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US 6,774,140 B1
MACAULEY, M. ET AL, "O-GlcNAcase Uses Substrate-assisted Catalysis, Kinetic Analysis and Development of Highly Selective Mechanism-Inspired Inhibitors." Journal of Biological Chemistry, 2005, 280(27), pages 25313-25322
RITTER, T ET AL, "Synthesis of N-acetylglucosamine thiazoline:lipid II hybrids." Tetrahedron Letters, 2001, 42, 615-618

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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

SELECTIVE GLYCOSIDASE INHIBITORS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional application Serial Nos. 60/841,196, filed on August 31, 2006, and 60/895,663, filed March 19, 2007, which are hereby incorporated by reference.

FIELD OF THE INVENTION

[0002] This application relates to compounds which selectively inhibit glycosidases and uses thereof.

BACKGROUND OF THE INVENTION

[0003] A wide range of cellular proteins, both nuclear and cytoplasmic, are post-translationally modified by the addition of the monosaccharide 2-acetamido-2-deoxy-ß-D-glucopyranoside (β-N-acetylglucosamine) which is attached via an O-glycosidic linkage.[1] This modification is generally referred to as O-linked N-acetylglucosamine or O-GlcNAc. The enzyme responsible for post-translationally linking β-N-acetylglucosamine (GlcNAc) to specific serine and threonine residues of numerous nucleocytoplasmic proteins is O-GlcNAc transferase (OGTase).[2-5] A second enzyme, known as O-GlcNAcase[6,7] removes this post-translational modification to liberate proteins making the O-GlcNAc-modification a dynamic cycle occurring several times during the lifetime of a protein.[8]

[0004] O-GlcNAc-modified proteins regulate a wide range of vital cellular functions including, for example, transcription,[9-12] proteasomal degradation,[13] and cellular signaling.[14] O-GlcNAc is also found on many structural proteins.[15-17] For example, it has been found on a number of cytoskeletal proteins, including neurofilament proteins,[18,19] synapsins,[6,20] synapsin-specific clathrin assembly protein AP-3,[7] and ankyrinG.[14] O-GlcNAc modification has been found to be abundant in the brain.[21,22] It has also been found on proteins clearly implicated in the etiology of several diseases including Alzheimer's disease (AD) and cancer.

[0005] For example, it is well established that AD and a number of related tauopathies including Downs' syndrome, Pick's disease, Niemann-Pick Type C disease, and amyotrophic lateral sclerosis (ALS) are characterized, in part, by the development of neurofibrillary tangles (NFTs). These NFTs are aggregates of paired helical filaments (PHFs) and are composed of an abnormal form of the cytoskeletal protein "tau". Normally

tau stabilizes a key cellular network of microtubules that is essential for distributing proteins and nutrients within neurons. In AD patients, however, tau becomes hyperphosphorylated, disrupting its normal functions, forming PHFs and ultimately aggregating to form NFTs. Six isoforms of tau are found in the human brain. In AD patients, all six isoforms of tau are found in NFTs, and all are markedly hyperphosphorylated.[23,24] Tau in healthy brain tissue bears only 2 or 3 phosphate groups, whereas those found in the brains of AD patients bear, on average, 8 phosphate groups.[25,26] A clear parallel between NFT levels in the brains of AD patients and the severity of dementia strongly supports a key role for tau dysfunction in AD.[27,28] The precise causes of this hyperphosphorylation of tau remain elusive. Accordingly, considerable effort has been dedicated toward: a) elucidating the molecular physiological basis of tau hyperphosphorylation;[29] and b) identifying strategies that could limit tau hyperphosphorylation in the hope that these might halt, or even reverse, the progression of Alzheimer's disease[30-33] Thus far, several lines of evidence suggest that up-regulation of a number of kinases may be involved in hyperphosphorylation of tau,[21,34,35] although very recently, an alternative basis for this hyperphosphorylation has been advanced.[21]

[0006] In particular, it has recently emerged that phosphate levels of tau are regulated by the levels of O-GlcNAc on tau. The presence of O-GlcNAc on tau has stimulated studies that correlate O-GlcNAc levels with tau phosphorylation levels. The recent interest in this field stems from the observation that O-GlcNAc modification has been found to occur on many proteins at amino acid residues that are also known to be phosphorylated.[36-38] Consistent with this observation, it has been found that increases in phosphorylation levels result in decreased O-GlcNAc levels and conversely, increased O-GlcNAc levels correlate with decreased phosphorylation levels.[39] This reciprocal relationship between O-GlcNAc and phosphorylation has been termed the "Yin-Yang hypothesis"[40] and has gained strong biochemical support by the recent discovery that the enzyme OGTase[4] forms a functional complex with phosphatases that act to remove phosphate groups from proteins.[41] Like phosphorylation, O-GlcNAc is a dynamic modification that can be removed and reinstalled several times during the lifespan of a protein. Suggestively, the gene encoding O-GlcNAcase has been mapped to a chromosomal locus that is linked to AD.[7,42] Hyperphosphorylated tau in human AD brains has markedly lower levels of O-GlcNAc than are found in healthy human brains.[21] Very recently, it has been shown that O-GlcNAc levels of soluble tau protein from human brains affected with AD are markedly lower than those

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

[0007] Neurons do not store glucose and therefore the brain relies on glucose supplied by blood to maintain its essential metabolic functions. Notably, it has been shown that within brain, glucose uptake and metabolism decreases with aging.[43] Within the brains of AD patients marked decreases in glucose utilization occur and are thought to be a potential cause of neurodegeneration.[44] The basis for this decreased glucose supply in AD brain[45-47] is thought to stem from any of decreased glucose transport,[48,49] impaired insulin signaling,[50,51] and decreased blood flow.[52]

[0008] In light of this impaired glucose metabolism, it is worth noting that of all glucose entering into cells, 2-5% is shunted into the hexosamine biosynthetic pathway, thereby regulating cellular concentrations of the end product of this pathway, uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc).[53] UDP-GlcNAc is a substrate of the nucleocytoplasmic enzyme O-GlcNAc transferase (OGTase),[2-5] which acts to post-translationally add GlcNAc to specific serine and threonine residues of numerous nucleocytoplasmic proteins. OGTase recognizes many of its substrates[54,55] and binding partners[41,56] through its tetratricopeptide repeat (TPR) domains.[57,58] As described above, O-GlcNAcase[6,7] removes this post-translational modification to liberate proteins making the O-GlcNAc-modification a dynamic cycle occurring several times during the lifetime of a protein.[8] O-GlcNAc has been found in several proteins on known phosphorylation sites,[10,37,38,59] including tau and neurofilaments.[60] Additionally, OGTase shows unusual

kinetic behaviour making it exquisitely sensitive to intracellular UDP-GlcNAc substrate concentrations and therefore glucose supply.[41]

[0009] Consistent with the known properties of the hexosamine biosynthetic pathway, the enzymatic properties of OGTase, and the reciprocal relationship between O-GlcNAc and phosphorylation, it has been shown that decreased glucose availability in brain leads to tau hyperphosphorylation.[44] Therefore the gradual impairment of glucose transport and metabolism, whatever its causes, leads to decreased O-GlcNAc and hyperphosphorylation of tau (and other proteins). Accordingly, the inhibition of O-GlcNAcase should compensate for the age related impairment of glucose metabolism within the brains of health individuals as well as patients suffering from AD or related neurodegenerative diseases.

[0010] 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[61] comes from recent studies showing that when transgenic mice harbouring human tau are treated with kinase inhibitors, they do not develop typical motor defects[33] and, in another case,[32] show decreased levels of insoluble tau. These studies provide a clear link between lowering tau phosphorylation levels and alleviating AD-like behavioural symptoms in a murine model of this disease.

[0011] There is also a large body of evidence indicating that increased levels of O-GlcNAc protein modification provides protection against pathogenic effects of stress in cardiac tissue, including stress caused by ischemia, hemorrhage, hypervolemic shock, and calcium paradox. For example, activation of the hexosamine biosynthetic pathway (HBP) by administration of glucosamine has been demonstrated to exert a protective effect in animals models of ischemia/reperfusion,[62-68] trauma hemorrhage,[69-71] hypervolemic shock,[72] and calcium paradox.[62,73] Moreover, strong evidence indicates that these cardioprotective effects are mediated by elevated levels of protein O-GlcNAc modification.[62,63,65,68,70,73-76] 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.[77]

[0012] Humans have three genes encoding enzymes that cleave terminal β-N-acetyl-glucosamine residues from glycoconjugates. The first of these encodes the enzyme O-glycoprotein 2-acetamido-2-deoxy-β-D-glucopyranosidase (O-GlcNAcase). O-GlcNAcase is a member of family 84 of glycoside hydrolases that includes enzymes from organisms as

diverse as prokaryotic pathogens to humans (for the family classification of glycoside hydrolases see Coutinho, P.M. & Henrissat, B. (1999) Carbohydrate-Active Enzymes server at URL: http://afmb.cnrs-mrs.fr/CAZY/.[27,28] O-GlcNAcase acts to hydrolyse O-GlcNAc off of serine and threonine residues of post-translationally modified proteins.[1,6,7,78,79] Consistent with the presence of O-GlcNAc on many intracellular proteins, the enzyme O-GlcNAcase appears to have a role in the etiology of several diseases including type II diabetes,[14,80] AD,[16,21,81] and cancer.[22,82] Although O-GlcNAcase was likely isolated earlier on,[18,19] about 20 years elapsed before its biochemical role in acting to cleave O-GlcNAc from serine and threonine residues of proteins was understood.[6] More recently O-GlcNAcase has been cloned,[7] partially characterized,[20] and suggested to have additional activity as a histone acetyltransferase.[20] However, little was known about the catalytic mechanism of this enzyme.

[0013] The other two genes, HEXA and HEXB, encode enzymes catalyzing the hydrolytic cleavage of terminal β-N-acetylglucosamine residues from glycoconjugates. The gene products of HEXA and HEXB predominantly yield two dimeric isozymes, hexosaminidase A and hexosaminidase B, respectively. Hexosaminidase A (αß), a heterodimeric isozyme, is composed of an α- and a ß-subunit. Hexosaminidase B (ßß), a homodimeric isozyme, is composed of two ß-subunits. The two subunits, α- and ß-, bear a high level of sequence identity. Both of these enzymes are classified as members of family 20 of glycoside hydrolases and are normally localized within lysosomes. The proper functioning of these lysosomal β-hexosaminidases is critical for human development, a fact that is underscored by the tragic genetic illnesses, Tay-Sach's and Sandhoff diseases which stem from a dysfunction in, respectively, hexosaminidase A and hexosaminidase B.[83] 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.[84]

[0014] As a result of the biological importance of these β-*N*-acetyl-glucosaminidases, small molecule inhibitors of glycosidases[85-88] have received a great deal of attention,[89] both as tools for elucidating the role of these enzymes in biological processes and in developing potential therapeutic applications. The control of glycosidase function using small molecules offers several advantages over genetic knockout studies including the ability to rapidly vary doses or to entirely withdraw treatment.

[0015] However, a major challenge in developing inhibitors for blocking the function of

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

[0016] A few of the better characterized inhibitors of β-*N*-acetyl-glucosaminidases which have been used in studies of O-GlcNAc post-translational modification within both cells and tissues are streptozotocin (STZ), 2′-methyl-α-D-glucopyrano-[2,1-*d*]-Δ2′-thiazoline (NAG-thiazoline) and *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamate (PUGNAc).[14,90-93]

[0017] STZ has long been used as a diabetogenic compound because it has a particularly detrimental effect on ß-islet cells.[94] STZ exerts its cytotoxic effects through both the alkylation of cellular DNA[94,95] as well as the generation of radical species including nitric oxide.[96] The resulting DNA strand breakage promotes the activation of poly(ADP-ribose) polymerase (PARP)[97] with the net effect of depleting cellular NAD+ levels and, ultimately, leading to cell death.[98,99] Other investigators have proposed instead that STZ toxicity is a consequence of the irreversible inhibition of O-GlcNAcase, which is highly expressed within ß-islet cells.[90,100] This hypothesis has, however, been brought into question by two independent research groups.[101,102] Because cellular O-GlcNAc levels on proteins increase in response to many forms of cellular stress[103] 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[104] and although it has been proposed by others that STZ acts to irreversibly inhibit O-GlcNAcase,[105] there has been no clear demonstration of this mode of action. Recently, it has been shown that STZ does not irreversibly inhibit O-GlcNAcase.[106]

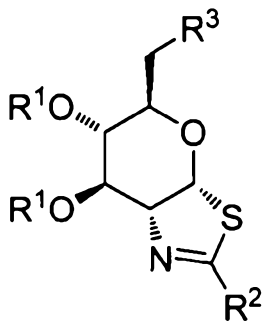
[0018] NAG-thiazoline has been found to be a potent inhibitor of family 20 hexosaminidases,[88,107] and more recently, the family 84 O-GlcNAcases.[106] Despite its potency, a downside to using NAG-thiazoline in a complex biological context is that it lacks selectivity and therefore perturbs multiple cellular processes.

[0019] PUGNAc is another compound that suffers from the same problem of lack of selectivity, yet has enjoyed use as an inhibitor of both human O-GlcNAcase[6,108] and the family 20 human β-hexosaminidases.[109] This molecule, developed by Vasella and coworkers, was found to be a potent competitive inhibitor of the β-*N*-acetyl-glucosaminidases from *Canavalia ensiformis*, *Mucor rouxii*, and the β-hexosaminidase from bovine kidney.[86] It has been demonstrated that administration of PUGNAc in a rat model of trauma hemorrhage decreases circulating levels of the pro-inflammatory cytokines TNF-α and IL-6.[110] It has also been shown that administration of PUGNAc in a cell-based model of lymphocyte activation decreases production of the cytokine IL-2.[111] Recent studies have indicated that PUGNAc can be used in an animal model to reduce myocardial infarct size after left coronary artery occlusions.[112] 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. [110,113] In addition, 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.[114]

[0020] International patent application PCT/CA2006/000300, filed 1 March 2006, published under No. WO 2006/092049 on 8 September 2006, which is hereby incorporated by reference, describe some more selective inhibitors of O-GlcNAcase, compared to NAG-thiazoline or PUGNAc.

Summary of the Invention

According to a first embodiment of the invention, there is provided a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



(I)

wherein

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

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent,

with the proviso that when each R^1 is H and R^3 is OH, R^2 excludes NH(phenyl), NH(4-methoxyphenyl), and $N(CH_3)_2$;

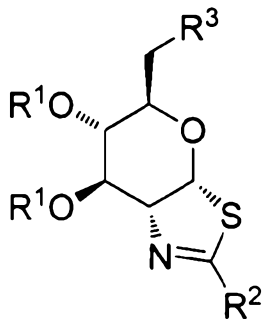
with the proviso that when each R^1 is $COCH_3$ and R^3 is $OC(O)CH_3$, R^2 excludes NH(phenyl), NH(4-methoxyphenyl), $N(CH_3)_2$, $NHCH_3$, $NH(CH_2)_2CH_3$, $NHCH(CH_3)_2$, $NH(CH_2)_3CH_3$, NH(cyclohexyl), NH(benzyl) $N(COCH_3)$(phenyl), and $N(COCH_3)$(4-methoxyphenyl); and

with the proviso that Formula (I) excludes compounds 74 and 75 described in Table 2.

According to a second embodiment of the invention, there is provided a pharmaceutical composition comprising the compound in accordance with the first embodiment of the present invention in combination with a pharmaceutically acceptable carrier.

According to a third embodiment of the invention, there is provided 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)

wherein

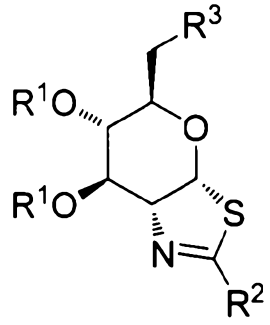
each R^1 is independently a non-interfering substituent;

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent.

According to a fourth embodiment of the invention, there is provided 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:



(I)

wherein

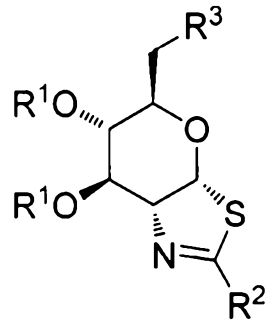
each R^1 is independently a non-interfering substituent;

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent.

According to a fifth embodiment of the invention, there is provided 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)

wherein

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

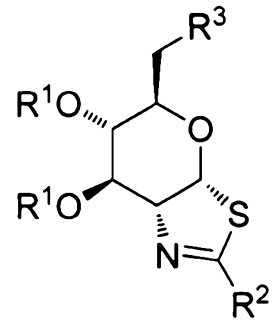
R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent,

with the proviso that the condition excludes a neurodegenerative disease, a tauopathy, cancer or stress.

According to a sixth embodiment of the invention, there is provided 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:



(I)

wherein

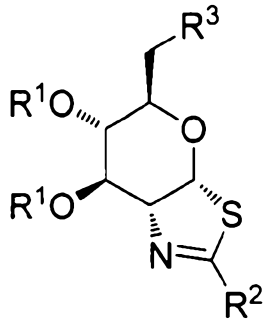
each R^1 is independently a non-interfering substituent;

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent.

According to a seventh embodiment of the invention, there is provided the use of a compound of an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



(I)

wherein

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

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

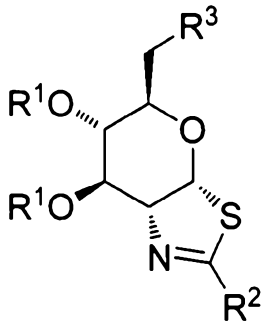
each R^4 is independently a non-interfering substituent,

with the proviso that the compound of Formula (I) excludes the compounds described in Tables 2 and 3, in the preparation of a medicament.

According to an eighth embodiment of the invention, there is provided a method for screening for a selective inhibitor of an O-GlcNAcase, the method comprising:

a) contacting a first sample with a test compound;

b) contacting a second sample with a compound of Formula (I)



(I)

wherein

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

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

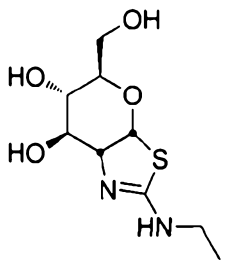
R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent,

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

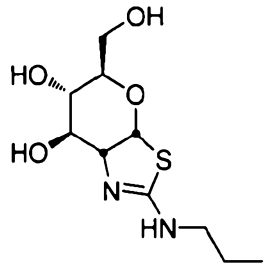
wherein the test compound is a selective inhibitor of a O-GlcNAcase if the test compound exhibits the same or greater inhibition of the O-GlcNAcase when compared to the compound of Formula (I).

According to a ninth embodiment of the invention, there is provided a (3aR,5R,6S,7R,7aR)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol compound having the structure:



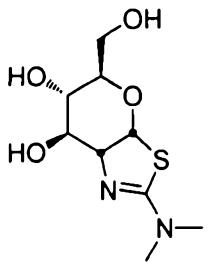
or a pharmaceutically acceptable salt thereof.

According to a tenth embodiment of the invention, there is provided a (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol compound having the structure:



or a pharmaceutically acceptable salt thereof.

According to an eleventh embodiment of the invention, there is provided a pharmaceutical composition comprising the compound (3aR,5R,6S,7R,7aR)-2-(dimethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol having the structure:

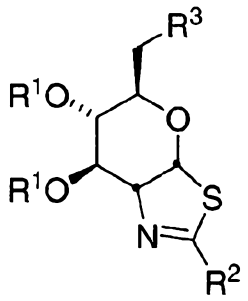


or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier.

[0021] The invention provides, in part, compounds for selectively inhibiting glycosidases, prodrugs of the compounds, uses of the compounds and the prodrugs, pharmaceutical compositions including the compounds or prodrugs of the compounds, amd methods of treating diseases and disorders related to deficiency or overexpression of O-GlcNAcase, accumulation or deficiency of O-GlcNAc.

[0022] In one aspect, the invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof:

[0023]



(I)

where each R^1 is independently a non-interfering substituent; R^2 is alkyl, aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent; R^3 is OR^4, N_3, or NR^4_2; and each R^4 is independently a non-interfering substituent, with the proviso that when each R^1 is H and R^3 is OH, R^2 excludes CH_3, CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_3CH_3$, $(CH_2)_4CH_3$, $(CH_2)_6CH_3$, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, NH(phenyl), NH(4-methoxyphenyl), $N(CH_3)_2$, $(CH_2)_2P(O)(OH)(OCH_3)$, and $(CH_2)_2P(O)(OH)(O(CH_2)_7CH_3)$; and with the proviso that when each R^1 is $COCH_3$ and R^3 is $OC(O)CH_3$, R^2 excludes CH_3, CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_3CH_3$, $(CH_2)_4CH_3$, $(CH_2)_6CH_3$, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, NH(phenyl), NH(4-methoxyphenyl), $N(CH_3)_2$, $(CH_2)_2P(O)(OH)(OCH_3)$, and $(CH_2)_2P(O)(OH)(O(CH_2)_7CH_3)$, $NHCH_3$, $NH(CH_2)_2CH_3$, $NHCH(CH_3)_2$, $NH(CH_2)_3CH_3$, NH(cyclohexyl), NH(benzyl), CH_2Br, $CHBr_2$, $CH_2P(O)(OCH_2CH_3)_2$, $(CH_2)_2P(O)(OCH_3)(O(CH_2)_7CH_3)$, $(CH_2)_2P(O)(OCH_3)_2$; $(CH_2)_2P(O)(OCH_3)_2$, $N(COCH_3)$(phenyl), and $N(COCH_3)$(4-methoxyphenyl); and with the proviso that Formula (I) excludes compounds 74 to 85 described in Table 2.

In alternative embodiments, each R^1 may be connected to form an additional ring structure; or when R^3 is OR^4, R^4 may be connected to either R^1 to form an additional ring structure.

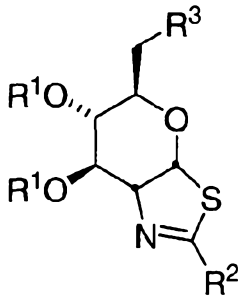
In alternative embodiments, the non-interfering substituent may be alkyl, alkenyl, alkynyl, aryl, arylalkyl, arylalkenyl, or arylalkynyl, or may include one or more heteroatoms selected from P, O, S, and N. The non-interfering substituent may be optionally substituted.

In alternative embodiments, R^1 may be H or $C(O)CH_3$; R^2 may be CH_2F, CHF_2, CF_3, $(CH_2)_2CH=CH_2$, $(CH_2)_2CH=CHCH_3$, CH_2OCH_3, $(CH_2)_2CF_3$, cyclopropylmethyl, phenyl, benzyl, NH_2, $NHCH_3$, $NHCH_2CH_3$, $NH(CH_2)_2CH_3$, $NH(CH_2)_3CH_3$, $NHCH_2CH=CH_2$, NHcyclopropyl, $NHCH_2CH_2F$, $NHCH_2CHF_2$, $NHCH_2CF_3$, $NHCH_2CH_2OH$, $NHCH_2CH_2OC(O)CH_3$, $N(CH_3)_2$, $N(CH_3)(CH_2CH_3)$, $NHOCH_3$, OCH_3, or $(CH_2)_2CH_3$; R^3 may be OH, $OC(O)CH_3$, N_3, or NH_2.

In alternative embodiments, the compound may be a compound described in Table 1; the compound may exclude one or more of the compounds described in Table 2 or Table 3; the compound may be a prodrug; the compound may selectively inhibit an O-glycoprotein 2-acetamido-2-deoxy-β-D-glucopyranosidase (O-GlcNAcase); the compound may selectively bind an O-GlcNAcase (e.g., a mammalian O-GlcNAcase); the compound may selectively inhibit the cleavage of a 2-acetamido-2-deoxy-β-D-glucopyranoside (O-GlcNAc); the compound may not substantially inhibit a mammalian β-hexosaminidase.

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

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:

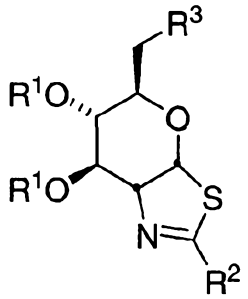


(I)

wherein each R^1 may be independently a non-interfering substituent; R^2 may be alkyl, aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent; R^3 may be OR^4, N_3, or NR^4_2; and each R^4 may be independently a non-interfering substituent, with the proviso that when each R^1 is H and R^3 is OH, R^2 excludes CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_3CH_3$, $(CH_2)_4CH_3$, $CH(CH_3)_2$, and $CH_2CH(CH_3)_2$; and with the proviso that when each R^1 is $COCH_3$ and R^3 is $OC(O)CH_3$, R^2 excludes CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_3CH_3$, $(CH_2)_4CH_3$, $CH(CH_3)_2$, and $CH_2CH(CH_3)_2$. The condition may be Alzheimer's disease, Amylotrophic lateral sclerosis (ALS), Amylotrophic lateral sclerosis with cognitive impairment (ALSci), Argyrophilic grain dementia, Bluit disease, Corticobasal degeneration (CBD), Dementia pugilistica, Diffuse neurofibrillary

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

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 Formula (I) or a pharmaceutically acceptable salt thereof:



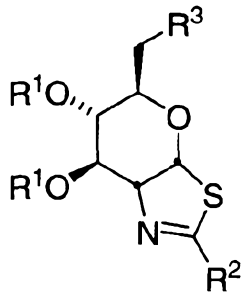
(I)

wherein each R^1 may be independently a non-interfering substituent; R^2 may be alkyl, aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent; R^3 may be OR^4, N_3, or NR^4_2; and each R^4 may be independently a non-interfering substituent. In some embodiments, the condition may be inflammatory or allergic diseases such as asthma, allergic rhinitis, hypersensitivity lung diseases, hypersensitivity pneumonitis, eosinophilic pneumonias, delayed-type hypersensitivity, atherosclerosis, interstitial lung disease (ILD) (*e.g.*, idiopathic pulmonary fibrosis, or ILD associated with rheumatoid arthritis, systemic lupus erythematosus,

ankylosing spondylitis, systemic sclerosis, Sjogren's syndrome, polymyositis or dermatomyositis); systemic anaphylaxis or hypersensitivity responses, drug allergies, insect sting allergies; autoimmune diseases, such as rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, systemic lupus erythematosus, myastenia gravis, glomerulonephritis, autoimmune thyroiditis, graft rejection, including allograft rejection or graft-versus-host disease; inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis; spondyloarthropathies; scleroderma; psoriasis (including T-cell mediated psoriasis) and inflammatory dermatoses such as dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria; vasculitis (*e.g.*, necrotizing, cutaneous, and hypersensitivity vasculitis); eosinphilic myotis, and eosiniphilic 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 lung allografts); epilepsy; pain; stroke, e.g., neuroprotection following a stroke.

In alternative embodiments, R^1 may be H or $C(O)CH_3$; R^2 may be CH_2F, CHF_2, CF_3, $(CH_2)_2CH=CH_2$, $(CH_2)_2CH=CHCH_3$, CH_2OCH_3, $(CH_2)_2CF_3$, cyclopropylmethyl, phenyl, benzyl, NH_2, $NHCH_3$, $NHCH_2CH_3$, $NH(CH_2)_2CH_3$, $NH(CH_2)_3CH_3$, $NHCH_2CH=CH_2$, NHcyclopropyl, $NHCH_2CH_2F$, $NHCH_2CHF_2$, $NHCH_2CF_3$, $NHCH_2CH_2OH$, $NHCH_2CH_2OC(O)CH_3$, $N(CH_3)_2$, $N(CH_3)(CH_2CH_3)$, $NHOCH_3$, OCH_3, or $(CH_2)_2CH_3$; R^3 may be OH, $OC(O)CH_3$, N_3, or NH_2; the compound may be selected from the group consisting of one or more of the compounds described in Table 2 and Table 3. The administering may increase the level of O-GlcNAc in the subject. The subject may be a human.

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:

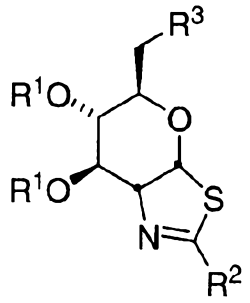


(I)

where each R^1 may be independently a non-interfering substituent; R^2 may be alkyl, aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with

a non-interfering substituent; R^3 may be OR^4, N_3, or NR^4_2; and each R^4 may be independently a non-interfering substituent, with the proviso that the compound of Formula (I) excludes the compounds described in Tables 2 and 3, in the preparation of a medicament. The medicament may be for selectively inhibiting an O-GlcNAcase, for increasing the level of O-GlcNAc, for treating a condition modulated by an O-GlcNAcase, for treating a neurodegenerative disease, a tauopathy, a cancer, or stress.

In alternative aspects, the inventio 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 alkyl, aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent; R^3 may be OR^4, N_3, or NR^4_2; and each R^4 may be independently a non-interfering substituent, c) determining the level of inhibition of the O-GlcNAcase in the first and second samples, where the test compound is a selective inhibitor of a O-GlcNAcase if the test compound exhibits the same or greater inhibition of the O-GlcNAcase when compared to the compound of Formula (I).

[0024] This summary of the invention does not necessarily describe all features of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] 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:

[0026] **FIGURES 1A-F** show Western blots of proteins from muscle and brain tissue of rats injected with various doses of (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (NAG-Bt) or vehicle alone (PBS). Equal amounts of homogenized muscle (A) and brain (B) tissue from animals treated with the indicated doses of NAG-Bt or vehicle alone (PBS; 0 mg/kg) were separated by SDS-PAGE followed by probing with the primary α-O-GlcNAc antibody and an anti-IgM-mouse IgG-HRP conjugate. Figures 1C (muscle) and 1D (brain) show Western blots of samples loaded in Figures 1A-B and probed using anti-β-actin mAb clone AC-40 followed by an anti-mouse IgG-HRP conjugate. Figures 1E (muscle) and 1F (brain) are graphs showing the analysis of the Western blot results by densitometry.

[0027] **FIGURES 2A-I** show Western blots of proteins from brain tissue of rats treated with or without NAG-Bt showing changes in brain tau phosphorylation at multiple sites following treatment. Equal amounts of homogenized brain tissue from an animal treated with and without NAG-Bt were separated by SDS-PAGE followed by probing with each of the primary antibodies, as indicated, and an appropriate secondary antibody (either an anti-mouse or anti-rabbit IgG-HRP conjugate, as appropriate). The lanes labeled "+" indicate samples from animals receiving NAG-Bt, while lanes labeled "-" indicate samples from animals receiving vehicle alone.

