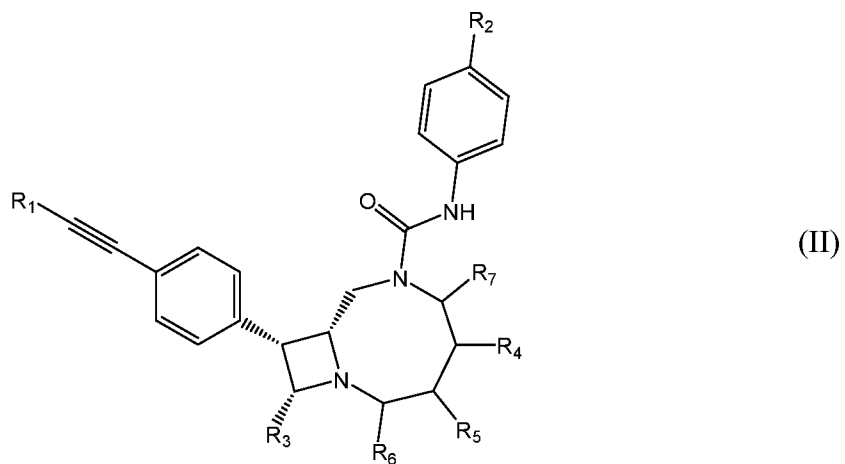
- 15 R<sub>1</sub> is optionally substituted aryl or heteroaryl;  
 R<sub>2</sub> is optionally substituted alkoxy, cycloalkoxy or heterocyclyl;  
 R<sub>3</sub> is hydrogen, or –CH<sub>2</sub>–X;  
 R<sub>4</sub> and R<sub>5</sub> are independently hydrogen, –X, or –CH<sub>2</sub>–X, R<sub>4</sub> and R<sub>5</sub> may together form a five- or six-membered fused ring, and at least one of R<sub>4</sub> and R<sub>5</sub> is not hydrogen;  
 20 R<sub>6</sub> and R<sub>7</sub> are independently hydrogen or R; and  
 z<sub>1</sub>–z<sub>8</sub> are independently selected at each occurrence from CH or N; where  
 –X is independently selected at each occurrence from –OH, –OR, –S(O)R, –S(O)<sub>2</sub>R, –N(R)–S(O)<sub>2</sub>R, –S(O)<sub>2</sub>–N(R)(R), –S(O)<sub>2</sub>–NHR, –N(R)–C(O)–R or –N(R)(R); and  
 R is independently at each occurrence an optionally substituted C<sub>1</sub>–C<sub>12</sub> alkyl;  
 25 or a pharmaceutically acceptable salt thereof. –X may be independently selected at each occurrence from –OH –NH<sub>2</sub> or –N(R)(R). In some embodiments –X groups in R<sub>4</sub> and/or R<sub>5</sub> (including –X groups when R<sub>4</sub> and/or R<sub>5</sub> is –CH<sub>2</sub>–X) may be selected from –OH, NH<sub>2</sub>, or –

5 N(R)(R). Also, R may be independently selected at each occurrence a C<sub>1-4</sub> linear or branched hydrocarbon.

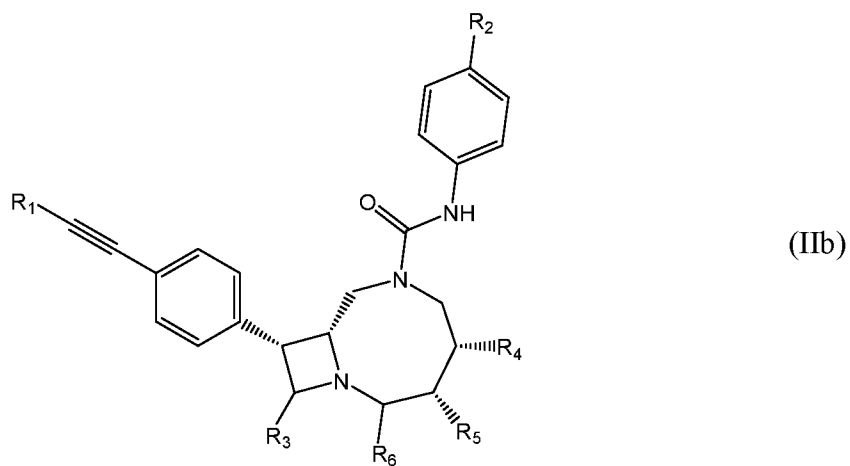
In some embodiments, R<sub>6</sub> and R<sub>7</sub> are each hydrogen In some embodiments, R<sub>4</sub> and R<sub>5</sub> may be the same functional group selected from -X or -CH<sub>2</sub>-X (*e.g.*, R<sub>4</sub> is -OH and R<sub>5</sub> is -OH, R<sub>4</sub> is -NH<sub>2</sub> and R<sub>5</sub> is -NH<sub>2</sub>, R<sub>4</sub> is -OCH<sub>3</sub> and R<sub>5</sub> is -OCH<sub>3</sub>, etc.). In other embodiments, 10 R<sub>5</sub> is hydrogen and R<sub>4</sub> is -X, or -(CH<sub>2</sub>)-X. In other embodiments, R<sub>4</sub> is hydrogen and R<sub>5</sub> is -X, or -(CH<sub>2</sub>)-X. In some embodiments, R<sub>4</sub> and R<sub>5</sub> are independently selected from -OH and -OR and R<sub>4</sub> and R<sub>5</sub> together form a 6-membered fused ring. In some embodiments, R<sub>6</sub> is a C<sub>1-4</sub> linear or branched hydrocarbon. In other embodiments, R<sub>6</sub> is hydrogen. R<sub>1</sub> may be an optionally substituted C<sub>6</sub> aryl or heteroaryl (*e.g.*, phenyl, fluorophenyl, difluorophenyl, 15 pyridyl, etc.). In some embodiments, R<sub>2</sub> is C<sub>1-4</sub> linear or branched alkoxy (*e.g.*, methoxy, ethoxy, propoxy, isopropoxy, etc.). In some embodiments, R<sub>2</sub> is C<sub>1-4</sub> linear or branched alkoxy substituted with one or more F (*e.g.*, -OCF<sub>3</sub>, -OCHF<sub>2</sub>, or -OCH<sub>2</sub>F). In other embodiments, R<sub>2</sub> is C<sub>3-6</sub> heterocyclyl (*e.g.*, aziridinyl, oxiranyl, thiranyl, oxetanyl, azetidyl, thietanyl, diazetidinyl, dioxetanyl, dithietanyl, pyrrolidinyl, tetrahydrofuranyl, imidazolyl, 20 pyrazolyl, oxazolyl, etc.). In preferred embodiments, R<sub>2</sub> is oxetanyl or azetidyl. In other embodiments, R<sub>2</sub> is C<sub>3-6</sub> cycloalkoxy (*e.g.*, cyclopropoxy). In some embodiments, R<sub>3</sub> is -CH<sub>2</sub>-X and -X is N(R)(R).

Each of z<sub>1</sub>-z<sub>8</sub> may be CH. In some embodiments, one of z<sub>1</sub>-z<sub>4</sub> is N and the rest are CH. In some embodiments, one of z<sub>5</sub>-z<sub>8</sub> is N and the rest are CH. In some embodiments, 25 z<sub>1</sub> and z<sub>4</sub> are each N and z<sub>3</sub> and z<sub>2</sub> are each CH. In some embodiments, z<sub>5</sub> and z<sub>7</sub> are each N and z<sub>6</sub> and z<sub>8</sub> are each CH.

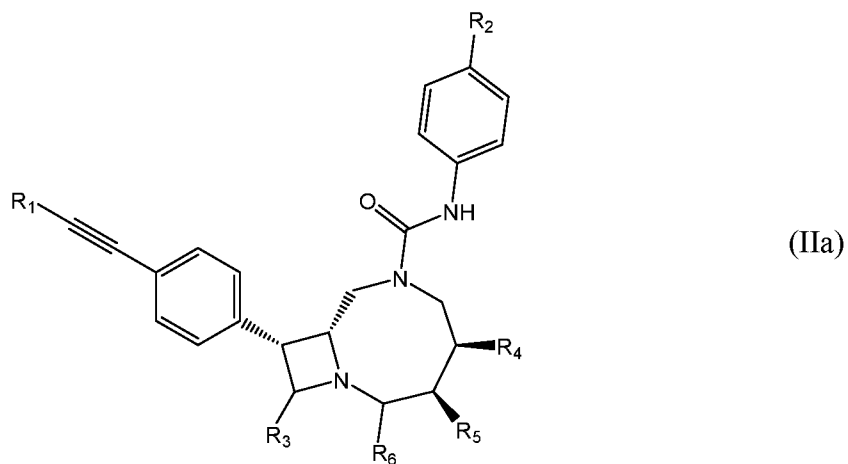
Any stereocenters in the structure of formula (I) may be in either configuration, or be present as a racemic mixture of each stereocenter (*e.g.*, stereoisomers, diastereomers, etc.). In some embodiments, the compounds have the structure of formula (II):



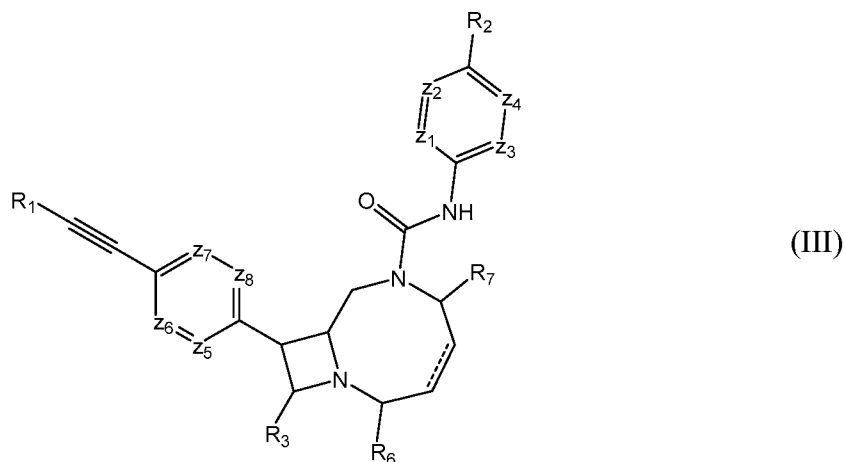
5 In some embodiments, the compounds have the structure of formula (IIb):



In other embodiments, the compounds have the structure of formula (IIa):



The compound may also have the structure of formula (III):



5

wherein the “dashed” bond may be a single or double bond;

R<sub>1</sub> is optionally substituted aryl or heteroaryl;

R<sub>2</sub> is optionally substituted alkoxy, cycloalkoxy or heterocyclyl;

R<sub>3</sub> is hydrogen or –CH<sub>2</sub>–X;

10 R<sub>6</sub> and R<sub>7</sub> are independently hydrogen or R; and

z<sub>1</sub>–z<sub>8</sub> are independently selected at each occurrence from CH or N; where

–X is independently selected at each occurrence from –OH, –OR, –S(O)R, –S(O)<sub>2</sub>R, –N(R)–S(O)<sub>2</sub>R, –S(O)<sub>2</sub>–N(R)(R), –S(O)<sub>2</sub>–NHR, –N(R)–C(O)–R or –N(R)(R); and

R is independently at each occurrence a C<sub>1</sub>–C<sub>12</sub> alkyl;

15 with the proviso that in the case where R<sub>6</sub> is hydrogen, R<sub>3</sub> is –CH<sub>2</sub>–N(R)(R) and said

“dashed” bond is a double bond. In some embodiments, the “dashed bond is a double bond.

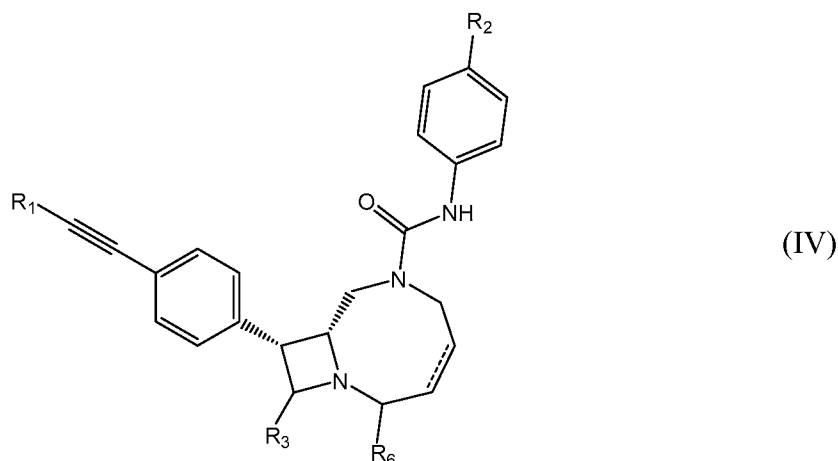
In other embodiments, the “dashed” bond is a single bond. –X may be independently selected at each occurrence from –OH –NH<sub>2</sub> or –N(R)(R). Also, R may be independently selected at each occurrence a C<sub>1-4</sub> linear or branched hydrocarbon.

20 Each of z<sub>1</sub>–z<sub>8</sub> may be CH. In some embodiments, one of z<sub>1</sub>–z<sub>4</sub> is N and the rest are CH. In some embodiments, one of z<sub>5</sub>–z<sub>8</sub> is N and the rest are CH. In some embodiments, z<sub>1</sub> and z<sub>4</sub> are each N and z<sub>3</sub> and z<sub>2</sub> are each CH. In some embodiments, z<sub>5</sub> and z<sub>7</sub> are each N and z<sub>6</sub> and z<sub>8</sub> are each CH.

In some embodiments, R<sub>6</sub> and R<sub>7</sub> are each hydrogen. In some embodiments, R<sub>6</sub> is lower alkyl (*e.g.*, methyl, ethyl, etc.). In some embodiments, R<sub>6</sub> is a C<sub>1-4</sub> linear or branched hydrocarbon. In other embodiments, R<sub>6</sub> is hydrogen. R<sub>1</sub> may be an optionally substituted C<sub>6</sub> aryl or heteroaryl (*e.g.*, phenyl, fluorophenyl, difluorophenyl, pyridyl, etc.). In some embodiments, R<sub>2</sub> is C<sub>1-4</sub> linear or branched alkoxy (*e.g.*, methoxy, ethoxy, propoxy, isopropoxy, etc.). In some embodiments, R<sub>2</sub> is C<sub>1-4</sub> linear or branched alkoxy substituted with

- 5 one or more F (*e.g.*,  $-\text{OCF}_3$ ,  $-\text{OCHF}_2$ , or  $-\text{OCH}_2\text{F}$ ). In other embodiments,  $\text{R}_2$  is  $\text{C}_{3-6}$  heterocyclyl (*e.g.*, aziridinyl, oxiranyl, thiranyl, oxetanyl, azetidynyl, thietanyl, diazetidinyl, dioxetanyl, dithietanyl, pyrrolidinyl, tetrahydrofuranyl, imidazolyl, pyrazolyl, oxazolyl, etc.). In preferred embodiments,  $\text{R}_2$  is oxetanyl or azetidynyl. In other embodiments,  $\text{R}_2$  is  $\text{C}_{3-6}$  cycloalkoxy (*e.g.*, cyclopropoxy).

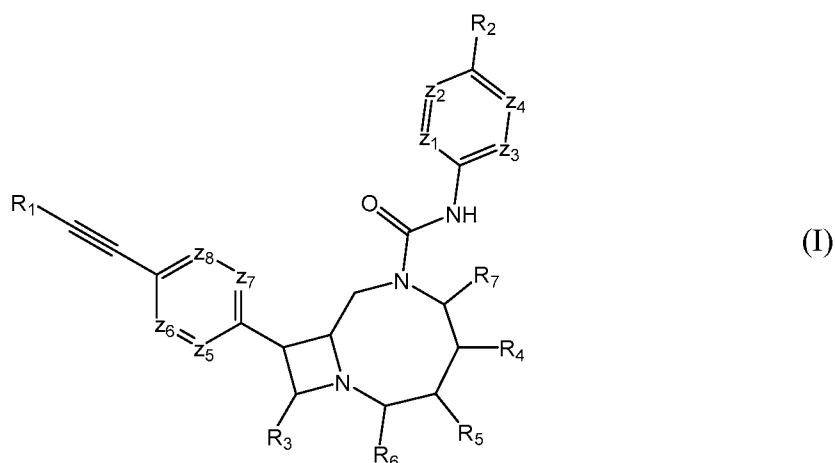
10 In some embodiments, the compound may have the structure of formula (IV):



In some embodiments, the compound is any of compounds E1-E38 of pharmaceutically acceptable salts thereof.

Any of the compounds described above may be used in pharmaceutical compositions.

- 15 In some embodiments, the pharmaceutical composition may comprise a pharmaceutically acceptable excipient and a compound having the structure of formula (I):



wherein the “dashed” bond may be a single or double bond;

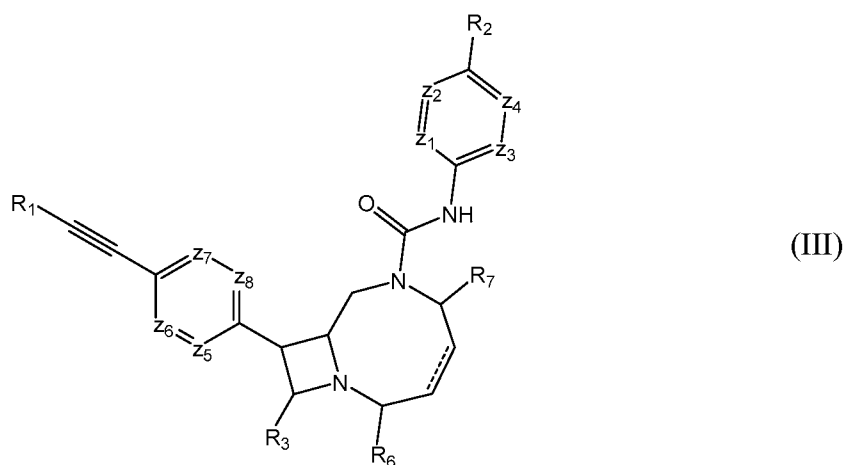
$\text{R}_1$  is optionally substituted aryl or heteroaryl;

$\text{R}_2$  is optionally substituted alkoxy, cycloalkoxy or heterocyclyl;

- 20  $\text{R}_3$  is hydrogen, or  $-\text{CH}_2-\text{X}$ ;

- 5  $R_4$  and  $R_5$  are independently hydrogen,  $-X$ , or  $-\text{CH}_2-X$ ,  $R_4$  and  $R_5$  may together form a five- or six-membered fused ring, and at least one of  $R_4$  and  $R_5$  is not hydrogen;  
 $R_6$  and  $R_7$  are independently hydrogen or  $R$ ; and  
 $z_1$ - $z_8$  are independently selected at each occurrence from CH or N; where  
 $-X$  is independently selected at each occurrence from  $-\text{OH}$ ,  $-\text{OR}$ ,  $-\text{S(O)R}$ ,  $-\text{S(O)}_2\text{R}$ ,  $-\text{N(R)}-$   
10  $\text{S(O)}_2\text{R}$ ,  $-\text{S(O)}_2-\text{N(R)(R)}$ ,  $-\text{S(O)}_2-\text{NHR}$ ,  $-\text{N(R)}-\text{C(O)}-\text{R}$  or  $-\text{N(R)(R)}$ ; and  
 $R$  is independently at each occurrence an optionally substituted  $\text{C}_1$ - $\text{C}_{12}$  alkyl;  
or a pharmaceutically acceptable salt thereof.  $-X$  may be independently selected at each  
occurrence from  $-\text{OH}$ ,  $-\text{NH}_2$  or  $-\text{N(R)(R)}$ . In some embodiments  $-X$  groups in  $R_4$  and/or  $R_5$   
(including  $-X$  groups when  $R_4$  and/or  $R_5$  is  $-\text{CH}_2-X$ ) may be selected from  $-\text{OH}$ ,  $\text{NH}_2$ , or  $-$   
15  $\text{N(R)(R)}$ . Also,  $R$  may be independently selected at each occurrence a  $\text{C}_{1-4}$  linear or branched  
hydrocarbon.

In some embodiments, the pharmaceutical composition may comprise a  
pharmaceutically acceptable excipient and a compound having the structure of formula (III):



- 20 wherein the “dashed” bond may be a single or double bond;  
 $R_1$  is optionally substituted aryl or heteroaryl;  
 $R_2$  is optionally substituted alkoxy, cycloalkoxy or heterocyclyl;  
 $R_3$  is hydrogen or  $-\text{CH}_2-X$ ;  
 $R_6$  and  $R_7$  are independently hydrogen or  $R$ ; and  
25  $z_1$ - $z_8$  are independently selected at each occurrence from CH or N; where  
 $-X$  is independently selected at each occurrence from  $-\text{OH}$ ,  $-\text{OR}$ ,  $-\text{S(O)R}$ ,  $-\text{S(O)}_2\text{R}$ ,  $-\text{N(R)}-$   
 $\text{S(O)}_2\text{R}$ ,  $-\text{S(O)}_2-\text{N(R)(R)}$ ,  $-\text{S(O)}_2-\text{NHR}$ ,  $-\text{N(R)}-\text{C(O)}-\text{R}$  or  $-\text{N(R)(R)}$ ; and  
 $R$  is independently at each occurrence a  $\text{C}_1$ - $\text{C}_{12}$  alkyl;

5 with the proviso that in the case where  $R_6$  is hydrogen,  $R_3$  is  $-\text{CH}_2-\text{N}(\text{R})(\text{R})$  and said “dashed” bond is a double bond. In some embodiments, the “dashed bond is a double bond. In other embodiments, the “dashed” bond is a single bond.  $-\text{X}$  may be independently selected at each occurrence from  $-\text{OH}$   $-\text{NH}_2$  or  $-\text{N}(\text{R})(\text{R})$ . Also,  $\text{R}$  may be independently selected at each occurrence a  $\text{C}_{1-4}$  linear or branched hydrocarbon. In some embodiments, the compound  
10 is present the pharmaceutical composition in an effective amount. For example, the compound may be present in an effective amount for the treatment or prophylaxis of malaria. In some embodiments, the compound may be present in an effective amount for the treatment or prophylaxis of a disease caused by a parasite from the genus *Cryptosporidium* (e.g., cryptosporidiosis). In some embodiments, the pharmaceutical composition may be  
15 formulated for treatment of malaria and cryptosporidiosis.

Related methods of the treatment or prophylaxis of a disease in a subject are also disclosed. In some embodiments, the method of treatment or prophylaxis of a parasitic disease in a subject, comprises the step of administering to the subject an effective amount of any compound disclosed herein. In some embodiments, the effective amount of compound is  
20 formulated in a pharmaceutical composition (e.g., veterinary composition, etc.).

The parasitic disease may be malaria. In some embodiments, the malaria is drug resistant malaria (e.g., malaria resistant to chloroquine, quinine, prymethamine, sulfadoxine, mefloquine, artemether, lumefantrine, artesunate, amodiaquine, dihydroartemisinin, piperazine, proguanil, doxycycline, clindamycin, artemisinin, atovaquone, or any  
25 combination thereof, etc.). In some embodiments, the malaria is blood stage malaria. In some embodiments, the malaria is transmission stage malaria. In some embodiments, the malaria is liver stage malaria. In some embodiments, the subject is infected with a malaria-causing parasite and said treatment prevents spread of said infection from their liver. In some embodiments, the malaria is carried in a mosquito species selected from *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi*, *P. berghei*, *P. chabaudi*, *P. vinckei*, or *P. yoelii*. In  
30 preferred embodiments, the mosquito speices is *P. falciparum* (particularly when the subject is human).

The parasitic disease may be cryptosporidiosis. In some embodiments, the cryptosporidiosis is carried by *C. parvum*.

35 In some embodiments, the subject is human. In other embodiments, the subject is not human (e.g., the pharmaceutical composition is formulated as a veterinary composition). In

5 some embodiments, the subject is a mouse, rat, rabbit, non-human primate, lizards, geckos, cow, calf, sheep, lamb, horse, foal, pig, or piglet.

These and other aspects of the invention will be apparent to those skilled in the art from the following detailed description, which is simply, by way of illustration, various modes contemplated for carrying out the invention. As will be realized, the invention is  
10 capable of additional, different obvious aspects, all without departing from the invention. Accordingly, the specification is illustrative in nature and not restrictive.

## DETAILED DESCRIPTION

### Definitions

It is to be understood that the terminology employed herein is for the purpose of  
15 describing particular embodiments, and is not intended to be limiting. Further, although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the disclosure, the preferred methods and materials are now described.

The term “acyl,” as used herein, represents a hydrogen or an alkyl group, as defined herein, that is attached to the parent molecular group through a carbonyl group, as defined  
20 herein, and is exemplified by formyl (i.e., a carboxyaldehyde group), acetyl, trifluoroacetyl, propionyl, and butanoyl. Exemplary unsubstituted acyl groups include from 1 to 6, from 1 to 11, or from 1 to 21 carbons. In some embodiments, the alkyl group is further substituted with 1, 2, 3, or 4 substituents as described herein.

As used herein, the term “alkyl,” alone or in combination with other groups, refers to  
25 a branched or straight-chain monovalent saturated aliphatic hydrocarbon radical of one to twenty carbon atoms (e.g., one to sixteen carbon atoms, one to twelve carbon atoms, one to ten carbon atoms, or one to six carbon atoms, etc.).

The term “alkylene” as used herein, represents a saturated divalent hydrocarbon group derived from a straight or branched chain saturated hydrocarbon by the removal of two  
30 hydrogen atoms, and is exemplified by methylene, ethylene, and isopropylene. In some embodiments, the alkylene can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for an alkyl group.

As used herein, the term “alkenyl,” alone or in combination with other groups, refers to a straight-chain or branched hydrocarbon residue having an olefinic bond.

35 The term “amino,” as used herein, represents  $-N(R^{N1})_2$ , wherein each  $R^{N1}$  is, independently, H, OH,  $NO_2$ ,  $N(R^{N2})_2$ ,  $SO_2OR^{N2}$ ,  $SO_2R^{N2}$ ,  $SOR^{N2}$ , an *N*-protecting group, alkyl, alkenyl, alkynyl, alkoxy, aryl, alkaryl, cycloalkyl, alkycycloalkyl, carboxyalkyl (e.g.,

5 optionally substituted with an *O*-protecting group, such as optionally substituted arylalkoxycarbonyl groups or any described herein), sulfoalkyl, acyl (e.g., acetyl, trifluoroacetyl, or others described herein), alkoxycarbonylalkyl (e.g., optionally substituted with an *O*-protecting group, such as optionally substituted arylalkoxycarbonyl groups or any described herein), heterocyclyl (e.g., heteroaryl), or heterocyclylalkyl (e.g., heteroarylalkyl),  
 10 wherein each of these recited  $R^{N1}$  groups can be optionally substituted, as defined herein for each group; or two  $R^{N1}$  combine to form a heterocyclyl or an *N*-protecting group, and wherein each  $R^{N2}$  is, independently, H, alkyl, or aryl. The amino groups can be an unsubstituted amino (i.e.,  $-NH_2$ ) or a substituted amino (i.e.,  $-N(R^{N1})_2$ ). In a preferred embodiment, amino is  $-NH_2$  or  $-NHR^{N1}$ , wherein  $R^{N1}$  is, independently, OH,  $NO_2$ ,  $NH_2$ ,  
 15  $NR^{N2}_2$ ,  $SO_2OR^{N2}$ ,  $SO_2R^{N2}$ ,  $SOR^{N2}$ , alkyl, carboxyalkyl, sulfoalkyl, acyl (e.g., acetyl, trifluoroacetyl, or others described herein), alkoxycarbonylalkyl (e.g., t-butoxycarbonylalkyl) or aryl, and each  $R^{N2}$  can be H,  $C_{1-20}$  alkyl (e.g.,  $C_{1-6}$  alkyl), or  $C_{6-10}$  aryl.

The term “aryl” refers to an aromatic mono- or polycyclic radical of 6 to 12 carbon atoms having at least one aromatic ring. Examples of such groups include, but are not limited to, phenyl, naphthyl, 1,2,3,4-tetrahydronaphthalyl, 1,2-dihydronaphthalyl, indanyl, and 1H-indenyl.  
 20 indenyl.

The “arylalkyl” group, which as used herein, represents an aryl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein. Exemplary unsubstituted arylalkyl groups are from 7 to 30 carbons (e.g., from 7 to 16 or from  
 25 7 to 20 carbons, such as  $C_{6-10}$  aryl  $C_{1-6}$  alkyl,  $C_{6-10}$  aryl  $C_{1-10}$  alkyl, or  $C_{6-10}$  aryl  $C_{1-20}$  alkyl). In some embodiments, the alkylene and the aryl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective groups.

The alkyl, carbocyclic, and aryl groups may be substituted or unsubstituted. When substituted, there will generally be, for example, 1 to 4 substituents present. These  
 30 substituents may optionally form a ring with the alkyl, carbocyclic, or aryl group with which they are connected. Substituents may include, for example: carbon-containing groups such as alkyl, aryl, arylalkyl (e.g., substituted and unsubstituted phenyl, substituted and unsubstituted benzyl); halogen atoms and halogen-containing groups such as haloalkyl (e.g., trifluoromethyl); oxygen-containing groups such as alcohols (e.g., hydroxyl, hydroxyalkyl, aryl(hydroxyl)alkyl), ethers (e.g., alkoxy, cycloalkoxy, aryloxy, alkoxyalkyl, aryloxyalkyl,  
 35 more preferably, for example, methoxy, ethoxy, propoxy, isopropoxy, cyclopropoxy, etc.), aldehydes (e.g., carboxaldehyde), ketones (e.g., alkylcarbonyl, alkylcarbonylalkyl,

5 arylcarbonyl, arylalkylcarbonyl, arylcarbonylalkyl), acids (e.g., carboxy, carboxyalkyl), acid derivatives such as esters (e.g., alkoxycarbonyl, alkoxycarbonylalkyl, alkylcarbonyloxy, alkylcarbonyloxyalkyl), amides (e.g., aminocarbonyl, mono- or di-alkylaminocarbonyl, aminocarbonylalkyl, mono- or di-alkylaminocarbonylalkyl, arylaminocarbonyl), carbamates (e.g., alkoxycarbonylamino, aryloxycarbonylamino, aminocarbonyloxy, mono- or di-  
10 alkylaminocarbonyloxy, arylaminocarbonyloxy) and ureas (e.g., mono- or di-alkylaminocarbonylamino or arylaminocarbonylamino); nitrogen-containing groups such as amines (e.g., amino, mono- or di-alkylamino, aminoalkyl, mono- or di-alkylaminoalkyl), azides, nitriles (e.g., cyano, cyanoalkyl), nitro; sulfur-containing groups such as thiols, thioethers, sulfoxides and sulfones (e.g., alkylthio, alkylsulfinyl, alkylsulfonyl, alkylthioalkyl, alkylsulfinylalkyl, alkylsulfonylalkyl, arylthio, arylsulfinyl, arylsulfonyl, arylthioalkyl, arylsulfinylalkyl, arylsulfonylalkyl); heterocyclyl heteroalkyl groups, and heterocyclic groups containing one or more heteroatoms, (e.g., thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, oxadiazolyl, thiadiazolyl, aziridinyl, azetidiny, pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, tetrahydrofuranyl, pyranyl, pyronyl,  
15 pyridyl, pyrazinyl, pyridazinyl, piperidyl, hexahydroazepinyl, piperazinyl, morpholinyl, thianaphthyl, benzofuranyl, isobenzofuranyl, indolyl, oxyindolyl, isoindolyl, indazolyl, indolinyl, 7-azaindolyl, benzopyranyl, coumarinyl, isocoumarinyl, quinolinyl, isoquinolinyl, naphthridinyl, cinnolinyl, quinazolinyl, pyridopyridyl, benzoxazinyl, quinoxalinyl, chromenyl, chromanyl, isochromanyl, phthalazinyl and carbolinyl).

25 The term “azido” represents an  $-N_3$  group, which can also be represented as  $-N=N=N$ .

The terms “carbocyclic” and “carbocyclyl,” as used herein, refer to an optionally substituted non-aromatic  $C_{3-12}$  monocyclic, bicyclic, or tricyclic structure in which the rings are formed by carbon atoms. Carbocyclic structures include cycloalkyl, cycloalkenyl, and  
30 cycloalkynyl groups.

The term “cycloalkyl” refers to a monovalent mono- or polycarbocyclic radical of three to ten, preferably three to six carbon atoms. This term is further exemplified by radicals such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, norbornyl, adamantyl, and indanyl. In a preferred embodiment, the “cycloalkyl” moieties can optionally be  
35 substituted with one, two, three or four substituents. Each substituent can independently be, alkyl, alkoxy, halogen, amino, hydroxyl or oxygen unless otherwise specifically indicated. Examples of cycloalkyl moieties include, but are not limited to, optionally substituted

5 cyclopropyl, optionally substituted cyclobutyl, optionally substituted cyclopentyl, optionally substituted cyclopentenyl, optionally substituted cyclohexyl, and optionally substituted cycloheptyl, or those which are specifically exemplified herein.

The term “cyano,” as used herein, represents a –CN group.

As used herein, the term “halo” or “halogen” means a fluorine (fluoro), chlorine  
10 (chloro), bromine (bromo) or iodine (iodo) radical.

The term “heteroalkyl,” as used herein, refers to an alkyl group, as defined herein, in which one or more of the constituent carbon atoms have each been replaced by nitrogen, oxygen, or sulfur. In some embodiments, the heteroalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkyl groups. Examples of  
15 heteroalkyl groups are an “alkoxy” which, as used herein, refers alkyl-O-; and “alkoyl” which, as used herein, refers to alkyl-CO-. Alkoxy substituent groups or alkoxy-containing substituent groups may be substituted by, for example, one or more alkyl groups.

The term “heteroaryl,” refers to an aromatic mono- or polycyclic radical of 5 to 12 atoms having at least one aromatic ring containing one, two, or three ring heteroatoms  
20 selected from N, O, and S, with the remaining ring atoms being C. One or two ring carbon atoms of the heteroaryl group may be replaced with a carbonyl group. Examples of heteroaryl groups are pyridyl, benzooxazolyl, benzoimidazolyl, and benzothiazolyl.

The term “heterocycle” or “heterocyclyl” denotes a mono- or polycyclic alkyl ring, wherein one, two or three of the carbon ring atoms is replaced by a heteroatom such as N, O  
25 or S. Examples of heterocyclyl groups include, but are not limited to, oxetanyl, morpholinyl, thiomorpholinyl, piperazinyl, piperidinyl, pyrrolidinyl, tetrahydropyranyl, tetrahydrofuranyl, and 1,3-dioxanyl. The heterocyclyl groups may be unsubstituted or substituted, and attachment may be through their carbon frame or through their heteroatom(s) where appropriate.

30 The term “heterocyclyl heteroalkyl” refers to a heterocyclic group, as defined herein, attached to the parent molecular group through a heteroalkyl group (e.g., an ether or alkoxy group). An example of a heterocyclyl heteroalkyl group is –OCH<sub>2</sub>CH<sub>2</sub>(morpholino) group.

The heterocyclyl and heteroaryl groups described above may be substituted independently with one, two, three, or more substituents. Substituents may include, for  
35 example: carbon-containing groups such as alkyl, aryl, arylalkyl (e.g., substituted and unsubstituted phenyl, substituted and unsubstituted benzyl); halogen atoms and halogen-containing groups such as haloalkyl (e.g., trifluoromethyl); oxygen-containing groups such as

5 alcohols (e.g., hydroxyl, hydroxyalkyl, aryl(hydroxyl)alkyl), ethers (e.g., alkoxy, aryloxy, alkoxyalkyl, aryloxyalkyl), aldehydes (e.g., carboxaldehyde), ketones (e.g., alkylcarbonyl, alkylcarbonylalkyl, arylcarbonyl, arylalkylcarbonyl, arylcarbonylalkyl), acids (e.g., carboxy, carboxyalkyl), acid derivatives such as esters (e.g., alkoxycarbonyl, alkoxycarbonylalkyl, alkylcarbonyloxy, alkylcarbonyloxyalkyl), amides (e.g., aminocarbonyl, mono- or di-  
10 alkylaminocarbonyl, aminocarbonylalkyl, mono- or di-alkylaminocarbonylalkyl, arylaminocarbonyl), carbamates (e.g., alkoxycarbonylamino, aryloxycarbonylamino, aminocarbonyloxy, mono- or di-alkylaminocarbonyloxy, arylaminocarbonyloxy) and ureas (e.g., mono- or di-alkylaminocarbonylamino or arylaminocarbonylamino); nitrogen-containing groups such as amines (e.g., amino, mono- or di-alkylamino, aminoalkyl, mono-  
15 or di-alkylaminoalkyl), azides, nitriles (e.g., cyano, cyanoalkyl), nitro; sulfur-containing groups such as thiols, thioethers, sulfoxides and sulfones (e.g., alkylthio, alkylsulfinyl, alkylsulfonyl, alkylthioalkyl, alkylsulfinylalkyl, alkylsulfonylalkyl, arylthio, arylsulfinyl, arylsulfonyl, arylthioalkyl, arylsulfinylalkyl, arylsulfonylalkyl); and heterocyclic groups containing one or more heteroatoms, (e.g., thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl,  
20 thiazolyl, isothiazolyl, oxazolyl, oxadiazolyl, thiadiazolyl, aziridinyl, azetidiny, pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, tetrahydrofuranyl, pyranal, pyronyl, pyridyl, pyrazinyl, pyridazinyl, piperidyl, hexahydroazepinyl, piperazinyl, morpholinyl, thianaphthyl, benzofuranyl, isobenzofuranyl, indolyl, oxyindolyl, isoindolyl, indazolyl, indolinyl, 7-azaindolyl, benzopyranal, coumarinyl, isocoumarinyl, quinolinyl, isoquinolinyl,  
25 naphthridinyl, cinnolinyl, quinazolinyl, pyridopyridyl, benzoxazinyl, quinoxalinyl, chromenyl, chromanyl, isochromanyl, phthalazinyl, benzothiazolyl and carbolinyl).

The term "hydroxyl," as used herein, represents an -OH group. In some embodiments, the hydroxyl group can be substituted with a *O*-protecting group as defined herein.

30 The term "*N*-protecting group," as used herein, represents those groups intended to protect an amino group against undesirable reactions during synthetic procedures. Commonly used *N*-protecting groups are disclosed in Greene, "Protective Groups in Organic Synthesis," 3<sup>rd</sup> Edition (John Wiley & Sons, New York, 1999), which is incorporated herein by reference. The term "*O*-protecting group," as used herein, represents those groups  
35 intended to protect an oxygen containing (e.g., phenol, hydroxyl, or carbonyl) group against undesirable reactions during synthetic procedures. Commonly used *O*-protecting groups are disclosed in Greene, "Protective Groups in Organic Synthesis," 3<sup>rd</sup> Edition (John Wiley &

5 Sons, New York, 1999), which is incorporated herein by reference. The term “perfluoroalkyl,” as used herein, represents alkyl group, as defined herein, where each hydrogen radical bound to the alkyl group has been replaced by a fluoride radical. For example, perfluoroalkyl groups are exemplified by trifluoromethyl and pentafluoroethyl.

The term “sulfonyl,” as used herein, represents an  $-S(O)_2-$  group.

10 A “substituted” hydrocarbon may have as a substituent one or more hydrocarbon radicals, substituted hydrocarbon radicals, or may comprise one or more heteroatoms. Examples of substituted hydrocarbon radicals include, without limitation, heterocycles, such as heteroaryls. Unless otherwise specified, a hydrocarbon substituted with one or more heteroatoms will comprise from 1-20 heteroatoms. In other embodiments, a hydrocarbon  
15 substituted with one or more heteroatoms will comprise from 1-12 or from 1-8 or from 1-6 or from 1-4 or from 1-3 or from 1-2 heteroatoms. Examples of heteroatoms include, but are not limited to, oxygen, nitrogen, sulfur, phosphorous, halogen (F, Cl, Br, I, etc.), boron, silicon, etc. In some embodiments, heteroatoms will be selected from the group consisting of oxygen, nitrogen, sulfur, phosphorous, and halogen (F, Cl, Br, I, etc.). In some embodiments,  
20 a heteroatom or group may substitute a carbon. In some embodiments, a heteroatom or group may substitute a hydrogen. In some embodiments, a substituted hydrocarbon may comprise one or more heteroatoms in the backbone or chain of the molecule (e.g., interposed between two carbon atoms, as in “oxa”). In some embodiments, a substituted hydrocarbon may comprise one or more heteroatoms pendant from the backbone or chain of the molecule (e.g.,  
25 covalently bound to a carbon atom in the chain or backbone, as in “oxo”, replacing a hydrogen in the backbone or chain, etc.).

The term “substituent” refers to a group “substituted” on, e.g., an alkyl, haloalkyl, cycloalkyl, heterocyclyl, heterocycloalkenyl, cycloalkenyl, aryl, or heteroaryl group at any atom of that group, replacing one or more hydrogen atoms therein. In one aspect, the  
30 substituent(s) on a group are independently any one single, or any combination of two or more of the permissible atoms or groups of atoms delineated for that substituent. In another aspect, a substituent may itself be substituted with any one of the above substituents. Further, as used herein, the phrase “optionally substituted” means unsubstituted (e.g., substituted with an H) or substituted. It is understood that substitution at a given atom is limited by valency.

35 Common substituents include halo,  $C_{1-12}$  straight chain or branched chain alkyl,  $C_{2-12}$  alkenyl,  $C_{2-12}$  alkynyl,  $C_{3-12}$  cycloalkyl,  $C_{6-12}$  aryl,  $C_{3-12}$  heteroaryl,  $C_{3-12}$  heterocyclyl,  $C_{1-12}$  alkylsulfonyl, nitro, cyano,  $-COOR$ ,  $-C(O)NRR'$ ,  $-OR$ ,  $-SR$ ,  $-NRR'$ , and oxo, such as mono-

5 or di- or tri-substitutions with moieties such as trifluoromethoxy, chlorine, bromine, fluorine, methyl, methoxy, pyridyl, furyl, triazyl, piperazinyl, pyrazoyl, imidazoyl, and the like, each optionally containing one or more heteroatoms such as halo, N, O, S, and P. R and R' are independently hydrogen, C<sub>1-12</sub> alkyl, C<sub>1-12</sub> haloalkyl, C<sub>2-12</sub> alkenyl, C<sub>2-12</sub> alkynyl, C<sub>3-12</sub> cycloalkyl, C<sub>4-24</sub> cycloalkylalkyl, C<sub>6-12</sub> aryl, C<sub>7-24</sub> aralkyl, C<sub>3-12</sub> heterocyclyl, C<sub>3-24</sub> heterocyclylalkyl, C<sub>3-12</sub> heteroaryl, or C<sub>4-24</sub> heteroarylalkyl. Unless otherwise noted, all groups described herein optionally contain one or more common substituents, to the extent permitted by valency. Further, as used herein, the phrase "optionally substituted" means unsubstituted (e.g., substituted with an H) or substituted. As used herein, the term "substituted" means that a hydrogen atom is removed and replaced by a substituent (e.g., a common substituent). It is understood by one of ordinary skill in the chemistry art that substitution at a given atom is limited by valency. The use of a substituent (radical) prefix names such as alkyl without the modifier "optionally substituted" or "substituted" is understood to mean that the particular substituent is unsubstituted. However, the use of "haloalkyl" without the modifier "optionally substituted" or "substituted" is still understood to mean an alkyl group, in which at least one hydrogen atom is replaced by halo.

It will be understood that the description of compounds herein is limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding with regard to valencies, etc., and to give compounds which are not inherently unstable.

Compounds provided herein can have one or more asymmetric carbon atoms and can exist in the form of optically pure enantiomers, mixtures of enantiomers such as racemates, optically pure diastereoisomers, mixtures of diastereoisomers, diastereoisomeric racemates or mixtures of diastereoisomeric racemates. The optically active forms can be obtained for example by resolution of the racemates, by asymmetric synthesis or asymmetric chromatography (chromatography with a chiral adsorbents or eluant). That is, certain of the disclosed compounds may exist in various stereoisomeric forms. Stereoisomers are compounds that differ only in their spatial arrangement. Enantiomers are pairs of stereoisomers whose mirror images are not superimposable, most commonly because they contain an asymmetrically substituted carbon atom that acts as a chiral center. "Enantiomer" means one of a pair of molecules that are mirror images of each other and are not superimposable. Diastereomers are stereoisomers that are not related as mirror images, most

5 commonly because they contain two or more asymmetrically substituted carbon atoms and represent the configuration of substituents around one or more chiral carbon atoms. Enantiomers of a compound can be prepared, for example, by separating an enantiomer from a racemate using one or more well-known techniques and methods, such as chiral chromatography and separation methods based thereon. The appropriate technique and/or

10 method for separating an enantiomer of a compound described herein from a racemic mixture can be readily determined by those of skill in the art. "Racemate" or "racemic mixture" means a mixture containing two enantiomers, wherein such mixtures exhibit no optical activity; i.e., they do not rotate the plane of polarized light. "Geometric isomer" means isomers that differ in the orientation of substituent atoms (*e.g.*, to a carbon-carbon double bond, to a cycloalkyl

15 ring, to a bridged bicyclic system, etc.). Atoms (other than H) on each side of a carbon-carbon double bond may be in an E (substituents are on opposite sides of the carbon-carbon double bond) or Z (substituents are oriented on the same side) configuration. "R," "S," "S\*," "R\*," "E," "Z," "cis," and "trans," indicate configurations relative to the core molecule. Certain of the disclosed compounds may exist in atropisomeric forms. Atropisomers are

20 stereoisomers resulting from hindered rotation about single bonds where the steric strain barrier to rotation is high enough to allow for the isolation of the conformers. The compounds disclosed herein may be prepared as individual isomers by either isomer-specific synthesis or resolved from an isomeric mixture. Conventional resolution techniques include forming the salt of a free base of each isomer of an isomeric pair using an optically active acid (followed

25 by fractional crystallization and regeneration of the free base), forming the salt of the acid form of each isomer of an isomeric pair using an optically active amine (followed by fractional crystallization and regeneration of the free acid), forming an ester or amide of each of the isomers of an isomeric pair using an optically pure acid, amine or alcohol (followed by chromatographic separation and removal of the chiral auxiliary), or resolving an isomeric

30 mixture of either a starting material or a final product using various well known chromatographic methods. When the stereochemistry of a disclosed compound is named or depicted by structure, the named or depicted stereoisomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9%) by weight relative to the other stereoisomers. When a single enantiomer is named or depicted by structure, the depicted or named enantiomer is at least 60%, 70%, 80%,

35 90%, 99%, or 99.9% by weight optically pure. When a single diastereomer is named or depicted by structure, the depicted or named diastereomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9% by weight pure. Percent optical purity is the ratio of the weight of the

5 enantiomer or over the weight of the enantiomer plus the weight of its optical isomer. Diastereomeric purity by weight is the ratio of the weight of one diastereomer or over the weight of all the diastereomers. When the stereochemistry of a disclosed compound is named or depicted by structure, the named or depicted stereoisomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9% by mole fraction pure relative to the other stereoisomers. When a single  
10 enantiomer is named or depicted by structure, the depicted or named enantiomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9% by mole fraction pure. When a single diastereomer is named or depicted by structure, the depicted or named diastereomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9% by mole fraction pure. Percent purity by mole fraction is the ratio of the moles of the enantiomer or over the moles of the enantiomer plus the moles of its  
15 optical isomer. Similarly, percent purity by moles fraction is the ratio of the moles of the diastereomer or over the moles of the diastereomer plus the moles of its isomer. When a disclosed compound is named or depicted by structure without indicating the stereochemistry, and the compound has at least one chiral center, it is to be understood that the name or structure encompasses either enantiomer of the compound free from the corresponding  
20 optical isomer, a racemic mixture of the compound or mixtures enriched in one enantiomer relative to its corresponding optical isomer. When a disclosed compound is named or depicted by structure without indicating the stereochemistry and has two or more chiral centers, it is to be understood that the name or structure encompasses a diastereomer free of other diastereomers, a number of diastereomers free from other diastereomeric pairs,  
25 mixtures of diastereomers, mixtures of diastereomeric pairs, mixtures of diastereomers in which one diastereomer is enriched relative to the other diastereomer(s) or mixtures of diastereomers in which one or more diastereomer is enriched relative to the other diastereomers. The disclosure embraces all of these forms.

The term “effective amount” or “therapeutically effective amount” of an agent, as  
30 used herein, is that amount sufficient to effect beneficial or desired results, such as clinical results, and, as such, an “effective amount” depends upon the context in which it is being applied. For example, in the context of administering an agent that is an antimalarial agent, an effective amount of an agent is, for example, an amount sufficient to achieve alleviation or amelioration or prevention or prophylaxis of one or more symptoms or conditions;  
35 diminishment of extent of disease, disorder, or condition; stabilized (i.e., not worsening) state of disease, disorder, or condition; preventing spread of disease, disorder, or condition (e.g., preventing the spread of *Plasmodium* infection beyond the liver, preventing systemic disease,

5 preventing the symptomatic stage of malaria, preventing establishment of *Plasmodium* infection and/or preventing further spread of the disease by preventing transmission back to the mosquito, etc.); delay or slowing the progress of the disease, disorder, or condition; amelioration or palliation of the disease, disorder, or condition; and remission (whether partial or total), whether detectable or undetectable, as compared to the response obtained  
10 without administration of the agent.

The term “pharmaceutical composition,” as used herein, represents a composition containing a compound described herein formulated with a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for  
15 the treatment of disease in a mammal. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (e.g., a tablet, capsule, caplet, gelcap, or syrup); for topical administration (e.g., as a cream, gel, lotion, or ointment); for intravenous administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use); or in any other formulation described herein (see below).

20 Useful pharmaceutical carriers for the preparation of the compositions hereof, can be solids, liquids, or gases. Thus, the compositions can take the form of tablets, pills, capsules, suppositories, powders, enterically coated or other protected formulations (e.g., binding on ion-exchange resins or packaging in lipid-protein vesicles), sustained release formulations, solutions, suspensions, elixirs, and aerosols. The carrier can be selected from the various oils  
25 including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, and sesame oil. Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly (when isotonic with the blood) for injectable solutions. For example, formulations for intravenous administration comprise sterile aqueous solutions of the active ingredient(s) which are prepared by dissolving solid active ingredient(s) in water to  
30 produce an aqueous solution, and rendering the solution sterile. Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, talc, gelatin, malt, rice, flour, chalk, silica, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, and ethanol. The compositions may be subjected to conventional pharmaceutical additives such as preservatives, stabilizing agents,  
35 wetting or emulsifying agents, salts for adjusting osmotic pressure, and buffers. Suitable pharmaceutical carriers and their formulation are described in Remington’s Pharmaceutical Sciences by E. W. Martin. Such compositions will, in any event, contain an effective amount

5 of the active compound together with a suitable carrier so as to prepare the proper dosage form for administration to the recipient.

As used herein, the term “pharmaceutically acceptable salt” refers to salts of any of the compounds described herein that within the scope of sound medical judgment, are suitable for use in contact with the tissues of humans and animals without undue toxicity,  
10 irritation, allergic response and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. Salts may be prepared from pharmaceutically acceptable non-  
15 toxic acids and bases including inorganic and organic acids and bases. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, dichloroacetate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glutamate, glycerophosphate, hemisulfate, heptonate,  
20 hexanoate, hippurate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, isethionate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, mandelate, methanesulfonate, mucate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pantothenate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate,  
25 toluenesulfonate, undecanoate, and valerate salts. Representative basic salts include alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, and magnesium, aluminum salts, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, caffeine, and ethylamine.

30 As used herein, the term “subject” refers to any organism to which a composition in accordance with the disclosure may be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include any animal (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans, lizards, geckos, etc.). The subject may be domesticated animals (e.g., cows, calves, sheep, lambs, horses, foals,  
35 pigs, piglets, etc.), or animals in the family *Muridae* (e.g., rats, mice, etc.). A subject may seek or be in need of treatment, require treatment, be receiving treatment, may be receiving

5 treatment in the future, or a human or animal that is under care by a trained professional for a particular disease or condition.

As used herein, and as well understood in the art, “to treat” a condition or “treatment” of the condition (e.g., the conditions described herein such as malaria) is an approach for obtaining beneficial or desired results, such as clinical results. Beneficial or desired results  
 10 can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions; diminishment of extent of disease, disorder, or condition; stabilized (i.e., not worsening) state of disease, disorder, or condition; preventing spread of disease, disorder, or condition (e.g., preventing the spread of *Plasmodium* infection beyond the liver or preventing transmission back to the mosquito, preventing systemic disease, preventing the symptomatic  
 15 stage of malaria, and/or preventing establishment of *Plasmodium* infection); delay or slowing the progress of the disease, disorder, or condition; amelioration or palliation of the disease, disorder, or condition; and remission (whether partial or total), whether detectable or undetectable. “Palliating” a disease, disorder, or condition means that the extent and/or undesirable clinical manifestations of the disease, disorder, or condition are lessened and/or  
 20 time course of the progression is slowed or lengthened, as compared to the extent or time course in the absence of treatment.

The term “unit dosage form” refers to a physically discrete unit suitable as a unitary dosage for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association  
 25 with any suitable pharmaceutical excipient or excipients. Exemplary, non-limiting unit dosage forms include a tablet (e.g., a chewable tablet), caplet, capsule (e.g., a hard capsule or a soft capsule), lozenge, film, strip, gelcap, and syrup (also see below).

Other features and advantages of the disclosure are described in the following detailed description and the claims.

30

## Compounds

The present disclosure provides for novel compounds and pharmaceutical compositions useful for the treatment of malaria. The disclosure also provides methods of using these compounds and compositions.

35 In some embodiments, the compounds may be any compound listed in Table 1.

Table 1

| Comp. | Structure | Name |
|-------|-----------|------|
|-------|-----------|------|

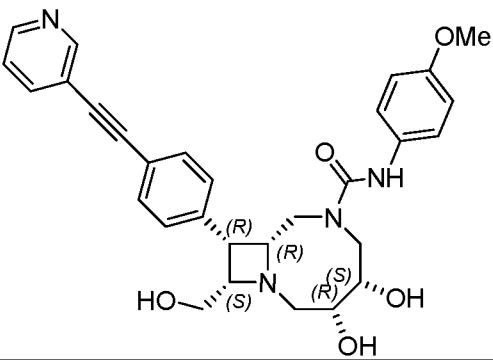
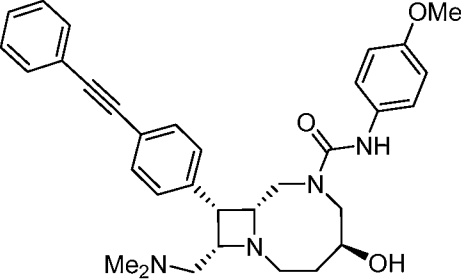
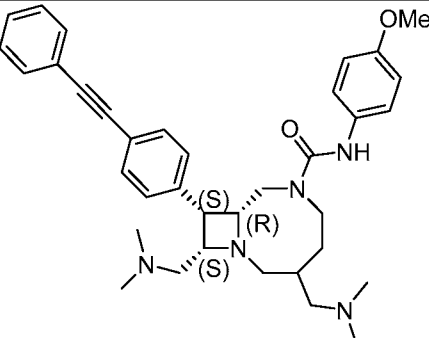
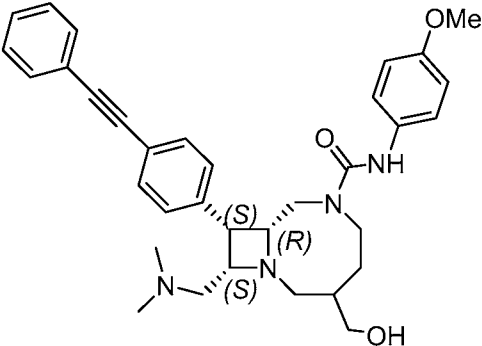
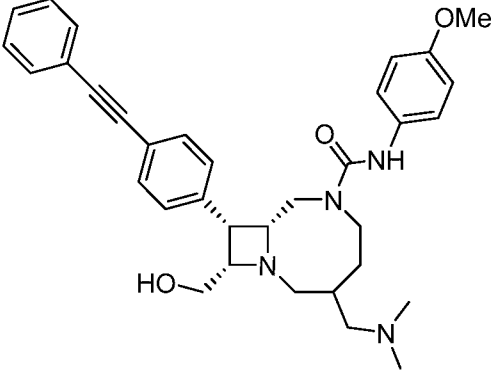
| Comp. | Structure | Name                                                                                                                                                                                                               |
|-------|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| E1    |           | (3 <i>S</i> ,4 <i>R</i> ,8 <i>R</i> ,9 <i>S</i> ,10 <i>S</i> )- <i>N</i> -(4-cyclopropoxyphenyl)-10-(((dimethylamino)methyl)-3,4-dihydroxy-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide |
| E2    |           | (3 <i>S</i> ,4 <i>R</i> ,8 <i>R</i> ,9 <i>S</i> ,10 <i>S</i> )-10-(((dimethylamino)methyl)-3,4-dimethoxy- <i>N</i> -(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide      |
| E3    |           | (4 <i>S</i> ,8 <i>R</i> ,9 <i>R</i> ,10 <i>S</i> )-4-(dimethylamino)-10-(hydroxymethyl)- <i>N</i> -(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide                       |
| E4    |           | (3 <i>Z</i> ,8 <i>R</i> ,9 <i>S</i> ,10 <i>S</i> )-10-[(dimethylamino)methyl]- <i>N</i> -(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide                            |
| E5    |           | (3 <i>S</i> ,4 <i>R</i> ,8 <i>R</i> ,9 <i>S</i> ,10 <i>S</i> )-10-(((dimethylamino)methyl)-3,4-dihydroxy- <i>N</i> -(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide      |

| Comp. | Structure | Name                                                                                                                                                                |
|-------|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| E6    |           | (3R,4S,8R,9S,10S)-10-(((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide             |
| E7    |           | (3S,4R,8R,9S,10S)-10-(((dimethylamino)methyl)-9-[4-[2-(2-fluorophenyl)ethynyl]phenyl]-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide |
| E8    |           | (3R,4S,8R,9S,10S)-10-(((dimethylamino)methyl)-9-[4-[2-(2-fluorophenyl)ethynyl]phenyl]-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide |
| E9    |           | (3S,4R,8R,9S,10S)-10-(diethylaminomethyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide               |

| Comp. | Structure | Name                                                                                                                                                                    |
|-------|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| E10   |           | (3S,4R,8R,9S,10S)-3,4-dihydroxy-10-[[isopropyl(methyl)amino]methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide       |
| E11   |           | (3R,4S,8R,9S,10S)-3,4-dihydroxy-10-[[isopropyl(methyl)amino]methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide       |
| E12   |           | (3S,4R,8R,9S,10S)-9-[4-(2-(2,3-difluorophenyl)ethynyl)phenyl]-10-(((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide |
| E13   |           | (3R,4S,8R,9S,10S)-9-[4-(2-(2,3-difluorophenyl)ethynyl)phenyl]-10-(((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide |

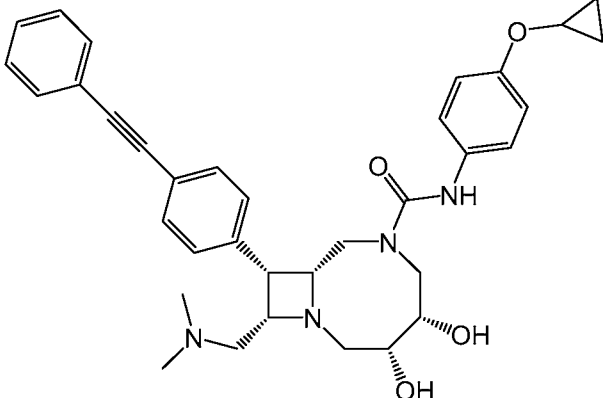
| Comp. | Structure | Name                                                                                                                                                                                                                                                                 |
|-------|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| E14   |           | (4a <i>R</i> ,7a <i>R</i> ,8 <i>S</i> ,9 <i>S</i> ,11a <i>S</i> )-9-((dimethylamino)methyl)- <i>N</i> -(4-methoxyphenyl)-8-(4-(phenylethynyl)phenyl)octahydro-2 <i>H</i> -azeto[1,2- $\alpha$ ][1,4]dioxino[2,3- <i>f</i> ][1,4]diazocine-6(3 <i>H</i> )-carboxamide |
| E15   |           | (3 <i>S</i> ,8 <i>R</i> ,9 <i>S</i> ,10 <i>S</i> )-10-[(dimethylamino)methyl]-3-hydroxy- <i>N</i> -(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide                                                                       |
| E16   |           | (8 <i>R</i> ,9 <i>S</i> ,10 <i>S</i> )-10-[(dimethylamino)methyl]-3-hydroxy- <i>N</i> -(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide, isomer 1*                                                                        |
| E17   |           | (8 <i>R</i> ,9 <i>S</i> ,10 <i>S</i> )-10-[(dimethylamino)methyl]-3-hydroxy- <i>N</i> -(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide, isomer 2*                                                                        |
| E18   |           | (8 <i>R</i> ,9 <i>R</i> ,10 <i>S</i> )-4-(dimethylamino)-10-(hydroxymethyl)- <i>N</i> -(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide, isomer 1*                                                                        |

| Comp. | Structure | Name                                                                                                                                                      |
|-------|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| E19   |           | (8R,9R,10S)-4-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide, isomer 2* |
| E20   |           | (8R,9S,10S)-9-(4-bromophenyl)-4-(dimethylamino)-10-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide, isomer 1*     |
| E21   |           | (8R,9S,10S)-9-(4-bromophenyl)-4-(dimethylamino)-10-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide, isomer 2*     |
| E22   |           | (3S,4R,8R,9R,10S)-3,4-dihydroxy-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(pyridin-3-ylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide      |

| Comp. | Structure                                                                           | Name                                                                                                                                                   |
|-------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| E23   |    | (3R,4S,8R,9R,10S)-3,4-dihydroxy-10-((4-methoxyphenyl)carbamoyl)-9-(4-(pyridin-3-ylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide          |
| E24   |    | (4S,8R,9S,10S)-10-((dimethylamino)methyl)-4-hydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide      |
| E25   |   | (8R,9S,10S)-3,10-bis((dimethylamino)methyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide              |
| E26   |  | (8R,9S,10S)-10-((dimethylamino)methyl)-3-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide |
| E27   |  | (8R,9R,10S)-3-((dimethylamino)methyl)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide |

| Comp. | Structure | Name                                                                                                                                                   |
|-------|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| E28   |           | (3R,8R,9R,10S)-3-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide      |
| E29   |           | (3S,8R,9R,10S)-3-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide        |
| E30   |           | (4R,8R,9R,10S)-4-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide        |
| E31   |           | (8R,9R,10S)-4-(((dimethylamino)methyl))-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide |
| E32   |           | (8R,9S,10S)-4,10-bis((dimethylamino)methyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide              |

| Comp. | Structure | Name                                                                                                                                                                                       |
|-------|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| E33   |           | (8 <i>R</i> ,9 <i>S</i> ,10 <i>S</i> )-10-[(dimethylamino)methyl]-4-(hydroxymethyl)- <i>N</i> -(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide |
| E34   |           | (8 <i>R</i> ,9 <i>S</i> ,10 <i>S</i> )-10-(((dimethylamino)methyl)- <i>N</i> -(4-methoxyphenyl)-2-methyl-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide           |
| E35   |           | (8 <i>R</i> ,9 <i>R</i> ,10 <i>S</i> )-10-(hydroxymethyl)- <i>N</i> -(4-methoxyphenyl)-2-methyl-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide                  |
| E36   |           | (3 <i>S</i> ,4 <i>R</i> ,8 <i>R</i> ,9 <i>S</i> )-3,4-dihydroxy- <i>N</i> -(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide                     |
| E37   |           | (3 <i>R</i> ,4 <i>S</i> ,8 <i>R</i> ,9 <i>S</i> )-3,4-dihydroxy- <i>N</i> -(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide                     |

| Comp. | Structure                                                                         | Name                                                                                                                                                                                                              |
|-------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| E38   |  | (3 <i>R</i> ,4 <i>S</i> ,8 <i>R</i> ,9 <i>S</i> ,10 <i>S</i> )- <i>N</i> -(4-cyclopropoxyphenyl)-10-((dimethylamino)methyl)-3,4-dihydroxy-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide |

5 \*Each stereoisomer was isolated as described in the exemplary synthesis and used in any biological experiments as described below. However, stereochemistry at the C3 or C4 position could not be determined - although each diastereomer (i.e., isomer 1 or isomer 2) was isolated as described and not present as a mixture.

