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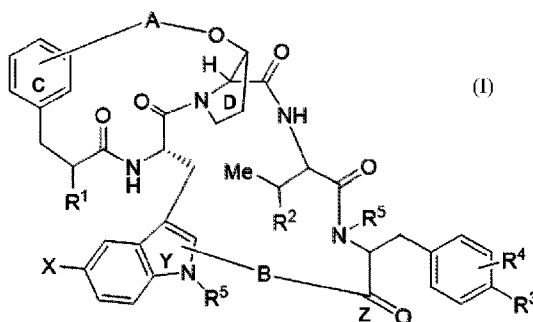
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(54) Title: PASSIVE PERMEABLE PCSK9 ANTAGONIST COMPOUNDS



(57) Abstract: Compounds of Formula (I), or a pharmaceutically acceptable salt thereof are disclosed (I), where A, B, R¹, R², R³, R⁴ and R⁵ are as defined herein. These compounds are antagonists of PCSK9 and have high passive (or intrinsic) permeability such that may be used without a permeability enhancing agent. Also described are pharmaceutical compositions comprising the compounds of Formula I or their salts, and methods of treating cardiovascular disease and conditions related to PCSK9 activity, e.g., atherosclerosis, hypercholesterolemia, coronary heart disease, metabolic syndrome, acute coronary syndrome, or related cardiovascular disease and cardiometabolic conditions.



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5 PASSIVE PERMEABLE PCSK9 ANTAGONIST COMPOUNDS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to U.S. Provisional Application No. 63/508,454 filed June 15, 2023, and U.S. Provisional Application No. 63/517,742 filed August 04, 2023. The contents of
10 each application are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0002] The invention relates to PCSK9 antagonist compounds that possess enhanced passive permeability.
15

BACKGROUND OF THE INVENTION

[0003] Reduction in LDL cholesterol levels have been directly related to the reduction in coronary events. Law et al., 2003 *BMJ* 326:1423-1427. Also, moderate lifelong reduction in plasma LDL cholesterol levels has been found to correlate with a substantial reduction in the
20 incidence of coronary events. Cohen et al., 2006 *N. Engl. J. Med.* 354:1264-1272. This was true even in populations with a high prevalence of non-lipid-related cardiovascular risk factors. *Supra*. Control of LDL cholesterol levels is therefore expected to of great benefit.

[0004] Proprotein convertase subtilisin-kexin type 9 (hereinafter called "PCSK9"), also known as neural apoptosis-regulated convertase 1 ("NARC-1"), is a proteinase K-like subtilase
25 identified as the 9th member of the secretory subtilase family. Seidah et al., 2003 *PNAS* 100:928-933. PCSK9 belongs to the mammalian proprotein convertase family of serine proteases and contains an N-terminal signal sequence, a prodomain, a catalytic domain, and a C-terminal domain. Seidah et al., 2012 *Nat. Rev. Drug Discov.* 11:367-383. PCSK9 is transcriptionally regulated by sterol regulatory element-binding proteins ("SREBP"), as found with other genes
30 involved in cholesterol metabolism (Maxwell et al., 2003 *J. Lipid Res.* 44:2109-2119) and as found with other genes implicated in lipoprotein metabolism (Dubuc et al., 2004 *Arterioscler. Thromb. Vasc. Biol.* 24:1454-1459). PCSK9 promoters possess two conserved sites involved in cholesterol regulation, a sterol regulatory element and an Sp1 site.

[0005] In the endoplasmic reticulum, PCSK9 catalyzes autocleavage between residues Gln-152
35 and Ser-153 (Naureckiene et al., 2003 *Arch. Biochem. Biophys.* 420:55-67; Seidah et al., 2003 *Proc. Natl. Acad. Sci. U.S.A.* 100:928-933). The prodomain remains tightly associated with the catalytic domain during subsequent trafficking through the trans-Golgi network. The maturation via autocleavage has been demonstrated to be critical for PCSK9 secretion and

5 subsequent extracellular function (see Benjannet et al., 2012 *J. Biol. Chem.* 287:33745-33755). Accordingly, several lines of evidence demonstrate that PCSK9, in particular, lowers the amount of hepatic LDLR protein and thus compromises the ability of the liver to remove LDL cholesterol from the circulation.

[0006] Adenovirus-mediated overexpression of PCSK9 in the liver of mice results in the
10 accumulation of circulating LDL-C due to a dramatic loss of hepatic LDLR protein, with no effect on LDLR mRNA levels (Benjannet et al., 2004 *J. Biol. Chem.* 279:48865-48875; Maxwell & Breslow, 2004 *PNAS* 101:7100-7105; Park et al., 2004 *J. Biol. Chem.* 279:50630-50638; and Lallanne et al., 2005 *J. Lipid Res.* 46:1312-1319). The effect of PCSK9 overexpression on raising circulating LDL-C levels in mice is completely dependent on the expression of LDLR, again,
15 indicating that the regulation of LDL-C by PCSK9 is mediated through downregulation of LDLR protein. In agreement with these findings, mice lacking PCSK9 or in which PCSK9 mRNA has been lowered by antisense oligonucleotide inhibitors have higher levels of hepatic LDLR protein and a greater ability to clear circulating LDL-C (Rashid et al., 2005 *PNAS* 102:5374-5379; and Graham et al., 2007 *J. Lipid Res.* 48(4):763-767). Additionally, lowering PCSK9 levels in
20 cultured human hepatocytes by siRNA was found to result in higher LDLR protein levels and an increased ability to take up LDL-C (Benjannet et al., 2004 *J. Biol. Chem.* 279:48865-48875; and Lallanne et al., 2005 *J. Lipid Res.* 46:1312-1319). Together, these data indicate that PCSK9 action leads to increased LDL-C by lowering LDLR protein levels.

[0007] Several mutations in the gene PCSK9 have been conclusively associated with autosomal
25 dominant hypercholesterolemia ("ADH"), an inherited metabolism disorder characterized by marked elevations of low-density lipoprotein ("LDL") particles in the plasma which can lead to premature cardiovascular failure (Abifadel et al., 2003 *Nature Genetics* 34:154-156; Timms et al., 2004 *Hum. Genet.* 114:349-353; and Leren, 2004 *Clin. Genet.* 65:419-422). A later-published study on the S127R mutation of Abifadel et al., supra, reported that patients carrying such a
30 mutation exhibited higher total cholesterol and apoB100 in the plasma attributed to (1) an overproduction of apoB100-containing lipoproteins, such as low density lipoprotein ("LDL"), very low density lipoprotein ("VLDL") and intermediate density lipoprotein ("IDL"), and (2) an associated reduction in clearance or conversion of said lipoproteins (Ouguerram et al., 2004 *Arterioscler. Thromb. Vasc. Biol.* 24:1448-1453).

[0008] Thus, PCSK9 plays a role in the regulation of LDL. The expression or upregulation of
35 PCSK9 is associated with increased plasma levels of LDL cholesterol, and the corresponding inhibition or lack of expression of PCSK9 is associated with reduced LDL cholesterol plasma levels. Decreased levels of LDL cholesterol associated with sequence variations in PCSK9 have

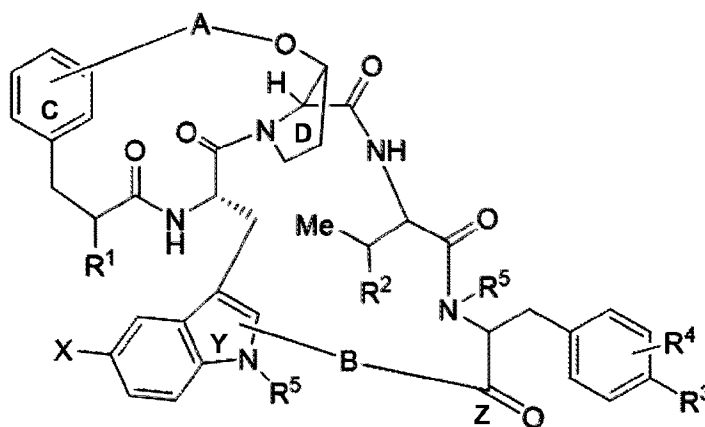
5 been found to confer protection against coronary heart disease (Cohen, 2006 *N. Engl. J. Med.* 354:1264-1272).

[0009] Thus, identification of compounds and/or agents that antagonize PCSK9's LDL regulating property. However, in general, because PCSK9 circulates in blood and has modest binding affinity to cell surface LDL receptors, attempts to utilize this mechanism in treatment of diseases related to high serum LDL levels have largely been focused on the use of large biomolecules, for example, antibodies. Cyclic peptides and related compounds that inhibit PCSK9's LDL regulating property have been disclosed, including WO 2019/246349, WO 2019/246387, WO 2019/246352, and Zhang et al., 2014 *J. Biol. Chemistry*, 289(2): 942-955.

[0010] There is a need for more effective small molecule PCSK9 antagonists.

SUMMARY OF THE INVENTION

[0011] The invention provides compounds (hereinafter referred to as compounds of the invention) which have been found to be antagonists of PCSK9 with enhanced intrinsic permeability. The compounds of the invention have a structure in accordance with the structural Formula (I):



(I),

or a pharmaceutically acceptable salt thereof, wherein ring A, B, R¹, R², R³, R⁴ and R⁵ are as defined below.

[0012] In another aspect, the invention provides pharmaceutical compositions comprising at least one compound of the invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. Such compositions according to the invention may optionally further include one or more additional therapeutic agents as described herein.

5 **[0013]** In another aspect, the invention provides a method for treating hypercholesterolemia. The method comprises administering to a patient in need thereof a therapeutically effective amount of one of the above compositions. These and other aspects and embodiments of the invention are described more fully below.

10 **DETAILED DESCRIPTION OF THE INVENTION**

15 **[0014]** In the description that follows, conventional structural representation is employed and includes conventional stereochemical notation for certain asymmetric carbon centers. Thus, structural representation of compounds of the invention includes conventional stereochemical notation for some asymmetric carbon centers shown in the example compounds. Accordingly, in such instances, solid black “wedge” bonds represent bonds projecting from the plane of the reproduction medium, “hashed wedge” bonds representing descending bonds into the plane of the reproduction medium, and a “wavy” line appended to a carbon bearing a double bond indicates both possible cis and trans orientations are included. As is conventional, plain solid lines represent all spatial configurations for the depicted bonding. Accordingly, where no specific stereochemical notation is supplied, the representation contemplates all stereochemical and spatial orientations of the structural features.

25 **[0015]** As is shown in the examples of the invention, and mentioned above, particular asymmetric carbon centers are structurally represented using conventional “Solid Wedge” and “Hash Wedge” bonding representation. Absolute configuration has not been determined for the example compounds but has been assigned by analogy to specific example compounds of known stereochemical configurations (determined by X-ray crystallography) prepared using the same or analogous reaction conditions and starting reagents and isolated under the same chromatographic conditions. Accordingly, specific assignment of the configurations structurally represented herein is meant to identify the specific compounds prepared as having an excess of one stereoisomer and is not put forth herein necessarily as being a statement of the absolute determination of the stereochemical structure of said compound unless otherwise noted in the data presented.

35 **[0016]** It will be appreciated that where isomeric mixtures are obtained, the preparation of individual stereoisomers in significant percentages of enantiomeric excess can be carried out, if desired, by separation of the mixture using customary methods, for example by chromatography or crystallization, or by the use of stereochemically uniform starting materials for the synthesis described, or by stereoselective synthesis. Optionally a derivatization can be carried out before a separation of stereoisomers. The separation of a mixture of stereoisomers can be carried out at an

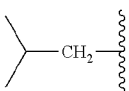
5 intermediate step during the synthesis of a compound of Formula I or it can be done on a final racemic product.

[0017] Where indicated herein, absolute stereochemistry is determined by X-ray crystallography of crystalline products or crystalline intermediates which are derivatized, if necessary, with a reagent containing a stereogenic center of known configuration. Unless a particular isomer, salt, solvate (including hydrates) or solvated salt of such racemate, enantiomer,
10 or diastereomer is indicated, the invention includes all such isomers, as well as salts, solvates (including hydrates) and solvated salts of such racemates, enantiomers, diastereomers, and mixtures thereof.

[0018] The invention also embraces isotopically-labeled compounds which are structurally identical to those recited herein, but for the fact that a statistically significant percentage of one or more atoms in that form of the compound are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number of the most abundant isotope usually found in nature, thus altering the naturally occurring abundance of that isotope present in a compound of the invention. The invention includes all suitable isotopic variations of the
15 compounds of Formula I.

[0019] Examples of isotopes that can be preferentially incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, iodine, fluorine and chlorine, for example, but not limited to ^2H , ^3H , ^{11}C , ^{13}C , ^{14}C , ^{13}N , ^{15}N , ^{15}O , ^{17}O , ^{18}O , ^{31}P , ^{32}P , ^{35}S , ^{18}F , ^{36}Cl , ^{123}I , and ^{125}I . It will be appreciated that other isotopes may be incorporated by
25 known means also. In particular, certain isotopically labeled compounds of the invention (e.g., those labeled with ^3H , ^{11}C , and ^{14}C) are recognized as being particularly useful in compound and/or substrate tissue distribution assays using a variety of known techniques. Additionally, compounds of the invention contemplate isotopic substitution with different isotopic forms of hydrogen (H), including protium (^1H) and deuterium (^2H or D). Protium is the predominant
30 hydrogen isotope found in nature. Enriching for deuterium may afford certain therapeutic advantages, such as increasing in vivo half-life or reducing dosage requirements, or may provide a compound useful as a standard for characterization of biological samples. Isotopically enriched compounds within Formula I can be prepared without undue experimentation by conventional techniques well known to those skilled in the art or by processes analogous to those described in
35 the Schemes and Examples herein using appropriate isotopically enriched reagents and/or intermediates.

[0020] Where a wavy line terminates a conventional bond (as opposed to connecting two atoms within a structure) it indicates a point of bonding to a structure, e.g.:

5 [0021]  indicates that a secondary-butyl moiety is bonded via the methylene group through the bond terminating with the wavy line. Where an alphabetical notation is used to depict a substituent moiety, a dash is employed to indicate the point of bonding to the indicated substrate, e.g.: —CH₂—C(O)—CH₂Cl indicates the acetyl chloride moiety is bonded via the methylene portion of the moiety.

10 [0022] Unless otherwise noted, when describing a linker throughout this disclosure, the first available bond on a linker group connects to the portion of the compound flanking the linker group to the left and the terminal available bond of the linker group connects to the terminal portion of the compound flanking the linker group to the right. For example, if the linker (L) is —CH₂—CF₂—, A and B are portions of the compound flanking the linker, respectively, on the left and
15 on the right, the definition of L includes only A—CH₂—CF₂— B, not A—CF₂—CH₂—B.

[0023] When any variable (e.g., n, R⁵, etc.) occurs more than one time in any constituent or in Formula I, its definition on each occurrence is independent of its definition at every other occurrence unless otherwise specified at the point of definition. One of ordinary skill in the art will recognize that choice of combinations of the various substituents defined in a structural
20 representation, i.e., R¹, R², etc., are to be chosen in conformity with well-known principles of chemical structure connectivity and stability, and combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

[0024] A “stable” compound is a compound which can be prepared and isolated and whose structure and properties remain or can be caused to remain essentially unchanged for a period of
25 time sufficient to allow use of the compound for the purposes described herein (e.g., therapeutic administration to a subject). The compounds of the invention are limited to stable compounds embraced by Formula I.

[0025] Where any variable or moiety is expressed in the form of a range, e.g. (—CH₂—)₁₋₄, both of the extremes of the specified range are included (i.e., 1 and 4 in the example) as well as
30 all of the whole number values in between (i.e., 2 and 3 in the example). As another example, where it is stated that m is an integer between 0 and 4, the range is inclusive of the numbers 0 and 4 and includes 0, 1, 2, 3, and 4.

[0026] The term “halogen” includes fluorine, chlorine, bromine and iodine unless specified otherwise at the point of use.

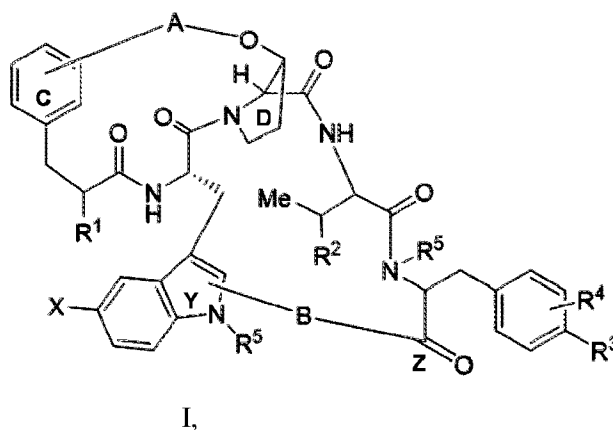
35 [0027] As the term is used herein, “subjects” (alternatively “patients”) refers to an animal, preferably a mammal, and in particular a human or a non-human animal including livestock

- 5 animals and domestic animals including, but not limited to, cattle, horses, sheep, swine, goats, rabbits, cats, dogs, and other mammals in need of treatment. In some embodiments the subject is preferably a human. As used herein, the term “administration” and variants thereof (e.g., “administering” a compound) in reference to a compound of Formula I means providing the compound, or a pharmaceutically acceptable salt thereof, to a subject in need of treatment.

10 **Embodiments**

[0028] Embodiments of the invention are as described below.

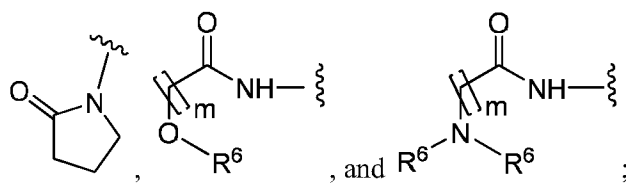
[0029] In embodiment 1, the invention provides a compound of Formula I



- 15 or a pharmaceutically acceptable salt thereof,
wherein

X is H, F, Cl, or Br;

R¹ is selected from the group consisting of -NR⁶CO(C₁-C₄)alkyl, pyrimidinyl-CONR⁵-,



- 20 R² is -OH, (C₁-C₄)alkyl or -O(C₁-C₄)alkyl;

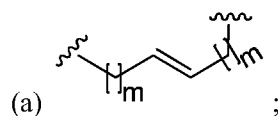
R³ is -O(C₁-C₄)alkyl, optionally substituted with one to six F;

R⁴ is H or -(C₁-C₄)alkyl;

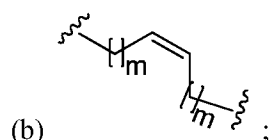
R⁵, at each occurrence, is independently selected from H, -(C₁-C₄)alkyl, and a bond;

R⁶, at each occurrence, is independently selected from H and -(C₁-C₄)alkyl;

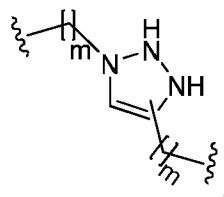
- 25 —A— is a linker selected from the group consisting of



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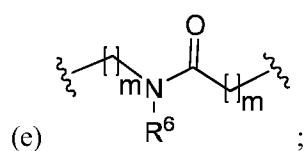


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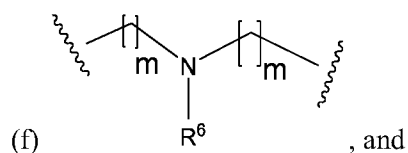


(c)

(d) $-(\text{CH}_2)_p-$;



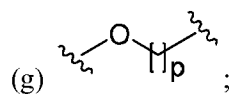
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(f)

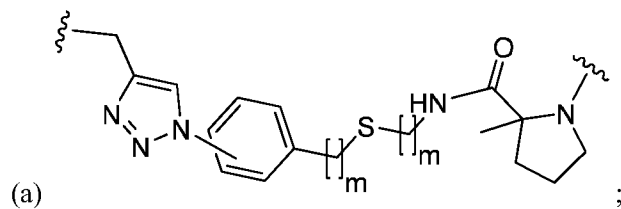
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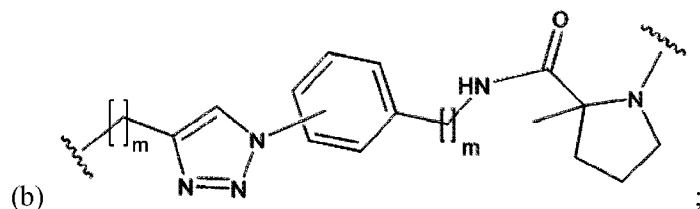


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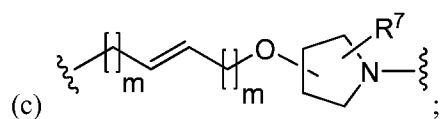
—B— is a linker selected from the group consisting of



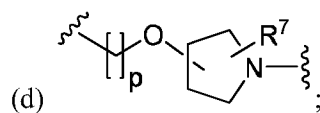
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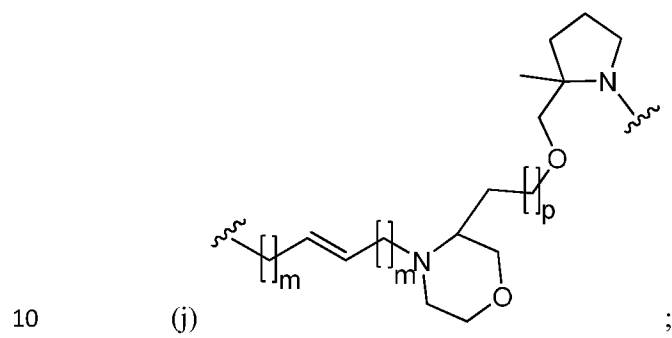
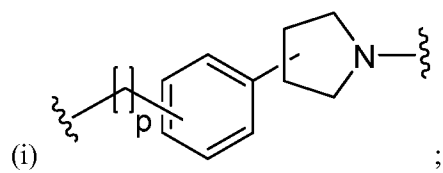
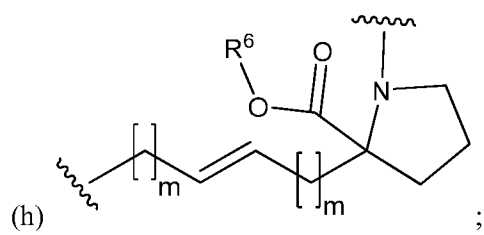
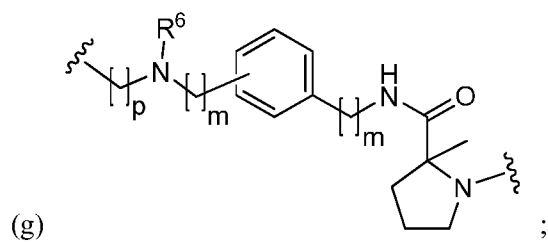
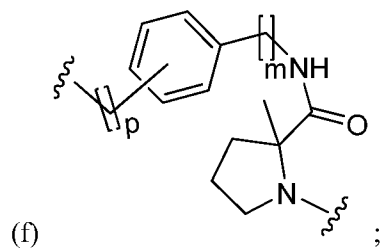
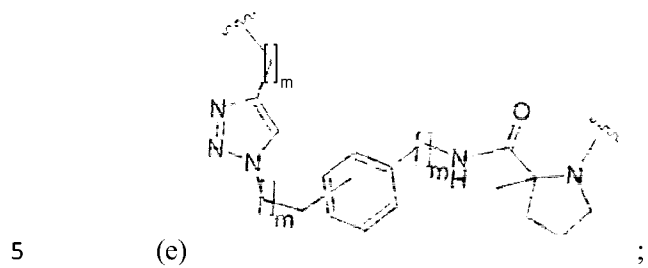


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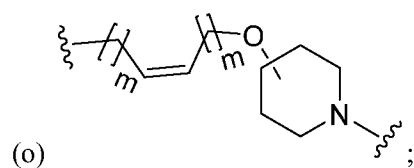
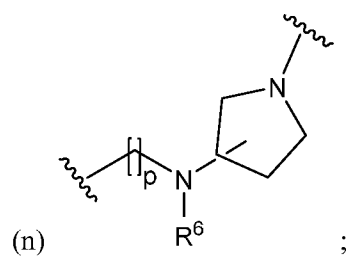
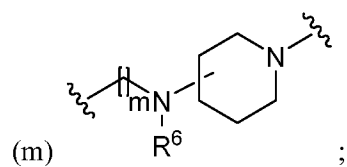
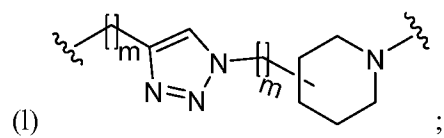
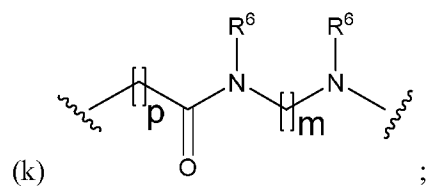


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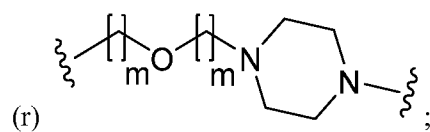
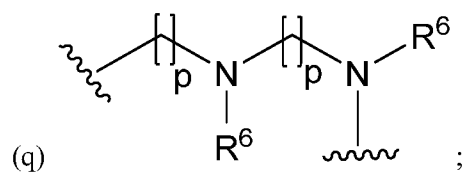
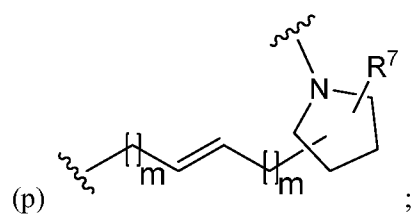
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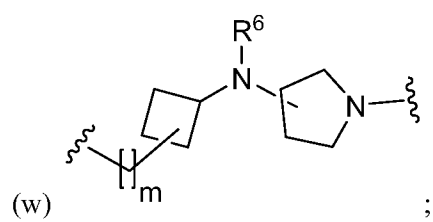
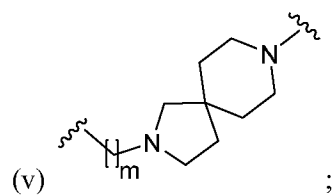
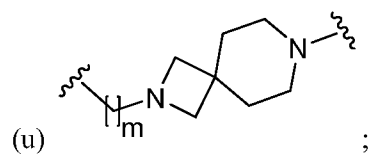
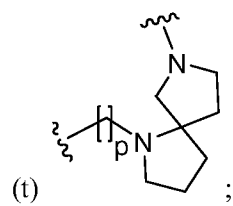
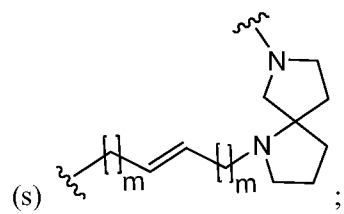
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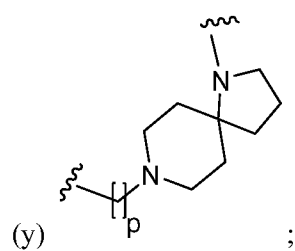
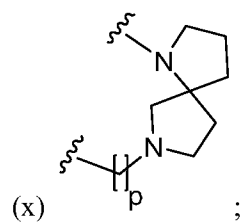
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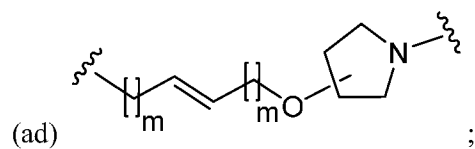
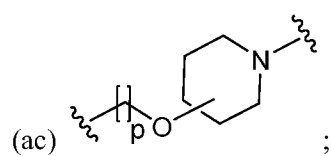
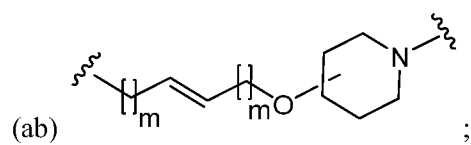
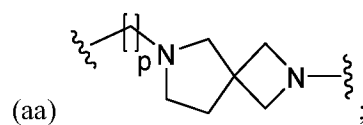
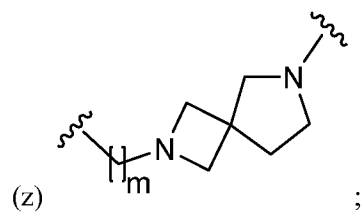
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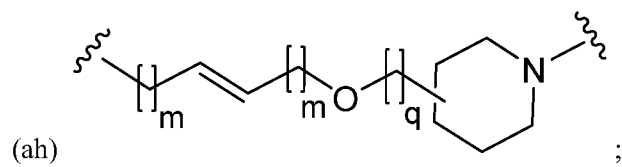
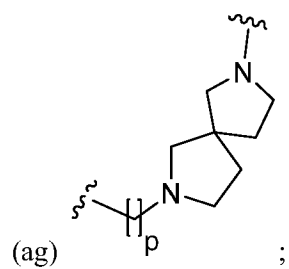
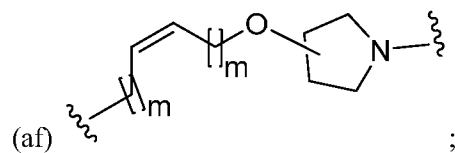
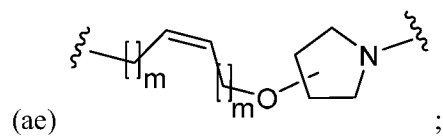
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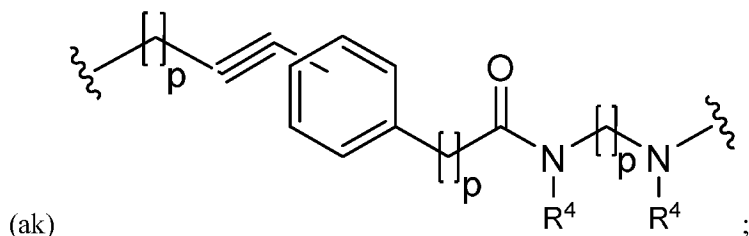
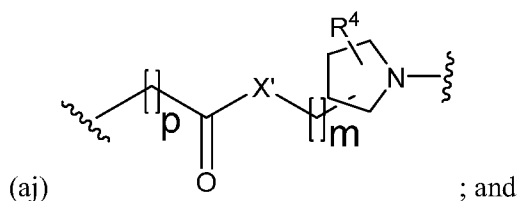
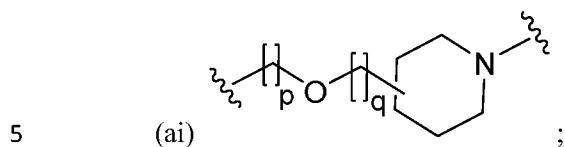


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wherein when B is connected to the nitrogen of the indole ring Y, R^5 is a bond;

n is an integer between 0 and 2;

10 m is an integer between 0 and 4;

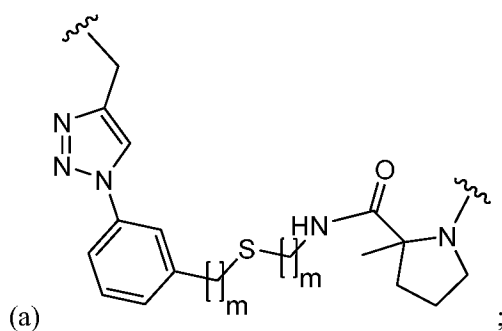
q is an integer between 1 and 4;

p is an integer between 1 and 5;

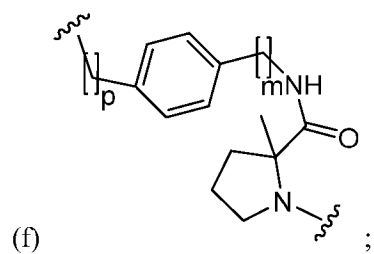
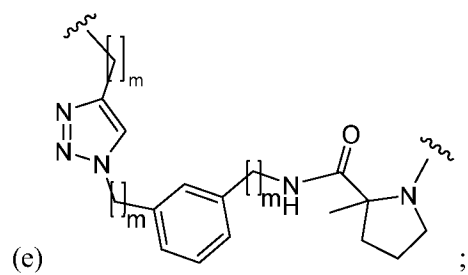
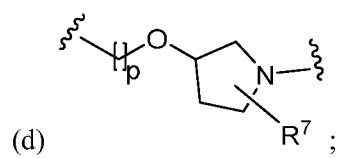
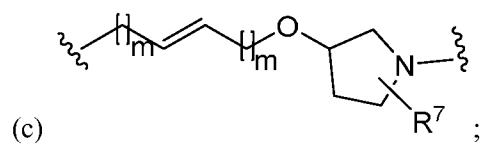
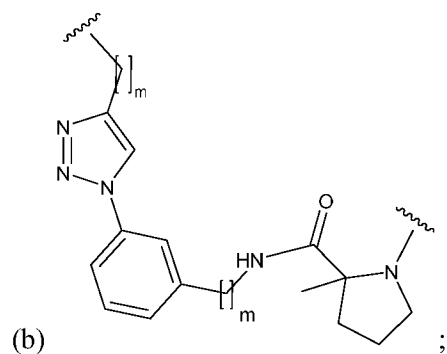
R^7 is H or $-\text{CON}(\text{R}^6)_2$; and

X' is N or O, wherein the N is optionally substituted with $-(\text{C}_1\text{-C}_4)\text{alkyl}$.

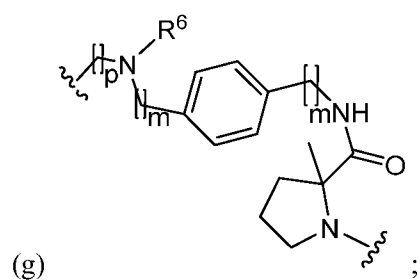
15 **[0030]** In embodiment 2, the invention provides the compound of embodiment 1, wherein the linker B is selected from the group consisting of:



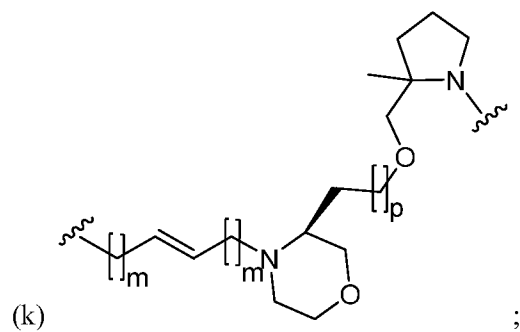
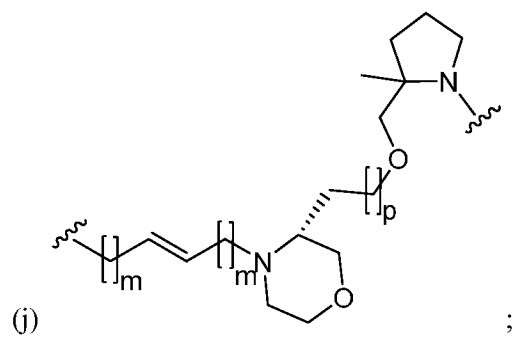
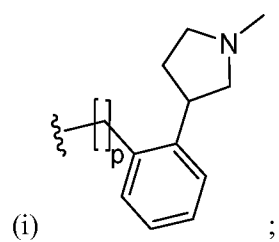
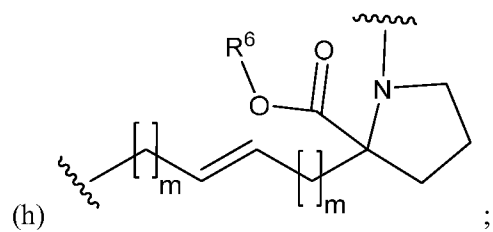
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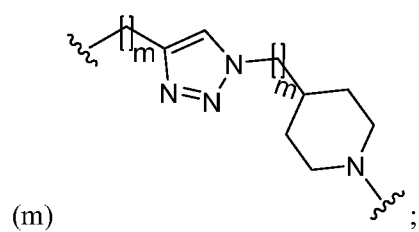
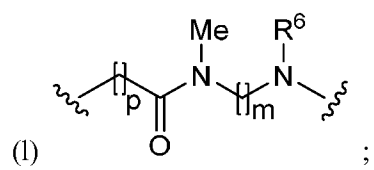
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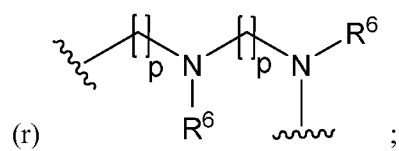
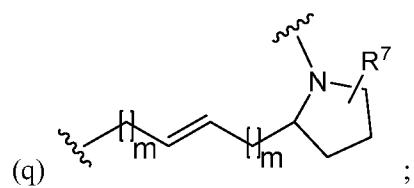
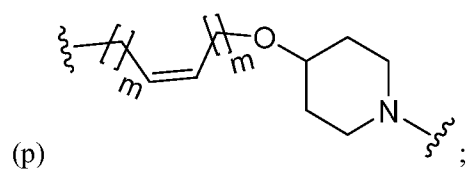
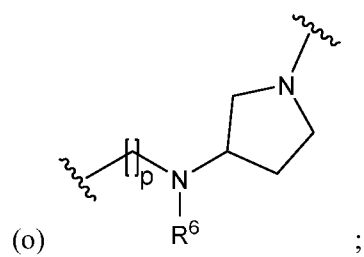
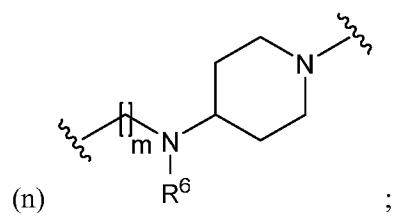
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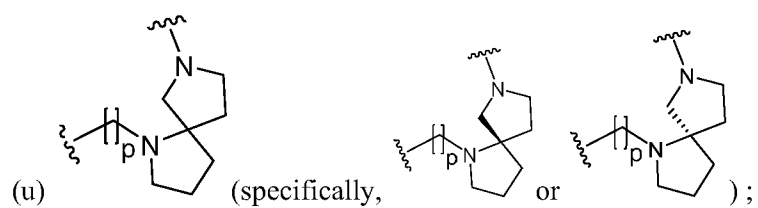
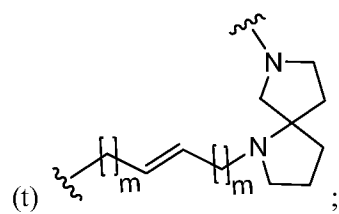
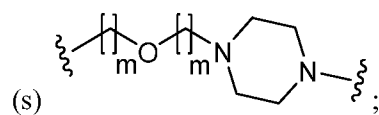
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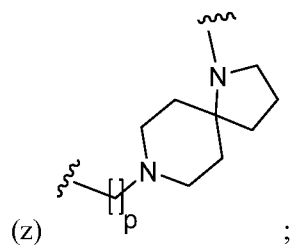
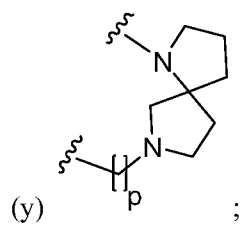
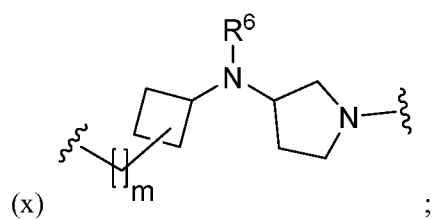
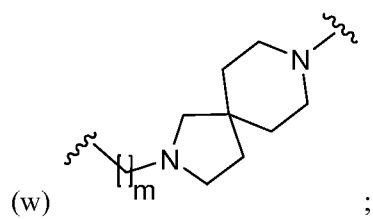
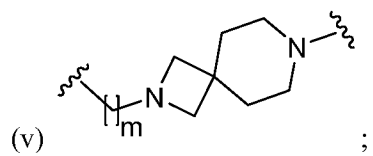
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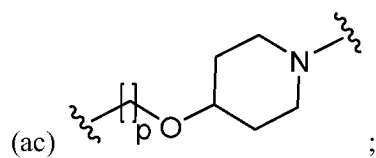
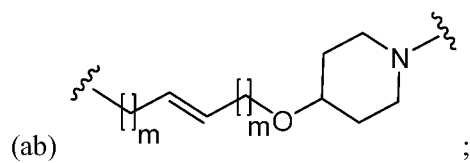
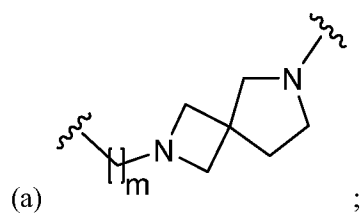
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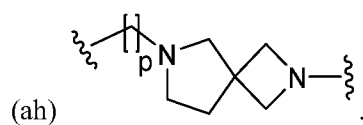
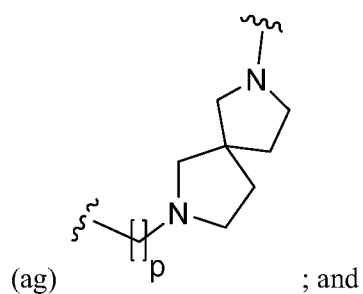
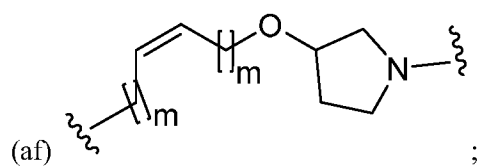
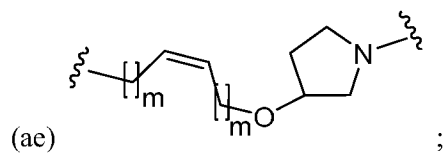
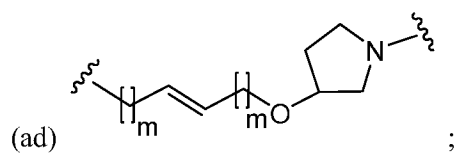
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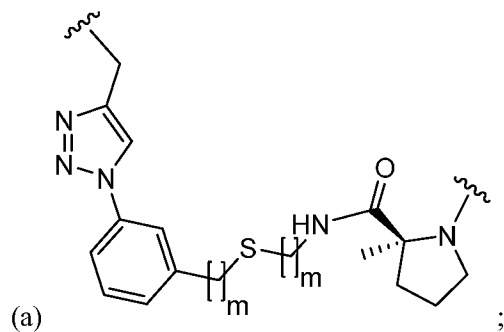
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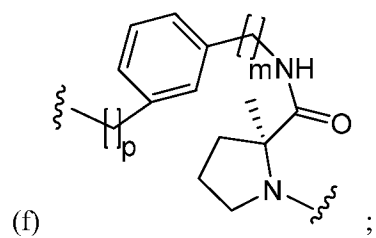
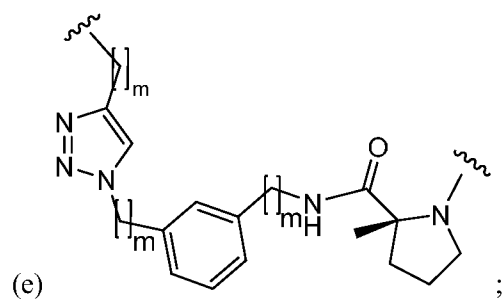
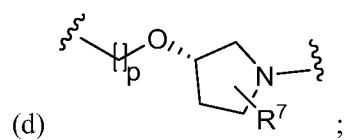
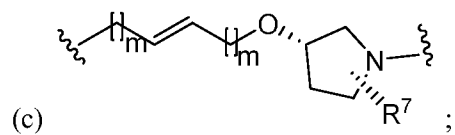
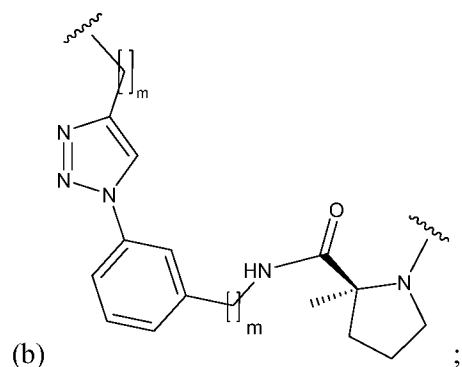
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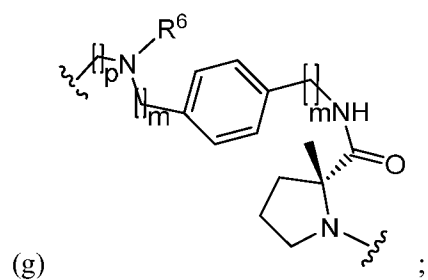
- 10 [0031] In embodiment 3, the invention provides the compound of any of embodiments 1 or 2, wherein the linker B is selected from the group consisting of:



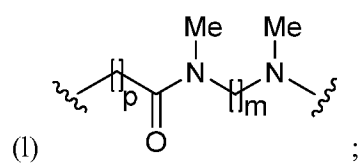
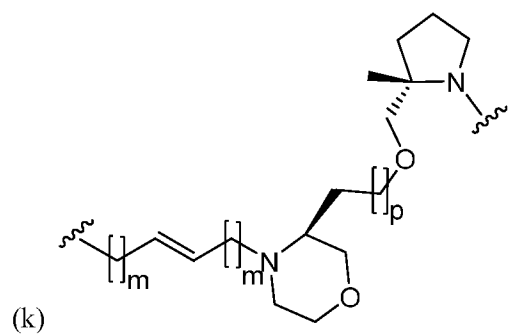
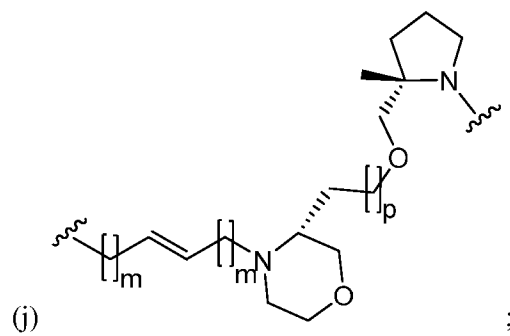
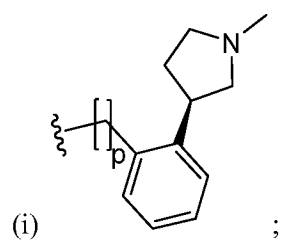
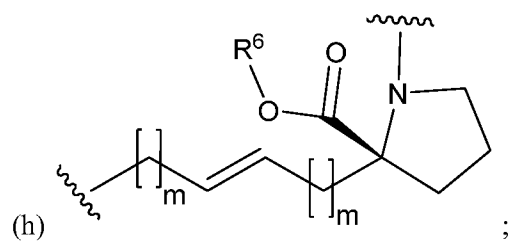
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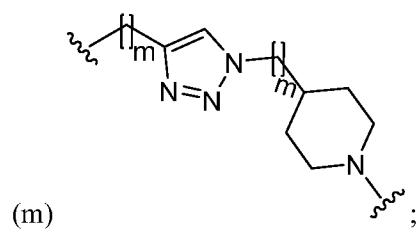
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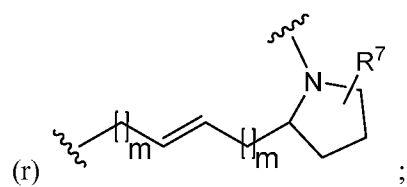
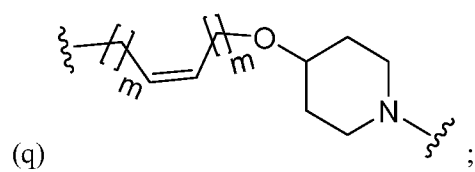
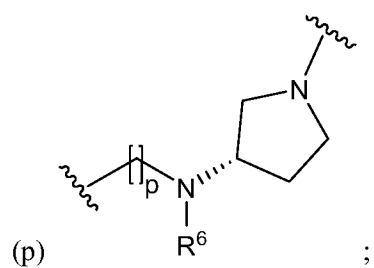
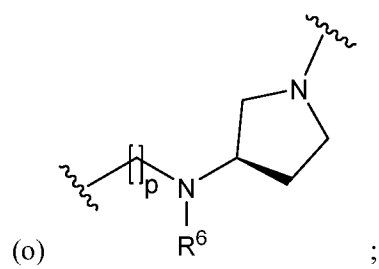
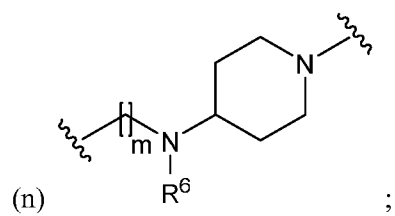
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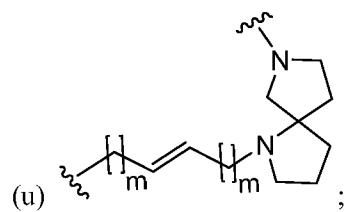
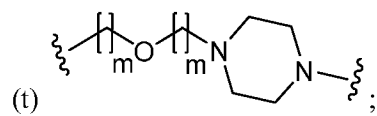
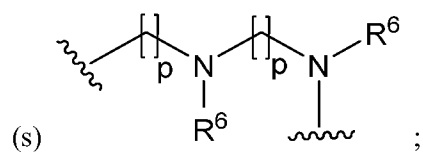
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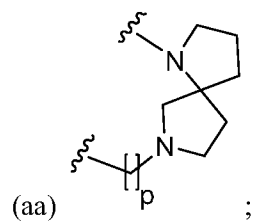
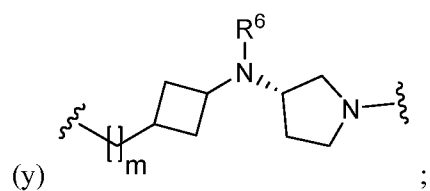
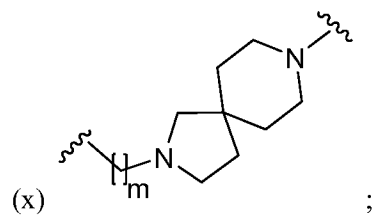
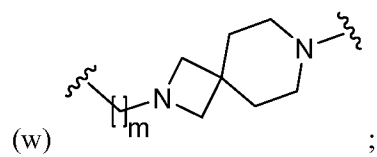
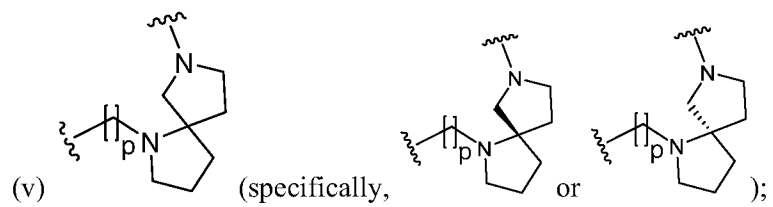
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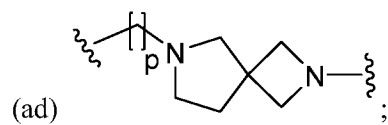
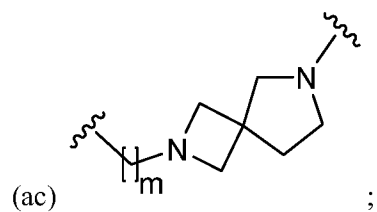
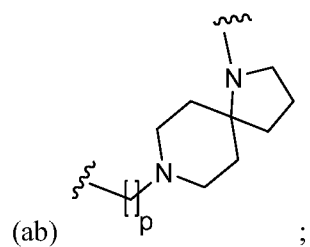
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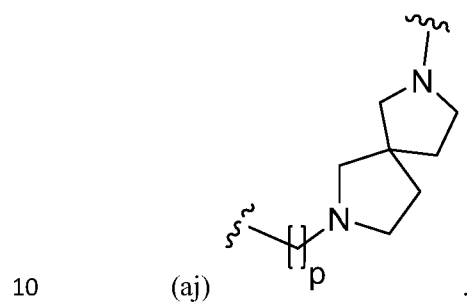
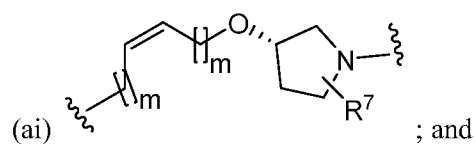
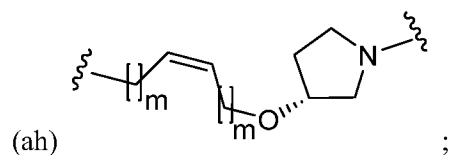
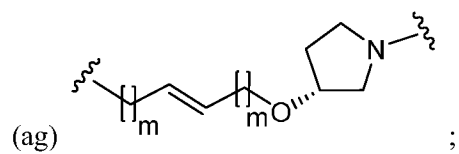
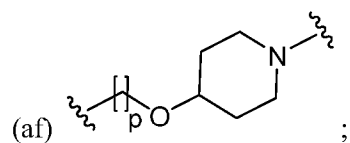
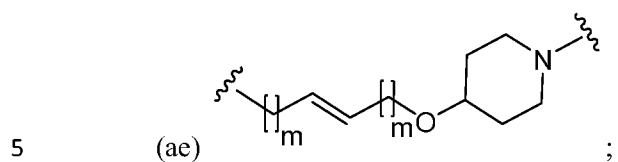


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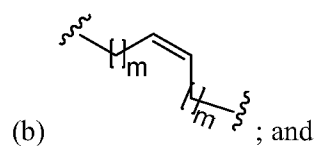
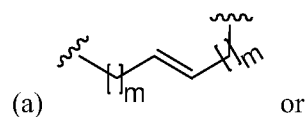


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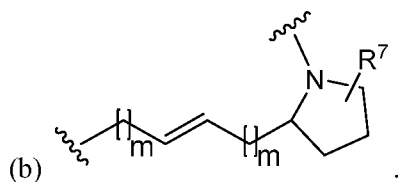
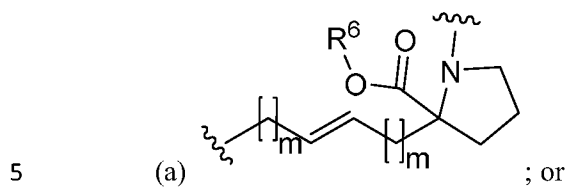




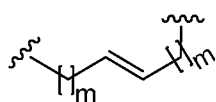
[0032] In embodiment 4, the invention provides the compound of the embodiment 1, wherein A is



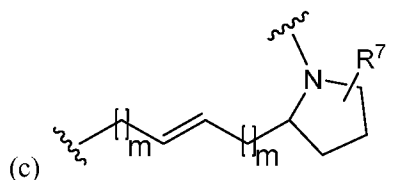
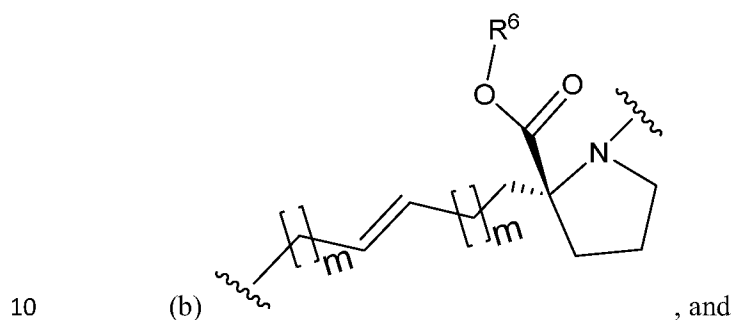
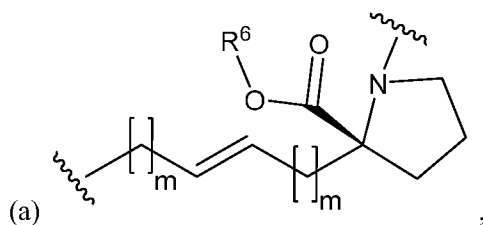
15 B is



[0033] In embodiment 5, the invention provides the compound of embodiment 4, wherein A is



and B is selected from the group consisting of



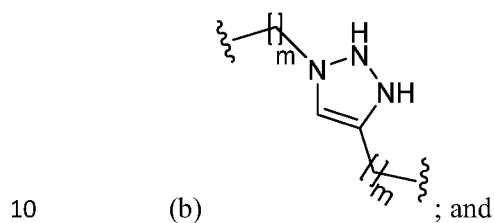
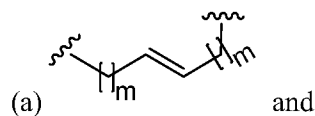
[0034] In embodiment 6, the invention provides the compound of embodiment 5, wherein at least one of the two R^5 groups is $-(C_1-C_4)\text{alkyl}$.

15 [0035] In embodiment 7, the invention provides the compound of embodiment 5, wherein both R^5 groups are $-(C_1-C_4)\text{alkyl}$.

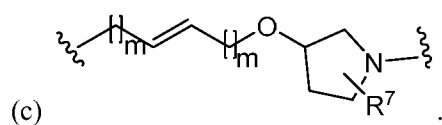
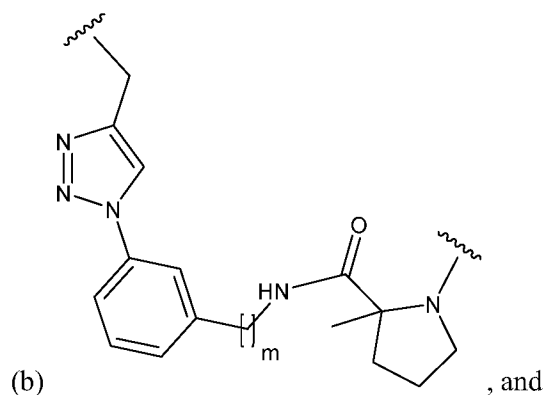
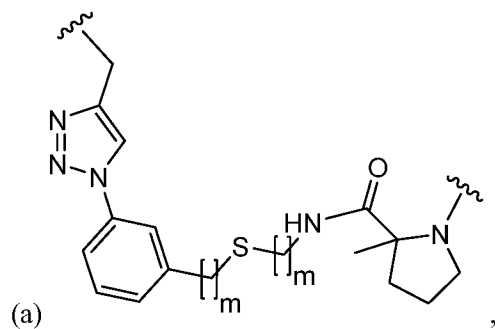
[0036] In embodiment 8, the invention provides the compound of any one of embodiments 6 or 7, wherein at least one occurrence of R^5 is $-\text{CH}_3$.

5 [0037] In embodiment 9, the invention provides the compound of any one of embodiments 6 or 7, wherein each occurrence of R^5 and R^6 is $-CH_3$, and R^7 is H.

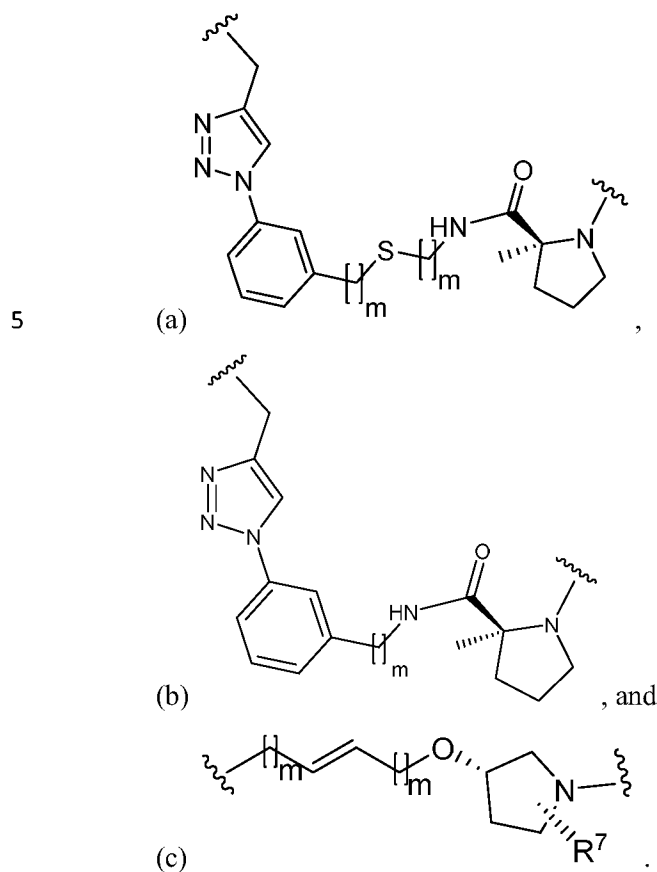
[0038] In embodiment 10, the invention provides the compound of embodiment 1, wherein A is selected from the group consisting of



B is selected from the group consisting of

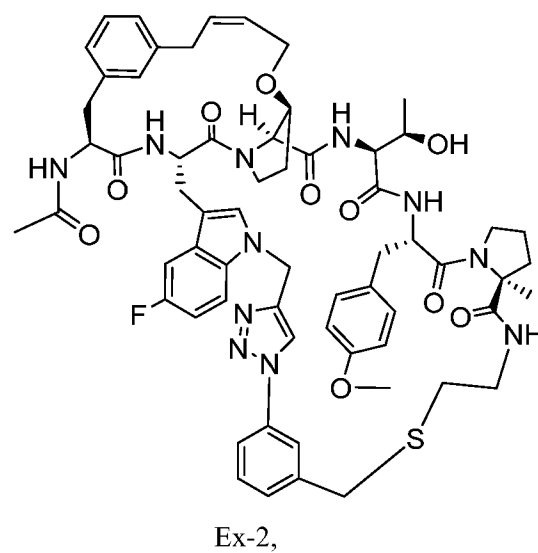
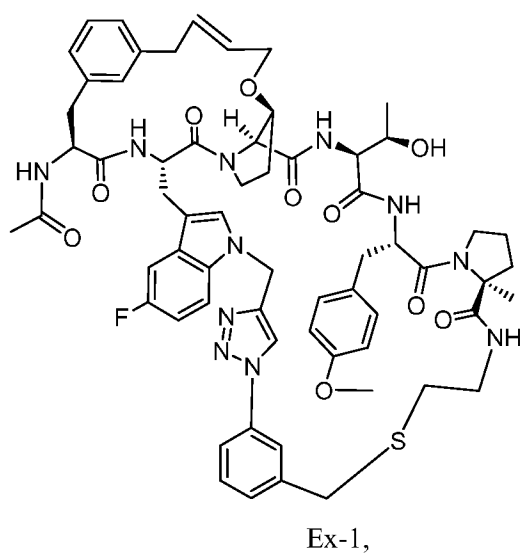


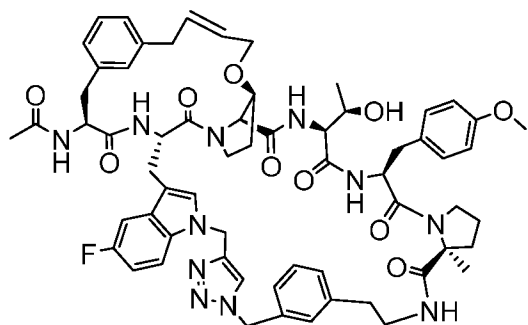
15 [0039] In embodiment 11, the invention provides the compound of embodiment 10, wherein B is selected from the group consisting of



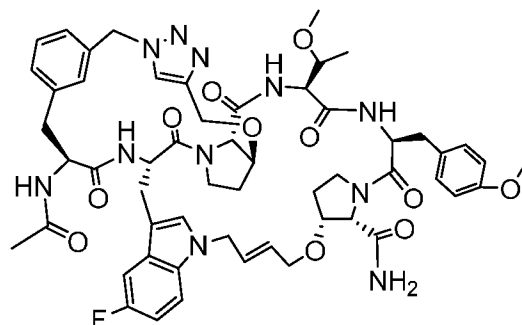
[0040] In embodiment 12, the invention provides the compound of any one of embodiments 1-11, wherein X is F.

10 [0041] In embodiment 13, the invention provides a compound of embodiment 1 selected from the group consisting of:

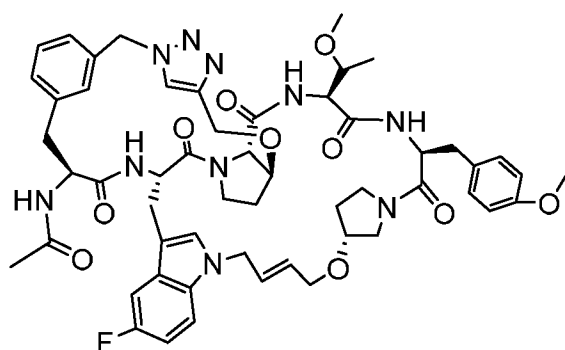




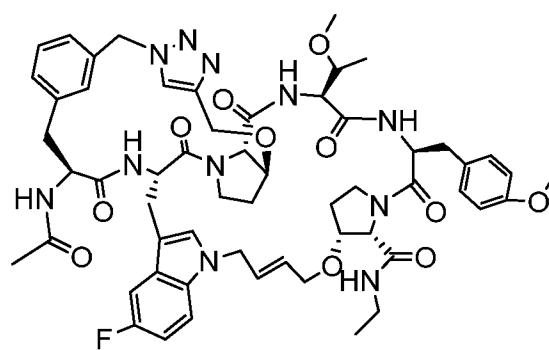
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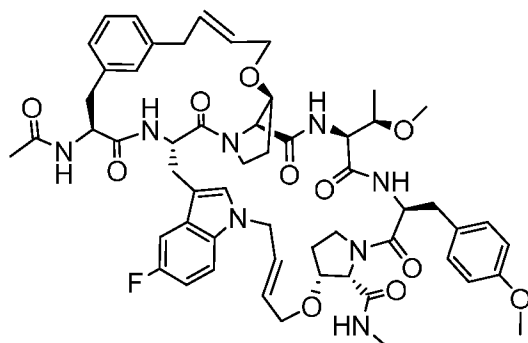
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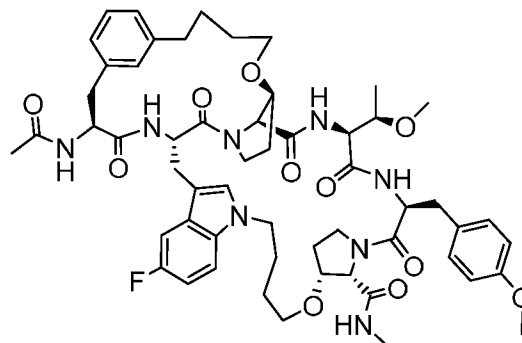
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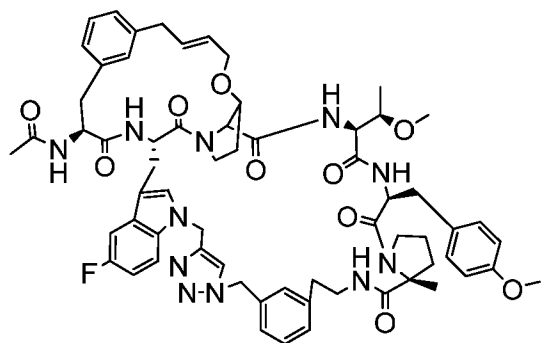
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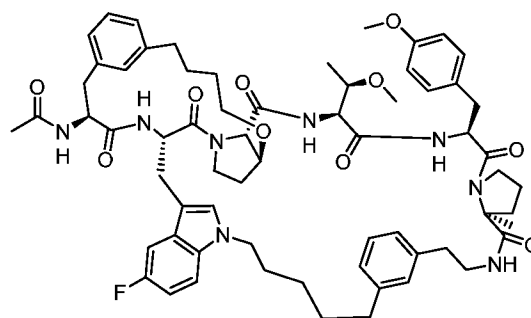
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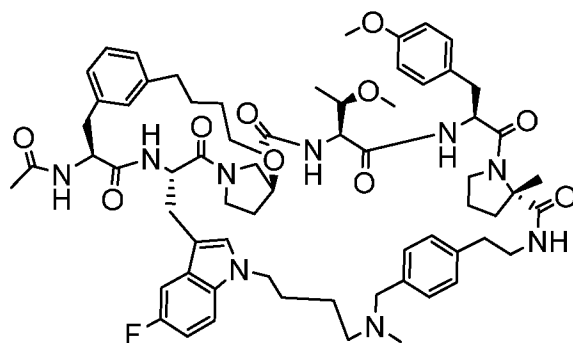
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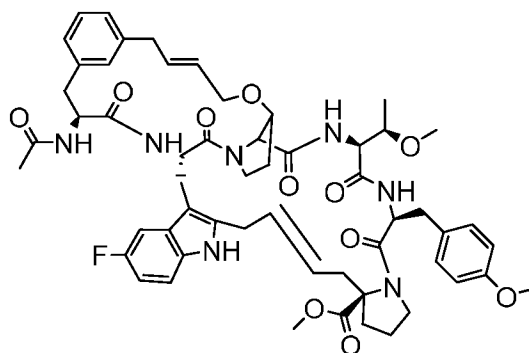
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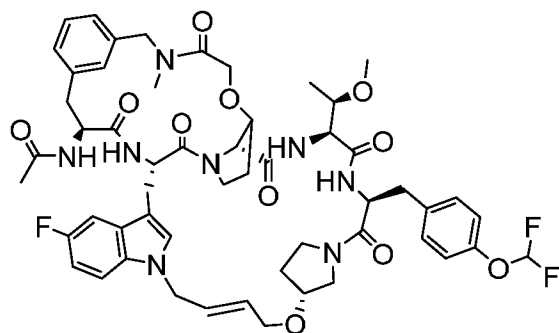
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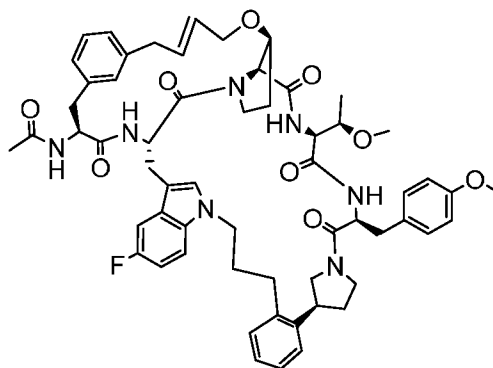
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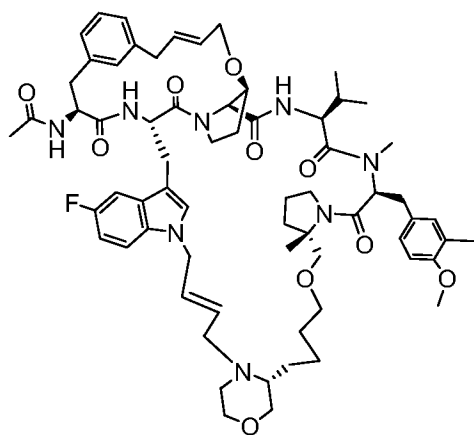
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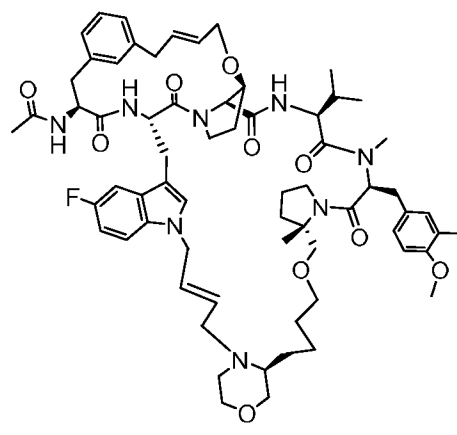
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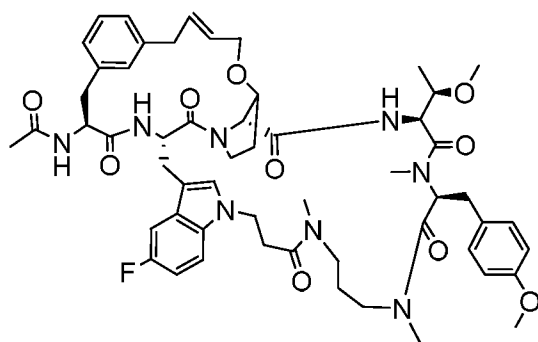
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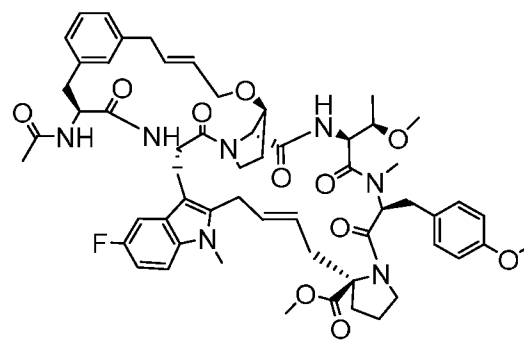
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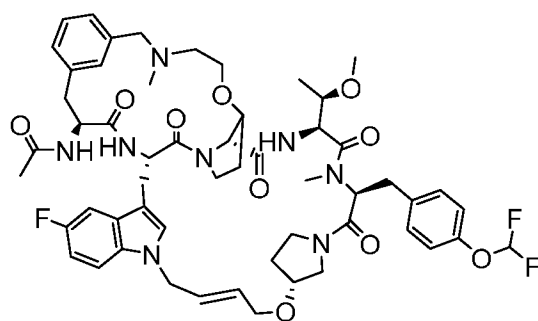
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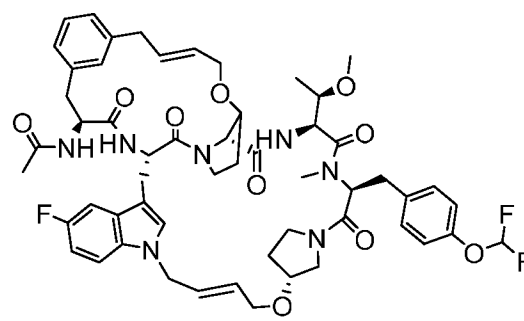
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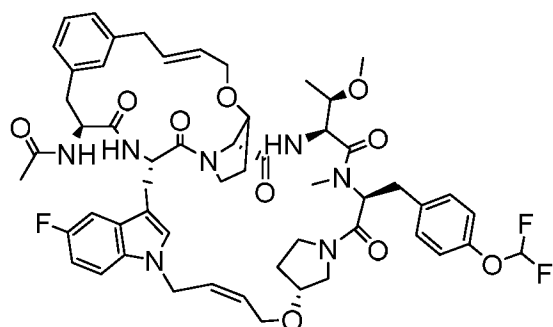
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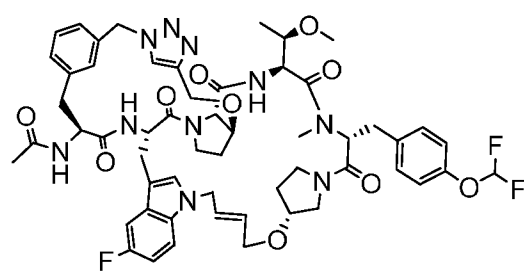
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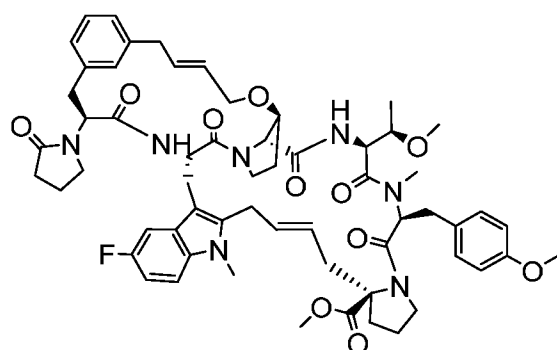
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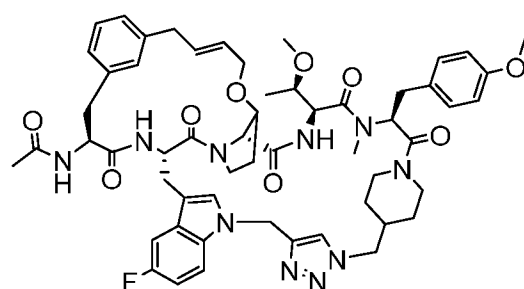
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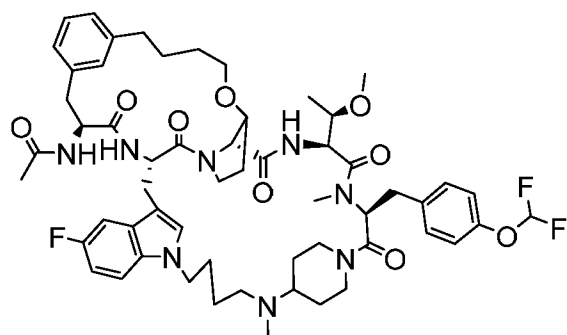
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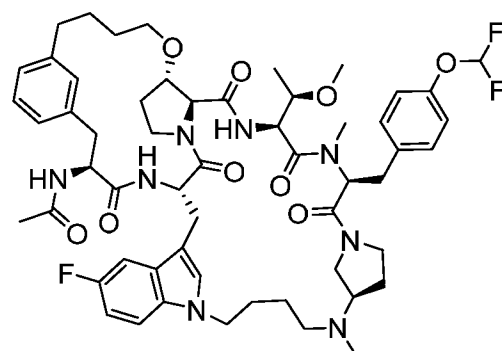
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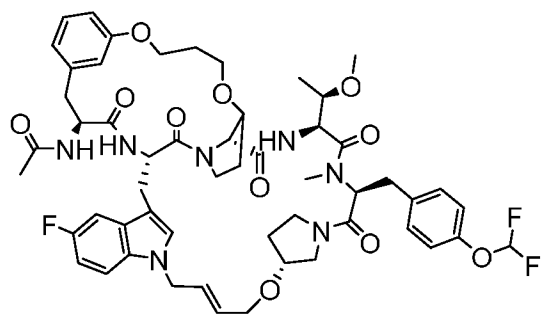
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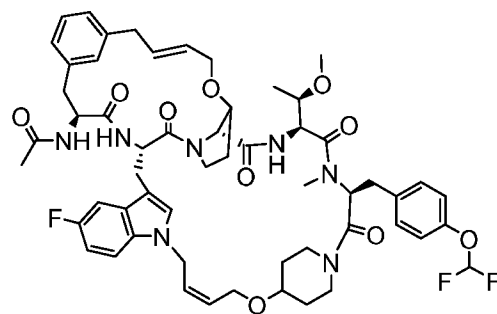
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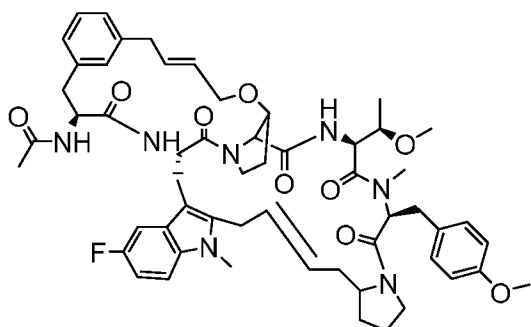
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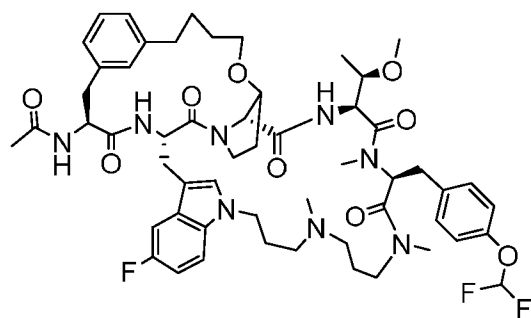
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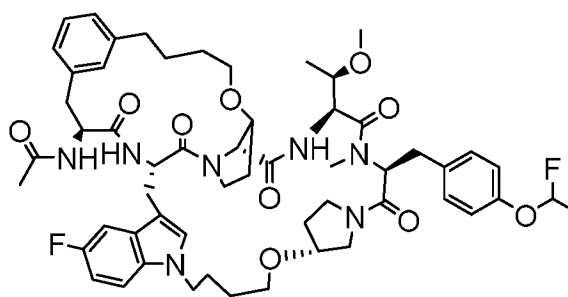
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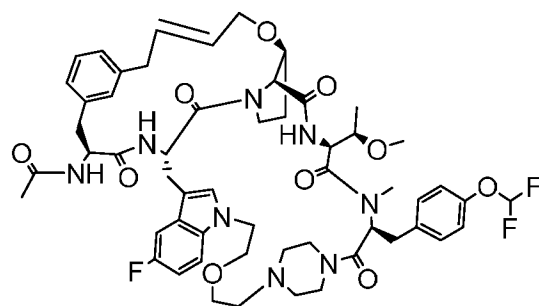
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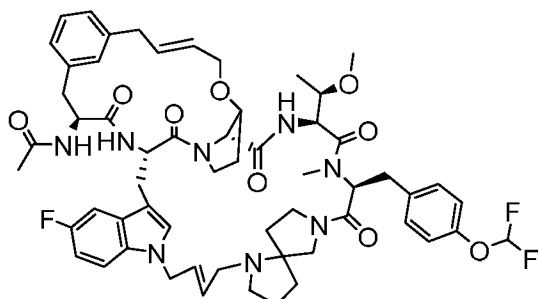
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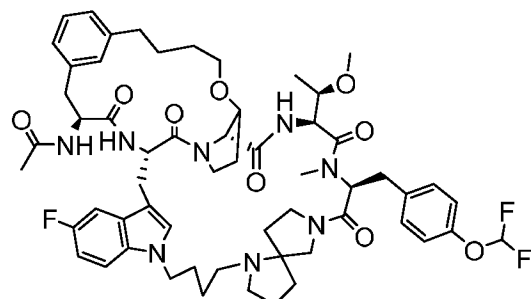
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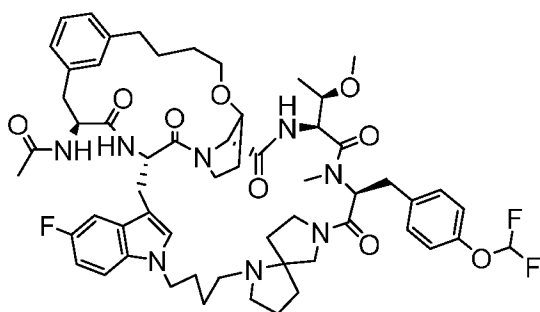
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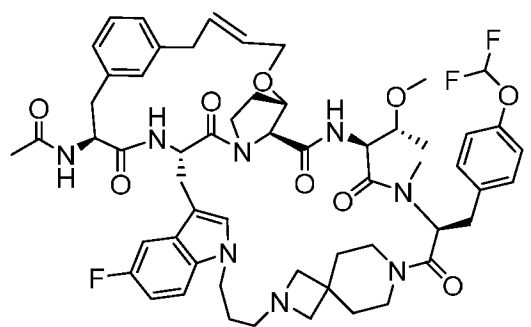
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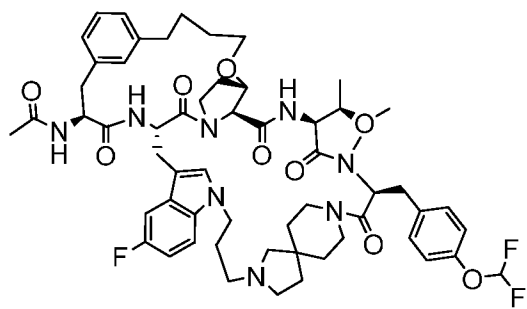
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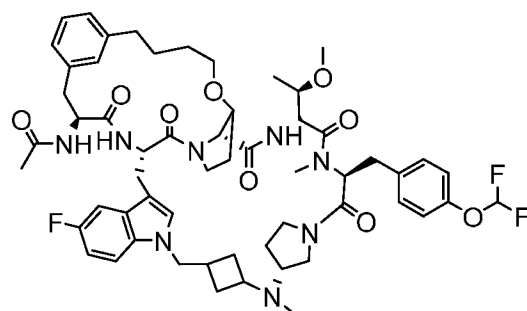
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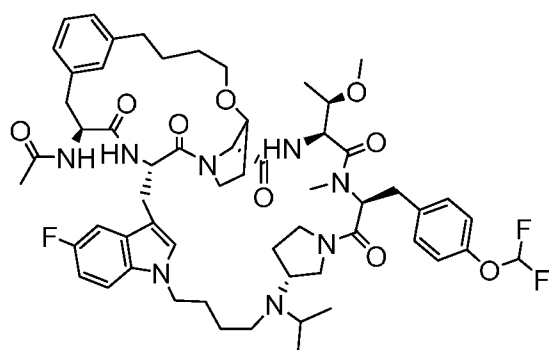
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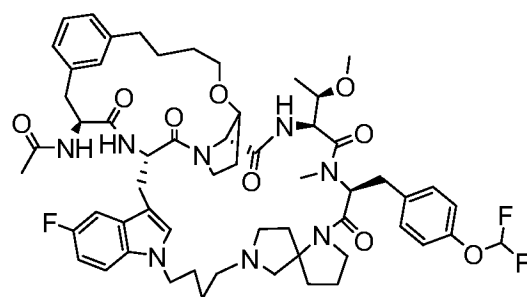
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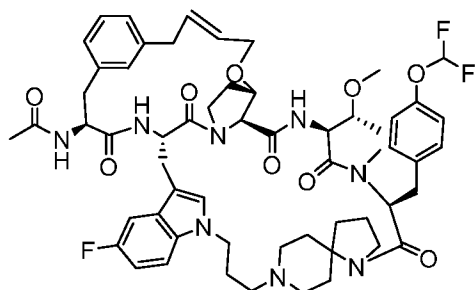
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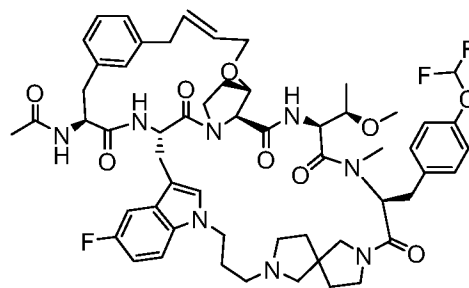
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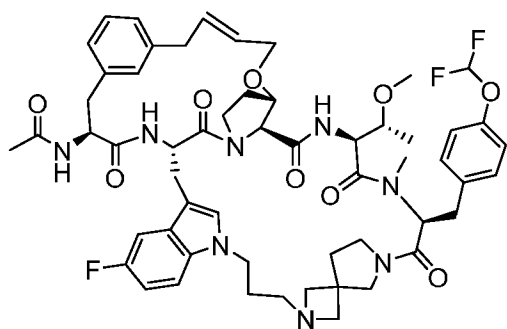
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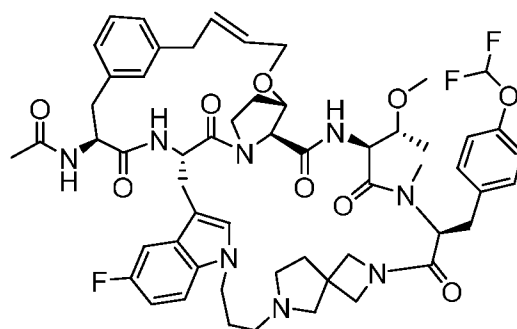
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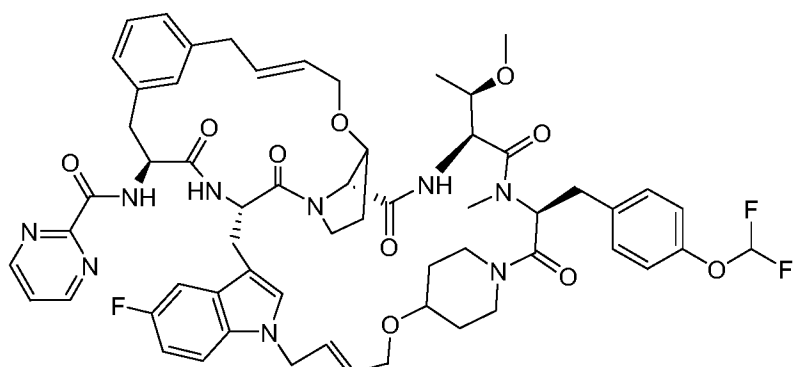
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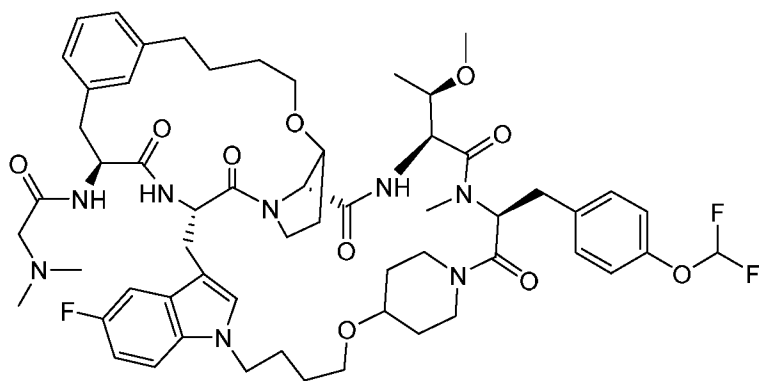
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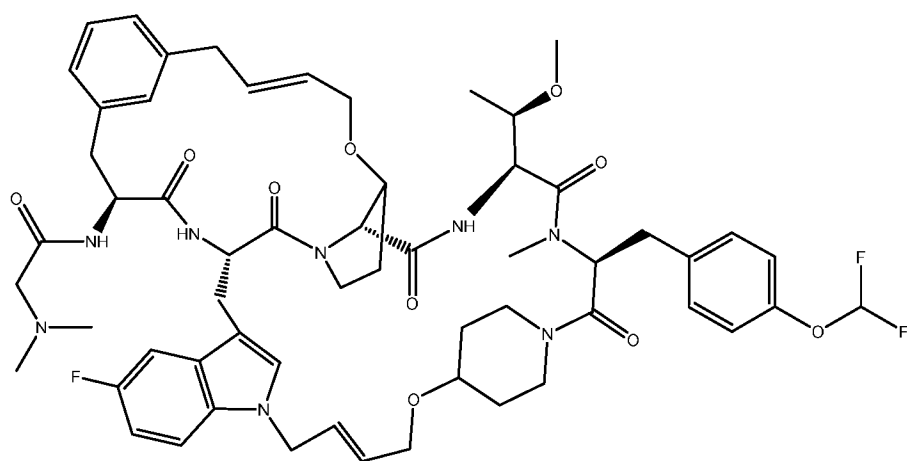
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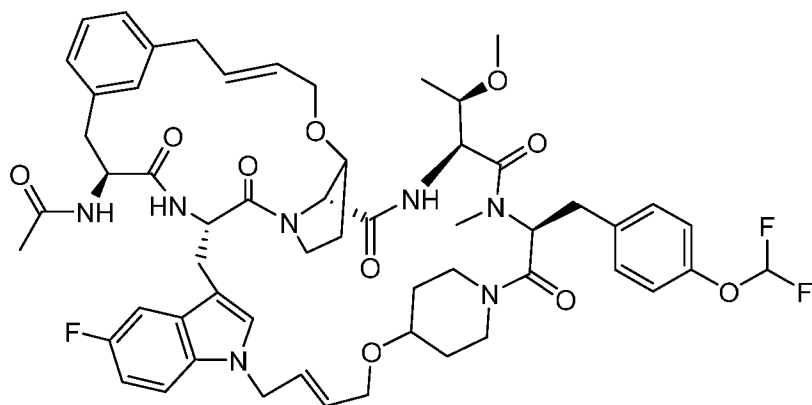
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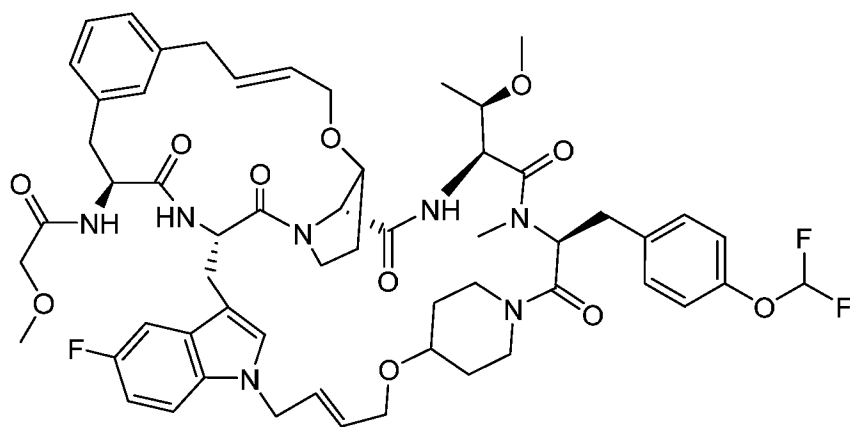
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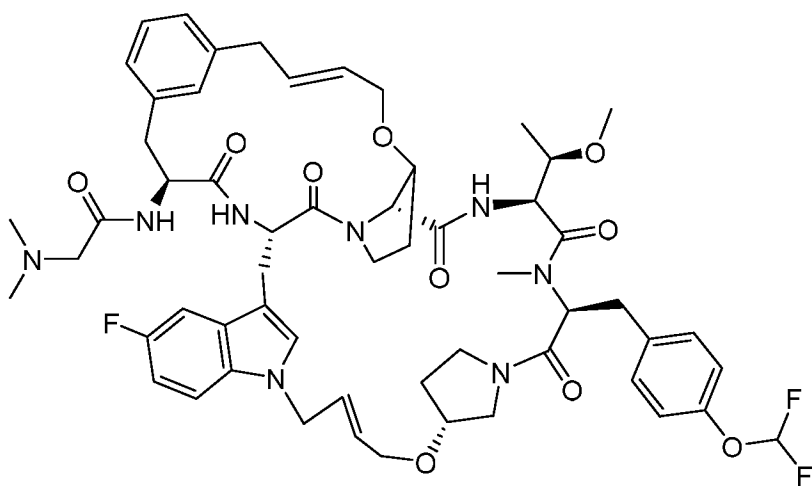
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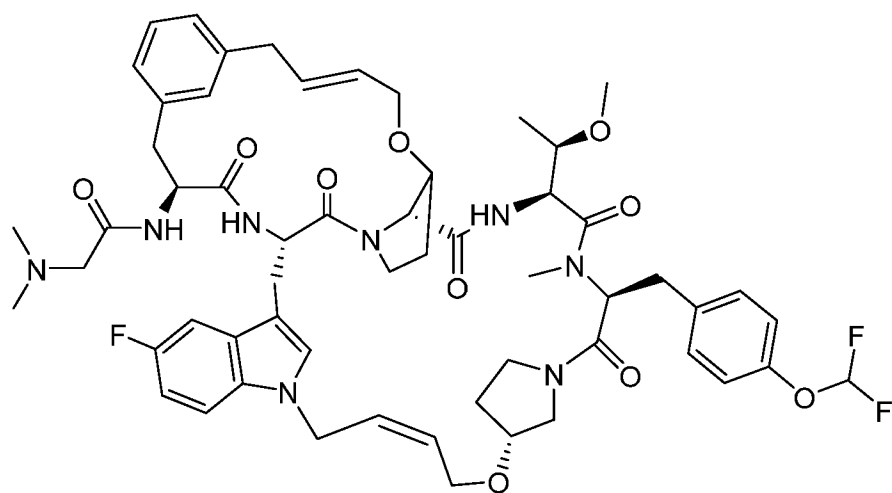
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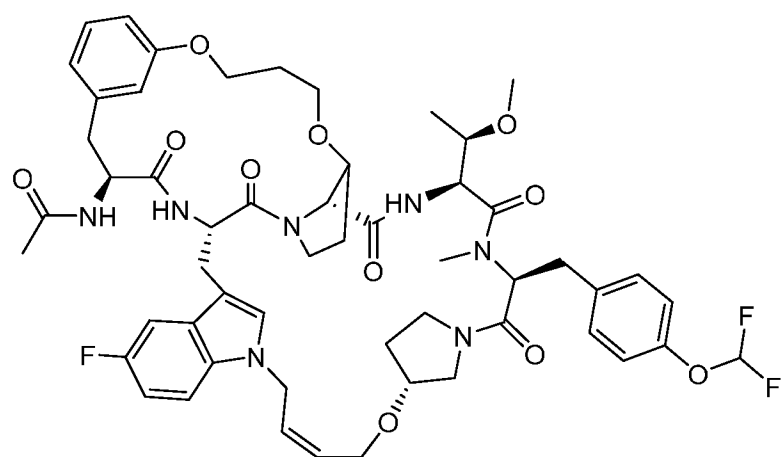
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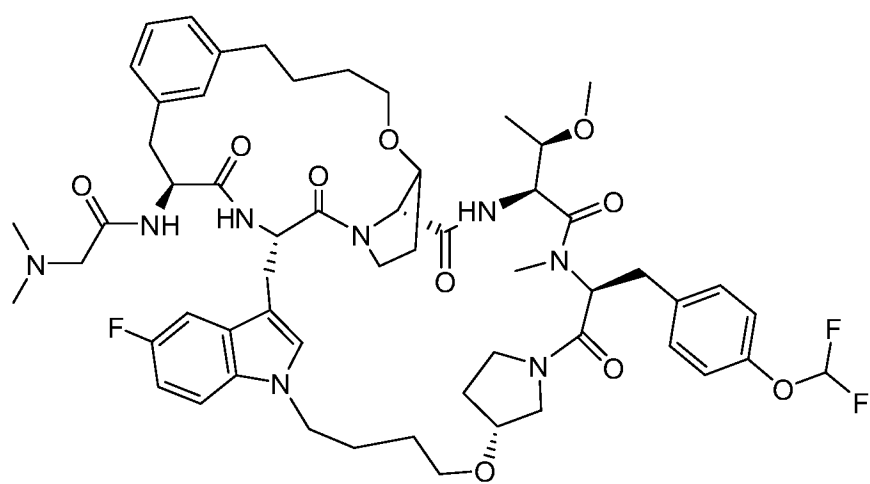
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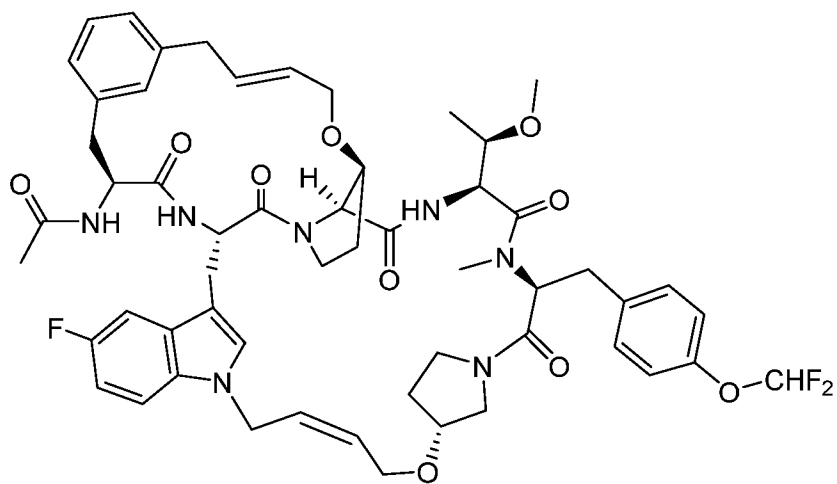
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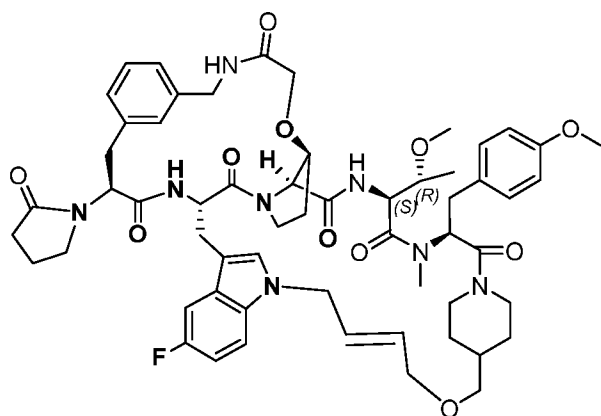
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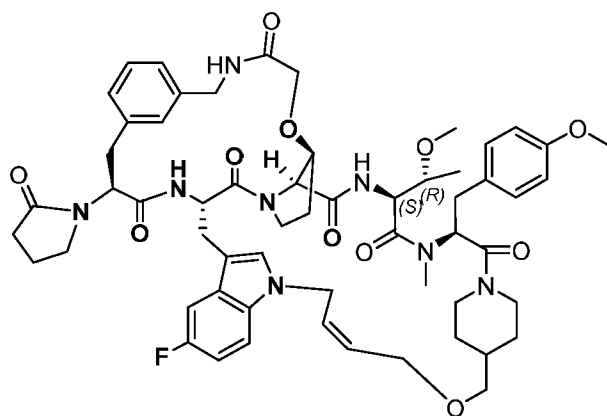
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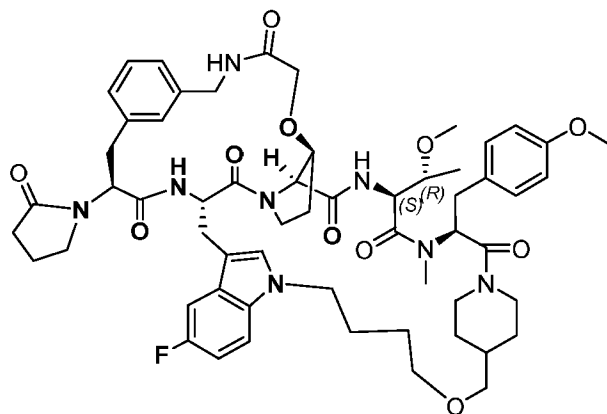
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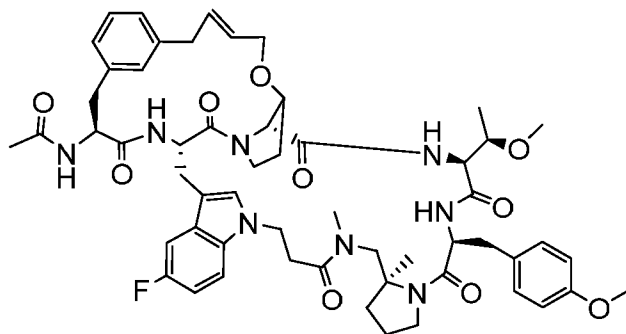
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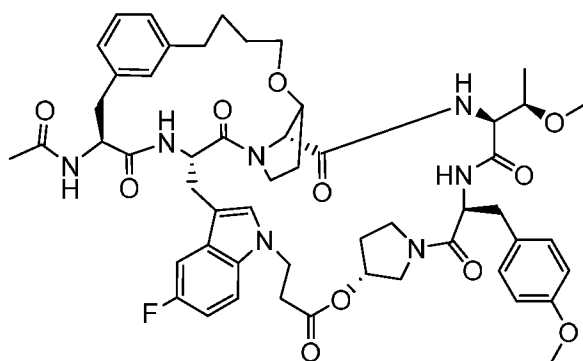
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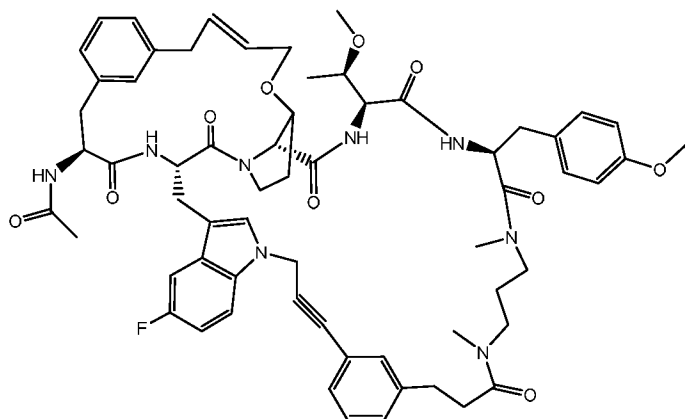
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Ex-58,



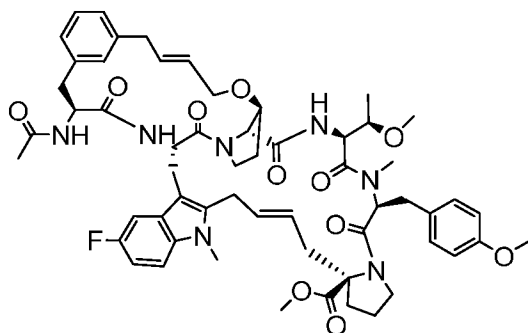
Ex-59,

5 or a pharmaceutically acceptable salt of any thereof.

[0042] In embodiment 14, the invention provides a composition comprising at least one compound of any of embodiments 1 to 13, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable excipient.

[0043] In embodiment 15, the invention provides a method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of embodiment 14.

[0044] In embodiment 16, the invention provides a compound having the structure

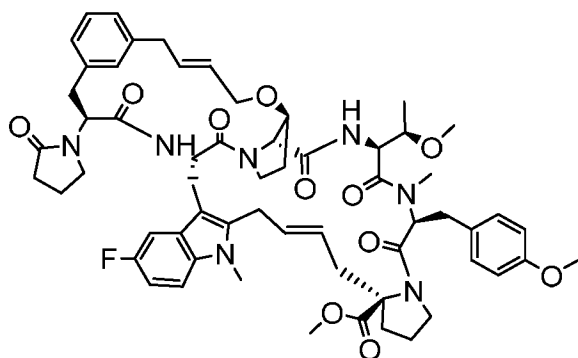


or a pharmaceutically acceptable salt thereof.

[0045] In embodiment 17, the invention provides a composition comprising the compound of embodiment 16 and at least one pharmaceutically acceptable excipient.

[0046] In embodiment 18, the invention provides a method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of embodiment 17.

[0047] In embodiment 19, the invention provides a compound having the structure



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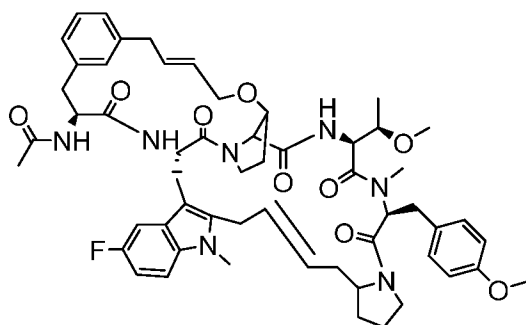
or a pharmaceutically acceptable salt thereof.

[0048] In embodiment 20, the invention provides a composition comprising the compound of embodiment 19 and at least one pharmaceutically acceptable excipient.

[0049] In embodiment 21, the invention provides a method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of embodiment 20.

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[0050] In embodiment 22, the invention provides a compound having the structure



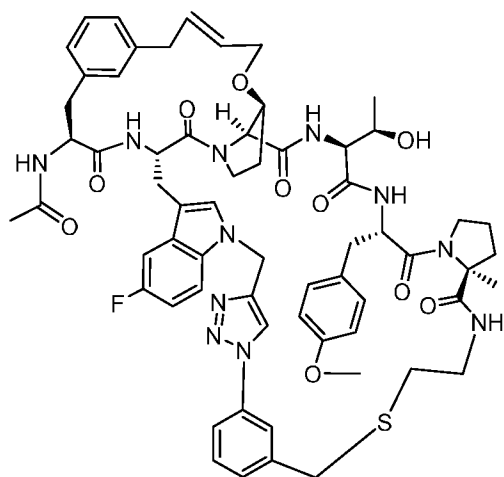
or a pharmaceutically acceptable salt thereof.

[0051] In embodiment 23, the invention provides a composition comprising the compound of embodiment 22 and at least one pharmaceutically acceptable excipient.

[0052] In embodiment 24, the invention provides a method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of embodiment 23.

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[0053] In embodiment 25, the invention provides a compound having the structure



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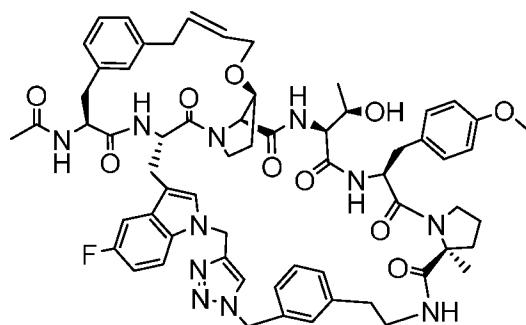
or a pharmaceutically acceptable salt thereof.

[0054] In embodiment 26, the invention provides a composition comprising the compound of the embodiment 25 and at least one pharmaceutically acceptable excipient.

[0055] In embodiment 27, the invention provides a method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of embodiment 26.

10

[0056] In embodiment 28, the invention provides a compound having the structure



or a pharmaceutically acceptable salt thereof.

[0057] In embodiment 29, the invention provides a composition comprising the compound of embodiment 28 and at least one pharmaceutically acceptable excipient.

[0058] In embodiment 30, the invention provides a method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of embodiment 29.

15

[0058] The term “salt(s)”, and its use in the phrase “pharmaceutically acceptable salts” employed herein, includes any of the following: acidic salts formed with inorganic and/or organic acids, basic salts formed with inorganic and/or organic bases, zwitterionic, and quaternary ammonium complexes. Salts of compounds of the invention may be formed by methods known to those of ordinary skill in the art, for example, by reacting a compound of the invention with an

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5 amount of acid or base, such as an equivalent amount, in a medium such as one in which the salt precipitates or in aqueous medium followed by lyophilization.

[0059] Compounds of the invention contain tri-coordinate nitrogen atoms, for example, primary, secondary or tertiary amino moieties, wherein, as is known, the lone pair of electrons residing on the nitrogen atom may be protonated with an appropriate acid or alkylated with an appropriate reagent, for example, alkyl bromide, under the appropriate reaction conditions to provide tetracoordinate charged nitrogen stabilized by an anion generated in the process, for example, a halogen ion or conjugate base. Accordingly, compounds of the invention may be prepared in the form of a free-base or isolated in the form of a quaternary complex or a salt complex. In some instances where there is an appropriate acidic proton proximal to a basic nitrogen formation of a zwitterionic complex is possible. As the term is employed herein, salts of the inventive compounds, whether acidic salts formed with inorganic and/or organic acids, basic salts formed with inorganic and/or organic bases, salts formed which include zwitterionic character, for example, where a compound contains both a basic moiety, for example, but not limited to, a nitrogen atom, for example, an amine, pyridine or imidazole, and an acidic moiety, for example, but not limited to a carboxylic acid, and quaternary ammonium complexes are included in the scope of the inventive compounds described herein.

