

**(12) STANDARD PATENT  
(19) AUSTRALIAN PATENT OFFICE**

(11) Application No. AU 2004247468 B2

(54) Title  
**2-hydroxymethyl-3,4,5-trihydroxy-1-(4-pentlyoxybenzyl) piperidine as glucosylceramide synthase (GCS)**

(51) International Patent Classification(s)  
**A61P 3/10** (2006.01)      **C07D 211/46** (2006.01)  
**A61P 35/00** (2006.01)

(21) Application No: **2004247468**      (22) Date of Filing: **2004.06.09**

(87) WIPO No: **WO04/111001**

(30) Priority Data

(31) Number **0313677.7**      (32) Date **2003.06.13**      (33) Country **GB**

(43) Publication Date: **2004.12.23**  
(44) Accepted Journal Date: **2009.11.19**

(71) Applicant(s)  
**Actelion Pharmaceuticals Ltd**

(72) Inventor(s)  
**Scopes, David Ian Carter;Orchard, Michael Glen**

(74) Agent / Attorney  
**A J PARK, Level 11 60 Marcus Clarke Street, Canberra, ACT, 2601**

(56) Related Art  
**WO 2002/055498 A1**

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
23 December 2004 (23.12.2004)

PCT

(10) International Publication Number  
**WO 2004/111001 A1**

(51) International Patent Classification<sup>7</sup>: **C07D 211/46**,  
A61K 31/445, A61P 3/10, 35/00

(21) International Application Number:  
PCT/GB2004/002450

(22) International Filing Date: 9 June 2004 (09.06.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
0313677.7 13 June 2003 (13.06.2003) GB

(71) Applicant (for all designated States except US): **OXFORD GLYCOSCIENCES (UK) LTD [GB/GB]**; 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ORCHARD, Michael, Glen [GB/GB]**; Celltech R & D Limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **SCOPES, David, Ian, Carter [GB/GB]**; Celltech R & D Limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

(74) Agent: **THOMPSON, John**; Celltech R & D limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/111001 A1

(54) Title: 2-HYDROXYMETHYL-3,4,5-TRIHYDROXY-1-(4-PENTLYOXYBENZYL) PIPERIDINE AS GLUCOSYLCE-  
RAMIDE SYNTHASE (GCS)

(57) Abstract: The compound 3,4,5-piperidinetriol, 2-(hydroxymethyl)-1-[(4-(pentyloxy)phenyl)methyl]-, (2S,3S,4R,5S), or a pharmaceutically acceptable salt or prodrug thereof, is useful as an inhibitor of glucosylceramide synthase.

2-HYDROXYMETHYL-3,4,5-TRIHYDROXY-1-(4-PENTLYOXYBENZYL) PIPERIDINE AS  
GLUCOSYLCERAMIDE SYNTHASE (GCS)

The present invention relates to a novel piperidine derivative useful as an inhibitor of glucosylceramide synthase (GCS; UDP-glucose:ceramide glucosyltransferase, UDP-glucose:N-acylsphingosine D-glucosyltransferase, EC 2.4.1.80), methods for its preparation and its use in medicine, specifically in the treatment and prevention of disease states mediated by GCS. The compound finds use in the treatment of glycolipid storage diseases, diseases associated with glycolipid accumulation, cancers in which glycolipid synthesis is abnormal, infectious diseases caused by microorganisms which use cell surface glycolipids as receptors, infectious diseases in which synthesis of glucosylceramide is essential or important, diseases in which excessive glycolipid synthesis occurs, neuronal disorders and neuronal injury.

GCS is an intracellular enzyme that catalyzes the assembly of uridine diphosphate-glucose and ceramide into the glycolipid, glucosylceramide. The role of GCS in regulating ceramide levels has been explored, since this molecule can induce apoptotic cell death (J. Biol. Chem., 2000, 275(10), 7138-43). The role of GCS in maintaining cholesterol/glycolipid 'rafts', cell-surface membrane domains of specialized permeability and functionality that appear to be involved in a variety of signal transduction events, has also been investigated (Nature, 1997, 387(6633), 569-72).

