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- (71) Applicant(s)
Amicus Therapeutics, Inc.
- (72) Inventor(s)
Boyd, Robert;Lee, Gary;Rybczynski, Philip
- (74) Agent / Attorney
Dark IP, PO BOX 2416, Fortitude Valley BC, QLD, 4006
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(71) Applicant (for all designated States except US): **AMICUS THERAPEUTICS, INC.** [US/US]; 6 Cedar Brook Drive, Cranbury, NJ 08512 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BOYD, Robert** [US/US]; 3 Danbridge Drive, Horsham, PA 19044 (US). **LEE, Gary** [GB/US]; 7 Leeds Court, West Windsor, NJ 08550 (US). **RYBCZYNSKI, Philip** [US/US]; 29 Tuscarora Trail, Branchburg, NJ 08876 (US).

(74) Agents: **SCOLA, Daniel A., Jr.** et al.; Hoffmann & Baron, LLP, 6900 Jericho Turnpike, Syosset, New York 11791 (US).

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(54) Title: NOVEL COMPOSITIONS FOR PREVENTING AND/OR TREATING LYSOSOMAL STORAGE DISORDERS

(57) Abstract: The present invention provides novel compositions as well as methods for preventing and/or treating lysosomal storage disorders. In particular, the present invention provides methods for preventing and/or treating Gaucher's disease.



WO 2011/049737 A1

**NOVEL COMPOSITIONS FOR PREVENTING AND/OR TREATING
LYSOSOMAL STORAGE DISORDERS**

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 61/252,806, filed October 19, 2009, the contents of which is incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present invention provides novel compounds, known as pharmacological chaperones, as well as methods using the same for preventing and/or treating lysosomal storage disorders. In particular, the present invention provides methods for preventing and/or treating Gaucher's disease.

BACKGROUND OF THE INVENTION

[0003] Lysosomal storage disorders are caused by a defect in lysosomal function that results in accumulation of substances within the lysosome of cells. This defect is usually a consequence of deficiency of a single enzyme required for the metabolism of lipid, glycogen, glycoprotein, or mucopolysaccharide. Gaucher's disease, the most common lysosomal storage disorder, is characterized by accumulation of the glycolipid glucocerebroside (also known as glucosylceramide). Symptoms of Gaucher's disease include enlarged spleen and liver, liver malfunction, skeletal disorders and bone lesions that may be painful, severe neurologic complications, swelling of lymph nodes and (occasionally) adjacent joints, distended abdomen, a brownish tint to the skin, anemia, low blood platelets and yellow fatty deposits on the sclera. In addition, persons affected with Gaucher's disease may also be more susceptible to infection.

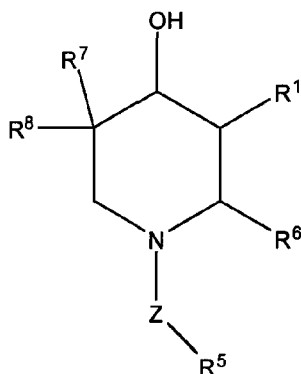
[0004] There is a need for methods to prevent and/or treat lysosomal storage disorders that provide patients with a higher quality of life and achieve a better clinical outcome. In particular, there is a need for methods to prevent

and/or treat Gaucher's disease that provide patients with a higher quality of life and achieve a better clinical outcome.

SUMMARY OF THE INVENTION

[0005] The present invention provides novel compounds as well as compositions and methods using the same to prevent and/or treat a lysosomal storage disorder in a patient at risk for developing or diagnosed with the same which includes administering to the patient in need thereof an effective amount of a compound described herein.

[0006] In one aspect, there is provided a compound as well as compositions and methods using the same to prevent and/or treat a lysosomal storage disorder in a patient at risk for developing or diagnosed with the same which includes administering to the patient in need thereof an effective amount of a compound defined by Formula I:



wherein:

R¹ is C(R²)(R³)(R⁴);

R² is hydrogen, -OH or halogen;

R³ is hydrogen, -OH, halogen or C₁₋₈ alkyl;

R⁴ is halogen, C₁₋₈ alkyl, substituted C₁₋₈ alkyl, aryl, substituted aryl, alkylcycloalkyl or substituted alkylcycloalkyl;

R³ and R⁴ may join with the carbon to which they are attached to form a cycloalkyl ring, which may be optionally substituted, preferably with halogen and more preferably with one or more fluorine atoms;

R^6 is hydrogen, C_{1-8} alkyl, substituted C_{1-8} alkyl, arylalkyl, substituted arylalkyl, alkylaryl, or substituted alkylaryl;

Z is optional, when present Z is $-(CH_2)_{1-8}-$, $-C(=O)-$, $-S(=O)_2NH-$, $-S(=O)_2-$,

$-C(=S)NH-$, $-S(=O)_2-CH_3$, $C(=O)-NH-$, $-S(=O)_2-NR^9R^{10}$, $-C(=O)C_{1-8}$ alkyl or

$-C(=O)CH(NH_2)CH_3$;

R^9 is hydrogen, C_{1-8} alkyl or substituted C_{1-8} alkyl;

R^{10} is hydrogen, C_{1-8} alkyl or substituted C_{1-8} alkyl;

R^5 is hydrogen, C_{1-8} alkyl, substituted C_{1-8} alkyl, aryl, substituted aryl, C_{1-8} alkenyl, substituted C_{1-8} alkenyl, arylalkyl, substituted arylalkyl, alkylaryl, substituted alkylaryl, aminoarylalkyl or substituted aminoarylalkyl;

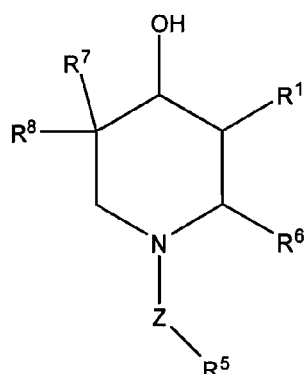
R^7 is $-OH$ or halogen; and

R^8 is hydrogen, halogen or C_{1-8} alkyl,

provided that R^2 and R^3 cannot both be hydrogen when R^4 is a halogen, Z is not present, R^7 is $-OH$, R^5 , R^6 and R^8 are hydrogen.

[0007] It is understood by a person of ordinary skill in the art that R^2 , R^3 and R^4 in aforementioned Formulas I, II, and III will not be selected such that an unstable molecule will result.

[0008] In another aspect, there is provided a compound as well as compositions and methods using the same to prevent and/or treat a lysosomal storage disorder in a patient at risk for developing or diagnosed with the same which includes administering to the patient in need thereof an effective amount of a compound defined by Formula II:



wherein:

R¹ is C(R²)(R³)(R⁴);

R² is hydrogen, -OH or halogen;

R³ is hydrogen, -OH, halogen or -CH₃;

R⁴ is halogen, -CH₃, phenyl, fluorophenyl, methylphenyl, cyclohexylmethyl, wherein when R⁴ is a halogen, both R² and R³ cannot be hydrogen;

R³ and R⁴ may join with the carbon to which they are attached to form a cycloalkyl ring, which may be optionally substituted with one or more halogen atoms;

R⁶ is hydrogen, phenylalkyl or substituted phenylalkyl;

Z is optional, when present Z is -(CH₂)-, -C(=O)-, -S(=O)₂NH-, -S(=O)₂-,

-S(=O)₂-CH₃, C(=O)-NH-, -S(=O)₂NR⁹R¹⁰, -C(=S)-NH- or -C(=O)₂-CH₃,

R⁹ is hydrogen or CH₃;

R¹⁰ is hydrogen or CH₃;

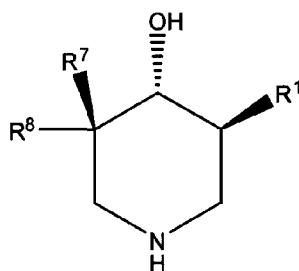
R⁵ is hydrogen or aminophenylalkyl;

R⁷ is -OH or halogen; and

R⁸ is hydrogen, halogen or -CH₃,

provided that R² and R³ cannot both be hydrogen when R⁴ is halogen, Z is not present, R⁷ is -OH, R⁵, R⁶ and R⁸ are hydrogen.

[0009] In yet another aspect, there is provided a compound as well as compositions and methods using the same to prevent and/or treat a lysosomal storage disorder in a patient at risk for developing or diagnosed with the same which includes administering to the patient in need thereof an effective amount of a compound defined by Formula III:



wherein:

R^1 is $C(R^2)(R^3)(R^4)$;

R^2 is hydrogen, -OH or halogen;

R^3 is hydrogen, -OH, halogen or $-CH_3$;

R^4 is halogen, $-CH_3$, phenyl, fluorophenyl, methylphenyl, cyclohexylmethyl, wherein when R^4 is a halogen, both R^2 and R^3 cannot be hydrogen;

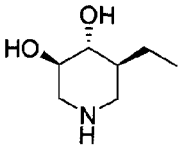
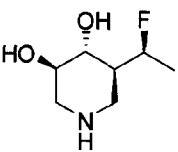
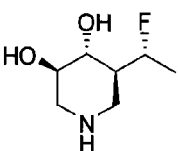
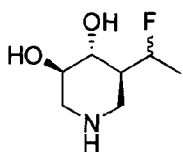
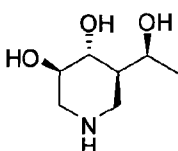
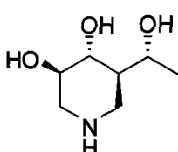
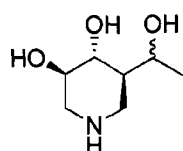
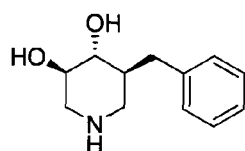
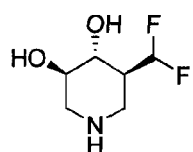
R^3 and R^4 may join with the carbon to which they are attached to form a cycloalkyl ring, which may be optionally substituted with one or more halogen atoms;

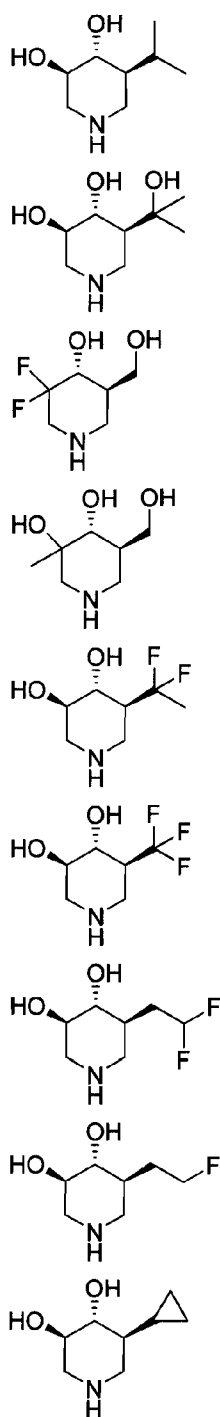
R^7 is -OH or halogen; and

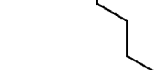
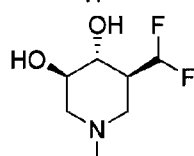
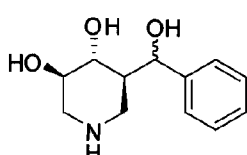
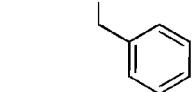
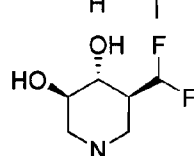
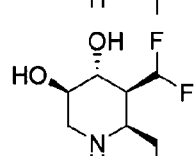
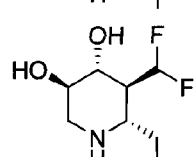
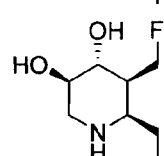
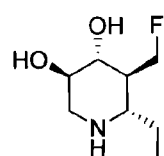
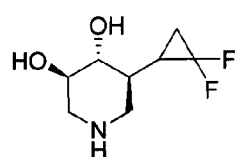
R^8 is hydrogen, halogen or $-CH_3$,

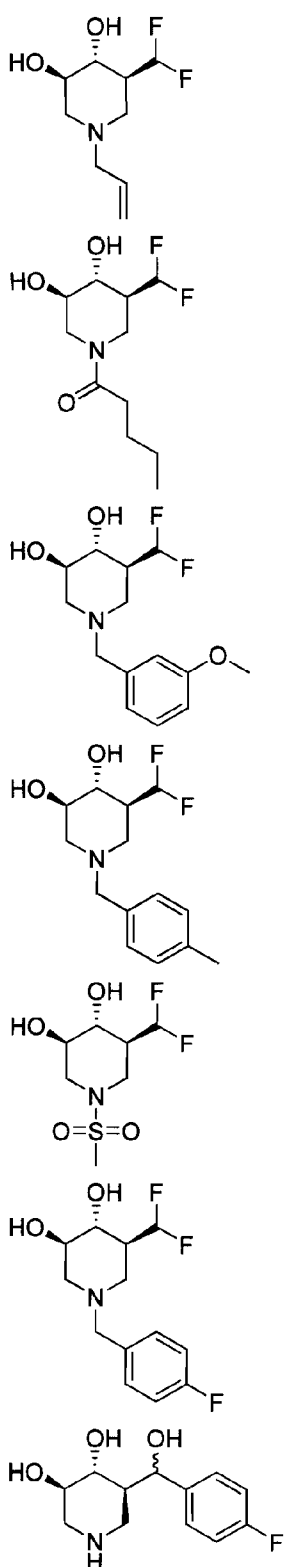
provided that R^2 and R^3 cannot both be hydrogen when R^4 is a halogen, R^7 is -OH and R^6 and R^8 are hydrogen.

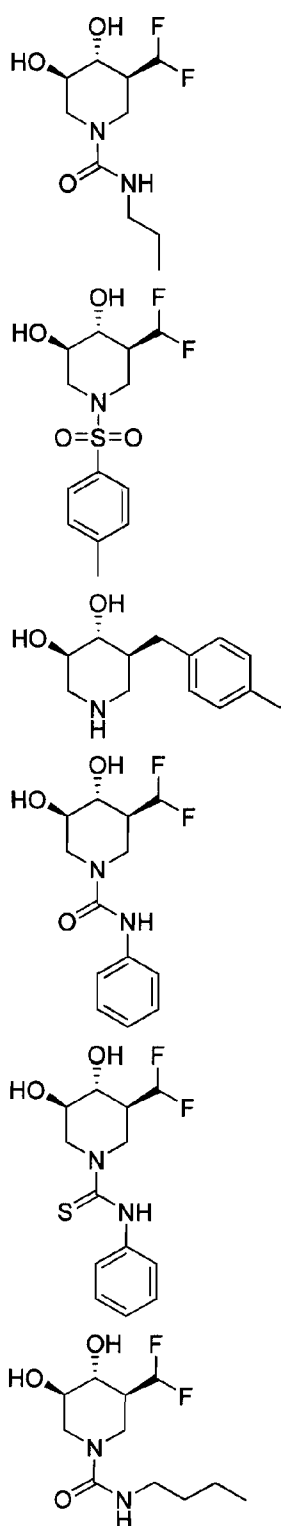
[0010] In still another aspect, there is provided a compound as well as compositions and methods using the same to prevent and/or treat a lysosomal storage disorder in a patient at risk for developing or diagnosed with the same which includes administering to the patient in need thereof an effective amount of a compound selected from the following:

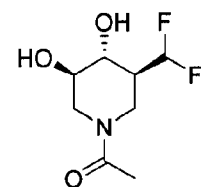
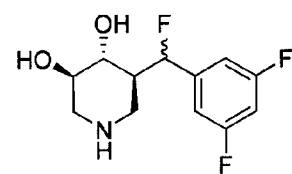
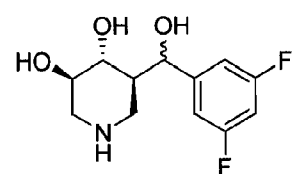
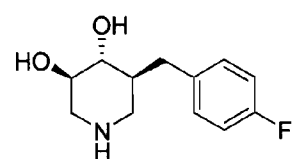
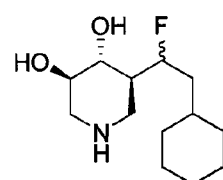
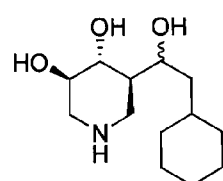
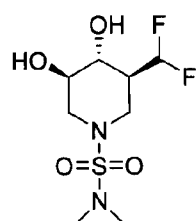
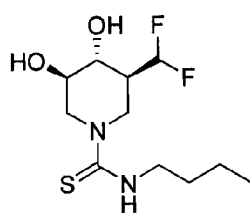


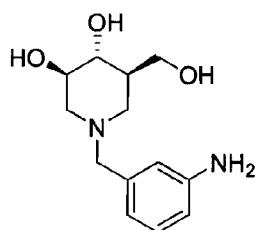
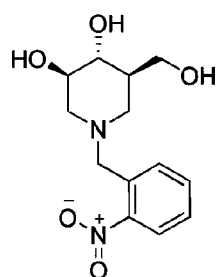
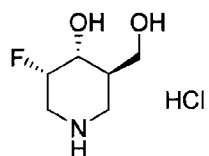
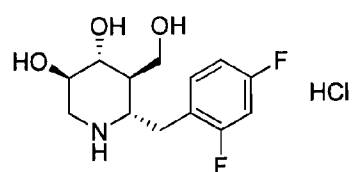
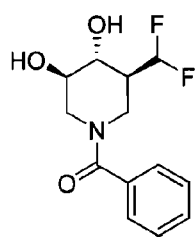


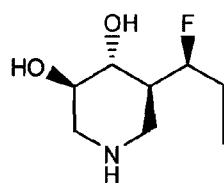
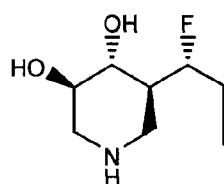
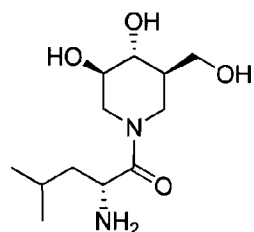
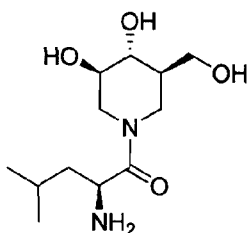
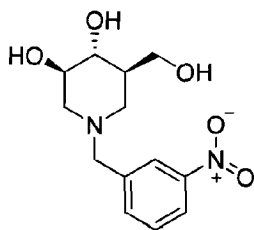












[0011]

or a pharmaceutically acceptable salt, solvate, or prodrug thereof.

