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[Continued on next page]

(54) Title: METHODS AND COMPOUNDS FOR INHIBITING GLYCOSYLTRANSFERASES

B.

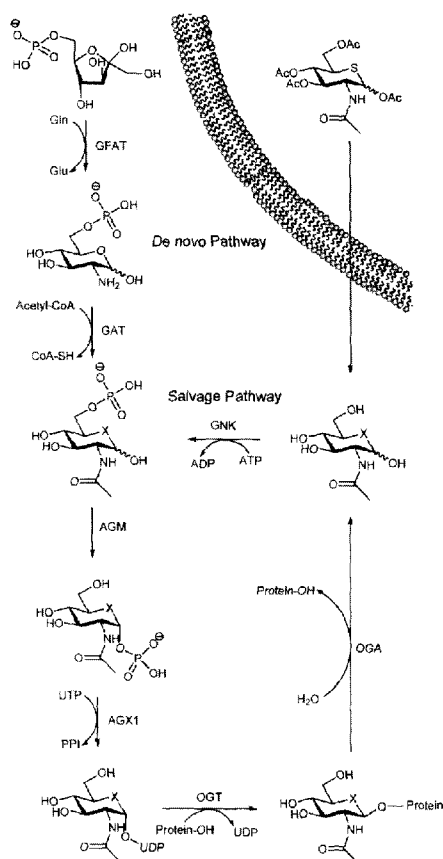


FIGURE 1

(57) Abstract: The invention provides, in part, compounds that are capable of inhibiting a glycosyltransferase, such as a uridine diphospho-*N*-acetylglucosamine:polypeptide β *N*-acetylglucosaminyltransferase, an alternative *N*-acetylglucosaminyltransferase, an *N*-acetylgalactosaminyltransferase, a fucosyltransferase, a xylotransferase or a sialyltransferase. The invention also provides methods for using the compounds.



(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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METHODS AND COMPOUNDS FOR INHIBITING GLYCOSYLTRANSFERASES

FIELD OF INVENTION

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

BACKGROUND OF THE INVENTION

[0002] Glycosyltransferases (GTs) are ubiquitous enzymes responsible for the
biosynthesis of myriad glycoconjugates. The formation of these glycoconjugates is
generally mediated by GT-catalyzed transfer of a sugar residue from an anionic
10 nucleotide sugar donor to various acceptors molecules, which may be proteins, lipids,
saccharides, or metabolites. Mammals have over 200 GTs that give rise to diverse
glycoconjugates and these structures are emerging as regulators of quality control,
cellular structure, signaling, gene transcription, and intercellular communication¹.

[0003] The post-translational installation of single 2-acetamido-2-deoxy-D-
15 glucopyranose (GlcNAc) residues β -glycosidically linked to serine or threonine
residues of proteins (*O*-GlcNAc)² is a nuclear and cytoplasmic modification
ubiquitously found in multicellular eukaryotes. Several hundred proteins, involved in
a wide range of cellular functions, have been shown to be modified³. *O*-GlcNAc can
be installed and removed several times during the lifetime of a given protein and its
20 cycling is regulated by two enzymes; a GT (uridine diphospho-*N*-
acetylglucosamine:polypeptide β -*N*-acetylglucosaminyltransferase; or "OGT")³, which
transfers *O*-GlcNAc onto proteins, and a glycoside hydrolase (*O*-GlcNAcase; or
"OGA")³ that removes *O*-GlcNAc (Figure 1A). *O*-GlcNAc has been proposed to
mediate cellular processes in nutrient signaling, regulation of gene transcription,
25 cellular stress response, and cell division³. The donor substrate used by OGT, uridine
diphospho-*N*-acetylglucosamine (UDP-GlcNAc), is biosynthesized through the action
of enzymes comprising the hexosamine biosynthetic pathway (HBP) (Figure 1B).

[0004] A number of chemical approaches have been used to reduce *O*-GlcNAc levels.
For example, 6-diazo-5-oxo-L-norleucine and L-azaserine block UDP-GlcNAc
30 biosynthesis but these compounds inhibit other amidotransferases including those

involved in purine and pyrimidine biosynthesis, leading to DNA damage⁴; and alloxan is a uracil analogue that is toxic and affects many cellular processes⁵. OGT inhibitors have also been recently discovered by library screening⁶ but have not been well characterized in cells⁷.

5 [0005] Selectin proteins recognize cell surface ligands that are carbohydrate structures. This recognition process mediates the rolling of leukocytes and other cell lineages, including cancer cells, along endothelial surfaces, which serves as the first step to the extravasation and invasion of these cells into the adjacent tissues. The three known selectins (P-selectin (CD62P), L-selectin (CD62L), and E-selectin (CD62E)
10 bind specific carbohydrate epitopes present on cell surface biomolecules including lipids and proteins⁶²⁻⁶⁵. These carbohydrate structures include sialyl Lewis A (sLe^a) and sialyl Lewis X (sLe^x)⁶²⁻⁶⁵. The biosynthesis of these carbohydrate structures is mediated by a number of glycosyltransferases including, as a last step, the attachment of a fucose residue onto *N*-acetylglucosamine. This last step is catalyzed by enzymes
15 known as fucosyltransferases (FUT). There are several different fucosyltransferases known in humans and various enzymes as reviewed in the scientific literature are involved in the synthesis of these carbohydrate structures^{62-64, 66, 67}. Increased levels of these carbohydrate structures permits cancer cells and leukocytes to bind more efficiently to selectins. These structures are therefore considered mediators of these
20 processes that can initiate the attachment of circulating cancer cells to tissues and thereby enable metastasis⁶⁶⁻⁶⁸. Likewise, increased expression of these carbohydrate structures can enable extravasation of leukocytes into tissues and thereby contribute to undesirable inflammatory processes^{63,64,69,70}. Studies in mice deficient in fucosyltransferase IV or VII showed decreased number of rolling leukocytes as well as
25 a higher leukocyte rolling velocity^{63,64,71,72}. Likewise, in several types of cancer some of these carbohydrate structures have been found to correlate with a poor outcome⁶⁶⁻⁶⁸. Further, some fucosyltransferases have been shown to be elevated in cancer tissues⁶⁶⁻⁶⁸. Accordingly, there have been attempts to design selectin antagonists based on the structure of sLe^x as potential therapeutics⁶². Fucosyltransferases and fucose
30 containing glycan structures have been implicated in a number of other diseases including *Helicobacter pylori* infections⁷, glaucoma⁶⁵, and atherosclerosis⁷⁰. Recently,

the recognition that therapeutic antibodies lacking fucose have been shown to have potential benefit⁷³.

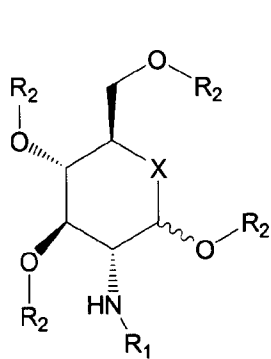
[0006] Biosynthetic pathways exist within cells and tissues to generate a range of nucleotide sugar donors such as UDP-GlcNAc, UDP-GalNAc, CMP-sialic acid, UDP-Xyl, and GDP-Fuc⁸. Furthermore, biosynthetic pathways, known as salvage pathways⁸, enable the assimilation and conversion of exogenously added sugars, such as GlcNAc⁹, GlcNH₂¹⁰, GalNAc¹¹, ManNAc^{12,13}, and Fuc^{14,15}, into the respective nucleotide sugars UDP-GlcNAc, UDP-GlcNAc, UDP-GalNAc or UDP-GlcNAc, CMP-sialic acid, and GDP-fucose. The enzymes in the salvage pathways and the biosynthetic pathways used by cells (including bacterial cells) to generate these nucleotide sugar donors are capable of enabling cells and living organisms to assimilate various sugar analogues to form the nucleotide sugar donors^{9,16-21}.

[0007] A number of sugar analogues have been synthesized²²⁻²⁸. Nucleotide sugar analogues in which the endocyclic sugar ring is replaced by sulphur appear to be poor substrates of GTs²⁹⁻³¹ and are turned over at only approximately 0.2 to 5% the rate as compared to the naturally occurring nucleotide sugar²⁹⁻³¹. In other cases when the endocyclic oxygen of the sugar ring is replaced by carbon the nucleotide sugar analogue does not appear to be turned over at all³². Derivatized metabolic intermediates, such as sugar-1-phosphates, can diffuse across the membrane and thereby be delivered to tissues where they are processed and assimilated into the biosynthetic pathways to generate the corresponding nucleotide sugar³³.

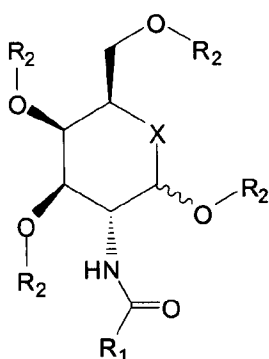
SUMMARY OF THE INVENTION

[0008] The invention provides, in part, inhibitors of glycosyltransferases and uses thereof.

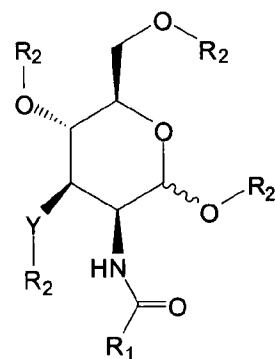
[0009] In one aspect, the invention provides a method of inhibiting a glycosyltransferase (GT) in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of any one of Formula (I-X) or a pharmaceutically acceptable salt thereof:



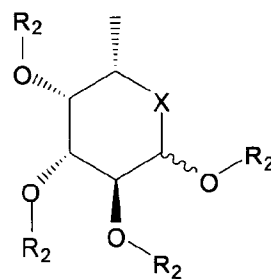
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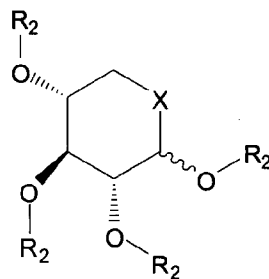
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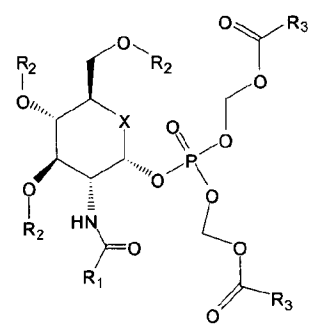
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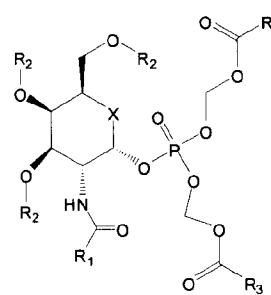
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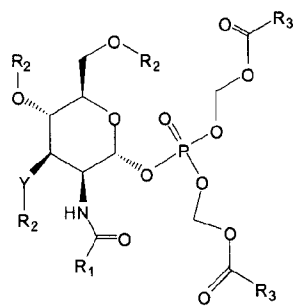
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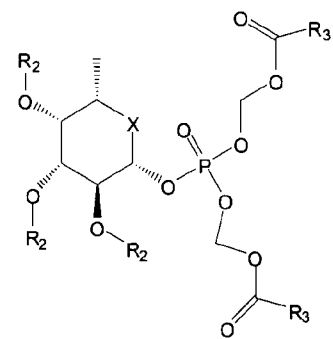
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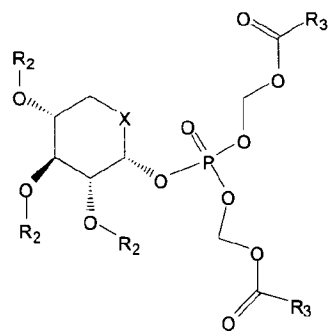


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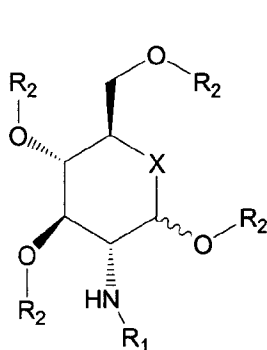
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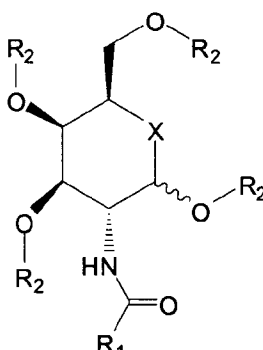
(X)

[0010] wherein X where present may be S, Se or CH₂; Y where present may be S or Se; R₁ may be either H or C(O)R₄, wherein R₄ may be H, CH₂OH, CH₂N₃, CH₂SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted; R₂ is H or C(O)R₅,
 5 wherein R₅ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; R₃ where present may be H or C(O)R₆, wherein R₆ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and ~~~~ may be the alpha anomer, the beta anomer, or both.

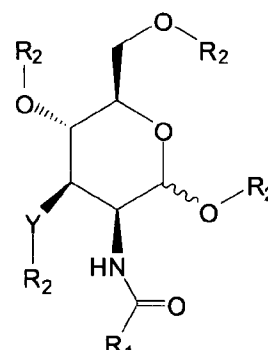
[0011] In an alternative aspect, the invention provides a method of reducing the level
 10 of a nucleotide sugar-modified biomolecule in a sample or in a subject in need thereof, the method comprising contacting the sample with, or administering to the subject, an effective amount of a compound of any one of Formula (I-X) or a pharmaceutically acceptable salt thereof:



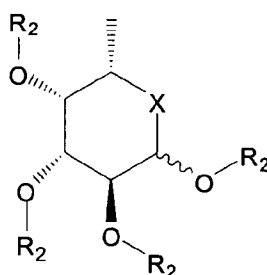
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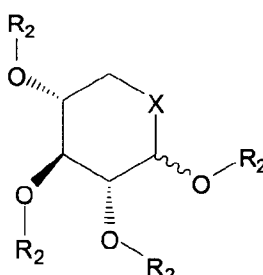
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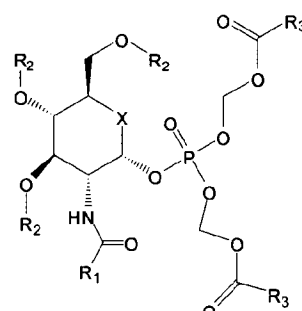
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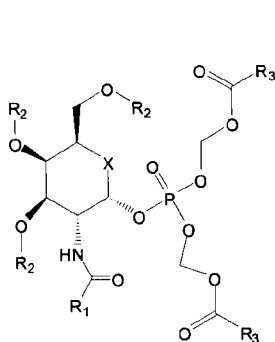
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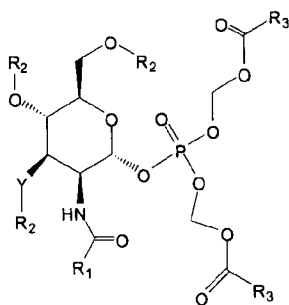
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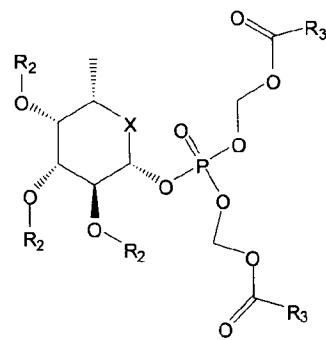
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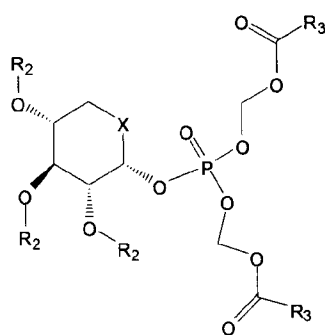
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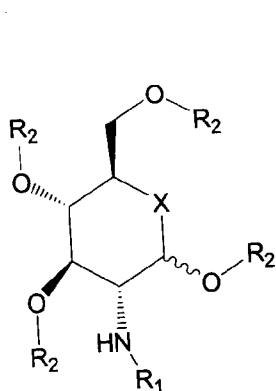
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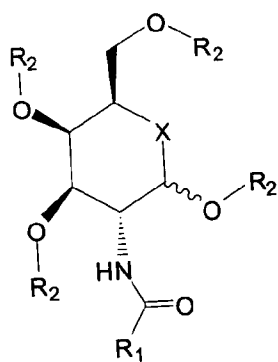
(X)

- 5 [0012] wherein X where present may be S, Se or CH₂; Y where present may be S or Se; R₁ may be either H or C(O)R₄, wherein R₄ may be H, CH₂OH, CH₂N₃, CH₂SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted; R₂ is H or C(O)R₅, wherein R₅ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; R₃
- 10 where present may be H or C(O)R₆, wherein R₆ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and ~~~~ may be the alpha anomer, the beta anomer, or both.

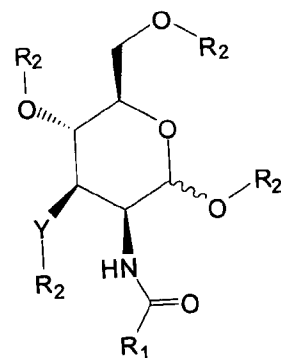
- [0013] In an alternative aspect, the invention provides a method of treating a condition that is modulated by a GT in a subject in need thereof, the method
- 15 comprising administering to the subject an effective amount of a compound of any one of Formula (I-X) or a pharmaceutically acceptable salt thereof:



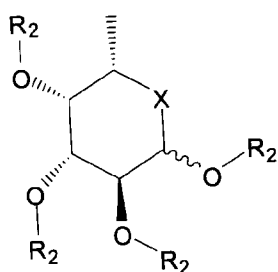
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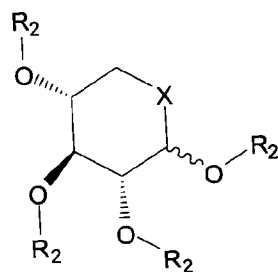
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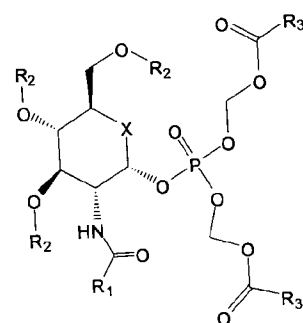
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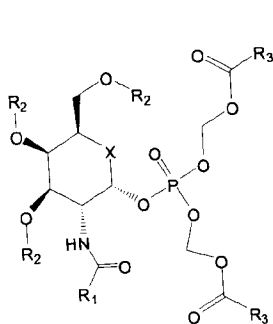
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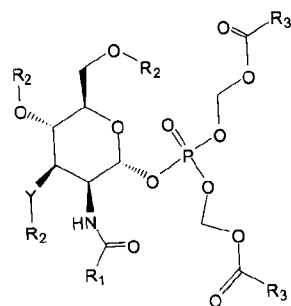
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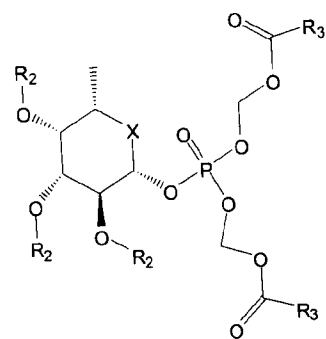
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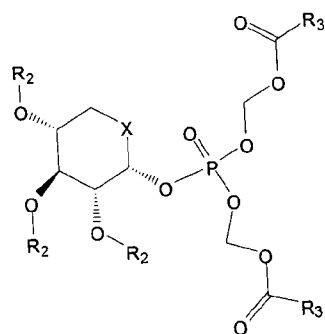
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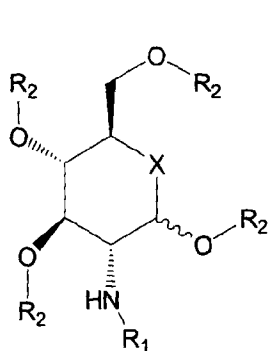
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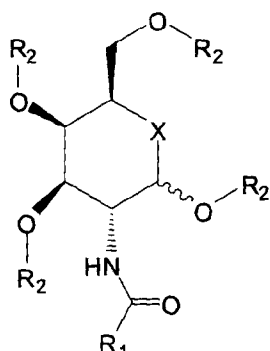
(X)

[0014] wherein X where present may be S, Se or CH₂; Y where present may be S or Se; R₁ may be either H or C(O)R₄, wherein R₄ may be H, CH₂OH, CH₂N₃, CH₂SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted; R₂ is H or C(O)R₅,
 5 wherein R₅ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; R₃ where present may be H or C(O)R₆, wherein R₆ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and $\sim$ may be the alpha anomer, the beta anomer, or both.

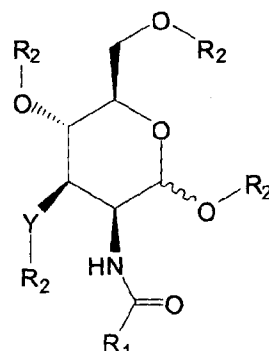
[0015] In an alternative aspect, the invention provides a method of inhibiting a GT in
 10 a cell or tissue, the method comprising administering to the cell or tissue an effective amount of a compound of any one of Formula (I-X) or a pharmaceutically acceptable salt thereof:



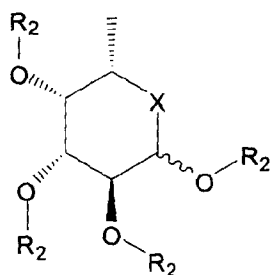
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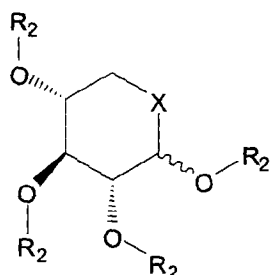
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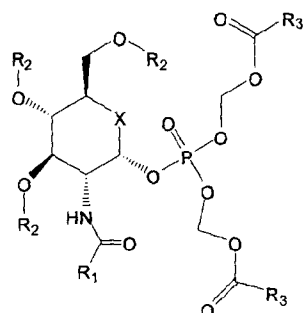
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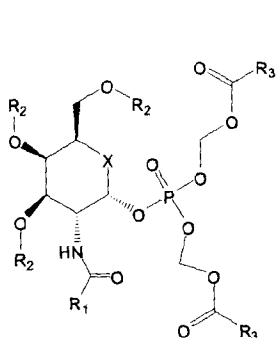
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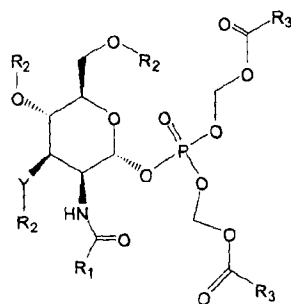
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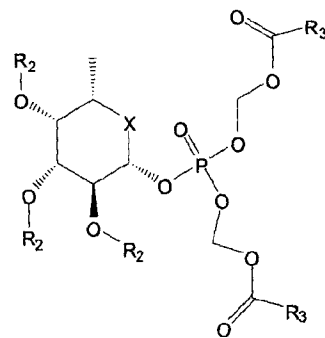
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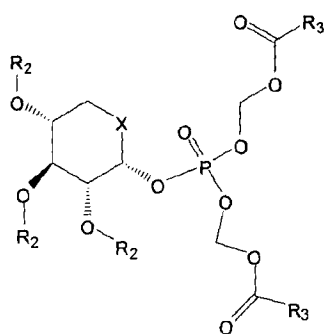
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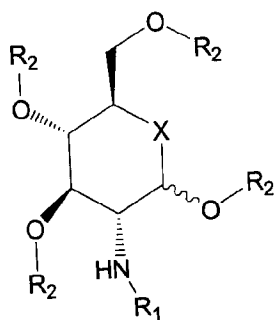
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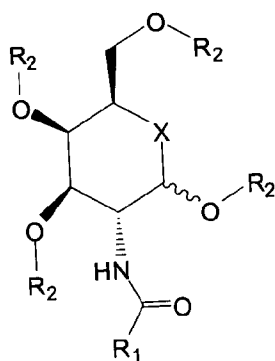
(X)

- 5 [0016] wherein X where present may be S, Se or CH₂; Y where present may be S or Se; R₁ may be either H or C(O)R₄, wherein R₄ may be H, CH₂OH, CH₂N₃, CH₂SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted; R₂ is H or C(O)R₅, wherein R₅ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; R₃ where present may be H or C(O)R₆, wherein R₆ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and ~~~~ may be the alpha anomer, the beta anomer, or both.
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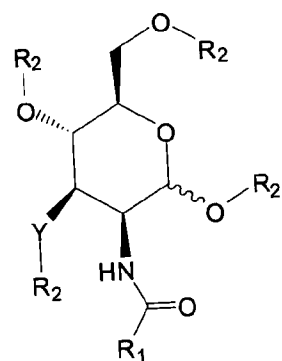
[0017] In alternative aspects, the invention provides the use of a compound of Formula (I-X) or a pharmaceutically acceptable salt thereof:



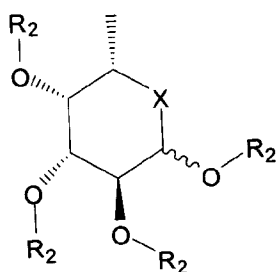
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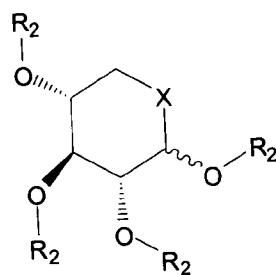
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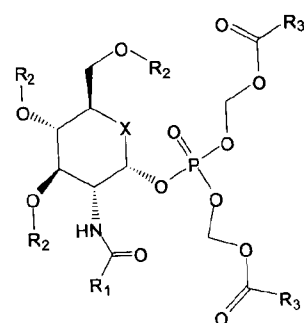
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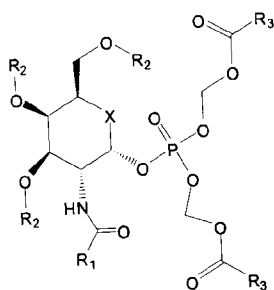
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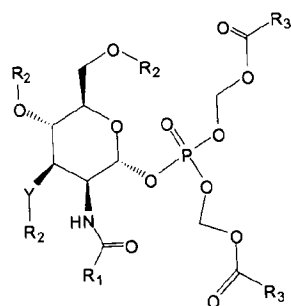
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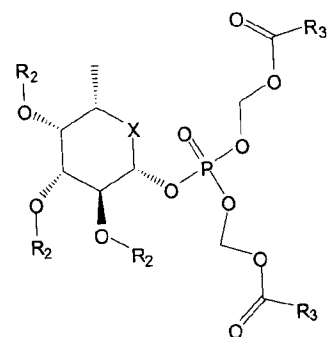
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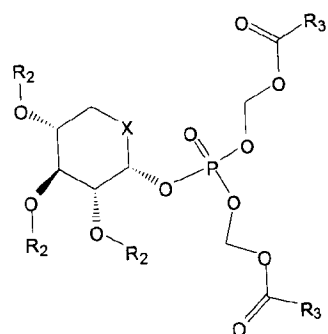
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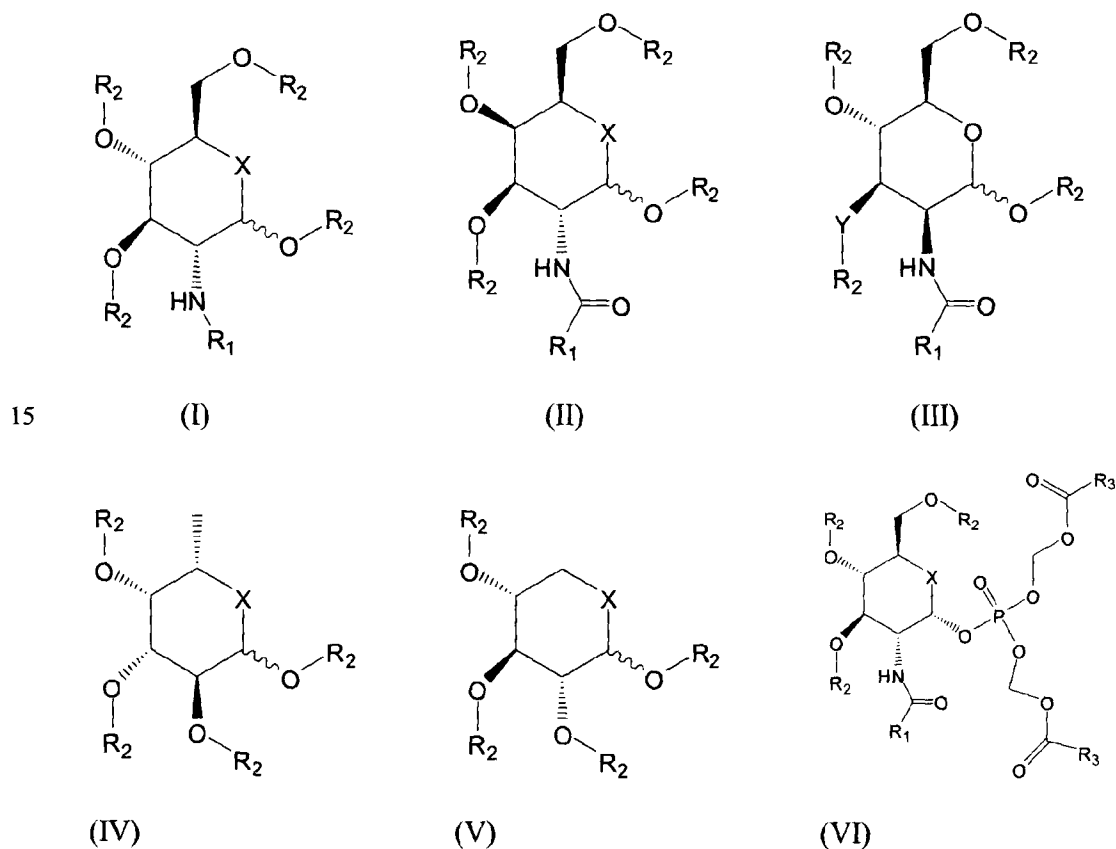
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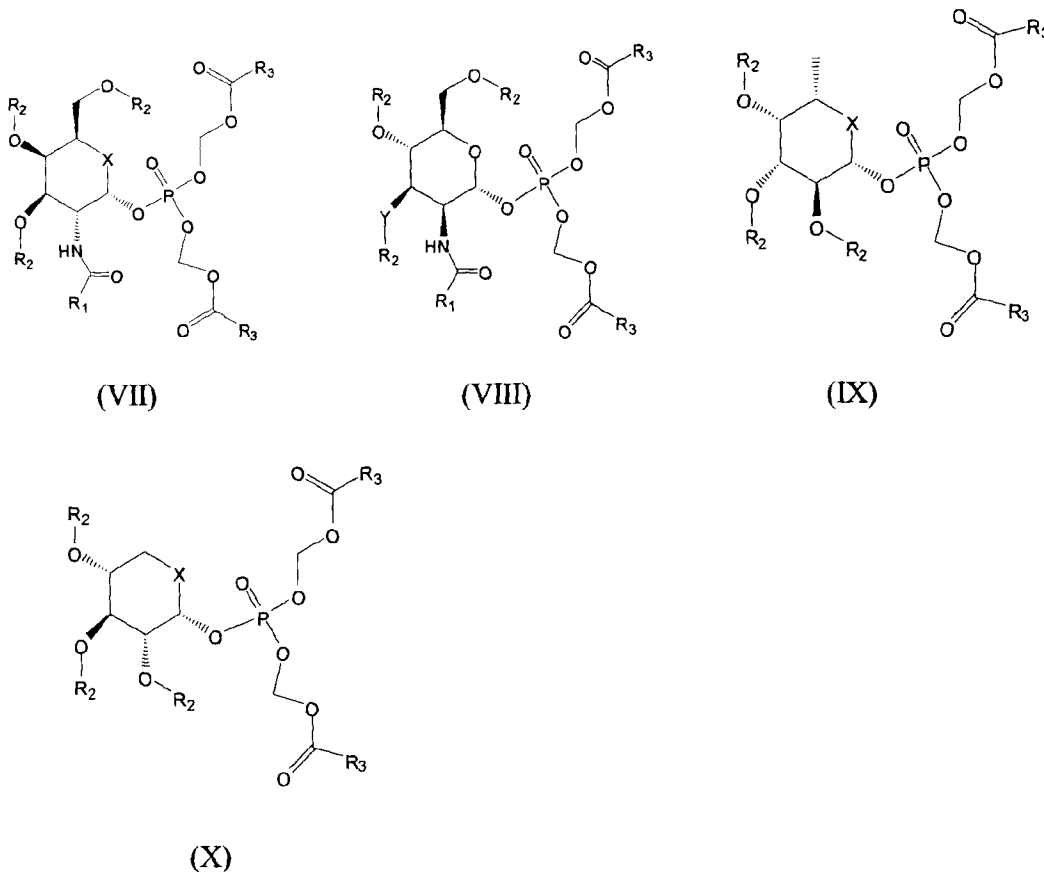


(X)

[0018] wherein X where present may be S, Se or CH₂; Y where present may be S or Se; R₁ may be either H or C(O)R₄, wherein R₄ may be H, CH₂OH, CH₂N₃, CH₂SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted; R₂ is H or C(O)R₅,
 5 wherein R₅ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; R₃ where present may be H or C(O)R₆, wherein R₆ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and ~~~~ may be the alpha anomer, the beta anomer, or both, for inhibiting a GT, reducing the level of a nucleotide sugar-modified biomolecule, or treating a condition that is modulated by a GT, in a subject in need
 10 thereof, or in a sample, cell or tissue.

[0019] In alternative aspects, the invention provides a compound of Formula (I-X) or a pharmaceutically acceptable salt thereof:





- 5 [0020] wherein X where present may be S, Se or CH₂; Y where present may be S or Se; R₁ may be either H or C(O)R₄, wherein R₄ may be H, CH₂OH, CH₂N₃, CH₂SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted; R₂ is H or C(O)R₅, wherein R₅ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; R₃
- 10 where present may be H or C(O)R₆, wherein R₆ may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and ~~~~ may be the alpha anomer, the beta anomer, or both, for inhibiting a GT, reducing the level of a nucleotide sugar-modified biomolecule, or treating a condition that is modulated by a GT, in a subject in need thereof, or in a sample, cell or tissue, wherein optionally said compound is not
- 15 5SGlcNAc, Ac-5SGlcNAc, 5CH₂GlcNAc, UDP-5CH₂GlcNAc, 5SGlcNAz, Ac-5SGlcNAz, or 5-T-Fuc.

[0021] In alternative aspects, the invention provides a kit comprising a composition or compound according to the invention together with instructions for use in for

inhibiting a GT, reducing the level of a nucleotide sugar-modified biomolecule, or treating a condition that is modulated by a GT.

[0022] In alternative embodiments, the subject may be a human. In some embodiments, the small alkyl may be methyl, ethyl, propyl, butyl, isopropyl, isobutyl, valeryl, or isovaleryl; the ether may be O-methyl, O-ethyl, O-propyl, O-isopropyl, O-butyl, or O-isobutyl; the thioether may be S-methyl, S-ethyl, S-propyl, S-isopropyl, S-butyl, or S-isobutyl; one, two, three or four of R₂ may be C(O)CH₃.

[0023] In some embodiments, the GT may be a fucosyltransferase (FUT), and the method may comprise administering an effective amount of a compound of Formula (IV) or (IX) or a pharmaceutically acceptable salt thereof.

[0024] In some embodiments, the GT may be a fucosyltransferase (FUT), and the method may comprise contacting a sample or cell comprising an antibody with an effective amount of a compound of Formula (IV) or (IX) or a pharmaceutically acceptable salt thereof.

[0025] In some embodiments, the GT may be a fucosyltransferase (FUT), and the method may comprise administering to the subject an effective amount of a compound of Formula (IV) or (IX) or a pharmaceutically acceptable salt thereof.

[0026] In some embodiments, the condition may be inflammation, an autoimmune disorder or a cancer.

[0027] In some embodiments, the GT may be a fucosyltransferase (FUT), and the method may comprises contacting the cell or tissue with an effective amount of a compound of Formula (IV) or (IX) or a pharmaceutically acceptable salt thereof.

[0028] In some embodiments, the compound may be 5-T-Fuc.

[0029] In some embodiments, the GT may be uridine diphospho-*N*-acetylglucosamine:polypeptide β -*N*-acetylglucosaminyltransferase (OGT), and the method may comprise administering an effective amount of a compound of Formula (I) or (VI) or a pharmaceutically acceptable salt thereof.

[0030] In some embodiments, the GT may be OGT, and the method may comprise administering to the subject an effective amount of a compound of Formula (I) or (VI) or a pharmaceutically acceptable salt thereof.

[0031] In some embodiments, the condition may be cancer, diabetes, complications of diabetes, inflammation, or autoimmune disease.

[0032] In some embodiments, the GT may be OGT and the method may comprise contacting the cell or tissue with an effective amount of a compound of Formula (I) or
5 (VI) or a pharmaceutically acceptable salt thereof.

[0033] In some embodiments, the cell may be a cancer cell or the tissue may be pancreatic tissue.

[0034] In some embodiments, the compound may increase the level of the OGT. In some embodiments, the compound may reduce the level of an *O*-GlcNAcase.

10 [0035] In some embodiments, the X may be S, R₂ may be H or C(O)CH₃, and R₄ may be H, CH₂N₃, CH₃, CH₂CH₃, (CH₂)₂CH₃, CH(CH₃)₂, (CH₂)₃CH₃, CH₂CH(CH₃)₂, (CH₂)₄CH₃, C(CH₃)₃, CH₂C₆H₁₁, CH₂C₁₀H₈, CH₂CH(CH₃)CH₂CH₃, or (CH₂)₃CH(CH₃)₂.

[0036] This summary of the invention does not necessarily describe all features of the
15 invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] 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:

[0038] **FIGURES 1A-B** are schematic diagrams showing (A) dynamic cycling of the
20 *O*-GlcNAc modification of nucleocytoplasmic proteins in eukaryotes. The modification is modulated by two enzymes: OGT transfers *O*-GlcNAc onto proteins from the UDP-GlcNAc sugar donor and OGA catalyzes hydrolysis of the sugar moiety and (B) the hexosamine biosynthetic pathway leading to the biosynthesis of UDP-GlcNAc, where endocyclic heteroatom is denoted X (O in nature and S in the
25 synthetic compounds according to some embodiments).

[0039] **FIGURE 1C** shows the structure of 5SGlcNAc, UDP-5SGlcNAc, *p*MP-5SGlcNAc, and Me-5SGlcNAc (X = O or S).

[0040] **FIGURE 1D** shows *in vitro* enzymatic synthesis of UDP-5SGlcNAc catalyzed by the human enzymes of the HBP and monitored by capillary electrophoresis; the

traces shows absorbance at 254 nm as a function of retention time. Upper trace shows the crude reaction mixture prior to purification and the lower trace shows UDP-5SGlcNAc following ion exchange and HPLC purification. Peak A corresponds to GDP-Glc (internal standard spiked into samples prior to analysis) and peak B corresponds to UDP-5SGlcNAc.

[0041] **FIGURE 1E** shows inhibition of OGT-catalyzed transfer of *O*-GlcNAc onto nup62 by UDP-5SGlcNAc. The K_i value, determined by Dixon analysis, is 8 μ M.

[0042] **Figure 1F-G.** ^1H NMR spectrum of UDP-5SGlcNAc. (F) ^1H NMR spectrum of UDP-5SGlcNAc. Peaks at 8.35, 3.10 and 1.18 ppm arise from the triethylammonium counterion. (G) Expansion of ^1H NMR spectrum of UDP-5SGlcNAc covering the region from 4.40 to 3.20 ppm.

[0043] **Figure 1H-I.** NMR spectra of UDP-5SGlcNAc. (H) $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of UDP-5SGlcNAc. Peaks at 8.1 (CH_3) and 46.6 (CH_2) are derived from the triethylammonium cation. The peak at 174.2 ppm is from the carbonyl of the acetyl group. (I) $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of UDP-5SGlcNAc referenced to 85% H_3PO_4 at 0 ppm.

[0044] **Figure 1J-K.** Two dimensional NMR spectra of UDP-5SGlcNAc. (K) HMQC spectrum and (L) COSY spectrum.

[0045] **FIGURES 2A-L** are a series of western blots showing that 5SGlcNAc acts in cells to decrease *O*-GlcNAc levels in a dose and time dependent manner.

[0046] **FIGURE 2A** shows Western blots of COS-7 cell lysates following Ac-5SGlcNAc administration at different doses (0-1000 μ M) for 24 h. Upper panel, probed with anti-*O*-GlcNAc antibody (CTD110.6); lower panel, probed with anti-actin antibody. The plot shows the densitometry analysis and yields an EC_{50} value for reduction of *O*-GlcNAc levels of 5 μ M.

[0047] **FIGURE 2B** shows Western blots of COS-7 cell lysates following Ac-5SGlcNAc administration at 50 μ M for different amounts of hours. Upper panel, probed with anti-*O*-GlcNAc antibody (CTD110.6); lower panel, probed with anti-actin antibody. The plot shows the densitometry analysis as a function of dose; *O*-GlcNAc levels diminish to a low level by 24 h.

[0048] **FIGURE 2C** shows Western blots of COS-7 cell lysates following Ac-5SGlcNAc administration at 50 μ M for different amounts of days. Probed with (from top to bottom) anti-*O*-GlcNAc antibody (CTD110.6), anti-actin antibody, anti-OGA antibody, and anti-OGT antibody.

5 [0049] **FIGURE 2D** shows Western blots of COS-7 cell lysates administered with specified agents at 50 μ M for 24 h; vehicle only (C), Ac-GlcNAc (G) or Ac-5SGlcNAc (5SG). Probed with (from top to bottom) anti-*O*-GlcNAc antibody (CTD110.6), anti-actin antibody, anti-OGA antibody, and anti-OGT antibody.