[0060] Accordingly, structural representation of compounds of the invention, whether in a free-base form, a salt form, a zwitterionic form or a quaternary ammonium form, also include all other forms of such compounds discussed above. Thus, one aspect of the invention is the provision of compounds of the invention in the form of a pharmaceutically acceptable salt, zwitterionic complex or quaternary ammonium complex. Those skilled in the art will recognize those instances in which the compounds of the invention may form such complexes, including where a tetracoordinate nitrogen can be quaternized or protonated and the charged nitrogen form stabilized by an associated anion. The term “pharmaceutically acceptable salt” refers to a salt (including a quaternary ammonium complex and an inner salt such as a zwitterion complex) which possesses effectiveness similar to or greater than a free-base form of the compound and which is not biologically or otherwise undesirable (e.g., is neither toxic nor otherwise deleterious to the recipient thereof).

[0061] The formation of pharmaceutically useful salts from basic (or acidic) pharmaceutical compounds are discussed, for example, by S. Berge et al., *Journal of Pharmaceutical Sciences* (1977) 66(1) 1-19; P. Gould, *International J. of Pharmaceutics* (1986) 33 201-217; Anderson et al, *The Practice of Medicinal Chemistry* (1996), Academic Press, New York; in *The Orange Book* (Food & Drug Administration, Washington, D.C. on their website); and P. Heinrich Stahl,

5 Camille G. Wermuth (Eds.), Handbook of Pharmaceutical Salts: Properties, Selection, and Use, (2002) Int'l Union of Pure and Applied Chemistry, pp. 330-331. These disclosures are incorporated herein by reference.

[0062] The invention contemplates both freebase forms of the compounds of the invention and all available salts, including salts which are generally recognized as safe for use in preparing
10 pharmaceutical formulations and those that may be formed presently within the ordinary skill in the art and are later classified as being “generally recognized as safe” for use in the preparation of pharmaceutical formulations, termed herein as “pharmaceutically acceptable salts.” As will be appreciated, freebase compounds may be prepared by controlling the conditions of isolation of the compound during synthesis or by neutralization and ion exchange from salt forms of
15 compounds of the invention.

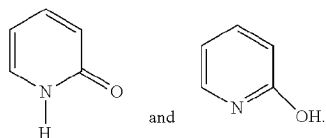
[0063] Examples of pharmaceutically acceptable acid salts include, but are not limited to, acetates, including trifluoroacetate salts, adipates, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, cyclopentanepropionates, digluconates, dodecylsulfates, ethanesulfonates, fumarates,
20 glucoheptanoates, glycerophosphates, hemisulfates, heptanoates, hexanoates, hydrochlorides, hydrobromides, hydroiodides, 2-hydroxyethanesulfonates, lactates, maleates, methanesulfonates, methyl sulfates, 2-naphthalenesulfonates, nicotines, nitrates, oxalates, pamoates, pectinates, persulfates, 3-phenylpropionates, phosphates, picrates, pivalates, propionates, salicylates, succinates, sulfates, sulfonates (such as those mentioned herein), tartarates, thiocyanates,
25 toluenesulfonates (also known as tosylates,) undecanoates, and the like.

[0064] Examples of pharmaceutically acceptable basic salts include, but are not limited to, ammonium salts, alkali metal salts such as sodium, lithium, and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, aluminum salts, zinc salts, salts with organic bases (for example, organic amines) such as benzathines, diethylamine, dicyclohexylamines,
30 hydrabamines (formed with N,N-bis(dehydroabietyl)ethylenediamine), N-methyl-D-glucamines, N-methyl-D-glucamides, t-butyl amines, piperazine, phenylcyclohexyl-amine, choline, tromethamine, and salts with amino acids such as arginine, lysine and the like. Basic nitrogen-containing groups may be converted to an ammonium ion or quaternized with agents such as lower alkyl halides (e.g., methyl, ethyl, propyl, and butyl chlorides, bromides and iodides),
35 dialkyl sulfates (e.g., dimethyl, diethyl, dibutyl, and diamyl sulfates), long chain halides (e.g. decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides), aralkyl halides (e.g. benzyl and phenethyl bromides), and others.

5 [0065] Further examples of pharmaceutically acceptable salts that may be used with the instant invention include, but are not limited to, fluoride, chloride, bromide and iodide. In general, salts of compounds are intended to be pharmaceutically acceptable salts within the scope of the invention.

[0066] The term “purified”, “in purified form” or “in isolated and purified form” for a
10 compound refers to the physical state of said compound after being isolated from a synthetic process or natural source or combination thereof. Thus, the term “purified”, “in purified form” or “in isolated and purified form” for a compound refers to the physical state of said compound after being obtained from a purification process or processes described herein or well known to the skilled artisan, and in sufficient purity to be characterized by standard analytical techniques
15 described herein or well known to the skilled artisan. Compounds of the invention include any form of the compound including in situ in a reaction mixture as well as in isolated and purified form obtained by routine techniques. Also included are polymorphic forms of the compounds of the invention and solvates and prodrugs thereof.

[0067] Certain compounds of the invention may exist in different tautomeric forms, for
20 example, but are not limited to, ketone/enol tautomeric forms, imine-enamine tautomeric forms, and for example heteroaromatic forms such as the following moieties:



[0068] In the same manner, unless indicated otherwise, presenting a structural representation of any tautomeric form of a compound which exhibits tautomerism is meant to include all such
25 tautomeric forms of the compound. Accordingly, where compounds of the invention, their salts, and solvates and prodrugs thereof, exist in different tautomeric forms or in equilibrium among such forms, all such forms of the compound are embraced by, and included within the scope of the invention.

[0069] In another aspect, the invention provides pharmaceutical compositions comprising one
30 or more compounds of the invention. As used herein, the term “pharmaceutical composition” comprises at least one pharmaceutically active compound and at least one excipient, and is intended to encompass both the combination of the specified ingredients in the specified amounts, and any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

35 [0070] As will be appreciated by the ordinarily skilled artisan, an excipient is any constituent which adapts the composition to a particular route of administration or aids the processing of a

5 composition into a dosage form without itself exerting an active pharmaceutical effect. In general compositions comprise more than one excipient depending upon the route of administration and the characteristics of the active being administered. Examples of excipients which impart to the composition properties which make it easier to handle or process include, but are not limited to, lubricants or pressing aids in powdered medicaments intended to be tableted, and emulsion
10 stabilizers in compositions in which the active is present in the form of an emulsion. Examples of excipients which adapt a composition to a desired route of administration are, for example, but not limited to, for oral administration, absorption enhancers promoting absorption from the gastrointestinal tract, for transdermal or transmucosal administration, penetration enhancers, for example, those employed in adhesive skin "patch" or compositions for buccal administration.

15 **[0071]** Notwithstanding the function excipients perform in a composition, excipients are collectively termed herein "a carrier." Typically, formulations may comprise up to about 95 percent active ingredient and the balance carrier, although formulations with different ratios may be prepared. In general, acceptable pharmaceutical compositions contain a suitable concentration of the active that an effective amount of the PCSK9 antagonist can be provided in an individual
20 dosage form of acceptable volume based upon the route of administration such that it can provide a therapeutic serum level of the active for an acceptable period of time in a subject to whom the composition is administered and the composition will retain biological activity during storage within an acceptable temperature range for an acceptable period of time.

[0072] Pharmaceutical composition, as used herein, refers both to a bulk composition, that is,
25 formulated material that has not yet been formed into individual dosage units for administration, and the composition contained within individual dosage units. While compositions of the invention may be employed in bulk form, it will be appreciated that for most applications compositions will be incorporated into a dosage form providing individual units suitable for administration to a patient, each dosage form comprising an amount of the selected composition
30 which contains an effective amount of said one or more compounds of Formula I. Examples of suitable dosage forms include, but are not limited to, dosage forms adapted for: (i) oral administration, e.g., a liquid, gel, powder, solid or semi-solid pharmaceutical composition which is loaded into a capsule or pressed into a tablet and may comprise additionally one or more coatings which modify its release properties, for example, coatings which impart delayed release
35 or formulations which have extended release properties; (ii) a dosage form adapted for administration through tissues of the oral cavity, for example, a rapidly dissolving tablet, a lozenge, a solution, a gel, a sachet or a needle array suitable for providing intramucosal administration; (iii) a dosage form adapted for administration via the mucosa of the nasal or

5 upper respiratory cavity, for example a solution, suspension or emulsion formulation for dispersion in the nose or airway; (iv) a dosage form adapted for transdermal administration, for example, a patch, cream or gel; (v) a dosage form adapted for intradermal administration, for example, a microneedle array; (vi) a dosage form adapted for intravenous (IV) infusion, for example, over a prolonged period using an I.V. infusion pump; (vii) a dosage form adapted for
10 intramuscular administration (IM), for example, an injectable solution or suspension, and which may be adapted to form a depot having extended release properties; (viii) a dosage form adapted for drip intravenous administration (IV), for example, a solution or suspension, for example, as an IV solution or a concentrate to be injected into a saline IV bag; (ix) a dosage form adapted for subcutaneous administration, including administration over an extended time period by
15 implanting a rod or other device which diffuses the compound into the surround tissue and thereby provides a continuous serum therapeutic level; or (x) a dosage form adapted for delivery via rectal or vaginal mucosa, for example, a suppository.

[0073] Pharmaceutical compositions can be solid, semi-solid or liquid. Solid, semi-solid and liquid form preparations can be adapted to a variety of modes of administration, examples of
20 which include, but are not limited to, powders, dispersible granules, mini-tablets, beads, which can be used, for example, for tableting, encapsulation, or direct administration. In addition, liquid form preparations include, but are not limited to, solutions, suspensions and emulsions which for example, but not exclusively, can be employed in the preparation of formulations intended for ingestion, inhalation or intravenous administration (IV), for example, but not limited to,
25 administration via drip IV or infusion pump, intramuscular injection (IM), for example, of a bolus which is released over an extended duration, direct IV injection, or adapted to subcutaneous routes of administration.

[0074] Other routes of administration that are contemplated include intranasal administration, or for administration to some other mucosal membrane. Formulations prepared for administration
30 to various mucosal membranes may also include additional components adapting them for such administration, for example, viscosity modifiers.

[0075] Although in some embodiments, compositions suitable for use in a solid oral dosage form, for example, a tablet or quick-melt mouth-dissolving formulation are preferable routes of administration for a compound of the invention or a salt thereof, a composition of the invention
35 may be formulated for administration via other routes mentioned above. Examples include aerosol preparations, for example, suitable for administration via inhalation or via nasal mucosa, may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable propellant, for example, an inert compressed gas, e.g., nitrogen.

5 Also included are solid form preparations which are intended to be converted, shortly before use, to a suspension or a solution, for example, for oral or parenteral administration. Examples of such solid forms include, but are not limited to, freeze dried formulations and liquid formulations adsorbed into a solid absorbent medium.

[0076] The compounds of the invention may also be deliverable transdermally or
10 transmucosally, for example, from a liquid, suppository, cream, foam, gel, or rapidly dissolving solid form. It will be appreciated that transdermal compositions can also take the form of creams, lotions, aerosols and/or emulsions and can be provided in a unit dosage form which includes a transdermal patch of any known in the art, for example, a patch which incorporates either a matrix comprising the pharmaceutically active compound or a reservoir which comprises a solid
15 or liquid form of the pharmaceutically active compound.

[0077] Examples of pharmaceutically acceptable carriers and methods of manufacture for various compositions mentioned above may be found in A. Gennaro (ed.), Remington: The Science and Practice of Pharmacy, 20th Edition, (2000), Lippincott Williams & Wilkins, Baltimore, Md. Additional examples of publications addressing formulation issues may be found
20 in: Pharmaceutical compositions may be formulated by any number of strategies known in the art, see, e.g., McGoff and Scher, 2000 *Solution Formulation of Proteins/Peptides*; McNally, E. J., ed. Protein Formulation and Delivery. New York, N.Y.: Marcel Dekker; pp. 139-158; Akers & Defilippis, 2000, *Peptides and Proteins as Parenteral Solutions*; Pharmaceutical Formulation Development of Peptides and Proteins. Philadelphia, Pa.: Taylor and Francis; pp. 145-177; Akers
25 et al., 2002, *Pharm. Biotechnol.* 14:47-127.

[0078] In another aspect the invention provides methods of employing PCSK9-specific antagonist compounds described herein for antagonizing PCSK9 function; said methods of which are further described below. Use of the term “antagonizing” throughout the present application refers to providing to the affected tissue(s) a substance which opposes the action of, inhibits,
30 counteracts, neutralizes, or curtails one or more functions of PCSK9 in the affected tissues. Inhibition or antagonism of one or more of PCSK9-associated functional properties can be readily determined according to methodologies known to the art (see, e.g., Barak & Webb, 1981 *J. Cell Biol.* 90:595-604; Stephan & Yurachek, 1993 *J. Lipid Res.* 34:325330; and McNamara et al., 2006 *Clinica Chimica Acta* 369:158-167) as well as those described herein.
35 Inhibition or antagonism will effectuate a decrease in PCSK9 activity relative to that seen in the absence of the antagonist or, for example, that seen relative to the activity observed when a control antagonist of irrelevant specificity is present. Preferably, a PCSK9-specific antagonist in accordance with the invention antagonizes PCSK9 functioning to the point that there is a

5 decrease of at least 10%, of the measured parameter including but not limited to the activities disclosed herein, and more preferably, a decrease of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 95% of the measured parameter. Such inhibition/antagonism of PCSK9 functioning is particularly effective in those instances where PCSK9 functioning is contributing at least in part to a particular phenotype, disease, disorder, or condition which is negatively
10 impacting the subject.

[0079] In one aspect, the invention provides a method for antagonizing the activity of PCSK9, which comprises contacting a cell, population of cells or tissue sample capable of being affected by PCSK9 (i.e., which expresses and/or comprises LDL receptors) with a PCSK9-specific antagonist disclosed herein under conditions that allow said antagonist to bind to PCSK9 when
15 present and inhibit PCSK9's inhibition of cellular LDL uptake. Some embodiments of the invention include such methods wherein the cell is a human cell. Additional embodiments of the invention include such methods wherein the cell is a murine cell.

[0080] In one aspect, the present invention provides a method for antagonizing the activity of PCSK9 in a subject, which comprises administering to the subject a therapeutically effective
20 amount of a PCSK9-specific antagonist of the present invention. In some embodiments, the methods for antagonizing PCSK9 function are for the treatment, as defined herein, of a PCSK9-associated disease, disorder, or condition or, alternatively, for providing therapy in a disease, disorder or condition that could benefit from the effects of a PCSK9 antagonist.

[0081] The invention, thus, contemplates the use of PCSK9-specific antagonists described
25 herein in various methods of treatment where antagonizing PCSK9 function is desirable. As used herein, the term "method of treatment" relates to a course of action resulting in a change in at least one symptom of a disease state which can be prophylactic or therapeutic in nature. In some embodiments, the invention relates to a method of treatment for a condition associated with and/or attributed to PCSK9 activity, or a condition where the functioning of PCSK9 is
30 contraindicated for a particular subject, the method comprising administering to the subject a therapeutically effective amount of a PCSK9-antagonist compound of Formula I, or pharmaceutically acceptable salt thereof. In some embodiments, the condition is atherosclerosis, hypercholesterolemia, coronary heart disease, metabolic syndrome, acute coronary syndrome or related cardiovascular disease or a cardiometabolic conditions. In some embodiments, the
35 condition is a disease state or condition in which PCSK9 activity is contraindicated.

[0082] Methods of treatment in accordance with the invention comprise administering to an individual a therapeutically (or prophylactically) effective amount of a PCSK9-specific antagonist of the invention. Use of the terms "therapeutically effective" or "prophylactically

5 effective” in reference to an amount refers to the amount necessary at the intended dosage to achieve the desired therapeutic and/or prophylactic effect for the period desired. The desired effect may be, for example, the alleviation, amelioration, reduction, or cessation of at least one symptom associated with the treated condition. These amounts will vary, as the skilled artisan will appreciate, according to various factors, including but not limited to the disease state, age, 10 sex, and weight of the individual, and the ability of the PCSK9-specific antagonist to elicit the desired effect in the individual. The response may be documented by *in vitro* assay, *in vivo* non-human animal studies, and/or further supported from clinical trials.

[0083] In some embodiments, a PCSK9 antagonist compound of the invention is administered in the form of a pharmaceutical composition as described herein.

15 [0084] Dosing of antagonist therapeutics is well within the realm of the skilled artisan, see, e.g., Lederman et al., 1991 *Int. J. Cancer* 47:659-664; Bagshawe et al., 1991 *Antibody, Immunoconjugates and Radiopharmaceuticals* 4:915-922, and will vary based on a number of factors, for example, but not limited to, those mentioned above, including the condition of the patient, the area being treated, the route of administration, and the treatment desired, for example, 20 prophylaxis or acute treatment and the like. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective therapeutic amount of the antagonist.

[0085] The subject may need, or desire, treatment for an existing disease or medical condition. As used herein, the subject “in need” of treatment of an existing condition encompasses both a determination of need by a medical professional as well as the desire of the subject for such 25 treatment. When a compound or a salt thereof is provided in combination with one or more other active agents, “administration” and its variants are each understood to include provision of the compound or its salt and the other agents contemporaneously or simultaneously or over a course of separate administrations over a period of time. When the agents of a combination are administered at the same time, they can be administered together in a single composition, or they 30 can be administered separately. It is understood that a “combination” of active agents can be a single composition containing all the active agents or multiple compositions each containing one or more of the active agents. In the case of two active agents a combination can be either a single composition comprising both agents or two separate compositions each comprising one of the agents. In the case of three active agents, a combination can be either a single composition 35 comprising all three agents, three separate compositions each comprising one of the agents, or two compositions one of which comprises two of the agents and the other comprises the third agent, and so forth.

5 **[0086]** The compositions and combinations of the invention are suitably administered in effective amounts. The term “effective amount” means the amount of active compound sufficient to antagonize PCSK9 and thereby elicit the response being sought (i.e., induce a therapeutic response in the treatment or management of conditions associated with or impacted by PCSK9 function, including, but not limited to atherosclerosis, hypercholesterolemia, coronary heart
10 disease, metabolic syndrome, acute coronary syndrome and related cardiovascular disease, and cardiometabolic conditions in an animal or human).

[0087] The actual dosage employed may be varied depending upon the requirements of the patient and the severity of the condition being treated. Determination of the proper dosage regimen for a particular situation is within the skill in the art, for example, as described in the
15 standard literature, for example, as described in the “Physicians' Desk Reference” (PDR), e.g., 1996 edition (Medical Economics Company, Montvale, N.J. 07645-1742, USA), the Physician's Desk Reference, 56th Edition, 2002 (published by Medical Economics company, Inc. Montvale, N.J. 07645-1742), or the Physician's Desk Reference, 57th Edition, 2003 (published by Thompson PDR, Montvale, N.J. 07645-1742); the disclosures of which is incorporated herein by
20 reference thereto. For convenience, the total daily dosage may be divided and administered in portions during the day as required or delivered continuously.

[0088] The PCSK9-specific antagonist may be administered to an individual by any route of administration appreciated in the art, including but not limited to oral administration, administration by injection (specific embodiments of which include intravenous, subcutaneous,
25 intraperitoneal, or intramuscular injection), or administration by inhalation, intranasal, or topical administration, either alone or in combination with other agents designed to assist in the treatment of the individual. The PCSK9-specific antagonist may also be administered by injection devices, injector pens, needleless devices, and subcutaneous patch delivery systems. The route of administration should be determined based on several considerations appreciated by
30 the skilled artisan including, but not limited to, the desired physiochemical characteristics of the treatment.

[0089] One or more additional pharmacologically active agents may be administered in combination with a compound of Formula I. An additional active agent (or agents) is intended to mean a pharmaceutically active agent (or agents) that is active in the body, including pro-drugs
35 that convert to pharmaceutically active form after administration, which are different from the compound of Formula I, and includes free acid, free base and pharmaceutically acceptable salts of said additional active agents. Generally, any suitable additional active agent or agents, including but not limited to anti-hypertensive agents, anti-atherosclerotic agents such as a lipid

5 modifying compound, anti-diabetic agents and/or anti-obesity agents may be used in any combination with the compound of Formula I in a single dosage formulation (a fixed dose drug combination), or may be administered to the subject in one or more separate dosage formulations which allows for concurrent or sequential administration of the active agents (co-administration of the separate active agents).

10 **[0090]** Examples of additional active agents that may be employed include but are not limited to angiotensin converting enzyme inhibitors (e.g., alacepril, benazepril, captopril, ceronapril, cilazapril, delapril, enalapril, enalaprilat, fosinopril, imidapril, lisinopril, moveltipril, perindopril, quinapril, ramipril, spirapril, temocapril, and trandolapril); angiotensin II receptor antagonists (e.g., losartan i.e., COZAAR®, valsartan, candesartan, olmesartan, telmesartan and any of these

15 drugs used in combination with hydrochlorothiazide such as HYZAAR®); neutral endopeptidase inhibitors (e.g., thiorphan and phosphoramidon); aldosterone antagonists; aldosterone synthase inhibitors; renin inhibitors including urea derivatives of di- and tri-peptides (see U.S. Pat. No. 5,116,835), amino acids and derivatives (U.S. Pat. Nos. 5,095,119 and 5,104,869), amino acid chains linked by non-peptidic bonds (U.S. Pat. No. 5,114,937), di- and tri-peptide derivatives,

20 peptidyl amino diols and peptidyl beta-aminoacyl aminodiol carbamates, small molecule renin inhibitors (including diol sulfonamides and sulfinyls, N-morpholino derivatives, N-heterocyclic alcohols and pyrrolimidazolones), pepstatin derivatives and fluoro- and chloro-derivatives of statone-containing peptides, enalkrein, RO 42-5892, A 65317, CP 80794, ES 1005, ES 8891, SQ 34017, aliskiren (2(S),4(S),5(S),7(S)—N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-

25 diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)-phenyl]-octanamid hemifumarate) SPP600, SPP630 and SPP635); endothelin receptor antagonists; phosphodiesterase-5 inhibitors (e.g. sildenafil, tadalafil and vardenafil); vasodilators; calcium channel blockers (e.g., amlodipine, nifedipine, verapamil, diltiazem, gallopamil, niludipine, nimodipins, nicardipine); potassium channel activators (e.g., nicorandil, pinacidil, cromakalim, minoxidil, aprilkalim, loprazolam);

30 diuretics (e.g., hydrochlorothiazide); sympatholitics; beta-adrenergic blocking drugs (e.g., propranolol, atenolol, bisoprolol, carvedilol, metoprolol, or metoprolol tartate); alpha adrenergic blocking drugs (e.g., doxazocin, prazosin or alpha methyl dopa); central alpha adrenergic agonists; peripheral vasodilators (e.g. hydralazine); lipid lowering agents, e.g., HMG-CoA reductase inhibitors such as simvastatin and lovastatin which are marketed as ZOCOR® and

35 MEVACOR® in lactone pro-drug form and function as inhibitors after administration; and pharmaceutically acceptable salts of dihydroxy open ring acid HMG-CoA reductase inhibitors such as atorvastatin (particularly the calcium salt sold in LIPITOR®), rosuvastatin (particularly the calcium salt sold in CRESTOR®), pravastatin (particularly the sodium salt sold in

5 PRAVACHOL®), fluvastatin (particularly the sodium salt sold in LESCOL®), crivastatin, and
 pitavastatin; a cholesterol absorption inhibitor such as ezetimibe (ZETIA®) and ezetimibe in
 combination with any other lipid lowering agents such as the HMG-CoA reductase inhibitors
 noted above and particularly with simvastatin (VYTORIN®) or with atorvastatin calcium; niacin
 in immediate-release or controlled release forms and/or with an HMG-CoA reductase inhibitor;
 10 niacin receptor agonists such as acipimox and acifran, as well as niacin receptor partial agonists;
 metabolic altering agents including insulin and insulin mimetics (e.g., insulin degludec, insulin
 glargine, insulin lispro), dipeptidyl peptidase-IV (DPP-4) inhibitors (e.g., sitagliptin, alogliptin,
 omarigliptin, linagliptin, vildagliptin); insulin sensitizers, including (i) PPAR γ agonists, such as
 the glitazones (e.g. pioglitazone, AMG 131, MBX2044, mitoglitazone, lobeglitazone, IDR-105,
 15 rosiglitazone, and balaglitazone), and other PPAR ligands, including (1) PPAR α/γ dual agonists
 (e.g., ZYH2, ZYH1, GFT505, chiglitazar, muraglitazar, aleglitazar, sodelglitazar, and
 naveglitazar); (2) PPAR α agonists such as fenofibric acid derivatives (e.g., gemfibrozil,
 clofibrate, ciprofibrate, fenofibrate, bezafibrate), (3) selective PPAR γ modulators (SPPAR γ M's),
 (e.g., such as those disclosed in WO 02/060388, WO 02/08188, WO 2004/019869, WO
 20 2004/020409, WO 2004/020408, and WO 2004/066963); and (4) PPAR γ partial agonists, (ii)
 biguanides, such as metformin and its pharmaceutically acceptable salts, in particular, metformin
 hydrochloride, and extended-release formulations thereof, such as Glumetza™, Fortamet™, and
 GlucophageXR™; and (iii) protein tyrosine phosphatase-1B (PTP-1B) inhibitors (e.g., ISIS-
 113715 and TTP814); insulin or insulin analogs (e.g., insulin detemir, insulin glulisine, insulin
 25 degludec, insulin glargine, insulin lispro and inhalable formulations of each); leptin and leptin
 derivatives and agonists; amylin and amylin analogs (e.g., pramlintide); sulfonylurea and non-
 sulfonylurea insulin secretagogues (e.g., tolbutamide, glyburide, glipizide, glimepiride,
 mitiglinide, meglitinides, nateglinide and repaglinide); α -glucosidase inhibitors (e.g., acarbose,
 voglibose and miglitol); glucagon receptor antagonists (e.g., MK-3577, MK-0893, LY-2409021
 30 and KT6-971); incretin mimetics, such as GLP-1, GLP-1 analogs, derivatives, and mimetics; and
 GLP-1 receptor agonists (e.g., dulaglutide, semaglutide, albiglutide, exenatide, liraglutide,
 lixisenatide, taspoglutide, CJC-1131, and BIM-51077, including intranasal, transdermal, and
 once-weekly formulations thereof); bile acid sequestering agents (e.g., colestilan, colestimide,
 colesevalam hydrochloride, colestipol, cholestyramine, and dialkylaminoalkyl derivatives of a
 35 cross-linked dextran), acyl CoA:cholesterol acyltransferase inhibitors, (e.g., avasimibe);
 antiobesity compounds; agents intended for use in inflammatory conditions, such as aspirin, non-
 steroidal anti-inflammatory drugs or NSAIDs, glucocorticoids, and selective cyclooxygenase-2
 or COX-2 inhibitors; glucokinase activators (GKAs) (e.g., AZD6370); inhibitors of 11 β -

5 hydroxysteroid dehydrogenase type 1, (e.g., such as those disclosed in U.S. Pat. No. 6,730,690, and LY-2523199), CETP inhibitors (e.g., anacetrapib, torcetrapib, and evacetrapib); inhibitors of fructose 1,6-bisphosphatase, (e.g., such as those disclosed in U.S. Pat. Nos. 6,054,587; 6,110,903; 6,284,748; 6,399,782; and 6,489,476); inhibitors of acetyl CoA carboxylase-1 or 2 (ACC1 or ACC2), AMP-activated Protein Kinase (AMPK) activators; other agonists of the G-
10 protein-coupled receptors: (i) GPR-109, (ii) GPR-119 (e.g., MBX2982 and PSN821), and (iii) GPR-40 (e.g., TAK875); SSTR3 antagonists (e.g., such as those disclosed in WO 2009/001836); neuromedin U receptor agonists (e.g., such as those disclosed in WO 2009/042053, including, but not limited to, neuromedin S (NMS)); SCD modulators; GPR-105 antagonists (e.g., such as those disclosed in WO 2009/000087); SGLT inhibitors (e.g., ASP1941, SGLT-3, empagliflozin,
15 dapagliflozin, canagliflozin, BI-10773, ertugliflozin, remogliflozin, TS-071, tofogliflozin, ipragliflozin, and LX-4211); inhibitors of acyl coenzyme A:diacylglycerol acyltransferase 1 and 2 (DGAT-1 and DGAT-2); inhibitors of fatty acid synthase; inhibitors of acyl coenzyme A:monoacylglycerol acyltransferase 1 and 2 (MGAT-1 and MGAT-2); agonists of the TGR5 receptor (also known as GPBAR1, BG37, GPCR19, GPR131, and M-BAR); ileal bile acid
20 transporter inhibitors; PACAP, PACAP mimetics, and PACAP receptor 3 agonists; PPAR agonists; protein tyrosine phosphatase-1B (PTP-1B) inhibitors; IL-1b antibodies, (e.g., XOMA052 and canakinumab); and bromocriptine mesylate and rapid-release formulations thereof; or with other drugs beneficial for the treatment of the above-mentioned conditions or disorders including the free-acid, free-base, and pharmaceutically acceptable salt forms of the
25 above active agents where chemically possible.

Experimental

[0091] The compounds of the invention can be prepared readily according to the schemes, descriptions, and specific examples provided below, or modifications thereof, using readily
30 available starting materials, reagents, and conventional synthetic procedures. In these reactions, it is also possible to make use of variants which are themselves known to those of ordinary skill in this art but are not mentioned in detail. The general procedures for making the compounds of the invention can be readily understood and appreciated by one skilled in the art from viewing these schemes and descriptions.

35 [0092] In the procedures that follow, after each starting material, reference to a certain synthesis or preparation is typically provided. This is provided merely for the assistance of the skilled chemist. The starting material may not necessarily have been prepared using the referenced procedure.

5 [0093] In the analytical characterization of the description compounds, “MS” refers to Mass spectrum associated with peaks obtained by UPLC-MS as described below.

[0094] Total ion current (TIC) and Dad UV chromatographic traces together with MS and UV spectra associated with peaks were obtained using a UPLC/MS Waters Acquity™ system equipped with UPLC-PDA extended λ detector coupled to a Waters Micromass ZQ™ Mass spectrometer operating in positive or negative electron spray ionization mode [LC/MS-ES(+or-)]. Chromatography details include Column A: Waters BEH C18 Column (130 Å, 1.7 μ m; column size: 2.1 * 100 mm or 2.1 * 50 mm; column temperature 45°C). Column B: Waters BEH C4 Column (300 Å, 1.7 μ m; column size: 2.1 * 100 mm or 2.1 * 50 mm, column temperature 45°C). Mobile phase 1: (A) 0.1% HCOOH in HPLC water and (B) 0.1% HCOOH in HPLC acetonitrile; or mobile phase 2: (A) 0.1% TFA in HPLC water and (B) 0.1% TFA in HPLC acetonitrile; injection volume: 1 μ L; flow rate: 0.4 mL/min; UV wavelength λ = 214 nm; Mass range: ES(+): 100-800 amu or 100-1000 amu or 100-1800 amu or 400-1800 amu; ES (-)100-800 amu. UV detection range: 400-1200 nm. The usage of this methodology is indicated by UPLC-MS in the analytic characterization of the described compounds.

20 [0095] Confirmation of identity and purity assessment of final compounds were performed by UPLC-MS using a reverse phase Waters Acquity™ UPLC-MS system. Column: Waters BEH C18 Column (130 Å, 1.7 μ m, column size 2.1 * 100 mm, column temperature 45°C). Mobile phase 1: (A) 0.1% HCOOH in HPLC water and (B) 0.1% HCOOH in HPLC acetonitrile; or mobile phase 2: (A) 0.1% TFA in HPLC water and (B) 0.1% TFA in HPLC acetonitrile; injection volume: 1 μ L; flow rate: 0.4 mL/min; UV wavelength λ = 214 nm;

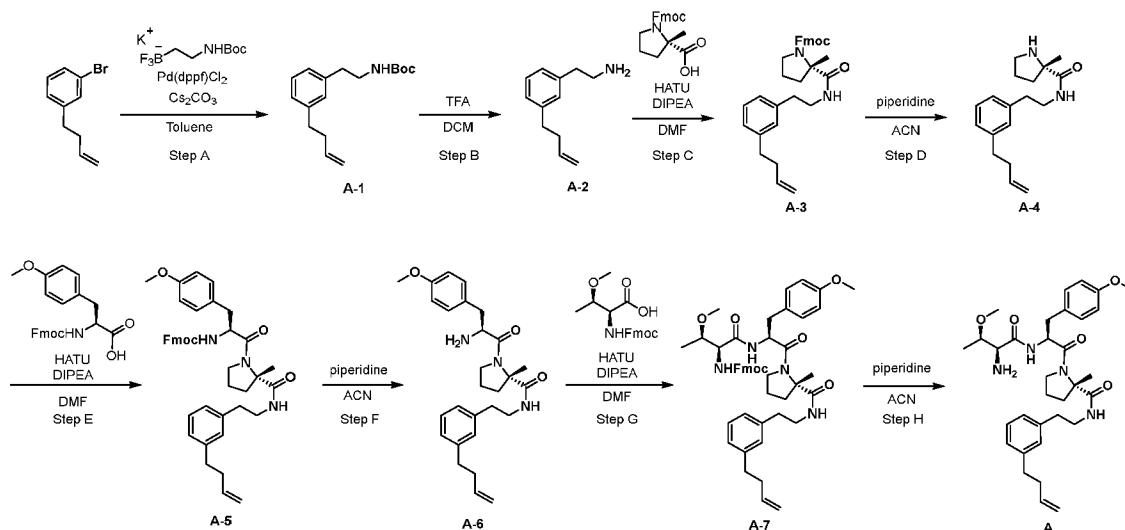
25 [0096] Throughout the synthetic schemes and examples, abbreviations and acronyms may be used with the following meanings unless otherwise indicated:

ACN	Acetonitrile
Boc ₂ O	Di- <i>tert</i> -butyl dicarbonate
BnBr	(Bromomethyl)benzene
BzCl	Benzoyl chloride
BBr ₃	Boron tribromide
CBr ₄	Perbromomethane
Cbz-Cl	Benzyl carbonochloridate
Cs ₂ CO ₃	Cesium carbonate
Cs ₂ CO ₃	Cesium fluoride
CsF	Cesium fluoride

DCM	Dichloromethane
DIEA	Diisopropylethylamine
DMF	<i>N,N</i> -dimethylformamide
DMAP	4-dimethylaminopyridine
DMSO	Dimethyl sulfoxide
DIAD	(<i>E</i>)-diisopropyl diazene-1,2-dicarboxylate
DEA	<i>N,N</i> -diethylamine
DCE	1,2-dichloroethane
DCC	Dicyclohexylcarbodiimide
DDQ	2,3-dicyano-5,6-dichlorobenzoquinone
DMP	Dess-Martin periodinane
EA	Ethyl acetate
EtOH	Ethanol
Fmoc	9H-fluoren-9-ylmethoxycarbonyl
Fmoc-Cl	(9H-fluoren-9-yl)methyl carbonochloridate
Fmoc-Osu	Fmoc <i>N</i> -hydroxysuccinimide este
Grubbs II	Grubbs II Dichloro[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene] (benzylidene)(tricyclohexylphosphine)ruthenium(II)
HATU	HATU 1-[Bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i>]pyridinium 3-oxid hexafluorophosphate
HOBt	1-Hydroxybenzotriazole
LiOH	Lithium hydroxide
LiHMDS	Lithium bis(trimethylsilyl)amide
MeOH	Methanol
MsCl	Methanesulfonyl chloride
Na ₂ SO ₄	Sodium sulfate
NaHCO ₃	Sodium bicarbonate
Na ₂ CO ₃	Sodium carbonate
NaBH ₃ CN	Sodium cyanoborohydride
NMP	<i>N</i> -Methyl-2-pyrrolidone
NaN ₃	Sodium azide

NH ₄ HCO ₃	NH ₄ HCO ₃ ammonium bicarbonate
NSCl	4-nitrobenzene-1-sulfonyl chloride
Osu	O-succinimide
PE	Petroleum ether
Pd ₂ (dba) ₃ (HCCl ₃)	Tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct
PPh ₃	PPh ₃ triphenylphosphine
Pd(dppf)Cl ₂ CH ₂ Cl ₂	Pd(dppf)Cl ₂ CH ₂ Cl ₂ Dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloromethane adduct
Pd(PPh ₃) ₄	Tetrakis(triphenylphosphine)palladium
RT	Room Temperature
RP-HPLC	Reverse phase High-performance liquid chromatography
SEM-Cl	(2-(chloromethoxy)ethyl)trimethylsilane
SPhos	2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
TBAF	Tetrabutylammonium fluoride
THF	Tetrahydropyran
TBTH	TBTH tributylstannane
TEA	Triethylamine
TFA	Trifluoroacetic acid
TF ₂ O	Trifluoromethanesulfonic anhydride
pTsOH	p-toluenesulfonic acid
UPLC	Ultra-Performance Liquid Chromatography or Ultrahigh Pressure Liquid Chromatography
2, 6-Lut	2,6-lutidine
Teoc-OSu	2,5-dioxopyrrolidin-1-yl (2-(trimethylsilyl)ethyl) carbonate
Zhan Catalyst-1B	Dichloro[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene][[5-[(dimethylamino)sulfonyl]-2-(1-methylethoxy-O)phenyl]methylene-C]ruthenium(II)

5 Preparation of Intermediate A



Step A - Synthesis of intermediate A-1

[0097] To a solution of 1-bromo-3-(but-3-en-1-yl)benzene (15 g, 71.1 mmol), potassium (2-((tert-butoxycarbonyl)amino)ethyl)trifluoroborate (19.63 g, 78 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (2.60 g, 3.55 mmol) in Toluene (300 ml), water (100 ml) and cesium carbonate (69.5 g, 213 mmol) were added at room temperature. The mixture was stirred at 80 °C for 16 h. The reaction solution was allowed to cool to room temperature and extracted with EA (3 x 100 mL). The combined organic layer was washed with brine (3 x 100 mL), dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure and purified by flash column chromatography (C18, 330g, 20-35 μ m; mobile phase A: water/10mM NH₄HCO₃, mobile phase B: ACN; Flow rate: 85 mL/min; gradient: 10 %B to 50 %B in 15 min; 50 %B to 70 %B in 15 min, 210nm, RT: 23 min) to afford A-1. LC/MS: [M+H]⁺ = 298.10 .

Step B - Synthesis of intermediate A-2

[0098] Intermediate A-1 (200 mg, 0.726 mmol) was dissolved in DCM (7263 μ l) and TFA (560 μ l, 7.26 mmol) was then added. After stirring at room temp for 1 hour, the reaction was quenched with sodium bicarbonate solution, extracted with DCM, washed with brine, dried over magnesium sulfate and concentrated to afford A-2. LC/MS: [M+H]⁺ = 176.25.

Step C - Synthesis of intermediate A-3

[0099] Intermediate A-2 (137.5 mg, 0.785 mmol) was dissolved in DMF (7845 μ l) and (S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-methylpyrrolidine-2-carboxylic acid (414 mg, 1.177 mmol), HATU (418 mg, 1.098 mmol), and DIPEA (410 μ l, 2.354 mmol) were added. After

5 stirring at room temp for 1 hour, the mixture was diluted with EtOAc, washed three times with water, then brine, dried over magnesium sulfate and concentrated. The residue was purified via flash column chromatography on a 12g silica gold column using 0-30-50% EtOAc in hexane gradient to afford A-3. LC/MS: $[M+H]^+ = 509.41$.

Step D - Synthesis of intermediate A-4

10 **[0100]** Intermediate A-3 (312.3 mg, 0.614 mmol) was dissolved in Acetonitrile (6140 μ l) and piperidine (608 μ l, 6.14 mmol) was added. After stirring at room temp for 1 hour, the mixture was washed three times with hexane and concentrated to afford A-4. LC/MS: $[M+H]^+ = 287.36$.

Step E - Synthesis of intermediate A-5

[0101] Intermediate A-4 (176 mg, 0.614 mmol) was dissolved in DMF (6145 μ l) and (S)-2-
15 (((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-methoxyphenyl)propanoic acid (385 mg, 0.922 mmol), HATU (327 mg, 0.860 mmol), and DIPEA (322 μ l, 1.843 mmol) were added in 1 mL DMF. After stirring at room temp for 1 hour, the mixture was diluted with EtOAc, washed three times with water, then brine, dried over magnesium sulfate, and concentrated. The residue was purified via flash column chromatography on a 12g silica gold column using 30-50-70%
20 EtOAc in hexane gradient to afford A-5. LC/MS: $[M+H]^+ = 686.40$.

Step F - Synthesis of intermediate A-6

[0102] Intermediate A-5 (505.2 mg, 0.737 mmol) was dissolved in Acetonitrile (7366 μ l) and piperidine (729 μ l, 7.37 mmol) was then added. After stirring at room temp for 1 hour, the mixture was washed three times with hexane and concentrated to afford A-6.
25 LC/MS: $[M+H]^+ = 464.41$.

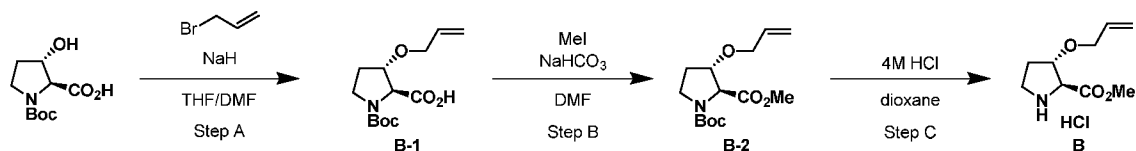
Step G - Synthesis of intermediate A-7

[0103] Intermediate A-6 (294.2 mg, 0.635 mmol) was dissolved in DMF (6346 μ l) and ((2S,3R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxybutanoic acid (338 mg, 0.952 mmol), HATU (338 mg, 0.888 mmol), and DIPEA (332 μ l, 1.904 mmol) were added in 1
30 mL DMF. After stirring at room temp 1.5 hours, the mixture was diluted with EtOAc, washed three times with water, then brine, dried over magnesium sulfate, and concentrated. The residue was purified via reverse phase flash column chromatography on a 30g C18 column using 10-100% [Acetonitrile with 0.05% TFA] in [water with 0.05% TFA], liquid loading in 1 mL DMF afforded A-7. LC/MS: $[M+H]^+ = 801.58$.

35 Step H - Synthesis of intermediate A

[0104] Intermediate A-7 (305.3 mg, 0.381 mmol) was dissolved in ACN (3812 μ l) and piperidine (377 μ l, 3.81 mmol) was then added. After stirring for 1 hour, the mixture was washed three times with hexane and concentrated to afford A. LC/MS: $[M+H]^+ = 579.39$.

5

Preparation of Intermediate B**Step A - Synthesis of intermediate B-1**

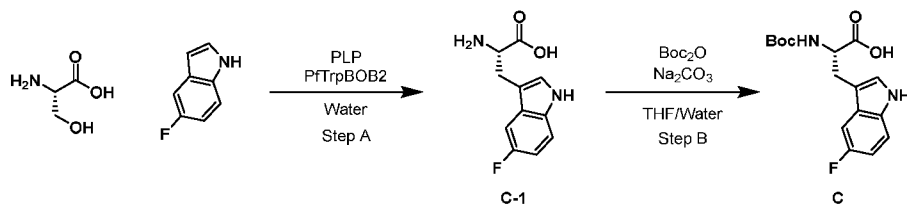
- 10 **[0105]** Sodium hydride (1.038 g, 25.9 mmol) in THF (60 ml)/DMF (12 ml) at 0°C was added to (2S,3S)-1-(tert-butoxycarbonyl)-3-hydroxypyrrolidine-2-carboxylic acid (2 g, 8.65 mmol) and stirred for 15 min. 3-bromoprop-1-ene (2.245 ml, 25.9 mmol) was added to the mixture and the mixture was allowed to warm to RT while stirring overnight. The reaction mixture was quenched with water and EtOAc was added. The solution was adjusted to pH 3-4 with 1N HCl. The
- 15 combined organic layers were washed with brine, dried over K₂CO₃, filtered and concentrated. The residue was purified by column chromatography on silica gel 80g and eluted with 0-10% MeOH/DCM to afford B-1. LC/MS: [M+H]⁺ = 271.00.

Step B - Synthesis of intermediate B-2

- [0106]** Intermediate B-1 (1.455 g, 5.36 mmol) was dissolved in the DMF (25 ml) and sodium hydrogen carbonate (0.901 g, 10.73 mmol) was added and followed by iodomethane (1.677 ml, 26.8 mmol). After stirring at RT for 18hrs, the mixture was poured into water, extracted twice with 100 ml MTBE, washed with 100ml water twice, washed with 100 ml brine, dried over magnesium sulfate, and concentrated to afford B-2. LC/MS: [M+H]⁺ = 286.15.

Step C - Synthesis of intermediate B

- 25 **[0107]** Intermediate B-2 (1.4 g, 4.91 mmol) was dissolved in 10 ml of 4M HCl in dioxane. After stirring at RT for 3 hours, the mixture was concentrated to dryness. Heptane was then added and the residue was re-concentrated to afford B. LC/MS: [M+H]⁺ = 187.07.

Preparation of Intermediate C

30

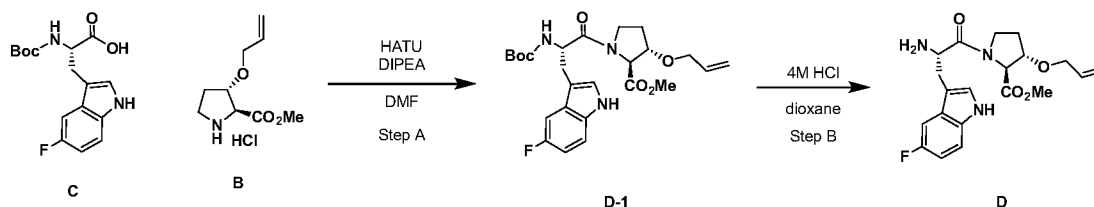
5 Step A - Synthesis of intermediate C-1

[0108] Pyridoxal 5'-phosphate (PLP) (0.14 g, 74.0 mmol) was dissolved in water (10.00 ml). PfTrpBOB2 (0.5 g, 74.0 mmol) (5wt%) was dissolved in 4.0 ml of 0.2M potassium phosphate buffer (pH= 8.0) over 10 minutes at room temperature without any agitation or stirring. Into a 3-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, 5-fluoro-1H-indole (10 g, 74.0 mmol) and (S)-2-amino-3-hydroxypropanoic acid (8.17 g, 78 mmol) were combined with DMSO (300 ml) and Phosphate buffer (300 ml). This mixture was heated to 65°C. To the mixture were then added 1/10 PLP stock and the entire PfTrpBOB2 solution. The resulting solution was stirred overnight, then cooled to room temperature and quenched by the addition of ACN (50.0 ml). The solution obtained was filtered to afford C-1. LC/MS: $[M+H]^+ = 223.1$.

Step B - Synthesis of intermediate C

[0109] A 200 mL RBF equipped with stir bar was charged with intermediate C-1 (2.0 g, 9.0 mmol). DI water was added to give a suspension which was stirred rapidly, then treated with sodium carbonate (1.91 g, 18.0 mmol). THF was added (10 mL) followed by dropwise addition of Di-tert-butyl dicarbonate (2.508 mL, 10.8 mmol) as a solution in THF (10 mL) via syringe pump over 27 minutes. After stirring for 1 hour, the reaction was diluted with EtOAc and 10% citric acid, and the organic and aqueous layers were allowed to separate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to afford C. LC/MS: $[M+H]^+ = 323.32$.

Preparation of Intermediate D



Step A - Synthesis of intermediate D-1

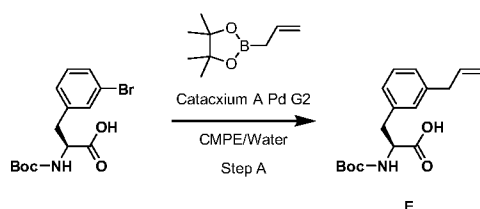
[0110] Intermediate B (344 mg, 1.552 mmol) was dissolved in DMF (5 mL) and intermediate C (500 mg, 1.552 mmol), HATU (590 mg, 1.552 mmol), and DIPEA (0.542 mL, 3.10 mmol) were added at -10 °C. The mixture was stirred at -10 °C for 1 h, diluted with water (10 mL), and extracted with EtOAc (10 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by a

- 5 silica gel column chromatography and eluted with 10% - 50% EtOAc in petroleum ether gradient to afford D-1. LC/MS: $[M+H]^+ = 490.35$.

Step B - Synthesis of intermediate D

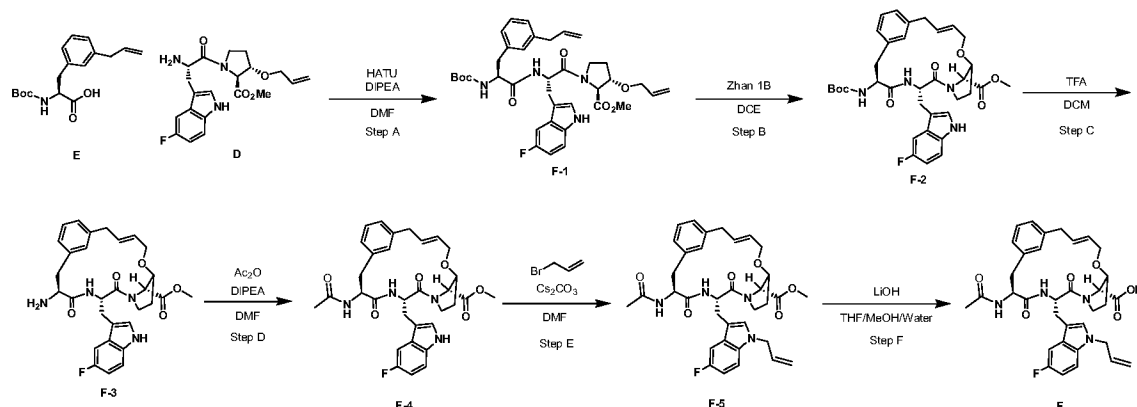
- [0111] Intermediate D-1 (4.2 g, 8.58 mmol) was dissolved in the solution of 4M HCl in 1,4-dioxane (40 mL) at room temperature. The reaction mixture was stirred at room temperature for 1
10 h and then concentrated under reduced pressure to afford D. LC/MS: $[M+H]^+ = 426.54$.

Preparation of Intermediate E



Step A - Synthesis of intermediate E

- 15 [0112] Potassium phosphate (4.63 g, 21.79 mmol) was added in a vial under nitrogen and water (6.05 ml) was then added. The resulting mixture was stirred for 10 min to dissolve solids. Thus, a base solution was obtained. This base solution was degassed for 10 min. (S)-3-(3-bromophenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (2.5 g, 7.26 mmol) and chloro[(di(1-Adamantyl)-N-butylphosphene)-2-(2-aminobiphenyl)]Palladium(II) (0.097 g, 0.145 mmol) were charged to a
20 round bottom flask and was placed under nitrogen. CPME (18.16 ml) was added to the contents of the flask, and the reaction was degassed for 10 min. During degassing, 2-allyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.831 g, 10.89 mmol) was added. The above base solution was then added to the reaction followed by degassing under nitrogen for 5 min. The reaction was then warmed to 80 °C and stirred under nitrogen for 90 minutes and the organic and aqueous layers
25 were allowed to separate. The organic layer was diluted with MTBE and washed with 1M HCl and brine. The organics were then dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography using a RediSep Rf Gold silica 120 g prepackaged column and eluted with a 0-100% gradient of EtOAc in hexane to afford E. LC/MS: $[M+H]^+ = 306.19$.

5 **Preparation of Intermediate F***Step A - Synthesis of intermediate F-1*

[0113] Intermediate E (4.75 g, 15.57 mmol) was dissolved in DMF (5 mL) and intermediate D (6.63 g, 15.57 mmol), HATU (7.70 g, 20.24 mmol), and DIPEA (5.44 mL, 31.1 mmol) were then added at 0 °C. The mixture was stirred at RT for 1 h and then diluted with water (120 mL). The aqueous phase was extracted with ethyl acetate (100 mL). The organic layer was washed with brine (100 x 2 mL), dried over anhydrous MgSO₄, filtered, and concentrated. The residue was purified by silica gel column chromatography and eluted with the gradient 1% - 60% EtOAc in petroleum ether to afford F-1. LC/MS: [M+H]⁺ = 677.4. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.09 (d, *J* = 16.9 Hz, 1H), 7.42 (dd, *J* = 9.4, 2.5 Hz, 1H), 7.32-7.15 (m, 2H), 7.07 (q, *J* = 7.8 Hz, 2H), 7.04-6.91 (m, 2H), 6.69 (d, *J* = 8.1 Hz, 1H), 6.03-5.81 (m, 1H), 5.35-5.18 (m, 2H), 5.13-5.03 (m, 1H), 4.88-4.77 (m, 1H), 4.54 (s, 1H), 4.35 (s, 1H), 4.14-3.93 (m, 2H), 3.76 (s, 3H), 3.49-3.32 (m, 2H), 3.13-3.03 (m, 3H), 2.87 (s, 3H), 1.99 (s, 2H), 1.43 (s, 1H), 1.39 (s, 9H).

Step B - Synthesis of intermediate F-2

[0114] Intermediate F-1 (2.00 g, 2.96 mmol) was dissolved in DCE (200 mL) and stirred at room temperature for 0.5 h, and Zhan catalyst-1B (0.217 g, 0.296 mmol) was added in 10 min. The reaction mixture was stirred at room temperature for 3 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a 1% - 60% EtOAc in petroleum ether gradient to afford F-2. LC/MS: [M+H]⁺ = 649.4. ¹H NMR (400 MHz, Methanol-*d*₄) δ 7.35 - 7.25 (m, 2H), 7.26-7.16 (m, 2H), 7.07 (d, *J* = 7.7 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 1H), 6.95-6.82 (m, 2H), 5.77-5.68 (m, 1H), 5.45 (d, *J* = 15.6 Hz, 1H), 4.92 (d, *J* = 7.6 Hz, 1H), 4.71 (s, 1H), 4.41 (t, *J* = 6.5 Hz, 1H), 4.24 (d, *J* = 14.8 Hz, 1H), 4.09 (d, *J* = 3.8 Hz, 1H), 3.95-3.86 (m, 1H), 3.71 (s, 3H), 3.30 (d, *J* = 7.2 Hz, 1H), 3.19 (dd, *J* = 14.2, 7.8 Hz, 1H), 3.17-3.05 (m, 1H), 3.03 (dd, *J* = 14.1, 6.2 Hz, 2H), 1.95 (dd, *J* = 13.6, 6.7 Hz, 1H), 1.83 (d, *J* = 11.6 Hz, 1H), 1.49 (s, 9H).

5 Step C - Synthesis of intermediate F-3

[0115] Intermediate F-2 (680 mg, 1.048 mmol) was dissolved in DCM (10.5 mL) and TFA (1.211 mL, 15.72 mmol) was added. The reaction was stirred at room temperature for 1.5 h. The reaction solution was diluted in 15 mL DCM and poured directly onto a 10 g Agilent Bond Elut SCX column. The column was flushed with MeOH to remove excess TFA, then eluted with 7N
10 NH₃/MeOH solution to afford F-3. LC/MS: [M+H]⁺ = 549.36.

Step D - Synthesis of intermediate F-4

[0116] Intermediate F-3 (453 mg, 0.826 mmol) was dissolved in DMF (8257 µl) and DIPEA (361 µl, 2.064 mmol) and acetic anhydride (93 µl, 0.991 mmol) were added. After stirring at room temp for 1.5 hours, the reaction solution was diluted with EtOAc, washed with water four
15 times, then washed with brine, dried over magnesium sulfate and concentrated to afford F-4. LC/MS: [M+H]⁺ = 591.4.

Step E - Synthesis of intermediate F-5

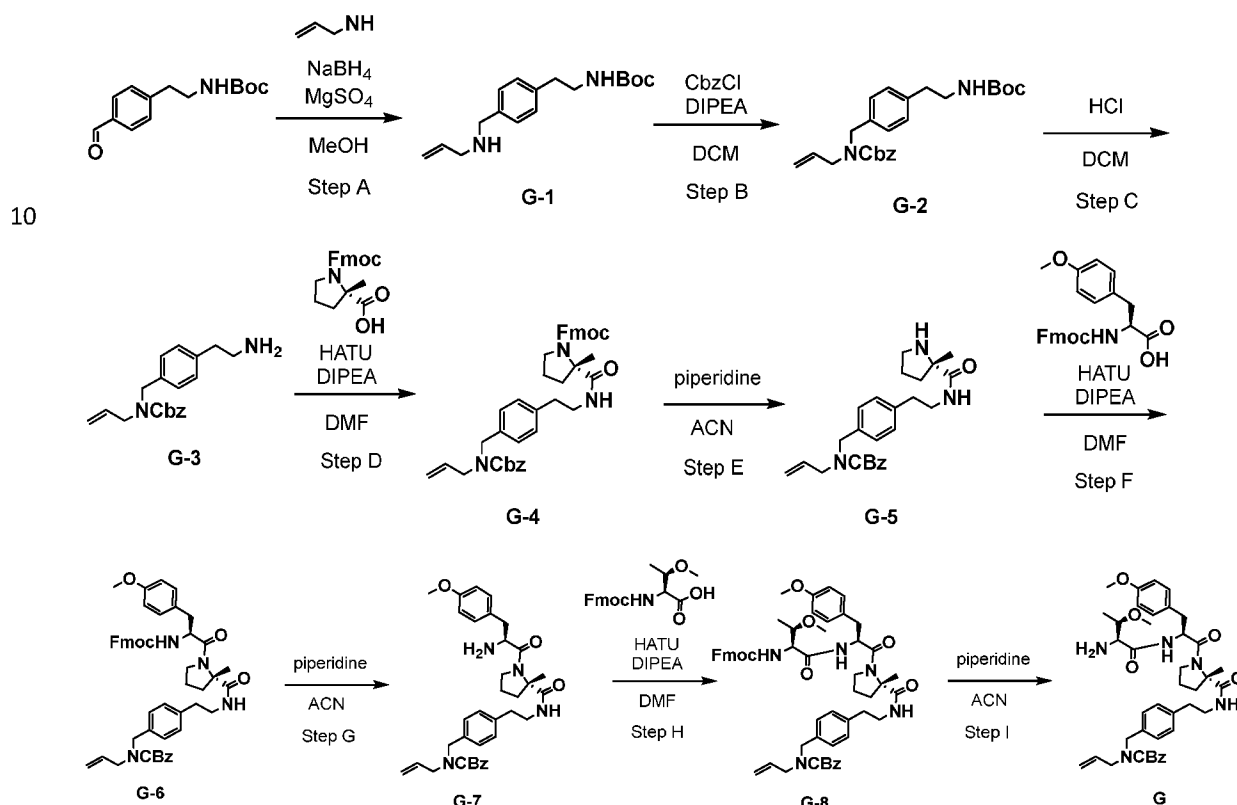
[0117] Intermediate F-4 (0.3391 g, 0.574 mmol) was dissolved in DMF (7 mL) and cesium carbonate (0.636 g, 1.952 mmol) was added followed by allyl bromide (0.176 mL, 2.038 mmol).
20 The reaction was stirred at room temperature for 18 h. Water was added, and precipitate was filtered to afford F-5. LC/MS: [M+H]⁺ = 631.39. ¹H NMR (500 MHz, Chloroform-*d*) δ 7.18 (dd, *J* = 10.2, 6.0 Hz, 2H), 7.00 (d, *J* = 7.9 Hz, 1H), 6.96 – 6.86 (m, 1H), 6.82 (s, 1H), 6.48 (d, *J* = 7.2 Hz, 1H), 6.01 – 5.90 (m, 2H), 5.64 – 5.42 (m, 3H), 5.18 (d, *J* = 10.2 Hz, 2H), 5.06 (d, *J* = 17.1 Hz, 2H), 4.81 (d, *J* = 4.2 Hz, 2H), 4.53 (d, *J* = 7.3 Hz, 1H), 4.32 (d, *J* = 15.1 Hz, 2H), 4.11 (d, *J* =
25 3.8 Hz, 1H), 3.91 (d, *J* = 15.1 Hz, 2H), 3.74 (s, 3H), 3.33 – 3.16 (m, 4H), 3.07 (dd, *J* = 14.6, 4.1 Hz, 2H), 2.81 – 2.70 (m, 1H), 2.10 (s, 3H), 2.06 – 1.97 (m, 1H), 1.94-1.85 (m, 2H).

Step F - Synthesis of intermediate F

[0118] Intermediate F-5 (0.291 g, 0.461 mmol) was dissolved in THF (8.8 mL), MeOH (2.2 mL), and water (4.4 mL), and LiOH (0.055 g, 2.307 mmol) was then added. After stirring at
30 room temperature for 7 h, the reaction was concentrated to ~1/2 vol and acidified with 1N HCl to pH 4-5. EtOAc was added and the layers were separated. The aqueous layer was extracted with EtOAc (3x15 mL). The combined organic layer was washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was dissolved in a small amount of DCM and triturated with hexane provided a solid. The solid was collected via filtration to afford F. LC/MS:
35 [M+H]⁺ = 617.34. ¹H NMR (500 MHz, Methanol-*d*₄) δ 7.96 (d, *J* = 37.6 Hz, 1H), 7.27 (s, 2H), 7.20 (s, 2H), 7.08 (d, *J* = 7.4 Hz, 1H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.93 (s, 1H), 6.92-6.87 (m, 1H), 6.09 – 5.91 (m, 2H), 5.83 – 5.64 (m, 2H), 5.48 (d, *J* = 14.9 Hz, 1H), 5.14 (d, *J* = 10.3 Hz, 2H),

- 5 5.02 (d, $J = 17.1$ Hz, 2H), 4.79 – 4.59 (m, 3H), 4.25 (d, $J = 14.7$ Hz, 2H), 4.16 (d, $J = 3.7$ Hz, 1H), 3.95 (d, $J = 14.4$ Hz, 2H), 3.75 (d, $J = 9.7$ Hz, 1H), 3.29 (d, $J = 7.8$ Hz, 2H), 3.22-3.15 (s, 1H), 3.05-2.96 (m, 4H), 1.99 (s, 3H).

Preparation of Intermediate G



Step A - Synthesis of intermediate G-1

- [0119] Tert-butyl 4-formylphenethylcarbamate (4.69 g, 18.81 mmol) was dissolved in MeOH (40 ml) and magnesium sulfate (4.53 g, 37.6 mmol) was added followed by allylamine (1.422 ml, 19.00 mmol). After stirring at room temperature for 16 h, the reaction mixture was cooled to 0 °C, and sodium borohydride (0.712 g, 18.81 mmol) was added. The resulting mixture was stirred at 0 °C for 20 min, followed by quenching by addition of water (4 mL). The mixture was filtered and the filtrate was concentrated. The residue obtained was resuspended in DCM (600 mL) and NaOH (0.1 N, 300 mL), the organic phase was dried over Na₂SO₄, concentrated, and the residue was purified over silica gel (Isco 220 g, eluted with DCM/MeOH 99:1 to 85:15) to afford G-1. LC/MS: $[M+H]^+ = 291.43$. ¹H NMR (500 MHz, Chloroform-*d*) δ 7.33 (t, $J = 8.3$ Hz, 2H), 7.20 (t, $J = 6.8$ Hz, 2H), 5.99 (ddt, $J = 16.5, 10.3, 6.2$ Hz, 1H), 5.36 – 5.19 (m, 2H), 4.56 (s, 1H), 3.84 (s, 2H), 3.40 – 3.36 (m, 3H), 3.33 (d, $J = 6.2$ Hz, 2H), 2.80 (t, $J = 6.8$ Hz, 2H), 1.46 (s, 9H).

5 Step B - Synthesis of intermediate G-2

[0120] Intermediate G-1 (3.39 g, 11.67 mmol) was dissolved in DCM (60 ml) and DIPEA (2.039 ml, 11.67 mmol) was added followed by dropwise addition of CBz-Cl (1.633 ml, 11.44 mmol) at 0 °C. After stirring for 30 min, the reaction mixture was quenched at 0 °C with saturated aqueous sodium bicarbonate and extracted with DCM. The organic fractions were
10 combined and washed with brine, dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by column chromatography over silica gel (Isco 220 g and eluted with Hexane/EtOAc 99:1 to 70:30) to afford G-2. LC/MS: [M+H]⁺ = 447.34. ¹H NMR (500 MHz, Chloroform-*d*) δ 7.38 (s, 2H), 7.34 (s, 2H), 7.22 (s, 1H), 7.14 (s, 2H), 5.78 (s, 1H), 5.21 (s, 2H), 5.18-5.07 (m, 2H), 4.55 (s, 1H), 4.48 (d, *J* = 8.5 Hz, 2H), 3.91 (s, 1H), 3.85 (s, 1H), 3.38 (s, 2H),
15 2.80 (t, *J* = 6.9 Hz, 2H), 1.46 (s, 9H).

Step C - Synthesis of intermediate G-3

[0121] Intermediate G-2 (4.30 g, 10.13 mmol) was dissolved in DCM (25 ml) at room temperature and HCl 4N in dioxane (10.13 ml, 40.5 mmol) was added. After stirring for 3 h, the reaction was concentrated. To the residue, DCM, toluene, and acetonitrile was added, and the
20 mixture was concentrated again. The mixture was dissolved in 30% acetonitrile and lyophilized to afford G-3. LC/MS: [M+H]⁺ = 325.28.

Step D - Synthesis of intermediate G-4

[0122] Intermediate G-3 (238 mg, 0.678 mmol) and benzyl allyl(4-(2-aminoethyl)benzyl)carbamate 0395898-0161 (200 mg, 0.616 mmol) were dissolved in DMF
25 (3082 μl), and HATU (258 mg, 0.678 mmol) was added, followed by DIPEA (323 μl, 1.849 mmol). After stirring at room temp for 2 hours, the mixture was diluted with EtOAc, washed thrice with water, then washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified via flash column chromatography on a 12g silica gold column using 0-50% EtOAc in hexane gradient to afford G-4. LC/MS: [M+H]⁺ = 658.42.

30 Step E - Synthesis of intermediate G-5

[0123] Intermediate G-4 (321 mg, 0.488 mmol) was dissolved in ACN (2440 μl) and piperidine (483 μl, 4.88 mmol) was added. After stirring for 1 hour, the reaction mixture was filtered to remove solids, washed thrice with hexane and concentrated to afford G-5. LC/MS: [M+H]⁺ = 436.35.

35 Step F - Synthesis of intermediate G-6

[0124] Intermediate G-5 (176.4 mg, 0.405 mmol) and (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-methoxyphenyl)propanoic acid (178 mg, 0.425 mmol) were dissolved in DMF (2025 μl) and HATU (159 mg, 0.417 mmol) and DIPEA (177 μl, 1.012 mmol)

5 were then added. After stirring at room temp for 1.5 hours, the reaction mixture was diluted with EtOAc and washed with water three times, then brine, then dried over magnesium sulfate and concentrated. The residue was purified via flash column chromatography on a 12g silica gold column using 0-60% EtOAc in hexane gradient to afford G-6. LC/MS: $[M+H]^+ = 835.47$.

Step G - Synthesis of intermediate G-7

10 **[0125]** Intermediate G-6 (277 mg, 0.332 mmol) was dissolved in ACN (1659 μ l) and piperidine (263 μ l, 2.65 mmol) was added. After stirring at room temp for 2 hours, the reaction mixture was washed four times with hexane and concentrated to afford G-7. LC/MS: $[M+H]^+ = 613.37$.

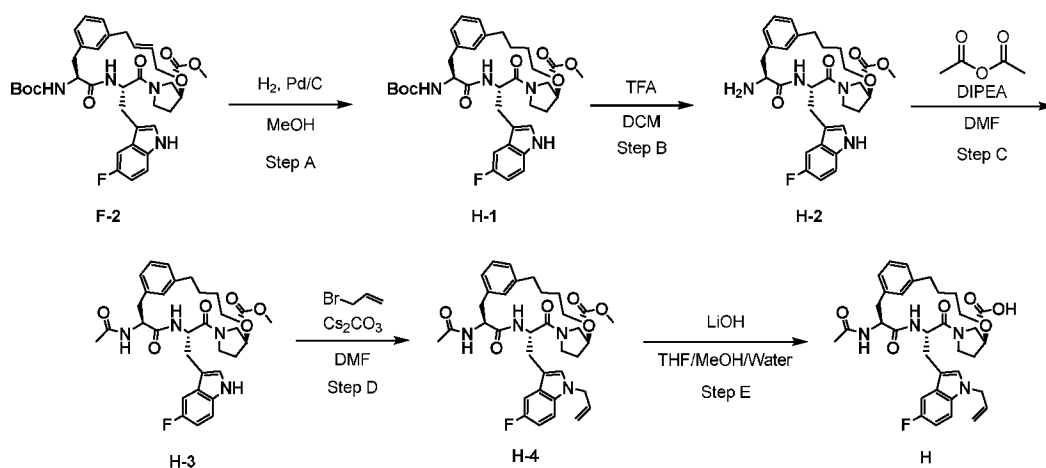
Step H - Synthesis of intermediate G-8

15 **[0126]** Intermediate G-7 (213 mg, 0.348 mmol) was dissolved in DMF (1738 μ l), and then (2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxybutanoic acid (130 mg, 0.365 mmol), HATU (139 mg, 0.365 mmol), and DIPEA (182 μ l, 1.043 mmol) were added. After stirring at room temp for 1.5 hours, the reaction mixture was diluted with EtOAc, washed thrice with water, then washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified via flash column chromatography on a 12g silica gold column (dry loading) 20 using 0-100% EtOAc in hexane gradient to afford G-8. LC/MS: $[M+H]^+ = 950.67$.

Step I - Synthesis of intermediate G

[0127] Intermediate G-8 (311 mg, 0.327 mmol) was dissolved in ACN (3273 μ l) and piperidine (194 μ l, 1.964 mmol) was added. After stirring at room temp 1 hour, the reaction mixture was filtered to remove solids. Next, it was washed thrice with hexane and concentrated to afford G. 25 LC/MS: $[M+H]^+ = 728.54$.

Preparation of Intermediate H



5 Step A - Synthesis of intermediate H-1

[0128] Intermediate F-2 (2g, 3.08 mmol) was dissolved in MeOH (30.8 ml) and Pd-C (0.033 g, 0.308 mmol) was added. The flask was evacuated and backfilled with H₂ (1 atm, from balloon) three times. After stirring at room temp for 2 hours, the reaction mixture was filtered through celite and concentrated to afford H-1. LC/MS: [M+H]⁺ = 651.36.

10 Step B - Synthesis of intermediate H-2

[0129] Intermediate H-1 (1.98 g, 3.04 mmol) was dissolved in DCM (15.21 ml) and TFA (2.344 ml, 30.4 mmol) was added. After stirring at room temp for 2 hours, the reaction mixture was concentrated, redissolved in ACN/Water and dried via lyophilization to afford H-2. LC/MS: [M+H]⁺ = 551.29.

15 Step C - Synthesis of intermediate H-3

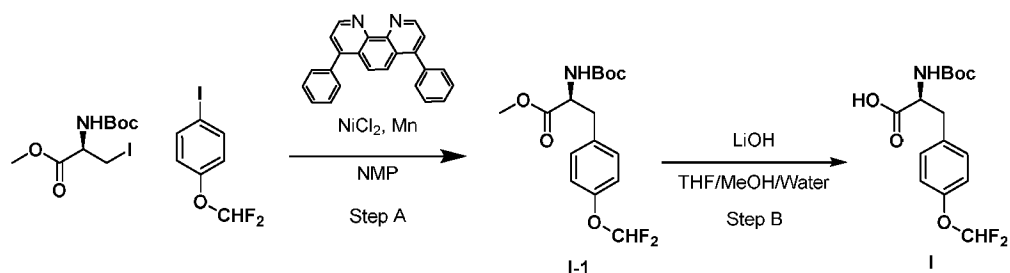
[0130] Intermediate H-2 (1.675 g, 3.04 mmol) was dissolved in DMF (15.21 ml) and DIPEA (1.063 ml, 6.08 mmol) and acetic anhydride (0.631 ml, 6.69 mmol) were added. After stirring at room temp for 1 hour, the reaction mixture was washed with water and filtered to remove precipitate to afford H-3. LC/MS: [M+H]⁺ = 593.32. ¹H NMR (500 MHz, Chloroform-*d*) δ 8.08 (s, 1H), 7.19 (s, 2H), 7.01 (d, *J* = 7.5 Hz, 2H), 6.94 (t, *J* = 8.9 Hz, 1H), 6.73 (s, 1H), 6.37 (d, *J* = 6.4 Hz, 1H), 6.24 (s, 1H), 5.07 (d, *J* = 6.5 Hz, 1H), 4.86 (s, 1H), 4.53 (s, 2H), 4.03 (s, 1H), 3.77 (d, *J* = 17.3 Hz, 4H), 3.47 (t, *J* = 10.2 Hz, 1H), 3.37 (t, *J* = 9.3 Hz, 1H), 3.30 (dd, *J* = 15.0, 6.8 Hz, 1H), 3.13 (d, *J* = 9.9 Hz, 1H), 3.01 (s, 1H), 2.95 (d, *J* = 36.3 Hz, 1H), 2.91 (s, 1H), 2.57 – 2.37 (m, 2H), 1.99 (d, *J* = 11.2 Hz, 2H), 1.69 (s, 2H), 1.58 (s, 3H), 1.53 – 1.41 (m, 3H).

25 Step D - Synthesis of intermediate H-4

[0131] Intermediate H-3 (1g, 1.687 mmol) was dissolved in DMF (5.62 ml) and cesium carbonate (1.869 g, 5.74 mmol) was added, followed by 3-bromoprop-1-ene (0.511 ml, 5.91 mmol). After stirring overnight at room temp, the reaction mixture was diluted with EtOAc, washed thrice with water, then washed with brine, dried over magnesium sulfate, and concentrated to obtain H-4. LC/MS: [M+H]⁺ = 633.36.

30 Step E - Synthesis of intermediate H

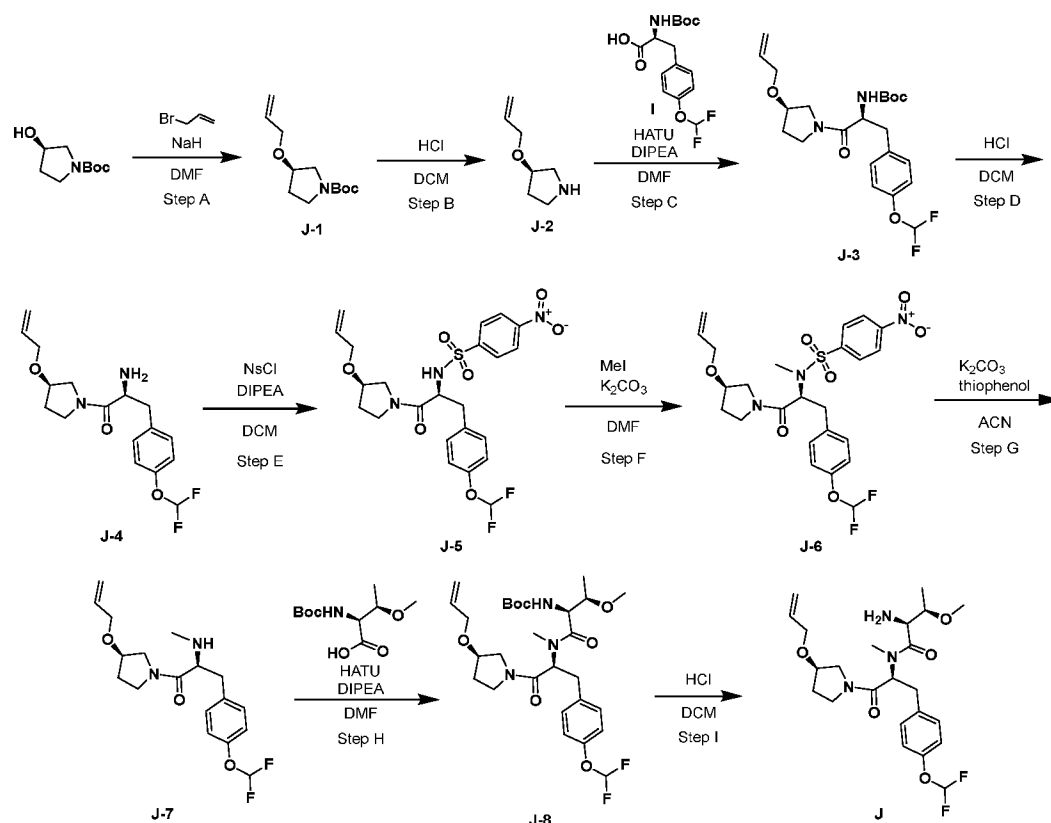
[0132] Intermediate H-4 (143.2 mg, 0.226 mmol) was dissolved in THF (4311 μl), MeOH (2155 μl), and water (1078 μl), and lithium hydroxide (27.1 mg, 1.132 mmol) was added. After stirring at room temp for 1.5 hours, the reaction mixture was acidified to pH ~2 with 1N HCl, extracted three times with EtOAc, washed with brine, dried over magnesium sulfate, and concentrated to afford H. LC/MS: [M+H]⁺ = 619.33.

5 **Preparation of Intermediate I***Step A - Synthesis of intermediate I-1*

[0133] Nickel(II) chloride (0.098 g, 0.760 mmol), manganese (1.252 g, 22.79 mmol), and 4,7-diphenyl-1,10-phenanthroline (0.252 g, 0.760 mmol) were added to a vial. After the vial was degassed by vacuum/N₂ three times, degassed NMP (26 ml) was added. The resulting mixture was heated at 80 °C for 20 min. After cooling to RT, (R)-methyl 2-((tert-butoxycarbonyl)amino)-3-iodopropanoate (2.5 g, 7.60 mmol) and 1-(difluoromethoxy)-4-iodobenzene (2.461 g, 9.11 mmol) in 0.9 mL NMP was added. The resulting mixture was further degassed thrice by vacuum/N₂, followed by stirring at RT under N for 48 hours. The mixture was filtered through celite, and the filtrate was partitioned between EtOAc/brine (300 mL/150 mL). The organic phase was washed with brine (3x100 mL), dried over sodium sulfate, and concentrated, and the residue was purified on silica gel column (120g) using 0-40%EtOAc/hexane as and eluted solvents to afford I-1. LC/MS: [M+H]⁺ = 346.17.

20 *Step B - Synthesis of intermediate I*

[0134] Intermediate I-1 (1.85 g, 5.36 mmol) was dissolved in tetrahydrofuran (20 ml), MeOH (7.00 ml), and water (7 ml) at 0 °C, and LiOH (10.71 ml, 10.71 mmol) was added. The resulting solution was stirred at 0 °C for 3h. Next the volatile phase was evaporated using a rotary evaporator. The aqueous phase was acidified to pH 4, then extracted with DCM (3x100 mL). The combined organic phase extract was dried over Na₂SO₄, and concentrated to afford I. LC/MS: [M+H]⁺ = 332.10.

5 **Preparation of Intermediate J****Step A - Synthesis of intermediate J-1**

[0135] (R)-tert-butyl 3-(allyloxy)pyrrolidine-1-carboxylate (3.72 g, 16.37 mmol) was dissolved in DMF (60 ml) and was cooled to 0 °C. Next, sodium hydride (1.031 g, 25.8 mmol) was added. After stirring at 0 °C for 30 min, allyl bromide (2.046 ml, 23.64 mmol) was added and the mixture was stirred at 0 °C for 30 min, then at RT for 1 h. The reaction was quenched by dropwise addition of sat. aq. sodium bicarbonate (20 mL) at 0 °C. The mixture was partitioned between EtOAc (300 mL) and water (200 mL). The aqueous phase was extracted with EtOAc (200 mL). Next, the organic phase was combined and washed with water (3x200 mL), dried over sodium sulfate, and concentrated. The residue was purified on silica gel column (220g) using 10-40% EtOAc/hexane as and eluted solvents to afford J-1. LC/MS: $[M+H]^+ = 228.17$.

Step B - Synthesis of intermediate J-2

[0136] Intermediate J-1 (3.72 g, 16.37 mmol) was dissolved in DCM (20 ml), and HCl (4N in dioxane) (40.9 ml, 164 mmol) was added. After stirring at RT for 4 h, the reaction mixture was concentrated and the residue was dissolved in ACN/water (20 mL, 1;1) and lyophilized to afford J-2. ^1H NMR (500 MHz, Methanol- d_4) δ 5.94 (ddt, $J = 16.2, 10.6, 5.4$ Hz, 1H), 5.33 (d, $J = 17.3$

5 Hz, 1H), 5.21 (d, $J = 10.4$ Hz, 1H), 4.12 (q, $J = 6.9$ Hz, 2H), 3.68 (s, 2H), 3.42 (dd, $J = 19.0, 10.5$ Hz, 3H), 3.27 (d, $J = 12.6$ Hz, 1H), 2.25 (d, $J = 13.9$ Hz, 1H), 2.08 (d, $J = 9.6$ Hz, 1H).

Step C- Synthesis of intermediate J-3

[0137] Intermediate J-2 (0.634 g, 3.87 mmol) and I (1.167 g, 3.52 mmol) was dissolved in DMF (10 ml), and HATU (1.406 g, 3.70 mmol) and DIPEA (1.846 ml, 10.57 mmol) were added. After stirring at RT for 30 min, the aqueous phase was partitioned between saturated sodium bicarbonate (100 mL) and DCM (100 mL). Next, the aqueous phase was further extracted with DCM (2x100 mL). The combined organic phase was dried over sodium sulfate, concentrated, and then purified on reverse phase MPLC on C18 275 g column using 5-100% ACN (0.05%TFA) as and eluted solvents to afford J-3. LC/MS: $[M+H]^+ = 441.2$.

15 Step D- Synthesis of intermediate J-4

[0138] Intermediate J-3 (1.56 g, 3.54 mmol) was dissolved in DCM (10 ml) and HCl (4N in dioxane) (26.6 ml, 106 mmol) was then added. After stirring at RT for 1.5 h, the reaction mixture was concentrated to afford J-4. LC/MS: $[M+H]^+ = 341.3$.

Step E- Synthesis of intermediate J-5

20 [0139] Intermediate J-4 (0.70 g, 1.858 mmol) was dissolved in DCM (30 ml) at 0 °C, and DIPEA (1.298 ml, 7.43 mmol) and 4-nitrobenzenesulfonyl chloride (0.453 g, 2.043 mmol) were added. After stirring at 0 °C for 0.5 h, the solution was partitioned between DCM (200 mL) and water. Next, the aqueous phase was further extracted with DCM (2x100 mL). The combined organic phase was dried over sodium sulfate, concentrated, and the residue was purified on silica gel column (120g) using 10-100% EtOAc/hexane as and eluted solvents to afford J-5. LC/MS: $[M+H]^+ = 526.1$.

Step F- Synthesis of intermediate J-6

30 [0140] Intermediate J-5 (0.88 g, 1.675 mmol) was dissolved in DMF (20 ml) at 0 °C, and potassium carbonate (0.694 g, 5.02 mmol) and iodomethane (0.178 ml, 2.85 mmol), in DMF (1 mL), was added dropwise. After stirring at 0 °C for 2 h, the mixture was partitioned between EtOAc (200 mL) and brine (100 ML). Next, the organic phase was washed with brine (2x100 mL), dried over sodium sulfate, concentrated, and the residue was purified on a silica gel column (80 g) using 10-80% EtOAc/hexane as and eluted solvents to afford J-6. LC/MS: $[M+H]^+ = 540.3$. ^1H NMR (500 MHz, Chloroform- d) δ 8.31 (dd, $J = 15.4, 8.8$ Hz, 1H), 7.91 (d, $J = 8.8$ Hz, 1H), 7.84 (d, $J = 8.8$ Hz, 1H), 7.22 (d, $J = 8.4$ Hz, 1H), 7.08 (td, $J = 24.5, 23.2, 8.4$ Hz, 2H), 6.66 (d, $J = 9.0$ Hz, 1H), 6.51 (d, $J = 9.2$ Hz, 1H), 6.36 (d, $J = 9.4$ Hz, 1H), 5.90 (dddt, $J = 33.1, 16.0, 10.6, 5.6$ Hz, 1H), 5.37 – 5.30 (m, 1H), 5.26 (d, $J = 15.7$ Hz, 1H), 5.21 (d, $J = 7.9$ Hz, 1H), 4.93 (ddd, $J = 22.6, 9.6, 5.9$ Hz, 1H), 4.15 (q, $J = 7.1$ Hz, 1H), 3.99 – 3.91 (m, 1H), 3.82 (d, $J = 10.9$

5 Hz, 1H), 3.53 (d, $J = 13.4$ Hz, 1H), 3.39 – 3.27 (m, 1H), 3.24 – 3.03 (m, 3H), 2.90 (dd, $J = 12.8$, 5.3 Hz, 1H), 2.66 (dd, $J = 13.4$, 6.3 Hz, 1H), 2.49 (dd, $J = 11.3$, 3.8 Hz, 1H), 2.03 (d, $J = 6.3$ Hz, 1H), 1.62 (d, $J = 9.6$ Hz, 1H).

Step G- Synthesis of intermediate J-7

[0141] Intermediate J-6 (0.85 g, 1.575 mmol) was dissolved in ACN (25 ml) and potassium carbonate (0.653 g, 4.73 mmol) and thiophenol (0.973 ml, 9.45 mmol) were then added. After stirring at rt for 4h, it was filtered. Next the filtrate was concentrated, and the residue was purified on silica gel column (80g) using 0-20% MeOH/DCM as and eluted solvents to afford J-7. LC/MS: $[M+H]^+ = 355.4$.

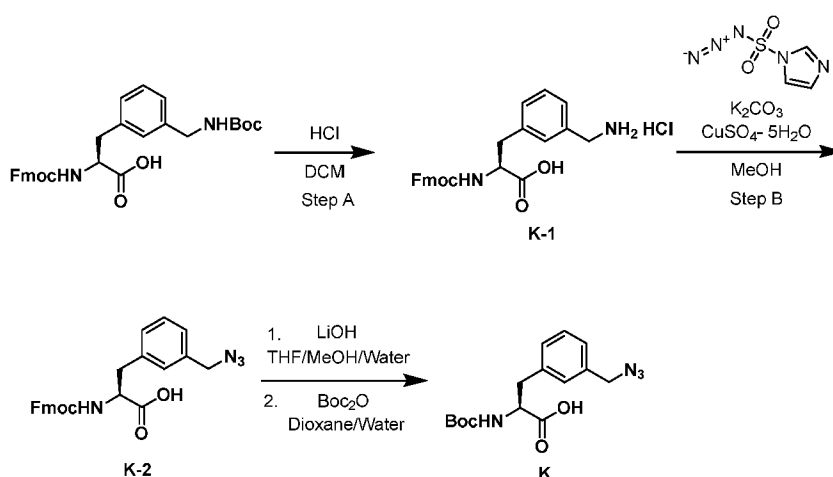
Step H- Synthesis of intermediate J-8

15 [0142] Intermediate J-7 (0.47g, 1.326 mmol) was dissolved in DMF (5 ml) and boc-O-methyl-L-threonine (0.325 g, 1.393 mmol), HATU (0.529 g, 1.393 mmol), and DIPEA (0.695 ml, 3.98 mmol) were then added. After stirring at RT for 1h, the residue was purified on reverse phase MPLC C18 column (130g) using 5-100% ACN (0.05%TFA) to afford J-8. LC/MS: $[M+H]^+ = 570.5$.

20 Step I- Synthesis of intermediate J

[0143] Intermediate J-8 (0.72 g, 1.264 mmol) was dissolved in DCM (6 ml) and HCl (9.48 ml, 37.9 mmol) were then added. After stirring at rt for 2h, the reaction mixture was concentrated to afford J. LC/MS: $[M+H]^+ = 470.4$.

25 Preparation of Intermediate K



5 Step A - Synthesis of intermediate K-1

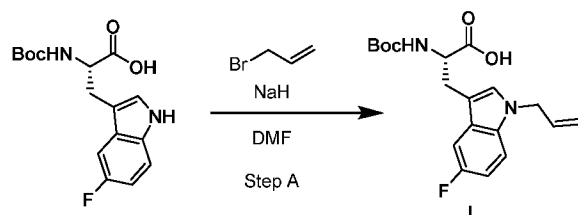
(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(3-((((tert-butoxycarbonyl)amino)methyl)phenyl)propanoic acid (750 mg, 1.452 mmol) was dissolved in HCl (4N in dioxane) (15 ml, 60.0 mmol) and stirred at RT overnight. Precipitate was filtered to afford K-1. LC/MS: $[M+H]^+ = 417.4$.

10 Step B- Synthesis of intermediate K-2

[0144] Intermediate K-1 was dissolved in dry MeOH (18.21 ml) under N₂. At 0 °C potassium carbonate (2.82 g, 20.40 mmol), 1H-imidazole-1-sulfonyl azide (1.262 g, 7.29 mmol) and copper (II) sulfate pentahydrate (9.10 mg, 0.036 mmol) were added. The reaction mixture was stirred at 0 °C for 2 hours. The reaction was diluted with EtOAc and washed with HCl (0.5N) 100mL. The organic layer was then dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash column chromatography using 7-70% gradient of EtOAc in petroleum ether (0.5% acetic acid) to afford K-2. LC/MS: $[M+H]^+ = 460.4$.

15 Step C- Synthesis of intermediate K

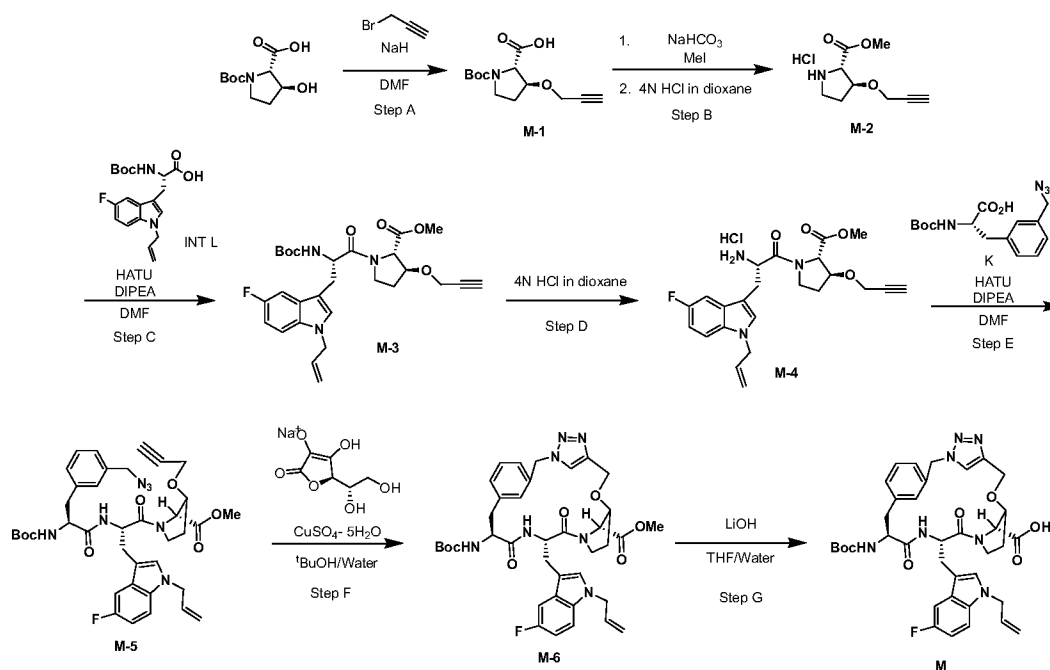
[0145] Aqueous 1N sodium hydroxide (13.56 ml, 13.56 mmol) was added dropwise to a solution of intermediate K-2 in THF (40 ml)/MeOH (20 ml)/ Water (40.0 ml) while cooled in an ice bath. The resulting mixture was stirred for a period of 2h. The THF and methanol were removed under reduced pressure and to the aqueous solution was added Dioxane (20.00 ml)/Water (20 ml). Di-tert-butyl dicarbonate (1.282 g, 5.88 mmol) was added at 0°C and stirring was continued overnight. The volatiles were removed under reduced pressure and the resulting aqueous mixture was washed with ether. Next dichloromethane was added, and the aqueous phase was acidified with 1N aq. HCl. The aqueous phase was separated and further extracted with dichloromethane. The combined organic phases were dried over magnesium sulfate and the volatiles removed under reduced pressure to afford K. LC/MS: $[M+H]^+ = 343.25$.

30 Preparation of Intermediate L

[0146] To the solution of (S)-2-((tert-butoxycarbonyl)amino)-3-(5-fluoro-1H-indol-3-yl)propanoic acid (2.08 g, 6.45 mmol) in DMF (40 ml) at 0 °C was added NaH (0.568 g, 14.19

5 mmol), the resulting solution was stirred at 0 °C for 50 min, followed by addition of allyl
bromide (1.172 ml, 13.54 mmol) dropwise. After stirring at 0 °C for 1.5h, it was quenched by
addition of 1N HCl. The solution was then partitioned between EtOAc and water. The organic
phase was washed with brine (2x100 mL), dried over sodium sulfate, concentrated and the
residue was purified on silica gel column (120 g) using 0-10%MeOH/DCM as and eluted
10 solvents to afford L. LC/MS: $[M+H]^+ = 363.33$.

Preparation of Intermediate M



Step A - Synthesis of intermediate M-1

15 **[0147]** Under argon atmosphere, to the solution of (2S,3S)-1-(tert-butoxycarbonyl)-3-
hydroxypyrrolidine-2-carboxylic acid (1.15 g, 4.72 mmol) in THF (50 mL) were added DMF (10
mL) and sodium hydride (660 mg, 16.50 mmol) at 0 °C. After stirring for 15 minutes, 3-
bromoprop-1-yne (1.20 g, 10.09 mmol) was added at 0 °C. After warming to 25 °C, the reaction
mixture was stirred for 16 hours. To the resulting mixture, saturated sodium bicarbonate (20 mL)
20 was added followed by extraction with ethyl ether (2 X 60 mL). Next the pH of the aqueous
phase was adjusted to 4 with aqueous HCl (1 M) and extracted with EA (100 mL X 5). The
combined organic layers were concentrated under reduced pressure to afford M-1. LC/MS:
 $[M+H]^+ = 269.00$.

5 Step B - Synthesis of intermediate M-2

[0148] Intermediate M-1 (1.12 g, 4.16 mmol) was dissolved in DMF (25 ml) and sodium hydrogen carbonate (0.699 g, 8.32 mmol) and iodomethane (1.300 ml, 20.80 mmol) were then added. After stirring at RT for 18 hrs, the reaction was poured into water, extracted twice with 100ml MTBE, washed with 100ml water twice, washed with 100ml brine, dried over magnesium sulfate, and concentrated to an oil. Next DCM was added, followed by 4N HCl in dioxane (1.040 ml, 4.16 mmol). The mixture was stirred under N₂ for 1h and then concentrated to afford M-2. LC/MS: [M+H]⁺ = 185.07.

Step C - Synthesis of intermediate M-3

[0149] Intermediate L (388 mg, 1.071 mmol) and M-2 (235 mg, 1.071 mmol) and HATU (489 mg, 1.285 mmol) were dissolved in DMF (10 ml) at 0°C and DIPEA (0.746 ml, 4.28 mmol) was then added. The mixture was allowed to warm up to rt and stirred for 4h. The reaction was quenched with water and extracted with EtOAc. The combined organic phase was washed with water (x2), dried over magnesium sulfate, and concentrated. The residue was purified by column chromatography on silica gel 12g and eluted with 0-100% EtAOAc/hexane to afford M-3. LC/MS: [M+H]⁺ = 529.37.

Step D - Synthesis of intermediate M-4

[0150] Intermediate M-3 (529 mg, 1.003 mmol) was dissolved in 4N hydrogen chloride (36.6 mg, 1.003 mmol). After stirring for 1h at room temperature, it was concentrated to afford M-4. LC/MS: [M+H]⁺ = 428.27.

25 Step E - Synthesis of intermediate M-5

[0151] DIPEA (698 µl, 4.01 mmol) was added dropwise to a stirred mixture of intermediate K (321 mg, 1.002 mmol) and M-4 (465mg, 1.002 mmol) and HATU (457 mg, 1.203 mmol) in DMF (13 mL). After stirring at 0°C, the reaction was then warmed up to room temperature for 1hr. The mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 80g and eluted with 0-100% EtOAc/hexane to afford M-5. LC/MS: [M+H]⁺ = 730.27.

Step F - Synthesis of intermediate M-6

[0152] Intermediate M-5 (577 mg, 0.791 mmol) and sodium (R)-5-((S)-1,2-dihydroxyethyl)-4-hydroxy-2-oxo-2,5-dihydrofuran-3-olate (2349 mg, 11.86 mmol) were dissolved in tBuOH (600 ml) and Water (300 ml) and copper (II) sulfate (1262 mg, 7.91 mmol) in Water (30 ml) was then added dropwise. After stirring at room temperature for 2h, the tBuOH was removed under reduced pressure and sat. aq. ammonium chloride and EtOAc were added. The aqueous phase

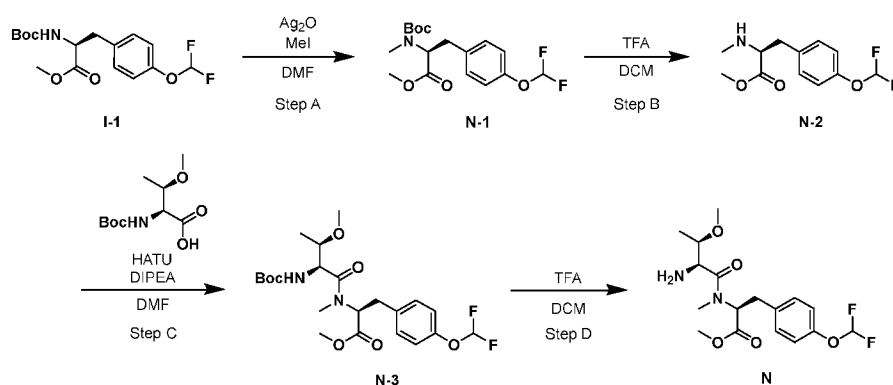
5 was separated and further extracted with EtOAc. The combined organic phase was washed with brine, dried (MgSO₄) and the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 4g and eluted with 0-10%MeOH/DCM to afford M-6. LC/MS: [M+H]⁺ = 730.51.

Step G - Synthesis of intermediate M

10 **[0153]** Intermediate M-6 (227 mg, 0.311 mmol) was dissolved in THF (2916 μ l) and aq 1M lithium hydroxide (467 μ l, 0.467 mmol) was then added. After stirring at RT overnight, DCM was added, and the reaction solution was acidified by 1N HCl. Next the mixture was extracted with DCM 2 times. Next the organic layers were combined and separated, washed with brine, separated, dried over MgSO₄, filtered and concentrated to afford M. LC/MS: [M+H]⁺ = 716.47.

15

Preparation of Intermediate N



Step A - Synthesis of intermediate N-1

20 **[0154]** To a solution of intermediate I-1 (5.7 g, 14.19 mmol) in DMF (30 ml) was added silver oxide (16.45 g, 71.0 mmol) and methyl iodide (11.54 ml, 185 mmol). The resulting mixture was heated at 45 °C for 4h. After cooling to 0 °C, the mixture was filtered through celite, washed with EtOAc. The filtrate was partitioned between EtOAc (300 mL) and brine (200 mL). Next the organic phase was washed with brine (2x200 mL), dried over sodium sulfate, concentrated and the residue was purified on silica gel column (80g) using 0-60%EtOAc/hexane as and eluted
 25 solvents to afford N-1. LC/MS: [M+H]⁺ = 360.2. ¹H NMR (500 MHz, Chloroform-*d*) δ 7.21 (dd, *J* = 18.1, 8.1 Hz, 2H), 7.07 (t, *J* = 8.9 Hz, 2H), 6.49 (t, *J* = 73.7 Hz, 1H), 4.95-4.53 (m, 1H), 3.77 (s, 3H), 3.38 – 3.22 (m, 1H), 3.02 (dd, *J* = 14.4, 10.8 Hz, 1H), 2.74 (d, *J* = 11.1 Hz, 3H), 1.38 (d, *J* = 16.6 Hz, 9H).

Step B - Synthesis of intermediate N-2

30 **[0155]** Intermediate N-1 (0.76 g, 2.115 mmol) was dissolved in DCM (3 ml) and TFA (3 ml, 38.9 mmol) was then added. After stirring at RT for 40 min, the reaction mixture was

5 concentrated and the residue was dissolved in DCM/toluene (1:1, 10 mL) and treated with HCl (2.64 mL, 10.57 mmol). The resulting solution was concentrated to afford N-2. LC/MS: $[M+H]^+ = 260.1$.

Step C - Synthesis of intermediate N-3

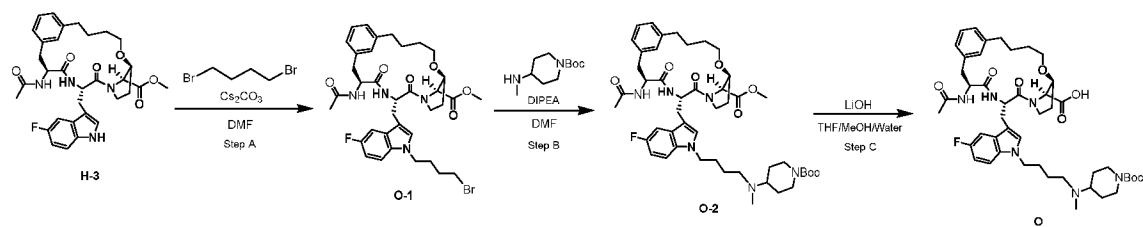
[0156] To the solution of intermediate N-2 (0.625 g, 2.115 mmol) and N-(tert-butoxycarbonyl)-
 10 O-methyl-L-threonine (0.518 g, 2.221 mmol) in DMF (8 mL) was added HATU (0.844 g, 2.221 mmol) and DIPEA (2.216 mL, 12.69 mmol). After stirring at RT for 1 h, the reaction mixture was partitioned between EtOAc (200 mL) and brine (100 mL). The organic phase was further washed with brine (2x100 mL), dried over sodium sulfate, concentrated. The residue was purified on silica gel column (80g) using 5-70% EtOAc/hexane as and eluted solvents to afford N-3. LC/MS:
 15 $[M+H]^+ = 475.4$. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.23 (d, $J = 8.4$ Hz, 2H), 7.04 (d, $J = 8.4$ Hz, 2H), 6.49 (t, $J = 74.1$ Hz, 1H), 5.41 – 5.22 (m, 2H), 4.57 (dd, $J = 8.5, 4.1$ Hz, 1H), 3.74 (s, 3H), 3.64 – 3.56 (m, 1H), 3.40 (d, $J = 5.8$ Hz, 1H), 3.35 (s, 3H), 3.03 (d, $J = 9.8$ Hz, 2H), 3.00 (s, 3H), 1.44 (s, 9H), 1.18 (d, $J = 6.3$ Hz, 2H).

Step D - Synthesis of intermediate N

20 [0157] Intermediate N-3 (0.780 g, 1.644 mmol) was dissolved in DCM (3 mL) and TFA (4 mL, 51.9 mmol) was then added. After stirring at RT for 30 min, the reaction mixture was concentrated and the residue was dissolved in ACN/water (3:1, 20 mL) and treated with HCl (3.29 mL, 3.29 mmol). The resulting solution was lyophilized to afford N. LC/MS: $[M+H]^+ = 375.3$.

25

Preparation of Intermediate O



Step A - Synthesis of intermediate O-1

[0158] Intermediate H-3 (80 mg, 0.135 mmol) was dissolved in dry DMF (1350 μL) and cesium carbonate (150 mg, 0.459 mmol) was then added, followed by 1,4-dibromobutane (117 mg, 0.540 mmol). After stirring at 50 $^\circ\text{C}$ for 1.5 hours, the mixture was filtered and directly injected for purification via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] 10 min gradient, 1 injection, 2 mL DMF, to afford O-1. LC/MS: $[M+H]^+ = 729.15$. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.27 (dd, $J = 9.5, 2.4$ Hz,

1H), 7.23 – 7.17 (m, 2H), 7.12 (s, 1H), 7.02 (d, $J = 7.6$ Hz, 2H), 6.96 (td, $J = 9.0, 2.5$ Hz, 1H), 6.72 (s, 1H), 6.45 (d, $J = 7.4$ Hz, 1H), 6.32 (d, $J = 8.0$ Hz, 1H), 5.11 – 4.99 (m, 1H), 4.85 (s, 1H), 4.61 – 4.48 (m, 1H), 4.10 (td, $J = 6.9, 2.4$ Hz, 2H), 4.03 (d, $J = 3.9$ Hz, 1H), 3.80 (s, 3H), 3.79 – 3.65 (m, 2H), 3.47 (t, $J = 9.4$ Hz, 1H), 3.40 (td, $J = 6.5, 2.5$ Hz, 2H), 3.34 (t, $J = 9.2$ Hz, 1H), 3.28 (dd, $J = 14.7, 7.0$ Hz, 1H), 3.13 (dd, $J = 14.8, 4.8$ Hz, 1H), 3.05 – 2.99 (m, 2H), 2.57 – 2.38 (m, 2H), 2.09 (s, 4H), 2.07 (s, 2H), 1.99 (p, $J = 7.2$ Hz, 3H), 1.89 – 1.78 (m, 2H), 1.59 (s, 1H), 1.54 – 1.45 (m, 2H).

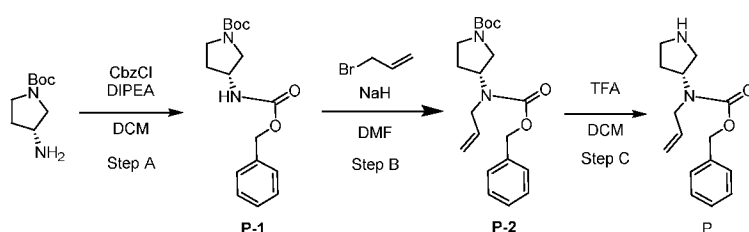
Step B - Synthesis of intermediate O-2

[0159] Intermediate O-1 (25 mg, 0.034 mmol) was dissolved in dry DMF (687 μ l) and tert-butyl 4-(methylamino)piperidine-1-carboxylate (14.73 mg, 0.069 mmol) and DIPEA (30.0 μ l, 0.172 mmol) were then added. After heating to 50 °C overnight, the reaction mixture was diluted with EtOAc, washed with water three times, then brine, dried over magnesium sulfate and concentrated to afford O-2. LC/MS: $[M+H]^+ = 861.66$.

Step C - Synthesis of intermediate O

[0160] Intermediate O-2 (70 mg, 0.081 mmol) was dissolved in THF (1548 μ l), MeOH (774 μ l), and Water (387 μ l) and LiOH (9.73 mg, 0.406 mmol) was then added. After stirring at room temp for 2 hours, the reaction mixture was evaporated under vacuum. Next it was acidified with 1N HCl to pH ~3 and extracted 3 times with EtOAc. washed with brine, dried over magnesium sulfate and concentrated to afford O. LC/MS: $[M+H]^+ = 847.56$.

25 Preparation of Intermediate P



Step A - Synthesis of intermediate P-1

[0161] Tert-butyl (R)-3-aminopyrrolidine-1-carboxylate (182 μ l, 1.074 mmol) was dissolved in DCM (7159 μ l) and cooled to 0 °C. DIPEA (563 μ l, 3.22 mmol) was then added, followed by benzyl chloroformate (184 μ l, 1.289 mmol). After stirring at 0 °C for 30 min, it was quenched with aqueous sodium carbonate, extracted with DCM, washed with brine, dried over magnesium sulfate and concentrated. The residue was purified via flash column chromatography on a 12g silica gold column using 0-30% [3:1 EtOAc:EtOH] in hexane gradient, loading directly onto column in DCM to afford P-1. LC/MS: $[M+H]^+ = 320.0$. ¹H NMR (500 MHz, Chloroform-*d*) δ

7.38 (s, 5H), 5.13 (s, 2H), 4.85 (s, 1H), 4.27 (s, 1H), 3.63 (dd, $J = 11.1, 6.1$ Hz, 1H), 3.43 (d, $J = 21.9$ Hz, 2H), 3.23 (d, $J = 26.3$ Hz, 1H), 2.15 (s, 1H), 1.85 (s, 1H), 1.48 (s, 9H).

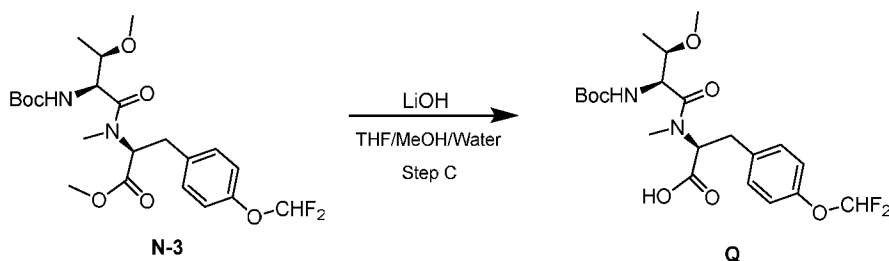
Step B - Synthesis of intermediate P-2

[0162] Intermediate P-1 (316 mg, 0.986 mmol) and allyl bromide (0.341 ml, 3.95 mmol) were dissolved in dry DMF (3 ml) and sodium hydride (71.0 mg, 1.775 mmol) was then added. After stirring at room temp overnight, it was added ~ 30 mL water, extracted 3 times with EtOAc, washed with water twice, then brine, dried over magnesium sulfate and concentrated to afford P-2. LC/MS: $[M+H]^+ = 361.25$. ^1H NMR (500 MHz, Chloroform- d) δ 7.35 (d, $J = 16.5$ Hz, 5H), 5.83 (s, 1H), 5.23 – 5.08 (m, 4H), 4.66 (s, 1H), 3.88 (s, 1H), 3.52 (d, $J = 35.0$ Hz, 2H), 3.24 (d, $J = 28.5$ Hz, 1H), 2.94 (d, $J = 36.2$ Hz, 1H), 2.04 (dd, $J = 15.2, 8.6$ Hz, 3H), 1.47 (s, 9H).