[0012]

In one embodiment, the compound is (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5S)-5-benzylpiperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the compound is

(3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the compound is (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the compound is (3R,4R,5S)-5-benzylpiperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof.

[0013] In one embodiment, the lysosomal storage disorder is associated with accumulation of at least one glycolipid. In one embodiment, the lysosomal storage disorder is associated with accumulation of at least one glycosphingolipid. In one embodiment, the lysosomal storage disorder is associated with accumulation of glucocerebroside. In one embodiment, the lysosomal storage disorder is associated with a deficiency in glucocerebrosidase. In one embodiment, the lysosomal storage disorder is associated with a mutation in glucocerebrosidase. In one embodiment, the lysosomal storage disease is Niemann-Pick disease. In one embodiment, the lysosomal storage disease is Gaucher's disease. In one embodiment, the method further comprises administering an effective amount of at least one other therapeutic agent. In one embodiment, the method comprises at least one other therapeutic agent is imiglucerase or 1,5-(butylimino)-1,5-dideoxy-D-glucitol.

[0014] The present invention also provides methods for preventing and/or treating Gaucher's disease in a patient at risk for developing or diagnosed with the same, which comprises administering to the patient in need thereof an effective amount of a composition comprising a compound of Formula I, or a pharmaceutically acceptable salt, solvate, or prodrug thereof.

[0015] In one embodiment, the method comprises administering the compound (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5S)-5-benzylpiperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the method comprises administering the compound (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the method comprises administering the

compound (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the method comprises administering the compound (3R,4R,5S)-5-benzylpiperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof.

[0016] In one embodiment, the method further comprises administering an effective amount of at least one other therapeutic agent. In one embodiment, at least one other therapeutic agent is imiglucerase or 1,5-(butylimino)-1,5-dideoxy-D-glucitol.

[0017] The present invention also provides kits comprising:

- a container having an effective amount of any of the compounds of the present invention, alone or in combination; and
- instructions for using the same to prevent and/or treat a lysosomal storage disorder.

[0018] In one embodiment, the lysosomal storage disorder is Gaucher's disease.

[0019] The present invention also provide methods for enhancing the activity of glucocerebrosidase in a cell ex vivo using 5-(fluoromethyl)piperidine-3,4-diol, 5-(chloromethyl)piperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof, or any combination of two or more thereof.

[0020] In addition, the present invention provides methods for diagnosing patients amenable to treatment comprising contacting ex vivo a cell from a patient at risk for developing or diagnosed with a lysosomal storage disorder with a therapeutic agent which is 5-(fluoromethyl)piperidine-3,4-diol, 5-(chloromethyl)piperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof, or any combination of two or more thereof and assaying a lysate of the cell for lysosomal glucocerebrosidase activity wherein an increase in lysosomal glucocerebrosidase activity relative to another cell that is not treated with the therapeutic agent indicates that the patient would be amenable to treatment. In one embodiment, the lysosomal storage disorder is Gaucher's disease.

DETAILED DESCRIPTION OF THE INVENTION

[0021] As used herein the following terms shall have the definitions set forth below.

[0022] As used herein the term "treating" means to ameliorate one or more symptoms associated with the referenced disorder.

[0023] As used herein, the term "preventing" means to mitigate a symptom of the referenced disorder.

[0024] As used herein the phrase "an effective amount" means an amount effective to prevent and/or treat a patient at risk for developing or diagnosed with the referenced disorder, and thus producing the desired therapeutic effect.

[0025] As used herein the term "patient" means a mammal (e.g., a human).

[0026] As used herein the phrase "lysosomal storage disorder" refers to any of a group of diseases resulting from abnormal metabolism resulting in accumulation of a substrate in the lysosome. Table 1 contains a non-limiting list of exemplary lysosomal storage disorders and their associated defective enzyme.

TABLE 1 Lysosomal storage disorders

<u>Lysosomal storage disorder</u>	<u>Defective enzyme</u>
Pompe disease	Acid α -glucosidase
Gaucher disease	Acid β -glucosidase or glucocerebrosidase
Fabry disease	α -Galactosidase A
G _{M1} -gangliosidosis	Acid β -galactosidase
Tay-Sachs disease	β -Hexosaminidase A
Sandhoff disease	β -Hexosaminidase B
Niemann-Pick disease	Acid sphingomyelinase
Krabbe disease	Galactocerebrosidase
Farber disease	Acid ceramidase
Metachromatic leukodystrophy	Arylsulfatase A
Hurler-Scheie disease	α -L-Iduronidase
Hunter disease	Iduronate-2-sulfatase
Sanfilippo disease A	Heparan N-sulfatase
Sanfilippo disease B	α -N-Acetylglucosaminidase
Sanfilippo disease C	Acetyl-CoA: α -glucosaminide N-acetyltransferase
Sanfilippo disease D	N-Acetylglucosamine-6-sulfate sulfatase
Morquio disease A	N-Acetylgalactosamine-6-sulfate sulfatase

Morquio disease B	Acid β -galactosidase
Maroteaux-Lamy disease	Arylsulfatase B
Sly disease	β -Glucuronidase
alpha.-Mannosidosis	Acid α -mannosidase
beta.-Mannosidosis Acid	β -mannosidase
Fucosidosis	Acid α -L-fucosidase
Sialidosis	Sialidase
Schindler-Kanzaki disease	α -N-acetylgalactosaminidase

[0027] The most common lysosomal storage disorder, Gaucher's disease, is characterized by accumulation of the glycolipid glucocerebroside (also known as glucosylceramide). Three phenotypes have been described for Gaucher's disease that are denoted by the absence (type 1) or presence of neurologic involvement during childhood (type 2) or adolescence (type 3). For example, see Grabowski, Gaucher's disease. *Adv Hum Genet* 1993; 21:377-441.

[0028] The three types of Gaucher's disease are inherited in an autosomal recessive fashion. Both parents must be carriers in order for a child to be affected. If both parents are carriers, there is a one in four, or 25%, chance with each pregnancy for an affected child. Genetic counseling and genetic testing is recommended for families who may be carriers of mutations. Each type has been linked to particular mutations. In all, there are about 80 known mutations that lead to Gaucher's disease (see, e.g., McKusick, V.A.: *Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders*. Baltimore: Johns Hopkins University Press, 1998 (12th edition)).

[0029] Type 1 Gaucher's disease is panethnic, but is especially prevalent among persons of Ashkenazi Jewish descent, with a carrier rate of 1 in 17 Ashkenazi Jews. The N370S and 84GG mutations are the most frequent mutations in the glucocerebrosidase gene among Ashkenazi Jews, with rates of 1 in 17.5 for N370S and 1 in 400 for 84GG in the general healthy Ashkenazi population, and are associated with mild and severe Gaucher's disease, respectively. The 84GG mutation occurs almost exclusively among Ashkenazi Jews. Other rare glucocerebrosidase gene variants identified in patients of Ashkenazi descent with Gaucher's disease include L444P, IVS2+1G→A, V394L,

and R496H. In contrast to presentation of Type 1 Gaucher's disease in Ashkenazi Jews, Type 1 Gaucher's disease tends to be severe and progressive in Japanese patients (see, Ida et al., Type 1 Gaucher Disease Patients: Phenotypic Expression and Natural History in Japanese Patients, *Blood Cells, Molecules and Diseases*, 1984, 24(5):73-81). In addition, Type 3 Gaucher's disease, associated with one or two copies of glucocerebrosidase gene variant L444P is prevalent in Swedish patients from the Norrbotten region.

[0030] A definitive diagnosis of Gaucher's disease is made with genetic testing. As there are numerous different mutations, sequencing of the glucocerebrosidase gene is sometimes necessary to confirm the diagnosis. Prenatal diagnosis is available, and is useful when there is a known genetic risk factor. However, a diagnosis of Gaucher's disease can also be implied by biochemical abnormalities such as high alkaline phosphatase, angiotensin-converting enzyme (ACE) and immunoglobulin levels, or by cell analysis showing "crinkled paper" cytoplasm and glycolipid-laden macrophages. Notably, Niemann-Pick disease is similar in that it is characterized by accumulation of G_{M2} -gangliosides and G_{M1} -gangliosides in addition to glucocerebroside (Vanier et al., *Brain Pathology*. 1998; 8: 163-74).

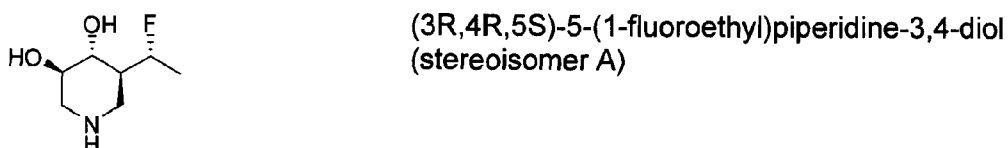
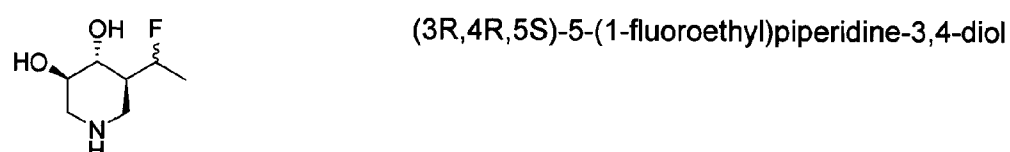
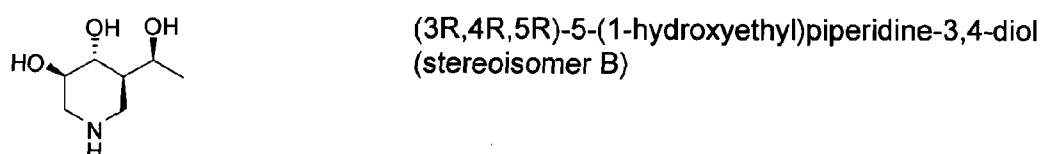
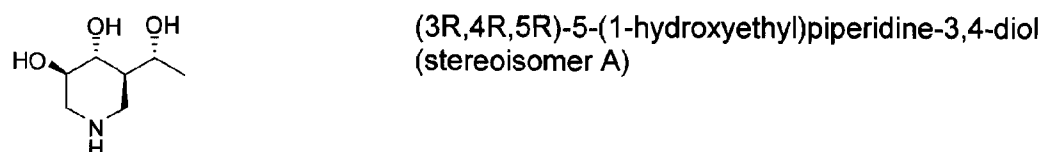
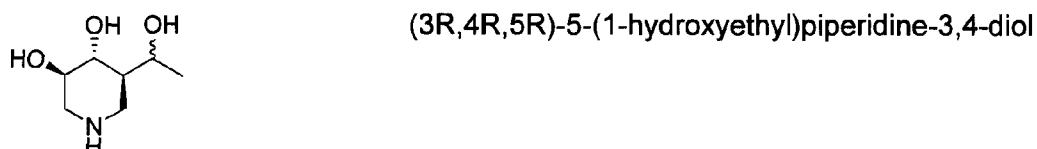
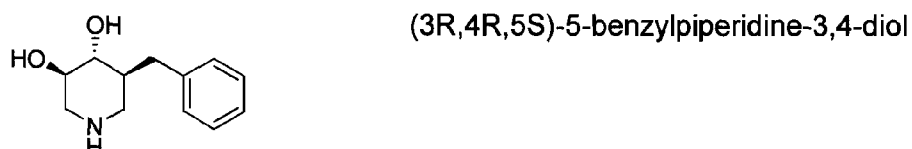
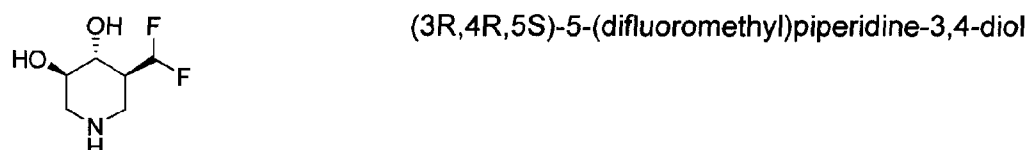
[0031] Symptoms of Gaucher's disease include the following:

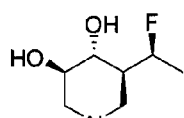
- Painless hepatomegaly and splenomegaly (the size of the spleen can be 1500-3000 ml, as opposed to the normal size of 50-200 ml)
- Hypersplenism: the rapid and premature destruction of blood cells, leading to anemia, neutropenia and thrombocytopenia (with an increased risk of infection and bleeding)
- Cirrhosis of the liver, though rare
- Neurological symptoms occur only in some types of Gaucher's (see below):
 - Type II: serious convulsions, hypertonia, mental retardation, apnea.
 - Type III: muscle twitches known as myoclonus, convulsions, dementia, ocular muscle apraxia.

- Osteoporosis: 75% develop visible bony abnormalities due to the accumulated glucosylceramide. A deformity of the distal femur in the shape of an Erlenmeyer flask is commonly described.
- Yellowish-brown skin pigmentation

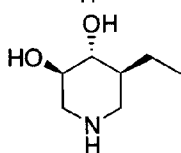
Compounds

[0032] Novel compounds of the present invention are provided below:

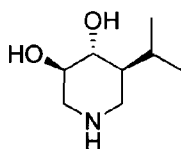




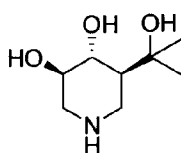
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(stereoisomer B)



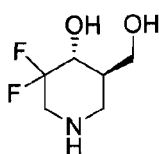
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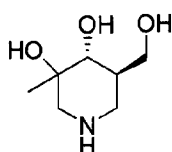
(3R,4R,5S)-5-isopropylpiperidine-3,4-diol



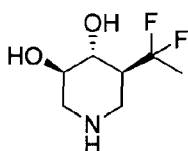
(3R,4R,5S)-5-(2-hydroxypropan-2-yl)piperidine-3,4-diol



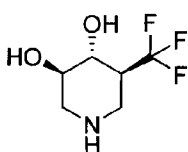
(4R,5R)-3,3-difluoro-5-(hydroxymethyl)piperidin-4-ol



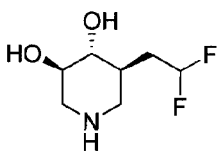
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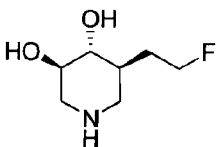
(3R,4R,5S)-5-(1,1-difluoroethyl)piperidine-3,4-diol



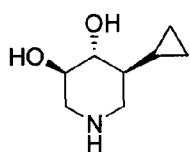
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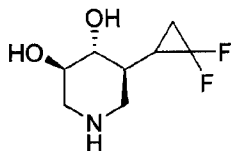
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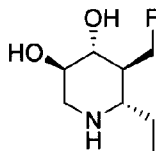
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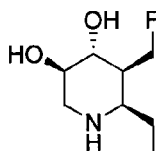
(3R,4R,5S)-5-cyclopropylpiperidine-3,4-diol



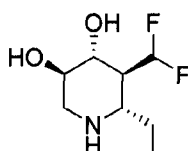
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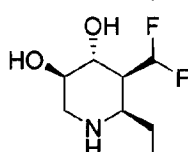
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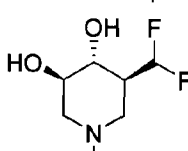
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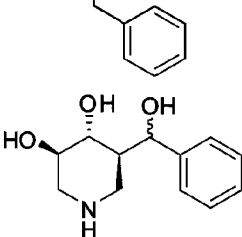
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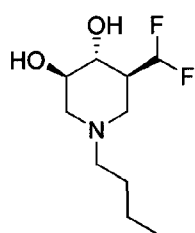
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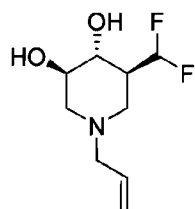
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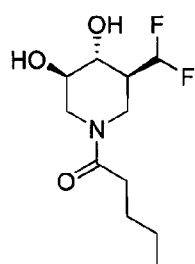
(3R,4R,5R)-5-((S)-hydroxy(phenyl)methyl)piperidine-3,4-diol



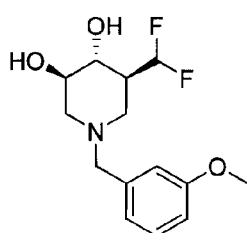
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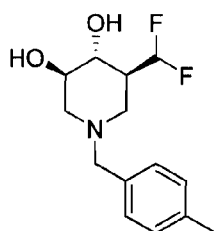
(3R,4R,5S)-1-allyl-5-(difluoromethyl)piperidine-3,4-diol



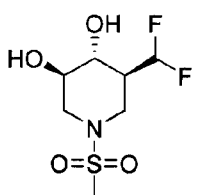
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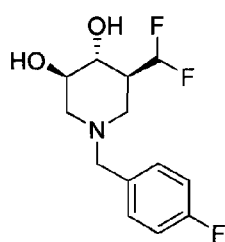
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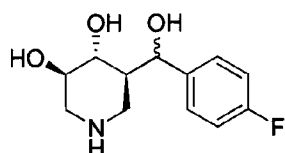
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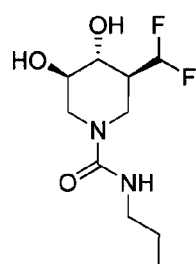
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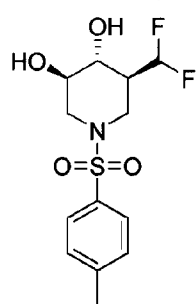
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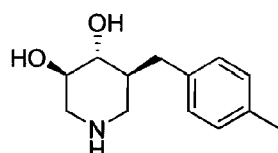
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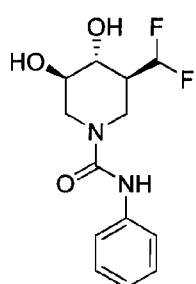
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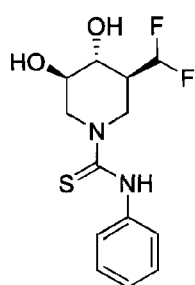
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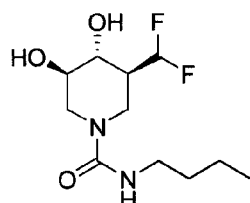
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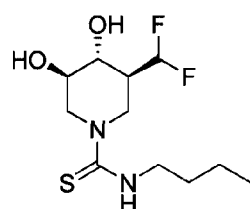
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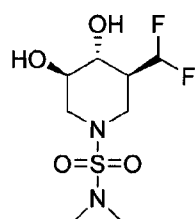
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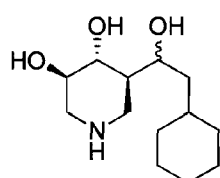
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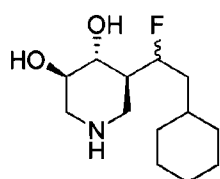
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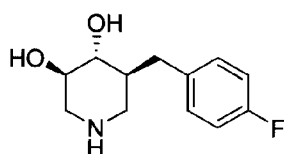
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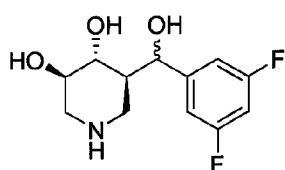
(3R,4R,5R)-5-(2-cyclohexyl-1-hydroxyethyl)piperidine-3,4-diol



