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. 61/757,934, filed on January 29, 2013, which is incorporated by reference in its entirety.

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 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 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 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 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

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 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 also been implicated in certain types of spatial learning.

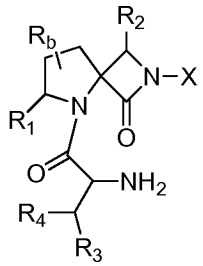
[0005] The NMDA receptor is believed to consist of several protein chains embedded in the postsynaptic membrane. The first two types of subunits discovered so far form a large extracellular region, which probably contains most of the allosteric binding sites, several transmembrane regions looped and folded so as to form a pore or channel, which is permeable 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.

[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 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.

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:



(I)

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

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

R_1 is H or C_1-C_6 alkyl;

R_2 is H or C_1-C_6 alkyl;

R_3 is selected from the group consisting of H, C_1-C_6alkyl, -OH, C_1-C_6alkoxy, -OC(O)-C_1-C_6 alkyl and -OC(O)-phenyl (optionally substituted by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

R_4 is H or C_1-C_6 alkyl; and

X is selected from the group consisting of hydrogen, -C_{1-6}alkylene-C_{3-6}cycloalkyl; C_{1-6}alkylene- heterocycle (optionally substituted by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy), and -C_{1-6}alkylene-heteroaryl (optionally substituted by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

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

R_b is selected from the group consisting of H, halogen, hydroxyl, cyano and C_1-C_6 alkyl (e.g., H);

R_1 is H or C_1-C_6 alkyl;

R_2 is H or C_1-C_6 alkyl;

R_3 is selected from the group consisting of H, C_1-C_6alkyl, -OH, C_1-C_6alkoxy, -OC(O)-C_1-C_6 alkyl and -OC(O)-phenyl (optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

R_4 is H or C_1-C_6 alkyl;

X is selected from the group consisting of:

(i) hydrogen;

(ii) -C_{1-6}alkylene-C_{3-6}cycloalkyl;

(iii) -C_{1-6}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heterocyclyl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

(iv) -C_{1-6}alkylene- C(O)-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(C_1-C_3 alkyl), O, and S; wherein the heterocyclyl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

(v) -C_{1-6}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heteroaryl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy;

(vi) branched unsubstituted C_3-C_6 alkyl; and

(vii) branched C_3-C_6 alkyl substituted with –C(O)NH_2 on one carbon and –OH on another carbon;

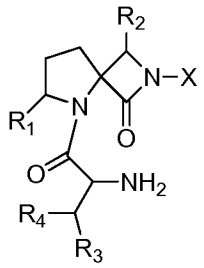
and wherein the –NH_2 group attached to the carbon adjacent to –CH(R_3)(R_4) is optionally substituted with a substituent selected from –C(O)OR_{31} and –C(O) R_{32}, wherein:

R_{31} is selected from the group consisting of: C_1-C_6 alkyl; C_1-C_6 haloalkyl; C_2-C_6 alkenyl; C_2-C_6 alkynyl; C_3-C_{10} cycloalkyl, wherein the C_3-C_{10} cycloalkyl is optionally

substituted with from 1-3 independently selected C_1-C_3 alkyl; -CH_2-C_3-C_{10} cycloalkyl wherein the C_3-C_{10} cycloalkyl is optionally substituted with from 1-3 independently selected C_1-C_3 alkyl; -CH_2-phenyl, wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C_1-C_3 alkyl; C_1-C_3 haloalkyl; C_1-C_3 alkoxy; C_1-C_3 haloalkoxy; nitro; halo; SO_2Me, cyano; and -OC(O)CH_3; and -CH_2-pyridyl; and

R_{32} is selected from the group consisting of: H; C_1-C_6 alkyl; C_1-C_6 haloalkyl; phenyl, wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C_1-C_3 alkyl; C_1-C_3 haloalkyl; C_1-C_3 alkoxy; C_1-C_3 haloalkoxy; nitro; halo; SO_2Me, cyano; and -OC(O)CH_3; and pyridyl.

[0009] In another aspect, disclosed herein are compounds represented by the formula:



(II)

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

R_1 is H or C_1-C_6 alkyl;

R_2 is H or C_1-C_6 alkyl;

R_3 is selected from the group consisting of H, C_1-C_6 alkyl, -OH, C_1-C_6 alkoxy, -OC(O)-C_1-C_6 alkyl and -OC(O)-phenyl;

R_4 is H or C_1-C_6 alkyl; and

X is –C_1-C_6alkylene-X′, wherein X′ is a 4- to 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from O, N, or S, where X' is optionally substituted on a free carbon by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy.

[0010] 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.

[0011] 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.

[0012] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **Figure 1** shows the potentiation of [^{3}H]MK-801 binding in the presence of Compound X.

DETAILED DESCRIPTION

[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

[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 C_2-C_6alkenyl, and C_3-C_4alkenyl, respectively. Exemplary alkenyl groups include, but are not limited to, vinyl, allyl, 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 C_1-C_6alkoxy, and C_2-C_6 alkoxy, respectively. Exemplary alkoxy groups include, but are not limited to methoxy, ethoxy, isopropoxy, etc.

[0018] The term "alkenyloxy" used herein refers to a straight or branched alkenyl group attached to an oxygen (alkenyl-O). Exemplary alkenoxy groupd include, but are not limited to, groups with an alkenyl group of 3-6 carbon atoms, (also e.g. referred to as C_3-C_6alkenyloxy). Exemplary "alkenoxy" groups include, but are not limited to allyloxy, butenyloxy, etc.

[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, C_3-C_6 alkynyloxy, e.g., propynyloxy.

[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 C_1-C_6 alkyl, C_1-C_4 alkyl, and C_1-C_3 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, CH_2F, CH_2CH_2Cl, CF_3, $CHFCH_2Cl$.

[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 C_2-C_6 alkynyl, and C_3-C_6 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 CH_2 or CH_2CH_2 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 $-NO_2$. 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 partically unsatured hydrocarbon group of for example 3-6, or 4-6 carbons, referred to herein, e.g., as "C_{3-6} cycloalkyl" or "C_{4-6} cycloalkyl," and derived from a cycloalkane. Exemplary cycloalkyl groups include, but are not limited to, cyclohexyl, cyclohexenyl, cyclopentyl, cyclobutyl, cyclopropyl or cyclopentyl.

[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 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 though carbon or nitrogen. Examples of heteroaryl rings include but are not limited to furyl, thienyl, pyrrolyl, thiazolyl, oxazolyl, isothiazolyl, isoxazolyl, imidazolyl, pyrazolyl, triazolyl, oxadiazolyl (e.g., 1,2,4- oxadiazolyl or 1,3,4- oxadiazolyl), pyridyl, and pyrimidinyl.

[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 pyrrolidinyl, piperidinyl, morpholino, thiomorpholino, and piperazinyl.

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

[0030] The term "heterocyclyloxyalkyl" refers to a heterocyclyl-O-alkyl- group.

[0031] The term "heterocycloxy" refers to a heterocyclyl-O- group. The term "cycloalkyloxy" refers to a cycloalkyl-O- group.

[0032] The term "heteroaryloxy" referes 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-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-methoxybenzyloxycarbonyl, acetyl, trifluoroacetyl, benzoyl, phthalimido, benzyl (Bn), p-methoxybenzyl, p-methoxyphenyl, 3,4-dimethoxybenzyl, triphenylmethyl, benzylidene, 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" 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 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 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.

[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, 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 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.

[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, glucaronate, 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 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 diasteriomers 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 chiral liquid chromatographic columns or (4) kinetic resolution using steroselective 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.

[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 ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively. 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 ^{3}H and ^{14}C) are useful in compound and/or substrate tissue distribution assays. Tritiated (*i.e.,* ^{3}H) and carbon-14 (*i.e.,* ^{14}C) isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (*i.e.,* ^{2}H) may afford certain therapeutic advantages resulting from greater metabolic stability (*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.

[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 (C_1-C_8)alkyl, (C_2-C_{12})alkanoyloxymethyl, 1-(alkanoyloxy)ethyl having from 4 to

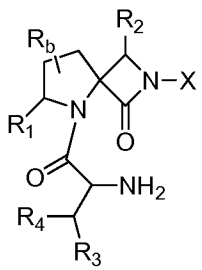
9 carbon atoms, 1-methyl-1-(alkanoyloxy)-ethyl having from 5 to 10 carbon atoms, alkoxycarbonyloxymethyl having from 3 to 6 carbon atoms, 1-(alkoxycarbonyloxy)ethyl having from 4 to 7 carbon atoms, 1-methyl-1-(alkoxycarbonyloxy)ethyl having from 5 to 8 carbon atoms, N-(alkoxycarbonyl)aminomethyl having from 3 to 9 carbon atoms, 1-(N-(alkoxycarbonyl)amino)ethyl having from 4 to 10 carbon atoms, 3-phthalidyl, 4-crotonolactonyl, gamma-butyrolacton-4-yl, di-N,N-(C_1-C_2)alkylamino(C_2-C_3)alkyl (such as β-dimethylaminoethyl), carbamoyl-(C_1-C_2)alkyl, N,N-di(C_1-C_2)alkylcarbamoyl-(C_1-C_2)alkyl and piperidino-, pyrrolidino- or morpholino(C_2-C_3)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 (C_1-C_6)alkanoyloxymethyl, 1-((C_1-C_6)alkanoyloxy)ethyl, 1-methyl-1-((C_1-C_6)alkanoyloxy)ethyl (C_1-C_6)alkoxycarbonyloxymethyl, N-(C_1-C_6)alkoxycarbonylaminomethyl, succinoyl, (C_1-C_6)alkanoyl, α-amino(C_1-C_4)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, $P(O)(OH)_2$, $-P(O)(O(C_1$-$C_6)alkyl)_2$ 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 secondary amine. For examples, see Simplício, *et al., Molecules* 2008, *13,* 519 and references therein.

Compounds

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



(I)

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

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

R_1 is H or C_1-C_6 alkyl;

R_2 is H or C_1-C_6 alkyl;

R_3 is selected from the group consisting of H, C_1-C_6 alkyl, -OH, C_1-C_6 alkoxy, -OC(O)-C_1-C_6 alkyl and -OC(O)-phenyl (optionally substituted by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6 alkyl, and C_1-C_6 alkoxy);

R_4 is H or C_1-C_6 alkyl; and

X is selected from the group consisting of hydrogen, -C_{1-6}alkylene-C_{3-6}cycloalkyl; C_{1-6}alkylene- heterocycle (optionally substituted by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6 alkyl, and C_1-C_6 alkoxy), and -C_{1-6}alkylene-heteroaryl (optionally substituted by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy));

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

R_b is selected from the group consisting of H, halogen, hydroxyl, cyano and C_1-C_6 alkyl (e.g., H);

R_1 is H or C_1-C_6 alkyl;

R_2 is H or C_1-C_6 alkyl;

R_3 is selected from the group consisting of H, C_1-C_6alkyl, -OH, C_1-C_6alkoxy, -OC(O)-C_1-C_6 alkyl and -OC(O)-phenyl (optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

R_4 is H or C_1-C_6 alkyl;

X is selected from the group consisting of:

(i) hydrogen;

(ii) -C_{1-6}alkylene-C_{3-6}cycloalkyl;

(iii) -C_{1-6}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heterocyclyl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

(iv) -C_{1-6}alkylene- C(O)-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(C_1-C_3 alkyl), O, and S; wherein the heterocyclyl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

(v) -C_{1-6}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heteroaryl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy;

(vi) branched unsubstituted C_3-C_6 alkyl; and

(vii) branched C_3-C_6 alkyl substituted with –C(O)NH_2 on one carbon and –OH on another carbon;

and wherein the –NH_2 group attached to the carbon adjacent to –CH(R_3)(R_4) is optionally substituted with a substituent selected from –C(O)OR_{31} and –C(O) R_{32}, wherein:

R_{31} is selected from the group consisting of: C_1-C_6 alkyl; C_1-C_6 haloalkyl; C_2-C_6 alkenyl; C_2-C_6 alkynyl; C_3-C_{10} cycloalkyl, wherein the C_3-C_{10} cycloalkyl is optionally substituted with from 1-3 independently selected C_1-C_3 alkyl; -CH_2-C_3-C_{10} cycloalkyl wherein the C_3-C_{10} cycloalkyl is optionally substituted with from 1-3 independently selected C_1-C_3 alkyl; -CH_2-phenyl, wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C_1-C_3 alkyl; C_1-C_3 haloalkyl; C_1-C_3 alkoxy; C_1-C_3 haloalkoxy; nitro; halo; SO_2Me, cyano; and -OC(O)CH_3; and -CH_2-pyridyl; and

R_{32} is selected from the group consisting of: H; C_1-C_6 alkyl; C_1-C_6 haloalkyl; phenyl, wherein the phenyl is optionally substituted with from 1-2 substituents independently

selected from C_1-C_3 alkyl; C_1-C_3 haloalkyl; C_1-C_3 alkoxy; C_1-C_3 haloalkoxy; nitro; halo; SO_2Me, cyano; and -$OC(O)CH_3$; and pyridyl.

[0054] In some embodiments, R_1 is H. In other embodiments , R_1 is C_1-C_6 alkyl (e.g., -CH_3).

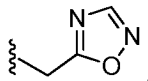
[0055] In some embodiments, R_2 is H. In other embodiments , R_2 is C_1-C_6 alkyl (e.g., -CH_3).

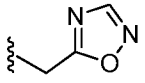
[0056] In certain other embodiments, R_3 is -OH.

[0057] In some embodiments, R_4 is C_1-C_6 alkyl. In certain embodiments, R_4 is methyl.

[0058] In some embodiments, X is as defined in definitions (i), (ii), (iii), and (v). In some embodiments, X is as defined in definitions (ii), (iii), (iv), and (v). In some embodiments, X is as defined in definitions (ii), (iii), and (v). Embodiments in which X is as defined above can include one or more of the following features: R_1 is H or methyl (e.g., H); R_2 is H; R_3 is –OH; R_4 is methyl; R_b is H; the $–NH_2$ group attached to the carbon adjacent to $–CH(R_3)(R_4)$ is not substituted, the $–NH_2$ group attached to the carbon adjacent to $–CH(R_3)(R_4)$ is substituted with $–C(O)OR_{31}$, or the $–NH_2$ group attached to the carbon adjacent to $–CH(R_3)(R_4)$ is substituted with $–C(O)R_{32}$.

[0059] In some embodiments, X is hydrogen.

[0060] In some embodiments, X is -C_{1-6}alkylene-heteroaryl (e.g., C_{1-2}alkylene- heteroaryl or C_1 alkylene-heteroaryl), the 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(C_1-C_3 alkyl), O, and S; wherein the heteroaryl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy. In certain embodiments, the heteroaryl is selected from the group consisting of 1,2,4- oxadiazolyl, 1,3,4- oxadiazolyl, pyridyl, and pyrimidinyl. In certain other embodiments, X is . Embodiments in which X is -C_{1-6}alkylene-heteroaryl can include one or more of the following features: R_1 is H or methyl (e.g., H); R_2 is H; R_3 is –OH; R_4 is methyl; R_b is H; the $–NH_2$ group attached to the carbon adjacent to $–CH(R_3)(R_4)$ is not substituted, the $–NH_2$ group attached to the carbon adjacent to $–CH(R_3)(R_4)$ is substituted with $–C(O)OR_{31}$ (e.g., R_{31} is C_1-C_6 alkyl (e.g., *tert*-butyl)), or the $–NH_2$ group attached to the carbon adjacent to $–CH(R_3)(R_4)$ is substituted with $–C(O)R_{32}$ (e.g., R_{32} is C_1-C_6 alkyl (e.g., $–CH_3$ or *iso*-propyl)).

[0061] In some embodiments, X is hydrogen. In other embodiments, X is C_{1-2}alkylene-heteroaryl. In certain other embodiments, X is .

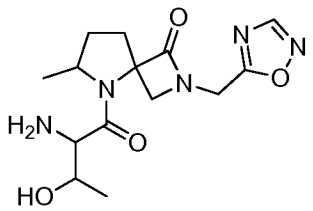
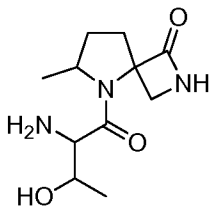
[0062] In some embodiments, the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is not substituted.

[0063] In other embodiments, the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is substituted with a substituent selected from $-C(O)OR_{31}$ and $-C(O)R_{32}$. In certain embodiments, the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is substituted with $-C(O)OR_{31}$. In embodiments, R_{31} is C_1-C_6 alkyl (e.g., *tert*-butyl). In other embodiments, the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is substituted with $-C(O)R_{32}$. In embodiments, R_{32} is C_1-C_6 alkyl (e.g., $-CH_3$ or *iso*-propyl).

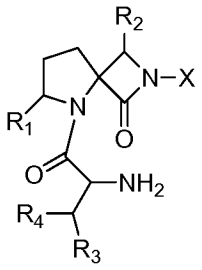
[0064] In some embodiments, R_1 is methyl; R_2 is H; R_3 is –OH; R_4 is methyl; and X is hydrogen. In certain embodiments, the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is not substituted, the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is substituted with $-C(O)OR_{31}$ (e.g., R_{31} is C_1-C_6 alkyl (e.g., *tert*-butyl)), or the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is substituted with $-C(O)R_{32}$ (e.g., R_{32} is C_1-C_6 alkyl (e.g., $-CH_3$ or *iso*-propyl)). In certain embodiments, R_b is H.

[0065] In some embodiments, R_1 is H; R_2 is H; R_3 is –OH; R_4 is methyl; and X is hydrogen. In certain embodiments, the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is not substituted, the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is substituted with $-C(O)OR_{31}$ (e.g., R_{31} is C_1-C_6 alkyl (e.g., *tert*-butyl)), or the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is substituted with $-C(O)R_{32}$ (e.g., R_{32} is C_1-C_6 alkyl (e.g., $-CH_3$ or *iso*-propyl)). In certain embodiments, R_b is H.

[0066] In some embodiments, a disclosed compound includes any of those delineated in Table 1 and/or the Examples, e.g., one having the formula:

 or .

[0067] In another aspect, disclosed compounds include those represented by the formula:



(II)

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

R_1 is H or C_1-C_6 alkyl;

R_2 is H or C_1-C_6 alkyl;

R_3 is selected from the group consisting of H, C_1-C_6 alkyl, -OH, C_1-C_6 alkoxy, -OC(O)-C_1-C_6 alkyl and -OC(O)-phenyl;

R_4 is H or C_1-C_6 alkyl; and

X is –C_1-C_6alkylene-X′, wherein X′ is a 4- to 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from O, N, or S, where X' is optionally substituted on a free carbon by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy.

[0068] 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 methane carbon (CH) that is

attached to three other substituents and has either the indicated R or S absolute configuration (see, e.g., Table 1).

[0069] 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.

[0070] 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.

[0071] 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

[0072] 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.

[0073] 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.

[0074] 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 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.

[0075] The specification for the dosage unit forms of the invention are dictated by and 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.

[0076] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, 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 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.

[0077] The compounds can be administered in a time release formulation, for example in a 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 copolymers (PLG). Many methods for the preparation of such formulations are generally known to those skilled in the art.

[0078] 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 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 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.

[0079] 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

[0080] 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.

[0081] 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.

[0082] 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.

[0083] In certain embodiments, methods for treating schizophrenia are provided. For example, paranoid type schizophrenia, disorganized type schizophrenia (i.e., hebephrenic 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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 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.

[0088] 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).

[0089] 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.

[0090] 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 $A\beta_{1-42}$), 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.

[0091] 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, 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.

[0092] 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.

[0093] 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.

[0094] 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,

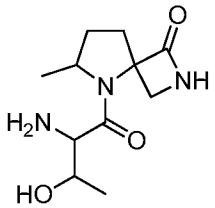
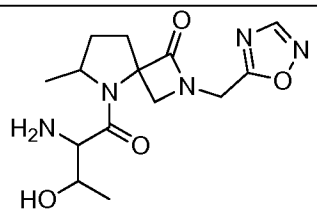
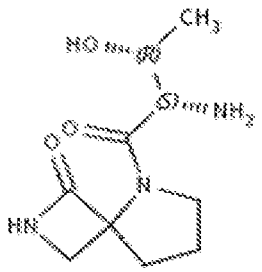
phenothiazines, thioxanthenes, clozapine, olanzapine, risperidone, quetiapine, ziprasidone, amisulpride, asenapine, paliperidone, iloperidone, zotepine, sertindole, lurasidone, and aripiprazole. It should be understood that combinations of a compound and one or more of the above therapeutics may be used for treatment of any suitable condition and are not limited to use as antidepressants or antipsychotics.