Step C - Synthesis of intermediate P

[0163] Intermediate P-2 (372 mg, 1.032 mmol) was dissolved in DCM (10 mL) and TFA (1193 μ l, 15.48 mmol) was then added. After stirring at room temp for 1 hour, the reaction mixture was concentrated and purified via flash column chromatography on a 12g silica gold column using 0-50-100% [3:1 EtOAc:EtOH] in hexane gradient to afford P. LC/MS: $[M+H]^+ = 261.28$. ^1H NMR (500 MHz, Chloroform- d) δ 7.56 – 7.35 (m, 5H), 5.77 (s, 1H), 5.19 (dd, $J = 28.5, 9.7$ Hz, 3H), 4.13 (s, 1H), 3.95 (s, 2H), 3.75-3.65 (m, 1H), 3.51 (d, $J = 29.8$ Hz, 3H), 3.29 (s, 1H), 2.44 (s, 1H), 2.21 (s, 2H).

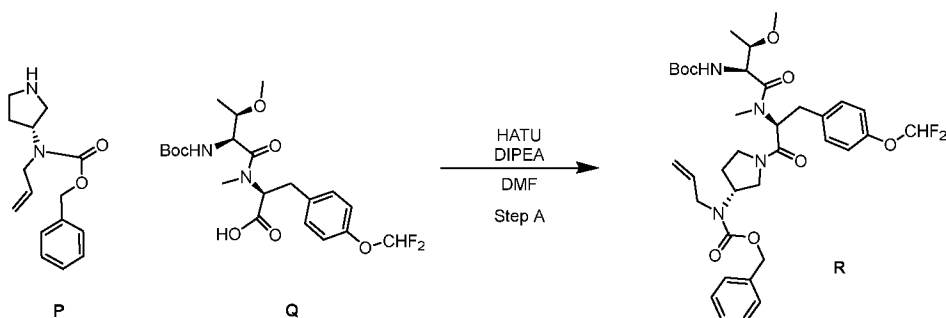
Preparation of Intermediate Q



Step A - Synthesis of intermediate Q

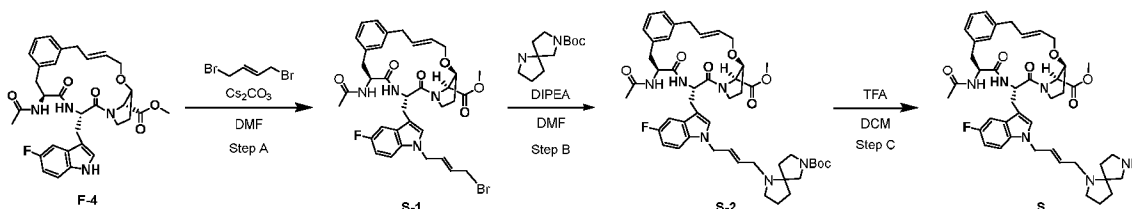
[0164] Intermediate N-3 (280 mg, 0.590 mmol) in THF (6 ml), MeOH (2 ml), and Water (2 ml) was dissolved at 0 °C and LiOH (4.72 ml, 4.72 mmol) was then added dropwise. After stirring at 0 °C for 1h, the mixture was neutralized by addition of 1N HCl (3 mL). Next the volatile was evaporated on rotary evaporator and the aqueous phase was acidified to pH 4, extracted with DCM (3x70 mL). The combined organic phase was dried over Na₂SO₄, and concentrated to afford Q. LC/MS: $[M+H]^+ = 461.36$.

5

Preparation of Intermediate RStep A - Synthesis of intermediate R

- 10 **[0165]** Intermediate Q (133 mg, 0.288 mmol) and P (75 mg, 0.288 mmol) were dissolved in dry DMF (2881 μ l) and cooled to 0 °C. HATU (120 mg, 0.317 mmol) and DIPEA (251 μ l, 1.440 mmol) were then added. After stirring at 0 °C for 1 hour, the reaction mixture was diluted with EtOAc, washed with water three times, then brine, dried over magnesium sulfate and concentrated to afford R. LC/MS: $[M+H]^+ = 703.41$.

15

Preparation of Intermediate S

- 20 Step A - Synthesis of intermediate S-1

- [0166]** Intermediate F -3 (1g, 1.693 mmol) was dissolved in dry DMF (10.58 ml) and cesium carbonate (1.876 g, 5.76 mmol) and (E)-1,4-dibromobut-2-ene (1.449 g, 6.77 mmol) were then added. After stirring at room temp for 4 hours, the reaction mixture was diluted with EtOAc, washed with water three times, then brine, dried over magnesium sulfate and concentrated. The residue was purified via flash column chromatography on a 40g silica gold column using [0-40% 3:1 EtOAc:EtOH] in hexane gradient to afford S-1. LC/MS: $[M+H]^+ = 723.20$. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.21 – 7.12 (m, 3H), 6.98 (m, 2H), 6.94 (td, $J = 9.0, 2.5$ Hz, 2H), 6.83 (s, 1H), 5.95 – 5.86 (m, 2H), 5.74 (dd, $J = 15.1, 7.5$ Hz, 2H), 5.54 – 5.45 (m, 2H), 4.83 – 4.76 (m,
- 25

2H), 4.71 – 4.64 (m, 3H), 4.52 (m, 1H), 4.18 – 4.09 (m, 2H), 3.99 – 3.87 (m, 2H), 3.75 (s, 3H), 3.31 – 3.17 (m, 4H), 3.08 (dd, $J = 14.7, 4.0$ Hz, 2H), 2.80 – 2.72 (m, 2H), 2.12 (s, 3H), 2.05 (d, $J = 18.2$ Hz, 2H)

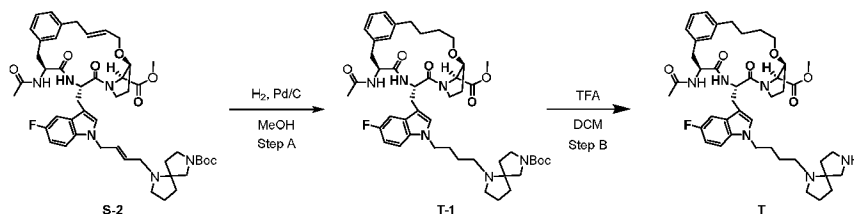
Step B - Synthesis of intermediate S-2

[0167] Intermediate S-1 (500 mg, 0.691 mmol) was dissolved in dry DMF (6910 μ l) and tert-butyl 1,7-diazaspiro[4.4]nonane-1-carboxylate (235 mg, 1.036 mmol) and DIPEA (603 μ l, 3.45 mmol) were then added. After heating to 50 °C for 3.5 hours, the reaction mixture was diluted with EtOAc, washed with water 3 times, then brine, dried over magnesium sulfate and concentrated. The residue was purified via flash column chromatography on a 12g silica gold column using 30-70% [3:1 EtOAc:EtOH] in hexane gradient to afford S-2. LC/MS: $[M+H]^+ =$ 869.93.

Step C - Synthesis of intermediate S

[0168] Intermediate S-2 (25.8 mg, 0.030 mmol) was dissolved in DCM (594 μ l) and TFA (70 μ l, 0.890 mmol) was then added. After stirring at room temp for 1 hour, the reaction mixture was concentrated, redissolved in DCM and concentrated four times to afford S. LC/MS: $[M+H]^+ =$ 769.55.

Preparation of Intermediate T



Step A - Synthesis of intermediate T-1

[0169] Intermediate S-2 (464 mg, 0.534 mmol) was dissolved in dry MeOH (10 mL) and Pd-C (11.36 mg, 0.107 mmol) was then added. After the flask was evacuated and backfilled with H₂ (1atm from balloon) three times, the reaction was stirred at room temp for 2 hours. Next the mixture was filtered through celite and concentrated. The residue was purified via flash column chromatography on a 24g silica gold column using 30-70% [3:1 EtOAc:EtOH] in hexane gradient to afford T-1. LC/MS: $[M+H]^+ = 873.95$.

Step B - Synthesis of intermediate T

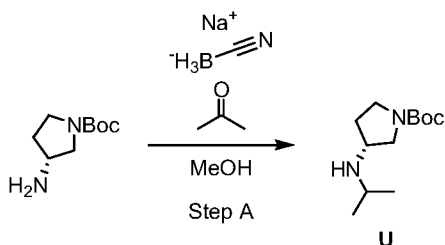
[0170] Intermediate T-1 (50 mg, 0.057 mmol) was dissolved in DCM (573 μ l) and TFA (66.2 μ l, 0.859 mmol) was then added. After stirring at room temp for 2 hours, the reaction mixture

- 5 was concentrated, re-dissolved in DCM and concentrated several times to afford T. LC/MS: $[M+H]^+ = 773.55$.

Preparation of Intermediate U

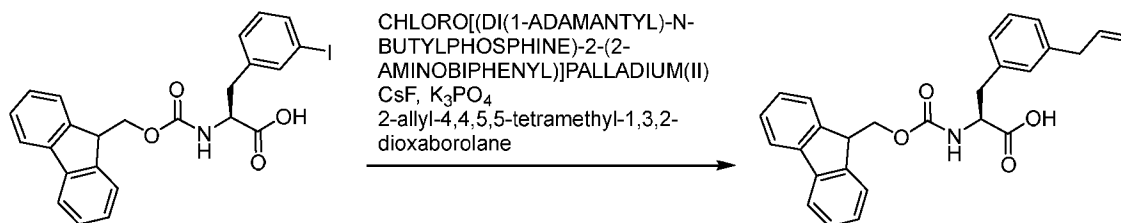
Step A - Synthesis of intermediate U

10



- [0171] Tert-butyl (R)-3-aminopyrrolidine-1-carboxylate (85 mg, 0.456 mmol) was dissolved in Acetone (2282 μ l) and MeOH (2282 μ l) with magnesium sulfate. After stirring for 15 min, it was added sodium cyanotrihydroborate (57.4 mg, 0.913 mmol) and adjusted pH to ~ 5 using AcOH.
- 15 After stirring at room temp overnight, the reaction was quenched with sat aq. sodium bicarbonate solution, evaporated volatiles, extracted with EtOAc three times, washed with brine, dried over magnesium sulfate and concentrated. The residue was purified via flash column chromatography on a 4g silica gold column using 0-100% [3:1 EtOAc in EtOH] in hexane gradient to afford U. LC/MS: $[M+H]^+ = 228.0$. ^1H NMR (500 MHz, Chloroform-*d*) δ 3.88 (dd, $J = 10.6, 7.2$ Hz, 2H),
- 20 3.78 – 3.65 (m, 3H), 3.42 – 3.33 (m, 2H), 2.44 (s, 2H), 1.55 (d, $J = 6.3$ Hz, 6H), 1.48 (s, 9H).

Preparation of Intermediate V



- [0172] To a pressure vial containing a solution of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(3-iodophenyl)propanoic acid (6000 mg, 11.69 mmol) in dioxane (72 ml), was added 2-allyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (7.67 ml, 40.9 mmol). The solution was bubbled with nitrogen gas. An aqueous solution (34 mL) of cesium fluoride (3551 mg, 23.38 mmol) and potassium phosphate (2853 mg, 13.44 mmol) was then added with continued bubbling of nitrogen gas. Next chloro[di(1-adamantyl)-N-
- 25

5 butylphosphine]palladium(II) (1172 mg, 1.753 mmol) was added and the vial was sealed and heated in preheated oil bath for 35 mins at 110°C. Next, the vial was removed from heat and put in a water bath to cool. To the resulting solution 1N HCl was added to lower pH of the reaction to 2-3 (34mL), and then dilute brine was added followed by extraction with EtOAc (3X). The combined organics phase was washed with brine, dried over Na₂SO₄, filtered, and concentrated
10 *in vacuo*. The residue was dissolved in toluene and purified by gradient elution on SiO₂ (220g SiO₂, 10-70% EtOAc/hexane) to afford intermediate V. LCMS: 428.2 (M+1)⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.88 (d, J = 7.5 Hz, 2H), 7.72 (d, J = 8.5 Hz, 1H), 7.68 – 7.61 (m, 2H), 7.44 – 7.39 (m, 2H), 7.30 (dt, J = 14.8, 7.3 Hz, 2H), 7.20 (t, J = 7.5 Hz, 1H), 7.13 – 7.08 (m, 2H), 7.03 (d, J = 7.8 Hz, 1H), 5.97 – 5.86 (m, 1H), 5.08 – 4.98 (m, 2H), 4.22 – 4.14 (m, 4H), 3.31 (d, J =
15 6.6 Hz, 2H), 3.11 – 3.01 (m, 1H), 2.91 – 2.81 (m, 1H).

Preparation of Intermediate W

Step A – Synthesis of Intermediate W-1

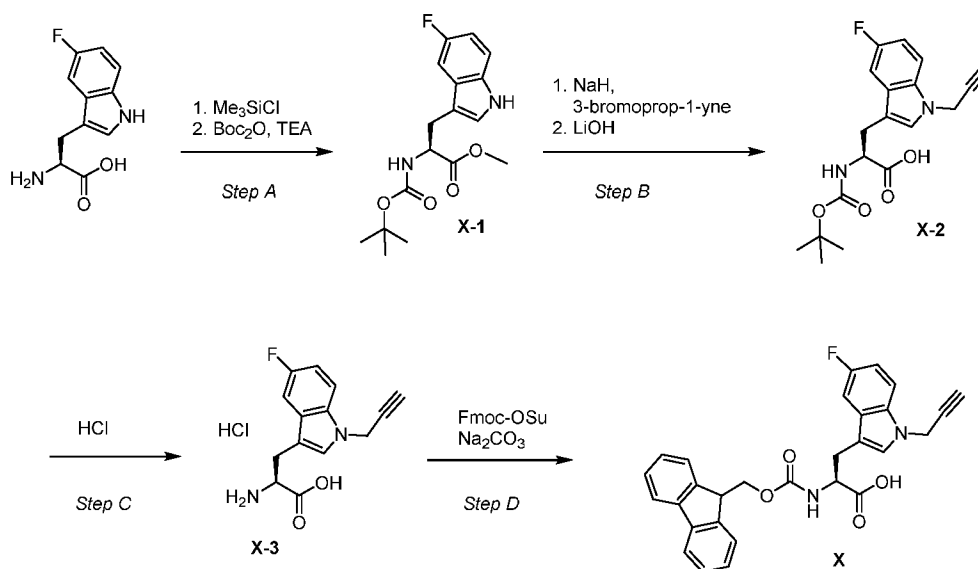
[0173] In a flame dried flask under an atmosphere of nitrogen, (2S,3S)-1-(tert-butoxycarbonyl)-
20 3-hydroxypyrrolidine-2-carboxylic acid (5 g, 21.62 mmol) was dissolved in anhydrous DMF (100 mL) and then cooled in ice bath. Next SODIUM HYDRIDE (1.365 g, 54.1 mmol) was added portion wise. After gas was evolved, reaction became too viscous with precipitate. It was diluted with more anhydrous DMF (200mL) and stirred at 0°C for a total of 45 minutes. ALLYL BROMIDE (2.058 ml, 23.78 mmol) was then added, and the reaction was stirred at room
25 temperature for 20 minutes, followed by addition of 1mL more allyl bromide. After 15 minutes, 1mL more allyl bromide was added and stirred for 20more minutes. After cooled in ice bath, it was quenched slowly with water to quench. 1N aq NaOH (20mL) was added after 20 minutes, followed by additional 1N aq HCl (~ 25 ml) after 30 minutes to adjust pH to 4-5. The mixture was concentrated, redissolved in water and EtOAc and then acidified with 1N aq HCl to pH 2-3,
30 then was diluted with brine and extracted 3X EtOAc. The combined phase was dried over Na₂SO₄, filtered, and concentrated *in vacuo* to afford intermediate W-1, which was used crude in the next step. LCMS: 294.1 (M+Na)⁺.

Step B – Synthesis of Intermediate W-2

[0174] Intermediate W-1 (5.87 g, 21.62 mmol) was dissolved in DCM (25 ml) and TFA (25 ml,
35 324 mmol) was then added. After stirring at room temperature for 20 minutes, the reaction was concentrated *in vacuo* and then concentrated *in vacuo* 2X from toluene to afford W-2, which was used crude in the next step. LCMS: 172.1 (M+1)⁺.

5 Step C – Synthesis of Intermediate W

[0175] Intermediate W-2 (6.17 g, 21.62 mmol) and SODIUM CARBONATE (4.58 g, 43.2 mmol) were dissolved in water (130 mL) and acetone (130 mL) and Fmoc-OSu (7.29 g, 21.62 mmol) was then added. The resultant suspension was diluted with water (40 mL) and acetone (40 mL). After stirring overnight at room temperature, the reaction was acidified reaction to pH 2-3
 10 with 1N aq HCl (~60mL) and concentrated *in vacuo*, then diluted with water and brine and extracted with DCM (3X). The combined organic phase was dried over Na₂SO₄, filtered, concentrated. The residue was dissolved in toluene and purified by gradient elution on SiO₂ (220g SiO₂, 0-70% EtOAc/hexane) to afford provide intermediate W. LCMS: 394.2 (M+1)⁺. ¹H NMR (500 MHz, Chloroform-*d*) δ 7.80 – 7.70 (m, 2H), 7.62 – 7.52 (m, 2H), 7.44 – 7.26 (m, 4H),
 15 5.95 – 5.84 (m, 1H), 5.31 (d, *J* = 17.2 Hz, 1H), 5.23 (dd, *J* = 10.3, 1.2 Hz, 1H), 4.50 – 4.36 (m, 3H), 4.36 – 4.25 (m, 2H), 4.22 – 3.98 (m, 3H), 3.70 – 3.60 (m, 2H), 2.15 – 2.00 (m, 2H).

Preparation of Intermediate X

20

Step A – Synthesis of Intermediate X-1

[0176] In flame dried flask under an atmosphere of nitrogen, (S)-2-amino-3-(5-fluoro-1H-indol-3-yl)propanoic acid (10.8 g, 48.6 mmol) dissolved in MeOH (200 ml) and cooled in ice bath. Next trimethylchlorosilane (35.6 ml, 279 mmol) was added dropwise. After stirring at
 25 room temperature overnight, more TMSCl (4 mL) was added and stirred overnight. After the reaction was cooled in ice bath, trimethylamine (38 ml, 273 mmol) was added to raise reaction pH to ~9, followed by a solution of BOC-Anhydride (12.98 ml, 55.9 mmol) in methanol (20mL).

5 After stirring at room temperature for 2 hours, the reaction mixture was concentrated and separated between water and ether and then extracted residue 3X ether. The combined organics phase was washed with brine and dried over Na₂SO₄, filtered solution through a plug of silica gel and concentrated in vacuo and dried. The resultant crystalline solid was triturated with hexane (3X) to afford **X-1**. LCMS: 673.5 (2M+1)⁺. ¹H NMR (600 MHz, Chloroform-d) δ 8.08 (s, 1H), 7.27 – 7.24 (m, 1H)(mixed with CHCl₃ peak), 7.17 (d, J = 9.4 Hz, 1H), 7.04 (s, 1H), 6.96 – 6.90 (m, 1H), 5.07 (d, J = 7.5 Hz, 1H), 4.65 – 4.61 (m, 1H), 3.69 (s, 3H), 3.30 – 3.18 (m, 2H), 1.43 (s, 9H).

Step B – Synthesis of Intermediate X-2

[0177] In flame dried flask under an atmosphere of nitrogen, intermediate **X-1** (1330 mg, 3.95 mmol) was dissolved in anhydrous DMF (30 ml) and cooled in ice bath. Next sodium hydride (125 mg, 4.94 mmol) was added portion wise. After stirring 20 minutes at 0°C, 3-bromoprop-1-yne (80% in xylene) (0.529 ml, 4.74 mmol) was added dropwise. After stirring at 0°C for an hour, lithium hydroxide (1M aq) (6 ml, 6.00 mmol) was added dropwise. After stirring at room temperature for 90 minutes, it was quenched with 6 mL 1N HCl to pH ~3, then extracted with EtOAc (3X). The combined organics phase was dried over Na₂SO₄, filtered, and concentrated in vacuo to afford **X-2**, which was used crude in the next step. LCMS: 721.5 (2M+1)⁺.

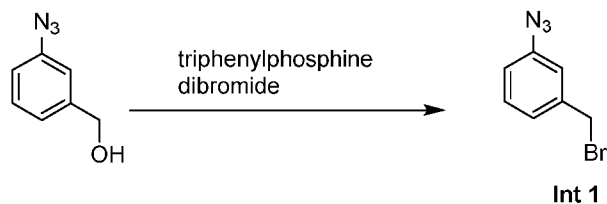
Step C – Synthesis of Intermediate X-3

[0178] Intermediate **X-2** (1425 mg, 3.95 mmol) was dissolved in HCl (4M HCl in dioxane) (20 ml, 80 mmol) at room temperature. After stirring for 45mins, it was concentrated in vacuo. The residue was concentrated in vacuo from ether (2X) to form a solid which was dried to afford **X-3**, which was used crude in the next step. LCMS: 261.2 (M+1)⁺.

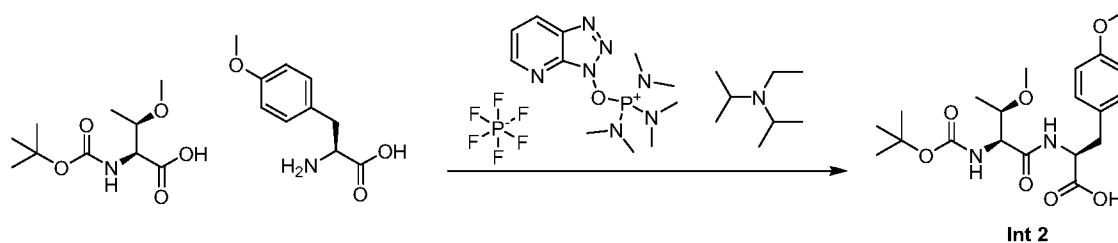
Step D – Synthesis of Intermediate X

[0179] Intermediate **X-3** (1173 mg, 3.95 mmol) and SODIUM CARBONATE (838 mg, 7.91 mmol) were dissolved in acetone (30 mL) and water (60 mL) and a solution of Fmoc-OSU (1334 mg, 3.95 mmol) in 30 mL acetone was then added. After stirring at room temperature for an hour, 1N aq HCl (~11 mL) was added dropwise to adjust pH~3. The reaction was extracted EtOAc (3x). The combined organics was washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was dissolved in DCM and purified by gradient elution on SiO₂ (120g SiO₂, 10-80% EtOAc/hexane to afford intermediate **X**. LCMS: 483.3 (M+1)⁺. ¹H NMR (500 MHz, DMSO-d₆) δ 12.71 (s, 1H), 7.88 (d, J = 7.5 Hz, 2H), 7.74 (d, J = 8.3 Hz, 1H), 7.66 (dd, J = 13.2, 7.5 Hz, 2H), 7.47 (dd, J = 8.9, 4.4 Hz, 1H), 7.44 – 7.36 (m, 3H), 7.34 – 7.23 (m, 3H), 7.08-7.00 (m, 1H), 5.08 – 5.00 (m, 2H), 4.27 – 4.15 (m, 4H), 3.38 (s, 1H), 3.15 (dd, J = 14.6, 4.6 Hz, 1H), 3.00 (dd, J = 14.6, 9.7 Hz, 1H).

5

Preparation of Intermediate Int 1**Synthesis of Intermediate Int 1**

- 10 **[0180]** (3-azidophenyl)methanol (500 mg, 3.35 mmol) was dissolved in DCM (50 ml) and cooled in ice bath and triphenylphosphine dibromide (1556 mg, 3.69 mmol) was then added, followed by 100 mg more triphenylphosphine dibromide after 30min at room temperature. After stirring for 30min, the reaction was filtered through a small column of silica gel and washed with 175mL DCM to fully elute product. The residue was concentrated in vacuo to afford intermediate
- 15 **Int 1**, which was stored in freezer. ¹H NMR (600 MHz, CDCl₃): δ 7.33 (t, J = 7.9 Hz, 1H), 7.16 (d, J = 7.6 Hz, 1H), 7.05 (s, 1H), 6.97 (m, 1H), 4.45 (s, 2H).

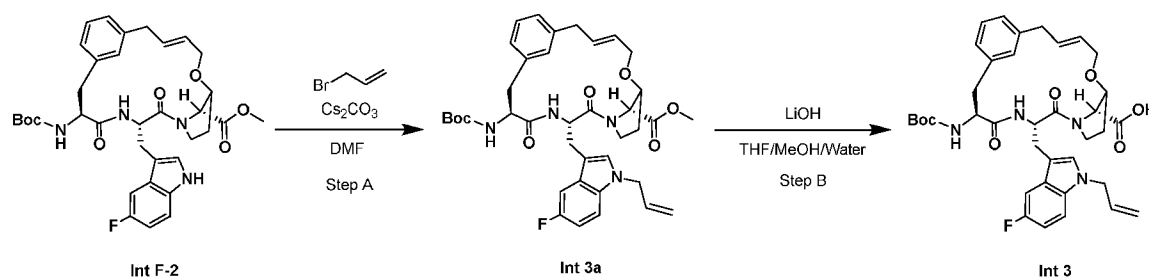
Preparation of Intermediate Int 2

20

Synthesis of Intermediate Int 2

- [0181]** (2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methoxybutanoic acid (1 g, 4.29 mmol) was dissolved in DCM (60 ml) and DIEA (1.497 ml, 8.57 mmol) was added, followed by AOP (CAS: 156311-85-2) (1.900 g, 4.29 mmol). After stirring at RT for 20 mins, solid (S)-2-amino-3-(4-methoxyphenyl)propanoic acid (0.921 g, 4.72 mmol) was added. After stirring for an hour, 3 mL DMF and DIEA (750 μL) was added. After stirring overnight at room temperature 12 mL 1N HCl was added to adjust pH to ~2, then diluted with water, and extracted with DCM (3x). The combined organic phase was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was redissolved in DCM and purified on silica
- 25
- 30 gel (120g, ISCO gel, 20-100% EtOAc-hexane) to afford **Int 2**. LCMS: 411.5 (M+1)⁺.

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Preparation of Intermediate Int 3**Step A: Synthesis of Intermediate Int 3a**

10 **[0182]** In a flame dried flask under an atmosphere of nitrogen gas, **Intermediate F-2** (400 mg, 0.617 mmol) was dissolved in anhydrous DMF (20 ml) and cesium carbonate (683 mg, 2.096 mmol) was then added, followed by 3-bromoprop-1-ene (0.187 ml, 2.158 mmol). After stirring vigorously at room temp for 90 mins, the reaction was heated at 40° C which helped reaction progress. Next more Cs₂CO₃ (340 mg) and allyl bromide (0.094 mL) were added and stirred at

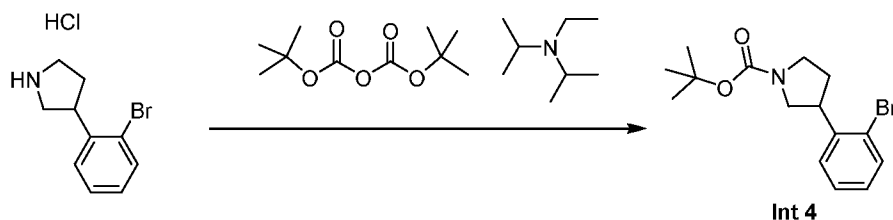
15 40° C for 8 hours, followed by 340 mg Cs₂CO₃ (340 mg) and allyl bromide (0.094 mL) and stirred at 40° C for 4 hours. The reaction was quenched with ~5mL 1N aq. HCl, diluted with water, and extracted with EtOAc (3X). The combined organics phase was washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to provide **Int 3a**, which was used crude in the next step. LCMS: 689.3 (M+1)⁺.

Step B: Synthesis of Intermediate Int 3

20 **[0183]** Intermediate **Int 3a** (425 mg, 0.617 mmol) was dissolved in THF (10 ml) and LiOH (1M, aq) (1.234 ml, 1.234 mmol) was then added, followed by methanol (5 ml). After stirring at RT for 45 mins, the reaction was quenched with 1.5 mL 1N aq. HCl, diluted with brine, and extracted with EtOAc (3X). The combined organic phase was washed with brine, dried over

25 sodium sulfate, filtered, and concentrated in vacuo. The residue was redissolved in DCM and purified on silica gel (40g ISCO gold, 40-100% EtOAc-hexane to afford **Int 3**. LCMS: 675.3 (M+1)⁺.

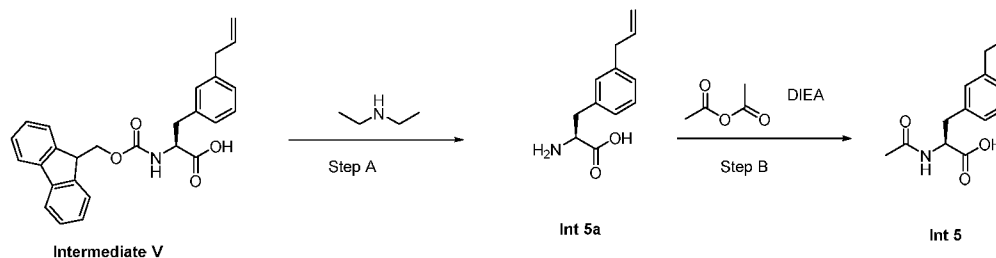
5 Preparation of Intermediate Int 4

*Synthesis of Intermediate Int 4*

- 10 **[0184]** 3-(2-bromophenyl)pyrrolidine hydrochloride (1000 mg, 3.81 mmol) was dissolved in DCM (20 ml) and DIEA (1.397 ml, 8.00 mmol) and BOC-Anhydride (873 mg, 4.00 mmol) was then added. After stirring overnight at RT, the reaction was loaded directly onto a silica gel column and purified using gradient elution (120g, ISCO gold, 0-50% EtOAc-hexane) to afford intermediate **Int 4**. LCMS: 326.2/ 328.2 (M+1)⁺.

15

Preparation of Intermediate Int 5

*Step A: Synthesis of Intermediate Int 5a*

- 20 **[0185]** Intermediate **V** (1000 mg, 2.339 mmol) was dissolved in DMF (6 ml) and DIETHYLAMINE (6 ml, 57.4 mmol) was then added. After stirring at RT for 30 mins, the reaction was concentrated in vacuo almost to dryness and then ether was added to precipitate a solid, which was filtered, rinsed with ether to afford intermediate **Int 5a** which was used as crude in the next step

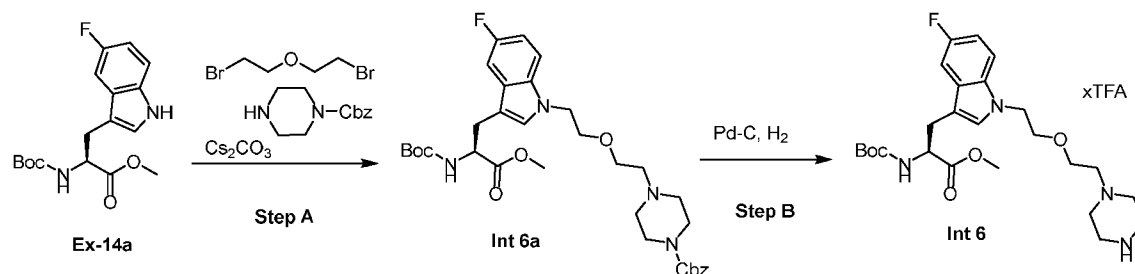
25 *Step B: Synthesis of Intermediate Int 5*

- [0186]** The above filtered solid **Int 5a** was added DCM (13 ml), DIEA (0.848 ml, 4.85 mmol), and ACETIC ANHYDRIDE (0.343 ml, 3.64 mmol). After stirring at RT for an hour, aq. 1N HCl was added to adjusted pH to ~2. The mixture was diluted with water and extracted with DCM (3x). The combined organic phase was washed with brine, dried over anhydrous Na₂SO₄, filtered,

- 5 and concentrated under reduced pressure. The residue was redissolved in DCM and purified on silica gel using gradient elution (24g, ISCO gold, 0-100% EtOAc-hexane to afford **Int 5**. LCMS: 248.2 (M+1)⁺.

Preparation of Intermediate Int 6

10



Step A: Synthesis of Intermediate Int 6a

- [0187] **Ex-14a** (125 mg, 0.372 mmol) was dissolved in anhydrous DMF (10 ml) under an atmosphere of nitrogen and 1-bromo-2-(2-bromoethoxy)ethane (157 mg, 0.677 mmol) and CESIUM CARBONATE (550 mg, 1.688 mmol) were then added. After stirring at RT for an hour, more 1-bromo-2-(2-bromoethoxy)ethane (157 mg, 0.677 mmol) was added, followed by addition of 1-bromo-2-(2-bromoethoxy)ethane (157 mg, 0.677 mmol) and cesium carbonate (450 mg) after another hour. After heated at 60°C for 2 hours and then heated at 70 °C for 2 more hours, benzyl piperazine-1-carboxylate (450 mg, 2.043 mmol) was added and heated at 50 °C overnight. The reaction was diluted with EtOAc and washed with water (3x) and then brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was redissolved in DCM and purified on silica gel (24g ISCO gold, 0-100% EtOAc-hexane followed by 0-20% EtOH-EtOAc) to afford **Int 6a**. LCMS: 627.7 (M+1)⁺.

Step B: Synthesis of Intermediate Int 6b

- [0188] Intermediate **Int 6a** (170 mg, 0.271 mmol) was dissolved in MeOH (10 ml) and bubbled with nitrogen gas. Next 10% Pd-C (30 mg, 0.282 mmol) was added, and the flask was evacuated with H₂ and back filled three times. After stirring at RT for 3 hours, the reaction was filtered through syringe filter and concentrated in vacuo. The residue was redissolved in 10:1 MeOH-water and purified using gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 15-65% ® /water w/ 0.1% TFA modifier over 18 min) to afford intermediate **Int 6b**. LCMS: 493.6 (M+1)⁺.

5

General ProtocolsGeneral cleavage protocol

[0189] Completed resin was transferred to a 20 mL fritted syringe. The peptides were cleaved from their solid support using 15 ml of trifluoroacetic acid / triisopropylsilane / water (92 / 4 / 2 (w/v)) mixture for every 0.1 mmol of resin. The suspended resin was rocked in cleavage solution for 1 hours at room temperature. The filtrate was collected in 50 mL polypropylene centrifuge tubes and precipitated with chilled diethyl ether (~50 mL per ~5 mL cleavage filtrate). The precipitated crude peptide was collected by centrifugation. The pellet was then subsequently suspended in chilled diethyl ether and collected by centrifugation two additional times. The resulting solid was air-dried to afford the crude peptide.

General RCM protocol

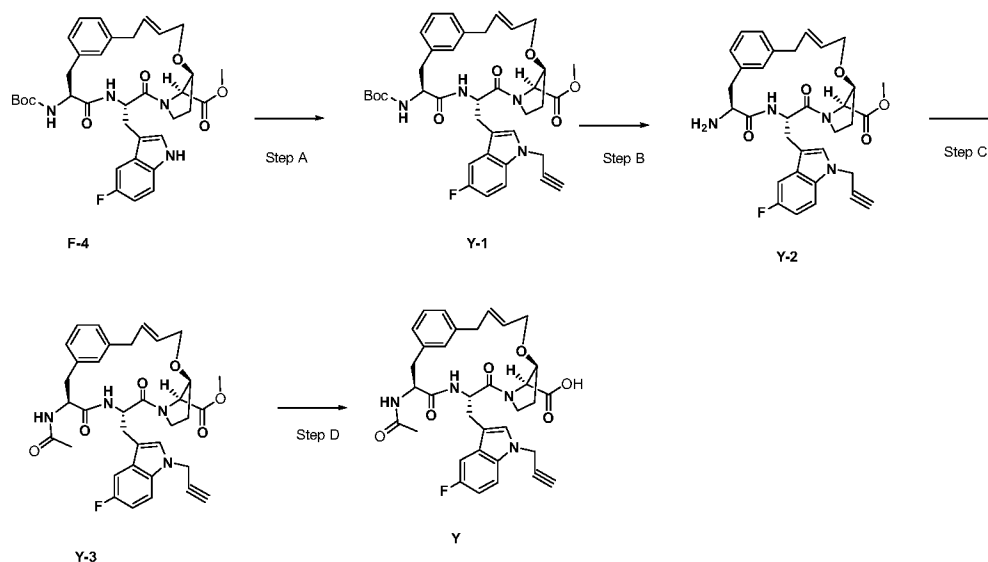
[0190] Compound (assume 0.1 mmol) was dissolved in 40 ml of mixture of DCM/acetic acid (19:1) for every 100 mg of material. Bubbled with N₂ for 20 min. Zhan catalyst (14.41 mg, 0.020 mmol) was added, and the reaction was refluxed in a preheated 60 °C oil bath, stirred under N₂ for 2 hours.

[0191] The reaction was cooled to RT and then was loaded the mixture directly onto a dry ISCO 80 g gold column and eluted with 0-80% EtOH/ (3:1 EtOAc: EtOH). Fractions were combined, solvent removed, and the residue was purified on RP-HPLC to afford clean material.

General on-resin click protocol

[0192] Peptidyl resin (0.125 mmol) was transferred to a high-pressure glass vial with a cap. Copper(I) iodide (0.095 g, 0.500 mmol) and N-ethyl-N-isopropylpropan-2-amine (0.129 g, 1.000 mmol) were dissolved in DMF (15 ml). Next sodium ®-2-((S)-1,2-dihydroxyethyl)-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate (0.198 g, 1.000 mmol) in Water (0.5 ml) was added. DMF solution and water solution were added to the resin, bubbled with N₂ for 5 min. The reaction was kept overnight with very gentle N₂ bubbling, at room temp. Then the above resin was transferred to a disposable syringe and the peptidyl resin was washed thoroughly with DMF, MeOH, DCM (3x). The resin was then washed with 0.5% sodium diethyldithio carbamate plus 0.5% DIEA in DMF (3x).

5 Preparation of Intermediate Y



Step A - Synthesis of intermediate Y-1

[0193] Intermediate **F-4** (500 mg, 0.771 mmol) was dissolved in DMF (5 mL) and Cs₂CO₃ (0.854 g, 2.62 mmol) was then added at -10 °C. After stirring at -10 °C for 0.5 hour, 3-bromoprop-1-yne (0.407 g, 2.74 mmol) was added. The reaction mixture was stirred at room temperature for overnight and then poured into 40 mL of saturated brine, extracted with EA (40 x 2 mL). The organic phase was washed with brine (40 x 2 mL), dried over anhydrous MgSO₄ and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel 12g and eluted with 0-10% MeOH/DCM to afford **Y-1** as a solid.

Step B - Synthesis of intermediate Y-2

[0194] Intermediate **Y-1** (1000 mg, 1.456 mmol) was dissolved in DCM (14 mL) and TFA (16.8 mL, 218 mmol) was then added. After stirring at room temperature for about 2.5 hour, the mixture was concentrated in vacuo to afford intermediate **Y-2** (TFA salt) as a thick oil. LC/MS 587.60 (M+1)⁺.

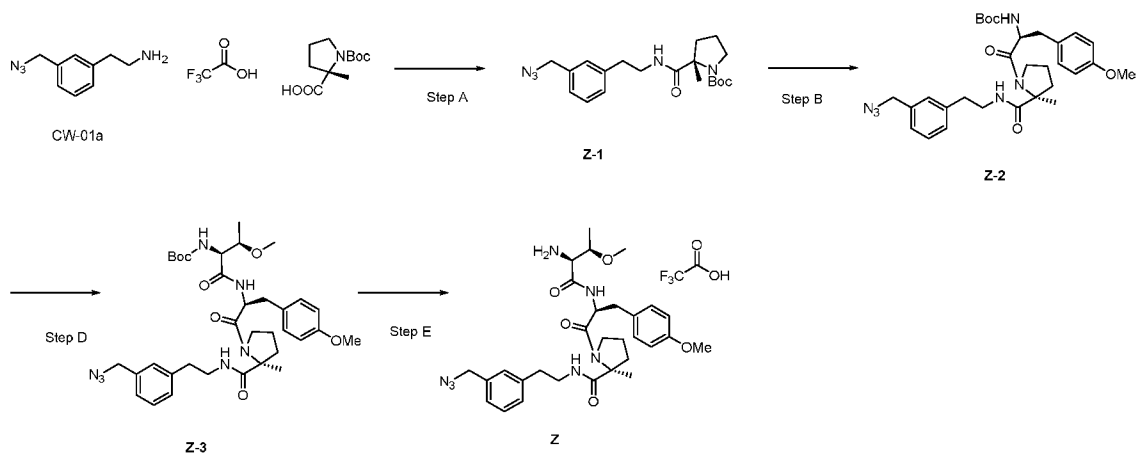
Step C - Synthesis of intermediate Y-3

[0195] Intermediate **Y-2** (510 mg, 0.728 mmol) was dissolved in DCM (10 mL) and DIEA (1272 μl, 7.28 mmol) and ACETIC ANHYDRIDE (137 μl, 1.456 mmol) were then added at 0°C. After stirring at room temperature for about 1.5 h, the mixture was purified by normal phase chromatography (Isco system using 40 g ISCO RediSep silica gold column and eluted with 0-100% EtOAc/hexane) to afford the intermediate **Y-3** as a solid (325 mg). LC/MS 629.49 (M+1)⁺.

5 Step D - Synthesis of intermediate Y

[0196] Intermediate **Y-3** (320 mg, 0.509 mmol) was dissolved in THF (2121 μ l) and MeOH (2121 μ l) and Water (2121 μ l) and LITHIUM HYDROXIDE MONOHYDRATE (85 mg, 2.036 mmol) were then added at room temperature. After stirring at room temperature for about 1.5 hour, the reaction was quenched with addition of HCl (1.0 M in water) (2036 μ l, 2.036 mmol) to adjust pH~5-6. Next the mixture was partitioned between EtOAc (50 ml) and water/ satd. NaCl (~ 10ml/10 ml). The aqueous was extracted with EtOAc for two more times (50 ml x3), and the combined organic phase was dried over Na₂SO₄, filtered, and concentrated in vacuo to afford the intermediate **Y** as solid. LC/MS 615.47 (M+1)⁺.

15 **Preparation of Intermediate Z**



Step A - Synthesis of intermediate Z-1

[0197] N-ethyl-N-isopropylpropan-2-amine (7248 μ l, 41.6 mmol) was added drop wise to the mixture of intermediate **EX-03a** (3355 mg, 11.56 mmol) (over mass crude) and (S)-1-(tert-butoxycarbonyl)-2-methylpyrrolidine-2-carboxylic acid (2650 mg, 11.56 mmol) and 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphinane 2,4,6-trioxide (8257 μ l, 13.87 mmol) in EtOAc (116 mL). After stirring at room temperature for 1 hour, the mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel 12g and eluted with 0-10% MeOH/DCM to afford intermediate **Z-1** as a solid.

5 Step B - Synthesis of intermediate Z-2

[0198] Intermediate **Z-2** (500 mg, 1.290 mmol) was dissolved in DCM (2mL) and 2,2,2-trifluoroacetic acid (2 ml, 1.290 mmol) was then added under N₂. The solution was concentrated and dried over high vacuum overnight.

[0199] N-ethyl-N-isopropylpropan-2-amine (0.899 ml, 5.16 mmol) was added dropwise to the mixture the above TFA salt and (S)-2-((tert-butoxycarbonyl)amino)-3-(4-methoxyphenyl)propanoic acid (381 mg, 1.290 mmol) and HATU (589 mg, 1.548 mmol) in DMF (12.90 ml). After stirring at room temperature for overnight, the mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel 24g and eluted with 0-10% MeOH/DCM to afford intermediate **Z-2** as a solid.

15 Step C - Synthesis of intermediate Z-3

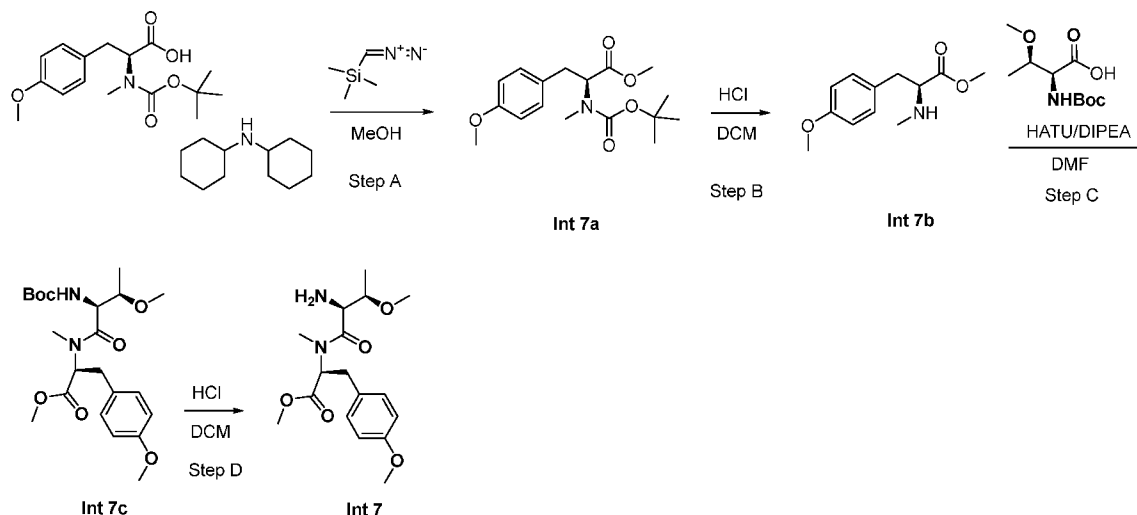
[0200] Intermediate **Z-2** (488 mg, 0.864 mmol) was dissolved in DCM (1mL) and 4N hydrogen chloride (1ml, 4.00 mmol) was added under N₂. After stirring at room temperature for 4 hours, the solution was concentrated and dried over high vacuum to dryness.

[0201] N-ethyl-N-isopropylpropan-2-amine (0.542 ml, 3.11 mmol) was added dropwise to the mixture the above HCl salt and (2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methoxybutanoic acid (202 mg, 0.864 mmol) and HATU (361 mg, 0.951 mmol) in DMF (4.32 ml) at 0°C and the mixture was allowed to warm up to room temperature and stirred for overnight. The mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 12g, and eluted with 0-10% MeOH/DCM to give tert-butyl ((2S,3R)-1-(((S)-1-((S)-2-((3-(azidomethyl)phenethyl)carbamoyl)-2-methylpyrrolidin-1-yl)-3-(4-methoxyphenyl)-1-oxopropan-2-yl)amino)-3-methoxy-1-oxobutan-2-yl)carbamate as a solid.

25 Step E - Synthesis of intermediate Z

[0202] Intermediate **Z** (426 mg, 0.627 mmol) was dissolved in DCM (2.5ml) and 2,2,2-trifluoroacetic acid (71.5 mg, 0.627 mmol) was then added under N₂. After stirring for 1h, the solution was concentrated to dried over high vacuum to dryness. The residue was added ether and allowed to sit for 10min, solid precipitate out. The solvent was decanted and the solid was dried to afford intermediate **Z** as a solid.

5 Preparation of Intermediate Int 7

*Step A - Synthesis of intermediate Int 7a*

[0203] To the solution of BOC-METYR(ME)-OH DCHA (500 mg, 1.019 mmol) in MeOH (5 ml) was added (trimethylsilyl) diazomethane (in hexane) (3.06 ml, 6.11 mmol) dropwise at 0 °C, the resulting solution was stirred at 0 °C for 10min, then quenched by addition of drops of acetic acid. The reaction mixture was concentrated and the residue was purified by flash column chromatography on silica gel (ISCO 40 g column, and eluted with EtOAc/Hexane(0-100% EtOAc in Hexane) to afford **Int 7a**. LC/MS 324.17 (M+1)⁺.

15 *Step B - Synthesis of intermediate Int 7b*

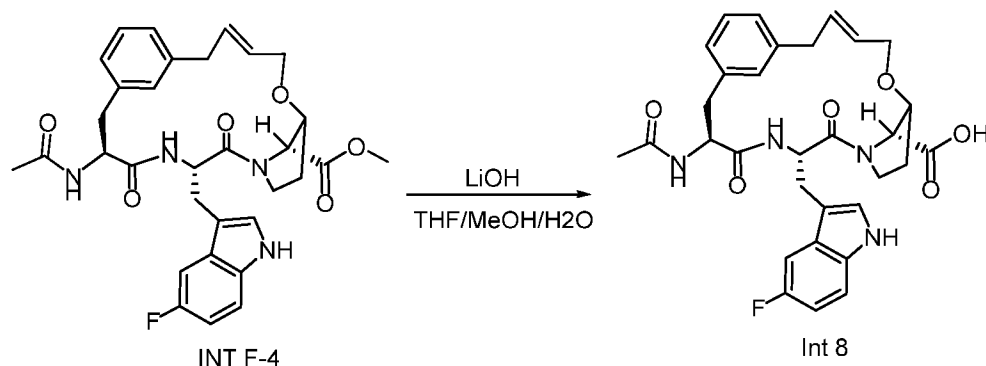
[0204] Intermediate **Int 7a** (0.330 g, 1.019 mmol) was dissolved in DCM (4 ml) and HCl (4N HCl in dioxane) (2.038 ml, 8.15 mmol) was then added. The resulting solution was stirred at RT for 1h. The solvent was concentrated to afford **Int 7b**. LC/MS 224.23 (M+1)⁺.

Step C - Synthesis of intermediate Int 7c

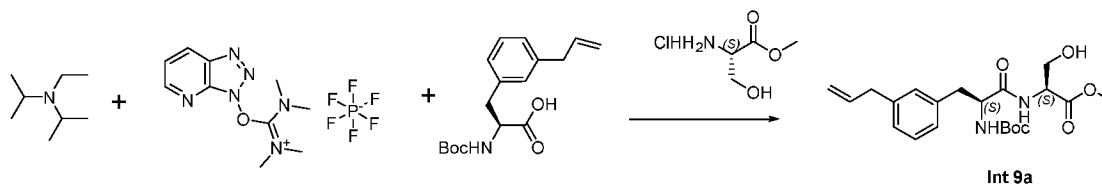
20 [0205] Intermediate **Int 7b** (0.132 g, 0.51 mmol) and (2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methoxybutanoic acid (0.125 g, 0.536 mmol) were dissolved in DMF (4 ml) and HATU (0.213 g, 0.561 mmol) and DIPEA (0.275 ml, 1.530 mmol) were then added. After stirring at RT for 2 hours, the reaction mixture was diluted with H₂O (4ml) and extracted with EtOAc (2x 6ml), the organic phase was dried over Na₂SO₄, concentrated and the residue was purified by flash
25 column chromatography on silica gel (ISCO 40 g column, and eluted with EtOAc/EtOH(3:1 v/v) and Hexane(0-60% EtOAc/EtOH(3:1 v:v) in Hexane) to afford **Int 7c**. LC/MS 439.3 (M+1)⁺.

5 Step D - Synthesis of intermediate **Int 7**

[0206] Intermediate **Int 7c** (0.224 g, 0.51 mmol) was dissolved in DCM (3 ml) and HCl (4M in dioxane) (0.638 ml, 2.55 mmol) was then added. After stirring at room temperature for 1 hour, the solvent was concentrated to afford intermediate **Int 7**. LC/MS 339.27 (M+1)⁺.

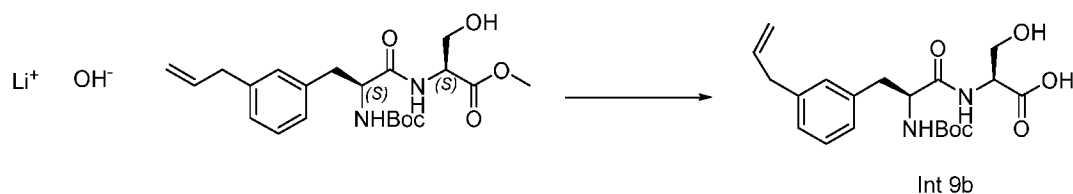
10 **Preparation of Intermediate Int 8**

[0207] Intermediate F-4 (0.49 g, 0.830 mmol) was dissolved in THF (6 ml) and MeOH (2ml) and lithium hydroxide (2.489 ml, 2.489 mmol) was added dropwise. After stirring at room temperature for 2 hours, the solvent was removed under reduced pressure and the residue was purified by preparative HPLC reverse phase (RediSepRf 130g C18 reverse phase) and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to afford **Int 8**. LC/MS 577.28 (M+H)⁺.

20 **Preparation of Intermediate Int 9**Step A - Synthesis of intermediate **Int 9a**

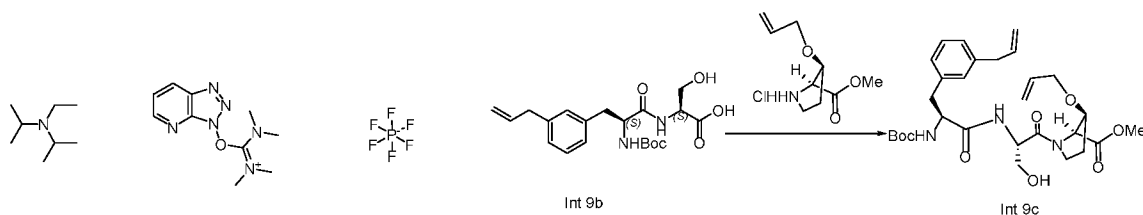
[0208] To a solution of (S)-3-(3-allylphenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (3.11 g, 10.18 mmol) in DMF (30 ml) was added HATU (4.26 g, 11.20 mmol) and (S)-methyl 2-amino-3-hydroxypropanoate hydrochloride (1.584 g, 10.18 mmol) at -20 °C. Then DIEA (7.11 ml, 40.7 mmol) was added at -20 °C. The solution was stirred at -20 °C for 3 h. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column

- 5 chromatography and eluted with 0 - 46 % PE in EA to afford **Int 9a** as a solid. LC/MS: $[M+H]^+ = 407.2$



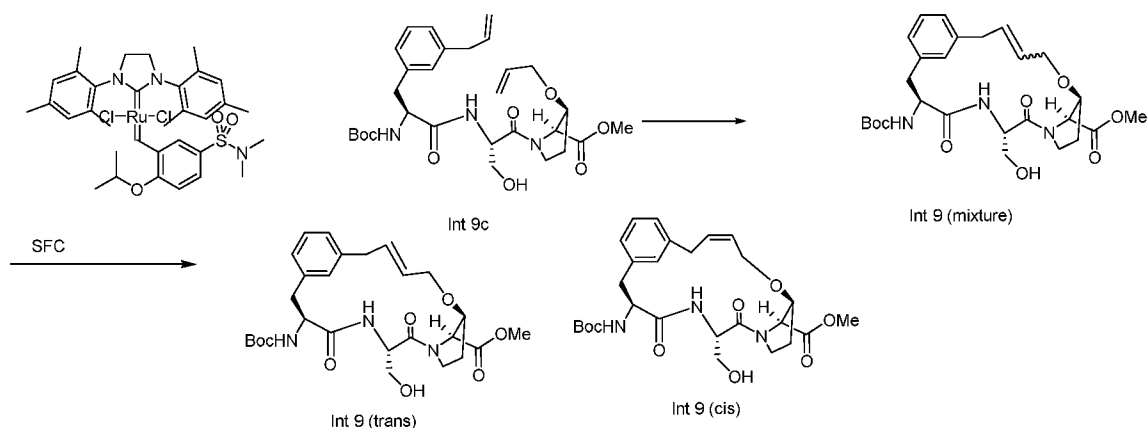
Step B - Synthesis of intermediate Int 9b

- 10 **[0209]** To a solution of **Int 9a** (600 mg, 1.476 mmol) in THF (6 ml) was added lithium hydroxide (70.7 mg, 2.95 mmol) at 0 °C. Then the resulted mixture was stirred for 3 h at 25 °C. The reaction solution was added with 1 M HCl (3 mL). To a solution of n-hexane was added the reaction solution at 25 °C for 20 mi. To afford **Int 9b** as a solid. LC/MS: $[M+H]^+ = 393.1$



- 15 **Step C - Synthesis of intermediate Int 9c**

- [0210]** To a solution of **Int 9b** (2.0 g, 5.10 mmol) in DMF (20 ml) was added 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (2.132 g, 5.61 mmol) and (2S,3S)-methyl 3-(allyloxy)pyrrolidine-2-carboxylate hydrochloride (1.130 g, 5.10 mmol) at -20 °C. Then N-ethyl-N-isopropylpropan-2-amine (2.63 g, 20.38 mmol) was added at -20 °C. The solution was stirred at -20 °C for 3 h. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography and eluted with 0 - 81 % EA in PE to afford **Int 9c** as a solid. LC/MS: $[M+H]^+ = 560.4$

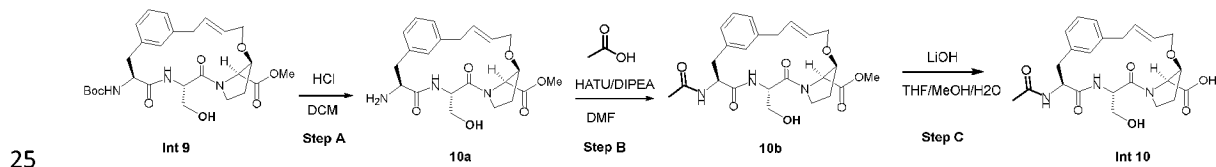


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Step D - Synthesis of intermediate **Int 9**

[0211] To a solution of **Int 9c** (1.25 g, 2.234 mmol) in DCE (1250 ml) was added (1,3-dimesitylimidazolidin-2-ylidene)(5-(N,N-dimethylsulfamoyl)-2-isopropoxybenzylidene)ruthenium(VI) chloride (0.164 g, 0.223 mmol) at room temperature under nitrogen atmosphere. After warming to 60 °C, the mixture was stirred at 60 °C for 4 h. 90% product was detected on LCMS. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography and eluted with 0 - 50% EA in PE, and PR Flash (Column: Flash C¹⁸ 330g; Mobile Phase A: water (0.05% TFA), Mobile Phase B: ACN; Flow rate: 100mL/min; Gradient: 0% B to 30% B in 10 min; 30% B to 50% B in 25 min; Detector: UV 220 nm; Rt = 30 min) to afford **Int 9 (mixture)** as a solid. LC/MS: [M+H]⁺ = 532.5

[0212] The residue of **Int 9 (mixture)** was purified by SFC separation with the following conditions (Column: (R,R) Whelk-01, 45x 25 mm, 5 um; Mobile Phase A: CO₂: 60%, Mobile Phase B : MeOH-Preparative:40%. Flow rate: 150 mL/min; 220 nm; Rt₁: 6.12 min; Rt₂: 7.76 min). The fractions at 6.12 min was collected and concentrated under reduced pressure to afford **Int 9 (trans)** as a solid. The fraction at 7.76 min was collected and concentrated under reduced pressure to afford **Int 9 (cis)** as a solid. LC/MS: [M+H]⁺ = 532.2.

Preparation of Intermediate **Int 10**Step A - Synthesis of intermediate **Int 10a**

[0213] Intermediate **Int 9** (108 mg, 0.203 mmol) was dissolved in DCM (1ml) and HCl (4N HCl in dioxane) (0.305 ml, 1.219 mmol) was then added at room temperature. After stirring at room temperature for 2 hours, the solvent was removed under reduced pressure to afford intermediate **Int 10a**. LC/MS 432.26 (M+1)⁺.

Step B - Synthesis of intermediate **Int 10b**

[0214] To the solution of intermediate **Int 10a** (95 mg, 0.203 mmol) and acetic acid (0.012 ml, 0.207 mmol) in DMF (2 ml) was added DIPEA (0.177 ml, 1.015 mmol) and HATU (79 mg, 0.207 mmol) at room temperature. After stirring at room temperature for 1 hour, the reaction mixture was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted

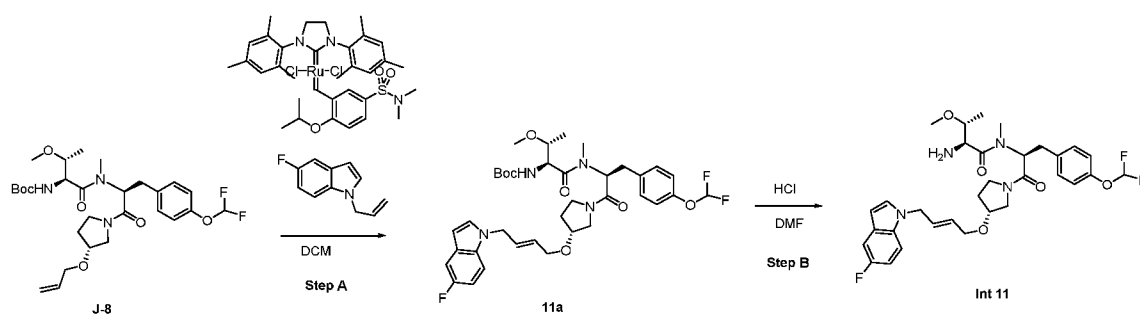
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5 with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to afford **Int 10b**. LC/MS 474.24 (M+1)⁺.

Step C - Synthesis of intermediate Int 10

[0215] Intermediate **Int 10b** (90 mg, 0.190 mmol) was dissolved in THF (1.8ml), MeOH (1.2 ml) and Water (0.6 ml) and LiOH (1M in H₂O) (0.570 ml, 0.570 mmol) was added. After stirring
 10 at room temperature for 2 hours, the solvent was concentrated under reduced pressure and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield product **Int 10**. LC/MS 460.24 (M+H)⁺.

15 **Preparation of Intermediate Int 11**



Step A - Synthesis of intermediate Int 11a

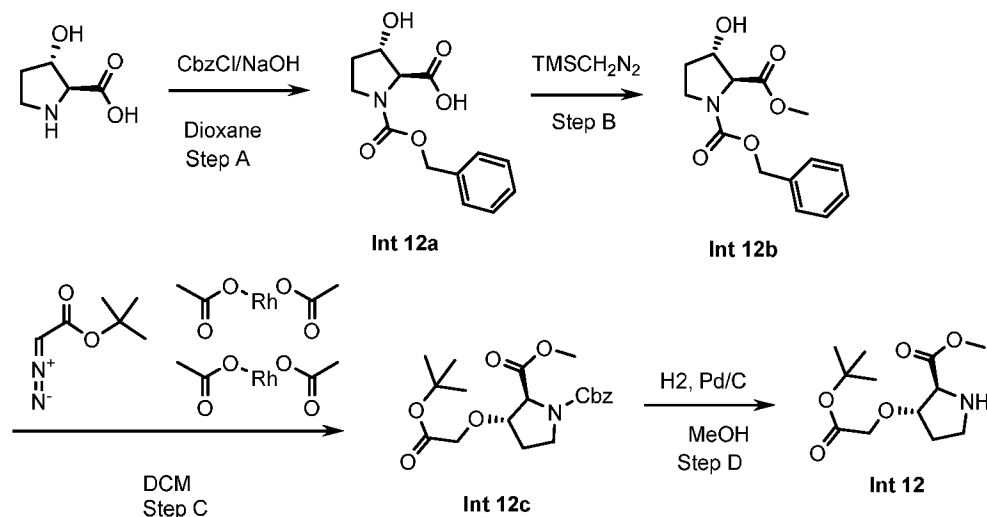
[0216] Intermediate **J-8** (35 mg, 0.061 mmol) was dissolved in CH₂Cl₂ (0.6 ml) and ZHAN
 20 CATALYST-1B (6.76 mg, 9.22 μmol) was then added at room temperature under N₂. After stirring at 40°C for overnight, the crude reaction mixture was purified by flash column chromatography on silica gel (ISCO 24 g column and eluted with MeOH/DCM (0-20% MeOH in DCM)) to give product as a solid. Then the solid was further purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-
 25 100% Acetonitrile in water) to yield product **Int 11a**. LC/MS 717.3 (M+H)⁺.

Step B - Synthesis of intermediate Int 11

[0217] Intermediate **Int 11a** (19 mg, 0.027 mmol) was dissolved in CH₂Cl₂ (0.3 ml) and HCl (4M in dioxane) (0.040 ml, 0.159 mmol) was then added at room temperature. After stirring at
 30 room temperature for 3 hours, the solvent was removed under reduced pressure and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to afford **Int 11**. LC/MS 617.73 (M+1)⁺.

5

Preparation of Intermediate Int 12



10 Step A: Synthesis of **Int 12a** (2S,3S)-1-((benzyloxy)carbonyl)-3-hydroxypyrrolidine-2-carboxylic acid

[0218] To the suspension of (2S,3S)-3-hydroxypyrrolidine-2-carboxylic acid (5.32 g, 40.6 mmol) in Dioxane (100 ml) at 0°C was added sodium hydroxide (122 ml, 122 mmol), followed by addition of benzyl carbonochloridate (6.50 ml, 44.6 mmol) dropwise, the resulting suspension was stirred at 0 °C for (8.56am) for 5h. After removing the volatile, the aqueous phase was acidified to pH 3, then partitioned between 30%IPA/DCM (200 mL) and brine (50 mL). The aqueous phase was further extracted with 30%IPA/DCM(2x100mL). The combined organic phase was dried over Na₂SO₄, concentrated to afford **Int 12a** (2S,3S)-1-((benzyloxy)carbonyl)-3-hydroxypyrrolidine-2-carboxylic acid as a crude solid. LC/MS 266.1 (M+1)⁺.

20 Step B: Synthesis of **Int 12b** (2S,3S)-1-benzyl 2-methyl 3-hydroxypyrrolidine-1,2-dicarboxylate

[0219] To the solution of **Int 12a** (7.48 g, 28.2 mmol) in MeOH (80 ml) was added TMS-Diazomethane (70.5 ml, 141 mmol) dropwise. After stirring at rt for 10min, the reaction was quenched by addition of acetic acid (ca. 400 μL) dropwise. The solution was concentrated, and the residue was purified on silica gel column using 10-90%EtOAc/hexane as and eluted solvents to afford **Int 12b** (2S,3S)-1-benzyl 2-methyl 3-hydroxypyrrolidine-1,2-dicarboxylate as an oil. LC/MS 280.1 (M+1)⁺.

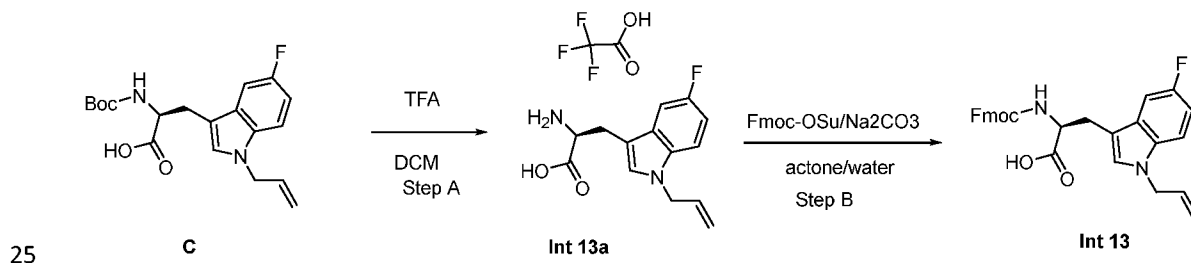
5 Step C: Synthesis of Int 12c

[0220] To solution of **Int 12b** (2.06 g, 7.38 mmol) in DCM (100 mL) was bubbled with N₂ for 30min, then RHODIUM(II) ACETATE DIMER (0.326 g, 0.738 mmol) was added. The mixture was cooled by ice-water-bath, and TERT-BUTYL DIAZOACETATE (1.533 mL, 11.06 mmol) were added at 0 °C. The resulting mixture was stirred at 0 °C for 1.5h. The reaction was
 10 quenched by addition of water (100 mL) and extracted with DCM (3x100 ML) The combined organic phase was dried over Na₂SO₄, concentrated and the residue was purified on reverse phase MPLC (275g) using 10-90% acetonitrile (0.05%TFA)/water (0.05%TFA) as and eluted solvents. The fraction was concentrated, and the aqueous phase was extracted with DCM
 (2x100mL). The combined organic phase was dried over Na₂SO₄, concentrated to afford
 15 (2S,3S)-1-benzyl 2-methyl 3-(2-(tert-butoxy)-2-oxoethoxy) pyrrolidine-1,2-dicarboxylate **Int 12c** as an oil. LC/MS 394.2 (M+1)⁺.

Step D: Synthesis of Int 12

[0221] To the solution of **Int 12c** (2.22 g, 5.64 mmol) in MeOH (100 ml) was added Pd/C (0.420 g, 0.395 mmol). The resulting mixture was subjected to hydrogenation via H₂ balloon at rt
 20 for 2h. The mixture was filtered through celite and the filtrate was concentrated to afford 2S,3S)-methyl 3-(2-(tert-butoxy)-2-oxoethoxy) pyrrolidine-2-carboxylate (**Int 12**) as a liquid. LC/MS 260.1 (M+1)⁺.

Preparation of intermediate Int 13



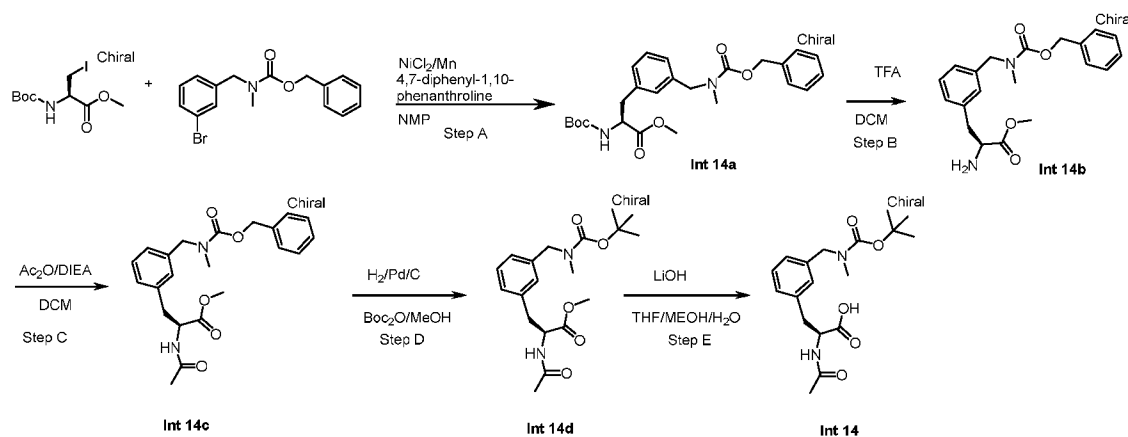
Step A: Synthesis of Int 13a (S)-3-(1-allyl-5-fluoro-1H-indol-3-yl)-2-aminopropanoic acid compound with 2,2,2-trifluoroacetic acid (1:1)

Intermediate C (1.73 g, 4.77 mmol) was dissolved in CH₂Cl₂ (6 ml) and TFA (6 ml, 78
 30 mmol) was then added. After stirring at rt for 30min, the reaction was concentrated to afford **Int 13a** (1:1 TFA salt) as an oil. LC/MS 263.2 (M+1)⁺.

5 Step B: Synthesis of Int 13

[0222] **Int 13a** (1.795 g, 4.77 mmol) was dissolved in Acetone (100 ml) and Water (100 ml) at 0 °C and sodium carbonate (1.011 g, 9.54 mmol) and Fmoc-OSU (1.770 g, 5.25 mmol) were then added. After stirring at RT for 2 hours, the volatile was removed on rotary evaporator, the aqueous phase was acidified to pH 3, the precipitate was extracted with EtOAc (3x100 mL) and the combined organic phase was dried over Na₂SO₄, concentrated. The residue was purified on silica gel column (120g) using 0-10%MeOH/DCM as a gradient to afford **Int 13** as a solid. LC/MS 485.3 (M+1)⁺.

15 **Preparation of intermediate Int 14**



Step A: Synthesis of Int 14a

[0223] To a vial was added nickel (II) chloride (0.088 g, 0.679 mmol), manganese (1.119 g, 20.38 mmol), and 4,7-diphenyl-1,10-phenanthroline (0.226 g, 0.679 mmol). After the vial was degassed by vacuum/N₂ three times, then N₂ degassed NMP (20 ml) was added. The resulting mixture was heated at 80 °C for 20min. After cooled to rt, ®-methyl 2-((tert-butoxycarbonyl)amino)-3-iodopropanoate (2.68 g, 8.15 mmol) and benzyl 3-bromobenzyl(methyl)carbamate (2.27 g, 6.79 mmol) in 0.9 mL NMP was added. The resulting mixture was further degassed by vacuum/N₂ three times, then stirred at rt under N₂ overnight. The mixture was filtered through celite and then the filtrate was partitioned between EtOAc/brine (300 mL/150 mL). The organic phase was washed with brine (3x100 mL), dried over Na₂SO₄, concentrated and the residue was purified on silica gel column (120g) using 0-70%EtOAc/hexane as and eluted solvents to **Int 14a** as a solid. LC/MS 457.3 (M+1)⁺.

5 Step B: Synthesis of Int 14b

[0224] Int 14a (517 mg, 1.132 mmol) was dissolved in CH₂Cl₂ (6 ml) and TFA (4 ml, 51.9 mmol) was then added. After stirring at rt for 1h, the reaction solution was concentrated to afford Int 14b as an oil. LC/MS 357.2 (M+1)⁺.

Step C: Synthesis of Int 14c

10 [0225] Int 14b (404 mg, 1.134 mmol) was dissolved in CH₂Cl₂ (20 ml) and DIEA (1.188 ml, 6.80 mmol) and acetic anhydride (0.160 ml, 1.700 mmol) were then added. After stirring at rt for 2h, the reaction solution was concentrated, and the residue was purified on reverse phase MPLC (130g) using 5-70%acetonitrile (0.05%TFA) to afford Int 14c as an oil. LC/MS 399.3 (M+1)⁺.

Step D: Synthesis of Int 14d

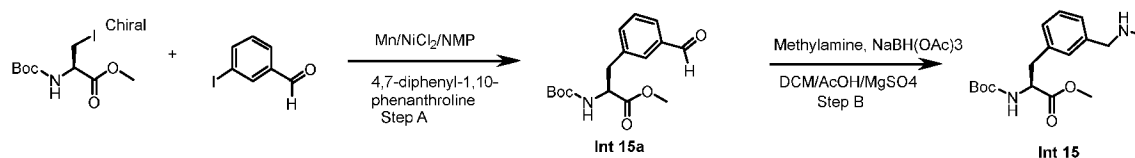
15 [0226] To the solution of Int 14d (438 mg, 1.099 mmol) in MeOH (20 ml) was added Pd/C (117 mg, 0.110 mmol), and BOC-Anhydride (0.383 ml, 1.649 mmol). The resulting mixture was hydrogenated at rt for 2h, then filtered through celite under N₂. Next the filtrate was concentrated, and the residue was purified on silica gel column (80g) using 10-100%EtOAc/hexane as and eluted solvents to afford Int 14d as an oil. LC/MS 365.2 (M+1)⁺.

20 Step E: Synthesis of Int 14

[0227] To the solution of Int 14d (0.33 g, 0.906 mmol) in THF (6 ml), MeOH (2 ml), and Water (2 ml) at 0 °C was added LiOH (3.62 ml, 3.62 mmol) dropwise. After stirring at 0 °C for 2h, the volatile was then evaporated on rotary evaporator and the aqueous phase was acidified to pH 4, then extracted with DCM (3x50 mL). The combined organic phase was dried over

25 Na₂SO₄, concentrated to afford Int 14 as an oil. LC/MS 351.2. (M+1)⁺.

Preparation of intermediate Int 15



30

Step A: Synthesis of Int 15a

[0228] Nickel (II) chloride (0.154 g, 1.185 mmol), manganese (1.953 g, 35.5 mmol), and 4,7-diphenyl-1,10-phenanthroline (0.394 g, 1.185 mmol) were added in a vial, which was degassed by vacuum/N₂ three times. Next N₂ degassed NMP (26 ml) was added. The resulting mixture

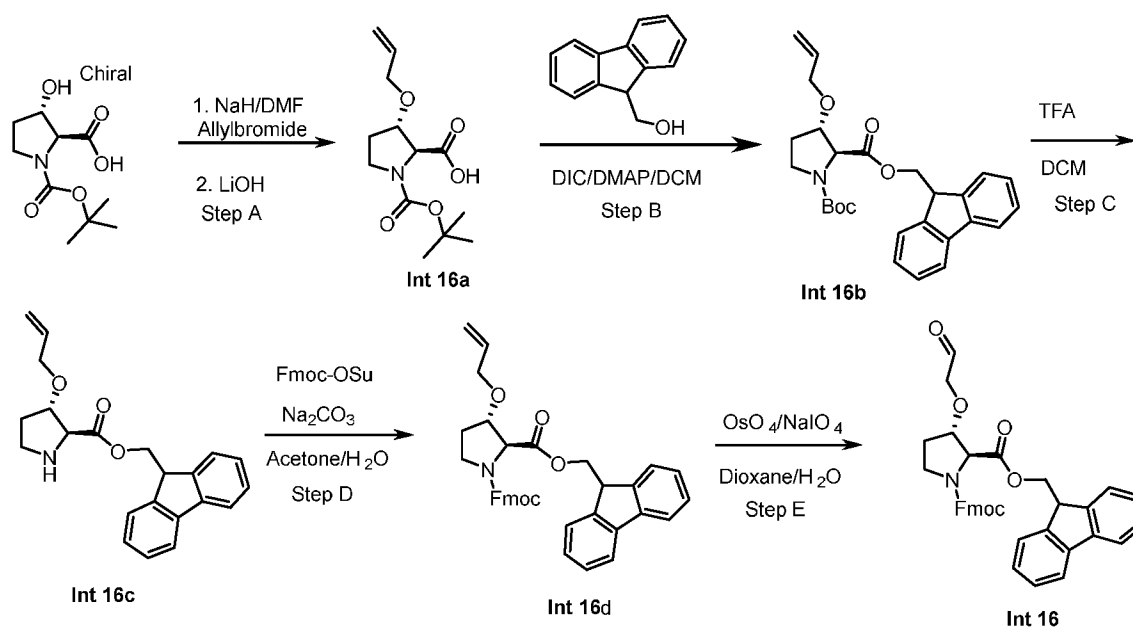
35 was heated at 80 °C for 20min. After cooled to rt, (R)-methyl 2-((tert-butoxycarbonyl)amino)-3-

5 iodopropanoate (3.9 g, 11.85 mmol) and 3-iodobenzaldehyde (3.02 g, 13.03 mmol) was added. The resulting mixture was further degassed by vacuum/N₂ three time, then stirred at RT under N₂ overnight. The mixture was filtered through celite, and the filtrate was partitioned between EtOAc/brine (300 mL/150 mL). The organic phase was washed with brine (3x100 mL), dried over Na₂SO₄, concentrated and the residue was purified on silica gel column (120g) using 0-40%EtOAc/hexane as and eluted solvents to afford **Int 15a** as an oil. LC/MS 330.3 (M+23)⁺.

Step B: Synthesis of **Int 15**

[0229] **Int 15a** (2.08 g, 6.77 mmol) was dissolved in CH₂Cl₂ (30 ml) and methanamine (40% in water) (0.791 ml, 10.15 mmol) was then added. Next drops of acetic acid, Magnesium sulphate (16 g) and sodium triacetoxyborohydride (4.30 g, 20.30 mmol) were added. After stirring at rt for 1h, the mixture was filtered, and the filtrate was concentrated. The residue was purified by reverse phase purification on MPLC (C18, 86 g), using 0-60%acetonitrile (0.05%TFA) as and eluted solvents to give the TFA salt product. The salt product (0.48g) was partitioned between DCM (100 mL) and sat. Na₂CO₃ (50 mL). The aqueous phase was extracted with DCM (3x50 mL). the combined organic phase was dried over Na₂SO₄, concentrated to afford **Int 15** as an oil. LC/MS 323.3 (M+1)⁺.

Preparation of intermediate **Int 16**



5 Step A: Synthesis of **Int 16a**

[0230] To the solution of (2S,3S)-1-(tert-butoxycarbonyl)-3-hydroxypyrrolidine-2-carboxylic acid (0.7 g, 3.03 mmol) in DMF (30 ml) at 0 °C was added NaH (0.303 g, 7.57 mmol). The resulting mixture was stirred at 0 °C for 30min. Then ALLYL BROMIDE (0.576 ml, 6.66 mmol) was added. After stirring at 0 °C for 1 hour, then rt for 2 hours, it was quenched by addition of
10 water dropwise (2 mL) at 0 °C. LiOH (3.03 ml, 3.03 mmol) was added to the above solution at 0 °C dropwise. The resulting solution was stirred at rt for 3h, then at 0 °C was acidified to pH 4 by addition of 0.1N HCl. The mixture was partitioned between EtOAc (400 mL) and water (400 mL). The organic phase was washed with water (2x400 mL), dried over MgSO₄, concentrated and the residue was purified on silica gel column using 0-5%MeOH/DCM as and eluted solvents
15 to afford **Int 16a** as an oil. LC/MS 272.2 (M+1)⁺.

Step B: Synthesis of **Int 16b**

[0231] **Int 16a** (2.099 g, 7.74 mmol) was dissolved in CH₂Cl₂ (20 ml) and (9H-fluoren-9-yl)methanol (1.822 g, 9.28 mmol), DIC (1.446 ml, 9.28 mmol), and DMAP (0.095 g, 0.774 mmol) were added. After stirring at rt for 2h, the mixture was filtered. The filtrate was
20 concentrated, and the residue was purified on silica gel column (120g) using 0-30-30%EtOAc/hexane as and eluted solvents to afford **Int 16b** as an oil. LC/MS 450.3 (M+1)⁺.

Step C: Synthesis of **Int 16c**

[0232] **Int 16b** (3.99 g, 7.63 mmol) was dissolved in CH₂Cl₂ (10 ml) and TFA (11.76 ml, 153 mmol) was then added. After stirring at rt for 1h, the reaction solution was concentrated and the
25 residue was dissolved in acetonitrile/water (1:1, 200 mL), and lyophilized to afford **Int 16c** as a powder. LC/MS 350.4 (M+1)⁺.

Step D: Synthesis of **Int 16d**

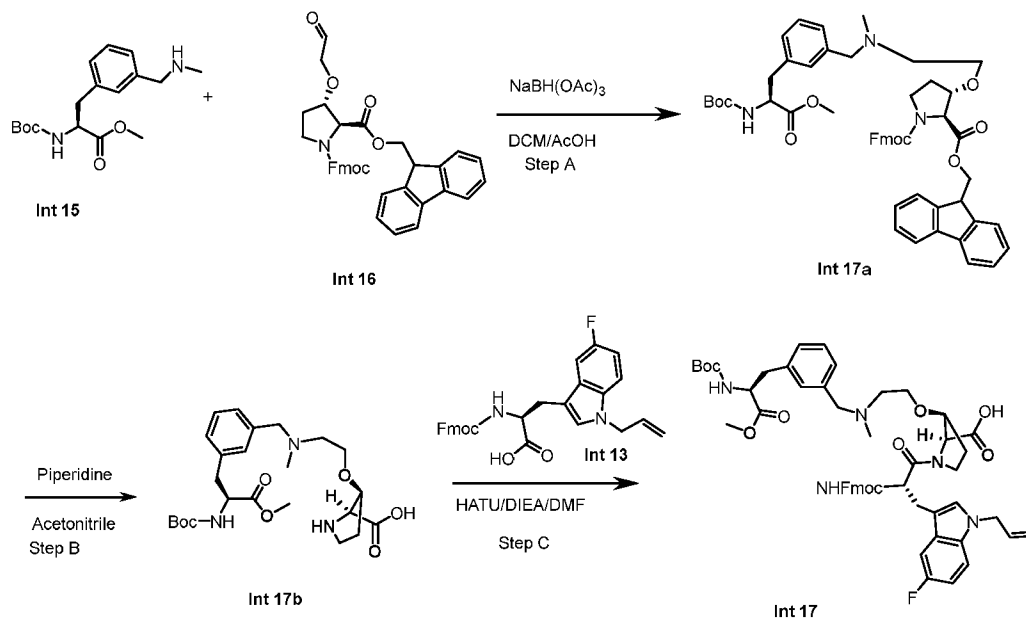
[0233] To the solution of **Int 16c** (3.54 g, 7.64 mmol) in Acetone (110 ml) and Water (100 ml) was added Fmoc-OSU (2.71 g, 8.02 mmol). The resulting mixture was stirred at rt for 2h. Next
30 the volatile was evaporated on rotary evaporator. The aqueous phase was extracted with EtOAc (3x150 mL), the combined organic phase was dried over Na₂SO₄, concentrated and the residue was purified on silica gel column using 0-30%EtOAc/hexane as and eluted solvents to afford **Int 16d** as an oil. LC/MS 572.3 (M+1)⁺.

Step E: Synthesis of **Int 16**

35 [0234] To the solution of **Int 16d** (4.23 g, 7.40 mmol) in Dioxane (80 ml) and Water (40 ml) was added sodium periodate (6.33 g, 29.6 mmol) and osmium tetroxide (2.351 g, 0.370 mmol).

- 5 After the mixture was filtered through celite and the volatile of the filtrate was evaporated and the residue was extracted with DCM (4x100 mL), the combined organic phase was dried over Na₂SO₄, concentrated and the residue was purified on silica gel column using 0-80%EtOAc/hexane as and eluted solvents to afford **Int 16** as an oil. LC/MS 574.3 (M+1)⁺.

10 **Preparation of intermediate Int 17**



Step A: Synthesis of **Int 17a**

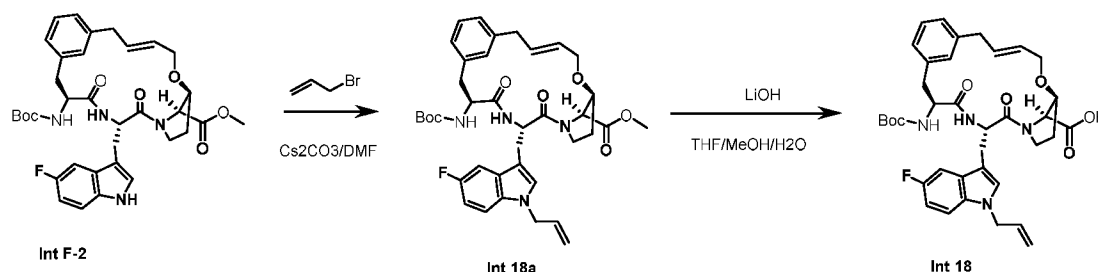
- [0235] To the solution of **Int 16** (458 mg, 1.421 mmol) and **Int 15** (978 mg, 1.705 mmol) in CH₂Cl₂ (20 ml) was added AcOH (50 μ L) and sodium triacetoxyborohydride (903 mg, 4.26 mmol). The resulting mixture was stirred at for 2h. Next the mixture was partitioned between sat. Na₂CO₃ (200 mL) and DCM (200 mL). The aqueous phase was further extracted with DCM (4x200 mL). The combined organic phase was dried over Na₂SO₄, concentrated and the residue was purified on silica gel column using 10-100%EtOAc/hexane as and eluted solvents to afford **Int 17a** as an oil. LC/MS 880.4 (M+1)⁺.

20 Step B: Synthesis of **Int 17b**

- [0236] To the solution of **Int 17a** (980 mg, 1.114 mmol) in Acetonitrile (20 ml) was added PIPERIDINE (0.551 ml, 5.57 mmol). The resulting solution was stirred at rt for 1.5h. Next the mixture was filtered, and the filtrate was concentrated. The residue was purified on reverse phase MPLC (C18, 86 g) using 0-50% acetonitrile (0.05%TFA) as and eluted solvents to afford **Int 17b** as an oil. LC/MS 480.3 (M+1)⁺.

5 Step C: Synthesis of **Int 17c**

[0237] To the solution of **Int 17b** (160 mg, 0.330 mmol) in DMF (2 ml) was added HATU (132 mg, 0.347 mmol) and DIEA (0.115 ml, 0.660 mmol). The resulting solution was stirred at rt for 30min, then was added to a solution of **Int 13** (234 mg, 0.330 mmol) and DIEA (0.346 ml, 1.981 mmol) in DMF (4 ml), the resulting solution was stirred at rt for 1h, then purified on reverse
 10 phase MPLC (C18, 86g) using 10-100%acetonitrile (0.05%TFA) as and eluted solvents to afford **Int 17** as a powder 1:1 (TFA salt). LC/MS 946.2 (M+1)⁺.

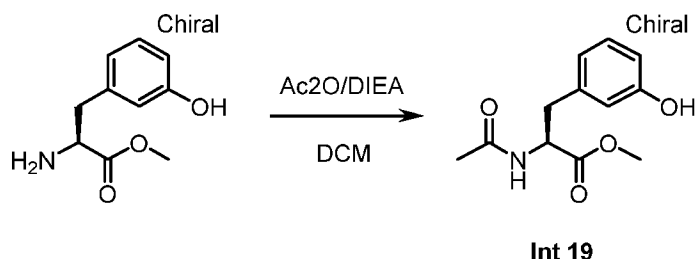
Preparation of intermediate Int 1815 Step A: Synthesis **Int 18a**

[0238] **Intermediate F-2** (2 g, 3.08 mmol) was dissolved in anhydrous DMF (20 ml) and cesium carbonate (3.42 g, 10.48 mmol) was then added, followed by 3-bromoprop-1-ene (0.934 ml, 10.79 mmol). The resulting mixture was stirred vigorously at overnight. The mixture was partitioned between EtOAc (200 mL) and water (100 mL). The organic phase was washed with
 20 water 2x100 mL, dried over magnesium sulfate and concentrated. The residue was purified by reverse phase MPLC (275g) C18 using 20-100% acetonitrile (0.05%TFA) as and eluted solvents to afford **Int 18a** as a powder. LC/MS 689.3 (M+1)⁺.

Step B: Synthesis of **Int 18**

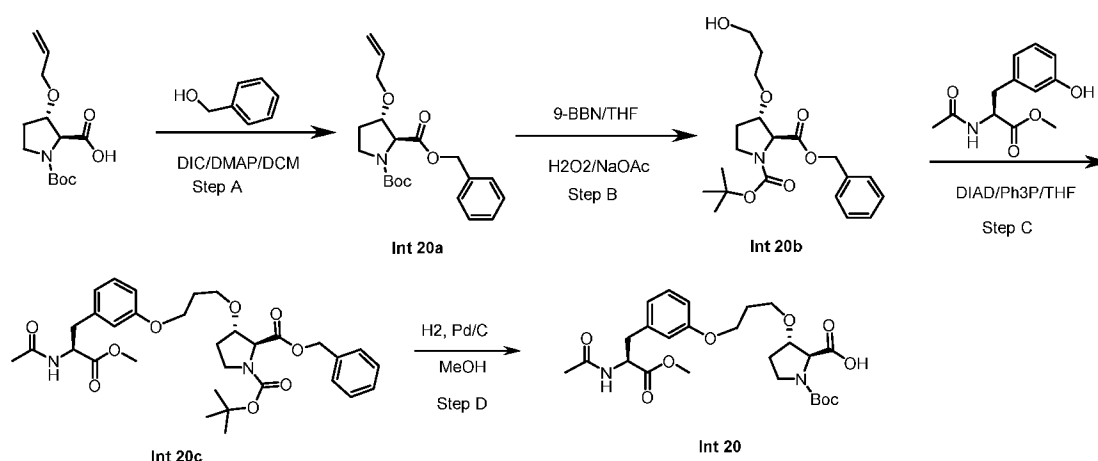
[0239] To the solution of **Int 18** (297 mg, 0.431 mmol) in THF (6 ml), MeOH (2 ml), and
 25 Water (2 ml) at 0 °C was added LiOH (1.725 ml, 1.725 mmol) dropwise. The resulting solution was stirred at 0 °C for 5h. The volatile was then evaporated on rotary evaporator and the aqueous phase was then acidified to pH 3, extracted with DCM (3x80 mL). The combined organic phase was dried over Na₂SO₄, concentrated to afford **Int 18** as a solid. LC/MS 675.2 (M+1)⁺.

5 Preparation of intermediate Int 19



[0240] To the solution of methyl (S)-2-amino-3-(3-hydroxyphenyl)propanoate hydrochloride (0.983 g, 4.24 mmol) in CH₂Cl₂ (60 ml) at 0 °C was added TEA (2.366 ml, 16.97 mmol) and Ac₂O (0.440 ml, 4.67 mmol). The resulting solution was stirred at 0 °C for 1h, then partitioned between DCM (200 mL) water (100 mL) and HCL (1N, 17 mL). The aqueous phase was further extracted with DCM (2x100 mL). The combined organic phase was dried over Na₂SO₄, concentrated and the residue was purified on silica gel column using 30-100%EtOAc/hexane as and eluted solvents to afford **Int 19** as an oil. LC/MS 238.2 (M+1)⁺.

15 Preparation of intermediate Int 20

Step A: Synthesis of Int 20a

[0241] To the solution of (2S,3S)-3-(allyloxy)-1-(tert-butoxycarbonyl)pyrrolidine-2-carboxylic acid (1.06 g, 3.91 mmol) in CH₂Cl₂ (20 ml) was added BENZYL ALCOHOL (0.487 ml, 4.69 mmol), DIC (0.730 ml, 4.69 mmol), and DMAP (0.048 g, 0.391 mmol). After stirring at rt for 2h, it was filtered, and the filtrate was concentrated. The residue was purified on a silica gel column (120g) using 0-50%EtOAc/hexane as and eluted solvents to afford **Int 20a** as an oil. LC/MS 362.2 (M+1)⁺.

5 Step B: Synthesis of Int 20b

[0242] To the solution of **Int 20a** (0.67 g, 1.854 mmol) in THF (10 ml) at 0 °C was added 9-BBN (0.5M in THF) (11.12 ml, 5.56 mmol) dropwise. The resulting solution was stirred at rt for 1h. After cooled to 0 °C, a solution of SODIUM ACETATE (0.928 g, 11.31 mmol) in water (5 mL) and HYDROGEN PEROXIDE (2.88 ml, 28.2 mmol) was added dropwise. The resulting
 10 solution was stirred at 0 °C for 1h, then partitioned between DCM (100 ml) and water (100 mL). Next the aqueous phase was extracted with DCM (2x100 mL). The combined organic phase was dried over Na₂SO₄, concentrated and the residue was purified on silica gel column using 0-80%EtOAc/hexane as and eluted solvents to afford **Int 20b** as an oil. LC/MS 380.4 (M+1)⁺.

Step C: Synthesis of Int 20c

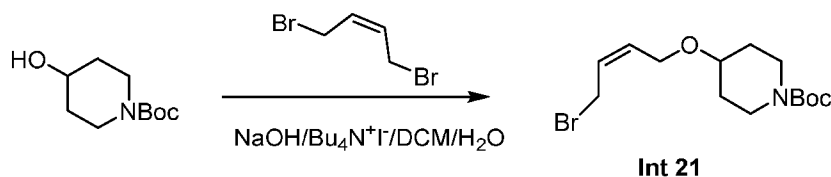
15 [0243] To the solution of **Int 20b** (358 mg, 0.943 mmol) and **Int 19** (328 mg, 1.132 mmol) in THF (10 ml) at 0 °C was added TRIPHENYLPHOSPHINE (371 mg, 1.415 mmol) and DIAD (0.275 mL, 1.415 mmol). The resulting solution was stirred from 0 °C to rt for 2h. The solution was concentrated, and the residue was purified on silica gel column using 0-60-100%-100% EtOAc/hexane as and eluted solvent to give a impure product which was repurified on reverse
 20 phase MPLC (86g) C18 column using 10-100% acetonitrile (0.05%TFA)/water (0.05%TFA) as and eluted solvents to afford **Int 20c** as an oil. LC/MS 599.6 (M+1)⁺.

Step D: Synthesis of Int 20

[0244] To the solution of **Int 20c** (155 mg, 0.259 mmol) in MeOH (10 ml) was added 10%Pd/C (27.6 mg, 0.026 mmol). The resulting mixture was hydrogenated at rt via a H₂ balloon for 2h,
 25 then filtered through celite. The filtrate was concentrated to afford **Int 20** as an oil. LC/MS 509.5 (M+1)⁺.

Preparation of intermediate Int 21

[0245]



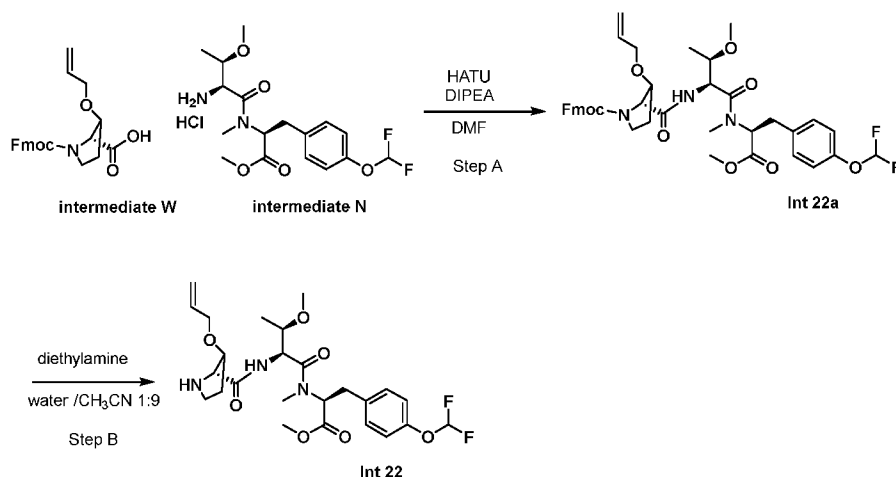
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[0246] To the solution of tert-butyl 4-hydroxypiperidine-1-carboxylate (1.285 g, 6.38 mmol) in DCM (29.0 ml) and Water (2.90 ml) was added TBAI (0.236 g, 0.638 mmol) and fresh ground NaOH (2.55 g, 63.8 mmol). The resulting mixture was stirred at rt until clear solution. Then (Z)-1,4-dibromobut-2-ene (4.10 g, 19.15 mmol) was added to the above solution via syringe. After

5 stirring at rt for 20h, it was partitioned between DCM (200 mL) and water (200 mL). The aqueous phase was further extracted with DCM (2x100 mL). The combined organic phase was dried over Na₂SO₄, concentrated and the residue was purified on a silica gel column (120g) using 0-30%EtOAc/hexane as and eluted solvents to afford **Int 21** as an oil. LC/MS 334.3; 336.3 (M+1)⁺.

10

Preparation of Intermediate Int 22



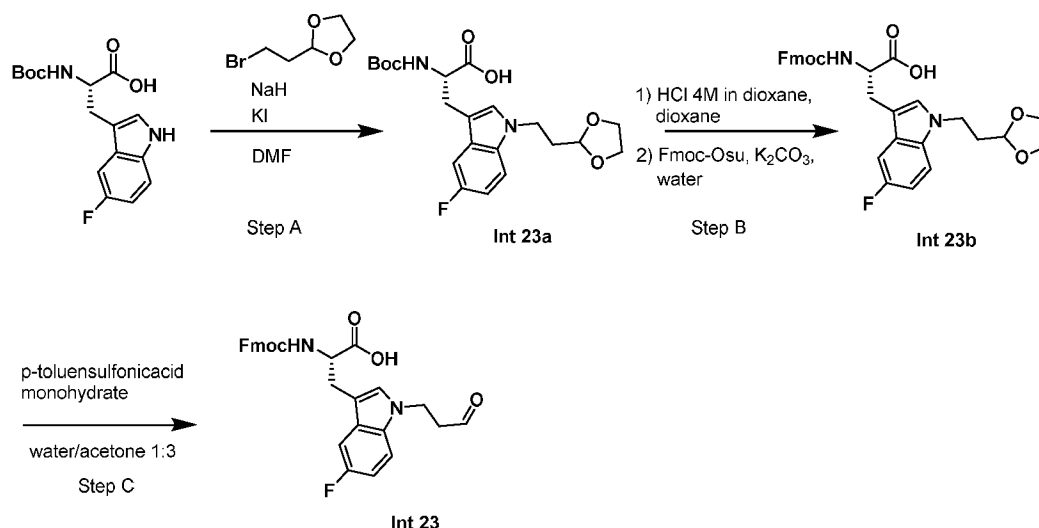
Step A - Synthesis of intermediate **Int 22a**

[0247] A solution of **intermediate N** (1.643 g, 4 mmol) and **intermediate W** (1.574 g, 4.00 mmol) in dry DMF (40 ml) was treated with HATU (1.521 g, 4.00 mmol) and DIPEA (2.79 ml, 16.00 mmol). After stirring at room for 3h, it was then added EtOAc and 0.1 M HCl. The phases were separated and the organic phase was washed with NaHCO₃ saturated aqueous solution and brine, the organic phase dried (Na₂SO₄) and filtered, evaporation of the solvent to afford the crude product which was purified by flash chromatography on silica (Luknova 40g, eluent: PE/EtOAc from 100:0 to 0:100 in 6 CV then EtOAc/MeOH 9:1 10CV) to afford **Int 22a**. UPLC-MS: [M+H]⁺ = 751.5.

Step B - Synthesis of intermediate **Int 22**

[0248] A solution of **intermediate Int 22** (2 g, 2.67 mmol) in CH₃CN (25 ml) was treated with diethylamine (3.90 ml, 37.3 mmol) for 16 h and then the solvents were removed to afford after lyophilization **Int 22** as gum. UPLC-MS: [M+H]⁺ = 529.2.

5 Preparation of Intermediate Int 23

Step A - Synthesis of intermediate Int 23a

[0249] NaH (3.24 g, 81 mmol) was suspended in DMF dry (180 ml) and (S)-2-((tert-butoxycarbonyl)amino)-3-(5-fluoro-1H-indol-3-yl)propanoic acid (8.70 g, 27 mmol) was added dissolved in dry DMF 20 ml. After stirring at room temperature for 20 minutes, KI (1.345 g, 8.10 mmol) and 1.5 equivalents of 2-(2-bromoethyl)-1,3-dioxolane were added and the reaction mixture was stirred at 50°C overnight. Next another 4 equivalents of 2-(2-bromoethyl)-1,3-dioxolane were added in two portions and the mixture was stirred at 50°C.

[0250] The mixture was diluted with EtOAc (250 mL) and washed with 0.1 N HCl (2 x 150 mL). The aqueous layer was back extracted with EtOAc (250 mL). Combined organic layer was washed with brine (250 mL), dried over Na₂SO₄, filtered, and concentrated to dryness to afford the crude material which was dissolved in THF/water 1:1 (30 mL) and treated with LiOH (0.168 g, 7 mmol) for 4 h at room temperature. The mixture was diluted with EtOAc (100 mL) and washed with 0.1 N HCl (2 x 150 mL). The aqueous layer was back extracted with EtOAc (150 mL). The combined organic phase was washed with brine and dried over Na₂SO₄, filtered, and concentrated to dryness to afford **Int 23a**. UPLC-MS: [M+H]⁺ = 423.0.

Step B - Synthesis of intermediate Int 23b

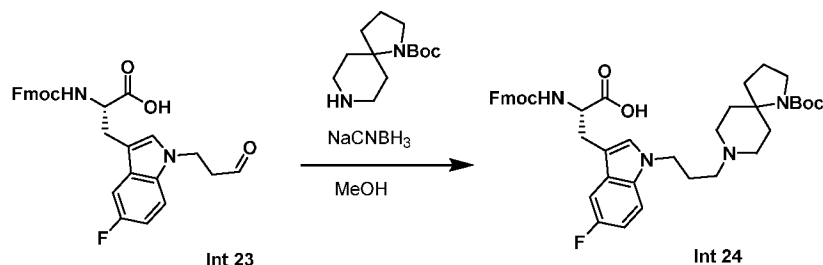
[0251] Intermediate **Int 23a** (11.41 g, 27 mmol) was dissolved in dioxane (30ml) and treated with 4M HCl in dioxane (27.0 ml, 108 mmol) at room temperature for overnight. The temperature was brought to 0 °C and a solution of Na₂CO₃ (22.89 g, 216 mmol) in water (20 ml) was added portion wise. After PH was checked ~ 7-8, Fmoc-Osu (8.20 g, 24.30 mmol) was added. The mixture was allowed to reach room temperature and stirred for 2 h. The mixture was

5 diluted with EtOAc (150 mL) and washed with 1N HCl (3 x 50mL). The aqueous layer was back
extracted with EtOAc (50 mL). Combined organic phase was washed with brine (2 x 50 mL),
dried over Na₂SO₄, filtered, and concentrated to dryness to afford the crude product, which was
purified by silica gel chromatography on Biotage, using Luknova SuperSep (240 g HP; gradient
(0.5% acetic acid): 10% to 80% AcOEt in PE over 12 CV. The collected fractions were
10 concentrated to dryness and lyophilized to afford **Int 23b** as solid. R_f (0.5%acetic acid/50%
AcOEt/50%PE) = 0.3. UPLC-MS: [M+H]⁺ = 542.9.

Step C - Synthesis of intermediate **Int 23**

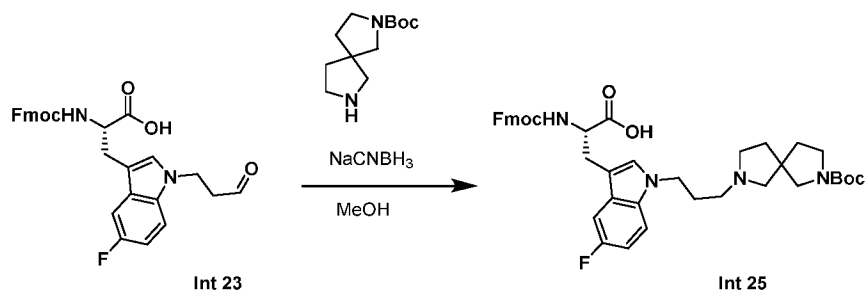
[0252] In a microwave vial **Int 23b** (1 g, 1.836 mmol) and p-toluensulfonic acid monohydrate (3.49 g, 18.36 mmol) were dissolved in water (4.67 ml) and acetone (14 ml). The mixture was heated at 80 °C for 1h under microwave irradiation. Then the mixture was extracted with Et₂O. The phases were separated and the organic washed with NaHCO₃ two times. Then the aqueous phase was basified with NaHCO₃ and extracted with EtOAc. The organic phases (Et₂O and EtOAc) were combined, dried over Na₂SO₄ and filtered and concentrated under reduced pressure to give **Int 23**. UPLC-MS: [M+H]⁺ = 501.0.

Preparation of Intermediate Int 24



[0253] Intermediate **Int 23** (280 mg, 0.559 mmol) and tert-butyl 1,8-diazaspiro[4.5]decane-1-decarboxylate (154 mg, 0.643 mmol) were dissolved in dry MeOH (3 ml). AcOH was added to
25 adjust pH to 5. After 20 min, sodiumcyanoborohydride (42.2 mg, 0.671 mmol) was added. After stirring at room temperature overnight, the reaction was concentrated to dryness. The crude product was dissolved with EtOAc and washed with NaHCO₃ saturated aqueous solution, 1M HCl and brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated to dryness to afford **Int 24**. UPLC-MS: [M+H]⁺ = 724.9.

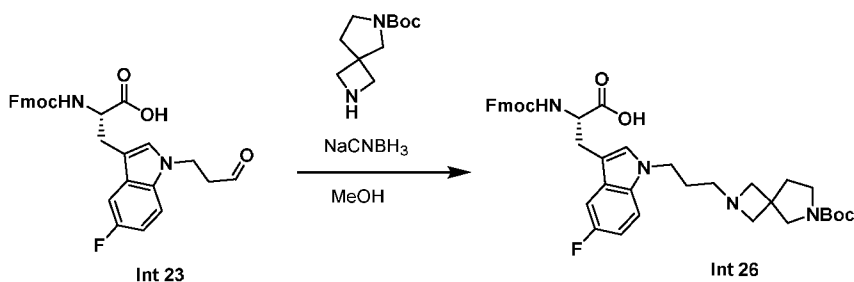
5 Preparation of Intermediate Int 25



[0254] Int 25 was prepared from Int 23 (280 mg) and 1,1-Dimethylethyl 2,7-diazaspiro[4.4]nonane-2-carboxylate (145 mg) according to the same procedure used for Int 24. UPLC-MS: $[M+H]^+ = 712.0$.

10

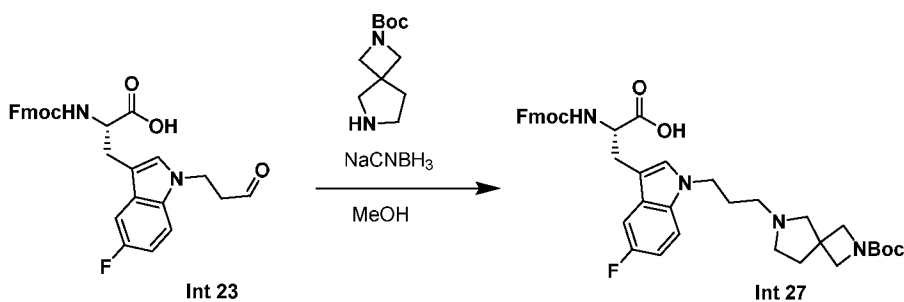
Preparation of Intermediate Int 26



[0255] Int 26 was prepared from Int 23 (500 mg) and tert-butyl 2,6-diazaspiro[3.4]octane-6-carboxylate hydrochloride (248 mg) according to the same procedure used for Int 24. UPLC-MS: $[M+H]^+ = 697.5$.

15

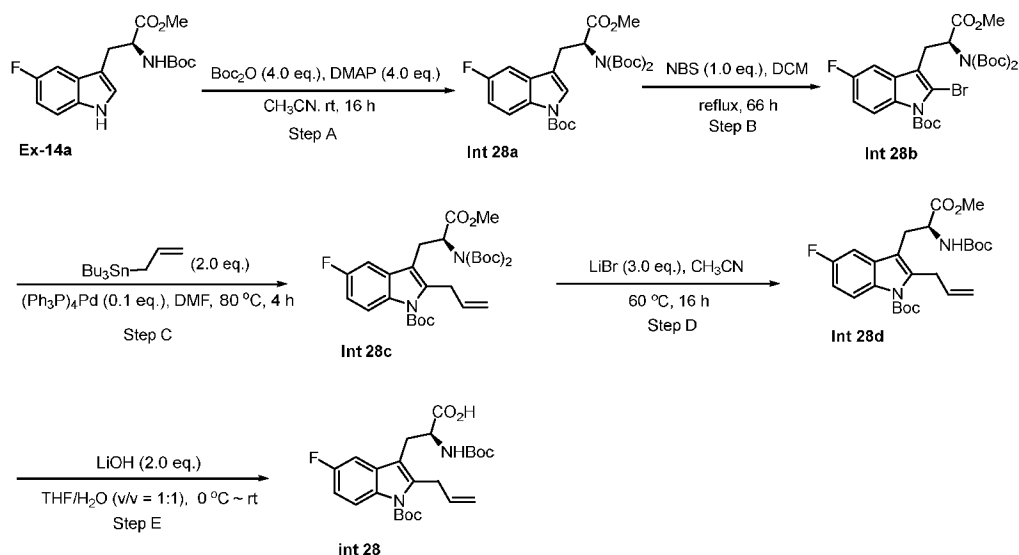
Preparation of Intermediate Int 27



[0256] Int 27 was prepared from Int 23 (500 mg, 0.999 mmol) and tert-butyl 2,6-diazaspiro[3.4]octane-2-carboxylate (244 mg) according to the same procedure used for Int 24. UPLC-MS: $[M+H]^+ = 698.1$.