[0050] **FIGURE 2E** shows Western blot of immunoprecipitated nup62 from cell
10 lysates following vehicle (C) or 250 μ M Ac-5SGlcNAc (5SG) treatment for 24 h. Following immunoprecipitation, nup62 was incubated with UDP-GalNAz in the absence (-) or presence (+) of GalT1 and chemoselectively labeled. Upper panel, probed with anti-nup62 antibody; lower panel, probed with streptavidin.

[0051] **FIGURE 2F** shows Western blot of immunoprecipitated nup62 from cell
15 lysates following vehicle (C) or 250 μ M Ac-5SGlcNAc (5SG) treatment for 24 h. Following immunoprecipitation, nup62 was incubated with buffer (-) or with *Bt*GH84 glucosaminidase for 2 h to remove *O*-GlcNAc. Upper panel, probed with anti-nup62 antibody; lower panel, probed with anti-*O*-GlcNAc antibody (long exposure time shown at top and short exposure shown below).

20 [0052] **FIGURE 2G.** Western blots of COS-7 cell lysates following 5SGlcNAc administration at different doses (0-5000 μ M) for 24 h. Upper panel, probed with anti-*O*-GlcNAc antibody (CTD110.6); lower panel, probed with anti-actin antibody. The plot shows the densitometry analysis converted to a % *O*-GlcNAc modification (corrected for actin levels) relative to the untreated control sample as a function of
25 dose; this gives an EC₅₀ of 700 μ M.

[0053] **FIGURE 2H.** Western blots of COS-7 cell lysates following no (C), Ac-GlcNAc (G) or Ac-5SGlcNAc (5SG) administration at 50 μ M for 24 h. Probed with different anti-*O*-GlcNAc antibodies: CTD110.6, RL2 and HGAC85, from left to right.

[0054] **FIGURE 2I.** Western blots of COS-7 cell lysates following no (C), 50 μ M
30 Ac-5SGlcNAc treatment for 48 h (5SG), 50 μ M Ac-5SGlcNAc treatment for 24 h,

followed by no treatment for 24 h (W). Upper panel, probed with anti-*O*-GlcNAc antibody (CTD110.6); lower panel, probed with anti-actin antibody.

[0055] **FIGURE 2J.** Western blots of CHO cell lysates following Ac-5SGlcNAc administration at different doses (0-250 μ M) for 24 h. Upper panel, probed with anti-*O*-GlcNAc antibody (CTD110.6); lower panel, probed with anti-actin antibody. The plot shows the densitometry analysis converted to a % *O*-GlcNAc modification (corrected for actin levels) relative to the untreated control sample as a function of dose; this gives an EC₅₀ of 0.8 μ M.

[0056] **FIGURE 2K.** Western blots of CHO cell lysates following Ac-5SGlcNAc administration at 50 μ M for different amounts of time (shown in hours). Upper panel, probed with anti-*O*-GlcNAc antibody (CTD110.6); lower panel, probed with anti-actin antibody. The plot shows the densitometry analysis converted to a % *O*-GlcNAc modification (corrected for actin levels) relative to the untreated control sample as a function of dose.

[0057] **FIGURE 2L.** Western blots of cell lysates from different cell lines following no (C), Ac-GlcNAc (G) or Ac-5SGlcNAc (5SG) administration at 50 μ M (or 100 μ M for PC12 cells) for 24 h. Upper panel, probed with anti-*O*-GlcNAc antibody (CTD110.6); lower panel, anti-actin antibody. Cell lines used: COS-7 (African green monkey kidney cell line), CHO (Chinese hamster ovary cell line), SK-N-SH (human neuroblastoma cell line), HepG2 (human liver carcinoma cell line), PC12 (rat adrenal medulla pheochromocytoma cell line, which terminally differentiate upon nerve growth factor treatment), mouse hybridoma cell line and EMEG32^{-/-} (mouse embryonic fibroblasts deficient in glucosamine-6-phosphate acetyltransferase).

[0058] **FIGURES 3A-D** show that 5SGlcNAc is converted in cells to generate intracellular UDP-5SGlcNAc, causing only small perturbations in UDP-sugar nucleotide pools, and leaving *N*-glycosylation unperturbed.

[0059] **FIGURE 3A** shows analysis of UDP-sugar pools from COS-7 cells treated with different concentrations of Ac-5SGlcNAc (0-1000 μ M from bottom to top) for 24 h; the CE trace shows absorbance at 254 nm as a function of retention time. (A) GDP-Glc (internal standard); (B) UDP-GlcNAc; (C) UDP-Glc; (D) UDP-5SGalNAc; (E) UDP-5SGlcNAc; (F) UDP-GalNAc; (G) UDP-Gal. UDP-5SGlcNAc and UDP-

GalNAc co-elute, but the amount of each could be estimated using the epimeric ratios determined from the standards (Figure 3G).

[0060] **FIGURE 3B** shows a bar chart showing relative concentrations of UDP-GlcNAc, UDP-5SGlcNAc, UDP-Gal, UDP-5SGal, UDP-Glc and UDP-Gal following treatment with 0-1000 μ M Ac-5SGlcNAc.

[0061] **FIGURE 3C** shows Western blots of COS-7 cell lysates following Ac-5SGlcNAc administration at different doses (0-1000 μ M) for 24 h. C+ denotes untreated cell lysate incubated with PNGase F and C- denotes untreated cell lysate incubated with vehicle. Blots are probed with (from top to bottom) anti-*O*-GlcNAc antibody (CTD110.6), anti-actin antibody, ConA lectin (recognizes α -D-mannose, α -D-glucose and branched mannose), GNA lectin (recognizes mannose), PHA-L (recognizes complex branched chain oligosaccharide structure), SNA lectin (recognizes NeuAc α (2,6)Gal/GalNAc) and MAA lectin (recognizes NeuAc α (2,3)Gal). Full blots are shown in Figure 3E.

[0062] **FIGURE 3D** shows Western blots of mouse hybridoma cell lysates (*O*-GlcNAc and actin) and immunoprecipitated mouse hybridoma antibody (IgG, ConA and GNA) following administration of Ac-5SGlcNAc at different doses (0-1000 μ M) for 24 h. C+ denotes untreated cell lysate incubated with PNGase F and C- denotes untreated cell lysate incubated with vehicle. Blots are probed with (from top to bottom) anti-*O*-GlcNAc antibody (CTD110.6) (full blot shown in Figure 3F), anti-actin antibody, anti-IgG antibody, ConA lectin and GNA lectin.

[0063] **FIGURE 3E** shows the monitoring UDP-5SGlcNAc and UDP-5SGalNAc *in vitro* by capillary electrophoresis; trace shows absorbance at 254 nm as a function of retention time. Run 1, UDP-5SGlcNAc; run 2, UDP-5SGlcNAc treatment with UDP-GlcNAc 4-epimerase; run 3, UDP-GlcNAc and UDP-GalNAc standards. (A) GDP-Glc (internal standard); (B) UDP-GlcNAc; (C) UDP-5SGalNAc; (D) UDP-5SGlcNAc; (E) UDP-GalNAc.

[0064] **FIGURE 3F** shows UDP-sugar analysis by CE to validate identity of peaks from cells; trace shows absorbance at 254 nm as a function of retention time. Run 1, UDP-5SGlcNAc and UDP-5SGalNAc standards; run 2, UDP-GlcNAc and UDP-GalNAc standards; run 3, UDP-sugars extracted from COS-7 cells following

treatment with 250 μ M Ac-5SGlcNAc for 24 h; run 4, sample from run 3 spiked with standards from run 1; run 5, sample from run 3 spiked with standards from run 2. (A) GDP-Glc (internal standard); (B) UDP-GlcNAc; (C) UDP-5SGalNAc; (D) UDP-5SGlcNAc; (E) UDP-GalNAc.

5 [0065] **FIGURE 3G** shows UDP-5SGlcNAc and UDP-GalNAc co-eluted during CE analysis. The contribution from each molecule to the peak could be calculated based on the concentration of UDP-GlcNAc and UDP-5SGalNAc, and the epimeric ratio determined from the standards of 2.1:1 GlcNAc:GalNAc. This graph shows a comparison of the total peak area compared to the sum of the estimated peak areas
10 from the epimeric ratios for each concentration of Ac-5SGlcNAc administered to cells.

[0066] **FIGURE 3H** shows western blots of COS-7 cell lysates following Ac-5SGlcNAc administration at different doses (0-1000 μ M) for 24 h. C-, untreated cell lysate not incubated with PNGase F, C+, untreated cell lysate incubated with PNGase
15 F. Blots are probed with (from top to bottom) anti-*O*-GlcNAc antibody (CTD110.6), anti-actin antibody, ConA lectin (recognizes α -D-mannose, α -D-glucose and branched mannose), GNA lectin (recognizes mannose), PHA-L (recognizes complex branched chain oligosaccharide structure), SNA lectin (recognizes sialic acid α (2,6)Gal/GalNAc) and MAA lectin (recognizes sialic acid α (2,3)Gal).

20 [0067] **FIGURE 3I** shows western blots of a mouse hybridoma cell lysates following Ac-5SGlcNAc administration at different doses (0-1000 μ M) for 24 h. C-, untreated cell lysate not incubated with PNGase F, C+, untreated cell lysate incubated with PNGase F. Blot is probed with anti-*O*-GlcNAc antibody (CTD110.6).

[0068] **FIGURE 4A** shows western blots of recombinantly modified p62 protein,
25 probed with the anti-*O*-GlcNAc antibody CTD110.6 in the presence of increasing concentrations (in mM) of either Me-GlcNAc or Me-5SGlcNAc.

[0069] **FIGURE 4B** shows Michaelis-Menten kinetics for OGA hydrolysis of *p*MP-GlcNAc performed at 37 °C. The fit gives a k_{cat} of 14.3 nmol s⁻¹ mg⁻¹ and a K_M of 350 μ M.

[0070] **FIGURE 4C** shows Michaelis-Menten kinetics for OGA hydrolysis of *p*MP-5SGlcNAc performed at 37 °C. The fit gives a k_{cat} of 0.28 nmol s⁻¹ mg⁻¹ and a K_M of 35 μM.

[0071] **FIGURE 5A-E** shows the effect of Ac-5SGlcNAc (9) on cell growth and Sp1 levels and testing the reversibility of Ac-5SGlcNAc (9) treatment. (A) Cell proliferation curves over the course of 5 days for CHO cells following no (circles), 50 μM Ac-GlcNAc (squares) or 50 μM Ac-5SGlcNAc (triangles) treatment. The error bars indicate the deviation from the mean for triplicate measurements. The western blots for the last time point indicate *O*-GlcNAc levels are still significantly decreased in cells treated with Ac-5SGlcNAc. Upper panel, probed with anti-*O*-GlcNAc antibody (CTD110.6); lower panel, probed with anti-actin antibody. (B) Cell proliferation curves over the course of 5 days for EMEG heterozygous mouse embryonic fibroblast cells following no (circles), 50 μM Ac-GlcNAc (squares) or 50 μM Ac-5SGlcNAc (triangles) treatment. The error bars indicate the deviation from the mean for triplicate measurements. The western blots for the last time point indicate *O*-GlcNAc levels are still significantly decreased in cells treated with Ac-5SGlcNAc. Upper panel, probed with anti-*O*-GlcNAc antibody (CTD110.6); lower panel, probed with anti-actin antibody. (C) Western blots of CHO cell lysates following no (C) or Ac-5SGlcNAc administration at 50 μM for 4 days. Probed with (from top to bottom) anti-Sp1 antibody, anti *O*-GlcNAc (CTD110.6) antibody and anti-actin antibody. Full blot of the anti-Sp1 is shown in panel (d). (D) Western blots of CHO cell lysates following no (C) or Ac-5SGlcNAc administration at 50 μM for 4 days. The full blot probed with anti-Sp1 antibody is shown. (E) Western blots of COS-7 cell lysates following no (C) or 50 μM Ac-5SGlcNAc treatment for 24 h. Following treatment, the media was replaced with no inhibitor, and cells were harvested at the time points indicated (between 0 and 24 h). Upper panel, probed with anti-*O*-GlcNAc antibody (CTD110.6); lower panel, probed with anti-actin antibody.

[0072] **FIGURE 6A-E** shows metabolic feeding of Ac-5SGlcNAz (41) to cells causes a decrease in *O*-GlcNAc levels, but chemoselective ligation demonstrates there is no accumulation of 5SGlcNAz on proteins. (A) Structures of the molecules used or generated during the chemoselective ligation study (X = O or S as indicated, R = H or Ac as indicated). (B) Western blots of COS-7 cell lysates administered specified

agents at 50 μ M for 24 h; vehicle only (C), Ac-GlcNAz or Ac-5SGlcNAz (41). Cells were harvested and then underwent the Staudinger ligation with biotin phosphine. Blots are probed with (from top to bottom) streptavidin-HRP, anti *O*-GlcNAc (CTD110.6) antibody and anti-actin antibody. (C) Western blot of immunoprecipitated nup62 from cell lysates following vehicle (C), 50 μ M Ac-GlcNAz or Ac-5SGlcNAz (41) treatment for 24 h. Following immunoprecipitation, nup62 was incubated with buffer (-) or with *Bt*GH84 (+) for 2 h to remove *O*-GlcNAc, and then underwent the Staudinger ligation with biotin phosphine. Blots are probed with streptavidin-HRP. (D) ^{1} H-NMR spectrum of Ac-5SGlcNAz (41). (E) ^{13} C-NMR spectrum of Ac-5SGlcNAz (41). Both were taken in CDCl₃. (F) Full western blot of immunoprecipitated nup62 from cell lysates following vehicle (C), 50 μ M Ac-GlcNAz or Ac-5SGlcNAz (41) treatment for 24 h. Following immunoprecipitation, nup62 was incubated with buffer (-) or with *Bt*GH84 (+) for 2 h to remove *O*-GlcNAc, and then underwent the Staudinger ligation with biotin phosphine. Blots are probed with streptavidin-HRP.

[0073] **FIGURE 7** shows western blots probed with anti-*O*-GlcNAc antibody (CTD110.6) following treatment of COS-7 cells with 50 μ M compound **9**, **27**, **28**, **37** or **36** or vehicle alone for 24 h.

[0074] **FIGURE 8** shows representative western blots from mouse studies. Either compound **9** or **28** (300 mg/kg intraperitoneally) or vehicle (75% DMSO or PBS) was dosed in triplicate for 16 hours, and subsequently tissues harvested. (a) Lung tissue following treatment with compound **9**; (b) Pancreatic tissue following treatment with compound **9**; (c) Lung tissue following treatment with compound **28**; (d) Pancreatic tissue following treatment with compound **28**. In all cases the top panel the blot is probed with anti-*O*-GlcNAc antibody (CTD110.6) and bottom panel with anti-actin antibody.

[0075] **FIGURE 9** shows western blot, probed with anti-*O*-GlcNAc antibody (CTD110.6) of primary CLL cells treated with a range of Ac-5SGlcNAc (**9**) concentrations (shown in μ M) in the presence or absence of cytokine stimulation.

[0076] **FIGURE 10** shows 5-thiofucose (5-T-Fuc) reduces sialyl-lewis X expression in HepG2 liver cells. Immunoblot analysis of HepG2 lysates was performed with (A)

anti-CD15s (sLe^X), (B) *Aleura aurantia* lectin (binds fucosylated glycans) and (C) the α 2,6-Neu5Ac-specific *Maackia amurensis* lectin.

[0077] **FIGURE 11** shows 5-T-Fuc reduces sLeX expression in a time-dependant fashion. Immunoblot analysis of sLeX expression in HepG2 cells cultured for an increasing length of time in the presence of 5-T-Fuc. (A) 0.5 min and (B) 3 min exposures. (C) Full recovery of sLeX expression after a 24h exposure to 5-T-Fuc occurs within 24 h.

[0078] **FIGURE 12** shows CHO K1 cells demonstrate a reduction in core (α 1,6)-fucosylation upon 5-T-Fuc-treatment.

10 **DETAILED DESCRIPTION**

[0079] The invention provides, in part, compounds that are capable of inhibiting a glycosyltransferase or “GT”. In some embodiments, the GT may be a uridine diphospho-*N*-acetylglucosamine:polypeptide β -*N*-acetylglucosaminyltransferase or “OGT”). In alternative embodiments, the GT may be an alternative *N*-acetylglucosaminyltransferase, an *N*-acetylgalactosaminyltransferase, a fucosyltransferase, a xylotransferase or a sialyltransferase.

[0080] In some embodiments, the invention provides for the design of an inhibitor of a GT (a “GT inhibitor”) by providing a nucleotide sugar substrate precursor or variant or analog thereof that is readily cell permeable and is capable of being utilized in an endogenous biosynthetic pathway to form the GT inhibitor.

[0081] In some embodiments, a GT inhibitor may be constructed by replacing the endocyclic oxygen atom found in sugars to a different group including, without limitation, sulphur, selenium, or CH₂, such that enzymes in biosynthetic pathways involved in the formation of a nucleotide sugar still process the sugar analogue to form, within tissues, an analogue of the natural nucleotide sugar in which the endocyclic ring oxygen is replaced by the group present in the sugar analogue precursor. Accordingly, by using sugar analogues in which the endocyclic oxygen is replaced by certain groups, the sugar analogue is assimilated by these salvage and biosynthetic pathways to form the unnatural nucleotide sugar analogue having the group present in place of the endocyclic sugar ring oxygen.

[0082] In some embodiments, the resulting nucleotide sugar analogue is either not a substrate or it is a worse substrate for the GT than is the naturally occurring nucleotide sugar used by the GT. In some embodiments, the unnatural nucleotide sugar analogue binds to and thereby inhibits the GT. In some embodiments, the activity of the GT is impaired and the levels of the glycoconjugates normally biosynthesized by the GT decrease. The levels of the GT may, in some cases, increase to compensate for its inhibition.

[0083] In the exemplary embodiment of OGT inhibition, as described herein, we show that the change from oxygen to sulphur was subtle enough for the enzymes in the salvage and biosynthetic pathways to tolerate the synthesis of the nucleotide sugar analogue, but sufficient to impair the ability of OGT to use the nucleotide sugar analogue and cause inhibition of OGT.

[0084] Other sugars having the endocyclic oxygen or other group replaced by a different group, as described herein, will also feed into the normal biosynthetic pathways of the cell to form nucleotide sugar analogues. These nucleotide sugar analogues are poor substrates for GTs that process their preferred natural nucleotide sugar substrates. Without being bound to any particular hypothesis, it is expected that because the nucleotide sugar analogues are turned over poorly by GTs, yet resemble the natural nucleotide sugar substrates used by these GTs, these nucleotide sugar analogues inhibit the normal functioning of these GTs both in vitro and in tissues and in vivo.

[0085] In alternative embodiments, unnatural sugar-1-phosphate analogues in which the endocyclic ring oxygen is replaced for example by CH₂, S, or Se, or which have other S or Se substitutions as described herein, may be used in the biosynthesis within cells of the corresponding unnatural nucleotide sugar which would, as discussed herein, inhibit GTs processing the corresponding natural nucleotide sugar phosphate. Several examples are provided herein, for example, formulae VI-X, wherein the sugar-1-phosphate is derivatized. Certain compounds described by these formulae are assimilated by intracellular biosynthetic pathways to form the unnatural nucleotide sugar that will inhibit the corresponding GT(s). Accordingly, a number of sugars,

including GlcNAc, GalNAc, ManNAc, Fuc, Xyl, or GlcNH₂, as well as their 1-phosphosugar derivatives, may be used as described herein to construct GT inhibitors.

[0086] A “glycosyltransferase” or “GT” is an enzyme that transfers a sugar residue from an anionic nucleotide sugar donor to various acceptor molecules, such as proteins, lipids, saccharides, metabolites, or other substrates. GTs include, without limitation, *N*-acetylglucosaminyltransferases, *N*-acetylgalactosaminyltransferases, fucosyltransferases, xylosyltransferases, sialyltransferases, mannosyltransferases, glucosyltransferases and galactosyltransferases.

[0087] A “uridine diphospho-*N*-acetylglucosamine:polypeptide β -*N*-acetylglucosaminyltransferase” or “OGT” is a GT that transfers *O*-GlcNAc onto proteins or polypeptides using the donor substrate uridine diphospho-*N*-acetylglucosamine (UDP-GlcNAc), which is biosynthesized through the action of enzymes comprising the hexosamine biosynthetic pathway (HBP) (Figure 1B). As used herein, an OGT may be derived from any source or subject or in different splice forms. Examples of OGTs include, without limitation human OGT having Accession number O15294, as well as OGT splice variants, for example those having Accession numbers NP_858058.1 or CAC86128.1 (isoform 1), NP_858059.1 or CAC86127.1 (isoform 2), or CAC86129 (mitochondrial variant). In alternative embodiments, OGTs include OGTs from various organisms, for example, rat, mouse, human, monkey, etc. In alternative embodiments, an OGT as used herein is substantially identical to the human OGT having Accession number O15294, or to OGT splice variants, for example those having Accession numbers NP_858058.1 or CAC86128.1 (isoform 1), NP_858059.1 or CAC86127.1 (isoform 2), or CAC86129 (mitochondrial variant), or to homologous sequences found in for example rat, mouse, monkey, etc.

[0088] A “fucosyltransferase” or “FUT” is a GT that transfers fucose onto proteins, polypeptides, or other saccharide units, using the donor substrate guanosine diphospho-fucose (GDP-Fuc), which is biosynthesized through the action of enzymes comprising the fucose biosynthetic pathway or salvaged via the fucose salvage pathway⁷⁴. There are currently thirteen known fucosyltransferase genes known within the human genome⁶⁷.

[0089] Some of these are involved in the synthesis of Lewis blood group antigens including sialyl Lewis X (sLe^x) while others synthesize Le^a, Le^b, sialyl Le^a⁶⁷. Some of these fucosyltransferases have other activities or the activities remain to be discovered. There are also two protein *O*-fucosyltransferases that are known as Pofut1 and Pofut2, and these transfer a fucose residue to serine or threonine within epidermal growth factor-like repeats or thrombospondin type 1 repeats to form an α -linkage⁶⁷. As used herein, a fucosyltransferase may be derived from any source or subject or in different splice forms. Examples of FUTs include, without limitation human FUT1 through FUT11 and includes poFUT1 and poFUT2 as well as FUT splice variants. In alternative embodiments, FUTs include FUTs from various organisms, for example, rat, mouse, human, monkey, etc.

[0090] A “biomolecule” refers to a molecule or compound produced by a living organism. Biomolecules include without limitation proteins, peptides, nucleic acids (e.g., DNA or RNA), polysaccharides, lipids, small molecules, etc. In some embodiments, a biomolecule as used herein may be modified with a nucleotide sugar molecule.

[0091] By “substantially identical” is meant an amino acid or nucleotide sequence that differs from a reference sequence only by one or more conservative substitutions, as discussed herein, or by one or more non-conservative substitutions, deletions, or insertions located at positions of the sequence that do not destroy the biological function of the amino acid or nucleic acid molecule. Such a sequence can be any integer from 10% to 99%, or more generally at least 10%, 20%, 30%, 40%, 50%, 55% or 60%, or at least 65%, 75%, 80%, 85%, 90%, or 95%, or as much as 96%, 97%, 98%, or 99% identical when optimally aligned at the amino acid or nucleotide level to the sequence used for comparison using, for example, the Align Program⁷⁵ or FASTA. For polypeptides, the length of comparison sequences may be at least 2, 5, 10, or 15 amino acids, or at least 20, 25, or 30 amino acids. In alternate embodiments, the length of comparison sequences may be at least 35, 40, or 50 amino acids, or over 60, 80, or 100 amino acids. For nucleic acid molecules, the length of comparison sequences may be at least 5, 10, 15, 20, or 25 nucleotides, or at least 30, 40, or 50 nucleotides. In alternate embodiments, the length of comparison sequences may be at least 60, 70, 80, or 90 nucleotides, or over 100, 200, or 500 nucleotides. Sequence

identity can be readily measured using publicly available sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, or BLAST software available from the National Library of Medicine, or as described herein). Examples of useful software include the programs Pile-up and PrettyBox. Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, substitutions, and other modifications.

[0092] Alternatively, or additionally, two nucleic acid sequences may be “substantially identical” if they hybridize under high stringency conditions. In some embodiments, high stringency conditions are, for example, conditions that allow hybridization comparable with the hybridization that occurs using a DNA probe of at least 500 nucleotides in length, in a buffer containing 0.5 M NaHPO₄, pH 7.2, 7% SDS, 1 mM EDTA, and 1% BSA (fraction V), at a temperature of 65°C, or a buffer containing 48% formamide, 4.8x SSC, 0.2 M Tris-Cl, pH 7.6, 1x Denhardt's solution, 10% dextran sulfate, and 0.1% SDS, at a temperature of 42°C. (These are typical conditions for high stringency northern or Southern hybridizations.) Hybridizations may be carried out over a period of about 20 to 30 minutes, or about 2 to 6 hours, or about 10 to 15 hours, or over 24 hours or more. High stringency hybridization is also relied upon for the success of numerous techniques routinely performed by molecular biologists, such as high stringency PCR, DNA sequencing, single strand conformational polymorphism analysis, and in situ hybridization. In contrast to northern and Southern hybridizations, these techniques are usually performed with relatively short probes (e.g., usually about 16 nucleotides or longer for PCR or sequencing and about 40 nucleotides or longer for in situ hybridization). The high stringency conditions used in these techniques are well known to those skilled in the art of molecular biology, and examples of them can be found, for example, in Ausubel et al.⁷⁶.

[0093] 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, an inhibitor of a GT reduces the levels of glycoconjugates biosynthesized by the GT. In some

embodiments, an inhibitor of an OGT decreases or reduces O-GlcNAc levels *e.g.*, O-GlcNAc-modified polypeptide or protein levels, in cells, tissues, or organs (*e.g.*, in pancreatic, brain, muscle, adipose, hepatic, blood, skin, eye, nervous system tissue) and in animals. By “reduced,” “reduces” or “reducing” is meant a decrease by any
5 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, 15-fold, 25-fold, 50-fold, 100-fold or more.

[0094] In some embodiments, the compounds of the present invention according to Formulae I or VI reduce O-GlcNAc levels on O-GlcNAc-modified polypeptides or
10 proteins *in vivo* specifically via interaction with an OGT, and are effective in treating conditions which require or respond to inhibition of OGT activity.

[0095] In some embodiments, the compounds of the present invention are useful as agents that produce a decrease in the levels of specific sets of glycoconjugates. In some embodiments, the compounds such as those described in Formulae II-V or VII-
15 X are therefore useful to treat conditions which require or respond to inhibition of GT activity, such as *N*-acetylglucosaminyltransferases, *N*-acetylgalactosaminyltransferases, fucosyltransferases, xylosyltransferases, sialyltransferases, mannosyltransferases, glucosyltransferases or galactosyltransferases.

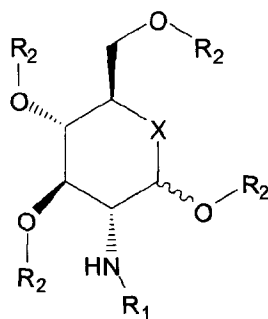
[0096] In some embodiments, the compounds of the present invention according to Formulae IV or IX reduce levels of fucose modified biomolecules *in vivo* via
20 interaction with an FUT, and/or through decreasing GDP-Fuc levels in cells, and are effective in treating conditions which require or respond to decreased FUT activity.

[0097] In some embodiments, the invention provides methods of producing an
25 antibody by contacting an antibody-producing cell with a fucosyltransferase inhibitor, such that an antibody with reduced levels of fucose is produced. By “reduced levels of fucose” is meant that the glycan structures present on the antibody contain decreased amounts of fucose. By “reduced,” “reduces” or “reducing” is meant a decrease by any value between 10% and 90%, or of any integer value between 30%
30 and 60%, or over 100%, or a decrease by 1-fold, 2-fold, 5-fold, 10-fold, 15-fold, 25-fold, 50-fold, 100-fold or more.

[0098] In some embodiments, the compounds produce a decrease in levels of O-GlcNAc modification on O-GlcNAc-modified polypeptides or proteins, and are therefore useful for treatment of disorders responsive to such decreases in O-GlcNAc modification; these disorders include without limitation cancer, diabetes, insulin resistance, complications of diabetes, inflammation, autoimmune disease or bacterial infections. In some embodiments, the compounds are also useful as a result of other biological activities related to their ability to inhibit the activity of glycosyltransferases. 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.

[0099] In alternative embodiments, the invention provides methods of reducing levels of protein O-GlcNAc modification in animal subjects, such as, veterinary and human subjects. In alternative embodiments, the invention provides methods of inhibiting an OGT in animal subjects, such as, veterinary and human subjects.

[00100] In specific embodiments, the invention provides compounds described generally by Formula (I) and the salts, prodrugs, and stereoisomeric forms thereof:



Formula (I)

[00101] As set forth in Formula (I):

[00102] X may be S, Se or CH₂;

[00103] R₁ may be either H or C(O)R₄, where R₄ may be H, CH₂OH, CH₂N₃, CH₂SH, small branched or unbranched alkyl groups (such as methyl, ethyl, propyl, butyl, isopropyl, isobutyl, tert-butyl, valeryl, isovaleryl, etc.), ethers such as O-methyl, O-ethyl, O-propyl, O-isopropyl, O-butyl, O-isobutyl, etc.); thioethers such as S-methyl, S-ethyl, S-propyl, S-isopropyl, S-butyl, S-isobutyl, etc.; ureas; thioureas; etc.,

where the alkyl groups, ethers, thioethers, ureas, or thioureas may be optionally substituted;

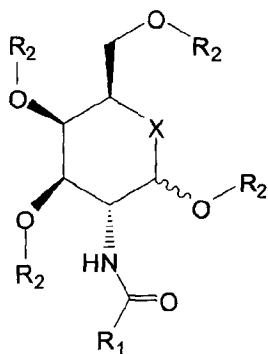
[00104] R_2 may be H or $C(O)R_5$, where R_5 may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl. In these cases, the ester groups may be hydrolyzed in vivo (e.g. in bodily fluids and/or within cells), releasing the active compounds in which R^2 is H. Prodrug embodiments of the invention include compounds where one, two, three or four of R_2 is $C(O)CH_3$;

[00105] $\sim$ may be the alpha anomer, the beta anomer, or both.

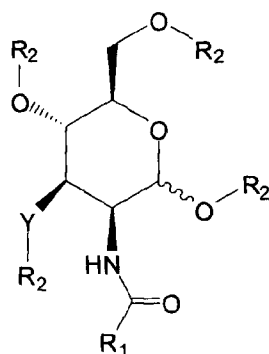
[00106] In some embodiments of the invention, X may be S, R_2 may be H or Ac, and R_4 may be CH_3 , CH_2CH_3 , $(CH_2)_2CH_3$, $CH(CH_3)_2$, $(CH_2)_3CH_3$, $CH_2CH(CH_3)_2$, $(CH_2)_4CH_3$, $C(CH_3)_3$, $CH_2C_6H_{11}$, $CH_2C_{10}H_8$, $CH_2CH(CH_3)CH_2CH_3$, $(CH_2)_3CH(CH_3)_2$, etc.

[00107] In some embodiments of the invention, one or more of the following compounds: 5SGlcNAc, Ac-5SGlcNAc, 5CH₂GlcNAc, UDP-5CH₂GlcNAc, 5SGlcNAz, Ac-5SGlcNAz, are specifically excluded from the compounds described in Formula (I). In some embodiments of the invention, specific stereoisomers or enantiomers of one or more of the following compounds: 5SGlcNAc, Ac-5SGlcNAc, 5CH₂GlcNAc, UDP-5CH₂GlcNAc, 5SGlcNAz, Ac-5SGlcNAz, are specifically excluded from the compounds described in Formula (I).

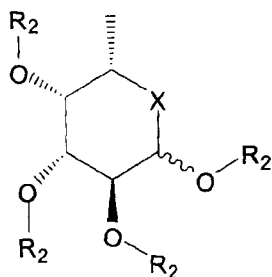
[00108] In alternative embodiments, Formulae II – V show sugar structures that can be used to target GTs:



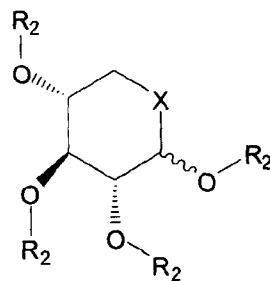
II



III

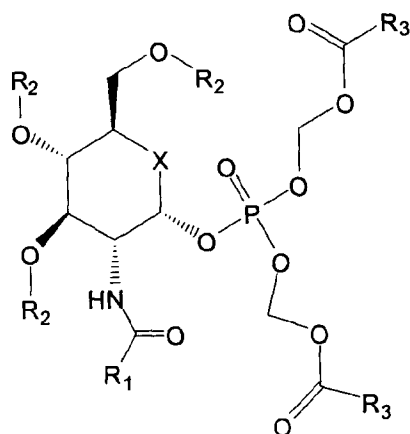


IV

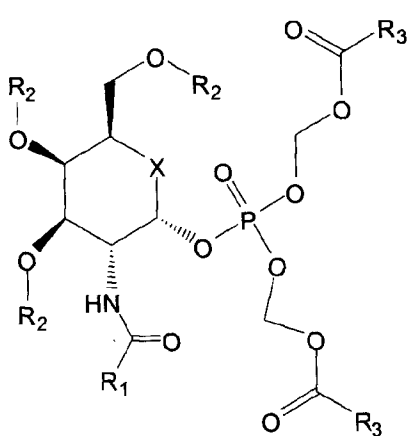


V

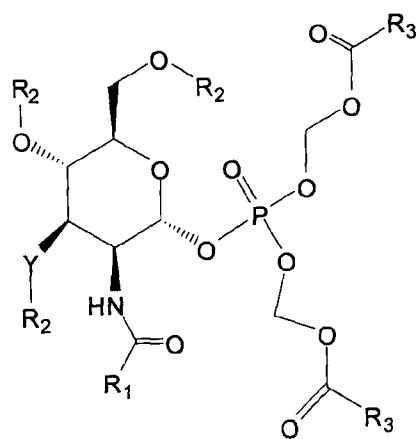
- [00109] R_1 may be either H or $C(O)R_4$, where R_4 may be H, CH_2OH , CH_2N_3 , CH_2SH , small branched or unbranched alkyl groups (such as methyl, ethyl, propyl, butyl, isopropyl, isobutyl, tert-butyl, valeryl, isovaleryl, etc.), ethers such as O-methyl, O-ethyl, O-propyl, O-isopropyl, O-butyl, O-isobutyl, etc.); thioethers such as S-methyl, S-ethyl, S-propyl, S-isopropyl, S-butyl, S-isobutyl, etc.; ureas; thioureas; etc., where the alkyl groups, ethers, thioethers, ureas, or thioureas may be optionally substituted;
- 10 [00110] R_2 may be H or $C(O)R_5$, where R_5 may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl. In these cases, the ester groups may be hydrolyzed in vivo (e.g. in bodily fluids and/or within cells), releasing the active compounds in which R^2 is H. Prodrug embodiments of the invention include compounds where one, two, three or four of R_2 is $C(O)CH_3$;
- 15 [00111] $\sim$ may be the alpha anomer, the beta anomer, or both.
- [00112] As set forth in Formulae (II, IV and V):
- [00113] X may be S, Se or CH_2 ;
- [00114] As set forth in Formula III:
- [00115] Y may be S or Se.
- 20 [00116] In alternative embodiments, Formulae VI–X show derivatized sugar phosphate structures that could be used to target GTs:



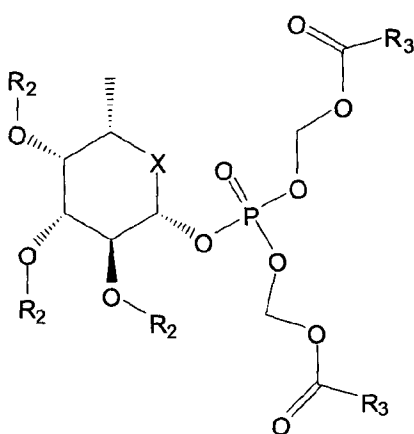
VI



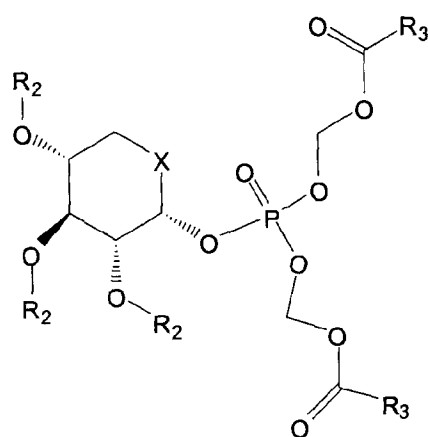
VII



VIII



IX



X

[00117] As set forth in Formulae (VI-X):

[00118] R_1 may be either H or $C(O)R_4$, where R_4 may be H, CH_2OH , CH_2N_3 , CH_2SH , small branched or unbranched alkyl groups (such as methyl, ethyl, propyl,

butyl, isopropyl, isobutyl, tert-butyl, valeryl, isovaleryl, etc.), ethers such as O-methyl, O-ethyl, O-propyl, O-isopropyl, O-butyl, O-isobutyl, etc.); thioethers such as S-methyl, S-ethyl, S-propyl, S-isopropyl, S-butyl, S-isobutyl, etc.; ureas; thioureas; etc., where the alkyl groups, ethers, thioethers, ureas, or thioureas may be optionally substituted;

[00119] R_2 may be H or $C(O)R_5$, where R_5 may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl. In these cases, the ester groups may be hydrolyzed in vivo (e.g. in bodily fluids and/or within cells), releasing the active compounds in which R_2 is H. Prodrug embodiments of the invention include compounds where one, two, or three of R_2 is $C(O)CH_3$;

[00120] R_3 may be H or $C(O)R_6$, where R_6 may be optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

[00121] As set forth in Formulae (VI, VII, IX and X):

[00122] X may be S, Se or CH_2 ;

[00123] As set forth in Formula VIII:

[00124] Y may be S or Se.

[00125] "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.

[00126] "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, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, etc. Unless otherwise stated specifically herein, the term "cycloalkyl" is meant to include cycloalkyl groups which are optionally substituted as described herein.

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[00127] “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.

[00128] “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 alkynyl group may be optionally substituted by one or more substituents as described herein.

[00129] “Aryl” refers to a a single or fused aromatic ring group , including for example, 5-12 members, such as a phenyl or naphthyl group. Unless stated otherwise specifically herein, the term “aryl” is meant to include aryl groups optionally substituted by one or more substituents as described herein.

[00130] “Heteroaryl” refers to a single or fused aromatic ring group containing one or more heteroatoms in the ring, for example N, O, S, including for example, 5-14 members. Examples of heteroaryl groups include furan, thiophene, pyrrole, oxazole, thiazole, imidazole, pyrazole, isoxazole, isothiazole, 1,2,3-oxadiazole, 1,2,3-triazole, 1,2,4-triazole, 1,3,4-thiadiazole, tetrazole, pyridine, pyridazine, pyrimidine, pyrazine, 1,3,5-triazine, imidazole, benzimidazole, benzoxazole, benzothiazole, indolizine, indole, isoindole, benzofuran, benzothiophene, 1H-indazole, purine, 4H-quinolizine, quinoline, isoquinoline, cinnoline, phthalazine, quinazoline, quinoxaline, 1,8-naphthyridine, pteridine. Unless stated otherwise specifically herein, the term “heteroaryl” is meant to include heteroaryl groups optionally substituted by one or more substituents as described herein.

[00131] “Ether” refers to a compound in which an oxygen atom is bonded to an alkyl or an aryl group, or to two alkyl or two aryl groups, as described herein.

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[00132] “Thioether” refers to a compound in which a sulphur atom is bonded to an alkyl or an aryl group, or to two alkyl or two aryl groups, as described herein.

[00133] “Optional” or “optionally” means that the subsequently described event of circumstances may or may not occur, and that the description includes instances
5 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. or including cycloalkyls such as
10 cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, etc., or including aryls such as phenyl or naphthyl; examples of optionally substituted alkenyl groups include allyl, crotyl, 2-pentenyl, 3-hexenyl, 2-cyclopentenyl, 2-cyclohexenyl, 2-cyclopentenylmethyl, 2-cyclohexenylmethyl, etc. In some embodiments, optionally substituted alkyl and alkenyl groups include C₁₋₆ alkyls or alkenyls.

15 [00134] 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” or “the glycosyltransferase” includes a particular enzyme as well as other family members and equivalents thereof as known to those skilled in the art.

20 [00135] 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
25 compositions including the compounds and a pharmaceutically acceptable carrier, and pharmaceutical compositions including prodrugs of the compounds and a pharmaceutically acceptable carrier.

[00136] In some embodiments, the formulations, preparation, and compositions including compounds according to the invention include mixtures of anomers (alpha
30 and beta) or include individual anomers (alpha or beta). In general, the compound may be supplied in any desired degree of purity.

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Therapeutic Indications

[00137] The invention provides methods of treating conditions that are modulated, directly or indirectly, by a GT such as *N*-acetylglucosaminyltransferases, *N*-acetylgalactosaminyltransferases, fucosyltransferases, xylosyltransferases, sialyltransferases, mannosyltransferases, glucosyltransferases or galactosyltransferases, or by sugar-modified protein levels, for example, a conditions which require or respond to inhibition of GT activity.

