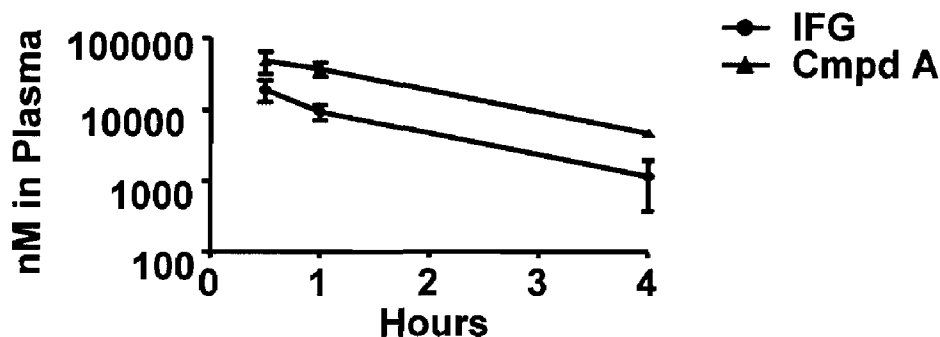




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(54) **Titre : METHODES POUR PREVENIR ET/OU TRAITER DES TROUBLES DEGENERATIFS DU SYSTEME NERVEUX CENTRAL**  
(54) **Title: METHODS FOR PREVENTING AND/OR TREATING DEGENERATIVE DISORDERS OF THE CENTRAL NERVOUS SYSTEM**



(57) **Abrégé/Abstract:**

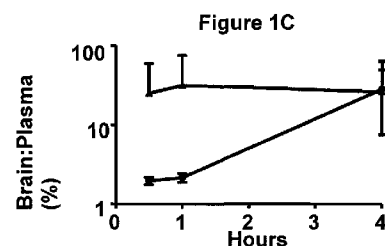
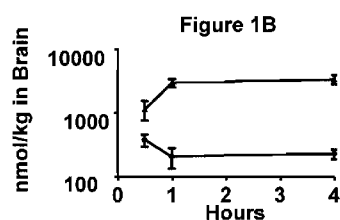
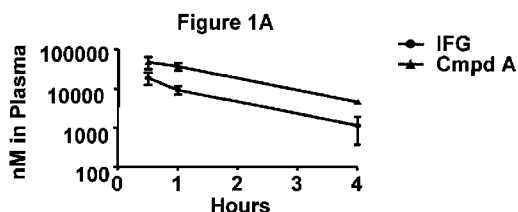
The present invention provides methods for preventing and/or treating degenerative disorders of the central nervous system using 5-(fluoromethyl)piperidine-3,4-diol, 5-(chloromethyl)piperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof, or any combination of two or more thereof. In particular, the present invention provides methods for preventing and/or treating Parkinson's disease.

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(54) **Title:** METHODS FOR PREVENTING AND/OR TREATING DEGENERATIVE DISORDERS OF THE CENTRAL NERVOUS SYSTEM

(57) **Abstract:** The present invention provides methods for preventing and/or treating degenerative disorders of the central nervous system 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. In particular, the present invention provides methods for preventing and/or treating Parkinson's disease.

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## METHODS FOR PREVENTING AND/OR TREATING DEGENERATIVE DISORDERS OF THE CENTRAL NERVOUS SYSTEM

### FIELD OF THE INVENTION

5 The present invention provides methods for preventing and/or treating degenerative disorders of the central nervous system using 5-(fluoromethyl)piperdine-3,4-diol, 5-(chloromethyl)piperdine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof, or any combination  
10 of two or more thereof. In particular, the present invention provides methods for preventing and/or treating Parkinson's disease.

### BACKGROUND OF THE INVENTION

Many degenerative disorders of the central nervous system are  
15 associated with pathologic aggregation of proteins or lipids. For example, synucleinopathies are a group of diseases that arise from disruption of synuclein protein homeostasis. In particular, alpha-synuclein aggregation is associated with pathological conditions characterized by Lewy bodies, such as Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. Likewise,  
20 alpha-synuclein fragment, non-Abeta component, is found in amyloid plaques of Alzheimer's disease. Recently, enhancement of glucocerebrosidase (beta-glucosidase; GCase) activity in the brain has been shown to prevent accumulation of synuclein in the brain (Sean Clark, Ying Sun, You-Hai Xu, Gregory Grabowski, and Brandon Wustman, "A biochemical link between  
25 Gaucher and Parkinson's disease and a potential new approach to treating synucleinopathies: a pharmacological chaperone for beta-glucocerebrosidase prevents accumulation of alpha-synuclein in a Parkinson's mouse model," Presented at the Society for Neuroscience Annual Meeting, San Diego, CA, 2007). Thus, agents that enhance GCase activity may provide relief for patients  
30 at risk for developing or diagnosed with degenerative disorders of the central nervous system.

There is a need for methods to prevent and/or treat degenerative disorders of the central nervous system 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 synucleinopathies such as Parkinson's disease and Alzheimer's disease that provide patients with a higher quality of life and achieve a better clinical outcome.

### **SUMMARY OF THE INVENTION**

The present invention provides methods for preventing and/or treating a degenerative disorder of the central nervous system 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 therapeutic agent which is 5-(fluoromethyl)piperdine-3,4-diol, 5-(chloromethyl)piperdine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof, or any combination of two or more thereof. In one embodiment, the method comprises administering 5-(fluoromethyl)piperdine-3,4-diol or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the method comprises administering (3*R*, 4*R*, 5*S*)-5-(fluoromethyl)piperdine-3,4-diol or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the method comprises administering (3*R*, 4*R*, 5*S*)-5-(fluoromethyl)piperdine-3,4-diol hydrochloride. In one embodiment, the method comprises administering 5-(chloromethyl)piperdine-3,4-diol or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the method comprises administering (3*R*, 4*R*, 5*S*)-5-(chloromethyl)piperdine-3,4-diol or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the method comprises administering (3*R*, 4*R*, 5*S*)-5-(chloromethyl)piperdine-3,4-diol. In one embodiment, the degenerative disorder is a synucleinopathy. In one embodiment, the degenerative disorder is characterized by Lewy bodies. In one embodiment, the degenerative disorder is Parkinson's disease, dementia with Lewy bodies, multiple system atrophy or Alzheimer's disease. In one embodiment, the degenerative disorder is associated with aggregation of at least

one protein. In one embodiment, the degenerative disorder is associated with aggregation of alpha-synuclein. In one embodiment, the degenerative disorder is associated with aggregation of non-Abeta component. In one embodiment, the degenerative disorder is associated with accumulation of at least one glycolipid.

5 In one embodiment, the degenerative disorder is associated with accumulation of at least one glycosphingolipid. In one embodiment, the degenerative disorder is associated with accumulation of glucocerebroside. In one embodiment, the degenerative disorder is associated with a mutation in glucocerebrosidase. In one embodiment, the method further comprises administering an effective  
10 amount of at least one other therapeutic agent. In one embodiment, at least one other therapeutic agent is levodopa, an anticholinergic, a catechol-O-methyl transferase inhibitor, a dopamine receptor agonist, a monoamine oxidase inhibitor, a peripheral decarboxylase inhibitor, or an anti-inflammatory agent.

The present invention also provides methods for preventing and/or  
15 treating Parkinson'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 (3*R*, 4*R*, 5*S*)-5-(fluoromethyl)piperidine-3,4-diol, (3*R*, 4*R*, 5*S*)-5-(chloromethyl)piperidine-3,4-diol, or a pharmaceutically acceptable salt, solvate, or prodrug thereof, or any combination of two or more thereof.

20 In one embodiment, the method comprises administering (3*R*, 4*R*, 5*S*)-5-(fluoromethyl)piperidine-3,4-diol or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the method comprises administering (3*R*, 4*R*, 5*S*)-5-(fluoromethyl)piperidine-3,4-diol hydrochloride. In one embodiment, the method comprises administering (3*R*, 4*R*, 5*S*)-5-(chloromethyl)piperidine-3,4-  
25 diol or a pharmaceutically acceptable salt, solvate, or prodrug thereof. In one embodiment, the method comprises administering (3*R*, 4*R*, 5*S*)-5-(chloromethyl)piperidine-3,4-diol. In one embodiment, the method comprises administering an effective amount of at least one other therapeutic agent. In one embodiment, at least one other therapeutic agent is levodopa, an anticholinergic,  
30 a catechol-O-methyl transferase inhibitor, a dopamine receptor agonist, a

monoamine oxidase inhibitor, a peripheral decarboxylase inhibitor, or an anti-inflammatory agent.

The present invention also provides kits comprising:

- a container having an effective amount of 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;
  - instructions for using the same to prevent and/or treat a degenerative disorder of the central nervous system.
- 10 In one embodiment, the degenerative disorder of the central nervous system is Parkinson's disease. In one embodiment, the degenerative disorder of the central nervous system is Alzheimer's disease.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

15 **Figure 1A** illustrates the plasma levels of reference compound, IFG, and test compound (3*R*, 4*R*, 5*S*)-5-(fluoromethyl)piperidine-3,4-diol (referred to herein as Compound A), in mice administered a single 30 mg/kg (free base equivalent) p.o. dose of IFG-tartrate or the hydrochloride salt form of Compound A.

**Figure 1B** illustrates the brain levels of reference compound, IFG, and test compound, Compound A, in mice administered a single 30 mg/kg (free base equivalent) p.o. dose of IFG-tartrate or the hydrochloride salt form of Compound A.

**Figure 1C** illustrates the ratio of brain to plasma levels of reference compound, IFG, and test compound, Compound A, in mice administered a single 30 mg/kg (free base equivalent) p.o. dose of IFG-tartrate or the hydrochloride salt form of Compound A.

**Figure 2** illustrates the brain levels of reference compound, IFG, and test compound, (3*R*,4*R*,5*S*)-5-(chloromethyl)piperidine-3,4-diol (referred to herein as Compound B), in mice administered a single 30 mg/kg (free base equivalent) p.o. dose of IFG-tartrate or free-base of Compound B.

**Figures 3A-D** illustrate the level of GCase in brain, spleen, liver and lung, respectively, of C57BL/6 mice administered a 2-week dosing regimen consisting of nine doses of (i) control vehicle; (ii) 100 mg/kg (free base equivalent) of reference compound, IFG-tartrate; or (iii) 10 or 100 mg/kg (free base equivalent) of test compound, the hydrochloride salt form of Compound A. Note that the values appearing above the bars in these figures represent the fold increase above control. Likewise, in these figures, the symbol “\*” represents  $p < 0.05$  t-test compared to control and the symbol “#” represents  $p < 0.05$  t-test compared to mice treated with reference compound, IFG-tartrate.

**Figures 4A-D** illustrate the level of GCase in brain, spleen, liver and lung, respectively, of C57BL/6 mice administered a 2-week dosing regimen consisting of nine doses of (i) control vehicle; (ii) 100 mg/kg (free base equivalent) of reference compound, IFG-tartrate; or (ii) 1, 3, 10, 30 or 100 mg/kg (free base equivalent) of test compound, the hydrochloride salt form of Compound A. Note that the values appearing above the bars in these figures represent the fold increase above control. Likewise, in these figures, the symbol “\*” represents  $p < 0.05$  t-test compared to control and the symbol “#” represents  $p < 0.05$  t-test compared to mice treated with reference compound, IFG-tartrate.

