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(54) Title: TREATMENT AND PREVENTION OF HBV DISEASES BY CYCLOSPORINE ANALOGUE MOLECULES MODIFIED AT AMINO ACIDES 1 AND 3

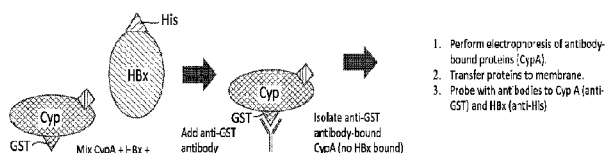
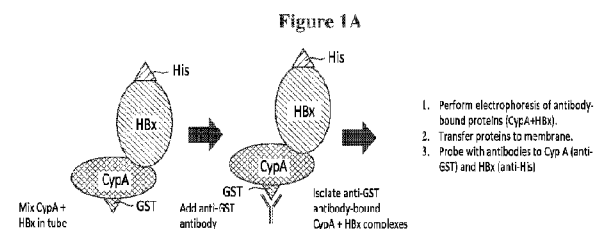
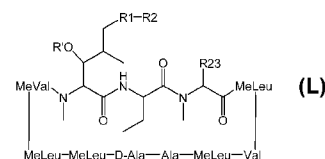
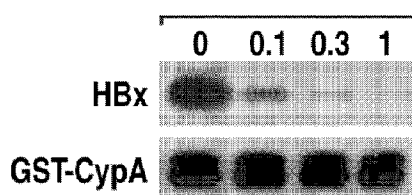


Figure 1B
Compound I
(μM)



(57) Abstract: The present application relates to a method of treating and/or preventing a hepatitis B virus (HBV) disease through inhibiting the interaction of CypA with HBV X protein (HBx) and/or Hepatitis B surface antigen (HBsAg), comprising administering to a subject in need thereof a compound of Formula L.



MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
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Published:

- *with international search report (Art. 21(3))*

TREATMENT AND PREVENTION OF HBV DISEASES BY CYCLOSPORINE ANALOGUE MOLECULES MODIFIED AT AMINO ACIDS 1 AND 3

RELATED APPLICATIONS

This application claims priority to, and the benefit of, U.S. Application No. 62/431,746, filed on December 8, 2016, and U.S. Application No. 62/479,683, filed on March 31, 2017, the entire contents of each of which are incorporated by reference.

5

BACKGROUND

Cyclosporines are a class of cyclic polypeptides having potent immunosuppressant activity. Among the many compounds in the cyclosporine family, Cyclosporine A (CsA) is the most widely used medically. Cyclosporines have three well established targets: calcineurin, the
10 CyP isoforms (*e.g.*, CyP-A, CyP-B, and CyP-D), and P-glycoprotein (PgP). The binding of cyclosporine to calcineurin results in significant immunosuppression.

Many non-naturally occurring cyclosporines have been prepared. For example, cyclosporine analogs modified at amino acid-1 (*e.g.*, “ISA_{TX}247”, “ISA247”, or “ISA”) are disclosed in WO 1999/018120 and WO 2003/033527, which are incorporated herein by
15 reference in their entirety. ISA247 is structurally identical to CsA and exhibits enhanced immunosuppression and reduced toxicity over many other cyclosporines.

HBx is a multifunctional viral protein known to be essential for HBV replication and for the maintenance and progression of chronic HBV disease. HBx is involved in the production and secretion of key viral proteins, such as HBsAg and HBeAg. HBx further disables host
20 factors that restrict the production of HBV and other viral products, including covalently closed circular DNA (cccDNA), which is key to the persistence of chronic HBV infection. (HBsAg) is another key HBV protein. High levels of HBsAg in HBV-infected patients is a predictor for progression of disease, including liver fibrosis, cirrhosis, and cancer.

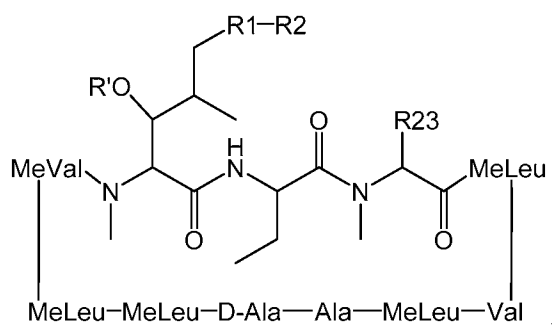
CyPs are involved in a multitude of cellular processes. Recent studies show that CyPs
25 may represent additional drug targets for hepatitis B treatment. Accordingly, cyclosporines that bind to CyPs can be useful in the treatment of many disease indications, such as hepatitis B. However, the concomitant effects of immunosuppression limit the utility of cyclosporines in clinical practice. Only a few CsA analogs have been proven to have little or reduced

immunosuppressive activity and still retain their ability to bind CyPs. Those CsA analogs generally require laborious and complex modification of amino acids 3 and 4 that involves disruption of the cyclosporine ring structure. Also, a single modification of amino acid 1 results in increased immunosuppression.

As such, there is a need for novel cyclosporine analogs that is readily synthesized, retains binding to CyPs, and displays reduced or no immunosuppression for the treatment and/or prevention of disease indications in which CyPs are involved (*e.g.*, HBV diseases). The present application addresses the need.

SUMMARY

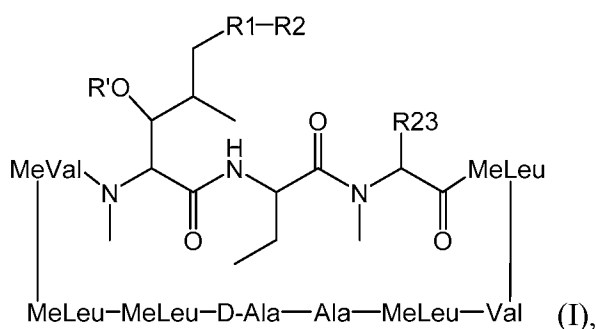
The present application relates to a method of treating and/or preventing a hepatitis B virus (HBV) disease, comprising administering to a subject in need thereof a compound of Formula L:



Formula L

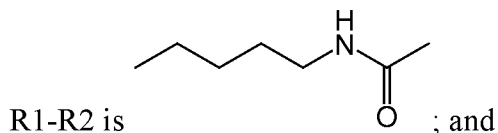
or a pharmaceutically acceptable salt thereof, wherein R', R1, R2, and R23 are each as defined herein below.

The present application relates to a method of treating and/or preventing a HBV disease, comprising administering Compound I, or a pharmaceutically acceptable salt thereof, to a subject in need thereof:



wherein:

R' is H;



R23 is methyl.

5 The present application also relates to a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, for use in treating and/or preventing a HBV disease in a subject in need thereof.

The present application also relates to a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament for
10 treatment and/or prevention of a HBV disease in a subject in need thereof.

The present application also relates to use of a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof.

The present application relates to a method of treating and/or preventing a HBV disease,
15 comprising administering to a subject in need thereof a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, in combination with a second therapeutic agent.

The present application also relates to a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, for use in treating and/or preventing a HBV disease in a subject in need thereof, wherein the subject is also administered a second therapeutic agent.

20 The present application also relates to a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof, wherein the subject is also administered a second therapeutic agent.

The present application also relates to use of a compound of Formula L or Compound I,
25 or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof, wherein the subject is also administered a second therapeutic agent.

In one embodiment, the present application relates to treating/treatment of a HBV disease in a subject in need thereof. In one embodiment, the present application relates to preventing/prevention of a HBV disease in a subject in need thereof.

In one embodiment, the treatment and/or prevention of a HBV disease is through modulation (*e.g.*, inhibition) of an interaction of HBV X protein (HBx) with cyclophilin A (CypA). In another embodiment, the treatment and/or prevention of a HBV disease is through modulation (*e.g.*, inhibition) of an interaction of Hepatitis B surface antigen (HBsAg) with cyclophilin A (CypA).

The present application relates to a method of modulating (*e.g.*, inhibiting) an interaction of HBx with CypA, comprising administering to a subject in need thereof a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof. The present application also relates to a method of modulating (*e.g.*, inhibiting) an interaction of HBsAg with CypA, comprising administering to a subject in need thereof a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof).

The present application also relates to a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, for use in modulating (*e.g.*, inhibiting) an interaction of HBx with CypA in a subject in need thereof. The present application also relates to a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, for use in modulating (*e.g.*, inhibiting) an interaction of HBsAg with CypA in a subject in need thereof.

The present application also relates to a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament for modulation (*e.g.*, inhibition) of an interaction of HBx with CypA in a subject in need thereof. The present application also relates to a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament for modulation (*e.g.*, inhibition) of an interaction of HBsAg with CypA in a subject in need thereof.

The present application also relates to use of a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for modulation (*e.g.*, inhibition) of an interaction of HBx with CypA in a subject in need thereof. The present application also relates to use of a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for modulation (*e.g.*, inhibition) of an interaction of HBsAg with CypA in a subject in need thereof.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this application belongs. In the case of conflict, the present specification, including definitions, will control. In the specification, the singular forms also include the plural unless the context clearly dictates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present application, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference. The references cited herein are not admitted to be prior art to the present application. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Other features and advantages of the present application will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A illustrates a GST pulldown assay which measures the inhibition of interaction of HBx with CypA by a compound of the present application.

Figure 1B is a Western blot showing inhibition of interaction of HBx with CypA by a compound of the present application at the indicated concentrations.

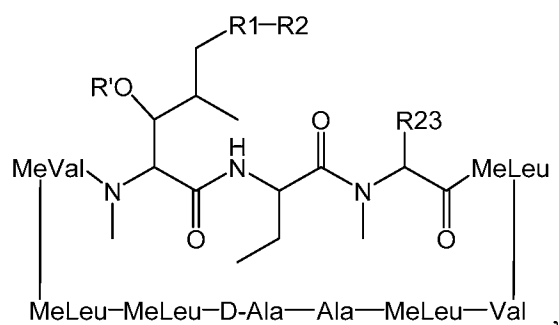
Figure 2A is a chart displaying inhibition of interaction of HBx with CypA as a function of concentration of a compound of the present application, Compound II, or Alisporivir.

Figure 2B is a chart displaying inhibition of cyclophilin isomerase activity as a function of concentration of a compound of the present application, Alisporivir, or CsA.

Figure 3 illustrates a microtiter plate binding assay which measures the inhibition of interaction of HBx with CypA by a compound of the present application.

DETAILED DESCRIPTION

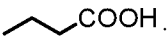
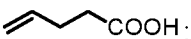
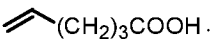
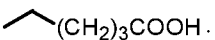
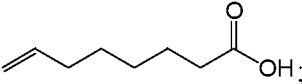
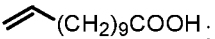
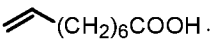
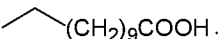
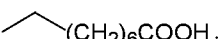
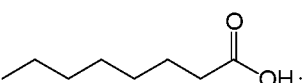
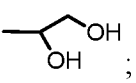
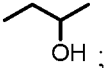
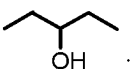
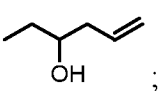
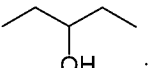
The present application relates to a method of treating and/or preventing a hepatitis B virus (HBV) disease, comprising administering to a subject in need thereof a compound of Formula L:

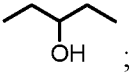
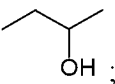
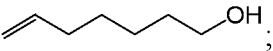
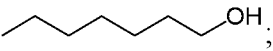
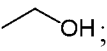
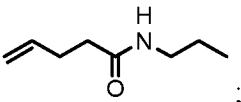
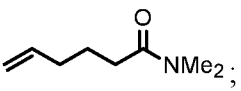
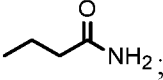
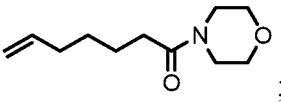
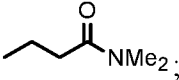
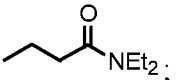
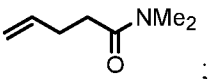
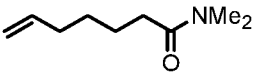
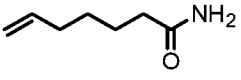
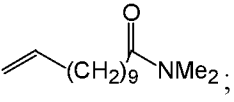
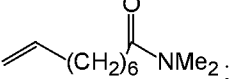
**Formula L**

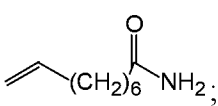
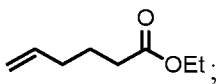
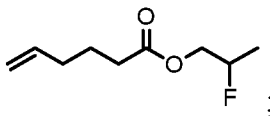
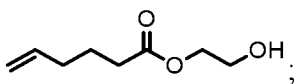
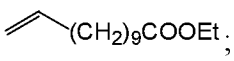
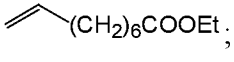
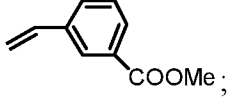
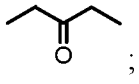
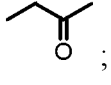
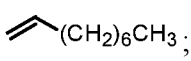
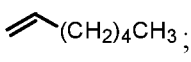
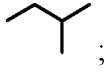
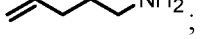
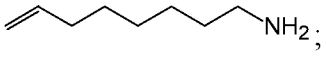
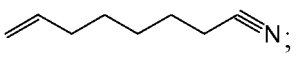
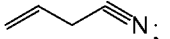
or a pharmaceutically acceptable salt thereof, wherein:

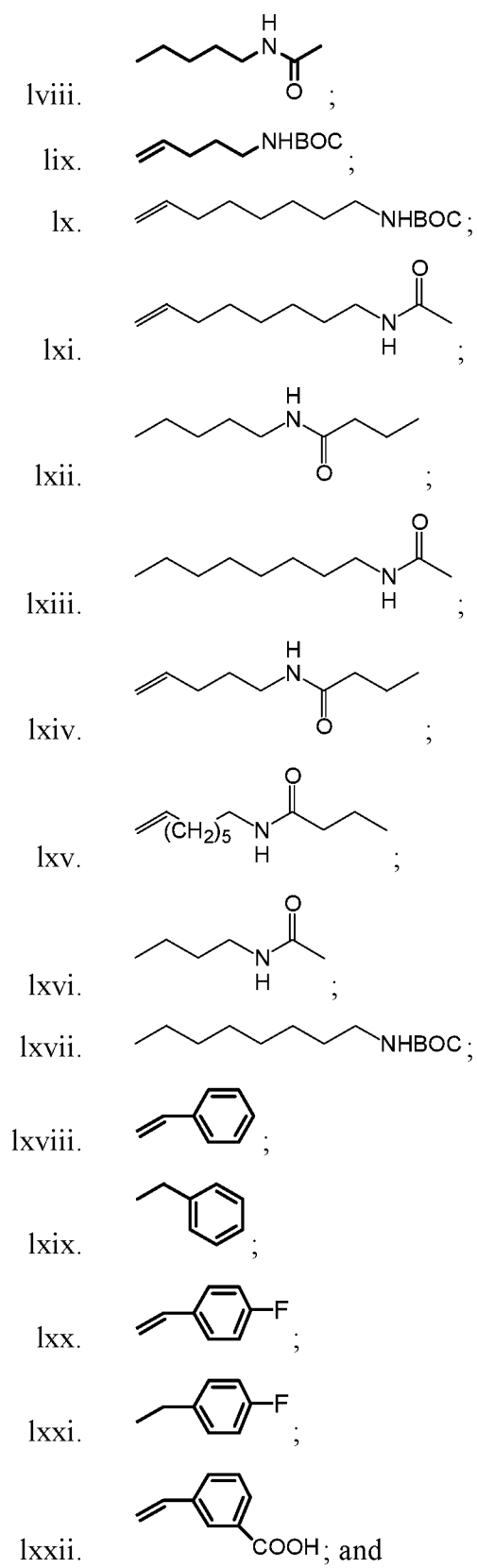
- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - i. H;
 - ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;
 - iii. a N-substituted or unsubstituted acyl protected amine;
 - iv. a carboxylic acid;
 - v. a N-substituted or unsubstituted amine;
 - vi. a nitrile;
 - vii. an ester;
 - viii. a ketone;
 - ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
 - x. a substituted or unsubstituted aryl;
 - xi. a saturated or unsaturated straight or branched aliphatic carbon chain optionally containing a substituent selected from the group consisting of ketones, hydroxyls, nitriles, carboxylic acids, esters, 1,3-dioxolanes, halogens, and oxo;
 - xii. an aromatic group containing a substituent selected from the group consisting of halides, esters and nitro; and
 - xiii. a combination of the saturated or unsaturated straight or branched aliphatic carbon chain of (xi) and the aromatic group of (xii); and
- d. R23 is a saturated or unsaturated straight or branched optionally substituted aliphatic carbon chain.

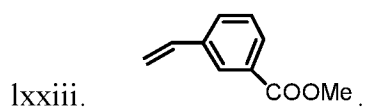
In one embodiment, R1-R2 is selected from the group consisting of:

- i. ;
- ii. ;
- iii. ;
- iv. ;
- v. ;
- vi. ;
- vii. ;
- viii. ;
- ix. ;
- x. ;
- xi. ;
- xii. ;
- xiii. ;
- xiv. ;
- xv. ;
- xvi. ;
- xvii. ;
- xviii. ;
- xix. ;
- xx. ;

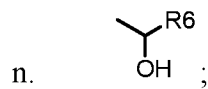
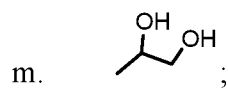
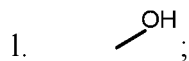
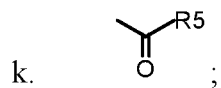
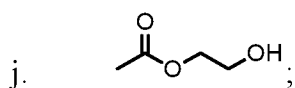
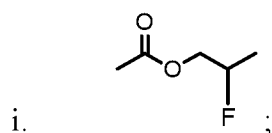
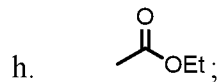
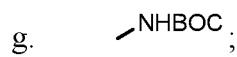
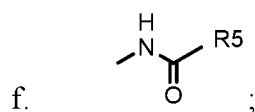
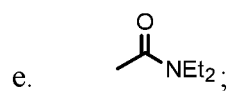
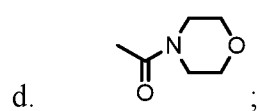
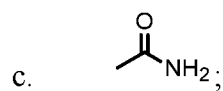
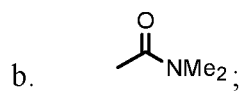
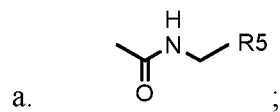
- xxi.  ;
- xxii.  ;
- xxiii.  ;
- xxiv.  ;
- xxv.  ;
- xxvi.  ;
- xxvii.  ;
- xxviii.  ;
- xxix.  ;
- xxx.  ;
- xxxi.  ;
- xxxii.  ;
- xxxiii.  ;
- xxxiv.  ;
- xxxv.  ;
- xxxvi.  ;
- xxxvii.  ;

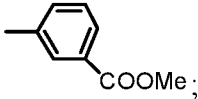
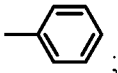
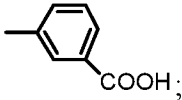
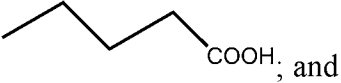
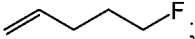
- xxxviii. ;
- xxxix. ;
- xl. ;
- xli. ;
- xl. ;
- xl. ;
- xliv. ;
- xl. ;
- xlvi. ;
- xl. ;
- xl. ;
- xl. ;
- i. ;
- li. ;
- lii. ;
- liii. ;
- liv. ;
- lv. ;
- lvi. ;
- lvii. ;





In another embodiment, R2 is selected from the group consisting of



- o. ;
- p. ;
- q. ;
- r. ; and
- s. .

wherein:

i.R5 is a saturated or unsaturated straight or branched aliphatic carbon chain between 1 and 10 carbons in length; and

5 ii.R6 is a monohydroxylated, dihydroxylated, trihydroxylated, or polyhydroxylated saturated or unsaturated straight or branched aliphatic carbon chain between 1 and 10 carbons in length.

10 In one embodiment, R1-R2 comprises a saturated or unsaturated straight or branched aliphatic carbon chain of between 2 and 5 carbons optionally substituted with a substituent selected from the group consisting of ketones, hydroxyls, nitriles, halogens, oxo, carboxylic acids, esters, and 1,3-dioxolanes.

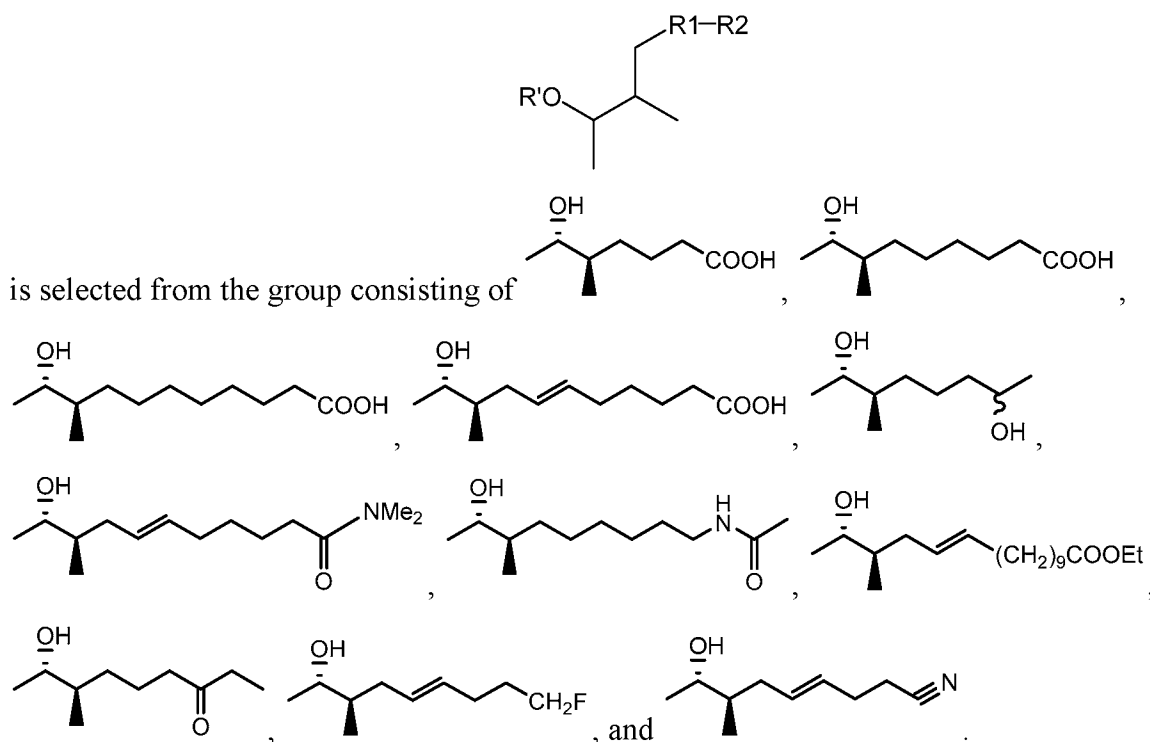
In one embodiment, R23 is selected from the group consisting of:

-
- i. $-\text{CH}_3$
 - ii. $-\text{CH}_2\text{CH}_3$
 - iii. $-\text{CH}_2\text{CHCH}_2$
 - iv. $-\text{CH}_2\text{CH}_2\text{CH}_2\text{I}$
 - v. $-(\text{CH}_2)_3\text{CH}_2\text{I}$
 - vi. $-(\text{CH}_2)_3\text{N}^+(\text{CH}_3)_3$
 - vii. $-\text{CH}_2\text{CCH}$
 - viii. $-\text{CH}_2\text{CO}_2(\text{t-Bu})$
-

ix.	$-\text{CH}_2\text{Ph}$
x.	$-\text{CH}_2\text{OH}$
xi.	$-\text{CH}(\text{OH})\text{CH}_3$
xii.	$-\text{CH}(\text{OH})(\text{t-Bu})$
xiii.	$-\text{CH}(\text{OH})\text{Ph}$
xiv.	$-\text{COOH}$
xv.	$-\text{SCH}_3$
xvi.	$-\text{S}(\text{p-Tol})$

In one embodiment, R23 comprises an optionally substituted alkyl, including optionally substituted C1-C3 alkyl. Said alkyl may be substituted with amino and may comprise a C1-C3-Ala wherein said compound comprises the D-epimer. In one embodiment, R23 can be MeAla.

In one embodiment, of the above Formula L,



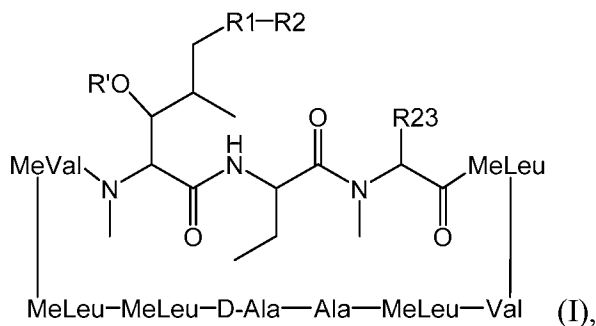
5 In one embodiment, R23 is a straight or branched aliphatic carbon chain of 1 to 6, 1 to 5, 1 to 4, 1 to 3 or 2 carbons in length.

Particularly, compounds of Formula I include the compounds wherein R' is H, R1 is an alkyl or alkenyl between 2 and 15 carbons (*e.g.*, between 2 and 12 carbons, between 2 and 10

carbons, between 2 and 9 carbons, between 2 and 8 carbons, between 2 and 7 carbons, between 2 and 6 carbons, or between 2 and 6 carbons,) in length, and R2 is selected from:

1. a carboxylic acid comprising a carboxyl group;
2. a N-substituted or N,N-disubstituted amide, wherein the substituents are independently selected from an alkyl between 1 and 7 carbons in length and a heterocyclic ring comprising 1-3 heteroatoms selected from O, N and S;
3. an ester of between 1 and 7 carbons in length;
4. an monohydroxylated or dihydroxylated alkyl of between 1 and 7 carbons in length;
5. a N-substituted or unsubstituted acyl protected amine of between 1 and 7 carbons in length;
6. a nitrile;
7. a ketone, wherein the carbonyl group of the ketone is connected to R1 and an alkyl or alkenyl chain between 1 and 7 carbons in length;
8. phenyl, optionally substituted with one or more substituents independently selected from nitrogen dioxide, a fluorine, an amine, an ester, and a carboxyl group.