20

5 Preparation of Int 28



Step A - Synthesis of intermediate **Int 28a**

[0257] To a solution of intermediate (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(5-fluoro-1H-indol-3-yl)propanoate **Ex-14a** (1.00 g, 2.82 mmol) in CH_3CN (30 mL) were added Boc_2O (2.47 g, 11.3 mmol) and DMAP (1.38 g, 11.3 mmol) at room temperature. The reaction solution was stirred at room temperature for 16 h. The resulting solution was concentrated under reduced pressure. The residue was purified by a silica gel column chromatography and eluted with gradient 1% - 20% EA in PE. The fractions containing the desired product were combined and concentrated under reduced pressure to afford (S)-tert-butyl 3-(2-(bis(tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-5-fluoro-1H-indole-1-carboxylate **Int 28a** as a solid. LCMS (ESI) calculated for $\text{C}_{27}\text{H}_{37}\text{FN}_2\text{O}_8$ $[\text{M} + \text{Na}]^+$: 559.2, found 559.3. ^1H NMR (300 MHz, CDCl_3) δ 8.16-7.99 (m, 1H), 7.43 (s, 1H), 7.18 (dd, $J = 8.9, 2.5$ Hz, 1H), 7.06-6.96 (m, 1H), 5.16 (dd, $J = 10.0, 5.0$ Hz, 1H), 3.77 (s, 3H), 3.56-3.25 (m, 2H), 1.64 (s, 9H), 1.35 (s, 18H).

Step B - Synthesis of intermediate **Int 28b**

[0258] Intermediate **Int 28a** (2.50 g, 4.43 mmol) was dissolved in DCM (2 mL) was NBS (0.79 g, 4.43 mmol) was added at room temperature. The reaction solution was refluxed at 50°C for 66 h. The resulting solution was allowed to warm to 25°C and concentrated under reduced pressure. The residue was purified by a silica gel column chromatography and eluted with gradient 1% - 20% EA in hexane. The fractions containing the desired product were combined and concentrated under reduced pressure to afford **Int 28b** as a solid. LCMS (ESI) calculated for $\text{C}_{27}\text{H}_{36}\text{BrFN}_2\text{O}_8$ $[\text{M} + \text{Na}]^+$: 639.2, found 639.3.

Step C - Synthesis of intermediate **Int 28c**

5 [0259] To a solution of **Int 28b** (200 mg, 0.031 mmol) in DMF (2 mL) were added allyltributylstannane (201 mg, 0.617 mmol) and Pd(PPh₃)₄ (35.7 mg, 0.309 mmol) at room temperature under argon atmosphere. The reaction solution was stirred at 80 °C for 4 h. The resulting solution was allowed to warm to 25 °C and saturated aqueous NaCl (20 mL) was added to the solution. The solution was extracted with EA (4 x 50 mL) and the organic layer was dried
10 over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by a gel column chromatography, and eluted with 0 - 60% PE in EA. The fractions containing the desired product was combined and concentrated under reduced pressure to afford **Int 28c** as a solid. LCMS [M + Na]⁺: 599.3. ¹H NMR (300 MHz, CDCl₃) δ 8.05 (dd, J = 9.1, 4.6 Hz, 1H), 7.15 (dd, J = 8.9, 2.6 Hz, 1H), 7.01-6.94 (m, 1H), 6.06-5.95 (m, 1H), 5.13 (dd, J = 9.7, 5.1 Hz, 1H), 5.09-5.02 (m, 1H), 4.99-4.92 (m, 1H), 3.86-3.66 (m, 5H), 3.50-3.35 (m, 2H),
15 1.66 (s, 9H), 1.32 (s, 18H).

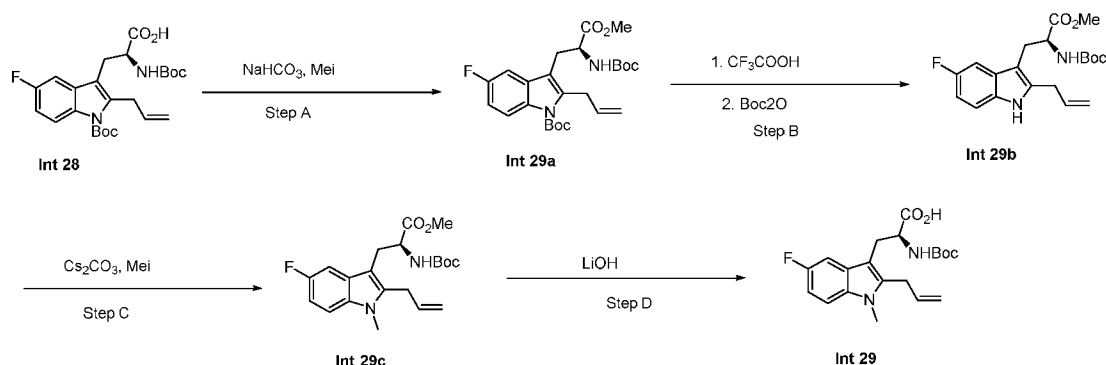
Step D - Synthesis of intermediate **Int 28d**

[0260] To a solution of intermediate **Int 28b** in CH₃CN (50 mL) was added LiBr (0.93 g, 10.73 mmol) at room temperature. The reaction solution was stirred at 60 °C for 16 h. The resulting
20 solution was concentrated under reduced pressure. The residue was purified by a silica gel column chromatography, and eluted with gradient 0 - 30% PE in EA. The fractions containing the desired product were combined and concentrated under reduced pressure to afford **Int 28d** as a solid. LC/MS [M + Na]⁺: 499.2.

Step E - Synthesis of intermediate **Int 28**

25 [0261] Intermediate **Int 28d** (2.20 g, 3.46 mmol) was dissolved in THF (90 mL) and LiOH (69 mL, 6.92 mmol, 0.1 N in water) was then added at 0 °C. The reaction solution was stirred for 4 h at room temperature. The resulting solution was adjusted pH to 4~5 with HCl (1M). The solution was extracted with EA (3 x 100 mL) and the combined organic layer was washed with brine (3 x 100 mL), dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced
30 pressure. The residue was purified by a silica gel column chromatography and eluted with gradient 1% - 70% CH₃CN in Water. The fractions containing the desired product were combined and concentrated under reduced pressure to afford **Int 28** as a solid. LCMS (ESI) [M + H]⁺: 463.2. ¹H NMR (400 MHz, CD₃OD) δ 8.07 (dd, J = 9.1, 4.6 Hz, 1H), 7.30 (dd, J = 9.2, 2.6 Hz, 1H), 7.04-6.95 (m, 1H), 6.12-5.98 (m, 1H), 5.09-5.02 (m, 1H), 4.97-4.89 (m, 1H), 4.37 (dd, J = 8.5, 5.2 Hz, 1H), 3.96-3.78 (m, 2H), 3.23 (dd, J = 14.6, 5.2 Hz, 1H), 3.10-2.94 (dd, J = 14.6,
35 8.5 Hz, 1H), 1.68 (s, 9H), 1.35 (s, 9H).

5 Preparation of Intermediate Int 29

*Step A - Synthesis of intermediate Int 29a*

[0262] Intermediate **Int 28** (2g, 4.32 mmol) was dissolved in DMF (20 ml) and iodomethane (1.346 ml, 21.62 mmol) was added. After stirring rt for 18 hours,

- 10 [0263] The reaction was poured into water, extracted twice with 100ml MTBE, washed with 100ml water twice, washed with 100ml brine, dried over MgSO₄, concentrated to yield **Int 29a** as solid. LCMS 477.33 (M+1)⁺.

Step B - Synthesis of intermediate Int 29b

- 15 [0264] TFA (8ml, 104 mmol) was added dropwise to a solution of **Int 29a** (2g, 4.20 mmol) in DCM (40 ml) while cooled in an ice bath. The resulting mixture was stirred at rt for a period of 2h. The solvents were removed under reduced pressure and to the aqueous solution was added THF (20.00 ml) and Water (20.00 ml). Next di-tert-butyl dicarbonate (1.374 g, 6.30 mmol) was added at 0°C and stirring was continued for overnight. The volatiles were removed under reduced pressure and the resulting aqueous mixture washed with ether (X2). Dichloromethane was added and the aqueous phase was acidified with 1N aq. HCl. The aqueous phase was separated and further extracted with dichloromethane. The combined organic phases were dried over MgSO₄, and the volatiles was removed under reduced pressure to give **Int 29b** as a solid. LCMS 377.30 (M+1)⁺.
- 20

Step C - Synthesis of intermediate Int 29c

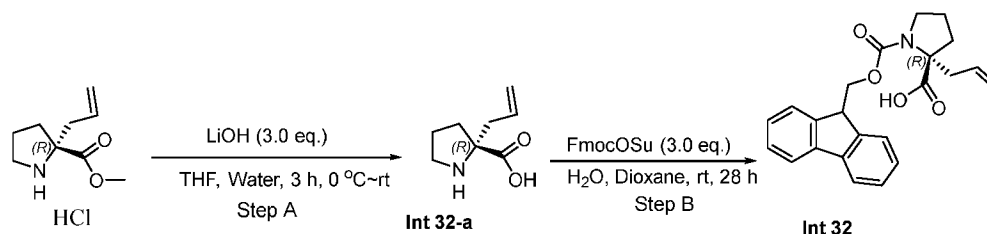
- 25 [0265] To the DMF (20 ml) solution of **Int 29b** (1.24g, 3.29 mmol) was added Cs₂CO₃ (3.22 g, 9.88 mmol) and iodomethane (0.615 ml, 9.88 mmol) at 0 °C under nitrogen atmosphere. After stirring at rt for 2 hours, the reaction solution was extracted with Et₂O (2 x 200 mL). The combined organic layer was washed with brine (3 x 100 mL), dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel 40gx2, and eluted with 0-30% EtOAc/Hexane to give
- 30

- 5 methyl (S)-3-(2-allyl-5-fluoro-1-methyl-1H-indol-3-yl)-2-((tert-butoxycarbonyl)amino)propanoate **Int 29c** as a solid. LCMS 391.30 (M+H)⁺.

Step D - Synthesis of intermediate Int 29d

- [0266] Intermediate **Int 29c** was dissolved in THF (5 ml) and aq 1M lithium hydroxide (4.23 ml, 4.23 mmol) was added and stirred at RT overnight. DCM was added and the reaction solution
 10 was acidified by 1N HCl. The mixture was extracted with DCM for 2 times. The organic layers were combined and separated, washed with brine, separated, dried over MgSO₄, filtered, and concentrated to afford **Int 29d** as a solid, which was use crude in the next step. LCMS 377.30 (M+H)⁺.

15 **Preparation of Intermediate Int 32**



Step A: Synthesis of intermediate Int 32-a

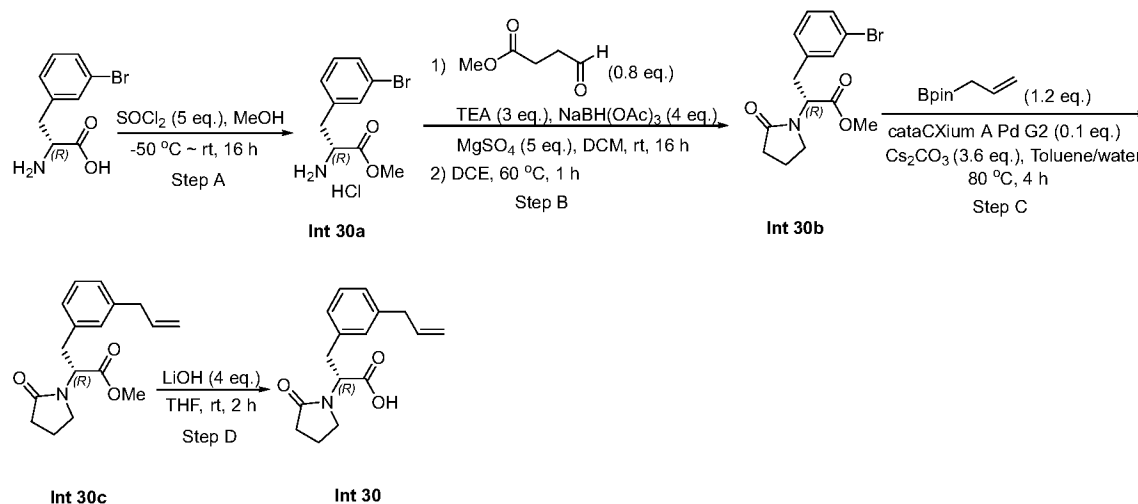
- [0267] To a solution of (R)-methyl 2-allylpyrrolidine-2-carboxylate hydrochloride (8.60 g, 41.8 mmol), in THF (75 mL) and water (75 mL) was added LiOH (4.01 g, 167 mmol) at 0 °C. After
 20 warmed to room temperature, the reaction solution was stirred for 5 h. The resulting solution was concentrated under reduce pressure. The pH value of the solution was adjusted to 8 with aqueous HCl (4 M), which was directly used for the next step without further purification. LCMS (ESI) calculated for C₈H₁₃NO₂ [M + H]⁺:156.1, found 156.2.

Step B: Synthesis of Intermediate Int 32

- 25 [0268] To a solution of **Int 32-a** (10.0 g, 64.4 mmol) in 1,4-dioxane (150 mL) and water (150 mL) was added Fmoc-OSu (65.2 g, 193 mmol). The reaction solution was stirred for 35 h at room temperature. The pH value of the solution was adjusted to 5 with aqueous HCl (4 M). The solution was extracted with EA (2 x 200 mL). The combined organic layers were washed with brine (2 x 200 mL) and concentrated under reduced pressure. The residue was purified by silica
 30 gel column chromatography and eluted with gradient 1% - 10% MeOH in DCM. The fractions containing desired product were combined and concentrated under reduced pressure to afford intermediate **Int 32** as a solid. LCMS (ESI) calculated for C₂₃H₂₃NO₄ [M + H]⁺:378.2, found 378.0; HTEM NMR (300 MHz, DMSO-d₆) δ 12.63 (s, 1H), 7.89 (m, 2H), 7.66 (m, 2H), 7.38 (m,

- 5 4H), 5.77-5.41 (m, 1H), 5.22-4.88 (m, 2H), 4.47-4.14 (m, 3H), 3.55 (m, 1H), 3.30 (m, 1H), 2.95-2.85 (m, 1H), 2.60-2.50 (m, 1H), 2.14-1.63 (m, 4H).

Preparation of Intermediate Int 30



10 Step A - Synthesis of intermediate Int 30a

[0269] To a solution of (R)-2-amino-3-(3-bromophenyl)propanoic acid (1.00 g, 4.10 mmol) in MeOH (10 mL) was added SOCl_2 (1.50 mL, 20.5 mmol) dropwise at -50°C . The reaction was stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure. The residue was washed with ether (50 mL) to afford intermediate Int 30a hydrochloride as a solid.

- 15 LCMS (ESI) calculated for $\text{C}_{10}\text{H}_{13}\text{BrClINO}_2$ $[\text{M} - \text{Cl} + \text{ACN}]^+$: 301.0, found 300.9. ^1H NMR (300 MHz, CD_3OD) δ 7.55-7.15 (m, 4H), 4.35 (t, $J = 6.8$ Hz, 1H), 3.88-3.74 (m, 3H), 3.29-3.10 (m, 2H).

Step B - Synthesis of intermediate Int 30b

- [0270] To a solution of intermediate Int 30a in DCM (10 mL) were added methyl 4-oxobutanoate (0.315 g, 2.72 mmol), TEA (1.42 mL, 10.2 mmol) and MgSO_4 (2.04 g, 17.0 mmol) at room temperature. The mixture was stirred at room temperature for 30 min. Then NaBH_3CN (0.853 g, 13.6 mmol) was added to the mixture at room temperature. The reaction solution was stirred at room temperature for 16 h. The reaction mixture was quenched by MeOH (5 mL) and filtered. The filtrate was concentrated under reduced pressure. The residue was re-
- 25 dissolved in DCE (3 mL) and stirred at 60°C for 1 h. The resulting solution was concentrated under reduced pressure and purified by a silica gel column chromatography and eluted with gradient 0% - 50% EA in PE. The fractions containing desired product were combined and concentrated under reduced pressure to afford Int 30b as an oil. LCMS (ESI) calculated for

- 5 $C_{14}H_{16}BrNO_3$ $[M + H]^+$: 328.0, found 328.0. ^1H NMR (400 MHz, $CDCl_3$) δ 7.45-7.35 (m, 2H), 7.24-7.14 (m, 2H), 5.11-5.02 (m, 1H), 3.76 (s, 3H), 3.49-2.93 (m, 4H), 2.45-2.22 (m, 2H), 2.13-1.84 (m, 2H).

Step C - Synthesis of intermediate **Int 30c**

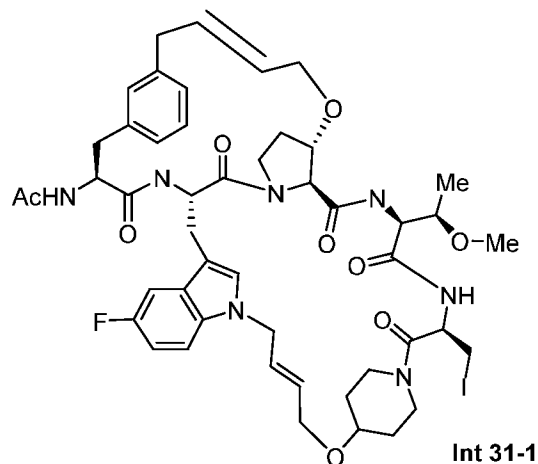
- 10 **[0271]** To a stirred solution of intermediate **Int 30b** (500 mg, 1.53 mmol) in toluene (1 mL) and water (100 μ L) were added allylboronic pinacol ester (309 mg, 1.84 mmol), cataCXium A Pd G2 (102 mg, 0.153 mmol) and Cs_2CO_3 (1.80 g, 5.52 mmol) at room temperature. The reaction solution was stirred at 80 $^{\circ}C$ for 4 h. The resulting solution was concentrated under reduced pressure and purified by a silica gel column chromatography and eluted with gradient 0% - 50% EA in PE. The fractions containing desired product were combined and concentrated under
- 15 reduced pressure to afford of **Int 30c** as an oil. LCMS (ESI) calculated for $C_{17}H_{21}NO_3$ $[M + H]^+$: 288.2, found 288.1; ^1H NMR (400 MHz, $CDCl_3$) δ 7.25-7.17 (m, 1H), 7.12-6.96 (m, 3H), 6.01-5.87 (m, 1H), 5.13-4.98 (m, 3H), 3.72 (s, 3H), 3.50-2.91 (m, 6H), 2.39-2.16 (m, 2H), 2.04-1.77 (m, 2H).

Step D - Synthesis of intermediate **Int 30**

- 20 **[0272]** To a stirred solution of intermediate **Int 30c** (400 mg, 1.39 mmol) in THF (4 mL) was added 1 N LiOH (5.57 mL, 5.57 mmol) at room temperature. The reaction solution was stirred at room temperature for 2 h. The reaction solution was adjusted pH to 2~3 with 1 M HCl. The resulting mixture was concentrated under reduced pressure. The residue was purified by a silica gel column chromatography and eluted with gradient 0% - 5% MeOH in DCM. The fractions
- 25 containing desired product were combined and concentrated under reduced pressure to afford **Int 30** as a solid. LCMS (ESI) calculated for $C_{16}H_{19}NO_3$ $[M + H]^+$: 274.2, found 274.2; ^1H NMR (300 MHz, CD_3OD) δ 7.26-7.14 (m, 1H), 7.11-6.99 (m, 3H), 6.04-5.86 (m, 1H), 5.09-4.87 (m, 3H), 3.48-2.96 (m, 6H), 2.37-2.06 (m, 2H), 2.06-1.72 (m, 2H).

5 Preparation of Intermediate Int 31-1

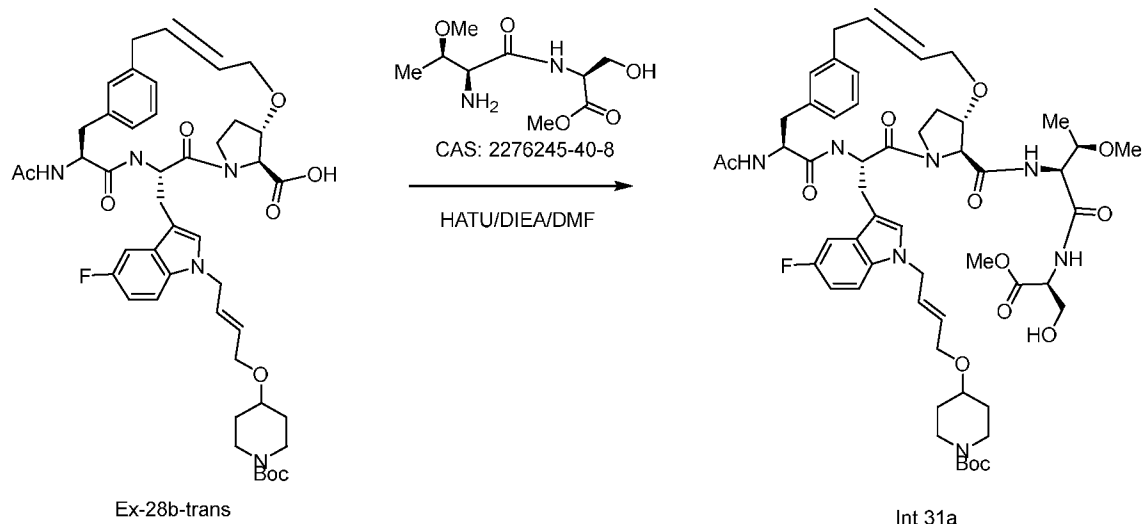
[0273] Intermediate Int 31-1 will be used as starting material in the following library synthesis.



[0274] N-(((3aS,6E,15S,18S,20Z,27E,36R,39S,41aS)-22-fluoro-36-(iodomethyl)-39-((R)-1-methoxyethyl)-16,35,38,41,45-pentaoxo-

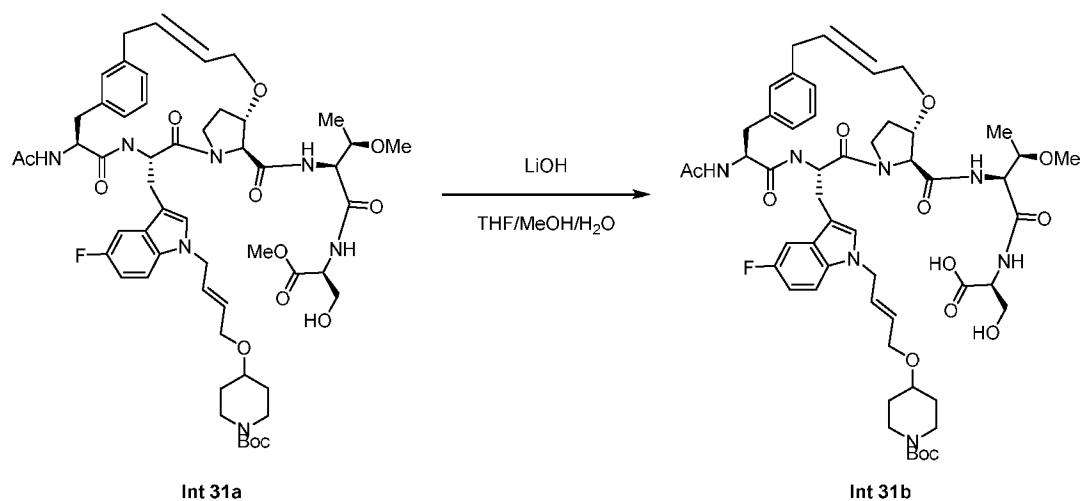
3,3a,5,8,14,15,16,17,18,19,26,29,32,33,35,36,37,38,39,40,41,41a-docosahydro-2H,31H-31,34-ethano-1,18-methano-9,13:20,25-di(metheno)benzo[u]pyrrolo[3,2-b][1,15]dioxo[5,8,11,20,26]pentaazacyclooctatriacontin-15-yl)acetamide

Step 1: tert-butyl 4-(((E)-4-(3-(((12S,13S,9S,12S,E)-9-acetamido-12-(((2S,3R)-1-(((S)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-methoxy-1-oxobutan-2-yl)carbamoyl)-10,13-dioxo-2-oxa-11-aza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphan-4-en-12-yl)methyl)-5-fluoro-1H-indol-1-yl)but-2-en-1-yl)oxy)piperidine-1-carboxylate



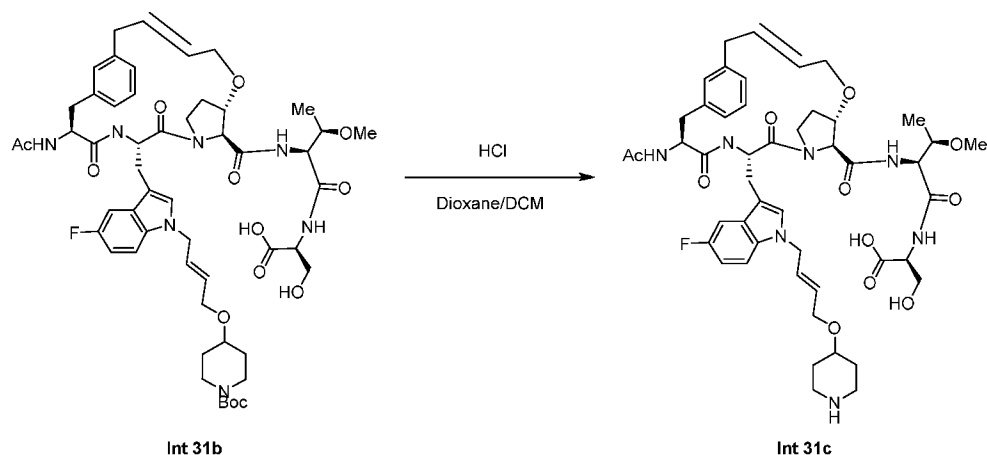
[0275] HATU (240 mg, 0.63 mmol) was added to a mixture of Ex-28b-trans (500 mg, 0.60 mmol), methyl O-methyl-L-threonyl-L-serinate (170 mg, 0.63 mmol), and DIEA (0.53 mL, 3.0

- 5 mmol) in DMF (6.0 mL). The reaction was stirred at 23 °C for 1 h. The reaction was diluted with EtOAc and washed with a saturated aqueous solution of NaHCO₃. The organic layer was dried over MgSO₄ and concentrated. The residue was purified by flash chromatography (silica gel, 0% MeOH/100% DCM to 10% MeOH/90% DCM) to provide compound **Int 31a**. LCMS 1047.1 (M+1)⁺.
- 10 **Step 2:** N-((12S,13S,9S,12S,E)-9-acetamido-12-((1-((E)-4-((1-(tert-butoxycarbonyl)piperidin-4-yl)oxy)but-2-en-1-yl)-5-fluoro-1H-indol-3-yl)methyl)-10,13-dioxo-2-oxa-11-aza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphan-4-ene-12-carbonyl)-O-methyl-L-threonyl-L-serine



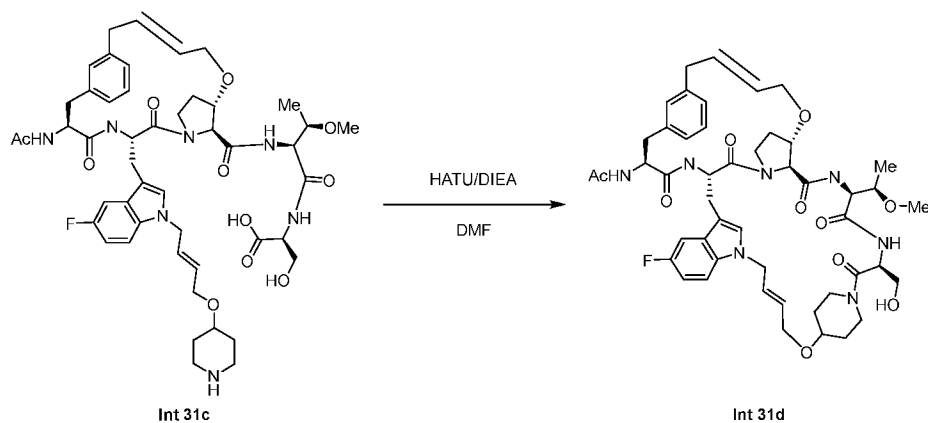
- [0276] LiOH•H₂O (45 mg, 1.1 mmol) was added to a solution of **Int 31a** (370 mg, 0.35 mmol) in 1:1:1 THF:MeOH:H₂O (3.5 mL). The reaction was stirred at 23 °C for 30 min. The reaction was diluted with 2 N aqueous solution of HCl. The resulting mixture was extracted with EtOAc. Combined organic layers were dried over MgSO₄ and concentrated to provide compound **Int 31b**. MS: m/z = 1033.0 (M+1).
- 15

- 5 **Step 3:** N-((12S,13S,9S,12S,E)-9-acetamido-12-((5-fluoro-1-((E)-4-(piperidin-4-yloxy)but-2-en-1-yl)-1H-indol-3-yl)methyl)-10,13-dioxo-2-oxa-11-aza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphan-4-ene-12-carbonyl)-O-methyl-L-threonyl-L-serine



- 10 **[0277]** HCl (4 M in dioxane, 0.89 mL, 3.5 mmol) was added to a solution of **Int 31b** (370 mg, 0.35 mmol) in DCM (1.8 mL). The reaction was stirred at 23 °C for 30 min. The reaction was concentrated to provide compound **Int 31c HCl salt**. MS: $m/z = 933.0$ (M+1).

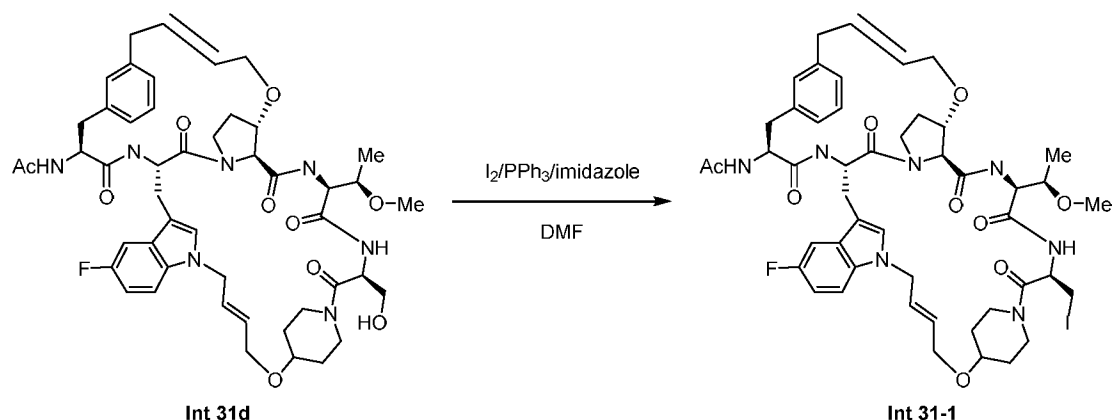
- 15 **Step 4:** N-((3aS,6E,15S,18S,20Z,27E,36S,39S,41aS)-22-fluoro-36-(hydroxymethyl)-39-((R)-1-methoxyethyl)-16,35,38,41,45-pentaoxo-3,3a,5,8,14,15,16,17,18,19,26,29,32,33,35,36,37,38,39,40,41,41a-docosahydro-2H,31H-31,34-ethano-1,18-methano-9,13:20,25-di(metheno)benzo[u]pyrrolo[3,2-b][1,15]dioxo[5,8,11,20,26]pentaazacyclooctatriacontin-15-yl)acetamide



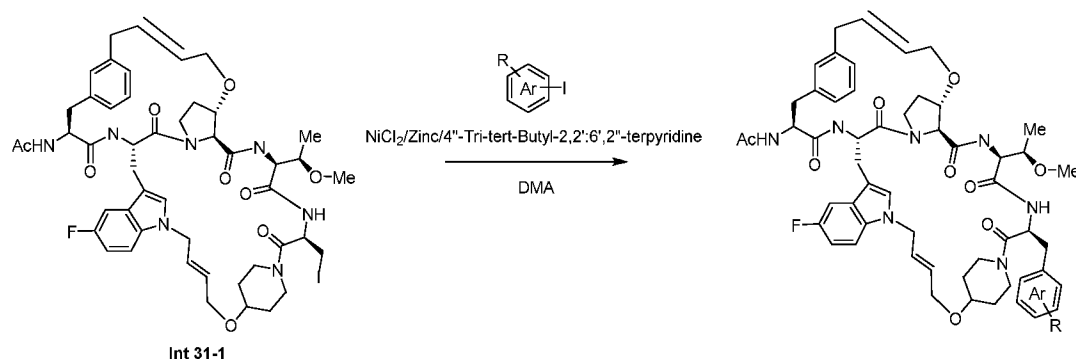
- 20 **[0278]** HATU (140 mg, 0.35 mmol) was added to a mixture of **Int 31c HCl salt** (340 mg, 0.35 mmol), and DIEA (0.19 mL, 1.1 mmol) in DMF (7.1 mL). The reaction was stirred at 23 °C for 2h. The reaction was diluted with EtOAc and washed with a saturated aqueous solution of NaHCO_3 . The organic layer was dried over MgSO_4 and concentrated. The residue was purified by flash chromatography (silica gel, 0% MeOH/100% DCM to 10% MeOH/90% DCM) to

5 provide compound **Int 31d**. dMS: m/z = 915.0 (M+1).

Step 5: N-((3aS,6E,15S,18S,20Z,27E,36R,39S,41aS)-22-fluoro-36-(iodomethyl)-39-((R)-1-methoxyethyl)-16,35,38,41,45-pentaoxo-3,3a,5,8,14,15,16,17,18,19,26,29,32,33,35,36,37,38,39,40,41,41a-docosahydro-2H,31H-31,34-ethano-1,18-methano-9,13:20,25-di(metheno)benzo[u]pyrrolo[3,2-b][1,15]dioxo[5,8,11,20,26]pentaazacyclooctatriacontin-15-yl)acetamide



[0279] Iodine (33 mg, 0.13 mmol) was added to a mixture of **Int 31d** (50 mg, 0.055 mmol), imidazole (11 mg, 0.16 mmol), and PPh₃ (40 mg, 0.15 mmol) in DCM (1.1 mL). The reaction was stirred at 23 °C for 4 h. The reaction was concentrated. The residue was purified by flash chromatography (silica gel, 0% MeOH/100% DCM to 10% MeOH/90% DCM) to provide compound **Int 31-1**. MS: m/z = 1024.9 (M+1).



Library Scheme 1.

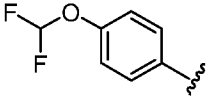
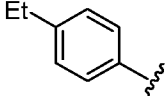
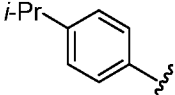
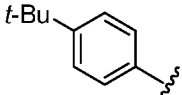
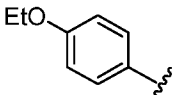
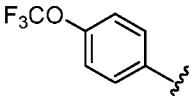
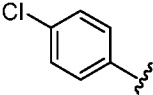
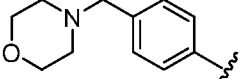
[0280] Analogs reported in the **Table 1-Library** below were prepared in a library format using the following general procedure:

[0281] Inside a nitrogen-filled glovebox, a stock solution of N-((3aS,6E,15S,18S,20Z,27E,36R,39S,41aS)-22-fluoro-36-(iodomethyl)-39-((R)-1-methoxyethyl)-16,35,38,41,45-pentaoxo-3,3a,5,8,14,15,16,17,18,19,26,29,32,33,35,36,37,38,39,40,41,41a-

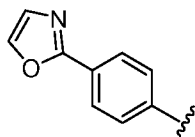
5 docosahydro-2H,31H-31,34-ethano-1,18-methano-9,13:20,25-di(metheno)benzo[u]pyrrolo[3,2-
b][1,15]dioxo[5,8,11,20,26]pentaazacyclooctatriacontin-15-yl)acetamide (**Int 31-1**) (100 mg,
0.098 mmol) in DMA (375 μ L), a suspension of Zn dust (19 mg, 0.39 mmol) in DMA (375 μ L),
NiCl₂•DME and 4''-Tri-tert-Butyl-2,2':6',2''-terpyridine in DMA (250 μ L) were prepared. 15 μ L
10 of **Int 31-1** solution, 15 μ L of Zn solution, and 10 μ L of NiCl₂•DME and 4''-Tri-tert-Butyl-
2,2':6',2''-terpyridine solution were dosed into 50 μ L micro vials charged with aryl iodide
monomers (0.008 mmol). The reaction block containing the vials was sealed, placed on a tumble
stirrer, and heated to 40 °C for 18 h. The reaction mixtures were allowed to cool to 23 °C,
quenched with 1% AcOH in DMSO, filtered. The resulting crude mixtures were purified by
reverse-phase high-performance liquid chromatography.

15

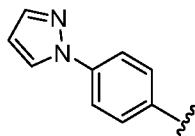
Table 1-Library

Compound Number	Structure of Aryl group
Int 31-2	
Int 31-3	
Int 31-4	
Int 31-5	
Int 31-6	
Int 31-7	
Int 31-8	
Int 31-9	

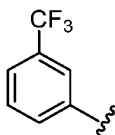
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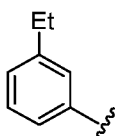
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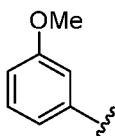
Int 31-12



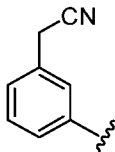
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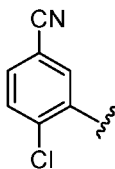
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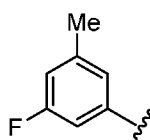
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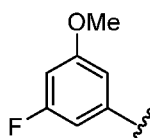
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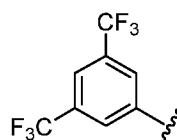
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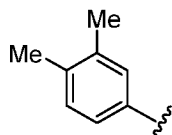
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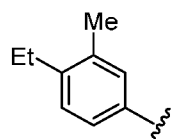
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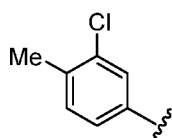
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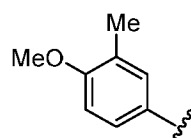
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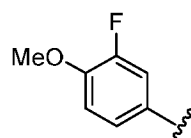
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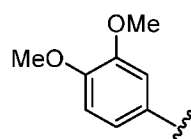
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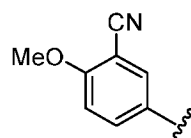
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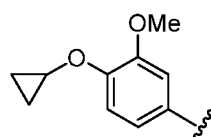
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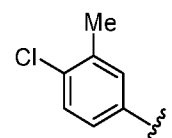
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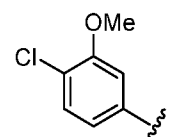
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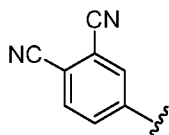
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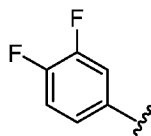
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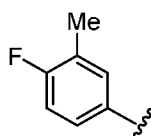
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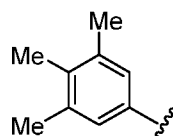
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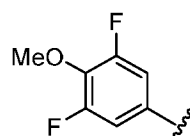
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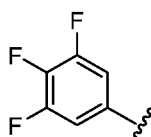
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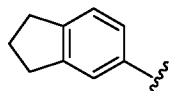
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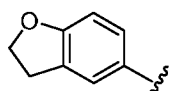
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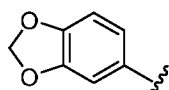
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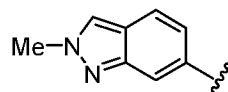
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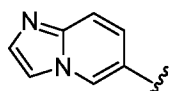
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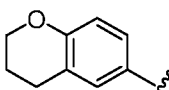
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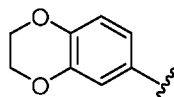
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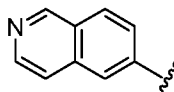
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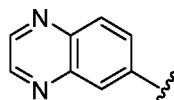
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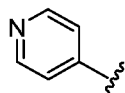
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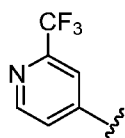
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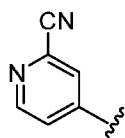
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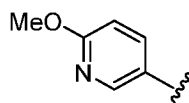
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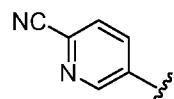
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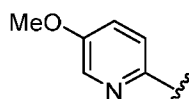
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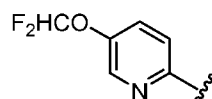
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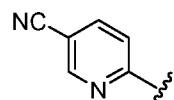
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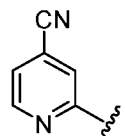
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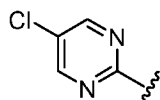
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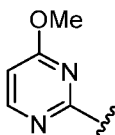
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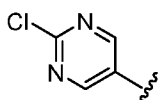
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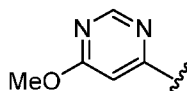
Int 31-55



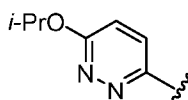
Int 31-56



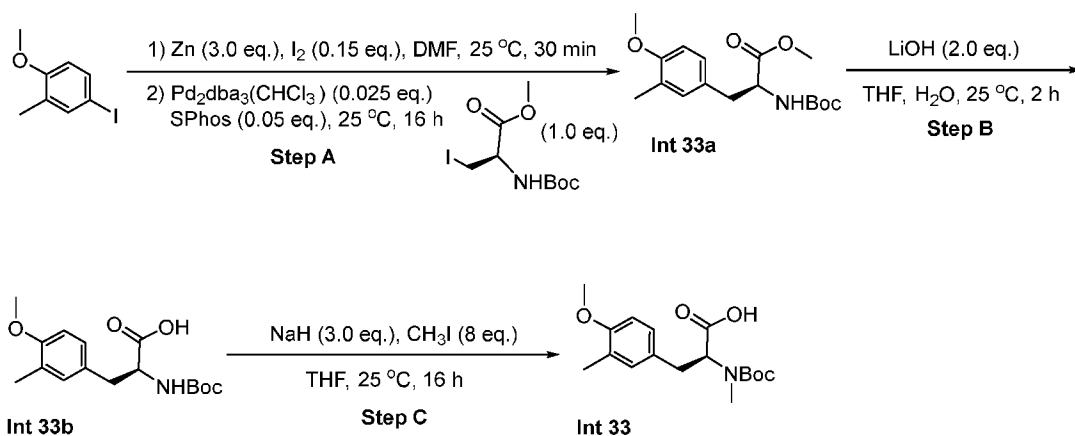
Int 31-57



Int 31-58



5

Preparatory intermediate Int 33

10

Step A: Synthesis of intermediate Int 33a

[0282] To a mixture of zinc dust (7.51 g, 115 mmol) in DMF (300 mL) was added I₂ (1.46 g, 5.74 mmol) at 25 °C under nitrogen atmosphere. After 5 min, (R)-methyl 2-((tert-butoxycarbonyl)amino)-3-iodopropanoate (12.6 g, 38.3 mmol) and I₂ (1.46 g, 5.74 mmol) were added to the mixture. The mixture was stirred at 25 °C for 30 min. Pd₂(dba)₃(HCCl₃) (900 mg, 0.981 mmol), SPhos (800 mg, 1.95 mmol) and 4-iodo-1-methoxy-2-methylbenzene (9.50 g, 38.3 mmol) were added to the above mixture and the mixture was stirred at 25 °C for 16 h. The reaction mixture was quenched with water (300 mL) and extracted with EA (3 x 300 mL). The combined organic layer was washed with brine (3 x 100 mL), dried over anhydrous Na₂SO₄, and

5 filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography and eluted with 0% - 30% EA in PE to afford **Int 33a** as an oil. LCMS (ESI) calculated for $C_{17}H_{25}NO_5$ $[M + H]^+$: 324.2, found 324.2. ^1H NMR (400 MHz, $CDCl_3$) δ 6.93-6.84 (m, 2H), 6.73 (d, $J = 8.2$ Hz, 1H), 4.95 (d, $J = 8.3$ Hz, 1H), 4.53-4.48 (m, 1H), 3.79 (s, 3H), 3.71 (s, 3H), 3.04-2.93 (m, 2H), 2.17 (s, 3H), 1.41 (s, 9H).

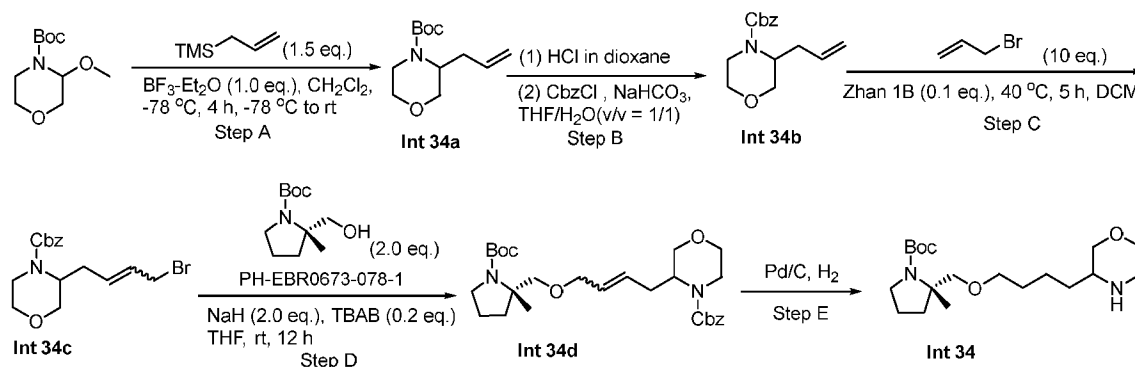
10 Step B: Synthesis of **Int 33b**

[0283] To a solution of **Int 33a** (10.0 g, 29.4 mmol) in THF (150 mL) was added LiOH (294 mL, 58.8 mmol, 0.2 N in water) at 0 °C. The reaction solution was stirred at 25 °C for 2 h. The reaction solution was extracted with EA (100 mL). The pH value of the aqueous phase was adjusted to 4 with aqueous HCl (1 N). The aqueous phase was extracted with DCM (5 x 100 mL). The combined organic phase was washed with brine (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to afford **Int 33b** as an oil. LCMS (ESI) calculated for $C_{16}H_{23}NO_5$ $[M + Na]^+$: 332.2, found 332.3. ^1H NMR (300 MHz, $CDCl_3$) δ 6.98-6.95 (m, 2H), 6.75 (d, $J = 9.0$ Hz, 1H), 4.93-4.89 (m, 1H), 4.57-4.48 (m, 1H), 3.80 (s, 3H), 3.13-2.97 (m, 2H), 2.19 (s, 3H), 1.46-1.42 (m, 9H).

20 Step C: Synthesis of **Int 33**

[0284] To a solution of **Int 33b** (1.30 g, 3.99 mmol) in dry THF (30 mL) was added NaH (0.479 g, 12.0 mmol, 60% in mineral oil) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 10 min. Iodomethane (4.53 g, 31.9 mmol) was added to the mixture in dropwise. The reaction solution was stirred at 25 °C for 16 h. The reaction was quenched with water (50 mL). The pH value of the reaction solution was adjusted to 3 with aqueous HCl (0.1 M). The solution was extracted with DCM (3 x 200 mL). The combined organic layer was washed with brine (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by a reverse phase column chromatography with following conditions: Column, X-bridge C^{18} , 330 g; mobile phase: ACN in water (5 mmol NH_4HCO_3), 40% - 70% in 30 min; Detector, UV 254 nm. RT: 25 min. The fractions containing the desired product were combined and concentrated under reduced pressure to afford **Int 33** as a solid. LCMS (ESI) calculated for $C_{17}H_{25}NO_5$ $[M + Na]^+$: 346.2, found 346.1. ^1H NMR (400 MHz, $CDCl_3$) δ 7.01-6.96 (m, 2H), 6.74 (d, $J = 8.4$ Hz, 1H), 4.72-4.59 (m, 1H), 3.80 (s, 3H), 3.23-3.20 (m, 1H), 3.28-2.90 (m, 1H), 2.76-2.68 (m, 3H), 2.19 (s, 3H), 1.42-1.35 (m, 9H).

5 **Preparatory intermediate Int 34**



Step A: Synthesis of Int 34a

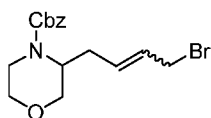
- 10 **[0285]** Tert-butyl 3-methoxymorpholine-4-carboxylate (1.09 g, 5.00 mmol) and allyltrimethylsilane (1.19 mL, 7.50 mmol) were dissolved in DCM (30 mL) in nitrogen atmosphere, it cooled to -78 °C. After the boron trifluoride ethyl ether (0.63 mL, 5.00 mmol) was dripped there over 10 minutes, it stirred for 4 hours, heating up naturally. Saturated sodium hydrogen carbonate solution (30 mL) was added after reaction, and chloroform (30 mL x 3)
- 15 extracted. The chloroform extract was evaporated under reduced pressure after drying, and the residue obtained was purified by flash column chromatography and eluted with gradient 0% - 30% EA in PE. The fractions containing the desired product were combined and concentrated under reduced pressure to afford **Int 34a** as an oil. LCMS (ESI) calculated for C₁₂H₂₁NO₃ [M + H]⁺: 228.1, found 228.0. ¹H NMR (300 MHz, CDCl₃) δ 5.77 (ddt, J = 17.2, 10.0, 7.2 Hz, 1H),
- 20 5.21-5.01 (m, 2H), 3.95 (s, 1H), 3.84 (dd, J = 11.4, 3.7 Hz, 1H), 3.74 (dd, J = 12.3, 7.4 Hz, 2H), 3.52 (dd, J = 11.5, 3.3 Hz, 1H), 3.44 (td, J = 11.8, 3.0 Hz, 1H), 3.12 (td, J = 12.7, 3.8 Hz, 1H), 2.46 (t, J = 7.2 Hz, 2H), 1.46 (s, 9H).

Step B: Synthesis of Int 34b

- 25 **[0286]** To a stirred solution of intermediate **Int 34a** (6.38 g, 28.1 mmol) in THF (20 mL) was added HCl in dioxane (4.0 M, 60 mL) at room temperature. The reaction mixture was stirred at 25 °C for 3 h. The solvent was concentrated under reduced pressure. The residue was dissolved in THF (100 mL) and Water (100 mL). Sodium hydrogen carbonate (9.43 g, 112 mmol) and benzyl carbonochloridate (7.18 g, 42.1 mmol) were added sequentially at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at 25 °C for 12 h. The reaction was diluted with
- 30 water (50 mL), extracted with EA (50 mL x 3). The combined organic layer was dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure. The

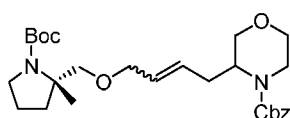
5 residue was purified by flash column chromatography and eluted with gradient 0% - 30% EA in PE. The fractions containing the desired product were combined and concentrated under reduced pressure to afford **Int 34b** as an oil. LCMS (ESI) calculated for $C_{15}H_{19}NO_3$ $[M + H]^+$: 262.1, found 262.2. ^1H NMR (300 MHz, $CDCl_3$) δ 7.38-7.34 (m, 5H), 5.84-5.63 (m, 1H), 5.14 (s, 2H), 5.12-4.98 (m, 2H), 4.04 (s, 1H), 3.93-3.68 (m, 3H), 3.48 (ddd, $J = 28.7, 11.6, 3.1$ Hz, 2H), 3.19 (td, $J = 13.0, 12.6, 3.7$ Hz, 1H), 2.48 (t, $J = 7.4$ Hz, 2H).

Step C: Synthesis of **Int 34c**



[0287] To a solution of intermediate **Int 34b** (1.0 g, 3.83 mmol) and 3-bromoprop-1-ene (3.33 mL, 38.3 mmol) in DCM (25 mL) was added (1,3-dimesitylimidazolidin-2-ylidene)(5-(N,N-dimethylsulfamoyl)-2-isopropoxybenzylidene)ruthenium(VI) chloride (0.28 g, 0.38 mmol) under nitrogen atmosphere at room temperature. The mixture was refluxed for 5 h then the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography and eluted with 0% - 40% EA in PE to afford **Int 34c** as an oil. LCMS (ESI) calculated for $C_{16}H_{20}BrNO_3$ $[M + H]^+$: found 354.1, 356.1, found 354.1, 356.1. ^1H NMR (400 MHz, $CDCl_3$) δ 7.42-7.30 (m, 5H), 5.75 (m, 2H), 5.14 (q, $J = 12.4$ Hz, 2H), 4.08 (b, 1H), 3.82 (d, $J = 8.2$ Hz, 4H), 3.73 (d, $J = 11.6$ Hz, 1H), 3.55 (dd, $J = 11.6, 3.3$ Hz, 1H), 3.45 (td, $J = 11.9, 3.1$ Hz, 1H), 3.25-3.11 (m, 1H), 2.55 (dt, $J = 14.4, 7.4$ Hz, 1H), 2.44 (dt, $J = 14.0, 7.0$ Hz, 1H).

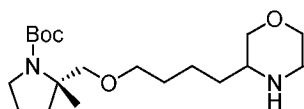
Step D: Synthesis of **Int 34d**



[0288] To a solution of (S)-tert-butyl 2-(hydroxymethyl)-2-methylpyrrolidine-1-carboxylate (1.86 g, 8.64 mmol) in THF (15 mL) was added sodium hydride (311 mg, 7.77 mmol) under nitrogen atmosphere at 0 °C. The solution was stirred for 15 min, then intermediate **Int 34c** (1.53 g, 4.32 mmol) in THF (15 mL) and tetrabutylammonium bromide (278 mg, 0.86 mmol) were added to the solution. The mixture was stirred at room temperature for 12 h and monitored periodically by LCMS. Upon completion, it was quenched with a saturated solution of NH_4Cl (20 mL). The aqueous layer was extracted with EA (3 x 50 mL) and the combined organic layer was washed with brine, dried over Na_2SO_4 , and filtered. The filtrate was evaporated under reduced pressure. The crude was purified by flash column chromatography and eluted with gradient 0% - 40% EA in PE to afford **Int 34d** as an oil. LCMS (ESI) calculated for $C_{27}H_{40}N_2O_6$ $[M + H]^+$:

- 5 489.3, found 489.3. ^1H NMR (300 MHz, CDCl_3) δ 7.34 (m, 5H), 5.60 (q, J = 5.0 Hz, 2H), 5.13 (s, 2H), 4.03 (d, J = 9.2 Hz, 1H), 3.95-3.67 (m, 5H), 3.57-3.29 (m, 6H), 3.20 (dt, J = 16.4, 8.2 Hz, 1H), 2.47 (d, J = 6.8 Hz, 2H), 2.13 (d, J = 10.8 Hz, 1H), 1.72 (m, 3H), 1.45 (s, 9H), 1.28 (s, 3H).

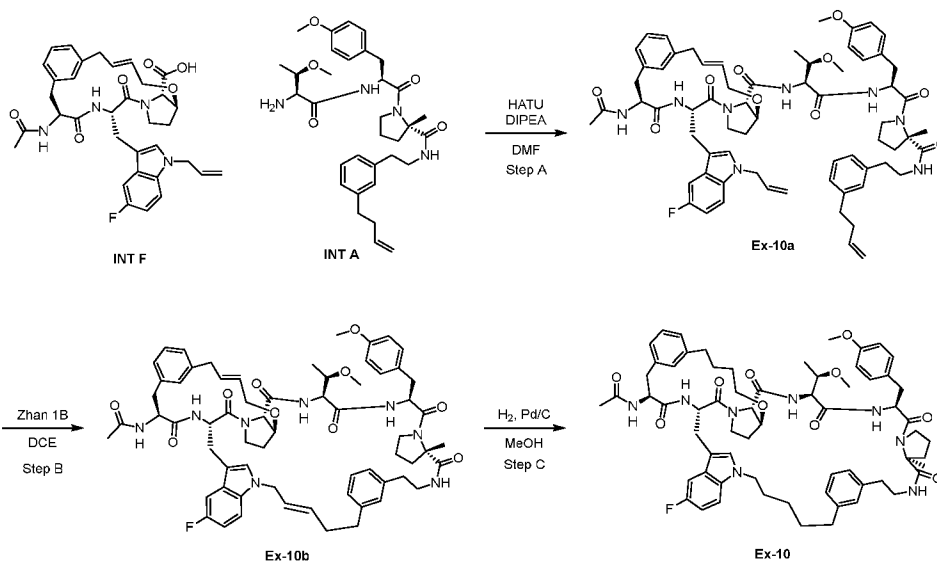
Step E: Synthesis of Int 34



- 10 **[0289]** To a stirred solution of **Int 34d** (1.46 g, 2.99 mmol) in THF (20 mL) was added 10% Palladium on activated carbon (354 mg, 2.99 mmol) under nitrogen atmosphere. The reaction mixture was degassed with hydrogen for three times and stirred at room temperature for 12 h under hydrogen. The resulting mixture was filtered, and the filtrate was concentrated under reduced pressure to afford **Int 34** as an oil. The crude product was used in the next step without
- 15 further purification. LCMS (ESI) calculated for $\text{C}_{19}\text{H}_{36}\text{N}_2\text{O}_4$ $[\text{M} + \text{H}]^+$: 357.3, found 357.3.

EXAMPLES

Example 1: Synthesis of Example compound 10 (Ex-10)



20

Step A - Synthesis of intermediate Ex-10a

- [0290]** Intermediate A (63 mg, 0.109 mmol) was dissolved in dry DMF (1089 μl) and intermediate F (67.1 mg, 0.109 mmol), HATU (43.5 mg, 0.114 mmol), and DIPEA (47.4 μl , 0.272 mmol) were then added. After stirring at room temp for 1 hour, the reaction mixture was
- 25 diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and

- 5 concentrated. The residue was purified via flash column chromatography on a 4g silica gold column with liquid loading in minimal DCM, using 30-100% EtOAc in hexane gradient to afford **Ex-10a**. LC/MS: $[M+H]^+ = 1178.78$.

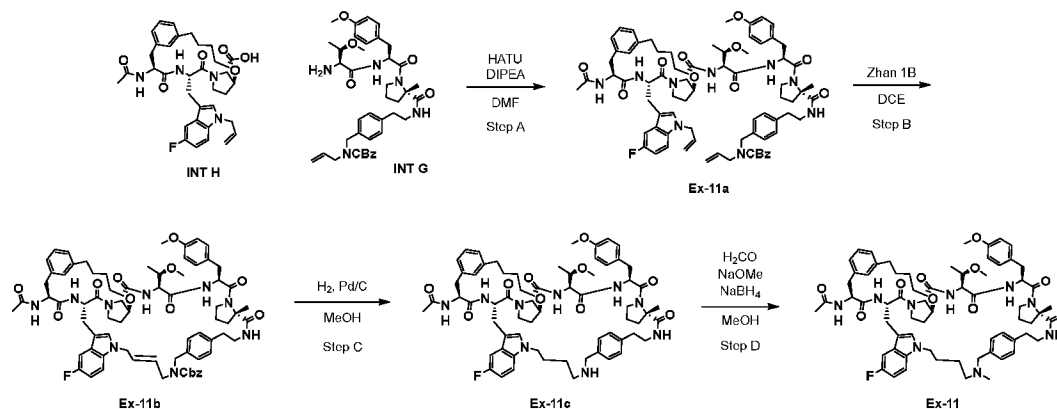
Step B - Synthesis of intermediate Ex-10b

- 10 **[0291]** Intermediate **Ex-10a** (71.7 mg, 0.061 mmol) was dissolved in dry DCE (5540 μ l) and Acetic Acid (5536 μ l), degassed for 15 min, was then added, followed by Zhan catalyst-1B (4.47 mg, 6.09 μ mol). After heating to 50 $^{\circ}$ C with nitrogen bubbling for 1 hour, the reaction was concentrated and purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient, loading in 2 mL DMSO, 2 injections to afford **Ex-10b**. LC/MS: $[M+H]^+ = 1149.0$.

15 *Step C - Synthesis of Ex-10*

- [0292]** Intermediate **Ex-10b** (38.9 mg, 0.034 mmol) was dissolved in MeOH (338 μ l) and excess Pd-C (3.60 mg, 0.034 mmol) was then added. After the flask was evacuated and backfilled with H_2 (1atm, by balloon) three times, the reaction was stirred for 1.5 hours and then filtered through celite, washed with MeOH and concentrated. The residue was purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient to afford **Ex-10**. LC/MS: $[M+H]^+ = 1154.73$.
- 20

Example 2: Synthesis of Example compound 11 (Ex-11)



25 *Step A - Synthesis of intermediate Ex-11a*

- [0293]** Intermediate **H** (93 mg, 0.151 mmol) and intermediate **G** (100 mg, 0.137 mmol) were dissolved in DMF (1374 μ l) and HATU (57.5 mg, 0.151 mmol) was then added, followed by DIPEA (71.8 μ l, 0.412 mmol). After stirring at room temp for 1 hour, the reaction mixture was diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and

5 concentrated. The residue was purified via flash column chromatography on a 12g silica gold column, and eluted with 10% MeOH in DCM to afford **Ex-11a**. LC/MS: $[M+H]^+ = 1328.90$.

Step B - Synthesis of intermediate **Ex-11b**

[0294] Intermediate **Ex-11a** (.119 g, 0.090 mmol) was dissolved in DCE (40.7 ml) and acetic
Acid (4.07 ml) was then added, followed by Zhan catalyst-1B (6.57 mg, 8.96 μ mol). After
10 heating to 50 °C with constant bubbling of N₂ for 1.5 hours, the reaction mixture was diluted
with EtOAc, washed with water three times, brine, dried over magnesium sulfate and
concentrated. The residue was purified via HPLC using a SunFire prep C18 OTD column, 19 x
100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient. 2 injection, 1 mL
DMSO each to afford **Ex-11b**. LC/MS: $[M+H]^+ = 1300.86$.

15 Step C - Synthesis of intermediate **Ex-11c**

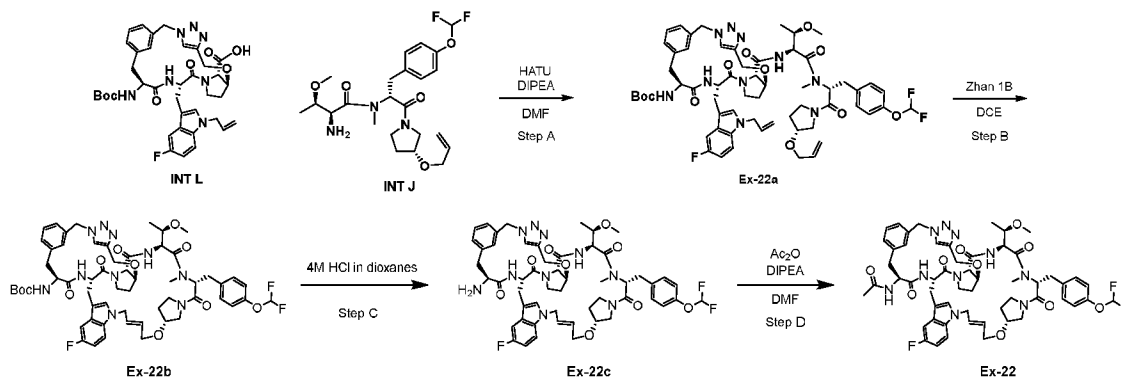
[0295] Intermediate **Ex-11b** (64.4 mg, 0.050 mmol) was dissolved in MeOH (1651 μ l) and Pd-
C (5.27 mg, 0.050 mmol) was then added. After the flask was evacuated and backfilled with
hydrogen (1 atm from balloon) twice, the reaction was stirred at room temp for 1 hour. Next it
was filtered through celite and concentrated to afford **Ex-11c**. LC/MS: $[M+H]^+ = 1168.86$.

20 Step D - Synthesis of **Ex-11**

[0296] Intermediate **Ex-11c** (55.4 mg, 0.047 mmol) was dissolved in MeOH (790 μ l) and
sodium methanolate (12.81 mg, 0.237 mmol) was then added, followed by formaldehyde (5.69
mg, 0.190 mmol). After stirring at 70 °C for 2 hours, it was cooled to 0°C, added sodium
tetrahydroborate (7.18 mg, 0.190 mmol) and warmed to RT for ~ 30 min. It was then heated to
25 70 °C for 2 hours and then cooled to RT and stirred overnight. The reaction mixture was
concentrated, redissolved in EtOAc, and washed with NH₄Cl solution and brine, dried over
magnesium sulfate and concentrated. The residue was purified via HPLC using a SunFire prep
C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA]
gradient. 1 mL DMSO single injection to afford **Ex-11**. LC/MS: $[M+H]^+ = 1182.81$.

30

5 **Example 3: Synthesis of Example compound 22 (Ex-22)**



Step A - Synthesis of intermediate **Ex-22a**

[0297] Intermediate **L** (37 mg, 0.052 mmol) and intermediate **J** (27.5 mg, 0.054 mmol) were dissolved in dry DMF (1397 μ l) and cooled to 0 °C. HATU (20.64 mg, 0.054 mmol) and DIPEA (45.0 μ l, 0.258 mmol) were added and the reaction was stirred at 0 °C for 1 hour. The mixture was loaded directly onto HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient to afford **Ex-22a**. LC/MS: $[M+H]^+ = 1167.60$.

Step B - Synthesis of intermediate **Ex-22b**

[0298] Intermediate **Ex-22a** (.0478 g, 0.041 mmol) was dissolved in dry DCE (13.65 ml) and degassed with N₂ for 30 min. After Zhan catalyst-1B (12.0 mg, 16.4 μ mol) was added, the reaction was heated to 40 °C with continued degassing for 1.5 hours. It was quenched by adding ~ 100-fold excess of ethyl vinyl ether and stirring for 30 min. The resulting mixture was concentrated and purified via HPLC, 1 injection, 1.5 mL DMSO, using a SunFire prep C18 OTD column 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient. to afford **Ex-22b**. LC/MS: $[M+H]^+ = 1139.66$.

Step C - Synthesis of intermediate **Ex-22c**

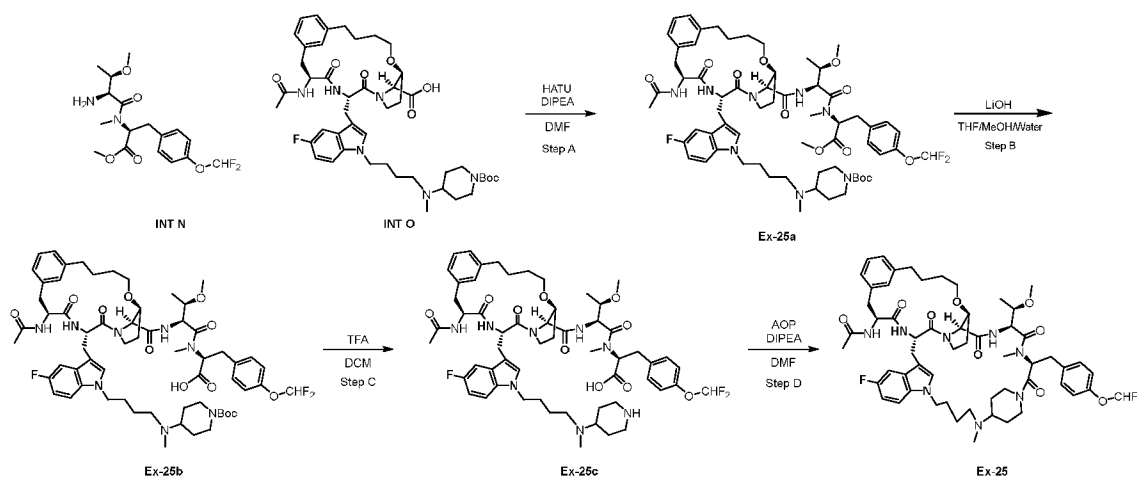
[0299] Intermediate **Ex-22b** (47 mg, 0.041 mmol) was dissolved in DCM (1250 μ l) and HCl (4M in dioxanes) (413 μ l, 1.650 mmol) was then added. After stirring at room temp for 3.5 hours, the reaction mixture was concentrated, redissolved in ACN/Water and dried via lyophilization to afford **Ex-22c**. LC/MS: $[M+H]^+ = 1039.89$.

Step D - Synthesis of Example **Ex-22**

[0300] Intermediate **Ex-22c** (36.3 mg, 0.035 mmol) was dissolved in dry DMF (1164 μ l) and DIPEA (36.6 μ l, 0.210 mmol) and acetic anhydride (6.59 μ l, 0.070 mmol) were then added. After stirring at room temp for 30 min, the mixture was loaded directly onto HPLC and purified via

- 5 HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 30-45% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient, 15 min gradient time, single injection, to afford **Ex-22**.
LC/MS: $[M+H]^+ = 1081.64$.

Example 4: Synthesis of Example compound 25 (Ex-25)



10

Step A - Synthesis of intermediate **Ex-25a**

- 15 **[0301]** Intermediate **O** (20 mg, 0.024 mmol) was dissolved in dry DMF (236 μ l) and cooled to 0 $^{\circ}$ C. Intermediate **N** (8.84 mg, 0.024 mmol), HATU (9.88 mg, 0.026 mmol) and DIPEA (20.62 μ l, 0.118 mmol) were then added. After warmed from 0 $^{\circ}$ C to RT overnight, the reaction mixture was diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and concentrated. The residue was purified via flash column chromatography on a 4g silica gold column using 0-100% [3:1 EtOAc:EtOH] in hexane gradient to obtain **Ex-25a**. LC/MS: $[M+H]^+ = 1203.88$.

Step B - Synthesis of intermediate **Ex-25b**

- 20 **[0302]** Intermediate **Ex-25a** (6 mg, 4.99 μ mol) was dissolved in THF (285 μ l), MeOH (142 μ l), and Water (71.2 μ l) and LiOH (0.597 mg, 0.025 mmol) was then added. After stirring at room temp for 2 hours, it was quenched with 1N HCl, extracted three times with EtOAc, dried over magnesium sulfate and concentrated to afford **Ex-25b**. LC/MS: $[M+H]^+ = 1189.45$.

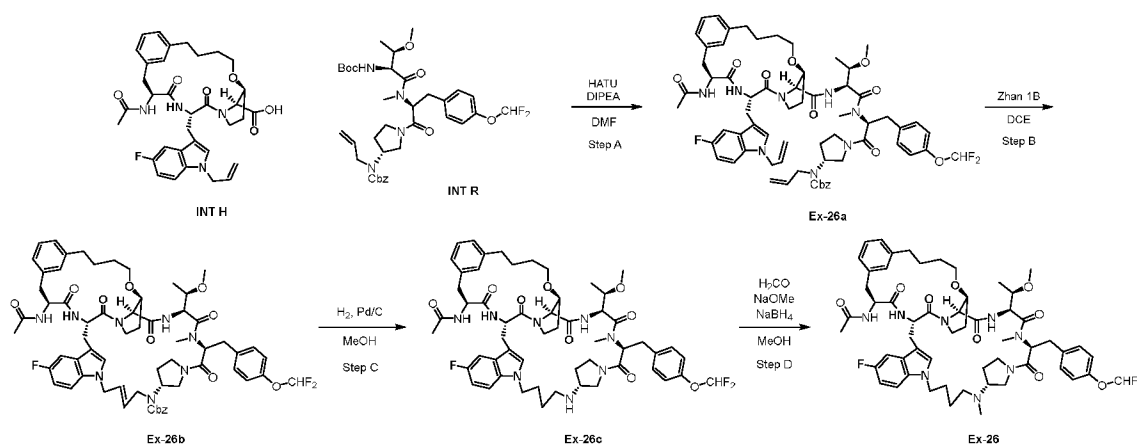
Step C - Synthesis of intermediate **Ex-25c**

- 25 **[0303]** Intermediate **Ex-25b** (5.9 mg, 4.96 μ mol) was dissolved in DCM (49.6 μ l) and TFA (3.82 μ l, 0.050 mmol) was then added. After stirring at room temp for 2 hour, it was concentrated, re-dissolved in DCM and concentrated several times to remove excess TFA to afford **Ex-25c**. LC/MS: $[M+H]^+ = 1089.53$.

5 Step D - Synthesis of Example Ex-25

[0304] AOP (4.27 mg, 9.64 μ mol) and DIPEA (7.86 μ l, 0.045 mmol) were dissolved in dry DCM (161 μ l) and intermediate **Ex-25c** (7 mg, 6.43 μ mol) in DMF (6603 μ l) was added dropwise. After stirring at room temp overnight, it was diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and concentrated. The residue was purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient to afford **Ex-25**. LC/MS: $[M+H]^+ = 1071.52$.

Example 5: Synthesis of Example compound 26 (Ex-26)



15 Step A - Synthesis of intermediate Ex-26a

[0305] Intermediate **H** (182 mg, 0.294 mmol) and intermediate **R** (161 mg, 0.267 mmol) were dissolved in dry DMF (2671 μ l), cooled to 0 °C and HATU (122 mg, 0.321 mmol) and DIPEA (233 μ l, 1.336 mmol) were then added. After stirring at 0 °C for 1 hour, it was loaded directly onto column for purification via reverse phase flash column chromatography on a 100g C18 column using 10-100% [Acetonitrile with 0.05% TFA] in [water with 0.05% TFA] to afford **Ex-26a**. LC/MS: $[M+H]^+ = 1203.43$.

Step B - Synthesis of intermediate Ex-26b

[0306] Intermediate **Ex-26a** (122 mg, 0.101 mmol) was dissolved in dry DCE (9120 μ l) and Acetic Acid (1010 μ l) was then added. After degassed for 20 min, Zhan catalyst-1B (14.88 mg, 0.020 mmol) was added and the reaction was stirred at 50 °C for 1.5 hours with degassing. It was then quenched with Ethyl vinyl ether (10 mL) and stirred and cooled to RT for 20 min. The reaction was concentrated and purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA], 15 min gradient. two 1 mL injections in DMSO to afford **Ex-26b**. LC/MS: $[M+H]^+ = 1175.41$.

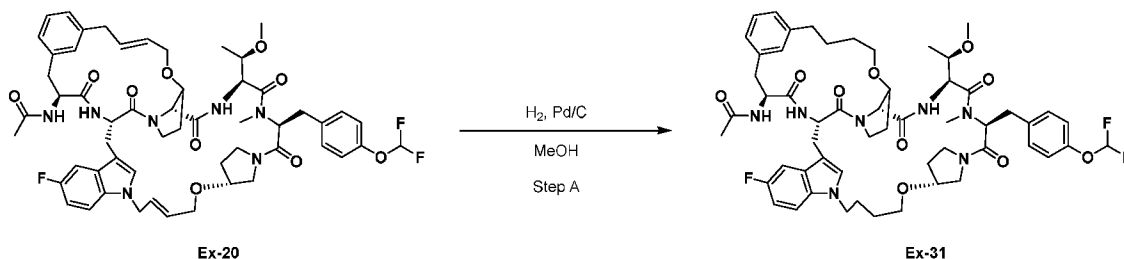
5 Step C - Synthesis of intermediate Ex-26c

[0307] Intermediate **Ex-26b** (110.4 mg, 0.094 mmol) was dissolved in dry Methanol (939 μ l) and Pd-C (10.00 mg, 0.094 mmol) was then added. After the flask was evacuated and backfilled with hydrogen (1 atm by balloon) three times, the reaction was stirred at room temp for 2 hours. The reaction mixture was filtered through celite and concentrated. The residue was purified via
 10 HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] 15 min gradient. 1 injection in 1.1 mL DMSO to afford **Ex-26c**.
 LC/MS: $[M+H]^+ = 1043.76$.

Step D - Synthesis of Example Ex-26

[0308] Intermediate **Ex-26c** (40 mg, 0.038 mmol) was dissolved in MeOH (1917 μ l) and
 15 formaldehyde (28.5 μ l, 0.383 mmol) and formic acid (7.35 μ l, 0.192 mmol) were then added. After stirring at room temp for 1 hour, sodium cyanotrihydroborate (19.28 mg, 0.307 mmol) was added. The reaction was stirred at room temp for 20 min, concentrated, redissolved in DCM, washed with saturated sodium bicarbonate solution, extracted twice with DCM, dried combined organic layers over magnesium sulfate and concentrated. The residue was dissolved in
 20 ACN/Water and dried via lyophilization to afford **Ex-26**. LC/MS: $[M+H]^+ = 1057.51$.

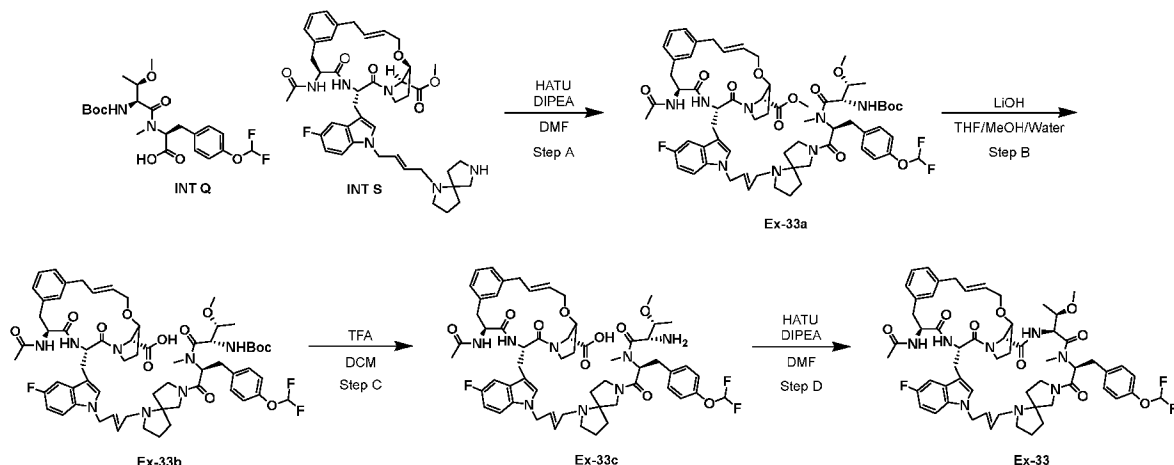
Example 6: Synthesis of Example compound 31 (Ex-31)



25 Step A - Synthesis of intermediate Ex-31

[0309] **Example Ex-20** (9.3 mg, 8.94 μ mol) was dissolved in MeOH (2980 μ l) and Pd-C (0.952 mg, 8.94 μ mol) was then added. After the flask was evacuated and backfilled with Hydrogen (1 atm via balloon) three times, the reaction was stirred at room temp for 2 hours and then filtered through celite. The residue was dissolved in ACN/Water and dried via lyophilization
 30 to afford **Ex-31**. LC/MS: $[M+H]^+ = 1044.59$.

5 **Example 7: Synthesis of Example compound 33 (Ex-33)**



Step A - Synthesis of intermediate Ex-33a

[0310] Intermediate **S** (22.8 mg, 0.030 mmol) was dissolved in dry DMF (297 μ l) and cooled to 0 $^{\circ}$ C. Intermediate **P** (20.48 mg, 0.044 mmol), HATU (16.91 mg, 0.044 mmol), and DIPEA (25.9 μ l, 0.148 mmol) were then added and the reaction was stirred at 0 $^{\circ}$ C for 30 min. Next the mixture was diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and concentrated. The residue was purified via flash column chromatography on a 4g silica gold column using [0-50% 3:1 EtOAc:EtOH] in hexane gradient to afford intermediate **Ex-33a**. LC/MS: $[M+H]^+ = 1211.61$.

15 **Step B - Synthesis of intermediate Ex-33b**

[0311] Intermediate **Ex-33a** (26 mg, 0.021 mmol) was dissolved in THF (613 μ l), MeOH (307 μ l), and Water (153 μ l) at room temp and LiOH (2.57 mg, 0.107 mmol) was then added. After stirring at room temp for 40 min, it was quenched with 1N HCl, extracted with EtOAc three times, washed with brine, dried over magnesium sulfate and concentrated to afford intermediate **Ex-33b**. LC/MS: $[M+H]^+ = 1197.92$.

Step C - Synthesis of intermediate Ex-33c

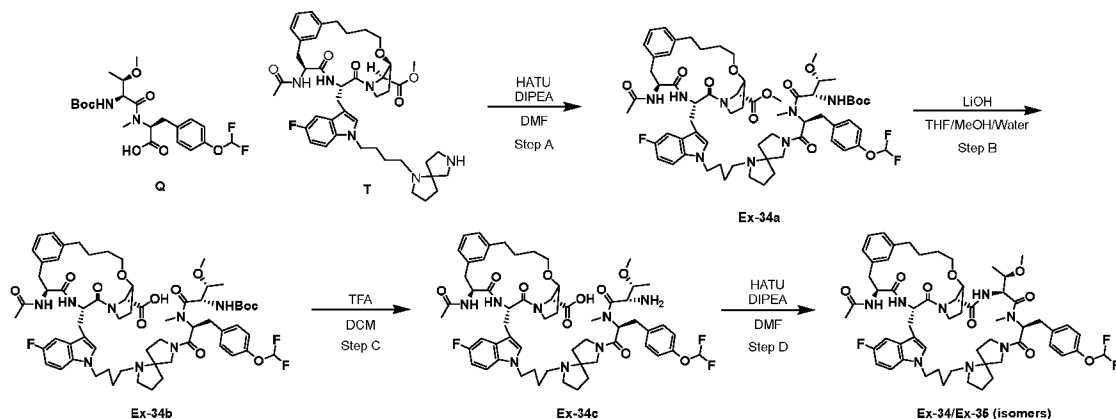
[0312] Intermediate **Ex-33b** (31.7 mg, 0.026 mmol) was dissolved in DCM (2647 μ l) and TFA (60.1 μ l, 0.794 mmol) was then added. After stirring for 1 hour, it was concentrated, redissolved in DCM and concentrated several times to remove excess TFA to afford intermediate **Ex-33c**. LC/MS: $[M+H]^+ = 1097.58$.

25 **Step D - Synthesis of Example Ex-33**

[0313] Intermediate **Ex-33c** (29 mg, 0.026 mmol) was dissolved in dry DMF (8810 μ l) and cooled to 0 $^{\circ}$ C. HATU (10.05 mg, 0.026 mmol) and DIPEA (27.7 μ l, 0.159 mmol) were then

- 5 added. After stirring for 1 hour, it was diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and concentrated. Purified via flash column chromatography on a 4g silica gold column using 0-100% [3:1 EtOAc:EtOH] in hexane gradient to afford **Ex-33**.
LC/MS: $[M+H]^+ = 1079.60$.

10 **Example 8: Synthesis of Example compounds Ex-34 and 35 (Ex-34 and Ex-35)**



Step A - Synthesis of intermediate EX-34a

- [0314] Intermediate **T** (44.3 mg, 0.057 mmol) was dissolved in dry DMF (573 μ l) and cooled to 0 °C. Intermediate **Q** (39.6 mg, 0.086 mmol), HATU (32.7 mg, 0.086 mmol), and DIPEA (50.0 μ l, 0.287 mmol) were then added. After stirring at 0 °C for 30 min, the reaction was diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and concentrated. The residue was purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient to afford intermediate **Ex-34a**.
LC/MS: $[M+H]^+ = 1215.62$.

Step B - Synthesis of intermediate EX-34b

- [0315] Intermediate **Ex-34a** (33.7 mg, 0.028 mmol) was dissolved in THF (792 μ l), MeOH (396 μ l), and Water (198 μ l) at room temp. Next LiOH (3.32 mg, 0.139 mmol) was added. After stirring at room temp for 40 min, it was quenched with 1N HCl, extracted with EtOAc three times, washed with brine, dried over magnesium sulfate and concentrated to afford intermediate **Ex-34b**. LC/MS: $[M+H]^+ = 1201.64$.

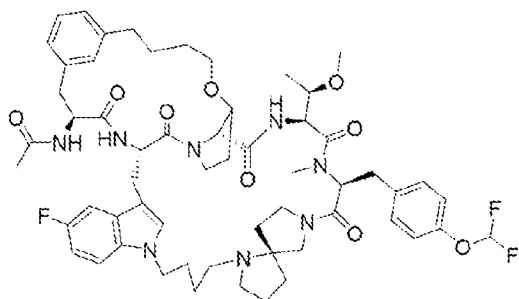
Step C - Synthesis of intermediate EX-34c

- [0316] Intermediate **Ex-34b** (33 mg, 0.027 mmol) was dissolved in DCM (1 mL) and TFA (0.021 mL, 0.275 mmol) was then added. After stirring at room temp for 2 hours, the reaction

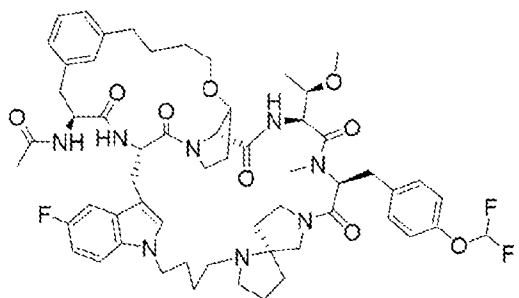
- 5 mixture was concentrated, redissolved in DCM and concentrated several times to remove TFA to afford intermediate **Ex-34c**. LC/MS: $[M+H]^+ = 1101.64$.

Step D - Synthesis of Example Ex-34/Ex-35

- [0317] Intermediate **Ex-34c** (30 mg, 0.027 mmol) was dissolved in dry DMF (2720 μ l) and cooled to 0 °C. HATU (10.36 mg, 0.027 mmol) and DIPEA (28.5 μ l, 0.163 mmol) were then
10 added. After stirring for 1 hour, the reaction was diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and concentrated. The residue was purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 30-70% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient, over 15 min to afford **Ex-34** and **Ex-35**, one having the structure

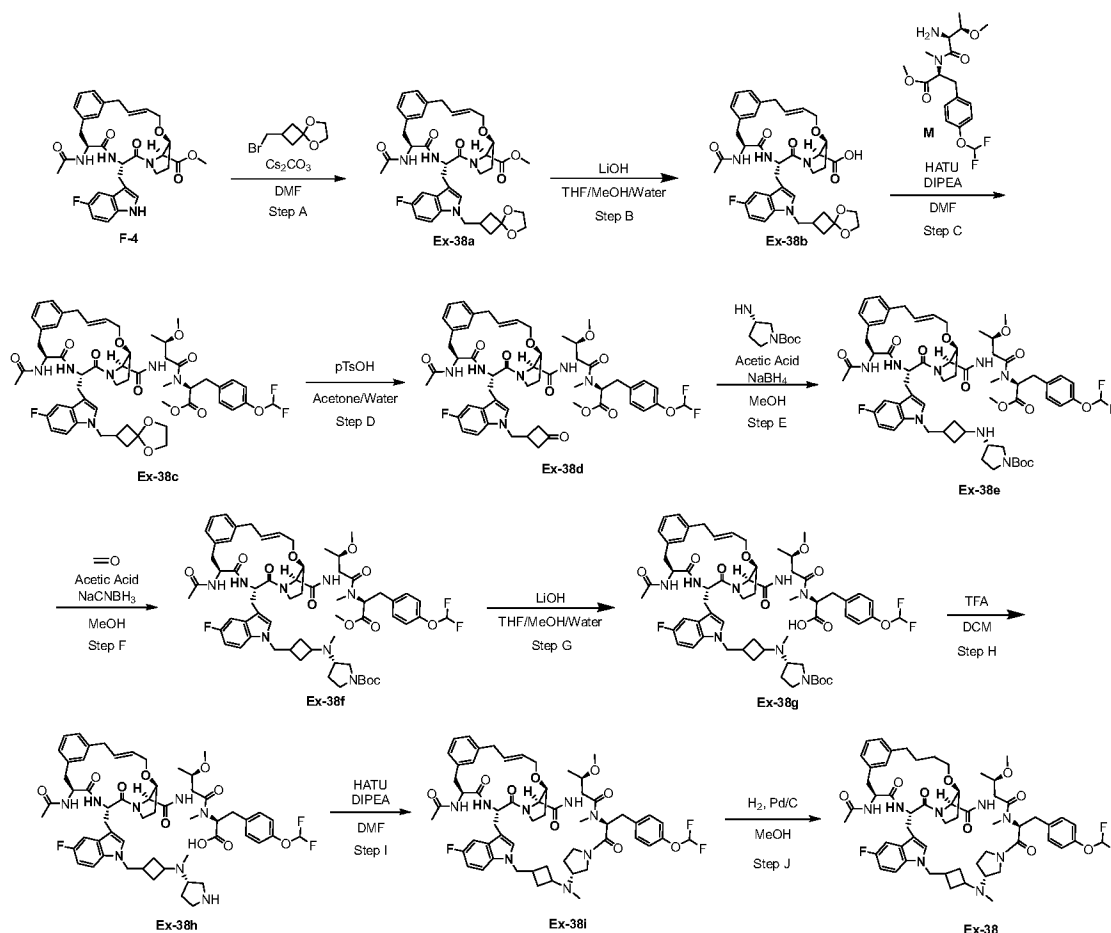


, and the other having the structure



- 15 . LC/MS: $[M+H]^+ = 1083.66$.

5 Example 9: Synthesis of Example compound 38 (Ex-38)



Step A - Synthesis of intermediate Ex-38a

[0318] Intermediate **F-4** (100 mg, 0.169 mmol) was dissolved in dry DMF (2116 μ l) and cesium carbonate (132 mg, 0.406 mmol) and 2-(bromomethyl)-5,8-dioxaspiro[3.4]octane (70.1 mg, 0.339 mmol) were then added. After stirring at room temp for 5 hours, the reaction was diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and concentrated. The residue was purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient to obtain **Ex-38a**. LC/MS: $[M+H]^+ = 717.5$.

15 *Step B - Synthesis of intermediate Ex-38b*

[0319] Intermediate **Ex-38a** (49 mg, 0.068 mmol) was dissolved in THF (1302 μ l), MeOH (651 μ l), and Water (326 μ l). Next LiOH (6.55 mg, 0.273 mmol) was added. After stirring for 1.5 hours, it was quenched with 1N HCl, extracted three times with EtOAc, washed with brine, dried over magnesium sulfate and concentrated to afford **Ex-38b**. LC/MS: $[M+H]^+ = 703.5$.

5 Step C - Synthesis of intermediate Ex-38c

[0320] Intermediate **Ex-38b** (48 mg, 0.068 mmol) was dissolved in dry DMF (683 μ l) and intermediate **M** (33.2 mg, 0.089 mmol) was then added, and the solution was cooled to 0 °C. Next HATU (33.8 mg, 0.089 mmol) and DIPEA (59.5 μ l, 0.342 mmol) were added. After stirring and warmed to RT overnight, the reaction was diluted with EtOAc, washed three times with
10 water, brine, dried over magnesium sulfate and concentrated. The residue was purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient to afford **Ex-38c**. LC/MS: $[M+H]^+ = 1059.7$.

Step D - Synthesis of intermediate Ex-38d

[0321] Intermediate **Ex-38c** (53 mg, 0.050 mmol) was dissolved in Acetone (1390 μ l) and
15 Water (278 μ l) and pTsOH (38.1 mg, 0.200 mmol) was then added. After heating to 56 °C and stirring in a sealed tube for 1 hour, the reaction was cooled to RT and quenched with sat aq sodium bicarbonate solution. The mixture was then extracted 3 times with 3:1 DCM:IPA, dried organic layers over magnesium sulfate and concentrated to afford **Ex-38d**. LC/MS: $[M+H]^+ = 1059.6$.

20 Step E - Synthesis of intermediate Ex-38e

[0322] Intermediate **Ex-38d** (57 mg, 0.056 mmol) was dissolved in dry MeOH (1872 μ l) and tert-butyl (R)-3-aminopyrrolidine-1-carboxylate (10.23 μ l, 0.056 mmol) was then added. After stirring for 30 min, the reaction was added sodium borohydride (2.124 mg, 0.056 mmol) and acetic acid (3.21 μ l, 0.056 mmol) and the mixture was stirred for 48 hours. The reaction was
25 quenched with water, removed MeOH, redissolved in EtOAc, and washed with water then brine, dried over magnesium sulfate and concentrated. Purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient to afford **Ex-38e**. LC/MS: $[M+H]^+ = 1085.6$.

Step F - Synthesis of intermediate Ex-38f

30 [0323] Intermediate **Ex-38e** (26.6 mg, 0.022 mmol) was dissolved in MeOH (748 μ l) and formaldehyde (16.71 μ l, 0.224 mmol) and acetic acid (6.42 μ l, 0.112 mmol) were then added. Next sodium cyanoborohydride (11.28 mg, 0.180 mmol) was added, followed by magnesium sulfate. After stirring at room temp for 2 hours, the reaction was quenched with sat aq. sodium bicarbonate solution. evaporated volatiles, extracted with EtOAc three times, washed with brine,
35 dried over magnesium sulfate and concentrated to afford **Ex-38f**. LC/MS: $[M+H]^+ = 1199.8$.

5 Step G - Synthesis of intermediate Ex-38g

[0324] Intermediate **Ex-38e** (26 mg, 0.022 mmol) was dissolved in THF (413 μ l), Methanol (206 μ l), and Water (103 μ l) and LiOH (2.60 mg, 0.108 mmol) was then added. After stirring at room temp for 1.5 hours, the reaction was quenched with 1N HCl, extracted with EtOAc three times, washed with brine, dried over magnesium sulfate and concentrated to afford **Ex-38g**.

10 LC/MS: $[M+H]^+ = 1185.6$.

Step H - Synthesis of intermediate Ex-38h

[0325] Intermediate **Ex-38g** (20.3 mg, 0.017 mmol) was dissolved in DCM (171 μ l) and TFA (13.19 μ l, 0.171 mmol) was then added. After stirring at room temp for 2 hours, the reaction mixture was concentrated, redissolved in DCM and concentrated several times to remove excess

15 TFA to afford **Ex-38h**. LC/MS: $[M+H]^+ = 1085.6$.

Step I - Synthesis of intermediate Ex-38i

[0326] Intermediate **Ex-38h** (18.6 mg, 0.017 mmol) was dissolved in dry DMF (3726 μ l) and cooled to 0 °C. Next HATU (6.52 mg, 0.017 mmol) and DIPEA (17.96 μ l, 0.103 mmol) were added. After stirring for 1 hour, the reaction was removed DCM in vacuo, diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and concentrated. The residue was purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient to afford **Ex-38i**. LC/MS: $[M+H]^+ = 1067.6$.

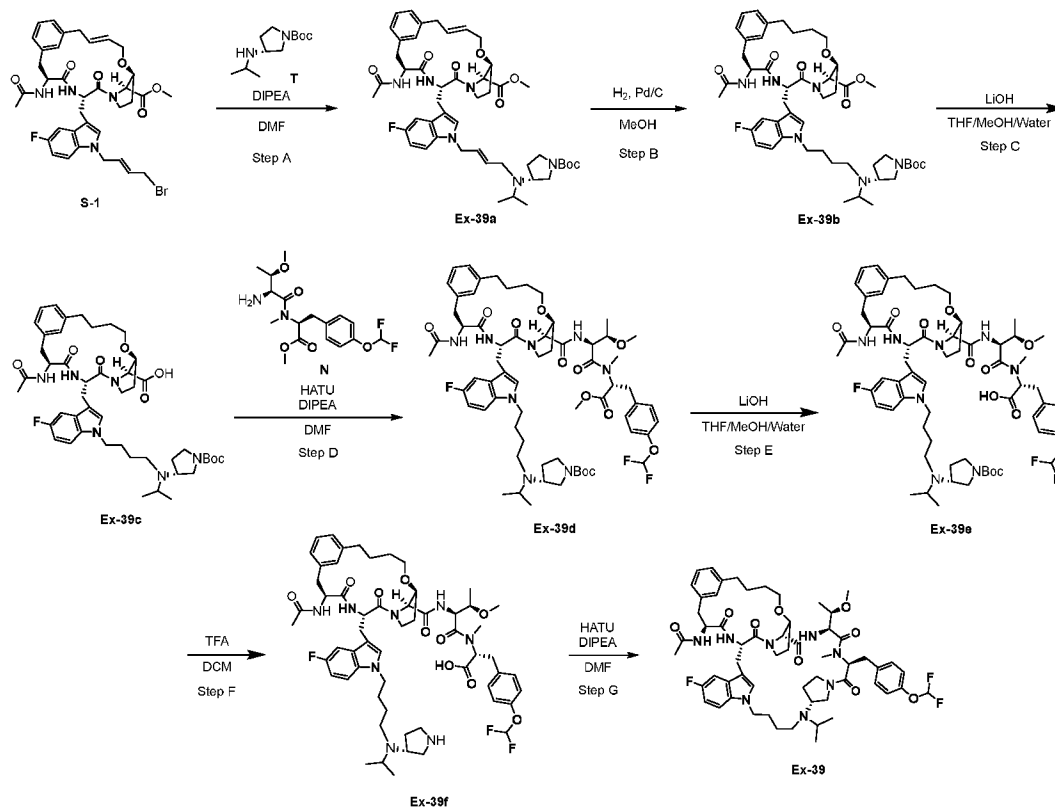
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Step J - Synthesis of Example Ex-38

[0327] Intermediate **Ex-38i** (3.8 mg, 3.56 μ mol) was dissolved in dry MeOH (119 μ l) and Pd-C (0.379 mg, 3.56 μ mol) was then added. After the flasks was evacuated and backfilled with H₂ (1 atm from balloon), three times, the reaction was stirred at room temp for 1.5 hours, filtered through celite, washed heavily with MeOH and concentrated. The residue was redissolved in ACN/Water and dried via lyophilization to afford **Ex-38**. LC/MS: $[M+H]^+ = 1069.7$.

30

5 **Example 10: Synthesis of Example compound 39 (Ex-39)**



Step A - Synthesis of intermediate Ex-39a

[0328] Intermediate S-4 (110 mg, 0.152 mmol) was dissolved in dry DMF (1520 μ l) and intermediate U (69.4 mg, 0.304 mmol) was then added, followed by DIPEA (133 μ l, 0.760 mmol). After heated to 50 $^{\circ}$ C for 1.5 hours, the reaction was diluted with EtOAc, washed with water 3 times, brine, dried over magnesium sulfate and concentrated. The residue was purified via flash column chromatography on a 4g silica gold column using 30-70% [3:1 EtOAc:EtOH] in hexane gradient to afford **Ex-39a**. LC/MS: $[M+H]^+ = 871.7$.

15 Step B - Synthesis of intermediate Ex-39b

[0329] Intermediate **Ex-39a** (17.6 mg, 0.020 mmol) was dissolved in dry MeOH (674 μ l) and Pd-C (0.215 mg, 2.021 μ mol) was then added. After the flask was evacuated and backfilled with H₂ (1 atm, by balloon) three times and then stirred at room temp for 1 hour, the reaction was filtered through celite, washed with MeOH and concentrated to afford **Ex-39b**. LC/MS: $[M+H]^+ = 875.9$.

5 Step C - Synthesis of intermediate Ex-39c

[0330] Intermediate **Ex-39b** (17.7 mg, 0.020 mmol) was dissolved in THF (385 μ l), MeOH (193 μ l), and Water (96 μ l). Next LiOH (2.422 mg, 0.101 mmol) was added. After stirring at room temp for 2 hours, the volatiles was evaporated and the mixture was diluted with EtOAc, washed with water, brine, dried over magnesium sulfate and concentrated to afford **Ex-39c**.

10 LC/MS: $[M+H]^+ = 861.4$.

Step D - Synthesis of intermediate Ex-39d

[0331] Intermediate **Ex-39c** (17.7 mg, 0.021 mmol) and intermediate **N** (11.54 mg, 0.031 mmol) were dissolved in DMF (685 μ l) and cooled to 0 °C. Next HATU (11.72 mg, 0.031 mmol) was added, followed by DIPEA (17.90 μ l, 0.103 mmol). After stirring at room temp for 40 min, the reaction was diluted with EtOAc, washed 3 times with water, brine, dried over magnesium sulfate and concentrated. The residue was purified via HPLC using a SunFire prep C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient. to afford **Ex-39d**. LC/MS: $[M+H]^+ = 1217.7$.

Step E - Synthesis of intermediate Ex-39e

20 [0332] Intermediate **Ex-39d** (4.2 mg, 3.45 μ mol) was dissolved in THF (65.7 μ l), Methanol (32.9 μ l), and Water (16.43 μ l) and LiOH (0.413 mg, 0.017 mmol) was then added. After stirring at room temp for 1 hour, the reaction was quenched with 1N HCl, extracted 3 times with EtOAc, washed with brine, dried over magnesium sulfate and concentrated to afford **Ex-39e**. LC/MS: $[M+H]^+ = 1203.8$.

25 Step F - Synthesis of intermediate Ex-39f

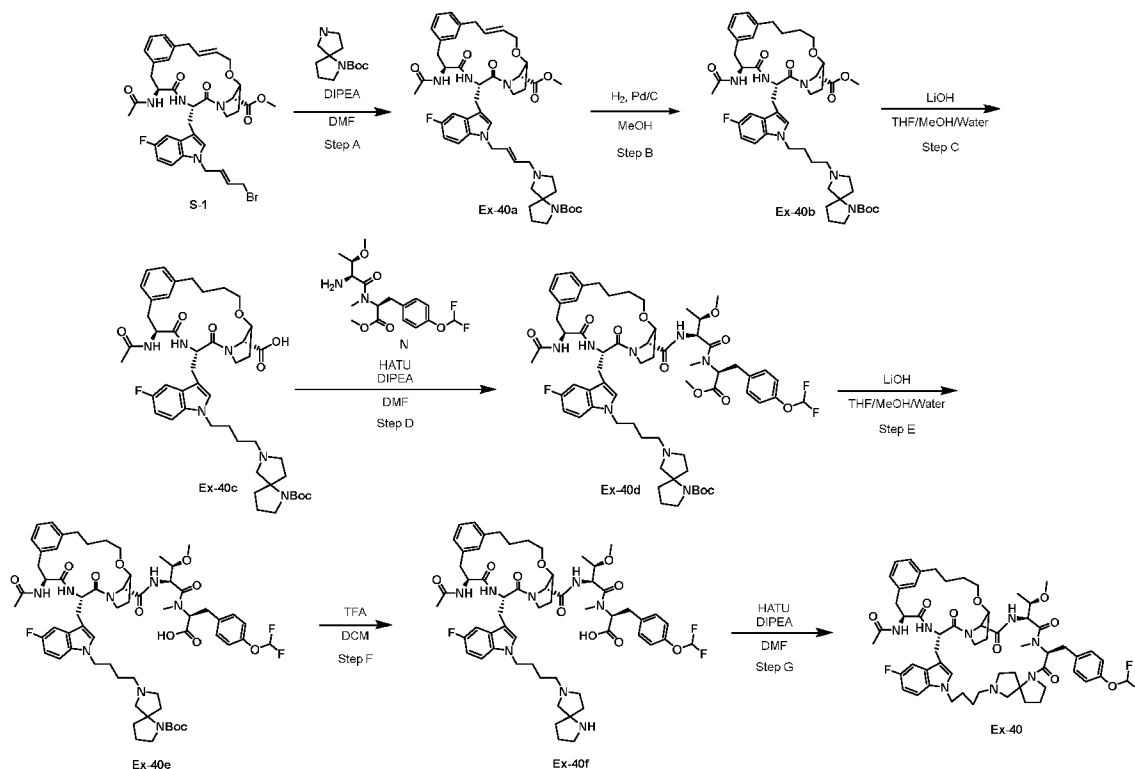
[0333] Intermediate **Ex-39e** (4.15 mg, 3.45 μ mol) was dissolved in DCM (34.5 μ l) and TFA (2.66 μ l, 0.034 mmol) was then added. After stirring at room temp for 1 hour, the reaction was concentrated, re-dissolved in DCM and concentrated several times to remove excess TFA to afford **Ex-39f**. LC/MS: $[M+H]^+ = 1103.7$.

30 Step G - Synthesis of Example Ex-39

[0334] Intermediate **Ex-39f** (3.8 mg, 3.44 μ mol) was dissolved in dry DMF (749 μ l) and DCM (6739 μ l) and cooled to 0 °C. Next HATU (1.310 mg, 3.44 μ mol) was added, followed by DIPEA (3.01 μ l, 0.017 mmol). After stirring at room temp for 1 hour, the reaction mixture was removed DCM in vacuo, diluted with EtOAc, washed with water three times, brine, dried over magnesium sulfate and concentrated. The residue was purified via HPLC using a SunFire prep

- 5 C18 OTD column, 19 x 100 mm, 10-100% [ACN with 0.05% TFA] in [water w/0.05% TFA] gradient to afford **Ex-39**. LC/MS: $[M+H]^+ = 1085.7$.

Example 11: Synthesis of Example compound 40 (Ex-40)



10

Step A - Synthesis of intermediate Ex-40a

- [0335] Intermediate **S-1** (500 mg, 0.691 mmol) was dissolved in dry DMF (6910 μ l) and tert-butyl 1,7-diazaspiro[4.4]nonane-1-carboxylate (235 mg, 1.036 mmol) and DIPEA (603 μ l, 3.45 mmol) were then added. After heating to 50 $^{\circ}$ C for 3.5 hours, the reaction was diluted with EtOAc, washed with water 3 times, brine, dried over magnesium sulfate and concentrated. The residue was purified via flash column chromatography on a 12g silica gold column using 30-70% [3:1 EtOAc:EtOH] in hexane gradient to afford **Ex-40a**. LC/MS: $[M+H]^+ = 869.93$.
- 15

Step B - Synthesis of intermediate Ex-40b

- [0336] Intermediate **Ex-40a** (464 mg, 0.534 mmol) was dissolved in dry MeOH (10 mL) and Pd-C (11.36 mg, 0.107 mmol) was then added. After the flask evacuated and backfilled with H_2 (1atm from balloon) three times, the reaction was stirred at room temp for 2 hour, filtered through celite and concentrated. The residue was purified via flash column chromatography on a
- 20

- 5 24g silica gold column using 30-70% [3:1 EtOAc:EtOH] in hexane gradient to afford **Ex-40b**.
LC/MS: $[M+H]^+ = 873.95$.

Step C - Synthesis of intermediate Ex-40c

- [0337] Intermediate **Ex-40b** (228.1 mg, 0.261 mmol) was dissolved in THF (4976 μ l),
Methanol (2488 μ l), and Water (1244 μ l). Next LiOH (31.3 mg, 1.306 mmol) was added. After
10 stirring at room temp for 1.5 hours, it was quenched with 1N HCl, diluted with EtOAc, washed
with brine, dried over magnesium sulfate and concentrated to afford **Ex-40c**. LC/MS: $[M+H]^+ =$
859.62.

Step D - Synthesis of intermediate Ex-40d

- [0338] Intermediate **Ex-40c** (236 mg, 0.275 mmol) was dissolved in dry DMF (2747 μ l) and
15 cooled to 0 °C. Next intermediate **N** (152 mg, 0.330 mmol), HATU (125 mg, 0.330 mmol), and
DIPEA (240 μ l, 1.374 mmol) were added. After stirring at 0 °C for 30 min, the reaction was
diluted with EtOAc, washed with water three times, then brine. dried over magnesium sulfate and
concentrated. The residue was purified by high pressure liquid chromatography (C18) to afford
Ex-40d. LC/MS: $[M+H]^+ = 1216.17$.

- 20 Step E - Synthesis of intermediate Ex-40e

- [0339] Intermediate **Ex-40d** (180.5 mg, 0.149 mmol) was dissolved in THF (2829 μ l), MeOH
(1414 μ l), and Water (707 μ l). Next LiOH (17.78 mg, 0.743 mmol) was added. After stirring for
1.5 hours, the reaction was adjusted to pH ~ 3 using 1N HCl, removed volatiles, then extracted
three times with EtOAc, washed with brine, dried over magnesium sulfate and concentrated to
25 afford **Ex-40e**. LC/MS: $[M+H]^+ = 1202.00$.

Step F - Synthesis of intermediate Ex-40f

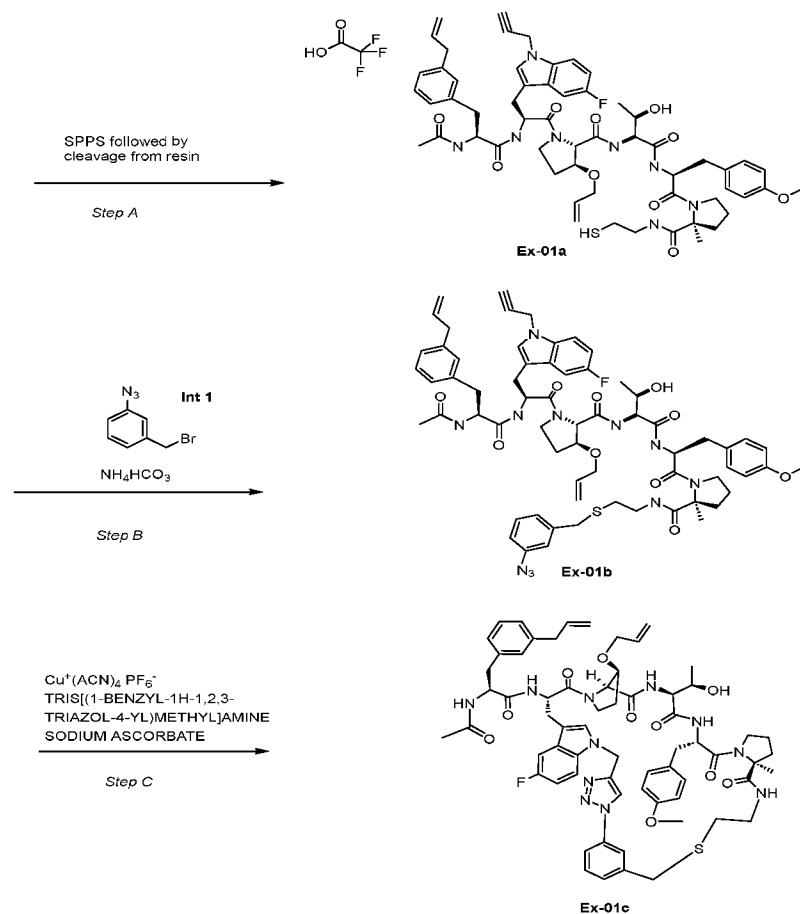
- [0340] Intermediate **Ex-40e** (151 mg, 0.126 mmol) was dissolved in DCM (1257 μ l) and TFA
(97 μ l, 1.257 mmol) was then added. After stirring at room temp for 1.5 hours, the reaction was
concentrated, dissolved in ACN/water and dried via lyophilization to remove excess acid to
30 afford **Ex-40f**. LC/MS: $[M+H]^+ = 1101.56$ $[M+1]$.