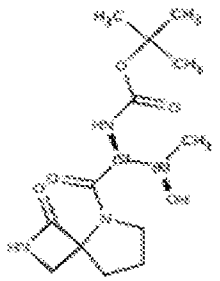
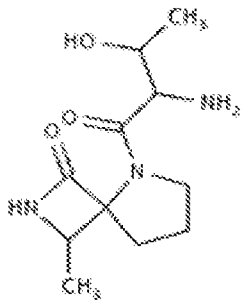
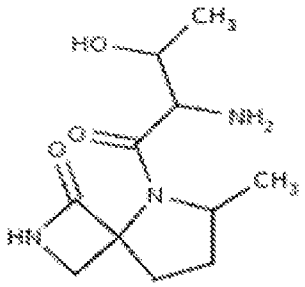
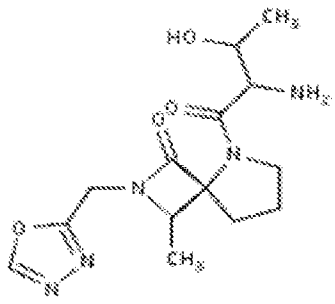
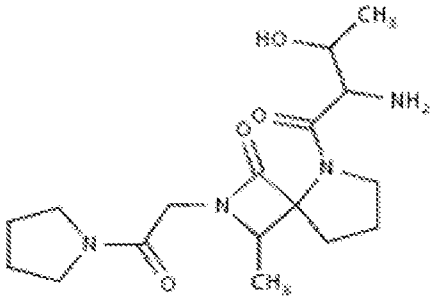
EXAMPLES

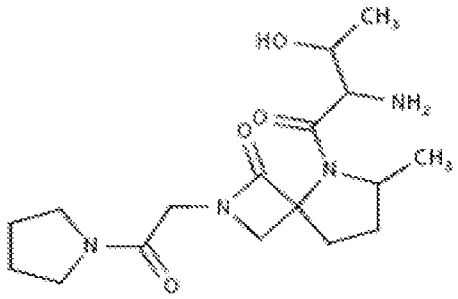
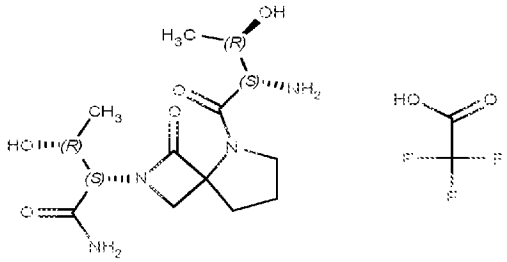
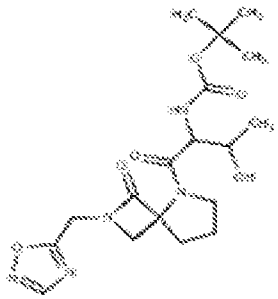
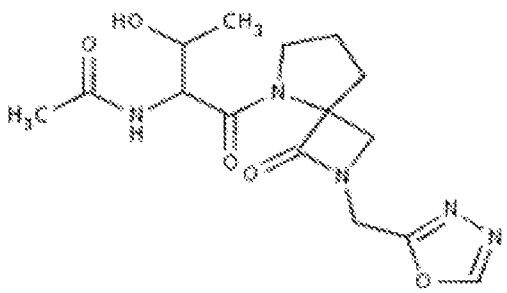
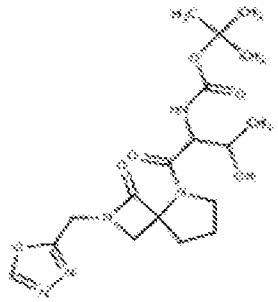
[0095] The following examples are provided for illustrative purposes only, and are not intended to limit the scope of the disclosure.

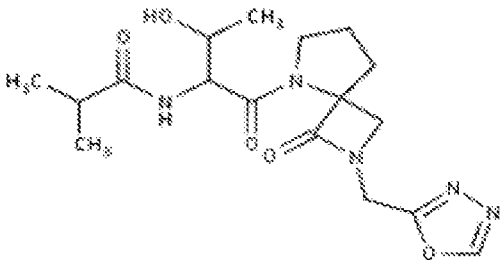
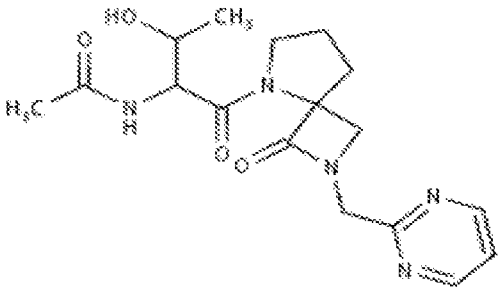
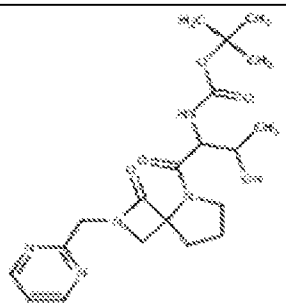
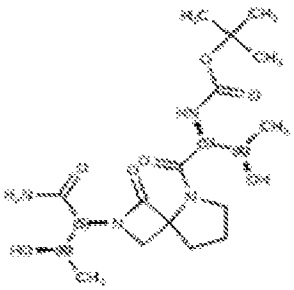
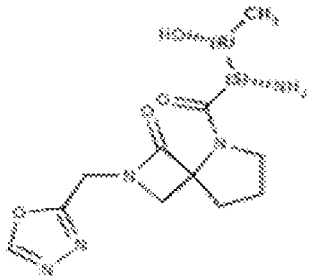
[0096] Table 1 below shows some exemplary compounds of the disclosure and provides physiochemical characteristics of the compounds.

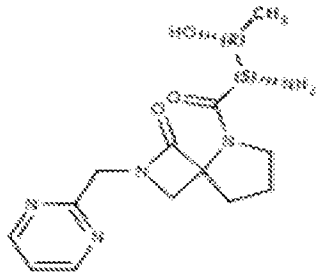
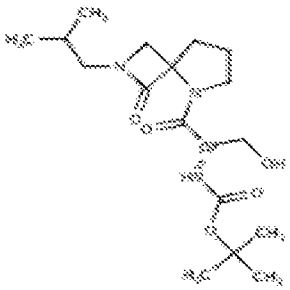
Table 1.

Compound	Structure	Molecular Weight	cLogP	tPSA
Compound X		241	-1.74	95.66
Compound Y		323	-2.04	125.79
4S-FNL-1		227.2603	-2.15173	95.66

4S-FNL-2		327.3761	-0.659636	107.97
4S-FNL-16		241.2869	-1.73516	95.66
4S-FNL-14		241.2869	-1.73516	95.66
4S-FNL-20		323.3476	-2.7324	125.79
4S-FNL-18		352.4286	-1.98729	107.18

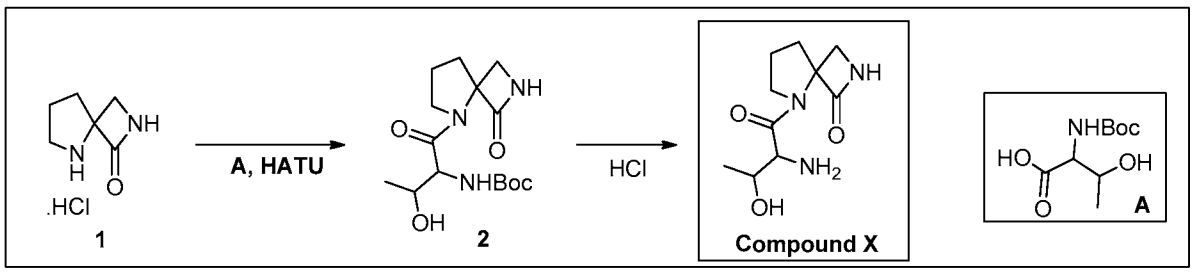
4S-FNL-15		352.4286	-1.98729	107.18
4S-FNL-3		442.3875	-3.31859	150.19
4S-FNL-19		409.4369	-0.960294	138.1
4S-FNL-10		351.3577	-3.33013	128.87
4S-FNL-7		409.4369	-1.65688	138.1

4S-FNL-9		379.4109	-2.08661	128.87
4S-FNL-13		361.3956	-1.88186	115.73
4S-FNL-12		419.4748	-0.35934	124.96
4S-7		428.48	-1.82649	162.5
4S-40		309.3211	-3.14898	125.79

4S-17		319.3589	-1.75921	112.65
4S-FNL-4		369.4558	0.391768	99.18

Example 1 – Synthesis of Compound X

Scheme 1.



Synthesis of tert-butyl (3-hydroxy-1-oxo-1-(1-oxo-2,5-diazaspiro[3.4]octan-5-yl)butan-2-yl)carbamate (2)

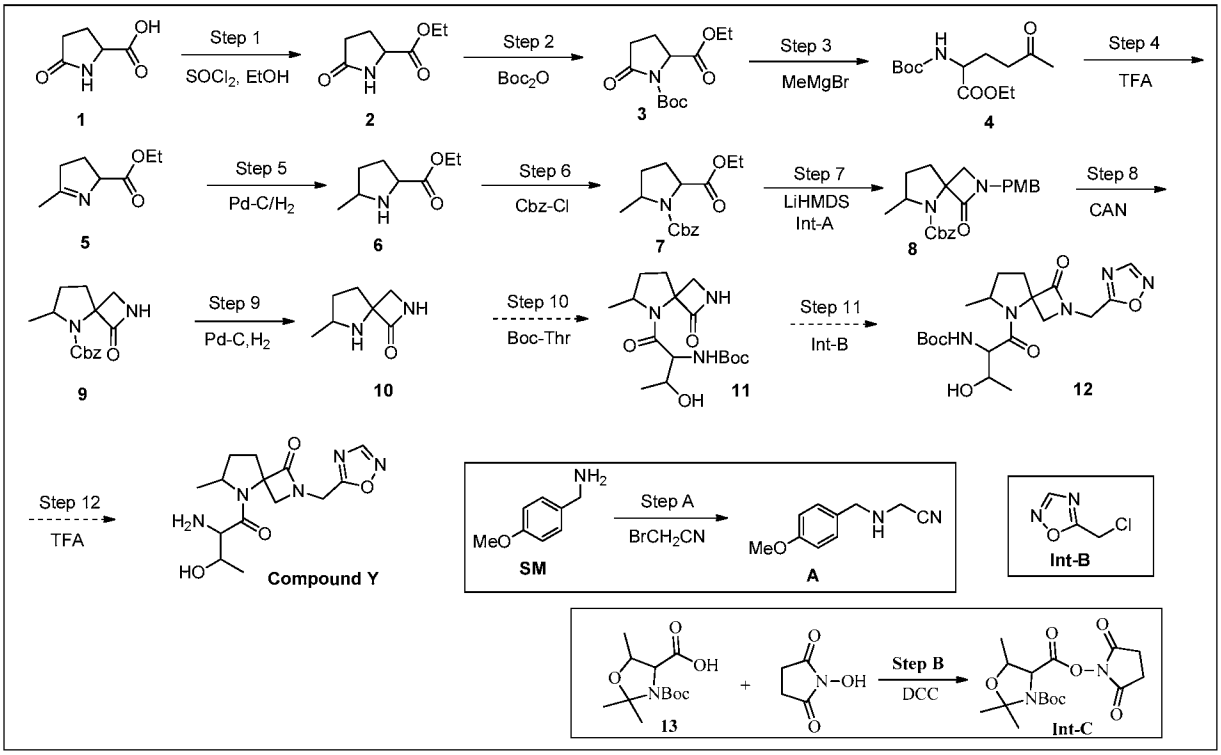
[0097] To a stirred solution of **1** (0.5 g, 3.96 mmol) in CH_2Cl_2 (20 mL) were added DIPEA (1.0 g, 7.92 mmol), **A** (0.869 mg, 3.96 mmol) and HATU (1.5 g, 3.96 mmol) at RT under inert atmosphere. The resulting reaction mixture was stirred for 2 h at RT; progress of the reaction was monitored by TLC. The reaction mixture was quenched with water and extracted with DCM. The organic layer was separated dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain the crude. The crude product was purified via preparative HPLC to afford **2** (30 mg, 2.3%) as syrup.

Synthesis of 5-(2-amino-3-hydroxybutanoyl)-2,5-diazaspiro[3.4]octan-1-one (Compound X)

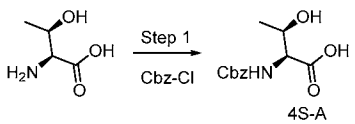
[0098] To a stirred solution of **2** (30 mg, 0.09 mmol) in DCM (5 mL) was added dioxane.HCl (2 mL) at RT and stirred for 2 h. The volatiles were evaporated under reduced pressure. The crude was triturated with ether and pentane to afford **Compound X** (15 mg, 75%) as brown thick syrup.

Example 2 - Synthesis of Compound Y

Scheme 2.



Scheme 4S-I-1



Synthesis of (2*S*, 3*R*)-2-(((benzyloxy) carbonyl) amino)-3-hydroxybutanoic acid (4S-A):

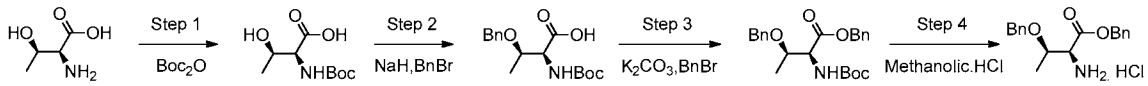
[0099] To a stirring solution of $NaHCO_3$ (529 g, 6.30 mol) in water (1 L) was added L-threonine (250 g, 2.10 mol) at RT and stirred for 30 min. The reaction mixture was cooled to 0 °C. To this Cbz-Cl (850 mL, 2.52 mol, 50% in $PhCH_3$) was added drop wise and stirred for 1 h. The reaction mixture was warmed to RT and again stirred for 28 h. To this MTBE (1L) was added and stirred for 20 min. Separated aqueous layer in toluene was stirred for 20 min.

Aqueous layer was acidified with 1*N* HCl (pH~1-2) and extracted with EtOAc (3 x 1.5 L). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was stirred with dicyclohexylamine (819 mL, 4.20 mol) for 4 h to get white solid, filtered and dried. Obtained solid was refluxed with EtOAc (1.5 L) for 1h and then filtered. The solid material was dissolved in water (1 L) and acidified with dilute H_2SO_4 and again stirred for 30 min. The aqueous layer was extracted with EtOAc (3 x 1 L). The separated organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Obtained crude material was triturated with *n*-hexane to afford **4S-A** (230 g, 43%) as white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 12.55 (br m, 1H), 7.37-7.30 (m, 5H), 6.94 (d, J = 8.8 Hz, 1H), 5.05 (s, 2H), 4.08-3.94 (m, 2H), 1.02 (d, J = 6.4 Hz, 3H).

ELSD purity: 84.66%.

Scheme 4S-I-2



Synthesis of (2*S*, 3*R*)-2-((tert-butoxycarbonyl) amino)-3-hydroxybutanoic acid (4S-B):

[0100] To a stirring solution of L-threonine (50 g, 420 mol) in THF/water (500 mL/500 mL) were added $NaHCO_3$ (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 Na_2SO_4, filtered and concentrated under reduced pressure to afford 4S-B (80 g, 87%) as thick syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ12.5 (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 (2*S*, 3*R*)-3-(benzyloxy)-2-((*tert*-butoxycarbonyl) amino) butanoic acid (4S-C)

[0101] To a stirring solution of compound 4S-B (40 g, 182 mmol) in DMF (400 mL) was added 60% NaH (18.2 g, 758 mmol) portion wise at -20 °C under N_2 atmosphere and stirred for

2 h. To this was added benzyl bromide (66.8 mL, 0.55 mol) dropwise 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 Na_2SO_4 and concentrated under reduced pressure to afford compound **4S-C** (45 g, 80%) as thick syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ12.64 (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 (4S-D):

[0102] To a stirring solution of compound **4S-C** (45 g, 146 mmol) in DMF (400 mL) was added K_2CO_3 (40 g, 292 mmol) under N_2 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 Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 20% EtOAc/*n*-hexane to afford compound **4S-D** (48 g, 82%) as thick syrup.

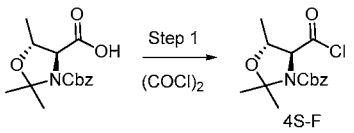
^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.37-7.18 (m, 10H), 6.81 (d, J = 9.0 Hz, 1H), 5.08 (s, 2H), 4.49 (d, J = 12.0 Hz, 1H), 4.32 (d, J = 12.0 Hz, 1H), 4.25-4.22 (m, 1H), 4.01-3.98 (m, 1H), 1.38 (s, 9H), 1.15 (d, J = 6.0 Hz, 3H).

Synthesis of (2*S*, 3*R*)-benzyl 2-amino-3-(benzyloxy) butanoate (4S-E):

[0103] To a stirring solution of compound **4S-D** (48 g, 120 mmol) in diethyl ether (50 mL) was added diethyl ether 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 **4S-E** (28 g, 77%) as semisolid (HCl salt).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ8.59 (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).

Scheme 4S-I-3



4S-F

Synthesis of (4S, 5R)-Benzyl 4-(chlorocarbonyl)-2, 2, 5-trimethyloxazolidine-3-carboxylate (4S-F):

[0104] To a stirring solution of (4S, 5R)-3-((benzyloxy) carbonyl)-2, 2, 5-trimethyloxazolidine-4-carboxylic acid (0.6 g, 2.04 mmol) in DCM (10 mL) was added oxalyl chloride (0.51 g, 4.08 mmol), at 0°C under N_2 atmosphere. The reaction mixture was stirred at RT for 30 min. After consumption of the starting material (by TLC), the reaction mixture was concentrated under N_2 atmosphere to afford **4S-F** (crude, 0.6 g) as yellow syrup was directly used for next step without purification.

Scheme 4S-I-4

4S-B 4S-G

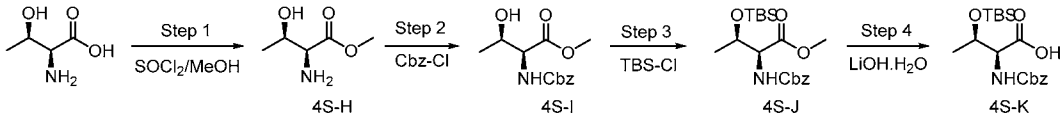
Synthesis of (4*S*, 5*R*)-3-(tert-butoxycarbonyl)-2, 2, 5-trimethyloxazolidine-4-carboxylic acid (4S-G):

[0105] To a stirring solution of **4S-B** (20 g, 91.3 mmol) in THF (200 mL) were added 2, 2-dimethoxy propane (100 mL) followed by PPTS (2.29 g, 9.13 mmol) at RT. The reaction mixture was heated to 80 °C for 16 h. After completion of starting material (by TLC), the reaction was evaporated under reduced pressure. The crude reaction mixture was diluted with water (150 mL) and EtOAc (300 mL). The separated organic layer was washed with (1 x 100 mL) of brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford **4S-G** (19 g, 80.1 %) as white solid.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 13.00 (br s, 1H), 4.07-4.04 (m, 1H), 3.78-3.74 (m, 1H), 1.51 (s, 6H), 1.17 (d, J = 7.0 Hz, 3H);

LCMS *m/z*: 260.3 [M^++1]

Scheme 4S-I-5



Synthesis of (2*S*, 3*R*)-methyl 2-amino-3-hydroxybutanoate (4S-H):

[0106] To a stirring solution of L-threonine (50 g, 420 mmol) in CH_3OH (250 mL) was added thionyl chloride (62.4 mL, 840 mmol) at 0 °C and stirred at 75 °C for 6 h. After completion of starting material (by TLC), the reaction mixture was concentrated under reduced pressure to afford **4S-H** (60 g, crude). This material was directly used for the next step without further purification.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.45 (s, 2H), 5.70 (s, 1H), 4.12-4.10 (m, 1H), 3.90 (s, 1H), 3.73 (s, 3H), 1.20 (d, J = 6.5 Hz, 3H).

Synthesis of (2*S*, 3*R*)-methyl 2-(((benzyloxy) carbonyl) amino)-3-hydroxybutanoate (4S-I):

[0107] To a stirring solution of **4S-H** (60 g, 353 mmol) in water/1,4 dioxane (150 mL/300 mL) The reaction mixture was cooled to 0 °C added $NaHCO_3$ (88.9 g, 1.059 mol) at 0 C and stirred for 15 min. Cbz-Cl (60.7 mL, 426 mmol) was added drop wise and stirred for 1 h. The reaction mixture was stirred to RT and stirred for 12 h. After completion of starting material (by TLC), diluted the reaction mass with EtOAc (300 ml). The separated organic layer was washed with (2x200 mL) of saturated $NaHCO_3$ solution followed by brine solution (2x100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford crude material was triturated with *n*-hexane (500mL) to afford **4S-I** (70 g, 74 %) as white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.37-7.30 (m, 5H), 7.20 (d, J = 8.4 Hz, 1H), 5.06 (s, 2H), 4.78 (d, J = 6.8 Hz, 1H), 4.09-4.05 (m, 2H), 3.64 (s, 3H), 1.09 (d, J = 6.0 Hz, 3H).