The present application relates to a method of treating and/or preventing a HBV disease, comprising administering to a subject in need thereof Compound I, or a pharmaceutically acceptable salt thereof:



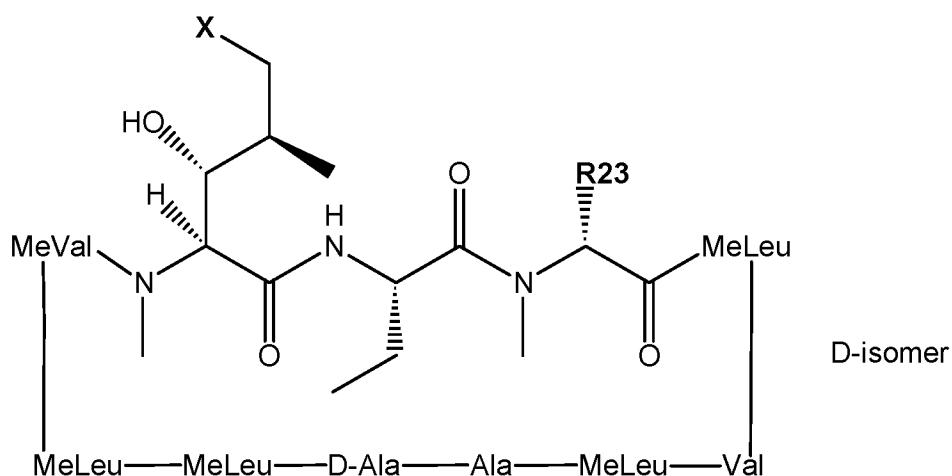
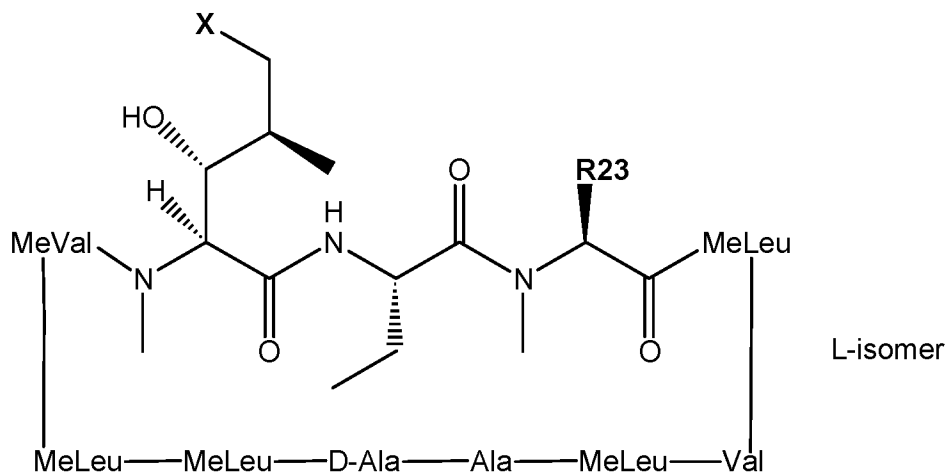
wherein:

R' is H;

R1-R2 is ; and

R23 is methyl.

In one embodiment, a compound of Formula L or Compound I is a D-epimer, wherein the chiral center of the D-epimer is the carbon atom to which R23 is attached. In one embodiment, a compound of Formula L or Compound I is an L-epimer, wherein the chiral center of the L-epimer is the carbon atom to which R23 is attached. In one embodiment, a compound of Formula L or Compound I is a mixture of D-epimer and L-epimer, wherein the chiral center of the D-epimer and the L-epimer is the carbon atom to which R23 is attached.



The present application also relates to a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) for use in treating and/or preventing a HBV disease in a subject in need thereof.

The present application also relates to a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) for use in the manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof.

The present application also relates to use of a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) in the manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof.

5 In one embodiment, the treatment and/or prevention of a HBV disease is through modulation (*e.g.*, inhibition) of an interaction of HBV X protein (HBx) with cyclophilin A (CypA). In another embodiment, the treatment and/or prevention of a HBV disease is through modulation (*e.g.*, inhibition) of an interaction of Hepatitis B surface antigen (HBsAg) with cyclophilin A (CypA).

10 The present application relates to a method of modulating (*e.g.*, inhibiting) an interaction of HBx with CypA, comprising administering to a subject in need thereof a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof). The present application also relates to a method of modulating (*e.g.*, inhibiting) an interaction of HBsAg with CypA, comprising administering to a subject in need thereof a
15 compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof).

 The present application also relates to a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) for use in modulating (*e.g.*, inhibiting) an interaction of HBx with CypA in a subject in need thereof. The
20 present application further relates to a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) for use in modulating (*e.g.*, inhibiting) an interaction of HBsAg with CypA in a subject in need thereof.

 The present application also relates to a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) for use in the
25 manufacture of a medicament for modulation (*e.g.*, inhibition) of an interaction of HBx with CypA in a subject in need thereof. The present application further relates to a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) for use in the manufacture of a medicament for modulation (*e.g.*, inhibition) of an interaction of HBsAg with CypA in a subject in need thereof.

30 The present application also relates to use of a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) in the

manufacture of a medicament for modulation (*e.g.*, inhibition) of an interaction of HBx with CypA in a subject in need thereof. The present application also relates to use of a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) in the manufacture of a medicament for modulation (*e.g.*, inhibition) of an

5 interaction of HBsAg with CypA in a subject in need thereof.

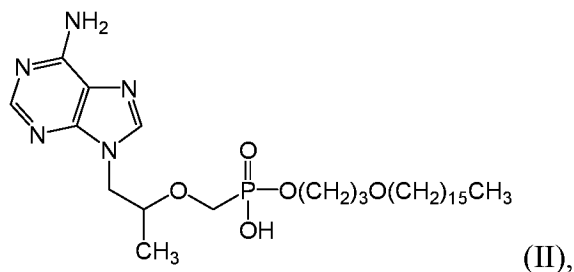
The present application relates to a method of treating and/or preventing a HBV disease, comprising administering to a subject in need thereof a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof), in combination with a second therapeutic agent.

10 The present application also relates to a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) for use in treating and/or preventing a HBV disease in a subject in need thereof, wherein the subject is also administered a second therapeutic agent.

The present application also relates to a compound of the application (*e.g.*, a compound
15 of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) for use in the manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof, wherein the subject is also administered a second therapeutic agent.

The present application also relates to use of a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) in the
20 manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof, wherein the subject is also administered a second therapeutic agent.

The second therapeutic agent can be any agent that is effective in treating and/or preventing a HBV disease, alone or in combination with another therapeutic agent. In one embodiment, the second therapeutic agent is a small molecule agent (*e.g.*, with a MW of less
25 than 2,000 Dalton, 1,000 Dalton, or 500 Dalton). In one embodiment, the second therapeutic agent is Compound II:



or a pharmaceutically acceptable salt thereof.

The present application relates to a method of treating and/or preventing a HBV disease, comprising administering to a subject in need thereof a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof), in combination with Compound II, or a pharmaceutically acceptable salt thereof. The present application relates to a method of treating and/or preventing a HBV disease, comprising administering to a subject in need thereof Compound I or a pharmaceutically acceptable salt thereof, in combination with Compound II or a pharmaceutically acceptable salt thereof.

The present application also relates to a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) for use in treating and/or preventing a HBV disease in a subject in need thereof, wherein the subject is also administered Compound II. The present application also relates to Compound I or a pharmaceutically acceptable salt thereof for use in treating and/or preventing a HBV disease in a subject in need thereof, wherein the subject is also administered Compound II or a pharmaceutically acceptable salt thereof.

The present application also relates to a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) for use in the manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof, wherein the subject is also administered Compound II. The present application also relates to Compound I or a pharmaceutically acceptable salt thereof for use in the manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof, wherein the subject is also administered Compound II or a pharmaceutically acceptable salt thereof.

The present application also relates to use of a compound of the application (*e.g.*, a compound of Formula L or Compound I, or a pharmaceutically acceptable salt thereof) in the manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof, wherein the subject is also administered Compound II. The present application also relates to use of Compound I or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for treatment and/or prevention of a HBV disease in a subject in need thereof, wherein the subject is also administered Compound II or a pharmaceutically acceptable salt thereof.

In one embodiment, the present application relates to treating a HBV disease in a subject in need thereof. In one embodiment, the present application relates to preventing a HBV disease in a subject in need thereof.

5 A HBV disease is a disease or condition caused by or associated with HBV infection. A HBV disease includes hepatitis B, cirrhosis, and hepatocellular carcinoma. In one embodiment, a HBV disease is hepatitis B.

In one embodiment, a HBV disease is mediated by an interaction of CypA with HBx. In one embodiment, a HBV disease is mediated by an interaction of CypA with HBsAg. In one embodiment, a HBV disease is mediated by an interaction of CypA with HBx or HBsAg. In one
10 embodiment, a HBV disease is mediated by an interaction of CypA with HBx and HBsAg. A HBV disease is mediated by an interaction of CypA with HBx and/or HBsAg when an interaction of CypA with HBx and/or HBsAg plays a certain role in the initiation, development, progression, persistence, and/or manifestation of the HBV disease. In one embodiment, a HBV
15 disease mediated by an interaction of CypA with HBx and/or HBsAg is different from a HBV disease not mediated by an interaction of CypA with HBx and/or HBsAg

HBx is a hepatitis B viral protein that is 154 amino acids long and interferes with transcription, signal transduction, cell cycle progress, protein degradation, apoptosis and chromosomal stability in the host.

20 HBsAg is the surface antigen of the hepatitis B virus (HBV). It indicates a current hepatitis B infection.

“Carboxylic acid” has the formula COOH , but may include a group in which the carboxyl moiety is connected to one of the following groups:

1. alkyl which may be substituted (for example, alkyl of 2 to 15 carbons);
2. alkenyl which may be substituted (for example, alkenyl of 2 to 15 carbons); and
- 25 3. alkynyl which may be substituted (for example, alkynyl of 2 to 15 carbons).

The substituents as described herein may include halogen (for example, fluorine, chlorine, bromine, iodine, *etc.*), nitro, cyano, hydroxy, thiol which may be substituted (for example, thiol, C1-C4 alkylthio, *etc.*), amino which may be substituted (for example, amino, mono-C1-C4 alkylamino, di-C1-C4 alkylamino, 5- to 6-membered cyclic amino such as
30 tetrahydropyrrole, piperazine, piperidine, morpholine, thiomorpholine, pyrrole, imidazole, *etc.*), C1-C4 alkoxy which may be halogenated (for example, methoxy, ethoxy, propoxy, butoxy,

trifluoromethoxy, trifluoroethoxy, *etc.*), C1-C4 alkoxy-C1-C4 alkoxy which may be halogenated (for example, methoxymethoxy, methoxyethoxy, ethoxyethoxy, trifluoromethoxyethoxy, trifluoroethoxyethoxy, *etc.*), formyl, C2-C4 alkanoyl (for example, acetyl, propionyl, *etc.*), C1-C4 alkylsulfonyl (for example, methanesulfonyl, ethanesulfonyl, *etc.*), and the like, and the number of the substituents is preferably 1 to 3.

Further, the substituents of the above "amino which may be substituted" may bind each other to form a cyclic amino group (for example, a group which is formed by subtracting a hydrogen atom from the ring constituting nitrogen atom of a 5- to 6-membered ring such as tetrahydropyrrole, piperazine, piperidine, morpholine, thiomorpholine, pyrrole, imidazole, *etc.* so that a substituent can be attached to the nitrogen atom, or the like). The cyclic amino group may be substituted and examples of the substituent include halogen (for example, fluorine, chlorine, bromine, iodine, *etc.*), nitro, cyano, hydroxy, thiol which may be substituted (for example, thiol, C1-C4 alkylthio, *etc.*), amino which may be substituted (for example, amino, mono-C1-C4 alkylamino, di-C1-C4 alkylamino, 5- to 6-membered cyclic amino such as tetrahydropyrrole, piperazine, piperidine, morpholine, thiomorpholine, pyrrole, imidazole, *etc.*), carboxyl which may be esterified or amidated (for example, carboxyl, C1-C4 alkoxy-carbonyl, carbamoyl, mono- C1-C4 alkyl-carbamoyl, di-C1-C4 alkyl-carbamoyl, *etc.*), C1-C4 alkoxy which may be halogenated (for example, methoxy, ethoxy, propoxy, butoxy, trifluoromethoxy, trifluoroethoxy, *etc.*), C1-C4 alkoxy-C1-C4 alkoxy which may halogenated (for example, methoxymethoxy, methoxyethoxy, ethoxyethoxy, trifluoromethoxyethoxy, trifluoroethoxyethoxy, *etc.*), formyl, C2-4 alkanoyl (for example, acetyl, propionyl, *etc.*), C1-C4 alkylsulfonyl (for example, methanesulfonyl, ethanesulfonyl), and the like, and the number of the substituents is preferably 1 to 3.

"Amine" includes a group which may be unsubstituted or in which the amine moiety is N-substituted or N,N disubstituted having one or two substituents which may be independently selected from:

1. alkyl which may be substituted (for example, alkyl of 2 to 15 carbons);
2. alkenyl which may be substituted (for example, alkenyl of 2 to 15 carbons);
3. alkynyl which may be substituted (for example, alkynyl of 2 to 15 carbons);

4. formyl or acyl which may be substituted (for example, alkanoyl of 2 to 4 carbons (for example, acetyl, propionyl, butyryl, isobutyryl, *etc.*), alkylsulfonyl of 1 to 4 carbons (for example, methanesulfonyl, ethanesulfonyl, *etc.*), and the like);

5. aryl which may be substituted (for example, phenyl, naphthyl, *etc.*); and the like;

5 and connected to a substituent independently selected from the substituents as defined above (*e.g.*, for “carboxylic acid”).

“Amide” includes a compound in which the carboxylic group of the amide moiety is connected to a substituent independently selected from the substituents as defined above (*e.g.*, for “carboxylic acid”), connected to the amino group of the amide moiety is an N-substituted or N,N disubstituted having one or two substituents, respectively, which may be independently selected from:

1. alkyl which may be substituted (for example, alkyl of 2 to 15 carbons);

2. alkenyl which may be substituted (for example, alkenyl of 2 to 15 carbons);

3. alkynyl which may be substituted (for example, alkynyl of 2 to 15 carbons);

15 4. formyl or acyl which may be substituted (for example, alkanoyl of 2 to 4 carbons (for example, acetyl, propionyl, butyryl, isobutyryl, *etc.*), alkylsulfonyl of 1 to 4 carbons (for example, methanesulfonyl, ethanesulfonyl, *etc.*) and the like);

5. aryl which may be substituted (for example, phenyl, naphthyl, *etc.*); and the like

20 “Aryl” may be exemplified by a monocyclic or fused polycyclic aromatic hydrocarbon group, and for example, a C₆-C₁₄ aryl group such as phenyl, naphthyl, anthryl, phenanthryl or acenaphthyl, and the like are preferred, with phenyl being preferred. Said aryl may be substituted with one or more substituents, such as lower alkoxy (*e.g.*, C₁-C₆ alkoxy such as methoxy, ethoxy or propoxy, *etc.*), a halogen atom (*e.g.*, fluorine, chlorine, bromine, iodine, *etc.*), lower alkyl (*e.g.*, C₁-C₆ alkyl such as methyl, ethyl or propyl, *etc.*), lower alkenyl (*e.g.*, C₂-C₆ alkenyl such as vinyl or allyl, *etc.*), lower alkynyl (*e.g.*, C₂-C₆ alkynyl such as ethynyl or propargyl, *etc.*), amino which may be substituted, hydroxyl which may be substituted, cyano, amidino which may be substituted, carboxyl, lower alkoxycarbonyl (*e.g.*, C₁-C₆ alkoxycarbonyl such as methoxycarbonyl or ethoxycarbonyl, *etc.*), carbamoyl which may be substituted (*e.g.*, carbamoyl which may be substituted with C₁-C₆ alkyl or acyl (*e.g.*, formyl, C₂-C₆ alkanoyl, benzoyl, C₁-C₆ alkoxycarbonyl which may be halogenated, C₁-C₆ alkylsulfonyl which may be halogenated, benzenesulfonyl, *etc.*) which may be substituted with a 5- to 6-membered aromatic

monocyclic heterocyclic group (*e.g.*, pyridinyl, *etc.*), 1-azetidinyldicarbonyl, 1-pyrrolidinylcarbonyl, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl (the sulfur atom may be oxidized), 1-piperazinylcarbonyl, *etc.*), and the like. Any of these substituents may be independently substituted at 1 to 3 substitutable positions.

5 “Ketone” includes a compound in which the carbonyl group of the ketone moiety is connected to one or two substituents independently selected from the substituents as defined above (*e.g.*, for “carboxylic acid”).

 “Ester” includes either a carboxylic or an alcohol ester wherein of the ester group is composed of one or two substituents independently selected from the substituents as defined
10 above (*e.g.*, for “carboxylic acid” or for “aryl”).

 “Alkyl” unless otherwise defined is an alkyl of 1 to 15 carbon units in length. In one embodiment, “alkyl” is an alkyl of 1 to 6 carbon units, 1 to 5 carbon units, 1 to 4 carbon units, or 1 to 3 carbon units (*e.g.*, methyl, ethyl, propyl, *i*-propyl, butyl, *i*-butyl, *s*-butyl, *t*-butyl, pentyl, or hexyl).

15 “Aromatic group” may be exemplified by aryl as defined above, or a 5- to 6-membered aromatic monocyclic heterocyclic group such as furyl, thienyl, pyrrolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, imidazolyl, pyrazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, furazanyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, tetrazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl or the like; and a
20 8- to 16-membered (*e.g.*, 10- to 12-membered) aromatic fused heterocyclic group.

 “Non-immunosuppressive” refers to the ability of a compound to exhibit a substantially reduced level of suppression of the immune system as compared with CsA, as measured by the compound’s ability to inhibit the proliferation of human lymphocytes in cell culture, for example, as measured by the method set out in the Examples.

25 “Analogue” or “analog” means a structural analogue of CsA that differs from CsA in one or more functional groups. For example, such analogues preserve at least a substantial portion of the ability of CsA to bind CyP.

 The term “subject” as used herein refers to a mammal. A subject therefore refers to, for example, dogs, cats, horses, cows, pigs, guinea pigs, and the like. Preferably the subject is a
30 human. When the subject is a human, the subject may be referred to herein as a patient.

"Treat", "treating" and "treatment" refer to a method of alleviating or abating a disease and/or its attendant symptoms.

As used herein, "preventing" or "prevent" describes reducing or eliminating the onset of the symptoms or complications of the disease, condition or disorder.

5 The terms "disease(s)", "disorder(s)", and "condition(s)" are used interchangeably, unless the context clearly dictates otherwise.

10 The term "therapeutically effective amount" of a compound or pharmaceutical composition of the application, as used herein, means a sufficient amount of the compound or pharmaceutical composition so as to decrease the symptoms of a disorder in a subject. As is well understood in the medical arts a therapeutically effective amount of a compound or pharmaceutical composition of this application will be at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the compounds and compositions of the present application will be decided by the attending physician within the scope of sound medical judgment. The specific inhibitory dose for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

20 As used herein, the term "pharmaceutically acceptable salt" refers to those salts of the compounds formed by the process of the present application which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 66: 1-19 (1977). The salts can be prepared in situ during the final isolation and purification of the compounds of the application, or separately by reacting the free base or acid function with a suitable acid or base.

30 Examples of pharmaceutically acceptable salts include, but are not limited to, nontoxic acid addition salts: salts formed with inorganic acids such as hydrochloric acid, hydrobromic

acid, phosphoric acid, sulfuric acid and perchloric acid, or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid. Other pharmaceutically acceptable salts include, but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate and aryl sulfonate.

The compounds of the present application may exist in the form of optically active compounds. The present application contemplates all enantiomers of optically active compounds within the scope of the above formulae, both individually and in mixtures of racemates. As well, the present application includes prodrugs of the compounds defined herein.

Optical isomers may be prepared from their respective optically active precursors by the procedures described herein, or by resolving the racemic mixtures. The resolution can be carried out in the presence of a resolving agent, by chromatography or by repeated crystallization or by some combination of these techniques which are known to those skilled in the art. Further details regarding resolutions can be found in Jacques, *et al.*, *Enantiomers, Racemates, and Resolutions* (John Wiley & Sons, 1981).

“Isomerism” means compounds that have identical molecular formulae but differ in the sequence of bonding of their atoms or in the arrangement of their atoms in space. Isomers that differ in the arrangement of their atoms in space are termed “stereoisomers”. Stereoisomers that are not mirror images of one another are termed “diastereoisomers”, and stereoisomers that are non-superimposable mirror images of each other are termed “enantiomers” or sometimes optical isomers. A mixture containing equal amounts of individual enantiomeric forms of opposite

chirality is termed a “racemic mixture”.

A carbon atom bonded to four non-identical substituents is termed a “chiral center”.

“Chiral isomer” means a compound with at least one chiral center. Compounds with more than one chiral center may exist either as an individual diastereomer or as a mixture of
5 diastereomers, termed “diastereomeric mixture”. When one chiral center is present, a stereoisomer may be characterized by the absolute configuration (R or S) of that chiral center. Absolute configuration refers to the arrangement in space of the substituents attached to the chiral center. The substituents attached to the chiral center under consideration are ranked in accordance with the *Sequence Rule* of Cahn, Ingold and Prelog. (Cahn *et al.*, *Angew. Chem.*

10 *Inter. Edit.* 1966, 5, 385; errata 511; Cahn *et al.*, *Angew. Chem.* 1966, 78, 413; Cahn and Ingold, *J. Chem. Soc.* 1951 (London), 612; Cahn *et al.*, *Experientia* 1956, 12, 81; Cahn, *J. Chem. Educ.* 1964, 41, 116).

“Epimer” means one member of a pair of stereoisomers wherein the two isomers differ in configuration at only one stereogenic center and all other stereocenters in the molecules, if any,
15 are the same in each.

“Geometric isomer” means the diastereomers that owe their existence to hindered rotation about double bonds. These configurations are differentiated in their names by the prefixes cis and trans, or Z and E, which indicate that the groups are on the same or opposite side of the double bond in the molecule according to the Cahn-Ingold-Prelog rules.

20 Furthermore, the structures and other compounds discussed in this application include all atropic isomers thereof. “Atropic isomers” are a type of stereoisomer in which the atoms of two isomers are arranged differently in space. Atropic isomers owe their existence to a restricted rotation caused by hindrance of rotation of large groups about a central bond. Such atropic isomers typically exist as a mixture, however as a result of recent advances in chromatography
25 techniques; it has been possible to separate mixtures of two atropic isomers in select cases.

“Tautomer” is one of two or more structural isomers that exist in equilibrium and is readily converted from one isomeric form to another. This conversion results in the formal migration of a hydrogen atom accompanied by a switch of adjacent conjugated double bonds. Tautomers exist as a mixture of a tautomeric set in solution. In solid form, usually one tautomer
30 predominates. In solutions where tautomerization is possible, a chemical equilibrium of the tautomers will be reached. The exact ratio of the tautomers depends on several factors, including

temperature, solvent and pH. The concept of tautomers that are interconvertible by tautomerizations is called tautomerism.

Of the various types of tautomerism that are possible, two are commonly observed. In keto-enol tautomerism a simultaneous shift of electrons and a hydrogen atom occurs. Ring-chain
5 tautomerism arises as a result of the aldehyde group (-CHO) in a sugar chain molecule reacting with one of the hydroxy groups (-OH) in the same molecule to give it a cyclic (ring-shaped) form as exhibited by glucose. Common tautomeric pairs are: ketone-enol, amide-nitrile, lactam-lactim, amide-imidic acid tautomerism in heterocyclic rings (*e.g.*, in nucleobases such as guanine, thymine and cytosine), amine-enamine and enamine-enamine. The compounds of this
10 application may also be represented in multiple tautomeric forms, in such instances, the application expressly includes all tautomeric forms of the compounds described herein (*e.g.*, alkylation of a ring system may result in alkylation at multiple sites, the application expressly includes all such reaction products).

In the present application, the structural formula of the compound represents a certain
15 isomer for convenience in some cases, but the present application includes all isomers, such as geometrical isomers, optical isomers based on an asymmetrical carbon, stereoisomers, tautomers, and the like. In the present specification, the structural formula of the compound represents a certain isomer for convenience in some cases, but the present application includes all isomers, such as geometrical isomers, optical isomers based on an asymmetrical carbon, stereoisomers,
20 tautomers, and the like.

According to one aspect, a compound of this application may be administered neat or with a pharmaceutical carrier to a warm-blooded animal in need thereof. The pharmaceutical carrier may be solid or liquid. The compound may be administered orally, topically, parenterally, by inhalation spray or rectally in dosage unit formulations containing conventional
25 non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral, as used herein, includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques.

The pharmaceutical compositions containing the inventive mixture may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions,
30 dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to methods known to the art for

the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparation. Tablets containing the active ingredient in admixture with non-toxic

5 pharmaceutically acceptable excipients may also be manufactured by known methods. The excipients used may be for example, (1) inert diluents such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents such as corn starch, or alginic acid; (3) binding agents such as starch, gelatin or acacia, and (4) lubricating agents such as magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may
10 be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the techniques described in the U.S. Patent Number 4,256,108; 4,160,452; and 4,265,874 to form osmotic therapeutic tablets for controlled release.

15 In some cases, formulations for oral use may be in the form of hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

20 Aqueous suspensions normally contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients may include: (1) suspending agents such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; or (2) dispersing or wetting agents which may be a naturally-occurring phosphatide such
25 as lecithin, a condensation product of an alkylene oxide with a fatty acid, for example, polyoxyethylene stearate, a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadecaethyleneoxycetanol, a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol such as polyoxyethylene sorbitol monooleate, or a condensation product of ethylene oxide with a partial ester derived from a fatty
30 acid and a hexitol anhydride, for example polyoxyethylene sorbitan monooleate.

The aqueous suspensions may also contain one or more preservatives, for example, ethyl or n-propyl p-hydroxybenzoate; one or more coloring agents; one or more flavoring agents; and one or more sweetening agents such as sucrose, aspartame or saccharin.

Oily suspension may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, a fish oil which contains omega 3 fatty acid, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules are suitable for the preparation of an aqueous suspension. They provide the active ingredient in a mixture with a dispersing or wetting agent, a suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, those sweetening, flavoring and coloring agents described above may also be present.

The pharmaceutical compositions containing the inventive mixture may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil such as olive oil or arachis oils, or a mineral oil such as liquid paraffin or a mixture thereof. Suitable emulsifying agents may be (1) naturally-occurring gums such as gum acacia and gum tragacanth, (2) naturally-occurring phosphatides such as soy bean and lecithin, (3) esters or partial ester derived from fatty acids and hexitol anhydrides, for example, sorbitan monooleate, (4) condensation products of said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example, glycerol, propylene glycol, sorbitol, aspartame or sucrose. Such formulations may also contain a demulcent, a preservative, and flavoring and coloring agents.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to known methods using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water,

Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

5 The inventive compound may also be administered in the form of suppositories for rectal administration of the drug. Suitable compositions can be prepared by mixing the compound with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

10 For topical use, suitable creams, ointments, jellies, solutions or suspensions, *etc.*, which normally are used with cyclosporine may be employed.

 In a particularly preferred embodiment, a liquid solution containing a surfactant, ethanol, a lipophilic and/or an amphiphilic solvent as non-active ingredients is used. Specifically, an oral multiple emulsion formula containing the isomeric analogue mixture and the following non-
15 medicinal ingredients: d-alpha Tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS), medium chain triglyceride (MCT) oil, Tween 40, and ethanol is used. A soft gelatin capsule (comprising gelatin, glycerin, water, and sorbitol) containing the compound and the same non-medicinal ingredients as the oral solution may also preferably be used.