[00138] In some embodiments, the invention provides methods of treating conditions that are modulated, directly or indirectly, by an OGT or by O-GlcNAc-modified protein levels, for example, a condition that is benefited by inhibition of an OGT or by a reduction of O-GlcNAc-modified protein levels. Such conditions include, without limitation, cancer, diabetes, insulin resistance, complications of diabetes, or autoimmune disease. The compounds of the invention are also useful in the treatment of diseases or disorders related to deficiency or over-expression of OGT or accumulation or depletion of O-GlcNAc, or any disease or disorder responsive to glycosyltransferase inhibition therapy. Such diseases and disorders include, but are not limited to, cancer, diabetes, insulin resistance, complications of diabetes, or autoimmune disease.

[00139] Such diseases and disorders may also include diseases or disorders related to the accumulation or deficiency in the enzyme O-GlcNAcase. 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.

[00140] In some embodiments, the invention provides methods of treating conditions that are modulated, directly or indirectly, by an FUT, for example, a condition that is benefited by inhibition of an FUT. Such conditions include, without limitation, inflammation, autoimmune disorders, cancer (e.g., tumour growth and/or metastasis), etc. The compounds of the invention are also useful in the treatment of diseases or disorders related to over-expression of an FUT, or any disease or disorder responsive to fucosyltransferase inhibition therapy. Such diseases and disorders include, but are not limited to, inflammation, autoimmune disorders, cancer (e.g.,

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tumour growth and/or metastasis), infections, (e.g., *Helicobacter pylori* infections,) glaucoma, atherosclerosis, etc.

[00141] By a “cancer” or “neoplasm” is meant any unwanted growth of cells serving no physiological function. In general, a cell of a neoplasm has been released
5 from its normal cell division control, i.e., a cell whose growth is not regulated by the ordinary biochemical and physical influences in the cellular environment. In most cases, a neoplastic cell proliferates to form a clone of cells which are either benign or malignant. Examples of cancers or neoplasms include, without limitation, transformed and immortalized cells, tumours, and carcinomas such as breast cell carcinomas and
10 prostate carcinomas. The term cancer includes cell growths that are technically benign but which carry the risk of becoming malignant. By “malignancy” is meant an abnormal growth of any cell type or tissue. The term malignancy includes cell growths that are technically benign but which carry the risk of becoming malignant. This term also includes any cancer, carcinoma, neoplasm, neoplasia, or tumor.

15 [00142] Most cancers fall within three broad histological classifications: carcinomas, which are the predominant cancers and are cancers of epithelial cells or cells covering the external or internal surfaces of organs, glands, or other body structures (e.g., skin, uterus, lung, breast, prostate, stomach, bowel), and which tend to metastasize; sarcomas, which are derived from connective or supportive tissue (e.g.,
20 bone, cartilage, tendons, ligaments, fat, muscle); and hematologic tumors, which are derived from bone marrow and lymphatic tissue. Carcinomas may be adenocarcinomas (which generally develop in organs or glands capable of secretion, such as breast, lung, colon, prostate or bladder) or may be squamous cell carcinomas (which originate in the squamous epithelium and generally develop in most areas of
25 the body). Sarcomas may be osteosarcomas or osteogenic sarcomas (bone), chondrosarcomas (cartilage), leiomyosarcomas (smooth muscle), rhabdomyosarcomas (skeletal muscle), mesothelial sarcomas or mesotheliomas (membranous lining of body cavities), fibrosarcomas (fibrous tissue), angiosarcomas or hemangioendotheliomas (blood vessels), liposarcomas (adipose tissue), gliomas or
30 astrocytomas (neurogenic connective tissue found in the brain), myxosarcomas (primitive embryonic connective tissue), or mesenchymous or mixed mesodermal tumors (mixed connective tissue types). Hematologic tumors may be myelomas,

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which originate in the plasma cells of bone marrow; leukemias which may be “liquid cancers” and are cancers of the bone marrow and may be myelogenous or granulocytic leukemia (myeloid and granulocytic white blood cells), lymphatic, lymphocytic, or lymphoblastic leukemias (lymphoid and lymphocytic blood cells) or polycythemia vera or erythremia (various blood cell products, but with red cells predominating); or lymphomas, which may be solid tumors and which develop in the glands or nodes of the lymphatic system, and which may be Hodgkin or Non-Hodgkin lymphomas. In addition, mixed type cancers, such as adenosquamous carcinomas, mixed mesodermal tumors, carcinosarcomas, or teratocarcinomas also exist.

10 [00143] Cancers may also be named based on the organ in which they originate i.e., the “primary site,” for example, cancer of the breast, brain, lung, liver, skin, prostate, testicle, bladder, colon and rectum, cervix, uterus, etc. This naming persists even if the cancer metastasizes to another part of the body, that is different from the primary site. Cancers named based on primary site may be correlated with histological

15 classifications. For example, lung cancers are generally small cell lung cancers or non-small cell lung cancers, which may be squamous cell carcinoma, adenocarcinoma, or large cell carcinoma; skin cancers are generally basal cell cancers, squamous cell cancers, or melanomas. Lymphomas may arise in the lymph nodes associated with the head, neck and chest, as well as in the abdominal lymph nodes or

20 in the axillary or inguinal lymph nodes. Identification and classification of types and stages of cancers may be performed by using for example information provided by the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute.

[00144] “Diabetes” or “diabetes mellitus” is a group of metabolic diseases

25 characterized by high blood sugar (glucose) levels, that result from defects in insulin secretion, or action, or both. Complications of diabetes are conditions or disorders as a consequence of diabetes or commonly found in diabetes patients and include without limitation, microvascular and macrovascular disease, blindness, kidney failure, nerve damage, atherosclerosis, leading to strokes, coronary heart disease, insulin resistance,

30 vascular damage, skin ulcers, circulatory damage, diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, etc.

WO

[00145] “Inflammation” refers to a non-specific immune response caused, for example, by infection, irritants (such as chemical irritants) or tissue or cell injury. Inflammation may be acute or chronic. Inflammatory diseases or disorders include, without limitation, atherosclerosis, allergy, inflammatory myopathy, cancer,
5 inflammation caused by drugs or chemical, etc.

[00146] Autoimmunity generally refers to a misdirected immune response that occurs when the immune system goes awry and attacks the body itself. While autoimmunity may be generally present in most individuals and may be benign, in some cases a progression to a pathogenic state causes autoimmune disease.

10 Autoimmunity is generally evidenced by the presence of antibodies or T lymphocytes reactive against host antigens. Autoimmune diseases include without limitation Type I diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, Grave’s disease, rheumatoid arthritis, chronic thyroiditis, etc.

[00147] The term “treating” as used herein includes treatment, prevention, and
15 amelioration.

[00148] In alternative embodiments, the invention provides methods of reducing levels of protein O-GlcNAc modification in animal subjects, such as, veterinary and human subjects. This reduction of O-GlcNAc levels can be useful, for example, for the prevention or treatment of cancer, diabetes, insulin resistance,
20 complications of diabetes, or autoimmune disease.

[00149] In alternative embodiments, the invention provides methods of inhibiting a GT, for example, an OGT or an FUT, in animal subjects, such as veterinary and human subjects.

[00150] In general, the methods of the invention are effected by administering a
25 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 Formulae (I-X). In some embodiments, compounds according to Formula I or VI are useful in the treatment of a disorder in which the regulation of
30 O-GlcNAc protein modification is implicated, or any condition as described herein. Disease states of interest include without limitation, cancer, diabetes, insulin

resistance, complications of diabetes, inflammation, automimmune disorders, cancer, infections, (e.g., *Helicobacter pylori* infections,) glaucoma, atherosclerosis, etc.

Pharmaceutical & Veterinary Compositions, Dosages, And Administration

[00151] 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 one or more of Formula (I-X) are provided.

[00152] The compounds of one or more of Formula (I-X) and their pharmaceutically acceptable salts, anomers, 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.

[00153] 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 OGT activity, for example, to treat cancer, diabetes, insulin resistance, complications of diabetes, inflammation, or autoimmune disease or any condition described herein or known as useful in the modulation of OGT activity.

[00154] 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 cancer, diabetes, insulin resistance, complications of diabetes, inflammation, automimmune disorders, cancer, infections, (e.g., *Helicobacter pylori* infections,) glaucoma, atherosclerosis, etc.

[00155] It is to be understood that combination of compounds according to the invention, or for use according to the invention, with GT inhibitors is not limited to the examples described herein, but includes combination with any agent useful for the treatment of cancer, diabetes, insulin resistance, complications of diabetes, inflammation, or autoimmune disease. Combination of compounds according to the invention, or for use according to the invention, and other cancer or diabetes therapies 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).

[00156] 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

5 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
10 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.

15 [00157] 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
20 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
25 acetamide, formamide, and benzamide derivatives of amine functional groups in the compounds of the invention and the like.

[00158] A discussion of prodrugs may be found in “Smith and Williams’ Introduction to the Principles of Drug Design,” H.J. Smith, Wright, Second Edition, London (1988); Bundgaard, H., *Design of Prodrugs* (1985), pp. 7-9, 21-24 (Elsevier,
30 Amsterdam); The Practice of Medicinal Chemistry, Camille G. Wermuth et al., Ch 31, (Academic Press, 1996); A Textbook of Drug Design and Development, P. Krosgaard-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.

5 [00159] Suitable prodrug forms of the compounds of the invention include embodiments in which R_2 is $C(O)R$, where R is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl. In these cases the ester groups may be hydrolyzed in vivo (e.g. in bodily fluids), releasing the active compounds in which R is H. Prodrug
embodiments of the invention include compounds of Formula (I-X) where one, two,
10 three or four of R_2 is $C(O)CH_3$. In some embodiments, suitable prodrug forms of the compounds of the invention include 1-phosphate derivatized compounds as described for example in Formula (VI-X).

[00160] Compounds according to the invention, or for use according to the invention, can be provided alone or in combination with other compounds in the
15 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
20 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
25 "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.

[00161] "Pharmaceutically acceptable carrier, diluent or excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent,
30 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.

[00162] 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 I-X 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.

[00163] 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.

[00164] 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, 5 piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine.

[00165] Thus, the term "pharmaceutically acceptable salt" encompasses all acceptable salts including but not limited to acetate, lactobionate, benzenesulfonate, 10 laurate, benzoate, malate, bicarbonate, maleate, bisulfate, mandelate, bitartrate, 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, 15 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.

[00166] Pharmaceutically acceptable salts of the compounds of the present 20 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- 25 glutamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylene-diamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethyl-amine, diethylamine, piperazine, tris(hydroxymethyl)aminomethane, and tetramethylammonium hydroxide.

[00167] Pharmaceutical formulations will typically include one or more carriers acceptable for the mode of administration of the preparation, be it by injection, 30 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.

[00168] 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*⁷⁷. 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.

[00169] 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, pessaries. In some embodiments, compounds or pharmaceutical compositions in accordance with this invention or for use in this invention may be administered by inhalation spray, nasal, vaginal, rectal, sublingual, or topical routes and may be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles appropriate for each route of administration.

[00170] 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 a GT activity, *e.g.*, O-GlcNAcase activity or inhibition of OGT or FUT activity.

[00171] 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 a GT, such as an FUT or OGT, reduction of O-GlcNAc levels, or treatment of 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.

[00172] 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
5 dosages and for periods of time necessary, to achieve the desired prophylactic result, such as inhibition of a GT, such as an FUT or OGT, reduction of O-GlcNAc levels, or prevention of 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
10 therapeutically or prophylactically effective amounts of a compound may be any value from 0.1 nM-0.1M, 0.1 nM-0.05M, 0.05 nM-15µM or 0.01 nM-10µM.

[00173] In alternative embodiments, in the treatment or prevention of conditions which require modulation of a GT activity, such as an FUT or OGT activity, an appropriate dosage level will generally be about 0.01 to 1000 mg per kg
15 subject body weight per day, and can be administered in single or multiple doses. In some embodiments, the dosage level will be about 0.1 to about 250 mg/kg per day. It will be understood that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound used, the metabolic stability and length of action
20 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.

[00174] 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
25 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
30 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.

[00175] 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.

15 [00176] The present invention will be further illustrated in the following examples.

[00177] EXAMPLE I: Materials and Methods

[00178] **Synthesis of (Ac-)5SGlcNAc:** Synthesis of (Ac-)5SGlcNAc was performed as described previously²⁷ with some minor modifications. A full description of the methods used and characterization of the compounds are in Example II.

[00179] **Chemoenzymatic synthesis of UDP-5SGlcNAc:** Over-expression and purification of GNK, AGM, and AGX1 were carried out as described previously³⁴⁻³⁶. The cloning, over-expression and purification of UDP-GlcNAc 4-epimerase was performed according to standard molecular biology procedures. Inorganic pyrophosphatase (PPA), ATP and UTP were purchased from Sigma. GNK, AGM, and AGX1 were buffer exchanged into 50 mM Tris, pH 7.5, 2 mM MgCl₂ using PD10 columns (GE Healthcare), and all substrates were dissolved in the same buffer. GDP-Glc, at a concentration of 68 μM was included as an internal standard to which CE peaks were normalized.

[00180] To synthesize UDP-5SGlcNAc, a one-pot reaction consisting of 2.5 mM ATP, 1 mM UTP, 0.6 mM 5SGlcNAc, 0.4 μ M GNK, 2.9 μ M AGM, 0.3 μ M AGX and 50 mU/mL PPA was incubated for 14 h at 37 °C. The reaction mixture was treated for an additional 4 h at 37 °C with calf alkaline phosphatase (Roche). Small aliquots (~100 μ L) of sample were run by CE (see below for details) to monitor UDP-5SGlcNAc production. Epimerization of UDP-5SGlcNAc was tested by spiking an aliquot with UDP-GlcNAc 4-epimerase and incubating for 14 h at 37 °C.

[00181] **Purification of UDP-5SGlcNAc:** UDP-5SGlcNAc was purified by ion exchange chromatography and HPLC. More specifically, enzymes were removed by passing the mixture through a centrifugal filtration device (10 kDa molecular weight cut-off; Centricon). The filtrate containing the desired product was desalted by passing it over a column packed with a 7 mL bed volume of Dowex AG 1-X4 ion exchange resin (BioRad), previously converted into the formate form and then pre-equilibrated in water. After loading, the column was washed, at a flow rate of ~2 mL/min, with 10 bed volumes of H₂O followed by 10 bed volumes 4 M formic acid, and eluted with 10 bed volumes of 550 mM ammonium formate, pH 4.0³⁷. The fraction containing UDP-5SGlcNAc was concentrated *in vacuo* and further purified by HPLC on a Hewlett Packard series 1100 instrument, equipped with an Eclipse XDB-C18 (5 μ m, 9.4 x 250 mm) column (Agilent Technologies), using ion-paired conditions (adapted from Ref. ³⁸). Compounds were detected by monitoring the UV absorbance at 254 nm and UV-active peaks were assessed for purity by HPLC and CE. Fractions containing the desired product were pooled and lyophilized.

[00182] **Capillary electrophoresis (CE):** CE was performed on a ProteomeLab PA800 (Beckman-Coulter) using fused silica capillaries of 50 μ M internal diameter x 44 cm (to detector). The running buffer was 40 mM Na₂B₄O₇ (Sigma), pH 9.5, containing 1.0% (w/v) polyethylene glycol (MW 20,000; Fluka) and filtered prior to use. The capillary was conditioned by washing with 1 N NaOH (2 min, 20 psi), 18 M Ω H₂O (3 min, 20 psi) and running buffer (5 min, 40 psi). After injecting a short (15 nL) H₂O plug, samples were electrostatically introduced and pre-concentrated at the anode by applying a potential of -0.5 kV for 10 s according to the field-amplified sample injection technique described by Chien and Burgi³⁹. Electrophoresis was carried out at a constant voltage of 30 kV and capillary temperature of 22 °C.

Electropherograms were derived by measuring the absorbance at 254 (+/- 10) nm at a rate of 4 Hz. Peaks were integrated using 32 Karat 5.0 software (Beckman-Coulter) and all peaks were normalized to the GDP-Glc internal standard.

[00183] **OGT transfer:** The ability of OGT (which was over-expressed and purified as described in Ref. ⁴⁰) to transfer UDP-5SGlcNAc was tested using recombinant nup62 as the acceptor. The sub-cloning, protein over-expression and purification of nup62 was performed according to standard molecular biology procedures. Assays contained 30 μ M nup62, 20 μ M UDP-(5S)GlcNAc and 0.4 μ M OGT in 20 mM phosphate, pH 7.4, 150 mM NaCl (phosphate buffered saline; PBS), in a total volume of 100 μ L, and were allowed to proceed for an appropriate time between 10 min and 2 h at 37 °C in order to maintain a constant rate without substrate depletion. Reactions were quenched upon addition of an equal volume of ethanol, frozen at -20 °C for 1 h to precipitate proteins and centrifuged at 13,000 rpm for 20 min. 5 μ M GDP-Glc, an internal standard, was added prior to freezing. The supernatant was removed and lyophilized. Nucleotides and nucleotide sugars were extracted as described below for the cell lysates, resuspended in 200 μ L H₂O, and run by CE. Production of UDP was monitored at 254 nm; the concentration was determined from a standard curve of UDP standards, which were prepared in triplicate using the same procedure as the reactions, at a concentration of between 1 and 20 μ M. Controls in the absence of nup62 were subtracted to account for OGT-catalyzed hydrolysis of the UDP-sugars.

[00184] **OGT inhibition:** The ability of UDP-5SGlcNAc to inhibit OGT activity was assessed using radiolabelled [³H]GlcNAc-UDP (American Radiolabel) as the donor and recombinant nup62 as the acceptor. Assays contained 18 μ M of nup62, 1 μ M of UDP-GlcNAc (constant specific activity of 0.5 Ci/mmol of [³H]-UDP-GlcNAc), 100 nM OGT, and various concentrations (0-25 μ M) of UDP-5SGlcNAc in PBS. The $V_{\max}$ for OGT was determined using high (30, 40 and 50 μ M) concentrations of UDP-GlcNAc (containing 0.033, 0.025, 0.02 Ci/mmol specific activity, respectively). Reactions were incubated at 37 °C for 1 h (over which time OGT has been shown to retain constant activity). The assay mixture was applied to a 1.5 x 3 cm piece of nitrocellulose membrane (Bio-Rad) and allowed to air dry. Membranes were rinsed with five washes (total 100 mL) of PBS and air dried. The

membranes were loaded into scintillation vials, and 4 mL of scintillation fluid (Amersham) was added. The tritium levels were quantified by liquid scintillation counter (BECKMAN LS6000). All reactions were performed in triplicate. The inhibitor concentration was plotted against the inverse of the rate, and the $-K_i$ value was taken where the line of best fit intersected $1/V_{\max}$.

[00185] **Cell culture:** Cells were grown in an incubator at 37 °C and 5% CO₂ atmosphere. Media and serum were purchased from Invitrogen. COS-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal bovine serum (FBS), and were typically 80-100% confluent prior to experimentation. HepG2, SK-N-SH, and EMEG32^{-/-} cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal bovine serum (FBS), CHO cells in DMEM/F-12 with 5% FBS and PC12 cells in DMEM with 5% FBS and 5% horse serum. Prior to experimentation, PC12 cells were plated at a density of $3-4 \times 10^5$ cells/10 cm plate, which had been pre-treated with 5 $\mu\text{g}/\text{cm}^2$ rat tail collagen (BD Biosciences). Cells were differentiated in DMEM containing 1% horse serum, in the presence of 2.5S mouse nerve growth factor (Chemicon) at a final concentration of 25 ng mL⁻¹, for 4-5 days prior to treatment. Other cell lines were 80-100% confluent prior to treatment. Following treatment with (Ac-)5SGlcNAc, using various conditions, cells were washed gently with PBS, lysed in SDS loading buffer and boiled for 10 minutes. Mouse hybridoma cells (obtained from the Developmental Studies Hybridoma Bank; antibody E7 for β -tubulin) were cultured in RPMI40 with 10% FBS. Prior to treatment with Ac-5SGlcNAc, hybridoma cells were cultured for 2 days in RPMI40 with 5% FBS, and during treatment in RPMI40 with no serum. Cells were collected, centrifuged (800 x g, 5 min) and the supernatant removed. Cells were washed with PBS, centrifuged, the pellet resuspended in SDS loading buffer and boiled. The supernatant after centrifugation, which contained the IgG antibody produced by the cells, was used for immunoprecipitation (see below for details).

[00186] **Western/lectin blots:** Samples were run on 10% SDS polyacrylamide gels and transferred onto nitrocellulose membranes (Biorad). Membranes were blocked for 1 h in PBS containing 0.1% Tween-20 (PBS-T) containing 1% (unless otherwise stated) bovine serum albumin (BSA), and probed with the appropriate primary antibody or lectin in 1% BSA in PBS-T overnight at 4 °C. Blots were washed

for 1 hr with PBS-T, and blocked for a further 30 min with 1% BSA in PBS-T at room temperature. They were then probed with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody/probe in 1% BSA in PBS-T at room temperature for 1 h, followed by washing in PBS-T for 1 h. Blots were developed with
 5 SuperSignal West Pico Chemiluminescent Substrate (Pierce) and exposed to CL-XPosure film (Pierce). Densitometry was performed using ImageQuant 5.2 (Molecular Dynamics), and fits to the data were made using GRAFIT⁴¹.

[00187] ***Antibodies/lectins:*** O-GlcNAc levels were assessed primarily using CTD110.6 (Covance), but RL2 (Abcam) and HGAC85 (Abcam) were also tested.

10 Actin was probed using JLA20 (Developmental Studies Hybridoma Bank). OGA levels were assessed using a polyclonal anti-OGA antibody which was a kind gift from Dr. Gerald Hart and OGT levels using H-300 (Santa Cruz Biotechnology). nup62 was probed using mAb 414 (Covance). Secondary antibodies employed (HRP-conjugated goat anti-mouse IgM, goat anti-mouse IgG, goat anti-chicken IgY and goat
 15 anti-rabbit IgG) were obtained from Santa Cruz Biotechnology. Biotinylated-ConA, PHA-L, SNA and MAA were purchased from EY Laboratories, and biotinylated-GNA from Vector Laboratories. HRP-conjugated streptavidin (Pierce) was used as a probe for the biotinylated lectins.

[00188] ***Blocking antibody interactions:*** Recombinantly modified nup62 was
 20 run on a gel and transferred onto nitrocellulose membrane. CTD110.6 at the appropriate dilution was incubated with 0-3 mM Me-GlcNAc or Me-5SGlcNAc for 1 h prior to applying to the membrane overnight at 4 °C. The standard procedure for western blots was subsequently followed.

[00189] ***OGA kinetics:*** Synthesis and characterization of *para*-methoxyphenyl-
 25 5SGlcNAc is described in Example II. Kinetics were performed at 37 °C in PBS, pH 7.4 in a total volume of 150 µL. Substrate concentrations of 25 µM to 1.5 mM were used for *p*MP-GlcNAc and 25 to 800 µM for *p*MP-5SGlcNAc. OGA (which was over-expressed and purified as described in Ref. ⁴²) was used at a concentration of 1 µM for *p*MP-GlcNAc and 3.6 µM for *p*MP-5SGlcNAc. Rates were monitored at a
 30 wavelength of 296 nm over the course of 10-20 min. Data were fit to the Michaelis-Menten equation in GRAFIT⁴¹ and the k_{cat} and K_M values determined.

[00190] **Immunoprecipitation of nup62:** nup62 was immunoprecipitated from COS-7 cell lysates using the procedure described in Ref.⁹. Following washing of the beads, and prior to elution, nup62 was incubated in the absence or presence of *BrGH84* (overexpressed and purified as described previously⁴³) shaking for 2 h at room temperature, which would allow *O*-GlcNAc levels on nup62 to be assessed. nup62 was subsequently eluted from the beads upon addition of SDS loading buffer and boiled for 10 min.

[00191] **Click-iT® kit for labelling *O*-GlcNAc residues:** The 'Click-iT® *O*-GlcNAc Enzymatic Labeling System' was purchased from Invitrogen, and essentially the manufacturer's protocol was followed with some alterations. nup62 was immunoprecipitated and eluted from the beads in 1% SDS in 20 mM Hepes, pH 7.9, and boiled for 10 min; this was used as the substrate for the chemoenzymatic labeling using UDP-GalNAz and GalT1. Following the overnight reaction, biotinylated phosphine (in 20% DMF) was added at a final concentration of 50 µM and incubated at 37 °C for 90 min. The reaction was stopped by the addition of SDS-PAGE loading buffer, and the samples were boiled for 10 min. Samples were run on a gel and transferred. The same procedure as described for the western blots was used, starting from the second blocking step. Blots were then probed with HRP-conjugated streptavidin (Pierce).

[00192] **Extraction of nucleotide sugars from cell lysates:** COS-7 cells, on 10 cm plates, were treated with 0, 50, 250 or 1000 µM Ac-5SGlcNAc for 24 h; each condition was performed in triplicate. Cells were washed gently with PBS prior to the addition of 1 mL PBS containing 10 mM EDTA. Cells were incubated at 37 °C for 4 min, the cells scraped from the plate, and pelleted by centrifugation (1000 rpm, 10 min, 4 °C). Cell pellets were immediately lysed by the addition of 750 µL 75% (v/v) ethanol (-20 °C) and sonicated using a W-375 ultrasonic processor (Heat Systems Ultrasonics, Inc.). Insoluble material was removed by centrifugation (14,000 rpm, 15 min, 4 °C) and the supernatant was snap frozen and lyophilized^{44,45}. The crude extract was spiked with GDP-Glc (200 pmol) as an internal standard, dissolved in 0.5 mL 18 MΩ H₂O and nucleotide sugars extracted using Envi-Carb graphite solid-phase extraction cartridges (200 mg) (Supelco) as described by Răbină *et al*³⁸. Briefly, Envi-Carb cartridges were conditioned with 80% (v/v) CH₃CN and 20% 0.1% (v/v) TFA (3

mL), followed by 18 MΩ H₂O (6 mL), before samples were applied. After passing the samples through the SPE cartridge, the cartridge was sequentially washed with 2 mL of each of 18 MΩ H₂O, 25% (v/v) CH₃CN, and 50 mM triethylammonium acetate (TEAA), pH 7.0. Sugar nucleotides were eluted with 3 x 1 mL 25% CH₃CN in 50 mM TEAA, pH 7.0, passed through a 0.22 μm filter (Millipore), lyophilized, and stored at -20 °C until analysis. Extracted sugar nucleotides were dissolved in 200 μL 18 MΩ H₂O and an aliquot was diluted 1:4 prior to characterization by CE using a method adapted from Feng *et al*⁴⁶.

[00193] **Precipitation of antibody:** Following collection of mouse hybridoma cells, cells were centrifuged and the supernatant (i.e. cell media containing the IgG antibody produced by the cells) was removed. 1 mL supernatant was incubated with 100 μL Protein G/Protein-A agarose beads (Calbiochem) shaking overnight at 4 °C. Beads were washed three times in PBS containing 0.1% NP-40. Antibody was eluted by the addition of SDS-PAGE loading buffer and boiling for 10 minutes. Assessment of total antibody precipitated was made by probing blots with secondary goat anti-mouse IgG antibody (using the western blot procedure starting from the second blocking step).

[00194] **PNGase F cleavage:** PNGase F was purchased from New England Biolabs and used as described in the manufacturer's protocol. Immunoprecipitated antibody (from the mouse hybridoma cells) was eluted from the beads in '10x denaturation buffer' prior to PNGase F treatment.

[00195] EXAMPLE II: Synthesis and characterization of Ac-5SGlcNAc, 5SGlcNAc, pMP-5SGlcNAc, Me-5SGlcNAc, and Ac-5SGlcNAz

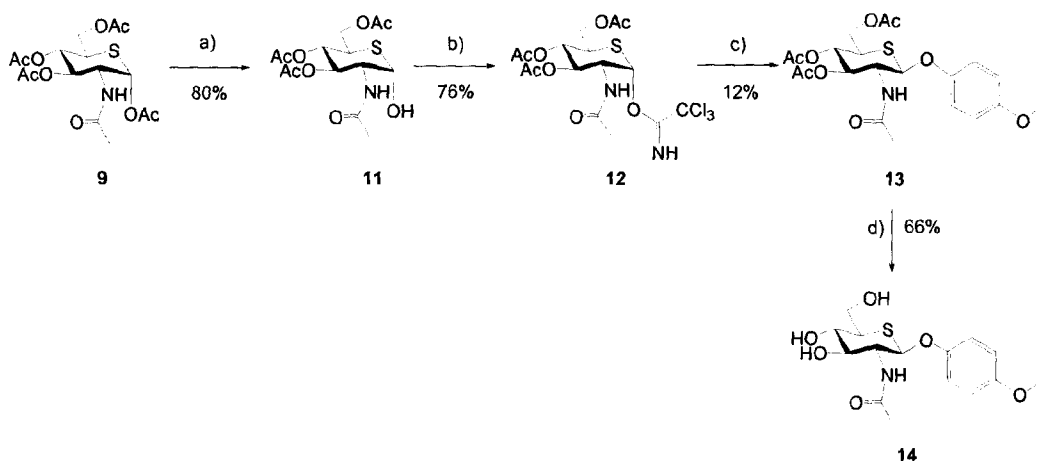
[00196] Synthesis of Ac-5SGlcNAc (9) and 5SGlcNAc (10) was carried out following literature procedures with some modifications^{37,38}.

[00197] *2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-5-thio-α-D-glucopyranose* (9)²⁷ –¹H-NMR (500 MHz, CDCl₃) δ (ppm) 5.92 (d, *J* = 3.04 Hz, 1H), 5.70 (d, *J* = 8.86 Hz, 1H), 5.37 (dd, *J* = 10.74, 9.64 Hz, 1H), 5.16 (dd, *J* = 10.89, 9.70 Hz, 1H), 4.63 (m, 1H), 4.34 (dd, *J* = 12.11, 4.95 Hz, 1H), 4.03 (dd, *J* = 12.11, 3.15 Hz, 1H), 3.50 (ddd, *J* = 10.8, 4.80, 3.20, 1H), 2.17 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s,

3H), 1.90 (s, 3H); ^{13}C -NMR (125 MHz, CDCl_3) δ (ppm) 171.73, 170.60, 169.52, 169.16, 168.77, 72.79, 71.78, 71.47, 61.10, 55.20, 39.75, 23.08, 21.14, 20.65, 20.53.

[00198] 2-Acetamido-2-deoxy-5-thio- α -D-glucopyranose (**10**)²⁷ ^1H -NMR (600 MHz, MeOH-d_4) δ (ppm) 4.89 (d, $J = 3.17$ Hz, 1H), 4.07 (dd, $J = 10.50, 2.82$ Hz, 1H), 3.87-3.79 (m, 2H), 3.65 (dd, $J = 10.46, 8.82$ Hz, 1H), 3.55 (dd, $J = 10.37, 8.93$ Hz, 1H), 3.25-3.22 (m, 1H), 1.96 (s, 3H); ^{13}C -NMR (150 MHz, MeOH-d_4) δ (ppm) 173.35, 76.88, 73.52, 73.50, 62.65, 60.01, 44.89, 22.72.

[00199] **Synthesis of pMP-5SGlcNAc (**14**)**



Scheme 1. Synthesis of pMP-5SGlcNAc (**14**). a) Hydrazine acetate, DMF, room temperature, 6 h. b) Trichloroacetoneitrile, DBU (cat.), DCM, $-20\text{ }^\circ\text{C} \rightarrow$ room temperature, 1h. c) Paramethoxyphenol, boron trifluoroetherate (cat.), DCM, $-20\text{ }^\circ\text{C} \rightarrow$ room temperature, 1h. d) Sodium methoxide (cat.) methanol, room temperature, 0.5 h.

[00200] 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-5-thio- α -D-glucopyranose (**11**) – Anomeric deacetylation was effected in a manner similar to that reported previously for different saccharides⁴⁸. To a solution of **9** (0.200 g, 0.494 mmol) in dimethylformamide (1 mL), stirring at room temperature, hydrazine acetate (0.050 g, 0.543 mmol) was added. The reaction was left to stir until all starting material was consumed (6 hours). The reaction was concentrated on the high vacuum rotary evaporator, and co-evaporated twice with toluene, without heating. The resulting residue was taken up in ethyl acetate and washed two times with saturated sodium bicarbonate, water (until a neutral pH was obtained) and brine. Each aqueous layer was back washed with ethyl acetate. The pooled organic layers were dried using magnesium sulphate, filtered and concentrated. The reaction was purified *via* flash

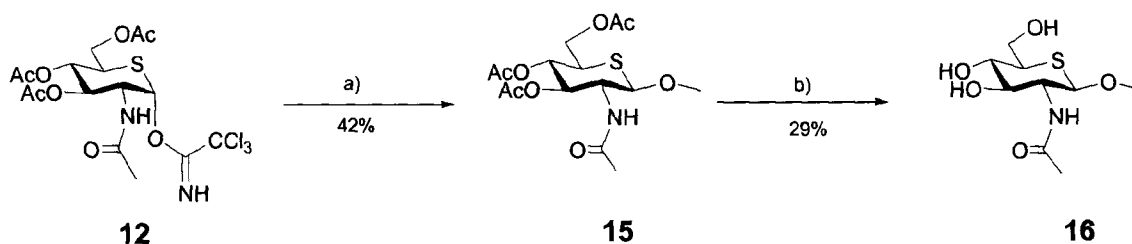
column chromatography with a solvent system of 70% ethyl acetate, 30% hexanes, and subsequently concentrated on the high vacuum rotary evaporator to reveal a white solid. This was recrystallized in ethyl acetate and hexanes (0.144 g, 80 %). ¹H-NMR (600 MHz, CDCl₃) δ (ppm) 4.89 (dd, *J* = 10.50, 2.82 Hz, 1H), 3.88 (m, 2H), 3.70 (dd, *J* = 10.46, 8.82 Hz, 1H), 3.60 (dd, *J* = 10.37, 8.93 Hz, 1H), 3.28 (m, 1H), 2.00 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ (ppm) 171.91, 75.43, 72.07, 72.05, 61.20, 58.56, 43.44, 21.27. HRMS (*m/z*): [M+H]⁺ calcd for C₁₄H₂₁NO₈S: 364.1066; found 364.1070

[00201] *2-Acetamido-3,4,6-tri-O-acetyl-1-trichloroacetimidate-2-deoxy-5-thio-α-D-glucopyranose (12)* - Formation of the trichloroacetimidate donor was carried out by modification of a previously described procedure used for different saccharides^{24,49-51}. **11** was dissolved in dichloromethane, and to this trichloroacetonitrile (0.181 g, 1.27 mmol) was added with a catalytic amount of DBU (~1 drop) at 0 °C. The reaction was allowed to stir at 0 °C until the reaction completion (0.5 hours). The reaction was diluted with benzene and concentrated on the high vacuum rotary evaporator to dryness. The crude mixture was purified using flash column chromatography using a solvent system of 60% ethyl acetate in hexanes. The pure product was isolated as a clear oil, which solidified to give a white solid (0.086 g, 76 %). ¹H-NMR (600 MHz, CDCl₃) δ (ppm) 8.77 (s, 1H), 6.08 (d, *J* = 3.00 Hz, 1H), 5.79 (d, *J* = 8.80 Hz, 1H), 5.37 (dd, *J* = 10.68, 9.79 Hz, 1H), 5.20 (dd, *J* = 10.68, 9.78 Hz, 1H), 4.65 (m, 1H), 4.29 (dd, *J* = 12.14, 4.98 Hz, 1H), 4.00 (dd, *J* = 12.11, 3.13 Hz, 1H), 3.49 (m, 1H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ (ppm) 171.51, 170.49, 169.59, 169.21, 159.20, 90.79, 77.82, 71.69, 71.31, 60.99, 55.80, 40.14, 23.06, 20.63, 20.60, 20.52. HRMS (*m/z*): [M+H]⁺ calcd for C₁₆H₁₂N₂O₈SCl₃: 507.0162; found 507.0173.

[00202] *para-Methoxyphenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-5-thio-α-D-glucopyranoside (13)* – Glycosylation was effected by adaptation of a reported method used for glycosylation of trichloroacetimidate donors of other saccharides^{24,51}. A solution of **12** (0.050 g, 0.100 mmol) in dichloromethane (0.77mL) and paramethoxyphenol (0.025 g, 0.20 mmol) was stirred at -20 °C. To this solution a catalytic amount of boron trifluoroetherate (~ 1 drop) was added, and the reaction was immediately allowed to warm to room temperature. The reaction was stirred for 1 hour, at which time the reaction was complete. To quench the borontrifluoroetherate,

2 equivalents of triethylamine (0.026 mL) was added to the reaction mixture. The reaction was concentrated on the high vacuum rotary evaporator without heating. Flash column chromatography was performed in order to isolate the spots observed by TLC that were presumed to be the alpha and beta anomers. Upon obtaining ¹H NMR, it was determined that the two products isolated were the alpha and beta anomers, in a ratio of 70% alpha and 30% beta. The two anomers were separated *via* flash column chromatography, using a solvent system of 60% ethyl acetate in hexanes to give rise to pure beta-glycosylated product (0.056 g, 12 %), and alpha-glycosylated product (0.096 g, 21 %), giving an overall yield of 34%. ¹H-NMR (600 MHz, CDCl₃) δ (ppm) 6.89 (d, *J* = 9.07 Hz, 2H), 6.77 (d, *J* = 9.09 Hz, 2H), 5.88 (d, *J* = 8.82 Hz, 1H), 5.31 (m, 1H), 5.13 (d, *J* = 6.33 Hz, 1H), 5.04 (m, 1H), 4.52 (m, 1H), 4.28 (m, 2H), 3.70 (s, 3H), 3.14 (m, 1H), 2.02 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.92 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ (ppm) 170.55, 170.15, 169.70, 168.95, 155.44, 150.46, 118.20, 114.71, 80.57, 71.04, 69.16, 63.44, 55.67, 40.60, 29.72, 23.41, 20.81, 20.72, 20.70. HRMS (*m/z*): [M+H]⁺ calcd for C₂₁H₂₇N₂O₉S: 470.1485; found 470.1478.

[00203] *para*-Methoxyphenyl 2-acetamido-2-deoxy-5-thio- α -D-glucopyranoside (**14**) – In a solution of **13** (0.0062 g, 0.013 mmol) and methanol (0.25 mL), a catalytic amount of sodium methoxide was added (enough to bring the reaction solution to pH 10). The reaction was allowed to stir at room temperature. After twenty minutes of reaction, a white solid began to precipitate out of solution. The reaction had reached completion after 30 minutes. The reaction was first diluted with additional methanol, and subsequently brought to a neutral pH by the drop-wise addition of a dilute mixture of acetic acid in methanol (pH 4). The reaction was concentrated on a high vacuum rotary evaporator without heating. **14** was purified by recrystallization with ethanol and ether, resulting in a white crystalline product (0.0033 g, 66 %). ¹H-NMR (600 MHz, MeOH, D₄) δ (ppm) 6.99 (d, *J* = 9.16 Hz, 2H), 6.83 (d, *J* = 9.16 Hz, 2H), 5.10 (d, *J* = 9.29 Hz, 1H), 4.19 (dd, *J* = 9.60 Hz, 1H), 3.96 (dd, *J* = 11.44, 3.73 Hz, 1H), 3.79 (dd, *J* = 11.44, 6.47 Hz, 1H), 3.74 (s, 3H), 3.60 (dd, *J* = 10.07 Hz, 1H), 3.38 (dd, *J* = 9.89 Hz, 1H), 2.89 (m, 1H), 1.96 (s, 3H); ¹³C-NMR (150 MHz, D₂O) δ (ppm) 172.44, 155.27, 151.78, 117.50, 114.14, 80.79, 75.08, 74.32, 61.23, 59.86, 54.64, 46.08, 21.58. HRMS (*m/z*): [M+H]⁺ calcd for C₁₅H₂₁NO₆S: 343.1168; found 344.1157.

[00204] **Synthesis of Me-5SGlcNAc (16).**

Scheme 2. Synthesis of Me-5SGlcNAc (**16**). a) Methanol (2 eq), boron trifluoroethyletherate (cat.), DCM, -20 °C→room temperature. b) NaOMe (cat.), methanol, room temperature.