LCMS *m/z*: 268.2[M^++1]

Synthesis of (2*S*, 3*R*)-methyl 2-(((benzyloxy) carbonyl) amino)-3-((tert-butyldimethylsilyl) oxy) butanoate (4S-J):

[0108] To a stirring solution of **4S-I** (50 g, 187 mmol) in DMF (400 mL) were added DIPEA (86 mL, 468 mmol) TBDMS-Cl (33.66 mL, 224 mmol) at 0 °C and stirred at RT for 12 h. After completion of starting material (by TLC), diluted the reaction mass with EtOAc (500 ml). The separated organic layer was washed with (2x200 mL) of water followed by brine solution (2x100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated

under reduced pressure to afford crude material, which was purified by column chromatography eluting 10% EtOAc/hexane to afford **4S-J** (50 g, 70.1%) as colorless syrup.

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.39-7.32 (m, 5H), 5.43 (d, *J* = 9.6 Hz, 1H), 5.14 (s, 2H), 4.45-4.43 (m, 1H), 4.29-4.26 (m, 1H), 3.72 (s, 3H), 1.21 (d, *J* = 6.0 Hz, 3H), 0.83 (s, 9H), 0.09 (s, 6H) **LCMS** *m/z*: 382.2[M^++1]

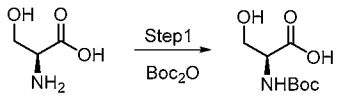
Synthesis of *N*-((benzyloxy) carbonyl)-*O*-(tert-butyldimethylsilyl)-L-threonine (4S-K):

[0109] To a stirring solution of **4S-J** (2 g, 5.24 mmol) in THF/H_2O (20 mL/20 mL) were added LiOH.H_2O (659 mg, 15.74 mmol) at RT and stirred for 16 h. After consumption of the starting material (by TLC), the solvent from the reaction mixture was evaporated under reduced pressure. The crude residue was acidified using citric acid solution (pH~4) and extracted with EtOAc (2 x 250 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to afford **4S-K** (1.7 g, 89.4%) as white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 12.75 (br s, 1H), 7.37-7.31 (m, 5H), 6.74 (d, *J* = 9.2 Hz, 1H), 5.05 (s, 2H), 4.32-4.30 (m, 1H), 4.05-4.02 (m, 1H), 1.11 (d, *J* = 6.4 Hz, 3H), 0.82 (s, 9H), 0.02 (s, 6H);

LCMS ***(m/z)*: 368.5 [M^++1].**

Scheme 4S-I-6



4S-L

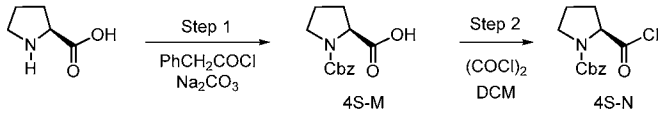
Synthesis of (tert-butoxycarbonyl)-L-serine (4S-L):

[0110] To a stirring solution of L-serine (30 g, 285 mmol) in 1, 4-dioxane (150 mL) / water (150 mL) were added NaOH (24 g, 599 mmol). After added Boc-anhydride (75 mL, 342 mmol) was added drop wise at 0 °C and stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was washed with EtOAc (2 x 150 mL). The aqueous layer pH was acidified by using 1*N* HCl (pH~4). The aqueous layer was extracted with EtOAc (2 x 250 mL). The separated organic extracts were washed with brine (1 x 150 mL) solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford compound 4S-L (38 g, 65%) as thick syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 6.67 (d, *J* = 8.5 Hz, 1H), 3.98-3.94 (m, 1H), 3.65-3.56 (m, 2H), 1.40 (s, 9H);

LCMS (ESI): *m/z* 206.1 [M^++1]

Scheme 4S-I-7



Synthesis of (*S*)-1-((benzyloxy) carbonyl) pyrrolidine-2-carboxylic acid (4S-M):

[0111] To a stirring solution of L-proline (250 g, 2.17 mol) in water (1 L) was added Na_2CO_3 (576 g, 5.43 mol) and stirred for 1 h. After being cooled to 0 °C, benzylchloroformate (50% in $PhCH_3$) (444 g, 2.61 mol) was added drop wise to the reaction mixture and again stirred for 1 h. The resulting reaction mixture was warmed to RT and further stirred for 24 h. After consumption of the starting material (by TLC), the reaction was diluted with water (1 L) and ether (1.5 L). The separated aqueous layer was treated with $PhCH_3$ (1.5 L) and acidified using 6*N*HCl. The aqueous layer was extracted with EtOAc (3 x 1.5 L) combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford **4S-M** (450 g, 84%) as light yellow syrup.

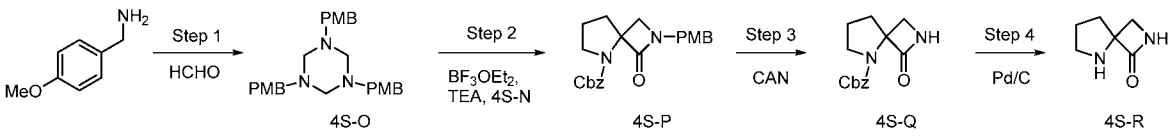
^{1}H-NMR: (400 MHz, DMSO-d_6): δ 12.71 (br s, 1H), 7.37-7.26 (m, 5H), 5.07-4.99 (m, 2H), 4.25-4.15 (m, 1H), 3.45-3.34 (m, 2H), 2.25-2.14 (m, 1H), 1.94-1.79 (m, 3H).

LCMS *m/z*: 250.4 [M^++1].

Synthesis of (*S*)-benzyl 2-(chlorocarbonyl) pyrrolidine-1-carboxylate (4S-N):

[0112] To a stirring solution of **4S-M** (90 g, 361 mmol) in CH_2Cl_2 (400 mL) was added oxalyl chloride (42 mL, 542 mmol) at 0 °C and stirred for 2 h. After complete formation of acid chloride, the reaction mixture was concentrated under reduced pressure to afford **4S-N** (95 g, crude). This material was directly used for the next step without further purification.

Scheme 4S-I-8



Synthesis of 1, 3, 5-Tris (4-methoxybenzyl)-1, 3, 5-triazinane (4S-O):

[0113] To a stirring solution of (4-methoxyphenyl) methanamine (100 g, 72.9 mmol) in EtOH (300 mL) at room temperature was added formaldehyde (33% aq, 56.2 mL) drop wise. The reaction mixture was stirred at room temperature for 1 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with EtOAc (1000 mL) and washed with water (1000 mL) followed by brine. The separated organic layer was concentrated under

reduced pressure to obtain crude; which was finally washed with *n*-hexane to afford compound **4S-O** (98 g, 90.15%) as white solid.

^{1}**H-NMR**: (500 MHz, DMSO-d_6): δ 7.18 (d, J = 8.0 Hz, 6H), 6.81 (d, J = 8.0 Hz, 6H), 3.71 (s, 9H), 3.50 (s, 6H), 3.29 (s, 6H).

Synthesis of Benzyl 2-(4-methoxybenzyl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-P):

[0114] To a stirring solution of **4S-N** (95.86 g (crude), 355 mmol) in dry CH_2Cl_2 (400 mL) was cooled to -40 °C and added Et_3N (200 mL, 1.45 mol) dropwise. The reaction mixture was stirred at -40 °C for 45 min. To this a mixture of **4S-O** (45 g, 100 mmol) in CH_2Cl_2 (150 mL) and BF_3OEt_2 (37 mL, 302 mmol) was added drop wise at -40 °C. The resulting reaction mixture was allowed to stirr at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was washed with saturated $NaHCO_3$ solution followed by brine. The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was triturated with EtOAc (100 mL) to afford compound **4S-P** (71 g, 51.6 %) as white crystalline solid.

^{1}H-NMR: (500 MHz, DMSO-*d6*): δ 7.36-7.30 (m, 5H), 7.24 (d, J = 8.0 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.90 (d, J = 7.5 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 5.09 (s, 2H), 4.29 (s, 1H), 4.13-3.96 (m, 1H), 3.73 (s, 3H), 3.11 (t, J = 5.0 Hz, 2H), 2.16-2.09 (m, 2H), 1.83-1.77 (m, 2H), 1.20-1.15 (m, 2H).

LCMS *m/z*: 381 [M^++1].

Synthesis of Benzyl 1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-Q):

[0115] To a stirring solution of compound **4S-P** (71 g, 186.8 mmol) in MeCN (710 mL) and H_2O (355 mL) were cooled to 0 °C and added a solution of CAN (204.7 g, 373.6 mmol) in H_2O (71 mL). The reaction mixture was stirred at 0 °C for 1 h. The resulting mass was poured into ice cold water and the aqueous layer was extracted with EtOAc (3 x 500 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (1 x 500 mL) followed by brine (1 x 500 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to get crude. Obtained material was purified by silica gel column chromatography eluting with 70% EtOAc/*n*-hexane to afford compound **4S-Q** (31 g, 48.6%) as yellow thick syrup.

^{1}**H-NMR**: (500 MHz, DMSO-d_6): δ 7.90 (s, 1H), 7.36-7.29 (m, 5H), 5.10 (s, 2H), 3.53 (d, J = 4.5 Hz, 2H), 3.36-3.30 (m, 1H), 3.17-3.13 (m, 1H), 2.17-2.10 (m, 2H), 1.82-1.76 (m, 2H).

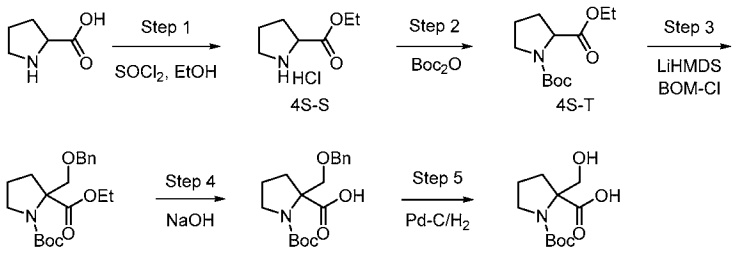
LCMS *m/z*: 261 [M^++1]

Synthesis of 2, 5-diazaspiro [3.4] octan-1-one (4S-R):

[0116] To a stirring solution of compound 4S-Q (2 g, 8.01 mmol) in EtOAc (30 mL) were added (50% wet) 10% Pd/C (600 mg) and stirred under H_2 atmosphere (balloon pressure) for 3 h at RT. After completion of reaction (by TLC), the reaction mixture was filtered through a pad of celite and washed with EtOAc (10 mL). The filtrate was concentrated under reduced pressure to afford compound 4S-R (250 mg, 25%) as colorless syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.67 (br s, 1H), 3.15-3.11 (m, 2H), 2.90-2.81 (m, 2H), 1.90-1.87 (m, 2H), 1.74-1.63 (m, 3H).

Scheme 4S-I-9



Synthesis of ethyl pyrrolidine-2-carboxylate hydrochloride (4S-S):

[0117] To a stirring solution of L-proline (110 g, 956.5 mmol) in ethanol was added thionyl chloride (141 ml, 1911.3 mmol) and heated to 80 °C for 16 h. The reaction mixture was brought to RT and concentrated under vacuum to afford compound 4S-S as hydrochloride salt (170 g, 99 %).

^{1}H-NMR: (400 MHz, $CDCl_3$): 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)*: 144 [M^++1].

Synthesis of 1-*tert*-butyl 2-ethyl pyrrolidine-1, 2-dicarboxylate (4S-T):

[0118] To a stirring solution of compound **4S-S** (70 g, 0.391 mol) in CH_2Cl_2 (700 mL) were added Et_3N (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 CH_2Cl_2 (2 x 200 mL). The organic layer was washed with water (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 compound **4S-T** (90 g, 90%) as thick syrup.

^{1}H-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].

HPLC: 96.11%.

Synthesis of 1-*tert*-butyl 2-ethyl 2-((benzyloxy) methyl) pyrrolidine-1, 2-dicarboxylate (4S-U):

[0119] To a stirring solution of compound **4S-T** (100 g, 436mmol) in THF (800 mL) was added LiHMDS (873 mL, 960 mmol) at -40 °C and stirred for 1 h. To this BOM-chloride (95 mL, 655 mmol) was added drop wise at -50 °C and stirred for 1 h at RT. After consumption of the starting material (by TLC), the reaction was quenched with aqueous NH_4Cl solution and extracted with EtOAc (2 x 200 mL). The combined organic layer was washed with citric acid solution (2 x 100 mL) followed by brine solution (2 x 100 mL). The separated organic layer was dried over Na_2SO_4 and concentrated to afford compound **4S-U** (150 g, 95%). This material was directly taken for the next step without further purification.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.32-7.30 (m, 5H), 4.59 (s, 2H), 4.10-4.02 (m, 2H), 4.09-4.00 (m, 2H), 3.30-3.24 (m, 2H), 2.21-2.10 (m, 1H), 1.82-1.75 (m, 3H), 1.39 (s, 9H), 1.18 (t, J = 3.6 Hz, 3H).

LCMS *(m/z)*: 263.4 [(M^++1)-Boc].

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

[0120] To a stirring solution of compound **4S-U** (150 g, 429 mmol) in methanol (200 mL), THF (100ml) was added NaOH solution (24g in 300 mL H_2O) at RT. The reaction mixture was heated to 65 °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 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 Na_2SO_4 and concentrated to afford compound **4S-V** (75 g, 52%).

^{1}H-NMR: (400 MHz, $CDCl_3$): 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).

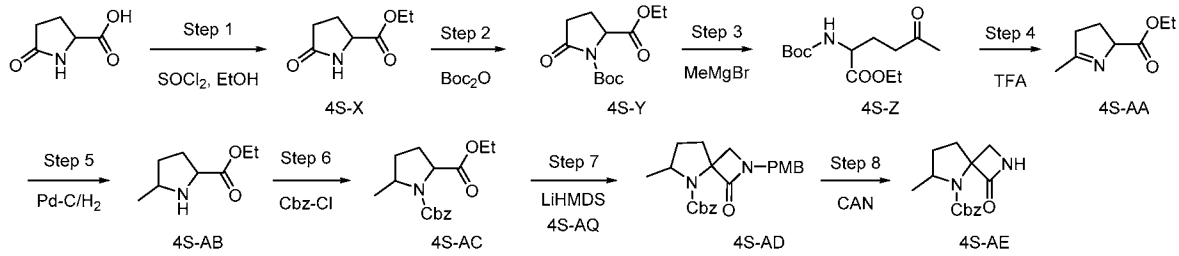
LCMS *(m/z)*: 335.3 [M^++1].

Synthesis of 1-(*tert*-butoxycarbonyl)-2-(hydroxymethyl) pyrrolidine-2-carboxylic acid (4S-W):

[0121] To a stirring solution of compound 4S-V (75 g, 223 mmol) in methanol (750 mL) was added 50% wet 10% Pd/C (20 g) at RT and stirred for 6 h under H_2 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 afford compound 4S-W (50 g, 91.1%) as pale yellow solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 12.55 (br s, 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).
LCMS *(m/z)*: 246.12 [M^++1].

Scheme 4S-I-10



Synthesis of benzyl 6-methyl-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-AE):

Synthesis of ethyl 5-oxopyrrolidine-2-carboxylate (4S-X)

[0122] 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 K_2CO_3. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography to afford compound **4S-X** (9 g, 74%).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ7.98 (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 (4S-Y):

[0123] To a stirring solution of compound 4S-X (9 g, 57.3 mmol) in CH_2Cl_2 (90 mL) was added DMAP (7.0 g, 57.3 mmol) followed by Et_3N (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 CH_2Cl_2 (50 mL) and washed with aqueous citric acid solution

followed by brine. The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. Obtained crude material was purified by column chromatography eluting with 50% EtOAc/Hexane to afford compound **4S-Y** (12 g, 82%).

^{1}**H-NMR**: (400 MHz, DMSO-d_6): δ4.61 (m, 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 (4S-Z):

[0124] To a stirring solution of compound **4S-Y** (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 NH_4Cl solution and the aqueous layer was extracted with EtOAc (2 x 200mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography eluting with 20% EtOAc/Hexane to afford compound **4S-Z** (10 g, 79%).

^{1}**H-NMR**: (400 MHz, $CDCl_3$): δ5.14 (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 (4S-AA & 4S-AB):

[0125] To a stirring solution of compound **4S-Z** (10 g, 36.7 mmol) in CH_2Cl_2 (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 **4S-AA** (crude). This was dissolved in ethanol (100 mL) and added 10% Pd/C (50% wet, 3 g) under N2 atmosphere. The reaction mixture was stirred under H_2 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 **4S-AB** (15 g, crude). This material was directly taken for the next step without further purification.

^{1}**H-NMR:** (500 MHz, DMSO-d_6): 4.4 (m, 1H), 4.2 (m, 2H), 3.6 (m,1H), 2.3 (m, 1H), 2.1 (m, 2H), 1.6 (m, 1H), 1.3 (d, 3H), 1.2 (t, 3H).

LCMS *m/z*: 158.1 [M^++1].

Synthesis of 1-benzyl 2-ethyl 5-methylpyrrolidine-1,2-dicarboxylate (4S-AC):

[0126] To a stirring solution of compound **4S-AB** (15 g, 95.4 mmol) in 1,4-dioxane/water (150 mL, 1: 1) was added $NaHCO_3$ (24 g, 286.3mmol) followed by Cbz-Cl (50% in $PhCH_3$, 143.1mmol) 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 (100 mL) and extracted

with EtOAc (2x 100mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography 20% EtOAc/hexane as eluent to afford compound **4S-AC** (9 g, 32%).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.41-7.25 (m, 5H), 4.52 (s, 2H), 4.35-4.32 (m, 1H), 4.16-4.10 (m, 3H), 2.24-2.20 (m, 1H), 2.11-2.05 (m, 1H), 1.97-1.93 (m, 1H), 1.64-1.60 (m, 1H), 1.26-1.22 (m, 4H), 1.12 (t, 2H).

LCMS *m/z*: 292 [M^++1].

Synthesis of benzyl 2-(4-methoxybenzyl)-6-methyl-1-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (4S-AD):

[0127] To a stirring solution of compound **4S-AC** (10 g, 34.1 mmol) in dry THF (50 mL) was added LiHMDS (1M in THF, 102.2 mL, 0.102 mol) at -10 °C under N_2 atmosphere and stirred for 1 h. To this a solution of **4S-AQ** (6.55 g, 37.4 mmol) in THF (50 mL) was added and stirred for 2 h. After consumption of the starting material (by TLC), the reaction was quenched with aqueous NH_4Cl solution and extracted with EtOAc (2 x 100 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 30% EtOAc/hexane to afford compound **4S-AD** (6 g, 45%).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.41-7.37 (m, 4H), 7.22 (d, 2H), 7.15-7.07 (m, 1H), 6.92-6.80 (m, 2H), 5.19-4.90 (m, 1H), 4.15-4.12 (m, 1H), 4.01-3.98 (m, 1H), 3.75 (d, 3H), 3.49-3.45 (m, 1H), 3.16-3.12 (m, 1H), 2.31-2.27 (m, 1H), 2.05-1.99 (m, 1H), 1.67-1.60 (m, 1H), 1.17 (d, 3H).

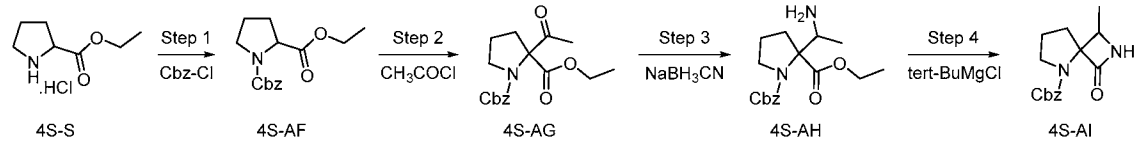
Synthesis of benzyl 6-methyl-1-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (4S-AE):

[0128] To a stirring solution of compound **4S-AD** (4 g, 10.14mmol) in H_2O/ACN (120 mL, 1: 1) was added CAN (16.6 g, 30.4mmol) at 0 °C. The reaction mixture was stirred at RT for 3 h. After consumption of the starting material (by TLC), the reaction was diluted with water (50 mL) and extracted with EtOAc (2 x50 mL). The separated organic layer was washed with aqueous $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated under vacuum. Obtained crude material was purified by column chromatography using 30% EtOAc/hexane as eluent to afford compound **4S-AE** (1 g, 36%).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.95 (d, 1H), 7.37-3.35 (m, 5H), 5.18-5.04 (m, 2H), 4.07-3.95 (m, 1H), 3.64-3.53 (m, 1H), 3.15-3.07 (m, 1H), 2.33-1.99 (m, 3H), 1.59-1.50 (m, 1H), 1.15 (t, 3H).