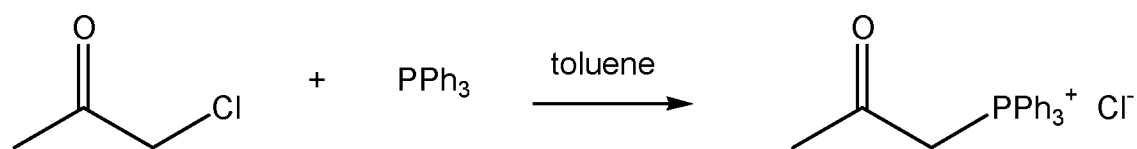
 It will be understood, however, that the specific dose level for any particular patient will
20 depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the nature and severity of the particular disease or condition undergoing therapy.

25 EXAMPLES

Example 1. Synthesis of compounds of the present application

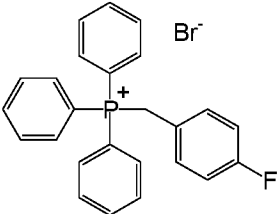
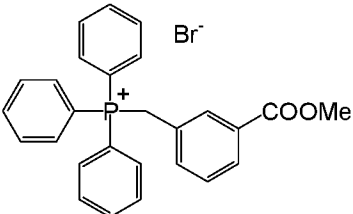
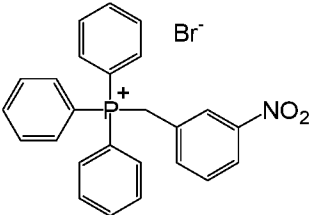
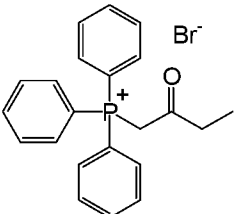
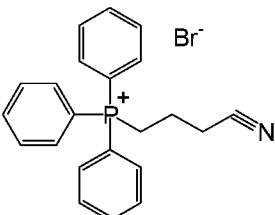
 Compounds of the present application may be prepared according to methods known in the art, for example WO 2012/079172, the contents of which are incorporated herein in their entirety.

30 *Reaction 1*



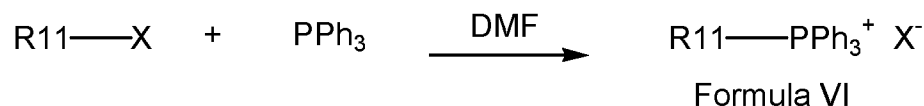
Using Reaction 1, the following compounds were synthesized.

Compound	Reactant (R10-X)	Conditions
404-08	benzyl bromide	4 hours at reflux
404-09	methyl iodide	RT over night
404-12	4-nitrobenzyl bromide	6 hours at reflux
404-15	chloroacetone	reflux over night

404-64	4-fluorobenzyl bromide	reflux over night
		
404-77	methyl 3-bromomethylbenzoate	6 hours at reflux
		
404-87	3-nitrobenzyl bromide	6 hours at reflux
		
404-161	1-bromo-2-butanone	RT over night
		
404-170	4-bromobutyronitrile	reflux over night
		

Suitable phosphonium salts may be synthesized through Reaction 2.

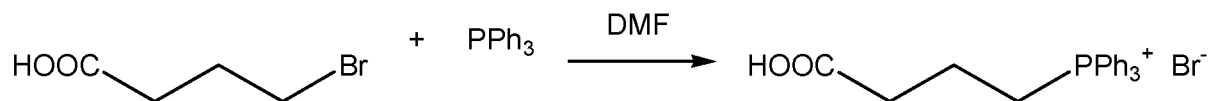
Reaction 2

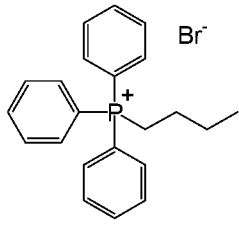
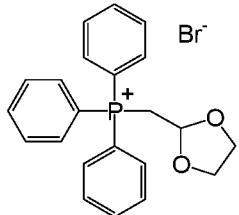


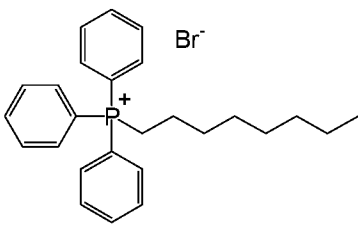
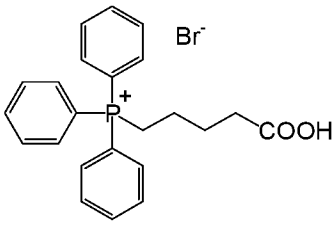
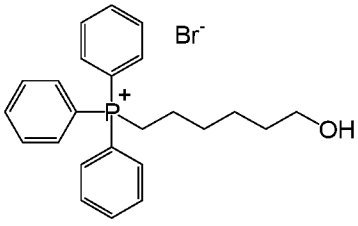
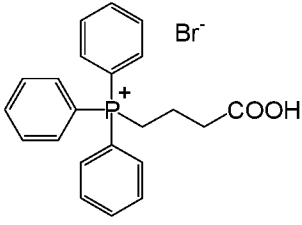
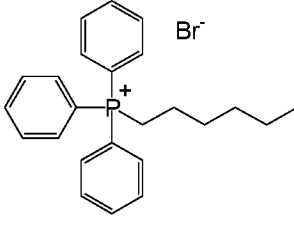
wherein X is a halide (including but not limited to Cl, Br, and I), and R10 is a saturated or unsaturated straight or branched aliphatic carbon chain, optionally containing a substituent selected from the group of ketones, hydroxyls, nitriles, carboxylic acids, esters, and 1,3-dioxolanes; an aromatic group, optionally containing a substituent selected from the group of halides, esters and nitro; or a combination of the aforementioned saturated or unsaturated straight or branched aliphatic carbon chain and the aforementioned aromatic groups.

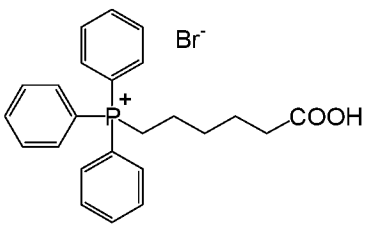
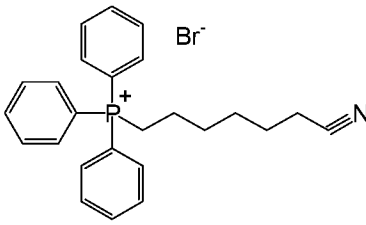
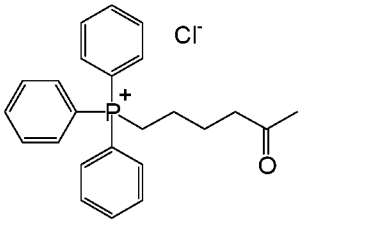
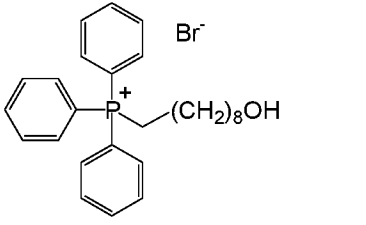
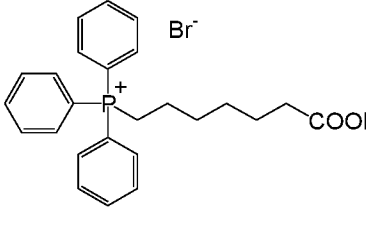
Using Reaction 2, the following compounds were synthesized.

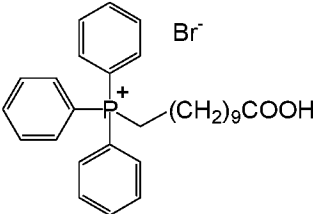
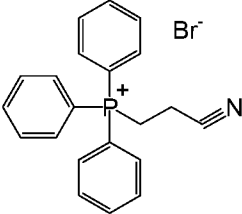
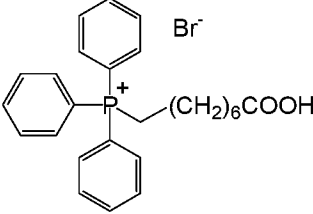
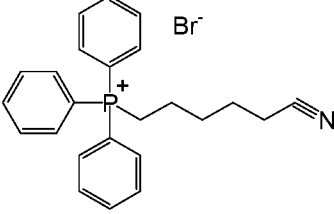
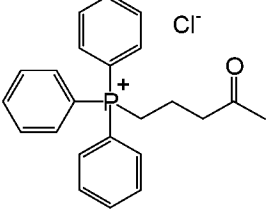
Synthesis of 404-51



Compound	Reactant (R11-X)	Conditions
404-14	1-bromobutane	6.5 hours at 120 °C
		
404-29	2-bromomethyl-1,3-dioxolane	120 °C over night
		

404-34	1-bromooctane	110 °C over night
		
404-51	5-bromovaleric acid	8 hours at 120 °C
		
404-78	6-bromohexanol	110 °C over night
		
404-116	4-bromobutyric acid	7 hours at 110 °C
		
416-01	1-bromohexane	110 °C over night
		

416-02	6-bromohexanoic acid	110 °C over night
		
419-132	7-bromoheptanenitrile	110 °C over night
		
419-134	6-chloro-2-hexanone	110 °C over night
		
419-136	9-bromo-1-nonanol	110 °C over night
		
420-32	methyl 7-bromohexanoate	110 °C over night
		

420-78	11-bromoundecanoic acid	110 °C over night
		
420-80	3-bromopropionitrile	110 °C over night
		
420-82	8-bromooctanoic acid	110 °C over night
		
420-90	6-bromohexanenitrile	110 °C over night
		
420-94	5-chloro-2-pentanone	110 °C over night
		

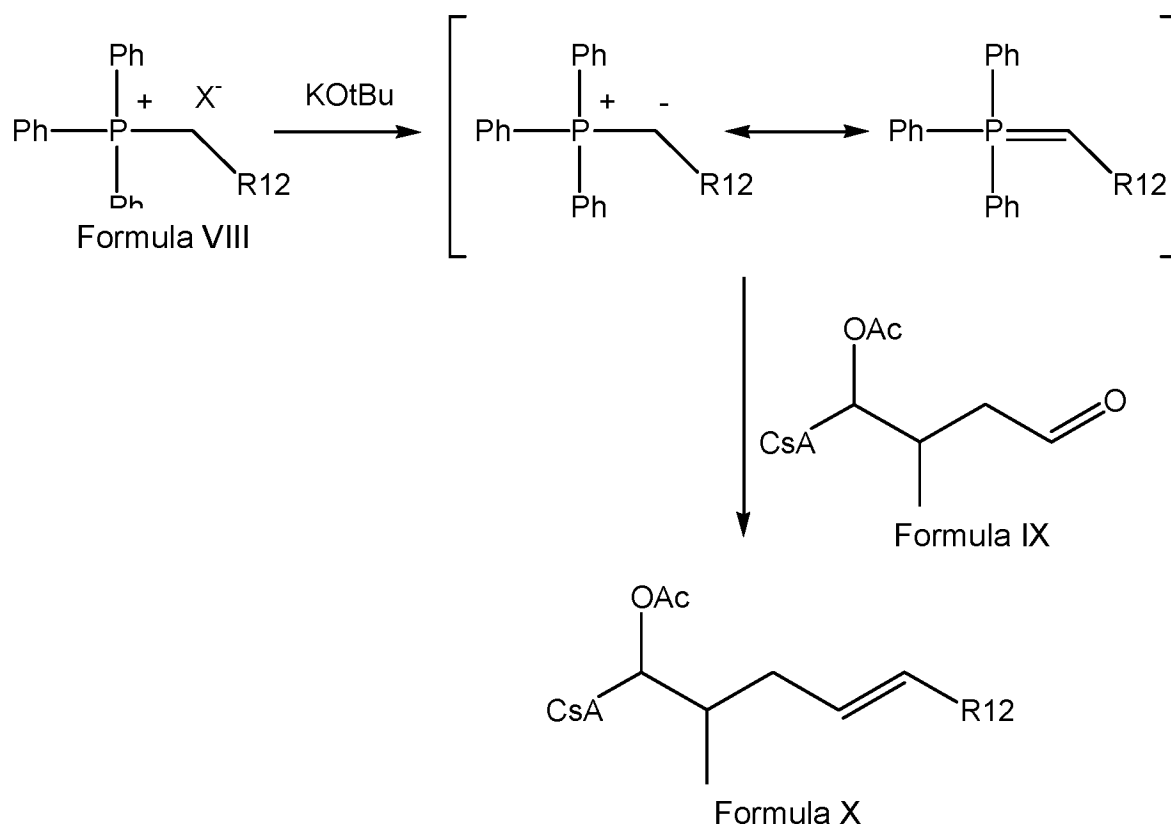
Wittig Reaction

The Wittig reaction is broadly applicable to a wide range of substrates and reactants. The side chain, which is introduced to the substrate in the reaction, can represent any number of

branched and unbranched, saturated and unsaturated aliphatic compounds of variable length (R') and may contain a broad range of functional groups. In the Wittig reaction, a base, such as potassium tert-butoxide (KOtBu) is used to generate an ylide from a phosphonium salt. The ylide reacts with the carbonyl group of the substrate, CsA-aldehyde, to form an alkene.

- 5 Phosphonium salts containing a carboxylic acid side chain require at least two equivalents of base to generate the ylide.

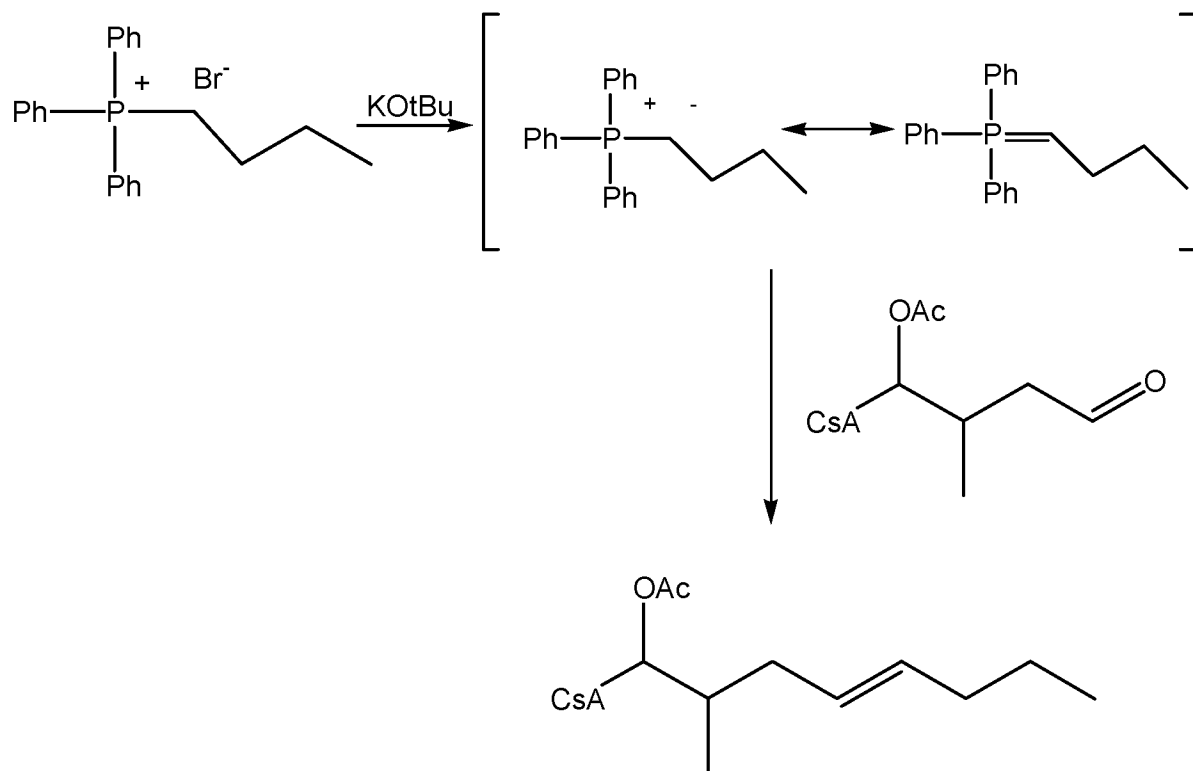
Reaction 3



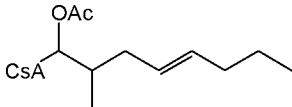
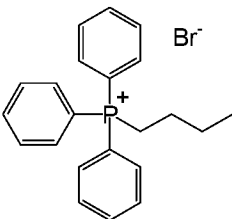
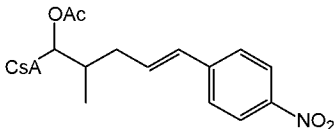
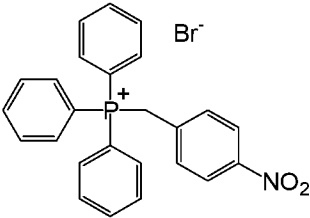
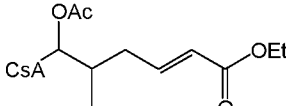
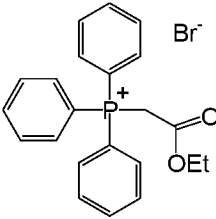
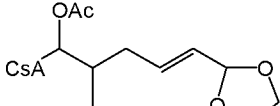
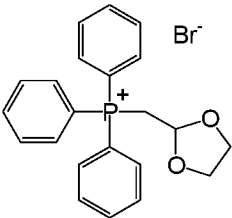
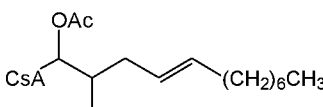
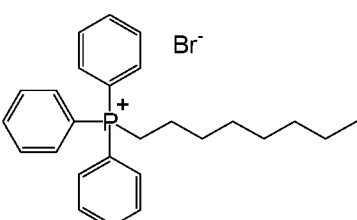
- 10 wherein X is a halide (including but not limited to Cl, Br, and I), and R12 is a saturated or unsaturated straight or branched aliphatic carbon chain, optionally containing a substituent selected from the group of ketones, hydroxyls, nitriles, carboxylic acids, esters, and 1,3-dioxolanes; an aromatic group, optionally containing a substituent selected from the group of halides, esters, and nitro; or a combination of the aforementioned saturated or unsaturated
- 15 straight or branched aliphatic carbon chain and the aforementioned aromatic groups.

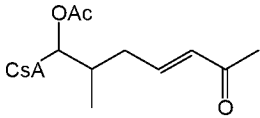
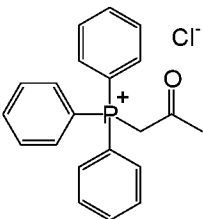
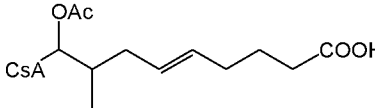
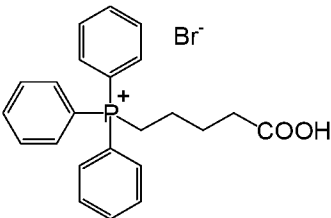
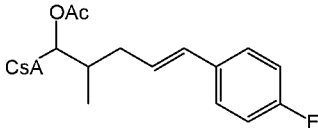
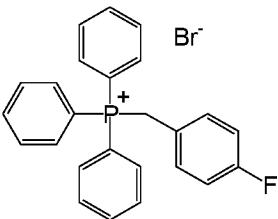
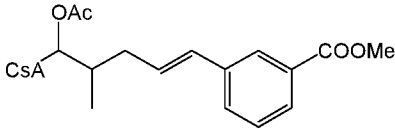
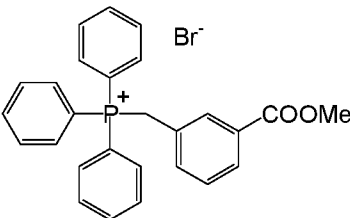
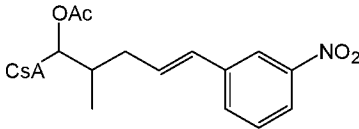
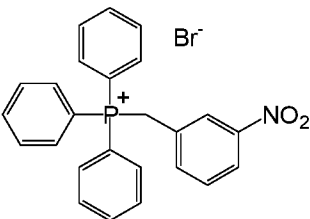
Using Reaction 3, the following compounds were synthesized.

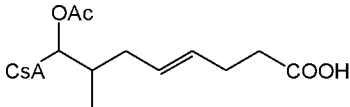
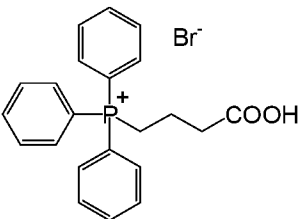
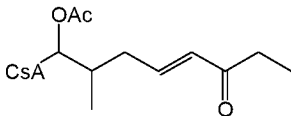
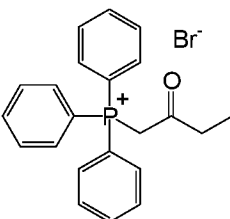
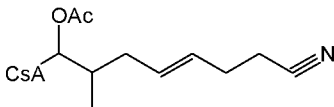
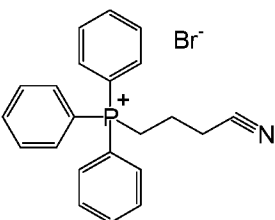
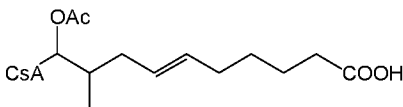
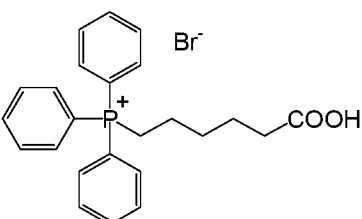
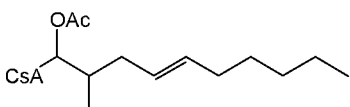
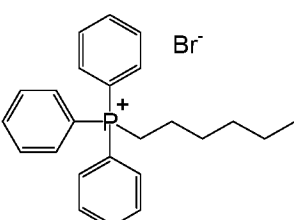
Synthesis of 404-20

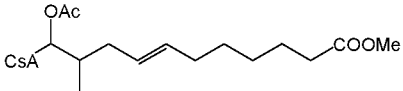
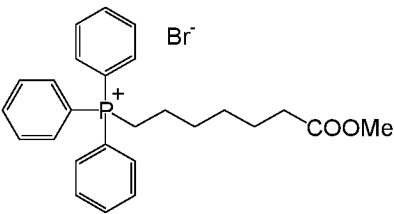
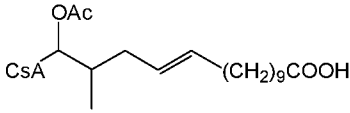
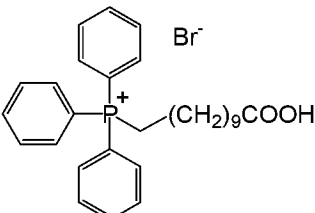
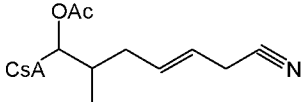
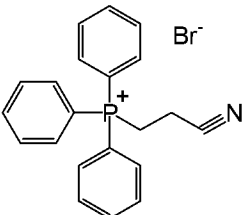
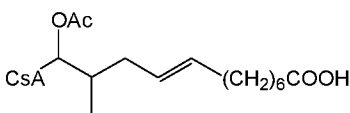
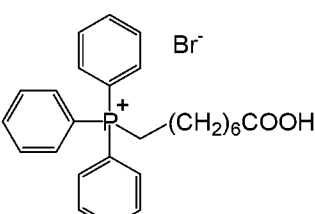
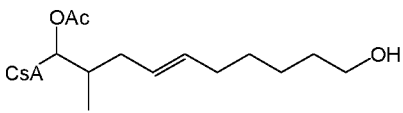
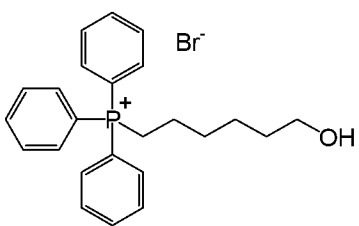


Compound	Starting Material	MS (Na ⁺)	Remarks
404-16	404-09	1252.9	
404-19	404-08	1328.9	

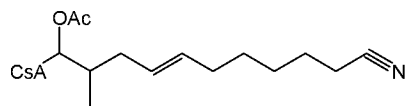
404-20	404-14	1294.9	
			
404-30	404-12	1373.9	stirred at 60 °C over night
			
404-31		1324.9	stirred at 60 °C for 2 days
			
404-33	404-29	1325.0	
			
404-40	404-34	1351.2	
			

404-43	404-15	1295.1	stirred at reflux for 10 days
			
404-59	404-51	1338.9	2 eq of KOtBu
			
404-65	404-64	1347.1	
			
404-79	404-77	1386.9	stirred at RT over night
			
404-89	404-87	1374.1	stirred at RT for 2 days
			

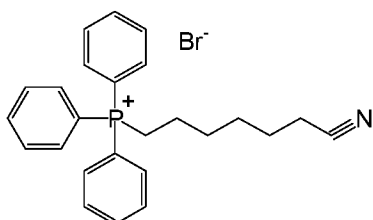
404-134	404-116	1325.0	2 eq of K ⁺ OT ⁻ Bu; stirred at RT for 2 days
			
404-163	404-161	1308.8	stirred at reflux for 15 days
			
404-187	404-170	1305.9	stirred at RT over night
			
416-04	416-02	1353.0	2 eq of K ⁺ OT ⁻ Bu
			
416-09	416-01	1323.1	
			

420-40	420-32	1381.0	stirred at RT over night
			
420-85	420-78	1423.1	2 eq of KOtBu
			
420-89	420-80	1291.9	
			
420-92	420-82	1381.1	2 eq of KOtBu
			
420-96	404-78	1338.9	
			

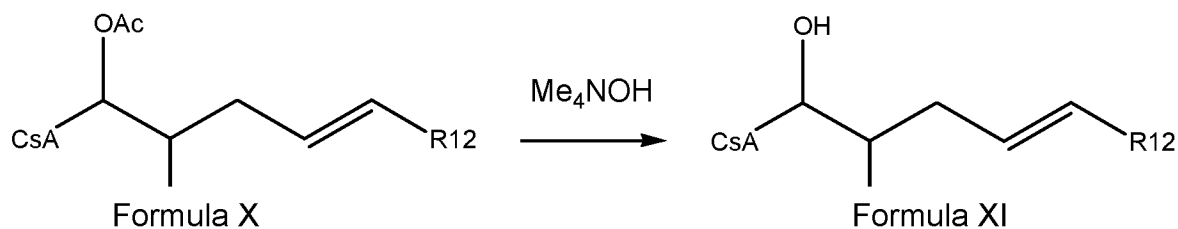
420-101



419-132



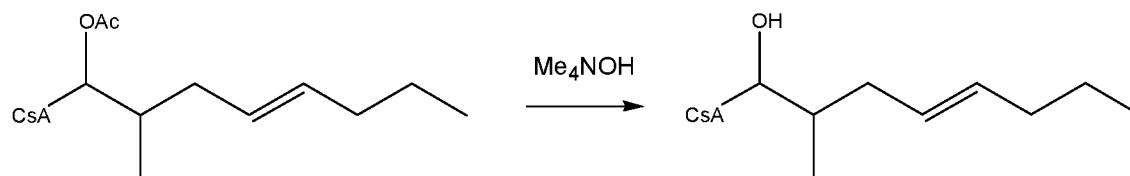
1347.9

Reaction 4

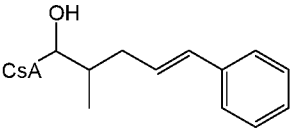
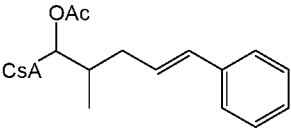
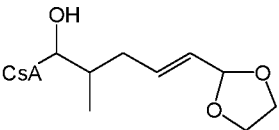
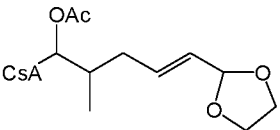
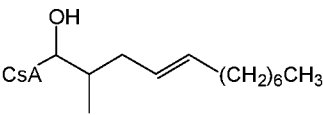
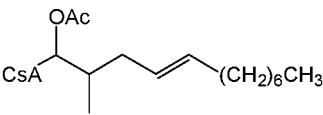
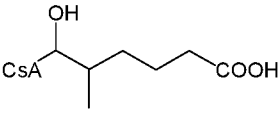
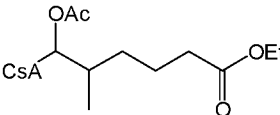
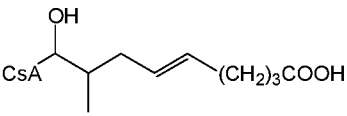
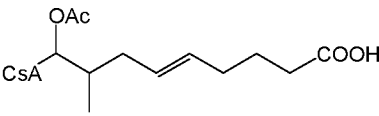
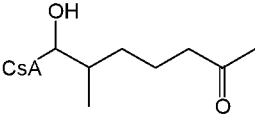
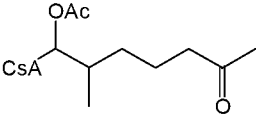
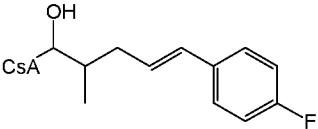
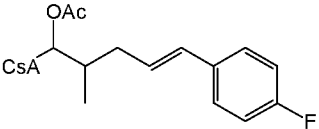
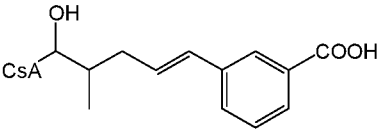
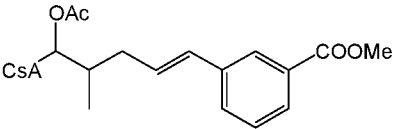
wherein R12 is a saturated or unsaturated straight or branched aliphatic carbon chain, optionally containing a substituent selected from the group of ketones, hydroxyls, nitriles, carboxylic acids, esters, amides, acyl-protected amines, and 1,3-dioxolanes; an aromatic group, optionally containing a substituent selected from the group of halides, esters, amines, and nitro; or a combination of the aforementioned saturated or unsaturated straight or branched aliphatic carbon chain and the aforementioned aromatic groups.