**Figures 5A-D** illustrate the level of GCase in brain, spleen, liver and lung, respectively, of C57BL/6 mice administered a 2-week dosing regimen consisting of nine doses of (i) control vehicle; (ii) 10 or 100 mg/kg (free base equivalent) of reference compound, IFG-tartrate; or (iii) 10 or 100 mg/kg (free base equivalent) of test compound, Compound B. Note that the values appearing above the bars in these figures represent the fold increase above control. Likewise, in these figures, the symbol “\*” represents  $p < 0.05$  t-test compared to control and the symbol “#” represents  $p < 0.05$  t-test compared to mice treated with reference compound, IFG-tartrate.

**Figure 6A** illustrates the plasma level of test compound, Compound A, in rats administered a single intravenous dose of 3 mg/kg (free base equivalent) of the hydrochloride salt form of Compound A.



**Figure 6B** illustrates the plasma level of Compound A in rats administered a single p.o. dose of 10, 30, or 300 mg/kg (free base equivalent) of the hydrochloride salt form of Compound A.

## **DETAILED DESCRIPTION OF THE INVENTION**

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

As used herein, the phrase "degenerative disorder of the central nervous system" means any disorder associated with the premature degeneration of any component of the central nervous system, such as neurons, myelin sheaths or axons. Such disorders include but are not limited to multi-infarct dementia, Huntington's disease, Pick's disease, amyotrophic lateral sclerosis, Creutzfeldt-Jakob's disease, frontal-lobe degeneration, corticobasal degeneration, progressive supranuclear palsy, Parkinson's disease, dementia with Lewy bodies, multiple system atrophy or Alzheimer's disease.

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

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

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.

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

Parkinson's disease may be diagnosed in patients according to the United Kingdom Parkinson's Disease Society brain-bank clinical diagnostic criteria (see, Hughes et al., Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 1992; 55:181-184) and/or the criteria described by Gelb et al., Diagnostic Criteria for Parkinson's Disease. *Arch Neurol.* 1999; 56(1):33-39. Likewise, the severity of Parkinson's disease may be ascertained using the Unified Parkinson's Disease Rating Scale. See, e.g., Fahn and Elton, Members of the Unified Parkinson's

Disease Rating Scale Development Committee. Unified Parkinson's Disease Rating Scale. In: Fahn et al., Recent developments in Parkinson's disease. New York: Macmillan, 1987: 153-163.

Alzheimer's disease may be diagnosed in patients according to the criteria  
5 for dementia of the Alzheimer's type of the Diagnostic and Statistical Manual of Mental Disorders, 4<sup>th</sup> ed.: DSM-IV. Washington, D.C.: American Psychiatric Association, 1994. Likewise, the criteria for probable Alzheimer's disease may be ascertained based on criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related  
10 Disorders Association. See also, McKhann et al., Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984; 34:939-944.

Multiple system atrophy (MSA) is characterized by glial cytoplasmic  
15 inclusion bodies (also known as Papp-Lantos bodies) in the movement, balance and automatic control centers of the brain. The most common first sign of MSA is the appearance of an "akinetic-rigid syndrome" (i.e., slowness of initiation of movement resembling Parkinson's disease) found in 62% at first presentation. Other common signs at onset include problems with balance (found in 22%),  
20 followed by genito-urinary problems (9%). For men, the first sign can be erectile dysfunction (unable to achieve or sustain an erection). Both men and women often experience problems with their bladders including urgency, frequency, incomplete bladder emptying or an inability to pass urine (retention). About 1 in 5 MSA patients will suffer a fall in their first year of disease. As the disease  
25 progresses three groups of symptoms predominate. These are: (i) parkinsonism (slow, stiff movement, writing becomes small and spidery); (ii) cerebellar dysfunction (difficulty coordinating movement and balance); and (iii) autonomic dysfunction (impaired automatic body functions) including: postural or orthostatic hypotension, resulting in dizziness or fainting upon standing up, urinary  
30 incontinence, impotence; constipation; dry mouth and skin; trouble regulating

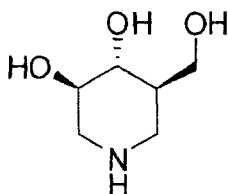
body temperature due to abnormal sweating; abnormal breathing during sleep. Notably, not all of these symptoms are experienced by all patients.

Dementia with Lewy bodies (DLB) is one of the most common types of progressive dementia. The central feature of DLB is progressive cognitive decline, combined with three additional defining features: (1) pronounced "fluctuations" in alertness and attention, such as frequent drowsiness, lethargy, lengthy periods of time spent staring into space, or disorganized speech; (2) recurrent visual hallucinations, and (3) parkinsonian motor symptoms, such as rigidity and the loss of spontaneous movement. People may also suffer from depression. The symptoms of DLB are caused by the build-up of Lewy bodies – accumulated bits of alpha-synuclein protein -- inside the nuclei of neurons in areas of the brain that control particular aspects of memory and motor control. Researchers don't know exactly why alpha-synuclein accumulates into Lewy bodies or how Lewy bodies cause the symptoms of DLB, but they do know that alpha-synuclein accumulation is also linked to Parkinson's disease, multiple system atrophy, and several other disorders, which are referred to as the "synucleinopathies." The similarity of symptoms between DLB and Parkinson's disease, and between DLB and Alzheimer's disease, can often make it difficult for a doctor to make a definitive diagnosis. In addition, Lewy bodies are often also found in the brains of people with Parkinson's and Alzheimer's diseases. These findings suggest that either DLB is related to these other causes of dementia or that an individual can have both diseases at the same time. DLB usually occurs sporadically, in people with no known family history of the disease. However, rare familial cases have occasionally been reported.

25

**Chemical syntheses****a. Isofagomine (IFG; (3R,4R,5R)-5-(hydroxymethyl)-3,4-piperidinediol))**

Isofagomine (IFG; (3R,4R,5R)-5-(hydroxymethyl)-3,4-piperidinediol)) refers to a compound having the following structure:



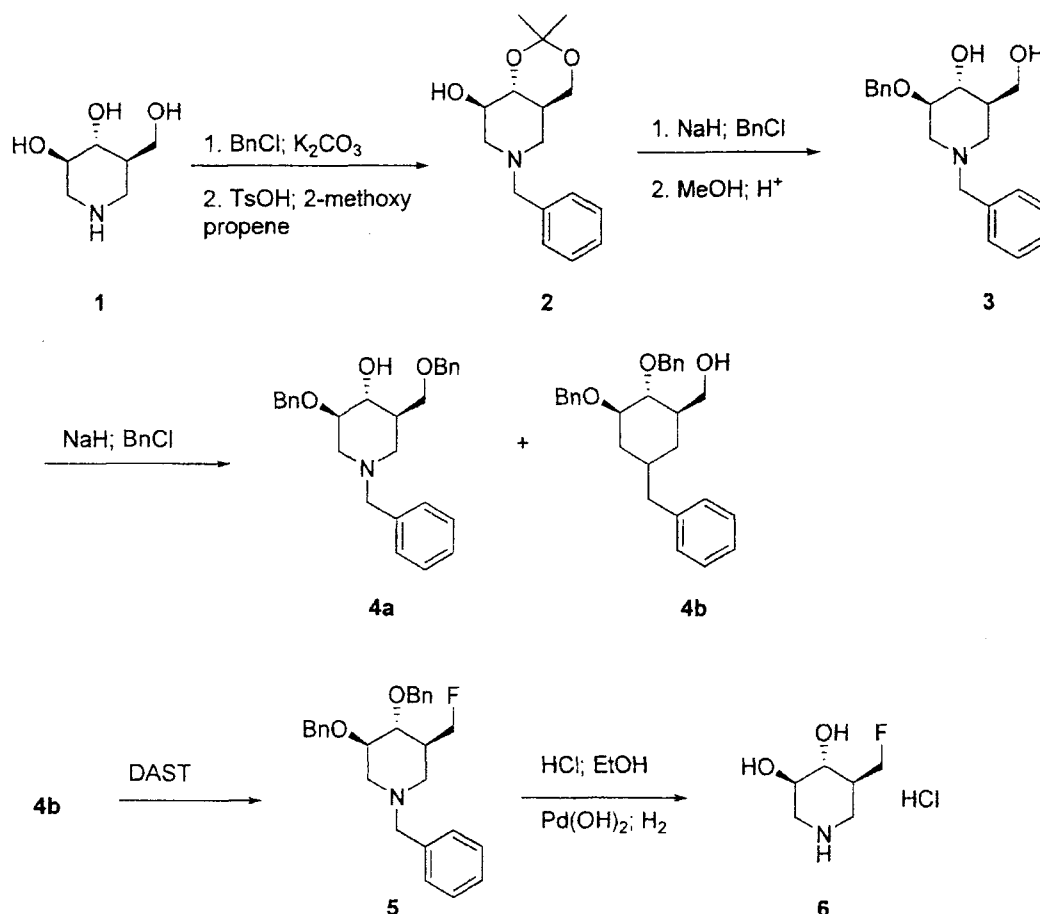
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Synthesis of isofagomine is described in U.S. Pat. Nos. 5,844,102 to Sierks et al. at column 17, line 53 to column 20, line 6, and 5,863,903 to Lundgren et al. in Example 1, at column 5, line 20 to column 7, line 33.

Isofagomine tartrate, also known as IFG and Plicera™, has been assigned CAS number 919364-56-0. Preparation of isofagomine tartrate is described in US2007/0281975I at paragraphs [0046] to [0050] and purification of isofagomine tartrate is described at paragraphs [0051] to [0053],

10

**b. Synthesis of (3R,4R,5S)-5-(fluoromethyl)piperidine-3,4-diol hydrochloride**



**5 (4aR,8R,8aR)-6-benzyl-2,2-dimethylhexahydro-4H-[1,3]dioxino[5,4,c]pyridin-8-ol (2).**

To a solution of (3R,4R,5R)-5-(hydroxymethyl)piperidine-3,4-diol (**1**) (5.9 g, 40.0 mmol) in dry DMF (75 mL) was added K<sub>2</sub>CO<sub>3</sub> (6.4 g, 46.0 mmol) followed by benzyl chloride (4.8 mL, 42.0 mmol) and the resulting mixture was heated to 70 °C for 14 hrs at which point the starting material could not be detected by tlc. The solvent was evaporated *in vacuo* and the residue was redissolved in a minimum amount of water. The solution was extracted 10-12x with EtOAc and the combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and filtered. The filtrate was evaporated *in vacuo* to give the title compound (7.5 g, 79%) as a tan solid which

could be used without further purification. The tan solid was dissolved in DMF (75 mL) and toluenesulfonic acid (6.7 g, 35.0 mmol) was added followed by 2-methoxy propene (7.2 mL, 75.0 mmol) and the mixture was stirred overnight at room temperature. After stirring overnight an additional 7.2 mL of 2-methoxy propene was added and the mixture was again stirred overnight at room temperature. At this point no starting material could be detected by tlc. Sodium hydroxide (50% aq, 5mL) was added to the reaction mixture and the solvent was evaporated *in vacuo*. The residue was dissolved in EtOAc and washed with water. The aqueous washes were combined and washed 1x with EtOAc. This was combined with original extracts, washed with brine and dried over MgSO<sub>4</sub>. The solution was filtered and the solvent evaporated to give a waxy solid. This solid can be directly used in the next step. An analytical sample can be obtained from chromatography (0 to 5% (9/1 MeOH/NH<sub>4</sub>OH) in CHCl<sub>3</sub>) to obtain the title compound. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.35 (s, 3H), 1.39 (s, 3H), 1.66 (t, 1H), 1.83 (m, 1H), 1.95 (t, 1H), 2.56 (ddd, 1H), 3.03 (ddd, 1H), 3.30 (t, 1H), 3.6-3.8 (m, 5H), 7.27 (m, 5H).

