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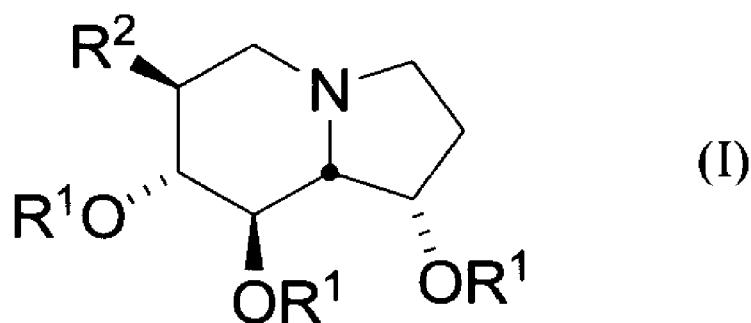
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**(54) Title: SELECTIVE GLYCOSIDASE INHIBITORS AND USES THEREOF**



**(57) Abstract:** The invention provides compounds of Formula (I) for selectively inhibiting glycosidases, prodrugs of the compounds, and pharmaceutical compositions including the compounds or prodrugs of the compounds. The invention also provides methods of treating diseases and disorders related to deficiency or overexpression of O-GlcNAcase, accumulation or deficiency of O-GlcNAc.

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## SELECTIVE GLYCOSIDASE INHIBITORS AND USES THEREOF

### FIELD OF THE INVENTION

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

### 5 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- $\beta$ -D-glucopyranoside ( $\beta$ -N-acetylglucosamine) which is attached via an O-glycosidic linkage.<sup>1</sup> This modification is generally referred to as O-linked N-acetylglucosamine or O-GlcNAc. The enzyme responsible for post-translationally linking  $\beta$ -N-acetylglucosamine (GlcNAc) to specific serine and threonine residues of numerous nucleocytoplasmic proteins is O-GlcNAc transferase (OGT).<sup>2-5</sup> A second enzyme, known as O-GlcNAcase<sup>6,7</sup> 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.<sup>8</sup>

15 [0003] O-GlcNAc-modified proteins regulate a wide range of vital cellular functions including, for example, transcription,<sup>9-12</sup> proteasomal degradation,<sup>13</sup> and cellular signaling.<sup>14</sup> O-GlcNAc is also found on many structural proteins.<sup>15-17</sup> For example, it has been found on a number of cytoskeletal proteins, including neurofilament proteins,<sup>18,19</sup> synapsins,<sup>6,20</sup> synapsin-specific clathrin assembly protein AP-3,<sup>7</sup> and ankyrinG.<sup>14</sup> O-GlcNAc modification has been found to be 20 abundant in the brain.<sup>21,22</sup> It has also been found on proteins clearly implicated in the etiology of several diseases including Alzheimer's disease (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). 25 These NFTs are aggregates of paired helical filaments (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 30 brain. In AD patients, all six isoforms of tau are found in NFTs, and all are markedly hyperphosphorylated.<sup>23,24</sup> 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.<sup>25,26</sup> 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.<sup>27,28</sup> 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;<sup>29</sup> and b) identifying strategies that could limit tau hyperphosphorylation in the hope that these 5 might halt, or even reverse, the progression of Alzheimer's disease<sup>30-33</sup> Thus far, several lines of evidence suggest that up-regulation of a number of kinases may be involved in hyperphosphorylation of tau,<sup>21,34,35</sup> although very recently, an alternative basis for this hyperphosphorylation has been advanced.<sup>21</sup>

[0005] In particular, it has recently emerged that phosphate levels of tau are regulated by the 10 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.<sup>36-38</sup> Consistent with this observation, it has been found that increases in phosphorylation levels result in decreased O- 15 GlcNAc levels and conversely, increased O-GlcNAc levels correlate with decreased phosphorylation levels.<sup>39</sup> This reciprocal relationship between O-GlcNAc and phosphorylation has been termed the "Yin-Yang hypothesis"<sup>40</sup> and has gained strong biochemical support by the recent discovery that the enzyme OGT<sup>4</sup> forms a functional complex with phosphatases that act to remove phosphate groups from proteins.<sup>41</sup> Like phosphorylation, O-GlcNAc is a dynamic 20 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.<sup>7,42</sup> Hyperphosphorylated tau in human AD brains has markedly lower levels of O- 25 GlcNAc than are found in healthy human brains.<sup>21</sup> 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.<sup>21</sup> Furthermore, PHF from diseased brain was suggested to lack completely any O-GlcNAc modification whatsoever.<sup>21</sup> 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 30 N-acetylglucosaminidase inhibitor was used to increase tau O-GlcNAc levels, whereupon it was observed that phosphorylation levels decreased.<sup>21</sup> 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  $\beta$ -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.<sup>43</sup> Within the brains of AD patients marked decreases in glucose utilization occur and are thought to be a potential cause of neurodegeneration.<sup>44</sup> The basis for this decreased glucose supply in AD brain<sup>45-47</sup> is thought to stem from any of decreased glucose transport,<sup>48,49</sup> impaired insulin signaling,<sup>50,51</sup> and decreased blood flow.<sup>52</sup>

[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).<sup>53</sup> UDP-GlcNAc is a substrate of the nucleocytoplasmic enzyme O-GlcNAc transferase (OGT),<sup>2-5</sup> which acts to post-translationally add GlcNAc to specific serine and threonine residues of numerous nucleocytoplasmic proteins. OGT recognizes many of its substrates<sup>54,55</sup> and binding partners<sup>41,56</sup> through its tetratricopeptide repeat (TPR) domains.<sup>57,58</sup> As described above, O-GlcNAcase<sup>6,7</sup> 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.<sup>8</sup> O-GlcNAc has been found in several proteins on known phosphorylation sites,<sup>10,37,38,59</sup> including tau and neurofilaments.<sup>60</sup> Additionally, OGT shows unusual kinetic behaviour making it exquisitely sensitive to intracellular UDP-GlcNAc substrate concentrations and therefore glucose supply.<sup>41</sup>

[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.<sup>44</sup> 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 intervention<sup>61</sup> comes from recent studies showing that when transgenic mice harbouring human tau are treated with kinase inhibitors, they do not develop typical motor defects<sup>33</sup> and, in another case,<sup>32</sup> show decreased levels of insoluble tau. These studies provide a clear link between lowering tau 5 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.<sup>62</sup>

[0010] Recent studies<sup>63</sup> support the therapeutic potential of small-molecule O-GlcNAcase inhibitors to limit tau hyperphosphorylation for treatment of AD and related tauopathies. 10 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.<sup>63</sup> 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.<sup>63</sup>

[0011] There is also a large body of evidence indicating that increased levels of O-GlcNAc 15 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,<sup>64-70</sup> trauma hemorrhage,<sup>71-73</sup> hypervolemic shock,<sup>74</sup> and calcium 20 paradox.<sup>64,75</sup> Moreover, strong evidence indicates that these cardioprotective effects are mediated by elevated levels of protein O-GlcNAc modification.<sup>64,65,67,70,72,75-78</sup> 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.<sup>79</sup>

[0012] Humans have three genes encoding enzymes that cleave terminal  $\beta$ -N-acetyl- 25 glucosamine residues from glycoconjugates. The first of these encodes the enzyme O-glycoprotein 2-acetamido-2-deoxy- $\beta$ -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: 30 <http://afmb.cnrs-mrs.fr/CAZY/>.<sup>27,28</sup> O-GlcNAcase acts to hydrolyse O-GlcNAc off of serine and threonine residues of post-translationally modified proteins.<sup>1,6,7,80,81</sup> 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,<sup>14,82</sup> AD,<sup>16,21,83</sup> and cancer.<sup>22,84</sup>

Although O-GlcNAcase was likely isolated earlier on,<sup>18,19</sup> about 20 years elapsed before its biochemical role in acting to cleave O-GlcNAc from serine and threonine residues of proteins was understood.<sup>6</sup> More recently O-GlcNAcase has been cloned,<sup>7</sup> partially characterized,<sup>20</sup> and suggested to have additional activity as a histone acetyltransferase.<sup>20</sup> However, little was known

5 about the catalytic mechanism of this enzyme.

[0013] The other two genes, HEXA and HEXB, encode enzymes catalyzing the hydrolytic cleavage of terminal  $\beta$ -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 10 of an  $\alpha$ - and a  $\beta$ -subunit. Hexosaminidase B ( $\beta\beta$ ), a homodimeric isozyme, is composed of two  $\beta$ -subunits. The two subunits,  $\alpha$ - and  $\beta$ -, 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  $\beta$ -hexosaminidases is critical for human development, a fact that is underscored by the tragic genetic illnesses, Tay- 15 Sach's and Sandhoff diseases which stem from a dysfunction in, respectively, hexosaminidase A and hexosaminidase B.<sup>85</sup> 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.<sup>86</sup>

20 [0014] As a result of the biological importance of these  $\beta$ -N-acetyl-glucosaminidases, small molecule inhibitors of glycosidases<sup>87-90</sup> have received a great deal of attention,<sup>91</sup> 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 25 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 30 in studying the cellular and organismal physiological role of one particular enzyme is complicated because complex phenotypes arise from the concomitant inhibition of such functionally related enzymes. In the case of  $\beta$ -N-acetylglucosaminidases, existing compounds that act to block O-GlcNAcase function are non-specific and act potently to inhibit the lysosomal  $\beta$ -hexosaminidases.

