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(54) **Title:** UREA AND GUANIDINIUM DERIVATIVES OF IMINOSUGARS

(57) **Abstract:** The invention relates to novel urea and guanidinium derivatives of deoxynojirimycin and related iminosugars, wherein the ring nitrogen in the iminosugars is derivatised, methods for the preparation urea and guanidinium derivatives of deoxynojirimycin, (protected) intermediates and the use of the urea and guanidinium derivatives of deoxynojirimycin in the modification of the activity of glycosidases and their use as a pharmaceutical.



Title: Urea and Guanidinium derivatives of iminosugars

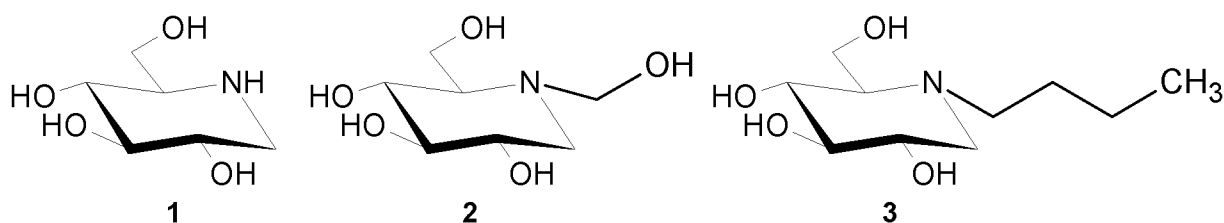
Description

Technical Field

5 [01] The present invention pertains to derivatives of iminosugars, in particular to urea and guanidinium derivatives of iminosugars, their salts, tautomers, enantiomers and stereoisomers. The invention further relates to methods for making guanidinium derivatives of iminosugars, the synthesis of relevant intermediates, and certain thioureas. The invention further relates to pharmaceutical compositions comprising the compounds of the invention and to methods of inhibiting or modifying the activity of glycosidase enzymes as well as
10 methods for the treatment of diseases using the compounds of the invention.

Background Art

[02] Glycosidases are important enzymes in nature that cleave carbohydrate units. Specifically, glycosidases cleave the glycosidic bonds in oligosaccharides and glycoconjugates. There are approximately 100 different glycosidases known in humans. Since they are involved in many areas of biology, their malfunction also can lead to disease. For this reason the inhibition of glycosidase has been an important goal in science and therapeutics (N. Asano, Cell. Mol. Life. Sci. 2009, 66, 1479-1492.). Major progress has been
15 made in this area by chemical synthesis and also compounds from natural sources have been discovered with special properties. The natural product deoxynojirimycin (DNJ) **1** from mulberry leaves was a major discovery and was found to be a potent inhibitor for numerous glycosidases. Alkylation of its nitrogen led to additional compounds with a more specific activity profile. This led to the registration of the drugs Miglitol® (Glyset) **2** against diabetes and Zavesca® (Miglustat) **3** against Gaucher's disease, both derivatives of deoxynojirimycin
20 However, due to the lack of selectivity, side effects are a major problem with these types of therapeutics.



[03] The blocking of glycosidases can aid in the treatment of diabetes (L. K. Campbell, D. E. Baker, R. K. Campbell, R. K., Ann. Pharmacother. 2000, 34, 1291–1301.) viral infections (M. von Itzstein, Curr. Opin. Chem. Biol. 2008, 12, 102-108.), lysosomal storage diseases (B.
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E. Smid, J. M. F. G. Aerts, R. G. Boot, G. E. Linthorst, C. E. M. Hollak, *Exp. Opin. on Investig. Drugs*, 2010, 19, 1367-1379.) and cancer (D. A. Kuntz, S. Nakayama, K. Shea, H. Hori, Y. Uto, H. Nagasawa, D. R. Rose, *ChemBioChem*, 2010, 11, 673-680.). Iminosugars have been shown to be highly effective inhibitors but their limited selectivity can lead to side effects when applied therapeutically. (G. Horne, F. X. Wilson, J. Tinsley, D. H. Williams, R. Storer, *Drug Disc. Today* 2011, 16, 107-118; E. Borges de Melo, A. da Silveira Gomes, I. Carvalho, *Tetrahedron*, 2006, 62, 10277-10302.) It is clear that exploring additional iminosugar derivatives while also improving upon the selectivity of iminosugars as glycosidase inhibitors are important challenges.

