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(54) Titre : INHIBITEURS SELECTIFS DE LA GLYCOSIDASE ET LEURS UTILISATIONS

(54) Title: SELECTIVE GLYCOSIDASE INHIBITORS AND USES THEREOF

(57) Abrégé/Abstract:

The application relates to an immoalditol compound for selectively inhibiting glycosidases, a prodrug thereof and a pharmaceutical composition comprising the compound or the prodrug. The application also relates to the use of the immoalditol compound for treating diseases and disorders related to deficiency or overexpression of O-GlcNAcase, accumulation or deficiency of O-GlcNAc. Such diseases and disorders include neurodegenerative diseases, tauopathy, cancers, and cardiac disorders.

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(54) Title: SELECTIVE GLYCOSIDASE INHIBITORS AND USES THEREOF

(57) Abstract: The application relates to an immoalditol compound for selectively inhibiting glycosidases, a prodrug thereof and a pharmaceutical composition comprising the compound or the prodrug. The application also relates to the use of the immoalditol compound for treating diseases and disorders related to deficiency or overexpression of O-GlcNAcase, accumulation or deficiency of O-GlcNAc. Such diseases and disorders include neurodegenerative diseases, tauopathy, cancers, and cardiac disorders.

SELECTIVE GLYCOSIDASE INHIBITORS AND USES THEREOF

FIELD OF THE INVENTION

[0001] This application relates to compounds which selectively inhibit glycosidases and
5 uses thereof.

BACKGROUND OF THE INVENTION

[0002] A wide range of cellular proteins, both nuclear and cytoplasmic, are post-
translationally modified by the addition of the monosaccharide 2-acetamido-2-deoxy- β -D-
10 glucopyranoside (β -N-acetylglucosamine) which is attached via an O-glycosidic linkage.¹
This modification is generally referred to as O-linked N-acetylglucosamine or O-GlcNAc.
The enzyme responsible for post-translationally linking β -N-acetylglucosamine (GlcNAc)
to specific serine and threonine residues of numerous nucleocytoplasmic proteins is O-
GlcNAc transferase (OGT).²⁻⁵ A second enzyme, known as O-GlcNAcase^{6,7} removes this
15 post-translational modification to liberate proteins making the O-GlcNAc-modification a
dynamic cycle occurring several times during the lifetime of a protein.⁸

[0003] O-GlcNAc-modified proteins regulate a wide range of vital cellular functions
including, for example, transcription,⁹⁻¹² proteasomal degradation,¹³ and cellular signaling.¹⁴
O-GlcNAc is also found on many structural proteins.¹⁵⁻¹⁷ For example, it has been found on
20 a number of cytoskeletal proteins, including neurofilament proteins,^{18,19} synapsins,^{6,20}
synapsin-specific clathrin assembly protein AP-3,⁷ and ankyrinG.¹⁴ O-GlcNAc
modification has been found to be abundant in the brain.^{21,22} It has also been found on
proteins clearly implicated in the etiology of several diseases including Alzheimer's disease
25 (AD) and cancer.

[0004] For example, it is well established that AD and a number of related tauopathies
including Downs' syndrome, Pick's disease, Niemann-Pick Type C disease, and
amyotrophic lateral sclerosis (ALS) are characterized, in part, by the development of
neurofibrillary tangles (NFTs). These NFTs are aggregates of paired helical filaments
30 (PHFs) and are composed of an abnormal form of the cytoskeletal protein "tau". Normally
tau stabilizes a key cellular network of microtubules that is essential for distributing proteins
and nutrients within neurons. In AD patients, however, tau becomes hyperphosphorylated,

disrupting its normal functions, forming PHFs and ultimately aggregating to form NFTs. Six isoforms of tau are found in the human brain. In AD patients, all six isoforms of tau are found in NFTs, and all are markedly hyperphosphorylated.^{23,24} Tau in healthy brain tissue bears only 2 or 3 phosphate groups, whereas those found in the brains of AD patients bear, on average, 8 phosphate groups.^{25,26} A clear parallel between NFT levels in the brains of AD patients and the severity of dementia strongly supports a key role for tau dysfunction in AD.^{27,28} The precise causes of this hyperphosphorylation of tau remain elusive.

Accordingly, considerable effort has been dedicated toward: a) elucidating the molecular physiological basis of tau hyperphosphorylation;²⁹ and b) identifying strategies that could limit tau hyperphosphorylation in the hope that these might halt, or even reverse, the progression of Alzheimer's disease³⁰⁻³³ Thus far, several lines of evidence suggest that up-regulation of a number of kinases may be involved in hyperphosphorylation of tau,^{21,34,35} although very recently, an alternative basis for this hyperphosphorylation has been advanced.²¹

[0005] In particular, it has recently emerged that phosphate levels of tau are regulated by the levels of O-GlcNAc on tau. The presence of O-GlcNAc on tau has stimulated studies that correlate O-GlcNAc levels with tau phosphorylation levels. The recent interest in this field stems from the observation that O-GlcNAc modification has been found to occur on many proteins at amino acid residues that are also known to be phosphorylated.³⁶⁻³⁸

Consistent with this observation, it has been found that increases in phosphorylation levels result in decreased O-GlcNAc levels and conversely, increased O-GlcNAc levels correlate with decreased phosphorylation levels.³⁹ This reciprocal relationship between O-GlcNAc and phosphorylation has been termed the "Yin-Yang hypothesis"⁴⁰ and has gained strong biochemical support by the recent discovery that the enzyme OGT⁴ forms a functional complex with phosphatases that act to remove phosphate groups from proteins.⁴¹ Like phosphorylation, O-GlcNAc is a dynamic modification that can be removed and reinstalled several times during the lifespan of a protein. Suggestively, the gene encoding O-GlcNAcase has been mapped to a chromosomal locus that is linked to AD.^{7,42}

Hyperphosphorylated tau in human AD brains has markedly lower levels of O-GlcNAc than are found in healthy human brains.²¹ Very recently, it has been shown that O-GlcNAc levels of soluble tau protein from human brains affected with AD are markedly lower than those from healthy brain.²¹ Furthermore, PHF from diseased brain was suggested to lack completely any O-GlcNAc modification whatsoever.²¹ The molecular basis of this

hypoglycosylation of tau is not known, although it may stem from increased activity of kinases and/or dysfunction of one of the enzymes involved in processing O-GlcNAc. Supporting this latter view, in both PC-12 neuronal cells and in brain tissue sections from mice, a nonselective N-acetylglucosaminidase inhibitor was used to increase tau O-GlcNAc levels, whereupon it was observed that phosphorylation levels decreased.²¹ The implication of these collective results is that by maintaining healthy O-GlcNAc levels in AD patients, such as by inhibiting the action of O-GlcNAcase, one should be able to block hyperphosphorylation of tau and all of the associated effects of tau hyperphosphorylation, including the formation of NFTs and downstream effects. However, because the proper functioning of the β -hexosaminidases is critical, any potential therapeutic intervention for the treatment of AD that blocks the action of O-GlcNAcase would have to avoid the concomitant inhibition of both hexosaminidases A and B.

[0006] Neurons do not store glucose and therefore the brain relies on glucose supplied by blood to maintain its essential metabolic functions. Notably, it has been shown that within brain, glucose uptake and metabolism decreases with aging.⁴³ Within the brains of AD patients marked decreases in glucose utilization occur and are thought to be a potential cause of neurodegeneration.⁴⁴ The basis for this decreased glucose supply in AD brain⁴⁵⁻⁴⁷ is thought to stem from any of decreased glucose transport,^{48,49} impaired insulin signaling,^{50,51} and decreased blood flow.⁵²

[0007] In light of this impaired glucose metabolism, it is worth noting that of all glucose entering into cells, 2-5% is shunted into the hexosamine biosynthetic pathway, thereby regulating cellular concentrations of the end product of this pathway, uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc).⁵³ UDP-GlcNAc is a substrate of the nucleocytoplasmic enzyme O-GlcNAc transferase (OGT),²⁻⁵ which acts to post-translationally add GlcNAc to specific serine and threonine residues of numerous nucleocytoplasmic proteins. OGT recognizes many of its substrates^{54,55} and binding partners^{41,56} through its tetratricopeptide repeat (TPR) domains.^{57,58} As described above, O-GlcNAcase^{6,7} removes this post-translational modification to liberate proteins making the O-GlcNAc-modification a dynamic cycle occurring several times during the lifetime of a protein.⁸ O-GlcNAc has been found in several proteins on known phosphorylation sites,^{10,37,38,59} including tau and neurofilaments.⁶⁰ Additionally, OGT shows unusual kinetic behaviour making it exquisitely sensitive to intracellular UDP-GlcNAc substrate concentrations and therefore glucose supply.⁴¹

[0008] Consistent with the known properties of the hexosamine biosynthetic pathway, the enzymatic properties of OGT, and the reciprocal relationship between O-GlcNAc and phosphorylation, it has been shown that decreased glucose availability in brain leads to tau hyperphosphorylation.⁴⁴ Therefore the gradual impairment of glucose transport and metabolism, whatever its causes, leads to decreased O-GlcNAc and hyperphosphorylation of tau (and other proteins). Accordingly, the inhibition of O-GlcNAcase should compensate for the age related impairment of glucose metabolism within the brains of health individuals as well as patients suffering from AD or related neurodegenerative diseases.

[0009] These results suggest that a malfunction in the mechanisms regulating tau O-GlcNAc levels may be vitally important in the formation of NFTs and associated neurodegeneration. Good support for blocking tau hyperphosphorylation as a therapeutically useful intervention⁶¹ comes from recent studies showing that when transgenic mice harbouring human tau are treated with kinase inhibitors, they do not develop typical motor defects³³ and, in another case,³² show decreased levels of insoluble tau. These studies provide a clear link between lowering tau phosphorylation levels and alleviating AD-like behavioural symptoms in a murine model of this disease. Indeed, pharmacological modulation of tau hyperphosphorylation is widely recognized as a valid therapeutic strategy for treating AD and other neurodegenerative disorders.⁶²

[0010] Recent studies⁶³ support the therapeutic potential of small-molecule O-GlcNAcase inhibitors to limit tau hyperphosphorylation for treatment of AD and related tauopathies. Specifically, the O-GlcNAcase inhibitor thiamet-G has been implicated in the reduction of tau phosphorylation in cultured PC-12 cells at pathologically relevant sites.⁶³ Moreover, oral administration of thiamet-G to healthy Sprague-Dawley rats has been implicated in reduced phosphorylation of tau at Thr231, Ser396 and Ser422 in both rat cortex and hippocampus.⁶³ .

[0011] There is also a large body of evidence indicating that increased levels of O-GlcNAc protein modification provides protection against pathogenic effects of stress in cardiac tissue, including stress caused by ischemia, hemorrhage, hypervolemic shock, and calcium paradox. For example, activation of the hexosamine biosynthetic pathway (HBP) by administration of glucosamine has been demonstrated to exert a protective effect in animals models of ischemia/reperfusion,⁶⁴⁻⁷⁰ trauma hemorrhage,⁷¹⁻⁷³ hypervolemic shock,⁷⁴ and calcium paradox.^{64,75} Moreover, strong evidence indicates that these cardioprotective effects are mediated by elevated levels of protein O-GlcNAc modification.^{64,65,67,70,72,75-78}

There is also evidence that the O-GlcNAc modification plays a role in a variety of neurodegenerative diseases, including Parkinson's disease and Huntington's disease.⁷⁹

[0012] Humans have three genes encoding enzymes that cleave terminal β -N-acetyl-glucosamine residues from glycoconjugates. The first of these encodes the enzyme O-glycoprotein 2-acetamido-2-deoxy- β -D-glucopyranosidase (O-GlcNAcase). O-GlcNAcase is a member of family 84 of glycoside hydrolases that includes enzymes from organisms as diverse as prokaryotic pathogens to humans (for the family classification of glycoside hydrolases see Coutinho, P.M. & Henrissat, B. (1999) Carbohydrate-Active Enzymes server at URL: <http://afmb.cnrs-mrs.fr/CAZY/>).^{27,28} O-GlcNAcase acts to hydrolyse O-GlcNAc off of serine and threonine residues of post-translationally modified proteins.^{1,6,7,80,81}

10 Consistent with the presence of O-GlcNAc on many intracellular proteins, the enzyme O-GlcNAcase appears to have a role in the etiology of several diseases including type II diabetes,^{14,82} AD,^{16,21,83} and cancer.^{22,84} Although O-GlcNAcase was likely isolated earlier on,^{18,19} about 20 years elapsed before its biochemical role in acting to cleave O-GlcNAc from serine and threonine residues of proteins was understood.⁶ More recently O-GlcNAcase has been cloned,⁷ partially characterized,²⁰ and suggested to have additional activity as a histone acetyltransferase.²⁰ However, little was known about the catalytic mechanism of this enzyme.

[0013] The other two genes, HEXA and HEXB, encode enzymes catalyzing the hydrolytic cleavage of terminal β -N-acetylglucosamine residues from glycoconjugates. The gene products of HEXA and HEXB predominantly yield two dimeric isozymes, hexosaminidase A and hexosaminidase B, respectively. Hexosaminidase A ($\alpha\beta$), a heterodimeric isozyme, is composed of an α - and a β -subunit. Hexosaminidase B ($\beta\beta$), a homodimeric isozyme, is composed of two β -subunits. The two subunits, α - and β -, bear a high level of sequence identity. Both of these enzymes are classified as members of family 20 of glycoside hydrolases and are normally localized within lysosomes. The proper functioning of these lysosomal β -hexosaminidases is critical for human development, a fact that is underscored by the tragic genetic illnesses, Tay-Sach's and Sandhoff diseases which stem from a dysfunction in, respectively, hexosaminidase A and hexosaminidase B.⁸⁵ These enzymatic deficiencies cause an accumulation of glycolipids and glycoconjugates in the lysosomes resulting in neurological impairment and deformation. The deleterious effects of accumulation of gangliosides at the organismal level are still being uncovered.⁸⁶

[0014] As a result of the biological importance of these β -N-acetyl-glucosaminidases, small

5 molecule inhibitors of glycosidases⁸⁷⁻⁹⁰ have received a great deal of attention,⁹¹ both as tools for elucidating the role of these enzymes in biological processes and in developing potential therapeutic applications. The control of glycosidase function using small molecules offers several advantages over genetic knockout studies including the ability to rapidly vary doses or to entirely withdraw treatment.

[0015] However, a major challenge in developing inhibitors for blocking the function of mammalian glycosidases, including O-GlcNAcase, is the large number of functionally related enzymes present in tissues of higher eukaryotes. Accordingly, the use of non-selective inhibitors in studying the cellular and organismal physiological role of one 10 particular enzyme is complicated because complex phenotypes arise from the concomitant inhibition of such functionally related enzymes. In the case of β -N-acetylglucosaminidases, existing compounds that act to block O-GlcNAcase function are non-specific and act potently to inhibit the lysosomal β -hexosaminidases.

[0016] A few of the better characterized inhibitors of β -N-acetyl-glucosaminidases which 15 have been used in studies of O-GlcNAc post-translational modification within both cells and tissues are streptozotocin (STZ), 2'-methyl- α -D-glucopyrano-[2,1-*d*]- Δ 2'-thiazoline (NAG-thiazoline) and *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamate (PUGNac).^{14,92-95}

[0017] STZ has long been used as a diabetogenic compound because it has a particularly 20 detrimental effect on β -islet cells.⁹⁶ STZ exerts its cytotoxic effects through both the alkylation of cellular DNA^{96,97} as well as the generation of radical species including nitric oxide.⁹⁸ The resulting DNA strand breakage promotes the activation of poly(ADP-ribose) polymerase (PARP)⁹⁹ with the net effect of depleting cellular NAD⁺ levels and, ultimately, leading to cell death.^{100,101} Other investigators have proposed instead that STZ toxicity is a 25 consequence of the irreversible inhibition of O-GlcNAcase, which is highly expressed within β -islet cells.^{92,102} This hypothesis has, however, been brought into question by two independent research groups.^{103,104} Because cellular O-GlcNAc levels on proteins increase in response to many forms of cellular stress¹⁰⁵ it seems possible that STZ results in increased O-GlcNAc-modification levels on proteins by inducing cellular stress rather than 30 through any specific and direct action on O-GlcNAcase. Indeed, Hanover and coworkers have shown that STZ functions as a poor and somewhat selective inhibitor of O-GlcNAcase¹⁰⁶ and although it has been proposed by others that STZ acts to irreversibly

inhibit O-GlcNAcase,¹⁰⁷ there has been no clear demonstration of this mode of action.

Recently, it has been shown that STZ does not irreversibly inhibit O-GlcNAcase.¹⁰⁸

[0018] NAG-thiazoline has been found to be a potent inhibitor of family 20

hexosaminidases,^{90,109} and more recently, the family 84 O-GlcNAcases.¹⁰⁸ Despite its

5 potency, a downside to using NAG-thiazoline in a complex biological context is that it lacks selectivity and therefore perturbs multiple cellular processes.

[0019] PUGNAc is another compound that suffers from the same problem of lack of

selectivity, yet has enjoyed use as an inhibitor of both human O-GlcNAcase^{6,110} and the

family 20 human β -hexosaminidases.¹¹¹ This molecule, developed by Vasella and

10 coworkers, was found to be a potent competitive inhibitor of the β -N-acetyl-

glucosaminidases from *Canavalia ensiformis*, *Mucor rouxii*, and the β -hexosaminidase from

bovine kidney.⁸⁸ It has been demonstrated that administration of PUGNAc in a rat model of

trauma hemorrhage decreases circulating levels of the pro-inflammatory cytokines TNF- α

and IL-6.¹¹² It has also been shown that administration of PUGNAc in a cell-based model

15 of lymphocyte activation decreases production of the cytokine IL-2.¹¹³ Recent studies have

indicated that PUGNAc can be used in an animal model to reduce myocardial infarct size

after left coronary artery occlusions.¹¹⁴ Of particular significance is the fact that elevation

of O-GlcNAc levels by administration of PUGNAc, an inhibitor of O-GlcNAcase, in a rat

model of trauma hemorrhage improves cardiac function.^{112,115} In addition, elevation of O-

20 GlcNAc levels by treatment with PUGNAc in a cellular model of ischemia/reperfusion

injury using neonatal rat ventricular myocytes improved cell viability and reduced necrosis

and apoptosis compared to untreated cells.¹¹⁶

[0020] More recently, it has been suggested that the selective O-GlcNAcase inhibitor

NButGT exhibits protective activity in cell-based models of ischemia/reperfusion and

25 cellular stresses, including oxidative stress.¹¹⁷ This study suggests the use of O-GlcNAcase

inhibitors to elevate protein O-GlcNAc levels and thereby prevent the pathogenic effects of

stress in cardiac tissue.

[0021] International patent applications PCT/CA2006/000300, filed 1 March 2006,

published under No. WO 2006/092049 on 8 September 2006, and PCT/CA2007/001554,

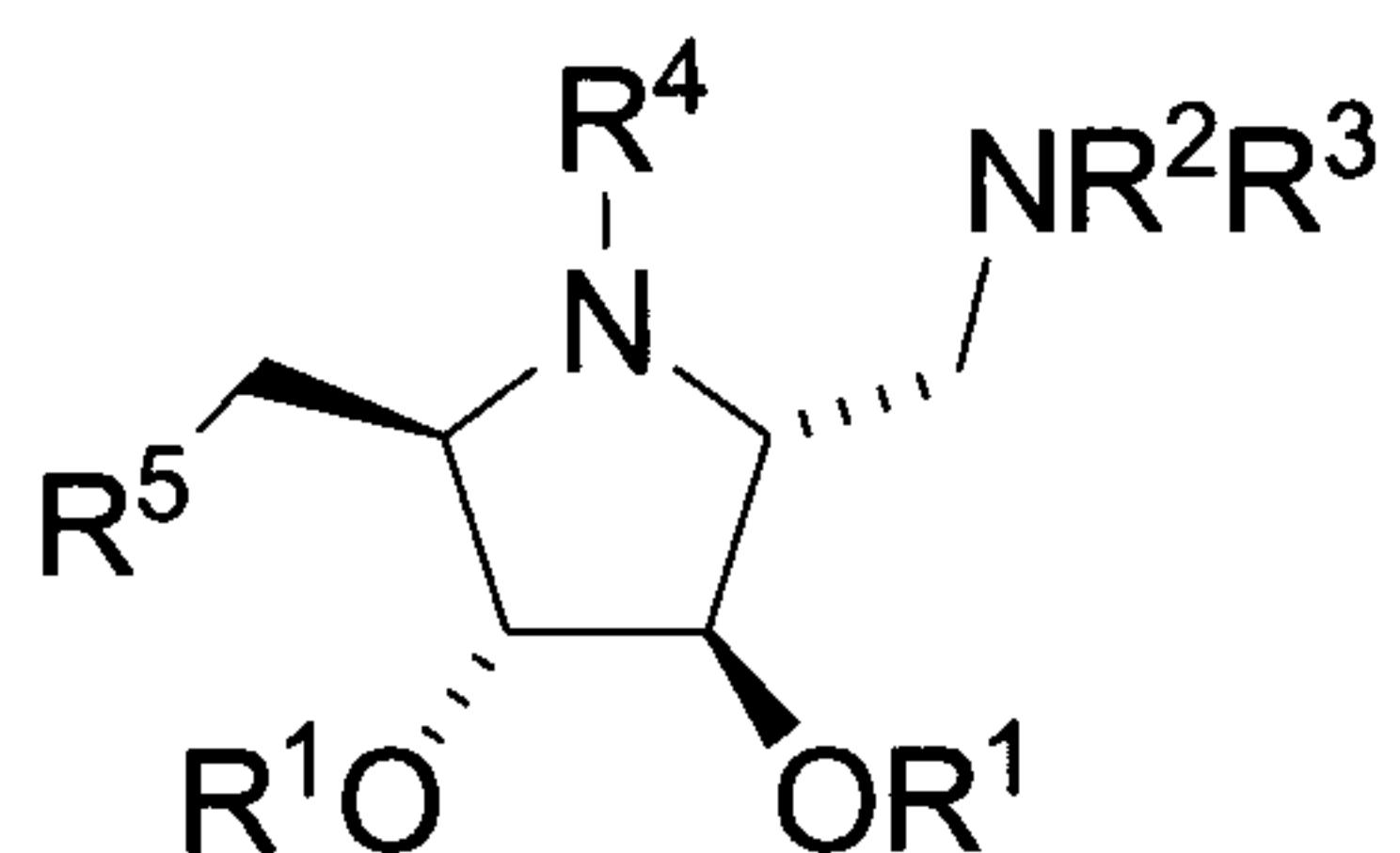
30 filed 31 August 2007, published under No. WO 2008/025170 on 6 March 2008, which are

hereby incorporated by reference, describe selective inhibitors of O-GlcNAcase.

SUMMARY OF THE INVENTION

[0022] The invention provides, in part, compounds for selectively inhibiting glycosidases, prodrugs of the compounds, uses of the compounds and the prodrugs, pharmaceutical compositions including the compounds or prodrugs of the compounds, and methods of 5 treating diseases and disorders related to deficiency or overexpression of O-GlcNAcase, and/or accumulation or deficiency of O-GlcNAc.