GCS is considered to be a target for treating certain human diseases. Glucosylceramide and structurally related glycolipids are stored in the lysosomes of patients with genetic diseases, which result from a mutation in one of the essential glycolipid-degrading enzymes (e.g. Gaucher, Tay Sachs, Sandhoff, GM1 gangliosidosis and Fabry diseases). Glycolipid storage also occurs as a secondary effect in some tissues (e.g. neuronal tissue) with genetic storage diseases such as Niemann-Pick C disease, mucopolysaccharidoses, mucolipidosis type IV (Proc. Natl. Acad. Sci. USA, 1998, May 26, 95(11), 6373-8) and  $\alpha$ -mannosidosis (Proc. Natl. Acad. Sci. USA, 1991, Dec 15, 88(24), 11330-4). GCS inhibitors may be applied to reduce the rate of glycolipid synthesis in diseased cells so that there is less glycolipid present to be stored, a treatment approach termed substrate deprivation. Studies have demonstrated that GCS inhibitors can be used to reduce the glycolipid accumulation seen in cell and animal models of glycolipid storage disorders (Proc. Natl. Acad. Sci. USA, 1999, 96(11), 6388-93; Science, 1997, 276(5311), 428-31; J. Clin. Invest., 2000, 105(11), 1563-71). Furthermore, clinical trials have shown that GCS inhibitors, such as, N-butyldeoxynojirimycin (NB-DNJ) are useful in treating human patients with Gaucher disease (Lancet, 2000, 355(9214), 1481-5). The use of the imino sugar NB-DNJ as a GCS inhibitor is disclosed in EP-A-0698012. EP-A-0536402 and EP-A-0698012 disclose that N-alkyl derivatives of deoxygalactonojirimycin, e.g. N-butyldeoxygalactonojirimycin (NB-DGJ), may also be of use in the treatment of glycolipid storage disorders. EP-A-0698012 also discloses that the corresponding N-butyl derivatives of mannose (NB-DMJ), fucose (NB-DFJ) and N-acetylglucosamine (NB-NAG) do not act as inhibitors of glycolipid biosynthesis.

The use of GCS inhibitors in the treatment of human malignancies has also been proposed. Tumours can synthesize abnormal quantities of glycolipids that are typically present/ absent in normal tissues. In addition glycolipids, or gangliosides, in particular are shed by tumour cells and released into the extracellular space and the bloodstream. Both tumour shed and cell surface bound tumour gangliosides can influence tumour host cell interactions such as cell-cell contacts or adhesion (Methods Enzymol., 2000, 312, 447-58), cell motility (Mol. Chem. Neuropathol., 1995, 24(2-3), 121-35), growth factor signalling events (J. Biol. Chem., 2000, 275(44), 34213-23), tumour stimulated angiogenesis (Acta. Oncol., 1997, 36(4), 383-7) and tumour specific immune responses (J. Immunol., 1999, Oct 1, 163(7), 3718-26). All these events can affect tumour development and progression. Glycolipids, glucosylceramide in particular, are known to accumulate in multidrug resistant (MDR) tumour cells

(Anticancer Res., 1998, 18(1B), 475-80) and in vitro treatment of these cells with GCS inhibitors can reverse the MDR phenotype (J. Biol. Chem., 1997, 272(3), 1682-7; Br. J. Cancer, 1999, 81(3), 423-30).

Cell surface glycolipids also have roles in infectious disease, serving as receptors for the binding of pathogenic bacteria (APMIS, 1990, Dec, 98(12), 1053-60, Review), fungi (Infect. Immun., 1990 Jul, 58(7), 2085-90) and viruses (FEBS Lett., 1984, May 7, 170(1), 15-8). In addition, glycolipids on the surface of cells are bound by bacterial toxins (Methods Enzymol., 2000, 312, 459-73) for instance, the B subunit of cholera toxin (ganglioside GM1) and verocytotoxin (globotriaosylceramide GB3) (J. Infect. Dis., 2001, suppl. 70-73, 183).

The use of GCS inhibitors may also be appropriate in a number of other clinical indications which are associated with abnormalities in glycolipid synthesis. Atherosclerotic lesions of human aorta have a higher ganglioside content than unaffected regions of the aorta and serum ganglioside concentrations in atherosclerotic patients are higher than in normal individuals (Lipids, 1994, 29(1), 1-5). Tissue derived from the kidneys of patients with polycystic kidney disease contains high levels of both glucosylceramide and lactosylceramide (J. Lipid. Res., 1996, Jun, 37(6), 1334-44). Renal hypertrophy in an animal model of diabetes is associated with increases in glycolipid synthesis, (J. Clin. Invest., 1993, Mar, 91(3), 797-803).

Glycolipid metabolism also plays a critical role in neuronal disorders, such as Alzheimer's disease and epilepsy. For instance, Niemann-Pick C (NPC) patient neurons present with fibrillar tangles reminiscent of the morphology seen in Alzheimer's disease.

GM1 ganglioside binding by amyloid beta-protein induces conformational changes that support its formation of fibrous polymers, and the fibrillar deposition of this protein is an early event in Alzheimer's disease (Yanagisawa et al., 1995, Nat. Med. 1, 1062-6; Choo-Smith et al., 1997, Biol. Chem., 272, 22987-90). Thus, decreasing GM1 synthesis by using agents such as GCS inhibitors, e.g. NB-DNJ, could inhibit the fibre formation seen in Alzheimer's disease.