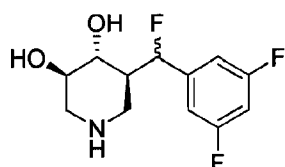
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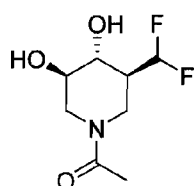
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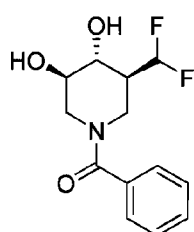
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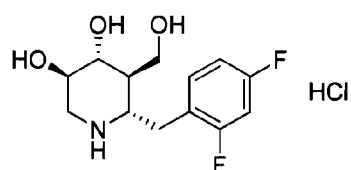
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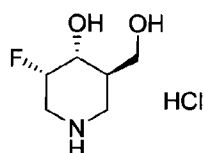
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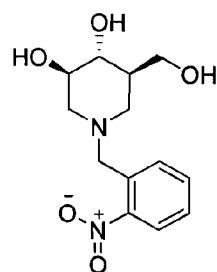
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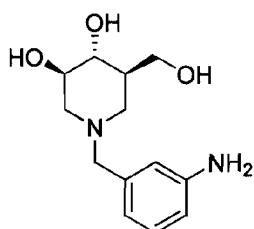
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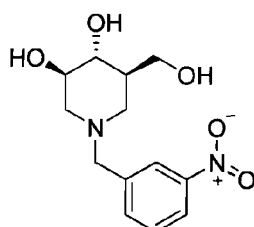
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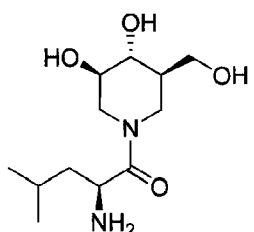
(3R,4R,5R)-5-(hydroxymethyl)-1-(2-nitrobenzyl)piperidine-3,4-diol



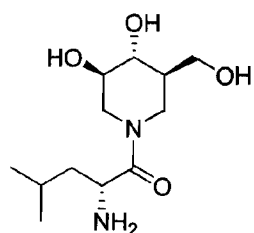
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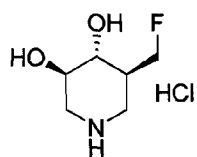
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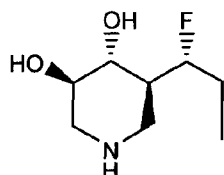
(S)-2-amino-1-((3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)piperidin-1-yl)-4-methylpentan-1-one



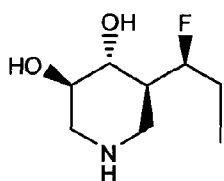
(R)-2-amino-1-((3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)piperidin-1-yl)-4-methylpentan-1-one



(3R,4R,5S)-5-(fluoromethyl)piperidine-3,4-diol
hydrochloride



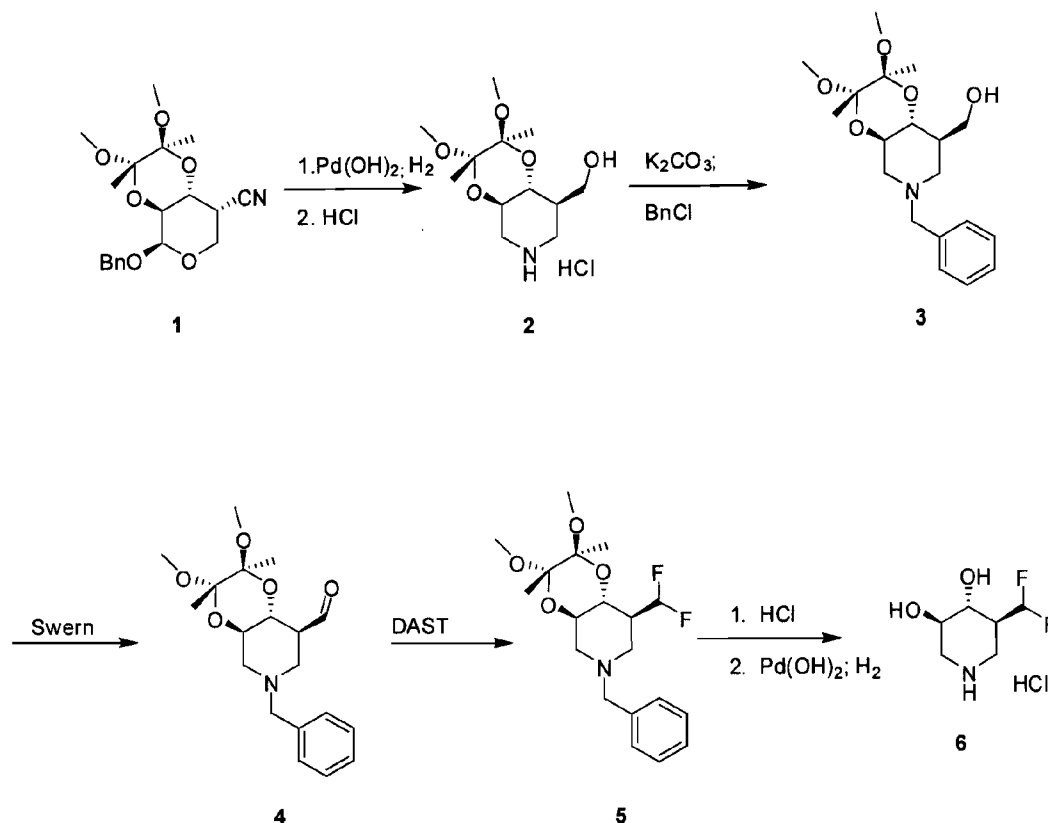
(3R,4R,5S)-5-((R)-1-fluoropropyl)piperidine-3,4-
diol hydrochloride



(3R,4R,5S)-5-((S)-1-fluoropropyl)piperidine-3,4-
diol hydrochloride

Chemical Process

[0033] Compositions of the present invention can be made in accordance of one or more of the following schemes.

Process Scheme 1:

[0034] ((2S,3S,4aR,8R,8aR)-2,3-Dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridin-8-yl)methanol Hydrochloride(2). A solution of 1 (20.0 g, 55.0 mmol) in MeOH (500 mL) was combined with Pd(OH)₂ (4-6 g) and ammonium formate (14 g, 220 mmol) and the mixture was heated at 50-55 °C. Additional amounts (3x100.0 mmol) of ammonium formate were added over the next 8 hrs. After the final addition, the reaction mixture was further stirred and heated an additional 16 hrs at 50-55 °C. The catalyst was removed by filtration and the filtrate was evaporated in vacuo. The crude product was dissolved in acetone (150 mL), filtered, and HCl in 2-PrOH was added. After seeding and

then cooling in an ice bath, the product was collected as a white crystalline solid (11.0 g, 71%). ¹H NMR (DMSO-d₆) 9.45 (s, 2H), 4.80 (t, 1H, ex), 3.85 (m, 1H), 3.0-3.75 (m, 11H), 2.8 (q, 2H), 1.95 (m, 1H), 1.2 (2, 6H).

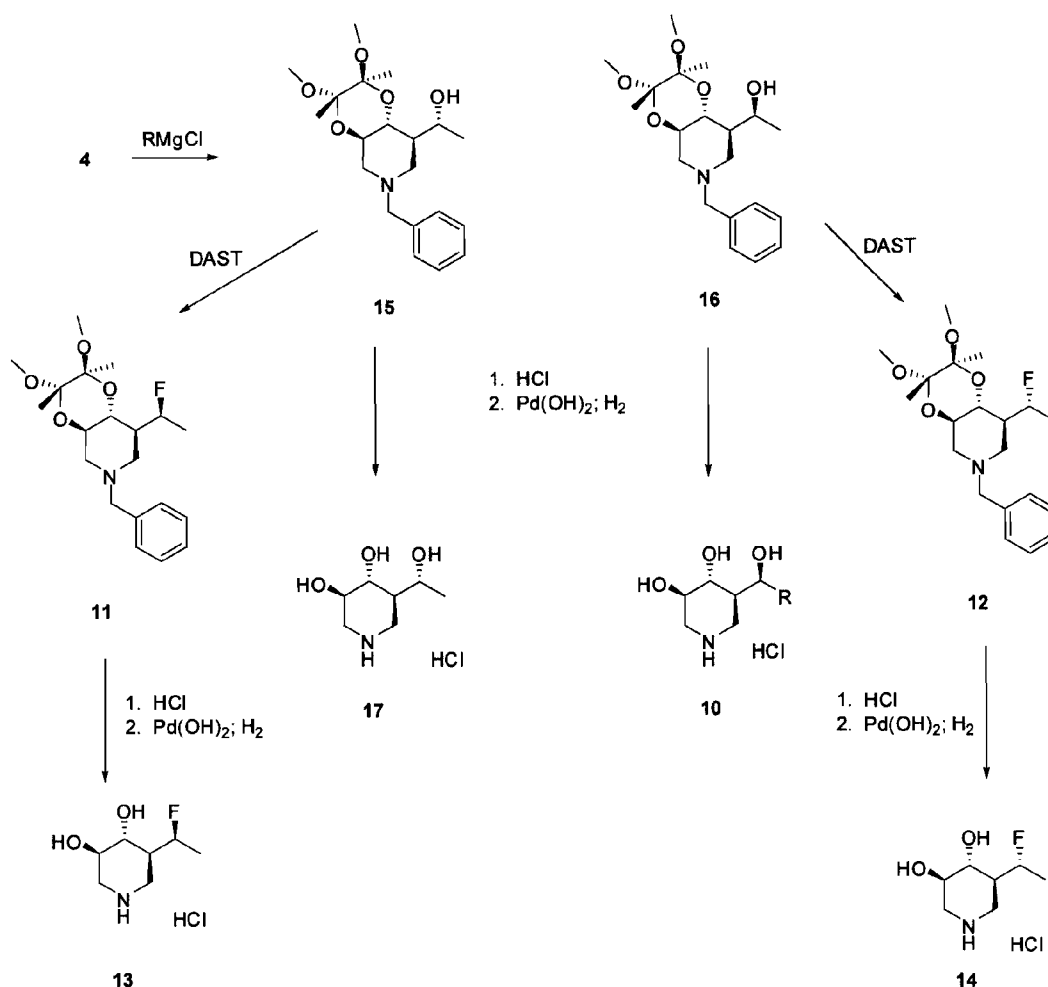
[0035] **((2S,3S,4aR,8R,8aR)-6-Benzyl-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridin-8-yl)methanol (3).** To a solution of 2 (14.85 g, 50.0 mmol) in DMF (200 mL) was added K₂CO₃ (17.25 g, 125 mmol) and the mixture was stirred at 40 °C for about 4 hrs. At this point, BnCl (5.7 mL, 50.0 mmol) was added in one portion and the reaction was stirred at 40 °C overnight. The solvent was evaporated in vacuo and the residue was suspended in water (600 mL) and HCl was added to dissolve the residue. The solution was washed with Et₂O and then basified with Na₂CO₃. The solution was extracted with EtOAc (2x) and the combined extracts were washed with water and then brine and then dried over MgSO₄. The solution was filtered and the filtrate evaporated in vacuo to give the title compound (17.2 g, >95%) as a colorless to very pale yellow viscous oil which was used without further purification. ¹H NMR (CDCl₃) 7.3 (m, 5H), 3.6-3.8 (m, 2H), 3.5 (s, 3H), 3.4 (t, 1H), 3.26 (s, 3H), 3.268 (s, 3H), 2.9 (m, 2H), 2.2 (br s, 1H), 2.05 (m, 1H), 1.85 (t, 1H), 1.28 (s, 3H), 1.26 (s, 3H).

[0036] **((2S,3S,4aR,8R,8aR)-6-Benzyl-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridin-8-yl)carboxaldehyde (General Procedure A) (4).** To a solution of DMSO (7.3 g, 96.9 mmol) in CH₂Cl₂ (150 mL) cooled to -78 °C was added a solution of oxalyl chloride (6.1 mL, 72.8 mmol) in CH₂Cl₂ dropwise. After the addition was complete the reaction mixture was stirred for an additional 30 min at which point a solution of 3 (17.0 g, 48.4 mmol) in CH₂Cl₂ was added dropwise. After addition was complete, the reaction was stirred for 1 hr at -78 °C and then diisopropylethylamine (34.4 mL, 193 mmol) was added dropwise. After this addition was complete, the cooling bath was removed and the reaction mixture was allowed to warm to 0 °C when saturated NaHCO₃ was added. The mixture was diluted with some additional CH₂Cl₂ and then the organic layer was separated and dried over MgSO₄. After filtering, the

solvent was evaporated in vacuo and the crude product was purified by silica gel chromatography (Hex/EtOAc) to give the title compound (12.7 g, 75%) as a viscous oil. ¹H NMR (CDCl₃) 9.73 (s, 1H), 7.2 (m, 5H), 3.75 (m, 2H), 3.5 (q, 2H), 3.2 (2s, 6H), 2.7-3.0 (m, 3H), 2.05 (m, 2H), 1.25 (2s, 6H).

[0037] **((2S,3S,4aR,8S,8aR)-6-Benzyl-8,8-difluoromethyl-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridine Hydrochloride (General Procedure B) (5).** To a solution of DAST (1.4 mL, 10.3 mmol) in CH₂Cl₂ (50 mL) cooled to -15 °C was added a solution of 4 (2.4 g, 6.9 mmol) dropwise. After 10 minutes, the ice bath was removed and the reaction was stirred at room temperature overnight. At this point the reaction mixture was again cooled in an ice bath and the reaction was quenched by addition of saturated NaHCO₃ (dropwise at first since this does produce a slight exotherm). The organic layer was separated and dried over Na₂SO₄, filtered and the solvent was evaporated in vacuo to give a yellow oil. The residue was purified by chromatography on silica gel (Hex/EtOAc) to give the title compound (1.6 g, 62%) as a colorless oil. ¹H NMR (CDCl₃) 7.2 (m, 5H), 6.0 (dt, 1H), 3.75 (m, 1H), 3.55 (m, 3H), 3.2 (2s, 6H), 2.95 (m, 1H), 2.85 (m, 1H), 2.3 (m, 2H), 1.5 (br s, 1H), 1.2 (2s, 6H).

[0038] **(3R,4R, 5S)-5-(Difluoromethyl)piperidine 3,4-diol Hydrochloride (General Procedure C) (6).** Compound 5 (1.6 g, 4.3 mmol) was heated at reflux in a mixture of EtOH/H₂O/HCl (40 mL/40 mL/5 mL) and the reaction monitored by HPLC until the starting material could no longer be detected. The solvent was evaporated in vacuo and then co-evaporated 2x with EtOH. The residue was dissolved in MeOH and hydrogenated over Pd(OH)₂. When complete, the catalyst was removed by filtration and the filtrate evaporated in vacuo. The residue was recrystallized from EtOH (50 mL) to the title compound (0.55 g, 66%) as a white solid (mp 168-170 °C). ¹H NMR (D₂O) 6.15 (dt, 1H), 4.3-4.8 (m, 2H), 3.0 (t, 1H), 2.85 (t, 1H), 2.3 (m, 1H).



[0039] (R)and(S)-1-((2S,3S,4aR,8R,8aR)-6-Benzyl-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridin-8-yl)ethanol **General Procedure D(15/16)**. To a solution of **4** (7.0 g, 20.0 mmol) in dry THF (100 mL) was added MeMgBr (20.0 mL, 1.4 M in 3:1 THF/toluene) and the reaction was stirred overnight at room temperature. The reaction was quenched with saturated NH_4Cl and the mixture was extracted with EtOAc (2x). The combined extracts were washed with brine, dried over Na_2SO_4 and the filtrate was evaporated in vacuo. The residue was purified by silica gel chromatography (hexane/2-PrOH) to give the major isomer (**15**) (1.6 g, 24.6%). ^1H NMR (CDCl_3). 7.3 (m, 5H), 4.15 (m, 1H), 3.5-3.9 (m, 3H), 3.3 (2s, 6H), 2.85 (m, 2H),

2.0 (2m, 4H), 1.3 (2s, 6H), 1.2 (d, 3H). The minor isomer (16) was also isolated (0.55 g, 7.5%) 7.3 (m, 5H), 3.75 (m, 2H), 3.5 (m, 2H), 3.2 (2s, 6H), 2.8 (m, 2H), 2.0 (t, 1H), 1.75 (m, 2H), 1.2 (2s, 6H), 1.0 (d, 3H).

[0040] **(3R,4R, 5R)-5((R)-1-Hydroxyethyl)piperidine 3,4-diol (17).** Compound 15 (0.55 g, 1.5 mmol) was stirred in a mixture of 9/1 TFA:H₂O (20 mL) until the starting material could no longer be detected by HPLC. The volatiles were removed and the residue was co-evaporated 2-3x with EtOH and then dissolved in EtOH and treated with solid K₂CO₃. After filtering the solid, the filtrate was evaporated in vacuo, and the residue was converted to an HCl salt and hydrogenated over Pd(OH)₂. The catalyst was filtered and the filtrate evaporated in vacuo. The crude product was purified using an ion exchange resin (Dowex 50WX8-200) eluting with 0.1 N NH₄OH. The appropriate fractions were combined and lyophilized to give the title compound (0.12 g, 50%). ¹H NMR (D₂O) 4.2 (q, 1H), 3.65 (m, 1H), 3.45 (m, 3H), 2.8 (m, 2H), 1.65 (m, 1H), 1.15 (d, 3H).