[0016] A few of the better characterized inhibitors of  $\beta$ -N-acetyl-glucosaminidases which have been used in studies of O-GlcNAc post-translational modification within both cells and tissues are streptozotocin (STZ), 2'-methyl- $\alpha$ -D-glucopyrano-[2,1-*d*]- $\Delta$ 2'-thiazoline (NAG-thiazoline) and *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamate (PUGNAC).<sup>14,92-95</sup>

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[0017] STZ has long been used as a diabetogenic compound because it has a particularly detrimental effect on  $\beta$ -islet cells.<sup>96</sup> STZ exerts its cytotoxic effects through both the alkylation of cellular DNA<sup>96,97</sup> as well as the generation of radical species including nitric oxide.<sup>98</sup> The resulting DNA strand breakage promotes the activation of poly(ADP-ribose) polymerase (PARP)<sup>99</sup> with the net effect of depleting cellular NAD<sup>+</sup> levels and, ultimately, leading to cell death.<sup>100,101</sup> Other investigators have proposed instead that STZ toxicity is a consequence of the irreversible inhibition of O-GlcNAcase, which is highly expressed within  $\beta$ -islet cells.<sup>92,102</sup> This hypothesis has, however, been brought into question by two independent research groups.<sup>103,104</sup> Because cellular O-GlcNAc levels on proteins increase in response to many forms of cellular stress<sup>105</sup> it seems possible that STZ results in increased O-GlcNAc-modification levels on proteins by inducing cellular stress rather than 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-GlcNAcase<sup>106</sup> and although it has been proposed by others that STZ acts to irreversibly inhibit O-GlcNAcase,<sup>107</sup> there has been no clear demonstration of this mode of action. Recently, it has been shown that STZ does not irreversibly inhibit O-GlcNAcase.<sup>108</sup>

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[0018] NAG-thiazoline has been found to be a potent inhibitor of family 20 hexosaminidases,<sup>90,109</sup> and more recently, the family 84 O-GlcNAcases.<sup>108</sup> Despite its potency, a downside to using NAG-thiazoline in a complex biological context is that it lacks selectivity and therefore perturbs multiple cellular processes.

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[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<sup>6,110</sup> and the family 20 human  $\beta$ -hexosaminidases.<sup>111</sup> This molecule, developed by Vasella and coworkers, was found to be a potent competitive inhibitor of the  $\beta$ -N-acetyl-glucosaminidases from *Canavalia ensiformis*, *Mucor rouxii*, and the  $\beta$ -hexosaminidase from bovine kidney.<sup>88</sup> It has been demonstrated that administration of PUGNAC in a rat model of trauma hemorrhage decreases circulating levels of the pro-inflammatory cytokines TNF- $\alpha$  and IL-6.<sup>112</sup> It has also been shown that administration of PUGNAC in a cell-based model of lymphocyte activation decreases production of the cytokine

IL-2.<sup>113</sup> Recent studies have indicated that PUGNAc can be used in an animal model to reduce myocardial infarct size after left coronary artery occlusions.<sup>114</sup> 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.<sup>112,115</sup> In addition, 5 elevation of O-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.<sup>116</sup>

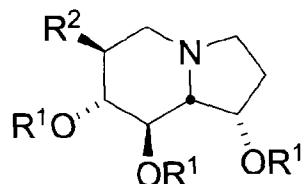
[0020] More recently, it has been suggested that the selective O-GlcNAcase inhibitor NButGT exhibits protective activity in cell-based models of ischemia/reperfusion and cellular stresses, 10 including oxidative stress.<sup>117</sup> This study suggests the use of O-GlcNAcase inhibitors to elevate protein 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, filed 31 August 2007, published under No. WO 2008/025170 on 6 March 2008, which are hereby incorporated 15 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 20 compositions including the compounds or prodrugs of the compounds, and methods of 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:



(I)

where each R<sup>1</sup> may be independently a non-interfering substituent; and R<sup>2</sup> may be NR<sup>3</sup><sub>2</sub>, where each R<sup>3</sup> may be optionally independently a non-interfering substituent,

with the proviso that when each  $R^1$  is H,  $R^2$  excludes NH(COCH<sub>3</sub>), NH(CO(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>), NH(benzoyl), NH<sub>2</sub>, NH(CH<sub>3</sub>), NH(CH<sub>2</sub>CH=CH<sub>2</sub>), NH((CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub>), NH(CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), NH(CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>3</sub>)), NH(CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)), NH(benzyl), NH(CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>, N(CH<sub>2</sub>CH<sub>3</sub>)((CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub>), NH(CH<sub>2</sub>)<sub>7</sub>CH<sub>3</sub>, N(COCH<sub>3</sub>)(benzyl), N<sub>3</sub>, and N(CH<sub>2</sub>CH<sub>2</sub>OH)<sub>2</sub>; or when each  $R^1$  is C(O)CH<sub>3</sub>,  $R^2$  excludes NH(COCH<sub>3</sub>), NH<sub>2</sub>, N<sub>3</sub>, and N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>.

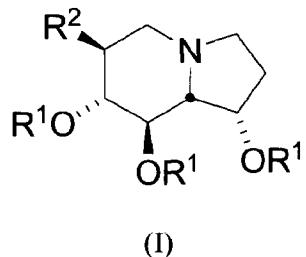
[0024] In alternative embodiments, each  $R^1$  may be connected to another  $R^1$  to form an additional ring structure.

[0025] In alternative embodiments, the non-interfering substituent may be alkyl, alkenyl, 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 selectively inhibit an O-glycoprotein 2-acetamido-2-deoxy- $\beta$ -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- $\beta$ -D-glucopyranoside (O-GlcNAc); the compound may not substantially inhibit a mammalian  $\beta$ -hexosaminidase.

[0027] In alternative aspects, the invention provides a pharmaceutical composition 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 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  $R^1$  may be independently a non-interfering substituent; and  $R^2$  may be NR<sup>3</sup><sub>2</sub>, where each  $R^3$  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 British dementia, Familial Danish dementia,

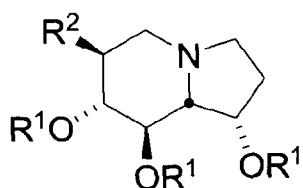
5 Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), Gerstmann-Straussler-Scheinker disease, Guadeloupean parkinsonism, Hallevorden-Spatz disease (neurodegeneration with brain iron accumulation type 1), Multiple system atrophy, Myotonic dystrophy, Niemann-Pick disease (type C), Pallido-ponto-nigral degeneration, Parkinsonism-dementia complex of Guam, Pick's disease (PiD), Post-encephalitic parkinsonism (PEP), Prion

10 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., ischemia; hemorrhage; hypovolemic shock; myocardial infarction; an interventional

15 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, in a subject in need thereof, by administering to the subject an effective amount of a compound of

20 Formula (I) or a pharmaceutically acceptable salt thereof:



(I)

wherein each R<sup>1</sup> may be independently a non-interfering substituent; R<sup>2</sup> may be NR<sup>3</sup><sub>2</sub>, where each R<sup>3</sup> may be optionally independently a non-interfering substituent. In some

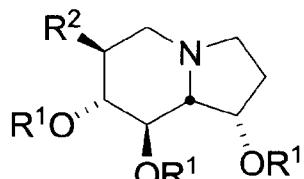
25 embodiments, the condition may be inflammatory or allergic diseases such as asthma, allergic rhinitis, hypersensitivity lung diseases, hypersensitivity pneumonitis, eosinophilic pneumonias, delayed-type hypersensitivity, atherosclerosis, interstitial lung disease (ILD) (e.g., idiopathic pulmonary fibrosis, or ILD associated with rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, systemic sclerosis, Sjogren's syndrome, polymyositis or

30 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; 5 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, lung, liver, kidney, and pancreas transplants (e.g. kidney and 10 lung allografts); epilepsy; pain; stroke, e.g., neuroprotection following a stroke.

[0030] In alternative embodiments, R<sup>1</sup> may be H or C(O)CH<sub>3</sub>. The administering may increase the level of O-GlcNAc in the subject. The subject may be a human.

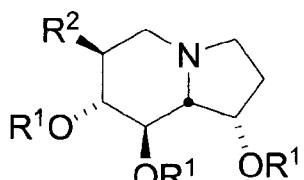
[0031] In alternative aspects, the invention provides use of a compound of an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



(I)

where each R<sup>1</sup> may be independently a non-interfering substituent; R<sup>2</sup> may be NR<sup>3</sup><sub>2</sub>, where each R<sup>3</sup> 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 20 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 a non-interfering substituent;  $R^2$  may be  $NR^3_2$ , where each  $R^3$  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 5 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.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0034] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

10 [0035] **FIGURES 1A-B** show Western blots of proteins from 3T3-L1 cells cultured for 16 hours in the presence or absence of 100  $\mu$ M of Compound 1 (Table 1). Incubation of 3T3-L1 cells with Compound 1 causes an increase in cellular levels of O-GlcNAc-modified proteins; plus signs (“+”) indicate presence of Compound 1, while minus signs (“-”) indicate absence of Compound 1. **Figure 1A:** Western blot analysis of cellular levels of O-GlcNAc-modified proteins using 15 anti-O-GlcNAc MAb CTD 110.6 followed by an anti-mouse IgG-HRP conjugate. **Figure 1B:** Western blot of samples loaded in Figure 1A treated with anti- $\beta$ -actin mAb Clone AC-40 followed by an anti-mouse IgG-HRP conjugate reveals equivalent  $\beta$ -actin levels in each sample.