In contrast, preliminary clinical trials have shown that neurodegenerative processes seen in Parkinson's disease, stroke and spinal cord injuries seem to improve by treating patients with GM1 ganglioside (Alter, (1998), Ann. NY Acad. Sci., 845, 391-4011; Schneider, 1998, Ann. NY. Acad. Sci., 845, 363-73; Geisler, (1998), Ann. NY. Acad. Sci., 845, 374-81). It is possible that co-administering glucosylceramide synthesis inhibitors would provide the clinician greater control over this treatment course. GCS inhibitors like NB-DNJ would limit patient-specific inconsistencies by blocking their neuronal glycolipid synthesis. In addition, inhibiting glucosylceramide synthesis would limit the metabolism of administered glycolipids into other, perhaps unproductive, forms. Thus, the ability to modulate glucosylceramide synthesis with GCS inhibitors may be useful in the treatment of a wide variety of neuronal disorders.

In addition, it has also been shown that imino sugars can reversibly induce male sterility and can, therefore, be used as male contraceptives. Also, GCS inhibitors could be used for the treatment of obesity.

A role for glycolipids in some aspects of inflammatory or immune responses has also been suggested. Following an inflammatory stimulus, such as that obtained with thioglycolate, the ganglioside profile of murine peritoneal macrophages changes from a simple profile (3 major species) in resting macrophage to a more complex profile (more than 14 species) in activated and recruited macrophage, see Ryan, J.L. et al., Yale J Biol Med, 1985, 58(2), 125-31; Yohe, H.C. et al., Biochim Biophys Acta, 1985, 818(1), 81-6; Yohe, H.C. et al., Immunol, 1991, 146(6), 1900-8. Furthermore, *in vivo* administration of an inflammatory agent, e.g. bacterial endotoxin, results in the increased expression of two enzymes, serine palmitoyltransferase and glucosylceramide synthase, which are key to the *de novo* synthesis of glycolipids, see Memon, R.A. et al., J Biol Chem, 1999, 274(28), 19707-13; Memon, R.A. et al., J Lipid

28 Oct 2009

2004247468

Res, 2001, 42(3), 452-9.

Such a role for glycolipids is further supported by the demonstration of changes in glycolipid expression in animals with genetic defects which result in hyper- or hyposensitive responses to inflammatory stimuli. For example, upon endotoxin treatment in C3H/HeJ mice, which have a toll-like receptor 4 mutation and are hypo-responsive to bacterial endotoxin, recruited macrophages were found to lack ganglioside GM<sub>1b</sub>, which is a major ganglioside found in recruited macrophages in normal mice, see Yohe, H.C. et al., Immunol, 1991, 146(6), 1900-8; Yohe, H.C. et al., Immunol, 1986, 137(12), 3921-7.

Hence, GCS inhibitors may be useful in the treatment of inflammatory diseases and other disorders associated with macrophage recruitment and activation, including, but not limited to, rheumatoid arthritis, Crohn's disease, asthma and sepsis.

WO02/055498 discloses piperidine derivatives useful as GCS inhibitors.

Given the importance of GCS in a wide spectrum of diseases, it is essential that new tools that provide a means for modulating this enzyme's function be developed. Towards this end, we have synthesized a novel compound that is useful in inhibiting GCS's catalytic activity.

In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

The term "comprising" as used in this specification means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner.

The compound of the invention exhibits improved potency and/or selectivity for GCS, relative to non-lysosomal- $\beta$ -glucocerebrosidase activity, over known hydroxylated piperidine derivatives.

The invention provides the compound 3,4,5-piperidinetriol, 2-(hydroxymethyl)-1-[(4-pentyloxy)phenyl]methyl-, (2S,3S,4R,5S) and pharmaceutically acceptable salts thereof.

As described herein, for all aspects of the invention, reference to the compound of the invention encompasses pharmaceutically acceptable salts and prodrugs thereof.

As described herein, the compound of the present invention can be used for the inhibition of GCS. Thus, in another aspect, the present invention provides the use of the compound of the invention in medicine.

Suitable, pharmaceutically acceptable salts of the compound of the invention include, but are not limited to, salts with inorganic acids such as hydrochloride, sulfate, phosphate, diphosphate, hydrobromide, and nitrate, or salts with an organic acid such as malate, maleate, fumarate, tartrate, succinate, citrate, acetate, lactate, methanesulfonate, p-toluenesulfonate, palmitate, salicylate, and stearate.

Suitable prodrugs of the compound of the invention include, but are not limited to, pharmaceutically acceptable esters such as C<sub>1-6</sub> alkyl esters.

The compound of the invention may be crystallised or recrystallised from solvents such as aqueous and organic solvents. In such cases solvates may be formed. This invention includes within its scope stoichiometric solvates including hydrates as well as compounds containing variable amounts of water that may be produced by processes such as lyophilisation.

28 Oct 2009

2004247468

Since the compound of the invention is intended for use in pharmaceutical compositions it will readily be understood that it is preferably provided in substantially pure form, for example at least 60% pure, more suitably at least 75% pure and preferably at least 85%, especially at least 98% pure (% are on a weight for weight basis). Impure preparations of the compound may be used for preparing the more pure forms used

5 in the pharmaceutical compositions; these less pure preparations of the compound should contain at least 1%, more suitably at least 5%, e.g. from 10 to 59%, of the compound of the invention or pharmaceutically acceptable derivative thereof.