[0023] In one aspect, the invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



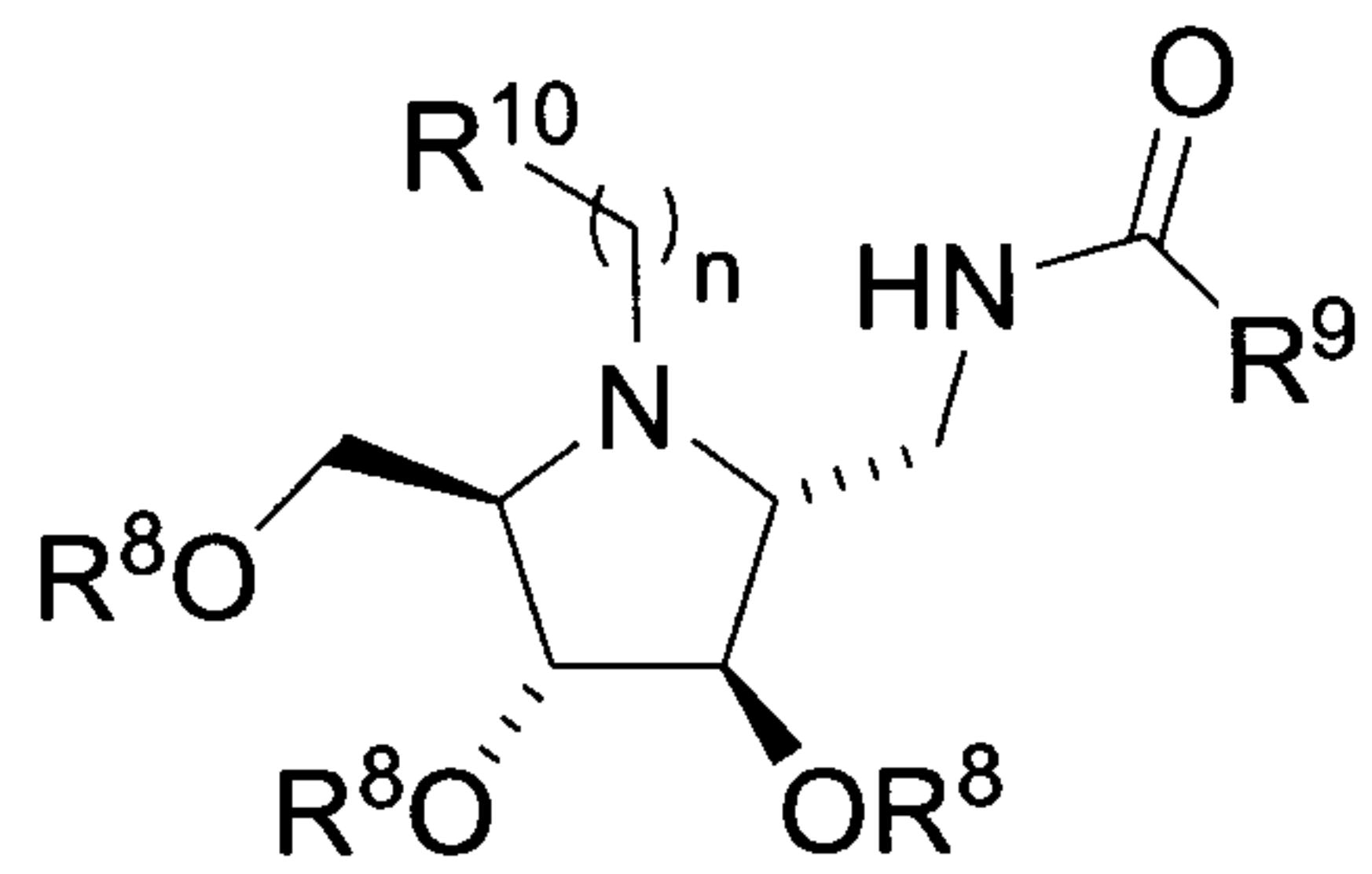
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(I)

where each R¹ may be independently H or C(O)R⁶; R² may be a non-interfering substituent; R³ may be H, C(O)R⁶, C(NR⁶)NR⁶₂, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl; R⁴ may 15 be a non-interfering substituent; R⁵ may be H, OR⁶, OC(O)R⁶, NR⁶C(O)R⁶, NR⁶₂, or an optionally substituted alkyl; wherein each R⁶ may be optionally independently a non-interfering substituent, with the proviso that when each R¹ is H, R² is H, R⁴ is H, and R⁵ is OH, R³ excludes H, C(O)CH₃, C(O)CH₂CH₃, C(O)(CH₂)₂CH₃, C(O)(CH₂)₃CH₃, C(O)(CH₂)₄CH₃, C(O)(CH₂)₇CH₃, C(O)(CH₂)₁₀CH₃, C(O)C≡CH, C(O)CH=CH₂, C(O)C(CH₃)=CH₂, C(O)(CH₂)₅OH, C(O)(1,3-benzodioxol-4-ylmethyl), C(O)(1,3-20 benzodioxol-5-ylmethyl), C(O)CH₂(3-indolyl), C(O)(CH₂)₂(phenyl), C(O)(2,3-dichlorophenyl), C(O)(2,3-dihydroxyphenyl), C(O)(2,3-dihydroxy-4-methylphenyl), C(O)(2-methyl-3-nitrophenyl), C(O)(2,3-dimethoxyphenyl), C(O)(2-hydroxy-3-methylphenyl), C(O)(2,6-dihydroxyphenyl), C(O)(2,3-dimethylphenyl), C(O)(2-hydroxy-3-methoxyphenyl), C(O)(2-naphthyl), C(O)(4-hydroxy-2-quinolyl), C(O)(4-benzoylphenyl), 25 C(O)(6-chloro-2H-chromene-3-yl), C(O)(1-naphthyl), C(O)(phenyl), C(O)(3-dimethylaminophenyl), C(O)(4-dimethylaminophenyl), C(O)(2-oxo-2H-chromene-3-yl), C(O)(7-diethylamino-2-oxo-2H-chromene-3-yl), S(O)₂(1-naphthyl), S(O)₂(5-dimethylamino-1-naphthyl), S(O)₂(phenyl), S(O)₂(CH₂)₇CH₃, C(O)(CH₂)₅NHSO₂(5-dimethylamino-1-naphthyl), (CH₂)₃NHSO₂(5-dimethylamino-1-naphthyl), C(O)(CH₂)₂(3-

indolyl), C(O)(5-indolyl), C(O)(CH₂)(1-naphthyl), C(O)(CH₂)(2-naphthyl), C(O)(2-methylthio(phenyl)), C(O)(E-(3-trifluoromethylphenyl-2-ethenyl)), C(O)(E-(3-chlorophenyl-2-ethenyl)), C(O)(E-(3-bromophenyl-2-ethenyl)), C(O)(E-(4-methylphenyl-2-ethenyl)), C(O)(E-(4-dimethylaminophenyl-2-ethenyl)), C(O)(E-(3-acetoxy-4-methoxyphenyl-2-ethenyl)), C(O)(E-(3-indolyl-2-ethenyl)), C(O)(3-(1-benzylindolyl)), C(O)((9-oxo-9H-fluorene)-2-yl), C(O)CH(CH₂CH₃)((CH₂)₄CH₃), C(O)CH₂OH, C(O)(cis-4-aminocyclohexyl), (CH₂)₃NH₂, (CH₂)₆OH; and/or with the proviso that when each R¹ is H, R² is H, R³ is C(O)CH₃, and R⁵ is OH, R⁴ excludes H, CH₃, CH₂CH₃, (CH₂)₃CH₃, (CH₂)₅CH₃, (CH₂)₆CH₃, (CH₂)₇CH₃, (CH₂)₈CH₃, (CH₂)₂O(CH₂)₂O(CH₂)₂N₃, (CH₂)₂O(CH₂)₂O(CH₂)₂NH₂, (CH₂)₃NH₂, (CH₂)₄NH₂, (CH₂)₅NH₂, (CH₂)₆NH₂, (CH₂)₇NH₂, (CH₂)₈NH₂, (CH₂)₈N₃, (CH₂)₇N₃, (CH₂)₆NHC(O)O^tBu, (CH₂)₅NHC(O)O^tBu, (CH₂)₄NHC(O)O^tBu, (CH₂)₃NHC(O)O^tBu, CH₂(4-(dimethylamino)phenyl), 1,3-benzodioxol-4-ylmethyl, CH₂(5-(4-chlorophenyl)-2-furanyl), CH₂(4-hydroxyphenyl), CH₂(4-(2-pyridyl)phenyl); and/or with the proviso that when each R¹ is H, R² is H, R³ is C(O)CH₃, and R⁴ is H, R⁵ excludes H and CH₃; and/or with the proviso that when each R¹ is H, R² is H, R³ is C(O)((1,2-dihydrocyclobutabenzene)-1-yl), and R⁵ is OH, R⁴ excludes H, (CH₂)₃CH₃, (CH₂)₄CH₃, (CH₂)₅CH₃, (CH₂)₆CH₃, (CH₂)₇CH₃, (CH₂)₈CH₃, (CH₂)₉CH₃, (CH₂)₁₀CH₃, (CH₂)₁₁CH₃; and/or with the proviso that when each R¹ is H, R² is H, R³ is H, and R⁵ is OH, R⁴ excludes (CH₂)₃CH₃, (CH₂)₉CH₃, (CH₂)₇CH₃, and (CH₂)₈CH₃; and/or with the proviso that Formula (I) excludes the following compounds: 5-{{[(2R,3R,4R,5R)-3,4-dihydroxy-5-hydroxymethyl-pyrrolidin-2-ylmethyl]-amino]-methylene}-1,3-dimethyl-pyrimidine-2,4,6-trione (CAS # 763122-23-2), (2R,2'R,3R,3'R,4R,4'R,5R,5'R)-2,2'-[iminobis(methylene)]bis[5-(hydroxymethyl)]-3,4-pyrrolidinediol (CAS # 231618-81-8), (2R,3R,4R,5R)-1-butyl-2-[(dibutylamino)methyl]-5-(hydroxymethyl)-3,4-pyrrolidinediol (CAS # 172936-43-5), (2R,3R,4R,5R)-2-(azidomethyl)-1-butyl-5-(hydroxymethyl)-3,4-pyrrolidinediol (CAS # 172936-41-3), methyl 2-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methylcarbamoyl)-1H-indole-5-carboxylate (CAS # 876751-91-6), and (R)-alpha-amino-N-[(2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-2-pyrrolidinyl]methyl]-4-oxo-1(4H)-pyridinepropanamide (CAS # 876751-85-8), (2R,3R,4R,5R)-2-(azidomethyl)-5-(hydroxymethyl)-3,4-pyrrolidinediol (CAS # 765308-83-6).

[0024] In alternative embodiments, the compound may be set forth in Formula (II):



(II)

wherein each R⁸ is independently H or C(O)R¹¹; R⁹ is a non-interfering substituent; R¹⁰ is a non-interfering substituent; n is an integer between 0 and 10; and wherein each R¹¹ is

5 optionally independently a non-interfering substituent; with the proviso that when each R⁸ is H, R¹⁰ is H, and n = 0, R⁹ excludes CH₃, CH₂CH₃, (CH₂)₂CH₃, (CH₂)₃CH₃, (CH₂)₄CH₃,
 (CH₂)₇CH₃, (CH₂)₁₀CH₃, C≡CH, CH=CH₂, C(CH₃)=CH₂, (CH₂)₅OH, 1,3-benzodioxol-4-ylmethyl, 1,3-benzodioxol-5-ylmethyl, CH₂(3-indolyl), (CH₂)₂(phenyl), 2,3-dichlorophenyl,
 10 2,3-dihydroxyphenyl, 2,3-dihydroxy-4-methylphenyl, 2-methyl-3-nitrophenyl, 2,3-dimethoxyphenyl, 2-hydroxy-3-methylphenyl, 2,6-dihydroxyphenyl, 2,3-dimethylphenyl, 2-hydroxy-3-methoxyphenyl, 2-naphthyl, 4-hydroxy-2-quinolyl, 4-benzoylphenyl, 6-chloro-2H-chromene-3-yl, 1-naphthyl, phenyl, 3-dimethylaminophenyl, 4-dimethylaminophenyl,
 15 2-oxo-2H-chromene-3-yl, 7-diethylamino-2-oxo-2H-chromene-3-yl, (CH₂)₅NHSO₂(5-dimethylamino-1-naphthyl), (CH₂)₂(3-indolyl), 5-indolyl, CH₂(1-naphthyl), CH₂(2-naphthyl), 2-methylthio(phenyl), E-(3-trifluoromethylphenyl-2-ethenyl), E-(3-chlorophenyl-2-ethenyl), E-(3-bromophenyl-2-ethenyl), E-(4-methylphenyl-2-ethenyl), E-(4-dimethylaminophenyl-2-ethenyl), E-(3-acetoxy-4-methoxyphenyl-2-ethenyl), E-(3-indolyl-2-ethenyl), 3-(1-benzylindolyl), (9-oxo-9H-fluorene)-2-yl, CH(CH₂CH₃)((CH₂)₄CH₃), CH₂OH, cis-4-aminocyclohexyl; and/or with the proviso that when each R⁸ is H, R⁹ is CH₃,
 20 and n = 0, R¹⁰ excludes H, CH₃, CH₂CH₃, (CH₂)₃CH₃, (CH₂)₅CH₃, (CH₂)₆CH₃, (CH₂)₇CH₃, (CH₂)₈CH₃, (CH₂)₂O(CH₂)₂O(CH₂)₂N₃, (CH₂)₂O(CH₂)₂O(CH₂)₂NH₂, (CH₂)₃NH₂,
 (CH₂)₄NH₂, (CH₂)₅NH₂, (CH₂)₆NH₂, (CH₂)₇NH₂, (CH₂)₈NH₂, (CH₂)₈N₃, (CH₂)₇N₃,
 (CH₂)₆NHC(O)O^tBu, (CH₂)₅NHC(O)O^tBu, (CH₂)₄NHC(O)O^tBu, (CH₂)₃NHC(O)O^tBu,
 25 CH₂(4-(dimethylamino)phenyl), 1,3-benzodioxol-4-ylmethyl, CH₂(5-(4-chlorophenyl)-2-furanyl), CH₂(4-hydroxyphenyl), CH₂(4-(2-pyridyl)phenyl); and/or with the proviso that when each R⁸ is H, R⁹ is (1,2-dihydrocyclobutabenzene)-1-yl, and n = 0, R¹⁰ excludes H, (CH₂)₃CH₃, (CH₂)₄CH₃, (CH₂)₅CH₃, (CH₂)₆CH₃, (CH₂)₇CH₃, (CH₂)₈CH₃, (CH₂)₉CH₃, (CH₂)₁₀CH₃, (CH₂)₁₁CH₃; and/or with the proviso that Formula (II) excludes the following compounds: methyl 2-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-

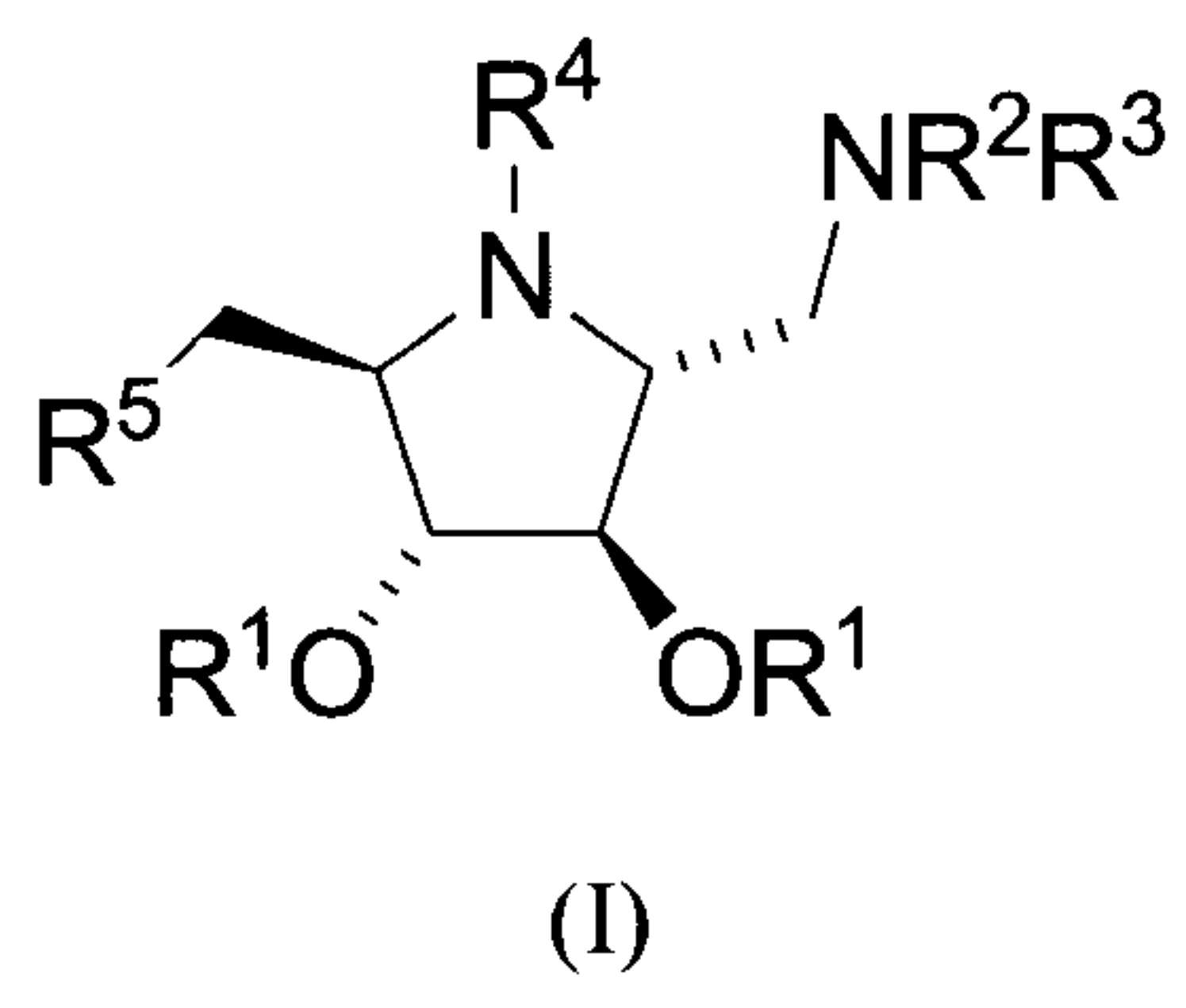
yl)methylcarbamoyl)-1H-indole-5-carboxylate (CAS # 876751-91-6), and (R)-alpha-amino-N-[(2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-2-pyrrolidinyl]methyl]-4-oxo-1(4H)-pyridinepropanamide (CAS # 876751-85-8).

[0025] In alternative embodiments, the non-interfering substituent may be alkyl, alkenyl, 5 alkynyl, aryl, arylalkyl, arylalkenyl, or arylalkynyl, or may include one or more heteroatoms selected from P, O, S, N, F, Cl, Br, I, or B. The non-interfering substituent may be optionally substituted.

[0026] In alternative embodiments, the compound may be a prodrug; the compound may 10 selectively inhibit an O-glycoprotein 2-acetamido-2-deoxy- β -D-glucopyranosidase (O-GlcNAcase); the compound may selectively bind an O-GlcNAcase (e.g., a mammalian O-GlcNAcase); the compound may selectively inhibit the cleavage of a 2-acetamido-2-deoxy- β -D-glucopyranoside (O-GlcNAc); the compound may not substantially inhibit a mammalian β -hexosaminidase.

[0027] In alternative aspects, the invention provides a pharmaceutical composition 15 including a compound according to the invention, in combination with a pharmaceutically acceptable carrier.

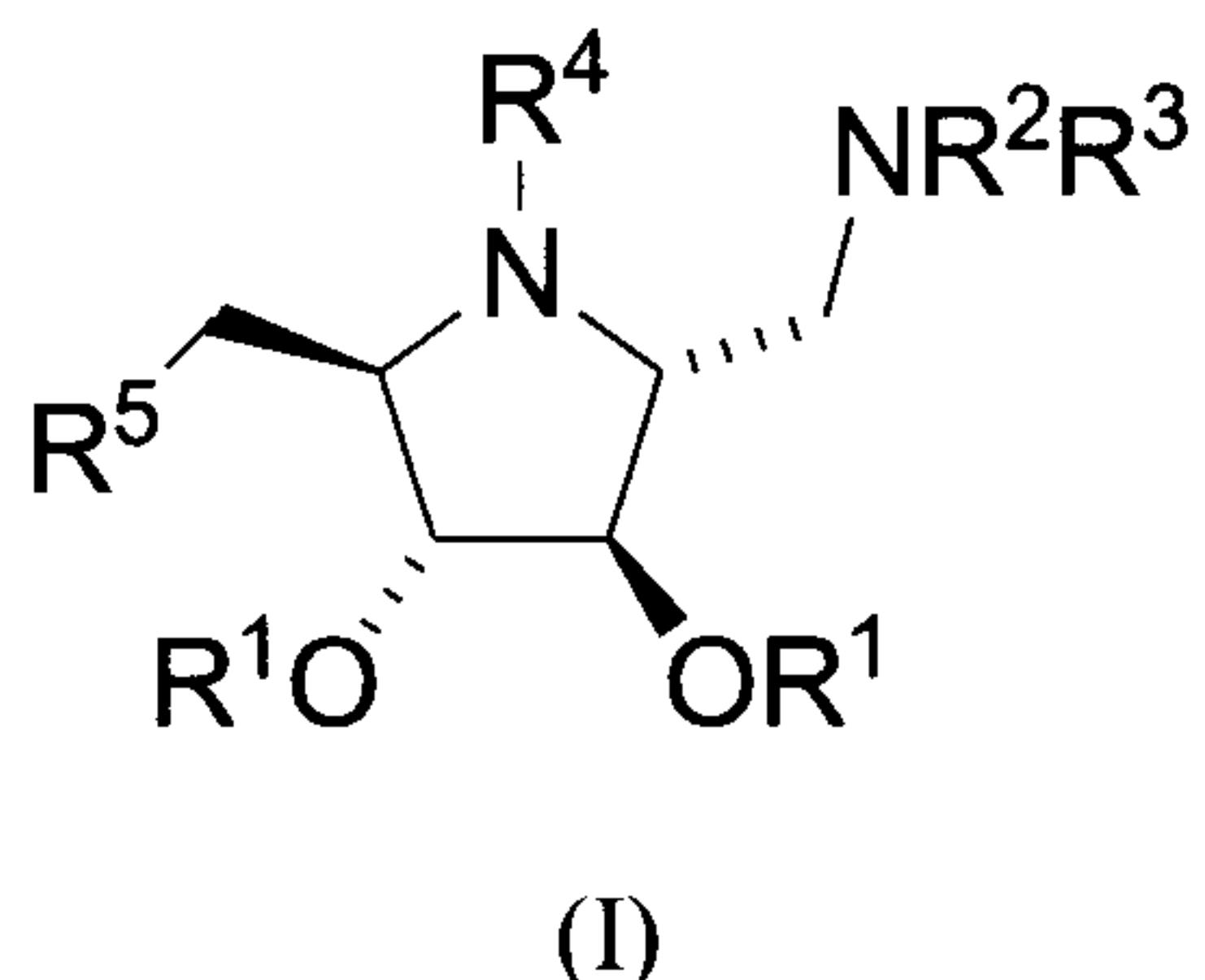
[0028] In alternative aspects, the invention provides methods of selectively inhibiting an O-GlcNAcase, or of inhibiting an O-GlcNAcase in a subject in need thereof, or of increasing 20 the level of O-GlcNAc, or of treating a neurodegenerative disease, a tauopathy, cancer or stress, in a subject in need thereof, by administering to the subject an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



where each R¹ may be independently H or C(O)R⁶; R² may be a non-interfering 25 substituent; R³ may be H, C(O)R⁶, C(NR⁶)NR⁶₂, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl; R⁴ may be a non-interfering substituent; R⁵ may be H, OR⁶, OC(O)R⁶, NR⁶C(O)R⁶, NR⁶₂, or an optionally substituted alkyl; wherein each R⁶ may be optionally independently a non-

interfering substituent. The condition may be Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Amyotrophic lateral sclerosis with cognitive impairment (ALSci), Argyrophilic grain dementia, Bluit disease, Corticobasal degeneration (CBD), Dementia pugilistica, Diffuse neurofibrillary tangles with calcification, Down's syndrome, Familial 5 British dementia, Familial Danish dementia, Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), Gerstmann-Straussler-Scheinker disease, Guadeloupean parkinsonism, Hallevorden-Spatz disease (neurodegeneration with brain iron accumulation type 1), Multiple system atrophy, Myotonic dystrophy, Niemann-Pick disease (type C), Pallido-ponto-nigral degeneration, Parkinsonism-dementia complex of Guam, 10 Pick's disease (PiD), Post-encephalitic parkinsonism (PEP), Prion diseases (including Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Fatal Familial Insomnia, and Kuru), Progressive supercortical gliosis, Progressive supranuclear palsy (PSP), Richardson's syndrome, Subacute sclerosing panencephalitis, Tangle-only dementia, Huntington's disease, or Parkinson's disease. The stress may be a cardiac disorder, e.g., 15 ischemia; hemorrhage; hypovolemic shock; myocardial infarction; an interventional cardiology procedure; cardiac bypass surgery; fibrinolytic therapy; angioplasty; or stent placement.