LCMS *m/z*: 273.2 [M^+-1].

Scheme 4S-I-11



Synthesis of (*S*)-1-benzyl 2-ethyl pyrrolidine-1, 2-dicarboxylate (4S-AF):

[0129] To a stirred solution of compound 4S-S (140 g, 782 mmol) in 1,4 dioxane/water (700 mL/700 mL) were added $NaHCO_3$ (197 g, 2.34 mol), Cbz-Cl (235 g, 1.17 mol) at 0 °C. After the reaction mixture was stirred at RT for 16 h. After completion of starting material (by TLC), the reaction mixture was diluted with EtOAc (300 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtained crude compound was purified by column chromatography to afford compound 4S-AF (130 g, 60 %) as pale yellow liquid.

^{1}H-NMR: (500 MHz, $CDCl_3$): δ 7.37-7.26(m, 5H), 5.05 (s, 2H), 4.39-4.31 (m, 1H), 4.22-4.18 (m, 1H), 4.07-4.04 (m, 1H), 3.62 (t, *J* = 4.5 Hz, 2H), 2.02-1.87(m, 4H), 1.27 (t, *J* = 7.5 Hz, 3H).

Mass *m/z*: 278 [M^++1].

Synthesis of 1-Benzyl 2-ethyl 2-acetylpyrrolidine-1, 2-dicarboxylate (4S-AG)

[0130] To a stirring solution of **4S-AF** (20 g, 72.1 mmol) in THF (100mL) was added LiHMDS (86.5 mL, 86.54 mmol, 1M solution in THF) at -20 °C and stirred for 1 h under inert atmosphere. To this acetyl chloride (5.66 mL, 79.33 mmol) was slowly added and stirring was continued for another 1 h at -20 °C. The reaction mixture was quenched with aqueous NH_4Cl solution and then extracted with EtOAc. Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography eluting with 10% EtOAc/Hexane to afford compound **4S-AG** (14 g, 60.8%) as colorless thick syrup.

Mass *m/z*: 320 [M^++1].

Synthesis of 1-Benzyl 2-ethyl 2-(1-aminoethyl) pyrrolidine-1, 2-dicarboxylate (4S-AH):

[0131] To a stirring solution of compound **4S-AG** (14 g, 43.88 mmol) in EtOH (140 mL) was added ammonium acetate (16.9 g, 219.4 mmol) followed by sodium cyanoborohydride (8.2 g, 131.6 mmol) at RT under inert atmosphere. The resultant reaction mixture was heated to 60 °C and stirred for 16 h. The progress of the reaction was monitored by TLC and the volatiles were evaporated under reduced pressure. The residue was diluted with EtOAc and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced

pressure. The crude was purified by silica gel column chromatography eluting with 2%MeOH/CH_2Cl_2 to afford compound **4S-AH** (0.6 g, 55%) as colorless syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.86 (br s, 2H), 7.37-7.28 (m, 5H), 5.16 (s, 2H), 4.10-4.01 (m, 1H), 3.95-3.90 (m, 2H), 3.73-3.69 (m, 1H), 3.47-3.42 (m, 1H), 2.10-1.90 (m, 4H), 1.31 (d, J = 12.0 Hz, 3H), 1.05 (t, J = 7.5 Hz, 3H).

LCMS (ESI): 321.3 [M^++1].

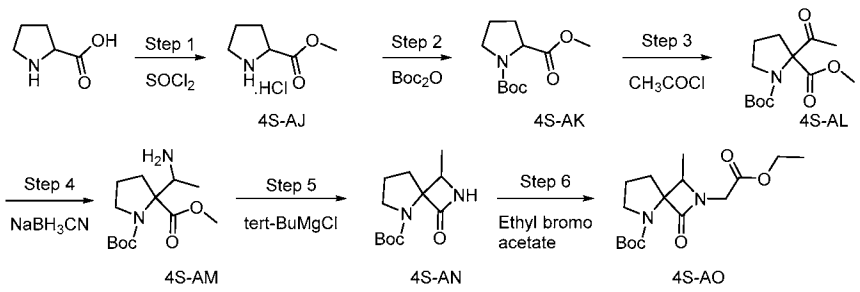
Synthesis of benzyl 1-methyl-3-oxo-2,5-diazaspiro[3.4]octane-5-carboxylate (4S-AI):

[0132] To a stirring solution of compound **4S-AH** (8.0 g, 25 mmol) in THF (150 mL) was added *t*-butylmagnesium chloride (1M in THF) (75 mL, 75 mmol) at 0 °C under nitrogen atmosphere slowly over a period of 15 min and stirred for 2 h at 0 °C. After completion of reaction by TLC, the reaction mixture was quenched with aqueous NH_4Cl solution and the aqueous layer was extracted with EtOAc (2x50 mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain crude product, which was purified by silica gel column chromatography eluting with 2% MeOH/DCM to afford compound **4S-AI** (4.5 g, 65.6%).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.08-8.03 (m, 1H), 7.39-7.30 (m, 5H), 5.13-5.00 (m, 2H), 4.05-4.02 (m, 1H), 3.53-3.47 (m, 1H), 3.41-3.36 (m, 1H), 2.10-2.05 (m, 1H), 1.96 (s, 1H), 1.90-1.85 (m, 2H), 1.22 (t, J = 6.8 Hz, 3H).

LCMS (ESI): 275.2 [M^++1].

Scheme 4S-I-12



Synthesis of methyl pyrrolidine-2-carboxylate (4S-AJ):

[0133] To a stirring solution of L-proline (100 g, 0.87 mol) in methanol (800 mL) was added thionyl chloride (76.9 mL, 1.04 mol) slowly dropwise at 0 °C. The reaction mixture was heated to 80°C for 12 h. After consumption of the starting material (by TLC), the reaction was concentrated under vacuum. Obtained residue was washed with *n*-Hexane (200 mL) and distilled off the solvent to afford compound **4S-AJ** (143.9 g, HCl salt).

^{1}H-NMR: (400 MHz, $CDCl_3$): 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 (ESI): 130 [M^++1].

Synthesis of 1-*tert*-butyl 2-methyl pyrrolidine-1, 2-dicarboxylate (4S-AK):

[0134] To a stirring solution of compound **4S-AJ** (35 g, 0.22 mol) in CH_2Cl_2 (175 mL) were added Et_3N (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 CH_2Cl_2 (2x 100 mL). The organic layer was washed with water (1 x 100 mL), brine (1 x 100 mL) and the separated organic layer was dried over Na_2SO_4 and concentrated. Obtained crude material was purified by silica gel column chromatography eluting with 30% EtOAc/Hexane to afford compound **4S-AK** (41 g, 95%).

^{1}H-NMR: (400 MHz, $CDCl_3$): 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 (ESI): 130 [(M^++1)-Boc]

Synthesis of 1-*tert*-butyl 2-methyl 2-acetylpyrrolidine-1, 2-dicarboxylate (4S-AL):

[0135] To a stirring solution of compound **4S-AK** (40g, 0.17mol) in THF (200 mL) was added LiHMDS (183.4mL, 0.18mol, 1M solution in THF) at -20 °C and stirred for 30 min under inert atmosphere. To this acetyl chloride (12.46mL, 0.17mol) was slowly added and stirring was continued for another 1 h at -20 °C. After completion of the reaction, quenched with aqueous NH_4Cl solution (100 mL) and then extracted with EtOAc (2 x 200 mL). Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford compound **4S-AL** (35 g, crude)(mixture of mono and di acetylated compound) as yellow thick syrup.

^{1}H-NMR: (500 MHz, $CDCl_3$): δ 3.79 (s, 3H), 3.64-3.38 (m, 2H), 2.43-2.17 (m, 3H), 2.03 (s, 3H), 1.48-1.42 (m, 1H), 1.39 (s, 9H).

Mass ***m/z*: 272 [M^++1].**

Synthesis of 1-*tert*-butyl 2-methyl 2-(1-aminoethyl) pyrrolidine-1, 2-dicarboxylate (4S-AM):

[0136] To a stirring solution of compound **4S-AL** (35 g, crude, 0.12 mol) in EtOH (350 mL) was added ammonium acetate (49.7 g, 0.64 mol) at RT and heated to 70 °C for 1 h. After reaction mass temperature was cooled to RT and added sodium cyanoborohydride (16.2 g, 0.25

mol) and the resultant reaction mixture was heated to 75 °C for 16 h. The progress of the reaction was monitored by TLC, and the volatiles were evaporated under reduced pressure. The residue was diluted with EtOAc (200 mL) and washed with water (2 x 100 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude was purified by silica gel column chromatography eluting with 2%MeOH/CH_2Cl_2 to afford compound **4S-AM** (17 g, 48.4 %) as colorless syrup.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.69 (br s, 2H), 4.05-3.99 (m, 2H), 3.87 (s, 3H), 3.71-3.60 (m, 1H), 2.24-2.11 (m, 1H), 1.99-1.71 (m, 3H), 1.42 (s, 9H), 1.07 (d,J = 6.8 Hz, 3H).

LCMS (ESI): 273 [M^++1].

Synthesis of *tert*-butyl 1-methyl-3-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-AN):

[0137] To a stirring solution of compound **4S-AM** (1.0 g, 3.67 mmol) in THF (15 mL) was added *t*-butylmagnesium chloride (1M in THF) (11 mL, 11.02 mmol) at 0 °C under nitrogen atmosphere slowly over a period of 15 min and stirred for 2 h at 0 °C. After completion of reaction by TLC, the reaction mixture was quenched with aqueous NH_4Cl solution (20 mL) and the aqueous layer was extracted with EtOAc (2x 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain compound **4S-AN** (800 mg, crude) which was used directly for next step without purification.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.97 (d, J = 9.2 Hz, 1H), 3.93-3.91 (m, 1H), 3.72-3.55 (m, 1H), 3.28-3.24 (m, 1H), 2.08-1.98 (m, 1H), 1.86-1.75 (m, 2H), 1.67-1.64 (m, 1H), 1.39 (s, 9H), 1.18 (d, J = 4.4 Hz, 3H).

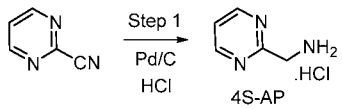
LCMS (ESI): 241 [M^++1].

Synthesis of *tert*-butyl 2-(2-ethoxy-2-oxoethyl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-AO):

[0138] To a stirring solution of compound 4S-AN (6.7 g, 27.8 mmol) in CH_3CN (70 mL) were added ethyl 2-bromoacetate (4.98 mL, 41.8 mmol), cesium carbonate (27.2 g, 83.6 mmol) and stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction was diluted with water (50 mL) and extracted with EtOAc (2 x 75 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford compound 4S-AO (8.6 g, crude), which was used directly for next step without any purification.

LCMS *m/z*: 327.3 [M^++1].

Scheme 4S-I-13



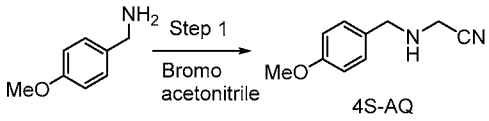
Synthesis of pyrimidin-2-ylmethanamine (4S-AP):

[0139] To a stirring solution of pyrimidine-2-carbonitrile (2.0 g, 19.0 mmol) in methanol (50 mL) were added 10% Pd/C (300 mg), 12 N HCl (1.5 mL) under N_2 atmosphere. The reaction mixture was stirred under H_2 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 to afford crude compound which was triturated with diethyl ether to obtained compound **4S-AP** (1.2 g, 44%) as white solid.

^{1}H-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) *(m/z)*: 110.3 [M^++1].

Scheme 4S-I-14

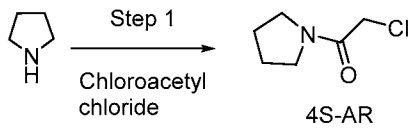


Synthesis of 2-((4-methoxybenzyl)amino)acetonitrile (4S-AQ):

[0140] To a stirring solution of (4-methoxyphenyl) methanamine (35 g, 255 mmol) in CH_2Cl_2 (350 mL) were added Et_3N (52.3 mL, 383 mmol) and bromoacetonitrile (21.2 mL, 306 mmol) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at RT for 16 h. The reaction was diluted with CH_2Cl_2 (150 mL) and washed with brine. The separated organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography using 30% EtOAc/Hexane as eluent to afford compound **4S-AQ** (22 g, 49%).

^{1}H-NMR: (500 MHz, $CDCl_3$): δ 7.27 (d, 2H), 6.90 (d, 2H), 3.87 (s, 2H), 3.84 (s, 3H), 3.56 (s, 2H).

Scheme 4S-I-15



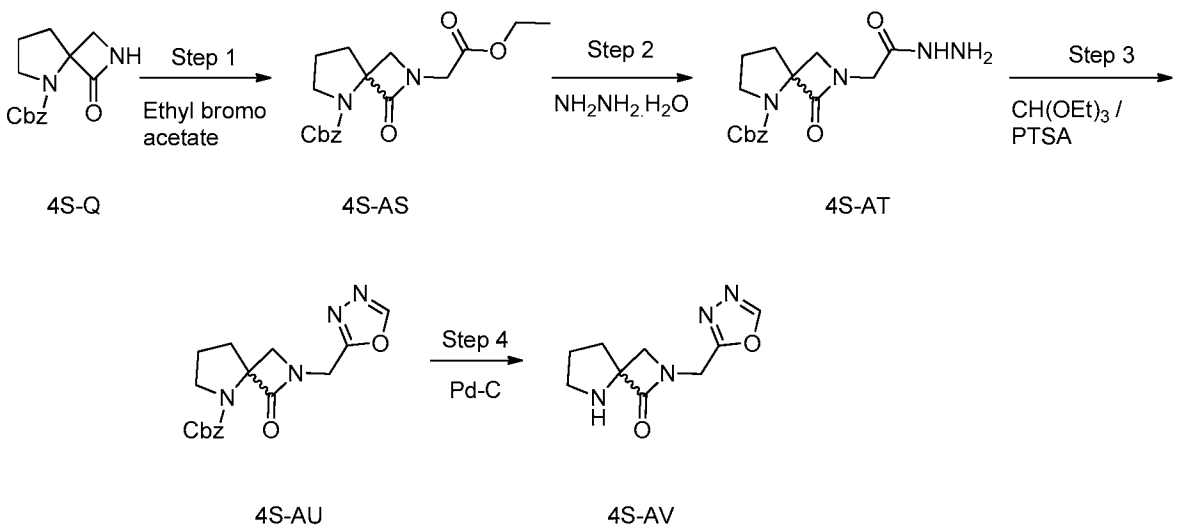
Synthesis of 2-chloro-1-(pyrrolidin-1-yl) ethanone (4S-AR):

[0141] To a stirring solution of pyrrolidine (5 g, 70.42 mmol) in DCM (30 mL) was added to chloroacetyl chloride (7.8 g, 70.42 mmol), at 0° C under N_2 atmosphere. The reaction mixture was stirred at RT for 12 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (25 mL). The organic layer was separated, washed with water (100 mL), and dried over anhydrous Na_2SO_4 concentrated under reduced pressure to afford 4S-AR as a reddish solid (6 g, 60%).

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 4.26 (s, 2H), 3.43 (t, J = 7.0 Hz, 2H), 3.29 (t, J = 7.5 Hz, 2H), 1.89-1.84 (m, 2H), 1.79-1.73 (m, 2H).

LCMS *m/z*: 148.3 [M^++1].

Scheme 4S-I-16



Synthesis of Benzyl 2-(2-ethoxy-2-oxoethyl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate 4S-AS:

[0142] To a stirred solution of compound **4S-Q** (31 g, 119 mmol) in acetonitrile (310 mL) were added Cs_2CO_3 (97 g, 297.8 mmol) and ethyl 2-bromoacetate (19.6 mL, 178.7 mmol) at RT and stirred for 16 h at RT. After completion of the reaction, volatiles were evaporated under reduced pressure. The residue was diluted with water (250 mL) and extracted with EtOAc (2 x 300 mL). The separated organic layer was washed with brine, dried over anhydrous Na_2SO_4

and concentrated under reduced pressure. The obtained crude material was purified by silica gel column chromatography eluting with 50% EtOAc/Hexane to afford compound **4S-AS** (29 g, 70.2%) as yellow thick syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.35-7.30 (m, 5H), 5.06 (s, 2H), 4.21 (s, 1H), 4.18 (s, 1H), 4.13-4.10 (m, 2H), 3.69 (d, J = 4.5 Hz, 1H), 3.47-3.44 (m, 3H), 2.16 (t, J = 6.0 Hz, 2H), 1.87-1.80 (m, 2H), 1.21-1.14 (m, 3H).

LCMS *m/z*: 347 [M^++1].

Synthesis of Benzyl 2-(2-hydrazinyl-2-oxoethyl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-AT):

[0143] To a stirred solution of compound **4S-AS** (29 g, 83.7 mmol) in EtOH (580 mL) was added hydrazine hydrate (12.2 mL, 251 mmol) at RT and stirred at 90 °C for 2 h. After completion of starting material (by TLC), ethanol was evaporated under reduced pressure. The crude residue was purified by column chromatography by eluting with 2% MeOH/DCM to afford compound **4S-AT** (19 g, 68%) as colorless thick syrup.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 7.38-7.32 (m, 5H), 5.10-4.99 (m, 2H), 4.30-4.15 (m, 2H), 3.81 (s, 2H), 3.66-3.42 (m, 2H), 3.40-3.30 (m, 2H), 2.22-2.08 (m, 2H), 1.87-1.84 (m, 2H).

LCMS *m/z*: 333.3 [M^++1].

Synthesis of Benzyl 2-((1, 3, 4-oxadiazol-2-yl) methyl)-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-AU):

[0144] To a stirred solution of compound **4S-AT** (19 g, 57.1 mmol) in triethyl orthoformate (190 mL) was added *p*-TSA (1.08 g, 5.71 mmol) at RT and stirred at 80 °C for 2 h. After completion of starting material (by TLC), triethyl orthoformate was evaporated under reduced pressure. The crude residue was purified by column chromatography eluting 1% MeOH/DCM to afford compound **4S-AU** (11 g, 56.2%) as thick syrup.

^{1}H-NMR: (400 MHz, DMSO-d_6): 9.17 (s, 1H), 7.37-7.30 (m, 5H), 5.06 (s,2H), 4.67-4.59 (m, 1H), 4.39-3.67 (m, 1H), 3.57-3.48 (m, 1H), 3.46-3.44 (m, 1H), 3.40-3.31 (m, 2H), 2.21-1.98 (m, 2H), 1.87-1.80 (m, 2H).

LCMS *m/z*: 343.4[M^++1].

Synthesis of 2-((1, 3, 4-oxadiazol-2-yl) methyl)-2, 5-diazaspiro [3.4] octan-1-one 4S-AV:

[0145] To a stirring solution of compound **4S-AU** (1 g, 2.92 mmol) in methanol (20 mL) was added 10% wet Pd/C (300 mg) and stirred under H_2 atmosphere (balloon pressure) for 12 h at

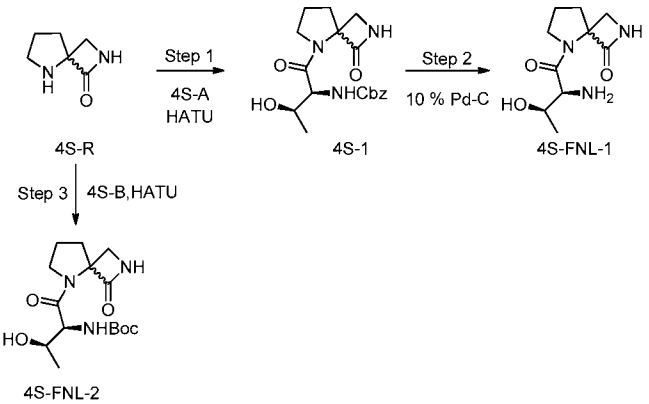
RT. The reaction mixture was filtered through a pad of celite and triturated with methanol (10 mL). The filtrate was concentrated under reduced pressure to afford **4S-AV** (280 mg, 46%) as white solid.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ9.22 (s, 1H), 4.67 (s, 2H), 3.36 (s, 2H), 3.29-3.23 (m, 1H), 3.07-2.84 (m, 2H), 1.93-1.90 (m, 2H), 1.76-1.65 (m, 2H).

LCMS *m/z*: 208.2 [M^++1].

HPLC: 97.32% (both isomers).