The compound of the invention can be prepared by art-recognized procedures from known or commercially available starting materials. If the starting materials are unavailable from a commercial 10 source, their synthesis is described herein, or they can be prepared by procedures known in the art.

---

2232736-1

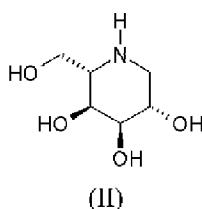
3a

28 Oct 2009

2004247468

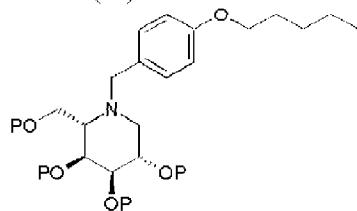
Specifically, the compound of the invention may be prepared by a process comprising:

(a) reacting a compound of formula (II):



5 with 4-(pentyloxy)benzaldehyde using NaBH<sub>3</sub>CN or a supported reagent, such as (polystyrylmethyl)-trimethylammonium cyanoborohydride, in acetic acid-methanol or HCl-methanol, or using NaBH(OAc)<sub>3</sub> in a solvent, such as dichloromethane, or

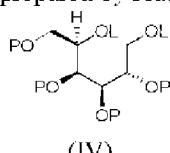
(b) deprotection of a compound of formula (III):



10 wherein P, which may be the same or different, are hydroxy protecting groups, e.g. benzyl (Bn). When P is CH<sub>2</sub>Ph the deprotection is conducted in the presence of hydrogen gas and a catalyst such as PdCl<sub>2</sub> or palladium on carbon in a suitable solvent such as an alcohol, e.g. ethanol. It will be understood that when P is CH<sub>2</sub>Ph, the nitrogen substituent may also be removed under these conditions to give the compound of 15 formula (II), thus the compound of the invention is preferably produced using process a) above.

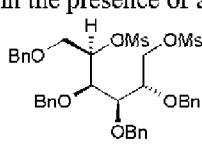
The compound of formula (II) is known, see e.g. *Tet. Lett.*, 1997, 38(45), 8009-12.

Compounds of formula (III) may be prepared by reacting a compound of formula (IV):



20 wherein L, which may be the same or different, are leaving groups, such as mesyl, and P is as defined for formula (III), with 4-(pentyloxy)benzylamine, either neat or in a solvent, such as tetrahydrofuran.

Compound (IVa), wherein L is mesyl and P is benzyl, may be prepared by reacting 2,3,4,6-tetra-O-benzyl-D-galactitol with mesyl chloride in the presence of a base such as pyridine.



25 Any novel intermediate compounds as described herein also fall within the scope of the present invention. Thus, also described herein is a compound of formula (III) as defined above.

During the synthesis of the compound of the invention labile functional groups in the intermediate compounds, e.g. hydroxy groups, may be protected. A comprehensive discussion of the 30 ways in which various labile functional groups may be protected and methods for cleaving the resulting protected derivatives is given in for example *Protective Groups in Organic Chemistry*, T.W. Greene and P.G.M. Wuts, (Wiley-Interscience, New York, 2nd edition, 1991).

Further details for the preparation of the compound of the invention are provided in the example.

5 The pharmaceutically effective compound of the invention may be administered in conventional dosage forms prepared by combining the compound of the invention ("active ingredient") with standard pharmaceutical carriers, excipients or diluents according to conventional procedures well known in the art. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation.

According to a further aspect, the present invention provides a pharmaceutical composition comprising the compound of the invention, together with one or more pharmaceutically acceptable carriers, excipients and/or diluents.

10 The active ingredient or pharmaceutical composition can be administered simultaneously, separately or sequentially with another treatment for the disorder to be treated.

15 The active ingredient or pharmaceutical composition may be administered to a subject by any of the routes conventionally used for drug administration, for example they may be adapted for oral (including buccal, sublingual), topical (including transdermal), nasal (including inhalation), rectal, vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) administration to mammals including humans. The most suitable route for administration in any given case will depend on the particular compound or pharmaceutical composition, the subject, and the nature and severity of the disease and the physical condition of the subject. Such compositions may be prepared by any method known in the art of pharmacy, for example by bringing into association the active ingredient with the 20 carrier(s), excipient(s) and/ or diluent(s).

Pharmaceutical compositions adapted for oral administration may be presented as discrete units such as capsules or tablets; powders or granules; solutions or suspensions in aqueous or non-aqueous liquids; edible foams or whips; or oil-in-water liquid emulsions or water-in-oil liquid emulsions.

25 Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulfate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral 30 liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for 35 example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl *p*-hydroxybenzoate or sorbic acid, and, if desired, conventional flavouring or colouring agents.