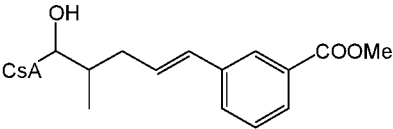
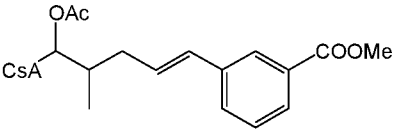
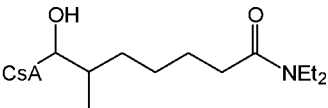
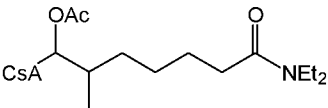
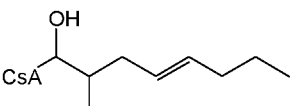
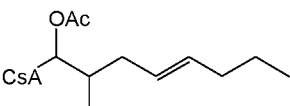
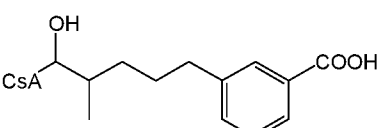
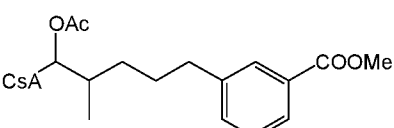
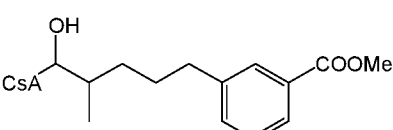
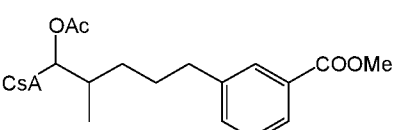
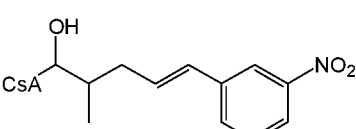
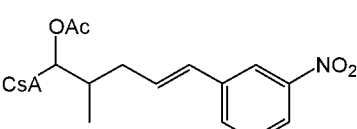
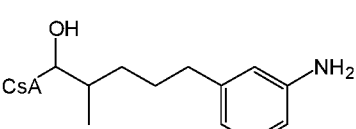
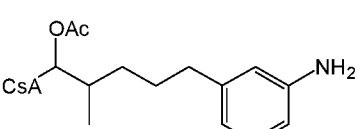
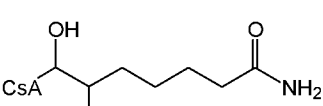
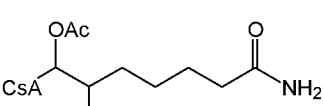
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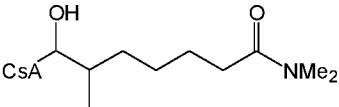
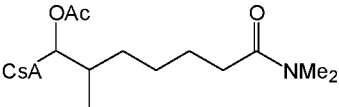
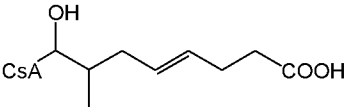
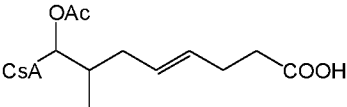
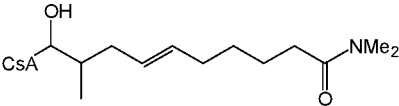
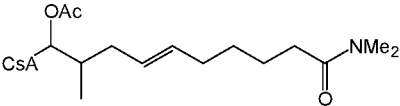
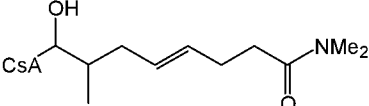
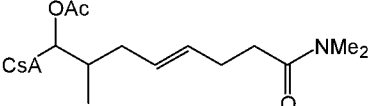
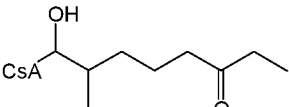
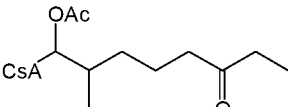
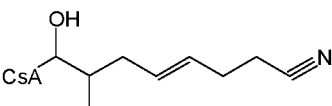
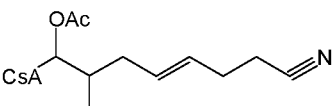
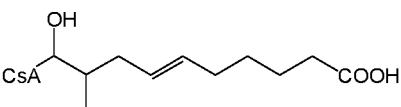
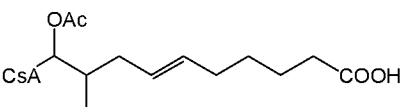
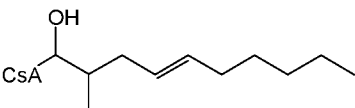
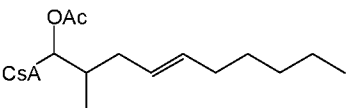
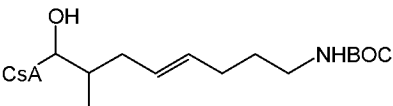
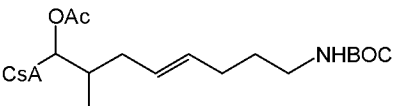
Using Reaction 4, the following compounds were synthesized.

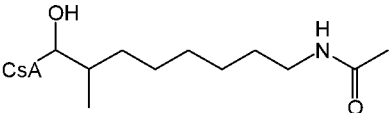
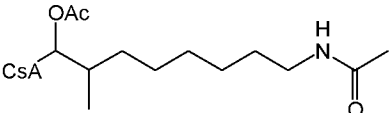
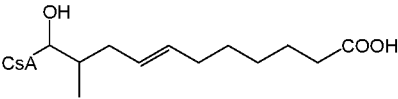
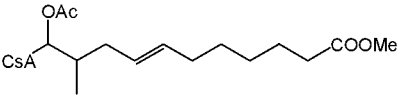
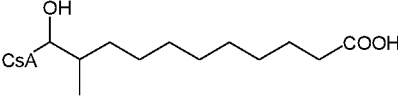
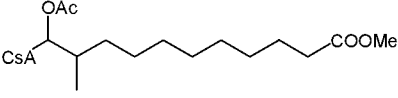
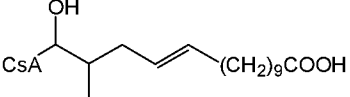
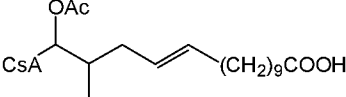
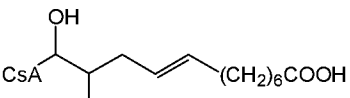
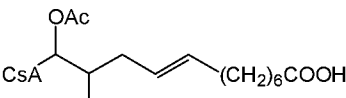
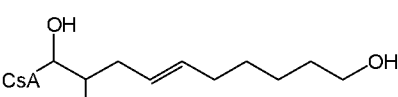
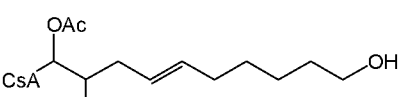
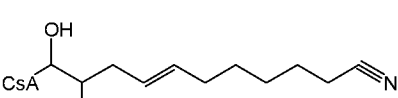
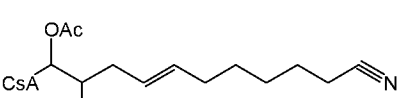
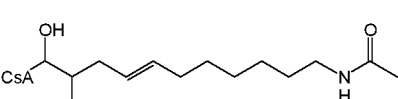
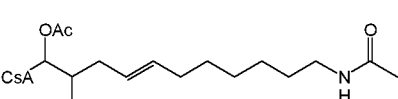
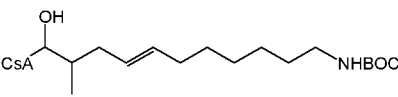
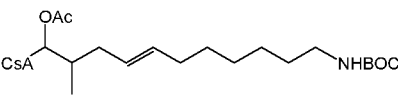
Synthesis of 404-90

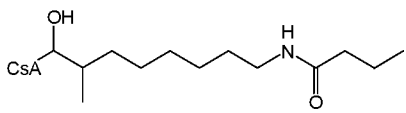
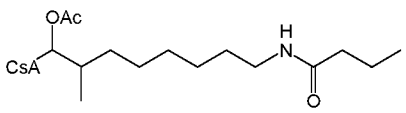
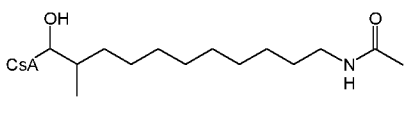
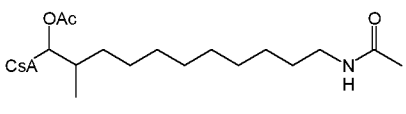
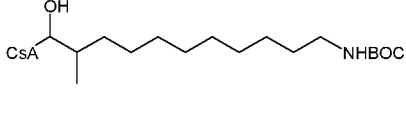
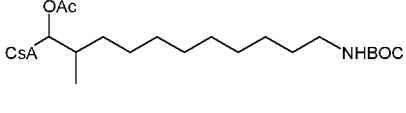
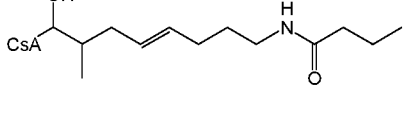
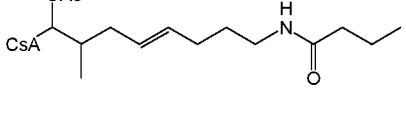
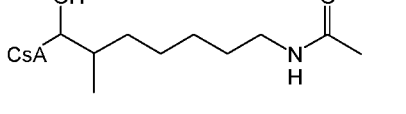
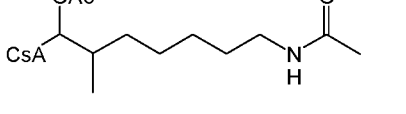
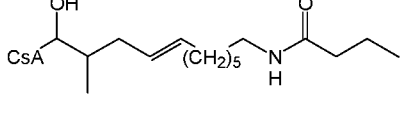
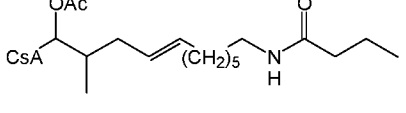
Compound	Starting Material	MS (Na ⁺)
404-22	404-16	1210.9

404-25	404-19	1287.0
		
404-36	404-33	1283.0
		
404-44	404-40	1309.1
		
404-58	404-57	1257.1
		
404-60	404-59	1297.1
		
404-61	404-56	1255.1
		
404-66	404-65	1305.1
		
404-81-1	404-79-1	1331.1
		

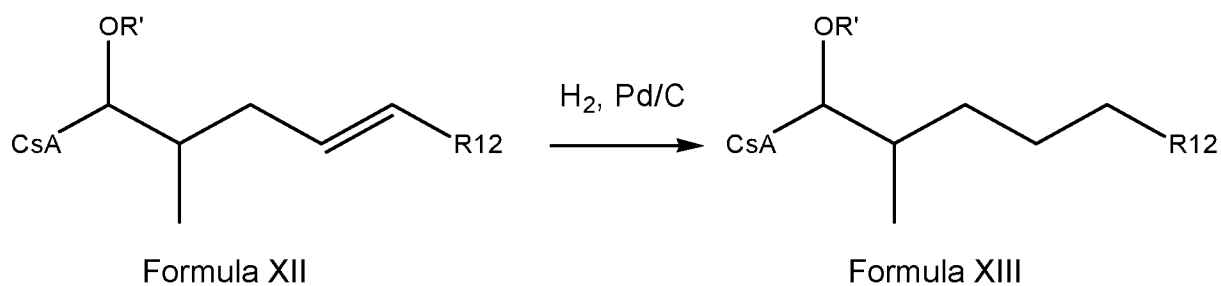
404-81-2	404-79-1	1345.1
		
404-85	404-83	1326.2
		
404-90	404-20	1253.0
		
404-96-1	404-94	1333.0
		
404-96-2	404-94	1347.0
		
404-97	404-89	1331.9
		
404-125	404-120	1304.0
		
404-130	404-128	1270.1
		

404-132	404-129	1298.0
		
404-137	404-134	1283.0
		
404-154	404-150	1338.1
		
404-157	404-155	1310.0
		
404-173	404-172	1268.9
		
404-194	404-187	1263.9
		
416-08	416-04	1311.0
		
416-13	416-09	1281.1
		
420-17	420-08-1	1368.0
		

420-30-1	420-27	1312.0
		
420-43	420-40	1324.9
		
420-47	420-46	1327.0
		
420-98	420-85	1381.1
		
420-100	420-92	1339.1
		
420-102	420-96	1297.0
		
420-108	420-101	1305.9
		
420-117	420-109-1	1352.1
		
420-120	420-110-1	1410.0
		

420-122	420-107-2	1340.0
		
420-124	420-109-2	1354.0
		
420-125	420-110-2	1412.0
		
420-126	420-107-1	1337.9
		
420-131	420-130	1297.9
		
420-132	420-128-1	1380.0
		

Reaction 5

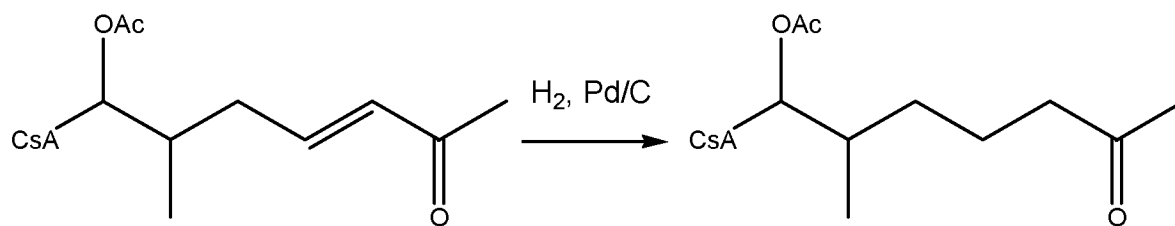


wherein R12 is a saturated or unsaturated straight or branched aliphatic carbon chain, optionally containing a substituent selected from the group of ketones, hydroxyls, nitriles, carboxylic acids, esters, amides, acyl-protected amines, and 1,3-dioxolanes; an aromatic group, optionally containing a substituent selected from the group of halides, esters, amines and nitro; or a

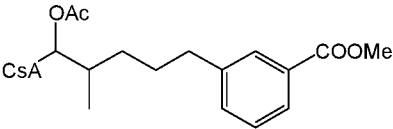
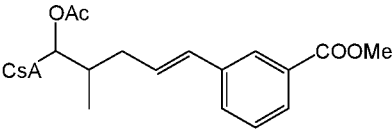
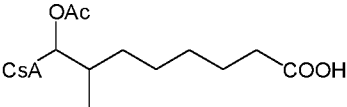
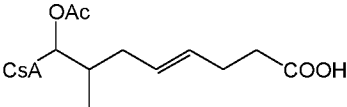
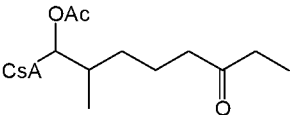
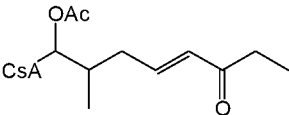
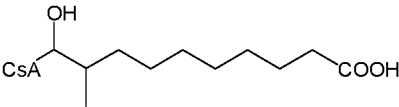
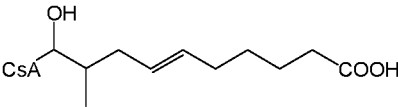
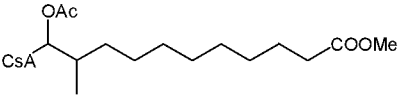
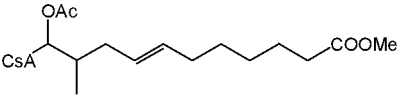
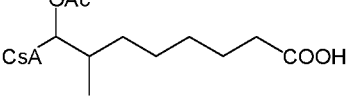
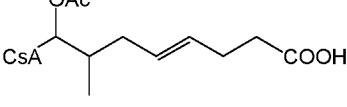
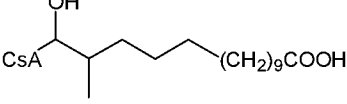
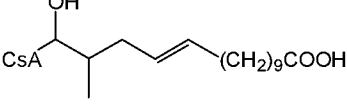
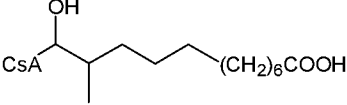
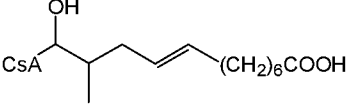
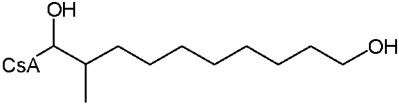
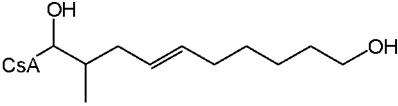
combination of the aforementioned saturated or unsaturated straight or branched aliphatic carbon chain and the aforementioned aromatic groups, and R' is H or acetyl.

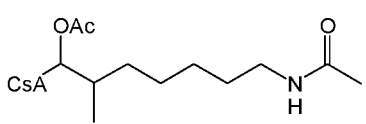
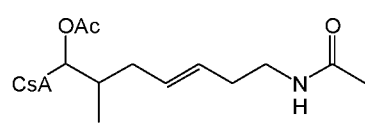
Using Reaction 5, the following compounds were synthesized.

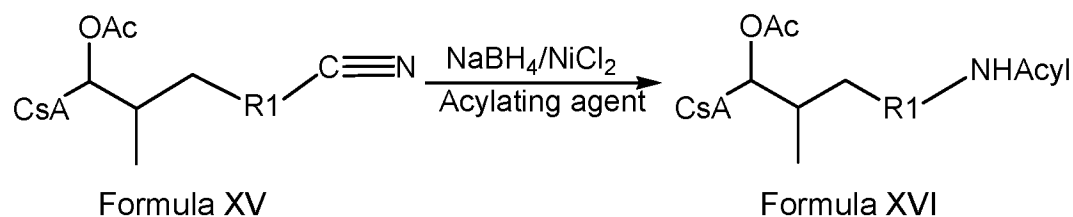
Synthesis of 404-56



Compound	Starting Material	MS (Na ⁺)
404-50	404-25	1289.1
404-56	404-43	1297.0
404-57	404-31	1327.1
404-63	404-60	1299.1
404-74	404-66	1307.1
404-92	404-90	1255.1

404-94	404-79	1388.9
		
404-168	404-134	1326.8
		
404-172	404-163	1310.9
		
420-19	416-08	1313.0
		
420-46	420-40	1383.1
		
420-68	420-134	1326.9
		
420-106	420-98	1383.1
		
420-111	420-100	1341.0
		
420-112	420-102	1298.9
		

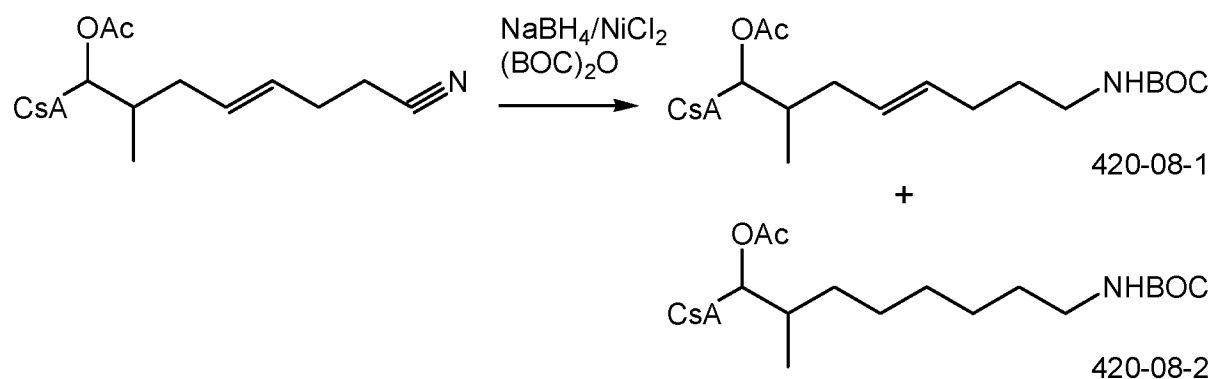
420-130	420-123	1340.0
		

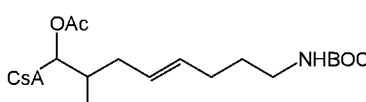
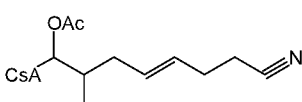
Reaction 6

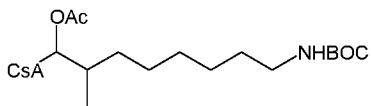
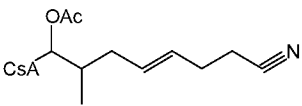
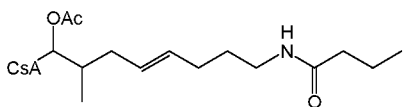
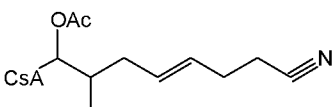
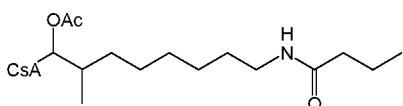
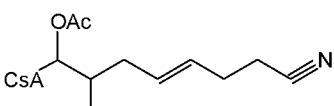
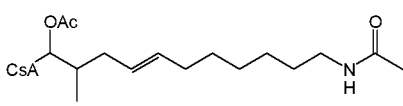
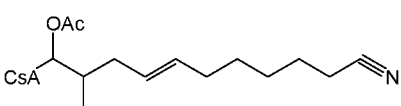
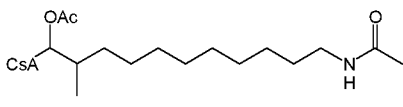
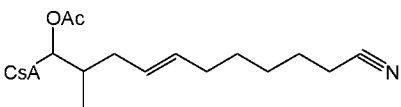
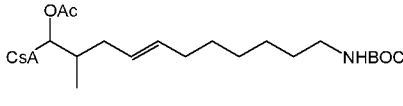
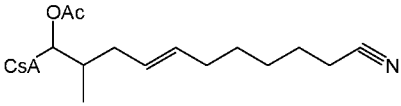
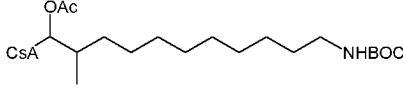
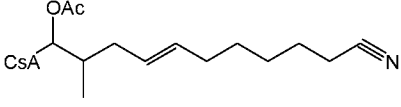
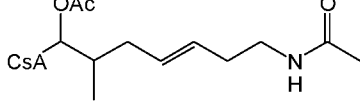
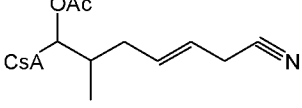
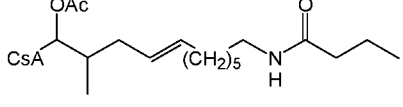
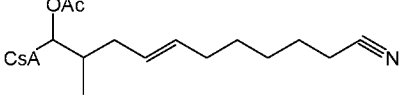
wherein Acyl is any one of BOC, acetyl, or butyryl, acylating agent is any one of di-tert-butylidicarbonate, acetic anhydride, and butyric anhydride and R1 is a saturated or unsaturated straight or branched aliphatic group. It would be understood by one skilled in the art that the acylating agents described above may be replaced with a broad range of acylating agents to produce a similarly broad range of acyl-protected amines.

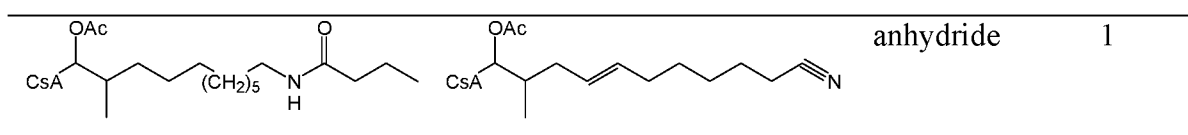
Using Reaction 6, the following compounds were synthesized.