Scheme 4S-1



Synthesis of benzyl ((2*S*, 3*R*)-3-hydroxy-1-oxo-1-(1-oxo-2, 5-diazaspiro [3.4] octan-5-yl)butan-2-yl) carbamate (4S-1):

[0146] To a stirring solution of compound **4S-R** (250 mg, 1.98 mmol) in DCM (20 mL) were added *N, N*-diisopropylethylamine (0.91 mL, 4.95 mmol), **4S-A** (602 mg, 2.38 mmol), followed by HOBt (321 mg, 2.38 mmol), EDCI (366 mg, 2.38 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 (10 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtained crude compound which was purified by column chromatography to afford compound **4S-1** (200 mg, 28%) as colorless syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ7.85 (s, 1H), 7.36-7.31 (m, 5H), 5.04 (s, 2H), 4.72 (d, *J* = 6.4 Hz, 2H), 4.11-4.09 (m, 1H), 4.03-3.99 (m, 1H), 3.82-3.75 (m, 1H), 3.70-3.63 (m, 1H), 3.02-2.75 (m, 2H), 1.97 (t, *J* = 2.8 Hz, 1H), 1.90-1.78 (m, 3H), 1.18-1.13 (m, 3H); LCMS *m/z*: 444.5 [M^++Na].

Synthesis of 5-((2*S*, 3*R*)-2-amino-3-hydroxybutanoyl)-2, 5-diazaspiro [3.4] octan-1-one (4S-FNL-1):

[0147] To a stirring solution of compound **4S-1** (400 mg, 1.10 mmol) in EtOAc (20 mL) was added (50% wet) 10% Pd/C (200 mg) and stirred under H_2 atmosphere (balloon pressure) for 16 h at RT. After completion of reaction (by TLC), the reaction mixture was filtered through a pad of celite and triturated with EtOAc (10 mL). The filtrate was concentrated under reduced pressure to afford compound **(4S-FNL-1)** (90 mg, 35.8%) as colorless thick syrup.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.37 (d, J = 2.4 Hz, 1H), 7.59 (s, 1H), 4.72 (d, J = 6.4 Hz, 2H), 4.11-4.09 (m, 1H), 4.03-3.99 (m, 1H), 3.82-3.75 (m, 1H), 3.70-3.63 (m, 1H), 3.02-2.75 (m, 2H), 1.97 (t, J = 2.8 Hz, 1H), 1.90-1.78 (m, 3H), 1.18-1.13 (m, 3H).

LCMS *m/z*: 228.2 [M^++1].

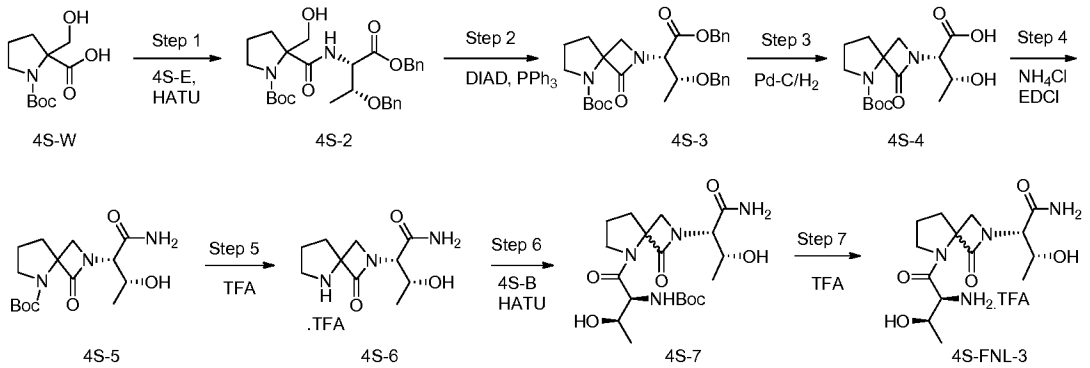
HPLC: 90.8%.

Synthesis of tert-butyl (3-hydroxy-1-oxo-1-(1-oxo-2, 5-diazaspiro[3.4]octan-5-yl)butan-2-yl)carbamate (4S-FNL-2):

[0148] To a stirred solution of **4S-R** (0.5 g, 3.96 mmol) in CH_2Cl_2 (20 mL) were added DIPEA (1.0 g, 7.92 mmol), **4S-B** (0.869 mg, 3.96 mmol) and HATU (1.5 g, 3.96 mmol) at RT under inert atmosphere. The resulting reaction mixture was stirred for 2 h at RT; progress of the reaction was monitored by TLC. The reaction mixture was quenched with water and extracted with DCM. The organic layer was separated dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain the crude. The crude product was purified via preparative HPLC to afford **(4S-FNL-2)** (30 mg, 2.3%) as syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.23 (br s, 1H), 8.03 (br s, 1H), 6.89 (br s, 1H), 3.98-3.96 (m, 2H), 3.76-3.74 (m, 2H), 3.62-3.57 (m, 1H), 2.92-2.87 (m, 1H), 1.78-1.74 (m, 2H), 1.68-1.62 (m, 2H), 1.38 (s, 9H), 1.14-0.92 (m, 3H).

Scheme 4S-2



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

[0149] To a stirring solution of compound **4S-W** (10 g, 40.8 mmol) in CH_2Cl_2 (100 mL) were added DIPEA (22 mL, 122 mmol), **4S-E** (12 g, 40.8 mmol), HATU (23 g, 61.2 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 CH_2Cl_2 (2 x 100 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, 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 **4S-2** (15g, 79.3%) as pale yellow liquid.

^{1}H-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 (4S-3):

[0150] To a stirring solution of triphenylphosphine (1.5 g, 5.69 mmol) in THF (10 mL) was added DIAD (0.91 g, 4.55 mmol) at RT and stirred for 30 min. To this added compound **4S-2** (1.2 g, 2.27 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 30% EtOAc/*n*-hexane to afford compound **4S-3** (1.0 g, 90%) as pale yellow liquid.

^{1}H-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].

Synthesis of (2*S*, 3*R*)-2-(5-(*tert*-butoxycarbonyl)-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-hydroxybutanoic acid (4S-4):

[0151] To a stirring solution of compound 4S-3 (10 g, 19.64mmol) in methanol (100 mL) was added 10% Pd/C (4 g) at RT and stirred for 24h under H_2 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 (50mL). Obtained filtrate was concentrated under reduced pressure to obtained crude, which was triturated with *n*-pentane (50 mL) to afford compound 4S-4 (6 g, 93.7%) as white solid.

^{1}H-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) *m/z*: 329.6 [M^++1].

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 (4S-5):

[0152] To a stirring solution of compound **4S-4** (500 mg, 1.52 mmol) in CH_2Cl_2 (10 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), NH_4Cl (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 CH_2Cl_2 (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 Na_2SO_4, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 2% MeOH/DCM to afford compound **4S-5** (200 mg, 40%) as colorless liquid.

^{1}H-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): *m/z* 328.3 [M^++1].

Synthesis of (2*S*, 3*R*)-3-hydroxy-2-(1-oxo-2, 5-diazaspiro [3.4] octan-2-yl) butanamide (4S-6):

[0153] To a stirring solution of compound 4S-5 (200 mg, 0.61 mmol) in CH_2Cl_2 (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/diethyl ether (5 mL/5 mL) to afford compound 4S-6 (100 mg, crude) as white solid (TFA salt).

^{1}H-NMR: (400 MHz,D_2O): δ 4.37-4.29 (m, 2H), 4.13-4.07 (m, 1H), 3.98-3.95 (m, 1H), 3.59-3.50 (m, 2H), 2.51-2.44 (m, 2H), 2.26-2.19 (m, 2H), 1.32 (d, J = 6.0 Hz, 3H).

Mass (ESI): *m/z* 228.2 [M^++1].

Synthesis of (2*S*, 3*R*)-3-hydroxy-2-(5-((2*S*, 3*R*)-3-hydroxy-2-methylbutanoyl)-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl) butanamide (4S-7):

[0154] To a stirring solution of compound **4S-6** (1.2 g, 3.70 mmol) in CH_2Cl_2 (20 mL) were added DIPEA (1.92 mL, 11.1 mmol), **4S-B** (810 mg, 3.70 mmol), HATU (2.1 g, 5.55 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 CH_2Cl_2 (2 x 40 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting 5% MeOH/DCM to afford compound **4S-7** (250 mg, crude) as semi solid.

^{1}H-NMR: (400 MHz, CD_3OD): δ 4.58-4.40 (m, 1H), 4.29-4.22 (m, 2H), 4.03-3.93 (m, 3H), 3.80-3.66 (m, 2H), 3.51-3.46 (m, 1H), 2.28-2.25 (m, 2H), 2.07-2.01 (m, 2H), 1.44 (s, 9H), 1.38 (d, J = 2.8 Hz, 2H), 1.20 (d, J = 6.0 Hz, 3H), 1.17 (d, J = 6.8 Hz, 3H).

Mass (ESI): *m/z* 328.3 [M^++1].

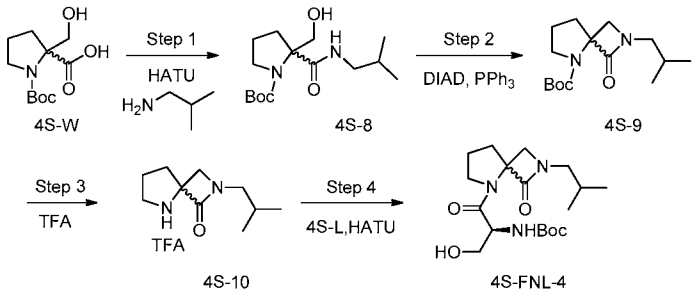
Synthesis of (2*S*, 3*R*)-2-(5-((2*S*, 3*R*)-2-amino-3-hydroxybutanoyl)-1-oxo-2, 5-diazaspiro [3.4] octan-2-yl)-3-hydroxybutanamide (4S-FNL-3):

[0155] To a stirring solution of compound **4S-7** (200 mg (crude), 0.46 mmol) in CH_2Cl_2 (10 mL) was added TFA (0.4 mL, 4.67 mmol) at 0 °C and stirred for 2 h at RT. After completion of reaction (by TLC), the reaction mixture was concentrated under reduced pressure to obtained crude compound which was triturated with DCM/diethyl ether (5 mL/5 mL) to afford **(4S-FNL-3)** (150 mg, 99.3%) as white solid (TFA salt).

^{1}H-NMR: (400 MHz, D_2O): δ 4.40-4.37 (m, 1H), 4.31-4.27 (m, 3H), 4.08 (d, J = 6.0 Hz, 1H), 3.88-3.68 (m, 3H), 2.43-2.34 (m, 2H), 2.31-2.26 (m, 2H), 1.33 (d, J = 6.4 Hz, 3H), 1.28 (d, J = 6.8 Hz, 3H).

LCMS (ESI): *m/z* 328.3 [M^++1].

Scheme 4S-3



Synthesis of tert-butyl 2-(hydroxymethyl)-2-(isobutylcarbamoyl) pyrrolidine-1-carboxylate (4S-8):

[0156] To a stirring solution of compound **4S-W** (2.0 g, 8.16 mmol) in CH_2Cl_2 (30 mL) was added DIPEA (4.25 mL, 24.4 mmol), 2-methylpropan-1-amine (0.97 mL, 9.79 mmol) followed

by HATU (4.65 g, 12.24 mmol) at 0°C. The reaction mixture was warmed to RT and stirred for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with H_2O (20 mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to get crude product, which was purified by silica gel column chromatography eluting with 50% EtOAc/hexane to afford compound **4S-8** (1.8 g, 73.46%).

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.65-7.56 (m, 1H), 5.03-4.94 (m, 1H), 4.04-4.00 (m, 2H), 3.85-3.81 (m, 1H), 3.66-3.50 (m, 3H), 2.28-2.23 (m, 1H), 1.87-1.69 (m, 4H), 1.38 (s, 9H), 0.83 (d, J = 7.0 Hz, 6H)

LCMS (ESI): *m/z* 301.4 [M^++1]

Synthesis of tert-butyl 2-isobutyl-1-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-9):

[0157] To a stirring solution of TPP (3.92 g, 14.98 mmol)) in THF (20 mL) was added DTAD (2.37 mL, 11.98 mmol) at RT and stirred for 20 min. Compound 4S-8 was added (1.8 g, 5.99 mmol) and the reaction stirred at RT for 4 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to get crude product, which was purified by silica gel column chromatography eluting with 40% EtOAc/hexane to afford compound 4S-9 (1.4 g, 82.8%) as thick syrup.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 4.91-4.73 (m, 2H), 3.59-3.43 (m, 1H), 3.37-3.26 (m, 2H), 3.17-3.11 (m, 1H), 2.14-2.07 (m, 2H), 1.84-1.71 (m, 3H), 1.39 (s, 9H), 0.90-0.82 (m, 6H)

LCMS *(m/z)*: 283.3 [M^++1]

Synthesis of 2-isobutyl-2, 5-diazaspiro [3.4] octan-1-one (4S-10):

[0158] To a stirring solution of compound 4S-9 (1.0 g, 3.54 mmol) in DCM (10 mL) was added TFA (2.72 mL, 35.41 mmol) at 0 °C under N_2 atmosphere and stirred at RT for 2 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to obtain crude product, which was triturated with *n*-pentane (10 mL) to afford compound 4S-10 (1 g, crude) was used directly for next step without any purification.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 4.90-4.75 (m, 1H), 3.67-3.50 (m, 2H), 3.37-3.23 (m, 2H), 3.04-2.94 (m, 2H), 2.28-2.13 (m, 2H), 2.03-1.96 (m, 2H), 1.87-1.80 (m, 1H), 0.86 (d, J = 6.4 Hz, 6H);

LCMS (ESI): *m/z* 183.3 [M^++1]

Synthesis of tert-butyl ((2*S*)-3-hydroxy-1-(2-isobutyl-1-oxo-2, 5-diazaspiro [3.4] octan-5-yl)-1-oxopropan-2-yl)carbamate (4S-FNL-4):

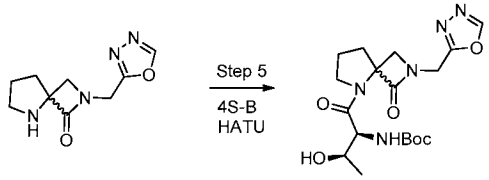
[0159] To a stirring solution of compound 4S-10 (1.0 g, 3.37 mmol) in CH_2Cl_2 (20 mL) was added DIPEA (2.9 mL, 16.87 mmol), 4S-L (899 mg, 4.38 mmol) followed by HATU (1.92 g, 5.06 mmol) at RT and stirred for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with H_2O (20 mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to get crude product, which was purified by silica gel column chromatography eluting with 2% MeOH/DCM to afford **(4S-FNL-4)** (350 mg, 28.2%).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 6.88-6.69 (m, 1H), 4.88-4.77 (m, 1H), 4.39-4.33 (m, 1H), 3.69-3.49 (m, 5H), 3.43-3.37 (m, 1H), 2.92-2.89 (m, 2H), 2.09-2.04 (m, 2H), 1.90-1.76 (m, 3H), 1.38 (s, 9H), 0.86 (d, J = 6.8 Hz, 6H);

HPLC: 93.12%

LCMS (ESI): *m/z* 370.4 [M^++1]

Scheme 4S-4



Synthesis of tert-butyl ((2*S*,3*R*)-1-(2-((1,3,4-oxadiazol-2-yl)methyl)-1-oxo-2,5-diazaspiro [3.4] octan-5-yl)-3-hydroxy-1-oxobutan-2-yl)carbamate (4S-FNL-7):

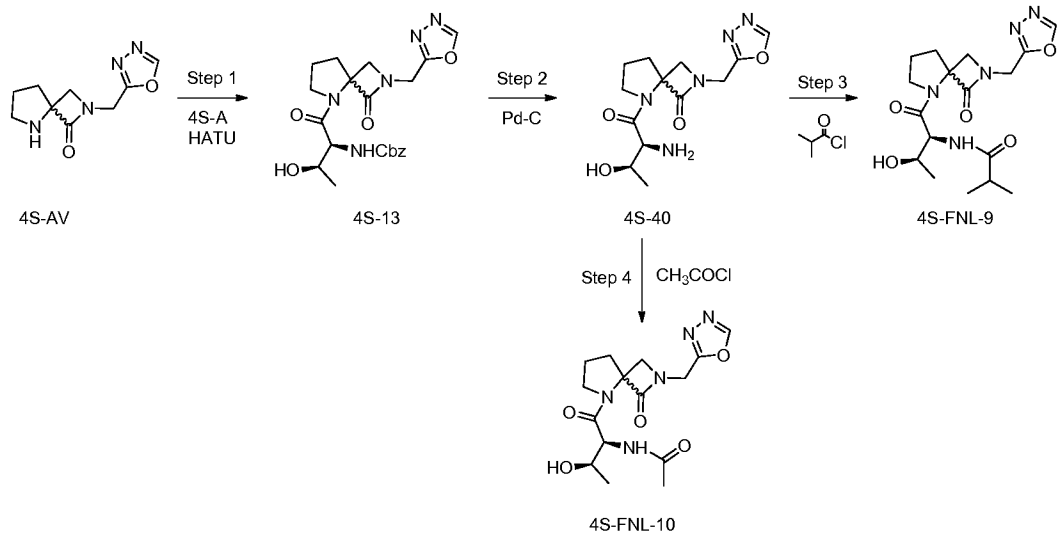
[0160] To a stirring solution of compound **4S-AV** (1 g, 4.8 mmol) in DMF (20 mL) were added DIPEA (4.4 mL, 24.03 mmol), **4S-B** (1.26 g, 5.76 mmol), HATU (2.73 g, 7.2 mmol) at RT and stirred for 16 h at RT. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (30 mL) and extracted with EtOAc (2 x 30 mL). The combined organic layer was washed with water (2 x 50 mL) followed by brine solution (2 x 50 mL). The separated organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 2% MeOH/DCM followed by preparative HPLC purification to afford **(4S-FNL-7)** (280 mg, 14.2%) as white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 9.24 (s, 1H), 6.67 (d, J = 6.4 Hz, 1H), 4.80 (s, 2H), 4.76-4.70 (m, 1H), 4.24-4.21 (m, 1H), 4.06-4.01 (m, 2H), 3.84-3.79 (m, 2H), 3.73-3.56 (m, 1H), 2.18-1.98 (m, 2H), 1.91-1.86 (m, 2H), 1.38 (s, 9H), 1.03 (d, J = 6.4 Hz, 3H).

Mass (ESI): *m/z* 410.4 [M^++1].

HPLC: 99.73% (both isomers).

Scheme 4S-5



Synthesis of benzyl ((2*S*,3*R*)-1-(2-((1,3,4-oxadiazol-2-yl)methyl)-1-oxo-2,5-diazaspiro[3.4] octan-5-yl)-3-hydroxy-1-oxobutan-2-yl)carbamate (4S-13):

[0161] To a stirring solution of **4S-AV** (1.2 g, 5.76 mmol) in DMF (15 mL) were added DIPEA (3 mL, 17.3 mmol), **4S-A** (1.75 g, 6.91 mmol), HATU (3.28 g, 8.64 mmol) at 0 °C and stirred to RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (100 mL) and EtOAc (100 mL). The organic layer was washed with brine solution (2 x 100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 2% MeOH/DCM to afford compound **4S-13** (800 mg, 31.3%) as an off-white solid.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 9.24 (s, 1H), 7.36-7.31 (m, 5H), 7.11 (d, *J* = 8.5 Hz, 1H), 5.07-4.99 (m, 2H), 4.81-4.74 (m, 2H), 4.60-4.57 (m, 1H), 4.29-4.10 (m, 1H), 3.85-3.71 (m, 2H), 3.63-3.55 (m, 2H), 2.16-2.09 (m, 2H), 2.07-1.86 (m, 2H), 1.19-1.12 (m, 3H).

LCMS (ESI): *m/z* 444.4 [M^+ +1].

Synthesis of 2-((1, 3, 4-oxadiazol-2-yl) methyl)-5-(L-threonyl)-2,5-diazaspiro[3.4]octan-1-one (4S-40):

[0162] To a stirring solution of compound 4S-13 (800mg, 1.8 mmol) in methanol (20 mL) was added 10% wet Pd/C (240 mg) and stirred under H_2 atmosphere (balloon pressure) for 12 h at RT. The reaction mixture was filtered through a pad of celite and triturated with methanol (10

mL). The filtrate was concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 8% MeOH/DCM to afford compound 4S-40 (400 mg, 72%) as white color semisolid.

^{1}H-NMR: (400 MHz, DMSO-d_6): 9.12 (s, 1H), 7.54 (s, 2H), 5.19 (d, J = 4.4 Hz, 1H), 4.09-3.97 (m, 1H), 3.90-3.72 (m, 1H), 3.66-3.60 (m, 2H), 3.26-3.13 (m, 2H), 2.88-2.74 (m, 2H), 2.12-1.92 (m, 1H), 1.89-1.74 (m, 3H), 1.16-1.08 (m, 3H).