### DETAILED DESCRIPTION

20 [0036] The invention provides, in part, novel compounds that are capable of inhibiting an O-glycoprotein 2-acetamido-2-deoxy- $\beta$ -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  $\beta$ -hexosaminidase is a mammalian  $\beta$ -hexosaminidase, such as a rat, mouse or human  $\beta$ -hexosaminidase.

25 [0037] 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  $\beta$ -hexosaminidase. In some embodiments, the compounds selectively inhibit the activity of a mammalian O-GlcNAcase over a mammalian  $\beta$ -hexosaminidase. In some embodiments, a selective inhibitor of an O-GlcNAcase does not substantially inhibit a  $\beta$ -hexosaminidase. A 30 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  $\beta$ -hexosaminidase. For example, in some embodiments, a selective

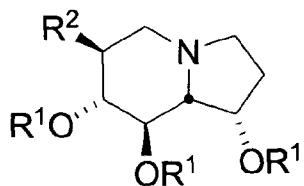
inhibitor of an O-GlcNAcase selectively inhibits the cleavage of 2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (O-GlcNAc) from polypeptides. In some embodiments, a selective inhibitor of an O-GlcNAcase selectively binds to an O-GlcNAcase. In some embodiments, a selective inhibitor of an O-GlcNAcase inhibits hyperphosphorylation of a tau protein and/or inhibits 5 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 10 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 15 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.

[0038] 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.

[0039] 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 25 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 30 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 cellular and organismal level.

[0040] 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 human subjects. In alternative 5 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.

[0041] In specific embodiments, the invention provides compounds described generally by Formula (I) and the salts, prodrugs, and stereoisomeric forms thereof:



10

(I)

[0042] As set forth in Formula (I): each R<sup>1</sup> can be independently a non-interfering substituent; R<sup>2</sup> can be NR<sup>3</sup><sub>2</sub>, where each R<sup>3</sup> may be optionally independently a non-interfering substituent. In some embodiments, each R<sup>1</sup> may be connected to another R<sup>1</sup> to form an additional ring structure.

15 [0043] 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; =NR<sup>4</sup>; OR; C<sub>1-10</sub> alkyl or C<sub>2-10</sub> alkenyl optionally containing one or more P, N, O, S, N, F, Cl, Br, I, or B and optionally substituted with halo; CN; optionally substituted carbonyl; NR<sup>4</sup><sub>2</sub>; C=NR<sup>4</sup>; an optionally substituted carbocyclic or heterocyclic ring; or an optionally substituted aryl or heteroaryl. R<sup>4</sup> 20 may be alkyl, branched alkyl, cycloalkyl, aryl, or heteroaryl.

[0044] In some embodiments, R<sup>1</sup> as set forth in Formula (I), may be either hydrogen or a substituent that includes 1-20 atoms that are other than hydrogen. In some embodiments, R<sup>1</sup> may be H, alkyl, or C(O)R<sup>4</sup>, where R<sup>4</sup> may be alkyl, branched alkyl, cycloalkyl, aryl, or heteroaryl.

25 In some embodiments, R<sup>1</sup> may be H or C(O)CH<sub>3</sub>.

[0045] In some embodiments, R<sup>2</sup> as set forth in Formula (I), may be optionally substituted NR<sup>5</sup><sub>2</sub>, where R<sup>5</sup> may be H, alkyl, branched alkyl, cycloalkyl, aryl, or heteroaryl.

[0046] In specific embodiments of the invention, compounds according to Formula (I) include the compounds described in Table 1.

**Table 1.**

5

Compound	Name	Structure
1	( <i>N</i> -((1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-1,7,8-trihydroxy-octahydroindolizin-6-yl)acetamide	
2	<i>N</i> -((1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-1,7,8-trihydroxy-octahydroindolizin-6-yl)butyramide	
3	<i>N</i> -((1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-1,7,8-trihydroxy-octahydroindolizin-6-yl)benzamide	
4	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-amino-octahydroindolizine-1,7,8-triol	
5	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(methylamino)-octahydroindolizine-1,7,8-triol	
6	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(allylamino)-octahydroindolizine-1,7,8-triol	
7	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(butylamino)-octahydroindolizine-1,7,8-triol	
8	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(isobutylamino)-octahydroindolizine-1,7,8-triol	

9	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(sec-butylamino)-octahydroindolizine-1,7,8-triol	
10	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(pentan-2-ylamino)-octahydroindolizine-1,7,8-triol	
11	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(benzylamino)-octahydroindolizine-1,7,8-triol	
12	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(2-methoxyethylamino)-octahydroindolizine-1,7,8-triol	
13	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(diethylamino)-octahydroindolizine-1,7,8-triol	
14	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(butyl(ethylamino)-octahydroindolizine-1,7,8-triol	
15	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(bis(2-hydroxyethyl)amino)-octahydroindolizine-1,7,8-triol	
16	(1 <i>S</i> ,6 <i>S</i> ,7 <i>R</i> ,8 <i>R</i> ,8 <i>aR</i> )-6-(octylamino)-octahydroindolizine-1,7,8-triol	

17	N-benzyl-N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)acetamide	
18	(1S,6S,7R,8R,8aR)-6-azido-octahydroindolizine-1,7,8-triol	
19	(1S,6S,7R,8R,8aR)-6-acetamido-octahydroindolizine-1,7,8-triyl triacetate	
20	(1S,6S,7R,8R,8aR)-6-amino-octahydroindolizine-1,7,8-triyl triacetate	
21	(1S,6S,7R,8R,8aR)-6-azido-octahydroindolizine-1,7,8-triyl triacetate	
22	(1S,6S,7R,8R,8aR)-6-(diethylamino)-octahydroindolizine-1,7,8-triyl triacetate	

[0047] 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
23	N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)formamide	

24	N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)propionamide	
25	N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)pentanamide	
26	N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)hexanamide	
27	N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)isobutyramide	
28	3-methyl-N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)butanamide	
29	N-methyl-N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)acetamide	
30	N-ethyl-N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)acetamide	
31	N-methyl-N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)propionamide	

32	1-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)urea	
33	1-methyl-3-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)urea	
34	1-ethyl-3-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)urea	
35	1-propyl-3-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)urea	
36	1-butyl-3-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)urea	
37	1-allyl-3-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)urea	
38	1,1-dimethyl-3-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)urea	

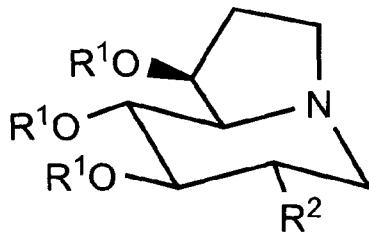
39	1-ethyl-1-methyl-3-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)urea	
40	N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)pyrrolidine-1-carboxamide	
41	1,3-dimethyl-1-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)urea	
42	1,1,3-trimethyl-3-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)urea	
43	(1S,6S,7R,8R,8aR)-6-(ethylamino)-octahydroindolizine-1,7,8-triol	
44	(1S,6S,7R,8R,8aR)-6-(propylamino)-octahydroindolizine-1,7,8-triol	
45	(1S,6S,7R,8R,8aR)-6-(isopropylamino)-octahydroindolizine-1,7,8-triol	
46	(1S,6S,7R,8R,8aR)-6-(dimethylamino)-octahydroindolizine-1,7,8-triol	

47	(1S,6S,7R,8R,8aR)-6-(ethyl(methyl)amino)-octahydroindolizine-1,7,8-triol	
48	(1S,6S,7R,8R,8aR)-6-(methyl(propyl)amino)-octahydroindolizine-1,7,8-triol	
49	(1S,6S,7R,8R,8aR)-6-(butyl(methyl)amino)-octahydroindolizine-1,7,8-triol	
50	(1S,6S,7R,8R,8aR)-6-(allyl(methyl)amino)-octahydroindolizine-1,7,8-triol	
51	(1S,6S,7R,8R,8aR)-6-(pyrrolidin-1-yl)-octahydroindolizine-1,7,8-triol	

[0048] 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).