**(3R,4R,5R)-1-benzyl-3-(benzyloxy)-5-(hydroxymethyl)piperidin-4-ol (3).**

To a solution of **2** (4.4 g, 15.9 mmol) in DMF (50mL) was added 95% NaH (0.43g, 17.9 mmol) and the resulting mixture was stirred 1 hr under N<sub>2</sub>. Then benzyl chloride (1.9mL, 16.3 mmol) was added and the mixture was stirred overnight at room temperature. After approximately 14 hrs the DMF was evaporated *in vacuo*. The residue was dissolved in EtOAc and washed with water and then brine and then dried over MgSO<sub>4</sub>. The solution was filtered and the filtrate evaporated *in vacuo* to give the crude product. Chromatography on silica gel eluting with 0-25% EtOAc/hexane gave 2.1 g of a white foam. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.15 (m, 1H), 1.19 (s, 3H), 1.39 (s, 3H), 1.9-2.05 (m, 4H), 2.53 (ddd, 1H), 3.04 (ddd, 1H), 3.3 (s, 1H), 3.45-3.8 (m, 4H), 7.2 (m, 10H). This was dissolved in MeOH (100mL) and 1.5 mL of 6N HCl in 2-PrOH was added and the mixture was stirred at room temperature. After 1 hr the starting material was gone as judged by tlc. The solvent was evaporated and the residue was dissolved in 10%

Na<sub>2</sub>CO<sub>3</sub> and extracted with EtOAc (2x). The combined extracts were washed with one small portion of water and then brine and dried over MgSO<sub>4</sub>. The solution was filtered and the filtrate was evaporated to give the title compound (1.7 g, 33%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.6-1.95 (m, 3H), 2.63 (ddd, 1H), 3.00 (ddd, 1H),  
5 3.25-3.65 (m, 5H), 4.35 (d, 1H), 4.5 (d, 1H), 7.2 (m, 10H).

**((3R,4R,5R)-1-benzyl-4,5-bis(benzyloxy)-piperdin-3-yl)methanol (4b).**

To a solution of **3** (1.1 g, 3.36 mmol) in DMF (10 mL), was added NaH (0.10 g, 4.0 mmol) and the reaction was stirred 30 minutes at room temperature  
10 at which point benzyl chloride (0.38 mL, 3.3 mmol) was added and the reaction was stirred overnight at room temperature. The solvent was evaporated *in vacuo* and the residue was dissolved in EtOAc and washed with water and then brine and dried over Na<sub>2</sub>SO<sub>4</sub>. The solution was filtered and the filtrate evaporated to give a mixture of **4a** and **4b** (roughly a 2/1 mixture). The mixture was  
15 chromatographed on silica gel eluting with 0-50% EtOAc/hexane. The fractions corresponding to the major regioisomer were combined to give **4a** (0.49 g, 35%) as a colorless oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.81 (t, 2H), 1.96 (m, 1H), 2.80 (m, 1H), 3.05 (ddd, 1H), 3.3-3.6 (m, 6H), 4.41 (s, 2H), 4.55 (q, 2H), 7.2 (m, 15H). The fractions of the minor regioisomer were combined to give **4b** (0.23 g, 16%) as a colorless  
20 oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 1.8-2.0 (m, 3H), 2.72 (dd, 1H), 3.05 (ddd, 1H), 3.27 (t, 1H), 3.4-3.6 (m, 4H), 3.65 (m, 1H), 4.56 (s, 2H), 4.60 (d, 1H), 4.90 (d, 1H), 7.2 (m, 15H).

**(3R,4R,5R)-1-benzyl-3,4-bis(benzyloxy)-5-(fluoromethyl)piperdin-4-ol (5).**

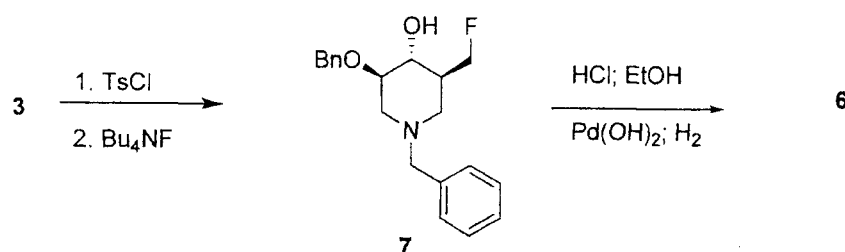
25 A solution of **4b** (0.32 g, 0.77 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40mL) was cooled to -15 to -20 °C in an ice/salt water bath. To this was added (diethylamino)sulfur trifluoride ( 0.15mL, 1.15 mmol) and the reaction was allowed to stir for about 20 minutes at which point the starting material was consumed as judged by tlc. The reaction was quenched by the addition of NaHCO<sub>3</sub> and the mixture was extracted  
30 with EtOAc (2x). The combined extracts were washed with brine and then dried over Na<sub>2</sub>SO<sub>4</sub>. The solution was filtered and the filtrate was evaporated to give

the crude product. Silica gel chromatography (0-25% EtOAc/hexane) afforded the desired product (0.2 g, 63%) as a colorless oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 1.9-2.15 (m, 3H), 2.88 (dd, 1H), 3.12 (ddd, 1H), 3.36 (t, 1H), 3.55 (d, 1H), 3.65 (m, 1H), 4.3-4.6 (m, 3H), 4.63-4.65 (m, 3H), 4.95 (d, 1H), 7.2 (m, 15H).

5

**(3R,4R,5S)-5-(fluoromethyl)piperidine-3,4-diol hydrochloride(6).**

To a solution of **5** (0.24 g, 0.57 mmol) in EtOH (40 mL) was added 0.5 mL of 6N HCl in 2-PrOH. The solvent was evaporated *in vacuo* and then co-evaporated 2x more with EtOH. The residue was dissolved in EtOH and  
 10 hydrogenated at 55 psi using  $\text{Pd}(\text{OH})_2$  as catalyst. After 14 hrs the starting material could no longer be detected and the solution was filtered and the filtrate was evaporated *in vacuo*. The residue was triturated with acetone and then filtered to give an off white solid (0.09 g, 85%) as the title compound.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ) 1.95-2.05 (m, 1H), 2.65 (t, 1H), 2.85 (t, 1H) 3.15-3.4 (m, 3H), 3.55 (m,  
 15 1H), 4.55 (m, 1H), 4.65 (m, 1H), 5.45-5.6 (dd, 2H), 9.05 (br s, 2H).



**(3R,4R,5R)-1-benzyl-3-(benzyloxy)-5-(fluoromethyl)piperidin-4-ol (7).**

To a solution of **3** (26.0 g, 79.5 mmol) dissolved in pyridine (300 mL) and cooled in an ice bath was added TsCl (16.6 g, 87.5 mmol) in portions. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirred overnight. The solvent was evaporated *in vacuo* and the residue dissolved in EtOAc. The solution was washed with water (2x) and then  
 25 brine and then dried over  $\text{MgSO}_4$ . The solution was filtered and the filtrate evaporated *in vacuo* to give the crude product which was dried under high vacuum. The residue was dissolved in THF (400 mL) and 1.0 M  $\text{Bu}_4\text{NF}$  (100



mmol, 100 mL was added and the mixture was heated at reflux. After 2 hrs, there was no starting material remaining. The reaction mixture was diluted with EtOAc and washed with water (2x) and then with brine and dried over MgSO<sub>4</sub>. The solution was filtered and the filtrate was evaporated *in vacuo*. The crude  
5 product was purified by silica gel chromatography (25% EtOAc/hexane) to give the title compound (7.5 g, 29% for 2 steps).

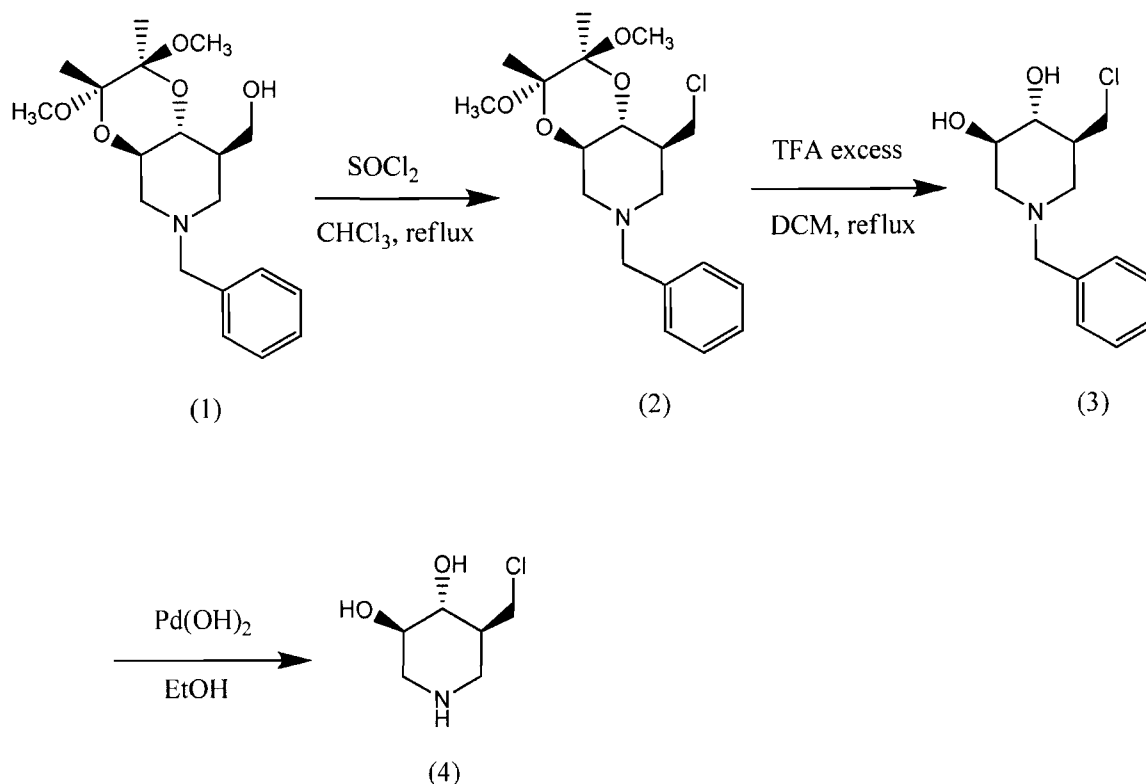
**(3R,4R,5S)- 5-(fluoromethyl)piperidine-3,4-diol hydrochloride(6).**

To a solution of **7** (7.5 g, 22.8 mmol) in EtOH (150 mL) was added 5N HCl  
10 in 2-PrOH (7 mL). The solution was evaporated *in vacuo*, then co-evaporated 2x more with EtOH. The resulting material was dissolved in EtOH (100 mL) and the resulting solution was hydrogenated overnight with Pd(OH)<sub>2</sub> at 50 psi. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was triturated with acetone and a pale yellow solid was collected. The  
15 resulting solid was recrystallized from EtOH to give the title compound as an off-white solid mp 200-202 °C. <sup>1</sup>H NMR DMSO *d*<sub>6</sub> consistent with previous spectra.

**c. Synthesis of (3R,4R,5S)- 5-(fluoromethyl)piperidine-3,4-diol**

One approach for obtaining the free base of (3R,4R,5S)- 5-  
20 (fluoromethyl)piperidine-3,4-diol is to synthesize (3R,4R,5S)- 5-(fluoromethyl)piperidine-3,4-diol hydrochloride as described in section "b" above followed by chromatography of (3R,4R,5S)- 5-(fluoromethyl)piperidine-3,4-diol hydrochloride using 5-15% MeOH/NH<sub>4</sub>OH (9:1) in CHCl<sub>3</sub> which converts the HCl salt form of (3R,4R,5S)- 5-(fluoromethyl)piperidine-3,4-diol to its free base.