LCMS *m/z*: 208.2 [M^++1].

HPLC: 97.63% (both isomers).

Synthesis of N-((2*S*,3*R*)-1-(2-((1,3,4-oxadiazol-2-yl)methyl)-1-oxo-2,5-diazaspiro[3.4]octan-5-yl)-3-hydroxy-1-oxobutan-2-yl)isobutyramide (4S-FNL-9):

[0163] To a stirred solution of **4S-40** (400 mg, 1.29 mmol) in DCM (5 mL) was added triethyl amine (0.36mL, 2.58 mmol) at 0 °C. After added isobutyryl chloride (0.15 mL, 1.41 mmol) at 0 °C and stirred at RT for 2 h. After completion of starting material (by TLC), reaction mass was evaporated under reduced pressure. The crude residue was purified by column chromatography eluting 4% MeOH/DCM followed by preparative HPLC purification to afford **(4S-FNL-9)** (55 mg, 11.2%) as white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): 9.20 (s, 1H), 8.28 (s, 1H), 5.26-5.09 (m, 1H), 4.99-4.48 (m, 2H), 4.10-3.91 (m, 1H), 3.89-3.71 (m, 3H), 3.69-3.61 (m, 1H), 3.41-3.31 (m, 1H), 2.82-2.49 (m, 1H), 2.24-2.18 (m, 1H), 1.98-1.73 (m, 3H), 1.10 (d, J = 6.4 Hz, 3H), 0.97-0.93 (m, 6H).

LCMS *m/z*: 380.4[M^++1].

HPLC: 97.84%.

Synthesis of *N*-((2*S*, 3*R*)-1-(2-((1, 3, 4-oxadiazol-2-yl) methyl)-1-oxo-2, 5-diazaspiro [3.4] octan-5-yl)-3-hydroxy-1-oxobutan-2-yl) acetamide (4S-FNL-10):

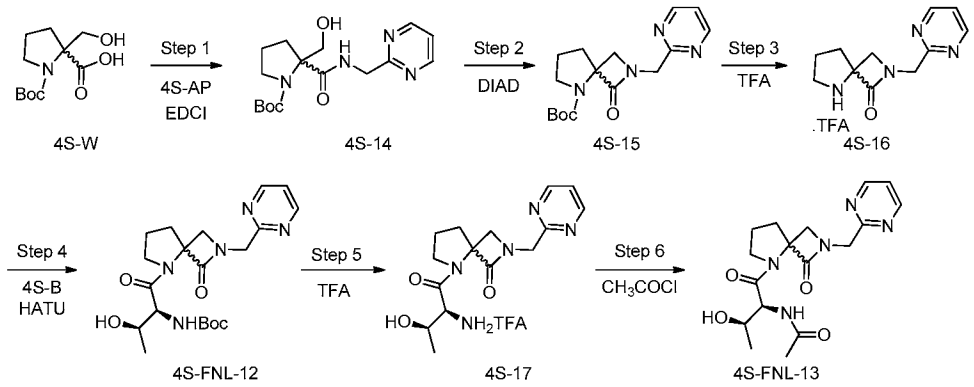
[0164] To a stirring solution of compound **(4S-40)** (800 mg, 2.58 mmol) in DCM (15 mL) was added TEA (783 mg, 7.74 mmol) followed by acetyl chloride (0.36 mL, 5.16 mmol) at 0 °C and stirred for 1 h. After consumption of the starting material (by TLC), the reaction mass was quenched with water (2 mL). The reaction mixture was concentrated under reduced pressure to obtain crude product which was purified by silica gel column chromatography eluting with 5% MeOH/DCM, preparative HPLC followed by chiral preparative HPLC purification to afford **(4S-FNL-10)** (130 mg, 14.3 %) as white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 9.20 (s, 1H), 5.25 (d, *J* = 4.4 Hz, 1H), 5.09-4.76 (m, 1H), 4.74-4.68 (m, 1H), 4.60-4.55 (m, 1H), 4.04-3.76 (m, 4H), 3.75-3.64 (m, 1H), 3.40-3.31 (m, 1H), 2.02 (s, 3H), 1.93-1.89 (m, 2H), 1.86-1.77 (m, 2H), 1.18-1.15 (m, 3H)

LCMS *m/z*: 352.3 [M^++1]

HPLC: 90.18%

Scheme 4S-6



Synthesis of tert-butyl 2-(hydroxymethyl)-2-((pyrimidin-2-ylmethyl) carbamoyl) pyrrolidine-1-carboxylate (4S-14):

[0165] To a stirring solution of compound **4S-W** (5 g, 20.4 mmol) in CH_2Cl_2 (50 mL) were added DIPEA (11 mL, 61.2 mmol), EDCI (5.84 g, 30.6 mmol), HOBT (4.68 g, 30.6 mmol), **4S-AP** (3.52 g, 24.4 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 (50 mL) and extracted with CH_2Cl_2 (2 x 100 mL). The combined organic layer was washed with citric acid (1 x 100 mL) followed by bicarbonate solution (1 x 100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Obtained crude material was purified by silica gel column chromatography eluting with 2% MeOH/DCM to afford compound **4S-14** (2.5 g, 36.5%) as an off-white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.75 (d, *J* = 4.8 Hz, 2H), 8.23 (d, *J* = 5.2 Hz, 1H), 7.38 (t, *J* = 5.2 Hz, 1H), 5.16 (t, *J* = 5.2 Hz, 1H), 4.53-4.41 (m, 2H), 3.92-3.82 (m, 2H), 3.67-3.63 (m, 1H), 3.54-3.48 (m, 1H), 2.32-2.19 (m, 2H), 2.15-1.97 (m, 2H), 1.39 (s, 9H).

Mass (ESI): *m/z* 337.4 [M^++1].

Synthesis of tert-butyl 1-oxo-2-(pyrimidin-2-ylmethyl)-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-15):

[0166] To a stirring solution of triphenylphosphine (4.87 g, 18.6 mmol) in THF (30 mL) was added DIAD (3.0 g, 14.88 mmol) at RT and stirred for 30 min. After added compound **4S-14** (2.5 g, 7.44 mmol) in (30 mL) THF slowly and reaction mixture was stirred at RT for 8 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 80% EtOAc/*n*-hexane to afford compound **4S-15** (1.2 g, 52%) as an off-white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.80 (d, J = 5.2 Hz, 2H), 7.44 (t, J = 4.8 Hz, 1H), 4.78-4.74 (m, 1H), 4.35-4.31 (m,1H), 3.69-3.60 (m, 1H), 3.42-3.33 (m, 2H), 3.28-3.23 (m, 1H), 2.18-2.14 (m, 2H), 1.90-1.76 (m, 2H), 1.40 (s, 9H).

Mass (ESI): *m/z* 319.3 [M^++1].

Synthesis of 2-(pyrimidin-2-ylmethyl)-2, 5-diazaspiro [3.4] octan-1-one (4S-16):

[0167] To a stirring solution of compound 4S-15 (1.5 g, 4.71 mmol) in CH_2Cl_2 (20 mL) was added TFA (1.5 mL, 18.86 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 4S-16 (1.4 g, 93%) as reddish syrup (TFA salt).

^{1}H-NMR: (400 MHz, D_2O): δ 8.84 (d, J = 5.2 Hz, 2H), 7.56 (t, J = 4.8 Hz, 1H), 4.88-4.73 (m, 2H), 3.97 (d, J = 6.8 Hz, 1H), 3.82 (d, J = 7.2 Hz, 1H), 3.55-3.51 (m, 2H), 2.57-2.40 (m, 2H), 2.28-2.15 (m, 2H).

LCMS (M/Z) *m/z*: 219.3 [M^++1].

HPLC: 94.4% (both isomers).

Synthesis of tert-butyl ((2*S*, 3*R*)-3-hydroxy-1-oxo-1-(1-oxo-2-(pyrimidin-2-ylmethyl)-2, 5-diazaspiro [3.4] octan-5-yl) butan-2-yl) carbamate (4S-FNL-12):

[0168] To a stirring solution of compound **4S-16** (200 mg, 0.63 mmol) in DMF (2 mL) were added DIPEA (0.33 mL, 1.89 mmol), **4S-B** (160 mg, 0.75 mmol) followed by HATU (289 mg, 0.75 mmol), at 0 °C and stirred for 12 h at RT. After consumption of the starting material (by TLC), the reaction mixture was evaporated under reduced pressure to Obtained crude material which was purified by silica gel column chromatography eluting 1% MeOH/DCM to afford **(4S-FNL-12)** (70 mg, 26.5 %) as white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.79 (d, J = 4.8 Hz, 2H), 7.43 (t, J = 4.8 Hz, 1H), 6.65 (d, J = 6.0 Hz, 1H), 6.36 (d, J = 8.8 Hz, 1H), 4.80 (s,2H), 4.30-4.22 (m, 2H), 4.06-3.83 (m, 2H), 3.73-3.57 (m, 1H), 3.36-3.34 (m, 1H), 2.17-2.08 (m, 2H), 1.90-1.88 (m, 2H), 1.38 (s, 9H), 1.06 (d, J = 6.8 Hz, 3H).

Mass (ESI): *m/z* 420.5 [M^++1].

HPLC: 95.8% (both isomers).

Synthesis of 5-((2*S*, 3*R*)-3-hydroxy-2-((2, 2, 2-trifluoroacetyl)-l4-azanyl) butanoyl)-2-(pyrimidin-2-ylmethyl)-2, 5-diazaspiro [3.4] octan-1-one (4S-17):

[0169] To a stirring solution of compound **(4S-FNL-12)** (500 mg, 1.19 mmol) in CH_2Cl_2 (5 mL) was added TFA (680 mg, 5.96 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 afford crude compound was triturated with 50 % EtOAc/*n*-hexane (10 mL) to obtained compound **4S-17** (400 mg, 77 %) as sticky solid (TFA salt).

^{1}H-NMR: (500 MHz, D_2O): δ 8.47 (d, *J* = 9.0 Hz, 1H), 7.71 (d, *J* = 8.5 Hz, 2H), 5.00-4.95 (m, 1H), 4.64-4.59 (m, 1H), 3.90-3.85 (m, 1H), 3.77-3.71 (m, 1H), 3.61-3.55 (m, 2H), 3.25-3.20 (m, 2H), 2.41-2.21 (m, 2H), 2.15-2.05 (m, 2H), 1.32-1.26 (m, 3H)

Synthesis of N-((2*S*, 3*R*)-3-hydroxy-1-oxo-1-(1-oxo-2-(pyrimidin-2-ylmethyl)-2, 5-diazaspiro [3.4] octan-5-yl) butan-2-yl) acetamide (4S-FNL-13):

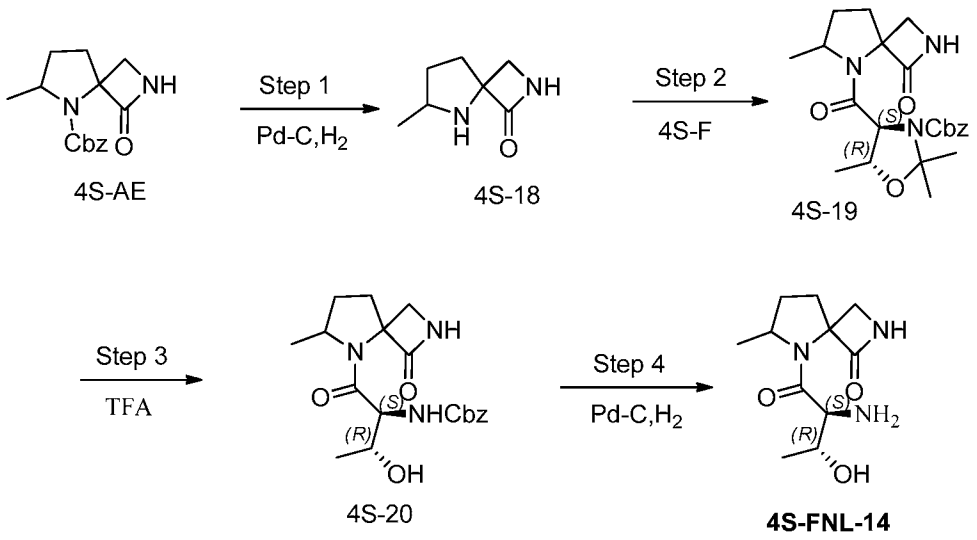
[0170] To a stirring solution of compound **4S-17** (500 mg, 1.56 mmol) in CH_2Cl_2/H_2O (5 mL/5 mL) were added $NaHCO_3$ (658 mg, 7.83 mmol), acetyl chloride (367 mg, 4.68 mmol) at 0 °C and stirred at RT for 16 h. Starting material was not completely consumed (observed by TLC). The reaction mixture was extracted by (2 x 20 mL) of 10% MeOH/DCM. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to afford crude compound which was purified by silica gel column chromatography eluting 7% MeOH/DCM followed by preparative HPLC purification to afford **(4S-FNL-13)** (25 mg, 4.4 %) as yellow solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.79 (d, *J* = 5.2 Hz, 2H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.43 (t, *J* = 4.8 Hz, 1H), 4.81-4.75 (m, 2H), 4.59-4.56 (m, 1H), 4.35-4.25 (m, 1H), 3.89-3.81 (m, 2H), 3.77-3.66 (m, 1H), 3.64-3.56 (m, 2H), 2.20-2.11 (m, 2H), 2.08-2.05 (m, 2H), 1.86 (s, 3H), 1.07-1.03 (m, 3H)

LCMS (m/z): 362.41 [M^++1]

HPLC: 90%

Scheme 4S-7



Synthesis of 6-methyl-2, 5-diazaspiro [3.4] octan-1-one (4S-18):

[0171] To a stirring solution of compound **4S-AE** (3 g, 7.79 mmol) in methanol (20 mL) was added 10% Pd/C (1.2 g) under N_2 atmosphere. The reaction mixture was stirred under H_2 atmosphere (balloon pressure) at RT for 2 h. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of ciliate and washed with methanol. Obtained filtrate was concentrated under reduced pressure to afford compound **4S-18** as yellow solid (1.5 g, 78%).

^{1}H-NMR: (500 MHz, DMSO-d_6): δ4.15-4.12 (m, 1H), 3.76-3.70 (m, 2H), 3.18-3.14 (m, 1H), 3.06 (d, J = 4.8 Hz, 1H), 1.33-1.28 (m, 4H), 1.18 (d, J = 3.2 Hz, 3H).

LCMS (ESI): 141.2 [M^++1].

Synthesis of (4*S*, 5*R*)-benzyl 2, 2, 5-trimethyl-4-(6-methyl-1-oxo-2, 5-diazaspiro [3.4] octane-5-carbonyl) oxazolidine-3-carboxylate (4S-19):

[0172] To a stirring solution of **4S-18** (6.3 g, 20.2 mmol) in DCM (30 mL) was added compound 4S-18 (2.58 g, 18.42 mmol), TEA (6.26 g, 61.43 mmol) at 0° C under N_2 atmosphere. The reaction mixture was stirred at RT for 2 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with water (25 mL).The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Obtained crude material was purified by column chromatography by using 2.5% MeOH/DCM to afford compound **4S-19** as green solid (1.5 g, 20%).

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.79 (s, 1H), 7.40-7.28 (m, 5H), 5.09-4.95 (m, 1H), 4.89-4.75 (m, 1H), 4.26-3.91 (m, 2H), 3.80-3.37 (m, 2H), 2.05-1.96 (m, 2H), 1.73-1.62 (m, 3H), 1.50 (s, 6H), 1.24-1.14 (m, 3H), 1.07-0.85 (m, 3H).

Synthesis of benzyl ((2*S*, 3*R*)-3-hydroxy-1-(6-methyl-1-oxo-2, 5-diazaspiro [3.4] octan-5-yl)-1-oxobutan-2-yl) carbamate (4S-20) :

[0173] To a stirring solution of compound **4S-19** (1.5 g, 3.61 mmol) in water (30 mL) was added TFA (15 mL) at 0 °C and reaction mixture was stirred at RT for 4 h. After consumption of the starting material (by TLC) reaction mixture was extracted with EtOAc (2 x 30 mL). Combined organic layer and washed with aqueous $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtained crude material was purified by column chromatography by using 5% MeOH/DCM to afford compound **4S-20** (350 mg, 26%) as off-white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.81 (s, 1H), 7.55-7.34 (m, 5H), 4.97 (s, 2H), 4.76 (d, J = 6.4 Hz, 6.4 Hz, 1H), 4.41-4.35 (m, 1H), 4.14-4.12 (m, 1H), 3.79-3.72 (m, 2H), 3.59 (d, J = 3.2 Hz, 1H), 2.99 (d, J = 4.4 Hz, 4.0 Hz, 1H), 2.24-2.16 (m, 1H), 2.06-1.88 (m, 2H), 1.63 (t, J = 5.2 Hz, 1H), 1.22 (d, J = 6.8 Hz, 3H), 1.10 (d, J = 6.8 Hz, 3H).

LCMS *m/z*: 376.4 [M^++1].

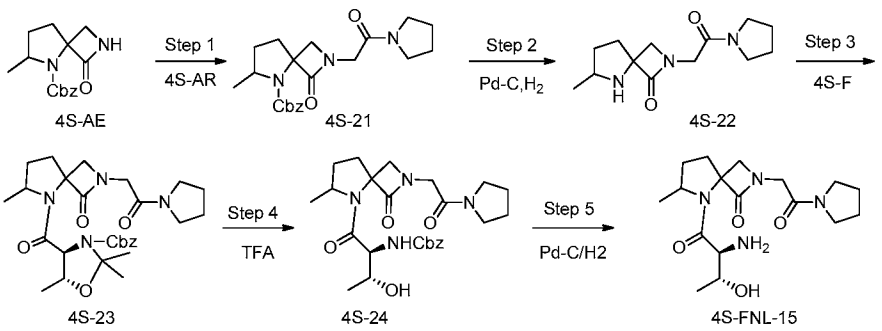
Synthesis of 6-methyl-2-(2-oxo-2-(pyrrolidin-1-yl) ethyl)-2, 5-diazaspiro [3.4] octan-1-one (4S-FNL-14):

[0174] To a stirring solution of compound **4S-20** (250 mg, 0.66 mmol) in EtOAc (10 mL) was added 10% Pd/C (100 mg) under N_2 atmosphere. The reaction mixture was stirred under H_2 atmosphere (balloon pressure) at RT for 8 h. After consumption of the starting material (by TLC), the reaction mixture was filtered through a pad of celite and washed with EtOAc (10 mL). Obtained filtrate was concentrated under reduced pressure to afford **(4S-FNL-14)** as yellow syrup (80 mg, 50%).

^{1}H-NMR : (400 MHz, DMSO-d_6): δ 7.98 (br s, 1H), 7.49 (br s, 1H), 4.70 (d, J = 7.2 Hz, 1H), 4.07-4.00 (m, 2H), 3.62 (t, J = 3.2 Hz, 1H), 2.92 (s, 1H), 2.77-2.69 (m, 2H), 2.14-2.03 (m, 1H), 1.95-1.83 (m, 2H), 1.47-1.40 (m, 1H), 1.23 (d, J = 6.4 Hz, 3H), 1.11 (d, J = 6.4 Hz, 3H).

LCMS *m/z*: 242.4 [M^++1] 92.6%.

Scheme 4S-8



Synthesis of benzyl 6-methyl-1-oxo-2-(2-oxo-2-(pyrrolidin-1-yl) ethyl)-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-21):

[0175] To a stirring solution of compound **4S-AE** (4 g, 14.59 mmol) in acetonitrile (20 mL) was added **4S-AR** (2.59 g, 17 mmol), cesium carbonate (11.86 g, 36.49 mmol) at 0° C under N_2 atmosphere. The reaction mixture was stirred at RT for 2 h. After consumption of the starting material (by TLC), reaction mixture was filtered through a pad of celite and washed with acetonitrile. Obtained filtrate was concentrated under reduced pressure to afford crude compound was purified by column chromatography by eluting 2% MeOH/DCM to afford compound **4S-21** (3.0 g, 40%) as yellow liquid.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.38-7.31 (m, 5H), 5.11-4.97 (m, 2H), 4.26 (s, 1H), 4.01-3.97 (m, 2H), 3.79-3.71 (m, 1H), 3.46-3.36 (m, 2H), 3.30-3.22 (m, 2H), 2.30-2.17 (m, 2H), 2.11-2.07 (m, 1H), 2.01-1.97 (m, 3H), 1.89-1.84 (m, 2H), 1.79-1.73 (m, 1H), 1.22-1.11 (m, 3H).

LCMS (ESI): 386 [M^++1].