[0049] In alternative embodiments, when each R<sup>1</sup> is H, R<sup>2</sup> excludes NH(COCH<sub>3</sub>), NH(CO(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>), NH(benzoyl), NH<sub>2</sub>, NH(CH<sub>3</sub>), NH(CH<sub>2</sub>CH=CH<sub>2</sub>), NH((CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub>), NH(CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), NH(CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>3</sub>)), NH(CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)), NH(benzyl), NH(CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>, N(CH<sub>2</sub>CH<sub>3</sub>)((CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub>), NH(CH<sub>2</sub>)<sub>7</sub>CH<sub>3</sub>, N(COCH<sub>3</sub>)(benzyl), N<sub>3</sub>, and N(CH<sub>2</sub>CH<sub>2</sub>OH)<sub>2</sub>; and when each R<sup>1</sup> is C(O)CH<sub>3</sub>, R<sup>2</sup> excludes NH(COCH<sub>3</sub>), NH<sub>2</sub>, N<sub>3</sub>, and N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>.

[0050] As will be appreciated by a person skilled in the art, Formula (I) above may also be represented alternatively as follows:



[0051] 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.

[0052] 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.

[0053] 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 purity.

[0054] In general, a “non-interfering substituent” is a substituent whose presence does not destroy the ability of the compound of Formula (I) 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 enzyme.

[0055] Suitable non-interfering substituents include: H, alkyl (C<sub>1-10</sub>), alkenyl (C<sub>2-10</sub>), alkynyl (C<sub>2-10</sub>), 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 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, CF<sub>3</sub>, CHF<sub>2</sub>, NO<sub>2</sub>, OR, SR, NR<sub>2</sub>, N<sub>3</sub>, COOR, and CONR<sub>2</sub>, 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 above, halo, OOCR, NROCR, where R is H or a substituent set forth above.

5 [0056] “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 substituted by one or more substituents as described herein. Unless stated otherwise specifically herein, it is  
10 understood that the substitution can occur on any carbon of the alkyl group.

[0057] “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 alkenyl group  
15 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.

[0058] “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 example, from  
20 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.

[0059] “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 optionally substituted by one or more substituents as described herein.

25 [0060] “Arylalkyl” refers to a group of the formula -R<sub>a</sub>R<sub>b</sub> where R<sub>a</sub> is an alkyl group as described herein and R<sub>b</sub> is one or more aryl moieties as described herein. The aryl group(s) may be optionally substituted as described herein.

[0061] “Arylalkenyl” refers to a group of the formula -R<sub>c</sub>R<sub>b</sub> where R<sub>c</sub> is an alkenyl moiety as described herein and R<sub>b</sub> is one or more aryl groups as described herein. The aryl group(s) and  
30 the alkenyl group may be optionally substituted as described herein.

[0062] “Acyl” refers to a group of the formula -C(O)R<sub>a</sub>, where R<sub>a</sub> is an alkyl group as described herein. The alkyl group(s) may be optionally substituted as described herein.

[0063] “Arylacyl” refers to a group of the formula -C(O)R<sub>b</sub>, where R<sub>b</sub> is an aryl group as described herein. The aryl group(s) may be optionally substituted as described herein.

[0064] “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 meant to include cycloalkyl groups which are optionally substituted as described herein.

[0065] By a “ring structure” is meant a cycloalkyl, aryl, heteroaryl, or any cyclic structure that may be optionally substituted.

[0066] “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 C<sub>1-6</sub> alkyls or alkenyls.

[0067] “Halo” refers to bromo, chloro, fluoro, iodo, etc. In some embodiments, suitable halogens include fluorine or chlorine.

[0068] 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 C<sub>1-10</sub>alkyl (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, C<sub>1-6</sub> alkyl, alkenyl and cycloalkyl are preferred. The amine group may also be optionally substituted with an aromatic or heterocyclic group, aralkyl (e.g., phenylC<sub>1-4</sub>alkyl) 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.

[0069] An amino group may be substituted with an optionally substituted C<sub>2-4</sub> alkanoyl, e.g., acetyl, propionyl, butyryl, isobutyryl etc., or a C<sub>1-4</sub>alkylsulfonyl (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.

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

[0070] 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, Glaucoma, Schizophrenia, tauopathies, such as Alzheimer's disease, neurodegenerative diseases, 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, Glaucoma, Schizophrenia, neurodegenerative disorders, such as Alzheimer's disease (AD), or 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 includes treatment, prevention, and amelioration.

[0071] 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 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.

[0072] In alternative embodiments, the invention provides methods of selectively inhibiting an O-GlcNAcase enzyme in animal subjects, such as veterinary and human subjects.

[0073] 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.

5 [0074] 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 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 10 modification is implicated, or any condition as described herein. Disease states of interest include Alzheimer's disease (AD) and related neurodegenerative 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 15 providing therapeutic benefit.

[0075] Tauopathies that may be treated with the compounds of the invention include: 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, 20 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 25 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, and Tangle-only dementia.

[0076] The compounds of this invention are also useful in the treatment of conditions associate 30 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.

[0077] 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 5 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 10 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; 15 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.

[0078] 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 20 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.

25 [0079] 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 30 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.

Pharmaceutical & Veterinary Compositions, Dosages, And Administration

5 [0080] 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.

10 [0081] 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.

15 [0082] 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 20 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.;
- 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.;
- beta-secretase inhibitors such as ATG-Z1, CTS-21166, etc.;
- alpha-secretase activators, such as NGX267, etc.;

- amyloid- $\beta$  aggregation and/or fibrillization inhibitors such as Alzhemed<sup>TM</sup> (3APS, Tramiprosate, 3-amino-1-propanesulfonic acid), AL-108, AL-208, AZD-103, PBT2, Cereact, ONO-2506PO, PPI-558, etc.;
- tau aggregation inhibitors such as methylene blue, etc.;
- 5
- microtubule stabilizers such as AL-108, AL-208, paclitaxel, etc.;
- RAGE inhibitors, such as TTP488, etc.;
- 10
- 5-HT1a receptor antagonists, such as Xaliproden, Lecozotan, etc.;
- 5-HT4 receptor antagonists, such as PRX-03410, etc.;
- kinase inhibitors such as SRN-003-556, amfurindamide, LiCl, AZD1080, NP031112, SAR-502250, etc.
- 15
- humanized monoclonal anti-A $\beta$  antibodies such as Bapineuzumab (AAB-001), LY2062430, RN1219, ACU-5A5, etc.;
- amyloid vaccines such as AN-1792, ACC-001
- neuroprotective agents such as Cerebrolysin, AL-108, AL-208, Huperzine A, etc.;
- 20
- 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<sup>®</sup> (Rosglitazone), etc.;
- 25
- 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<sup>®</sup>), TVP-1012, etc.;
- AMPA receptor modulators such as Ampalex (CX 516), etc.;
- 30
- 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.

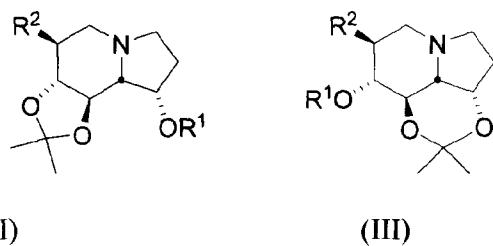
[0083] It is to be understood that combination of compounds according to the invention, or for use according to the invention, with Alzheimer's agents is not limited to the examples described

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).

5 [0084] 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  
10 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  
15 hydrolysis in blood. The prodrug compound often offers advantages of solubility, tissue compatibility or delayed release in a subject.

[0085] 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  
20 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. 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  
25 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.

[0086] Additional examples of prodrugs for the compounds of the invention include acetonide derivatives (also known as isopropylidene derivatives) in which two OR<sup>1</sup> groups in Formula (I) may  
30 be linked in a ring, for example, as in Formulae (II) and (III) shown below. Such acetonide groups may be cleaved *in vivo* to liberate the parent compound of the invention, making these acetonide derivatives prodrugs.



5 [0087] 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. Ch 5, pgs 113 191  
10 (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.

[0088] Suitable prodrug forms of the compounds of the invention include embodiments in which R<sup>1</sup> 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<sup>1</sup> is H. Preferred prodrug embodiments of the invention are the compounds of Formula (I) where R<sup>1</sup> is C(O)CH<sub>3</sub>.

[0089] 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 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.

[0090] “Pharmaceutically acceptable carrier, diluent or excipient” includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor 5 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 humans or domestic animals.

[0091] The compounds of the present invention may be administered in the form of 10 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 term “pharmaceutically acceptable salt” as used herein means an active ingredient comprising compounds of Formula 1 used in the form of a salt thereof, particularly where the salt form confers on the active ingredient 15 improved pharmacokinetic properties as compared to the free form of the active ingredient or other previously disclosed salt form.

[0092] A “pharmaceutically acceptable salt” includes both acid and base addition salts. A “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, 20 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, 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.

[0093] 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 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 30 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, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, 5 polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine.

[0094] 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, 10 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, 15 hydrobromide, tannate, hydrochloride, tartrate, hydroxynaphthoate, teoclate, iodide, tosylate, isothionate, triethiodide, lactate, panoate, valerate, and the like.

[0095] 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 20 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.

25 [0096] 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.

30 [0097] 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 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 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

5 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, 20<sup>th</sup> ed., 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, 10 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 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 15 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.

[0098] 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.

20 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 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.

25 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, 30 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.

[0099] 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. However, the 5 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.