25

**d. Synthesis of (3R,4R,5S)-5-(chloromethyl)piperidine-3,4-diol**

**(2S,3S,4aR,8S,8aR)-6-benzyl-8-(chloromethyl)-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridine (2).**

To a solution of ((2S,3S,4aR,8R,8aR)-6-benzyl-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridine-8-yl)methanol (1) (2.5 g, 7.1 mmol) in chloroform (35 mL) was added thionyl chloride (1.3 mL, 18 mmol). The resulting mixture was heated at reflux for 4-8 hours until reaction was judged complete by TLC (98:2  $\text{CH}_2\text{Cl}_2$ /2-PrOH). Solvent and excess reagent were evaporated *in vacuo* and the residue was chromatographed (98:2  $\text{CH}_2\text{Cl}_2$ /2-PrOH) to give the title compound (2.2 g, 85%). Product was characterized via HPLC/MS ( $\text{MH}^+ = 369$ ). Purity was judged as >95%.

**(3R,4R,5S)-1-benzyl-5-(chloromethyl)piperidine-3,4-diol (3).**

To a solution of (2S,3S,4aR,8S,8aR)-6-benzyl-8-(chloromethyl)-2,3-dimethoxy-2,3-dimethyloctahydro-[1,4]dioxino[2,3-c]pyridine (2) (2.2 g, 5.9 mmol) in dichloromethane (15 mL) was added trifluoroacetic acid (0.35 mol, 26 mL).

Reaction mixture was heated at reflux for about 1 hour at which point reaction was judged complete by TLC. Excess solvent and TFA were evaporated *in vacuo* and the residue was chromatographed using silica gel (2-8% MeOH in CHCl<sub>3</sub>). Fractions were combined and evaporated to give (1.3 g, 87 %) as the title compound. Product was characterized via HPLC/MS (MH<sup>+</sup> = 255) and judged to be >95% pure.

**(3R,4R,5S)-5-(chloromethyl)piperidine-3,4-diol (4).**

5N HCl (4.7 mmol, 0.94 mL) was added to (3R,4R,5S)-1-benzyl-5-(chloromethyl)piperidine-3,4-diol (3) (5.1 mmol, 1.3g) dissolved in EtOH. Solution was evaporated *in vacuo* and then co-evaporated 2-3x with EtOH. The residue was redissolved in EtOH and combined with Pd(OH)<sub>2</sub> (0.27 g), and hydrogenated at 55 psi for 12 hrs. The catalyst was subsequently filtered through dicalite and the filtrate was evaporated to obtain crude (4) as an HCl salt. This was then chromatographed (5-15% MeOH/NH<sub>4</sub>OH (9:1) in CHCl<sub>3</sub>) to give 0.4 g of (4) as a white solid with MH<sup>+</sup> of 165 and <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) 1.6 (m, 1H), 2.2 (m, 3H), 2.9 (m, 3H), 3.15 (m, 1H), 3.6(m, 1H), 3.8 (dd, 1H), 4.68 (d, 1H), 4.88 (d, 1H).

**e. Synthesis of (3R,4R,5S)-5-(chloromethyl)piperidine-3,4-diol hydrochloride**

One approach for obtaining the hydrochloride salt of (3R,4R,5S)-5-(chloromethyl)piperidine-3,4-diol is to synthesize (3R,4R,5S)-5-(chloromethyl)piperidine-3,4-diol as described in section "d" above followed by reacidification with HCl (either as an aqueous solution or in 2-PrOH as it is often sold) whereby the free base is converted to the HCl salt form of (3R,4R,5S)-5-(chloromethyl)piperidine-3,4-diol and subsequent isolation of (3R,4R,5S)-5-(chloromethyl)piperidine-3,4-diol hydrochloride.

Alternatively, the synthesis of (3R,4R,5S)-5-(chloromethyl)piperidine-3,4-diol may be followed as described in section "d" above with the exception of the last chromatographic step whereby the HCl salt is chromatographed using 5-15%

MeOH/NH<sub>4</sub>OH (9:1) in CHCl<sub>3</sub> as this step results in the conversion of the crude HCl salt form of (3*R*,4*R*,5*S*)-5-(chloromethyl)piperidine-3,4-diol to the free base. Rather, the crude HCl salt form of (3*R*,4*R*,5*S*)-5-(chloromethyl)piperidine-3,4-diol may be purified by crystallization.

5

**Salts, solvates and prodrugs**

Compounds of the present invention include pharmaceutically acceptable salts, solvates and pro-drugs of (3*R*,4*R*,5*S*)-5-(fluoromethyl)piperidine-3,4-diol (also referred to herein as Compound A) and (3*R*,4*R*,5*S*)-5-(chloromethyl)piperidine-3,4-diol (also referred to herein as Compound B).

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'-diacetythylenediamine, 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 (3*R*,4*R*,5*S*)-5-(fluoromethyl)piperidine-3,4-diol is the hydrochloride salt (also referred to herein as Compound A-HCl).

"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 H<sub>2</sub>O. Other non-limiting examples of suitable  
5 solvates include alcohols (e.g., ethanolates, methanolates, and the like).

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).

Additionally, a discussion of prodrugs is provided in T. Higuchi and V. Stella, Pro-  
10 drugs 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.

Prodrugs can be used to alter the biodistribution (e.g., to allow compounds which would not typically enter the  
15 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  
20 with moieties (e.g., acyloxymethyl esters) which are cleaved to reveal an intermediate compound which subsequently decomposes to yield the active compound.

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  
25 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,  
30 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.

All stereoisomers (for example, geometric isomers, optical isomers and the like) of 5-(fluoromethyl)piperdine-3,4-diol and 5-(chloromethyl)piperdine-3,4-diol (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 5-(fluoromethyl)piperdine-3,4-diol and 5-(chloromethyl)piperdine-3,4-diol.

### **Formulations**

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).

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.

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.

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); 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.

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  $\alpha$ -glucoside, Octyl  $\beta$ -glucoside, Brij 35™, Pluronic™, and Tween™ 20.



**Routes of Administration**

The therapeutic agent(s) may be administered orally or parenterally, including intravenously, subcutaneously, intra-arterially, intraperitoneally, ophthalmically, intramuscularly, buccally, rectally, vaginally, intraorbitally, 5 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.

Administration of therapeutic agent(s) may be by periodic injections of a 10 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.

Intrapulmonary delivery methods and apparatus are described, for example, in U.S. Pat. Nos. 5,654,007, 5,780,014, 15 and 5,814,607. 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 20 are described in U.S. Pat. Nos. 5,879,327; 5,520,639; 5,846,233 and 5,704,911.

Any of the formulations described above can be administered using these methods.

Subcutaneous injections have the advantages allowing self-administration, while also resulting in a prolonged plasma half-life as compared to intravenous 25 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**

30 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  
5 intermittently.

Exemplary dosing regimens are described in International patent application PCT/US08/61764 published as WO 2008/134628 on June 11, 2008 and U.S. patent application 12/604,855, filed on October 23, 2009,

In one embodiment, the  
10 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.

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  
15 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.

20

### ***Combination Drug Therapy***

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  
25 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.

In certain embodiments, the therapeutic agent(s) of the present invention  
30 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).

5           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®).

10           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.

15           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).

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

          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. Following is a non-limiting list  
25           of therapeutic agents for lysosomal enzymes.

**Table 1**

<b>LYSOSOMAL ENZYME</b>	<b>THERAPEUTIC AGENT</b>
$\alpha$ -Glucosidase GenBank Accession No. Y00839	1-deoxynojirimycin (DNJ) $\alpha$ -homonojirimycin castanospermine
Acid $\beta$ -Glucosidase ( $\beta$ -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- $\beta$ -valeinamines Fluphenazine calystegines A <sub>3</sub> , B <sub>1</sub> , B <sub>2</sub> and C <sub>1</sub>
$\alpha$ -Galactosidase A GenBank Accession No. NM000169	1-deoxygalactonojirimycin (DGJ) $\alpha$ - <i>allo</i> -homonojirimycin $\alpha$ - <i>galacto</i> -homonojirimycin $\beta$ -1-C-butyl-deoxynojirimycin calystegines A <sub>2</sub> and B <sub>2</sub> N-methyl calystegines A <sub>2</sub> and B <sub>2</sub>
Acid $\beta$ -Galactosidase GenBank Accession No. M34423	4- <i>epi</i> -isofagomine 1-deoxygalactonojirimycin
Galactocerebrosidase (Acid $\beta$ -Galactosidase) GenBank Accession No. D25283	4- <i>epi</i> -isofagomine 1-deoxygalactonojirimycin

Acid $\alpha$ -Mannosidase GenBank Accession No. U68567	1-deoxymannojirimycin Swainsonine Mannostatin A
Acid $\beta$ -Mannosidase GenBank Accession No. U60337	2-hydroxy-isofagomine
Acid $\alpha$ -L-fucosidase GenBank Accession No. NM_000147	1-deoxyfuconojirimycin $\beta$ -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
$\alpha$ -N-Acetylglucosaminidase GenBank Accession No. U40846	1,2-dideoxy-2-N-acetamido-nojirimycin
$\alpha$ -N-Acetylgalactosaminidase GenBank Accession No. M62783	1,2-dideoxy-2-N-acetamido-galactonojirimycin
$\beta$ -Hexosaminidase A GenBank Accession No. NM_000520	2-N-acetylamino-isofagomine 1,2-dideoxy-2-acetamido-nojirimycin Nagstatin
$\beta$ -Hexosaminidase B GenBank Accession No. NM_000521	2-N-acetamido-isofagomine 1,2-dideoxy-2-acetamido-nojirimycin Nagstatin
$\alpha$ -L-Iduronidase GenBank Accession No. NM_000203	1-deoxyiduronojirimycin 2-carboxy-3,4,5-trideoxypiperidine
$\beta$ -Glucuronidase	6-carboxy-isofagomine

GenBank Accession No. NM_000181	2-carboxy-3,4,5-trideoxypiperidine
Sialidase	2,6-dideoxy-2,6, imino-sialic acid
GenBank Accession No. U84246	Siastatin B
Iduronate sulfatase	2,5-anhydromannitol-6-sulphate
GenBank Accession No. AF_011889	
Acid sphingomyelinase	desipramine, phosphatidylinositol-4,5-diphosphate
GenBank Accession No. M59916	

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).

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  $\beta$ -hexosaminidase A and/or a therapeutic agent for acid  $\beta$ -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).

The therapeutic agent(s) of the present invention can be administered in combination with at least one other therapeutic agent which includes but is not limited to, RNAi, dopamine replacement (e.g., levadopa (L-DOPA)), dopamine

replacement stabilizer (e.g., carbidopa, and entacapone), anticholinergic (e.g., trihexyphenidyl, benzotropine mesylate (Cogentin®), trihexyphenidyl HCL (Artane®), and procyclidine), catechol-O-methyltransferase (COMT) inhibitor (e.g., entacapone (Comtan®) and tolcapone (Tasmar®)), dopamine receptor  
5 agonist (e.g., bromocriptine (Parlodel®), pramipexole (Mirapex®), ropinirole (Requip®)), pergolide (Permax), and APOKYN™ injection (apomorphine hydrochloride), monoamine oxidase (MAO) inhibitor (i.e., MAO-A and/or MAO-B inhibitors, e.g., selegiline (Deprenyl, Eldepryl®, Carbox®), selegiline HCl orally disintegrating tablet (Zelapar®), and rasagiline (Azilect®)), peripheral  
10 decarboxylase inhibitor, amantadine (Symmetrel®), and rivastigmine tartrate (Exelon®).