[0041] **(3R,4R, 5R)-5((S)-1-Hydroxyethyl)piperidine 3,4-diol (10).** Compound 16 (0.34 g, 0.93 mmol) was deprotected as described above to give the title compound (0.11 g, 75%). ¹H NMR (D₂O) 4.15 (m, 2H), 3.5 (m, 1H), 3.35 (t, 1H), 3.15 (m, 2H), 1.8 (m, 1H), 1.1 (d, 3H).

[0042] **((2S,3S,4aR,8R,8aR)-6-Benzyl-8(S)-(1fluoroethyl)-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridine (11).** Compound 15 (1.8 g, 5.0 mmol) was fluorinated using General Procedure B. Silica gel chromatography (Hex/EtOAc) gave the title compound (0.42 g, 23%). ¹H NMR (CDCl₃) 7.25 (m, 5H), 4.7-4.9 (dq, 1H), 3.75 (m, 2H), 3.4 (m, 2H), 3.2 (2s, 6H), 2.8 (m, 2H), 2.0 (m, 3H), 1.35 (dd, 3H), 1.2 (2s, 6H).

[0043] **(3R,4R, 5R)-5((S)-1-Fluoroethyl)piperidine 3,4-diol Hydrochloride (13).** Compound 11 (0.42 g, 1.14 mmol) was deprotected as described in General Procedure C. After catalyst was removed, the filtrate was evaporated in vacuo and then co-evaporated with EtOH (2x). The resulting residue was triturated with acetone to give the title compound (0.20 g, 88%) as a white solid. ¹H NMR (DMSO-d₆) 9.0 (br s, 2H), 5.6 (d, 1H, ex), 5.4 (d, 1H, ex),

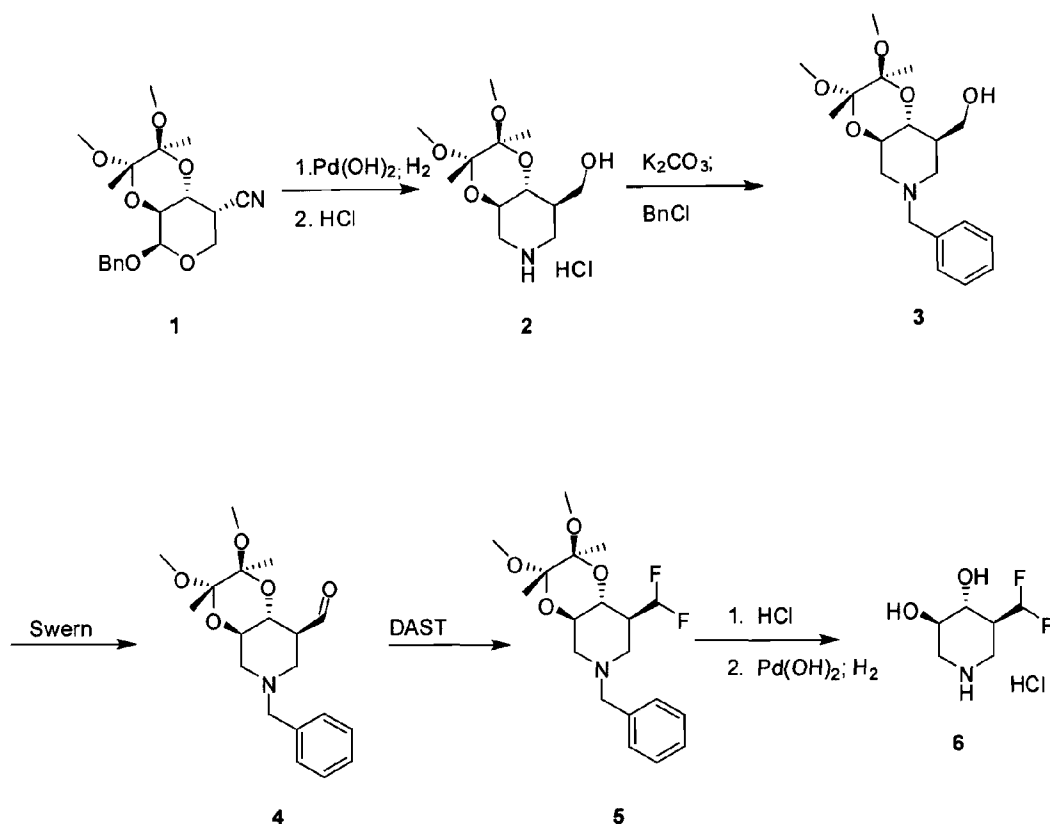
5.0-5.2 (dq, 1H), 3.55 (m, 1H), 3.2 (m, 2H), 2.9 (t, 1H), 2.7 (t, 1H), 2.2 (m, 1H), 1.3 (dd, 3H).

[0044] **((2S,3S,4aR,8R,8aR)-6-Benzyl-8(R)-(1fluoroethyl)-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridine (12).**

Compound 16 (0.55 g, 1.5 mmol) was fluorinated using General Procedure B to give the title compound (0.22 g, 40%). ¹H NMR (CDCl₃) 7.3 (m, 5H), 5.0 (dq, 1H), 3.8 (m, 1H), 3.5-3.75 (m, 3H), 3.3 (2s, 6H), 3.0 (d, 1H), 2.9 (m, 1H), 2.1 (m, 2H), 1.85 (m, 1H), 1.3 (2s, 6H).

[0045] **(3R,4R, 5R)-5((R)-(1-Fluoroethyl)piperdine 3,4-diol Hydrochloride (14).**

Compound 12 (0.22 g, 0.6 mmol) was deprotected as described in General Procedure C. After catalyst was removed, the filtrate was evaporated in vacuo and then co-evaporated with EtOH (2x). The resulting residue was triturated with acetone to give the title compound (0.08 g, 67%) as a white solid. ¹H NMR (D₂O) 5.1 (dq, 1H), 3.5 (m, 4H), 2.8 (m, 2H), 1.8 (m, 1H), 1.3 (dd, 3H).

Process Scheme 2:

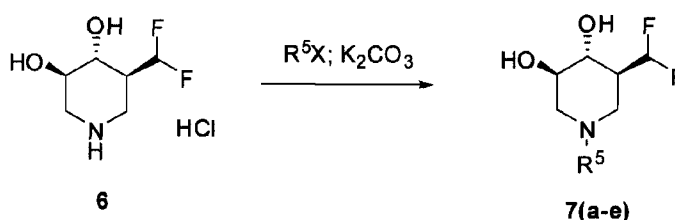
[0046] ((2*S*,3*S*,4*aR*,8*R*,8*aR*)-2,3-Dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-*c*]pyridin-8-yl)methanol Hydrochloride(2). A solution of 1 (20.0 g, 55.0 mmol) in MeOH (500 mL) was combined with $\text{Pd}(\text{OH})_2$ (4-6 g) and ammonium formate (14 g, 220 mmol) and the mixture was heated at 50-55 °C. Additional amounts (3x100.0 mmol) of ammonium formate were added over the next 8 hrs. After the final addition, the reaction mixture was further stirred and heated an additional 16 hrs at 50-55 °C. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The crude product was dissolved in acetone (150 mL), filtered, and HCl in 2-PrOH was added. After seeding and then cooling in an ice bath, the product was collected as a white crystalline solid (11.0 g, 71%). ^1H NMR ($\text{DMSO}-d_6$) 9.45 (s, 2H), 4.80 (t, 1H, ex), 3.85 (m, 1H), 3.0-3.75 (m, 11H), 2.8 (q, 2H), 1.95 (m, 1H), 1.2 (2, 6H).

[0047] **((2S,3S,4aR,8R,8aR)-6-Benzyl-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridin-8-yl)methanol (3).** To a solution of **2** (14.85 g, 50.0 mmol) in DMF (200 mL) was added K₂CO₃ (17.25 g, 125 mmol) and the mixture was stirred at 40 °C for about 4 hrs. At this point, BnCl (5.7 mL, 50.0 mmol) was added in one portion and the reaction was stirred at 40 °C overnight. The solvent was evaporated *in vacuo* and the residue was suspended in water (600 mL) and HCl was added to dissolve the residue. The solution was washed with Et₂O and then basified with Na₂CO₃. The solution was extracted with EtOAc (2x) and the combined extracts were washed with water and then brine and then dried over MgSO₄. The solution was filtered and the filtrate evaporated *in vacuo* to give the title compound (17.2 g, >95%) as a colorless to very pale yellow viscous oil which was used without further purification. ¹H NMR (CDCl₃) 7.3 (m, 5H), 3.6-3.8 (m, 2H), 3.5 (s, 3H), 3.4 (t, 1H), 3.26 (s, 3H), 3.268 (s, 3H), 2.9 (m, 2H), 2.2 (br s, 1H), 2.05 (m, 1H), 1.85 (t, 1H), 1.28 (s, 3H), 1.26 (s, 3H).

[0048] **((2S,3S,4aR,8R,8aR)-6-Benzyl-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridin-8-yl)carboxaldehyde (General Procedure A) (4).** To a solution of DMSO (7.3 g, 96.9 mmol) in CH₂Cl₂ (150 mL) cooled to -78 °C was added a solution of oxalyl chloride (6.1 mL, 72.8 mmol) in CH₂Cl₂ dropwise. After the addition was complete the reaction mixture was stirred for an additional 30 min at which point a solution of **3** (17.0 g, 48.4 mmol) in CH₂Cl₂ was added dropwise. After addition was complete, the reaction was stirred for 1 hr at -78 °C and then diisopropylethylamine (34.4 mL, 193 mmol) was added dropwise. After this addition was complete, the cooling bath was removed and the reaction mixture was allowed to warm to 0 °C when saturated NaHCO₃ was added. The mixture was diluted with some additional CH₂Cl₂ and then the organic layer was separated and dried over MgSO₄. After filtering, the solvent was evaporated *in vacuo* and the crude product was purified by silica gel chromatography (Hex/EtOAc) to give the title compound (12.7 g, 75%) as a viscous oil. ¹H NMR (CDCl₃) 9.73 (s, 1H), 7.2 (m, 5H), 3.75 (m, 2H), 3.5 (q, 2H), 3.2 (2s, 6H), 2.7-3.0 (m, 3H), 2.05 (m, 2H), 1.25 (2s, 6H).

[0049] **((2S,3S,4aR,8S,8aR)-6-Benzyl-8,8-difluoromethyl-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridine Hydrochloride (General Procedure B) (5).** To a solution of DAST (1.4 mL, 10.3 mmol) in CH_2Cl_2 (50 mL) cooled to $-15\text{ }^\circ\text{C}$ was added a solution of **4** (2.4 g, 6.9 mmol) dropwise. After 10 minutes, the ice bath was removed and the reaction was stirred at room temperature overnight. At this point the reaction mixture was again cooled in an ice bath and the reaction was quenched by addition of saturated NaHCO_3 (dropwise at first since this does produce a slight exotherm). The organic layer was separated and dried over Na_2SO_4 , filtered and the solvent was evaporated *in vacuo* to give a yellow oil. The residue was purified by chromatography on silica gel (Hex/EtOAc) to give the title compound (1.6 g, 62%) as a colorless oil. ^1H NMR (CDCl_3) 7.2 (m, 5H), 6.0 (dt, 1H), 3.75 (m, 1H), 3.55 (m, 3H), 3.2 (2s, 6H), 2.95 (m, 1H), 2.85 (m, 1H), 2.3 (m, 2H), 1.5 (br s, 1H), 1.2 (2s, 6H).

[0050] **(3R,4R, 5S)-5-(Difluoromethyl)piperidine 3,4-diol Hydrochloride (General Procedure C) (6).** Compound **5** (1.6 g, 4.3 mmol) was heated at reflux in a mixture of EtOH/ H_2O /HCl (40 mL/40 mL/5 mL) and the reaction monitored by HPLC until the starting material could no longer be detected. The solvent was evaporated *in vacuo* and then co-evaporated 2x with EtOH. The residue was dissolved in MeOH and hydrogenated over $\text{Pd}(\text{OH})_2$. When complete, the catalyst was removed by filtration and the filtrate evaporated *in vacuo*. The residue was recrystallized from EtOH (50 mL) to the title compound (0.55 g, 66%) as a white solid (mp $168\text{--}170\text{ }^\circ\text{C}$). ^1H NMR (D_2O) 6.15 (dt, 1H), 4.3–4.8 (m, 2H), 3.0 (t, 1H), 2.85 (t, 1H), 2.3 (m, 1H).



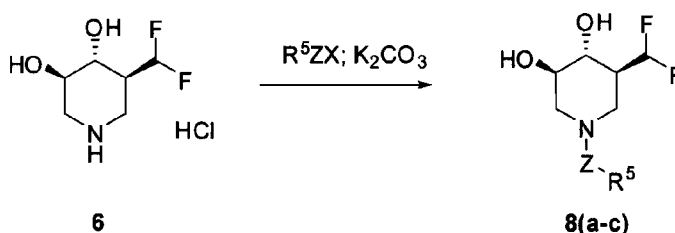
[0051] **(3R,4R, 5S)-1-Butyl-5-(difluoromethyl)piperidine 3,4-diol (General Procedure D) (7a; R⁵ = Bu).** A mixture of **6** (0.30 g, 1.4 mmol), K₂CO₃ (0.48 g, 3.5 mmol) and BuBr (0.20 g, 1.4 mmol) was combined in DMF (10 mL) and heated overnight at 60 °C. The solvent was evaporated *in vacuo* and the residue was dissolved in EtOAc, washed with water and then brine and dried over Na₂SO₄. After filtration, the filtrate was evaporated in vacuo to give the crude product which was purified by chromatography (CH₂Cl₂/(9:1) MeOH/NH₄OH) to give the title compound (0.25g, 80%) as a colorless sirup. MH⁺ = 224. ¹H NMR (DMSO-*d*₆) 6.2 (t, 1H, *J* = 57 Hz), 5.13 (d, 1H, *ex*), 4.91 (d, 1H, *ex*), 3.3 (m, 1H), 3.1 (m, 1H), 2.9 (m, 2H), 2.3 (m, 2H), 1.95 (m, 2H), 1.75 (t, 1H), 1.2-1.5 (2m, 4H), 0.9 (t, 3H).

[0052] **(3R,4R, 5S)-1-Allyl-5-(difluoromethyl)piperidine 3,4-diol (7b; R⁵ = allyl).** Following General Procedure D using allyl bromide (0.17 g, 1.4mmol) the tile compound was obtained as a white solid (0.22 g, 76%). MH⁺ = 208. ¹H NMR (DMSO-*d*₆) 6.2 (t, 1H, *J* = 57 Hz), 5.8 (m, 1H), 5.2 (m, 3H), 4.92 (d, 1H), 3.3 (m, 1H), 3.1 (1H), 2.95 (d, 2H), 2.85 (d, 2H), 1.9 (br m, 2H), 1.75 (t, 1H).

[0053] **(3R,4R, 5S)-5-(Difluoromethyl)-1-(4-fluorobenzyl)piperidine 3,4-diol (7c; R⁵ = 4-fluorobenzyl).** Following General Procedure D except that reaction was run at room temperature and using 4-fluorobenzyl bromide (0.26 g, 1.4mmol) the tile compound was obtained as a white solid (0.22 g, 56%). MH⁺ = 276. ¹H NMR (DMSO-*d*₆) 7.4 (m, 2H), 7.15 (m, 2H), 6.2 (t, 1H, *J* = 57 Hz), 5.2 (d, 1H, *ex*), 4.9 (d, 1H, *ex*), 3.5 (q, 2H), 3.3 (m, 1H), 3.1 (m, 1H), 2.8 (m, 2H), 2.0 (m, 2H), 1.8 (t, 1H).

[0054] **(3R,4R, 5S)-5-(Difluoromethyl)-1-(4-methylbenzyl)piperidine 3,4-diol (7d; R⁵ = 4-methylbenzyl).** Following General Procedure D except that reaction was run at room temperature and using 4-methylbenzyl bromide (0.26 g, 1.4mmol) the tile compound was obtained as a white solid (0.30, 81%). MH⁺ = 272. ¹H NMR (DMSO-*d*₆) 7.2 (m, 4H), 6.2 (t, 1H, *J* = 57 Hz), 5.2 (d, 1H, *ex*), 4.9 (d, 1H, *ex*), 3.5 (q, 2H), 3.3 (1H), 3.05 (m, 1H), 2.8 (m, 2H), 2.5 (s, 3H), 1.95 (m, 2H), 1.8 (t, 1H).

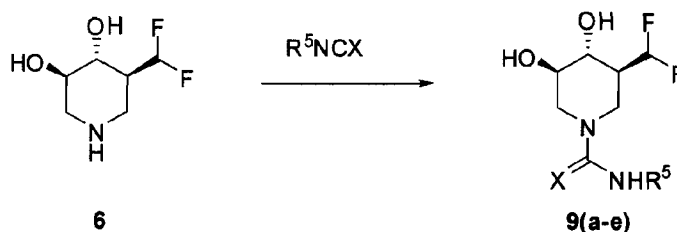
[0055] **(3*R*,4*R*, 5*S*)-5-(Difluoromethyl)-1-(4-methoxybenzyl)piperidine 3,4-diol (7e; R⁵ = 4-methoxybenzyl).** Following General Procedure D except that reaction was run at room temperature and using 4-methoxybenzyl chloride (0.26 g, 1.4mmol) the title compound was obtained as a colorless sirup (0.19 g, 49%). $MH^+ = 288$. ¹H NMR (DMSO-*d*₆) 7.3 (m, 1H), 6.85 (m, 3H) 6.2 (t, 1H, *J* = 57 Hz), 5.2 (d, 1H, *ex*), 4.9 (d, 1H, *ex*), 3.75 (s, 3H), 3.5 (q, 2H), 3.4 (m, 1H), 3.1 (m, 1H), 2.85(m, 2H), 1.95 (m, 2H), 1.8 (t, 1H).