[0028] **FIGURES 3A-B** show Western blots of proteins from cardiac tissue of rats injected with 50 mg/kg of NAG-AE or vehicle alone (PBS) and sacrificed at various lengths of time following injection. Equal amounts of homogenized cardiac tissue from animals treated with NAG-AE for the indicated lengths of time were separated by SDS-PAGE followed by probing with the primary α-O-GlcNAc antibody and an anti-IgM-mouse IgG-HRP conjugate (A). Figure 3B shows a Western blot of samples loaded in Figure 3A and probed using an anti-β-actin mAb clone AC-40 followed by an anti-mouse IgG-HRP conjugate.

[0029] **FIGURES 4A-H** show stained hippocampus brain tissue sections collected from P301L JNPL3 mice, a transgenic model that develops hyperphosphorylated tau protein and NFTs. Group E are wild-type control mice that received vehicle alone; Group A are transgenic mice that received vehicle alone; Group B are transgenic mice that received 100 mg/kg/day NAG-Bt orally for 16 weeks, then 1000 mg/kg/day NAG-Bt orally for 16 weeks; Group D are transgenic mice that received 500 mg/kg/day NAG-AE orally for 16 weeks; all mice were 42-44 weeks of age at the time of sacrifice. Panels on the right (Figures 4E-H)

show sections stained with anti-OGlcNAc antibody (a marker for protein O-GlcNAc levels), while panels on the left (Figures A-D) show sections stained with anti-phospho Tau-Ser404 antibody (a marker for levels of tau phosphorylation and NFT formation). Animals receiving either NAG-Bt or NAG-AE (Groups B and D) shown elevated protein O-GlcNAc levels and significantly decreased formation of hyperphosphorylated tau and NFTs when compared to the untreated transgenic group (Group A). The boxed area in each panel indicates similar regions from each brain section in the transgenic animals, highlighted for comparative purposes.

[0030] **FIGURES 5A-D** show long-term effects of oral dosing (100 mg/kg/day) of Sprague-Dawley rats with NAG-Bt on weight (A), food consumption (B), water consumption (C), and blood glucose levels (D) determined over four months. Data for eight control rats (squares) versus eight dosed rats (circles) are plotted on all graphs; no significant differences were noted.

[0031] **FIGURE 6** is a bar graph showing results for organ weight measurements (brain, liver, pancreas, spleen, heart, fat, muscle) on rats dosed orally with NAG-Bt for 8 months at 100 mg/kg/day versus controls. Measurements were made on 6 rats per group, which were averaged. No significant differences were observed.

[0032] **FIGURES 7A-G** show Western blots of proteins from brain tissue of rats treated orally with NAG-AE (200 mg/kg/day in drinking water) or with vehicle alone (normal drinking water, 0 mg/kg/day), showing decreases in brain tau phosphorylation at multiple sites following treatment, as well as global increases in protein O-GlcNAc levels. Equal amounts of homogenized brain tissue from animals treated with and without NAG-AE (3 animals each) were separated by SDS-PAGE followed by probing with each of the primary antibodies and an appropriate secondary antibody. Figure 7A shows the Western blot of samples probed with Tau-5 (a phospho-independent tau antibody), and demonstrates equal loading of tau protein. Figures 7B-D show Western blots of the same samples probed with specific anti-phospho-tau antibodies, while Figure 7E shows analysis of the Western blot results by densitometry. Figure 7F shows a Western blot of whole brain lysates from these animals probed with the primary α-O-GlcNAc antibody, showing increases the global levels of O-GlcNAc in the brains of animals receiving NAG-AE. Figure 7G shows the same samples as in Figure 7F, probed using an anti-β-actin antibody and demonstrates equal sample loading.

DETAILED DESCRIPTION

[0033] The invention provides, in part, novel compounds that are capable of inhibiting an O-glycoprotein 2-acetamido-2-deoxy-β-D-glucopyranosidase (O-GlcNAcase). In some embodiments, the O-GlcNAcase is a mammalian O-GlcNAcase, such as a rat, mouse or human O-GlcNAcase. In some embodiments, the β-hexosaminidase is a mammalian β-hexosaminidase, such as a rat, mouse or human β-hexosaminidase.

[0034] In some embodiments, compounds according to the invention exhibit a surprising and unexpected selectivity in inhibiting an O-GlcNAcase. For example, when compared to the compounds described in Table 3 herein, the compounds according to the invention are surprisingly effective inhibitors of an O-GlcNAcase. In some embodiments, the compounds according to the invention are surprisingly more selective for an O-GlcNAcase over a β-hexosaminidase. In some embodiments, the compounds selectively inhibit the activity of a mammalian O-GlcNAcase over a mammalian β-hexosaminidase. In some embodiments, a selective inhibitor of an O-GlcNAcase does not substantially inhibit a β-hexosaminidase. A compound that "selectively" inhibits an O-GlcNAcase is a compound that inhibits the activity or biological function of an O-GlcNAcase, but does not substantially inhibit the activity or biological function of a β-hexosaminidase. For example, in some embodiments, a selective inhibitor of an O-GlcNAcase selectively inhibits the cleavage of 2-acetamido-2-deoxy-β-D-glucopyranoside (O-GlcNAc) from polypeptides. In some embodiments, a selective inhibitor of an O-GlcNAcase selectively binds to an O-GlcNAcase. In some embodiments, a selective inhibitor of an O-GlcNAcase inhibits hyperphosphorylation of a tau protein and/or inhibits formations of NFTs. By "inhibits," "inhibition" or "inhibiting" means a decrease by any value between 10% and 90%, or of any integer value between 30% and 60%, or over 100%, or a decrease by 1-fold, 2-fold, 5-fold, 10-fold or more. It is to be understood that the inhibiting does not require full inhibition. In some embodiments, a selective inhibitor of an O-GlcNAcase elevates or enhances O-GlcNAc levels e.g., O-GlcNAc-modified polypeptide or protein levels, in cells, tissues, or organs (e.g., in brain, muscle, or heart (cardiac) tissue) and in animals. By "elevating" or "enhancing" is meant an increase by any value between 10% and 90%, or of any integer value between 30% and 60%, or over 100%, or an increase by 1-fold, 2-fold, 5-fold, 10-fold, 15-fold, 25-fold, 50-fold, 100-fold or more. In some embodiments, a selective inhibitor of an O-GlcNAcase exhibits a selectivity ratio, as described herein, in the range 100 to 100000, or in the range

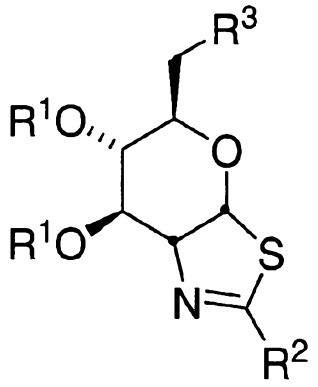
1000 to 100000, or at least 100, 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 6000, 7000, 10,000, 25,000, 50,000, 75,000, or any value within or about the described range.

[0035] 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.

[0036] In some embodiments, the compounds of the present invention are useful as agents that produce a decrease in tau phosphorylation and NFT formation. In some embodiments, the compounds are therefore useful to treat Alzheimer's disease and related tauopathies. In some embodiments, the compounds are thus capable of treating Alzheimer's disease and related tauopathies by lowering tau phosphorylation and reducing NFT formation as a result of increasing tau O-GlcNAc levels. In some embodiments, the compounds produce an increase in levels of O-GlcNAc modification on O-GlcNAc-modified polypeptides or proteins, and are therefore useful for treatment of disorders responsive to such increases in O-GlcNAc modification; these disorders include without limitation neurodegenerative, inflammatory, cardiovascular, and immunoregulatory diseases. In some embodiments, the compounds are also useful as a result of other biological activites 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.

[0037] 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 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.

[0038] In specific embodiments, the invention provides compounds described generally by Formula (I) and the salts, prodrugs, and stereoisomeric forms thereof:



(I)

[0039] As set forth in Formula (I): each R^1 can be independently a non-interfering substituent; R^2 can be alkyl, aryl, heteroaryl, OR^4, NR^4_2, or NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent, and where each R^4 may be independently a non-interfering substituent; R^3 can be OR^4, N_3, or NR^4_2, where each R^4 may be independently a non-interfering substituent. In some embodiments, each R^1 may be connected to form an additional ring structure. In alternative embodiments, when R^3 is OR^4, the OR^4 group may be connected to either R^1 to form an additional ring structure.

[0040] 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; $=NR^5$; OR; $C_{1\text{-}10}$ alkyl or $C_{2\text{-}10}$ alkenyl optionally containing one or more P, N, O, or S, and optionally substituted with halo; CN; optionally substituted carbonyl; NR^5_2; $C=NR^5$; an optionally substituted carbocyclic or heterocyclic ring; or an optionally substituted aryl or heteroaryl. R^5 may be alkyl, branched alkyl, cycloalkyl, aryl, or heteroaryl.

[0041] In some embodiments, R^1 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^1 may be H, alkyl, or $C(O)R^5$, where R^5 may be alkyl, branched alkyl, cycloalkyl, aryl, or heteroaryl. In some embodiments, R^1 may be H or $C(O)CH_3$.

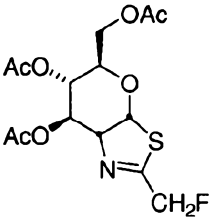
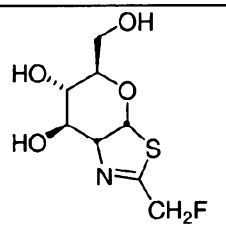
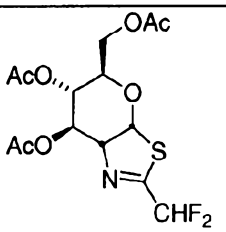
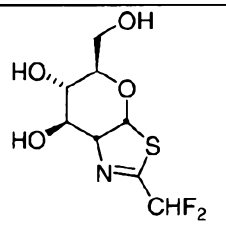
[0042] In some embodiments, R^2 as set forth in Formula (I), may be optionally substituted alkyl, OR, NR_2, or NR^6OR^6, where R^6 may be H, alkyl, branched alkyl, cycloalkyl, aryl, or heteroaryl. In some embodiments, R^2 may be CH_2F, CHF_2, CF_3, $(CH_2)_2CH=CH_2$, $(CH_2)_2CH=CHCH_3$, CH_2OCH_3, $(CH_2)_2CF_3$, cyclopropylmethyl, phenyl, benzyl, NH_2, $NHCH_3$, $NHCH_2CH_3$, $NH(CH_2)_2CH_3$, $NH(CH_2)_3CH_3$, $NHCH_2CH=CH_2$, NHcyclopropyl,

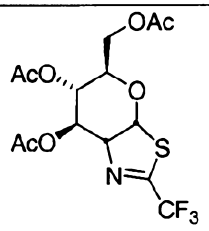
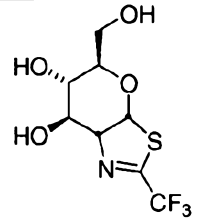
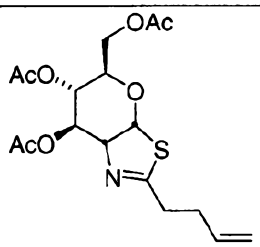
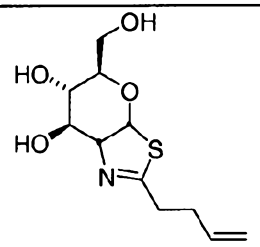
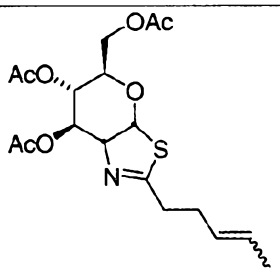
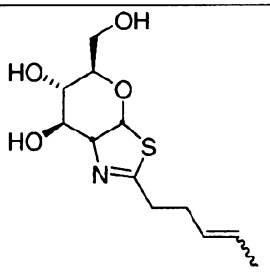
$NHCH_2CH_2F$, $NHCH_2CHF_2$, $NHCH_2CF_3$, $NHCH_2CH_2OH$, $NHCH_2CH_2OC(O)CH_3$, $N(CH_3)_2$, $N(CH_3)(CH_2CH_3)$, $NHOCH_3$, OCH_3, or $(CH_2)_2CH_3$.

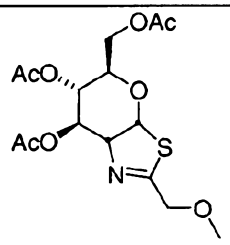
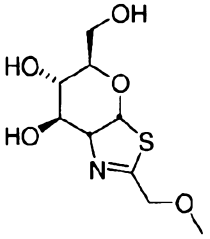
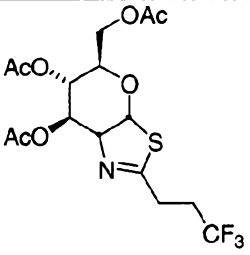
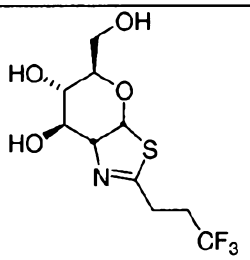
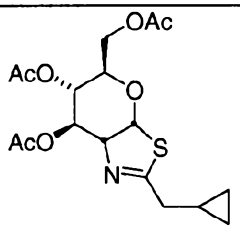
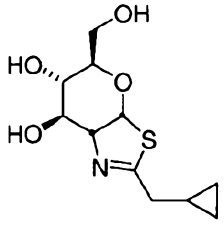
[0043] In some embodiments, R^3 as set forth in Formula (I), may be OR, N_3, or NR^7_2, where R^7 may be H, alkyl, branched alkyl, cycloalkyl, aryl, or heteroaryl. In some embodiments, R^3 may be OH, $OC(O)CH_3$, N_3, or NH_2.

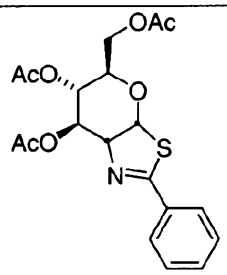
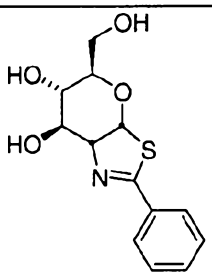
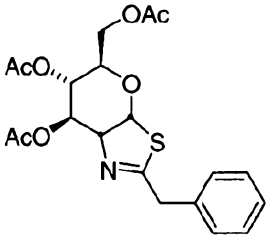
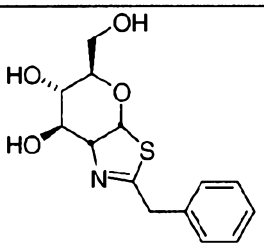
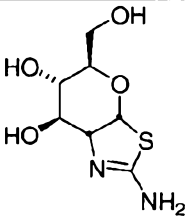
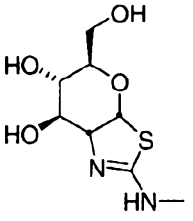
[0044] In specific embodiments of the invention, compounds according to Formula (I) include one or more of the compounds described in Table 1.

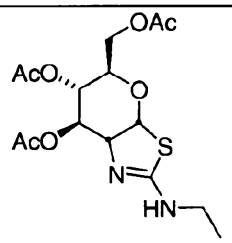
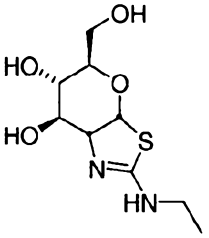
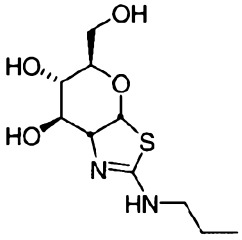
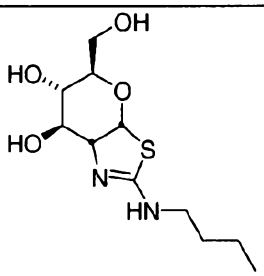
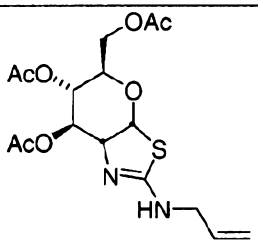
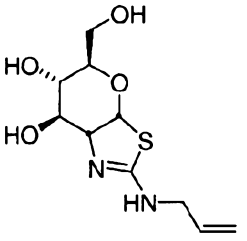
Table 1.

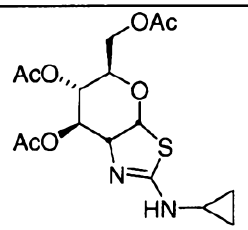
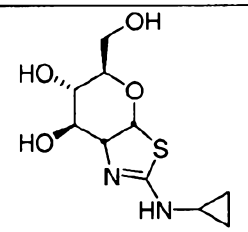
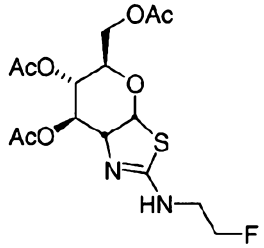
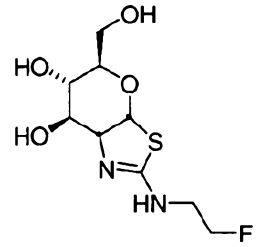
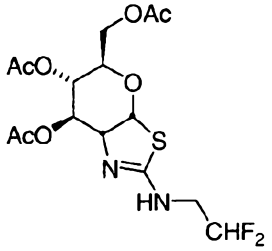
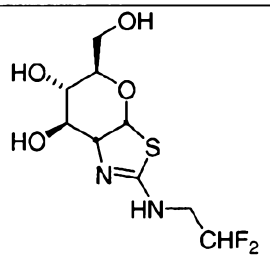
Compound	Name	Structure
1	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(fluoromethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
2	(3aR,5R,6S,7R,7aR)-2-(fluoromethyl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
3	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(difluoromethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
4	(3aR,5R,6S,7R,7aR)-2-(difluoromethyl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

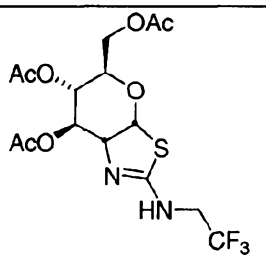
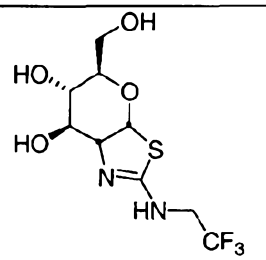
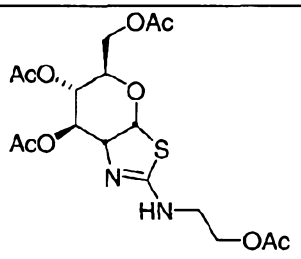
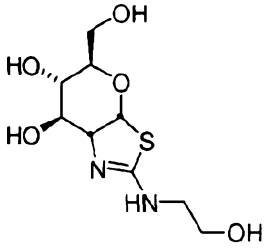
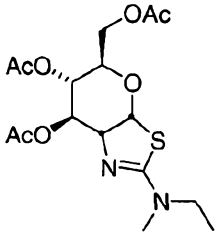
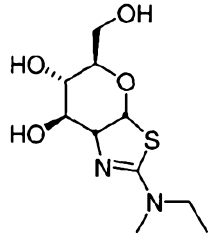
5	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(trifluoromethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
6	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(trifluoromethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
7	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(but-3-enyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
8	(3aR,5R,6S,7R,7aR)-2-(but-3-enyl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
9	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(E,Z)-(pent-3-enyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
10	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(E,Z)-(pent-3-enyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

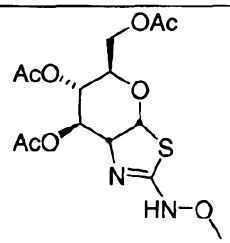
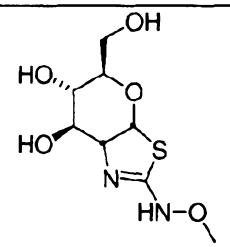
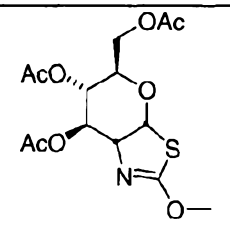
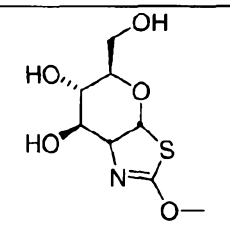
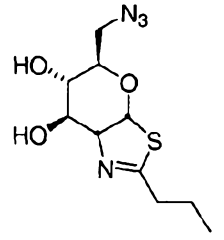
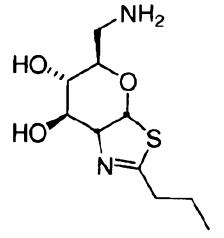
11	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(methoxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
12	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(methoxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
13	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(3,3,3-trifluoropropyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
14	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(3,3,3-trifluoropropyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
15	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(cyclopropylmethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
16	(3aR,5R,6S,7R,7aR)-2-(cyclopropylmethyl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

17	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-phenyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
18	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-phenyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
19	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-benzyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
20	(3aR,5R,6S,7R,7aR)-2-benzyl-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
21	(3aR,5R,6S,7R,7aR)-2-amino-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
23	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

24	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(ethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
25	(3aR,5R,6S,7R,7aR)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
27	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
29	(3aR,5R,6S,7R,7aR)-2-(butylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
30	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(allylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
31	(3aR,5R,6S,7R,7aR)-2-(allylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

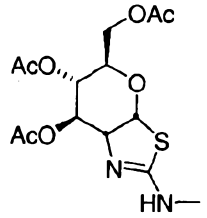
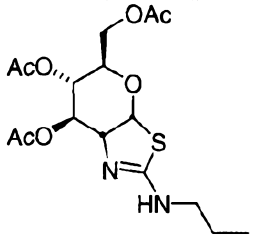
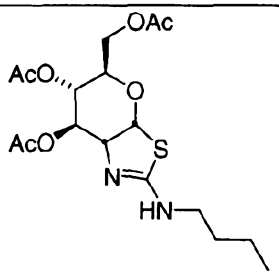
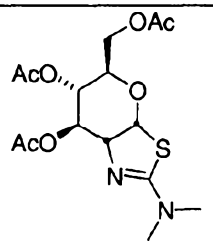
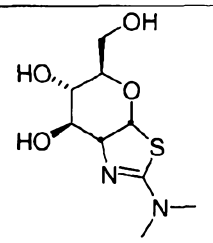
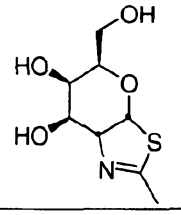
32	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(cyclopropylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
33	(3aR,5R,6S,7R,7aR)-2-(cyclopropylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
34	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2-fluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
35	(3aR,5R,6S,7R,7aR)-2-(2-fluoroethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
36	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2,2-difluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
37	(3aR,5R,6S,7R,7aR)-2-(2,2-difluoroethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

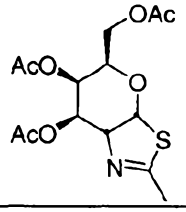
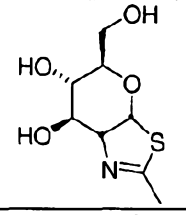
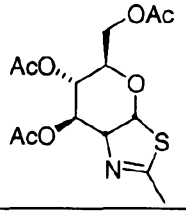
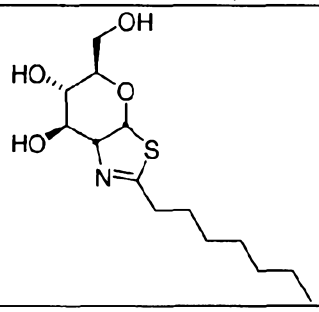
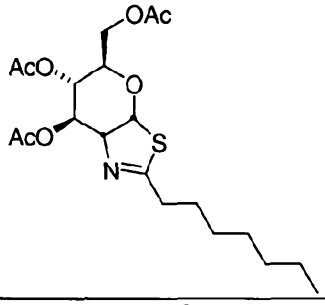
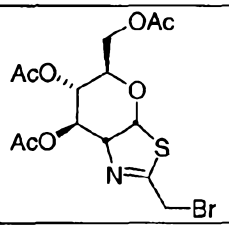
38	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2,2,2-trifluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
39	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(2,2,2-trifluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
40	(3aR,5R,6S,7R,7aR)-2-(2-acetoxyethylamino)-5-(acetoxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
41	(3aR,5R,6S,7R,7aR)-2-(2-hydroxyethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
44	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(ethyl(methyl)amino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
45	(3aR,5R,6S,7R,7aR)-2-(ethyl(methyl)amino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

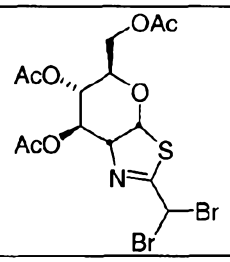
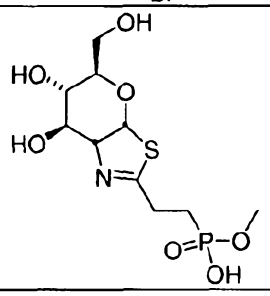
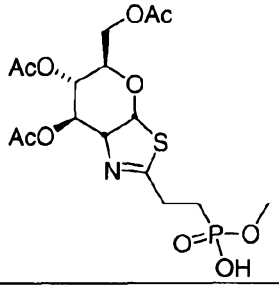
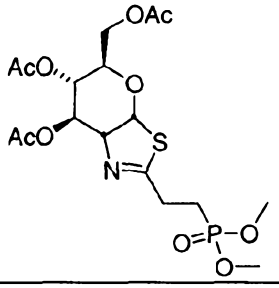
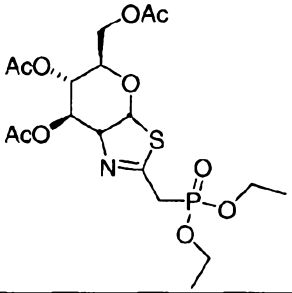
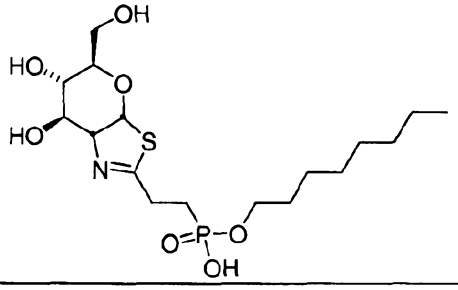
46	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(methoxyamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
47	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(methoxyamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
48	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-methoxy-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
49	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-methoxy-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
50	(3aR,5R,6S,7R,7aR)-5-(azidomethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
51	(3aR,5R,6S,7R,7aR)-5-(aminomethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

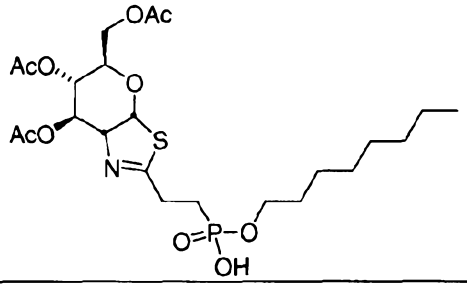
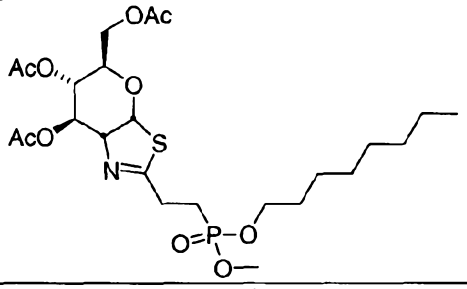
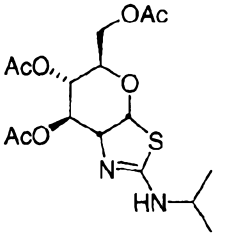
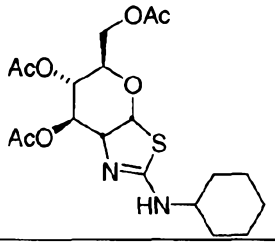
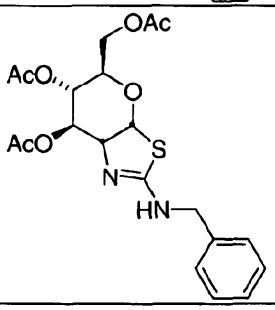
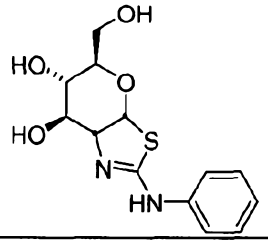
[0045] In alternative embodiments of the invention, compounds according to Formula (I) include one or more of the compounds described in Table 2.

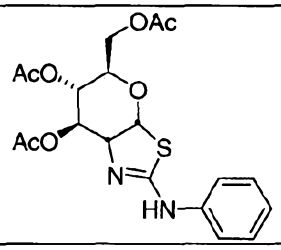
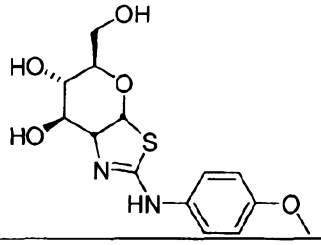
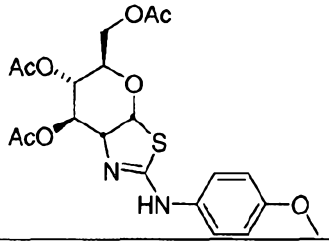
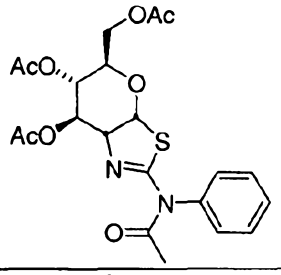
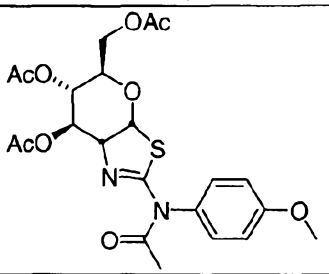
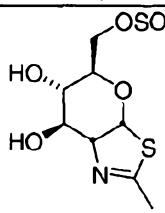
Table 2.