40 Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, impregnated dressings, sprays, aerosols or oils and may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams. Such applications include those to the eye or other external tissues, for example the mouth and skin and the compositions are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-45 miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-

water cream base or a water-in-oil base. The composition may also contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions.

Pharmaceutical compositions adapted for topical administration to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent.

5 Pharmaceutical compositions adapted for topical administration in the mouth include lozenges, pastilles and mouth washes.

10 Pharmaceutical compositions adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis as generally described in *Pharmaceutical Research*, 3(6), 318, (1986).

15 Pharmaceutical compositions adapted for nasal administration wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable compositions wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient.

20 Pharmaceutical compositions adapted for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose pressurised aerosols, nebulizers or insufflators.

25 Pharmaceutical compositions adapted for rectal administration may be presented as suppositories or enemas. Suppositories will contain conventional suppository bases, e.g. cocoa-butter or other glyceride.

Pharmaceutical compositions adapted for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray compositions.

30 Pharmaceutical compositions adapted for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

35 For parenteral administration, fluid unit dosage forms are prepared utilizing the active ingredient and a sterile vehicle, e.g. water. The active ingredient, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the active ingredient can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing.

40 Advantageously, agents such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilized powder is then sealed in the vial and an accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions are prepared in substantially the same manner except that the active ingredient is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The active ingredient can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the active ingredient.

The pharmaceutical compositions according to the invention are preferably adapted for oral administration.

It should be understood that in addition to the ingredients particularly mentioned above, the compositions may also include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents. They may also contain therapeutically active agents in addition to the compounds of the present invention. Such carriers may be present as from about 1% up to about 98% of the formulation. More usually they will form up to about 80% of the formulation.

The compositions may contain from 0.1% by weight, e.g. 10-60% by weight, of the active material, depending on the method of administration.

Pharmaceutical compositions may be presented in unit dose forms containing a predetermined amount of active ingredient per dose. Such a unit may contain for example 0.1mg/kg to 750mg/kg, more preferably 0.1mg/kg to 10mg/kg, depending on the condition being treated, the route of administration and the age, weight and condition of the patient. Preferred unit dosage compositions are those containing a daily dose or sub-dose, or an appropriate fraction thereof, of an active ingredient.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of active ingredients will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular subject being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e. the number of doses of the active ingredients given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

No toxicological effects are indicated when the compound of the invention is administered in the above mentioned dosage range.

The compound of the invention is useful in that it is capable of inhibiting glucosylceramide synthase. Thus, the compound of the invention can be used in the treatment of various glycolipid storage diseases such as Gaucher's disease, Sandhoff's disease, Tay-Sachs disease, Fabry disease, GM1 gangliosidosis, etc. In addition, the compound can also find use in the treatment of conditions in which glycolipid accumulation occurs such as Niemann-Pick disease, mucopolysaccharidoses (MPS I, MPS IIIA, MPS IIIB, MPS VI and MPS VII, preferably MPS I), mucolipidosis type IV and  $\alpha$ -mannosidosis.

The compound of the present invention can also be used in the treatment of cancer in which glycolipid synthesis is abnormal such as brain tumours, neuroblastoma, malignant melanoma, renal adenocarcinoma and multi-drug resistant cancers in general.

The compound of the present invention can also be used in the treatment of diseases caused by infectious microorganisms which use cell surface glycolipids as receptors for either the infectious organism itself or for a toxin produced by the infectious organism (e.g. for attachment and/or invasion onto/into the host cell).

The compound of the present invention can also be used in the treatment of disease caused by infectious microorganisms for which the synthesis of glucosylceramide is an essential or important process, e.g. the pathogenic fungus *Cryptococcus neoformans*.

The compound of the present invention can also be used in the treatment of disease in which excessive glycolipid synthesis occurs, such as, but not limited to, atherosclerosis, polycystic kidney disease and diabetic renal hypertrophy.

The compound of the present invention can also be used in the treatment of neuronal disorders, such as Alzheimer's disease and epilepsy; and neuronal degenerative disease, such as, Parkinsons' disease

The compound of the present invention can also be used in the treatment of neuronal injury such as spinal cord injuries or stroke.

The compound of the present invention can also be used in the treatment of obesity.

5 The compound of the present invention can also be used in the treatment of inflammatory diseases or disorders associated with macrophage recruitment and activation, including, but not limited to, rheumatoid arthritis, Crohn's disease, asthma and sepsis.

In additional aspects, therefore, the present invention provides:

(i) use of the compound of the invention in the manufacture of a medicament for use as an inhibitor of glucosylceramide synthase.

10 (ii) use of the compound of the invention in the manufacture of a medicament for the treatment of a glycolipid storage disease. Examples of glycolipid storage disease which can be treated include, but are not limited to, Gaucher disease, Sandhoff's disease, Tay-Sachs disease, Fabry disease or GM1 gangliosidosis.