[0056] **1-((3*S*,4*R*, 5*R*)-3-(Difluoromethyl)-4,5-dihydroxypiperidine-1-yl)pentane-1-one (8a; Z = CO; R⁵ = butyl).** Following General Procedure D, except that the reaction was run at room temperature and using pentanoyl chloride (0.17 g, 1.4 mmol), the title compound was obtained as a white solid (0.26 g, 71%). $MH^+ = 252$. ¹H NMR (DMSO-*d*₆) 5.9-6.5 (dt, 1H), 5.35 (m, 1H, *ex*), 5.25 (m, 1H, *ex*), 4.2 (dd, 1H), 3.75 (dd, 1H), 3.35 (m, 2H), 3.1 (m, 1H), 2.85 (m, 1H), 2.3 (t, 2H), 1.9 br m, 1H), 1.4 (m, 2H), 1.25 (m, 2H), 0.85 (t, 3H).

[0057] **(3*R*,4*R*, 5*S*)-5-(Difluoromethyl)-1-(methanesulfonyl)piperidine 3,4-diol (8b; Z = SO₂; R⁵ = Me)** Following General Procedure D except that the reaction was run at room temperature and using methanesulfonyl chloride (0.16 g, 1.4 mmol), the title compound was obtained as a white solid (0.17 g, 51%). ¹H NMR (DMSO-*d*₆) 6.2 (t, 1H, *J* = 53 Hz), 5.43 (d, 1H, *ex*), 5.38 (d, 1H, *ex*), 3.2-3.7 (m, 4H), 2.95 (s, 3H), 2.85 (m, 1H), 2.7 (t, 1H), 2.1 (br s, 1H). **(3*R*,4*R*, 5*S*)-5-(Difluoromethyl)-1-tosylpiperidine 3,4-diol (8b; Z = SO₂; R⁵ = Ph)** Following General Procedure D except that the reaction was run at room temperature and using toluenesulfonyl chloride (0.26, 1.4 mmol), the title compound was obtained

as a white solid (0.35 g, 67%). ^1H NMR ($\text{DMSO}-d_6$) 7.6 (d, 2H), 7.45 (d, 2H), 6.25 (t, 1H, $J = 53$ Hz), 5.4 (2d, 2H, ex), 3.3-3.55 (m, 4H), 3.2 (m, 1H), 2.5 (m, 3H), 2.4 (t, 1H), 2.1 (m, 1H).



[0058] **(3S,4R, 5R)-3-(Difluoromethyl)-4,5-dihydroxy-N-propylpiperidine-1-carboxamide (General Procedure E) (9a; X = O; R⁵ = propyl).** To a solution of **6** (free base) (0.29 g, 1.2mmol) in dry DMF (5mL), was added propyl isocyanate (0.10 g, 1.2 mmol) and the reaction was stirred at room temperature overnight. The solvent was evaporated in vacuo and the residue was purified by chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$) to give the title compound as a white solid (0.14 g, 48%). $\text{MH}^+ = 253$. ^1H NMR ($\text{DMSO}-d_6$) 6.7 (t, 1H), 6.22 (t, 1H, $J = 53$ Hz), 5.25 (d, 1H, ex), 5.15 (d, 1H, ex), 4.05 (d, 1H), 3.9 (d, 1H), 3.3 (m, 2H), 3.0 (q, 2H), 2.5 (m, 1H), 1.8 (br d, 1H), 1.4 (m, 2H), 0.85 (t, 3H).

[0059] **(3S,4R, 5R)-3-(Difluoromethyl)-4,5-dihydroxy-N-phenylpiperidine-1-carboxamide (9b; X = O; R⁵ = phenyl).** Following General Procedure E and using phenyl isocyanate (0.14 g, 1.2 mmol) the title compound was obtained as a white solid (0.21 g, 62%). $\text{MH}^+ = 287$. ^1H NMR ($\text{DMSO}-d_6$) 8.7 (s, 1H), 7.45 (d, 2H), 7.3 (t, 2H), 6.95 (t, 1H), 6.3 (t, 1H, $J = 53$ Hz), 5.35 (d, 1H), 5.25 (d, 1H), 4.1 (t, 2H), 3.3 (m, 2H), 2.85 (t, 1H), 2.75 (t, 1H), 1.95 (br d, 1H).

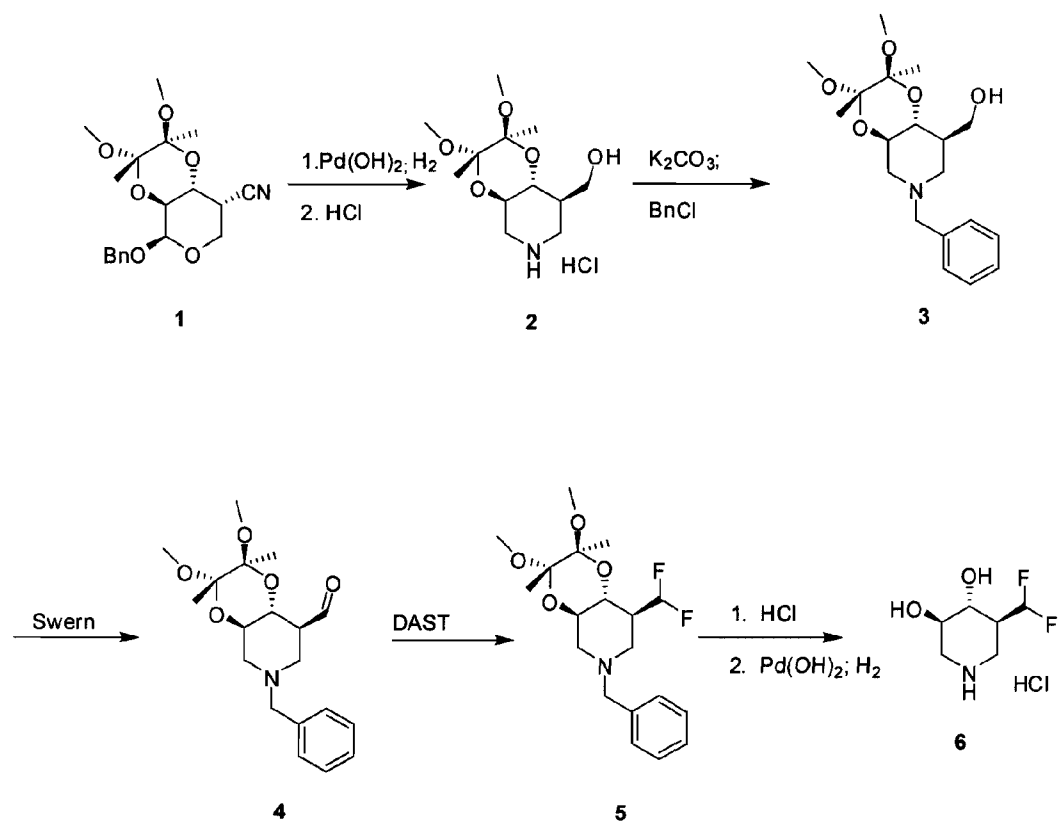
[0060] **(3S,4R, 5R)-3-(Difluoromethyl)-4,5-dihydroxy-N-butylpiperidine-1-carboxamide (9c; X = O; R⁵ = butyl).** Following General Procedure E and using butyl isocyanate (0.12 g, 1.2 mmol) the title compound was obtained as a white solid (0.24 g, 76%). $\text{MH}^+ = 267$. ^1H NMR ($\text{DMSO}-d_6$) 6.6 (t, 1H), 6.2 (t, 1H, $J = 53$ Hz), 5.25 (d, 1H), 5.1 (d, 1H), 4.05 (d, 1H), 3.9 (d, 1H), 3.35 (m, 2H),

3.05 (q, 2H), 2.65 (t, 1H), 2.45 (m, 1H), 1.8 (br d, 1H), 1.2-1.4 (2m, 4H), 0.85 (t, 3H).

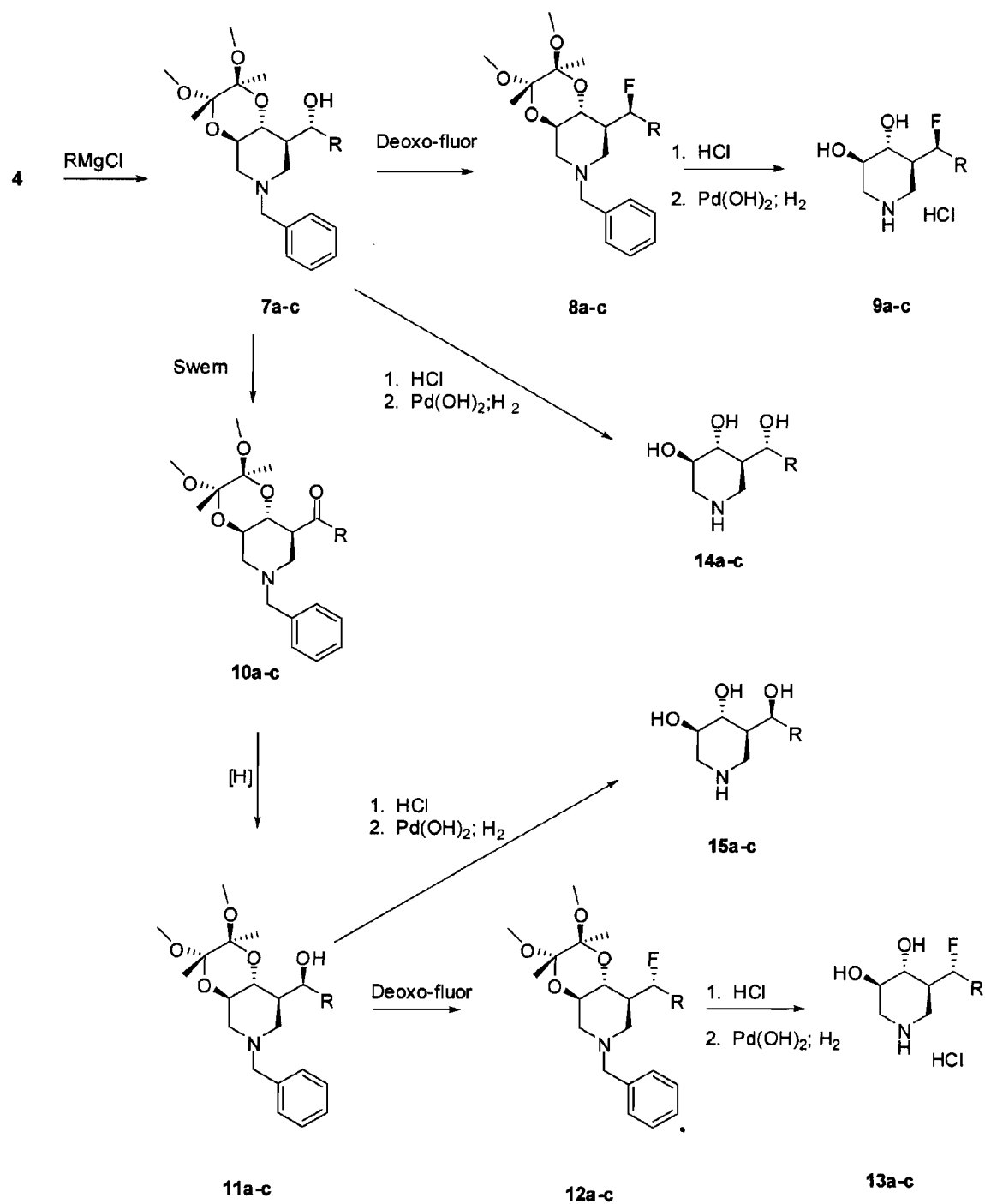
[0061] **(3*S*,4*R*, 5*R*)-3-(Difluoromethyl)-4,5-dihydroxy-*N*-butylpiperdine-1-carbthioamide (9d; X = S; R⁵ = butyl).** Following General Procedure E and using butyl isothiocyanate (0.14 g, 1.2 mmol) the title compound was obtained as a colorless sirup (0.21 g, 63%). $MH^+ = 283$. ¹H NMR (DMSO-*d*₆) 7.85 (t, 1H), 6.25 (t, 1H), 5.35 (2d, 2H), 4.8 (d, 1H), 4.45 (d, 1H), 3.45 (m, 2H), 3.25 (m, 1H), 3.05 (t, 1H), 2.8 (t, 1H), 1.85 (br d, 1H), 1.4 (m, 2H), 1.35 (m, 2H), 1.1 (m, 1H), 0.95 (t, 3H).

[0062] **(3*S*,4*R*, 5*R*)-3-(Difluoromethyl)-4,5-dihydroxy-*N*-phenylpiperdine-1-carbthioamide (9e; X = S; R⁵ = phenyl).** Following General Procedure E and using phenyl isothiocyanate (0.16 g, 1.2 mmol) the title compound was obtained as a white solid (0.31 g, 86%). $MH^+ = 303$. ¹H NMR (DMSO-*d*₆) 9.5 (s, 1H), 7.3 (m, 4H), 7.1 (t, 1H), 6.35 (t, 1H), 5.35 (2d, 2H), 4.85 (d, 1H), 4.55 (d, 1H), 3.45 (m, 2H), 3.2 (t, 1H), 3.0 (t, 1H), 2.05 (br d, 1H).

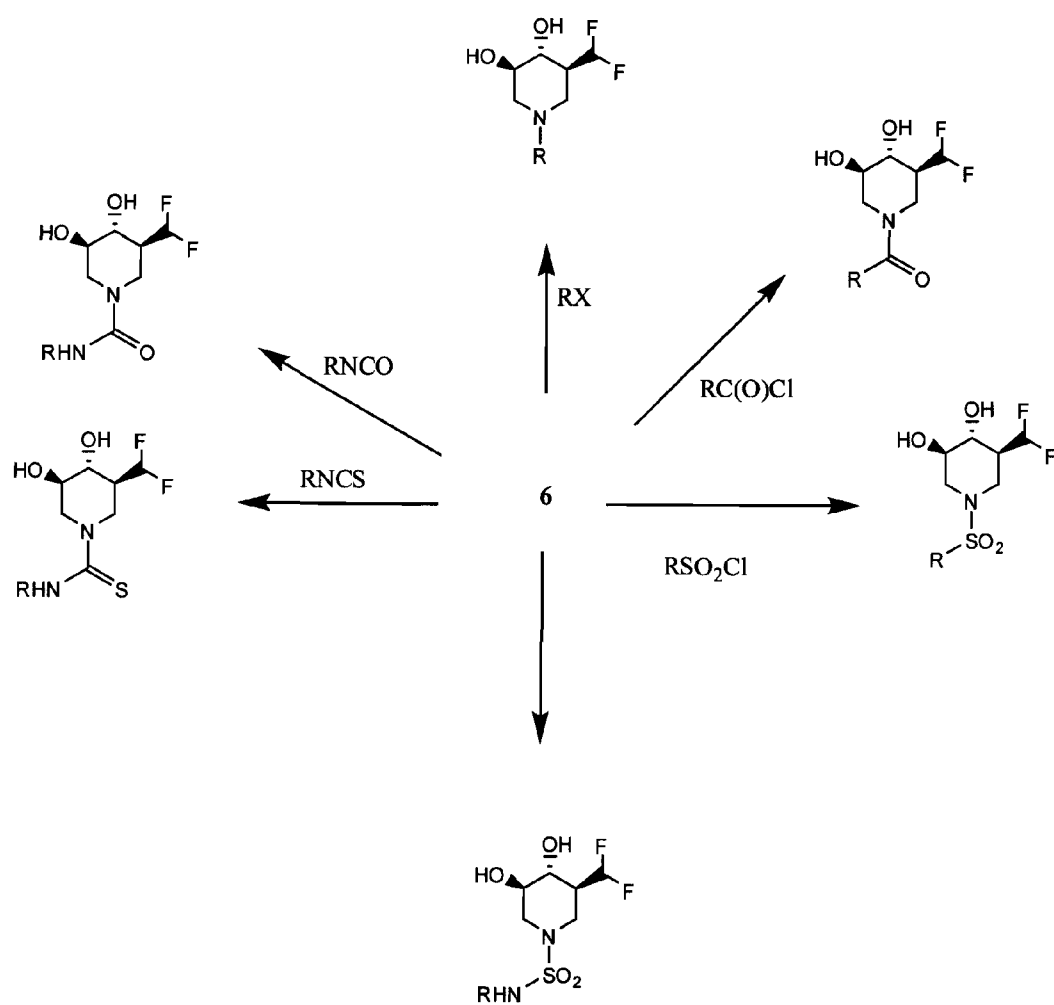
[0063] Compounds of the present invention can also be made by one skilled in the art using the following general schemes:



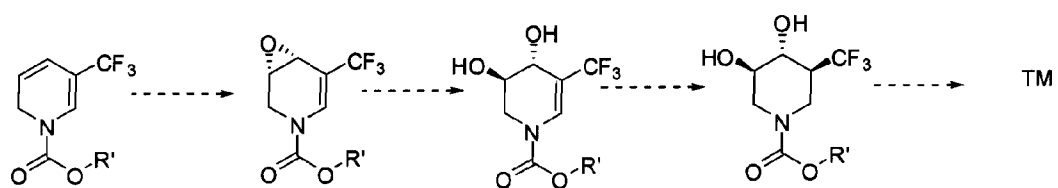
Scheme 3



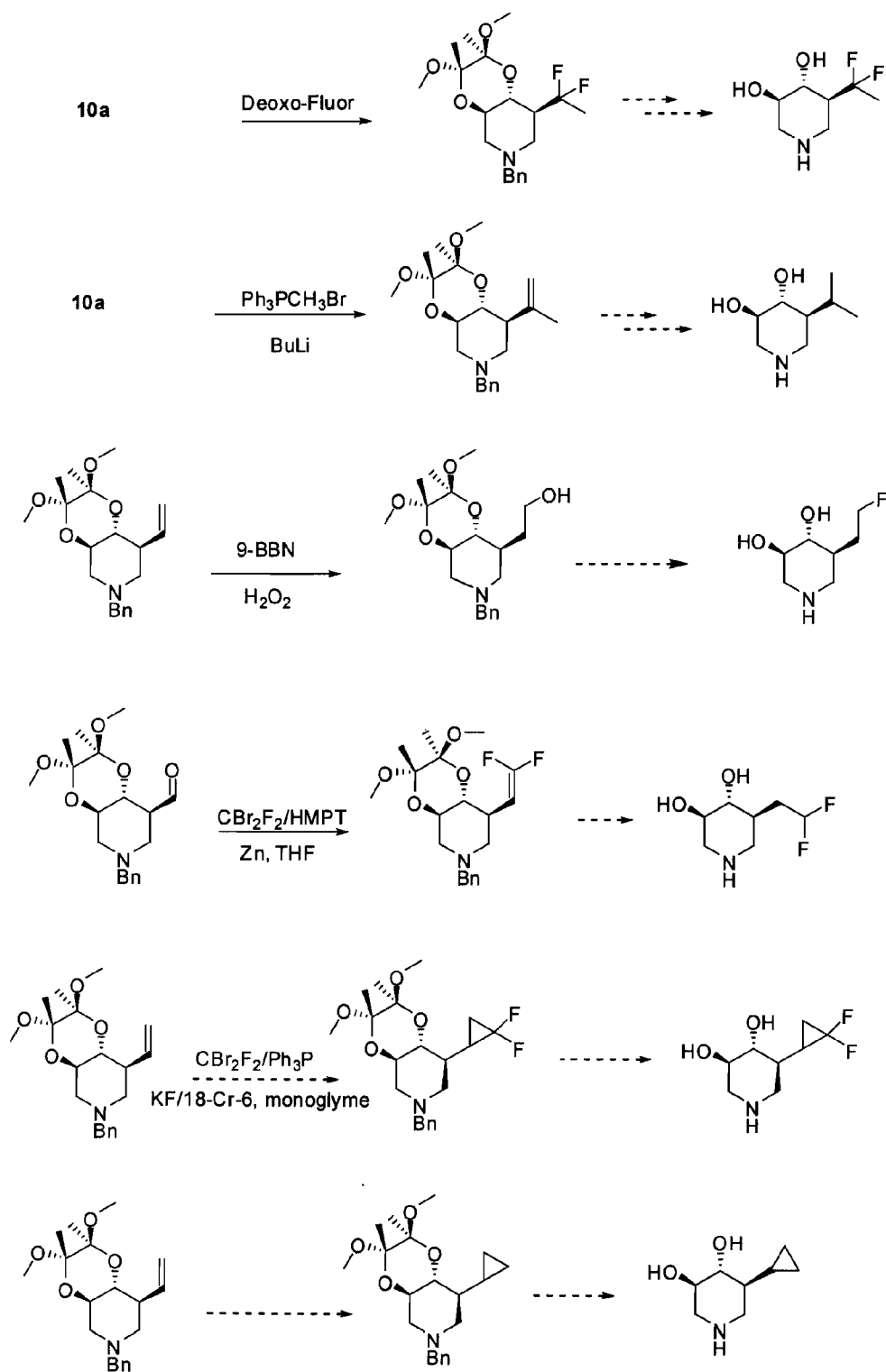
Scheme 4



Scheme 5



Scheme 6



Scheme 7

Salts, solvates and prodrugs

[0064] Compounds of the present invention include pharmaceutically acceptable salts, solvates and pro-drugs of the compounds disclosed herein. Pharmaceutically acceptable salts include salts derived from inorganic bases such as Li, Na, K, Ca, Mg, Fe, Cu, Zn, Mn; salts of organic bases such as N,N'-diacetylenediamine, glucamine, triethylamine, choline, hydroxide, dicyclohexylamine, metformin, benzylamine, trialkylamine, thiamine; chiral bases like alkylphenylamine, glycinol, phenyl glycinol, salts of natural amino acids such as glycine, alanine, valine, leucine, isoleucine, norleucine, tyrosine, cystine, cysteine, methionine, proline, hydroxy proline, histidine, ornithine, lysine, arginine, serine; non-natural amino acids such as D-isomers or substituted amino acids; guanidine, substituted guanidine wherein the substituents are selected from nitro, amino, alkyl, alkenyl, alkynyl, ammonium or substituted ammonium salts and aluminum salts. Salts may include acid addition salts where appropriate which are, hydrochlorides, sulphates, nitrates, phosphates, perchlorates, borates, hydrohalides, acetates, tartrates, maleates, citrates, succinates, palmoates, methanesulphonates, benzoates, salicylates, benzenesulfonates, ascorbates, glycerophosphates, ketoglutarates. In one embodiment, the pharmaceutically acceptable salt of the compounds disclosed herein is the hydrochloride salt.

[0065] "Solvate" denotes a physical association of a compound with one or more solvent molecules. This physical association involves varying degrees of ionic and covalent bonding, including hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolatable solvates. "Hydrate" is a solvate wherein the solvent molecule is H₂O. Other non-limiting examples of suitable solvates include alcohols (e.g., ethanولات, methanولات, and the like).

[0066] Prodrugs are compounds which are converted *in vivo* to active forms (see, e.g., R. B. Silverman, 1992, "The Organic Chemistry of Drug Design and Drug Action", Academic Press, Chapter 8, incorporated herein by reference).

Additionally, a discussion of prodrugs is provided in T. Higuchi and V. Stella, Prodrugs as Novel Delivery Systems, Volume 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, Edward B. Roche, ed., American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference thereto. Prodrugs can be used to alter the biodistribution (e.g., to allow compounds which would not typically enter the reactive site of the protease) or the pharmacokinetics for a particular compound. For example, a carboxylic acid group, can be esterified, e.g., with a methyl group or an ethyl group to yield an ester. When the ester is administered to a subject, the ester is cleaved, enzymatically or non-enzymatically, reductively, oxidatively, or hydrolytically, to reveal the anionic group. An anionic group can be esterified with moieties (e.g., acyloxymethyl esters) which are cleaved to reveal an intermediate compound which subsequently decomposes to yield the active compound.

[0067] Examples of prodrugs and their uses are well known in the art (See, e.g., Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19). The prodrugs can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound with a suitable derivatizing agent. For example hydroxy groups can be converted into esters via treatment with a carboxylic acid in the presence of a catalyst. Examples of cleavable alcohol prodrug moieties include substituted and unsubstituted, branched or unbranched lower alkyl ester moieties, (e.g., ethyl esters), lower alkenyl esters, di-lower alkyl-amino lower-alkyl esters (e.g., dimethylaminoethyl ester), acylamino lower alkyl esters, acyloxy lower alkyl esters (e.g., pivaloyloxymethyl ester), aryl esters (phenyl ester), aryl-lower alkyl esters (e.g., benzyl ester), substituted (e.g., with methyl, halo, or methoxy substituents) aryl and aryl-lower alkyl esters, amides, lower-alkyl amides, di-lower alkyl amides, and hydroxy amides.

[0068] All stereoisomers (for example, geometric isomers, optical isomers and the like) of the compounds disclosed herein (including those of the salts, solvates and prodrugs of these compounds as well as the salts and solvates of

the prodrugs), such as those which may exist due to asymmetric carbons on various substituents, including enantiomeric forms (which may exist even in the absence of asymmetric carbons), rotameric forms, atropisomers, and diastereomeric forms, are contemplated within the scope of this invention. Individual stereoisomers of these compounds may, for example, be substantially free of other isomers, or may be admixed, for example, as racemates or with all other, or other selected, stereoisomers. The chiral centers of the aforementioned compounds can have the S or R configuration as defined by the IUPAC 1974 Recommendations. The use of the terms "salt", "solvate" "prodrug" and the like, is intended to equally apply to the salt, solvate and prodrug of enantiomers, stereoisomers, rotamers, tautomers, racemates or prodrugs of the compounds of the present invention disclosed herein.

Formulations

[0069] The therapeutic agent(s) can be formulated to be suitable for any route of administration, including *e.g.*, orally in the form of tablets or capsules or liquid, or in sterile aqueous solution for injection. When the therapeutic agent(s) is formulated for oral administration, tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or another suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters,

ethyl alcohol or fractionated vegetable oils); or preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The liquid preparations may also contain buffer salts, flavoring, coloring or sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled or sustained release of the therapeutic agent(s).

[0070] In certain embodiments of the present invention, the therapeutic agent(s) is administered in a dosage form that permits systemic uptake, such that the therapeutic agent(s) may cross the blood-brain barrier so as to exert effects on neuronal cells. For example, pharmaceutical formulations of the therapeutic agent(s) suitable for parenteral/injectable use generally include sterile aqueous solutions (where water soluble), or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, polyethylene glycol, and the like), suitable mixtures thereof, or vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, benzyl alcohol, sorbic acid, and the like. In many cases, it will be reasonable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate or gelatin.

[0071] Sterile injectable solutions are prepared by incorporating the therapeutic agent(s) in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter or terminal sterilization. Generally, dispersions are prepared by incorporating the

various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

[0072] The formulation can contain an excipient. Pharmaceutically acceptable excipients which may be included in the formulation are buffers such as citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer, amino acids, urea, alcohols, ascorbic acid, phospholipids; proteins, such as serum albumin, collagen, and gelatin; salts such as EDTA or EGTA, and sodium chloride; liposomes; polyvinylpyrrolidone; sugars, such as dextran, mannitol, sorbitol, and glycerol; propylene glycol and polyethylene glycol (e.g., PEG-4000, PEG-6000); glycerol; glycine or other amino acids; and lipids. Buffer systems for use with the formulations include citrate; acetate; bicarbonate; and phosphate buffers. Phosphate buffer is a preferred embodiment.

[0073] The formulation can also contain a non-ionic detergent. Preferred non-ionic detergents include Polysorbate 20, Polysorbate 80, Triton X-100, Triton X-114, Nonidet P-40, Octyl α -glucoside, Octyl β -glucoside, Brij 35, Pluronic, and Tween 20.

Routes of Administration

[0074] The therapeutic agent(s) may be administered orally or parenterally, including intravenously, subcutaneously, intra-arterially, intraperitoneally, ophthalmically, intramuscularly, buccally, rectally, vaginally, intraorbitally, intracerebrally, intradermally, intracranially, intraspinally, intraventricularly, intrathecally, intracisternally, intracapsularly, intrapulmonarily, intranasally, transmucosally, transdermally, or via inhalation. In one preferred embodiment, the therapeutic agent(s) is administered orally.

[0075] Administration of therapeutic agent(s) may be by periodic injections of a bolus of the formulation, or may be administered by intravenous or

intraperitoneal administration from a reservoir which is external (e.g., an i.v. bag) or internal (e.g., a bioerodable implant). See, e.g., U.S. Pat. Nos. 4,407,957 and 5,798,113, each incorporated herein by reference. Intrapulmonary delivery methods and apparatus are described, for example, in U.S. Pat. Nos. 5,654,007, 5,780,014, and 5,814,607, each incorporated herein by reference. Other useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, pump delivery, encapsulated cell delivery, liposomal delivery, needle-delivered injection, needle-less injection, nebulizer, aerosolizer, electroporation, and transdermal patch. Needle-less injector devices are described in U.S. Pat. Nos. 5,879,327; 5,520,639; 5,846,233 and 5,704,911, the specifications of which are herein incorporated by reference. Any of the formulations described above can be administered using these methods.

[0076] Subcutaneous injections have the advantages allowing self-administration, while also resulting in a prolonged plasma half-life as compared to intravenous administration. Furthermore, a variety of devices designed for patient convenience, such as refillable injection pens and needle-less injection devices, may be used with the formulations of the present invention as discussed herein.

Dosage

[0077] A suitable pharmaceutical preparation is in a unit dosage form. In such form, the preparation is subdivided into suitably sized unit doses containing appropriate quantities of the active component, e.g., an effective amount to achieve the desired purpose. In certain embodiments, the therapeutic agent(s) is administered in one or more daily doses (e.g., once-a-day, twice-a-day, thrice-a-day). In certain embodiments, the therapeutic agent(s) is administered in intermittently.

[0078] Exemplary dosing regimens are described in International patent application PCT/US08/61764 published as WO 2008/134628 on June 11, 2008 and U.S. provisional patent application 61/108,192, filed on October 24, 2008,

both of which are incorporated by reference herein in their entirety. In one embodiment, the therapeutic agent(s) is administered in an intermittent dosing regimen that includes an initial "loading dose" given daily, followed by a period of non-daily interval dosing.

[0079] The amount of effective therapeutic agent(s) for preventing or treating the referenced disorder can be determined on a case-by-case basis by those skilled in the art. The amount and frequency of administration of the therapeutic agent(s) will be regulated according to the judgment of the attending clinician (physician) considering such factors as age, condition and size of the patient as well as risk for developing disorder or severity of the symptoms of the referenced disorder being treated.

Combination Drug Therapy

[0080] The therapeutic agent(s) of the present invention can be administered in combination with at least one other therapeutic agent. Administration of the therapeutic agent(s) of the present invention with at least one other therapeutic agent is understood to encompass administration that is sequential or concurrent. In one embodiment, the therapeutic agents are administered in separate dosage forms. In another embodiment, two or more therapeutic agents are administered concurrently in the same dosage form.

[0081] In certain embodiments, the therapeutic agent(s) of the present invention are administered in combination with at least one other therapeutic agent which is an anti-dyskinesia Agent (e.g., Carbidopa, Levodopa), an anti-infective agent (e.g., Miglustat), an antineoplastic agent (e.g., Busulfan, Cyclophosphamide), a gastrointestinal agent (e.g., Methylprednisolone), a micronutrient (e.g., Calcitriol, Cholecalciferol, Ergocalciferols, Vitamin D), a vasoconstrictor agent (e.g., Calcitriol). In one preferred embodiment, the aforementioned other therapeutic agents are administered when the disorder is Gaucher's disease.

[0082] In certain embodiments, the therapeutic agent(s) of the present invention are administered in combination with allopregnanolone, a low-

cholesterol diet, or cholesterol-lowering agents such as statins (e.g., Lipitor®); fibrates such as fenofibrate (Lipidil®); niacin; and/or binding resins such as cholestyramine (Questran®).

[0083] In one embodiment, the therapeutic agent(s) of the present invention is administered in combination with gene therapy. Gene therapy is contemplated both with replacement genes such as glucocerebrosidase or with inhibitory RNA (siRNA) for the SNCA gene. Gene therapy is described in more detail in U.S. Patent No. 7,446,098, filed on February 17, 2004.

[0084] In one embodiment, the therapeutic agent(s) of the present invention is administered in combination with at least one other therapeutic agent which is an anti-inflammatory agent (e.g., ibuprofen or other NSAID).

[0085] In one embodiment, the therapeutic agent(s) of the present invention is administered in combination with a substrate inhibitor for glucocerebrosidase, such as N-butyl-deoxynojirimycin (Zavesca®; miglustat available from Actelion Pharmaceuticals, US, Inc., South San Francisco, CA, US).

[0086] Combinations of the therapeutic agent(s) of the present invention with at least one other therapeutic agent which is a therapeutic agent for one or more other lysosomal enzymes are also contemplated. Table 2 contains a non-limiting list of therapeutic agents for lysosomal enzymes.

Table 2

LYSOSOMAL ENZYME	THERAPEUTIC AGENT
α -Glucosidase GenBank Accession No. Y00839	1-deoxynojirimycin (DNJ) α -homonojirimycin castanospermine
Acid β -Glucosidase (β -glucocerebrosidase) GenBank Accession No. J03059	isofagomine C-benzyl isofagomine and derivatives N-alkyl (C9-12)-DNJ Glucoimidazole (and derivatives) C-alkyl-IFG (and derivatives)

	N-alkyl- β -valeinamines Fluphenazine calystegines A ₃ , B ₁ , B ₂ and C ₁
α -Galactosidase A GenBank Accession No. NM000169	1-deoxygalactonojirimycin (DGJ) α - <i>allo</i> -homonojirimycin α - <i>galacto</i> -homonojirimycin β -1-C-butyl-deoxynojirimycin calystegines A ₂ and B ₂ N-methyl calystegines A ₂ and B ₂
Acid β -Galactosidase GenBank Accession No. M34423	4- <i>epi</i> -isofagomine 1-deoxygalactonojirimycin
Galactocerebrosidase (Acid β -Galactosidase) GenBank Accession No. D25283	4- <i>epi</i> -isofagomine 1-deoxygalactonojirimycin
Acid α -Mannosidase GenBank Accession No. U68567	1-deoxymannojirimycin Swainsonine Mannostatin A
Acid β -Mannosidase GenBank Accession No. U60337	2-hydroxy-isofagomine
Acid α -L-fucosidase GenBank Accession No. NM_000147	1-deoxyfuconojirimycin β -homofuconojirimycin 2,5-imino-1,2,5-trideoxy-L-glucitol 2,5-deoxy-2,5-imino-D-fucitol 2,5-imino-1,2,5-trideoxy-D-altritol
α -N-Acetylglucosaminidase GenBank Accession No. U40846	1,2-dideoxy-2-N-acetamido-nojirimycin
α -N-Acetylgalactosaminidase GenBank Accession No. M62783	1,2-dideoxy-2-N-acetamido-galactonojirimycin
β -Hexosaminidase A GenBank Accession No. NM_000520	2-N-acetylamino-isofagomine 1,2-dideoxy-2-acetamido-nojirimycin Nagstatin
β -Hexosaminidase B	2-N-acetamido-isofagomine

GenBank Accession No. NM_000521	1,2-dideoxy-2-acetamido-nojirimycin Nagstatin
α -L-Iduronidase GenBank Accession No. NM_000203	1-deoxyiduronojirimycin 2-carboxy-3,4,5-trideoxypiperidine
β -Glucuronidase GenBank Accession No. NM_000181	6-carboxy-isofagomine 2-carboxy-3,4,5-trideoxypiperidine
Sialidase GenBank Accession No. U84246	2,6-dideoxy-2,6, imino-sialic acid Siastatin B
Iduronate sulfatase GenBank Accession No. AF_011889	2,5-anhydromannitol-6-sulphate
Acid sphingomyelinase GenBank Accession No. M59916	desipramine, phosphatidylinositol- 4,5-diphosphate

[0087] In certain embodiments, the therapeutic agent(s) of the present invention are administered in combination with at least one therapeutic agent which is an anti-dyskinesia Agent (e.g., Carbidopa, Levodopa), an anti-infective agent (e.g., Cyclosporine, Miglustat, Pyrimethamine), an antineoplastic agent (e.g., Alemtuzumab, Azathioprine, Busulfan, Clofarabine, Cyclophosphamide, Melphalan, Methotrexate, Rituximab), an antirheumatic agent (e.g., Rituximab) a gastrointestinal agent (e.g., Methylprednisolone), a micronutrient (e.g., Calcitriol, Cholecalciferol, Ergocalciferols, Folic Acid, Vitamin D), a reproductive control agent (e.g., Methotrexate), a respiratory system agent (e.g., Tetrahydrozoline), vasoconstrictor agent (e.g., Calcitriol, Tetrahydrozoline).