[04] Several solutions have been proposed in the art. JP60-224675 describes certain iminosugars that are substituted at the nitrogen with a carboxamide group. US 4806650 describes processes for preparing alkyl or aralkyl N-substituted 1-deoxynojirimycins based on converting D-glucose to aminosorbitol. US6177447 describes deoxynojirimicin derivatives substituted (optionally via spacers) with large hydrophilic groups. WO00/33843 describes long chain N-alkyl derivatives of 1-deoxynojirimycin in the treatment of glycolipid storage diseases. Further derivatives are described in US2006/01114000, US5273981, EP536402, WO2006/125141. Aguilar *et al.* (*Chem. Comm.* 2012, 48, 6514-6516) describe conformationally locked sp²-iminosugars such as 5-N, 6-S-[N'-(n-octyliminomethylidene)]-6-thionojirimycin as an inhibitor of lysosomal beta glucosidase. Aguilar *et al.* (*Tetrahedron* 68, 2012, 681-689) describes isourea-type bicyclic sp²-iminosugars as potential glycosidase inhibitors. Aguilar *et al.* (*J. Org. Chem* 2008, 73, 1995-1998) describes guanidine-type iminosugars as glucosidase inhibitors and describes a method for the synthesis of the N-amidinoylpiperidine azasugars. The method is complex and laborious and is not suitable for the preparation of a wide variety of guanidine-type iminosugars, preferred for a qualitative broad evaluation of the potential pharmaceutical properties. Bini D. *et al.*, *Beilstein Journal of Organic Chemistry*, (8), 2012, 514-521, April 5, 2012 describes certain iminosugars including a nojiromicin-based inhibitor having a monosubstituted urea (DNJ(CO)NH₂) that are tested as porcine and insect trehalases but not as a glycosidase inhibitor.

[05] Given the variations in activity and selectivity of known iminosugars vis-à-vis various glycosidases, there remains a need for further variations in the principal structure of the iminosugars-based compounds to further enhance the effectiveness, applicability and therapeutic feasibility of iminosugars.

Summary of Invention

[06] The present inventors have now found new urea and guanidinium derivatives of iminosugars and in particular of new urea and guanidinium derivatives of deoxynojirimicin.

Furthermore, they have found an efficient method for the synthesis of a wide variety of these compounds as well as methods for modifying the specific inhibitory activity towards various glycosidases using the compounds of the invention.

[07] Furthermore the inventors have successfully devised a straightforward route to a new class of iminosugar analogues and investigated their ability to inhibit a variety of glycosidases. The derivatized urea, and guanidinium derivatives prepared were screened against a series of glycosidases and showed advantageous inhibition profiles. Incorporation of the guanidine moiety in iminosugars led to enhanced potency against several glycosidases whereas the neutral urea counterpart also proved to be a very selective inhibitor.

Incorporation of the guanidinium-group in iminosugars alters both the steric and electronic properties of the iminosugar in an advantageous way. Without being bound by theory, it is thought that its charge is likely more delocalized than in the case of the N-alkylated iminosugars. Furthermore, due to the degree of sp² hybridisation of the ring nitrogen, the ring conformation is likely slightly altered. Taken together these effects result in a potent inhibitory motif for a range of glycosidases that can be introduced using a concise and straightforward synthetic approach. It is also worth noting that some of the compounds bear a relatively simple alkyl substituent at the guanidine.

Brief Description of Drawings

[08] Figure 1. IC₅₀ determination of Guanidinium (13) (N-1-butylguanidine-1-deoxynojirimycin, 1-DNJ (1), Urea (10) (N-1-butylurea-1-deoxynojirimycin) and Alkylated (8) (N-Hexyl-1-deoxynojirimycin) against alpha-galactosidase (from green coffee beans; G8507).