Synthesis of 6-methyl-2-(2-oxo-2-(pyrrolidin-1-yl) ethyl)-2, 5-diazaspiro [3.4] octan-1-one (4S-22):

[0176] To a stirring solution of compound **4S-21** (3 g, 7.79 mmol) in methanol (20 mL) was added 10% Pd/C (1.2 g) under N_2 atmosphere. The reaction mixture was stirred under H_2 atmosphere (balloon pressure) at RT for 2 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 **4S-22** as yellow solid (1.5 g, 78%).

^{1}H-NMR: (500 MHz, DMSO-d_6): δ4.01-3.96 (m, 1H), 3.91-3.72 (m, 1H), 3.38-3.18 (m, 8H), 2.01-1.83 (m, 6H), 1.77-1.72 (m, 2H), 1.02 (d, J = 6.0 Hz, 3H).

LCMS (ESI): 251 [M^++1].

Synthesis of (4*S*, 5*R*)-benzyl 2, 2, 5-trimethyl-4-(6-methyl-1-oxo-2-(2-oxo-2-(pyrrolidin-1-yl) ethyl)-2, 5-diazaspiro [3.4] octane-5-carbonyl) oxazolidine-3-carboxylate (4S-23):

[0177] To a stirring solution of **4S-F** (0.6 g, 1.92 mmol) in DCM (10 mL) was added to compound 4S-22 (0.48 g, 1.92 mmol), TEA (0.58 g, 5.76 mmol) at 0° C under N_2 atmosphere. Reaction mixture was stirred at RT for 1 h. After consumption of the starting material (by TLC), Reaction mixture was diluted with water (5 mL).The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtained crude material was purified by column chromatography by using 3% MeOH/DCM to afford compound **4S-23** as brown solid (0.60 g, 56%).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.38-7.34 (m, 5H), 5.09-4.74 (m, 2H), 4.30-3.92 (m, 4H), 3.85-3.64 (m, 2H), 3.46-3.38 (m, 3H), 2.22-2.01 (m, 3H), 1.88-1.74 (m, 5H), 1.58-1.50 (m, 6H), 1.35-1.20 (m, 5H), 1.12-1.07 (m, 1H), 0.87-0.78 (m, 2H).

LCMS (ESI): 527 [M^++1].

Synthesis of benzyl ((2*S*,3*R*)-3-hydroxy-1-(6-methyl-1-oxo-2-(2-oxo-2-(pyrrolidin-1-yl)ethyl) -2,5-diazaspiro[3.4]octan-5-yl)-1-oxobutan-2-yl)carbamate (4S-24):

[0178] To a stirring solution of compound **4S-23** (0.8 g, 1.52 mmol) in water (5 mL) was added TFA (5mL) at 0 °C, Reaction mixture was stirred at RT for 4 h. After consumption of the starting material (by TLC).Reaction mixture was extracted with EtOAc (2 x20 mL). Separated organic layer and washed with aqueous $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtained crude material was purified by column chromatography by using 2% MeOH/DCM to afford compound **4S-24** (0.25 g, 34%) as yellow solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.56 (d,J = 7.6 Hz, 1H), 7.37-7.30 (m, 5H), 5.08-4.97 (m, 2H), 4.67 (d, J = 6.0 Hz, 1H), 4.52 (t,J = 6.4 Hz, 1H), 4.42-4.36 (m, 1H), 4.24-4.18 (m, 1H), 4.07 (t,J = 8.0 Hz, 1H), 3.80-3.56 (m, 4H), 3.38 (t, J = 6.8 Hz, 2H), 3.30 (s, 2H), 2.25-2.20 (m, 1H), 2.11-2.04 (m, 2H), 1.90-1.82 (m, 2H), 1.78-1.71 (m, 1H), 1.67-1.63 (m, 1H), 1.24 (t, J = 6.8 Hz, 3H), 1.09-1.02 (m, 3H).

LCMS *m/z*: 487.4 [M^++1].

Synthesis of 5-((2*S*, 3*R*)-2-Amino-3-hydroxybutanoyl)-6-methyl-2-(2-oxo-2-(pyrrolidin-1-yl) ethyl)-2, 5-diazaspiro [3.4] octan-1-one (4S-FNL-15):

[0179] To a stirring solution of compound **4S-24** (0.15 g, 0.308 mmol) in methanol (5 mL) was added 10% Pd/C (0.06 g) under N_2 atmosphere. Reaction mixture was stirred under H_2

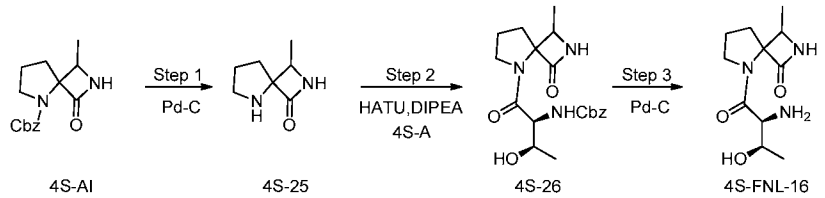
atmosphere (balloon pressure) for 6 h at RT. After consumption of the starting material (by TLC), reaction mixture was filtered through a pad of celite and washed with methanol and concentrated under reduced pressure to afford **(4S-FNL-15)** as yellow solid (0.075g, 69%).

^{1}H-NMR: (400 MHz, D_2O): δ4.52-4.46 (m, 3H), 4.12-3.81 (m, 1H), 3.59-3.52 (m, 1H), 3.47-3.43 (m, 5H), 3.06-2.87 (m, 1H), 2.35-2.12 (m, 8H), 1.30 (s, 6H).

LCMS (ESI): 353.2 [M^++1];

HPLC: 99.72%

Scheme 4S-9



Synthesis of 3-methyl-2, 5-diazaspiro [3.4] octan-1-one (4S-25):

[0180] To a stirring solution of compound **4S-AI** (2.50 g, 9.12 mmol) in EtOAc (50 mL) was added 10% Pd/C (500 mg) under N_2 atmosphere. The reaction mixture was stirred under H_2 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 EtOAc (30 mL). Obtained filtrate was concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 4% MeOH/DCM to afford compound **4S-25** as pale yellow thick syrup (1.0 g, 78.7%).

^{1}H-NMR: (500 MHz, DMSO-d_6): δ7.79(br s, 1H), 3.39-3.35 (m, 1H), 2.92-2.85 (m, 2H), 2.80-2.49 (m, 1H), 1.75-1.64 (m, 2H), 1.62-1.60 (m, 2H), 1.10-1.06 (m, 3H).

LCMS (ESI): 141.3 [M^++1].

Synthesis of benzyl ((2*S*, 3*R*)-3-hydroxy-1-(1-methyl-3-oxo-2, 5-diazaspiro [3.4] octan-5-yl)-1-oxobutan-2-yl) carbamate (4S-26):

[0181] To a stirring solution of compound **4S-25** (1.0 g, 7.13 mmol) in DMF (20 mL) were added *N, N*-diisopropylethylamine (3.94 mL, 21.3 mmol), **4S-A** (2.70 g, 10.67 mmol), followed by HATU (5.42 g, 14.26 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 (30 mL) and EtOAc (50 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtained crude compound which was purified by column chromatography eluting 2% MeOH/DCM to afford compound **4S-26** (500 mg, 18.7%) as colorless syrup.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ7.95 (br s, 1H), 7.37-7.28 (m, 5H), 7.05 (d, J = 8.4 Hz, 1H) 5.08-4.98 (m, 2H), 4.78-4.71 (m, 1H), 4.33-4.11 (m, 1H), 3.99-3.72 (m, 2H), 3.68-3.44 (m, 2H), 1.97-1.74 (m, 4H), 1.12-1.11 (m, 3H), 1.06 (t, J = 6.4 Hz, 3H).

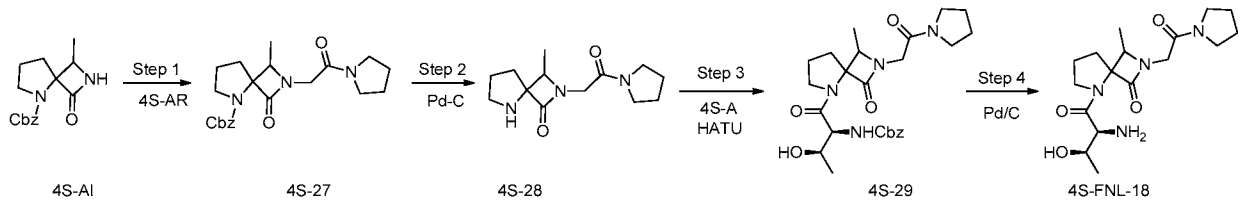
Synthesis of 5-((2*S*, 3*R*)-2-amino-3-hydroxybutanoyl)-3-methyl-2, 5-diazaspiro [3.4] octan-1-one (4S-FNL-16):

[0182] To a stirring solution of compound **4S-26** (500 mg, 1.33 mmol) in EtOAc (20 mL) was added 10% Pd/C (100 mg) under N_2 atmosphere. The reaction mixture was stirred under H_2 atmosphere (balloon pressure) at RT for 8 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 EtOAc (20 mL). Obtained filtrate was concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 2% MeOH/DCM to afford **(4S-FNL-16)** as pale yellow thick syrup (170 mg, 52.8%).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ7.42 (s, 1H), 4.68 (d, J = 6.4 Hz, 1H), 4.10-4.00 (m, 1H) 3.79-3.76 (m, 1H), 3.38-3.33 (m, 1H), 3.04-2.97 (m, 1H), 3.38-3.33 (m, 1H), 3.04-2.97 (m, 1H), 2.16-2.14 (m, 1H), 1.97-1.64 (m, 4H), 1.20-1.04 (m, 6H).

LCMS (ESI): 242.1 [M^++1].

Scheme 4S-10



Synthesis of benzyl 1-methyl-3-oxo-2-(2-oxo-2-(pyrrolidin-1-yl) ethyl)-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-27):

[0183] To a stirring solution of compound **4S-AI** (500 mg, 1.82 mmol) in acetonitrile (10 mL) were added **4S-AR** (377 mg, 2.55 mmol), cesium carbonate (1.48 g, 4.56 mmol) at 0° C under N_2 atmosphere. 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 (20 mL) and extracted with EtOAc (30 mL). The separated organic layer was dried over Na_2SO_4, concentrated under reduced pressure to afford crude compound, which was purified by column chromatography eluting 2% MeOH/DCM to afford compound **4S-27** (400 mg, 56.8%) as yellow liquid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.39-7.31 (m, 5H), 5.08-5.04 (m, 2H), 4.11-3.95 (m, 2H), 3.87 (s, 1H), 3.60-3.47 (m, 4H), 2.18-2.14 (m, 2H), 1.86-1.74 (m, 7H), 1.23-1.11 (m, 1H), 1.06 (d, J = 6.4 Hz, 3H).

LCMS (ESI): 386.4 [M^++1].

Synthesis of 3-methyl-2-(2-oxo-2-(pyrrolidin-1-yl) ethyl)-2, 5-diazaspiro [3.4] octan-1-one 4S-28:

[0184] To a stirring solution of compound **4S-7** (400 mg, 1.03 mmol) in methanol (10 mL) was added 10% Pd/C (80 mg) under N_2 atmosphere. The reaction mixture was stirred under H_2 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 (10 mL). Obtained filtrate was concentrated under reduced pressure to afford crude compound was purified by column chromatography by eluting 2% MeOH/DCM to afford **4S-28** as pale yellow thick syrup (160 mg, 61.3%).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ4.06-3.99 (m, 1H), 3.77-3.70 (m, 1H), 3.63-3.58 (m, 1H), 3.39 (s, 2H), 3.27 (t, J = 7.2 Hz, 2H), 2.94-2.76 (m, 3H), 1.98-1.59 (m, 8H), 1.06 (d, J = 6.4 Hz, 3H).

LCMS (ESI): 252.3 [M^++1].

ELSD: 32.06 and 67.69%.

Synthesis of benzyl (3-hydroxy-1-(1-methyl-3-oxo-2-(2-oxo-2-(pyrrolidin-1-yl) ethyl)-2, 5-diazaspiro [3.4] octan-5-yl)-1-oxobutan-2-yl) carbamate (4S-29):

[0185] To a stirring solution of **4S-28** (600 mg, 2.38 mmol) in CH_2Cl_2 (20 mL) were added *N, N*-diisopropylethylamine (1.31 mL, 7.16 mmol), 4S-A (Cbz-threonine, 906 mg, 3.58 mmol), HATU (1.81 g, 4.77 mmol), at 0 °C and stirring was continued at RT for 16 h. The reaction mixture was treated with saturated aqueous $NaHCO_3$ solution for 15 min. The aqueous layer was extracted with CH_2Cl_2 (2x20 mL), the organic layer was separated and washed with saturated citric acid solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtained crude product, which was eluted with 2% MeOH/DCM to afford compound **4S-29** (280 mg, 24%) as pale yellow syrup.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 7.37-7.27 (m,5H), 7.04 (s, 1H), 5.07-4.98 (m, 2H), 4.77-4.70 (m, 1H), 4.29-4.26 (m, 1H), 4.15-3.96 (m, 2H), 3.86-3.56 (m, 4H), 3.48-3.35 (m, 2H), 3.30-3.25 (m, 2H), 2.13-2.04 (m, 1H), 1.88-1.74 (m, 7H), 1.14 (d, J = 6.4 Hz, 3H), 1.07-1.05 (m, 3H). **LCMS (ESI):** 487.6 [M^++1]

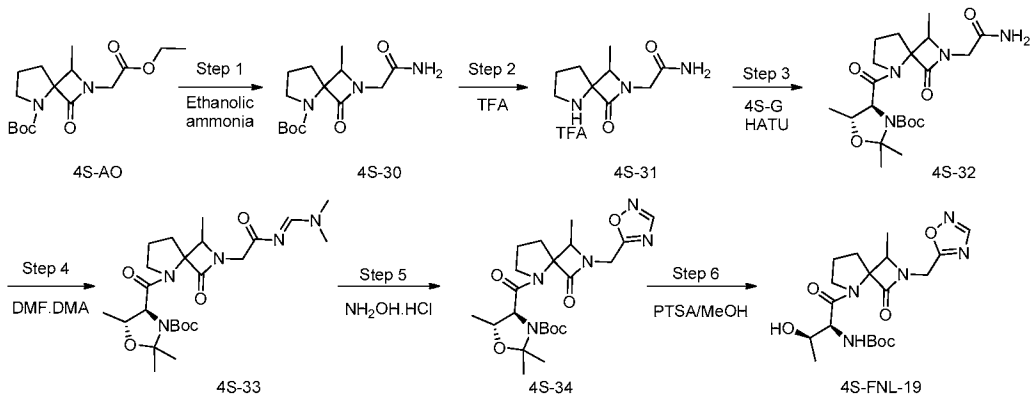
Synthesis of 5-(2-amino-3-hydroxybutanoyl)-3-methyl-2-(2-oxo-2-(pyrrolidin-1-yl) ethyl)-2, 5-diazaspiro [3.4] octan-1-one (4S-FNL-18):

[0186] To a stirring solution of compound **4S-29** (140 mg, 0.28 mmol) in methanol (10 mL) was added 10% Pd/C (80 mg) under N_2 atmosphere. The reaction mixture was stirred under H_2 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 (10 mL). Obtained filtrate was concentrated under reduced pressure to afford crude compound was purified by column chromatography by eluting 4% MeOH/DCM to afford compound **(4S-FNL-18)** as off-white sticky solid (160 mg, 61.3%).

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 5.27 (d, J =4.4 Hz, 1H), 4.71-4.66 (m, 1H), 4.13-4.05 (m, 2H), 3.65-3.55 (m, 1H), 3.27-3.18 (m, 7H), 2.22-2.18 (m, 1H), 1.97-1.71 (m, 8H), 1.16-0.98 (m, 6H).

LCMS (ESI): 353.3 [M^++1].

Scheme 4S-11



Synthesis of tert-butyl 2-(2-amino-2-oxoethyl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octane-5-carboxylate (4S-30):

[0187] To a stirring solution of **4S-AO** (2 g, 6.13 mmol) in ethanol (5 mL) was added ethanolic ammonia (20 mL) at 0 °C in sealed tube. The reaction mixture was stirred at RT for 16 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to afford compound 4S-30 (1.5 g, 83%) as white sticky solid used directly for next step without purification.

^{1}H-NMR: (400 MHz, DMSO-d_6) δ 7.52 (s, 2H), 3.98-3.81 (m, 1H), 3.73-3.52 (m, 2H), 3.49-3.31 (m, 2H), 2.12-1.70 (m, 4H), 1.40 (s, 9H), 1.18 (d, J = 6.4 Hz, 3H);

LCMS (ESI): *m/z* 298.3 [M^++1]

Synthesis of 2-(1-methyl-3-oxo-2, 5-diazaspiro [3.4] octan-2-yl) acetamide (4S-31):

[0188] To a stirring solution of compound **4S-30** (1.5 g, 5.05 mmol) in DCM (40 mL) was added TFA (3.85 mL, 50.5 mol) slowly and stirred to RT for 3 h. After consumption of the starting material (by TLC), the reaction was concentrated under reduced pressure to afford compound **4S-31** (1.5 g, crude, TFA salt) was used directly for next step without any purification.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 9.45 (s, 2H), 7.49 (d, J = 15.5 Hz, 1H), 3.99-3.40 (m, 3H), 3.39-3.26 (m, 2H), 2.27-1.90 (m, 4H), 1.17-1.11 (m, 3H);

LCMS (ESI): ***m/z*** **198.2 [M$^+$+1]**

Synthesis of tert-butyl (4*S*, 5*R*)-4-(2-(2-amino-2-oxoethyl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octane-5-carbonyl)-2, 2, 5-trimethyloxazolidine-3-carboxylate (4S-32):

[0189] To a stirring solution of compound **4S-31** (1.5 g, 4.82 mmol) in DMF (20 mL) were added *N, N*-diisopropylethylamine (4.19 mL, 24.1 mmol), 4S-G (1.62 g, 6.26 mmol) followed by HATU (2.74 g, 7.23 mmol) at 0 °C and stirred at RT for 16 h. After consumption of the starting material (by TLC), the residue was diluted with water (100 mL) and EtOAc (100 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 2% MeOH/DCM to afford compound **4S-32** (1.2 g) as brown thick syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.61 (s, 2H), 4.26-4.06 (m, 1H), 4.00-3.93 (m, 2H), 3.92-3.74 (m, 2H), 3.63-3.43 (m, 2H), 2.36-1.99 (m, 1H), 1.92-1.67 (m, 3H), 1.52 (s, 6H), 1.48-1.39 (m, 3H), 1.39 (s, 9H), 1.20-1.17 (m, 3H);

LCMS (ESI): *m/z* 439.5 [M$^+$+1]

Synthesis of tert-butyl (4*S*, 5*R*)-4-(2-(2-(((*E*)-(dimethylamino) methylene) amino)-2-oxoethyl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octane-5-carbonyl)-2, 2, 5-trimethyloxazolidine-3-carboxylate (4S-33):

[0190] To a stirring solution of compound **4S-32** (2.3 g, 5.24 mmol) in DMF.DMA (11.5 mL) was heated to 80 °C for 4 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to afford compound **4S-33** (2.6 g, crude) as brown thick syrup was used directly for next step without purification.