[0029] In alternative aspects, the invention provides a method of treating an O-GlcNAcase-mediated condition that excludes a neurodegenerative disease, a tauopathy, cancer or stress, 20 in a subject in need thereof, by administering to the subject an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



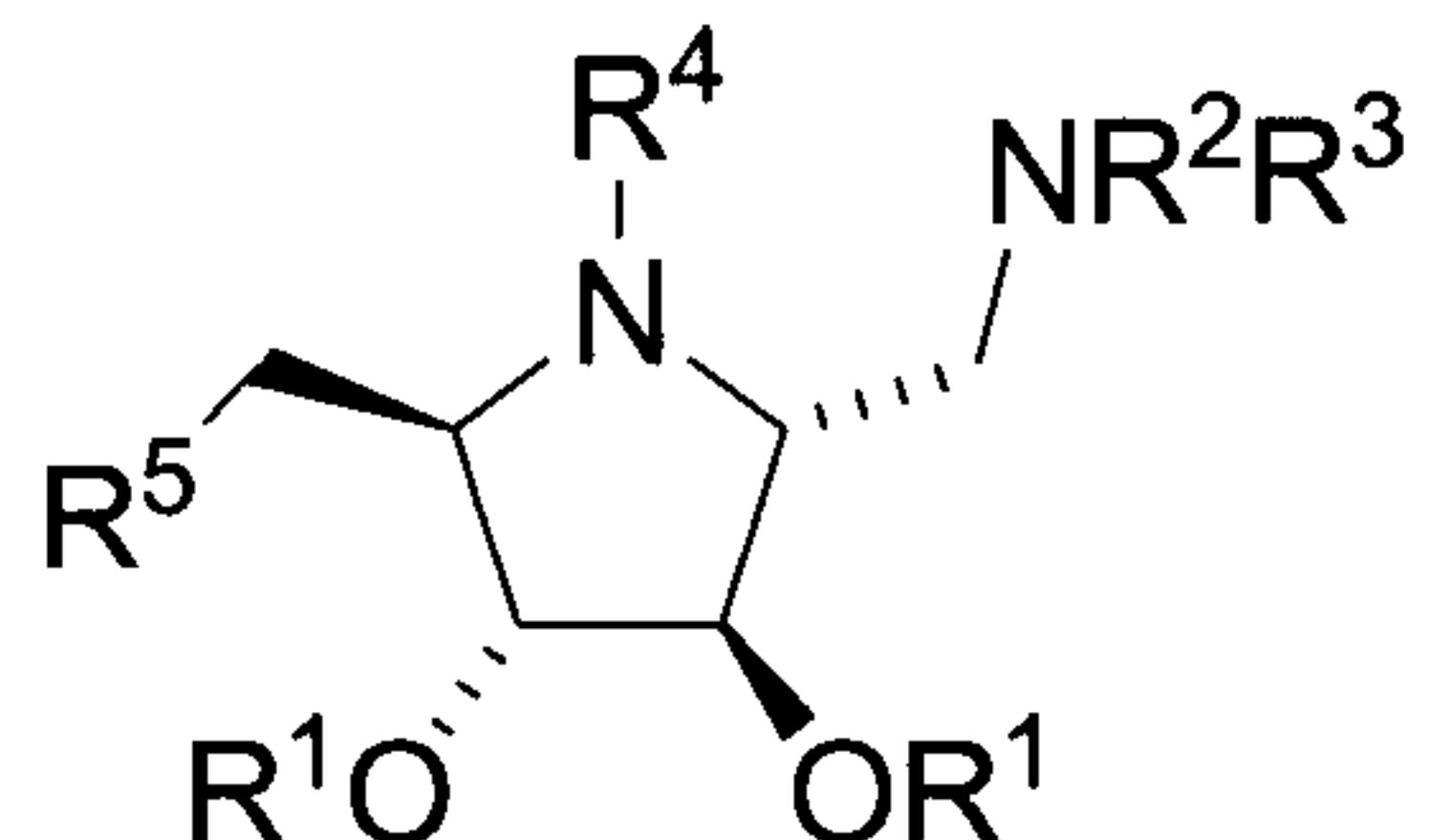
(I)

where each R¹ may be independently H or C(O)R⁶; R² may be a non-interfering 25 substituent; R³ may be H, C(O)R⁶, C(NR⁶)NR⁶₂, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl; R⁴ may be a non-interfering substituent; R⁵ may be H, OR⁶, OC(O)R⁶, NR⁶C(O)R⁶, NR⁶₂, or an optionally substituted alkyl; wherein each R⁶ may be optionally independently a non-interfering substituent. In some embodiments, the condition may be inflammatory or

allergic diseases such as asthma, allergic rhinitis, hypersensitivity lung diseases, hypersensitivity pneumonitis, eosinophilic pneumonias, delayed-type hypersensitivity, atherosclerosis, interstitial lung disease (ILD) (e.g., idiopathic pulmonary fibrosis, or ILD associated with rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, 5 systemic sclerosis, Sjogren's syndrome, polymyositis or dermatomyositis); systemic anaphylaxis or hypersensitivity responses, drug allergies, insect sting allergies; autoimmune diseases, such as rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, systemic lupus erythematosus, myastenia gravis, glomerulonephritis, autoimmune thyroiditis, graft rejection, including allograft rejection or graft-versus-host disease; inflammatory bowel 10 diseases, such as Crohn's disease and ulcerative colitis; spondyloarthropathies; scleroderma; psoriasis (including T-cell mediated psoriasis) and inflammatory dermatoses such as dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria; vasculitis (e.g., necrotizing, cutaneous, and hypersensitivity vasculitis); eosinophilic myositis, and eosinophilic fasciitis; graft rejection, in particular but not limited to solid organ transplants, such as heart, 15 lung, liver, kidney, and pancreas transplants (e.g. kidney and lung allografts); epilepsy; pain; stroke, e.g., neuroprotection following a stroke.

[0030] In alternative embodiments, R¹ or R⁸ may be H or C(O)CH₃, R² may be H, or R⁵ may be OH or OC(O)CH₃. The administering may increase the level of O-GlcNAc in the subject. The subject may be a human.

20 [0031] In alternative aspects, the invention provides use of a compound of an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof:

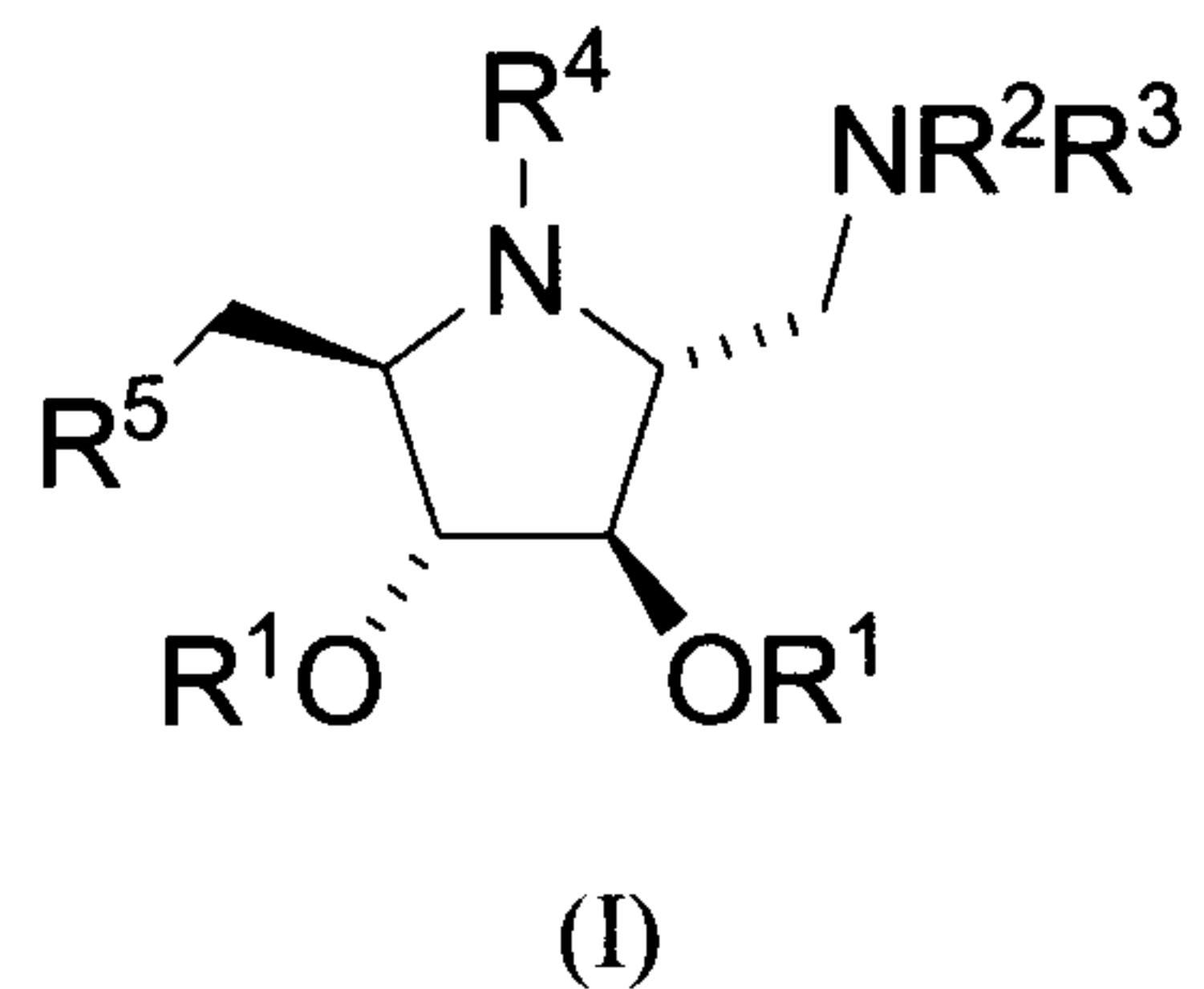


(I)

where each R¹ may be independently H or C(O)R⁶; R² may be a non-interfering 25 substituent; R³ may be H, C(O)R⁶, C(NR⁶)NR⁶₂, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl; R⁴ may be a non-interfering substituent; R⁵ may be H, OR⁶, OC(O)R⁶, NR⁶C(O)R⁶, NR⁶₂, or an optionally substituted alkyl; wherein each R⁶ may be optionally independently a non-interfering substituent, in the preparation of a medicament. The medicament may be for

selectively inhibiting an O-GlcNAcase, for increasing the level of O-GlcNAc, for treating a condition modulated by an O-GlcNAcase, for treating a neurodegenerative disease, a tauopathy, a cancer, or stress.

[0032] In alternative aspects, the invention provides a method for screening for a selective inhibitor of an O-GlcNAcase, by a) contacting a first sample with a test compound; b) contacting a second sample with a compound of Formula (I)



(I)

where each R^1 may be independently H or $C(O)R^6$; R^2 may be a non-interfering substituent; R^3 may be H, $C(O)R^6$, $C(NR^6)NR^6_2$, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl; R^4 may be a non-interfering substituent; R^5 may be H, OR^6 , $OC(O)R^6$, $NR^6C(O)R^6$, NR^6_2 , or an optionally substituted alkyl; wherein each R^6 may be optionally independently a non-interfering substituent, c) determining the level of inhibition of the O-GlcNAcase in the first and second samples, where the test compound is a selective inhibitor of a O-GlcNAcase if the test compound exhibits the same or greater inhibition of the O-GlcNAcase when compared to the compound of Formula (I).

[0033] This summary of the invention does not necessarily describe all features of the invention.

20

DETAILED DESCRIPTION

[0034] The invention provides, in part, novel compounds that are capable of inhibiting an O-glycoprotein 2-acetamido-2-deoxy- β -D-glucopyranosidase (O-GlcNAcase). In some embodiments, the O-GlcNAcase is a mammalian O-GlcNAcase, such as a rat, mouse or human O-GlcNAcase. In some embodiments, the β -hexosaminidase is a mammalian β -hexosaminidase, such as a rat, mouse or human β -hexosaminidase.

[0035] In some embodiments, compounds according to the invention exhibit a surprising and unexpected selectivity in inhibiting an O-GlcNAcase. In some embodiments, the

compounds according to the invention are surprisingly more selective for an O-GlcNAcase over a β -hexosaminidase. In some embodiments, the compounds selectively inhibit the activity of a mammalian O-GlcNAcase over a mammalian β -hexosaminidase. In some embodiments, a selective inhibitor of an O-GlcNAcase does not substantially inhibit a β -hexosaminidase. A compound that “selectively” inhibits an O-GlcNAcase is a compound that inhibits the activity or biological function of an O-GlcNAcase, but does not substantially inhibit the activity or biological function of a β -hexosaminidase. For example, in some embodiments, a selective inhibitor of an O-GlcNAcase selectively inhibits the cleavage of 2-acetamido-2-deoxy- β -D-glucopyranoside (O-GlcNAc) from polypeptides. In some embodiments, a selective inhibitor of an O-GlcNAcase selectively binds to an O-GlcNAcase. In some embodiments, a selective inhibitor of an O-GlcNAcase inhibits hyperphosphorylation of a tau protein and/or inhibits formations of NFTs. By “inhibits,” “inhibition” or “inhibiting” means a decrease by any value between 10% and 90%, or of any integer value between 30% and 60%, or over 100%, or a decrease by 1-fold, 2-fold, 5-fold, 10-fold or more. It is to be understood that the inhibiting does not require full inhibition. In some embodiments, a selective inhibitor of an O-GlcNAcase elevates or enhances O-GlcNAc levels e.g., O-GlcNAc-modified polypeptide or protein levels, in cells, tissues, or organs (e.g., in brain, muscle, or heart (cardiac) tissue) and in animals. By “elevating” or “enhancing” is meant an increase by any value between 10% and 90%, or of any integer value between 30% and 60%, or over 100%, or an increase by 1-fold, 2-fold, 5-fold, 10-fold, 15-fold, 25-fold, 50-fold, 100-fold or more. In some embodiments, a selective inhibitor of an O-GlcNAcase exhibits a selectivity ratio, as described herein, in the range 100 to 100000, or in the range 1000 to 100000, or at least 100, 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 6000, 7000, 10,000, 25,000, 50,000, 75,000, or any value within or about the described range.

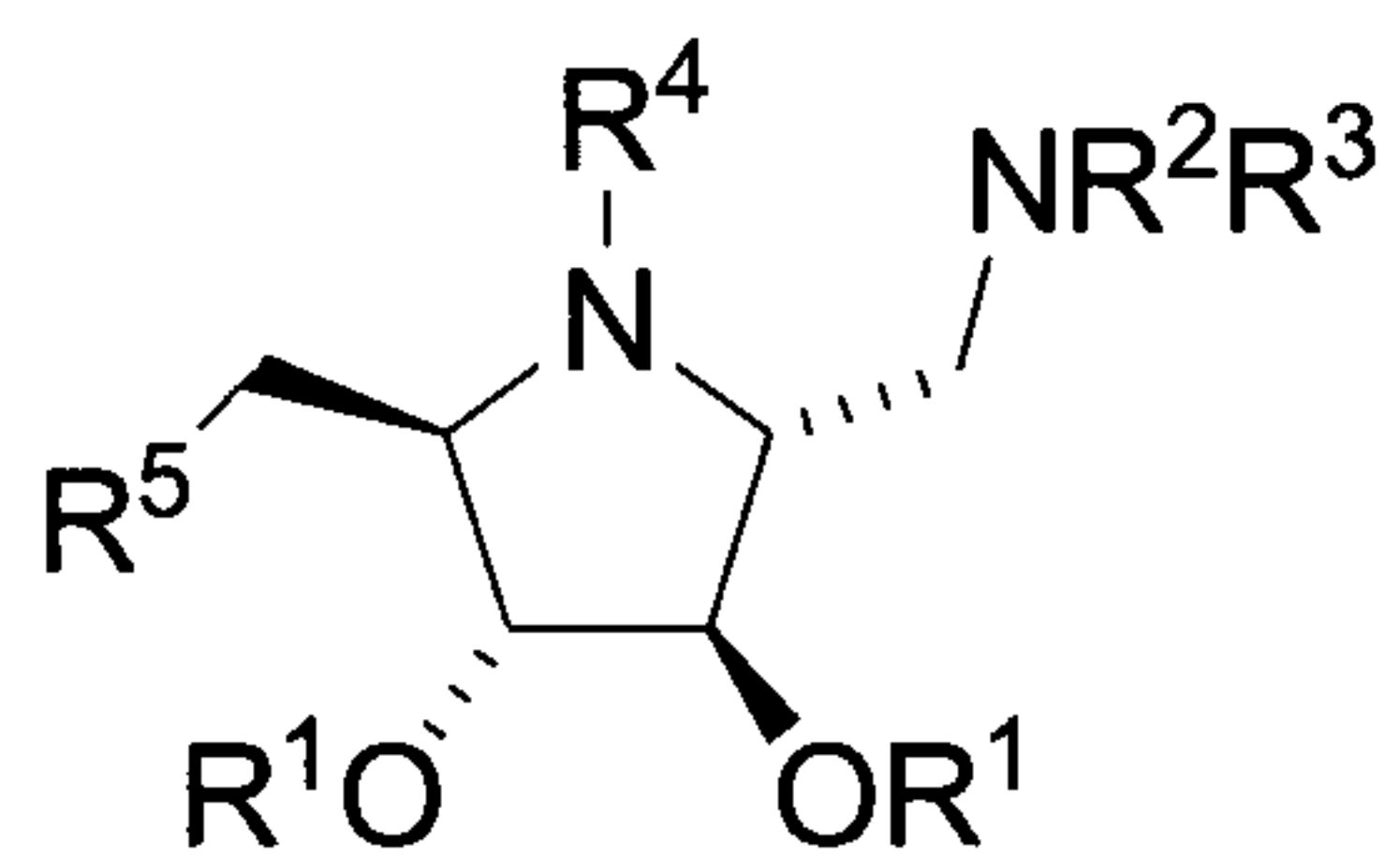
[0036] The compounds of the present invention elevate O-GlcNAc levels on O-GlcNAc-modified polypeptides or proteins *in vivo* specifically via interaction with an O-GlcNAcase enzyme, and are effective in treating conditions which require or respond to inhibition of O-GlcNAcase activity.

[0037] In some embodiments, the compounds of the present invention are useful as agents that produce a decrease in tau phosphorylation and NFT formation. In some embodiments, the compounds are therefore useful to treat Alzheimer’s disease and related tauopathies. In some embodiments, the compounds are thus capable of treating Alzheimer’s disease and

related tauopathies by lowering tau phosphorylation and reducing NFT formation as a result of increasing tau O-GlcNAc levels. In some embodiments, the compounds produce an increase in levels of O-GlcNAc modification on O-GlcNAc-modified polypeptides or proteins, and are therefore useful for treatment of disorders responsive to such increases in O-GlcNAc modification; these disorders include without limitation neurodegenerative, inflammatory, cardiovascular, and immunoregulatory diseases. In some embodiments, the compounds are also useful as a result of other biological activities related to their ability to inhibit the activity of glycosidase enzymes. In alternative embodiments, the compounds of the invention are valuable tools in studying the physiological role of O-GlcNAc at the 10 cellular and organismal level.

[0038] In alternative embodiments, the invention provides methods of enhancing or elevating levels of protein O-GlcNAc modification in animal subjects, such as, veterinary and human subjects. In alternative embodiments, the invention provides methods of selectively inhibiting an O-GlcNAcase enzyme in animal subjects, such as, veterinary and 15 human subjects. In alternative embodiments, the invention provides methods of inhibiting phosphorylation of tau polypeptides, or inhibiting formation of NFTs, in animal subjects, such as, veterinary and human subjects.

[0039] In specific embodiments, the invention provides compounds described generally by Formula (I) and the salts, prodrugs, and stereoisomeric forms thereof:



20

(I)

[0040] As set forth in Formula (I): each R¹ can be independently H or C(O)R⁶; R² may be a non-interfering substituent; R³ may be H, C(O)R⁶, C(NR⁶)NR⁶₂, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or 25 branched alkynyl; R⁴ may be a non-interfering substituent; R⁵ may be H, OR⁶, OC(O)R⁶, NR⁶C(O)R⁶, NR⁶₂, or an optionally substituted alkyl; where each R⁶ may be optionally independently a non-interfering substituent.

[0041] In the above Formula (I), each optionally substituted moiety may be substituted with one or more non-interfering substituents. For example, each optionally substituted moiety may be substituted with one or more inorganic substituents; phosphoryl; halo; =O; =NR⁷; OR; C₁₋₁₀ alkyl or C₂₋₁₀ alkenyl optionally containing one or more P, N, O, S, N, F, Cl, Br, I, 5 or B, and optionally substituted with halo; CN; optionally substituted carbonyl; NR⁷₂; C=NR⁷; an optionally substituted carbocyclic or heterocyclic ring; or an optionally substituted aryl or heteroaryl. R⁷ may be alkyl, branched alkyl, cycloalkyl, aryl, or heteroaryl.

[0042] In some embodiments, R¹ as set forth in Formula (I), may be either hydrogen or a 10 substituent that includes 1-20 atoms that are other than hydrogen. In some embodiments, R¹ may be H, or C(O)R⁷, where R⁷ may be alkyl, branched alkyl, cycloalkyl, aryl, or heteroaryl. In some embodiments, R¹ may be H or C(O)CH₃.