Also contemplated are combinations of the therapeutic agent(s) of the present invention with more than one other therapeutic agent. Exemplary combinations of other therapeutic agents include, but not are not limited to,  
15 carbidopa/levodopa (Sinemet® or Parcopa®), carbidopa, levodopa and entacapone (Stalevo®), levodopa with a dopamine receptor agonist such as bromocriptine (Parlodel®), pramipexole (Mirapex®), ropinirole (Requip®), pergolide (Permax), or APOKYN™ injection (apomorphine hydrochloride).

In one embodiment, the therapeutic agent(s) of the present invention is  
20 administered in combination with vaccine therapy, such as a vaccine comprising alpha-synuclein and an adjuvant (Pilcher et al., *Lancet Neurol.* 2005; 4(8):458-9).

In one embodiment, the therapeutic agent(s) of the present invention is administered in combination with at least one other therapeutic agent that may be protective such as dextromethorphan (Li et al., *FASEB J.* 2005;  
25 Apr;19(6):489-96); genistein (Wang et al., *Neuroreport.* 2005; Feb 28;16(3):267-70), or minocycline (Blum et al., *Neurobiol Dis.* 2004; Dec;17(3):359-66).

In one embodiment, the therapeutic agent(s) of the present invention is administered in combination with at least one other therapeutic agent which is therapeutic agent for alpha-synuclein (e.g., Hsp70).

30 Patients having Parkinson's disease experience tremor, rigidity, bradykinesia, and postural imbalance. Patients having Lewy Body Dementia

experience strong psychotic symptoms (visual hallucinations) in addition to mental decline such as memory loss and an inability to carry out simple tasks. Observable improvements in symptoms, or a delay of onset of certain symptoms in patients at risk of developing a disorder, or a delay in progression of the disorder will be evidence of a favorable response to the therapies provided herein.

In addition, measurable surrogate markers also may be useful for evaluating response to therapy. For instance, some investigators have reported detecting higher levels of alpha-synuclein or oligomeric forms of alpha-synuclein have been detected in the plasma of patients with Parkinson's disease (Lee et al., *J Neural Transm.* 2006;113(10):1435-9; El-Agnaf et al., *FASEB J.* 2006;20(3):419-25), while some have reported decreased plasma alpha-synuclein in Parkinson's patients compared with normal controls (Li et al., *Exp Neurol.* 2007;204(2):583-8).

In certain embodiments, the therapeutic agent(s) of the present invention is administered in combination with at least one other therapeutic agent which is an alcohol deterrent (e.g., Acamprosate), a narcotic analgesic (e.g., Remifentanyl), an anti-dyskinesia agent (e.g., Amantadine, Apomorphine, Benserazide, Bromocriptine, Cabergoline, Carbidopa, Dexetimide, Droxidopa, Entacapone, Levodopa, Lisuride, Memantine, Piribedil, Pramipexol, Ropinirole, Selegiline, Sinemet), an anti-infective agent (e.g., Amantadine, Amoxicillin, Clarithromycin, Ethanol, Interferons, Minocycline, PS-K), an anti-obesity agent (e.g., Phenylpropanolamine, Topiramate), an anticonvulsant (e.g., Etiracetam, Topiramate), an antiemetic (e.g., Trimethobenzamide), an antihypertensive agent (e.g., Trandolapril), an antineoplastic agent (e.g., Cabergoline, PS-K), central nervous system depressant (e.g., Aripiprazole, Benzocaine, Clozapine, Cocaine, Dexmedetomidine, Diphenhydramine, Isoflurane, Lithium, Lithium Carbonate, Methylperon, Morphine, Propofol, Quetiapine, Raclopride, Remifentanyl, Sodium Oxybate), a central nervous system stimulant (e.g., Caffeine citrate, Modafinil, Nicotine polacrilex), a coagulant (e.g., Arginine Vasopressin, Deamino Arginine Vasopressin, Vasopressins), a dermatologic agent (e.g., Loratadine,



Promethazine), a gastrointestinal agent (e.g., Diphenhydramine, Domperidone, Omeprazole, Trimethobenzamide), a hypnotic and/or sedative (e.g., Remifentanyl), a micronutrient (e.g., Alpha-Tocopherol, Coenzyme Q10, Ergocalciferols, Hydroxocobalamin, Iron, Tocopherol acetate, Tocopherols, Vitamin B 12, Vitamin D, Vitamin E), a neuroprotective agent (e.g., Eliprodil, Modafinil, Rasagiline, Rivastigmine, Topiramate), a nootropic agent (e.g., Donepezil, Etiracetam), a psychotropic drug (e.g., Aripiprazole, Citalopram, Clozapine, Duloxetine, Lithium, Lithium Carbonate, Metylperon, Nortriptyline, Paroxetine, Quetiapine, Raclopride, Venlafaxine), a respiratory system agent (e.g., Dextromethorphan, Guaifenesin, Ipratropium, Naphazoline, Oxymetazoline, Phenylephrine, Phenylpropanolamine), a vasoconstrictor agent (e.g., Naphazoline, Oxymetazoline, Phenylephrine, Phenylpropanolamine).

In one preferred embodiment, the aforementioned other therapeutic agents are administered when the disorder is Parkinson's disease.

In certain embodiments, the therapeutic agent(s) of the present invention is administered in combination with at least one other therapeutic agent which is a nicotinic alpha-7 agonist (e.g., MEM 3454 or MEM 63908 both of which are available from Memory Pharmaceuticals). In certain embodiments, the therapeutic agent(s) of the present invention is administered in combination with at least one other therapeutic agent which is R3487 and/or R4996 (both of which are available from Roche). Also contemplated are combinations of the therapeutic agent(s) of the present invention with more than one other therapeutic agents. Exemplary combinations of other therapeutic agents include, but not are not limited to, R3487/MEM 3454 and R4996/MEM 63908.

In certain embodiments, the therapeutic agent(s) of the present invention is administered in combination with at least one cholinesterase inhibitor (e.g., donepezil (brand name *Aricept*), galantamine (brand name *Razadyne*), and rivastigmine (branded as *Exelon* and *Exelon Patch*).

In certain embodiments, the therapeutic agent(s) of the present invention is administered in combination with at least one noncompetitive NMDA receptor

antagonist (e.g., memantine (brand names *Akatinol*, *Axura*, *Ebixa/Abixa*, *Memox* and *Namenda*)).

In certain embodiments, the therapeutic agent(s) of the present invention is administered in combination with at least one other therapeutic agent which is

5 a non-narcotic analgesic (e.g., Celecoxib, Resveratrol, Rofecoxib, TNFR-Fc fusion protein), an anti-dyskinesia agent (e.g., Dexetimide, Gabapentin, Levodopa, Memantine), an anti-infective agent (e.g., Acetylcysteine, Acyclovir, Benzoates, Deoxyglucose, Doxycycline, Interferon Alfa-2a, Interferon-alpha, Interferons, Moxifloxacin, PS-K, Quinacrine, Rifampin, Salicylic Acid,

10 Valacyclovir), an anti-Inflammatory agent (e.g., Aspirin, Celecoxib, Curcumin, Ibuprofen, Indomethacin, Naproxen, Resveratrol, Rofecoxib, TNFR-Fc fusion protein), an anti-obesity agent (e.g., Phenylpropanolamine), an anticonvulsant agent (e.g., Gabapentin, Homotaurine, Lamotrigine), an antiemetic (e.g., Olanzapine), an antihypertensive agent (e.g., Trandolapril), an antilipemic agent

15 (e.g., Atorvastatin, Choline, Clofibrilic Acid, Pravastatin, Simvastatin), an antineoplastic agent (e.g., Bryostatin 1, Carmustine, Cyclophosphamide, Interferon Alfa-2a, Leuprolide, Medroxyprogesterone 17-Acetate, Methyltestosterone, PK 11195, Prednisone, PS-K, Resveratrol, 2,3-dihydro-1H-imidazo(1,2-b)pyrazole), an antirheumatic agent (e.g., Aspirin, Celecoxib,

20 Curcumin, Ibuprofen, Indomethacin, Naproxen, Resveratrol, Rofecoxib, TNFR-Fc fusion protein), a central nervous system depressant (e.g., Aripiprazole, Benzocaine, Cocaine, Gabapentin, Haloperidol, Haloperidol decanoate, Lithium, Lithium Carbonate, Lorazepam, Midazolam, Olanzapine, Perphenazine, Propofol, Quetiapine, Risperidone, Sodium Oxybate, Trazodone, Valproic Acid,

25 Zolpidem), a central nervous system stimulant (e.g., Caffeine citrate, Modafinil, Nicotine polacrilex), a channel blocker (e.g., Gabapentin, Lamotrigine), a coagulant (e.g., Antiplasmin, Vitamin K), a dermatologic agent (e.g., Mineral Oil, Salicylic Acid), a gastrointestinal agent (e.g., Choline, Haloperidol, Lorazepam, Olanzapine, Omeprazole, TNFR-Fc fusion protein), a hypnotic and/or sedative

30 agent (e.g., Zolpidem), a hypoglycemic agent (e.g., Insulin, Asp(B28)-, Rosiglitazone), a micronutrient (e.g., Alpha-Tocopherol, Ascorbic Acid,

Coenzyme Q10, Copper, Folic Acid, Hydroxocobalamin, Inositol, Iron, Niacin, Niacinamide, Nicotinic Acids, Pyridoxine, Selenium, Thiocctic Acid, Tocopherol acetate, Tocopherols, Vitamin B 12, Vitamin B 6, Vitamin E, Vitamin K), a neuroprotective agent (e.g., Huperzine A, Modafinil, Nefiracetam, Rasagiline, 5 Rivastigmine, (3-aminopropyl)(n-butyl)phosphinic acid), a nootropic agent (e.g., Donepezil, Nefiracetam), a platelet aggregation inhibitor (e.g., Resveratrol), a psychotropic drug (e.g., Aripiprazole, Bupropion, Citalopram, Duloxetine, Gabapentin, Haloperidol, Haloperidol decanoate, Lithium, Lithium Carbonate, Lorazepam, Midazolam, Nefiracetam, Olanzapine, Paroxetine, Perphenazine, 10 Quetiapine, Risperidone, Sertraline, Trazodone, Tryptophan, Valproic Acid, Venlafaxine), a reproductive control agent (e.g., Estradiol 17 beta-cypionate, Estradiol 3-benzoate, Estradiol valerate, Indomethacin, Leuprolide, Medroxyprogesterone, Medroxyprogesterone 17-Acetate, Mifepristone), a respiratory system agent (e.g., Acetylcysteine, Dextromethorphan, Guaifenesin, 15 Naphazoline, Oxymetazoline, Phenylephrine, Phenylpropanolamine), or a vasoconstrictor agent (e.g., Naphazoline, Oxymetazoline, Phenylephrine, Phenylpropanolamine).

In one preferred embodiment, the aforementioned other therapeutic agents are administered when the disorder is Alzheimer's disease.

20

### **EXAMPLES**

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.

25 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.

30

**EXAMPLE 1 Determination of Inhibition Constants**

The binding affinity (defined here by  $K_i$  binding constant) of GCase for small molecule pharmacological chaperones described in this document were empirically determined using enzyme inhibition assays. In brief, the enzyme inhibition assays used monitored the ability of 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- $\beta$ -D-glucopyranoside (4-MU- $\beta$ -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***

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  $\beta$ -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  $\beta$ -D-glucopyranoside in buffer "M" with 0.15% Triton X-100 at both pH's. Five  $\mu$ l of diluted enzyme was added to 15  $\mu$ l of the various inhibitor concentrations or

buffer "M" alone and incubated at 37° C for 1 hour with 50 µl 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 this inhibition assay for reference compound, IFG-tartrate, and test compounds, the hydrochloride salt form of Compound A and Compound B, are summarized below in **Table 2A**.