[00100] An “effective amount” of a compound according to the invention includes a 10 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 herein. A therapeutically effective amount of a compound may vary according to factors such as the 15 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 therapeutically beneficial effects. A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of 20 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 25 any integer from 0.1 nM-0.1M, 0.1 nM-0.05M, 0.05 nM-15μM or 0.01 nM-10μM.

[00101] 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 30 500 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 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.

[00102] 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 5 only and do not limit the dosage ranges that may be selected by 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 10 increased as indicated by the exigencies of the 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, 15 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.

20 Other Uses and Assays

[00103] 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 25 skill in the art. For example, a fluorescence or UV-based assay known in the art 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, 30 interfering with inhibition of O-GlcNAcase-dependent cleavage of O-GlcNAc or by interfering with any biological response induced by a compound of Formula (I).

[00104] 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 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 5 general decrease modulation relative to a known compound, while a compound that is a positive modulator will in general increase modulation relative to a known compound.

[00105] 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 10 source of test extracts or compounds is not critical to the 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 15 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. 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), 20 Xenova (Slough, UK), Harbor Branch Oceanographic Institute (Ft. Pierce, FL, USA), and PharmaMar, MA, USA. In addition, natural and synthetically produced 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.

[00106] When a crude extract is found to modulate inhibition of O-GlcNAcase-dependent 25 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 purification process is the careful characterization and identification of a chemical entity within 30 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 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 or known in the art.

[00107] In some embodiments, the compounds described herein (e.g., the compounds of Formula I) or test compounds may be analyzed using established cellular<sup>118-120</sup> and/or transgenic animal models of disease<sup>32,33</sup> and the ability of the compounds to, for example, block the formation of toxic tau species determined. Such analyses may be used for example to determine or confirm the efficacy of the compounds in treating or preventing pathology associated with the accumulation of toxic tau species (for example, Alzheimer's disease and other tauopathies).

[00108] In some embodiments, the compounds described herein (e.g., the compounds of Formula I) or test compounds may be analyzed using established cellular stress assays<sup>105,116,117</sup> and/or animal models of ischemia-reperfusion<sup>70,114</sup> or trauma-hemorrhage.<sup>72,112,115</sup> Such analyses may be used for example to determine or confirm the efficacy of the compounds in treating or preventing pathology associated with cellular stress (including ischemia, hemorrhage, hypovolemic shock, myocardial infarction, and other cardiovascular disorders) or in treating or preventing tissue damage or promoting functional recovery.

[00109] In some embodiments, the compounds are useful in the development of animal 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.

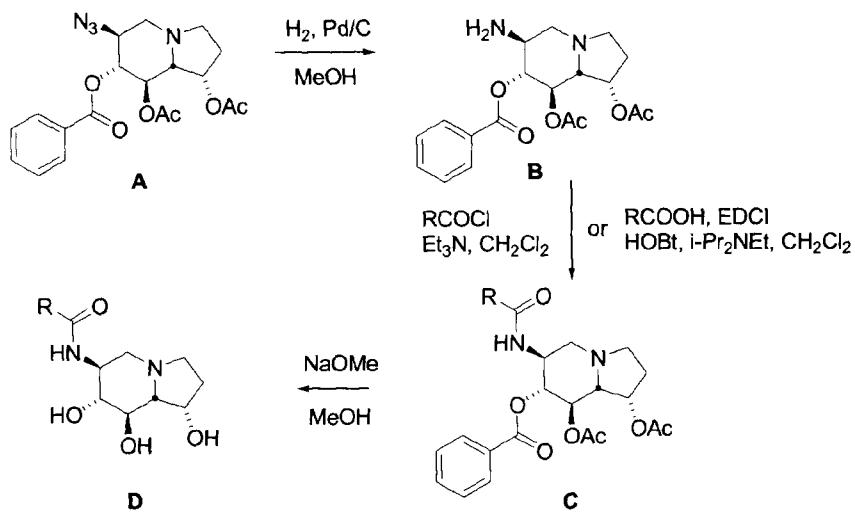
[00110] Various alternative embodiments and examples of the invention are described herein. These embodiments and examples are illustrative and should not be construed as limiting the scope of the invention.

EXAMPLES

[00111] The following examples are intended to illustrate embodiments of the invention and are not intended to be construed in a limiting manner.

Example 1

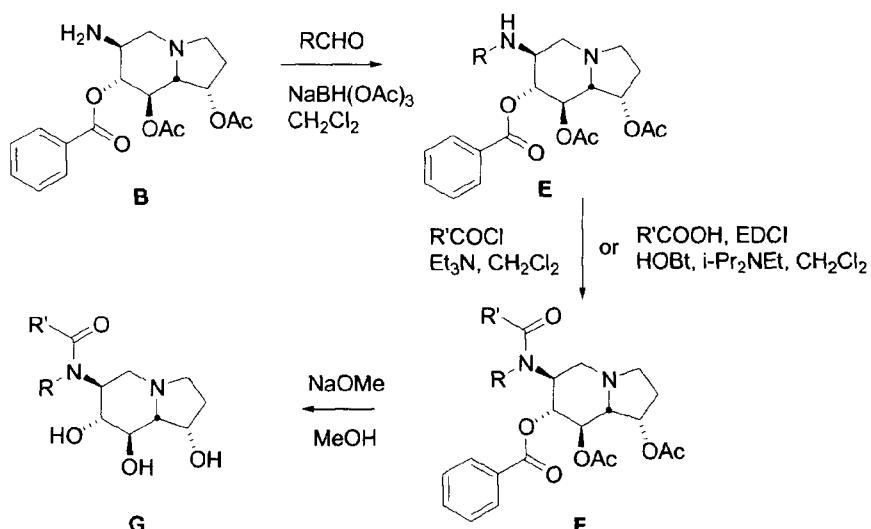
5 [00112] Compounds of the invention having general structure **D** are prepared according to the sequence described in Scheme 1. Thus, hydrogenation of the known intermediate **A**<sup>121,122</sup> in the presence of catalytic Pd/C in MeOH provides the amine **B**. Coupling of this material with the appropriate acid chloride followed by deprotection with sodium methoxide in methanol provides the desired products **D**. Alternatively, conversion of **B** to **C** is also effected by reaction of **B** with 10 the appropriate carboxylic acid in the presence of EDCI, HOBr, and Hunig's base in dichloromethane.



Scheme 1

Example 2

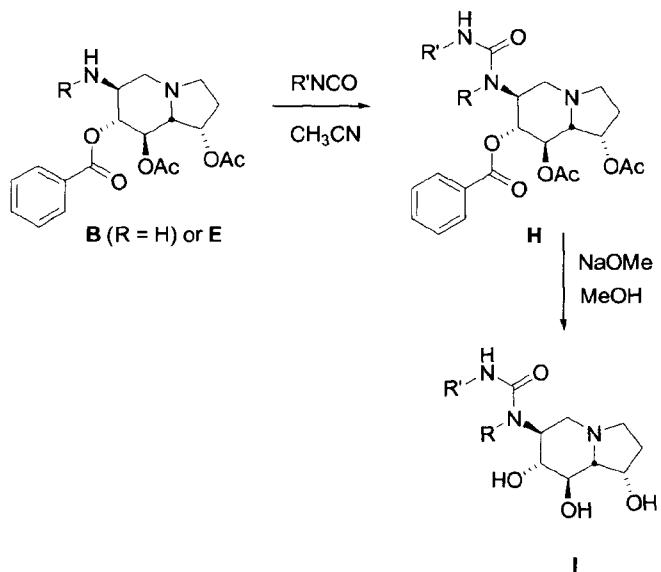
15 [00113] Compounds of the invention having general structure **G** are prepared according to the sequence described in Scheme 2. Thus, reductive amination of intermediate **B** (Scheme 1) with the appropriate aldehyde in the presence of NaBH(OAc)<sub>3</sub> provides the secondary amine **E**. Coupling of this material with the appropriate acid chloride followed by deprotection with sodium methoxide in methanol provides the desired products **G**. Alternatively, conversion of **E** to **F** is also effected by reaction of **B** with the appropriate carboxylic acid in the presence of 20 EDCI, HOBr, and Hunig's base in dichloromethane.



**Scheme 2**

### Example 3

[00114] Compounds of the invention having general structure **I** is prepared according to the sequence described in Scheme 3. Thus, reaction of either intermediate **B** (Scheme 1) or **E** (Scheme 2) with the appropriate isocyanate provides the ureas **H**. Deprotection with sodium methoxide in methanol then furnishes the desired products **I**.