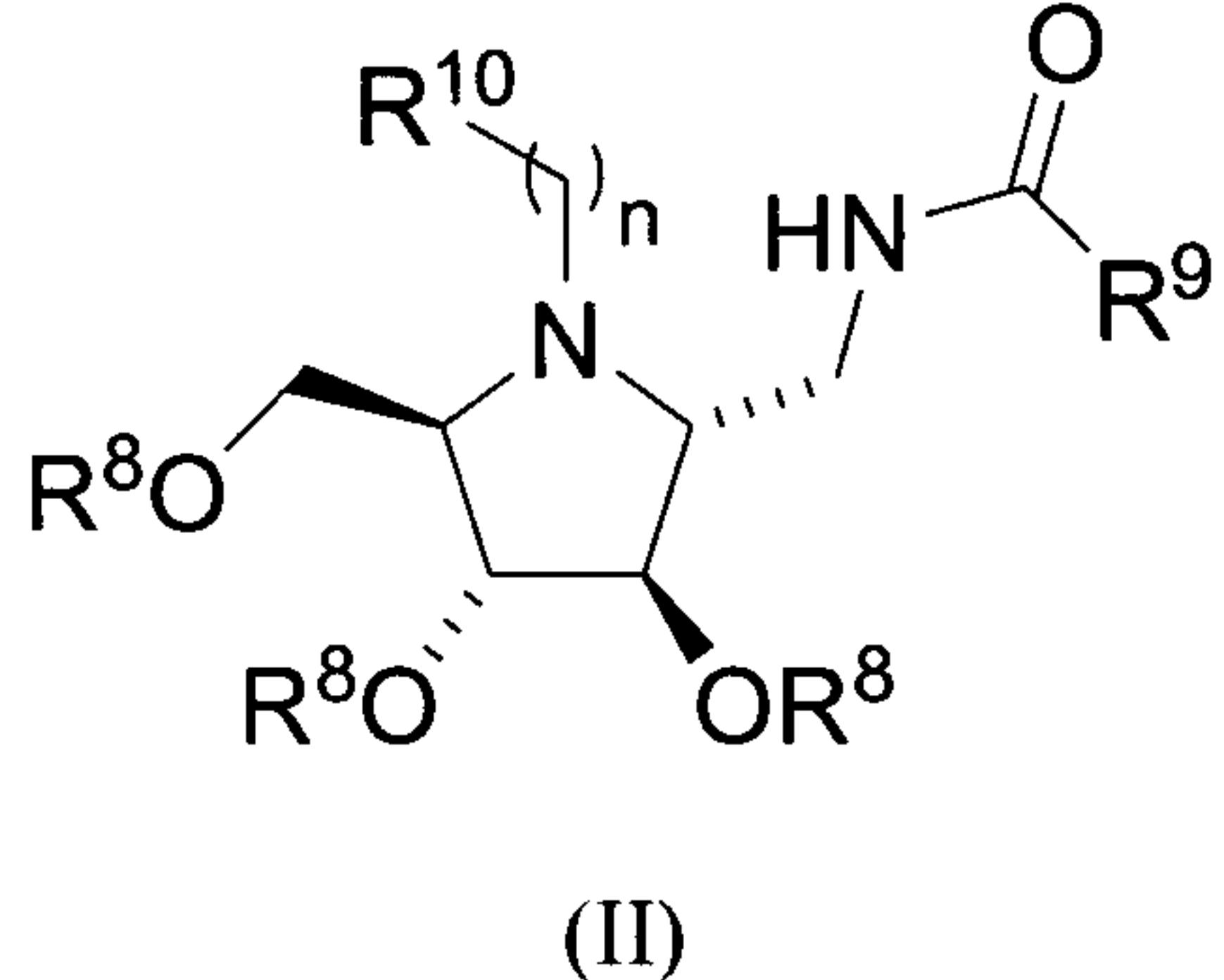
[0043] In some embodiments, R² as set forth in Formula (I), may be hydrogen or optionally substituted alkyl, branched alkyl, cycloalkyl, aryl, or heteroaryl. In some embodiments, R² 15 may be H or CH₃.

[0044] In some embodiments, R³ as set forth in Formula (I), may be hydrogen, optionally substituted alkyl, or C(O)R⁷, where R⁷ may be alkyl, branched alkyl, or cycloalkyl. In some embodiments, R³ may be H, CH₃, or C(O)CH₃.

[0045] In some embodiments, R⁴ as set forth in Formula (I), may be hydrogen or optionally substituted alkyl. In some embodiments, R⁴ may be H or CH₃. 20

[0046] In some embodiments, R⁵ as set forth in Formula (I), may be hydrogen, optionally substituted alkyl, OR⁷, or C(O)R⁷, where R⁷ may be alkyl, branched alkyl, or cycloalkyl. In some embodiments, R⁵ may be H, CH₃, OH, or OC(O)CH₃.

[0047] In alternative embodiments of the invention, compounds according to Formula (I) 25 may include compounds as set forth in Formula (II):



[0048] wherein each R⁸ is independently H or C(O)R¹¹; R⁹ is a non-interfering substituent; R¹⁰ is a non-interfering substituent; n is an integer between 0 and 10; and wherein each R¹¹ is optionally independently a non-interfering substituent. In some embodiments, R⁸ may be H or C(O)CH₃.

5 [0049] In specific embodiments of the invention, compounds according to Formula (I) include the compounds described in Table 1.

Table 1.

Compound	Name	Structure
1	(2R,3R,4R,5R)-2-(aminomethyl)-5-(hydroxymethyl)pyrrolidine-3,4-diol	
2	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide	
3	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)propionamide	
4	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)butyramide	
5	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)pentanamide	
6	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)hexanamide	

7	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)-2-hydroxyacetamide	
8	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-1-methylpyrrolidin-2-yl)methyl)acetamide	
9	N-(((2R,3R,4R,5R)-1-butyl-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide	
10	N-(((2R,3R,4R,5R)-1-hexyl-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide	
11	N-(((2R,3R,4R,5R)-1-heptyl-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide	
12	N-(((2R,3R,4R,5R)-1-(3-aminopropyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide	
13	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-methylpyrrolidin-2-yl)methyl)acetamide	

14	N-(((2R,3R,4R,5R)-5-ethyl-3,4-dihydroxypyrrolidin-2-yl)methyl)acetamide	
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[0050] In alternative embodiments of the invention, compounds according to Formula (I) include one or more of the compounds described in Table 2.

5 **Table 2.**

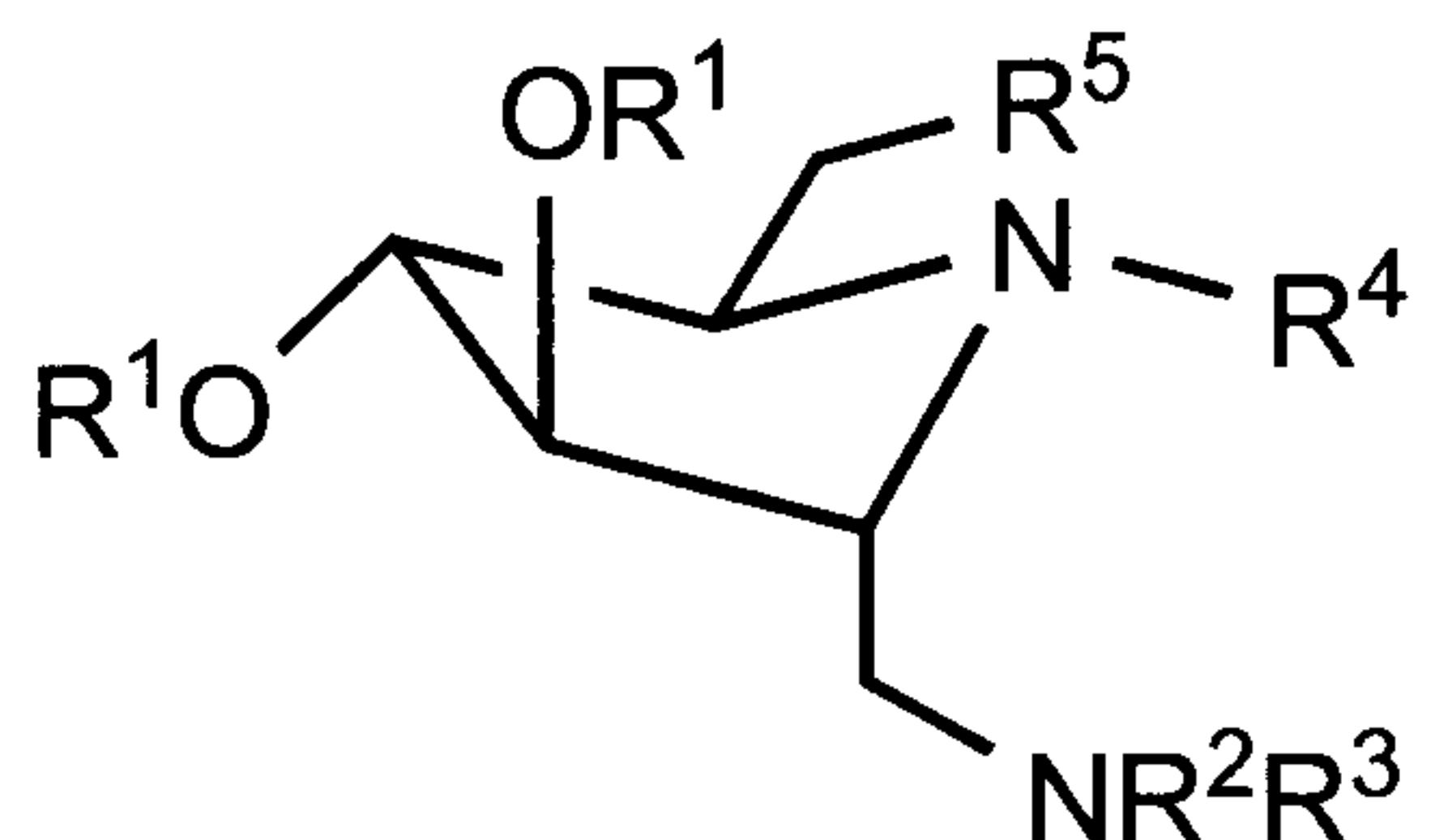
Compound	Name	Structure
15	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)isobutyramide	
16	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)-3-methylbutanamide	
17	N-(((2R,3R,4R,5R)-1-ethyl-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide	
18	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-1-propylpyrrolidin-2-yl)methyl)acetamide	
19	N-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-1-isopropylpyrrolidin-2-yl)methyl)acetamide	

20	N-((2R,3R,4R,5R)-1-benzyl-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide	
21	N-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-1-phenethylpyrrolidin-2-yl)methyl)acetamide	
22	N-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-1-methylpyrrolidin-2-yl)methyl)propionamide	
23	N-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)-N-methylacetamide	
24	N-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-1-methylpyrrolidin-2-yl)methyl)-N-methylacetamide	
25	N-((2R,3R,4R,5R)-3,4-dihydroxy-5-(methoxymethyl)pyrrolidin-2-yl)methyl)acetamide	
26	N-((2R,3R,4R,5R)-3,4-dihydroxy-5-((methylamino)methyl)pyrrolidin-2-yl)methyl)acetamide	
27	N,N'-(2R,3R,4R,5R)-3,4-dihydroxypyrrolidine-2,5-diyldis(methylene)diacetamide	

28	(2R,3R,4R,5R)-2-(hydroxymethyl)-5-((methylamino)methyl)pyrrolidine-3,4-diol	
29	(2R,3R,4R,5R)-2-((dimethylamino)methyl)-5-(hydroxymethyl)pyrrolidine-3,4-diol	
30	(2R,3R,4R,5R)-2-((dimethylamino)methyl)-5-(hydroxymethyl)-1-methylpyrrolidine-3,4-diol	

[0051] In alternative embodiments of the invention, one or more of the compounds described in Table 1 are specifically excluded from the compounds described in Formula (I). In alternative embodiments of the invention, specific stereoisomers or enantiomers of one or more of the compounds described in Table 1 are specifically excluded from the compounds described in Formula (I). In alternative embodiments of the invention, specific precursors of one or more of the compounds described in Table 1 are specifically excluded from the compounds described in Formula (I).

5 [0052] As will be appreciated by a person skilled in the art, Formula (I) above may also be represented alternatively as follows:



10 [0053] As used herein the singular forms “a”, “and”, and “the” include plural referents unless the context clearly dictates otherwise. For example, “a compound” refers to one or more of such compounds, while “the enzyme” includes a particular enzyme as well as other family members and equivalents thereof as known to those skilled in the art.

15 [0054] Throughout this application, it is contemplated that the term “compound” or “compounds” refers to the compounds discussed herein and includes precursors and derivatives of the compounds, including acyl-protected derivatives, and pharmaceutically

acceptable salts of the compounds, precursors, and derivatives. The invention also includes prodrugs of the compounds, pharmaceutical compositions including the compounds and a pharmaceutically acceptable carrier, and pharmaceutical compositions including prodrugs of the compounds and a pharmaceutically acceptable carrier.

5 [0055] In some embodiments, all of the compounds of the invention contain at least one chiral center. In some embodiments, the formulations, preparation, and compositions including compounds according to the invention include mixtures of stereoisomers, individual stereoisomers, and enantiomeric mixtures, and mixtures of multiple stereoisomers. In general, the compound may be supplied in any desired degree of chiral 10 purity.

[0056] In general, a “non-interfering substituent” is a substituent whose presence does not destroy the ability of the compound of Formula (I) or Formula (II) to modulate the activity of the O-GlcNAcase enzyme. Specifically, the presence of the substituent does not destroy the effectiveness of the compound as a modulator of the activity of the O-GlcNAcase 15 enzyme.

[0057] Suitable non-interfering substituents include: H, alkyl (C₁₋₁₀), alkenyl (C₂₋₁₀), alkynyl (C₂₋₁₀), aryl (5-12 members), arylalkyl, arylalkenyl, or arylalkynyl, each of which may optionally contain one or more heteroatoms selected from O, S, P, N, F, Cl, Br, I, or B , and each of which may be further substituted, for example, by =O; or optionally substituted 20 forms of acyl, arylacyl, alkyl- alkenyl-, alkynyl- or arylsulfonyl and forms thereof which contain heteroatoms in the alkyl, alkenyl, alkynyl or aryl moieties. Other noninterfering substituents include =O, =NR, halo, CN, CF₃, CHF₂, NO₂, OR, SR, NR₂, N₃, COOR, and CONR₂, where R is H or alkyl, cycloalkyl, alkenyl, alkynyl, aryl, or heteroaryl. Where the substituted atom is C, the substituents may include, in addition to the substituents listed 25 above, halo, OOCR, NROCR, where R is H or a substituent set forth above.

[0058] “Alkyl” refers to a straight or branched hydrocarbon chain group consisting solely of carbon and hydrogen atoms, containing no unsaturation and including, for example, from one to ten carbon atoms, and which is attached to the rest of the molecule by a single bond. Unless stated otherwise specifically in the specification, the alkyl group may be optionally 30 substituted by one or more substituents as described herein. Unless stated otherwise specifically herein, it is understood that the substitution can occur on any carbon of the alkyl group.

[0059] “Alkenyl” refers to a straight or branched hydrocarbon chain group consisting solely of carbon and hydrogen atoms, containing at least one double bond and including, for example, from two to ten carbon atoms, and which is attached to the rest of the molecule by a single bond or a double bond. Unless stated otherwise specifically in the specification, the 5 alkenyl group may be optionally substituted by one or more substituents as described herein. Unless stated otherwise specifically herein, it is understood that the substitution can occur on any carbon of the alkenyl group.

[0060] “Alkynyl” refers to a straight or branched hydrocarbon chain group consisting solely of carbon and hydrogen atoms, containing at least one triple bond and including, for 10 example, from two to ten carbon atoms. Unless stated otherwise specifically in the specification, the alkenyl group may be optionally substituted by one or more substituents as described herein.

[0061] “Aryl” refers to a phenyl or naphthyl group, including for example, 5-12 members. Unless stated otherwise specifically herein, the term “aryl” is meant to include aryl groups 15 optionally substituted by one or more substituents as described herein.

[0062] “Arylalkyl” refers to a group of the formula $-R_aR_b$ where R_a is an alkyl group as described herein and R_b is one or more aryl moieties as described herein. The aryl group(s) may be optionally substituted as described herein.

[0063] “Arylalkenyl” refers to a group of the formula $-R_cR_b$ where R_c is an alkenyl moiety 20 as described herein and R_b is one or more aryl groups as described herein. The aryl group(s) and the alkenyl group may be optionally substituted as described herein.

[0064] “Acyl” refers to a group of the formula $-C(O)R_a$, where R_a is an alkyl group as described herein. The alkyl group(s) may be optionally substituted as described herein.

[0065] “Arylacyl” refers to a group of the formula $-C(O)R_b$, where R_b is an aryl group as 25 described herein. The aryl group(s) may be optionally substituted as described herein.

[0066] “Cycloalkyl” refers to a stable monovalent monocyclic, bicyclic or tricyclic hydrocarbon group consisting solely of carbon and hydrogen atoms, having for example from 3 to 15 carbon atoms, and which is saturated and attached to the rest of the molecule by a single bond. Unless otherwise stated specifically herein, the term “cycloalkyl” is 30 meant to include cycloalkyl groups which are optionally substituted as described herein.

[0067] By a “ring structure” is meant a cycloalkyl, aryl, heteroaryl, or any cyclic structure that may be optionally substituted.

[0068] “Optional” or “optionally” means that the subsequently described event of circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, “optionally substituted alkyl” means that the alkyl group may or may not be substituted and that the description includes both substituted alkyl groups and alkyl groups having no substitution. Examples of optionally substituted alkyl groups include, without limitation, methyl, ethyl, propyl, etc. and including cycloalkyls such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, etc.; examples of optionally substituted alkenyl groups include allyl, crotyl, 2-pentenyl, 3-hexenyl, 2-cyclopentenyl, 2-cyclohexenyl, 2-cyclopentenylmethyl, 2-cyclohexenylmethyl, etc. In some embodiments, optionally substituted alkyl and alkenyl groups include C₁₋₆ alkyls or alkenyls.

[0069] “Halo” refers to bromo, chloro, fluoro, iodo, etc. In some embodiments, suitable halogens include fluorine or chlorine.

[0070] An amino group may also be substituted once or twice (to form a secondary or tertiary amine) with a group such as an optionally substituted alkyl group including C₁₋₁₀alkyl (e.g., methyl, ethyl propyl etc.); an optionally substituted alkenyl group such as allyl, crotyl, 2-pentenyl, 3-hexenyl, etc., or an optionally substituted cycloalkyl group such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, etc. In these cases, C₁₋₆ alkyl, alkenyl and cycloalkyl are preferred. The amine group may also be optionally substituted with an aromatic or heterocyclic group, aralkyl (e.g., phenylC₁₋₄alkyl) or heteroalkyl for example, phenyl, pyridine, phenylmethyl (benzyl), phenethyl, pyridinylmethyl, pyridinylethyl, etc. The heterocyclic group may be a 5 or 6 membered ring containing 1-4 heteroatoms.

[0071] An amino group may be substituted with an optionally substituted C₂₋₄ alkanoyl, e.g., acetyl, propionyl, butyryl, isobutyryl etc., or a C₁₋₄alkylsulfonyl (e.g., methanesulfonyl, ethanesulfonyl, etc.) or a carbonyl or sulfonyl substituted aromatic or heterocyclic ring, e.g., benzenesulfonyl, benzoyl, pyridinesulfonyl, pyridinecarbonyl etc. The heterocycles are as described herein.

[0072] Examples of optionally substituted carbonyl groups, or sulfonyl groups include optionally substituted forms of such groups formed from various hydrocarbyls such as

alkyl, alkenyl and 5- to 6-membered monocyclic aromatic group (e.g., phenyl, pyridyl, etc.), as described herein.

Therapeutic Indications

5 [0073] The invention provides methods of treating conditions that are modulated, directly or indirectly, by an O-GlcNAcase enzyme or by O-GlcNAc-modified protein levels, for example, a condition that is benefited by inhibition of an O-GlcNAcase enzyme or by an elevation of O-GlcNAc-modified protein levels. Such conditions include, without limitation, tauopathies, such as Alzheimer's disease, neurodegenerative diseases, 10 cardiovascular diseases, diseases associated with inflammation, diseases associated with immunosuppression and cancers. The compounds of the invention are also useful in the treatment of diseases or disorders related to deficiency or over-expression of O-GlcNAcase or accumulation or depletion of O-GlcNAc, or any disease or disorder responsive to glycosidase inhibition therapy. Such diseases and disorders include, but are not limited to, 15 neurodegenerative disorders, such as Alzheimer's disease (AD), and cancer. Such diseases and disorders may also include diseases or disorders related to the accumulation or deficiency in the enzyme OGT. Also included is a method of protecting or treating target cells expressing proteins that are modified by O-GlcNAc residues, the dysregulation of which modification results in disease or pathology. The term "treating" as used herein 20 includes treatment, prevention, and amelioration.

[0074] In alternative embodiments, the invention provides methods of enhancing or elevating levels of protein O-GlcNAc modification in animal subjects, such as, veterinary and human subjects. This elevation of O-GlcNAc levels can be useful for the prevention or treatment of Alzheimer's disease; prevention or treatment of other neurodegenerative 25 diseases (e.g. Parkinson's disease, Huntington's disease); providing neuroprotective effects; preventing damage to cardiac tissue; and treating diseases associated with inflammation or immunosuppression.

[0075] In alternative embodiments, the invention provides methods of selectively inhibiting an O-GlcNAcase enzyme in animal subjects, such as veterinary and human subjects.

30 [0076] In alternative embodiments, the invention provides methods of inhibiting phosphorylation of tau polypeptides, or inhibiting formation of NFTs, in animal subjects,

such as, veterinary and human subjects. Accordingly, the compounds of the invention may be used to study and treat AD and other tauopathies.

[0077] In general, the methods of the invention are effected by administering a compound according to the invention to a subject in need thereof, or by contacting a cell or a sample 5 with a compound according to the invention, for example, a pharmaceutical composition comprising a therapeutically effective amount of the compound according to Formula (I). More particularly, they are useful in the treatment of a disorder in which the regulation of O-GlcNAc protein modification is implicated, or any condition as described herein.

Disease states of interest include Alzheimer's disease (AD) and related neurodegenerative 10 tauopathies, in which abnormal hyperphosphorylation of the microtubule-associated protein tau is involved in disease pathogenesis. In some embodiments, the compounds may be used to block hyperphosphorylation of tau by maintaining elevated levels of O-GlcNAc on tau, thereby providing therapeutic benefit.

[0078] The effectiveness of the compounds in treating pathology associated with the 15 accumulation of toxic tau species (for example, Alzheimer's disease and other tauopathies) may be confirmed by testing the ability of the compounds to block the formation of toxic tau species in established cellular¹¹⁸⁻¹²⁰ and/or transgenic animal models of disease.^{32,33}

[0079] Tauopathies that may be treated with the compounds of the invention include: Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Amyotrophic lateral sclerosis 20 with cognitive impairment (ALSci), Argyrophilic grain dementia, Bluit disease, Corticobasal degeneration (CBD), Dementia pugilistica, Diffuse neurofibrillary tangles with calcification, Down's syndrome, Familial British dementia, Familial Danish dementia, Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), Gerstmann-Straussler-Scheinker disease, Guadeloupean parkinsonism, Hallevorden-Spatz 25 disease (neurodegeneration with brain iron accumulation type 1), Multiple system atrophy, Myotonic dystrophy, Niemann-Pick disease (type C), Pallido-ponto-nigral degeneration, Parkinsonism-dementia complex of Guam, Pick's disease (PiD), Post-encephalitic parkinsonism (PEP), Prion diseases (including Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Fatal Familial Insomnia, and Kuru), Progressive 30 supercortical gliosis, Progressive supranuclear palsy (PSP), Richardson's syndrome, Subacute sclerosing panencephalitis, and Tangle-only dementia.

[0080] The compounds of this invention are also useful in the treatment of conditions associate with tissue damage or stress, stimulating cells, or promoting differentiation of cells. Accordingly, in some embodiments, the compounds of this invention may be used to provide therapeutic benefit in a variety of conditions or medical procedures involving stress in cardiac tissue, including but not limited to: ischemia; hemorrhage; hypovolemic shock; myocardial infarction; an interventional cardiology procedure; cardiac bypass surgery; fibrinolytic therapy; angioplasty; and stent placement.