5

[00205] *Methyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-5-thio- α -D-*

glucopyranoside – (**15**). **12** (0.050 g, 0.11 mmol) was dissolved in dry dichloromethane (0.762 mL). Two equivalents of dry methanol (0.009 mL, 0.211 mmol) was added to the solution at -20°C. A catalytic amount of boron

10 trifluoroethyletherate was added to the reaction mixture and allowed to warm to room temperature. Once the reaction was complete, two equivalents of triethylamine (0.03 mL) was added to the solution to quench the trifluoroethyletherate. The reaction was concentrated directly on a high vacuum rotary evaporator and run immediately through a silica gel column in a solvent system of ethyl acetate. The ratio of α to β

15 anomers was found to be 1:1 by ^1H NMR, and the β anomer was slightly more polar than the α anomer by TLC. The final β -product was isolated through a second purification using flash column chromatography in ethyl acetate in order to separate the two anomers. **15** was isolated (0.0154 g, 42%) with an overall yield of α and β anomers of 0.0354 g, 96%. ^1H -NMR (600 MHz, MeOH, D_4) δ (ppm) 5.22 (dd, J = 9.74 Hz, 1H), 5.06 (dd, J = 9.53 Hz, 1H), 4.64 (d, J = 8.76 Hz, 1H), 4.40 (dd, J = 11.86, 5.23 Hz, 1H), 4.26 (dd, J = 9.21 Hz, 1H), 4.14 (dd, J = 11.82, 3.87 Hz, 1H), 3.49 (s, 3H), 3.31 (m, 1H), 2.06 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.93 (s, 3H); ^{13}C -NMR (150 MHz, MeOH, D_4) δ (ppm) 171.93, 170.81, 170.12, 169.87, 82.96, 73.38, 71.96, 62.19, 57.76, 57.20, 40.46, 21.27, 19.19, 19.16, 19.09. HRMS

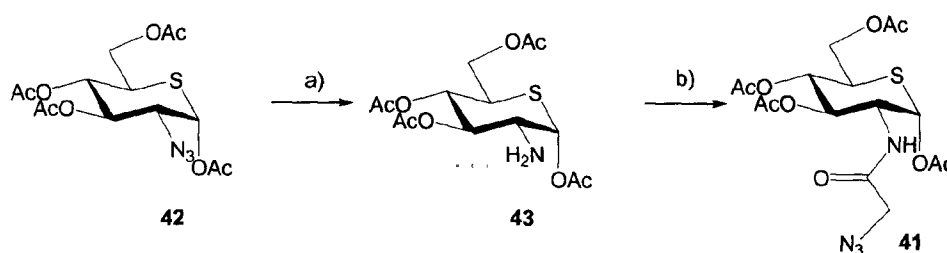
25 (m/z): [$\text{M}+\text{H}$] $^+$ calcd for $\text{C}_{15}\text{H}_{23}\text{NO}_8\text{S}$: 378.1223; found 378.1214.

[00206] *Methyl 2-acetamido-2-deoxy-5-thio- α -D-glucopyranoside* – (**16**). **15**

(0.0088 g, 0.023 mmol) was dissolved in methanol (0.35 mL) at room temperature. To this solution a catalytic amount of sodium methoxide was added (the pH of the reaction solution was 8-9). The reaction was followed closely by TLC and upon

completion was neutralized (to pH 7) with a dilute solution of acetic acid in methanol (pH 4). The neutralized mixture was concentrated on the high vacuum rotary evaporator without heating. The reaction was purified using column chromatography in a solvent system of 15% methanol in chloroform. The reaction was crystallized in ethanol with ether. The final material was isolated as a white residue, which appeared solid (1.68 mg, 29%). $^1\text{H-NMR}$ (600 MHz, MeOH, D_4) δ (ppm) 4.31 (d, $J = 8.89$ Hz, 1H), 3.90 (dd, $J = 9.26$ Hz, 1H), 3.86 (dd, $J = 11.35, 3.93$ Hz, 1H), 3.66 (dd, $J = 11.35, 6.59$ Hz, 1H), 3.45 (dd, $J = 8.95$ Hz, 1H), 3.34 (s, 3H), 2.68 (m, 1H), 1.88 (s, 3H). $^{13}\text{C-NMR}$ (150 MHz, MeOH, D_4) δ (ppm) 172.32, 83.30, 75.44, 74.17, 61.68, 59.50, 56.90, 46.03, 21.51. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_9\text{H}_{17}\text{NO}_5\text{S}$: 252.0906; found 252.0913.

[00207] Synthesis of Ac-5SGlcNAz (**41**).



Scheme 3. Synthesis of Ac-5SGlcNAz (**41**). a) H_2 , PtO_2 , MeOH/HCl/ H_2O , r.t., 2 h; b) Azidoacetic acid (3.0 eq.), HBTU (3.8 eq.), DIPEA (5.9 eq), DCM, $0^\circ\text{C} \rightarrow \text{r.t.}$, overnight, 30% over 2 steps.

[00208] *1,3,4,6-Tetra-O-acetyl-2-azido-2-deoxy-5-thio- α -D-glucopyranoside* (**42**) – Synthesized from D-glucofurano-3,6-lactone using literature procedures with minor modifications in 13 steps⁵⁸⁻⁶⁰. Careful flash silica gel column chromatography using a gradient of hexanes:ethyl acetate in a ratio of 5:1 to 4:1 to 3:1, followed by preparative thin layer chromatography using a solvent system of hexanes:ethyl acetate in a ratio of 4:1 yielded pure α -anomer as a colourless syrup (for which the characterisation is described) and impure β -anomer which was not used further. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ (ppm) 6.10 (d, $J = 3.0$ Hz, 1H), 5.40 (t, $J = 10.0$ Hz, 1H), 5.32 (t, $J = 10.0$ Hz, 1H), 4.39 (dd, $J = 12.0, 5.0$ Hz, 1H), 4.03 (dd, $J = 12.0, 3.0$ Hz, 1H), 3.88 (dd, $J = 10.0, 3.0$ Hz, 1H), 3.56 (ddd, $J = 11.0, 5.0, 3.0$ Hz, 1H), 2.19 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ (ppm)

170.45, 169.71, 169.51, 168.67, 71.74, 71.70, 64.88, 60.88, 39.80, 20.98, 20.60, 20.59, 20.50. HRMS (m/z): $[M+NH_4]^+$ calcd for $C_{16}H_{26}NO_{10}S$: 424.1272; found 424.1267. $[M+Na]^+$ calcd for $C_{16}H_{22}NaO_{10}S$: 429.0286; found 429.0280.

[00209] *1,3,4,6-Tetra-O-acetyl-2-azidoacetamido-2-deoxy-5-thio- α -D-*

- 5 *glucopyranose (41)* – 2-Azido sugar **42** (42 mg, 0.11 mmol) and PtO_2 (22 mg, 0.097 mmol) were suspended in MeOH (5.0 mL) and 1 N HCl (aq., 1.0 mL) was added. The resulting reaction mixture was stirred under an atmosphere of H_2 (1 atm) at r.t. for 2 hours and then filtered through Celite. The filtrate was concentrated under reduced vacuum, the residue was co-evaporated with toluene (2 x 5.0 mL), and was then dried
- 10 under high vacuum for 2 hours to afford crude intermediate 2-amino-1,3,4,6-tetra-*O*-acetyl-5-thio- α -D-glucopyranose hydrochloride (**43**) as a brown syrup (39 mg). This material was used in the next step directly without purification. To a solution of **43** (39 mg, 0.11 mmol) in dry CH_2Cl_2 (4.0 mL), was added HBTU (140 mg, 0.37 mmol), azidoacetic acid⁶¹ (30 mg, 0.30 mmol), and then DIPEA (0.10 mL, 0.58
- 15 mmol) was added drop-wise at 0 °C. The resultant reaction mixture was stirred overnight before being quenched by addition of sat. $NaHCO_3$ (aq.) (10 mL), and the reaction mixture was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extracts were washed with brine (1 x 10 mL) and dried ($MgSO_4$). After filtration and concentration under reduced pressure, the residue was purified by flash column silica
- 20 chromatography (hexanes:ethyl acetate, 3:1 to 2:1 then 1:1), followed by preparative TLC (CH_2Cl_2 :MeOH, 20:1) to afford a pale yellow syrup (13 mg, 30 %). ^1H -NMR (500 MHz, $CDCl_3$) δ (ppm) 6.57 (brd, $J = 8.5$ Hz, 1H, NH), 6.00 (d, $J = 2.5$ Hz, 1H, H-1), 5.40 (t, $J = 10.0$ Hz, 1H, H-4), 5.24 (t, $J = 10.0$ Hz, 1H, H-3), 4.61 (m, 1H, H-2), 4.37 (dd, $J = 12.0, 5.0$ Hz, 1H, H-6), 4.05 (dd, $J = 12.0, 3.0$ Hz, 1H, H-6'), 3.92 (s,
- 25 2H, N_3CH_2), 3.51 (ddd, $J = 10.5, 5.0, 3.0$ Hz, 1H, H-5), 2.21 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H); ^{13}C -NMR (125 MHz, $CDCl_3$) δ (ppm) 171.43, 170.53, 169.17, 168.83, 168.51, 72.20 (C-1), 71.34 (C-4), 71.30 (C-3), 61.02 (C-6), 55.42 (C-2), 52.47 (N_3CH_2), 39.82 (C-5), 21.04, 20.63, 20.59, 20.52. HRMS (m/z): $[M+H]^+$ calcd for $C_{16}H_{23}N_4O_9S$: 447.1180; found 447.1180. $[M+NH_4]^+$ calcd for
- 30 $C_{16}H_{26}N_5O_9S$: 464.1446; found 464.1440. $[M+Na]^+$ calcd for $C_{16}H_{22}N_4NaO_9S$: 469.1000; found 469.0999.

[00210] **Characterization of UDP-5SGlcNAc: 5'-(2-acetamido-2-deoxy-5-thio- α -D-glucopyranosyl)diphosphate triethylammonium salt** - The enzymatic synthesis was carried out as described in the methods section. Uridine ^1H NMR (600 MHz, D_2O): δ (ppm) 2.08 (s, 3H, $\text{NH}(\text{CO})\text{CH}_3$), 3.38 (ddd, 1H, $J_{5^*,6a^*} = 5.4$ Hz, $J_{5^*,6b^*} = 3.0$ Hz, H-5"), 3.71 (dd, 1H, $J_{4^*,5^*} = 10.2$ Hz, H-4"), 3.75 (dd, 1H, $J_{3^*,2^*} = 9.6$ Hz, $J_{3^*,4^*} = 9.0$ Hz, H-3"), 3.89 (dd, 1H, $J_{6b^*,6a^*} = 12.0$ Hz, H-6G"), 3.97 (dd, 1H, H-6a"), 4.91 (m, 1H, H-2"), 4.20 (m, 1H, H-5b"), 4.25 (m, 1H, $J_{5a^*,5b^*} = 11.4$ Hz, H-5a'), 4.29 (m, 1H, H-4'), ^{13}C NMR (150 MHz, D_2O): δ (ppm) 22.06 ($\text{NH}(\text{CO})\text{CH}_3$), 43.59 (C-5"), 57.74 (C-2"), 59.91 (C-6"), 64.89 (C-5'), 69.61 (C-3"), 72.03 (C-3"), 73.62 (C-4"), 73.75 (C-2'), 76.62 (C-1"), 83.17 (C-4'), 88.39 (C-1'), 102.63 (C-5), 141.63 (C-6), 151.78 (C-2), 166.20 (C-4), 169.13 ($\text{NH}(\text{CO})\text{CH}_3$). ^{31}P NMR (242 MHz, D_2O): δ (ppm) -10.66 (d, $J_{\text{p,p}} = 21.8$ Hz), -12.09 (d). HRMS (m/z): $[\text{M-H}]^-$ calcd for $\text{C}_{17}\text{H}_{26}\text{N}_3\text{O}_{16}\text{P}_2\text{S}$: 622.0519, found 622.0514. (**Figures 1F-K**).

[00211] **EXAMPLE III: Biosynthesis and Characterization of UDP-5SGlcNAc**

[00212] The end product of the hexosamine biosynthetic pathway (HBP) is UDP-GlcNAc, the donor substrate used by OGT, as depicted in Figure 1B. The first and rate-limiting step in the *de novo* pathway, where fructose-6-phosphate (diverted from glycolysis) is converted to glutamine-6-phosphate, is catalyzed by glutamine: fructose-6-phosphate amidotransferase (GFAT). Glutamine-6-phosphate is transformed into GlcNAc-6-phosphate by acetyl-CoA:D-glucosamine-6-phosphate *N*-acetyltransferase (GAT). The salvage pathway recycles cellular GlcNAc, which is converted into GlcNAc-6-phosphate by GlcNAc kinase (GNK). GlcNAc-6-phosphate is converted into GlcNAc-1-phosphate by GlcNAc mutase (AGM), and then to the end product, UDP-GlcNAc, by UDP-GlcNAc pyrophosphorylase (AGX1). The 5-thio analogue is converted *via* the salvage pathway to generate intracellular UDP-5SGlcNAc. Ac-5SGlcNAc is deacetylated by cell esterases.

[00213] We evaluated whether 5SGlcNAc (**Figure 1C**) could be transformed into UDP-5SGlcNAc by human HBP enzymes. 5SGlcNAc was synthesized as described herein. Using this material, UDP-5SGlcNAc (**Figure 1C**) was prepared in a reaction containing 5SGlcNAc, ATP, UTP, and recombinant human GlcNAc kinase (GNK), GlcNAc mutase (AGM) and UDP-GlcNAc pyrophosphorylase (AGX1). The

reaction, monitored by capillary electrophoresis (CE), showed the production of a UDP-sugar (**Figure 1D**), which was purified and shown to be UDP-5SGlcNAc. Therefore, UDP-5SGlcNAc can be biosynthesized from 5SGlcNAc by the mammalian enzymes of the HBP.

5 [00214] EXAMPLE IV: Inhibition of OGT by UDP-5SGlcNAc

[00215] We then assayed the ability of OGT to use UDP-5SGlcNAc as a donor using nup62 as an acceptor^{52,53} and found it was an approximately 14-fold worse substrate as compared to UDP-GlcNAc. Since UDP-5SGlcNAc was found to be a relative poor substrate in this assay, we assessed its inhibitory activity toward OGT.

10 Using radiolabelled UDP-GlcNAc as the donor, and nup62 as the acceptor, the amount of *O*-GlcNAc transferred was evaluated in the presence of increasing concentrations of UDP-5SGlcNAc. UDP-5SGlcNAc is an effective inhibitor of OGT with a K_i value of 8 μ M (**Figure 1E**), indicating that it binds as well as the natural substrate UDP-GlcNAc ($K_m = 2-7 \mu$ M)^{40,53}. Therefore, UDP-5SGlcNAc is a poor

15 substrate for OGT, and is a good inhibitor of OGT *in vitro*.

[00216] EXAMPLE V: Treatment of Cells with 5SGlcNAc

[00217] With these results in hand we evaluated the effect of treating cells with 5SGlcNAc. We treated cultured COS-7 cells for 24 hours with 5SGlcNAc as well as its peracetylated analogue (Ac-5SGlcNAc). Both compounds decreased *O*-GlcNAc

20 levels in a dose-dependent fashion as evaluated by western blots using an *O*-GlcNAc-directed antibody (CTD110.6) (**Figures 2A and 2G**). Other commercial *O*-GlcNAc antibodies (RL2 and HGAC85) reveal similar decreases in *O*-GlcNAc levels (**Figure 2H**). The western blots reveal the EC_{50} values for Ac-5SGlcNAc and 5SGlcNAc to be 5 μ M and 700 μ M respectively, which is consistent with acetylation facilitating entry

25 of 5SGlcNAc into cells. Hence in subsequent experiments we used Ac-5SGlcNAc.

[00218] We also observed a time-dependent decrease in *O*-GlcNAc levels, with almost no *O*-GlcNAc being detectable after 24 hours (**Figure 2B**). Dosing for 5 days showed *O*-GlcNAc levels dropped in the first day and remained low (**Figure 2C**). In these studies there were no apparent changes in cell morphology or rate of

30 proliferation at the doses assayed. We observed Ac-5SGlcNAc administration induced a compensatory decrease in OGA levels and an increase in OGT levels (**Figure 2D**).

Despite OGA levels continuing to drop and OGT levels rising over 5 days of dosing, *O*-GlcNAc levels remained low and unchanged after 24 hours (**Figure 2C**).

[00219] We next investigated the reversibility of the effect of Ac-5SGlcNAc treatment and found that following a change of media containing no compound, *O*-GlcNAc returned to basal levels within 24 hours (**Figure 2I**). A time course and dose response study in CHO cells (Figures 2J-K), revealed a similar EC₅₀ value (0.8 μM). Various other cell lines showed equivalent responses (**Figure 2L**).

[00220] EXAMPLE VI: *O*-GlcNAc Levels on nup62 After Treatment with Ac-5SGlcNAc

10 [00221] We next probed *O*-GlcNAc levels on an individual protein after treatment with Ac-5SGlcNAc by immunoprecipitating nup62 from cell lysates. To probe for *O*-GlcNAc on immunoprecipitated nup62 we used a commercial chemoenzymatic method⁵⁴ that involves chemoselective Staudinger ligation⁵⁵ of biotin to terminal GlcNAc residues. Labeled and unlabeled nup62 from cell lysates
15 were probed with streptavidin (**Figure 2E**); nup62 from untreated cells shows clear *O*-GlcNAc modification, whereas nup62 derived from cells treated with Ac-5SGlcNAc shows almost no modification. Previous studies revealed that nup62 can be observed as two bands under certain SDS-PAGE conditions; the upper band corresponds to *O*-GlcNAc modified nup62 whereas the lower band corresponds to deglycosylated
20 nup62^{9,52}. We therefore analyzed immunoprecipitated nup62 in this way and found that untreated cells yielded only the upper band in western blots and this species was heavily *O*-GlcNAc modified (**Figure 2F**). Treating this nup62 precipitate with a bacterial homologue of OGA (*BtGH84*⁴³) greatly diminished *O*-GlcNAc immunoreactivity and the resulting deglycosylated nup62 appeared as a lower band.
25 nup62 from cells treated with Ac-5SGlcNAc, however, appeared as a doublet of bands consistent with there being a mixture of two species; an upper band, which is slightly *O*-GlcNAc modified, and a lower band, which is an essentially unmodified species of nup62. *BtGH84* digestion of nup62 derived from Ac-5SGlcNAc treated cells resulted in near complete loss of remaining *O*-GlcNAc and only the lower band remained,
30 consistent with remaining *O*-GlcNAc on the partly modified upper band of nup62 being removed. These results collectively show that 5SGlcNAc dramatically decreases

O-GlcNAc levels even on proteins that are constitutively and heavily *O*-GlcNAc modified in cells.

[00222] EXAMPLE VII: Immunoreactivity of GlcNAc and 5SGlcNAc

[00223] We tested the immunoreactivity of GlcNAc and 5SGlcNAc with
 5 CTD110.6 through blocking experiments using different concentrations of methyl 2-acetamido-2-deoxy- β -D-glucopyranoside (Me-GlcNAc) and methyl 2-acetamido-2-deoxy-5-thio- β -D-glucopyranoside (Me-5SGlcNAc; **Figures 1C and 4A**). We find that Me-5SGlcNAc blocks binding almost as well as Me-GlcNAc, supporting the view that 5SGlcNAc does not accumulate to a significant extent on proteins.
 10 Nevertheless, we assayed the ability of OGA to process 5SGlcNAc by synthesizing *para*-methoxyphenyl 5SGlcNAc (*p*MP-5SGlcNAc, **Figure 1C**) and evaluating the OGA-catalyzed hydrolysis of this compound compared to *para*-methoxyphenyl-GlcNAc⁵⁶ (*p*MP-GlcNAc). Michaelis-Menten kinetics (**Figures 4B-C**) reveal human OGA processes *p*MP-5SGlcNAc ($V_{\max}/E_0K_M = 8.1 \mu\text{mol s}^{-1} \text{mg}^{-1} \text{M}^{-1}$) only 5-fold less
 15 efficiently than *p*MP-GlcNAc ($V_{\max}/E_0K_M = 40.7 \mu\text{mol s}^{-1} \text{mg}^{-1} \text{M}^{-1}$).

[00224] An alternate process that could cause decreased *O*-GlcNAc levels in cells treated with Ac-5SGlcNAc is that the pool of UDP-GlcNAc in cells be greatly diminished. To verify that UDP-5SGlcNAc is being biosynthesized within cells, and to evaluate the cellular levels of UDP-GlcNAc and other nucleotide sugar donors, we
 20 treated COS-7 cells with different concentrations of Ac-5SGlcNAc. Analysis of the extracted UDP-sugars using CE (**Figures 3A-B, F-G**) revealed a dose dependent increase in the amount of UDP-5SGlcNAc in cells and a small decrease in UDP-GlcNAc levels. An additional peak corresponding to a synthetic standard of UDP-5SGalNAc is also observed (**Figures 3E-F**), while uridine diphosphoglucose and
 25 uridine diphosphogalactose levels are unaffected. The marked accumulation of UDP-5SGlcNAc is consistent with OGT being unable to use it as a substrate. At 50 μM we find near complete loss of *O*-GlcNAc in the cell lines tested, yet UDP-GlcNAc levels are still more than 60% of basal levels; a decrease that is unlikely to account for the low *O*-GlcNAc levels observed in treated cells. Therefore, 5SGlcNAc can efficiently
 30 traverse the HBP to form levels of UDP-5SGlcNAc that proficiently inhibit OGT in cells.

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[00225] EXAMPLE VIII: Cell Surface Glycosylation

[00226] To assess whether cell surface glycosylation could be affected through changes in UDP-GlcNAc availability or inhibition of GlcNAc transferases within the secretory pathway, we treated COS-7 cells with a range of Ac-5SGlcNAc concentrations and evaluated the effect on various glycans using lectin blots. All lectins tested showed no change in glycosylation, even at the high doses of Ac-5SGlcNAc assayed (**Figures 3C and H**). These lectins probed for both high mannose (ConA and GNA) and complex *N*-glycans (PHA-L, SNA and MAA). We also examined the effect of Ac-5SGlcNAc on *N*-glycosylation of an individual protein. Immunoprecipitated IgG produced by a mouse hybridoma cell line appeared to possess only high mannose glycans. There appeared to be a slight decrease in GNA and ConA reactivity when using a very high dose of Ac-5SGlcNAc (**Figures 3D and I**). These collective observations are consistent with reports showing that EMEG32^{-/-} cells, which are deficient in the biosynthesis of GlcNAc, have very low UDP-GlcNAc levels but *N*-glycosylation is virtually unaffected⁵⁷. Apparently, ~5% of the normal UDP-GlcNAc levels found in cells is sufficient for *N*-glycosylation⁵⁷, a level much lower than that observed when using inhibitory concentrations of 5SGlcNAc to block OGT.

[00227] EXAMPLE IX: Cellular Responses to Ac-5SGlcNAc

[00228] Cellular responses to Ac-5SGlcNAc (**9**) treatment and consequent decreases in *O*-GlcNAc levels were evaluated. In the studies and cell lines examined, there were qualitatively no apparent changes in cell morphology or rate of proliferation at the doses assayed. We quantitatively evaluated cell proliferation and established growth curves over the course of 5 days in both CHO and EMEG32 heterozygous mouse embryonic fibroblast cells treated with 50 μ M Ac-5SGlcNAc (**9**), 50 μ M Ac-GlcNAc (**G**), or vehicle (**Figures 5A-B**). For CHO cells the growth rate of Ac-5SGlcNAc (**9**) treated cells was indistinguishable from those of Ac-GlcNAc (**G**) and vehicle treated cells. In EMEG32 heterozygotes, the growth rates of the Ac-5SGlcNAc (**9**) and Ac-GlcNAc (**G**) treated cells were indistinguishable but were both lower than vehicle treated cells. Interestingly, despite the absence of Ac-5SGlcNAc

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(9) specific effects on growth rate, decreases were observed in the levels of transcription factor Sp1 (**Figures 5C-D**).

[00229] EXAMPLE X: Metabolic feeding of 5SGlcNAz

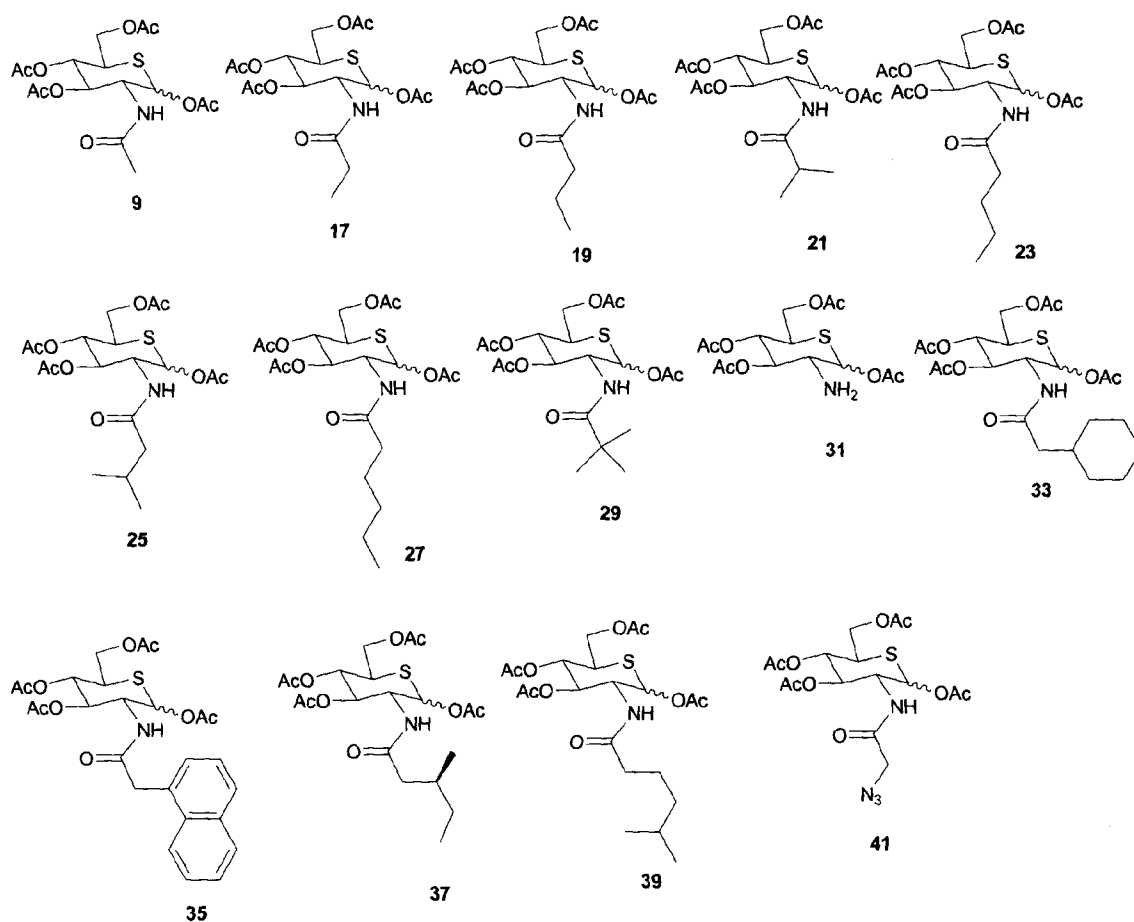
[00230] To probe the possibility that 5SGlcNAc is being incorporated onto
5 proteins, we used a metabolic feeding and chemoselective ligation strategy using 2-
azidoacetamido-2-deoxy-D-glucopyranose (GlcNAz), which can be assimilated by the
HBP to form uridine diphospho-*N*-azidoacetylglucosamine (UDP-GlcNAz) and then
be installed onto proteins as *O*-GlcNAz⁹. Using a chemoselective ligation to install a
reporter group allows sensitive detection of *O*-GlcNAz modified proteins⁹.
10 Accordingly, we evaluated whether 2-azidoacetamido-2-deoxy-5-thio-D-
glucopyranose (5SGlcNAz) is incorporated onto proteins to determine whether
5SGlcNAc might be transferred onto proteins in cells. This approach has the
advantage that it relies on neither recognition of potential *O*-5SGlcNAc by the three
antibodies tested nor by the GalT used for the chemoenzymatic labelling approach.
15 We therefore carried out the synthesis of 1,3,4,6-tetra-*O*-acetyl-2-azidoacetamido-2-
deoxy-5-thio- α -D-glucopyranose (Ac-5SGlcNAz, **41**, **Figure 6A**) in 15 steps, the last
two steps of which diverge from a previously known compound⁶⁰ (see Scheme 3, and
Figure 6D-E for details of the synthesis and characterization). Briefly, these two steps
involved catalytic hydrogenation followed by DCC coupling, and furnished Ac-
20 5SGlcNAz (**41**) in reasonable yield. Treating cells with either vehicle, 1,3,4,6-tetra-*O*-
acetyl-2-azidoacetamido-2-deoxy- α -D-glucopyranose⁹ (Ac-GlcNAz) or Ac-5SGlcNAz
(**41**) revealed that only 5SGlcNAz decreased *O*-GlcNAc levels. Proteins from cells
treated with vehicle, Ac-GlcNAz, or Ac-5SGlcNAz (**41**) were collected and subjected
to Staudinger ligation⁵⁵ with biotin phosphine (**Figure 6A**). Following the ligation,
25 proteins were blotted onto nitrocellulose and probed using streptavidin-horse radish
peroxidase. Only proteins from cells treated with Ac-GlcNAz revealed any signal,
whereas the signal from cells treated with vehicle were indistinguishable from those
treated with Ac-5SGlcNAz (**41**) (**Figure 6B**). Similarly, we immunoprecipitated
nup62 from cells treated with vehicle, Ac-GlcNAz, or Ac-5SGlcNAz (**41**), and prior
30 to Staudinger ligation, incubated half of each sample with BtGH84⁴³. The resulting
blot, probed with streptavidin-HRP, once again only showed signal from nup62
immunoprecipitated from cells treated with Ac-GlcNAz, and only in the sample that

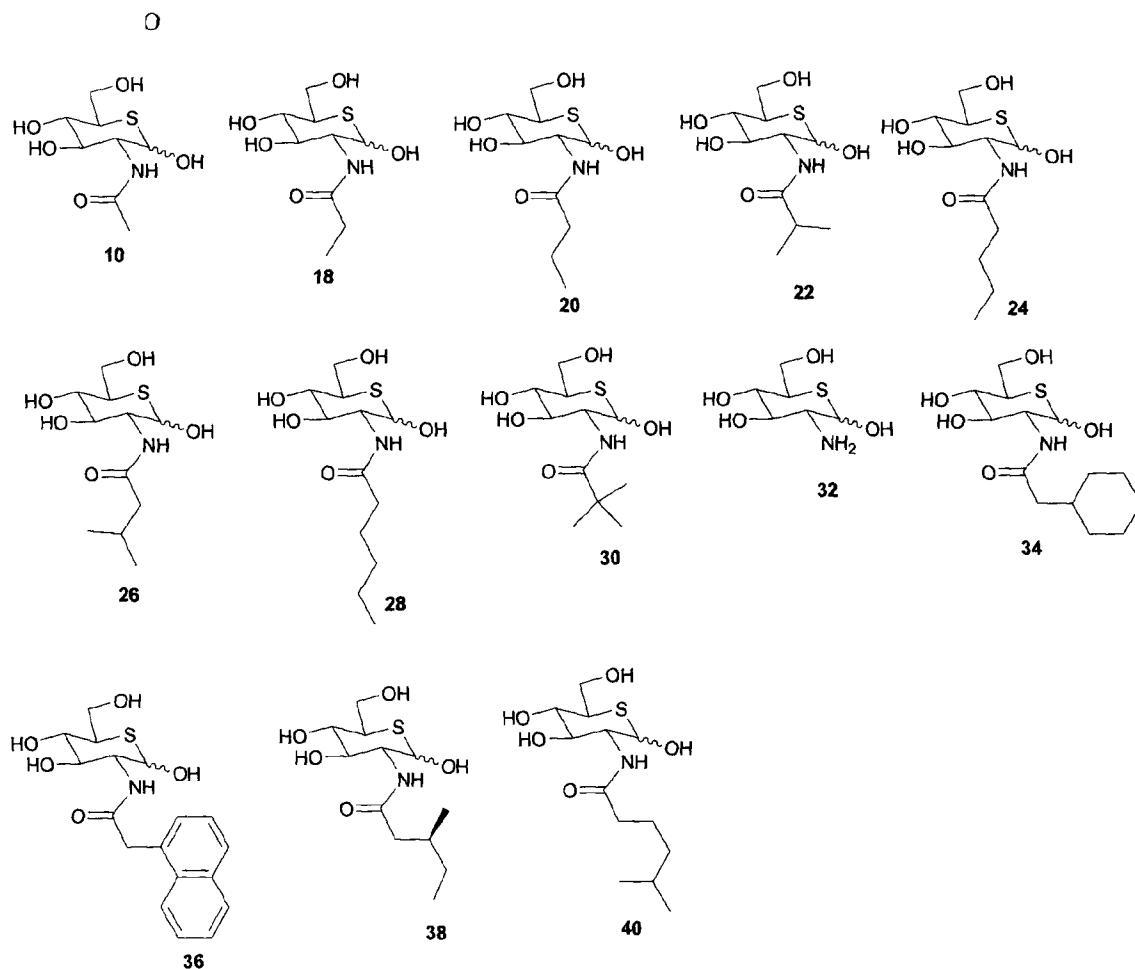
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had not been subjected to *BtGH84* hydrolysis (**Figure 6C**). The observation that Ac-5SGlcNAz (**41**) decreases *O*-GlcNAc levels indicates that uridine diphospho-5-thio-*N*-azidoacetylglucosamine (UDP-5SGlcNAz) is formed within cells where it acts to inhibit OGT. The chemoselective ligation data, however, indicates that 5SGlcNAz does not accumulate on proteins to any measurable level. These data indicated that 5SGlcNAc does not accumulate on proteins, and that decreased *O*-GlcNAc levels arise from inhibition of OGT by UDP-5SGlcNAc.

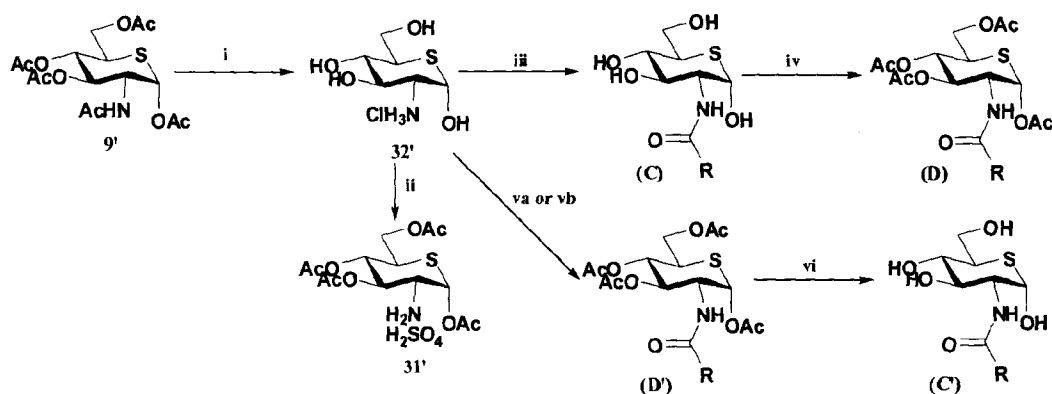
[00231] EXAMPLE XI: Synthesis of Ac-5SGlcNAc analogues

[00232] A total of 27 analogues, as shown below, were synthesized based on the Ac-5SGlcNAc (**9**) template. These analogues incorporate different alkyl substituents at the C2 position, and were synthesized in a protected (acetylated at C1, C3, C4 and C6) and deprotected (free hydroxyl) form.





[00233] In general, the compounds were prepared according to the synthetic route outlined in **Scheme 4**.



i. 2N HCl, reflux, 4 hrs; ii. Ac₂O, H₂SO₄, r.t.; iii. (RCO)₂O, Et₃N, H₂O/EtOH (1:1), r.t.;
iv. Ac₂O, pyridine, r.t.; va. RCOOH, HBTU, EDIPA, 1,4-dioxane, r.t., then Ac₂O, pyridine;
vb. RCOCl, EDIPA, 1,4-dioxane, r.t., then Ac₂O, pyridine; vi. NaOMe, MeOH.

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Scheme 4

[00234] General Procedure A: Synthesis of *N*-acyl- α -D-5-thio-glucosamine (C)
using acid anhydrides. Acid anhydrides (1.5 eq.) and Et₃N (1.1 eq.) were added into

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the solution of 5-thio-*D*-glucosamine hydrochloride (**32'**) in solvent H₂O/EtOH (1:1), the resultant reaction mixture was stirred at r.t. for 18-20 hrs. The solvent was then removed in vacuum, and the desired materials were isolated as solids by flash silica chromatography using a solvent system of EtOAc:MeOH:H₂O in ratios ranging from 15:1:0.5 to 10:1:0.5 as appropriate.

[00235] General Procedure B: Synthesis of *N*-acyl-1,3,4,6-tetra-*O*-acetyl- α -*D*-5-thio-glucosamine (**D**). *N*-acyl- α -*D*-5-thio-glucosamine (**C**) was dissolved in pyridine and Ac₂O (10 eq.) was added at r.t. The resultant reaction mixture was stirred at r.t. for 18-20 hrs. The solvent was then removed in vacuum, and the desired materials were isolated as solids by flash silica chromatography using a solvent system of Hex:EtOAc in ratios ranging from 3:1 to 1:1 as appropriate.

[00236] General Procedure C: Synthesis of *N*-acyl-1,3,4,6-tetra-*O*-acetyl- α -*D*-5-thio-glucosamine (**D'**) using acids. To the suspension of 5-thio-*D*-glucosamine hydrochloride (**32'**) in 1,4-dioxane was added acids (1.5-1.8 eq.), EDIPA (2.0 eq.) and HBTU (2.0 eq.), the resultant reaction mixture was stirred at r.t. for 18-20 hrs. The solvent was then removed in vacuum, and the *N*-acylated intermediates were isolated as solids by flash silica chromatography using a solvent system of EtOAc:MeOH:H₂O in ratios ranging from 15:1:0.5 to 10:1:0.5 as appropriate. Then the intermediates were dissolved in pyridine, and Ac₂O (10 eq.) was added at r.t.. The resultant reaction mixture was stirred at r.t. for 18-20 hrs. The solvent was then removed in vacuum, and the desired materials were isolated as solids by flash silica chromatography using a solvent system of Hex:EtOAc in ratios ranging from 3:1 to 1:1 as appropriate.

[00237] General Procedure D: Synthesis of *N*-acyl-1,3,4,6-tetra-*O*-acetyl- α -*D*-5-thio-glucosamine (**D'**) using acid chloride. To the suspension of 5-thio-*D*-glucosamine hydrochloride (**32'**) in 1,4-dioxane was added acids (1.5-2.0 eq.) and EDIPA (1.5-2.0 eq.), the resultant reaction mixture was stirred at r.t. for 18-20 hrs. The solvent was then removed in vacuum, and the *N*-acylated intermediates were isolated as solids by flash silica chromatography using a solvent system of EtOAc:MeOH:H₂O in ratios ranging from 15:1:0.5 to 10:1:0.5 as appropriate. Then the intermediates were dissolved in pyridine, and Ac₂O (10 eq.) was added at r.t.. The resultant reaction mixture was stirred at r.t. for 18-20 hrs. The solvent was then

removed in vacuum, and the desired materials were isolated as solids by flash silica chromatography using a solvent system of Hex:EtOAc in ratios ranging from 3:1 to 1:1 as appropriate.

[00238] General Procedure E: Synthesis of *N*-acyl- α -D-5-thio-glucosamine (C')
 5 (C'). Sodium methoxide was added into the solution of *N*-acyl-1,3,4,6-tetra-*O*-acetyl- α -D-5-thio-glucosamine (D') in MeOH to keep pH~10, and the resultant reaction mixture was stirred at r.t. for 2 hrs. The reaction was then neutralized with Amberlite IR 120 (H⁺) resin. After filtration and removal of the solvent, the desired materials were isolated as solids by flash silica chromatography using a solvent system of
 10 EtOAc:MeOH:H₂O in ratios ranging from 15:1:0.5 to 10:1:0.5 as appropriate.

[00239] **Compound 9 and 10:** *N*-Acetyl-1,3,4,6-tetra-*O*-acetyl- α -D-5-thio-glucosamine (9) and *N*-acetyl- α -D-5-thio-glucosamine (10)

[00240] The title compound 9 and 10 was synthesized from *N*-acetyl-D-glucosamine in 8 and 9 steps respectively (ref: *Nature Chem. Biol.* **2011**, 7, 174-181.).

15 [00241] **Compound 32':** α -D-5-thio-glucosamine hydrochloride (32')

[00242] Compound *N*-acetyl-1,3,4,6-tetra-*O*-acetyl- α -D-5-thio-glucosamine (1, 945 mg, 2.33 mmol) was suspended in 2 N HCl (aq., 15 mL), and the resultant reaction mixture was stirred under reflux for 4 hrs. After cooling down to r.t., the reaction mixture was concentrated under high vacuum, and was co-evaporated with
 20 toluene (8 X 15 mL). The residue was then re-dissolved in MeOH (100 mL), and activated carbon was added to the solution. After stirring for 30 mins, the mixture was filtered through Celite, and concentrated to dryness. The residue was dried under high vacuum to afford 32' as a solid (520 mg, 96 %), which was used directly to the next reaction without purification. ¹H-NMR (500 M Hz, D₂O): δ 5.23 (d, *J* = 3.00 Hz, 1 H),
 25 3.94 (dd, *J* = 12.0, 5.50 Hz, 1 H), 3.89 (dd, *J* = 12.0, 3.00 Hz, 1 H), 3.84 (dd, *J* = 10.0, 9.00 Hz, 1 H), 3.67 (dd, *J* = 10.5, 9.00 Hz, 1 H), 3.61 (dd, *J* = 10.5, 3.00 Hz, 1 H), 3.30 (ddd, *J* = 10.0, 5.50, 3.00 Hz, 1 H). ¹³C-NMR (125 M Hz, D₂O): δ 73.98, 71.16, 70.51, 60.45, 59.06, 43.84. HRMS (*m/z*): C₆H₁₄NO₄S (M+H)⁺: Calcd, 196.0638; Found, 196.0634. C₆H₁₃NNaO₄S (M+Na)⁺: Calcd, 218.0457; Found, 218.0457.
 30 C₆H₁₃KNO₄S (M+K)⁺: Calcd, 234.0197; Found, 234.0201.