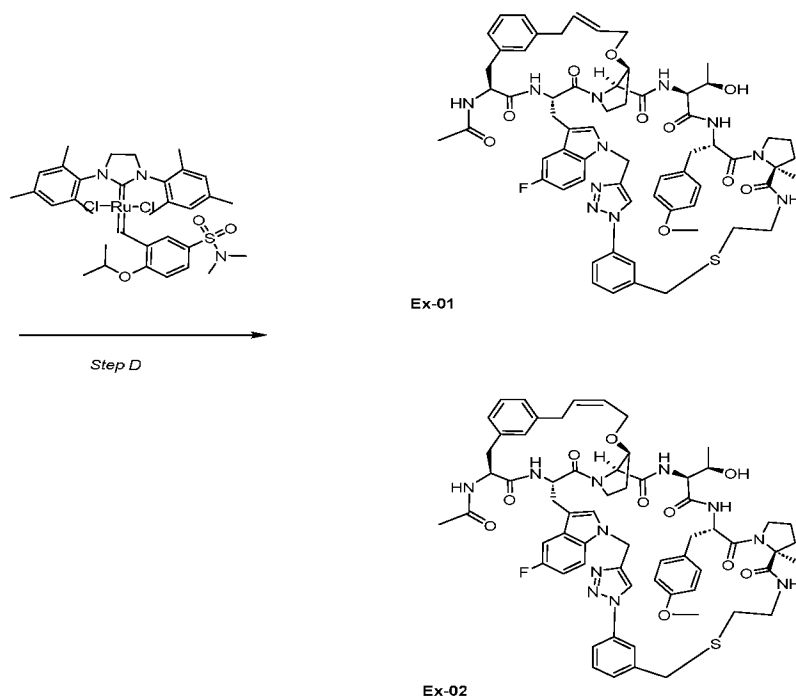
Step G - Synthesis of Example Ex-40

- [0341] Intermediate **Ex-40f** (0.174 g, 0.158 mmol) was dissolved in dry DMF (34.4 ml) and
DCM (310 ml) and then cooled to 0 °C. Next PYAOP (0.083 g, 0.158 mmol) and DIPEA (0.138
ml, 0.792 mmol) were added. After stirring for 1 hour, the reaction was removed DCM in vacuo,
35 diluted with EtOAc, washed with water three times, then brine, dried over magnesium sulfate and

- 5 concentrated. The residue was purified by high pressure liquid chromatography (C18) under basic conditions (NH₄OH) to afford **Ex-40**. LC/MS: [M+H]⁺ = 1083.6.

Example 12: Synthesis of Example compounds 1 and 2 (Ex-01 and Ex-02)





5

Step A – Synthesis of Intermediate **Ex-01a**

[0342] The peptide was synthesized using Fmoc/t-Bu chemistry on cysteamine 4-methoxytrityl resin with a CEM Liberty automated microwave peptide synthesizer. The peptide sequence was synthesized on a 0.125 mmol scale, using single-couplings of 4 equivalents of Fmoc protected amino acids as a 0.2M DMF solution along with 3.6 eq of HATU as a 0.45 M DMF solution and 8 eq of 2M DIEA in NMP, and heated at 75C for 300 seconds. Fmoc deprotections were performed using 20%(V/V) piperidine in DMF at 90C for 90 seconds.

[0343] Fmoc-AAs were coupled to the resin in this order: (S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-methylpyrrolidine-2-carboxylic acid, (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-methoxyphenyl)propanoic acid, N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-(tert-butyl)-L-threonine, (2S,3S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-(allyloxy)pyrrolidine-2-carboxylic acid (Intermediate W), (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-fluoro-1-(prop-2-yn-1-yl)-1H-indol-3-yl)propanoic acid (Intermediate X), (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(3-allylphenyl)propanoic acid (Intermediate V), and acetic acid for capping.

[0344] After the resin was isolated via filtration, the peptide was cleaved from solid support using 11 ml of TFA solution (v/v) (90% TFA: 2.5% DODT: 2.5% phenol: 2.5% triisopropylsilane: 2.5% water) for approximately 3 hours at room temperature. The resin was filtered and washed with 4 ml of cleavage solution. The combined filtrate was concentrated and

5 then precipitated in approximately 60 ml of cold ethyl ether (-78C). Crude peptide pellet was collected by centrifugation, washed in cold ethyl ether and centrifuged once more to provide ME01-a, which was used crude in the next step. LCMS: 1091.4 (M+1)+.

Step B – Synthesis of Intermediate Ex-01b

[0345] Intermediate **Ex-01a** (68.2 mg, 0.063 mmol) was dissolved in a degassed solution of acetonitrile (62 ml) and 20mM aq. NH_4HCO_3 (28 ml). A degassed solution of intermediate **Int 1** (19.88 mg, 0.094 mmol) in 2 mL acetonitrile was added. After stirring at RT under an atmosphere of nitrogen gas overnight. A total of 24 mg more intermediate **Int 1** was added portion wise through day to drive to completion. The reaction was then lyophilized to afford **Ex-01b**, which was used crude in the next step. LCMS: 1222.5 (M+1)⁺.

15 Step C – Synthesis of Intermediate Ex-01c

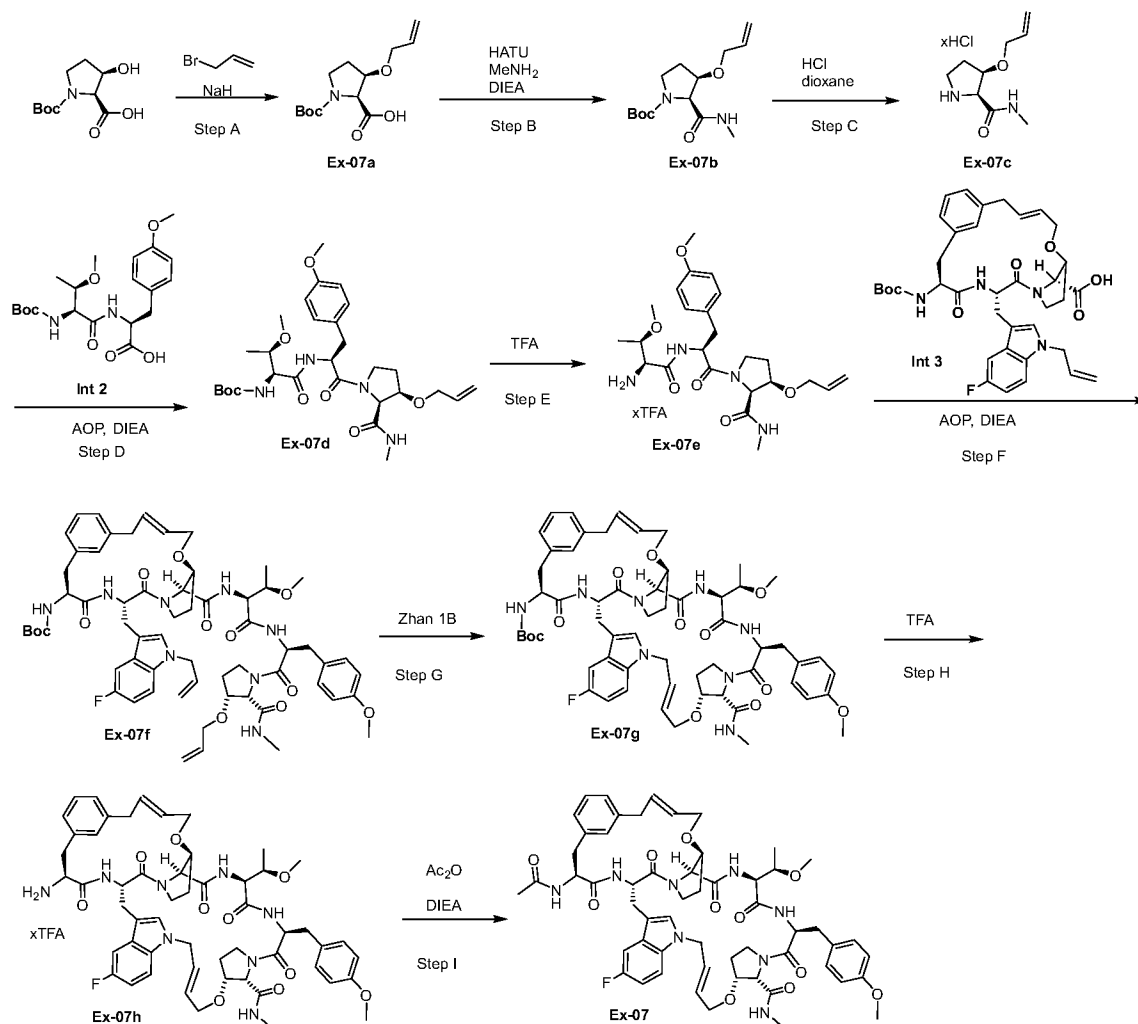
[0346] Intermediate **Ex-01b** (77 mg, 0.063 mmol) was dissolved in degassed t-BuOH (50 ml) and water (25 ml) under an atmosphere of nitrogen gas and put into a preheated oil bath at 50°C to raise to target temperature. Next tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (7.5 mg, 0.014 mmol) and sodium ascorbate (22 mg, 0.111 mmol) were then added. After all solid was dissolved, tetrakis(acetonitrile)copper(I) hexafluorophosphate (5.3 mg, 0.014 mmol) was added. After stirring at 50°C under an atmosphere of nitrogen for two hours, the reaction was cooled and concentrated in vacuo. The residue was partially dissolved in a 2 mL solution of ACN/MeOH/DCM and filtered onto a 40g ISCO-gold column, rinsed with ~20% ACN/DCM and purified using gradient elution (0-20% EtOH in EtOAc) to afford compound **ME-01c**.
25 LCMS: 1222.5 (M+1)⁺.

Step D – Synthesis of Example Ex-01 and Ex-02

[0347] Intermediate **Ex-01c** (37 mg, 0.030 mmol) was dissolved in degassed acetic acid (2 ml) and DCM (40 ml) with continued bubbling of nitrogen and 1,3-Bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene[2-(i-propoxy)-5-(N,N-dimethylaminosulfonyl)phenyl] methyleneruthenium(II) dichloride (Zhan Catalyst-1B) (16.4 mg, 0.023 mmol) was then added. The reaction was sealed and put in preheated oil bath at 50 °C for 100 minutes. The reaction was cooled and loaded directly onto a dry ISCO 40g gold column, rinsed with DCM. The mixture of isomers was separated from impurities using gradient elution (0-26% EtOH in EtOAc). The combined fractions were then concentrated in vacuo and the isomers were separated using
35 gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 32-65% CH_3CN /water w/ 0.1% TFA modifier over 20 min).

- 5 [0348] The fractions for the major isomer (trans) were lyophilized to afford compound **Ex-01** (3mg, 8%). LCMS anal. calculated. for C₆₃H₇₂FN₁₁O₁₀S: 1193.5; Found: 1194.5 (M+1)⁺. The fraction for the minor isomer (cis) (70:30 mix with ME01) was lyophilized to afford compound **Ex-02**. LCMS: 1194.5 (M+1)⁺.

10 **Example 13: Synthesis of Example compound 03 (Ex-07)**



Step A – Synthesis of Intermediate Ex-07a

- [0349] In a flame dried flask under an atmosphere of nitrogen gas, (2S,3R)-1-(tert-butoxycarbonyl)-3-hydroxypyrrolidine-2-carboxylic acid (2 g, 8.65 mmol) was dissolved in anhydrous THF (100 ml) and anhydrous DMF (30 ml) and the solution cooled in ice bath. Next, sodium hydride (0.655 g, 25.9 mmol) was added portion wise. After stirring at 0°C for 30 mins, allyl bromide (1.3 ml, 15.02 mmol) was added, and the solution was stirred at RT overnight. The reaction was diluted with water and aq. NaHCO₃ was added to adjust pH to 9-10 and then
- 15

- 5 extracted with ether (2X). 1N aq HCl (~25mL) was added to the aqueous layer to adjust pH to 1-2. To the resulting solution, brine was added followed by extraction with EtOAc (3X). The combined organic phase was dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was dissolved in DCM and purified on silica gel using gradient elution (80g-ISCO gold, 50-100% EtOAc-hexane) to afford intermediate **Ex-07a**.
- 10 **[0350]** ¹H NMR (600 MHz, CDCl₃) δ 5.9-5.8 (m, 1H), 5.27 (dd, J = 17.2, 1.5 Hz, 1H), 5.17 (d, J = 10.3 Hz, 1H), 4.55-4.40 (m, 1H), 4.32 – 4.27 (m, 1H), 4.15-4.02 (m, 2H), 3.68-3.33 (m, 2H), 2.13 – 2.03 (m, 2H), 1.45-1.42 (m, 9H).

Step B – Synthesis of Intermediate **Ex-07b**

- [0351]** Intermediate **Ex-07a** (693 mg, 2.55 mmol) was dissolved in DMF (17 ml) and DIEA (1.784 ml, 10.22 mmol) was added, followed by methylamine (2M THF) (2.55 ml, 5.11 mmol) and then HATU (1020 mg, 2.68 mmol). After stirring the reaction mixture at room temperature for several hours, 1N aq. HCl was added to neutralize the mixture, followed by dilution with water and extraction with DCM (3X). The combined organic phase was dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was dissolved in DCM and purified on silica gel using gradient elution (40g-ISCO gold, 15-100% EtOAc-hexane) to afford **Ex-07b** (as a mixture with residual HOAT.) LCMS: 285 (M+1)⁺.
- 15
20

Step C – Synthesis of Intermediate **Ex-07c**

- [0352]** Intermediate **Ex-07b** ((as a mixture with residual HOAT) (720 mg, 1.722 mmol) was dissolved in HCl (4M in dioxane) (20 ml, 80 mmol). After stirring for 1 hour at RT, the reaction mixture was concentrated in vacuo. The residue was redissolved in toluene-ACN and concentrated in vacuo (2X) to afford intermediate **Ex-07c** (as a mixture with residual HOAT.) LCMS: 185 (M+1)⁺.
- 25

Step D – Synthesis of Intermediate **Ex-07d**

- [0353]** Intermediate **Int 2** (100 mg, 0.244 mmol) and intermediate **Ex-07c** (87 mg, 0.268 mmol) were dissolved in DCM (20 ml) along with DIEA (0.170 ml, 0.975 mmol) and DMF (5 ml) and then AOP (CAS: 156311-85-2) (108 mg, 0.244 mmol) was added. After stirring at RT overnight, ~1mL 1N aq. HCl was added to the reaction to adjust pH to 1-2 and then washed with brine and extracted with DCM (3X.) The combined organic phase was dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was dissolved in DCM and purified on silica gel using gradient elution (40g-ISCO gold, 15-100% EtOAc-hexane). The combined fractions were then concentrated in vacuo and the residue was then dissolved in DMSO (with ~10% MeOH and water) and purified using gradient elution on reverse phase (50 x 250 mm SunFire Prep C18; 25-
- 30
35

- 5 75% CH₃CN /water w/ 0.1% TFA modifier over 25 min) to afford intermediate **Ex-07d**. LCMS: 577.4 (M+1)⁺.

Step E – Synthesis of Intermediate Ex-07e

- [0354] Intermediate **Ex-07d** (41 mg, 0.071 mmol) was dissolved in DCM (1 ml), and TFA (1 ml, 12.98 mmol) was added. After stirring at RT for 30mins, the reaction mixture was evaporated
10 under a stream of nitrogen gas, and was redissolved in 1:1 ACN-water and lyophilized to afford intermediate **Ex-07e**. LCMS: 975.5 (2M+Na)⁺.

Step F – Synthesis of Intermediate Ex-07f

- [0355] Intermediate **Int 3** (44.6 mg, 0.066 mmol) and **Ex-07e** (39 mg, 0.066 mmol) were dissolved in DMF (2 ml) and DIEA (29 µL, 0.166 mmol), and then AOP (CAS: 156311-85-2)
15 (30.7 mg, 0.069 mmol) was added, followed by 20 µL DIEA to adjust pH to 9-10. After stirring at RT for 90 mins, the reaction mixture was diluted with 9:1 DMSO/water and purified using gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 5-95% CH₃CN /water w/ 0.1% TFA modifier over 25 min) to afford intermediate **Ex-07f**. LCMS: 1133.5 (M+1)⁺.

Step G – Synthesis of Intermediate Ex-07g

- 20 [0356] Intermediate **Ex-07f** (50 mg, 0.044 mmol) was dissolved in degassed DCE (50 ml) and Acetic Acid (2.5 ml). After it was bubbled with nitrogen gas and put in a preheated oil bath at 80 °C, 1,3-Bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene[2-(i-propoxy)-5-(N,N-dimethylaminosulfonyl)phenyl] methylenruthenium(II) dichloride (Zhan Catalyst-1B) (28 mg, 0.038 mmol) was added. After heated under an atmosphere of nitrogen at 80°C for 60 mins, the
25 reaction was cooled and loaded directly onto a dry ISCO 40g gold column, rinsed with DCM, separated from impurities using gradient elution (0-26% EtOH in EtOAc) and the cleanest fraction isolated and concentrated in vacuo to afford intermediate **Ex-07g**. LCMS: 1127.6 (M+Na)⁺.

Step H – Synthesis of Intermediate Ex-07h

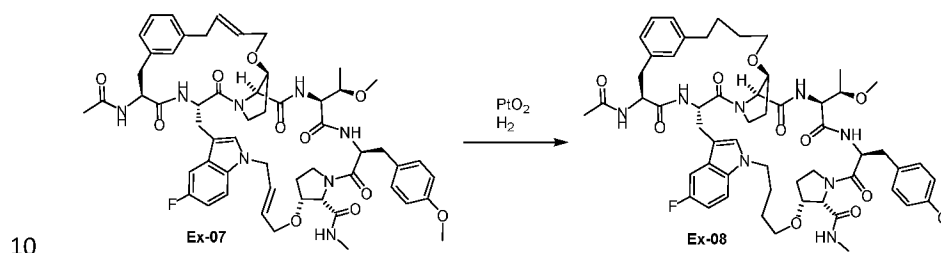
- 30 [0357] Intermediate **Ex-07g** (20 mg, 0.018 mmol) was dissolved in DCM (1 ml) and TFA (1ml, 12.98 mmol). After stirring for 20 mins, the reaction was evaporated under a stream of nitrogen gas and then redissolved in 1:1 ACN-water and lyophilized to afford intermediate **Ex-07h**. LCMS: 1005.5 (M+1)⁺.

Step I – Synthesis of Compound Ex-07

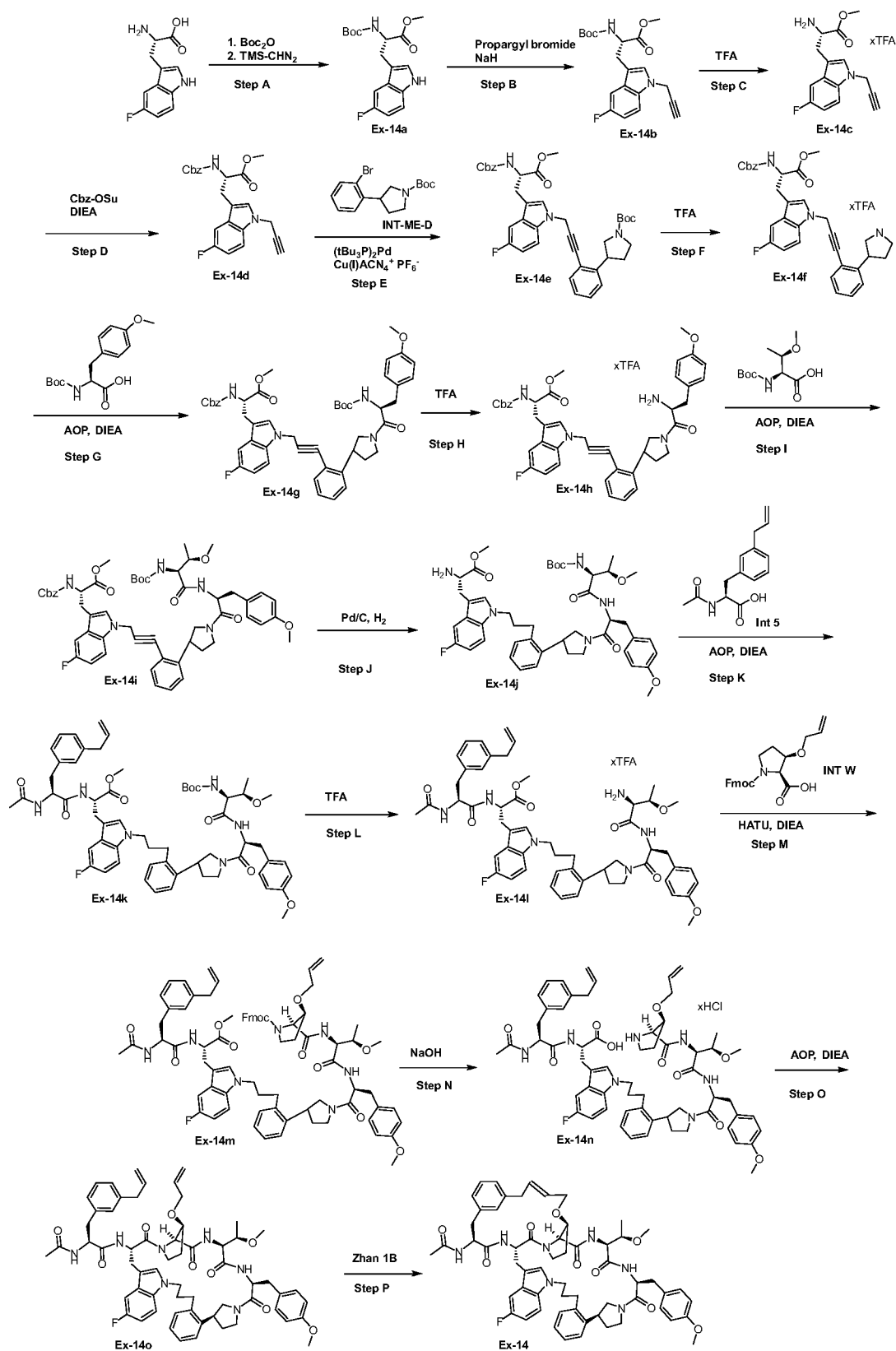
- 35 [0358] Intermediate **Ex-07h** (12 mg, 10.72 µmol) was dissolved in DMF (0.5 ml), and DIEA (5.62 µl, 0.032 mmol) and acetic anhydride (2.023 µl, 0.021 mmol) were then added. After

5 stirring at RT for one hour, the reaction mixture was diluted with 10:1 DMSO-water and purified using gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 30-65% CH₃CN /water w/ 0.1% TFA modifier over 16 min) to afford **Ex-07**. LCMS: 1047.4 (M+1)⁺.

Example 14: Synthesis of Example compound 08 (Ex-08)



[0359] Example **Ex-07** (isomeric mixture) (2.8 mg, 2.67 μmol) was dissolved in ethanol (4 ml) and nitrogen gas was bubbled through the solution. Next, platinum (IV) oxide (1.4 mg, 6.17 μmol) was added, and the flask was put on H_2 balloon, and evacuated and back filled with H_2 three times. After stirring at RT for one hour, the reaction was filtered through a syringe filter, rinsed with ethanol and the filtrate was concentrated in vacuo. The residue was redissolved in ~5:1:1 MeOH-DMSO-water and purified using gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 30-70% CH_3CN /water w/ 0.1% TFA modifier over 14 min to afford Example **Ex-08**. LCMS: 1051.5 ($\text{M}+1$)⁺.

5 **Example 15: Synthesis of Example compound 14 (Ex-14)**

5 Step A – Synthesis of Intermediate Ex-14a

[0360] (S)-2-amino-3-(5-fluoro-1H-indol-3-yl)propanoic acid (3000 mg, 13.50 mmol) and BOC-Anhydride (3.29 ml, 14.18 mmol) were dissolved in DMA (30 ml) and Methanol (10 ml) and triethylamine (2.070 ml, 14.85 mmol) was then added. After stirring reaction at RT for an hour, the mixture was cooled in ice bath and a solution of conc. HCl (1.220 ml, 14.85 mmol) in 10 mL of methanol was added to adjust pH to 3. Next TMS-Diazomethane (2M in hexane) (10.13 ml, 20.25 mmol) was added over 10mins followed by more TMS- Diazomethane (3 X 5mL) through day to drive to completion. The reaction was quenched with 2 mL acetic acid, diluted with aq. NaHCO₃ to raise pH to 9-10 and extracted with ether (3X.) The combined organics were washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was dissolved in toluene/DCM and purified on silica gel using gradient elution (120g-ISCO gold, 0-60% EtOAc-hexane to afford intermediate **Ex-14a**. LCMS: 673.5 (2M+1)⁺.

Step B – Synthesis of Intermediate Ex-14b

[0361] Intermediate **Ex-14a** (500 mg, 1.487 mmol) was dissolved in DMF (6.00 ml) and sodium hydride (62.4 mg, 1.561 mmol) was added at -10 °C. After stirring at -10 °C for 20 min, 3-bromoprop-1-yne (195 mg, 1.635 mmol) was added to the solution. The reaction solution was stirred at -10 °C for 0.5 h. The reaction was quenched with saturated aq. NH₄Cl (10 mL). The reaction solution was diluted with EtOAc (100 mL), washed with brine (3 x 100 mL), dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated in vacuo. The residue was purified on silica gel using gradient elution (0-30% EtOAc-PE) to afford intermediate **Ex-14b**. LCMS: 397.2 (M+Na)⁺.

Step C – Synthesis of Intermediate Ex-14c

[0362] Intermediate **Ex-14b** (1.558 g, 4.16 mmol) was dissolved in DCM (5 ml) and cooled in ice bath. TFA (5 ml, 64.9 mmol) was then added. After stirring for 20mins, the reaction was concentrated in vacuo. The residue was redissolved in ACN and concentrated in vacuo (3X). Small amt of ether was added and solid precipitated. The resulting mixture was concentrated in vacuo to afford **Ex-14c** which was used crude into the next step. LCMS: 275.3 (M+1)⁺.

Step D – Synthesis of Intermediate Ex-14d

[0363] Intermediate **Ex-14c** (1.615 g, 4.16 mmol) was dissolved in DCM (23 ml) and DIEA (1.816 ml, 10.40 mmol) and N-(benzyloxycarbonyloxy)succinimide (1.140 g, 4.58 mmol) were added. After stirring at RT for 2 hours, the reaction was loaded directly onto silica gel column and purified using gradient elution (40g ISCO gold, 0-60% EtOAc-hexane) to afford intermediate **Ex-14d**. LCMS: 409.3 (M+1)⁺.

5 Step E – Synthesis of Intermediate Ex-14e

[0364] Intermediate **Ex-14d** (600 mg, 1.469 mmol) and intermediate **AD** (839 mg, 2.57 mmol) were dissolved in N-methylimidazole (20 ml) and was bubbled with nitrogen gas and put in heating block preheated to 80°C. Next Bis(tri-t-butylphosphine) palladium(0) (405 mg, 0.793 mmol) and tetrakis(acetonitrile)copper(I)hexafluorophosphate (219 mg, 0.588 mmol) were added. The vial was sealed and heated at 80°C for 150 minutes. Bis(tri-t-butylphosphine) palladium(0) (100mg) and tetrakis(acetonitrile)copper(I)hexafluorophosphate (55 mg) were added and was then heated at 85°C for 2 hours and then stirred at RT overnight. The reaction was partitioned between EtOAc (~180mL) and water and washed with aq 1N HCl (2X) and then with brine. The organics were dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was dissolved in DCM and purified on silica gel using gradient elution (120g-ISCO gold, 0-60% EtOAc-hexane) to afford intermediate **Ex-14e**. LCMS: 676.3 (M+Na)⁺.

15 Step F – Synthesis of Intermediate Ex-14f

[0365] Intermediate **Ex-14e** (110 mg, 0.168 mmol) was dissolved in DCM (2 ml) and TFA (2 ml, 26.0 mmol) was then added. After stirring at RT for 20mins, the reaction was concentrated in vacuo. The residue was redissolved in ACN and concentrated in vacuo (3X) to afford intermediate **Ex-14f**, which was used crude into the next step. LCMS: 554.3 (M+1)⁺.

20 Step G – Synthesis of Intermediate Ex-14g

[0366] Intermediate **Ex-14f** (112 mg, 0.168 mmol) and (S)-2-((tert-butoxycarbonyl)amino)-3-(4-methoxyphenyl)propanoic acid (64.5 mg, 0.218 mmol) were dissolved in DCM (5 ml) and DIEA (0.088 ml, 0.504 mmol) was then added, followed by AOP (CAS: 156311-85-2) (89 mg, 0.202 mmol). Next DIEA (44uL) was added to adjust pH to >8. After stirring at RT for 90 mins, the reaction was loaded directly onto silica gel column and purified using gradient elution (24g ISCO gold, 0-80% EtOAc-hexane) to afford **Ex-14g**. LCMS: 831.4 (M+1)⁺.

25 Step H – Synthesis of Intermediate Ex-14h

[0367] Intermediate **Ex-14g** (122 mg, 0.147 mmol) was dissolved in DCM (2 ml) and TFA (2 ml, 26.0 mmol) was then added. After stirring at RT for 25 mins, the reaction was concentrated in vacuo. The residue was redissolved in ACN and concentrated in vacuo (3X) to afford intermediate **Ex-14h**, which was used crude into the next step. LCMS: 731.3 (M+1)⁺.

30 Step I – Synthesis of Intermediate Ex-14i

[0368] Intermediate **Ex-14h** (124 mg, 0.147 mmol) and (2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methoxybutanoic acid (44.6 mg, 0.191 mmol) were dissolved in DCM

5 (5 ml) and DIEA (0.116 ml, 0.662 mmol) was then added, followed by AOP (CAS: 156311-85-2) (78 mg, 0.176 mmol). Next DIEA (40 μ L) was added to adjust pH to >8. After stirring at RT for 90 mins, the reaction was loaded directly onto silica gel column and purified using gradient elution (24g ISCO gold, 20-90% EtOAc-hexane) to afford intermediate **Ex-14i**. LCMS: 946.5 (M+1)⁺.

10 Step J – Synthesis of Intermediate **Ex-14j**

[0369] Intermediate **Ex-14i** (128 mg, 0.135 mmol) was dissolved in MeOH (10 ml). After bubbled with nitrogen gas, 10% Pd-C (35 mg, 0.329 mmol) was added and then H₂ balloon (purged 3X). after stirring at RT for 2 hours, the reaction was filtered, rinsed with methanol, and concentrated in vacuo. The residue was redissolved in ACN and concentrated in vacuo (2X) to afford intermediate **Ex-14j**. LCMS: 816.5 (M+1)⁺.

Step K – Synthesis of Intermediate **Ex-14k**

[0370] Intermediate **Ex-14j** (100 mg, 0.123 mmol) and **INT-ME-E** (42.4 mg, 0.172 mmol) were dissolved in DCM (5 ml) and DIEA (0.064 ml, 0.368 mmol) was added, followed by AOP (CAS: 156311-85-2) (67.9 mg, 0.153 mmol). The reaction was stirred at RT (pH~9.) for 45mins, 20 2mL DMF was added to dissolve hazy solid. **Intermediate Int 5** (42.4 mg, 0.172 mmol), AOP (CAS: 156311-85-2) (67.9 mg, 0.153 mmol) and DIEA (0.064 ml, 0.368 mmol) were premixed in a 1.5mL DMF and ~1 mL of this premixed solution was added. After stirring at RT for 15 mins, it was diluted by brine and 1mL 1N aq. HCl and extracted with DCM (1x) and EtOAc (2x.) The combined organics were dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue 25 was dissolved in DCM and purified on silica gel using gradient elution (24g-ISCO gold, 20-100% EtOAc-hex and then 5% EtOH-EtOAc flush) to afford intermediate **Ex-14k**. LCMS: 1045.4 (M+1)⁺.

Step L – Synthesis of Intermediate **Ex-14l**

[0371] Intermediate **Ex-14k** (100 mg, 0.096 mmol) was dissolved in DCM (1.5 ml) and TFA (2 30 ml, 26.0 mmol) was then added. After stirring at RT for 15 mins, the reaction was concentrated in vacuo. The residue was redissolved in ACN and concentrated in vacuo (3X) to afford intermediate **Ex-14l**, which was used crude into the next step. LCMS: 945.5 (M+1)⁺.

Step M – Synthesis of Intermediate **Ex-14m**

[0372] Intermediate **Ex-14l** (100 mg, 0.094 mmol) was dissolved in DMF (4 mL) and DIEA 35 (80 μ L, 0.458 mmol) and a premixed solution of Intermediate **W** (60 mg, 0.153 mmol), HATU (50 mg, 0.131 mmol), DIEA (30 μ L, 0.172 mmol) in DMF (1 mL) were added, followed by more

5 DIEA (60 μ L) to raise pH to >8. After stirring at RT for an hour, the reaction was concentrated in vacuo. Next it was diluted with 10:1:1 DMF-water-MeOH and purified using gradient elution on reverse phase (50 x 250 mm SunFire Prep C18; 40-93% CH₃CN /water w/ 0.1% TFA modifier over 20 min and then holding at 93% ACN) to afford intermediate **Ex-14m**. LCMS: 1320.6 (M+1)⁺.

10 Step N– Synthesis of Intermediate **Ex-14n**

[0373] Intermediate **Ex-14m** (60 mg, 0.045 mmol) was dissolved in THF (5 ml) and MeOH (5 ml) and 1N aq. NaOH (0.5 ml, 0.500 mmol) was added. After stirring at RT, 1N aq. NaOH was added through day (2 x 100 μ L and 1X 60 μ L) to drive to completion. Next the reaction was quenched by 1200 μ L 1N aq. HCl, concentrated in vacuo. The residue was redissolved in ACN and concentrated in vacuo (2X.) The resulting residue was triturated with ether and ether was pipetted off (4X) to remove soluble impurities. The residual was dissolved in 1:1 ACN-water and lyophilized to afford **Ex-14n** which was used crude in the next step (contains residual NaCl). LCMS: 1084.6 (M+1)⁺.

Step O– Synthesis of Intermediate **Ex-14o**

20 [0374] A mixture of intermediate **Ex-14n** (0.045 mmol) and DIEA (20 μ L, 0.115 mmol) in DMF (2 mL) (pH >9) was added via syringe pump over an hour to a DCM (55 ml) and DMF (1mL) solution of AOP (CAS: 156311-85-2) (39.9 mg, 0.090 mmol) and DIEA (60 μ L, 0.344 mmol). The syringe was rinsed with DMF (2 x 1 mL). After stirring for 8 hours, more AOP (20mg) was added and was stirred overnight at RT.

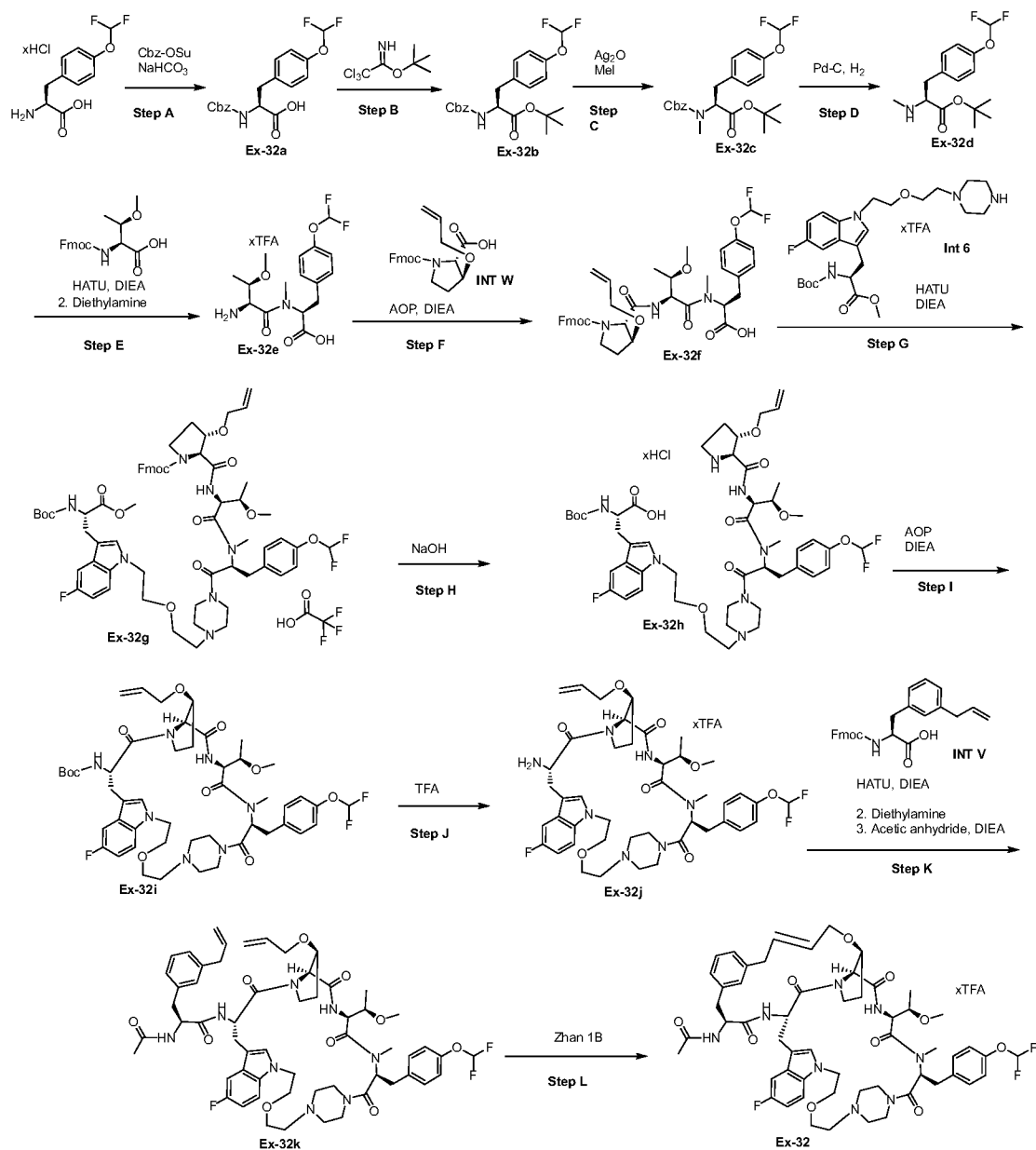
25 [0375] The reaction was acidified with 600 μ L 1N aq. HCl and concentrated in vacuo to ~1 mL suspension, diluted to a ~10:1 DMF/ aqueous mixture and filtered through a 45 μ M syringe filter to remove residual (water soluble) ppt. It was purified using gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 45-85% ACN /water w/ 0.1% TFA modifier over 20 min.) The mixed fractions for the 2 major isomers were lyophilized and then redissolved in 10:1:1 MeOH-DMF-water and repurified using gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 45-75% ACN /water w/ 0.1% TFA modifier over 14 min and then held at 75% acetonitrile) to afford intermediate **Ex-14o**. LCMS: 1066.2 (M+1)⁺.

Step P– Synthesis of **Ex-14**

35 [0376] Intermediate **Ex-14o** (5.3 mg, 4.97 μ mol) was dissolved in degassed DCE (8 ml) and Acetic Acid (0.4 ml) and was then bubbled with nitrogen gas and put in preheated oil bath at 65°C. 1,3-Bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene[2-(i-propoxy)-5-(N,N-dimethylaminosulfonyl)phenyl] methylenruthenium(II) dichloride (Zhan Catalyst-1B) (3.58 mg,

- 5 4.97 μmol) was then added and heated under an atmosphere of nitrogen for one hour. The reaction was cooled and concentrated in vacuo. The residue was redissolved in 10:1 MeOH-water and purified using gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 40-77% ACN /water w/ 0.1% TFA modifier over 14 min) to afford **Ex-14**. LCMS: 1038.1 (M+1)⁺.

10 **Example 16: Synthesis of Example compound 32 (Ex-32)**



5 Step A – Synthesis of Intermediate Ex-32a

[0377] (S)-2-amino-3-(4-(difluoromethoxy)phenyl)propanoic acid hydrochloride (1530 mg, 5.72 mmol) was dissolved in THF (15 mL) along with a solution of SODIUM BICARBONATE (1201 mg, 14.29 mmol) dissolved in water (35 mL). Next a solution of benzyl (2,5-dioxopyrrolidin-1-yl) carbonate (1522 mg, 6.11 mmol) dissolved in THF was added dropwise. After stirring at RT for an hour, the reaction was acidified with ~10mL 1N aq. HCl and extracted with EtOAc (3x), dried organics over Na₂SO₄, filtered and concentrated in vacuo to afford intermediate **Ex-32a** which was used crude into the next step. LCMS: 366.4 (M+1)⁺.

Step B – Synthesis of Intermediate Ex-32b

[0378] Intermediate **Ex-32a** (5.72 mmol) was dissolved in DCM (50 mL) and a solution of TERT-BUTYL 2,2,2-TRICHLOROACETIMIDATE tert-butyl 2,2,2-trichloroacetimidate (2.62 mL, 14.65 mmol) in Cyclohexane (15 mL) was then added. After stirring at RT for 45mins, more tert-butyl 2,2,2-trichloroacetimidate (700 µL) was added to the reaction, and after 30 more mins at RT tert-butyl 2,2,2-trichloroacetimidate (400 µL) was added once again. Next, after 30 mins, the reaction was filtered through syringe filter to remove trichloroacetamide by-product, rinsed with DCM, diluted filtrate with water, brine, and ~ 2mL aq. bicarb (pH~9) and extracted with DCM (3x.) The combined organics were dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was dissolved in DCM and purified on silica gel using gradient elution (80g-ISCO gold, 0-60% EtOAc-hexane). The appropriate fractions were concentrated in vacuo. The sample was rinsed with cyclohexane, concentrated in vacuo to afford intermediate **Ex-32b**. LCMS: 467.6 (M+2Na)⁺. ¹H NMR (600 MHz, CDCl₃) δ 7.36-7.26 (m, 5H), 7.11 (d, J = 7.4 Hz, 2H), 7.01-6.97 (m, 2H), 6.44 (t, J = 73.9 Hz, 1H), 5.25-5.21 (m, 1H), 5.13-5.02 (m, 2H), 4.52-4.46 (m, 1H), 3.09-2.98 (m, 2H), 1.42-1.32 (m, 9H).

Step C – Synthesis of Intermediate Ex-32c

[0379] Intermediate **Ex-32b** (2000 mg, 4.75 mmol) was dissolved in anhydrous DMF (25 mL) under an atmosphere of nitrogen gas and IODOMETHANE (4 mL, 64.0 mmol) and SILVER OXIDE (5600 mg, 24.17 mmol) were then added. [0380] The reaction vial was sealed and heated at 45°C for 2 hours to completion. The reaction was diluted with EtOAc (100 mL) and filtered through a 12g silica gel pad, rinsed with EtOAc, concentrated filtrate in vacuo. The residue was redissolved oil in ether and filtered through syringe filter with ether rinse and concentrated filtrate in vacuo to afford intermediate **Ex-32c**. LCMS: 481.6 (M+2Na)⁺.

5 **[0381]** Step D– Synthesis of Intermediate **Ex-32d**

[0382] Intermediate **Ex-32c** (2.05 g, 4.71 mmol) was dissolved in methanol (40 ml) and then was bubbled solution with nitrogen gas. 10% Pd-C (275 mg, 0.258 mmol) was added and then H₂ balloon (purged 3X.) After stirring at RT for 4 hours, the reaction was filtered through syringe filter with methanol rinse and concentrated filtrate in vacuo to afford intermediate **Ex-32d**.

10 LCMS: 302.4 (M+1)⁺.Step E– Synthesis of Intermediate **Ex-32e**

[0383] N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-methyl-L-threonine (1751 mg, 4.93 mmol) and Intermediate **Ex-32d** (1350 mg, 4.48 mmol) were dissolved in DMF (30 ml) and DIEA (1.956 ml, 11.20 mmol). Next HATU (1840 mg, 4.84 mmol) was added, and reaction was stirred
15 at RT for 10 mins then heated at 40°C. After 90min, more HATU (130 mg) was added and stirred at 40° C for 15 more mins. Then the reaction cooled to RT and DIETHYLAMINE (32.8 ml, 314 mmol) was added. After stirring at RT for 10 mins, the reaction was concentrated in vacuo down to ~15 mL. A solution of TFA (0.7 ml, 9.09 mmol) in 30mL MeOH was added to acidify to pH 2-3, concentrated in vacuo to an oil which was stored in the freezer overnight. The
20 residue was then dissolved in a mixture of DMF-water-MeOH, filtered through syringe filter, and purified using gradient elution over 2 runs on reverse phase (50 x 250 mm SunFire Prep C18; 20-70% ACN /water w/ 0.1% TFA modifier over 25 min. (20-65% for 2nd run)). The appropriate fractions were lyophilized. Analysis of lyophilized solid showed a 1:1 mixture of the expected product containing the t-butyl ester and **Ex-32e**, which was used as the mixture in the next step.

25 LCMS: 361.4 (M+1)⁺.Step F– Synthesis of Intermediate **EX-32f**

[0384] Intermediate **W** (423 mg, 1.075 mmol) was dissolved in CH₂Cl₂ (30 ml) and DIEA (0.76 ml, 4.35 mmol). After stirring for 10 mins at RT, a solution of **Ex-32e** (mixture with analog containing t-butyl ester) (485 mg, 0.914 mmol) in DMF (7 ml) was added dropwise over
30 15 mins. After stirring for 30 mins, it was added 4 mL 1N aq. HCl to lower pH to ~2, diluted with water and extracted with DCM (3x.) The combined organics were dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was dissolved in DCM and purified on silica gel using gradient elution (80g-ISCO gold, 0-100% EtOAc-hexane and then 1:3 EtOH-EtOAc). The appropriate fractions were concentrated in vacuo, to afford intermediate **Ex-32f**. LCMS: 736.3
35 (M+1)⁺.

5 Step G– Synthesis of Intermediate Ex-32g

[0385] Intermediate **Ex-32f** (92 mg, 0.125 mmol) and Intermediate **Int 6** (99 mg, 0.138 mmol) were dissolved in DMF (3 ml) and DIEA (0.109 ml, 0.625 mmol) and HATU (47.5 mg, 0.125 mmol) was added. After stirring at RT for 3 hours, it was added 300uL water and purified using gradient elution on reverse phase (50 x 250 mm SunFire Prep C18; 35-75% ACN /water w/ 0.1% TFA modifier over 20 mins) to afford intermediate **Ex-32g**. LCMS: 1210.6 (M+1)⁺.
10

Step H– Synthesis of Intermediate Ex-32h

[0386] Intermediate **Ex-32g** (40 mg, 0.030 mmol) was dissolved in MeOH (8 ml) and THF (2 ml) and NaOH (1M aq) (0.604 ml, 0.604 mmol) was then added. After stirring at RT for 3.5 hours, it was added 660uL 1N aq. HCl to acidify to pH3, concentrated in vacuo. The residue was trituated with ether (3x) to remove soluble impurities and then dried in vacuo to afford intermediate **Ex-32h** which was used crude in the next step (contains residual NaCl). LCMS: 974.5 (M+1)⁺.
15

Step I– Synthesis of Intermediate Ex-32i

[0387] To a DCM (45 ml) and DMF (2 mL) solution of AOP (CAS: 156311-85-2) (66.5 mg, 0.150 mmol) and DIEA (0.052 ml, 0.300 mmol) was added via syringe pump over 90 mins, a mixture of intermediate **Ex-32h** (0.030 mmol) in DMF (3 mL). After stirring at RT (pH >8) overnight, the reaction was heated at 50°C for 2 hours. Next the reaction was acidified with 300uL 1N aq. HCl and concentrated in vacuo to ~1mL suspension. The resulting mixture was dissolved with DMF, MeOH and water (~2.7 mL 5:2:1 MeOH-DMF-water total) and purified using gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 30-75% ACN /water w/ 0.1% TFA modifier over 18 mins) to afford intermediate **Ex-32i**. LCMS: 956.5 (M+1)⁺.
20
25

Step J– Synthesis of Intermediate Ex-32j

[0388] Intermediate **Ex-32i** (12.5 mg, 0.013 mmol) was dissolved in a solution of TFA (2 ml, 26.0 mmol) and DCM (2 ml). After stirring at RT for 15 mins, the reaction was concentrated in vacuo. The residue was redissolved in ACN and concentrated in vacuo (2X) to afford intermediate **Ex-32j** which was used crude in the next step. LCMS: 856.5 (M+1)⁺.
30

Step K– Synthesis of Intermediate Ex-32k

[0389] Intermediate **Ex-32j** (0.013 mmol) was dissolved in DMF (2 ml) and DIEA (13.70 µl, 0.078 mmol), intermediate **V** (8.38 mg, 0.020 mmol) and HATU (6.46 mg, 0.017 mmol) were then added. After stirring at RT for 2 hours, the reaction was added DIETHYLAMINE (200 µl, 1.914 mmol) and stirred at RT for 10 mins and then concentrated in vacuo. The residue was
35

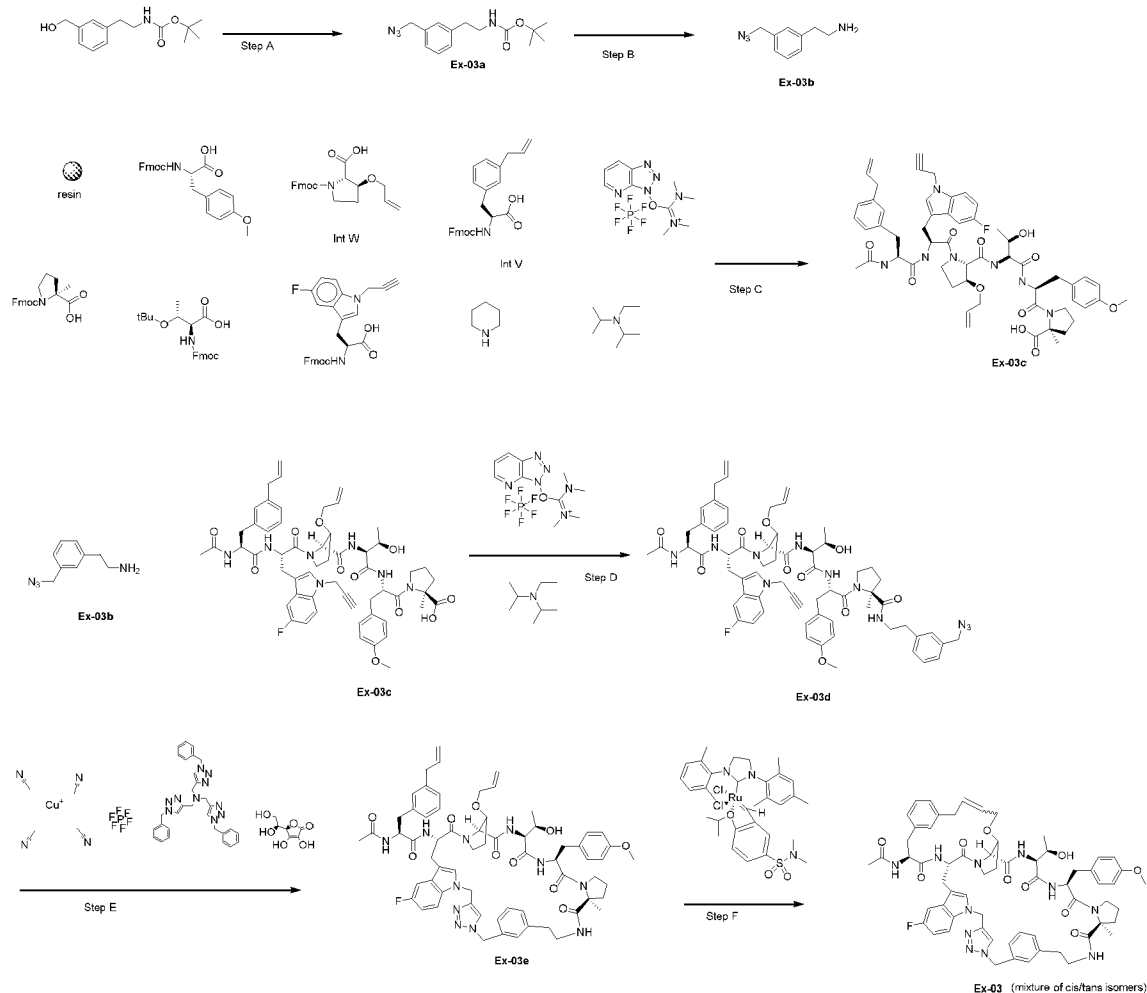
5 redissolved in DMF (1 mL) and DIEA (25 μ l, 0.14 mmol) and Ac₂O (12.33 μ l, 0.131 mmol) were added. After stirring at RT for 10 mins, it was diluted with DMF, MeOH and water (~2.7 mL 5:5:1 MeOH-DMF-water total) and purified using gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 30-75% ACN /water w/ 0.1% TFA modifier over 14 mins) to afford intermediate **Ex-32k**. LCMS: 1085.5 (M+1)⁺.

10 Step L— Synthesis of Intermediate **Ex-32**

[0390] Intermediate **Ex-32k** ((11.5 mg, 10.60 μ mol) was dissolved in degassed DCE (12 ml) and Acetic Acid (0.6 ml) and was then bubbled with nitrogen gas and put in preheated block at 50°C. 1,3-Bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene[2-(i-propoxy)-5-(N,N-dimethylaminosulfonyl)phenyl] methyleneruthenium(II) dichloride (Zhan Catalyst-1B) (7.64 mg, 10.60 μ mol) was then added. The vial was sealed and heated at 50°C for 90 minutes to completion. The reaction was cooled and concentrated in vacuo. The residue was redissolved in 12:1 MeOH-water and purified using gradient elution on reverse phase (30 x 150 mm SunFire Prep C18; 20-65% ACN /water w/ 0.1% TFA modifier over 13 min) to afford **Ex-32**. LCMS: 1057.6 (M+1)⁺.

20

5 **Example 17: Synthesis of Example compound 03 (Ex-03)**



Step A - Synthesis of Intermediate **Ex-03a** (tert-butyl 3-(azidomethyl)phenethylcarbamate)

[0391] To a solution of tert-butyl 3-(hydroxymethyl)phenethylcarbamate (2 g, 7.96 mmol) in THF 40 ml, 2-azido-1,3-dimethyl-1H-imidazol-3-ium hexafluorophosphate(V) (3.15 g, 11.14 mmol), and DBU (2.79 g, 11.14 mmol) were added. After stirring at room temperature for 10 min, the reaction was quenched with sat. aq NH₄Cl, and org. materials were extracted with CH₂Cl₂. The combined extracts were washed with brine, and dried over Na₂SO₄ and concentrated. The crude compounds were purified through 80-gram size silica column by hexane /EtOAc 0-70% to afford intermediate **Ex-03a**. LC/MS 277.0 (M+1)⁺.

Step B - Synthesis of Intermediate **Ex-03b** (2-(3-(azidomethyl)phenyl)ethanamine)

[0392] Intermediate **Ex-03a** (2.211 g, 8mmol) was dissolved in 20 ml of 1:1 (v/v) TFA/DCM. After stirring at room temp for 30 min, it was removed the solvent to afford intermediate **Ex-03b** (1.5 g) as an oily material, which was used directly without purification. LC/MS 177.05 (M+1)⁺.

5 Step C - Synthesis of Intermediate Ex-03c

[0393] The linear peptide intermediate **Ex-03c** was synthesized using Fmoc-protected amino acids on a solid-phase 2-chlorotriyl chloride resin (0.2 mmol, 100-200 mesh, 0.6 mmol/g, CreoSalus). Triyl resin was first loaded with 2 eq of (S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-methylpyrrolidine-2-carboxylic acid and 4 eq DIEA. It was heated at 50°C in microwave for 10 min for 2 times. Then, the synthesis was continued by standard SPPS using Fmoc/t-Bu chemistry on a CEM Liberty Blue automated peptide synthesizer (CEM Corp.). Reaction conditions were as follows: Deprotection Conditions: 20% piperidine (v/v) in DMF (2 X 2 min at 75 °C); Residue Coupling Conditions: 5 eq (relative to resin) of activated amino acid (5 mL of a 0.2 M amino acid stock solution in DMF) was delivered to the resin, followed by 5 eq of HATU activator (2 mL of a 0.45 M solution in DMF), and 10 eq of DIEA (1 mL of a 2M solution in NMP) and allowed to react for 5 min at 75 °C. Fmoc-AAs were coupled to the resin in this order: (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-methoxyphenyl)propanoic acid (Y01), (2S,3R)-2-(((9H-fluoren-9-yl)methoxy) carbonyl) amino)-3-(tert-butoxy)butanoic acid (Thr), (2S,3S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-(allyloxy)pyrrolidine-2-carboxylic acid (P42), (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-fluoro-1-((prop-2-yn-1-yl)-1H-indol-3-yl)propanoic acid (W18), (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(3-allylphenyl)propanoic acid, and acetic acid for capping.

[0394] The completed resin was cleaved by following the general cleavage protocol to afford the intermediate **Ex-03c**. LC/MS 1032.87 (M+1)⁺.

Step D - Synthesis of Intermediate Ex-03d

[0395] Intermediate **Ex-03b** (137 mg, 0.775 mmol), **Ex-03c** (200 mg, assume 0.194 mmol-not purified), HATU (295 mg, 0.775 mmol), and DIEA (200 mg, 1.550 mmol) were dissolved in 5 ml of DMF. After stirring at room temp for 30 min, the reaction was concentrated under high vacuum to afford intermediate **Ex-03d**, which was used directly in the next reaction without purification. LC/MS 1190.7 (M+1)⁺.

Step E - Synthesis of Intermediate Ex-03e

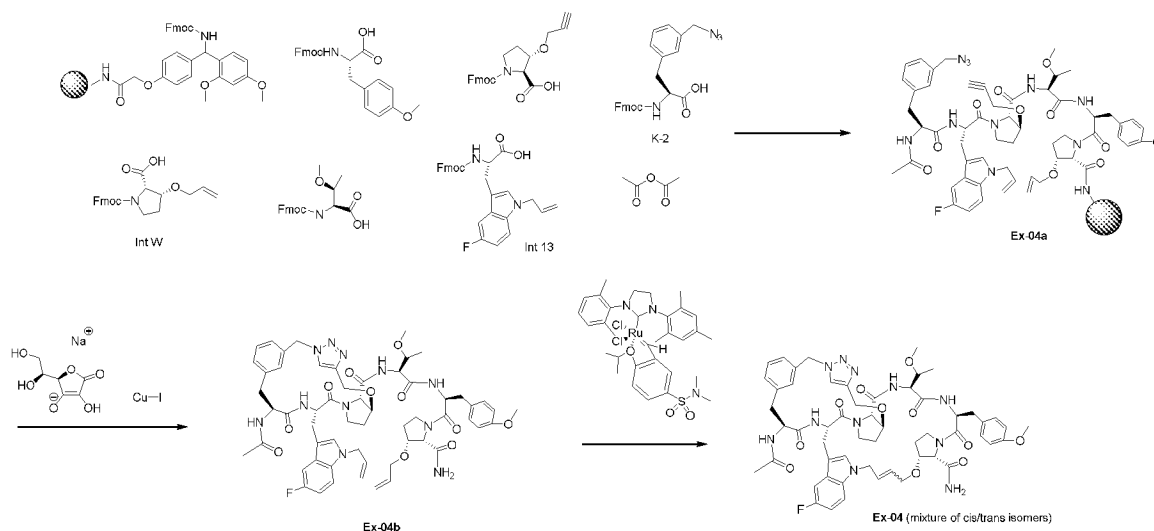
[0396] Intermediate **Ex-03d** (200 mg, assume 0.16 mmol) was dissolved in t-BuOH 50 ml and Water (50.0 ml) with some sonication. 1M NH₄HCO₃ was then added to adjust pH to 8-9 and it was bubbled with N₂. Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (17.83 mg, 0.034 mmol) and (R)-5-((S)-1,2-dihydroxyethyl)-3,4-dihydroxyfuran-2(5H)-one (29.6 mg, 0.168 mmol) and tetrakis(acetonitrile)copper(I)hexafluorophosphate (12.52 mg, 0.034 mmol) were then

- 5 added. After capped and stirring at 50 °C for 30 min. the reaction was concentrated to thick oily material, re-dissolved in water and EtOAc, and adjusted PH to 3-4. The reaction mixture was extracted 2x with EtOAc, washed orgs with brine. The combined organic phase was dried on Na₂SO₄, concentrated to an oily material, and purified on a flash column: size 24 gram, EtOAc: (3:1 EtOH: EtOAc) 0-80%, UV 215nm to afford intermediate **Ex-03e** as whitish material.
- 10 LC/MS 1190.7 (M+1)⁺.

Synthesis of Example Ex-03

[0397] Intermediate **Ex-03e** (100 mg, assume 0.1 mmol) was converted to **Ex-03** (32 mg) By following General RCM protocol. LC/MS 1161.7 (M+1)⁺.

15 Example 18: Synthesis of Example compound 04 (Ex-04)



Synthesis of peptidyl resin EX-04a

- [0398] Peptidyl resin **Ex-04a** was synthesized by standard SPPS using Fmoc/t-Bu chemistry.
- 20 The first residue Fmoc-(2S,3R)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-(allyloxy)pyrrolidine-2-carboxylic acid was coupled manually to Fmoc-MBHA-ProTide resin (0.19 mmol/g, CEM), using 2 eq of AA:2 eq HATU: 4 eq of DIEA, room temp. stirred for 2 hours, two times. Then, it was switched to a Liberty Blue to extend the sequence with the following AAs in order: (S)-2-
- 25 (((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-methoxyphenyl)propanoic acid, (2S,3S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxybutanoic acid, (2S,3S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-(prop-2-yn-1-yloxy)pyrrolidine-2-carboxylic acid, (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-allyl-5-fluoro-1H-indol-3-yl)propanoic acid, (S)-

- 5 (9H-fluoren-9-yl)methyl (1-(3-(azidomethyl)phenyl)-3-oxopropan-2-yl)carbamate, and acetic anhydride for capping. Acylation was performed by 4 eq of DIC/Oxymapure as activators. 0.1M DIEA was added to the Oxyma pure solution.

[0399] Completed resin was sampled and fully cleaved with 95% TFA :2.5% TIS: 2.5% water to afford the peptidyl resin **Ex-04a**. LC/MS 1102.3 (M+1)⁺.

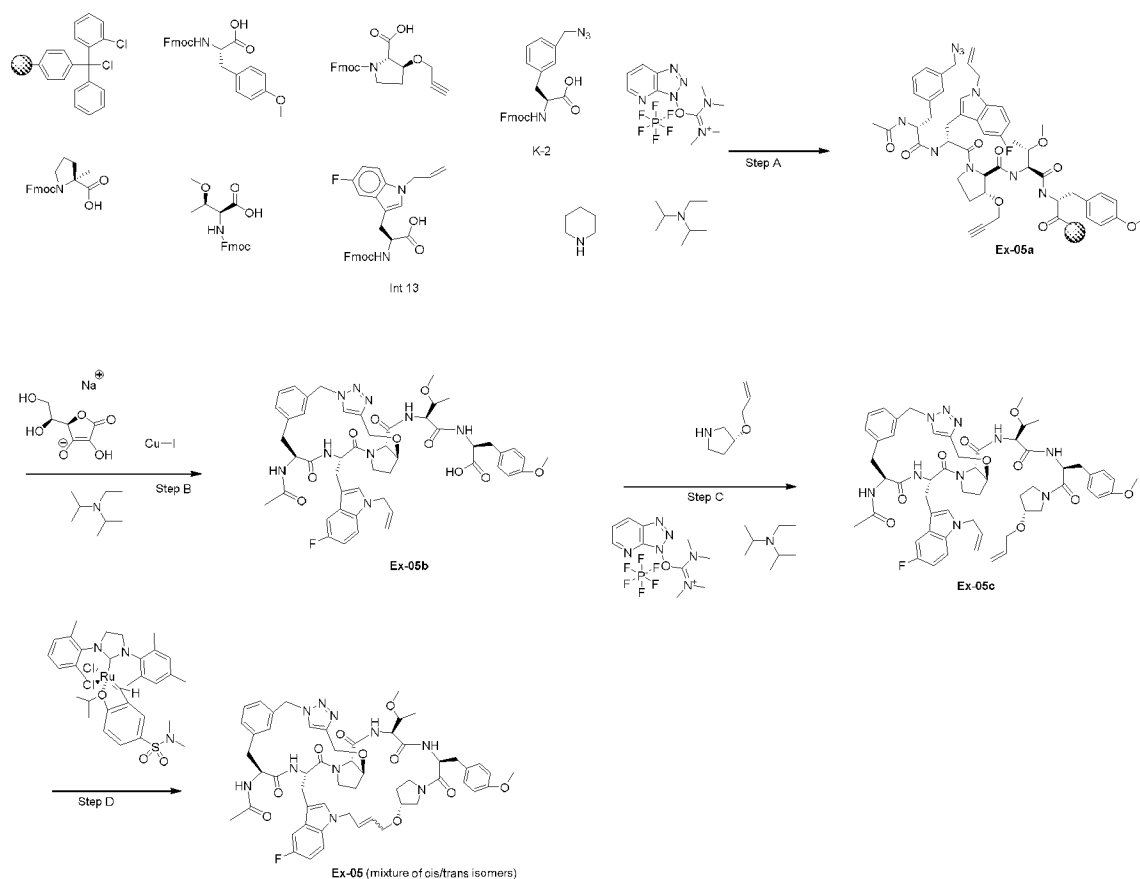
10 Synthesis of Intermediate **Ex-04b**

[0400] The above peptidyl resin **Ex-04a** (0.125 mmol) was converted to intermediate **Ex-04b** (120 mg) by following general on-resin click protocol, and general cleavage protocol. The crude material was used directly without purification in the next step. LC/MS 1102.7 (M+1)⁺.

Synthesis of Intermediate **Ex-04**

- 15 [0401] The above crude intermediate **Ex-04b** (100 mg) was added Zhan catalyst (26.2 mg, 0.036 mmol) and converted to afford Example **Ex-04** (7.8 mg) by following General RCM Protocol. LC/MS 1074.2 (M+1)⁺.

Example 19: Synthesis of Example compound 05 (Ex-05)



5 Synthesis of Intermediate **Ex-05a**

[0402] The peptidyl resin **Ex-05a** was synthesized using Fmoc-protected amino acids on a solid-phase 2-chlorotrityl chloride resin (0.16 mmol, 0.6 mmol/g, CreoSalus). Trityl resin was first loaded with 2 eq of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-methoxyphenyl)propanoic acid using and 4 eq DIEA and was heated at 50°C microwave for 10 min for 2 times. Then, the loaded resin was synthesized by standard SPPS using Fmoc/t-Bu chemistry on a CEM Liberty Blue automated peptide synthesizer (CEM Corp.). Reaction conditions were as follows: Deprotection Conditions: 20% piperidine (v/v) in DMF (2 X 2 min at 75 °C); Residue Coupling Conditions: 6 eq (relative to resin) of activated amino acid (5 mL of a 0.2 M amino acid stock solution in DMF) was delivered to the resin, followed by 6 eq of HATU activator (2 mL of a 0.45 M solution in DMF), and 12 eq of DIEA (1 mL of a 2M solution in NMP) and allowed to react for 5 min at 75 °C. Fmoc-AAs were coupled to the resin in this order: (2S,3S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxybutanoic acid, (2S,3S)-1-((((9H-fluoren-9-yl)methoxy)carbonyl)-3-(prop-2-yn-1-yloxy)pyrrolidine-2-carboxylic acid, (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-allyl-5-fluoro-1H-indol-3-yl)propanoic acid, (S)-(9H-fluoren-9-yl)methyl (1-(3-(azidomethyl)phenyl)-3-oxopropan-2-yl)carbamate, and acetic anhydride for capping.

[0403] Completed resin was sampled for cleavage in 95% TFA :2.5% TIS: 2.5% water to afford **Ex-05a**. LC/MS 950.2 (M+1)⁺.

Synthesis of Intermediate **Ex-05b**

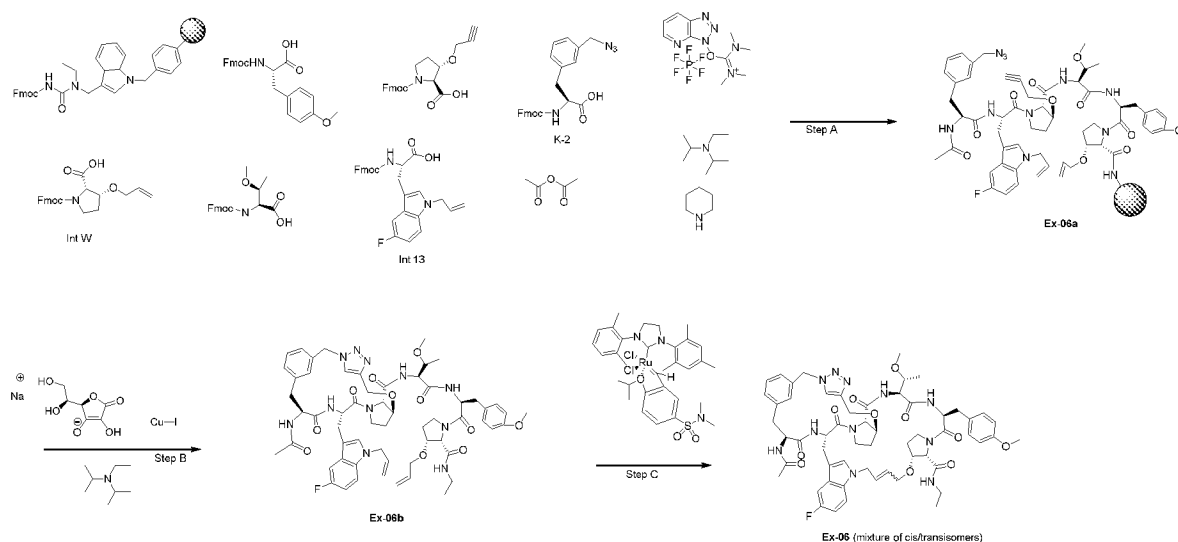
25 [0404] The above peptidyl resin **Ex-05a** (0.16 mmol) was converted to the intermediate **Ex-05b** (130 mg) as a dry powder by following general on-resin click protocol. The sample was cleaved by following cleavage protocol and was used directly without purification in the next step. LC/MS 950.2 (M+1)⁺.

Synthesis of Intermediate **Ex-05c**

30 [0405] Intermediate **Ex05b** (130 mg) was dissolved in 2 ml of DMF and (R)-3-(allyloxy)pyrrolidine (25.4 mg, 0.200 mmol) in 1 ml of DMF was then dissolved. Next 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (76 mg, 0.200 mmol) and N-ethyl-N-isopropylpropan-2-amine (25.8 mg, 0.200 mmol) were added to the mixture. After stirring at room temperature for 30 min, the reaction solvent was evaporated to dryness and then was purified on RP-HPLC to afford 16 mg of intermediate **Ex-05c**. LC/MS 1059.6 (M+1)⁺.

5 Synthesis of Example Ex-05

[0406] Example **Ex-05** (16 mg, 0.015 mmol) was synthesized with Zhan catalyst (6.53 mg, 9.06 μ mol) by following general RCM protocol to afford **Ex-05** (7.8 mg). LC/MS 1031.3 (M+1)⁺.

10 Example 20: Synthesis of Example compound 6 (Ex-06)Synthesis of Intermediate Ex-06a

[0407] The peptidyl resin **Ex-06a** was synthesized by standard SPPS using Fmoc/t-Bu chemistry on a CEM Liberty Blue automated peptide synthesizer (CEM Corp.) on Ethyl indole AM resin (0.1 mmol, Novabiochem, 0.73 mmol/g). Reaction conditions were as follows:

Deprotection Conditions: 20% piperidine (v/v) in DMF (2 X 2 min at 75 °C); Residue Coupling Conditions: 5 eq (relative to resin) of activated amino acid (5 mL of a 0.2 M amino acid stock solution in DMF) was delivered to the resin, followed by 4.5 eq of HATU activator (1 mL of a 0.45 M solution in DMF), and 10 eq of DIEA (0.5 mL of a 2M solution in NMP) and allowed to react for 2 min at 90 °C. Fmoc-AAs were coupled to the resin in this order: (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-methoxyphenyl)propanoic acid, (2S,3S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxybutanoic acid, (2S,3S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-(prop-2-yn-1-yloxy)pyrrolidine-2-carboxylic acid, (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-allyl-5-fluoro-1H-indol-3-yl)propanoic acid, (S)-(9H-fluoren-9-yl)methyl (1-(3-(azidomethyl)phenyl)-3-oxopropan-2-yl)carbamate, and acetic anhydride for capping.

- 5 [0408] Completed resin was sampled for cleavage in 95% TFA :2.5% TIS: 2.5% water to afford the title peptidyl resin **Ex-06a**. LC/MS 1130.3 (M+1)⁺.

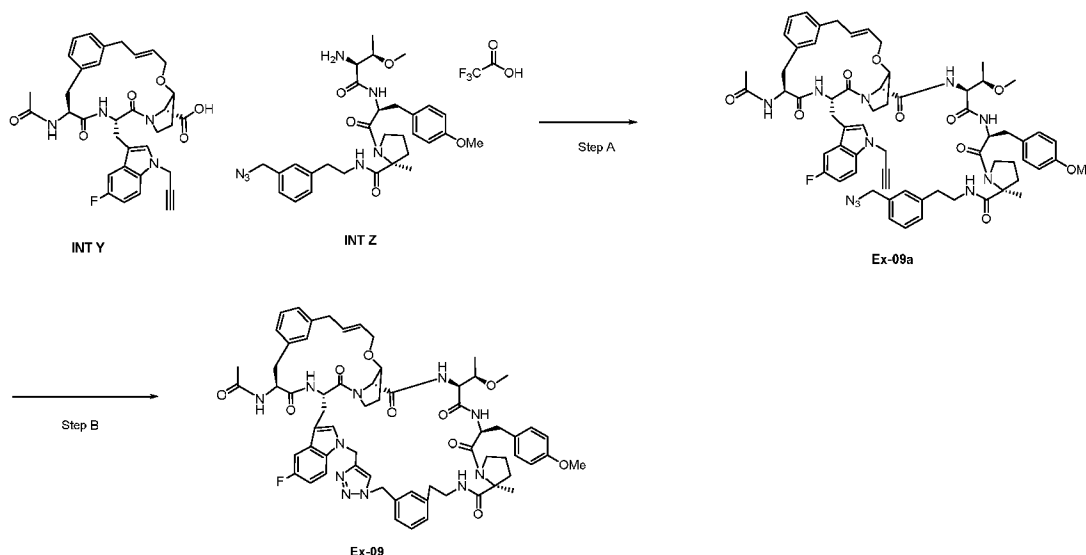
Synthesis of Intermediate Ex-06b

- [0409] The peptidyl resin **Ex-06a** (0.1 mmol) was converted to Compound **Ex-06b** by following general on-resin click protocol, and general cleavage protocol. The crude material was
10 purified by RP-HPLC to afford 16 mg of the intermediate **Ex-06b**. LC/MS 1130.3 (M+1)⁺.

Synthesis of Example Ex-06

- [0410] Intermediate **Ex-06b** (16 mg, 0.014 mmol) was converted to **Ex-06** (12 mg) by following general RCM protocol. LC/MS 1102.3 (M+1)⁺.

15 **Example 21: Synthesis of Example compound 9 (Ex-09)**

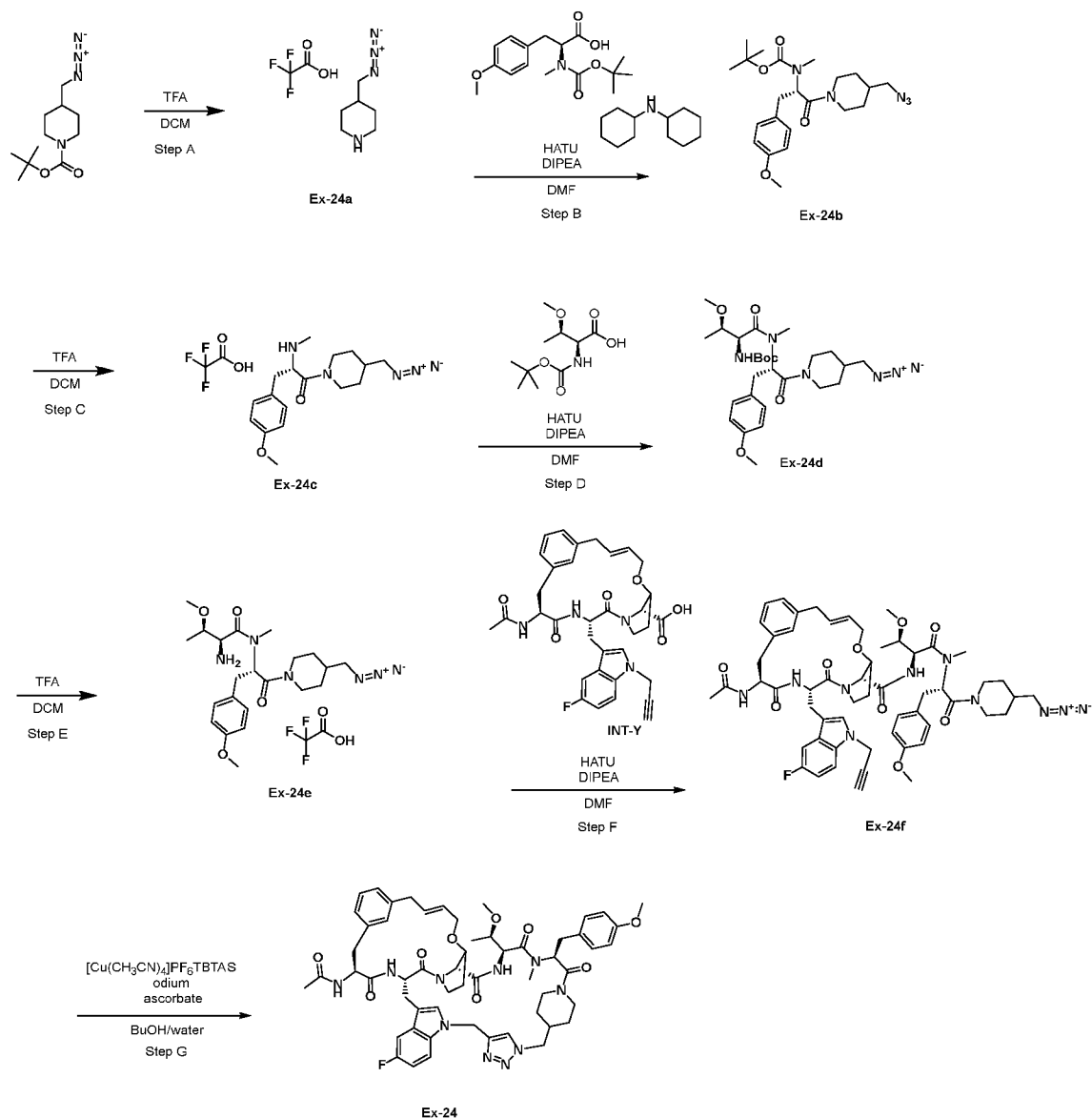


Step A - Synthesis of intermediate Ex-09a

- [0411] N-ethyl-N-isopropylpropan-2-amine (0.052 ml, 0.296 mmol) was added dropwise to
20 intermediate **Y** (50.5 mg, 0.082 mmol), intermediate **Z** (57 mg, 0.082 mmol), and HATU (37.5 mg, 0.099 mmol) in DMF (0.5 ml) at 0°C and the mixture was allowed to warm up to room temperature and stirred for 1h. The mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel 12g and
25 eluted with 0-10% MeOH/DCM to afford **EX-09a** as a solid.

5 Step B - Synthesis of Example Ex-09

[0412] Copper(II) sulfate (74.6 mg, 0.468 mmol) in Water (1 ml) was added dropwise to a mixture of intermediate **Ex-09a** and sodium (R)-5-((S)-1,2-dihydroxyethyl)-4-hydroxy-2-oxo-2,5-dihydrofuran-3-olate (185 mg, 0.935 mmol) in tBuOH (20 ml) and Water (10 ml) and the resulting reaction mixture was stirred at room temperature for 2h. The tBuOH was removed
10 under reduced pressure and sat. aq. ammonium chloride and EtOAc were added. The aqueous phase was separated and further extracted with EtOAc. The combined organic phase was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by high throughput purification to afford **Ex-09** as a solid.

5 **Example 22: Synthesis of Example compound 24 (Ex-24)****Step A - Synthesis of intermediate Ex-24a**

[0413] Tert-butyl 4-(azidomethyl)piperidine-1-carboxylate (363.4 mg, 1.512 mmol) was dissolved in DCM (5 mL) and TFA (2 mL, 26.0 mmol) was then added. After stirring at room temperature for 2 hours, the reaction solution was concentrated under reduced pressure to afford Ex-24a as an oil. LC/MS 140.94 (M+1)⁺.

5 Step B - Synthesis of intermediate **Ex-24b**

[0414] Boc-N-methyl-O-methyl-L-tyrosine dicyclohexylammonium salt (741 mg, 1.511 mmol) and HATU (574 mg, 1.511 mmol) were dissolved in DMF (8 mL) in a methanol/ice bath and **Ex-24a** (384 mg, 1.511 mmol) and Hunig's base (0.264 mL, 1.511 mmol) were then added. The reaction was allowed to warm to ambient overnight. The reaction crude was purified by reverse
10 phase chromatography (C18, 86 g cartridge), and eluted with an acetonitrile/water/0.1% v/v formic acid mixture (0% to 100%) to afford **Ex-24b**. LC/MS 454.33 (M+Na)⁺.

Step C - Synthesis of intermediate **Ex-24c**

[0415] Intermediate **Ex-24b** (343 mg, 0.795 mmol) was dissolved in DCM (5 mL) and TFA (0.061 mL, 0.795 mmol) was added. After stirring at room temperature for 2 hours, the reaction
15 solution was concentrated under reduced pressure to afford **Ex-24c** as an oil. LC/MS 333.33 (M+1)⁺.

Step D - Synthesis of intermediate **Ex-24d**

[0416] Boc-O-Methyl-L-threonine (199 mg, 0.852 mmol) and HATU (442 mg, 1.162 mmol) were dissolved in DMF (5 mL) in a methanol/ice bath. Next **Ex-24c** (345 mg, 0.775 mmol) and
20 Hunig's base (0.406 mL, 2.324 mmol) were added. After stirring overnight at room temperature, the reaction crude was purified by reverse phase chromatography (C18, 86 g cartridge), and eluted with an acetonitrile/water/0.1% v/v formic acid mixture (0% to 73%) to afford **Ex-24d**. LC/MS 569.28 (M+Na)⁺.

Step E - Synthesis of intermediate **Ex-24e**

25 [0417] Intermediate **Ex-24d** (345 mg, 0.631 mmol) was dissolved in CH₂Cl₂ (5 mL) and TFA (3 mL, 38.9 mmol) was added. After stirring at room temperature for 2 h, the reaction solution was concentrated under reduced pressure to afford **Ex-24e** as an oil. LC/MS 447.41 (M+H)⁺.

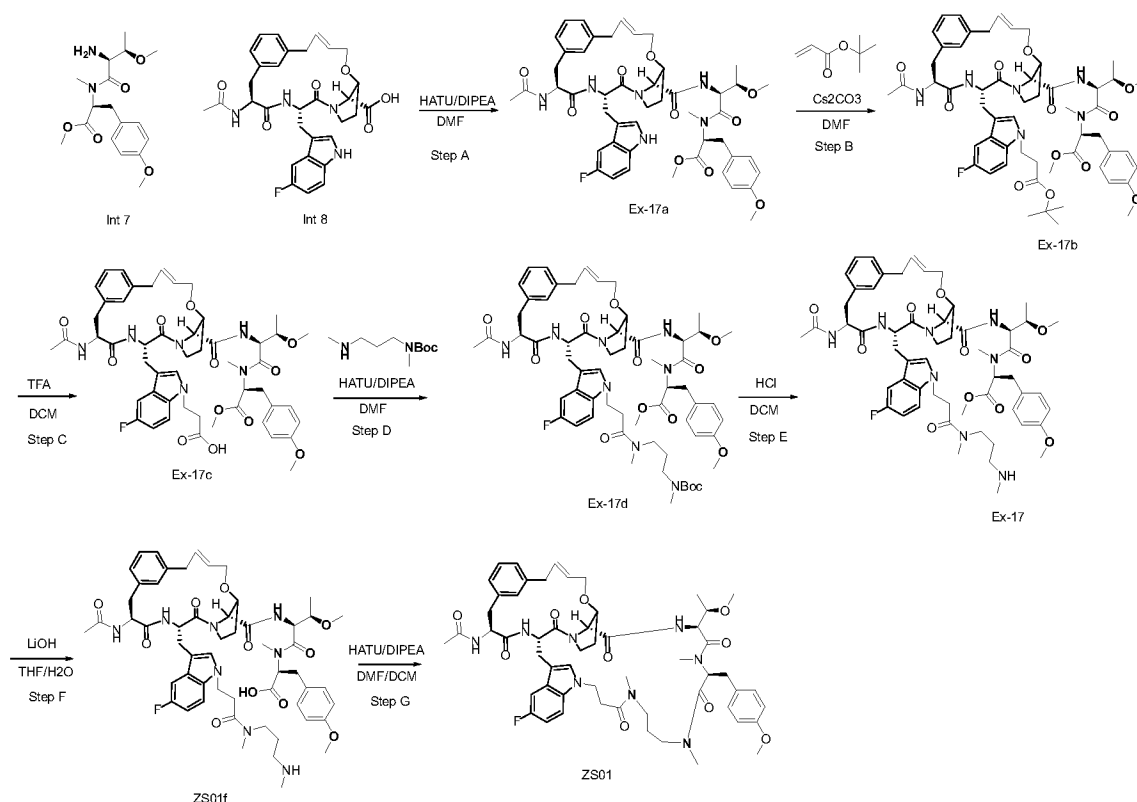
Step F - Synthesis of intermediate **Ex-24f**

[0418] Intermediate **Y** (94 mg, 0.153 mmol) and HATU (116 mg, 0.306 mmol) were dissolved
30 in DMF (3 mL) in a methanol/ice bath and intermediate **Ex-24e** and Hunig's Base (0.080 mL, 0.459 mmol) were then added. After stirring at room temperature overnight, the reaction crude was purified by reverse phase chromatography (C18, 43 g cartridge), and eluted with an acetonitrile/water/0.1% v/v formic acid mixture (0% to 100 %) to afford **Ex-24f**. LC/MS 1065.65 (M+Na)⁺.

5 Step G - Synthesis of intermediate **Ex-24**

[0419] Tetrakis(acetonitrile)copper(I) hexafluorophosphate (10.18 mg, 0.027 mmol), tris[(1-benzyl-1h-1,2,3-triazol-4-yl)methyl]amine (14.50 mg, 0.027 mmol) and sodium ascorbate (27.1 mg, 0.137 mmol) in BuOH (2 mL)/water (1.000 mL) were bubbled with N₂ and then placed in a 50 °C oil bath. **Ex-24f** (57 mg, 0.055 mmol) in DCM solution (1 mL) was added into the

10 reaction. The reaction was stirred for 1 h at 50 °C. The reaction crude was evaporated in vacuo. The residue was purified by reverse phase chromatography (C18, 43 g cartridge), and eluted with an acetonitrile/water/0.1% v/v formic acid mixture (0% to 50%) to afford **Example Ex-24**. LC/MS 1065.63 (M+Na)⁺.

15 Example 23: Synthesis of Example compound 17 (Ex-17)Step A - Synthesis of intermediate **Ex-17a**

[0420] To the solution of intermediate **Int 7** (80 mg, 0.213 mmol) and **Int 8** (123 mg, 0.213 mmol) in DMF (2ml) at room temperature was added HATU (85 mg, 0.224 mmol) and DIPEA (0.149 ml, 0.854 mmol). After stirring at room temperature for 1 hour, the reaction mixture was

20 diluted with EtOAc, washed with H₂O, the organic phase was dried over Na₂SO₄, concentrated

- 5 and the residue was purified by flash column chromatography on silica gel (ISCO 40 g column, and eluted with MeOH/DCM(0-20% MeOH in DCM) to afford **Ex-17a**. LC/MS 897.4 (M+H)⁺.

Step B - Synthesis of intermediate Ex-17b

- [0421] Intermediate **Ex-17a** (170 mg, 0.190 mmol) and cesium carbonate (185 mg, 0.569 mmol) were dissolved in DMF (2 ml) and tert-butyl acrylate (0.110 ml, 0.758 mmol) was then
10 added. After stirring at 50°C for 2 hours, the reaction mixture was diluted with EtOAc and washed with water, the organic phase was dried over Na₂SO₄, concentrated and the residue was purified by flash column chromatography on silica gel (ISCO 40 g column, and eluted with MeOH/DCM(0-10% MeOH in DCM) to afford intermediate **Ex-17b**. LC/MS 1025.43 (M+1)⁺.

Step C - Synthesis of intermediate Ex-17c

- 15 [0422] Intermediate **Ex-17b** (160 mg, 0.156 mmol) was dissolved in DCM (1.5 ml) and TFA (0.481 ml, 6.24 mmol) was then added. After stirring for 2 hours, the reaction solution was concentrated under reduced pressure and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield product **Ex-17c**. LC/MS 969.4 (M+H)⁺.

20 Step D - Synthesis of intermediate Ex-17d

- [0423] To the solution of intermediate **Ex-17c** (35 mg, 0.036 mmol) and tert-butyl methyl(3-(methylamino)propyl)carbamate (7.67 mg, 0.038 mmol) in DMF (0.5ml) at room temperature was added HATU (14.42 mg, 0.038 mmol) and DIPEA (0.025 ml, 0.144 mmol). After stirring at room temperature for 2 hours, the reaction mixture was diluted with EtOAc and washed with
25 water. the organic phase was dried over Na₂SO₄, concentrated and the residue was purified by flash column chromatography on silica gel (ISCO 24 g column, and eluted with MeOH/DCM (0-20% MeOH in DCM) to afford intermediate **Ex-17d**. LC/MS 1154.08 (M+H)⁺.

Step E - Synthesis of intermediate Ex-17e

- [0424] Intermediate **Ex-17d** (38 mg, 0.033 mmol) was dissolved in DCM (0.4ml) and HCl (4M in dioxane) (0.329 ml, 1.318 mmol) was then added. After stirring at room temperature for 2
30 hours, the reaction mixture was concentrated to afford intermediate **Ex-17e**. LC/MS 1054.03 (M+H)⁺.

Step F - Synthesis of intermediate Ex-17f

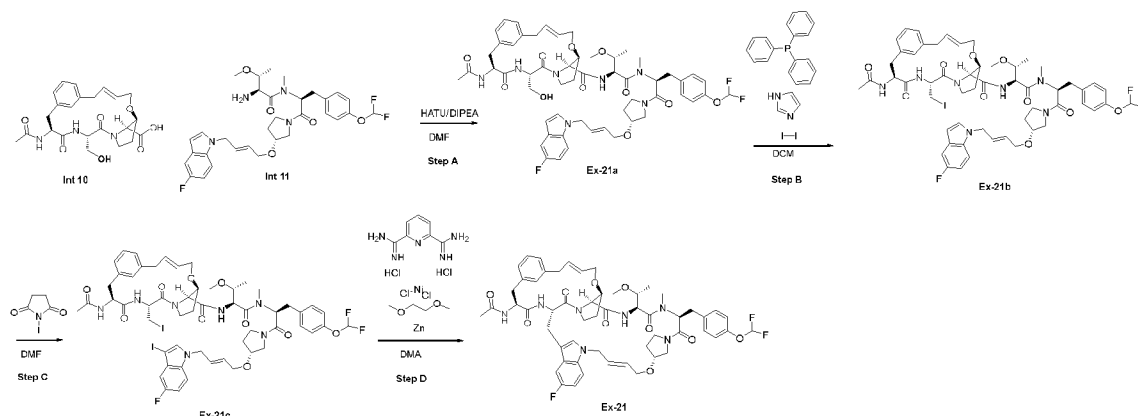
- [0425] Intermediate **Ex-17e** (36.0 mg, 0.033 mmol) was dissolved in THF (0.4ml) and LiOH
35 (0.330 ml, 0.330 mmol) was then added. After stirring at room temperature for 2 hours, the solvent was removed under reduced pressure and the residue was purified by preparative HPLC

- 5 reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield **Ex-17f**. LC/MS 1039.99 (M+1)⁺.

Step G - Synthesis of Example Ex-17

- [0426] To the solution of intermediate **Ex-17f** (20 mg, 0.018 mmol) in DMF (2.5 ml) and DCM (30 ml) was added HATU (7.03 mg, 0.018 mmol) and DIPEA (0.015 ml, 0.088 mmol). The
10 resulting solution was stirred at RT for 2 hours. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (20-60% Acetonitrile in water) to afford **Ex-17**. LC/MS 1021.37 (M+1)⁺.

15 Example 24: Synthesis of Example compound 21 (Ex-21)



Step A - Synthesis of intermediate Ex-21a

- [0427] To the solution of **Int 10** (11.48 mg, 0.025 mmol) and **Int 11** (17 mg, 0.024 mmol) in DMF (0.4 ml) was added DIPEA (0.021 ml, 0.119 mmol) and HATU (9.50 mg, 0.025 mmol) at
20 room temperature. After stirring for 1 hour, the crude mixture was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to afford intermediate **Ex-21a**. LC/MS 1058.37 (M+1)⁺.

Step B - Synthesis of intermediate Ex-21b

- [0428] To a stirred solution of **Ex-21a** (15 mg, 0.014 mmol) in DCM (0.2ml) was added
25 imidazole (3.86 mg, 0.057 mmol), triphenylphosphine (13.87 mg, 0.053 mmol), and iodine (11.51 mg, 0.045 mmol) at room temperature. After stirring at room temperature for 2 hours, the mixture was directly loaded on silica gel column (ISCO 24 g column and eluted with MeOH/DCM (0-10 % MeOH in DCM) to afford intermediate **Ex-21b** as a solid. LC/MS 1168.21 (M+1)⁺.

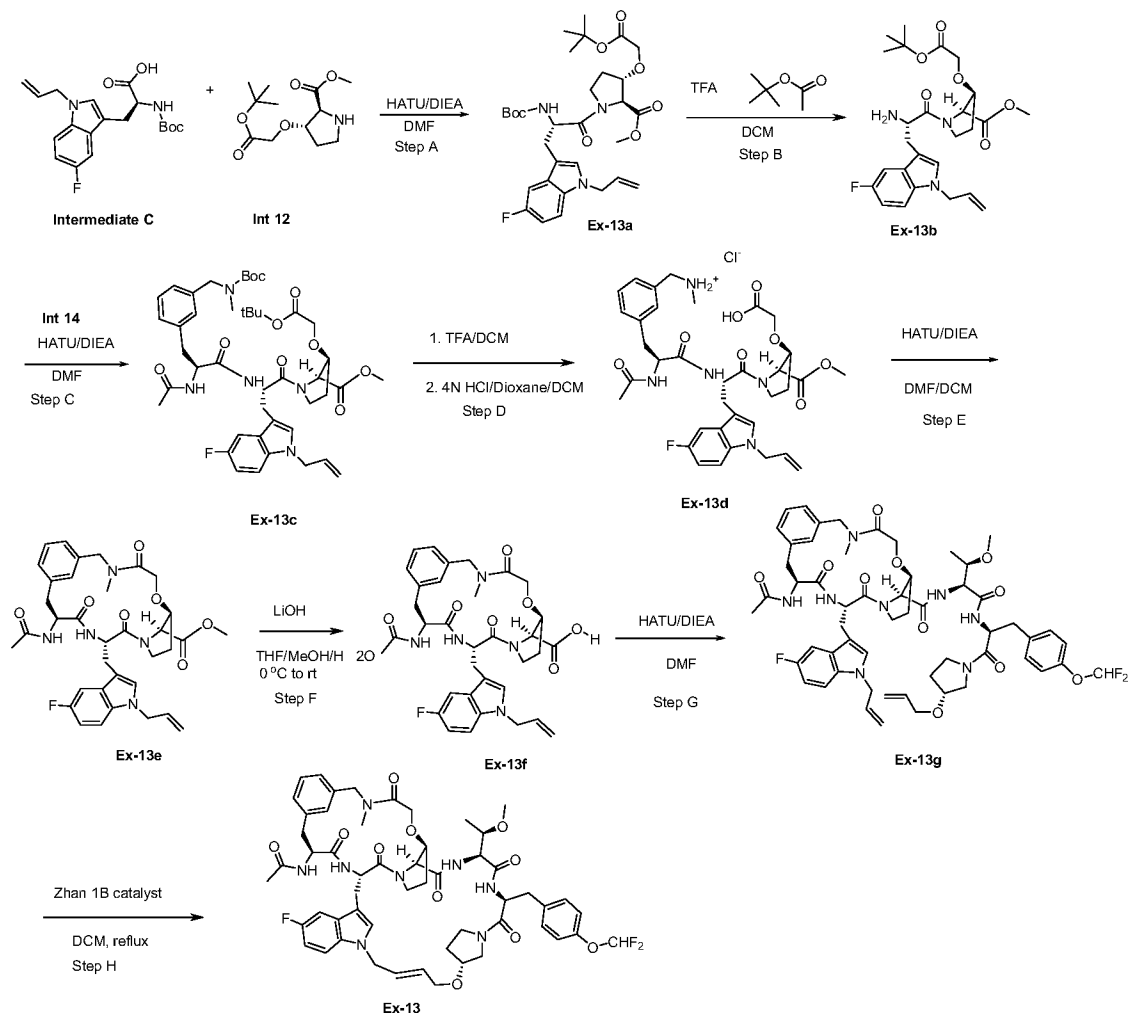
5 Step C - Synthesis of intermediate **Ex-21c**

[0429] To a stirred solution of **Ex-21b** (14mg, 0.012 mmol) in DMF (0.3 ml) was added N-iodosuccinimide (3.51 mg, 0.016 mmol) at 0 °C under nitrogen atmosphere. The reaction solution was stirred at 0 °C for 30 min. The reaction was quenched with water (5mL), extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (5 mL),
10 dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography silica gel (ISCO 24 g column, and eluted with MeOH/DCM (0-15% MeOH in DCM)) to afford **Ex-21c** as a solid. LC/MS 1294.4 (M+1)⁺.

Step D - Synthesis of **Example Ex-21**

15 [0430] To a vial was added intermediate **Ex-21c** (5.7 mg, 4.41 μmol), nickel (II) chloride ethylene glycol dimethyl ether complex (1.936 mg, 8.81 μmol) and pyridine-2,6-bis(carboximidamide) dihydrochloride (2.080 mg, 8.81 μmol), then followed by DMA (176 μl) and ZINC (1.267 mg, 0.019 mmol) in glovebox. The reaction solution was stirred at 45°C for 20h in the glovebox. The reaction mixture was concentrated under reduced pressure, and the
20 residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to afford **Ex-21**. LC/MS 1040.5 (M+1)⁺.

5 **Example 25: Synthesis of Example compound 13 (Ex-13)**



Step A: Synthesis of intermediate Ex-13a

[0431] To the solution of **Intermediate C** (1.297 g, 3.58 mmol) and **Int 12** (1.021 g, 3.94 mmol) in DMF (30 ml) was added HATU (1.497 g, 3.94 mmol) and DIEA (1.251 ml, 7.16 mmol). After stirring at rt for 1h, the mixture was partitioned between EtOAc (200 mL) and brine (100 mL). The organic phase was washed with brine (3x100 mL), dried over Na₂SO₄, concentrated and the residue was purified on silica gel column using 10-50%EtOAc/hexane as and eluted solvents to **Ex-13a** as a foamy solid. LC/MS 604.2 (M+1)⁺.

15 Step B: Synthesis of Ex-13b

[0432] To the solution of **Ex-13a** (0.55 g, 0.911 mmol) in DCM (4 ml) was added tert-butyl acetate (2 ml, 14.98 mmol), and TFA (3 ml, 38.9 mmol). After stirring at rt for 2h, it was then concentrated, and the residue was purified on reverse phase MPLC (C18, 86g) using 5-60-100-

5 100%acetonitrile (0.05%TFA) as gradient to give **Ex-13b (TFA salt)** as a powder. LC/MS 504.2 (M+1)⁺. To the solution of **Ex-13b** (0.53 g, 0.858 mmol) in Acetonitrile (100 ml) and Water (50 ml) at 0 °C was added HCl (17.16 ml, 1.716 mmol) dropwise. After stirring at 0 °C for 2min, it was frozen and lyophilized to give **Ex-13b (hydrochloride salt)** as a powder. LC/MS 504.2 (M+1)⁺.

10 Step C: Synthesis of **Ex-13c**

[0433] To the solution of **Int 14** (0.316 g, 0.901 mmol) and **Ex-13a** (0.463 g, 0.858 mmol) in DMF (8 ml) at 0 °C was added HATU (0.343 g, 0.901 mmol) and DIEA (0.599 ml, 3.43 mmol). After stirring at 0 °C for 40min, it was purified on reverse phase MPLC (275g) using 10-100% acetonitrile (0.05%TFA) to afford **Ex-13c** as a powder. LC/MS 836.4 (M+1)⁺.

15 Step D: Synthesis of **Ex-13d**

[0434] Intermediate **Ex-13c** (0.64 g, 0.766 mmol) was dissolved in CH₂Cl₂ (6 ml) and TFA (6 ml, 78 mmol) was then added. After stirring at rt for 1h, it was concentrated and the residue was dissolved in DCM (10 mL) and toluene (6 mL), then concentrated again. The resulting residue was redissolved in DCM (10 mL) and treated with HCl (0.957 ml, 3.83 mmol), then concentrated
20 to afford **Ex-13d (hydrochloride salt)** as an oil. LC/MS 680.3 (M+1)⁺.

Step E: Synthesis of **Ex-13e**

[0435] To the solution of **Ex-13d** (549 mg, 0.766 mmol) in DMF (25 ml) and CH₂Cl₂ (500 ml) was added HATU (350 mg, 0.919 mmol), followed by addition of DIEA (0.803 ml, 4.60 mmol) dropwise. After stirring at rt for 1h, the reaction solution was concentrated and the residue was
25 purified on reverse phase MPLC (C18, 275 g column), using 0-60%acetonitrile (0.05%TFA) as and eluted solvents to afford **Ex-13e** as a powder. LC/MS 662.2 (M+1)⁺.

Step F: Synthesis of **Ex-13f**

[0436] To the solution of **Ex-13e** (383 mg, 0.579 mmol) in THF (6 ml), Water (2 ml), and MeOH (2 ml) at 0 °C was added LiOH (1.736 ml, 1.736 mmol) dropwise. After stirring at 0 °C
30 for 4h, then rt for 1h, the volatile was evaporated, and the aqueous phase was acidified to pH 3, extracted with 30%Isopropanol/DCM (3x100 mL). The combined organic phase was dried over Na₂SO₄, concentrated and the residue was lyophilized from acetonitrile/water (2:1, 30 mL) to **Ex-13f** as a powder. LC/MS 648.3 (M+1)⁺.

Step G: Synthesis of **Ex-13g**

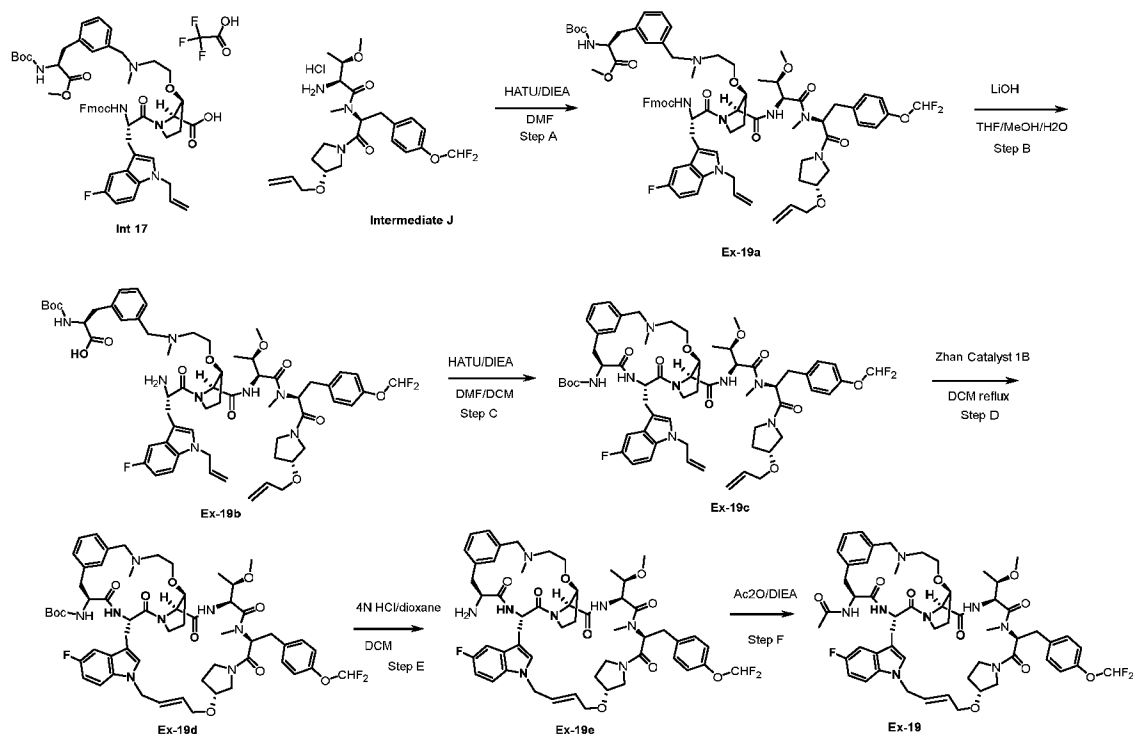
35 [0437] To the solution of **Ex-13f** (50.1 mg, 0.102 mmol) in DMF (3 ml) at 0 °C was added HATU (38.7 mg, 0.102 mmol) and DIEA (0.065 ml, 0.371 mmol). After stirring at 0 °C for 1h,

- 5 the reaction solution was purified on reverse MPLC (C18, 86g) using 5-80% acetonitrile (0.05%TFA) as and eluted solvents to afford **Ex-13g** as a powder. LC/MS 1085.2 (M+1)⁺.

Step H: Synthesis of Example Ex-13

- [0438] The solution of **Ex-13g** (84 mg, 0.077 mmol) in CH₂Cl₂ (140 ml) was bubbled with N₂ for 30min, then was added ZHAN CATALYST-1B (28.4 mg, 0.039 mmol). Next the resulting
 10 solution was further bubbled with N₂ for 30min, then heated at 50 °C for 5h. It was then concentrated, and the residue was purified on Gilson using 5-75%acetonitrile (0.05%TFA), and the product was repurified on silica gel column (40 g) using 0-10%MeOH/DCM as and eluted solvents to **Ex-13** as a solid. LC/MS 1057.2 (M+1)⁺.

15 **Example 26: Synthesis of Example compound (Ex-19)**



Step A: Synthesis of Ex-19

- [0439] To the solution of **Int 17** (trifluoroacetic acid 1:1) (275 mg, 0.259 mmol) and
 20 **Intermediate J** (138 mg, 0.272 mmol) in DMF (4 ml) at 0 °C was added HATU (104 mg, 0.272 mmol) and DIEA (0.272 ml, 1.556 mmol). After stirring at 0 °C for 45 min, the reaction solution was partitioned between EtOAc (200 mL) and brine (100 mL) and then washed with sat. Na₂CO₃ (50 mL). The organic phase was washed with brine (2x100 mL), dried over Na₂SO₄,

5 concentrated and the residue was purified on silica gel column (80g) using 0-10%MeOH/DCM as and eluted solvents to afford **Ex-19a** as an oil. LC/MS 1398.5 (M+1)⁺.

Step B: Synthesis **Ex-19b**

[0440] To the solution of **Ex-19a** (363 mg, 0.260 mmol) in THF (9 ml), MeOH (3 ml), and Water (3 ml) at 0 °C was added LiOH (2.078 ml, 2.078 mmol) dropwise. After stirring at 0°C for
10 2h, the reaction solution was neutralized by addition of 1N HCl (1.8 mL). Then the volatile was evaporated, and the aqueous phase was acidified to pH 5, then lyophilized to **Ex-19b** as a crude solid. LC/MS 1161.4 (M+1)⁺.

Step C: Synthesis of **Ex-19c**

[0441] To the solution of **Ex-19b** (302 mg, 0.260 mmol) in DMF (25 ml) and CH₂Cl₂ (500 ml)
15 was added DIEA (0.273 ml, 1.560 mmol) and HATU (94 mg, 0.247 mmol). After stirring at rt for 2h, the volatile was evaporated and the DMF solution was partitioned between EtOAc (200 mL) and brine (100 mL) and sat. Na₂CO₃ (50 mL). The organic phase was further washed with brine (2x200 mL), dried over Na₂SO₄, concentrated and the residue was purified on silica gel column using 0-20%MeOH/DCM as and eluted solvents to afford **Ex-19c** as a solid. LC/MS
20 1143.4 (M+1)⁺.