<b>Table 2A <i>In vitro</i> Determination of Inhibition Constants</b>				
<b>Compound</b>	<b>IC<sub>50</sub> (mM) pH 5.2</b>	<b>Ki (µM) pH 5.2</b>	<b>IC<sub>50</sub> (µM) pH 7</b>	<b>Ki (µM) pH 7</b>
<b>IFG-Tartrate</b>	<b>0.0437 ± 0.0018</b>	<b>0.023 ± 0.001</b>	<b>0.00626 ± 0.00018</b>	<b>0.0033 ± 0.00013</b>
<b>Compound A-HCl</b>	<b>0.014±0.006</b>	<b>0.006±0.003</b>	<b>0.006±0.0005</b>	<b>0.003±0.0003</b>
<b>Compound A-HCl*</b>	<b>0.014±0.001</b>	<b>0.006±0.0004</b>	<b>0.007±0.0004</b>	<b>0.004±0.0002</b>
<b>Compound B</b>	<b>0.0729±0.004</b>	<b>0.384±0.0021</b>	<b>0.0098±0.0005</b>	<b>0.0051±0.0003</b>
<b>Note:</b> * independent synthesis of the hydrochloride salt form of Compound A				

### ***In situ* assay**

The effect of IFG and its derivatives 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 IC<sub>50</sub>.

Specifically, the following concentration ranges were employed: Compound A:  $1 \times 10^{-5}$  to  $3.33 \times 10^{-11}$ ; IFG:  $3.33 \times 10^{-5}$  to  $1 \times 10^{-10}$ ; Compound B:  $3 \times 10^{-5}$  to  $9 \times 10^{-11}$ ; 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 IC<sub>90</sub>. 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 inhibition assays are summarized below in **Table 2B**.

<b>Table 2B <i>In situ</i> Determination of Inhibition Constants</b>				
<b>Compound</b>	<b><i>In Situ</i> IC<sub>50</sub> (<math>\mu</math>M)</b>	<b><i>In Situ</i> Washout (hr)</b>	<b>EC<sub>50</sub> (<math>\mu</math>M)</b>	<b>E<sub>max</sub> (%)</b>
IFG-Tartrate	0.271 $\pm$ 0.012	8.2 $\pm$ 0.04	0.9 $\pm$ 0.2	93.6 $\pm$ 2.5
Compound A-HCl	0.017 $\pm$ 0.008	6.6 $\pm$ 0.12	0.00138 $\pm$ 0.00063	96.8 $\pm$ 7.7
Compound A-HCl*			0.0056 $\pm$ 0.0015	104 $\pm$ 4.58
Compound B	0.121 $\pm$ 0.007	2.9 $\pm$ 0.22	0.0096 $\pm$ 0.001	104.2 $\pm$ 3.4
<b>Notes:</b> * independent synthesis of the hydrochloride salt form of Compound A  <i>Cheng-Prusoff equation:</i> $K_i = IC_{50} / (1 + [S]/K_m)$ where [S] = substrate concentration; 2.5 mM 4-MU- $\beta$ -D-Glc was used K <sub>m</sub> = Michaelis constant that defines substrate affinity; $1.8 \pm 0.6$ mM for 4-MU- $\beta$ -D-Glc (Liou et al., (2006) <i>J Biol. Chem.</i> 281 (7), 4242-53)				

Notably, test compounds, the hydrochloride salt form of Compound A and Compound B, were found to cause a concentration-dependent increase in GCase activity. Moreover, when compared to IFG-tartrate, test compounds, the hydrochloride salt form of Compound A and Compound B, enhanced enzyme activity to the same maximum level at much lower concentration.

**EXAMPLE 2: Blood brain barrier penetration**

The blood-brain barrier (BBB) penetration of reference compound (IFG-tartrate) and test compounds (IFG-derivatives, the hydrochloride salt form of Compound A and Compound B) were assayed after oral administration to mice.

- 5 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 CO<sub>2</sub> at the following time points: 0-, 0.5-, 1-, and 4-hour post-dose. After euthanization, whole blood was collected from
- 10 the inferior vena cava into lithium heparin tubes. Similarly, brains were collected from each mouse. Plasma was derived by spinning whole blood at 2700g 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,
- 15 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 13C2-15N in 0.5% formic acid in (70:30)
- 20 acetonitrile:methanol. Samples were again clarified by centrifugation and 75 µl of the supernatant was combined with 75 µl 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
- 25 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 µl sample was injected for analysis. Notably, the retention times for IFG, Compound A and Compound B are 4.91, 4.33, and 4.32 minutes, respectively. For MS/MS, the analytes were monitored by MRM with the following ion masses
- 30 (Q1/Q3, amu): IFG 13C2-15N isotopically-labeled internal standard (151.1/115.1), IFG (148.1/112.1), Compound A (150.1/103.1), Compound B

(166.2/130.1). When Compound B was analyzed, Compound A was used as internal standard (150.1/130.1). 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.

The plasma levels and brain levels of reference compound, IFG, and test compound, Compound A, detected in mice administered a single 30 mg/kg (free base equivalent) p.o. dose of IFG-tartrate or the hydrochloride salt form of Compound A are illustrated in **Figures 1A** and **1B**, respectively. Similarly, the ratio of brain to plasma levels of Compound A and IFG detected in these mice is illustrated in Figure 1C. Surprisingly, these results reflect that Compound A crossed the blood brain barrier more readily as compared to IFG.

The brain levels of reference compound, IFG, and test compound, Compound B, detected in mice administered a single 30 mg/kg (free base equivalent) p.o. dose of IFG-tartrate or free base form of Compound B is illustrated in **Figure 2**. Notably, even higher levels of Compound B were detected in brain than that observed following administration of the hydrochloride salt form of Compound A.

### **EXAMPLE 3: GCase enhancement**

The ability of orally administered test compounds (the hydrochloride salt form of Compound A and Compound B) to elevate GCase levels as compared to reference compound, IFG-tartrate, was assessed in mice. For this purpose, 8-week old wild-type male mice (C57/BL6) were administered a single p.o. (gavage) dose (detailed in **Figures 3A-D**, **4A-D**, and **5A-D**) of control, reference compound (IFG-tartrate) or test compound (the hydrochloride salt form of Compound A or Compound B). Seven animals were used per dose. 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.

After completion of dosing, mice were euthanized with CO<sub>2</sub> 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 Mcllvane (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-methylumbelliferryl-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-methylumbelliferone (4-MU) standard curve ranging from 1.0 nM to 50 µM 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.

**Figures 3A-D** illustrate the level of GCase in brain, spleen, liver and lung, respectively, of C57BL/6 mice administered a 2-week dosing regimen consisting of nine doses of (i) control vehicle; (ii) 100 mg/kg (free base equivalent) of reference compound, IFG-tartrate; or (iii) 10 or 100 mg/kg (free base equivalent) of test compound, the hydrochloride salt form of Compound A. Furthermore, **Tables 3A-C** detail the level of GCase enhancement in brain, spleen, and plasma, respectively, of mice treated as described above.

Table 3A Brain samples of GCase enhancement study					
Compound	Dose (mg/kg)	Dosing Solution	GCase increase (-fold)	Concentration (nM)	IC50 pH 5.2 (nM)
Compound A	10	Water	2.5	124±11 (0.5)	10±1
Compound A	100	Water	3.5	726±51 (3)	
IFG	100	Water	2.2	182±13 (0.7)	50±3
Notes: BLQ < 5 ng/g Values in parenthesis indicate concentrations in GCase assays after dilution IC50 data is average of three independent experiments					

Table 3B Spleen samples of GCase enhancement study					
Compound	Dose (mg/kg)	Dosing solution	GCase Increase (-fold)	Concentration (nM)	IC50 pH 5.2 (nM)
Compound A	10	Water	1.9	69±6 (0.3)	10±1
Compound A	100	Water	2.8	304±63 (1.1)	
IFG	100	Water	1.9	711±100 (2.6)	50±3
Notes: BLQ < 8 ng/g Values in parenthesis indicate concentrations in GCase assays after dilution IC50 data is average of three independent experiments					

Table 3C Plasma samples of GCase enhancement study			
Compound	Dose (mg/kg)	Dosing solution	Concentration (nM)
Compound A	10	Water	32±2.4
Compound A	100	Water	254±73.5
IFG	100	Water	330±36
Note: BLQ < 1ng/mL			

Similarly, **Figures 4A-D** illustrate the level of GCase in brain, spleen, liver and lung, respectively, of C57BL/6 mice administered a 2-week dosing regimen consisting of nine doses of (i) control vehicle; (ii) 100 mg/kg (free base equivalent) of reference compound, IFG-tartrate; or (ii) 1, 3, 10, 30 or 100 mg/kg (free base equivalent) of test compound, the hydrochloride salt form of Compound A.

As reflected in **Figures 3A-D** and **4A-D** as well as **Tables 3A-3C**, mice administered the hydrochloride salt form of Compound A demonstrated statistically significant greater GCase enhancement in brain, spleen, liver and lung as compared to mice administered control or IFG-tartrate. Moreover, GCase enhancement in mice administered the hydrochloride salt form of Compound A was unexpectedly greater than that observed with IFG-tartrate even when far lower doses of the hydrochloride salt form of Compound A were administered than IFG-tartrate.

Likewise, **Figures 5A-D** illustrate the level of GCase detected in brain, spleen, liver, and lung, respectively, of mice treated with Compound B and IFG-tartrate as described above. Furthermore, **Tables 4A-4C** detail the level of GCase enhancement in brain, spleen, and plasma, respectively, of mice treated as described above.

Table 4A Brain samples of GCase enhancement study					
Compound	Dose (mg/kg)	Dosing solution	GCase Increase (-fold)	Concentration (nM)	IC50 pH 5.2 (nM)
Compound B	10	Water	2.3	246±42 (0.9)	73±9
Compound B	100	Water	3.5	2066±231 (7.6)	
IFG	10	Water	1.5	49±2.4 (0.2)	50±3
IFG	100	Water	2.1	287±74 (1.1)	
Notes: BLQ < 5 ng/g Values in parenthesis indicate concentrations in GCase assays after dilution IC50 data is average of three independent experiments					

Table 4B Spleen samples of GCase enhancement study					
Compound	Dose (mg/kg)	Dosing solution	GCase Increase (-fold)	Concentration (nM)	IC50 pH 5.2 (nM)
Compound B	10	Water	1.4	645±214 (2.4)	73±9
Compound B	100	Water	2.2	2156±464 (8)	
IFG	10	Water	1.6	150±12 (0.5)	50±3
IFG	100	Water	2.0	1139±220 (4.2)	
Notes: BLQ <5 ng/g Values in parenthesis indicate concentrations in GCase assays after dilution IC50 data is average of three independent experiments					

<b>Table 4C Plasma samples of GCase enhancement study</b>			
<b>Compound</b>	<b>Dose (mg/kg)</b>	<b>Dosing solution</b>	<b>Concentration (nM)</b>
<b>Compound B</b>	<b>10</b>	<b>Water</b>	<b>104.3±15</b>
<b>Compound B</b>	<b>100</b>	<b>Water</b>	<b>1529±601</b>
<b>IFG</b>	<b>10</b>	<b>Water</b>	<b>67±10</b>
<b>IFG</b>	<b>100</b>	<b>water</b>	<b>852±134</b>
<b>Note:</b> BLQ < 1ng/mL			

As reflected in **Figures 5A-D** as well as **Tables 4A-4C**, mice administered Compound B demonstrated statistically significant greater GCase enhancement in brain, spleen, liver and lung as compared to mice administered control or IFG-tartrate. Moreover, GCase enhancement in mice administered Compound B was unexpectedly greater than that observed with IFG-tartrate even when far lower doses of Compound B were administered than IFG-tartrate.