10 It will be understood that in the event of any inconsistency between a chemical name and formula, both compounds with the indicated chemical name and compounds with the indicated chemical structure will be considered as embraced by the invention.

The compounds of the present invention include the compounds themselves, as well as their salts and their prodrugs, if applicable. A salt, for example, can be formed between an anion and a positively charged substituent (e.g., amino) on a compound described herein.

15 Suitable anions include chloride, bromide, iodide, sulfate, nitrate, phosphate, citrate, methanesulfonate, trifluoroacetate, and acetate. Likewise, a salt can also be formed between a cation and a negatively charged substituent (e.g., carboxylate) on a compound described herein. Suitable cations include sodium ion, potassium ion, magnesium ion, calcium ion, and an ammonium cation such as tetramethylammonium ion. Examples of prodrugs include C<sub>1-6</sub> alkyl esters of carboxylic acid groups, which, upon administration to a subject, are capable of providing active compounds.

Pharmaceutically acceptable salts of the compounds of the present disclosure include those derived from pharmaceutically acceptable inorganic and organic acids and bases. As used herein, the term “pharmaceutically acceptable salt” refers to a salt formed by the addition of a pharmaceutically acceptable acid or base to a compound disclosed herein. As used herein, the phrase “pharmaceutically acceptable” refers to a substance that is acceptable for use in pharmaceutical applications from a toxicological perspective and does not adversely interact with the active ingredient.

30 Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, digluconate,

5 dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, 10 tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the present invention and their pharmaceutically acceptable acid addition salts. Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and N-(alkyl)<sub>4</sub><sup>+</sup> salts. The 15 present invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization. Salt forms of the compounds of any of the formulae herein can be amino acid salts of carboxyl groups (e.g., L-arginine, -lysine, -histidine salts).

Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., 20 Mack Publishing Company, Easton, Pa., 1985, p. 1418; Journal of Pharmaceutical Science, 66, 2 (1977); and "Pharmaceutical Salts: Properties, Selection, and Use A Handbook; Wermuth, C. G. and Stahl, P. H. (eds.) Verlag Helvetica Chimica Acta, Zurich, 2002 [ISBN 3-906390-26-8] each of which is incorporated herein by reference in their entireties.

The neutral forms of the compounds may be regenerated by contacting the salt with a 25 base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

In addition to salt forms, the present invention provides compounds which are in a 30 prodrug form. Prodrugs of the compounds described herein are those compounds that undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an ex vivo environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a 35 transdermal patch reservoir with a suitable enzyme or chemical reagent. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be more bioavailable by oral administration than the parent drug. The

5 prodrug may also have improved solubility in pharmacological compositions over the parent drug. A wide variety of prodrug derivatives are known in the art, such as those that rely on hydrolytic cleavage or oxidative activation of the prodrug. An example, without limitation, of a prodrug would be a compound of the present invention which is administered as an ester (the "prodrug"), but then is metabolically hydrolyzed to the carboxylic acid, the active entity.  
10 Additional examples include peptidyl derivatives of a compound of the present invention.

The present invention also includes various hydrate and solvate forms of the compounds.

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the  
15 compounds may be radiolabeled with radioactive isotopes, such as for example tritium ( $^3\text{H}$ ), iodine-125 ( $^{125}\text{I}$ ) or carbon-14 ( $^{14}\text{C}$ ). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

## 20 **Methods**

The compounds described herein are useful in the methods provided herein and, while not bound by any particular theory, are believed to exert their desirable effects through their ability to inhibit the growth of or kill a parasitic protozoan that causes malaria (e.g., *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and/or *P. knowlesi*) and/or cryptosporidiosis (e.g.,  
25 *C. parvum*, *C. hominis*, *C. canis*, *C. felis*, *C. meleagridis*, and *C. muris*). In some embodiments, the treatment includes causative prophylaxis, such as preventing the spread of *Plasmodium* and/or *Cryptosporidium* infection beyond the liver, preventing systemic disease, preventing the symptomatic stage of malaria, preventing the establishment of the infection, and/or preventing further transmission (e.g., to a mosquito). In some embodiments, the  
30 treatment of malaria refers to treatment intended to achieve cure (e.g., of *P. vivax* or *P. malariae*), e.g., treatment for radical cure (i.e., clearing hypnozoites from the liver). In various examples, the methods include preventing spread of infection of a malaria-causing parasite from the liver. In some embodiments, the treatment of cryptosporidiosis includes causative prophylaxis, such as preventing the spread of *Cryptosporidium* beyond infected  
35 portions of a subject (e.g. liver, intestines, respiratory tract, etc.).

The compounds may be useful in the treatment of drug resistant malaria, such as malaria resistant to chloroquine, quinine, pyrimethamine, sulfadoxine, mefloquine,

5 artemether, lumefantrine, artesunate, amodiaquine, dihydroartemisinin, piperazine, proguanil, doxycycline, clindamycin, artemisinin, atovaquone, and any combination thereof.

## Pharmaceutical Compositions

### 1. Formulations

10 For use in the methods described herein, the compounds can be formulated as pharmaceutical or veterinary compositions. The formulation selected can vary depending on the subject to be treated, the mode of administration, and the type of treatment desired (e.g., prevention, prophylaxis, or therapy). A summary of formulation techniques is found in *Remington: The Science and Practice of Pharmacy, 21<sup>st</sup> Edition*, Lippincott Williams & 15 Wilkins, (2005); and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York, each of which is incorporated herein by reference. Exemplary routes of administration and formulations are described as follows.

In the practice of the disclosed methods, the compounds (or pharmaceutically acceptable salts thereof) or compositions can be administered by any of the usual and 20 acceptable routes and methods known in the art. The compounds or compositions can thus be administered, for example, by the enteral or gastrointestinal route (e.g., orally or rectally), topically (e.g., to the skin or an accessible mucous membrane (e.g., an intraoral (e.g., sublingual or buccal), intranasal, intrarectal, or genitourinary surface)), parenterally (e.g., by intramuscular, intravenous, subcutaneous, intraarticular, intravesicular, intrathecal, epidural, 25 ocular, or aural application or injection), transdermally, or by inhalation (e.g., by aerosol).

The compositions can be in the form of a solid, liquid, or gas, as determined to be appropriate by those of skill in the art. Thus, as general examples, the pharmaceutical compositions may be in the form of tablets, capsules, syrups, pills, enterically coated or other protected formulations, sustained release formulations, elixirs, powders, granulates, 30 suspensions, emulsions, solutions, gels (e.g., hydrogels), pastes, ointments, creams, plasters, transdermal patches, drenches, suppositories, enemas, injectables, implants, sprays, or aerosols.

The compositions, in general, include an effective amount of a compound described herein and one or more pharmaceutically acceptable carriers or excipients, as is well known 35 in the art. The compositions can thus include one or more diluents, buffers, preservatives, salts, carbohydrates, amino acids, carrier proteins, fatty acids, lipids, etc. The compounds

5 described herein may be present in amounts totaling, for example, 1-95% by weight of the total weight of the composition.

For injection, formulations can be prepared in conventional forms as liquid solutions or suspensions, or as solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients for these formulations include, for example,  
10 water, saline, dextrose, and glycerol. Such compositions can also contain nontoxic auxiliary substances, such as wetting or emulsifying agents, and pH buffering agents, such as sodium acetate, sorbitan monolaurate, and so forth.

Formulations for oral use include tablets containing a compound in a mixture with one or more non-toxic pharmaceutically acceptable excipients. These excipients may be, for  
15 example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose,  
20 glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other  
25 pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, and buffering agents.

Formulations for oral use may also be provided as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin), or as  
30 soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders, granulates, and pellets may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus or a spray drying equipment.

Dissolution or diffusion controlled release can be achieved by appropriate coating of a  
35 tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above and/or, e.g., shellac, beeswax, glycowax, castor

5 wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-poly(lactic acid), cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-hydroxymethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, and/or polyethylene glycols. In a controlled release matrix  
10 formulation, the matrix material may also include, e.g., hydrated methylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, and/or halogenated fluorocarbon.

The liquid forms in which the compounds and compositions can be incorporated for administration orally include aqueous solutions, suitably flavored syrups, aqueous or oil  
15 suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

The pharmaceutical composition may also be formulated as a veterinary composition, intended for use with subjects other than humans. The veterinary compositions according to the present invention can be in any appropriate forms to suit the requested administration  
20 modes, for instance nasal, oral, intradermic, cutaneous or parenteral. In a preferred embodiment, the composition is in a form intended for an oral administration and, for instance when the domestic animal eating, either mixed to the food ration, or directly into the mouth after meal. The veterinary compositions of the invention are in the form of a nasal, oral or injectable liquid suspension or solution, or in solid or semi-solid form, powders,  
25 pellets, capsules, granules, sugar-coated pills, gelules, sprays, cachets, pills, tablets, pastes, implants or gels. In a particular embodiment, the compositions are in the form of an oral solid form, preferably tablets. In some embodiments, the veterinary compositions may have an effective amount of the compound for a specific species of animal (e.g., cow, lamb, goat, horse, etc.).

30 In a preferred embodiment, the compositions of the invention are formulated in pellets or tablets for an oral administration. According to this type of formulation, they comprise lactose monohydrate, cellulose microcrystalline, crospovidone/povidone, aroma, compressible sugar and magnesium stearate as excipients. When the compositions are in the form of pellets or tablets, they are for instance 1 mg, 2 mg, or 4 mg torasemide pellets or  
35 tablets. Such pellets or tablets are divisible so that they can be cut to suit the posology according to the invention in one or two daily takes. In a further preferred embodiment, the compositions of the invention are formulated in injectable solutions or suspensions for a

parenteral administration. The injectable compositions are produced by mixing therapeutically efficient quantity of torasemide with a pH regulator, a buffer agent, a suspension agent, a solubilisation agent, a stabilizer, a tonicity agent and/or a preservative, and by transformation of the mixture into an intravenous, sub-cutaneous, intramuscular injection or perfusion according to a conventional method. Possibly, the injectable compositions may be lyophilized according to a conventional method. Examples of suspension agents include methylcellulose, polysorbate 80, hydroxyethylcellulose, xanthan gum, sodic carboxymethylcellulose and polyethoxylated sorbitan monolaurate. Examples of solubilisation agent include polyoxy ethylene- solidified castor oil, polysorbate 80, nicotinamide, polyethoxylated sorbitan monolaurate, macrogol and ethyl ester of castor oil fatty acid. Moreover, the stabilizer includes sodium sulfite, sodium metabisulfite and ether, while the preservative includes methyl p-hydroxybenzoate, ethyl p- hydroxybenzoate, sorbic acid, phenol, cresol and chlorocresol. An example of tonicity agent is mannitol. When preparing injectable suspensions or solutions, it is desirable to make sure that they are blood isotonic.

## **2. Kits**

The compounds and compositions can be packaged in a kit, optionally with one or more other pharmaceutical agents (see below). Non-limiting examples of the kits include those that contain, e.g., two or more pills, a pill and a powder, a suppository and a liquid in a vial, or two topical creams. The kits can include optional components that aid in the administration of the unit dose to subjects, such as vials for reconstituting powder forms, syringes for injection, customized IV delivery systems, or inhalers. Additionally, the unit dose kits can contain instructions for preparation and administration of the compositions. The kits can be manufactured as a single use unit dose for one subject, multiple uses for a particular subject (at a constant dose or in which the individual compounds may vary in potency as therapy progresses); or the kits can contain multiple doses suitable for administration to multiple subjects ("bulk packaging"). The kit components can be assembled in cartons, blister packs, bottles, and tubes.

## **3. Dosage**

The dose of a compound depends on a number of factors, such as the manner of administration, the age and the body weight of the subject, and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such an amount of the compound, as determined by the attending physician or veterinarian, is referred

5 to herein, and in the claims, as a “therapeutically effective amount.” For example, the dose of a compound disclosed herein is typically in the range of about 1 to about 1000 mg per day. Preferably, the therapeutically effective amount is in an amount of from about 1 mg to about 500 mg per day.

Administration of each drug, as described herein, can, independently, be one to four  
10 times daily for one day to one year, and may even be for the life of the subject. Chronic, long-term administration may be indicated.

#### 4. Combination Therapies

The compounds and pharmaceutical compositions can be formulated and employed in combination therapies, that is, the compounds and pharmaceutical compositions can be  
15 formulated with or administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired  
20 effect for the same disorder, or they may achieve different effects (e.g., control of any adverse effects).

Examples of other drugs to combine with the compounds described herein include pharmaceuticals for the treatment of malaria (e.g., chloroquine, quinine, pyrimethamine, sulfadoxine, mefloquine, artemether, lumefantrine, artesunate, amodiaquine,  
25 dihydroartemisinin, piperaquine, proguanil, doxycycline, clindamycin, artemisinin, atovaquone, any other therapeutic approved for the treatment of malaria, and any combination thereof) and/or pharmaceuticals for the treatment of cryptosporidiosis (e.g., nitazoxanide). Other examples of drugs to combine with the compounds described herein include pharmaceuticals for the treatment of different, yet associated or related symptoms or  
30 indications. Combination methods can involve the use of the two (or more) agents formulated together or separately, as determined to be appropriate by those of skill in the art. In one example, two or more drugs are formulated together for the simultaneous or near simultaneous administration of the agents.

#### EXAMPLES

35 The following Examples illustrate the synthesis of a representative number of compounds and the use of these compounds in the treatment of malaria. Accordingly, the Examples are intended to illustrate but not to limit the disclosure. Additional compounds not

5 specifically exemplified may be synthesized using conventional methods in combination with the methods described herein.

## Synthesis of Compounds

### Materials and Methods

10 All reactions were carried out under N<sub>2</sub> or argon atmosphere. All reagents and solvents were purchased from commercial vendors and used as received, or synthesized according to the footnoted references. NMR spectra were recorded on a Bruker 300 (300 MHz <sup>1</sup>H, 75 MHz <sup>13</sup>C) and Bruker 400 (400 MHz <sup>1</sup>H, 100 MHz <sup>13</sup>C) spectrometer. Proton chemical shifts are reported in ppm (δ) referenced to the NMR solvent. Data are reported as follows: chemical shifts, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q =  
15 quartet, p = pentet, m = multiplet; coupling constant(s) in Hz; integration). Unless otherwise indicated NMR data were collected at 25°C. Flash chromatography was performed using 40-60 µm Silica Gel (60 Å mesh) on a Teledyne Isco Combiflash Rf. For purity analysis, purity was measured by UV absorbance at 210 nm for all examples, and identity was determined on a SQ mass spectrometer by positive electrospray ionization. The following method was used:  
20 Tandem Liquid Chromatography/Mass Spectrometry (LCMS) was performed on a Waters 2795 separations module and 3100 mass detector. Mobile phase A consisted of 0.01% formic acid in water, while mobile phase B consisted of 0.01% formic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 2.5, 5 or 7.5 min at 1.75 mL/min. An Agilent Poroshell 120 EC-C18, 2.7 µm, 3.0x30 mm column was used with column  
25 temperature maintained at 40°C. 2.1 µL of sample solution were injected. Analytical TLC was performed on EM Reagent 0.25 mm silica gel 60-F plates. Visualization was accomplished with UV light and aqueous potassium permanganate (KMnO<sub>4</sub>) stain followed by heating. Accurate mass measurements were obtained on an Agilent 6230 Time-of-Flight mass spectrometer as the (M+H)<sup>+</sup>. Compound purity and identity were also determined by  
30 UPLC-MS. Purity was measured by UV absorbance at 210 nm. Identity was determined on a SQ mass spectrometer by positive and negative electrospray ionization. Mobile phase A consisted of either 0.1% ammonium hydroxide or 0.05% trifluoroacetic acid in water, while mobile phase B consisted of either 0.1% ammonium hydroxide or 0.06% trifluoroacetic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 2.65 min at 0.9  
35 mL/min. An Acquity BEH C18, 1.7 µm, 2.1x50 mm column was used with column temperature maintained at 65°C. Compounds were dissolved in DMSO at a nominal concentration of 1 mg/mL, and 1.0 µL of this solution was injected. Chiral separations were

5 performed by SFC-MS. A Berger G600 supercritical fluid chromatograph was coupled with a Waters ZQ single quadrupole mass spectrometer operating in positive APCI mode. Using liquefied CO<sub>2</sub> modified with 20% isopropanol, an isocratic separation was performed for 5.0 minutes at 4.0 mL/min on a 4.6x100 mm Chiralpak AD-H column maintained at 40°C. Compounds were dissolved in methanol at a nominal concentration of 1 mg/mL, and 10 µL of this solution was injected. The synthetic examples below are not particularly limiting, and other methodologies to synthesize the compounds of the present invention are well known to persons of skill in the art.

### Abbreviations

15 Throughout the synthetic examples below, various abbreviations are employed and are well known to persons of ordinary skill in the art. Table 2 illustrates various examples of abbreviations used throughout the examples and the corresponding reference.

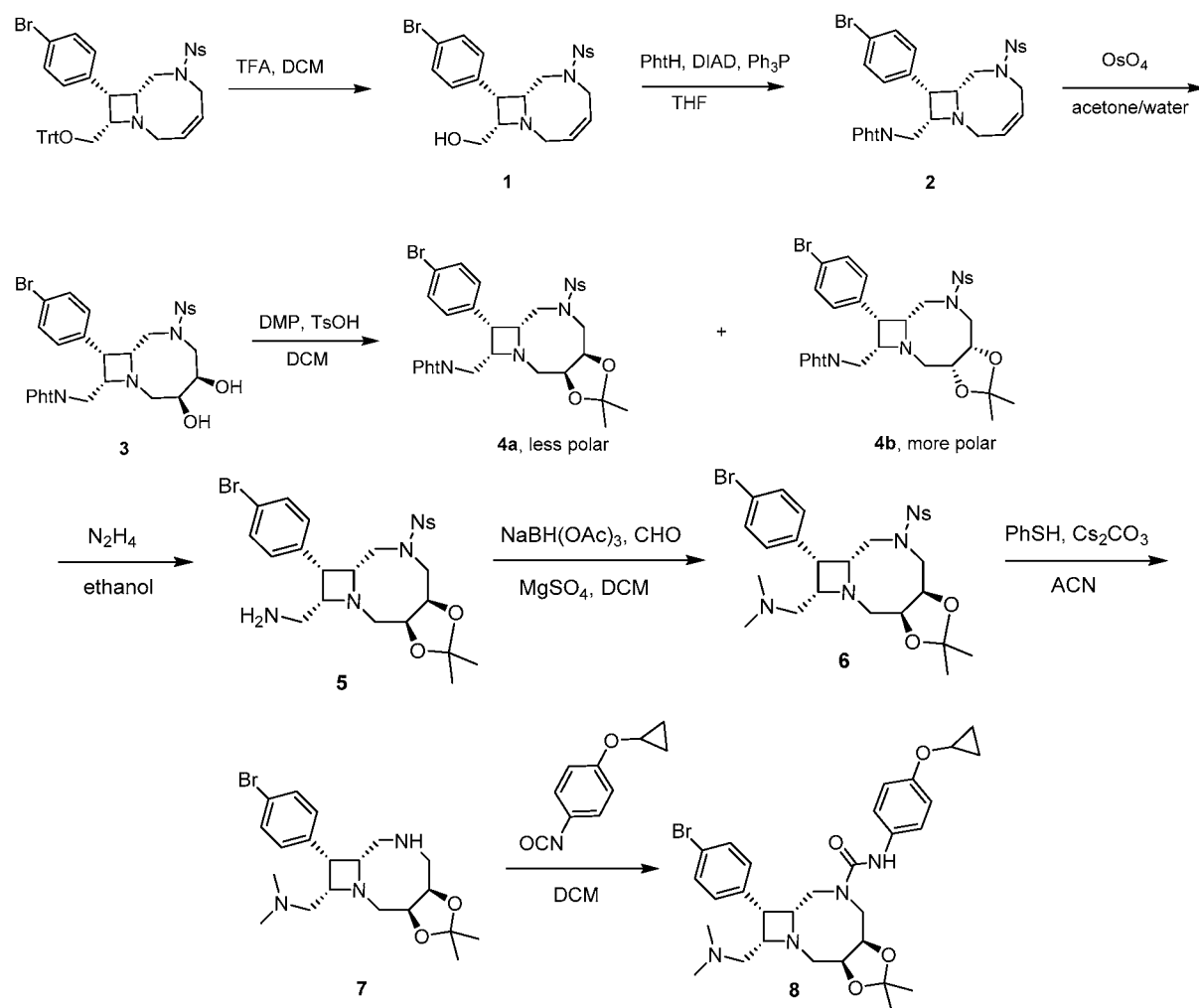
Table 2

| Abbreviation                                       | Reference                                                                                                                                                                                     |
|----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ACN                                                | acetonitrile                                                                                                                                                                                  |
| DCM                                                | dichlormethane                                                                                                                                                                                |
| DMF                                                | dimethylformamide                                                                                                                                                                             |
| EtOAc                                              | ethyl acetate                                                                                                                                                                                 |
| HPLC                                               | High Performance Liquid Chromatography                                                                                                                                                        |
| MeOH                                               | Methanol                                                                                                                                                                                      |
| PBS                                                | Phosphate Buffered Saline                                                                                                                                                                     |
| THF                                                | Tetrahydrofuran                                                                                                                                                                               |
| TFA                                                | Trifluoroacetic Acid                                                                                                                                                                          |
| TLD                                                | Thin Layer Chromatography                                                                                                                                                                     |
| UPLC                                               | Ultra Performance Liquid Chromatography                                                                                                                                                       |
| DBU                                                | 1,8-diazabicyclo[5.4.0]undec-7-ene                                                                                                                                                            |
| XPhos Pd G3                                        | XPhos Precatalyst (i.e., 2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) methanesulfonate, CAS Number 1445085-55-1)                     |
| TMSOTf                                             | trimethylsilyl trifluoromethanesulfonate                                                                                                                                                      |
| DIAD                                               | diisopropyl azodicarboxylate                                                                                                                                                                  |
| NMO                                                | N-methylmorpholine N-oxide                                                                                                                                                                    |
| TsOH                                               | p-toluenesulfonic acid                                                                                                                                                                        |
| TBAB/F                                             | tetra-N-butylammonium bromide/fluoride                                                                                                                                                        |
| DCE                                                | dichloroethylene                                                                                                                                                                              |
| TFAA                                               | trifluoroacetic anhydride                                                                                                                                                                     |
| DMP                                                | 2,2-dimethoxypropane                                                                                                                                                                          |
| Dess-Martin                                        | Dess–Martin periodinane (i.e., 1,1,1-Tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1H)-one, CAS Number 87413-09-0)                                                                           |
| Hoyveda-Grubbs 2 <sup>nd</sup> Generation Catalyst | Hoyveyda-Grubbs 2nd Generation ruthenium based catalyst (i.e., (1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(o-isopropoxyphenylmethylene)ruthenium, Dichloro[1,3-bis(2,4,6- |

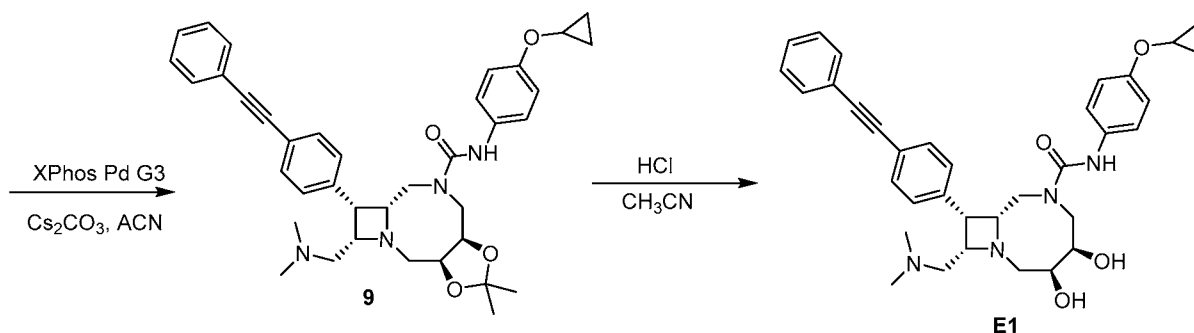
|                                                    |                                                                                                                                                                                                                                                |
|----------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                    | trimethylphenyl)-2-imidazolidinylidene](2-isopropoxyphenylmethylene)ruthenium(II), CAS Number 301224-40-8).                                                                                                                                    |
| Hoyveda-Grubbs 1 <sup>st</sup> Generation Catalyst | Hoyveda-Grubbs 1st Generation ruthenium based catalyst (i.e., dichloro(2-isopropoxyphenylmethylene)(tricyclohexylphosphine)ruthenium(II), Dichloro(o-isopropoxyphenylmethylene)(tricyclohexylphosphine)ruthenium(II), CAS Number 203714-71-0). |
| NBSH                                               | 2-nitrobenzenesulfonohydrazide                                                                                                                                                                                                                 |
| PhSH                                               | Benzenethiol                                                                                                                                                                                                                                   |
| DIBAL-H                                            | Diisobutylaluminium Hydride                                                                                                                                                                                                                    |

5

**Example 1:** (3S,4R,8R,9S,10S)-N-(4-cyclopropoxyphenyl)-10-((dimethylamino)methyl)-3,4-dihydroxy-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E1")



10



**Intermediate 1:** ((8*R*,9*R*,10*S*,*Z*)-9-(4-bromophenyl)-6-((2-nitrophenyl)sulfonyl)-1,6-diazabicyclo[6.2.0]dec-3-en-10-yl)methanol.

To a solution of (8*R*,9*R*,10*S*,*Z*)-10-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-9-(4-bromophenyl)-6-((2-nitrophenyl)sulfonyl)-1,6-diazabicyclo[6.2.0]dec-3-ene (2.00 g, 2.66 mmol) (Journal of Organic Chemistry (2012), 77(17), 7187-7211; WO 2015070204) in DCM (27.00 mL) was added TFA (3.03 g, 26.60 mmol, 1.97 mL). The mixture was stirred at 15°C for 20 hours. The reaction mixture was quenched by addition NaHCO<sub>3</sub> solution (100 mL), and then extracted with DCM 180 mL (3 X 60 mL). The combined organic layers were dried over [NaSO<sub>4</sub>], filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether: DCM=1:1 to DCM:MeOH=10:1 yielding Intermediate 1 (1.47g, crude) as a black solid. Presence of the reaction product was confirmed by NMR and LCMS.

**Intermediate 2:** 2-(((8*R*,9*S*,10*S*,*Z*)-9-(4-bromophenyl)-6-((2-nitrophenyl)sulfonyl)-1,6-diazabicyclo[6.2.0]dec-3-en-10-yl)methyl)isoindoline-1,3-dione.

To a mixture of triphenylphosphine (1.52 g, 5.78 mmol) in THF (5 mL) degassed and purged with N<sub>2</sub> was added diisopropyl azodicarboxylate (1.17 g, 5.78 mmol, 1.12 mL). To this mixture stirred at 0°C under N<sub>2</sub> atmosphere was added a mixture of Intermediate 1 (1.47 g, 2.89 mmol, 1.00 eq) and isoindoline-1,3-dione (637.81 mg, 4.34 mmol, 1.50 eq) in THF (5 mL) degassed and purged with N<sub>2</sub>. The mixture was stirred at 16°C for 18 hours under N<sub>2</sub> atmosphere. The reaction mixture was quenched by addition H<sub>2</sub>O (20 mL), and then extracted with DCM (3 X 20 mL). The combined organic layers were dried over [Na<sub>2</sub>SO<sub>4</sub>], filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether: ethylacetate =5:1 to 2:1) yielding Intermediate 2 (1.26 g, 1.98 mmol, 68.39% yield) as a brown solid as confirmed by NMR and LCMS.

**Intermediate 3:** 2-(((8*R*,9*S*,10*S*)-9-(4-bromophenyl)-3,4-dihydroxy-6-((2-nitrophenyl)sulfonyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)methyl)isoindoline-1,3-dione

To a solution of Intermediate 2 (1.26 g, 1.98 mmol) in acetone/water (15.00 mL) was added N-methylmorpholine N-oxide (463.09 mg, 3.95 mmol, 417.20  $\mu$ L) and then OsO<sub>4</sub> (5.02 mg, 19.76  $\mu$ mol, 1.03  $\mu$ L). The mixture was stirred at 15°C for 1.5 hour. The reaction mixture was dried over [Na<sub>2</sub>SO<sub>4</sub>], filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=3/1 to 0:1) to yield Intermediate 3 (1.11 g, 1.65 mmol, 83.48% yield) as a white solid as confirmed by LCMS and NMR.

**Intermediates 4a and 4b:** 2-(((3*aR*,6*aR*,7*S*,8*R*,10*aS*)-7-(4-bromophenyl)-2,2-dimethyl-5-((2-nitrophenyl)sulfonyl)octahydro-3*aH*-azeto[1,2-*a*][1,3]dioxolo[4,5-*f*][1,4]diazocin-8-yl)methyl)isoindoline-1,3-dione (Intermediate 4a) and 2-(((3*aS*,6*aS*,7*S*,8*R*,10*aS*)-7-(4-bromophenyl)-2,2-dimethyl-5-((2-nitrophenyl)sulfonyl)octahydro-3*aH*-azeto[1,2-*a*][1,3]dioxolo[4,5-*f*][1,4]diazocin-8-yl)methyl)isoindoline-1,3-dione (Intermediate 4b).

To a solution of Intermediate 3 (880.00 mg, 1.31 mmol) and 2,2-dimethoxypropane (272.97 mg, 2.62 mmol, 321.14  $\mu$ L) in DCM (10.00 mL) was added TsOH (225.66 mg, 1.31 mmol). The mixture was stirred at 15°C. After 20 hours, the reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, petroleum ether/ethyl acetate= 1:1) yielding each cis-diol, Intermediate 4a (less polar, 53.00 mg, 74.48  $\mu$ mol, 5.68% yield) as a white solid and Intermediate 4b (more polar, 260.00 mg, 365.38  $\mu$ mol, 27.88% yield) as a white solid as confirmed by NMR and LCMS.

**Intermediate 5:** ((3*aR*,6*aR*,7*S*,8*S*,10*aS*)-7-(4-bromophenyl)-2,2-dimethyl-5-((2-nitrophenyl)sulfonyl)octahydro-3*aH*-azeto[1,2-*a*][1,3]dioxolo[4,5-*f*][1,4]diazocin-8-yl)methanamine.

To a solution of Intermediate 4b (360.00 mg, 505.92  $\mu$ mol) in ethanol (5.00 mL) was added hydrazine hydrate (37.99 mg, 758.88  $\mu$ mol, 36.88  $\mu$ L). After stirring at 70°C for 1.5 hour, the reaction mixture was filtered through celite and concentrated under reduced pressure to yield Intermediate 5 (239.00 mg, 411.02  $\mu$ mol, 81.24% yield) as a yellow solid as confirmed by NMR and LCMS.

**Intermediate 6:** 1-((3*aR*,6*aR*,7*S*,8*S*,10*aS*)-7-(4-bromophenyl)-2,2-dimethyl-5-((2-nitrophenyl)sulfonyl)octahydro-3*aH*-azeto[1,2-*a*][1,3]dioxolo[4,5-*f*][1,4]diazocin-8-yl)-*N,N*-dimethylmethanamine.

To a solution of Intermediate 5 (239.00 mg, 411.02  $\mu$ mol) and formaldehyde (200.47 mg, 2.47 mmol, 183.92  $\mu$ L) in DCM (500  $\mu$ L) was added MgSO<sub>4</sub> (494.74 mg, 4.11 mmol) and acetic acid (49.36 mg, 822.04  $\mu$ mol, 47.01  $\mu$ L). After 20 mins, sodium

5 triacetoxyborohydride (60.32 mg, 1.23 mmol) was added and the mixture was stirred at 15°C for 23 hour. The reaction mixture was filtered by celite and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1) yielding Intermediate **6** (210.00 mg, 344.53 μmol, 83.82% yield) as a yellow oil as confirmed by LCMS.

10 **Intermediate 7:** 1-((3*aR*,6*aR*,7*R*,8*S*,10*aS*)-7-(4-bromophenyl)-2,2-dimethyloctahydro-3*aH*-azeto[1,2-*a*][1,3]dioxolo[4,5-*f*][1,4]diazocin-8-yl)-*N,N*-dimethylmethanamine.

To a solution of Intermediate **6** (210.00 mg, 344.53 μmol) in CH<sub>3</sub>CN (2.00 mL) was added benzenethiol (17.08 mg, 155.04 μmol, 15.82 μL) and then added Cs<sub>2</sub>CO<sub>3</sub> (224.51 mg, 689.06 μmol). The mixture was stirred at 40°C for 1.5 hour under N<sub>2</sub> atmosphere. The  
15 reaction mixture was quenched by addition H<sub>2</sub>O (5 mL), and extracted with DCM 15 mL (3 X 5 mL). The combined organic layers were dried over [Na<sub>2</sub>SO<sub>4</sub>], filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1) yielding Intermediate **7** (72.00 mg, 169.66 μmol, 49.24% yield) as a yellow oil as confirmed by NMR and LCMS.

20 **Intermediate 8:** (3*aR*,6*aR*,7*S*,8*S*,10*aS*)-7-(4-bromophenyl)-*N*-(4-cyclopropoxyphenyl)-8-((dimethylamino)methyl)-2,2-dimethylhexahydro-3*aH*-azeto[1,2-*a*][1,3]dioxolo[4,5-*f*][1,4]diazocine-5(4*H*)-carboxamide.

To a solution of Intermediate **7** (48.00 mg, 113.11 μmol) in DCM (2.00 mL) was added TEA (5.72 mg, 56.55 μmol, 7.84 μL) and 1-(cyclopropoxy)-4-isocyanato-benzene  
25 (23.78 mg, 135.73 μmol) under N<sub>2</sub> atmosphere. After stirring at 15°C for 22 hour, the reaction mixture was quenched by addition H<sub>2</sub>O (5 mL), and then extracted with DCM 15 mL (3 x 5 mL). The combined organic layers were concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1) yielding Intermediate **8** (29.40 mg, 49.04 μmol, 43.35% yield) as a pale oil as confirmed by LCMS.

30 **Intermediate 9:** (3*aR*,6*aR*,7*S*,8*S*,10*aS*)-*N*-(4-cyclopropoxyphenyl)-8-((dimethylamino)methyl)-2,2-dimethyl-7-(4-(phenylethynyl)phenyl)hexahydro-3*aH*-azeto[1,2-*a*][1,3]dioxolo[4,5-*f*][1,4]diazocine-5(4*H*)-carboxamide.

To a solution of Intermediate **8** (29.00 mg, 48.37 μmol) in CH<sub>3</sub>CN (1.00 mL) under N<sub>2</sub> atmosphere mixture was added TEA (97.89 mg, 967.38 μmol, 134.09 μL) and  
35 ethynylbenzene (24.70 mg, 241.84 μmol, 26.56 μL) and XPhos Pd G3 (6.14 mg, 7.26 μmol). After stirring at 70°C for 1h, the reaction mixture was quenched by addition H<sub>2</sub>O (4 mL), and then extracted with DCM 15 mL (3 x 5 mL). The combined organic layers were dried over

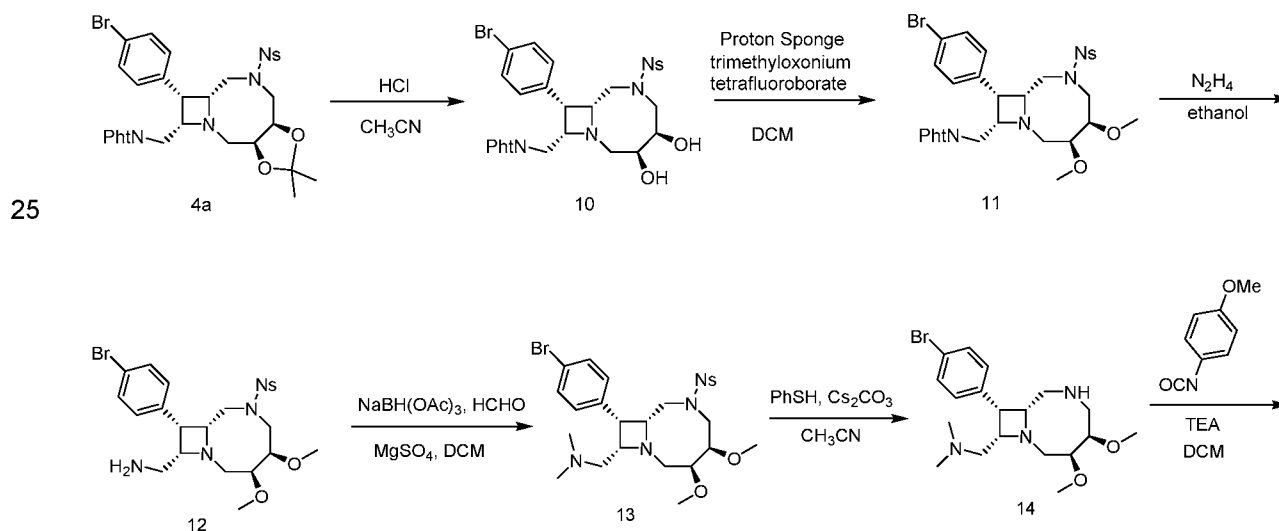
- 5  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give Intermediate 9 (30.00 mg) as a pale oil as confirmed by LCMS.

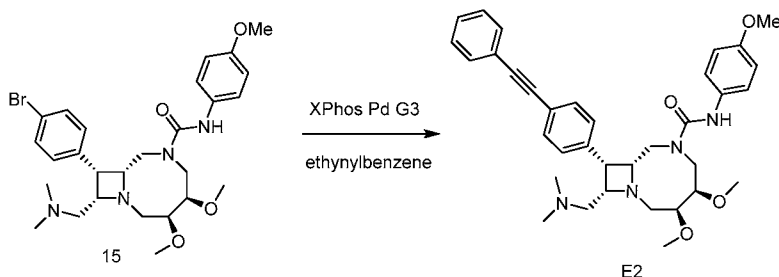
**Synthesis of E1:** (3*S*,4*R*,8*R*,9*S*,10*S*)-*N*-(4-cyclopropoxyphenyl)-10-((dimethylamino)methyl)-3,4-dihydroxy-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

- 10 To a solution of Intermediate 9 (20.00 mg, 32.22  $\mu\text{mol}$ ) in  $\text{CH}_3\text{CN}$  (500.00  $\mu\text{L}$ ) was added 1 M HCl (500.00  $\mu\text{L}$ ). After stirring at 15°C for 18h, the reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (0.225% formic acid) B: acetonitrile) yielding compound E1 (8.20 mg, 14.12  $\mu\text{mol}$ , 43.83% yield) as a yellow
- 15 solid.

- $^1\text{H}$  NMR (400 MHz,  $\text{CHLOROFORM-}d$ )  $\delta$  ppm 0.73 (d,  $J=4.52$  Hz, 4 H) 1.26 (s, 1 H) 2.32 (s, 6 H) 2.60 - 2.70 (m, 2 H) 2.86 - 2.92 (m, 1 H) 2.96 - 3.05 (m, 2 H) 3.22 (br d,  $J=15.56$  Hz, 1 H) 3.52 - 3.65 (m, 3 H) 3.65 - 3.75 (m, 2 H) 3.86 (br s, 2 H) 3.97 - 4.05 (m, 1 H) 4.13 (br s, 1 H) 4.31 (br d,  $J=9.54$  Hz, 1 H) 6.95 (d,  $J=9.03$  Hz, 2 H) 7.24 (d,  $J=9.03$  Hz, 2 H) 7.32 - 7.46 (m, 6 H) 7.49 - 7.57 (m, 4 H) 8.37 (br s, 1 H) 8.56 (br s, 1 H).
- 20

**Example 2:** (3*S*,4*R*,8*R*,9*S*,10*S*)-10-((dimethylamino)methyl)-3,4-dimethoxy-*N*-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E2")





**Intermediate 10:** 2-(((3*S*,4*R*,8*R*,9*S*,10*S*)-9-(4-bromophenyl)-3,4-dihydroxy-6-((2-nitrophenyl)sulfonyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)methyl)isoindoline-1,3-dione.

A solution of Intermediate 4a (170.00 mg, 238.90  $\mu\text{mol}$ ) in  $\text{CH}_3\text{CN}$  (2.00 mL) and 1 M HCl (2.00 mL) was stirred at 20°C for 18 hours. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC ( $\text{SiO}_2$ , DCM: MeOH = 10:1) yielding Intermediate 10 (140.00 mg, 208.49  $\mu\text{mol}$ , 87.27% yield) as a white solid as confirmed by NMR.

**Intermediate 11:** 2-(((3*S*,4*R*,8*R*,9*S*,10*S*)-9-(4-bromophenyl)-3,4-dimethoxy-6-((2-nitrophenyl)sulfonyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)methyl)isoindoline-1,3-dione.

To a solution of Intermediate 10 (125.00 mg, 186.15  $\mu\text{mol}$ ) in DCM (4.00 mL) was added trimethyloxonium tetrafluoroborate (137.67 mg, 930.75  $\mu\text{mol}$ ) and N1,N1,N8,N8-tetramethylnaphthalene-1,8-diamine (239.36 mg, 1.12 mmol) at 0°C under N<sub>2</sub> atmosphere. After stirring at RT (15°C) for 18h, the reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, ethyl acetate) yielding Intermediate 11 (100.00 mg, 142.94  $\mu\text{mol}$ , 76.79% yield) as a white solid as confirmed by NMR.

**Intermediate 12:** ((3*S*,4*R*,8*R*,9*S*,10*S*)-9-(4-bromophenyl)-3,4-dimethoxy-6-((2-nitrophenyl)sulfonyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)methanamine.

To a solution of Intermediate 11 (100.00 mg, 142.94  $\mu\text{mol}$ ) in ethanol (3.00 mL) was added  $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$  (10.73 mg, 214.42  $\mu\text{mol}$ , 10.42  $\mu\text{L}$ ). After stirring at 70°C for 1 hour, the reaction mixture was filtered through celite and concentrated under reduced pressure to give crude Intermediate 12 (87.00 mg) as a yellow solid as confirmed by NMR.

**Intermediate 13:** 1-((3*S*,4*R*,8*R*,9*S*,10*S*)-9-(4-bromophenyl)-3,4-dimethoxy-6-((2-nitrophenyl)sulfonyl)-1,6-diazabicyclo[6.2.0]decan-10-yl)-*N,N*-dimethylmethanamine.

To a solution of Intermediate 12 (87.00 mg, 152.77  $\mu\text{mol}$ ) in DCM (2.00 mL) was added HCHO (27.53 mg, 916.64  $\mu\text{mol}$ , 25.25  $\mu\text{L}$ ),  $\text{MgSO}_4$  (183.89 mg, 1.53 mmol),  $\text{NaBH}(\text{OAc})_3$  (97.14 mg, 458.32  $\mu\text{mol}$ ) and AcOH (18.35 mg, 305.55  $\mu\text{mol}$ , 17.47  $\mu\text{L}$ ). After stirring at 15°C for 3 h, the reaction mixture was filtered through celite and concentrated

5 under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1) to give Intermediate 13 (46.00 mg, 76.98 μmol, 50.39% yield) as a white oil.

**Intermediate 14:** ((3*S*,4*R*,8*R*,9*S*,10*S*)-9-(4-bromophenyl)-3,4-dimethoxy-1,6-diazabicyclo[6.2.0]decan-10-yl)methanamine.

To a solution of Intermediate 13 (46.00 mg, 76.98 μmol) in CH<sub>3</sub>CN (2.00 mL) was  
10 added benzenethiol (12.72 mg, 115.48 μmol, 11.78 μL) and Cs<sub>2</sub>CO<sub>3</sub> (50.17 mg, 153.97 μmol). After stirring at 40°C for 1.5 hour under N<sub>2</sub> atmosphere, the reaction mixture was quenched by addition H<sub>2</sub>O (2 mL), and extracted with DCM 15 mL (3 x 5 mL). The combined organic layers were dried over [Na<sub>2</sub>SO<sub>4</sub>], filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH =  
15 10:1) yielding Intermediate 14 (17.00 mg, 41.23 μmol, 53.55% yield) as a white oil as confirmed by LCMS.

**Intermediate 15:** (3*S*,4*R*,8*R*,9*S*,10*S*)-10-(aminomethyl)-9-(4-bromophenyl)-3,4-dimethoxy-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 14 (17.00 mg, 41.23 μmol) in DCM (2.00 mL) was  
20 added TEA (2.09 mg, 20.61 μmol, 2.86 μL) and 1-isocyanato-4-methoxy-benzene (6.15 mg, 41.23 μmol, 5.30 μL). After stirring under N<sub>2</sub> atmosphere mixture at 15°C for 2.5 h, the reaction mixture was quenched by addition H<sub>2</sub>O (5 mL), and then extracted with DCM 15 mL (3 x 5 mL). The combined organic layers were concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (DCM:MeOH=10:1) to give Intermediate 15  
25 (33.00 mg, crude) as a clear oil as confirmed by LCMS.

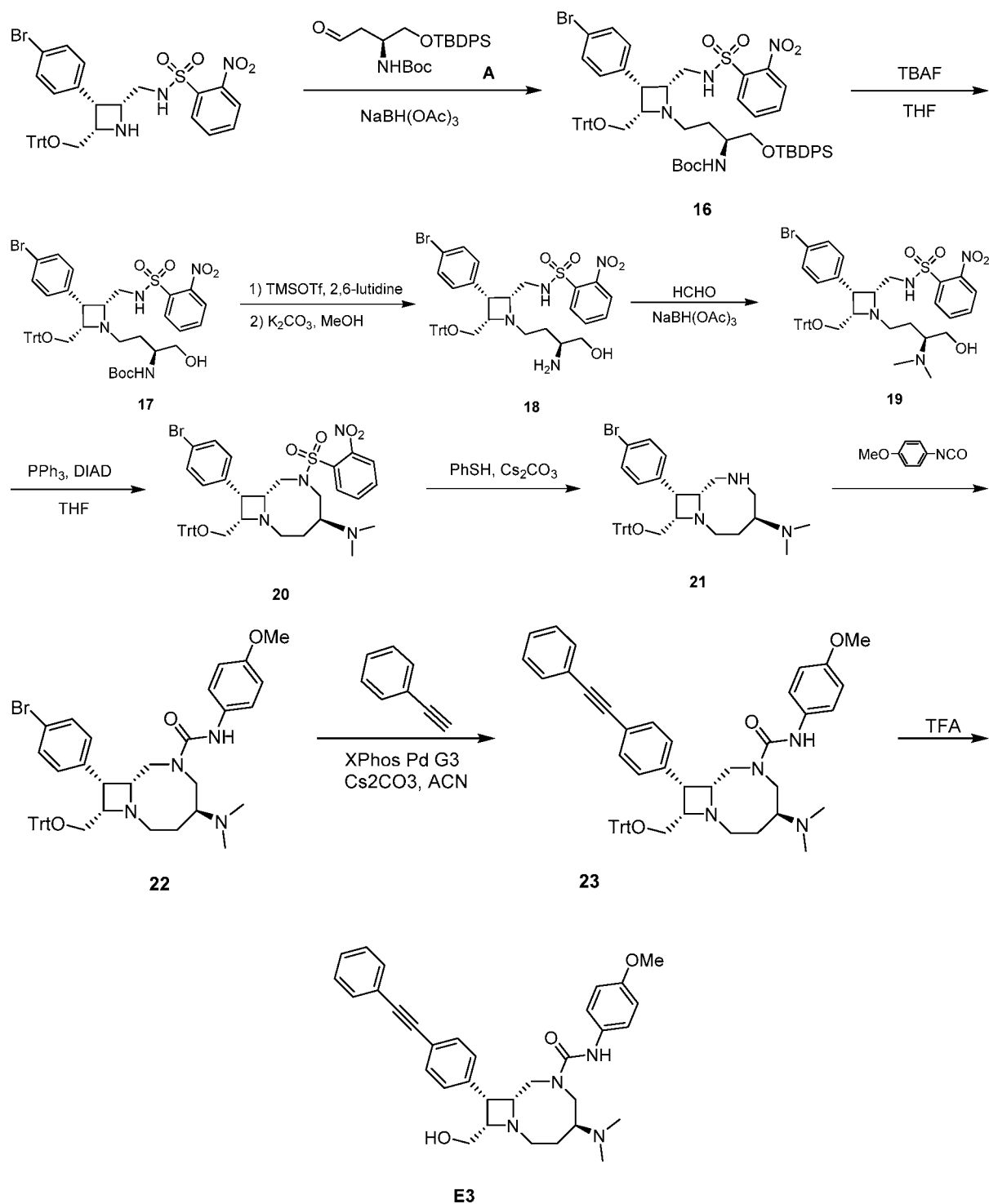
**Synthesis of E2:** (3*S*,4*R*,8*R*,9*S*,10*S*)-10-((dimethylamino)methyl)-3,4-dimethoxy-*N*-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide

To a solution of Intermediate 15 (33.00 mg, 58.77 μmol) in acetonitrile (2.00 mL) was  
added TEA (118.94 mg, 1.18 mmol, 162.93 μL) and XPhos Pd G3 (7.46 mg, 8.82 μmol)  
30 under N<sub>2</sub> atmosphere. After stirring at 70°C for 1 hour, the reaction mixture was quenched by addition H<sub>2</sub>O (2 mL), and then extracted with DCM 15 mL (3 x 5 mL). The combined organic layers were dried over [Na<sub>2</sub>SO<sub>4</sub>], filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1) followed by prep-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (0.225% formic acid) B: acetonitrile) to give 4.4 mg, 7.55 μmol, 12.85% yield as a white solid. The structure  
35 was confirmed by LCMS and NMR.

<sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.06 (s, 6 H) 2.39 (br s, 2 H) 2.58 (br s, 2 H)

5 3.03 - 3.14 (m, 1 H) 3.30 (br s, 1 H) 3.48 (s, 4 H) 3.51 - 3.58 (m, 2 H) 3.61 - 3.67 (m, 4 H)  
 3.78 (s, 3 H) 3.89 - 4.12 (m, 3 H) 6.83 (d,  $J=8.91$  Hz, 2 H) 7.18 (d,  $J=8.78$  Hz, 2 H) 7.36 (br  
 d,  $J=5.40$  Hz, 3 H) 7.43 - 7.47 (m, 2 H) 7.48 - 7.51 (m, 2 H) 7.52 - 7.56 (m, 2 H) 7.99 (br s, 1  
 H).

**Example 3:** (4*S*,8*R*,9*R*,10*S*)-4-(dimethylamino)-10-(hydroxymethyl)-*N*-(4-methoxyphenyl)-  
 10 9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E3")



15

5 **Intermediate 16:** *tert*-butyl N-[(1*S*)-3-[(2*R*,3*R*,4*S*)-3-(4-bromophenyl)-2-[(2-nitrophenyl)sulfonylamino]methyl]-4-(trityloxymethyl)azetidin-1-yl]-1-[[*tert*-butyl(diphenyl)silyl]oxymethyl]propyl]carbamate.

To a solution of *N*-(((2*R*,3*R*,4*S*)-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidin-2-yl)methyl)-2-nitrobenzenesulfonamide (WO2015070204) (1.20 g, 1.72 mmol) and (*S*)-*tert*-butyl (1-((*tert*-butyldiphenylsilyl)oxy)-4-oxobutan-2-yl)carbamate (US 20150266867) (911.52 mg, 2.06 mmol) in DCM (40.00 mL) was added MgSO<sub>4</sub> (2.07 g, 17.20 mmol) and NaBH(OAc)<sub>3</sub> (729.07 mg, 3.44 mmol). After stirring at 15°C for 2 hours, the reaction mixture was quenched by addition sat. NaHCO<sub>3</sub> aqueous (15 mL) and extracted with DCM (2 x 5 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=20:1 to 3:1) to afford Intermediate 16 (2.00 g) as a white solid: <sup>1</sup>H NMR (400MHz, CHLOROFORM-*d*) δ = 7.81 - 7.69 (m, 3H), 7.66 - 7.58 (m, 6H), 7.45 - 7.34 (m, 8H), 7.15 - 7.04 (m, 18H), 4.87 - 4.76 (m, 2H), 3.68 - 3.59 (m, 3H), 3.50 - 3.42 (m, 3H), 3.14 (br. s., 1H), 3.03 - 2.97 (m, 1H), 2.95 - 2.82 (m, 2H), 2.46 (br. s., 2H), 1.40 (s, 9H), 1.05 (s, 9H).

**Intermediate 17:** *tert*-butyl ((*S*)-4-((2*R*,3*R*,4*S*)-3-(4-bromophenyl)-2-((2-nitrophenyl)sulfonamido)methyl)-4-((trityloxy)methyl)azetidin-1-yl)-1-hydroxybutan-2-yl]carbamate

To a solution of Intermediate 16 (500.00 mg, 444.74 μmol) in THF (6 mL) was added TBAF (174.42 mg, 667.11 μmol) at 15°C. After stirring at 15°C for 1 hour, the reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH =100:1 to 20:1) to afford Intermediate 17 (300.00 mg, 338.65 μmol, 76.15% yield) as a white solid: <sup>1</sup>H NMR (400MHz, CHLOROFORM-*d*) δ = 7.83 (dd, J=7.8, 17.3 Hz, 2H), 7.76 - 7.63 (m, 2H), 7.22 - 7.12 (m, 15H), 7.01 (d, J=8.0 Hz, 2H), 5.05 (br. s., 1H), 4.45 (br. s., 1H), 3.78 - 3.70 (m, 1H), 3.59 (br. s., 3H), 3.43 (d, J=9.5 Hz, 1H), 3.33 - 3.16 (m, 2H), 3.05 (d, J=6.0 Hz, 2H), 2.96 - 2.87 (m, 1H), 2.83 - 2.72 (m, 1H), 2.58 - 2.48 (m, 1H), 1.74 (dd, J=4.8, 14.8 Hz, 1H), 1.43 (s, 9H).

**Intermediate 18:** N-[(2*R*,3*R*,4*S*)-1-[(3*S*)-3-amino-4-hydroxy-butyl]-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-2-yl]methyl]-2-nitro-benzenesulfonamide.

