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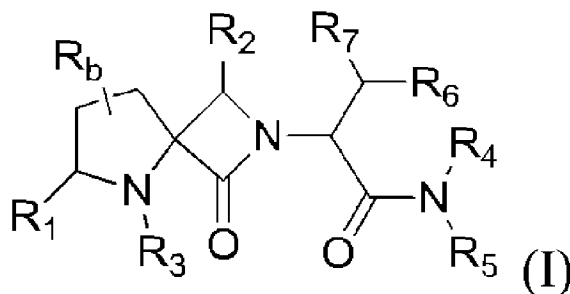
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(54) Titre : MODULATEURS SPIROLACTAMES D'UN RECEPTEUR NMDA ET LEURS UTILISATIONS

(54) Title: SPIRO-LACTAM NMDA RECEPTOR MODULATORS AND USES THEREOF



(57) **Abrégé/Abstract:**

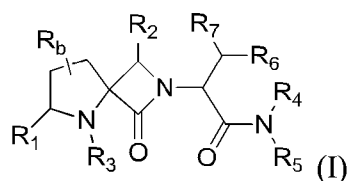
Disclosed are compounds having enhanced potency in the modulation of NMDA receptor activity. Such compounds are contemplated for use in the treatment of conditions such as depression and related disorders. Orally available formulations and other pharmaceutically acceptable delivery forms of the compounds, including intravenous formulations, are also disclosed. Useful compounds include compounds of the Formula (I):

(see formula I)

or a pharmaceutically acceptable salt, a stereoisomer, or an N-oxide thereof.

ABSTRACT

Disclosed are compounds having enhanced potency in the modulation of NMDA receptor activity. Such compounds are contemplated for use in the treatment of conditions such as depression and related disorders. Orally available formulations and other pharmaceutically acceptable delivery forms of the compounds, including intravenous formulations, are also disclosed. Useful compounds include compounds of the Formula (I):



or a pharmaceutically acceptable salt, a stereoisomer, or an N-oxide thereof.

- 1 -

SPIRO-LACTAM NMDA RECEPTOR MODULATORS AND USES THEREOF**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of United States Provisional Application No. 61/757,903, filed on January 29, 2013.

BACKGROUND

[0002] An N-methyl-d-aspartate (NMDA) receptor is a postsynaptic, ionotropic receptor that is responsive to, *inter alia*, the excitatory amino acids glutamate and glycine and the
5 synthetic compound NMDA. The NMDA receptor controls the flow of both divalent and monovalent ions into the postsynaptic neural cell through a receptor associated channel (Foster *et al.*, Nature 1987, 329:395-396; Mayer *et al.*, Trends in Pharmacol. Sci. 1990, 11:254-260). The NMDA receptor has been implicated during development in specifying neuronal architecture and synaptic connectivity, and may be involved in experience-dependent synaptic
10 modifications. In addition, NMDA receptors are also thought to be involved in long term potentiation and central nervous system disorders.

[0003] The NMDA receptor plays a major role in the synaptic plasticity that underlies many higher cognitive functions, such as memory acquisition, retention and learning, as well as in certain cognitive pathways and in the perception of pain (Collingridge *et al.*, The NMDA
15 Receptor, Oxford University Press, 1994). In addition, certain properties of NMDA receptors suggest that they may be involved in the information-processing in the brain that underlies consciousness itself.

[0004] The NMDA receptor has drawn particular interest since it appears to be involved in a broad spectrum of CNS disorders. For instance, during brain ischemia caused by stroke or
20 traumatic injury, excessive amounts of the excitatory amino acid glutamate are released from damaged or oxygen deprived neurons. This excess glutamate binds to the NMDA receptors which opens their ligand-gated ion channels; in turn the calcium influx produces a high level of intracellular calcium which activates a biochemical cascade resulting in protein degradation and cell death. This phenomenon, known as excitotoxicity, is also thought to be responsible for

- 2 -

the neurological damage associated with other disorders ranging from hypoglycemia and cardiac arrest to epilepsy. In addition, there are preliminary reports indicating similar involvement in the chronic neurodegeneration of Huntington's, Parkinson's, and Alzheimer's diseases. Activation of the NMDA receptor has been shown to be responsible for post-stroke
5 convulsions, and, in certain models of epilepsy, activation of the NMDA receptor has been shown to be necessary for the generation of seizures. Neuropsychiatric involvement of the NMDA receptor has also been recognized since blockage of the NMDA receptor Ca^{++} channel by the animal anesthetic PCP (phencyclidine) produces a psychotic state in humans similar to schizophrenia (reviewed in Johnson, K. and Jones, S., 1990). Further, NMDA receptors have
10 also been implicated in certain types of spatial learning.

[0005] The NMDA receptor is believed to consist of several protein chains embedded in the postsynaptic membrane. The first two types of subunits discovered so far form a large extracellular region, which probably contains most of the allosteric binding sites, several transmembrane regions looped and folded so as to form a pore or channel, which is permeable
15 to Ca^{++} , and a carboxyl terminal region. The opening and closing of the channel is regulated by the binding of various ligands to domains (allosteric sites) of the protein residing on the extracellular surface. The binding of the ligands is thought to affect a conformational change in the overall structure of the protein which is ultimately reflected in the channel opening, partially opening, partially closing, or closing.

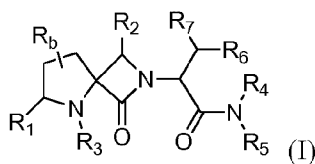
20 **[0006]** NMDA receptor compounds may exert dual (agonist/antagonist) effect on the NMDA receptor through the allosteric sites. These compounds are typically termed "partial agonists". In the presence of the principal site ligand, a partial agonist will displace some of the ligand and thus decrease Ca^{++} flow through the receptor. In the absence of or lowered level of the principal site ligand, the partial agonist acts to increase Ca^{++} flow through the receptor
25 channel.

[0007] A need continues to exist in the art for novel and more specific/potent compounds that are capable of binding the glycine binding site of NMDA receptors, and provide pharmaceutical benefits. In addition, a need continues to exist in the medical arts for orally deliverable forms of such compounds.

- 3 -

SUMMARY

[0008] Provided herein, at least in part, are compounds that are NMDA modulators, for example, partial agonists of NMDA. For example, disclosed herein are compounds represented by the formula:



5 and pharmaceutically acceptable salts, stereoisomers, and N-oxides thereof, wherein

R_b is selected from the group consisting of H, halogen, hydroxyl, cyano and C₁-C₆ alkyl;

R₁ is H or C₁-C₆ alkyl;

R₂ is H or C₁-C₆ alkyl;

10 R₃ is selected from the group consisting of H, C₁-C₆ alkyl and a nitrogen protecting group;

R₄ and R₅ are independently H or C₁-C₆ alkyl, or R₄ and R₅ taken together with the nitrogen to which they are attached form a 4-, 5- or 6-membered heterocyclic or heteroaryl ring optionally substituted with one or more substituents selected from the group consisting of halogen, cyano, oxo, C₁-C₆ alkyl, -OH, C₁-C₆ alkoxy, and -N(R')R',
 15 wherein R' is independently selected for each occurrence from H or C₁-C₆ alkyl;

R₆ is selected from the group consisting of -OH, C₁-C₆ alkoxy, -OC(O)-C₁-C₆ alkyl, and -OC(O)phenyl; and

R₇ is H or C₁-C₆ alkyl;

20 or in other embodiments, the variables set forth in formula (I) are defined as follows:

R_b is selected from the group consisting of H, halogen, hydroxyl, cyano and C₁-C₆ alkyl (e.g., H);

R₁ is H or C₁-C₆ alkyl;

R₂ is H or C₁-C₆ alkyl;

- 4 -

R₃ is selected from the group consisting of H, C₁-C₆ alkyl and a nitrogen protecting group;

R₄ and R₅ are each independently selected from the group consisting of H, C₁-C₆ alkyl, X, and -C₁-C₆ alkylene-X, wherein X is selected from the group consisting of:

- 5 (i) C₃-C₆ cycloalkyl;
- (ii) heteroaryl including from 5 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S;
- 10 (iii) heterocyclyl including from 3 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and
- (iv) phenyl;

wherein C₃-C₆ cycloalkyl and heterocyclyl are each optionally substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, oxo, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R', wherein R' is independently selected for each occurrence from H and C₁-C₆ alkyl; and heteroaryl and phenyl are each optionally substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'; or R₄ and R₅ together with the nitrogen to which they are attached form:

20 heterocyclyl including from 4 to 6 ring atoms; wherein the heterocyclyl includes not more than two ring heteroatoms (including the nitrogen atom attached to R₄ and R₅), and the second ring heteroatom, when present, is independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and wherein the heterocyclyl is optionally substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, oxo, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'; or

25 heteroaryl including from 5 to 6 ring atoms; wherein the heteroaryl includes not more than four ring heteroatoms (including the nitrogen atom attached to R₄ and R₅), and each additional ring heteroatom, when present, is independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and wherein the heteroaryl is optionally substituted with from 1-3 substituents independently

- 5 -

selected from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R';

R₆ is selected from the group consisting of -OH, C₁-C₆ alkoxy, -OC(O)-C₁-C₆ alkyl, -OC(O)phenyl, and -N(R')R'; and

5 R₇ is H or C₁-C₆ alkyl.

[0009] Also provided herein are pharmaceutically acceptable compositions comprising a disclosed compound, and a pharmaceutically acceptable excipient. For example, such compositions may be suitable for oral or intravenous administration to a patient.

[0010] In another aspect, a method of treating a condition selected from the group consisting of autism, anxiety, depression, bipolar disorder, attention deficit disorder, attention deficit hyperactivity disorder (ADHD), schizophrenia, a psychotic disorder, a psychotic symptom, social withdrawal, obsessive-compulsive disorder, phobia, post-traumatic stress syndrome, a behavior disorder, an impulse control disorder, a substance abuse disorder, a sleep disorder, a memory disorder, a learning disorder, urinary incontinence, multiple system atrophy, progressive supra-nuclear palsy, Friedrich's ataxia, Down's syndrome, fragile X syndrome, tuberous sclerosis, olivio-ponto-cerebellar atrophy, cerebral palsy, drug-induced optic neuritis, ischemic retinopathy, diabetic retinopathy, glaucoma, dementia, AIDS dementia, Alzheimer's disease, Huntington's chorea, spasticity, myoclonus, muscle spasm, Tourette's syndrome, epilepsy, cerebral ischemia, stroke, a brain tumor, traumatic brain injury, cardiac arrest, myelopathy, spinal cord injury, peripheral neuropathy, acute neuropathic pain, and chronic neuropathic, in a patient in need thereof is provided. Such methods may comprise administering to the patient a pharmaceutically effective amount of a disclosed compound or pharmaceutically acceptable salts, stereoisomers, N-oxides, and hydrates thereof.

[0011] In some embodiments, a contemplated method includes treating depression. For example, depression may include one or more of major depressive disorder, dysthymic disorder, psychotic depression, postpartum depression, seasonal affective disorder, bipolar disorder, mood disorder, or depression caused by a chronic medical condition. In other embodiments, a contemplated method may treat schizophrenia. Such schizophrenia may be, for example, paranoid type schizophrenia, disorganized type schizophrenia, catatonic type schizophrenia, undifferentiated type schizophrenia, residual type schizophrenia, post-schizophrenic depression, or simple schizophrenia.

- 6 -

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] **Figure 1** shows the potentiation of [³H]MK-801 binding in the presence of Compound X.

[0013] **Figure 2** shows results of long term potentiation in hippocampal slices using Compound X.

DETAILED DESCRIPTION

5 [0014] This disclosure is generally directed to compounds that are capable of modulating NMDA, e.g., NMDA antagonists or partial agonists, and compositions and/or methods of using the disclosed compounds.

Definitions

10 [0015] “Treating” includes any effect, e.g., lessening, reducing, modulating, or eliminating, that results in the improvement of the condition, disease, disorder and the like.

[0016] The term “alkenyl” as used herein refers to an unsaturated straight or branched hydrocarbon having at least one carbon-carbon double bond, such as a straight or branched group of 2-6 or 3-4 carbon atoms, referred to herein for example as C₂-C₆ alkenyl, and C₃-C₄ alkenyl, respectively. Exemplary alkenyl groups include, but are not limited to, vinyl, allyl, 15 butenyl, pentenyl, etc.

[0017] The term “alkoxy” as used herein refers to a straight or branched alkyl group attached to an oxygen (alkyl-O-). Exemplary alkoxy groups include, but are not limited to, alkoxys of 1-6 or 2-6 carbon atoms, referred to herein as C₁-C₆ alkoxy, and C₂-C₆ alkoxy, respectively. Exemplary alkoxy groups include, but are not limited to methoxy, ethoxy, 20 isopropoxy, etc.

[0018] The term “alkenyloxy” used herein refers to a straight or branched alkenyl group attached to an oxygen (alkenyl-O). Exemplary alkenoxy group include, but are not limited to, groups with an alkenyl group of 3-6 carbon atoms, (also e.g. referred to as C₃-C₆ alkenyloxy). Exemplary “alkenoxo” groups include, but are not limited to allyloxy, butenyloxy, etc.

25 [0019] The term “alkynyloxy” used herein refers to a straight or branched alkynyl group attached to an oxygen (alkynyl-O)). Exemplary alkynyloxy groups include, but are not limited to, C₃-C₆ alkynyloxy, e.g., propynyloxy.

- 7 -

[0020] The term “alkyl” as used herein refers to a saturated straight or branched hydrocarbon, such as a straight or branched group of 1-6, 1-4, or 1-3 carbon atoms, referred to herein as C₁-C₆ alkyl, C₁-C₄ alkyl, and C₁-C₃ alkyl, respectively. Exemplary alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, 2-methyl-1-propyl, 2-methyl-2-propyl, 2-methyl-1-butyl, 3-methyl-1-butyl, 3-methyl-2-butyl, 2,2-dimethyl-1-propyl, 2-methyl-1-pentyl, 3-methyl-1-pentyl, 4-methyl-1-pentyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 2,2-dimethyl-1-butyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, neopentyl, hexyl, etc. The term “haloalkyl” as used herein refers to a saturated straight or branched alkyl groups, in which one or more hydrogen atoms of the alkyl group are replaced with one or more independently selected halogens. The term “haloalkyl” encompasses alkyl groups in which all of hydrogen atoms of the alkyl group are replaced independently selected halogens (sometimes referred to as “perhalo” alkyl groups. Exemplary haloalkyl groups include, but are not limited to, CH₂F, CH₂CH₂Cl, CF₃, CHFCH₂Cl.

[0021] The term “alkynyl” as used herein refers to an unsaturated straight or branched hydrocarbon having at least one carbon-carbon triple bond, such as a straight or branched group of 2-6, or 3-6 carbon atoms, referred to herein as C₂-C₆ alkynyl, and C₃-C₆ alkynyl, respectively. Exemplary alkynyl groups include, but are not limited to, ethynyl, propynyl, butynyl, pentynyl, hexynyl, methylpropynyl, etc.

[0022] The term “bridged cycloalkyl”, as used herein, is defined as a monocyclic 4- to 7-membered cycloalkyl group in which two non-adjacent atoms are linked by a CH₂ or CH₂CH₂ group. A “bridged cycloalkyl” may be fused to one or more phenyl, partially unsaturated, or saturated rings. Examples of bridged carbocyclic groups include but are not limited to bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, bicyclo[2.2.2]octene etc.

[0023] The term “carbonyl” as used herein refers to the radical -C(O)-. The term “cyano” as used herein refers to the radical -CN. The term “nitro” refers to the radical -NO₂. The term “H” refers to hydrogen.

[0024] The term “cycloalkoxy” as used herein refers to a cycloalkyl group attached to an oxygen (cycloalkyl-O-).

[0025] The term “cycloalkyl” as used herein refers to a monocyclic saturated or partially unsaturated hydrocarbon group of for example 3-6, or 4-6 carbons, referred to herein, e.g., as “C₃₋₆ cycloalkyl” or “C₄₋₆ cycloalkyl,” and derived from a cycloalkane. Exemplary cycloalkyl

- 8 -

groups include, but are not limited to, cyclohexane, cyclohexene, cyclopentane, cyclobutane, cyclopropane or cyclopentane.

[0026] The terms “halo” or “halogen” as used herein refer to F, Cl, Br, or I.

[0027] The terms “heteroaryl” as used herein refers to a monocyclic aromatic 4-6
5 membered ring system containing one or more heteroatoms, for example one to three heteroatoms, such as nitrogen, oxygen, and sulfur. Where possible, said heteroaryl ring may be linked to the adjacent radical through carbon or nitrogen. Examples of heteroaryl rings include but are not limited to furan, thiophene, pyrrole, thiazole, oxazole, isothiazole, isoxazole, imidazole, pyrazole, triazole, pyridine, and pyrimidine.

10 [0028] The terms “heterocyclyl” or “heterocyclic group” are art-recognized and refer to saturated or partially unsaturated 4- to 7-membered ring structures, whose ring structures include one to three heteroatoms, such as nitrogen, oxygen, and sulfur. A heterocycle may be fused to one or more phenyl, partially unsaturated, or saturated rings. Examples of heterocyclyl groups include but are not limited to pyrrolidine, piperidine, morpholine, thiomorpholine, and
15 piperazine.

[0029] The term “heterocyclylalkoxy” as used herein refers to a heterocyclyl- alkyl-O- group.

[0030] The term “heterocycloxyalkyl” refers to a heterocyclyl-O-alkyl- group.

[0031] The term “heterocycloxy” refers to a heterocyclyl-O- group. The term
20 “cycloalkoxy” refers to a cycloalkyl-O- group.

[0032] The term “heteroaryloxy” refers to a heteroaryl-O- group.

[0033] The terms “hydroxy” and “hydroxyl” as used herein refers to the radical -OH.

[0034] The term “oxo” as used herein refers to the radical =O.

[0035] The term “nitrogen protecting group” or “amino protecting group” is art-
25 recognized and as used herein refers to a chemical moiety that is covalently linked to a nitrogen atom of an amino (primary or secondary) group and that temporarily blocks the reactivity of the amino group during a synthetic step and is selectively removed once the synthetic step is complete. Nitrogen protecting groups include, for example, 9-Fluorenylmethyloxycarbonyl (Fmoc), tert-butoxycarbonyl (Boc), carbobenzyloxycarbonyl (Cbz), p-
30 methoxybenzyloxycarbonyl, acetyl, trifluoroacetyl, benzoyl, phthalimido, benzyl (Bn), p-

- 9 -

methoxybenzyl, p-methoxyphenyl, 3,4-dimethoxybenzyl, triphenylmethyl, benzyldiene, and p-toluenesulfonyl (Ts). In some embodiments, the nitrogen protecting group can have one of the following formulas: $-C(O)OR_{31}$ or $-C(O)R_{32}$ as defined herein.

[0036] As used in the present disclosure, the term “partial NMDA receptor agonist”

5 generally refers to a compound that is capable of binding to a glycine binding site of an NMDA receptor; at low concentrations a NMDA receptor agonist acts substantially as agonist and at high concentrations it acts substantially as an antagonist. These concentrations are experimentally determined for each and every “partial agonist.”

[0037] “Pharmaceutically or pharmacologically acceptable” include molecular entities and

10 compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, or a human, as appropriate. For human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

[0038] The term “pharmaceutically acceptable carrier” or “pharmaceutically acceptable

15 excipient” as used herein refers to any and all solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. The compositions may also contain other active compounds providing supplemental, additional, or enhanced therapeutic functions.

20 [0039] The term “pharmaceutical composition” as used herein refers to a composition comprising at least one compound as disclosed herein formulated together with one or more pharmaceutically acceptable carriers.

[0040] “Individual,” “patient,” or “subject” are used interchangeably and include any animal, including mammals, preferably mice, rats, other rodents, rabbits, dogs, cats, swine,

25 cattle, sheep, horses, or primates, and most preferably humans. The compounds of the invention can be administered to a mammal, such as a human, but can also be administered to other mammals such as an animal in need of veterinary treatment, *e.g.*, domestic animals (*e.g.*, dogs, cats, and the like), farm animals (*e.g.*, cows, sheep, pigs, horses, and the like) and laboratory animals (*e.g.*, rats, mice, guinea pigs, and the like). The mammal treated in the
30 methods of the invention is desirably a mammal in which treatment *e.g.*, of pain or depression is desired. “Modulation” includes antagonism (*e.g.*, inhibition), agonism, partial antagonism and/or partial agonism.

- 10 -


[0041] In the present specification, the term “therapeutically effective amount” means the amount of the subject compound that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician. The compounds of the invention are administered in therapeutically effective amounts to treat a disease. Alternatively, a therapeutically effective amount of a compound is the quantity required to achieve a desired therapeutic and/or prophylactic effect, such as an amount which results in lessening a symptom of depression.

[0042] The term "pharmaceutically acceptable salt(s)" as used herein refers to salts of acidic or basic groups that may be present in compounds used in the present compositions. Compounds included in the present compositions that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions, including but not limited to malate, oxalate, chloride, bromide, iodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Compounds included in the present compositions that are acidic in nature are capable of forming base salts with various pharmacologically acceptable cations. Examples of such salts include alkali metal or alkaline earth metal salts and, particularly, calcium, magnesium, sodium, lithium, zinc, potassium, and iron salts. Compounds included in the present compositions that include a basic or acidic moiety may also form pharmaceutically acceptable salts with various amino acids. The compounds of the disclosure may contain both acidic and basic groups; for example, one amino and one carboxylic acid group. In such a case, the compound can exist as an acid addition salt, a zwitterion, or a base salt.

[0043] The compounds of the disclosure may contain one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as geometric isomers, enantiomers or diastereomers. The term “stereoisomers” when used herein consist of all geometric isomers, enantiomers or diastereomers. These compounds may be designated by the symbols “R” or “S,” depending on the configuration of substituents around the stereogenic carbon atom. The present invention encompasses various stereoisomers of these compounds and mixtures thereof. Stereoisomers include enantiomers and diastereomers. Mixtures of enantiomers or

- 11 -

diastereomers may be designated “(±)” in nomenclature, but the skilled artisan will recognize that a structure may denote a chiral center implicitly.

[0044] The compounds of the disclosure may contain one or more chiral centers and/or double bonds and, therefore, exist as geometric isomers, enantiomers or diastereomers. The enantiomer and diastereomers may be designated by the symbols “(+),” “(-).” “R” or “S,” depending on the configuration of substituents around the stereogenic carbon atom, but the skilled artisan will recognize that a structure may denote a chiral center implicitly. Geometric isomers, resulting from the arrangement of substituents around a carbon-carbon double bond or arrangement of substituents around a cycloalkyl or heterocyclic ring, can also exist in the compounds of the present invention. The symbol  denotes a bond that may be a single, double or triple bond as described herein. Substituents around a carbon-carbon double bond are designated as being in the “Z” or “E” configuration wherein the terms “Z” and “E” are used in accordance with IUPAC standards. Unless otherwise specified, structures depicting double bonds encompass both the “E” and “Z” isomers. Substituents around a carbon-carbon double bond alternatively can be referred to as “cis” or “trans,” where “cis” represents substituents on the same side of the double bond and “trans” represents substituents on opposite sides of the double bond. The arrangement of substituents around a carbocyclic ring can also be designated as “cis” or “trans.” The term “cis” represents substituents on the same side of the plane of the ring and the term “trans” represents substituents on opposite sides of the plane of the ring. Mixtures of compounds wherein the substituents are disposed on both the same and opposite sides of plane of the ring are designated “cis/trans.”

[0045] The term “stereoisomers” when used herein consist of all geometric isomers, enantiomers or diastereomers. The present invention encompasses various stereoisomers of these compounds and mixtures thereof.

[0046] Individual enantiomers and diastereomers of compounds of the present invention can be prepared synthetically from commercially available starting materials that contain asymmetric or stereogenic centers, or by preparation of racemic mixtures followed by resolution methods well known to those of ordinary skill in the art. These methods of resolution are exemplified by (1) attachment of a mixture of enantiomers to a chiral auxiliary, separation of the resulting mixture of diastereomers by recrystallization or chromatography and liberation of the optically pure product from the auxiliary, (2) salt formation employing an optically active resolving agent, (3) direct separation of the mixture of optical enantiomers on

- 12 -

chiral liquid chromatographic columns or (4) kinetic resolution using stereoselective chemical or enzymatic reagents. Racemic mixtures can also be resolved into their component enantiomers by well-known methods, such as chiral-phase gas chromatography or crystallizing the compound in a chiral solvent. Stereoselective syntheses, a chemical or enzymatic reaction in which a single reactant forms an unequal mixture of stereoisomers during the creation of a new stereocenter or during the transformation of a pre-existing one, are well known in the art. Stereoselective syntheses encompass both enantio- and diastereoselective transformations. For examples, see Carreira and Kvaerno, *Classics in Stereoselective Synthesis*, Wiley-VCH: Weinheim, 2009.

10 [0047] The compounds disclosed herein can exist in solvated as well as unsolvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like, and it is intended that the invention embrace both solvated and unsolvated forms. In one embodiment, the compound is amorphous. In one embodiment, the compound is a single polymorph. In another embodiment, the compound is a mixture of polymorphs. In another embodiment, the compound is in a crystalline form.

[0048] The invention also embraces isotopically labeled compounds of the invention which are identical to those recited herein, except that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, fluorine and chlorine, such as ^2H , ^3H , ^{13}C , ^{14}C , ^{15}N , ^{18}O , ^{17}O , ^{31}P , ^{32}P , ^{35}S , ^{18}F , and ^{36}Cl , respectively. For example, a compound of the invention may have one or more H atom replaced with deuterium.

[0049] Certain isotopically-labeled disclosed compounds (*e.g.*, those labeled with ^3H and ^{14}C) are useful in compound and/or substrate tissue distribution assays. Tritiated (*i.e.*, ^3H) and carbon-14 (*i.e.*, ^{14}C) isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (*i.e.*, ^2H) may afford certain therapeutic advantages resulting from greater metabolic stability (*e.g.*, increased *in vivo* half-life or reduced dosage requirements) and hence may be preferred in some circumstances. Isotopically labeled compounds of the invention can generally be prepared by following procedures analogous to those disclosed in the *e.g.*, Examples herein by substituting an isotopically labeled reagent for a non-isotopically labeled reagent.

- 13 -

[0050] The term “prodrug” refers to compounds that are transformed *in vivo* to yield a disclosed compound or a pharmaceutically acceptable salt, hydrate or solvate of the compound. The transformation may occur by various mechanisms (such as by esterase, amidase, phosphatase, oxidative and or reductive metabolism) in various locations (such as in the intestinal lumen or upon transit of the intestine, blood or liver). Prodrugs are well known in the art (for example, see Rautio, Kumpulainen, *et al*, Nature Reviews Drug Discovery 2008, 7, 255). For example, if a compound of the invention or a pharmaceutically acceptable salt, hydrate or solvate of the compound contains a carboxylic acid functional group, a prodrug can comprise an ester formed by the replacement of the hydrogen atom of the acid group with a group such as (C₁-C₈)alkyl, (C₂-C₁₂)alkanoyloxymethyl, 1-(alkanoyloxy)ethyl having from 4 to 9 carbon atoms, 1-methyl-1-(alkanoyloxy)-ethyl having from 5 to 10 carbon atoms, alkoxy-carbonyloxymethyl having from 3 to 6 carbon atoms, 1-(alkoxy-carbonyloxy)ethyl having from 4 to 7 carbon atoms, 1-methyl-1-(alkoxy-carbonyloxy)ethyl having from 5 to 8 carbon atoms, N-(alkoxy-carbonyl)aminomethyl having from 3 to 9 carbon atoms, 1-(N-(alkoxy-carbonyl)amino)ethyl having from 4 to 10 carbon atoms, 3-phthalidyl, 4-crotonolactonyl, gamma-butyrolacton-4-yl, di-N,N-(C₁-C₂)alkylamino(C₂-C₃)alkyl (such as β-dimethylaminoethyl), carbamoyl-(C₁-C₂)alkyl, N,N-di(C₁-C₂)alkylcarbamoyl-(C₁-C₂)alkyl and piperidino-, pyrrolidino- or morpholino(C₂-C₃)alkyl.

[0051] Similarly, if a compound of the invention contains an alcohol functional group, a prodrug can be formed by the replacement of the hydrogen atom of the alcohol group with a group such as (C₁-C₆)alkanoyloxymethyl, 1-((C₁-C₆)alkanoyloxy)ethyl, 1-methyl-1-((C₁-C₆)alkanoyloxy)ethyl (C₁-C₆)alkoxy-carbonyloxymethyl, N-(C₁-C₆)alkoxy-carbonylaminomethyl, succinoyl, (C₁-C₆)alkanoyl, α-amino(C₁-C₄)alkanoyl, arylacyl and α-aminoacyl, or α-aminoacyl-α-aminoacyl, where each α-aminoacyl group is independently selected from the naturally occurring L-amino acids, P(O)(OH)₂, -P(O)(O(C₁-C₆)alkyl)₂ or glycosyl (the radical resulting from the removal of a hydroxyl group of the hemiacetal form of a carbohydrate).

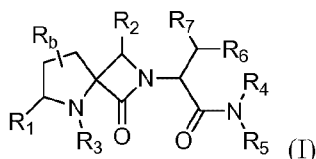
[0052] If a compound of the invention incorporates an amine functional group, a prodrug can be formed, for example, by creation of an amide or carbamate, an N-acyloxyakyl derivative, an (oxodioxolenyl)methyl derivative, an N-Mannich base, imine or enamine. In addition, a secondary amine can be metabolically cleaved to generate a bioactive primary amine, or a tertiary amine can metabolically cleaved to generate a bioactive primary or

- 14 -

secondary amine. For examples, see Simplicio, *et al.*, *Molecules* 2008, 13, 519 and references therein.

Compounds

[0053] Disclosed compounds include those represented by the formula:



5

and pharmaceutically acceptable salts, stereoisomers, and N-oxides thereof, wherein

R_b is selected from the group consisting of H, halogen, hydroxyl, cyano and C₁-C₆ alkyl;

R₁ is H or C₁-C₆ alkyl;

10

R₂ is H or C₁-C₆ alkyl;

R₃ is selected from the group consisting of H, C₁-C₆ alkyl and a nitrogen protecting group;

R₄ and R₅ are independently H or C₁-C₆ alkyl, or R₄ and R₅ taken together with the nitrogen to which they are attached form a 4-, 5- or 6-membered heterocyclic or heteroaryl ring optionally substituted with one or more substituents selected from the group consisting of halogen, cyano, oxo, C₁-C₆ alkyl, -OH, C₁-C₆ alkoxy, and -N(R')R', wherein R' is independently selected for each occurrence from H or C₁-C₆ alkyl;

15

R₆ is selected from the group consisting of -OH, C₁-C₆ alkoxy, -OC(O)-C₁-C₆ alkyl, and -OC(O)phenyl; and

20

R₇ is H or C₁-C₆ alkyl;

or in other embodiments, the variables set forth in formula (I) are defined as follows:

R_b is selected from the group consisting of H, halogen, hydroxyl, cyano and C₁-C₆ alkyl (e.g., H);

R₁ is H or C₁-C₆ alkyl;

25

R₂ is H or C₁-C₆ alkyl;

- 15 -

R₃ is selected from the group consisting of H, C₁-C₆ alkyl and a nitrogen protecting group;

R₄ and R₅ are each independently selected from the group consisting of H, C₁-C₆ alkyl, X, and -C₁-C₆ alkylene-X, wherein X is selected from the group consisting of:

- 5 (i) C₃-C₆ cycloalkyl;
- (ii) heteroaryl including from 5 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S;
- (iii) heterocyclyl including from 3 to 6 ring atoms wherein 1, 2, or 3 of the ring
10 atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and
- (iv) phenyl;

wherein C₃-C₆ cycloalkyl and heterocyclyl are each optionally substituted with from 1-3
substituents independently selected from the group consisting of halogen, cyano, oxo,
15 C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R', wherein R' is independently
selected for each occurrence from H and C₁-C₆ alkyl; and heteroaryl and phenyl are
each optionally substituted with from 1-3 substituents independently selected from the
group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R';
or R₄ and R₅ together with the nitrogen to which they are attached form:

20 heterocyclyl including from 4 to 6 ring atoms; wherein the heterocyclyl includes not
more than two ring heteroatoms (including the nitrogen atom attached to R₄ and R₅),
and the second ring heteroatom, when present, is independently selected from the
group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and wherein the heterocyclyl
is optionally substituted with from 1-3 substituents independently selected from the
25 group consisting of halogen, cyano, oxo, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -
N(R')R'; or

heteroaryl including from 5 to 6 ring atoms; wherein the heteroaryl includes not
more than four ring heteroatoms (including the nitrogen atom attached to R₄ and
R₅), and each additional ring heteroatom, when present, is independently selected
30 from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and wherein the
heteroaryl is optionally substituted with from 1-3 substituents independently

- 16 -

selected from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R';

R₆ is selected from the group consisting of -OH, C₁-C₆ alkoxy, -OC(O)-C₁-C₆ alkyl, -OC(O)phenyl, and -N(R')R'; and

5 R₇ is H or C₁-C₆ alkyl.

[0054] In some embodiments, R₁ is H. In other embodiments, R₁ is C₁-C₆ alkyl, e.g., CH₃.

[0055] In some embodiments, R₂ is H. In other embodiments, R₂ is C₁-C₆ alkyl, e.g., CH₃.

[0056] In some embodiments, R₃ is H.

[0057] In some embodiments, R₃ is a nitrogen protecting group. In some embodiments, R₃ has formula -C(O)OR₃₁, wherein R₃₁ is selected from the group consisting of: C₁-C₆ alkyl; C₁-C₆ haloalkyl; C₂-C₆ alkenyl; C₂-C₆ alkynyl; C₃-C₁₀ cycloalkyl, wherein the C₃-C₁₀ cycloalkyl is optionally substituted with from 1-3 independently selected C₁-C₃ alkyl; -CH₂-C₃-C₁₀ cycloalkyl wherein the C₃-C₁₀ cycloalkyl is optionally substituted with from 1-3 independently selected C₁-C₃ alkyl; -CH₂-phenyl, wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C₁-C₃ alkyl, C₁-C₃ haloalkyl, C₁-C₃ alkoxy, C₁-C₃ haloalkoxy, nitro, halo, SO₂Me, cyano, and -OC(O)CH₃; and -CH₂-pyridyl. In certain embodiments, R₃₁ is C₁-C₆ alkyl (e.g., *tert*-butyl). In other embodiments, R₃ has formula -C(O)R₃₂, wherein R₃₂ is selected from the group consisting of: H; C₁-C₆ alkyl; C₁-C₆ haloalkyl; phenyl, wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C₁-C₃ alkyl; C₁-C₃ haloalkyl; C₁-C₃ alkoxy; C₁-C₃ haloalkoxy; nitro; halo; SO₂Me, cyano; and -OC(O)CH₃; and pyridyl. In certain embodiments, R₃₂ is C₁-C₆ alkyl (e.g., -CH₃ or *iso*-propyl).

[0058] In some embodiments, R₄ and R₅ are each independently selected from the group consisting of H, C₁-C₆ alkyl, X, and -C₁-C₆ alkylene-X. In certain embodiments, R₄ and R₅ are each independently selected from the group consisting of H and C₁-C₆ alkyl. In other embodiments, R₄ and R₅ are each independently selected from the group consisting of H and -C₁-C₆ alkylene-X. In certain embodiments, R₄ and R₅ are H. In other embodiments, one of R₄ and R₅ is H, and the other is -C₁-C₆ alkylene-X. In certain of these embodiments, the compounds can include one or both (e.g., both) of the following features: (i) -C₁-C₆ alkylene-X is -CH₂-X; and (ii) X is phenyl or heteroaryl including from 5 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; each optionally substituted with from 1-3 substituents independently selected

- 17 -

from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'.

[0059] In other embodiments, R₄ and R₅ taken together form a heterocyclic or heteroaryl ring as defined previously and anywhere herein. In certain embodiments, R₄ and R₅ taken together form a heterocyclic ring, e.g., a ring selected from the group consisting of azetidiny, pyrrolidinyl, pyrazolidinyl, isooxazolidinyl, imidazolidinyl, oxazolidinyl, thiazolidinyl, and isothiazolidinyl. In a particular embodiment, R₄ and R₅ taken together form a pyrrolidinyl ring. In certain embodiments, R₄ and R₅ taken together form a heteroaryl ring, e.g., a ring selected from the group consisting of imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, pyridinyl, diazinyl, oxazinyl, and thiazinyl.

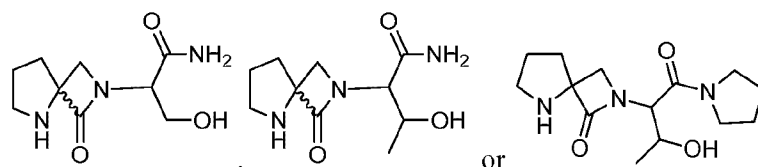
[0060] In some embodiments, R₁ is H; R₂ is H; R₃ is H; and R₄ and R₅ taken together form a pyrrolidine ring. In some embodiments, R₁ is H; R₂ is H; R₃ is H; and R₄ and R₅ are H. In some embodiments, R₁ is H or CH₃; R₂ is H or CH₃; R₃ is H; and R₄ and R₅ taken together form a pyrrolidinyl ring. In some embodiments, R₁ is H or CH₃; R₂ is H or CH₃; R₃ is H; and R₄ and R₅ are H. In some embodiments, R₁ is H or CH₃; R₂ is H or CH₃; R₃ is H; and one of R₄ and R₅ is H, and the other is -CH₂-X, wherein X is phenyl or heteroaryl including from 5 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; each optionally substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'. In some embodiments, R₁ is H or CH₃; R₂ is H or CH₃; R₃ is nitrogen protecting group (e.g., -C(O)OR₃₁ or -C(O)R₃₂); and R₄ and R₅ taken together form a pyrrolidinyl ring. In some embodiments, R₁ is H or CH₃; R₂ is H or CH₃; R₃ is nitrogen protecting group (e.g., -C(O)OR₃₁ or -C(O)R₃₂); and R₄ and R₅ are H. In some embodiments, R₁ is H or CH₃; R₂ is H or CH₃; R₃ is nitrogen protecting group (e.g., -C(O)OR₃₁ or -C(O)R₃₂); and one of R₄ and R₅ is H, and the other is -CH₂-X, wherein X is phenyl or heteroaryl including from 5 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; each optionally substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'.

[0061] In some embodiments (including any of the foregoing embodiments described above), R₆ is selected from the group consisting of -OH, C₁-C₆ alkoxy, -OC(O)-C₁-C₆ alkyl, and -OC(O)phenyl. In certain embodiments (including any of the foregoing embodiments described above), R₆ is -OH. In other embodiments (including any of the foregoing

- 18 -

embodiments described above), R_6 is $-\text{NH}_2$. In some embodiments (including any of the foregoing embodiments described above), R_7 is $\text{C}_1\text{-C}_6$ alkyl, e.g., CH_3 . In some embodiments (including any of the foregoing embodiments described above), R_6 is $-\text{OH}$ or $-\text{NH}_2$, and R_7 is $\text{C}_1\text{-C}_6$ alkyl, e.g., CH_3 . In some embodiments (including any of the foregoing embodiments described above), R_6 is H.

[0062] In some embodiments, the compound is selected from the compounds delineated in Table 1 and/or the Examples. In certain embodiments, a disclosed compound includes one having the formula:



[0063] The compounds of the present disclosure and formulations thereof may have a plurality of chiral centers. Each chiral center may be independently *R*, *S*, or any mixture of *R* and *S*. For example, in some embodiments, a chiral center may have an *R:S* ratio of between about 100:0 and about 50:50, between about 100:0 and about 75:25, between about 100:0 and about 85:15, between about 100:0 and about 90:10, between about 100:0 and about 95:5, between about 100:0 and about 98:2, between about 100:0 and about 99:1, between about 0:100 and 50:50, between about 0:100 and about 25:75, between about 0:100 and about 15:85, between about 0:100 and about 10:90, between about 0:100 and about 5:95, between about 0:100 and about 2:98, between about 0:100 and about 1:99, between about 75:25 and 25:75, and about 50:50. Formulations of the disclosed compounds comprising a greater ratio of one or more isomers (i.e., *R* and/or *S*) may possess enhanced therapeutic characteristic relative to racemic formulations of a disclosed compounds or mixture of compounds. In some instances, chemical formulas contain the descriptor “-(*R*)-” or “-(*S*)-” that is further attached to solid wedge or dashed wedge. This descriptor is intended to show a methine carbon (CH) that is attached to three other substituents and has either the indicated *R* or *S* configuration (see, e.g., Table 1).