[09] Figure 2. IC₅₀ determination of Guanidinium (13) (N-1-butylguanidine-1-deoxynojirimycin, 1-DNJ (1), Urea (10) (N-1-butylurea-1-deoxynojirimycin) and Alkylated (8) (N-Hexyl-1-deoxynojirimycin) against beta-galactosidase (from Bovine liver G1875).

[10] Figure 3. IC₅₀ determination of Guanidinium (13) (N-1-butylguanidine-1-deoxynojirimycin, 1-DNJ (1), Urea (10) (N-1-butylurea-1-deoxynojirimycin) and Alkylated (8) (N-Hexyl-1-deoxynojirimycin) against alpha-glucosidase (from Bakers yeast G5003).

[11] Figure 4. IC₅₀ determination of Guanidinium (13) (N-1-butylguanidine-1-deoxynojirimycin, 1-DNJ (1), Urea (10) (N-1-butylurea-1-deoxynojirimycin) and Alkylated (8) (N-Hexyl-1-deoxynojirimycin) against beta-glucosidase (from Almonds G4511).

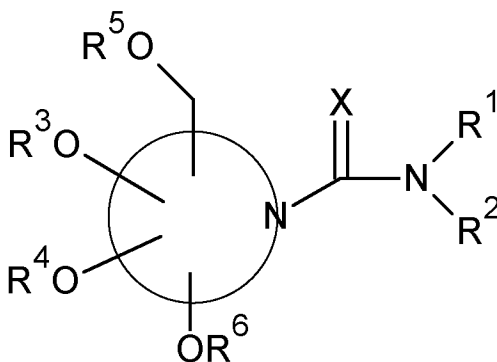
[12] Figure 5. IC₅₀ determination of Guanidinium (13) (N-1-butylguanidine-1-deoxynojirimycin, 1-DNJ (1), Urea (10) (N-1-butylurea-1-deoxynojirimycin) and Alkylated (8) (N-Hexyl-1-deoxynojirimycin) against alpha-mannosidase (from Jack Beans M7257).

[13] Figure 6. IC₅₀ determination of Guanidinium (13) (N-1-butylguanidine-1-deoxynojirimycin, 1-DNJ (1), Urea (10) (N-1-butylurea-1-deoxynojirimycin) and Alkylated (8) (N-Hexyl-1-deoxynojirimycin) against beta-mannosidase (from *Helix pomatia* M9400).

[14] Figure 7. IC50 determination of Guanidinium (13) (N-1-butylguanidine-1-deoxynojirimycin, 1-DNJ (1), Urea (10) (N-1-butylurea-1-deoxynojirimycin) and Alkylated (8) (N-Hexyl-1-deoxynojirimycin) against Naringinase (from *Penicillium decimbens* N1385).

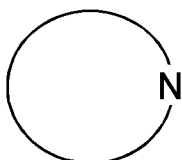
5 Detailed description of the invention

[15] Thus, in a first aspect the invention pertains to a compound represented by the general formula



wherein

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is a 5, 6, 7 or 8-membered (saturated) nitrogen-containing cyclic compound;

wherein X is O or NR⁷;

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wherein R¹ is H or a straight or branched alkyl, cycloalkyl, alkenyl, alkynyl, alkoxyalkyl or aminoalkyl containing 1-20 carbon atoms, an aryl, alkylaryl, heteroaryl, heteroalkyl, alkoxy(het)aryl containing 5-12 ring atoms, wherein R¹ is optionally substituted with one or more -OH, -COOH, -Cl, -F, -CF₃, -OCF₃, -O-C(=O)N-(alkyl)₂;

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wherein R² is H or a straight or branched alkyl, cycloalkyl, alkenyl, alkynyl, alkoxyalkyl or aminoalkyl containing 1-12 carbon atoms, an aryl, alkylaryl, heteroaryl, heteroalkyl, alkoxy(het)aryl containing 5-12 ring atoms, wherein R¹ is optionally substituted with one or more -OH, -COOH, -Cl, -F, -CF₃, -OCF₃, -O-C(=O)N-(alkyl)₂;

wherein R³, R⁴, R⁵, R⁶ are independently H or a hydroxyl protecting group,

wherein R⁷ is H, C1-C6 alkyl;