Synthesis of tert-butyl (4*S*, 5*R*)-4-(2-((1, 2, 4-oxadiazol-5-yl) methyl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octane-5-carbonyl)-2, 2, 5-trimethyloxazolidine-3-carboxylate (4S-34):

[0191] To a stirring solution of compound **4S-33** (2.6 g, 5.26 mmol) in ethanol was added hydroxyl amine.HCl (732 mg, 10.53 mmol) and heated to 80 °C for 6 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to afford crude compound, which was diluted with H_2O (30 mL) and EtOAc (50 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford crude residue which was purified by column chromatography by eluting 1% MeOH/DCM to afford compound **4S-34** (620 mg, 25.4%) as yellow thick syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 9.00 (s, 1H), 4.95-4.62 (m, 2H), 4.35-4.01 (m, 1H), 3.94-3.42 (m, 2H), 2.89-2.63 (m, 2H), 2.17-1.86 (m, 4H), 1.51 (s, 6H), 1.41 (s, 9H), 1.28-1.10 (m, 6H)

LCMS: *m/z* 464.5 [M^++1]

Synthesis of tert-butyl ((2*S*, 3*R*)-1-(2-((1, 2, 4-oxadiazol-5-yl) methyl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octan-5-yl)-3-hydroxy-1-oxobutan-2-yl) carbamate (4S-FNL-19):

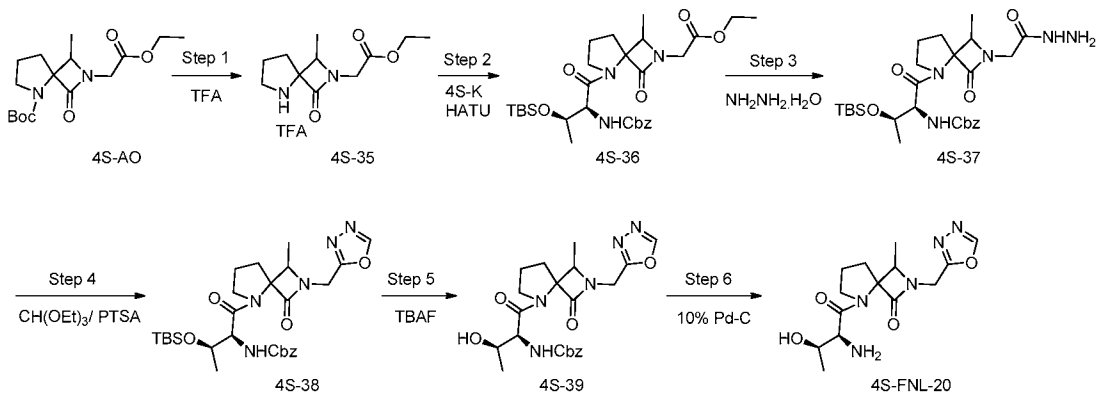
[0192] To a stirring solution of compound **4S-34** (620 mg, 1.33 mmol) in methanol (10 mL) was added PTSA (254 mg, 1.33 mmol) at RT and stirred for 16 h. After consumption of the starting material (by TLC), the reaction mixture was quenched with $NaHCO_3$ and stirred for 15 minutes. After filtered the reaction mass and methanol was concentrated under reduced pressure to afford crude compound, which was purified by column chromatography by eluting 1% MeOH/DCM to afford **(4S-FNL-19)** (240 mg, 42.6%) as an off-white semi solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.98 (s, 1H), 6.77 (d, J = 7.6 Hz, 1H), 4.70-4.62 (m, 2H), 4.23-4.20 (m, 1H), 4.08-4.01 (m, 2H), 3.72-3.55 (m, 3H), 2.15-2.06 (m, 1H), 1.90-1.81 (m, 3H), 1.38 (s, 9H), 1.16-1.04 (m, 6H);

HPLC:95.96%

LCMS (ESI): *m/z* 424.4 [M^++1]

Scheme 4S-12



Synthesis of ethyl 2-(1-methyl-3-oxo-2, 5-diazaspiro [3.4] octan-2-yl) acetate (4S-35):

[0193] To a stirring solution of **4S-AO** (2 g, 6.13 mmol) in DCM (20 mL) was added trifluoroacetic acid (4.71 mL, 61.2 mmol) at 0 °C under N_2 atmosphere. The reaction mixture was stirred at RT for 1 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 *n*-pentane (10 mL) to afford compound **4S-35** (2.08 g, crude) as brown thick syrup (TFA salt).

^{1}H-NMR: (500 MHz, DMSO-d_6) δ 9.20 (s, 1H), 4.22-3.98 (m, 3H), 3.45-3.33 (m, 2H), 3.31-3.26 (m, 2H), 2.23-2.05 (m, 3H), 2.04-1.50 (m, 2H), 1.44-1.31 (m, 2H), 1.30-1.22 (m, 3H)

LCMS *m/z*: 227.3 [M^++1]

Synthesis of ethyl 2-(5-(*N*-((benzyloxy) carbonyl)-*O*-(tert-butyldimethylsilyl)-L-threonyl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octan-2-yl) acetate (4S-36):

[0194] To a stirring solution of compound **4S-35** (2 g, 5.87 mmol) in DMF (10 mL) were added *N, N*-diisopropylethylamine (2.55 mL, 14.6 mmol), 4S-K (2.58 g, 7.03) followed by HATU (3.34 g, 8.8 mmol) at 0 °C and stirred at RT for 16 h. After consumption of the starting material (by TLC), the residue was diluted with water (100 mL) and EtOAc (100 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtained crude; which was purified by column chromatography eluting 50% EtOAc/*n*-hexane to afford compound **4S-36** (1 g, 29.6%) as yellow thick syrup.

^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.37-7.31 (m, 5H), 7.19 (d, J = 9.5 Hz, 1H), 5.01 (s, 2H), 4.35-4.10 (m, 2H), 4.09-4.00 (m, 2H), 3.99-3.64 (m, 4H), 2.71-2.60 (m, 4H), 2.10-1.78 (m, 4H), 1.23-1.12 (m, 6H), 1.10 (s, 9H), 0.04 (s, 6H)

LCMS *m/z*: 576.7 [M^++1]

Synthesis of benzyl ((2*S*, 3*R*)-3-((tert-butyldimethylsilyl) oxy)-1-(2-(2-hydrazinyl-2-oxoethyl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octan-5-yl)-1-oxobutan-2-yl) carbamate (4S-37):

[0195] To a stirring solution of compound **4S-36** (1 g, 1.73 mmol) in ethanol (15 mL) was added hydrazine hydrate (0.25 mL, 5.21 mmol) and heated to 80 °C for 3 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 2% MeOH/DCM to afford compound **4S-37** (850 mg, 87.6%) as an off-white semi solid.

^{1}**H-NMR:** (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 7.50 (s, 1H), 7.36-7.26 (m, 5H), 4.99 (s, 2H), 4.42-4.38 (m, 3H), 4.03-3.92 (m, 2H), 3.87-3.81 (m, 1H), 3.70-3.62 (m, 3H), 2.13-2.09 (m, 1H), 1.98-1.74 (m, 3H), 1.23-1.10 (m, 6H), 0.81 (s, 9H), 0.05 (s, 6H)

LCMS (ESI): **562.7 [M^++1]**

Synthesis of benzyl ((2*S*, 3*R*)-3-((tert-butyldimethylsilyl) oxy)-1-(2-(2-hydrazinyl-2-oxoethyl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octan-5-yl)-1-oxobutan-2-yl) carbamate (4S-38):

[0196] To a stirring solution of compound **4S-37** (850 mg, 1.51 mmol) in triethyl orthoformate (8.5 mL) was added PTSA (28.7 mg, 0.15 mmol) and heated to 80 °C for 6 h. After consumption of the starting material (by TLC), the reaction mixture was concentrated under reduced pressure to afford crude compound, which was purified by column chromatography by eluting 1% MeOH/DCM to afford compound **4S-38** (650 mg, 75.4%) as yellow thick syrup.

^{1}**H-NMR**: (400 MHz, DMSO-d_6): δ 9.24 (s, 1H), 7.35-7.30 (m, 5H), 5.01 (s, 2H), 4.64-4.58 (m, 2H), 4.22-3.89 (m, 4H), 3.67-3.60 (m, 2H), 2.12-2.07 (m, 1H), 2.01-1.78 (m, 3H), 1.33-1.12 (m, 6H), 0.82 (s, 9H), 0.01 (s, 6H).

LCMS *m/z*: 571.3 [M^++1].

Synthesis of benzyl ((2*S*, 3*R*)-1-(2-((1, 3, 4-oxadiazol-2-yl) methyl)-1-methyl-3-oxo-2, 5-diazaspiro [3.4] octan-5-yl)-3-hydroxy-1-oxobutan-2-yl) carbamate (4S-39):

[0197] To a stirring solution of compound **4S-38** (650 mg, 1.13 mmol) in THF (10 mL) was added TBAF (1M in THF) (1.7 mL, 1.7 mmol) at 0 °C under N_2 atmosphere. The reaction mixture was stirred at RT for 1 h. After consumption of the starting material (by TLC), the reaction mixture was diluted with NH_4Cl solution (20 mL) and EtOAc (30 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to

obtained crude; which was purified by column chromatography eluting 2% MeOH/DCM to afford compound **4S-39** (300 mg, 58.1%) as white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ 9.24 (s, 1H), 7.37-7.30 (m, 5H), 7.07 (t, J = 8.8 Hz, 1H), 5.07 (s, 2H), 4.81-4.72 (m, 1H), 4.69-4.61 (m, 1H), 4.33-4.25 (m, 2H), 3.99-3.85 (m, 2H), 3.40-3.15 (m, 2H), 2.13-2.05 (m, 1H), 1.85-1.80 (m, 3H), 1.13-1.03 (m, 6H).

Synthesis of 2-((1, 3, 4-oxadiazol-2-yl) methyl)-5-(L-threonyl)-3-methyl-2, 5-diazaspiro [3.4] octan-1-one (4S-FNL-20):

[0198] To a stirring solution of compound 4S-39 (300 mg, 0.65 mmol) in methanol (10 mL) was added 10% wet Pd/C (90 mg) and stirred under H_2 atmosphere (balloon pressure) for 12 h at RT. After consumption of the starting material (by TLC), reaction mixture was filtered through a pad of celite and triturated with methanol (5 mL). The filtrate was concentrated under reduced pressure to afford crude compound which was purified by column chromatography by eluting 5% MeOH/DCM to afford **(4S-FNL-20)** (100 mg, 47.1%) as white solid.

^{1}H-NMR: (400 MHz, DMSO-d_6): 9.14 (s, 1H), 7.99-7.94 (m, 1H), 5.16-5.09 (m, 1H), 4.11-4.05 (m, 1H), 3.99-3.74 (m, 3H), 3.55-3.52 (m, 1H), 3.36-3.27 (m, 1H), 3.17-2.79 (m, 1H), 2.68-2.64 (m, 1H), 2.33-2.18 (m, 1H), 1.96-1.63 (m, 3H), 1.17-1.01 (m, 6H);
LCMS *m/z*: 324.3 [M^++1];
HPLC: 95.52% .

Example 3 - [^{3}H] MK-801 binding assay

Methods

[00199] 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. GLYX-13: a monoclonal antibody-derived peptide that acts as an N-methyl-D-aspartate receptor modulator. Neuropharmacology. 49, 1077-87) The potentiation of [^{3}H]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 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 [^{3}H]MK-801 binding by tests compounds was calculated by using

a 3 parameter log agonist vs. response equation (Graph pad Prism, USA) and potency (EC_{50}, expressed in pM) and maximal activity (% maximal stimulation) were calculated for the test compound.

Results

[00200] As shown in Table 2, Figure 1, the potency and maximal activity for Compound X is 0.3 and 31%.

Table 2.

Compounds	pEC50	Activity (%)
X	0.3	31

Table 3. Additional Biological Data

Compound	[3H] MK-801 binding assay: EC50 (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)
4S-FNL-1	2.43E-13	80	1	NS	70	3	IV	1
4S-FNL-2	5.53E-11							

EQUIVALENTS

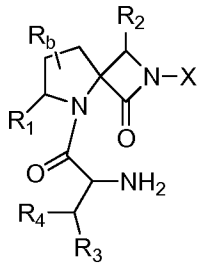
[00201] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

INCORPORATION BY REFERENCE

[00202] The entire contents of all patents, published patent applications, websites, and other references cited herein are hereby expressly incorporated herein in their entireties by reference.

What is claimed is:

1. A compound represented by formula I:



(I)

or a pharmaceutically acceptable salt thereof, wherein

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

R_1 is H or C_1-C_6 alkyl;

R_2 is H or C_1-C_6 alkyl;

R_3 is selected from the group consisting of H, C_1-C_6alkyl, -OH, C_1-C_6alkoxy, -OC(O)-C_1-C_6 alkyl and -OC(O)-phenyl (optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

R_4 is H or C_1-C_6 alkyl;

X is selected from the group consisting of:

(i) hydrogen;

(ii) -C_{1-6}alkylene-C_{3-6}cycloalkyl;

(iii) -C_{1-6}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heterocyclyl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

(iv) -C_{1-6}alkylene- C(O)-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(C_1-C_3 alkyl), O, and S; wherein the heterocyclyl is optionally substituted by

one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

(v) -C_{1-6}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heteroaryl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy;

(vi) branched unsubstituted C_3-C_6 alkyl; and

(vii) branched C_3-C_6 alkyl substituted with –C(O)NH_2 on one carbon and –OH on another carbon;

and wherein the –NH_2 group attached to the carbon adjacent to –CH(R_3)(R_4) is optionally substituted with a substituent selected from –C(O)OR_{31} and –C(O) R_{32}, wherein:

R_{31} is selected from the group consisting of: C_1-C_6 alkyl; C_1-C_6 haloalkyl; C_2-C_6 alkenyl; C_2-C_6 alkynyl; C_3-C_{10} cycloalkyl, wherein the C_3-C_{10} cycloalkyl is optionally substituted with from 1-3 independently selected C_1-C_3 alkyl; -CH_2-C_3-C_{10} cycloalkyl wherein the C_3-C_{10} cycloalkyl is optionally substituted with from 1-3 independently selected C_1-C_3 alkyl; -CH_2-phenyl, wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C_1-C_3 alkyl; C_1-C_3 haloalkyl; C_1-C_3 alkoxy; C_1-C_3 haloalkoxy; nitro; halo; SO_2Me, cyano; and -OC(O)CH_3; and -CH_2-pyridyl; and

R_{32} is selected from the group consisting of: H; C_1-C_6 alkyl; C_1-C_6 haloalkyl; phenyl, wherein the phenyl is optionally substituted with from 1-2 substituents independently selected from C_1-C_3 alkyl; C_1-C_3 haloalkyl; C_1-C_3 alkoxy; C_1-C_3 haloalkoxy; nitro; halo; SO_2Me, cyano; and -OC(O)CH_3; and pyridyl.

2. The compound of claim 1, wherein R_1 is H.

3. The compound of claim 1, wherein R_1 is C_1-C_6 alkyl.

4. The compound of claim 1, wherein R_1 is methyl.

5. The compound of any one of claims 1-4, wherein R_2 is H.

6. The compound of any one of claims 1-5, wherein R_3 is –OH.

7. The compound of any one of claims 1-6, wherein R_4 is C_1-C_6 alkyl.

8. The compound of any one of claims 1-6, wherein R_4 is methyl.

9. The compound of any one of claims 1-8, wherein X is hydrogen.

10. The compound of any one of claims 1-8, wherein X is selected from the group consisting of:

(ii) -C_{1-6}alkylene-C_{3-6}cycloalkyl;

(iii) -C_{1-6}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heterocyclyl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

(iv) -C_{1-6}alkylene- C(O)-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(C_1-C_3 alkyl), O, and S; wherein the heterocyclyl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy); and

(v) -C_{1-6}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heteroaryl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy.

11. The compound of any one of claims 1-8, wherein X is selected from the group consisting of:

(ii) -C_{1-6}alkylene-C_{3-6}cycloalkyl;

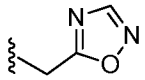
(iii) -C_{1-6}alkylene- heterocyclyl including from 3 to 6 ring atoms wherein 1, 2, or 3 of the ring atoms are independently selected from the group consisting of N, NH, N(C_1-C_3 alkyl), O, and S; wherein the heterocyclyl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy); and

(v) -C_{1-6}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heteroaryl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy.

12. The compound of any one of claims 1-8, wherein X is -C_{1-6}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heteroaryl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy.

13. The compound of any one of claims 1-8, wherein X is C_{1-2}alkylene- 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(C_1-C_3 alkyl), O, and S; wherein the heteroaryl is optionally substituted by one, two or three substituents independently selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy.

14. The compound of any one of claims 1-8 and 10-13, wherein the heteroaryl is selected from the group consisting of 1,2,4- oxadiazolyl, 1,3,4- oxadiazolyl, pyridyl, and pyrimidinyl.

15. The compound of any one of claims 1-8 and 10-14, wherein X is .

16. The compound of claim 1, wherein R_1 is H; R_2 is H; R_3 is –OH; R_4 is methyl; and X is hydrogen.

17. The compound of claim 1, wherein R_1 is methyl; R_2 is H; R_3 is –OH; R_4 is methyl; and X is hydrogen.

18. The compound of any one of claims 1-17, wherein the –NH_2 group attached to the carbon adjacent to –CH(R_3)(R_4) is not substituted.

19. The compound of any one of claims 1-17, wherein the –NH_2 group attached to the carbon adjacent to –CH(R_3)(R_4) is substituted with a substituent selected from –C(O)OR_{31} and –C(O) R_{32}.

20. The compound of claim 19, wherein the –NH_2 group attached to the carbon adjacent to –CH(R_3)(R_4) is substituted with –C(O)OR_{31}.

21. The compound of claim 20, wherein R_{31} is C_1-C_6 alkyl.

22. The compound of claim 21, wherein R_{31} is *tert*-butyl.

23. The compound of claim 19, wherein the $-NH_2$ group attached to the carbon adjacent to $-CH(R_3)(R_4)$ is substituted with $-C(O)R_{32}$.

24. The compound of claim 23, wherein R_{32} is C_1-C_6 alkyl.

25. The compound of claim 23, wherein R_{32} is $-CH_3$ or *iso*-propyl.

26. The compound of claim 1, wherein the compound is selected from the compounds delineated in Table 1.

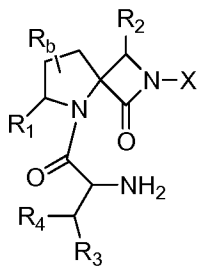
27. A pharmaceutical composition comprising a compound of any one of claims 1-26, and a pharmaceutically acceptable excipient.

28. The pharmaceutical composition of claim 27, suitable for oral administration.

29. The pharmaceutical composition of claim 27, suitable for intravenous administration.

30. A method of treating of treating depression, Alzheimer's disease, attention deficit disorder, schizophrenia, or anxiety, in a patient in need thereof, comprising administering to said patient: a pharmaceutically effective amount of a compound of any one of claims 1-26.

31. A compound represented by formula I:



(I)

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

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

R_1 is H or C_1-C_6 alkyl;

R_2 is H or C_1-C_6 alkyl;

R_3 is selected from the group consisting of H, C_1-C_6alkyl, -OH, C_1-C_6alkoxy, -OC(O)-C_1-C_6 alkyl and -OC(O)-phenyl (optionally substituted by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy);

R_4 is H or C_1-C_6 alkyl; and

X is selected from the group consisting of hydrogen, -C_{1-6}alkylene-C_{3-6}cycloalkyl; C_{1-6}alkylene- heterocycle (optionally substituted by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy), and -C_{1-6}alkylene- heteroaryl (optionally substituted by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy).

32. The compound of claim 31, wherein R_1 is C_1-C_6 alkyl.

33. The compound of claim 31, wherein R_1 is methyl.

34. The compound of any one of claims 31-33, wherein R_2 is H.

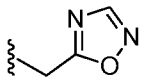
35. The compound of any one of claims 31-34, wherein R_3 is –OH.

36. The compound of any one of claims 31-35, wherein R_4 is C_1-C_6 alkyl.

37. The compound of any one of claims 30-35, wherein R_4 is methyl.

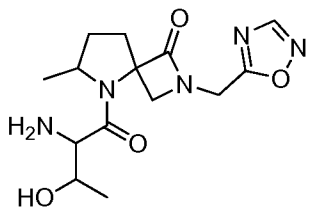
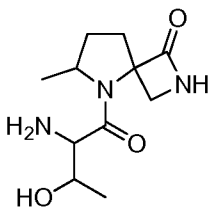
38. The compound of any one of claims 31-37, wherein X is hydrogen.

39. The compound of any one of claims 31-37, wherein X is C_{1-2}alkylene- heteroaryl.

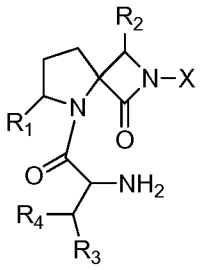
40. The compound of any one of claims 31-37, wherein X is .

41. The compound of claim 31, wherein R_1 is methyl; R_2 is H; R_3 is –OH; R_4 is methyl; and X is hydrogen.

42. The compound of claim 1, wherein the compound is

 or .

43. A compound represented by formula II:



(II)

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

R_1 is H or C_1-C_6 alkyl;

R_2 is H or C_1-C_6 alkyl;

R_3 is selected from the group consisting of H, C_1-C_6 alkyl, -OH, C_1-C_6 alkoxy, -OC(O)-C_1-C_6 alkyl and -OC(O)-phenyl;

R_4 is H or C_1-C_6 alkyl; and

X is $-C_1$-C_6alkylene-X′, wherein X′ is a 4- to 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from O, N, or S, where X' is optionally substituted on a free carbon by one, two or three substituents selected from the group consisting of halogen, hydroxyl, C_1-C_6alkyl, and C_1-C_6alkoxy.

44. A pharmaceutical composition comprising a compound of any one of claims 31-43, and a pharmaceutically acceptable excipient.

45. The pharmaceutical composition of claim 44, suitable for oral administration.

46. The pharmaceutical composition of claim 44, suitable or intravenous administration.

47. A method of treating of treating depression, Alzheimer's disease, attention deficit disorder, schizophrenia, or anxiety, in a patient in need thereof, comprising administering to said patient:

a pharmaceutically effective amount of a compound of any one of claims 31-43.