To a solution of Intermediate 17 (800.00 mg, 903.08 μmol) in DCM (16.00 mL) was added TMSOTf (602.15 mg, 2.71 mmol, 489.55 μL) and 2,6-lutidine (387.06 mg, 3.61 mmol,

5 420.72  $\mu$ L, 4 eq). After stirring at 15°C for 16h, the reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1:0 to 20:1) to afford Intermediate 18 (700 mg, crude) as a white solid (containing TEA): <sup>1</sup>H NMR (400MHz, CHLOROFORM-d)  $\delta$  = 7.85 (td, J=2.3, 4.7 Hz, 1H), 7.76 - 7.72 (m, 1H), 7.70 - 7.63 (m, 2H), 7.25 - 7.16 (m, 15H), 6.98 (d, J=8.4 Hz, 2H), 3.72 - 3.60 (m, 3H), 3.52 - 3.42 (m, 3H), 3.27 (dd, J=6.6, 14.1 Hz, 1H), 3.14 - 3.00 (m, 3H), 2.83 - 2.68 (m, 2H), 1.85 - 1.62 (m, 2H)

**Intermediate 19:** N-[(2R,3R,4S)-3-(4-bromophenyl)-1-[(3S)-3-(dimethylamino)-4-hydroxybutyl]-4-(trityloxymethyl)azetidin-2-yl]methyl]-2-nitro-benzenesulfonamide.

To a solution of Intermediate 18 (700.00 mg, 890.88  $\mu$ mol) in DCM (15.00 mL) was added HCHO (86.77 mg, 1.07 mmol, 79.61  $\mu$ L, 37% purity), MgSO<sub>4</sub> (1.07 g, 8.91 mmol) and then NaBH(OAc)<sub>3</sub> (377.63 mg, 1.78 mmol) added in portions. After stirring at 15°C for 1 hour, the reaction mixture was quenched by addition saturated NaHCO<sub>3</sub> aqueous (100 mL) and extracted with DCM (2 x 30 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1:0 to 15:1) to afford Intermediate 19 (500 mg, 614.40  $\mu$ mol, 68.97% yield) as a white solid: <sup>1</sup>H NMR (400MHz, CHLOROFORM-d)  $\delta$  = 7.87 (dd, J=1.5, 7.5 Hz, 1H), 7.78 - 7.73 (m, 1H), 7.72 - 7.61 (m, 2H), 7.23 - 7.12 (m, 18H), 7.05 (d, J=8.0 Hz, 2H), 3.64 - 3.54 (m, 3H), 3.22 - 3.06 (m, 4H), 3.03 - 2.98 (m, 1H), 2.87 - 2.70 (m, 2H), 2.56 (td, J=6.0, 12.0 Hz, 1H), 2.45 (br. s., 1H), 2.22 (s, 6H), 1.57 (d, J=14.1 Hz, 1H), 1.41 (d, J=6.0 Hz, 1H)

**Intermediate 20:** (4S,8R,9R,10S)-9-(4-bromophenyl)-N,N-dimethyl-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-amine.

To a solution of triphenylphosphine (1 g, 3.76 mmol) in THF (19 mL) at 0°C was slowly added diisopropyl azodicarboxylate (0.72 mL, 3.76 mmol). This mixture was then added to a solution of Intermediate 19 (500 mg, 614.40  $\mu$ mol) in THF (75 mL) at 0°C. After stirring at 15°C for 4h, the reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 20:1) to afford Intermediate 20 (200.00 mg, 251.33  $\mu$ mol, 40.91% yield) as a white solid: <sup>1</sup>H NMR (400MHz, CHLOROFORM-d)  $\delta$  = 8.14 (d, J=7.5 Hz, 1H), 7.65 - 7.55 (m, 3H), 7.25 - 7.19 (m, 19H), 3.62 - 3.50 (m, 4H), 3.42 (br. s., 1H), 3.17 - 2.98 (m, 4H), 2.85 (dd, J=7.1, 9.3 Hz, 1H), 2.69 - 2.60 (m, 1H), 2.44 - 2.31 (m, 2H), 2.04 (br. s., 6H), 1.89 (br. s., 1H)

**Intermediate 21:** (4S,8R,9R,10S)-9-(4-bromophenyl)-N,N-dimethyl-10-(trityloxymethyl)-

## 5 1,6-diazabicyclo[6.2.0]decan-4-amine.

To a solution of Intermediate 20 (150.00 mg, 188.49  $\mu$ mol) in acetonitrile (3.00 mL) was added  $\text{Cs}_2\text{CO}_3$  (368.49 mg, 1.13 mmol) and benzenethiol (83.07 mg, 753.96  $\mu$ mol, 76.92  $\mu$ L). After stirring at 40°C for 16h, the reaction mixture was quenched by addition water (15 mL) and extracted with ethyl acetate (2 x 5 mL). The combined organic layers were washed with brine (2 x 5 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC ( $\text{SiO}_2$ , DCM: MeOH = 10:1) to afford Intermediate 21 (90.00 mg, 147.39  $\mu$ mol, 78.20% yield) as a white solid used directly.

15 **Intermediate 22:** (4S,8R,9R,10S)-9-(4-bromophenyl)-4-(dimethylamino)-N-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 21 (90.00 mg, 147.39  $\mu$ mol) in DCM (3.00 mL) was added 1-isocyanato-4-methoxy-benzene (26.38 mg, 176.87  $\mu$ mol). After stirring at 15°C for 1 hour, the reaction mixture was quenched by addition water (15 mL) and extracted with DCM (2 x 5 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC ( $\text{SiO}_2$ , DCM: MeOH = 20:1) to afford Intermediate 22 (90.00 mg, 118.46  $\mu$ mol, 80.37% yield) as a white solid:  $^1\text{H}$  NMR (400MHz,  $\text{CHCl}_3$ -d)  $\delta$  = 7.28 - 7.18 (m, 21H), 6.82 (d,  $J$ =8.8 Hz, 2H), 4.15 - 4.06 (m, 1H), 3.77 (s, 3H), 3.64 - 3.47 (m, 4H), 3.18 - 2.98 (m, 3H), 2.92 - 2.82 (m, 2H), 2.70 (d,  $J$ =13.2 Hz, 1H), 2.43 - 2.33 (m, 7H), 2.10 (br. s., 1H), 1.95 (br. s., 1H)

25 **Intermediate 23:** (4S,8R,9R,10S)-4-(dimethylamino)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

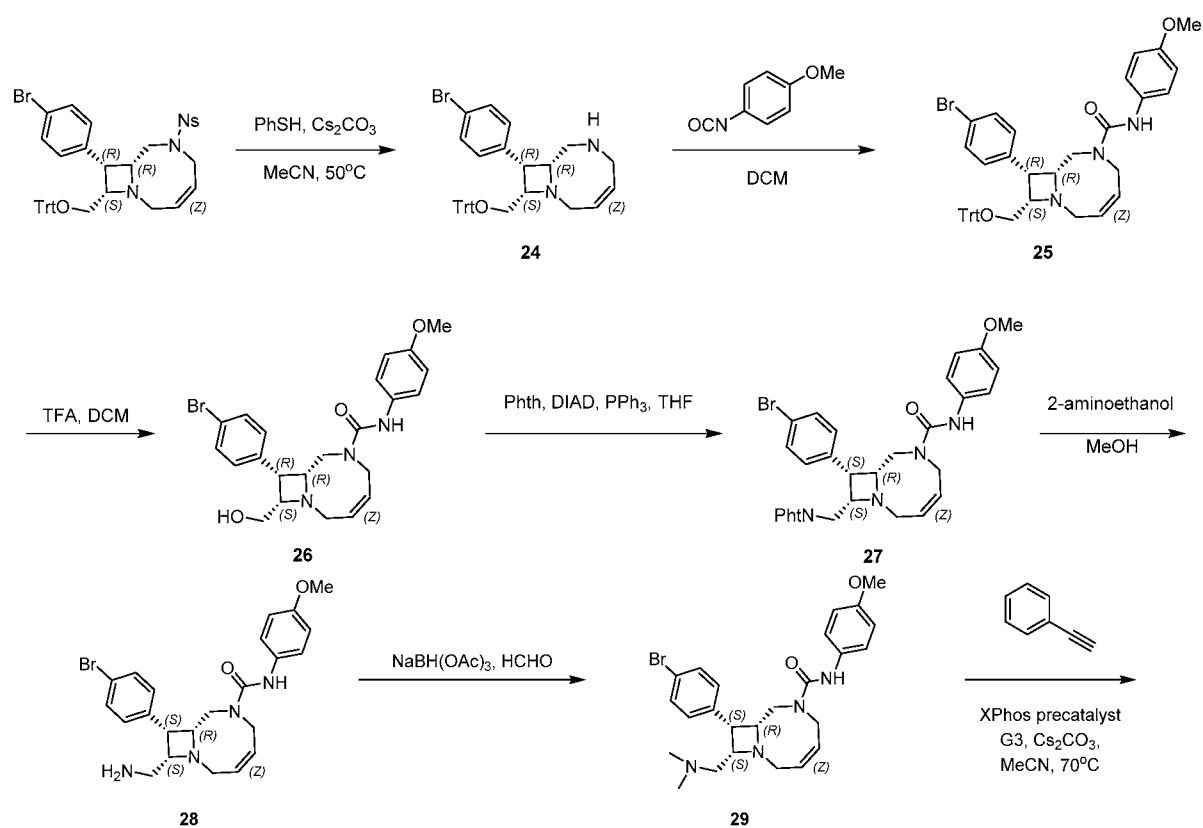
To a solution of Intermediate 22 (64.00 mg, 84.24  $\mu$ mol) and ethynylbenzene (25.81 mg, 252.71  $\mu$ mol) in acetonitrile (1 mL) was added XPhos Pd G3 (7.13 mg, 8.42  $\mu$ mol) and  $\text{Cs}_2\text{CO}_3$  (54.89 mg, 168.47  $\mu$ mol). After stirring at 70°C for 2 h, the reaction mixture was concentrated to give the residue. The residue was purified by pre-TLC (ethyl acetate: methanol = 10 :1) to the give Intermediate 23 (47.00 mg, 60.18  $\mu$ mol, 71.44% yield) as a brown solid.  $^1\text{H}$  NMR (400MHz,  $\text{MeOD-d}_4$ )  $\delta$  7.54 (d,  $J$  = 4.8 Hz, 2H), 7.38-7.35(m, 7H), 7.33-7.21 (m, 18H), 6.83 (d,  $J$  = 8.8 Hz, 2H), 4.13 (s, 1H), 3.77 (s, 3H), 3.61-3.52 (m, 4H), 3.19-3.17 (m, 2H), 2.94-2.90 (m, 2H), 2.73-2.44 (m, 1H), 2.17-1.97 (m, 1H).

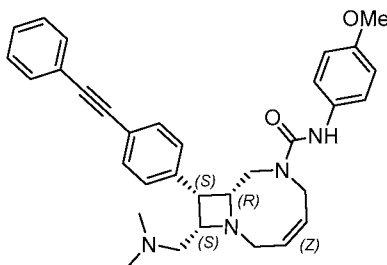
35 **Synthesis of E3:** (4S,8R,9R,10S)-4-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-

5 carboxamide.

To a solution of Intermediate 23 (40.00 mg, 51.22  $\mu\text{mol}$ ) in DCM (1 mL) was added TFA (58.40 mg, 512.17  $\mu\text{mol}$ , 37.92  $\mu\text{L}$ ). After stirring at 25°C for 12 h, the reaction mixture was quenched by sat.  $\text{NaHCO}_3$  and extracted with DCM (3 x 5 mL to give organic layer. The layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give the residue. The residue was  
 10 purified by prep-HPLC(column: Waters Xbridge Prep OBD C18 150\*30 5 $\mu$ ; A: water (0.225% formic acid) B: acetonitrile) to the give compound E3 (21.70 mg, 40.28  $\mu\text{mol}$ , 78.6% yield) as a white solid. HRMS (ESI): calcd for  $\text{C}_{33}\text{H}_{38}\text{N}_4\text{O}_3$   $[\text{M} + \text{H}]^+$  539.29, found 539.30.  $^1\text{H}$  NMR: (400MHz,  $\text{MeOD-d}_4$ )  $\delta$  7.60(d,  $J$  = 4.8 Hz, 2H), 7.49-7.46 (m, 4H), 7.38-7.36 (m, 2H), 7.23-7.24 (d,  $J$  = 8.8 Hz, 2H), 6.87 (d,  $J$  = 8.8 Hz, 2H), 3.76 (s, 3H), 3.55-3.52  
 15 (m, 2H), 3.48-3.342 (m, 6H), 3.34-3.31 (m, 1H), 3.19 (s, 1H), 2.89 (s, 6H), 2.62 (s, 1H), 2.04 (m, 2H).

**Example 4:** (3Z,8R,9S,10S)-10-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide ("E4")





E4

**Intermediate 24:** (3Z,8R,9R,10S)-9-(4-bromophenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]dec-3-ene.

To a solution of (8R,9R,10S,Z)-10-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-9-(4-bromophenyl)-6-((2-nitrophenyl)sulfonyl)-1,6-diazabicyclo[6.2.0]dec-3-ene and benzenethiol (132.09 mg, 1.20 mmol, 122.31  $\mu$ L) in acetonitrile (6 mL) was added  $\text{Cs}_2\text{CO}_3$  (260.41 mg, 799.25  $\mu$ mol) at 25°C. After stirring at 50°C for 12 h, the reaction mixture was quenched with  $\text{H}_2\text{O}$  (5 mL) and extracted with DCM (10 mL) to give the organic phase. The organic phase was concentrated and purified by prep-TLC (petroleum ether: ethyl acetate=1:1) to Intermediate 24 (450 mg, 795.70  $\mu$ mol, 99.56% yield) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ -d1)  $\delta$ 7.26-7.16 (m, 19H), 5.90-5.84 (m, 1H), 5.67-5.60 (m, 1H), 3.61-3.53 (m, 4H), 3.61-3.52 (m, 4H), 3.11-3.09 (m, 2H), 2.86-2.84 (m, 2H), 2.48 (d,  $J$  = 5.2 Hz, 1H).

**Intermediate 25:** (3Z,8R,9R,10S)-9-(4-bromophenyl)-N-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide.

To a solution of Intermediate 24 (400 mg, 707.29  $\mu$ mol) and  $\text{Et}_3\text{N}$  (196.08  $\mu$ L, 1.41 mmol) in DCM (2 mL) was added 1-isocyanato-4-methoxy-benzene (116.04 mg, 778.02  $\mu$ mol) dropwise at 0°C. After stirring at 25°C for 5 h, the reaction mixture was concentrated to give the residue. The residue was purified prep-TLC (petroleum ether: ethyl acetate=1:1) to give Intermediate 25 (400 mg, 559.68  $\mu$ mol, 79.13% yield) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ -d1)  $\delta$ 7.31-7.19 (m, 19H), 6.85-6.81 (m, 2H), 6.04 (s, 1H), 5.81-5.80 (m, 1H), 5.73-5.68 (m, 1H), 4.15 (d,  $J$  = 16 Hz, 1H), 5.72 (d,  $J$  = 16 Hz, 1H), 4.15-4.00 (m, 1H), 3.99-3.70 (m, 1H), 3.66-3.56 (m, 5H), 3.21-3.19 (m, 2H), 3.19-3.00 (m, 1H), 2.81-2.77 (m, 1H).

**Intermediate 26:** (3Z,8R,9R,10S)-9-(4-bromophenyl)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide.

To a solution of Intermediate 25 (360.00 mg, 503.71  $\mu$ mol) in DCM (1 mL) was added TFA (3 mL, 5037  $\mu$ mol) dropwise at 0°C. After stirring at 25°C for 1 h, the reaction mixture was

5 quenched with saturated  $\text{NaHCO}_3$  (3 mL) and extracted with DCM (5 mL) to give the organic phase. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , concentrated and purified pre-TLC (petroleum ether: ethyl acetate = 1:2) to give Intermediate 26 (210 mg, 444.57  $\mu\text{mol}$ , 88.26% yield) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$  7.48 (d,  $J$  = 8.4 Hz, 2H), 7.38 (d,  $J$  = 8.4 Hz, 2H), 7.21 (d,  $J$  = 9.6 Hz, 2H), 6.83 (d,  $J$  = 8.8 Hz, 2H), 6.06 (s, 2H), 5.85  
10 (s, 1H), 5.74-5.71 (m, 1H), 4.26 (m, 1H), 4.03-3.95 (m, 1H), 3.77-3.59 (m, 6H), 3.28 (m, 1H), 2.90 (s, 1H).

**Intermediate 27:** (3Z,8R,9S,10S)-9-(4-bromophenyl)-10-[(1,3-dioxoisindolin-2-yl)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide.

To a solution of Intermediate 26 (210.00 mg, 444.57  $\mu\text{mol}$ ), isoindoline-1,3-dione  
15 (71.95 mg, 489.03  $\mu\text{mol}$ ) and  $\text{PPh}_3$  (233.21 mg, 889.13  $\mu\text{mol}$ ) in THF (2 mL) was added DIAD (179.79 mg, 889.13  $\mu\text{mol}$ ) dropwise at 25°C. After stirring at 25°C for 5 h, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (petroleum ether: ethyl acetate = 1:1) to give Intermediate 27 (200 mg, 332.51  $\mu\text{mol}$ , 74.79% yield) as a light brown solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$  7.80-7.67 (m, 17H), 7.54-7.67  
20 (m, 22H), 7.25-7.20 (m, 2H), 6.80 (s, 2H), 6.06 (s, 2H), 5.85 (s, 1H), 5.74 (s, 1H), 4.22 (s, 1H) 4.02 (m, 1H), 3.77-3.75 (m, 4H), 3.70-3.66 (m, 3H), 3.50 (s, 3H), 3.12 (m, 1H), 2.88 (s, 1H).

**Intermediate 28:** (3Z,8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide.

25 To a solution of Intermediate 27 (60.00 mg, 99.75  $\mu\text{mol}$ ) in MeOH (1mL) was added 2-aminoethanol (60.33  $\mu\text{L}$ , 997.52  $\mu\text{mol}$ ) dropwise. After stirring at 25°C for 3 h, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol = 12:1) to give Intermediate 28 (33.00 mg, 70.01  $\mu\text{mol}$ , 70.18% yield) as a white solid  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$  7.50 (d,  $J$  = 13.6 Hz, 2H), 7.46 (m,  
30 2H), 7.21 (d,  $J$  = 5.5 Hz, 2H), 6.82 (d,  $J$  = 9.6 Hz, 1H), 6.08 (s, 1H), 5.83 (s, 1H), 5.73(s, 1H), 4.22 (m, 2H), 4.02-4.00 (m, 1H), 3.77(s, 3H), 3.71-3.63 (m, 2H), 3.55-3.50 (m, 1H), 3.36-3.17 (m, 1H), 2.81-2.76 (m, 3H).

**Intermediate 29:** (3Z,8R,9S,10S)-9-(4-bromophenyl)-10-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide.

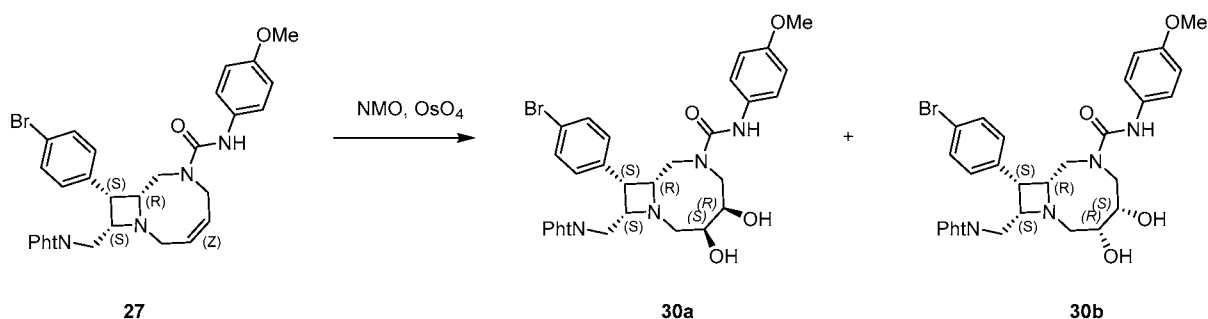
35 To a stirred solution of Intermediate 28 (23.00 mg, 48.79  $\mu\text{mol}$ ) in DCM (2 mL) was added  $\text{NaBH}(\text{OAc})_3$  (51.70 mg, 243.96  $\mu\text{mol}$ ) at 25°C. After stirring at 25°C for 2 h, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC

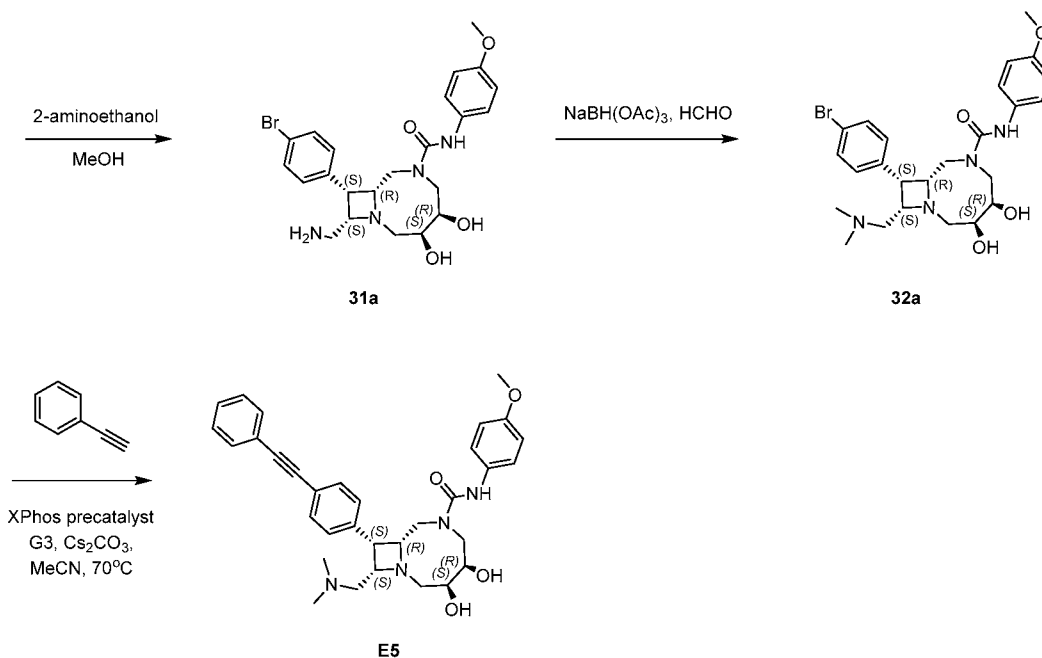
5 (dichloromethane: methanol=12:1) to give Intermediate 29 (20 mg, 40.04  $\mu$ mol, 82% yield) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$  7.53 (d,  $J$  = 8.4 Hz, 2H), 7.31-7.26 (m, 2H), 7.19 (d,  $J$  = 8.8 Hz, 3H), 6.82 (d,  $J$  = 8.8 Hz, 3H), 6.14 (s, 1H), 5.83-5.86 (m, 2H), 4.12-4.02 (m, 3H), 3.90-3.59 (m, 9H), 3.19 (m, 1H), 3.01-2.80 (m, 2H), 2.38 (s, 6H).

**Synthesis of E4:** (3Z,8R,9S,10S)-10-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide.

To a solution of Intermediate 29 (20.00 mg, 40.04  $\mu$ mol) and ethynylbenzene (12.27 mg, 120.13  $\mu$ mol, 13.19  $\mu$ L) in acetonitrile (1 mL) was added XPhos Pd G3 (3.39 mg, 4.00  $\mu$ mol) and  $\text{Cs}_2\text{CO}_3$  (26.09 mg, 80.09  $\mu$ mol). After stirring at 70°C for 2 hr, the reaction mixture was concentrated to give the residue. The residue was purified by prep-HPLC  
 15 (0.04% HCl/acetonitrile/ $\text{H}_2\text{O}$  system) to give (3Z,8R,9S,10S)-10-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide (2.00 mg, 3.84  $\mu$ mol, 9.59% yield) as a white solid. HRMS (ESI) calcd for  $\text{C}_{32}\text{H}_{33}\text{N}_4\text{O}$   $[\text{M} + \text{H}]^+$  489.27, found 520.10  $^1\text{H}$  NMR (400MHz,  $\text{MeOD-d}_4$ )  $\delta$  7.74 (d,  $J$  = 5.5 Hz, 2H), 7.67 (d,  $J$  = 8.8 Hz, 2H), 7.53 (d,  $J$  = 3.6 Hz, 2H), 7.20 (d,  $J$  = 8.8 Hz, 2H), 7.18 (d,  $J$  = 8.8 Hz, 2H), 6.13 (d,  $J$  = 5.6 Hz, 1H), 5.78 (d,  $J$  = 12.4 Hz, 1H), 4.33 (s, 2H), 4.30-4.21 (m, 9H), 3.90-3.75 (m, 5H), 3.50-3.31 (m, 3H), 2.77 (s, 6H).

**Example 5:** (3S,4R,8R,9S,10S)-10-((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E5")





5

**Intermediate 30a and 30b:** (3*S*,4*R*,8*R*,9*S*,10*S*)-9-(4-bromophenyl)-10-((1,3-dioxoisindolin-2-yl)methyl)-3,4-dihydroxy-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (30a) and (3*R*,4*S*,8*R*,9*S*,10*S*)-9-(4-bromophenyl)-10-((1,3-dioxoisindolin-2-yl)methyl)-3,4-dihydroxy-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (30b).

10

To a stirred solution of Intermediate 27 (60 mg, 99.75  $\mu$ mol) in acetone/H<sub>2</sub>O=10:1 (1 mL) was added NMO (17.53 mg, 149.62  $\mu$ mol) and OsO<sub>4</sub> (2.54 mg, 9.98  $\mu$ mol) at room temperature. After stirring at 25°C for 2 h, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (petroleum ether: ethyl acetate=1:5) to give

Intermediate 30a (less polar) (25 mg, 39.34  $\mu$ mol, 39.44% yield) as a light brown solid and Intermediate 30b (more polar) (25 mg, 39.34  $\mu$ mol, 39.44% yield) as a light brown solid.

15

Intermediate 30a <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>)  $\delta$ 7.82 (d, *J* = 2.8 Hz, 2H), 7.81 (d, *J* = 2.0 Hz, 2H), 7.71-7.02 (m, 4H), 7.26 (m, 2H), 6.80 (d, *J* = 1.2 Hz, 2H), 4.24-4.07 (m, 2H), 3.93-3.81 (m, 2H), 3.74(s, 3H), 3.62-3.54 (m, 3H), 3.28-2.88 (m, 1H), 2.65-2.49 (m, 2H).

20

Intermediate 30b <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>)  $\delta$ 7.83 (d, *J* = 2.8 Hz, 2H), 7.81 (d, *J* = 2.0 Hz, 2H), 7.73-7.47 (m, 4H), 7.24 (m, 2H), 6.80 (d, *J* = 8.8 Hz, 2H), 6.58 (s, 1H), 3.96-3.90 (m, 2H), 3.70-3.68 (m, 4H), 3.56-3.50 (m, 3H), 3.00 (s, 1H), 2.87-2.76 (m, 2H).

**Intermediate 31a:** (3*S*,4*R*,8*R*,9*S*,10*S*)-10-(aminomethyl)-9-(4-bromophenyl)-3,4-dihydroxy-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

25

To a stirred solution of Intermediate 30a (120 mg, 188.83  $\mu$ mol) in MeOH (2 mL) was added 2-aminoethanol (115.34 mg, 1.89 mmol). After stirring at 25°C for 2 h, the reaction mixture

5 was concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol=8:1) to give Intermediate 31a (70 mg, 138.50  $\mu$ mol, 73.35% yield) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.47-7.43 (m, 3H), 7.42-7.40 (m, 1H), 7.35-7.20 (m, 3H), 7.18-6.79 (m, 2H), 4.31-4.27 (m, 1H), 3.89 (s, 1H), 3.89-3.79 (m, 5H), 3.76-3.23 (m, 2H), 2.91-2.86 (m, 2H), 2.72-2.61 (m, 2H).

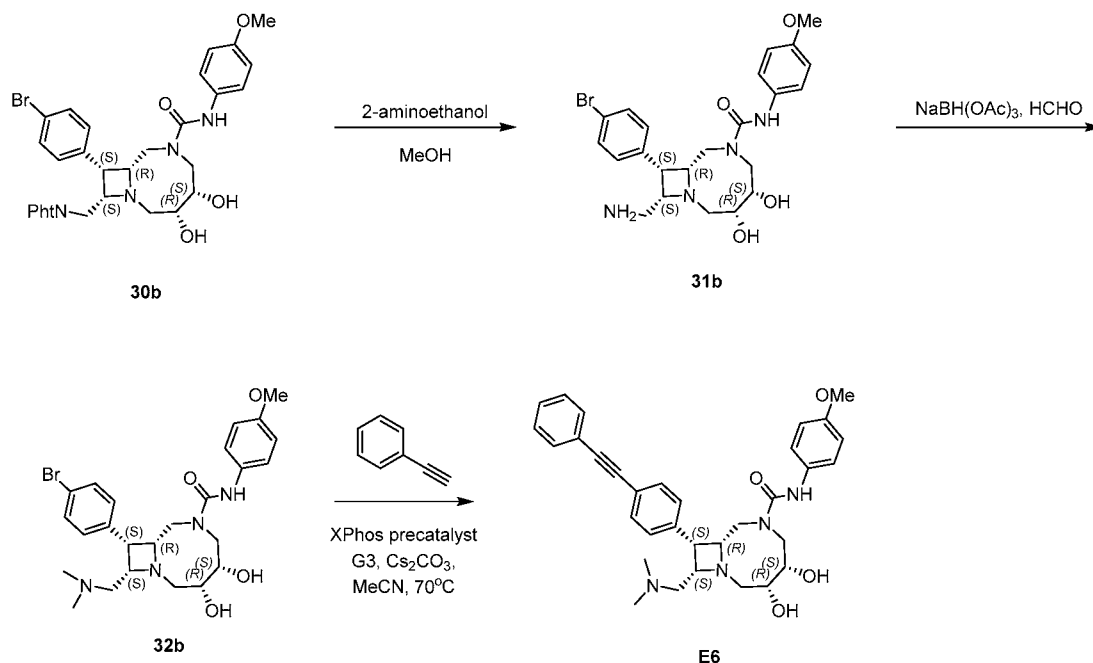
10 **Intermediate 32a:** (3S,4R,8R,9S,10S)-9-(4-bromophenyl)-10-((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a stirred solution of 31a (70.00 mg, 138.50  $\mu$ mol) in DCM (2 mL) was added HCHO (112.41 mg, 1.39 mmol, 103.13  $\mu$ L, 37% solution) and  $\text{MgSO}_4$  (166.72 mg, 1.39 mmol). After stirring at 25°C for 0.5 h, additional  $\text{NaBH}(\text{OAc})_3$  (146.77 mg, 692.52  $\mu$ mol)  
15 was added. After an additional 1.5 h, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol=5:1) to give Intermediate 32a (60 mg, 112.47  $\mu$ mol, 81% yield) as light brown solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.48-7.43 (m, 4H), 7.42-7.40 (m, 1H), 7.27-7.20 (m, 2H), 6.81-6.78 (m, 2H), 4.26-4.03 (m, 3H), 3.64-3.54 (m, 5H), 3.27-3.24 (m, 1H).

20 **Synthesis of E5:** (3S,4R,8R,9S,10S)-10-((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 32a (20.00 mg, 40.04  $\mu$ mol) and ethynylbenzene (12.27 mg, 120.13  $\mu$ mol, 13.19  $\mu$ L) in acetonitrile (1 mL) was added XPhos Pd G3 (3.39 mg, 4.00  $\mu$ mol, 0.10 eq) and  $\text{Cs}_2\text{CO}_3$  (26.09 mg, 80.09  $\mu$ mol). After stirring at 70°C for 2 hr, the  
25 reaction mixture was concentrated to give the residue. The residue was purified by prep-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5 $\mu$ ; A: water (0.225% formic acid) B: acetonitrile) to give (3S,4R,8R,9S,10S)-10-((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (2.00 mg, 3.84  $\mu$ mol, 9.59% yield) as a white solid. HRMS (ESI) calcd for  $\text{C}_{33}\text{H}_{38}\text{N}_4\text{O}_4$   $[\text{M} + \text{H}]^+$  554.29, found 554.20  $^1\text{H}$  NMR (400MHz,  $\text{MeOD-d}_4$ )  $\delta$ 7.59 (s, 4H), 7.51 (d,  $J$  = 3.6 Hz, 2H), 7.38 (s, 3H), 7.16 (d,  $J$  = 8.8 Hz, 2H), 6.85 (d,  $J$  = 8.8 Hz, 2H), 4.20-4.11 (s, 3H), 3.85-3.82 (m, 4H), 3.75 (s, 3H), 3.57-3.44 (m, 3H), 3.12-2.97 (m, 4H), 2.97-2.56 (m, 6H).

- 5 **Example 6:** (3R,4S,8R,9S,10S)-10-((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E6")



- 10 **Intermediate 31b:** (3R,4S,8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

- To a stirred solution of Intermediate 30b (30 mg, 47.21  $\mu\text{mol}$ ) in MeOH (2 mL) was added 2-aminoethanol (28.84 mg, 472.10  $\mu\text{mol}$ ) at 25°C. After stirring at 25°C for 2 h, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol=8:1) to give Intermediate 31b (20 mg, 39.57  $\mu\text{mol}$ , 83.82% yield) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.51-7.48 (m, 3H), 7.46-7.43 (m, 4H), 6.83 (d,  $J$  = 8.8 Hz, 2H), 7.77-3.73 (m, 3H), 3.65-3.51 (m, 2H), 3.23-3.15 (m, 1H), 2.80-2.73 (m, 3H).

- Intermediate 32b:** (3R,4S,8R,9S,10S)-9-(4-bromophenyl)-10-((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

- 20 To a stirred solution of Intermediate 31a (10 mg, 19.79  $\mu\text{mol}$ ) in DCM (2 mL) was added HCHO (16.06 mg, 197.90  $\mu\text{mol}$ , 14.73  $\mu\text{L}$ , 37% solution) and  $\text{MgSO}_4$  (23.82 mg, 197.90  $\mu\text{mol}$ ) at 25°C. After stirring at 25°C for 0.5 h, additional  $\text{NaBH}(\text{OAc})_3$  (20.97 mg, 98.95  $\mu\text{mol}$ ) was added. After stirring for at 25°C for 1.5 h additionally, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol=5:1) to give Intermediate 32b (10 mg, 18.75  $\mu\text{mol}$ , 47.36% yield) as light brown solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.54-7.46 (m, 4H), 7.30-7.25

- 5 (m, 7H), 6.83-6.80 (d,  $J = 8.8$  Hz, 2H), 3.89-3.78 (m, 2H), 3.67-3.63 (m, 6H), 3.56-3.48 (m, 3H), 2.74-2.64 (m, 3H), 2.28 (s, 6H).

**Synthesis of E6:** (3R,4S,8R,9S,10S)-10-((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

- To a solution of Intermediate 32b (10 mg, 18.75  $\mu$ mol) and ethynylbenzene (5.74 mg, 56.24  $\mu$ mol, 6.18  $\mu$ L) in acetonitrile (1 mL) was added XPhos Pd G3 (1.59 mg, 1.87  $\mu$ mol) and Cs<sub>2</sub>CO<sub>3</sub> (12.22 mg, 37.49  $\mu$ mol). After stirring at 70°C for 2 hr, the reaction mixture was concentrated to give the residue. The residue was purified by prep-HPLC (0.04%

HCl/ACN/H<sub>2</sub>O system) to give (3R,4S,8R,9S,10S)-10-((dimethylamino)methyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-

- 15 diazabicyclo[6.2.0]decane-6-carboxamide (2 mg, 3.61  $\mu$ mol, 19.23% yield) as a white solid. HRMS (ESI) calcd for C<sub>33</sub>H<sub>38</sub>N<sub>4</sub>O<sub>4</sub> [M + H]<sup>+</sup> 554.29, found 554.2. <sup>1</sup>H NMR (400MHz, MeOD-d<sub>4</sub>)  $\delta$  7.62 (s, 4H), 7.53-7.51 (m, 2H), 7.40 (d,  $J = 4.8$  Hz, 3H), 7.24 (d,  $J = 4.8$  Hz, 2H), 6.87 (d,  $J = 8.8$  Hz, 2H), 4.18 (s, 3H), 4.08 (s, 1H), 3.94-3.93 (m, 3H), 3.76 (s, 4H), 3.56-3.52 (m, 2H), 3.42-3.40 (m, 2H), 3.23-3.21 (m, 2H), 2.99-2.95 (m, 1H), 3.70 (s, 6H).

## 20 Examples 7-9

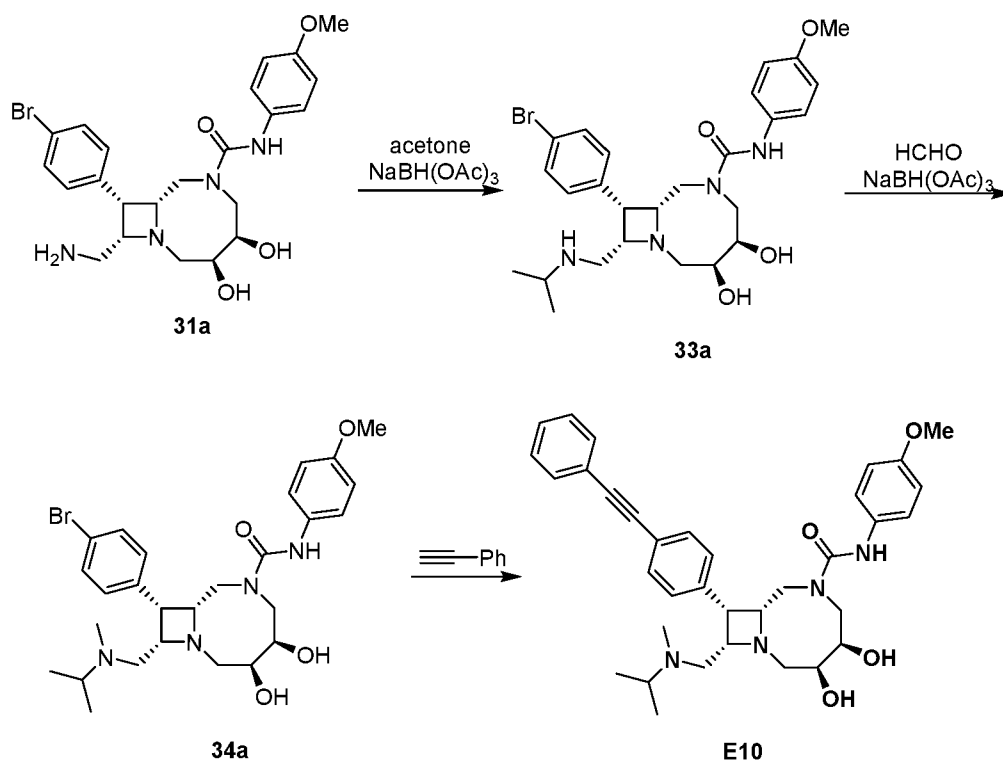
The following example compounds (E7-E9) were prepared according to example 6 with the listed alkynes and the specified aldehyde (where indicated) in Table 3 starting with either Intermediate ("Int.") 32a or 32b.

Table 3

|                                                                                                                                                                                               | Alkyne                    | Aldehyde     | Int. | NMR                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|--------------|------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Example 7</b><br>(3S,4R,8R,9S,10S)-10-[(dimethylamino)methyl]-9-[4-[2-(2-fluorophenyl)ethynyl]phenyl]-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E7") | 1-ethynyl-2-fluorobenzene | formaldehyde | 32a  | <sup>1</sup> H NMR (400MHz, CHLOROFORM-d) $\delta$ = 8.65 - 8.29 (m, 1H), 7.58 - 7.49 (m, 3H), 7.43 (d, $J=8.0$ Hz, 2H), 7.38 - 7.32 (m, 1H), 7.27 (d, $J=9.0$ Hz, 2H), 7.21 - 7.08 (m, 2H), 6.83 (d, $J=9.0$ Hz, 2H), 4.31 (d, $J=11.0$ Hz, 2H), 4.14 (br. s., 1H), 4.02 (d, $J=13.1$ Hz, 1H), 3.85 - 3.69 (m, 5H), 3.61 (br. s., 1H), 3.54 (d, $J=8.0$ Hz, 1H), 3.23 (d, $J=16.1$ Hz, 1H), 2.99 (br. s., 1H), 2.82 - 2.48 (m, 4H), 2.11 (br. s., 6H) |
| <b>Example 8</b>                                                                                                                                                                              | 1-ethynyl-2-              | formaldehyde | 32b  | <sup>1</sup> H NMR (400MHz,                                                                                                                                                                                                                                                                                                                                                                                                                            |

|                                                                                                                                                                                                  |                |              |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|--------------|-----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>(3R,4S,8R,9S,10S)-10-[(dimethylamino)methyl]-9-[4-[2-(2-fluorophenyl)ethynyl]phenyl]-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (“E8”)</b>                 | fluorobenzene  |              |     | CHLOROFORM-d) $\delta$ = 8.52 (br. s., 1H), 7.61 - 7.52 (m, 3H), 7.40 (d, $J$ =8.0 Hz, 2H), 7.37 - 7.30 (m, 2H), 7.21 - 7.10 (m, 2H), 6.84 (d, $J$ =9.0 Hz, 2H), 3.89 (d, $J$ =5.0 Hz, 2H), 3.79 (s, 6H), 3.73 - 3.64 (m, 3H), 3.63 - 3.50 (m, 2H), 3.24 (dd, $J$ =6.0, 14.1 Hz, 1H), 2.90 (br. s., 1H), 2.78 (d, $J$ =14.1 Hz, 1H), 2.67 (d, $J$ =10.0 Hz, 1H), 2.49 (dd, $J$ =7.0, 13.1 Hz, 1H), 2.16 (s, 6H)                                                                                                                                                                                   |
| <b>Example 9</b><br><b>(3S,4R,8R,9S,10S)-10-(diethylaminomethyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (“E9”) BIMH2W60</b> | ethynylbenzene | acetaldehyde | 32a | <sup>1</sup> H NMR (400MHz, CHLOROFORM-d) $\delta$ = 8.61 (br s, 1H), 8.46 (br s, 1H), 7.55 (br d, $J$ =3.5 Hz, 2H), 7.52 - 7.47 (m, 1H), 7.50 (br d, $J$ =8.2 Hz, 1H), 7.44 - 7.33 (m, 5H), 7.24 (br d, $J$ =8.8 Hz, 2H), 6.81 (br d, $J$ =8.8 Hz, 2H), 5.14 (br s, 2H), 4.34 - 4.21 (m, 1H), 4.12 (br s, 1H), 3.96 (br d, $J$ =14.1 Hz, 1H), 3.81 (br d, $J$ =7.9 Hz, 1H), 3.75 (s, 4H), 3.55 (br t, $J$ =7.1 Hz, 1H), 3.48 (br d, $J$ =9.0 Hz, 1H), 3.21 (br d, $J$ =15.7 Hz, 1H), 2.93 - 2.71 (m, 4H), 2.60 (br dd, $J$ =7.7, 12.6 Hz, 3H), 2.52 - 2.38 (m, 2H), 0.85 (br t, $J$ =6.7 Hz, 6H) |

- 5 **Example 10:** (3S,4R,8R,9S,10S)-3,4-dihydroxy-10-[[isopropyl(methyl)amino]methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E10")



- 10 **Intermediate 33a:** (3S,4R,8R,9S,10S)-9-(4-bromophenyl)-3,4-dihydroxy-10-[[isopropyl(methyl)amino]methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 31a (100.00 mg, 197.86  $\mu$ mol), acetone (68.95 mg, 1.19 mmol, 87.28  $\mu$ L), AcOH (11.88 mg, 197.86  $\mu$ mol, 11.31  $\mu$ L) and MgSO<sub>4</sub> (238.16 mg, 1.98 mmol) in DCM (1.00 mL) was added NaBH(OAc)<sub>3</sub> (125.80 mg, 593.58  $\mu$ mol). After stirring at 20°C for 12 h, the reaction mixture filtered through a celite and concentrated under reduced pressure to give the crude Intermediate 33a (116.00 mg) as a pale yellow brown solid.

- 15 **Intermediate 34a:** (3S,4R,8R,9S,10S)-9-(4-bromophenyl)-3,4-dihydroxy-10-[[isopropyl(methyl)amino]methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

20 To a solution of Intermediate 33a (116.00 mg, 211.88  $\mu$ mol), formaldehyde (103.08 mg, 1.27 mmol, 94.57  $\mu$ L), MgSO<sub>4</sub> (255.04 mg, 2.12 mmol) and AcOH (12.72 mg, 211.88  $\mu$ mol, 12.11  $\mu$ L) in DCM (1.00 mL) was added NaBH(OAc)<sub>3</sub> (134.72 mg, 635.64  $\mu$ mol). After stirring at 20°C for 2 h, the reaction mixture was filtered through a celite and concentrated under reduced pressure to give the crude Intermediate 34b (138.00 mg,

5 crude) as a dark yellow solid.

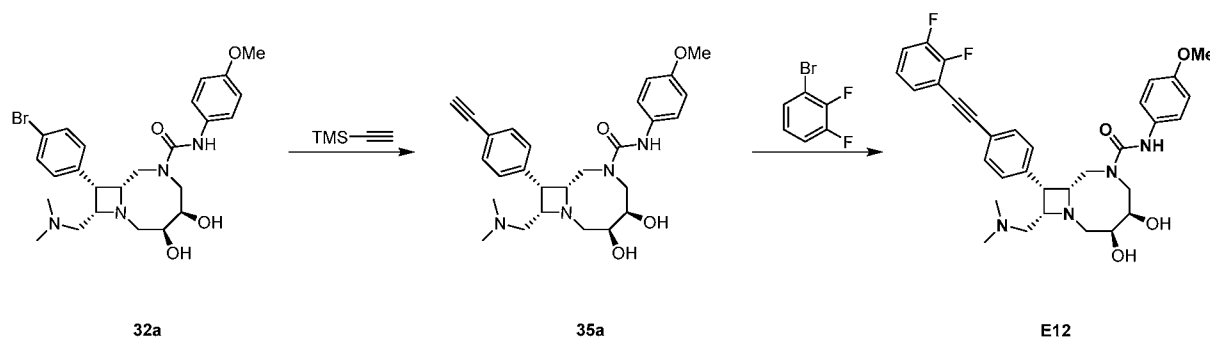
**Synthesis of E10:** (3S,4R,8R,9S,10S)-3,4-dihydroxy-10-[[isopropyl(methyl)amino]methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 34a (30.00 mg, 53.43  $\mu$ mol) and ethynylbenzene (10.91 mg, 106.86  $\mu$ mol, 11.73  $\mu$ L) in CH<sub>3</sub>CN (1.00 mL) was added Et<sub>3</sub>N (16.22 mg, 160.29  $\mu$ mol, 22.22  $\mu$ L) and XPhos Pd G3 (4.52 mg, 5.34  $\mu$ mol). After stirring at 70°C for 2 h, the reaction mixture was filtered through a celite and concentrated under reduced pressure to give a residue, which was purified by prep-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (0.225% formic acid) B: acetonitrile) to give (3S,4R,8R,9S,10S)-3,4-dihydroxy-10-[[isopropyl(methyl)amino]methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (11.20 mg, 17.81  $\mu$ mol, 33.34% yield, formic acid salt) as a light yellow solid. <sup>1</sup>H NMR (400MHz, CHLOROFORM-d)  $\delta$  = 8.65 - 8.37 (m, 1H), 7.57 - 7.52 (m, 2H), 7.49 (d, *J*=8.0 Hz, 2H), 7.42 - 7.34 (m, 5H), 7.25 (d, *J*=9.0 Hz, 2H), 6.81 (d, *J*=9.0 Hz, 2H), 4.36 - 4.21 (m, 1H), 4.13 (br. s., 1H), 3.96 (d, *J*=14.1 Hz, 1H), 3.84 - 3.73 (m, 5H), 3.62 - 3.46 (m, 2H), 3.21 (d, *J*=15.6 Hz, 1H), 2.83 (d, *J*=14.1 Hz, 4H), 2.75 - 2.54 (m, 2H), 2.11 (br. s., 3H), 0.99 (d, *J*=5.5 Hz, 3H), 0.71 (d, *J*=5.0 Hz, 3H)

**Example 11:** (3R,4S,8R,9S,10S)-3,4-dihydroxy-10-[[isopropyl(methyl)amino]methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E11")

Example E11 was prepared using the synthesis described in Example 10 beginning with Intermediate 31b. <sup>1</sup>H NMR (400MHz, CHLOROFORM-d)  $\delta$  = 8.73 - 8.47 (m, 1H), 7.56 - 7.51 (m, 4H), 7.41 - 7.34 (m, 5H), 7.28 (d, *J*=8.9 Hz, 2H), 6.81 (d, *J*=8.9 Hz, 2H), 3.88 (d, *J*=5.4 Hz, 1H), 3.80 - 3.74 (m, 5H), 3.72 - 3.48 (m, 5H), 3.24 (dd, *J*=6.8, 14.1 Hz, 1H), 2.88 (t, *J*=11.9 Hz, 1H), 2.82 - 2.75 (m, 1H), 2.72 (d, *J*=14.2 Hz, 2H), 2.65 - 2.56 (m, 1H), 2.09 (s, 3H), 0.95 (d, *J*=6.5 Hz, 3H), 0.71 (d, *J*=6.4 Hz, 3H).

- 5 **Example 12:** (3S,4R,8R,9S,10S)-9-[4-[2-(2,3-difluorophenyl)ethynyl]phenyl]-10-[(dimethylamino)methyl]-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E12")



- 10 **Intermediate 35a:** (3S,4R,8R,9S,10S)-10-[(dimethylamino)methyl]-9-(4-ethynylphenyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

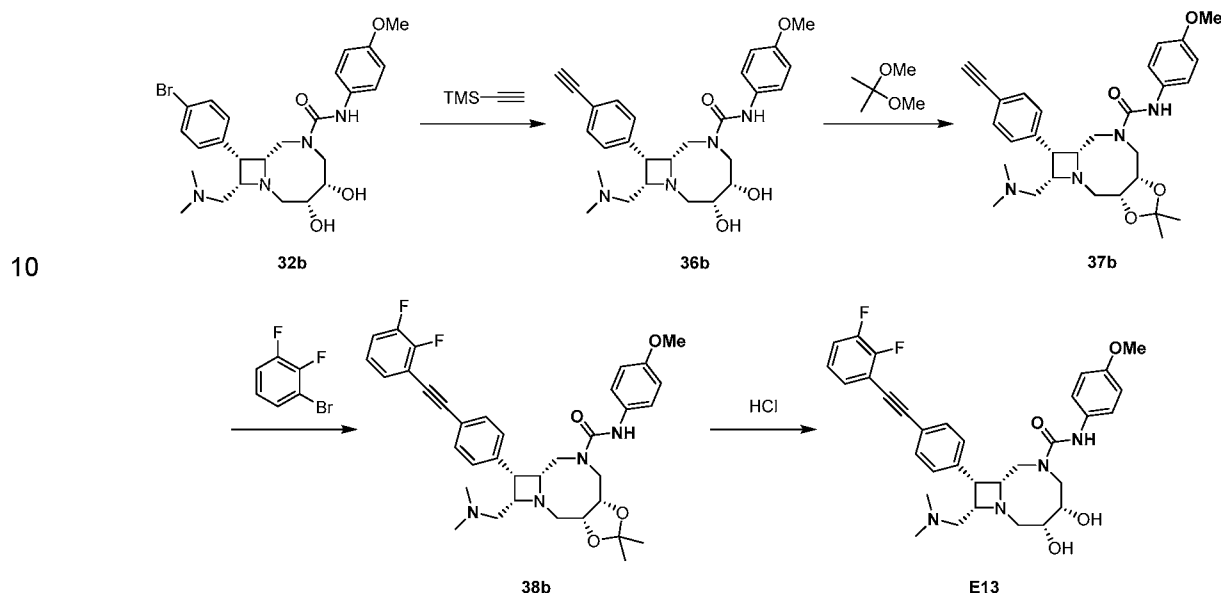
To a solution of Intermediate 32a (54.00 mg, 101.23  $\mu$ mol) and ethynyltrimethylsilane (29.83 mg, 303.69  $\mu$ mol, 42.01  $\mu$ L) in  $\text{CH}_3\text{CN}$  (1.00 mL) was added  $\text{Cs}_2\text{CO}_3$  (131.93 mg, 404.92  $\mu$ mol) and XPhos Pd G3 (8.57 mg, 10.12  $\mu$ mol). After stirring with  $\text{N}_2$  atmosphere at 70°C for two hours, the reaction mixture was filtered through a celite and concentrated under reduced pressure to give the crude product 2 (78.00 mg, crude) as a dark yellow solid.

**Synthesis of E12:** (3S,4R,8R,9S,10S)-9-[4-[2-(2,3-difluorophenyl)ethynyl]phenyl]-10-[(dimethylamino)methyl]-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of (3R,4S,8R,9S,10S)-10-[(dimethylamino)methyl]-9-(4-ethynylphenyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (Intermediate 35a) (21.60 mg, 45.13  $\mu$ mol) and 1-bromo-2,3-difluorobenzene (8.71 mg, 45.13  $\mu$ mol, 5.06  $\mu$ L) in  $\text{CH}_3\text{CN}$  (500  $\mu$ L) was added  $\text{Cs}_2\text{CO}_3$  (29.41 mg, 90.26  $\mu$ mol) and XPhos Pd G3 (3.82 mg, 4.51  $\mu$ mol). After stirring with  $\text{N}_2$  atmosphere at 40°C for 1 h, the residue was purified by prep-TLC ( $\text{SiO}_2$ , DCM: MeOH = 8:1) to remove the catalyst and then purified by prep-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (0.225% formic acid) B: acetonitrile) to give (3S,4R,8R,9S,10S)-9-[4-[2-(2,3-difluorophenyl)ethynyl]phenyl]-10-[(dimethylamino)methyl]-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (2.00 mg, 3.14  $\mu$ mol, 6.96% yield, formic acid salt)) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CHLOROFORM-d}$ )  $\delta$  = 8.48 - 8.27 (m, 1H), 7.58 (d,  $J$ =7.9 Hz, 2H), 7.40 (d,  $J$ =7.9 Hz, 2H), 7.29 (d,  $J$ =8.4 Hz, 3H), 7.22 - 7.13 (m, 1H), 7.11 - 7.03 (m, 1H), 6.83 (d,  $J$ =8.8 Hz, 2H), 6.73 (br. s., 1H), 3.89 (br. s., 2H),

5 3.77 (s, 4H), 3.75 - 3.62 (m, 3H), 3.61 - 3.48 (m, 2H), 3.28 (dd,  $J=6.6$ , 14.1 Hz, 1H), 2.89 (d,  $J=12.8$  Hz, 3H), 2.80 (d,  $J=14.6$  Hz, 2H), 2.68 (d,  $J=7.5$  Hz, 1H), 2.27 (s, 6H)

**Example 13:** (3*R*,4*S*,8*R*,9*S*,10*S*)-9-(4-((2,3-difluorophenyl)ethynyl)phenyl)-10-((dimethylamino)methyl)-3,4-dihydroxy-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E13")



**Intermediate 36b:** (3*R*,4*S*,8*R*,9*S*,10*S*)-10-((dimethylamino)methyl)-9-(4-ethynylphenyl)-3,4-dihydroxy-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of (3*R*,4*S*,8*R*,9*S*,10*S*)-9-(4-bromophenyl)-10-[(dimethylamino)methyl]-3,4-dihydroxy-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (Intermediate 32b) (40.00 mg, 74.98  $\mu$ mol) and ethynyl(trimethyl)silane (22.09 mg, 224.95  $\mu$ mol, 31.12  $\mu$ L) in CH<sub>3</sub>CN (1.00 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (97.72 mg, 299.93  $\mu$ mol) and XPhos Pd G3 (6.35 mg, 7.50  $\mu$ mol). After stirring with N<sub>2</sub> atmosphere at 70°C for 1 h, the reaction mixture was filtered through a celite and concentrated under reduced pressure to give a residue which was purified by prep-TLC (DCM: MeOH = 10:1, R<sub>f</sub> = 0.2) to give the Intermediate 36b (30.00 mg, 62.69  $\mu$ mol, 83.60% yield) as a light yellow solid.

**Intermediate 37b:** (3*aS*,6*aR*,7*S*,8*S*,10*aR*)-8-((dimethylamino)methyl)-7-(4-ethynylphenyl)-*N*-(4-methoxyphenyl)-2,2-dimethylhexahydro-3*aH*-azeto[1,2-*a*][1,3]dioxolo[4,5-*f*][1,4]diazocine-5(4*H*)-carboxamide.

To a solution of Intermediate 36b (30.00 mg, 62.69  $\mu$ mol) in 2,2-dimethoxypropane (500  $\mu$ L) and DCM (500  $\mu$ L) was added TsOH (1.08 mg, 6.27  $\mu$ mol). After stirring at 20°C for 1 h, the reaction was concentrated and purified by prep-TLC (DCM: MeOH = 10:1) to give the Intermediate 37b (20.00 mg, 38.56  $\mu$ mol, 61.51% yield) as a yellow oil.

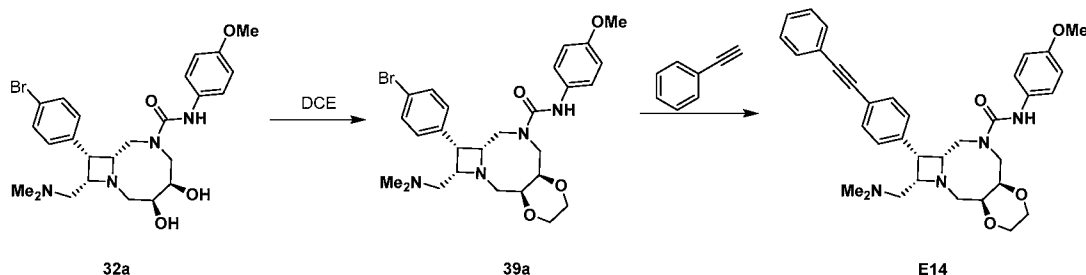
5 **Intermediate 38b:** (3*aS*,6*aR*,7*S*,8*S*,10*aR*)-7-(4-((2,3-difluorophenyl)ethynyl)phenyl)-8-((dimethylamino)methyl)-*N*-(4-methoxyphenyl)-2,2-dimethylhexahydro-3*aH*-azeto[1,2-*a*][1,3]dioxolo[4,5-*f*][1,4]diazocine-5(4*H*)-carboxamide.

To a solution of Intermediate 37b (20.00 mg, 38.56  $\mu$ mol) and 1-bromo-2,3-difluorobenzene (22.33 mg, 115.68  $\mu$ mol, 12.98  $\mu$ L) in CH<sub>3</sub>CN (500  $\mu$ L) was added Cs<sub>2</sub>CO<sub>3</sub> (50.25 mg, 154.24  $\mu$ mol) and XPhos Pd G3 (3.26 mg, 3.86  $\mu$ mol). After stirring under N<sub>2</sub> atmosphere at 70°C for one hour, the reaction mixture was purified by prep-TLC (DCM: MeOH = 10:1) to give the Intermediate 38b (15.00 mg, 23.78  $\mu$ mol, 61.68% yield) as a yellow oil.