[0081] The effectiveness of the compounds in treating pathology associated with cellular stress (including ischemia, hemorrhage, hypovolemic shock, myocardial infarction, and other cardiovascular disorders) may be confirmed by testing the ability of the compounds to prevent cellular damage in established cellular stress assays,^{105,116,117} and to prevent tissue damage and promote functional recovery in animal models of ischemia-reperfusion,^{70,114} and trauma-hemorrhage.^{72,112,115}

[0082] Compounds that selectively inhibit O-GlcNAcase activity may be used for the treatment of diseases that are associated with inflammation, including but not limited to, inflammatory or allergic diseases such as asthma, allergic rhinitis, hypersensitivity lung diseases, hypersensitivity pneumonitis, eosinophilic pneumonias, delayed-type hypersensitivity, atherosclerosis, interstitial lung disease (ILD) (e.g., idiopathic pulmonary fibrosis, or ILD associated with rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, systemic sclerosis, Sjogren's syndrome, polymyositis or dermatomyositis); systemic anaphylaxis or hypersensitivity responses, drug allergies, insect sting allergies; autoimmune diseases, such as rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, systemic lupus erythematosus, myastenia gravis, glomerulonephritis, autoimmune thyroiditis, graft rejection, including allograft rejection or graft-versus-host disease; inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis; spondyloarthropathies; scleroderma; psoriasis (including T-cell mediated psoriasis) and inflammatory dermatoses such as dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria; vasculitis (e.g., necrotizing, cutaneous, and hypersensitivity vasculitis); eosinophilic myositis, eosinophilic fasciitis; and cancers.

[0083] In addition, compounds that affects levels of protein O-GlcNAc modification may be used for the treatment of diseases associated with immunosuppression, such as in individuals undergoing chemotherapy, radiation therapy, enhanced wound healing and burn treatment, therapy for autoimmune disease or other drug therapy (e.g., corticosteroid

therapy) or combination of conventional drugs used in the treatment of autoimmune diseases and graft/transplantation rejection, which causes immunosuppression; or immunosuppression due to congenital deficiency in receptor function or other causes.

[0084] The compounds of the invention may be useful for treatment of neurodegenerative diseases, including Parkinson's disease and Huntington's disease. Other conditions that may be treated are those triggered, affected, or in any other way correlated with levels of O-GlcNAc post-translational protein modification. It is expected that the compounds of this invention may be useful for the treatment of such conditions and in particular, but not limited to, the following for which a association with O-GlcNAc levels on proteins has been established: graft rejection, in particular but not limited to solid organ transplants, such as heart, lung, liver, kidney, and pancreas transplants (e.g. kidney and lung allografts); cancer, in particular but not limited to cancer of the breast, lung, prostate, pancreas, colon, rectum, bladder, kidney, ovary; as well as non-Hodgkin's lymphoma and melanoma; epilepsy, pain, or stroke, e.g., for neuroprotection following a stroke.

15

Pharmaceutical & Veterinary Compositions, Dosages, And Administration

[0085] Pharmaceutical compositions including compounds according to the invention, or for use according to the invention, are contemplated as being within the scope of the invention. In some embodiments, pharmaceutical compositions including an effective amount of a compound of Formula (I) are provided.

[0086] The compounds of Formula (I) and their pharmaceutically acceptable salts, stereoisomers, solvates, and derivatives are useful because they have pharmacological activity in animals, including humans. In some embodiments, the compounds according to the invention are stable in plasma, when administered to a subject.

[0087] In some embodiments, compounds according to the invention, or for use according to the invention, may be provided in combination with any other active agents or pharmaceutical compositions where such combined therapy is useful to modulate O-GlcNAcase activity, for example, to treat neurodegenerative, inflammatory, cardiovascular, or immunoregulatory diseases, or any condition described herein. In some embodiments, compounds according to the invention, or for use according to the invention, may be provided in combination with one or more agents useful in the prevention or treatment of Alzheimer's disease. Examples of such agents include, without limitation,

- acetylcholine esterase inhibitors (AChEIs) such as Aricept® (Donepezil), Exelon® (Rivastigmine), Razadyne® (Razadyne ER®, Reminyl®, Nivalin®, Galantamine), Cognex® (Tacrine), Dimebon, Huperzine A, Phenserine, Debio-9902 SR (ZT-1 SR), Zanapezil (TAK0147), ganstigmine, NP7557, etc.;
- 5 • NMDA receptor antagonists such as Namenda® (Axura®, Akatinol®, Ebixa®, Memantine), Dimebon, SGS-742, Neramexane, Debio-9902 SR (ZT-1 SR), etc.;
- gamma-secretase inhibitors and/or modulators such as Flurizan™ (Tarenflurbil, MPC-7869, R-flurbiprofen), LY450139, MK 0752, E2101, BMS-289948, BMS-299897, BMS-433796, LY-411575, GSI-136, etc.;
- 10 • beta-secretase inhibitors such as ATG-Z1, CTS-21166, etc.;
- alpha-secretase activators, such as NGX267, etc.;
- amyloid- β aggregation and/or fibrillization inhibitors such as Alzhemed™ (3APS, Tramiprosate, 3-amino-1-propanesulfonic acid), AL-108, AL-208, AZD-103, PBT2, Cereact, ONO-2506PO, PPI-558, etc.;
- 15 • tau aggregation inhibitors such as methylene blue, etc.;
- microtubule stabilizers such as AL-108, AL-208, paclitaxel, etc.;
- RAGE inhibitors, such as TTP488, etc.;
- 5-HT1a receptor antagonists, such as Xaliproden, Lecozotan, etc.;
- 5-HT4 receptor antagonists, such as PRX-03410, etc.;
- 20 • kinase inhibitors such as SRN-003-556, amfurindamide, LiCl, AZD1080, NP031112, SAR-502250, etc.;
- humanized monoclonal anti-A β antibodies such as Bapineuzumab (AAB-001), LY2062430, RN1219, ACU-5A5, etc.;
- amyloid vaccines such as AN-1792, ACC-001
- 25 • neuroprotective agents such as Cerebrolysin, AL-108, AL-208, Huperzine A, etc.;
- L-type calcium channel antagonists such as MEM-1003, etc.;
- nicotinic receptor antagonists, such as AZD3480, GTS-21, etc.;
- nicotinic receptor agonists, such as MEM 3454, Nefiracetam, etc.;
- peroxisome proliferator-activated receptor (PPAR) gamma agonists such as Avandia® (Rosglitazone), etc.;
- 30 • phosphodiesterase IV (PDE4) inhibitors, such as MK-0952, etc.;
- hormone replacement therapy such as estrogen (Premarin), etc.;

- monoamine oxidase (MAO) inhibitors such as NS2330, Rasagiline (Azilect®), TVP-1012, etc.;
- AMPA receptor modulators such as Ampalex (CX 516), etc.;
- nerve growth factors or NGF potentiators, such as CERE-110 (AAV-NGF), T-588, T-817MA, etc.;
- agents that prevent the release of luteinizing hormone (LH) by the pituitary gland, such as leuoprolide (VP-4896), etc.;
- GABA receptor modulators such as AC-3933, NGD 97-1, CP-457920, etc.;
- benzodiazepine receptor inverse agonists such as SB-737552 (S-8510), AC-3933, etc.;
- noradrenaline-releasing agents such as T-588, T-817MA, etc.

[0088] It is to be understood that combination of compounds according to the invention, or for use according to the invention, with Alzheimer's agents is not limited to the examples described herein, but includes combination with any agent useful for the treatment of Alzheimer's disease. Combination of compounds according to the invention, or for use according to the invention, and other Alzheimer's agents may be administered separately or in conjunction. The administration of one agent may be prior to, concurrent to, or subsequent to the administration of other agent(s).

[0089] In alternative embodiments, the compounds may be supplied as "prodrugs" or protected forms, which release the compound after administration to a subject. For example, the compound may carry a protective group which is split off by hydrolysis in body fluids, *e.g.*, in the bloodstream, thus releasing the active compound or is oxidized or reduced in body fluids to release the compound. Accordingly, a "prodrug" is meant to indicate a compound that may be converted under physiological conditions or by solvolysis to a biologically active compound of the invention. Thus, the term "prodrug" refers to a metabolic precursor of a compound of the invention that is pharmaceutically acceptable. A prodrug may be inactive when administered to a subject in need thereof, but is converted *in vivo* to an active compound of the invention. Prodrugs are typically rapidly transformed *in vivo* to yield the parent compound of the invention, for example, by hydrolysis in blood. The prodrug compound often offers advantages of solubility, tissue compatibility or delayed release in a subject.

[0090] The term "prodrug" is also meant to include any covalently bonded carriers which release the active compound of the invention *in vivo* when such prodrug is administered to a

subject. Prodrugs of a compound of the invention may be prepared by modifying functional groups present in the compound of the invention in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compound of the invention.

5 Prodrugs include compounds of the invention wherein a hydroxy, amino or mercapto group is bonded to any group that, when the prodrug of the compound of the invention is administered to a mammalian subject, cleaves to form a free hydroxy, free amino or free mercapto group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and acetamide, formamide, and benzamide derivatives of amine functional groups in the compounds of the invention and the like.

10 [0091] A discussion of prodrugs may be found in "Smith and Williams' Introduction to the Principles of Drug Design," H.J. Smith, Wright, Second Edition, London (1988); Bundgaard, H., *Design of Prodrugs* (1985), pp. 7-9, 21-24 (Elsevier, Amsterdam); The Practice of Medicinal Chemistry, Camille G. Wermuth et al., Ch 31, (Academic Press, 1996); A Textbook of Drug Design and Development, P. Krogsgaard-Larson and H. Bundgaard, eds. 15 Ch 5, pgs 113 191 (Harwood Academic Publishers, 1991); Higuchi, T., *et al.*, "Pro-drugs as Novel Delivery Systems," A.C.S. Symposium Series, Vol. 14; or in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, all of which are incorporated in full by reference herein.

20 [0092] Suitable prodrug forms of the compounds of the invention include embodiments in which R^1 is $C(O)R$, where R is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl. In these cases the ester groups may be hydrolyzed *in vivo* (e.g. in bodily fluids), releasing the active compounds in which R^1 is H. Preferred prodrug embodiments of the invention include compounds of Formula (I) where one or both of R^1 is $C(O)CH_3$ and R^5 is $OC(O)CH_3$.

25 [0093] Compounds according to the invention, or for use according to the invention, can be provided alone or in combination with other compounds in the presence of a liposome, an adjuvant, or any pharmaceutically acceptable carrier, diluent or excipient, in a form suitable for administration to a subject such as a mammal, for example, humans, cattle, sheep, etc. If desired, treatment with a compound according to the invention may be combined with more 30 traditional and existing therapies for the therapeutic indications described herein.

Compounds according to the invention may be provided chronically or intermittently. "Chronic" administration refers to administration of the compound(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an

extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature. The terms "administration," "administrable," or "administering" as used herein should be understood to mean providing a compound of the invention to the subject in need of treatment.

5 [0094] "Pharmaceutically acceptable carrier, diluent or excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, or emulsifier that has been approved, for example, by the United States Food and Drug Administration or other governmental agency as being acceptable for use in
10 humans or domestic animals.

[0095] The compounds of the present invention may be administered in the form of pharmaceutically acceptable salts. In such cases, pharmaceutical compositions in accordance with this invention may comprise a salt of such a compound, preferably a physiologically acceptable salt, which are known in the art. In some embodiments, the
15 term "pharmaceutically acceptable salt" as used herein means an active ingredient comprising compounds of Formula I used in the form of a salt thereof, particularly where the salt form confers on the active ingredient improved pharmacokinetic properties as compared to the free form of the active ingredient or other previously disclosed salt form.

[0096] A "pharmaceutically acceptable salt" includes both acid and base addition salts. A
20 "pharmaceutically acceptable acid addition salt" refers to those salts which retain the biological effectiveness and properties of the free bases, which are not biologically or otherwise undesirable, and which are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, trifluoroacetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid,
25 maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like.

[0097] A "pharmaceutically acceptable base addition salt" refers to those salts which retain the biological effectiveness and properties of the free acids, which are not biologically or
30 otherwise undesirable. These salts are prepared from addition of an inorganic base or an organic base to the free acid. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper,

manganese, aluminum salts and the like. Preferred inorganic salts are the ammonium, sodium, potassium, calcium, and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, cocaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine.

[0098] Thus, the term “pharmaceutically acceptable salt” encompasses all acceptable salts including but not limited to acetate, lactobionate, benzenesulfonate, laurate, benzoate, malate, bicarbonate, maleate, bisulfate, mandelate, bitartarate, mesylate, borate, methylbromide, bromide, methylnitrite, calcium edetate, methylsulfate, camsylate, mucate, carbonate, napsylate, chloride, nitrate, clavulanate, N-methylglucamine, citrate, ammonium salt, dihydrochloride, oleate, edetate, oxalate, edisylate, pamoate (embonate), estolate, palmitate, esylate, pantothenate, fumarate, phosphate/diphosphate, gluceptate, polygalacturonate, gluconate, salicylate, glutame, stearate, glycolylarsanilate, sulfate, hexylresorcinate, subacetate, hydramidine, succinate, hydrobromide, tannate, hydrochloride, tartrate, hydroxynaphthoate, teoclate, iodide, tosylate, isothionate, triethiodide, lactate, panoate, valerate, and the like.

[0099] Pharmaceutically acceptable salts of the compounds of the present invention can be used as a dosage for modifying solubility or hydrolysis characteristics, or can be used in sustained release or prodrug formulations. Also, pharmaceutically acceptable salts of the compounds of this invention may include those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc, and from bases such as ammonia, ethylenediamine, N-methyl-glutamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylene-diamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethyl-amine, diethylamine, piperazine, tris(hydroxymethyl)aminomethane, and tetramethylammonium hydroxide.

[00100] Pharmaceutical formulations will typically include one or more carriers acceptable for the mode of administration of the preparation, be it by injection, inhalation, topical

administration, lavage, or other modes suitable for the selected treatment. Suitable carriers are those known in the art for use in such modes of administration.

[00101] Suitable pharmaceutical compositions may be formulated by means known in the art and their mode of administration and dose determined by the skilled practitioner. For 5 parenteral administration, a compound may be dissolved in sterile water or saline or a pharmaceutically acceptable vehicle used for administration of non-water soluble compounds such as those used for vitamin K. For enteral administration, the compound may be administered in a tablet, capsule or dissolved in liquid form. The table or capsule may be enteric coated, or in a formulation for sustained release. Many suitable formulations 10 are known, including, polymeric or protein microparticles encapsulating a compound to be released, ointments, gels, hydrogels, or solutions which can be used topically or locally to administer a compound. A sustained release patch or implant may be employed to provide release over a prolonged period of time. Many techniques known to skilled practitioners are described in *Remington: the Science & Practice of Pharmacy* by Alfonso Gennaro, 20th ed., 15 Williams & Wilkins, (2000). Formulations for parenteral administration may, for example, contain excipients, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery 20 systems for modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

25 [00102] The compounds or pharmaceutical compositions according to the present invention may be administered by oral or non-oral, e.g., intramuscular, intraperitoneal, intravenous, intracisternal injection or infusion, subcutaneous injection, transdermal or transmucosal routes. In some embodiments, compounds or pharmaceutical compositions in accordance with this invention or for use in this invention may be administered by means of a medical 30 device or appliance such as an implant, graft, prosthesis, stent, etc. Implants may be devised which are intended to contain and release such compounds or compositions. An example would be an implant made of a polymeric material adapted to release the compound over a period of time. The compounds may be administered alone or as a mixture

with a pharmaceutically acceptable carrier *e.g.*, as solid formulations such as tablets, capsules, granules, powders, etc.; liquid formulations such as syrups, injections, etc.; injections, drops, suppositories, pessaries. In some embodiments, compounds or pharmaceutical compositions in accordance with this invention or for use in this invention may be administered by inhalation spray, nasal, vaginal, rectal, sublingual, or topical routes and may be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles appropriate for each route of administration.

[00103] The compounds of the invention may be used to treat animals, including mice, rats, horses, cattle, sheep, dogs, cats, and monkeys. However, compounds of the invention can also be used in other organisms, such as avian species (*e.g.*, chickens). The compounds of the invention may also be effective for use in humans. The term “subject” or alternatively referred to herein as “patient” is intended to be referred to an animal, preferably a mammal, most preferably a human, who has been the object of treatment, observation or experiment.

15 However, the compounds, methods and pharmaceutical compositions of the present invention may be used in the treatment of animals. Accordingly, as used herein, a “subject” may be a human, non-human primate, rat, mouse, cow, horse, pig, sheep, goat, dog, cat, etc. The subject may be suspected of having or at risk for having a condition requiring modulation of O-GlcNAcase activity.

20 [00104] An “effective amount” of a compound according to the invention includes a therapeutically effective amount or a prophylactically effective amount. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as inhibition of an O-GlcNAcase, elevation of O-GlcNAc levels, inhibition of tau phosphorylation, or any condition described

25 herein. A therapeutically effective amount of a compound may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the compound are outweighed by the

30 therapeutically beneficial effects. A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as inhibition of an O-GlcNAcase, elevation of O-GlcNAc levels, inhibition of tau phosphorylation, or any condition described herein. Typically, a

prophylactic dose is used in subjects prior to or at an earlier stage of disease, so that a prophylactically effective amount may be less than a therapeutically effective amount. A suitable range for therapeutically or prophylactically effective amounts of a compound may be any integer from 0.1 nM-0.1M, 0.1 nM-0.05M, 0.05 nM-15μM or 0.01 nM-10μM.

5 [00105] In alternative embodiments, in the treatment or prevention of conditions which require modulation of O-GlcNAcase activity, an appropriate dosage level will generally be about 0.01 to 500 mg per kg subject body weight per day, and can be administered in single or multiple doses. In some embodiments, the dosage level will be about 0.1 to about 250 mg/kg per day. It will be understood that the specific dose level and frequency of dosage
10 for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound used, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the patient undergoing therapy.

15 [00106] It is to be noted that dosage values may vary with the severity of the condition to be alleviated. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgement of the person administering or supervising the administration of the compositions. Dosage ranges set forth herein are exemplary only and do not limit the dosage ranges that may be selected by
20 medical practitioners. The amount of active compound(s) in the composition may vary according to factors such as the disease state, age, sex, and weight of the subject. 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
25 therapeutic situation. It may be advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. In general, compounds of the invention should be used without causing substantial toxicity, and as described herein, the compounds exhibit a suitable safety profile for therapeutic use. Toxicity of the compounds of the invention can be determined using standard techniques,
30 for example, by testing in cell cultures or experimental animals and determining the therapeutic index, i.e., the ratio between the LD50 (the dose lethal to 50% of the population) and the LD100 (the dose lethal to 100% of the population). In some circumstances

however, such as in severe disease conditions, it may be necessary to administer substantial excesses of the compositions.

Other Uses and Assays

5 [00107] A compound of Formula (I) may be used in screening assays for compounds which modulate the activity of glycosidase enzymes, preferably the O-GlcNAcase enzyme. The ability of a test compound to inhibit O-GlcNAcase-dependent cleavage of O-GlcNAc from a model substrate may be measured using any assays, as described herein or known to one of ordinary skill in the art. For example, a fluorescence or UV-based assay known in the art 10 may be used. A “test compound” is any naturally-occurring or artificially-derived chemical compound. Test compounds may include, without limitation, peptides, polypeptides, synthesised organic molecules, naturally occurring organic molecules, and nucleic acid molecules. A test compound can “compete” with a known compound such as a compound of Formula (I) by, for example, interfering with inhibition of O-GlcNAcase-dependent 15 cleavage of O-GlcNAc or by interfering with any biological response induced by a compound of Formula (I).

[00108] Generally, a test compound can exhibit any value between 10% and 200%, or over 500%, modulation when compared to a compound of Formula (I) or other reference compound. For example, a test compound may exhibit at least any positive or negative 20 integer from 10% to 200% modulation, or at least any positive or negative integer from 30% to 150% modulation, or at least any positive or negative integer from 60% to 100% modulation, or any positive or negative integer over 100% modulation. A compound that is a negative modulator will in general decrease modulation relative to a known compound, while a compound that is a positive modulator will in general increase modulation relative 25 to a known compound.

[00109] In general, test compounds are identified from large libraries of both natural products or synthetic (or semi-synthetic) extracts or chemical libraries according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the 30 method(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or

animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available.

5 Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceanographic Institute (Ft. Pierce, FL, USA), and PharmaMar, MA, USA. In addition, natural and synthetically produced 10 libraries are produced, if desired, according to methods known in the art, e.g., by standard extraction and fractionation methods. Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

[00110] When a crude extract is found to modulate inhibition of O-GlcNAcase-dependent 15 cleavage of O-GlcNAc, or any biological response induced by a compound of Formula (I), further fractionation of the positive lead extract is necessary to isolate chemical constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and 20 purification process is the careful characterization and identification of a chemical entity within the crude extract having O-GlcNAcase- inhibitory activities. The same assays described herein for the detection of activities in mixtures of compounds can be used to purify the active component and to test derivatives thereof. Methods of fractionation and 25 purification of such heterogeneous extracts are known in the art. If desired, compounds shown to be useful agents for treatment are chemically modified according to methods known in the art. Compounds identified as being of therapeutic, prophylactic, diagnostic, or other value may be subsequently analyzed using a suitable animal model, as described herein on known in the art.

[00111] In some embodiments, the compounds are useful in the development of animal 30 models for studying diseases or disorders related to deficiencies in O-GlcNAcase, over-expression of O-GlcNAcase, accumulation of O-GlcNAc, depletion of O-GlcNAc, and for studying treatment of diseases and disorders related to deficiency or over-expression of O-GlcNAcase, or accumulation or depletion of O-GlcNAc. Such diseases and disorders include neurodegenerative diseases, including Alzheimer's disease, and cancer.

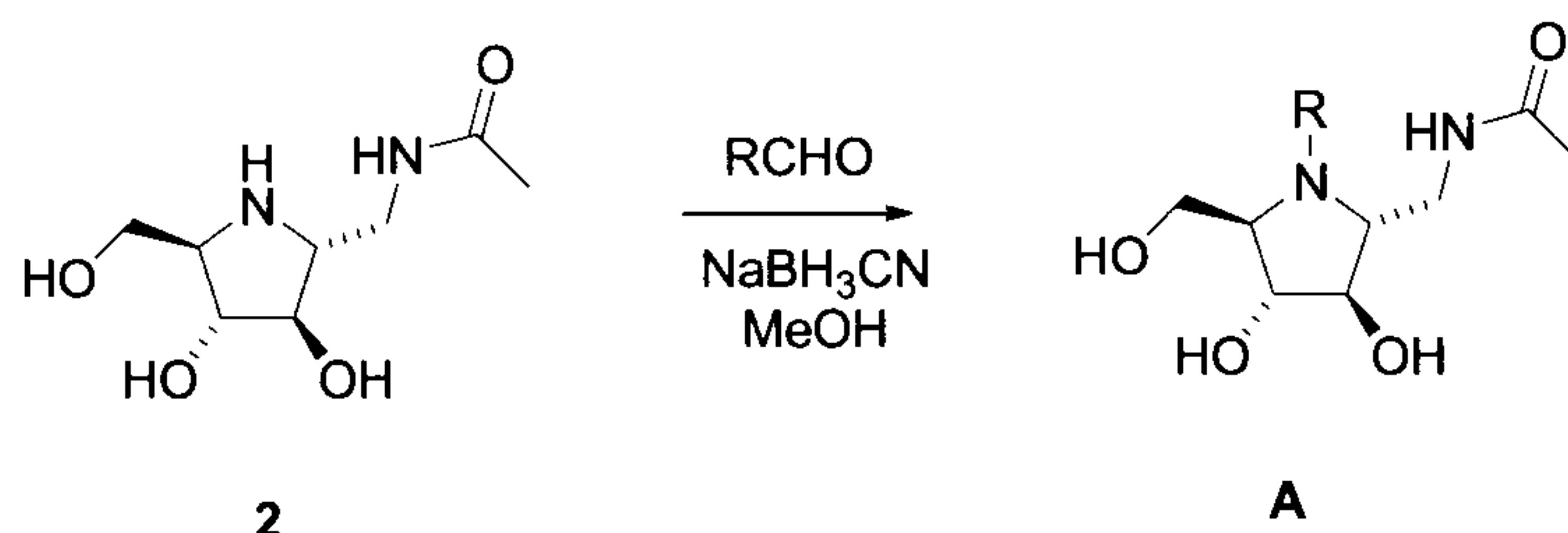
[00112] Various alternative embodiments and examples of the invention are described herein. These embodiments and examples are illustrative and should not be construed as limiting the scope of the invention.