(iii) use of the compound of the invention in the manufacture of a medicament for the treatment of Niemann-Pick disease, types A and C.

15 (iv) use of the compound of the invention in the manufacture of a medicament for the treatment of mucopolysaccharidosis type I, mucopolysaccharidosis type IIIA, mucopolysaccharidosis type IIIB, mucopolysaccharidosis type VI or mucopolysaccharidosis type VII. Preferably the compound is used in the treatment of mucopolysaccharidosis type I.

20 (v) use of the compound of the invention in the manufacture of a medicament for the treatment of  $\alpha$ -mannosidosis or mucolipidosis type IV.

(vi) use of the compound of the invention in the manufacture of a medicament for the treatment of cancer in which glycolipid synthesis is abnormal, including, but not limited to, brain cancer, neuronal cancer, neuroblastoma, renal adenocarcinoma, malignant melanoma, multiple myeloma and multi-drug resistant cancers.

25 (vii) use of the compound of the invention in the manufacture of a medicament for use in the treatment of Alzheimer's disease, epilepsy or stroke.

(viii) use of the compound of the invention in the manufacture of a medicament for use in the treatment of Parkinson's disease.

30 (ix) use of the compound of the invention in the manufacture of a medicament in the treatment of spinal injury.

(x) use of the compound of the invention in the manufacture of a medicament for use in the treatment of diseases caused by infectious microorganisms which utilize glycolipids on the surface of cells as receptors for either the organism itself or for toxins produced by the organism.

35 (xi) use of the compound of the invention in the manufacture of a medicament for use in the treatment of diseases caused by infectious microorganisms, e.g. pathogenic fungi, for which the synthesis of glucosylceramide is an essential or important process, such as, but not limited to, pathologies associated with *Cryptococcus neoformans* infection.

40 (xii) use of the compound of the invention in the manufacture of a medicament for use in the treatment of diseases associated with abnormal glycolipid synthesis, including, but not limited to, polycystic kidney disease, diabetic renal hypertrophy and atherosclerosis.

(xiii) use of the compound of the invention in the manufacture of a medicament for the treatment of a condition treatable by the administration of a ganglioside, such as GM1 ganglioside. Examples of such conditions are Parkinson's disease, stroke and spinal cord injuries.

45 (xiv) use of the compound of the invention in the manufacture of a medicament for reversibly rendering a male mammal infertile.

(xv) use of the compound of the invention in the manufacture of a medicament for the treatment of obesity, e.g. as an appetite suppressant.

5 (xvi) use of the compound of the invention in the manufacture of a medicament for the treatment of inflammatory diseases or disorders associated with macrophage recruitment and activation, including, but not limited to, rheumatoid arthritis, Crohn's disease, asthma and sepsis.

(xvii) a method for the treatment of a glycolipid storage disease, e.g. Gaucher's disease, Sandhoff's disease, Tay-Sachs disease or GM1 gangliosidosis, which comprises the step of administering to a patient an effective amount of the compound of the invention.

10 (xviii) a method for the treatment of Niemann-Pick disease, types A and C, which comprises the step of administering to a patient an effective amount of the compound of the invention.

(xix) a method for the treatment of mucopolysaccharidosis type I, mucopolysaccharidosis type IIIA, mucopolysaccharidosis type IIIB, mucopolysaccharidosis type VI or mucopolysaccharidosis type VII which comprises the step of administering to a patient an effective amount of the compound of the invention.

15 (xx) a method for the treatment of  $\alpha$ -mannosidosis or mucolipidosis type IV which comprises the step of administering to a patient an effective amount of the compound of the invention.

(xxi) a method for the treatment of cancer in which glycolipid synthesis is abnormal, including but not limited to brain cancer, neuronal cancer, renal adenocarcinoma, malignant melanoma, multiple myeloma and multi-drug resistant cancers, which comprises the step of administering to a patient an effective amount of the compound of the invention.

20 (xxii) a method for the treatment of Alzheimer's disease, epilepsy or stroke which comprises the step of administering to a patient an effective amount of the compound of the invention.

(xxiii) a method for the treatment of Parkinson's disease, which comprises the step of administering to a patient an effective amount of the compound of the invention.

25 (xxiv) a method for the treatment of spinal injury which comprises the step of administering to a patient an effective amount of the compound of the invention.

(xxv) a method for the treatment of diseases caused by infectious microorganisms, which utilize glycolipids on the surface of cells as receptors for either the organism itself or for toxins produced by the organism, which comprises the step of administering to a patient an effective amount of the compound of the invention.

30 (xxvi) a method for the treatment of diseases caused by infectious microorganisms, e.g. pathogenic fungi, for which the synthesis of glucosylceramide is an essential or important process, such as, but not limited to, pathologies associated with *Cryptococcus neoformans* infection, which comprises the step of administering to a patient an effective amount of the compound of the invention.