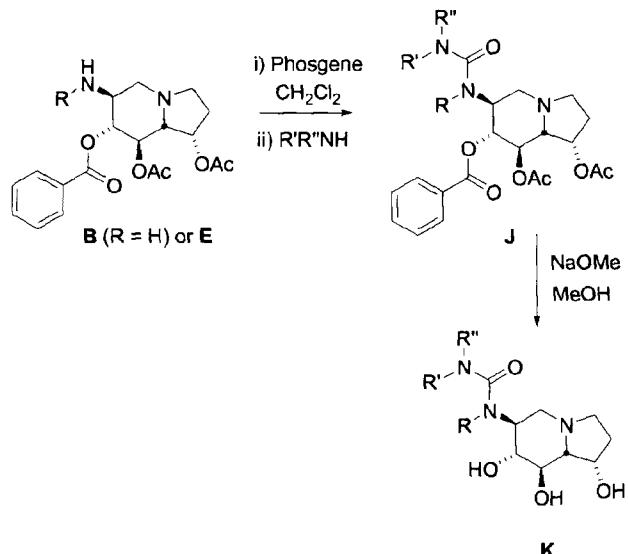


**Scheme 3**

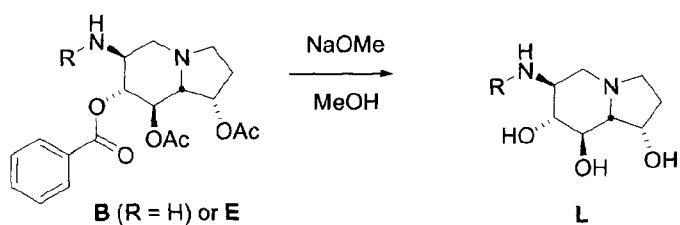
Example 4

[00115] Compounds of the invention having general structure **K** is prepared according to the sequence described in Scheme 4. Thus, reaction of either intermediate **B** (Scheme 1) or **E** (Scheme 2) with phosgene followed by the appropriate secondary amine provides the ureas **J**.

5 Deprotection with sodium methoxide in methanol then furnishes the desired products **K**.

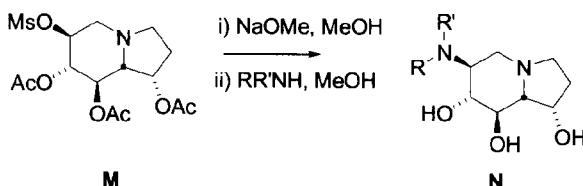
**Scheme 4**Example 5

[00116] Compounds of the invention having general structure **L** are prepared according to the sequence described in Scheme 5. Thus, deprotection of either intermediate **B** (Scheme 1) or **E** (Scheme 2) with sodium methoxide in methanol provides the desired products **L**.

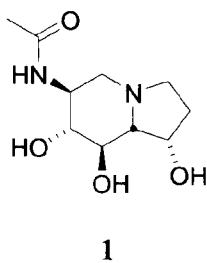
**Scheme 5**

Example 6

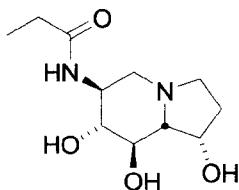
[00117] Compounds of the invention having general structure **N** are prepared according to the sequence described by Furneaux et al.<sup>123</sup> and in Scheme 6. Following this route, deprotection of intermediate **M**<sup>123</sup> with sodium methoxide in methanol followed by nucleophilic addition of the appropriate amine (to the resulting intermediate epoxide) provides the desired products **N**.

**Scheme 6**Example 7**Compound 1: (N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolin-6-yl)acetamide**

10

**1**

[00118] Compound **1** was prepared using literature procedures<sup>123</sup> and exhibited spectral characteristics consistent with those reported.<sup>123</sup>

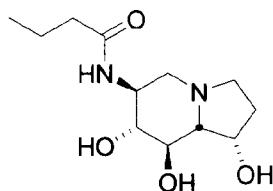
Example 8**15 Compound 24: N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolin-6-yl)propionamide****24**

[00119] Compound **24** was prepared following standard procedures (e.g. Scheme 1). <sup>1</sup>H NMR (500 MHz, methanol-d<sub>4</sub>) δ 1.12 (t, 3H, *J* = 7.6 Hz), 1.70-1.90 (m, 3H), 2.08 (q, 1H, *J* = 8.8 Hz), 2.17-2.25 (m, 3H), 3.14-3.07 (m, 2H), 3.27 (dd, 1H, *J* = 8.8, 10.2 Hz), 3.65 (dd, 1H, *J* = 8.9, 8.9 Hz), 3.92 (dd, 1H, *J* = 4.9, 9.4, 9.4 Hz), 4.28-4.32 (m, 1H); <sup>13</sup>C NMR (125 MHz, methanol-d<sub>4</sub>) δ

10.40, 30.24, 34.48, 52.70, 53.09, 55.84, 71.24, 71.54, 73.73, 78.25, 177.36; MS (HRMS): m/z 244.1408 ( $M + H$ )<sup>+</sup>; Calcd. for  $C_{11}H_{20}N_2O_4$ : 244.1423. Anal. Calcd for  $C_{11}H_{20}N_2O_4$ : C, 54.08; H, 8.25; N, 11.47; Found: C, 54.02; H, 8.28; N, 11.41.

Example 9

5 **Compound 2: N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)butyramide**



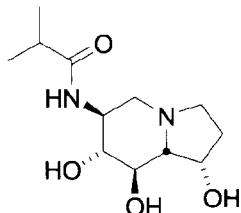
2

[00120] Compound 2 was prepared using literature procedures<sup>123</sup> and exhibited spectral characteristics consistent with those reported.<sup>123</sup>

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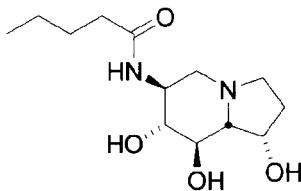
Example 10

**Compound 27: N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)isobutyramide**



27

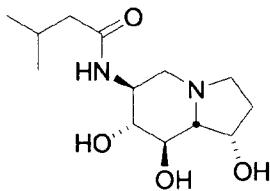
[00121] Compound 27 was prepared following standard procedures (e.g. Scheme 1). <sup>1</sup>H NMR (500 MHz, methanol-d<sub>4</sub>)  $\delta$  1.09 (d, 3H,  $J$  = 6.9 Hz), 1.12 (d, 3H,  $J$  = 6.9 Hz), 1.70-1.87 (m, 3H), 2.09 (q, 1H,  $J$  = 8.8 Hz), 2.19-2.25 (m, 1H), 2.45 (m,  $J$  = 6.9 Hz), 3.07-3.14 (m, 2H), 3.28 (dd, 1H,  $J$  = 8.8, 10.6 Hz), 3.65 (dd, 1H,  $J$  = 8.9, 8.9 Hz), 3.90 (ddd, 1H,  $J$  = 4.8, 10.6, 10.6 Hz), 4.27-4.31 (m, 1H); <sup>13</sup>C NMR (125 MHz, methanol-d<sub>4</sub>)  $\delta$  19.77, 20.07, 34.48, 36.35, 52.59, 53.10, 55.84, 71.25, 71.61, 73.75, 78.20, 180.56; MS (HRMS): m/z 258.1559 ( $M + H$ )<sup>+</sup>; Calcd. for  $C_{12}H_{22}N_2O_4$ : 258.1580. Anal. Calcd for  $C_{12}H_{22}N_2O_4$ : C, 55.80; H, 8.58; N, 10.84; Found: C, 55.92; H, 8.63; N, 10.79.

Example 11Compound 25: N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)pentanamide**25**

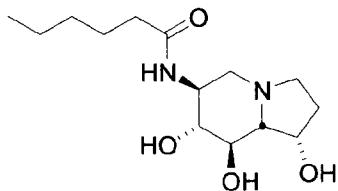
5 [00122] Compound **25** was prepared following standard procedures (e.g. Scheme 1).  $^1\text{H}$  NMR (500 MHz, methanol-d<sub>4</sub>)  $\delta$  0.93 (t, 3H,  $J$  = 7.3 Hz), 1.31-1.39 (m, 2H), 1.55-1.62 (m, 2H), 1.70-1.87 (m, 3H, H<sub>2</sub>, H<sub>5</sub> $\square$ , H<sub>8a</sub>), 2.08 (q,  $J$  = 8.7 Hz), 2.17-2.25 (m, 3H), 3.07-3.14 (m, 2H), 3.26 (dd, 1H,  $J$  = 8.8, 10.7 Hz), 3.65 (dd, 1H,  $J$  = 8.9, 8.9 Hz), 3.92 (dd, 1H,  $J$  = 4.9, 10.7, 10.7 Hz, H<sub>6</sub>), 4.28-4.32 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz, methanol-d<sub>4</sub>)  $\delta$  14.14, 23.36, 29.16, 34.48, 36.98, 52.70, 53.09, 55.86, 71.24, 71.57, 73.73, 78.23, 176.64; MS (HRMS): m/z 272.1759 (M +H)<sup>+</sup>; Calcd. for C<sub>13</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>: 272.1736. Anal. Calcd for C<sub>13</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>: C, 57.33; H, 8.88; N, 10.29; Found: C, 57.29; H, 8.83; N, 10.34.