**EXAMPLE 4: Rat pharmacokinetics**

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 Vd, %F, CL, and  $t_{1/2}$ .

Plasma levels in rats following a single 3 mg/kg (free base equivalent) intravenous dose of the hydrochloride salt form of Compound A are illustrated in Figure 6A. Similarly, plasma levels in rats administered a single dose of 10, 30, and 300 mg/kg (free base equivalent) p.o. dose of the hydrochloride salt form of Compound A are illustrated in Figure 6B. Various pharmacokinetic parameters for Compound A based on the aforementioned study are detailed in Table 5 below.

Table 5 Rat PK data for Compound A								
Dose (mg/kg)		Route	AUC <sub>last</sub> (hr*ng/mL)	%F	t <sub>1/2</sub> (h)	C <sub>max</sub> (ng/mL)	CL (mL/hr/kg)	V <sub>D</sub> (mL/kg)
Salt	Free Base							
3.72	3	IV	1798 ± 42.7	N/A	1.4 ± 0.1	7274 ± 139	1666 ± 38.5	3481 ± 232
12.4	10	PO	2982 ± 228	50 ± 3.8	1.2 ± 0.4	1289 ± 271	3387 ± 277	13283 ± 1929
37.2	30	PO	8251 ± 388	46 ± 2.5	2.3 ± 0.3	2870 ± 355	4009 ± 509	13532 ± 3291
124	100	PO	29606 ± 1748	49 ± 2.7	2.6 ± 0.1	9393 ± 742	3393 ± 209	13101 ± 1518
<b>Notes:</b> <i>Non compartmental analysis mean values ( N=3 rats)</i> <i>BLD Below Limit of Detection (&lt;0.5ng/mL)</i> <i>BLQ Below Limit of Quantitation</i> <i>%F = <math>\frac{AUC\ PO \times 100\ dose\ normalized}{AUC\ IV}</math></i> <i>AUC<sub>last</sub> = Area under the Concentration/Time curve to the last data point</i>								

As reflected in Figures 6A and 6B as well as Table 5, the hydrochloride salt form of Compound A has a favorable pharmacokinetic profile for drug development as a pharmacological chaperone. In particular, the hydrochloride salt form of Compound A shows good oral bioavailability (~50%) and dose proportionality, a half-life of 1.0 to 2.5 hours, and a volume of distribution suggesting adequate penetration into peripheral tissues.

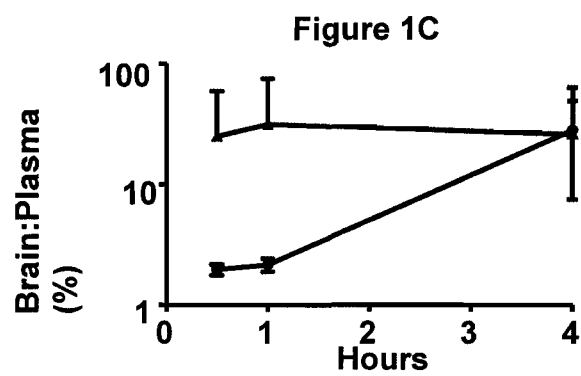
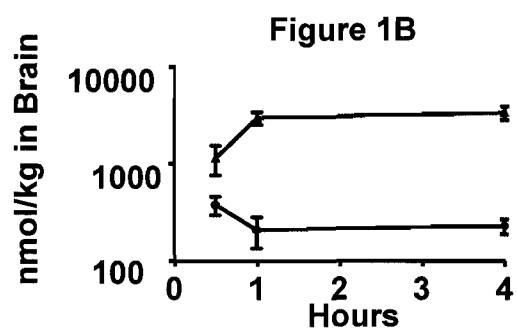
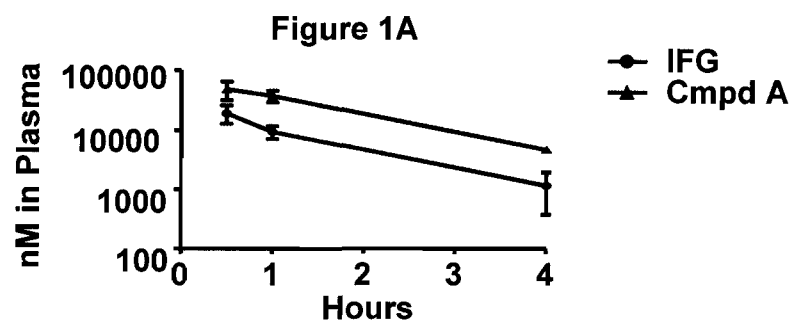
## Claims:

1. Use of the therapeutic agent 5-(fluoromethyl)piperdine-3,4-diol, 5-(chloromethyl)piperdine-3,4-diol, or a pharmaceutically acceptable salt or solvate thereof, or any combination of two or more thereof for preventing and/or treating a degenerative disorder of the central nervous system in a patient at risk for developing or diagnosed with the same.
2. Use of the therapeutic agent 5-(fluoromethyl)piperdine-3,4-diol, 5-(chloromethyl)piperdine-3,4-diol, or a pharmaceutically acceptable salt or solvate thereof, or any combination of two or more thereof for the manufacture of a medicament for preventing and/or treating a degenerative disorder of the central nervous system in a patient at risk for developing or diagnosed with the same.
3. The use of claim 1 or claim 2 wherein the therapeutic agent is (3R, 4R, 5S)-5-(fluoromethyl)piperdine-3,4-diol or a pharmaceutically acceptable salt or solvate thereof.
4. The use of claim 1 or claim 2, wherein the therapeutic agent is (3R, 4R, 5S)-5-(chloromethyl)piperdine-3,4-diol or a pharmaceutically acceptable salt or solvate thereof.
5. The use of claim 3 or claim 4, wherein the therapeutic agent is in the form of the hydrochloride salt.
6. The use of any one of claims 1 to 5, wherein the degenerative disorder is Parkinson's disease, dementia with Lewy bodies, multiple system atrophy or Alzheimer's disease.
7. The use of any one of claims 1 to 5, wherein the degenerative disorder is characterized by Lewy bodies.
8. The use of any one of claims 1 to 5, wherein the degenerative disorder is associated with aggregation of non-Abeta component or alpha-synuclein.

9. The use of any one of claims 1 to 5, wherein the degenerative disorder is associated with accumulation of at least one of glucocerebroside, glycosphingolipid, or glycolipid.
10. Use of the therapeutic agent 5-(fluoromethyl)piperdine-3,4-diol, 5-(chloromethyl)piperdine-3,4-diol, or a pharmaceutically acceptable salt or solvate thereof, or any combination of two or more thereof for treating a degenerative disorder of the central nervous system in a patient at risk for developing or diagnosed with the same.
11. Use of the therapeutic agent 5-(fluoromethyl)piperdine-3,4-diol, 5-(chloromethyl)piperdine-3,4-diol, or a pharmaceutically acceptable salt or solvate thereof, or any combination of two or more thereof for the manufacture of a medicament for treating a degenerative disorder of the central nervous system in a patient at risk for developing or diagnosed with the same.
12. The use of claim 10 or claim 11 wherein the therapeutic agent is (3R, 4R, 5S)-5-(fluoromethyl)piperdine-3,4-diol or a pharmaceutically acceptable salt or solvate thereof.
13. The use of claim 10 or claim 11, wherein the therapeutic agent is (3R, 4R, 5S)-5-(chloromethyl)piperdine-3,4-diol or a pharmaceutically acceptable salt or solvate thereof.
14. The use of claim 12 or claim 13, wherein the therapeutic agent is in the form of the hydrochloride salt.
15. The use of any one of claims 6 or 10 to 14, wherein the degenerative disorder is Parkinson's disease.
16. The use of any one of claims 6 or 10 to 14, wherein the degenerative disorder is dementia with Lewy bodies.
17. The use of any one of claims 6 or 10 to 14, wherein the degenerative disorder is multiple system atrophy.



18. The use of any one of claims 6 or 10 to 14, wherein the degenerative disorder is Alzheimer's disease.
19. The use of any one of claims 10 to 14, wherein the degenerative disorder is characterized by Lewy bodies.
20. The use of any one of claims 10 to 14, wherein the degenerative disorder is associated with aggregation of non-Abeta component or alpha-synuclein.
21. The use of any one of claims 10 to 14, wherein the degenerative disorder is associated with accumulation of at least one of glucocerebroside, glycosphingolipid, or glycolipid.
22. The use of any one of claims 1 to 21 further comprising administering an effective amount of at least one other therapeutic agent.
23. The use of claim 22, wherein at least one other therapeutic agent is levodopa, an anticholinergic, a catechol-O-methyl transferase inhibitor, a dopamine receptor agonist, a monoamine oxidase inhibitor, a peripheral decarboxylase inhibitor, or an anti-inflammatory agent.