[0064] Disclosed compounds may provide for efficient cation channel opening at the NMDA receptor, e.g. may bind or associate with the glutamate site of the NMDA receptor to assist in opening the cation channel. The disclosed compounds may be used to regulate (turn on or turn off) the NMDA receptor through action as an agonist.

- 19 -

[0065] The compounds as described herein may be glycine site NMDA receptor partial agonists. A partial agonist as used in this context will be understood to mean that at a low concentration, the analog acts as an agonist and at a high concentration, the analog acts as an antagonist. Glycine binding is not inhibited by glutamate or by competitive inhibitors of glutamate, and also does not bind at the same site as glutamate on the NMDA receptor. A second and separate binding site for glycine exists at the NMDA receptor. The ligand-gated ion channel of the NMDA receptor is, thus, under the control of at least these two distinct allosteric sites. Disclosed compounds may be capable of binding or associating with the glycine binding site of the NMDA receptor. In some embodiments, disclosed compounds may possess a potency that is 10-fold or greater than the activity of existing NMDA receptor glycine site partial agonists.

[0066] The disclosed compounds may exhibit a high therapeutic index. The therapeutic index, as used herein, refers to the ratio of the dose that produces a toxicity in 50% of the population (i.e., TD_{50}) to the minimum effective dose for 50% of the population (i.e., ED_{50}). Thus, the therapeutic index = $(TD_{50})/(ED_{50})$. In some embodiments, a disclosed compound may have a therapeutic index of at least about 10:1, at least about 50:1, at least about 100:1, at least about 200:1, at least about 500:1, or at least about 1000:1.

Compositions

[0067] In other aspects, formulations and compositions comprising the disclosed compounds and optionally a pharmaceutically acceptable excipient are provided. In some embodiments, a contemplated formulation comprises a racemic mixture of one or more of the disclosed compounds.

[0068] Contemplated formulations may be prepared in any of a variety of forms for use. By way of example, and not limitation, the compounds may be prepared in a formulation suitable for oral administration, subcutaneous injection, or other methods for administering an active agent to an animal known in the pharmaceutical arts.

[0069] Amounts of a disclosed compound as described herein in a formulation may vary according to factors such as the disease state, age, sex, and weight of the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein

- 20 -

refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0070] The specification for the dosage unit forms of the invention are dictated by and
5 directly dependent on (a) the unique characteristics of the compound selected and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0071] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion,
10 liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use
15 of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin.

[0072] The compounds can be administered in a time release formulation, for example in a
20 composition which includes a slow release polymer. The compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic
25 copolymers (PLG). Many methods for the preparation of such formulations are generally known to those skilled in the art.

[0073] Sterile injectable solutions can be prepared by incorporating the compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by
30 incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation

- 21 -

are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0074] In accordance with an alternative aspect of the invention, a compound may be formulated with one or more additional compounds that enhance the solubility of the compound.

Methods

[0075] Methods for treating a condition in a patient in need thereof by administering a therapeutically effective dose of a compound described herein are provided. In some embodiments, the condition may be a mental condition. For example, a mental illness may be treated. In another aspect, a nervous system condition may be treated. For example, a condition that affects the central nervous system, the peripheral nervous system, and/or the eye may be treated. In some embodiments, neurodegenerative diseases may be treated.

[0076] In some embodiments, the methods include administering a compound to treat patients suffering from autism, anxiety, depression, bipolar disorder, attention deficit disorder, attention deficit hyperactivity disorder (ADHD), schizophrenia, a psychotic disorder, a psychotic symptom, social withdrawal, obsessive-compulsive disorder (OCD), phobia, post-traumatic stress syndrome, a behavior disorder, an impulse control disorder, a substance abuse disorder (e.g., a withdrawal symptom, opiate addiction, nicotine addiction, and ethanol addition), a sleep disorder, a memory disorder (e.g., a deficit, loss, or reduced ability to make new memories), a learning disorder, urinary incontinence, multiple system atrophy, progressive supra-nuclear palsy, Friedrich's ataxia, Down's syndrome, fragile X syndrome, tuberous sclerosis, olivio-ponto-cerebellar atrophy, cerebral palsy, drug-induced optic neuritis, ischemic retinopathy, diabetic retinopathy, glaucoma, dementia, AIDS dementia, Alzheimer's disease, Huntington's chorea, spasticity, myoclonus, muscle spasm, Tourette's syndrome, epilepsy, cerebral ischemia, stroke, a brain tumor, traumatic brain injury, cardiac arrest, myelopathy, spinal cord injury, peripheral neuropathy, acute neuropathic pain, and chronic neuropathic pain.

[0077] In some embodiments, methods of treating a memory disorder associated with aging, schizophrenia, special learning disorders, seizures, post-stroke convulsions, brain ischemia, hypoglycemia, cardiac arrest, epilepsy, migraine, AIDS dementia, Huntington's chorea, Parkinson's disease, early stage Alzheimer's disease, and Alzheimer's disease are contemplated.

[0078] In certain embodiments, methods for treating schizophrenia are provided. For example, paranoid type schizophrenia, disorganized type schizophrenia (i.e., hebephrenic

- 22 -

schizophrenia), catatonic type schizophrenia, undifferentiated type schizophrenia, residual type schizophrenia, post-schizophrenic depression, and simple schizophrenia may be treated using the methods and compositions contemplated herein. Psychotic disorders such as schizoaffective disorders, delusional disorders, brief psychotic disorders, shared psychotic disorders, and psychotic disorders with delusions or hallucinations may also be treated using the compositions contemplated herein.

[0079] Paranoid schizophrenia may be characterized where delusions or auditory hallucinations are present, but thought disorder, disorganized behavior, or affective flattening are not. Delusions may be persecutory and/or grandiose, but in addition to these, other themes such as jealousy, religiosity, or somatization may also be present. Disorganized type schizophrenia may be characterized where thought disorder and flat affect are present together. Catatonic type schizophrenia may be characterized where the patient may be almost immobile or exhibit agitated, purposeless movement. Symptoms can include catatonic stupor and waxy flexibility. Undifferentiated type schizophrenia may be characterized where psychotic symptoms are present but the criteria for paranoid, disorganized, or catatonic types have not been met. Residual type schizophrenia may be characterized where positive symptoms are present at a low intensity only. Post-schizophrenic depression may be characterized where a depressive episode arises in the aftermath of a schizophrenic illness where some low-level schizophrenic symptoms may still be present. Simple schizophrenia may be characterized by insidious and progressive development of prominent negative symptoms with no history of psychotic episodes.

[0080] In some embodiments, methods are provided for treating psychotic symptoms that may be present in other mental disorders, including, but not limited to, bipolar disorder, borderline personality disorder, drug intoxication, and drug-induced psychosis. In another embodiment, methods for treating delusions (e.g., "non-bizarre") that may be present in, for example, delusional disorder are provided.

[0081] Also provided are methods for treating social withdrawal in conditions including, but not limited to, social anxiety disorder, avoidant personality disorder, and schizotypal personality disorder.

[0082] In some embodiments, methods are provided for treating neuropathic pain. The neuropathic pain may be acute or chronic. In some cases, the neuropathic pain may be associated with a condition such as herpes, HIV, traumatic nerve injury, stroke, post-ischemia, fibromyalgia, reflex sympathetic dystrophy, complex regional pain syndrome, spinal cord

- 23 -

injury, sciatica, phantom limb pain, diabetic neuropathy, and cancer chemotherapeutic-induced neuropathic pain. Methods for enhancing pain relief and for providing analgesia to a patient are also contemplated.

[0083] Further contemplated methods include a method of treating autism and/or an autism spectrum disorder in a patient need thereof, comprising administering an effective amount of a compound to the patient. In an embodiment, a method for reducing the symptoms of autism in a patient in need thereof is contemplated, comprising administering an effective amount of a disclosed compound to the patient. For example, upon administration, the compound may decrease the incidence of one or more symptoms of autism such as eye contact avoidance, failure to socialize, attention deficit, poor mood, hyperactivity, abnormal sound sensitivity, inappropriate speech, disrupted sleep, and perseveration. Such decreased incidence may be measured relative to the incidence in the untreated individual or an untreated individual(s).

[0084] Also provided herein is a method of modulating an autism target gene expression in a cell comprising contacting a cell with an effective amount of a compound described herein. The autism gene expression may be for example, selected from ABAT, APOE, CHRNA4, GABRA5, GFAP, GRIN2A, PDYN, and PENK. In another embodiment, a method of modulating synaptic plasticity in a patient suffering from a synaptic plasticity related disorder is provided, comprising administering to the patient an effective amount of a compound.

[0085] In another embodiment, a method of treating Alzheimer's disease, or e.g., treatment of memory loss that e.g., accompanies early stage Alzheimer's disease, in a patient in need thereof is provided, comprising administering a compound. Also provided herein is a method of modulating an Alzheimer's amyloid protein (e.g., beta amyloid peptide, e.g. the isoform A β ₁₋₄₂), in-vitro or in-vivo (e.g. in a cell) comprising contacting the protein with an effective amount of a compound is disclosed. For example, in some embodiments, a compound may block the ability of such amyloid protein to inhibit long-term potentiation in hippocampal slices as well as apoptotic neuronal cell death. In some embodiments, a disclosed compound may provide neuroprotective properties to a Alzheimer's patient in need thereof, for example, may provide a therapeutic effect on later stage Alzheimer's -associated neuronal cell death.

[0086] In a further embodiment, a method of treating depression comprising administering a compound described herein is provided. In some embodiments, the treatment may relieve depression or a symptom of depression without affecting behavior or motor coordination and without inducing or promoting seizure activity. Exemplary depression conditions that are expected to be treated according to this aspect of the invention include, but are not limited to,

- 24 -

major depressive disorder, dysthymic disorder, psychotic depression, postpartum depression, premenstrual syndrome, premenstrual dysphoric disorder, seasonal affective disorder (SAD), bipolar disorder (or manic depressive disorder), mood disorder, and depressions caused by chronic medical conditions such as cancer or chronic pain, chemotherapy, chronic stress, and post traumatic stress disorders. In addition, patients suffering from any form of depression often experience anxiety. Various symptoms associated with anxiety include fear, panic, heart palpitations, shortness of breath, fatigue, nausea, and headaches among others. Anxiety or any of the symptoms thereof may be treated by administering a compound as described herein.

[0087] Also provided herein are methods of treating a condition in treatment-resistant patients, e.g., patients suffering from a mental or central nervous system condition that does not, and/or has not, responded to adequate courses of at least one, or at least two, other compounds or therapeutics. For example, provided herein is a method of treating depression in a treatment resistant patient, comprising a) optionally identifying the patient as treatment resistant and b) administering an effective dose of a compound to said patient.

[0088] In some embodiments, a compound described herein may be used for acute care of a patient. For example, a compound may be administered to a patient to treat a particular episode (e.g., a severe episode) of a condition contemplated herein.

[0089] Also contemplated herein are combination therapies comprising a compound in combination with one or more other active agents. For example, a compound may be combined with one or more antidepressants, such as tricyclic antidepressants, MAO-I's, SSRI's, and double and triple uptake inhibitors and/or anxiolytic drugs. Exemplary drugs that may be used in combination with a compound include Anafranil, Adapin, Aventyl, Elavil, Norpramin, Pamelor, Pertofrane, Sinequan, Surmontil, Tofranil, Vivactil, Parnate, Nardil, Marplan, Celexa, Lexapro, Luvox, Paxil, Prozac, Zoloft, Wellbutrin, Effexor, Remeron, Cymbalta, Desyrel (trazodone), and Ludiomill. In another example, a compound may be combined with an antipsychotic medication. Non-limiting examples of antipsychotics include butyrophenones, phenothiazines, thioxanthenes, clozapine, olanzapine, risperidone, quetiapine, ziprasidone, amisulpride, asenapine, paliperidone, iloperidone, zotepine, sertindole, lurasidone, and aripiprazole. It should be understood that combinations of a compound and one or more of the above therapeutics may be used for treatment of any suitable condition and are not limited to use as antidepressants or antipsychotics.

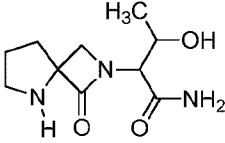
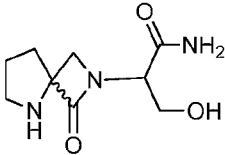
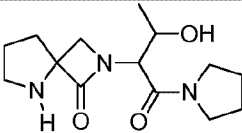
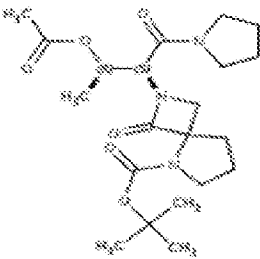
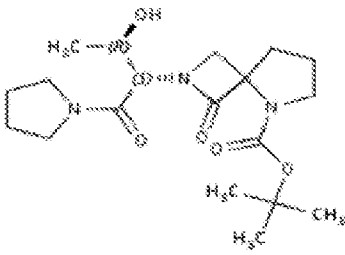
- 25 -

EXAMPLES

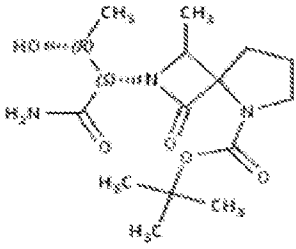
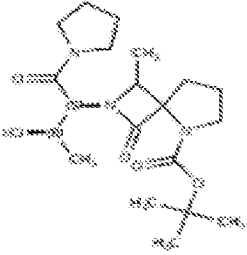
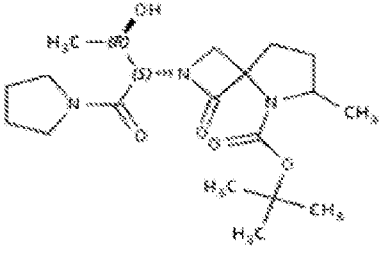
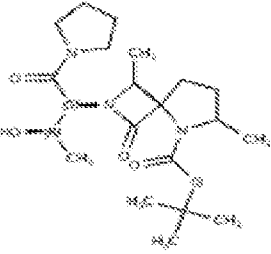
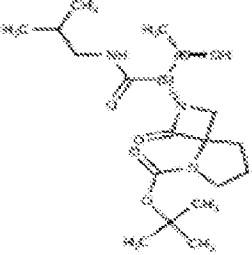
[0090] The following examples are provided for illustrative purposes only, and are not intended to limit the scope of the disclosure.

[0091] Table 1 below shows some exemplary compounds of the disclosure and provides physiochemical characteristics of the compounds.

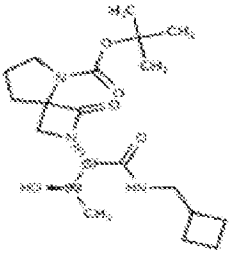
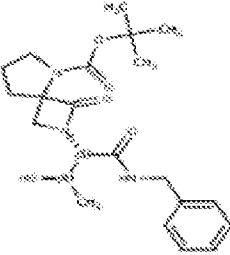
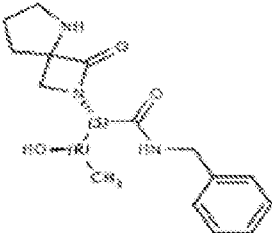
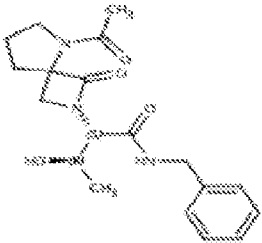
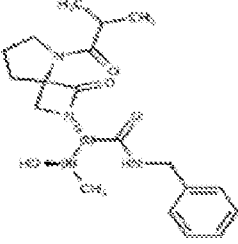
5 **Table 1.**

Compound	Structure	Molecular Weight (Da)	cLogP	tPSA
Compound X		227	-1.94	96.7
Compound Y		213	-2.36	96.7
Compound Z		281	-1.09	72.9
2S-19		423.5032	0.634639	96.46
2S-20		381.4665	0.193514	90.39

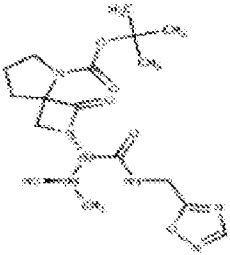
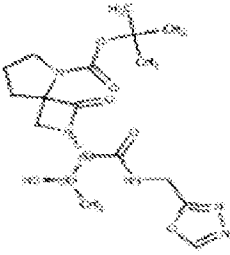
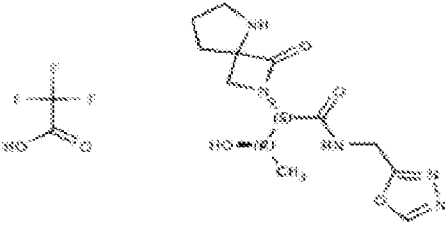
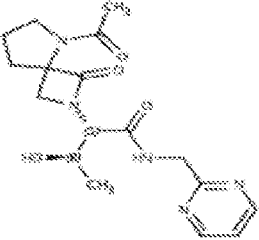
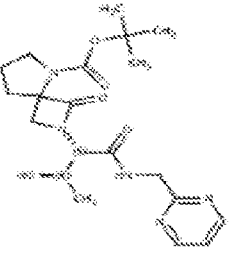
- 26 -

2S-21		341.4027	-0.243061	113.17
2S-24		395.4931	0.610089	90.39
2S-27		395.4931	0.610089	90.39
2S-30		409.5197	1.02666	90.39
2S-8		383.4824	0.808343	99.18

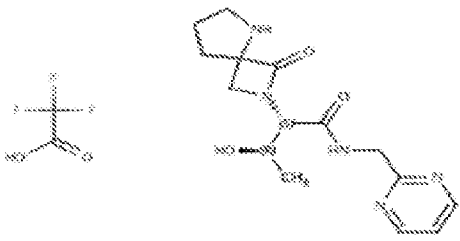
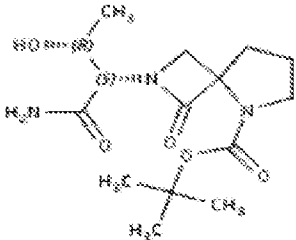
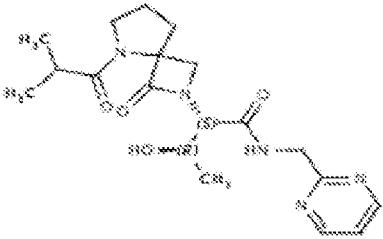
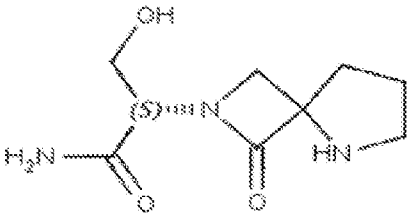
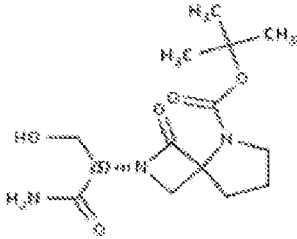
- 27 -

2S-9		395.4931	0.789186	99.18
2S-FNL-10		417.4986	1.28851	99.18
2S-FNL-11		317.3828	0.005320 27	81.67
2S-FNL-12		359.4195	-0.384737	89.95
2S-FNL-13		387.4727	0.858785	89.95

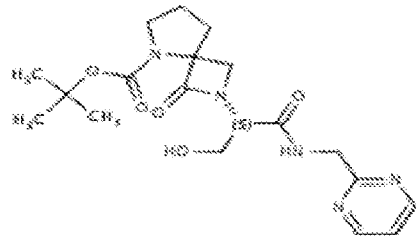
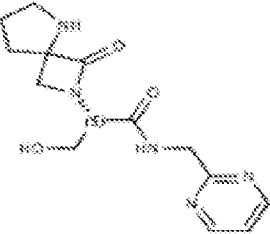
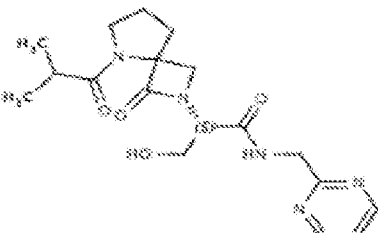
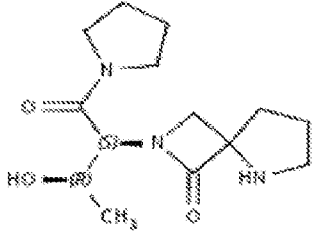
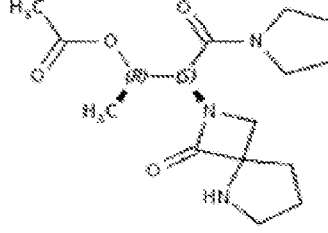
- 28 -

2S-FNL-14		409.4369	-0.960294	138.1
2S-FNL-15		409.4369	-1.65688	138.1
2S-FNL-16		423.3444	-2.94007	120.59
2S-FNL-17		361.3956	-1.88186	115.73
2S-FNL-18		419.4748	-0.35934	124.96

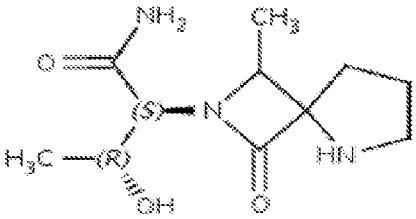
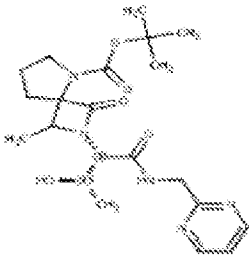
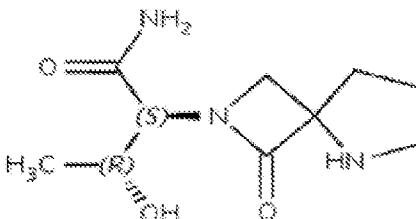
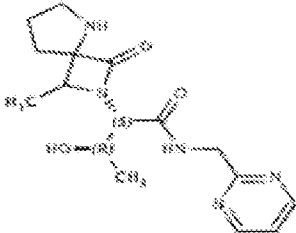
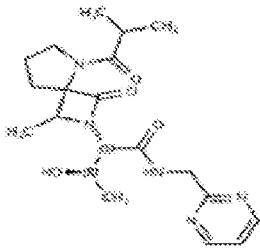
- 29 -

2S-FNL-19		433.3823	-1.6029	107.45
2S-FNL-2		327.3761	-0.659636	113.17
2S-FNL-20		389.4488	-0.696233	115.73
2S-FNL-21		213.2337	-2.3594	95.66
2S-FNL-22, 2S-16		313.3495	-1.07621	113.17

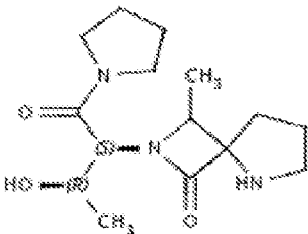
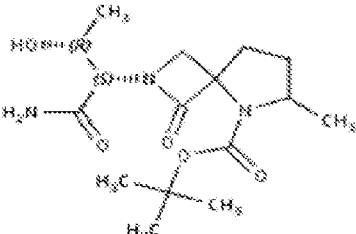
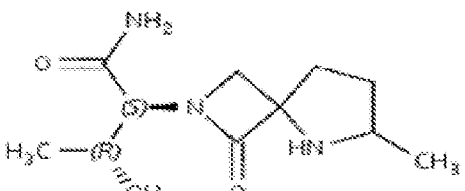
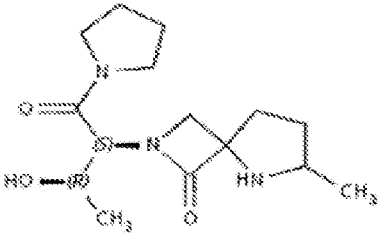
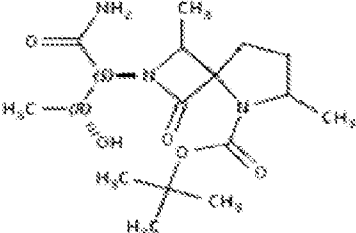
- 30 -

2S-FNL-23		405.4482	-0.769835	124.96
2S-FNL-24		305.3324	-2.01339	107.45
2S-FNL-25		375.4222	-1.10673	115.73
2S-FNL-26		281.3507	-1.08968	72.88
2S-FNL-27	HCl 	359.848	-0.648554	78.95

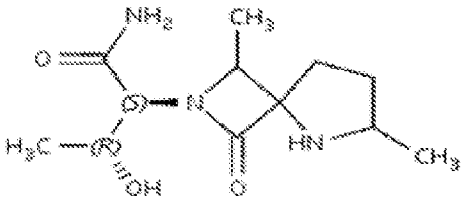
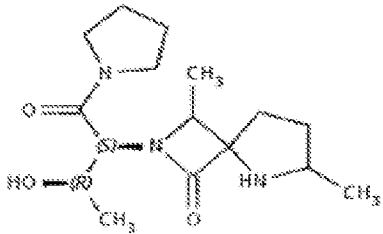
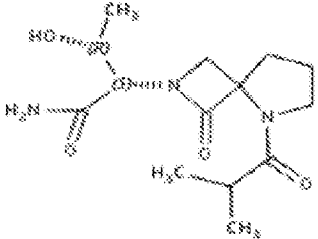
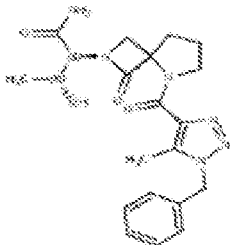
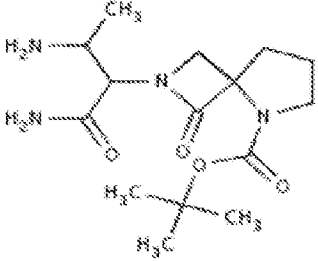
- 31 -

2S-FNL-28		241.2869	-1.52625	95.66
2S-FNL-29		433.5013	0.051154 4	124.96
2S-FNL-3		227.2603	-1.94283	95.66
2S-FNL-30		333.3855	-1.1924	107.45
2S-FNL-31		403.4754	-0.285738	115.73

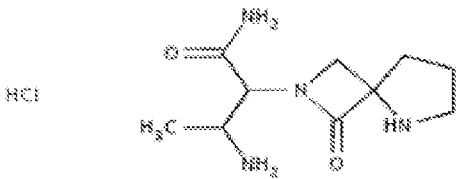
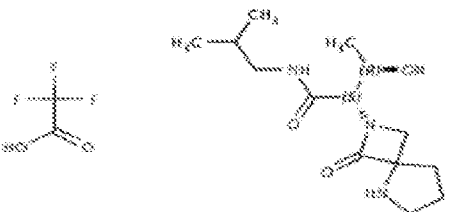
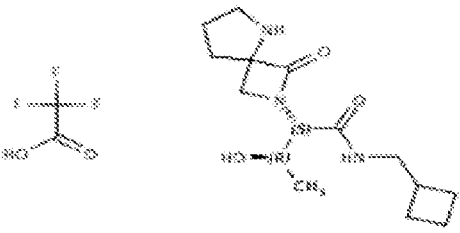
- 32 -

2S-FNL-32		295.3773	-0.673104	72.88
2S-FNL-33		341.4027	-0.243061	113.17
2S-FNL-34		241.2869	-1.52625	95.66
2S-FNL-35		295.3773	-0.673104	72.88
2S-FNL-36		355.4293	0.173514	113.17

- 33 -

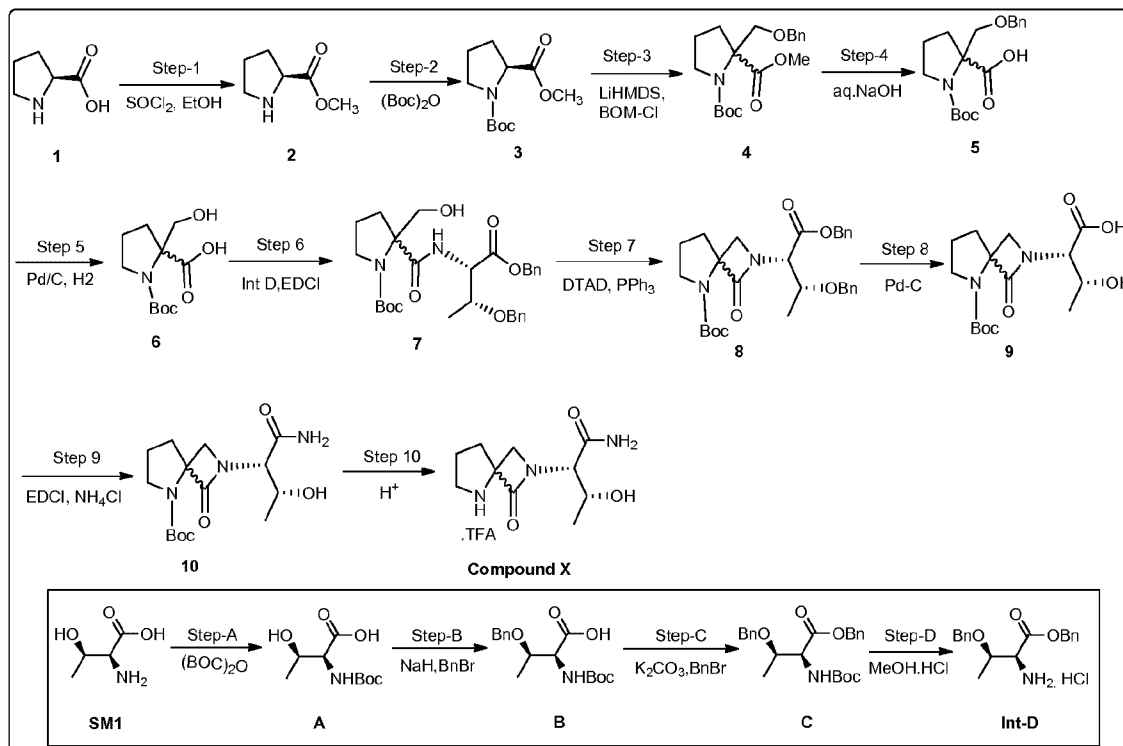
2S-FNL-37		255.3134	-1.10968	95.66
2S-FNL-38		309.4039	-0.256529	72.88
2S-FNL-4		297.3501	-1.08936	103.94
2S-FNL-5		426.4689	-0.121843	134.65
2S-FNL-6		326.3913	-0.766518	118.96

- 34 -

2S-FNL-7	 HCl	262.736	-2.04971	101.45
2S-FNL-8		397.3899	-0.47485	81.67
2S-FNL-9		409.4006	-0.494007	81.67

Example 1 – Synthesis of Compound X***Scheme 1.***

- 35 -



Synthesis of (2S, 3R)-2-((tert-butoxycarbonyl) amino)-3-hydroxybutanoic acid (A)

- [0092] To a stirred solution of L-threonine (SM1) (100 g, 0.84 mol) in 1,4-dioxane (500 mL) and water (800 mL) was added Na₂CO₃ (178 g, 1.67 mol) and stirred at RT for 30 min. The reaction mixture was cooled to 0°C, Boc-anhydride (219.6 g, 1.007 mol) was added drop wise and stirring was continued for 16 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure and obtained residue was neutralized using 1N HCl (pH~4). The aqueous layer was extracted with EtOAc (2 x 250 mL). The separated organic extracts were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford A (160 g, 87%).

¹H-NMR: (500 MHz, DMSO-d₆): δ 6.30 (d, 1H), 4.07-4.01 (m, 1H), 3.90 (d, 1H), 1.99 (s, 1H), 1.42 (s, 9H), 1.09 (d, 3H).

LCMS (m/z): 218.1 [M⁺-1]

15 Synthesis of (2S, 3R)-3-(benzyloxy)-2-((tert-butoxycarbonyl) amino) butanoic acid (B)

[0093] To a stirred solution of A (100 g, 0.45 mol) in DMF (600 mL) was added 60% NaH (36.5 g, 0.91 mol) portion wise at -20°C under N₂ atmosphere and stirred for 2 h. To this was

- 36 -

added benzyl bromide (66.8 mL, 0.55 mol) drop wise and the reaction mixture was stirred at RT for 12 h. After consumption of the starting material (by TLC), the reaction mixture was quenched with ice cold water and washed with diethyl ether (2 x 250 mL). The separated aqueous layer was acidified using 1N HCl and extracted with EtOAc (2 x 250 mL). The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford **B** (100 g, 71%).

Synthesis of (2S, 3R)-benzyl 3-(benzyloxy)-2-((tert-butoxycarbonyl) amino) butanoate (C)

[0094] To a stirred solution of **B** (100 g, 0.32 mol) in DMF (400 mL) was added K₂CO₃ (111.6 g, 0.81 mol) under N₂ atmosphere and stirred for 30 min. To this was added benzyl bromide (47.4 mL, 0.38 mol) drop wise and stirred at RT for 12 h. The reaction mixture was quenched with ice cold water and extracted with diethyl ether (2 x 250 mL). The separated the organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 5% EtOAc/*n*-hexane to afford **C** (80 g, 62%).

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.41-7.25 (m, 10H), 5.09 (s, 2H), 4.55-4.50 (m, 1H), 4.34-4.30 (m, 1H), 2.09 (s, 3H), 1.42 (s, 9H), 1.15 (d, 3H).

Synthesis of (2S, 3R)-benzyl 2-amino-3-(benzyloxy) butanoate (Int-D)

[0095] To a stirred solution of **C** (80 g, 0.20 mol) in methanol (100 mL) was added methanolic HCl (70 mL) under N₂ atmosphere and stirred for 12 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure. The crude material was washed with *n*-hexane and dried under reduced pressure to afford **Int-D** (45 g, 75%) as HCl salt.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.35-7.30 (m, 10H), 5.25 (q, 2H), 4.58-4.52 (m, 3H), 4.37 (d, 1H), 4.27 (br s, 1H), 4.15-4.10 (m, 1H), 1.30 (d, 3H).

LCMS (*m/z*): 300.2 [*M*⁺+1]

Synthesis of (S)-methyl pyrrolidine-2-carboxylate (2)

[0096] To a stirred solution of L-proline **1** (100 g, 0.87 mol) in methanol (800 mL) was added thionyl chloride (76.9 mL, 1.04 mol) slowly drop wise at 0°C. The reaction mixture was heated to reflux for 12 h. After consumption of the starting material (by TLC), the reaction was concentrated under reduced pressure. The residue was washed with *n*-hexane to afford **2** (143.9 g, HCl salt).

- 37 -

¹H-NMR: (400 MHz, CDCl₃): δ 3.89 (s, 3H), 3.68-3.62 (m, 2H), 3.59-3.47 (m, 2H), 2.49-2.37 (m, 1H), 2.27-2.05 (m, 3H).

LCMS (m/z): 166 [M⁺+1]

Synthesis of (S)-1-tert-butyl 2-methyl pyrrolidine-1,2-dicarboxylate (3)

- 5 [0097] To a stirred solution of **2** (35 g, 0.22 mol) in CH₂Cl₂ (175 mL) was added Et₃N (90 mL, 0.65 mol) followed by Boc-anhydride (56.9 mL, 0.26 mol) at 0°C. The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction was diluted with water (100 mL) and extracted with CH₂Cl₂ (2x 100 mL). The organic layer was washed with water, brine, dried over Na₂SO₄ and concentrated. The crude material was
- 10 purified by silica gel column chromatography eluting with 30% EtOAc/*n*-hexane to afford **3** (41 g, 95%).

¹H-NMR: (400 MHz, CDCl₃): δ 4.25-4.21 (m, 1H), 3.75 (s, 3H), 3.57-3.26 (m, 2H), 2.29-2.10 (m, 1H), 1.99-1.75 (m, 3H), 1.45 (s, 9H).

LCMS (m/z): 130 [(M⁺+1)-Boc]

- 15 **Synthesis of 1-tert-butyl 2-methyl 2-((benzyloxy) methyl) pyrrolidine-1, 2-dicarboxylate (4)**

- [0098] To a stirred solution of **3** (100 g, 0.43 mol) in THF (800 mL) was added LiHMDS (873 mL, 0.87 mol) at -78°C and stirred for 1 h. To this BOM-chloride (93.2 mL, 0.65 mol) was added drop wise at -78°C and stirred for 2 h at -20°C. After consumption of the starting
- 20 material (by TLC), the reaction was quenched with aqueous NH₄Cl solution and extracted with EtOAc. The separated organic layer was washed with water, dried over Na₂SO₄ and concentrated to afford **4** (180 g, crude). This material was directly taken for the next step without further purification.

LCMS (m/z): 250 [(M⁺+1)-Boc]

- 25 **Synthesis of 2-((benzyloxy) methyl)-1-(tert-butoxycarbonyl) pyrrolidine-2-carboxylic acid (5)**

- [0099] To a stirred solution of **4** (100 g, 0.28 mol) in methanol (200 mL) was added 2N NaOH solution (300 mL) at RT. The reaction mixture was heated to reflux for 4 h. After consumption of the starting material (by TLC), the solvent from the reaction was evaporated
- 30 under reduced pressure and diluted with EtOAc (100 mL). The aqueous layer was acidified

- 38 -

using citric acid solution and extracted with CH₂Cl₂ (2x 250 mL). The separated organic layer was washed with water, dried over Na₂SO₄ and concentrated to afford **5** (60 g, 63%).

¹H-NMR: (400 MHz, CDCl₃): δ 7.37-7.32 (m, 5H), 4.61 (s, 2H), 4.05-3.88 (m, 2H), 3.65-3.42 (m, 2H), 2.54-2.46 (m, 2H), 1.95 (br s, 2H), 1.57 (s, 9H).

5 LCMS (m/z): 334 [M⁺-1]

Synthesis of 1-(tert-butoxycarbonyl)-2-(hydroxymethyl)pyrrolidine-2-carboxylic acid (6)

[00100] To a stirred solution of **5** (10 g, 29.81 mmol) in methanol (300 mL) was added 50% wet 10% Pd/C (5 g) at RT and stirred for 24 h under H₂ atmosphere (balloon pressure). After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad
10 of celite and the pad was washed with methanol. Obtained filtrate was concentrated under reduced pressure to afford **6** (6 g, 82%).

¹H-NMR: (400 MHz, DMSO-d₆): δ 12.55 (br m, 1H), 3.99 (d, 1H), 3.88 (d, 1H), 7.65-7.60 (m, 1H), 3.51-3.45 (m, 1H), 3.39-3.34 (m, 1H), 2.32-2.14 (m, 1H), 1.98-1.69 (m, 3H), 1.39 (s, 9H).

15 **Synthesis of tert-butyl 2-(((2S, 3R)-1, 3-bis(benzyloxy)-1-oxobutan-2-yl) carbamoyl)-2-(hydroxymethyl) pyrrolidine-1-carboxylate (7)**

[00101] To a stirred solution of **6** (3 g, 12.2 mmol) in CH₂Cl₂ (100 mL) was added **Int-D** (5.8 g, 14.6 mmol), EDCl.HCl (2.8 g, 14.6 mmol) followed by HOBt (1.99 g, 14.6 mmol) and DIPEA (4.8 g, 36.7 mmol) at RT and stirred for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (100 mL) and extracted with
20 CH₂Cl₂ (2 x 100 mL). The separated organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography to afford **7** (1.6 g, 25%).

25 ¹H-NMR: (400 MHz, DMSO-d₆): δ 8.25-8.12 (m, 1H), 7.31-7.27 (m, 10H), 5.85 (t, 1H), 5.14 (s, 2H), 4.54-4.49 (m, 2H), 4.31 (dd, 1H), 4.15-4.07 (m, 1H), 3.91-3.50 (m, 1H), 3.52-3.37 (m, 1H), 3.31-3.27 (m, 2H), 2.35-2.07 (m, 1H), 1.95-1.90 (m, 1H), 1.73-1.52 (m, 2H), 1.39-1.27 (m, 9H), 1.19-1.12 (m, 3H).

Mass (ESI): m/z 527.4 [M⁺+1]

Synthesis of tert-butyl 2-((2S,3R)-1,3-bis(benzyloxy)-1-oxobutan-2-yl)-1-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (8)

30 [00102] To a stirred solution of **7** (1.4 g, 2.65 mmol) in THF (20 mL) was added triphenylphosphine (1.1 g, 3.98 mmol) and DTAD (1.2 g, 3.98 mmol). The reaction mixture

- 39 -

was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography to afford **8-F1** (0.6 g) and **8-F2** (0.55 g).

Synthesis of (2S, 3R)-2-(5-(tert-butoxycarbonyl)-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-hydroxybutanoic acid (9)

[00103] To a stirred solution of **8-F1** and **8-F2** (0.6 g) in methanol (50 mL) was added 10% Pd/C (120 mg) at RT and stirred for 6 h under H₂ atmosphere (balloon pressure). After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol. The filtrate was concentrated under reduced pressure to give crude, trituration using diethyl ether afforded **9** (0.3 g, 82%) as an off-white solid.