Synthesis of 420-08



Compound	Starting Material	Protecting Reagent	MS (Na ⁺)
420-08-1	404-197	di-tert-butylidicarbonate	1410.0
			

420-08-2		404-197		di-tert-butyl-	1412.
				carbonate	1
420-107-1		404-197		butyric	1379.
				anhydride	9
420-107-2		404-197		butyric	1382.
				anhydride	1
420-109-1		420-101		acetic	1394.
				anhydride	1
420-109-2		420-101		acetic	1396.
				anhydride	1
420-110-1		420-101		di-tert-butyl-	1452.
				carbonate	1
420-110-2		420-101		di-tert-butyl-	1454.
				dicarbonate	1
420-123 ¹		420-89		acetic	1337.9/
				anhydride	1339.9
420-128-1		420-101		butyric	1422.
				anhydride	1
420-128-2		420-101		butyric	1424.



¹ mixture not separated

Reaction 7



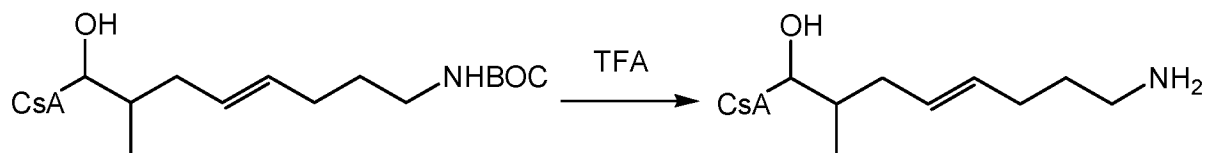
Formula XX

Formula XXI

wherein R1 is a saturated or unsaturated straight or branched aliphatic carbon chain, and R' is H or acetyl.

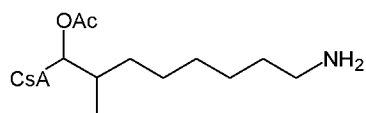
Using Reaction 7, the following compounds were synthesized.

Synthesis of 420-23

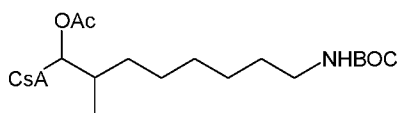


Compound	Starting Material	MS (M+1)
420-23	420-17	1246.0

420-25

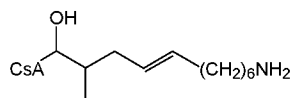


420-13

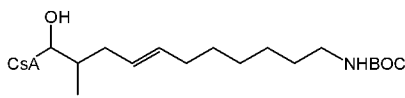


1290.0

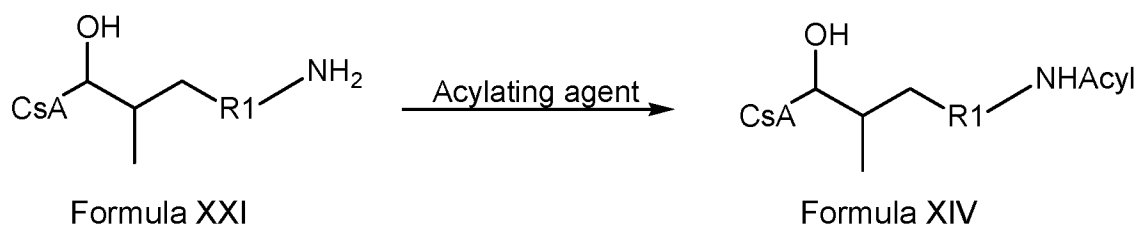
420-129



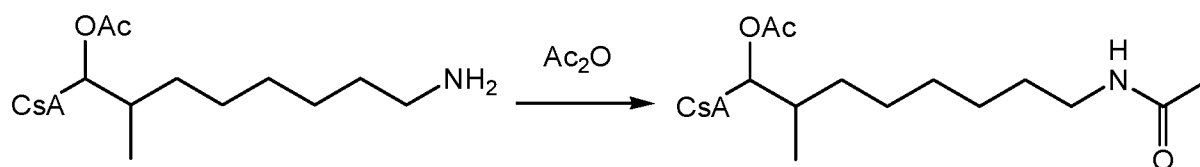
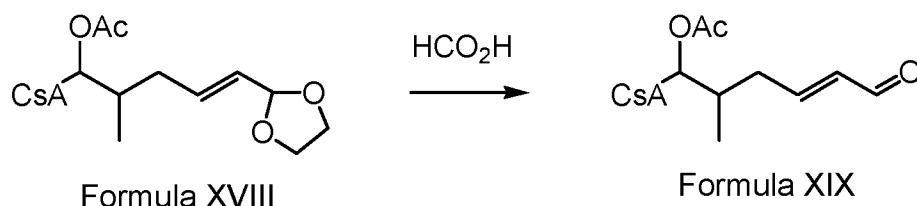
420-120

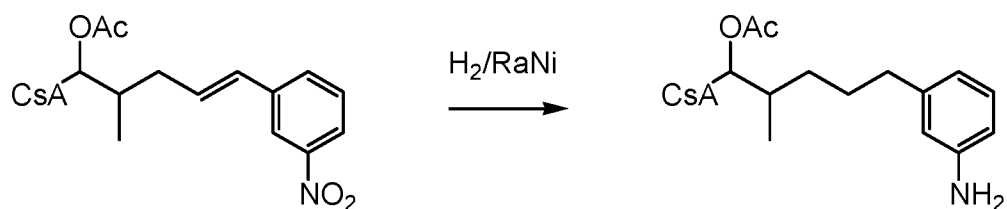


1288.0

Reaction 8

wherein Acyl is any one of BOC, acetyl or butyryl, acylating agent is any one of di-tert-butylidicarbonate, acetic anhydride, or butyric anhydride. It would be understood by one skilled in the art that a broad range of acylating agents including, dicarbonates, anhydrides and acyl halides can be employed to produce a broad range of acyl-protected amines, and R1 is a saturated or unsaturated straight or branched aliphatic group.

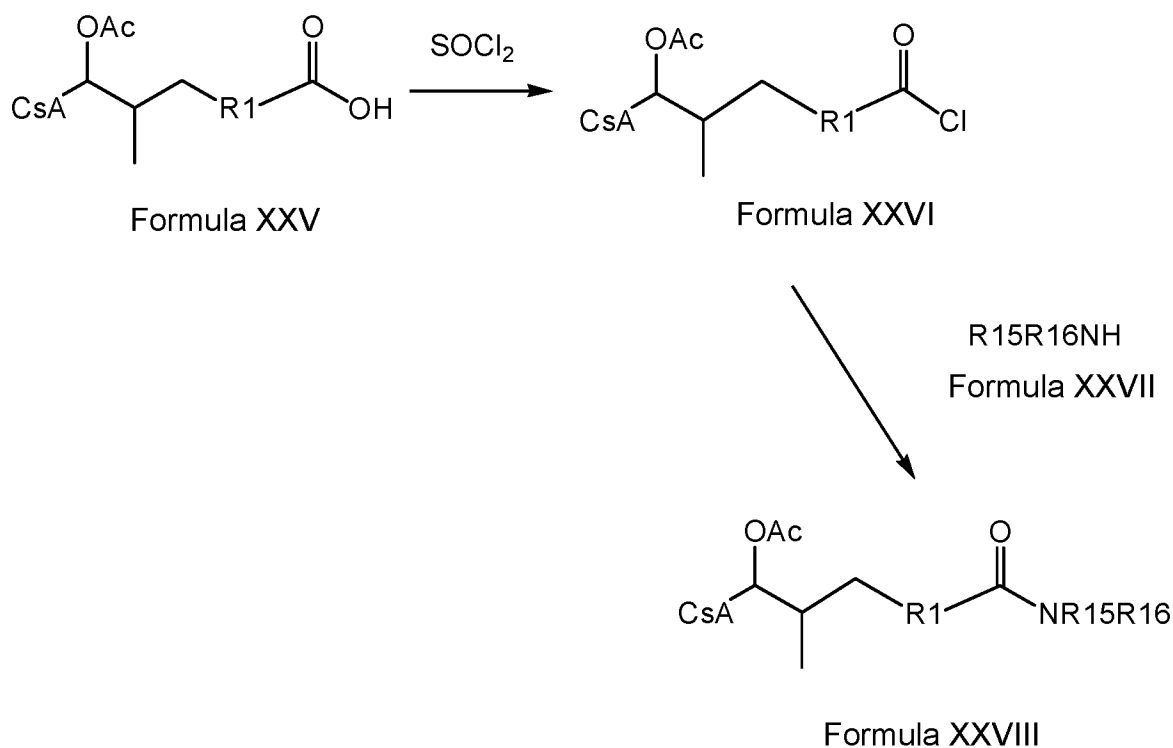
Synthesis of 420-27*Reaction 9*Synthesis of 404-47*Reaction 10*Synthesis of 404-120



Formula XXII

Formula XXIII

Reaction 11



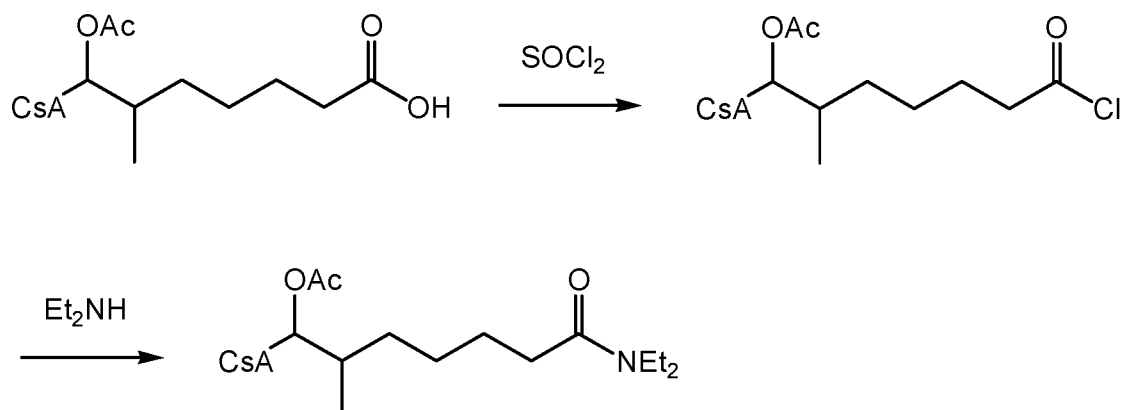
5

wherein R1 is a saturated or unsaturated straight or branched aliphatic carbon chain, R15 and R16 are independently hydrogen or a saturated or unsaturated straight or branched aliphatic carbon chain, or NR15R16 together forms a morpholinyl moiety.

Using Reaction 11, the following compounds were synthesized.

10

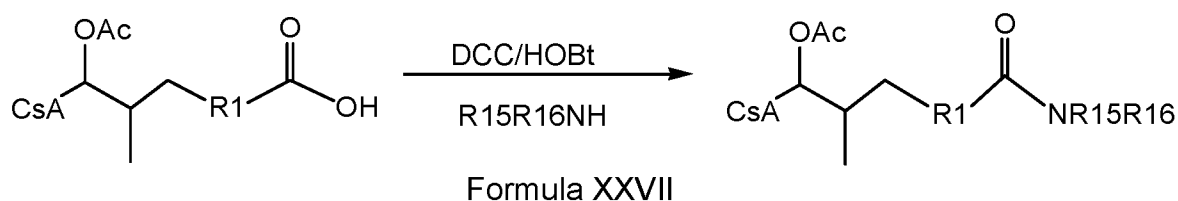
Synthesis of 404-85



Compound	Starting Material	MS (Na^+)	Amine
404-83	365-73	1368.2	diethylamine
404-128	404-124	1311.9	anhydrous ammonia ¹
404-129	404-124	1340.1	Dimethylamine ²

¹ passed through reaction for 10 min at 0 °C; ² 2 M solution In THF

Reaction 12



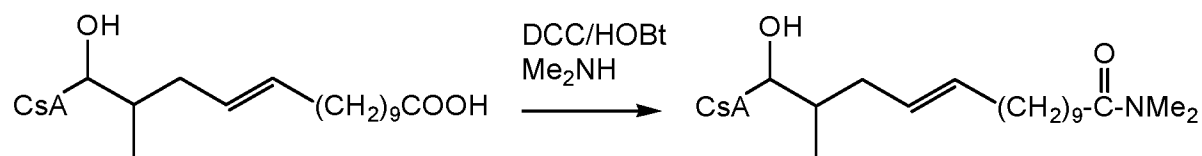
Formula XXV

Formula XXVIII

- 5 wherein R1 is a saturated or unsaturated straight or branched aliphatic carbon chain, R15 and R16 are independently hydrogen or a saturated or unsaturated straight or branched aliphatic carbon chain, or NR15R16 together forms a morpholinyl moiety.

Using Reaction 12, the following compounds were synthesized.

Synthesis of 420-104

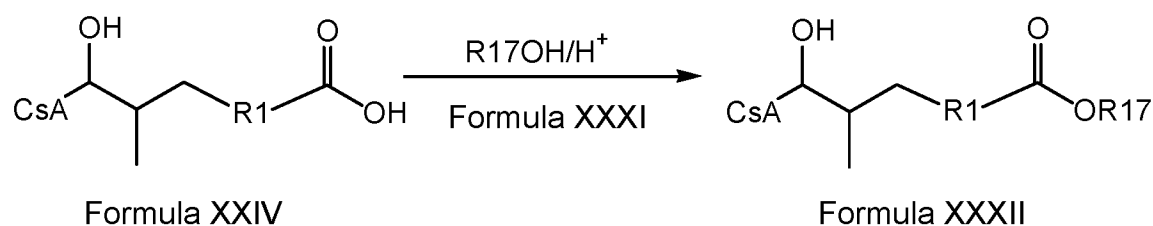


Compound	Starting Material	MS (Na^+)	Amine
404-150	416-04	1380.1	Dimethyl-amine ²
404-155	404-134	1352.1	Dimethyl-amine ²
404-156	404-60	1324.1	Dimethyl-amine ²
404-162	416-08	1379.9	Morpholine
404-164	416-08	1309.8	anhydrous ammonia ¹
404-178	404-137	1323.9	Propylamine

420-104	420-98	1408.1	Dimethyl- amine ²
420-114	420-100	1366.0	Dimethyl- amine ²
420-121	420-100	1338.0	anhydrous ammonia ¹

¹ passed through reaction for 10 min at 0 °C; ² 2 M solution in THF

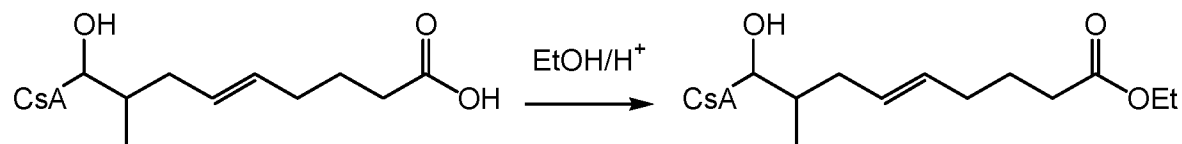
Reaction 13



wherein R1 is a saturated or unsaturated straight or branched aliphatic carbon chain, and R17 is a saturated or unsaturated straight or branched aliphatic carbon chain, optionally containing a halogen or hydroxyl substituent.

Using Reaction 13, the following compounds were synthesized.

Synthesis of 404-171



Compound	Starting Material	MS (Na ⁺)	Reagent
404-171	404-60	1368.2	ethanol

404-182	404-60	1311.9	ethylene glycol ¹
420-103	420-98	1409.1	ethanol
420-113	420-100	1366.9	ethano 1

¹ 3 hours at 90 °C; product extracted with EtOAc

Reaction 14



Formula XXIV

Formula XXXI

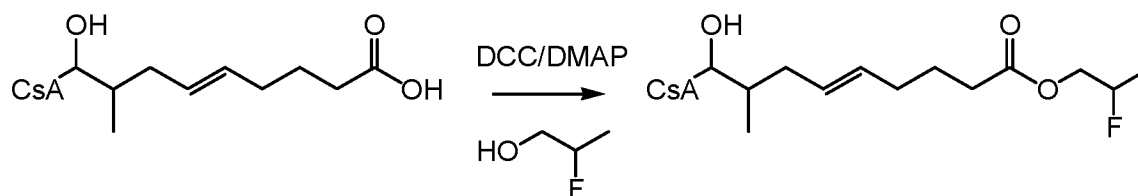
Formula XXXII

5

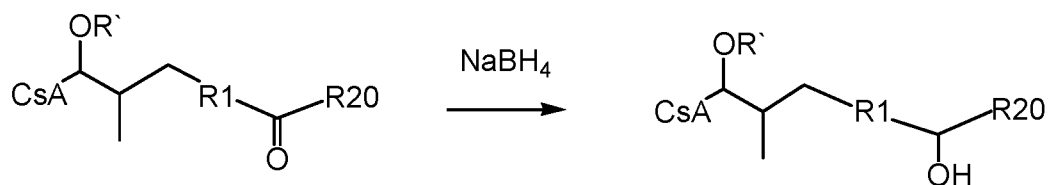
wherein R1 is a saturated or unsaturated straight or branched aliphatic carbon chain, and R17 is a saturated or unsaturated straight or branched aliphatic carbon chain, optionally containing a halogen or hydroxyl substituent.

Using Reaction 14, the following compounds were synthesized.

10 Synthesis of 420-24



Reaction 15



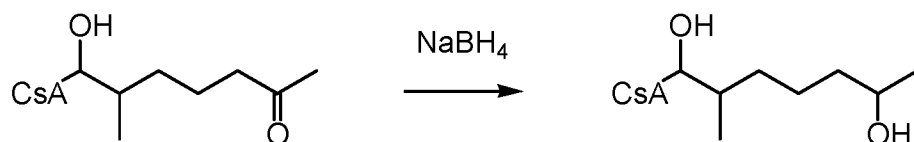
Formula XXXV

Formula XXXVI

wherein R' is a H or acetyl, R1 is a saturated or unsaturated straight or branched aliphatic carbon chain, and R20 is a saturated or unsaturated straight or branched aliphatic carbon chain.

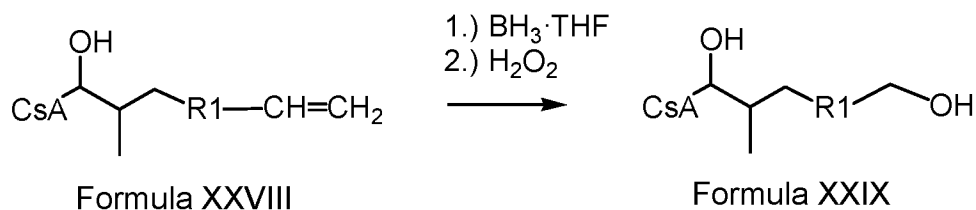
5

Using Reaction 15, the following compounds were synthesized.

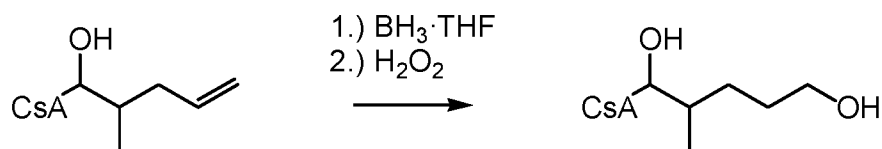
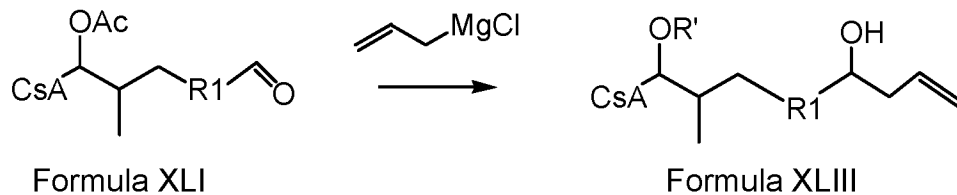
Synthesis of 404-98

Compound	Starting Material	MS (Na ⁺)
404-98	404-61	1256.9
		1271.0
404-195	404-173	
		1313.0
404-198	404-172	

420-09	404-56	1298.9

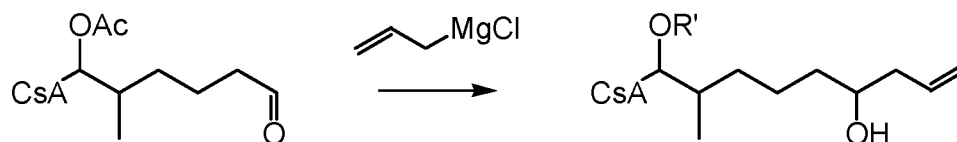
Reaction 16

5 wherein R1 is a saturated or unsaturated straight or branched aliphatic carbon chain.

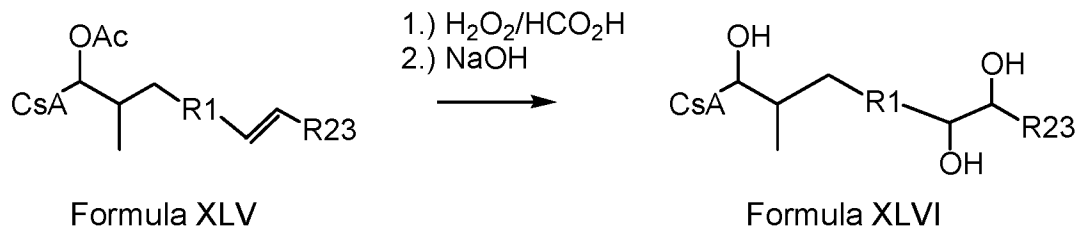
Synthesis of 420-28-1*Reaction 17*

10

wherein R1 is a saturated or unsaturated straight or branched aliphatic carbon chain, R' is either a H or an acetyl group.

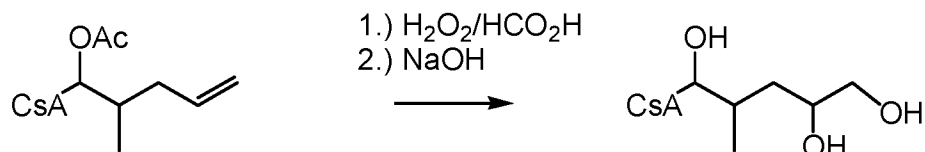
Synthesis of 420-49

15

Reaction 18

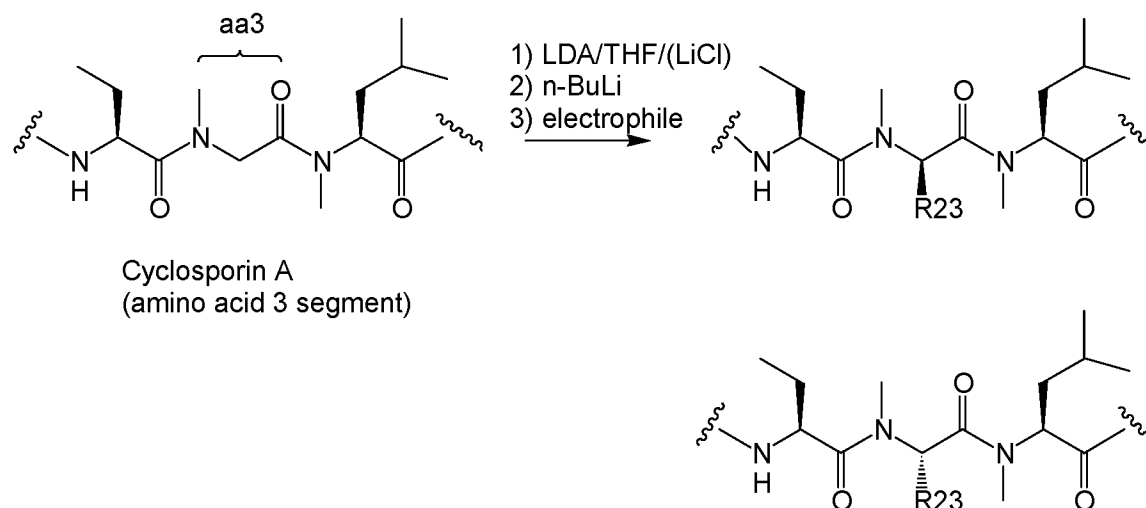
wherein R1 is a saturated or unsaturated straight or branched aliphatic carbon chain, and R23 is a saturated or unsaturated straight or branched aliphatic carbon chain.

Synthesis of 404-126



5 Reaction 19 - Modification of Amino Acid 3

CsA undergoes substitution on AA3 as outlined below.



Route A: [D-MeSar]³-CsA

An oven dried flask is charged under argon atmosphere with 160 mL anhydrous THF and diisopropylamine (2.07 mL, 14.8 mmol). The solution is cooled to -78 °C and n-butyl lithium (2.5 M in hexane, 5.4 mL, 13.5 mmol) is added. After stirring for 30 minutes, CsA (2.40 g, 2.0 mmol, dissolved in 40 mL anhydrous THF) is added. The reaction is stirred for 1 hour at -78 °C. Additional n-butyl lithium (3.2 mL, 8.0 mmol) is added, followed by addition of methyl iodide (1.25 mL, 20.0 mmol). Stirring is continued at -78 °C for 1.5 hours, and then the reaction is allowed to warm to room temperature over an additional 1.5 hours. 20 mL H₂O are added and the THF is removed in vacuum. Additional 50 mL H₂O are added and an extraction is carried out with 150 mL EtOAc. The extract is washed with brine and dried over Na₂SO₄. The solvent is removed in vacuum and the crude product is purified over silica gel (hexane/acetone 3:1). Yield: 0.74 g (0.61 mmol, 30 %).

20 Route B: [MeSar]³-CsA

A dry 100 mL flask is charged under argon atmosphere with 7.5 mL anhydrous THF and

diisopropylamine (0.46 mL, 3.3 mmol). The solution is cooled to 0 °C and n-butyl lithium (1.32 mL, 2.5 M solution in hexane, 3.3 mmol) is added. The reaction is stirred for 20 minutes at 0 °C and is then cooled to -78 °C. A solution of CsA (601 mg, 0.5 mmol) and lithium chloride (636 mg, 15 mmol) in 12 mL anhydrous THF is prepared and cooled to -78 °C under argon atmosphere. The LDA solution is then transferred into this mixture through a cannula. The reaction is stirred at -78 °C for 2 hours. Additional n-butyl lithium (1.20 mL, 3.0 mmol) is added, followed by methyl iodide (0.62 mL, 10 mmol). The mixture is allowed to warm to -20 °C and stirred at this temperature for 3 hours. The reaction is allowed to warm to room temperature, quenched with saturated NH₄Cl solution, extracted with EtOAc (2 x 20 mL), washed with brine and dried over Na₂SO₄. The solvent is removed in vacuum and the crude product is purified over silica gel (hexane/acetone 3:1). Yield: [L-MeAla³]-CsA: 302 mg (0.25 mmol, 50 %). [D-MeAla³]-CsA: 76 mg (0.06 mmol, 12 %).

Table 1: Examples of possible electrophiles used for the alkylation of the 3-position of Cyclosporin.