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[00243] **Compound 31'**: *1,3,4,6-tetra-O-acetyl- α -D-5-thio-glucosamine sulphuric acid salt (31')*

[00244] *α -D-5-thio-Glucosamine hydrochloride 9* (20.0 mg, 0.086 mmol) was dissolved in Ac₂O (0.30 mL), and con. H₂SO₄ (1 drop, about 0.025 mL) was added, the resulting mixture was stirred at r.t. for 40 hrs. After addition of MeOH (2 drops), the mixture became clear. To the resultant reaction mixture was added EtOAc (about 3.0 mL) under ice-bath (0°C) and stirred for several minutes. White solid was precipitated. Filtration and washing with EtOAc (20 mL) afford **31'** as a white solid, which was purified by recrystallization from EtOAc/MeOH system. The yield was 10 mg (25 %). ¹H-NMR (400 M Hz, MeOD): δ 6.12 (d, J = 3.20 Hz, 1 H), 5.40 (t, J = 10.0 Hz, 1 H), 5.31 (t, J = 10.0 Hz, 1 H), 4.46 (dd, J = 12.4, 4.80 Hz, 1 H), 4.12 (dd, J = 10.8, 3.20 Hz, 1 H), 4.04 (dd, J = 12.4, 3.20 Hz, 1 H), 3.72 (ddd, J = 10.8, 4.40, 3.20 Hz, 1 H), 2.23 (s, 3 H), 2.12 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H). ¹³C-NMR (100 M Hz, MeOD): δ 172.01, 171.58, 171.16, 170.17, 73.03, 72.10, 71.96, 62.01, 56.88, 40.84, 20.82, 20.77, 20.51, 20.48. HRMS (m/z): C₁₄H₂₂NO₈S (M+H)⁺: Calcd, 364.1061; Found, 364.1068. C₁₄H₂₁NNaO₈S (M+Na)⁺: Calcd, 386.0880; Found, 386.0890.

[00245] **Compound 18 and 17**: *N-Propionyl- α -D-5-thio-glucosamine (18) and N-propionyl-1,3,4,6-tetra-O-acetyl- α -D-5-thio-glucosamine (17)*

[00246] The title compounds were prepared from the material thus obtained following General Procedures A and B. For **18**: ¹H-NMR (400 M Hz, D₂O): δ 4.99 (d, J = 2.80 Hz, 1 H, H-1), 4.11 (dd, J = 10.4, 2.80 Hz, 1 H, H-2), 3.95 (dd, J = 12.0, 5.60 Hz, 1 H, H-6a), 3.89 (dd, J = 12.0, 3.20 Hz, 1 H, H-6b), 3.73 (t, J = 9.20 Hz, 1 H, H-3), 3.68 (t, J = 9.20 Hz, 1 H, H-4), 3.28 (ddd, J = 9.60, 5.60, 3.60 Hz, 1 H, H-5), 2.31 (q, J = 7.60 Hz, 2 H, H-2'), 1.13 (t, J = 7.60 Hz, 3 H, H-3'). ¹³C-NMR (100 M Hz, D₂O): δ 178.00 (C-1'), 74.04 (C-4), 71.75 (C-1), 71.52 (C-3), 60.20 (C-6), 58.05 (C-2), 43.15 (C-5), 29.08 (C-2'), 9.49 (C-3'). HRMS (m/z): C₉H₁₈NO₅S (M+H)⁺: Calcd, 252.0900; Found, 252.0895. C₉H₁₇NNaO₅S (M+Na)⁺: Calcd, 274.0720; Found, 274.0718. C₉H₁₇KNO₅S (M+K)⁺: Calcd, 290.0459; Found, 290.0454.

[00247] For **17**: ¹H-NMR (500 M Hz, CDCl₃): δ 5.93 (d, J = 3.00 Hz, 1 H), 5.71 (d, J = 9.00 Hz, 1 H), 5.37 (t, J = 10.0 Hz, 1 H), 5.18 (t, J = 10.5 Hz, 1 H), 4.66-

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4.61 (m, 1 H), 4.32 (dd, $J = 12.0, 5.00$ Hz), 4.03 (dd, $J = 12.0, 3.00$ Hz), 3.46 (ddd, $J = 11.0, 5.00, 3.50$ Hz, 1 H), 2.17 (s, 3 H), 2.15-2.05 (m, 2 H), 2.06 (s, 3 H), 2.03 (s, 3 H), 2.01 (s, 3 H), 1.06 (t, $J = 7.50$ Hz, 3 H). ^{13}C -NMR (125 M Hz, CDCl_3): δ 173.21, 171.57, 170.51, 169.08, 168.68, 72.69, 71.69, 71.43, 61.07, 54.99, 39.70, 29.50, 21.03, 20.58, 20.46, 9.63. HRMS (m/z): $\text{C}_{17}\text{H}_{26}\text{NO}_9\text{S}$ ($\text{M}+\text{H}$) $^+$: Calcd, 420.1323; Found, 420.1324. $\text{C}_{17}\text{H}_{29}\text{N}_2\text{O}_9\text{S}$ ($\text{M}+\text{NH}_4$) $^+$: Calcd, 437.1588; Found, 437.1591. $\text{C}_{17}\text{H}_{25}\text{NNaO}_9\text{S}$ ($\text{M}+\text{Na}$) $^+$: Calcd, 442.1142; Found, 442.1143. $\text{C}_{17}\text{H}_{25}\text{KNO}_5\text{S}$ ($\text{M}+\text{K}$) $^+$: Calcd, 458.0882; Found, 458.0883.

[00248] **Compound 20 and 19:** *N*-(*n*-Butyryl)- α -D-5-thio-glucosamine (**20**)
and *N*-(*n*-butyryl)-1,3,4,6-tetra-*O*-acetyl- α -D-5-thio-glucosamine (**19**)

[00249] The title compounds were prepared from the material thus obtained following General Procedures A and B. For **20**: ^1H -NMR (500 M Hz, D_2O): δ 4.97 (d, $J = 3.00$ Hz, 1 H), 4.10 (dd, $J = 10.5, 3.00$ Hz, 1 H), 3.93 (dd, $J = 12.0, 5.50$ Hz, 1 H), 3.89 (dd, $J = 12.0, 3.50$ Hz, 1 H), 3.70 (t, $J = 9.50$ Hz, 1 H), 3.66 (t, $J = 9.50$ Hz, 1 H), 3.27 (ddd, $J = 10.0, 5.50, 3.00$ Hz, 1 H), 2.26 (t, $J = 7.50$ Hz, 2 H), 1.65-1.57 (m, 2 H), 0.90 (t, $J = 7.50$ Hz, 3 H). ^{13}C -NMR (125 M Hz, D_2O): δ 177.07, 74.02, 71.79, 71.41, 60.15, 58.02, 43.11, 37.56, 18.99, 12.63. HRMS (m/z): $\text{C}_{10}\text{H}_{20}\text{NO}_5\text{S}$ ($\text{M}+\text{H}$) $^+$: Calcd, 266.1057; Found, 266.1052. $\text{C}_{10}\text{H}_{19}\text{NNaO}_5\text{S}$ ($\text{M}+\text{Na}$) $^+$: Calcd, 288.0876; Found, 288.0876. $\text{C}_{10}\text{H}_{19}\text{KNO}_5\text{S}$ ($\text{M}+\text{K}$) $^+$: Calcd, 304.0616; Found, 304.0614.

[00250] For **19**: ^1H -NMR (500 M Hz, CDCl_3): δ 5.94 (d, $J = 3.00$ Hz, 1 H), 5.71 (d, $J = 9.00$ Hz, 1 H), 5.37 (t, $J = 10.0$ Hz, 1 H), 5.18 (t, $J = 10.0$ Hz, 1 H), 4.66-4.61 (m, 1 H), 4.32 (dd, $J = 12.5, 5.00$ Hz, 1 H), 4.03 (dd, $J = 12.0, 3.00$ Hz, 1 H), 3.47 (ddd, $J = 11.0, 5.00, 3.50$ Hz, 1 H), 2.17 (s, 3 H), 2.10-2.06 (m, 2 H), 2.06 (s, 3 H), 2.03 (s, 3 H), 2.02 (s, 3 H), 1.60-1.53 (m, 2 H), 0.88 (t, $J = 7.50$ Hz, 3 H). ^{13}C -NMR (125 M Hz, CDCl_3): δ 172.39, 171.63, 170.51, 169.11, 168.66, 72.69, 71.64, 71.46, 61.08, 55.01, 39.70, 38.71, 21.04, 20.64, 20.60, 20.49, 18.94, 13.51. HRMS (m/z): $\text{C}_{18}\text{H}_{28}\text{NO}_9\text{S}$ ($\text{M}+\text{H}$) $^+$: Calcd, 434.1479; Found, 434.1484. $\text{C}_{18}\text{H}_{31}\text{N}_2\text{O}_9\text{S}$ ($\text{M}+\text{NH}_4$) $^+$: Calcd, 451.1745; Found, 451.1752. $\text{C}_{18}\text{H}_{27}\text{NNaO}_9\text{S}$ ($\text{M}+\text{Na}$) $^+$: Calcd, 456.1299; Found, 456.1304. $\text{C}_{18}\text{H}_{27}\text{KNO}_5\text{S}$ ($\text{M}+\text{K}$) $^+$: Calcd, 472.1038; Found, 472.1044.

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[00251] **Compound 22 and 21:** *N*-(*i*-Butyryl)- α -D-5-thio-glucosamine (22) and *N*-(*i*-butyryl)-1,3,4,6-tetra-*O*-acetyl- α -D-5-thio-glucosamine (21)

[00252] The title compounds were prepared from the material thus obtained following General Procedures A and B. For **22**: $^1\text{H-NMR}$ (400 M Hz, D_2O): δ 4.98 (d, $J = 2.80$ Hz, 1 H), 4.12 (dd, $J = 10.4, 2.80$ Hz, 1 H), 3.94 (dd, $J = 11.6, 5.60$ Hz, 1 H), 3.90 (dd, $J = 12.0, 3.20$ Hz, 1 H), 3.73 (t, $J = 9.60$ Hz, 1 H), 3.68 (t, $J = 9.60$ Hz, 1 H), 3.29 (ddd, $J = 9.60, 5.60, 3.20$ Hz, 1 H), 2.63-2.52 (m, 1 H), 1.12 (d, $J = 6.80$ Hz, 6 H). $^{13}\text{C-NMR}$ (100 M Hz, D_2O): δ 181.11, 74.07, 71.80, 71.48, 60.22, 57.93, 43.17, 34.97, 18.72, 18.55. HRMS (m/z): $\text{C}_{10}\text{H}_{20}\text{NO}_5\text{S}$ ($\text{M}+\text{H}$) $^+$: Calcd, 266.1057; Found, 266.1052. $\text{C}_{10}\text{H}_{19}\text{NNaO}_5\text{S}$ ($\text{M}+\text{Na}$) $^+$: Calcd, 288.0876; Found, 288.0874. $\text{C}_{10}\text{H}_{19}\text{KNO}_5\text{S}$ ($\text{M}+\text{K}$) $^+$: Calcd, 304.0616; Found, 304.0612.

[00253] For **21**: $^1\text{H-NMR}$ (500 M Hz, CDCl_3): δ 5.95 (d, $J = 3.00$ Hz, 1 H), 5.69 (d, $J = 9.00$ Hz, 1 H), 5.38 (t, $J = 10.0$ Hz, 1 H), 5.18 (t, $J = 10.0$ Hz, 1 H), 4.65-4.59 (m, 1 H), 4.34 (dd, $J = 12.0, 5.00$ Hz, 1 H), 4.04 (dd, $J = 12.0, 3.00$ Hz, 1 H), 3.48 (ddd, $J = 11.0, 5.00, 3.50$ Hz, 1 H), 2.30-2.21 (m, 1 H), 2.18 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 1.06 (d, $J = 5.00$ Hz, 3 H), 1.05 (d, $J = 5.00$ Hz, 3 H). $^{13}\text{C-NMR}$ (125 M Hz, CDCl_3): δ 176.32, 171.63, 170.54, 169.12, 168.65, 72.61, 71.66, 71.44, 61.10, 54.93, 39.73, 35.41, 21.00, 20.62, 20.49, 19.38, 19.17. HRMS (m/z): $\text{C}_{18}\text{H}_{28}\text{NO}_9\text{S}$ ($\text{M}+\text{H}$) $^+$: Calcd, 434.1479; Found, 434.1482. $\text{C}_{18}\text{H}_{31}\text{N}_2\text{O}_9\text{S}$ ($\text{M}+\text{NH}_4$) $^+$: Calcd, 451.1745; Found, 451.1743. $\text{C}_{18}\text{H}_{27}\text{NNaO}_9\text{S}$ ($\text{M}+\text{Na}$) $^+$: Calcd, 456.1299; Found, 456.1300. $\text{C}_{18}\text{H}_{27}\text{KNO}_5\text{S}$ ($\text{M}+\text{K}$) $^+$: Calcd, 472.1038; Found, 472.1038.

[00254] **Compound 24 and 23:** *N*-(*n*-Valeryl)- α -D-5-thio-glucosamine (24) and *N*-(*n*-valeryl)-1,3,4,6-tetra-*O*-acetyl- α -D-5-thio-glucosamine (23)

[00255] The title compounds were prepared from the material thus obtained following General Procedures A and B. For **24**: $^1\text{H-NMR}$ (500 M Hz, D_2O): δ 4.96 (d, $J = 2.50$ Hz, 1 H), 4.10 (dd, $J = 10.0, 3.00$ Hz, 1 H), 3.93 (dd, $J = 12.0, 5.50$ Hz, 1 H), 3.87 (dd, $J = 12.0, 3.00$ Hz, 1 H), 3.70 (t, $J = 9.50$ Hz, 1 H), 3.66 (t, $J = 10.0$ Hz, 1 H), 3.27 (ddd, $J = 9.50, 5.50, 3.00$ Hz, 1 H), 2.29 (t, $J = 7.50$ Hz, 2 H), 1.60-1.54 (m, 2 H), 1.34-1.27 (m, 2 H), 0.88 (t, $J = 7.50$ Hz, 3 H). $^{13}\text{C-NMR}$ (125 M Hz, D_2O): δ 177.19, 73.88, 71.66, 71.30, 60.00, 57.93, 43.00, 35.34, 27.47, 21.40, 12.90. HRMS (m/z):

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$C_{11}H_{22}NO_5S$ (M+H)⁺: Calcd, 280.1213; Found, 280.1200. $C_{11}H_{21}NNaO_5S$ (M+Na)⁺: Calcd, 302.1033; Found, 302.1023. $C_{11}H_{21}KNO_5S$ (M+K)⁺: Calcd, 318.0772; Found, 318.0767.

[00256] For **23**: ¹H-NMR (500 M Hz, CDCl₃): δ 5.93 (d, *J* = 3.50 Hz, 1 H),
 5.71 (d, *J* = 8.50 Hz, 1 H), 5.38 (dd, *J* = 11.0, 10.0 Hz, 1 H), 5.18 (dd, *J* = 11.0, 10.0
 Hz, 1 H), 4.65-4.60 (m, 1 H), 4.34 (dd, *J* = 12.0, 5.00 Hz, 1 H), 4.02 (dd, *J* = 12.0,
 3.50 Hz, 1 H), 3.47 (ddd, *J* = 10.5, 4.50, 3.00 Hz, 1 H), 2.17 (s, 3 H), 2.10-2.05 (m, 2
 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 1.53-1.47 (m, 2 H), 1.29-1.22 (m, 2 H),
 0.87 (t, *J* = 7.50 Hz, 3 H). ¹³C-NMR (125 M Hz, CDCl₃): δ 172.56, 171.57, 170.50,
 169.09, 168.63, 72.66, 71.62, 71.46, 61.07, 54.99, 39.68, 36.18, 27.53, 22.12, 21.00,
 20.59, 20.57, 20.46, 13.63. HRMS (*m/z*): $C_{19}H_{30}NO_9S$ (M+H)⁺: Calcd, 448.1636;
 Found, 448.1640. $C_{19}H_{29}NNaO_9S$ (M+Na)⁺: Calcd, 470.1455; Found, 470.1459.
 $C_{19}H_{29}KNO_9S$ (M+K)⁺: Calcd, 486.1195; Found, 486.1197.

[00257] **Compound 26 and 25**: *N*-(*i*-Valeryl)-α-*D*-5-thio-glucosamine (**26**) and
N-(*i*-valeryl)-1,3,4,6-tetra-*O*-acetyl-α-*D*-5-thio-glucosamine (**25**)

[00258] The title compounds were prepared from the material thus obtained
 following General Procedures A and B. For **26**: ¹H-NMR (500 M Hz, D₂O): δ 4.97 (d,
J = 2.50 Hz, 1 H), 4.10 (dd, *J* = 10.0, 3.00 Hz, 1 H), 3.93 (dd, *J* = 12.0, 5.50 Hz, 1 H),
 3.90 (dd, *J* = 12.0, 3.50 Hz, 1 H), 3.70 (t, *J* = 9.50 Hz, 1 H), 3.66 (t, *J* = 9.50 Hz, 1 H),
 3.26 (ddd, *J* = 9.50, 5.00, 3.00 Hz, 1 H), 2.15 (d, *J* = 7.50 Hz, 2 H), 2.03-1.95 (m, 1
 H), 0.92 (d, *J* = 6.50 Hz, 3 H), 0.91 (d, *J* = 6.50 Hz, 3 H). ¹³C-NMR (125 M Hz, D₂O):
 δ 176.45, 74.06, 71.84, 71.38, 60.16, 58.01, 44.86, 43.12, 26.20, 21.54, 21.36. HRMS
 (*m/z*): $C_{11}H_{22}NO_5S$ (M+H)⁺: Calcd, 280.1213; Found, 280.1201. $C_{11}H_{21}NNaO_5S$
 (M+Na)⁺: Calcd, 302.1033; Found, 302.1022. $C_{11}H_{21}KNO_5S$ (M+K)⁺: Calcd,
 318.0772; Found, 318.0759.

[00259] For **25**: ¹H-NMR (500 M Hz, CDCl₃): δ 5.95 (d, *J* = 3.00 Hz, 1 H),
 5.71 (d, *J* = 8.50 Hz, 1 H), 5.37 (dd, *J* = 10.5, 9.50 Hz, 1 H), 5.18 (dd, *J* = 11.0, 10.0
 Hz, 1 H), 4.65-4.60 (m, 1 H), 4.34 (dd, *J* = 12.0, 5.00 Hz, 1 H), 4.03 (dd, *J* = 12.0,
 3.00 Hz, 1 H), 3.47 (ddd, *J* = 11.0, 5.00, 3.00 Hz, 1 H), 2.16 (s, 3 H), 2.06 (s, 3 H),
 2.04 (s, 3 H), 2.02 (s, 3 H), 2.01-1.92 (m, 3 H), 0.87 (d, *J* = 6.50 Hz, 6 H). ¹³C-NMR
 (125 M Hz, CDCl₃): δ 171.88, 171.66, 170.52, 169.11, 168.62, 72.64, 71.56, 71.50,

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61.08, 55.07, 45.77, 39.69, 26.02, 22.20, 22.11, 21.01, 20.67, 20.60, 20.48. HRMS (m/z): $C_{19}H_{30}NO_9S$ ($M+H$)⁺: Calcd, 448.1636; Found, 448.1641. $C_{19}H_{29}NNaO_9S$ ($M+Na$)⁺: Calcd, 470.1455; Found, 470.1460. $C_{19}H_{29}KNO_5S$ ($M+K$)⁺: Calcd, 486.1195; Found, 486.1200.

5 [00260] **Compound 28 and 27: *N*-Hexonoyl- α -D-5-thio-glucosamine (28) and *N*-hexonoyl -1,3,4,6-tetra-O-acetyl- α -D-5-thio-glucosamine (27)**

[00261] The title compounds were prepared from the material thus obtained following General Procedures A and B. For **28**: ^1H -NMR (500 M Hz, D_2O): δ 4.96 (d, $J = 2.50$ Hz, 1 H), 4.10 (dd, $J = 10.0, 3.00$ Hz, 1 H), 3.93 (dd, $J = 12.0, 5.50$ Hz, 1 H),
 10 3.88 (dd, $J = 12.0, 3.00$ Hz, 1 H), 3.70 (t, $J = 9.50$ Hz, 1 H), 3.66 (t, $J = 9.50$ Hz, 1 H), 3.27 (ddd, $J = 9.50, 5.50, 3.50$ Hz, 1 H), 2.28 (t, $J = 7.50$ Hz, 2 H), 1.63-1.56 (m, 2 H), 1.30-1.27 (m, 4 H), 0.86 (t, $J = 7.00$ Hz, 3 H). ^{13}C -NMR (125 M Hz, D_2O): δ 177.26, 74.05, 71.81, 71.42, 60.18, 58.02, 43.12, 35.66, 30.40, 25.04, 21.65, 13.20. HRMS (m/z): $C_{12}H_{24}NO_5S$ ($M+H$)⁺: Calcd, 294.1370; Found, 294.1370. $C_{12}H_{23}NNaO_5S$ ($M+Na$)⁺: Calcd, 316.1189; Found, 316.1190. $C_{12}H_{23}KNO_5S$ ($M+K$)⁺: Calcd,
 15 332.0929; Found, 332.0927.

[00262] For **27**: ^1H -NMR (500 M Hz, $CDCl_3$): δ 5.92 (d, $J = 3.00$ Hz, 1 H, H-1), 5.72 (d, $J = 8.50$ Hz, 1 H, N-H), 5.38 (dd, $J = 11.0, 10.0$ Hz, 1 H), 5.18 (dd, $J = 11.0, 10.0$ Hz, 1 H), 4.66-4.60 (m, 1 H), 4.35 (dd, $J = 12.0, 5.00$ Hz, 1 H), 4.01 (dd, $J = 12.0, 3.00$ Hz, 1 H), 3.46 (ddd, $J = 11.0, 5.00, 3.00$ Hz, 1 H), 2.17 (s, 3 H), 2.10-
 20 2.05 (m, 2 H), 2.06 (s, 3 H), 2.03 (s, 3 H), 2.02 (s, 3 H), 1.55-1.48 (m, 2 H), 1.31-1.25 (m, 2 H), 1.25-1.17 (m, 2 H), 0.85 (t, $J = 7.50$ Hz, 3 H). ^{13}C -NMR (125 M Hz, $CDCl_3$): δ 172.61, 171.62, 170.59, 169.13, 168.69, 72.65, 71.57, 71.32, 61.00, 54.90, 39.61, 36.46, 31.15, 25.20, 22.28, 21.08, 20.65, 20.51, 13.84. HRMS (m/z):
 25 $C_{20}H_{32}NO_9S$ ($M+H$)⁺: Calcd, 462.1792; Found, 462.1791. $C_{20}H_{35}N_2O_9S$ ($M+NH_4$)⁺: Calcd, 479.2058; Found, 479.2063. $C_{20}H_{31}NNaO_9S$ ($M+Na$)⁺: Calcd, 484.1612; Found, 484.1612. $C_{20}H_{31}KNO_5S$ ($M+K$)⁺: Calcd, 500.1351; Found, 500.1351.

[00263] **Compound 30 and 29: *N*-Pivaloyl- α -D-5-thio-glucosamine (30) and *N*-pivaloyl-1,3,4,6-tetra-O-acetyl- α -D-5-thio-glucosamine (29)**

30 [00264] The title compounds were prepared from the material thus obtained following General Procedures A and B.

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[00265] For **30**: $^1\text{H-NMR}$ (500 M Hz, D_2O): δ 4.96 (d, $J = 3.00$ Hz, 1 H), 4.13 (dd, $J = 10.5, 3.00$ Hz, 1 H), 3.93 (dd, $J = 12.0, 5.50$ Hz, 1 H), 3.88 (dd, $J = 12.0, 3.50$ Hz, 1 H), 3.76 (t, $J = 10.0$ Hz, 1 H), 3.67 (t, $J = 10.0$ Hz, 1 H), 3.26 (ddd, $J = 10.0, 5.50, 3.50$ Hz, 1 H), 1.19 (s, 9 H). $^{13}\text{C-NMR}$ (125 M Hz, D_2O): δ 182.40, 74.05, 71.75, 71.34, 60.18, 58.16, 43.19, 38.57, 26.47. HRMS (m/z): $\text{C}_{11}\text{H}_{22}\text{NO}_5\text{S}$ ($\text{M}+\text{H}$) $^+$: Calcd, 280.1213; Found, 280.1203. $\text{C}_{11}\text{H}_{21}\text{NNaO}_5\text{S}$ ($\text{M}+\text{Na}$) $^+$: Calcd, 302.1033; Found, 302.1025. $\text{C}_{11}\text{H}_{21}\text{KNO}_5\text{S}$ ($\text{M}+\text{K}$) $^+$: Calcd, 318.0772; Found, 318.0767.

[00266] For **29**: $^1\text{H-NMR}$ (500 M Hz, CDCl_3): δ 6.01 (d, $J = 3.50$ Hz, 1 H), 5.90 (d, $J = 8.50$ Hz, 1 H), 5.38 (dd, $J = 10.5, 9.50$ Hz, 1 H), 5.18 (dd, $J = 10.5, 10.0$ Hz, 1 H), 4.58-4.54 (m, 1 H), 4.34 (dd, $J = 12.0, 5.00$ Hz, 1 H), 4.03 (dd, $J = 12.0, 3.00$ Hz, 1 H), 3.48 (ddd, $J = 11.0, 5.00, 3.00$ Hz, 1 H), 2.17 (s, 3 H), 2.07 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 1.09 (s, 9 H). $^{13}\text{C-NMR}$ (125 M Hz, CDCl_3): δ 177.87, 171.76, 170.54, 169.13, 168.59, 72.24, 71.59, 71.34, 61.14, 55.44, 39.81, 38.58, 27.19, 20.86, 20.62 (2 C), 20.50. HRMS (m/z): $\text{C}_{19}\text{H}_{30}\text{NO}_9\text{S}$ ($\text{M}+\text{H}$) $^+$: Calcd, 448.1636; Found, 448.1636. $\text{C}_{19}\text{H}_{29}\text{NNaO}_9\text{S}$ ($\text{M}+\text{Na}$) $^+$: Calcd, 470.1455; Found, 470.1453. $\text{C}_{19}\text{H}_{29}\text{KNO}_9\text{S}$ ($\text{M}+\text{K}$) $^+$: Calcd, 486.1195; Found, 486.1191.

[00267] **Compound 33 and 34**: *N*-(2-Cyclohexylacetyl)-1,3,4,6-tetra-*O*-acetyl- α -D-5-thio-glucosamine (**33**) and *N*-(2-cyclohexylacetyl)- α -D-5-thio-glucosamine (**34**)

[00268] The title compounds were prepared from the material thus obtained following General Procedures D and E.

[00269] For **33**: $^1\text{H-NMR}$ (600 M Hz, CDCl_3): δ 5.95 (d, $J = 3.00$ Hz, 1 H), 5.68 (d, $J = 9.00$ Hz, 1 H), 5.37 (t, $J = 10.2$ Hz, 1 H), 5.18 (t, $J = 10.2$ Hz, 1 H), 4.66-4.60 (m, 1 H), 4.35 (dd, $J = 12.0, 4.80$ Hz, 1 H), 4.04 (dd, $J = 12.0, 3.00$ Hz, 1 H), 3.47 (ddd, $J = 10.8, 4.80, 3.00$ Hz, 1 H), 2.17 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 1.99 (dd, $J = 13.8, 7.20$ Hz, 1 H), 1.92 (dd, $J = 13.8, 7.20$ Hz, 1 H), 1.74-1.60 (m, 6 H), 1.28-1.20 (m, 2 H), 1.19-1.11 (m, 1 H), 0.88-0.81 (m, 2 H). $^{13}\text{C-NMR}$ (150 M Hz, CDCl_3): δ 171.81, 171.66, 170.54, 169.12, 168.61, 72.66, 71.54, 71.50, 61.09, 55.06, 44.56, 39.68, 35.20, 32.85, 32.79, 26.02, 25.91, 21.02, 20.67, 20.61, 20.50. HRMS (m/z): $\text{C}_{22}\text{H}_{34}\text{NO}_9\text{S}$ ($\text{M}+\text{H}$) $^+$: Calcd, 488.1949; Found, 488.1951. $\text{C}_{22}\text{H}_{37}\text{N}_2\text{O}_9\text{S}$ ($\text{M}+\text{NH}_4$) $^+$: Calcd, 505.2214; Found, 505.2222. $\text{C}_{22}\text{H}_{33}\text{NNaO}_9\text{S}$ ($\text{M}+\text{Na}$) $^+$: Calcd,

510.1768; Found, 510.1765. $C_{22}H_{33}KNO_5S$ (M+K)⁺: Calcd, 526.1508; Found, 526.1456.

[00270] For **34**: ¹H-NMR (600 M Hz, MeOD): δ 4.88 (d, J = 2.40 Hz, 1 H), 4.10 (dd, J = 10.8, 3.00 Hz, 1 H), 3.89 (dd, J = 11.4, 3.60 Hz, 1 H), 3.82 (dd, J = 11.4, 6.00 Hz, 1 H), 3.67 (dd, J = 10.8, 9.0 Hz, 1 H), 3.57 (dd, J = 10.2, 9.0 Hz, 1 H), 3.26 (ddd, J = 10.2, 6.00, 4.20 Hz, 1 H), 2.11 (d, J = 7.20 Hz, 2 H), 1.80-1.68 (m, 5 H), 1.68-1.64 (m, 1 H), 1.32-1.25 (m, 2 H), 1.22-1.12 (m, 1 H), 1.03-0.96 (m, 2 H). ¹³C-NMR (150 M Hz, MeOD): δ 175.57, 76.98, 73.65, 73.38, 62.69, 59.88, 45.11, 44.90, 36.89, 34.24, 27.42, 27.33, 27.32. HRMS (m/z): $C_{14}H_{26}NO_5S$ (M+H)⁺: Calcd, 320.1526; Found, 320.1528. $C_{14}H_{25}NNaO_5S$ (M+Na)⁺: Calcd, 342.1346; Found, 342.1347. $C_{14}H_{25}KNO_5S$ (M+K)⁺: Calcd, 358.1085; Found, 358.1042.

[00271] **Compound 36 and 35**: *N*-(1-Naphthylacetyl)- α -D-5-thio-glucosamine (**36**) and *N*-(1-naphthylacetyl)-1,3,4,6-tetra-O-acetyl- α -D-5-thio-glucosamine (**35**)

[00272] The title compounds were prepared from the material thus obtained following General Procedures A and B. For **36**: ¹H-NMR (400 M Hz, Acetone-D-6 with some drops of MeOD): δ 8.11 (d, J = 8.00 Hz, 1 H), 7.86 (d, J = 8.00 Hz, 1 H), 7.78 (d, J = 8.00 Hz, 1 H), 7.52-7.38 (m, 4 H), 4.87 (d, J = 2.40 Hz, 1 H), 4.12 (dd, J = 10.0, 2.40 Hz, 1 H), 4.05 (d, J = 2.80 Hz, 2 H), 3.86-3.74 (m, 2 H), 3.66 (t, J = 9.60 Hz, 1 H), 3.58 (t, J = 9.60 Hz, 1 H), 3.23-3.19 (m, 1 H). ¹³C-NMR (100 M Hz, Acetone-D-6 with some drops of MeOD): δ 173.45, 135.90, 134.40, 134.26, 130.35, 129.73, 129.37, 127.98, 127.53, 127.38, 126.12, 78.00, 74.28, 74.22, 63.63, 60.42, 45.45, 42.13. HRMS (m/z): $C_{18}H_{22}NO_5S$ (M+H)⁺: Calcd, 364.1213; Found, 364.1220. $C_{18}H_{21}NNaO_5S$ (M+Na)⁺: Calcd, 386.1033; Found, 386.1035.

[00273] For **35**: ¹H-NMR (400 M Hz, CDCl₃): δ 7.90-7.80 (m, 3 H), 7.56-7.51 (m, 2 H), 7.47 (t, J = 7.60 Hz, 1 H), 7.30 (d, J = 6.80 Hz, 1 H), 5.83 (d, J = 3.20 Hz, 1 H), 5.54 (d, J = 8.40 Hz, 1 H), 5.25 (t, J = 10.4 Hz, 1 H), 4.82 (t, J = 10.4 Hz, 1 H), 4.52-4.47 (m, 1 H), 4.26 (dd, J = 12.0, 4.80 Hz, 1 H), 4.00-3.90 (m, 3 H), 3.28 (ddd, J = 10.8, 4.80, 3.20 Hz, 1 H), 2.02 (s, 3 H), 1.94 (s, 3 H), 1.67 (s, 3 H), 1.66 (s, 3 H). ¹³C-NMR (100 M Hz, CDCl₃): δ 170.79, 170.45, 170.44, 169.03, 168.17, 133.92, 131.77, 130.25, 128.80, 128.69, 128.34, 127.11, 126.34, 125.66, 123.33, 71.95, 71.28, 70.97, 60.99, 55.30, 41.59, 39.57, 20.55, 20.37, 20.33, 20.03. HRMS (m/z):

$C_{26}H_{30}NO_9S$ (M+H)⁺: Calcd, 532.1636; Found, 532.1633. $C_{26}H_{33}N_2O_9S$ (M+NH₄)⁺: Calcd, 549.1901; Found, 549.1887. $C_{26}H_{29}NNaO_9S$ (M+Na)⁺: Calcd, 554.1455; Found, 554.1450. $C_{26}H_{29}KNO_5S$ (M+K)⁺: Calcd, 570.1195; Found, 570.1200.

[00274] **Compound 37 and 38:** *N*-[(*S*)-methylvaleryl]-1,3,4,6-tetra-*O*-acetyl-
5 *α*-*D*-5-thio-glucosamine (37) and *N*-[(*S*)-2-methylvaleryl]-*α*-*D*-5-thio-glucosamine (38)

[00275] The title compounds were prepared from the material thus obtained following General Procedures C and E. For **37**: ¹H-NMR (400 M Hz, CDCl₃): δ 5.96 (d, *J* = 3.20 Hz, 1 H), 5.71 (d, *J* = 8.40 Hz, 1 H), 5.38 (t, *J* = 10.8 Hz, 1 H), 5.19 (t, *J* =
10 10.8 Hz, 1 H), 4.67-4.61 (m, 1 H), 4.35 (dd, *J* = 12.0, 5.20 Hz, 1 H), 4.04 (dd, *J* = 12.0, 3.20 Hz, 1 H), 3.46 (ddd, *J* = 10.8, 4.80, 3.20 Hz, 1 H), 2.17 (s, 3 H), 2.15-2.12 (m, 2 H), 2.07 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 1.75-1.68 (m, 2 H), 1.33-1.23 (m, 1 H), 1.21-1.10 (m, 1 H), 0.88-0.83 (m, 6 H). ¹³C-NMR (100 M Hz, CDCl₃): δ 172.08, 171.68, 170.54, 169.12, 168.62, 72.67, 71.59, 71.55, 61.11, 55.09, 43.86, 39.72,
15 32.10, 29.13, 21.02, 20.70, 20.61, 20.50, 18.92, 11.17. HRMS (*m/z*): $C_{20}H_{32}NO_9S$ (M+H)⁺: Calcd, 462.1792; Found, 462.1793. $C_{20}H_{35}N_2O_9S$ (M+NH₄)⁺: Calcd, 479.2058; Found, 479.2048. $C_{20}H_{31}NNaO_9S$ (M+Na)⁺: Calcd, 484.1612; Found, 484.1609. $C_{20}H_{31}KNO_5S$ (M+K)⁺: Calcd, 500.1351; Found, 500.1319.

[00276] For **38**: ¹H-NMR (600 M Hz, MeOD): δ 4.88 (d, *J* = 2.40 Hz, 1 H),
20 4.11 (dd, *J* = 10.2, 3.00 Hz, 1 H), 3.89 (dd, *J* = 11.4, 3.60 Hz, 1 H), 3.83 (dd, *J* = 11.4, 6.00 Hz, 1 H), 3.68 (dd, *J* = 10.2, 9.0 Hz, 1 H), 3.57 (dd, *J* = 10.2, 9.0 Hz, 1 H), 3.27 (ddd, *J* = 9.60, 6.00, 4.20 Hz, 1 H), 2.24 (dd, *J* = 13.2, 6.0 Hz, 1 H), 2.04 (dd, *J* = 13.2, 7.8 Hz, 1 H), 1.89-1.83 (m, 1 H), 1.44-1.37 (m, 1 H), 1.26-1.19 (m, 1 H), 0.94 (d, *J* = 6.60 Hz, 3 H), 0.92 (t, *J* = 7.20 Hz, 3 H). ¹³C-NMR (150 M Hz, MeOD): δ
25 175.83, 76.98, 73.62, 73.38, 62.68, 59.86, 44.90, 44.48, 33.75, 30.49, 19.54, 11.73. HRMS (*m/z*): $C_{12}H_{24}NO_5S$ (M+H)⁺: Calcd, 294.1370; Found, 294.1378. $C_{12}H_{23}NNaO_5S$ (M+Na)⁺: Calcd, 316.1189; Found, 316.1193. $C_{12}H_{23}KNO_5S$ (M+K)⁺: Calcd, 332.0929; Found, 332.0909.

[00277] **Compound 39 and 40:** *N*-(5-Methylhexonoyl)-1,3,4,6-tetra-*O*-acetyl-
30 *α*-*D*-5-thio-glucosamine (39) and *N*-(5-methylhexonoyl)-*α*-*D*-5-thio-glucosamine (40)

[00278] The title compounds were prepared from the material thus obtained following General Procedures C and E. For **39**: ^1H -NMR (400 M Hz, CDCl_3): δ 5.95 (d, $J = 2.80$ Hz, 1 H), 5.68 (d, $J = 8.80$ Hz, 1 H), 5.38 (t, $J = 10.4$ Hz, 1 H), 5.18 (t, $J = 10.4$ Hz, 1 H), 4.67-4.61 (m, 1 H), 4.35 (dd, $J = 12.0, 5.20$ Hz, 1 H), 4.04 (dd, $J = 12.0, 3.20$ Hz, 1 H), 3.48 (ddd, $J = 10.8, 4.80, 3.20$ Hz, 1 H), 2.18 (s, 3 H), 2.10-2.00 (m, 2 H), 2.07 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 1.56-1.48 (m, 3 H), 1.14-1.06 (m, 2 H), 0.86 (d, $J = 6.80$ Hz, 6 H). ^{13}C -NMR (100 M Hz, CDCl_3): δ 172.57, 171.65, 170.55, 169.12, 168.62, 72.72, 71.72, 71.50, 61.12, 55.07, 39.74, 38.26, 36.77, 27.78, 23.44, 22.41(2 C), 21.06, 20.67, 20.62, 20.50. HRMS (m/z): $\text{C}_{21}\text{H}_{34}\text{NO}_5\text{S}$ ($\text{M}+\text{H}$) $^+$: Calcd, 476.1949; Found, 476.1942. $\text{C}_{21}\text{H}_{37}\text{N}_2\text{O}_9\text{S}$ ($\text{M}+\text{NH}_4$) $^+$: Calcd, 493.2214; Found, 493.2189. $\text{C}_{21}\text{H}_{33}\text{NNaO}_9\text{S}$ ($\text{M}+\text{Na}$) $^+$: Calcd, 498.1768; Found, 498.1764. $\text{C}_{21}\text{H}_{33}\text{KNO}_5\text{S}$ ($\text{M}+\text{K}$) $^+$: Calcd, 514.1508; Found, 514.1484.

[00279] For **40**: ^1H -NMR (600 M Hz, MeOD): δ 4.88 (d, $J = 3.00$ Hz, 1 H), 4.10 (dd, $J = 10.2, 3.00$ Hz, 1 H), 3.89 (dd, $J = 11.4, 3.60$ Hz, 1 H), 3.82 (dd, $J = 11.4, 6.00$ Hz, 1 H), 3.67 (dd, $J = 10.2, 9.0$ Hz, 1 H), 3.58 (dd, $J = 10.2, 9.0$ Hz, 1 H), 3.26 (ddd, $J = 9.60, 6.00, 4.20$ Hz, 1 H), 2.22 (d, $J = 7.80$ Hz, 2 H), 1.65-1.60 (m, 2 H), 1.59-1.53 (m, 1 H), 1.25-1.21 (m, 2 H), 0.89 (t, $J = 6.60$ Hz, 6 H). ^{13}C -NMR (150 M Hz, MeOD): δ 176.36, 76.93, 73.60, 73.43, 62.68, 59.87, 44.90, 39.64, 37.34, 29.05, 24.92, 22.96. HRMS (m/z): $\text{C}_{13}\text{H}_{26}\text{NO}_5\text{S}$ ($\text{M}+\text{H}$) $^+$: Calcd, 308.1526; Found, 308.1528. $\text{C}_{13}\text{H}_{25}\text{NNaO}_5\text{S}$ ($\text{M}+\text{Na}$) $^+$: Calcd, 330.1346; Found, 330.1349. $\text{C}_{13}\text{H}_{25}\text{KNO}_5\text{S}$ ($\text{M}+\text{K}$) $^+$: Calcd, 346.1085; Found, 346.1049.

[00280] EXAMPLE XII: Characterization of Analogues

[00281] Analogues (**9**, **10**, **17-41**) were tested in COS-7 cells, dosed at a final concentration of 50 μM in DMSO for 24 h. Following treatment, cells were washed with PBS, harvested into SDS-PAGE loading buffer and analyzed by western blot using standard procedures; blots were probed with the anti-*O*-GlcNAc antibody (CTD110.6).