¹H-NMR: (500 MHz, DMSO-d₆): δ 12.95 (br s, 1H), 4.97 (br s, 1H), 4.24-4.20 (m, 1H), 4.14-4.07 (m, 1H), 3.84 (d, 1H), 3.53 (t, 1H), 3.41-3.35 (m, 1H), 3.27-3.22 (m, 1H), 2.14-2.08 (m, 2H), 1.84-1.80 (m, 2H), 1.42 (s, 9H), 1.24 (d, 3H).

LCMS (m/z): 329.6 [M⁺+1]

Synthesis of tert-butyl 2-((2S, 3R)-1-amino-3-hydroxy-1-oxobutan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (10)

[00104] To a stirred solution of **9** (5 g, 15.2 mmol) in CH₂Cl₂ (100 mL) was added ammonium chloride (2 g, 38.1 mmol), EDCI.HCl (3.5 g, 18.2 mmol) followed by HOBt (5.9 g, 45.7 mmol) and DIPEA (5.9 g, 45.7 mmol) at RT and stirred for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (100 mL) and extracted with CH₂Cl₂ (2 x 100 mL). The separated organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude material was trituated with Et₂O (50 mL) and n-pentane (50 mL) to afford **10** (2.5 g, 51%) as an off-white solid.

¹H-NMR: (400 MHz, DMSO-d₆): δ 7.51 (br s, 1H), 7.19 (br s, 1H), 4.64 (d, 1H), 4.07-3.95 (m, 2H), 3.78 (m, 1H), 3.62-3.35 (m, 2H), 3.27-3.25 (m, 1H), 2.18-2.05 (m, 2H), 1.86-1.74 (m, 2H), 1.41 (s, 9H), 1.12 (d, 3H).

LCMS (m/z): 328.2 [M⁺+1]

Synthesis of (2S, 3R)-3-hydroxy-2-(1-oxo-2, 5-diazaspiro[3.4]octan-2-yl)butanamide (Compound X)

- 40 -

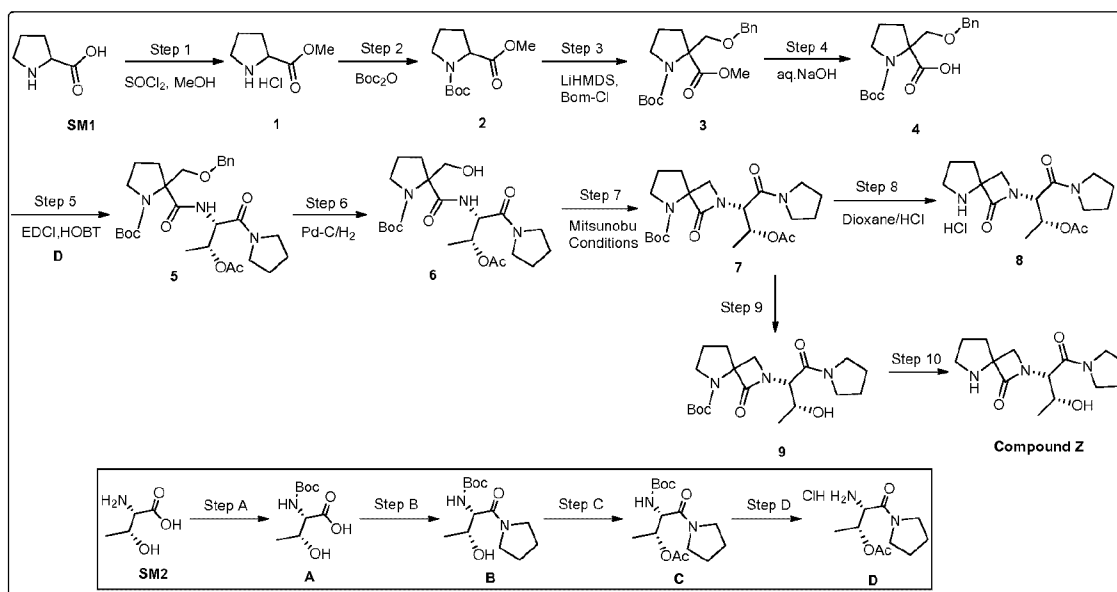
[00105] To a stirred solution of **10** (2.2 g, 6.70 mmol) in CH_2Cl_2 (25 mL) was added TFA (7.6 g, 67 mmol) at 0°C and stirred at RT for 2 h. The reaction mixture was concentrated under reduced pressure to afford **Compound X** (2 g, 87%) as TFA salt.

$^1\text{H-NMR}$: (400 MHz, D_2O): δ 4.33-4.29 (m, 2H), 4.09 (d, 1H), 3.95 (d, 1H), 3.57-3.48 (m, 2H), 2.51-2.46 (m, 2H), 2.25-2.19 (m, 2H), 1.31 (d, 3H).

LCMS (m/z): 455 [$2\text{M}^+ + 1$]

Example 2- Synthesis of Compound Z

Scheme 3.



Synthesis of (2S, 3R)-2-((tert-butoxycarbonyl) amino)-3-hydroxybutanoic acid (A)

[00106] To a stirred solution of (2S, 3R)-2-amino-3-hydroxybutanoic acid (SM2) (30 g, 0.25 mol) in THF (150 mL) and water (150 mL) was added NaHCO_3 (65 g, 0.75 mol) followed by Boc-anhydride (66 mL, 0.302 mol) at 0°C . The reaction mixture was stirred at RT for 16 h.

After consumption of the starting material (by TLC), the reaction mixture was extracted with EtOAc (2 x 150 mL). The aqueous layer was acidified using 2N HCl and then extracted with 10% MeOH/ CH_2Cl_2 . The separated organic extracts were dried over anhydrous Na_2SO_4 , filtered and concentrated under vacuum to afford A (30 g, 63%).

$^1\text{H-NMR}$: (400 MHz, CDCl_3): δ 5.92-5.70 (m, 2H), 5.55 (d, 1H), 4.42 (br s, 1H), 4.29 (d, 1H),

1.47 (s, 9H), 1.25 (d, 3H)

- 41 -

LCMS (m/z): 218 [M⁺-1]

Synthesis of *tert*-butyl ((2*S*, 3*R*)-3-hydroxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl) carbamate (B)

[00107] To a stirred solution of **A** (13 g, 59.36 mmol) in DMF (65 mL) was added
 5 EDCl.HCl (12.5 g, 65.2 mmol) followed by HOBT (8.8 g, 65.2 mmol) at 0°C. After stirring for
 5 min, DIPEA (30.6 mL, 0.17 mol) followed by pyrrolidine (4.6 g, 65.2 mmol) was added to
 the reaction mixture and stirring was continued for another 16 h at RT. The reaction mixture
 was washed with water and extracted with EtOAc (2x 100 mL). The organic layer was washed
 with brine, dried over anhydrous Na₂SO₄ and concentrated under vacuum. The crude was
 10 purified by column chromatography to afford **B** (5 g, 31%).

¹H-NMR: (400 MHz, CDCl₃): δ 5.51 (br s, 1H), 4.32 (d, 1H), 4.15-4.10 (m, 1H), 3.77-3.74 (m, 1H), 3.55-3.46 (m, 3H), 1.99-1.94 (m, 2H), 1.91-1.85 (m, 2H), 1.47 (s, 9H), 1.26 (t, 1H), 1.29 (d, 3H).

Synthesis of (2*R*, 3*S*)-3-((*tert*-butoxycarbonyl) amino)-4-oxo-4-(pyrrolidin-1-yl) butan-2-yl acetate (D)

[00108] To a stirred solution of **B** (4 g, 14.7 mmol) in CH₂Cl₂ (40 mL) was added Et₃N (5.1 mL, 36.7 mmol) followed by acetic anhydride (1.7 g, 17.6 mmol) and catalytic amount of DMAP at 0 °C. The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water and separated the
 20 organic layer. Organic layer was washed with water, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude residue obtained was purified by silica gel column chromatography to give **C**. To this 1,4-dioxane/HCl (20 mL) was added and stirred at RT for 2 h. The reaction mixture was concentrated under vacuum and the residue was washed with Et₂O (2x 15 mL) to afford **D** (3.5 g, 97%) as HCl salt.

25 ¹H-NMR: (500 MHz, DMSO-*d*₆) (Rotamers): δ 8.49 (br s, 3H), 8.15 (br s, 1H), 5.14-5.10 (m, 1H), 4.26-4.22 (m, 1H), 3.97-3.95 (m, 1H), 3.59 (s, 2H), 2.09 (s, 3H), 1.98 (s, 2H), 1.87-1.80 (m, 2H), 1.26 (d, 3H).

LCMS (m/z): 215.1 [M⁺+1]

Synthesis of methyl pyrrolidine-2-carboxylate (1)

30 [00109] To a stirred solution of pyrrolidine-2-carboxylic acid (**SM1**) (100 g, 0.87 mol) in methanol (800 mL) was added thionyl chloride (76.9 mL, 1.04 mol) slowly drop wise at 0°C.

- 42 -

The reaction mixture was heated to reflux for 12 h. After consumption of the starting material (by TLC), the reaction was concentrated under vacuum. The residue was washed with *n*-Hexane and distilled off the solvent to afford **1** (143.9 g, HCl salt).

¹H-NMR: (400 MHz, CDCl₃) (Rotamers): δ 3.89 (s, 3H), 3.68-3.62 (m, 2H), 3.59-3.47 (m, 2H), 2.49-2.37 (m, 1H), 2.27-2.05 (m, 3H).

LCMS (m/z): 166 [M⁺+1]

Synthesis of 1-*tert*-butyl 2-methyl pyrrolidine-1,2-dicarboxylate (2)

[00110] To a stirred solution of **1** (35 g, 0.22 mol) in CH₂Cl₂ (175 mL) were added Et₃N (90 mL, 0.65 mol) followed by Boc-anhydride (56.9 mL, 0.26 mol) at 0 °C. The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction was diluted with water (100 mL) and extracted with CH₂Cl₂ (2x 100 mL). The organic layer was washed with water, brine, dried over Na₂SO₄ and concentrated. The crude material was purified by silica gel column chromatography eluting with 30% EtOAc/Hexane to afford **2** (41 g, 95%).

¹H-NMR: (400 MHz, CDCl₃) (Rotamers): δ 4.25-4.21 (m, 1H), 3.75 (s, 3H), 3.57-3.26 (m, 2H), 2.29-2.10 (m, 1H), 1.99-1.75 (m, 3H), 1.45 (s, 9H).

LCMS (m/z): 130 [(M⁺+1)-Boc]

Synthesis of 1-*tert*-butyl 2-methyl 2-((benzyloxy) methyl) pyrrolidine-1, 2-dicarboxylate (3)

[00111] To a stirred solution of **2** (100 g, 0.43 mol) in THF (800 mL) was added LiHMDS (873 mL, 0.87 mol) at -78°C and stirred for 1 h. To this BOM-chloride (93.2 mL, 0.65 mol) was added drop wise at -78°C and stirred for 2 h at -20°C. After consumption of the starting material (by TLC), the reaction was quenched with NH₄Cl at 0°C. The separated organic layer was washed with water, dried over Na₂SO₄ and concentrated to afford **3** (180 g, crude). This material was directly taken for the next step without further purification.

LCMS (m/z): 250 [(M⁺+1)-Boc]

Synthesis of 2-((benzyloxy) methyl)-1-(*tert*-butoxycarbonyl) pyrrolidine-2-carboxylic acid (4)

[00112] To a stirred solution of **3** (100 g, 0.28 mol) in methanol (200 mL) was added 2N NaOH solution (300 mL) at RT. The reaction mixture was heated to reflux for 4 h. After consumption of the starting material (by TLC), the solvent from the reaction was evaporated

- 43 -

under vacuum and diluted with EtOAc (100 mL). The aqueous layer was acidified using citric acid solution and extracted with CH₂Cl₂ (2x 250 mL). The separated organic layer was washed with water, dried over Na₂SO₄ and concentrated to afford **4** (60 g, 63%).

¹H-NMR: (400 MHz, CDCl₃) (Rotamers): δ 7.37-7.32 (m, 5H), 4.61 (s, 2H), 4.05-3.88 (m, 2H), 3.65-3.42 (m, 2H), 2.54-2.46 (m, 2H), 1.95 (br s, 2H), 1.57 (s, 9H).

LCMS (m/z): 334 [M⁺-1]

Synthesis of *tert*-butyl 2-(((2*S*, 3*R*)-3-acetoxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl) carbamoyl)-2-((benzyloxy) methyl) pyrrolidine-1-carboxylate (5**)**

[00113] To a stirred solution of **D** (1 g, 2.90 mmol) in DMF (8 mL) was added EDCl.HCl (0.63 g, 3.28 mmol) followed by HOBT (0.44 g, 3.28 mmol) at 0°C. After being stirred for 5 min, DIPEA (1.3 mL, 7.46 mmol) followed by compound **4** (0.74 g, 3.58 mmol) was added to the reaction mixture and stirring was continued for another 16 h at RT. The reaction mixture was washed with water and extracted with EtOAc (2x 500 mL). The organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under vacuum. The crude was purified by column chromatography to afford **5** (0.6 g, 38%).

LCMS (m/z): 532 [M⁺+1]

Synthesis of *tert*-butyl 2-(((2*S*, 3*R*)-3-acetoxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl) carbamoyl)-2-(hydroxymethyl) pyrrolidine-1-carboxylate (6**)**

[00114] To a stirred solution of **5** (4.5 g, 8.40 mmol) in MeOH (40 mL) was added wet 10% Pd/C (1.5 g) under inert atmosphere and stirred for 4 h under H₂ atmosphere (balloon pressure). The reaction mixture was filtered through celite pad and concentrated under reduced pressure to afford **6** (3.0 g, 81%).

LCMS (m/z): 442.5 [M⁺+1]

Synthesis of *tert*-butyl 2-(((2*S*, 3*R*)-3-acetoxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl)-1-oxo-2, 5-diazaspiro[3.4] octane-5-carboxylate (7**)**

[00115] To a stirred solution of **6** (3 g, 6.70 mmol) in THF (25 mL) was added triphenylphosphine (2 g, 7.40 mmol) followed by DTAD (2.5 g, 10.2 mmol). The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 10% MeOH/CH₂Cl₂ to afford **7** (1.2 g with TPPO, 43%).

- 44 -

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 5.25-5.19 (m, 1H), 4.65 (d, 1H), 3.61-3.57 (m, 3H), 3.47-3.42 (m, 2H), 3.41-3.25 (m, 4H), 2.05 (s, 4H), 1.95-1.71 (m, 7H), 1.42 (s, 10H).

LCMS (m/z): 424.4 [M⁺+1]

Synthesis of (2R, 3S)-4-oxo-3-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-4-(pyrrolidin-1-yl) butan-2-yl acetate (8)

[00116] A stirred solution of 7 (0.4 g, 0.94 mmol) in 1,4-dioxane/HCl (5 mL) was cooled to 0°C and stirred at RT for 1 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure. The crude material was washed with *n*-pentane followed by EtOAc to afford **8** (0.22 g, 65%).

¹H-NMR: (400 MHz, D₂O): δ 4.62 (d, 1H), 4.41-4.29 (m, 2H), 4.24 (d, 1H), 3.89-3.77 (m, 3H), 3.54-3.49 (m, 3H), 2.57-2.52 (m, 1H), 2.49 (s, 3H), 2.42-2.00 (m, 8H), 1.30 (d, 3H).

LCMS (m/z): 324.3 [M⁺+1]

UPLC Purity: 99.37%

Synthesis of *tert*-butyl 2-((2S, 3R)-3-hydroxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (9)

[00117] A solution of 7 (0.15 g, 0.41 mmol) in aqueous NH₃ (2 mL) was stirred at RT for 4 h. After consumption of the starting material (by TLC), the reaction diluted with CH₂Cl₂ (75 mL). The separated organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford **9** (0.1 g, 76%).

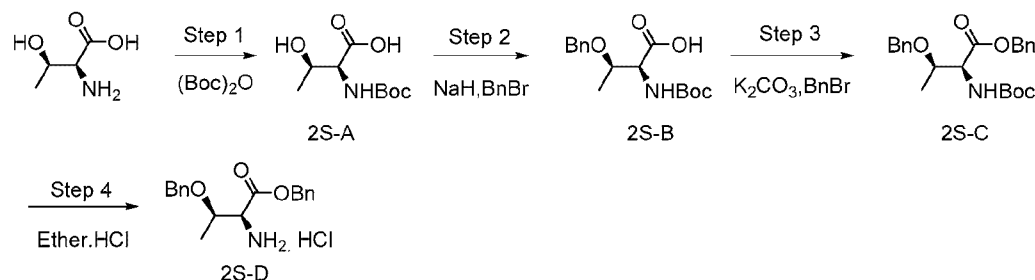
LCMS (m/z): 382 [M⁺+1]

Synthesis of 2-((2S, 3R)-3-hydroxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl)-2, 5-diazaspiro [3.4] octan-1-one (Compound Z)

[00118] To a stirred solution of **9** (0.2 g, 0.63 mmol) in CH₂Cl₂ (2 mL) was added TFA (0.3 mL) at 0 °C and stirred at RT for 1 h. The reaction mixture was concentrated under vacuum and the residue was diluted with water and extracted with CH₂Cl₂ (2x 25 mL). The separated organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under vacuum to afford **Compound Z** (0.2 g, 80%) as TFA salt.

¹H-NMR: (400 MHz, D₂O): δ 4.64 (t, 1H), 4.25-4.21 (m, 1H), 4.09 (d, 1H), 3.99-3.87 (m, 1H), 3.70 (t, 2H), 3.55-3.47 (m, 5H), 2.52-2.34 (m, 2H), 2.25-2.22 (m, 2H), 2.08-1.98 (m, 5H), 1.25 (t, 3H).

- 45 -

LCMS (*m/z*): 282.4 [*M*⁺+1]**Scheme 2S-I-1**

5

Synthesis of (2S, 3R)-2-((tert-butoxycarbonyl) amino)-3-hydroxybutanoic acid (2S-A):

[00119] To a stirring solution of L-threonine (50 g, 420 mol) in THF/water (500 mL/500 mL) were added NaHCO₃ (111 g, 1.05 mol) and stirred at RT for 30 min. The reaction mixture was cooled to 0 °C and Boc-anhydride (137 mL, 630 mmol) was added drop wise and the stirring was continued at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure and obtained residue was diluted with water (100 mL) and acidified by using 1N HCl (pH~3). The aqueous layer was extracted with EtOAc (2 x 250 mL). The combined organic layer was washed with brine (1 x 200 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford compound **2S-A** (80 g, 87%) as thick syrup.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 12.5 (br s, 1H), 6.30 (d, *J* = 8.5 Hz, 1H), 4.50 (br s, 1H), 4.05-4.02 (m, 1H), 3.88-3.86 (m, 1H), 1.39 (s, 9H), 1.08 (d, *J* = 6.0 Hz, 3H);

LCMS *m/z*: 218.1 [*M*⁺-1]

Synthesis of (2S, 3R)-3-(benzyloxy)-2-((tert-butoxycarbonyl) amino) butanoic acid (2S-B):

[00120] To a stirring solution of 2S-A (40 g, 182 mmol) in DMF (400 mL) was added 60% NaH (18.2 g, 758 mmol) portion wise at -20 °C under N₂ atmosphere and stirred for 2 h. To this added benzyl bromide (66.8 mL, 0.55mol) drop wise and the reaction mixture was stirred at RT for 3 h. After consumption of the starting material (by TLC), the reaction mixture was quenched with ice cold water and washed with diethyl ether (2 x 250 mL). The separated aqueous layer was acidified using citric acid solution (100 mL) and extracted with EtOAc (2 x 250 mL). The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford compound **2S-B** (45 g, 80%) as thick syrup.

- 46 -

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 12.64 (br s, 1H), 7.34-7.25 (m, 5H), 6.46 (d, *J* = 8.5 Hz, 1H), 4.53 (d, *J* = 11.5 Hz, 1H), 4.39 (d, *J* = 12.0 Hz, 1H), 4.00-3.98 (m, 2H), 1.39 (s, 9H), 1.15 (d, *J* = 6.0 Hz, 3H);

Synthesis of (2*S*, 3*R*)-benzyl 3-(benzyloxy)-2-((*tert*-butoxycarbonyl) amino) butanoate (2*S*-C):

[00121] To a stirring solution of compound **2*S*-B** (45 g, 146 mmol) in DMF (400 mL) was added K₂CO₃ (40 g, 292 mmol) under N₂ atmosphere and stirred for 30 min. To this benzyl bromide (21 mL, 175 mmol) was added drop wise at 0 °C and stirred at RT for 16 h. The reaction mixture was quenched with ice cold water and extracted with diethyl ether (2 x 250 mL). The separated organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 20% EtOAc/*n*-hexane to afford compound **2*S*-C** (48 g, 82%) as thick syrup.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.37-7.18 (m, 10H), 6.81 (d, *J* = 9.0 Hz, 1H), 5.08 (s, 2H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.32 (d, *J* = 12.0 Hz, 1H), 4.25-4.22 (m, 1H), 4.01-3.98 (m, 1H), 1.38 (s, 9H), 1.15 (d, *J* = 6.0 Hz, 3H)

Mass (ESI): *m/z* 399.4[M⁺+1];

Synthesis of (2*S*, 3*R*)-benzyl 2-amino-3-(benzyloxy) butanoate (2*S*-D):

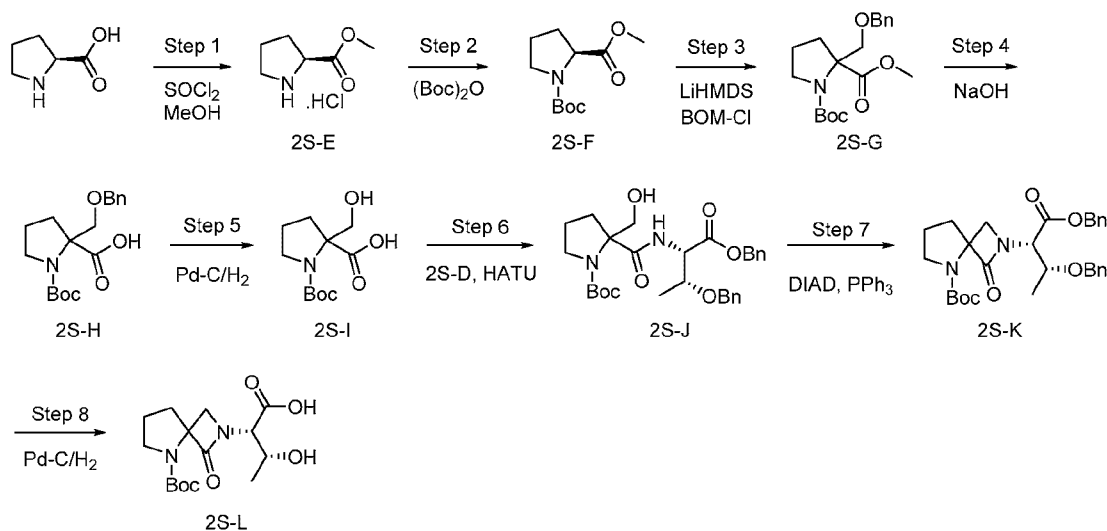
[00122] To a stirring solution of compound **2*S*-C** (48 g, 120 mmol) in diethylether (50 mL) was added diethylether saturated with HCl (350 mL) at 0 °C and stirred at RT for 10 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure. The crude material was triturated with diethyl ether/*n*-pentane (50 mL/50 mL) and dried under reduced pressure to afford compound **2*S*-D** (28 g, 77%) as semisolid (HCl salt).

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 8.59 (s, 2H), 7.50-7.25 (m, 10H), 5.23 (d, *J* = 12.5 Hz, 1H), 5.16 (d, *J* = 12.5 Hz, 1H), 4.54 (d, *J* = 12.0 Hz, 1H), 4.36 (d, *J* = 12.0 Hz, 1H), 4.12-4.09 (m, 1H), 4.09-3.99 (m, 1H), 1.29 (d, *J* = 6.5 Hz, 3H)

Mass (ESI): *m/z* 299.4[M⁺+1];

30 ***Scheme 2*S*-I-2***

- 47 -

**Synthesis of methyl pyrrolidine-2-carboxylate (2S-E):**

- 5 [00123] To a stirring solution of L-proline (50 g, 434 mmol) in methanol was added thionyl chloride (37.5 ml, 521 mmol) at 0 °C and heated to 70 °C for 16 h. The reaction mixture was brought to RT and concentrated under vacuum to afford compound **2S-E** as (70 g, 99 %) as thick syrup (hydrochloride salt).

10 ¹H-NMR: (500 MHz, DMSO-*d*₆): δ 4.15-4.13 (m, 1H), 3.65 (s, 3H), 3.35-3.30 (m, 2H), 2.23-2.15 (m, 1H), 1.86-1.78 (m, 3H), 1.41 (s, 9H);

LCMS *m/z*: 129 [$\text{M}^+ + 1$]

Synthesis of 1-tert-butyl 2-methyl pyrrolidine-1, 2-dicarboxylate (2S-F):

- [00124] To a stirring solution of compound **2S-E** (70 g, 422 mmol) in CH_2Cl_2 (700 mL) were added Et_3N (183 mL, 1.26 mol) at 0 °C and stirred for 10 min. After added Boc-anhydride (184 mL, 845 mmol) at 0 °C and the reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction was diluted with water (200 mL) and extracted with CH_2Cl_2 (2 x 200 mL). The combined organic layer was washed with citric acid (1 x 150 mL), brine (1 x 200 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 50% EtOAC/*n*-hexane to obtain compound **2S-F** (80 g, 83%) as thick syrup.

- 48 -

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 4.15-4.13 (m, 1H), 3.65 (s, 3H), 3.35-3.30 (m, 2H), 2.23-2.15 (m, 1H), 1.86-1.78 (m, 3H), 1.41 (s, 9H);

LCMS *m/z*: 229 [(M⁺+1)-Boc].

Synthesis of 1-tert-butyl 2-methyl 2-((benzyloxy) methyl) pyrrolidine-1, 2-dicarboxylate

5 **(2S-G):**

[00125] To a stirring solution of compound **2S-F** (25 g, 109 mmol) in THF (250 mL) was added LiHMDS (240 mL, 240 mmol) at -20 °C and stirred for 2 h. To this BOM-chloride (23 mL, 163 mmol) was added drop wise at -30 °C and stirred for 2 h. After consumption of the starting material (by TLC), the reaction was quenched with aqueous NH₄Cl solution (100 mL) and extracted with EtOAc (2 x 200 mL). The combined organic layer was washed with water (2 x 150 mL) followed by brine solution (2 x 100 mL). The organic layer was dried over Na₂SO₄ and concentrated to obtain crude compound which was purified by column chromatography by eluting 10% EtOAc/*n*-hexane to afford compound **2S-G** (30 g, 79%) as thick syrup.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.36-7.22 (m, 5H), 4.59-4.48 (m, 2H), 4.02-3.88 (m, 1H), 3.63 (s, 3H), 3.49-3.35 (m, 2H), 3.34-3.30 (m, 1H), 2.31-2.23 (m, 1H), 2.04-1.89 (m, 2H), 1.82-1.78 (m, 1H);

LCMS *m/z*: 349.4 [(M⁺+1)-Boc]

Synthesis of 2-((benzyloxy) methyl)-1-(tert-butoxycarbonyl) pyrrolidine-2-carboxylic acid

(2S-H):

20 [00126] To a stirring solution of compound **2S-G** (30 g, 86 mmol) in methanol (70 mL) was added NaOH solution (6.88 g in 70 mL H₂O) at RT. The reaction mixture was heated to 70 °C for 16 h. After consumption of the starting material (by TLC), the solvent from the reaction was evaporated under reduced pressure and diluted with EtOAc (2 x 200 mL). The separated aqueous layer was acidified using citric acid solution (pH~3) and extracted with EtOAc (2 x 250 mL). The combined organic layer was dried over Na₂SO₄ and concentrated to afford crude was triturated with *n*-hexane to obtain compound **2S-H** (25 g, 86.8%) as an off-white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 12.35 (br s, 1H), 7.37-7.29 (m, 5H), 4.56-4.48 (m, 2H), 4.06-4.00 (m, 1H), 3.92-3.89 (m, 1H), 3.66-3.45 (m, 1H), 3.37-3.28 (m, 1H), 2.31-2.20 (m, 1H), 2.05-1.97 (m, 1H), 1.87-1.75 (m, 2H), 1.38 (s, 9H);

30 **LCMS *m/z*:** 335.3 [M⁺+1]

Synthesis of 1-(tert-butoxycarbonyl)-2-(hydroxymethyl) pyrrolidine-2-carboxylic acid

(2S-I):

- 49 -

[00127] To a stirring solution of compound 2S-H (25 g, 74 mmol) in methanol (150 mL) was added 50% wet 10% Pd/C (7 g) at RT and stirred for 10 h under H₂ atmosphere. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol (100 mL). Obtained filtrate was concentrated under reduced pressure to afford compound 2S-I (15 g, 82.8%) as white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 4.66 (br s, 1H), 3.96-3.83 (m, 1H), 3.63-3.59 (m, 1H), 3.49-3.41 (m, 1H), 3.34-3.25 (m, 1H), 2.30-2.17 (m, 1H), 1.95-1.72 (m, 3H), 1.38 (s, 9H).

Mass (ESI): *m/z* 245 [M⁺+1]

Synthesis of *tert*-butyl 2-(((2*S*, 3*R*)-1, 3-bis (benzyloxy)-1-oxobutan-2-yl) carbamoyl)-2-(hydroxymethyl) pyrrolidine-1-carboxylate (2S-J):

[00128] To a stirring solution of compound 2S-I (18 g, 73.4 mmol) in CH₂Cl₂ (180 mL) were added DIPEA (40 mL, 220 mmol), 2S-D (21.9 g, 73.4 mmol), HATU (41.8 g, 110 mmol) at RT and stirred for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (50 mL) and extracted with CH₂Cl₂ (2 x 100 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 30% EtOAc/*n*-hexane to afford compound 2S-J (20 g, 52%) as pale yellow thick syrup.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 8.25-8.12 (m, 1H), 7.31-7.27 (m, 10H), 5.85 (t, *J* = 4.8 Hz, 1H), 5.14 (s, 2H), 4.54-4.49 (m, 2H), 4.31-4.20 (m, 1H), 4.15-4.07 (m, 1H), 3.91-3.50 (m, 1H), 3.52-3.37 (m, 1H), 3.31-3.27 (m, 2H), 2.35-2.07 (m, 1H), 1.95-1.90 (m, 1H), 1.73-1.52 (m, 2H), 1.39 (s, 9H), 1.19 (d, *J* = 6.4 Hz, 3H);

Mass (ESI): *m/z* 527.4 [M⁺+1]

Synthesis of *tert*-butyl 2-(((2*S*, 3*R*)-1, 3-bis (benzyloxy)-1-oxobutan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-K):

[00129] To a stirring solution of triphenylphosphine (24.7 g, 94 mmol) in THF (100 mL) was added DIAD (15.3 g, 75 mmol) at RT and stirred for 30 min. To this added compound 2S-J (20 g, 37.9 mmol) in (10 mL) THF slowly and reaction mixture was stirred at RT for 2 h. After consumption of the starting material (by TLC), the reaction was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting 25% EtOAc/*n*-hexane to afford compound 2S-K (17 g, 88%) as pale yellow thick syrup.

- 50 -

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.33-7.26 (m, 5H), 7.23-7.18 (m, 5H), 5.10 (s, 2H), 4.80-4.73 (m, 2H), 4.60 (s, 2H), 4.31 (s, 2H), 4.05-4.00 (m, 2H), 1.80-1.68 (m, 4H), 1.39 (s, 9H), 1.18 (d, *J* = 6.0 Hz, 3H);

Mass (ESI): *m/z* 509.4 [*M*⁺+1]

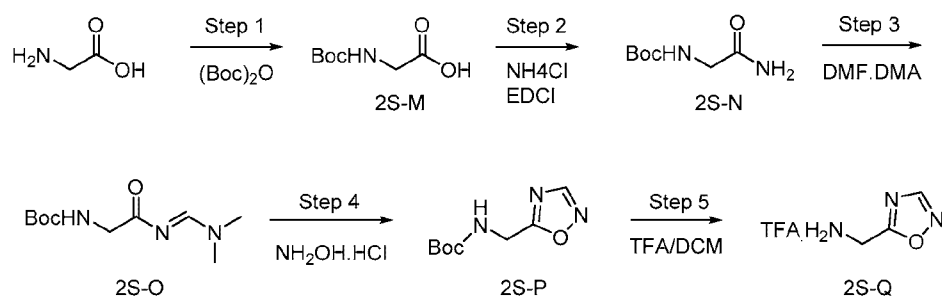
5 **Synthesis of (2*S*, 3*R*)-2-(5-(*tert*-butoxycarbonyl)-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-hydroxybutanoic acid (2*S*-L):**

[00130] To a stirring solution of compound **2*S*-K** (7 g, 13.7 mmol) in methanol (100 mL) was added 10% Pd/C (4 g) at RT and stirred for 6 h under H₂ atmosphere. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol (50 mL). Obtained filtrate was concentrated under reduced pressure to obtained crude, which was triturated with *n*-pentane (50 mL) to afford compound **2*S*-L** (4 g, 88%) as white solid.

15 **¹H-NMR:** (500 MHz, DMSO-*d*₆): δ 12.80 (br s, 1H), 4.78-4.73 (m, 1H), 4.21-4.19 (m, 1H), 4.09 (s, 2H), 3.55-3.46 (m, 2H), 2.09-2.05 (m, 2H), 1.80 (d, *J* = 7.0 Hz, 1H), 1.38 (s, 9H), 1.35-1.28 (m, 2H), 1.17 (d, *J* = 6.5 Hz, 3H)

LCMS *m/z*: 329.6 [*M*⁺+1]

Scheme 2*S*-I-3



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Synthesis of 2-((*tert*-butoxycarbonyl) amino) acetic acid (2*S*-M):

[00131] To a stirring solution of glycine (15 g, 200 mmol) in 1,4-dioxane/water (150 mL/75 mL) were added Na₂CO₃ (53 g, 500 mmol). After added Boc-anhydride (109 mL, 500 mmol) slowly at 0 °C. The reaction mixture was stirred at RT for 12 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure. The crude residue was acidified (pH~4) by using citric acid solution and aqueous layer was extracted with EtOAc (2 x 150 mL). The combined organic layer was washed with brine

25

- 51 -

solution (2 x 100 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under vacuum to afford compound **2S-M** (30 g, 85.7%) as white solid. This material was directly used for the next step without further purification.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 12.41 (br s, 1H), 7.04 (t, *J* = 5.5 Hz, 1H), 3.57 (d, *J* = 5.5 Hz, 2H), 1.37 (s, 9H);

Synthesis of tert-butyl (2-amino-2-oxoethyl) carbamate (2S-N):

[00132] To a stirring solution of **2S-M** (10 g, 57.14 mmol) in CH₂Cl₂ (100 mL) were added HOBt (15.43 g, 114 mmol), EDCI.HCl (21.8 g, 114 mmol) followed by NH₄Cl (4.54 g, 85.71 mmol) and DIPEA (30.7 mL, 171 mmol) at 0 °C. The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was washed with water (2 x 100 mL). Organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give crude; which was purified by silica gel column chromatography eluting with 2% MeOH/CH₂Cl₂. After compound was triturated with ether (25 mL) and the precipitated solid was filtered to afford **2S-N** (2 g, 20%) as white solid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.52 (br s, 1H), 7.17 (br s, 1H), 3.46 (d, *J* = 6.5 Hz, 2H), 1.38 (s, 9H);

Synthesis of (E)-tert-butyl (2-(((dimethylamino) methylene) amino)-2-oxoethyl) carbamate (2S-O):

[00133] To a stirring solution of **2S-N** (7 g, 40.22 mmol) in THF (70 mL) was added DMF.DMA (10.7 mL, 80.44 mmol) at RT and heated to 80 °C for 2 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to afford **2S-O** (9 g, crude) as brown syrup. This crude material was directly taken for the next step without further purification.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 6.72 (br s, 1H), 4.35 (s, 1H), 3.64 (d, *J* = 5.5 Hz, 2H), 3.09 (s, 1H), 1.42 (s, 9H);

Mass (ESI): *m/z* 230.2 [M⁺+1];

Synthesis of tert-butyl ((1, 2, 4-oxadiazol-5-yl) methyl) carbamate (2S-P):

[00134] To a stirring solution of **2S-O** (9 g (crude), 39.30 mmol) in ethanol (80 mL) was added hydroxylamine hydrochloride (5.45 g, 78.60 mmol) under N₂ atmosphere. The reaction mixture was heated to 90 °C and stirred for 2 h. After consumption of the starting material (by TLC) evaporated solvent under reduced pressure and crude residue was diluted with water (75 mL). The aqueous layer was extracted by DCM (3 x 100 mL). The combined organic layer was

- 52 -

washed by brine solution (1 x 100 mL). The organic layer was dried over anhydrous Na₂SO₄ and solvent was concentrated under reduced pressure to give crude; which was purified by silica gel column chromatography eluting with 25% EtOAc/hexane to afford **2S-P** (4 g, 51%).

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 8.90 (s, 1H), 7.64 (s, 1H), 4.44 (s, 2H), 1.39 (s, 9H);

5 LCMS *m/z*: 198.4 [M⁻-1]

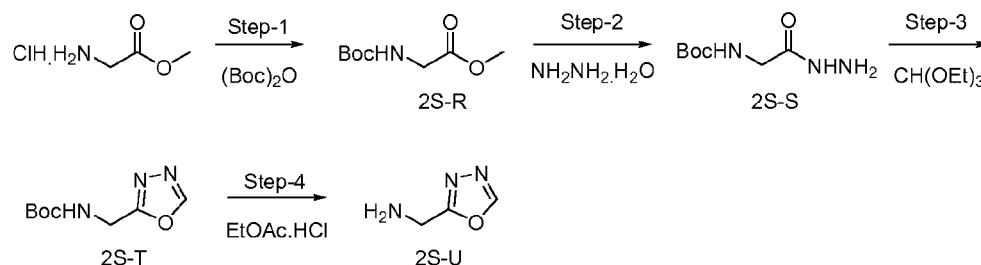
Synthesis of (1, 2, 4-oxadiazol-5-yl) methanamine (2S-Q):

[00135] To a stirring solution of **2S-P** (1.1 g, 5.52 mmol) in DCM (30 mL) was added trifluoroacetic acid (2.1 mL, 27.63 mmol) at 0 °C for 30 min. The reaction mixture was stirred at RT for 4 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under vacuum. The crude residue was triturated with ether (20 mL) to afford **2S-Q** (850 mg, 72.6%) as white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 9.13 (s, 1H), 8.90 (br s, 2H), 4.56 (s, 2H);

LCMS (ESI): 100.4 [M⁺+1]

Scheme 2S-I-4



Synthesis of methyl (tert-butoxycarbonyl) glycinate (2S-R):

[00136] To a stirring solution of glycine methyl ester hydrochloride (50 g, 400 mmol) in 1,4 dioxane/water (300 mL/200 mL) were added Na₂CO₃ (84.8 g, 800 mmol) and stirred at RT for 10 min. The reaction mixture was cooled to 0 °C and Boc-anhydride (104 mL, 480 mmol) was added drop wise and the stirring was continued at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure and obtained residue was diluted with water (100 mL) and extracted with EtOAc (2 x 250 mL). The combined organic layer was washed with brine (1 x 200 mL) and organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford **2S-R** (64 g, 84%) as thick syrup.

- 53 -

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.19 (t, *J* = 5.5 Hz, 1H), 3.67 (d, *J* = 6.0 Hz, 2H), 3.62 (s, 3H), 1.38 (s, 9H);

LCMS *m/z*: 190.2 [*M*⁺+1]

Synthesis of tert-butyl (2-hydrazinyl-2-oxoethyl) carbamate (2S-S):

5 [00137] A solution of **2S-R** (20 g, 105 mmol) in EtOH (100 mL) was added hydrazine hydrate (15.8 g, 315 mmol) at RT and after stirred at 100 °C for 6 h. After consumption of the starting material (by TLC), ethanol was evaporated under reduced pressure. Obtained crude material was triturated with *n*-pentane/diethyl ether (20 mL/20 mL) to afford **2S-S** as white solid.