Step D: Synthesis of **Ex-19d**

[0442] To the solution of **Ex-19c** (195 mg, 0.171 mmol) in CH₂Cl₂ (300 ml) was bubbled with N₂ for 30min, then was added ZHAN CATALYST-1B (62.6 mg, 0.085 mmol). The resulting solution was further bubbled with N₂ for 30min, then heated at 50 °C for 5.5h. It was then
25 concentrated, and the residue was partitioned between DCM (200 mL) and sat. Na₂CO₃ (50 mL) and brine (100 mL). The aqueous phase was extracted with DCM (2x100 mL) and the combined organic phase was dried over Na₂SO₄, concentrated and the residue was purified on silica gel column using 0-20%%MeOH/DCM as and eluted solvents to afford **Ex-19d** as a solid. LC/MS 1115.4 (M+1)⁺.

30 Step E: Synthesis of **Ex-19e**

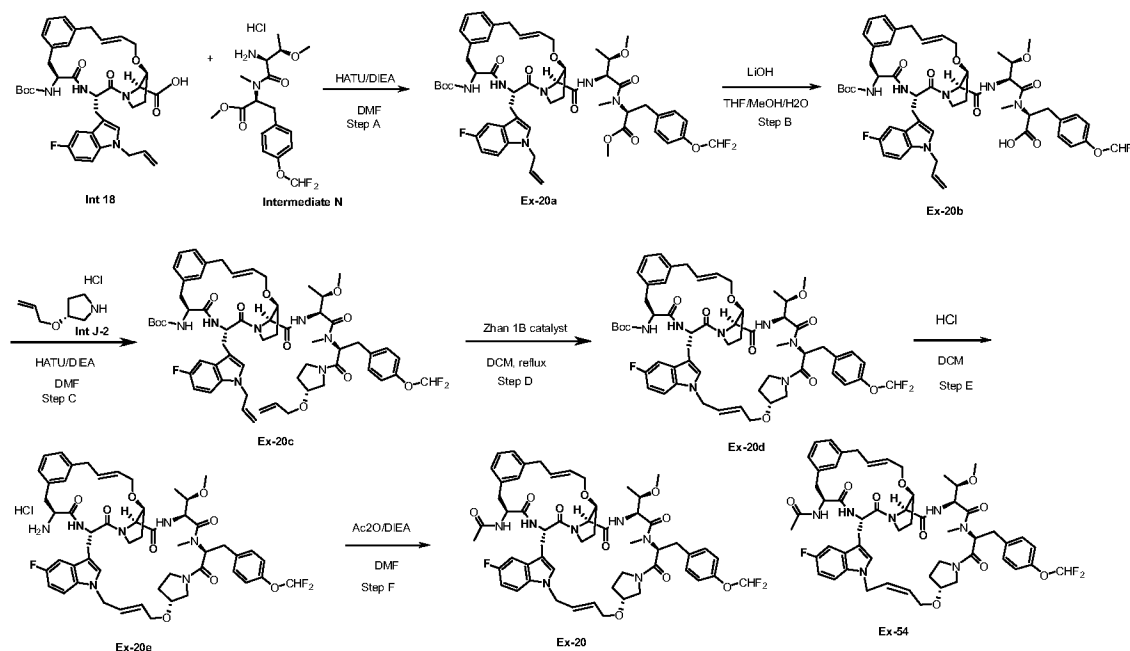
[0443] To the solution of **Ex-19e** (190 mg, 0.170 mmol) in CH₂Cl₂ (4 ml) was added HCl (4N in dioxane) (1.278 ml, 5.11 mmol). After stirring at rt for 2h, the reaction solution was concentrated and the residue was dissolved in acetonitrile/water (10 mL, 2:1) to afford **Ex-19e** as a solid. LC/MS 1015.9 (M+1)⁺.

5 Step F: Synthesis of Ex-19

[0444] To the solution of **Ex-19e** (48.8 mg, 0.045 mmol) in DMF (1 ml) was added ACETIC ANHYDRIDE (8.46 μ l, 0.090 mmol) and DIEA (0.047 ml, 0.269 mmol). After stirring at rt for 30min, the reaction solution was then purified on Gilson (C18 column) using 25-43%acetonitrile (0.05%TFA) as and eluted solvents to afford **Ex-19** as a powder. LC/MS 1057.8 (M+1)⁺.

10

Example 27: Synthesis of Example compounds 20 and 54 (Ex-20 and Ex-54)



Step A: Synthesis of Ex-20a

[0445] To the solution of **Int 18** (520 mg, 0.771 mmol) and **Intermediate N** (380 mg, 0.925 mmol) in DMF (12 ml) at 0 °C was added 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate (322 mg, 0.848 mmol) and DIEA (0.942 ml, 5.39 mmol). After stirring at 0 °C for 3h, the reaction solution was partitioned between EtOAc (500 mL) and water (300 mL), the organic phase was washed with brine (2x300 mL), dried over Na₂SO₄, concentrated and the residue was purified on silica gel column using 0-5%MeOH/DCM as and eluted solvents to afford **Ex-20a** as a solid. LC/MS 1031.8 (M+1)⁺.

20

Step B: Synthesis of Ex-20b

[0446] To the solution of **Ex-20a** (883 mg, 0.856 mmol) in THF (21 ml), MeOH (7.00 ml), and Water (7 ml) at 0 °C was added LiOH (3.43 ml, 3.43 mmol). After stirring at 0 °C for 2h, the volatile was evaporated, and the aqueous phase was acidified to pH 4. The mixture was extracted

5 with DCM (3x100 mL). The combined organic phase was dried over Na₂SO₄, concentrated to afford **Ex-20b** as a solid. LC/MS 1017.8 (M+1)⁺.

Step C: Synthesis of Ex-20c

[0447] To the solution of **Intermediate J-2** (38.6 mg, 0.236 mmol) and **Ex-20b** (200 mg, 0.197 mmol) in DMF (4 ml) at 0 °C was added HATU (90 mg, 0.236 mmol) and DIEA (0.275 ml,
10 1.573 mmol). After stirring at 0 °C for 30min, it was purified on reverse phase MPLC (C18, 86 g) using 5-100%acetonitrile (0.05%TFA) as and eluted solvents to afford **Ex-20c** as a powder. LC/MS 1126.9 (M+1)⁺.

Step D: Synthesis of Ex-20d

[0448] The solution of **Ex-20c** (180 mg, 0.160 mmol) in CH₂Cl₂ (400 ml) was bubbled with
15 N₂ for 1h, then was added ZHAN CATALYST-1B (58.6 mg, 0.080 mmol). The resulting solution was further bubbled with N₂ for 30min, then heated at 50 °C for 3h. It was then quenched by addition of vinyl ethyl ether (1 mL). After stirring at rt open to air for 10min, it was concentrated, and the residue was purified on silica gel column using 0-10%MeOH/DCM as and eluted solvents to afford **Ex-20d** as a solid. LC/MS 1098.8 (M+1)⁺.

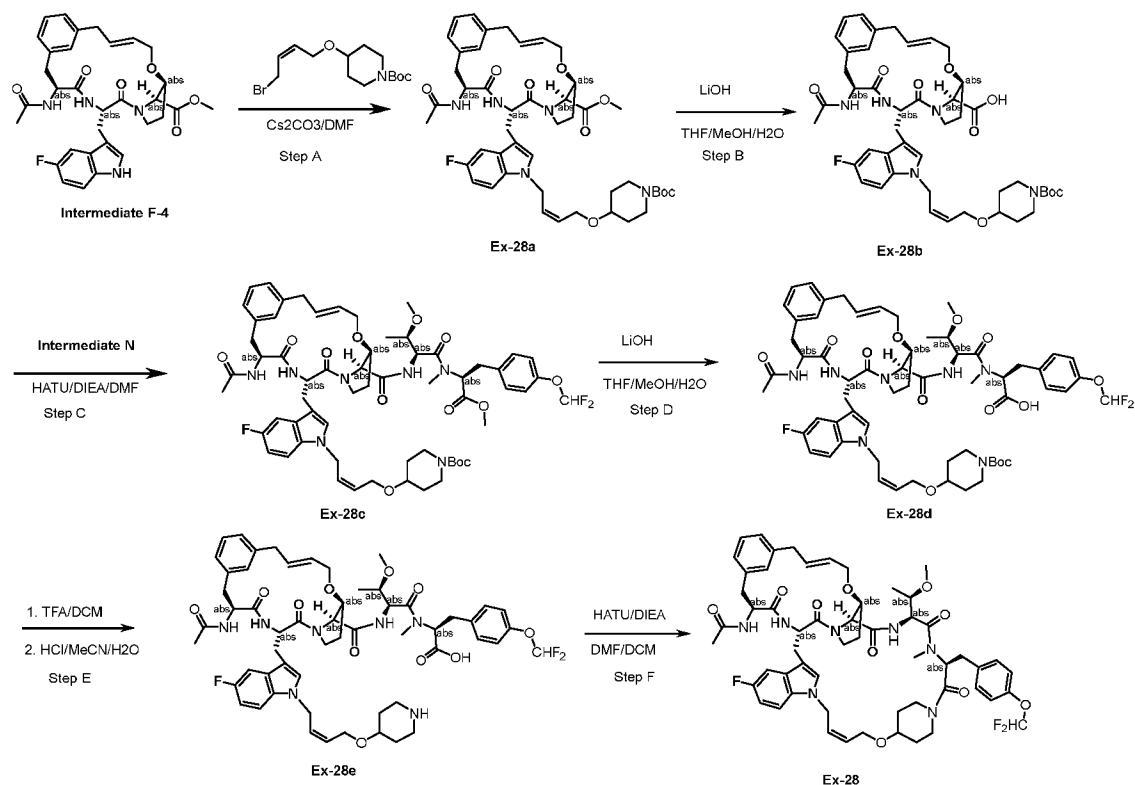
20 Step E: Synthesis of Ex-20e

[0449] **Intermediate Ex-20d** (143 mg, 0.130 mmol) was dissolved in CH₂Cl₂ (4 ml) and HCl (4N in dioxane) (1.302 ml, 5.21 mmol) was then added. After stirring at rt for 2.5h, it was then concentrated, and the residue was dissolved in 15 mL acetonitrile/water (1:1) and lyophilized to **Ex-20e** as a solid. LC/MS 998.8 (M+1)⁺.

25 Step F: Synthesis of Ex-20 and Ex-54

[0450] To the solution of **Ex-20e** (29 mg, 0.028 mmol) in DMF (1 ml) was added DIEA (0.029 ml, 0.168 mmol) and acetic anhydride (5.29 µl, 0.056 mmol), the resulting solution was stirred at rt for 30min, then purified on reverse phase Gilson C18 using 35-64% acetonitrile(0.05%TFA)/water(0.05%TFA) as and eluted solvents to give **Ex-20** as a powder
30 LC/MS 1040.7 (M+1)⁺; and slow eluant **Ex-54**. LC/MS 1040.8 (M+1)⁺.

5 **Example 28: Synthesis of Example compound 28 (Ex-28)**



Step A: Synthesis of Ex-28a

[0451] To the solution of **Int 21** and **Intermediate F-4** (100 mg, 0.169 mmol) in DMF (1 ml) was added Cs_2CO_3 (165 mg, 0.508 mmol). After stirring at rt overnight, the mixture was partitioned between EtOAc (150 mL) and water (100 mL). The organic phase was further washed with water (2x100 mL), dried over Na_2SO_4 , concentrated and the residue was purified on silica gel column using 0-10% MeOH/DCM as and eluted solvents to afford **Ex-28a** as an oil. LC/MS 844.8 (M+1)⁺.

Step B: Synthesis of Ex-28b

[0452] To the solution of **Ex-28a** (136 mg, 0.161 mmol) in THF (3 ml), MeOH (1 ml), and Water (1 ml) at 0 °C was added LiOH (0.500 ml, 0.500 mmol) dropwise. After stirring at 0 °C for 3h, the volatile was evaporated and the mixture was acidified to pH 3-4, extracted with DCM (3x70 mL). The combined organic phase was dried over Na_2SO_4 , concentrated to afford **Ex-28b** as an oil. LC/MS 830.7 (M+1)⁺.

5 Step C: Synthesis of Ex-28c

[0453] To the solution of **Ex-28b** (60.6 mg, 0.073 mmol) **Intermediate N** (30 mg, 0.073 mmol) in DMF (2 ml) was added HATU (28.3 mg, 0.074 mmol) and DIEA (0.077 ml, 0.438 mmol). After stirring at rt for 45min, the mixture was partitioned between EtOAc (150 mL) and brine (100 mL). The organic phase was washed with brine (2x100 mL), dried over MgSO₄,
10 concentrated and the residue was purified on silica gel column (40g) using 0-10%MeOH/DCM as and eluted solvents to afford **Ex-28c** as an oil. LC/MS 1186.9 (M+1)⁺.

Step D: Synthesis of Ex-28d

[0454] To the solution of **Ex-28c** (73.8 mg, 0.062 mmol) in THF (6 ml), MeOH (2 ml), and Water (2 ml) at 0 °C was added LiOH (0.249 ml, 0.249 mmol) dropwise. After at 0 °C for 2h, the
15 volatile was evaporated on rotary evaporator. Next the aqueous phase was acidified to pH 3-4, extracted with DCM (2x50 mL), then 10%IPA/DCM (2x50mL). The combined organic phase was dried over MgSO₄, concentrated to afford **Ex-28d** as a solid. LC/MS 1172.9 (M+1)⁺.

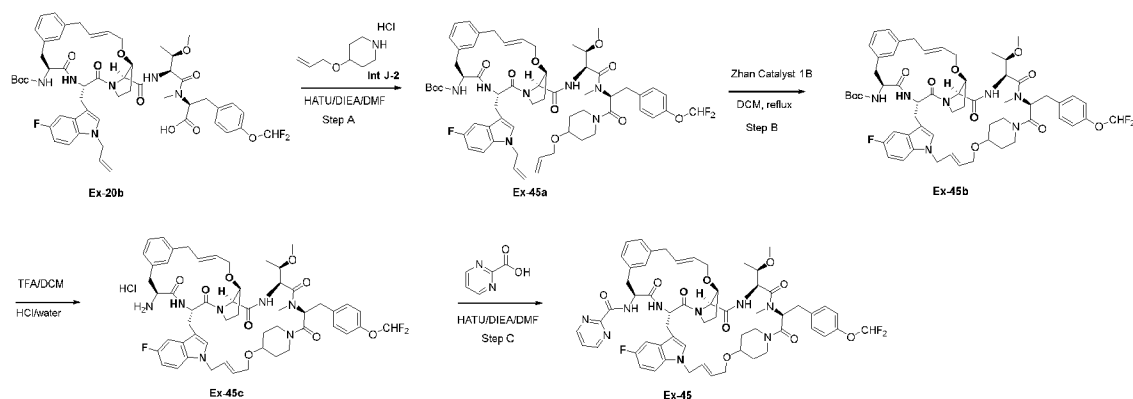
Step E: Synthesis of Ex-28e

[0455] **Intermediate Ex-28d** (73 mg, 0.062 mmol) was dissolved in DCM (1 ml) and TFA (1
20 mL, 12.98 mmol) was then added. After stirring at rt for 1h, then concentrated and the residue was dissolved in acetonitrile/water (2:1, 10 mL) and treated with HCl (0.311 mL, 0.311 mmol). The resulting solution was lyophilized to afford **Ex-28e** as a crude solid. LC/MS 1072.8 (M+1)⁺.

Step F: Synthesis of Ex-28

[0456] To the solution of **Ex-28e** (69 mg, 0.062 mmol) in DMF (6 ml) and CH₂Cl₂ (120 ml) at
25 0 °C was added HATU (23.66 mg, 0.062 mmol) and DIEA (0.065 ml, 0.373 mmol). After stirring at 0 °C for 2h, the reaction mixture was concentrated, and the residue was purified on reverse phase Gilson using 30-65% acetonitrile (0.05%TFA)/water (0.05%TFA) as gradient over 15 min to afford **Ex-28** as a powder. LC/MS 1054.8 (M+1)⁺.

5 **Example 29: Synthesis of Example compound 45 (Ex-45)**



Step A: Synthesis of Ex-45a

10 **[0457]** To the solution of intermediate **Ex-20b** in DMF (6 ml) at 0 °C was added HATU (101 mg, 0.265 mmol) and DIEA (0.232 ml, 1.327 mmol). After stirring at 0 °C for 60min, the mixture was partitioned between EtOAc (250 mL) and brine (200 mL). Next the organic phase was washed with brine (2x200 mL), dried over Na₂SO₄, concentrated and the residue was purified on silica gel column (80g) using 0-10% MeOH/DCM as and eluted solvents to afford **Ex-45a** as an oil. LC/MS 1140.9 (M+1)⁺.

15 **Step B: Synthesis of Ex-45b**

20 **[0458]** To the solution of **Ex-45a** (247 mg, 0.217 mmol) in CH₂Cl₂ (650 ml) was bubbled with N₂ for 40min, then ZHAN CATALYST-1B (79 mg, 0.108 mmol) was added. The resulting mixture was further bubbled with N₂ for 30min, then heated at reflux for 2.0 h. It was then quenched by addition of 1,4-bis(2-isocyanopropyl)piperazine (105 mg, 0.477 mmol) and the resulting mixture was stirred at rt for 1h. The reaction mixture was then concentrated, and the residue was purified on silica gel column (80g) using 0-10% MeOH/DCM as and eluted solvents to give a mixture of TRANS/CIS product, which was further separated on Gilson using 55-75% acetonitrile (0.05% TFA)/water (0.05% TFA) to **Ex-45b** as a powder. LC/MS 1112.7 (M+1)⁺.

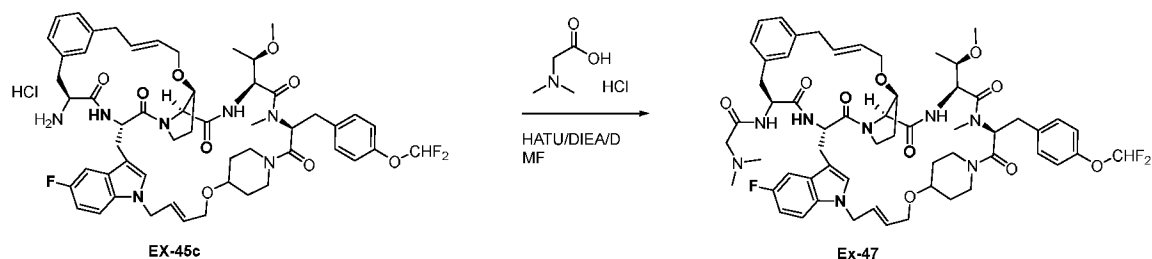
Step C: Synthesis of Ex-45c

25 **[0459]** Intermediate **Ex-45b** (123 mg, 0.111 mmol) was dissolved in CH₂Cl₂ (2 ml) and TFA (2 mL, 26.0 mmol) was then added. After stirring at rt for 1h, the mixture was concentrated and the residue was dissolved in acetonitrile/water (1:1, 10 mL) and treated with HCl (0.553 mL, 0.553 mmol). The resulting solution was then lyophilized to afford **Ex-45c** as a solid. LC/MS 1012.8 (M+1)⁺.

5 Step D: Synthesis of Ex-45

[0460] To the solution of **Ex-45c** (5 mg, 4.77 μ mol) and pyrimidine-2-carboxylic acid (1.183 mg, 9.54 μ mol) in DMF (0.5 ml) was added HATU (3.63 mg, 9.54 μ mol) and DIEA (6.66 μ l, 0.038 mmol). After stirring at rt for 1h, the mixture was then purified on reverse phase Gilson using 10-100%acetonitrile (0.05%TFA)/water (0.05%TFA) as and eluted solvents to afford **Ex-**
 10 **45** as a powder. LC/MS 1118.8 (M+1)⁺.

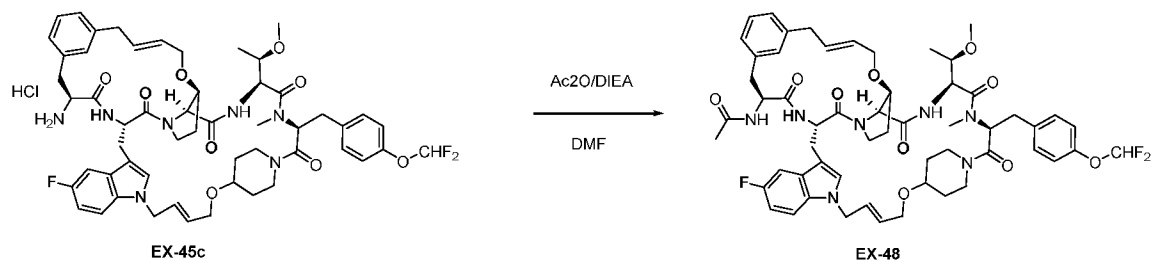
Example 30: Synthesis of Example compound 47 (Ex-47)



15 Synthesis of Ex-47

[0461] To the solution of dimethylglycine hydrochloride (7.72 mg, 0.055 mmol) in DMF (0.5 ml) was added HATU (21.03 mg, 0.055 mmol) and DIEA (0.039 ml, 0.221 mmol). After stirring at rt for 30min, 0.3 mL of the above solution was added to a solution of **Ex-45c** (14.5 mg, 0.014 mmol) and DIEA (9.66 μ l, 0.055 mmol) in DMF (0.5 ml). After stirring at rt for 30min, the
 20 mixture was then purified on reverse phase Gilson using 20-60% acetonitrile (0.05%TFA) /water (0.05%TFA) as and eluted solvents to afford **Ex-47** as a powder. LC/MS 1097.9 (M+1)⁺.

Example 31: Synthesis of Example compound 48 (Ex-48)



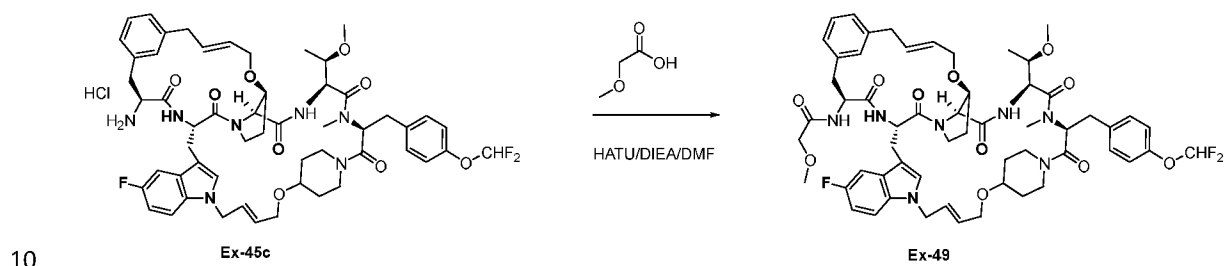
25

Synthesis of Ex-48

[0462] Intermediate **Ex-45c** (8 mg, 7.63 μ mol) was dissolved in DMF (1 ml) and DIEA (8.00 μ l, 0.046 mmol) and ACETIC ANHYDRIDE (1.440 μ l, 0.015 mmol) were then added. After

- 5 stirring at rt for 30min, the mixture was purified on reverse phase Gilson using 10-90% acetonitrile (0.05%TFA)/water (0.05%TFA) as and eluted solvents to afford **Ex-48** as a powder. LC/MS 1054.7 (M+1)⁺.

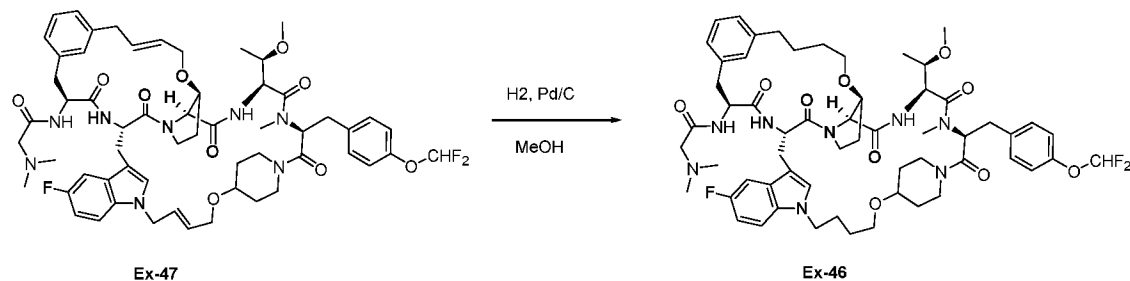
Example 32: Synthesis of Example compound 49 (Ex-49)



Synthesis of Ex-49

- 15 **[0463]** To the solution of 2-methoxyacetic acid (11.00 mg, 0.122 mmol) in DMF (1 ml) was added HATU (46.4 mg, 0.122 mmol) and DIEA (0.043 ml, 0.244 mmol). After stirring at rt for 30min, 0.2 mL of the above solution was added to a solution of **Ex-45c** (16 mg, 0.015 mmol) and DIEA (8.00 μ l, 0.046 mmol) in DMF (0.5 ml). The resulting solution was stirred at rt for 30min, then purified on reverse phase Gilson using 30-90% acetonitrile (0.05%TFA) /water (0.05%TFA) as and eluted solvents to afford **Ex-49** as a powder. LC/MS 1084.9 (M+1)⁺.

20 **Example 33: Synthesis of Example compound 46 (Ex-46)**

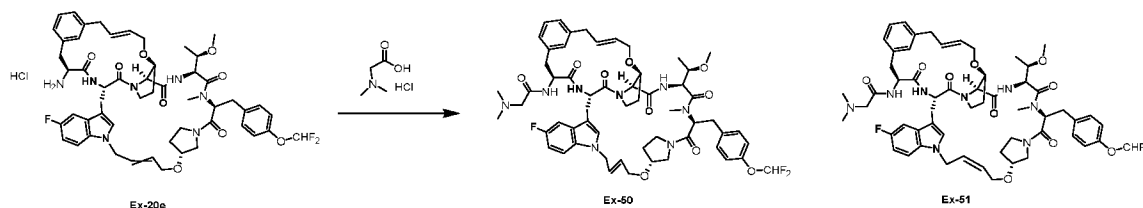


Synthesis of Ex-46

- 25 **[0464]** To the solution of **Ex-47** (3.7 mg, 3.05 μ mol) in MeOH (4 ml) was added Pd/C (1.300 mg, 1.222 μ mol). The resulting mixture was hydrogenated at rt via H2 Balloon for 2h. then the mixture was filtered through celite. The filtrate was concentrated, and the residue was partitioned between DCM (20 mL) and sat. NaHCO₃ (10 mL). The aqueous phase was extracted with DCM (2x20 mL). The combined organic phase was dried over Na₂SO₄, concentrated and the residue

- 5 was purified on silica gel column (12g) using 0-10%MeOH/DCM as and eluted solvents to afford **Ex-46** as a solid. LC/MS 1101.9 (M+1)⁺.

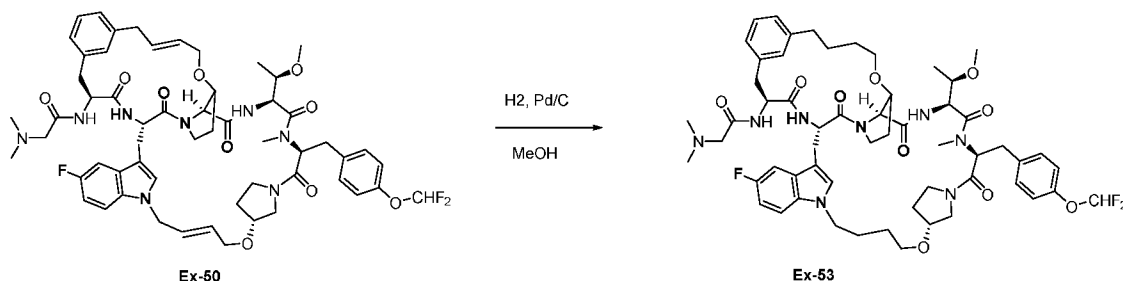
Example 34: Synthesis of Example compounds 50 and 51 (Ex-50 and Ex-51)



10 **Synthesis of Ex-50 and Ex-51**

- [0465] To the solution of **Ex-20e** (33.8 mg, 0.033 mmol) and dimethylglycine hydrochloride (5.47 mg, 0.039 mmol) in DMF (1.5 ml) was added HATU (14.91 mg, 0.039 mmol) and DIEA (0.046 ml, 0.261 mmol). The resulting solution was stirred at rt for 30min, then purified on Gilson (C18 column) using 26-42%acetonitrile (0.05%TFA) as a and eluted solvents to afford
- 15 **Ex-50** as a powder LC/MS 1083.9 (M+1)⁺, and Peak 2 (less polar) **Ex-51** as a powder. LC/MS 1083.8 (M+1)⁺.

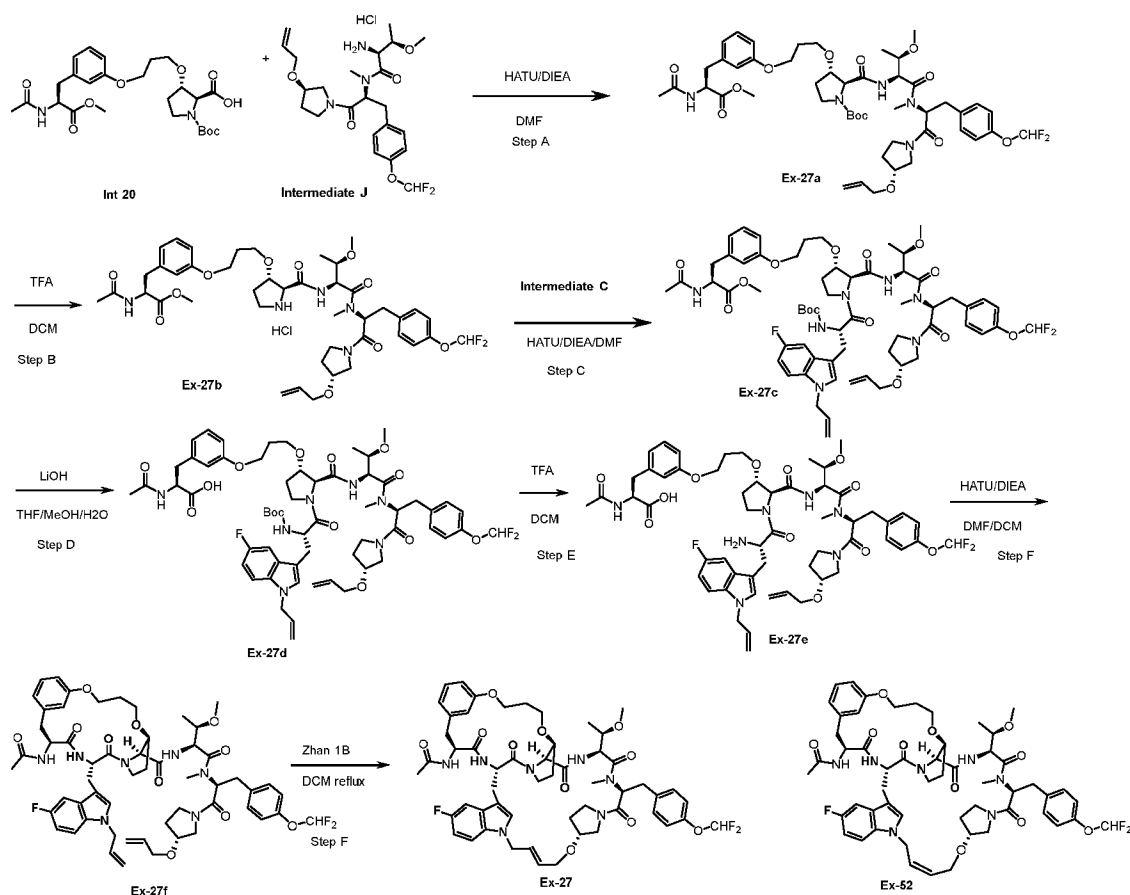
Example 35: Synthesis of Example compound 53 (Ex-53)



20 **Synthesis of Ex-53**

- [0466] To the solution of **Ex-50** (68 mg, 0.057 mmol) in MeOH (20 ml) was added Pd/C (19.04 mg, 0.036 mmol). The resulting mixture was hydrogenated at rt under H2 Balloon for 1h then filtered through celite. The filtrate was concentrated and the residue was purified on reverse phase Gilson using 25-50% acetonitrile (0.05%TFA)/water(0.05%TFA) over 15min as gradient
- 25 to **Ex-53** as a TFA salt in powder form. LC/MS 1087.9 (M+1)⁺.

5 **Example 36: Synthesis of Example compounds 27 and 52 (Ex-27 and Ex-52)**



Step A: Synthesis of Ex-27a

10 **[0467]** To the solution of intermediate **Int 20** (75 mg, 0.147 mmol) and **Intermediate J** (82 mg, 0.162 mmol) in DMF (4 ml) was added HATU (61.7 mg, 0.162 mmol) and DIEA (0.129 ml, 0.737 mmol). After stirring at rt for 1h, the solution was partitioned between EtOAc (150 mL) and brine (100 mL). The organic phase was washed with brine (2x100 mL), dried over Na₂SO₄, concentrated and the residue was purified on silica gel column using 0-10%MeOH/DCM as and
15 eluted solvents to afford **Ex-27a** as an oil. LC/MS 960.8 (M+1)⁺.

Step B: Synthesis of Ex-27b

[0468] Intermediate **Ex-27a** (121.8 mg, 0.127 mmol) was dissolved in CH₂Cl₂ (2 ml) and TFA (2 mL, 26.0 mmol) was then added. After stirring at rt for 30min, the mixture was then concentrated. The residue was dissolved in acetonitrile/water (1:1, 10 mL) and treated with HCl
20 (0.634 mL, 0.634 mmol) and lyophilized to afford **Ex-27b** as a powder. LC/MS 860.7 (M+1)⁺.

5 Step C: Synthesis of **Ex-27c**

[0469] To the solution of **Ex-27b** (114 mg, 0.127 mmol) in DMF (5 ml) was added **Intermediate C** (50.7 mg, 0.140 mmol), HATU (53.2 mg, 0.140 mmol), and DIEA (0.178 ml, 1.017 mmol). The resulting solution was stirred at rt for 40min, then partitioned between EtOAc (150 mL) and brine (100 mL). The organic phase was washed with brine (2x100 mL), dried
10 over Na₂SO₄, concentrated and the residue was purified on silica gel column (40 g) using 0-10% MeOH/DCM as and eluted solvents to afford **Ex-27c** as an oil. LC/MS 1204.9 (M+1)⁺.

Step D: Synthesis of **Ex-27d**

[0470] To the solution of **Ex-27c** (173 mg, 0.144 mmol) in THF (6 ml), Water (2 ml) and MeOH (2 ml) at 0 °C was added LiOH (0.575 ml, 0.575 mmol) dropwise. After stirring at 0 °C
15 for 2h, the volatile was evaporated, and the aqueous phase was acidified to pH 3-4 with 1 N HCl. The mixture was extracted with DCM (3x80 mL). The combined organic phase was concentrated to afford **Ex-27d** as an oil. LC/MS 1190.9 (M+1)⁺.

Step E: Synthesis of **Ex-27e**

[0471] **Intermediate Ex-27d** (0.150 g, 0.126 mmol) was dissolved in CH₂Cl₂ (2 ml) and TFA
20 (2 ml, 26.0 mmol) was then added. After stirring at rt for 45min, the mixture was concentrated and the residue was dissolved in acetonitrile/water (3:1, 30 mL), and treated with HCl (0.630 ml, 0.630 mmol). The resulting mixture was lyophilized to afford **Ex-27e** as a powder. LC/MS 1090.9 (M+1)⁺.

Step F: Synthesis of **Ex-27f**

25 [0472] To the solution of **Ex-27e** (142 mg, 0.126 mmol) in DMF (10 ml) and CH₂Cl₂ (200.00 ml) at 0 °C was added HATU (47.9 mg, 0.126 mmol) and DIEA (0.132 ml, 0.756 mmol). After stirring at 0 °C for 2.5h, the volatile was evaporated, and the residue was then partitioned between EtOAc (200 mL) and brine (100 mL). The organic phase washed with brine (2x100 mL), dried over Na₂SO₄, concentrated and the residue was purified on silica gel column (40 g)
30 using 0-10% MeOH/DCM as and eluted solvents to afford **Ex-27f** as a solid. LC/MS 1072.8 (M+1)⁺.

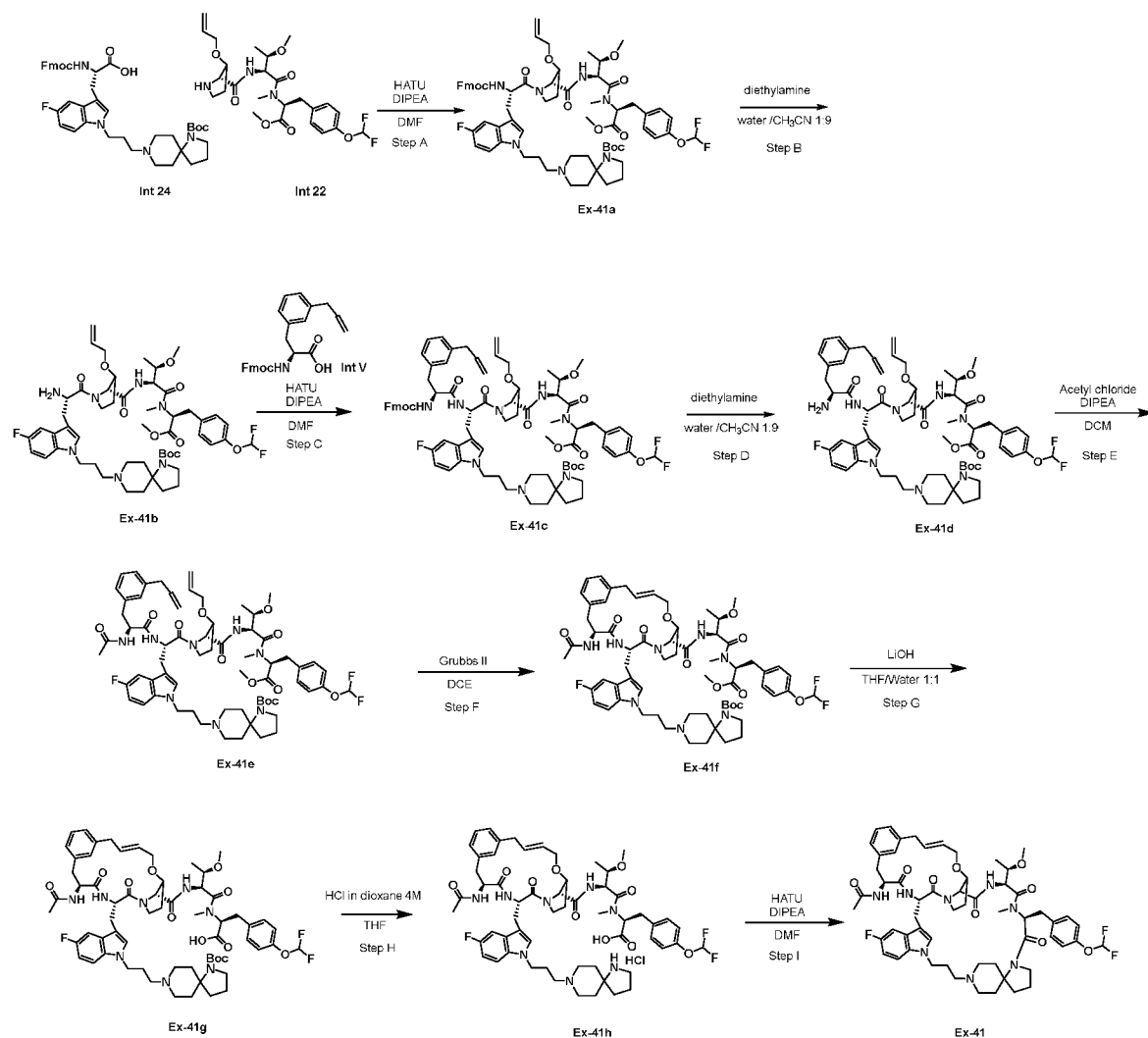
Step G: Synthesis of **Example Ex-27** and **Ex-52**

[0473] The solution of **Ex-27f** (59 mg, 0.055 mmol) in CH₂Cl₂ (120 ml) was bubbled with N₂ for 30min, then Zhan Catalyst-1B (20.19 mg, 0.028 mmol) was added. The resulting solution was
35 further bubbled with N₂ for 20min, then heated to reflux for 2h. After cooled to rt, 1,4-bis(2-isocyanopropyl)piperazine) (26.7 mg, 0.121 mmol) was added and the resulting solution was

- 5 stirred at rt for 15min, then concentrated. The residue was purified on silica gel column using 0-10%MeOH/DCM as and eluted solvents to give a mixture of trans/cis isomers (53 mg) as solid. The solid mixture was further purified on Gilson using 35-60% acetonitrile (0.05%TFA) /water (0.05%TFA) as gradient over 15 min to give more polar eluant **Ex-27** as a powder, LC/MS 1044.9 (M+1)⁺, and less polar eluant **Ex-52**. LC/MS 1044.8 (M+1)⁺.

10

Example 37: Synthesis of Example compound 41 (Ex-41) (HCl salt)



Step A - Synthesis of intermediate Ex-41a

- 15 **[0474]** A solution of **Int 22** (214 mg, 0.379 mmol) and **Int 24** (275 mg, 0.379 mmol) in DMF (3 ml) was treated with HATU (144 mg, 0.379 mmol) and DIPEA (0.199 mL, 1.138 mmol). After stirring for 2h, the reaction mixture was diluted with EtOAc and washed with 0.1N HCl

5 and then with saturated aqueous solution of NaHCO₃ and brine. The Organic layer was dried over Na₂SO₄, filtered and concentrated to afford after lyophilization **Ex-41a**. UPLC-MS: [M+H]⁺ = 1235.4.

Step B - Synthesis of intermediate **Ex-41b**

[0475] A solution of Ex-41a (110 mg, 0.089 mmol) in CH₃CN/H₂O 9:1 (3 ml) was treated
10 with diethylamine (9.30 µl, 0.089 mmol) and the mixture was stirred at room temperature for 16 h. After the volatiles were removed under reduced pressure, the residue was taken up with a mixture of CH₃CN/water 9:1 (10 ml) and washed 6 times with heptane (8 ml). The CH₃CN phase was partially evaporated under reduced pressure and then water was added and the mixture lyophilized to give Ex-41b. UPLC-MS: [M+H]⁺ = 1012.5.

15 Step C - Synthesis of intermediate **Ex-41c**

[0476] A solution of Ex-41b (0.090 g, 0.089 mmol) and Intermediate V (0.036 g, 0.085 mmol) in DMF (1.5 ml) was treated with HATU (0.032 g, 0.085 mmol) and DIPEA (0.047 ml, 0.267 mmol). After stirring for 2h, the reaction mixture was diluted with EtOAc and washed with 0.1 M HCl, saturated aqueous solution of NaHCO₃ and brine. Organic layer was dried over Na₂SO₄,
20 filtered and concentrated to obtain after lyophilization Ex-41c. UPLC-MS: [M+H]⁺ = 1422.3.

Step D - Synthesis of intermediate **Ex-41d**

[0477] Intermediate **Int-41c** (126 mg, 0.089 mmol) was dissolved in CH₃CN/H₂O 9:1 (5 ml) and treated with diethylamine (150 µl, 1.436 mmol). After stirring for 16 h, the solvents were removed to give **Ex-41d**. UPLC-MS: [M+H]⁺ = 1199.5.

25 Step E - Synthesis of intermediate **Ex-41e**

[0478] Intermediate **Int-41d** (107 mg, 0.089 mmol) was dissolved in DCM (2 ml) and treated with acetyl chloride (6.96 µl, 0.098 mmol) and DIPEA (0.062 ml, 0.356 mmol). After stirring for 16 hours, the solvents were removed under reduced pressure to afford the crude product which was purified by reverse phase flash chromatography (120 g C18, elution water
30 0.1%TFA/CH₃CN 0.1%TFA from 100:0 to 50:50 in 10CV, then to 50:50 in 6CV) to give after lyophilization **Ex-41e** as beige solid. UPLC-MS: [M+H]⁺ = 1242.6.

Step F - Synthesis of intermediate **Ex-41f**

[0479] A solution of **Ex-41e** (63mg, 0.051 mmol) in DCE (60 ml) was degassed under nitrogen and heated at 60°C and treated with Grubbs II (4.31 mg, 5.07 µmol) then heated at 80°C for 30
35 minutes. The reaction mixture was allowed to reach room temperature then SiliaMet_DMT-resin (200 mg) was added. After stirring for 1 h, the reaction was filtered and concentrated to give **Ex-41f**. UPLC-MS: [M+H]⁺ = 1213.8.

5 Step G - Synthesis of intermediate Ex-41g

[0480] **Ex-41f** (81 mg, 0.071 mmol) dissolved in THF/H₂O 1.1 (2 ml) and treated with LiOH (1.135 mg, 0.047 mmol) for 2h. Then the solvents were removed under reduced pressure to give **Ex-41g**. UPLC-MS: [M+H]⁺ = 1199.8.

Step H - Synthesis of intermediate Ex-41h

10 [0481] Intermediate **Ex-41g** was dissolved in THF (1 ml) and treated with 4M HCl in dioxane (0.019 ml, 0.075 mmol). After stirring for 5 h, the solvents were removed under reduced pressure to give **Ex-41h**. UPLC-MS: [M+H]⁺ = 1099.9.

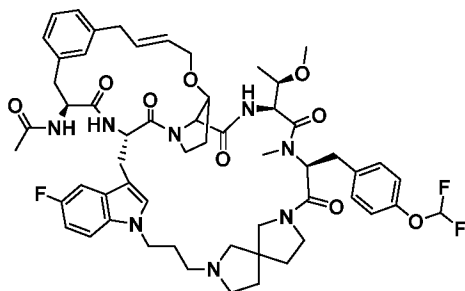
Step I - Synthesis of example Ex-41

15 [0482] Intermediate **Ex-41h** (21.58 mg, 0.019 mmol) was dissolved in DMF (80ml) and HATU (7.22 mg, 0.019 mmol) and DIPEA (0.013 ml, 0.076 mmol) were added. After stirring for 5 min, the reaction was quenched with TFA, concentrated in high vacuum.

[0483] The crude cyclic peptide was purified by preparative reversed-phase high performance liquid chromatography (RP-HPLC) [Luna C18 (Phenomenex) (column size 30 * 250 mm, 100Å, 5 μm); mobile phase: (A) 0.1% TFA in HPLC water and (B) 0.1% TFA in HPLC acetonitrile, 20 flow rate: 30 mL/min; UV wavelength λ = 214 nm; gradient: 30% B over 5 min, 30 to 55 % B over 5-25 min, 50 to 60 % B over 25-30 min. Sample load: (%B): 20, 5 ml/min, DMSO] to give purified cyclic peptide as TFA salt then salt exchange was made to HCl salt and lyophilized to afford **Ex-41** (major isomer) as HCl salt (solid). UPLC-MS: [M+H]⁺ = 1081.9 (mass of the free base, C₅₈H₇₁F₃N₈O₉, is 1081.25).

25

Example 38: Synthesis of Example compound 42 (Ex-42) (HCl salt)

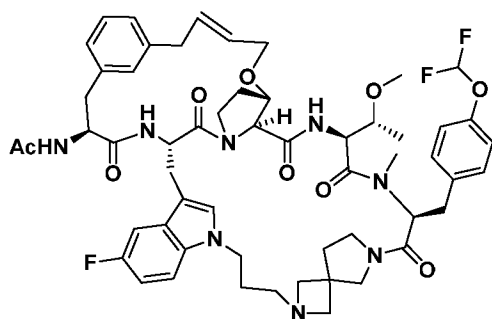


Ex-42

[0484] Compound **Ex-42** was prepared from **Int 25** and **Int 22** by applying the synthetic procedure used for **Ex-41**.

- 5 [0485] The crude cyclic peptide was purified by preparative reversed-phase high performance liquid chromatography (RP-HPLC) [Luna C18 (Phenomenex) (column size 30 * 250 mm, 100Å, 5 μm); mobile phase: (A) 0.1% TFA in HPLC water and (B) 0.1% TFA in HPLC acetonitrile, flow rate: 30 mL/min; UV wavelength λ = 214 nm; gradient: 35% B over 5 min, 35 to 55 % B over 5-25 min. Sample load: (%B): 35, 5 ml/min, DMSO] to give purified cyclic peptide as TFA salt then salt exchange was made to HCl salt and lyophilized to afford **Ex-42** (major isomer) as HCl salt (solid). UPLC-MS: $[M+H]^+ = 1067.9$ (mass of the free base, $C_{57}H_{69}F_3N_8O_9$ is 1067.22).
- 10

Example 39: Synthesis of Example compound 43 (Ex-43)

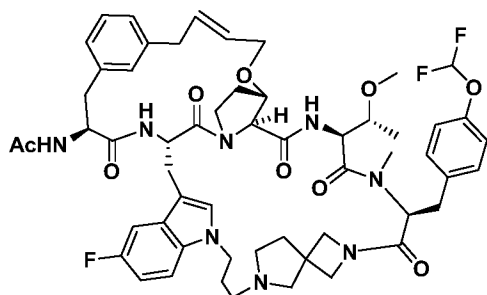


Ex-43

- 15 [0486] Compound **Ex-43** was prepared from **Int 26** and **Int 22** by applying the synthetic procedure used for **Ex-41**.
- [0487] The crude cyclic peptide was purified by preparative reversed-phase high performance liquid chromatography (RP-HPLC) [Luna C18 (Phenomenex) (column size 30 * 250 mm, 100Å, 5 μm), mobile phase: (A) 0.1% TFA in HPLC water and (B) 0.1% TFA in HPLC acetonitrile, flow rate: 30 mL/min; UV wavelength λ = 214 nm; gradient: 35% B over 10 min, 35 to 50 % B over 10-35 min. Sample load: (%B): 35, 5 ml/min, DMSO] to give purified cyclic peptide as TFA salt then salt exchange was made to HCl salt and lyophilized to afford **Ex-43** as HCl salt in solid form. Free base $C_{56}H_{67}F_3N_8O_9$, requires 1053.19; UPLC-MS: $[M+H]^+ = 1053.8$ (mass of the free base, $C_{56}H_{67}F_3N_8O_9$, is 1053.19).
- 20

25

5 **Example 40: Synthesis of Example compound 44 (Ex-44) (HCl salt)**

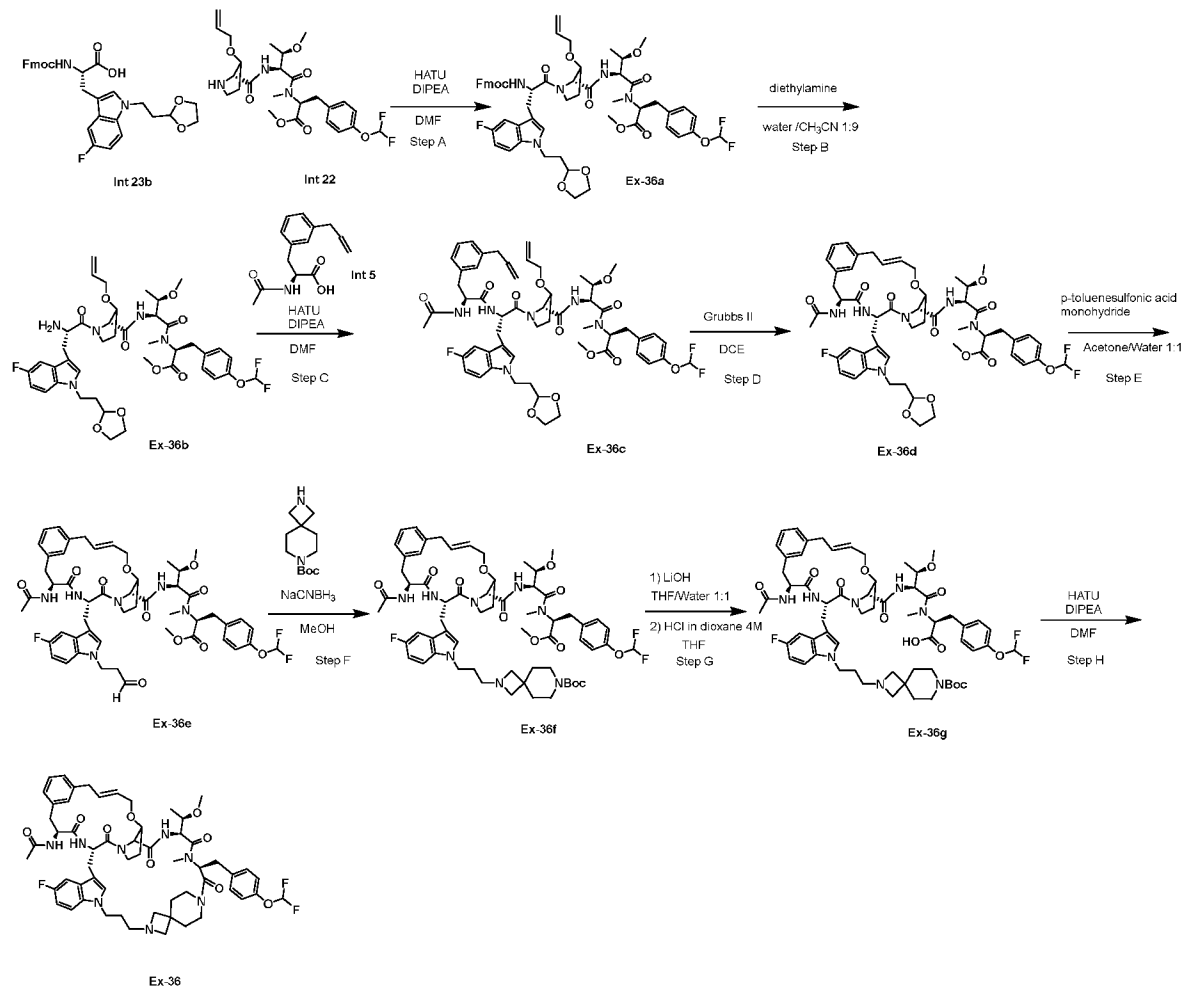


Ex-44

[0488] Compound **Ex-44** was prepared from **Ex-27** and **Int 22** by applying the synthetic procedure used for **Ex-41**.

[0489] The crude cyclic peptide was purified by preparative reversed-phase high performance liquid chromatography (RP-HPLC) [Luna C18 (Phenomenex) (column size 30 * 250 mm, 100Å, 5 μm). mobile phase: (A) 0.1% TFA in HPLC water and (B) 0.1% TFA in HPLC acetonitrile, flow rate: 30 mL/min; UV wavelength $\lambda = 214$ nm; gradient: 30% B over 5 min, 30 to 55 % B over 5-25 min, 50 to 60 % B over 25-30 min. Sample load: (%B): 20, 5 ml/min, DMSO] to give purified cyclic peptide as TFA salt then salt exchange was made to HCl salt and lyophilized to afford **Ex-44** (major isomer) as HCl salt in solid form. UPLC-MS: $[M+H]^+ = 1053.8$ (mass of the salt free form, $C_{56}H_{67}F_3N_8O_9$, is 1053.19).

5 **Example 41: Synthesis of Example compound 36 (Ex-36) (HCl salt)**



Step A - Synthesis of intermediate Ex-36a

[0490] Intermediate **Int 23b** (216 mg, 0.396 mmol), **Int 22** (235 mg, 0.417 mmol) and HATU (151 mg, 0.396 mmol) were dissolved in DMF (4 ml) and DIPEA (0.218 ml, 1.250 mmol) was added. After stirring for 5 min, the reaction was quenched with AcOH and concentrated to dryness. The crude product was dissolved with EtOAc and washed with 0.1 M HCl, NaHCO₃ saturated aqueous solution and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated to dryness to afford the crude title compound which was purified by silica gel chromatography on Biotage (Luknova Supersep 12g. gradient: 0% to 100% DCM/MeOH 95:5 in DCM, over 15 CV) to afford **Ex-36a**. UPLC-MS: [M+H]⁺ = 1054.3.

Step B - Synthesis of intermediate Ex-36b

[0491] Intermediate **Ex-36a** (274 mg, 0.260 mmol) was dissolved in acetonitrile (2.6 ml) and diethylamine (0.272 ml, 2.60 mmol) was added. After stirring at room temperature for 60 min the

5 reaction was concentrated to dryness. The crude product was dissolved with CH₃CN/H₂O 9:1 and washed several times with heptane. The CH₃CN layer was diluted with H₂O, acidified by HCl (2.60 ml, 0.260 mmol) and lyophilized to afford **Ex-36b**. UPLC-MS: [M+H]⁺ = 832.4.

Step C - Synthesis of intermediate Ex-36c

[0492] Intermediate **Int 5** (62.1 mg, 0.251 mmol), **Ex-36b** (209 mg, 0.251 mmol) and HATU
10 (96 mg, 0.251 mmol) were dissolved in DMF (2.5 ml) and DIPEA (0.176 ml, 1.005 mmol) was added. After stirring for 5 min, the reaction was quenched with AcOH and concentrated to dryness.

[0493] The crude product was dissolved with EtOAc and washed with 0.1M HCl, NaHCO₃ saturated aqueous solution and brine. The organic layer was dried over Na₂SO₄, filtered and
15 concentrated to dryness to afford the crude product which was purified by silica gel chromatography on Biotage, (Luknova Supersep 12g; gradient: 0% to 100% DCM/MeOH 95:5 in DCM, over 15CV. Collected fractions were concentrated to dryness and lyophilized to afford **Ex-36c**. UPLC-MS: [M+H]⁺ = 1061.3.

Step D - Synthesis of intermediate Ex-36d

20 [0494] Intermediate **Ex-36c** (128 mg, 0.121 mmol) was dissolved in dry DCE (128 ml) under N₂. GrubbsII (20.48 mg, 0.024 mmol) was added and the reaction was stirred at 60 °C under N₂ for 45 min. SiliaMetS DMT resin (212 mg, 0.121 mmol) was added and stirred at room temperature for 1h, filtered and evaporated to afford **Ex-36d**. UPLC-MS: [M+H]⁺ = 1033.3.

Step E - Synthesis of intermediate Ex-36e

25 [0495] Intermediate **Ex-36d** (101 mg, 0.098 mmol) was dissolved in acetone/H₂O 2:1 (3 ml) together with pTsOH monohydrate (186 mg, 0.978 mmol) and was heated at 80 °C by microwave irradiation for 2h. The reaction was concentrated to dryness. The residue was dissolved with EtOAc and washed with NaHCO₃ saturated aqueous solution and brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated to dryness to afford **Ex-36e**.
30 UPLC-MS: [M+H]⁺ = 989.4.

Step F - Synthesis of intermediate Ex-36f

[0496] Intermediate **Ex-36e** (83 mg, 0.084 mmol) and tert-butyl 2,7-diazaspiro[3.5]nonane-7-carboxylate (26.6 mg, 0.117 mmol) were dissolved in dry MeOH (2 ml). AcOH was added to adjust pH to 5. After stirring for 20 min, sodium cyanoborohydride (7.38 mg, 0.117 mmol) was
35 added and stirred at room temperature overnight. Then the reaction mixture was concentrated to dryness. The residue was dissolved with EtOAc and washed with NaHCO₃ saturated aqueous

- 5 solution and brine. The organic layer was dried over Na₂SO₄, filtered and concentrated to dryness to afford the crude product which was purified by flash chromatography on silica (12g column, elution DCM/MeOH 0.5% TEA from 100:0 to 80:20 in 6CV, then to 80:20 in 6CV) to afford **Ex-36f**; UPLC-MS: [M+H]⁺ = 1199.9.

Step G - Synthesis of intermediate Ex-36g

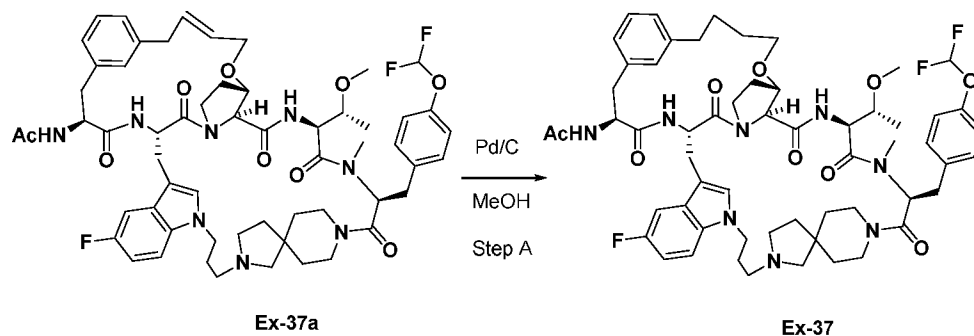
- 10 **[0497]** A solution of **Ex-36f** (150 mg, 0.125 mmol) in THF/MeOH/water 1.6 ml/0.1ml/0.1ml was treated with LiOH (30.0 mg, 1.251 mmol) for 2h. The mixture was then heated at 50 °C for 2h. Then the solvents were removed, the residue was treated with 4M HCl in dioxane (2ml, 24.35 mmol). After stirring for 16h, the solvents were removed to afford **Ex-36g**; UPLC-MS: [M+H]⁺ = 1085.8.

15 *Step H - Synthesis of intermediate Ex-36*

[0498] Intermediate **Ex-36g** was dissolved in DMF (120 ml) and HATU (31.9 mg, 0.084 mmol) and DIPEA (0.044 ml, 0.252 mmol) were added. After stirring for 5 min, the reaction was quenched with TFA and concentrated in high vacuum.

- 20 **[0499]** The crude cyclic peptide was purified by preparative reversed-phase high performance liquid chromatography (RP-HPLC) [Luna (Phenomenex) (column size 30 * 250 mm, 100Å, 5 μm). mobile phase: (A) 0.1% TFA in HPLC water and (B) 0.1% TFA in HPLC acetonitrile, flow rate: 30 mL/min; UV wavelength λ = 214 nm; gradient: 35% B over 5 min, 35 to 50 % B over 5-20 min, 50 to 55 % B over 20-25 min. Sample load: (%B): 35, 5 ml/min, DMSO] to give purified cyclic peptide as TFA salt then salt exchange was made to HCl salt and lyophilized to afford **Ex-36** (major isomer) as HCl salt. UPLC-MS: [M+H]⁺ = 1067.7 (mass of the free base, C₅₇H₆₉F₃N₈O₉ is 1067.22).

Example 42: Synthesis of Example compound 37 (Ex-37) (HCl salt)



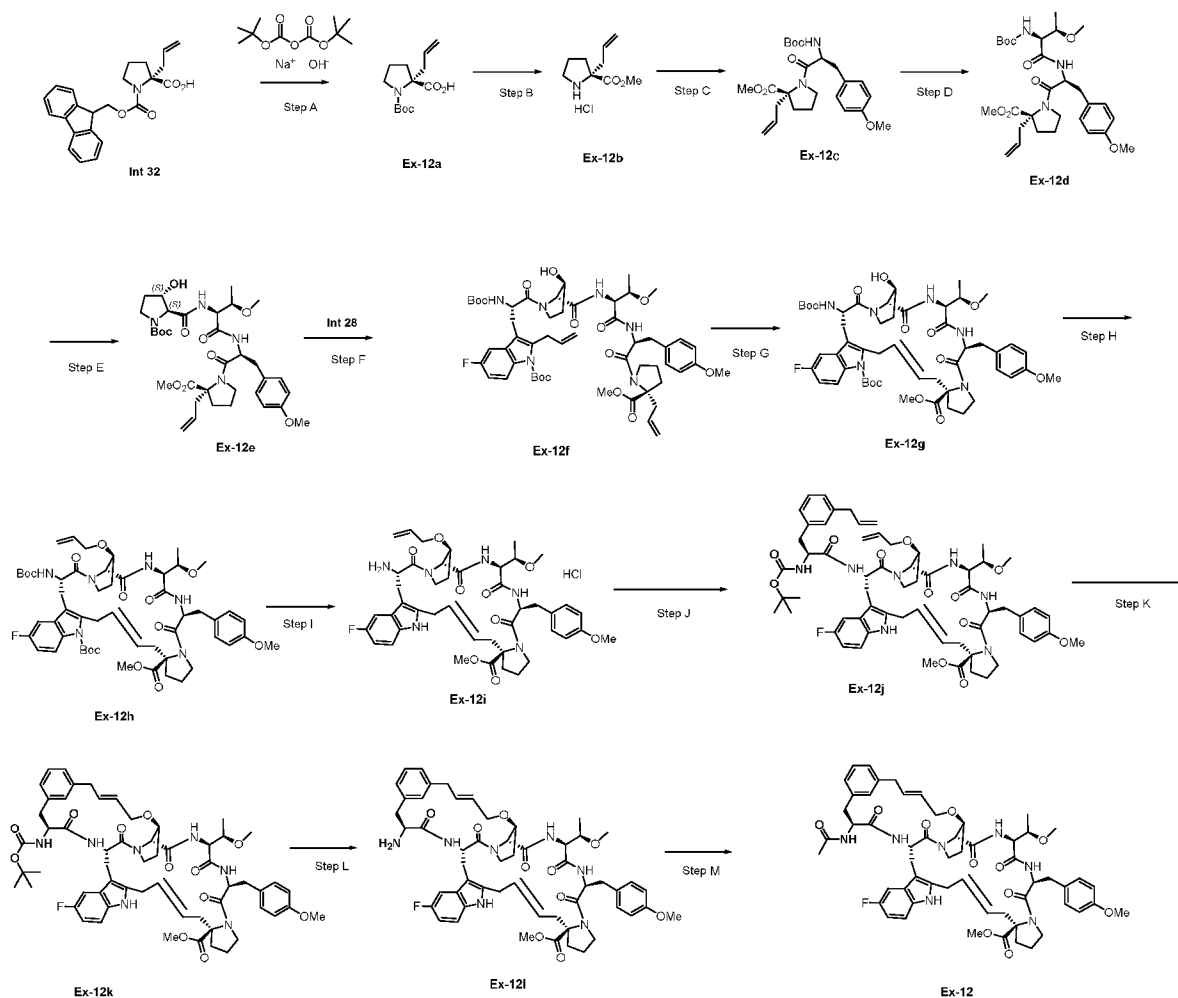
5 Synthesis of intermediate **Ex-37a**

[0500] Intermediate **Ex-37a** was prepared from **Int 23b** and **Int 22** by applying the synthetic procedure used for **Ex-36**.

[0501] The crude cyclic peptide purified by preparative reversed-phase high performance liquid chromatography (RP-HPLC) [Deltapak C18, (40 * 100 mm, 15um, 100Å); mobile phase: (A) 0.1% TFA in HPLC water and (B) 0.1% TFA in HPLC acetonitrile, flow rate: 30 mL/min; UV wavelength $\lambda = 214$ nm; gradient: 40% B 5 min, 40 to 60 % B over 5-25 min. Sample load: 5 ml/min, DMSO] to afford **Ex-37a** as TFA salt. UPLC-MS: $[M+H]^+ = 1081.7$ (mass of the free base, $C_{58}H_{71}F_3N_8O_9$, is 1081.25).

[0502] Intermediate **Ex-37a** (13 mg, 0.012 mmol) was dissolved in dry MeOH (10 mL) and N_2 atm. was applied, then Pd/C (2.6 mg, 2.443 μ mol) was added. H_2 atm was applied and the mixture was stirred at room temperature overnight, 90 % conversion observed. The catalyst was filtered, and the reaction was repeated. Then the reaction mixture was concentrated to dryness under reduced pressure to afford the crude cyclic peptide which was purified by preparative reversed-phase high performance liquid chromatography (RP-HPLC) [Reprosil Gold C4 Prep column, (column size 10 * 150 mm, 10 μ m, 120Å); mobile phase: (A) 0.1% TFA in HPLC water and (B) 0.1% TFA in HPLC acetonitrile, flow rate: 30 mL/min; UV wavelength $\lambda = 214$ nm;. Sample load: 5 ml/min, DMSO] to give purified saturated cyclic peptide as TFA salt then salt exchange was made to HCl salt and lyophilized to afford **Ex-37**. UPLC-MS: $[M+H]^+ = 1083.9$ (mass of the free base, $C_{58}H_{73}F_3N_8O_9$, is 1083.26).

25

5 **Example 43: Synthesis of Example compound 12 (Ex-12)****Step A - Synthesis of intermediate Ex-12a**

[0503] Aqueous 1N sodium hydroxide (7.95 ml, 7.95 mmol) was added dropwise to a solution of intermediate **Int 32** (1g, 2.65 mmol) in THF (20 ml)/MeOH (10 ml)/Water (20.00 ml) while cooled in an ice bath. The resulting mixture was stirred for a period of 2h. The THF and methanol were removed under reduced pressure and to the aqueous solution was added dioxane (20.00 ml) and Water (20.00 ml). Next di-tert-butyl dicarbonate (0.752 g, 3.44 mmol) was added at 0°C and stirring was continued for overnight. The volatiles were removed under reduced pressure and the resulting aqueous mixture washed with ether (X2). Dichloromethane was added and the aqueous phase was acidified with 1N aq. HCl. The aqueous phase was separated and further extracted with dichloromethane. The combined organic phases were dried (MgSO₄) and the volatiles removed under reduced pressure to afford **Ex-12a** as a solid. LCMS 278.85 (M+Na)⁺.

5 Step B - Synthesis of intermediate **Ex-12b**

[0504] Intermediate **Ex-12a** was dissolved in DMF (10 ml) and sodium hydrogencarbonate (0.329 g, 3.92 mmol) and iodomethane (0.612 ml, 9.79 mmol) were then added. After stirring for 18 hours, the reaction was poured into water, extracted twice with 100ml MTBE, washed with 100ml water twice, washed with 100ml brine, dried over MgSO₄, concentrated to give an oil, 10 which was dissolved in DCM (10.00 ml) and then added 4N HCl in dioxane (0.490 ml, 1.958 mmol). After stirring under N₂ for 1h, the reaction was concentrated to afford **Ex-12b** as a solid. LCMS 170.18 (M+1)⁺.

Step C - Synthesis of intermediate **Ex-12c**

[0505] N-ethyl-N-isopropylpropan-2-amine (0.799 ml, 4.59 mmol) was added to the mixture of 15 (S)-2-((tert-butoxycarbonyl)amino)-3-(4-methoxyphenyl)propanoic acid (339 mg, 1.147 mmol) and intermediate **Ex-12b** (236mg, 1.147 mmol) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (524 mg, 1.377 mmol) in DCM (9 ml)/DMF (1 ml) at 0°C. The reaction was stirred for 1hr. The mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and 20 the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 12g, and eluted with 0-100%EtOAc/hexane, then 0-10% MeOH/DCM to give **Ex-12c** as a solid. LCMS 448.38 (M+1)⁺.

Step D - Synthesis of intermediate **Ex-12d**

[0506] Intermediate **Ex-12c** (128 mg, 0.296 mmol) was dissolved in DCM (10.00 ml) and then 25 added 4N HCl in dioxane (0.490 ml, 1.958 mmol). After stirring under N₂ for 1h, the reaction was concentrated to afford an HCl salt solid. N-ethyl-N-isopropylpropan-2-amine (0.532 ml, 3.05 mmol) was added dropwise to the above HCl salt (341 mg, 0.764 mmol) and (2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methoxybutanoic acid (178 mg, 0.764 mmol) and HATU (348 mg, 0.916 mmol) in DMF (1 ml) / DCM (4 ml) at 0°C and allowed to warm up to room temperature 30 and stirred for overnight. The mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 12g, and eluted with 0-10% MeOH/DCM to give **Ex-12d** as a solid. LCMS 563.45 (M+1)⁺.

Step E - Synthesis of intermediate **Ex-12e**

35 [0507] N-ethyl-N-isopropylpropan-2-amine (0.388 ml, 2.229 mmol) was added to a mixture of **Ex-12d** and (2S,3S)-1-(tert-butoxycarbonyl)-3-hydroxypyrrolidine-2-carboxylic acid (129 mg, 0.557 mmol) and HATU (254 mg, 0.669 mmol) in DMF (10 ml) at 0°C and allowed to warm up

5 to rt and stirred for 4h. The reaction was quenched with water and extracted with EtOAc. The mixture was washed with water (x2), the org layer was separated and dried over MgSO₄, filtered, and concentrated. The residue was purified by column chromatography on silica gel 12g, and eluted with 0-100% EtAOAc/hexane to give **Ex-12e** as a solid. LCMS 675.49 (M+1)⁺.

Step F - Synthesis of intermediate Ex-12f

10 **[0508]** Intermediate **Ex-12e** (128 mg, 0.296 mmol) was dissolved in DCM (10.00 ml) and then added 4N HCl in dioxane (0.490 ml, 1.958 mmol). After stirring under N₂ for 1h, the reaction was concentrated to afford an HCl salt solid. N-ethyl-N-isopropylpropan-2-amine (0.362 ml, 2.081 mmol) was added to a mixture of **Int 28** (241 mg, 0.520 mmol) and the above formed HCl salt of **Ex-12e** and HATU (237 mg, 0.624 mmol) in DCM (1.000 ml)/ DMF (1ml) at 0°C and
15 allowed to warm up to rt and stirred for 4h. The reaction was quenched with water and extracted with EtOAc. The mixture was washed with water (x2). The org layer was separated and dried over MgSO₄, filtered, and concentrated. The residue was purified by column chromatography on silica gel 12g and eluted with 0-100% EtAOAc/hexane to give Intermediate **Ex-12f** (128 mg, 0.296 mmol) was dissolved in DCM (10.00 ml) and then added 4N HCl in dioxane (0.490 ml,
20 1.958 mmol). After stirring under N₂ for 1h, the reaction was concentrated to afford an HCl salt solid as a solid. LCMS 1020.23 (M+1)⁺.

Step G - Synthesis of intermediate Ex-12g

[0509] Intermediate **Ex-12f** (400 mg, 0.392 mmol) in anhydrous CH₂Cl₂ (100ml) was added dropwise to a solution of Zhan catalyst (115 mg, 0.157 mmol) in anhydrous DCM (800 ml) in 90
25 min and the resulting reaction mixture was stirred at room temperature under an atmosphere of nitrogen for a period of 3h then was heated at 40°C for 3h. After stirring at rt overnight, more Zhan catalyst (0.4eq) was added and heated at 45°C for 3 hr then was stirred at RT overnight.
[0510] The reaction mixture was filtered through celite and then removed solvent under reduced pressure and the residue was purified by silica gel column chromatography (ISCO 24g
30 column) using 0 to 10% MeOH in dichloromethane as eluent to afford **Ex-12g** as a solid. LCMS 991.64 (M+1)⁺.

Step H - Synthesis of intermediate Ex-12h

[0511] Tetrabutylammonium bromide (38.4 mg, 0.119 mmol) was added into the mixture of DCM (1.000 ml) solution of **Ex-12g** (118 mg, 0.119 mmol) and aq 1M sodium hydroxide (1.191 ml, 1.191 mmol) and followed by 3-bromoprop-1-ene (17.28 mg, 0.143 mmol). After stirring for
35 3h at rt, the reaction mixture was quenched with water. It was extracted with ethyl acetate (3 x). The combined organic layers were washed with brine (saturated), dried (MgSO₄), filtered and

5 concentrated in vacuo to afford a crude product. The residue was purified by column chromatography on silica gel 4g, and eluted with 0-15% MeOH/DCM to give **Ex-12h** as a solid. LCMS 1032.30 (M+1)⁺.

Step I - Synthesis of intermediate **Ex-12i**

[0512] Intermediate **Ex-12h** (54 mg, 0.052 mmol) was dissolved in DCM (2 ml) and 4M
10 hydrogen chloride (0.013 ml, 0.052 mmol) in dioxane was added. After stirring at rt for 2hr, the mixture was concentrated to dryness to afford **Ex-12i** which was use as crude in the next step. LCMS 831.34 (M+1)⁺.

Step J - Synthesis of intermediate **Ex-12j**

[0513] N-ethyl-N-isopropylpropan-2-amine (0.036 ml, 0.207 mmol) was added to the mixture
15 of **Ex-12i** (43 mg, 0.052 mmol) and (S)-3-(3-allylphenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (15.80 mg, 0.052 mmol) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (23.61 mg, 0.062 mmol) in DCM (1 ml)/DMF (1ml) and stirred at rt overnight. The mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and
20 the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 4g, and eluted with 0-100%EtOAc/hexane, then 0-10% MeOH/DCM to afford **Ex-12j** as a solid. LCMS 1118.45 (M+1)⁺.

Step K - Synthesis of intermediate **Ex-12k**

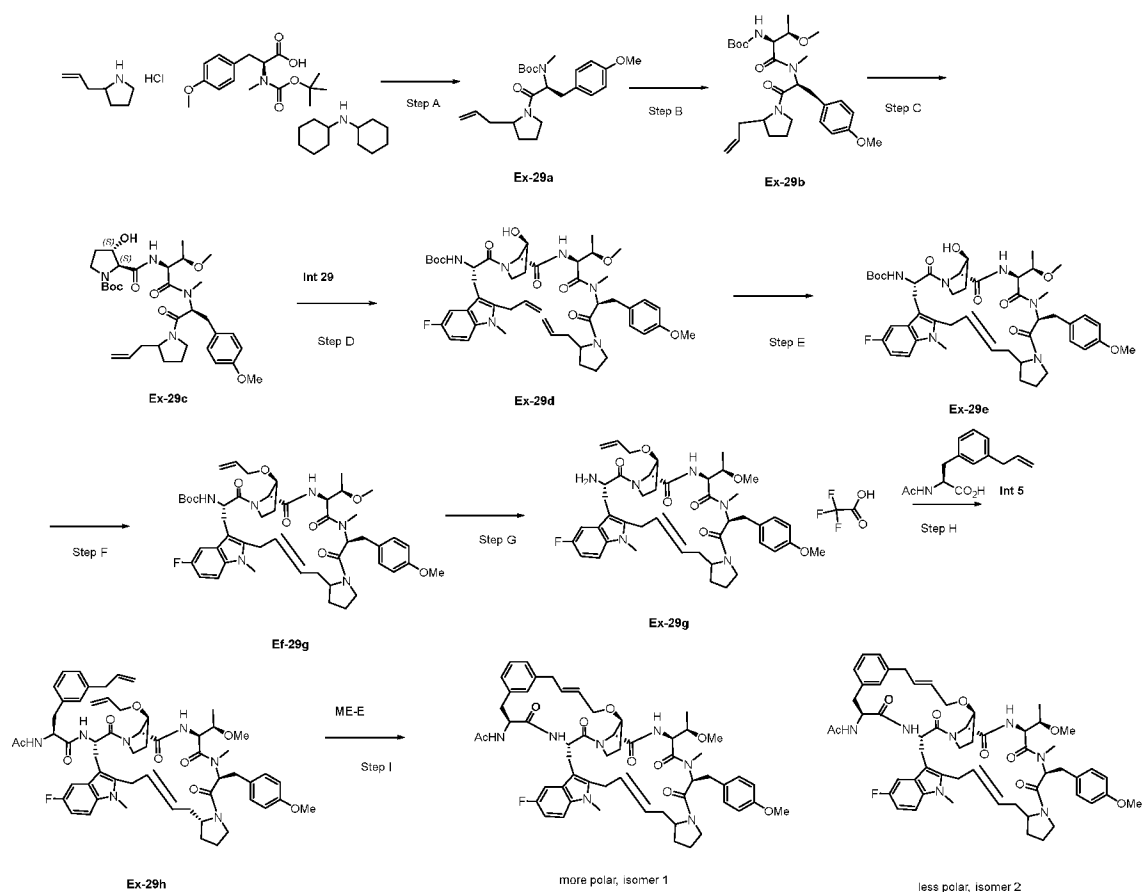
[0514] Intermediate **Ex-12j** (57 mg, 0.051 mmol) was dissolved in anhydrous CH₂Cl₂ (10ml)
25 and to a solution of Zhan catalyst(1,3-dimesitylimidazolidin-2-ylidene)(5-(N,N-dimethylsulfamoyl)-2-isopropoxybenzylidene)ruthenium(VI) chloride (14 mg, 0.019 mmol) in anhydrous DCM (80 ml) was added dropwise in 30minx3 and the resulting reaction mixture was stirred at room temperature under an atmosphere of nitrogen for a period of 3h. The reaction mixture was filtered through celite and then removed solvent under reduced pressure and the
30 residue purified by silica gel column chromatography (ISCO 24g column) using 0 to 10% MeOH in dichloromethane as eluent to give **Ex-12k** as a solid. LCMS 1090.62 (M+1)⁺.

Step L - Synthesis of intermediate **Ex-12l**

[0515] Intermediate **Ex-12k** (42 mg, 0.039 mmol) was dissolved in DCM (2ml) and TFA (2.97
μl, 0.039 mmol) was then added. After stirring at rt for 3hr, the mixture was concentrated. The
35 crude was sent to HTP for purification to give **Ex-12l** as solid. LCMS 990.36 (M+1)⁺.

5 Step M - Synthesis of Example Ex-12

[0516] To a flask containing intermediate **Ex-12I** (20 mg, 0.018 mmol) in DCM (2 ml) was added N-ethyl-N-isopropylpropan-2-amine (0.013 ml, 0.072 mmol) followed by N-acetoxysuccinimide (4.27 mg, 0.027 mmol). The reaction was stirred at room temperature for overnight. EtOAc and water were added to the reaction. The aqueous layer was extracted with
 10 EtOAc. The combined organic layer was washed with Brine, dried over Na₂SO₄, and concentrated in vacuo to give a solid. The crude was sent to HTP for purification to afford Example Ex-12 as a solid. LCMS 1032.30 (M+1)⁺.

Example 44: Synthesis of Example compound 29 (EX-29)

15

Step A - Synthesis of intermediate Ex-29

[0517] N-ethyl-N-isopropylpropan-2-amine (2.64 ml, 15.17 mmol) was added to the mixture of 2-allylpyrrolidine hydrochloride (560 mg, 3.79 mmol) and BOC-METYR(ME)-OH DCHA (2047mg, 4.17 mmol) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-

5 tetramethylisouronium hexafluorophosphate(V) (1731 mg, 4.55 mmol) in DMF (1 ml) / DCM (10 ml) at 0°C. The mixture was allowed to warm up to room temperature and stirred overnight. The mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 24g and eluted with 0-10% MeOH/DCM to give **Ex-29a** as a solid. LCMS 403.22 (M+H)⁺.

Step B - Synthesis of intermediate **Ex-29b**

[0518] Intermediate **Ex-29a** (1.359 g, 3.38 mmol) was dissolved in DCM (10.00 ml) and then added TFA (5mL). After stirring under N₂ for 1h, the reaction was concentrated to afford an TFA salt solid. N-ethyl-N-isopropylpropan-2-amine (1.071 ml, 6.15 mmol) was added to the mixture of TFA salt of N-(tert-butoxycarbonyl)-O-methyl-L-threonine (395 mg, 1.692 mmol) and the TFA salt of **Ex-29a** (619 mg, 1.538 mmol) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (643 mg, 1.692 mmol) in DMF (1 ml) / DCM (10 ml) at 0°C. The mixture was allowed to warm up to room temperature and stirred overnight. The mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 4g and eluted with 0-10% MeOH/DCM to give **Ex-29b** as a solid. LCMS 519.49 (M+H)⁺.

Step C - Synthesis of intermediate **Ex-29c**

[0519] Intermediate **Ex-29b** (739 mg, 1.428 mmol) was dissolved in DCM (10.00 ml) and then added TFA (5mL). After stirring under N₂ for 1h, the reaction was concentrated to afford an TFA salt solid. N-ethyl-N-isopropylpropan-2-amine (0.995 ml, 5.71 mmol) was added to a mixture of the above obtained TFA salt of **Ex-29b** and (2S,3S)-1-(tert-butoxycarbonyl)-3-hydroxypyrrolidine-2-carboxylic acid (396 mg, 1.713 mmol) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (651 mg, 1.713 mmol) in DCM (10 ml)/DMF (1 ml) at 0°C. The mixture was allowed to warm up to rt and stirred for 4h. The reaction was quenched with water and extracted with EtOAc. Next the mixture was washed with water (x2). The org layer was separated and dried over MgSO₄, filtered, and concentrated. The residue was purified by column chromatography on silica gel 12g and eluted with 0-100% EtAOAc/hexane to give **Ex-29c** as a solid. LCMS 631.54 (M+H)⁺.

35 Step D - Synthesis of intermediate **Ex-29d**

[0520] Intermediate **Ex-29c** (740 mg, 1.173 mmol) was dissolved in DCM (10.00 ml) and then added TFA (5mL). After stirring under N₂ for 1h, the reaction was concentrated to afford an

5 TFA salt solid. N-ethyl-N-isopropylpropan-2-amine (0.442 ml, 2.54 mmol) was added to the mixture of the above obtained TFA salt of **Ex-29c** (400 mg, 0.634 mmol) and intermediate **Int 29** (286 mg, 0.761 mmol) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (289 mg, 0.761 mmol) in DCM (1 ml)/DMF (1ml). The mixture was stirred at rt overnight. The mixture was partitioned between EtOAc and
10 water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 4g, and eluted with 0-100%EtOAc/hexane, then 0-10% MeOH/DCM to give **Ex-29d** as a solid. LCMS 889.44 (M+H)⁺.

Step E - Synthesis of intermediate **Ex-29e**

15 **[0521]** Intermediate **Ex-29d** (350 mg, 0.394 mmol) in anhydrous CH₂Cl₂ (25ml) was added dropwise to a solution of Zhan catalyst(1,3-dimesitylimidazolidin-2-ylidene)(5-(N,N-dimethylsulfamoyl)-2-isopropoxybenzylidene)ruthenium(VI) chloride (116 mg, 0.157 mmol) in anhydrous DCM (800 ml) in 30minx3 and the resulting reaction mixture was stirred at rt under an atmosphere of nitrogen for overnight. 1,4-bis(3-isocyanopropyl)piperazine as Ru scavenger
20 (173 mg, 0.787 mmol) was added to the reaction solution. After stirring at rt for 30min, the reaction mixture was concentrated, filtered through celite, and then removed solvent under reduced pressure and the residue was purified by silica gel column chromatography (ISCO 24g column) using 0 to 10% MeOH in dichloromethane as eluent to give **Ex-29e** (260 mg) as a solid. LCMS 861.52 (M+H)⁺.

25 Step F - Synthesis of intermediate **Ex-29f**

[0522] Tetrabutylammonium bromide (97 mg, 0.302 mmol) was added into the mixture of DCM (1 ml) solution of **Ex-29e** (260 mg, 0.302 mmol) and aq 10M sodium hydroxide (0.604 ml, 6.04 mmol) and followed by 3-bromoprop-1-ene (110 mg, 0.906 mmol). The reaction mixture was vigorously stirred at rt overnight and quenched with water. It was extracted with ethyl
30 acetate (3 x). The combined organic layers were washed with brine (saturated), dried (MgSO₄), filtered and concentrated in vacuo to afford a crude product. The residue was purified by column chromatography on silica gel 4g and eluted with 0-15% MeOH/DCM to give **Ex-29f** as a solid. LCMS 901.58 (M+H)⁺.

Step G - Synthesis of intermediate **Ex-29g**

35 **[0523]** Intermediate **Ex-29g** (167 mg, 0.185 mmol) was dissolved in DCM (3 ml) and 2,2,2-trifluoroacetic acid (0.3ml, 0.185 mmol) was added. After stirring at rt for 3 hours, the mixture

5 was concentrated to dryness. Next 3ml of Acetonitrile/water (1:1) was added and cooled to -78°C and dried over two days to give **Ex-29g** as a solid. LCMS 801.48 (M+H)⁺.

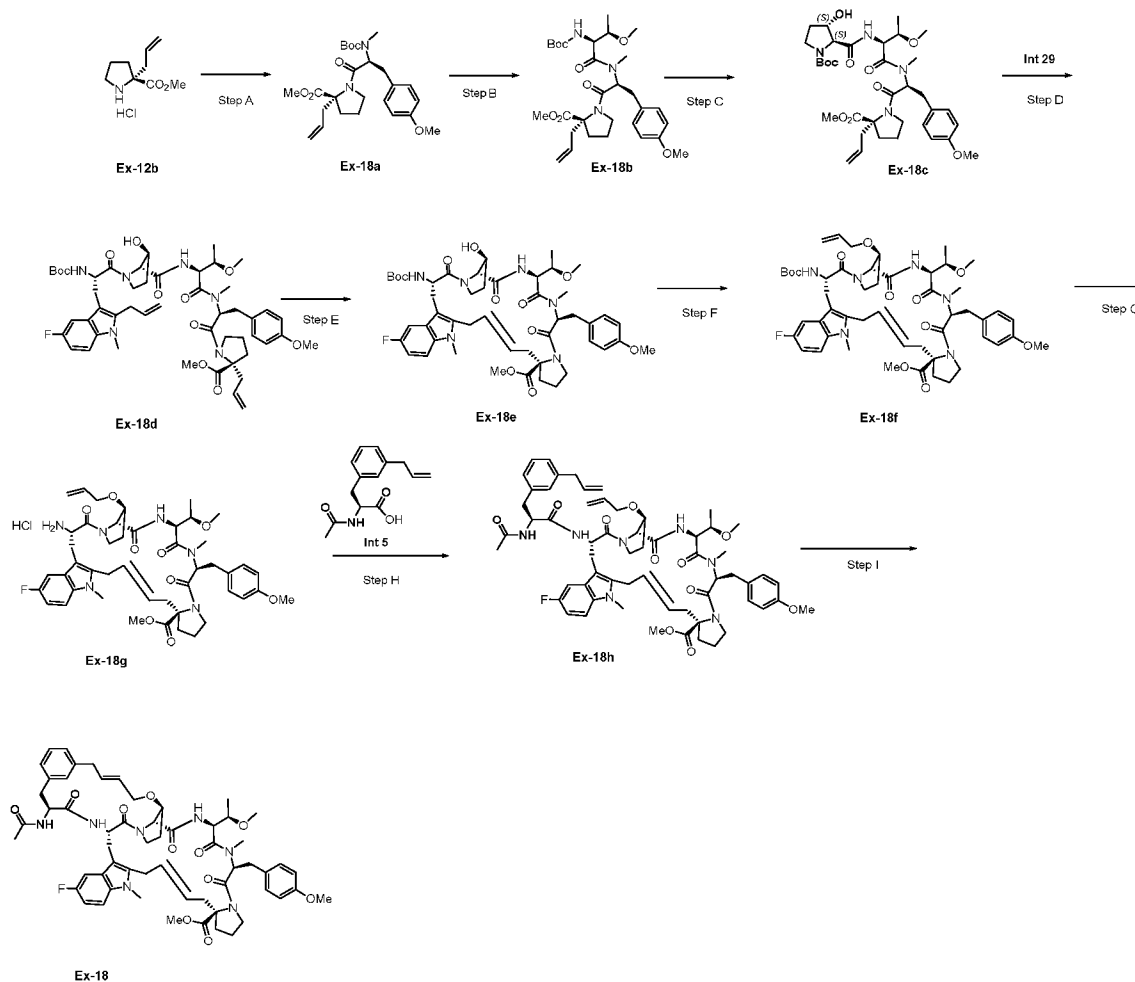
Step H - Synthesis of intermediate **Ex-29h**

[0524] N-ethyl-N-isopropylpropan-2-amine (0.067 ml, 0.385 mmol) was added to the mixture of the above TFA salt of **Ex-29g** and **Int 5** (28.5 mg, 0.115 mmol), 2-(3H-[1,2,3]triazolo[4,5-
10 b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (43.9 mg, 0.115 mmol) in DCM (3 ml)/DMF (1ml) and stirred at rt overnight. The mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water (X2), dried (MgSO₄) and the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 4g, and eluted with 0-100%EtOAc/hexane, then 0-10% MeOH/DCM to give **Ex-29h**
15 as solid. LCMS 1030.61 (M+H)⁺.

Step I - Synthesis of Example **Ex-29**

[0525] Intermediate **Ex-29h** (66 mg, 0.064 mmol) in anhydrous DCM (20 ml) was added dropwise to a solution of Zhan's catalyst (23.5 mg, 0.032 mmol) in anhydrous in DCM (200 ml) over 30minx3 and the resulting reaction mixture was stirred at 45°C under an atmosphere of
20 nitrogen for a period of 4h. 1,4-bis(3-isocyanopropyl)piperazine as Ru scavenger 1,4-bis(3-isocyanopropyl)piperazine (28.2 mg, 0.128 mmol) was added to the reaction solution and stirred at rt for 30min. The reaction mixture was concentrated, filtered through celite and then removed solvent under reduced pressure and the residue was purified by silica gel column chromatography (ISCO 24g column) using 0 to 10% MeOH in dichloromethane as eluent to give **Ex-29** isomer 1
25 as a solid, and isomer 2 . LCMS 1002.64 (M+H)⁺.

5 **Example 45: Synthesis of Example compound 18 (Ex-18)**



Step A - Synthesis of intermediate **Ex-18a**

[0526] To the solution of intermediate **Ex-12b** (1.105 g, 3.90 mmol) and N-BOC-N-METHYL-4-METHOXY-L-PHENYLALANINE (1.568 g, 5.07 mmol) in DMF (10 ml) was added HATU (1.928 g, 5.07 mmol) and DIEA (4.09 ml, 23.40 mmol). After stirring at rt overnight, the solution was partitioned between EtOAc (200 mL) and brine (100 mL). The organic phase was further washed with brine (2x200 mL), dried over Na₂SO₄, concentrated and the residue was purified on silica gel column (120 g) using 10-80%EtOAc/hexane to afford **Ex-18a** (1.42 g, 3.08 mmol) as an oil. LCMS 460.0 (M+H)⁺.

Step B - Synthesis of intermediate **Ex-18b**

[0527] To a stirred solution of (2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methoxybutanoic acid (3.14 g, 13.45 mmol) in DMF (60 ml) was added HATU (5.11 g, 13.45 mmol) at 0 °C under

5 nitrogen atmosphere. The reaction solution was stirred at 0 °C for 10 min. Then intermediate **Ex-18a** and DIEA (8.54 ml, 48.9 mmol) were added to the solution and stirred at 0 °C for 1 h. The reaction was quenched with water (100 mL), extracted with ether acetate (2 x 300 mL). The combined organic layer was washed with brine (3 x 100 mL), dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by
10 silica gel column chromatography and eluted with 0-26% EA in PE to give **Ex-18b** as an oil. LCMS 576.39 (M+H)⁺.

Step C - Synthesis of intermediate **Ex-18c**

[0528] N-ethyl-N-isopropylpropan-2-amine (0.680 ml, 3.91 mmol) was added to a mixture of intermediate **Ex-18b** and (2S,3S)-1-(tert-butoxycarbonyl)-3-hydroxypyrrolidine-2-carboxylic
15 acid (271 mg, 1.172 mmol) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (446 mg, 1.172 mmol) in DCM (10 ml)/DMF (1 ml) at 0°C and allowed to warm up to rt and stirred for 4h. The reaction was quenched the reaction with water and extracted with EtOAc. The mixture was washed with water (x2), the org layer was separated and dried over MgSO₄, filtered, and concentrated. The residue was purified
20 by column chromatography on silica gel 12g and eluted with 0-100% EtAOAc/hexane to give **Ex-18c** as a solid. LCMS 689.36 (M+H)⁺.

Step D – Synthesis of intermediate **Ex-18d**

[0529] Intermediate **Ex-18c** (698 mg, 1.013 mmol) was dissolved in DCM (2ml) and 4N HCl in dioxide was added. After stirring for 3hr, the reaction was concentrated to give crude HCl salt as
25 a solid.

[0530] N-ethyl-N-isopropylpropan-2-amine (0.251 ml, 1.440 mmol) was added to the mixture of **above HCl salt** and intermediate **Int 29** and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (164 mg, 0.432 mmol) in DCM (1 ml)/DMF (1ml) and stirred at rt overnight. The mixture was partitioned between EtOAc and water. The
30 organic phase was separated, washed with water (X2), dried (MgSO₄) and the volatiles removed under reduced pressure. The residue was purified by column chromatography on silica gel 4g, and eluted with 0-100%EtOAc/hexane, then 0-10% MeOH/DCM to give **Ex-18d** as solid. LCMS 947.6 (M+H)⁺.

Step E – Synthesis of intermediate **Ex-18e**

35 [0531] Intermediate **Ex-18d** (194 mg, 0.188 mmol) in anhydrous CH₂Cl₂ (100ml) was added dropwise to a solution of Zhan catalyst(1,3-dimesitylimidazolidin-2-ylidene)(5-(N,N-dimethylsulfamoyl)-2-isopropoxybenzylidene)ruthenium(VI) chloride (138 mg, 0.188 mmol) in

5 anhydrous DCM (400 ml) over 30minx3 and the resulting reaction mixture was stirred at room temperature under an atmosphere of nitrogen for a period of 3h. The reaction mixture was filtered through celite and then removed solvent under reduced pressure and the residue was purified by silica gel column chromatography (ISCO 24g column) using 0 to 10% MeOH in dichloromethane as eluent to afford **Ex-18e** as a solid. LCMS 1006.23 (M+H)⁺.

10 Step F – Synthesis of intermediate **Ex-18f**

[0532] Palladium-tetrakis(triphenylphosphine) (44.0 mg, 0.038 mmol) was added to a mixture of intermediate **Ex-18e** (175 mg, 0.190 mmol) and allyl methyl carbonate (0.043 ml, 0.381 mmol) in anhydrous THF (3 ml) and the resulting reaction mixture was heated to 65°C (oil bath temperature) under an atmosphere of nitrogen for overnight. After cooled, the volatiles were removed under reduced pressure and the residue purified by silica gel column chromatography (ISCO 12g column) using a 0-50% gradient of 70/30 ethyl acetate/ethanol in hexane over 30 minutes to give **Ex-18f** (131 mg, 0.130 mmol). LCMS 1045.69 (M+H)⁺.

Step G – Synthesis of intermediate **Ex-18g**

[0533] Intermediate **Ex-18f** (63 mg, 0.066 mmol) was dissolved in DCM (2 ml) and 4M hydrogen chloride (0.066 mmol) in dioxane was added. After stirring for 1 hr, the mixture was concentrated and lyophilized overnight to the dryness. The crude was used as it is.

Step H – Synthesis of intermediate **Ex-18h**

[0534] The crude intermediate **Ex-18g** above and **Int 5** were dissolved in DMF (657 µl) with HATU (30.0 mg, 0.079 mmol). The reaction was evacuated and purged with N₂. DIEA (41.3 µl, 0.236 mmol) was added and the reaction was stirred at ambient temperature for 90 minutes. The reaction was diluted with ethyl acetate and washed with 10% aq LiCl solution. The organics were dried with sodium sulfate, filtered, and concentrated. The crude material was purified by column chromatography using a RediSep Rf Gold silica 12 g prepackaged column on an Isco and eluted with a 0-100% gradient of 70/30 ethyl acetate/ethanol in hexane over the course of 30 min to give **Ex-18h** (35 mg, 0.032 mmol). LCMS 1088.92 (M+H)⁺.

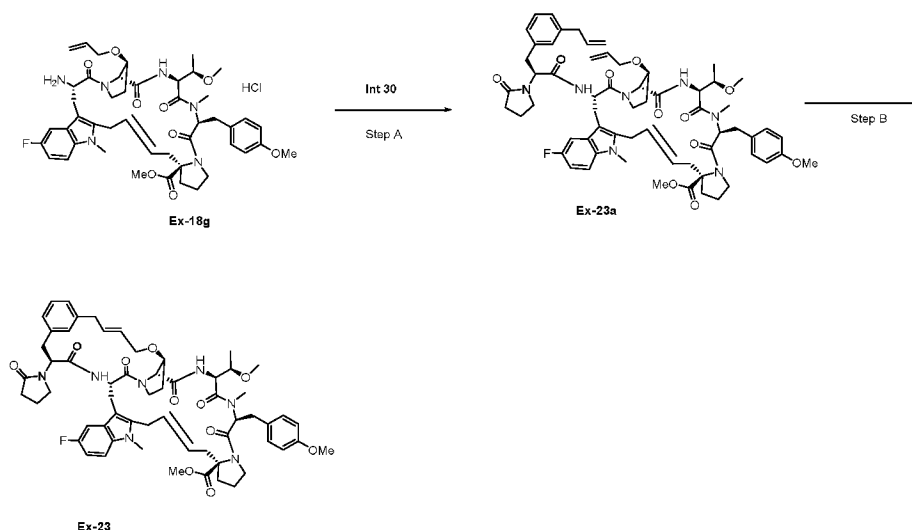
Step I – Synthesis of Example **Ex-18**

[0535] Intermediate **Ex-18h** (35 mg, 0.032 mmol) in anhydrous CH₂Cl₂ (6 ml) was added dropwise to a solution of Zhan catalyst(1,3-dimesitylimidazolidin-2-ylidene)(5-(N,N-dimethylsulfamoyl)-2-isopropoxybenzylidene)ruthenium(VI) chloride (9.44 mg, 0.013 mmol) in anhydrous DCM (56.300 ml) over 30minx3 and the resulting reaction mixture was stirred at room temperature under an atmosphere of nitrogen for a period of 2h. Then the reaction was

5 stirred at ambient temperature for an additional 3 hours. The reaction mixture was filtered through celite and then removed solvent under reduced pressure. The crude residue was diluted in DMSO and sent to HTP for purification. HTP used reverse phase chromatography and a TFA_50mL_45_51_25m_V3_C1 method to give **Ex-18** (13.2 mg, 0.01 mmol). LCMS 1060.66 (M+H)⁺.

10

Example 46: Synthesis of Example compound 23 (Ex-23)



Step A – Synthesis of intermediate Ex-23a

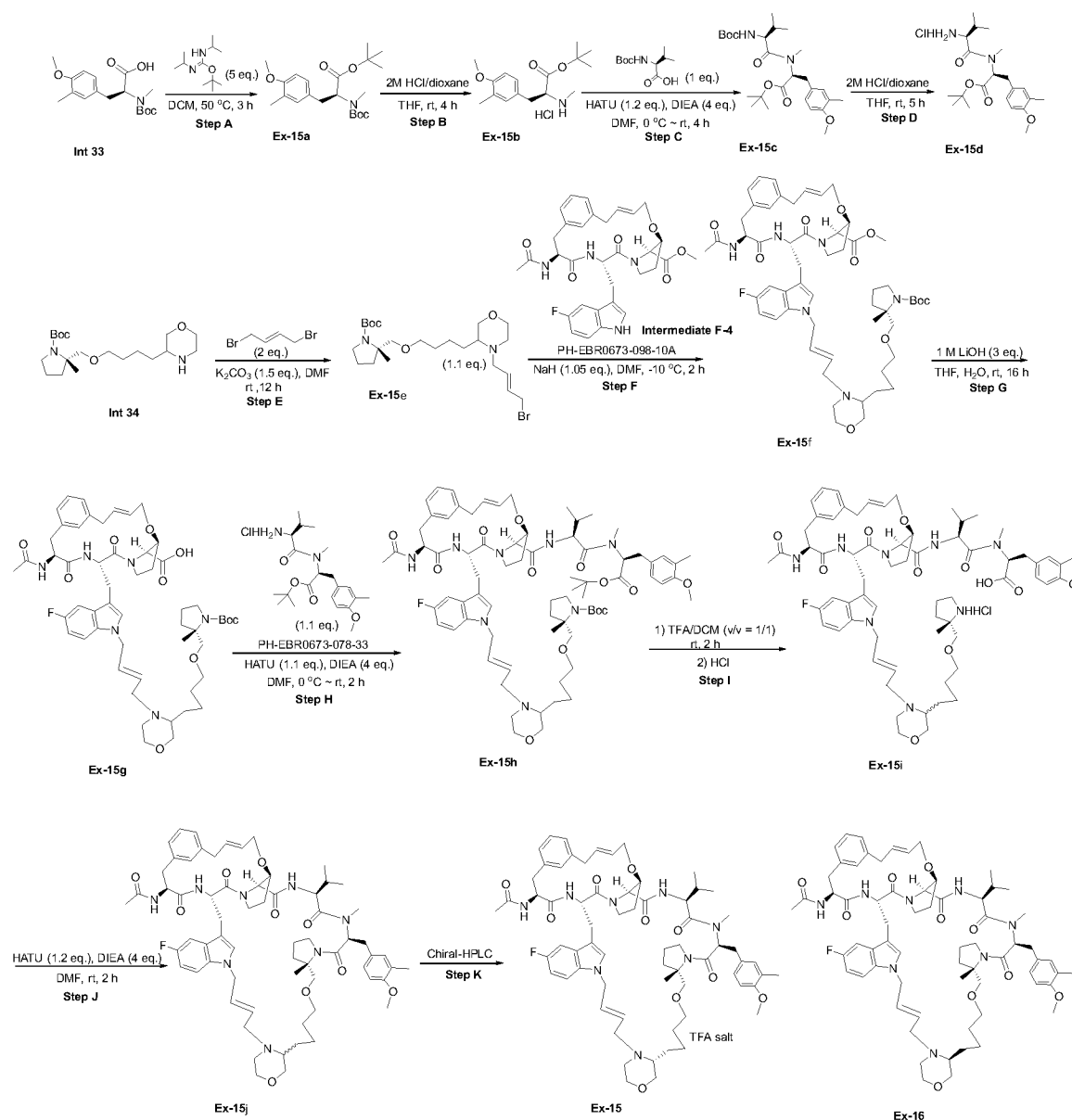
15 **[0536]** Intermediate **Ex-18g** (30mg, 0.031 mmol) and **Int 30** were dissolved in DMF (44.7 μ l) with HATU (14.27 mg, 0.038 mmol). The reaction was evacuated and purged with N₂. Next DIEA (19.67 μ l, 0.113 mmol) was added and the reaction was stirred at ambient temperature for 90 minutes. The reaction was diluted with ethyl acetate and washed with 10% aq LiCl solution. The organics were dried with sodium sulfate, filtered, and concentrated. The crude material was
 20 purified by column chromatography using a RediSep Rf Gold silica 12 g prepackaged column on an Isco, and eluted with a 0-100% gradient of 70/30 ethyl acetate/ethanol in hexane over the course of 30 min to give **Ex-23a**. LCMS 1115.11 (M+H)⁺.

Step B – Synthesis of intermediate Ex-23

25 **[0537]** Intermediate **Ex-23a** (10 mg, 8.97 μ mol) in anhydrous CH₂Cl₂ (1.5 ml) was added dropwise to a solution of Zhan catalyst(1,3-dimesitylimidazolidin-2-ylidene)(5-(N,N-dimethylsulfamoyl)-2-isopropoxybenzylidene)ruthenium(VI) chloride (2.63 mg, 3.59 μ mol) in anhydrous DCM (13.500 ml) over 15 min and the resulting reaction mixture was stirred at room

5 temperature under an atmosphere of nitrogen for 6h. The reaction mixture was filtered through
celite and then removed solvent under reduced pressure. The crude residue was diluted in DMSO
and was purified by reversed mobile phase Flash chromatography (Column: C¹⁸, 80 g; Mobile
Phase A: water (10 mmol TFA), Mobile Phase B: ACN; Flow rate: 60 mL/min; Gradient: 10% B
to 40% B in 5 min; 40% B to 70% B in 30 min; 210 nm, RT: 25 min). The fractions were
10 collected and concentrated under reduced pressure to afford **Ex-23**. LCMS 1086.91 (M+H)⁺.

Example 47: Synthesis of Example compounds 15 and 16 (Ex-15 and Ex-16)



5 Step A: Synthesis of Ex-15a

[0538] To a stirred solution of **Int 33** (200 mg, 0.62 mmol) in DCM (2 mL) was added (*E*)-tert-butyl N,N'-diisopropylcarbamimidate (619 mg, 3.09 mmol) at room temperature. The reaction solution was stirred at 50 °C for 3 h. The resulting solution was quenched with water (30 mL) and extracted with EA (3 x 60 mL). The combined organic layer was washed with brine (3 x 50 mL), dried over anhydrous Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography and eluted with 0% - 20% EA in PE to afford **Ex-15a** as an oil. LCMS (ESI) [M + Na]⁺ 402.1. ¹H NMR (300 MHz, CD₃OD) δ 6.98 (d, J = 8.2 Hz, 2H), 6.80 (d, J = 8.2 Hz, 1H), 4.63-4.55 (m, 1H), 3.79 (s, 3H), 3.12 (dd, J = 14.4, 5.1 Hz, 1H), 2.96-2.82 (m, 1H), 2.70 (d, J = 4.9 Hz, 3H), 2.16 (s, 3H), 1.48 (s, 9H), 1.45-1.30 (m, 9H).

Step B: Synthesis of Ex-15b

[0539] To a solution of **Ex-15a** (150 mg, 0.39 mmol) in THF (1.5 mL) was added HCl (4 M) in 1,4-dioxane (1.5 mL). The reaction solution was stirred for 4 h at room temperature. The resulting solution was concentrated under reduced pressure to afford **Ex-15b** hydrochloride salt as an oil. LCMS 280.3 –M - HCl + H]⁺.

Step C: Synthesis of Ex-15c

[0540] To a stirred solution of (S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanoic acid (76.0 mg, 0.350 mmol) in DMF (2 mL) were added HATU (160 mg, 0.420 mmol), **Ex-15b** (110 mg, 0.35 mmol) and DIEA (0.24 mL, 1.40 mmol) at 0 °C under nitrogen atmosphere. The reaction solution was stirred at room temperature for 4 h. The resulting solution was quenched with water (50 mL), extracted with EA (3 x 50 mL). The combined organic layer was washed with brine (3 x 50 mL), dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography and eluted with 0% - 20% EA in PE to afford **Ex-15c** as an oil. LCMS 479.3 [M + H]⁺. ¹H NMR (300 MHz, CD₃OD) δ 7.08-6.93 (m, 2H), 6.88-6.74 (m, 1H), 5.02-4.95 (m, 1H), 4.35-4.24 (m, 1H), 3.79 (s, 3H), 3.26-3.15(m, 1H), 3.02-2.84 (m, 5H), 2.16 (s, 3H), 1.56-1.25 (m, 18H), 1.01-0.86 (m, 6H).