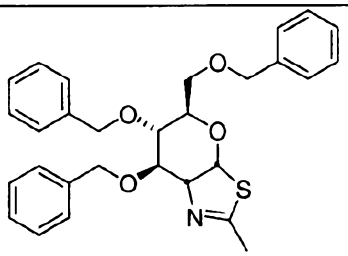
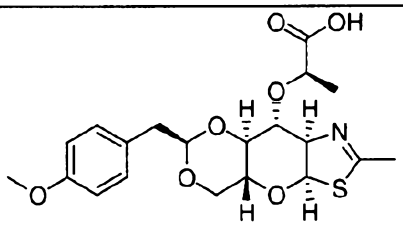
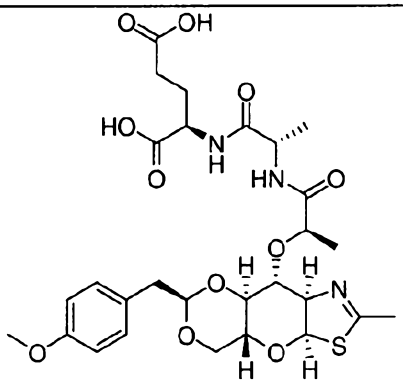
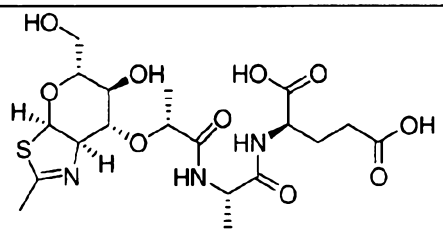
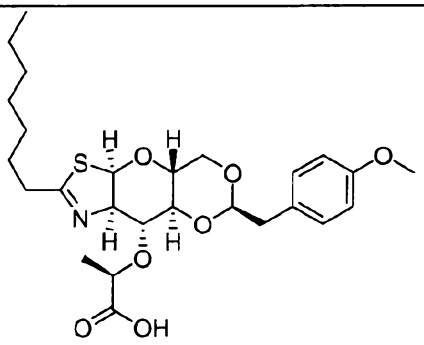
Compound	Name	Structure
22	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
26	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
28	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(butylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
42	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
43	(3aR,5R,6S,7R,7aR)-2-(dimethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
52	(3aR,5R,6R,7R,7aR)-5-(hydroxymethyl)-2-methyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

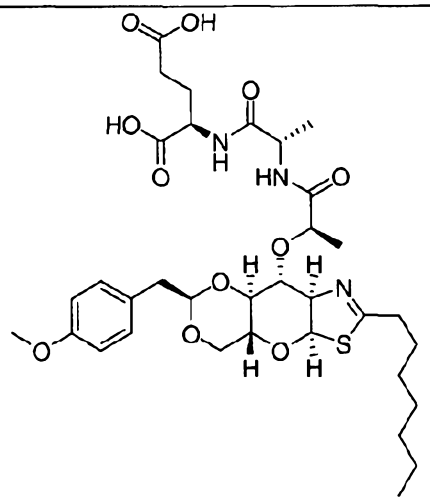
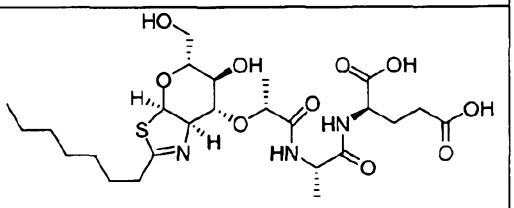
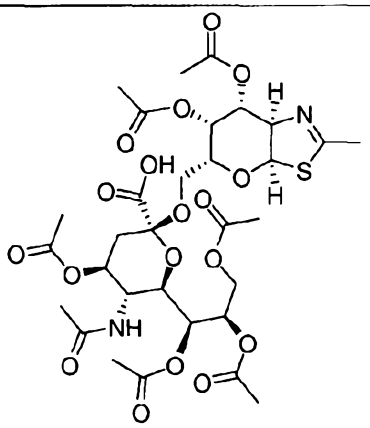
53	(3aR,5R,6R,7R,7aR)-5-(acctoxymethyl)-2-methyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
54	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-methyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
55	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-methyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
56	(3aR,5R,6S,7R,7aR)-2-heptyl-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
57	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-heptyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
58	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(bromomethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	

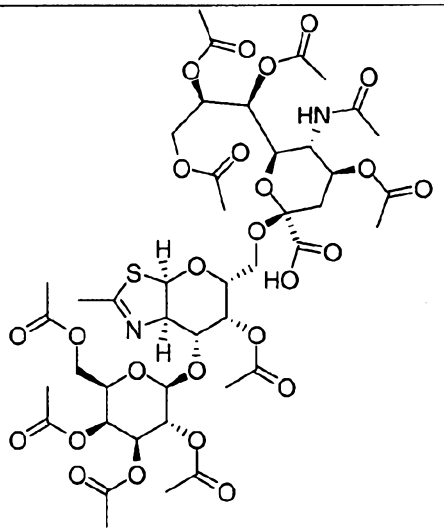
59	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(dibromomethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
60	methyl hydrogen 2-((3aR,5R,6S,7R,7aR)-6,7-dihydroxy-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazol-2-yl)ethylphosphonate	
61	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2-(hydroxy(methoxy)phosphoryl)ethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
62	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2-(dimethoxyphosphoryl)ethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
63	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-((diethoxyphosphoryl)methyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
64	octyl hydrogen 2-((3aR,5R,6S,7R,7aR)-6,7-dihydroxy-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazol-2-yl)ethylphosphonate	

65	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2-(hydroxy(octyloxy)phosphoryl)ethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
66	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2-(methoxy(octyloxy)phosphoryl)ethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
67	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(isopropylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
68	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(cyclohexylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
69	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(benzylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
70	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(phenylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

71	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(phenylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
72	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(4-methoxyphenylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
73	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(4-methoxyphenylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
74	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(N-phenylacetamido)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
75	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(N-(4-methoxyphenyl)acetamido)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
76	((3aR,5R,6S,7R,7aR)-6,7-dihydroxy-2-methyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazol-5-yl)methyl hydrogen sulfate	

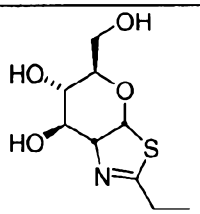
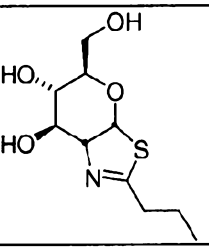
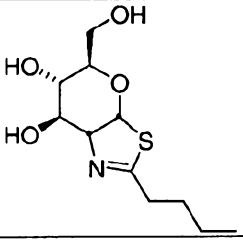
77	(3aR,5R,6S,7R,7aR)-6,7-bis(benzyloxy)-5-(benzyloxymethyl)-2-methyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole	
78	(2R)-2-[[(3aR,4aR,7R,8aS,9R,9aR)-3a,4a,5,8a,9,9a-hexahydro-7-[(4-methoxyphenyl)methyl]-2-methyl[1,3]dioxino[4',5':5,6]pyrano[3,2-d]thiazol-9-yl]oxy]-propanoic acid	
79	N-[(2R)-2-[[(3aR,4aR,7R,8aS,9R,9aR)-3a,4a,5,8a,9,9a-hexahydro-7-[(4-methoxyphenyl)methyl]-2-methyl[1,3]dioxino[4',5':5,6]pyrano[3,2-d]thiazol-9-yl]oxy]-1-oxopropyl]-L-alanyl-D-Glutamic acid	
80	N-[(2R)-1-oxo-2-[[(3aR,5R,6S,7R,7aR)-3a,6,7,7a-tetrahydro-6-hydroxy-5-(hydroxymethyl)-2-methyl-5H-pyrano[3,2-d]thiazol-7-yl]oxy]propyl]-L-alanyl-D-Glutamic acid	
81	(2R)-2-[[(3aR,4aR,7R,8aS,9R,9aR)-2-heptyl-3a,4a,5,8a,9,9a-hexahydro-7-[(4-methoxyphenyl)methyl][1,3]dioxino[4',5':5,6]pyrano[3,2-d]thiazol-9-yl]oxy]-Propanoic acid	

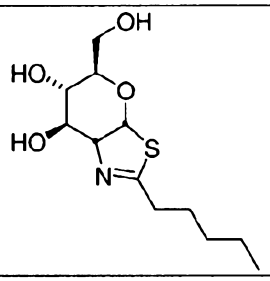
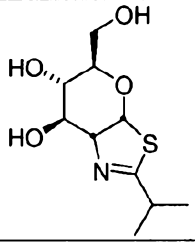
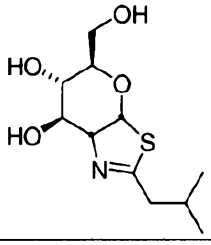
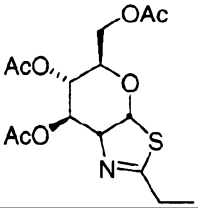
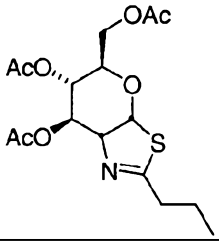
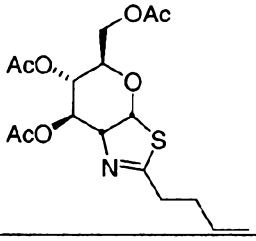
82	N-[(2R)-2-[[(3aR,4aR,7R,8aS,9R,9aR)-2-heptyl-3a,4a,5,8a,9,9a-hexahydro-7-[(4-methoxyphenyl)methyl][1,3]dioxino[4',5':5,6]pyrano[3,2-d]thiazol-9-yl]oxy]-1-oxopropyl]-L-alanyl- D-Glutamic acid	
83	N-[(2R)-2-[[(3aR,5R,6S,7R,7aR)-2-heptyl-3a,6,7,7a-tetrahydro-6-hydroxy-5-(hydroxymethyl)-5H-pyrano[3,2-d]thiazol-7-yl]oxy]-1-oxopropyl]-L-alanyl-D-Glutamic acid	
84	N-acetyl-2-O-[[(3aR,5R,6R,7R,7aR)-6,7-bis(acetyloxy)-3a,6,7,7a-tetrahydro-2-methyl-5H-pyrano[3,2-d]thiazol-5-yl]methyl]-α-Neuraminic acid, 4,7,8,9-tetraacetate	

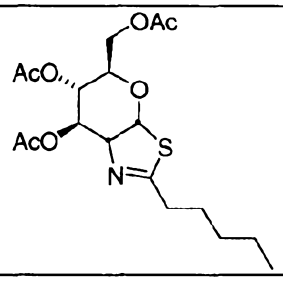
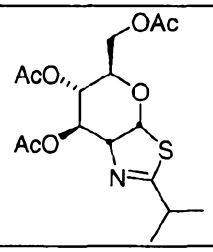
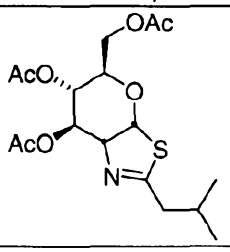
85	N-acetyl-2-O-[[(3aR,5R,6R,7R,7aR)-6-(acetyloxy)-3a,6,7,7a-tetrahydro-2-methyl-7-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)oxy]-5H-pyrano[3,2-d]thiazol-5-yl]methyl]-α-Neuraminic acid, 4,7,8,9-tetraacetate	

[0046] In alternative embodiments of the invention, compounds according to Formula (I) include one or more of the compounds described in Table 3.

Table 3.

Compound	Name	Structure
86	(3aR,5R,6S,7R,7aR)-2-ethyl-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
87	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
88	(3aR,5R,6S,7R,7aR)-2-butyl-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	

89	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-pentyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
90	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-isopropyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
91	(3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-isobutyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol	
92	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-ethyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
93	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
94	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-butyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	

95	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-pentyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
96	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-isopropyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	
97	(3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-isobutyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate	

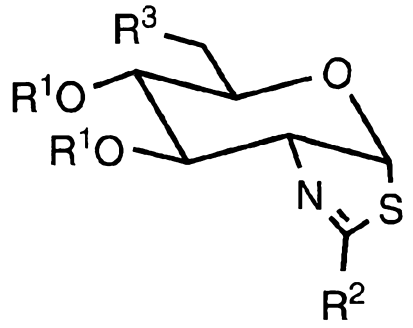
[0047] In alternative embodiments of the invention, one or more of the compounds described in Tables 1, 2 or 3 are specifically excluded from the compounds described in Formula (I). In alternative embodiments of the invention, specific stereoisomers or enantiomers of one or more of the compounds described in Tables 1, 2 or 3 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 Tables 1, 2 or 3 are specifically excluded from the compounds described in Formula (I).

[0048] In some embodiments, when each R^1 is H and R^3 is OH, R^2 is not CH_3, CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_3CH_3$, $(CH_2)_4CH_3$, $(CH_2)_6CH_3$, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, NH(phenyl), NH(4-methoxyphenyl), $N(CH_3)_2$, $(CH_2)_2P(O)(OH)(OCH_3)$, or $(CH_2)_2P(O)(OH)(O(CH_2)_7CH_3)$.

[0049] In alternative embodiments, when each R^1 is $COCH_3$ and R^3 is $OC(O)CH_3$, R^2 excludes CH_3, CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_3CH_3$, $(CH_2)_4CH_3$, $(CH_2)_6CH_3$, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, NH(phenyl), NH(4-methoxyphenyl), $N(CH_3)_2$, $(CH_2)_2P(O)(OH)(OCH_3)$, and $(CH_2)_2P(O)(OH)(O(CH_2)_7CH_3)$, $NHCH_3$, $NH(CH_2)_2CH_3$, $NHCH(CH_3)_2$, $NH(CH_2)_3CH_3$, NH(cyclohexyl), NH(benzyl), CH_2Br, $CHBr_2$, $CH_2P(O)(OCH_2CH_3)_2$,

$(CH_2)_2P(O)(OCH_3)(O(CH_2)_7CH_3)$, $(CH_2)_2P(O)(OCH_3)_2$; $(CH_2)_2P(O)(OCH_3)_2$, $N(COCH_3)$(phenyl), and $N(COCH_3)$(4-methoxyphenyl).

[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_{1-10}), alkenyl (C_{2-10}), alkynyl (C_{2-10}), aryl (5-12 members), arylalkyl, arylalkenyl, or arylalkynyl, each of which may optionally contain one or more heteroatoms selected from O, S, P, and N, 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_3, CHF_2, NO_2, OR, SR, NR_2, N_3, COOR, and $CONR_2$, 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.

[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 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 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 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.

[0060] "Arylalkyl" refers to a group of the formula $-R_aR_b$ where R_a is an alkyl group as described herein and R_b is one or more aryl moieties as described herein. The aryl group(s) may be optionally substituted as described herein.

[0061] "Arylalkenyl" refers to a group of the formula $-R_cR_b$ where R_c is an alkenyl moiety as described herein and R_b is one or more aryl groups as described herein. The aryl group(s) and the alkenyl group may be optionally substituted as described herein.

[0062] "Acyl" refers to a group of the formula $-C(O)R_a$, where R_a is an alkyl group as described herein. The alkyl group(s) may be optionally substituted as described herein.

[0063] "Arylacyl" refers to a group of the formula $-C(O)R_b$, where R_b 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_{1-6} 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_{1-10}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_{1-6} alkyl, alkenyl and cycloalkyl are preferred. The amine group may also be optionally substituted with an aromatic or heterocyclic group, aralkyl (*e.g.*, phenylC_{1-4}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_{2-4} alkanoyl, *e.g.*, acetyl, propionyl, butyryl, isobutyryl etc., or a C_{1-4}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.

[0070] 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

[0071] The invention provides methods of treating conditions that are modulated, directly or indirectly, by an O-GlcNAcase enzyme or by O-GlcNAc-modified protein levels, for example, a condition that is benefited by inhibition of an O-GlcNAcase enzyme or by an elevation of O-GlcNAc-modified protein levels. Such conditions include, without limitation, tauopathies, such as Alzheimer's disease, neurodegenerative diseases, 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, neurodegenerative disorders, such as Alzheimer's disease (AD), and cancer. Such diseases and disorders may also include diseases or disorders related to the accumulation or

deficiency in the enzyme OGTase. 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.

[0072] 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.

[0073] In alternative embodiments, the invention provides methods of selectively inhibiting an O-GlcNAcase enzyme in animal subjects, such as veterinary and human subjects.

[0074] 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.

[0075] 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 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 providing therapeutic benefit.

[0076] Tauopathies that may be treated with the compounds of the invention include: Alzheimer's disease, Amylotrophic lateral sclerosis (ALS), Amylotrophic lateral sclerosis with cognitive impairment (ALSci), Argyrophilic grain dementia, Bluit disease, Corticobasal degeneration (CBD), Dementia pugilistica, Diffuse neurofibrillary tangles with

calcification, Down's syndrome, Familial British dementia, Familial Danish dementia, Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), Gerstmann-Straussler-Scheinker disease, Guadeloupean parkinsonism, Hallevorden-Spatz disease (neurodegeneration with brain iron accumulation type 1), Multiple system atrophy, Myotonic dystrophy, Niemann-Pick disease (type C), Pallido-ponto-nigral degeneration, Parkinsonism-dementia complex of Guam, Pick's disease (PiD), Post-encephalitic parkinsonism (PEP), Prion diseases (including Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Fatal Familial Insomnia, and Kuru), Progressive supercortical gliosis, Progressive supranuclear palsy (PSP), Richardson's syndrome, Subacute sclerosing panencephalitis, and Tangle-only dementia.

[0077] The compounds of this invention are also useful in the treatment of conditions associate with tissue damage or stress, stimulating cells, or promoting differentiation of cells. Accordingly, in some embodiments, the compounds of this invention may be used to provide therapeutic benefit in a variety of conditions or medical procedures involving stress in cardiac tissue, including but not limited to: ischemia; hemorrhage; hypovolemic shock; myocardial infarction; an interventional cardiology procedure; cardiac bypass surgery; fibrinolytic therapy; angioplasty; and stent placement.

[0078] Compounds that selectively inhibit O-GlcNAcase activity may be used for the treatment of diseases that are associated with inflammation, including but not limited to, inflammatory or allergic diseases such as asthma, allergic rhinitis, hypersensitivity lung diseases, hypersensitivity pneumonitis, eosinophilic pneumonias, delayed-type hypersensitivity, atherosclerosis, interstitial lung disease (ILD) (*e.g.*, idiopathic pulmonary fibrosis, or ILD associated with rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, systemic sclerosis, Sjogren's syndrome, polymyositis or dermatomyositis); systemic anaphylaxis or hypersensitivity responses, drug allergies, insect sting allergies; autoimmune diseases, such as rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, systemic lupus erythematosus, myastenia gravis, glomerulonephritis, autoimmune thyroiditis, graft rejection, including allograft rejection or graft-versus-host disease; inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis; spondyloarthropathies; scleroderma; psoriasis (including T-cell mediated psoriasis) and inflammatory dermatoses such as dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria; vasculitis (*e.g.*, necrotizing, cutaneous, and hypersensitivity vasculitis); eosinphilic myotis, eosiniphilic fasciitis; and cancers.

[0079] In addition, compounds that affects levels of protein O-GlcNAc modification may be used for the treatment of diseases associated with immunosuppression, such as in individuals undergoing chemotherapy, radiation therapy, enhanced wound healing and burn treatment, therapy for autoimmune disease or other drug therapy (*e.g.*, corticosteroid therapy) or combination of conventional drugs used in the treatment of autoimmune diseases and graft/transplantation rejection, which causes immunosuppression; or immunosuppression due to congenital deficiency in receptor function or other causes.

[0080] The compounds of the invention may be useful for treatment of neurodegenerative diseases, including Parkinson's disease and Huntington's disease. Other conditions that may be treated are those triggered, affected, or in any other way correlated with levels of O-GlcNAc post-translational protein modification. It is expected that the compounds of this invention may be useful for the treatment of such conditions and in particular, but not limited to, the following for which a association with O-GlcNAc levels on proteins has been established: graft rejection, in particular but not limited to solid organ transplants, such as heart, lung, liver, kidney, and pancreas transplants (e.g. kidney and lung allografts); cancer, in particular but not limited to cancer of the breast, lung, prostate, pancreas, colon, rectum, bladder, kidney, ovary; as well as non-Hodgkin's lymphoma and melanoma; epilepsy, pain, or stroke, e.g., for neuroprotection following a stroke.

Pharmaceutical & Veterinary Compositions, Dosages, And Administration

[0081] 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.

[0082] 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.

[0083] In some embodiments, compounds according to the invention, or for use according to the invention, may be provided in combination with any other active agents or pharmaceutical compositions where such combined therapy is useful to modulate O-GlcNAcase activity, for example, to treat neurodegenerative, inflammatory, cardiovascular,

or immunoregulatory diseases, or any condition described herein. In some embodiments, compounds according to the invention, or for use according to the invention, may be provided in combination with one or more agents useful in the prevention or treatment of Alzheimer's disease. Examples of such agents include, without limitation,

- acetylcholine esterase inhibitors (AChEIs) such as Aricept® (Donepezil), Exelon® (Rivastigmine), Razadyne® (Razadyne ER®, Reminyl®, Nivalin®, Galantamine), Cognex® (Tacrine), Dimebon, Huperzine A, Phenserine, Debio-9902 SR (ZT-1 SR), Zanapezil (TAK0147), ganstigmine, NP7557, etc.;
- 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, etc.;
- beta-secretase inhibitors such as ATG-Z1, etc.;
- alpha-secretase activators, such as NGX267, etc;
- amyloid-β aggregation and/or fibrillization inhibitors such as Alzhemed™ (3APS, Tramiprosate, 3-amino-1-propanesulfonic acid), AL-108, AL-208, AZD-103, PBT2, Cereact, ONO-2506PO, PPI-558, etc.;
- tau aggregation inhibitors such as methylene blue, etc.;
- microtubule stabilizers such as AL-108, AL-208, paclitaxel, etc.;
- RAGE inhibitors, such as TTP488, etc.;
- 5-HT1a receptor antagonists, such as Xaliproden, Lecozotan, etc.;
- 5-HT4 receptor antagonists, such as PRX-03410, etc.;
- kinase inhibitors such as SRN-003-556, amfurindamide, LiCl, AZD1080, NP031112, SAR-502250, etc.
- humanized monoclonal anti-Aβ 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.;
- L-type calcium channel antagonists such as MEM-1003, etc.;
- nicotinic receptor antagonists, such as AZD3480, GTS-21, etc.;
- nicotinic receptor agonists, such as MEM 3454, Nefiracetam, etc.;

- peroxisome proliferator-activated receptor (PPAR) gamma agonists such as Avandia® (Rosglitazone), etc.;
- phosphodiesterase IV (PDE4) inhibitors, such as MK-0952, etc.;
- hormone replacement therapy such as estrogen (Premarin), etc.;
- monoamine oxidase (MAO) inhibitors such as NS2330, Rasagiline (Azilect®), TVP-1012, etc.;
- AMPA receptor modulators such as Ampalex (CX 516), etc.;
- nerve growth factors or NGF potentiators, such as CERE-110 (AAV-NGF), T-588, T-817MA, etc.;
- agents that prevent the release of luteinizing hormone (LH) by the pituitary gland, such as leuoprolide (VP-4896), etc.;
- GABA receptor modulators such as AC-3933, NGD 97-1, CP-457920, etc.;
- benzodiazepine receptor inverse agonists such as SB-737552 (S-8510), AC-3933, etc.;
- noradrenaline-releasing agents such as T-588, T-817MA, etc.

[0084] 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).

[0085] In alternative embodiments, the compounds may be supplied as "prodrugs" or protected forms, which release the compound after administration to a subject. For example, the compound may carry a protective group which is split off by hydrolysis in body fluids, *e.g.*, in the bloodstream, thus releasing the active compound or is oxidized or reduced in body fluids to release the compound. Accordingly, a "prodrug" is meant to indicate a compound that may be converted under physiological conditions or by solvolysis to a biologically active compound of the invention. Thus, the term "prodrug" refers to a metabolic precursor of a compound of the invention that is pharmaceutically acceptable. A prodrug may be inactive when administered to a subject in need thereof, but is converted in vivo to an active compound of the invention. Prodrugs are typically rapidly transformed *in vivo* to yield the parent compound of the invention, for example, by hydrolysis in blood. The

prodrug compound often offers advantages of solubility, tissue compatibility or delayed release in a subject.

[0086] The term "prodrug" is also meant to include any covalently bonded carriers which release the active compound of the invention *in vivo* when such prodrug is administered to a subject. Prodrugs of a compound of the invention may be prepared by modifying functional groups present in the compound of the invention in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compound of the invention. Prodrugs include compounds of the invention wherein a hydroxy, amino or mercapto group is bonded to any group that, when the prodrug of the compound of the invention is administered to a mammalian subject, cleaves to form a free hydroxy, free amino or free mercapto group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and acetamide, formamide, and benzamide derivatives of amine functional groups in the compounds of the invention and the like.

[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); Bundgard, 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 (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^1 is C(O)R and R^3 is OC(O)R, where R is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl. In these cases the ester groups may be hydrolyzed in vivo (e.g. in bodily fluids), releasing the active compounds in which R^1 is H and R^3 is OH. Preferred prodrug embodiments of the invention are the compounds of Formula (I) where R^1 is $C(O)CH_3$ and R^3 is $OC(O)CH_3$.

[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 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 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 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, 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 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, 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, 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, glycollylarsanilate, sulfate, hexylresorcinate, subacetate, hydradamine, succinate, 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 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.

[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.

[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 patch or implant may be employed to provide release over a prolonged period of time. Many techniques known to skilled practitioners are described in *Remington: the Science & Practice of Pharmacy* by Alfonso Gennaro, 20th ed., Williams & Wilkins, (2000). Formulations for parenteral administration may, for example, contain excipients, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

[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. 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. 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, pessaryies. In some embodiments, compounds or pharmaceutical compositions in accordance with this invention or for use in this invention may be administered by inhalation spray, nasal, vaginal, rectal, sublingual, or topical routes and may be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles appropriate for each route of administration.

[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 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 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 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 time necessary, to achieve the desired prophylactic result, such as inhibition of an O-GlcNAcase, elevation of O-GlcNAc levels, inhibition of tau phosphorylation, or any condition described herein. Typically, a prophylactic dose is used in subjects prior to or at an earlier stage of disease, so that a prophylactically effective amount may be less than a therapeutically effective amount. A suitable range for therapeutically or prophylactically effective amounts of a compound may be any integer from 0.1 nM-0.1M, 0.1 nM-0.05M, 0.05 nM-15μM or 0.01 nM-10μM.

[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 500 mg per kg subject body weight per day, and can be administered in singe 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 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 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, 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.

[00103]

[00104] Other Uses and Assays

[00105] A compound of Formula (I) may be used in screening assays for compounds which modulate the activity of glycosidase enzymes, preferably the O-GlcNAcase enzyme. The ability of a test compound to inhibit O-GlcNAcase-dependent cleavage of O-GlcNAc from a model substrate may be measured using any assays, as described herein or known to one of ordinary skill in the art. For example, a fluoresence 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, 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).

[00106] 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 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.

[00107] In general, test compounds are identified from large libraries of both natural products or synthetic (or semi-synthetic) extracts or chemical libraries according to methods known in the art. Those skilled in the field of drug discovery and development will

understand that the precise source of test extracts or compounds is not critical to the method(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceanographic Institute (Ft. Pierce, FL, USA), and PharmaMar, MA, USA. In addition, natural and synthetically produced 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.

[00108] When a crude extract is found to modulate inhibition of O-GlcNAcase-dependent 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 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 on known in the art.

[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-

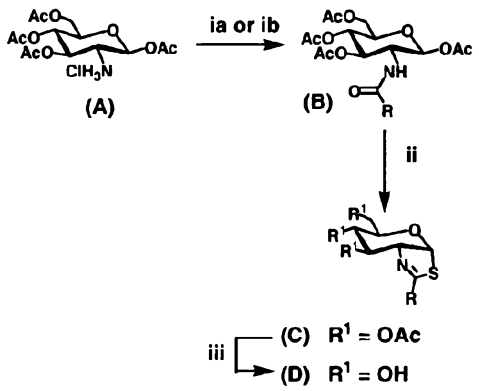
GlcNAcase, or accumulation or depletion of O-GlcNAc. Such diseases and disorders include neurodegenerative diseases, including Alzheimer's disease, and cancer.

[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.

[00111]

EXAMPLES

[00112] The following examples are intended to illustrate embodiments of the invention and are not intended to be construed in a limiting manner. Many compounds in the following examples were prepared according to the synthetic route outlined in Scheme 1.



ia) RCOCl, Et_3N, CH_2Cl_2, 0 °C; ib) EDC or DCC, RCOOH ii) Lawesson's Reagent, Tol, Δ; iii) a) NaOMe, MeOH, b) AcOH, MeOH

Scheme 1: Preparation of compounds

General Procedures:

[00113] General Procedure A: Synthesis of 2-amido sugars (**B**). To a suspension of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride (2.0 g, 5.2 mmol) in CH_2Cl_2 (50 mL) was added triethylamine (1.45 mL, 10.4 mmol), at which time the starting material dissolved. The reaction mixture was cooled to 0 °C, and 1.5 equiv of the appropriate acid chloride (7.8 mmol) was added via syringe. The resultant mixture was stirred for 2 h at room temperature. When the reaction mixture was judged complete by TLC analysis, EtOAc (200 mL) was added. The organic phase was washed successively with water, 1 M

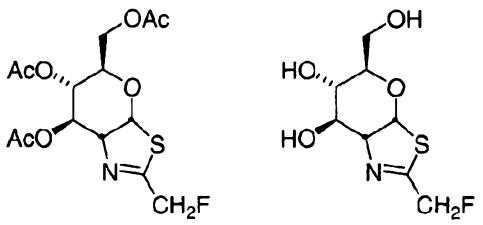
aqueous NaOH, and brine. The organic phase was dried ($MgSO_4$) and concentrated to yield a white crystalline solid. The material thus obtained was recrystallized (EtOAc/hexanes) to yield the desired *N*-acylated materials.

[00114] General Procedure B: Synthesis of tri-O-acetyl-protected thiazolines (**C**). Lawesson's reagent (0.6 eq) was added to a solution of the appropriate amide (**B**) in anhydrous toluene, and the reaction mixture was refluxed for 2-8 h. The reaction stopped when conversion was judged complete by TLC analysis, at which time the solution was cooled to room temperature, and the solvent was removed in vacuo. The desired material was isolated by flash column silica chromatography using a solvent system of hexanes and EtOAc in ratios ranging from 5:1 to 10:1 as appropriate. Products were isolated and used in the next step without further purification.

[00115] General Procedure C: Synthesis of deprotected thiazolines (**D**). A spatula tip of anhydrous sodium methoxide was added to a solution of the appropriate protected thiazoline in MeOH. The basic solution was stirred until the reaction was judged complete by TLC analysis (typically 1 h). A solution of glacial acetic acid in MeOH (1:20) was added dropwise to the reaction mixture until the pH of the solution was found to be neutral. The solvent was then removed in vacuo, and the desired materials were isolated as syrups by flash silica chromatography using a solvent system of EtOAc and MeOH in ratios ranging from 5:1 to 10:1 as appropriate.