10 ¹H-NMR: (400 MHz, DMSO-*d*₆): δ 8.91 (s, 1H), 6.88 (t, *J* = 5.5 Hz, 1H), 4.16 (s, 2H), 3.47 (d, *J* = 6.0 Hz, 2H), 1.37 (s, 9H);

LCMS *m/z*: 190.2 [*M*⁺+1]

Synthesis of tert-butyl ((1, 3, 4-oxadiazol-2-yl) methyl) carbamate (2S-T):

15 [00138] A solution of **2S-S** (14 g, 74 mmol) in triethyl orthoformate (140 mL) was added *p*-TSA (catalytic, 140 mg) at RT and after stirred at 80 °C for 4 h. After consumption of starting material (by TLC), triethyl orthoformate was evaporated under reduced pressure. The crude residue was purified by column chromatography eluting 20% EtOAc/hexane to afford **2S-T** (6.1 g, 41.5%) as an off-white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 10.74 (s, 1H), 7.45 (s, 1H), 4.03 (s, 2H), 1.47 (s, 9H);

20 LCMS *m/z*: 200.2 [*M*⁺+1]

Synthesis of (1, 3, 4-oxadiazol-2-yl) methanamine (2S-U):

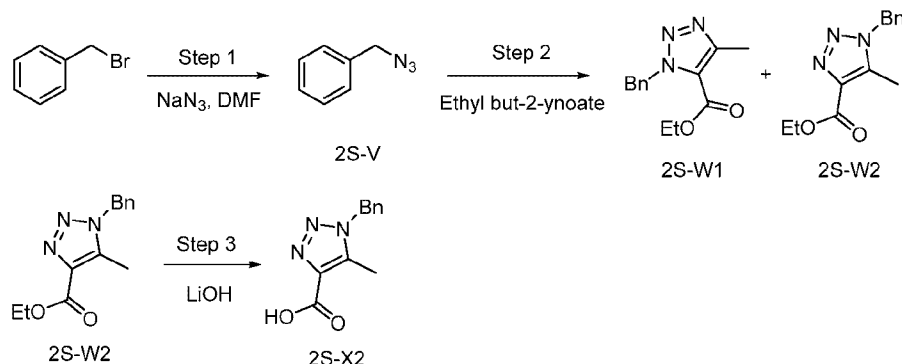
25 [00139] To a stirring solution of **2S-T** (5 g, 25 mmol) in EtOAc (10 mL) was added EtOAc saturated with HCl (60 mL) at 0 °C and stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure. The crude material was triturated with diethylether/*n*-pentane (25 mL/25 mL) and dried under reduced pressure to afford **2S-U** (3 g, 88.7%) as an off-white solid (HCl salt).

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 9.55 (br s, 2H), 7.99 (s, 1H), 3.90 (s, 2H);

LCMS *m/z*: 100 [*M*⁺+1]

Scheme 2S-I-5

- 54 -



Synthesis of (azidomethyl) benzene (2S-V):

[00140] To a stirring solution of benzyl bromide (30 g, 175 mmol) in dimethyl formamide (300 mL) was added sodium azide (45.6 g, 701 mmol) at RT under inert atmosphere. The resultant reaction mixture was stirred at 70 °C for 16 h. After completion of reaction monitored (by TLC), the reaction mixture was allowed to RT; the volatiles were diluted with water (300 mL) and ether (200 mL). The separated organic layer was washed by (3 x 200 mL) of chilled water. The separated organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford compound **2S-V** (18 g, crude) as an off-white solid.

¹H-NMR: (400 MHz, CDCl₃): δ 7.40-7.29 (m, 5H), 4.32 (s, 2H).

Synthesis of ethyl 1-benzyl-5-methyl-1H-1, 2, 3-triazole-4-carboxylate (2S-W2)

[00141] To a stirring solution of ethyl but-2-ynoate (8.0 g, 71.3 mmol) in toluene (80 mL) was added **2S-V** (12.0 g, 107 mmol) at RT under inert atmosphere. The resultant reaction mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was allowed to RT; the volatiles were evaporated under reduced pressure to which, crude residue was purified by column chromatography by eluting 40% EtOAc/hexane to afford **2S-W1** and **2S-W2** (8.2 g, 47.1%) (separable by column chromatography)

¹H-NMR: (400 MHz, CDCl₃): δ 7.36-7.31 (m, 3H), 7.16 (t, *J* = 6.0 Hz, 2H), 5.53 (s, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 2.45 (s, 3H), 1.41 (t, *J* = 7.2 Hz, 3H);

Mass *m/z*: 246.3 [*M*⁺+1]

Synthesis of 1-benzyl-5-methyl-1H-1, 2, 3-triazole-4-carboxylic acid (2S-X2)

[00142] To a stirring solution of compound **2S-W2** (8.2 g, 33.4 mmol) in THF/H₂O (82 mL/82 mL, 1:1) was added LiOH·H₂O (4.2 g, 0.4 mmol) at RT and stirred for 16 h. After completion of reaction (by TLC), the volatiles were evaporated under reduced pressure. The residue was acidified with aqueous 2*N* HCl and the precipitated solid was filtered and washed

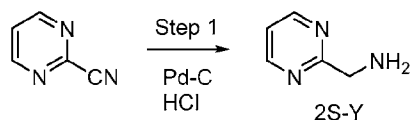
- 55 -

with water (25 mL), dried under reduced pressure to afford compound **2S-X2** (7.0 g, 96.6%) as an off-white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 13.01 (br s, 1H), 7.40-7.32 (m, 5H), 5.63 (s, 2H), 2.45 (s, 3H);

5 **Mass *m/z*:** 218.3 [*M*⁺+1];

Scheme 2S-I-6



Synthesis of pyrimidin-2-ylmethanamine (2S-Y):

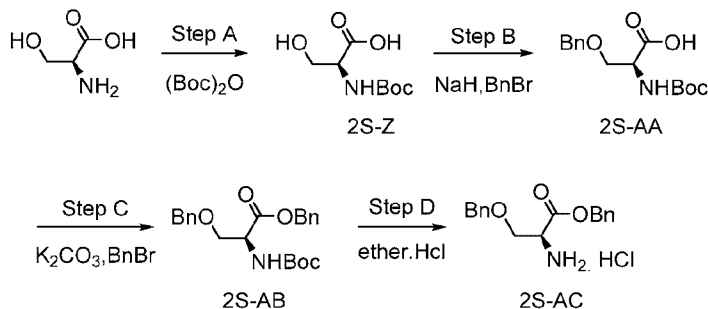
10 **[00143]** To a stirring solution of 2-cyanopyrimidine (2.0 g, 19.0 mmol) in methanol (50 mL) were added 10%Pd/C (300 mg), 12 N HCl (1.5 mL) under N₂ atmosphere. The reaction mixture was stirred under H₂ atmosphere (balloon pressure) at RT for 3 h. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol. Obtained filtrate was concentrated under reduced pressure
15 to afford crude compound which was triturated with diethyl ether to obtained compound **2S-Y** (1.2 g, 44%) as white solid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 8.87 (d, *J* = 5.0 Hz, 2H), 8.69 (br s, 2H), 7.52 (t, *J* = 5.0 Hz, 1H), 4.24 (s, 2H);

Mass (ESI): 110.3 [*M*⁺+1]

20

Scheme 2S-I-7



Synthesis of (S)-2-((tert-butoxycarbonyl) amino)-3-hydroxypropanoic acid (2S-Z)

- 56 -

[00144] To a stirring solution of L-serine (76 g, 723 mmol) in 1, 4 dioxane/H₂O (350 mL/300 mL) were added NaOH (61 g, 1.51mol), Boc-anhydride (190 mL, 868 mmol) at 0 °C. The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was acidified with 2N HCl (pH~4) and extracted with EtOAc (5x500 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford **2S-Z** (100 g, 67.5%) as yellow syrup.

¹H-NMR: (400 MHz, CDCl₃): δ 6.54 (br s, 1H), 5.77 (br s, 1H), 4.35-4.04 (m, 1H), 3.87-3.84 (m, 2H), 1.45 (s, 9H).

Synthesis of (S)-3-(benzyloxy)-2-((tert-butoxycarbonyl) amino) propanoic acid (2S-AA)

10 [00145] To a stirring solution of **2S-Z** (50 g, 245 mmol) in DMF (650 mL) was added NaH (60%) (23 g, 563 mmol) at -15 °C and stirred for 2 h. Benzyl bromide (32.8 mL, 269 mmol) was slowly added. The reaction mixture temperature was warmed to RT and stirred for 12 h. After consumption of the starting material (by TLC), the reaction mixture was poured into chilled water (200 mL) and extracted with diethylether (2x 250 mL). The aqueous layer was acidified with citric acid (pH~4) and extracted with EtOAc (2x500 mL). The combined organic layers were washed with water (3x250 mL). The organic extracts were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford **2S-AA** (54 g, 75%) as brown syrup.

15 ¹H-NMR: (400 MHz, CDCl₃): δ 7.32-7.26 (m, 5H), 5.43 (d, *J* = 7.6 Hz, 1H), 4.70-4.46 (m, 1H), 4.45 (s, 2H), 4.13-3.91 (m, 1H), 3.73-3.70 (m, 1H), 1.44 (s, 9H).

Synthesis of (S)-benzyl 3-(benzyloxy)-2-((tert-butoxycarbonyl) amino) propanoate (2S-AB)

[00146] To a stirring solution of **2S-AA** (36 g, 122 mmol) in DMF (250 mL) was added Na₂CO₃ (20 g, 183 mmol) at 0 °C and added benzyl bromide (18 mL, 146 mmol) slowly. The reaction mixture temperature was warmed to RT and stirred for 12 h. After consumption of the starting material (by TLC), the reaction mixture was poured into chilled water (200 mL) and extracted with diethylether (2x 250 mL). The combined organic layers were washed with water (3x250 mL). The organic extracts were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford **2S-AB** (42 g, 91%) as brown syrup was used directly for next step without any purification.

30 **Synthesis of (S)-benzyl 2-amino-3-(benzyloxy) propanoate hydrochloride (2S-AC):**

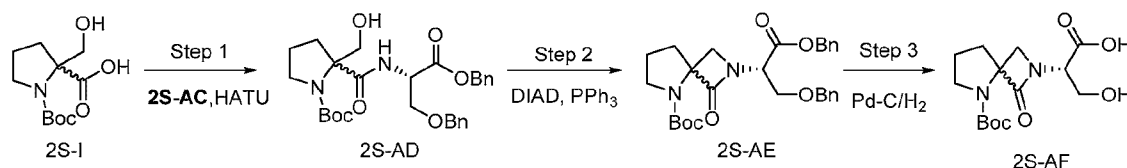
[00147] To a stirring solution of **2S-AB** (10 g, 25.9 mmol) in ether saturated with HCl (50 mL) was added at 0 °C and stirred at RT for 12 h. The obtained precipitate was filtered and

- 57 -

trituated with diethylether (2x100 mL). The filtered compound was dried under vacuum to afford **2S-AC** (5 g, 60%) as white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 8.66 (s, 2H), 7.38-7.27 (m, 10H), 5.29-5.22 (m, 2H), 4.57-4.44 (m, 3H), 3.91-3.81 (m, 2H)

5 **Scheme 2S-I-8**



Synthesis of tert-butyl 2-(((S)-1, 3-bis (benzyloxy)-1-oxopropan-2-yl) carbamoyl)-2-(hydroxymethyl) pyrrolidine-1-carboxylate (2S-AD):

[00148] To a stirring solution of compound **2S-I** (5 g, 20.4 mmol) in CH₂Cl₂ (50 mL) were added DIPEA (10.7 mL, 61.2 mmol), **2S-AC** (5.8 g, 20.4 mmol), HATU (11.6 g, 30.6 mmol) at 0 °C and stirred to RT for 12 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (100 mL) and extracted with CH₂Cl₂ (2 x 100 mL). The combined organic layer was washed with citric acid (1 x 100 mL) followed by brine solution (1 x 100 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 50% EtOAc/*n*-hexane to afford compound **2S-AD** (8 g, 76.5%) as yellow thick syrup.

¹H-NMR: (400 MHz, CD₃OD): δ 7.33-7.24 (m, 10H), 5.23-5.11 (m, 2H), 4.72-4.66 (m, 2H), 4.50-4.44 (m, 1H), 4.18-3.91 (m, 2H), 3.75-3.70 (m, 2H), 3.65-3.40 (m, 2H), 2.34-2.03 (m, 2H), 1.81-1.78 (m, 2H), 1.41 (s, 9H);

Mass (ESI): *m/z* 512.6 [M⁺+1]

Synthesis of tert-butyl 2-(((S)-1, 3-bis (benzyloxy)-1-oxopropan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-AE):

[00149] To a stirring solution of triphenylphosphine (640 mg, 2.44 mmol) in THF (5 mL) was added DIAD (392 mg, 1.94 mmol) at RT and stirred for 15 min, then compound **2S-AD** (500 mg, 0.97 mmol) in (5 mL) THF was slowly added and reaction mixture was stirred at RT for 2 h. After consumption of the starting material (by TLC), the reaction was concentrated

- 58 -

under reduced pressure. The crude material was purified by column chromatography by eluting 2% MeOH/DCM to afford compound **2S-AE** (450 mg, 93%) as yellow liquid.

¹H-NMR: (400 MHz, CD₃OD): δ 7.34-7.27 (m, 10H), 5.25-5.14 (m, 2H), 4.78-4.73 (m, 1H), 4.70-4.42 (m, 2H), 4.04-3.98 (m, 1H), 3.93-3.78 (m, 1H), 3.89-3.78 (m, 1H), 3.76-3.68 (m, 1H), 3.45-3.35 (m, 2H), 2.20-2.09 (m, 2H), 1.90-1.78 (m, 2H), 1.46 (s, 9H)

LCMS (ESI): *m/z* 495.5 [M⁺+1]

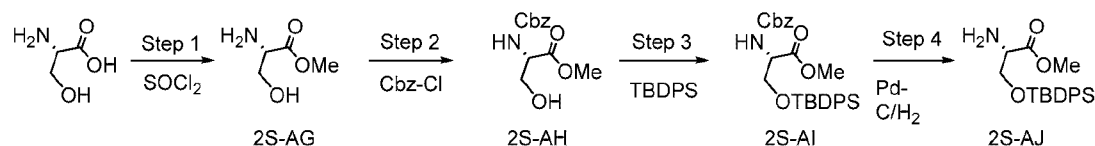
Synthesis of 1-(tert-butoxycarbonyl)-2-(hydroxymethyl) pyrrolidine-2-carboxylic acid (2S-AF):

[00150] To a stirring solution of compound **2S-AE** (500 mg, 1.01 mmol) in methanol (25 mL) was added 50% wet 10% Pd/C (250 mg) at RT and stirred for 24 h under H₂ atmosphere. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol (20 mL). Obtained filtrate was concentrated under reduced pressure to afford compound **2S-AF** (400 mg, crude) as white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 4.92-4.87 (m, 1H), 4.28-4.07 (m, 3H), 3.63-3.60 (m, 1H), 3.55-3.40 (m, 2H), 2.30-2.25 (m, 2H), 1.95-1.87 (m, 2H), 1.47 (s, 9H);

LCMS: 315.3 [M+1]

Scheme 2S-I-9



Synthesis of (S)-methyl 2-amino-3-hydroxypropanoate (2S-AG):

[00151] To a stirring solution of L-serine (40 g, 0.38 mol) in methanol (300 mL) was added SOCl₂ (33.6 mL, 0.45 mol) drop wise at 0 °C and stirred for 1 h. The resulting reaction mixture was refluxed for 24 h. After consumption of the starting material (by TLC), the reaction mixture was warmed to RT and concentrated under vacuum and decanted with *n*-hexane (2 x 200 mL) to afford compound **2S-AG** (59.18 g, crude).

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 8.62 (s, 3H), 4.08 (d, *J* = 3.2 Hz, 1H), 3.83 (d, *J* = 3.6 Hz, 2H), 3.78 (s, 3H);

LCMS, *m/z*: 120.2 [M⁺-1]

- 59 -

Synthesis of (S)-methyl 2-(((benzyloxy)carbonyl)amino)-3-hydroxypropanoate(2S-AH):

[00152] To a stirring solution of compound **2S-AG** (40 g, 0.33 mol) in 1, 4-dioxane (300 mL) and water (100 mL) was added Na₂CO₃ (71.18 g, 0.67 mol) and stirred at RT for 30 min. The reaction mixture was cooled to 0 °C, benzyl chloroformate (68.5 g, 0.40mol) was added drop wise and the stirring was continued at RT for 8 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with EtOAc (200 mL). The aqueous layer was extracted with EtOAc (2 x 200 mL). The separated organic extracts were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 20% EtOAc/*n*-hexane to afford compound **2S-AH** (53 g, 62%);

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.49 (d, *J* = 8 Hz, 1H), 7.37-7.29 (m, 5H), 5.04 (s, 2 H), 4.93 (t, *J* = 6 Hz, 1H), 4.18-4.13 (m, 1H), 3.78 (s, 3H), 3.67-3.56 (m, 2H)

Synthesis of (S)-methyl 2-(((benzyloxy)carbonyl)amino)-3-((tert-butyldiphenylsilyl)oxy)propanoate (2S-AI):

[00153] To a stirring solution of compound **2S-AH** (20 g, 79.20 mmol) in DCM (700 mL) was added imidazole(16g, 237.6mmol) at 0°C followed by TBDPS (25.9 g, 95.04 mmol) under N₂ atmosphere and stirred at RT for 8 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (100 mL) and the aqueous layer was extracted with DCM (2 x 200 mL). The separated organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 20% EtOAc/*n*-hexane to afford compound **2S-AI** (25 g, 64%).

¹H-NMR: (500 MHz, CDCl₃): δ 7.23-7.68 (m, 1H), 7.58 (d, *J* = 7 Hz, 3H), 7.44-7.37 (m, 9H), 7.34 (d, *J* = 7.5 Hz, 2H), 5.65 (d, *J* = 9 Hz, 1H), 5.12 (d, *J* = 2, 2H), 4.45 (d, *J* = 9 Hz, 1H), 4.10-4.07 (m, 1H), 3.91-3.88 (m, 1H), 3.74 (s, 3H), 1.04 (s, 9H);

LCMS (*m/z*): 492.1 [M⁺-1]

Synthesis of (S)-methyl 2-amino-3-((tert-butyldiphenylsilyl)oxy)propanoate (2S-AJ):

[00154] To a stirring solution of compound **2S-AI** (25 g, 51.12 mmol) in ethanol (250 mL) was added 50% wet 10% Pd/C (15 g) at RT and stirred for 8 h under H₂ atmosphere (balloon pressure). After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with ethanol. Obtained filtrate was concentrated under reduced pressure to afford compound **2S-AJ** (18 g, 97%) as yellow liquid.

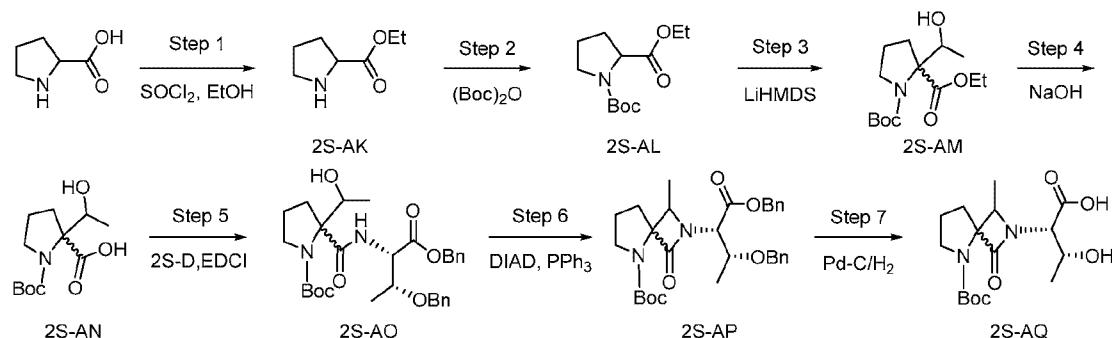
- 60 -

¹H-NMR: (400 MHz, CDCl₃): δ 7.66-7.61 (m, 4H), 7.43-7.36 (m, 6H), 4.00-3.97 (m, 2H), 3.74(s, 3H), 3.64 (t, *J* = 4 Hz, 1H), 2.65 (s, 2H), 1.04 (s, 9H);

LCMS *m/z*: 358 [M⁺-1]

Scheme 2S-I-10

5



Synthesis of ethyl pyrrolidine-2-carboxylate hydrochloride (2S-AK):

[00155] To a stirring solution of L-proline (110 g, 956.5 mmol) in ethanol was added thionyl chloride (141 ml, 1911.3 mmol) and refluxed for 16 h. The reaction mixture was brought to RT and concentrated under vacuum to afford compound **2S-AK** as the hydrochloride salt (170 g, 99 %).

¹H-NMR: (400 MHz, CDCl₃): δ 4.15-4.10 (m, 2H), 3.68-3.62 (m, 2H), 3.59-3.47 (m, 2H), 2.49-2.37 (m, 1H), 2.27-2.05 (m, 3H), 1.18 (t, *J* = 3.6 Hz, 3H);

LCMS, *m/z*: 143 [M⁺+1]

Synthesis of 1-tert-butyl 2-ethyl pyrrolidine-1, 2-dicarboxylate (2S-AL):

[00156] To a stirring solution of compound **2S-AK** (70 g, 0.391 mol) in CH₂Cl₂ (700 mL) were added Et₃N (170.7 mL, 1.22 mol) followed by Boc-anhydride (133 g, 0.61 mol) at 0 °C. The reaction mixture was stirred at RT for 12 h. After consumption of the starting material (by TLC), the reaction was diluted with water (100 mL) and extracted with CH₂Cl₂ (2x 200 mL). The organic layer was washed with water (1 x 150 mL), brine (1 x 200 mL), dried over Na₂SO₄ and concentrated under reduced pressure to afford compound **2S-AL** (90 g, 90%) as thick syrup.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 4.15-4.10 (m, 2H), 4.09-4.02 (m, 1H), 3.36-3.29 (m, 2H), 2.25-2.13 (m, 1H), 1.87-1.76 (m, 3H), 1.40 (s, 9H), 1.18 (t, *J* = 3.6 Hz, 3H);

LCMS, *m/z*: 144 [(M⁺+1)-Boc];

- 61 -

HPLC: 96.11%

Synthesis of 1-tert-butyl 2-ethyl 2-(1-hydroxyethyl) pyrrolidine-1, 2-dicarboxylate (2S-AM):

[00157] To a stirring solution of compound **2S-AL** (5 g, 20.5 mmol) in THF (50 mL) was added LiHMDS (20.3 mL, 20.5 mmol) at -20 °C and stirred for 1 h. To this acetaldehyde (1.2 mL, 20.5 mmol) was added dropwise at -20 °C and stirred for 1 h at -20 °C. After consumption of the starting material (by TLC), the reaction was quenched with aqueous NH₄Cl solution and extracted with EtOAc (1 x 50 mL). The separated organic layer was dried over Na₂SO₄ and concentrated to afford crude compound was purified by column chromatography eluting 10% EtOAc/hexane to afford compound **2S-AM** (1.8 g, 30%) as pale yellow syrup.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 5.10 (d, *J* = 8.5 Hz, 1H), 4.54-4.36 (m, 2H), 4.05-3.99 (m, 2H), 3.60-3.49 (m, 1H), 1.97-1.74 (m, 4H), 1.40 (s, 9H), 1.18, 1.15 (dd, *J* = 7.5 Hz, 6.5 Hz, 3H), 0.96 (d, *J* = 9.5 Hz, 3H);

LCMS, *m/z*: 188 [(M⁺+1)-Boc]

Synthesis of 1-(tert-butoxycarbonyl)-2-(1-hydroxyethyl) pyrrolidine-2-carboxylic acid (2S-AN):

[00158] To a stirring solution of compound **2S-AM** (10 g, 34.8 mmol) in methanol (30 mL) were added NaOH (2.7 g, 69.6 mmol), H₂O/THF (30 mL/30 mL) at 0 °C. The reaction mixture was heated to 80 °C for 5 h. After consumption of the starting material (by TLC), the solvent was evaporated under reduced pressure. The aqueous layer was acidified using citric acid solution and extracted with EtOAc (2x 100 mL). The separated organic layer was washed with water (1 x 50 mL), dried over Na₂SO₄ and concentrated to afford compound **2S-AN** (4.8 g, 53.3%) as brown sticky solid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 4.60-4.54 (m, 1H), 3.98 (d, *J* = 10.0 Hz, 1H), 3.90-3.77 (m, 2H), 3.44-3.34 (m, 1H), 2.01-1.68 (m, 4H), 1.40 (s, 9H), 1.26 (d, *J* = 10.0 Hz, 3H);

LCMS, *m/z*: 258 (M⁺-1);

HPLC (purity): 91.7%

Synthesis of tert-butyl 2-(((2S,3R)-1,3-bis(benzyloxy)-1-oxobutan-2-yl)carbamoyl)-2-(1-hydroxyethyl)pyrrolidine-1-carboxylate (2S-AO):

[00159] To a stirring solution of compound **2S-AN** (2.0 g, 7.72 mmol) in CH₂Cl₂ (50 mL) were added DIPEA (4.2 mL, 22.4 mmol), EDCI.HCl (2.2 g, 11.5 mmol) followed by HOBt (1.5 g, 11.5 mmol), compound D (2.8 g, 8.35 mmol) at 0 °C and stirred for 12 h. After

- 62 -

consumption of the starting material (by TLC), the reaction mixture was diluted with water (30 mL) and extracted with CH₂Cl₂ (2 x 50 mL). The combined organic layer was washed with brine (2 x 50 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 25% EtOAc/*n*-hexane to afford compound **2S-AO** (1.5 g, 36%) as colorless liquid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 8.47 (t, *J* = 8.8 Hz, 1H), 7.31-7.19 (m, 10H), 5.73-5.58 (m, 1H), 5.18 (s, 2H), 4.64 (s, 2H), 4.60-4.49 (m, 1H), 4.29 (d, *J* = 12.0 Hz, 1H), 4.15-4.12 (m, 1H), 3.59-3.59 (m, 1H), 3.24-3.13 (m, 1H), 1.71-1.60 (m, 2H), 1.43-1.38 (m, 2H), 1.35 (s, 9H), 1.18 (d, *J* = 6.0 Hz, 3H), 1.04 (d, *J* = 6.4 Hz, 3H);

Mass (ESI): *m/z* 540 [M⁻¹]

Synthesis of *tert*-butyl 2-((2*S*,3*R*)-1,3-bis(benzyloxy)-1-oxobutan-2-yl)-1-methyl-3-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-AP):

[00160] To a stirring solution of triphenylphosphine (1.45 g, 5.53 mmol) in THF (30 mL) was added DIAD (1.12 g, 5.53 mmol) at RT and stirred for 30 min. To this added compound **2S-AO** (1.5 g, 2.77 mmol) in (10 mL) THF slowly and reaction mixture was stirred at RT for 2 h. After consumption of the starting material (by TLC), the reaction was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting 20% EtOAc/hexane to afford compound **2S-AP** (800 mg, 57%) as pale yellow syrup.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.33-7.18 (m, 10H), 5.07 (s, 2H), 4.61 (s, 2H), 4.38-4.31 (m, 1H), 3.77-3.75 (m, 1H), 3.28-3.24 (m, 1H), 2.67-2.66 (m, 1H), 2.22-2.12 (m, 1H), 1.98-1.92 (m, 3H), 1.72-1.60 (m, 1H), 1.40 (s, 9H), 1.18 (d, *J* = 5.6 Hz, 3H), 1.13 (d, *J* = 6.4 Hz, 3H)

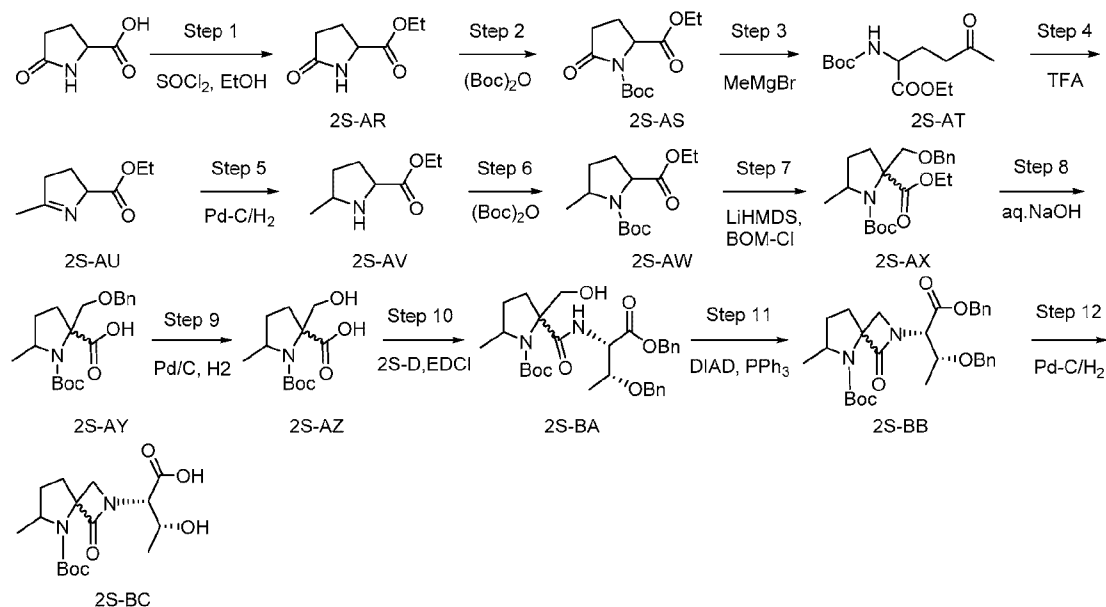
Mass (ESI): *m/z* 523 [M⁺+1]

Synthesis of (2*S*,3*R*)-2-(5-(*tert*-butoxycarbonyl)-1-methyl-3-oxo-2,5-diazaspiro[3.4]octan-2-yl)-3-hydroxybutanoic acid (2S-AQ):

[00161] To a stirring solution of compound **2S-AP** (900mg) in methanol (30 mL) was added 10% Pd/C (300 mg) at RT and stirred for 16 h under H₂ atmosphere (balloon pressure). After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and washed with methanol (10 mL). Obtained filtrate was concentrated under reduced pressure to afford compound **2S-AQ** (480 mg, 82%) as yellow thick syrup.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 12.80 (br s, 1H), 5.11-4.96 (m, 1H), 4.83-4.04 (m, 3H), 3.40-3.35 (m, 1H), 2.11 (s, 3H), 2.10-2.03 (m, 2H), 1.46 (s, 9H), 1.43-1.39 (m, 6H).

- 63 -

LCMS: 342 [M⁻-1]**Scheme 2S-I-11**

5

Synthesis of ethyl 5-oxopyrrolidine-2-carboxylate (2S-AR):

[00162] To a stirring solution of 5-oxopyrrolidine-2-carboxylic acid (10 g, 77.4 mmol) in ethanol (100 mL) was added thionyl chloride (6.7 mL, 92.9 mmol) at 0 °C. The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the solvents from the reaction mixture were removed under vacuum. The residue was diluted with EtOAc (50 mL) and stirred over K₂CO₃. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography to afford compound **2S-AR** (9 g, 74%).

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.98 (br s, 1H), 4.16 (t, 3H), 2.37-2.30 (m, 1H), 2.15 (q, 2H), 2.03-1.97 (m, 1H), 1.22 (t, 3H);

LCMS, *m/z*: 157.9 [M⁺+1]

Synthesis of 1-tert-butyl 2-ethyl 5-oxopyrrolidine-1,2-dicarboxylate (2S-AS):

[00163] To a stirring solution of compound **2S-AR** (9 g, 57.3 mmol) in CH₂Cl₂ (90 mL) was added DMAP (7.0 g, 57.3 mmol) followed by Et₃N (15.9 mL, 114.6 mmol) and Boc-anhydride (36.7 mL, 171.9 mmol) at 0 °C. The reaction mixture was stirred at RT for 16 h. The reaction mixture was diluted with CH₂Cl₂ (50 mL) and washed with aqueous 1N HCl solution

20

- 64 -

followed by brine. The separated organic layer was dried over anhydrous Na₂SO₄ and concentrated under vacuum. Obtained crude material was purified by column chromatography eluting with 50% EtOAc/Hexane to afford compound 2S-AS (12 g, 82%).

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 4.61 (dd, 1H), 4.19 (q, 2H), 2.46-2.40 (m, 2H), 2.37-2.25 (m, 1H), 1.91-1.85 (m, 1H), 1.42 (s, 9H), 1.22 (t, 3H).

Synthesis of ethyl 2-((tert-butoxycarbonyl) amino)-5-oxohexanoate (2S-AT):

[00164] To a stirring solution of compound 2S-AS (12 g, 46.6 mmol) in THF (120 mL) under inert atmosphere was added MeMgBr (3M in ether) (20.2 mL, 60.6 mmol) at 0 °C and stirred for 2 h. After consumption of the starting material (by TLC), the reaction mixture was quenched with aqueous NH₄Cl solution and the aqueous layer was extracted with EtOAc (2 x 200 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude residue obtained was purified by silica gel column chromatography eluting with 20% EtOAc/Hexane to afford compound 2S-AT (10 g, 79%).

¹H-NMR: (400 MHz, CDCl₃): δ 5.14 (br s, 1H), 4.23 (q, 2H), 2.62-2.47 (m, 2H), 2.17 (s, 4H), 1.91-1.82 (m, 1H), 1.45 (s, 10H), 1.26 (t, 3H).

Synthesis of ethyl 5-methylpyrrolidine-2-carboxylate (2S-AU & 2S-AV):

[00165] To a stirring solution of compound 2S-AT (10 g, 36.7mmol) in CH₂Cl₂ (100 mL) was added TFA (14.89 mL, 194.6 mmol) at 0°C. After being stirred for 2 h at RT, the reaction mixture was concentrated under reduced pressure to get compound 2S-AU. Obtained material was dissolved in ethanol (100 mL) and 10% Pd/C (50% wet, 3 g) under N₂ atmosphere. The reaction mixture was stirred under H₂ atmosphere (balloon pressure) for 16 h. The reaction mixture was filtered through a pad of celite and filtrate was concentrated under reduced pressure to afford compound 2S-AV (15 g, crude). This material was directly taken for the next step without further purification.

25 LCMS, *m/z*: 158.1 [M⁺+1]

Synthesis of 1-tert-butyl 2-ethyl 5-methylpyrrolidine-1,2-dicarboxylate (2S-AW):

[00166] To a stirring solution of compound 2S-AV (30 g, 191 mmol) in CH₂Cl₂ (150 mL) was added DMAP (23.3 g, 191 mmol) followed by Et₃N (79.8 mL, 573 mmol) and Boc-anhydride (104 mL, 477 mmol) at 0 °C. The reaction mixture was stirred at RT for 16 h. The reaction mixture was diluted with CH₂Cl₂ (50 mL) and washed with water (2x150 mL) followed by brine. The separated organic layer was dried over anhydrous Na₂SO₄ and concentrated under vacuum. Obtained crude material was purified by column chromatography

- 65 -

eluting with 6% EtOAc/hexane to afford compound **2S-AW** (30 g, 61.22%) as pale yellow liquid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 4.13-3.86 (m, 4H), 2.15 (d, *J* = 3.5 Hz, 1H), 1.99-1.82 (m, 2H), 1.52 (t, *J* = 4.5 Hz, 1H), 1.38 (s, 9H), 1.24 (t, *J* = 5.5 Hz, 3H), 1.16 (d, *J* = 6.5 Hz, 3H).

5 LCMS, *m/z*: 258 [(M⁺+1)]

Synthesis of 1-tert-butyl 2-ethyl 2-((benzyloxy) methyl)-5-methylpyrrolidine-1, 2-dicarboxylate (2S-AX):

[00167] To a stirring solution of compound **2S-AW** (8.0 g, 31.12 mmol) in THF (70 mL) was added LiHMDS (59 mL, 41.72 mmol) at -78 °C and stirred for 2 h. To this BOM-chloride
10 (6.56 mL, 41.72 mmol) was added dropwise and stirred for 2 h at -30 °C. After consumption of the starting material (by TLC), the reaction was quenched with aqueous NH₄Cl solution (20 mL) and extracted with DCM (30 mL). The separated organic layer was dried over Na₂SO₄ and concentrated to afford crude material was purified by column chromatography eluting with 10% EtOAc/Hexane to afford compound **2S-AX** (11 g, 94.2%) as pale yellow liquid.

15 **¹H-NMR:** (500 MHz, DMSO-*d*₆): δ 7.33-7.25 (m, 5H), 4.38 (d, *J* = 10.5 Hz, 2H), 4.08-3.98 (m, 1H), 3.88 (d, *J* = 9.5 Hz, 2H), 2.20-2.08 (m, 2H), 1.38 (s, 9H), 1.37-1.29 (m, 4H), 1.19 (t, *J* = 7.5 Hz, 3H), 1.14-1.10 (m, 3H);

LCMS, *m/z*: 378 (M⁺+1)

20 **Synthesis of 2-((benzyloxy) methyl)-1-(tert-butoxycarbonyl)-5-methylpyrrolidine-2-carboxylic acid (2S-AY):**

[00168] To a stirring solution of compound **2S-AX** (11 g, 29.17 mmol) in CH₃OH/THF (22 mL/20 mL) were added 2N NaOH solution (33 mL) at RT. The reaction mixture was heated to 65 °C for 8 h. After consumption of the starting material (by TLC), the solvent from the reaction was evaporated under reduced pressure and diluted with EtOAc (50 mL). The
25 aqueous layer was acidified using citric acid solution and extracted with CH₂Cl₂ (2x 100 mL). The separated organic layer was washed with water (1 x 50 mL), dried over Na₂SO₄ and concentrated to afford compound **2S-AY** (8 g, 80%).