15 **Synthesis of E13:** (3*R*,4*S*,8*R*,9*S*,10*S*)-9-(4-((2,3-difluorophenyl)ethynyl)phenyl)-10-((dimethylamino)methyl)-3,4-dihydroxy-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a mixture of Intermediate 38b (15.00 mg, 23.78  $\mu$ mol, 1.00 *eq*) in CH<sub>3</sub>CN (500.00  $\mu$ L) was added 1 M HCl (500.00  $\mu$ L). After stirring at 15°C for 12 h, the reaction mixture was concentrated under reduced pressure to give a residue which was purified by prep-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (0.225% formic acid) B: acetonitrile) to give (3*S*,4*R*,8*R*,9*S*,10*S*)-9-(4-((2,3-difluorophenyl)ethynyl)phenyl)-10-((dimethylamino)methyl)-3,4-dihydroxy-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (11.00 mg, 17.28  $\mu$ mol, 72.65% yield, formic acid salt) as a white solid. <sup>1</sup>H NMR (400MHz, METHANOL-*d*<sub>4</sub>)  $\delta$  = 8.69 - 8.45 (m, 1H), 7.61 (s, 4H), 7.40 - 7.29 (m, 2H), 7.25 - 7.13 (m, 3H), 6.85 (d, *J*=9.0 Hz, 2H), 4.22 (dd, *J*=6.0, 15.6 Hz, 1H), 4.12 (d, *J*=6.0 Hz, 1H), 3.87 - 3.80 (m, 2H), 3.80 - 3.72 (m, 5H), 3.53 - 3.46 (m, 1H), 3.36 (br. s., 1H), 3.00 - 2.88 (m, 3H), 2.84 - 2.73 (m, 2H), 2.41 (s, 6H)

30 **Example 14: (4*aR*,7*aR*,8*S*,9*S*,11*aS*)-9-((dimethylamino)methyl)-*N*-(4-methoxyphenyl)-8-(4-(phenylethynyl)phenyl)octahydro-2*H*-azeto[1,2-*a*][1,4]dioxino[2,3-*f*][1,4]diazocine-6(3*H*)-carboxamide ("E14")**



**Intermediate 39a:** (4*aR*,7*aR*,8*S*,9*S*,11*aS*)-8-(4-bromophenyl)-9-((dimethylamino)methyl)-*N*-(4-methoxyphenyl)octahydro-2*H*-azeto[1,2-*a*][1,4]dioxino[2,3-*f*][1,4]diazocine-6(3*H*)-

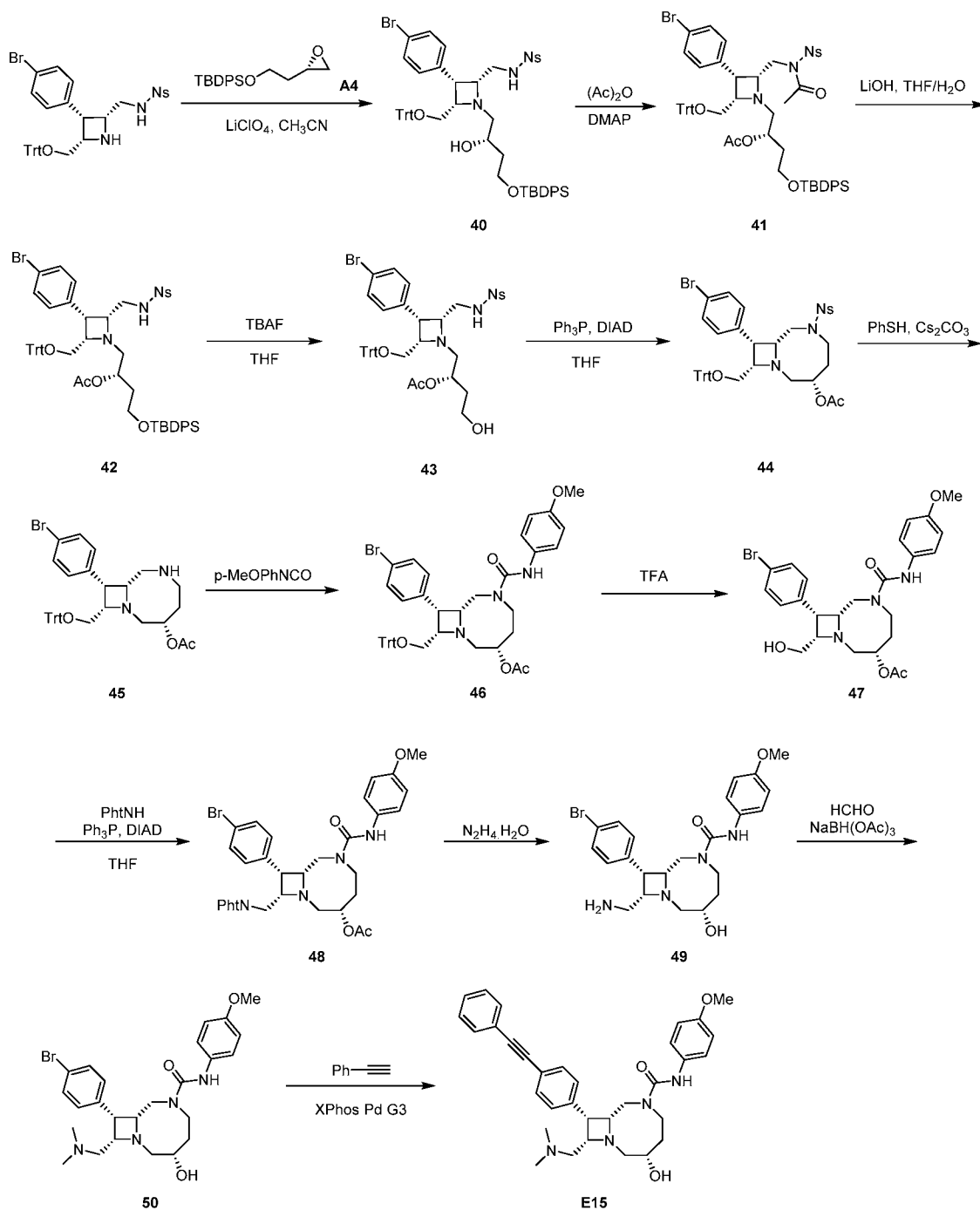
5 carboxamide.

A mixture of Intermediate 32a (30.00 mg, 56.24  $\mu$ mol), NaOH (15.00 mg, 112.48  $\mu$ mol, 180.30  $\mu$ L, 30% m/v) and TBAB (3.63 mg, 11.25  $\mu$ mol) in DCE (1.00 mL) was stirred at 55°C for 12 h. LC-MS showed ~40% of Reactant 1 was remained. One new peak with desired Intermediate MS was detected. The reaction mixture was diluted with DCM 10 mL and washed with H<sub>2</sub>O 15 mL \* 5. Then washed with brine 3 mL \* 2, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue which was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10: 1) to give the Intermediate 39 (10.00 mg, 17.87  $\mu$ mol, 31.78% yield) as a colorless oil.

**Synthesis of E14:** (4a*R*,7a*R*,8*S*,9*S*,11a*S*)-9-((dimethylamino)methyl)-*N*-(4-methoxyphenyl)-8-(4-(phenylethynyl)phenyl)octahydro-2*H*-azeto[1,2-*a*][1,4]dioxino[2,3-*f*][1,4]diazocine-6(3*H*)-carboxamide.

To a solution of Intermediate 39a (20.00 mg, 35.75  $\mu$ mol) and ethynylbenzene (10.95 mg, 107.25  $\mu$ mol, 11.77  $\mu$ L) in CH<sub>3</sub>CN (500  $\mu$ L) was added Cs<sub>2</sub>CO<sub>3</sub> (46.59 mg, 143.00  $\mu$ mol) and XPhos Pd G3 (3.03 mg, 3.58  $\mu$ mol). After stirring under N<sub>2</sub> atmosphere at 70°C for 1 hour under N<sub>2</sub> atmosphere, the reaction mixture was concentrated, purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1) to remove the catalyst and then purified by pre-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5 $\mu$ ; A: water (0.225% formic acid) B: acetonitrile) to give (4a*R*,7a*R*,8*S*,9*S*,11a*S*)-9-((dimethylamino)methyl)-*N*-(4-methoxyphenyl)-8-(4-(phenylethynyl)phenyl)octahydro-2*H*-azeto[1,2-*a*][1,4]dioxino[2,3-*f*][1,4]diazocine-6(3*H*)-carboxamide (formic acid salt), 6.00 mg, 10.33  $\mu$ mol, 28.90% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CHLOROFORM-*d*)  $\delta$  = 8.26 (br s, 1H), 7.55 (dd, *J*=2.0, 7.0 Hz, 2H), 7.51 - 7.42 (m, 4H), 7.40 - 7.33 (m, 3H), 7.20 (d, *J*=9.0 Hz, 2H), 6.83 (d, *J*=9.0 Hz, 2H), 4.21 (br d, *J*=15.6 Hz, 1H), 4.10 (br d, *J*=14.1 Hz, 1H), 4.06 - 3.94 (m, 3H), 3.86 - 3.76 (m, 5H), 3.66 (br s, 1H), 3.59 - 3.52 (m, 2H), 3.48 - 3.29 (m, 3H), 2.89 (br d, *J*=13.1 Hz, 1H), 2.60 - 2.33 (m, 3H), 2.03 (s, 6H), 1.83 (br s, 1H).

5 **Example 15:** (3S,8R, 9S,10S)-10-[(dimethylamino)methyl]-3-hydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E15")



10 **Intermediate 40:** N-[[[(2R,3R,4S)-3-(4-bromophenyl)-1-[(2S)-4-[tert-butyl(diphenyl)silyl]oxy-2-hydroxy-butyl]-4-(trityloxymethyl)azetidin-2-yl]methyl]-2-nitro-benzenesulfonamide.

To a solution of N-[[[(2R,3R,4S)-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-2-yl]methyl]-2-nitro-benzenesulfonamide (400.00 mg, 572.56 μmol) and LiClO<sub>4</sub> (121.83 mg,

5 1.15 mmol, 50.34  $\mu$ L) in  $\text{CH}_3\text{CN}$  (4.00 mL) was added tert-butyl-[2-[(2R)-oxiran-2-yl]ethoxy]-diphenyl-silane (Organic Letters (2016), 18(3), 468-471) (373.88 mg, 1.15 mmol). After stirring at 80°C for 16 h, the reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with  $\text{H}_2\text{O}$  (20 mL) and extracted with DCM (3 x 20 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over  
10  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give a residue which was purified by prep-TLC ( $\text{SiO}_2$ , petroleum ether/ethyl acetate=2/1) to give the Intermediate 40 (250.00 mg, 243.87  $\mu$ mol, 42.59% yield) as a light yellow solid.

**Intermediate 41:** [(1S)-1-[[[(2R,3R,4S)-2-[[acetyl-(2-nitrophenyl)sulfonyl-amino]methyl]-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-1-yl]methyl]-3-[tert-butyl(diphenyl)silyl]oxy-propyl] acetate.  
15

To a solution of N-[[[(2R,3R,4S)-3-(4-bromophenyl)-1-[(2S)-4-[tert-butyl(diphenyl)silyl]oxy-2-hydroxy-butyl]-4-(trityloxymethyl)azetidin-2-yl]methyl]-2-nitrobenzenesulfonamide (Intermediate 40) (250.00 mg, 243.87  $\mu$ mol),  $\text{Et}_3\text{N}$  (24.68 mg, 243.87  $\mu$ mol, 33.81  $\mu$ L) and DMAP (2.98 mg, 24.39  $\mu$ mol) in DCM (3.00 mL) was added  $\text{Ac}_2\text{O}$   
20 (62.24 mg, 609.68  $\mu$ mol, 57.10  $\mu$ L). After stirring at 15°C for 16 h, the reaction mixture was quenched by addition  $\text{H}_2\text{O}$  (20 mL), and then extracted with DCM (3 x 15 mL). The combined organic layers were washed with brine (10 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give a residue which was purified by prep-TLC ( $\text{SiO}_2$ , petroleum ether/ethyl acetate=3/1, plate 2) to give Intermediate 41 (270.00 mg, 243.42  $\mu$ mol,  
25 99.81% yield) as a white solid.

**Intermediate 42:** [(1S)-1-[[[(2R,3R,4S)-3-(4-bromophenyl)-2-[[2-nitrophenyl)sulfonylamino]methyl]-4-(trityloxymethyl)azetidin-1-yl]methyl]-3-[tert-butyl(diphenyl)silyl]oxy-propyl] acetate.

To a solution of [(1S)-1-[[[(2R,3R,4S)-2-[[acetyl-(2-nitrophenyl)sulfonyl-amino]methyl]-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-1-yl]methyl]-3-[tert-butyl(diphenyl)silyl]oxy-propyl] acetate (Intermediate 41) (270.00 mg, 243.42  $\mu$ mol) in THF  
30 (2 mL) and  $\text{H}_2\text{O}$  (2 mL) was added  $\text{LiOH}\cdot\text{H}_2\text{O}$  (20.43 mg, 486.84  $\mu$ mol). After stirring at 15°C for 1 h, the reaction mixture was diluted with  $\text{H}_2\text{O}$  (30 mL) and extracted with DCM (20 mL\* 4). The combined organic layers were washed with brine (20 mL\* 3), dried over  
35  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give a residue which was purified by prep-TLC ( $\text{SiO}_2$ , petroleum ether/ethyl acetate=3/1, plate 1) to give Intermediate 42 (200.00 mg, 187.41  $\mu$ mol, 76.99% yield) as a white solid.

5 **Intermediate 43:** [(1S)-1-[[[(2R,3R,4S)-3-(4-bromophenyl)-2-[(2-nitrophenyl)sulfonylamino]methyl]-4-(trityloxymethyl)azetidin-1-yl]methyl]-3-hydroxy-propyl] acetate.

To a solution of [(1S)-1-[[[(2R,3R,4S)-3-(4-bromophenyl)-2-[(2-nitrophenyl)sulfonylamino]methyl]-4-(trityloxymethyl)azetidin-1-yl]methyl]-3-[tert-butyl(diphenyl)silyl]oxy-propyl] acetate (Intermediate 42) (200.00 mg, 187.41  $\mu$ mol) in THF  
10 (3 mL) was added TBAF (98.00 mg, 374.82  $\mu$ mol). After stirring at 15°C for 2 h, the reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with DCM (30 mL) and washed with H<sub>2</sub>O (15 mL \* 5), then brine (10 mL \* 3), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue which was  
15 purified by prep-TLC (SiO<sub>2</sub>, petroleum ether/ethyl acetate=1/1, plate 1) to give Intermediate 43 (150.00 mg, 180.99  $\mu$ mol, 96.57% yield) as a white solid.

**Intermediate 44:** [(3S,8R,9R,10S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate.

A mixture of PPh<sub>3</sub> (94.94 mg, 361.98  $\mu$ mol, 2.00 *eq*) and DIAD (73.20 mg, 361.98  
20  $\mu$ mol, 70.38  $\mu$ L, 2.00 *eq*) in THF (2 mL) was stirred at 0°C under N<sub>2</sub> atmosphere to give the milky mixture. To this mixture was added a solution of [(1S)-1-[[[(2R,3R,4S)-3-(4-bromophenyl)-2-[(2-nitrophenyl)sulfonylamino]methyl]-4-(trityloxymethyl)azetidin-1-yl]methyl]-3-hydroxy-propyl] acetate (Intermediate 43) (150.00 mg, 180.99  $\mu$ mol) in THF (1 mL). After stirring at 15°C for 16 h, the reaction mixture was concentrated under reduced  
25 pressure to remove solvent. The residue was diluted with DCM (30 mL) washed with H<sub>2</sub>O (10 mL \* 2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue which was purified by prep-TLC (SiO<sub>2</sub>, Petroleum ether/Ethyl acetate=1/1, plate 1) to give Intermediate 44 (150.00 mg, crude) as a white solid.

30 **Intermediate 45:** [(3S,8R,9R,10S)-9-(4-bromophenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate.

To a solution of [(3S,8R,9R,10S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate (Intermediate 44) (150 mg, 185.01  $\mu$ mol) in CH<sub>3</sub>CN (3 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (120.56 mg, 370.02  $\mu$ mol) and benzenethiol (30.58 mg, 277.52  $\mu$ mol, 28.31  $\mu$ L). After stirring at 40°C for 2 h, the reaction  
35 mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with H<sub>2</sub>O (30 mL) and extracted with DCM (20 mL \* 5). The combined organic layers were

5 washed with brine (20 mL \* 3), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue which was purified by prep-TLC (SiO<sub>2</sub>, Petroleum ether/Ethyl acetate=1/1, plate 1) to give the Intermediate 45 (180.00 mg, crude) as a yellow solid.

**Intermediate 46:** [(3S,8R,9R,10S)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate.

10 To a solution of [(3S,8R,9R,10S)-9-(4-bromophenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate (Intermediate 45) (180 mg, 287.73 μmol) in DCM (3 mL) was added 1-isocyanato-4-methoxybenzene (42.91 mg, 287.73 μmol, 36.99 μL). After stirring at 15°C for 1 h, the reaction mixture was quenched by addition H<sub>2</sub>O (15 mL), and then extracted with DCM (20 mL \* 4). The combined organic layers were washed with  
15 NaHCO<sub>3</sub> solution (15 mL), brine (15 mL\*2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue which was purified by prep-TLC (SiO<sub>2</sub>, petroleum ether/ethyl acetate=1/1, plate 1) to give the Intermediate 46 (200 mg, 258.15 μmol, 89.72% yield) as a white solid.

**Intermediate 47:** [(3S, 8R,9R,10S)-9-(4-bromophenyl)-10-(hydroxymethyl)-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate.

20 To a solution of [(3S, 8R,9R,10S)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate (Intermediate 46) (200 mg, 258.15 μmol, 1.00 eq) in DCM (3 mL) was added TFA (294.34 mg, 2.58 mmol, 191.13 μL). After stirring at 15°C for 2 h., the reaction mixture was  
25 quenched by addition NaHCO<sub>3</sub> solution (20 mL), and extracted with DCM (20 mL \* 5). The combined organic layers were washed with brine (10 mL\* 3), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue which was purified by prep-TLC (SiO<sub>2</sub>, petroleum ether/ethyl acetate=1/1, plate 1) to give the Intermediate 47 (130.00 mg, 244.16 μmol, 94.58% yield) as a white solid.

30 **Intermediate 48:** [(3S, 8R,9S,10S)-9-(4-bromophenyl)-10-[(1,3-dioxoisindolin-2-yl)methyl]-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate.

A mixture of PPh<sub>3</sub> (128.08 mg, 488.32 μmol, 2.00 eq) and DIAD (98.74 mg, 488.32 μmol, 94.94 μL, 2.00 eq) in THF (1 mL) was stirred at 0°C under N<sub>2</sub> atmosphere to give a milky mixture. To this milky mixture was added to the solution of (3S,8R,9R,10S)-9-(4-bromophenyl)-10-(hydroxymethyl)-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate (Intermediate 47) (130 mg, 244.16 μmol) and isoindoline-1,3-dione (53.88 mg, 366.24 μmol) in THF (1 mL) at 0°C. After  
35

5 stirring at 15°C for 12 h, the reaction mixture was concentrated under reduced pressure to remove solvent and then purified by prep-TLC (SiO<sub>2</sub>, petroleum ether/ethyl acetate=1/1, plate 1) to give Intermediate 48 (200 mg, crude) as a yellow oil.

**Intermediate 49:** (3S, 8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-3-hydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

10 To a solution of [(3S, 8R,9S,10S)-9-(4-bromophenyl)-10-[(1,3-dioxoisindolin-2-yl)methyl]-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate (Intermediate 48) (200 mg, 302.32 µmol) in EtOH (2 mL) was added N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O (29.39 µL, 604.64 µmol). After stirring at 70°C for 2 h, the reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with H<sub>2</sub>O (10 mL) and extracted  
15 with DCM (10 mL \* 5). The combined organic layers were washed with brine (10 mL \* 2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue which was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1, plate 1) to give the Intermediate 49 (60 mg, 122.60 µmol, 40.55% yield) as a white solid.

**Intermediate 50:** (3S,8R,9S,10S)-9-(4-bromophenyl)-10-[(dimethylamino)methyl]-3-hydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of (3S, 8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-3-hydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (Intermediate 49) (60 mg, 122.60 µmol), formaldehyde (54.77 µL, 735.60 µmol), MgSO<sub>4</sub> (147.57 mg, 1.23 mmol) and AcOH (0.70 µL, 12.26 µmol) in DCM (3 mL) was added NaBH(OAc)<sub>3</sub> (77.95 mg, 367.80  
25 µmol). After stirring at 15°C for 2 h, the reaction mixture was filtered through a celite and washed with NaHCO<sub>3</sub> solution (10 mL \* 3). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give Intermediate 50 (50 mg, 96.63 µmol, 78.81% yield) as a white solid.

**Synthesis of E15:** (3S,8R,9S,10S)-10-[(dimethylamino)methyl]-3-hydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

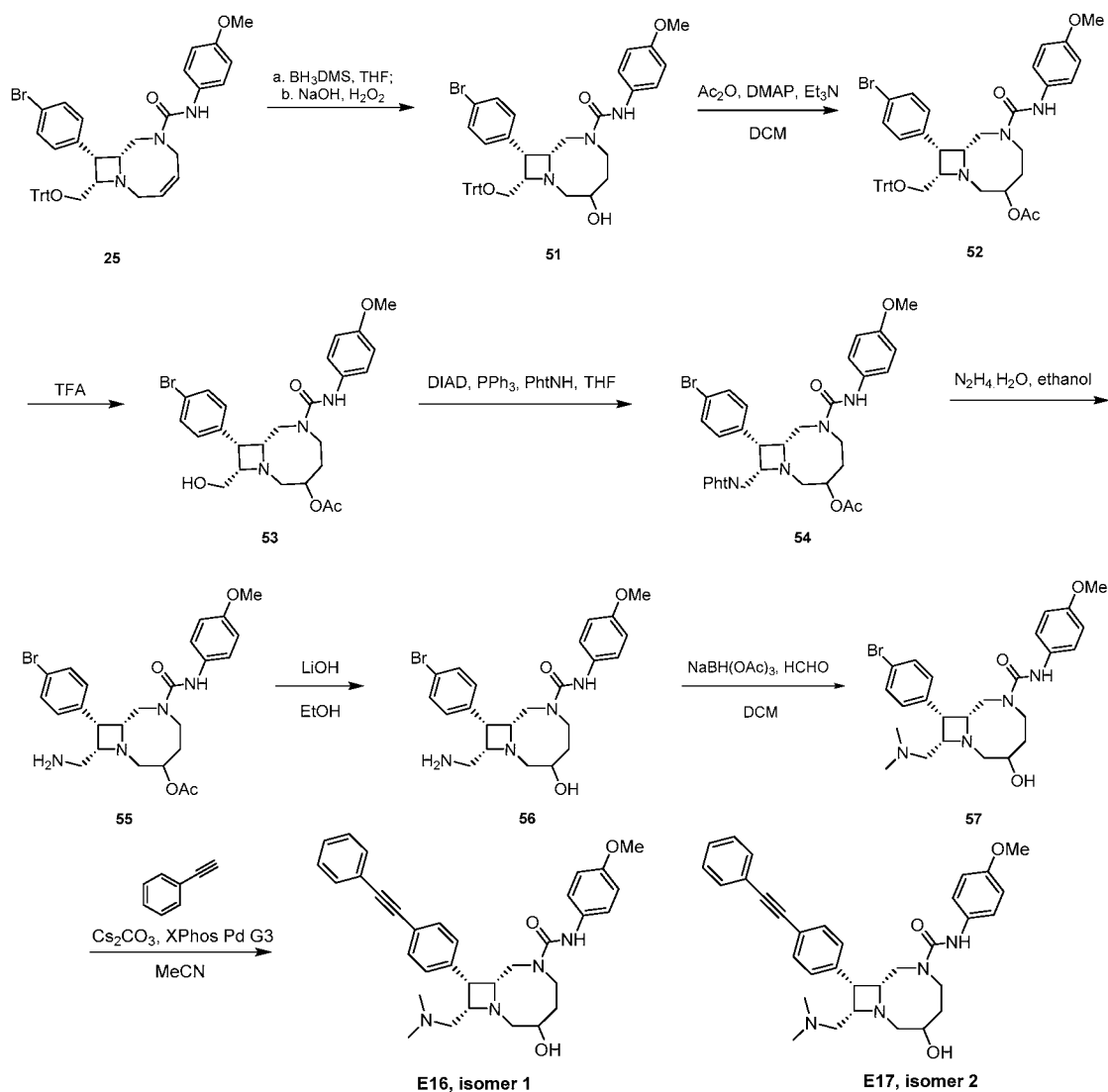
To a solution of (3S, 8R,9S,10S)-9-(4-bromophenyl)-10-[(dimethylamino)methyl]-3-hydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (Intermediate 50) (50 mg, 96.63 µmol) and ethynylbenzene (29.61 mg, 289.89 µmol, 31.84 µL) in CH<sub>3</sub>CN  
35 (1 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (125.94 mg, 386.52 µmol) and XPhos Pd G3 catalyst (8.18 mg, 9.66 µmol). After stirring under N<sub>2</sub> atmosphere at 70°C for 1 h, the reaction mixture was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1, plate 1) to remove the catalyst giving the

5 crude product which was further purified by prep-HPLC (prep-HPLC (column: Luna C18 150\*25 5u; mobile phase: A: water (0.225% formic acid) B: acetonitrile) to give the product phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (10 mg, 17.10  $\mu$ mol, 17.7% yield, formic acid salt)) as a white solid.  $^1\text{H}$  NMR (400MHz, CHLOROFORM- $d$ )  $\delta$  = 8.44 (br s, 1H), 7.54 (br d,  $J$ =7.0 Hz, 4H), 7.44 - 7.34 (m, 5H), 7.29 - 7.24 (m, 2H), 6.83 (br d,  $J$ =8.8 Hz, 2H), 6.29 (s, 1H), 5.71 (br s, 1H), 3.87 (br s, 2H), 3.77 (s, 3H), 3.75 - 3.62 (m, 4H), 3.52 - 3.38 (m, 1H), 3.18 (br dd,  $J$ =6.1, 13.6 Hz, 1H), 2.93 - 2.79 (m, 1H), 2.77 - 2.66 (m, 2H), 2.66 - 2.58 (m, 1H), 2.19 (s, 6H), 2.08 - 1.97 (m, 1H), 1.96 - 1.85 (m, 1H)

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**Examples 16-17: Isomers of (8R,9S,10S)-10-[(dimethylamino)methyl]-3-hydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-**

15 **carboxamide**



5 **Intermediate 51:** (8R,9S,10S)-10-trityloxymethyl-3-hydroxy-N-(4-methoxyphenyl)-9-[4-bromophenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of (3Z,8R,9R,10S)-9-(4-bromophenyl)-N-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide (Intermediate 25, 980.00 mg, 1.37 mmol, 1.00 eq) in THF (1.00 mL) was added  $\text{BH}_3\text{-Me}_2\text{S}$  (10 M, 686  $\mu\text{L}$ , 5.00 eq) at 10  $0^\circ\text{C}$  and the reaction mixture was stirred at  $25^\circ\text{C}$  for 3 h.  $\text{H}_2\text{O}$  (1.24 g, 68.56 mmol, 1.24 mL, 50.00 eq) was added slowly and the reaction mixture was stirred at  $25^\circ\text{C}$  for 30 min. Then to the reaction solution was added  $\text{NaOH}/\text{H}_2\text{O}$  (18.27 g, 68.50 mmol, 15% purity, 50.00 eq) and  $\text{H}_2\text{O}_2$  (6.66 g, 68.56 mmol, 5.65 mL, 35% purity, 50.00 eq). The resulting reaction mixture was stirred at  $25^\circ\text{C}$  for 3 h. The reaction was quenched with  $\text{H}_2\text{O}$  (10 mL) and extracted with 15 DCM (50 mL). The combined organic layers were dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give a crude product as a white solid (mixtures), which was used into next step without further purification.

**Intermediate 52:** [(8R,9R,10S)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl] acetate.

20 To a solution of Intermediate 51 (1.2 g, 1.64 mmol),  $\text{Et}_3\text{N}$  (497.85 mg, 4.92 mmol) and DMAP (20.04 mg, 164  $\mu\text{mol}$ ) in DCM (2 mL) at  $0^\circ\text{C}$  was added  $\text{Ac}_2\text{O}$  (334.86 mg, 3.28 mmol). After stirring at  $25^\circ\text{C}$  for 3 h, the reaction was quenched with  $\text{H}_2\text{O}$  (10 mL) and extracted with DCM (20 mL) to give the organic layer. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give the residue. The residue was purified by prep- 25 TLC (petroleum ether: ethyl acetate = 1:1) to give two isomers of [(8R,9R,10S)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl] acetate. Each isomer was separated by prep-TLC to produce Isomer 1 (126 mg, 9.92% yield,  $R_f = 0.5$ ) as a white solid and Isomer 2 (197 mg, 15.50% yield,  $R_f = 0.4$ ) as a white solid and mixed fractions [(8R,9R,10S)-9-(4-bromophenyl)-6-[(4-methoxy phenyl)carbamoyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl] acetate 30 (624 mg, 49.11% yield,  $R_f = 0.3$ ) as a white solid. Although each stereoisomer was separated individually, the stereochemistry of the 4 position for each compound could not be determined.

Isomer 1:  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$  8.25 (s, 1H), 7.47 (d,  $J = 8.8$  Hz, 2H), 7.19-7.11 (m, 23H), 6.77 (d,  $J = 8.8$  Hz, 2H), 4.62 (s, 1H), 3.92-3.88 (m, 1H), 3.74-3.70 (m, 1H), 3.56-3.55 (m, 4H), 3.45-3.41 (m, 1H), 3.30-3.29 (m, 1H), 3.08-3.07 (m, 3H), 2.89-2.87 (m, 1H), 2.73-2.70 (m, 1H), 2.55-2.52 (m, 1H), 2.43 (s, 1H), 2.06 (s, 3H), 1.92-1.88 (m, 2H), 1.12-

5 1.1.10 (m, 3H), 0.9-0.87 (m, 3H).

Isomer 2: <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.34-7.22 (m, 23H), 7.09 (d, *J* = 8 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 5.07-5.04 (m, 1H), 3.92-3.88 (m, 1H), 3.77 (m, 5H), 3.65-3.58 (m, 3H), 3.25-3.27 (m, 2H), 3.03-3.00 (m, 1H), 3.02-2.99 (m, 1H), 2.61-2.57 (m, 1H), 2.45-2.44 (m, 1H), 2.16 (s, 3H), 2.08-2.05 (m, 1H), 1.74-1.71 (m, 2H), 1.00-0.95 (m, 1H).

10 Mixed Fractions <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.52-7.17 (m, 21H), 6.84-6.82 (m, 3H), 4.81 (s, 1H), 4.29-4.16 (m, 1H), 3.99-3.77 (m, 1H), 3.67-3.66 (m, 1H), 3.65-3.63 (m, 6H), 3.63-3.56 (m, 5H), 3.40-3.39 (m, 3H), 3.16-3.12 (m, 2H), 2.96-2.94 (m, 1H), 2.63-.59 (m, 1H), 2.28-2.25 (m, 1H), 1.97 (s, 3H), 1.89-1.86 (m, 1H), 1.28-1.25 (m, 2H), 0.88-0.84 (m, 1).

**Intermediate 53:** (8R,9R,10S)-9-(4-bromophenyl)-10-(hydroxymethyl)-6-((4-

15 methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl acetate.

To a solution of [(8R,9R,10S)-9-(4-bromophenyl)-6-[(4-methoxy phenyl)carbamoyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl] acetate (624 mg, 805.43 μmol) in DCM (2 mL) was added TFA (918.35 mg, 8.05 mmol, 596.33 μL). After stirring at 25°C for 3 h, the reaction mixture was quenched by NaHCO<sub>3</sub> (10 mL) and extracted with DCM (20  
20 mL) to give the organic layer. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue and purified by prep-TLC (petroleum ether: ethyl acetate = 1:1) to Intermediate 53 (265 mg, 61.80% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.46 (d, *J* = 8 Hz, 2H), 7.38 (d, *J* = 8 Hz, 2H), 7.26 (d, *J* = 8 Hz, 2H), 6.83 (d, *J* = 8 Hz, 2H), 6.47 (s, 1H), 4.80 (s, 1H), 3.81-3.77 (m, 4H), 3.64-3.57 (m, 5H), 3.52-3.47 (m, 3H),  
25 3.25-3.22 (m, 1H), 3.21 (s, 1H), 2.97-2.84 (m, 1H), 2.23-2.22 (m, 2H), 2.24 (s, 2H), 2.15-1.96 (m, 2H).

**Intermediate 54:** [(8R,9S,10S)-9-(4-bromophenyl)-10-[(1,3-dioxoisindolin-2-yl)methyl]-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate.

To a solution of Intermediate 53 (275 mg, 516.5 μmol), isoindoline-1,3-dione (83.59  
30 mg, 568.15 μmol) and PPh<sub>3</sub> (203.21 mg, 774.75 μmol) in THF (2 mL) was added DIAD (156.66 mg, 774.75 μmol) dropwise at 25°C. After stirring at 25°C for 5 h, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (petroleum ether: ethyl acetate = 1:1) to give Intermediate 54 (460 mg, crude) as a light yellow solid. <sup>1</sup>H NMR (4400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.79 (s, 2H), 7.70-7.65 (m, 20H), 7.55-7.53  
35 (m, 20H), 7.48-7.45 (m, 30H), 6.86 (m, 3H), 6.62 (s, 1H), 4.76 (s, 2H), 3.86-3.83 (m, 6H), 3.79-7.77 (m, 3H), 3.65-3.64 (m, 3H), 3.54-3.49 (m, 1H), 3.09-3.04 (m, 1H), 2.85-2.81 (m, 1H), 2.56-2.53 (m, 1H), 3.37-3.14 (m, 1H), 2.21-2.14 (m, 1H).

5 **Intermediate 55:** [(8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl] acetate.

To a solution of Intermediate 54 (460 mg, 695.35  $\mu$ mol) in EtOH (1 mL) was added hydrazine hydrate (53.28 mg, 1.04 mmol, 51.73  $\mu$ L) dropwise. After stirring at 80°C for 2 h, the reaction mixture was filtered and concentrated to give Intermediate 55 (500 mg, crude) as a brown liquid without purification and will be used directly in next step. HRMS (ESI): calcd for C<sub>24</sub>H<sub>28</sub>BrN<sub>4</sub>O<sub>3</sub> [M + H]<sup>+</sup> 531.15, found 531.1

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**Intermediate 56:** (8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-3-hydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 55 (300 mg, 564.50  $\mu$ mol) in EtOH : H<sub>2</sub>O = 2:1 (1 mL) was added LiOH.H<sub>2</sub>O (47.37 mg, 1.13 mmol). After stirring at 40°C for 2 h, the reaction mixture was diluted with H<sub>2</sub>O (3 mL) and extracted with DCM (10 mL) to give organic layer. The organic layer was concentrated and purified by pre-TLC (dichloromethane: methanol=10:1) to give Intermediate 56 (90 mg, 32.58% yield) as a white solid. <sup>1</sup>H NMR (4400MHz, CDCl<sub>3</sub>-d<sub>1</sub>)  $\delta$ 7.52-7.47 (m, 2H), 7.34-7.32 (m, 18H), 7.27-7.22 (m, 3H), 6.84 (d, *J* = 9.2 Hz, 2H), 6.17 (s, 1H), 3.87-3.84 (m, 1H), 3.78-3.74 (m, 3H), 3.70-3.66 (m, 3H), 3.57-3.55 (m, 2H), 3.13-3.10 (m, 1H), 2.77-2.71 (m, 1H), 2.04-1.88 (m, 5H).

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**Intermediate 57:** (8R,9S,10S)-9-(4-bromophenyl)-10-[(dimethylamino)methyl]-3-hydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a stirred solution of Intermediate 56 (90 mg, 183.9  $\mu$ mol) in DCM (2 mL) was added HCHO (149.26 mg, 1.84 mmol, 136.93  $\mu$ L, 37% solution) and MgSO<sub>4</sub> (442.72 mg, 3.68 mmol). After stirring at 25°C for 0.5 h, NaBH(OAc)<sub>3</sub> (194.88 mg, 919.49  $\mu$ mol) was added. After stirring at 25°C for 1.5 h, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol= 10: 1) to give Intermediate 57 (58 mg, 60.95% yield) as light brown solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>)  $\delta$ 7.51-7.44 (m, 2H), 7.30-7.23 (m, 5H), 6.85 (d, *J* = 8.8 Hz, 2H), 6.15 (s, 1H), 3.89-3.87 (m, 2H), 3.83-3.78 (m, 3H), 3.73-3.67 (m, 4H), 3.20-3.15 (m, 1H), 2.72-2.69 (m, 2H), 2.60-2.51 (m, 2H), 2.14 (s, 5H), 2.05-2.026 (m, 4H), 2.00-1.91 (m, 1H).

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**Synthesis of E16 and E17:** (8R,9S,10S)-10-[(dimethylamino)methyl]-3-hydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide isomer 1 (E16) and isomer 2 (E17).

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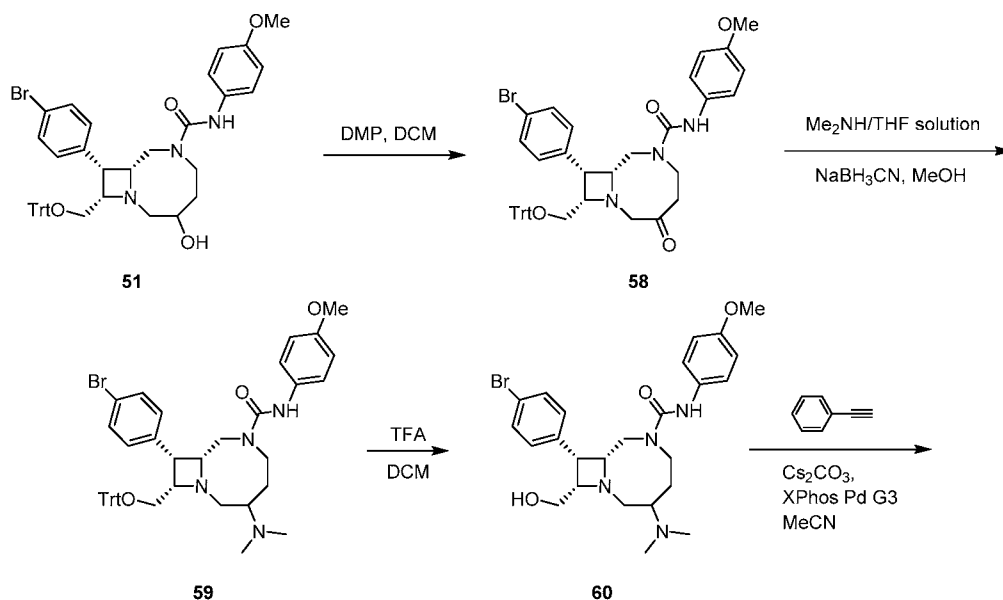
To a solution of Intermediate 57 (50 mg, 96.63  $\mu$ mol) and ethynylbenzene (29.61 mg, 289.89  $\mu$ mol) in Acetonitrile (1 mL) was added XPhos Pd G3 (8.18 mg, 9.66  $\mu$ mol) and

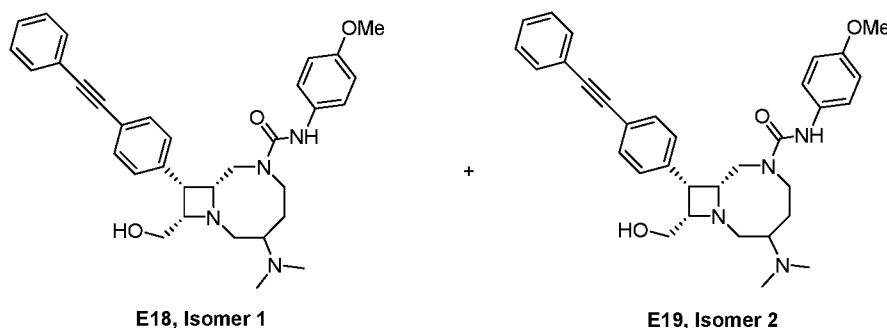
5 Cs<sub>2</sub>CO<sub>3</sub> (62.97 mg, 193.26 μmol). After stirring at 70°C for 2 h, the reaction mixture was concentrated to give the residue. The residue was purified by pre-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5μ; A: water (0.225% formic acid) B: acetonitrile) to give each stereoisomer of (8R,9S,10S)-10-[(dimethylamino)methyl]-3-hydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide. Each isomer was separated to produce Isomer 1 (5.1 mg, 9.80% yield) and Isomer 2 (17.1 mg, 32.85% yield) as a white solid.

E16: Isomer 1: HRMS (ESI): calcd for C<sub>32</sub>H<sub>35</sub>N<sub>4</sub>O<sub>2</sub> [M + H]<sup>+</sup> 539.29, found 529. <sup>1</sup>H NMR (400MHz, MeOD-d<sub>4</sub>) δ7.60-7.50 (m, 6H), 7.39-7.37 (m, 3H), 7.36-7.18 (m, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 3.83-3.80 (m, 2H), 3.76 (s, 3H), 3.69-3.68 (m, 2H), 3.55-3.52 (m, 3H), 3.15-3.09 (m, 2H), 3.01-3.00 (m, 2H), 3.41-3.34 (m, 2H), 2.26 (m, 6H), 1.75-1.72 (m, 1H).

E17: Isomer 2: HRMS (ESI): calcd for C<sub>32</sub>H<sub>35</sub>N<sub>4</sub>O<sub>2</sub> [M + H]<sup>+</sup> 539.29, found 529.40 <sup>1</sup>H NMR (400MHz, MeOD-d<sub>4</sub>) δ7.61-7.54 (m, 6H), 7.50 (d, *J* = 1.6 Hz, 3H), 7.38-7.36 (m, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 3.84-3.80 (m, 3H), 3.75 (s, 3H), 3.67-3.61 (m, 2H), 3.55-3.51 (m, 1H), 3.08-3.05 (m, 2H), 2.83-2.80 (m, 1H), 2.74-2.68 (m, 3H), 2.26 (s, 6H), 2.03-1.94 (m, 2H).

20 **Examples 18-19:** (8R,9R,10S)-4-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide isomer 1 ("E18") and isomer 2 ("E19")





**Intermediate 58:** (8R,9R,10S)-9-(4-bromophenyl)-N-(4-methoxyphenyl)-3-oxo-10-((trityloxy)methyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 51 (500.00 mg, 682.41  $\mu\text{mol}$ , 1 eq) in DCM (5 mL) was added Dess-Martin (347.32 mg, 818.89  $\mu\text{mol}$ ). After stirring at 25°C for 3 h, the reaction mixture was quenched by saturated  $\text{Na}_2\text{SO}_3$  (5 mL) and extracted with DCM (10 mL) to give the organic layer. The organic layer was concentrated and prep-TLC (petroleum ether: ethyl acetate = 1:1) to give Intermediate 58 (280 mg, 56.15% yield) as a brown solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.37-7.19 (m, 30H), 6.86-6.82 (m, 2H), 6.20 (s, 1H), 4.27-4.20 (m, 3H), 4.07-3.81 (m, 1H), 3.79-3.77 (m, 3H), 3.70-3.66 (m, 3H), 3.54-3.31 (m, 1H), 3.06-3.04 (m, 1H), 2.98-2.94 (m, 2H), 2.76-2.73 (m, 1H), 2.72-2.45 (m, 1H).

**Intermediate 59:** (8R,9R,10S)-9-(4-bromophenyl)-3-(dimethylamino)-N-(4-methoxyphenyl)-10-((trityloxy)methyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a stirred solution of Intermediate 58 (250 mg, 342.14  $\mu\text{mol}$ ) in MeOH (2 mL) was added N-methylmethanamine (2 M, 1.71 mL) and  $\text{CH}_3\text{COOH}$  (205.46 mg, 3.42 mmol). After stirring at 25°C for 0.5 h,  $\text{NaBH}_3\text{CN}$  (21.50 mg, 342.14  $\mu\text{mol}$ ) was added. After stirring at 25°C for 1.5 h, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol = 15 : 1) to give Intermediate 59 (120 mg, 46.16% yield) as light brown solid. HRMS (ESI) calcd for  $\text{C}_{32}\text{H}_{33}\text{N}_4\text{O}$   $[\text{M} + \text{H}]^+$  759.28, found 759.2.

**Intermediate 60:** (8R,9R,10S)-9-(4-bromophenyl)-3-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a stirred solution of Intermediate 59 (115 mg, 151.36  $\mu\text{mol}$ ) in DCM (1 mL) was added TFA (172.58 mg, 1.51 mmol). After stirring at 25°C for 12 h, the reaction mixture was quenched with saturated  $\text{NaHCO}_3$  (5 mL) and extracted with DCM (5 mL) to give organic layer. The organic solution was collected, concentrated and then purified by prep-TLC (dichloromethane: methanol = 10: 1) to give Intermediate 60 (45.00 mg, 57.45% yield) as light brown solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.47-7.43 (m, 2H), 7.38-7.31 (m, 4H),

5 6.83 (d,  $J = 8.8$  Hz, 2H), 3.99 (s, 1H), 3.77 (s, 5H), 3.63-3.61 (m, 5H), 3.59-3.57 (m, 5H), 3.48-3.46 (m, 2H), 3.32-3.13 (m, 3H), 2.76 (s, 6H), 2.48-2.34 (m, 4H), 2.04-2.00 (m, 1H).

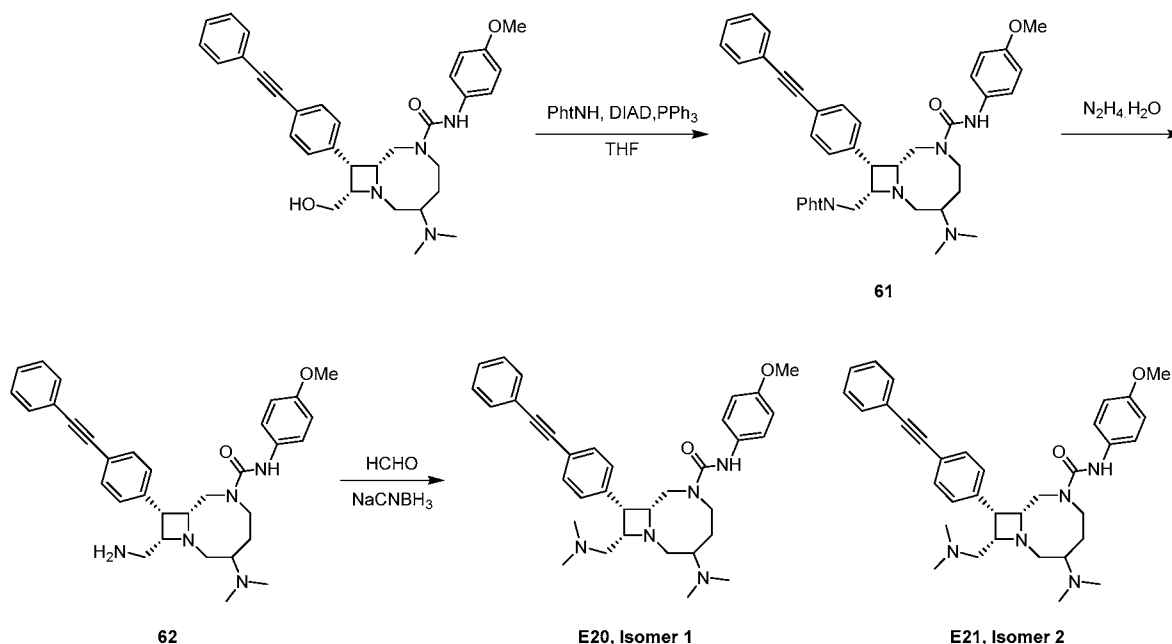
**Synthesis of E18 and E19:** (8R,9R,10S)-4-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide isomer 1 (E18) and isomer 2 (E19)

10 To a solution of Intermediate 59 (30 mg, 57.98  $\mu$ mol) and ethynylbenzene (17.76 mg, 173.94  $\mu$ mol) in acetonitrile (1 mL) was added XPhos Pd G3 (4.91 mg, 5.80  $\mu$ mol) and  $\text{Cs}_2\text{CO}_3$  (37.78 mg, 115.96  $\mu$ mol). After stirring at 70°C for 2 h, the reaction mixture was concentrated to give a residue. The residue was purified by pre-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5 $\mu$ ; A: water (0.225% formic acid) B: acetonitrile) to the  
15 give each stereoisomer of (8R,9R,10S)-4-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide separated as isomer 1 (E18) (2.60 mg, 8.32% yield) and isomer 2 (E19) (11.40 mg, 36.50% yield) as a white solid.

E18: Isomer 1, HRMS (ESI) calcd for:  $\text{C}_{33}\text{H}_{38}\text{N}_4\text{O}_3$   $[\text{M} + \text{H}]^+$  539.29, found 539.4  $^1\text{H}$  NMR (400MHz,  $\text{MeOD}-d_4$ )  $\delta$  8.34 (s, 1H), 7.56-7.51 (m, 2H), 7.50-7.48 (m, 4H), 7.37 (d,  $J = 5.2$  Hz, 2H), 7.26 (d,  $J = 9.6$  Hz, 2H), 6.85 (d,  $J = 8.8$  Hz, 1H), 4.09-4.05 (m, 1H), 3.75-3.72 (m, 3H), 3.66-3.65 (m, 1H), 3.52-3.46 (m, 4H), 3.26-3.20 (m, 2H), 2.64 (s, 6H), 2.55-2.52 (m, 1H), 1.97 (s, 2H).

E19: Isomer 2, HRMS (ESI) calcd for:  $\text{C}_{33}\text{H}_{38}\text{N}_4\text{O}_3$   $[\text{M} + \text{H}]^+$  539.29, found 539.4  $^1\text{H}$  NMR (400MHz,  $\text{MeOD}-d_4$ )  $\delta$  8.55 (s, 1H), 7.61 (d,  $J = 8$  Hz, 2H), 7.51-7.49 (m, 4H), 7.38-7.36 (m, 3H), 7.23 (d,  $J = 8.8$  Hz, 2H), 6.86 (d,  $J = 8.8$  Hz, 2H), 4.21-4.11 (m, 1H), 3.76-3.73 (m, 5H), 3.76-3.73 (m, 2H), 3.62-3.40 (m, 3H), 3.31-3.13 (m, 3H), 2.81-2.77 (s, 6H), 2.64 (s, 1H), 2.46 (s, 1H), 2.06-2.03 (m, 1H).

- 5 **Examples 20-21:** (8*R*,9*S*,10*S*)-9-(4-bromophenyl)-4-(dimethylamino)-10-[(dimethylamino)methyl]-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide isomer 1 ("E20") and isomer 2 Example 21 ("E21")



- 10 **Intermediate 61:** (8*R*,9*S*,10*S*)-3-(dimethylamino)-10-((1,3-dioxoisindolin-2-yl)methyl)-*N*-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

- To a solution of a mixture of Examples 18 and 19 (165 mg, 318.87  $\mu$ mol, 1 eq), isoindoline-1,3-dione (51.61 mg, 350.75  $\mu$ mol) and  $PPh_3$  (125.45 mg, 478.30  $\mu$ mol) in THF (2 mL) was added DIAD (96.72 mg, 478.30  $\mu$ mol) dropwise. After stirring at 25°C for 12 h, the residue was purified by prep-TLC (petroleum ether: ethyl acetate = 0:1) to give
- 15 Intermediate 61 (184 mg, 89.25% yield) as a light yellow solid.  $^1H$  NMR (400MHz,  $CDCl_3$ -d1)  $\delta$  7.83-7.81 (m, 1H), 7.73-7.72 (m, 2H), 7.55-7.26 (m, 5H), 6.82-6.79 (m, 2H), 3.94-3.74 (m, 2H), 3.72 (s, 3H), 3.67-3.56 (m, 5H), 3.54-3.55 (m, 2H), 3.35-3.06 (m, 1H), 2.77 (s, 6H), 1.26 (s, 1H).

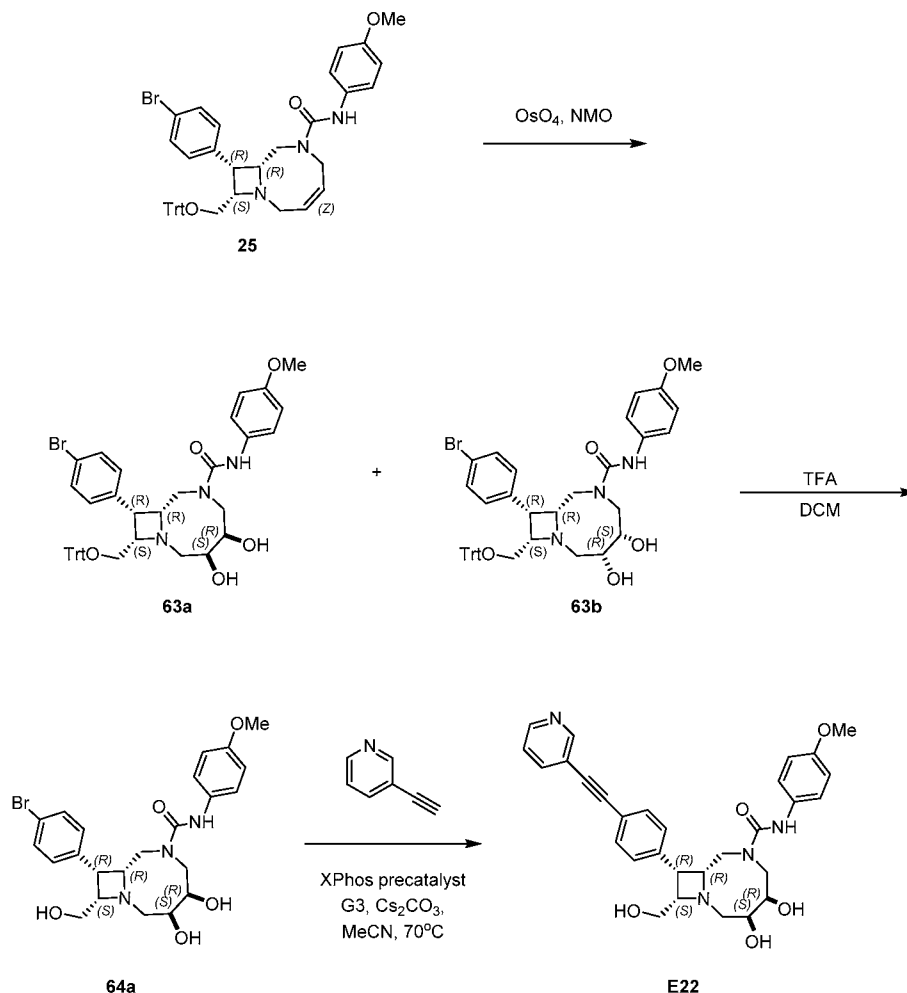
- 20 **Intermediate 62:** (8*R*,9*S*,10*S*)-10-(aminomethyl)-3-(dimethylamino)-*N*-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide

- To a solution of Intermediate 61 (174 mg, 260.56  $\mu$ mol) in EtOH (1 mL) was added hydrazine hydrate (19.96 mg, 390.84  $\mu$ mol) dropwise. After stirring at 80°C for 2 h, the reaction mixture was filtered and concentrated to give the residue. The residue was purified
- 25 by prep-TLC (dichloromethane: methanol=6:1) to give Intermediate 62 (50.00 mg, 35.69% yield) as a brown liquid. HRMS (ESI) calcd for  $C_{33}H_{39}N_5O_2$   $[M + H]^+$  538.31, found 538.2

5 **Synthesis of E20 and E21:** (4S,8R,9S,10S)-9-(4-bromophenyl)-4-(dimethylamino)-10-  
[(dimethylamino)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-  
carboxamide (E20) and (4R,8R,9S,10S)-9-(4-bromophenyl)-4-(dimethylamino)-10-  
[(dimethylamino)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-  
carboxamide (E21).

10 To a stirred solution of Intermediate 61 (50 mg, 96.81  $\mu$ mol) in DCM (2.00 mL) was  
added HCHO (78.57 mg, 968.10  $\mu$ mol, 72.08  $\mu$ L) and  $\text{MgSO}_4$  (116.53 mg, 968.10  $\mu$ mol).  
After stirring at 25°C for 0.5 h,  $\text{CH}_3\text{COOH}$  (5.81 mg, 96.81  $\mu$ mol) and  $\text{NaBH}(\text{OAc})_3$  (102.59  
mg, 484.05  $\mu$ mol) were added. After stirring at 25°C for 1.5 h, the reaction mixture was  
quenched by  $\text{H}_2\text{O}$  (5 mL) and extract with DCM (20 mL \* 3) to give organic layer. The  
15 organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give the residue. The  
residue was purified by prep-HPLC to give and separate each isomer of (8R,9S,10S)-9-(4-  
bromophenyl)-4-(dimethylamino)-10-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-1,6-  
diazabicyclo[6.2.0]decane-6-carboxamide as isomer 1 (E20) (2.90 mg, 5.33  $\mu$ mol, 5.51%  
yield) as light brown solid (HRMS (ESI) calcd for:  $\text{C}_{35}\text{H}_{43}\text{N}_5\text{O}_2$   $[\text{M} + \text{H}]^+$  566.34, found  
20 566.4;  $^1\text{H}$  NMR (400MHz,  $\text{MeOD-d}_4$ )  $\delta$ 7.61-7.51 (m, 5H), 7.44-7.38 (m, 4H), 7.37-7.23 (m,  
2H), 6.87-6.85 (m, 2H), 4.05-3.77 (m, 1H), 3.77 (s, 3H), 3.66-3.51 (m, 2H), 3.19-3.16 (m,  
1H), 2.50-2.47 (m, 1H), 2.35-2.19 (m, 1H), 1.86-1.80 (m, 1H))) and isomer 2 (E21) (15.20  
mg, 27.91  $\mu$ mol, 28.83% yield) as light brown solid ( $^1\text{H}$  NMR (400MHz,  $\text{MeOD-d}_4$ )  $\delta$ 7.56-  
7.54 (m, 6H), 7.52-7.51 (m, 3H), 7.23-7.22 (m, 2H), 6.83-6.84 (m, 2H), 4.14-4.12 (m, 1H),  
25 3.87-3.81 (m, 1H), 3.76 (s, 3H), 3.67-3.48 (m, 2H), 3.24-3.22 (m, 2H), 2.77 (s, 6H), 2.71-  
2.60 (m, 4H), 2.32-2.31 (m, 2H), 2.31-2.30 (m, 1H)).

5 **Example 22:** (3*S*,4*R*,8*R*,9*R*,10*S*)-3,4-dihydroxy-10-(hydroxymethyl)-*N*-(4-methoxyphenyl)-9-(4-(pyridin-3-ylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E22")



10 **Intermediate 63a and 63b:** (3*S*,4*R*,8*R*,9*R*,10*S*)-9-(4-bromophenyl)-3,4-dihydroxy-*N*-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (63a) and (3*R*,4*S*,8*R*,9*R*,10*S*)-9-(4-bromophenyl)-3,4-dihydroxy-*N*-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (63b)

To a solution of Intermediate 25 (220 mg, 307.83  $\mu$ mol) and NMO (43.27 mg, 369.4  $\mu$ mol) in acetone/H<sub>2</sub>O = 10:1 (1 mL) was added OsO<sub>4</sub> (7.83 mg, 30.78  $\mu$ mol) at 25°C. After stirring at 25°C for 12 h, the reaction mixture was concentrated to give a residue. The residue was purified by pre-TLC (petroleum ether: ethyl acetate = 1 : 5) to give Intermediate 63a (120 mg, 52.07% yield) as a white solid (<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>)  $\delta$ 7.31-7.05 (m, 23H), 6.85-6.81 (m, 2H), 6.82 (d, *J* = 8 Hz, 2H), 4.23-4.19 (m, 1H), 3.99 (s, 1H), 3.76-3.73 (m, 6H), 3.50-3.43 (m, 2H), 3.19-3.13 (m, 2H), 2.98-2.96 (m, 1H), 2.86-2.84 (m, 1H), 2.78-2.59 (m, 1H)) and Intermediate 63b (134 mg, 58.14% yield) as a white solid (<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>)  $\delta$ 7.39-7.24 (m, 23H), 7.09-7.05 (m, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 3.85-3.78 (m,

15

20

5 5H), 3.69-3.61 (m, 4H), 3.45-3.40 (m, 3H), 3.14-3.13 (m, 1H), 3.05-3.04 (m, 1H), 2.78-2.75 (m, 1H)).