Example 1

Compounds 1 and 2: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(fluoromethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**1**) and (3aR,5R,6S,7R,7aR)-2-(fluoromethyl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**2**)



1 **2**

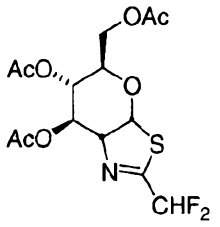
[00116] Triethylamine (0.8 mL) and dry pyridine (20 mL) were added to a cooled (0 ^{0}C) solution of 2-amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose hydrochloride (1 g) in a solution of DMF (100 mL). Sodium fluoroacetate (1.8 g) was added to a stirred mixture of dry DMF (90 mL) containing dried Dowex 50-H^+ resin (12 g). After 1 h, DCC (3.2 g) and 30 mL of the fluoroacetic acid solution were added via cannula to the reaction vessel containing the hydrochloride salt. The

resulting solution was allowed to stand for 16 h at 0 ^{0}C, after which time the reaction was judged complete by TLC analysis. The solvent was partially removed *in vacuo* and EtOAc (300 mL) and a solution of saturated sodium chloride (100 mL) were added. The organic layer was collected, and the aqueous layer was extracted twice with EtOAc. The combined organic extracts were washed successively with water, twice with saturated aqueous $NaHCO_3$, and finally with a solution of brine. The organic extracts were dried over $MgSO_4$ and filtered, and the solvent was removed *in vacuo* to yield colorless syrup. The desired product was purified using flash chromatography on silica gel (2:1; hexanes/EtOAc) to yield the partially purified amide that was used in the next step without further purification.

[00117] The title compounds were prepared from the material thus obtained following General Procedures B and C. For **2**: ^{1}H NMR (500 MHz, methanol-d_4) δ 3.28 (dd, 1H, J = 2.5, 6.4 Hz), 3.54 (m, 1H), 3.57 (m, 1H), 3.70 (m, 1H), 4.14 (t, 1H, J = 4.1 Hz), 4.38 (m, 1H), 5.17 (tdd, 2H, J = 2.2, 13.1, 53.4 Hz), 6.41 (d, 1H, J = 7.0 Hz).

Example 2

Compounds 3 and 4: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(difluoromethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**3**) and (3aR,5R,6S,7R,7aR)-2-(difluoromethyl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**4**)



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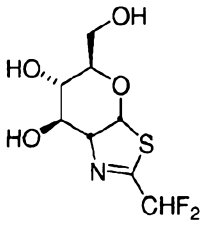


4

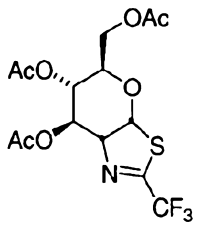
[00118] Triethylamine (0.8 mL) and dry pyridine (20 mL) were added to a cooled (0 °C) solution of 2-amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose hydrochloride (1 g) in a solution of DMF (100 mL). Dicyclohexylcarbodiimide (DCC, 3 g) and difluoroacetic acid (1.2 mL) were added to the reaction mixture via syringe. The resulting solution was allowed to stand for 16 h at 0 °C, after which time another 0.5 mL of difluoroacetic acid were added. After a further 3.5 h at room temperature, the reaction was judged complete by TLC analysis. The solvent was partially removed *in vacuo* and EtOAc (300 mL) and a solution of saturated sodium chloride (100 mL) were added. The organic layer was collected, and the aqueous layer was extracted twice with EtOAc. The combined organic extracts were washed successively with water, twice with saturated aqueous

$NaHCO_3$, and finally with a solution of brine. The organic extracts were dried over $MgSO_4$ and filtered, and the solvent was removed *in vacuo* to yield colorless syrup. The desired product was purified using flash chromatography on silica gel (3:1; hexanes/EtOAc) to yield the partially purified amide that was used in the next step without further purification.

[00119] The title compounds were prepared from the material thus obtained following General Procedures B and C. For **4**: ^{1}H NMR (500 MHz, methanol-d_4) δ 3.27 (dd, 1H, J = 2.5, 6.3 Hz), 3.55 (m, 1H), 3.58 (m, 1H), 3.71 (m, 1H), 4.14 (t, 1H, J = 4.6 Hz), 4.44 (m, 1H), 6.43 (t, 1H, J = 54.3 Hz), 6.50 (d, 1H, J = 7.1 Hz).

Example 3

Compounds 5 and 6: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(trifluoromethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**5**) and (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(trifluoromethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**6**)



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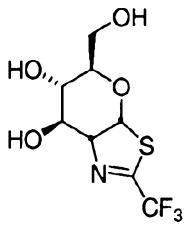


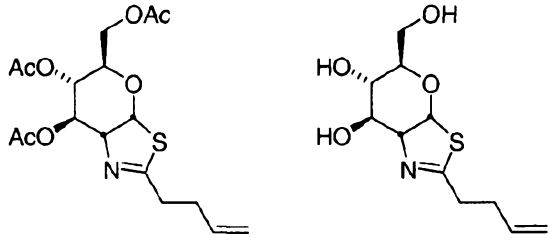
6

[00120] Triethylamine (0.8 mL) was added to a solution of 2-amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose hydrochloride (1 g) dissolved in dry dichloromethane (20 mL) and cooled (0 ^{0}C). Trifluoroacetic anhydride (0.6 mL) was added via syringe and the resulting solution was allowed to stand for 16 h at 0 ^{0}C, after which time the reaction was judged complete by TLC analysis. The solution was diluted in 50 mL of EtOAc and washed successively with water, twice with saturated aqueous $NaHCO_3$, and finally with a solution of brine. The organic extracts were dried over $MgSO_4$ and filtered, and the solvent was removed *in vacuo* to yield colorless syrup. The desired product was purified using flash chromatography on silica gel (4:1; hexanes/EtOAc) to yield the partially purified amide that was used in the next step without further purification.

[00121] The title compounds were prepared from the material thus obtained following General Procedures B and C. For **6**: ^{1}H NMR (500 MHz, methanol-d_4) δ: 3.29 (m, 1H), 3.55 (m, 1H), 3.59 (m, 1H), 3.72 (m, 1H), 4.12 (t, 1H, J = 4.5 Hz), 4.38 (m, 1H), 6.64 (d, 1H, J = 7.1 Hz).

Example 4

Compounds 7 and 8: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(but-3-enyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**7**) and (3aR,5R,6S,7R,7aR)-2-(but-3-enyl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**8**)



7 **8**

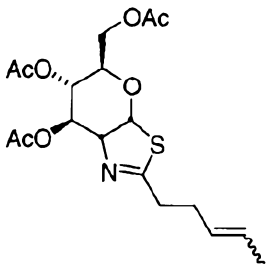
[00122] Following General Procedure A, (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride and pent-4-enoyl chloride were converted to (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-pent-4-enamido-tetrahydro-2H-pyran-2,4,5-triyl triacetate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.01 (s, 3H), 2.02 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H), 2.18-2.22 (m, 2H), 2.28-2.32 (m, 2H), 3.80 (ddd, 1H, J = 2.1, 4.6, 9.5 Hz), 4.10 (dd, 1H, J = 2.1, 12.5 Hz), 4.24 (dd, 1H, J = 4.6, 12.5 Hz), 4.30 (dd, 1H, J = 9.2, 19.3 Hz), 4.95 (ddd, 1H, J = 1.6, 3.1, 10.2 Hz), 5.01 (ddd, 1H, J = 1.6, 2.6, 17.2 Hz), 5.10 (dd, 1H, J = 9.5, 9.5 Hz), 5.16 (dd, 1H, J = 9.5, 9.5 Hz), 5.68 (d, 1H, J = 8.8 Hz), 5.74 (dddd, 1H, J = 2.6, 3.1, 10.2, 17.2 Hz), 5.98 (d, 1H, J = 9.5 Hz).

[00123] Following General Procedure B, the amide obtained above was converted to (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(but-3-enyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**7**). ^{1}H NMR (500 MHz, methanol-d_4) δ 2.08 (s, 3H), 2.09 (s, 3H), 2.14 (s, 3H), 2.45 (m, 2H), 2.68 (m, 2H), 3.55 (ddd, 1H, J = 3.2, 5.8, 12.3 Hz), 4.09 (dd, 1H, J = 5.9, 12.3 Hz), 4.12 (dd, 1H, J = 3.2, 12.3 Hz), 4.48 (ddd, 1H, J = 1.5, 3.2, 7.0 Hz), 4.94 (m, 1H), 5.02 (m, 1H), 5.10 (m, 1H), 5.58 (dd, 1H, J = 1.6, 3.2 Hz), 5.86 (ddd, 1H, J = 6.5, 10.3, 17.1 Hz), 6.22 (d, 1H, J = 7.2 Hz).

[00124] Following General Procedure C, the thiazoline obtained above was converted to the title compound (**8**). ^{1}H NMR (500 MHz, methanol-d_4) δ 2.42 (m, 2H), 2.65 (m, 2H), 3.35 (ddd, 1H, J = 2.5, 6.4, 12.1), 3.56 (dd, 1H, J = 3.6, 9.1 Hz), 3.61 (dd, 1H, J = 6.4, 12.1 Hz), 3.73 (dd, 1H, J = 2.5, 12.1 Hz), 4.12 (t, 1H, J = 4.2 Hz), 4.32 (m, 1H), 5.02 (m, 1H), 5.10 (m, 1H), 5.86 (ddd, 1H, J = 6.5, 10.2, 17.1 Hz), 6.35 (d, 1H, J = 7.0 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 31.42, 34.08, 62.26, 70.02, 73.10, 75.08, 79.01, 89.05, 115.22, 136.71, 173.51.

Example 5

Compounds 9 and 10: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(E,Z)-(pent-3-enyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**9**) and (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(E,Z)-(pent-3-enyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**10**)



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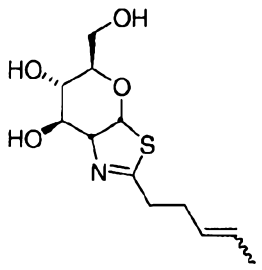

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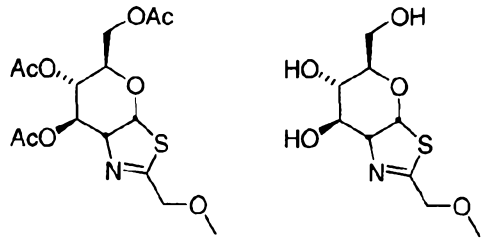
[00125] Following General Procedure A, (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride and (E,Z)-hex-4-enoyl chloride were converted to (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-((E,Z)-hex-4-enamido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.61-1.65 (m, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.11 (s, 3H), 2.14-2.20 (m, 2H), 2.22-2.28 (m, 2H), 3.78-3.82 (m, 1H), 4.13 (dd, 1H, *J* = 2.2, 12.5 Hz), 4.27 (dd, 1H, *J* = 4.6, 12.5 Hz), 4.28-4.36 (m, 1H), 5.10-5.18 (m, 2H), 5.32-5.40 (m, 1H), 5.42-5.52 (m, 1H), 5.51-5.54 (m, 1H), 5.67-5.70 (m, 1H).

[00126] Following General Procedure B, the amide obtained above was converted to (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-((E,Z)-pent-3-enyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**9**). ^{1}H NMR (500 MHz, methanol-d_4) δ 1.60 (m, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.35 (m, 2H), 2.61 (m, 2H), 3.5 (m, 1H), 4.08 (d, 2H, *J* = 4.5 Hz), 4.45 (m, 1H), 4.91 (d, 1H, *J* = 9.5 Hz), 5.45 (m, 2H), 5.55 (dd, 1H, *J* = 1.3, 3.1 Hz), 6.18 (d, 1H, *J* = 7.2 Hz).

[00127] Following General Procedure C, the thiazoline obtained above was converted to the title compound (**10**). ^{1}H NMR (500 MHz, methanol-d_4) δ 1.64 (d, 3H, *J* = 6.0 Hz), 2.35 (m, 2H), 2.60 (m, 2H), 3.34 (ddd, 1H, *J* = 2.0, 6.3, 12.0), 3.56 (dd, 1H, *J* = 3.7, 9.2 Hz), 3.61 (dd, 1H, *J* = 6.3, 12.0 Hz), 3.73 (dd, 1H, *J* = 2.0, 12.0 Hz), 4.12 (t, 1H, *J* = 4.2 Hz), 4.30 (t, 1H, *J* = 5.9 Hz), 5.50 (m, 2H), 6.35 (d, 1H, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 16.88, 30.36, 34.72, 62.30, 70.14, 73.12, 75.04, 78.97, 88.88, 126.41, 129.06, 173.81.

Example 6

Compounds 11 and 12: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(methoxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**11**) and (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(methoxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**12**)



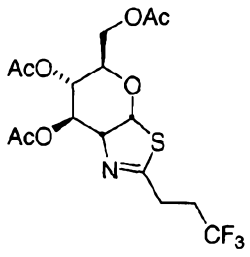
11 **12**

[00128] To a suspension of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride (0.500 g, 1.31 mmol) in CH_2Cl_2 (20 mL) was added triethylamine (0.544 mL, 3.915 mmol), followed by 2-methoxyacetyl chloride (0.13 mL, 1.44mmol). The reaction was stirred at room temperature for 18 h. The reaction mixture was washed once with saturated aqueous $NaHCO_3$ (3 mL) and once with brine (3 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification of the crude material by flash chromatography on silica gel (5:1 EtOAc:hexanes) afforded (2S, 3R, 4R, 5S, 6R)-6-(acetoxymethyl)-3-(2-methoxyacetamido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white solid (0.380 g, 70% yield).

[00129] The amide obtained above was converted to the title compounds following General Procedures B and C. For **12**: ^{1}H NMR (500 MHz, methanol-d_4) δ 3.35 (s, 1H), 3.37 (dd, 1H, J = 2.5, 12.1 Hz), 3.41 (s, 3H), 3.59 (m, 1H), 4.16 (t, 1H, J = 4.0 Hz), 4.29 (m, 2H), 4.38 (m, 1H), 6.36 (d, 1H, J = 7.0 Hz).

Example 7

Compounds 13 and 14: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(3,3,3-trifluoropropyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**13**) and (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(3,3,3-trifluoropropyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**14**)



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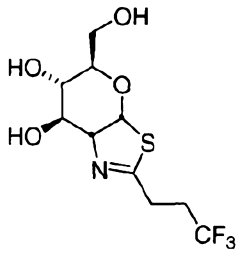

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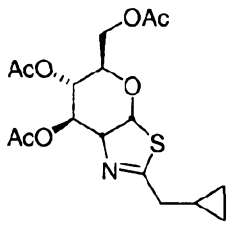
[00130] To a suspension of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride (0.500 g, 1.31 mmol) in CH_2Cl_2 (20 mL) was added 4-(dimethylamino)pyridine (0.478 g, 3.91 mmol), followed by 1- (3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.300 g, 1.57 mmol), and 4,4,4-trifluorobutyric acid (0.222 g, 1.56 mmol). The reaction was stirred at room temperature overnight. The reaction was diluted with CH_2Cl_2 (80 mL) and the organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated in vacuo. Crystallization of the crude material thus obtained (EtOAc/hexanes) afforded (2S, 3R, 4R, 5S, 6R)- 6-(acetoxymethyl)-3-(4,4,4-trifluorobutanamido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white solid (0.398, 68% yield).

[00131] The amide obtained above was converted to the title compounds following General Procedures B and C. For **13**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.09 (s, 3H), 2.10 (s, 3H), 2.15 (s, 3H), 2.48-2.65 (m, 2H), 3.49-3.54 (m, 1H), 4.12-4.13 (m, 2H), 4.48-4.50 (m, 1H), 4.95 (dt, 1H, J = 1.5, 4.9 Hz), 5.56 (dd, 1H, J = 1.8, 5.6 Hz), 6.28 (d, 1H, J = 7.2 Hz). For **14**: ^{1}H NMR (500 MHz, methanol-d_4) δ 2.61 (m, 2H), 2.81 (m, 2H), 3.59 (m, 2H), 3.74 (dd, 1H, J = 2.4, 12.1 Hz), 4.15 (t, 1H, J = 3.9 Hz), 4.35 (m, 1H), 5.49 (s, 1H), 6.40 (d, 1H, J = 7.0 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 27.22, 30.94, 62.29, 70.09, 70.89, 75.13, 79.25, 89.53, 170, 207.59.

Example 8

Compounds 15 and 16: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(cyclopropylmethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**15**) and (3aR,5R,6S,7R,7aR)-2-(cyclopropylmethyl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**16**)



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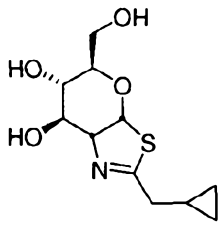


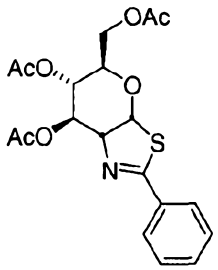
16

[00132] To a suspension of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride (0.500 g, 1.31 mmol) in CH_2Cl_2 (20 mL) was added 4-(dimethylamino)pyridine (0.478 g, 3.91 mmol), followed by 1- (3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.300 g, 1.57 mmol), and 2-cyclopropylacetic acid (0.146 mL, 1.57 mmol). The reaction was stirred for 12 h. Additional CH_2Cl_2 (80 mL) was added and the organic layer was washed once with saturated aqueous $NaHCO_3$ (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography of the crude material on silica gel (3:2 EtOAc:hexanes) afforded (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(2-cyclopropylacetamido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white solid (0.256 g, 56% yield).

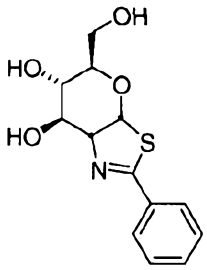
[00133] The amide obtained above was converted to the title compounds following General Procedures B and C. For **15**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.00-0.03 (m, 2H), 0.30-0.37 (m, 2H), 0.73-0.81 (m, 1H), 1.82 (s, 3H), 1.83 (s, 3H), 1.89 (s, 3H), 2.14-2.28 (m, 2H), 3.31-3.34 (m, 1H), 3.81-3.91 (m, 2H), 4.23-4.26 (m, 1H), 4.70 (d, 1H, J = 9.5 Hz), 5.33-5.34 (m, 1H), 5.97 (d, 1H, J = 7.1 Hz). ^{13}C NMR (500 MHz, $CDCl_3$) δ 4.92, 5.32, 9.48, 20.96, 21.10, 39.74, 63.63, 68.58, 69.55, 70.85, 76.34, 88.14, 169.51, 169.77, 170.78, 172.95. For **16**: ^{1}H NMR (500 MHz, methanol-d_4) δ 0.03 (m, 2H), 0.35 (m, 2H), 0.77 (m, 1H), 2.23 (d, 2H, J = 7.2 Hz), 3.15 (m, 1H), 3.44 (m, 2H), 3.52 (dd, 1H, J = 2.7, 12.0 Hz), 3.94 (t, 1H, J = 3.9 Hz), 4.12 (t, 1H, J = 5.1 Hz), 6.15 (d, 1H, J = 7.0 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 4.39, 8.96, 39.41, 62.27, 70.10, 73.04, 75.07, 78.70, 88.56, 174.41.

Example 9

Compounds 17 and 18: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-phenyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**17**) and (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-phenyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**18**)



17



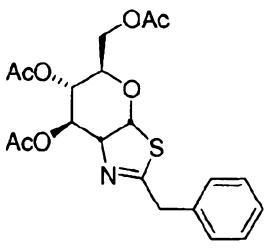
18

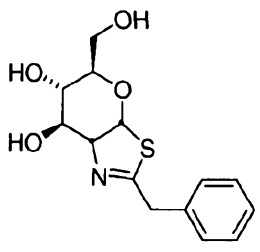
[00134] To a suspension of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride (0.500 g, 1.31 mmol) in CH_2Cl_2 (20 mL) was added 4-(dimethylamino)pyridine (0.478 g, 3.91 mmol), followed by benzoyl chloride (0.198 g, 1.57 mmol). The reaction was stirred for 2 h. Additional CH_2Cl_2 (80 mL) was added and the organic layer was washed once with saturated aqueous $NaHCO_3$ (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated in vacuo. Crystallization of the crude material thus obtained (EtOAc/hexanes) afforded (2S, 3R, 4R, 5S, 6R)-6-(acetoxymethyl)-3-benzamido-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white solid (0.418, 69% yield).

[00135] The amide obtained above was converted to the title compounds following General Procedures B and C. For **17**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.06 (s, 3H), 2.07 (s, 3H), 2.18 (s, 3H), 3.61-3.64 (m, 1H), 4.73-4.75 (m, 1H), 5.00 (d, 1H, J = 9.3 Hz), 5.73-5.74 (m, 1H), 6.37 (d, 1H, J = 7.1 Hz), 7.14-7.18 (m, 1H), 7.25 (t, 1H, J = 8.0 Hz), 7.46 (t, 2H, J = 7.7 Hz), 7.52 (t, 1H, J = 7.4 Hz), 7.86 (d, 2H, J = 7.2 Hz). For **18**: ^{1}H NMR (500 MHz, methanol-d_4) δ 3.63 (m, 2H), 3.76 (dd, 1H, J = 2.5, 12.1 Hz), 4.28 (t, 1H, J = 4.3 Hz), 4.57 (dd, 1H, J = 5.0, 11.8 Hz), 4.62 (m, 1H),6.49 (d, 1H, J = 6.9 Hz), 7.48 (m, 3H) ,7.85 (m, 2H). ^{13}C NMR (125 MHz, methanol-d_4) δ 62.27, 70.27, 73.35, 75.38, 80.21, 88.85, 128.18, 128.52, 131.57, 133.32, 169.25.

Example 10

Compounds 19 and 20: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-benzyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**19**) and (3aR,5R,6S,7R,7aR)-2-benzyl-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**20**)



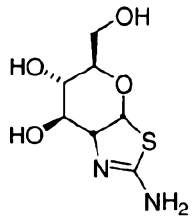


[00136] To a suspension of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride (0.500 g, 1.31 mmol) in CH_2Cl_2 (20 mL) was added 4-(dimethylamino)pyridine (0.478 g, 3.91 mmol) followed by 1- (3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.300 g, 1.57 mmol) and 2-phenylacetic acid (0.2132 g, 1.57 mmol). The reaction was stirred for 12 h. Additional CH_2Cl_2 (80 mL) was added and the organic layer was washed once with saturated aqueous $NaHCO_3$ (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography of the crude material on silica gel (3:2 EtOAc:hexanes) followed by crystallization (EtOAc/hexanes) afforded (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(2-phenylacetamido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white solid (0.418 g, 69% yield).

[00137] The amide obtained above was converted to the title compounds following General Procedures B and C. For **19**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.06 (s, 3H), 2.07 (s, 3H), 2.18 (s, 3H), 3.61-3.64 (m, 1H), 4.73-4.75 (m, 1H), 5.00 (d, 1H, J = 9.3 Hz), 5.73-5.74 (m, 1H), 6.37 (d, 1H, J = 7.1 Hz), 7.14-7.18 (m, 1H), 7.25 (t, 1H, J = 8.0 Hz), 7.46 (t, 2H, J = 7.7 Hz), 7.52 (t, 1H, J = 7.4 Hz), 7.86 (d, 2H, J = 7.2 Hz). For **20**: ^{1}H NMR (500 MHz, methanol-d_4) δ 3.35 (m, 2H), 3.62 (m, 2H), 3.70 (dd, 1H, J = 2.6, 12.1 Hz), 3.85 (m, 1H), 4.15 (t, 1H, J = 4.48 Hz), 4.36 (t, 1H, J = 5.5 Hz), 6.34 (d, 1H, J = 7.0 Hz), 7.27 (m, 5H). ^{13}C NMR (125 MHz, methanol-d_4) δ 40.96, 62.12, 69.94, 73.17, 75.13, 78.89, 89.27, 127.14, 128.59, 129.01, 135.89, 173.17.

Example 11

Compound 21: (3aR,5R,6S,7R,7aR)-2-amino-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol



21

[00138] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride (250 mg, 0.65 mmol) in CH_2Cl_2 (5 mL) was added triethylamine (90 μL, 0.65 mmol). The solution was diluted saturated aqueous $NaHCO_3$ (20 mL),

then the resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated to give presumably (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate (220 mg) which was used without further purification.

[00139] The amine from above (220 mg) was dissolved in pyridine (5 mL) and 9-Fluorenylmethoxycarbonyl isothiocyanate (180 mg, 0.65 mmol) and triethylamine (0.02 mL) was added. Then the resulting mixture was stirred at room temperature for 16 h. The solution was concentrated and the residue taken up in CH_2Cl_2 (20 mL) and diluted with saturated aqueous $NaHCO_3$ (20 mL), then the resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (EtOAc:hexanes 2:3) gave (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)thioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white foam (360 mg, 89% yield over two steps). ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.06 (s, 3H), 2.07 (s, 3H), 2.12 (s, 3H), 2.14 (s, 3H), 3.89 (ddd, 1H, J = 2.4, 4.8, 9.6 Hz), 4.17 (dd, 1H, J = 2.4, 12.5 Hz), 4.24 (dd, 1H, J = 6.6, 6.6 Hz), 4.33 (dd, 1H, J = 4.8, 12.5 Hz), 4.52 (s, 1H), 4.54 (s, 1H), 5.08-5.12 (m, 1H), 5.22 (dd, 1H, J = 9.5, 9.6 Hz), 5.34 (dd, 1H, J = 6.6, 9.5 Hz), 5.88 (d, 1H, J = 6.6 Hz), 7.36 (dd, 2H, J = 7.2, 7.8 Hz), 7.45 (dd, 2H, J = 7.2, 7.8 Hz), 7.57 (d, 2H, J = 7.8 Hz), 7.80 (d, 2H, J = 7.2 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.62, 20.72, 20.77, 21.05, 46.49, 57.59, 61.65, 67.47, 68.40, 72.21, 72.87, 92.21, 120.09, 120.29, 124.84, 124.96, 125.32, 127.30, 127.83, 128.15, 128.25, 129.06, 141.37, 142.80, 152.16, 169.27, 169.34, 170.46, 170.72, 180.22.

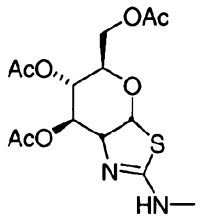
[00140] The thiourea from above (200 mg, 0.32 mmol) was dissolved in CH_2Cl_2 (4 mL) and $SnCl_4$ (0.5 mL, 4.0 mmol) was added. Then the resulting mixture was stirred at room temperature for 16 h. The solution was diluted saturated aqueous $NaHCO_3$ (20 mL), then the resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated. Flash chromatography of the residue (EtOAc:hexanes 2:3) gave (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate as a pale colourless foam (125 mg, 69% yield). ^{1}H NMR 500 MHz, $CDCl_3$) δ 1.88 (s, 3H), 2.04 (s, 3H), 2.12 (s, 3H), 3.69 (m, 1H), 3.81 (ddd, 1H, J = 2.5, 5.5, 6.5 Hz), 4.12 (dd, 1H, J = 2.5, 12.5 Hz), 4.21-4.26 (m, 2H), 4.55 (dd, 1H, J = 6.0, 12.5 Hz), 4.72-4.75 (dd, 1H, J = 6.5, 6.5 Hz), 4.95 (dd, 1H J = 5.5, 9.5 Hz), 5.24 (dd, 1H J = 6.5, 9.5 Hz), 6.01 (d, 1H, J = 6.5 Hz), 7.35 (dd, 2H, J = 7.1, 7.8 Hz), 7.44 (dd, 2H, J = 7.0, 7.8 Hz), 7.59 (d, 2H, J = 7.1 Hz), 7.81 (d, 2H, J = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.62, 20.71, 21.35, 46.78, 57.91, 63.70, 67.92, 69.46, 71.88, 72.52, 90.23, 120.71, 120.89, 123.83, 124.04, 125.15, 127.76,

127.97, 129.61, 129.72, 130.08, 141.31, 142.86, 152.11, 161.43, 169.11, 169.63, 170.23.

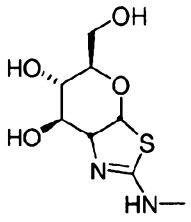
[00141] The triacetate from above (114 mg, 0.20 mmol) was dissolved in MeOH (2.0 mL) and then NaOMe (14 mg, 0.25 mmol) was added. The resulting mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of AcOH. Concentration gave a colourless oil which was dissolved in pyridine (3 mL) and then piperidine (0.6 mL) was added. The resulting mixture was stirred at room temperature for 2 h. The mixture was then concentrated and any remaining piperidine was co-evaporated with pyridine. The resultant residue was triturated with EtOAc to give the title compound (38 mg, 81% yield) as a white solid. ^{1}H NMR (600 MHz, methanol-d_4) δ 3.47 (dd, 1H, J = 5.0, 9.0 Hz), 3.57-3.66 (m, 2H), 3.78 (dd, 1H, J = 2.0, 12.5 Hz), 3.90 (dd, 1H, J = 5.5, 5.5 Hz), 4.04 (dd, 1H, J = 6.0, 6.0 Hz), 6.31 (d, 1H, J = 6.0 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 61.15, 69.14, 73.45, 74.14, 74.52, 89.62, 161.08. Anal. Calcd. for $C_7H_{12}N_2O_4S$: C, 38.17; H, 5.49; N, 12.72; Found: C, 38.05; H, 5.37; N, 12.66.

Example 12

Compounds 22 and 23: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**22**) and (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**23**)



22



23

[00142] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.51 g, 1.32 mmol) (Jochims, J.C. et al, *Tetrahedron*, **1965**, *21(9)*, 2611-16) in CH_3CN, was added neat methylamine hydrochloride (0.18 g, 2.64 mmol). The reaction was stirred at room temperature until complete by TLC (1.5 h). The reaction was washed with a minimal amount of saturated aqueous $NaHCO_3$ (15 mL). The aqueous layer was then extracted three times with DCM, and the organic layers were combined, dried with $MgSO_4$, filtered and concentrated. The concentrated mixture was purified via flash column chromatography on silica gel (EtOAc:hexanes, 1:1), providing (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-methylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.35 g, 62% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.06 (s, 3H), 2.08 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 2.98 (s, 3H), 3.82-3.88 (m, 1H), 4.10-4.16 (m, 2H),

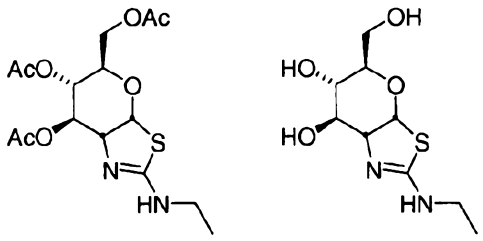
4.28 (dd, 1H, J = 4.6, 12.5 Hz), 5.17-5.22 (m, 2H), 5.74 (d, 1H, J = 8.1 Hz), 5.92 (s, 1H), 6.21 (s, 1H).