Example 12Compound 28: 3-methyl-N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)butanamide

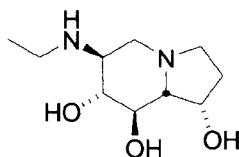
15

**28**

15 [00123] Compound **28** was prepared following standard procedures (e.g. Scheme 1).  $^1\text{H}$  NMR (500 MHz, methanol-d<sub>4</sub>)  $\delta$  0.93 (d, 3H,  $J$  = 6.2 Hz), 0.97 (d, 3H,  $J$  = 6.2 Hz), 1.70-1.87 (m, 3H), 2.03-2.11, 2.18-2.24 (2m, 5H), 3.07-3.14 (m, 2H), 3.26 (dd, 1H), 3.64 (dd, 1H,  $J$  = 9.3, 9.3 Hz), 3.93 (dd, 1H,  $J$  = 4.7, 10.5, 10.5 Hz), 4.26-4.30 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz, methanol-d<sub>4</sub>)  $\delta$  22.62, 22.81, 27.42, 34.49, 46.49, 52.69, 53.09, 55.91, 71.26, 71.62, 73.74, 78.23, 175.91; MS (HRMS): m/z 272.1748 (M +H)<sup>+</sup>; Calcd. for C<sub>13</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>: 272.1736. Anal. Calcd for C<sub>13</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>: C, 57.33; H, 8.88; N, 10.29; Found: C, 57.29; H, 8.78; N, 10.21.

Example 13Compound 26: N-((1S,6S,7R,8R,8aR)-1,7,8-trihydroxy-octahydroindolizin-6-yl)hexanamide**26**

5 [00124] Compound **26** was prepared following standard procedures (e.g. Scheme 1).  $^1\text{H}$  NMR (500 MHz, methanol-d<sub>4</sub>)  $\delta$  0.92 (t, 3H,  $J$  = 6.9 Hz), 1.27-1.38 (m, 4H), 1.57-1.64 (m, 2H), 1.70-1.89 (m, 3H), 2.09 (q, 1H,  $J$  = 8.9 Hz), 2.17-2.25 (m, 3H), 3.07-3.15 (m, 2H), 3.27 (dd, 1H), 3.65 (dd, 1H,  $J$  = 9.1, 9.1 Hz), 3.93 (dd, 1H,  $J$  = 4.8, 10.6 Hz), 4.28-4.32 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz, methanol-d<sub>4</sub>)  $\delta$  14.28, 23.45, 26.69, 32.52, 34.46, 37.20, 52.67, 53.09, 55.82, 71.21, 71.54, 10 73.72, 78.19, 176.64; MS (HRMS): m/z 286.1888 (M +H)<sup>+</sup>; Calcd. for C<sub>14</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>: 286.1893. Anal. Calcd for C<sub>14</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>: C, 58.72; H, 9.15; N, 9.78; Found: C, 58.88; H, 9.08; N, 9.75.

Example 14Compound 43: (1S,6S,7R,8R,8aR)-6-(ethylamino)-octahydroindolizine-1,7,8-triol

15

**43**

[00125] Compound **43** was prepared following standard procedures (e.g. Schemes 1, 2, 5).  $^1\text{H}$  NMR (500 MHz, methanol-d<sub>4</sub>)  $\delta$  0.92 (t, 3H,  $J$  = 6.9 Hz), 1.27-1.38 (m, 4H), 1.57-1.64 (m, 2H), 1.70-1.89 (m, 3H), 2.09 (q, 1H,  $J$  = 8.9 Hz), 2.17-2.25 (m, 3H), 3.07-3.15 (m, 2H), 3.27 (dd, 1H), 3.65 (dd, 1H,  $J$  = 9.1, 9.1 Hz), 3.93 (dd, 1H,  $J$  = 4.8, 10.6 Hz), 4.28-4.32 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz, methanol-d<sub>4</sub>)  $\delta$  14.28, 23.45, 26.69, 32.52, 34.46, 37.20, 52.67, 53.09, 55.82, 71.21, 71.54, 20 73.72, 78.19, 176.64; MS (HRMS): m/z 286.1888 (M +H)<sup>+</sup>; Calcd. for C<sub>14</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>: 286.1893. Anal. Calcd for C<sub>14</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>: C, 58.72; H, 9.15; N, 9.78; Found: C, 58.88; H, 9.08; N, 9.75.

Example 15Assay for determination of  $K_I$  values for inhibition of O-GlcNAcase activity

[00126] Experimental procedure for kinetic analyses: Enzymatic reactions were carried out in PBS buffer (pH 7.4) using pNP-GlcNAc as a substrate (0.5 mM) and monitored continuously at 5  $37^{\circ}\text{C}$  at 400 nm using a Cary 3E UV-VIS spectrophotometer equipped with a Peltier temperature controller. Reactions were pre-heated in a 500  $\mu\text{L}$  quartz cuvette for approximately 5 minutes followed by addition of 10  $\mu\text{L}$  enzyme via syringe (final enzyme concentration 0.002 mg/mL). Reaction velocities were determined by linear regression of the linear region of the reaction progress curve between the first and third minutes. An inhibitor concentration range of 10 1/5 to 5 times  $K_I$  was used in each case.

When tested in this assay, compound 1 exhibited a  $K_I$  value for inhibition of O-GlcNAcase of 0.67  $\mu\text{M}$ .

When tested in this assay, many of the compounds described herein exhibit  $K_I$  values for inhibition of O-GlcNAcase in the range 1 nM - 50  $\mu\text{M}$ . All  $K_I$  values are determined using linear 15 regression of Dixon plots.

Example 16Assay for determination of  $K_I$  values for inhibition of  $\beta$ -hexosaminidase activity

[00127] Experimental procedure for kinetic analyses: All enzymatic assays are carried out in triplicate at  $37^{\circ}\text{C}$  using a stopped assay procedure by measuring the amount of 4-nitrophenolate 20 liberated as determined by absorption measurements at 400 nm. Reactions (50  $\mu\text{L}$ ) are initiated by the addition, *via* syringe, of enzyme (3  $\mu\text{L}$ ). Time-dependent assay of  $\beta$ -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.  $\beta$ -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 25 concentrations ranging from 5 times to 1/5  $K_I$ .  $K_I$  values are determined by linear regression of data from Dixon plots.

[00128] When tested in this assay, many of the compounds described herein exhibit  $K_I$  values for inhibition of  $\beta$ -hexosaminidase in the range 1  $\mu\text{M}$  - 10 mM.

[00129] The selectivity ratio for inhibition of O-GlcNAcase over  $\beta$ -hexosaminidase is defined 30 here as:

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

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  $\beta$ -hexosaminidase.

Example 17

5 [00130] Western Blot Analyses: 3T3-L1 adipocytes were treated with Compound 1 at 100  $\mu$ M for 16 hours. Cells were harvested by adding 400  $\mu$ L of 1% SDS with 50 mM beta-ME, boiling this for 10 minutes, using these lysates in subsequent Western blots. Briefly, samples were separated by SDS-PAGE (10% gels), transferred to nitrocellulose membrane (Bio-Rad), blocked for one hour at room temperature (RT) with 1% bovine serum albumin (BSA) in PBS containing 10 0.1% Tween-20 (PBS-T) and then subsequently probed with the appropriate primary antibody delivered in 1% BSA in PBS-T for overnight at 4 °C. Membranes were then extensively washed with PBS-T, blocked again for 30 minutes with 1% BSA in PBS-T at RT and then probed with the appropriate HRP conjugated secondary antibody for one at RT delivered in 1% BSA in PBS-T. Finally, the membranes were washed extensively and then developed with SuperSignal West 15 Pico Chemiluminescence substrate.

[00131] Results from Cell Studies: Cellular levels of O-GlcNAc-modified proteins within cells cultured for 16 hours in the presence of Compound 1, or in its absence was carried out using the O-GlcNAc directed monoclonal antibody mAbCTD110.6. Marked increases in cellular levels of O-GlcNAc-modified proteins within the cells were observed as compared to the control 20 (Figure 1) indicating that this compound readily gains access to the interior of the cell where it acts to block O-GlcNAcase function.

[00132] The present invention has been described with regard to one or more embodiments. 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 25 claims.

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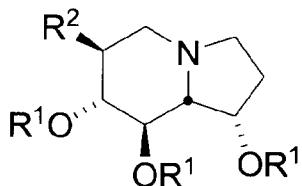
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30

All citations are hereby incorporated by reference.

## WHAT IS CLAIMED IS:

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



5

(I)

wherein

each R<sup>1</sup> is independently a non-interfering substituent; andR<sup>2</sup> is NR<sup>3</sup><sub>2</sub>, wherein each R<sup>3</sup> is optionally independently a non-interfering substituent,with the proviso that when each R<sup>1</sup> is H, R<sup>2</sup> excludes NH(COCH<sub>3</sub>), NH(CO(CH<sub>2</sub>)<sub>2</sub>CH<sub>3</sub>),

10 NH(benzoyl), NH<sub>2</sub>, NH(CH<sub>3</sub>), NH(CH<sub>2</sub>CH=CH<sub>2</sub>), NH((CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub>), NH(CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>),  
 NH(CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>3</sub>)), NH(CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)), NH(benzyl), NH(CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>),  
 N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>, N(CH<sub>2</sub>CH<sub>3</sub>)((CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub>), NH(CH<sub>2</sub>)<sub>7</sub>CH<sub>3</sub>, N(COCH<sub>3</sub>)(benzyl), N<sub>3</sub>, and  
 N(CH<sub>2</sub>CH<sub>2</sub>OH)<sub>2</sub>; or when each R<sup>1</sup> is C(O)CH<sub>3</sub>, R<sup>2</sup> excludes NH(COCH<sub>3</sub>), NH<sub>2</sub>, N<sub>3</sub>, and  
 N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>;

15 2. The compound of claim 1 wherein each R<sup>1</sup> may be connected to another R<sup>1</sup> to form an additional ring structure.