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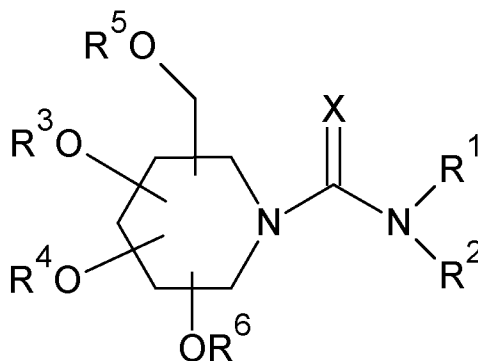
and pharmaceutically acceptable salts, tautomers, enantiomers stereoisomers and/or esters thereof.

[16] In one embodiment, the invention does not encompass a compound selected from the group consisting of ((2*R*,3*R*,4*S*)-1-(*N'*-Phenylamidino)-3,4,5,5-tetrahydroxy-2-hydroxymethylpiperidine; (2*R*,3*R*,4*S*)-1-(*N'*-Benzyl-*N''*-phenylamidino)-3,4,5,5-tetrahydroxy-2-hydroxymethylpiperidine and (2*R*,3*R*,4*S*)-1-(*N'*,*N''*-Dibenzylamidino)-3,4,5,5-tetrahydroxy-2-hydroxymethylpiperidine; such as described in Aguilar *et al.* J. Org. Chem 2008, 73, 1995-

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1998 wherein two hydroxyl groups are bound to the same carbon atom of the nitrogen-containing heterocycle, preferably at a position located beta from the ring nitrogen, more preferably the hydrochloride salts of these compounds.

[17] In certain embodiments, the nitrogen-containing heterocycle in the compound is a six-membered nitrogen-containing heterocycle and more preferably is a piperidine-based structure, the compound preferably having the general structure



wherein X, R¹, R², R³, R⁴, R⁵, R⁶ and R⁷ are as described herein elsewhere.

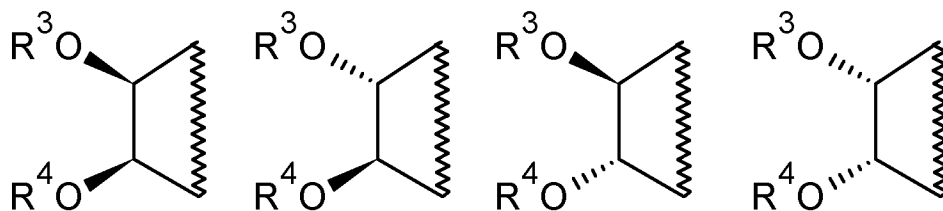
[18] Preferably, the substituents R¹ and R² are, independently, selected from H or a straight or branched alkyl, cycloalkyl, alkenyl, alkynyl, alkoxyalkyl or aminoalkyl containing 1-20 carbon atoms, an aryl, alkylaryl, heteroaryl, heteroalkyl, alkoxy(het)aryl containing 5-12 ring atoms.

[19] In certain embodiments, the compounds of the invention do not encompass a compound wherein X=O and R¹ and R² are both H. Thus, in a preferred embodiment of the invention, when X=O, then one of R¹ and R² is not H.

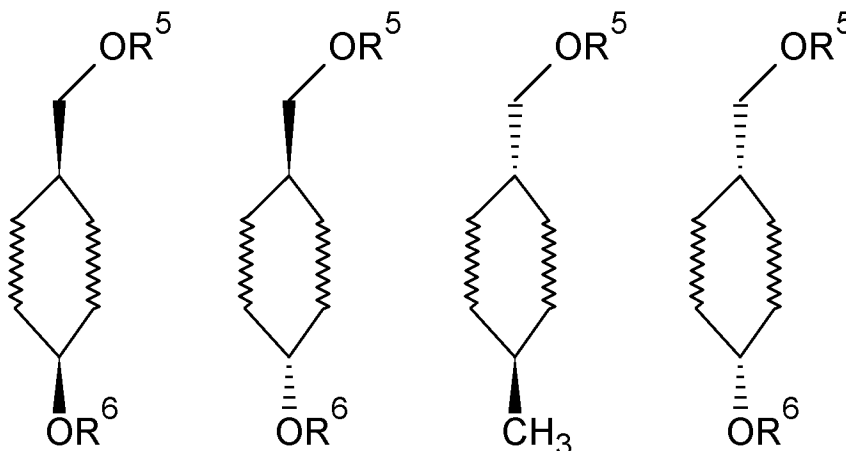
[20] In certain embodiments, the compounds of the invention do not encompass a compound wherein X=O, R¹=H, and R² is -(CH₂)_n-Ph-R, wherein R=H, halogen, lower alkyl, lower alkoxy and n=0-5, a ring-substituted (alkyl)phenyl. Thus, in a preferred embodiment, when X=O and R¹=H then R² is not -(CH₂)_n-Ph-R, wherein R=H, halogen, lower alkyl, lower alkoxy and n=0-5