[00143] (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-methylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.457 g, 1.09 mmol) was added to dry DCM, and $SnCl_4$ (1.13 g, 4.33 mmol) was added dropwise. The reaction was stirred at room temperature overnight (16 h). The reaction was quenched with saturated aqueous $NaHCO_3$ until the solution was basic and no more gas was evolved. The aqueous layer was extracted three times with DCM and the combined organic layers were dried with $MgSO_4$, filtered and concentrated. The crude material was purified by flash chromatography (silica gel, EtOAc), providing (5R,6S,7R)-5-(acetoxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**22**) as an oil (0.30 g, 77% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 2.90 (s, 3H), 3.84 (m, 1H), 4.12 (m, 2H), 4.34 (dd, 1H, J = 4.3, 6.2 Hz), 4.90 (ddd, 1H, J = 0.8, 2.8, 9.6 Hz), 5.39 (dd, 1H, J = 2.9, 4.1 Hz), 6.21 (d, 1H, J = 6.5 Hz).. ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.00, 21.10, 21.23, 31.19, 63.40, 68.67, 69.40, 72.24, 73.01, 90.03, 161.21, 169.72, 169.90, 170.89.

[00144] The product isolated above (0.090 g, 0.250 mmol) was dissolved in anhydrous MeOH. Solid K_2CO_3 was added to the solution until it was basic, and the reaction was stirred at room temperature (5 h). A white solid, the desired product, precipitated out of solution. The final product, (5R,6S,7R)-5-(hydroxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**23**), was purified by isolating this solid and washing it several times with MeOH (0.038 g, 64% yield). ^{1}H NMR (500 MHz, D_2O) δ 2.67 (s, 3H), 3.40-3.43 (m, 1H), 3.48-3.54 (m, 2H), 3.65-3.68 (m, 1H), 3.90 (t, 1H, J = 5.1 Hz), 4.04 (t, 1H, J = 5.8 Hz), 6.14 (d, 1H, J = 6.4 Hz). ^{13}C NMR (125 MHz, D_2O) δ 29.83, 61.39, 69.28, 73.24, 73.61, 74.19, 88.52, 163.73. MS (CI): m/z 235 (M+1). Anal. Calcd. for $C_8H_{14}N_2O_4S$: C, 41.01; H, 6.02; N, 11.96; Found: C, 40.60; H, 5.56; N, 10.99.

Example 13

Compounds 24 and 25: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(ethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**24**) and (3aR,5R,6S,7R,7aR)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**25**)



24 25

[00145] To a suspension of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-amino-tetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride (2.04 g, 5.19 mmol) in CH_3CN (80 mL) was added ethyl isothiocyanate (1.36 g, 15.57 mmol), followed by triethylamine (0.94 g, 9.31 mmol). The reaction mixture was heated to reflux and stirred for 3 h. The organic layer was concentrated, and redissolved in CH_2Cl_2. The reaction was then washed with a minimal amount of saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted an additional two times with CH_2Cl_2. The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The desired (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-ethylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate was obtained as a yellow oil (2.21 g, 98% yield) following flash chromatography (EtOAc/hexanes, 1:1). ^1H NMR (500 MHz, $CDCl_3$) δ 1.19 (m, 3H), 1.81 (d, 1H, J = 4.0 Hz), 2.05 (s, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 2.14 (s, 3H), 3.40 (s, 1H), 3.85 (m, 1H), 4.12 (m, 1H), 4.28 (m, 1H), 4.81 (s, 1H), 5.19 (m, 1H), 5.73 (d, 1H, J = 7.7 Hz), 6.00 (s, 1H), 6.12 (d, 1H, J = 15.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.39, 20.81, 20.96, 21.07, 21.25, 57.84, 60.67, 61.95, 68.08, 72.99, 73.31, 93.12, 163.03, 169.60, 170.97, 171.88.

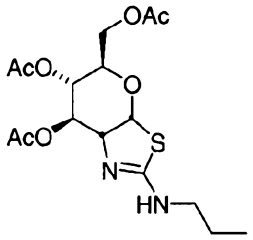
[00146] The thiourea isolated above (1.74 g, 4.01mmol) was dissolved in dry CH_2Cl_2. $SnCl_4$ (1.88 mL, 16.05 mmol) was added dropwise and the reaction turned slightly yellow. The reaction was allowed to stir overnight. The reaction was then quenched using a saturated aqueous solution of $NaHCO_3$ until the solution was neutral and no further CO_2 gas was evolved. The aqueous layer was extracted with CH_2Cl_2 three times, and the combined organic fractions were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the crude material via flash chromatography (silica gel, EtOAc) provided **24** as a pale yellow solid (1.35 g, 90% yield). ^1H NMR (500 MHz, $CDCl_3$) δ 1.22 (t, 3H, J = 7.1 Hz), 2.08 (s, 3H), 2.09 (s, 3H), 2.12 (s, 3H), 3.34 (m, 2H), 3.85 (m, 1H), 4.14 (d, 2H, J = 4.3 Hz), 4.37 (ddd, 1H, J = 0.8, 4.1, 6.4 Hz), 4.96 (ddd, 1H, J = 0.9, 2.6, 9.6 Hz), 5.43 (m, 1H), 6.24

(d, 1H, J = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.28, 20.81, 20.96, 21.07, 21.25, 57.84, 61.95, 68.08, 72.99, 73.31, 93.12, 169.60, 170.97, 171.88.

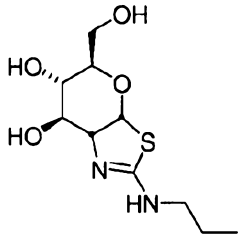
[00147] Deprotection of the thiazoline **24** described above following General Procedure C provided the title compound (**25**) as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 1.22 (t, 3H, J = 7.3 Hz), 3.35 (m, 2H), 3.49 (dd, 1H, J = 6.1, 9.0 Hz), 3.66, (m, 2H), 3.82 (dd, 1H, J = 1.8, 11.7 Hz), 3.89 (t, 1H, J = 6.2 Hz), 4.09 (t, 1H, J = 6.4 Hz), 6.44 (d, 1H, J = 6.4 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 13.73, 38.35, 62.07, 69.99, 74.57, 75.11, 89.72, 161.92. MS (CI): m/z 249 (M+1). Anal. Calcd. for $C_9H_{16}N_2O_4S$: C, 43.53; H, 6.49; N, 11.28; Found: C, 43.82; H, 6.62; N, 11.02.

Example 14

Compounds 26 and 27: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**26**) and (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**27**)



26



27

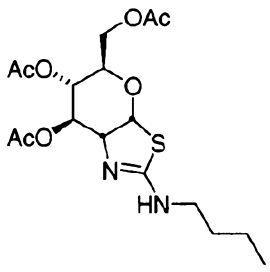
[00148] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (530 mg, 1.36 mmol) in CH_3CN (7 mL) was added neat propylamine hydrochloride (260 mg, 2.72 mmol) followed by triethylamine (378 μL, 2.72 mmol) and the resulting mixture was stirred for 1 h. Saturated aqueous $NaHCO_3$ (20 mL) was added, then the resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated. The resulting crude material was purified by flash chromatography on silica gel (hexane/EtOAc 1:1) to give (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-propylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white foam (532 mg, 87% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (t, 3H, J = 7.5 Hz), 1.59-1.64 (m, 2H), 1.53-1.59 (m, 2H), 2.06 (s, 3H), 2.08 (s, 3H), 2.12 (s, 3H), 2.15 (s, 3H), 3.35 (br s, 2H), 3.81-3.85 (m, 1H), 4.13-4.17 (m, 2H), 4.20-4.25 (m, 1H), 4.29 (dd, 1H, J = 4.5, 12.5 Hz), 5.17-5.23 (m, 2H), 5.73 (d, 1H, J = 8.5 Hz), 6.07 (br s, 1H).

[00149] The thiourea from above (230 mg, 0.51 mmol) was dissolved in CH_2Cl_2 (2.6 mL) and $SnCl_4$ (240 μL, 2.1 mmol) was added. Then the resulting mixture was stirred for 4 h. The solution was diluted saturated aqueous $NaHCO_3$ (50 mL), then the resulting mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated. The resulting crude material was purified by flash chromatography on silica gel (hexane/EtOAc 1:1 to 1:1.5) to give (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydropyrano[3,2-d]thiazole-6,7-diyl diacetate (**26**) as a pale yellow foam (150 mg, 75% yield). ^{1}H NMR (500 MHz) δ 0.93 (t, 3H, J = 7.5 Hz), 1.56-1.63 (m, 2H), 2.06 (s, 3H), 2.07 (s, 3H), 2.10 (s, 3H), 3.15-3.20 (m, 1H), 3.26 -3.31 (m, 1H), 4.12 (d, 2H, J = 4.0 Hz), 4.33-4.35 (m, 1H), 4.91-4.94 (m, 1H), 5.41 (dd, 1H, J = 3.0, 4.0 Hz), 6.21 (d, 1H, J = 6.5 Hz).

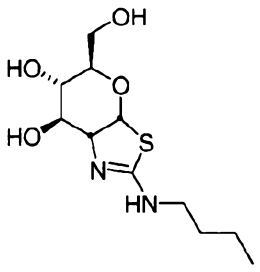
[00150] The triacetate from above (150 mg, 0.39 mmol) was dissolved in MeOH (2.5 mL) and then K_2CO_3 (55 mg, 0.39 mmol) was added. Then the resulting mixture was stirred at room temperature for 2 h. The mixture was diluted with CH_2Cl_2 (9 mL) and then poured onto the top of a basic Al_2O_3 (1 g) column. The column was eluted with 10-25% MeOH in CH_2Cl_2 to give the title compound **27** (57.4 mg, 57% yield) as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 0.94 (t, 3H, J = 7.5 Hz), 1.54-1.60 (m, 2H), 3.14-3.24 (m, 2H), 3.47 (dd, 1H, J = 5.0, 8.5 Hz), 3.58-3.66 (m, 2H), 3.78 (dd, 1H, J = 2.0, 11.5 Hz), 3.91 (t, 1H, J = 6.0 Hz), 4.03 (t, 1H, J = 6.0 Hz), 6.28 (d, 1H, J = 6.5 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 10.59, 22.47, 45.56, 62.03, 69.98, 74.54, 75.10, 89.62, 89.66, 162.17. MS (EI): m/z 263 (M+1). Anal. Calcd. for $C_{10}H_{18}N_2O_4S$: C, 45.79; H, 6.92; N, 10.68; Found: C, 45.58; H, 6.86; N, 10.77.

Example 15

Compounds 28 and 29: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(butylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**28**) and (3aR,5R,6S,7R,7aR)-2-(butylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**29**)



28



29

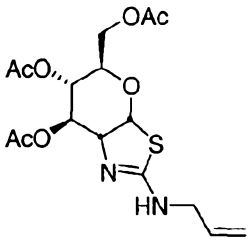
[00151] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (489 mg, 1.26 mmol) in CH_2Cl_2 (5 mL) was added neat butylamine (197 μL, 2.00 mmol) and the resulting mixture was stirred for 30 min. Saturated aqueous $NaHCO_3$ (20 mL) was added, then the resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated. The resulting crude material was purified by flash chromatography on silica gel (hexane/EtOAc 1:1) to give (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-butylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white foam (566 mg, 97% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, 3H, J = 7.5 Hz), 1.35-1.41 (m, 2H), 1.53-1.59 (m, 2H), 2. 06 (s, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 2.14 (s, 3H), 3.38 (br s, 2H), 3.82-3.85 (m, 1H), 4.13-4.16 (m, 2H), 4.20-4.25 (m, 1H), 4.29 (dd, 1H, J = 4.5, 12.5 Hz), 5.17-5.22 (m, 2H), 5.73 (d, 1H, J = 8.0 Hz), 6.10 (br s, 1H).

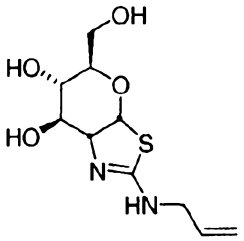
[00152] The thiourea from above (560 mg, 1.21 mmol) was dissolved in CH_2Cl_2 (6 mL) and $SnCl_4$ (567 μL, 4.84 mmol) was added, then the resulting mixture was stirred for 16 h. The solution was diluted saturated aqueous $NaHCO_3$ (50 mL), then the resulting mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated. The resulting crude material was purified by flash chromatography on silica gel (hexane/EtOAc 1:1 to 1:1.5) to give (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(butylamino)-5,6,7,7a-tetrahydropyrano[3,2-d]thiazole-6,7-diyl diacetate (**28**) as a pale yellow oil (320 mg, 66% yield). ^{1}H NMR (500 MHz) δ 0.93 (t, 3H, J = 7.5 Hz), 1.34-1.40 (m, 2H), 1.53-1.58 (m, 2H), 2.07 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 3.20-3.26 (m, 1H), 3.31-3.36 (m, 1H), 3.82-3.86 (m, 1H), 4.14 (d, 2H, J = 4.5 Hz), 4.35-4.37 (m, 1H), 4.56 (br s, 1H), 4.94-4.96 (m, 1H), 5.43 (t, 1H, J = 3.2 Hz), 6.22 (d, 1H, J = 6.5 Hz).

[00153] The triacetate from above (130 mg, 0.32 mmol) was dissolved in MeOH (2 mL) and then K_2CO_3 (50 mg, 0.36 mmol) was added, then the resulting mixture was stirred at room temperature for 2 h. The mixture was diluted with CH_2Cl_2 (8 mL) and then poured onto the top of a basic Al_2O_3 (1 g) column. The column was eluted with 10-25% MeOH in CH_2Cl_2 to give the title compound **29** (18.1 mg, 20% yield) as a white solid. ^{1}H NMR (500 MHz, methanol-d_4) δ 0.94 (t, 3H, J = 7.5 Hz), 1.34-1.41 (m, 2H), 1.51-1.56 (m, 2H), 3.18-3.27 (m, 1H), 3.47 (dd, 1H, J = 5.0, 8.5 Hz), 3.50-3.66 (m, 2H), 3.78 (dd, 1H, J = 2.0, 11.5 Hz), 3.91 (t, 1H, J = 5.5 Hz), 4.03 (t, 1H, J = 5.5 Hz), 6.28 (d, 1H, J = 6.5 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 12.98, 19.96, 31.37, 43.52, 62.06, 70.01, 74.55, 74.59, 75.14, 89.66, 162.13. MS(EI): m/z 277 (M+1). Anal. Calcd. for $C_{11}H_{20}N_2O_4S \cdot 0.2(CH_4O) \cdot 0.1(C_6H_{14})$: C, 48.65; H, 7.68; N, 9.61; Found: C, 48.30; H, 7.96; N, 9.64.

Example 16

Compounds 30 and 31: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(allylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**30**) and (3aR,5R,6S,7R,7aR)-2-(allylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**31**)





30 **31**

[00154] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.50 g, 1.31 mmol) in CH_3CN, was added neat 3-isothiocyanatoprop-1-ene (0.155 g, 1.2 mmol), dropwise. The reaction was stirred at room temperature until complete by TLC (3 h). The reaction was washed with a minimal amount of saturated aqueous $NaHCO_3$ (15 mL). The aqueous layer was then extracted three times with DCM, and the organic layers were combined, dried with $MgSO_4$, filtered and concentrated. The concentrated mixture was purified via flash column chromatography in a solvent system of 1:1 EtOAc and hexanes, providing (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-allylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.410 g, 81% yield) ^1H NMR (500 MHz, $CDCl_3$) δ 1.98 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 3.81-3.83 (m, 1H), 4.02-4.08 (m, 3H), 4.21 (dd, 1H, J = 4.6, 12.5 Hz), 5.06-5.15 (m, 3H), 5.20-5.30 (m, 2H), 5.72 (d, 1H, J = 8.6 Hz), 5.75-5.8 (s, 1H), 6.42-6.52 (m, 2H).

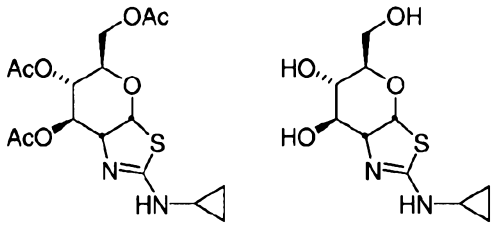
[00155] The product isolated above (0.410 g, 0.92 mmol) was dissolved in DCM. To this solution, trifluoroacetic acid (0.80 g, 7.0 mmol) was added, and the reaction was allowed to stir overnight (16 h). The reaction was worked up by washing the reaction mixture with saturated aqueous $NaHCO_3$ (20 mL). The aqueous layer was extracted three times with DCM, and the combined organic layers were dried with $MgSO_4$, filtered and concentrated. The concentrated mixture was purified via flash column chromatography in a solvent system of EtOAc. The product, (5R,6S,7R)-5-(acetoxymethyl)-2-(allylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**30**), was isolated (0.281 g, 90% yield). ^1H NMR (500 MHz, $CDCl_3$) δ 1.97 (s, 3H), 1.98 (s, 3H), 2.01 (s, 3H), 3.76-3.90 (m, 3H), 4.04- 4.05 (m, 2H), 4.27- 4.29 (m, 1H), 4.84 (dd, 1H, J = 2.4, 9.4 Hz), 5.06 (d, 1H, J = 11.3 Hz), 5.16 (d, 1H, J = 17.2 Hz), 5.30 (t, 1H, J = 3.3 Hz), 5.77-5.84 (m,

1H), 6.17 (d, 1H, J = 6.6 Hz), 6.34 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.87, 21.00, 21.16, 47.43, 63.26, 68.75, 68.85, 68.98, 71.26, 71.43, 88.82, 117.08, 133.64, 169.61, 161.84, 170.76.

[00156] (5R,6S,7R)-5-(acetoxymethyl)-2-(allylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (0.281 g, 0.73 mmol) was dissolved in anhydrous MeOH. Solid K_2CO_3 was added to the solution until it was basic, and the reaction was stirred at room temperature (1.5 h). The reaction was filtered and then concentrated *in vacuo*. The crude material was purified via flash column chromatography (DCM:MeOH, 5:2) providing (5R,6S,7R)-2-(allylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**31**) (0.048 g, 18% yield). ^{1}H NMR: (500 MHz, methanol-d_4) δ 3.50 (dd, 1H, J = 5.5, 8.9 Hz), 3.6-3.7 (m, 2H), 3.81 (d, 1H, J = 10.7 Hz), 3.87-3.95 (m, 3H), 4.09 (t, 1H, J = 6.2 Hz), 5.15 (d, 1H, J = 10.3 Hz), 5.25 (d, 1H, J = 17.2 Hz), 5.88-5.96 (m, 1H), 6.36 (d, 1H, J = 6.4 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ: 39.23, 61.80, 69.52, 73.52, 74.51, 75.39, 90.67, 161.87, 165.33.

Example 17

Compounds 32 and 33: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(cyclopropylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**32**) and (3aR,5R,6S,7R,7aR)-2-(cyclopropylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**33**)



32 **33**

[00157] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate[115] (300 mg, 0.77 mmol) in CH_2Cl_2 (10 mL) was added neat cyclopropylamine (107 μL, 1.54 mmol) and the resulting mixture was stirred for 1 h. Saturated aqueous $NaHCO_3$ (10 mL) was added, then the resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated. The resulting crude material was purified by flash chromatography on silica gel (hexane/EtOAc 1:1) to give (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-cyclopropylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a pale yellow oil (306 mg, 89% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.55-0.59 (m, 2H), 0.77-0.80 (m, 2H), 2.02 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 2.11 (s, 3H), 2.38 (br s, 1H), 3.79-

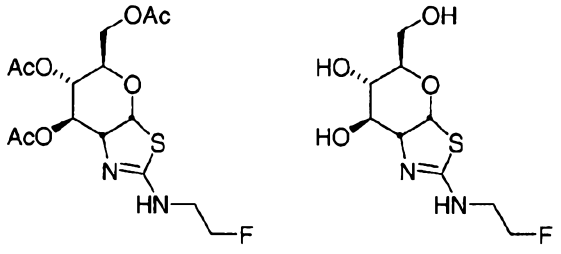
3.83 (m, 1H), 4.11 (dd, 1H, J = 2.0, 12.5 Hz), 4.24 (dd, 1H, J = 4.5, 12.5 Hz), 5.20 (t, 1H, J = 9.5 Hz), 5.26 (t, 1H, J = 10.0 Hz), 5.84 (d, 1H, J = 8.5 Hz), 6.34 (d, 1H, J = 10.0 Hz), 6.75 (br s, 1H). ^{13}C NMR (125 MHz, methanol-d_4) δ: 6.47, 26.00, 61.43, 65.04, 68.57, 73.36, 76.68, 87.89.

[00158] The thiourea from above (306 mg, 0.69 mmol) was dissolved in CH_2Cl_2 (10 mL) and TFA (153 μL, 2.06 mmol) was added, then the resulting mixture was stirred for 18 h. At this time, the solvent was removed under reduced pressure and the residue was redissolved in CH_2Cl_2 (5 mL). Solid K_2CO_3 (215 mg, 1.55 mmol) was added, then the mixture was filtered and concentrated. The resulting crude material was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 5:1) to give (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(cyclopropylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**32**) as a pale yellow oil (188 mg, 71% yield). ^{1}H NMR (500 MHz) δ 0.61-0.64 (m, 2H), 0.75-0.78 (m, 2H), 2.09 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 2.65-2.69 (m, 1H), 3.85 (dt, 1H, J = 4.5, 9.5 Hz), 4.15 (d, 2H, J = 4.0 Hz), 4.34 (dd, 1H, J = 4.2, 6.4 Hz), 4.95 (ddd, 1H, J = 0.5, 2.0, 9.6 Hz), 5.31 (br s, 1H), 5.41 (dd, 1H, J = 2.9, 4.0 Hz), 6.21 (d, 1H, J = 6.5 Hz).

[00159] The triacetate from above (188 mg, 0.49 mmol) was dissolved in MeOH (5 mL) and K_2CO_3 (3 mg, 0.02 mmol) was added, then the resulting mixture was stirred vigorously for 1 h. At this time, the mixture was filtered and concentrated. The crude material thus obtained was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 5:2) to give the title compound **33** (66 mg, 52% yield) as a white foam. ^{1}H NMR (500 MHz, methanol-d_4) δ 0.74-0.77 (m, 2H), 0.90-0.94 (m, 2H), 2.76-2.80 (m, 1H), 3.52 (dd, 1H, J = 6.5, 9.0 Hz), 3.65-3.68 (m, 1H), 3.72 (dd, 1H, J = 6.0, 12.0 Hz), 3.85 (dd, 1H, J = 2.5, 12.0 Hz), 3.91 (t, 1H, J = 6.5 Hz), 4.19 (t, 1H, J = 6.5 Hz), 6.62 (d, 1H, J = 7.0 Hz).

Example 18

Compounds 34 and 35: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2-fluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**34**) and (3aR,5R,6S,7R,7aR)-2-(2-fluoroethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**35**)



[00160] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.54 g, 1.39 mmol) in CH_3CN, was added neat 2-fluoroethanamine (0.28 g, 2.79 mmol), dropwise. The reaction was stirred at room temperature until complete by TLC (3 h). The reaction was washed with a minimal amount of saturated aqueous $NaHCO_3$ (15 mL). The aqueous layer was then extracted three times with DCM, and the organic layers were combined, dried with $MgSO_4$, filtered and concentrated. The concentrated mixture was purified via flash column chromatography in a solvent system of EtOAc and hexanes (1:1), providing (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-(2-fluoroethyl)thioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.358 g, 57% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.23 (t, 2H, J = 7.3 Hz), 2.02 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.11 (s, 3H), 3.85-3.88 (m, 2H), 4.07-4.12 (m, 1H), 4.25 (dd, 1H, J = 4.6, 12.5 Hz), 4.47-4.53 (m, 1H), 4.54-4.63 (m, 1H), 5.14 (t, 1H, J = 9.7 Hz), 5.25 (t, 1H, J = 5.4 Hz), 5.73 (d, 1H, J = 8.6 Hz), 6.50 (d, 1H, J = 9.3 Hz), 6.68 (t, 1H, J = 5.4 Hz).

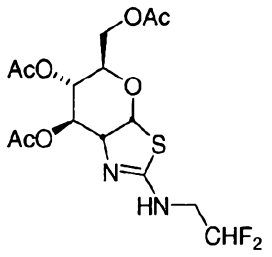
[00161] The product isolated above (0.276 g, 0.61 mmol) was added to dry DCM, and $SnCl_4$ (0.64 g, 2.46 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight (16 h). The reaction was quenched with saturated aqueous $NaHCO_3$ until the solution was basic and no more gas was evolved. The aqueous layer was extracted three times with DCM. The combined organic layers were dried with $MgSO_4$, filtered and concentrated. The crude material was purified by flash chromatography (EtOAc), providing (5R,6S,7R)-5-(acetoxymethyl)-2-(2-fluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**34**) (0.100 g, 42% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.04 (s, 3H), 2.05 (s, 3H), 2.08 (s, 3H), 3.47-3.66 (m, 2H), 3.7-3.8 (m, 1H), 4.00-4.14 (m, 3H), 4.32 (t, 1H, J = 6.3 Hz), 4.42-4.46 (m, 1H), 4.52-4.57 (m, 1H), 4.62-4.66 (m, 1H), 4.92 (d, 1H, J = 9.5 Hz), 5.37 (t, 1H, J = 3.1 Hz), 6.21 (d, 1H, J = 6.5 Hz).

[00162] (5R,6S,7R)-5-(acetoxymethyl)-2-(2-fluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (0.074 g, 0.19 mmol) was dissolved in anhydrous MeOH. K_2CO_3 was added to the solution until it was basic, and the reaction was stirred at room temperature (1.5 h). The reaction was filtered and then concentrated *in vacuo*. The crude material was purified via flash column chromatography with a solvent system of 5:1 DCM and MeOH, providing (5R,6S,7R)-2-(2-fluoroethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**35**) (0.045 g, 90% yield). ^{1}H NMR (500 MHz, methanol-d_4) δ 3.47-3.67 (m, 5H), 3.78 (dd, 1H, J = 2.1, 11.8 Hz), 3.92 (t, 1H, J = 5.6 Hz), 4.06 (t, 1H, J = 6.1

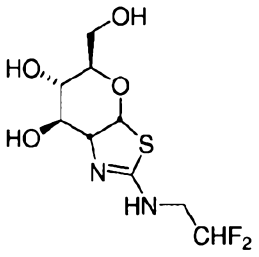
Hz), 4.40- 4.50 (m, 1H), 4.50-4.56 (m, 1H), 6.31 (d, 1H, J = 6.4 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 62.02, 69.93, 74.41, 75.15, 81.31, 82.64, 89.84, 89.87, 162.00.

Example 19

Compounds 36 and 37: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2,2-difluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**36**) and (3aR,5R,6S,7R,7aR)-2-(2,2-difluoroethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**37**)



36



37

[00163] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.64 g, 1.63 mmol) in CH_3CN, was added neat 2,2-difluoroethylamine (0.23 g, 1.97 mmol), dropwise. The reaction was stirred at room temperature until complete by TLC (3.5 h). The reaction was washed with a minimal amount of saturated aqueous $NaHCO_3$ (15 mL). The aqueous layer was then extracted three times with DCM, and the organic layers were combined, dried with $MgSO_4$, filtered and concentrated. The concentrated mixture was purified via flash column chromatography in a solvent system of EtOAc and hexanes (1:1, 2:1. then pure EtOAc, respectively), providing (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-(2,2-difluoroethyl) thioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.433 g, 56% yield) ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.99 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H), 3.83-3.86 (m, 2H), 3.95 (s, 1H), 4.02-4.09 (m, 2H), 4.18-4.23 (m, 1H), 5.05-5.11 (m, 1H), 5.21-5.30 (m, 1H), 5.72 (d, 1H, J = 8.6 Hz), 5.94 (t, 1H, J = 56.1 Hz), 6.63-6.73 (m, 2H).

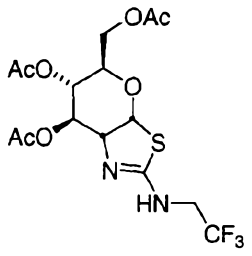
[00164] The product isolated above (0.320 g, 0.68 mmol) was dissolved in dry DCM, and $SnCl_4$ (0.71 g, 2.73 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight (16 h). The reaction was quenched with saturated aqueous $NaHCO_3$ until the solution was basic and no more gas was evolved. The aqueous layer was extracted three times with DCM. The combined organic layers were dried with $MgSO_4$, filtered and concentrated. The crude material was purified by flash chromatography (EtOAc:hexanes 1:1, then 2:1, then pure EtOAc) providing

(5R,6S,7R)-5-(acetoxymethyl)-2-(2,2-difluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**36**) (0.209 g, 75% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.04 (s, 3H), 2.05 (s, 3H) 2.09 (s, 3H), 3.45-3.55 (m, 1H), 3.67-3.77 (m, 2H), 4.06-4.14 (m, 2H), 4.31-4.34 (m, 1H), 4.91-4.93 (d, 1H, J = 9.4 Hz), 5.27 (s, 1H), 5.35-5.37 (m, 1H), 6.00 (tt, 1H, J = 3.7, 57.5 Hz), 6.24 (d, 1H, J = 6.5 Hz)..

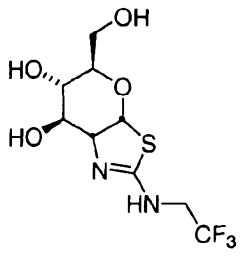
[00165] (5R,6S,7R)-5-(acetoxymethyl)-2-(2,2-difluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (0.209 g, 0.51 mmol) was dissolved in anhydrous MeOH. Solid K_2CO_3 was added to the solution until it was basic, and the reaction was stirred at room temperature (1.5 h). The reaction was filtered and then concentrated *in vacuo*. The resulting oil was purified via flash column chromatography with a solvent system of 5:1 DCM and MeOH, providing (5R,6S,7R)-2-(2-fluoroethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**37**) (0.106 g, 74% yield). ^{1}H NMR (500 MHz, methanol-d_4) δ 3.26-3.27 (m, 1H), 3.43-3.46 (m, 1H), 3.51-3.56 (m, 2H), 3.58-3.61 (m, 1H), 3.74 (dd, 1H, J = 2.3, 11.9 Hz), 3.93 (t, 1H, J = 5.4 Hz), 4.08 (t, 1H, J = 6.0 Hz), 6.01 (tt, 1H, J = 4.3, 56.4 Hz), 6.34 (d, 1H, J = 6.4 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 58.84, 69.85, 74.23, 75.17, 90.09, 114.19 (t, $J_{C\text{-}F}$ = 241 Hz), 161.92.