3. The compound of claim 1 or 2 wherein R<sup>1</sup> is H or C(O)CH<sub>3</sub>.

4. The compound of any one of claims 1 to 3 wherein said non-interfering substituent is selected from one or more of the group consisting of alkyl, branched alkyl, alkenyl, branched alkenyl,  
 20 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.

5. The compound of any one of claims 1 to 4 wherein said non-interfering substituent comprises one or more heteroatoms selected from P, O, S, N, F, Cl, Br, I, or B.

25 6. The compound of any one of claims 1 to 5 wherein said non-interfering substituent is optionally substituted.

7. The compound of any one of claims 1 to 6 with the proviso that the compound excludes one or more of the compounds described in Table 1.

8. The compound of claim 1 wherein the compound is a prodrug.

9. The compound of any one of claims 1 to 8 wherein the compound selectively inhibits an O-glycoprotein 2-acetamido-2-deoxy- $\beta$ -D-glucopyranosidase (O-GlcNAcase).

10. The compound of any one of claims 1 to 9 wherein the compound selectively binds an O-GlcNAcase.

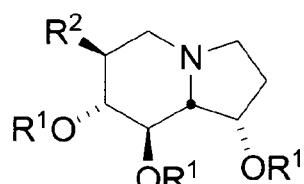
11. The compound of any one of claims 1 to 10 wherein the compound selectively inhibits the cleavage of 2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (O-GlcNAc).

10 12. The compound of claim 9 wherein the O-GlcNAcase is a mammalian O-GlcNAcase.

13. The compound of any one of claims 1 to 12 wherein the compound does not substantially inhibit a mammalian  $\beta$ -hexosaminidase.

14. A pharmaceutical composition comprising the compound of any one of claims 1 to 13 in combination with a pharmaceutically acceptable carrier.

15 15. 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:



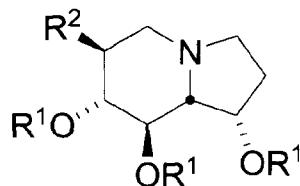
(I)

20 wherein

each R<sup>1</sup> is independently a non-interfering substituent; and

R<sup>2</sup> is NR<sup>3</sup><sub>2</sub>, wherein each R<sup>3</sup> is optionally independently a non-interfering substituent.

16. A method of elevating the level of O-GlcNAc 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:



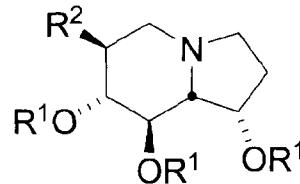
5 (I)

wherein

each R<sup>1</sup> is independently a non-interfering substituent; and

R<sup>2</sup> is NR<sup>3</sup><sub>2</sub>, wherein each R<sup>3</sup> is optionally independently a non-interfering substituent.

10 17. 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:



(I)

15 wherein

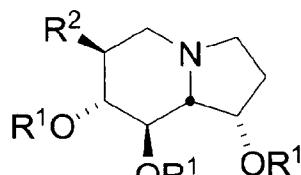
each R<sup>1</sup> is independently a non-interfering substituent; and

R<sup>2</sup> is NR<sup>3</sup><sub>2</sub>, wherein each R<sup>3</sup> is optionally independently a non-interfering substituent.

20 18. The method of claim 17 wherein the condition is selected from one or more of the 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 dermatomyositis, systemic anaphylaxis or

hypersensitivity response, drug allergy, insect sting allergy, autoimmune disease, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, systemic lupus erythematosus, myasthenia gravis, glomerulonephritis, autoimmune thyroiditis, graft rejection, allograft rejection, graft-versus-host disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, spondyloarthropathy, 5 scleroderma, psoriasis, T-cell mediated psoriasis, inflammatory dermatosis, dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria, vasculitis, necrotizing, cutaneous, and hypersensitivity vasculitis, eosinophilic myositis, 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, 10 stroke, neuroprotection.

19. 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:



15 (I)

wherein

each R<sup>1</sup> is independently a non-interfering substituent; and

R<sup>2</sup> is NR<sup>3</sup><sub>2</sub>, wherein each R<sup>3</sup> is optionally independently a non-interfering substituent.

20

20. The method of claim 19 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, 25 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.

21. The method of claim 19 wherein the stress is a cardiac disorder.

22. The method of claim 21 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.

10 23. The method of any one of claims 15 to 22 wherein R<sup>1</sup> is H or C(O)CH<sub>3</sub>.

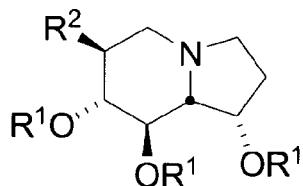
24. The method of any one of claims 15 to 23 wherein R<sup>2</sup> is NH(COCH<sub>3</sub>)

25. The method of any one of claims 15 to 22 wherein the compound is selected from the group consisting of one or more of the compounds described in Table 1.

15 26. The method of any one of claims 15 to 25 wherein said administering increases the level of O-GlcNAc in the subject.

27. The method of any one of claims 15 to 26 wherein the subject is a human.

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



(I)

wherein

each R<sup>1</sup> is independently a non-interfering substituent; and

R<sup>2</sup> is NR<sup>3</sup><sub>2</sub>, wherein each R<sup>3</sup> is optionally independently a non-interfering substituent, in the preparation of a medicament.

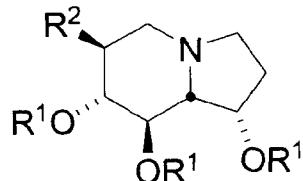
25

29. The use of claim 28 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.

30. A method for screening for a selective inhibitor of an O-GlcNAcase, the method

5 comprising:

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



(I)

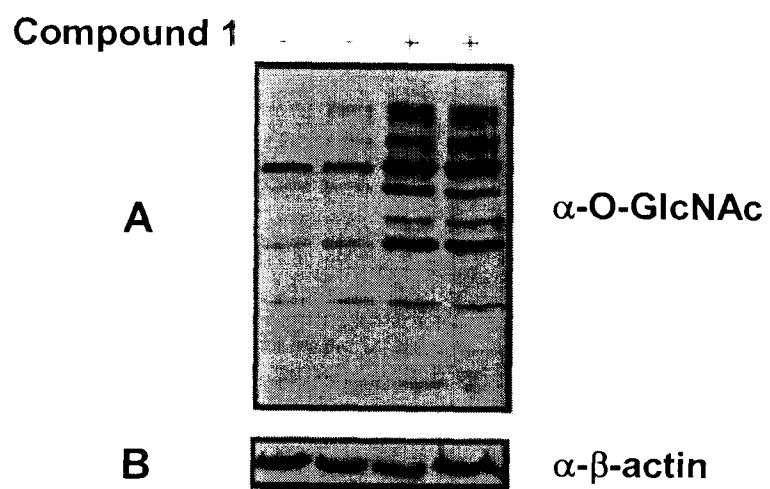
10 wherein

each  $R^1$  is independently a non-interfering substituent; and

$R^2$  is  $NR^3_2$ , wherein each  $R^3$  is optionally independently a non-interfering substituent;

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

15 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).



**Figure 1**

**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/CA2009/001087

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC: **C07D 471/04** (2006.01), **A61K 31/437** (2006.01), **G01N 33/53** (2006.01)  
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC: C07D 471/04 (2006.01), A61K 31/437 (2006.01), G01N 33/53 (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)  
Canadian Patent Database, Scopus, Google Patent, SureChem, STN, google for non-patent prior disclosures (search terms: indoliz\*, glycosid\*)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Furneaux et. al.; The Chemistry of Castanospermine, Part I: Synthetic Modifications at C-6; <u>Tetrahedron</u> (1994), 50(7), 2131-60. Compounds 86, 87, 90-93, p. 2138.	1-29
Y	Entire Document	30
X	Liu et. al.; A Potent Inhibitor of b-N-Acetylglucosaminidases: 6-Acetamido-6-deoxycastanospermine; <u>Tetrahedron Letters</u> (1991), 32(6), 719-720. Compound 10, p. 720.	1-29
X	WO1991/18598 (LIU et. al.) 12 December 1991 (12.12.1991) Example 8	1-29

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :	
“A” document defining the general state of the art which is not considered to be of particular relevance	“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
“E” earlier application or patent but published on or after the international filing date	“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
“O” document referring to an oral disclosure, use, exhibition or other means	“&” document member of the same patent family
“P” document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

5 September 2009 (05-09-2009)

Date of mailing of the international search report

22 October 2009 (22-10-2009)

Name and mailing address of the ISA/CA  
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Authorized officer

Daniel Black

**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/CA2009/001087

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1.  Claim Nos. : 15-27

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 15-27 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 15-27.

2.  Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3.  Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows :

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

**Remark on Protest**  The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.  
**PCT/CA2009/001087**

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)		Publication Date
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		NO	924670D0	03-12-1992
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