[0088] In certain embodiments, the therapeutic agent(s) of the present invention are administered in combination with at least one therapeutic agent which is a therapeutic agent for β -hexosaminidase A and/or a therapeutic agent for acid β -galactosidase. In certain embodiments, the therapeutic agent(s) of the present invention are administered in combination with at least one therapeutic agent which is an anti-infective agent (e.g., Miglustat), an antineoplastic agent (e.g., Alemtuzumab, Busulfan, Cyclophosphamide), a gastrointestinal agent (e.g., Methylprednisolone). In one embodiment, the aforementioned combination is

administered to subjects at risk or diagnosed with Niemann-Pick disease (e.g., Niemann-Pick disease type C).

EXAMPLES

[0089] The present invention is further described by means of the examples, presented below. The use of such examples is illustrative only and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to any particular preferred embodiments described herein. Indeed, many modifications and variations of the invention will be apparent to those skilled in the art upon reading this specification. The invention is therefore to be limited only by the terms of the appended claims along with the full scope of equivalents to which the claims are entitled.

EXAMPLE 1 Determination of Inhibition Constants

[0089] The binding affinity (defined here by K_i binding constant) of GCase for novel compounds of the present invention were empirically determined using enzyme inhibition assays. In brief, the enzyme inhibition assays used monitored the ability of a test compound to bind and prevent the hydrolysis of a fluorogenic substrate in a concentration-dependent manner. Specifically, the enzyme activity of recombinant human GCase (rhGCase; Cerezyme®, Genzyme Corp.) was measured using the 4-methylumbelliferyl- β -D-glucopyranoside (4-MU- β -D-Glc) fluorogenic substrate in the absence or in the presence of varying amounts of each test compound. The resultant data were analyzed by comparing all test samples to the no inhibition control sample (no compound; corresponding to 100% enzyme activity) to determine the residual enzyme activity in the presence of test compound. The normalized residual activity data were subsequently graphed (on y-axis) relative to the concentration of test compound (on x-axis) to extrapolate the test compound concentration which leads to 50% inhibition of enzyme activity (defined as IC_{50}). The IC_{50} value for each test compound was then inserted into the Cheng-Prusoff equation (detailed below) to derive the absolute inhibition constant K_i that accurately reflects the binding affinity of

GCase for the test compound. The enzyme inhibition assays were performed at both pH 7.0 (endoplasmic reticulum pH) and at pH 5.2 (lysosomal pH) to gain insight into the binding affinity (i.e., potency) of compounds for GCase in the endoplasmic reticulum and lysosome.

In vitro assay

[0090] Various concentrations of test compounds were prepared in buffer "M" consisting of 50mM sodium phosphate buffer with 0.25% sodium taurocholate at pH 7.0 and pH 5.2. Enzyme (Cerezyme®, a recombinant form of the human enzyme β -glucocerebrosidase) was also diluted in the same buffer "M" at pH 7.0 and pH 5.2. The substrate solution consisted of 3 mM 4-methylumbelliferone β -D-glucopyranoside in buffer "M" with 0.15% Triton X-100 at both pH's. Five microliters of diluted enzyme was added to 15 μ l of the various inhibitor concentrations or buffer "M" alone and incubated at 37° C for 1 hour with 50 μ l of the substrate preparation to assess β -glucosidase activity at pH 7.0 and pH 5.2. Reactions were stopped by addition of an equal volume of 0.4 M glycine, pH 10.6. Fluorescence was measured on a plate reader for 1 sec/well using 355 nm excitation and 460 nm emission. Incubations without added enzyme or without added inhibitors were used to define no enzyme activity and maximum activity, respectively, and normalize % inhibition for a given assay. The results of such *in vitro* inhibition assays for reference compound IFG-tartrate and several test compounds are summarized below in **Table 2A**

Table 2A: <i>In vitro</i> Determination of Inhibition Constants					
Cmpd #	Compound Name	IC₅₀ (μM) pH 5.2	K_i (μM) pH 5.2	IC₅₀ (μM) pH 7.0	K_i (μM) pH 7.0
6	(3R,4R,5S)-5-(difluoromethyl)-piperidine-3,4-diol	0.0259±0.0014	0.0136±0.0008	0.0058±0.00023	0.00306±0.00012
13	(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	0.0946±0.0028	0.0498±0.0015	0.0171±0.0008	0.009±0.0004
9	(3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol*	0.107±0.0041	0.044±0.0017	0.020±0.0008	0.010±0.0004
10	(3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol*	0.343±0.021	0.142±0.0088	0.066±0.0041	0.035±0.0021
14	(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	0.038±0.0016	0.016±0.0007	0.007±0.0003	0.004±0.0001
none	(3R,4R,5S)-5-((R)-1-fluoropropyl)-piperidine-3,4-diol hydrochloride	0.291±0.006	0.121±0.0026	0.060±0.0029	0.031±0.0015
none	(3R,4R,5S)-5-benzyl-piperidine-3,4-diol	0.659±0.028	0.273±0.012	0.127±0.01	0.067±0.005
none	(3R,4R,5R)-5-((S)-hydroxy(phenyl)methyl)-piperidine-3,4-diol	3.29±0.25	1.36±0.10	0.017±0.0035	0.0089±0.0018
none	(3R,4R,5S)-5-(2-hydroxypropan-2-yl)piperidine-3,4-diol	0.234±0.0037	0.097±0.0015	0.029±0.0013	0.015±0.0007
none	IFG-tartrate	0.049±0.0029	0.026±0.0015	0.0074±0.00007	0.0039±0.000037
Notes:					
* Stereoisomer A and/or B					

***In situ* assay**

[0091] The effect of the novel compounds of the present invention on lysosomal GCase activity was assayed *in situ* using fibroblasts established from a normal subject. Cells seeded in 48-well plates were incubated with the indicated concentrations of compound for 16-24 hours. For the dose-response assays, cells were incubated with the *in situ* substrate 5-(pentafluorobenzoylamino)fluorescein di-β-D-glucopyranoside (PFBFDβGlu) for 1 hour and subsequently lysed to determine the extent of substrate hydrolysis in the presence of compound. The assay employed a range of 12 concentrations encompassing 5 orders of magnitude, centered on the IC₅₀. Specifically, the following concentration ranges were employed: (3R,4R,5S)-5-

(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol, (3R,4R,5S)-5-((R)-1-fluoropropyl)piperidine-3,4-diol hydrochloride, and (3R,4R,5S)-5-benzylpiperidine-3,4-diol: 1.0×10^{-3} to 3.0×10^{-9} M; (3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol: 1.0×10^{-4} to 3.0×10^{-10} M; and (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol: 1.0×10^{-3} to 3.0×10^{-11} M; wherein compound was serially diluted 1:3 from the highest concentration in the ranges specified.

Inhibition was determined as the ratio of activity in the presence of compound to that in the absence of compound. For the washout assays, cells were treated with compound for 16-24 hours at a concentration equal to the IC₉₀. Cells were washed extensively and incubated in drug-free medium to allow net compound efflux from cells. Cells were then tested for lysosomal GCase activity at 2 hour intervals over a total period of 8 hours following compound removal. The increase in activity over time was fitted with a single exponential function to determine the compound's washout time. The results of these *in situ* inhibition assays for reference compound IFG-tartrate and several test compounds are summarized below in **Table 2B**.

Table 2B: <i>In situ</i> Determination of Inhibition Constants					
Cmpd #	Compound Name	In situ IC₅₀ (μM)	In situ washout (hr)	EC₅₀ (μM)	E_{max} (%)
6	(3R,4R,5S)-5-(difluoromethyl)-piperidine-3,4-diol	0.408 ± 0.046	2.1 ± 0.30	0.018 ± 0.008	105.6 ± 8.7
13	(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	0.650 ± 0.172	2.7 ± 0.12	0.044 ± 0.005	92.8 ± 6.6
9	(3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol*	0.518 ± 0.022	10.5 ± 1.75	0.49 ± 0.06	83.7 ± 2.9
10	(3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol*	0.798 ± 0.043	12 ± 1.65	1.06 ± 0.12	99.3 ± 4.9
14	(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	0.061 ± 0.019	3.7 ± 0.63	0.026 ± 0.003	89.7 ± 3.5
none	(3R,4R,5S)-5-((R)-1-fluoropropyl)-piperidine-3,4-diol hydrochloride	0.972 ± 0.201	ND	0.086 ± 0.002	84.0 ± 4.1
none	(3R,4R,5S)-5-benzyl-piperidine-3,4-diol	1.299 ± 0.323	1.2 ± 0.13	0.18 ± 0.01	98.0 ± 4.5
none	(3R,4R,5R)-5-((S)-hydroxy(phenyl)methyl)-piperidine-3,4-diol	ND	ND	4.99 ± 0.86	72.1 ± 3.5
none	(3R,4R,5S)-5-(2-hydroxypropan-2-yl)piperidine-3,4-diol	ND	ND	0.791 ± 0.162	109.3 ± 3.6
none	IFG-tartrate	0.271 ± 0.012	8.2 ± 0.04	0.31 ± 0.11	105.5 ± 12.8
Notes: * Stereoisomer A and/or B <i>Cheng-Prusoff equation:</i> $K_i = IC_{50} / (1 + [S]/K_m)$ where [S] = substrate concentration; 2.5 mM 4-MU-β-D-Glc was used K _m = Michaelis constant that defines substrate affinity; 1.8 ± 0.6 mM for 4-MU-β-D-Glc (Liou et al., (2006) <i>J Biol. Chem.</i> 281 (7), 4242-53)					

[0092] When compared to reference compound IFG-tartrate, the following is notable: (i) test compounds (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol, (3R,4R,5S)-5-((R)-1-fluoropropyl)piperidine-3,4-diol hydrochloride, and (3R,4R,5S)-5-benzylpiperidine-3,4-diol, were found to cause a concentration-dependent increase in GCase activity and enhanced enzyme activity to the same maximum level as reference compound IFG-tartrate at much lower concentration; (ii) test compounds (3R,4R,5S)-5-(difluoromethyl)piperidine-

3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, and (3R,4R,5S)-5-benzylpiperidine-3,4-diol, washed out of the lysosomal compartment (*in situ* washout) considerably faster than reference compound IFG-tartrate; and (iii), test compounds (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol, (3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol, (3R,4R,5S)-5-((R)-1-fluoropropyl)piperidine-3,4-diol hydrochloride, and (3R,4R,5S)-5-benzylpiperidine-3,4-diol, inhibited GCCase activity.

EXAMPLE 2: Blood brain barrier penetration

[0093] The blood-brain barrier (BBB) penetration of reference compound IFG-tartrate and several compounds of the present invention (i.e., (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol, (3R,4R,5S)-5-((R)-1-fluoropropyl)piperidine-3,4-diol hydrochloride, and (3R,4R,5S)-5-benzylpiperidine-3,4-diol) were assayed after oral administration to mice. For this purpose, 8-week old wild-type male mice (C57BL/6) were administered a single 30 mg/kg (free base equivalent) p.o. dose of reference or test compound by gavage (n = 3 mice per time point). Dosing solutions were prepared in water. After dosing, mice were euthanized with CO₂ at the following time points: 0-, 0.5-, 1-, and 4-hour post-dose. After euthanization, whole blood was collected from the inferior vena cava into lithium heparin tubes. Similarly, brains were collected from each mouse. Plasma was derived by spinning whole blood at 2,700 x g for 10 minutes at 4°C followed by storage on dry ice. Whole brains were washed in cold PBS to remove contaminating blood, blotted dry, flash frozen on dry ice, and ultimately stored at -80°C until analysis. To prepare brain samples for analysis, 50-100 mg of tissue was homogenized in 400 µl of water/mg tissue. Samples were then clarified by centrifugation. Next, 25 µl of the brain homogenate supernatant or 25 µl of plasma were combined with 25 µl of acetonitrile:water (95/5). This was supplemented with 25 µl of acetonitrile and 50 µL of internal standard (100 ng/mL IFG-tartrate 13C2-15N in 0.5% formic acid in (70:30)

acetonitrile:methanol). Samples were again clarified by centrifugation and 75 μ l of the supernatant was combined with 75 μ l of acetonitrile. Samples were then analyzed for compound levels by LC-MS/MS at PPD Inc. (3230 Deming Way, Middleton, WI 53562). In brief, a Thermo Betasil, Silica-100, 50 x 3mm, 5 μ column equilibrated with a mixture of mobile phase consisting of 5 mM ammonium formate and 0.05% formic acid in (A) 95:5 acetonitrile:water or (B) 70:20:10 methanol:water:acetonitrile was employed. Between 20 and 30 μ l sample was injected for analysis. For calculating drug concentrations, raw data for plasma (ng/mL) and brain (ng/g) was converted to nM using the molecular weight of respective compounds and assuming 1 g of tissue is equivalent to 1 mL volume. Concentration as a function of time was plotted in GraphPad Prism version 4.02.

[0094] The plasma levels and brain levels detected in mice administered a single 30 mg/kg (free base equivalent) p.o. dose of reference compound (i.e., IFG-tartrate) or test compound (i.e., (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5R)-5-(1-hydroxyethyl)piperidine-3,4-diol, (3R,4R,5S)-5-((R)-1-fluoropropyl)piperidine-3,4-diol hydrochloride, or (3R,4R,5S)-5-benzylpiperidine-3,4-diol) reflect that (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5S)-5-((R)-1-fluoropropyl)piperidine-3,4-diol hydrochloride, and (3R,4R,5S)-5-benzylpiperidine-3,4-diol crossed the blood brain barrier more readily as compared to IFG-tartrate. Additionally, higher levels of (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5S)-5-((R)-1-fluoropropyl)piperidine-3,4-diol hydrochloride, and (3R,4R,5S)-5-benzylpiperidine-3,4-diol were detected in brain than that observed following administration of IFG-tartrate.

EXAMPLE 3: GCase enhancement

[0095] The ability of orally administered test compounds ((3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5S)-5-((R)-1-fluoropropyl)piperidine-3,4-diol hydrochloride, or (3R,4R,5S)-5-benzylpiperidine-3,4-diol) to elevate GCase levels was assessed in mice. For this purpose, 8-week old wild-type male mice (C57BL/6) were administered a single p.o. (gavage) dose of a compound of the present invention (i.e., (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, (3R,4R,5S)-5-((R)-1-fluoropropyl)piperidine-3,4-diol hydrochloride, or (3R,4R,5S)-5-benzylpiperidine-3,4-diol). Details of the dose administered for each compound are provided in **Tables 3A** and **3B**. The dosing solutions were prepared in water. Compounds were administered over 2 weeks as follows: week 1, Mon-Fri (On), Sat-Sun (Off); week 2, Mon-Thu (On); necropsy on Friday. Thus, a total of 9 doses (dosing solutions prepared fresh every day) were given to each mouse, with a 24-hour washout between the last dose and necropsy.

[0096] After completion of dosing, mice were euthanized with CO₂ and whole blood was drawn into lithium heparin tubes from the inferior vena cava. Plasma was collected by spinning blood at 2700g for 10 minutes at 4°C. Liver, spleen, lung, and brain tissues were removed, washed in cold PBS, blotted dry, flash frozen on dry ice, and stored at -80°C until analysis. GCase levels were measured by homogenizing approximately 50 mg tissue in 500 µL McIlvane (MI) buffer (100 mM sodium citrate, 200 mM sodium phosphate dibasic, 0.25% sodium taurocholate, and 0.1% Triton X-100, pH 5.2) at pH 5.2 for 3-5 seconds on ice with a micro homogenizer. Homogenates were then incubated at room temperature without and with 2.5 mM conduritol-B-epoxide (CBE) for 30 min. Finally, 3.7 mM 4-methylumbelliferyl-β-glucoside (4-MUG) substrate was added and incubated at 37°C for 60 min. Reactions were stopped by addition of 0.4 M glycine, pH 10.6. Fluorescence was measured on a plate reader for 1 sec/well using 355 nm excitation and 460 nm emission. Total protein was determined in lysates using the MicroBCA kit according to the manufacturer's instructions. A 4-

methyumbelliferone (4-MU) standard curve ranging from 1.0 nM to 50 μ M was run in parallel for conversion of raw fluorescence data to absolute GCase activity (in the presence and absence of CBE) and expressed as nanomoles of 4-MU released per milligram of protein per hour (nmol/mg protein/hr). GCase levels and protein levels were calculated using Microsoft Excel (Redmond, WA) and GraphPad Prism version 4.02.

[0097] **Tables 3A and 3B** summarize the dose administered for each compound examined in mice as described above as well as the resultant level of GCase enhancement in brain and spleen, respectively, compound concentration in tissue, compound concentration in GCase assay and inhibition constant (K_i).