[00282] Compounds 17, 19, 21, 23, 25, 27, 28, 31, 33, 34, 35, 36, 37, 38, and 41 decreased *O*-GlcNAc levels in cells at a dose of 50 μM , with the hexyl (**27/28**) and 3-methyl pentyl (**37/38**) substituents showing the greatest promise at decreasing *O*-

GlcNAc levels to a comparable extent to the parent compound Ac-5SGlcNAc **9**.

Representative western blots are shown in **Figure 7**.

[00283] EXAMPLE XIII: Animal studies

[00284] Ac-5SGlcNAc **9** and the deprotected analogue with the hexyl
5 substituent at the C2 position **28** have been analyzed for effectiveness at decreasing *O*-
GlcNAc levels in mice. Pilot studies were conducted with mice dosed in triplicate
with either compound or vehicle alone. Compounds were dosed at 300 mg/kg
intraperitoneally using 75% DMSO as vehicle for delivery of **9** and phosphate-
buffered saline (PBS) for delivery of **28**. Mice were euthanized using a CO₂ chamber
10 after 16 hours exposure to either compound, and tissues (liver, lung, heart, kidney,
spleen, pancreas, brain, blood, fat and muscle) harvested and flash frozen in liquid
nitrogen. Frozen tissues were ground using a pestle and mortar, and resuspended in
cell lysis buffer (50 mM NaH₂PO₄, 150 mM NaCl, 0.1% SDS, 1% NP-40, 0.25%
sodium deoxycholate, 1 mM EDTA, 30 mM sodium fluoride, 30 mM β-
15 glycerophosphate, 5 mM sodium pyrophosphate, 1 mM Thiamet-G, protease inhibitor
cocktail (Roche)) at a concentration of 100 mg tissue/1 mL buffer prior to
homogenization. Homogenates were centrifuged at 13,000 rpm for 20 min, the
supernatant removed, added to 2x SDS-PAGE loading buffer and boiled for 10 min.
SDS-PAGE and western blots were performed using standard procedures. Blots were
20 probed using anti-*O*-GlcNAc antibody (CTD110.6, Santa Cruz Biotechnology) and
anti-actin antibody for a loading control.

[00285] Compounds **9** and **28** were both effective at decreasing *O*-GlcNAc
levels during the 16 hour dosing period in the liver, lung, heart, kidney, spleen,
pancreas, fat and muscle. Representative western blots are shown in **Figure 8**.

25 [00286] EXAMPLE XIV: Chronic lymphoid leukaemia (CLL) cells

[00287] Primary CLL cells, obtained from Dr. David Spaner at the University
of Toronto, were tested for their response to Ac-5SGlcNAc (**9**). Cells were plated at a
confluency of 1 million cells/mL, and treated with between 0-250 μM **9**, in the
presence or absence of cytokine (1000 U/mL IL-2 and 1 μg/mL Resiquimod)
30 stimulation to mimic cancer progression. Cells were observed over the course of 5

days, and harvested after this time into SDS-PAGE loading buffer before being subjected to western blotting.

[00288] At concentrations of 50 μ M Ac-5SGlcNAc and higher in stimulated cells, the proliferation that was obvious in untreated stimulated cells was absent. Ac-5SGlcNAc was not toxic to either unstimulated or stimulated cells, but instead appeared to prevent proliferation of stimulated cells. Western blots (**Figure 9**) showed that O-GlcNAc levels were decreased in a dose-dependent fashion.

[00289] EXAMPLE XV: Sialyl-lewis X (sLe^X) expression in HepG2 cells

[00290] 5-T-Fuc and/or Ac-5-T-Fuc were tested to determine whether these compounds would inhibit intracellular fucose transferases. A dose-dependent decrease in the level of the tetrasaccharide glycan sialyl-Lewis^X (sLe^X) was found in the hepatocyte cell line HepG2, indicating the use of this compound in treatment or prevention of inflammation.

[00291] More specifically, monolayers of Hep G2 cells were grown to about 75% confluency in DMEM containing 10% FBS before they were switched to serum free media for 10 h. FBS-free conditions were utilized to limit the amount of fucose which could be made available to cells upon degradation of serum glycoproteins. Cells were subsequently exposed to peracetylated 5-thio-L-fucose (5-T-Fuc) in serum free media for 48 h after which the media were collected, gently centrifuged (800rpm, 10min, 4°C) to remove dead cells, and concentrated (~10-fold) by centrifugal filtration (Amicon, NMWCO 3000 daltons). Cells were washed with cold PBS prior to harvesting them in PBS+ 0.5% SDS. Proteins were solubilised upon sonication (2 blasts, 10 sec, 15% power) and protein concentrations were determined for all samples by the DC protein concentration assay (BioRad). Samples were normalized prior to analysis. Identical amounts of proteins were analyzed for all lectin-blotting experiments. Cell extracts and conditioned media containing secreted proteins were all analyzed on 8% polyacrylamide gels (**Figure 10**).

[00292] 5-T-Fuc caused a significant, dose-dependent decrease in sLe^X expression on proteins obtained from treated cells (**Figure 10A**) though no decrease in *Aleura aurantia* lectin reactivity was observed (**Figure 10B**). This suggests that not all fucosylated antigens are affected by 5-T-Fuc. In contrast, there was dose-dependent

increase in *Maackia amurensis* lectin binding in 5-T-Fuc-treated cells (Figure 10C) which suggests that this inhibitor may allow for an increase in the rate of formation of sialylated oligosaccharides by limiting the activity of competing fucosyltransferases. Monolayers of HepG2 cells were exposed to 50µM 5-T-Fuc for varying lengths of time before they were harvested as described above. When necessary, fresh media and inhibitors were added after 24 h. Alternatively, cells were exposed to 5-T-Fuc for 24 h after which the media was replaced and cells were harvested at the indicated time-points (**Figure 11**).

[00293] CHO K1 cells exposed to 5-T-Fuc demonstrate a “switch-like” response in their expression of core-fucosylated antigens as assessed by lectin-blotting with the *Aleura aurantia* lectin: no further decrease in lectin reactivity was observed above inhibitor concentrations of 5 µM (**Figure 12**). Cells were exposed to 5-T-Fuc for 48 h prior to harvesting as described above.

[00294] 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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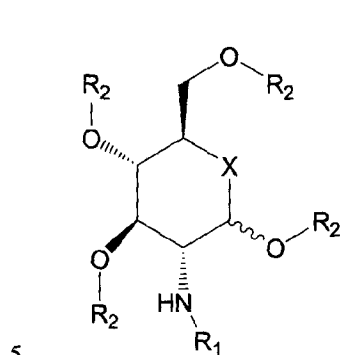
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10 prostate cancer cell trafficking. *Proc Natl Acad Sci U S A* 106, 19491-6 (2009).
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20 fucosyltransferase-IV and FucT-VII during leukocyte rolling in dermal microvessels. *Immunity* 12, 665-76 (2000).
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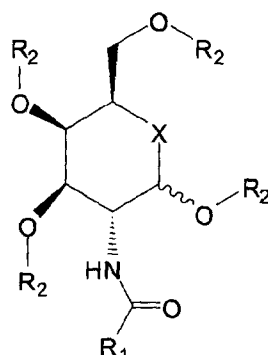
All citations are hereby incorporated by reference.

WHAT IS CLAIMED IS:

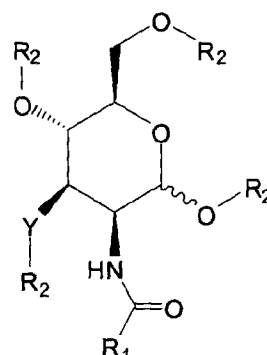
1. A method of inhibiting a glycosyltransferase (GT) in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of any one of Formula (I-X) or a pharmaceutically acceptable salt thereof:



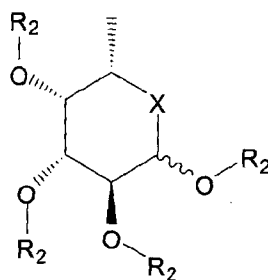
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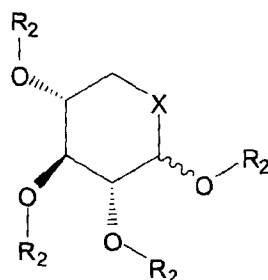
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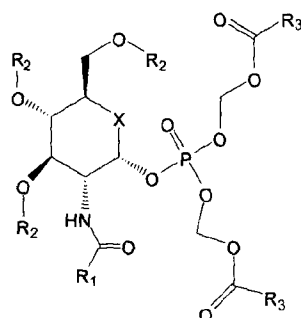
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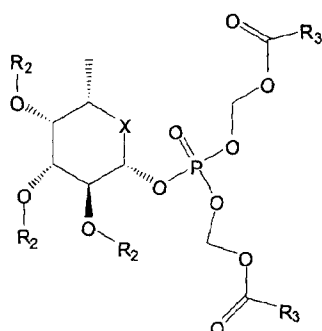
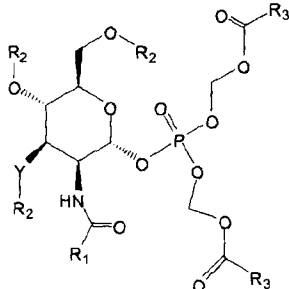
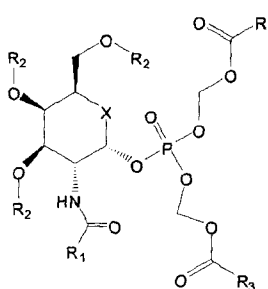
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(V)



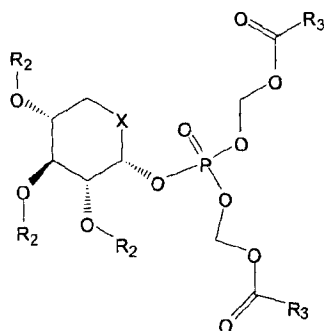
(VI)



(VII)

(VIII)

(IX)



(X)

wherein:

5 X where present is S, Se or CH₂;

Y where present is S or Se;

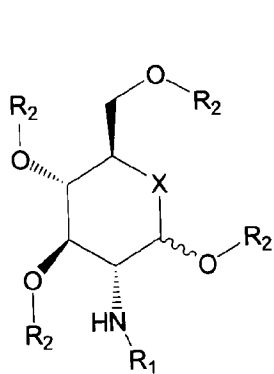
R₁ is either H or C(O)R₄, wherein R₄ is H, CH₂OH, CH₂N₃, CH₂SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted;

10 R₂ is H or C(O)R₅, wherein R₅ is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl;

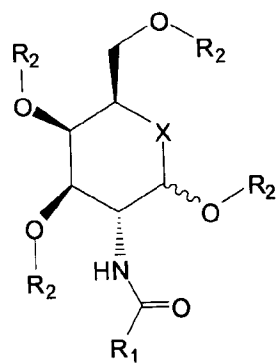
R₃ where present is H or C(O)R₆, wherein R₆ is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and

~~~~~ may be the alpha anomer, the beta anomer, or both.

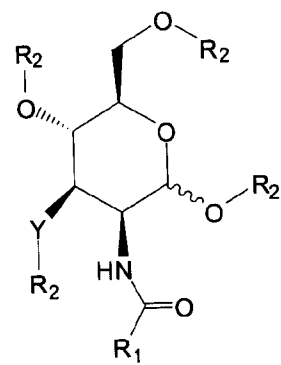
15 2. A method of reducing the level of a nucleotide sugar-modified biomolecule in a sample or in a subject in need thereof, the method comprising contacting the sample with, or administering to the subject, an effective amount of a compound of any one of Formula (I-X) or a pharmaceutically acceptable salt thereof:



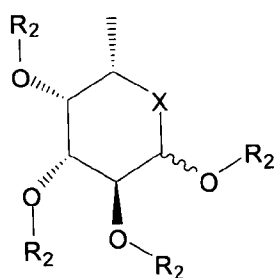
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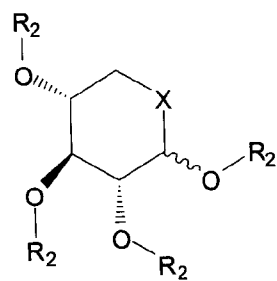
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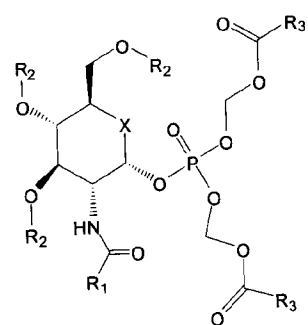
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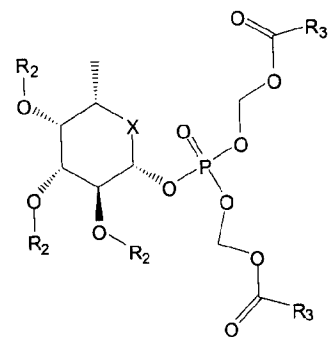
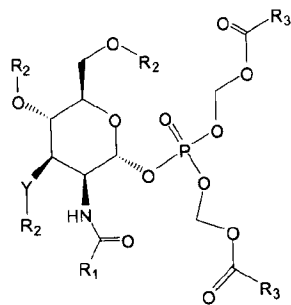
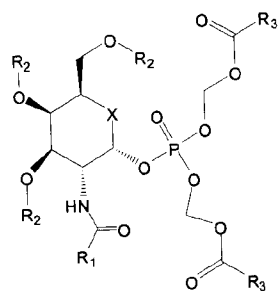
(IV)



(V)



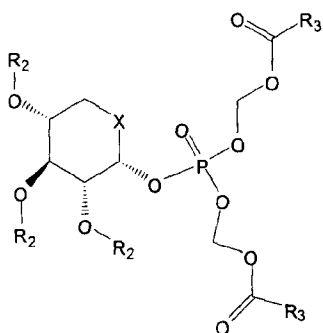
(VI)



(VII)

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(X)

wherein:

5 X where present is S, Se or CH<sub>2</sub>;

Y where present is S or Se;

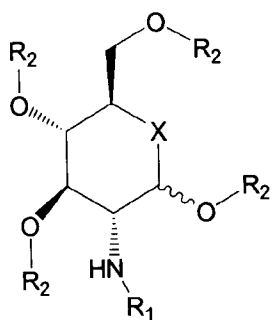
R<sub>1</sub> is either H or C(O)R<sub>4</sub>, wherein R<sub>4</sub> is H, CH<sub>2</sub>OH, CH<sub>2</sub>N<sub>3</sub>, CH<sub>2</sub>SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted;

10 R<sub>2</sub> is H or C(O)R<sub>5</sub>, wherein R<sub>5</sub> is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and

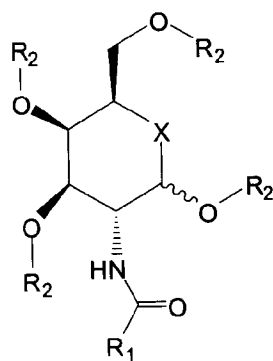
R<sub>3</sub> where present is H or C(O)R<sub>6</sub>, wherein R<sub>6</sub> is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and

~~~~ may be the alpha anomer, the beta anomer, or both. .

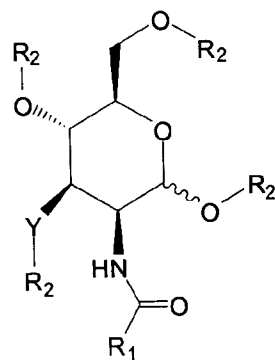
15 3. A method of treating a condition that is modulated by a GT in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of any one of Formula (I-X) or a pharmaceutically acceptable salt thereof:



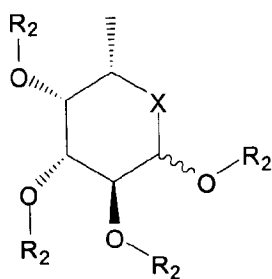
(I)



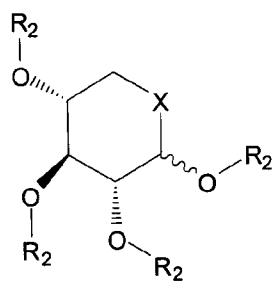
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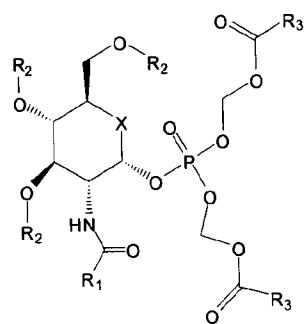
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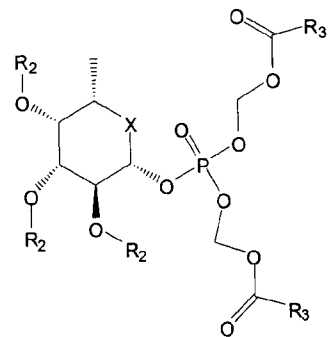
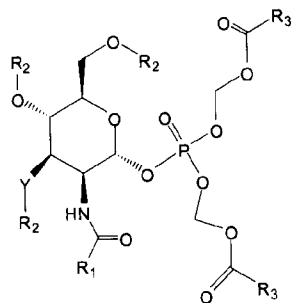
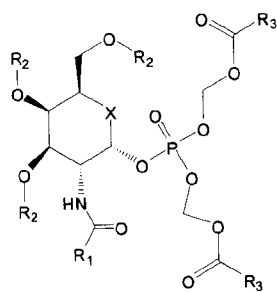
(IV)



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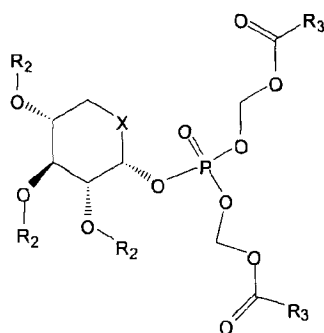
(VI)



(VII)

(VIII)

(IX)



(X)

wherein:

5 X where present is S, Se or CH₂;

Y where present is S or Se;

R₁ is either H or C(O)R₄, wherein R₄ is H, CH₂OH, CH₂N₃, CH₂SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted;

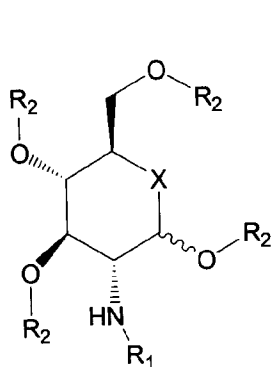
10 R₂ is H or C(O)R₅, wherein R₅ is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl;

R₃ where present is H or C(O)R₆, wherein R₆ is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and

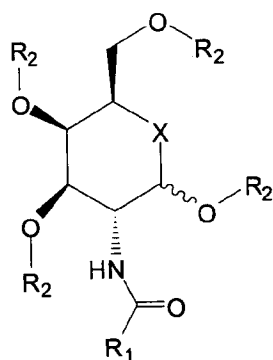
~~~~~ may be the alpha anomer, the beta anomer, or both.

15 4. The method of any one of claims 1 to 3 wherein the subject is a human.

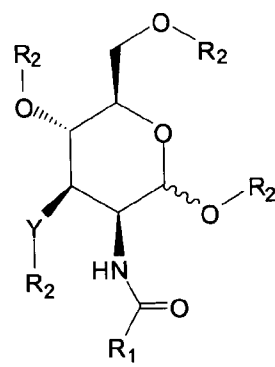
5. A method of inhibiting a GT in a cell or tissue, the method comprising administering to the cell or tissue an effective amount of a compound of any one of Formula (I-X) or a pharmaceutically acceptable salt thereof:



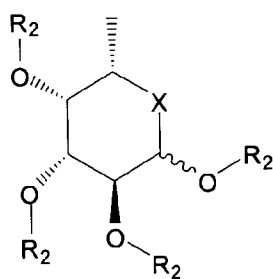
(I)



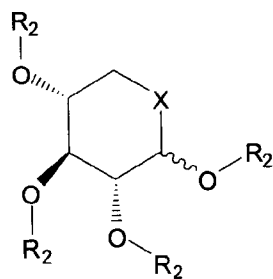
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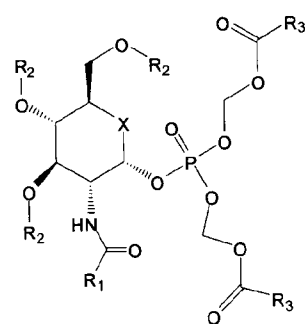
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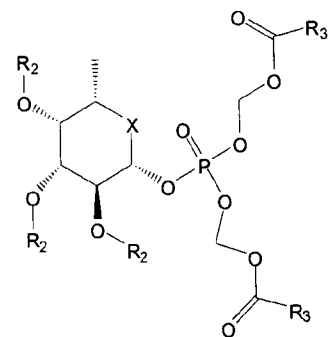
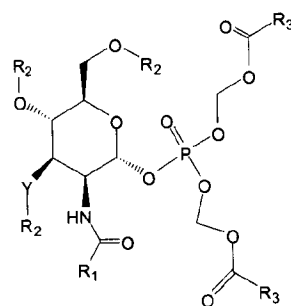
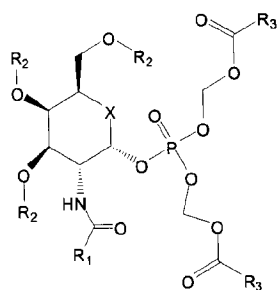
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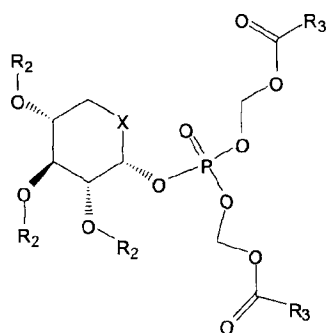


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(VII)

(VIII)

(IX)



(X)

wherein:

5 X where present is S, Se or CH<sub>2</sub>;

Y where present is S or Se;

R<sub>1</sub> is either H or C(O)R<sub>4</sub>, wherein R<sub>4</sub> is H, CH<sub>2</sub>OH, CH<sub>2</sub>N<sub>3</sub>, CH<sub>2</sub>SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted;

10 R<sub>2</sub> is H or C(O)R<sub>5</sub>, wherein R<sub>5</sub> is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl;

R<sub>3</sub> where present is H or C(O)R<sub>6</sub>, wherein R<sub>6</sub> is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and

~~~~~ is the alpha anomer, the beta anomer, or both.

15 6. The method of any one of claims 1 to 5 wherein the small alkyl is methyl, ethyl, propyl, butyl, isopropyl, isobutyl, valeryl, or isovaleryl.

7. The method of any one of claims 1 to 5 wherein the ether is O-methyl, O-ethyl, O-propyl, O-isopropyl, O-butyl, or O-isobutyl.

20 8. The method of any one of claims 1 to 5 wherein the thioether is S-methyl, S-ethyl, S-propyl, S-isopropyl, S-butyl, or S-isobutyl.

-)
9. The method of any one of claims 1 to 10 wherein one, two, three or four of R₂ is C(O)CH₃.
10. The method of any one of claims 1 to 9 wherein the GT is a fucosyltransferase (FUT), and the method comprises administering an effective amount of a compound
5 of Formula (IV) or (IX) or a pharmaceutically acceptable salt thereof.
11. The method of claim 2 wherein the GT is a fucosyltransferase (FUT), and the method comprises contacting the sample comprising an antibody with an effective amount of a compound of Formula (IV) or (IX) or a pharmaceutically acceptable salt thereof.
- 10 12. The method of claim 3 wherein the GT is a fucosyltransferase (FUT), and the method comprises administering to the subject an effective amount of a compound of Formula (IV) or (IX) or a pharmaceutically acceptable salt thereof.
13. The method of claim 12 wherein the condition is inflammation, an autoimmune disorder or cancer.
- 15 14. The method of claim 9 wherein the GT is a fucosyltransferase (FUT), and the method comprises contacting the cell or tissue with an effective amount of a compound of Formula (IV) or (IX) or a pharmaceutically acceptable salt thereof.
15. The method of any one of claims 10 to 14 wherein the compound is 5-T-Fuc.
16. The method of any one of claims 1 to 9 wherein the GT is uridine diphospho-
20 *N*-acetylglucosamine:polypeptide β -*N*-acetylglucosaminyltransferase (OGT), and the method comprises administering an effective amount of a compound of Formula (I) or (VI) or a pharmaceutically acceptable salt thereof:
17. The method of claim 3 wherein the GT is OGT, and the method comprises administering to the subject an effective amount of a compound of Formula (I) or (VI)
25 or a pharmaceutically acceptable salt thereof.
18. The method of claim 14 wherein the condition is cancer, diabetes, complications of diabetes, inflammation, or autoimmune disease.

19. The method of claim 5 wherein the GT is OGT and the method comprises contacting the cell or tissue with an effective amount of a compound of Formula (I) or (VI) or a pharmaceutically acceptable salt thereof.

20. The method of claim 19 wherein cell is a cancer cell or the tissue is pancreatic tissue.

21. The method of any one of claims 16 to 20 wherein the compound increases the level of the OGT.

22. The method of any one of claims 16 to 20 wherein the compound reduces the level of an *O*-GlcNAcase.

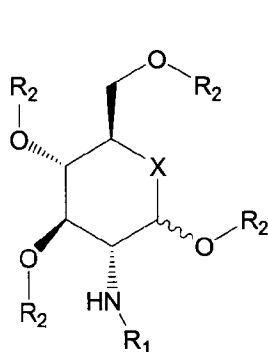
23. The method of any one of claims 16 to 22 wherein:

X is S,

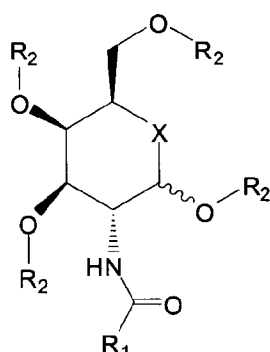
R₂ is H or C(O)CH₃, and

R₄ is H, CH₂N₃, CH₃, CH₂CH₃, (CH₂)₂CH₃, CH(CH₃)₂, (CH₂)₃CH₃, CH₂CH(CH₃)₂, (CH₂)₄CH₃, C(CH₃)₃, CH₂C₆H₁₁, CH₂C₁₀H₈, CH₂CH(CH₃)CH₂CH₃, or (CH₂)₃CH(CH₃)₂.

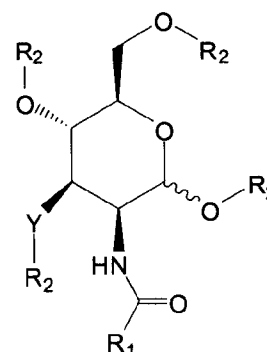
24. Use of a compound of Formula (I-X) or a pharmaceutically acceptable salt thereof:



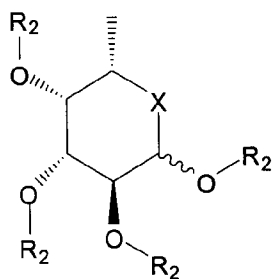
(I)



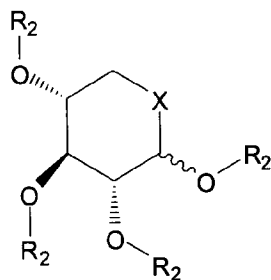
(II)



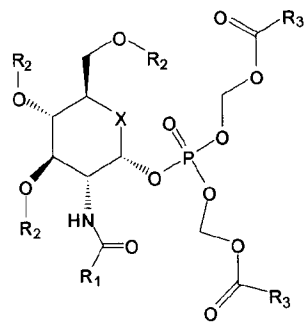
(III)



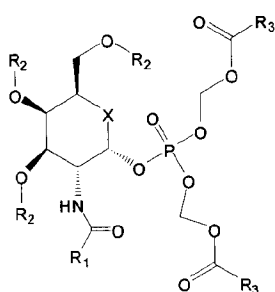
(IV)



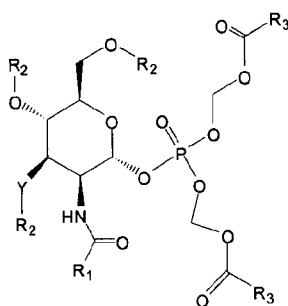
(V)



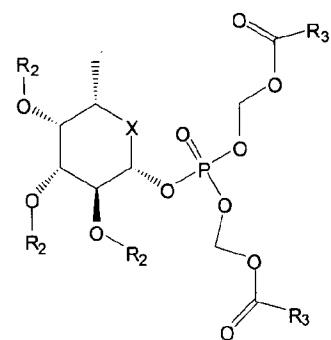
(VI)



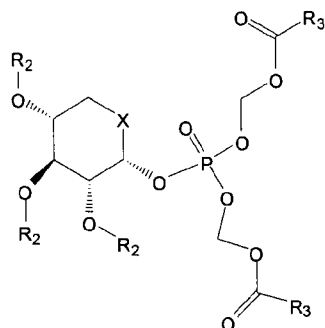
(VII)



(VIII)



(IX)



(X)

wherein:

X where present is S, Se or CH₂;

Y where present is S or Se;

- 10 R₁ is either H or C(O)R₄, wherein R₄ is H, CH₂OH, CH₂N₃, CH₂SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted;

R_2 is H or $C(O)R_5$, wherein R_5 is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl;

R_3 where present is H or $C(O)R_6$, wherein R_6 is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and

5 $\sim$ may be the alpha anomer, the beta anomer, or both,

for inhibiting a GT, reducing the level of a nucleotide sugar-modified biomolecule, or treating a condition that is modulated by a GT, in a subject in need thereof, or in a sample, cell or tissue.

25. The use of claim 24 wherein the small alkyl is methyl, ethyl, propyl, butyl,
10 isopropyl, isobutyl, valeryl, or isovaleryl.

26. The use of claim 24 wherein the ether is O-methyl, O-ethyl, O-propyl, O-isopropyl, O-butyl, or O-isobutyl.

27. The use of claim 24 wherein the thioether is S-methyl, S-ethyl, S-propyl, S-isopropyl, S-butyl, or S-isobutyl.

15 28. The use of any one of claims 24 to 27 wherein one, two, three or four of R_2 is $C(O)CH_3$.

29. The use of any one of claims 24 to 28 wherein the GT is a fucosyltransferase (FUT), and the compound is of Formula (IV) or (IX) or a pharmaceutically acceptable salt thereof.

20 30. The use of any one of claim 29 wherein the compound is 5-T-Fuc.

31. The use of any one of claims 24 to 28 wherein the GT is uridine diphospho-*N*-acetylglucosamine:polypeptide β -*N*-acetylglucosaminyltransferase (OGT), and the compound is of Formula (I) or (VI) or a pharmaceutically acceptable salt thereof:

32. The use of any one of claims 24 to 31 wherein the condition is cancer,
25 diabetes, complications of diabetes, inflammation, or autoimmune disease.

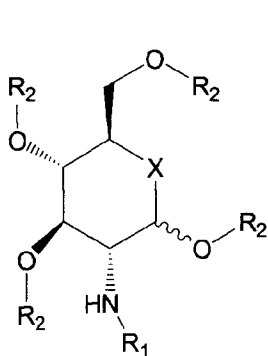
33. The use of any one of claims 24 to 32 wherein:

X is S,

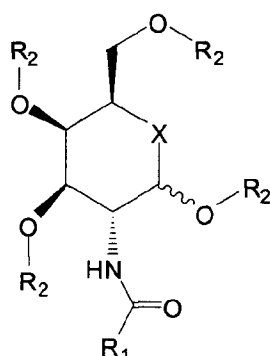
R₂ is H or C(O)CH₃, and

R₄ is H, CH₂N₃, CH₃, CH₂CH₃, (CH₂)₂CH₃, CH(CH₃)₂, (CH₂)₃CH₃,
CH₂CH(CH₃)₂, (CH₂)₄CH₃, C(CH₃)₃, CH₂C₆H₁₁, CH₂C₁₀H₈, CH₂CH(CH₃)CH₂CH₃, or
5 (CH₂)₃CH(CH₃)₂.

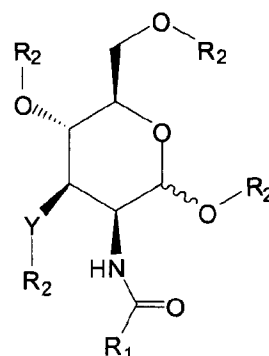
34. A pharmaceutical composition comprising a compound of Formula (I-X) or a pharmaceutically acceptable salt thereof:



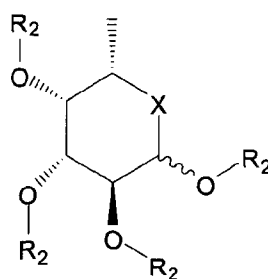
(I)



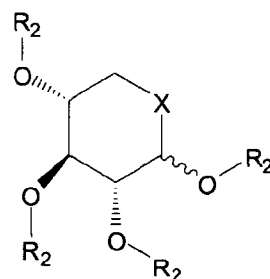
(II)



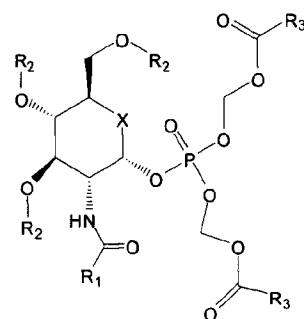
(III)



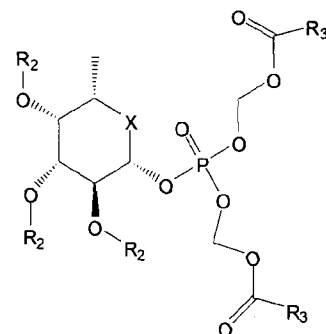
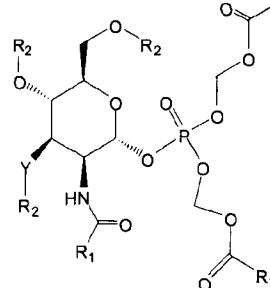
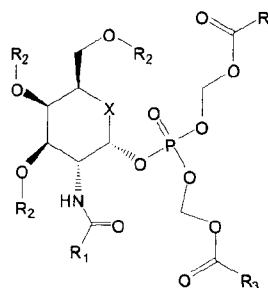
(IV)



(V)



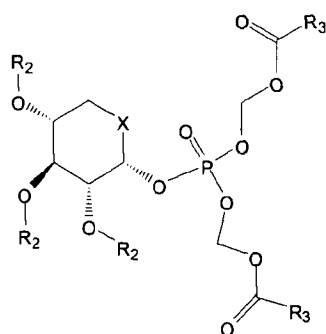
(VI)



(VII)

(VIII)

(IX)



(X)

wherein:

5 X where present is S, Se or CH_2 ;

Y where present is S or Se;

R_1 is either H or $\text{C}(\text{O})\text{R}_4$, wherein R_4 is H, CH_2OH , CH_2N_3 , CH_2SH , small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted;

10 R_2 is H or $\text{C}(\text{O})\text{R}_5$, wherein R_5 is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl;

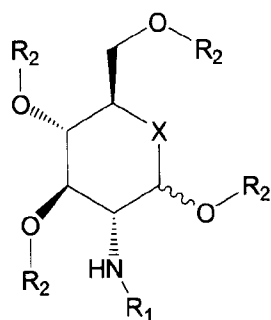
R_3 where present is H or $\text{C}(\text{O})\text{R}_6$, wherein R_6 is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and

~~~~~ may be the alpha anomer, the beta anomer, or both,

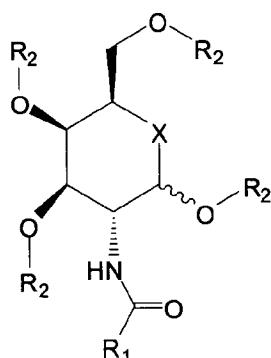
15 in combination with a pharmaceutically acceptable carrier,

wherein optionally said compound is not 5SGlcNAc, Ac-5SGlcNAc, 5CH<sub>2</sub>GlcNAc, UDP-5CH<sub>2</sub>GlcNAc, 5SGlcNAz, Ac-5SGlcNAz, or 5-T-Fuc.

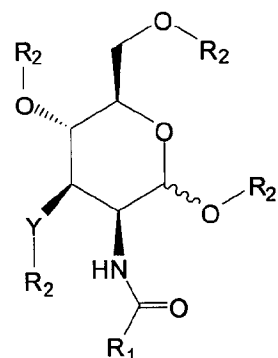
35. A compound of Formula (I-X) or a pharmaceutically acceptable salt thereof:



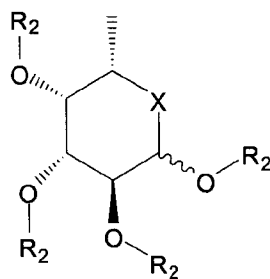
(I)



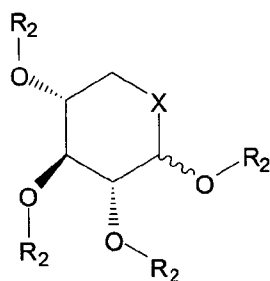
(II)



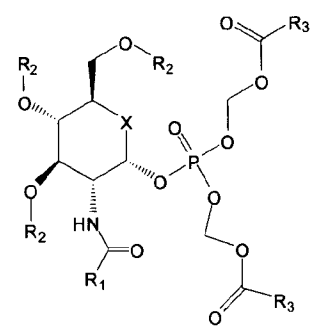
(III)



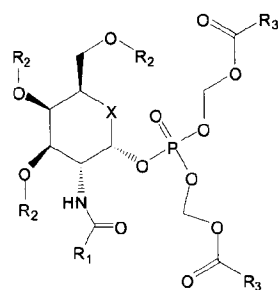
(IV)



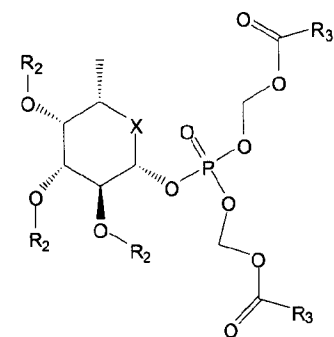
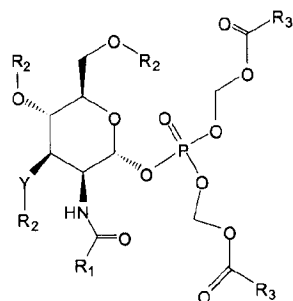
(V)



(VI)



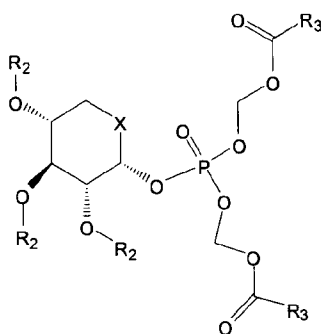
5



(VII)

(VIII)

(IX)



(X)

wherein:

5 X where present is S, Se or CH<sub>2</sub>;

Y where present is S or Se;

R<sub>1</sub> is either H or C(O)R<sub>4</sub>, wherein R<sub>4</sub> is H, CH<sub>2</sub>OH, CH<sub>2</sub>N<sub>3</sub>, CH<sub>2</sub>SH, small branched or unbranched alkyl, ether, thioether, urea, or thiourea, wherein the alkyl, ether, thioether, urea, or thiourea are optionally substituted;

10 R<sub>2</sub> is H or C(O)R<sub>5</sub>, wherein R<sub>5</sub> is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl;

R<sub>3</sub> where present is H or C(O)R<sub>6</sub>, wherein R<sub>6</sub> is optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; and

~~~~~ may be the alpha anomer, the beta anomer, or both,

15 wherein optionally said compound is not 5SGlcNAc, Ac-5SGlcNAc, 5CH₂GlcNAc, UDP-5CH₂GlcNAc, 5SGlcNAz, Ac-5SGlcNAz, or 5-T-Fuc.

36. The composition of claim 34 or the compound of claim 36 wherein the small alkyl is methyl, ethyl, propyl, butyl, isopropyl, isobutyl, valeryl, or isovaleryl.

37. The composition of claim 34 or the compound of claim 36 wherein the ether is
20 O-methyl, O-ethyl, O-propyl, O-isopropyl, O-butyl, or O-isobutyl.

38. The composition of claim 34 or the compound of claim 36 wherein the thioether is S-methyl, S-ethyl, S-propyl, S-isopropyl, S-butyl, or S-isobutyl.

39. The composition of claim 34 or the compound of claim 36 wherein one, two, three or four of R₂ is C(O)CH₃.

5 40. The composition of claim 34 or the compound of claim 36 wherein:

X is S,

R₂ is H or C(O)CH₃, and

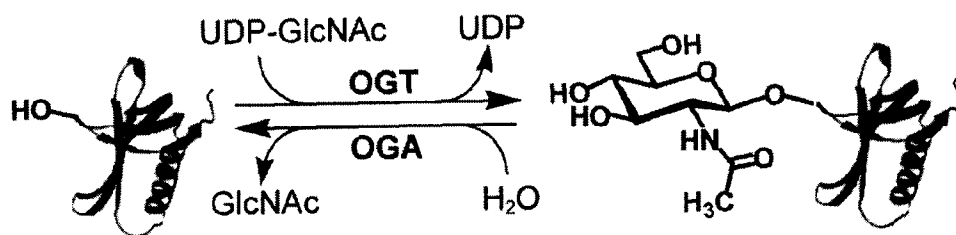
R₄ is H, CH₂N₃, CH₃, CH₂CH₃, (CH₂)₂CH₃, CH(CH₃)₂, (CH₂)₃CH₃,
CH₂CH(CH₃)₂, (CH₂)₄CH₃, C(CH₃)₃, CH₂C₆H₁₁, CH₂C₁₀H₈, CH₂CH(CH₃)CH₂CH₃, or
10 (CH₂)₃CH(CH₃)₂.