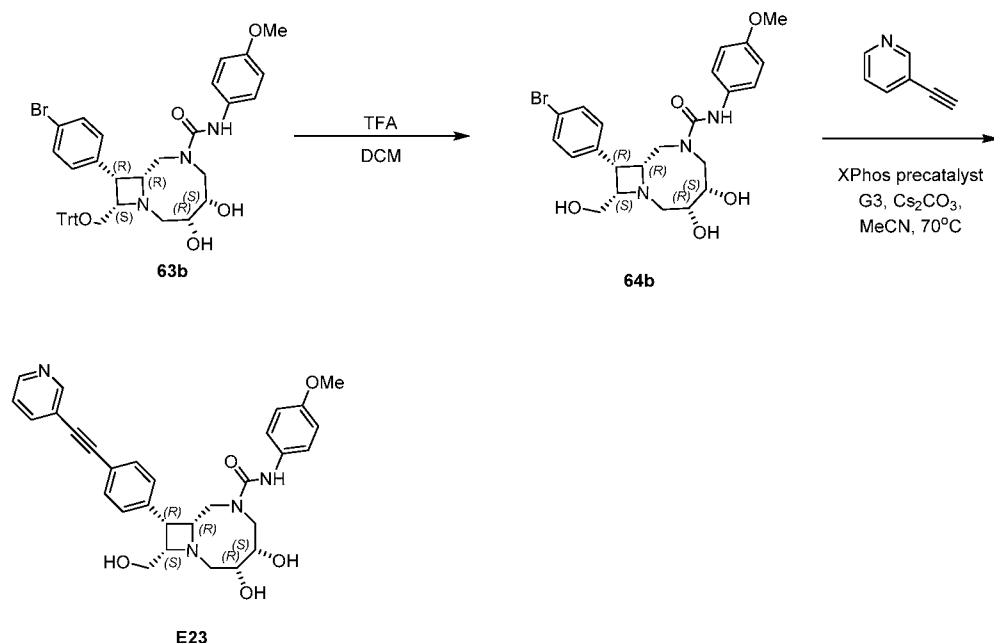
**Intermediate 64a:** (3S,4R,8R,9R,10S)-9-(4-bromophenyl)-3,4-dihydroxy-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 63a (60 mg, 80.14  $\mu$ mol) in DCM (2 mL) was added  
10 TFA (91.37 mg, 801.39  $\mu$ mol). After stirring at 25°C for 3 h, the reaction was quenched with NaHCO<sub>3</sub> (10 mL) and extracted with DCM (20 mL) to give the organic layer. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by prep-TLC (petroleum ether: ethyl acetate) to give the Intermediate 64a (35 mg, 86.24% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>)  $\delta$ 7.43 (d, *J* = 7.6 Hz, 2H),  
15 7.30 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 4.32-4.28 (m, 1H), 4.13-4.06 (m, 2H), 3.85 (m, 1H), 3.76 (s, 3H), 3.61 (s, 1H), 3.48 (s, 3H), 3.22 (m, 1H), 2.98-2.88 (m, 2H), 2.77-2.70 (m, 2H), 2.0 (s, 1H).

**Synthesis of E22:** (3S,4R,8R,9R,10S)-3,4-dihydroxy-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(pyridin-3-ylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-  
20 carboxamide.

To a solution of Intermediate 64a (20 mg, 39.5  $\mu$ mol) and 3-ethynylpyridine (8.15 mg, 78.99  $\mu$ mol) in acetonitrile (1 mL) was added XPhos Pd G3 (3.34 mg, 3.95  $\mu$ mol) and Cs<sub>2</sub>CO<sub>3</sub> (25.74 mg, 78.99  $\mu$ mol). After stirring at 70°C for 1 h, the reaction mixture was concentrated to give the residue. The residue was purified by pre-HPLC (Column: Waters  
25 Xbridge Prep OBD C18 150\*30 5 $\mu$ ; A: water (0.225% formic acid) B: acetonitrile) to the give (3S,4R,8R,9R,10S)-3,4-dihydroxy-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(pyridin-3-ylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (6.8 mg, 29.69% yield) as a white solid (HRMS (ESI) calcd for C<sub>30</sub>H<sub>32</sub>N<sub>4</sub>O<sub>5</sub> [M+H]<sup>+</sup> 529.24, found 529.30; <sup>1</sup>H NMR (400MHz, MeOD-d<sub>4</sub>)  $\delta$ 8.69 (s, 1H), 8.51 (d, *J* = 4 Hz, 1H), 7.98 (d, *J* = 8 Hz, 1H),  
30 7.60-7.52 (m, 4H), 7.48-7.45 (m, 1H), 7.16 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 4.16-4.11 (m, 2H), 3.84-3.82 (m, 2H), 3.75 (s, 3H), 3.64-3.58 (m, 4H), 3.39-3.60 (m, 2H), 2.88-2.79 (m, 3H)).

- 5 **Example 23:** (3R,4S,8R,9R,10S)-3,4-dihydroxy-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(pyridin-3-ylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E23")



**Intermediate 64b:** (3R,4S,8R,9R,10S)-9-(4-bromophenyl)-3,4-dihydroxy-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

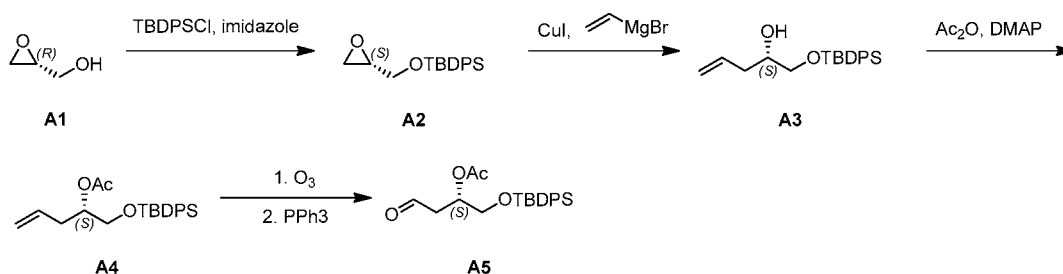
- 10 To a solution of Intermediate 63b (130 mg, 173.63  $\mu\text{mol}$ ) in DCM (2 mL) was added TFA (197.98 mg, 1.74 mmol). After stirring at 25°C for 3 h, the reaction was quenched with NaHCO<sub>3</sub> (10 mL) and extracted with DCM (20 mL) to give the organic layer. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by prep-TLC (petroleum ether: ethyl acetate) to give the Intermediate 64b (75 mg
- 15 85.30% yield) as a white solid (<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>)  $\delta$  7.48 (d,  $J$  = 7.6 Hz, 2H), 7.09-7.28 (m, 7H), 6.84 (d,  $J$  = 8.8 Hz, 2H), 3.78 (d,  $J$  = 10.8 Hz, 1H), 3.72-3.66 (m, 4H), 3.59-3.57 (m, 5H), 3.53-3.51 (m, 4H), 3.27-3.25 (m, 1H), 2.88-2.74 (m, 2H)).

**Synthesis of E23:** (3R,4S,8R,9R,10S)-3,4-dihydroxy-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(pyridin-3-ylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

- 20 To a solution of Intermediate 64b (20 mg, 39.50  $\mu\text{mol}$ ) and 3-ethynylpyridine (8.15 mg, 78.99  $\mu\text{mol}$ ) in acetonitrile (1 mL) was added XPhos Pd G3 (3.34 mg, 3.95  $\mu\text{mol}$ ) and Cs<sub>2</sub>CO<sub>3</sub> (25.74 mg, 78.99  $\mu\text{mol}$ ). After stirring at 70°C for 2 h, the reaction mixture was concentrated to give the residue. The residue was purified by pre-HPLC (Column: Waters
- 25 Xbridge Prep OBD C18 150\*30 5 $\mu$ ; A: water (0.225% formic acid) B: acetonitrile) to give (3R,4S,8R,9R,10S)-3,4-dihydroxy-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-

- 5 (pyridin-3-ylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (7.4 mg, 29.69% yield) as a white solid. HRMS (ESI) calcd for  $C_{30}H_{32}N_4O_5$   $[M + H]^+$  529.24, found 529.30.  $^1H$  NMR (400MHz, MeOD- $d_4$ )  $\delta$ 8.69 (s, 1H), 8.52 (d,  $J$  = 5.2 Hz, 1H), 7.98 (d,  $J$  = 8 Hz, 1H), 7.61-7.56 (m, 4H), 7.48-7.46 (m, 1H), 7.23 (d,  $J$  = 8.8 Hz, 2H), 6.86 (d,  $J$  = 9.2 Hz, 2H), 3.84-3.80 (m, 3H), 3.76 (s, 3H), 3.65-3.50 (m, 7H), 3.31-3.28 (m, 1H), 3.08-3.26 (m, 1H),  
10 2.74-2.71 (m, 1H).

**Synthesis of Intermediate A5:** [(1S)-1-[[tert-butyl(diphenyl)silyl]oxymethyl]-3-oxo-propyl] acetate.



**Intermediate A2:** tert-butyl-[[[(2S)-oxiran-2-yl]methoxy]-diphenyl-silane

- 15 To a solution of (*R*)-oxiran-2-ylmethanol (3.00 g, 40.50 mmol, 2.68 mL) and imidazole (5.51 g, 80.99 mmol) in DCM (80 mL) at 0°C was added TBDPSCl (13.36 g, 48.60 mmol, 12.48 mL). After stirring at 25°C for 12 h, the reaction mixture was quenched with H<sub>2</sub>O (20 mL) and extracted by DCM (60 mL \*2) to give the organic layer. The layer was  
20 dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 10:1) to give Intermediate A2 (12.66 g, 100.00% yield) as a colorless liquid.  $^1H$  NMR (400MHz, CDCl<sub>3</sub>-  
d<sub>1</sub>)  $\delta$ 7.69-7.68 (m, 4H), 7.46-7.40 (m, 5H), 8.87-3.85(m, 1H), 3.73-3.70 (m, 1H), 3.14-3.13 (m, 1H), 2.76-2.74 (m, 1H), 1.05 (s, 9H).

**Intermediate A3:** (2S)-1-[tert-butyl(diphenyl)silyl]oxypent-4-en-2-ol.

- 25 CuI (1.83 g, 9.60 mmol, 1.5 eq) was placed in a three-necked flask under nitrogen and then anhydrous THF (20 mL) was added. The resulting mixture was cooled to -78°C, and then vinyl magnesium bromide (1 M, 22.40 mL, 3.5 eq) was added dropwise while maintaining the internal temperature below -78°C. The heterogeneous mixture was warmed to -20°C and stirred at this temperature for 30 min. After cooling the solution back to -78°C,  
30 C, Intermediate A2 (2.00 g, 6.40 mmol, 1 eq) was added dropwise. The mixture was stirred, and allowed to gradually warm to 20°C for 12 h. The reaction mixture was quenched with H<sub>2</sub>O (20 mL) and extracted by DCM (60 mL \*2) to give the organic layer. The layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified

5 by column chromatography on silica gel (petroleum ether: ethyl acetate = 7:1) to give Intermediate A3 (2.18 g, 100.00% yield) as a colorless liquid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.67-7.65 (m, 4H), 7.44-7.37 (m, 7H), 5.82-5.76 (m, 1H), 5.10-4.90 (m, 2H), 3.81-3.78 (m, 1H), 3.68-3.65 (m, 1H), 3.57-3.54 (m, 1H), 2.46-2.44 (m, 1H), 2.25-2.22 (m, 1H), 1.05 (m, 9H).

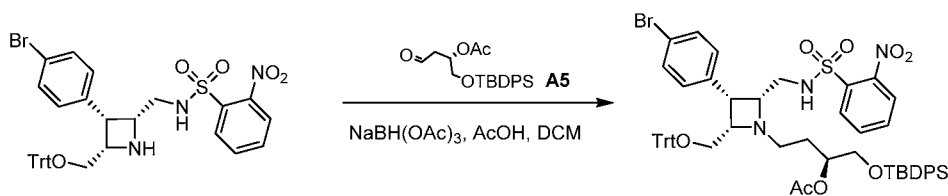
10 **Intermediate A4:** [(1S)-1-[[tert-butyl(diphenyl)silyl]oxymethyl]but-3-enyl] acetate.

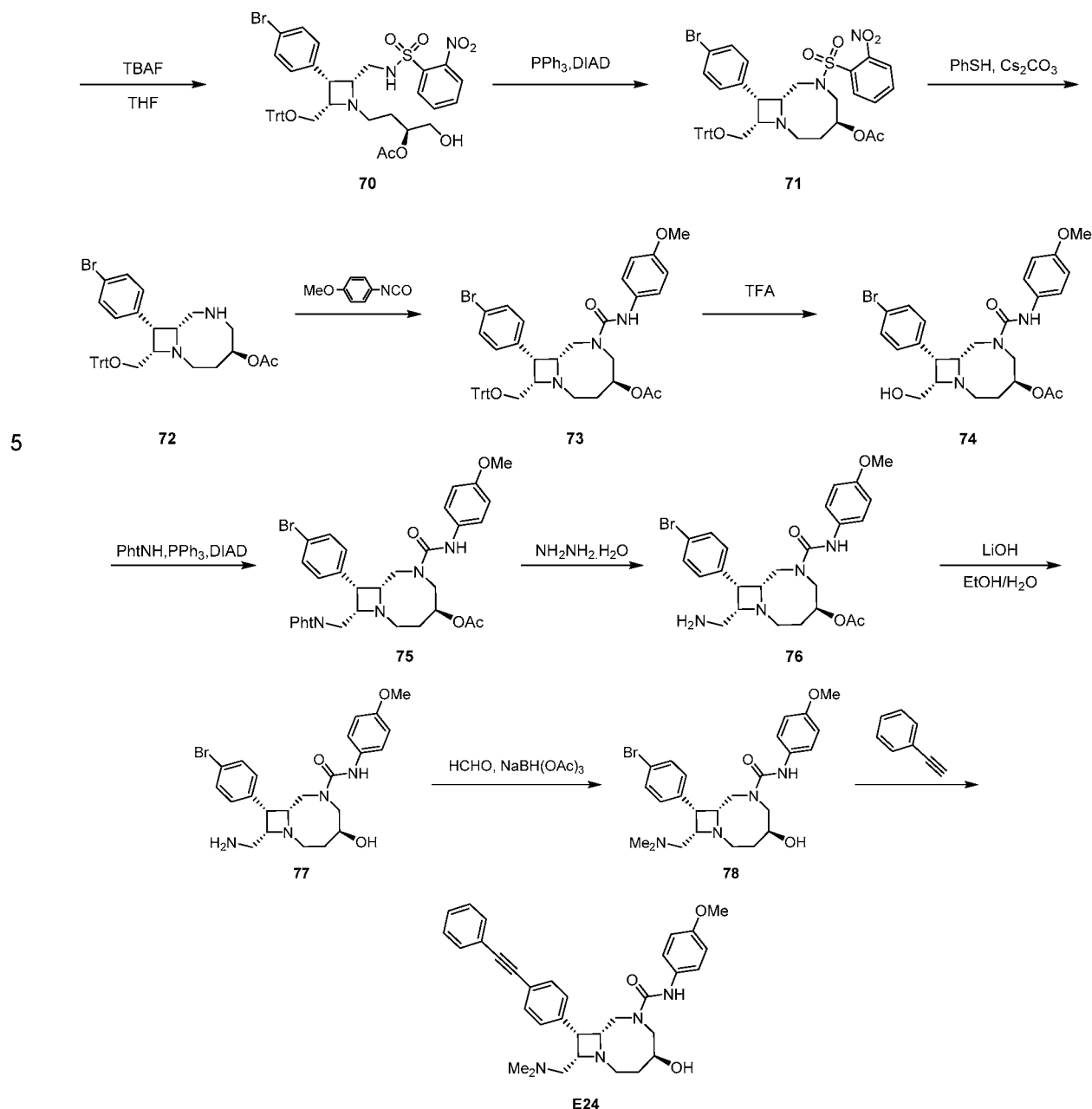
To a solution of Intermediate A3 (2.18 g, 6.40 mmol, 1 eq), Et<sub>3</sub>N (1.94 g, 19.21 mmol, 3 eq) and DMAP (78.21 mg, 640.18 μmol, 0.1 eq) in DCM (2 mL) at 0°C was added acetic anhydride (980.06 mg, 9.60 mmol, 1.5 eq). The resulting reaction mixture was stirred at 25°C for 3 h. TLC (petroleum ether: ethyl acetate = 10:1) showed the reaction was  
15 complete, the reaction mixture was quenched with H<sub>2</sub>O (10 mL) and extracted with DCM (20 mL \* 3) to give organic layer. The layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate=10:1) to give Intermediate A4 (2.30 g, 93.94% yield) as a colorless liquid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.67-7.65 (m, 4H), 7.40-7.26 (m, 6H), 5.77-5.69 (m, 1H), 5.11-5.01 (m, 3H), 3.73-3.66 (m, 2H), 2.48-2.32 (m, 1H), 2.02 (m, 3H), 1.09 (m, 9H).

**Intermediate A5:** [(1S)-1-[[tert-butyl(diphenyl)silyl]oxymethyl]-3-oxo-propyl] acetate.

A -78°C solution of Intermediate A4 (1.70 g, 4.44 mmol, 1 eq) in DCM/MeOH = 1:1 (30 mL) was treated with a stream of O<sub>3</sub> in O<sub>2</sub> until the solution turned blue. N<sub>2</sub> was then  
25 bubbled through the solution until the color disappeared. The resulting solution was treated with Me<sub>2</sub>S (2.76 g, 44.44 mmol, 10 eq), warmed to 20°C and stirred for 2 h. TLC (petroleum ether: ethyl acetate = 5:1) showed the reaction was complete, the reaction mixture was diluted with DCM (50 mL) and washed with H<sub>2</sub>O (10 mL) to give the organic layer. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give Intermediate A5 (1.70 g, crude) as a colorless liquid without further purification.

**Example 24:** (4S,8R,9S,10S)-10-[(dimethylamino)methyl]-4-hydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E24")





10 **Intermediate 69:** [(1*S*)-3-[(2*R*,3*R*,4*S*)-3-(4-bromophenyl)-2-[(2-nitrophenyl)sulfonylamino]methyl]-4-(trityloxymethyl)azetidin-1-yl]-1-[[tert-butyl(diphenyl)silyl]oxymethyl]propyl] acetate.

To a stirred solution of *N*-(((2*R*,3*R*,4*S*)-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidin-2-yl)methyl)-2-nitrobenzenesulfonamide (WO2015070204) (1.20 g, 1.72 mmol, 1 eq) in DCM (12 mL) was added Intermediate A5 (1.46 g, 3.78 mmol, 2.2 eq) at 25°C and CH<sub>3</sub>COOH (103.29 mg, 1.72 mmol, 1 eq). The resulting reaction mixture was stirred at 25°C for 0.5 h. To the mixture was added NaBH(OAc)<sub>3</sub> (1.09 g, 5.16 mmol, 3 eq). The resulting reaction mixture was stirred at 25°C for 1.5 h. LCMS showed the reaction was complete, the reaction mixture was quenched by H<sub>2</sub>O (5 mL) and extracted with DCM (10

5 mL\*3) to give the organic layer. The layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 1: 1) to give Intermediate 69 (1.67 g, crude) as light brown solid.

<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 5.15-5.11 (m, 1H), 4.96-4.95 (m, 1H), 4.10-4.07 (m, 1H),  
10 3.62-3.44(m, 4H), 3.17-3.12 (m, 1H), 2.88-2.81 (m, 3H), 2.51-2.44 (m, 2H), 2.06-2.08 (m, 3H).

**Intermediate 70:** [(1S)-3-[(2R,3R,4S)-3-(4-bromophenyl)-2-[(2-nitrophenyl)sulfonylamino]methyl]-4-(trityloxymethyl)azetidin-1-yl]-1-(hydroxymethyl)propyl] acetate.

15 To a stirred solution of Intermediate 69 (1.67 g, 1.56 mmol, 1 eq) in THF (16 mL) was TBAF (1.22 g, 4.68 mmol, 3 eq). The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture concentrated to give the residue. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 1: 1) to give Intermediate 70 (920.00 mg, 71.16% yield) as a white solid. <sup>1</sup>H  
20 NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 7.85-7.66 (m, 10), 7.26-6.97 (m, 20H), 5.02 (m, 1H), 4.85-4.82 (m, 1H), 4.05-3.76 (m, 1H), 3.70-3.52 (m, 3H), 3.12-2.86 (m, 3H), 2.03 (s, 5H), 1.52-1.46 (m, 2H).

**Intermediate 71:** [(4S,8R,9R,10S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl] acetate.

25 To a stirred solution of Intermediate 70 (660.00 mg, 796.36 μmol, 1 eq) in THF (20 mL) was a mixture of PPh<sub>3</sub> (835.51 mg, 3.19 mmol, 4 eq) and DIAD (644.13 mg, 3.19 mmol, 619.35 μL, 4 eq). The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (petroleum ether: ethyl acetate = 1 : 1) to give Intermediate  
30 71 (445.00 mg, crude) as a white solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 7.92-7.90 (m, 1H), 7.69-7.61 (m, 3H), 7.26-7.16 (m, 20H), 4.42-4.40 (m, 2H), 4.14-4.10 (m, 1H), 3.57 (s, 3H), 3.56-3.45 (m, 1H), 3.10-3.03 (m, 3H), 2.85-2.84 (m, 1H), 2.37-2.31 (m, 1H), 2.13-1.98 (m, 1H), 1.87-1.54 (m, 1H), 0.128-1.26 (m, 3H).

**Intermediate 72:** [(4S,8R,9R,10S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl] acetate.

35 To a stirred solution of Intermediate 71 (660.00 mg, 796.36 μmol, 1 eq) in THF (20.00 mL) was a mixture of PPh<sub>3</sub> (835.51 mg, 3.19 mmol, 4 eq) and DIAD (644.13 mg, 3.19

5 mmol, 619.35  $\mu$ L, 4 eq). The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was concentrated to give the residue. The residue was purified by prep-TLC (petroleum ether: ethyl acetate = 1: 1) to give Intermediate 72

(445.00 mg, crude) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.26-7.22 (m, 25H),  
10 4.01-4.00 (m, 1H), 3.83-3.81 (m, 1H), 3.62-3.47 (m, 1H), 3.51-3.47 (m, 1H), 3.49-3.48 (m, 1H), 3.21-3.11 (m, 1H), 2.92-2.88 (m, 1H), 2.49-2.47 (m, 2H), 2.05 (s, 3H), 1.85-1.084 (m, 1H), 1.60 (s, 3H).

**Intermediate 73:** [(4S,8R,9R,10S)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl] acetate.

15 To a stirred solution of Intermediate 72 (154.00 mg, 246.17  $\mu$ mol, 1 eq) and  $\text{Et}_3\text{N}$  (49.82 mg, 492.34  $\mu$ mol, 68.25  $\mu$ L, 2 eq) in DCM (2 mL) was 1-isocyanato-4-methoxybenzene (40.39 mg, 270.78  $\mu$ mol, 34.82  $\mu$ L, 1.1 eq). The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was quenched by  $\text{H}_2\text{O}$  (2 mL) and extracted with DCM (10 mL\*3) to give the organic layer.  
20 The layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give the residue. The residue was purified by prep-TLC (petroleum ether: ethyl acetate = 1: 1) to give Intermediate 73 (169 mg, 88.61% yield) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.33-7.23 (m, 20H), 6.83-6.82 (m, 2H), 4.59-4.57 (m, 1H), 4.30-4.27 (m, 1H), 3.78-3.75 (m, 4), 3.63-3.3.62 (m, 1H), 3.54-3.53 (m, 1H), 3.15-3.13 (m, 2H), 2.92-2.90 (m, 2H), 2.08-2.00 (m, 3H), 1.89-1.85  
25 (s, 2H), 1.57 (m, 1H).

**Intermediate 74:** [(4S,8R,9R,10S)-9-(4-bromophenyl)-10-(hydroxymethyl)-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-4-yl] acetate.

To a stirred solution of Intermediate 73 (169.00 mg, 218.14  $\mu$ mol, 1 eq) in DCM (2 mL) was TFA (248.72 mg, 2.18 mmol, 10 eq). The resulting reaction mixture was stirred at  
30 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was quenched by  $\text{NaHCO}_3$  and extracted with DCM (10 mL\*3) to give the organic layer. The layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give the residue. The residue was purified by prep-TLC (petroleum ether: ethyl acetate = 1 : 2) to give Intermediate 74 (81.00 mg, 152.13  $\mu$ mol, 69.74% yield) as a white solid. HRMS (ESI): calcd for  $\text{C}_{25}\text{H}_{30}\text{BrN}_3\text{O}_5$   $[\text{M} + \text{H}]^+$   
35 532.14, found 534.1

**Intermediate 75:** [(4S,8R,9S,10S)-9-(4-bromophenyl)-10-[(1,3-dioxoisindolin-2-yl)methyl]-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-4-yl] acetate.

To a solution of Intermediate 74 (81.00 mg, 104.55  $\mu$ mol, 1 eq), isoindoline-1,3-dione (16.92 mg, 115.01  $\mu$ mol, 1.1 eq) and  $\text{PPh}_3$  (41.13 mg, 156.82  $\mu$ mol, 1.5 eq) in THF (2 mL) was added DIAD (31.71 mg, 156.82  $\mu$ mol, 30.49  $\mu$ L, 1.5 eq) dropwise at 25°C under  $\text{N}_2$ . The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was concentrated to give the residue. The residue was purified by pre-TLC (Petroleum ether: Ethyl acetate = 1:1) to give Intermediate 75 (58.00 mg, 83.86% yield) as a yellow solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$  7.83-7.81 (m, 2H), 7.73-7.71 (m, 2H), 7.51-7.49 (m, 4H), 7.25-7.23 (m, 2H), 6.89-6.84 (m, 2H), 6.56 (s, 1H), 4.59-4.55 (m, 1H), 4.28-4.27 (m, 1H), 3.82-3.78 (m, 4H), 3.58-3.55 (m, 3.50H), 3.50-3.25 (m, 2H), 2.99-2.98 (m, 1H), 2.37-2.12 (m, 1H), 2.08 (s, 5H).

**Intermediate 76:** (4S,8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-6-((4-methoxyphenyl)carbamoyl)-1,6-diazabicyclo[6.2.0]decan-4-yl acetate.

To a solution of Intermediate 75 (58.00 mg, 87.67  $\mu$ mol, 1 eq) in EtOH (1 mL) was added hydrazine hydrate (6.72 mg, 131.51  $\mu$ mol, 6.52  $\mu$ L, 98% purity, 1.5 eq) dropwise at 25°C under  $\text{N}_2$ . The resulting reaction mixture was stirred at 70°C for 1 h. LCMS showed the reaction was complete, the reaction mixture was concentrated to give the residue Intermediate 76 without further purification. HRMS (ESI): calcd for  $\text{C}_{25}\text{H}_{31}\text{BrN}_4\text{O}_4$   $[\text{M} + \text{H}]^+$  531.15, found 531.1

**Intermediate 77:** (4S,8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-4-hydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 76 (46.00 mg, 86.56  $\mu$ mol, 1 eq) in EtOH: $\text{H}_2\text{O}$ =1:1 (1 mL) was added LiOH. $\text{H}_2\text{O}$  (7.26 mg, 173.12  $\mu$ mol, 2 eq) at 25°C. The resulting reaction mixture was stirred at 25°C for 2 h. LCMS showed the reaction was complete, the reaction mixture was quenched by  $\text{H}_2\text{O}$  (2 mL) and extracted with DCM (10 mL\*3) to give the organic layer. The layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give Intermediate 77 (80.00 mg, crude) as a white solid. HRMS (ESI): ET6538-397-P1P calcd for  $\text{C}_{23}\text{H}_{29}\text{BrN}_4\text{O}_3$   $[\text{M} + \text{H}]^+$  489.14, found 491.1.

**Intermediate 78:** (4S,8R,9S,10S)-9-(4-bromophenyl)-10-((dimethylamino)methyl)-4-hydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

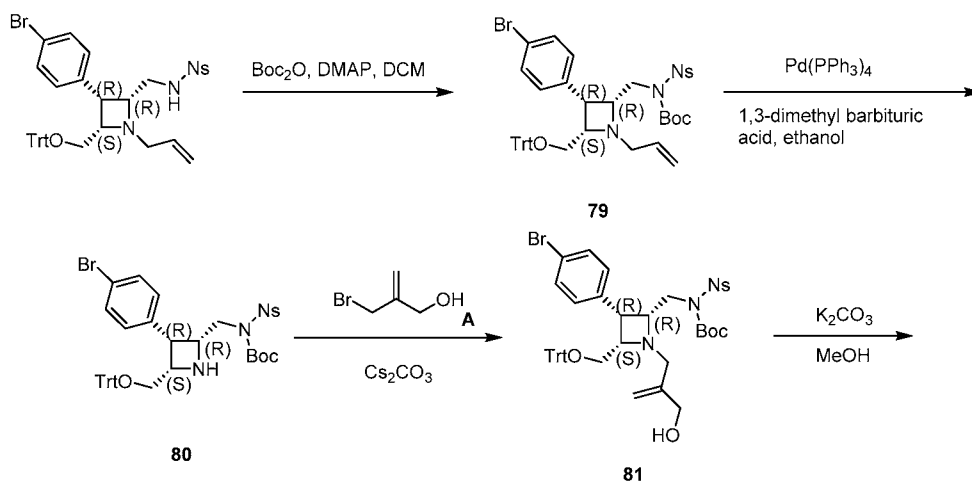
To a solution of Intermediate 77 (757.00 mg, 1.01 mmol, 1 eq), isoindoline-1,3-dione (164.07 mg, 1.12 mmol, 1.1 eq) and  $\text{PPh}_3$  (398.85 mg, 1.52 mmol, 1.5 eq) in THF (2 mL) was added DIAD (307.49 mg, 1.52 mmol, 1.5 eq) dropwise at 25°C under  $\text{N}_2$ . The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the

5 reaction mixture was concentrated to give the residue. The residue was purified by pre-TLC (petroleum ether: ethyl acetate = 1:1) to give Intermediate 78 (1.13 g, crude) as a yellow solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.45-7.44 (m, 3H), 7.39-7.37 (m, 2H), 6.83-6.81 (m, 2H), 4.08-3.97 (m, 2H), 3.77-3.76 (m, 2H), 3.45-3.44 (m, 1H), 3.29-3.10 (m, 3H), 2.02 (s, 6H), 0.87 (s, 2H).

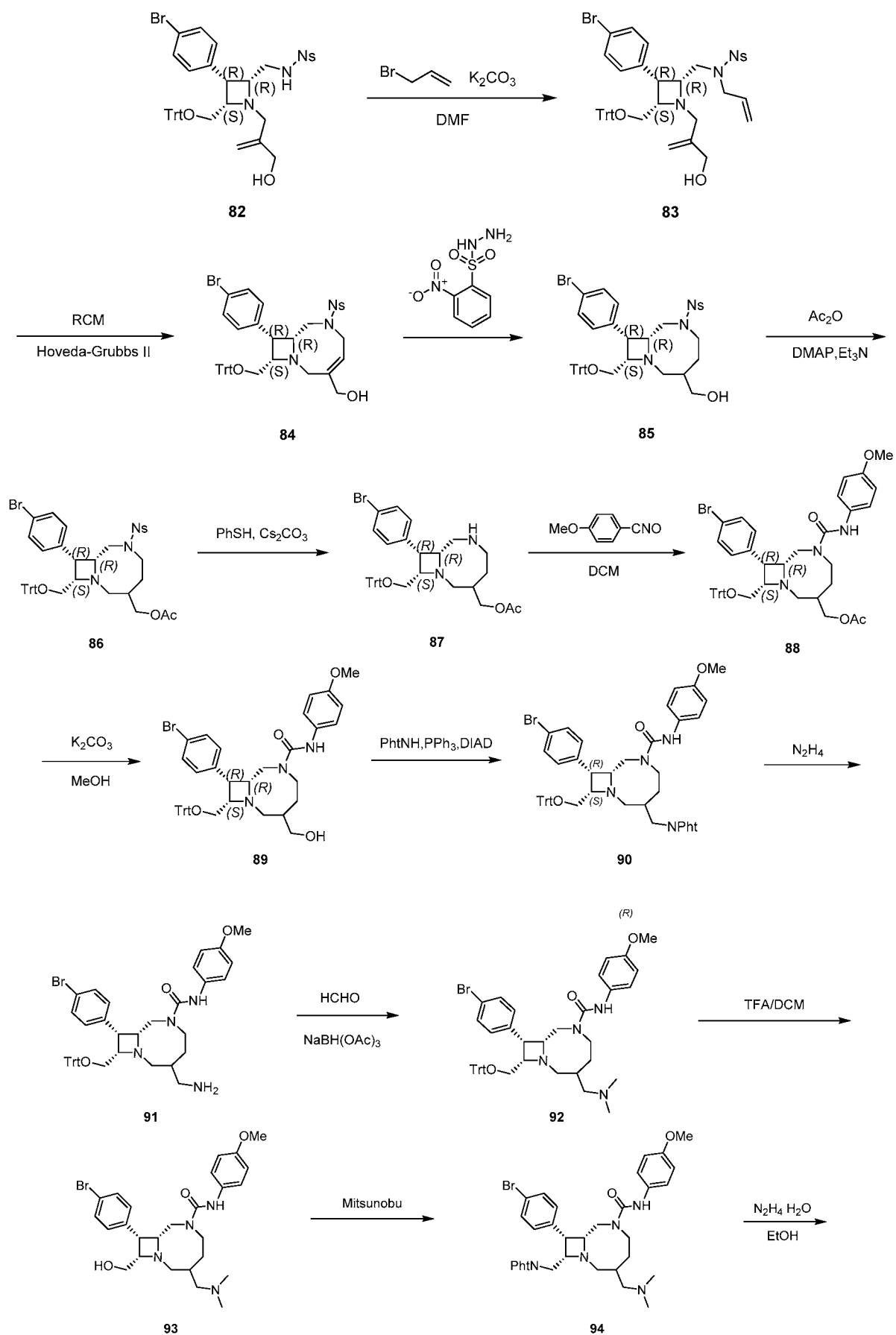
10 **Synthesis of E24:** (4S,8R,9S,10S)-10-[(dimethylamino)methyl]-4-hydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E24).

To a solution of Intermediate 78 (40.00 mg, 77.30 μmol, 1 eq) and ethynylbenzene (23.68 mg, 231.90 μmol, 25.47 μL, 3.00 eq) in acetonitrile (1 mL) was added XPhos Pd G3 (6.54 mg, 7.73 μmol, 0.1 eq) and Cs<sub>2</sub>CO<sub>3</sub> (50.37 mg, 154.60 μmol, 2 eq). The resulting reaction mixture was stirred at 70°C for 2 h. LCMS showed the reaction was complete, the reaction mixture was concentrated to give the residue. The residue was purified by prep-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (0.225% formic acid) B: acetonitrile) to give (4S,8R,9S,10S)-10-[(dimethylamino)methyl]-4-hydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (6.10 mg, 14.65% yield) as a white solid. HRMS (ESI): calcd for C<sub>33</sub>H<sub>38</sub>N<sub>4</sub>O<sub>3</sub> [M + H]<sup>+</sup> 539.29, found 539.4 <sup>1</sup>H NMR (400MHz, MeOD-d<sub>4</sub>) δ7.55-7.50 (m, 2H), 7.49-7.37 (m, 4H), 6.86-6.84 (m, 2H), 4.18-4.05 (m, 1H), 4.02-4.00 (m, 1H), 3.75 (s, 3H), 3.64-3.31 (m, 1H), 2.90-2.85 (m, 3H), 2.43 (s, 6H), 2.35-2.34 (m, 1H), 2.34-2.06 (m, 1H).

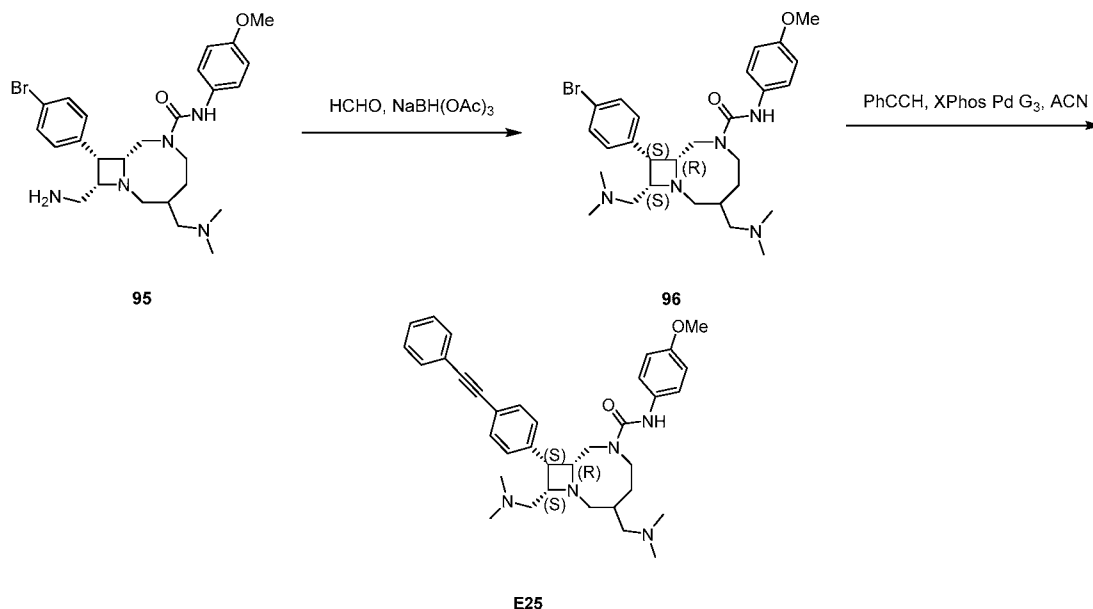
25 **Example 25:** (8R,9S,10S)-3,10-bis[(dimethylamino)methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E25")



5



10



**Intermediate 79:** tert-butyl N-[[[(2R,3R,4S)-1-allyl-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-2-yl]methyl]-N-(2-nitrophenyl)sulfonyl-carbamate.

To a solution of *N*-(((2R,3R,4S)-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidin-2-yl)methyl)-2-nitrobenzenesulfonamide (WO2015070204) (8.50 g, 11.51 mmol, 1 eq), DMAP (140.58 mg, 1.15 mmol, 0.1 eq) and Et<sub>3</sub>N (3.49 g, 34.52 mmol, 4.79 mL, 3 eq) in DCM (60 mL) was added BOC<sub>2</sub>O (2.76 g, 12.66 mmol, 1.1 eq) and the reaction mixture was stirred at 25°C for 4 h. TLC (petroleum ether : DCM = 3:1) showed the reaction was complete, the reaction was quenched with H<sub>2</sub>O (100 mL) and extracted with DCM (300 mL) to give the organic layer. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by chromatography on silica gel (petroleum ether: ethyl acetate = 3:1) to give Intermediate 79 (8.19 g, 84.83% yield) as a brown solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 8.20 (d, *J* = 4.4 Hz, 1H), 7.70 (s, 3H), 7.35-7.32 (m, 5H), 7.19-7.13 (m, 10H), 7.11 (m, 7H), 5.73-5.63 (m, 1H), 5.09-5.05 (m, 1H), 4.90-4.87 (m, 1H), 3.95-3.91 (m, 1H), 3.65-3.64 (m, 3H), 3.54-3.49 (m, 1H), 3.09-3.04 (m, 1H), 2.85-2.81 (m, 1H), 2.56-2.53 (m, 1H), 3.06-3.05 (m, 1H), 2.94-2.89 (m, 1H).

**Intermediate 80:** tert-butyl N-[[[(2R,3R,4S)-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-2-yl]methyl]-N-(2-nitrophenyl)sulfonyl-carbamate.

To a solution of Intermediate 79 (7.70 g, 9.18 mmol, 1 eq) in EtOH (10 mL) was added 1,3-dimethylhexahydropyrimidine-2,4,6-trione (2.15 g, 13.77 mmol, 1.5 eq) and Pd(PPh<sub>3</sub>)<sub>4</sub> (1.06 g, 917.98 μmol, 0.1 eq) and the reaction mixture was stirred at 40°C for 2 h. LCMS showed the reaction was complete, the reaction was quenched with 10% NaOH (20 mL) and extracted with DCM (500 mL) to give the organic layer. The organic layer was dried

5 over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 3:1) to give Intermediate 80 (6.44 g, 8.06 mmol, 87.83% yield) as a light brown solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 8.24-8.22 (m, 1H), 7.72-7.65 (m, 3H), 7.33 (s, 4H), 7.22-7.19 (m, 16H), 7.42-7.34 (m, 2H), 3.95-3.89 (m, 1H), 3.06-3.04 (m, 1H), 3.02-2.95 (m, 1H), 2.05 (s, 1H),  
10 1.03 (s, 9H).

**Intermediate 81:** tert-butyl N-[[[(2R,3R,4S)-3-(4-bromophenyl)-1-[2-(hydroxymethyl)allyl]-4-(trityloxymethyl) azetidin-2-yl]methyl]-N-(2-nitrophenyl)sulfonyl-carbamate.

To a solution of Intermediate 80 (6.44 g, 8.06 mmol, 1 eq) and K<sub>2</sub>CO<sub>3</sub> (3.34 g, 24.18 mmol, 3 eq) in DMF (60 mL) was added 2-(bromomethyl)prop-2-en-1-ol (1.83 g, 12.09  
15 mmol, 1.5 eq) at 25°C and the reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction was quenched with H<sub>2</sub>O (50 mL) and extracted with DCM (100 mL) to give the organic layer. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by chromatography on silica gel (petroleum ether: ethyl acetate = 2:1) to give Intermediate 81 (5.52 g, 78.83% yield)  
20 as a white solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 8.20 (d, *J* = 4.8 Hz, 2H), 8.02 (s, 1H), 7.72-7.67 (m, 3H), 7.42-7.40 (m, 2H), 7.30-7.26 (m, 2H), 7.18-7.16 (m, 10H), 7.08-7.07 (m, 7H), 4.80 (s, 1H), 4.65 (s, 1H), 4.06-3.98 (m, 3H), 3.84 (m, 1H), 3.65-3.62 (m, 2H), 3.55-3.51 (m, 2H), 3.03-3.00 (m, 2H), 1.30 (s, 9H).

**Intermediate 82:** N-[[[(2R,3R,4S)-3-(4-bromophenyl)-1-[2-(hydroxymethyl)allyl]-4-(trityloxymethyl)azetidin-2-yl]methyl]-2-nitro-benzenesulfonamide.  
25

To a solution of Intermediate 81 (5.00 g, 5.75 mmol, 1 eq) in MeOH (50 mL) was added K<sub>2</sub>CO<sub>3</sub> (3.97 g, 28.75 mmol, 5 eq) and the reaction mixture was stirred at 80°C for 3 h. LCMS showed the reaction was complete, the reaction was quenched with H<sub>2</sub>O (100 mL) and extracted with DCM (300 mL) to give the organic layer. The organic layer was dried over  
30 anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by chromatography on silica gel (petroleum ether: ethyl acetate = 1:1) to give Intermediate 82 (2.50 g, 3.25 mmol, 56.56% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 7.82-7.78 (m, 2H), 7.71-7.69 (m, 1H), 7.18-7.13 (m, 18H), 5.10 (s, 1H), 5.01-4.97 (m, 2H), 4.12-4.07 (m, 2H), 3.70-3.58 (m, 3H), 3.34-3.23 (m, 1H), 3.07-3.06 (m, 1H), 2.93-2.89 (m, 2H).

**Intermediate 83:** N-allyl-N-[[[(2R,3R,4S)-3-(4-bromophenyl)-1-[2-(hydroxymethyl)allyl]-4-(trityloxymethyl)azetidin-2-yl]methyl]-2-nitro-benzenesulfonamide.  
35

To a solution of Intermediate 82 (2.50 g, 3.25 mmol, 1 eq) in DMF (1.00 mL) was

5 added 3-bromoprop-1-ene (590.18 mg, 4.88 mmol, 1.5 eq) and  $K_2CO_3$  (1.35 g, 9.76 mmol, 3 eq) at 25°C and the reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction was quenched with  $H_2O$  (100 mL) and extracted with DCM (500 mL) to give the organic layer. The organic layer was dried over anhydrous  $Na_2SO_4$  and concentrated to give the residue. The residue was purified by chromatography on silica gel  
10 (petroleum ether: ethyl acetate = 1:1) to give Intermediate 83 (2.62 g, 99.68% yield) as a white solid.  $^1H$  NMR (400MHz,  $CDCl_3$ -d1)  $\delta$ 8.02 (s, 1H), 7.66-7.53 (m, 1H), 7.37-7.35 (m, 3H), 7.15-7.11 (m, 19H), 5.43-5.36 (m, 1H), 5.00-4.98 (m, 1H), 4.88 (s, 1H), 4.80-4.76 (m, 2H), 4.04- (s, 2H), 3.82-3.79 (m, 2H), 3.52-3.32 (m, 3H), 3.16 (m, 1H), 3.06-3.02 (m, 2H), 2.96-2.88 (m, 2H).

15 **Intermediate 84:** [(3E,8R,9R,10S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]dec-3-en-3-yl]methanol.

To a solution of Intermediate 83 (2.20 g, 2.72 mmol, 1 eq) in DCM (20 mL) was added [1,3-bis(2,4,6-trimethylphenyl)imidazolidin-2-ylidene]-dichloro-[(2-isopropoxyphenyl) methylene]ruthenium (Hoyveda-Grubbs 2<sup>nd</sup> Generation Catalyst) (426.12  
20 mg, 680.04  $\mu$ mol, 0.25 eq) and the reaction mixture was stirred at 40°C for 12 h. LCMS showed the reaction was complete, the reaction was concentrated to give the residue. The residue was purified by column chromatography on silica (petroleum ether: ethyl acetate = 1:1) to give Intermediate 84 (2.12 g, 100.00% yield) as a white solid.  $^1H$  NMR (400MHz,  $CDCl_3$ -d1)  $\delta$ 7.89-7.87 (m, 1H), 7.70-7.61 (m, 3H), 7.26-7.22 (m, 17H), 7.11-7.08 (m, 2H),  
25 5.68-5.64 (m, 1H), 4.15-4.05 (m, 1H), 3.99-3.64 (m, 2H), 3.53-3.52 (m, 1H), 3.31-3.22 (m, 2H), 3.12-3.09 (m, 2H), 2.85-2.84 (m, 1H), 2.17 (s, 1H).

**Intermediate 85:** [(8R,9R,10S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl]methanol.

To a solution of Intermediate 84 (750.00 mg, 960.65  $\mu$ mol, 1 eq) and  $Et_3N$  (1.94 g,  
30 19.21 mmol, 2.66 mL, 20 eq) in THF (15 mL) was added 2-nitrobenzenesulfonylhydrazide (2.09 g, 9.61 mmol, 10 eq) and the reaction mixture was stirred at 40°C for 12 h. LCMS showed the reaction was complete, the reaction was quenched with  $H_2O$  (3 mL) and extracted with DCM (10 mL\*2) to give the organic layer. The organic layer was dried over anhydrous  $Na_2SO_4$  and concentrated to give the residue. The residue was purified by column  
35 chromatography on silica gel (petroleum ether: ethyl acetate = 1:1) to give Intermediate 85 (1.50 g, 100.00% yield) as a yellow solid.  $^1H$  NMR (400MHz,  $CDCl_3$ -d1)  $\delta$ 7.76-7.74 (d,  $J$  = 5.6 Hz, 2H), 7.65-7.55 (m, 4H), 7.26-7.22 (m, 19H), 7.10-7.08 (d,  $J$  = 8.4 Hz, 2H), 3.75-3.61

5 (m, 6H), 3.26-3.22 (m, 1H), 3.16-3.12 (m, 1H), 3.00-2.99 (m, 2H), 2.89-2.87 (m, 2H), 2.72-2.69 (m, 1H), 2.17 (s, 1H), 1.97-1.90 (m, 3H), 1.40-1.26 (m, 2H).

**Intermediate 86:** [(8R,9R,10S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl]methyl acetate.

To a solution of Intermediate 85 (1.49 g, 1.90 mmol, 1 eq), Et<sub>3</sub>N (576.78 mg, 5.70 mmol, 3 eq) and DMAP (23.21 mg, 190.00 μmol, 0.1 eq) in DCM (15 mL) at 0°C was added acetic anhydride (387.94 mg, 3.80 mmol, 355.91 μL, 2 eq). The resulting reaction mixture was stirred at 25°C for 3 h. LCMS showed the reaction was complete, the reaction mixture was quenched with H<sub>2</sub>O (10 mL) and extracted with DCM (30 mL\*2) to give the organic layer. The layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by column chromatography on silica gel (petroleum ether : ethyl acetate = 1:1) to give Intermediate 86 (1.51 g, 96.32% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 7.80-7.76 (m, 1H), 7.61-7.56 (m, 3H), 7.27-7.22 (m, 15H), 7.13-7.11 (d, *J* = 8.4 Hz, 2H), 4.01-3.99 (m, 2H), 3.69-3.63 (m, 4H), 3.22-3.12 (m, 3H), 2.94-2.88 (m, 2H), 2.62-2.61 (m, 1H), 2.20-2.05 (m, 1H), 1.92 (s, 1H), 1.82 (s, 3H), 1.32-1.27 (m, 1H).

20 **Intermediate 87:** [(8R,9R,10S)-9-(4-bromophenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl]methyl acetate.

To a solution of Intermediate 86 (1.51 g, 1.83 mmol, 1. eq) and benzenethiol (302.57 mg, 2.75 mmol, 280.16 μL, 1.5 eq) in acetonitrile (15 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (1.19 g, 3.66 mmol, 2 eq) dropwise at 25°C. The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was quenched with H<sub>2</sub>O (10 mL) and extracted with DCM (20 mL\*3) to give the organic layer. The organic was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by column chromatography on silica gel (dichloromethane: methanol = 10:1) to give Intermediate 87 (1.09 g, crude) as a white solid. HRMS (ESI): calcd for C<sub>37</sub>H<sub>39</sub>BrN<sub>2</sub>O<sub>3</sub> [M + H]<sup>+</sup> 639.21, found 639.1.

30 **Intermediate 88:** [(8R,9R,10S)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl]methyl acetate.

To a solution of Intermediate 87 and Et<sub>3</sub>N (344.05 mg, 3.40 mmol, 2 eq) in DCM (10 mL) was added 1-isocyanato-4-methoxy-benzene (278.91 mg, 1.87 mmol, 1.1 eq) dropwise at 0°C. The resulting reaction mixture was stirred at 25°C for 3 h. LCMS showed the reaction was complete, the reaction mixture was quenched with H<sub>2</sub>O (20 mL) and extracted with DCM (50 mL \*3) to give the organic layer. The layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>

5 and concentrated to give the residue. The residue was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 1:1) to give Intermediate 87 (1.10 g, 81.76% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 7.30-7.23 (m, 20H), 6.84-6.82 (m, 2H), 6.09 (s, 1H), 4.07-4.03 (m, 1H), 3.79-3.78 (m, 3H), 3.72 (m, 1H), 3.62-3.55 (m, 3H), 3.19-3.17 (m, 1H), 3.00-2.94 (m, 2H), 2.55-2.54 (m, 1H), 2.52-2.50 (m, 1H), 2.00 (s, 1H), 1.87 (s, 3H), 1.86-1.77 (m, 1H).

**Intermediate 89:** (8R,9R,10S)-9-(4-bromophenyl)-3-(hydroxymethyl)-N-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 88 (750.00 mg, 950.85 μmol, 1 eq) in MeOH (7 mL) was added K<sub>2</sub>CO<sub>3</sub> (394.25 mg, 2.85 mmol, 3 eq). The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was filtered to give the filtrate and concentrated to give the residue. The residue was purified by pre-TLC (petroleum ether: ethyl acetate = 1:1) to give Intermediate 88 (757.00 mg, crude) as a white solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 7.29-7.23 (m, 24H), 7.09-7.07 (m, 1H), 6.84-6.81 (m, 2H), 3.77 (s, 3H), 3.66-3.63 (m, 2H), 3.62 (m, 4H), 3.15-3.07 (m, 1H), 2.82-2.79 (m, 1H), 2.66-2.64 (m, 1H), 1.77 (s, 1H), 1.28-1.25 (s, 2H).

**Intermediate 90:** (8R,9R,10S)-9-(4-bromophenyl)-3-[(1,3-dioxoisindolin-2-yl)methyl]-N-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 89 (757.00 mg, 1.01 mmol, 1 eq), isoindoline-1,3-dione (164.07 mg, 1.12 mmol, 1.1 eq) and PPh<sub>3</sub> (398.85 mg, 1.52 mmol, 1.5 eq) in THF (2 mL) was added DIAD (307.49 mg, 1.52 mmol, 1.5 eq) dropwise at 25°C under N<sub>2</sub>. The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was concentrated to give the residue. The residue was purified by pre-TLC (petroleum ether: ethyl acetate = 1:1) to give Intermediate 90 (1.13 g, crude) as a yellow solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ 7.70-7.68 (m, 3H), 7.68-7.65 (m, 8H), 7.49-7.47 (m, 3H), 7.22-7.17 (m, 3H), 6.74-6.72 (m, 2H), 5.99 (s, 1H), 3.84-3.77 (m, 2H), 3.75 (s, 3H), 3.64-3.60 (m, 4H), 3.54-3.50 (m, 3H), 3.16 (s, 1H), 3.05 (s, 1H), 2.94 (s, 1H), 2.64-2.62 (m, 1H), 1.88 (s, 1H).

**Intermediate 91:** (8R,9R,10S)-3-(aminomethyl)-9-(4-bromophenyl)-N-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 90 (1.13 g, 1.29 mmol, 1 eq) in EtOH (2 mL) was added NH<sub>2</sub>NH<sub>2</sub>·H<sub>2</sub>O (98.86 mg, 1.94 mmol, 98% purity, 1.5 eq) dropwise. The resulting reaction mixture was stirred at 70°C for 1 h. LCMS showed the reaction was complete, the reaction

5 mixture was quenched by H<sub>2</sub>O (2 mL) and extracted with DCM (10 mL\*3) to give the organic layer. The layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol = 12:1) to give Intermediate 91 (586.00 mg, 60.91% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.21-7.14 (m, 20H), 7.12-7.09 (m, 3H), 6.81-6.78 (m, 2H), 3.76-3.70 (m, 5H), 3.59-3.51 (m, 5H), 3.17-3.14 (m, 1H), 3.00-2.99 (m, 1H), 2.77-2.72 (m, 1H), 2.68-2.46 (m, 3H), 1.91 (m, 2H), 1.69 (s, 1H), 1.63 (s, 1H).

**Intermediate 92:** (8R,9R,10S)-9-(4-bromophenyl)-3-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a stirred solution of Intermediate 91 (586.00 mg, 785.80 μmol, 1 eq) in DCM (6 mL) was added HCHO (637.77 mg, 7.86 mmol, 585.11 μL, 10 eq) and MgSO<sub>4</sub> (945.86 mg, 7.86 mmol, 10 eq). The resulting reaction mixture was stirred at 25°C for 0.5 h. To the mixture was added CH<sub>3</sub>COOH (47.19 mg, 785.80 μmol, 1 eq) and NaBH(OAc)<sub>3</sub> (832.71 mg, 3.93 mmol, 5 eq). The resulting reaction mixture was stirred at 25°C for 1.5 h. LCMS showed the reaction was complete, the reaction mixture was quenched by H<sub>2</sub>O (5 mL) and extract with DCM (20 mL \* 3) to give organic layer. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol = 12:1) to give Intermediate 92 (370.00 mg, 60.85% yield) as light brown solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.34-7.29 (m, 2H), 7.76-7.22 (m, 10H), 7.14-7.12 (m, 2H), 6.83 (s, 1H), 6.83-6.79 (m, 2H), 3.76-3.67 (m, 4H), 3.63-3.60 (m, 4H), 3.15-3.14 (m, 1H), 2.97-2.96 (m, 1H), 2.73-2.64 (m, 3H), 2.73-2.65 (m, 1H), 2.02-1.88 (m, 3H).

**Intermediate 93:** (8R,9R,10S)-9-(4-bromophenyl)-3-[(dimethylamino)methyl]-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a stirred solution of Intermediate 92 (370.00 mg, 478.16 μmol, 1 eq) in DCM (1 mL) was TFA (545.20 mg, 4.78 mmol, 10 eq). The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was quenched by NaHCO<sub>3</sub> (5 mL) and extracted with DCM (10 mL\*3) to give the organic layer. The layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol = 8 : 1) to give (172.00 mg, 67.68% yield) as a light brown solid.

<sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.44 (d, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.28-7.25 (m, 3H), 6.84 (d, *J* = 8.8 Hz, 2H), 3.78-3.73 (m, 4H), 3.70-3.61 (m, 1H), 3.60-3.56 (m, 4H),

5 3.51-3.39 (m, 1H), 3.23-3.14 (m, 1H), 2.89-2.63 (m, 1H), 2.29 (m, 6H), 2.17-2.12 (m, 1H), 1.89 (s, 2H), 1.64-1.61 (m, 1H).

**Intermediate 94:** (8R,9S,10S)-9-(4-bromophenyl)-3-[(dimethylamino)methyl]-10-[(1,3-dioxoisindolin-2-yl)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

10 To a solution of Intermediate 93 (172.00 mg, 323.62  $\mu$ mol, 1 eq), isoindoline-1,3-dione (52.38 mg, 355.98  $\mu$ mol, 1.1 eq) and PPh<sub>3</sub> (127.32 mg, 485.43  $\mu$ mol, 1.5 eq) in THF (2 mL) was added DIAD (98.16 mg, 485.43  $\mu$ mol, 1.5 eq) dropwise at 25°C under N<sub>2</sub>. The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was concentrated to give the residue. The residue was purified  
15 by pre-TLC (dichloromethane: methanol = 10:1) to give Intermediate 94 (152.00 mg, 71.10% yield) as a yellow solid. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>)  $\delta$  7.81 (d, *J* = 4.8 Hz, 2H), 7.70 (d, *J* = 4.8 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.26-7.23 (m, 3H), 6.82 (d, *J* = 8.8 Hz, 2H), 3.92-3.89 (m, 1H), 3.76 (s, 3H), 3.70-3.68 (m, 2H), 3.66-3.64 (m, 2H), 3.57-3.53 (m, 4H), 2.79 (m, 1H), 2.67-2.61 (m, 1H), 2.39-2.38 (m, 1H), 2.36 (m, 3H), 2.16-1.99  
20 (m, 1H), 1.26 (s, 1H).