35 (xxvii) a method for the treatment of diseases associated with abnormal glycolipid synthesis including but not limited to polycystic kidney disease, diabetic renal hypertrophy and atherosclerosis, which comprises the step of administering to a patient an effective amount of the compound of the invention.

(xxviii) a method for the treatment of a condition treatable by the administration of a ganglioside, such as GM1 ganglioside, which comprises the step of administering to a patient an effective amount of the compound of the invention. Examples of such conditions are Parkinson's disease, stroke and spinal cord injuries.

40 (xxix) a method for reversibly rendering a male mammal infertile, which comprises the step of administering to said male mammal an effective amount of the compound of the invention.

(xxx) a method for the treatment of obesity, which comprises the step of administering to a patient an effective amount of the compound of the invention.

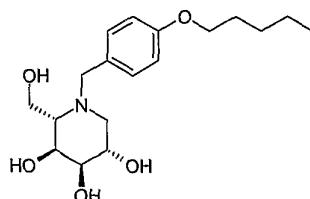
(xxxi) a method for the treatment of inflammatory diseases or disorders associated with macrophage recruitment and activation, including, but not limited to, rheumatoid arthritis, Crohn's disease, asthma and sepsis which comprises the step of administering to a patient an effective amount of the compound of the invention.

5 The invention also provides for the use of the compound of the invention for the treatment of the above mentioned diseases and conditions.

All publications, including, but not limited to, patents and patent applications, cited in this specification, are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

10 The invention will now be described by reference to the following example which is merely illustrative and is not to be construed as a limitation of the scope of the present invention.

**Example 1** 3,4,5-Piperidinetriol, 2-(hydroxymethyl)-1-[(4-(pentyloxy)phenyl)methyl]-, (2S,3S,4R,5S)



15 To a mixture of 3,4,5-piperidinetriol, 2-(hydroxymethyl)-, (2S,3S,4R,5S) (50 mg, 0.31 mmol) and (polystyrylmethyl)trimethylammonium cyanoborohydride (178 mg, 0.78 mmol) in 10% acetic acid in methanol (2 ml) was added 4-(pentyloxy)benzaldehyde (146 mg, 0.76 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was purified using a plug of acidic Dowex 50X4-200 resin (3 g) (which had been pre-washed with 10% aqueous hydrochloric acid). The 20 resin was eluted with methanol (25 ml) to remove all non-basic side products. The desired compound was then eluted using a solution of 2:2:1 methanol/water/ammonium hydroxide (100 ml). The resulting solution was concentrated to a small volume (1 ml) and freeze-dried to afford the title compound as a white solid (30 mg, 29%). <sup>1</sup>H NMR (d4-methanol) δ 0.94 (3H, t, *J* = 6.8Hz), 1.45 (4H, m), 1.78 (2H, m), 2.52 (1H dd, *J* = 6.4, 12.0Hz), 2.65 (1H dd, *J* = 3.4, 12.0Hz), 2.75 (1H, m), 3.52-4.05 (9H, m), 6.72 (2H, d, *J* = 8.7 Hz), 7.35 (2H, d, *J* = 8.7 Hz). MS *m/z* 340.1 (M+H)<sup>+</sup>.

25

#### Biological Assays

The compound of the invention may be tested for biological activity in the following assays:

##### Inhibition of GCS

30 The assay for inhibition of GCS was performed essentially as described in Platt et al., J. Biol. Chem., 1994, 269, 27108, the enzyme source being human recombinant GCS expressed in insect cells.

##### Inhibition of non-lysosomal-β-glucocerebrosidase

35 The assay for inhibition of non-lysosomal-β-glucocerebrosidase was essentially carried out as described in Overkleeft, H. S. et al., J. Biol. Chem., 1998, 273, 26522-26527, with the following differences: whole cell extracts of MCF7 (a human breast carcinoma cell line) was used as the source of the enzyme instead of splenic membrane suspensions; 5 mM instead of 3 mM, 4-MU β-glucoside was used as substrate and 0.2M citrate/phosphate (pH 5.8) was used instead of McIlvaine buffer.

Table I shows IC<sub>50</sub> data for the compound of the invention against human GCS and non-lysosomal-β-glucocerebrosidase enzymes.

**Table I**

Example	Inhibition of glucosylceramide synthase (IC <sub>50</sub> $\mu$ M)	Inhibition of non-lysosomal- $\beta$ -glucocerebrosidase (IC <sub>50</sub> $\mu$ M)
Example 1	4.8	2.5

Estimating the cell-based IC<sub>50</sub> for GCS inhibition by measuring glucosylceramide (GlcCer) depletion

Human mammary epithelial cells (MCF-7) were cultured for 5-7 days, with varying concentrations of the compound of the invention (0; 0.01; 0.05; 0.25; 1.25 and 6.25  $\mu$ M). The cells were harvested and the total cellular lipids extracted. Neutral glycolipids were separated by partitioning in a DIPE/1-butanol/saline suspension, according to methods well known to those skilled in the art. The neutral glycolipid extracts were then separated by High-Performance Thin Layer Chromatography (HPTLC), using non-polar TLC conditions (chloroform: methanol: 0.2% CaCl<sub>2</sub>; 65:35:8), according to methods well known to those skilled in the art. GlcCer bands were visualized and the TLC plates were scanned immediately. Scion Image software was then used to quantify GlcCer in the samples relative to a GlcCer standard. This enabled a cell-based IC<sub>50</sub> to be calculated for the compound of the invention for GCS inhibition.