Example 20

Compounds 38 and 39: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2,2,2-trifluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**38**) and (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(2,2,2-trifluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**39**)



38



39

[00166] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.56 g, 1.44 mmol) in CH_3CN, was added neat 2,2,2-trifluoroethylamine (0.236 g, 1.74 mmol), dropwise. The reaction was stirred at room temperature until complete by TLC (3 h). The reaction was quenched with saturated aqueous $NaHCO_3$ (15 mL).

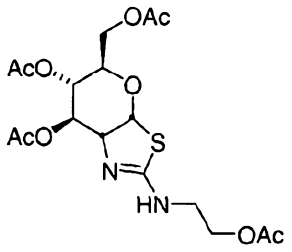
The aqueous layer was then extracted three times with DCM, and the organic layers were combined, dried with $MgSO_4$, filtered and concentrated. The concentrated mixture was purified via flash column chromatography in a solvent system of 1:1 EtOAc and hexanes, providing (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-(2,2,2-trifluoroethyl) thioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.576 g, 81% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.00 (6H, s), 2.04 (s, 3H), 3.87-3.90 (m, 1H), 4.03-4.11 (m, 1H), 4.20-4.26 (m, 2H), 4.36 (s, 1H), 5.07 (t, 1H, J = 9.6 Hz), 5.27 (t, 1H, J = 9.8 Hz), 5.73 (d, 1H, J = 8.5 Hz), 6.75 (s, 2H).

[00167] The product isolated above (0.576 g, 1.18 mmol) was added to dry DCM, and $SnCl_4$ (1.23 g, 4.72 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight (16 h). The reaction was quenched with saturated aqueous $NaHCO_3$ until the solution was basic and no more gas was evolved. The aqueous layer was extracted three times with DCM. The combined organic layers were dried with $MgSO_4$, filtered and concentrated. The crude material was purified by flash chromatography (EtOAc:hexanes 1:1, then 2:1), providing (5R,6S,7R)-5-(acetoxymethyl)-2-(2,2,2-trifluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**38**) (0.328 g, 65% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.00 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 3.71-4.75 (m, 1H), 3.77-3.84 (m, 1H), 3.99-4.11 (m, 3H), 4.29-4.31(m, 1H), 4.87 (d, 1H, J = 10.4 Hz), 5.33-5.34 (m, 1H), 6.58 (d, 1H, J = 6.6 Hz).

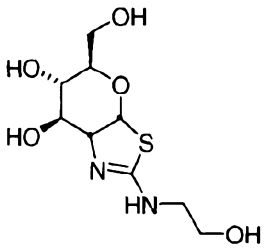
[00168] (5R,6S,7R)-5-(acetoxymethyl)-2-(2,2,2-trifluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (0.328 g, 0.776 mmol) was dissolved in anhydrous MeOH. Solid K_2CO_3 was added to the solution until it was basic, and the reaction was stirred at room temperature (1 h). The reaction was filtered and then concentrated *in vacuo*. The final reaction mixture was purified via flash column chromatography with a solvent system of 5:1 DCM and MeOH, providing (5R,6S,7R)-5-(hydroxymethyl)-2-(2,2,2-trifluoroethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**39**) (0.110 g, 47% yield). ^{1}H NMR (500 MHz, methanol-d_4) δ 3.47-3.50 (m, 1H), 3.61-3.69 (m, 2H), 3.81 (d, 1H, J = 11.8 Hz), 3.92 (m, 3H), 4.03 (s, 1H), 4.10 (q, 1H, J = 6.9 Hz), 6.35 (d, 1H, J = 6.0 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 61.94, 69.79, 74.31, 75.35, 123.82, 126.04, 175.25, 225.56.

Example 21

Compounds 40 and 41: (3aR,5R,6S,7R,7aR)-2-(2-acetoxyethylamino)-5-(acetoxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**40**) and (3aR,5R,6S,7R,7aR)-2-(2-hydroxyethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**41**)



40



41

[00169] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (500 mg, 1.3 mmol) in CH_3CN (10 mL) was added 2-aminoethyl acetate trifluoroacetate (600 mg, 3 mmol) and triethylamine (0.5 mL, 3.5 mmol). The mixture was stirred at room temperature for 1 h. The solution was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL), and the organic extract was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on silica gel (hexane/EtOAc 1:1) to give (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-(2-acetoxyethyl)thioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white foam (580 mg, 92% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.06 (s, 3H), 2.08 (s, 3H), 2.11 (s, 3H), 2.12 (s, 3H), 2.15 (s, 3H), 3.66-3.80 (m, 2H), 3.85 (ddd, 1H, J = 2.5, 4.5, 9.0 Hz), 4.14 (dd, 1H, J = 2.0, 12.5 Hz), 4.22 (dd, 1H, J = 5.0, 5.0 Hz), 4.29 (dd, 1H, J = 4.5, 12.5 Hz), 4.65-4.75 (m, 1H), 5.13-5.22 (m, 2H), 5.73 (d, 1H, J = 8.5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.82, 20.86, 20.98, 21.04, 21.19, 40.51, 57.21, 61.85, 62.75, 68.25, 71.84, 73.12, 92.65, 169.34, 169.73, 170.13, 170.49, 170.77, 184.26.

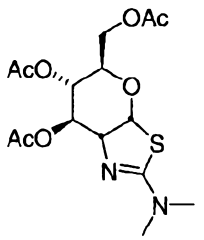
[00170] The thiourea from above (300 mg, 0.35 mmol) was dissolved in CH_2Cl_2 (5 mL) and TFA (0.4 mL, 5.4 mmol) was added. Then the resulting mixture was stirred at room temperature for 5 h. The solution was diluted with saturated aqueous $NaHCO_3$ (20 mL), then the resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated to give (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(2-acetoxyethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**40**) as a colourless oil (248 mg, 93% yield). This was pure enough for the next reaction. ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.11 (s, 9H), 2.14 (s, 3H), 3.52 (ddd, 1H, J = 4.2, 7.2, 14.0 Hz), 3.63 (ddd, 1H, J = 3.6, 4.2, 14.0 Hz), 3.83 (ddd, 1H, J = 3.0, 5.5, 9.0 Hz), 4.13-4.18 (m, 2H), 3.63 (ddd, 1H, J = 3.6, 4.2, 11.0 Hz), 4.31 (ddd, 1H, J = 4.2, 7.2, 11.0 Hz), 4.38 (dd, 1H, J = 3.5, 6.6 Hz), 4.97 (dd, 1H, J = 5.5, 9.0 Hz), 5.44 (dd, 1H, J = 3.5, 5.5 Hz), 6.26 (d, 1H, J = 6.6 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.81, 20.86, 20.91, 21.03, 43.34, 62.72, 63.27, 68.51, 69.08, 71.71, 72.69, 89.98, 159.71, 169.53, 169.75, 170.68, 171.04.

[00171] The tetraacetate from above (195 mg, 0.45 mmol) was dissolved in MeOH (10 mL) and

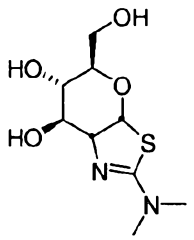
then K_2CO_3 (10 mg, 0.07 mmol) was added. The resulting mixture was stirred at room temperature for 1 h. The mixture was concentrated and then diluted with CH_2Cl_2 (9 mL) and then poured onto the top of a silica gel column The column was eluted (MeOH:EtOAc 1:1) to give the title compound **41** (105 mg, 88% yield) as a colourless oil. ^{1}H NMR (600 MHz, methanol-d_4) δ 3.35 (dd, 1H, J = 4.8, 6.0 Hz), 3.40-3.45 (m, 1H), 3.49 (dd, 1H, J = 5.4, 9.0 Hz), 3.61-3.69 (m, 4H), 3.79 (dd, 1H, J = 1.8, 11.4 Hz), 3.95 (dd, 1H, J = 5.4, 5.4 Hz), 4.08 (dd, 1H, J = 6.0, 6.0 Hz), 6.32 (d, 1H, J = 6.0 Hz). ^{13}C NMR (150 MHz, methanol-d_4) δ 47.21, 62.08, 63.36, 71.25, 75.62, 75.72, 76.32, 91.11, 163.68.

Example 22

Compounds 42 and 43: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**42**) and (3aR,5R,6S,7R,7aR)-2-(dimethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**43**)



42



43

[00172] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.51 g, 1.32 mmol) in CH_3CN, was added solid dimethylamine hydrochloride. The reaction was stirred at room temperature until complete by TLC (1 h). The reaction was washed with a minimal amount of saturated aqueous $NaHCO_3$ (15 mL). The aqueous layer was then extracted three times with DCM, and the organic layers were combined, dried with $MgSO_4$, filtered and concentrated. The concentrated mixture was purified via flash column chromatography in a solvent system of EtOAc and hexanes (1:1), providing (2S,3R,4R,5S,6R)- 6-(acetoxymethyl)-3-(3,3-dimethylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate (0.51 g, 91% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.00 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 3.17 (s, 6H), 3.79-3.82 (m, 1H), 4.06-4.13 (m, 1H), 4.22 (dd, 1H, J = 4.7, 12.5 Hz), 5.16-5.24 (m, 2H), 5.31 (dd, 1H, J = 9.4 Hz), 5.72 (d, 1H, J = 9.2 Hz), 5.77 (d, 1H, J = 8.4 Hz).

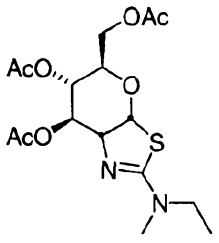
[00173] The product isolated above (0.51 g, 1.17 mmol) was dissolved in DCM. To this solution, trifluoroacetic acid (1.0 g, 8.76 mmol) was added, and the reaction was allowed to stir overnight

(16 h). The reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL). The aqueous layer was extracted three times with DCM, and the combined organic layers were dried with $MgSO_4$, filtered and concentrated. The concentrated mixture was purified via flash column chromatography (EtOAc:hexanes, 1:1) to provide (5R,6S,7R)- 5-(acetoxymethyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**42**) (0.19 g, 42% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.99 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 3.10 (s, 6H), 3.90-3.94 (m, 1H), 4.00-4.16 (m, 2H), 4.42 (t, 1H, J = 6.1 Hz), 4.92 (dd, 1H, J = 5.1, 9.5 Hz), 5.32 (t, 1H, J = 5.3 Hz), 6.35 (d, 1H, J = 6.7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.28, 20.80, 41.62, 50.22, 60.60, 62.53, 66.08, 67.65, 70.15, 71.15, 88.07, 88.12, 167.37, 169.77, 170.27, 170.86.

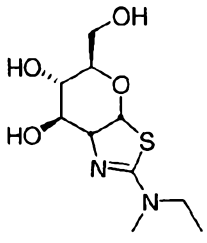
[00174] The product isolated above (0.185 g, 0.494 mmol) was dissolved in anhydrous MeOH. K_2CO_3 was added to the solution until it was basic, and the reaction was stirred at room temperature (1.5 h). The reaction was filtered and then concentrated *in vacuo*. The final reaction mixture was purified via flash column chromatography with a solvent system of 5:1 DCM and MeOH, providing (5R,6S,7R)-2-(dimethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**43**) (0.092 g, 75% yield). ^{1}H NMR (500 MHz, methanol-d_4) δ 3.04 (s, 6H), 3.48 (dd, 1H, J = 6.0 Hz), 3.62-3.69 (m, 2H), 3.81 (d, 1H, J = 11.7 Hz), 3.87 (t, 1H, J = 6.1 Hz), 4.07 (t, 1H, J = 6.3 Hz), 6.38 (d, 1H, J = 6.5 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 39.23, 69.80, 69.52, 73.43, 73.52, 74.51, 79.39, 90.67, 161.87, 165.33.

Example 23

Compounds 44 and 45: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(ethyl(methyl)amino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**44**) and (3aR,5R,6S,7R,7aR)-2-(ethyl(methyl)amino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**45**)



44



45

[00175] To a stirred solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (1.10 g, 2.8 mmol) in CH_2Cl_2 (10 mL) was added neat

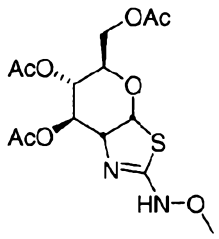
ethyl(methyl)amine (310 μL, 3.6 mmol) dropwise. The mixture was stirred at room temperature for 1 h. Solvents were removed by concentration. The residue was purified by flash chromatography on silica gel (hexane/EtOAc 1:1) to give (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-ethyl-3-methylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white foam (1.09 g, 86% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15 (t, 3H, J = 7.0 Hz), 2.05 (s, 3H), 2.06 (s, 3H), 2.11 (s, 3H), 2.13 (s, 3H), 3.08 (s, 3H), 3.74-3.81 (m, 3H), 4.16 (dd, 1H, J = 2.0, 12.5 Hz), 4.27 (dd, 1H, J = 4.5, 12.5 Hz), 5.14 (t, 1H, J = 10.0 Hz), 5.26 (t, 1H, J = 10.0 Hz), 5.34-5.40 (m, 2H), 5.78 (d, 1H, J = 8.0 Hz).

[00176] The thiourea from above (155 mg, 0.35 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and TFA (20 μL, 2.63 mmol) was added. Then the resulting mixture was stirred at room temperature for 16 h. The solution was diluted saturated aqueous $NaHCO_3$ (20 mL), then the resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated to give (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(ethyl(methyl)amino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**44**) as a pale yellow foam (134 mg, 100% yield). This was pure enough for the next reaction. ^{1}H NMR (500 MHz) δ 1.16 (t, 3H, J = 7.0 Hz), 2.06 (s, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 2.98 (s, 3H), 3.24-3.31 (m, 1H), 3.39-3.45 (m, 1H), 3.83-3.86 (m, 1H), 4.14 (d, 2H, J = 4.5 Hz), 4.34 (dd, 1H, J = 4.5, 6.5 Hz), 4.93 (dd, 1H, J = 3.0, 10.0 Hz), 5.40 (dd, 1H, J = 3.0, 4.5 Hz), 6.21 (d, 1H, J = 6.5 Hz).

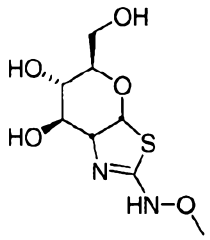
[00177] The triacetate from above (134 mg, 0.35 mmol) was dissolved in MeOH (2.0 mL) and then K_2CO_3 (72 mg, 0.52 mmol) was added. The resulting mixture was stirred at room temperature for 2 h. The mixture was diluted with CH_2Cl_2 (9 mL) and then poured onto the top of a basic Al_2O_3 (1 g) column. The column was eluted with 10-25% MeOH in CH_2Cl_2 to give the title compound **45** (57.4 mg, 57% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.07 (t, 3H, J = 7.0 Hz), 2.88 (s, 3H), 3.21-3.28 (m, 2H), 3.58-3.60 (m, 2H), 3.67-3.73 (m, 2H), 3.79 (dd, 1H, J = 3.5, 7.0 Hz), 3.88 (t, 1H, J = 6.5 Hz), 4.07 (t, 1H, J = 6.5 Hz), 4.60 (br s, 3H), 6.28 (d, 1H, J = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.88, 35.70, 46.93, 60.82, 68.02, 73.03, 73.76, 74.02, 90.19, 162.40. MS (EI): m/z 263 (M+1). Anal. Calcd. for $C_{10}H_{18}N_2O_4S$: C, 45.79 ; H, 6.92; N, 10.68; Found: C, 46.01; H, 7.18; N, 10.46.

Example 24

Compounds 46 and 47: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(methoxyamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**46**) and (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(methoxyamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**47**)



46



47

[00178] To a suspension of methoxyamine hydrochloride (180 mg, 2.16 mmol) in acetonitrile (7 mL) was added (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (560 mg, 1.44 mmol) followed by triethylamine (300 μL, 2.16 mmol). The mixture was stirred at room temperature for 2 h. Solvents were removed by concentration. The residue was purified by flash chromatography on silica gel (hexane/EtOAc 1:1) to give (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(3-methoxythioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white solid (545 mg, 87% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.09 (s, 3H), 2.10 (s, 6H), 2.13 (s, 3H), 3.67 (s, 3H), 3.82-3.85 (m, 1H), 4.15 (dd, 1H, J = 2.5, 12.5 Hz), 4.28 (dd, 1H, J = 4.5, 12.5 Hz), 5.03 (dd, 1H, J = 10.0, 13.0 Hz), 5.20-5.30 (m, 2H), 5.84 (d, 1H, J = 8.5 Hz), 7.01 (d, 1H, J = 10.0 Hz).

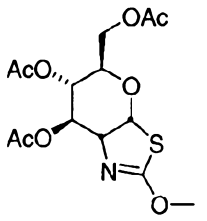
[00179] The thiourea from above (210 mg, 0.48 mmol) was dissolved in CH_2Cl_2 (2 mL) and TFA (180 μL, 2.41 mmol) was added. The mixture was stirred at room temperature for 16 h. The solution was diluted saturated aqueous $NaHCO_3$ (10 mL), extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated to give the crude product. This was purified by silica gel column, eluted with 1:1 hexane/EtOAc to give (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-(methoxyamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**46**) as a white foam (122 mg, 67% yield). The ^{1}H NMR spectrum showed this was a *ca.* 1:1 mixture of rotamers. ^{1}H NMR (500 MHz) δ 2.05 (s, 3H), 2.07 (s, 3H), 2.08 (s, 6H), 2.09 (s, 3H), 2.11 (s, 3H), 3.75 (s, 3H), 3.77 (s, 3H), 3.88 (t, 1H, J = 6.5 Hz), 3.99 (td, 1H, J = 6.5, 1.5 Hz), 4.11-4.14 (m, 2H), 4.23-4.31 (m, 4H), 4.98 (d, 1H, J = 6.5 Hz), 5.00 (d, 1H, J = 6.5 Hz), 5.16 (d, 1H, J = 7.0 Hz), 5.19 (d, 1H, J = 5.5 Hz), 5.20 (br s, 1H), 5.60 (br s, 1H), 6.10 (d, 1H, J = 6.5 Hz), 6.16 (d, 1H, J = 6.0 Hz).

[00180] The triacetate from above (63 mg, 0.17 mmol) was dissolved in MeOH (1.0 mL) and then K_2CO_3 (54 mg, 0.39 mmol) was added. The resulting mixture was stirred at room temperature for 30 min. The mixture was diluted with CH_2Cl_2 (9 mL) and then poured onto the top of a silica gel column. The column was eluted with 5-15% methanol in CH_2Cl_2 to give the title compound **47** (40

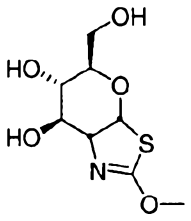
mg, 95% yield) as a white solid. The ^{1}H NMR spectrum showed this was a *ca.* 2:1 mixture of rotamers. ^{1}H NMR (500 MHz, methanol-d_4) δ 3.35-3.41 (m, 1H), 3.54-3.85 (m, 5H), 3.65 (s, 1H), 3.69 (s, 2 H), 6.16 (d, 0.34H, J = 6.0 Hz), 6.31 (d, 0.66H, J = 6.0 Hz). ^{13}C NMR (125 MHz, methanol-d_4) δ 60.65, 60.76, 61.14, 61.73, 62.45, 68.75, 68.88, 74.18, 74.83, 75.33, 75.66, 84.21, 84.70, 94.99, 157.65, 160.96. MS(CI): m/z 251 (M+1). Anal. Calcd. for $C_8H_{14}N_2O_5S$: C, 38.39; H, 5.64; N, 11.19; Found: C, 38.20; H, 5.89; N, 11.06.

Example 25

Compounds 48 and 49: (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-methoxy-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate (**48**) and (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-methoxy-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**49**)



48



49

[00181] A solution of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-isothiocyanato-tetrahydro-2H-pyran-2,4,5-triyl triacetate (1.0 g, 2.57 mmol) in anhydrous methanol (10 mL) was heated to reflux. The reaction was complete after 1 h as determined by TLC. The solvent was removed *in vacuo*. The product was then purified by flash column silica chromatography using a solvent system of 3:1 hexanes/EtOAc, which provided (2R,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(methoxycarbonothioylamino)-tetrahydro-2H-pyran-2,4,5-triyl triacetate (1.0 g, 92% yield) as a slightly yellow syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.11 (s, 3H), 2.12 (s, 6H), 3.81 (ddd, 1H), 3.95 (s, 3H), 4.1 (m, 2H), 4.31 (m, 1H), 5.17 (m, 2H), 5.73 (d, 1H, J = 8.6 Hz), 6.21 (d, 1H, J = 10.0 Hz).

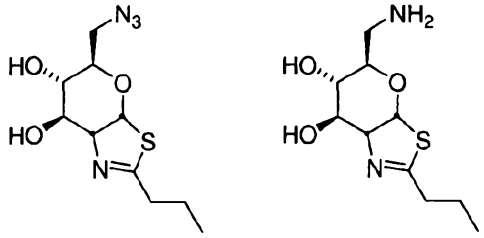
[00182] The thiocarbamate from above (1.0 g, 2.37 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) and $SnCl_4$ (2.47 g, 9.49 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight (16 h). The reaction was quenched with saturated aqueous $NaHCO_3$ until the solution was basic and no more gas was evolved. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were dried with $MgSO_4$, filtered and concentrated. The crude material was purified by flash chromatography (EtOAc/hexanes, 1:1), providing (3aR,5R,6S,7R,7aR)-5-(acetoxymethyl)-2-methoxy-

5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diyl diacetate **48** (0.65 g, 76% yield) as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.08 (s, 3H), 2.09 (s, 3H), 2.13 (s, 3H), 3.89 (ddd, 1H, *J* = 3.7, 4.6, 9.8 Hz), 3.92 (s, 3H), 4.12 (m, 2H), 4.36 (ddd, 1H, *J* = 1.0, 4.0, 6.9 Hz), 4.96 (ddd, 1H, *J* = 1.0, 2.9, 9.4 Hz), 5.40 (dd, 1H, *J* = 2.9, 4.0 Hz), 6.30 (d, 1H, *J* = 6.9 Hz).

[00183] Following General Procedure C, the material obtained above was converted to the title compound **49** which was isolated as a colourless oil after purification (0.33 g, 78% yield). In this case purification was carried out using flash chromatography on silica gel (EtOAc). ^{1}H NMR (500 MHz, methanol-d_4) δ 3.45 (dd, 1H, *J* = 5.5, 9.2 Hz), 3.56 (ddd, 1H, *J* = 1.8, 8.7, 9.3 Hz), 3.62 (dd, 1H, *J* = 6.1, 11.8 Hz), 3.75 (dd, 1H, *J* = 1.7, 11.8 Hz), 3.84 (dd, 1H, *J* = 5.7, 5.8 Hz), 3.86 (s, 3H), 4.07 (dd, 1H, *J* = 6.2, 6.4 Hz), 6.39 (d, 1H, *J* = 6.7 Hz).

Example 26

Compounds 50 and 51: (3aR,5R,6S,7R,7aR)-5-(azidomethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**50**) and (3aR,5R,6S,7R,7aR)-5-(aminomethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**51**)



50 **51**

[00184] (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (200 mg, 0.81 mmol) was dissolved in pyridine (2 mL) and CH_2Cl_2 (2 mL) and cooled to 0 °C. Tosyl chloride (230 mg, 1.2 mmol) was then added and the solution allowed to warm to room temperature over 1 h. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with water (2 x 5 mL), dried ($MgSO_4$), filtered and concentrated. The resultant colourless residue (280 mg) was taken up in DMF (3 mL) and NaN_3 (158 mg, 2.4 mmol) was added. The resultant mixture was stirred at 55 °C for 2 days. Concentration of the mixture gave a residue which was taken up in CH_2Cl_2 (20 mL) and washed with water (2 x 5 mL), dried ($MgSO_4$), filtered and concentrated. Flash chromatography of the residue on silica gel (MeOH:EtOAc, 1:9) gave 180 mg (82% yield) of (3aR,5R,6S,7R,7aR)-5-(azidomethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**50**) as a colourless oil. ^{1}H NMR (500 MHz, methanol-d_4): δ 0.98 (t, 3H, *J* =

7.3 Hz), 1.63-1.67 (m, 2H), 2.46-2.50 (m, 2H), 3.33 (m, 1H), 3.54 (dd, 1H, J = 4.3, 9.5 Hz), 3.44 (dd, 1H, 2.3, 12.0 Hz), 3.55 (dd, 1H, J = 6.2, 12.0 Hz), 4.06 (dd, 1H, J = 4.4, 4.6 Hz), 4.29 (m, 1H, J = 4.3 Hz), 6.31 (d, 1H, J = 6.9 Hz).

[00185] The azide obtained as described above (200 mg, 0.81 mmol) was dissolved in 3:1 THF:H_2O (5 mL) and triphenylphosphine (310 mg, 1.2 mmol) was added. The solution was then stirred overnight at room temperature. Concentration of the mixture followed by flash chromatography of the resultant residue on silica gel (MeOH:EtOAc 2:3) gave 130 mg (75% yield) of (3aR,5R,6S,7R,7aR)-5-(aminomethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**51**) as a colourless oil. This material was observed to decompose slowly with time when stored neat at 0 °C (7 d). ^{1}H NMR (500 MHz, methanol-d_4): δ 0.97 (t, 3H, J = 7.4 Hz), 1.63-1.67 (m, 2H), 2.43-2.49 (m, 2H), 3.39 (ddd, 1H, J = 2.5, 4.1, 9.0 Hz), 3.51 (dd, 1H, J = 4.1, 9.5 Hz), 3.64 (dd, 1H, J = 2.5, 12.5 Hz), 3.75 (dd, 1H, J = 6.1, 12.5 Hz), 4.04 (dd, 1H, J = 4.4, 4.5 Hz), 4.28 (m, 1H), 6.35 (d, 1H, J = 7.0 Hz).

Example 27

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

[00186] 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 37 ^{0}C at 400 nm using a Cary 3E UV-VIS spectrophotometer equipped with a Peltier temperature controller. Reactions were pre-heated in a 500 µL quartz cuvette for approximately 5 minutes followed by addition of 10 µL 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 1/5 to 5 times K_I was used in each case.

When tested in the assay described above, many of the compounds described in the Examples herein exhibit K_I values for inhibition of O-GlcNAcase in the range 1 nM - 50 µM. For example, the K_I values for inhibition of O-GlcNAcase shown in Table 4 were obtained for compounds **2, 4,** and **6**. All K_I values were determined using linear regression of a Dixon plots.

Table 4: Inhibition constants for *O*-GlcNAcase.

Compound	*O*-GlcNAcase K_I (μM)
2	10
4	7.7
6	5.6

Example 28

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

[00187] Experimental procedure for kinetic analyses: All enzymatic assays were carried out in triplicate at 37 °C using a stopped assay procedure by measuring the amount of 4-nitrophenolate liberated as determined by absorption measurements at 400 nm. Reactions (50 μL) were initiated by the addition, *via* syringe, of enzyme (3 μL). Time-dependent assay of β-hexosaminidase revealed that the enzyme was stable in the buffer over the period of the assay: 50 mM citrate, 100 mM NaCl, 0.1% BSA, pH 4.25. β-hexosaminidase was used at a concentration of 0.036 mg/mL with *p*NP-GlcNAc as a substrate at a concentration of 0.5 mM. The inhibitor was tested at five concentrations ranging from 5 times to 1/5 K_I. K_I values were determined by linear regression of data from Dixon plots.

[00188] When tested in the assay described above, many of the compounds described in the Examples hereinexhibit K_I values for inhibition of β-hexosaminidase in the range 5 μM - 10 mM.

[00189] The selectivity ratio for inhibition of O-GlcNAcase over β-hexosaminidase is defined here as:

$$K_{I\,(\beta\text{-hexosaminidase})}/K_{I\,(\text{O-GlcNAcase})}$$

In general, the compounds described in the Examples hereinexhibit a selectivity ratio in the range of about 1000 to 100000. When compared, for example, to the compounds of Table 3, many of the compounds described in the Examples herein exhibit greater selectivity towards O-GlcNAcase. Thus, the compounds of the invention show exhibit high selectivity for inhibition of O-GlcNAcase over β-hexosaminidase.

Example 29

Dose-dependent elevation of rat brain and muscle O-GlcNAc levels

[00190] The effect of intravenous (IV) administration of (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**Compound 54**; hereinafter referred to as NAG-Bt) on levels of O-GlcNAc modification in brain and muscle tissue in Sprague-Dawley rats was measured. Animals were obtained from Charles-River as 5-week old healthy male Sprague-Dawley rats. Animals were given one week to acclimatize and at six weeks of age the appropriate treatment was initiated. Eight animals were given intravenous tail vein injections of various concentrations of NAG-Bt or vehicle alone (PBS); doses of NAG-Bt included 0, 2, 5, 10, 25, 50, 100, and 250 mg/kg. Seven hours later animals were sacrificed and tissues were removed from animals as quickly as possible to minimize post-mortem delay. Tissues were immediately frozen in liquid nitrogen and stored at -80°C until further use. Homogenization of the tissues was carried out by manual grinding followed by homogenization in cell lysis buffer (50 mM Tris, pH 8.0, 1 mM PMSF, 0.1% NP-40, 1 mM NAG-Bt) using a tissue homogenizer (IKA) at 4°C. Insoluble cell debris was removed by centrifugation at 17,900 x g for 20 minutes at 4°C and the resulting supernatant was stored at -20°C until use.

[00191] Western blotting of the samples thus obtained was carried out using the α-O-GlcNAc antibody (CTD110.6; Covance) and the α-actin antibody as described previously.[106] Equal amounts of homogenized brain and muscle tissue from animals treated with NAG-Bt or vehicle alone were separated by SDS-PAGE followed by probing with the primary α-O-GlcNAc antibody and an anti-IgM-mouse IgG-HRP conjugate. The resulting Western blots are shown in Figures 1A-F, and clearly reveal a dose-dependent elevation of O-GlcNAc levels in both brain and muscle tissue. Figures 1C and 1D are Western blots of samples loaded in the upper panels (Figures 1A and 1B) probed using anti-β-actin mAb clone AC-40 followed by an anti-mouse IgG-HRP conjugate, and reveal equivalent sample loading. Analysis of the Western blot results by densitometry (Figures 1E-F) reveals a more pronounced effect in brain tissue at the 250 mg/kg dose (roughly 25-fold elevation of O-GlcNAc levels over baseline) as compared to the effect in muscle tissue (roughly 10-fold elevation of O-GlcNAc levels over baseline). These results demonstrate there a dose-response for elevation of brain and muscle O-GlcNAc levels by IV administration of NAG-Bt, and that the minimum IV dose required for an observable effect in brain is roughly 5 mg/kg, under the conditions used.