Electrophile	R23 ¹	Route	Remarks
methyl iodide	—CH ₃	A/B	-
ethyl iodide	—CH ₂ CH ₃	A/B	-
allyl bromide	—CH ₂ CHCH ₂	B	-
1,3-diiodopropane	—CH ₂ CH ₂ CH ₂ I	B	-
1,4-diiodobutane	—(CH ₂) ₃ CH ₂ I	B	-
trimethylammonium-3-iodopropane hexafluorophosphate	—(CH ₂) ₃ N ⁺ (CH ₃) ₃	B	-
propargyl bromide	—CH ₂ CCH	A	10 equiv electrophile; stirred for 1 h at room temperature after electrophile addition
tert-butyl bromoacetate	—CH ₂ CO ₂ (t-Bu)	A	5 equiv electrophile; stirred for 1 h at room temperature after electrophile addition
benzyl bromide	—CH ₂ Ph ²	A	15 equiv LDA and 30 equiv

			electrophile; stirred for 6 h at -75 °C and 10 h at room temperature after electrophile addition
formaldehyde	—CH ₂ OH	A	formaldehyde prepared from paraformaldehyde at 170 °C prior to addition
acetaldehyde	—CH(OH)CH ₃	B	stirred for 2.5 h at -78 °C after electrophile addition
pivalaldehyde	—CH(OH)(t-Bu) ³	B	stirred for 70 min at -78 °C after electrophile addition
benzaldehyde	—CH(OH)Ph	B	stirred for 2.5 h at -78 °C after electrophile addition
carbon dioxide	—COOH	A	CO ₂ gas passed for 15 min through reaction mixture at -78 °C; stirred for 1 h at -78 °C after electrophile addition
dimethyl disulfide	—SCH ₃	B	stirred for 18 h at 0 °C after electrophile addition
p-tolyl disulfide	—S(p-Tol) ⁴	B	stirred for 18 h at 0 °C after electrophile addition

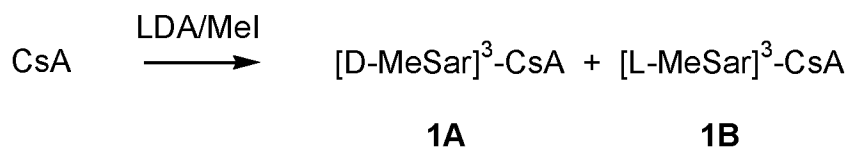
¹ according to Reaction 19 ² Ph = phenyl; ³ t-Bu = tert-butyl; ⁴ Tol = tolyl.

Reaction 20 - AA1 Modification of Alkylated CsA

Following the 3-alkylation, a 2 step procedure leads to the acetylated aldehyde (compound **3** in the example below), which is a suitable substrate for the modification of the 1-
5 position via Wittig reaction. This method allows introduction of residues to the AA1 side-chain that have limited stability under the reaction conditions used in steps 1-3, such as strong base and oxidizing agents.

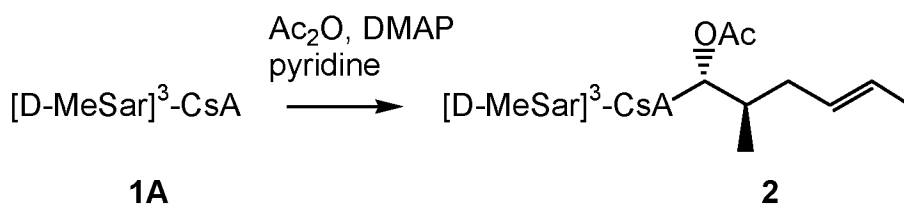
Further examples of compounds prepared using this sequence is summarized in Table 2.

Step 1: Alkylation of AA3 side-chain



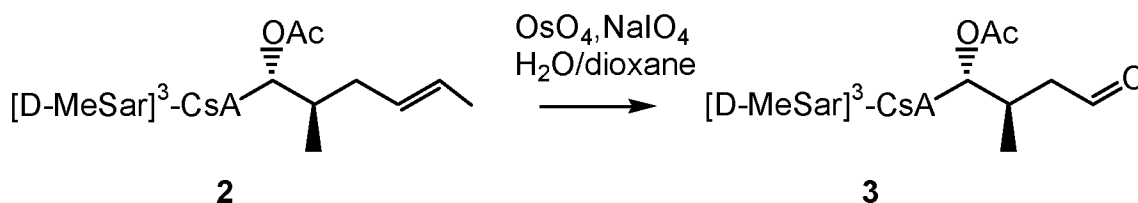
Synthesis is carried out according to Route A or B, respectively, as described above.

Step 2: Acetylation of the hydroxy-group on AA1 side-chain



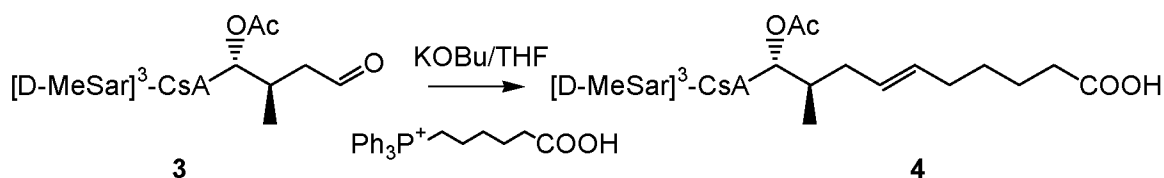
5 An oven dried flask is charged under nitrogen with [D-MeSar]³-CsA (1.84 g, 1.51 mmol), N,N-dimethylaminopyridine (19 mg, 0.15 mmol) and 20 mL anhydrous pyridine, followed by acetic anhydride (10 mL, 0.1 mol). The reaction is stirred at ambient temperature overnight. The mixture is poured into 100 mL ice-water and is stirred until all ice has melted. A solid is collected by filtration and dried in air. The solid is dissolved in 50 mL EtOAc and is washed
10 with 1 M HCl (2x), sat. NaHCO₃ solution and brine. The organic phase is dried over Na₂SO₄ and evaporated. The crude product is purified over silica gel (hexane/EtOAc/MeOH 10:10:0.5).

Step 3: Aldehyde formation



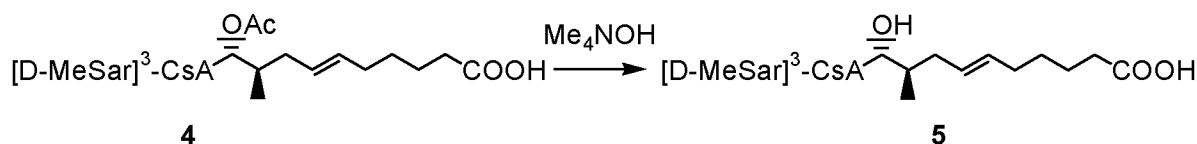
To a flask containing compound **2** (800 mg, 0.636 mmol) are added 10 mL dioxane and
15 10 mL H₂O. NaIO₄ (544 mg, 2.54 mmol) and OsO₄ (7.9 mM solution in water/dioxane 1:1, 4.05 mL, 32 mmol) are added and the reaction is stirred at room temperature overnight. 75 mL H₂O is added and the reaction is extracted with 3 x 25 mL EtOAc. The extracts are washed with water, sat. NaHCO₃ solution, water and brine (25 mL each) and are dried over MgSO₄. The solvent is removed in vacuum and the crude product is purified over silica gel (hexane/EtOAc
20 3:1).

Step 4: Wittig Reaction



An oven dried flask is charged under argon atmosphere with triphenyl-6-hexanoic acid phosphonium bromide (90 mg, 0.195 mmol) and 5 mL anhydrous THF. Potassium t-butoxide (1 M solution in THF, 0.39 mL, 0.39 mmol) is added at 0 °C and the solution is stirred for 30 minutes to give a bright orange color. Compound **3** (81 mg, 0.065 mmol, dissolved in 1 mL anhydrous THF) is added to the reaction drop-wise and stirring is continued at room temperature overnight. The reaction is quenched with sat. NH₄Cl solution and is extracted with EtOAc. The extract is washed with brine and dried over Na₂SO₄. The solvent is removed in vacuum and the crude product is purified over silica gel (toluene/acetone 3:1).

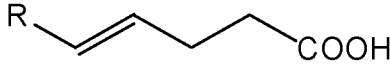
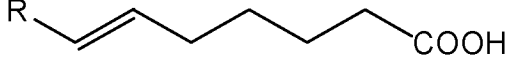
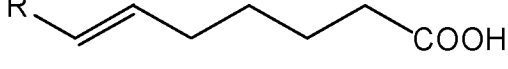
Step 5: Deacetylation

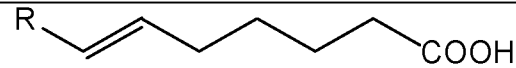
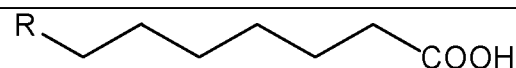
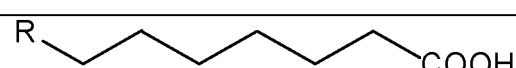
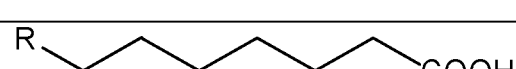
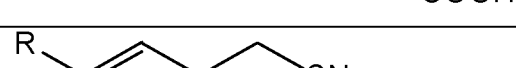
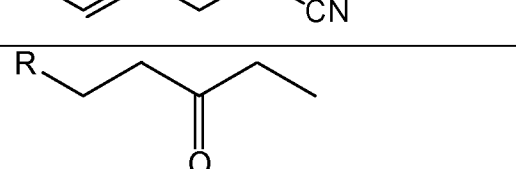
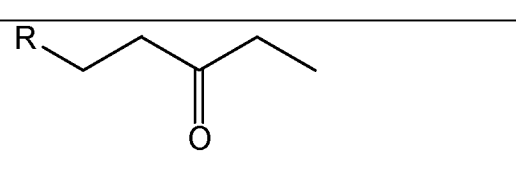
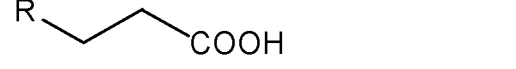
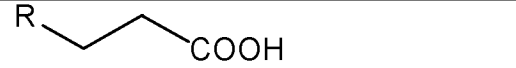
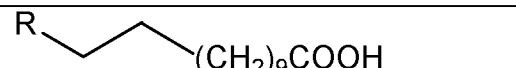


Compound **4** (30 mg, 0.022 mmol) is dissolved in 2 mL methanol and 0.5 mL water and tetramethylammonium hydroxide pentahydrate (12 mg, 0.066 mmol) is added. The reaction is stirred at room temperature for several days until HPLC confirms deprotection is complete. The reaction is acidified to pH 2 with 1 M HCl and concentrated in a vacuum. The residue is taken up in EtOAc, is washed with water and dried over Na₂SO₄. The solvent is evaporated and the crude product is purified by preparative HPLC.

Using the method above, the following compounds were synthesized (X and Y in reference to the above schematic representation; and reference of R in X is to indicate attachment of structure to AA1 of CsA).

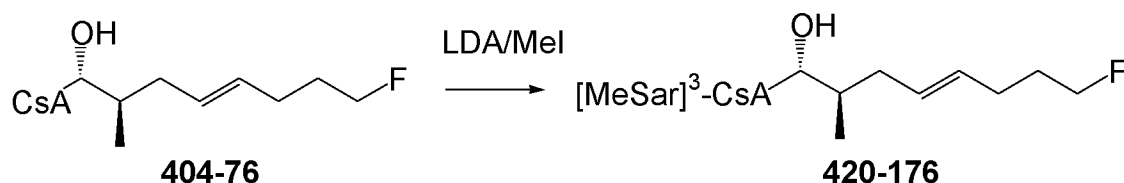
Table 2

Compound	X	R23	Isomer	MS (Na ⁺)
431-13	R- 	-CH ₃	L	1296.8
414-64	R- 	-CH ₃	D	1325.0
431-19	R- 	-CH ₃	L	1324.8

431-40		-CH ₂ CH ₃	D	1338.8
440-02		-CH ₃	D	1327.1
431-20		-CH ₃	L	1326.9
440-13		-CH ₂ CH ₃	D	1341.1
431-21		-CH ₃	D	1277.9
431-44		-CH ₂ CH ₃	L	1291.9
440-14		-CH ₂ CH ₃	D	1292.0
431-136		-CH ₃	L	1283.1
440-24		-CH ₃	D	1283.1
440-10-1		-CH ₃	D	1270.9
440-22-1		-CH ₂ CH ₃	D	1285.0
440-20		-CH ₃	D	1397.2

Reaction 21 - Alkylation of AA1 Modified Compounds

Reaction substituents to the AA3 residue of compounds previously modified on the AA1 side-chain. In addition to the groups available through *Reaction 19*, this route allows the introduction of substituents at AA3 that are unstable under the reaction conditions used in *Reaction 20*, e.g., a thiomethyl residue could undergo oxidation during the formation of the aldehyde in step 3 of this method.



A dry 25 mL flask is charged under argon atmosphere with 1.5 mL anhydrous THF and diisopropylamine (87 μL , 0.62 mmol). The solution is cooled to 0 $^{\circ}\text{C}$ and n-butyl lithium (2.5 M in hexane, 0.25 mL, 0.62 mmol) is added. The mixture is stirred for 20 minutes at 0 $^{\circ}\text{C}$ and is then cooled to -70 $^{\circ}\text{C}$. The clear LDA solution is transferred into a solution of 404-76 (118 mg, 0.095 mmol) and lithium chloride (120 mg, 2.84 mmol) in 1.5 mL anhydrous THF at -70 $^{\circ}\text{C}$. Stirring is continued for 2 hours at -70 $^{\circ}\text{C}$. Additional n-butyl lithium (0.23 mL, 0.58 mmol) is added, followed by methyl iodide (118 μL , 1.89 mmol). The reaction is allowed to warm to -20 $^{\circ}\text{C}$ and is kept at this temperature overnight. The reaction is quenched with sat. NH_4Cl solution and is extracted with EtOAc. The extract is washed with brine, dried over Na_2SO_4 and evaporated to dryness. The crude product is purified over silica gel (hexane/acetone 3:1 $\rightarrow$ 2:1).

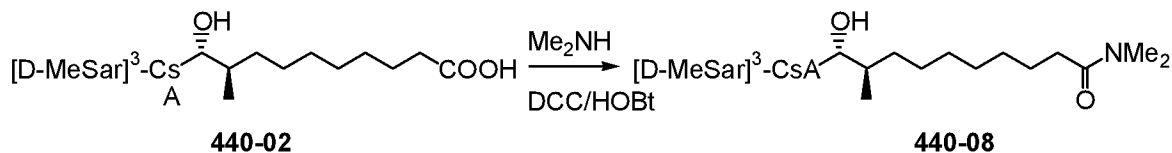
Table 3: Examples of compounds prepared by Reaction 21 (X and R23 according to the above schematic representation of 1,3 modified cyclosporine derivatives; and reference of R in X is to indicate attachment of structure to AA1 of CsA)

Compound	X	R23	Isomer	MS (Na^+)
420-176-1		-CH ₃	D	1284.9
420-176-2		-CH ₃	L	1284.9
420-177-1		-CH ₃	D	1298.8
420-177-2		-CH ₃	L	1298.8
420-180-1		-CH ₃	D	1302.8
420-182-1		-CH ₃	D	1270.8

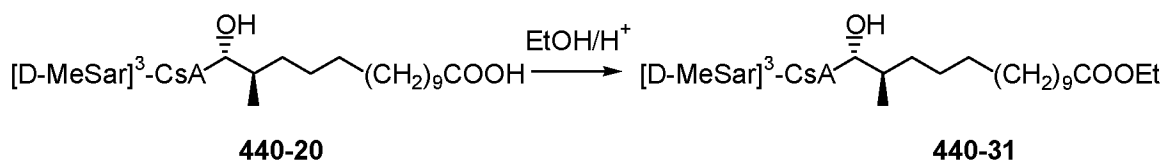
420-182-2		-CH ₃	L	1270.8
420-186		-CH ₂ CH ₃	L	1409.0
431-42		-SCH ₃	D/L ¹	1328.8
440-03		-SCH ₃	D	1319.1
440-78		-(CH ₂) ₃ N ⁺ (CH ₃) ₃	D/L ¹	1359.9 ²
440-34-3		-CH ₂ -	D	1416.2
440-36		-CH ₃	D	1251.1

¹ isomers not separated; ² m⁺ signal.

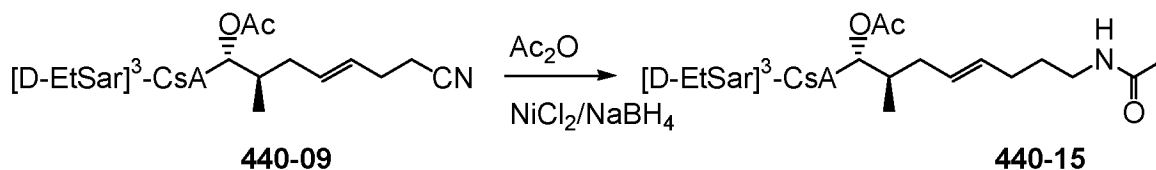
Amide Formation from Carboxylic Acid – Synthesis of 440-08



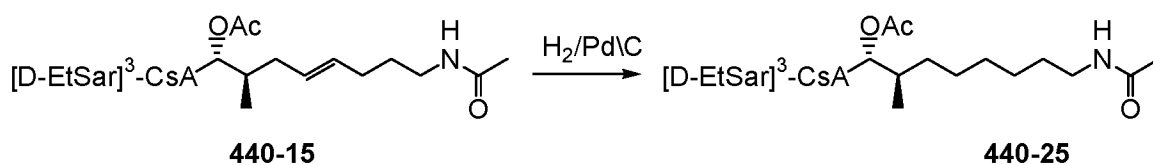
5 *Ester Formation – Synthesis of 440-31*



Amide Formation from Nitrile Compound (Reversed Amide) – Synthesis of 440-15



Synthesis of 440-25



Synthesis of 440-32

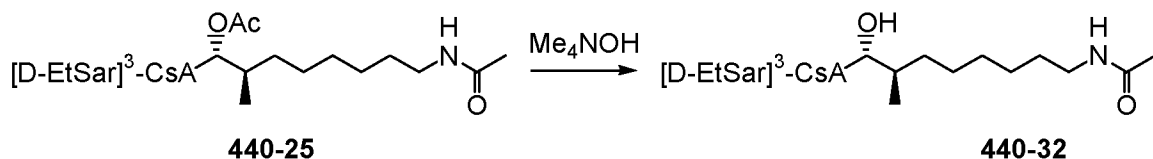
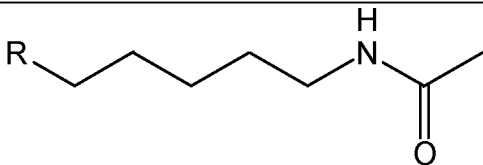
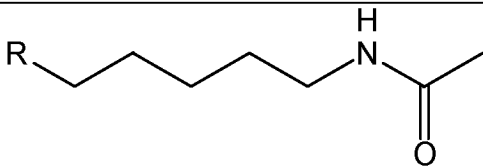
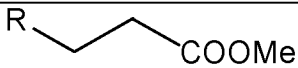
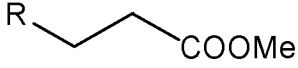
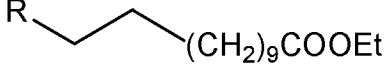


Table 4: Examples of 1,3-modified cyclosporine compounds obtained by reducing the double bond created in the Wittig reaction

Compound	X	R23	Isomer	MS (Na ⁺)
431-23		-CH ₃	L	1323.8
431-29		-CH ₃	L	1393.9
440-08		-CH ₃	D	1354.1
440-27		-CH ₂ CH ₃	D	1368.1
440-23		-CH ₃	D	1424.2
431-32		-CH ₃	D	1325.9

431-53		-CH ₂ CH ₃	L	1339.9
440-32		-CH ₂ CH ₃	D	1340.1
440-10-2		-CH ₃	D	1285.0
440-22-2		-CH ₂ CH ₃	D	1299.1
440-31		-CH ₃	D	1425.2

Example 2. Cyclophilin A Isomerase Inhibition Assay

An enzymatic assay was used to measure the inhibition of CyP-A activity by 1,3 CsA analogs of the present application, according to a protocol described in the scientific literature with minor modifications. The assay is based on the ability of CyP-A to catalyze a conformational change in proline-containing peptides from *cis* to *trans* isomeric conformations. Briefly, a peptide substrate that includes a nitroanilide moiety was supplied to a reaction mixture containing CyP-A, test compound (CsA analog, CsA, or dimethylsulfoxide vehicle), and a second enzyme, alpha-chymotrypsin. Each test compound was tested at 10 concentrations in triplicate or quadruplicate. The peptide was converted from the *cis* conformation to the *trans* conformation both by non-catalytic and CyP catalytic processes. The *trans* isomer of the peptide, but not the *cis* isomer, is a substrate for alpha-chymotrypsin. Alpha-chymotrypsin immediately cleaved nitroanilide from the rest of the peptide, and free nitroanilide accumulated at a rate proportional to *cis-trans* isomerization. Since free nitroanilide is a colored product, its accumulation was quantified by measuring its absorbance with a spectrophotometer. Nitroanilide accumulation was measured for 6 minutes, and first order rate constants for each reaction were calculated using Graphpad Prism software. The CyP-A catalytic rate constant of each reaction was determined by subtracting the non-catalytic rate constant (derived from the reaction without CyP-A) from the total reaction rate constant. Plots of the catalytic rate constants as a function of inhibitor concentrations demonstrated the compounds' potencies,

defined by their IC₅₀ values.

A. Peptide

The assay peptide was N-succinyl-alanine-alanine-proline-phenylalanine-p-nitroanilide. It was dissolved to a concentration of 3 mM in a solution of trifluoroethanolamine and lithium chloride (TFE/LiCl). TFE/LiCl was prepared fresh each day by dissolving lithium chloride in trifluoroethanolamine to a concentration of 17 mg/ml. Following dissolution of LiCl, the water content of the TFE/LiCl solution was reduced by adding heat-dried molecular sieves and gentle mixing the solution for at least 30 minutes. The peptide was then dissolved in TFE/LiCl, and the solution cooled to 4°C – 8°C prior to the assays. Dissolution of the peptide in dry TFE/LiCl promoted more peptide to exist in the *cis* conformation at the beginning of each assay reaction. Data analysis showed that approximately 60% of the peptide in our assays began as a *cis* isomer which is consistent with reported data in the scientific literature. In the enzyme reactions the peptide was diluted 20-fold to a final assay concentration of 150 µM.

B. Test Compounds

The test compounds consisted of CsA, CsA analogs, or dimethylsulfoxide (DMSO). Stock solutions of CsA and CsA analogs were made by dissolution in DMSO to a concentration of 10 mg/ml in sterile microcentrifuge tubes. Stock solutions were stored at -20°C when not in use. Further dilutions of the test compounds were made on each day of the assays. DMSO and CsA were tested in every experiment to serve as the vehicle control and reference compound, respectively.

The 10 mg/ml stock solutions of CsA and CsA analogs were diluted with DMSO to 50 µM in microcentrifuge tubes, based on the molecular weights of the compounds. Nine 3-fold serial dilutions of each compound in DMSO were then made in a 96-well polystyrene plate. An aliquot of DMSO-solution or DMSO vehicle alone was diluted 50-fold in reaction buffer (see below for recipe) to make final concentrations of CsA or CSA ANALOGs of 1000, 333, 111, 37, 12, 4.1, 1.4, 0.46, 0.15, and 0.05 nM. The reaction buffer solutions were stored at 4°C – 8°C for at least one hour prior to the assays.

C. Reaction Buffer

The starting solution (saline buffer) for the reaction buffer consisted of Hepes 50 mM, sodium chloride 100 mM, and human serum albumin 1 mg/ml, adjusted to pH 8.0 with sodium hydroxide. The saline buffer was stored at 4°C when not in use. On each assay day bovine

alpha-chymotrypsin was dissolved in a volume of saline buffer to a concentration of 1 mg/ml. An aliquot of the alpha-chymotrypsin solution was removed to serve as the noncatalytic control reaction buffer. Recombinant human CyP-A was added to the remainder of the chymotrypsin solution to a concentration of 5 nM. The solution containing alpha-chymotrypsin and CyP-A was designated the reaction buffer and was used for preparation of the reaction solutions.

D. Reaction Protocol

All assay reactions were conducted in a cold room within a temperature range of 4°C – 8°C. All solutions and equipment were stored in the cold room for at least 1 hour prior to the assays. The low temperature was necessary for reactions to proceed at a sufficiently slow rate to measure with the available equipment. The measuring device was a BMG Polarstar microplate reader configured for absorbance readings at OD 405 nm. Reactions were performed in 96-well, flat-bottom, polystyrene assay plates. Each assay run consisted of 12 separate reactions in one row of the plate. Peptide was aliquoted at 5 µl per well with a single-channel pipettor in one row of the plate, then the plate placed in the plate holder of the microplate reader. Reactions were begun by dispensing 95 µl of reaction buffer into each peptide-containing well using a 12-channel pipettor and mixing each reaction thoroughly by repeat pipetting to ensure uniform dissolution of the peptide. The 12 reactions in each assay run were represented by the following:

- a) 10 reactions, representing one replicate for each of the 10 concentrations of one test compound (CyP-A in reaction buffer)
- b) 1 reaction with 5 µl DMSO vehicle (CyP-A in reaction buffer)
- c) 1 reaction with 5 µl DMSO vehicle (CyP-A absent from reaction buffer)

Absorbance recordings were begun immediately after mixing. Approximately 15 seconds elapsed from addition of the reaction buffer to the first OD₄₀₅ recording due to mixing time and instrument setup. Subsequent readings were made at 6-second intervals for a total of 60 readings over 360 seconds. Three or four reaction runs were made for each test compound to provide data replicates.

E. Data Analysis

The raw data consisted of a time-dependent increase in OD₄₀₅. In the presence of CyP-A and the absence of inhibitor the peptide was completely converted to the *trans* isomer within approximately 150 seconds as demonstrated by a plateau in the OD₄₀₅. OD₄₀₅ vs. time data were plotted with Graphpad Prism software and fitted with a one phase exponential equation to derive

a first order rate constant K for each reaction. In reactions without CyP-A, the rate constant entirely represented the spontaneous noncatalytic, thermal *cis*-to-*trans* isomerization of the peptide and was defined as the noncatalytic rate constant K_0 . In reactions containing CyP-A, isomerization occurred both through noncatalytic and enzyme-catalyzed processes. Thus, the rate constant K in CyP-A-containing reactions represented the sum of the noncatalytic rate constant K_0 and the catalytic rate constant K_{cat} . K_{cat} was calculated by subtracting K_0 (obtained from the reaction without CyP-A) from the total rate constant K . K_{cat} typically was 3-fold higher than K_0 in reactions with 5 nM CyP-A, 150 μ M peptide substrate, and no inhibitor.

Plots of K_{cat} versus inhibitor concentration were fitted with sigmoidal dose-response nonlinear regressions to demonstrate inhibitor potencies. Software-calculated EC_{50} values represented the test compound concentrations that inhibited K_{cat} by 50%. To normalize for inter-experiment variability in assay conditions, CsA was run in every experiment as a reference compound, and CsA analog potency was expressed as a fold-potency relative to CsA based on EC_{50} values. For example, a CsA analog EC_{50} that was $\frac{1}{2}$ of CsA represented a 2-fold potency compared to CsA, whereas a CSA analog IC_{50} that was 5-fold higher than CsA represented a 0.2-fold potency compared to CsA.