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 12.58 (s, 1H), 7.34-7.28 (m, 5H), 4.54-4.47 (m, 2H), 4.05-3.87 (m, 2H), 3.70-3.62 (m, 1H), 2.28-2.08 (m, 3H), 1.46-1.37 (m, 1H), 1.28 (s, 9H);

30 LCMS, *m/z*: 350 [M⁺+1].

Synthesis of 1-(tert-butoxycarbonyl)-2-(hydroxymethyl)-5-methylpyrrolidine-2-carboxylic acid (2S-AZ):

- 66 -

[00169] To a stirring solution of compound **2S-AY** (8 g, 1.45 mmol) in methanol (40 mL) was added 10%Pd/C (4 g) under N₂ atmosphere. The reaction mixture was stirred under H₂ atmosphere (balloon pressure) at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol. Obtained filtrate was concentrated under reduced pressure to afford crude compound which was triturated with n-pentane to obtained compound **2S-AZ** (4.5 g, 75.2%) as white solid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 12.37 (br s, 1H), 4.61 (br s, 1H), 3.95-3.85 (m, 3H), 2.18-2.06 (m, 3H), 1.44-1.41 (m, 1H), 1.38 (s, 9H), 1.09 (d, *J* = 6.0 Hz, 3H);

10 LCMS (ESI): *m/z* 260 [M⁺+1]

Synthesis of tert-butyl 2-(((2*S*, 3*R*)-1, 3-bis (benzyloxy)-1-oxobutan-2-yl) carbamoyl)-2-(hydroxymethyl)-5-methylpyrrolidine-1-carboxylate (2S-BA):

[00170] To a stirring solution of compound **2S-AZ** (3 g, 11.58 mmol) in DCM (30 mL) were added *N, N*-diisopropylethylamine (6 mL, 34.7 mmol), Int D (5 g, 13.8 mmol), followed by EDCI (2.7 g, 13.8 mmol), HOBT (1.9 g, 13.8 mmol) at 0 °C and stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (20 mL). The separated organic layer was washed with saturated NaHCO₃ solution (1x50 mL), 2N HCl solution (30 mL) followed by brine solution (1x40 mL). The separated organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by column chromatography to obtained compound **2S-BA** (2 g, 32.5%) as pale yellow liquid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.30-7.17 (m, 10H), 5.16-5.10 (m, 2H), 4.50 (t, *J* = 5.2 Hz, 2H), 4.28 (t, *J* = 12.0 Hz, 1H), 4.13-4.07 (m, 1H), 3.95 (s, 2H), 2.10-1.85 (m, 4H), 1.40-1.35 (m, 1H), 1.30 (s, 9H), 1.18, 1.16 (dd, *J* = 6.4 Hz, 6H);

25 LCMS (ESI): *m/z* 440.3 [M⁺+1]

Synthesis of tert-butyl 2-((2*S*, 3*R*)-1, 3-bis(benzyloxy)-1-oxobutan-2-yl)-6-methyl-1-oxo-2,5-diazaspiro [3.4] octane-5-carboxylate (2S-BB):

[00171] To a stirring solution of compound **2S-BA** (1.0 g, 1.85 mmol) in THF (10 mL) was added triphenylphosphine (0.935 g, 4.62 mmol) and DIAD (0.75 g, 3.70 mmol). The reaction mixture was stirred at RT for 8 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure. The crude material was

30

- 67 -

purified by silica gel column chromatography eluting 20% EtOAc/hexane to afford compound **2S-BB** (0.8 g, 51%) as yellow liquid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.30-7.17 (m, 10H), 5.17(s, 2H), 4.79, 4.76 (dd, *J* = 6.0 Hz, 2H), 4.73 (s, 2H), 4.31-4.18 (m, 2H), 3.84 (t, *J* = 6.8 Hz, 2H), 2.12 (t, *J* = 6.8 Hz, 1H),
 1.98-1.91 (m, 2H), 1.39 (s, 9H), 1.32, 1.25 (dd, *J* = 6.0 Hz, 6.4 Hz, 3H), 1.18, 1.09 (dd, *J* = 6.0 Hz, 6.4 Hz, 3H);

LCMS (ESI) : *m/z* 523.3 [*M*⁺+1]

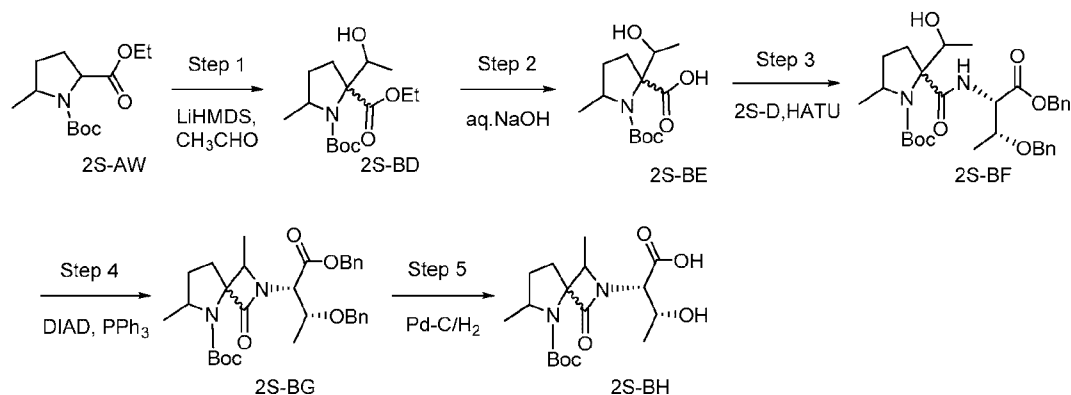
Synthesis of (2*S*, 3*R*)-2-(5-(tert-butoxycarbonyl)-6-methyl-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-hydroxybutanoic acid (2S-BC):

[00172] To a stirring solution of compound **2S-BB** (1 g, 1.91 mmol) in methanol (20 mL) was added 10%Pd/C (400 mg) under N₂ atmosphere. The reaction mixture was stirred under H₂ atmosphere (balloon pressure) at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was
 washed with methanol. Obtained filtrate was concentrated under reduced pressure to afford compound **2S-BC** (0.80 g, crude) as white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 12.75 (br s, 1H), 4.80-4.73 (m, 3H), 4.20-4.05 (m, 2H), 3.40, 3.36 (dd, *J* = 6.8 Hz, 1H), 2.21-2.13 (m, 1H), 2.06-1.99 (m, 2H), 1.53 (t, *J* = 6.0 Hz, 1H), 1.40 (s, 9H), 1.11, 1.10 (dd, *J* = 4.8 Hz, 5.2 Hz, 6H);

LCMS (ESI) : *m/z* 343.3 [*M*⁺+1]

Scheme 2S-I-12



Synthesis of 1-tert-butyl 2-ethyl 2-(1-hydroxyethyl)-5-methylpyrrolidine-1, 2-dicarboxylate (2S-BD):

- 68 -

[00173] To a stirring solution of compound **2S-AW** (20 g, 77.8 mmol) in THF (200 mL) was added LiHMDS (84 mL, 155 mmol) dropwise at -20 °C and stirred for 30 min. To this acetaldehyde (8.77 mL, 155mmol) was added drop wise and stirred at RT for 3h. After consumption of the starting material (by TLC), the reaction was quenched with aqueous NH₄Cl solution (100 mL) and extracted with DCM (2 x 150 mL). The combined organic layer was washed with brine solution (1 x 150 mL). The separated organic layer was dried over Na₂SO₄ and concentrated to afford crude material was purified by column chromatography eluting with 30% EtOAc/Hexane to afford compound **2S-BD** (16 g, 23.1%) as colorless syrup.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.33-7.25 (m, 5H), 4.38 (d, *J* = 10.5 Hz, 2H), 4.08-3.98 (m, 1H), 3.88 (d, *J* = 9.5 Hz, 2H), 2.20-2.08 (m, 2H), 1.38 (s, 9H), 1.37-1.29 (m, 4H), 1.19 (t, *J* = 7.5 Hz, 3H), 1.14-1.10 (m, 3H);

LCMS *m/z*: 378 (M⁺+1)

Synthesis of 1-(tert-butoxycarbonyl)-2-(1-hydroxyethyl)-5-methylpyrrolidine-2-carboxylic acid (2S-BE):

[00174] To a stirring solution of compound **2S-BD** (15 g, 49 mmol) in EtOH/THF (10 mL/20 mL) were added NaOH (3.98 g, 99 mmol) in water (10 mL) at RT. The reaction mixture was heated to 90 °C for 4 h. After consumption of the starting material (by TLC), the solvent from the reaction was evaporated under reduced pressure and acidified by using citric acid (pH~4). The aqueous layer was extracted with DCM (2 x 200 mL) and the combined organic layer was washed with brine solution (1 x 150 mL). The separated organic layer was dried over Na₂SO₄ and concentrated to obtained crude compound, which was purified by column chromatography eluting 40% EtOAc/*n*-hexane to afford compound **2S-BE** (8.2 g, 60.7%) as brown syrup.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 12.15 (br s, 2H), 4.54-4.50 (m, 1H), 4.03-4.02 (m, 1H), 2.17-1.77 (m, 3H), 1.41(s, 9H), 1.39-1.09 (m, 3H), 0.99-0.94 (m, 3H);

LCMS *m/z*: 272.4 [M⁻-1]

Synthesis of tert-butyl 2-(((2S,3R)-1,3-bis(benzyloxy)-1-oxobutan-2-yl)carbamoyl)-2-(1-hydroxyethyl)-5-methylpyrrolidine-1-carboxylate (2S-BF):

[00175] To a stirring solution of compound **2S-BE** (8 g, 29.3 mmol) in DCM (100 mL) were added *N,N*-diisopropylethylamine (15.12 mL, 87 mmol), 2S-D (12.13 g, 40.6 mmol) followed by HATU (16.5g, 43.5 mmol) at 0 °C and stirred at RT for 12 h. After consumption of

- 69 -

the starting material (by TLC), the reaction mixture was diluted with water (100 mL). The separated organic layer was washed with citric acid solution (1 x 75 mL) followed by brine solution (1x100 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting with 40% EtOAc/*n*-hexane to obtain compound **2S-BF** (11g, 68.4%) as pale yellow liquid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.31-7.19 (m, 10H), 5.14-5.06 (m, 2H), 4.59-4.48 (m, 3H), 4.31-4.26 (m, 1H), 4.05-4.00 (m, 2H), 1.98-1.89 (m, 2H), 1.41 (s, 9H), 1.39-1.35 (m, 3H), 1.28-1.17 (m, 6H), 1.16-1.00 (m, 3H);

10 LCMS (ESI): 555.6 [M⁺+1]

Synthesis of tert-butyl 2-((2*S*,3*R*)-1,3-bis(benzyloxy)-1-oxobutan-2-yl)-1,6-dimethyl-3-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-BG):

[00176] To a stirring solution of triphenylphosphine (3.5 g, 13.5 mmol) in THF (10 mL) was added DIAD (2.72 g, 13.5 mmol) as portion-wise and stirred for 20 min at RT. To this added compound **2S-BF** (3 g, 5.4 mmol) in THF (10 mL) slowly at RT and stirred for 3 h. After consumption of the starting material (by LCMS), the reaction mixture was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting 20% EtOAc/hexane to afford compound **2S-BG** (2.5 g, 86.5%) as yellow liquid.

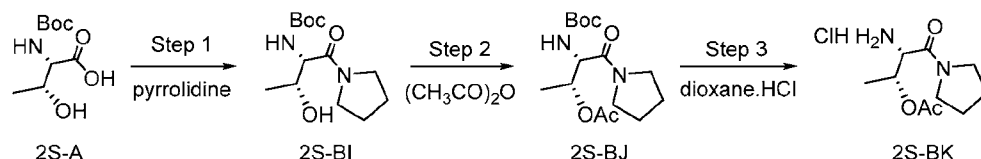
15 ¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.39-7.18 (m, 10H), 5.19-5.10 (m, 2H), 4.78-4.49 (m, 3H), 4.34-4.25 (m, 2H), 3.85-3.76 (m, 1H), 2.10-1.69 (m, 4H), 1.40 (s, 9H), 1.35-1.26 (m, 3H), 1.18-1.12 (m, 6H);

Synthesis of (2*S*,3*R*)-2-(5-(tert-butoxycarbonyl)-1,6-dimethyl-3-oxo-2,5-diazaspiro [3.4]octan-2-yl)-3-hydroxybutanoic acid (2S-BH):

[00177] To a stirring solution of compound **2S-BG** (2 g, 3.72 mmol) in methanol (20 mL) was added 10% dry Pd/C (200 mg) under N₂ atmosphere. The reaction mixture was stirred under H₂ atmosphere at RT for 12 h. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol (10 mL). Obtained filtrate was concentrated under reduced pressure to afford compound **2S-BH** (1.8 g, 60.4%) as yellow solid.

30 ¹H-NMR: (400 MHz, DMSO-*d*₆): δ 12.72 (br s, 1H), 5.11-4.97 (m, 1H), 4.30-4.15 (m, 2H), 3.91-3.76 (m, 2H), 2.18-1.90 (m, 3H), 1.40 (s, 9H), 1.37-1.29 (m, 1H), 1.26-1.22 (m, 3H), 1.21-1.10 (m, 6H);

- 70 -

LCMS (ESI): 357.5 [$M^+ + 1$]**Scheme 2S-I-13**

5 **Synthesis of tert-butyl ((2S, 3R)-3-hydroxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl) carbamate (2S-BI)**

[00178] To a stirring solution of compound 2S-A (13 g, 59.36 mmol) in DMF (65 mL) was added EDCl.HCl (12.5 g, 65.2 mmol) followed by HOBT (8.8 g, 65.2 mmol) at 0 °C. After being stirred for 5 min, DIPEA (30.6 mL, 0.17 mol) followed by pyrrolidine (4.6 g, 65.2 mmol) was added to the reaction mixture and stirring was continued for another 16 h at RT. The reaction mixture was washed with water and extracted with EtOAc (2x 100 mL). The organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under vacuum. The crude was purified by column chromatography to afford compound 2S-BI (5 g, 31%).

¹H-NMR: (400 MHz, CDCl₃): δ 5.51 (br s, 1H), 4.32 (d, 1H), 4.15-4.10 (m, 1H), 3.77-3.74 (m, 1H), 3.55-3.46 (m, 3H), 1.99-1.94 (m, 2H), 1.91-1.85 (m, 2H), 1.47 (s, 9H), 1.26 (t, 1H), 1.29 (d, 3H).

15 **Synthesis of (2R, 3S)-3-((tert-butoxycarbonyl) amino)-4-oxo-4-(pyrrolidin-1-yl) butan-2-yl acetate (2S-BJ and 2S-BK):**

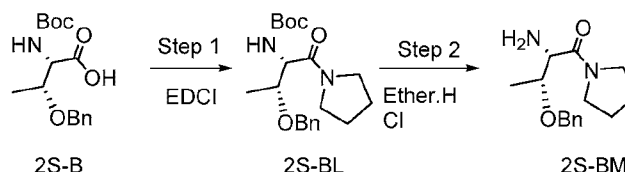
[00179] To a stirring solution of compound 2S-BI (4 g, 14.7 mmol) in CH₂Cl₂ (40 mL) was added Et₃N (5.1 mL, 36.7 mmol) followed by acetic anhydride (1.7 g, 17.6 mmol) and catalytic amount of DMAP at 0 °C. The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water and separated the organic layer. Organic layer was washed with water, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude residue obtained was purified by silica gel column chromatography to give compound 2S-BJ. To this 1, 4-dioxane/HCl (20 mL) was added and stirred at RT for 2 h. The reaction mixture was concentrated under vacuum and obtained material was washed with Et₂O (2x 15 mL) to afford compound 2S-BK (3.5 g, 97%) as HCl salt.

- 71 -

¹H-NMR: (500 MHz, DMSO-*d*₆) (Rotamers): δ 8.49 (br s, 3H), 8.15 (br s, 1H), 5.14-5.10 (m, 1H), 4.26-4.22 (m, 1H), 3.97-3.95 (m, 1H), 3.59 (s, 2H), 2.09 (s, 3H), 1.98 (s, 2H), 1.87-1.80 (m, 2H), 1.26 (d, 3H).

LCMS (ESI): 215.1 [M^+ +1].

5 **Scheme 2S-I-14**



Synthesis of tert-butyl ((2S, 3R)-3-(benzyloxy)-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl) carbamate (2S-BL):

10 **[00180]** To a stirring solution of compound **2S-B** (8 g, 25.8 mmol) in DCM (80 mL) were added *N, N*-diisopropylethylamine (11 mL, 87.4 mmol), pyrrolidine (2.5 mL, 35.4 mmol), followed by EDCI (7.39 g, 38.7 mmol), HOBT (5.2 g, 38.7 mmol) at 0 °C and stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (20 mL). The separated organic layer was washed with saturated NaHCO₃ solution
 15 (1x25 mL), followed by brine solution (1x30 mL). The separated organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by column chromatography eluting 1% MeOH/DCM to obtained compound **2S-BL** (8 g, 86%) as white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.34-7.24 (m, 5H), 6.57 (d, *J* = 4.0 Hz, 1H), 4.53, 4.44 (dd, *J* = 12.0 Hz, 12.0 Hz, 2H), 4.32-4.28 (m, 1H), 3.74 (t, *J* = 6.0 Hz, 1H), 3.58-3.53 (m, 1H), 3.42-3.38 (m, 1H), 3.28-3.24 (m, 2H), 1.82-1.70 (m, 4H), 1.37 (s, 9H), 1.11 (d, *J* = 6.4 Hz, 3H)

Mass (ESI): *m/z* 363.4 [M^+ +1].

Synthesis of (2S, 3R)-2-amino-3-(benzyloxy)-1-(pyrrolidin-1-yl) butan-1-one (2S-BM):

25 **[00181]** To a stirring solution of compound **2S-BL** (6 g, 16.52 mmol) in ether saturated with HCl (30 mL) was added at 0 °C and stirred at RT for 2 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to afford crude compound which was triturated with pentane (15 mL) to obtained compound **2S-BM** (4 g, 93%) as white solid.

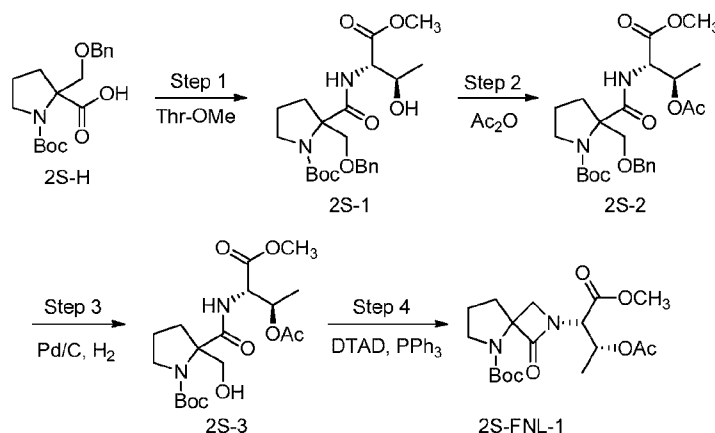
- 72 -

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 8.25 (s, 2H), 7.36-7.29 (m, 5H), 4.58, 4.48 (dd, *J* = 12.0, 11.5 Hz, 2H), 4.12 (t, *J* = 4.5 Hz, 1H), 3.87 (t, *J* = 5.5 Hz, 1H), 3.60-3.57 (m, 1H), 3.37-3.33 (m, 3H), 1.78-1.70 (m, 4H), 1.22 (d, *J* = 6.5 Hz, 3H).

Mass (ESI): 263.3[M⁺+1].

5

Scheme 2S-1



Synthesis of *tert*-Butyl-2-((benzyloxy)methyl)-2-(((2S,3R)-3-hydroxy-1-methoxy-1-oxobutan-2-yl)carbamoyl) pyrrolidine-1-carboxylate (2S-1):

10 **[00182]** To a stirred solution of compound **2S-H** (2.0 g, 5.97 mmol) in CH₂Cl₂ (10 mL) was added DIPEA (2.6 mL, 14.92 mmol) followed by HATU (2.26 g, 5.94 mmol) at 0 °C and stirred for 10 minutes. A solution of methyl 2-amino-3-hydroxybutanoate hydrochloride (1 g, 5.97 mmol) in CH₂Cl₂ (10mL) was added to the reaction mixture at 0 °C. The resultant reaction mixture was allowed to warm to RT and stirring was continued for another 3 h. The volatiles were evaporated under reduced pressure. The obtained residue was diluted with water (25 mL) and extracted with CH₂Cl₂ (2 x 75mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude was purified by silica gel column chromatography eluting with 40%EtOAc/Hexane to afford compound **2S-1** (1.8 g, 67%) as a liquid.

20 **¹H-NMR:** (400 MHz, DMSO-*d*₆): δ 7.38-7.31 (m, 5H), 5.16 (br s, 1H), 4.54 (s, 2H), 4.28-4.26 (m, 1H), 4.17-4.12 (m, 1H), 3.84-3.82 (m, 1H), 3.62 (s, 3H), 3.54-3.51 (m, 1H), 2.32-2.27 (m, 4H), 1.84-1.78 (m, 2H), 1.42 (s, 9H), 1.06 (d, 3H);

LCMS *m/z*: 451.6 [M⁺+1]

Synthesis of *tert*-Butyl-2-(((2S,3R)-3-acetoxy-1-methoxy-1-oxobutan-2-yl)carbamoyl)-2-((benzyloxy)methyl) pyrrolidine-1-carboxylate (2S-2):

25

- 73 -

[00183] To a stirred solution of compound **2S-1** (1.0 g, 2.22 mmol) in CH₂Cl₂ (15 mL) was added Et₃N (0.34 mL, 2.44 mmol) drop wise at 0 °C under inert atmosphere. To this Ac₂O (0.27 mL, 2.64 mmol) followed by DMAP (50 mg, 0.40 mmol) was added at 0 °C and allowed to stir at RT for 2 h. The reaction mixture was diluted with water and the aqueous layer was
 5 extracted with CH₂Cl₂ (2 x 25 mL). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford compound **2S-2** (0.8 g, 73%) as a liquid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.41-7.34 (m, 5H), 5.26-5.24 (m, 1H), 4.54 (s, 2H), 4.06-3.97 (m, 1H), 3.78-3.72 (m, 1H), 3.62 (s, 3H), 3.52-3.49 (m, 1H), 2.68 (s, 6H), 2.34-2.31 (m,
 10 1H), 1.87 (s, 3H), 1.78-1.74 (m, 2H), 1.42 (s, 6H), 1.14 (d, 3H).

Mass *m/z*: 493.8 [M⁺+1]

Synthesis of *tert*-Butyl-2-(((2*S*, 3*R*)-3-acetoxy-1-methoxy-1-oxobutan-2-yl)carbamoyl)-2-(hydroxymethyl) pyrrolidine-1-carboxylate (2S-3):

[00184] To a stirred solution of compound **2S-2** (0.8 g, 1.62 mmol) in EtOAc (15 mL) was added 10% Pd-C (0.15 g) and stirred at RT for 24 h under H₂ atmosphere (balloon
 15 pressure). The reaction mixture was filtered through a celite pad and washed with EtOAc. The filtrate was concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography eluting with 40% EtOAc/hexane to afford compound **2S-3** (0.5 g, 77%) as a liquid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 8.16 (br s, 1H), 5.78-5.74 (m, 1H), 5.23 (d, *J* = 9.5 Hz, 1H), 4.64-4.58 (m, 1H), 4.03-3.98 (m, 1H), 3.61 (s, 3H), 3.52 (d, *J* = 10.0 Hz, 1H), 3.43 (d, *J* = 6.5 Hz, 1H), 2.29-2.27 (m, 1H), 1.96-1.94 (m, 4H), 1.74-1.68 (m, 2H), 1.38-1.30 (m, 9H), 1.14 (d, *J* = 6.5 Hz, 3H);

LCMS *m/z*: 403.6 [M⁺+1]

Synthesis of *tert*-Butyl-2-(((2*S*,3*R*)-3-acetoxy-1-methoxy-1-oxobutan-2-yl)-1-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-FNL-1):

[00185] To a stirred solution of compound **2S-3** (0.35 g, 0.87mmol) in THF (15 mL) was added PPh₃ (274 mg, 1.04 mmol) at RT and stirred for 30 minutes under inert atmosphere. Then the reaction mixture was cooled 0 °C, DTAD (0.22 g, 0.95 mmol) was added to the
 30 reaction mixture and allowed to warm to room temperature and stirring was continued for another 20 h. It was quenched with saturated citric acid and washed with saturated NaCl solution and extracted with EtOAc (2 x 20mL). The combined organic extract were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The obtained crude material was

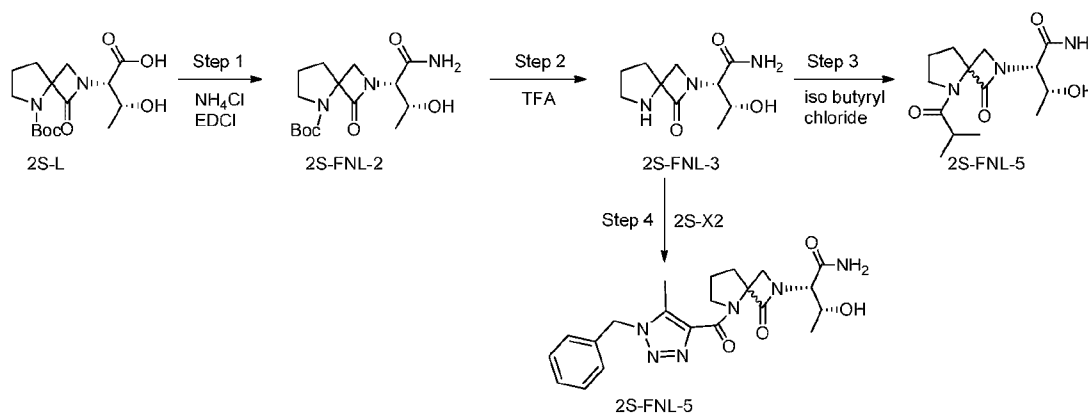
- 74 -

purified by silica gel column chromatography eluting with 40% EtOAc/Hexane to afford **NRX-1076 (2S-FNL-1)** (160 mg, 48%) as a liquid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 5.21-5.18 (m, 1H), 4.57 (d, 1H), 3.82 (d, 1H), 3.64 (d, 3H), 3.42 (d, 2H), 3.24-3.21 (m, 1H), 2.14-2.11 (m, 2H), 1.97 (s, 3H), 1.84-1.78 (m, 2H), 1.37 (s, 9H), 1.18 (d, 3H);

Mass m/z: 383.1 [M-1]

Scheme 2S-2:



Synthesis of *tert*-butyl 2-((2*S*, 3*R*)-1-amino-3-hydroxy-1-oxobutan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-FNL-2):

[00186] To a stirring solution of compound **2S-L** (500 mg, 1.52 mmol) in CH₂Cl₂ (5 mL) were added DIPEA (0.8 mL, 4.57 mmol), EDCI.HCl (350 mg, 1.82 mmol) followed by HOBt (280 mg, 1.82 mmol), NH₄Cl (161 mg, 3.04 mmol) at 0 °C and stirred for 16 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL) and extracted with CH₂Cl₂ (2 x 30 mL). The combined organic layer was washed with citric acid solution (2 x 30 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 2% MeOH/DCM to afford compound **(2S-FNL-2)** (200 mg, 40%) as colorless liquid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.53 (s, 2H), 4.59 (s, 1H), 4.02 (s, 1H), 3.77-3.70 (m, 2H), 3.62-3.53 (m, 2H), 3.46-3.33 (m, 1H), 2.17-2.03 (m, 2H), 1.88-1.71 (m, 2H), 1.38 (s, 9H), 1.18 (d, *J* = 6.5 Hz, 3H);

Mass (ESI): 328.3 [M⁺+1]

- 75 -

Synthesis of (2S, 3R)-3-hydroxy-2-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl) butanamide (2S-FNL-3):

[00187] To a stirring solution of compound (2S-FNL-2) (200 mg, 0.61 mmol) in CH₂Cl₂ (5 mL) was added TFA (0.5 mL, 6.1 mmol) at 0 °C and stirred at RT for 3 h. After completion of reaction (by TLC), the reaction mixture was concentrated under reduced pressure to obtained crude compound which was triturated with *n*-pentane/diethylether (5 mL/5 mL) to afford compound (2S-FNL-3) (100 mg) as white solid (TFA salt).

¹H-NMR: (400 MHz, D₂O): δ 4.33-4.29 (m, 2H), 4.09 (d, 1H), 3.95 (d, 1H), 3.57-3.48 (m, 2H), 2.51-2.46 (m, 2H), 2.25-2.19 (m, 2H), 1.31 (d, 3H);

LCMS, *m/z*: 455 [2M⁺+1]

Synthesis of (2S, 3R)-3-hydroxy-2-(5-isobutyl-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl) butanamide (2S-FNL-4):

[00188] To a stirring solution of (2S-FNL-3) (500 mg (crude), 2.20 mmol) in CH₂Cl₂ (10 mL) was added TEA (1 mL, 7.70 mmol) followed by SM3 (256 mg, 2.42 mmol) at 0 °C and stirred for 16 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL) and extracted with CH₂Cl₂ (2 x 30 mL). The combined organic layer was washed with citric acid solution (2 x 30 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 2% MeOH/DCM to afford (2S-FNL-4) (100 mg, 15.2%) as white solid.

¹H-NMR: (500 MHz, D₂O): δ 4.54-4.52 (m, 1H), 4.41-4.37 (m, 1H), 4.27 (d, *J* = 3.6 Hz, 1H), 4.04 (t, *J* = 6.5 Hz, 1H), 3.85-3.72 (m, 1H), 3.71-3.66 (m, 1H), 2.92-2.87 (m, 1H), 2.38-2.27 (m, 2H), 2.12-2.05 (m, 2H), 1.30 (d, *J* = 6.5 Hz, 3H), 1.14 (d, *J* = 6.5 Hz, 6H);

Mass (ESI): 298.3 [M⁺+1]

Synthesis of (2S, 3R)-2-(5-(1-benzyl-5-methyl-1H-1, 2, 3-triazole-4-carbonyl)-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-hydroxybutanamide (2S-FNL-5):

[00189] To a stirring solution of 2S-X2 (200 mg, 0.92 mmol) in CH₂Cl₂ (10 mL), DMF (0.1 mL) were added oxalyl chloride (0.16 mL, 1.84 mmol) at 0 °C. The reaction mixture was warmed to RT and stirred for 2 h. The volatiles were evaporated under reduced pressure in presence of N₂ atmosphere to afford acid chloride (300 mg, crude). To a stirred solution of acid chloride (300 mg, crude) in DCM (5 mL) was added (2S-FNL-3) (220 mg, 0.92 mmol), *N*, *N*-

- 76 -

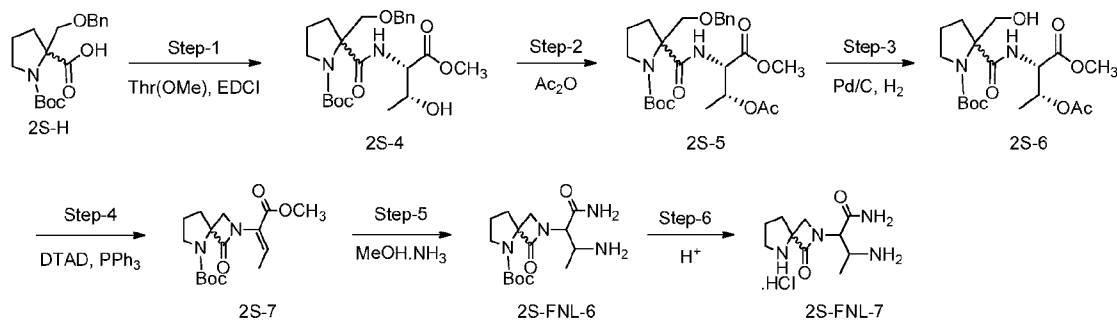
diisopropylethylamine (0.53 mL, 2.76 mmol) at 0 °C. The resulting reaction mixture was stirred at RT for 1 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL) and extracted with CH₂Cl₂ (2 x 20 mL). Combined organic extracts were washed by brine solution (2 x 10 mL) and dried over anhydrous Na₂SO₄ concentrated under reduced pressure to obtain crude product, which was purified by silica gel column chromatography eluting with 3% MeOH/CH₂Cl₂ to afford compound (2S-FNL-5) (200 mg, 48.6%) as pale brown solid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.77 (s, 2H), 7.38-7.31 (m, 3H), 7.18 (d, *J* = 7.5 Hz, 2H), 5.65 (s, 2H), 4.89 (d, *J* = 5.5 Hz, 1H), 4.76 (d, *J* = 4.0 Hz, 1H), 4.07-3.91 (m, 4H), 3.62-3.48 (m, 1H), 2.39 (s, 3H), 2.26-2.12 (m, 2H), 1.98-1.91 (m, 2H), 1.13 (d, *J* = 6.5 Hz, 3H);

LCMS *m/z*: 427.6 [*M*⁺+1];

HPLC: 95.5% (both enantiomers)

Scheme 2S-2:



Synthesis of *tert*-butyl 2-((benzyloxy)methyl)-2-(((2S,3R)-3-hydroxy-1-methoxy-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carboxylate(2S-4):

[00190] To a stirring solution of compound 2S-H (50 g, 0.15 mol) in CH₂Cl₂ (500 mL) was added methyl 2-amino-3-hydroxybutanoate (23.8 g, 0.18 mol), EDCI.HCl (34.2 g, 0.18 mol) followed by HOBt (24.1 g, 0.18 mol) and DIPEA (57.8 g, 0.45 mol) at RT and stirred for 2 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (250 mL) and extracted with CH₂Cl₂ (2 x 250 mL). The separated organic layers were washed with water, brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 50% EtOAc/Hexane to afford compound 2S-4 (53 g, 78.9%) as light green liquid.

- 77 -

Synthesis of *tert*-butyl 2-(((2*S*,3*R*)-3-acetoxy-1-methoxy-1-oxobutan-2-yl)carbamoyl)-2-((benzyloxy)methyl)pyrrolidine-1-carboxylate (2*S*-5):

[00191] To a stirring solution of compound **2*S*-4** (15 g, 33.3 mmol) in CH₂Cl₂ (150 mL) was added DIPEA (6.4 g, 49.9 mmol) followed by acetic anhydride (4 g, 39.9 mmol) and DMAP (408 mg, 3.33 mmol) at RT and stirred for 2 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (100 mL) and extracted with CH₂Cl₂ (2 x 100 mL). The separated organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford compound **2*S*-5** (16 g, crude) as light brown liquid.

¹H-NMR: (400 MHz, CDCl₃): δ 7.35 (d, 5H), 5.47-5.44 (m, 1H), 4.80 (dd, 1H), 4.64-4.61 (m, 2H), 4.15-4.11 (m, 1H), 3.86-3.83 (m, 1H), 3.75 (s, 4H), 3.54-3.50 (m, 2H), 2.42-3.38 (m, 1H), 1.91-1.85 (m, 5H), 1.45-1.41 (m, 10H), 1.27 (d, 2H);

LCMS (ESI): 492 [M⁺]

Synthesis of *tert*-butyl 2-(((2*S*,3*R*)-3-acetoxy-1-methoxy-1-oxobutan-2-yl)carbamoyl)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (2*S*-6):

[00192] To a stirring solution of compound **2*S*-5** (16 g, 32.5 mmol) in methanol (100 mL) and EtOAc (100 mL) was added 10% Pd on Charcoal (3 g) at RT and stirred for 4 h under H₂ atmosphere (balloon pressure). After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol. Obtained filtrate was concentrated under reduced pressure to afford compound **2*S*-6** (10 g, crude) as brown thick syrup. This material was directly used for the next step without further purification.

Synthesis of (Z)-*tert*-butyl 2-(1-methoxy-1-oxobut-2-en-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2*S*-7):

[00193] To a stirring solution of compound **2*S*-6** (10 g, 24.8 mmol) in THF (50 mL) was added triphenylphosphine (13 g, 49.7 mmol) and DTAD (11.15 g, 37.3 mmol). The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 40% EtOAc/hexane to afford compound **2*S*-7** (2 g, 24.8%).

Synthesis of *tert*-butyl 2-(1, 3-diamino-1-oxobutan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2*S*-FNL-6):

- 78 -

[00194] A solution of compound **2S-7** (2 g, 6.16 mmol) in methanolc.NH₃ (50 mL) was stirred at RT for 4 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure. Obtained crude material was washed with Et₂O (25 mL) and *n*-pentane (25 mL) to afford (**2S-FNL-6**) (0.35 g, 16.6%) as white solid.

5 ¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.70 (br s, 1H), 7.07 (s, 1H), 3.85 (d, 1H), 3.73 (d, 1H), 3.42-3.38 (m, 2H), 3.29-3.25 (m, 1H), 3.12-3.07 (m, 1H), 2.09 (t, 2H), 1.95 (br s, 1H), 1.84-1.81 (m, 2H), 1.93 (s, 9H), 1.12 (d, 1H), 0.99 (d, 2H);

LCMS (ESI) *m/z*: 327.3 [M⁺+1]

Synthesis of 3-amino-2-(1-oxo-2,5-diazaspiro[3.4]octan-2-yl)butanamide (2S-FNL-7):

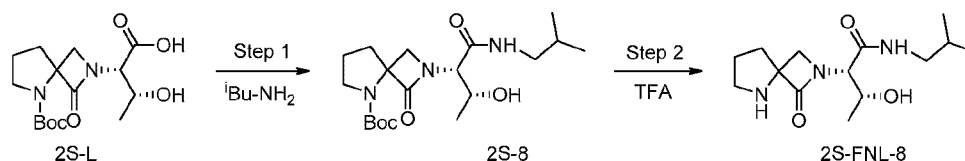
10 [00195] To a stirring solution of (**2S-FNL-6**) (0.25 g, 0.76 mmol) in CH₂Cl₂ (10 mL) was added ether.HCl (5 mL) at RT and stirred for 4 h. To this was added 1, 4-dioxane-HCl (5 mL) and stirring was continued for 2 h. After consumption of starting material (by TLC), the solvent from the reaction was removed under reduced pressure and obtained crude material was washed with ACN (25 mL) and Et₂O (25 mL) to afford (**2S-FNL-7**) (0.11 g, 63.5%) as an off-
15 white solid.

¹H-NMR: (400 MHz, D₂O): δ 4.69-4.55 (m, 1H), 4.12-3.86 (m, 3H), 3.62-3.51 (m, 2H), 2.56-2.23 (m, 2H), 2.25-2.21 (m, 2H), 1.52-1.43 (m, 3H).

LCMS (ESI) *m/z*: 227.2 [M⁺+1];

UPLC (Purity): 97.96%

20 **Scheme 2S-3:**



Synthesis of tert-butyl 2-((2S, 3R)-3-hydroxy-1-(isobutylamino)-1-oxobutan-2-yl)-1-oxo-2,5-diazaspiro [3.4] octane-5-carboxylate (2S-8):

[00196] To a stirring solution of compound **2S-L** (300 mg, 0.91 mmol) in CH₂Cl₂ (10 mL) were added DIPEA (354 mg, 2.74 mmol), EDCI.HCl (210 mg, 1.09 mmol) followed by HOBt (165 mg, 1.09 mmol), isobutylamine (80 mg, 1.09 mmol) at 0 °C and stirred for 16 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with
30 water (10 mL) and extracted with CH₂Cl₂ (2 x 30 mL). The combined organic layer was

- 79 -

washed with brine (2 x 30 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 2% MeOH/DCM to afford compound **2S-8** (250 mg, 71.5 %) as white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 8.02 (t, *J* = 5.2 Hz, 1H), 4.37 (s, 2H), 4.04 (t, *J* = 5.6 Hz, 1H), 4.00 (d, *J* = 5.6 Hz, 1H), 3.77-3.72 (m, 1H), 3.61 (d, *J* = 6.0 Hz, 1H), 3.44-3.38 (m, 1H), 2.98-2.84 (m, 3H), 2.19-2.08 (m, 3H), 1.84-1.79 (m, 1H), 1.42 (s, 9H), 1.39 (d, *J* = 5.5 Hz, 3H), 0.84 (d, *J* = 6.4 Hz, 6H);

Mass (ESI): *m/z* 384.4 [*M*⁺+1]

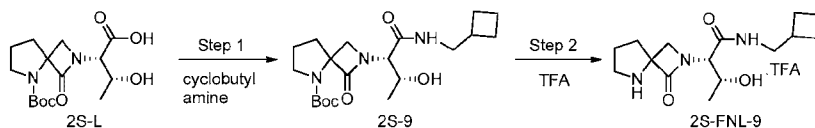
Synthesis of (2*S*, 3*R*)-3-hydroxy-*N*-isobutyl-2-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl) butanamide (2S-FNL-8):

[00197] To a stirring solution of compound **2S-8** (250 mg, 0.65 mmol) in CH₂Cl₂ (5 mL) was added TFA (0.6 mL, 6.52 mmol) at 0 °C and stirred at RT for 3 h. After completion of reaction (by TLC), the reaction mixture was concentrated under reduced pressure to obtained crude compound which was triturated with *n*-pentane/diethylether (3 x 5 mL) to afford (**2S-FNL-8**) (125 mg, 67.9 %) as an white solid (TFA salt).

¹H-NMR: (500 MHz, D₂O): δ 4.26 (s, 2H), 4.09 (t, *J* = 8.0 Hz, 1H), 3.95 (t, *J* = 7.5 Hz, 1H), 3.56-3.51 (m, 2H), 3.14-3.06 (m, 2H), 2.50-2.43 (m, 2H), 2.25-2.21 (m, 2H), 1.85-1.82 (m, 1H), 1.30 (d, *J* = 5.5 Hz, 3H), 0.96 (d, *J* = 7.0 Hz, 6H);

Mass (ESI): *m/z* 284.3 [*M*⁺+1]

Scheme 2S-4:



Synthesis of tert-butyl 2-((2*S*, 3*R*)-1-((cyclobutylmethyl) amino)-3-hydroxy-1-oxobutan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-9):

[00198] To a stirring solution of compound **2S-L** (500 mg, 1.52 mmol) in CH₂Cl₂ (10 mL) were added DIPEA (0.8 mL, 4.57 mmol), EDCLHCl (350 mg, 1.82 mmol) followed by HOBt (280 mg, 1.82 mmol), cyclobutylamine (155 mg, 1.82 mmol) at 0 °C and stirred for 16 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL) and the separated organic layer was washed with citric acid (2 x 20 mL),

- 80 -

brine (2 x 20 mL). The combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 80% EtOAc/*n*-hexane to afford compound **2S-9** (250 mg, 41.5 %) as colorless syrup.