Table 3A: GCase enhancement in Brain

Compound Name	Dose (mg/kg) FBE	GCase Increase (fold)	Compound concentration in tissue ^{2.2} nmol/kg	Compound concentration in GCase assay (μM)	Ki pH 5.2 (uM)
(3R,4R,5S)-5-(difluoromethyl)-piperidine-3,4-diol	10	2.1	55	0.0002	0.0136±0.0008
(3R,4R,5S)-5-(difluoromethyl)-piperidine-3,4-diol	100	2.6	301	0.0010	
(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	10	1.5	50	0.0002	0.0498±0.0015
(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	100	2.4	415	0.0014	
(3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol*	ND	ND	ND	ND	0.044±0.0017
(3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol*	ND	ND	ND	ND	0.142±0.0088
(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	10	1.5	BLQ (1)	BLQ	0.016±0.0007
(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	100	2.2	41	0.0001	
(3R,4R,5S)-5-((R)-1-fluoropropyl)-piperidine-3,4-diol hydrochloride	10	0.9	BLQ (2)	BLQ	0.121±0.0026
(3R,4R,5S)-5-((R)-1-fluoropropyl)-piperidine-3,4-diol hydrochloride	100	1.1	38	0.0001	
(3R,4R,5S)-5-benzyl-piperidine-3,4-diol	10	1.2	ND	ND	0.273±0.012
(3R,4R,5S)-5-benzyl-piperidine-3,4-diol	100	1.4	ND	ND	
(3R,4R,5R)-5-((S)-hydroxy(phenyl)methyl)-piperidine-3,4-diol	ND	ND	ND	ND	1.36±0.10
(3R,4R,5S)-5-(2-hydroxypropan-2-yl)piperidine-3,4-diol	ND	ND	ND	ND	0.097±0.0015
Notes:					
* Stereoisomer A and/or B					
(1) BLQ < 7.4 nmol/kg; (2) BLQ < 2.2 nmol/kg					
ND: Not determined					

Table 3B: GCase enhancement in Spleen					
Compound Name	Dose (mg/kg) FBE	GCase increase (fold)	Compound concentration in tissue	Compound concentration in GCase assay	Ki pH 5.2 (uM)
(3R,4R,5S)-5-(difluoromethyl)-piperidine-3,4-diol	10	1.9	100	0.0003	0.0136±0.0008
(3R,4R,5S)-5-(difluoromethyl)-piperidine-3,4-diol	100	2.4	435	0.0015	
(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	10	1.0	BLQ (1)	BLQ	0.0498±0.0015
(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	100	1.5	948	0.0032	
(3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol*	ND	ND	ND	ND	0.044±0.0017
(3R,4R,5R)-5-(1-hydroxyethyl)-piperidine-3,4-diol*	ND	ND	ND	ND	0.142±0.0088
(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	10	1.6	BLQ (2)	BLQ	0.016±0.0007
(3R,4R,5S)-5-(1-fluoroethyl)-piperidine-3,4-diol*	100	2.3	99	0.0003	
(3R,4R,5S)-5-((R)-1-fluoropropyl)-piperidine-3,4-diol hydrochloride	10	0.7	21	0.0001	0.121±0.0026
(3R,4R,5S)-5-((R)-1-fluoropropyl)-piperidine-3,4-diol hydrochloride	100	0.7	60	0.0002	
(3R,4R,5S)-5-benzyl-piperidine-3,4-diol	10	1.0	ND	ND	0.273±0.012
(3R,4R,5S)-5-benzyl-piperidine-3,4-diol	100	1.2	ND	ND	
(3R,4R,5R)-5-((S)-hydroxy(phenyl)methyl)-piperidine-3,4-diol	ND	ND	ND	ND	1.36±0.10
(3R,4R,5S)-5-(2-hydroxypropan-2-yl)piperidine-3,4-diol	ND	ND	ND	ND	0.097±0.0015
Notes:					
* Stereoisomer A and/or B					
(1) BLQ < 6.8 nmol/kg; (2) BLQ < 7.9 nmol/kg					
ND: Not determined					

[0098] As reflected in **Tables 3A and 3B**, mice administered (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, or (3R,4R,5S)-5-benzylpiperidine-3,4-diol demonstrated significant GCase enhancement in brain and spleen.

EXAMPLE 4: Rat pharmacokinetics

[0099] Pharmacokinetic (PK) data was obtained in rats to assess the bioavailability of test compound. In particular, the following PK parameters were calculated: bioavailability as measured by area under the Concentration/Time curve (AUC), fraction of dose available (%F; further defined below), clearance (CL), volume of distribution (Vd), and half-life ($t_{1/2}$). For this purpose, 8-week old Sprague-Dawley male rats were given either a single intravenous (IV) dose equivalent to 3 mg/kg of free base or single escalating p.o. (gavage) doses of test compound equivalent to 10, 30, and 100 mg/kg of free base. Three rats were used per dosing group. Blood was collected over a 24-hr period. The time points for blood collection after intravenous administration were: 0, 2.5, 5, 10, 15, 30, 45 min, 1, 2, 4, 8, 12, and 24 hrs; time points for blood collection after p.o. administrations were: 0, 5, 15, 30, 45 min, 1, 2, 3, 4, 8, 12, and 24 hrs. Plasma samples were analyzed for compound levels by LC-MS/MS at PPD. Raw data was analyzed by non-compartmental analysis in Win-nonLin to calculate V_D , %F, CL, and $t_{1/2}$.

[00100] Various pharmacokinetic parameters for (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, and (3R,4R,5S)-5-benzylpiperidine-3,4-diol based on the aforementioned study are detailed below in **Tables 4A-D**.

Table 4A: Rat PK for (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol-HCl								
Dose (mg/kg)		Route	AUC _{last} (hr*ng/ml)	%F	t _{1/2} (h)	C _{max} (ng/mL)	CL (mL/hr/kg)	V _D (mL/kg)
Salt	Free Base							
3.65	3	IV	AUC _{0-12 hr} 2044 ± 294	N/A	1.1 ± 0.05	2323 ± 348	1555 ± 218	2612 ± 269
12.18	10	PO	AUC _{0-12hr} 6714 ± 524	106 ± 8.6	2.58 ± 0.78	3363 ± 219	N/A	N/A
36.54	30	PO	AUC _{0-24hr} 21685 ± 1515	101 ± 6.9	2.75 ± 0.36	10037 ± 865	N/A	N/A
121.81	100	PO	AUC _{0-24hr} 79389 ± 8570	121 ± 12.9	2.41 ± 0.16	33200 ± 4990	N/A	N/A
Notes: Non compartmental analysis mean values (N=3 rats) BLD Below Limit of Detection (<0.5ng/mL) BLQ Below Limit of Quantitation %F = $\frac{AUC\ PO \times 100}{AUC\ IV}$ dose normalized AUC_{last} = Area under the Concentration/Time curve to the last data point								

Table 4B: Rat PK for (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol-HCl*								
Dose (mg/kg)		Route	AUC _{last} (hr*ng/ml)	%F	t _{1/2} (h)	C _{max} (ng/mL)	CL (mL/hr/kg)	V _D (mL/kg)
Salt	Free Base							
3.67	3	IV	AUC _{0-24hr} 1421 ± 188.1	N/A	2.6 ± 0.64	2328 ± 373	2708 ± 410	9774 ± 1551
12.23	10	PO	AUC _{0-24hr} 7097 ± 606	148 ± 12.5	2.8 ± 0.50	2680 ± 167	N/A	N/A
36.70	30	PO	AUC _{0-24hr} 21664 ± 1708	155 ± 12.2	2.7 ± 0.12	6917 ± 451	N/A	N/A
122.34	100	PO	AUC _{0-24hr} 59481 ± 1005	142 ± 2.5	2.5 ± 0.19	19433 ± 3031	N/A	N/A
Note: * Stereoisomer A and/or B								

Table 4C: Rat PK for (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol-HCl*

Dose (mg/kg)		Route	AUC _{last} (hr*ng/ml)	%F	t _{1/2} (h)	C _{max} (ng/mL)	CL (mL/hr/kg)	V _D (mL/kg)
Salt	Free Base							
3.67	3	IV	AUC _{0-24hr} 1370 ± 109	N/A	2.06 ± 0.47	2427 ± 192	2188 ± 173	6304 ± 927
12.23	10	PO	AUC _{0-24hr} 4251 ± 88	98 ± 1.85	3.0 ± 0.22	1127 ± 60	N/A	N/A
36.70	30	PO	AUC _{0-24hr} 14229 ± 127	104 ± 0.88	2.6 ± 0.16	4680 ± 369	N/A	N/A
122.34	100	PO	AUC _{0-24hr} 50946 ± 713	104 ± 1.5	2.4 ± 0.16	15733 ± 622	N/A	N/A

Note:
* Stereoisomer A and/or B

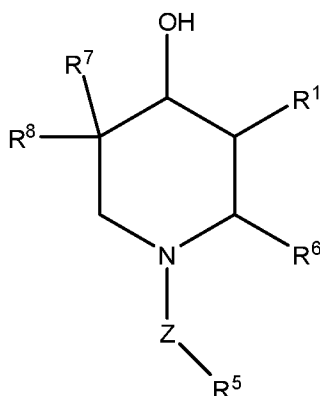
Table 4D: Rat PK for (3R,4R,5S)-5-benzylpiperidine-3,4-diol-HCl

Dose (mg/kg)		Route	AUC _{last} (hr*ng/ml)	%F	t _{1/2} (h)	C _{max} (ng/mL)	CL (mL/hr/kg)	V _D (mL/kg)
Salt	Free Base							
3.53	3	IV	AUC _{0-12hr} 592 ± 60.9	N/A	1.7 ± 1.5	969 ± 104	5145 ± 532	12570 ± 1792
11.76	10	PO	AUC _{0-24hr} 1200 ± 46.4	61.7 ± 2.4	3.86 ± 0.6	641 ± 48.7	N/A	N/A
35.28	30	PO	AUC _{0-24hr} 3690 ± 71.5	62.3 ± 1.2	3.8 ± 0.19	1703 ± 133	N/A	N/A
117.59	100	PO	AUC _{0-24hr} 13519 ± 2177	68.3 ± 10.8	2.9 ± 0.11	7140 ± 1357	N/A	N/A

[00101] As reflected in Tables 4A-D, (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, and (3R,4R,5S)-5-benzylpiperidine-3,4-diol have favorable pharmacokinetic profiles for drug development. In particular, (3R,4R,5S)-5-(difluoromethyl)piperidine-3,4-diol, (3R,4R,5S)-5-(1-fluoroethyl)piperidine-3,4-diol, and (3R,4R,5S)-5-benzylpiperidine-3,4-diol show excellent oral bioavailability (approximately 50-100%) and dose proportionality, a half-life of 1.0 to 4.0 hours, and a volume of distribution suggesting adequate penetration into peripheral tissues.

What is claimed:

1. A method for preventing and/or treating a lysosomal storage disorder in a patient at risk for developing or diagnosed with the same, which comprises administering to the patient in need thereof an effective amount of a compound of Formula II:



wherein:

R¹ is C(R²)(R³)(R⁴);

R² is hydrogen, -OH or halogen;

R³ is hydrogen, -OH, halogen or -CH₃;

R⁴ is halogen, -CH₃, phenyl, fluorophenyl, methylphenyl, cyclohexylmethyl, wherein when R⁴ is a halogen, both R² and R³ cannot be hydrogen;

R³ and R⁴ may join with the carbon to which they are attached to form a cycloalkyl ring, which may be optionally substituted with one or more halogen atoms;

R⁶ is hydrogen or phenylalkyl;

Z is optional, when present Z is -(CH₂)-, -C(=O)-, -S(=O)₂NH-, -S(=O)₂-, -S(=O)₂-CH₂-, C(=O)-NH-, -S(=O)₂NR⁹-, -C(=S)NH- or -C(=O)₂-CH₂-;

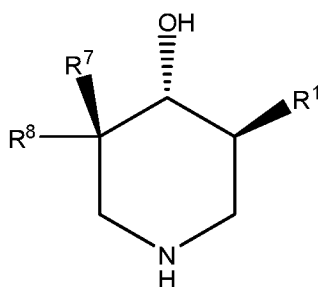
R⁹ is hydrogen or CH₃;

R⁵ is hydrogen or aminophenylalkyl;

R⁷ is -OH or halogen; and

R^8 is hydrogen, halogen or $-\text{CH}_3$,
 provided that R^2 and R^3 cannot both be hydrogen when R^4 is halogen, and
 when Z is not present, R^7 is $-\text{OH}$, R^5 , R^6 and R^8 are hydrogen
 or a pharmaceutically acceptable salt or solvate thereof.

2. A method according to claim 1, wherein the compound is of Formula III:



wherein:

R^1 is $\text{C}(\text{R}^2)(\text{R}^3)(\text{R}^4)$;

R^2 is hydrogen, $-\text{OH}$ or halogen;

R^3 is hydrogen, $-\text{OH}$, halogen or $-\text{CH}_3$;

R^4 is halogen, $-\text{CH}_3$, phenyl, fluorophenyl, methylphenyl, cyclohexylmethyl, wherein when R^4 is a halogen, both R^2 and R^3 cannot be hydrogen;

R^3 and R^4 may join with the carbon to which they are attached to form a cycloalkyl ring, which may be optionally substituted with one or more halogen atoms;

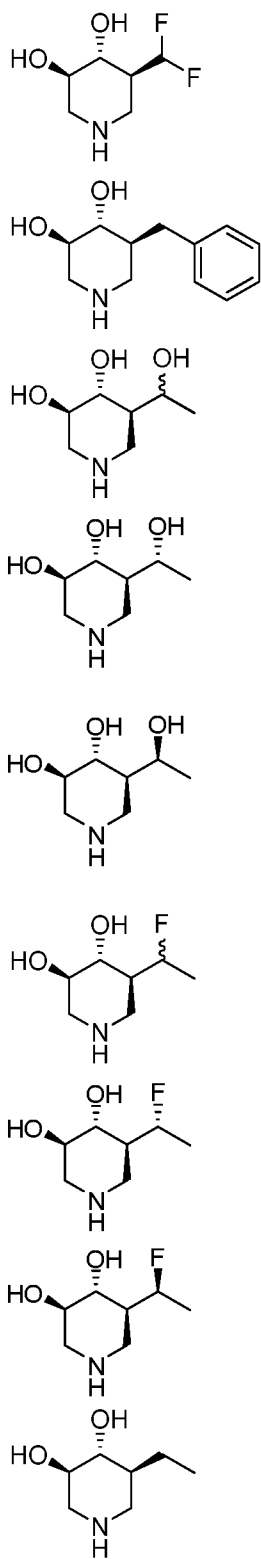
R^7 is $-\text{OH}$;

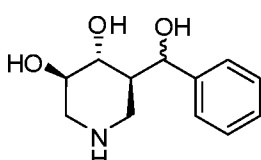
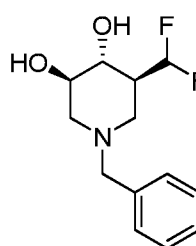
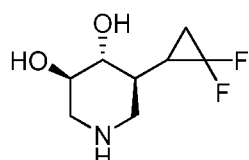
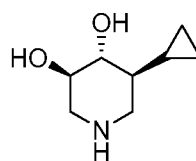
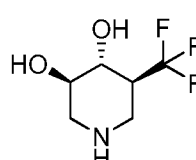
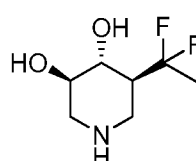
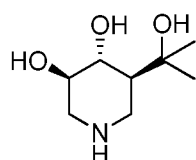
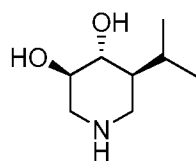
R^6 is hydrogen; and

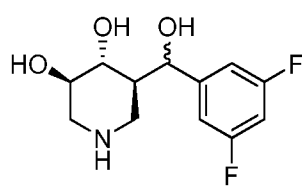
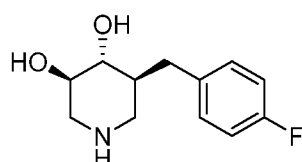
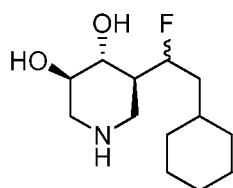
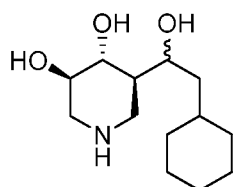
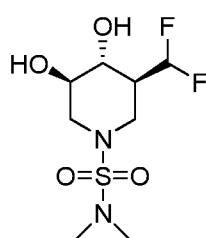
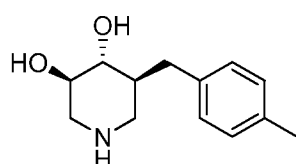
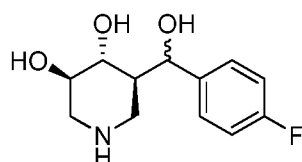
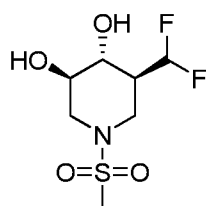
R^8 is hydrogen,

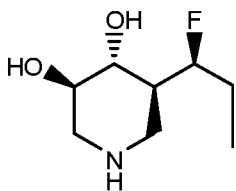
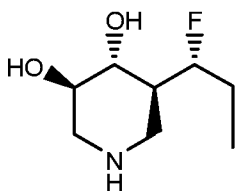
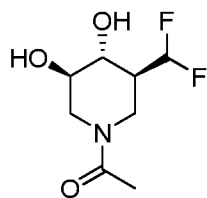
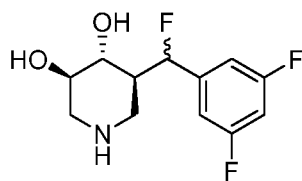
provided that R^2 and R^3 cannot both be hydrogen when R^4 is a halogen.

3. A method for preventing and/or treating a lysosomal storage disorder in a patient at risk for developing or diagnosed with the same, which comprises administering to the patient in need thereof an effective amount of a compound selected from:



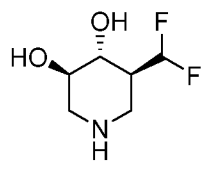






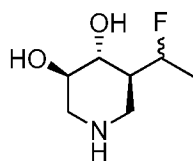
or a pharmaceutically acceptable salt or solvate thereof, or any combination of two or more thereof.

4. The method of Claim 3, wherein the compound is:



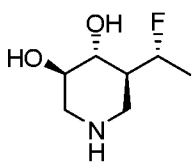
or a pharmaceutically acceptable salt or solvate thereof.

5. The method of Claim 3, wherein the compound is:



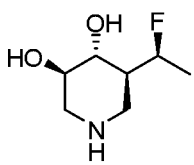
or a pharmaceutically acceptable salt or solvate thereof.

6. The method of Claim 3, wherein the compound is:



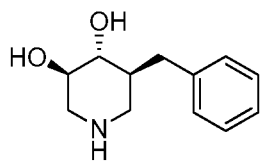
or a pharmaceutically acceptable salt or solvate thereof.

7. The method of Claim 3, wherein the compound is:



or a pharmaceutically acceptable salt or solvate thereof.

8. The method of Claim 3, wherein the compound is:



or a pharmaceutically acceptable salt or solvate thereof.

9. The method of any one of Claims 1 to 8, wherein the lysosomal storage disease is Niemann-Pick disease.

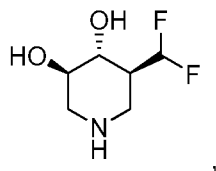
10. The method of any one of Claims 1 to 8, wherein the lysosomal storage disease is Gaucher's disease.

11. The method of any one of Claims 1 to 10, further comprising administering an effective amount of at least one other therapeutic agent.

12. The method of Claim 11, wherein at least one other therapeutic agent is imiglucerase or 1,5-(butylimino)-1,5-dideoxy-D-glucitol.

13. A kit when used in a method for preventing and/or treating a lysosomal storage disorder in a patient at risk for developing or diagnosed with the same, the kit comprising:

- a container having an effective amount of a compound of the Formula:



or a pharmaceutically acceptable salt or solvate thereof; and

- instructions for using the same to prevent or treat a lysosomal storage disorder.