Example 30

Decrease of rat brain tau phosphorylation levels

[00192] The effect of oral administration of NAG-Bt on tau phosphorylation levels in brain tissue in Sprague-Dawley rats was measured. All animals were obtained from Charles-River as 5-week old healthy male Sprague-Dawley rats. Animals were given one week to acclimatize and at six weeks of age the appropriate treatment was initiated. Four animals were fed food containing 100 mg/kg/day of NAG-Bt for five days. Four additional animals fed with food containing no inhibitor were used as controls. At the end of five days, the animals were fasted for 11 hours, then the four animals that received NAG-Bt in food were each given an intravenous tail vein injection of 50 mg/kg NAG-Bt. All animals were fasted for a further 5 hours, then they were sacrificed and brains were removed, stored, and processed as described in Example 29.

[00193]

Western blotting of the samples thus obtained was carried out using the α-O-GlcNAc antibody (CTD110.6; Covance) and α-Actin antibody as described previously.[106] For Tau blotting, pS199, pS214, pS217, pS262, pS396, and pS422 (Biosource), Tau-5 (Lab Vision; a non-PTM dependent tau antibody), Tau-1 (Chemicon; selective for nonphosphorylated Ser195, Ser198, Ser199, and Ser202), and pS404 (Sigma) antibodies were used according to the manufacturer's protocols. Equal amounts of homogenized brain tissue from an animal treated with and without NAG-Bt were separated by SDS-PAGE followed by probing with each of the primary antibodies and an appropriate secondary antibody (either an anti-mouse or anti-rabbit IgG-HRP conjugate, as appropriate). The resulting Western blots are shown in Figures 2A-I, and reveal decreases of brain tau phosphorylation at multiple sites following treatment with NAG-Bt; lanes labeled "+" indicate samples from animals receiving NAG-Bt, while lanes labeled "-" indicate samples from animals receiving vehicle alone. Treated animals exhibit decreases in phosphorylation at the Tau-1 epitope (including Ser195, Ser198, Ser199, and Ser202), Ser199, Ser262, Ser396, Ser422, and Thr231; brain lysates probed using the Tau-5 primary antibody show equivalent sample loading. Treatment with NAG-Bt increases phosphorylation at Ser214 and Ser404, a result consistent with observations made in cultured cells using a non-selective O-GlcNAcase inhibitor.[16] Dosing with NAG-Bt essentially blocks phosphorylation of two critically important sites (Thr231 and Ser396) involved in the toxic self-assembly of tau.[116,117] These data demonstrate that oral administration of NAG-Bt has the overall effect of reducing tau phosphorylation levels in the brain.

Example 31

Elevation of rat cardiac O-GlcNAc levels

[00194] The effect of intravenous (IV) administration of (3aR,5R,6S,7R,7aR)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**Compound 25**, hereinafter referred to as NAG-AE) on O-GlcNAc levels in cardiac tissue in Sprague-Dawley rats was measured. Animals were obtained from Charles-River as 5-week old healthy male Sprague-Dawley rats. Animals were given one week to acclimatize and at six weeks of age the appropriate treatment was initiated. Nine animals were given intravenous tail vein injections of 50 mg/kg NAG-AE; following injection, one animal was sacrificed at each of the following time points: 0, 1, 2, 4, 7, 10, 13, 16, and 20 h. In addition, one animal was given an injection of vehicle (PBS – pH 7.4) and sacrificed two hours later for use as a control. Tissues were removed from sacrificed animals as quickly as possible to minimize post-mortem delay. The hearts from each animal were immediately frozen in liquid nitrogen and stored at -80°C until further use. Homogenization of the cardiac tissue was carried out by manual grinding followed by homogenization in cell lysis buffer (50 mM Tris, pH 8.0, 1 mM PMSF, 0.1% NP-40, 1 mM NAG-Bt) using a tissue homogenizer (IKA) at 4°C. Insoluble cell debris was removed by centrifugation at 17,900 x g for 20 minutes at 4°C and the resulting supernatant was stored at -20°C until use.

[00195] Western blotting of the samples thus obtained was carried out using the α-O-GlcNAc antibody (CTD110.6; Covance) and the α-actin antibody as described previously.[106] Equal amounts of homogenized cardiac tissue from animals treated with NAG-AE for the varying lengths of time were separated by SDS-PAGE followed by probing with the primary α-O-GlcNAc antibody and an anti-IgM-mouse IgG-HRP conjugate. The resulting Western blots are shown in Figures 3A-B, and clearly reveal a time-dependent elevation of O-GlcNAc levels in cardiac tissue, with a maximum effect being obtained between 4 and 13 h. Figure 3B shows a Western blot of samples loaded in the upper panel (Figure 3A) probed using an anti-β-actin mAb clone AC-40 followed by an anti-mouse IgG-HRP conjugate, and reveals equivalent sample loading. These results demonstrate there an elevation of cardiac O-GlcNAc levels resulting from IV administration of NAG-AE. The other compounds of the invention behave in a similar manner to NAG-AE.

Example 32

Reduction of NFT formation in transgenic P301L JNPL3 mice

[00196] Animal dosing and tissue collection. Hemizygous transgenic female JNPL3 mice overexpressing human mutant tau protein (P301L) and wild type control mice were obtained from Taconic Farms, Inc. (model numbers 001638-T-F and 001638-W-F, respectively). The mice were 10-12 weeks of age at delivery, designated "week 1" of the study. In week 1, the mice were split into four groups: Group A (transgenic mice) received vehicle alone throughout the study; Group B (transgenic mice) received 100 mg/kg/day NAG-Bt in their food from week 1 to week 15, then were switched to 1000 mg/kg/day NAG-Bt in their drinking water from week 16 to week 32; Group D (transgenic mice) received vehicle alone from weeks 1 to 15, and then 500 mg/kg/day NAG-AE in their drinking water from weeks 16 to 32; Group E (wild-type) received vehicle alone throughout the study. In week 32, three animals per group were sacrificed and tissues were collected. Transgenic mice and controls were sacrificed using a CO_2 chamber. About 45 seconds after they stopped breathing, they were perfused transcardially with 30 mL of 0.9% NaCl buffer followed by 30 mL of 4% paraformaldehyde (w/v in 1x phosphate-buffered saline, PBS, pH 7.4). The brains were then carefully dissected and post-fixed in 4% parformaldehyde before being cryoprotected in 20% sucrose (w/v in 1x PBS) for 24 hours at 4°C.

[00197] Cryostat sectioning. The brains were then mounted with optimal cutting temperature (OCT) medium (Tissue Tek) and sectioned sagittally on a Reichert-Jung Cryocut 1800 (Leica) using Feather microtome blades (Tissue Tek) at 50 μm and placed in 1X PBS for further processing. The cryostat temperature was set at –17 °C to -19°C.

[00198] Immunohistochemical staining. Free-floating 50 μm sagittal sections (lateral about 0.6 mm from midline, Mouse Brain in Stereotaxic Coordinates, second edition, by George Paxinos and Leith B.J. Franklin) were permeabilized with 1x PBS containing 0.3% Triton X-100 for 15 min. After blocking with 10% goat serum and 2.5% BSA for 1 h at room temperature, sections were incubated with specific antibodies overnight at 4 °C (primary antibodies: anti-OGlcNAc, Covance; anti-phospho Tau-ser404, Santa Cruz). The sections then were washed three times with 0.3% Triton X-100 in PBS for 15 min each and incubated with specific secondary antibody conjugated with Cy3 or FITC for 1.5 h at room temperature in the dark. After several washes in 1x PBS, sections were mounted on slides and air-dried in the dark. Once dry, Vectashield Mounting Medium (Vector Laboratories, Inc.) was added to the slide before applying coverslips. Coverslips were sealed with clear

nail enamel and slides were stored in dark at 4°C. For negative control staining, sections were incubated without primary antibodies.

[00199] Imaging. Sections were visualized using a Leica fluorescent microscope (DM4000B). A filter set (excitation peak: 480 nm, emission peak: 520 nm, Leica) was used for O-GlcNAc/FITC imaging and another filter set (excitation filter: 530-550 nm, emission filter: 570 nm, Leica) was used for phospho-Tau-ser404/Cy3 imaging. 10x Images from the hippocampus regions of brains were acquired using a Spot digital camera (Diagnostic Instruments, Sterling Heights, MI, USA).and processed with LAS (Leica Application Suite) software.

[00200] The images of hippocampal brain sections from representative mice in each group are shown in Figures 4A-H. The images on the right-hand side (Figures 4E-H) are sections stained with anti-OGlcNAc antibody, and light areas correspond to regions with high levels of protein O-GlcNAc modification. The images on the left-hand side (Figures 4A-D) are sections stained with anti-phospho Tau-Ser404 antibody, and light areas correspond to regions with high levels of phosphorylated tau protein; in particular, the bright dots correspond to aggregates of hyperphosphorylated tau protein, or neurofibrillary tangles (NFTs). The grey boxes highlight similar section areas for comparative purposes. It is clear that the groups receiving vehicle alone (Groups E and A, Figures 4E-F) exhibit low levels of protein O-GlcNAc modification, while those groups receiving either NAG-Bt or NAG-AE (Groups B and D, Figures 4G-H) show dramatically elevated levels of O-GlcNAc modification (right-hand panels). More striking are the differences in hyperphosphorylated tau and NFT formation between groups. As expected, the wild-type mice (Group E, Figure 4A) show low levels of phosphorylated tau, while the untreated transgenic animals (Group A) exhibit extensive tau phosphorylation and NFT formation. However, groups receiving either NAG-Bt or NAG-AE (Groups B and D, Figures 4C and 4D, respectively) show dramatically reduced levels of tau phosphorylation and NFT formation compared to the untreated transgenic animals (Group A, Figure 4B). These images provide compelling evidence that the compounds of the invention have the desired effect of reducing the number of NFTs and overall tau phosphorylation in a murine model of Alzheimer's disease.

Example 33

Eight-month repeat dose toxicology study in rats

[00201] The toxicological effects of repeated oral administration of (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (NAG-Bt) to Sprague-Dawley rats over eight months was measured. All animals were obtained from Charles-River as 5-week old healthy male Sprague-Dawley rats. Animals were given one week to acclimatize and at six weeks of age the appropriate treatment was initiated. Eight animals were fed food containing 100 mg/kg/day of NAG-Bt for eight months. Eight additional animals fed with food containing no inhibitor were used as controls. During this time, body weight, food consumption, water consumption, and blood glucose levels were monitored for animals in each group (Figures 5A-D, respectively; data for control rats represented by squares, data for dosed rats represented by circles); no significant differences were noted between the two groups. No gross pathological abnormalities or behavioural differences were observed in the group receiving NAG-Bt during this time. After four months of dosing, blood and urine samples were collected from four rats in each group. These samples were analyzed by hematology (CBC), serum chemistry and urinalysis (Tables 5 and 6); no statistically significant differences were detected between groups.

[00202] **Table 5** shows results from hematology (CBC) and serum chemistry tests on rats dosed orally with NAG-Bt for 4 months at 100 mg/kg/day versus controls

CBC / Serum Measurement	Untreated (n = 4 rats)		Treated (n = 4 rats)	
	average	sd	average	sd
Nonesterified Fatty Acids (mM)	1.8	0.6	1.8	0.8
Red cell cnt(L-1)	9.3	1.0	10.1	0.7
White cell cnt (1e9 /l)	4.5	1.7	3.8	0.8
Lymphs%	88.3	3.3	82.3	6.8
Monocytes%	4.3	2.2	4.3	1.9
Polys %	7.5	2.4	10.8	5.0
Hemoglobin (g/L)	157.8	13.4	169.5	10.3
Hematocrit (l/l)	0.5	0.0	0.5	0.0
Mean Corp Vol. (fL)	53.8	1.5	53.3	1.3
Mean Corp Hemoglobin (pg)	16.9	0.6	16.9	0.6
Mean Corp Hemoglobin Conc. (g/l)	314.5	6.0	318.5	4.4
RDW	16.6	1.5	17.4	0.8
Platelet cnt (10e9/L)	441.8	173.0	561.8	360.0
Mean Platelet Vol (fL)	11.5	2.1	9.3	2.3
Glucose (mM)	1.9	0.6	2.0	1.0
Blood Urea Nitrogen (mM)	8.1	2.4	8.9	0.8

Creatinine (uM)	23.8	11.0	29.0	9.1
Sodium (mM)	147.0	0.8	145.8	2.1
Potassium (mM)	6.4	0.6	5.9	0.6
Calcium (mM)	2.7	0.0	2.7	0.1
Phosphorus (mM)	2.3	0.2	2.2	0.2
Total Protein (g/l)	78.0	2.7	78.0	4.5
Albumin (g/l)	49.8	1.5	47.8	4.7
Globulin (g/l)	28.3	2.5	30.3	1.0
Albumin/Globulin	1.8	0.2	1.6	0.2
Bilirubin total (uM)	0.0	0.0	0.0	0.0
Alkaline phsphatase (iu/l)	152.5	28.2	168.3	27.8
AST (iu/l)	255.8	56.2	202.3	37.9
Gamma gt (iu/l)	12.0	4.3	17.5	4.2
Chloride (mM)	106.0	0.0	104.5	1.9
Carbon Dioxide (mM)	15.8	1.3	17.5	1.3
Osmolality (mmol/kg)	295.3	3.5	292.9	3.4
Anion Gap	31.8	1.7	29.8	2.2
Creatine Phosphokinase (iu/l)	1591.0	325.5	1324.5	321.6
ALT (iu/l)	124.3	29.5	109.3	18.4
Sorbitol Dehydrogenase (iU/L)	15.8	7.6	22.3	7.3
Cholesterol (mM)	1.9	0.3	2.9	0.6
Triglyceride (mM)	1.8	0.5	2.7	1.4

Table 6 shows results from urinalysis tests on rats dosed orally with NAG-Bt for 4 months at 100 mg/kg/day versus controls

Urine Measurement	**Untreated (-)**				**Treated (+)**		
SP. GRV.	1.06	1.05	1.05	1.05	1.042	1.046	1.036
Appear	Clear	Slcld	Clear	Slcld	Turbid	Turbid	Clear
Color	Yellow	Yellow	Pale	Yellow	Yellow	Yellow	Yellow
Protein (g/L)	Trace	1	Trace	Trace	1	neg	neg
Glucose	neg	neg	neg	neg	neg	neg	neg
Blood	2+	trace	neg	1+	2+	neg	2+
Bilirubin	neg	neg	neg	neg	neg	neg	neg
Urobil	Norm	Norm	Norm	Norm	Norm	Norm	Norm
WBC	0-3	0-3	neg	neg	0-3	neg	neg
RBC	6.0-10	0-3	0-3	0-3	0-3	0-3	neg
Epith	Few	Few	Few	neg	neg	neg	neg
Hyal cast	neg	neg	neg	neg	neg	neg	neg

Gran Cast	neg	neg	neg	neg	neg	neg	neg
RBC Cast	neg	neg	neg	neg	neg	neg	neg
Bacteria	neg	neg	neg	neg	Moderate	neg	neg
Mucus	neg	few	neg	neg	neg	few	neg
Crystals	PO4	PO4	PO4	PO4	PO4	PO4	
Amount	Many	Moderate	Few	Many	Many	Many	

[00203]

[00204] Notably, no changes in ALT, AST, bilirubin or sorbitol dehydrogenase were observed in the dosed group, indicating the absence of liver toxicity. At the end of eight months, all animals were sacrificed and their organs removed and weighed. Organ weights (brain, liver, pancreas, spleen, heart, fat, muscle) for 6 animals from each group are shown in Figure 6; no significant differences were observed between groups. These results indicate that there are no serious toxicological consequences resulting from long-term dosing of NAG-Bt in rats. This evidence supports the use of the compounds of the invention in humans to safely treat disease conditions responsive to modulation of protein O-GlcNAc levels; specifically, these data indicate the compounds of the invention have a suitable safety profile to be used for therapeutic purposes.

Example 34

Decrease of rat brain tau phosphorylation levels

[00205] The effect of oral administration of (3aR,5R,6S,7R,7aR)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (**Compound 25**, NAG-AE) on tau phosphorylation levels in brain tissue in Sprague-Dawley rats was measured. All animals were obtained from Charles-River as 5-week old healthy male Sprague-Dawley rats. Animals were given one week to acclimatize and at six weeks of age the appropriate treatment was initiated. Three animals received 200 mg/kg/day of NAG-AE in their drinking water for one day. Three additional animals received drinking water containing no NAG-AE and were used as controls. Immediately following the dosing period, all animals were sacrificed and brains were removed, stored, and processed as described in Example 29.

[00206] Western Blots. Samples were separated through 10% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) and then transferred to nitrocellulose (Bio-Rad) membranes. Membranes were then blocked for 1 h at room temperature (RT) with 1 % bovine serum albumin (BSA) in PBS containing 0.1 % Tween-20 (Sigma) (PBS-T) and then

subsequently probed with appropriate primary antibody delivered in 1 % BSA in PBS-T for either 1 h at RT or overnight at 4 °C. Membranes were then extensively washed with PBS-T, blocked again for 30 min with 1 % BSA in PBS-T at RT and then probed with the appropriate HRP conjugated secondary antibody for 1 h at RT delivered in 1 % BSA in PBS-T. Finally, the membranes were washed extensively and then developed with SuperSignal West Pico Chemiluminesence substrate (Pierce) and exposed to CL-XPosure Film (Pierce).

[00207] Antibodies. Mouse monoclonal α-Tau-5 which recognizes the central region of tau in a phosphorylation state independent manner was purchased from Lab Vision Corporation and used at a dilution of 1:500. Rabbit polyclonal α-Tau [pS^{231}], α-Tau [pT^{396}], and α-Tau [pS^{422}] recognize phosphorylated Thr-231, Ser-396, and Ser-422 respectively, and were purchased from Biosource International and used at a dilution of 1:1000. Mouse monoclonal α-O-GlcNAc (CTD110.6) which recognizes the O-GlcNAc monosaccharide modification was purchased from Covance and used at a dilution of 1:2500. Mouse monoclonal α-actin (clone AC-40) was purchased from Sigma and was used at a dilution of 1:1000.

[00208] As can be seen in Figures 7B-D, treatment of healthy rats with NAG-AE causes a reduction in tau phosphorylation at Ser-396, Thr-231, and Ser-422, respectively. By densitometry the phosphorylation at these residues is reduced by ~3.1, ~2.7 and ~1.8-fold respectively (Figure 7E). Western blots with the Tau-5 antibody demonstrates that there is an equal amount of total tau protein in each lane (Figure 7A) and thus the observed differences between groups cannot be attributed to differences in total tau loading. These same samples were then immunoblotted with an *O*-GlcNAc specific antibody which reveals that the global levels of O-GlcNAc are increased in the NAG-AE treated animals, as shown in Figure7F. Figure 7G shows a Western blot of samples loaded in the upper panel (Figure 7F) probed using an anti-β-actin mAb antibody, and reveals equivalent sample loading.

Example 35

[00209] Nine-month repeat dose toxicology study in mice

The toxicological effects of repeated oral administration of (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-propyl-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (NAG-Bt) and (3aR,5R,6S,7R,7aR)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol (NAG-AE) to wild-type JNPL3 mice over nine months was

measured. All animals were obtained from Taconic Farms, Inc. (model number 001638-W-F), and were 10-12 weeks of age at delivery, designated "week 1" of the study. In week 1, the mice were split into three groups: Group E received vehicle alone throughout the study; Group F1 received 100 mg/kg/day NAG-Bt in their food from week 1 to week 15, then were switched to 500 mg/kg/day NAG-AE in their drinking water from week 16 to week 40; Group F2 received 100 mg/kg/day NAG-Bt in their food from week 1 to week 15 then were switched to 1000 mg/kg/day NAG-Bt in their drinking water from week 16 to week 40. During this time, body weight, food consumption, and water consumption were monitored for animals in each group; no significant differences were noted between groups. No gross pathological abnormalities or behavioural differences were observed in the groups receiving NAG-Bt or NAG-AE during this time. In week 40, blood and urine samples were collected from animals in each group; the urine samples for animals in each group were pooled prior to analysis, while the blood samples were analyzed individually. These samples were analyzed by hematology (CBC), serum chemistry and urinalysis (Tables 7 and 8); no statistically significant differences were detected between groups.

Table 7 shows results from hematology (CBC) and serum chemistry tests on mice dosed orally with NAG-Bt or NAG-AE for nine months versus controls

CBC / Serum Measurement	**Group E Untreated (-) (n = 4 mice)**		**Group F2 NAG-Bt treated (n = 2 mice)**		**Group F1 NAG-AE treated (n = 4 mice)**	
	average	sd	average	sd	average	sd
Hematology						
White cell cnt (1e9 /L)	3.3	1.3	1.8	0.3	3.0	0.9
Differential						
Eosinophils%	0	-	3	3	2	3
Neutrophils%	7	3	37	33	6	3
Lymphocytes%	91	3	52	32	86	6
Monocytes%	3	3	8	2	6	2
Morphology						
Platelets	adequate		adequate		adequate	
RBC Morph	normal		normal		normal	
Manual pcv L/L	0.45	0.01	0.42	0.02	0.44	0.02
Chemistry						
Glucose (mM)	0.8	-	1.5	0.2	1.3	0.9
Blood Urea Nitrogen (mM)	7	3	6.3	0.2	6	3
Creatinine (uM)	35	24	6.2	0.4	13	6
Bun/Cr Ratio	87	59	256	9	114	68
Sodium (mM)	122	25	136	24	148	13
Potassium (mM)	13	2	13	2	15	4

Na/K Ratio	9.0	0.7	10.5	0.5	11	2
Chloride (mM)	89	20	100	17	112	13
Carbon Dioxide (mM)	0.6	0.3	0.75	0.05	1.3	0.7
Anion Gap	46	7	49	9	50	4
Calcium (mM)	2.6	0.2	2.5	0.1	2.4	0.2
Phosphorus (mM)	7	3	6.2	1.6	6.7	1.3
Total Protein (g/L)	70	33	72	3	76	5
Albumin (g/L)	29	13	33	11	38	8
Globulin (g/L)	41	20	39	9	38	11
Albumin/Globulin Ratio	0.8	0.2	1	0.5	1.3	0.8
Alkaline phsphatase (IU/L)	54	31	70	13	116	43
AST (IU/L)	272	136	250	93	201	62
Gamma gt (IU/L)	37	25	39	15	30	9
Creatine Phosphokinase (IU/L)	2448	1236	1452	776	1981	1229
Osmolality (mmol/kg)	204	-	286	48	142	143
ALT (IU/L)	18	1.3	26	11	15	1
Sorbitol Dehydrogenase (IU/L)	297	24	184	71	234	99

Table 8 shows results from urinalysis tests on mice dosed orally with NAG-Bt or NAG-AE for nine months versus controls

Urine Measurement	Group E Untreated (-) (n = 3 mice, pooled)	Group F2 Treated (NAG-Bt) (n = 2 mice, pooled)	Group F1 Treated (NAG-AE) (n = 3 mice, pooled)
Sp. Grv.	1.050	1.05	1.039
Appear	Clear	Clear	Slcloudy
Color	Yellow	Yellow	Yellow
Protein (g/L)	Neg	Neg	Neg
Glucose	Neg	Neg	Neg
Ketones	Neg	Neg	Neg
Blood	4+	NSQ	Neg
Bilirubin	Neg	Neg	Neg
Urobil	Normal	Normal	Normal
WBC	0-3	Neg	Neg
RBC	6-10	Neg	Neg
Epith	Few	Few	Few
Rods	Neg	Neg	Neg
Cocci	Neg	Neg	Neg
pH	5	5	6
Urine Creatinine	5693.6	7790.9	3105.4

[00210] Notably, no changes in ALT, AST, or sorbitol dehydrogenase were observed in the dosed group, indicating the absence of liver toxicity. These results indicate that there are no serious toxicological consequences resulting from long-term dosing of NAG-Bt or NAG-AE in mice at comparatively high levels. This evidence supports the use of the compounds of the invention in humans to safely treat disease conditions responsive to modulation of protein O-GlcNAc levels; specifically, these data indicate the compounds of the invention have a suitable safety profile to be used for therapeutic purposes.

[00211] 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 claims.

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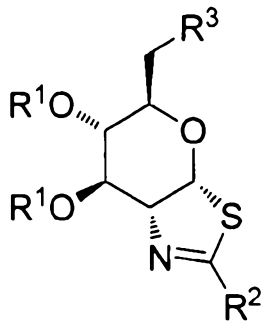
All citations are hereby incorporated by reference.

The claims defining the invention are as follows:

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



(I)

wherein

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

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent,

with the proviso that when each R^1 is H and R^3 is OH, R^2 excludes NH(phenyl), NH(4-methoxyphenyl), and $N(CH_3)_2$;

with the proviso that when each R^1 is $COCH_3$ and R^3 is $OC(O)CH_3$, R^2 excludes NH(phenyl), NH(4-methoxyphenyl), $N(CH_3)_2$, $NHCH_3$, $NH(CH_2)_2CH_3$, $NHCH(CH_3)_2$, $NH(CH_2)_3CH_3$, NH(cyclohexyl), NH(benzyl), $N(COCH_3)$(phenyl), and $N(COCH_3)$(4-methoxyphenyl); and

with the proviso that Formula (I) excludes compounds 74 and 75 described in Table 2.

2. The compound of claim 1 wherein each R^1 may be connected to form an additional ring structure.

3. The compound of claim 1 or 2 wherein when R^3 is OR^4, R^4 may be connected to either R^1 to form an additional ring structure.

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, alkenyl, alkynyl, aryl, arylalkyl, arylalkenyl, and arylalkynyl.

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, and N.

6. The compound of any one of claims 1 to 4 wherein said non-interfering substituent is optionally substituted.

7. The compound of any one of claims 1 to 3 wherein R^1 is H or $C(O)CH_3$.

8. The compound of any one of claims 1 to 3 wherein R^2 is cyclopropylmethyl, phenyl, benzyl, NH_2, $NHCH_3$, $NHCH_2CH_3$, $NH(CH_2)_2CH_3$, $NH(CH_2)_3CH_3$, $NHCH_2CH{=}CH_2$, NHcyclopropyl, $NHCH_2CH_2F$, $NHCH_2CHF_2$, $NHCH_2CF_3$, $NHCH_2CH_2OH$, $NHCH_2CH_2OC(O)CH_3$, $N(CH_3)_2$, $N(CH_3)(CH_2CH_3)$, $NHOCH_3$, or OCH_3.

9. The compound of any one of claims 1 to 3 wherein R^3 is OH, $OC(O)CH_3$, N_3, or NH_2.

10. The compound of claim 1 wherein the compound is a compound described in Table 1.

11. The compound of claim 1 with the proviso that that Formula (I) excludes Compounds 22, 26, 28, 42, 43, and 67 to 73 described in Table 2.

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

13. The compound of any one of claims 1 to 12 wherein the compound selectively inhibits an O-glycoprotein 2-acetamido-2-deoxy-β-D-glucopyranosidase (O-GlcNAcase).

14. The compound of any one of claims 1 to 13 wherein the compound selectively binds an O-GlcNAcase.

15. The compound of any one of claims 1 to 14 wherein the compound selectively inhibits the cleavage of 2-acetamido-2-deoxy-β-D-glucopyranoside (O-GlcNAc).

16. The compound of claim 13 wherein the O-GlcNAcase is a mammalian O-GlcNAcase.

17. The compound of any one of claims 1 to 16 wherein the compound does not substantially inhibit a mammalian β-hexosaminidase.

18. A compound as defined in claim 1, substantially as hereinbefore described with reference to any one of the examples.

19. A pharmaceutical composition comprising the compound of any one of claims 1 to 18 in combination with a pharmaceutically acceptable carrier.

20. 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:

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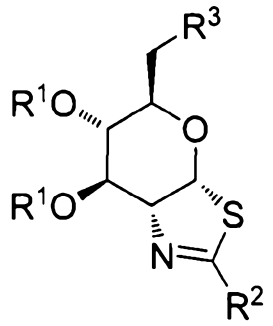


(I)

wherein

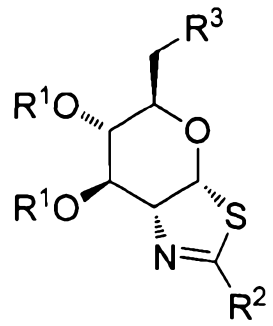
each R^1 is independently a non-interfering substituent;

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent.

21. 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:



(I)

wherein

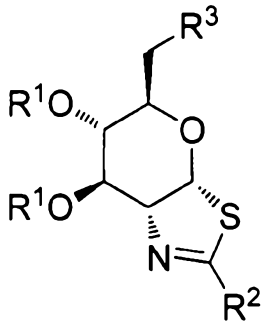
each R^1 is independently a non-interfering substituent;

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent.

22. 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)

wherein

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

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

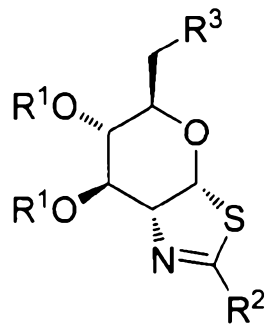
each R^4 is independently a non-interfering substituent,

with the proviso that the condition excludes a neurodegenerative disease, a tauopathy, cancer or stress.

23. The method of claim 22 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, myastenia gravis, glomerulonephritis, autoimmune thyroiditis, graft rejection, allograft rejection, graft-versus-host disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, spondyloarthropathy, scleroderma, psoriasis, T-cell mediated psoriasis, inflammatory dermatosis, dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria, vasculitis, necrotizing, cutaneous, and hypersensitivity vasculitis, eosinphilic myotis, eosiniphilic 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, stroke, neuroprotection.

24. 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:



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wherein

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

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent.