- 5 **¹H-NMR:** (500 MHz, DMSO-*d*₆): δ 8.20 (t, *J* = 11.5 Hz, 1H), 4.53 (s, 2H), 4.03 (t, *J* = 7.5 Hz, 1H), 3.88 (t, *J* = 8.5 Hz, 2H), 3.41-3.33 (m, 2H), 3.32-3.24 (m, 2H), 2.41-2.33 (m, 3H), 2.32-2.27 (m, 2H), 2.24-2.17 (m, 2H), 2.10-1.90 (m, 2H), 1.68 (t, *J* = 8.5 Hz, 2H), 1.40 (s, 9H), 1.18 (d, *J* = 6.4 Hz, 3H);

Mass (ESI): *m/z* 396.4 [M⁺+1]

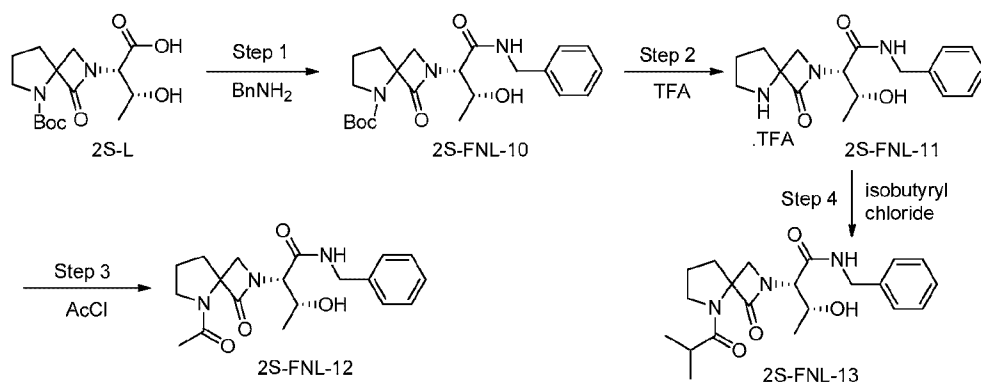
10 **Synthesis of (2S, 3R)-N-(cyclobutylmethyl)-3-hydroxy-2-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl) butanamide (2S-FNL-9):**

[00199] To a stirring solution of compound **2S-9** (250 mg, 0.63 mmol) in CH₂Cl₂ (5 mL) was added TFA (0.5 mL, 5.06 mmol) at 0 °C and stirred at RT for 3 h. After completion of reaction (by TLC), the reaction mixture was concentrated under reduced pressure to obtained
15 crude compound which was triturated with diethylether (5 mL) to afford (**2S-FNL-9**) (90 mg, 48.3%) as hygroscopic white solid (TFA salt).

¹H-NMR: (500 MHz, D₂O): δ 4.23 (s, 2H), 4.08 (t, *J* = 7.0 Hz, 1H), 3.94 (t, *J* = 8.5 Hz, 1H), 3.56-3.51 (m, 2H), 3.32-3.24 (m, 2H), 2.56-2.53 (m, 3H), 2.48-2.43 (m, 2H), 2.25-2.21 (m, 2H), 2.07-1.88 (m, 2H), 1.71 (t, *J* = 8.5 Hz, 2H), 1.28 (d, *J* = 6.4 Hz, 3H);

- 20 **Mass (ESI):** *m/z* 296.3 [M⁺+1];

Scheme 2S-5:



- 81 -

Synthesis of tert-butyl 2-((2*S*, 3*R*)-1-(benzylamino)-3-hydroxy-1-oxobutan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2*S*-FNL-10):

[00200] To a stirring solution of compound 2*S*-L (1 g, 3.04 mmol) in CH₂Cl₂ (15 mL) were added DIPEA (1.6 mL, 9.14 mmol), EDCI.HCl (700 mg, 3.66 mmol) followed by HOBT (560 mg, 3.66 mmol), benzylamine (325 mg, 3.04 mmol) at 0 °C and stirred for 16 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (20 mL) and extracted with CH₂Cl₂ (2 x 30 mL). The combined organic layer was washed with citric acid solution (2 x 30 mL) and organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 5% MeOH/DCM to afford (2*S*-FNL-10) (800 mg, 63.5 %) as white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 7.38-7.20 (m, 5H), 4.62-4.60 (m, 1H), 4.49-4.43 (m, 2H), 4.33-4.25 (m, 1H), 4.04 (d, *J* = 5.6 Hz, 1H), 3.96-3.92 (m, 1H), 3.51-3.45 (m, 1H), 3.43-3.31 (m, 1H), 2.31-2.21 (m, 2H), 1.98-1.86 (m, 2H), 1.39 (s, 9H), 1.24-1.22 (m, 3H);

Mass (ESI): *m/z* 418.4 [M⁺+1];

HPLC: 91.8% (both isomers)

Synthesis of (2*S*, 3*R*)-N-benzyl-3-hydroxy-2-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl) butanamide (2*S*-FNL-11):

[00201] To a stirring solution of (2*S*-FNL-10) (700 mg, 1.67 mmol) in CH₂Cl₂ (10 mL) was added TFA (1.9 mL, 16.7 mmol) at 0 °C and stirred at RT for 4 h. After completion of reaction (by TLC), the reaction mixture was concentrated under reduced pressure to obtained crude compound which was triturated with *n*-pentane/diethylether (5 mL/5 mL) to afford (2*S*-FNL-11) (400 mg, 75.6 %) as an white solid (TFA salt).

¹H-NMR: (400 MHz, D₂O): δ 7.45-7.34 (m, 5H), 4.45 (s, 2H), 4.29-4.21 (m, 2H), 4.06-3.85 (m, 2H), 3.52-3.47 (m, 2H), 2.45-2.35 (m, 2H), 2.22-2.16 (m, 2H), 1.24-1.20 (m, 3H);

Mass (ESI): *m/z* 318.4 [M⁺+1];

HPLC: 89.1% (both isomers).

Synthesis of (2*S*, 3*R*)-2-(5-acetyl-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-N-benzyl-3-hydroxybutanamide (2*S*-FNL-12):

[00202] To a stirring solution of (2*S*-FNL-11) (240 mg, 0.75 mmol) in CH₂Cl₂ (10 mL) were added TEA (0.31 mL, 2.25 mmol) at RT. After added acetyl chloride (0.1 mL, 0.9 mmol) slowly at 0 °C and stirred to RT for 2 h. After consumption of the starting material (by TLC),

- 82 -

the reaction mixture was diluted with water (5 mL) and extracted with CH₂Cl₂ (2 x 20 mL). The combined organic layer was washed with citric acid solution (1 x 20 mL), brine (1 x 20 mL). The separated organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 2% MeOH/DCM to afford **(2S-FNL-12)** (90 mg, 33.4%) as an off-white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 7.32-7.20 (m, 5H), 4.58-4.55 (m, 1H), 4.52-4.42 (m, 2H), 4.36-4.22 (m, 1H), 4.08-3.93 (m, 1H), 3.70-3.65 (m, 2H), 3.64-3.53 (m, 2H), 2.32-2.22 (m, 2H), 2.20 (s, 3H), 2.04-1.95 (m, 2H), 1.22-1.20 (m, 3H);

Mass (ESI): *m/z* 360.3 [M⁺+1];

HPLC: 97.5% (both isomers)

Synthesis of (2S, 3R)-N-benzyl-3-hydroxy-2-(5-isobutyryl-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl) butanamide (NRX-2563) (2S-FNL-13):

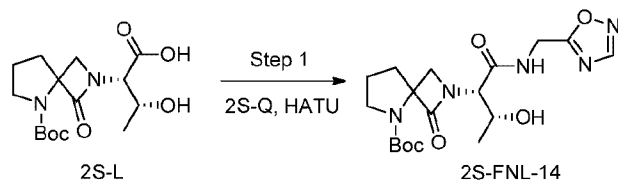
[00203] To a stirring solution of **(2S-FNL-11)** (244 mg, 0.76 mmol) in CH₂Cl₂ (10 mL) was added TEA (0.37 mL, 2.66 mmol) at 0 °C. After added Int-F (89 mg, 0.84 mmol) and stirred at RT for 2 h. After completion of reaction (by TLC), the reaction mixture was diluted with water (10 mL) and extracted with CH₂Cl₂ (2 x 20 mL). The combined organic layer was washed with citric acid solution (2 x 30 mL) and organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 60% EtOAc/*n*-hexane to afford **(2S-FNL-13)** (150 mg, 51 %) as an off-white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 7.35-7.20 (m, 5H), 4.82-4.49 (m, 1H), 4.46-4.31 (m, 1H), 4.29-4.03 (m, 1H), 3.90-3.70 (m, 2H), 3.69-3.57 (m, 2H), 3.46-3.31 (m, 1H), 2.77-2.73 (m, 1H), 2.28-2.21 (m, 2H), 2.06-1.97 (m, 2H), 1.22 (d, *J* = 6.8 Hz, 3H), 1.08-0.98 (m, 6H);

Mass (ESI): *m/z* 388.4 [M⁺+1];

HPLC: 95.2% (both isomers)

- 83 -

Scheme 2S-6:

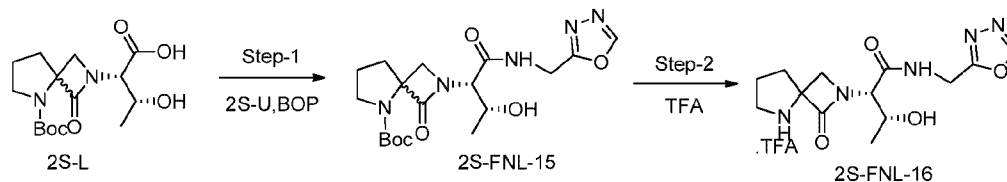
Synthesis of tert-butyl 2-((2S, 3R)-1-(((1, 2, 4-oxadiazol-5-yl) methyl) amino)-3-hydroxy-1-oxobutan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-FNL-14):

[00204] To a stirring solution of compound **2S-L** (600 mg, 1.82 mmol) in DMF (10 mL) were added DIPEA (708 mg, 5.48 mmol), **2S-Q** (290 mg, 1.82 mmol) HATU (761 mg, 2.00 mmol) at 0 °C and stirred for 16 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (50 mL) and EtOAc (100 mL). The organic layer was washed with water (2 x 50 mL) followed by brine solution (2 x 30 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 2% MeOH/DCM. The obtained solid was triturated with ether/n-pentane (5 mL/5 mL) to afford (**2S-FNL-14**) (100 mg, 13.5 %) as white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 8.61 (s, 1H), 4.85 (s, 2H), 4.78-4.63 (m, 1H), 4.59-4.55 (m, 1H), 4.30-4.25 (m, 1H), 3.52-3.46 (m, 2H), 3.43-3.29 (m, 1H), 2.31-2.23 (m, 2H), 1.96-1.88 (m, 2H), 1.45 (s, 9H), 1.26-1.20 (m, 3H);

Mass (ESI): *m/z* 410.4 [M⁺+1];

HPLC: 98.14% (both isomers)

Scheme 2S-7:

Synthesis of tert-butyl 2-((2S, 3R)-1-(((1, 3, 4-oxadiazol-2-yl) methyl) amino)-3-hydroxy-1-oxobutan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-FNL-15):

[00205] To a stirring solution of compound **2S-L** (1 g, 3.04 mmol) in DMF (10 mL) were added DIPEA (1.58 mL, 9.12 mmol), BOP reagent (2.01 g, 4.56 mmol) followed by **2S-U** (496 mg, 3.64 mmol) at 0 °C and stirred for 16 h at RT. After consumption of the starting

- 84 -

material (by TLC), the reaction mixture was diluted with water (100 mL) and extracted with EtOAc (2 x 30 mL). The combined organic layer was washed with brine solution (2 x 50 mL) and organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting
 5 80% EtOAc/n-hexane followed by preparative HPLC purification to afford **(2S-FNL-15)** (67 mg, 5.4 %) as an off-white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 10.92 (s, 1H), 7.83 (s, 1H), 4.97-4.88 (m, 2H), 4.07 (d, *J* = 7.2 Hz, 2H), 3.83-3.65 (m, 2H), 3.57-3.40 (m, 1H), 3.38-3.25 (m, 2H), 2.15-2.01 (m, 2H), 1.83-1.80 (m, 2H), 1.40 (s, 9H), 1.22 (d, *J* = 6.4 Hz, 3H)

10 **Mass (ESI):** *m/z* 410.4 [M⁺+1];

HPLC: 90.6%

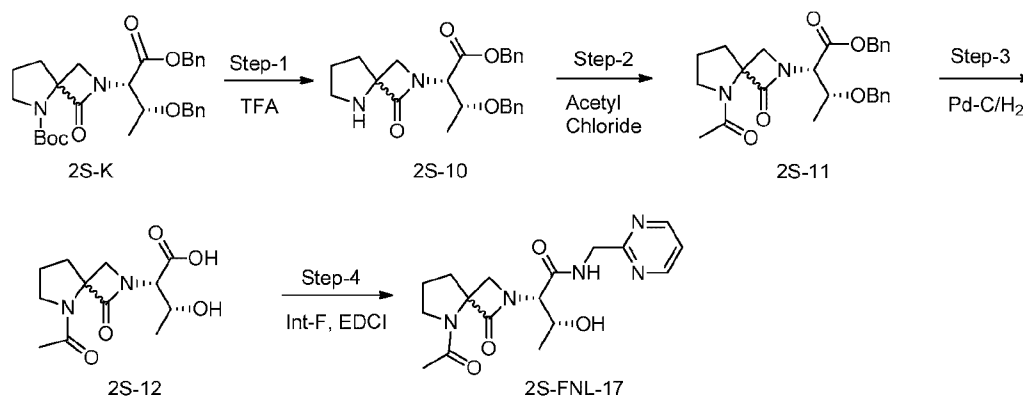
Synthesis of (2S, 3R)-N-((1, 3, 4-oxadiazol-2-yl) methyl)-3-hydroxy-2-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl) butanamide (2S-FNL-16):

[00206] To a stirring solution of compound **(2S-FNL-15)** (70 mg, 0.71 mmol) in CH₂Cl₂
 15 (5 mL) was added TFA (195 mg, 1.71 mmol) at 0 °C and stirred at RT for 1 h. After completion of reaction (by TLC), the reaction mixture was concentrated under reduced pressure to obtained crude compound which was triturated with *n*-pentane/diethylether (5 mL/5 mL) to afford compound **(2S-FNL-16)** (60 mg, 84.5%) as an off-white solid (TFA salt).

¹H-NMR: (400 MHz, D₂O): δ 7.83 (s, 1H), 5.20-5.10 (m, 1H), 4.80 (s, 1H), 4.39-4.30 (m, 2H),
 20 4.13-4.04 (m, 2H), 3.53-3.48 (m, 2H), 2.44-2.41 (m, 2H), 2.21-2.16 (m, 2H), 1.31 (d, *J* = 6.4 Hz, 3H); **Mass (ESI):** *m/z* 310.1 [M⁺+1];

HPLC: 90.99%

Scheme 2S-8:



25

- 85 -

Synthesis of benzyl (2*S*, 3*R*)-3-(benzyloxy)-2-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl) butanoate (8):

[00207] To a stirring solution of compound **2S-K** (800 mg, 1.57 mmol) in DCM (10 mL) was added TFA (1.2 mL) at 0 °C and stirred at RT for 2 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to afford compound **2S-10** (500 mg, 78%) as an off-white solid (HCl salt) was used directly for next step.

¹H-NMR: (500 MHz, D₂O): δ 7.48 (m, 5H), 7.24-7.21 (m, 5H), 5.29 (s, 2H), 4.96 (s, 2H), 4.80-4.62 (m, 1H), 4.29-4.18 (m, 2H), 4.01-3.89 (m, 1H), 3.52-3.46 (m, 2H), 2.43-2.38 (m, 2H), 2.24-2.14 (m, 2H), 1.35-1.28 (m, 3H);

LCMS: 408 [M⁺+1]

Synthesis of benzyl (2*S*, 3*R*)-2-(5-acetyl-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-(benzyloxy) butanoate (2S-11):

[00208] To a stirring solution of compound **2S-10** (500 mg, 1.22 mmol) in DCM (5 mL) was added TEA (0.46 mL, 3.36 mmol) followed by acetyl chloride (0.1 mL, 1.47 mmol) at 0 °C and stirred at RT for 2 h. After consumption of the starting material (by TLC), reaction mixture was diluted with water (10 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 2% MeOH/DCM to afford compound **2S-11** (300 mg, 54.5 %) as white solid.

¹H-NMR: (400 MHz, CD₃OD): 7.36-7.29 (m, 5H), 7.26-7.16 (m, 5H), 5.13 (s, 2H), 4.59 (s, 2H), 4.32-4.29 (m, 2H), 4.16-4.13 (m, 1H), 3.65-3.61 (m, 1H), 3.60-3.46 (m, 2H), 2.21-2.09 (m, 2H), 2.02 (s, 3H), 2.01-1.91 (m, 2H), 1.21 (d, *J* = 6.4 Hz, 3H);

LCMS: 451.3 [M⁺+1]

Synthesis of (2*S*, 3*R*)-2-(5-acetyl-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-hydroxybutanoic acid (2S-12):

[00209] To a stirring solution of compound **2S-11** (1 g, 2.22 mmol) in methanol (30 mL) was added 10% Pd/C (500 mg) at RT and stirred for 24 h under H₂ atmosphere. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol (20 mL). Obtained filtrate was concentrated under reduced pressure to afford compound **2S-12** (500 mg, 83.3%) as an off-white solid.

- 86 -

¹H-NMR: (400 MHz, CD₃OD): δ 4.35-4.30 (m, 1H), 4.29-4.17 (m, 1H), 4.09-4.04 (m, 1H), 3.76-3.67 (m, 1H), 3.59-3.48 (m, 1H), 3.34-3.31 (m, 1H), 2.29-2.24 (m, 2H), 2.15 (s, 3H), 2.04-1.96 (m, 2H), 1.28 (d, *J* = 6.4 Hz, 3H);

LCMS *m/z*: 270.4 [*M*⁺+1]

5 **Synthesis of (2*S*, 3*R*)-2-(5-acetyl-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-hydroxy-N-(pyrimidin-2-ylmethyl) butanamide (2*S*-FNL-17):**

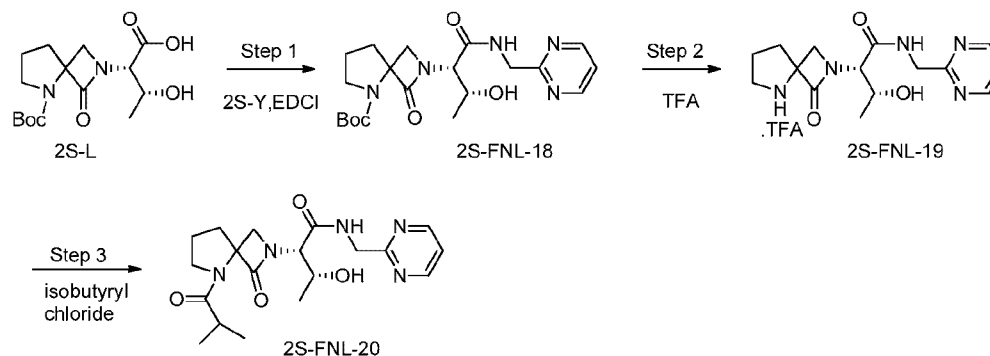
[00210] To a stirring solution of compound **2*S*-12** (700 mg, 2.59 mmol) in DCM (15 mL) were added DIPEA (1.35 mL, 7.77 mmol), **2*S*-Y** (410 mg, 2.84 mmol), EDCI (593 mg, 3.1 mmol) followed by HOBT (474 mg, 3.1 mmol) at 0 °C and stirred for 16 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (40 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 3% MeOH/DCM to afford (**2*S*-FNL-17**) (100 mg, 10.7 %) as white solid.

15 **¹H-NMR:** (400 MHz, D₂O): δ 8.78 (d, *J* = 5.2 Hz, 2H), 7.49 (t, *J* = 5.2 Hz, 1H), 4.79 (s, 2H), 4.55-4.47 (m, 1H), 4.40-4.37 (m, 2H), 3.79-3.56 (m, 3H), 2.37-2.26 (m, 2H), 2.14-2.03 (m, 2H), 2.01 (s, 3H), 1.28 (d, *J* = 6.4 Hz, 3H);

Mass (ESI): *m/z* 362.4 [*M*⁺+1];

HPLC: 92.3% (both isomers)

20 ***Scheme 2*S*-9:***



25 **Synthesis of tert-butyl 2-((2*S*, 3*R*)-3-hydroxy-1-oxo-1-((pyrimidin-2-ylmethyl) amino) butan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2*S*-FNL-18):**

- 87 -

[00211] To a stirring solution of compound **2S-L** (1 g, 3.04 mmol) in CH₂Cl₂ (30 mL) were added DIPEA (1.63 mL, 9.14 mmol), EDCl.HCl (696 mg, 3.64 mmol) followed by HOBt (558 mg, 3.64 mmol), **2S-Y** (241 mg, 3.34 mmol) at 0 °C and stirred for 16 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (30 mL). The organic layer was washed with citric acid solution (2 x 30 mL) followed by brine solution (2 x 25 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 5% MeOH/DCM to afford (**2S-FNL-18**) (800 mg, 63 %) as white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 8.72 (t, *J* = 4.8 Hz, 2H), 7.36 (t, *J* = 4.8 Hz, 1H), 4.81-4.76 (m, 1H), 4.62-4.49 (m, 1H), 4.34-4.29 (m, 1H), 4.18-4.03 (m, 2H), 3.56 (d, *J* = 5.6 Hz, 2H), 3.52-3.46 (m, 1H), 2.30-2.25 (m, 2H), 1.97-1.88 (m, 2H), 1.46 (s, 9H), 1.31-1.28 (m, 3H);

Mass (ESI): *m/z* 420.4 [M⁺+1];

HPLC: 99.6% (both isomers)

Synthesis of (2S, 3R)-3-hydroxy-2-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-N-(pyrimidin-2-ylmethyl) butanamide (2S-FNL-19):

[00212] To a stirring solution of (**2S-FNL-18**) (280 mg, 0.66 mmol) in CH₂Cl₂ (5 mL) was added TFA (0.3 mL, 4.0 mmol) at 0 °C and stirred at RT for 4 h. After completion of reaction (by TLC), the reaction mixture was concentrated under reduced pressure to obtained crude compound that was triturated with *n*-pentane/diethyl ether (5 mL/5 mL) to afford (**2S-FNL-19**) (95 mg, 44.6 %) as an white solid (TFA salt).

¹H-NMR: (500 MHz, D₂O): δ 8.81 (d, *J* = 4.5 Hz, 2H), 7.53 (t, *J* = 5.0 Hz, 1H), 4.80-4.65 (m, 2H), 4.46 (d, *J* = 6.0 Hz, 1H), 4.36-4.31 (m, 2H), 4.10 (d, *J* = 7.5 Hz, 1H), 3.95 (t, *J* = 8.0 Hz, 1H), 3.58-3.49 (m, 1H), 2.51-2.40 (m, 2H), 2.26-2.17 (m, 2H), 1.34 (d, *J* = 6.0 Hz, 3H);

Mass (ESI): *m/z* 320.3 [M⁺+1]

Synthesis of (2S, 3R)-3-hydroxy-2-(5-isobutyryl-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-N-(pyrimidin-2-ylmethyl) butanamide (2S-FNL-20):

[00213] To a stirring solution of (**2S-FNL-19**) (300 mg, 0.94 mmol) in CH₂Cl₂ (5 mL) was added TEA (0.4 mL, 2.82 mmol) followed by SM-4 (120 mg, 1.12 mmol) at 0 °C and stirred at RT for 2 h. After completion of reaction (by TLC), the reaction mixture was diluted with water (10 mL) and extracted with CH₂Cl₂ (2 x 20 mL). The combined organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained

- 88 -

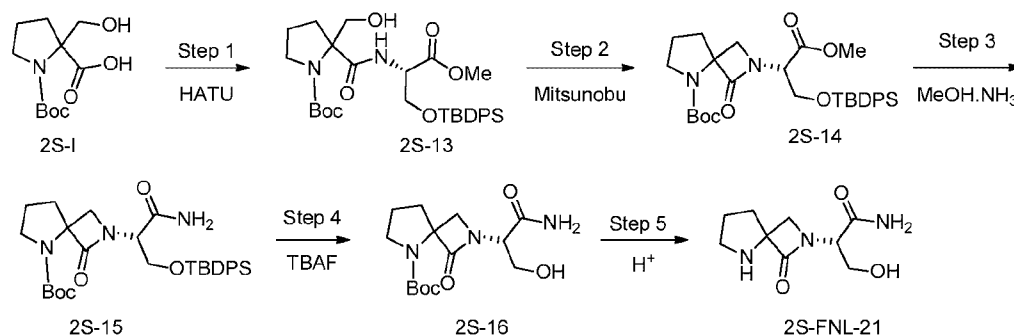
crude material was purified by silica gel column chromatography eluting 2% MeOH/DCM to afford **(2S-FNL-20)** (100 mg, 27.3 %) as white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 8.73 (t, *J* = 5.2 Hz, 2H), 7.36 (t, *J* = 4.8 Hz, 1H), 4.83-4.55 (m, 1H), 4.51-4.29 (m, 3H), 4.21-4.02 (m, 1H), 3.75-3.69 (m, 1H), 3.64-3.60 (m, 1H), 3.31-3.30 (m, 1H), 2.79-2.72 (m, 1H), 2.28-2.25 (m, 2H), 2.08-1.97 (m, 2H), 1.31 (d, *J* = 6.4 Hz, 3H), 1.07-1.02 (m, 6H).

Mass (ESI): *m/z* 390.4 [*M*⁺+1],

HPLC: 97.75%

Scheme 2S-10:



tert-Butyl 2-(((S)-3-((tert-butyldiphenylsilyl)oxy)-1-methoxy-1-oxopropan-2-yl)carbamoyl)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (2S-13):

[00214] To a stirring solution of compound **2S-I** (11 g, 44.89 mmol) in CH₂Cl₂ (110 mL) was added compound **2S-AJ** (16.07 g, 44.89 mmol), HATU (20.4 g, 53.68 mmol) followed by DIPEA (17.37 g, 0.13 mol) at RT and stirred for 10 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (100 mL) and extracted with CH₂Cl₂ (2 x 100 mL). The separated organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography to afford compound **2S-13** (16 g, 61%) as yellow liquid.

¹H-NMR: (500 MHz, CDCl₃): δ 7.58-7.37 (m, 10H), 4.67 (s, 1H), 4.12-4.08 (m, 2H), 3.93 (s, 1H), 3.75 (s, 3H), 3.72-3.64 (m, 2H), 2.8 (s, 1H), 2.35 (s, 1H), 2.04 (s, 1H), 1.98-1.82 (m, 3H), 1.25 (s, 9H), 1.03 (s, 9H);

Mass (ESI): *m/z* 583.5 [*M*⁺-1].

Synthesis of *tert*-butyl 2-((*S*)-3-((*tert*-butyldiphenylsilyl)oxy)-1-methoxy-1-oxopropan-2-yl)-1-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-14):

[00215] To a stirring solution of compound **2S-13** (1.6 g, 2.73 mmol) in THF (20 mL) was added triphenylphosphine (0.994 g, 4.10 mmol) and DTAD (0.788 g, 3.00 mmol). The reaction mixture was stirred at RT for 8 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water and extracted with EtOAc (2x 30 mL). The separated organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography to afford compound **2S-14** (0.8 g, 51%) as yellow sticky compound.

¹H-NMR: (400 MHz, CDCl₃): δ 7.63-7.58 (m, 4H), 7.45-7.30 (m, 6H), 4.1 (s, 3H), 3.80-3.67 (m, 4H), 3.56-3.44 (m, 3H), 2.04-1.95 (m, 4H), 1.59 (s, 9H), 1.04 (s, 9H).

Mass (ESI): *m/z* 567.4 [M⁺+1]

Synthesis of *tert*-butyl 2-((*S*)-1-amino-3-((*tert*-butyldiphenylsilyl)oxy)-1-oxopropan-2-yl)-1-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-15):

[00216] To a stirring solution of compound **2S-14** (6 g) in methanol (50 mL) was added methanolic ammonia (50 mL) at 0°C and stirred for 12 h at RT. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure and purified the crude residue by silica gel column chromatography eluting 40% EtOAc:hexane to afford compound-**2S-15** (1 g, 17%) as an pale yellow solid.

¹H-NMR: (400 MHz, CDCl₃): δ 8.27 (s, 1H), 7.67-7.63 (m, 4H), 7.45-7.36 (m, 7H), 5.37 (s, 1H), 4.56-4.54 (m, 1H), 3.82 (d, *J* = 5.2 Hz, 1H), 3.44 (t, *J* = 7.6 Hz, 2H), 3.35 (d, *J* = 5.2 Hz, 1H), 3.21 (s, 1H), 2.09-2.06 (m, 2H), 2.03 (d, *J* = 4.8 Hz, 2H), 1.44 (s, 9H), 1.08 (s, 9H).

LCMS (M/Z) *m/z*: 214 [M⁺+1].

Synthesis of *tert*-butyl 2-((*S*)-1-amino-3-hydroxy-1-oxopropan-2-yl)-1-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-16):

[00217] To a stirring solution of compound **2S-15** (1g, 1.81 mmol) in THF (10 mL) was added TBAF (0.943 g, 3.62 mmol) at 0°C and the reaction mixture was slowly warmed to RT and stirred for 2 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (5 mL) and extracted with EtOAc (2x 15 mL). The separated organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography by eluting 3% MeOH:DCM to afford **2S-16** (0.13g, 23%) as white solid.

¹H-NMR: (400 MHz, D₂O): δ 4.53 (t, *J* = 6.8 Hz, 1H), 4.03 (d, *J* = 4.8, 1H), 3.96-3.91 (m, 2H), 3.85 (t, *J* = 5.8 Hz, 1H), 3.82 (s, 2H), 2.30 (t, *J* = 4Hz, 2H), 2.15-1.82 (m, 2H), 1.49 (s, 9H).

LCMS (M/Z) m/z : 314.2 [$M^+ + 1$]

5 **Synthesis of (2*S*)-3-hydroxy-2-(1-oxo-2, 5-diazaspiro[3.4]octan-2-yl)propanamide (2S-FNL-21):**

[00218] To a stirring solution of 2S-16 (0.13 g, 0.415 mmol) in CH₂Cl₂ (3 mL) was added TFA (1 mL) at 0 °C and stirred at RT for 2 h. The reaction mixture was concentrated under reduced pressure to afford (**2S-FNL-21**) (100 mg, crude) as TFA salt.

10 **¹H-NMR:** (400 MHz, D₂O): δ 4.58 (t, *J* = 5.8 Hz, 1H), 4.09-4.03 (m, 3H), 3.92 (d, *J* = 7.2 Hz, 1H), 3.57-3.52 (m, 2H), 2.55-2.41 (m, 2H), 2.28-2.19 (m, 2H);

LCMS (M/Z) m/z : 214[M⁺+1].

Scheme 2S-11:



15 **Synthesis of tert-butyl 2-((*S*)-1-amino-3-hydroxy-1-oxopropan-2-yl)-1-oxo-2, 5-diazaspiro**
[3.4] octane-5-carboxylate (2S-FNL-22):

[00219] To a stirring solution of compound **2S-AF** (250 mg, 0.79 mmol) in DCM (10 mL) were added DIPEA (0.5 mL, 2.38 mmol), EDCI (181 mg, 0.94 mmol), HOBT (127 mg, 0.94 mmol) followed by NH₄Cl (84.5 mg, 1.58 mmol) at 0 °C and stirred to RT for 12 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (20 mL) and washed with citric acid (1 x 30 mL) followed by brine solution (1 x 30 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 5% MeOH/DCM to afford (**2S-FNL-22**) (150 mg, 60.7%) as yellow thick syrup.

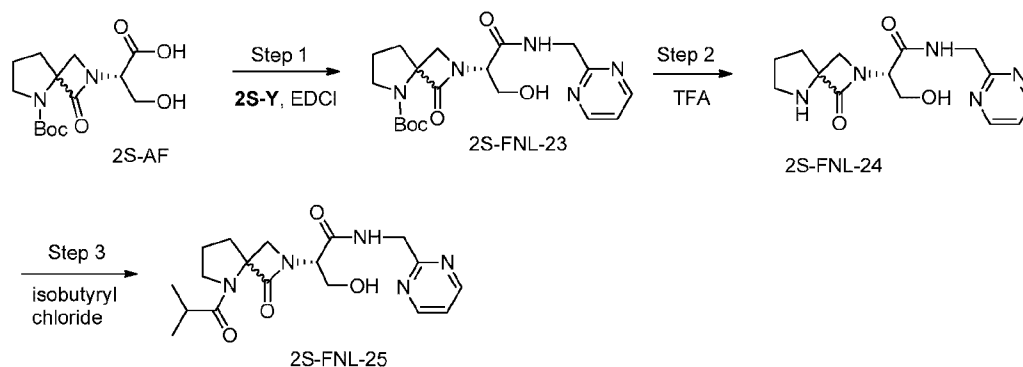
25 **¹H-NMR:** (400 MHz, CD₃OD): δ 4.13-4.07 (m, 2H), 3.96-3.88 (m, 1H), 3.87-3.77 (m, 1H), 3.63-3.47 (m, 2H), 3.44-3.30 (m, 1H), 2.31-2.26 (m, 2H), 1.97-1.88 (m, 2H), 1.47 (s, 9H);

LCMS (ESI): m/z 314.3 $[M^++1]$:

HPLC: 98.38%

Scheme 2S-12:

- 91 -



Synthesis of tert-butyl 2-((S)-3-hydroxy-1-oxo-1-((pyrimidin-2-ylmethyl) amino) propan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-FNL-23):

[00220] To a stirring solution of compound **2S-AF** (1.3 g, 4.14 mmol) in DCM (25 mL) were added DIPEA (2.15 mL, 12.42 mmol), HOBT (760 mg, 4.96 mmol), EDCI (1 g, 4.96 mmol) followed by **2S-Y** (715 mg, 4.96 mmol) at 0 °C and stirred for 16 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (50 mL). The organic layer was washed with citric acid (1 x 30 mL) followed by bicarbonate solution (1 x 30 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 2% MeOH/DCM to afford (**2S-FNL-23**) (800 mg, 50%) as white solid.

[00221] **¹H-NMR:** (400 MHz, CD₃OD): δ 8.75-8.71 (m, 2H), 7.37-7.34 (m, 1H), 4.66-4.49 (m, 2H), 4.27-4.24 (m, 1H), 4.19-4.14 (m, 1H), 4.03-3.99 (m, 1H), 3.97-3.92 (m, 1H), 3.66-3.54 (m, 1H), 3.49-3.45 (m, 1H), 3.40-3.36 (m, 1H), 2.32-2.27 (m, 2H), 1.97-1.88 (m, 2H), 1.47 (s, 9H); **Mass (ESI):** *m/z* 406.4 [M⁺+1].

HPLC: 97.1%

Synthesis of (2S)-3-hydroxy-2-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-N-(pyrimidin-2-ylmethyl) propanamide (2S-FNL-24):

[00221] To a stirring solution of compound (**2S-FNL-23**) (350 mg, 0.86 mmol) in DCM (5 mL) was added TFA (985 mg, 0.86 mmol) at 0 °C and stirred to RT for 3 h. The reaction mixture was brought to RT and concentrated under vacuum to afford (**2S-FNL-24**) (250 mg, 95.4%) as white solid.

- 92 -

¹H-NMR: (400 MHz, D₂O): δ 8.84 (d, *J* = 5.2 Hz, 2H), 7.55 (t, *J* = 4.8 Hz, 1H), 4.90-4.67 (m, 3H), 4.10-4.06 (m, 3H), 3.94-3.92 (m, 1H), 3.57-3.51 (m, 2H), 2.54-2.43 (m, 2H), 2.28-2.19 (m, 2H);

LCMS: *m/z* 306.4 [*M*⁺+1];

5 **HPLC:** 90.07%.

Synthesis of (2*S*)-3-hydroxy-2-(5-isobutyryl-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-*N*-(pyrimidin-2-ylmethyl) propanamide (2*S*-FNL-25):

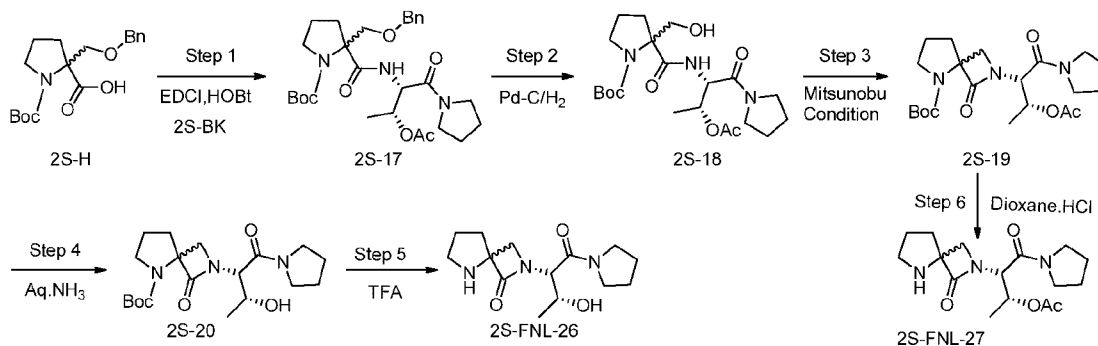
[00222] To a stirring solution of compound (2*S*-FNL-24) (500 mg, 1.63 mmol) in DCM (5 mL) was added TEA (0.7 mL, 4.91 mmol) at 0 °C. After added Int-F (207 mg, 1.95 mmol) slowly and stirred to RT for 3 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (20 mL). The organic layer was washed with citric acid (1 x 30 mL) followed by brine solution (1 x 30 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 5% MeOH/DCM to afford (2*S*-FNL-25) (100 mg, 16.3%) as white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 8.73 (t, *J* = 4.8 Hz, 2H), 7.37 (d, *J* = 5.2 Hz, 1H), 4.56-4.51 (m, 2H), 4.32-4.29 (m, 1H), 4.17-4.12 (m, 1H), 4.05-3.98 (m, 2H), 3.74-3.68 (m, 1H), 3.63-3.58 (m, 1H), 3.57-3.51 (m, 1H), 2.77-2.69 (m, 1H), 2.31-2.26 (m, 2H), 2.08-1.95 (m, 2H), 1.05-0.98 (m, 6H);

20 **LCMS:** *m/z* 376.4 [*M*⁺+1];

HPLC: 89.6% (both isomers)

Scheme 2S-13:



25

Synthesis of *tert*-butyl 2-(((2*S*,3*R*)-3-acetoxy-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)carbamoyl)-2-((benzyloxy)methyl)pyrrolidine-1-carboxylate (2*S*-17):

[00223] To a stirring solution of compound 2*S*-BK (1 g, 2.90 mmol) in DMF (8 mL) was added EDCL.HCl (0.63 g, 3.28 mmol) followed by HOBt (0.44 g, 3.28 mmol) at 0 °C. After being stirred for 5 min, DIPEA (1.3 mL, 7.46 mmol) followed by compound 2*S*-H (0.74 g, 3.58 mmol) was added to the reaction mixture and stirring was continued for another 16 h at RT. The reaction mixture was washed with water and extracted with EtOAc (2x 500 mL). The organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under vacuum. The crude was purified by column chromatography to afford compound 2*S*-17 (0.6 g, 38%).

¹H-NMR: (400 MHz, CDCl₃) (Rotamers): δ 7.34 (s, 5H), 5.37-5.34 (m, 1H), 4.84-4.80 (m, 1H), 4.72-4.65 (m, 2H), 4.09-4.02 (m, 1H), 3.91-3.87 (m, 1H), 3.65-3.61 (m, 3H), 3.52-3.46 (m, 3H), 2.41 (br s, 1H), 2.22-2.15 (m, 1H), 1.98 (d, 5H), 1.87-1.84 (m, 4H), 1.50-1.42 (m, 9H).

LCMS *m/z*: 532 [M⁺+1].

Synthesis of *tert*-butyl 2-(((2*S*,3*R*)-3-acetoxy-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)carbamoyl)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (2*S*-18):

[00224] To a stirring solution of compound 2*S*-17 (4.5 g, 8.40 mmol) in MeOH (40 mL) was added wet 10% Pd/C (1.5 g) under inert atmosphere and stirred for 4 h under H₂ atmosphere (balloon pressure). The reaction mixture was filtered through celite pad and concentrated under reduced pressure to afford compound 2*S*-18 (3.0 g, 81%).

LCMS *m/z*: 442.5 [M⁺+1].

Synthesis of *tert*-butyl 2-(((2*S*,3*R*)-3-acetoxy-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)-1-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2*S*-19):

[00225] To a stirring solution of compound 2*S*-18 (3 g, 6.70 mmol) in THF (25 mL) was added triphenylphosphine (2 g, 7.40 mmol) followed by DTAD (2.5 g, 10.2 mmol). The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 10% MeOH/CH₂Cl₂ to afford compound 2*S*-19 (1.2 g with TPPO, 43%).

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 5.25-5.19 (m, 1H), 4.65 (d, 1H), 3.61-3.57 (m, 3H), 3.47-3.42 (m, 2H), 3.41-3.25 (m, 4H), 2.05 (s, 4H), 1.95-1.71 (m, 7H), 1.42 (s, 10H).