Example 3. Inhibition of interaction of HBx with CypA

GST Pulldown Assay

A GST pulldown assay was conducted to measure the inhibition of interaction of HBx with CypA by a compound of the present application. The assay is illustrated in Figure 1A. As shown in Figure 1B, a compound of the present application inhibited the interaction of HBx with CypA.

ELISA Assay

An ELISA assay was conducted to measure the inhibition of interaction of HBx with CypA by a compound of the present application, Compound II, or Alisporivir. As shown in Figure 2A, a compound of the present application inhibited the interaction of HBx with CypA, with a higher potency as compared to Alisporivir. Also, Figure 2B shows that a compound of the present application potently inhibited cyclophilin isomerase activity.

Microtiter Plate Binding Assay

A microtiter plate binding assay was conducted to measure the inhibition of interaction of HBx with CypA by a compound of the present application, as follows.

1. Bind anti-GST antibody to plate.
2. Add GST-CypA fusion protein to bind to plate-bound antibody. Rinse away excess GST-CypA.
3. Add poly-histidine-HBx fusion protein ("His-tagged") to bind to CypA. Wash away
5 excess HBx.
4. Add anti-His antibody (coupled to alkaline phosphatase or horseradish peroxidase). Rinse away excess antibody.
5. Add HRP substrate and begin reaction to colorimetric product.

10 The assay is illustrated in Figure 3. As demonstrated in the microtiter plate assay, a compound of the present application inhibited the interaction of HBx with CypA.

EQUIVALENTS

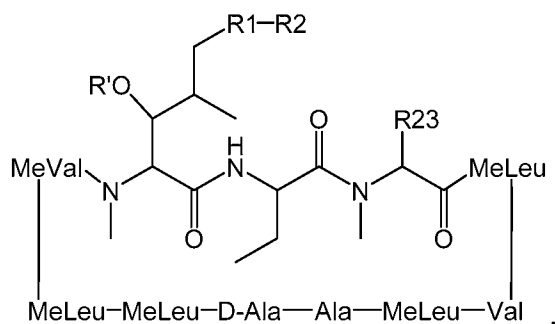
Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the present application.

5 All patents, patent applications, and literature references cited herein are hereby expressly incorporated by reference.

10

CLAIMS

1. A compound of Formula L:



Formula L

- 5 or a pharmaceutically acceptable salt thereof, wherein:

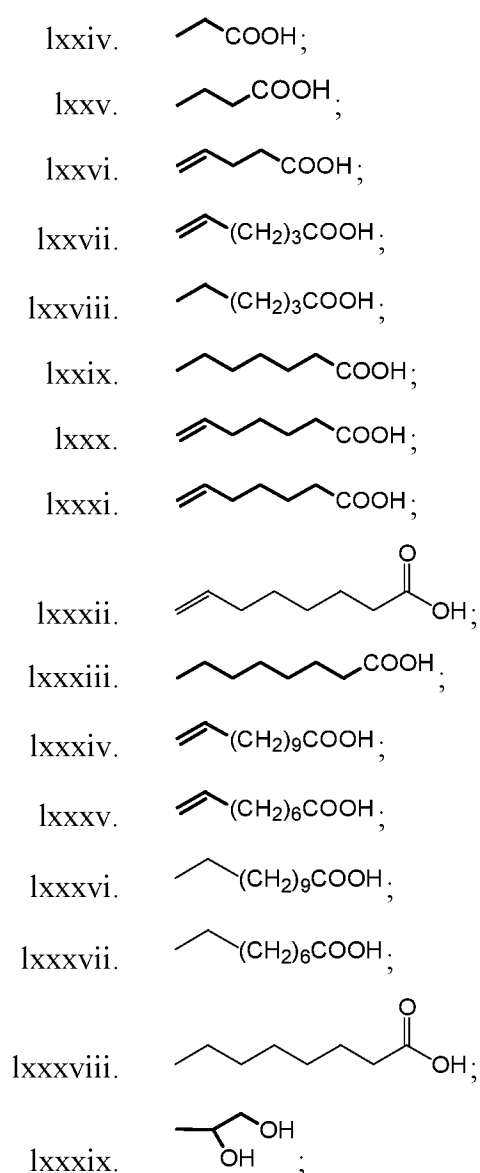
- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - 10 i. H;
 - ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;
 - iii. a N-substituted or unsubstituted acyl protected amine;
 - iv. a carboxylic acid;
 - v. a N-substituted or unsubstituted amine;
 - 15 vi. a nitrile;
 - vii. an ester;
 - viii. a ketone;
 - ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
 - x. a substituted or unsubstituted aryl;
 - 20 xi. a saturated or unsaturated straight or branched aliphatic carbon chain optionally containing a substituent selected from the group consisting of ketones, hydroxyls, nitriles, carboxylic acids, esters, 1,3-dioxolanes, halogens, and oxo;
 - xii. an aromatic group containing a substituent selected from the group consisting of halides, esters and nitro; and
 - 25 xiii. a combination of the saturated or unsaturated straight or branched aliphatic carbon chain of (xi) and the aromatic group of (xii); and

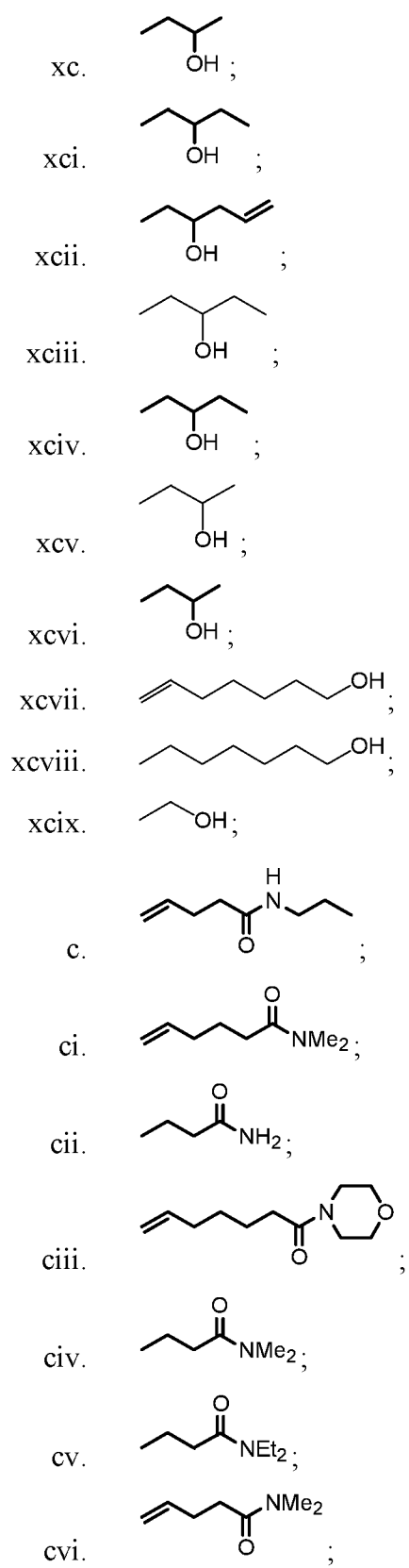
d. R23 is a saturated or unsaturated straight or branched optionally substituted aliphatic carbon chain,

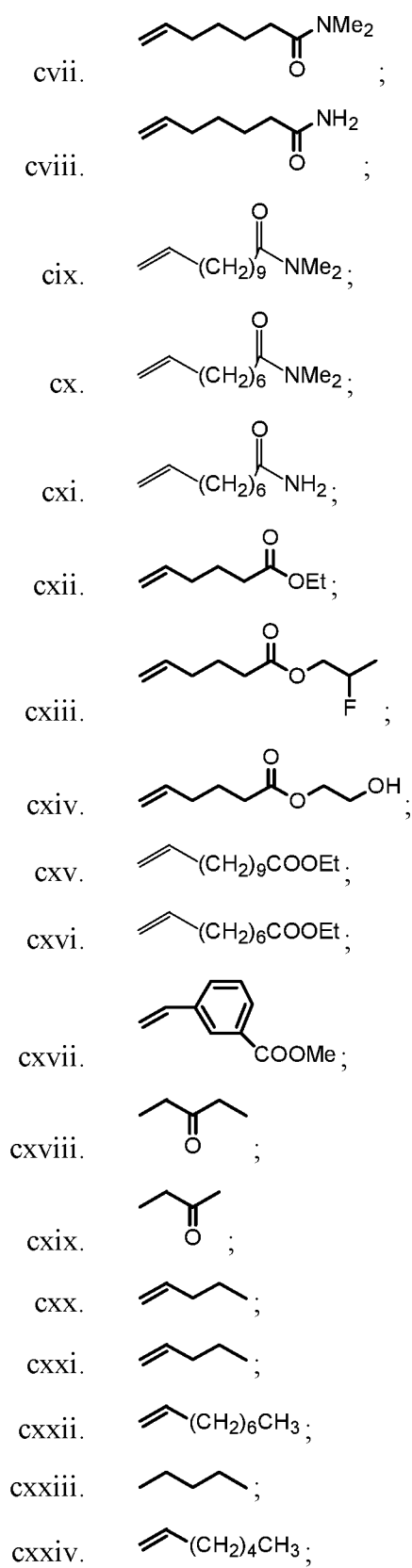
or a pharmaceutically acceptable salt thereof, for use in treating and/or preventing a hepatitis B virus (HBV) disease through inhibiting the interaction of CypA with HBV X protein (HBx)

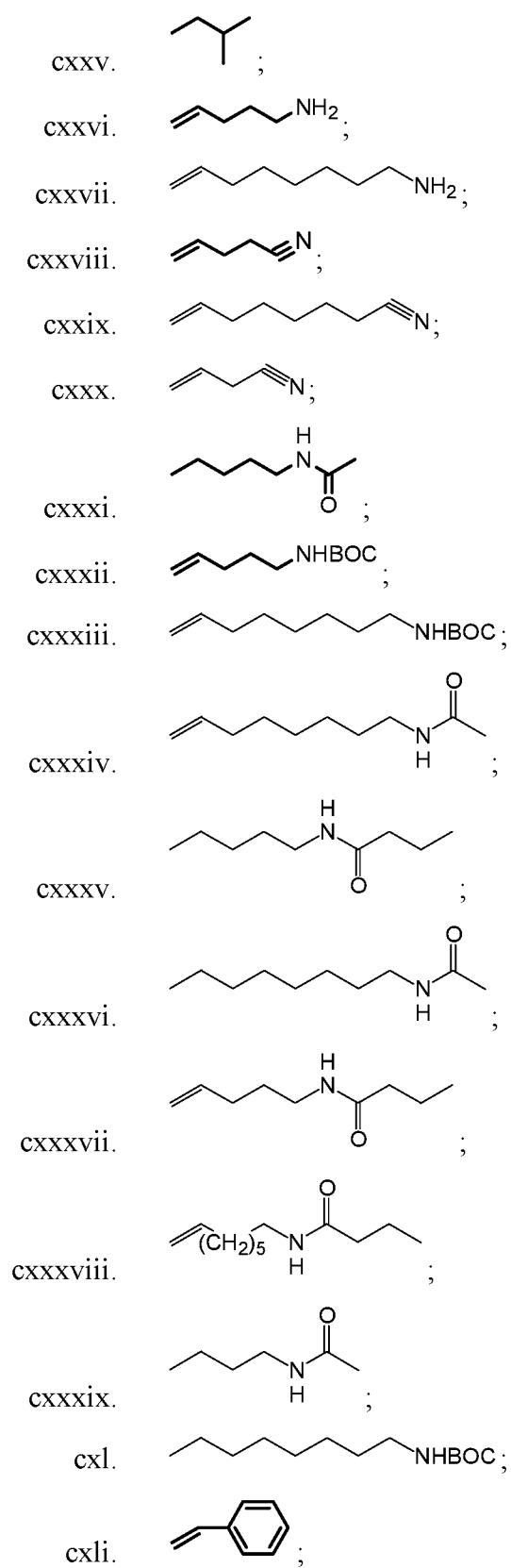
5 and/or Hepatitis B surface antigen (HBsAg), or in modulating an interaction of HBx or HBsAg with CypA, in a subject in need thereof.

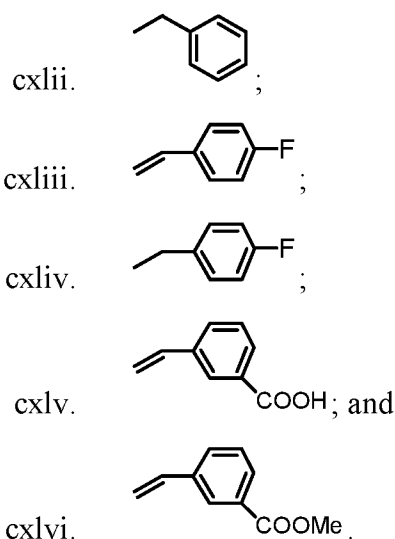
2. The compound for use of claim 1, wherein R1-R2 is selected from the group consisting of:











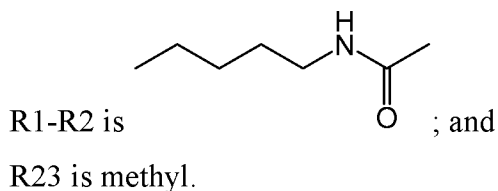
3. The compound for use of claim 1, wherein R1-R2 comprises a saturated or unsaturated straight or branched aliphatic carbon chain of between 2 and 5 carbons optionally substituted with a substituent selected from the group consisting of ketones, hydroxyls, nitriles, halogens, oxo, carboxylic acids, esters, and 1,3-dioxolanes.

4. The compound for use of claim 1, wherein R23 is a straight or branched aliphatic carbon chain of 1 to 6, 1 to 5, 1 to 4, 1 to 3 or 2 carbons in length.

5. The compound for use of claim 1, wherein R23 is selected from the group consisting of:

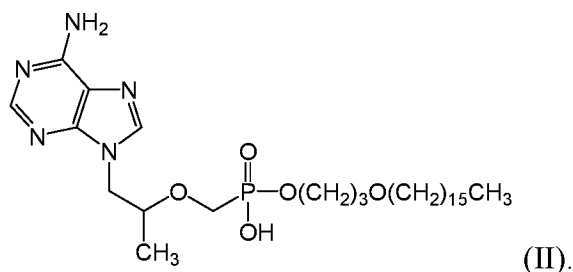
-
- xvii. $-\text{CH}_3$
- xviii. $-\text{CH}_2\text{CH}_3$
- xix. $-\text{CH}_2\text{CHCH}_2$
- xx. $-\text{CH}_2\text{CH}_2\text{CH}_2\text{I}$
- xxi. $-(\text{CH}_2)_3\text{CH}_2\text{I}$
- xxii. $-(\text{CH}_2)_3\text{N}^+(\text{CH}_3)_3$
- xxiii. $-\text{CH}_2\text{CCH}$
- xxiv. $-\text{CH}_2\text{CO}_2(\text{t-Bu})$
- xxv. $-\text{CH}_2\text{Ph}$
- xxvi. $-\text{CH}_2\text{OH}$
-

R' is H;



5 8. The compound for use of claim 1, wherein the use is in combination with a second therapeutic agent.

9. The compound for use of claim 8, wherein the second therapeutic agent is Compound II:



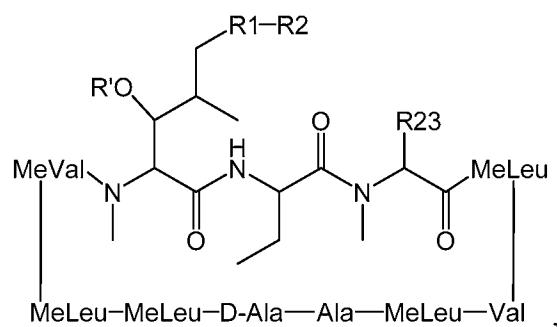
10 or a pharmaceutically acceptable salt thereof.

10. The compound for use of claim 1, wherein the HBV disease is mediated by an interaction of CypA with HBx and/or HBsAg.

15 11. The compound for use of claim 1, wherein the HBV disease is hepatitis B, cirrhosis, or hepatocellular carcinoma.

12. The compound for use of claim 11, wherein the HBV disease hepatitis B.

20 13. A method of treating and/or preventing a hepatitis B virus (HBV) disease through inhibiting the interaction of CypA with HBV X protein (HBx) and/or Hepatitis B surface antigen (HBsAg), comprising administering to a subject in need thereof a compound of Formula L:

**Formula L**

or a pharmaceutically acceptable salt thereof, wherein:

e. R' is H or acetyl;

f. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;

g. R2 is selected from the group consisting of:

xiv. H;

xv. an unsubstituted, N-substituted, or N,N-disubstituted amide;

xvi. a N-substituted or unsubstituted acyl protected amine;

xvii. a carboxylic acid;

xviii. a N-substituted or unsubstituted amine;

xix. a nitrile;

xx. an ester;

xxi. a ketone;

xxii. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and

xxiii. a substituted or unsubstituted aryl;

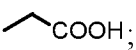
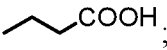
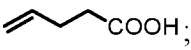
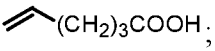
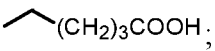
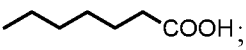
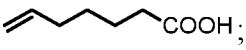
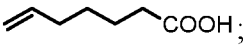
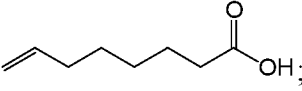
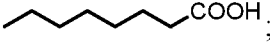
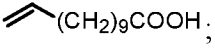
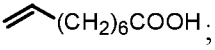
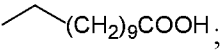
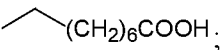
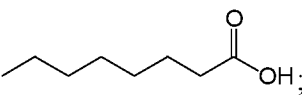
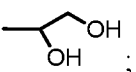
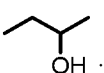
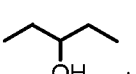
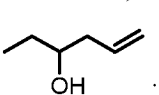
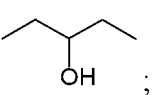
xxiv. a saturated or unsaturated straight or branched aliphatic carbon chain optionally containing a substituent selected from the group consisting of ketones, hydroxyls, nitriles, carboxylic acids, esters, 1,3-dioxolanes, halogens, and oxo;

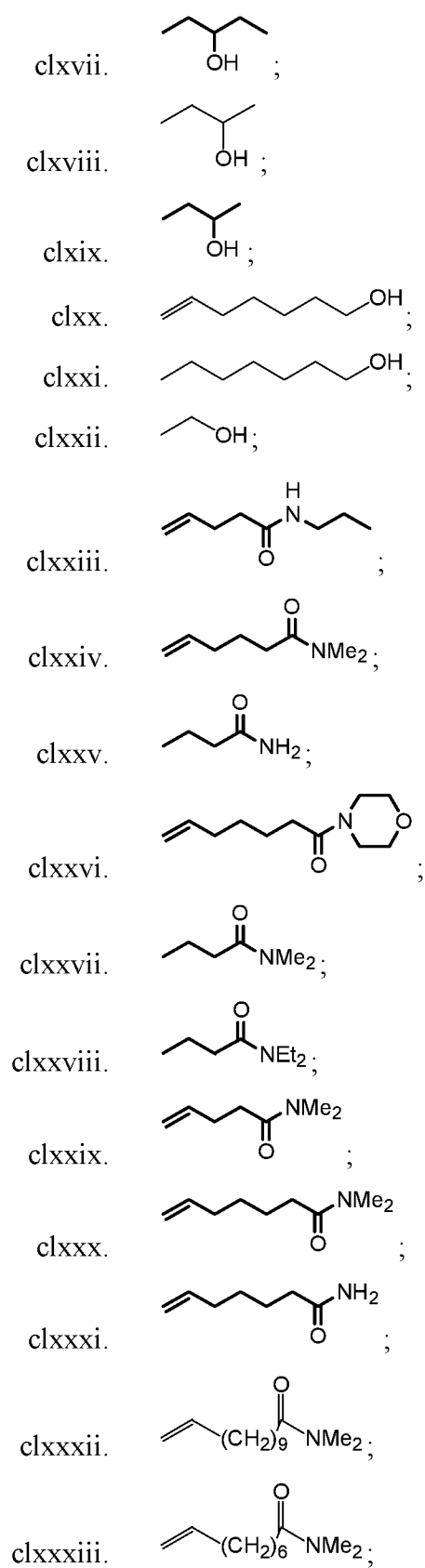
xxv. an aromatic group containing a substituent selected from the group consisting of halides, esters and nitro; and

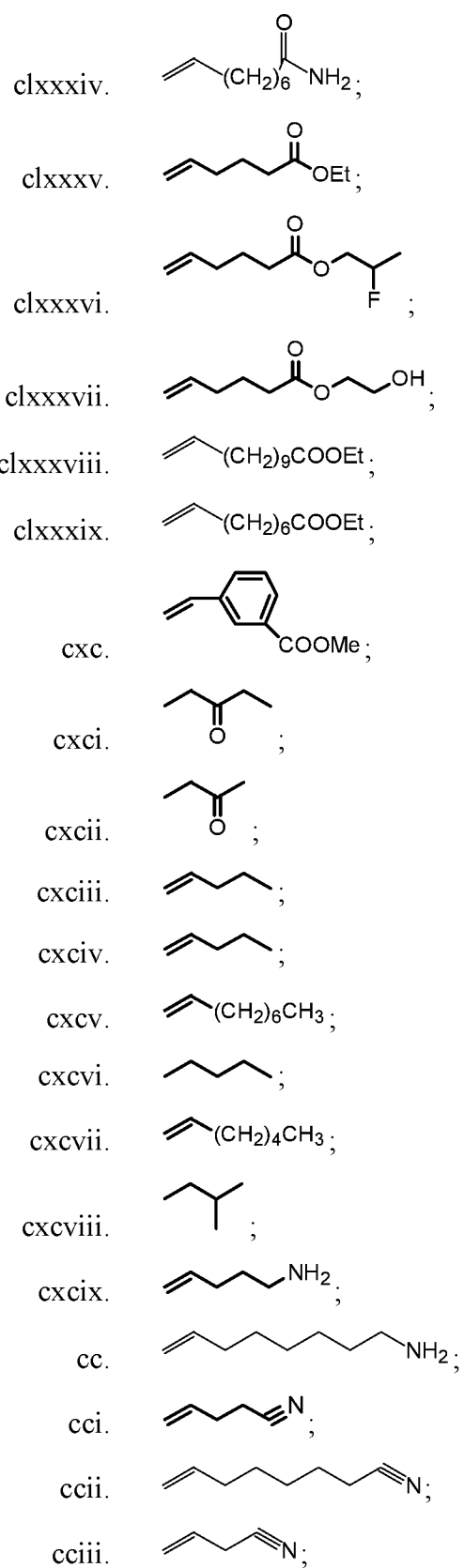
xxvi. a combination of the saturated or unsaturated straight or branched aliphatic carbon chain of (xi) and the aromatic group of (xii); and

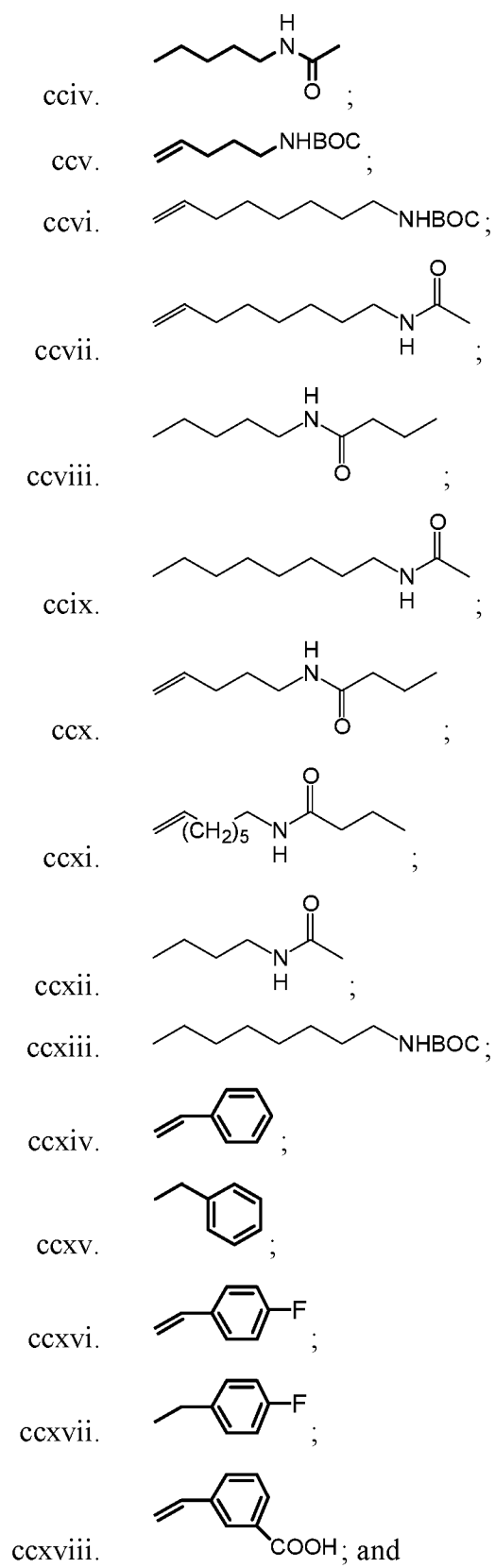
h. R23 is a saturated or unsaturated straight or branched optionally substituted aliphatic carbon chain.

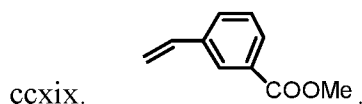
14. The method of claim 13, wherein R1-R2 is selected from the group consisting of:

- cxlvii. ;
- cxlviii. ;
- cxlix. ;
- cl. ;
- cli. ;
- clii. ;
- cliii. ;
- cliv. ;
- clv. ;
- clvi. ;
- clvii. ;
- clviii. ;
- clix. ;
- clx. ;
- clxi. ;
- clxii. ;
- clxiii. ;
- clxiv. ;
- clxv. ;
- clxvi. ;







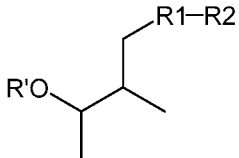


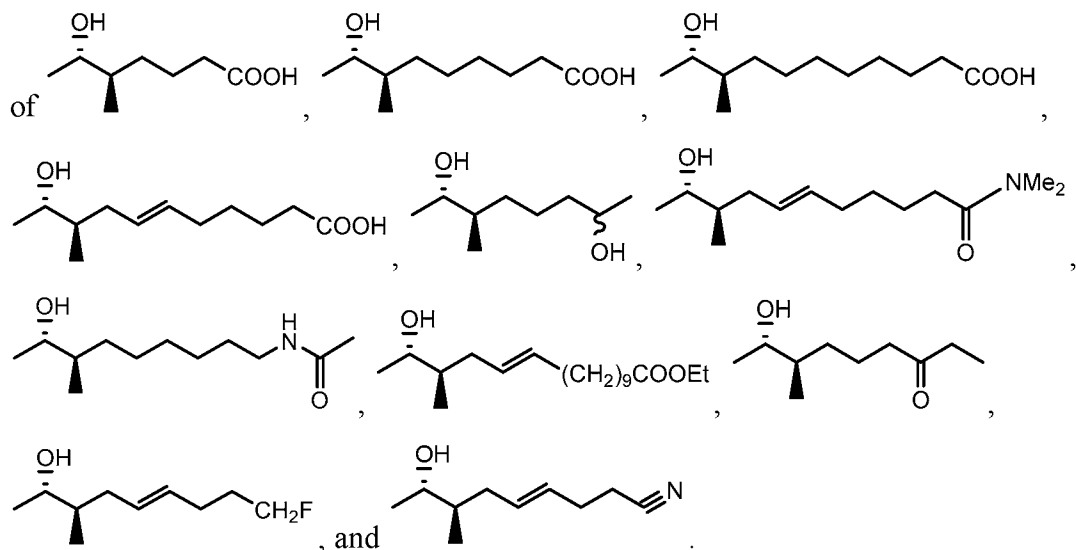
15. The method of claim 13, wherein R1-R2 comprises a saturated or unsaturated straight or branched aliphatic carbon chain of between 2 and 5 carbons optionally substituted with a substituent selected from the group consisting of ketones, hydroxyls, nitriles, halogens, oxo, carboxylic acids, esters, and 1,3-dioxolanes.