25. The method of claim 24 wherein the condition is selected from one or more of the group consisting of Alzheimer's disease, Amylotrophic lateral sclerosis (ALS), Amylotrophic lateral sclerosis with cognitive impairment (ALSci), Argyrophilic grain dementia, Bluit disease, Corticobasal degeneration (CBD), Dementia pugilistica, Diffuse neurofibrillary tangles with calcification, Down's syndrome, Familial British dementia, Familial Danish dementia, Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), Gerstmann-Straussler-Scheinker disease, Guadeloupean parkinsonism, Hallevorden-Spatz disease (neurodegeneration with brain iron accumulation type 1), Multiple system atrophy, Myotonic dystrophy, Niemann-Pick disease (type C), Pallido-ponto-nigral degeneration, Parkinsonism-dementia complex of Guam, Pick's disease (PiD), Post-encephalitic parkinsonism (PEP), Prion diseases (including Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Fatal Familial Insomnia, and Kuru), Progressive supercortical gliosis, Progressive supranuclear palsy (PSP), Richardson's syndrome, Subacute sclerosing panencephalitis, Tangle-only dementia, Huntington's disease, and Parkinson's disease.

26. The method of claim 24 wherein the stress is a cardiac disorder.

27. The method of claim 26 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.

28. The method of any one of claims 20 to 27 wherein R^1 is H or $C(O)CH_3$.

29. The method of any one of claims 20 to 28 wherein R^2 is cyclopropylmethyl, phenyl, benzyl, NH_2, $NHCH_3$, $NHCH_2CH_3$, $NH(CH_2)_2CH_3$, $NH(CH_2)_3CH_3$, $NHCH_2CH{=}CH_2$, NHcyclopropyl, $NHCH_2CH_2F$, $NHCH_2CHF_2$, NHCH2CF3, $NHCH_2CH_2OH$, $NHCH_2CH_2OC(O)CH_3$, $N(CH_3)_2$, $N(CH_3)(CH_2CH_3)$, $NHOCH_3$, or OCH_3.

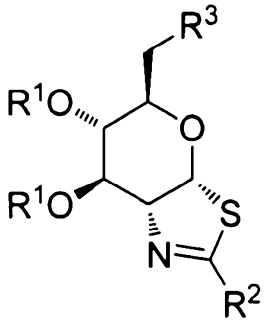
30. The method of any one of claims 20 to 29 wherein R^3 is OH, $OC(O)CH_3$, N_3, or NH_2.

31. The method of any one of claims 20 to 27 wherein the compound is selected from the group consisting of one or more of the compounds described in Table 2 and Table 3.

32. The method of any one of claims 20 to 31 wherein said administering increases the level of O-GlcNAc in the subject.

33. The method of any one of claims 20 to 32 wherein the subject is a human.

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



(I)

wherein

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

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent,

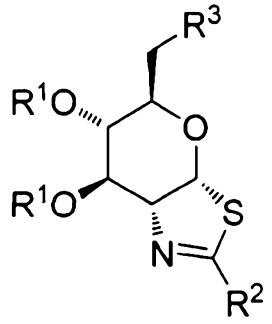
with the proviso that the compound of Formula (I) excludes the compounds described in Tables 2 and 3, in the preparation of a medicament.

35. The use of claim 34 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, for treating a neurodegenerative disease, a tauopathy, a cancer, or stress.

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

a) contacting a first sample with a test compound;

b) contacting a second sample with a compound of Formula (I)



(I)

wherein

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

R^2 is aryl, heteroaryl, OR^4, NR^4_2, and NR^4OR^4, each of which may be optionally substituted with a non-interfering substituent;

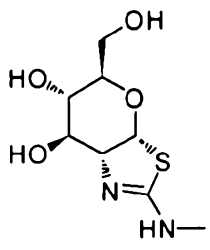
R^3 is OR^4, N_3, or NR^4_2; and

each R^4 is independently a non-interfering substituent,

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

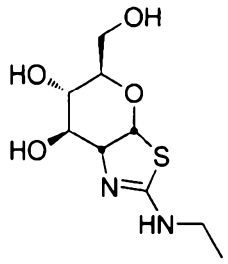
wherein the test compound is a selective inhibitor of a O-GlcNAcase if the test compound exhibits the same or greater inhibition of the O-GlcNAcase when compared to the compound of Formula (I).

37. The compound of claim 1 where the compound is:



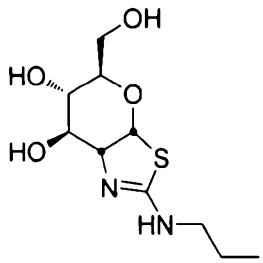
((3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol), or a pharmaceutically acceptable salt thereof.

38. A (3aR,5R,6S,7R,7aR)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol compound having the structure:



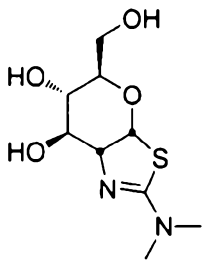
or a pharmaceutically acceptable salt thereof.

39. A (3aR,5R,6S,7R,7aR)-5-(hydroxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol compound having the structure:



or a pharmaceutically acceptable salt thereof.

40. A pharmaceutical composition comprising the compound (3aR,5R,6S,7R,7aR)-2-(dimethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazole-6,7-diol having the structure:



or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier.

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41. A pharmaceutical composition comprising one or more of Compounds 22, 26, 28, 42, 43, 67, 68, 69, 70, 71, 72, 73, 74, 75, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier.

Dated 22 October, 2012
Simon Fraser University

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

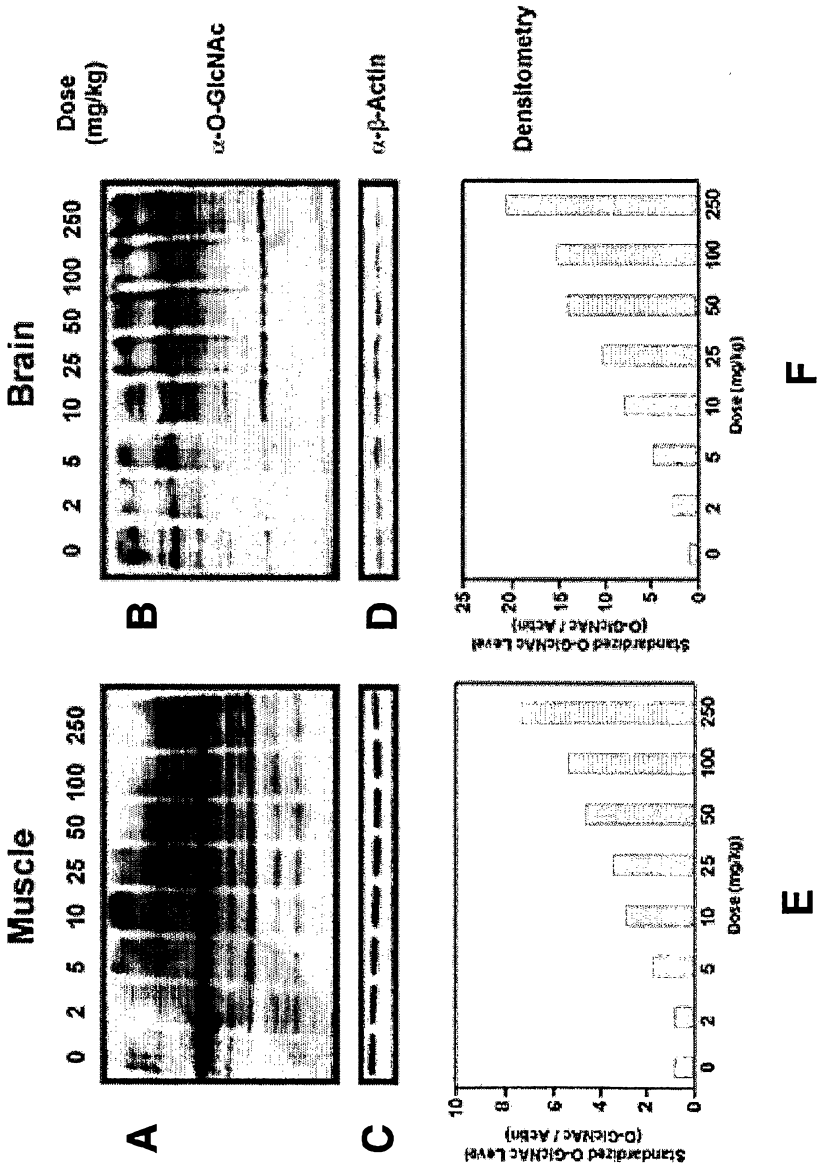
Muscle
Brain
Dose (mg/kg)
0 2 5 10 25 50 100 250
α-O-GlcNAc
α-β-Actin
Densitometry
A
B
C
D
E
F
Standardized O-GlcNAc Level (O-GlcNAc / Actin)
Dose (mg/kg)

FIG. 1

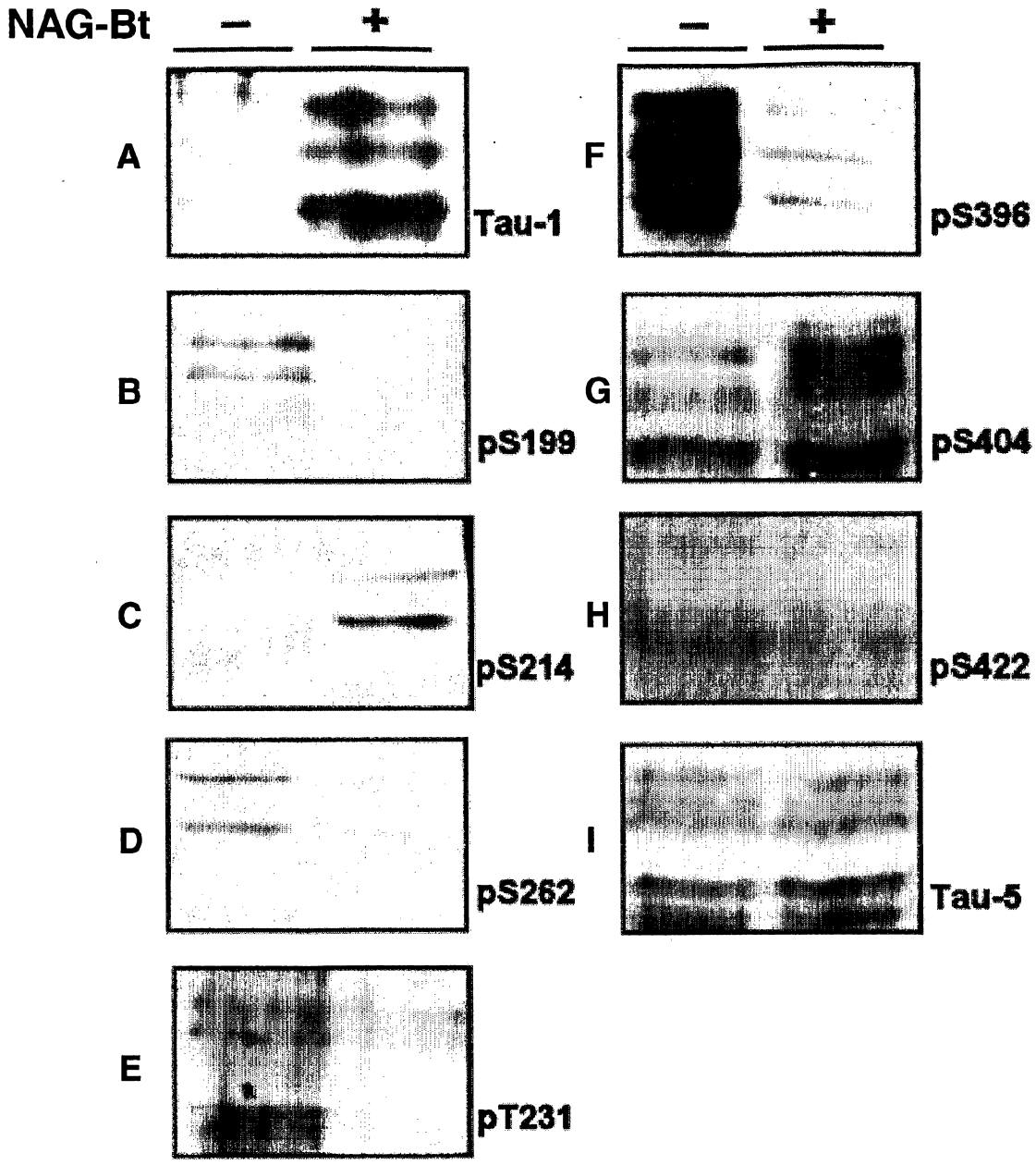
NAG-Bt
–
+
–
+
A
Tau-1
F
pS396
B
pS199
G
pS404
C
pS214
H
pS422
D
pS262
I
Tau-5
E
pT231

FIG. 2

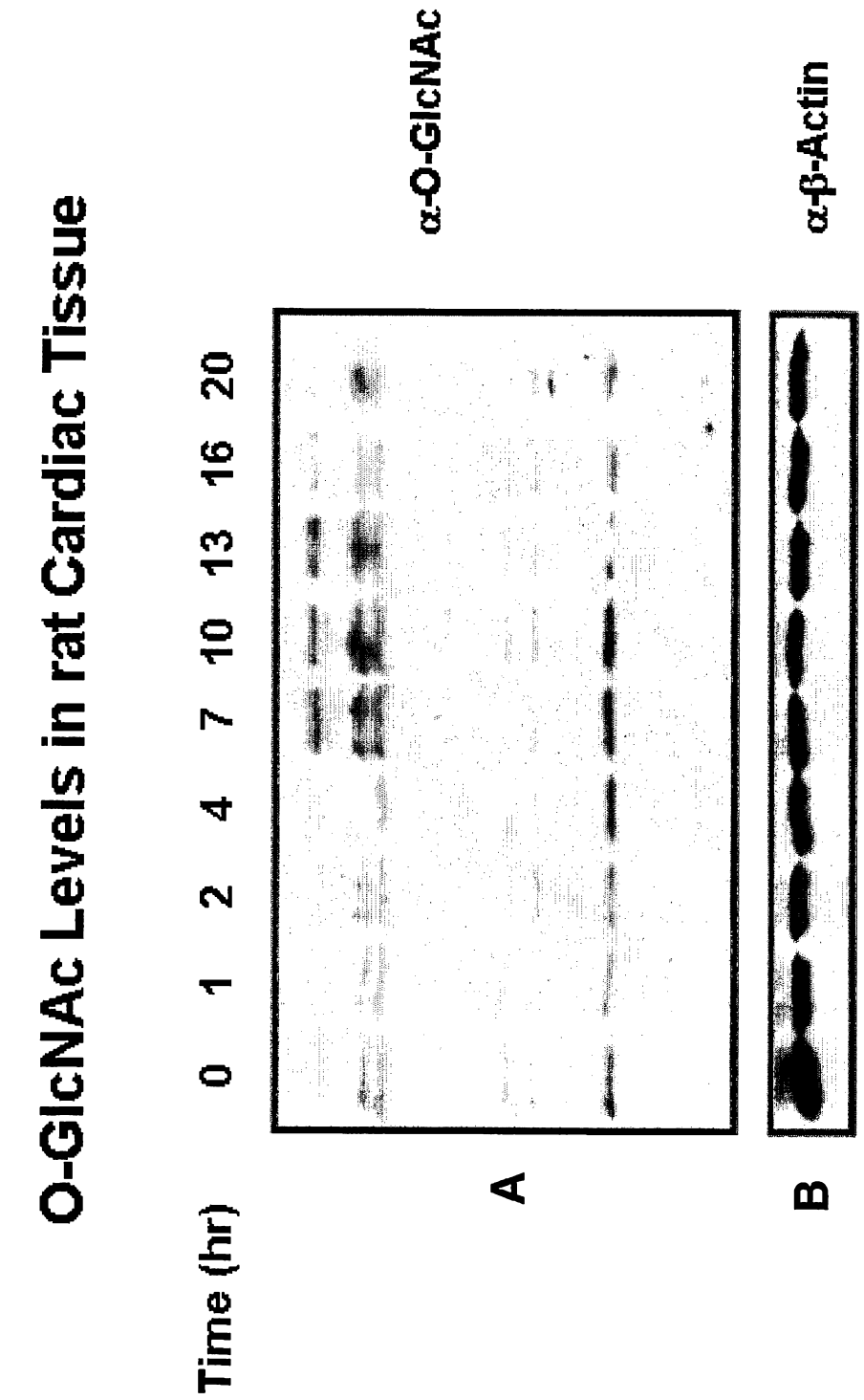
O-GlcNAc Levels in rat Cardiac Tissue
Time (hr)
0 1 2 4 7 10 13 16 20
α-O-GlcNAc
α-β-Actin
A
B

FIG. 3

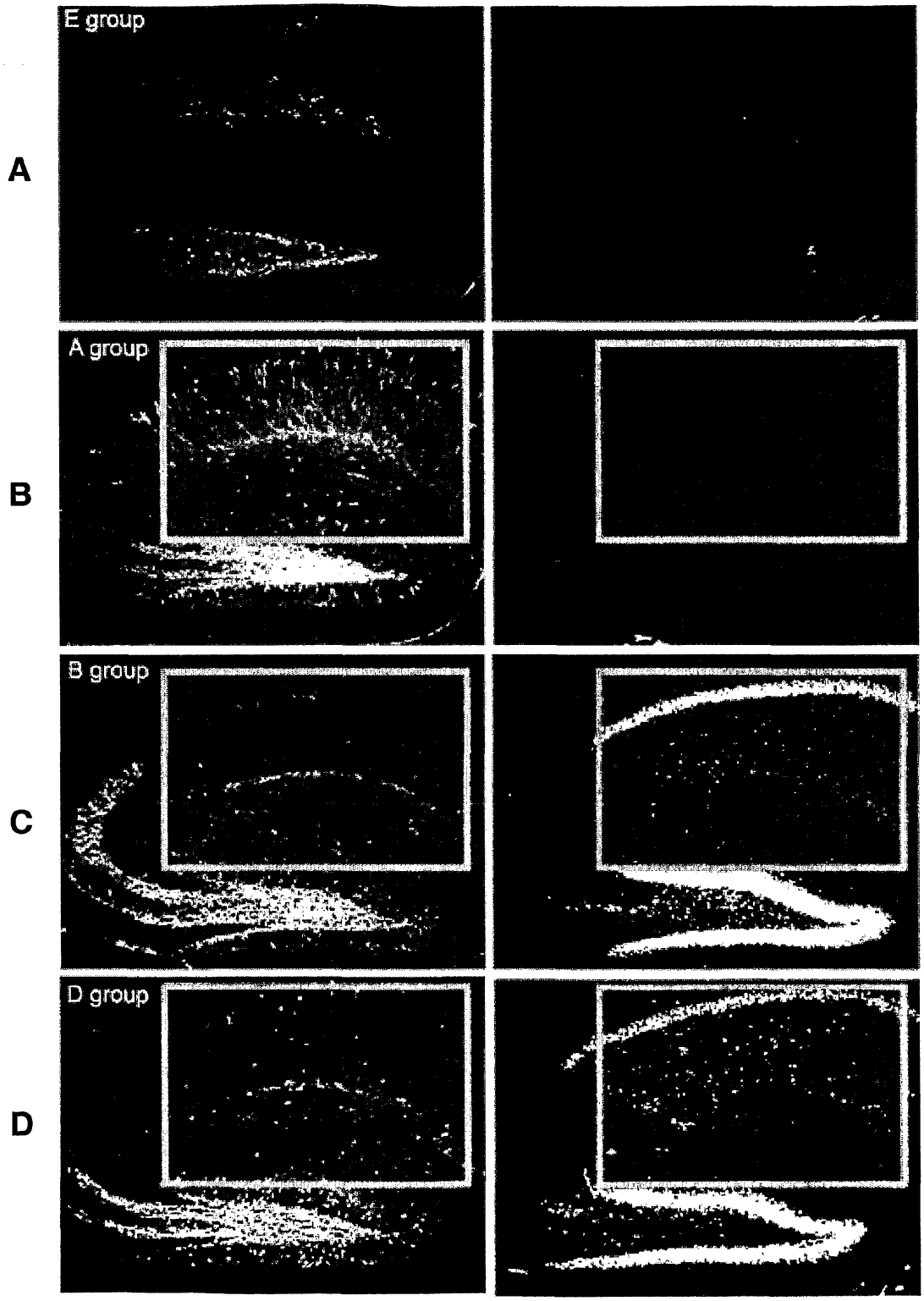

FIG. 4

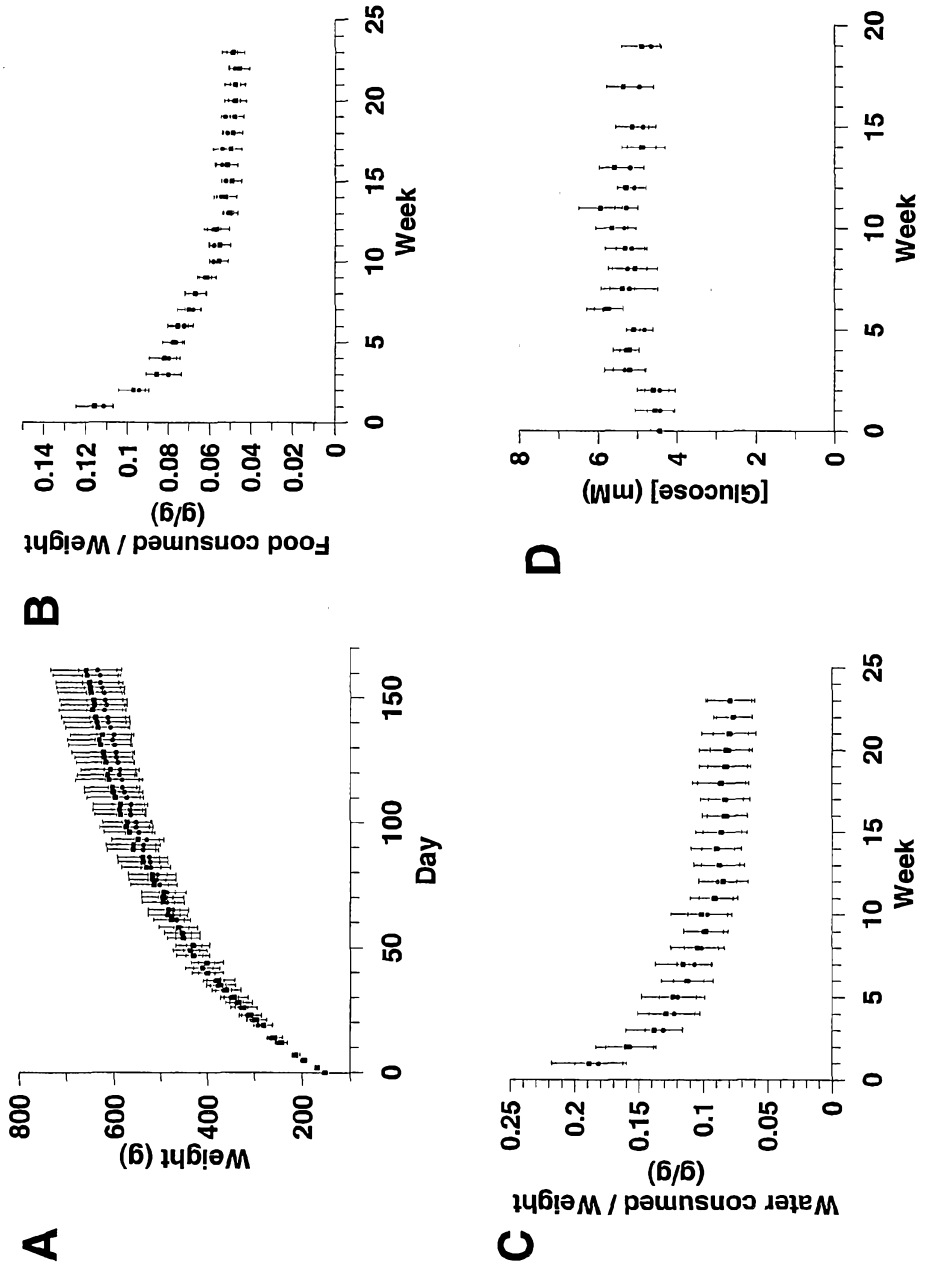
A
Weight (g)
800
600
400
200
0
50
100
150
Day
B
Food consumed / Weight
(g/g)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
5
10
15
20
25
Week
C
Water consumed / Weight
(g/g)
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
Week
D
[Glucose] (mM)
8
6
4
2
0
0
5
10
15
20
Week

FIG. 5

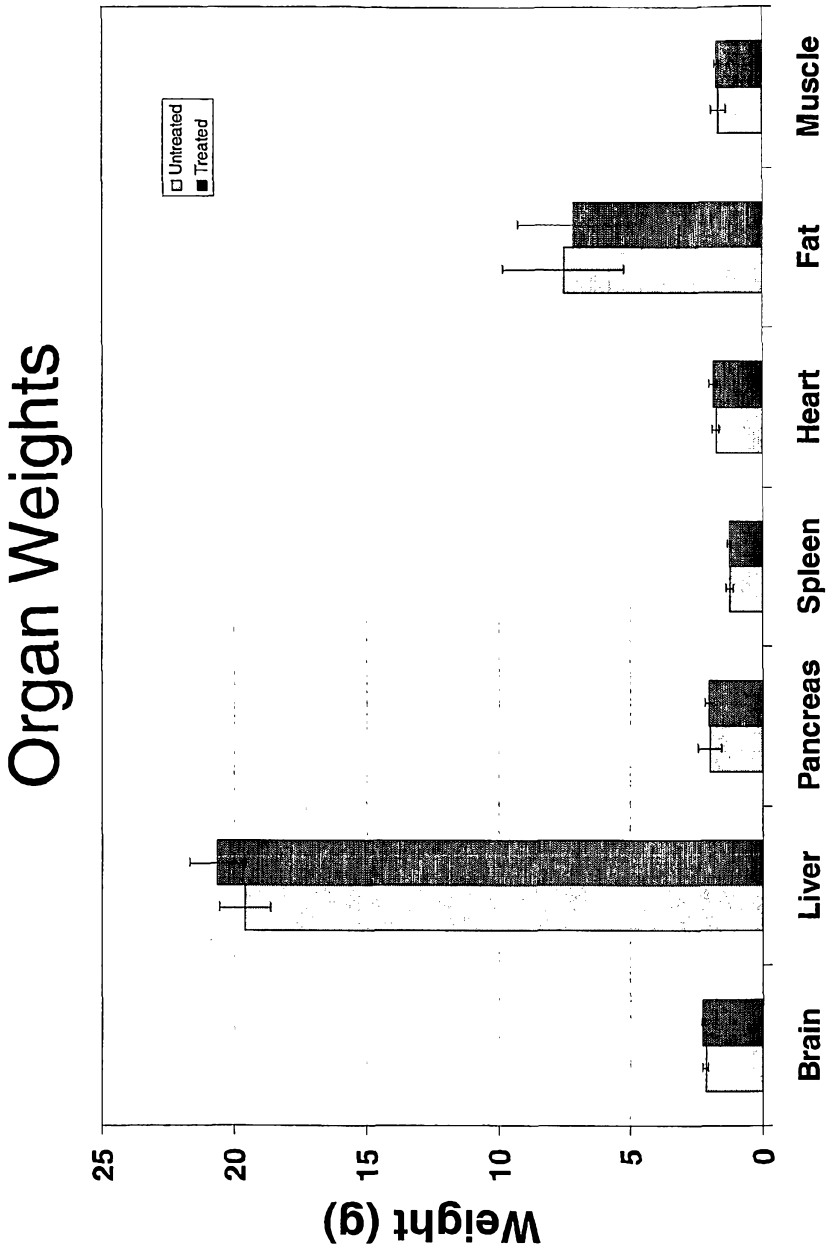
Organ Weights
Weight (g)
0
5
10
15
20
25
Untreated
Treated
Brain
Liver
Pancreas
Spleen
Heart
Fat
Muscle
Tissue

FIG. 6

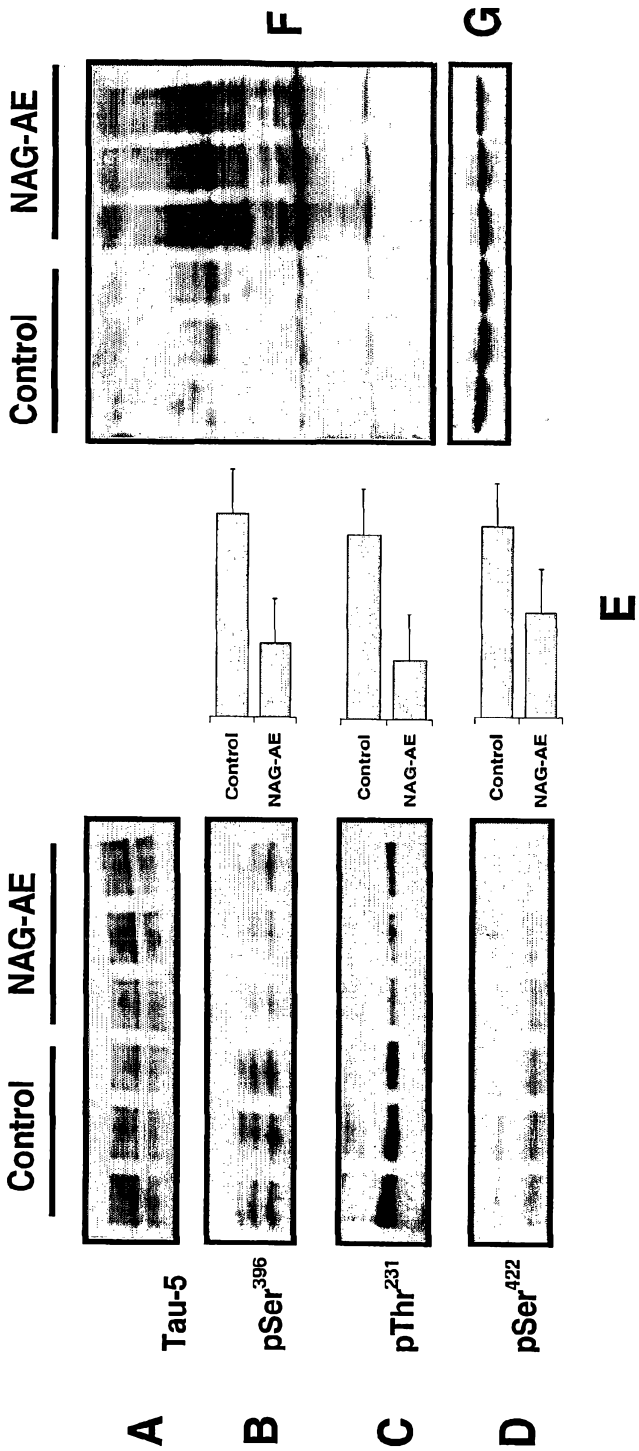
A
Tau-5
Control
NAG-AE
B
pSer396
C
pThr231
D
pSer422
E
Control
NAG-AE
F
G

FIG. 7