[21] The nitrogen containing heterocycle of the present invention is a 5, 6, 7 or 8-membered (saturated) nitrogen-containing cyclic compound. The chemistry for these nitrogen containing cyclic compound is described in Stocker, B. L.; Dangerfield, E. M.; Win-Mason, A. L.; Haslett, G. W.; Timmer, M. S. M. Eur. J. Org. Chem. 2010, 1615-1637 and in Dragutan, I.; Dragutan, V.; Mitan, C.; Vosloo, H. C. M.; Delaude, L.; Demonceau, A. Beilstein J. Org. Chem. 2011, 7, 699-716.

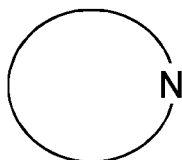
[22] It is commonplace with cyclic carbohydrate structures that they have different stereochemistries, i.e. with the hydroxyl-groups below or above the ring structure. Thus, in certain embodiments, the R³ and R⁴ substituted hydroxyl groups on the nitrogen-containing cyclic compound have a orientation selected from the group consisting of



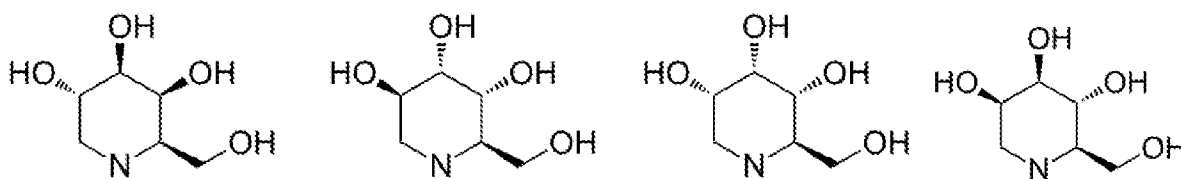
[23] Furthermore, in certain embodiments, the R^5 and R^6 substituted hydroxyl groups on the nitrogen-containing cyclic compound may also have orientations selected from the group consisting of



[24] Of particular preference in the present invention as the nitrogen containing heterocycle



are the piperidine-based iminocyclitols such as 1-deoxymannonojiromycin, 1-deoxyaltronojiromycin, 1-deoxygalactonojiromycin and 1-deoxyallonojiromycin



[25] In certain preferred embodiments, R^1 is H or a straight or branched alkyl or cycloalkyl, containing 1-20 carbon atoms, preferably H or a straight alkyl containing 1-12 carbon atoms, more preferably H, ethyl, butyl, hexyl, octyl and their structural isomers.

[26] In certain preferred embodiments, R² is H or a straight or branched alkyl or cycloalkyl, containing 1-20 carbon atoms, preferably H or a straight alkyl containing 1-12 carbon atoms, more preferably H.

[27] The hydroxyl-protecting group in the general formula of the compounds of the invention is typically a hydrolysable group that is capable of forming an ester or ether bond with the hydroxyls on the ring or the CH₂OH. The protecting group can either shield the hydroxyl or serve as a prodrug which can be hydrolysed by the organism. Suitable protecting groups are for instance described in Greene's Protective Groups in Organic Synthesis, Fourth Edition John Wiley & Sons, Inc., 2007, chapter 2.