41. A kit comprising a composition according to claim 34 or the compound of claim 36 together with instructions for use in for inhibiting a GT, reducing the level of a nucleotide sugar-modified biomolecule, or treating a condition that is modulated by a GT,

15

V8

A.



B.

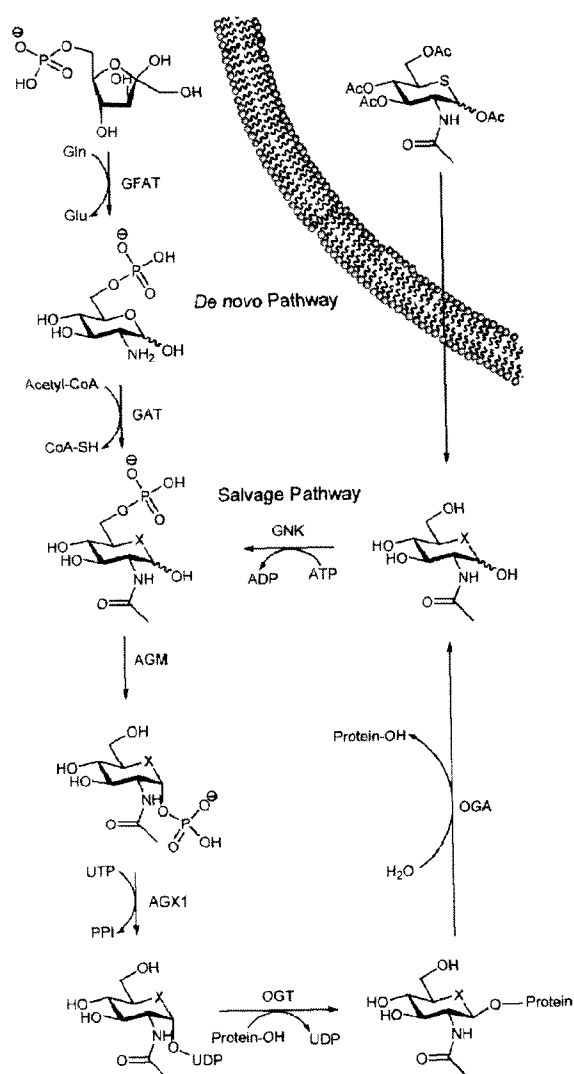
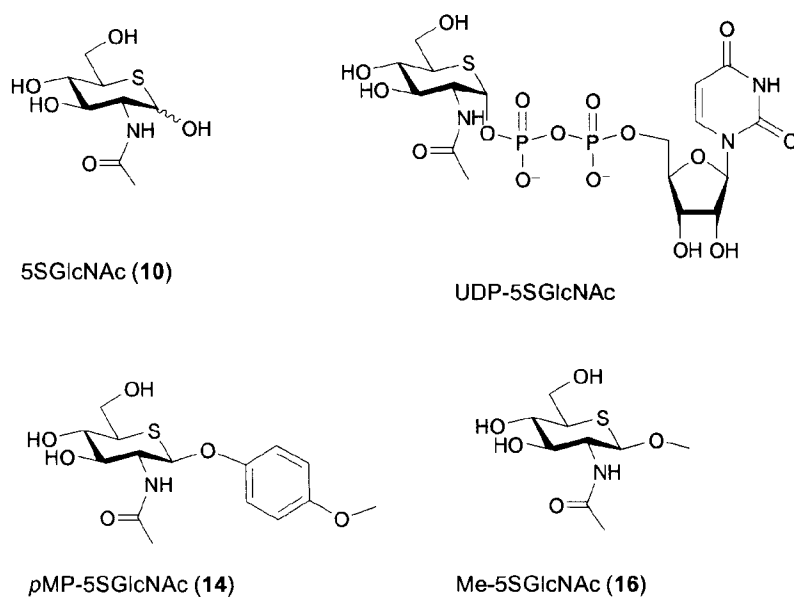


FIGURE 1

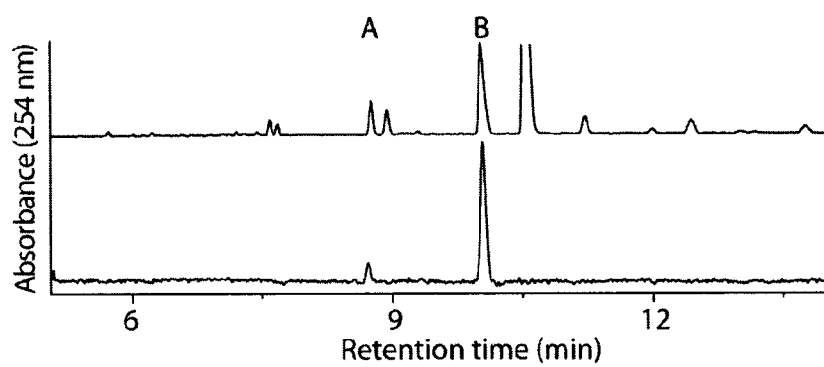
V

C.

**FIGURE 1**

V

D.



E.

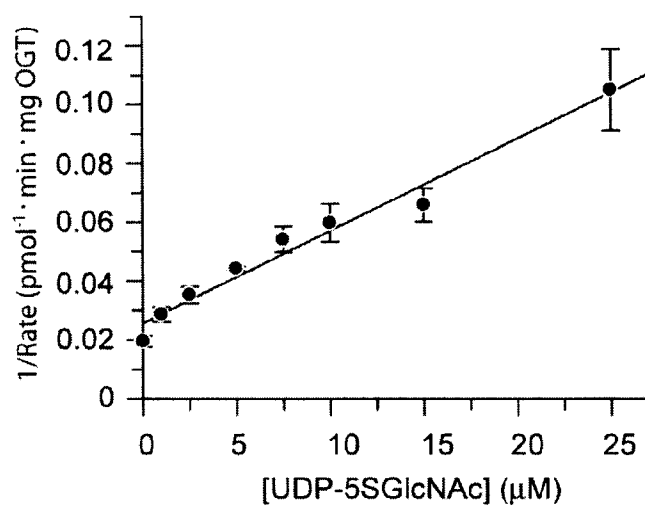
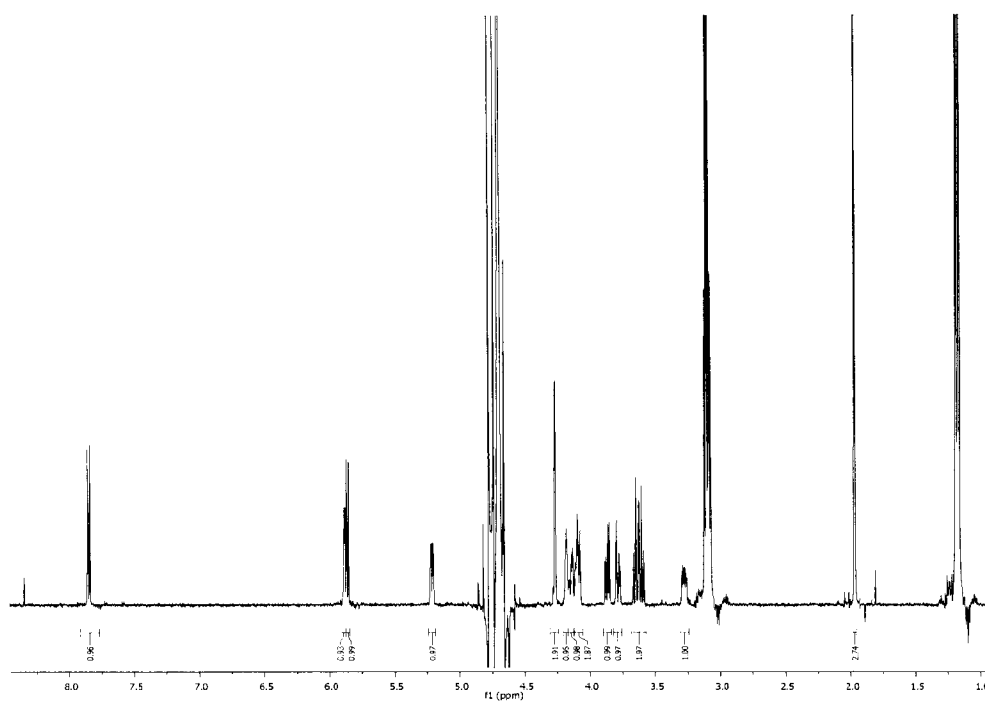


FIGURE 1

V

F.



G.

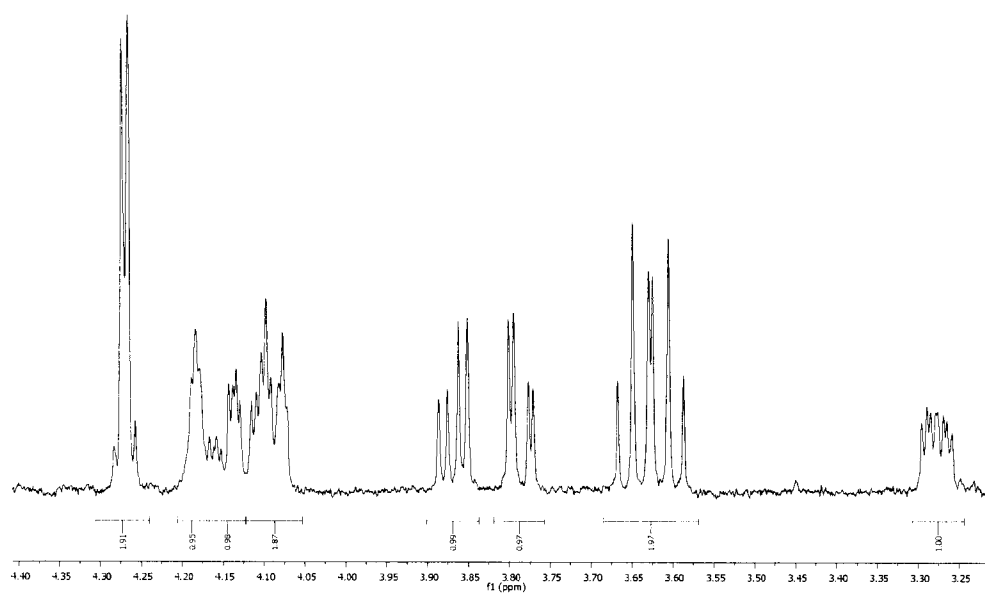
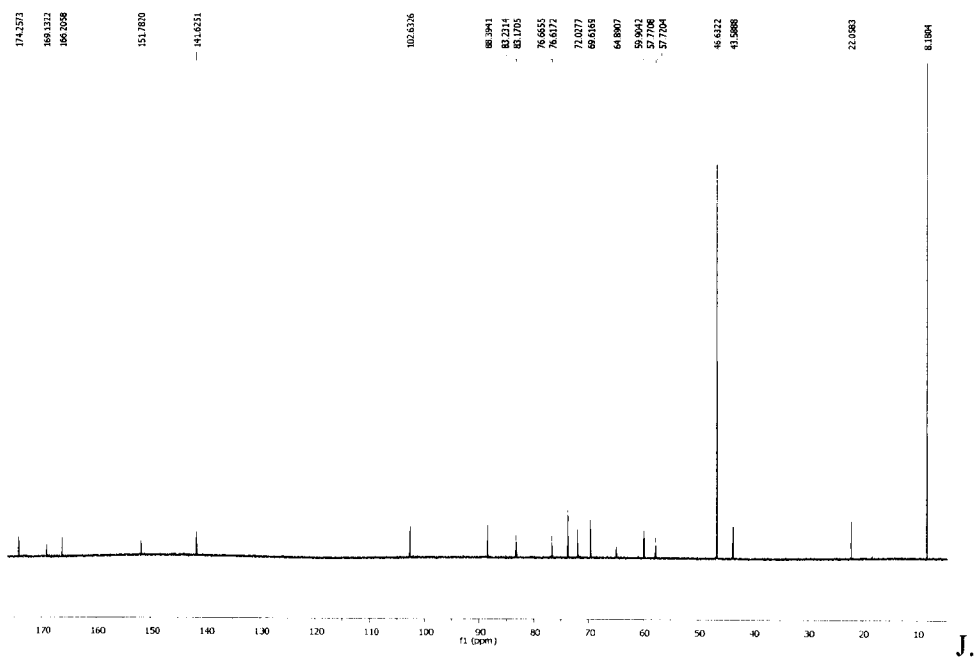


FIGURE 1

V

H.



I.

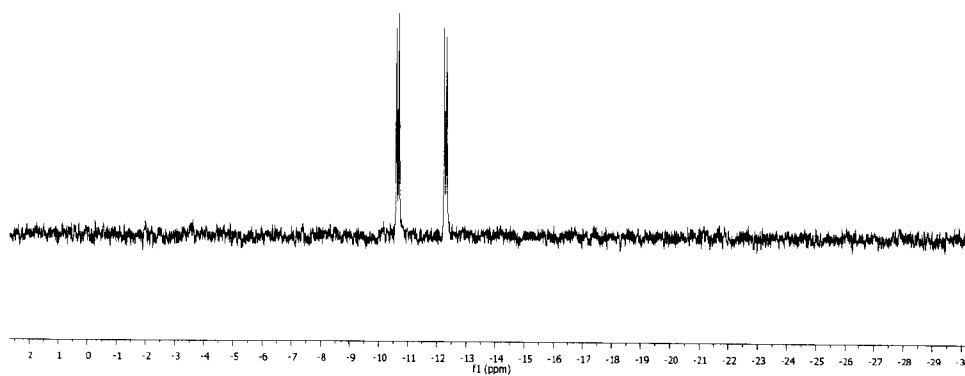
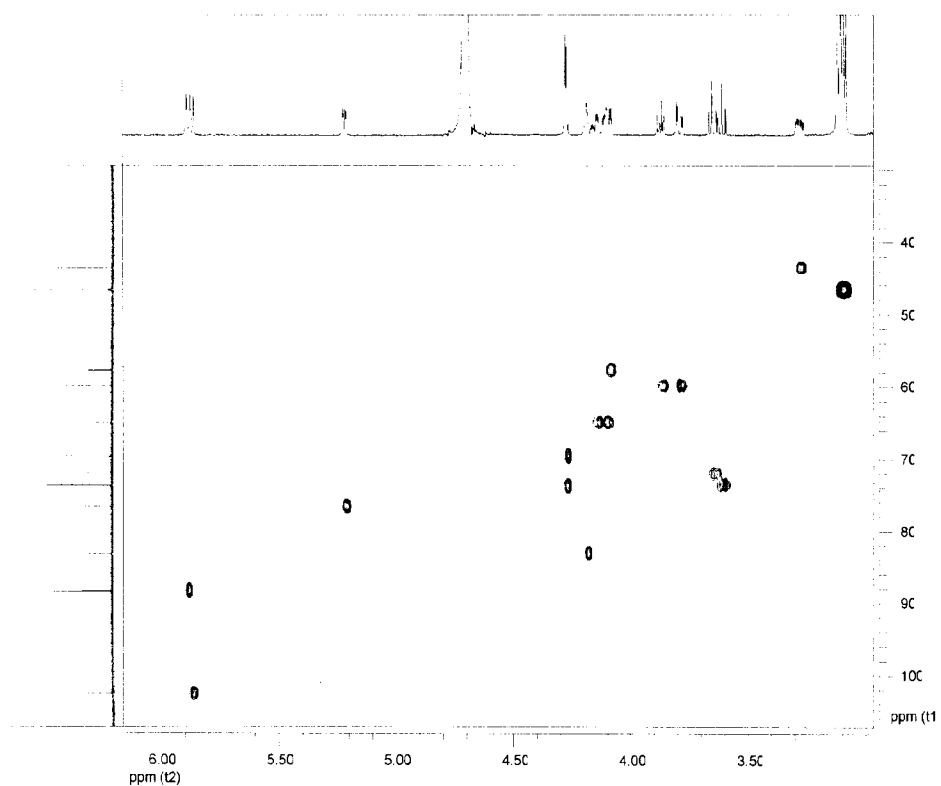


FIGURE 1

V

J.



K.

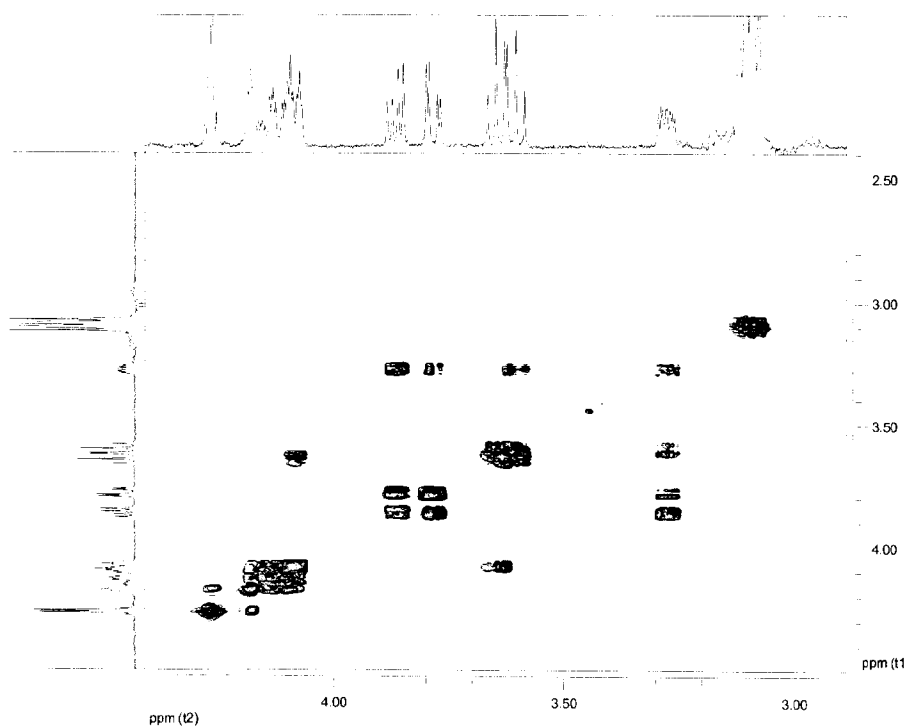


FIGURE 1

V{

A.

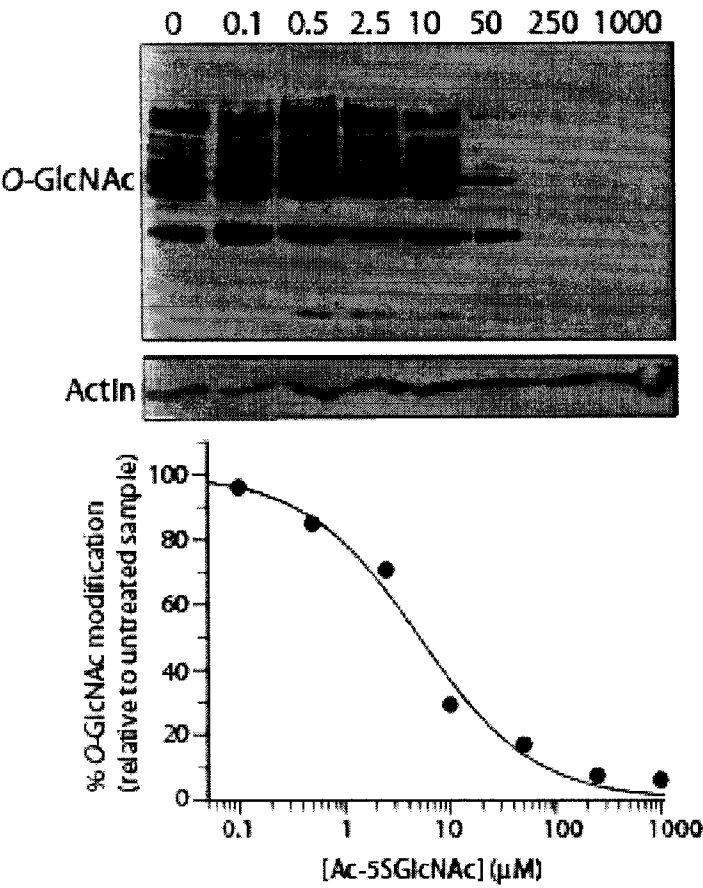


FIGURE 2

V8

B.

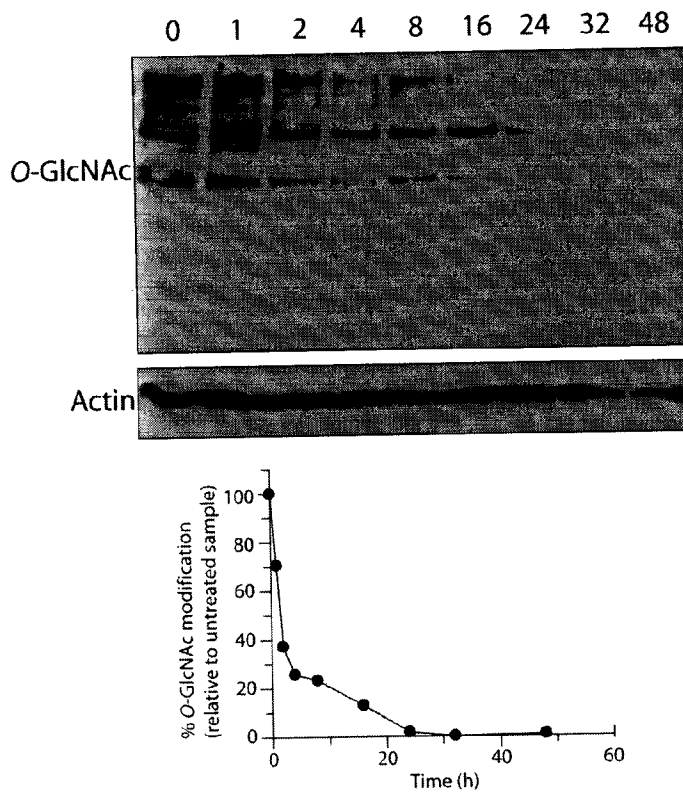
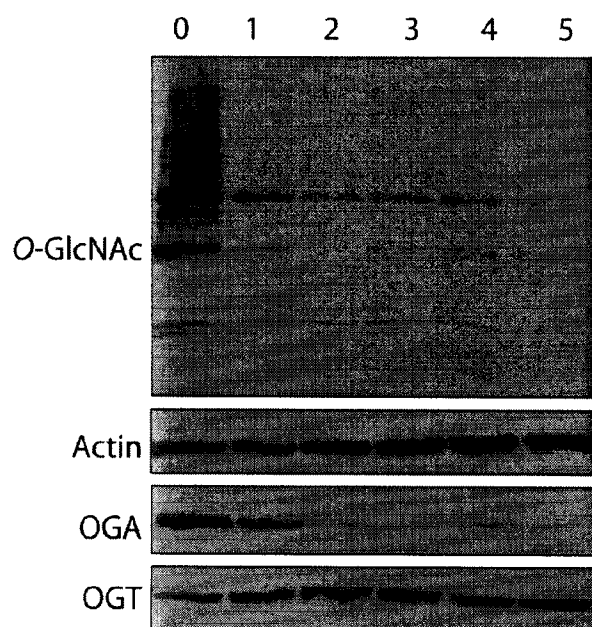


FIGURE 2

C.



D.

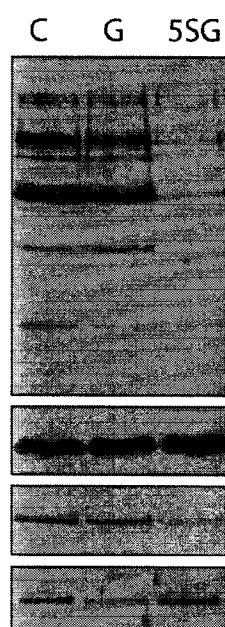
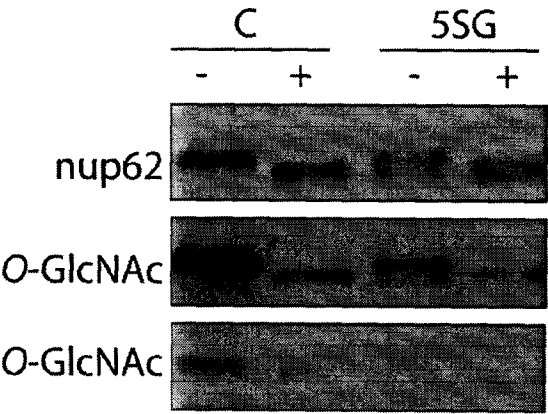


FIGURE 2

V

E.



F.

FIGURE 2

V{
G.

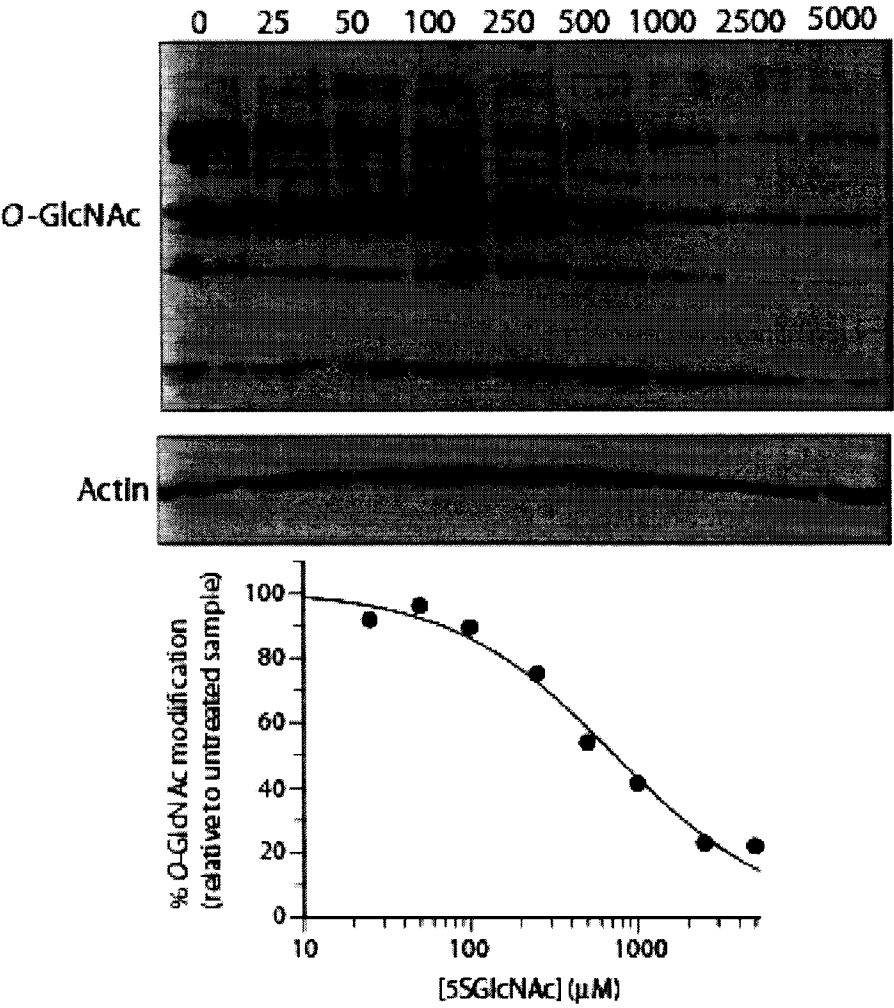
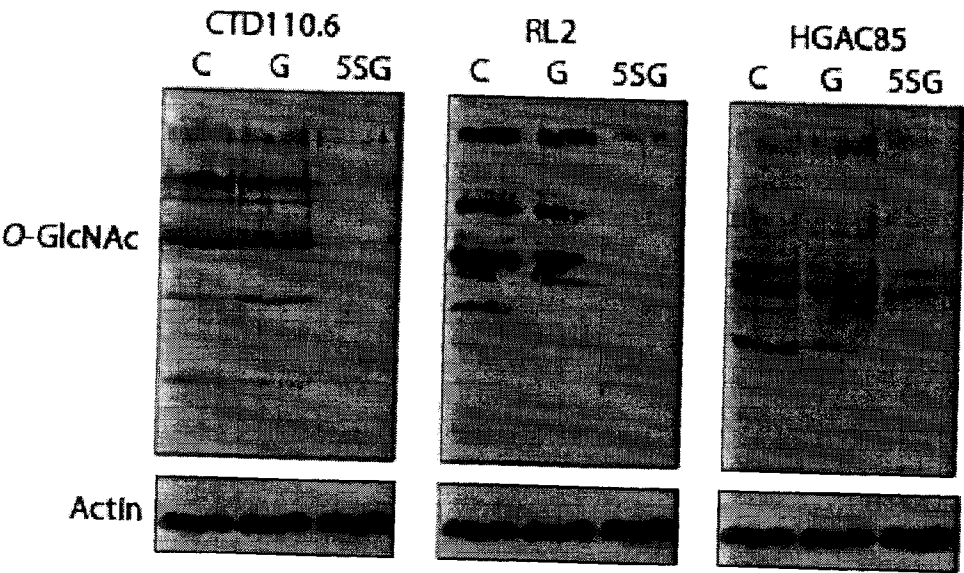


FIGURE 2

11.



I.

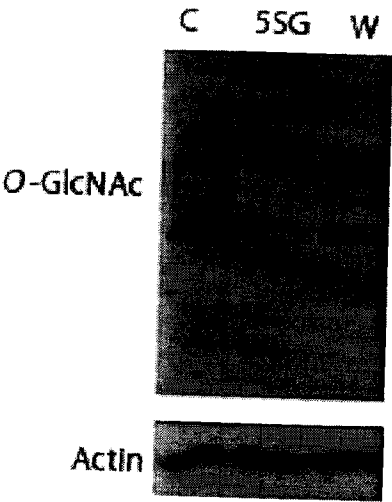


FIGURE 2

V

J.

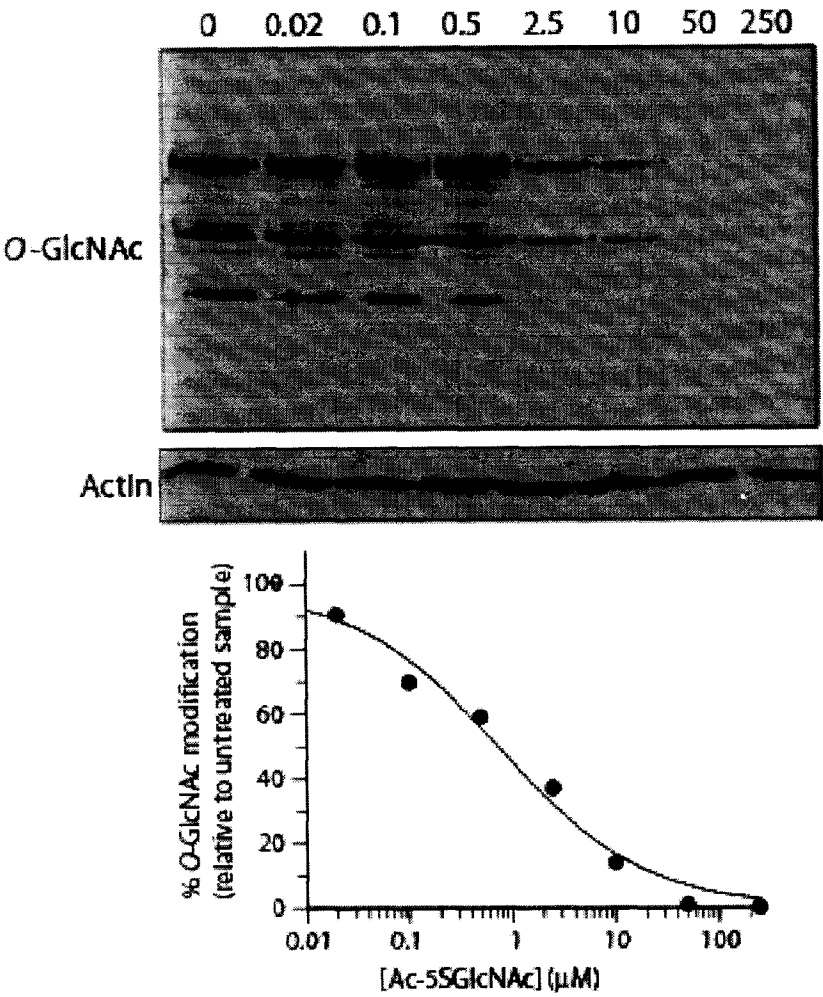


FIGURE 2

VI

K.

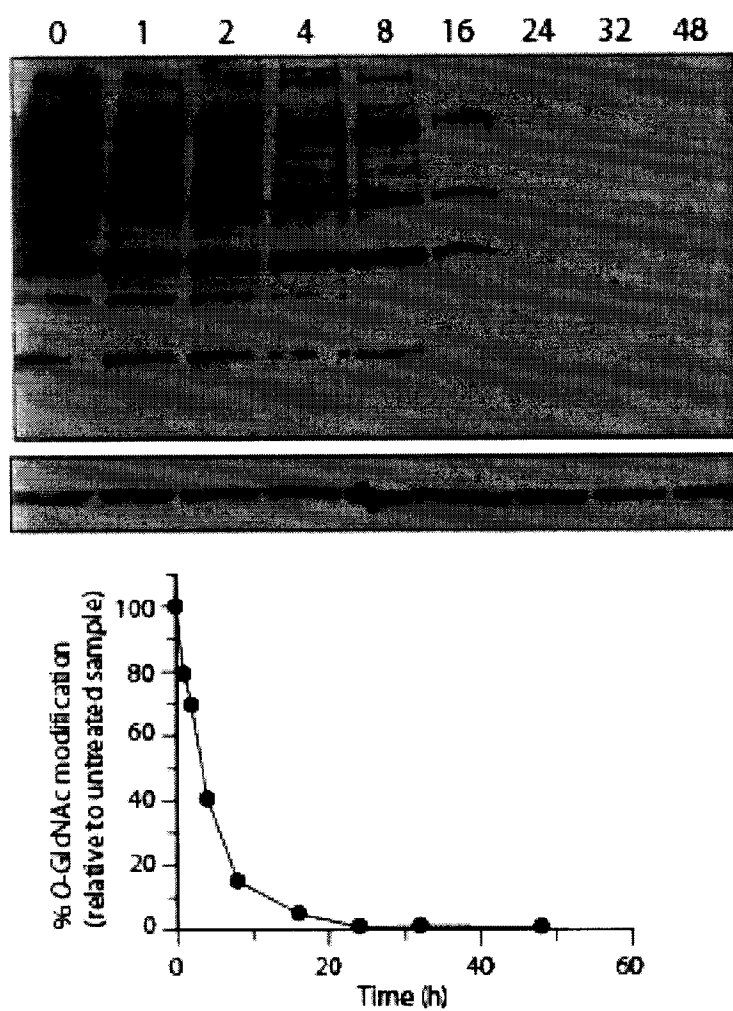


FIGURE 2

V

L.

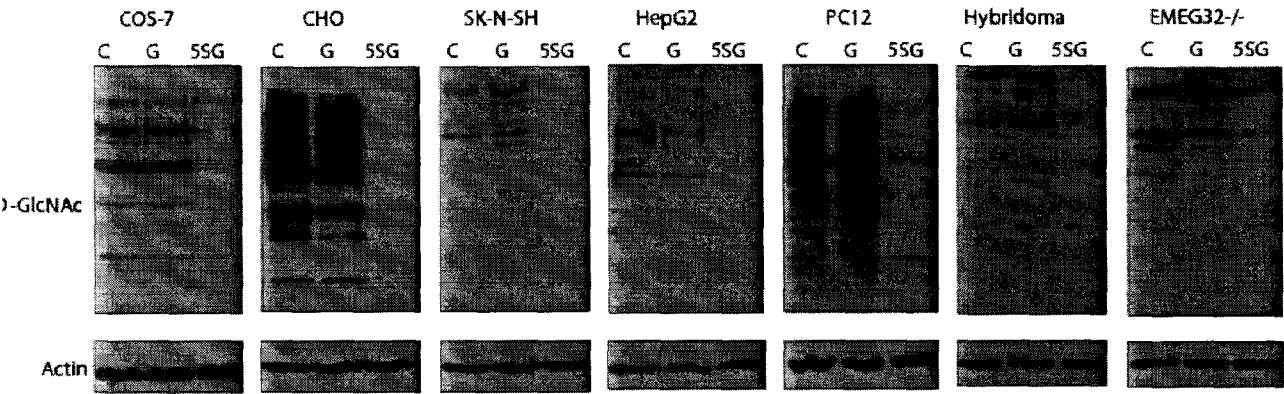
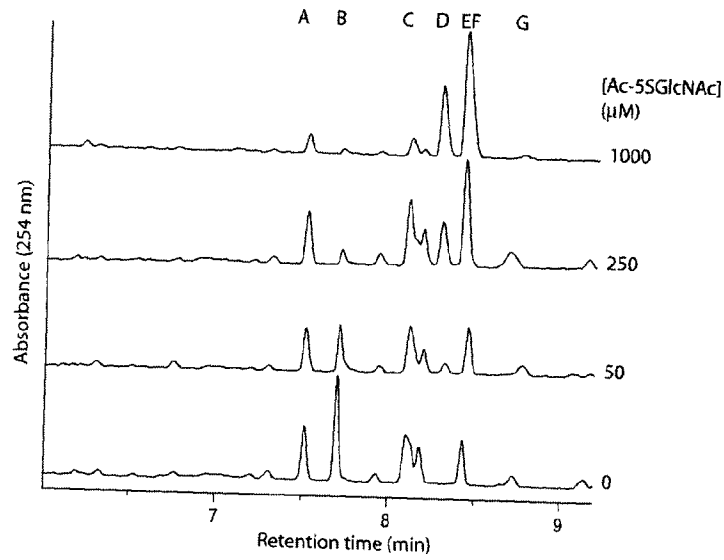


FIGURE 2

A.



B.

Fig 3b

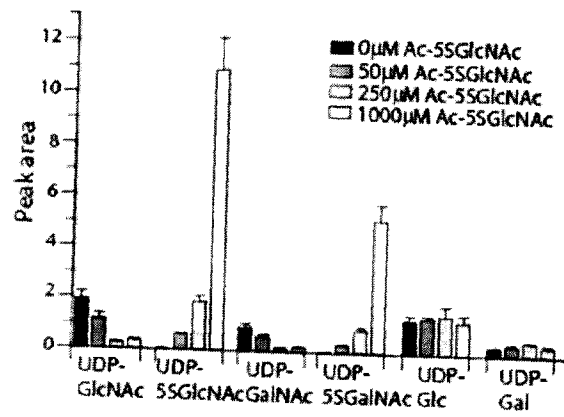
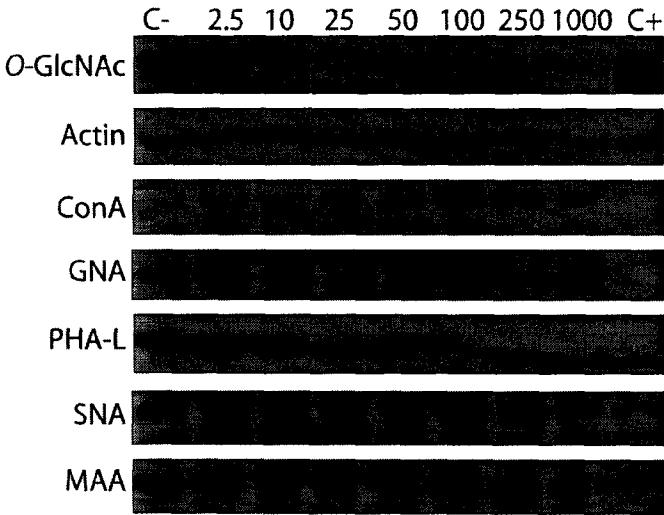


FIGURE 3

v

C.



D.

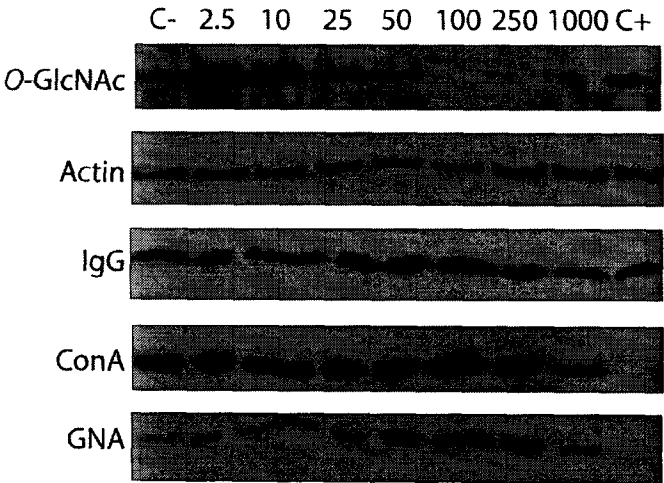
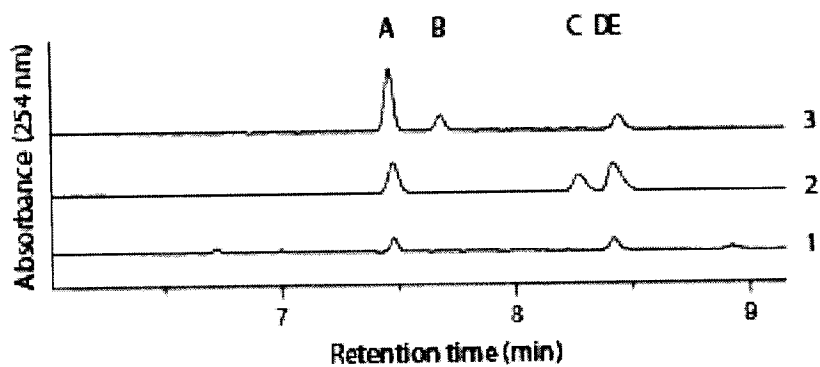


FIGURE 3

V:
E.