Step D: Synthesis of Ex-15d

[0541] To a solution of **Ex-15c** (150 mg, 0.310 mmol) in THF (2 mL) was added HCl (4 M) in 1,4-dioxane (2 mL). The reaction solution was stirred at room temperature for 5 h. The resulting solution was concentrated under reduced pressure to afford **Ex-15d** as an oil. LCMS 379.3 –M - HCl + H]⁺.

5 Step E: Synthesis of Ex-15e

[0542] To a stirred solution of intermediate **Int 34** (330 mg, 0.930 mmol) in DMF (3 mL) were added (E)-1,4-dibromobut-2-ene (396 mg, 1.85 mmol) and K₂CO₃ (192 mg, 1.39 mmol) at room temperature. The reaction solution was stirred at room temperature for 12 h. The resulting solution was quenched with water (50 mL) and extracted with EA (3 x 50 mL). The combined organic layer was washed with brine (3 x 50 mL), dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography and eluted with 0% - 3% MeOH in DCM to afford **Ex-15e** as an oil. LCMS (ESI) calculated for C₂₃H₄₁BrN₂O₄ [M + H]⁺ found 489.3, 491.3. ¹H NMR (300 MHz, CD₃OD) δ 6.04-5.81 (m, 2H), 4.05 (d, J = 6.8, 2.8 Hz, 2H), 3.87-3.55 (m, 4H), 3.51-3.35 (m, 7H), 3.05-2.94 (m, 1H), 2.81-2.72 (m, 1H), 2.45-2.31 (m, 2H), 2.27-2.15 (m, 1H), 1.88-1.59 (m, 5H), 1.54-1.20 (m, 16H).

Step F: Synthesis of Ex-15f

[0543] To a solution of **Intermediate F-4** (50.0 mg, 0.085 mmol) in DMF (1 mL) was added NaH (3.56 mg, 0.089 mmol, 60% in mineral oil) at -10 °C. The reaction solution was stirred at -10 °C for 0.5 h. Then intermediate **Ex-15e** (45.6 mg, 0.093 mmol) was added to the solution at -10 °C. The reaction solution was stirred at -10 °C for 2 h. The resulting solution was quenched with NH₄Cl (1 mL) and the residue was purified by Flash (Column: Flash C¹⁸ 40 g; Mobile Phase A: water (0.05% TFA), Mobile Phase B: ACN; Flow rate: 45 mL/min; Gradient: 5% B to 35% B in 15 min; 35% B to 55% B in 20 min; Detector: UV 220 nm; Rt = 25 min) to afford **Ex-15f** as a solid. LCMS (ESI) calculated for C₅₅H₇₅FN₆O₁₀ [M + H]⁺: 999.6, found 999.5. ¹H NMR (300 MHz, CD₃OD) δ 7.35-7.25 (m, 2H), 7.19 (d, J = 7.5 Hz, 2H), 7.07 (d, J = 7.6 Hz, 1H), 7.01-6.86 (m, 3H), 6.27 (d, J = 15.2 Hz, 1H), 5.73-5.62 (m, 1H), 5.47 (d, J = 15.4 Hz, 1H), 5.31 (s, 1H), 4.87 (s, 3H), 4.68-4.56 (m, 2H), 4.24 (d, J = 15.1 Hz, 1H), 4.13 (d, J = 3.8 Hz, 1H), 3.90 (d, J = 17.2 Hz, 3H), 3.83-3.62 (m, 7H), 3.48-3.35 (m, 5H), 3.27 (s, 2H), 3.24-3.12 (m, 1H), 3.08-2.94 (m, 3H), 2.18 (s, 2H), 1.98 (s, 3H), 1.80-1.72 (m, 9H), 1.45 (d, J = 9.8 Hz, 15H), 1.29 (s, 4H).

Step G: Synthesis of Ex-15g

[0544] To a stirred solution of **Ex-15f** (30.0 mg, 0.03 mmol) in THF (1 mL) and water (0.1 mL) was added LiOH (2.16 mg, 0.09 mmol) at 0 °C. The reaction solution was stirred at room temperature for 16 h. The pH value of the solution was adjusted to 5 with 1 N HCl and the resulting solution was lyophilized to afford **Ex-15g** as a solid. LCMS (ESI) calculated for C₅₄H₇₃FN₆O₁₀ [M + H]⁺: 985.5, found 985.5.

5 Step H: Synthesis of Ex-15h

[0545] To a stirred solution of **Ex-15g** (400 mg, 0.410 mmol) in DMF (5 mL) were added HATU (170 mg, 0.450 mmol), (S)-tert-butyl 2-((S)-2-amino-N,3-dimethylbutanamido)-3-(4-methoxy-3-methylphenyl)propanoate hydrochloride (185 mg, 0.450 mmol) and DIEA (0.28 mL, 1.62 mmol) at 0 °C under nitrogen atmosphere. The reaction solution was stirred at room
10 temperature for 2 h. The resulting solution was quenched with water (2 mL) and the residue was purified by Flash (Column: Flash C¹⁸ 80 g; Mobile Phase A: water (0.05% TFA), Mobile Phase B: ACN; Flow rate: 65 mL/min; Gradient: 5% B to 45% B in 15 min; 45% B to 70% B in 20 min; Detector: UV 220 nm; Rt = 28 min) to afford **Ex-15h** as a solid. LCMS (ESI) calculated for C₇₅H₁₀₅FN₈O₁₃ [M + H]⁺: 1345.8, found 1345.8.

15 Step I: Synthesis of Ex-15i

[0546] To a stirred solution of **Ex-15h** (200 mg, 0.150 mmol) in DCM (3 mL) was added TFA (3 mL) at room temperature. The reaction solution was stirred at room temperature for 2 h. The solution was concentrated under reduced pressure and co-evaporated with toluene and DCM. The solution was dissolved in DCM (2 mL) and treated with HCl (4 N) in dioxane (0.3 mL). The
20 solution was concentrated under reduced pressure. The residue was re-dissolved in ACN (2 mL) and water (5 mL), and 1 N HCl (0.2 mL) added at 0 °C, and lyophilized to afford **Ex-15i** as a solid. LCMS (ESI) calculated for C₆₆H₉₀ClN₈O₋₁ [M - HCl + H]⁺: 1189.7, found 1189.6.

Step J: Synthesis of Ex-15j

[0547] To a stirred solution of **Ex-15i** (20.0 mg, 0.016 mmol) in DMF (3 mL) was added
25 HATU (7.44 mg, 0.020 mmol) under nitrogen atmosphere. The reaction solution was stirred at room temperature for 0.5 h. Then DCM (9 mL) and DIEA (8.43 mg, 0.065 mmol) were added to the solution. The reaction solution was stirred at room temperature for 2 h. The resulting solution was quenched with water (1 mL) and the residue was purified by Flash (Column: Flash C¹⁸ 20 g; Mobile Phase A: water (0.05% TFA), Mobile Phase B: ACN; Flow rate: 30 mL/min; Gradient:
30 5% B to 40% B in 15 min; 40% B to 60% B in 20 min; Detector: UV 220 nm; Rt = 25 min) to afford **Ex-15j** as a solid. LCMS (ESI) calculated for C₆₆H₈₇FN₈O₁₀ [M + H]⁺: 1171.7, found 1171.6.

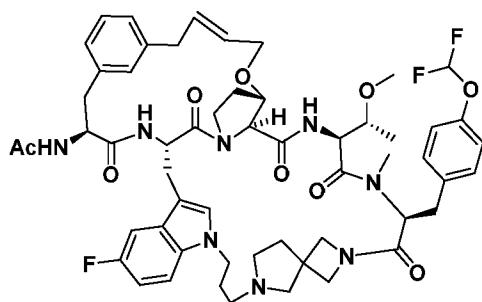
Step K: Synthesis of Ex-15 and Ex-16

[0548] Intermediate **Ex-15j** (220 mg, 0.188 mmol) was separated by Chiral-HPLC with the
35 following conditions: Column: CHIRALPAK IG, 20 x 250 mm, 5 µm; Mobile Phase A: Hex (8 mmol/L NH₃-MeOH), Mobile Phase B: MeOH : EtOH = 1 : 1; Flow rate: 20 mL/min; Gradient: 30% B in 27 min; Detector: UV 254/220 nm; Injection Volume: 0.5 mL; Number of Runs: 16.

5 The fractions at 18.41 min was collected and concentrated under reduced pressure to afford **Ex-15** as a solid. LCMS (ESI) calculated for $C_{68}H_{88}F_4N_8O_{10}$ $[M - TFA + H]^+$: 1171.7, found 1171.7. ^1H NMR (400 MHz, CD_3OD) δ 7.40 (d, $J = 12.6$ Hz, 2H), 7.28-7.11 (m, 3H), 7.03-6.91 (m, 3H), 6.86 (s, 1H), 6.67 (d, $J = 8.0$ Hz, 1H), 6.34 (d, $J = 7.4$ Hz, 1H), 6.17 (s, 1H), 5.71 (s, 3H), 5.55 (s, 1H), 5.39 (d, $J = 15.3$ Hz, 1H), 5.25 (s, 1H), 5.00 (d, $J = 6.7$ Hz, 1H), 4.83 (s, 1H), 4.79 (s, 1H), 10 4.73 (s, 2H), 4.51 (s, 1H), 4.33 (t, $J = 6.7$ Hz, 1H), 4.28 (s, 1H), 4.22 (s, 1H), 4.05-3.91 (m, 2H), 3.86 (s, 1H), 3.79 (s, 4H), 3.67 (d, $J = 28.5$ Hz, 2H), 3.52 (s, 1H), 3.34 (s, 2H), 3.32-3.20 (m, 6H), 3.06 (d, $J = 11.2$ Hz, 6H), 2.90 (t, $J = 11.7$ Hz, 1H), 2.67 (s, 3H), 2.18 (d, $J = 14.3$ Hz, 5H), 2.03 (s, 3H), 1.79-1.70 (m, 1H), 1.52-1.42 (m, 1H), 1.40 (s, 3H), 1.38-1.30 (m, 1H), 1.28 (s, 5H), 1.16 (s, 2H), 1.03-0.92 (m, 5H), 0.77 (d, $J = 6.7$ Hz, 3H). The fraction at 22.75 min was collected and concentrated under reduced pressure to afford **Ex-16** as a solid. LCMS (ESI) calculated for $C_{66}H_{87}FN_8O_{10}$ $[M + H]^+$: 1171.7, found 1171.6. ^1H NMR (400 MHz, CD_3OD) δ 7.48 (d, $J = 14.1$ Hz, 2H), 7.29-7.22 (m, 1H), 7.21 (s, 1H), 7.15 (d, $J = 7.7$ Hz, 1H), 7.04-6.92 (m, 4H), 6.88-6.79 (m, 2H), 6.65 (d, $J = 8.1$ Hz, 1H), 6.37 (d, $J = 7.6$ Hz, 1H), 6.20 (s, 1H), 5.77 (s, 1H), 5.67 (d, $J = 14.2$ Hz, 1H), 5.54 (s, 1H), 5.38 (d, $J = 15.5$ Hz, 1H), 5.18 (s, 1H), 5.00 (d, $J = 6.0$ Hz, 1H), 4.79 20 (d, $J = 16.6$ Hz, 4H), 4.55 (s, 1H), 4.24 (d, $J = 17.1$ Hz, 2H), 4.05-3.91 (m, 1H), 3.78 (s, 2H), 3.66-3.51 (m, 1H), 3.41 (s, 3H), 3.36-3.25 (m, 4H), 3.18 (s, 4H), 3.08 (s, 4H), 3.01 (d, $J = 9.5$ Hz, 1H), 2.96-2.85 (m, 1H), 2.71 (d, $J = 11.8$ Hz, 1H), 2.63 (d, $J = 12.3$ Hz, 1H), 2.55 (d, $J = 14.8$ Hz, 1H), 2.20 (s, 1H), 2.14 (s, 3H), 2.04 (s, 4H), 1.87 (s, 2H), 1.67 (s, 3H), 1.39 (d, $J = 36.6$ Hz, 8H), 1.27 (s, 4H), 1.11 (s, 2H), 0.96 (d, $J = 6.7$ Hz, 3H), 0.80 (d, $J = 6.6$ Hz, 3H).

25

Example 48: Synthesis of Example compound 44 (Ex-44) (HCl salt)



Ex-44

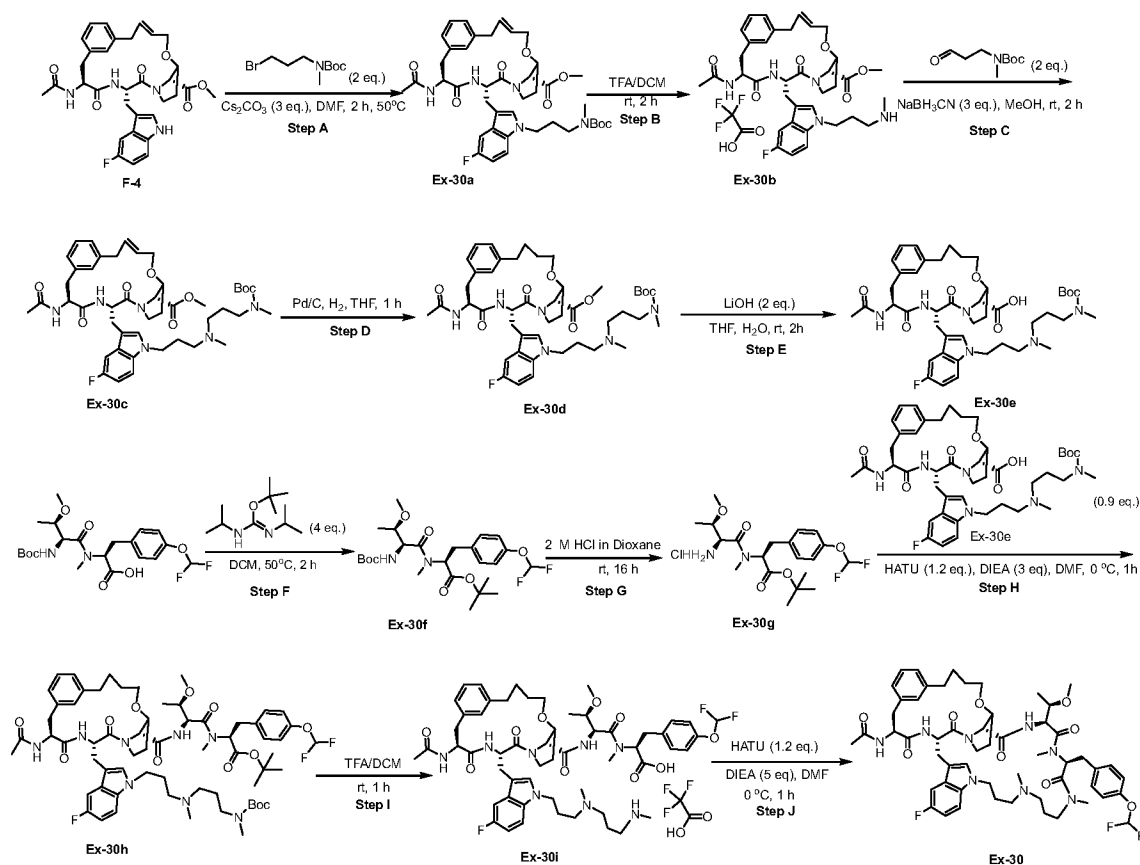
[0549] Compound **Ex-44** was prepared from **Ex-27** and **Int 22** by applying the synthetic procedure used for **Ex-41**.

30 [0550] The crude cyclic peptide was purified by preparative reversed-phase high performance liquid chromatography (RP-HPLC) [Luna C18 (Phenomenex) (column size 30 * 250 mm, 100Å,

5 5 μ m). mobile phase: (A) 0.1% TFA in HPLC water and (B) 0.1% TFA in HPLC acetonitrile, flow rate: 30 mL/min; UV wavelength λ = 214 nm; gradient: 30% B over 5 min, 30 to 55 % B over 5-25 min, 50 to 60 % B over 25-30 min. Sample load: (%B): 20, 5 ml/min, DMSO] to give purified cyclic peptide as TFA salt then salt exchange was made to HCl salt and lyophilized to afford **Ex-44** (major isomer) as HCl salt solid. UPLC-MS: $[M+H]^+ = 1053.8$ (mass of the salt

10 free form, $C_{56}H_{67}F_3N_8O_9$, is 1053.19).

Example 49: Synthesis of Example compound 30 (Ex-30)



Step A-Synthesis of Ex-30a

[0551] To a stirred solution of **Intermediate F-4** (200 mg, 0.339 mmol) in DMF (4 mL) were added Cs_2CO_3 (331 mg, 1.016 mmol) and $tert$ -butyl (3-bromopropyl)(methyl)carbamate (256 mg, 1.016 mmol) at 25 °C under nitrogen atmosphere. The reaction solution was stirred at 50 °C

20 for 2 h. The reaction was quenched with water (60 mL). The reaction solution was extracted with EA (2 x 100 mL). The combined organic layer was washed with brine (3 x 50 mL), dried over

5 anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure to afford 210 mg of **Ex-30a** as an oil. LCMS 762.5 (M + H)⁺. ¹H NMR (300 MHz, CD₃OD) δ 7.99 (s, 1H), 7.57 (s, 1H), 7.42-6.81 (m, 5H), 5.80-5.59 (m, 1H), 5.58-5.34 (m, 1H), 4.95 (t, *J* = 7.1 Hz, 1H), 4.64 (s, 2H), 4.35-3.97 (m, 3H), 3.97-3.62 (m, 4H), 3.51-3.06 (m, 7H), 3.00 (s, 6H), 2.86 (d, *J* = 3.4 Hz, 6H), 2.12-1.78 (m, 4H), 1.56 (d, *J* = 61.6 Hz, 11H).

10 Step B-Synthesis of **Ex-30b**

[0552] To a solution of **Ex-30a** (255 mg, 0.335 mmol) in DCM (3 mL) at 0 °C was added TFA (1 mL, 15.1 mmol). The reaction solution was stirred at room temperature for 2 h. The solvent was concentrated under reduced pressure to afford 260 mg of **Ex-30b** as an oil. LCMS 662.4 (M – TFA + H)⁺.

15 Step C-Synthesis of **Ex-30c**

[0553] To a solution of **Ex-30b** (180 mg, 0.237 mmol) in MeOH (5 mL) were added *tert*-butyl methyl(3-oxopropyl)carbamate (89 mg, 0.474 mmol) and Et₃N (0.099 mL, 0.712 mmol) at room temperature and the mixture was stirred at room temperature for 30 min. Then NaBH₃CN (44.7 mg, 0.712 mmol) was added at 0 °C. The mixture was stirred at room temperature for 16 h. The reaction was quenched with water (20 mL). The reaction mixture was extracted with EA (2 x 50 mL). The combined organic layer was washed with brine (20 mL), dried over anhydrous Na₂SO₄ and filtered. The filtrate was purified by reversed mobile phase Flash (Column: C¹⁸, 40 g; Mobile Phase A: water (10 mmol NH₄HCO₃), Mobile Phase B: ACN; Flow rate: 40 mL/min; Gradient: 10% B to 30% B in 10 min; 40% B to 80% B in 30 min; 210 nm, RT: 19 min; The fractions were collected and concentrated under reduced pressure to afford 150 mg of **Ex-30c** a solid. LCMS 833.5 (M + H)⁺. ¹H NMR (300 MHz, CD₃OD) δ 7.44-7.34 (m, 1H), 7.34-7.25 (m, 1H), 7.24-7.14 (m, 2H), 7.07 (d, *J* = 7.6 Hz, 1H), 7.01-6.89 (m, 3H), 5.73-5.62 (m, 1H), 5.50 (s, 1H), 4.63 (d, *J* = 7.0 Hz, 2H), 4.24 (d, *J* = 14.1 Hz, 3H), 4.12 (d, *J* = 3.4 Hz, 1H), 3.90 (d, *J* = 15.3 Hz, 1H), 3.73 (s, 3H), 3.27 (s, 4H), 3.28-3.11 (m, 1H), 3.09-2.94 (m, 6H), 2.84 (s, 3H), 2.74 (s, 3H), 2.23 (s, 2H), 2.01 (d, *J* = 7.7 Hz, 4H), 1.86 (s, 3H), 1.45 (d, *J* = 9.9 Hz, 11H), 1.33-1.19 (m, 1H).

30 Step D: Synthesis of **Ex-30d**

To a stirred solution of **Ex-30c** (150 mg, 0.180 mmol) in THF (10 mL) under nitrogen was added Pd-C (192 mg, 0.180 mmol, 10%) at 25 °C. The mixture was degassed with hydrogen for 3 times at room temperature for 1 h. The solid was filtered out and the filtrate was concentrated under reduced pressure to afford 120 mg of **Ex-30d** as a solid. LCMS 835.6 (M + H)⁺.

5 Step E-Synthesis of Ex-30e

[0554] To a solution of **Ex-30d** (120 mg, 0.144 mmol) in THF (2 mL) and Water (2mL) was added LiOH (0.287 ml, 0.287 mmol) at 0 °C. The reaction solution was stirred at room temperature for 16 h. The pH value of the reaction mixture was adjusted to 4 with HCl (1 M). The solution was extracted with EA (2 x 50 mL). The combined organic layer was washed with
10 brine (30 mL), dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure to afford 110 mg of **Ex-30e** as a solid. LCMS 821.7 (M + H)⁺.

Step F-Synthesis of Ex-30f

[0555] To a solution of (S)-2-((2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methoxy-N-methylbutanamido)-3-(4-(difluoromethoxy)phenyl)propanoic acid (300 mg, 0.652 mmol) and
15 (Z)-tert-butyl N,N'-diisopropylcarbamidate (522 mg, 2.61 mmol) in DCM (10 mL) at room temperature and the mixture was stirred at 50°C for 2 h. The reaction solution was cooled to room temperature, the solid was filtered out. The filtrate was partitioned between water (50 mL) and EA (60 mL x 2). The organic layer was washed with brine (50 mL), dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure and the residue was
20 purified by a silica gel column chromatography, eluted with 0-16% EA in PE to afford 210 mg of **Ex-30f** as an oil. LCMS 517.2 (M + H)⁺.

Step G-Synthesis of Ex-30g

[0556] **Ex-30f** (160 mg, 0.310 mmol) was dissolved in HCl(2M) in 1,4-dioxane (10 ml) at 25°C. The reaction solution was stirred at room temperature for 16 h. The reaction solution was
25 concentrated under reduced pressure. The fractions containing desired product were combined and concentrated under reduced pressure to afford 150 mg of **Ex-30g** as a solid. LCMS 417.2 (M – HCl + H)⁺.

Step H-Synthesis of Ex-30h

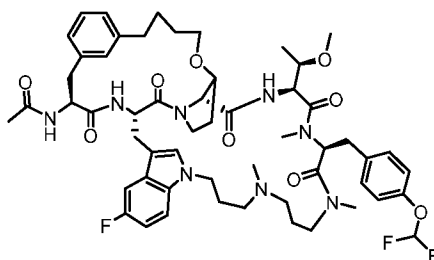
[0557] To a stirred solution of **Ex-30e** (20 mg, 0.024 mmol) in DMF (1 mL) was added HATU
30 (11.1 mg, 0.029 mmol) at 0 °C under nitrogen atmosphere. The reaction solution was stirred at 0 °C for 10 min. **Ex-30g** (22.1 mg, 0.049 mmol) and DIEA (0.013 mL, 0.073 mmol) were added to the solution and stirred at 0 °C for 1 h. The reaction was quenched with water (1 mL). The solvent was concentrated under reduced pressure and the residue was purified by reversed mobile phase Flash (Column: C¹⁸, 40 g; Mobile Phase A: water (10 mmol NH₄HCO₃), Mobile Phase B: ACN; Flow rate: 40 mL/min; Gradient: 10% B to 30% B in 10 min; 40% B to 70% B in 30 min;
35 210 nm, RT: 25 min; The fractions were collected and concentrated under reduced pressure to afford 19 mg of **Ex-30h** as a solid. LCMS 1219.6 (M + H)⁺. ¹H NMR (300 MHz, CD₃OD) δ 7.35 (s, 4H), 7.31-7.18 (m, 2H), 7.04 (d, J = 8.0 Hz, 3H), 6.92 (s, 2H), 6.79 (d, J = 9.2 Hz, 2H), 5.04

(s, 2H), 4.79 (s, 1H), 4.62 (s, 2H), 4.19 (s, 3H), 3.89 (s, 1H), 3.66 (d, $J = 10.7$ Hz, 3H), 3.37 (s, 3H), 3.20 (t, $J = 7.0$ Hz, 2H), 3.08 (s, 6H), 2.80 (s, 4H), 2.38 (s, 7H), 2.25 (s, 4H), 2.02 (s, 5H), 1.92 (s, 4H), 1.66 (s, 6H), 1.45 (d, $J = 11.7$ Hz, 18H), 0.90 (s, 3H).

Step I-Synthesis of Ex-30i

[0558] To a solution of **Ex-30h** (130 mg, 0.107 mmol) in DCM (1 mL) was added TFA (1 mL) at 25°C. The reaction solution was stirred at room temperature for 1 h. The reaction solution was concentrated under reduced pressure. The fractions containing desired product were combined and concentrated under reduced pressure to afford 130 mg of **Ex-30i** (1:1 2,2,2-trifluoroacetic acid salt) as a solid. LCMS 1061–8 (M - TFA -H)⁺.

Step J- Synthesis of Ex-30



15

[0559] To a stirred solution of Ex-30i (2,2,2-trifluoroacetic acid salt) (130 mg, 0.110 mmol) in DMF (1.6 mL) was added DIEA (0.096 mL, 0.552 mmol) at 0 °C under nitrogen atmosphere. The reaction solution was stirred at 0 °C for 30 min. DCM (4.9 mL) and HATU (50.4 mg, 0.133 mmol) were added to the solution and stirred at 0 °C for 1 h. The reaction was quenched with water (1 mL). The solvent was concentrated under reduced pressure and the residue was purified by reversed mobile phase Flash (Column: C18, 40 g; Mobile Phase A: water (10 mmol NH₄HCO₃), Mobile Phase B: ACN; Flow rate: 40 mL/min; Gradient: 10% B to 30% B in 10 min; 40% B to 70% B in 30 min; 210 nm, RT: 33 min; The fractions were collected and concentrated under reduced pressure to afford 35.6 mg of Ex-30 as a solid. LCMS 1045.6 (M + H)⁺. ¹H NMR (400 MHz, CD₃OD) δ 7.49-7.29 (m, 3H), 7.31-7.13 (m, 5H), 7.06-6.88 (m, 4H), 6.88-6.61 (m, 2H), 5.25 (d, $J = 30.3$ Hz, 1H), 4.70 (s, 1H), 4.55 (s, 2H), 4.29 (s, 4H), 4.16 (s, 1H), 4.10 (s, 1H), 3.99 (s, 2H), 3.76 (d, $J = 6.5$ Hz, 1H), 3.71 (s, 2H), 3.59 (d, $J = 15.4$ Hz, 2H), 3.47 (t, $J = 10.0$ Hz, 1H), 3.33 (d, $J = 10.6$ Hz, 2H), 3.26 (s, 1H), 3.12 (s, 4H), 3.10-3.00 (m, 1H), 2.89 (d, $J = 4.6$ Hz, 3H), 2.81 (s, 1H), 2.75 (d, $J = 1.7$ Hz, 3H), 2.45 (t, $J = 8.5$ Hz, 4H), 2.29 (s, 1H), 2.17 (s, 2H), 2.04 (d, $J = 1.8$ Hz, 3H), 1.94 (s, 1H), 1.69 (s, 1H), 1.60 (s, 1H), 1.31 (s, 5H), 1.29 (d, $J = 1.6$ Hz, 1H), 1.09 (s, 1H), 0.92 (s, 1H).

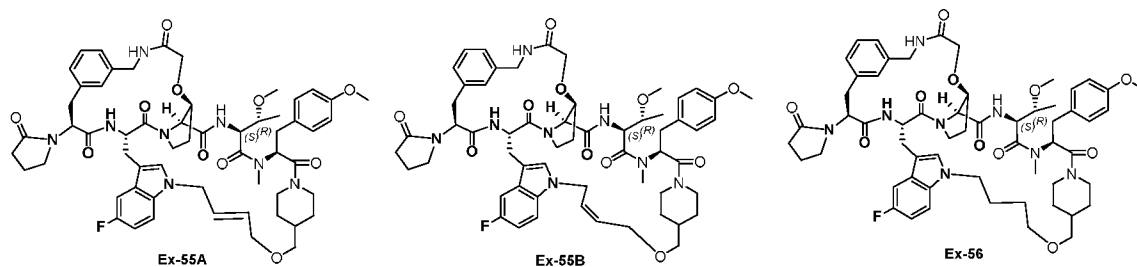
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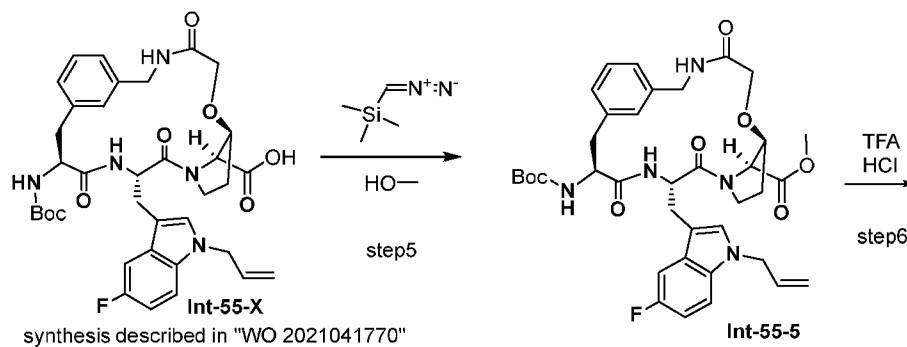
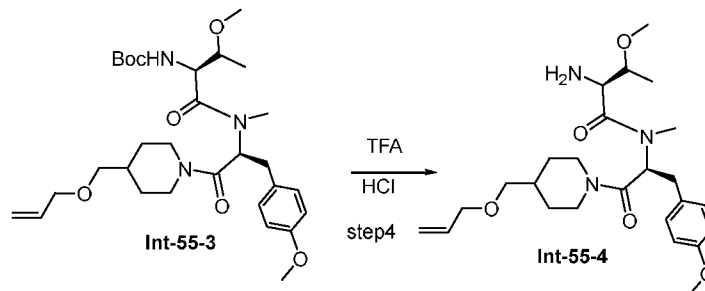
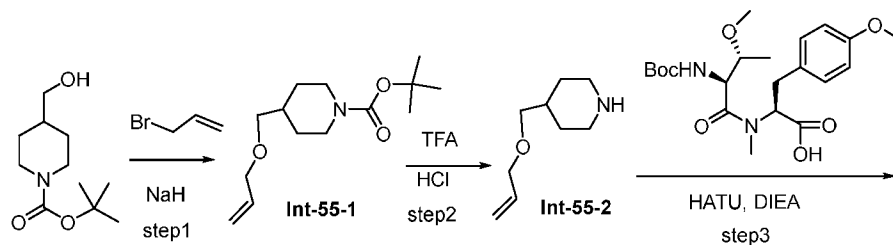
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5 [0560] **Example 50:** Synthesis of Example compounds 55A, 55B, and 56

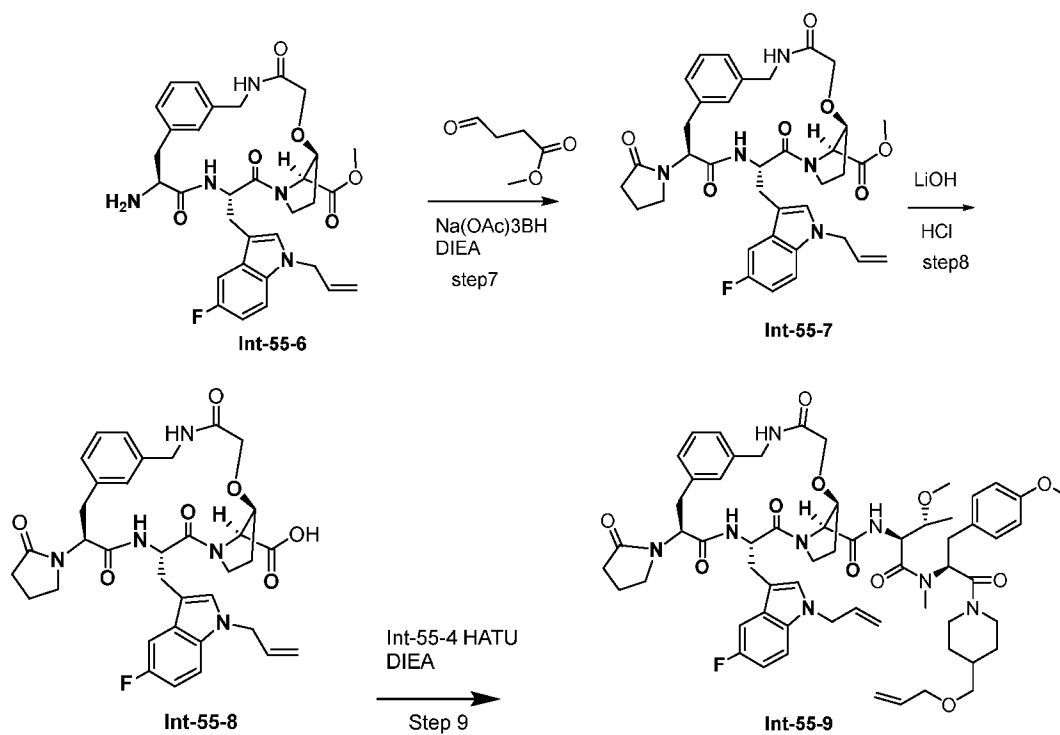
The structures of compounds 55A, 55B, and 56 are as follows.



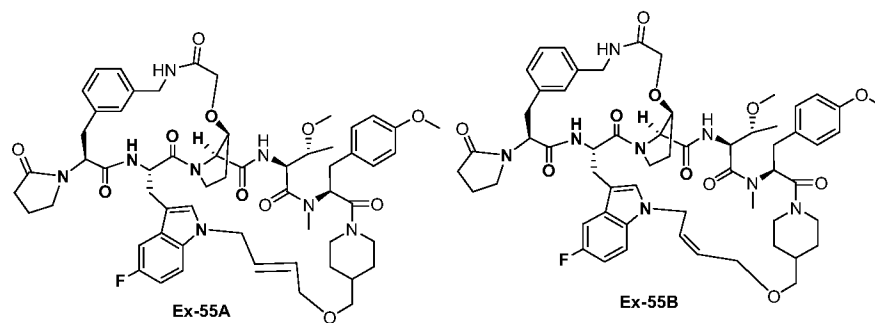
These compounds were synthesized using the following reaction scheme.



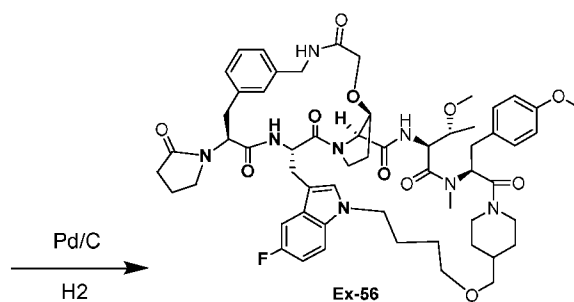
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ZHAN
 CATALYST-1B
 DCM



10



5 *Step 1: Synthesis of Int-55-1*

[0561] To a 40- ml vial with a magnetic stir bar, tert-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (Aldrich) (500 mg, 2.322 mmol) and SODIUM HYDRIDE (60% in oil) (139 mg, 3.48 mmol) was charged dry THF (11.6 ml) under N₂ atmosphere. The mixture was stirred at room temperature for about 5 min or until bubbling ceased. Next, ALLYL BROMIDE (322 µl, 10 3.72 mmol) was added. The mixture was stirred at room temperature for about 1h. LCMS check showed desired product, but the reaction was not complete. The mixture was left stirring at room temperature overnight. LCMS check showed desired product as the major product.

[0562] The resulting reaction mixture was quenched with addition of MeOH (~ 5 ml), and concentrated in vacuo. The residue was purified by normal phase chromatography (Isco system 15 using 40 g ISCO RediSep silica gold column and eluted with 0-100% EtOAc/hexane). The major fractions were combined and concentrated to afford tert-butyl 4-((allyloxy)methyl)piperidine-1-carboxylate (Int-55-1) as an oil (529 mg). For C₁₄H₂₅NO₃, calc. [M+H] = 256.2; [M-tBu (-56) +H = 200.2]; [M-Boc (-100)+H = 156.2]; found on LCMS: [M+H] = 256; [M-tBu (-56) +H] = 200.1; [M-Boc (-100)+H] = 156.1

20 ¹H NMR (500 MHz, Methanol-*d*₄) δ 5.92 (ddt, *J* = 16.8, 10.8, 5.5 Hz, 1H), 5.35 – 5.24 (m, 1H), 5.17 (d, *J* = 10.4 Hz, 1H), 4.09 (d, *J* = 13.2 Hz, 2H), 4.01 – 3.90 (m, 2H), 3.33 – 3.29 (m, 2H, under solvent peak), 2.77 (s,br, 2H), 1.90 – 1.65 (m, 3H), 1.47 (s, 9H), 1.14 (qd, *J* = 12.5, 4.3 Hz, 2H).

25 *Step 2: Synthesis of Int-55-2*

[0563] To a stirred solution of tert-butyl 4-((allyloxy)methyl)piperidine-1-carboxylate (Int-1, 520 mg, 2.036 mmol) in CH₂Cl₂ (17 ml) was added TFA (18.8 ml, 244 mmol). The mixture was stirred at room temperature for about 65 min. LCMS check showed reaction completed. The mixture was concentrated. The residue was redissolved in hplc grade acetonitrile-water (2:1, 20 30 ml), HCl (2.0 M in water) (Aldrich) (3055 µl, 6.11 mmol) added, mixed well, and lyophilized to give 4-((allyloxy)methyl)piperidine HCl salt (Int-55-2). For C₉H₁₇NO·HCl; calc. [M+H] = 156.13; found on LCMS: [M+H] = 156.1.

35 ¹H NMR (500 MHz, Methanol-*d*₄) δ 5.90 (ddt, *J* = 16.9, 10.8, 5.4 Hz, 1H), 5.30 - 5.23 (m, 1H), 5.16 (d, *J* = 10.5 Hz, 1H), 4.00 - 3.96 (m, 2H), 3.41 (s, 1H), 3.39 - 3.34 (m, 3H), 2.99 (t, *J* = 12.8 Hz, 2H), 1.98 (s, 1H), 1.97 - 1.88 (m, 2H), 1.50 (s, 1H), 1.47 (d, *J* = 10.6 Hz, 1H).

5 *Step 3: Synthesis of Int-55-3*

[0564] To a stirred solution of (S)-2-((2S,3R)-2-((tert-butoxycarbonyl)amino)-3-methoxy-N-methylbutanamido)-3-(4-methoxyphenyl)propanoic acid (155 mg, 0.365 mmol) and HATU (Aldrich) (146 mg, 0.383 mmol) in DMF (3651 μ l) were added DIEA (Aldrich) (255 μ l, 1.461 mmol) and 4-((allyloxy)methyl)piperidine HCl (Int-55-2, 77 mg, 0.402 mmol) at 0°C. The mixture was stirred at room temperature for about 1h. LCMS check showed desired product as the major product. The mixture was diluted with DMSO (~ 2 ml) and purified by reverse phase HPLC purification using Isco system and RediSepRf Gold C18 column (50 g) using 0-100% acetonitrile-water (buffering with 0.05 TFA%) to afford tert-butyl ((2S,3R)-1-(((S)-1-(4-((allyloxy)methyl)piperidin-1-yl)-3-(4-methoxyphenyl)-1-oxopropan-2-yl)(methyl)amino)-3-methoxy-1-oxobutan-2-yl)carbamate (Int-55-3, 148 mg) as a solid after lyophilization. For C₃₀H₄₇N₃O₇, calc. [M+H] = 562.34; [M+Na] = 584.34; found on LCMS: [M+H] = 562.2; [M+Na] = 584.2

Step 4: Synthesis of Int-55-4

[0565] To a stirred solution of tert-butyl ((2S,3R)-1-(((S)-1-(4-((allyloxy)methyl)piperidin-1-yl)-3-(4-methoxyphenyl)-1-oxopropan-2-yl)(methyl)amino)-3-methoxy-1-oxobutan-2-yl)carbamate (Int-55-3, 146 mg, 0.260 mmol) in CH₂Cl₂ (2166 μ l), TFA (TFA) (2403 μ l, 31.2 mmol) was added. The mixture was stirred at room temperature for about 65 min. LCMS check showed reaction completed. The mixture was concentrated. The residue was redissolved in hplc grade acetonitrile- water (2:1, 20 ml), added HCl (2.0 M in water) (Aldrich) (390 μ l, 0.780 mmol), mixed well and lyophilized to afford (2S,3R)-N-((S)-1-(4-((allyloxy)methyl)piperidin-1-yl)-3-(4-methoxyphenyl)-1-oxopropan-2-yl)-2-amino-3-methoxy-N-methylbutanamide HCl (Int-55-4). For C₂₅H₃₉N₃O₅·HCl, calc. [M+H] = 462.29, found on LCMS: [M+H] = 462.3.

30 *Step 5: Synthesis of Int-55-5*

[0566] To a stirred solution of (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-9-((tert-butoxycarbonyl)amino)-4,10,13-trioxo-2-oxa-5,11-diaza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxylic acid (Int-55-X, 2000 mg, 2.89 mmol; synthesis was described in WO 2021041770,) in CH₂Cl₂ (19.3 ml) and MeOH (anhydrous, Aldrich) (17.5 ml, 434 mmol), TMS-Diazomethane (2.0 M in hexane) (Aldrich) (2891 μ l, 5.78 mmol) was added. The mixture was stirred at room temperature for about 2 h. LCMS check showed that the reaction was not complete. More TMS-Diazomethane (2.0 M in hexane) (Aldrich) (2891 μ l, 5.78 mmol),

5 was added followed by stirring overnight at room temperature. The reaction was then quenched with addition of acetic acid (~ 2 ml). The reaction mixture was then concentrated and purified by normal phase chromatography using 40 g Isco silica gel gold column and 0-100% EtOAc-EtOH (3:1)/hexane as eluent to give the desired product methyl (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-9-((tert-butoxycarbonyl)amino)-4,10,13-trioxo-2-oxa-5,11-diaza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxylate (Int-55-5, 1861 mg) a solid. For C₃₇H₄₄FN₅O₈, calc. [M+H] = 706.32; found on LCMS: [M+H] = 706.4.

Step 6: Synthesis of Int-55-6

[0567] To a stirred solution of methyl (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-9-((tert-butoxycarbonyl)amino)-4,10,13-trioxo-2-oxa-5,11-diaza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxylate (Int-55-5, 940 mg, 1.332 mmol) in CH₂Cl₂ (16.6 ml), TFA (16.4 ml, 213 mmol) was added. The mixture was stirred at room temperature for about 30 min. LCMS check showed that the reaction was complete. The mixture was concentrated. The residue was re-dissolved in hplc grade acetonitrile-water (2:1, 20 ml), and HCl (2.0 M in water) (Aldrich) (1998 µl, 4.00 mmol) was added and mixed well, followed lyophilization to give the desired product methyl (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-9-amino-4,10,13-trioxo-2-oxa-5,11-diaza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxylate HCl as a solid. For C₃₂H₃₆FN₅O₆·HCl, calc. [M+H] = 606.26; found on LCMS: [M+H] = 606.3.

25

Step 7: Synthesis of Int-55-7

[0568] To a stirred solution of methyl (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-9-amino-4,10,13-trioxo-2-oxa-5,11-diaza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxylate HCl (Int-55-6, 500 mg, 0.779 mmol) in dry CH₂Cl₂ (9733 µl), DIEA (Aldrich) (204 µl, 1.168 mmol) and METHYL 4-OXOBUTANOATE (Aldrich) (98 µl, 0.934 mmol) were added, followed by addition of 4A molecular sieve (~ 1000 mg, Aldrich). The mixture was stirred at room temperature for about 10 min. Next, SODIUM TRIACETOXYBOROHYDRIDE (Aldrich) (825 mg, 3.89 mmol) and more DIEA (340 µl, 1.947 mmol) were added. The mixture was stirred at room temperature overnight. LCMS check showed the starting material mostly consumed. The uncyclized alkylation amine product (0133-A) and cyclized product were both observed on the crude LCMS.) The reaction was quenched with addition of MeOH (~ 20 ml). The mixture was filtered, and the filtrate was concentrated. The residue was redissolved in DCM and stirred overnight at 50 °C. The mixture was cooled to room

35

5 temperature, and crude LCMS check showed the desired product ($M+H = 674$). The mixture was concentrated, and the residue was redissolved in DMSO-water (4:1, 20 ml), filtered, and purified by reverse phase HPLC using Isco system and RediSepRf Gold C18 column (275 g) using 0-100% acetonitrile-water (each buffering with 0.05 % TFA) to afford methyl (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-4,10,13-trioxo-9-(2-oxopyrrolidin-1-yl)-2-oxa-5,11-diaza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxylate (Int-55-7, 72 mg) as a solid after lyophilization.

For C₃₆H₄₀FN₅O₇, calc. $[M+H] = 674.29$; found on LCMS: $[M+H] = 674.4$

Step 8: Synthesis of Int-55-8

15 **[0569]** To a stirred solution of methyl (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-4,10,13-trioxo-9-(2-oxopyrrolidin-1-yl)-2-oxa-5,11-diaza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxylate (Int-55-7, 70 mg, 0.104 mmol) in MeOH (649 μ l), THF (649 μ l), LITHIUM HYDROXIDE MONOHYDRATE (1.0 M in water) (416 μ l, 0.416 mmol) and water (233 μ l) were added. The mixture was stirred at room temperature for 2.5 h.

20 LCMS check showed that the reaction was complete. The mixture was neutralized with addition of HCl (2.0 M in water) (Aldrich) (208 μ l, 0.416 mmol), then partitioned between EtOAc-satd. NaCl-water (5 ml-2ml-2-ml). The aqueous was extracted with EtOAc for three times (5ml x 3). Organic phases were combined, dried over Na₂SO₄, filtered and concentrated to give (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-4,10,13-trioxo-9-(2-oxopyrrolidin-1-yl)-2-oxa-5,11-diaza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxylic acid (Int-55-8, 68 mg) as a solid.

For C₃₅H₃₈FN₅O₇, calc. $[M+H] = 660.28$; found on LCMS: $[M+H] = 660.2$

Step 9: Synthesis of Int-55-9

30 **[0570]** To the stirred solution of (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-4,10,13-trioxo-9-(2-oxopyrrolidin-1-yl)-2-oxa-5,11-diaza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxylic acid (Int-55-8, 65 mg, 0.099 mmol), HATU (Aldrich) (41.2 mg, 0.108 mmol) and (2S,3R)-N-((S)-1-(4-((allyloxy)methyl)piperidin-1-yl)-3-(4-methoxyphenyl)-1-oxopropan-2-yl)-2-amino-3-methoxy-N-methylbutanamide, HCl (Int-55-4, 58.9 mg, 0.118 mmol) in DMF (985 μ l) was added DIEA (Aldrich) (138 μ l, 0.788 mmol) at 0°C. The mixture was stirred at room temperature for about 3h. The mixture was purified by reverse HPLC using Teledyne Isco system, RediSepRf Gold C18 column (50 g) and 0-100% acetonitrile-

5 water (buffering with 0.05 % TFA for each solvent) as eluent to afford the desired product
 (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-N-((2S,3R)-1-(((S)-1-(4-
 ((allyloxy)methyl)piperidin-1-yl)-3-(4-methoxyphenyl)-1-oxopropan-2-yl)(methyl)amino)-3-
 methoxy-1-oxobutan-2-yl)-4,10,13-trioxo-9-(2-oxopyrrolidin-1-yl)-2-oxa-5,11-diaza-1(3,1)-
 pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxamide (Int-55-9, 102 mg) as a solid after
 10 lyophilization. For C₆₀H₇₅FN₈O₁₁, calc. [M+H] = 1103.55, found on LCMS: [M+H] = 1103.7

Step 10: Synthesis of Ex-55A, Ex-55B

[0571] The solution of (12S,13S,9S,12S)-12-((1-allyl-5-fluoro-1H-indol-3-yl)methyl)-N-
 ((2S,3R)-1-(((S)-1-(4-((allyloxy)methyl)piperidin-1-yl)-3-(4-methoxyphenyl)-1-oxopropan-2-
 15 yl)(methyl)amino)-3-methoxy-1-oxobutan-2-yl)-4,10,13-trioxo-9-(2-oxopyrrolidin-1-yl)-2-oxa-
 5,11-diaza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphane-12-carboxamide (Int-55-9, 100
 mg, 0.091 mmol) in dry CH₂Cl₂ (181 ml) was bubbled with N₂ for 20 min, then was added
 ZHAN CATALYST-1B (Strem) (33.3 mg, 0.045 mmol). The resulting mixture was further
 bubbled with N₂ for additional 20min, followed by stirring at room temperature overnight under
 20 N₂. LCMS check showed the desired mass as the major peak. The mixture was concentrated in
 rotavap (water bath was kept ~ 25 °C), and the residue was purified by normal phase
 chromatography (Isco system using 40 g ISCO RediSep silica gold column and eluted with 0-
 100% EtOAc-EtOH (3:1)/hexane). Major fractions were concentrated to give the desired product
 as a solid (51 mg). The product was redissolved in DMSO (~ 2 ml) and then submitted to HTP
 25 group for further purification (isomers separation). It was purified by mass directed reverse
 HPLC using the following conditions (TFA_50mL_39_44_25m_V3_C1): column: Waters CSH
 C18, 5u, 30x150 mm Flow rate: 50 ml/min gradient and eluted with: 39%-44% acetonitrile-
 water (both buffering with 0.16% TFA) for 25 min. The collected fractions with desired mass
 were lyophilized to afford Ex-55A (faster and eluted, more polar, 25.8 mg) as a solid and Ex-55B
 30 (slower and eluted, less polar, 2.3 mg) as a solid (2.3 mg).
 For C₅₈H₇₁FN₈O₁₁, calc. [M+H] = 1075.52; found Ex-55A: [M+H] = 1075.5 (rt = 1.12 min by
 2-min positive high mass method on uplc); found Ex-55B: [M+H] = 1075.5 (rt = 1.17 min by 2-
 min positive high mass method on uplc)

35 *Step 11: Synthesis of Ex-56*

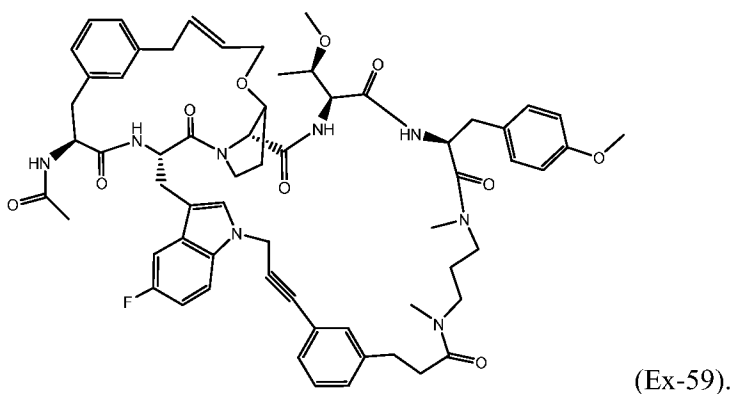
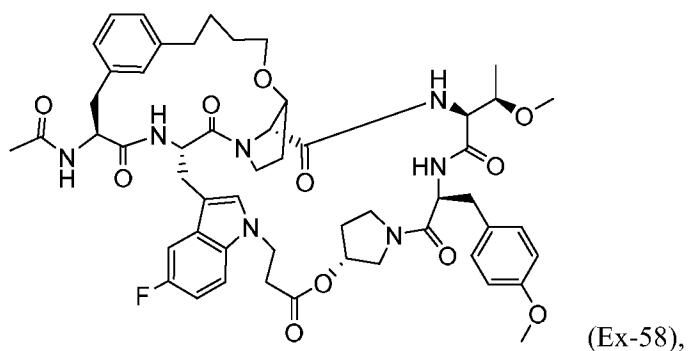
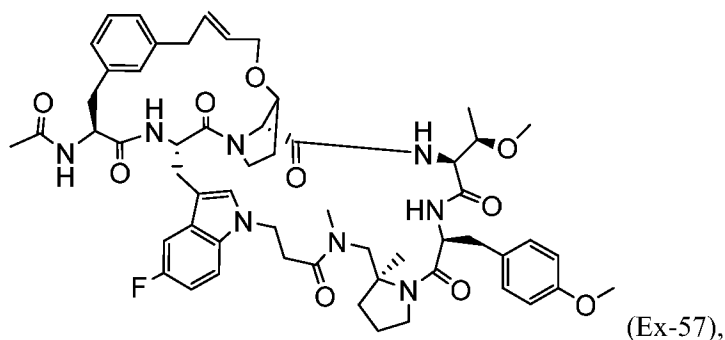
[0572] To a round bottom flask (50 ml) was added PALLADIUM ON CARBON (10%) (1.979
 mg, 1.860 μmol), followed by addition of (Ex-55A, 4.0 mg, 3.72 μmol) in Ethanol (3720 μl).

- 5 The flask was equipped with a hydrogen balloon. Next, the system was degassed and flushed with H₂ twice. The mixture was stirred at room temperature overnight under H₂ overnight. LCMS check showed reaction completed. The catalyst was filtered off, and the filtrate was evaporated to dryness to give the desired product as a solid.
- For C₅₈H₇₃FN₈O₁₁, calc. [M+H] = 1077.54; found on LCMS: M+H = 1077.1; M+Na = 1099.5.

10

[0573] Example 51: Synthesis of Example compounds 57, 58, and 59

The structures of Example Compounds 57, 58, and 59 are:

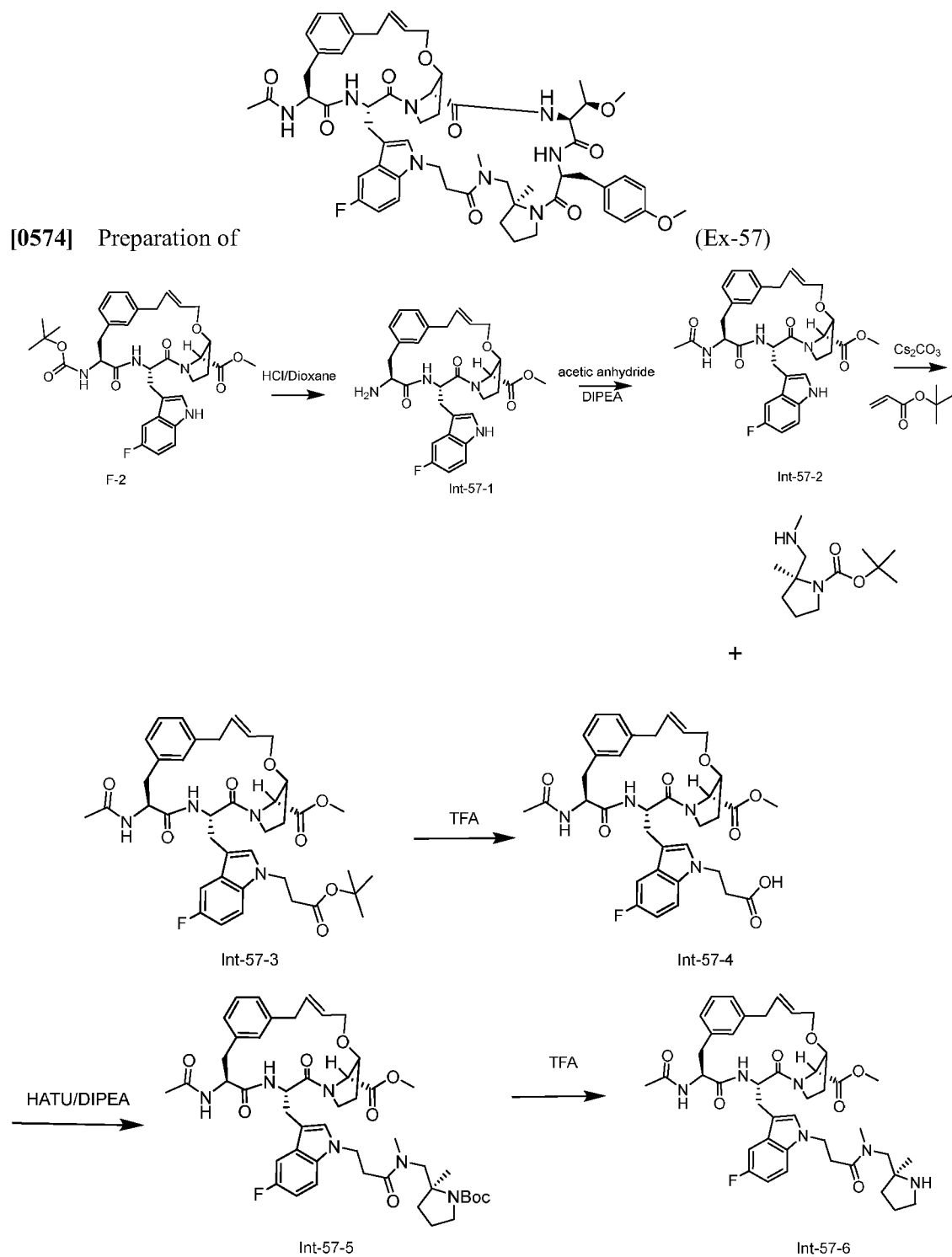


15 and

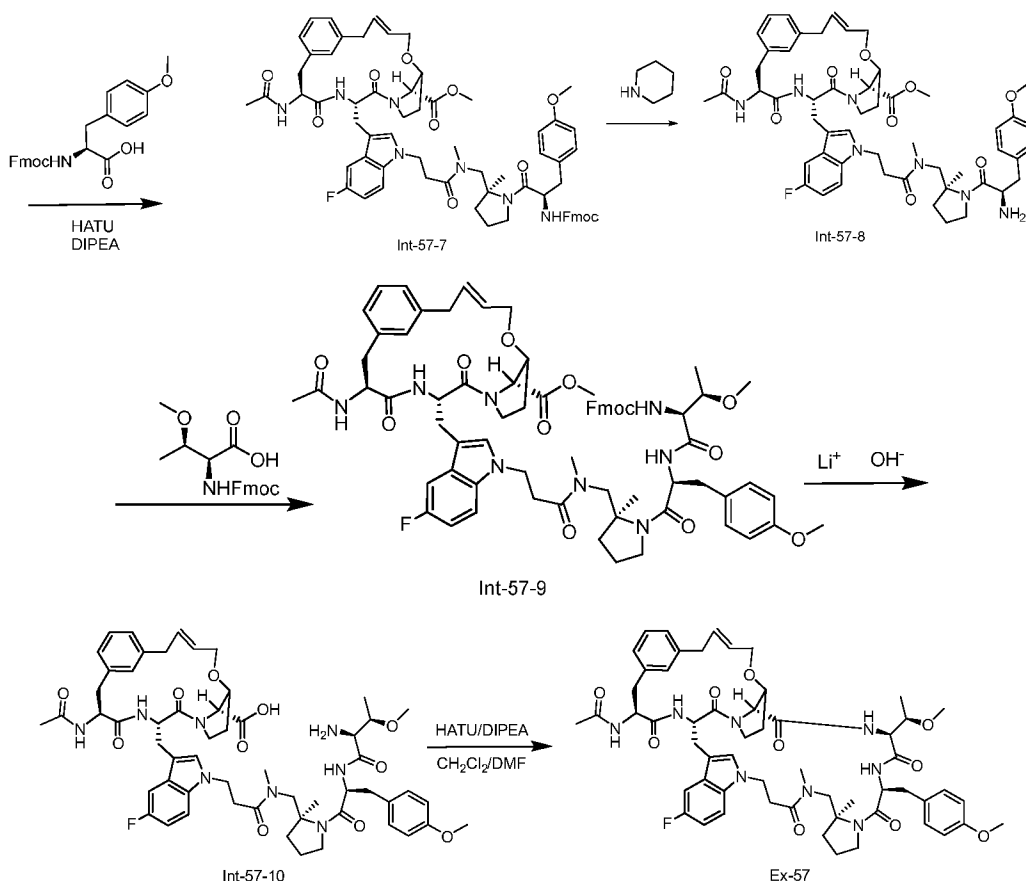
5

These compounds were synthesized as described below.

[0574] Preparation of



5



Compound Int-57-1:

[0575] To the solution of Intermediate F-2 (see the section, "Preparation of Intermediate F"; 1 g, 1.541 mmol) in MeOH (5 ml) was added 4 N HCl in dioxane (7.71 ml, 30.8 mmol) at r.t. and the reaction was stirred at r.t. for 2h. LCMS showed the desired mass. The reaction mixture was concentrated to dryness to yield compound Int-57-1 as a solid without further purification. M/Z (ESI): 549.39 $[\text{M}+\text{H}]^+$.

Compound Int-57-2

[0576] To a solution of compound Int-57-1 (845 mg, 1.541 mmol) in THF (12 ml) was added DIPEA (0.538 ml, 3.08 mmol) at r.t., followed by addition of acetic anhydride (0.276 ml, 2.93 mmol). The reaction was stirred at r.t. for 1h. LCMS showed the desired mass. The reaction mixture was quenched with water and extracted with EtOAc(3x20ml). The combined organic layers were washed with brine and separated, dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel 80g and eluted with 0-5% MeOH/DCM to give compound Int-57-2 as a solid. M/Z (ESI): 591.42 $[\text{M}+\text{H}]^+$.

5 Compound Int-57-3:

[0577] To a mixture of compound Int-57-2 (120 mg, 0.203 mmol) and Cs_2CO_3 (199 mg, 0.610 mmol) was added DMF (7 ml) and tert-butyl acrylate (104 mg, 0.813 mmol). The reaction mixture was stirred at 55°C for 3h. LCMS showed that the reaction was complete, and the desired mass of the product was observed as a major peak. The reaction mixture was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (20-100% Acetonitrile in water) to yield compound Int-57-3 as a solid. M/Z (ESI): 719.42 $[\text{M}+\text{H}]^+$

Compound Int-57-4:

[0578] To the solution of compound Int-57-3 (64 mg, 0.089 mmol) in DCM (2 ml) was added TFA (0.206 ml, 2.67 mmol). The resulting solution was stirred at r.t. for 3h. The desired mass was observed by LCMS indicating that the reaction was complete. The solvent was removed under reduced pressure to afford the crude product as a solid. The crude product was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield compound Int-57-4 as a solid. M/Z (ESI): 663.38 $[\text{M}+\text{H}]^+$

Compound Int-57-5:

[0579] To the mixture of compound Int-57-4 (20mg, 0.030 mmol) and (2S)-1-boc-2-methyl-2-(methylaminomethyl)pyrrolidine (7.24 mg, 0.032 mmol; commercially available) in DMF (0.3 ml) was added HATU (12.62 mg, 0.033 mmol) and N,N-diisopropylethylamine (10.51 μl , 0.060 mmol). The reaction was stirred at r.t. for 2h. LCMS showed that the desired mass was present. The reaction mixture was diluted with EtOAc(6ml) and washed with water(2x2ml). The organic phase was dried over Na_2SO_4 , concentrated to give compound Int-57-5 as a solid. M/Z (ESI): 873.49 $[\text{M}+\text{H}]^+$

Compound Int-57-6:

[0580] To the mixture of compound Int-57-5 (20mg, 0.030 mmol) and (2S)-1-boc-2-methyl-2-(methylaminomethyl)pyrrolidine (7.24 mg, 0.032 mmol) in DMF (0.3 ml) was added HATU (12.62 mg, 0.033 mmol) and N,N-diisopropylethylamine (10.51 μl , 0.060 mmol). The reaction was stirred at r.t. for 2h. LCMS showed that the desired mass was present. The reaction mixture was diluted with EtOAc(6ml) and washed with water(2x2ml). The resulting organic phase was dried over Na_2SO_4 , and concentrated to give compound Int-57-6 as a solid (23 mg, 0.03 mmol, 100%) M/Z (ESI): 773.60 $[\text{M}+\text{H}]^+$

5 Compound Int-57-7:

[0581] To the mixture of compound Int-57-6 (0.023 g, 0.03 mmol) and (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-methoxyphenyl)propanoic acid (0.013 g, 0.032 mmol) in DMF (0.5 ml), HATU (0.013 g, 0.033 mmol) and N,N-diisopropylethylamine (0.016 ml, 0.090 mmol) were added. The reaction mixture was stirred at r.t. for 2h. LCMS showed that the desired mass was present. The reaction mixture was diluted with EtOAc (6ml) and washed with water(2x2ml). The resulting organic phase was dried over Na₂SO₄ and concentrated to give compound Int-57-7 as a solid. M/Z (ESI): 1172.46 [M+H]⁺

Compound Int-57-8:

[0582] To the solution of compound Int-57-7 (0.035 g, 0.03 mmol) in acetonitrile (0.5 ml), piperidine (0.015 ml, 0.150 mmol) was added. The resulting solution was stirred at rt for 1h. The desired mass was observed in LCMS. The reaction mixture was concentrated, and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water), to yield compound Int-57-8 as a solid. M/Z (ESI): 950.47 [M+H]⁺

20 Compound Int-57-9:

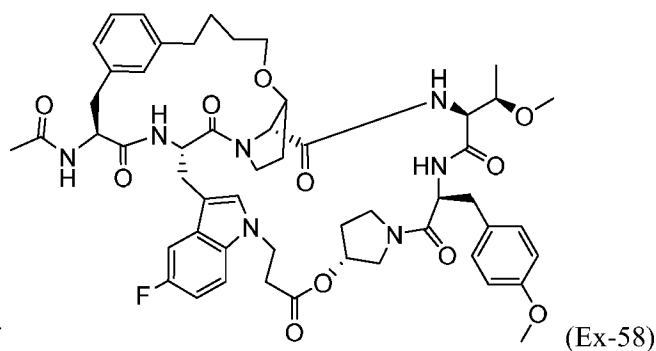
[0583] To the mixture of compound Int-57-8 (29 mg, 0.03 mmol) and (2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxybutanoic acid (0.012 g, 0.033 mmol) in DMF (0.4 ml), HATU (0.013 g, 0.035 mmol) and N,N-diisopropylethylamine (0.016 ml, 0.090 mmol) were added. The reaction mixture was stirred at r.t. for 2h. LCMS showed that the desired mass was present. The reaction mixture was diluted with EtOAc (6ml) and washed with water(2x2ml). The resulting organic phase was dried over Na₂SO₄ and concentrated to give compound Int-57-9 as a solid (39 mg, 0.03 mmol 100%). M/Z (ESI): 1287.44 [M+H]⁺

Compound Int-57-10:

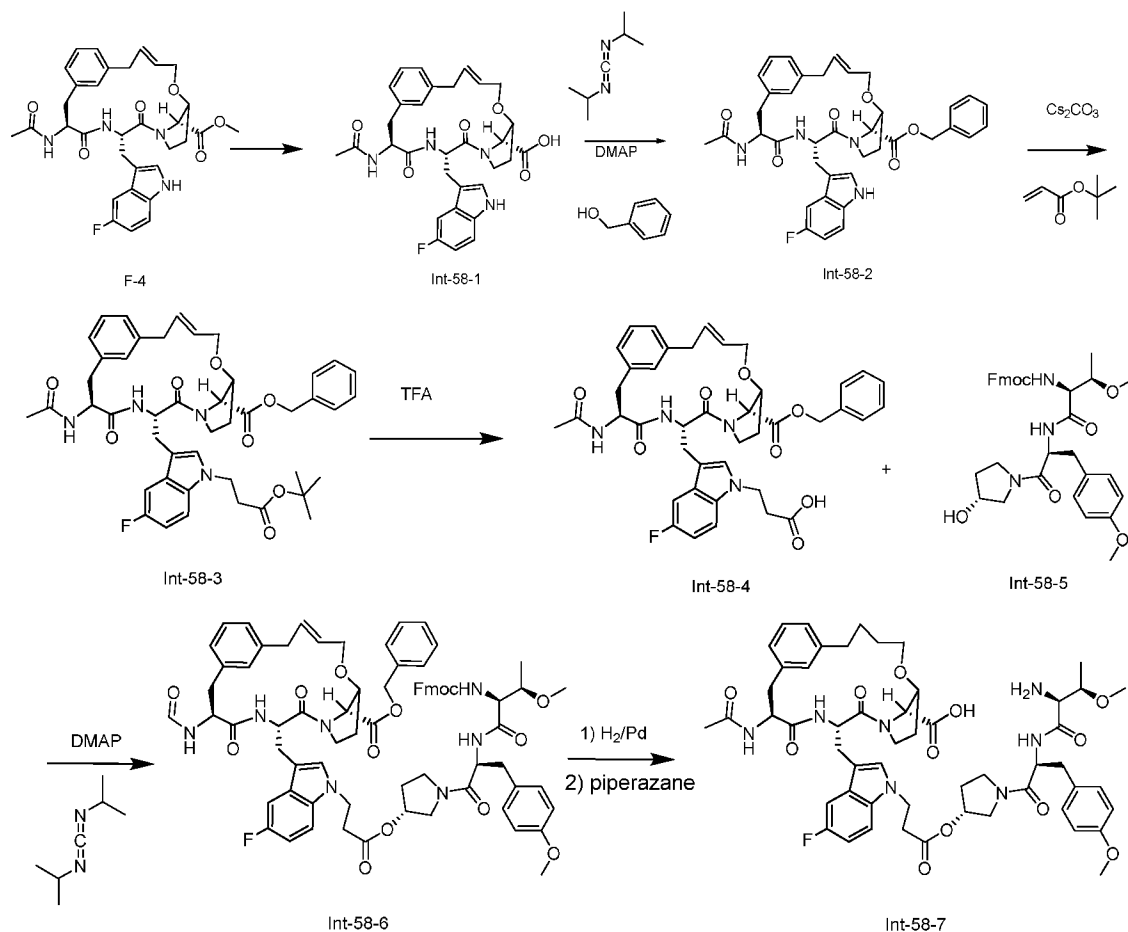
[0584] To a solution of compound Int-57-9 (39 mg, 0.03 mmol) in THF (0.4 ml) and MeOH (0.1 ml) at r.t. lithium hydroxide (0.300 ml, 0.300 mmol) was added dropwise. The resulting solution was stirred at r.t for 1h. LCMS showed that the desired mass was present. The volatile solvent was removed under reduced pressure and the aqueous solution was neutralized by adding 1N HCl in water to PH ~5. The solvent was removed, and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield compound Int-57-10 as a solid. M/Z (ESI): 1051.41 [M+H]⁺

5 Example compound 57 (Ex-57):

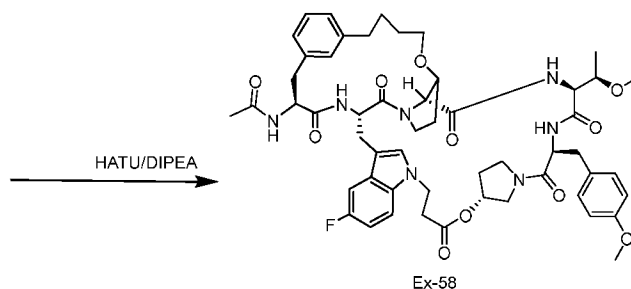
[0585] To a solution of compound Int-57-10 (30 mg, 0.029 mmol) and DIPEA (0.020 ml, 0.114 mmol) in DMF (2ml) and CH₂Cl₂ (40ml) at r.t. was added HATU (13.02 mg, 0.034 mmol). The resulting solution was stirred at r.t. for 1h. LCMS showed that the desired mass was present. The solvent was removed under reduced pressure, and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield Ex-57 as a solid. M/Z (ESI): 1033.35 [M+H]⁺.



[0586] Preparation of



15



5

Compound Int-58-1

[0587] To a solution of compound F-4 (see Preparation of Intermediate F; 50mg, 0.085 mmol) in THF (0.6 ml) and MeOH (0.3 ml) at r.t. lithium hydroxide (0.508 ml, 0.508 mmol) was added dropwise. The resulting solution was stirred at r.t for 1h. The desired mass was observed in LCMS. Volatile solvent was removed under reduced pressure and the aqueous solution was neutralized by adding 1N HCl in water to pH ~5. The solvent was removed, and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield compound Int-58-1 as a solid. M/Z (ESI): 577.32 [M+H]⁺.

15

Compound Int-58-2

[0588] To the mixture of compound Int-58-1(44.5mg, 0.077 mmol) and phenylmethanol (9.58 μl, 0.093 mmol) in DCM (1 ml) and DMF (0.3ml), N,N'-diisopropylcarbodiimide (0.022 ml, 0.139 mmol) and 4-dimethylaminopyridine (1.414 mg, 0.012 mmol) were added. The reaction was stirred at r.t. for 2h. LCMS showed that the desired mass was present. The reaction was diluted with DCM (8ml), washed with H₂O(2x2ml), the organic phase dried over Na₂SO₄, filtered, and concentrated to dryness. The residue was purified by flash column chromatography on silica gel (ISCO 24g column, and eluted with MeOH/DCM(0-10% MeOH in DCM) to give compound Int-58-2 as solid. M/Z (ESI): 667.32 [M+H]⁺.

20

Compound Int-58-3:

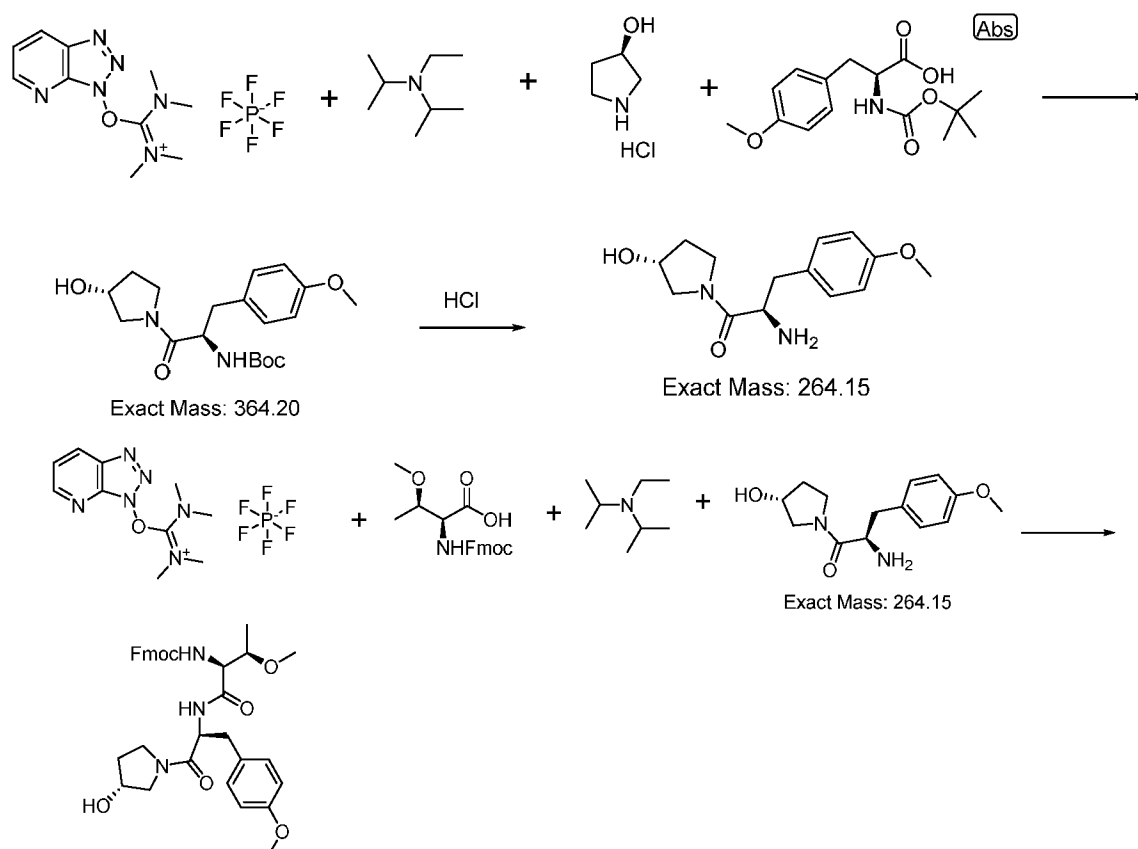
[0589] To the mixture of compound Int-58-2 (47.6 mg, 0.071 mmol) and Cs₂CO₃ (69.8 mg, 0.214 mmol) in DMF (1.5ml) was added tert-butyl acrylate (0.041 ml, 0.286 mmol). The mixture was stirred at 50°C for 2h. LCMS showed that the desired mass was present. The reaction was diluted with EtOAc (8ml) and washed with H₂O (3x3ml). The organic phase was dried over Na₂SO₄, filtered, and concentrated to give compound Int-58-3 as solid. M/Z (ESI): 795.41 [M+H]⁺.

30

5 Compound Int-58-4:

[0590] To the solution of compound Int-58-3 (56.4 mg, 0.071 mmol) in DCM (1ml) was added TFA (0.164 ml, 2.130 mmol). The resulting solution was stirred at r.t. for 3h. LCMS showed that the desired mass was present, and that the reaction was complete. The solvent was removed under reduced pressure, and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield compound Int-58-4 as a solid. M/Z (ESI): 739.35 [M+H]⁺.

Compound Int-58-5:

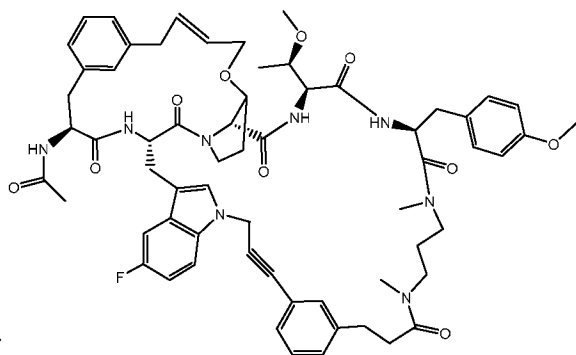


15 [0591] To the mixture of (R)-pyrrolidin-3-ol hydrochloride (50 mg, 0.405 mmol) and BOC-TYR(ME)-OH (125 mg, 0.425 mmol) in DMF (4ml) and Water (0.4 ml) was added HATU (169 mg, 0.445 mmol) and N,N-DIISOPROPYLETHYLAMINE (0.214 ml, 1.214 mmol). The reaction was stirred at r.t. for 1h. LCMS showed the presence of the desired mass. The reaction mixture was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (5-80% Acetonitrile in water) to yield tert-butyl ((R)-1-((R)-3-hydroxypyrrolidin-1-yl)-3-(4-methoxyphenyl)-1-oxopropan-2-yl)carbamate as a solid (exact mass of 364.20). To a solution of this compound (exact mass of 364.20) in DCM (1.5 ml),

- 5 HCl (4N HCl in dioxane) (0.500 ml, 2.000 mmol) was added and the resulting solution was stirred at r.t. for 1h. LCMS showed the presence of the desired mass and completion of the reaction. The reaction mixture was removed under reduced pressure to afford (R)-2-amino-1-((R)-3-hydroxypyrrolidin-1-yl)-3-(4-methoxyphenyl)propan-1-one as a solid (exact mass of 264.15).
- 10 **[0592]** Next, to the mixture of (R)-2-amino-1-((R)-3-hydroxypyrrolidin-1-yl)-3-(4-methoxyphenyl)propan-1-one (52.9 mg, 0.2 mmol) and (2S,3R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxybutanoic acid (85 mg, 0.240 mmol) in DMF (2 ml) was added HATU (95 mg, 0.250 mmol) and N,N-DIISOPROPYLETHYLAMINE (0.106 ml, 0.600 mmol). The reaction was stirred at r.t. for 2h. LCMS showed the presence of the desired mass.
- 15 The reaction mixture was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield compound Int-58-5 as a solid.
- Compound Int-58-6:
- [0593]** To the mixture of compound Int-58-4 (23.4 mg, 0.032 mmol) and (9H-fluoren-9-yl)methyl ((2S,3R)-1-(((S)-1-((R)-3-hydroxypyrrolidin-1-yl)-3-(4-methoxyphenyl)-1-oxopropan-2-yl)amino)-3-methoxy-1-oxobutan-2-yl)carbamate(compound Int-58-5) (22.87 mg, 0.038 mmol) in DCM (1 ml), N,N'-diisopropylcarbodiimide (8.83 μ l, 0.057 mmol) and 4-dimethylaminopyridine (0.464 mg, 3.80 μ mol) were added. The reaction was stirred at r.t. for 3 h. LCMS showed the desired mass was present. The solvent was removed under reduced
- 25 pressure, and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield compound Int-58-6 as a solid. M/Z (ESI): 1322.32 [M+H]⁺
- Compound Int-58-7:
- [0594]** To the solution of compound Int-58-6 (34 mg, 0.026 mmol) in MeOH (0.8 ml) was
- 30 added Pd/C (27.4 mg, 0.026 mmol). The reaction was charged with H₂ balloon and stirred at r.t. for 1 h. LCMS showed the desired mass was present. The mixture was filtered through celite, washed with MeOH, and concentrated to give the crude product a solid. This material was dissolved in Acetonitrile (0.6 ml) followed by addition of piperidine (0.013 ml, 0.130 mmol). The resulting solution was stirred at r.t. for 1h. LCMS showed the desired mass. The reaction was
- 35 concentrated, and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield compound Int-58-7 as a solid. M/Z (ESI): 1322.32 [M+H]⁺.

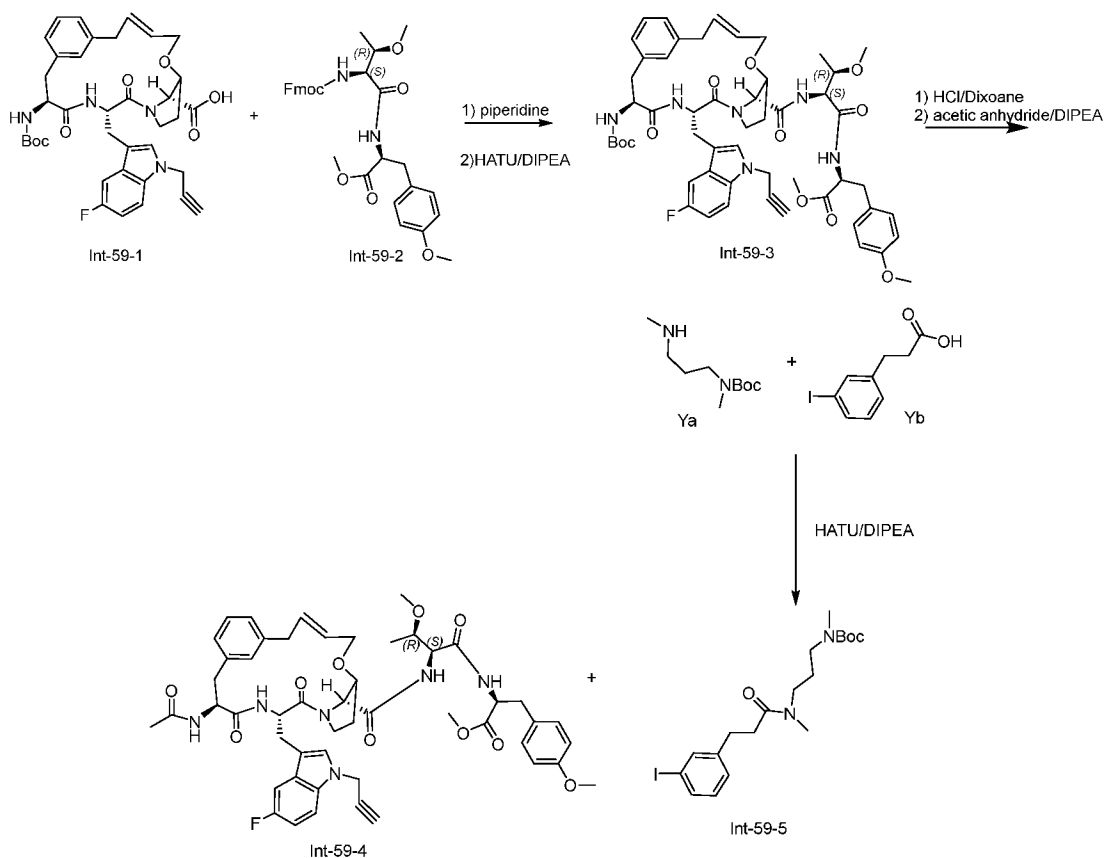
5 Ex-58:

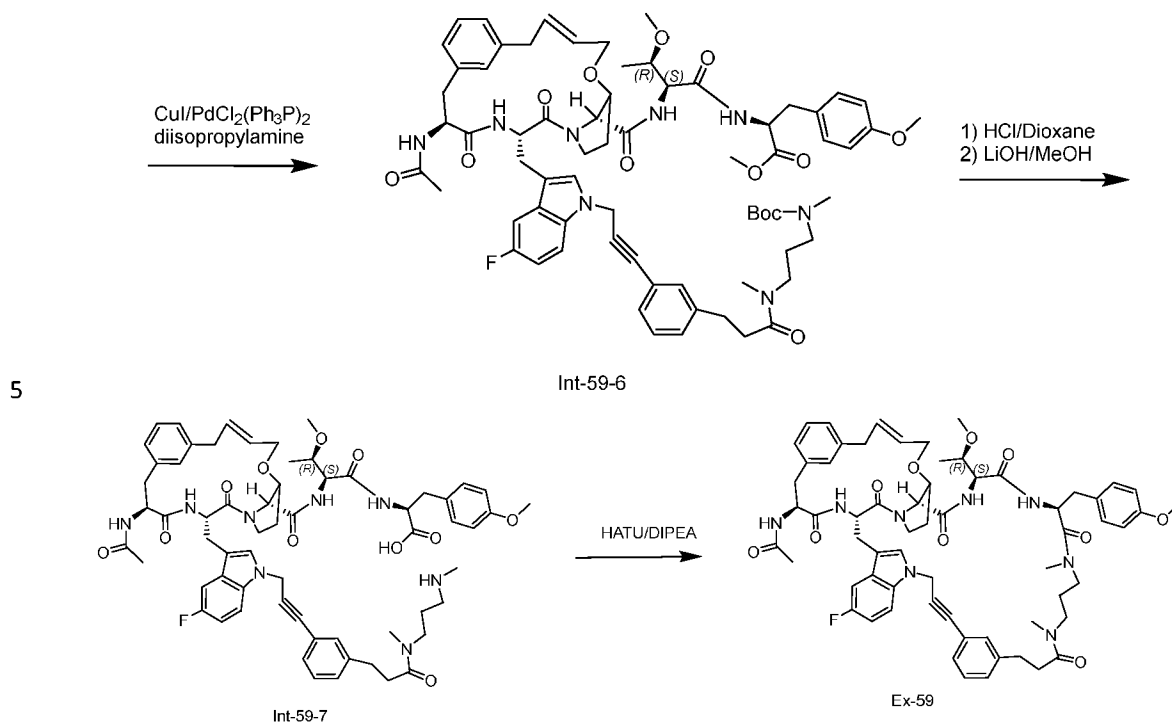
[0595] To the solution of compound Int-58-7 (10.5 mg, 10.37 μ mol) in DMF (2 ml) and CH_2Cl_2 (6ml) was added HATU (4.34 mg, 0.011 mmol). The resulting solution was stirred at r.t. for 5 min. Then another 14 ml of DCM was added to the above reaction mixture, followed by DIPEA (7.25 μ l, 0.041 mmol). The reaction was stirred at r.t. for 1h. LCMS showed the desired mass. The solvent was removed under reduced pressure, and the residue was purified by preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA (10-100% Acetonitrile in water) to yield Ex-58 as solid.



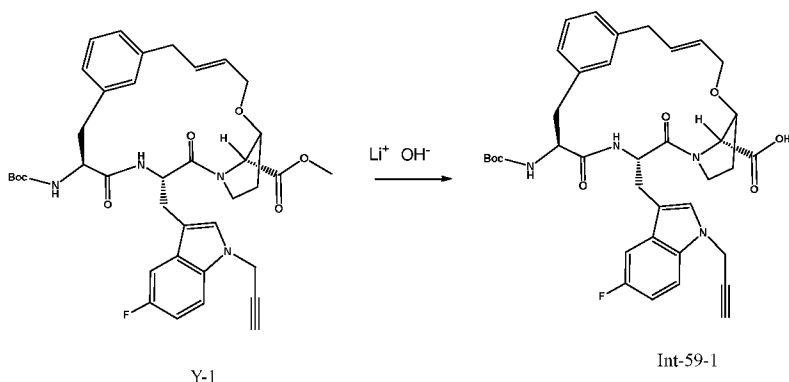
[0596] Preparation of

(Ex-59)





Compound Int-59-1:



- 10 **[0597]** To a solution of Intermediate Y-1 (methyl (12S,13S,9S,12S,E)-9-((tert-butoxycarbonyl)amino)-12-((5-fluoro-1-(prop-2-yn-1-yl)-1H-indol-3-yl)methyl)-10,13-dioxo-2-oxa-11-aza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphan-4-ene-12-carboxylate (1.796 g, 2.62 mmol; see above under “Preparation of Intermediate Y”) in THF (37.4 ml) at r.t. was added lithium hydroxide (3.92 ml, 3.92 mmol). The mixture was stirred at rt for 4h. LCMS showed that
- 15 the reaction was complete. The reaction was partitioned with 200ml EtOAc and 200ml of pH 4 Phosphate buffer (1M KH_2PO_4), layers separated, and organics were washed twice with 50ml brine, dried over MgSO_4 and concentrated to give compound Int-59-1 (12S,13S,9S,12S,E)-9-((tert-butoxycarbonyl)amino)-12-((5-fluoro-1-(prop-2-yn-1-yl)-1H-indol-3-yl)methyl)-10,13-

5 dioxo-2-oxa-11-aza-1(3,1)-pyrrolidina-7(1,3)-benzenacyclotridecaphan-4-ene-12-carboxylic acid as a foam.

Compound Int-59-3

[0598] (S)-methyl 2-((2S,3R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxybutanamido)-3-(4-methoxyphenyl)propanoate (Int-59-2) (406 mg, 0.743 mmol) was dissolved in CH₂Cl₂ (0.3 ml) and Acetonitrile (1 ml) was added followed by addition of
10 piperidine (0.587 ml, 5.94 mmol), and the mixture was stirred at 23 °C for 30 min. LCMS showed complete deprotection of the Fmoc group. The solvent was removed, and the mixture was dried under vacuum. This crude material was dissolved in DMF (2.5 mL) to make solution 1. A mixture of Int-59-1 (500 mg, 0.743 mmol) and HATU (367 mg, 0.966 mmol) in DMF (2.5 ml)
15 was stirred at 23 °C for 15 min. followed by the addition of solution 1 and N,N-diisopropylethylamine (0.647 ml, 3.71 mmol). The mixture was stirred at 23 °C for 1 hr. LCMS showed completion of the reaction. The crude material was purified by ISCO reverse phase C-18 column (150 g) and eluted with Acetonitrile with TFA (0.05%) in H₂O with TFA (0.05%) (5 % to 100%) to give compound Int-59-3 as a solid. M/Z (ESI): 979.62 [M+H]⁺.

20 Compound Int-59-4

[0599] To the solution of compound Int-59-3 (317.2 mg, 0.324 mmol) in CH₂Cl₂ (1.0 ml) was added HCl in dioxane (3228 mg, 25.9 mmol). The mixture was stirred at 23 °C for 1 hr. LCMS showed complete deprotection of Boc group. The solvent was removed, and the crude material was dried under vacuum. The de-Boced material was then dissolved in THF (1 ml), and DIPEA
25 (0.283 ml, 1.620 mmol) was added at 23 °C, followed by the addition of acetic anhydride (0.061 ml, 0.648 mmol). The reaction was stirred at 23 °C for 1h. LCMS showed the desired mass. The solvent was removed, and the crude material was purified with preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05%TFA(10-100% Acetonitrile in water) to give compound Int-59-4 as a solid. M/Z (ESI): 921.36 [M+H]⁺.

30 Compound Int-59-5:

[0600] 3-(3-iodophenyl)propanoic acid (compound Yb) (100 mg, 0.362 mmol) was dissolved in DMF (2 ml) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (179 mg, 0.471 mmol) was added and the mixture was stirred at 23 °C for 15 min. before addition of tert-butyl methyl(3-(methylamino)propyl)carbamate (compound
35 Ya) (73.3 mg, 0.362 mmol) and N-ethyl-N-isopropylpropan-2-amine (0.315 ml, 1.811 mmol). The reaction was then stirred at 23 °C for 2 hr. LCMS showed no more starting material and complete conversion to product. The crude material was purified on a reverse phase C-18 column (50 g) and eluted with Acetonitrile with TFA(0.05%) in water with TFA (0.05%) 5% to

5 80% then to 100% to give tert-butyl (3-(3-(3-iodophenyl)-N-methylpropanamido)propyl)(methyl)carbamate (compound Int-59-5) as an oil. M/Z (ESI): 461.14 [M+H]⁺.

Compound Int-59-6:

[0601] To the solution of compound Int-59-5 (20 mg, 0.022 mmol) and tert-butyl (3-(3-(3-iodophenyl)-N-methylpropanamido)propyl)(methyl)carbamate (compound Y) (30.0 mg, 0.065 mmol) in THF (0.4 ml) at 23 °C was added bis-(triphenylphosphino)-palladium chloride (1.447 mg, 2.172 μmol), copper(I) iodide (0.414 mg, 2.172 μmol). The mixture was degassed three times before the addition of diisopropylamine (0.8 ml, 5.71 mmol). The mixture was stirred at 23 °C for 2h. LCMS showed the reaction completed. The reaction mixture was diluted with EtOAc (2 mL) and extracted with NaCl (aq. 2 ml). The organic layer was isolated and concentrated to dryness to give crude compound Int-59-6. M/Z (ESI): 1253.41 [M+H]⁺.

Compound Int-59-7:

[0602] To compound Int-59-6 (crude material) (27.2 mg, 0.022 mmol) was added HCl in 1,4-dioxane (434 μL, 1.736 mmol, 4N). The resulting solution was stirred at 23 °C for 30 min. LCMS showed that the reaction was complete. The volatile solvent was removed under reduced pressure, and the residue was lyophilized to give crude de-Boced material. This material was used without further purification. It was then dissolved in THF (500 μL), and to the resulting solution lithium hydroxide (174 μL, 0.174 mmol) was added. The mixture was stirred at 23 °C for 1 h. The crude material was purified with preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05% TFA (10-100% Acetonitrile in water) to give compound Int-59-7. M/Z (ESI): 1139.27 [M+H]⁺.

Ex-59:

[0603] To the solution of compound Int-59-7 (7.6 mg, 6.67 μmol) and HATU (3.04 mg, 8.00 μmol) in DMF (0.5 ml) and CH₂Cl₂ (12 mL) at 23 °C was added DIPEA (4.66 μl, 0.027 mmol). The resulting solution was stirred at 23 °C for 30 min. LCMS showed that the reaction was complete. Solvent was removed and the crude material was purified with preparative HPLC reverse phase (SunFire C-18, 19x150mm), and eluted with Acetonitrile/Water + 0.05% TFA (20-100% Acetonitrile in water) to give Ex-59 as a solid. M/Z (ESI): 1121.28 [M+H]⁺.

35 Biological Assays

Example 52: Activity Determination

[0604] Selected compounds of the invention were subjected to one or more of the following procedures to assay their activity for antagonism of PCSK9 activity. The following is a

5 description of the assays used to determine activity of compounds of the invention, and any comparator compounds reported, toward PCSK9 antagonism. Biotinylated PCSK9 was obtained commercially.

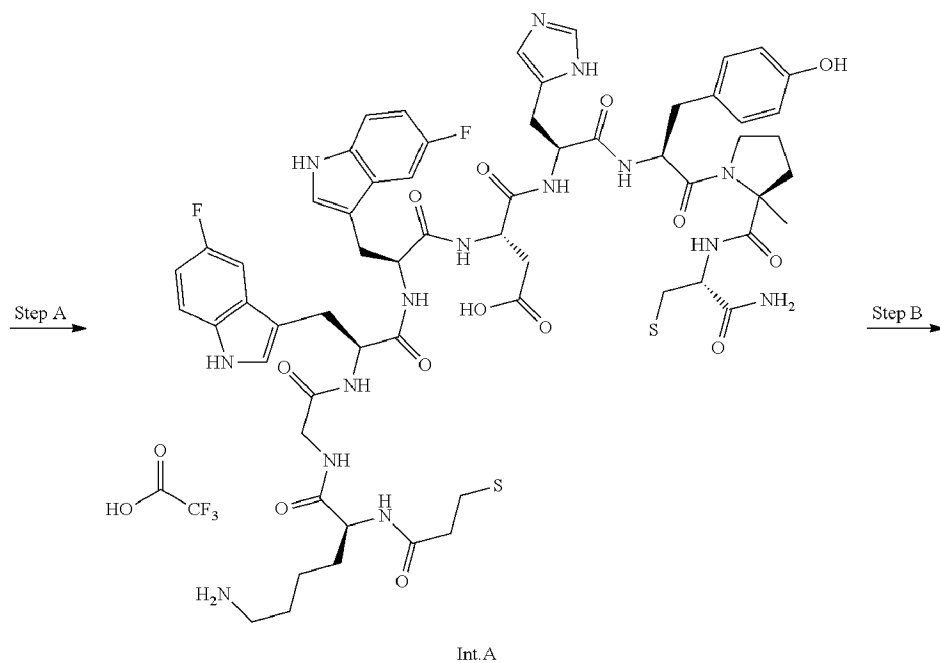
LDLR TR-FRET

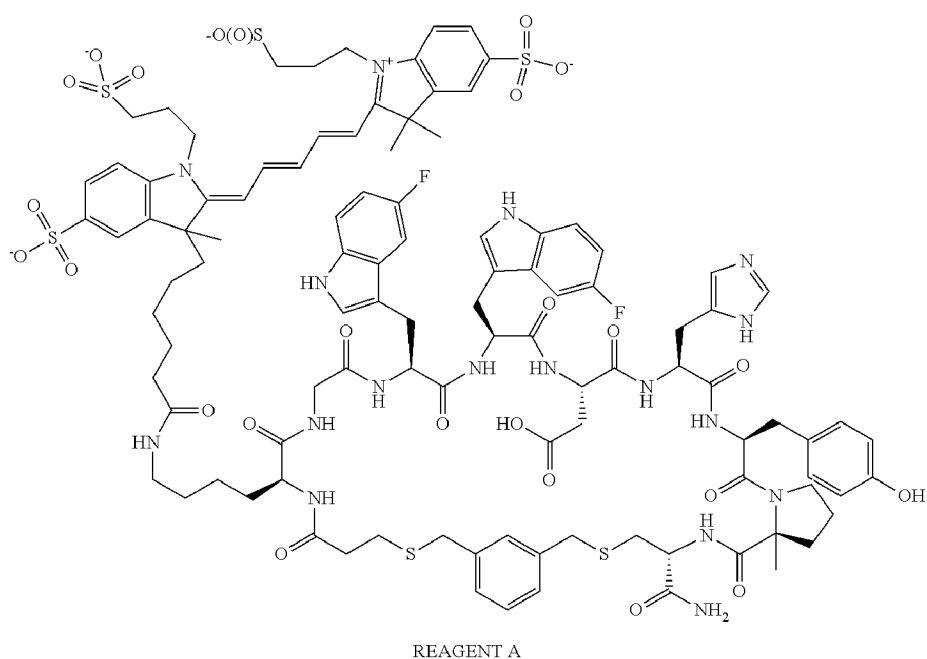
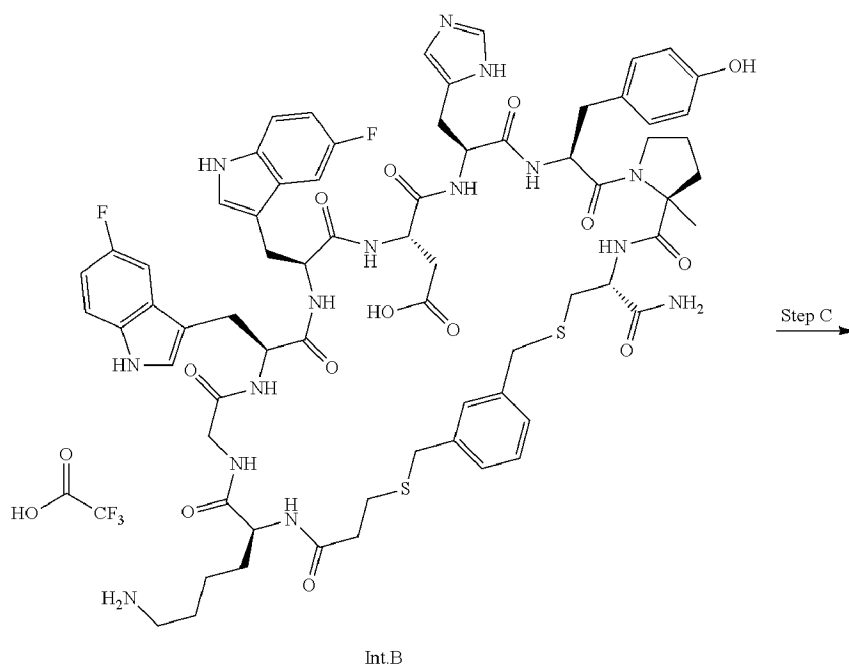
[0605] The PCSK9 TR-FRET assay measures the interaction between PCSK9 and LDLR. A solution containing 40 nM biotinylated PCSK9 + 10 nM Lance ULight Streptavidin is made in 10 50 mM HEPES pH 7.4, 0.15 M NaCl, 5 mM CaCl₂, 0.01% BSA, and 0.01% Surfactant P20. A separate solution containing 40 nM rhLDLR-6×His+10 nM Eu-W1024 anti-6×His is made in the same buffer system. An Echo is used to transfer 0.750 µl of compound to an assay plate followed by the addition of 15 µl of PCSK9+ULight and 15 µl of LDLR+Eu. The final assay volume is 15 30.750 µl containing 20 nM PCSK9, 5 nM Ulight, 20 nM LDLR, and 5 nM Eu. The reaction is incubated at room temperature for at least two hours prior to fluorescence measurements using an Envision Multilabel Reader. 1050 values are determined by fitting data to a sigmoidal dose-response curve using nonlinear regression. Counts (B-counts) of the europium-labeled LDLR are followed to observe if compounds are adversely affecting LDLR. A fall off of the B-counts is 20 likely indicates a false positive of inhibition.

Alexa FRET Standard TR-FRET

[0606] The PCSK9 Alexa FRET Standard assay measures the interaction between PCSK9 and an AlexaFluor647 (AF) tagged cyclic peptide, Reagent A (K_D=83 nM). A solution containing 1 25 nM biotinylated PCSK9+2.5 nM Lance Streptavidin Europium (Strep-Eu) is made in 50 mM HEPES pH 7.4, 0.15 M NaCl, 5 mM CaCl₂, 0.01% BSA, and 0.01% Surfactant P20. A separate solution containing 40 nM of the AlexaFluor tagged cyclic peptide is made in the same buffer system. An Echo is used to transfer 0.750 µl of compound to an assay plate followed by the addition of 15 µl of PCSK9+Strep-Eu and 15 µl of AF peptide. The final assay volume is 30.750 30 µl containing 0.5 nM PCSK9, 1.25 nM Strep-Eu, and 20 nM AF cyclic peptide. The reaction is incubated at room temperature for at least two hours prior to fluorescence measurements using an Envision Multilabel Reader. IC₅₀ values are determined by fitting data to a sigmoidal dose-response curve using nonlinear regression. K_i is then calculated from the IC₅₀ and the K_D of AF cyclic peptide. Counts (B-counts) of the europium-labeled PCSK9 are followed to observe if 35 compounds are adversely PCSK9. A fall from the B-counts likely indicates a false positive of inhibition. Data from this procedure is reported as “A=‘numerical value’ (nanomolar)”

- 5 [0607] Reagent A was prepared in accordance with the following method:





5

Step A—Synthesis of Intermediate Compound Int-A

[0608] The peptide was synthesized on a 0.250 mmol scale on CEM Liberty Blue, Microwave synthesizer using Fmoc/tBu chemistry on PS Rink-Amide MBHA resin, 0.32 mmol g⁻¹. The

- 5 assembly was performed using single couplings using 4 eq of Fmoc protected amino acid 0.2M in DMF, 4 eq of 0.5M HATU in DMF, 4 eq of 2M DIPEA (double coupling for Tyr). Fmoc deprotection cycles were performed using 20% (V/V) piperidine in DMF.
- [0609] The sequence of Fmoc protected amino acids and building blocks used are:
1. N-(((9H-fluoren-9-yl)methoxy)carbonyl)-S-trityl-L-cysteine
 - 10 2. (S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpyrrolidine-2-carboxylic acid
 3. (((9H-fluoren-9-yl)methoxy)carbonyl)-L-tyrosine
 4. N-(((9H-fluoren-9-yl)methoxy)carbonyl)-N-trityl-L-histidine
 5. (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(tert-butoxy)-4-oxobutanoic acid
 - 15 6. (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-fluoro-1H-indol-3-yl)propanoic acid
 7. (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-fluoro-1H-indol-3-yl)propanoic acid
 - 20 8. (((9H-fluoren-9-yl)methoxy)carbonyl)glycine
 9. N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(tert-butoxycarbonyl)-L-lysine
 10. 3-(tritylthio)propanoic acid. At the end of the assembly, the resin was washed with DMF, MeOH, DCM, Et₂O. The peptide was cleaved from solid support using 50 ml of TFA solution (v/v) (91% TFA, 5% H₂O, 4% TIPS) for approximately 1.5 hours, at room temperature.
 - 25 The resin was filtered, washed with TFA and solution concentrated to dryness and lyophilized. Lyophilization afforded Intermediate Compound Int. A (399 mg), which was used as crude in the next step. LCMS Anal. calculated. C₆₁H₇₅F₂N₁₅O₁₃S₂: 1328.48, found: 1328.2 (M+1)⁺.

Step B—Synthesis of Intermediate Compound Int-B: As Described for Reagent B

- 30 [0610] Purified by RP-HPLC (Waters Deltapak C4, double cartridge, 40×100 mm, 15μm, 300Å; 15% to 35% ACN/water+0.1% TFA modifier over 20 min). Collected fractions lyophilized to afford 35 mg of Intermediate Compound Int-B. LCMS Anal. calculated for C₆₉H₈₁F₂N₁₅O₁₃S₂: 1430.62; found: 1430.9 (M+1)⁺.