[28] Hydroxyl protecting groups can be selected from amongst Acetyl (Ac), Benzoyl (Bz), Benzyl (Bn), β -Methoxyethoxymethyl ether (MEM), Dimethoxytrityl, [bis-(4-methoxyphenyl)phenylmethyl] (DMT), Methoxymethyl ether (MOM), Methoxytrityl [(4-methoxyphenyl)diphenylmethyl, MMT), p-Methoxybenzyl ether (PMB), Methylthiomethyl ether, Pivaloyl (Piv), Tetrahydropyranyl (THP), Trityl (Tr), Silyl ether (most popular ones include trimethylsilyl (TMS), tert-butyldimethylsilyl (TBDMS), tri-iso-propylsilyloxymethyl (TOM), and triisopropylsilyl (TIPS) ethers), Methyl Ethers, Ethoxyethyl ethers (EE). Preferred hydroxyl protecting group is benzyl (bn).

[29] The preferred compounds of the present invention are:

N-1-butylurea-1-deoxynojirimycin;

(2*R*, 3*R*, 4*R*, 5*S*)-*N*-butyl-3,4,5-trihydroxy-2-(hydroxymethyl)piperidine-1-carboximidamide;

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide;

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-hydroxyethyl)-2-(hydroxymethyl)-piperidine-1-carboximidamide;

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-(2-hydroxyethoxy)ethyl) 2-(hydroxymethyl)-piperidine-1-carboximidamide;

(2*R*, 3*R*, 4*R*, 5*S*)-*N*-benzyl-3,4,5-trihydroxy- 2-(hydroxymethyl)-piperidine-1-carboximidamide;

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-(3-phenylpropyl)piperidine-1-carboximidamide;

(2*R*, 3*R*, 4*R*, 5*S*)-*N*-(3-(adamantylmethoxy)propyl)-3,4,5-trihydroxy- 2-

(hydroxymethyl)piperidine-1-carboximidamide;

(2*R*, 3*S*, 4*R*, 5*S*)-*N*-butyl -3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide;

(2*R*, 3*S*, 4*R*, 5*S*)-*N*-hexyl -3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide;

(2*R*, 3*S*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide;

[30] The compounds of the present invention contain one or more nitrogen atoms that may be transformed into their pharmaceutically acceptable salts by contacting the compound with a suitable acid as a salt forming compound.

5 [31] Preferred compounds of the present invention are the hydrochloride salts of the guanidinium compounds of the present invention and in particular:

(2*R*, 3*R*, 4*R*, 5*S*)-*N*-butyl-3,4,5-trihydroxy-2-(hydroxymethyl)piperidine-1-carboximidamide hydrochloride;

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide hydrochloride;

10 (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-hydroxyethyl)-2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride;

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-(2-hydroxyethoxy)ethyl) 2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride;

15 (2*R*, 3*R*, 4*R*, 5*S*)-*N*-benzyl-3,4,5-trihydroxy- 2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride;

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-(3-phenylpropyl)piperidine-1-carboximidamide hydrochloride;

(2*R*, 3*R*, 4*R*, 5*S*)-*N*-(3-(adamantylmethoxy)propyl)-3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide hydrochloride;

20 (2*R*, 3*S*, 4*R*, 5*S*)-*N*-butyl -3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide hydrochloride;

(2*R*, 3*S*, 4*R*, 5*S*)-*N*-hexyl -3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide hydrochloride;

25 (2*R*, 3*S*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide hydrochloride;

[32] The hydrochloride compounds of the present invention can be converted into other pharmaceutically acceptable salts using conventional procedures wherein one salt of the compound is exchanged for another salt, usually via the free base of the compound as an intermediate.

[33] Pharmaceutical salts, their preparation and properties are described in Stahl et al. 'Handbook of pharmaceutical salts, properties, selection and use', Wiley-VCH, 2008.