**Intermediate 95:** (8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-3-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 94 (152.00 mg, 230.09  $\mu$ mol, 1 eq) in EtOH (2 mL) was  
25 added NH<sub>2</sub>NH<sub>2</sub>.H<sub>2</sub>O (17.63 mg, 345.14  $\mu$ mol, 17.12  $\mu$ L, 98% purity, 1.5 eq) dropwise. The resulting reaction mixture was stirred at 70°C for 1 h. LCMS showed the reaction was complete, the reaction mixture was quenched by H<sub>2</sub>O (2 mL) and extracted with DCM (10 mL\*3) to give the organic layer. The layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane:  
30 methanol = 5:1) to give Intermediate 95 (80.00 mg, 65.54% yield) as a white solid. HRMS (ESI): calcd for C<sub>26</sub>H<sub>36</sub>BrN<sub>5</sub>O<sub>2</sub> [M + H]<sup>+</sup> 530.21, found 530.1

**Intermediate 96:** (8R,9S,10S)-9-(4-bromophenyl)-3,10-bis[(dimethylamino)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

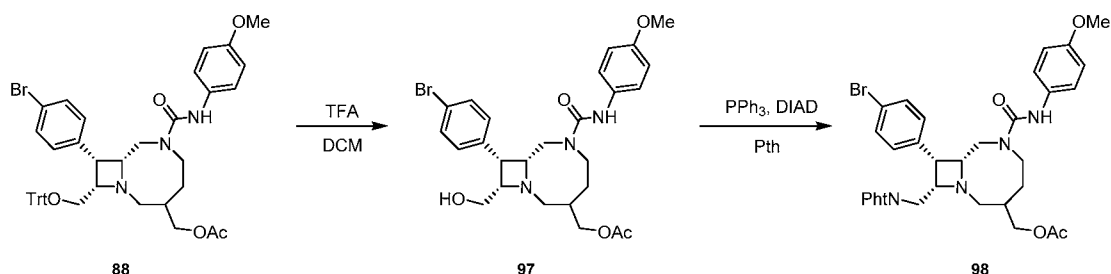
To a stirred solution of Intermediate 95 (120.00 mg, 226.20  $\mu$ mol, 1 eq) in DCM (2  
35 mL) was added HCHO (183.43 mg, 2.26 mmol, 168.28  $\mu$ L, 10 eq) and MgSO<sub>4</sub> (272.28 mg, 2.26 mmol, 10 eq). The resulting reaction mixture was stirred at 25°C for 0.5h. To the mixture was added CH<sub>3</sub>COOH (13.58 mg, 226.20  $\mu$ mol, 1 eq) and NaBH(OAc)<sub>3</sub> (239.71 mg,

10 NMR (400MHz, CDCl<sub>3</sub>-d<sub>1</sub>) δ7.46 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 12.8 Hz, 2H), 6.83 (d, *J* = 8.4 Hz, 2H), 6.12 (s, 1H), 3.77 (s, 3H), 3.61-3.59 (m, 2H), 3.57-3.55 (m, 2H), 3.41-3.39 (m, 2H), 2.78-2.75 (m, 1H), 2.42-2.38 (m, 1H), 2.28-2.26 (m, 2H), 2.16 (s, 6H), 2.00 (m, 1H), 1.76 (m, 1H).

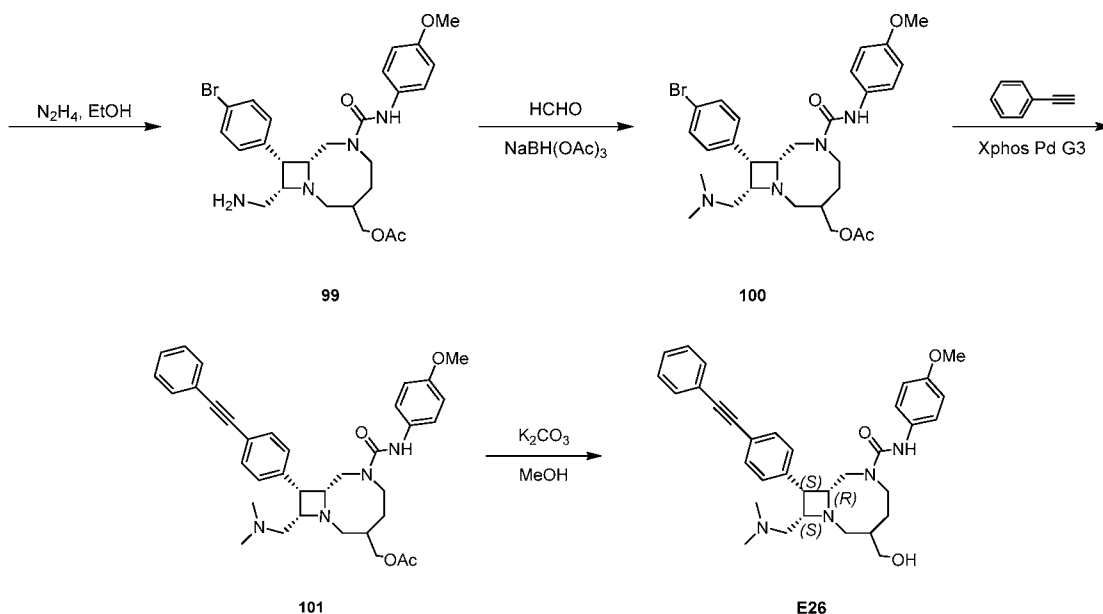
15 [4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E25).

reaction mixture was quenched by H<sub>2</sub>O (5 mL) and extracted with DCM (10 mL\*3) to give the organic layer. The layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the residue. The residue was purified by prep-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (0.225% formic acid) B: acetonitrile) to give Example 25 (10.00 mg, 17.25 μmol, 19.27% yield) as a white solid. HRMS (ESI): calcd for C<sub>36</sub>H<sub>45</sub>N<sub>5</sub>O<sub>2</sub> [M + H]<sup>+</sup> 580.36, found 580.5 <sup>1</sup>H NMR (400MHz, MeOD-d<sub>4</sub>) δ 7.56-7.51 (m, 6H), 7.38-7.37 (m, 2H), 7.37 (d, *J* = 13.2 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 4.02-3.98 (m, 2H), 3.853-3.82 (m, 2H), 3.75 (s, 3H), 3.54-3.51 (m, 1H), 2.99-2.91 (m, 3H), 2.87 (m, 1H), 2.71 (s, 6H), 2.44 (s, 6H), 2.02 (m, 2H), 1.84-1.81 (m, 1H).

**Example 26:** (8R,9S,10S)-10-[(dimethylamino)methyl]-3-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (“E26”)



5



**Intermediate 97:** [(8R,9R,10S)-9-(4-bromophenyl)-10-(hydroxymethyl)-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl]methyl acetate.

To a stirred solution of Intermediate 88 (180.00 mg, 228.20  $\mu$ mol, 1 eq) in DCM (3.6 mL) was TFA (2.28 mmol, 168.96  $\mu$ L, 10 eq). The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was quenched with saturated  $\text{NaHCO}_3$  (10 mL) and extracted with DCM (50 mL \*3) to give organic layer and the layer was concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol = 12:1) to give Intermediate 97 (300.00 mg, crude) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.47-7.45 (m, 1H), 7.38-7.32 (m, 2H), 7.26-7.22 (m, 3H), 6.84-6.82 (d,  $J$  = 8.8 Hz, 2H), 6.08 (s, 1H), 4.24-4.20 (m, 1H), 4.11-4.07 (m, 1H), 3.80-3.77 (m, 4H), 3.65 (s, 4H), 3.55-3.54 (m, 2H), 3.48-3.43 (m, 1H), 2.97 (s, 1H), 2.83 (s, 1H), 2.08-2.03 (m, 4H), 1.96-1.94 (m, 1H), 1.86-1.84 (m, 1H).

**Intermediate 98:** [(8R,9S,10S)-9-(4-bromophenyl)-10-[(1,3-dioxoisindolin-2-yl)methyl]-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl]methyl acetate.

To a solution of Intermediate 97 (300.00 mg, 549.00  $\mu$ mol, 1 eq), isoindoline-1,3-dione (88.85 mg, 603.90  $\mu$ mol, 1.1 eq) and  $\text{PPh}_3$  (216.00 mg, 823.50  $\mu$ mol, 1.5 eq) in THF (2 mL) was added DIAD (166.52 mg, 823.50  $\mu$ mol, 1.5 eq) dropwise at 25°C. The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was concentrated to give the residue. The residue was purified by pre-TLC (petroleum ether: ethyl acetate = 1:1) to give Intermediate 98 (458.00 mg, crude) as a yellow solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.80-7.70 (m, 2H), 7.68-7.65 (m, 12H), 7.49-7.46 (m, 19H), 7.46-7.44 (m, 3H), 6.85-6.80 (m, 3H), 6.06 (s, 1H), 4.12-4.06 (m, 2H), 3.84-3.77 (m,

5 1H), 3.67-3.65 (m, 4H), 3.59-3.55 (m, 5H), 2.87-2.83 (m, 2H), 2.46-2.42 (m, 1H), 1.98 (s, 3H), 1.30-1.26 (m, 3H).

**Intermediate 99:** [(8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl]methyl acetate.

To a solution of Intermediate 98 (458.00 mg, 677.95  $\mu$ mol, 1 eq) in EtOH (2 mL) was  
10 added  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$  (51.95 mg, 1.02 mmol, 98% purity, 1.5 eq) dropwise. The resulting reaction mixture was stirred at 70°C for 1 h. LCMS showed the reaction was complete, the reaction mixture was quenched by  $\text{H}_2\text{O}$  (2 mL) and extracted with DCM (10 mL\*3) to give the organic layer. The layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give the residue. The residue was purified by prep-TLC (dichloromethane: methanol = 12:1) to give  
15 Intermediate 99 (180.00 mg, 48.67% yield) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.47-7.45 (m, 3H), 7.38-7.34 (m, 2H), 7.24-7.22 (m, 2H), 6.84-6.81 (m, 2H), 6.07 (s, 1H), 4.20-4.18 (m, 1H), 4.10-4.09 (m, 1H), 3.77-3.62 (m, 5H), 3.60-3.57 (m, 3H), 3.45-3.33 (m, 2H), 3.35-3.32 (m, 2H), 2.95-2.92 (m, 1H), 2.77-2.76 (m, 2H), 2.55-2.54 (m, 1H), 2.03 (s, 3H), 1.94-1.74 (m, 2H), 1.27-1.24 (m, 2H).

20 **Intermediate 100:** [(8R,9S,10S)-9-(4-bromophenyl)-10-[(dimethylamino)methyl]-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-3-yl]methyl acetate.

To a stirred solution of Intermediate 99 (180.00 mg, 329.99  $\mu$ mol, 1 eq) in DCM (2 mL) was added HCHO (267.83 mg, 3.30 mmol, 245.71  $\mu$ L, 10 eq) and  $\text{MgSO}_4$  (397.21 mg, 3.30 mmol, 10 eq). The resulting reaction mixture was stirred at 25°C for 0.5 h. To the  
25 mixture was added  $\text{CH}_3\text{COOH}$  (19.82 mg, 329.99  $\mu$ mol, 1 eq) and  $\text{NaBH}(\text{OAc})_3$  (349.69 mg, 1.65 mmol, 5 eq). The resulting reaction mixture was stirred at 25°C for 1.5 h. LCMS showed the reaction was complete, the reaction mixture was quenched by  $\text{H}_2\text{O}$  (5 mL) and extract with DCM (20 mL \* 3 ) to give organic layer. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give the residue. The residue was purified by prep-TLC  
30 (dichloromethane: methanol = 12:1) to give Intermediate 100 (134.00 mg, 70.80% yield) as a white solid.  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3\text{-d}_1$ )  $\delta$ 7.49-7.47 (m, 2H), 7.35-7.26 (m, 2H), 7.24-7.22 (m, 2H), 6.84-6.81 (m, 2H), 6.08 (s, 1H), 4.23-4.19 (m, 1H), 3.77 (s, 4H), 3.73-3.58 (m, 4H), 3.60-3.58 (m, 1H), 2.98-2.93(m, 1H), 2.95-2.93 (m, 1H), 2.76 (m, 2H), 2.56-2.51 (m, 2H), 2.05 (s, 6H), 1.92-1.88 (m, 2H).

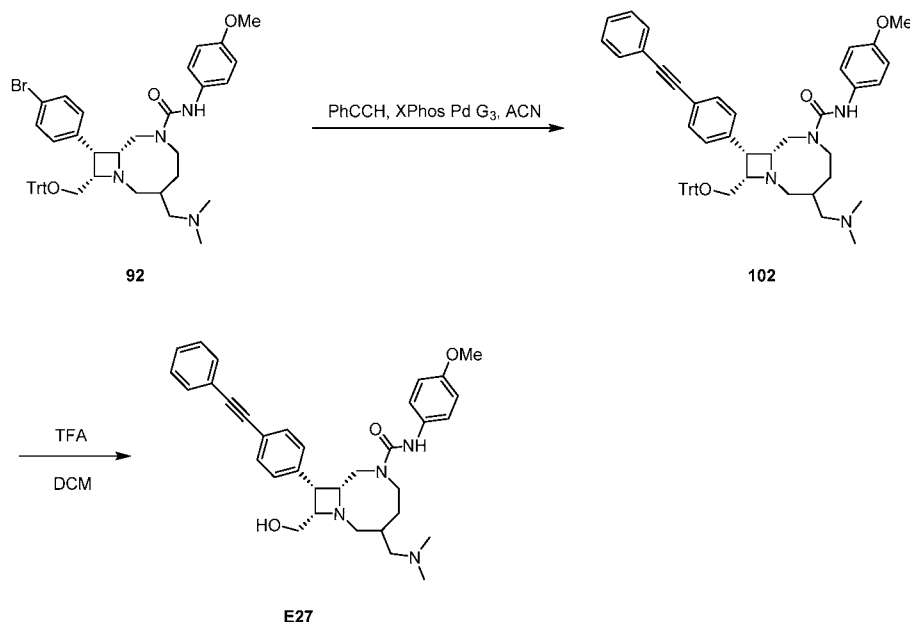
35 **Intermediate 101:** [(8R,9S,10S)-10-[(dimethylamino)methyl]-6-[(4-methoxyphenyl)carbamoyl]-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decan-3-yl]methyl acetate.

5 To a solution of Intermediate 100 (60.00 mg, 104.62  $\mu$ mol, 1 eq) and ethynylbenzene (32.05 mg, 313.85  $\mu$ mol, 34.47  $\mu$ L, 3 eq) in acetonitrile (1 mL) was added XPhos Pd G3 (8.86 mg, 10.46  $\mu$ mol, 0.1 eq) and  $\text{Cs}_2\text{CO}_3$  (68.17 mg, 209.23  $\mu$ mol, 2 eq). The resulting reaction mixture was stirred at 70°C for 2 h. LCMS showed the reaction was complete, the reaction mixture was concentrated to give the residue. The residue was purified by pre-TLC (dichloromethane: methanol=7:1) to give Intermediate 101 (42.00 mg, 67.50% yield) as a brown solid. HRMS (ESI): calcd for  $\text{C}_{36}\text{H}_{42}\text{N}_4\text{O}_4$   $[\text{M} + \text{H}]^+$  595.32, found 565.2

10 **Synthesis of E26:** (8R,9S,10S)-10-[(dimethylamino)methyl]-3-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E26).

15 To a solution of Intermediate 101 (42.00 mg, 70.62  $\mu$ mol, 1 eq) in MeOH (2 mL) was added  $\text{K}_2\text{CO}_3$  (29.28 mg, 211.86  $\mu$ mol, 3 eq). The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was quenched by  $\text{H}_2\text{O}$  (5 mL) and extracted with DCM (10 mL\*3) to give the organic layer. The layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give the residue. The residue was purified by prep-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (0.225% formic acid) B: acetonitrile) to give compound E26 (10.80 mg, 27.67% yield) as a white solid. HRMS (ESI): calcd for  $\text{C}_{34}\text{H}_{40}\text{N}_4\text{O}_3$   $[\text{M} + \text{H}]^+$  553.31, found 553.4  $^1\text{H}$  NMR (400MHz, MeOD- $d_4$ )  $\delta$  7.57-7.51 (m, 6H), 7.39-7.38 (m, 3H), 6.85-6.83 (m, 2H), 3.91-3.82 (m, 1H), 3.76-3.71 (m, 1H), 3.71-3.69 (m, 4H), 3.76 (m, 2H), 3.03-2.94 (m, 3H), 2.59-2.47 (m, 4H), 25 1.95-1.92 (m, 1H), 1.91-1.82 (m, 2H).

- 5 **Example 27:** (8R,9R,10S)-3-[(dimethylamino)methyl]-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E27")



- 10 **Intermediate 102:** (8R,9R,10S)-3-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

- To a solution of Intermediate 92 (30.00 mg, 38.77  $\mu\text{mol}$ , 1 eq) and ethynylbenzene (11.88 mg, 116.31  $\mu\text{mol}$ , 12.77  $\mu\text{L}$ , 3 eq) in acetonitrile (1 mL) was added XPhos Pd G3 (3.28 mg, 3.88  $\mu\text{mol}$ , 0.1 eq) and  $\text{Cs}_2\text{CO}_3$  (25.26 mg, 77.54  $\mu\text{mol}$ , 2 eq). The resulting reaction mixture was stirred at 70°C for 2 h. LCMS showed the reaction was complete, the reaction mixture was concentrated to give the residue. The residue was purified by pre-TLC (dichloromethane: methanol = 7:1) to give Intermediate 102 (50.00 mg, crude) as a brown solid. HRMS (ESI): calcd for  $\text{C}_{53}\text{H}_{54}\text{N}_4\text{O}_3$   $[\text{M} + \text{H}]^+$  795.42, found 795.4
- 15

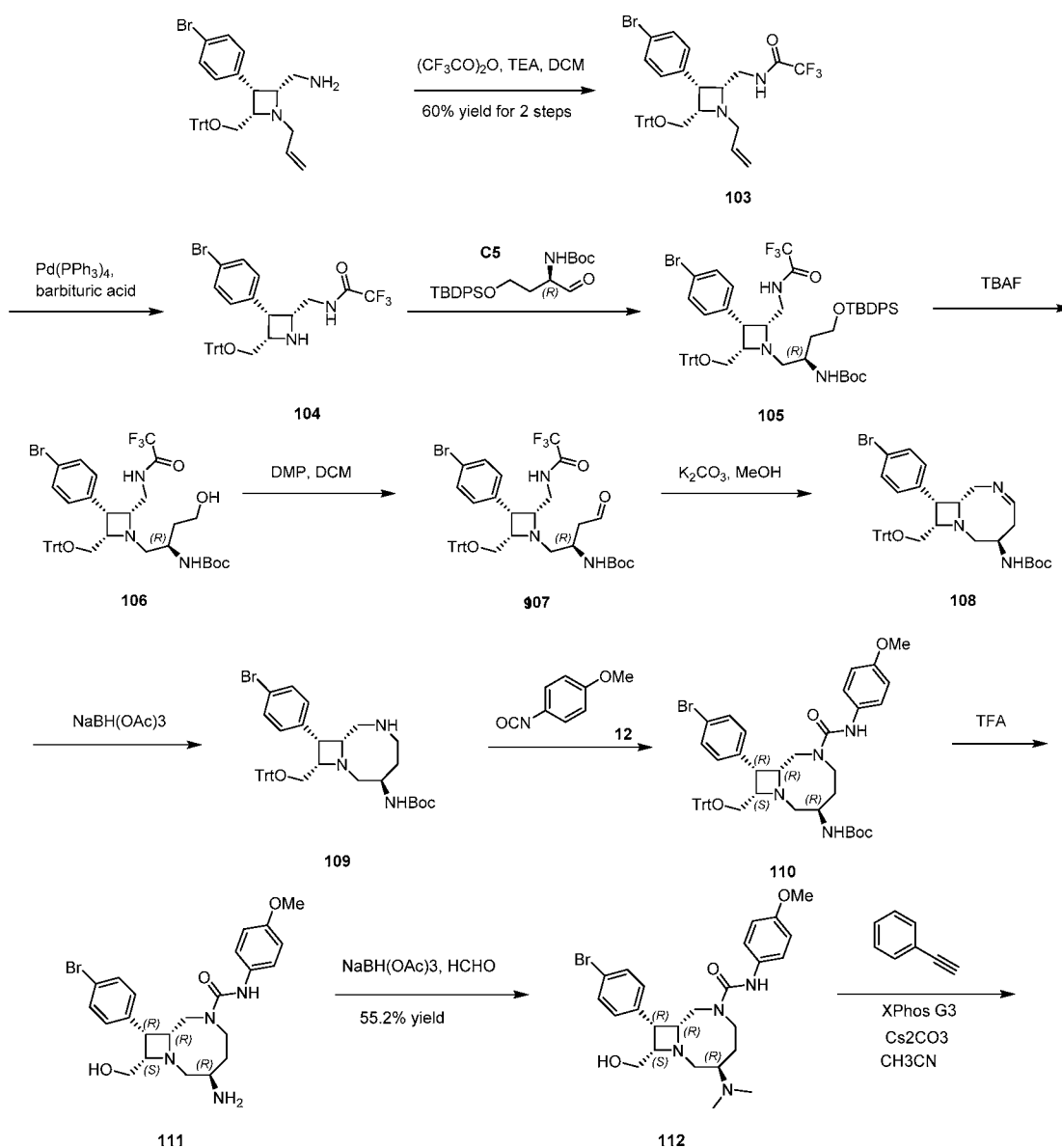
- Synthesis of E27:** (8R,9R,10S)-3-[(dimethylamino)methyl]-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E27).
- 20

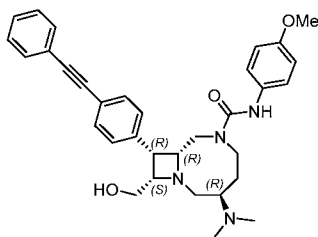
- To a stirred solution of Intermediate 102 (50.00 mg, 62.89  $\mu\text{mol}$ , 1 eq) in DCM (1 mL) was added TFA (71.71 mg, 628.91  $\mu\text{mol}$ , 46.56  $\mu\text{L}$ , 10.00 eq). The resulting reaction mixture was stirred at 25°C for 12 h. LCMS showed the reaction was complete, the reaction mixture was quenched by  $\text{NaHCO}_3$  (5 mL) and extracted with DCM (10 mL\*3) to give the organic layer. The layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to give the residue. The residue was purified by prep-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5 $\mu$ ; A: water (0.225% formic acid) B: acetonitrile) to give compound E27 (4.70 mg,
- 25

5 13.52% yield) as a white solid. HRMS (ESI): calcd for  $C_{33}H_{37}N_4O_2$   $[M + H]^+$  553.29, found 553.4

$^1H$  NMR (400MHz, MeOD- $d_4$ )  $\delta$  7.544-7.51 (m, 6H), 7.39-7.37 (m, 3H), 7.34-7.22 (m, 2H), 6.86-6.81 (m, 2H), 4.02-3.99 (m, 1H), 3.76-3.72 (m, 4H), 3.70-3.66 (m, 1H), 3.41-3.40 (m, 1H), 3.38-3.35 (m, 1H), 3.12 (m, 1H), 2.85-2.75 (m, 1H), 2.16-2.09 (m, 2H), 1.81-1.77 (m, 1H).

**Example 28:** (3R,8R,9R,10S)-3-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E28")





E28

**Intermediate 103:** N-[[[(2R,3R,4S)-1-allyl-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-2-yl]methyl]-2,2,2-trifluoro-acetamide.

To a solution of [(2R,3R,4S)-1-allyl-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-2-yl]methanamine (J.O.C. (2012), 77(17), 7187-7211) (25.00 g, 31.62 mmol, 1.00 *eq*) in DCM (250.00 mL) was added TEA (25.59 g, 252.92 mmol, 35.06 mL, 8.00 *eq*), then it was cooled to 0° C. TFAA (26.56 g, 126.46 mmol, 17.59 mL, 4.00 *eq*) was added to the reaction mixture dropwise. After stirring at 20°C for 16 h, the reaction mixture was quenched by NaHCO<sub>3</sub> solution (100 mL), extracted with DCM (50 mL x 3), the combined organic layer was concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=20/1 to 1:1) yielding Intermediate 103 (11.00 g, 16.94 mmol, 53.56% yield) as a yellow solid. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.87 - 3.08 (m, 2 H) 3.09 - 3.24 (m, 2 H) 3.30 - 3.51 (m, 3 H) 3.57 - 3.69 (m, 2 H) 5.10 (br d, *J*=10.14 Hz, 1 H) 5.18 - 5.33 (m, 1 H) 5.80 (dddd, *J*=17.28, 10.01, 7.44, 5.40 Hz, 1 H) 6.11 (br s, 1 H) 7.17 - 7.23 (m, 16 H) 7.33 (d, *J*=8.38 Hz, 2 H)

**Intermediate 104:** N-[[[(2R,3R,4S)-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-2-yl]methyl]-2,2,2-trifluoro-acetamide.

To a solution of Intermediate 103 (11.00 g, 16.94 mmol, 1.00 *eq*) in EtOH (169.00 mL) was added 1,3-dimethylbarbituric acid (3.97 g, 25.41 mmol, 1.50 *eq*) and Pd(PPh<sub>3</sub>)<sub>4</sub> (1.96 g, 1.69 mmol, 0.10 *eq*) under N<sub>2</sub> atmosphere. The mixture was stirred at 40°C for 16 h. The reaction mixture was quenched by NaHCO<sub>3</sub> solution (200 mL), extracted with DCM (50 mL x 3), the combined organic layer was concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=10/1 to 5:1) yielding Intermediate 104 (7.80 g, 12.80 mmol, 75.55% yield as a yellow solid. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.26 (br s, 1 H) 2.20 - 2.31 (m, 1 H) 2.99 - 3.07 (m, 1 H) 3.10 - 3.23 (m, 2 H) 3.30 - 3.39 (m, 1 H) 3.76 (br t, *J*=7.28 Hz, 1 H) 4.27 - 4.49 (m, 2 H) 6.10 (br s, 1 H) 7.17 - 7.24 (m, 16 H) 7.32 - 7.38 (m, 2 H)

**Intermediate 105:** tert-butyl N-[(1R)-1-[[[(2R,3R,4S)-3-(4-bromophenyl)-2-[[[(2,2,2-trifluoroacetyl)amino]methyl]-4-(trityloxymethyl)azetidin-1-yl]methyl]-3-[tert-

5 butyl(diphenyl)silyl]oxy-propyl]carbamate.

To a solution of Intermediate 104 (4.00 g, 6.56 mmol, 1.00 *eq*), tert-butyl N-[(1R)-3-[tert-butyl(diphenyl)silyl]oxy-1-formyl-propyl]carbamate (synthesized as described in Bioorganic & Medicinal Chemistry (2006), 14(1), 214-236, hereby incorporated by reference in its entirety) (2.90 g, 6.56 mmol, 1.00 *eq*) in DCM (50.00 mL) was added MgSO<sub>4</sub> (158.00  
10 mg, 1.31 mmol, 20.00 *eq*) and NaBH(OAc)<sub>3</sub> (139.10 mg, 656.30 μmol, 10.00 *eq*). After stirring at 20°C for 16 h, additional tert-butyl N-[(1R)-3-[tert-butyl(diphenyl)silyl]oxy-1-formyl-propyl]carbamate (1 g) was added. After an additional 0.5h, the reaction mixture was quenched by NaHCO<sub>3</sub> solution (200 mL), extracted with DCM (50 mL x 3), the combined organic layer was concentrated to give a residue. The residue was purified by  
15 column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=20/1 to 10:1) to give Intermediate 105 (4.20 g, 4.06 mmol, 61.85% yield as a yellow gum. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.01 - 1.05 (m, 9 H) 1.41 - 1.47 (m, 10 H) 1.63 - 1.78 (m, 1 H) 1.82 - 1.92 (m, 1 H) 2.65 - 2.76 (m, 1 H) 2.82 - 3.01 (m, 2 H) 3.13 (br dd, *J*=9.60, 6.09 Hz, 1 H) 3.29 - 3.79 (m, 7 H) 4.67 (br d, *J*=9.29 Hz, 1 H) 7.11 - 7.25 (m, 17 H) 7.31 - 7.50 (m, 8 H)  
20 7.63 (td, *J*=7.37, 1.44 Hz, 4 H) 8.40 - 8.73 (m, 1 H)

**Intermediate 106:** tert-butyl N-[(1R)-1-[(2R,3R,4S)-3-(4-bromophenyl)-2-[(2,2,2-trifluoroacetyl)amino]methyl]-4-(trityloxymethyl)azetidin-1-yl]methyl]-3-hydroxy-propyl]carbamate.

To a solution of Intermediate 105 (4.20 g, 4.06 mmol, 1.00 *eq*) in THF (50.00  
25 mL) was added TBAF (1.59 g, 6.09 mmol, 1.50 *eq*). After stirring at 20°C for 16 hour, the reaction mixture was concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=10/1 to 2:1) to give Intermediate 106 (2.30 g, 2.89 mmol, 71.23% yield as a yellow solid. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.41 (s, 3 H) 1.45 - 1.53 (m, 9 H) 1.58 - 1.77 (m, 2 H) 2.49 (br dd, *J*=12.74, 4.33 Hz, 2  
30 H) 2.82 (t, *J*=12.17 Hz, 1 H) 2.88 - 2.98 (m, 2 H) 3.08 - 3.29 (m, 3 H) 3.47 (td, *J*=7.87, 3.20 Hz, 1 H) 3.60 - 3.72 (m, 1 H) 4.51 (br d, *J*=9.41 Hz, 1 H) 7.12 (d, *J*=8.41 Hz, 1 H) 7.09 - 7.14 (m, 1 H) 7.16 - 7.26 (m, 17 H) 7.34 (d, *J*=8.41 Hz, 1 H) 8.13 (br s, 1 H)

**Intermediate 107:** tert-butyl N-[(1R)-1-[(2R,3R,4S)-3-(4-bromophenyl)-2-[(2,2,2-trifluoroacetyl)amino]methyl]-4-(trityloxymethyl)azetidin-1-yl]methyl]-3-oxo-propyl]carbamate.  
35

To a solution of Intermediate 106 (2.30 g, 2.89 mmol, 1.00 *eq*) in DCM (30.00 mL) was added DMP (2.45 g, 5.78 mmol, 1.79 mL, 2.00 *eq*) at 0°C under N<sub>2</sub>. The mixture

5 was stirred at 0°C for 1 hour. LCMS showed reactant 106 was consumed completely and the majority was the desired product, the reaction mixture was quenched by a solution of NaHCO<sub>3</sub> (2.5 g) and Na<sub>2</sub>SO<sub>3</sub> (2.5 g) in H<sub>2</sub>O (20 mL), extracted with DCM (20 mL x 3), the combined organic layer was concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=2/1 to 0:1) to give Intermediate 107 (1.80 g, 2.27 mmol, 78.37% yield as a yellow solid. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.47 (s, 9 H) 1.51 - 1.69 (m, 3 H) 2.46 - 2.54 (m, 2 H) 2.78 - 2.88 (m, 1 H) 2.89 - 3.00 (m, 2 H) 3.08 - 3.20 (m, 2 H) 3.49 - 3.69 (m, 4 H) 3.91 - 4.07 (m, 1 H) 4.50 - 4.76 (m, 1 H) 7.08 - 7.13 (m, 3 H) 7.17 - 7.26 (m, 22 H) 8.25 (br s, 1 H) 9.63 (s, 1 H)

10 **Intermediate 108:** tert-butyl N-[(3R,5Z,8R,9R,10S)-9-(4-bromophenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]dec-5-en-3-yl]carbamate.

15 To a solution of Intermediate 107 (1.70 g, 2.14 mmol, 1.00 *eq*) in MeOH (20.00 mL) and H<sub>2</sub>O (77.13 mg, 4.28 mmol, 77.13 μL, 2.00 *eq*) was added K<sub>2</sub>CO<sub>3</sub> (591.32 mg, 4.28 mmol, 2.00 *eq*). After stirring at 20°C for 16 h, additional K<sub>2</sub>CO<sub>3</sub> (200 mg) was added. After 2 h additional, the reaction mixture was dissolved in water (30 mL), extracted with DCM (20 mL x 3), the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, then concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=5/1 to 0:1) to give Intermediate 108 (750.00 mg, 771.30 μmol, 45.05% yield, 70% purity) as a yellow solid.

20 **Intermediate 109:** tert-butyl N-[(3R,8R,9R,10S)-9-(4-bromophenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl]carbamate.

25 To a solution of Intermediate 108 (750.00 mg, 1.10 mmol, 1.00 *eq*) in DCM (18.00 mL) was added AcOH (66.17 mg, 1.10 mmol, 63.02 μL, 1.00 *eq*) and NaBH(OAc)<sub>3</sub> (2.34 g, 11.02 mmol, 10.00 *eq*). The mixture was stirred at 20°C for 0.5 hour. LCMS showed reactant 108 was consumed completely, the majority was the desired product, the reaction mixture was quenched by NaHCO<sub>3</sub> solution (20 mL), extracted with DCM (10 mL x 3), the combined organic layer was concentrated to give Intermediate 109 as a yellow solid (750 mg, crude), which was used directly in next step.

30 **Intermediate 110:** tert-butyl N-[(3R,8R,9R,10S)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-3-yl]carbamate.

35 To a solution of Intermediate 109 (750.00 mg, 769.02 μmol, 1.00 *eq*) in DCM (10.00 mL) was added TEA (38.91 mg, 384.51 μmol, 53.30 μL, 0.50 *eq*) and 1-isocyanato-4-

5 methoxy-benzene (137.64 mg, 922.82  $\mu\text{mol}$ , 118.66  $\mu\text{L}$ , 1.20 *eq*). The mixture was stirred at 20°C for 0.5 hour. LCMS showed reactant 109 was consumed completely, and the majority was the desired product. The reaction mixture was concentrated to give a residue. The residue was purified by column chromatography ( $\text{SiO}_2$ , petroleum ether/ethyl acetate), further purified by pre-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5 $\mu$ ; A: water  
10 (0.225% formic acid) B: acetonitrile). Intermediate 110 (150.00 mg, 180.33  $\mu\text{mol}$ , 23.45% yield) was obtained as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CHLOROFORM-}d$ )  $\delta$  ppm 1.45 (br d,  $J=6.15$  Hz, 9 H) 2.18 (br s, 2 H) 3.20 - 3.68 (m, 3 H) 3.72 - 4.51 (m, 11 H) 5.14 - 5.42 (m, 1 H) 6.78 (br s, 1 H) 6.88 (br t,  $J=7.97$  Hz, 2 H) 7.22 - 7.38 (m, 20 H) 7.47 - 7.53 (m, 1 H).  
**Intermediate 111:** (3R,8R,9R,10S)-3-amino-9-(4-bromophenyl)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.  
15

To a solution of Intermediate 110 (120.00 mg, 144.26  $\mu\text{mol}$ , 1.00 *eq*) in DCM (3.00 mL) was added TFA (1.54 g, 13.51 mmol, 1.00 mL, 93.63 *eq*) at 0°C. The mixture was stirred at 20°C for 1 hour. LCMS showed reactant 110 consumed completely, the majority was the desired product, the reaction mixture was quenched by  $\text{NaHCO}_3$  solution (20 mL),  
20 then washed with  $\text{NH}_3\cdot\text{H}_2\text{O}$  (1 mL), extracted with DCM (10 mL x 3), the combined organic layer was concentrated to give a residue. The residue was purified by prep-TLC ( $\text{SiO}_2$ , DCM: MeOH = 10:1) to give Intermediate 111 (60.00 mg, 122.60  $\mu\text{mol}$ , 84.98% yield) as a white solid.

**Intermediate 112:** (3R,8R,9R,10S)-9-(4-bromophenyl)-3-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.  
25

To a solution of Intermediate 111 (60.00 mg, 122.60  $\mu\text{mol}$ , 1.00 *eq*), HCHO (99.50 mg, 1.23 mmol, 91.28  $\mu\text{L}$ , 10.00 *eq*) in DCM (3.00 mL) was added  $\text{MgSO}_4$  (147.57 mg, 1.23 mmol, 10.00 *eq*) and  $\text{NaBH}(\text{OAc})_3$  (259.84 mg, 1.23 mmol, 10.00 *eq*) at 20°C. The mixture was stirred at 20°C for 1 hour. LCMS showed reactant 111 consumed completely, the  
30 majority was the desired product, the reaction mixture was quenched by  $\text{NaHCO}_3$  solution (10 mL), extracted with DCM (5 mL x 3), the combined organic layer was concentrated to give a residue. The residue was purified by prep-TLC ( $\text{SiO}_2$ , DCM: MeOH = 10:1) to give Intermediate 112 (38.00 mg, 73.44  $\mu\text{mol}$ , 59.90% yield) as a yellow solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CHLOROFORM-}d$ )  $\delta$  ppm 1.84 - 1.99 (m, 1 H) 2.49 (br s, 6 H) 2.55 (br d,  $J=9.26$  Hz, 2 H) 2.88 (br d,  $J=11.69$  Hz, 1 H) 3.02 - 3.16 (m, 3 H) 3.41 (br dd,  $J=11.58$ , 4.52 Hz, 1 H) 3.51 - 3.61 (m, 3 H) 3.62 - 3.86 (m, 6 H) 3.98 - 4.17 (m, 1 H) 4.73 (s, 1 H) 6.78 - 6.91 (m, 2 H) 7.23 (d,  $J=8.82$  Hz, 2 H) 7.34 - 7.53 (m, 4 H).  
35

5 **Synthesis of E28:** (3R,8R,9R,10S)-3-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E28).

To a solution of Intermediate 112 (25.00 mg, 48.31  $\mu$ mol, 1.00 *eq*), ethynylbenzene (14.80 mg, 144.93  $\mu$ mol, 15.91  $\mu$ L, 3.00 *eq*) in CH<sub>3</sub>CN (350.00  $\mu$ L) was added Cs<sub>2</sub>CO<sub>3</sub> (47.22 mg, 144.93  $\mu$ mol, 3.00 *eq*) and XPhos Pd G3 (4.09 mg, 4.83  $\mu$ mol, 0.10 *eq*) under N<sub>2</sub> atmosphere. The mixture was stirred at 70°C for 3 hour. LCMS showed reactant 1 consumed completely, the majority was the desired product, the reaction mixture was dissolved in water (2 mL), extracted with DCM(2 mL x 3), the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, then concentrated to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1), then further purified by pre-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (0.225% formic acid) B: acetonitrile) to give compound E28 (13.00 mg, 24.13  $\mu$ mol, 49.95% yield as a formic acid salt) as a white solid. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*)  $\delta$  ppm 1.88 (br d, *J*=7.65 Hz, 1 H) 2.38 (s, 7 H) 2.49 (br dd, *J*=13.30, 9.29 Hz, 3 H) 2.71 (br s, 1 H) 2.79 - 2.96 (m, 1 H) 3.09 (br d, *J*=15.94 Hz, 1 H) 3.25 (br dd, *J*=13.30, 5.02 Hz, 1 H) 3.41 - 3.73 (m, 6 H) 3.78 (s, 3 H) 3.90 - 4.03 (m, 1 H) 6.84 (d, *J*=8.91 Hz, 2 H) 7.21 (d, *J*=8.91 Hz, 2 H) 7.32 - 7.40 (m, 3 H) 7.46 - 7.57 (m, 6 H).

**Example 29:** (3S,8R,9R,10S)-3-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E29")

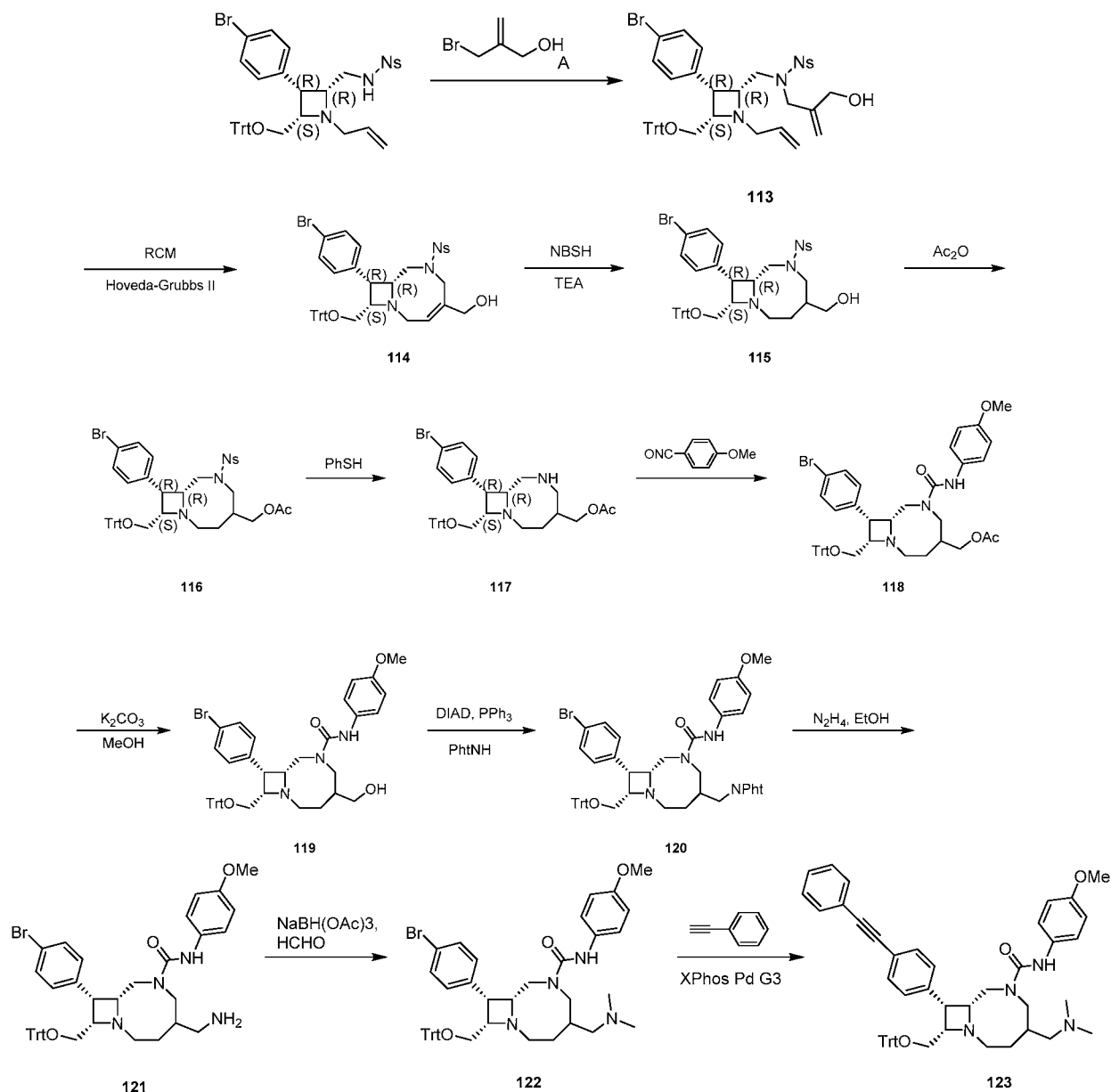
25 Synthesis was performed similarly to that described in Example 28 after the synthesis of Intermediate 105 (Intermediate 105) utilizing the enantiomeric *tert*-butyl N-[(1S)-3-[*tert*-butyl(diphenyl)silyl]oxy-1-formyl-propyl]carbamate (Bioorganic & Medicinal Chemistry (2006)) in place of Intermediate 105. <sup>1</sup>H NMR (400MHz, CHLOROFORM-*d*)  $\delta$  = 7.64 - 7.46 (m, 6H), 7.44 - 7.34 (m, 3H), 7.33 - 7.29 (m, 1H), 7.29 - 7.25 (m, 2H), 6.85 (d, *J*=8.9 Hz, 2H), 6.29 (br s, 1H), 3.86 (br d, *J*=10.8 Hz, 1H), 3.80 (s, 3H), 3.78 - 3.72 (m, 2H), 3.72 - 3.65 (m, 1H), 3.65 - 3.54 (m, 4H), 3.21 - 3.11 (m, 1H), 2.99 - 2.87 (m, 1H), 2.72 - 2.60 (m, 2H), 2.37 (s, 6H), 2.15 (br s, 1H), 2.01 (br d, *J*=7.9 Hz, 1H).

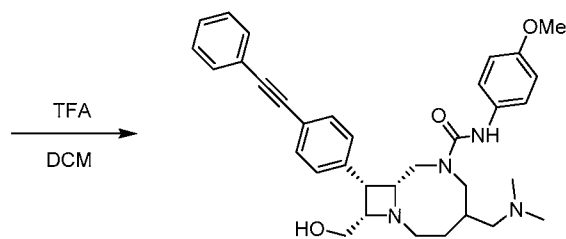
**Example 30:** (4R,8R,9R,10S)-4-(dimethylamino)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E30")

Synthesis was performed as shown in Example 3 utilizing the enantiomeric (*R*)-*tert*-butyl (1-((*tert*-butyldiphenylsilyl)oxy)-4-oxobutan-2-yl)carbamate (US 20150266867) as the

- 5 starting material.  $^1\text{H}$  NMR (400MHz,  $\text{CHLOROFORM-d}$ )  $\delta$  = 7.59 - 7.54 (m, 2H), 7.52 - 7.45 (m, 4H), 7.41 - 7.30 (m, 5H), 6.89 - 6.80 (m, 2H), 3.80 (s, 3H), 3.77 - 3.58 (m, 5H), 3.57 (br d,  $J=8.2$  Hz, 1H), 3.51 - 3.43 (m, 2H), 3.29 (br d,  $J=13.6$  Hz, 1H), 3.02 (br s, 1H), 2.67 - 2.44 (m, 2H), 2.34 (s, 6H), 1.81 (br s, 1H), 1.66 (br s, 1H).

**Example 31:** (8R,9R,10S)-4-((dimethylamino)methyl)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (“E31”)





E31

**Intermediate 113:** N-[[[(2R,3R,4S)-1-allyl-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-2-yl]methyl]-N-[2-(hydroxymethyl)allyl]-2-nitro-benzenesulfonamide.

To a solution of N-[[[(2R,3R,4S)-1-allyl-3-(4-bromophenyl)-4-(trityloxymethyl)azetidin-2-yl]methyl]-2-nitro-benzenesulfonamide (WO2015070204) (5.00 g, 6.77 mmol, 1.00 eq) in DMF (62.5 mL) was added K<sub>2</sub>CO<sub>3</sub> (2.11 g, 15.23 mmol, 2.25 eq) and cooled to 0°C, whereby 2-(bromomethyl)prop-2-en-1-ol (2.04 g, 13.54 mmol, 2.00eq) in DMF (7.5 mL) was added. The mixture was stirred at 20°C for 16 hour. LC-MS showed Reactant 113 was consumed completely and desired MS was detected. The reaction mixture was quenched by addition water (300 mL) and extracted with ethyl acetate (100mL \* 2). The combined organic layers were washed with brine (100 mL \* 2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=20:1 to 2:1) to afford Intermediate 113 (5.00 g, 6.18 mmol, 91.32% yield) as a white solid: <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ = 8.00 (s, 1H), 7.68 - 7.62 (m, 1H), 7.60 - 7.46 (m, 3H), 7.30 - 7.23 (m, 3H), 7.19 - 7.12 (m, 10H), 7.07 (dd, J=2.9, 6.8 Hz, 6H), 5.57 (tdd, J=6.6, 10.4, 17.0 Hz, 1H), 5.10 - 5.00 (m, 2H), 4.89 (d, J=10.1 Hz, 1H), 4.69 (s, 1H), 3.91 (d, J=8.8 Hz, 3H), 3.70 - 3.61 (m, 1H), 3.54 - 3.38 (m, 3H), 3.26 - 3.14 (m, 3H), 2.98 (dd, J=4.4, 9.3 Hz, 1H), 2.85 - 2.73 (m, 2H)

**Intermediate 114:** [(3E,8R,9R,10S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]dec-3-en-4-yl]methanol.

To a solution of Intermediate 113 (5.00 g, 6.18 mmol, 1.00 eq) in DCM (500.00 mL) was added Hoveyda-Grubbs Catalyst 2<sup>nd</sup> Generation (774.77 mg, 1.24 mmol, 0.20 eq). The mixture was stirred at 55°C for 2 hour. TLC (petroleum ether: ethylacetate=1:1) showed Intermediate 113 was consumed completely and desired MS was detected. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=20:1 to 1:1) to afford Intermediate 114 (4.00 g, 5.12 mmol, 82.90% yield) as a brown solid: <sup>1</sup>H NMR (400MHz,

5 CHLOROFORM-d)  $\delta$  = 7.88 - 7.83 (m, 1H), 7.64 - 7.51 (m, 3H), 7.18 (d, J=6.0 Hz, 2H), 7.13 (s, 14H), 7.06 (d, J=8.5 Hz, 2H), 5.81 (t, J=6.3 Hz, 1H), 4.07 - 4.02 (m, 3H), 3.96 - 3.88 (m, 1H), 3.59 - 3.32 (m, 6H), 3.19 (d, J=14.1 Hz, 1H), 3.09 - 2.93 (m, 3H), 2.83 - 2.79 (m, 1H).

**Intermediate 115:** [(8R,9R,10S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl]methanol.

To a solution of Intermediate 114 (4.00 g, 5.12 mmol, 1.00 eq) in THF (51.00 mL) was added 2-nitrobenzenesulfonohydrazide (5.56 g, 25.60 mmol, 4.00eq) and TEA (5.18 g, 51.23 mmol, 7.10 mL, 10.00 eq). After stirring at 40°C for 16 hour, the reaction mixture was quenched by addition water (100 mL) and extracted with ethyl acetate (50 mL \* 2). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=10/1 to 1:1) to afford Intermediate 115 (3.60 g, crude) as a white solid.

**Intermediate 116:** [(8R,9R,10S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl]methyl acetate.

To a solution of Intermediate 115 (3.60 g, 4.60 mmol, 1.00 eq) in DCM (80.00 mL) was added DMAP (56.19 mg, 460.00  $\mu$ mol, 0.1 eq) and TEA (465.40 mg, 4.60 mmol, 637.53  $\mu$ L, 1.00 eq), then Ac<sub>2</sub>O (939.07 mg, 9.20 mmol, 861.53  $\mu$ L, 2.00 eq) at 0°C. After stirring at 20°C for 2 h, the reaction mixture was quenched by addition sat. NaHCO<sub>3</sub> aqueous (150 mL) and extracted with DCM (50mL \* 2). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=30/1 to 1:1) to afford Intermediate 116 (3.00 g, 3.64 mmol, 79.07% yield) as a white solid.

**Intermediate 117:** [(8R,9R,10S)-9-(4-bromophenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl]methyl acetate.

To a solution of Intermediate 116 (3.00 g, 3.64 mmol, 1.00 eq) in acetonitrile (80.00 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (1.42 g, 4.37 mmol, 1.20 eq) and benzenethiol ("PhSH") (601.14 mg, 5.46 mmol, 1.50 eq) at 0°C. After stirring at 20°C for 16 h, the reaction mixture was quenched by addition water (100 mL) and extracted with ethyl acetate (30 mL \* 2). The combined organic layers were washed with brine (50 mL \* 2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by

5 column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=10:1 to 1:1) to afford Intermediate 117 (2.00 g, 3.13 mmol, 85.90% yield) as a white solid.

**Intermediate 118:** [(8R,9R,10S)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decan-4-yl]methyl acetate.

To a solution of Intermediate 117 (2.00 g, 3.13 mmol, 1.00 eq) in DCM (50.00 mL) was added 1-isocyanato-4-methoxy-benzene (560.21 mg, 3.76 mmol, 1.20 eq) at 0°C. The mixture was stirred at 15°C for 2 hour. LC-MS showed Intermediate 117 was consumed completely and desired MS was detected. The reaction mixture was quenched by addition water (100 mL) and extracted with DCM (30mL \* 2). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 100: 1 to 20:1) to afford Intermediate 118 (2.20 g, 2.79 mmol, 89.11% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ = 7.28 - 7.16 (m, 23H), 6.83 (d, J=9.0 Hz, 2H), 4.01 - 3.95 (m, 1H), 3.92 - 3.85 (m, 1H), 3.80 - 3.73 (m, 5H), 3.63 - 3.54 (m, 2H), 3.42 (t, J=8.0 Hz, 1H), 3.20 - 2.90 (m, 5H), 2.74 (dd, J=11.0, 14.1 Hz, 1H), 2.50 - 2.40 (m, 1H), 2.06 (s, 3H), 1.66 (dd, J=6.8, 13.8 Hz, 1H), 1.53 - 1.45 (m, 1H).

**Intermediate 119:** (8R,9R,10S)-9-(4-bromophenyl)-4-(hydroxymethyl)-N-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 118 (200.00 mg, 253.56 μmol, 1.00 eq) in DCM (1.00 mL) and MeOH (1 mL) was added K<sub>2</sub>CO<sub>3</sub> (175.22 mg, 1.27 mmol, 5.00 eq). The mixture was stirred at 15°C for 4 hour. LC-MS showed Intermediate 118 was consumed completely and one main peak with desired MS was detected. The reaction mixture was quenched by addition water (10 mL) and extracted with DCM (5 mL \* 2). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=10:1 to 1:1) to afford Intermediate 119 (150.00 mg, 200.88 μmol, 79.22% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ = 7.37 (d, J=8.5 Hz, 2H), 7.27 (s, 4H), 7.22 (s, 15H), 6.80 (d, J=8.5 Hz, 2H), 3.90 (d, J=16.1 Hz, 2H), 3.77 (s, 3H), 3.70 - 3.57 (m, 3H), 3.43 - 3.32 (m, 2H), 3.19 - 3.07 (m, 2H), 3.05 - 2.94 (m, 2H), 2.75 (d, J=11.5 Hz, 1H), 2.47 (t, J=12.0 Hz, 1H), 1.89 (br. s., 1H), 1.52 - 1.38 (m, 2H).

**Intermediate 120:** (8R,9R,10S)-9-(4-bromophenyl)-4-[(1,3-dioxoisindolin-2-yl)methyl]-N-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of PPh<sub>3</sub> (0.15 g) in 3 mL THF was added DIAD (0.11 mL) at 0°C. After 5 minutes, this mixture was added to a solution of Intermediate 119 (20.00 mg, 26.78 μmol, 1.00 eq) and isoindoline-1,3-dione (5.91 mg, 40.17 μmol, 1.50 eq) in THF (1 mL) at 0°C. The mixture was stirred at 15°C for 16h. LC-MS showed Intermediate 9 was consumed completely and desired MS was detected. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, petroleum ether/ethyl acetate=1:1) to afford Intermediate 120 (10.00 mg, 11.42 μmol, 42.63% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ = 7.87 (dd, J=3.0, 5.5 Hz, 1H), 7.75 (dd, J=3.3, 5.3 Hz, 2H), 7.32 - 7.28 (m, 7H), 7.27 - 7.20 (m, 15H), 6.80 (d, J=9.0 Hz, 2H), 6.17 (s, 1H), 3.83 - 3.75 (m, 3H), 3.74 - 3.56 (m, 5H), 3.52 (d, J=7.5 Hz, 1H), 3.24 - 3.12 (m, 3H), 3.03 - 2.96 (m, 1H), 2.83 - 2.74 (m, 1H), 2.45 - 2.30 (m, 2H), 1.83 (d, J=7.5 Hz, 1H).

**Intermediate 121:** (8R,9R,10S)-4-(aminomethyl)-9-(4-bromophenyl)-N-(4-methoxyphenyl)-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 120 (500.00 mg, 570.87 μmol, 1.00 eq) in EtOH (10.00 mL) was added N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O (28.58 mg, 570.87 μmol, 27.75 μL, 1.00 eq). The mixture was stirred at 70°C for 1 hour. LC-MS showed Intermediate 120 was consumed and desired MS was detected. The reaction mixture was quenched by addition water (30 mL) and extracted with DCM (10 mL \* 2). The combined organic layers were washed with brine (10 mL \* 2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 100:1 to 10:1) to afford Intermediate 121 (150.00 mg, crude) as a yellow solid contain Ph<sub>3</sub>PO. <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ = 7.64 - 7.56 (m, 4H), 7.51 - 7.45 (m, 3H), 7.39 (dt, J=2.8, 7.4 Hz, 5H), 7.14 (s, 13H), 6.71 (d, J=9.0 Hz, 2H), 3.79 - 3.62 (m, 4H), 3.56 - 3.46 (m, 2H), 3.31 (br. s., 1H), 3.11 - 2.85 (m, 5H), 2.74 (d, J=12.5 Hz, 1H), 2.44 - 2.18 (m, 2H), 1.62 - 1.39 (m, 3H).

**Intermediate 122:** (8R,9R,10S)-9-(4-bromophenyl)-4-((dimethylamino)methyl)-N-(4-methoxyphenyl)-10-((trityloxy)methyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 121 (150.00 mg, 201.14 μmol, 1.00 eq) in DCM (5.00 mL) was added MgSO<sub>4</sub> (290.53 mg, 2.41 mmol, 12.00eq) and HCHO (98.21 mg, 1.21 mmol, 90.10 μL, 37% purity, 6.02 eq) then NaBH(OAc)<sub>3</sub> (639.44 mg, 3.02 mmol, 15.00 eq). The mixture was stirred at 15°C for 16 hour. LC-MS showed Intermediate 121 was consumed and one desired MS was detected. The reaction mixture was quenched by addition sat. NaHCO<sub>3</sub> aqueous (20 mL) and extracted with DCM (5 mL \* 2). The combined organic layers were

5 dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 20:1) to afford Intermediate 122 (60.00 mg, 77.54 μmol, 38.55% yield) as a yellow solid. <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ = 7.24 - 7.08 (m, 24H), 6.72 (d, J=9.0 Hz, 2H), 3.88 (d, J=14.1 Hz, 1H), 3.67 (s, 3H), 3.57 - 3.48 (m, 3H), 3.33 - 3.25 (m, 1H), 3.10 - 2.86 (m, 5H), 2.64 (dd, J=10.8, 14.3 Hz, 1H), 2.44 - 2.36 (m, 1H), 2.21 (s, 6H), 1.90 (d, J=12.0 Hz, 1H), 1.51 (br. s., 2H), 1.36 (d, J=4.5 Hz, 2H).