EXAMPLES

5 [00113] The following examples are intended to illustrate embodiments of the invention and are not intended to be construed in a limiting manner.

[00114] Example 1

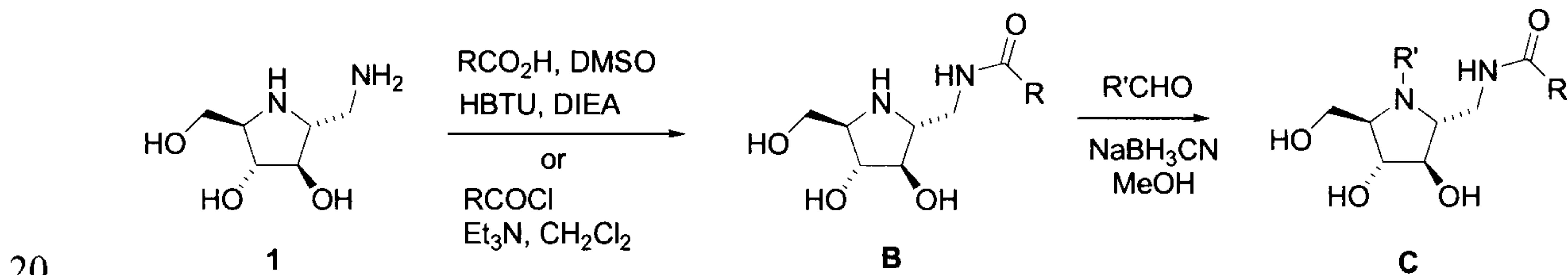
[00115] Compounds of the invention having general structure **A** are prepared via known synthetic methods.^{121,122} For example, following the synthetic route of Liu, Liang et al. (Scheme 1), reductive amination of the known intermediate **2**¹²³ with the appropriate aldehyde provides the desired N-substituted materials **A**.^{121,122}



Scheme 1

Example 2

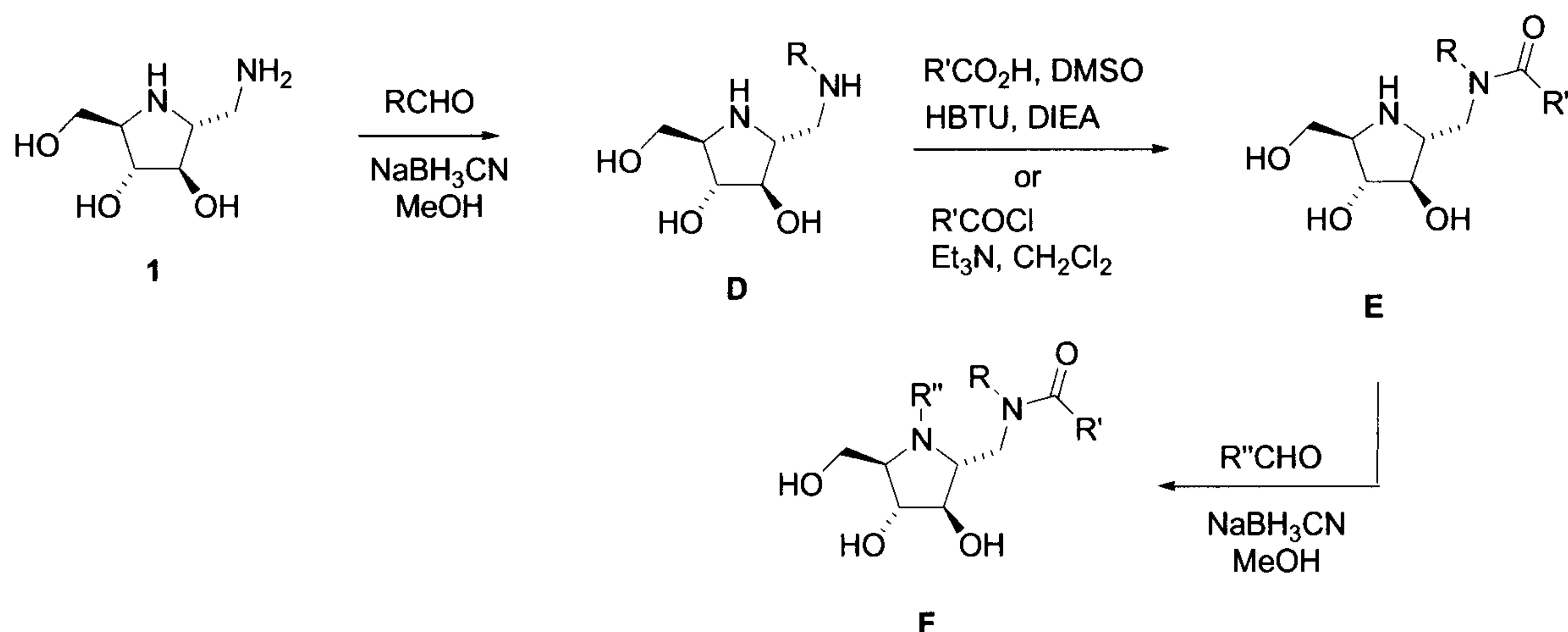
15 [00116] Compounds of the invention having general structures **B** and **C** are also prepared via known synthetic methods.^{121,122} For example, following the synthetic route of Liu, Liang et al. (Scheme 2), coupling of the known intermediate **1**¹²⁴ with the appropriate carboxylic acid or acid chloride provides the desired amides **B**.^{121,122} Reductive amination of **B** with the appropriate aldehyde provides the desired N-substituted materials **C**.¹²¹



Scheme 2

Example 3

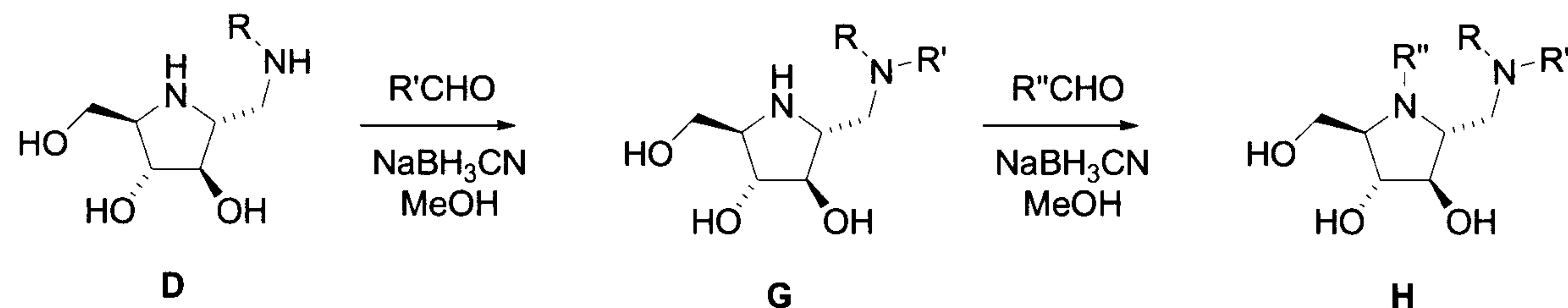
[00117] Compounds of the invention having general structures **D**, **E**, and **F** are prepared according to the sequence described in Scheme 3. Thus, starting from intermediate **1**¹²⁴, reductive amination with the appropriate aldehyde provides structures **C**. Coupling of **C** with the appropriate carboxylic acid or acid chloride provides the desired amides **D**.^{121,122} A second reductive amination of **D** with the appropriate aldehyde furnishes structures **F**.¹²¹

**Scheme 3**

10

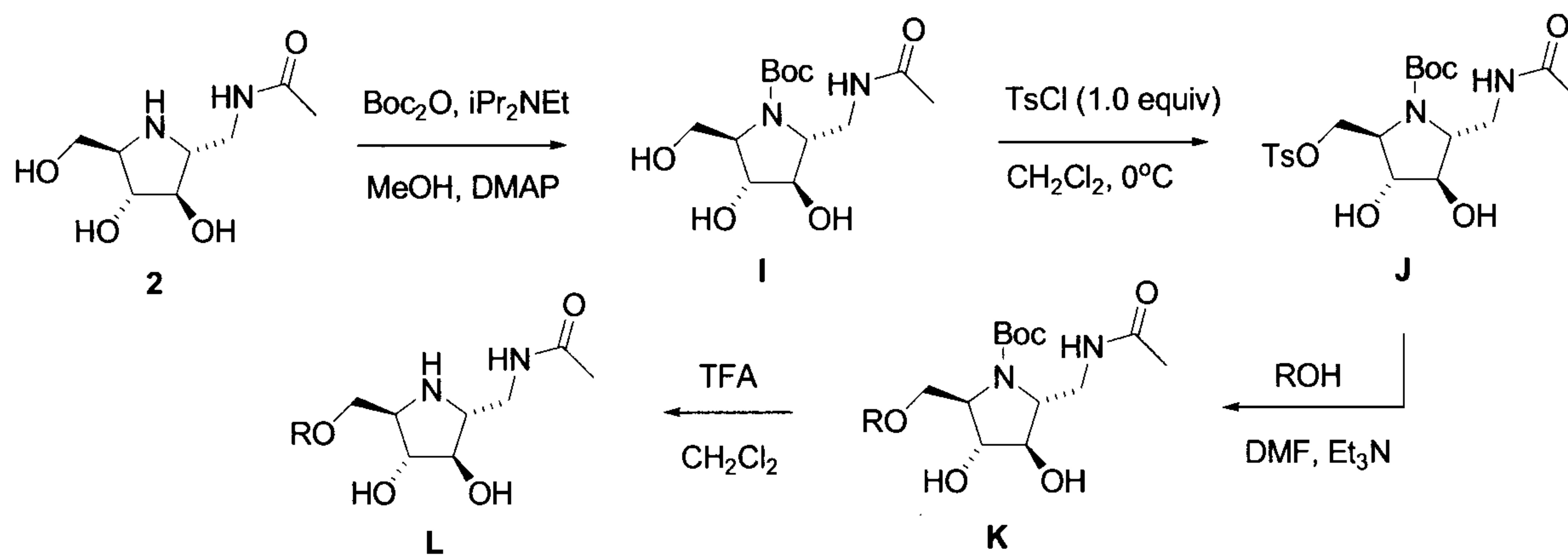
Example 4

[00118] Compounds of the invention having general structures **G** and **H** are prepared according to the sequence described in Scheme 4. Thus, starting from intermediate **D**, reductive amination with the appropriate aldehyde provides structures **G**. Subsequent reductive amination of **G** with the appropriate aldehyde furnishes structures **H**.

**Scheme 4**

Example 5

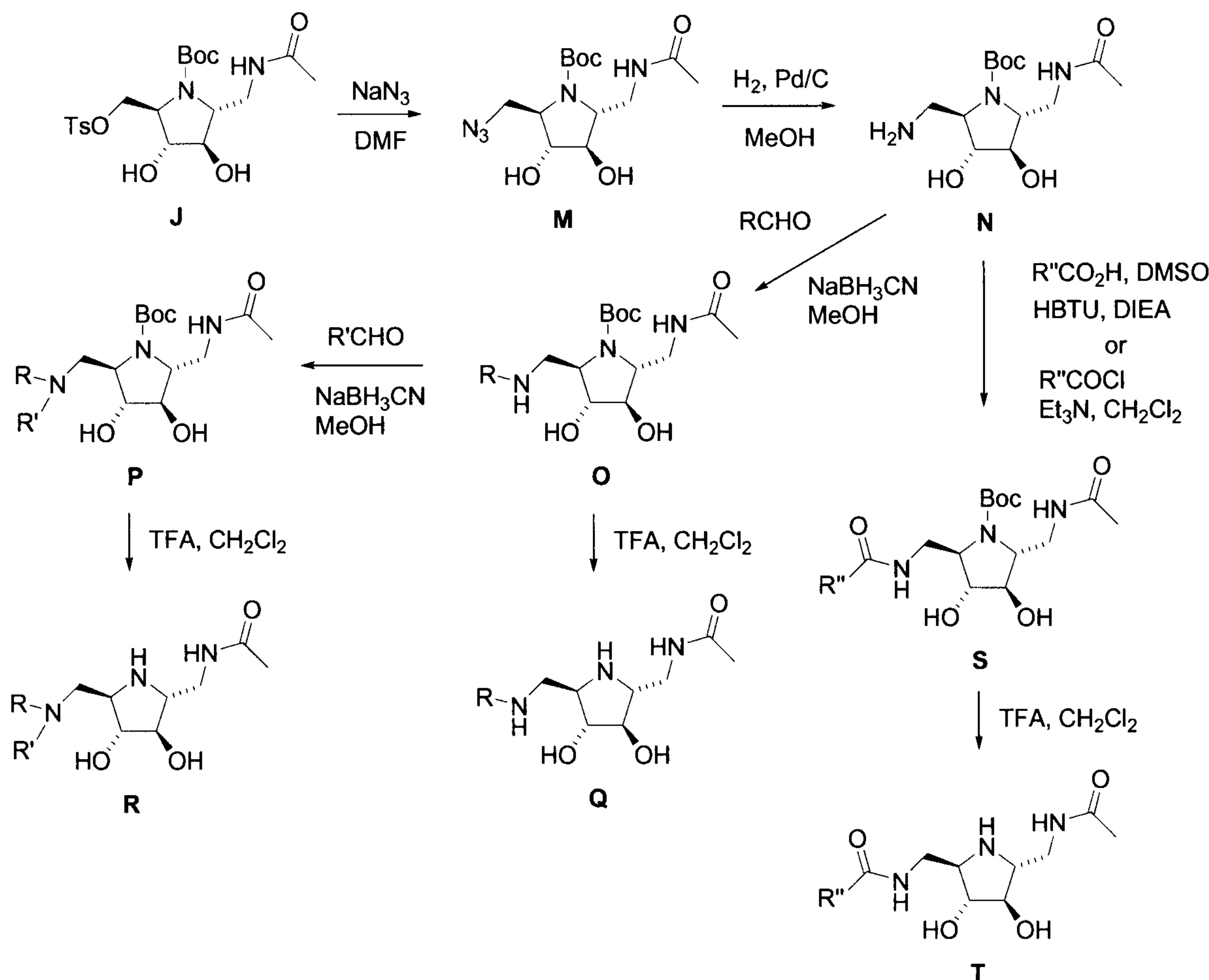
[00119] Compounds of the invention having general structures **L** are prepared according to the sequence described in Scheme 5. Thus, starting from intermediate **2**¹²³, Boc-protection of the secondary amine provides **I**. Selective tosylation of the primary alcohol in **I** furnishes **J**. Displacement of the tosyl group in **J** with the appropriate alcohol provides **K**, which, after TFA deprotection of the Boc group, gives the desired materials **L**.

**Scheme 5**

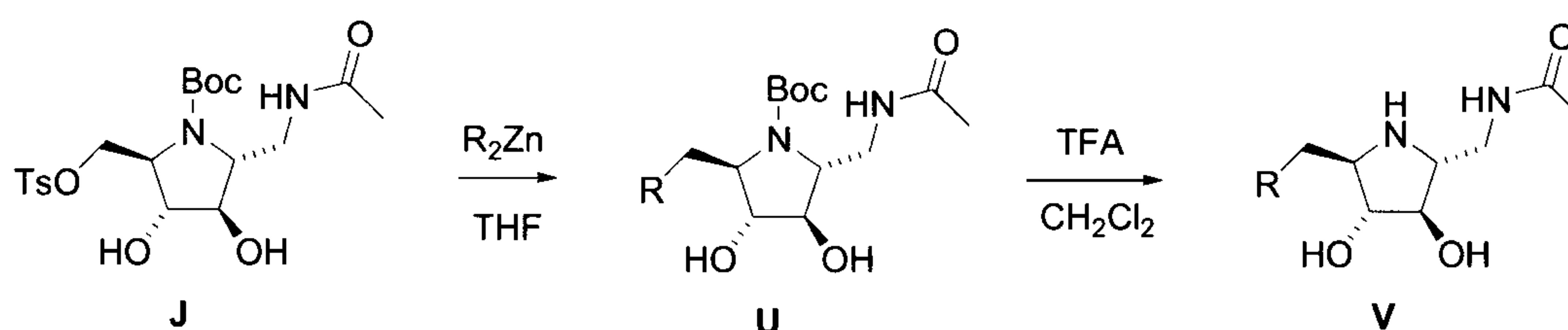
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Example 6

[00120] Compounds of the invention having general structures **Q**, **R**, and **T** are prepared according to the sequence described in Scheme 6. Thus, starting from intermediate **J**, azide displacement of the tosyl group provides **M**, with subsequent hydrogenation furnishing **N**.
 15 Reductive amination of **N** with the appropriate aldehyde gives **O** which is then deprotected with TFA to provide the desired compounds **Q**. Alternatively, a second reductive amination of **O** with the appropriate aldehyde gives **P**, which is then deprotected with TFA to give the bis-alkylated products **R**. Coupling of intermediates **N** with the appropriate carboxylic acid provides **S** with subsequent TFA deprotection giving products **T**.

**Scheme 6**Example 7

[00121] Compounds of the invention having general structure **V** are prepared according to the sequence described in Scheme 7. Thus, reaction of intermediate **J** with the appropriate dialkylzinc reagent provides **U**. Subsequent TFA deprotection of the Boc group in **U** provides the desired structures **V**.

**Scheme 7**

Example 8Assay for determination of K_I values for inhibition of O-GlcNAcase activity

[00122] Experimental procedure for kinetic analyses: Enzymatic reactions are carried out in PBS buffer (pH 7.4) using pNP-GlcNAc as a substrate (0.5 mM) and monitored continuously at 37 °C at 400 nm using a Cary 3E UV-VIS spectrophotometer equipped with a Peltier temperature controller. Reactions are pre-heated in a 500 μ L quartz cuvette for approximately 5 minutes followed by addition of 10 μ L enzyme via syringe (final enzyme concentration 0.002 mg/mL). Reaction velocities are determined by linear regression of the linear region of the reaction progress curve between the first and third minutes. An inhibitor concentration range of 1/5 to 5 times K_I is used in each case.

[00123] When tested in the assay described above, compound **2** exhibited a K_I value for inhibition of O-GlcNAcase of 0.85 μ M. When tested in the assay described above, many of the compounds described herein exhibit K_I values for inhibition of O-GlcNAcase in the range 1 nM - 50 μ M. All K_I values are determined using linear regression of Dixon plots.

15

Example 9Assay for determination of K_I values for inhibition of β -hexosaminidase activity

[00124] Experimental procedure for kinetic analyses: All enzymatic assays are carried out in triplicate at 37 °C using a stopped assay procedure by measuring the amount of 4-nitrophenolate liberated as determined by absorption measurements at 400 nm. Reactions (50 μ L) are initiated by the addition, *via* syringe, of enzyme (3 μ L). Time-dependent assay of β -hexosaminidase has revealed that the enzyme is stable in the buffer over the period of the assay: 50 mM citrate, 100 mM NaCl, 0.1% BSA, pH 4.25. β -hexosaminidase is used at a concentration of 0.036 mg/mL with pNP-GlcNAc as a substrate at a concentration of 0.5 mM. The inhibitor is tested at five concentrations ranging from 5 times to 1/5 K_I . K_I values are determined by linear regression of data from Dixon plots.

[00125] When tested in this assay, many of the compounds described herein exhibit K_I values for inhibition of β -hexosaminidase in the range 1 μ M - 10 mM.

[00126] The selectivity ratio for inhibition of O-GlcNAcase over β -hexosaminidase is defined here as:

30

$$K_I(\beta\text{-hexosaminidase})/K_I(\text{O-GlcNAcase})$$

In general, the compounds described herein should exhibit a selectivity ratio in the range of about 10 to 100000. Thus, many compounds of the invention exhibit high selectivity for inhibition of O-GlcNAcase over β -hexosaminidase.

[00127] The present invention has been described with regard to one or more embodiments.

5 However, it will be apparent to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as defined in the claims.

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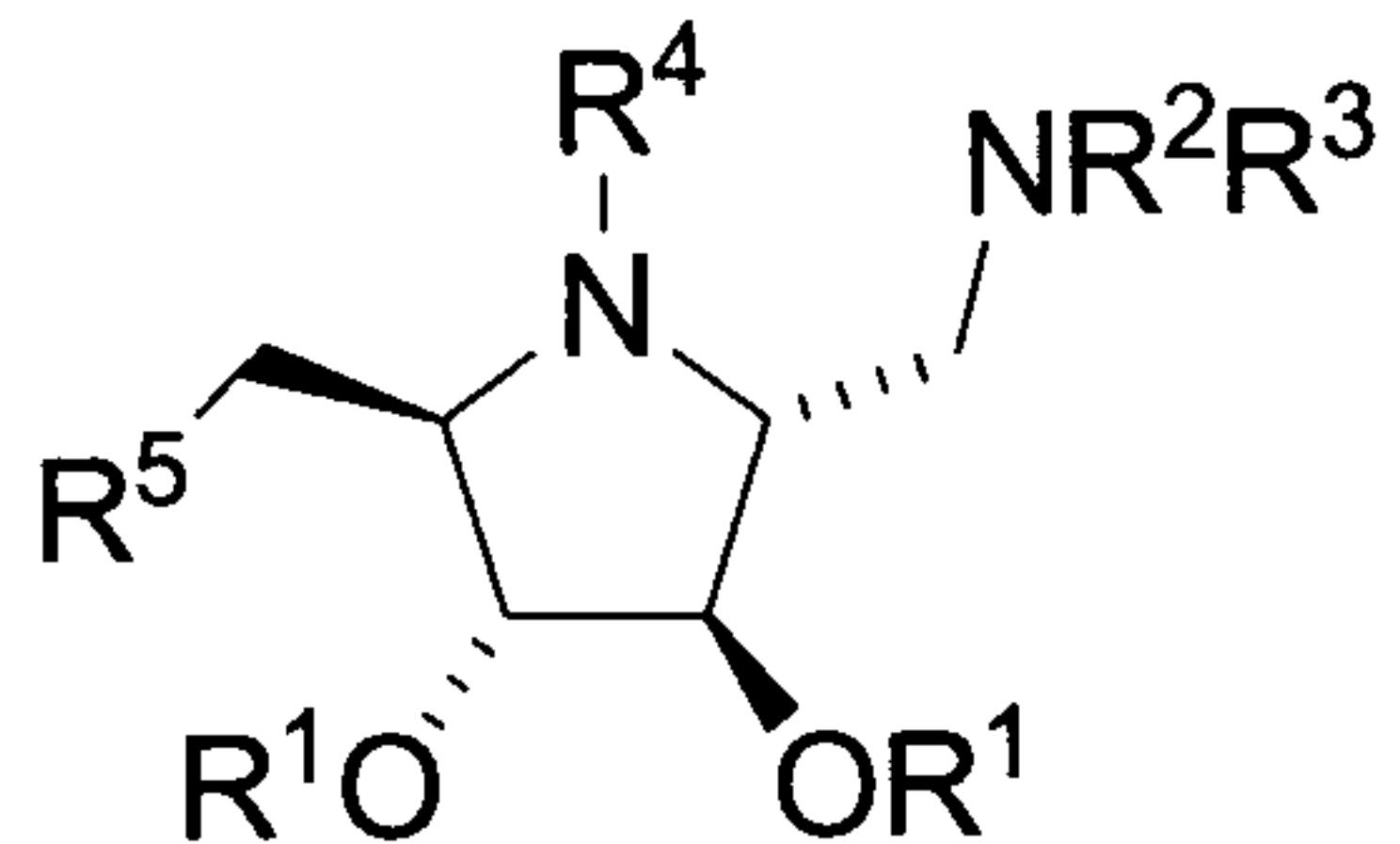
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All citations are hereby incorporated by reference.