28 Oct 2009

2004247468

## THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. The compound 3,4,5-piperidinetriol, 2-(hydroxymethyl)-1-[(4-(pentyloxy)phenyl)methyl]-, (2S,3S,4R,5S) or a pharmaceutically acceptable salt thereof.

5

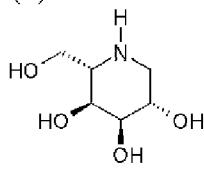
2. A compound as defined in claim 1 for use in medicine.

3. A pharmaceutical composition comprising a compound, as defined in claim 1, together with one or more pharmaceutically acceptable carriers, excipients and/or diluents.

10

4. A process for the preparation of a compound, as defined in claim 1, which comprises:

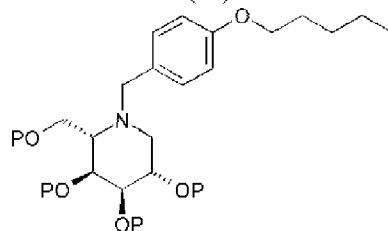
a) reacting a compound of formula (II):



(II)

15 15. with 4-(pentyloxy)benzaldehyde using NaBH<sub>3</sub>CN or a supported reagent in acetic acid-methanol or HCl-methanol, or using NaBH(OAc)<sub>3</sub> in a solvent, or

b) deprotection of a compound of formula (III):



(III)

20 20. wherein P, which may be the same or different, are hydroxy protecting groups.

5. The use of a compound, as defined in claim 1, in the manufacture of an inhibitor of glucosylceramide synthase.

25 6. A method of treating a glycolipid storage disease comprising the step of administering to a patient in need thereof an effective amount of a compound of claim 1.

7. The method as claimed in claim 6, wherein the glycolipid storage disease is Gaucher disease, Sandhoff's disease, Tay-Sachs disease, Fabry disease or GM1 gangliosidosis.

30

8. A method of treating Niemann-Pick disease type C, mucopolysaccharidosis type I, mucopolysaccharidosis type IIIA, mucopolysaccharidosis type IIIB, mucopolysaccharidosis type VI or mucopolysaccharidosis type VII,  $\alpha$ -mannosidosis or mucolipidosis type IV, comprising the step of administering to a patient in need thereof an effective amount of a compound of claim 1.

35

9. A method of treating cancer in which glycolipid synthesis is abnormal, comprising the step of administering to a patient in need thereof an effective amount of a compound of claim 1.

28 Oct 2009

2004247468

10. The method according to claim 9, wherein the cancer in which glycolipid synthesis is abnormal is selected from brain cancer, neuronal cancer, neuroblastoma, renal adenocarcinoma, malignant melanoma, multiple myeloma and multi-drug resistant cancers.  
5
11. The method of treating Alzheimer's disease, epilepsy, stroke, Parkinson's disease or spinal injury, comprising the step of administering to a patient in need thereof an effective amount of a compound of claim 1.
- 10 12. The method of treating diseases caused by infectious microorganisms which utilize glycolipids on the surface of cells as receptors for either the organism itself or for toxins produced by the organism, or infectious microorganisms for which the synthesis of glucosylceramide is an essential or important process, the method comprising the step of administering to a patient in need thereof an effective amount of a compound of claim 1.  
15
13. The method of treating diseases associated with abnormal glycolipid synthesis comprising the step of administering to a patient in need thereof an effective amount of a compound of claim 1.
14. The method according to claim 13, wherein the diseases associated with abnormal glycolipid synthesis are selected from polycystic kidney disease, diabetic renal hypertrophy and atherosclerosis.  
20
15. A method of treating a condition treatable by the administration of a ganglioside such as GM1 ganglioside, comprising the step of administering to a patient in need thereof an effective amount of a compound of claim 1.  
25
16. A method of reversibly rendering a male mammal infertile, comprising the step of administering to a patient in need thereof an effective amount of a compound of claim 1.
17. A method of treating obesity, comprising the step of administering to a patient in need thereof an effective amount of a compound of claim 1.  
30
18. A method of treating inflammatory diseases or disorders associated with macrophage recruitment and activation, comprising the step of administering to a patient in need thereof an effective amount of a compound of claim 1.  
35
19. The method according to claim 18, wherein the inflammatory disease or disorder associated with macrophage recruitment and activation is selected from rheumatoid arthritis, Crohn's disease, asthma and sepsis.  
40