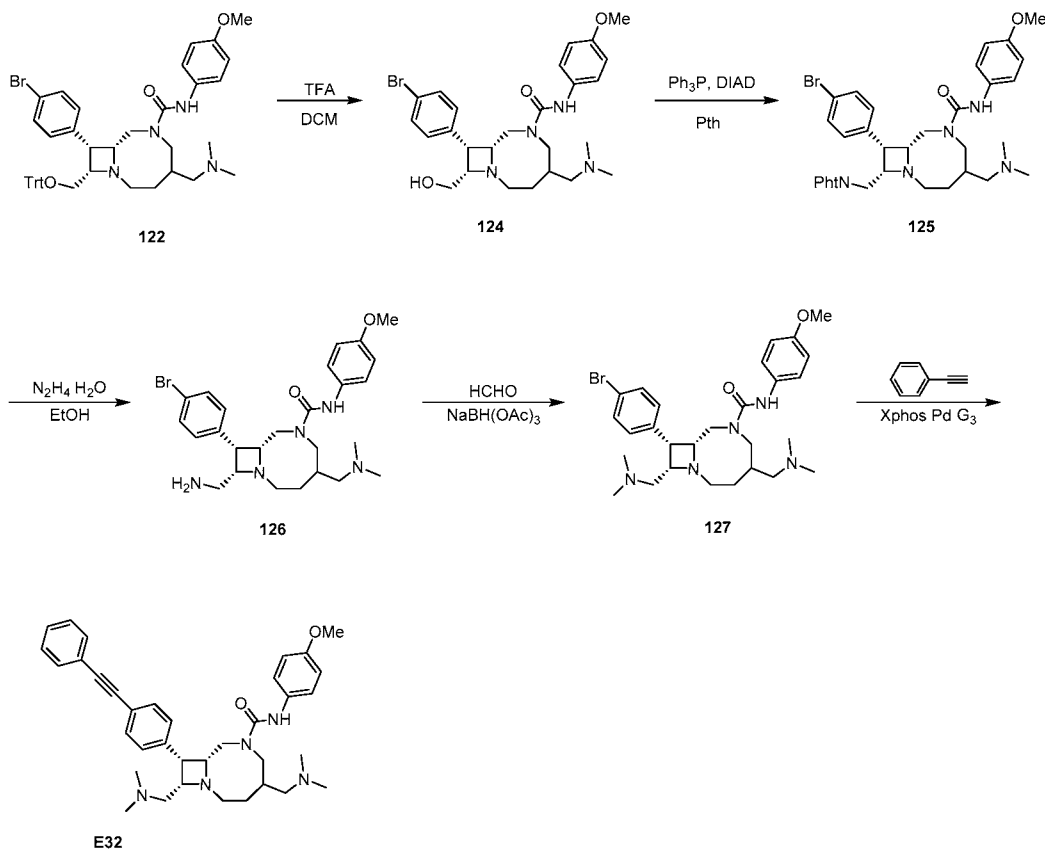
**Intermediate 123:** (8R,9R,10S)-4-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-10-(trityloxymethyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 122 (60.00 mg, 77.54 μmol, 1.00 eq) in acetonitrile (1.00 mL) was added ethynylbenzene (23.76 mg, 232.62 μmol, 25.55 μL, 3.00 eq), Cs<sub>2</sub>CO<sub>3</sub> (101.06 mg, 310.16 μmol, 4.00 eq) and Xphos Pd G3 (6.56 mg, 7.75 μmol, 0.1 eq). The mixture was stirred at 70°C for 2 hour. LC-MS showed reactant was consumed completely and desired MS was detected. The reaction mixture was quenched by addition water (10 mL) and extracted with DCM (5 mL \* 2). The combined organic layers were washed with brine (5 mL \* 2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 20:1) to afford Intermediate 123 (50.00 mg, 62.89 μmol, 81.11% yield) as a yellow solid.

**Synthesis of E31:** (8R,9R,10S)-4-((dimethylamino)methyl)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E31).

To a solution of Intermediate 123 (50.00 mg, 62.89 μmol, 1.00 eq) in DCM (1.00 mL) was added TFA (71.71 mg, 628.90 μmol, 46.56 μL, 10.00 eq). The mixture was stirred at 25°C for 1 hour. LC-MS showed Intermediate 123 was consumed completely and desired MS was detected. The reaction mixture was quenched by addition sat. NaHCO<sub>3</sub> aqueous (10 mL) and extracted with DCM (5 mL \* 2). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1) to afford compound E31 (4.50 mg, 8.14 μmol, 12.95% yield) as a white solid. <sup>1</sup>H NMR (400MHz, CHLOROFORM-d) δ = 7.54 (dd, J=2.0, 7.5 Hz, 2H), 7.48 (s, 4H), 7.37 - 7.29 (m, 5H), 6.82 (d, J=9.0 Hz, 2H), 4.05 (d, J=14.1 Hz, 1H), 3.78 (s, 3H), 3.73 - 3.62 (m, 4H), 3.60 - 3.52 (m, 2H), 3.46 (t, J=8.5 Hz, 1H), 3.15 (dd, J=5.3, 16.3 Hz, 2H), 2.86 (dd, J=10.5, 14.6 Hz, 1H), 2.58 (br. s., 1H), 2.32 (s, 6H), 2.25 (s, 6H), 2.03 (d, J=12.5 Hz, 1H), 1.59 (br. s., 2H), 1.53 (br. s., 1H)

5 **Example 32:** (8R,9S,10S)-10-[(dimethylamino)methyl]-4-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E32")



10 **Intermediate 124:** (8R,9R,10S)-9-(4-bromophenyl)-4-[(dimethylamino)methyl]-10-(hydroxymethyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 122 (400.00 mg, 516.93  $\mu\text{mol}$ , 1.00 eq) in DCM (10.00 mL) was added TFA (589.40 mg, 5.17 mmol, 382.73  $\mu\text{L}$ , 10.00 eq). The mixture was stirred at 25°C for 1 hour. LC-MS showed Intermediate 122 was consumed completely and desired MS was detected. The reaction mixture was quenched by addition sat.  $\text{NaHCO}_3$  aqueous (10 mL) and extracted with DCM (5 mL \* 2). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography ( $\text{SiO}_2$ , DCM: MeOH = 100:1 to 10:1) to afford Intermediate 124 (130.00 mg, crude) as a yellow solid.  $^1\text{H}$  NMR (400MHz, CHLOROFORM- $d$ )  $\delta$  = 7.47 (d,  $J$ =8.8 Hz, 1H), 7.39 - 7.32 (m, 3H), 7.32 - 7.26 (m, 9H), 7.23 (d,  $J$ =9.3 Hz, 3H), 6.91 (d,  $J$ =8.4 Hz, 1H), 3.93 (d,  $J$ =14.1 Hz, 1H), 3.69 (s, 3H), 3.60 - 3.52 (m, 4H), 3.04 (d,  $J$ =14.1 Hz, 2H), 2.75 - 2.63 (m, 2H), 2.23 (s, 6H), 2.15 (d,  $J$ =11.9 Hz, 1H), 1.65 - 1.46 (m, 4H).

5 **Intermediate 125:** (8R,9S,10S)-9-(4-bromophenyl)-4-[(dimethylamino)methyl]-10-[(1,3-dioxoisindolin-2-yl)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of PPh<sub>3</sub> (0.15 g) in 3 mL THF was added DIAD (0.11 mL) at 0°C. This mixture (1.16 mL) were added to a solution of Intermediate 124 (30.00 mg, 56.45 μmol, 1.00 eq) and isoindoline-1,3-dione (12.46 mg, 84.67 μmol, 1.50 eq) in THF (1.00 mL) at 0°C. The mixture was stirred at 25°C for 16 hour. LC-MS showed Intermediate 124 was consumed completely and desired MS was detected. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 20:1) to afford Intermediate 125 (30.00 mg, crude) as a white solid contain Ph<sub>3</sub>PO.

15 **Intermediate 126:** (8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-4-[(dimethylamino)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 125 (100.00 mg, 151.38 μmol, 1.00 eq) in EtOH (2.00 mL) was added N<sub>2</sub>H<sub>4</sub>.H<sub>2</sub>O (7.58 mg, 151.38 μmol, 7.36 μL, 1.00 eq). The mixture was stirred at 70°C for 1 hour. LC-MS showed Intermediate 125 was consumed completely and desired MS was detected. The reaction mixture was quenched by addition water (10 mL) and extracted with DCM (5 mL \* 2). The combined organic layers were washed with brine (5 mL \* 2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 8:1) to afford Intermediate 126 (90.00 mg, crude) as a yellow solid.

**Intermediate 127:** (8R,9S,10S)-9-(4-bromophenyl)-4,10-bis[(dimethylamino)methyl]-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

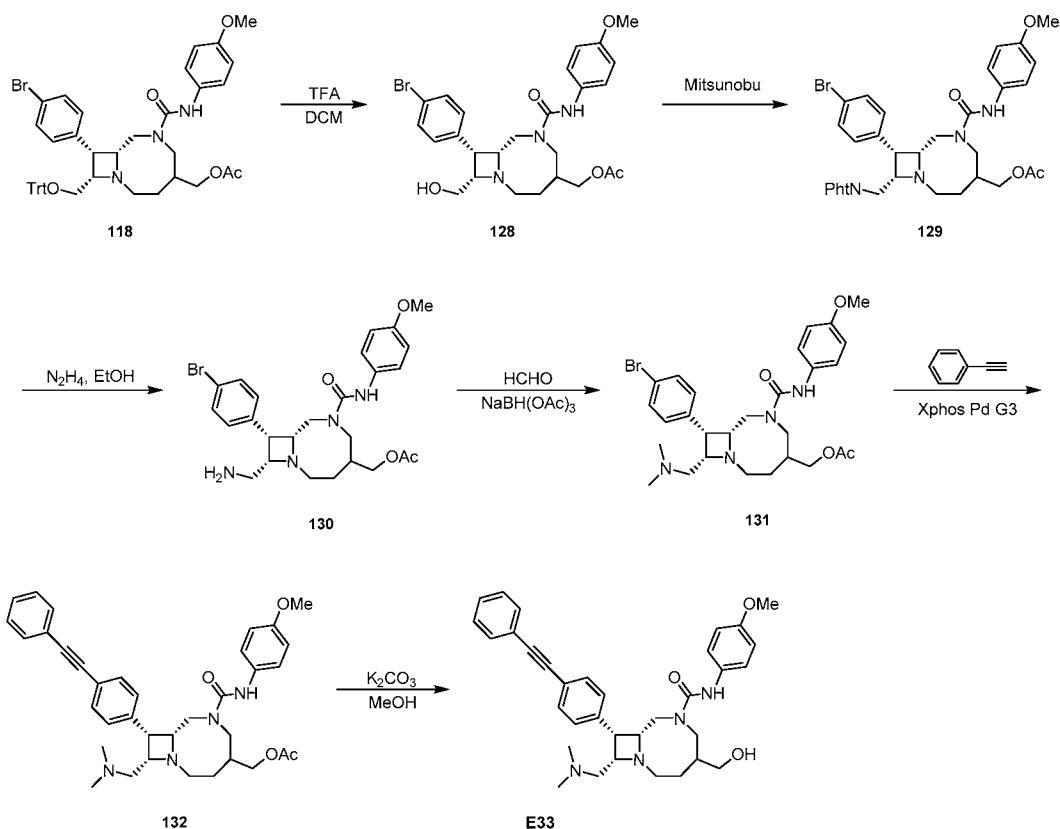
To a solution of Intermediate 126 (45.00 mg, 84.83 μmol, 1.00 eq) in DCM (2.00 mL) was added HCHO (41.31 mg, 508.98 μmol, 37.90 μL, 37% solution, 6.00 eq) and MgSO<sub>4</sub> (122.53 mg, 1.02 mmol, 12.00 eq), after stirred for 30min, NaBH(OAc)<sub>3</sub> (269.68 mg, 1.27 mmol, 15.00eq) was added. The mixture was stirred at 15°C for 16 hour. LC-MS showed Intermediate 126 was consumed completely and desired MS was detected. The reaction mixture was quenched by addition sat. NaHCO<sub>3</sub> aqueous (10 mL) and extracted with DCM (3 mL \* 2). The combined organic layers were washed with brine (3 mL \* 2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 10:1) to afford Intermediate 127 (20.00 mg,

5 35.81  $\mu$ mol, 42.21% yield) as a yellow solid.  $^1\text{H}$  NMR (400MHz, CHLOROFORM- $d$ )  $\delta$  = 7.46 - 7.40 (m, 2H), 7.37 - 7.33 (m, 2H), 7.30 (d,  $J$ =9.0 Hz, 2H), 6.81 (d,  $J$ =9.0 Hz, 2H), 3.97 (d,  $J$ =14.1 Hz, 1H), 3.77 (s, 3H), 3.67 (d,  $J$ =15.6 Hz, 1H), 3.60 - 3.54 (m, 1H), 3.45 - 3.32 (m, 2H), 3.15 - 3.03 (m, 2H), 2.74 (dd,  $J$ =10.8, 14.3 Hz, 1H), 2.47 - 2.34 (m, 4H), 2.30 (s, 6H), 2.25 - 2.17 (m, 1H), 2.02 (s, 6H), 1.62 - 1.48 (m, 3H).

10 **Synthesis of E32:** (8R,9S,10S)-4,10-bis[(dimethylamino)methyl]-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E32).

To a solution of Intermediate 127 (15.00 mg, 26.86  $\mu$ mol, 1.00 eq) in acetonitrile (1.00 mL) was added ethynylbenzene (10.97 mg, 107.42  $\mu$ mol, 3.00eq), Xphos Pd G3 (2.27 mg, 2.69  $\mu$ mol, 0.10 eq) and  $\text{Cs}_2\text{CO}_3$  (35.00 mg, 107.42  $\mu$ mol, 4.00 eq). The mixture was  
15 stirred at 70°C for 1 hour. LC-MS showed Intermediate 127 was consumed completely and desired MS was detected. The reaction mixture was quenched by addition water (10 mL) and extracted with ethyl acetate (3 mL \* 2). The combined organic layers were washed with brine (3 mL \* 2), dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC ( $\text{SiO}_2$ , DCM: MeOH = 8:1) to afford  
20 compound E32 (4.30 mg, 7.42  $\mu$ mol, 27.61% yield) as a yellow solid.  $^1\text{H}$  NMR (400MHz, CHLOROFORM- $d$ )  $\delta$  = 7.50 - 7.44 (m, 2H), 7.44 - 7.36 (m, 4H), 7.31 - 7.21 (m, 5H), 6.74 (d,  $J$ =8.5 Hz, 2H), 3.93 (d,  $J$ =14.6 Hz, 1H), 3.70 (s, 3H), 3.63 - 3.52 (m, 2H), 3.42 - 3.28 (m, 2H), 3.09 - 2.99 (m, 2H), 2.72 (dd,  $J$ =10.5, 14.1 Hz, 1H), 2.36 (d,  $J$ =10.0 Hz, 3H), 2.23 (s, 6H), 2.16 (t,  $J$ =12.5 Hz, 2H), 1.96 (br. s., 6H), 1.54 (br. s., 1H), 1.45 (br. s., 2H).

5 **Example 33:** (8R,9S,10S)-10-[(dimethylamino)methyl]-4-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E33")



10 **Intermediate 128:** [(8R,9R,10S)-9-(4-bromophenyl)-10-(hydroxymethyl)-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-4-yl]methyl acetate.

To a solution of Intermediate 118 (600.00 mg, 760.68  $\mu$ mol, 1.00 eq) in DCM (10.00 mL) was added TFA (867.33 mg, 7.61 mmol, 563.20  $\mu$ L, 10.00 eq). The mixture was stirred at 20°C for 2 hour. LC-MS showed Intermediate 118 was consumed and desired MS was detected. The reaction mixture was quenched by addition sat. NaHCO<sub>3</sub> aqueous (30 mL) and  
 15 extracted with DCM (10 mL \* 2). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM /MeOH=1:0 to 20:1) to afford Intermediate 128 (300.00 mg, 549.00  $\mu$ mol, 72.17% yield) as a white solid.

20 **Intermediate 129:** [(8R,9S,10S)-9-(4-bromophenyl)-10-[(1,3-dioxoisindolin-2-yl)methyl]-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-4-yl]methyl acetate.

To a solution of triphenylphosphine (1 g, 3.76 mmol) in THF (19 ml) at 0°C was slowly added DIAD (0.73 mL, 3.76 mmol). This prepared mixture (11 mL) was added to a

5 mixture of Intermediate 128 (300.00 mg, 549.00  $\mu$ mol, 1.00 eq) and isoindoline-1,3-dione (121.16 mg, 823.50  $\mu$ mol, 1.50 eq) in THF (15.00 mL) at 0°C and then the mixture was stirred at 25°C for 16 hour under N<sub>2</sub> atmosphere. LC-MS showed Intermediate 128 was consumed completely and desired MS was detected. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column  
10 chromatography (SiO<sub>2</sub>, DCM: MeOH = 1:0 to 10:1) to afford Intermediate 129 (400.00 mg, crude) as a yellow solid contain Ph<sub>3</sub>PO.

**Intermediate 130:** [(8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-4-yl]methyl acetate.

To a solution of Intermediate 129 (400.00 mg, 592.09  $\mu$ mol, 1.00 eq) in EtOH (10.00  
15 mL) was added N<sub>2</sub>H<sub>4</sub>.H<sub>2</sub>O (29.64 mg, 592.09  $\mu$ mol, 28.78  $\mu$ L, 1.00 eq). The mixture was stirred at 70°C for 2h. The reaction mixture was quenched by addition water (30 mL) and extracted with DCM (30 mL \* 2). The combined organic layers were washed with brine (30 mL \* 2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH  
20 =1:0 to 20:1) to afford Intermediate 130 (280.00 mg, 513.32  $\mu$ mol, 86.70% yield) as a white solid.

**Intermediate 131:** [(8R,9S,10S)-9-(4-bromophenyl)-10-[(dimethylamino)methyl]-6-[(4-methoxyphenyl)carbamoyl]-1,6-diazabicyclo[6.2.0]decan-4-yl]methyl acetate.

To a solution of Intermediate 130 (140.00 mg, 256.66  $\mu$ mol, 1.00 eq) in DCM (5.00  
25 mL) was added MgSO<sub>4</sub> (370.73 mg, 3.08 mmol, 12.00 eq) and HCHO (124.99 mg, 1.54 mmol, 114.67  $\mu$ L, 37% solution, 6.00 eq) then NaBH(OAc)<sub>3</sub> (815.95 mg, 3.85 mmol, 15.00 eq) was added in portions. The mixture was stirred at 15°C for 16 hour. LC-MS showed Intermediate 130 was consumed and one desired MS was detected. The reaction mixture was quenched by addition sat. NaHCO<sub>3</sub> aqueous (20 mL) and extracted with DCM (5 mL \* 2).  
30 The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, DCM: MeOH = 20:1) to afford Intermediate 131 (73.00 mg, 127.28  $\mu$ mol, 49.59% yield) as a white solid.

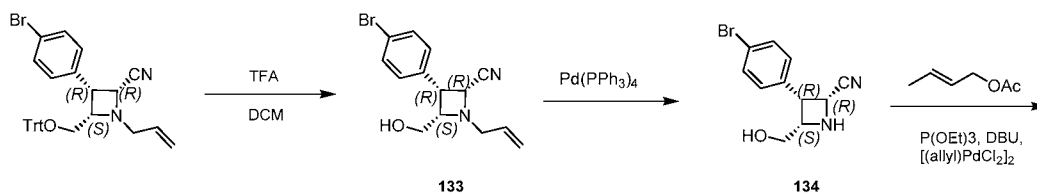
**Intermediate 132:** [(8R,9S,10S)-10-[(dimethylamino)methyl]-6-[(4-methoxyphenyl)carbamoyl]-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decan-4-yl]methyl acetate.  
35

To a solution of Intermediate 131 (73.00 mg, 127.28  $\mu\text{mol}$ , 1.00 eq) in acetonitrile (4.00 mL) was added ethynylbenzene (39 mg, 381.85  $\mu\text{mol}$ , 3.00eq), Xphos Pd G3 (10.77 mg, 12.73  $\mu\text{mol}$ , 0.10 eq) and  $\text{Cs}_2\text{CO}_3$  (165.89 mg, 509.14  $\mu\text{mol}$ , 4.00 eq). The mixture was stirred at 70°C for 1 hour. LC-MS showed Intermediate 131 was consumed completely and desired MS was detected. The reaction mixture was quenched by addition water (10 mL) and extracted with ethyl acetate (3 mL \* 2). The combined organic layers were washed with brine (3 mL \* 2), dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC ( $\text{SiO}_2$ , DCM: MeOH = 10:1) to afford Intermediate 132 (30.00 mg, 50.44  $\mu\text{mol}$ , 39.63% yield) as a brown solid.

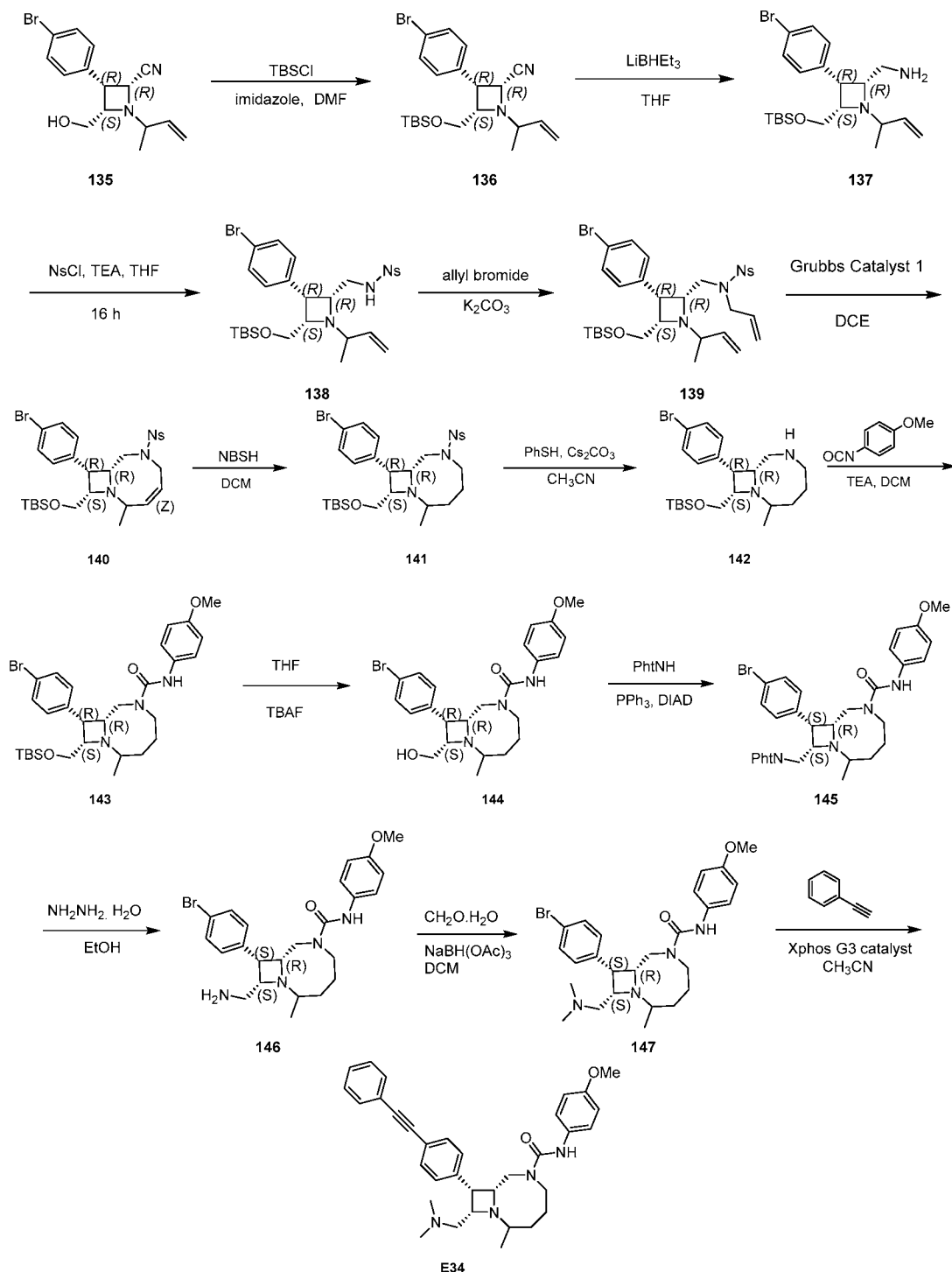
**Synthesis of E33:** (8R,9S,10S)-10-[(dimethylamino)methyl]-4-(hydroxymethyl)-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E33).

To a solution of Intermediate 132 (30.00 mg, 50.44  $\mu$ mol, 1.00 eq) in MeOH (500.00  $\mu$ L) /DCM(0.5 mL) was added  $K_2CO_3$  (34.86 mg, 252.20  $\mu$ mol, 5.00 eq). The mixture was stirred at 15°C for 1 hour. LC-MS showed Intermediate 132 was consumed completely and one main peak with desired MS was detected. The reaction mixture was quenched by addition water (10 mL) and extracted with DCM (5 mL \* 2). The combined organic layers were dried over  $Na_2SO_4$ , filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Luna C18 150\*25 5 $\mu$ ;mobile phase: [water(0.225% formic acid)-acetonitrile];B%: 25%-45%,12min) to afford compound E33 (11.00 mg, 18.37  $\mu$ mol, 36.42% yield, formic acid salt) as a white solid:  $^1H$  NMR (400MHz, METHANOL- $d_4$ )  $\delta$  = 7.58 - 7.49 (m, 6H), 7.38 (d, J=3.5 Hz, 3H), 7.29 (d, J=8.8 Hz, 2H), 6.81 (d, J=9.3 Hz, 2H), 3.92 (d, J=15.4 Hz, 1H), 3.82 - 3.71 (m, 5H), 3.59 (d, J=6.2 Hz, 2H), 3.45 (t, J=9.0 Hz, 1H), 3.20 - 3.05 (m, 2H), 3.01 - 2.91 (m, 2H), 2.88 - 2.81 (m, 1H), 2.48 (t, J=12.1 Hz, 1H), 2.40 - 2.29 (m, 6H), 1.83 (br. s., 1H), 1.67 - 1.48 (m, 2H).

**Example 34:** (8*R*,9*S*,10*S*)-10-(((dimethylamino)methyl)-*N*-(4-methoxyphenyl)-2-methyl-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (“E34”)



5



**Intermediate 133:** (2R,3R,4S)-1-allyl-3-(4-bromophenyl)-4-(hydroxymethyl)azetidine-2-carbonitrile.

To a solution of (2R,3R,4S)-1-allyl-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (J.O.C (2012), 77(17), 7187-7211) (16.00 g, 29.12 mmol, 1.00 *eq*) in DCM (291.00 mL) was added TFA (33.20 g, 291.20 mmol, 21.56 mL, 10.00 *eq*) at 25°C. The mixture was stirred at 25°C for 16 hour. TLC showed reactant consumed completely, desired product was observed, quenched with NaHCO<sub>3</sub> solution (500 mL), extracted with DCM

5 (200 mL x 3), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give a residue (8.95 g, crude) as a yellow solid, which was used directly in next step.

**Intermediate 134:** (2R,3R,4S)-3-(4-bromophenyl)-4-(hydroxymethyl)azetidine-2-carbonitrile.

A mixture of Intermediate 133 (8.95 g, 29.14 mmol, 1.00 *eq*), 1, 3-dimethyl barbituric acid (6.82 g, 43.71 mmol, 1.50 *eq*), Pd(PPh<sub>3</sub>)<sub>4</sub> (3.37 g, 2.91 mmol, 0.10 *eq*) in EtOH (290.00 mL) was degassed and purged with N<sub>2</sub> for 3 times, and then the mixture was stirred at 40°C for 16 hour under N<sub>2</sub> atmosphere. LCMS showed reactant 133 consumed completely, desired MS was observed, concentrated to give a residue. The residue was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (10mM NH<sub>4</sub>HCO<sub>3</sub>); B: acetonitrile) to give Intermediate 134 (4.70 g, 17.60 mmol, 60.38% yield) as an orange solid.

**Intermediate 135:** (2R,3R,4S)-3-(4-bromophenyl)-4-(hydroxymethyl)-1-(1-methylallyl)azetidine-2-carbonitrile

To a solution of Intermediate 134 (1.60 g, 5.99 mmol, 1.00 *eq*) in THF (3.50 mL) was added P(OEt)<sub>3</sub> (39.81 mg, 239.59 μmol, 41.04 μL, 0.04 *eq*), (*E*)-but-2-en-1-yl acetate (724.70 mg, 6.35 mmol, 1.06 *eq*), allyl(chloro)palladium (21.92 mg, 59.90 μmol, 0.01 *eq*) and DBU (911.89 mg, 5.99 mmol, 902.86 μL, 1.00 *eq*), the mixture was degassed and purged with N<sub>2</sub> for 3 times, and then the mixture was stirred at 40°C for 16hr under N<sub>2</sub> atmosphere. The reaction was quenched with water (15 mL), extracted with DCM (20 mL x 3), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=10/1 to 0:1) to give Intermediate 135 (150.00 mg, 466.98 μmol, 7.80% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, METHANOL-*d*<sub>4</sub>) δ=1.20 (dd, *J*=11.04, 6.53 Hz, 3 H) 3.11 (quin, *J*=7.03 Hz, 1 H) 3.43 - 3.55 (m, 2 H) 3.59 - 3.67 (m, 1 H) 3.80 - 3.92 (m, 1 H) 4.04 - 4.15 (m, 1 H) 4.35 (d, *J*=8.53 Hz, 1 H) 4.41 (d, *J*=8.53 Hz, 1 H) 5.05 - 5.41 (m, 2 H) 5.64 - 5.91 (m, 1 H) 7.47 - 7.52 (m, 2 H) 7.52 - 7.56 (m, 2 H).

**Intermediate 136:** (2R,3R,4S)-3-(4-bromophenyl)-4-[[tert-butyl(dimethyl)silyl]oxymethyl]-1-(1-methylallyl)azetidine-2-carbonitrile.

To a solution of Intermediate 135 (1.23 g, 3.83 mmol, 1.00 *eq*) in DMF (6.00 mL) was added imidazole (1.56 g, 22.98 mmol, 6.00 *eq*) and TBSCl (2.31 g, 15.32 mmol, 1.88 mL, 4.00 *eq*). The mixture was stirred at 25°C for 16 hour. LCMS showed reactant consumed completely, desired MS was observed, washed with water (30 mL), extracted with ethyl acetate (30 mL x 3), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=100/1 to 10:1) to

5 give Intermediate 136 (1.60 g, 3.67 mmol, 95.93% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, METHANOL-*d*<sub>4</sub>) δ ppm -0.36 - -0.13 (m, 7 H) 0.69 - 0.77 (m, 9 H) 1.19 (dd, *J*=18.57, 6.53 Hz, 3 H) 3.04 - 3.17 (m, 1 H) 3.39 - 3.47 (m, 1 H) 3.53 - 3.59 (m, 1 H) 3.61 - 3.72 (m, 1 H) 3.82 - 3.92 (m, 1 H) 4.33 - 4.44 (m, 1 H) 5.07 - 5.35 (m, 2 H) 5.61 - 5.85 (m, 1 H) 7.46 - 7.50 (m, 2 H) 7.52 - 7.56 (m, 2 H).

10 **Intermediate 137:** [(2R,3R,4S)-3-(4-bromophenyl)-4-[[tert-butyl(dimethyl)silyl]oxymethyl]-1-(1-methylallyl)azetidin-2-yl]methanamine.

To a solution of Intermediate 136 (1.85 g, 4.25 mmol, 1.00 *eq*) in THF (200.00 mL) was added LiBHEt<sub>3</sub> (1 M, 5.06 mL, 10.00 *eq*) at 0°C under N<sub>2</sub> atmosphere. The mixture was stirred at 25°C for 2 hours. LCMS showed reactant 136 consumed completely, desired  
15 MS was observed, quenched with H<sub>2</sub>O (100 mL), extracted with ethyl acetate (300 mL x 3), concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM/MeOH=1/0 to 10:1) to give Intermediate 137 (1.40 g, 3.19 mmol, 74.95% yield) as an oil.

**Intermediate 138:** N-[[[(2R,3R,4S)-3-(4-bromophenyl)-4-[[tert-butyl(dimethyl)silyl]oxymethyl]-1-(1-methylallyl)azetidin-2-yl]methyl]-2-nitrobenzenesulfonamide.

To a solution of Intermediate 138 (1.36 g, 3.09 mmol, 1.00 *eq*) in THF (55.00 mL) was added TEA (9.91 g, 97.95 mmol, 13.58 mL, 31.70 *eq*) and 2-nitrobenzenesulfonyl chloride (2.05 g, 9.27 mmol, 3.00 *eq*) at 0°C. The mixture was stirred at 25°C for 2  
25 hours. LCMS showed reactant 1 consumed completely, desired MS was observed, concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=20/1 to 5:1) to give Intermediate 138 (1.93 g, crude) as a yellow oil.

**Intermediate 139:** N-allyl-N-[[[(2R,3R,4S)-3-(4-bromophenyl)-4-[[tert-butyl(dimethyl)silyl]oxymethyl]-1-(1-methylallyl)azetidin-2-yl]methyl]-2-nitrobenzenesulfonamide.

To a solution of Intermediate 138 (1.95 g, 3.12 mmol, 1.00 *eq*) in DMF (15.00 mL) was added K<sub>2</sub>CO<sub>3</sub> (647.17 mg, 4.68 mmol, 1.50 *eq*), then 3-bromoprop-1-ene (1.13 g, 9.36 mmol, 808.84 μL, 3.00 *eq*) was added. The mixture was stirred at 25°C for 16 hour. LCMS showed  
35 reactant 138 consumed completely, desired MS was observed, washed with brine (20 mL x 3), extracted with ethyl acetate (20 mL x 3), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl

5 acetate=20/1 to 5:1) to give Intermediate 139 (1.90 g, 2.86 mmol, 91.61% yield as a yellow gum. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ = ppm -0.34 - -0.27 (m, 3 H) -0.23 - -0.18 (m, 3 H) 0.70 - 0.75 (m, 9 H) 1.14 (dd, *J*=16.76, 6.62 Hz, 3 H) 3.11 - 3.42 (m, 5 H) 3.15 - 3.79 (m, 6 H) 4.69 - 4.82 (m, 1 H) 4.93 - 5.04 (m, 1 H) 5.05 - 5.22 (m, 2 H) 5.28 - 5.50 (m, 1 H) 5.67 - 5.81 (m, 1 H) 7.32 (d, *J*=8.38 Hz, 2 H) 7.43 (dd, *J*=8.38, 1.32 Hz, 2 H) 7.52 - 7.62 (m, 3 H) 7.62 - 7.70 (m, 1 H).

**Intermediate 140:** [(3*Z*,8*R*,9*R*,10*S*)-9-(4-bromophenyl)-2-methyl-6-(2-nitrophenyl)sulfonyl-1,6-diazabicyclo[6.2.0]dec-3-en-10-yl]methoxy-tert-butyl-dimethyl-silane.

To a solution of Intermediate 139 (1.84 g, 2.77 mmol, 1.00 *eq*) in DCE (368.00 mL) was added Hoveyda-Grubbs Catalyst 1<sup>st</sup> Generation (569.51 mg, 692.50 μmol, 0.25 *eq*). The mixture was stirred at 90°C for 16 hour. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, petroleum ether/ethyl acetate= 5:1). Intermediate 140 (1.25 g, 1.96 mmol, 70.88% yield) as a light black solid.

**Intermediate 141:** [(8*R*,9*R*,10*S*)-9-(4-bromophenyl)-2-methyl-6-(2-nitrophenyl)sulfonyl-1,6-diazabicyclo[6.2.0]decan-10-yl]methoxy-tert-butyl-dimethyl-silane.

To a solution of Intermediate 140 (1.00 g, 1.57 mmol, 1.00 *eq*) in THF (40.00 mL) was added TEA (1.43 g, 14.13 mmol, 1.96 mL, 9.00 *eq*) and 2-nitrobenzenesulfonylhydrazide (1.02 g, 4.71 mmol, 3.00 *eq*). The mixture was stirred at 40°C in an oil bath for 16h. LCMS showed reactant 140 consumed completely, the majority was the desired product. The reaction mixture was quenched with a saturated solution of NaHCO<sub>3</sub> (20 mL) and extracted three times with ethyl acetate (30 mL x3). The combined organic extracts were dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure to provide the crude product. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=20/1 to 5:1) to give Intermediate 141 (830.00 mg, 1.04 mmol, 66.22% yield, 80% purity) as a yellow solid. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ = ppm -0.29 - -0.08 (m, 6 H) 0.75 (d, *J*=14.05 Hz, 9 H) 1.03 - 1.17 (m, 2 H) 1.36 - 1.48 (m, 1 H) 1.71 - 2.17 (m, 3 H) 2.62 (br. s., 1 H) 2.75 - 2.94 (m, 1 H) 3.00 - 3.29 (m, 2 H) 3.37 - 3.48 (m, 1 H) 3.49 - 3.67 (m, 3 H) 3.69 - 3.87 (m, 2 H) 7.28 - 7.46 (m, 4 H) 7.53 - 7.66 (m, 3 H) 7.78 (d, *J*=6.02 Hz, 1 H)

**Intermediate 142:** [(8*R*,9*R*,10*S*)-9-(4-bromophenyl)-2-methyl-1,6-diazabicyclo[6.2.0]decan-10-yl]methoxy-tert-butyl-dimethyl-silane.

To a solution of Intermediate 141 (830.00 mg, 1.30 mmol, 1.00 *eq*) in CH<sub>3</sub>CN (8.00 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (508.28 mg, 1.56 mmol, 1.20 *eq*) and benzenethiol (214.77 mg, 1.95

5 mmol, 198.86  $\mu$ L, 1.50 *eq*). After stirring at 20°C for 16 hour, the reaction was diluted with water (15 mL), extracted with ethyl acetate (15 mL x 3), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give a residue. The residue was purified by prep-TLC (SiO<sub>2</sub>, petroleum ether/ethyl acetate=5:1) to give Intermediate 142 (580.00 mg, 1.02 mmol, 78.70% yield, 80% purity) as a yellow solid

10 **Intermediate 143:** (8R,9R,10S)-9-(4-bromophenyl)-10-[[tert-butyl(dimethyl)silyl]oxymethyl]-N-(4-methoxyphenyl)-2-methyl-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 142 (550.00 mg, 1.21 mmol, 1.00 *eq*) in DCM (10.00 mL) was added TEA (61.36 mg, 606.35  $\mu$ mol, 84.05  $\mu$ L, 0.50 *eq*) and 1-isocyanato-4-methoxy-benzene (180.88 mg, 1.21 mmol, 155.93  $\mu$ L, 1.00 *eq*) at 0°C. After stirring at 20°C  
15 for 3 hour, the reaction was diluted with water (10 mL), extracted with DCM (20 mL x 3), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=10/1 to 5:1) to give Intermediate 143 (700.00 mg, 1.16 mmol, 95.99% yield) as a yellow solid.

20 **Intermediate 144:** (8R,9R,10S)-9-(4-bromophenyl)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-2-methyl-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 143 (690.00 mg, 1.14 mmol, 1.00 *eq*) in THF (10.00 mL) was added TBAF (596.13 mg, 2.28 mmol, 2.00 *eq*). The mixture was stirred at 50°C for 16 hour. TLC showed reactant 1 consumed completely, desired product was observed,  
25 washed with water (10 mL x3), extracted with ethyl acetate (20 mL x 3), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM/MeOH=1/0 to 20:1) to give Intermediate 144 (440.00 mg, 900.86  $\mu$ mol, 79.02% yield) as a yellow solid. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*)  $\delta$ = ppm 1.09 (d, *J*=6.53 Hz, 3 H) 1.40 (d, *J*=16.56 Hz, 1 H) 1.77 - 1.99 (m, 2 H) 2.72 - 2.90 (m, 2 H) 3.51 (d, *J*=6.02 Hz, 1 H)  
30 3.57 - 3.69 (m, 4 H) 3.71 - 3.80 (m, 4 H) 3.81 - 3.94 (m, 2 H) 6.02 - 6.08 (m, 1 H) 6.79 - 6.87 (m, 2 H) 7.24 (d, *J*=9.03 Hz, 2 H) 7.36 - 7.43 (m, 2 H) 7.43 - 7.49 (m, 2 H)

**Intermediate 145:** (8R,9S,10S)-9-(4-bromophenyl)-10-[(1,3-dioxo-3a,7a-dihydroisoindol-2-yl)methyl]-N-(4-methoxyphenyl)-2-methyl-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 144 (420.00 mg, 859.92  $\mu$ mol, 1.00 *eq*) in THF (18.00  
35 mL) was added isoindoline-1,3-dione (189.78 mg, 1.29 mmol, 1.50 *eq*), PPh<sub>3</sub> (451.09 mg, 1.72 mmol, 2.00 *eq*) and DIAD (347.77 mg, 1.72 mmol, 334.39  $\mu$ L, 2.00 *eq*) at 0°C. The mixture was stirred at 25°C for 16 hour. LCMS showed reactant 145 consumed completely,

5 the majority was the desired product, washed with water (10 mL), extracted with ethyl acetate (10 mL x 3), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=5/1 to 1:1) to give Intermediate 145 (800.00 mg, 645.63 μmol, 75.08% yield, 50% purity) as a brown gum.

**Intermediate 146:** (8R,9S,10S)-10-(aminomethyl)-9-(4-bromophenyl)-N-(4-methoxyphenyl)-2-methyl-1,6-diazabicyclo[6.2.0]decane-6-carboxamide.

To a solution of Intermediate 145 (750.00 mg, 605.28 μmol, 1.00 eq) in EtOH (14.00 mL) was added NH<sub>2</sub>NH<sub>2</sub>.H<sub>2</sub>O (30.30 mg, 605.28 μmol, 29.42 μL, 1.00 eq). The mixture was stirred at 80°C for 2 hour. LCMS showed Intermediate 145 consumed completely, the majority was the desired product (**Intermediate 146**) washed with water (40 mL), extracted with ethyl acetate (30 mL x 3), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM/MeOH=0/1 to 10:1) to give (10S,10aR)-9-amino-10-(4-bromophenyl)-N-(4-methoxyphenyl)-6-methyloctahydropyrrolo[1,2-a][1,4]diazocine-2(1H)-carboxamide ("R146") (190 mg) (<sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ=ppm 0.97 (d, *J*=6.02 Hz, 1 H) 1.14 (d, *J*=8.03 Hz, 2 H) 1.25 - 1.48 (m, 2 H) 1.87 - 2.09 (m, 2 H) 2.72 - 2.84 (m, 1 H) 3.01 (d, *J*=10.04 Hz, 1 H) 3.15 - 3.23 (m, 1 H) 3.28 (d, *J*=6.02 Hz, 1 H) 3.45 (br. s., 1 H) 3.62 - 4.11 (m, 7 H) 6.82 (d, *J*=10.04 Hz, 2 H) 7.18 - 7.25 (m, 2 H) 7.29 (d, *J*=8.03 Hz, 2 H) 7.49 (d, *J*=8.03 Hz, 2 H)) as a yellow mixture solid and Intermediate 146 (40 mg) as a yellow solid (<sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) =ppm 0.99 - 1.16 (m, 3 H) 1.44 (d, *J*=11.04 Hz, 1 H) 1.87 (br. s., 3 H) 2.66 (br. s., 1 H) 2.80 - 2.91 (m, 1 H) 2.92 - 3.00 (m, 1 H) 3.01 - 3.15 (m, 1 H) 3.38 (br. s., 1 H) 3.59 (d, *J*=12.55 Hz, 2 H) 3.67 - 3.84 (m, 6 H) 6.14 (s, 1 H) 6.81 (d, *J*=8.53 Hz, 2 H) 7.20 (d, *J*=8.53 Hz, 2 H) 7.34 (d, *J*=8.03 Hz, 2 H) 7.46 (d, *J*=8.03 Hz, 2 H)). H).

**Intermediate 147:** (8R,9S,10S)-9-(4-bromophenyl)-10-((dimethylamino)methyl)-N-(4-methoxyphenyl)-2-methyl-1,6-diazabicyclo[6.2.0]decane-6-carboxamide

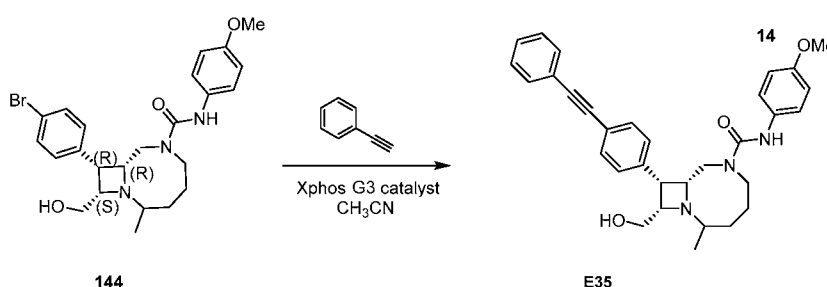
30 To a solution of Compound 146 (15.00 mg, 21.54 μmol, 1.00 eq) in DCM (3.00 mL) was added HCHO (17.48 mg, 215.42 μmol, 16.04 μL, 37% purity, 10.00 eq) and MgSO<sub>4</sub> (51.86 mg, 430.83 μmol, 20.00 eq) followed by NaBH(OAc)<sub>3</sub> (45.66 mg, 215.42 μmol, 10.00 eq). After the mixture was stirred at 20 °C for 2 hour, the resulting mixture was filtered and the filtrate was concentrated. The residue was purified by prep-TLC (SiO<sub>2</sub>, dichloromethane:methanol = 20:1) to give compound 147 (5.00 mg, 7.76 μmol, 36.02% yield, 80% purity) as a light yellow solid.

**Synthesis of E34:** (8R,9S,10S)-10-((dimethylamino)methyl)-N-(4-methoxyphenyl)-2-

5 methyl-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E34).

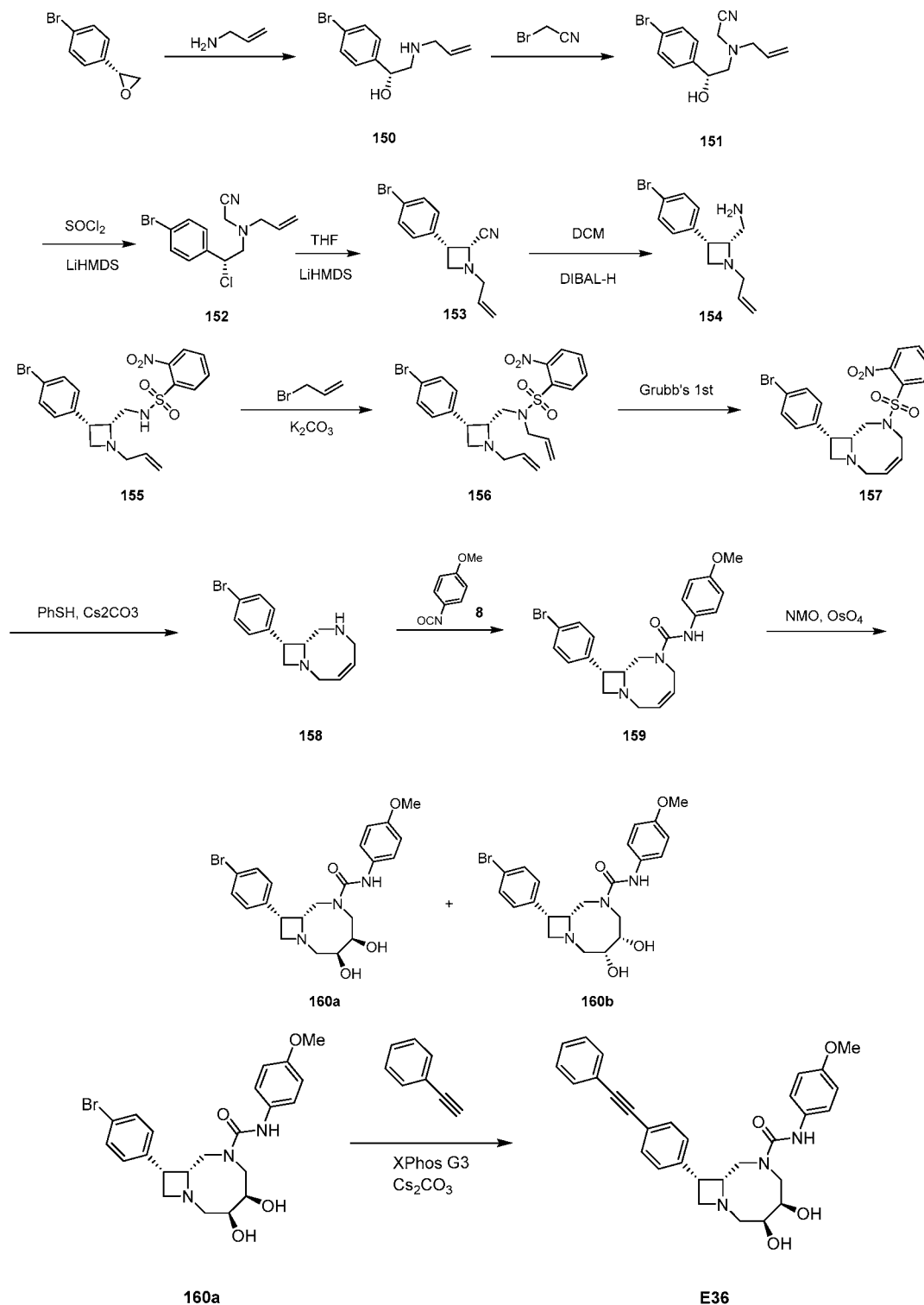
To a solution of Intermediate 147 (5.00 mg, 9.70  $\mu\text{mol}$ , 1.00 *eq*) in  $\text{CH}_3\text{CN}$  (500.00  $\mu\text{L}$ ) was added ethynylbenzene (2.97 mg, 29.10  $\mu\text{mol}$ , 3.19  $\mu\text{L}$ , 3.00 *eq*),  $\text{Cs}_2\text{CO}_3$  (12.64 mg, 38.80  $\mu\text{mol}$ , 4.00 *eq*), degassed with  $\text{N}_2$  for 3 times, and then XPhos Pd G3 (821.04  $\mu\text{g}$ , 0.97  $\mu\text{mol}$ , 0.10 *eq*). After stirring at  $70^\circ\text{C}$  for 3h, the reaction was diluted with water (3 mL),  
 10 extracted with ethyl acetate (5 mL x 3), dried over  $\text{Na}_2\text{SO}_4$ , concentrated to give a residue. The residue was purified by prep-TLC ( $\text{SiO}_2$ , petroleum ether/ethyl acetate= 1:1) to give compound E34 (5.00 mg, 9.32  $\mu\text{mol}$ , 96.04% yield) as a light yellow solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CHLOROFORM-}d$ )  $\delta$ =ppm 1.16 (d,  $J$ =6.17 Hz, 3 H) 1.70 - 1.94 (m, 4 H) 2.01 (br. s., 6 H) 2.42 - 2.66 (m, 3 H) 2.83 - 2.94 (m, 1 H) 3.47 (br. s., 2 H) 3.53 - 3.62 (m, 1 H)  
 15 3.65 - 3.82 (m, 6 H) 6.04 (s, 1 H) 6.83 (d,  $J$ =8.82 Hz, 2 H) 7.24 (d,  $J$ =8.82 Hz, 2 H) 7.33 - 7.39 (m, 3 H) 7.39 - 7.44 (m, 2 H) 7.50 (d,  $J$ =7.94 Hz, 2 H) 7.53 - 7.57 (m, 2 H).

**Example 35:** (8R,9R,10S)-10-(hydroxymethyl)-N-(4-methoxyphenyl)-2-methyl-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E35")



20 To a solution of Intermediate 144 (20.00 mg, 40.95  $\mu\text{mol}$ , 1.00 *eq*) in  $\text{CH}_3\text{CN}$  (1.00 mL) was added ethynylbenzene (12.55 mg, 122.85  $\mu\text{mol}$ , 13.49  $\mu\text{L}$ , 3.00 *eq*),  $\text{Cs}_2\text{CO}_3$  (53.37 mg, 163.80  $\mu\text{mol}$ , 4.00 *eq*), degassed with  $\text{N}_2$  for 3 times and then XPhos Pd G3 (3.47 mg, 4.10  $\mu\text{mol}$ , 0.10 *eq*). The mixture was stirred at  $70^\circ\text{C}$  for 16 hour. LCMS showed reactant 144 consumed completely, the majority was the desired product, washed with water (10 mL),  
 25 extracted with ethyl acetate (10 mL x 3), dried over  $\text{Na}_2\text{SO}_4$ , concentrated to give a residue. The residue was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (10mM  $\text{NH}_4\text{HCO}_3$ ); B: acetonitrile) to give compound E35 (8.30 mg, 16.29  $\mu\text{mol}$ , 39.77% yield) as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CHLOROFORM-}d$ )  $\delta$  = 1.10 (d,  $J$ =6.02 Hz, 3 H) 1.40 (d,  $J$ =13.55 Hz, 1 H) 1.76 - 2.01 (m, 3 H) 2.72 - 3.03 (m, 1 H) 2.76 (br. s., 1 H) 3.43 - 3.81 (m, 9 H) 3.82 - 3.96 (m, 2 H) 6.03 - 6.11 (m, 1 H) 6.80 - 6.88 (m, 2 H)  
 30 7.24 (d,  $J$ =9.03 Hz, 2 H) 7.32 - 7.40 (m, 3 H) 7.48 - 7.56 (m, 6 H).

5 **Example 36:** (3*S*,4*R*,8*R*,9*S*)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E36")



10 **Intermediate 150:** (R)-2-(allylamino)-1-(4-bromophenyl)ethan-1-ol

To a flask containing (R)-2-bromophenyl oxirane (8.0g, 40.2 mmol) was added allylamine (12.03 ml, 161 mmol). The mixture was stirred at 55°C for 20 h. After this time analysis by LC/MS indicated the reaction was complete and the mixture was cooled to room

5 temperature and the organic solvent was evaporated under reduced pressure. Upon addition of hexane to the residue a white solid formed and was filtered and dried under high vacuum for 2 hours to afford the desired product (*R*)-2-(allylamino)-1-(4-bromophenyl)ethan-1-ol (9.66 g, 37.7 mmol, 94% yield) as a white solid. The reaction was carried on to the next step without further purification. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.53 – 7.36 (m, 2H), 7.29 – 7.13 (m, 2H), 5.96 – 5.72 (m, 1H), 5.28 – 5.02 (m, 2H), 4.67 (dd, J = 9.0, 3.6 Hz, 1H), 3.41 – 3.18 (m, 2H), 2.87 (dd, J = 12.2, 3.6 Hz, 1H), 2.66 (dd, J = 12.2, 8.9 Hz, 1H).

**Intermediate 151:** (R)-2-(allyl(2-(4-bromophenyl)-2-hydroxyethyl)amino)acetonitrile

To a solution of (*R*)-2-(allylamino)-1-(4-bromophenyl)ethan-1-ol (9.66 g, 37.7 mmol) in anhydrous acetonitrile (60 ml) at room temperature was added K<sub>2</sub>CO<sub>3</sub> (7.82 g, 56.6 mmol) and 2-bromoacetonitrile (7.88 ml, 113 mmol). The heterogeneous mixture was stirred at 85°C under a nitrogen atmosphere for 18 h. After this time analysis by LCMS indicated the reaction was complete and the mixture was cooled to room temperature. The organic solvent was evaporated under reduced pressure, diluted with water (50 ml) and extracted with ethyl acetate. The combined organic components were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, ethyl acetate/hexane=20-30%) to give (R)-2-(allyl(2-(4-bromophenyl)-2-hydroxyethyl)amino)acetonitrile (9 g, 30.5 mmol, 81% yield) as a pale yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.54–7.40 (m, 2H), 7.31–7.16 (m, 2H), 5.91–5.66 (m, 1H), 5.43–5.22 (m, 2H), 4.78–4.65 (m, 1H), 3.79–3.54 (m, 2H), 3.45–3.30 (m, 1H), 3.27–3.11 (m, 2H), 2.86 – 2.74 (m, 1H), 2.68 – 2.54 (m, 1H).

**Intermediate 152:** (R)-2-(allyl(2-(4-bromophenyl)-2-chloroethyl)amino)acetonitrile

To a solution of pyridine (12.1 ml, 151 mmol) in anhydrous DMF (100 ml) at 0°C was added thionyl chloride (4.38 ml, 60.3 mmol) dropwise. The mixture was stirred at 0°C for 15 minutes. To this solution was added (R)-2-(allyl(2-(4-bromophenyl)-2-hydroxyethyl)amino)acetonitrile (8.9 g, 30.2 mmol) in DCM (10 ml) over 5 minutes. This mixture was stirred at 0°C for 30 minutes upon which TLC analysis indicated that the reaction was complete. To the reaction mixture was added saturated aq. NaHCO<sub>3</sub> and the resulting solution was stirred vigorously for 10 min before being transferred to a separatory funnel. The layers were separated and the aqueous phase was extracted DCM and the combined organic extracts were washed with brine, dried with MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure to afford the crude product. The residue was purified by column chromatography (SiO<sub>2</sub>, ethyl acetate/hexane = 0-20%) to give the desired

5 intermediate (5.00 g, 9.87 mmol, 92% yield) as a brown solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ ppm 7.57 – 7.42 (m, 2H), 7.32 – 7.23 (m, 2H), 5.84 – 5.58 (m, 1H), 5.37 – 5.14 (m, 2H), 4.91 – 4.71 (m, 1H), 3.65 – 3.38 (m, 2H), 3.26 – 2.94 (m, 4H).

**Intermediate 153:** (2*R*,3*S*)-1-allyl-3-(4-bromophenyl)azetidine-2-carbonitrile

A 100 ml round-bottomed flask was charged with (R)-2-(allyl(2-(4-bromophenyl)-2-chloroethyl)amino)acetonitrile (0.55 g, 1.754 mmol) and anhydrous THF (10 ml) to give a  
10 pale yellow solution. The flask was completely submerged in a dry ice/acetone bath and cooled to -50°C. LiHMDS (1M, THF solution) was added dropwise to this chilled solution. After complete addition, the reaction was stirred for 1 h at -50°C. LCMS and TLC analysis (20% EtOAc in hexanes, KMnO<sub>4</sub> staining) indicated complete disappearance of starting  
15 material. The reaction mixture was treated with saturated aq. NH<sub>4</sub>Cl and the resulting solution was stirred vigorously for 10 min before being transferred to a separatory funnel. The layers were separated, the aqueous phase was extracted EtOAc and the combined organic extracts were washed with brine, dried with MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure to afford the crude product. The residue was purified by column chromatography  
20 (SiO<sub>2</sub>, ethyl acetate/hexane = 0-20%) to give two main sets of fractions. The first set of fractions (0.16g, 0.577 mmol, 33% yield) gave the (2*S*,3*S*)-1-allyl-3-(4-bromophenyl)azetidine-2-carbonitrile side product. The second set of fractions (0.17g, 0.613 mmol, 35% yield) solidified upon standing to give the desired product (2*R*,3*S*)-1-allyl-3-(4-bromophenyl)azetidine-2-carbonitrile. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.59 – 7.45 (m, 2H),  
25 7.36 – 7.28 (m, 2H), 5.91 – 5.69 (m, 1H), 5.36 – 5.14 (m, 2H), 4.39 – 4.26 (m, 1H), 3.87 – 3.75 (m, 1H), 3.60 – 3.40 (m, 2H), 3.31 – 3.18 (m, 2H).

**Intermediate 154:** ((2*R*,3*S*)-1-allyl-3-(4-bromophenyl)azetidin-2-yl)methanamine

To a solution of (2*R*,3*S*)-1-allyl-3-(4-bromophenyl)azetidine-2-carbonitrile (0.62 g, 2.25 mmol) in DCM (23 ml) and cooled to 0°C with stirring was added DIBAL-H (2.41 ml, 13.51 mmol) slowly *via* syringe. After 15 minutes, the reaction was allowed to warm to room  
30 temperature over 45 min. After this time analysis by LCMS indicated the reaction was complete and the reaction was cooled to 0°C and methanol (1.366 ml, 33.8 mmol) was added slowly. To this mixture was added Rochelle's solution (100 mL) slowly (care needed, exothermic). The aqueous phase was extracted with DCM and the combined organic phases  
35 were dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give (2*R*,3*S*)-1-allyl-3-(4-bromophenyl)azetidine-2-carbonitrile as a yellow oil that was carried on to the next step without purification.