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Figure 2

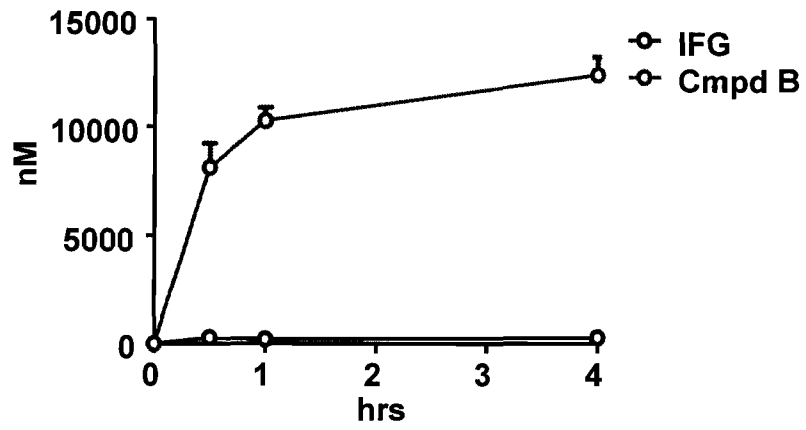


Figure 3

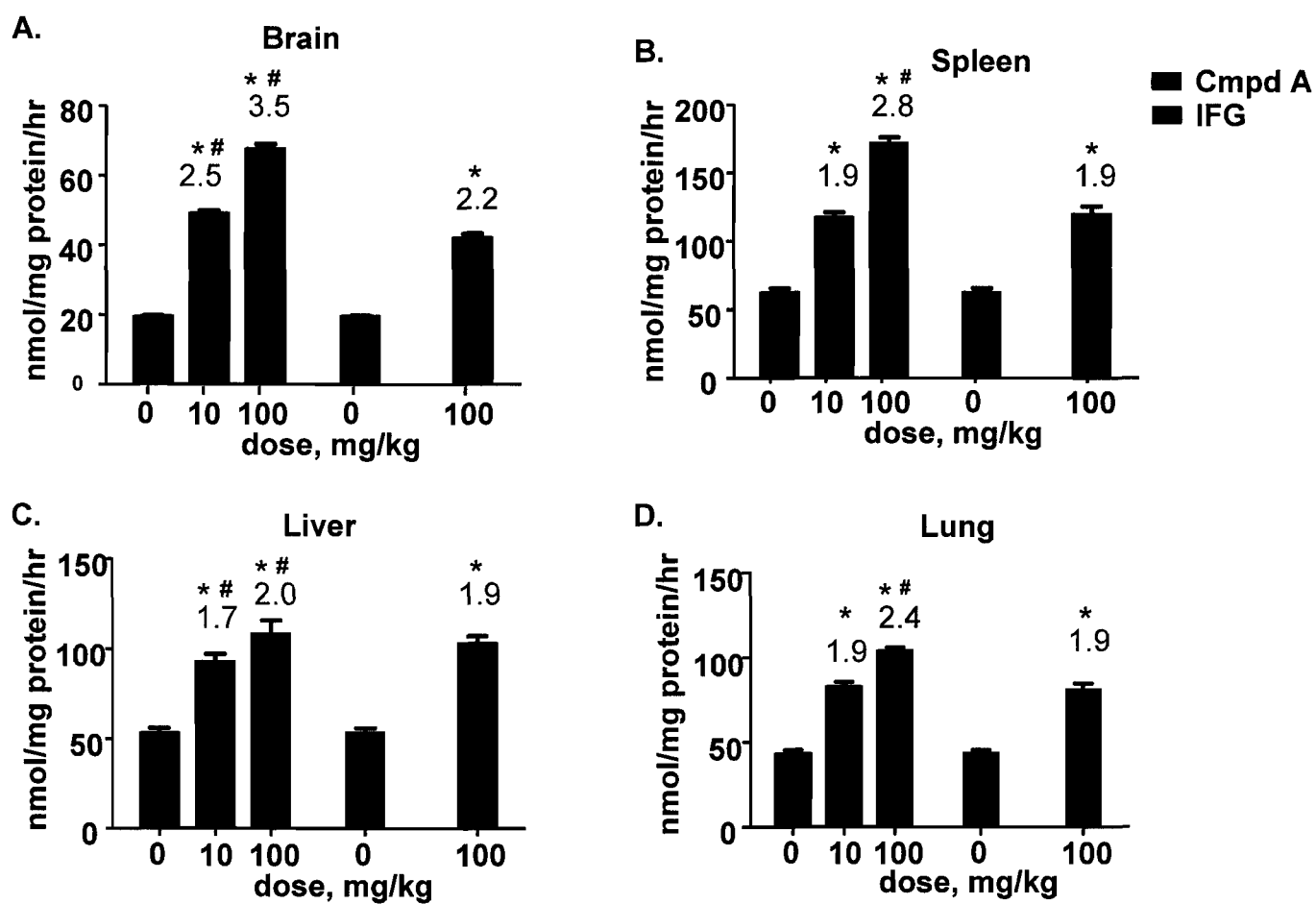


Figure 4

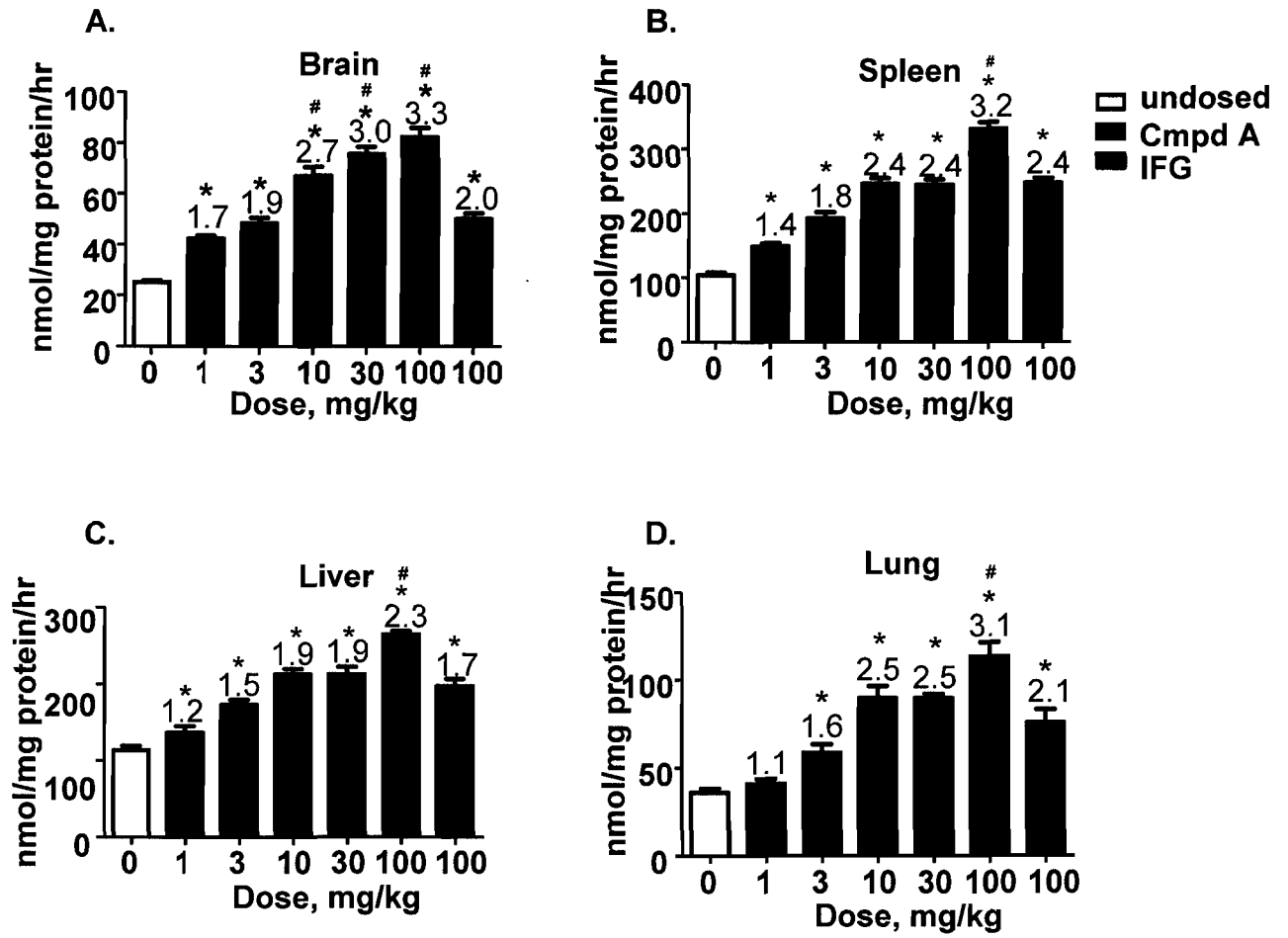


Figure 5

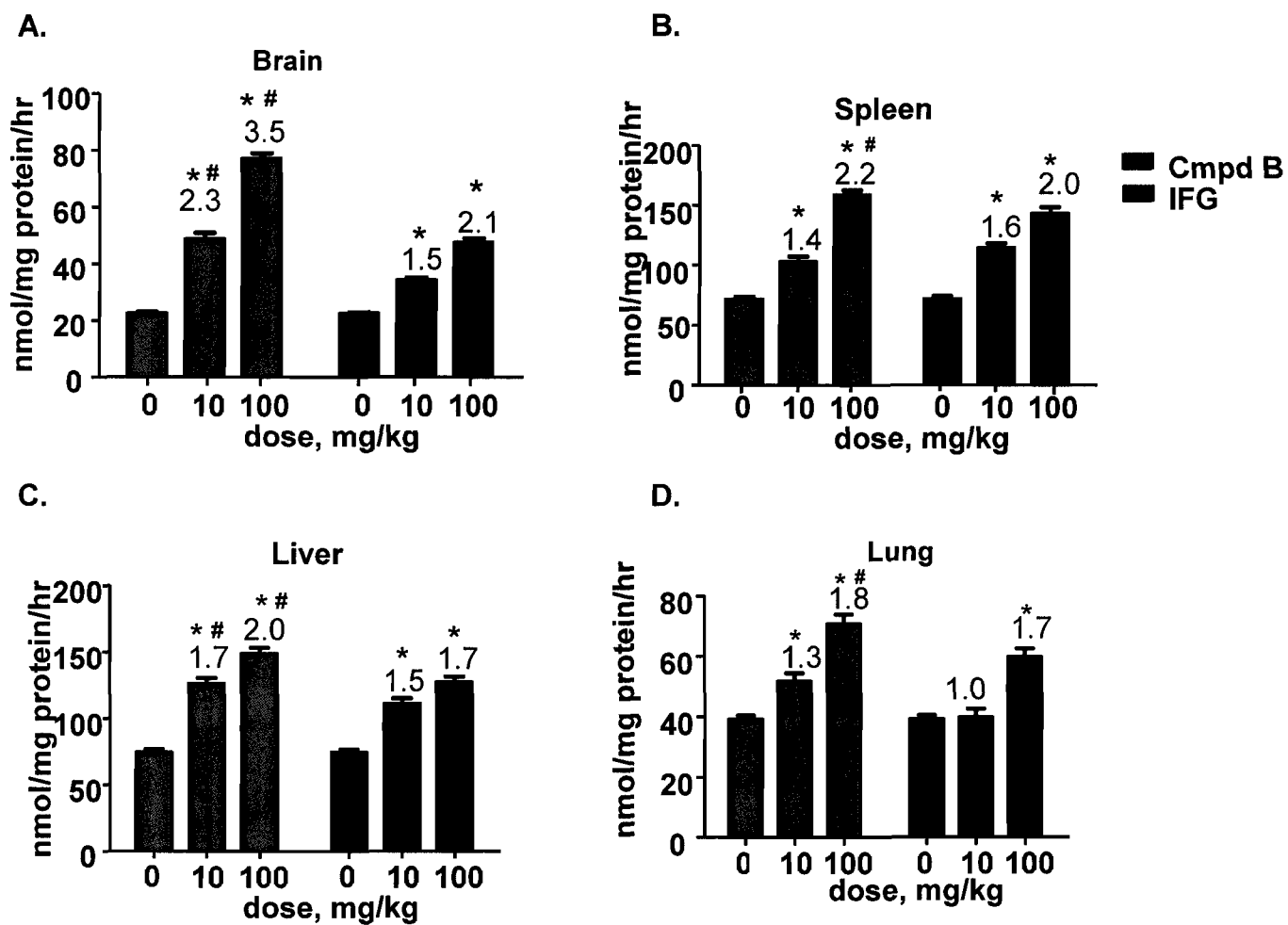


Figure 6A

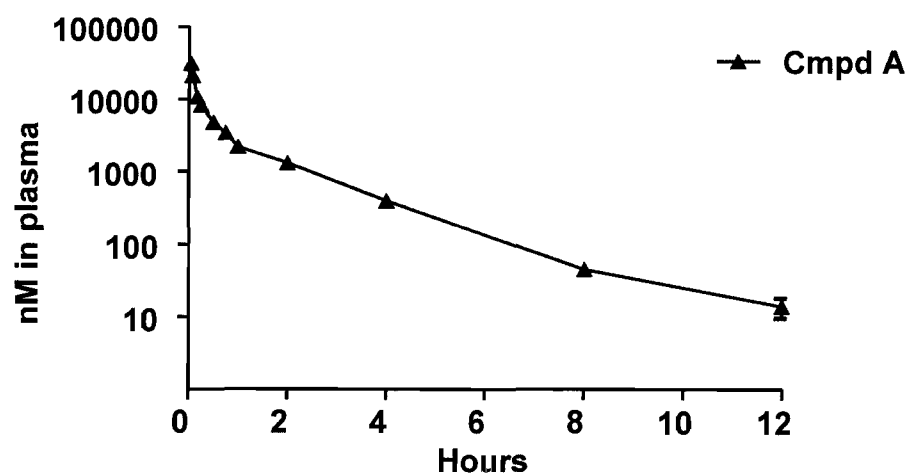


Figure 6B