16. The method of claim 13, wherein R23 is a straight or branched aliphatic carbon chain of 1 to 6, 1 to 5, 1 to 4, 1 to 3 or 2 carbons in length.

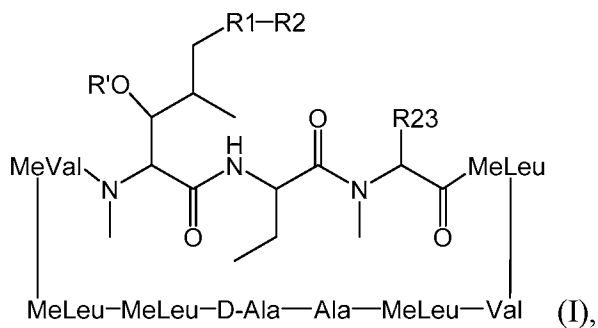
17. The method of claim 13, wherein R23 is selected from the group consisting of:

-
- xxiii. $-\text{CH}_3$
 - xxiv. $-\text{CH}_2\text{CH}_3$
 - xxv. $-\text{CH}_2\text{CHCH}_2$
 - xxvi. $-\text{CH}_2\text{CH}_2\text{CH}_2\text{I}$
 - xxvii. $-(\text{CH}_2)_3\text{CH}_2\text{I}$
 - xxviii. $-(\text{CH}_2)_3\text{N}^+(\text{CH}_3)_3$
 - xxix. $-\text{CH}_2\text{CCH}$
 - xl. $-\text{CH}_2\text{CO}_2(\text{t-Bu})$
 - xli. $-\text{CH}_2\text{Ph}$
 - xl. $-\text{CH}_2\text{OH}$
 - xl. $-\text{CH}(\text{OH})\text{CH}_3$
 - xl. $-\text{CH}(\text{OH})(\text{t-Bu})$
 - xl. $-\text{CH}(\text{OH})\text{Ph}$
 - xlvi. $-\text{COOH}$
 - xlvi. $-\text{SCH}_3$
 - lviii. $-\text{S}(\text{p-Tol})$
-

18. The method of claim 13, wherein  is selected from the group consisting

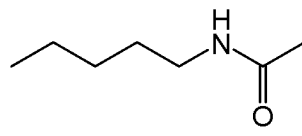


19. The method of claim 13, wherein the compound of Formula L is Compound I:



5 wherein:

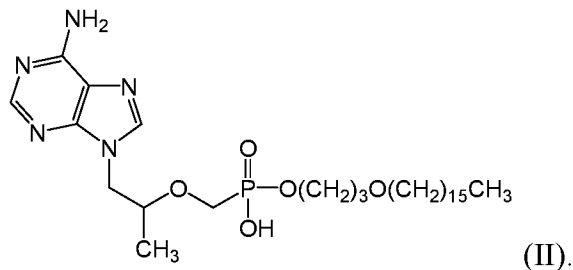
R' is H;

R1-R2 is ; and

R23 is methyl.

- 10 20. The method of claim 13, further comprising administering a second therapeutic agent.

21. The method of claim 20, wherein the second therapeutic agent is Compound II:



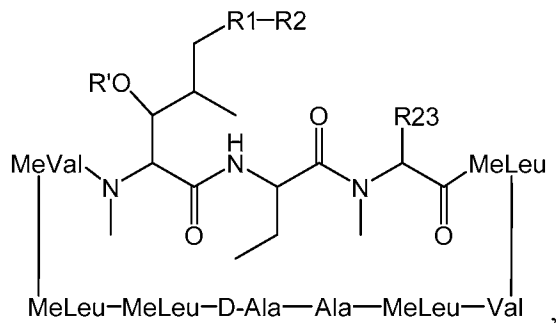
or a pharmaceutically acceptable salt thereof.

22. The method of claim 13, wherein the HBV disease is mediated by an interaction of CypA with HBx and/or HBsAg.

23. The method of claim 13, wherein the HBV disease is hepatitis B, cirrhosis, or hepatocellular carcinoma.

24. The method of claim 23, wherein the HBV disease hepatitis B.

25. A method of modulating an interaction of HBx or HBsAg with CypA, comprising administering to a subject in need thereof a compound of Formula L:



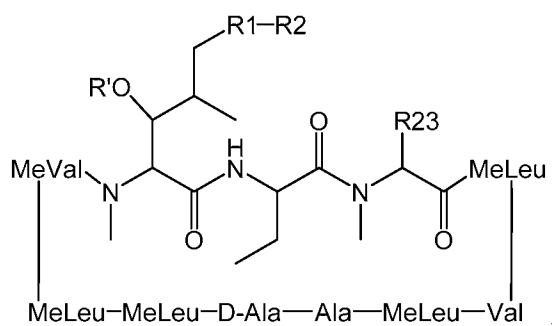
Formula L

or a pharmaceutically acceptable salt thereof, wherein:

- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - i. H;

- ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;
- iii. a N-substituted or unsubstituted acyl protected amine;
- iv. a carboxylic acid;
- v. a N-substituted or unsubstituted amine;
- 5 vi. a nitrile;
- vii. an ester;
- viii. a ketone;
- ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
- x. a substituted or unsubstituted aryl;
- 10 xi. a saturated or unsaturated straight or branched aliphatic carbon chain optionally containing a substituent selected from the group consisting of ketones, hydroxyls, nitriles, carboxylic acids, esters, 1,3-dioxolanes, halogens, and oxo;
- xii. an aromatic group containing a substituent selected from the group consisting of halides, esters and nitro; and
- 15 xiii. a combination of the saturated or unsaturated straight or branched aliphatic carbon chain of (xi) and the aromatic group of (xii); and
- d. R23 is a saturated or unsaturated straight or branched optionally substituted aliphatic carbon chain.

20 26. A compound of Formula L:



Formula L

or a pharmaceutically acceptable salt thereof, wherein:

- a. R' is H or acetyl;
- 25 b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;

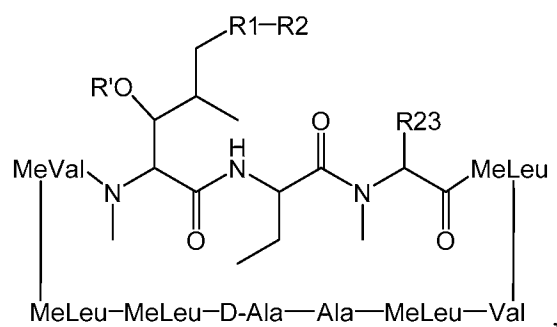
c. R2 is selected from the group consisting of:

- i. H;
- ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;
- iii. a N-substituted or unsubstituted acyl protected amine;
- iv. a carboxylic acid;
- v. a N-substituted or unsubstituted amine;
- vi. a nitrile;
- vii. an ester;
- viii. a ketone;
- ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
- x. a substituted or unsubstituted aryl;
- xi. a saturated or unsaturated straight or branched aliphatic carbon chain optionally containing a substituent selected from the group consisting of ketones, hydroxyls, nitriles, carboxylic acids, esters, 1,3-dioxolanes, halogens, and oxo;
- xii. an aromatic group containing a substituent selected from the group consisting of halides, esters and nitro; and
- xiii. a combination of the saturated or unsaturated straight or branched aliphatic carbon chain of (xi) and the aromatic group of (xii); and

d. R23 is a saturated or unsaturated straight or branched optionally substituted aliphatic carbon chain,

or a pharmaceutically acceptable salt thereof, for use in the manufacture of a medicament for treatment and/or prevention of a hepatitis B virus (HBV) disease through inhibiting the interaction of CypA with HBV X protein (HBx) and/or Hepatitis B surface antigen (HBsAg), or for modulation of an interaction of HBx or HBsAg with CypA in a subject in need thereof.

27. Use of a compound of Formula L:

**Formula L**

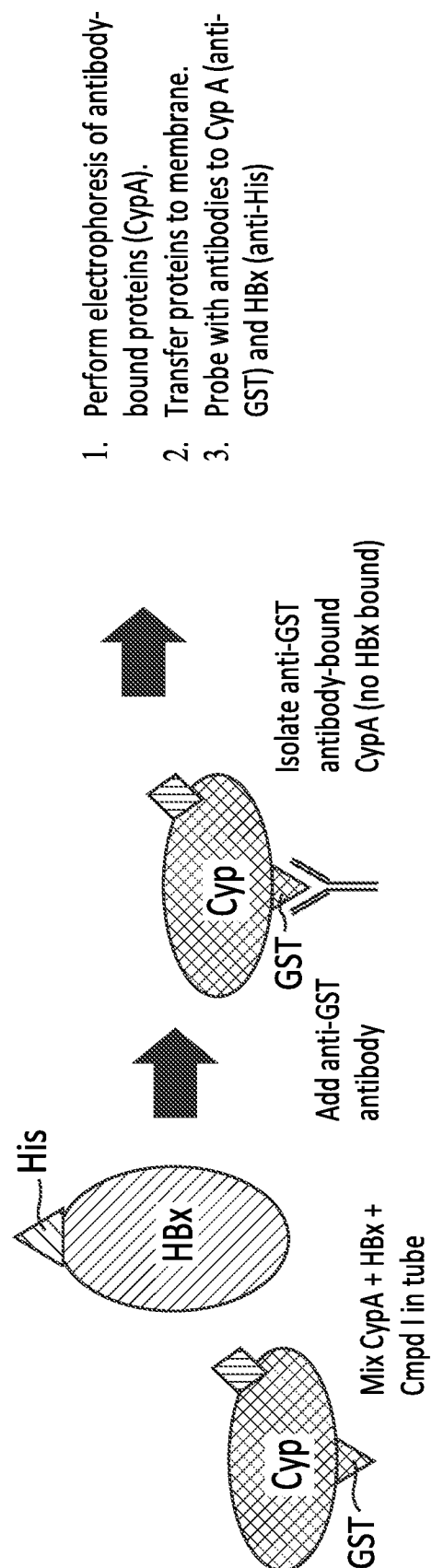
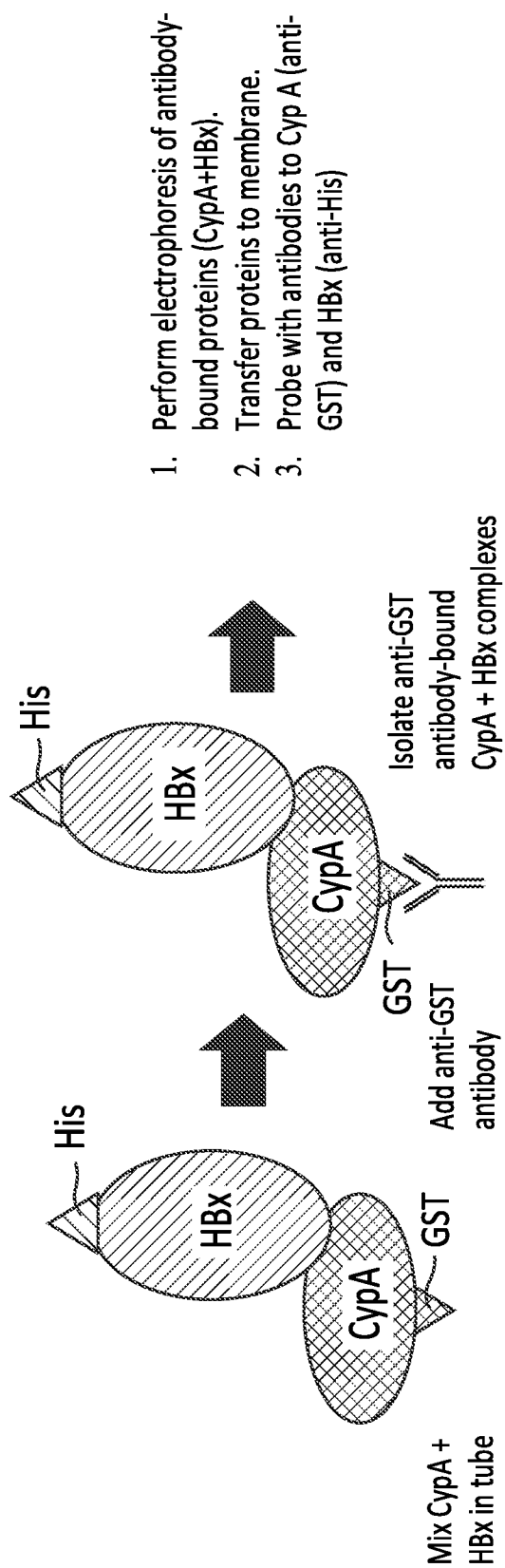
or a pharmaceutically acceptable salt thereof, wherein:

- a. R' is H or acetyl;
- b. R1 is a saturated or unsaturated straight or branched aliphatic carbon chain from 2 to 15 carbon atoms in length;
- c. R2 is selected from the group consisting of:
 - i. H;
 - ii. an unsubstituted, N-substituted, or N,N-disubstituted amide;
 - iii. a N-substituted or unsubstituted acyl protected amine;
 - iv. a carboxylic acid;
 - v. a N-substituted or unsubstituted amine;
 - vi. a nitrile;
 - vii. an ester;
 - viii. a ketone;
 - ix. a hydroxy, dihydroxy, trihydroxy, or polyhydroxy alkyl; and
 - x. a substituted or unsubstituted aryl;
 - xi. a saturated or unsaturated straight or branched aliphatic carbon chain optionally containing a substituent selected from the group consisting of ketones, hydroxyls, nitriles, carboxylic acids, esters, 1,3-dioxolanes, halogens, and oxo;
 - xii. an aromatic group containing a substituent selected from the group consisting of halides, esters and nitro; and
 - xiii. a combination of the saturated or unsaturated straight or branched aliphatic carbon chain of (xi) and the aromatic group of (xii); and
- d. R23 is a saturated or unsaturated straight or branched optionally substituted aliphatic carbon chain,

or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treatment and/or prevention of a hepatitis B virus (HBV) disease through inhibiting the interaction of CypA with HBV X protein (HBx) and/or Hepatitis B surface antigen (HBsAg), or for modulation of an interaction of HBx or HBsAg with CypA in a subject in need thereof.

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Figure 1A



2/5

Figure 1B

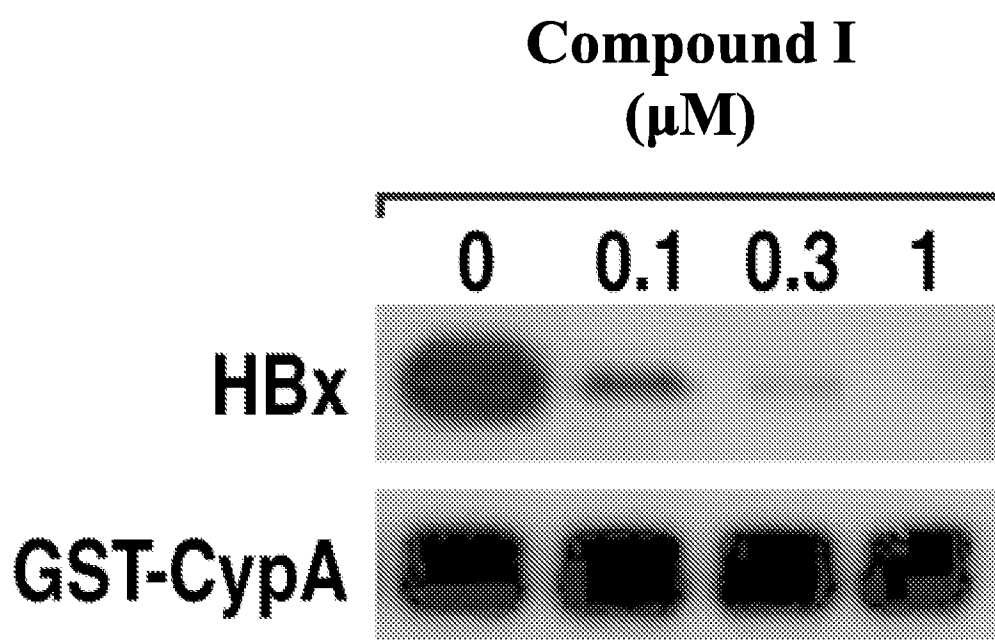


Figure 2A

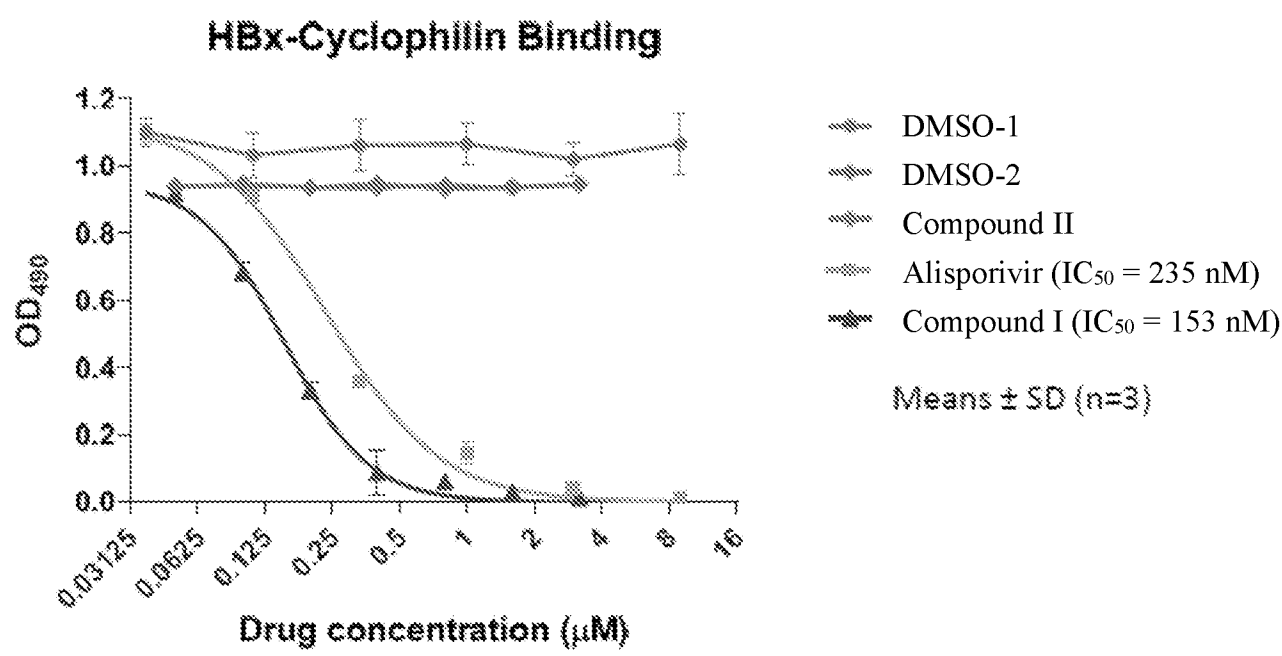
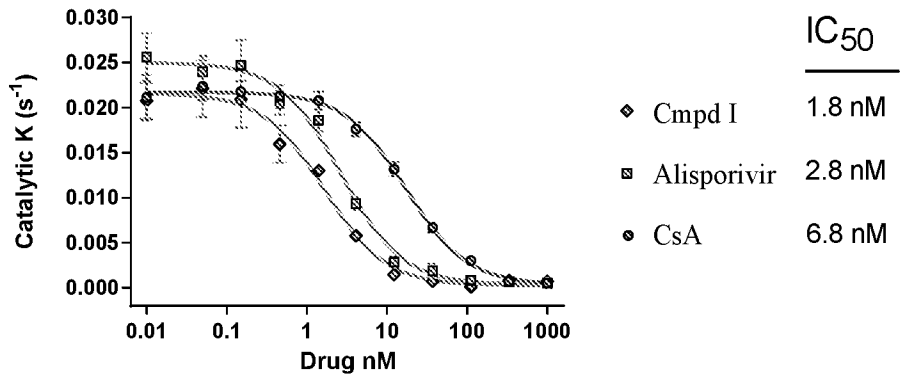


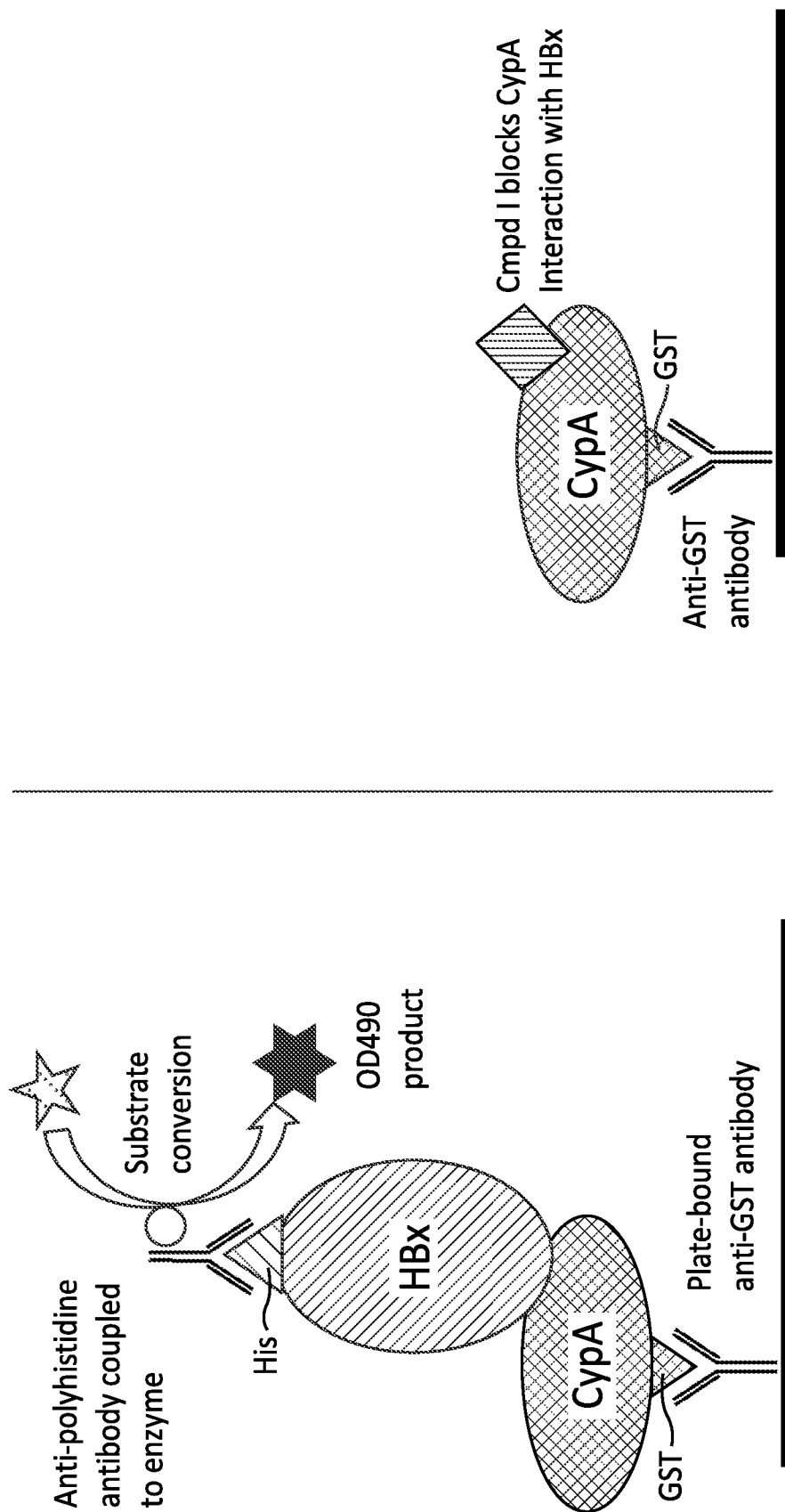
Figure 2B

Cyclophilin Isomerase Inhibition (D Ure)



5/5

Figure 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/065132

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/13 A61K31/675 A61P31/14
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

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

EPO-Internal, BIOSIS, INSPEC, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Robert Foster ET AL: "CRV431: An Optimized Cyclophilin Inhibitor with Multiple Anti-HBV Activities, High Selectivity Index, and Synergy with CMX157", AASLD-EASL HBV Treatment endpoints workshop: From discovery to regulatory approval, 9 September 2016 (2016-09-09), XP055460893, Retrieved from the Internet: URL: http://lzpkyu38mbmzo6c6947efbfe-wpengi ne.netdna-ssl.com/wp-content/uploads/2016/09/AASLD-Endpoints-Workshop-Aug-22-2016.pdf f [retrieved on 2018-03-20]	1-27
Y	the whole document ----- -/--	1-27



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

20 March 2018

Date of mailing of the international search report

28/03/2018

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax: (+31-70) 340-3016

Authorized officer

Orlando, Michele

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/065132

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	----- GALLAY P ET AL: "P0890 : Novel cyclophilin inhibitor CPI-431-32 shows broad spectrum antiviral activity by blocking replication of HCV, HBV and HIV-1 viruses", JOURNAL OF HEPATOLOGY, vol. 62, 2015, XP029162239, ISSN: 0168-8278, DOI: 10.1016/S0168-8278(15)31093-X abstract	1-27
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Y	----- WO 2012/079172 A1 (ISOTECHNIKA PHARMA INC [CA]; HEGMANS ALEXANDER [CA]; FENSKE BRUCE W [C] 21 June 2012 (2012-06-21) example 25	1-27
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/065132

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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T	Anonymous: "ContraVir's Cyclophilin Inhibitor CRV431 Potently Inhibits Essential Pathway in Hepatitis B", 8 December 2016 (2016-12-08), XP055460865, Retrieved from the Internet: URL:http://ir.contravir.com/news-releases/news-release-details/contravir-cyclophilin-inhibitor-crv431-potently-inhibits [retrieved on 2018-03-20] the whole document -----	1-27
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/065132

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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