WHAT IS CLAIMED IS:

1. A compound of Formula (I) or a pharmaceutically acceptable salt thereof:



(I)

wherein

each R¹ is independently H or C(O)R⁶;R² is a non-interfering substituent;R³ is H, C(O)R⁶, C(NR⁶)NR⁶₂, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl;R⁴ is a non-interfering substituent;R⁵ is H, OR⁶, OC(O)R⁶, NR⁶C(O)R⁶, NR⁶₂, or an optionally substituted alkyl; andwherein each R⁶ is optionally independently a non-interfering substituent;with the proviso that when each R¹ is H, R² is H, R⁴ is H, and R⁵ is OH, R³ excludes

15 H, C(O)CH₃, C(O)CH₂CH₃, C(O)(CH₂)₂CH₃, C(O)(CH₂)₃CH₃, C(O)(CH₂)₄CH₃,
 C(O)(CH₂)₇CH₃, C(O)(CH₂)₁₀CH₃, C(O)C≡CH, C(O)CH=CH₂, C(O)C(CH₃)=CH₂,
 C(O)(CH₂)₅OH, C(O)(1,3-benzodioxol-4-ylmethyl), C(O)(1,3-benzodioxol-5-
 ylmethyl), C(O)CH₂(3-indolyl), C(O)(CH₂)₂(phenyl), C(O)(2,3-dichlorophenyl),
 C(O)(2,3-dihydroxyphenyl), C(O)(2,3-dihydroxy-4-methylphenyl), C(O)(2-methyl-
 20 3-nitrophenyl), C(O)(2,3-dimethoxyphenyl), C(O)(2-hydroxy-3-methylphenyl),
 C(O)(2,6-dihydroxyphenyl), C(O)(2,3-dimethylphenyl), C(O)(2-hydroxy-3-
 methoxyphenyl), C(O)(2-naphthyl), C(O)(4-hydroxy-2-quinolyl), C(O)(4-
 benzoylphenyl), C(O)(6-chloro-2H-chromene-3-yl), C(O)(1-naphthyl),

C(O)(phenyl), C(O)(3-dimethylaminophenyl), C(O)(4-dimethylaminophenyl),
 C(O)(2-oxo-2H-chromene-3-yl), C(O)(7-diethylamino-2-oxo-2H-chromene-3-yl),
 S(O)₂(1-naphthyl), S(O)₂(5-dimethylamino-1-naphthyl), S(O)₂(phenyl),
 S(O)₂(CH₂)₇CH₃, C(O)(CH₂)₅NHSO₂(5-dimethylamino-1-naphthyl),

(CH₂)₃NHSO₂(5-dimethylamino-1-naphthyl), C(O)(CH₂)₂(3-indolyl), C(O)(5-indolyl), C(O)(CH₂)(1-naphthyl), C(O)(CH₂)(2-naphthyl), C(O)(2-methylthio(phenyl)), C(O)(E-(3-trifluoromethylphenyl-2-ethenyl)), C(O)(E-(3-chlorophenyl-2-ethenyl)), C(O)(E-(3-bromophenyl-2-ethenyl)), C(O)(E-(4-methylphenyl-2-ethenyl)), C(O)(E-(4-dimethylaminophenyl-2-ethenyl)), C(O)(E-(3-acetoxy-4-methoxyphenyl-2-ethenyl)), C(O)(E-(3-indolyl-2-ethenyl)), C(O)(3-(1-benzylindolyl)), C(O)((9-oxo-9H-fluorene)-2-yl), C(O)CH(CH₂CH₃)((CH₂)₄CH₃), C(O)CH₂OH, C(O)(cis-4-aminocyclohexyl), (CH₂)₃NH₂, (CH₂)₆OH; or

5 with the proviso that when each R¹ is H, R² is H, R³ is C(O)CH₃, and R⁵ is OH, R⁴ excludes H, CH₃, CH₂CH₃, (CH₂)₃CH₃, (CH₂)₅CH₃, (CH₂)₆CH₃, (CH₂)₇CH₃, (CH₂)₈CH₃, (CH₂)₂O(CH₂)₂O(CH₂)₂N₃, (CH₂)₂O(CH₂)₂O(CH₂)₂NH₂, (CH₂)₃NH₂, (CH₂)₄NH₂, (CH₂)₅NH₂, (CH₂)₆NH₂, (CH₂)₇NH₂, (CH₂)₈NH₂, (CH₂)₈N₃, (CH₂)₇N₃, (CH₂)₆NHC(O)O^tBu, (CH₂)₅NHC(O)O^tBu, (CH₂)₄NHC(O)O^tBu, (CH₂)₃NHC(O)O^tBu, CH₂(4-(dimethylamino)phenyl), 1,3-benzodioxol-4-ylmethyl, CH₂(5-(4-chlorophenyl)-2-furanyl), CH₂(4-hydroxyphenyl), CH₂(4-(2-pyridyl)phenyl); or

10 with the proviso that when each R¹ is H, R² is H, R³ is C(O)CH₃, and R⁵ is H, R⁴ excludes H and CH₃; or

15 with the proviso that when each R¹ is H, R² is H, R³ is C(O)((1,2-dihydrocyclobutabenzene)-1-yl), and R⁵ is OH, R⁴ excludes H, (CH₂)₃CH₃, (CH₂)₄CH₃, (CH₂)₅CH₃, (CH₂)₆CH₃, (CH₂)₇CH₃, (CH₂)₈CH₃, (CH₂)₉CH₃, (CH₂)₁₀CH₃, (CH₂)₁₁CH₃; or

20 with the proviso that when each R¹ is H, R² is H, R³ is H, and R⁵ is OH, R⁴ excludes (CH₂)₃CH₃, (CH₂)₉CH₃, (CH₂)₇CH₃, and (CH₂)₈CH₃; or

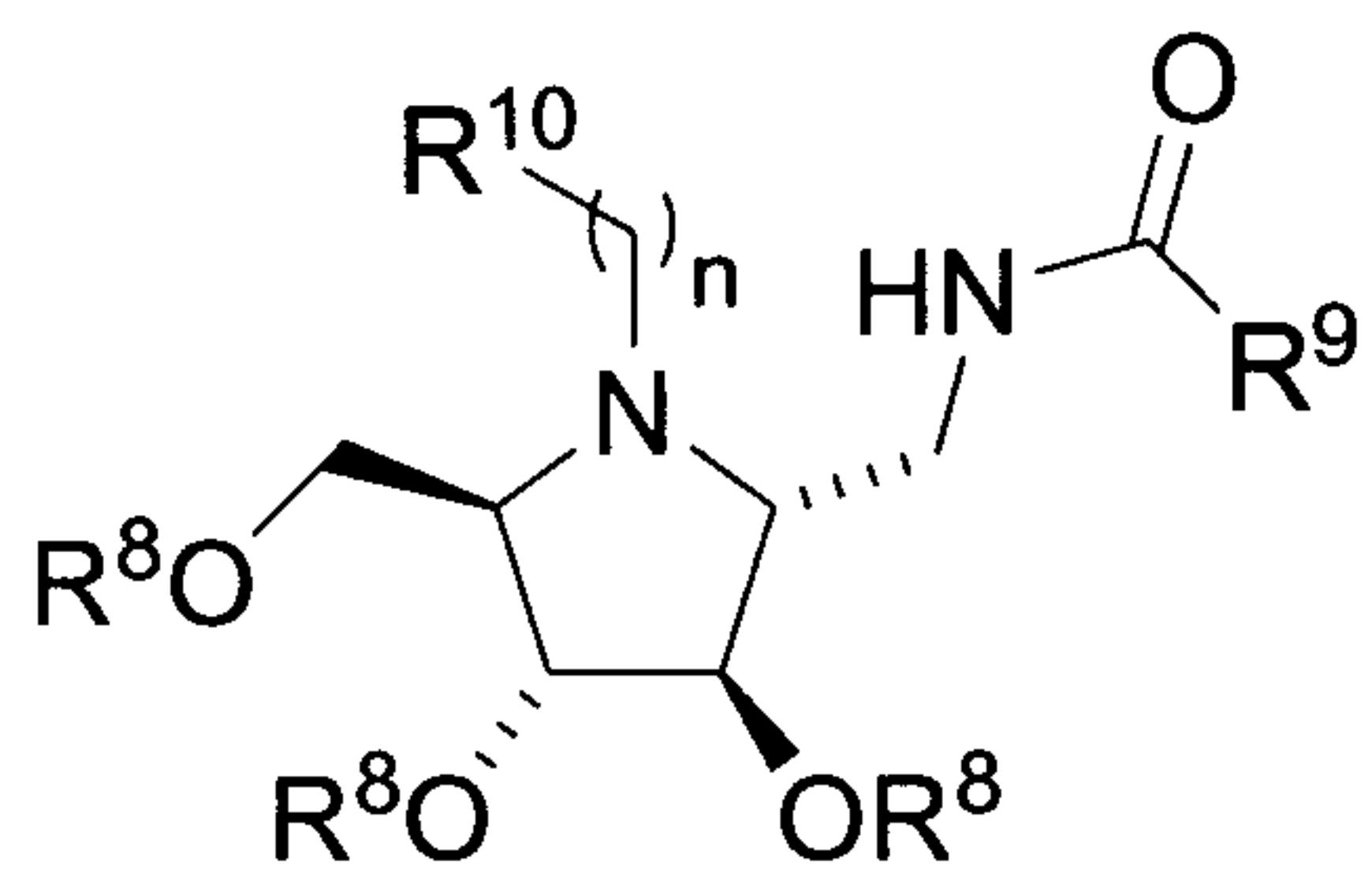
25 with the proviso that Formula (I) excludes the following compounds: 5-{{[(2R,3R,4R,5R)-3,4-dihydroxy-5-hydroxymethyl-pyrrolidin-2-ylmethyl]-amino}-methylene}-1,3-dimethyl-pyrimidine-2,4,6-trione (CAS # 763122-23-2), (2R,2'R,3R,3'R,4R,4'R,5R,5'R)-2,2'-[iminobis(methylene)]bis[5-(hydroxymethyl)-3,4-pyrrolidinediol (CAS # 231618-81-8), (2R,3R,4R,5R)-1-butyl-2-[(dibutylamino)methyl]-5-(hydroxymethyl)-3,4-pyrrolidinediol (CAS # 172936-43-5), (2R,3R,4R,5R)-2-(azidomethyl)-1-butyl-5-(hydroxymethyl)-3,4-pyrrolidinediol (CAS # 172936-41-3), methyl 2-(((2R,3R,4R,5R)-3,4-dihydroxy-5-

30

(hydroxymethyl)pyrrolidin-2-yl)methylcarbamoyl)-1H-indole-5-carboxylate (CAS # 876751-91-6), and (R)-alpha-amino-N-[(2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-2-pyrrolidinyl]methyl]-4-oxo-1(4H)-pyridinepropanamide (CAS # 876751-85-8), (2R,3R,4R,5R)-2-(azidomethyl)-5-(hydroxymethyl)-3,4-pyrrolidinediol (CAS # 765308-83-6).

5

2. The compound of claim 1 wherein R^5 is OH or $OC(O)CH_3$.
3. The compound of claim 1 wherein R^1 is H or $C(O)CH_3$.
4. The compound of claim 1 as set forth in Formula (II):



10 (II)

wherein

each R^8 is independently H or $C(O)R^{11}$;

R^9 is a non-interfering substituent;

R^{10} is a non-interfering substituent;

15 n is an integer between 0 and 10; and

wherein each R^{11} is optionally independently a non-interfering substituent;

with the proviso that when each R^8 is H, R^{10} is H, and $n = 0$, R^9 excludes CH_3 , CH_2CH_3 , $(CH_2)_2CH_3$, $(CH_2)_3CH_3$, $(CH_2)_4CH_3$, $(CH_2)_7CH_3$, $(CH_2)_{10}CH_3$, $C\equiv CH$, $CH=CH_2$, $C(CH_3)=CH_2$, $(CH_2)_5OH$, 1,3-benzodioxol-4-ylmethyl, 1,3-benzodioxol-5-ylmethyl, $CH_2(3\text{-indolyl})$, $(CH_2)_2(\text{phenyl})$, 2,3-dichlorophenyl, 2,3-dihydroxyphenyl, 2,3-dihydroxy-4-methylphenyl, 2-methyl-3-nitrophenyl, 2,3-dimethoxyphenyl, 2-hydroxy-3-methylphenyl, 2,6-dihydroxyphenyl, 2,3-dimethylphenyl, 2-hydroxy-3-methoxyphenyl, 2-naphthyl, 4-hydroxy-2-quinolyl, 4-benzoylphenyl, 6-chloro-2H-chromene-3-yl, 1-naphthyl, phenyl, 3-dimethylaminophenyl, 4-dimethylaminophenyl, 2-oxo-2H-chromene-3-yl, 7-diethylamino-2-oxo-2H-chromene-3-yl, $(CH_2)_5NHSO_2(5\text{-dimethylamino-1-}$

20

25

naphthyl), (CH₂)₂(3-indolyl), 5-indolyl, CH₂(1-naphthyl), CH₂(2-naphthyl), 2-methylthio(phenyl), E-(3-trifluoromethylphenyl-2-ethenyl), E-(3-chlorophenyl-2-ethenyl), E-(3-bromophenyl-2-ethenyl), E-(4-methylphenyl-2-ethenyl), E-(4-dimethylaminophenyl-2-ethenyl), E-(3-acetoxy-4-methoxyphenyl-2-ethenyl), E-(3-indolyl-2-ethenyl), 3-(1-benzylindolyl), (9-oxo-9H-fluorene)-2-yl, CH(CH₂CH₃)((CH₂)₄CH₃), CH₂OH, cis-4-aminocyclohexyl; or

with the proviso that when each R⁸ is H, R⁹ is CH₃, and n = 0, R¹⁰ excludes H, CH₃, CH₂CH₃, (CH₂)₃CH₃, (CH₂)₅CH₃, (CH₂)₆CH₃, (CH₂)₇CH₃, (CH₂)₈CH₃, (CH₂)₂O(CH₂)₂O(CH₂)₂N₃, (CH₂)₂O(CH₂)₂O(CH₂)₂NH₂, (CH₂)₃NH₂, (CH₂)₄NH₂, (CH₂)₅NH₂, (CH₂)₆NH₂, (CH₂)₇NH₂, (CH₂)₈NH₂, (CH₂)₈N₃, (CH₂)₇N₃, (CH₂)₆NHC(O)O^tBu, (CH₂)₅NHC(O)O^tBu, (CH₂)₄NHC(O)O^tBu, (CH₂)₃NHC(O)O^tBu, CH₂(4-(dimethylamino)phenyl), 1,3-benzodioxol-4-ylmethyl, CH₂(5-(4-chlorophenyl)-2-furanyl), CH₂(4-hydroxyphenyl), CH₂(4-(2-pyridyl)phenyl); or

with the proviso that when each R⁸ is H, R⁹ is (1,2-dihydrocyclobutabenzeno)-1-yl, and n = 0, R¹⁰ excludes H, (CH₂)₃CH₃, (CH₂)₄CH₃, (CH₂)₅CH₃, (CH₂)₆CH₃, (CH₂)₇CH₃, (CH₂)₈CH₃, (CH₂)₉CH₃, (CH₂)₁₀CH₃, (CH₂)₁₁CH₃; or

with the proviso that Formula (II) excludes the following compounds: methyl 2-(((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methylcarbamoyl)-1H-indole-5-carboxylate (CAS # 876751-91-6), and (R)-alpha-amino-N-[(2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-2-pyrrolidinyl]methyl]-4-oxo-1(4H)-pyridinepropanamide (CAS # 876751-85-8).

5. The compound of claim 4 wherein R⁸ is H or C(O)CH₃.
6. The compound of any one of claims 1 to 5 wherein said non-interfering substituent is selected from one or more of the group consisting of alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, branched alkynyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, arylalkenyl, heteroarylalkenyl, arylalkynyl, and heteroarylalkynyl, each of which may be optionally substituted with one or more heteroatoms or additional non-interfering substituents.
7. The compound of any one of claims 1 to 6 wherein said non-interfering substituent comprises one or more heteroatoms selected from P, O, S, N, F, Cl, Br, I, and B.

8. The compound of any one of claims 1 to 7 wherein said non-interfering substituent is optionally substituted.

9. The compound of any one of claims 1 to 8 with the proviso that the compound excludes one or more of the compounds described in Table 1.

5 10. The compound of claim 1 or 4 wherein the compound is a prodrug.

11. The compound of any one of claims 1 to 10 wherein the compound selectively inhibits an O-glycoprotein 2-acetamido-2-deoxy- β -D-glucopyranosidase (O-GlcNAcase).

12. The compound of any one of claims 1 to 11 wherein the compound selectively binds an O-GlcNAcase.

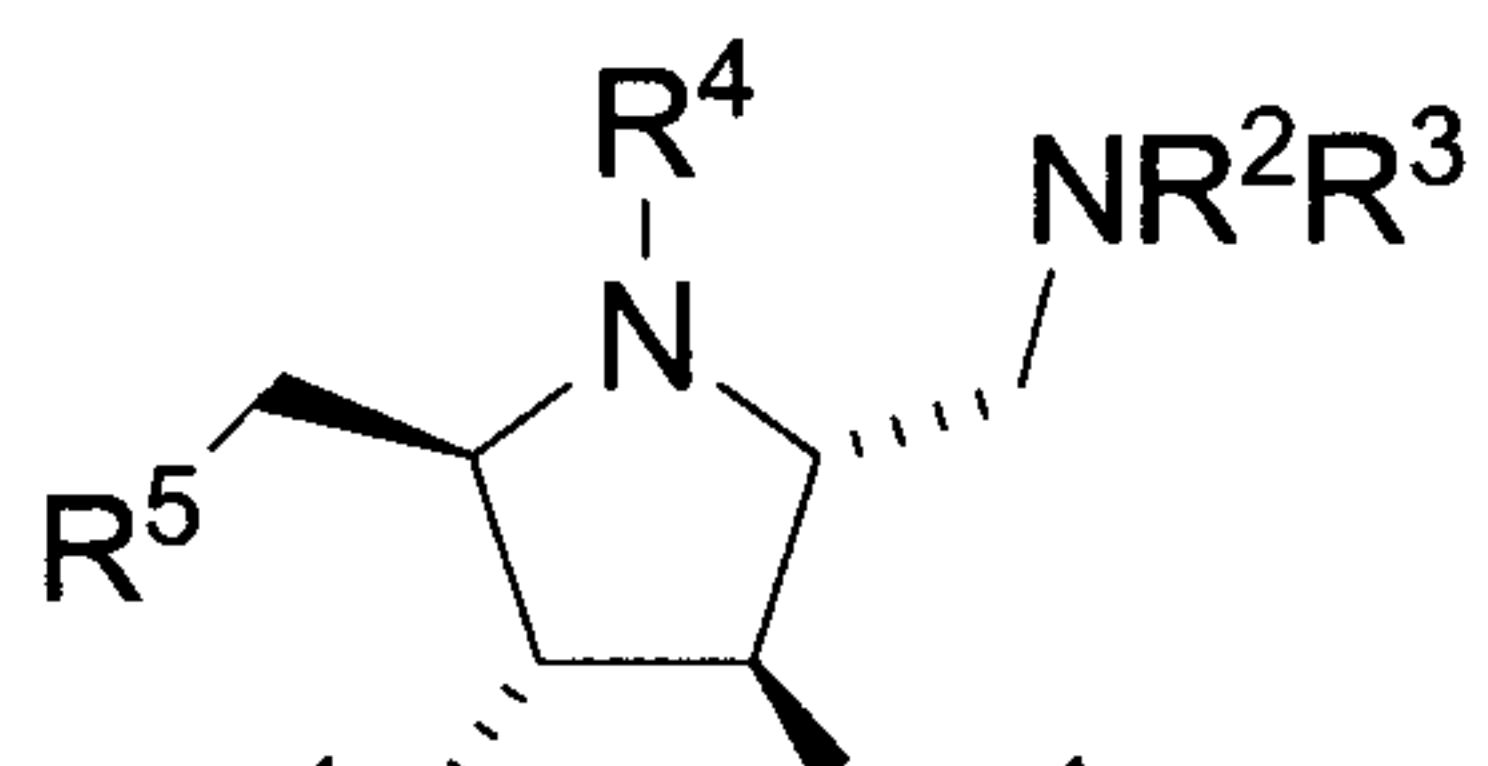
10 13. The compound of any one of claims 1 to 12 wherein the compound selectively inhibits the cleavage of 2-acetamido-2-deoxy- β -D-glucopyranoside (O-GlcNAc).

14. The compound of claim 12 wherein the O-GlcNAcase is a mammalian O-GlcNAcase.

15. The compound of any one of claims 1 to 14 wherein the compound does not substantially inhibit a mammalian β -hexosaminidase.

15 16. A pharmaceutical composition comprising the compound of any one of claims 1 to 15 in combination with a pharmaceutically acceptable carrier.

17. A method of selectively inhibiting an O-GlcNAcase in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



20

(I)

wherein

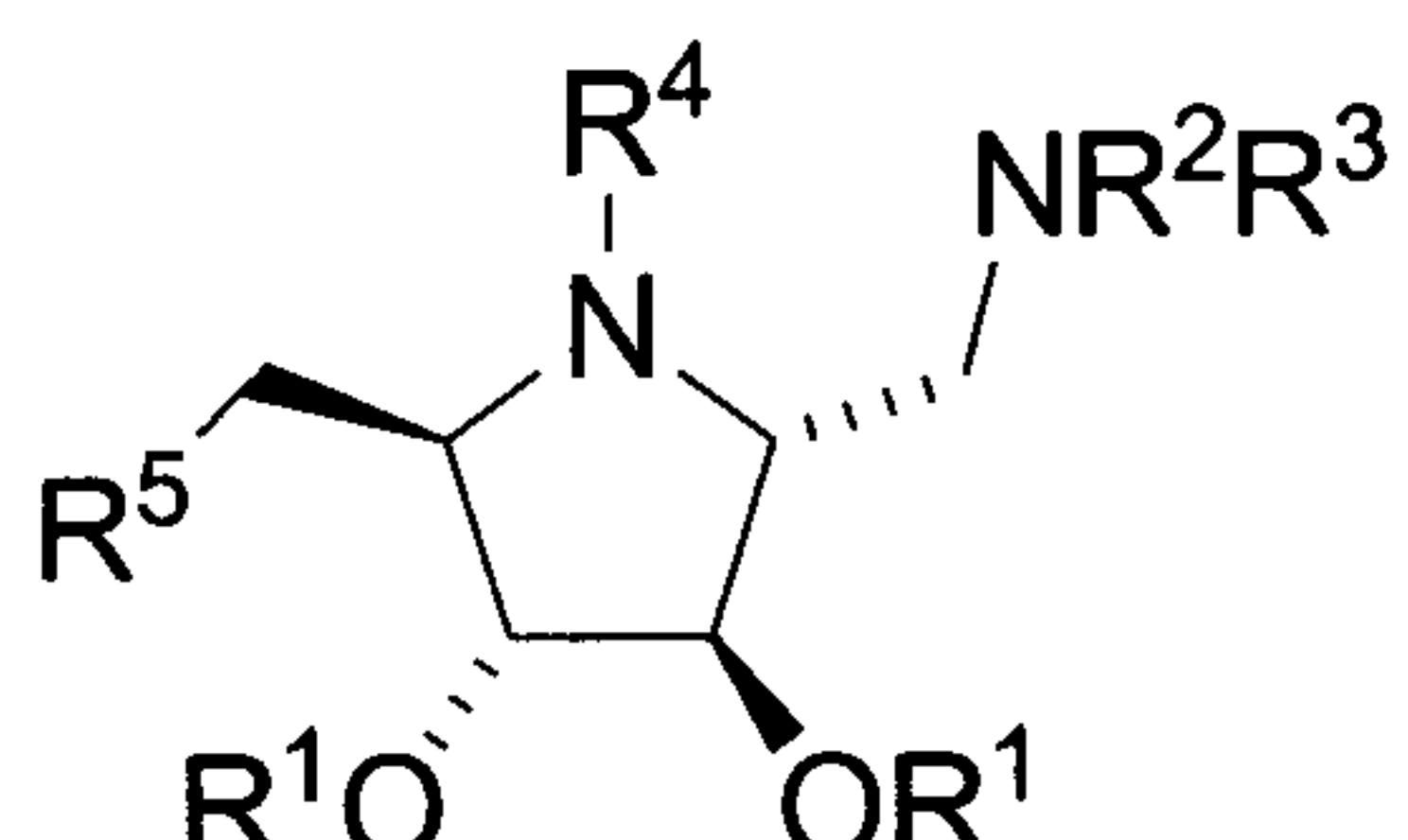
each R¹ is independently H or C(O)R⁶;R² is a non-interfering substituent;

25 R³ is H, C(O)R⁶, C(NR⁶)NR⁶₂, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl;

R^4 is a non-interfering substituent;

R^5 is H, OR^6 , $OC(O)R^6$, $NR^6C(O)R^6$, NR^6_2 , or an optionally substituted alkyl; and
wherein each R^6 is optionally independently a non-interfering substituent.