LCMS *m/z*: 424.4 [M⁺+1].

- 94 -

Synthesis of *tert*-butyl 2-((2*S*, 3*R*)-3-hydroxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2*S*-20):

[00226] A solution of compound **2*S*-19** (0.15 g, 0.41 mmol) in aqueous NH₃ (2 mL) was stirred at RT for 4 h. After consumption of the starting material (by TLC), the reaction diluted
5 with CH₂Cl₂ (75 mL). The separated organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford compound **2*S*-20** (0.1 g, 76%).

LCMS *m/z*: 382 [M⁺+1] .

Synthesis of 2-((2*S*,3*R*)-3-hydroxy-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)-2,5 diazaspiro[3.4] octan-1-one. (2*S*-FNL-26):

10 [00227] To a stirring solution of compound **2*S*-20** (0.2 g, 0.63 mmol) in CH₂Cl₂ (2 mL) was added TFA (0.3 mL) at 0 °C and stirred at RT for 1 h. The reaction mixture was concentrated under vacuum. Obtained residue was diluted with water and extracted with CH₂Cl₂ (2x 25 mL). The separated organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under vacuum to afford (**2*S*-FNL-26**) (0.2 g, 80%) as TFA salt.

15 ¹H-NMR: (400 MHz, D₂O): δ 4.64 (t, 1H), 4.25-4.21 (m, 1H), 4.09 (d, 1H), 3.99-3.87 (m, 1H), 3.70 (t, 2H), 3.55-3.47 (m, 5H), 2.52-2.34 (m, 2H), 2.25-2.22 (m, 2H), 2.08-1.98 (m, 5H), 1.25 (t, 3H).

LCMS (ESI) *m/z*: 282.4 [M⁺+1].

Synthesis of (2*R*, 3*S*)-4-oxo-3-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-4-(pyrrolidin-1-yl) butan-2-yl acetate (2*S*-FNL-27):

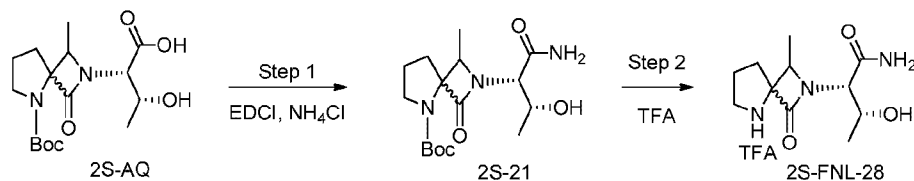
20 [00228] A stirring solution of compound **2*S*-19** (0.4 g, 0.94 mmol) in 1,4-dioxane/HCl (5 mL) was cooled to 0 °C and stirred at RT for 1 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure. Obtained crude material was washed with *n*-pentane followed by EtOAc to afford (**2*S*-FNL-27**) (0.22 g, 65%).

25 ¹H-NMR: (400 MHz, D₂O): δ 4.62 (d, 1H), 4.41-4.29 (m, 2H), 4.24 (d, 1H), 3.89-3.77 (m, 3H), 3.54-3.49 (m, 3H), 2.57-2.52 (m, 1H), 2.49 (s, 3H), 2.42-2.00 (m, 8H), 1.30 (d, 3H).

LCMS *m/z*: 324.3 [M⁺+1].

HPLC Purity: 99.37%.

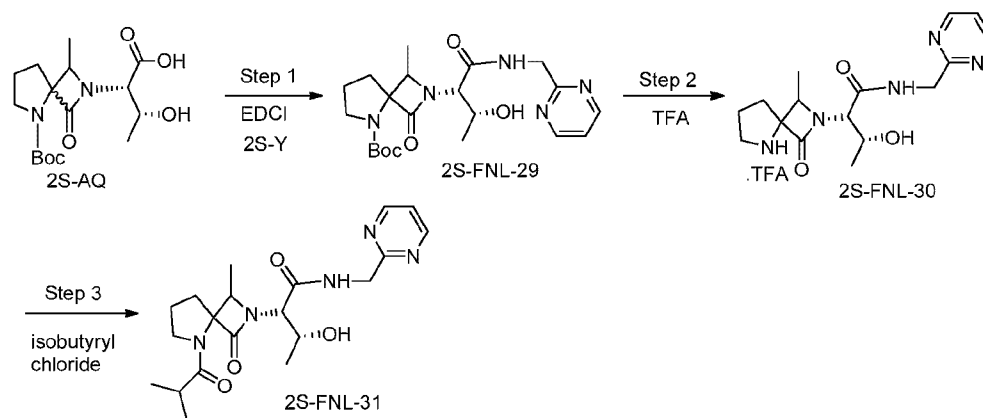
*Scheme 2*S*-14:*



Mass (ESI): m/z 483.1 $[2M^+ + 1]$

- 96 -

Scheme 2S-15:



Synthesis of tert-butyl 2-((2R, 3S)-3-hydroxy-1-oxo-1-((pyrimidin-2-ylmethyl) amino) butan-2-yl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-FNL-29):

[00231] To a stirring solution of compound 2S-AQ (500 mg, 1.46 mmol) in CH₂Cl₂ (15 mL) were added DIPEA (0.76 mL, 4.38 mmol), EDCI.HCl (334 mg, 1.75 mmol), HOBt (334 mg, 1.75 mmol) followed by 2S-Y (252 mg, 1.75 mmol) at 0 °C and stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (20 mL) and extracted with CH₂Cl₂ (2 x 30 mL). The combined organic layer was washed with citric acid solution (20 mL), NaHCO₃ (1 x 30 mL) followed by brine (1 x 50 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 2% MeOH/DCM to afford (2S-FNL-29) (200 mg, 31.6%) as white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 8.74-8.70 (m, 2H), 7.37-7.32 (m, 1H), 4.72-4.43 (m, 3H), 4.24-4.14 (m, 1H), 4.10-3.88 (m, 2H), 3.52-3.36 (m, 2H), 2.22-2.19 (m, 2H), 2.01-1.94 (m, 1H), 1.88-1.79 (m, 1H), 1.45-1.41 (m, 3H), 1.40 (s, 9H), 1.29-1.26 (m, 3H),

Mass (ESI): 434.5 [M⁺+1], HPLC:92.8%

Synthesis of (2S, 3R)-3-hydroxy-2-(1-methyl-3-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-N-(pyrimidin-2-ylmethyl) butanamide (2S-FNL-30):

[00232] To a stirring solution of compound (2S-FNL-29) (250 mg, 0.57 mmol) in DCM (10 mL) was added TFA (0.44 mL) under N₂ atmosphere and stirred for 2 h at RT. After consumption of the starting material (by TLC), the reaction mixture was concentrated under

- 97 -

reduced pressure. The obtained crude material was triturated with diethylether/n-pentane (5 mL/5 mL) and dried under reduced pressure to afford **(2S-FNL-30)** (180 mg, 94.7%) as semi solid (TFA salt).

¹H-NMR: (400 MHz, D₂O): δ 8.82 (d, *J* = 2.0 Hz, 2H), 7.53 (t, *J* = 4.8 Hz, 1H), 4.67-4.62 (m, 2H), 4.44-4.40 (m, 1H), 4.34-4.32 (m, 2H), 3.61-3.56 (m, 2H), 2.51-2.20 (m, 4H), 1.55-1.46 (m, 3H), 1.32-1.29 (m, 3H)

LCMS (ESI) : *m/z* 333.3

HPLC: 90.7%

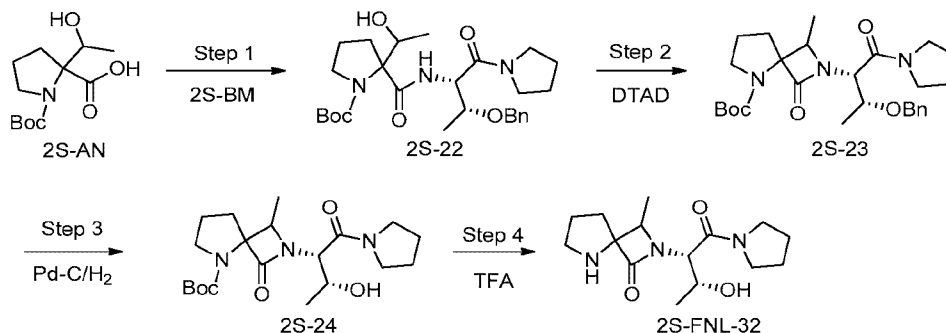
Synthesis of (2*S*, 3*R*)-3-hydroxy-2-(5-isobutyryl-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-*N*-(pyrimidin-2-ylmethyl) butanamide (2S-FNL-31):

[00233] To a stirring solution of compound **(2S-FNL-30)** (150 mg, 0.45 mmol) in DCM (5 mL) was added TEA (0.18 mL, 1.35 mmol) followed by isobutyryl chloride (57 mg, 0.54 mmol) at 0 °C under N₂ atmosphere and stirred for 2 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (5 mL) and extracted with CH₂Cl₂ (2 x 10 mL). The combined organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 2% MeOH/DCM to afford **(2S-FNL-31)** (85 mg, 47%) as semi solid.

¹H-NMR: (400 MHz, CD₃OD): δ 8.73 (d, *J* = 4.8 Hz, 2H), 7.36 (t, *J* = 4.8 Hz, 1H), 4.83-4.54 (m, 3H), 4.35-4.32 (m, 1H), 4.22-4.11 (m, 1H), 3.93-3.88 (m, 1H), 3.76-3.71 (m, 1H), 3.67-3.60 (m, 2H), 2.81-2.76 (m, 1H), 2.21-2.07 (m, 3H), 1.96-1.91 (m, 1H), 1.29-1.26 (m, 6H), 1.05-1.02 (m, 6H)

LCMS (ESI) : *m/z* 404.4

HPLC: 93.57%

Scheme 2S-16:

Synthesis of tert-butyl 2-(((2S, 3R)-3-(benzyloxy)-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl) carbamoyl)-2-(1-hydroxyethyl) pyrrolidine-1-carboxylate (2S-22):

5 [00234] To a stirring solution of compound **2S-AN** (2.5 g, 9.65 mmol) in CH₂Cl₂ (50 mL) were added compound **2S-BM** (2.7 g, 10.6 mmol), EDCI.HCl (2.7 g, 14.4 mmol) followed by HOBT (1.9 g, 14.4 mmol) and DIPEA (5.3 mL, 28.9 mmol) at 0 °C and stirred for 12 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (30 mL) and extracted with CH₂Cl₂ (2 x 50 mL). The separated organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 2% MeOH/DCM to afford compound **2S-22** (3.5 g, 73%) as colorless liquid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 8.11 (d, *J* = 9.0 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.33-7.26 (m, 5H), 6.56 (s, 1H), 4.68-4.63 (m, 1H), 4.56 (s, 2H), 3.80-3.74 (m, 1H), 3.55-3.33 (m, 5H), 1.76-1.66 (m, 7H), 1.40 (s, 9H), 1.37-1.24 (m, 2H), 1.08-0.97 (m, 6H).

Mass (ESI): *m/z* 504 [M⁺+1].

Synthesis of tert-butyl 2-(((2S, 3R)-3-(benzyloxy)-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-23):

20 [00235] To a stirring solution of compound **2S-22** (3.5 g, 6.95 mmol) in THF (50 mL) was added triphenylphosphine (3.6 g, 13.9 mmol) and DTAD (3.2 g, 13.9 mmol). The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting 30% EtOAc/hexane to afford compound **2S-23** (1.0 g, 30%) as pale yellow liquid.

- 99 -

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.63-7.54 (m, 1H), 7.41-7.24 (m, 4H), 4.60-4.37 (m, 3H), 3.98 (d, *J* = 10.0 Hz, 1H), 3.91 (d, *J* = 7.0 Hz, 1H), 3.77 (d, *J* = 7.0 Hz, 2H), 3.44-3.34 (m, 4H), 2.01-1.91 (m, 2H), 1.85-1.68 (m, 6H), 1.40 (s, 9H), 1.20-1.11 (m, 6H).

Mass (ESI): *m/z* 486.6 [*M*⁺+1].

5 **Synthesis of (2*S*, 3*R*)-2-(5-(tert-butoxycarbonyl)-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-hydroxybutanoic acid (2*S*-24):**

[00236] To a stirring solution of compound **2*S*-23** (1 g) in methanol (30 mL) was added 10% Pd/C (400 mg) at RT and stirred for 12 h under H₂ atmosphere (balloon pressure). After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol. Obtained filtrate was concentrated under reduced pressure to afford compound **2*S*-24** (230 mg, 28%) as white solid.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 4.79 (br s, 1H), 4.34 (br s, 1H), 4.27 (d, *J* = 8.5 Hz, 1H), 4.03-3.95 (m, 1H), 3.78 (d, *J* = 6.5 Hz, 1H), 3.67-3.63 (m, 1H), 3.53-3.49 (m, 2H), 3.39 (t, *J* = 9.0 Hz, 2H), 2.04-1.67 (m, 8H), 1.36 (s, 9H), 1.26 (d, *J* = 6.0 Hz, 3H), 1.08, 1.06 (dd, *J* = 6.5 Hz, 3H).

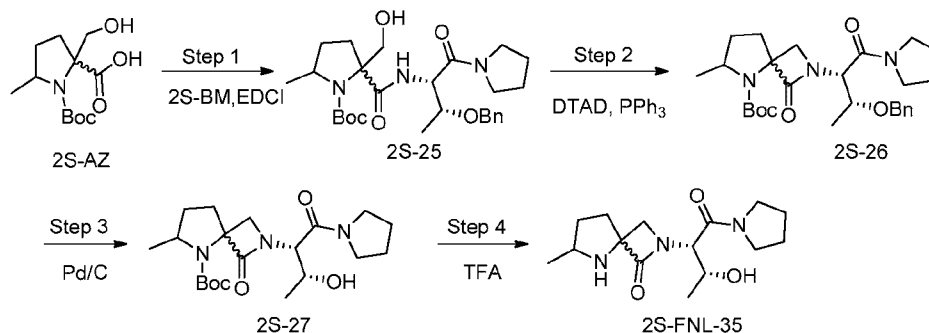
LCMS: 396.4 [*M*⁺+1].

Synthesis of 2-((2*S*, 3*R*)-3-hydroxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl)-3-methyl-2, 5-diazaspiro [3.4] octan-1-one (2*S*-FNL-32):

[00237] To a stirring solution of compound **2*S*-24** (230 mg, 0.58 mmol) in CH₂Cl₂ (2 mL) was added TFA (0.44 mL, 5.82 mmol) at 0 °C and stirred at RT for 2 h. After completion of reaction (by TLC), the reaction mixture was concentrated under reduced pressure to afford (**2*S*-FNL-32**) was triturated with pentane and diethyl ether (5 mL/5 mL) (210 mg, 92%) as sticky solid (TFA salt).

¹H-NMR: (400 MHz, D₂O): δ 4.27 (d, *J* = 8.0 Hz, 1H), 4.07-4.03 (m, 1H), 4.01-3.97 (m, 1H), 3.52-3.48 (m, 1H), 3.39-3.35 (m, 2H), 3.32-3.20 (m, 3H), 2.16 (t, *J* = 7.6 Hz, 2H), 2.06-1.96 (m, 2H), 1.89-1.80 (m, 2H), 1.78-1.74 (m, 2H), 1.43 (d, *J* = 6.4 Hz, 3H), 1.06 (d, *J* = 6.0 Hz, 3H);

- 101 -



Synthesis of tert-butyl 2-(((2S, 3R)-3-(benzyloxy)-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl) carbamoyl)-2-(hydroxymethyl)-5-methylpyrrolidine-1-carboxylate (2S-25):

[00240] To a stirring solution of compound **2S-AZ** (1.1 g, 4.28 mmol) in DCM (20 mL) were added *N, N*-diisopropylethylamine (2.2 mL, 12.8 mmol), **2S-BM** (1.2 g, 4.78 mmol), followed by EDCI (2.45 g, 12.8 mmol), HOBT (1.7 g, 12.8 mmol) at 0 °C and stirred at RT for 12 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL). The separated organic layer was washed with saturated NaHCO₃ solution (1x25 mL), followed by brine solution (1x30 mL). The separated organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by column chromatography eluting 5% MeOH/DCM to obtained compound **2S-25** (1.2 g, 57%) as a thick white syrup.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 7.93 (t, *J* = 7.6 Hz, 1H), 7.71-7.26 (m, 5H), 5.30 (br s, 1H), 4.65-4.61 (m, 1H), 4.57 (s, 2H), 3.93-3.85 (m, 2H), 3.57-3.34 (m, 2H), 3.17-3.09 (m, 2H), 2.07-1.94 (m, 2H), 1.77-1.73 (m, 4H), 1.36-1.28 (m, 10H), 1.20 (s, 9H);

LCMS: *m/z* 504.7 [M⁺+1].

Synthesis of tert-butyl 2-(((2S, 3R)-3-(benzyloxy)-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl)-6-methyl-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-26):

[00241] To a stirring solution of compound **2S-25** (0.6 g, 1.19 mmol) in THF (10 mL) was added triphenylphosphine (0.46 g, 1.78 mmol) and DTAD (0.4 g, 1.78 mmol). The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL). The separated organic layer was washed with brine solution (1x30 mL). The separated organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by column chromatography eluting 2% MeOH/DCM to obtained compound **2S-26** (0.2 g, 35%) as white solid.

- 102 -

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.63-7.26 (m, 5H), 4.52 (s, 2H), 3.91-3.77 (m, 3H), 3.56-3.36 (m, 4H), 2.35-2.11 (m, 4H), 1.94-1.68 (m, 6H), 1.39 (s, 9H), 1.13, 1.09 (dd, *J* = 6.0 Hz, 5.5 Hz, 3H), 1.04 (d, *J* = 6.5 Hz, 3H);

LCMS: *m/z* 486.6 [*M*⁺+1].

5 **Synthesis of tert-butyl 2-((2*S*, 3*R*)-3-(benzyloxy)-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl)-6-methyl-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2*S*-27):**

[00242] To a stirring solution of compound **2*S*-26** (1.5 g, 3.09 mmol) in methanol (20 mL) was added 10%Pd/C (200 mg) under N₂ atmosphere. The reaction mixture was stirred under H₂ atmosphere (balloon pressure) at RT for 4 h. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol. Obtained filtrate was concentrated under reduced pressure to afford crude compound which was purified by column chromatography to obtained compound **2*S*-27** (0.9 g, 90.9%) as white solid.

¹H-NMR: (500 MHz, CDCl₃): δ 4.11 (d, *J* = 7.0 Hz, 1H), 3.96-3.90 (m, 1H), 3.73 (s, 2H), 3.49 (d, *J* = 13.0 Hz, 2H), 3.40-3.34 (m, 2H), 2.50-2.28 (m, 4H), 2.17-1.82 (m, 6H), 1.53 (s, 9H), 1.52-1.41 (m, 3H), 1.36-1.18 (m, 3H);

LCMS: 396.5 [*M*⁺+1].

Synthesis of 2-((2*S*, 3*R*)-3-hydroxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl)-6-methyl-2, 5-diazaspiro [3.4] octan-1-one (2*S*-FNL-35):

20 [00243] To a stirring solution of compound **2*S*-27** (0.18 g, 0.45 mmol) in methanol (10 mL) was added TFA (3 mL) under N₂ atmosphere at 0 °C. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to afford crude compound which was triturated with n-pentane (10 mL) to obtained (**2*S*-FNL-35**) (0.1 g, 74.6%) as white solid.

25 **¹H-NMR:** (400 MHz, DMSO-*d*₆): δ 9.99 (br s, 1H), 9.57 (br s, 1H), 4.32 (d, *J* = 7.6 Hz, 1H), 3.93 (t, *J* = 6.0 Hz, 1H), 3.81-3.75 (m, 3H), 3.51-3.46 (m, 2H), 3.30 (t, *J* = 6.8 Hz, 2H), 2.29-2.20 (m, 3H), 1.93-1.75 (m, 4H), 1.68-1.63 (m, 1H), 1.34 (d, *J* = 6.8 Hz, 3H), 1.13 (d, *J* = 6.4 Hz, 3H);

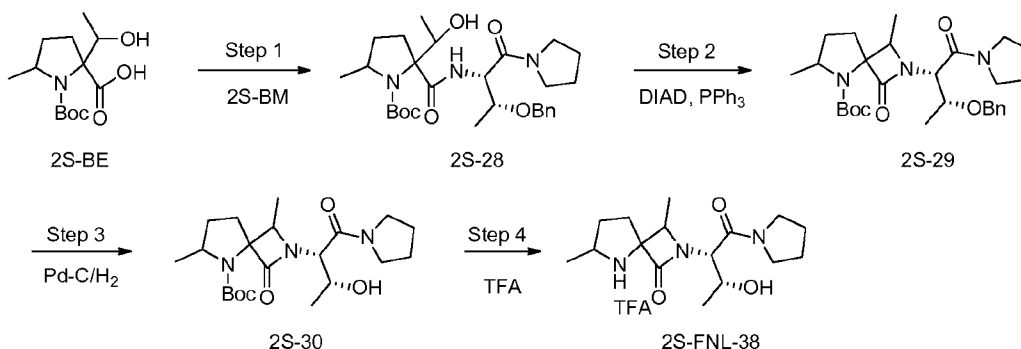
Mass (ESI): *m/z* 296.3 [*M*⁺+1]

30 ***Scheme 2*S*-19:***

LCMS (ESI): 256.4 $[M^+ + 1]$;

- 104 -

HPLC (ELSD):93.86%.

Scheme 2S-20:

5

Synthesis of *tert*-butyl 2-(((2*S*,3*R*)-3-(benzyloxy)-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)-1,6-dimethyl-3-oxo-2,5-diazaspiro [3.4] octane-5-carboxylate (2S-28):

[00246] To a stirring solution of compound **2S-BE** (2 g, 7.32 mmol) in DMF (20 mL) were added *N,N*-diisopropylethylamine (6.7 mL, 36.5 mmol), **2S-BM** (2.6 g, 8.7 mmol), followed by HATU (3.3g, 8.7 mmol) at 0 °C and stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (100 mL) and EtOAc (200 mL). The separated organic layer was washed with sodium bicarbonate solution (2 x 75 mL), citric acid solution (2 x 50 mL) followed by brine solution (1x 50 mL). The separated organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 40% EtOAc/*n*-hexane to obtain compound **2S-28** (1 g, 27%) as pale yellow liquid;

LCMS (ESI):518 [M⁺+1]

Synthesis of *tert*-butyl 2-((2*S*,3*R*)-3-(benzyloxy)-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)-1,6-dimethyl-3-oxo-2,5-diazaspiro [3.4] octane-5-carboxylate (2S-29):

[00247] To a stirring solution of triphenylphosphine (1.5 g, 5.7 mmol) in THF (10 mL) was added DIAD (976 mg, 4.8 mmol) as portion-wise and stirred for 20 min at RT. To this was added compound **2S-28** (1 g, 1.93 mmol) in THF (10 mL) slowly at RT and stirred for 4 h. After consumption of the starting material (by LCMS), the reaction mixture was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting 30% EtOAc/hexane to afford compound **2S-29** (500 mg, 63%) as yellow liquid.

- 105 -

¹H-NMR: (400 MHz, CDCl₃): δ 7.69-7.66 (m, 1H), 7.48-7.43 (m, 1H), 7.32-7.29 (m, 3H), 4.68 (s, 2H), 4.46-4.40 (m, 1H), 4.26-4.05 (m, 2H), 3.97-3.91 (m, 0.5H), 3.87-3.81 (m, 0.5H), 3.58-3.53 (m, 1H), 3.40-3.32 (m, 2H), 2.16-2.11 (m, 1H), 2.04-1.90 (m, 2H), 1.80-1.71 (m, 2H), 1.41 (s, 9H), 1.32-1.21 (m, 10H), 1.17-1.15 (m, 3H).

5 LCMS (ESI): 500 [M⁺+1].

Synthesis of *tert*-butyl 2-((2*S*, 3*R*)-3-hydroxy-1-oxo-1-(pyrrolidin-1-yl) butan-2-yl)-1, 6-dimethyl-3-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2*S*-30):

[00248] To a stirring solution of compound 2*S*-29 (200 mg, 0.40 mmol) in methanol (5 mL) was added 10% Pd/C (50 mg) under N₂ atmosphere. The reaction mixture was stirred under H₂ atmosphere at RT for 4 h. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol (10 mL). Obtained filtrate was concentrated under reduced pressure to obtain crude compound, which was purified by column chromatography eluting 1% MeOH/DCM to afford compound 2*S*-30 (100 g, 61%) as yellow syrup.

15 ¹H-NMR: (400 MHz, DMSO-*d*₆): δ 4.93 (d, *J* = 5.6 Hz, 1H), 4.26 (d, *J* = 9.2 Hz, 0.5 H), 4.17 (d, *J* = 7.2 Hz, 0.5 H), 4.02-3.99 (m, 1H), 3.91-3.66 (m, 3H), 3.33-3.30 (m, 1H), 3.55-3.50 (m, 1H), 3.19-3.16 (m, 1H), 2.69 (s, 1H), 2.13-2.03 (m, 1H), 1.99-1.87 (m, 3H), 1.81-1.75 (m, 2H), 1.56-1.50 (m, 1H), 1.39 (s, 9H), 1.19 (d, *J* = 5.6 Hz, 3H), 1.13 (d, *J* = 6.4 Hz, 6H).

LCMS: 410.5 [M⁺+1].

20 **Synthesis of 2-((2*S*, 3*R*)-3-hydroxy-1-oxo-1-(pyrrolidin-1-yl)butan-2-yl)-3,6-dimethyl-2,5-diazaspiro[3.4]octan-1-one (2*S*-FNL-38):**

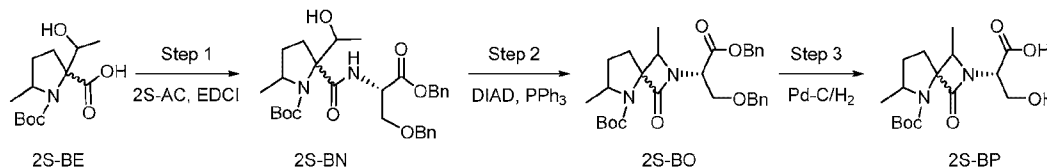
[00249] To a stirring solution of compound 2*S*-30 (300 mg, 0.73 mmol) in DCM (20 mL) was added TFA (418 mg, 3.66 mmol) at 0 °C under N₂ atmosphere. The reaction mixture was stirred at RT for 4 h. After consumption of the starting material (by TLC), the reaction mixture was evaporated under reduced pressure to afford crude, which was purified by preparative HPLC method to afford (2*S*-FNL-38) (140 mg, 46%) as thick syrup.

¹H-NMR: (400 MHz, D₂O): δ 4.53-4.46 (m, 1H), 4.34-4.22 (m, 2H), 4.03 (d, *J* = 6.4 Hz, 1H), 3.68 (s, 2H), 3.52-3.41 (m, 2H), 2.44-2.37 (m, 3H), 2.03-1.94 (m, 5H), 1.56 (d, *J* = 6.4 Hz, 6H), 1.27 (d, *J* = 6.0 Hz, 3H).

30 LCMS (ESI): 310 [M⁺+1].

Scheme 2*S*-I-15

- 106 -



Synthesis of *tert*-butyl 2-(((*S*)-1, 3-bis (benzyloxy)-1-oxopropan-2-yl) carbamoyl)-2-(1-hydroxyethyl)-5-methylpyrrolidine-1-carboxylate (2S-BN):

5 [00250] To a stirring solution of **2S-BE** (3 g, 10.98 mmol) in DCM (30 mL) were added *N, N*-diisopropylethylamine (5.73 mL, 32.96 mmol), 2S-AC (3.75 g, 13.17 mmol) followed by HATU (5 g, 13.17 mmol) at 0 °C and stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (20 mL). The separated organic layer was washed with brine solution (30 mL). The organic layer was dried over anhydrous

10 Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 20% EtOAc/*n*-hexane to obtained compound **2S-BN** (2.9 g, 49%) as brown thick syrup.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 8.50 (m, 1H), 7.33-7.27 (m, 10H), 5.68-5.60 (m, 1H), 5.22-5.09 (m, 2H), 4.72-4.43 (m, 3H), 3.89-3.63 (m, 3H), 2.28-1.78 (m, 3H), 1.45-1.42 (m,

15 1H), 1.36 (s, 9H), 1.26-1.04 (m, 6H);

LCMS (ESI): *m/z* 541.6 [M⁺+1]

Synthesis of *tert*-butyl 2-((*S*)-1,3-bis(benzyloxy)-1-oxopropan-2-yl)-1,6-dimethyl-3-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-BO):

[00251] To a stirring solution of triphenylphosphine (3.51 g, 13.42 mmol) in dry THF

20 (30 mL) was added DIAD (2.21 g, 10.74 mmol) as portionwise and stirred for 15 min at RT. To this precipitated solution added 2S-BN (2.9 g, 5.37 mmol) in dry THF (15 mL) slowly at RT and stirred for 16 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure. The crude material was triturated with 30% di ethylether/*n*-pentane. The filtrate was concentrated under reduced pressure to obtained crude

25 compound which was purified by silica gel column chromatography eluting 30% EtOAc/hexane to afford **2S-BO** (2.5 g, 89.2%) as brown thick syrup.

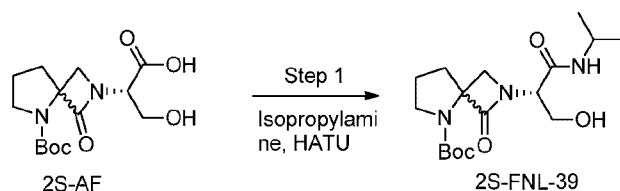
¹H-NMR: (500 MHz, DMSO-*d*₆): δ 7.38-7.25 (m, 10H), 5.22-5.15 (m, 2H), 4.80-4.73 (m, 2H), 4.56-4.43 (m, 2H), 3.92-3.60 (m, 3H), 1.89-1.83 (m, 3H), 1.50-1.44 (m, 1H), 1.40 (s, 9H), 1.22-1.18 (s, 3H), 1.16-1.13 (m, 3H);

- 107 -

LCMS (ESI): m/z 523.6 [$M^+ + 1$]**Synthesis of (2S)-2-(5-(*tert*-butoxycarbonyl)-1, 6-dimethyl-3-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-hydroxypropanoic acid (2S-BP):**

[00252] To a stirring solution of **2S-BO** (2.5 g, 4.78 mmol) in methanol (50 mL) was added 10% Pd/C (800 mg) under N_2 atmosphere. The reaction mixture was stirred under H_2 atmosphere at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and the pad was washed with methanol (30 mL). Obtained filtrate was concentrated under reduced pressure to afford crude which was triturated with *n*-pentane (30 mL) to afford **2S-BP** (900 mg, 56.2%) as sticky solid.

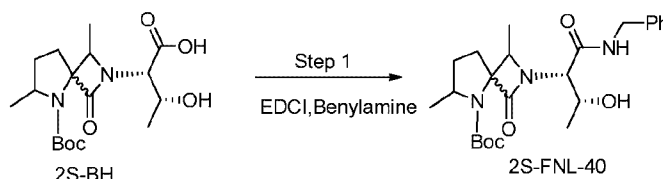
1H -NMR: (400 MHz, DMSO- d_6): δ 4.78-4.75 (m, 1H), 4.24-4.18 (m, 1H), 3.86-3.81 (m, 1H), 3.80-3.72 (m, 2H), 3.64-3.59 (m, 1H), 2.15-1.93 (m, 3H), 1.55-1.50 (m, 1H), 1.39 (s, 9H), 1.24-1.10 (m, 6H);

LCMS (ESI): m/z 343.3 [$M^+ + 1$]**Scheme 2S-21:****Synthesis of *tert*-butyl 2-((*S*)-3-hydroxy-1-(isopropylamino)-1-oxopropan-2-yl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (2S-FNL-39):**

[00253] To a stirring solution of **2S-AF** (200 mg, 0.63 mmol) in CH_2Cl_2 (10 mL) were added DIPEA (0.32 mL, 1.90 mmol), isopropyl amine (0.08 mL, 0.94 mmol), HATU (287 mg, 0.75 mmol) at 0 °C and stirred to RT for 5 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL). The separated organic layer was washed with citric acid (1 x 20 mL) followed by brine solution (1 x 20 mL). The organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure. Obtained crude material was purified by preparative HPLC purification to afford (**2S-FNL-39**) (150 mg, 67.2%) as white solid.

1H -NMR: (400 MHz, DMSO- d_6): δ 7.76 (d, J = 8.4 Hz, 1H), 5.01-4.91 (m, 1H), 4.70 (t, J = 6.0 Hz, 1H), 4.14-4.07 (m, 1H), 3.99-3.80 (m, 2H), 3.78-3.61 (m, 2H), 3.58-3.35 (m, 2H), 2.20-2.05 (m, 2H), 1.85-1.77 (m, 2H), 1.43 (s, 9H), 1.10-1.00 (m, 6H)

- 108 -

Mass (ESI): m/z 356.6 [$M^+ + 1$]**HPLC:** 99.27%**Scheme 2S-22:**

5

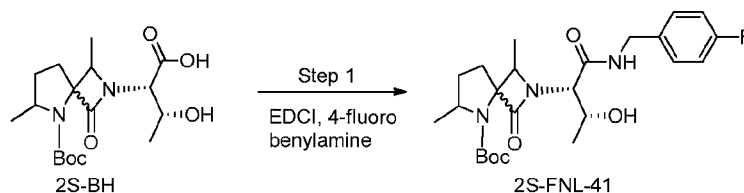
Synthesis of *tert*-butyl 2-((2*S*,3*R*)-1-(benzylamino)-3-hydroxy-1-oxobutan-2-yl)-1,6-dimethyl-3-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-FNL-40):

[00254] To a stirring solution of **2S-BH** (250 mg, 0.70 mmol) in CH_2Cl_2 (10 mL) were added DIPEA (0.36 mL, 2.11 mmol), EDCI (161 mg, 0.84 mmol), HOBt (129 mg, 0.84 mmol) followed by benzylamine (82 mg, 0.77 mmol) at 0 °C and stirred for 12 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 5% MeOH/DCM to afford (**2S-FNL-40**) (55 mg, 16%) as an off-white solid.

15 **$^1\text{H-NMR}$** : (400 MHz, $\text{DMSO-}d_6$): δ 7.31-7.20 (m, 5H), 4.92-4.87 (m, 1H), 4.64-4.55 (m, 1H), 4.46-4.37 (m, 2H), 4.22-4.10 (m, 1H), 4.02-3.90 (m, 2H), 2.39-1.95 (m, 3H), 1.70-1.60 (m, 1H), 1.37 (s, 9H), 1.30-1.22 (m, 9H);

LCMS (ESI): m/z 446.56 [$M^+ + 1$];**HPLC:** 89.54%

20

Scheme 2S-23:

Synthesis of *tert*-butyl 2-((2*S*,3*R*)-1-((4-fluorobenzyl)amino)-3-hydroxy-1-oxobutan-2-yl)-1,6-dimethyl-3-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-FNL-41):

- 109 -

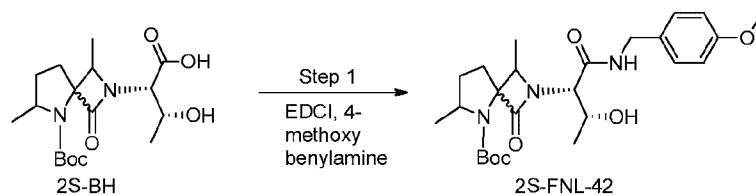
[00255] To a stirring solution of **2S-BH** (500 mg, 1.40 mmol) in CH₂Cl₂ (10 mL) were added DIPEA (0.73 mL, 4.21 mmol), EDCI (321 mg, 1.68 mmol), HOBt (257 mg, 1.68 mmol) followed by 4-fluoro benzylamine (175 mg, 1.40 mmol) at 0 °C and stirred for 12 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (15 mL). The separated organic layer was washed with citric acid solution (1 x 25 mL) followed by brine solution (1 x 25 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 5% MeOH/DCM followed by preparative HPLC purification to afford (**2S-FNL-41**) (150 mg, 23.07%) as white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 7.34-7.30 (m, 2H), 7.02-6.98 (m, 2H), 4.65-4.59 (m, 1H), 4.55-4.36 (m, 2H), 4.34-4.20 (m, 1H), 4.12-3.99 (m, 2H), 2.39-2.31 (m, 1H), 2.19-2.01 (m, 2H), 1.71-1.62 (m, 1H), 1.40 (s, 9H), 1.29-1.13 (m, 9H);

LCMS (ESI): *m/z* 464.5 [M⁺+1];

HPLC: 96.32%

Scheme 2S-24:



Synthesis of *tert*-butyl 2-((2*S*, 3*R*)-3-hydroxy-1-((4-methoxybenzyl)amino)-1-oxobutan-2-yl)-1, 6-dimethyl-3-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (**2S-FNL-42**):

[00256] To a stirring solution of **2S-BH** (250 mg, 0.70 mmol) in CH₂Cl₂ (10 mL) were added DIPEA (0.36 mL, 2.11 mmol), EDCI (161 mg, 0.84 mmol), HOBt (129 mg, 0.84 mmol) followed by 4-methoxy benzylamine (106 mg, 0.77 mmol) at 0 °C and stirred for 12 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL). The separated organic layer was washed with citric acid solution (1 x 20 mL) followed by brine solution (1 x 25 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by

- 110 -

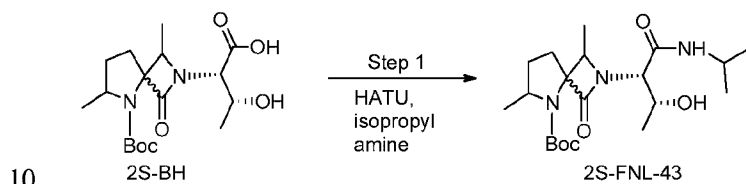
column chromatography by eluting 5% MeOH/DCM to afford **(2S-FNL-42)** (60 mg, 17.9%) as an off-white solid.

¹H-NMR: (400 MHz, CD₃OD): δ 7.24 (d, *J* = 1.6 Hz, 2H), 6.85 (d, *J* = 1.6 Hz, 2H), 4.64-4.58 (m, 1H), 4.39-4.28 (m, 1H), 4.21-4.08 (m, 2H), 4.06-3.99 (m, 1H), 3.98-3.88 (m, 1H), 3.83 (s, 3H), 2.39-2.28 (m, 1H), 2.22-2.13 (m, 1H), 2.09-1.97 (m, 1H), 1.71-1.61 (m, 1H), 1.40 (s, 9H), 1.31-1.22 (m, 9H);

LCMS (ESI): *m/z* 476.6 [*M*⁺+1];

HPLC: 90.29%

Scheme 2S-25:



Synthesis of *tert*-butyl 2-((2*S*,3*R*)-3-hydroxy-1-(isopropylamino)-1-oxobutan-2-yl)-1,6-dimethyl-3-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-FNL-43):

[00257] To a stirring solution of **2S-BH** (500 mg, 1.40 mmol) in CH₂Cl₂ (10 mL) were added DIPEA (0.73 mL, 4.21 mmol), isopropyl amine (100 mg, 1.68 mmol), HATU (798 mg, 2.1 mmol) at 0 °C and stirred for 12 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL). The separated organic layer was washed with citric acid solution (15 mL) followed by brine solution (15 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 2% MeOH/DCM followed by preparative HPLC purification to afford **(2S-FNL-43)** (100 mg, 18%) as white solid.