F.

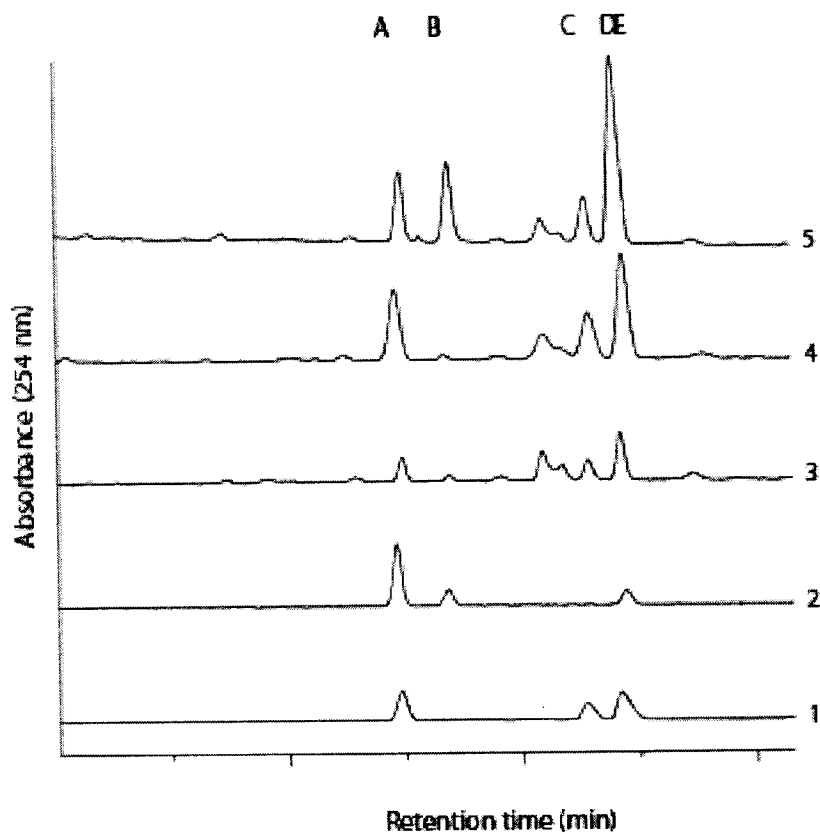


FIGURE 3

V

G.

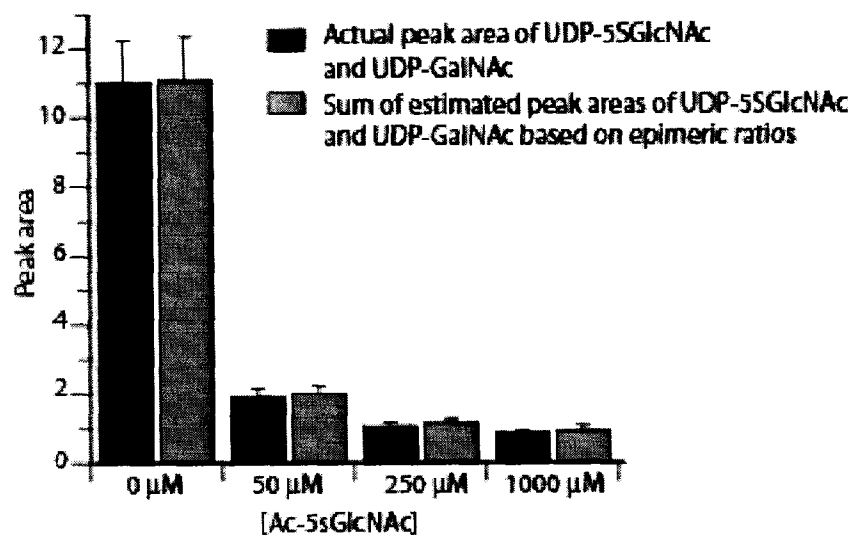


FIGURE 3

V:
H.

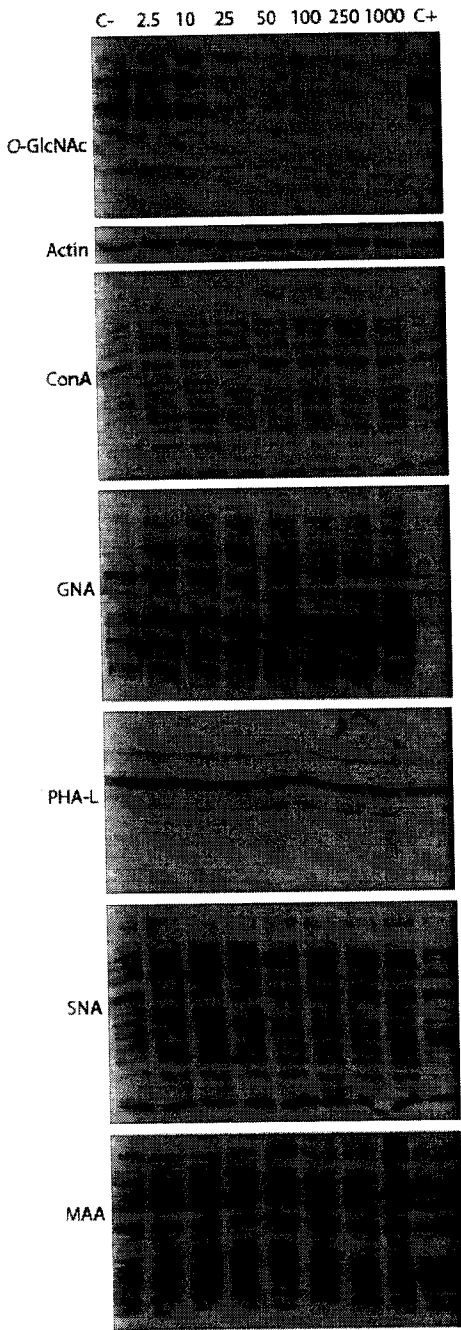


FIGURE 3

V:

I.

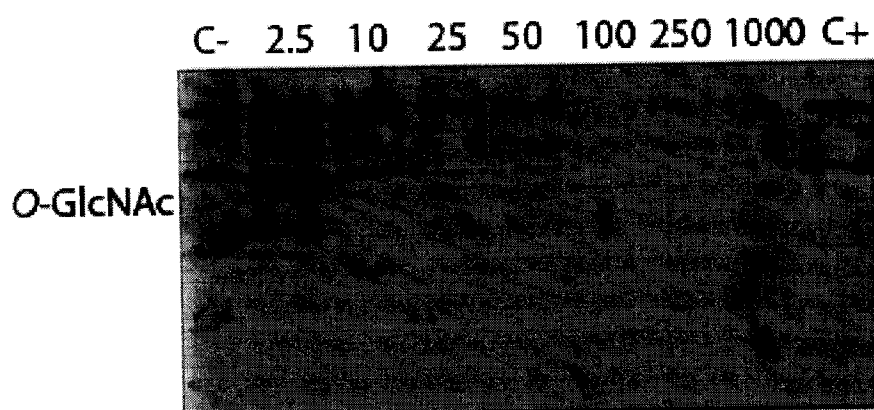
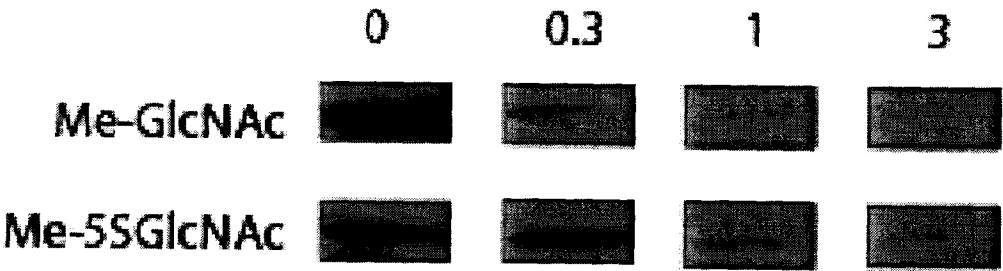
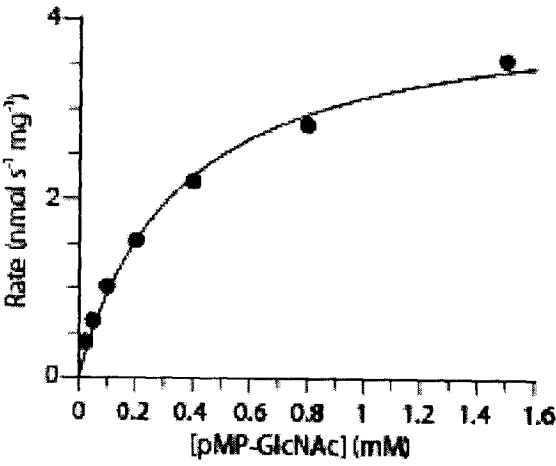


FIGURE 3

A.



B.



C.

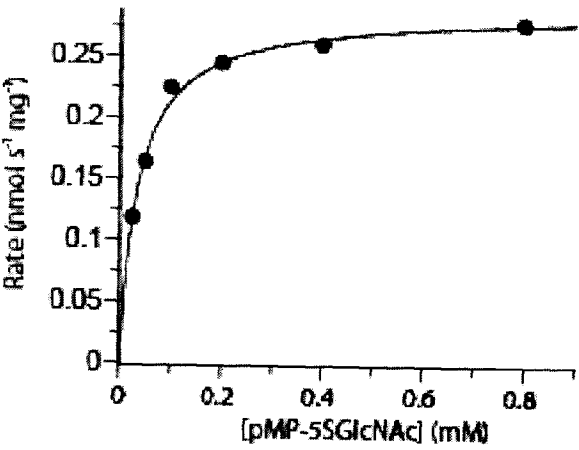
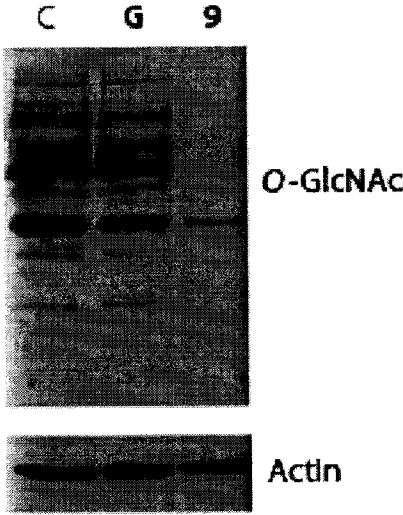
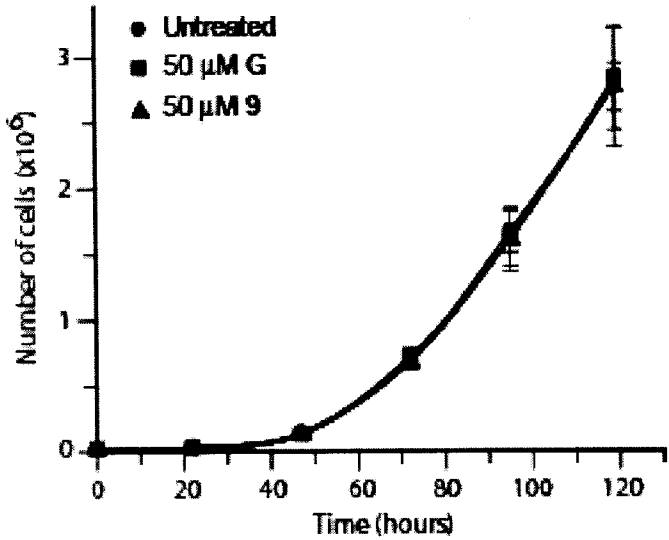


FIGURE 4

V

A.



B.

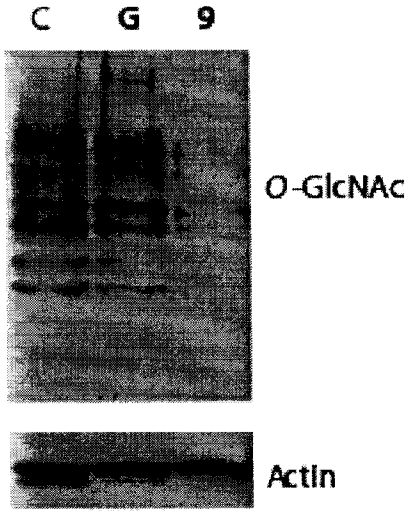
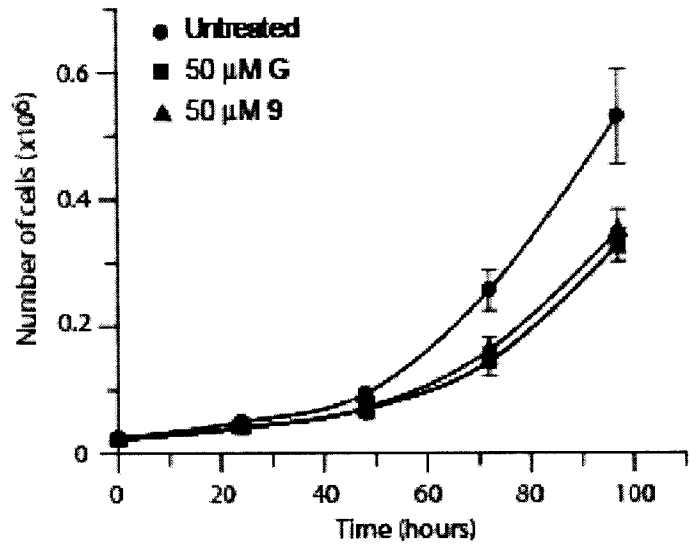


FIGURE 5

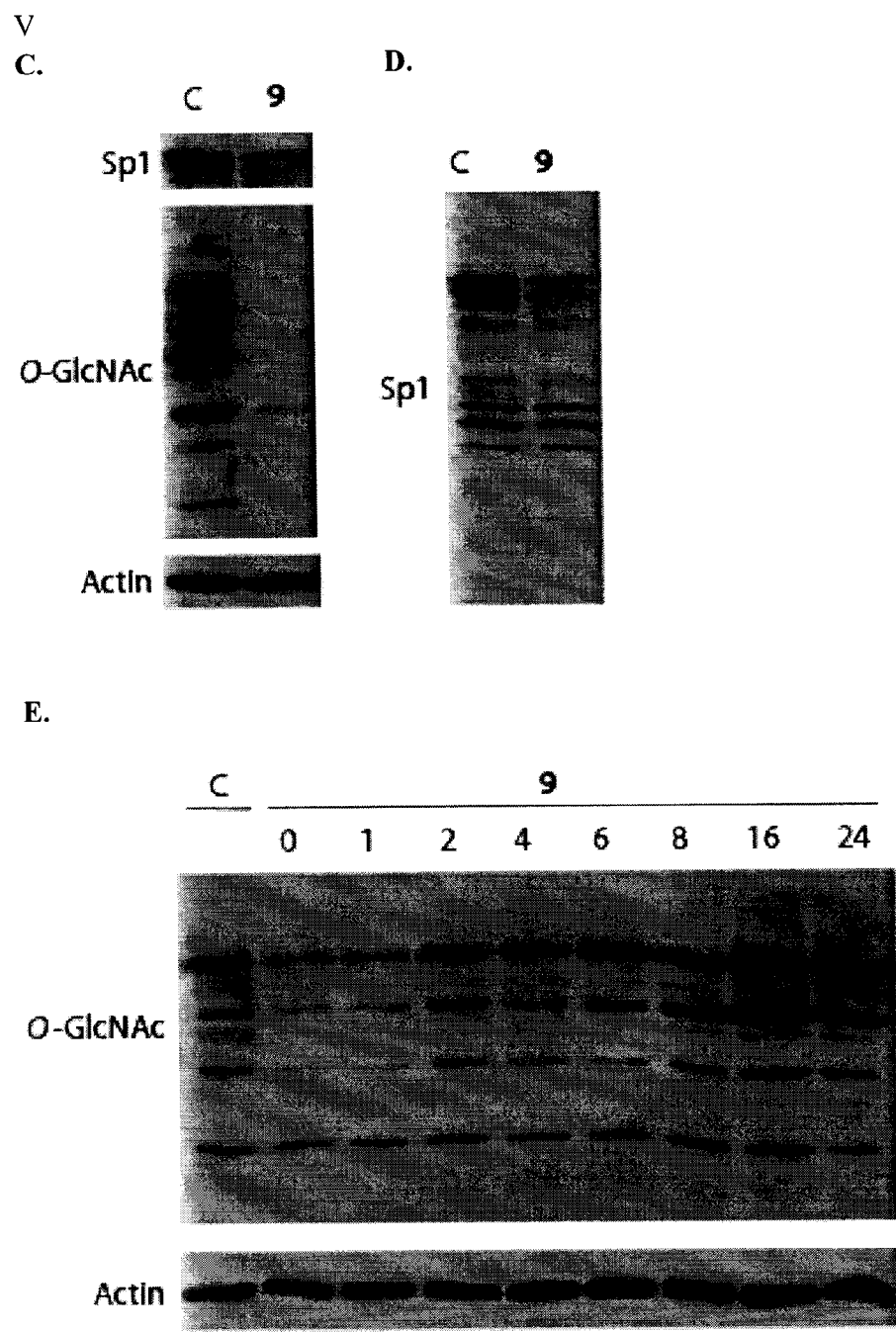
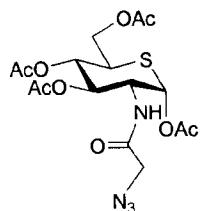


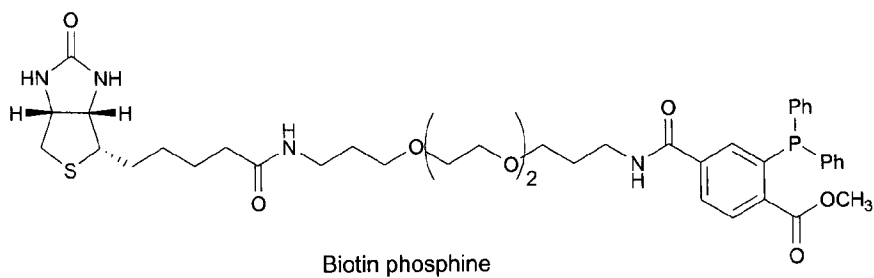
FIGURE 5

V

A.



Ac-5SGlcNAz (41)



Biotin phosphine

FIGURE 6

V

B.

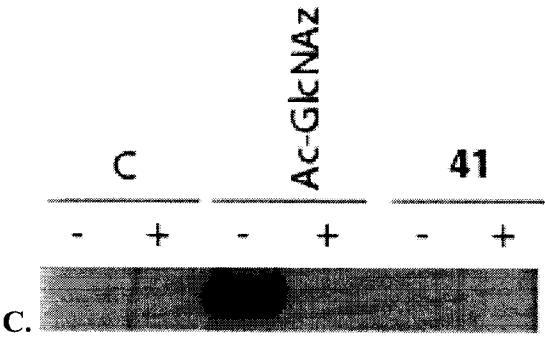
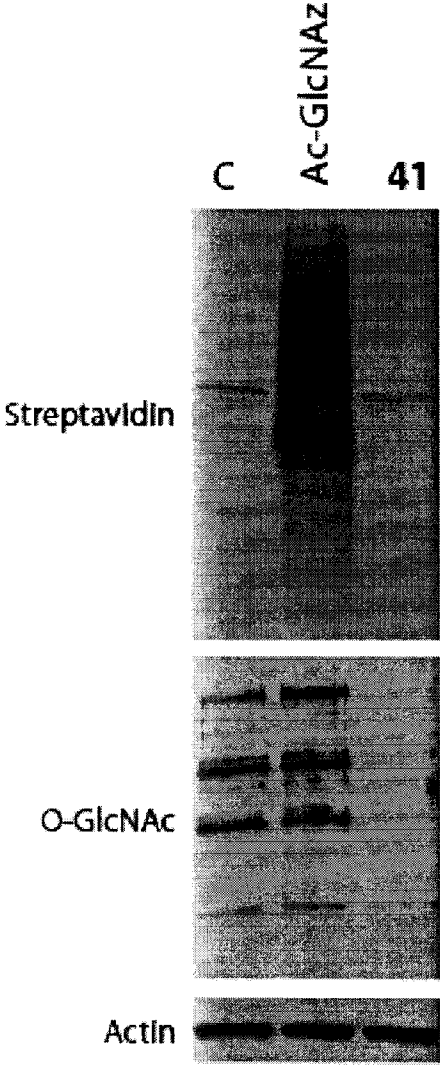


FIGURE 6

V

D.

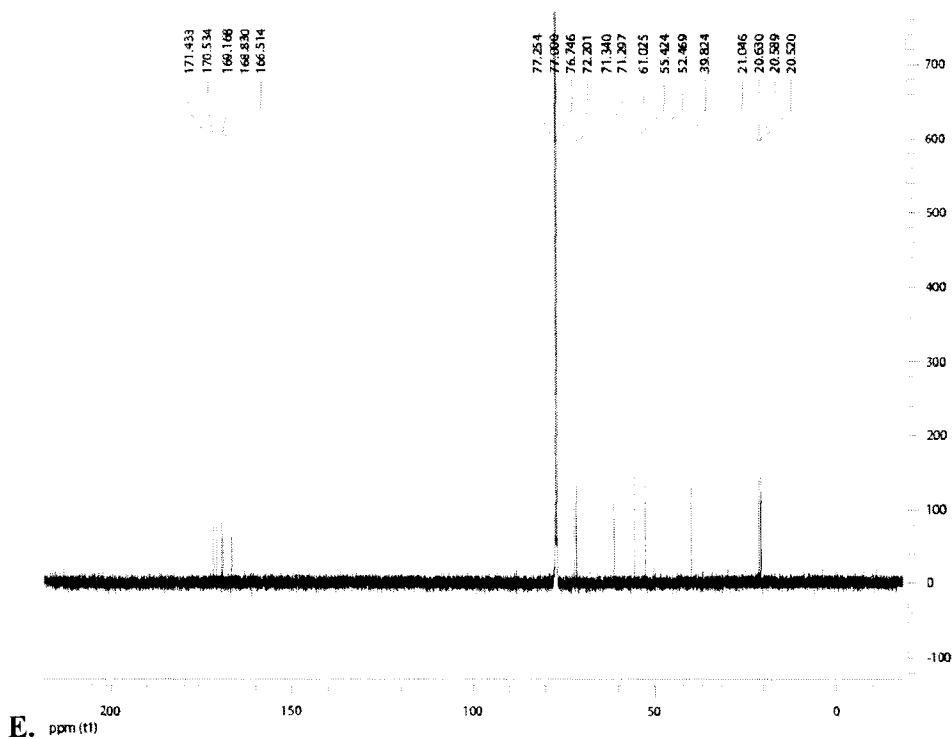
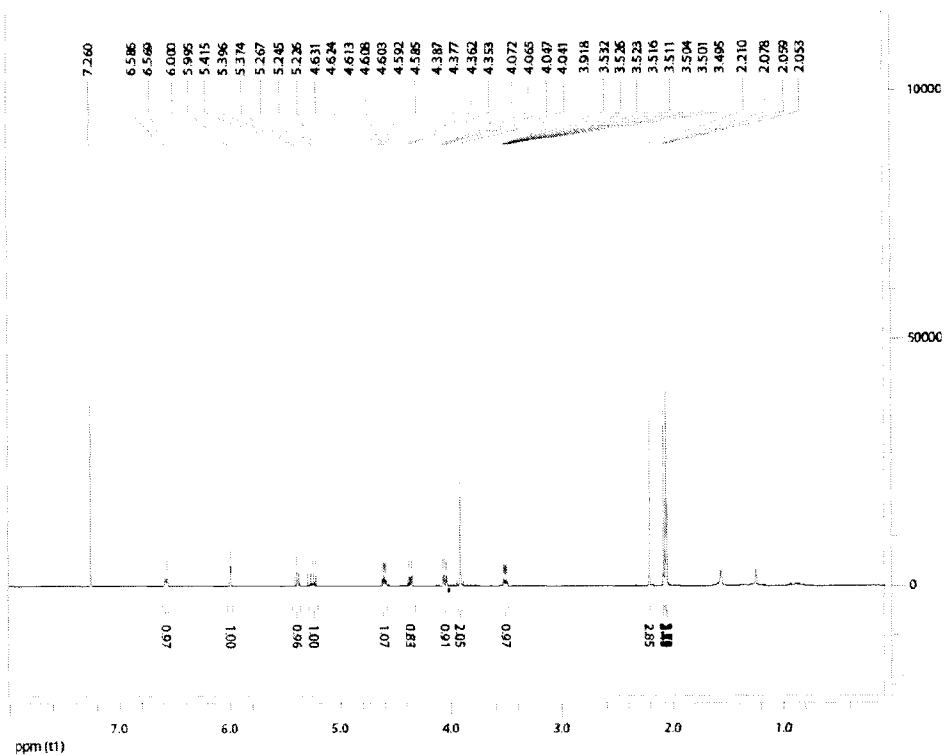


FIGURE 6

5

F.

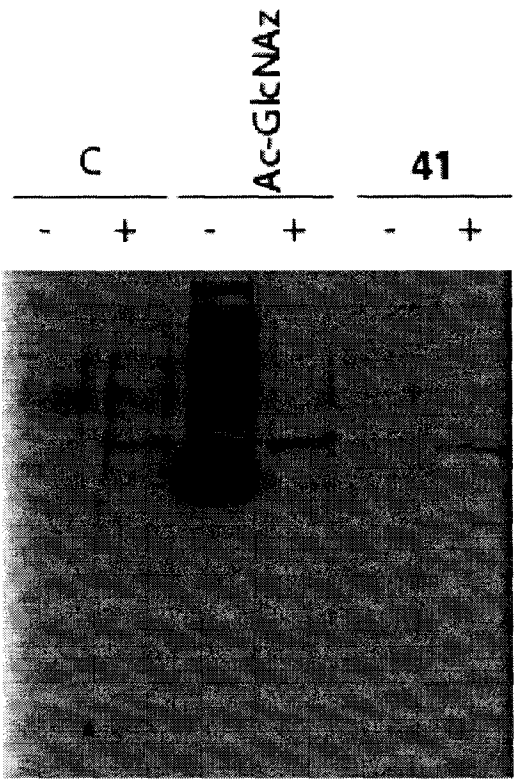
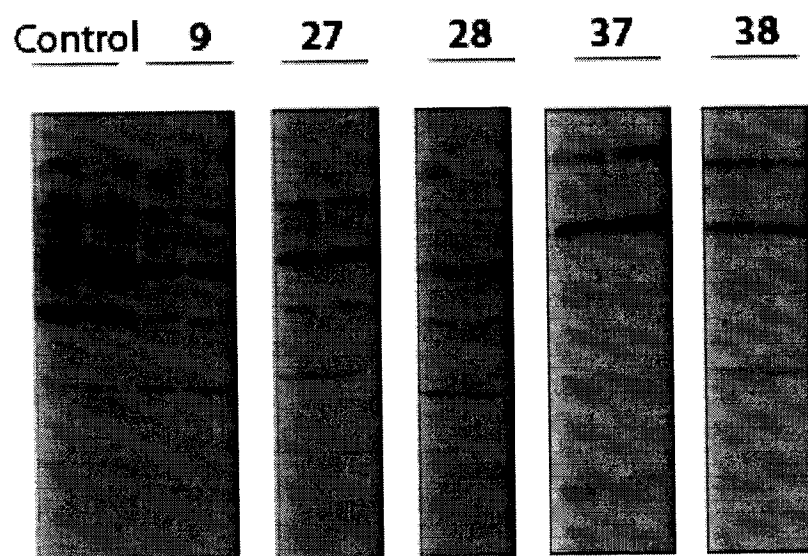


FIGURE 6

V

**FIGURE 7**

v

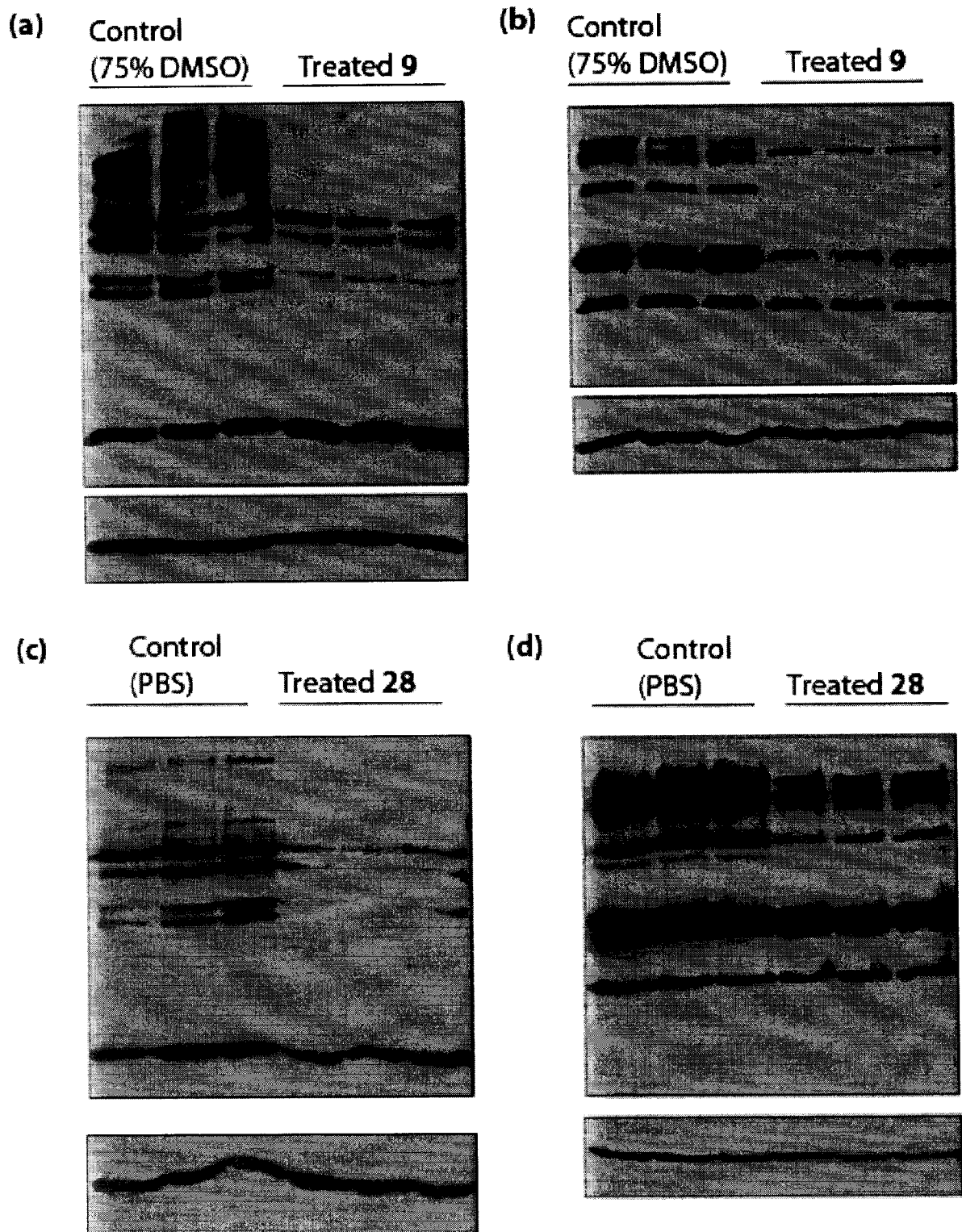


FIGURE 8

v

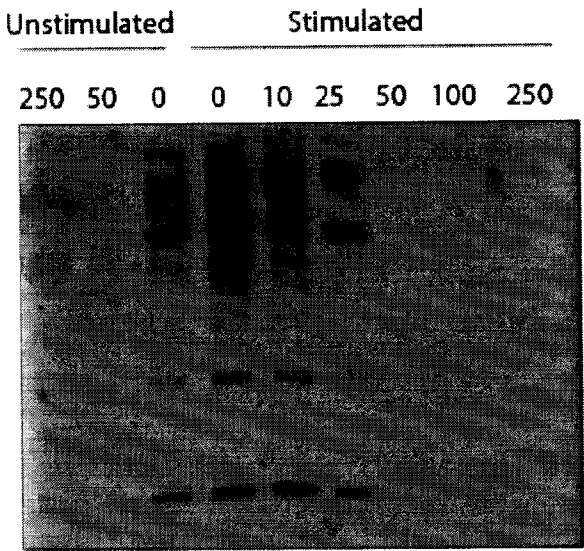


FIGURE 9

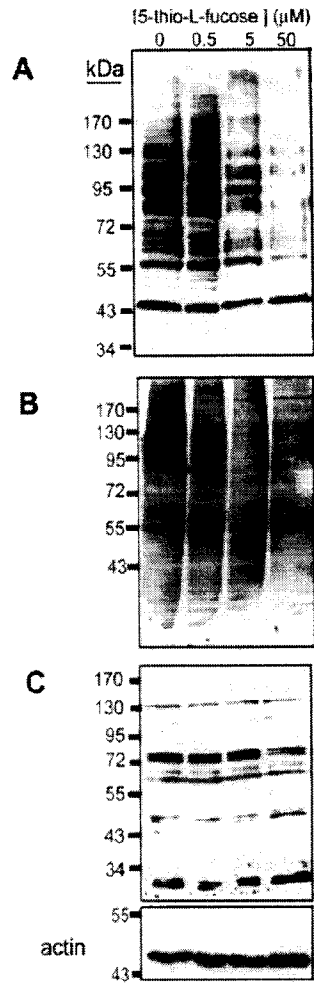
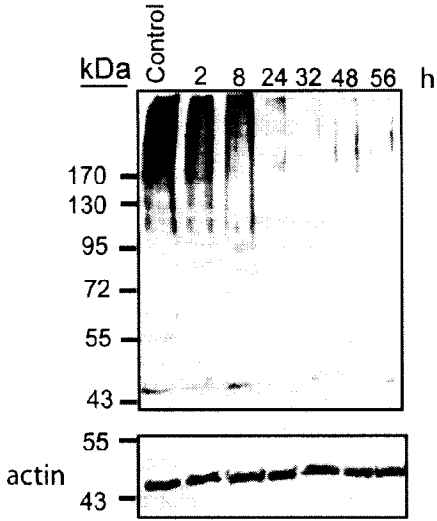


Figure 10

V

A



B



C

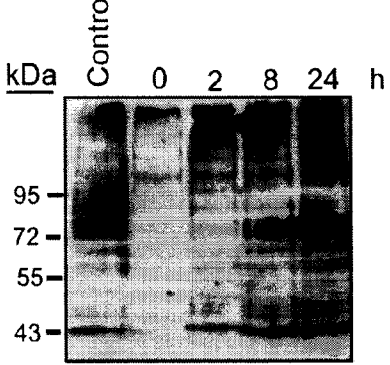


Figure 11

v

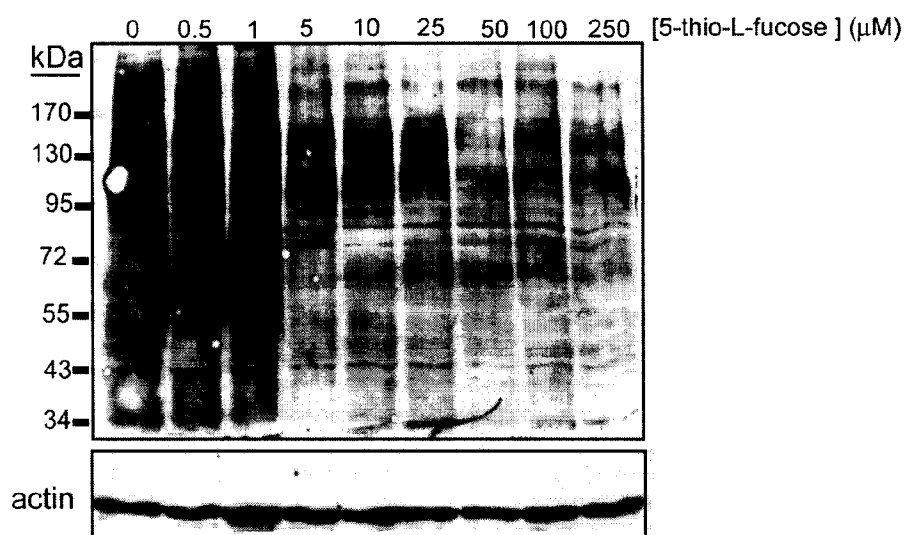


Figure 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2011/000538

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07H 13/04** (2006.01) , **A61K 31/7024** (2006.01) , **A61P 29/00** (2006.01) , **A61P 35/00** (2006.01) , **A61P 37/00** (2006.01) , **C07H 13/00** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: **C07D**, **A61K**

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
STN (structure search in Registry for Formulae (I)-(X), CAPlus, Keyword: glycosyltransferase), EPOQUE (EPODOC: glycosyltransferase, thiosugar, carbasugar, Inventor search), Canadian Patent Database (glycosyltransferase, thiosugar, carbasugar, Inventor search)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| P, X | T. M. GLOSTER et al: "Hijacking a biosynthetic pathway yields a glycosyltransferase inhibitor within cells"; <i>Nature Chemical Biology</i> (2011), 7 (3), 174-181.
See the whole document. | 1-41 |
| X | S. AUGY-DOREY et al: "Synthesis and biological evaluation of carbocyclic analogues of Lipid X: new nonpolar antagonists of LPS induced TNF production"; <i>Drug Design and Discovery</i> , 1999, 16, 41-48.
See compounds 7 and 8. | 35, 41 |
| X | A. HASEGAWA et al: "Synthesis of 2-acetamido-2-deoxy-5-thio-D-glucopyranose"; <i>Carbohydrate Research</i> , 1978, 63, 131-137.
See compounds 12 and 13. | 35-36, 39-41 |
| X | K. BOCK et al: "Proton and carbon-13 n.m.r. spectra of six pseudo-2-acetamido-2-deoxy-DL-hexopyranoses"; <i>Carbohydrate Research</i> , 1989, 191, 357-363.
See compounds 3 and 4. | 35, 41 |

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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

Date of the actual completion of the international search

23 June 2011 (23-06-2011)

Date of mailing of the international search report

4 August 2011 (04-08-2011)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer

Lu Jiang (819) 934-6738

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2011/000538**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

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

1. ☒ Claim Nos. : 1-23
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 1-23 are directed to a method for treatment of the human or animal body by surgery or therapy, are not required to be searched nor is a written opinion required by this Authority. Regardless, this Authority has established a written opinion based on the alleged effect or purpose/use of the product defined in claims 1-23.
2. ☐ Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :
3. ☐ Claim Nos. :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

- Remark on Protest** ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2011/000538

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| X | S. OGAWA et al: "New synthesis of 2-amino-5a-carba-2-deoxy- α -DL-glucopyranose and its transformation into valienamine and valioamine analogues"; <i>Liebigs Ann. Chem.</i> 1992 , 637-641.
See compounds 2 and 10 . | 35-36, 39, 41 |
| X | S. OGAWA et al: "Synthesis of seven penta-N,O-acetyl-pseudo-2-amino-2-deoxy-DL-hexopyranoses"; <i>Carbohydrate Research</i> , 1988, 177 , 191-212.
See compound 29 . | 35-36, 39-41 |
| X | S. OGAWA et al: "Synthesis and biological evaluation of α -L-fucosidase inhibitors: 5a-carba- α -L-fucopyranosylamine and related compounds"; <i>Eur. J. Org. Chem.</i> 2001 , 967-974.
See compound 4 . | 34-35, 41 |
| X | H. HASHIMOTO et al: "Synthesis of 5-thio-L-fucose and its inhibitory effect on fucosidase"; <i>J. Carbohydrate Chem.</i> (1990), 9 (5), 683-694.
See compounds 1 and 11 . | 34-36, 39-41 |
| X | C. LE DRIAN et al: "17. Total synthesis of (-)-conduritol B ((-)-1L-cyclohex-5-ene-1,3/2,4-tetrol) and of (+)-conduritol F ((+)-1D-cyclohex-5-ene-1,2,4/3-tetrol). Determination of the absolute configuration of (+)-leucanthemitol"; <i>Helvetica Chimica Acta</i> (1990), 73 , 161-168.
See compounds (+)- 2 , (+)- 16 and (-)- 17 . | 35-36, 39, 41 |
| X | P. RENAUT et al: "5a-Carba- β -D-, 5a-Carba- β -L- and 5-thio- β -L-xylopyranosides as new orally active venous antithrombotic agents"; <i>Helvetica Chimica Acta</i> (1998), 81 , 2043-2052.
See compound (-)- 25 . | 35-36, 39-41 |
| X | K. BLUMBERG et al: "Selenium derivatives of L-arabinose, D-ribose, and D-xylose"; <i>Carbohydrate Research</i> (1977), 59 , 351-362.
See compound 35 . | 35-36, 39, 41 |
| A | W. ZOU "C-glycosides and aza-C-glycosides as potential glycosidase and glycosyltransferase inhibitors"; <i>Current Topics in Medicinal Chemistry</i> , 2005, 5 , 1363-1391.
See the whole document. | 1-41 |
| A | P. COMPAIN et al: "Carbohydrate mimetics-based glycosyltransferase inhibitors"; <i>Bioorg. Med. Chem.</i> (2001), 9 , 3077-3092.
See the whole document. | 1-41 |