5 **Intermediate 155:** N-(((2R,3S)-1-allyl-3-(4-bromophenyl)azetidin-2-yl)methyl)-2-nitrobenzenesulfonamide

To a solution of Intermediate 154 (1.454 g, 5.171 mmol) in DCM (26 ml) and cooled to 0°C were added 2,6-lutidine (1.797 ml, 15.51 mmol) followed by 2-nitrobenzene-1-sulfonyl chloride (1.261 g, 5.69 mmol) in one portion. The solution was then allowed to warm to room temperature and stir for 16 h. To the reaction mixture was added water and the aqueous layer was extracted with DCM. The combined organic extracts were dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure to provide the crude product. The residue was purified by column chromatography (SiO<sub>2</sub>, ethyl acetate/hexane = 0-20%) to give Intermediate 155 (2.4 g, 100% yield).

15 **Intermediate 156:** N-allyl-N-(((2R,3S)-1-allyl-3-(4-bromophenyl)azetidin-2-yl)methyl)-2-nitro-benzenesulfonamide.

To a solution of Intermediate 155 (5.00 g, 10.72 mmol, 1.00 *eq*) in DMF (10.00 mL) was added K<sub>2</sub>CO<sub>3</sub> (2.22 g, 16.08 mmol, 1.50 *eq*) and 3-bromoprop-1-ene (1.95 g, 16.08 mmol, 1.39 mL, 1.50 *eq*). The mixture was stirred at 25°C for 16 hour. LCMS showed reactant 156 consumed completely, the majority was the desired product, the reaction mixture was dissolved in water (100 mL), extracted with DCM (20 mL x 3), the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, then concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=10/1 to 5:1) to give Intermediate 156 (5.00 g, 9.87 mmol, 92.10% yield) was obtained as a brown solid. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.53 (s, 1 H) 2.90 (dd, *J*=13.80, 6.65 Hz, 1 H) 2.94 - 3.11 (m, 2 H) 3.18 - 3.26 (m, 1 H) 3.33 (dd, *J*=7.34, 2.20 Hz, 1 H) 3.41 (dd, *J*=13.80, 5.14 Hz, 1 H) 3.47 - 3.63 (m, 2 H) 3.65 - 3.75 (m, 1 H) 3.88 (dd, *J*=16.00, 5.96 Hz, 1 H) 4.95 (dd, *J*=17.07, 1.13 Hz, 1 H) 5.03 - 5.14 (m, 2 H) 5.21 (dd, *J*=17.19, 1.51 Hz, 1 H) 5.48 (ddt, *J*=16.89, 10.43, 6.23, 6.23 Hz, 1 H) 5.64 - 5.87 (m, 1 H) 7.35 - 7.40 (m, 2 H) 7.42 - 7.49 (m, 2 H) 7.55 - 7.62 (m, 2 H) 7.64 - 7.72 (m, 2 H)

30 **Intermediate 157:** (3Z,8R,9S)-9-(4-bromophenyl)-6-(2-nitrophenyl)sulfonyl-1,6-diazabicyclo[6.2.0]dec-3-ene.

To a solution of Intermediate 156 (5.00 g, 9.87 mmol, 1.00 *eq*) in toluene (500.00 mL) was added Hoveyda-Grubbs Catalyst 1<sup>st</sup> Generation (812 mg) under N<sub>2</sub> atmosphere. The mixture was stirred at 60°C in an oil bath for 16 h. Additional Hoveyda-Grubbs Catalyst 1<sup>st</sup> Generation (1218 mg) was added, stirred for 16 h more. The reaction mixture was concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>,

5 petroleum ether/ethyl acetate=5/1 to 0:1) to give Intermediate 157 (1.20 g, 1.25 mmol, 12.71% yield, 50% purity) as a black solid along with recovered SM (2.2 g). <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.62 (br s, 1 H) 3.15 - 3.31 (m, 2 H) 3.38 (br s, 1 H) 3.52 (br d, *J*=16.98 Hz, 1 H) 3.60 - 3.79 (m, 3 H) 3.82 - 3.95 (m, 1 H) 4.03 (br dd, *J*=14.33, 6.62 Hz, 1 H) 5.58 - 5.67 (m, 1 H) 5.68 - 5.81 (m, 1 H) 7.30 (br d, *J*=7.94 Hz, 2 H) 7.45 (br d, *J*=8.16 Hz, 2 H) 7.56 (br d, *J*=7.28 Hz, 1 H) 7.61 - 7.67 (m, 2 H) 7.83 (br d, *J*=7.50 Hz, 1 H).

**Intermediate 158:** (3Z,8R,9S)-9-(4-bromophenyl)-1,6-diazabicyclo[6.2.0]dec-3-ene.

To a solution of Intermediate 157 (1.70 g, 1.78 mmol, 1.00 *eq*) in CH<sub>3</sub>CN (17.00 mL) was added benzenethiol (293.67 mg, 2.67 mmol, 271.92 μL, 1.50 *eq*) and Cs<sub>2</sub>CO<sub>3</sub> (694.74 mg, 2.13 mmol, 1.20 *eq*). After stirring at 40°C for 16 hour, the reaction mixture  
15 was dissolved in water (100 mL), extracted with DCM (30 mL x 3), the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, then concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=1/1 to dichloromethane: methanol=10:1) to give Intermediate 158 (650.00 mg, 1.55 mmol, 87.18% yield, 70% purity) as a black oil. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.42 - 2.50 (m, 1 H) 2.52 -  
20 2.61 (m, 1 H) 2.77 - 3.10 (m, 3 H) 3.22 (br dd, *J*=16.22, 4.38 Hz, 1 H) 3.36 - 3.50 (m, 3 H) 3.53 - 3.69 (m, 3 H) 3.77 (br t, *J*=8.99 Hz, 1 H) 5.67 - 5.86 (m, 2 H) 7.21 - 7.28 (m, 2 H) 7.44 (d, *J*=8.33 Hz, 2 H).

**Intermediate 159:** (3Z,8R,9S)-9-(4-bromophenyl)-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]dec-3-ene-6-carboxamide.

25 To a solution of Intermediate 158 (650.00 mg, 1.55 mmol, 1.00 *eq*) in DCM (10.00 mL) was added TEA (78.52 mg, 775.92 μmol, 107.56 μL, 0.50 *eq*) and then 1-isocyanato-4-methoxy-benzene (277.75 mg, 1.86 mmol, 239.44 μL, 1.20 *eq*) was added at 20°C under N<sub>2</sub> atmosphere. The mixture was stirred at 20°C for 1 hour. LCMS showed reactant 158 consumed completely, the majority was the desired product, the reaction mixture  
30 was dissolved in water (10 mL), extracted with DCM (5 mL x 3), the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, then concentrated to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, petroleum ether/ethyl acetate=5/1 to 1:1) to give Intermediate 159 (560.00 mg, 1.27 mmol, 81.84% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 2.37 - 2.56 (m, 1 H) 3.24 - 3.33 (m, 1 H) 3.34 - 3.41 (m, 1 H) 3.42  
35 - 3.53 (m, 1 H) 3.54 - 3.64 (m, 2 H) 3.68 - 3.74 (m, 2 H) 3.77 (s, 3 H) 3.86 (br dd, *J*=15.88, 7.28 Hz, 1 H) 4.17 (br d, *J*=15.44 Hz, 1 H) 5.64 (br d, *J*=11.69 Hz, 1 H) 5.74 - 5.91 (m, 1 H) 6.06 (s, 1 H) 6.82 (d, *J*=8.82 Hz, 2 H) 7.22 (d, *J*=9.04 Hz, 2 H) 7.34 (d, *J*=8.16 Hz, 2 H) 7.48

5 (d,  $J=8.38$  Hz, 2 H).

**Intermediate 160a and 160b:** (3S,4R,8R,9S)-9-(4-bromophenyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (160a); (3R,4S,8R,9S)-9-(4-bromophenyl)-3,4-dihydroxy-N-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (160b).

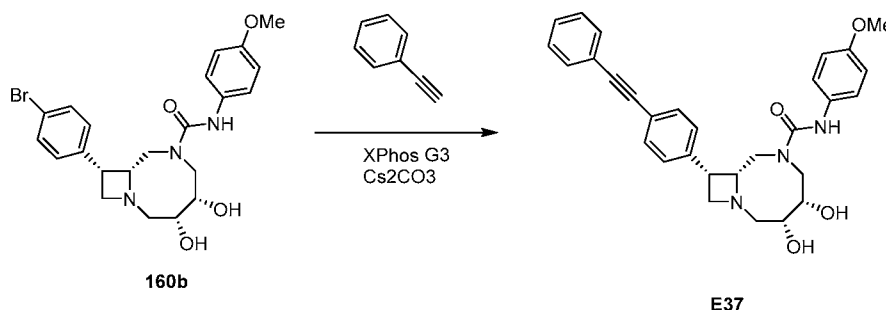
10 To a solution of Intermediate 159 (500.00 mg, 1.13 mmol, 1.00 *eq*) in acetone (5.00 mL) and H<sub>2</sub>O (500.00  $\mu$ L) was added NMO (198.63 mg, 1.69 mmol, 178.95  $\mu$ L, 1.50 *eq*) and OsO<sub>4</sub> (2.87 mg, 11.30  $\mu$ mol, 0.59  $\mu$ L, 0.01 *eq*) at -78°C. The resulting reaction mixture was then allowed to stir at 20°C for 0.5 h. TLC showed reactant consumed completely, two desired products were observed, the reaction mixture was concentrated to give a residue. The  
15 residue was purified by prep-TLC (SiO<sub>2</sub>, petroleum ether/ethyl acetate). Intermediate 160a (100.00 mg, 209.93  $\mu$ mol, 61.92% yield) was obtained as a white solid (<sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*)  $\delta$  ppm 2.17 - 2.37 (m, 1 H) 2.63 (br d,  $J=13.16$  Hz, 1 H) 2.90 (br dd,  $J=13.37$ , 8.55 Hz, 1 H) 3.23 (br d,  $J=15.79$  Hz, 1 H) 3.39 - 3.45 (m, 1 H) 3.46 - 3.52 (m, 2 H) 3.58 (br d,  $J=5.26$  Hz, 2 H) 3.73 - 3.83 (m, 5 H) 3.95 (br s, 1 H) 4.11 (br dd,  $J=15.57$ , 5.04  
20 Hz, 1 H) 4.25 (br s, 1 H) 6.81 (d,  $J=8.77$  Hz, 2 H) 7.16 (d,  $J=8.77$  Hz, 2 H) 7.32 (d,  $J=8.33$  Hz, 2 H) 7.44 (d,  $J=8.33$  Hz, 2 H) 7.92 (br s, 1 H)) and Intermediate 160b (275.00 mg, 577.29  $\mu$ mol, 72.98% yield) as a white solid (<sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*)  $\delta$  ppm 2.27 (br s, 1 H) 2.79 (br d,  $J=14.03$  Hz, 1 H) 2.93 - 3.05 (m, 1 H) 3.27 - 3.52 (m, 1 H) 3.45 - 3.52 (m, 3 H) 3.54 - 3.70 (m, 5 H) 3.73 - 3.82 (m, 4 H) 6.79 (d,  $J=9.21$  Hz, 2 H) 7.21 (d,  $J=8.77$   
25 Hz, 2 H) 7.25 - 7.30 (m, 1 H) 7.27 (d,  $J=6.82$  Hz, 1 H) 7.28 - 7.31 (m, 1 H) 7.47 (d,  $J=8.33$  Hz, 2 H)).

**Synthesis of E36:** (3S,4R,8R,9S)-3,4-dihydroxy-N-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E36).

To a solution of Intermediate 160a (10.00 mg, 20.99  $\mu$ mol, 1.00 *eq*), ethynylbenzene  
30 (6.43 mg, 62.97  $\mu$ mol, 6.91  $\mu$ L, 3.00 *eq*) in CH<sub>3</sub>CN (150.00  $\mu$ L) was added Cs<sub>2</sub>CO<sub>3</sub> (27.36 mg, 83.96  $\mu$ mol, 4.00 *eq*) and Xphos Pd G3 (1.78 mg, 2.10  $\mu$ mol, 0.10 *eq*) under N<sub>2</sub> atmosphere. The mixture was stirred at 70°C for 1 hour. LCMS showed reactant 1 consumed completely, the majority was the desired product. The reaction mixture was filtered, the filter cake was washed with DCM (10 mL), the filtrate was concentrated to give a residue. The  
35 residue was purified by prep-TLC (SiO<sub>2</sub>, petroleum ether/ethyl acetate= 1:1) and then further purified by pre-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5 $\mu$ ; A: water (0.225% formic acid) B: acetonitrile) to give compound E36 (4.00 mg, 8.04  $\mu$ mol, 38.30%

5 yield) as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CHLOROFORM-}d$ )  $\delta$  ppm 2.31 - 2.44 (m, 1 H) 2.73 (br d,  $J=13.45$  Hz, 1 H) 2.92 - 2.99 (m, 1 H) 3.33 (br d,  $J=15.66$  Hz, 1 H) 3.57 (br t,  $J=7.39$  Hz, 2 H) 3.70 (br d,  $J=4.63$  Hz, 1 H) 3.75 - 3.85 (m, 4 H) 3.87 - 3.94 (m, 1 H) 4.00 (br s, 1 H) 4.16 (br d,  $J=16.32$  Hz, 1 H) 6.83 (br d,  $J=8.82$  Hz, 2 H) 7.21 (br d,  $J=8.82$  Hz, 2 H) 7.35 (br s, 3 H) 7.44 (br d,  $J=7.94$  Hz, 2 H) 7.49 - 7.59 (m, 4 H) 7.82 (br s, 1 H).

10 **Example 37:** (3*R*,4*S*,8*R*,9*S*)-3,4-dihydroxy-*N*-(4-methoxyphenyl)-9-[4-(2-phenylethynyl)phenyl]-1,6-diazabicyclo[6.2.0]decane-6-carboxamide ("E37").



To a stirred solution of Intermediate 160b (10.00 mg, 20.99  $\mu\text{mol}$ , 1.00 *eq*), ethynylbenzene (6.43 mg, 62.98  $\mu\text{mol}$ , 6.92  $\mu\text{L}$ , 3.00 *eq*) in  $\text{CH}_3\text{CN}$  (150.00  $\mu\text{L}$ ) was added  $\text{Cs}_2\text{CO}_3$  (27.36 mg, 83.97  $\mu\text{mol}$ , 4.00 *eq*) and Xphos Pd G3 (1.78 mg, 2.10  $\mu\text{mol}$ , 0.10 *eq*) under  $\text{N}_2$  atmosphere. The mixture was stirred at 70°C for 1 hour. LCMS showed reactant 160b consumed completely, the majority was the desired product. The reaction mixture was filtered, the filter cake was washed with DCM (5 mL), the filtrate was concentrated to give a residue. The residue was purified by prep-TLC ( $\text{SiO}_2$ , petroleum ether/ethyl acetate= 1:1), then further purified by pre-HPLC (Column: Waters Xbridge Prep OBD C18 150\*30 5u; A: water (0.225% formic acid) B: acetonitrile) to give compound E37 (5.00 mg, 10.05  $\mu\text{mol}$ , 47.87% yield) as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CHLOROFORM-}d$ )  $\delta$  ppm 2.32 (br s, 1 H) 2.60 (br s, 2 H) 2.87 (br d,  $J=14.11$  Hz, 1 H) 3.06 (dd,  $J=14.00$ , 6.95 Hz, 1 H) 3.29 - 3.48 (m, 2 H) 3.57 - 3.65 (m, 2 H) 3.71 (br d,  $J=5.51$  Hz, 4 H) 3.77 (s, 3 H) 3.80 - 3.84 (m, 1 H) 6.66 (br s, 1 H) 6.82 (d,  $J=8.82$  Hz, 2 H) 7.20 - 7.26 (m, 2 H) 7.27 (s, 1 H) 7.33 - 7.43 (m, 5 H) 7.49 - 7.57 (m, 4 H).

**Example 38:** (3*R*,4*S*,8*R*,9*S*,10*S*)-*N*-(4-cyclopropoxyphenyl)-10-((dimethylamino)methyl)-3,4-dihydroxy-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (E38)

The synthesis of compound E38 was performed in an otherwise identical synthetic route to the synthesis of compound E1 where Intermediate 4b was used in place of Intermediate 4a. The same reactions steps as described above were then followed

5

**Measurements of Biological Activity****Example 39:** *In vitro* *P. falciparum* blood-stage culture and assay.

Strains of *P. falciparum* (Dd2, 3D7, D6, K1, NF54, V1/3, HB3, 7G8, FCB and TM90C2B) were obtained from the Malaria Research and Reference Reagent Resource Center (MR4). *P. falciparum* isolates were maintained with O-positive human blood in an atmosphere of 93% N<sub>2</sub>, 4% CO<sub>2</sub>, 3% O<sub>2</sub> at 37°C in complete culturing medium (10.4 g/L RPMI 1640, 5.94 g/L HEPES, 5 g/L albumax II, 50 mg/L hypoxanthine, 2.1 g/L sodium bicarbonate, 10% human serum and 43 mg /L gentamicin). Parasites were cultured in medium until parasitaemia reached 3–8%. Parasitaemia was determined by checking at least 500 red blood cells from a Giemsa-stained blood smear. For the compound screening, a parasite dilution at 2.0% parasitaemia and 2.0% haematocrit was created with medium. 25 µl of medium was dispensed into 384-well black, clear-bottom plates and 100 nl of each compound in DMSO was transferred into assay plates along with the control compound (mefloquine). Next, 25 µl of the parasite suspension in medium was dispensed into the assay plates giving a final parasitaemia of 1% and a final haematocrit of 1%. The assay plates were incubated for 72 h at 37°C. 10 µl of detection reagent consisting of 10× SYBR Green I (Invitrogen; supplied in 10,000× concentration) in lysis buffer (20 mM Tris-HCl, 5 mM EDTA, 0.16% (w/v) Saponin, 1.6% (v/v) Triton X-100) was dispensed into the assay plates. For optimal staining, the assay plates were left at room temperature for 24 h in the dark. The assay plates were read with 505 dichroic mirrors with 485 nm excitation and 530 nm emission settings in an Envision (PerkinElmer).

By following the above-described protocol, the 50% effective concentration (EC<sub>50</sub>) for compounds E1-E38 was measured against *P. falciparum* Dd2 and the results are shown in Table 4 below as +++ for an EC<sub>50</sub> less than 50 nM, ++ for an EC<sub>50</sub> from 50 nM to 250 nM and + for an EC<sub>50</sub> >250 nM.

Table 4

| Example | Pf Dd2<br>(nM) |     |     |     |     |     |     |
|---------|----------------|-----|-----|-----|-----|-----|-----|
| E1      | +++            | E11 | ++  | E21 | +   | E31 | +   |
| E2      | +++            | E12 | ++  | E22 | +   | E32 | +   |
| E3      | +              | E13 | +++ | E23 | ++  | E33 | +++ |
| E4      | +++            | E14 | +++ | E24 | +   | E34 | +++ |
| E5      | +++            | E15 | +++ | E25 | ++  | E35 | +++ |
| E6      | +++            | E16 | +   | E26 | +++ | E36 | ++  |

|     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|
| E7  | +++ | E17 | +++ | E27 | +++ | E37 | ++  |
| E8  | ++  | E18 | +   | E28 | ++  | E38 | +++ |
| E9  | +++ | E19 | ++  | E29 | ++  |     |     |
| E10 | +++ | E20 | +   | E30 | +   |     |     |

5

**Example 40:** *In vitro* *P. berghei* liver-stage assay.

HepG2 cells (ATCC) were maintained in DMEM, 10% (v/v) FBS (Sigma), and 1% (v/v) antibiotic–antimycotic in a standard tissue culture incubator (37°C, 5% CO<sub>2</sub>). *P. berghei* (ANKA GFP–luc) infected *A. stephensi* mosquitoes were obtained from the New York University Langone Medical Center Insectary. For assays, ~17,500 HepG2 cells per well were added to a 384-well microtitre plate in duplicate. After 18–24 h at 37°C the media was exchanged and compounds were added. After 1 h, parasites obtained from freshly dissected mosquitoes were added to the plates (4,000 parasites per well), the plates were spun for 10 min at 1,000 r.p.m. and then incubated at 37°C. The final assay volume was 30 µl. After a 48-h incubation at 37°C, Bright-Glo (Promega) was added to the parasite plate to measure relative luminescence. The relative signal intensity of each plate was evaluated with an EnVision (PerkinElmer) system.

**Example 41:** *In vitro* *P. falciparum* liver-stage assay.

Micropatterned co-culture (MPCC) is an *in vitro* co-culture system of primary human hepatocytes organized into colonies and surrounded by supportive stromal cells. Hepatocytes in this format maintain a functional phenotype for up to 4–6 weeks without proliferation, as assessed by major liver-specific functions and gene expression. In brief, 96-well plates were coated homogenously with rat-tail type I collagen (50 µg /mL) and subjected to soft-lithographic techniques to pattern the collagen into 500-µm-island microdomains that mediate selective hepatocyte adhesion. To create MPCCs, cryopreserved primary human hepatocytes (BioreclamationIVT) were pelleted by centrifugation at 100g for 6 min at 4°C, assessed for viability using Trypan blue exclusion (typically 70–90%), and seeded on micropatterned collagen plates (each well contained ~10,000 hepatocytes organized into colonies of 500 µM) in serum-free DMEM with 1% penicillin–streptomycin. The cells were washed with serum-free DMEM with 1% penicillin–streptomycin 2–3 h later and replaced with human hepatocyte culture medium. 3T3-J2 mouse embryonic fibroblasts were seeded (7,000 cells per well) 24 h after hepatocyte seeding.

MPCCs were infected with 75,000 sporozoites (NF54) (Johns Hopkins University) 1 day after hepatocytes were seeded. After incubation at 37°C and 5% CO<sub>2</sub> for 3 h, wells were

5 washed once with PBS, and the respective compounds were added. Cultures were dosed daily. Samples were fixed on day 3.5 after infection. For immunofluorescence staining, MPCCs were fixed with  $-20^{\circ}\text{C}$  methanol for 10 min at  $4^{\circ}\text{C}$ , washed twice with PBS, blocked with 2% BSA in PBS, and incubated with mouse anti-*P. falciparum* Hsp70 antibodies (clone 4C9, 2  $\mu\text{g}/\text{mL}$ ) for 1 h at room temperature. Samples were washed with PBS then incubated  
10 with Alexa 488-conjugated secondary goat anti-mouse for 1 h at room temperature. Samples were washed with PBS, counterstained with the DNA dye Hoechst 33258 (Invitrogen; 1:1,000), and mounted on glass slides with fluoromount G (Southern Biotech). Images were captured on a Nikon Eclipse Ti fluorescence microscope. Diameters of developing liver stage parasites were measured and used to calculate the corresponding area.

15 **Example 42:** *P. falciparum* cytoplasmic PheRS biochemical assay.

Protein sequences of both  $\alpha$ - (PF3D7\_0109800) and  $\beta$ - (PF3D7\_1104000) subunits of cytoplasmic *P. falciparum* PheRS were obtained from PlasmoDB (<http://plasmodb.org/plasmo/>). Full length  $\alpha$ - and  $\beta$ -subunit gene sequences optimized for expression in *E. coli* were cloned into pETM11 (Kanamycin resistance) and pETM20 (ampicillin resistance)  
20 expression vectors using Nco1 and Kpn1 sites and co-transformed into *E. coli* B834 cells. Protein expression was induced by addition of 0.5 mM isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG) and cells were grown until an OD<sub>600</sub> of 0.6–0.8 was reached at  $37^{\circ}\text{C}$ . They were then allowed to grow at  $18^{\circ}\text{C}$  for 20 h after induction. Cells were separated by centrifugation at 5,000g for 20 min and the bacterial pellets were suspended in a buffer consisting of 50 mM  
25 Tris–HCl (pH 7.5), 200 mM NaCl, 4 mM  $\beta$ -mercaptoethanol, 15% (v/v) glycerol, 0.1 mg/mL lysozyme and 1 mM phenylmethylsulfonyl fluoride (PMSF). Cells were lysed by sonication and cleared by centrifugation at 20,000g for 1 h. The supernatant was applied on to prepacked NiNTA column (GE Healthcare), and bound proteins were eluted by gradient-mixing with elution buffer (50 mM Tris–HCl (pH 7.5), 80 mM NaCl, 4 mM  $\beta$ -  
30 mercaptoethanol, 15% (v/v) glycerol, 1 M imidazole). Pure fractions were pooled and loaded on to heparin column for further purification. Again, bound proteins were eluted using gradient of heparin elution buffer 50 mM Tris–HCl (pH 7.5), 1 M NaCl, 4 mM  $\beta$ -mercaptoethanol, 15% (v/v) glycerol). Pure fractions were again pooled and dialysed overnight into a buffer containing 50 mM Tris–HCl (pH 7.5), 200 mM NaCl, 4 mM  $\beta$ -  
35 mercaptoethanol, 1 mM DTT and 0.5 mM EDTA. TEV protease (1:50 ratio of protease:protein) was added to the protein sample and incubated at  $20^{\circ}\text{C}$  for 24 h to remove the polyhistidine tag. Protein was further purified via gel-filtration chromatography on a GE

5 HiLoad 60/600 Superdex column in 50 mM Tris-HCl (pH 7.5), 200 mM NaCl, 4 mM  $\beta$ -mercaptoethanol, 1 mM  $MgCl_2$ . The eluted protein (a heterodimer of *P. falciparum* cPheRS) were collected, assessed for purity via SDS-PAGE and stored at  $-80^\circ C$ .

Nuclear encoded tRNAPhe from *P. falciparum* was synthesized using an *in vitro* transcription method as described in *Nature* 538, 344–349 (20 October 2016)  
10 doi:10.1038/nature19804. Aminoacylation and enzyme inhibition assays for *P. falciparum* cytosolic PheRS were performed as described in *Biochem. J.* 465, 459–469 (2015). Enzymatic assays were performed in buffer containing 30 mM HEPES (pH 7.5), 150 mM NaCl, 30 mM KCl, 50 mM  $MgCl_2$ , 1 mM DTT, 100  $\mu M$  ATP, 100  $\mu M$  L-phenylalanine, 15  $\mu M$  *P. falciparum* tRNAPhe, 2 U /mL *E. coli* inorganic pyrophosphatase (NEB) and 500 nM  
15 recombinant *P. falciparum* PheRS at  $3^\circ C$ . Reactions at different time points were stopped by the addition of 40 mM EDTA and subsequent transfer to ice. Recombinant maltose binding protein was used as negative control. The cPheRS inhibition assays were performed using inhibitor concentrations of 0.01 nM, 0.1 nM, 1 nM, 10 nM, 100 nM, 1  $\mu M$ , 5  $\mu M$  and 10  $\mu M$  for strong binders and 1 nM, 10 nM, 100 nM, 1  $\mu M$ , 10  $\mu M$ , 100  $\mu M$  and 500  $\mu M$  for weaker  
20 binders in the assay buffer. Enzymatic and inhibition experiments were performed twice in triplicate.

**Example 43:** Mammalian cell cytotoxicity assays.

Mammalian cells (HepG2, A549, and HEK293) were obtained from the ATCC and cultured routinely in DMEM with 10% FBS and 1% (v/v) antibiotic-antimycotic. For  
25 cytotoxicity assays,  $1 \times 10^6$  cells were seeded into 384-well plates 1 day before compound treatment. Cells were treated with ascending doses of compound for 72 h, and viability was measured using Cell-Titer Glo (Promega). All cell lines were tested for *Mycoplasma* contamination using Universal mycoplasma Detection Kit (ATCC).

**Example 44:** *In vitro* ADME/PK and safety assays.

30 *In vitro* characterization assays (protein binding, microsomal stability, hepatocyte stability, cytochrome P450 (CYP) inhibition, and aqueous solubility) were performed according to industry-standard techniques. Ion channel inhibition studies were performed using the Q-Patch system using standard techniques.

**Example 45:** *In vivo* *P. berghei* blood-stage assay.

35 CD-1 mice ( $n = 4$  per experimental group; female; 6–7-week-old; 20–24 g, Charles River) were intravenously inoculated with approximately  $1 \times 10^5$  *P. berghei* (ANKA GFP-luc) blood-stage parasites 24 h before treatment and compounds were administered orally (at

0 h). Parasitaemia was monitored by the *in vivo* imaging system (IVIS SpectrumCT, PerkinElmer) to acquire the bioluminescence signal (150 mg kg<sup>-1</sup> of luciferin was intraperitoneally injected approximately 10 min before imaging). In addition, blood smear samples were obtained from each mouse periodically, stained with Giemsa, and viewed under a microscope for visual detection of blood parasitaemia. Animals with parasitaemia exceeding 25% were humanely euthanized.

**Example 46:** *In vivo* *P. berghei* causal prophylaxis assay.

CD-1 mice ( $n = 4$  per experimental group; female; 6–7-week-old; 20–24 g, Charles River) were inoculated intravenously with approximately  $1 \times 10^5$  *P. berghei* (ANKA GFP-luc) sporozoites freshly dissected from *A. stephensi* mosquitoes. Immediately after infection, the mice were treated with single oral doses of Compound; infection was monitored as described for the *P. berghei* erythrocytic-stage assay. For time-course experiments, the time of compound treatment (single oral dose of 10 mg kg<sup>-1</sup>) was varied from 5 days before infection to 2 days after infection.

**Example 47:** *In vivo* *P. berghei* transmission-stage assay.

CD-1 ( $n = 3$  per experimental group; female; 6–7-week-old; 21–24 g, Charles River) mice were infected with *P. berghei* (ANKA GFP-luc) for 96 h before treatment with vehicle or compound (day 0). On day 2, female *A. stephensi* mosquitoes were allowed to feed on the mice for 20 min. After 1 week (day 9), the midguts of the mosquitoes were dissected out and oocysts were enumerated microscopically (12.5× magnification).

**Example 48:** *In vivo* *P. falciparum* blood-stage assay.

*In vivo* adapted *P. falciparum* (3D7HLH/BRD) were selected as described in *PLoS One* **3**, e2252 (2008). In brief, NSG mice ( $n = 2$  per experimental group; female; 4–5-week-old; 19–21 g; The Jackson Laboratory) were intraperitoneally injected with 1 ml of human erythrocytes (O-positive, 50% haematocrit, 50% RPMI 1640 with 5% albumax) daily to generate mice with humanized circulating erythrocytes (huRBC NSG). Approximately  $2 \times 10^7$  blood-stage *P. falciparum* 3D7HLH/BRD (*FASEB J.* **25**, 3583–3593 (2011)) were intravenously infected to huRBC NSG mice and >1% parasitaemia was achieved 5 weeks after infection. After three *in vivo* passages, the parasites were frozen and used experimentally. Approximately 48 h after infection with  $1 \times 10^7$  blood-stage of *P. falciparum* 3D7HLH/BRD, the mean parasitaemia was approximately 0.4%. huRBC NSG mice were orally treated with a single dose of compound and parasitaemia was monitored for 30 days by IVIS to acquire the bioluminescence signal (150 mg kg<sup>-1</sup> of luciferin was intraperitoneally

5 injected approximately 10 min before imaging).

**Example 49:** *In vivo P. falciparum* transmission-stage assay.

huRBCNSGmice ( $n=2$  per experimental group; female; 4–5-week-old; 18–20 g; Jackson Laboratory) were infected with blood-stage *P. falciparum* 3D7HLH/BRD for 2 weeks to allow the development of mature gametocytes. Subsequently, the mice were treated  
 10 with a single oral dose of compound. Blood samples were collected for 11 days. For molecular detection of parasite stages, 40  $\mu$ l of blood was obtained from control and treated mice. In brief, total RNA was isolated from blood samples using RNeasy Plus Kit with genomic DNA eliminator columns (Qiagen). First-strand cDNA synthesis was performed from extracted RNA using SuperScript III First- Strand Synthesis System (Life  
 15 Technologies). Parasite stages were quantified using a stage-specific qRT–PCR assay as described in *Sci. Transl. Med.* 6, 244re5 (2014). Primers were designed to measure transcript levels of PF3D7\_0501300 (ring stage parasites), PF3D7\_1477700 (immature gametocytes) and PF3D7\_1031000 (mature gametocytes). Primers for PF3D7\_1120200 (*P. falciparum* UCE) transcript were used as a constitutively expressed parasite marker. The assay was  
 20 performed using cDNA in a total reaction volume of 20  $\mu$ l, containing primers for each gene at a final concentration of 250 nM. Amplification was performed on a Viia7 qRT–PCR machine (Life Technologies) using SYBR Green Master Mix (Applied Biosystems) with the following reaction conditions: 1 cycle  $\times$  10 min at 95°C and 40 cycles  $\times$  1 s at 95°C and 20 s at 60°C. Each cDNA sample was run in triplicate and the mean  $C_t$  value was used for the  
 25 analysis.  $C_t$  values obtained above the cut-off (negative control) for each marker were considered negative for the presence of specific transcripts. Blood samples from each mouse before parasite inoculation were also tested for ‘background noise’ using the same primer sets. No amplification was detected from any samples.

**Example 50:** *In vivo P. falciparum* liver-stage assay.

30 FRG knockout on C57BL/6 (human repopulated, >70%) mice (huHep FRG knockout;  $n = 2$  per experimental group; female; 5.5–6-month-old; 19–21 g; Yecuris) were inoculated intravenously with approximately  $1 \times 10^5$  *P. falciparum* (NF54HT-GFP-luc) sporozoites and Compound was administered as a single 10 mg kg<sup>–1</sup> oral dose one day after inoculation. Infection was monitored daily by IVIS. Daily engraftment of human erythro- cytes (0.4 ml,  
 35 O-positive, 50% haematocrit, 50% RPMI 1640 with 5% albumax) was initiated 5 days after inoculation. For qPCR analysis, blood samples (40  $\mu$ l) were collected 7 days after inoculation. For molecular detection of the blood- stage parasite, 40  $\mu$ l of blood was obtained

5 from control and treated mice. In brief, total RNA was isolated from blood samples using RNeasy Plus Kit with genomic DNA eliminator columns (Qiagen). First-strand cDNA synthesis was performed from extracted RNA using SuperScript III First-Strand Synthesis System (Life Technologies). The presence of the blood-stage parasites was quantified using a highly stage-specific qRT-PCR assay as described in *PLOS Comput. Biol.* 9, e1003392  
10 (2013). Primers were designed to measure transcript levels of PF3D7\_1120200 (*P. falciparum* UCE). The assay was performed using cDNA in a 20 µl total reaction volume containing primers for each gene at a final concentration of 250 nM. Amplification was performed on a Viia7 qRT-PCR machine (Life Technologies) using SYBR Green Master Mix (Applied Biosystems) and the reaction conditions are as follows: 1 cycle×10min at 95°C  
15 and 40 cycles×1 s at 95°C and 20 s at 60°C. Each cDNA sample was run in triplicate and the mean *Ct* value was used for the analysis. *Ct* values obtained above the cut-off (negative control) for each marker were considered negative for presence of specific transcripts. Blood samples from each mouse were also tested for background noise using the same primer sets before parasite inoculation. No amplification was detected from any samples.

20 **Example 51: *In vivo* *C. parvum* assay.**

The NOD SCID gamma mouse model of chronic, asymptomatic *C. parvum* infection was used to test *in vivo* compound efficacy. NOD SCID gamma mice were infected with ~1×10<sup>5</sup> *C. parvum* oocysts by oral gavage 5-7 days after weaning. The infected animals begin shedding oocysts in the feces 1 week after infection, which is measured by quantitative  
25 PCR (qPCR). Based on experience with the positive control compound paromomycin, four mice are required per experimental group to achieve 80% power to detect an 80% percent reduction in parasite shedding after four days of drug compound. In addition to the experimental drug regimen groups, additional negative (gavage with DMSO/methylcellulose carrier) and positive (paromomycin 2000 mg/kg once daily) control groups are included in  
30 each experiment. Mice are infected 5-7 days after weaning (day -6), infection is confirmed 1 week later (day 0), and experimental compounds are dosed by oral gavage on days 1-4. The dosing frequency was as indicated. Treatment efficacy was assessed by measurement of fecal oocyst shedding by qPCR on day 5.

**Example 52: *C. parvum* *in vitro* EC<sub>50</sub> assay.**

35 Human ileocecal adenocarcinoma (HCT-8) cells were obtained from ATCC and maintained in T-75 tissue culture flasks with RPMI 1640 medium with HEPES, sodium pyruvate (1 mM), and L-glutamine (ATCC) supplemented with 10% horse serum (ATCC)

5 and 120 U/ml penicillin and 120 µg/ml streptomycin. Cells were plated into 384-well, tissue culture-treated, black-walled, clearbottom microwell plates (BD Falcon) at a density of 8,850 cells/well and allowed to grow to confluence. They were then inoculated with  $5.5 \times 10^3$  primed *C. parvum* oocysts (Bunchgrass Farms, Deary, ID) suspended in inoculation medium (RPMI 1640 without horse serum). Oocysts were primed for excystation by following a  
 10 previously described protocol (J. Eukaryot. Microbiol. 46:56S–57S). Briefly, oocysts were treated for 10 min with 10mM HCl at 37°C, centrifuged, and treated with a 2 mM solution of sodium taurocholate (Sigma-Aldrich) in phosphate-buffered saline (PBS) with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . The suspension was incubated for 10 min at 16°C and then diluted in inoculation medium and added to each well. Infected cells were incubated at 37°C for 3 h, at which point  
 15 an equal volume of growth medium containing 20% horse serum (total serum concentration of 10%). Compounds were diluted and assayed at fixed doses of 0.12, 0.37, 1.1, 3.3, and 10 µM (each concentration, n =14) for the generation of EC<sub>50</sub> curves. In the case of final EC<sub>50</sub> curves, three wells were left uninfected but treated with each of the corresponding concentrations of the compound to assess for background staining. All curves were generated  
 20 using the log[inhibitor] versus response \_ variable slope equation in GraphPad Prism (equation 3), with the bottom constraint set equal to 0.

By following the above-described protocol, the 50% effective concentration (EC<sub>50</sub>) for several compounds having the structure of formula (I) was measured against *C. parvum* and the results are shown in Table 5 below.

Table 5

| Compound | EC <sub>50</sub> (nM) |
|----------|-----------------------|
| E26      | 98                    |
| E27      | 134                   |
| E15      | 33                    |
| E36      | 6                     |
| E6       | 95                    |
| E4       | 68                    |

### Other Embodiments

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. Also incorporated by  
 30 reference is the disclosure of US Publication No. 2016/0289235. In particular embodiments,

- 5 a compound of the invention is not a compound described in Table 1 of US Publication No. 2016/0289235.

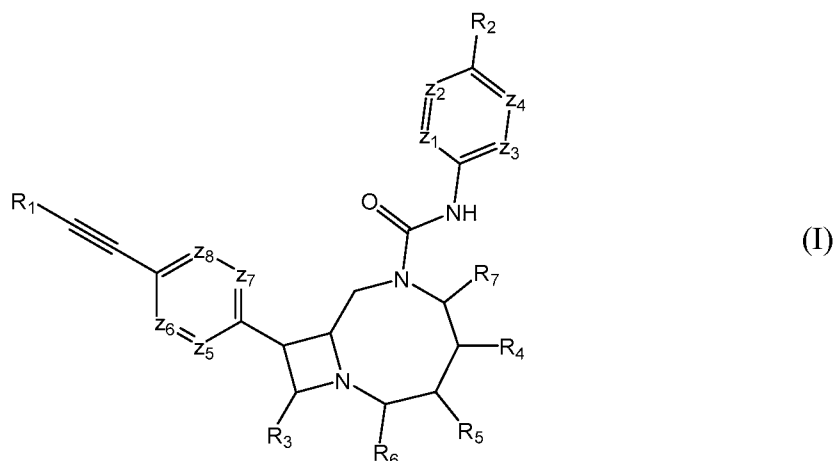
While the disclosure provides specific embodiments, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the following, in general, the principles described herein and including such  
10 departures from the present disclosure come within known or customary practice within the art to which the disclosure pertains and may be applied to the essential features hereinbefore set forth.

5

## CLAIMS

What is claimed is:

1. A compound having the structure of formula (I):



wherein the “dashed” bond may be a single or double bond;

R<sub>1</sub> is optionally substituted aryl or heteroaryl;

10 R<sub>2</sub> is optionally substituted alkoxy, cycloalkoxy or heterocyclyl;

R<sub>3</sub> is hydrogen, or -CH<sub>2</sub>-X;

R<sub>4</sub> and R<sub>5</sub> are independently hydrogen, -X, or -CH<sub>2</sub>-X, R<sub>4</sub> and R<sub>5</sub> may together form a five- or six-membered fused ring, and at least one of R<sub>4</sub> and R<sub>5</sub> is not hydrogen;

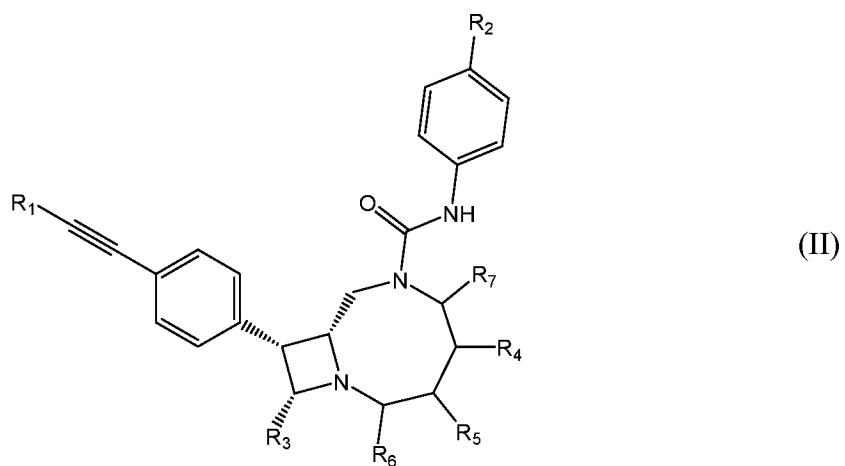
R<sub>6</sub> and R<sub>7</sub> are independently hydrogen or R; and

15 z<sub>1</sub>-z<sub>8</sub> are independently selected at each occurrence from CH or N; where

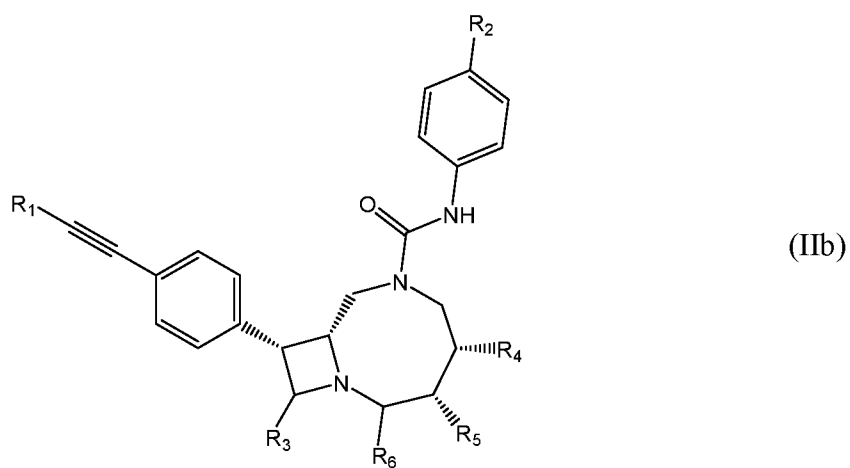
-X is independently selected at each occurrence from -OH, -OR, -S(O)R, -S(O)<sub>2</sub>R, -N(R)-S(O)<sub>2</sub>R, -S(O)<sub>2</sub>-N(R)(R), -S(O)<sub>2</sub>-NHR, -N(R)-C(O)-R or -N(R)(R); and

R is independently at each occurrence an optionally substituted C<sub>1</sub>-C<sub>12</sub> alkyl; or a pharmaceutically acceptable salt thereof.

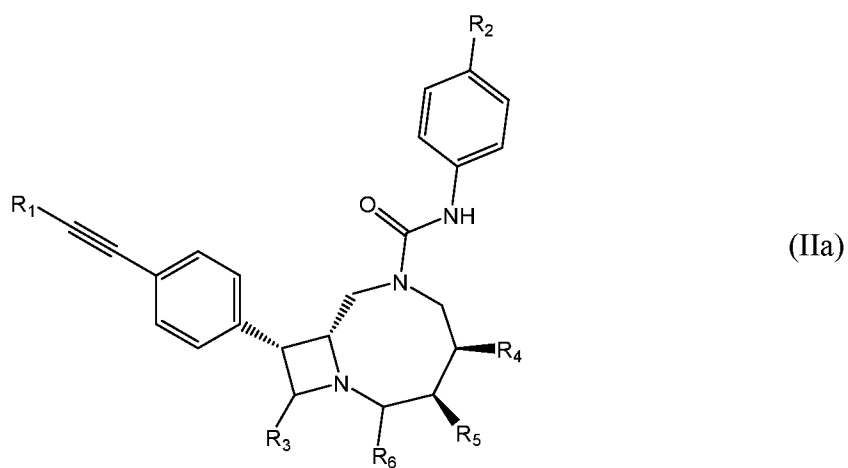
20 2. The compound according to claim 1 having the structure of formula (II):



- 5 3. The compound according to claim 1 or 2 wherein R<sub>7</sub> is hydrogen.
4. The compound according to claim 1 having the structure of formula (IIb):



5. The compound according to claim 1 having the structure of formula (IIa):

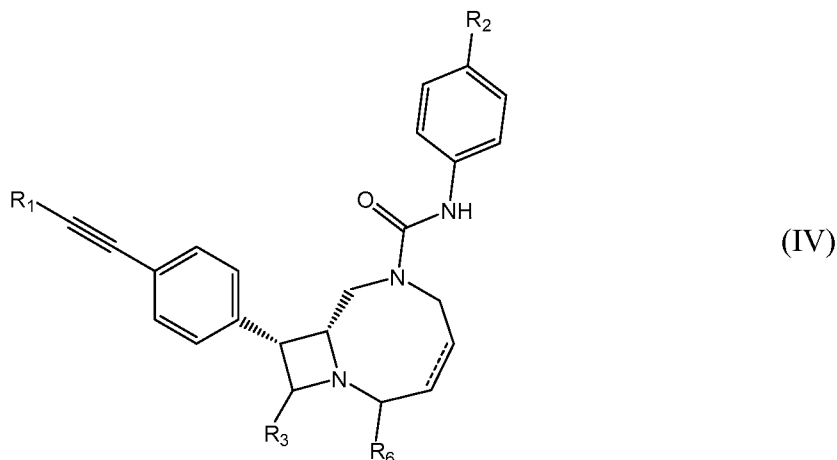


- 5 6. The compound according to any one of claims 1-5, wherein  $R_4$  and  $R_5$  are the same functional group selected from  $-X$  or  $-(CH_2)-X$ .
7. The compound according to any one of claims 1-5, wherein  $R_5$  is hydrogen and  $R_4$  is  $-X$ , or  $-(CH_2)-X$ .
8. The compound according to any one of claims 1-5, wherein  $R_4$  is hydrogen and  $R_5$  is  $-X$ ,  
10 or  $-(CH_2)-X$ .
9. The compound according to any one of claims 6-8, wherein  $-X$  in the group  $-X$  or  $-(CH_2)-X$  of  $R_4$  or  $R_5$  is  $-OH$ ,  $-NH_2$ , or  $-N(R)(R)$ .
10. The compound according to any one of claims 1-5, wherein  $R_4$  and  $R_5$  are independently selected from  $-OH$  and  $-OR$  and  $R_4$  and  $R_5$  together form a 6-membered fused ring.
- 15 11. The compound according to any one of claims 1-10, wherein  $R_6$  is a  $C_{1-4}$  linear or branched hydrocarbon.
12. The compound according to any one of claims 1-10, wherein  $R_6$  is hydrogen.
13. The compound according any one of claims 1-11, wherein  $R$  independently at each occurrence a  $C_{1-4}$  linear or branched hydrocarbon.
- 20 14. The compound according to any one of claims 1-13, wherein  $R_1$  is an optionally substituted  $C_6$  aryl or heteroaryl.
15. The compound according to any one of claims 1-14, wherein  $R_1$  is phenyl, fluorophenyl, difluorophenyl, or pyridyl.
16. The compound according to any one of claims 1-15, wherein  $R_2$  is  $C_{1-4}$  linear or  
25 branched alkoxy.
17. The compound according to any one of claims 1-16, wherein  $R_2$  is methoxy, ethoxy, propoxy, or isopropoxy.
18. The compound according to any one of claims 1-15, wherein  $R_2$  is  $C_{1-4}$  linear or branched alkoxy substituted with one or more F.
- 30 19. The compound according to any one of claims 1-15, wherein  $R_2$  is  $-OCF_3$ ,  $-OCHF_2$ , or  $-OCH_2F$ .



- 5     $-X$  is independently selected at each occurrence from  $-OH$ ,  $-OR$ ,  $-S(O)R$ ,  $-S(O)_2R$ ,  $-N(R)-S(O)_2R$ ,  $-S(O)_2-N(R)(R)$ ,  $-S(O)_2-NHR$ ,  $-N(R)-C(O)-R$  or  $-N(R)(R)$ ; and  
 $R$  is independently at each occurrence a  $C_1-C_{12}$  alkyl;  
 with the proviso that in the case where  $R_6$  is hydrogen,  $R_3$  is  $-CH_2-N(R)(R)$  and said  
 “dashed” bond is a double bond.

- 10    29. The compound according to claim 28 having the structure of formula (IV):



30. The compound according to claim 28 or 29, wherein said “dashed” bond is a double bond.
31. The compound according to claim 28 or 29, wherein  $R_6$  is a  $C_{1-4}$  linear or branched hydrocarbon.
32. The compound according to any one of claims 28-31, wherein  $R_1$  is an optionally substituted  $C_6$  aryl or heteroaryl.
33. The compound according to any one of claims 28-32, wherein  $R_1$  is phenyl, fluorophenyl, difluorophenyl, or pyridyl.
34. The compound according to any one of claims 28-33, wherein  $R_2$  is  $C_{1-4}$  linear or branched alkoxy.
35. The compound according to any one of claims 28-23, wherein  $R_2$  is methoxy, ethoxy, propoxy, or isopropoxy.
36. The compound according to claim 35, wherein  $R_2$  is  $C_{3-6}$  cycloalkoxy.
37. The compound according to claim 35, wherein  $R_2$  is cyclopropoxy.

- 5 38. The compound according to any one of claims 1-15, wherein R<sub>2</sub> is C<sub>1-4</sub> linear or branched alkoxy substituted with one or more F.
39. The compound according to any one of claims 1-15, wherein R<sub>2</sub> is -OCF<sub>3</sub>, -OCHF<sub>2</sub>, or -OCH<sub>2</sub>F.
- 10 40. The compound according to any one of claims 1-15, wherein R<sub>2</sub> is C<sub>3-6</sub> heterocyclyl (e.g., aziridinyl, oxiranyl, thiranyl, oxetanyl, azetidyl, thietanyl, diazetidinyl, dioxetanyl, dithietanyl, pyrrolidinyl, tetrahydrofuranyl, imidazolyl, pyrazolyl, oxazolyl, etc.).
41. The compound according to any one of claims 1-15, wherein R<sub>2</sub> is oxetanyl.
42. The compound according to any one of claims 28 or 30-37, wherein one of z<sub>1</sub>-z<sub>4</sub> is N and the rest are CH.
- 15 43. The compound according to any one of claim 28 or 30-42, wherein one of z<sub>5</sub>-z<sub>8</sub> is N and the rest are CH.
44. The compound according to any one of claims 28, 30-37 or 43, wherein z<sub>1</sub> and z<sub>4</sub> are each N and z<sub>3</sub> and z<sub>2</sub> are each CH.
45. The compound according to any one of claims 28, 30-37, or 44, wherein z<sub>5</sub> and z<sub>7</sub> are  
20 each N and z<sub>6</sub> and z<sub>8</sub> are each CH.
46. The compound according to claims 1 or 28, wherein said compound is selected from the group consisting of compounds E1-E38.
47. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a compound of any one of claims 1-46
- 25 48. The pharmaceutical composition according to claim 47, wherein said compound is present in an effective amount.
49. The pharmaceutical composition according to claim 47, formulated as a veterinary composition.
50. The pharmaceutical composition according to any one of claims 47-49, wherein said  
30 compound is present in an effective amount to treat malaria.
51. The pharmaceutical composition according to any one of claims 47-49, wherein said compound is present in a therapeutically effective amount to treat a disease caused by a parasite from the genus *Cryptosporidium*.

- 5 52. The pharmaceutical composition according to claim 51, wherein said disease is cryptosporidiosis.
53. A method of treatment or prophylaxis of a parasitic disease in a subject, comprising the step of administering to the subject an effective amount of a compound of any one of claims 1-46.
- 10 54. The method of claim 53, wherein said parasitic disease is malaria.
55. The method of claim 53, wherein said malaria is drug resistant malaria.
56. The method of claim 55, wherein drug resistant malaria is resistant to chloroquine, quinine, prymethamine, sulfadoxine, mefloquine, artemether, lumefantrine, artesunate, amodiaquine, dihydroartemisinin, piperaquine, proguanil, doxycycline, clindamycin,
- 15 artemisinin, atovaquone, or any combination thereof.
57. The method of claim 53, wherein said malaria is liver stage malaria.
58. The method of claim 57, wherein the liver of said subject is infected with a malaria-causing parasite and said treatment prevents spread of said infection from their liver.
59. The method of claims 53, wherein said malaria is blood stage malaria.
- 20 60. The method of claim 53, wherein said malaria is transmission stage malaria.
61. The method of claim 53, wherein said malaria is carried in a mosquito species selected from *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi*, *P. berghei*, *P. chabaudi*, *P. vinckei*, or *P. yoelii*.
62. The method of claim 61, witherein said mosquito speices is *P. falciparum*.
- 25 63. The method of claim 53, wherein said parasitic disease is cryptosporidiosis.
64. The method of claim 63, wherein said cryptosporidiosis is carried by *C. parvum*.
65. The method of claim 53, wherein said subject is human.
66. The method of claim 53, wherein said subject is not human.
67. The method of claim 66, wherein said subject is a mouse, rat, rabbit, non-human primate,
- 30 lizards, geckos, cow, calf, sheep, lamb, horse, foal, pig, or piglet.
68. A method of treatment or prophylaxis of a parasitic disease in a subject, comprising the step of administering to a subject the pharmaceutical composition of any one of claims 47-52.

- 5 69. The method of claim 68, wherein said parasitic disease is malaria.
70. The method of claim 68, wherein said malaria is drug resistant malaria.
71. The method of claim 70, wherein drug resistant malaria is resistant to chloroquine, quinine, prymethamine, sulfadoxine, mefloquine, artemether, lumefantrine, artesunate, amodiaquine, dihydroartemisinin, piperaquine, proguanil, doxycycline, clindamycin,  
10 artemisinin, atovaquone, or any combination thereof.
72. The method of claim 68, wherein said malaria is liver stage malaria.
73. The method of claim 72, wherein the liver of said subject is infected with a malaria-causing parasite and said treatment prevents spread of said infection from their liver.
74. The method of claims 68, wherein said malaria is blood stage malaria.
- 15 75. The method of claim 68, wherein said malaria is transmission stage malaria.
76. The method of claim 68, wherein said malaria is carried in a mosquito species selected from *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi*, *P. berghei*, *P. chabaudi*, *P. vinckei*, or *P. yoelii*.
77. The method of claim 68, witherein said mosquito speices is *P. falciparum*.
- 20 78. The method of claim 68, wherein said parasitic disease is cryptosporidiosis.
79. The method of claim 78, wherein said cryptosporidiosis is carried by *C. parvum*.
80. The method of claim 68, wherein said subject is human.
81. The method of claim 68, wherein said subject is not human.
82. The method of claim 81, wherein said subject is a mouse, rat, rabbit, non-human primate,  
25 lizards, geckos, cow, calf, sheep, lamb, horse, foal, pig, or piglet.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/23270

## A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 31/395; C07D 487/04 (2018.01)

CPC - A61K 31/395; C07D 487/04

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)

See Search History document

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

See Search History document

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

See Search History document

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                      | Relevant to claim No.  |
|-----------|-------------------------------------------------------------------------------------------------------------------------|------------------------|
| X         | US 2016/0289235 A1 (THE BROAD INSTITUTE, INC.) 06 October 2016; paragraphs [0021]-[0027], [0039], [0042]-[0043], [0139] | 1-2, 3/1-2, 4-5, 28-29 |
| A         | US 3,176,017 A (FREED, ME) 30 March 1965; entire document                                                               | 1-2, 3/1-2, 4-5, 28-29 |

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

## \* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&amp;" document member of the same patent family

Date of the actual completion of the international search

26 April 2018 (26.04.2018)

Date of mailing of the international search report

20 JUN 2018

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/23270

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

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

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 6-27, 32-45, 47-82  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

---Please See Within the Next Supplemental Box---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-2, 3/1-2, 4-5, 28-29

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

\*\*\*-Continued from Box No. III Observations where unity of invention is lacking \*\*\*-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid. Groups I+, Claims 1-5 (in-part), 28-31 (in-part), and 46 (in-part); compound of Formula (I), wherein the "dashed" bond is a single bond; R1 is optionally substituted aryl; R2 is optionally substituted alkoxy; R3 is -CH<sub>2</sub>-X wherein X is -OH; R4 and R5 are hydrogen; R6 and R7 are hydrogen; and Z1-Z8 are CH (first exemplary compound structure).

The compound, compositions and methods will be searched to the extent the compound encompasses a compound of Formula (I), wherein the "dashed" bond is a single bond; R1 is optionally substituted aryl; R2 is optionally substituted alkoxy; R3 is -CH<sub>2</sub>-X wherein X is -OH; R4 and R5 are hydrogen; R6 and R7 are hydrogen; and Z1-Z8 are CH (first exemplary compound structure). Applicant is invited to elect additional compound(s), with fully specified structure (e.g. no optional or variable atoms or substituents) for each, to be searched. Additional compound(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 4 (in-part), 5 (in-part), 28 (in-part), and 29 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass a compound of Formula (I), wherein the "dashed" bond is a single bond; R1 is optionally substituted aryl; R2 is optionally substituted alkoxy; R3 is -CH<sub>2</sub>-X wherein X is -OH; R4 and R5 are hydrogen; R6 and R7 are hydrogen; and Z1-Z8 are CH (first exemplary compound structure). Applicants must specify the claims that encompass any additionally elected compound structure(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a compound of Formula (I), wherein the "dashed" bond is a double bond; R1 is optionally substituted aryl; R2 is optionally substituted alkoxy; R3 is -CH<sub>2</sub>-X wherein X is -OH; R4 and R5 are hydrogen; R6 and R7 are hydrogen; and Z1-Z8 are CH (first exemplary elected compound structure).

Groups I+ share the technical features including: a compound of Formula (I), wherein the "dashed" bond is a single bond; R1 is optionally substituted aryl; R2 is optionally substituted alkoxy; R3 is -CH<sub>2</sub>-X wherein X is -OH; R4 and R5 are hydrogen; R6 and R7 are hydrogen; and Z1-Z8 are CH.

However, these shared technical features are previously disclosed by US 2016/0289235 A1 (THE BROAD INSTITUTE, INC.) (hereinafter 'Broad').

Broad discloses a compound of Formula (I), as shown, wherein the "dashed" bond is a single bond; R1 is optionally substituted aryl; R2 is optionally substituted alkoxy; R3 is -CH<sub>2</sub>-X wherein X is -OH; R4 and R5 are hydrogen; R6 and R7 are hydrogen; and Z1-Z8 are CH (compound 27 on page 9 of table 1; paragraph [0043]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Broad reference, unity of invention is lacking.