18. A method of elevating the level of O-GlcNAc in a subject in need thereof, the method
5 comprising administering to the subject an effective amount of a compound of Formula (I)
or a pharmaceutically acceptable salt thereof:



(I)

wherein

10 each R^1 is independently H or $C(O)R^6$;

R^2 is a non-interfering substituent;

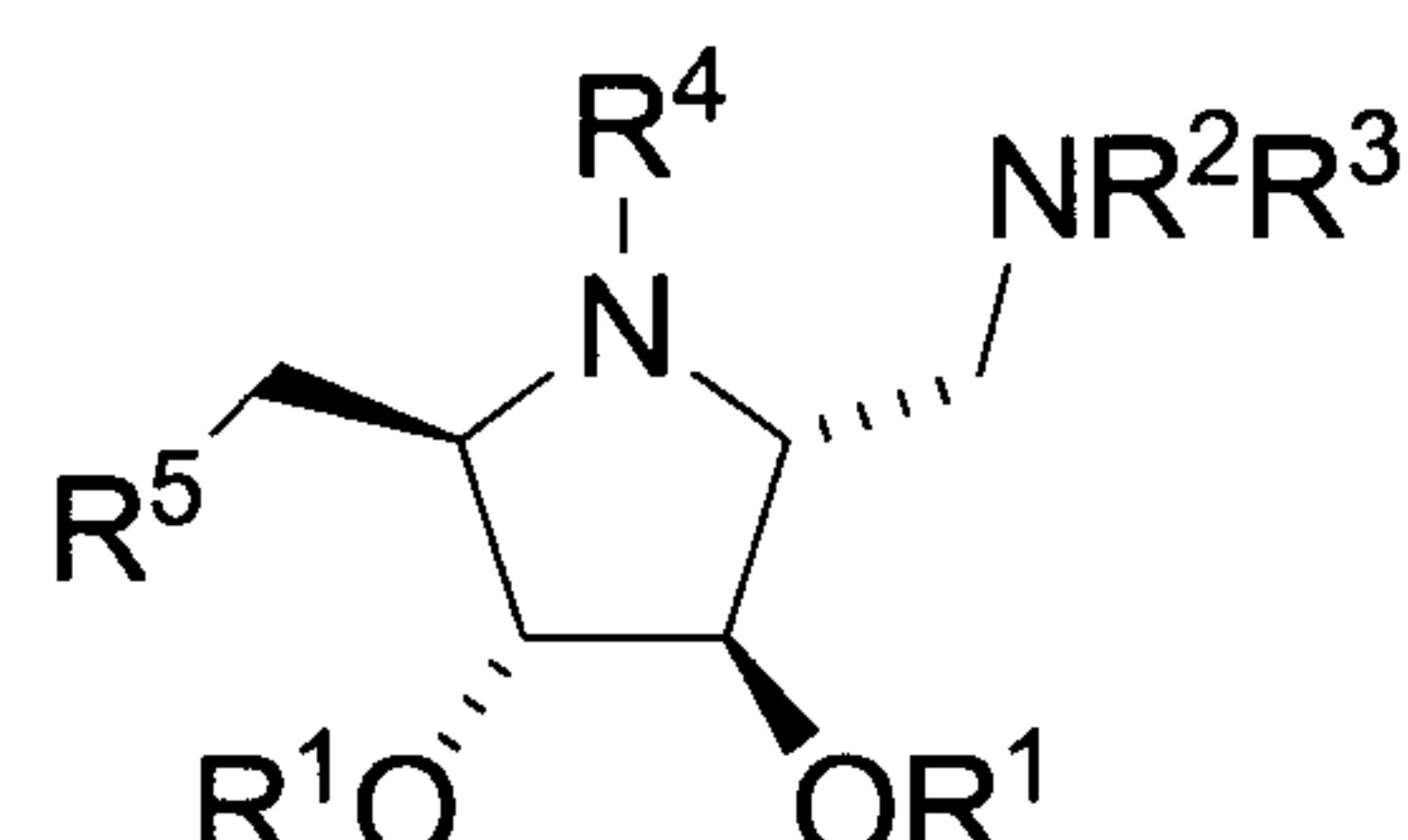
R^3 is H, $C(O)R^6$, $C(NR^6)NR^6_2$, or an optionally substituted alkyl, branched alkyl,
cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl;

R^4 is a non-interfering substituent;

15 R^5 is H, OR^6 , $OC(O)R^6$, $NR^6C(O)R^6$, NR^6_2 , or an optionally substituted alkyl; and

wherein each R^6 is optionally independently a non-interfering substituent.

19. A method of treating a condition that is modulated by an O-GlcNAcase, in a subject in
need thereof, the method comprising administering to the subject an effective amount of a
compound of Formula (I) or a pharmaceutically acceptable salt thereof:



20

(I)

wherein

each R¹ is independently H or C(O)R⁶;

R² is a non-interfering substituent;

R³ is H, C(O)R⁶, C(NR⁶)NR⁶₂, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl;

5

R⁴ is a non-interfering substituent;

R⁵ is H, OR⁶, OC(O)R⁶, NR⁶C(O)R⁶, NR⁶₂, or an optionally substituted alkyl; and

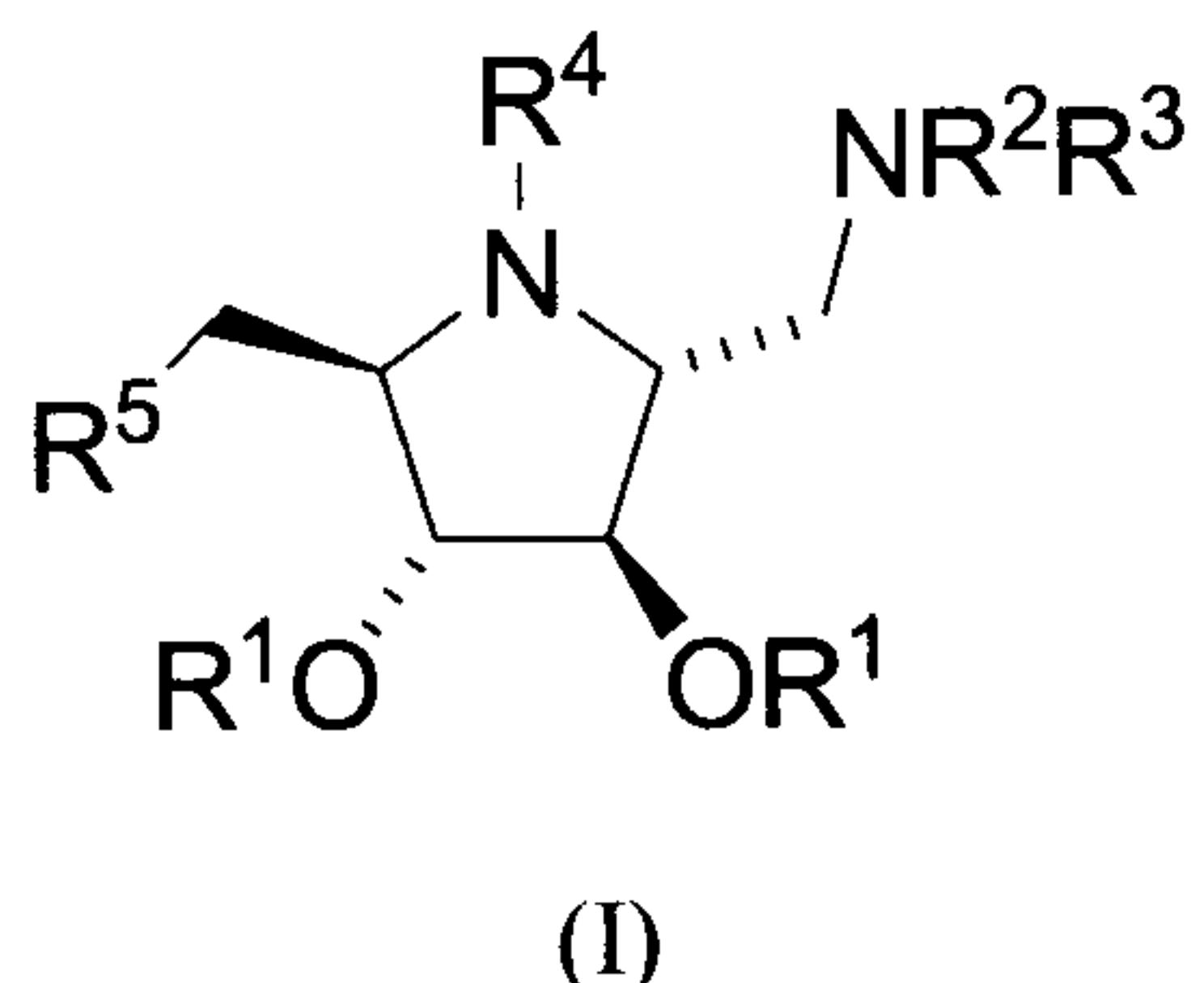
wherein each R⁶ is optionally independently a non-interfering substituent.

20. The method of claim 19 wherein the condition is selected from one or more of the

10 group consisting of an inflammatory disease, an allergy, asthma, allergic rhinitis, hypersensitivity lung diseases, hypersensitivity pneumonitis, eosinophilic pneumonias, delayed-type hypersensitivity, atherosclerosis, interstitial lung disease (ILD), idiopathic pulmonary fibrosis, ILD associated with rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, systemic sclerosis, Sjogren's syndrome, polymyositis or
15 dermatomyositis, systemic anaphylaxis or hypersensitivity response, drug allergy, insect sting allergy, autoimmune disease, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, systemic lupus erythematosus, myastenia gravis, glomerulonephritis, autoimmune thyroiditis, graft rejection, allograft rejection, graft-versus-host disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, spondyloarthropathy, scleroderma, psoriasis, T-
20 cell mediated psoriasis, inflammatory dermatosis, dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria, vasculitis, necrotizing, cutaneous, and hypersensitivity vasculitis, eosinophilic myitis, eosinophilic fasciitis, solid organ transplant rejection, heart transplant rejection, lung transplant rejection, liver transplant rejection, kidney transplant rejection, pancreas transplant rejection, kidney allograft, lung allograft, epilepsy, pain,
25 stroke, neuroprotection.

21. A method of treating a condition selected from the group consisting of a

neurodegenerative disease, a tauopathy, cancer and stress, in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



wherein

each R¹ is independently H or C(O)R⁶;

5 R² is a non-interfering substituent;

R³ is H, C(O)R⁶, C(NR⁶)NR⁶₂, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl;

R⁴ is a non-interfering substituent;

R⁵ is H, OR⁶, OC(O)R⁶, NR⁶C(O)R⁶, NR⁶₂, or an optionally substituted alkyl; and

10 wherein each R⁶ is optionally independently a non-interfering substituent.

22. The method of claim 21 wherein the condition is selected from one or more of the group consisting of Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Amyotrophic lateral sclerosis with cognitive impairment (ALSci), Argyrophilic grain dementia, Bluit disease, Corticobasal degeneration (CBD), Dementia pugilistica, Diffuse neurofibrillary tangles with calcification, Down's syndrome, Familial British dementia, Familial Danish dementia, Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), Gerstmann-Straussler-Scheinker disease, Guadeloupean parkinsonism, Hallevorden-Spatz disease (neurodegeneration with brain iron accumulation type 1), Multiple system atrophy, Myotonic dystrophy, Niemann-Pick disease (type C), Pallido-ponto-nigral degeneration, Parkinsonism-dementia complex of Guam, Pick's disease (PiD), Post-encephalitic parkinsonism (PEP), Prion diseases (including Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Fatal Familial Insomnia, and Kuru), Progressive supercortical gliosis, Progressive supranuclear palsy (PSP), Richardson's syndrome, Subacute sclerosing panencephalitis, Tangle-only dementia, Huntington's disease, and Parkinson's disease.

23. The method of claim 21 wherein the stress is a cardiac disorder.

24. The method of claim 23 wherein the cardiac disorder is selected from one or more of the group consisting of ischemia; hemorrhage; hypovolemic shock; myocardial infarction; an interventional cardiology procedure; cardiac bypass surgery; fibrinolytic therapy; angioplasty; and stent placement.

5 25. The method of any one of claims 17 to 24 wherein R^1 is H or $C(O)CH_3$.

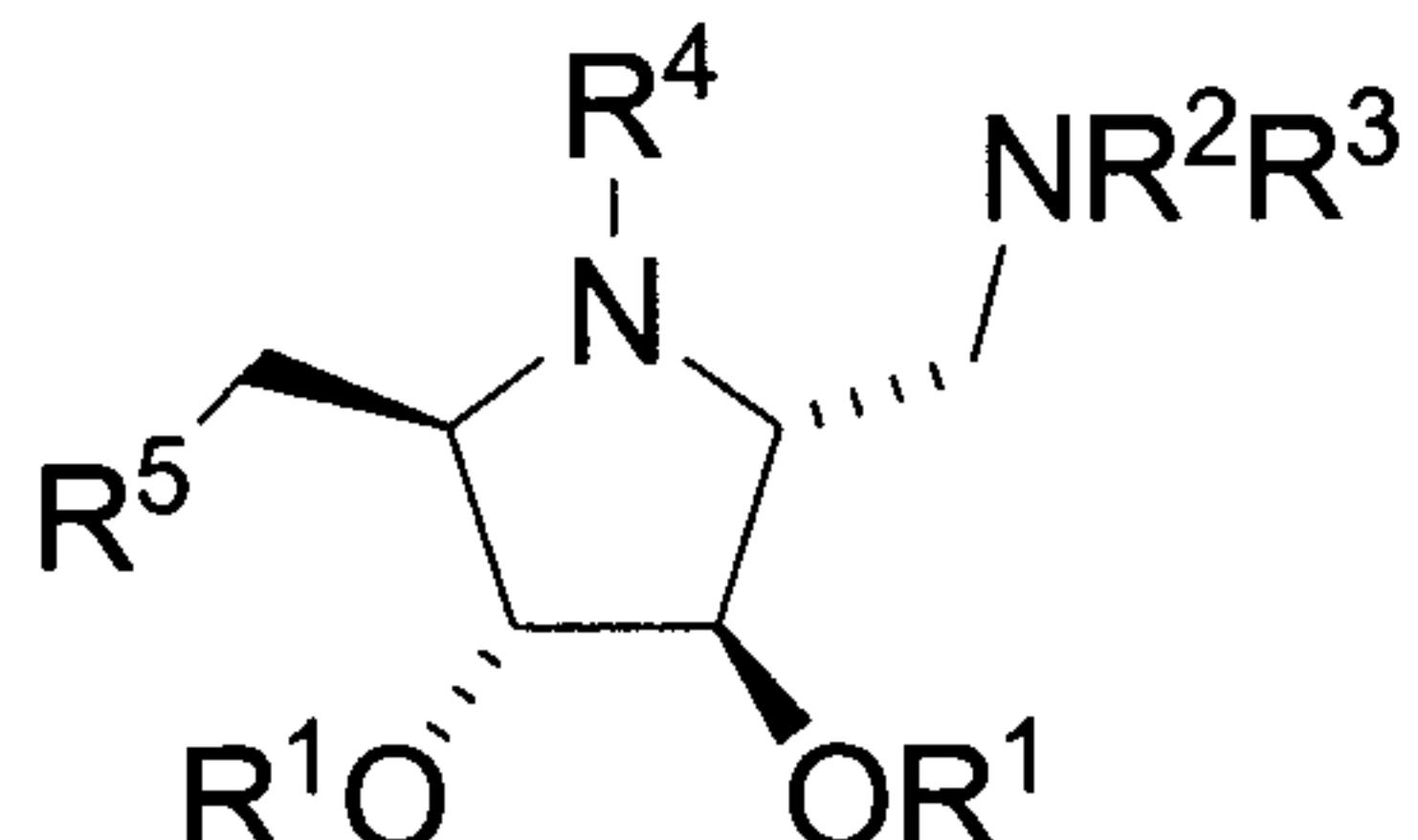
26. The method of any one of claims 17 to 25 wherein R^2 is $N(CH_3)_2$

27. The method of any one of claims 17 to 26 wherein the compound is selected from the group consisting of one or more of the compounds described in Table 1.

28. The method of any one of claims 17 to 27 wherein said administering increases the 10 level of O-GlcNAc in the subject.

29. The method of any one of claims 17 to 28 wherein the subject is a human.

30. Use of a compound of an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



15

(I)

wherein

each R^1 is independently H or $C(O)R^6$;

R^2 is a non-interfering substituent;

20 R^3 is H, $C(O)R^6$, $C(NR^6)NR^6_2$, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl;

R^4 is a non-interfering substituent;

R^5 is H, OR^6 , $OC(O)R^6$, $NR^6C(O)R^6$, NR^6_2 , or an optionally substituted alkyl; and

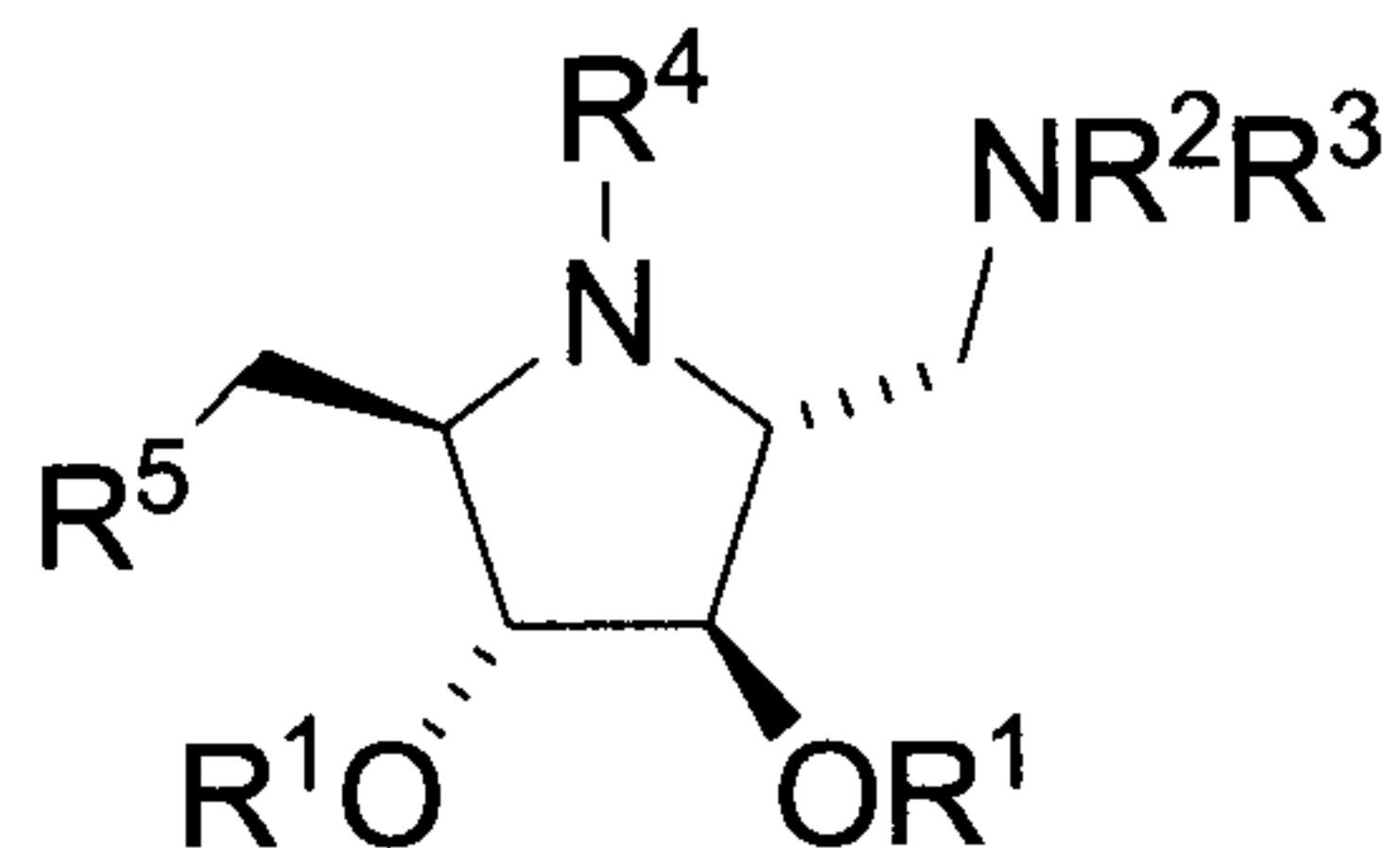
wherein each R^6 is optionally independently a non-interfering substituent,

in the preparation of a medicament.

31. The use of claim 30 wherein said medicament is for selectively inhibiting an O-GlcNAcase, for increasing the level of O-GlcNAc, for treating a condition modulated by an O-GlcNAcase, or for treating a neurodegenerative disease, a tauopathy, a cancer, or stress.

5 32. A method for screening for a selective inhibitor of an O-GlcNAcase, the method comprising:

- a) contacting a first sample with a test compound;
- b) contacting a second sample with a compound of Formula (I)



(I)

10 wherein

each R¹ is independently H or C(O)R⁶;

R² is a non-interfering substituent;

R³ is H, C(O)R⁶, C(NR⁶)NR⁶₂, or an optionally substituted alkyl, branched alkyl, cycloalkyl, alkenyl, branched alkenyl, cycloalkenyl, alkynyl, or branched alkynyl;

15 R⁴ is a non-interfering substituent;

R⁵ is H, OR⁶, OC(O)R⁶, NR⁶C(O)R⁶, NR⁶₂, or an optionally substituted alkyl; and

wherein each R⁶ is optionally independently a non-interfering substituent;

c) determining the level of inhibition of the O-GlcNAcase in the first and second samples,

20 wherein the test compound is a selective inhibitor of a O-GlcNAcase if the test compound exhibits the same or greater inhibition of the O-GlcNAcase when compared to the compound of Formula (I).