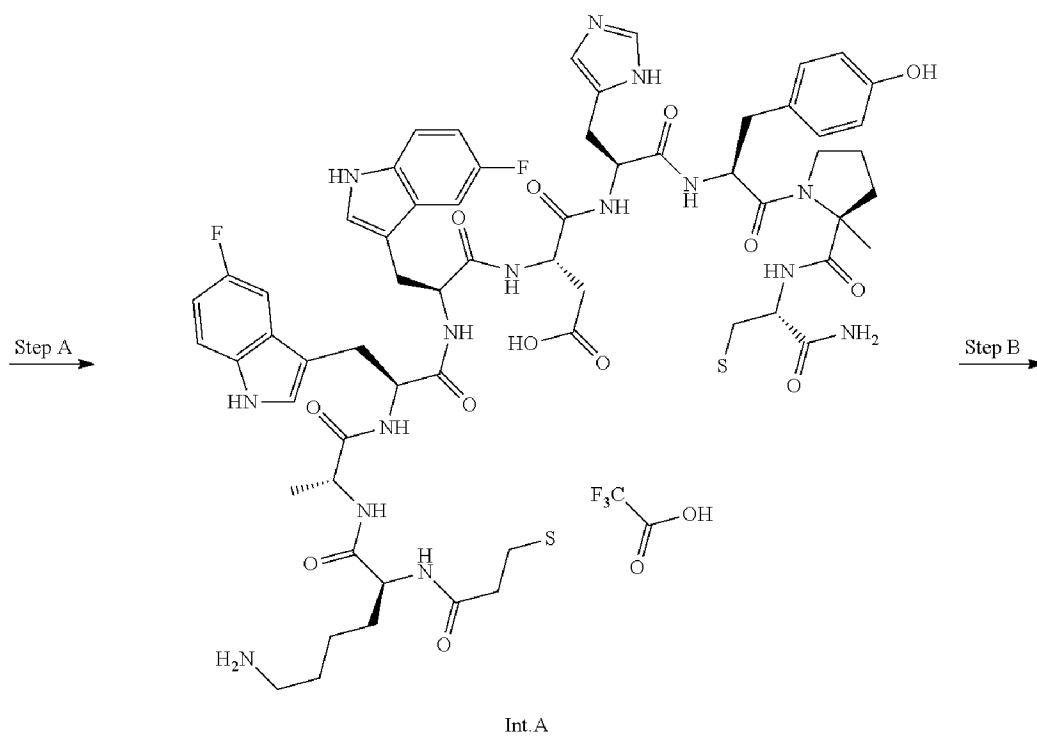
5 Step C—Synthesis of Compound Reagent A: As Described for Reagent B

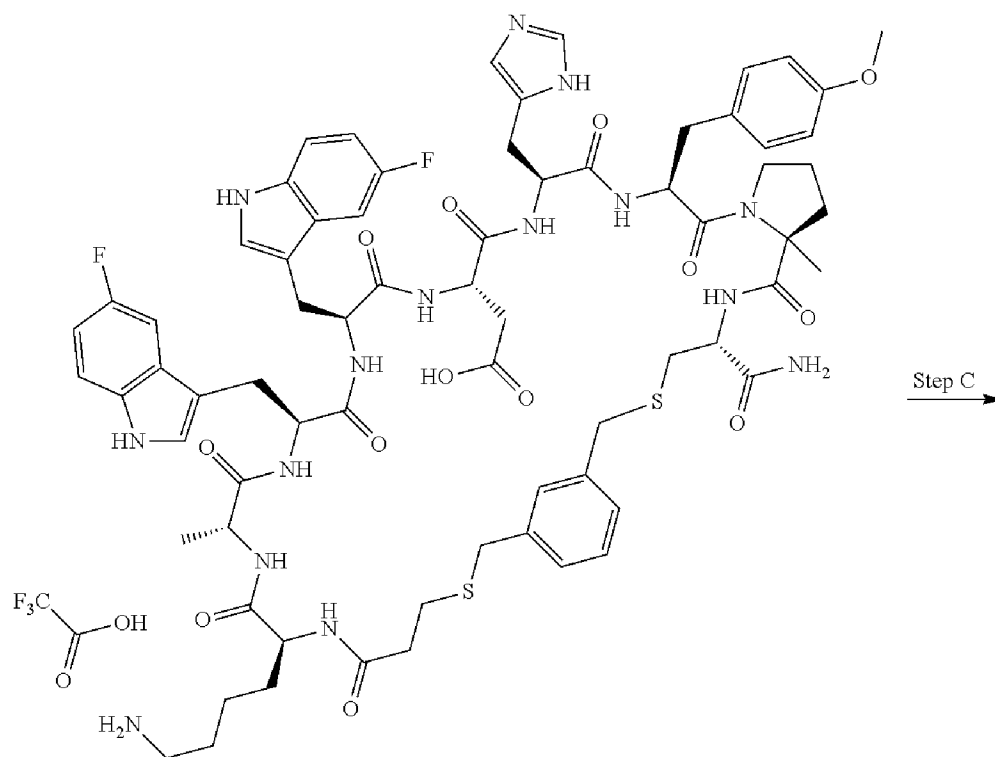
[0611] LCMS Anal. calculated for $C_{105}H_{122}F_2N_{17}O_{26}S_6^{3-}$: 2268.58; 1135.8 $(M+2)^{2+}$

Alexa FRET Plus TR-FRET

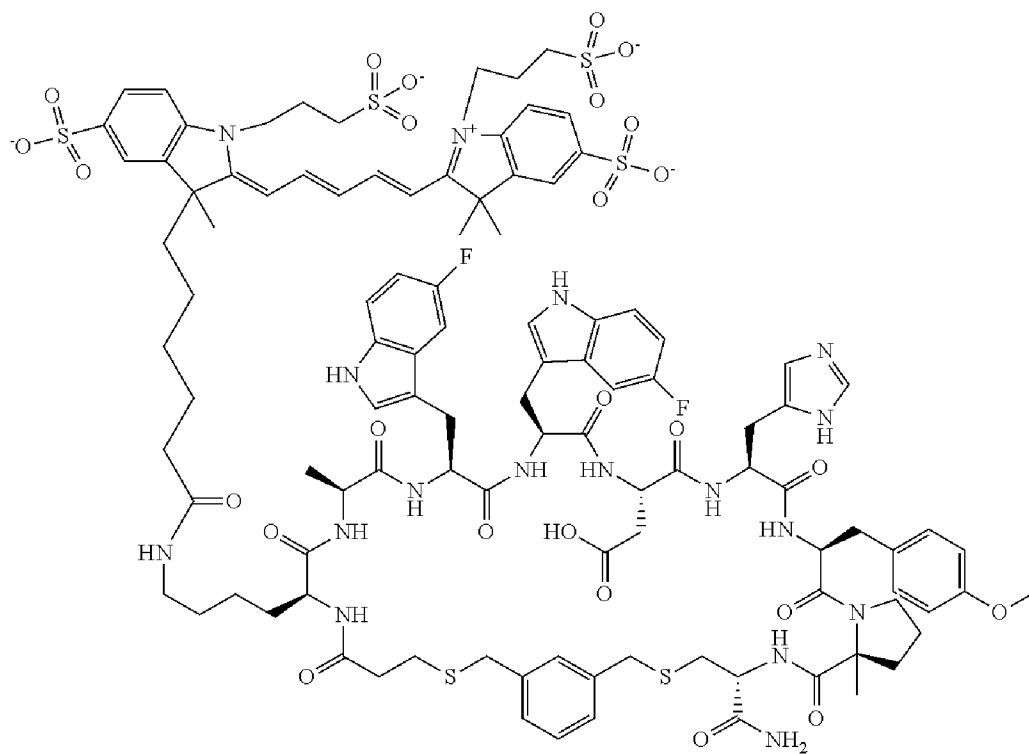
[0612] The PCSK9 Alexa FRET Plus assay measures the interaction between PCSK9 and an
10 AlexaFluor647 (AF) tagged cyclic peptide, Reagent B ($K_D=35$ nM). A solution containing 1 nM
biotinylated PCSK9 + 2.5 nM Lance Streptavidin Europium (Strep-Eu) is made in 50 mM
HEPES pH 7.4, 0.15 M NaCl, 5 mM $CaCl_2$, 0.01% BSA, and 0.01% Surfactant P20. A separate
solution containing 1920 nM of the AlexaFluor tagged cyclic peptide is made in the same buffer
system. An Echo is used to transfer 0.075 μ l of compound plus 0.675 μ l of DMSO to each well
15 of an assay plate followed by the addition of 15 μ l of PCSK9+Strep-Eu and 15 μ l of AF peptide.
The final assay volume is 30.750 μ l containing 0.5 nM PCSK9, 1.25 nM Strep-Eu, and 960 nM
AF cyclic peptide. The reaction is incubated at room temperature for at least two hours prior to
fluorescence measurements using an Envision Multilabel Reader. IC_{50} values are determined by
fitting data to a sigmoidal dose-response curve using nonlinear regression. K_i is then calculated
20 from the IC_{50} and the K_D of AF cyclic peptide. Counts (B-counts) of the europium-labeled
PCSK9 are followed to observe if compounds are adversely affecting PCSK9. A fall from the B-
counts is likely indicates a false positive of inhibition. Data from this procedure is reported as
“P=‘numerical value’ (nanomolar)”

5 [0613] Reagent B was prepared by the following procedure.





Int.B



Reagent B
- 248 -

5 Step A—Synthesis of Intermediate Compound Int-A

[0614] The peptide was synthesized on a 0.250 mmol scale on CEM Liberty Blue, Microwave synthesizer using Fmoc/tBu chemistry on PS Rink-Amide MBHA resin, 0.32 mmol g⁻¹. The assembly was performed using single-couplings using 4 eq of Fmoc protected amino acid 0.2M in DMF, 4 eq of 1M Oxyme in DMF, 4 eq of 0.5M N,N-diisopropylcarbodiimide (DIC) (double
10 coupling for Y01). Fmoc deprotection cycles were performed using 20% (V/V) piperidine in DMF.

[0615] The sequence of Fmoc protected amino acids and building blocks used are:

1. N-(((9H-fluoren-9-yl)methoxy)carbonyl)-S-trityl-L-cysteine
2. (S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-methylpyrrolidine-2-carboxylic
15 acid
3. (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-methoxyphenyl)propanoic acid
4. N-(((9H-fluoren-9-yl)methoxy)carbonyl)-N-trityl-L-histidine
5. (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(tert-butoxy)-4-oxobutanoic
20 acid
6. (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-fluoro-1H-indol-3-yl)propanoic acid
7. (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-fluoro-1H-indol-3-yl)propanoic acid
- 25 8. (((9H-fluoren-9-yl)methoxy)carbonyl)-D-alanine
9. N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(tert-butoxycarbonyl)-L-lysine
10. 3-(tritylthio)propanoic acid

[0616] At the end of the assembly, the resin was washed with DMF, MeOH, DCM, Et₂O. The peptide was cleaved from solid support using 50 ml of TFA solution (v/v) (91% TFA, 5% H₂O, 30 4% TIPS) for approximately 1.5 hours, at room temperature. The resin was filtered, washed with TFA and solution concentrated to dryness and lyophilized. Lyophilization afforded Intermediate Compound Int. A (300 mg), which was used as crude in the next step. LCMS anal. calculated. C₆₃H₇₉F₂N₁₅O₁₃S₂: 1356.53, found: 1356.9 (M+1)⁺.

Step B—Synthesis of Intermediate Compound Int-B

35 [0617] Crude Int-A (0.22 mmol) was redissolved in 24 ml of DMF. 6 ml of 1M aqueous solution of sodium bicarbonate was added to raise the pH to 7. Then 0.26 mmol of 1,3-bis(bromomethyl)benzene (0.1 M in DMF) was added dropwise. Reaction was left under stirring

5 at room temperature for 20 min, quenched with TFA (pH to 3-4) and then concentrated in vacuo to provide crude Int-B, which was purified by RP-HPLC (Waters XBridge, C18, 50×150 mm, 5 μm, 130 Å; 25% to 40% ACN/water+0.1% TFA modifier over 20 min). Collected fractions were lyophilized to afford 35 mg of Intermediate Compound Int-B. LCMS anal. calculated for C71H85F2N15O13S2: 1458.67; found: 1458.8 (M+1)⁺.

10 Step C—Synthesis of Compound Reagent B

[0618] Intermediate Compound Int-B (15 mg) was dissolved in 0.2 ml of dry DMSO. Then 15 mg of ALEXAFLUOR 647NHS Ester (A37566, Life technology) dissolved in 1.5 ml of dry DMSO was added. 20 μL of dry DIPEA was added. Reaction was left under stirring at room temperature for 12 h under Nitrogen atmosphere in the dark, quenched with TFA (pH to 3-4) and
15 purified by R-HPLC (Dr Maish, Reprosil Gold C18, 250×20 mm, 120 Å, 10 μm; 20% to 35% of 0.1% TFA in ACN/0.1% TFA in H₂O, over 20 min, then 35% to 40% over 5 min at 20 mL/min flow rate). Collected fractions were lyophilized to afford 16.1 mg of Compound Reagent B. LCMS anal. for C107H126F2N17O26S6³⁺: 2296.64, found: 1150.6 (M+2)²⁺.

20 Passive permeability of Example compound across MDCKII cell monolayers

[0619] The passive permeability of test compounds across MDCKII cell monolayers was measured. MDCKII cells were seeded onto 96-well transwell culture plates and used in experiments after four days in culture. [³H]Test compound (5 μM), [³H]verapamil (1 μM) or [³H]mannitol (5 μM) was prepared in HBSS with 0.1% w/v BSA, 10 mM HEPES (pH 7.4), 10
25 μM CsA, a known P-gp inhibitor, and 1.2 μM dextran Texas red (to confirm monolayer integrity). Substrate solution (150 μL) was added to either the apical (A) or the basolateral (B) compartment of the culture plate and buffer (150 μL; HBSS, with 0.1% w/v BSA, 10 mM HEPES, 10 μM CsA, pH 7.4) was added to the compartment opposite to that containing the substrate. At t=3 hr, 50 μL of sample was taken out from both sides, transferred to Costar 96-well
30 white plates with clear bottom, and 200 μL of UltimaGold scintillation fluid was added. Radioactivity was determined by liquid scintillation counting in a MicroBeta Wallac Trilux scintillation counter.

Parallel Artificial Membrane Passive Permeability (PAMPA)

35 [0620] PAMPA permeability of Example compounds was measured using a Pion Inc Stirwell 96-well sandwich plate with a 0.45μm PVDF membrane. Example compound was diluted from DMSO stock into Pion Donor buffer (pH adjusted to 7.4 with Pion Prisma-HT aqueous buffer

- 5 and NaOH) to an incubation concentration of 5 μ M, and 200 μ L of this solution was added to the donor plate wells in triplicate. A volume of 5 μ L of GIT-0 phospholipid (Pion Inc.) was applied to the acceptor plate PVDF membrane and each acceptor well was filled with 200 μ L of Acceptor Solution Buffer (ASB; Pion Inc). The apparatus was assembled and incubated for 20 hours at room temperature.
- 10 **[0621]** MS analysis was performed on a Waters I-Class Acquity Ultra High-Pressure Liquid Chromatography system (UHPLC) coupled with a Waters Xevo TQ-S triple quadrupole mass spectrometer. A rapid gradient elution (2-minute run time) was performed with mobile phase A = water + 1% formic acid and mobile phase B = acetonitrile + 0.1% formic acid at a flow rate of 0.75 ml/min. The gradient was held at 98% A for 0.1 minutes then ramped linearly to 90%
- 15 mobile phase B over 0.9 minutes, held for 0.5 minutes before returning to the initial conditions. A Waters HSS T3 2.1x50 mm 1.8 μ m particle size column was used and held at 50 °C. Parent to product transitions monitored m/z 776.2 to m/z 678.4 and m/z 523.9 to m/z 784.5 for MK-0616 and the angiotensin II internal standard, respectively.
- [0622]** A MK-0616 standard curve (1.6nM- 5 μ M) and was prepared by dilution of 2mM DMSO stock into donor buffer using a Hewlett Packard D300 system. Initial, acceptor, donor and standard curve plates were matrix matched and a 4x volume of internal standard solution added (250ng/mL Angiotensin II in acetonitrile). MK-0616 concentration data was imported into BioAssay (Perkin Elmer) for calculation of P_{eff} and percentage recovery. P_{eff} and % recovery were calculated using the equations below:

25

$$P_{eff} = -2.303 \times \frac{-2.303 \times V_d}{e \times A \times (t - t_0)} \times \text{Log} \left\{ \frac{C_d(t)}{C_d(0) \times (1 - R)} \right\}$$

Where:

$$R = 1 - \frac{(V_a \times C_a(t)) + (V_d \times C_d(t))}{V_d \times C_d(0)}$$

- 30 V = Volume, C = Concentration, A = Membrane area, a = Acceptor, d = Donor, t = Time, e = Apparent porosity (0.7 for the Pion system)

$$\% \text{ Recovery} = 100 \times \frac{C_a(t) + C_d(t)}{C_d(0)}$$

35

5

Example compound	Activity (nM)	Type of Alexa FRET Assay	Permeability (MDCKII Cell) P_{app} ($\times 10^6$ cm/s)	Permeability (PAMPA)
Ex-1	1.43	Standard	na	0
Ex-2	1.9	Standard	na	na
Ex-3	0.328	Plus	0.7	0
Ex-4	22.56	Standard	na	0
Ex-5	901.4	Standard	0.895	0.3125
Ex-6	69.62	Standard	0.71	0
Ex-7	46.49	Standard	0.4	0.306
Ex-8	132.7	Standard	na	na
Ex-9	0.28	Plus	0.6	0.61
Ex-10	73.59	Standard	1.265	17.2
Ex-11	37.12	Standard	1.435	3.45/11.76
Ex-12	34.68	Standard	2.02	19.27
Ex-13	5368	Standard	0.8	0.87
Ex-14	36.14	Standard	2.36	14.31
Ex-15	144.7	Standard	7.05	37.68
Ex-16	102.6	Standard	8.56	27.68
Ex-17	145.4	Standard	1.85	16.46
Ex-18	15.96	Standard	6.31	38.43/20.67
Ex-19	534.4	Standard	1.27	2.5
Ex-20	331.3	Standard	5.46	32.06
Ex-21	364.7	Standard	9.97	-999
Ex-22	381.4	Standard	0.87	1.25/0.925
Ex-23	166.7	Standard	8.887	11.26/21.43
Ex-24	14670	Standard	1.71	24.68
Ex-25	658.8	Standard	1.23	0.99/2.125
Ex-26	315.3	Standard	9.295	33.79/40.65
Ex-27	354.3	Standard	6.84	-999
Ex-28	37.96	Standard	6.41	13.55
Ex-29	16.57	Standard	6.44	40.27

Ex-30	330.3	Standard	6.96	39.29
Ex-31	632	Standard	9.35	43.44
Ex-32	160.8	Standard	1.12	17.87/16.09
Ex-33	54.5	Standard	5.58	13.73
Ex-34	18.32	Standard	7.36	20.53
Ex-35	5071	Standard	4.37	58.18
Ex-36	15.2	Standard	0.75	0.01
Ex-37	143.7	Standard	1.13	2.19
Ex-38	349.8	Standard	6.28	38.16
Ex-39	5100	Standard	na	-999
Ex-40	27.6	Standard	5.23	31.74
Ex-41	25190	Standard	na	na
Ex-42	72.6	Standard	3.35	4.47
Ex-43	79.6	Standard	1.6	4.18
FP-44	64.7	Standard	1.61	-999
Ex-45	15.9	Standard	2.43	10.24
Ex-46	175.6	Standard	5.43	31.06
Ex-47	65.3	Standard	5.54	37.16
Ex-48	97.5	Standard	4.805	40
Ex-49	171.2	Standard	5.69	30.4
Ex-50	212.2	Standard	6.15	33.69
Ex-51	111.5	Standard	10.24	33.62/41.04
Ex-52	192.4	Standard	12.17	21.7
Ex-53	555.9	Standard	6.695	51.65
EX-54	324.2			
EX-55A	152.0	Standard		
EX-55B	199.0	Standard		
EX-56	569.0	Standard		3.01
EX-57	91.69	Plus	1.51	19.9
EX-58	1074	Standard	1.92	27
EX-59	424.5	Standard	1.62	6.9

5 Passive permeability of test compound across MDCKII cell monolayers

[0623] The passive permeability of test compound across MDCKII cell monolayers was measured. MDCKII cells were seeded onto 96-well transwell culture plates and used in experiments after four days in culture. [³H]Test compound (5 μM), [³H]verapamil (1 μM) or [³H]mannitol (5 μM) was prepared in HBSS with 0.1% w/v BSA, 10 mM HEPES (pH 7.4), 10 μM CsA, a known P-gp inhibitor, and 1.2 μM dextran Texas red (to confirm monolayer integrity). Substrate solution (150 μL) was added to either the apical (A) or the basolateral (B) compartment of the culture plate and buffer (150 μL; HBSS, with 0.1% w/v BSA, 10 mM HEPES, 10 μM CsA, pH 7.4) was added to the compartment opposite to that containing the substrate. At t=3 hr, 50 μL of sample was taken out from both sides, transferred to Costar 96-well plates with clear bottom, and 200 μL of UltimaGold scintillation fluid was added. Radioactivity was determined by liquid scintillation counting in a MicroBeta Wallac Trilux scintillation counter.

Incorporation by reference

20 [0624] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

Equivalents

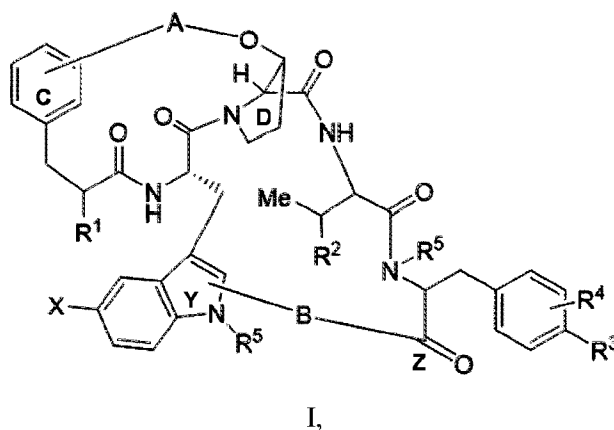
[0625] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Various structural elements of the different embodiments and various disclosed method steps may be utilized in various combinations and permutations, and all such variants are to be considered forms of the invention. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

30

CLAIMS

What is claimed is:

1. A compound of Formula I

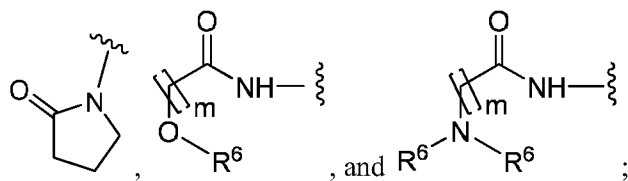


or a pharmaceutically acceptable salt thereof,

wherein

X is H, F, Cl, or Br;

R¹ is selected from the group consisting of -NR⁶CO(C₁-C₄)alkyl, pyrimidinyl-CONR⁶-,



R² is -OH, (C₁-C₄)alkyl or -O(C₁-C₄)alkyl;

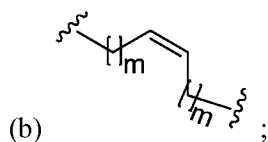
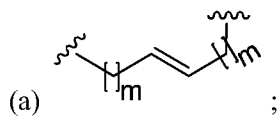
R³ is -O(C₁-C₄)alkyl, optionally substituted with one to six F;

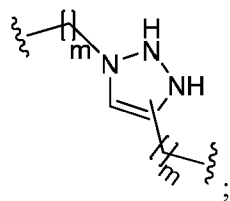
R⁴ is H or -(C₁-C₄)alkyl;

R⁵, at each occurrence, is independently selected from H, -(C₁-C₄)alkyl, and a bond;

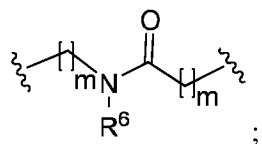
R⁶, at each occurrence, is independently selected from H and -(C₁-C₄)alkyl;

—A— is a linker selected from the group consisting of

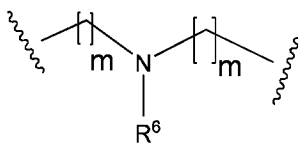




(c)

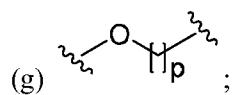
(d) $-(CH_2)_p-$;

(e)



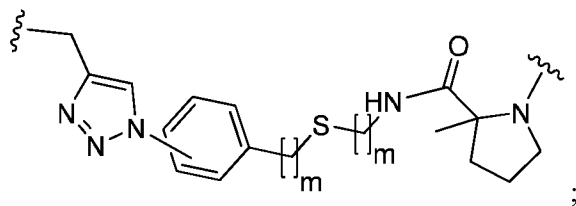
(f)

, and

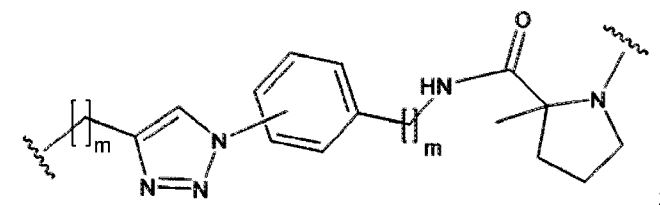


(g)

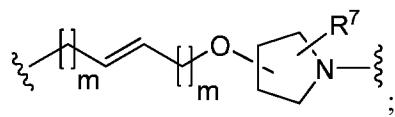
— **B** — is a linker selected from the group consisting of



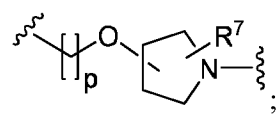
(a)



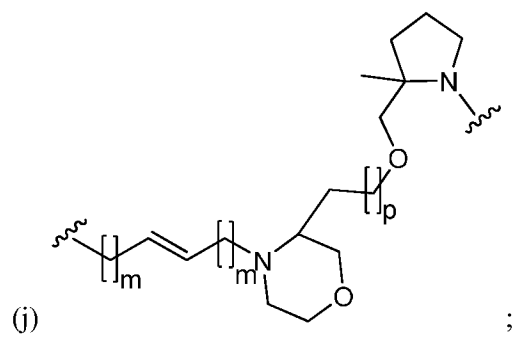
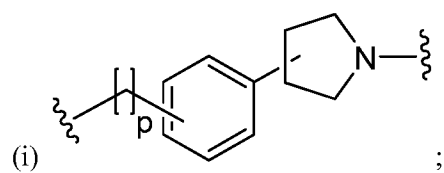
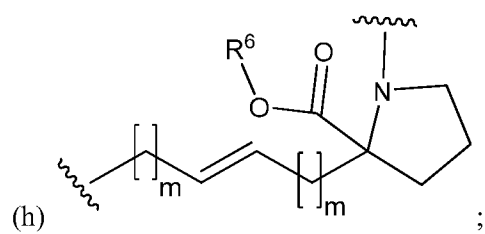
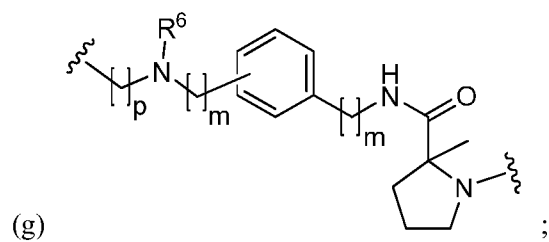
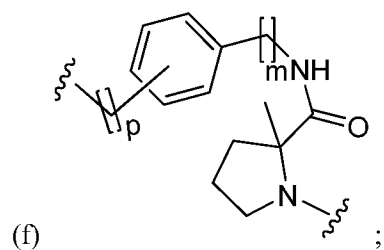
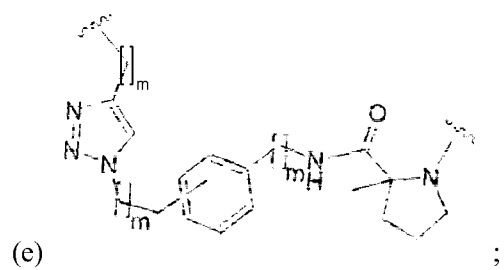
(b)

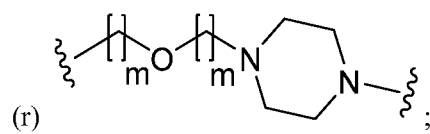
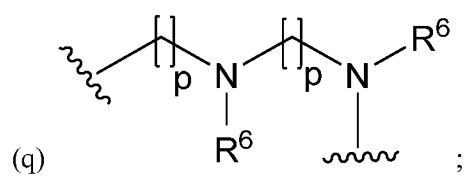
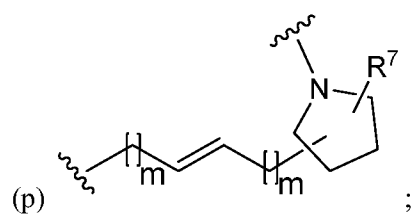
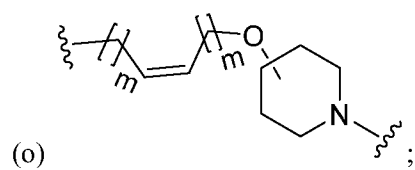
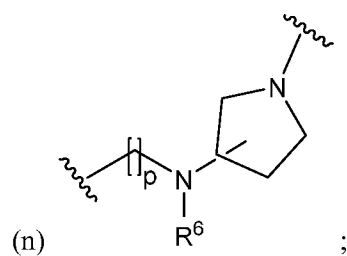
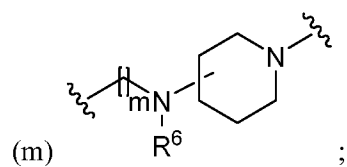
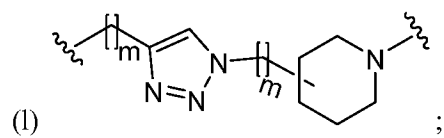
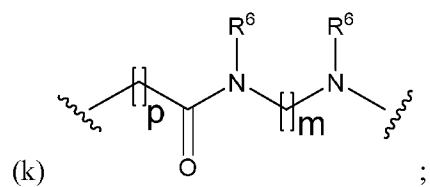


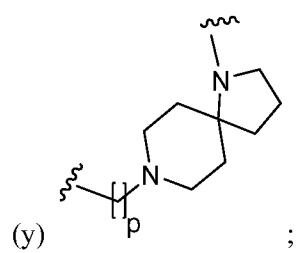
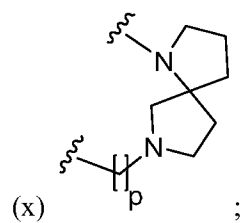
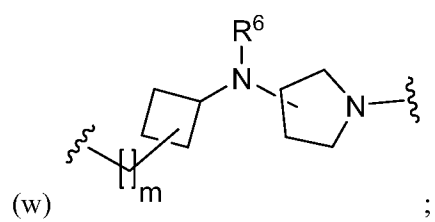
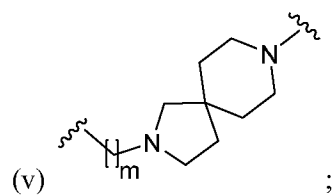
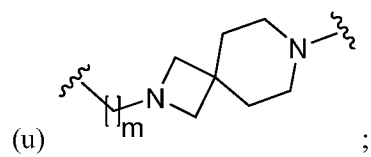
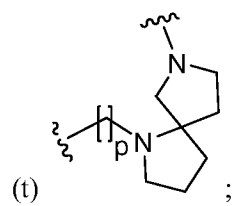
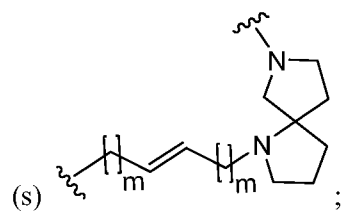
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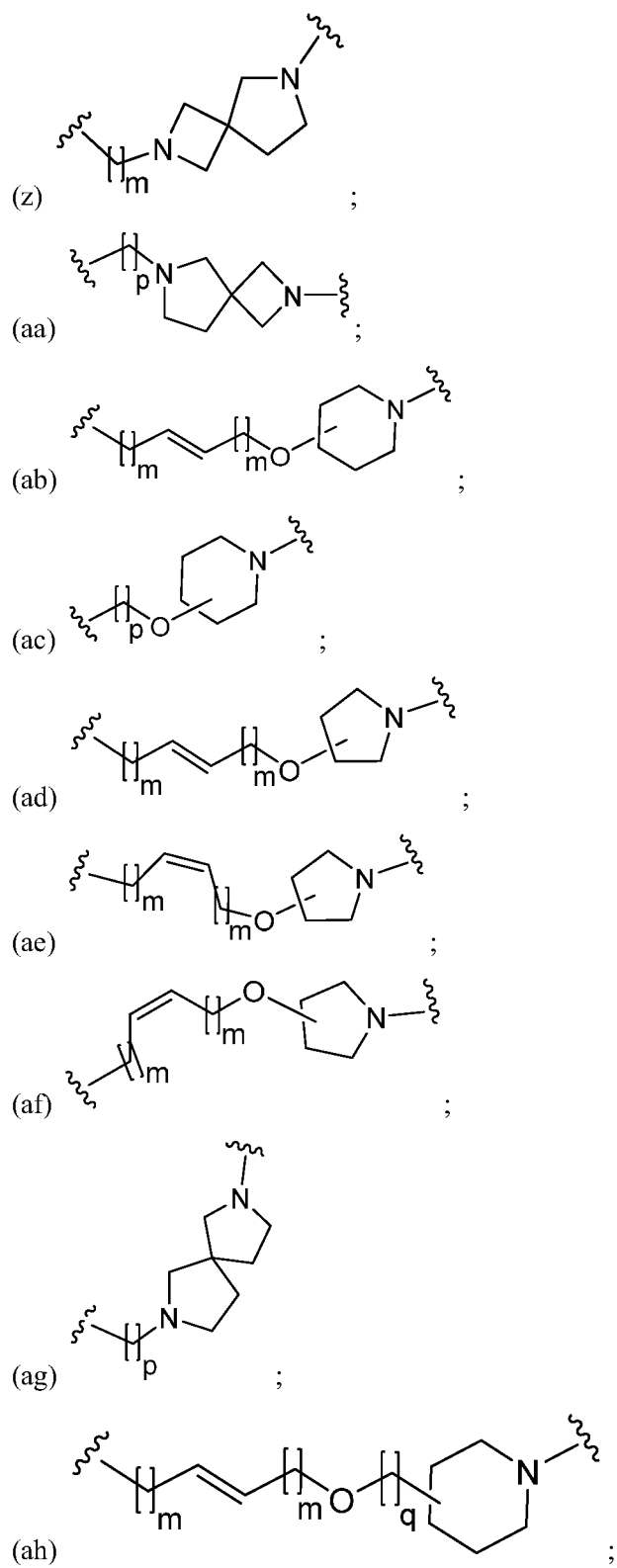


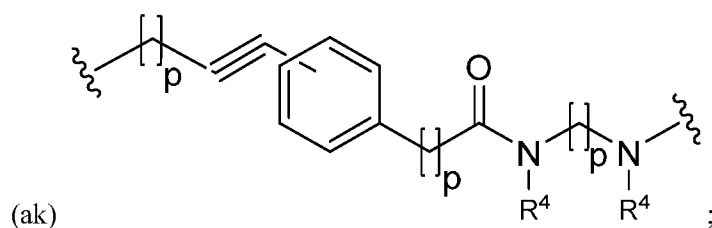
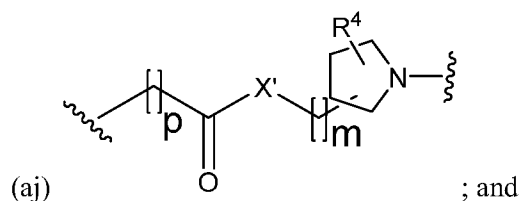
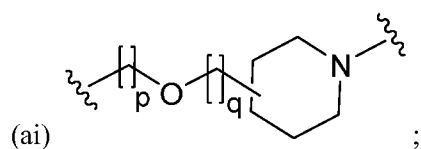
(d)











wherein when B is connected to the nitrogen of the indole ring Y, R^5 is a bond;

n is an integer between 0 and 2;

m is an integer between 0 and 4;

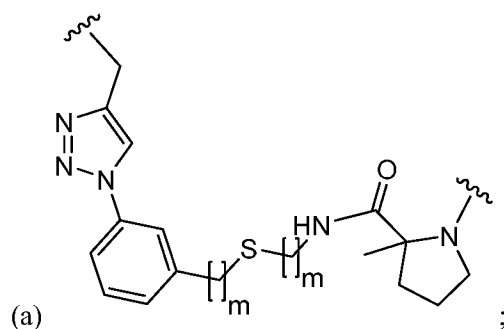
q is an integer between 1 and 4;

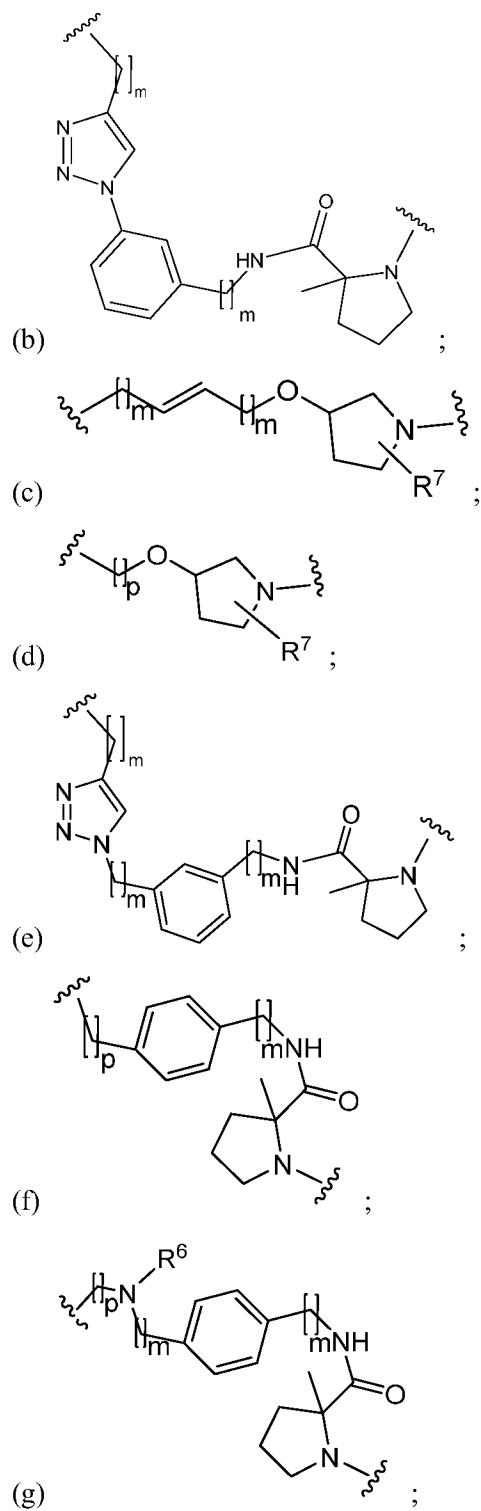
p is an integer between 1 and 5;

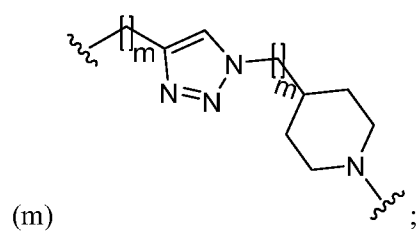
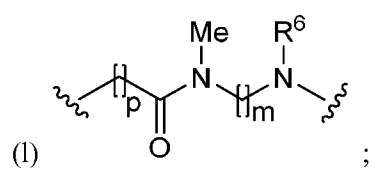
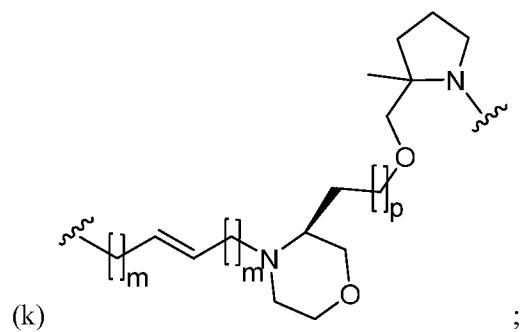
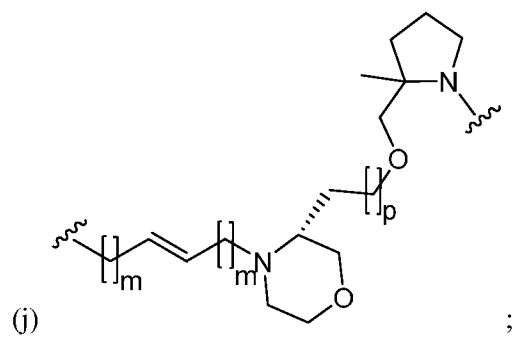
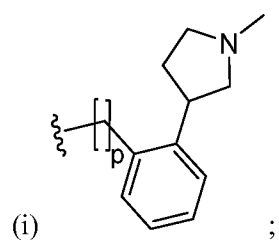
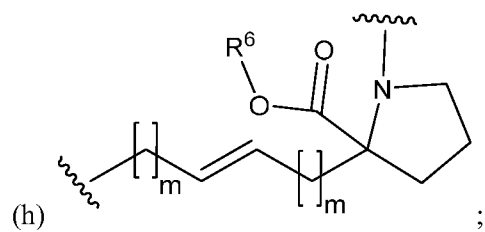
R^7 is H or $-\text{CON}(R^6)_2$; and

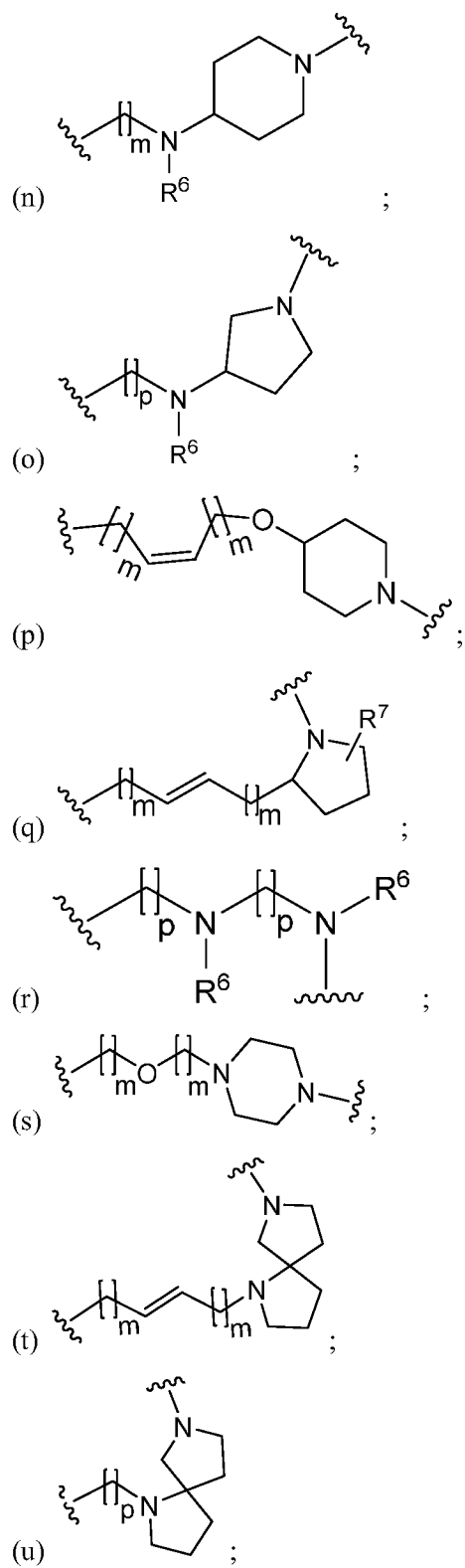
X' is N or O, wherein the N is optionally substituted with $-(\text{C}_1\text{-C}_4)\text{alkyl}$.

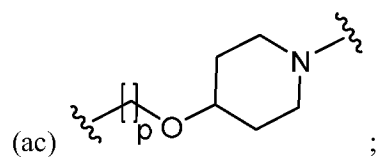
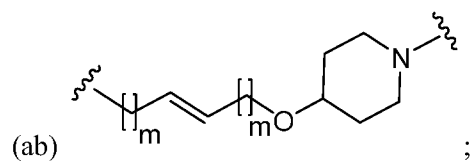
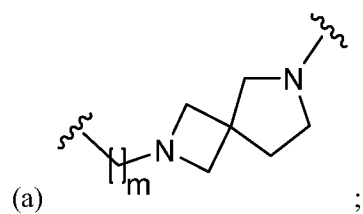
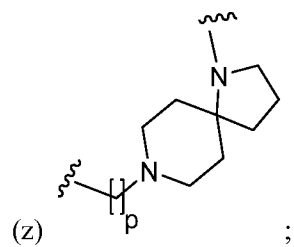
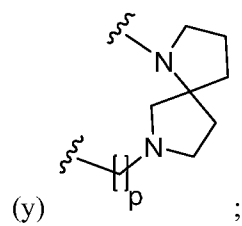
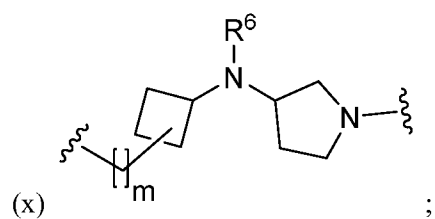
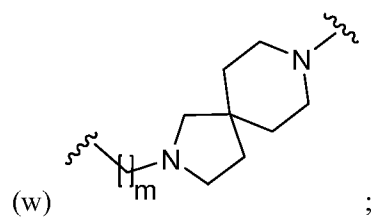
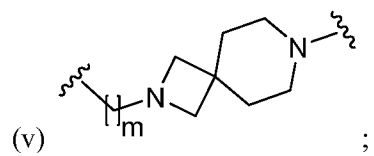
2. The compound of claim 1, wherein the linker B is selected from the group consisting of:

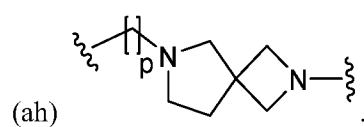
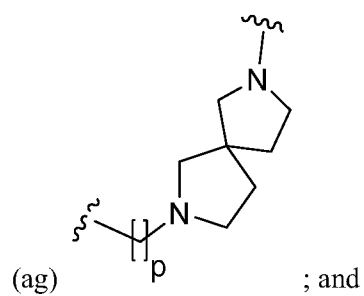
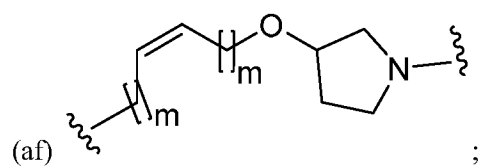
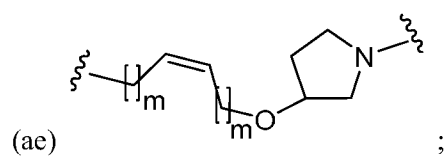
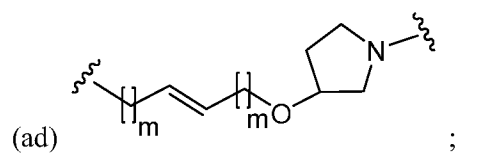




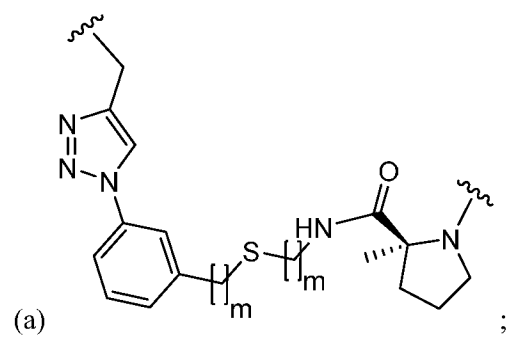


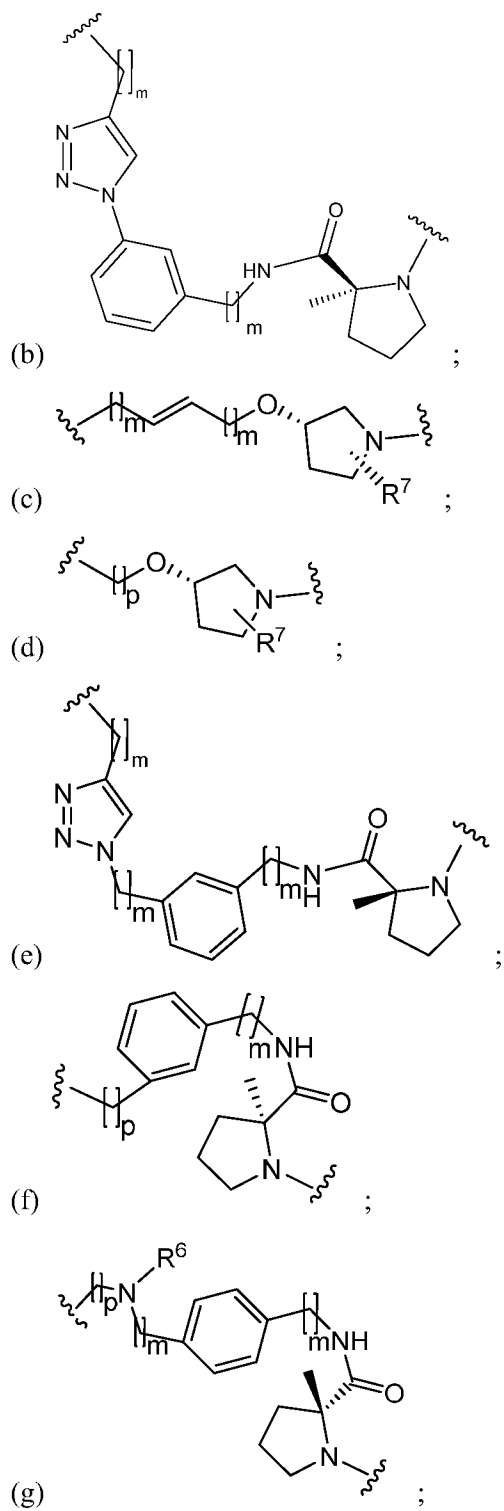


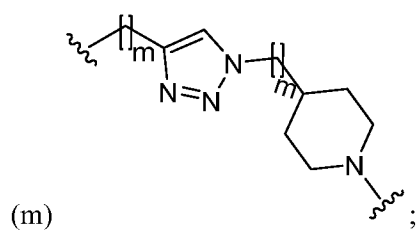
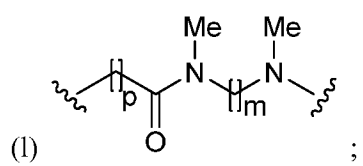
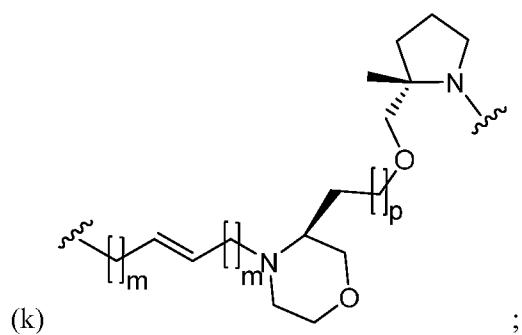
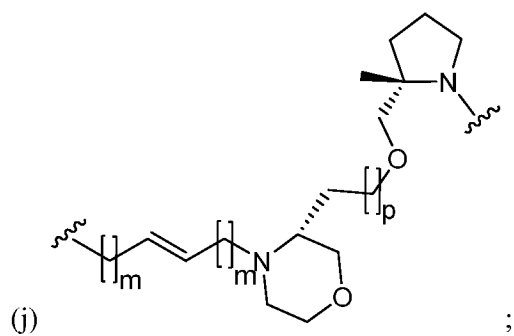
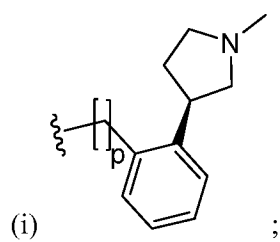
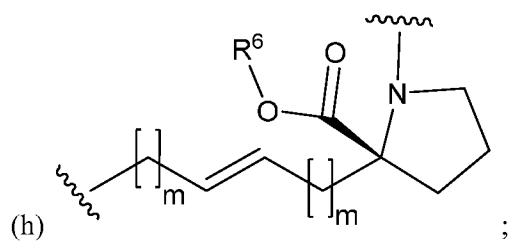


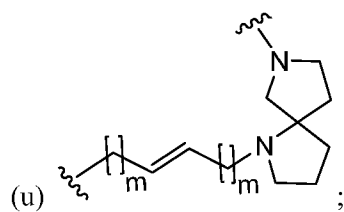
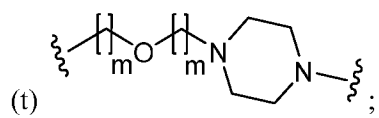
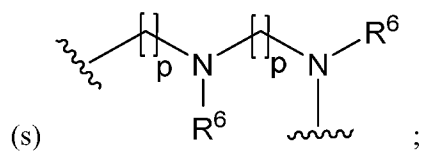
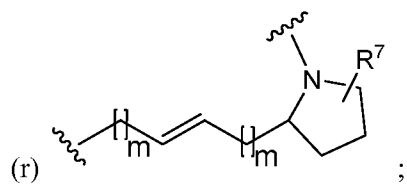
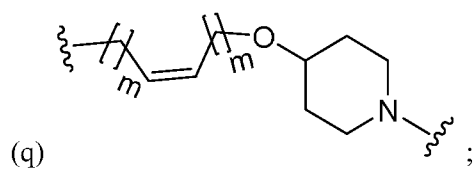
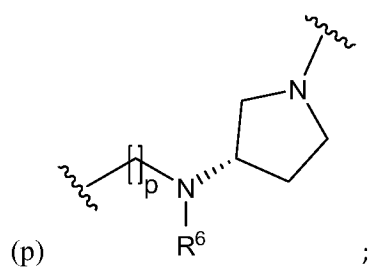
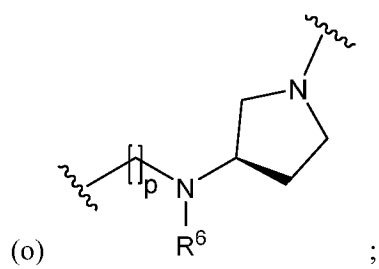
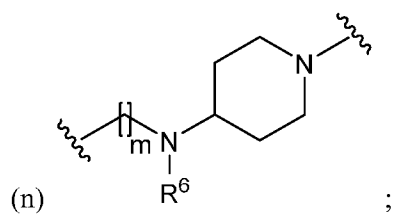


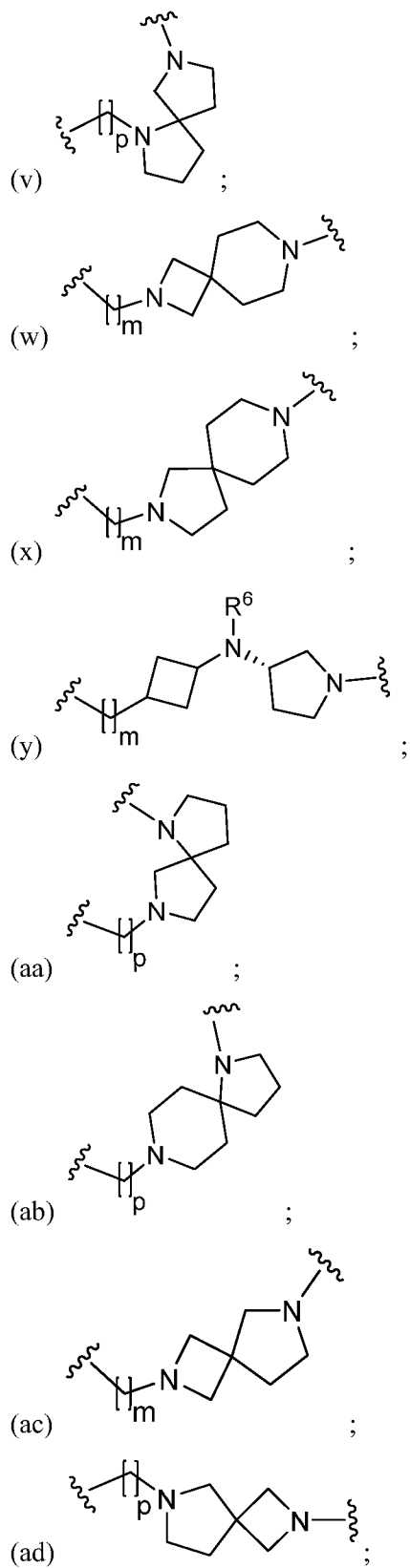
3. The compound of any of claim 1 or claim 2, wherein the linker B is selected from the group consisting of:

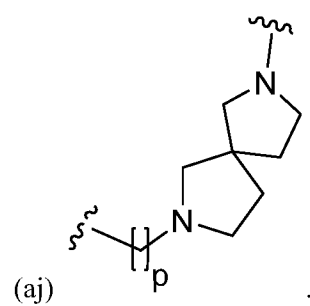
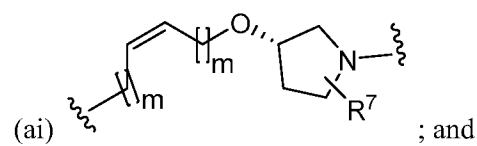
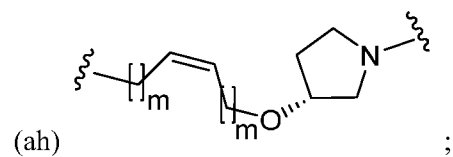
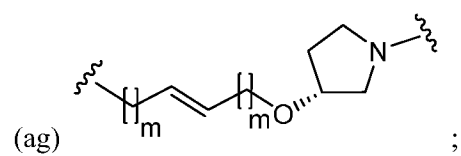
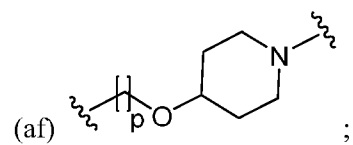
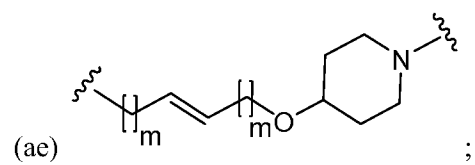




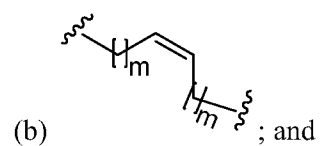
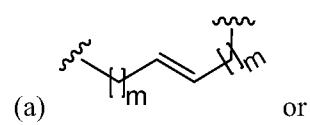




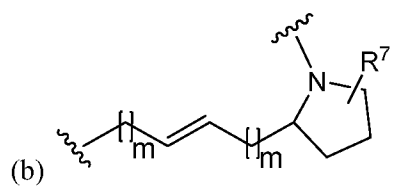
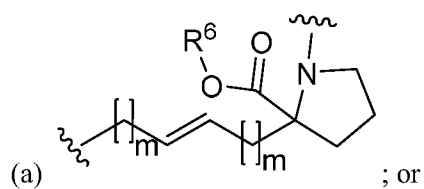




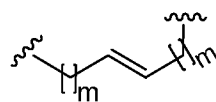
4. The compound of claim 1, wherein
A is



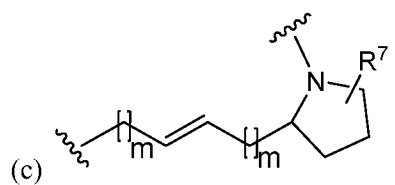
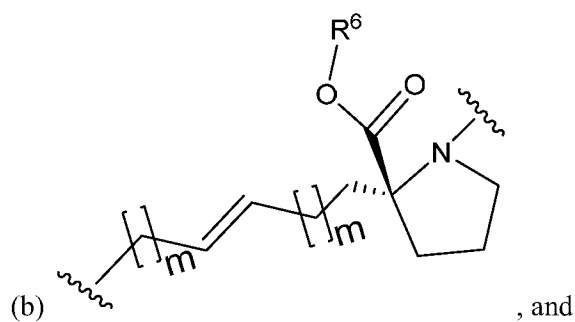
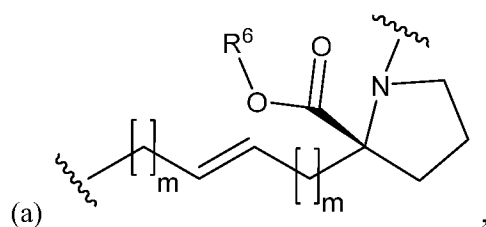
B is



5. The compound of claim 4, wherein A is



and B is selected from the group consisting of



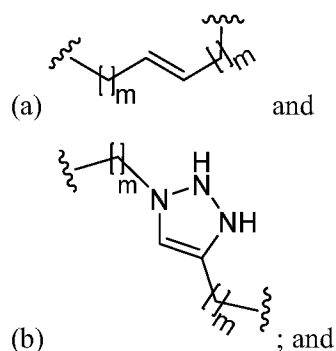
6. The compound of claim 5, wherein at least one of the two R^5 groups is $-(C_1-C_4)\text{alkyl}$.

7. The compound of claim 5, wherein both R^5 groups are $-(C_1-C_4)\text{alkyl}$.

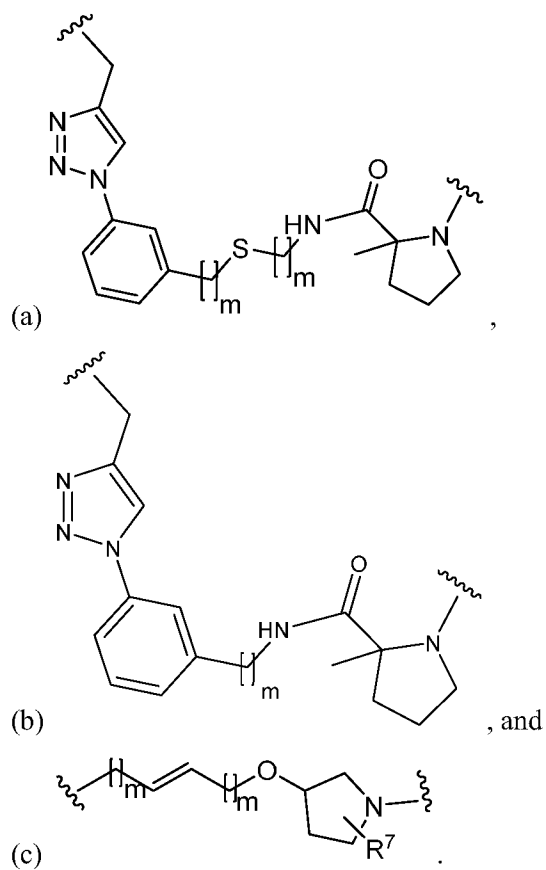
8. The compound of any one of claims 6 or 7, wherein at least one occurrence of R^5 is $-\text{CH}_3$.

9. The compound of any one of claims 6 or 7, wherein each occurrence of R^5 and R^6 is - CH_3 , and R^7 is H.

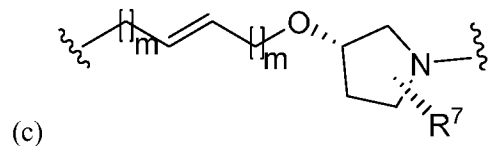
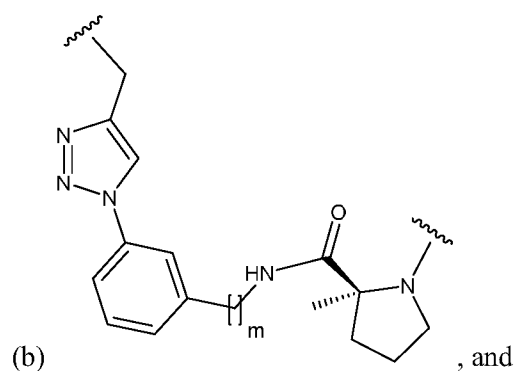
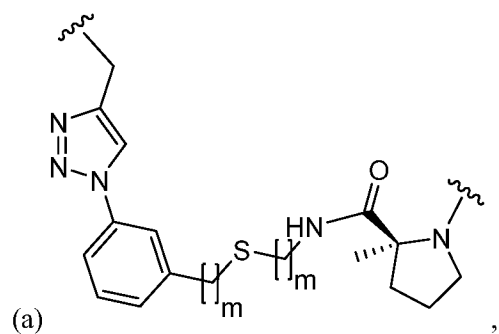
10. The compound of claim 1, wherein
A is selected from the group consisting of



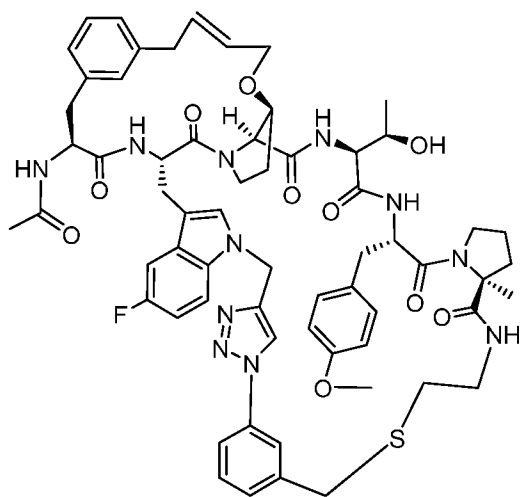
B is selected from the group consisting of



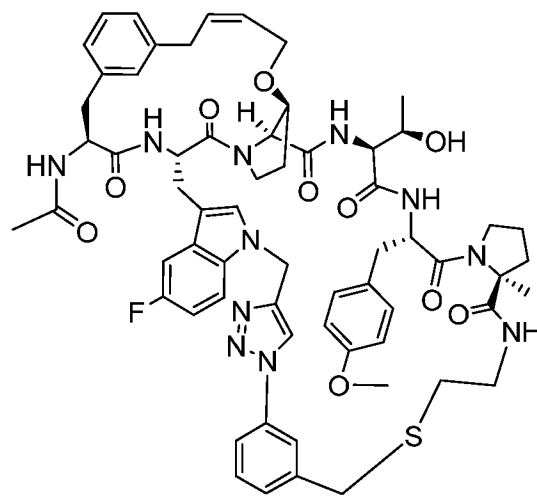
11. The compound of claim 10, wherein B is selected from the group consisting of



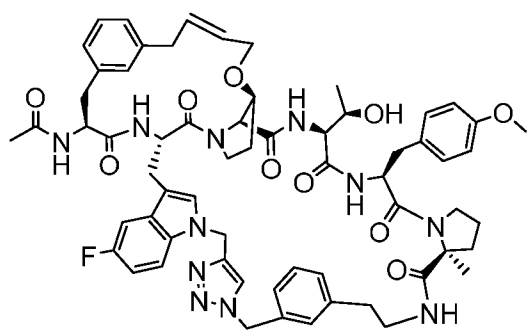
12. The compound of any one of claims 1-11, wherein X is F.
13. A compound of claim 1 selected from the group consisting of



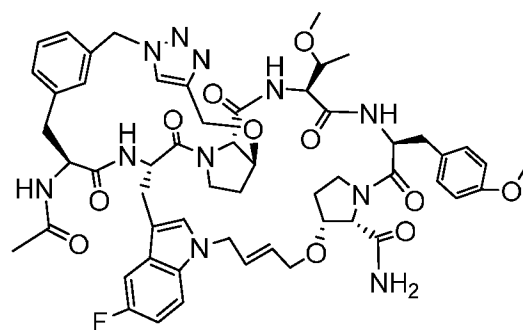
Ex-1,



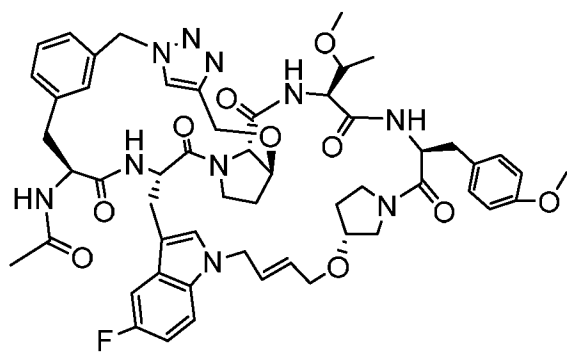
Ex-2,



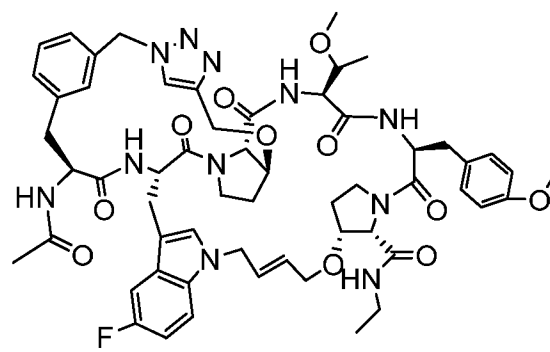
Ex-3,



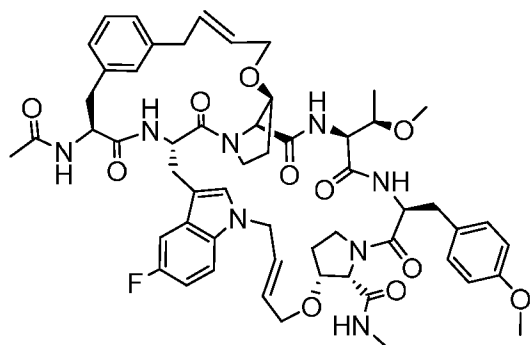
Ex-4,



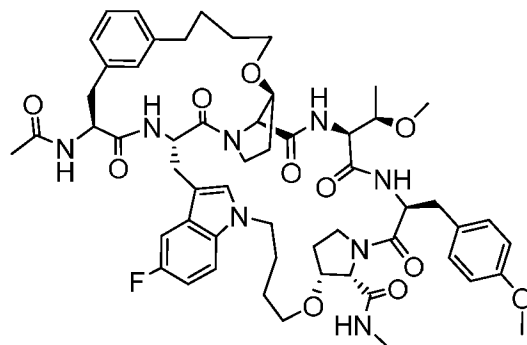
Ex-5,



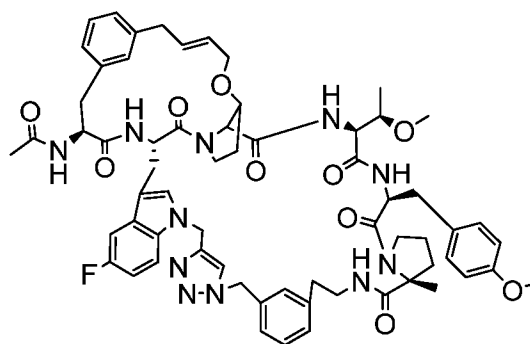
Ex-6,



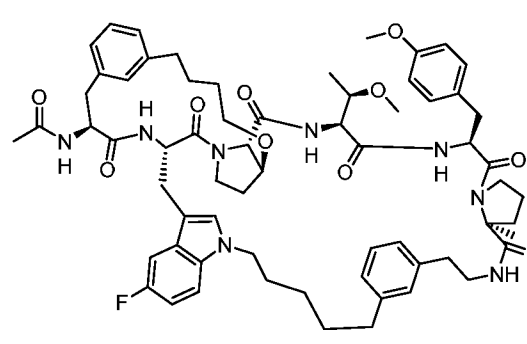
Ex-7,



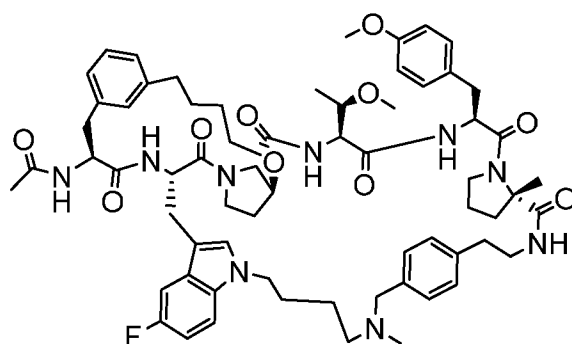
Ex-8,



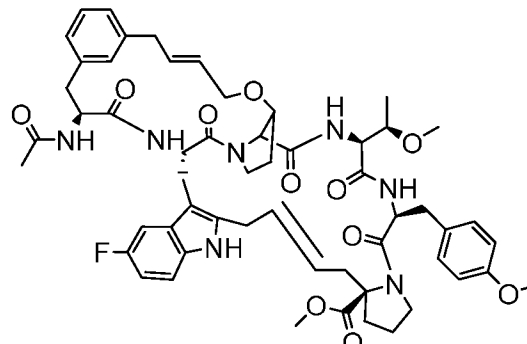
Ex-9,



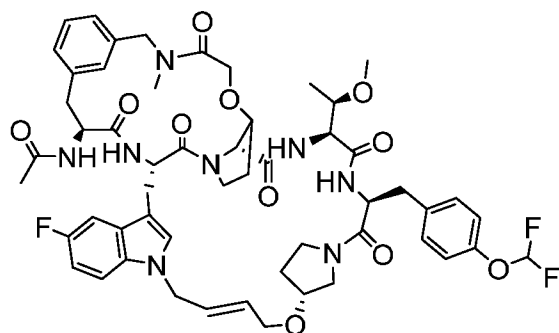
Ex-10,



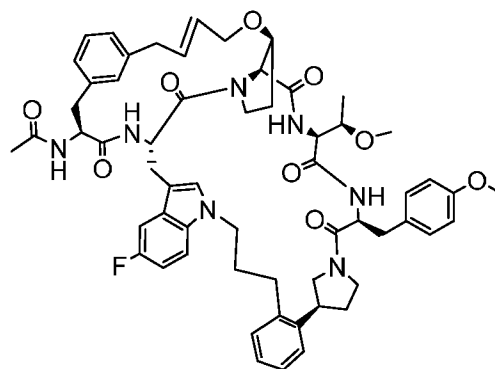
Ex-11,



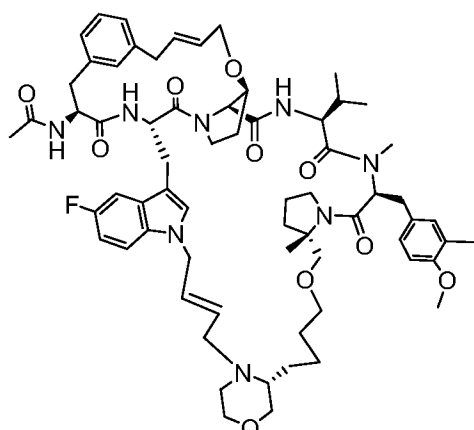
Ex-12,



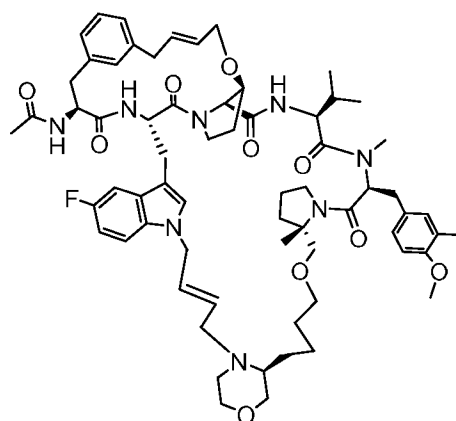
Ex-13,



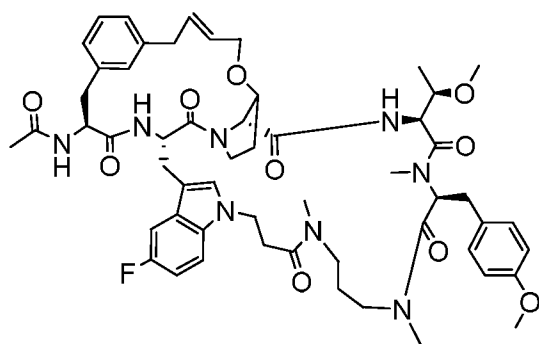
Ex-14,



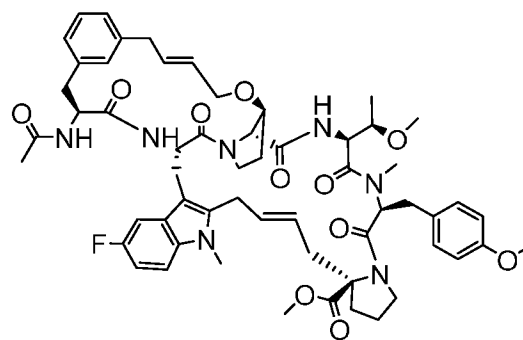
Ex-15,



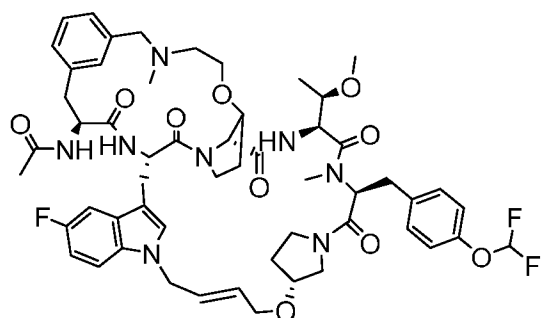
Ex-16,



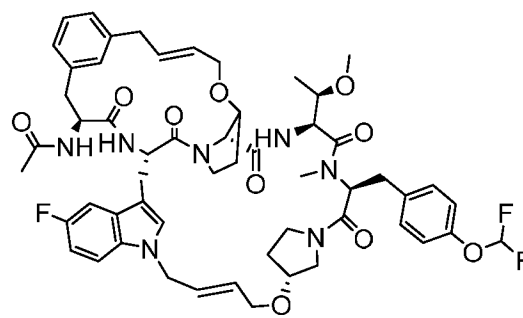
Ex-17,



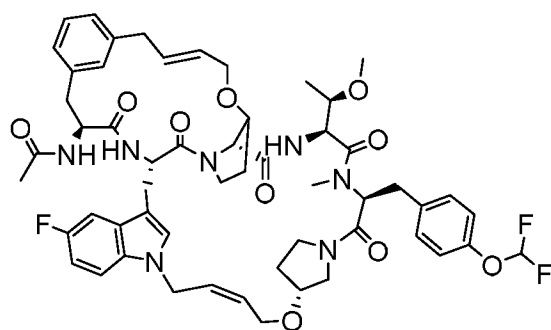
Ex-18,



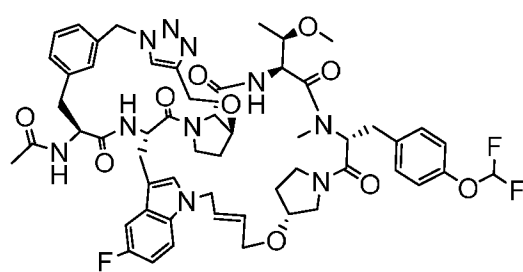
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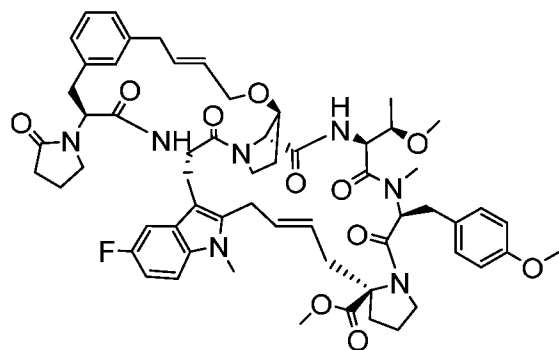
Ex-20,



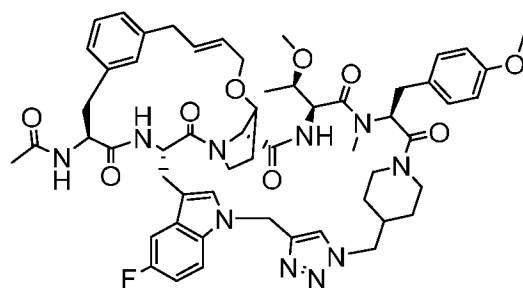
Ex-21,



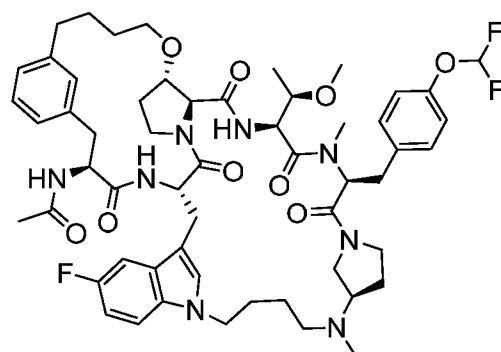
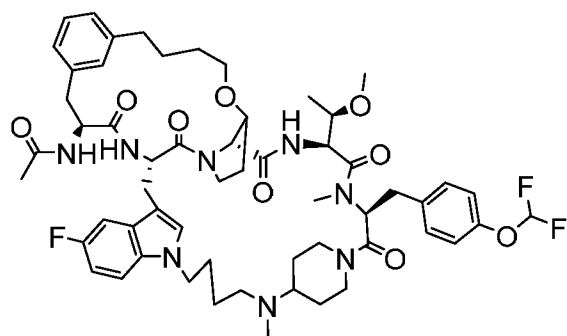
Ex-22,



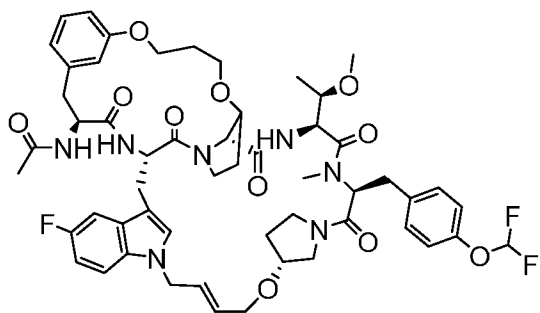
Ex-23,



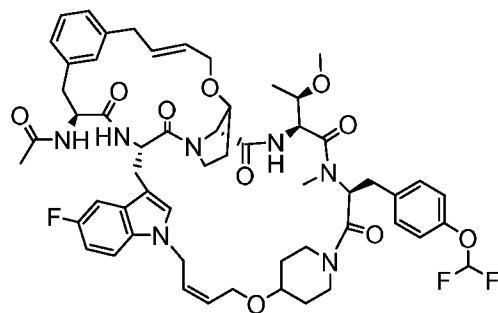
Ex-24,



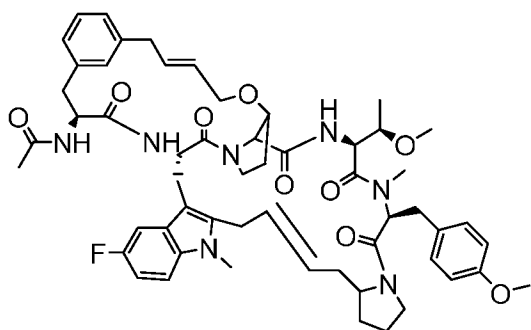
Ex-25,



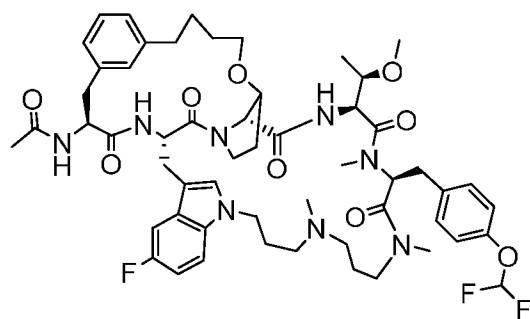
Ex-26



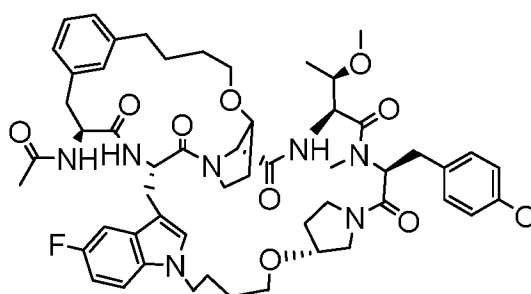
Ex-27,



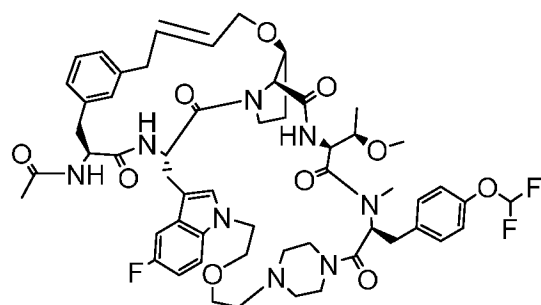
Ex-28,



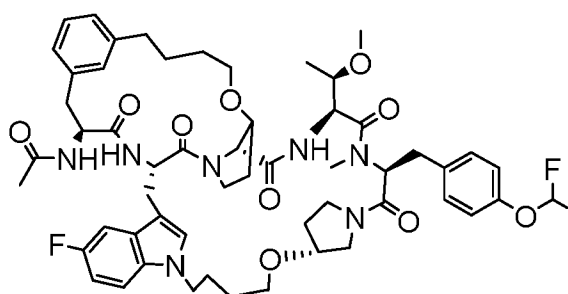
Ex-29,



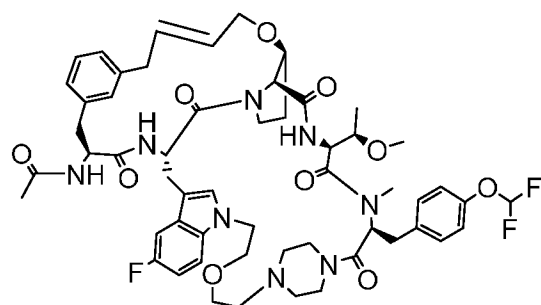
Ex-30,



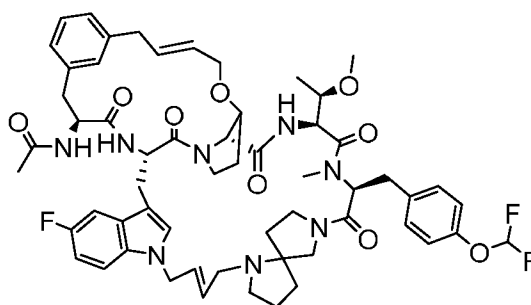
Ex-31,



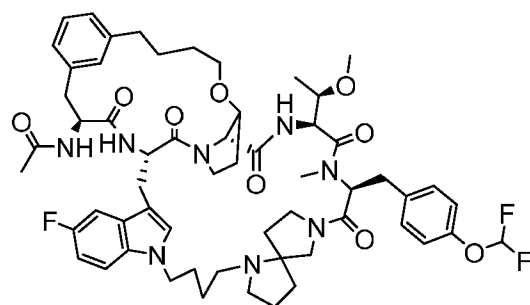
Ex-32,

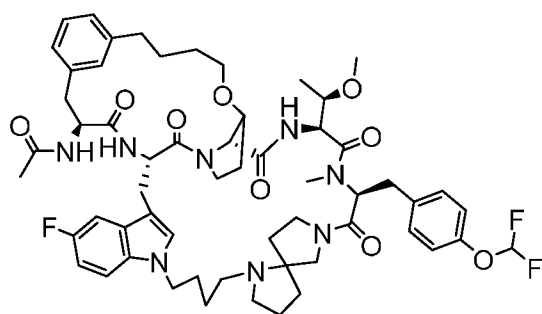


Ex-33,

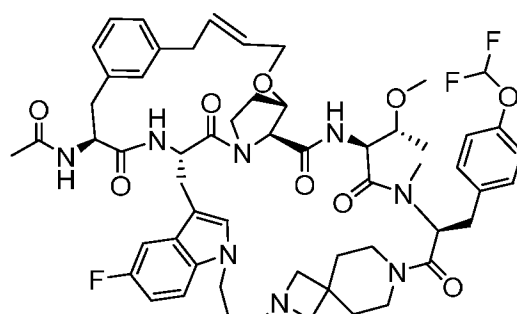


Ex-34,

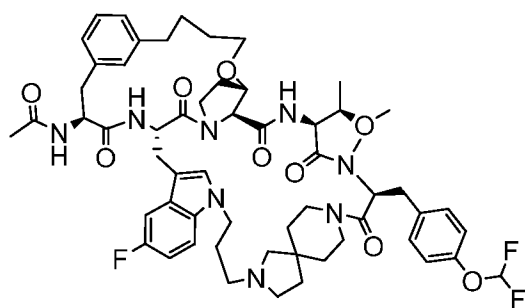




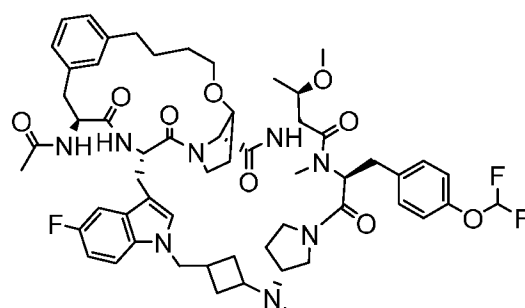
Ex-35,



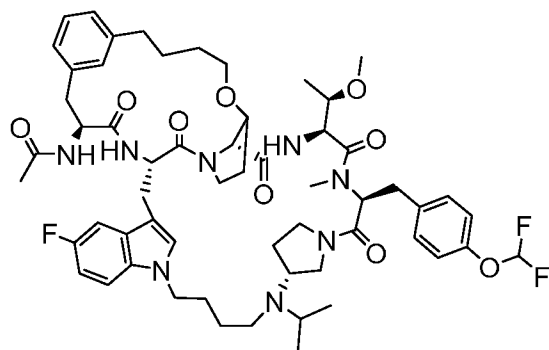
Ex-36,



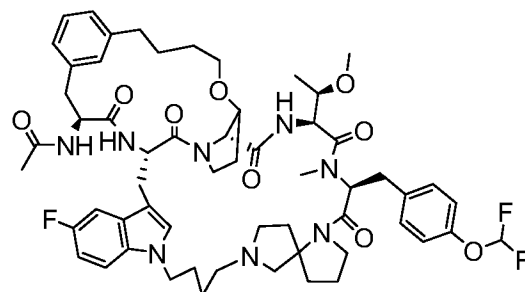
Ex-37,



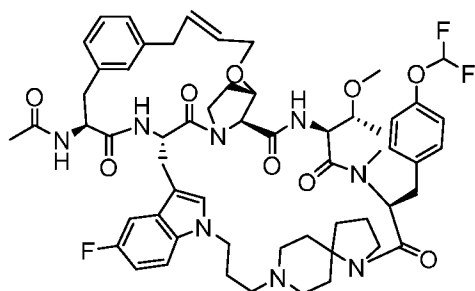
Ex-38,



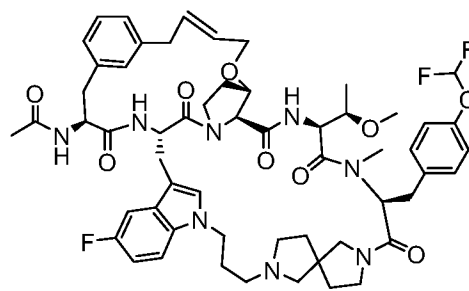
Ex-39,



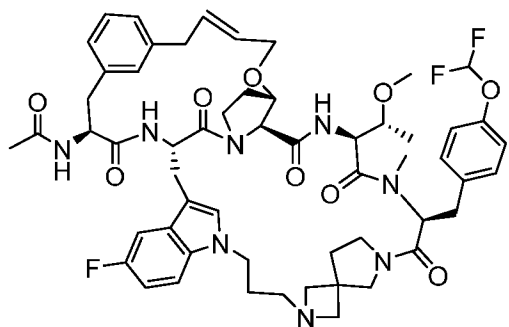
Ex-40



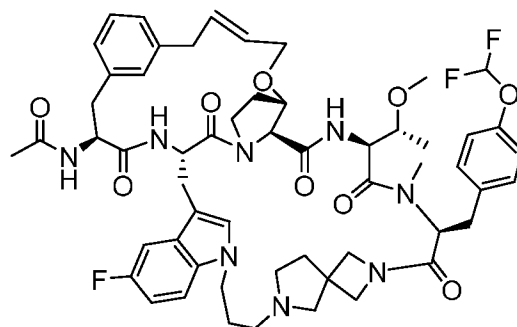
Ex-41



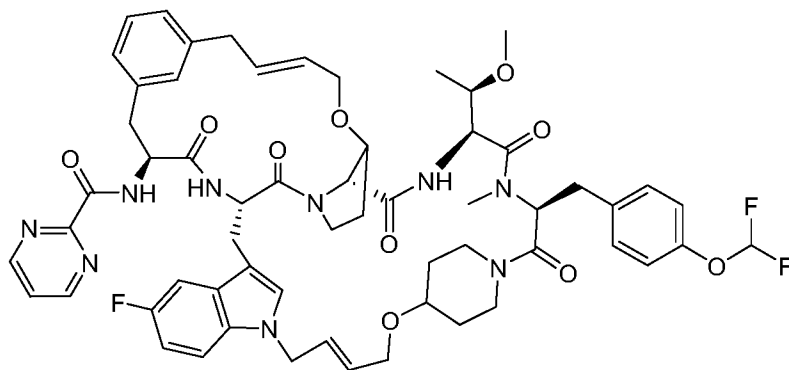
Ex-42



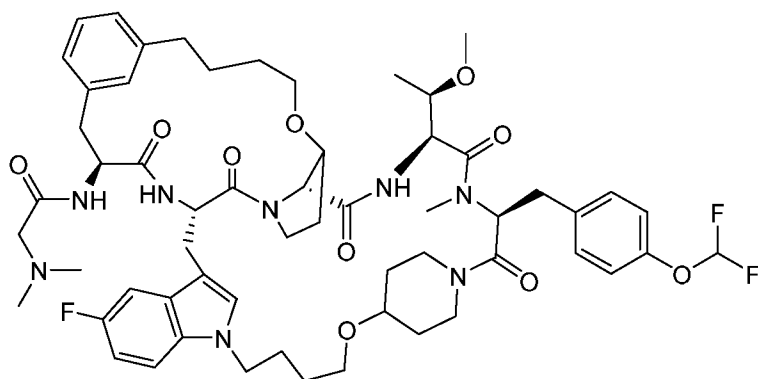
Ex-43,



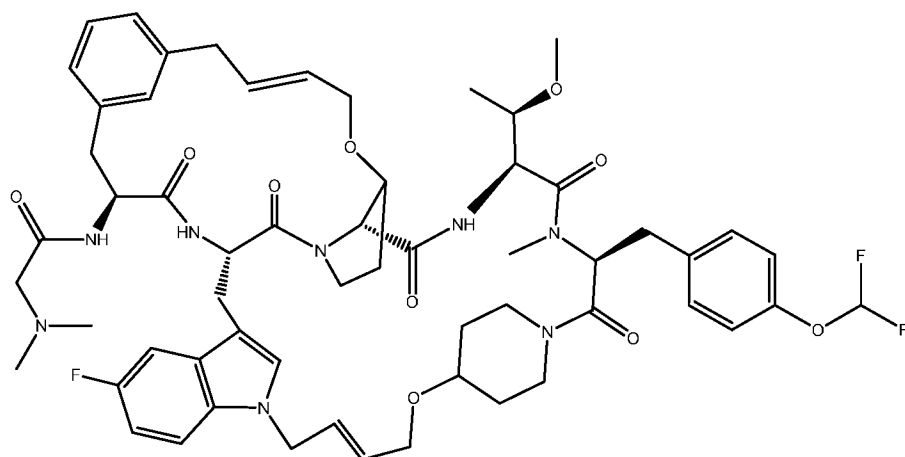
Ex-44,



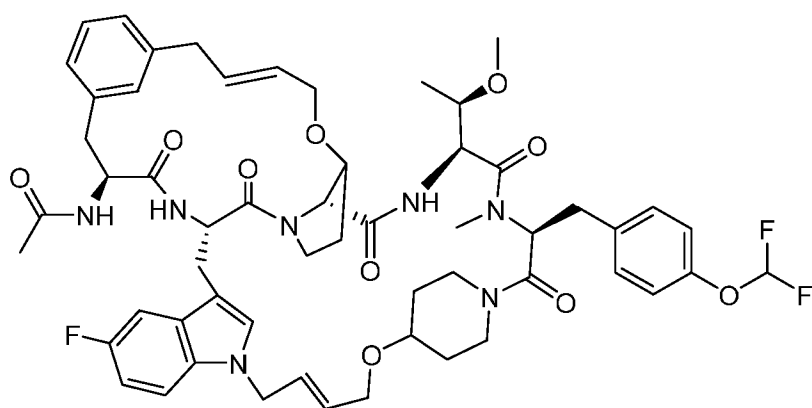
Ex-45,



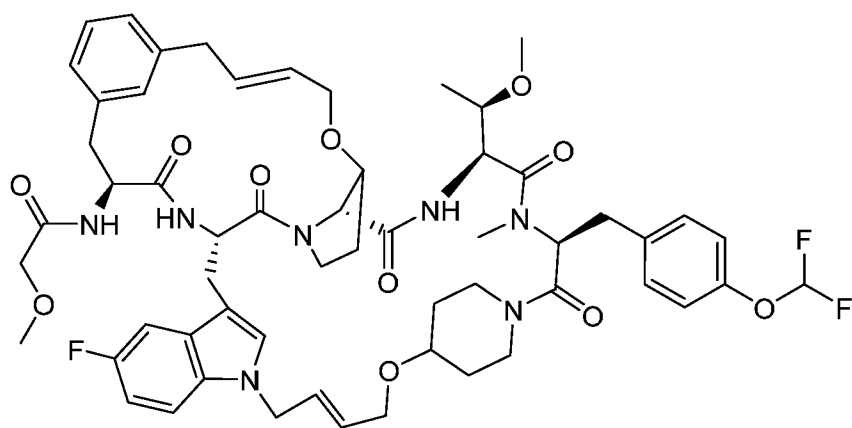
Ex-46,



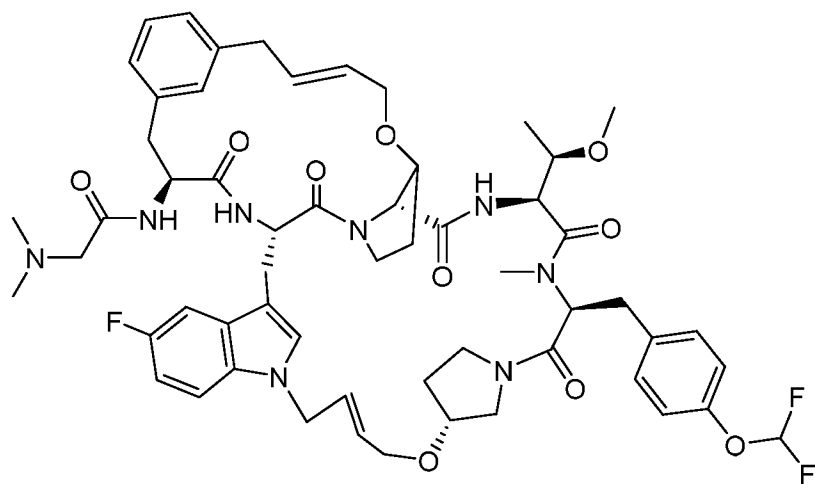
Ex-47,



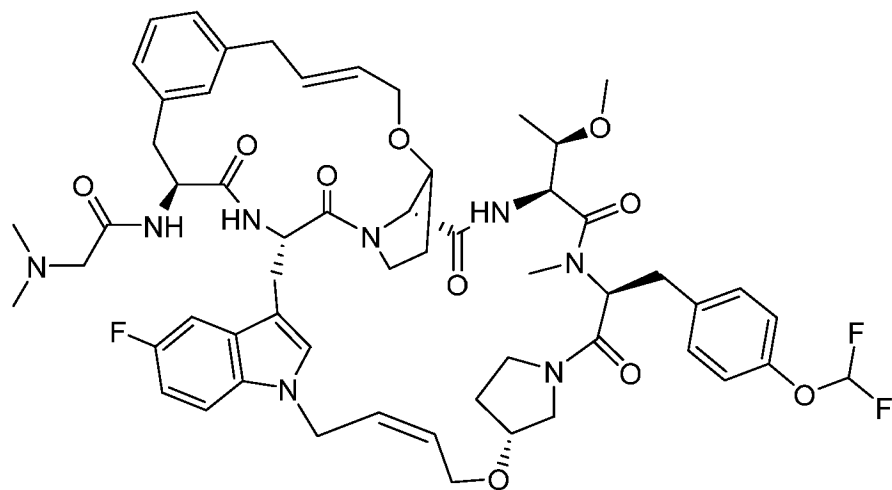
Ex-48,



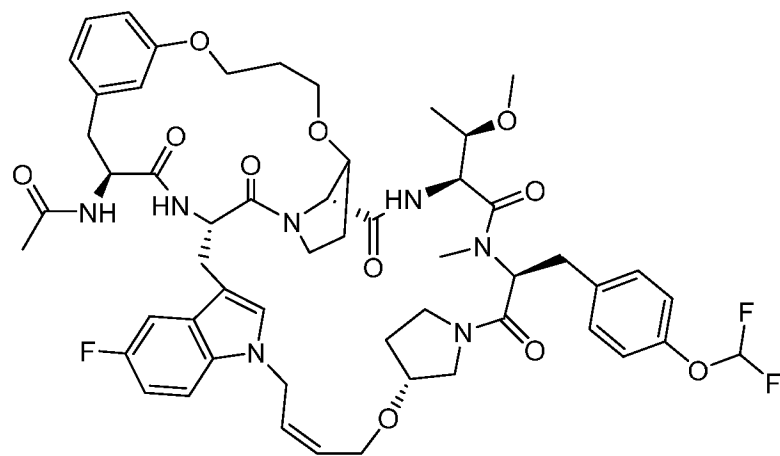
Ex-49,



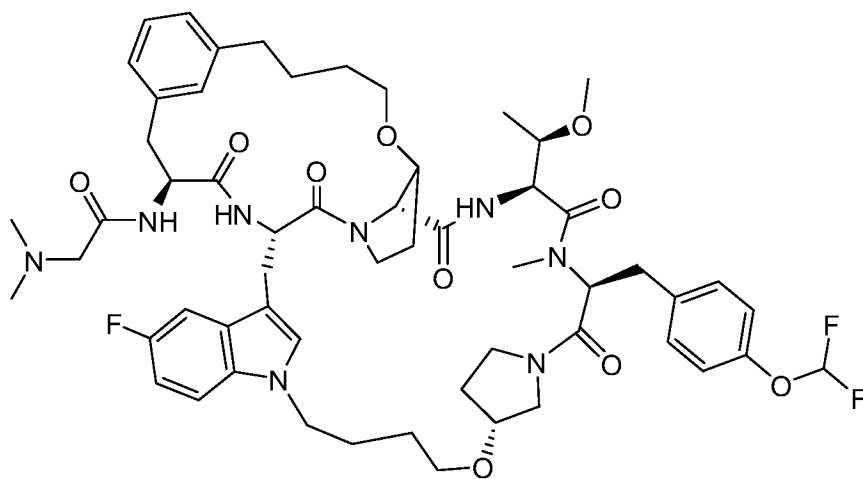
Ex-50,



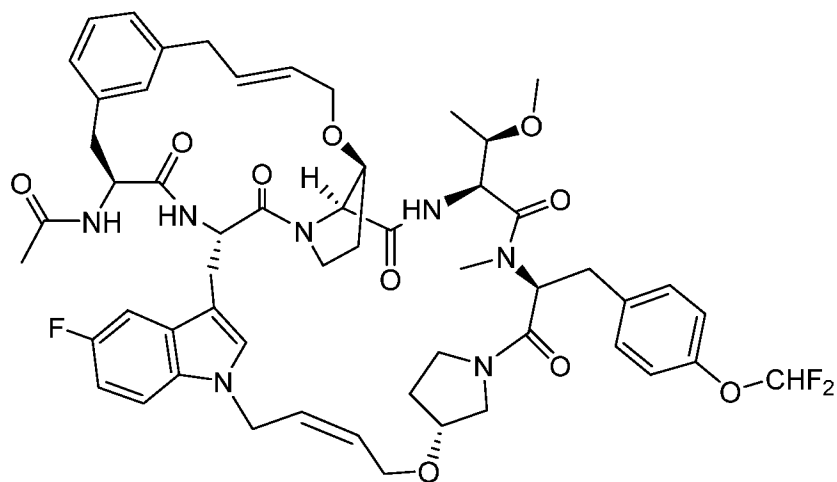
Ex-51,



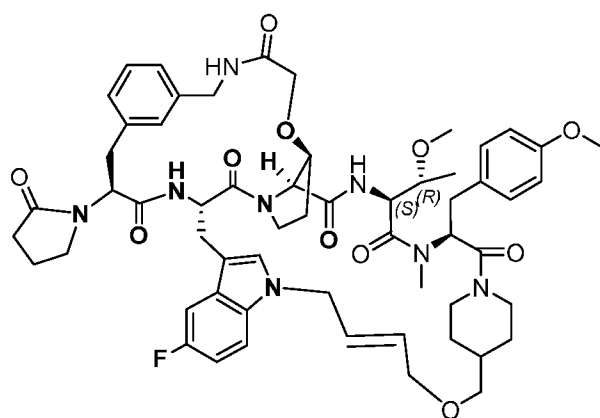
Ex 52,



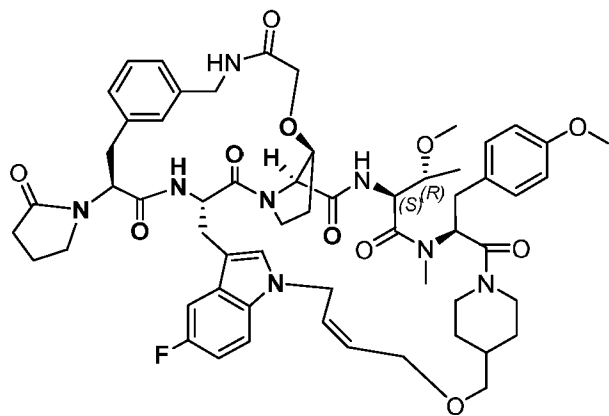
Ex-53, and



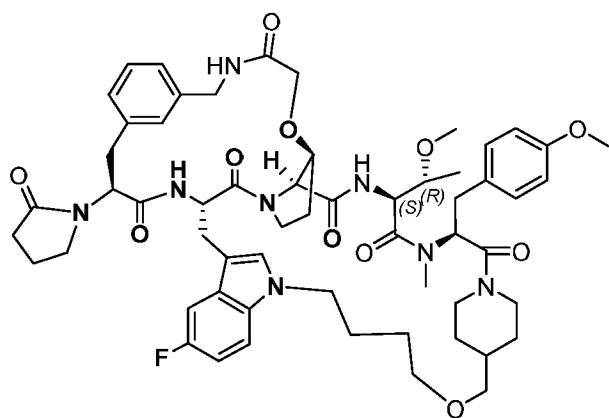
Ex-54.



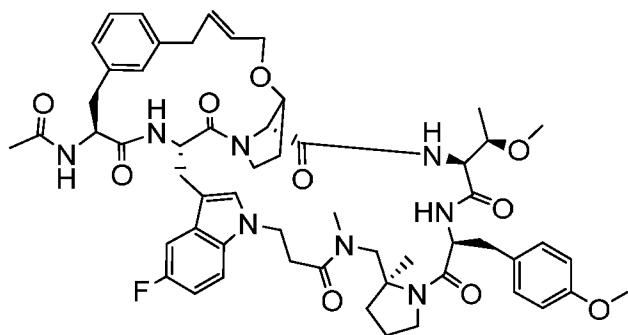
Ex-55A,



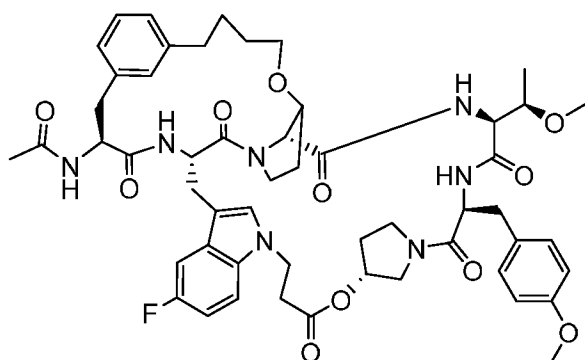
Ex-55B,



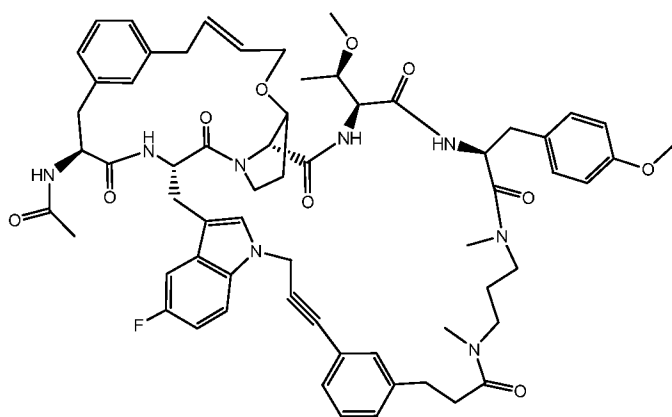
Ex-56,



Ex-57,



Ex-58, and



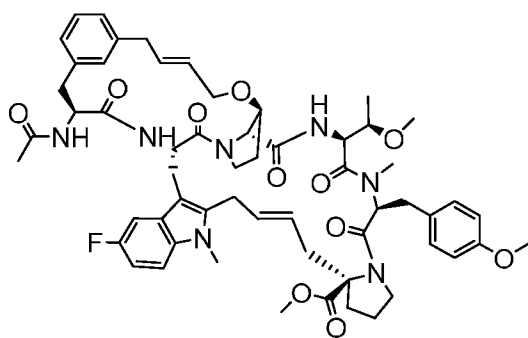
Ex-59,

or a pharmaceutically acceptable salt of any thereof.

14. A composition comprising at least one compound of any of claims 1 to 13, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable excipient.

15. A method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of claim 14.

16. A compound having the structure

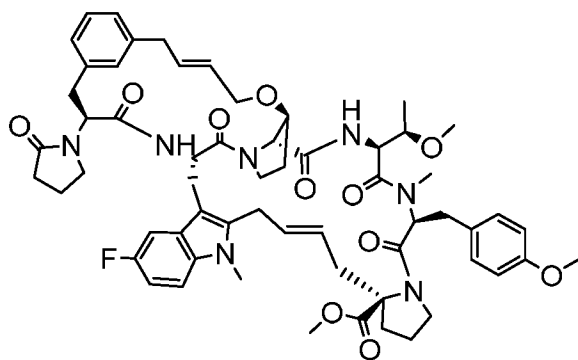


or a pharmaceutically acceptable salt thereof.

17. A composition comprising the compound of claim 16 and at least one pharmaceutically acceptable excipient.

18. A method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of claim 17.

19. A compound having the structure

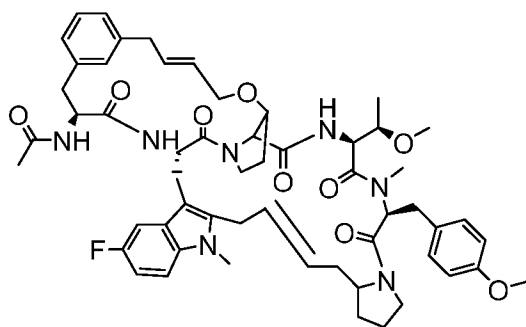


or a pharmaceutically acceptable salt thereof.

20. A composition comprising the compound of claim 19 and at least one pharmaceutically acceptable excipient.

21. A method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of claim 20.

22. A compound having the structure

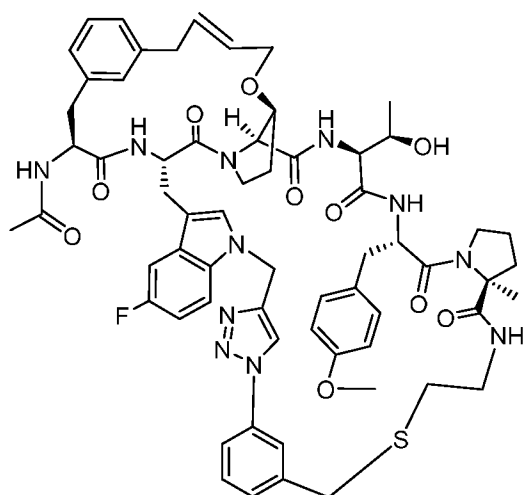


or a pharmaceutically acceptable salt thereof.

23. A composition comprising the compound of claim 22 and at least one pharmaceutically acceptable excipient.

24. A method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of claim 23.

25. A compound having the structure

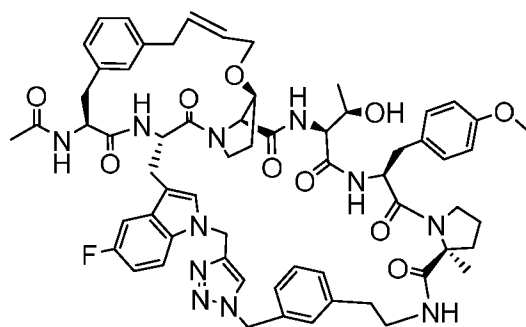


or a pharmaceutically acceptable salt thereof.

26. A composition comprising the compound of claim 25 and at least one pharmaceutically acceptable excipient.

27. A method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of claim 26.

28. A compound having the structure



or a pharmaceutically acceptable salt thereof.

29. A composition comprising the compound of claim 28 and at least one pharmaceutically acceptable excipient.

30. A method of treating hypercholesterolemia, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of claim 29.