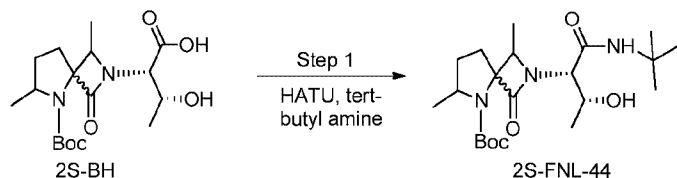
¹H-NMR: (400 MHz, D₂O): δ 4.42-3.89 (m, 5H), 2.38-2.04 (m, 3H), 1.77-1.72 (m, 1H), 1.40 (s, 9H), 1.36-1.17 (m, 15H)

LCMS (ESI): *m/z* 398.5 [*M*⁺+1];

HPLC: 93.36%

Scheme 2S-26:

- 111 -



Synthesis of *tert*-butyl 2-((2*S*,3*R*)-1-(*tert*-butylamino)-3-hydroxy-1-oxobutan-2-yl)-1,6-dimethyl-3-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-FNL-44):

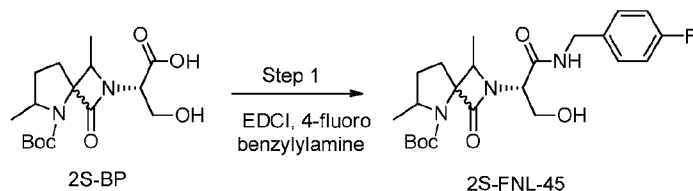
[00258] To a stirring solution of **2S-BH** (500 mg, 1.40 mmol) in CH₂Cl₂ (10 mL) were
 5 added DIPEA (0.62 mL, 3.51 mmol), *tert*-butyl amine (125 mg, 1.68 mmol), HATU (798 mg, 2.1 mmol) at 0 °C and stirred for 12 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (10 mL). The separated organic layer was washed with citric acid solution (15 mL) followed by brine solution (15 mL). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford
 10 crude compound which was purified by column chromatography by eluting 2% MeOH/DCM followed by preparative HPLC purification to afford (**2S-FNL-44**) (100 mg, 17.3%) as white solid.

¹H-NMR: (500 MHz, CD₃OD): δ 4.53-4.50 (m, 1H), 4.08-3.99 (m, 2H), 3.82-3.79 (m, 1H), 2.38-2.34 (m, 1H), 2.20-2.17 (m, 2H), 2.09-2.01 (m, 1H), 1.71-1.67 (m, 1H), 1.40 (s, 9H), 1.38
 15 (s, 9H), 1.33-1.21 (m, 9H);

LCMS (ESI): *m/z* 412.5 [M⁺+1];

HPLC: 93.91%

Scheme 2S-27:



Synthesis of *tert*-butyl 2-((*S*)-1-((4-fluorobenzyl) amino)-3-hydroxy-1-oxopropan-2-yl)-1,6-dimethyl-3-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (2S-FNL-45):

[00259] To a stirring solution of **2S-BP** (200 mg, 0.58 mmol) in DCM (10 mL) were
 added *N,N*-diisopropylethylamine (0.3 mL, 1.75 mmol), EDCI (133 mg, 0.69 mmol), HOBT
 (93 mg, 0.69 mmol) followed by 4-fluoro benzylamine (79.7 mg, 0.63 mmol) at 0 °C and

- 112 -

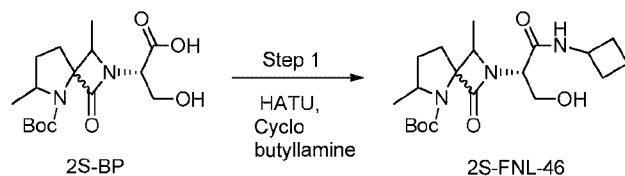
stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (20 mL). The separated organic layer was washed with citric acid (20 mL) followed by brine solution (30 mL). The separated organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 3% MeOH/DCM to obtained **(2S-FNL-45)** (46 mg, 17.7%) as thick syrup.

¹H-NMR: (500 MHz, DMSO-*d*₆): δ 8.63-8.59 (m, 1H), 7.30-7.26 (m, 2H), 7.15-7.07 (m, 2H), 5.07-5.00 (m, 1H), 4.31-4.21 (m, 3H), 3.89-3.62 (m, 4H), 2.13-1.84 (m, 3H), 1.58-1.52 (m, 1H), 1.36 (s, 9H), 1.32-1.20 (m, 3H), 1.18-1.13 (m, 3H);

LCMS (ESI): *m/z* 450.5 [M⁺+1]

HPLC: 93%

Scheme 2S-28:



Synthesis of *tert*-butyl 2-((*S*)-1-((4-fluorobenzyl) amino)-3-hydroxy-1-oxopropan-2-yl)-1,6-dimethyl-3-oxo-2,5-diazaspiro [3.4] octane-5-carboxylate (2S-FNL-46):

[00260] To a stirring solution of **2S-BP** (500 mg, 1.46 mmol) in DCM (15 mL) were added *N, N*-diisopropylethylamine (0.76 mL, 4.38 mmol), cyclobutylamine (124 mg, 1.75 mmol) followed by HATU (665 mg, 1.75 mmol) at 0 °C and stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (20 mL). The separated organic layer was washed with citric acid (20 mL) followed by brine solution (30 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 3% MeOH/DCM to obtained **(2S-FNL-46)** (110 mg, 19%) as an off-white solid.

¹H-NMR: (400 MHz, DMSO-*d*₆): δ 8.23 (d, *J* = 8.0 Hz, 1H), 4.98-4.83 (m, 1H), 4.30-4.13 (m, 2H), 3.95-3.76 (m, 2H), 3.72-3.66 (m, 2H), 2.49-1.89 (m, 3H), 1.64-1.54 (m, 3H), 1.48 (s, 9H), 1.19-1.12 (m, 10H);

LCMS (ESI): *m/z* 396.5 [M⁺+1]

HPLC: 96.6%

- 113 -

Example 3 - [³H] MK-801 binding assayMethods

[00261] Assays were conducted as described in Moskal et al. (Moskal, J.R., Kuo, A.G., Weiss, C., Wood, P.L., O'Connor Hanson, A., Kelso, S., Harris, R.B., Disterhoft, J.F., 2005.

- 5 GLYX-13; a monoclonal antibody-derived peptide that acts as an N-methyl-D-aspartate receptor modulator. *Neuropharmacology*. 49, 1077-87) The potentiation of [³H]MK-801 binding (5 nM; 22.5 Ci / mmol) to well washed rat cortical membranes (200 µg) was measured under non-equilibrium conditions (15 min @ 25 °C) in the presence of increasing concentrations of test compounds and 50µM glutamate. Zero levels were determined in the
- 10 absence of any glycine ligand and in the presence of 30µM 5,7 DCKA. Maximal stimulation was measured in the presence of 1 mM glycine, and 50µM glutamate was present in all samples. The facilitation of [³H]MK-801 binding by tests compounds was calculated by using a 3 parameter log agonist vs. response equation (Graph pad Prism, USA) and potency (EC₅₀, expressed in pM) and maximal activity (% maximal stimulation) were calculated for the test
- 15 compound.

Results

[00262] As shown in Table 2 and Figure 1, the pEC₅₀ and maximal activity for Compound X are -7.4 and 38%.

20 **Table 2.**

Compound	pEC ₅₀	Activity (%)
X	-7.4	38

Table 3. Additional Biological Data

Compound	[³ H] MK-801 binding assay: EC ₅₀ (M)	Unified Activity Data: LTP Augmentation (Percent)	Unified Activity Data: LTP Concentration (uM)	Unified Activity Data: LTP, Significant (S) or Non-significant (NS)	Unified Activity Data: Porsolt Floating Time Inhibition (Percent)	Unified Activity Data: Porsolt Dose (mg/kg)	Unified Activity Data: Porsolt Dose, route	Unified Activity Data: Porsolt Time Post Dose (Hours)
25-FNL-3	5.43E-08	130	1	S	80	3	IV	1
25-		80	0.1	NS				

- 114 -

FNL-21								
25-FNL-7	1.1E-08							
25-FNL-27	3.49E-12							
25-FNL-34		100	0.1	S	73	1	PO	1

Example 4- Long Term Potentiation in Hippocampal Slices

Methods

[00263] Assays were conducted as described in Zhang et al. (Zhang, X.L., Sullivan, J.A., Moskal, J.R., Stanton, P.K., 2008. A NMDA receptor glycine site partial agonist, GLYX-13, simultaneously enhances LTP and reduces LTD at Schaffer collateral-CA1 synapses in hippocampus. *Neuropharmacology*. 55, 1238-50) Sprague-Dawley rats (12–18 days old; Taconic Farms) were deeply anesthetized with isoflurane and decapitated. Rat brains were removed rapidly, submerged in ice-cold artificial cerebrospinal fluid (ACSF, 2–4 °C), which contained (in mM): 124 NaCl, 4 KCl, 2 MgSO₄, 2 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 glucose; at pH 7.4, gassed continuously with 95% O₂/5% CO₂). The rat brains were hemisected, the frontal lobes cut off, and individual hemispheres glued using cyanoacrylate adhesive onto a stage immersed in ice-cold ACSF gassed continuously with 95% O₂/5% CO₂ during slicing. Coronal slices (400 µm thick) were cut using a Vibratome (Leica VT1200S), and transferred to an interface holding chamber for incubation at room temperature for a minimum of one hour before transferring to a Haas-style interface recording chamber continuously perfused at 3 ml/min with oxygenated ACSF at 32 ± 0.5 °C. Low resistance recording electrodes were made from thin-walled borosilicate glass (1–2 MΩ after filling with ACSF) and inserted into the apical dendritic region of the Schaffer collateral termination field in stratum radiatum of field CA1 region to record field excitatory postsynaptic potentials (fEPSPs). A bipolar stainless steel stimulating electrode (FHC Co.) was placed on Schaffer collateral-commissural fibers in CA3 stratum radiatum, and constant current stimulus intensity adjusted to evoke approximately half-maximal fEPSPs once each 30 s (50–100 pA; 100 µs duration). fEPSP slope was measured before and after induction of LTP by linear interpolation from 20 to 80% of maximum negative deflection, and slopes confirmed to be stable to within ± 10% for at least 15 min before commencing an experiment. Bath application of the test compound (1 µM) was applied 30 min prior to application of Schaffer collateral stimulus trains

- 115 -

to elicit LTP. LTP was induced by stimulation of Schaffer collateral axons with four high frequency theta burst stimulus trains of 10×100 Hz/5 pulse bursts each, applied at an inter-burst interval of 200 ms. Each train was 2 seconds in duration, and trains were applied 15 seconds apart. The signals were recorded using a Multiclamp 700B amplifier and digitized with

5 a Digidata 1322 (Axon Instruments, USA). Data were analyzed using pClamp software (version 9, Axon Instruments) on an IBM-compatible personal computer.

Results

[00264] As shown in Figure 2, Compound X tested at 1 μ M increased long-term potentiation after high frequency stimulation of rat Schaffer collateral-evoked NMDA e.p.s.c.s recorded in

10 CA1 pyramidal neurons.

Table 4. Additional Biological Data

Compound	MK-801 Glycine Site Binding Assay: Rat Cortex EC50 (M)	LTP: LTP Augmentation (%)	LTP: LTP Concentration (uM)	LTP: LTP Significance, S or NS
25-FNL-38	3.313E-09			
25-FNL-2	2.002E-08			
25-FNL-10	1.188E-12	90	1	NS
25-FNL-14	6.133E-11	120	1	NS
25-FNL-33	1.89E-08	140	1	S

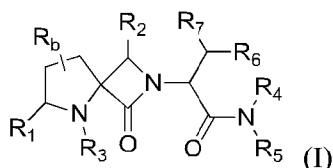
EQUIVALENTS

[00265] Those skilled in the art will recognize, or be able to ascertain using no more than

15 routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. A compound represented by formula I:



or a pharmaceutically acceptable salt, a stereoisomer, or an N-oxide thereof, wherein

5 R_b is selected from the group consisting of H, halogen, hydroxyl, cyano and C₁-C₆ alkyl;

R₁ is H or C₁-C₆ alkyl;

R₂ is H or C₁-C₆ alkyl;

10 R₃ is selected from the group consisting of H, C₁-C₆ alkyl and a nitrogen protecting group;

wherein the nitrogen protecting group is selected from the group consisting of 9-fluorenylmethyloxycarbonyl, tert-butoxycarbonyl, p-methoxybenzyloxycarbonyl, acetyl, trifluoroacetyl, benzoyl, benzyl, p-methoxybenzyl, p-methoxyphenyl, 3,4-dimethoxybenzyl, triphenylmethyl, p-toluenesulfonyl, -C(O)OR₃₁ and -C(O)R₃₂; wherein

15 R₃₁ is selected from the group consisting of C₁-C₆ alkyl, C₁-C₆ haloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₁₀ cycloalkyl, -CH₂-C₃-C₁₀ cycloalkyl, -CH₂-phenyl, and -CH₂-pyridyl, wherein any aforementioned cycloalkyl is optionally substituted with from 1-3 independently selected C₁-C₃ alkyl, and wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C₁-C₃ alkyl, C₁-C₃ haloalkyl, C₁-C₃ alkoxy, C₁-C₃ haloalkoxy, nitro, halo, SO₂Me, cyano, and -OC(O)CH₃; and

20 R₃₂ is selected from the group consisting of H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, phenyl, and pyridyl, wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C₁-C₃ alkyl, C₁-C₃ haloalkyl, C₁-C₃ alkoxy, C₁-C₃ haloalkoxy, nitro, halo, SO₂Me, cyano, and -OC(O)CH₃;

25 R₄ and R₅ are each independently selected from the group consisting of H, C₁-C₆ alkyl, X, and -C₁-C₆ alkylene-X, wherein X is selected from the group consisting of:

(i) C₃-C₆ cycloalkyl;

(ii) heteroaryl including from 5 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S;

(iii) heterocyclyl including from 3 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and

(iv) phenyl;

5 wherein C₃-C₆ cycloalkyl and heterocyclyl are each unsubstituted or substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, oxo, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'; and heteroaryl and phenyl are each unsubstituted or substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -
10 N(R')R';

or R₄ and R₅ together with the nitrogen to which they are attached form:

heterocyclyl including from 4 to 6 ring atoms; wherein the heterocyclyl includes not more than two ring heteroatoms, and the second ring heteroatom, when present, is independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and
15 wherein the heterocyclyl is unsubstituted or substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, oxo, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'; or

heteroaryl including from 5 to 6 ring atoms; wherein the heteroaryl includes not more than four ring heteroatoms, and each additional ring heteroatom, when present, is independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and
20 wherein the heteroaryl is unsubstituted or substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R';

R₆ is selected from the group consisting of -OH, C₁-C₆ alkoxy, -OC(O)-C₁-C₆ alkyl, -OC(O)phenyl, and -N(R')R';
25 alkyl, -OC(O)phenyl, and -N(R')R';

R₇ is H or C₁-C₆ alkyl; and

R' is independently selected for each occurrence from H and C₁-C₆ alkyl.

2. The compound of claim 1, wherein R₁ is H.
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3. The compound of claim 1 or 2, wherein R₂ is H.

4. The compound of any one of claims 1-3, wherein R₃ is H.

5. The compound of any one of claims 1-3, wherein R₃ is a nitrogen protecting group.
6. The compound of claim 5, wherein R₃ has formula –C(O)OR₃₁.
- 5 7. The compound of claim 6, wherein R₃₁ is C₁-C₆ alkyl.
8. The compound of claim 7, wherein R₃₁ is *tert*-butyl.
- 10 9. The compound of claim 5, wherein R₃ has formula –C(O)R₃₂.
10. The compound of claim 9, wherein R₃₂ is C₁-C₆ alkyl.
11. The compound of claim 10, wherein R₃₂ is –CH₃ or *iso*-propyl.
- 15 12. The compound of any one of claims 1-11, wherein R₄ and R₅ are each independently selected from the group consisting of H, C₁-C₆ alkyl, and –C₁-C₆ alkylene-X.
- 20 13. The compound of claim 12, wherein R₄ and R₅ are each independently selected from the group consisting of H and –C₁-C₆ alkylene-X.
14. The compound of claim 13, wherein one of R₄ and R₅ is H, and the other is –C₁-C₆ alkylene-X.
- 25 15. The compound of any one of claims 1 and 12-14, wherein –C₁-C₆ alkylene-X is –CH₂-X.
16. The compound of any one of claims 1 and 12-15, wherein X is phenyl or
30 heteroaryl including from 5 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; each unsubstituted or substituted with from 1-3 substituents independently selected from

the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'.

17. The compound of any one of claims 1-13, wherein R₄ and R₅ are H.
18. The compound of any one of claims 1-11, wherein R₄ and R₅ together with the nitrogen to which they are attached form:

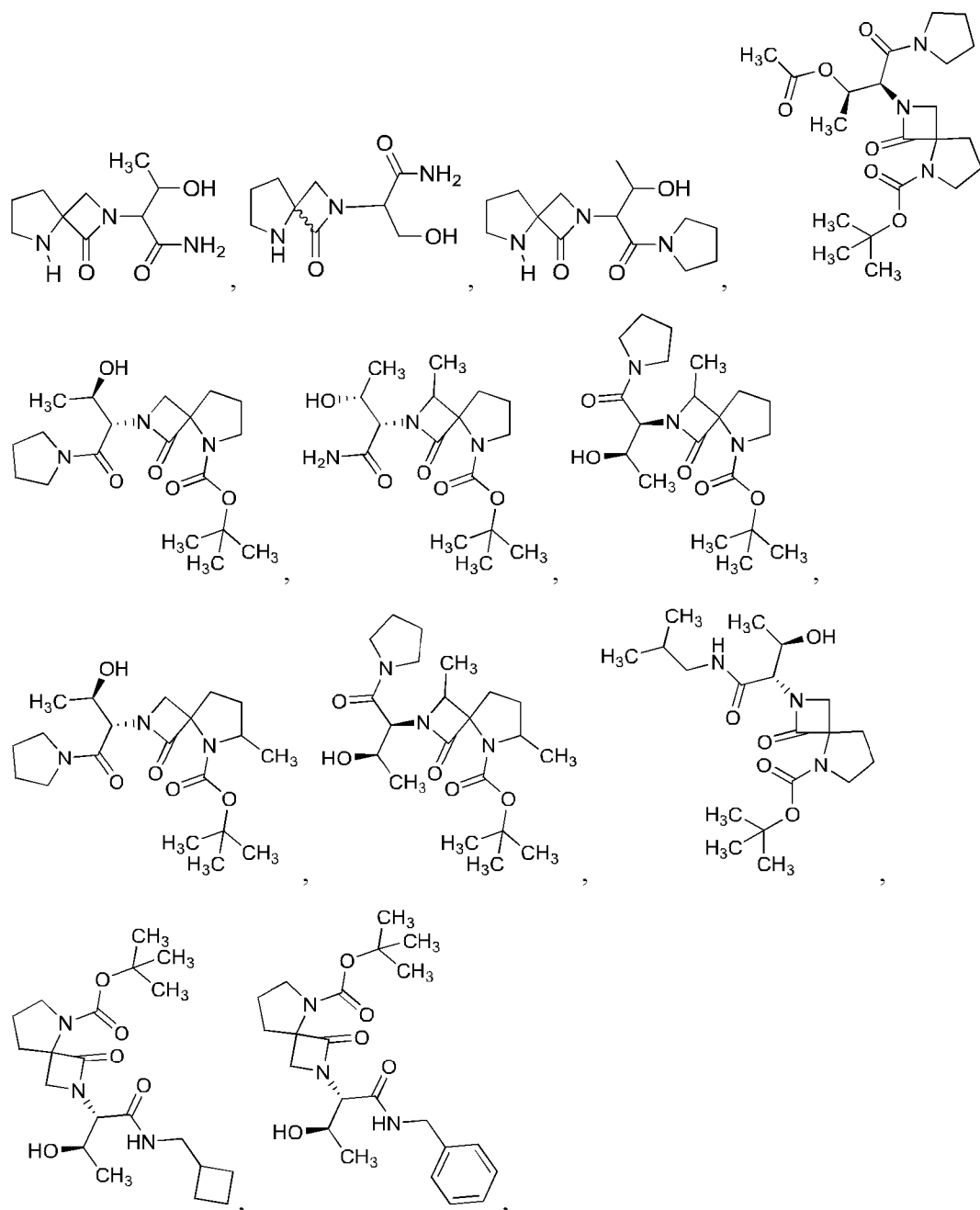
heterocyclyl including from 4 to 6 ring atoms; wherein the heterocyclyl includes not more than two ring heteroatoms, and the second ring heteroatom, when present, is independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and wherein the heterocyclyl is unsubstituted or substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, oxo, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'; or

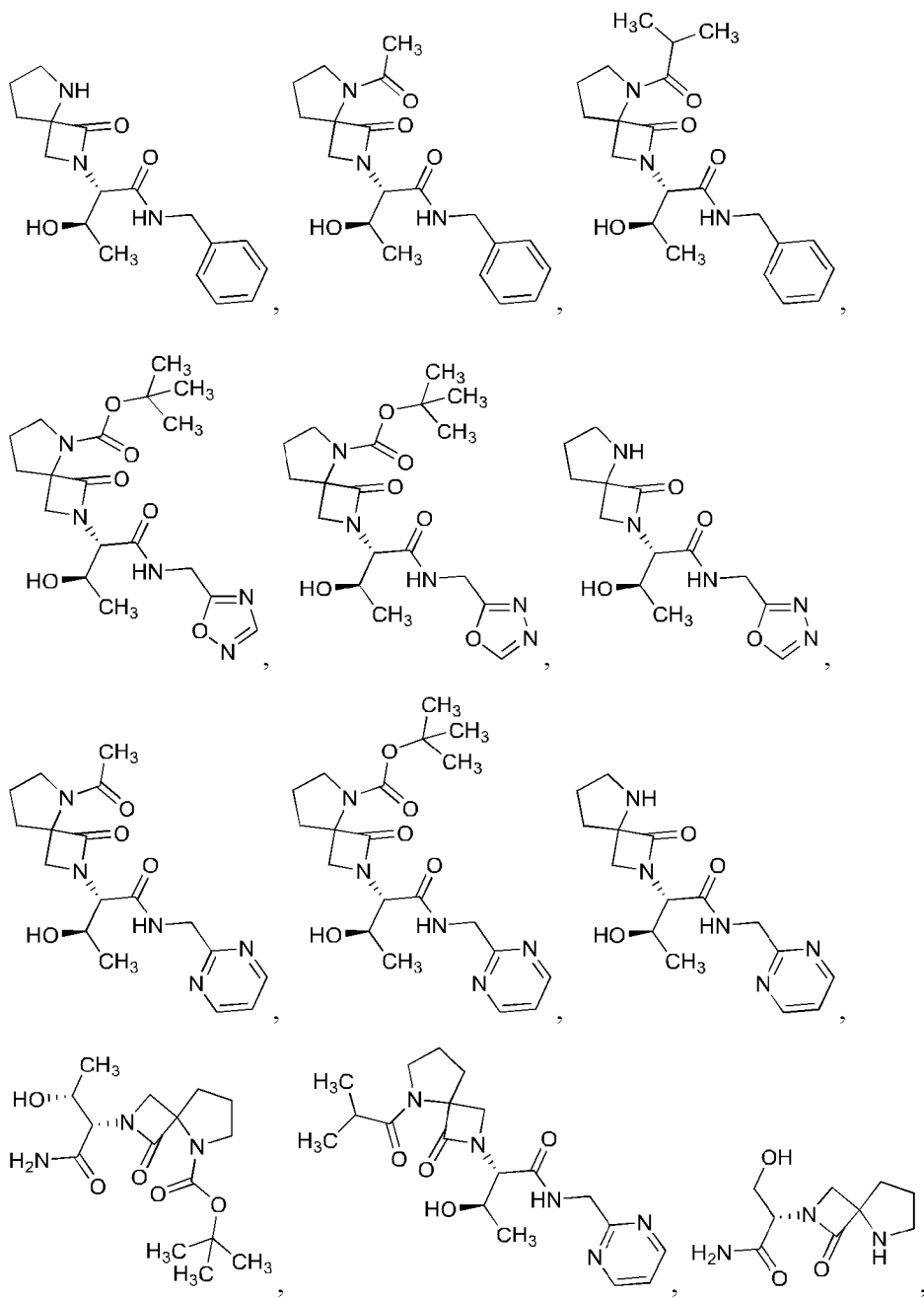
heteroaryl including from 5 to 6 ring atoms; wherein the heteroaryl includes not more than four ring heteroatoms, and each additional ring heteroatom, when present, is independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; and wherein the heteroaryl is unsubstituted or substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'.
19. The compound of claim 18, wherein R₄ and R₅ taken together with the nitrogen to which they are attached form a ring selected from the group consisting of azetidiny, pyrrolidiny, pyrazolidiny, isooxazolidiny, imidazolidiny, oxazolidiny, thiazolidiny, and isothiazolidiny.
20. The compound of claim 19, wherein R₄ and R₅ taken together with the nitrogen to which they are attached form a pyrrolidiny ring.
21. The compound of claim 18, wherein R₄ and R₅ taken together with the nitrogen to which they are attached form a ring selected from the group consisting of imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, pyridinyl, diazinyl, oxazinyl, and thiazinyl.

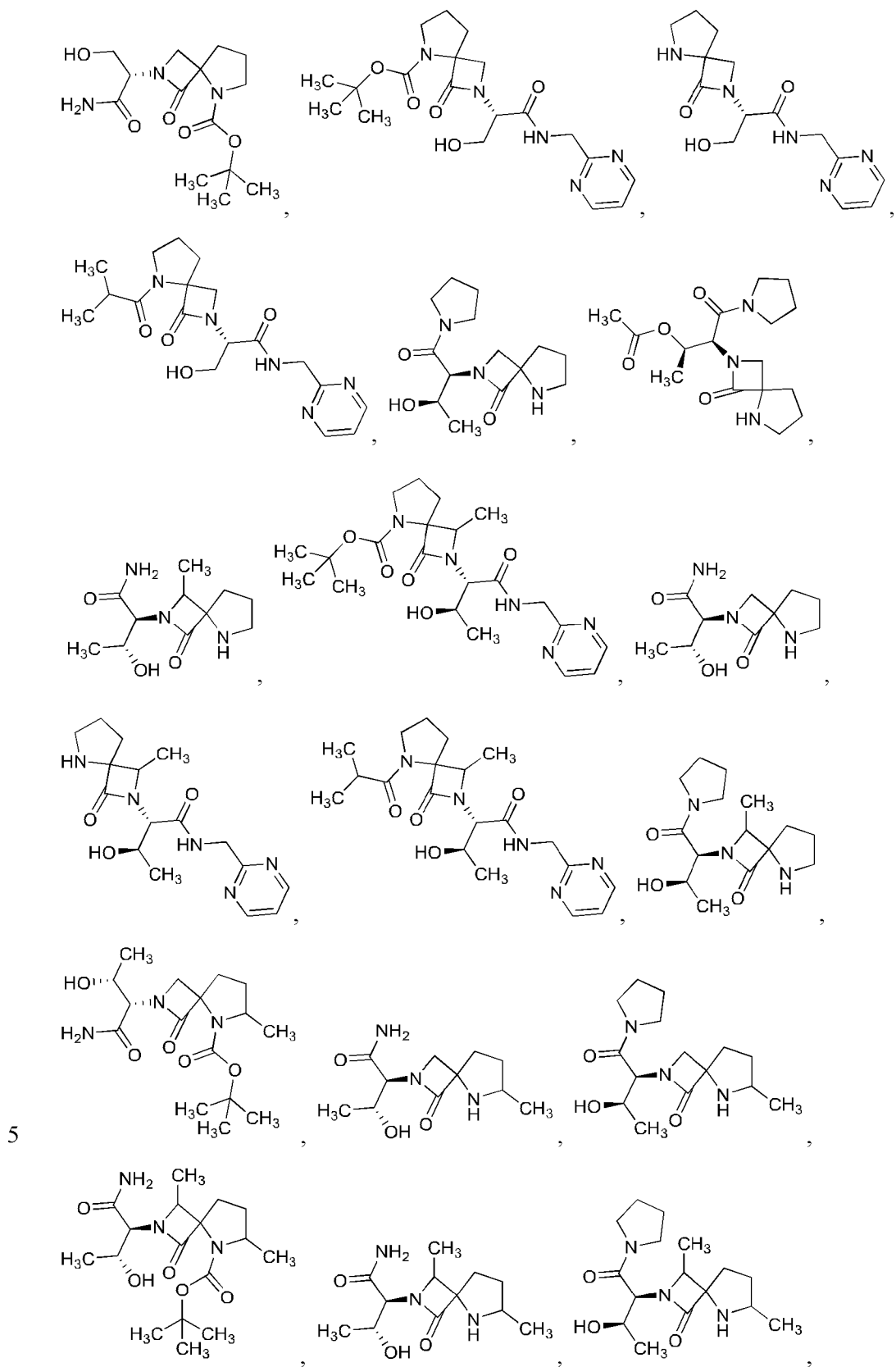
22. The compound of claim 1, wherein R₁ is H or CH₃; R₂ is H or CH₃; R₃ is H; and R₄ and R₅ taken together form a pyrrolidinyl ring.
23. The compound of claim 1, wherein R₁ is H or CH₃; R₂ is H or CH₃; R₃ is H; and R₄ and R₅ are H.
24. The compound of claim 1, wherein R₁ is H or CH₃; R₂ is H or CH₃; R₃ is H; and one of R₄ and R₅ is H, and the other is -CH₂-X, wherein X is phenyl or heteroaryl including from 5 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; each unsubstituted or substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'.
25. The compound of claim 1, wherein R₁ is H or CH₃; R₂ is H or CH₃; R₃ is nitrogen protecting group; and R₄ and R₅ taken together form a pyrrolidinyl ring.
26. The compound of claim 1, wherein R₁ is H or CH₃; R₂ is H or CH₃; R₃ is nitrogen protecting group; and R₄ and R₅ are H.
27. The compound of claim 1, wherein R₁ is H or CH₃; R₂ is H or CH₃; R₃ is nitrogen protecting group; and one of R₄ and R₅ is H, and the other is -CH₂-X, wherein X is phenyl or heteroaryl including from 5 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C₁-C₃ alkyl), O, and S; each unsubstituted or substituted with from 1-3 substituents independently selected from the group consisting of halogen, cyano, C₁-C₆ alkyl, hydroxyl, C₁-C₆ alkoxy, and -N(R')R'.
28. The compound of any one of claims 1-27, wherein R₆ is selected from the group consisting of -OH, C₁-C₆ alkoxy, -OC(O)-C₁-C₆ alkyl, and -OC(O)phenyl.
29. The compound of claim 28, wherein R₆ is -OH.
30. The compound of any one of claims 1-29, wherein R₇ is C₁-C₆ alkyl.

31. The compound of claim 30, wherein R_7 is $-\text{CH}_3$.

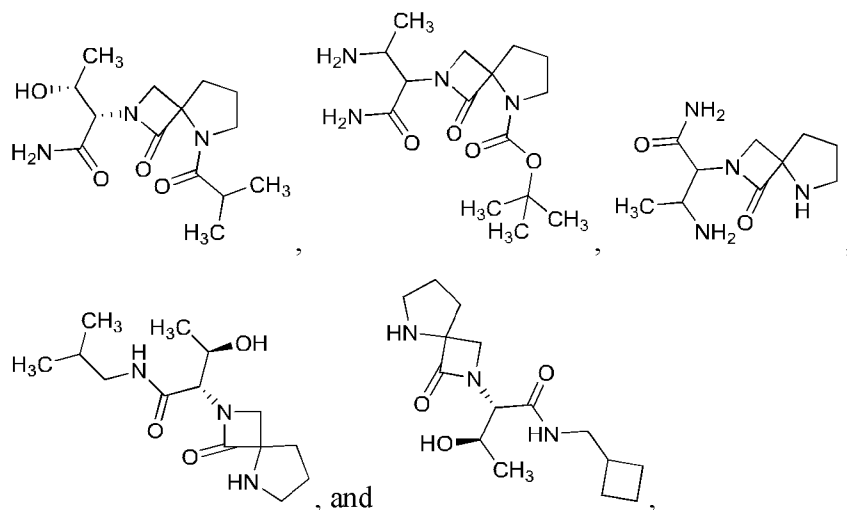
32. The compound of claim 1, wherein the compound is selected from the group
5 consisting of





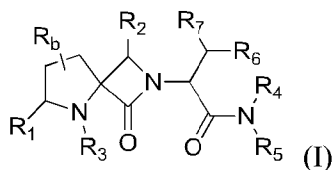


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or a pharmaceutically acceptable salt, a stereoisomer, or an N-oxide thereof.

- 5 33. A pharmaceutical composition comprising a compound of any one of claims 1-32, and a pharmaceutically acceptable excipient.
34. The pharmaceutical composition of claim 33, suitable for oral administration.
- 10 35. The pharmaceutical composition of claim 33, suitable for intravenous administration.
36. A use of a pharmaceutically effective amount of a compound of any one of claims 1-32 for treating depression, Alzheimer's disease, attention deficit disorder, schizophrenia,
- 15 or anxiety, in a patient in need thereof.
37. A compound represented by formula I:



or a pharmaceutically acceptable salt, a stereoisomer, or an N-oxides thereof, wherein

- 20 R_b is selected from the group consisting of H, halogen, hydroxyl, cyano and C_1 - C_6 alkyl;

R₁ is H or C₁-C₆ alkyl;

R₂ is H or C₁-C₆ alkyl;

R₃ is selected from the group consisting of H, C₁-C₆ alkyl and a nitrogen protecting group;

5 wherein the nitrogen protecting group is selected from the group consisting of 9-fluorenylmethyloxycarbonyl, tert-butoxycarbonyl, p-methoxybenzyloxycarbonyl, acetyl, trifluoroacetyl, benzoyl, benzyl, p-methoxybenzyl, p-methoxyphenyl, 3,4-dimethoxybenzyl, triphenylmethyl, p-toluenesulfonyl, -C(O)OR₃₁ and -C(O)R₃₂; wherein

10 R₃₁ is selected from the group consisting of C₁-C₆ alkyl, C₁-C₆ haloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₁₀ cycloalkyl, -CH₂-C₃-C₁₀ cycloalkyl, -CH₂-phenyl, and -CH₂-pyridyl, wherein any aforementioned cycloalkyl is optionally substituted with from 1-3 independently selected C₁-C₃ alkyl, and wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C₁-C₃ alkyl, C₁-C₃ haloalkyl, C₁-C₃ alkoxy, C₁-C₃ haloalkoxy, nitro, halo, SO₂Me, cyano, and -OC(O)CH₃; and

15 R₃₂ is selected from the group consisting of H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, phenyl, and pyridyl, wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C₁-C₃ alkyl, C₁-C₃ haloalkyl, C₁-C₃ alkoxy, C₁-C₃ haloalkoxy, nitro, halo, SO₂Me, cyano, and -OC(O)CH₃;

20 R₄ and R₅ are independently H or C₁-C₆ alkyl, or R₄ and R₅ taken together with the nitrogen to which they are attached form a 4-, 5- or 6-membered heterocyclic or heteroaryl ring, unsubstituted or substituted with one or more substituents selected from the group consisting of halogen, cyano, oxo, C₁-C₆ alkyl, -OH, C₁-C₆ alkoxy, and -N(R')R', wherein R' is independently selected for each occurrence from H or C₁-C₆ alkyl;

25 R₆ is selected from the group consisting of -OH, C₁-C₆ alkoxy, -OC(O)-C₁-C₆ alkyl, and -OC(O)phenyl; and

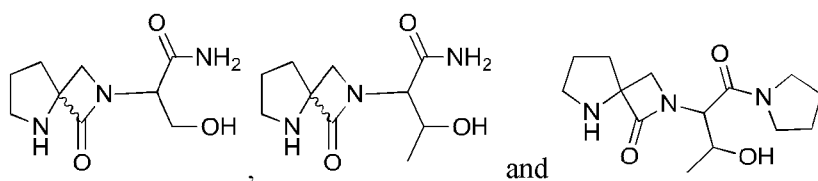
R₇ is H or C₁-C₆ alkyl.

38. The compound of claim 37, wherein R₁ is H.

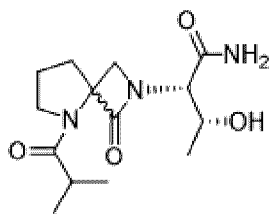
30 39. The compound of claim 37 or 38, wherein R₂ is H.

40. The compound of any one of claims 37-39, wherein R₃ is H.

41. The compound of any one of claims 37-40, wherein R₄ and R₅ are H.
42. The compound of any one of claims 37-40, wherein R₄ and R₅ taken together form a 4 or 5-membered heterocyclic ring selected from the group consisting of azetidiny, pyrrolidiny, pyrazolidiny, isooxazolidiny, imidazolidiny, oxazolidiny, thiazolidiny, and isothiazolidiny.
43. The compound of any one of claims 37-40, wherein R₄ and R₅ taken together form a pyrrolidine ring.
44. The compound of any one of claims 37-40, wherein R₄ and R₅ taken together form a heteroaromatic ring selected from the group consisting of imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, pyridinyl, diazinyl, oxazinyl, and thiazinyl.
45. The compound of claim 37, wherein R₁ is H; R₂ is H; R₃ is H; and R₄ and R₅ taken together form a pyrrolidiny ring.
46. The compound of claim 37, wherein R₁ is H; R₂ is H; R₃ is H; and R₄ and R₅ are H.
47. The compound of claim 37, wherein the compound is selected from the group consisting of:

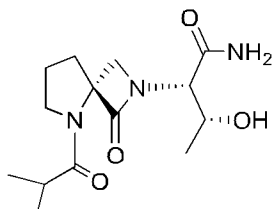


48. The compound of claim 37, wherein the compound is:



or a pharmaceutically acceptable salt, a stereoisomer, or an N-oxide thereof.

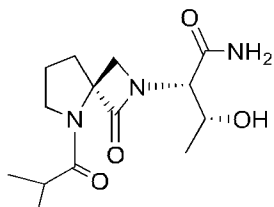
49. The compound of claim 48, wherein the compound is:



or a pharmaceutically acceptable salt thereof.

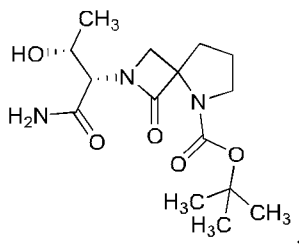
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50. The compound of claim 48, wherein the compound is:



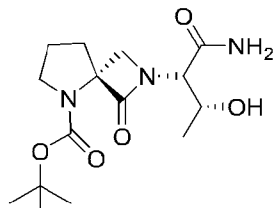
or a pharmaceutically acceptable salt thereof.

10 51. The compound of claim 37, wherein the compound is:



or a pharmaceutically acceptable salt, a stereoisomer, or an N-oxide thereof.

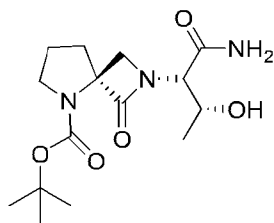
52. The compound of claim 51, wherein the compound is:



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or a pharmaceutically acceptable salt thereof.

53. The compound of claim 51, wherein the compound is:



or a pharmaceutically acceptable salt thereof.

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54. A pharmaceutical composition comprising a compound of any one of claims 37-53, and a pharmaceutically acceptable excipient.

55. The pharmaceutical composition of claim 54, suitable for oral administration.

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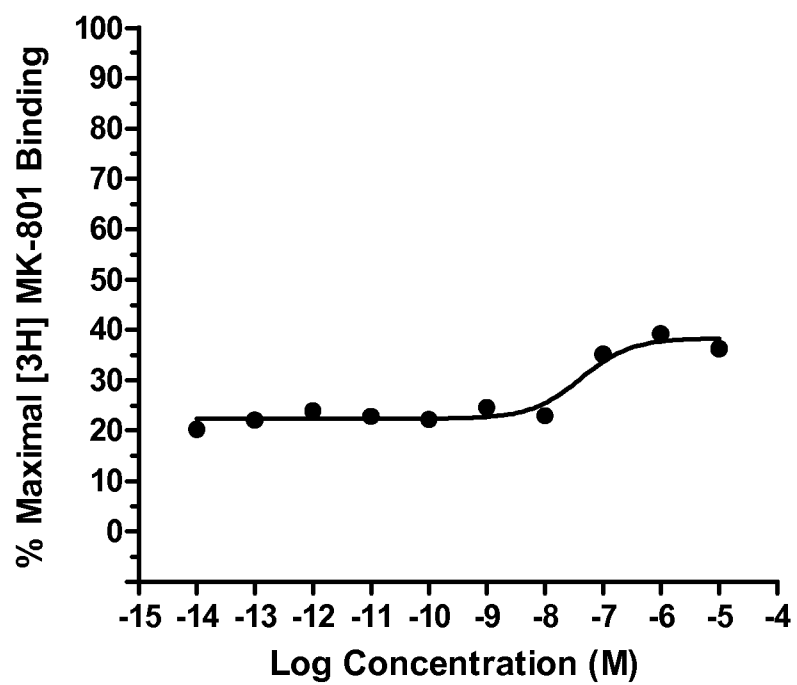
56. The pharmaceutical composition of claim 54, suitable for intravenous administration.

57. A use of a pharmaceutically effective amount of a compound of any one of claims 37-53 for treating depression, Alzheimer's disease, attention deficit disorder, schizophrenia, or anxiety, in a patient in need thereof.

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Figure 1



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Figure 2