Pharmaceutical salts can be made using a variety of salt-forming components, usually acids such as those selected from the group consisting of 1-hydroxy-2-naphthoic acid, 2,2-dichloroacetic acid, 2-hydroxyethanesulfonic acid, 2-oxoglutaric acid, 4-acetamidobenzoic acid, 4-aminosalicylic acid, acetic acid, adipic acid, ascorbic acid (L), aspartic acid (L),

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benzenesulfonic acid, benzoic acid, camphoric acid (+), camphor-10-sulfonic acid (+), capric acid (decanoic acid), caproic acid (hexanoic acid), caprylic acid (octanoic acid), carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid (D), gluconic acid (D), glucuronic acid (D), glutamic acid, glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, isobutyric acid, lactic acid (DL), lactobionic acid, lauric acid, maleic acid, malic acid (- L), malonic acid, mandelic acid (DL), methanesulfonic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, nicotinic acid, nitric acid, oleic acid, oxalic acid, palmitic acid, palmoic acid, phosphoric acid, proprionic acid, pyroglutamic acid (- L), salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, tartaric acid (+ L), thiocyanic acid, toluenesulfonic acid (p), undecylenic acid, preferably the hydrochloride or hydrobromide.

Chemical Definitions

[34] The term 'alkyl' refers to a straight or branched C1-C20 hydrocarbon group consisting solely of carbon and hydrogen atoms, containing no unsaturation, and which is attached to the rest of the molecule by a single bond, e.g., methyl, ethyl, n-propyl, 1-methylethyl (isopropyl), n-butyl, i-butyl, 1,1-dimethylethyl (t-butyl), n-pentyl, 2-methylbutyl, 3-methylbutyl, 2,2-dimethylpropyl, 3,3 dimethylpropyl, hexyl, 2-methylpentyl, 3-methyl pentyl, 4-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 3,2-dimethylbutyl, 1,1-dimethylbutyl heptyl, octyl, nonyl, decyl, undecyl and dodecyl. The alkyls used herein are preferably C1 – C12 alkyls.

[35] The term "alkenyl" refers to a C2-C20 aliphatic hydrocarbon group containing at least one carbon-carbon double bond and which may be a straight or branched chain, e.g., ethenyl, 1-propenyl, 2-propenyl (allyl), iso-propenyl, 2-methyl- 1-propenyl, 1-butenyl, 2-butenyl.

[36] The term "alkynyl" refers to a C2-C20 aliphatic hydrocarbon group containing at least one carbon-carbon triple bond and which may be a straight or branched chain, e.g., ethynyl, 1-propynyl, 2-propynyl (propargyl).

[37] The term "cycloalkyl" denotes an unsaturated, non-aromatic mono- or multicyclic hydrocarbon ring system such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl. Examples of multicyclic cycloalkyl groups include perhydronaphthyl, adamantyl and norbornyl groups bridged cyclic group or spirobicyclic groups, e.g., spiro (4,4) non-2-yl.

[38] The term "cycloalkalkyl" refers to a cycloalkyl as defined above directly attached to an alkyl group as defined above, that results in the creation of a stable structure such as cyclopropylmethyl, cyclobutylethyl, cyclopentylethyl.

[39] The term "alkyl ether" or "alkoxyalkyl" refers to an alkyl group or cycloalkyl group as defined above having at least one oxygen incorporated into the alkyl chain, e.g., methyl ethyl ether, diethyl ether, tetrahydrofuran.

[40] The term "aminoalkyl" refers to an alkyl group or a cycloalkyl group as defined above having at least one nitrogen atom, e.g., n-butyl amine and tetrahydrooxazine.

[41] The term "aryl" refers to aromatic radicals having in the range of about 6 to about 14 carbon atoms such as phenyl, naphthyl, tetrahydronaphthyl, indanyl, biphenyl. The term "arylalkyl" refers to an aryl group as defined above directly bonded to an alkyl group as defined above, e.g., -CH₂C₆H₅, and -C₂H₄C₆H₅. The term "alkylaryl" sometimes refers to an alkyl group as defined above directly bonded to an aryl group as defined above, e.g., -C₆H₄CH₃, and -C₆H₄C₂H₅.

[42] The term "heterocyclic" refers to a stable 3- to 15-membered ring radical which consists of carbon atoms and from one to five heteroatoms selected from the group consisting of nitrogen, phosphorus, oxygen and sulfur. For purposes of this invention, the heterocyclic ring radical may be a monocyclic, bicyclic or tricyclic ring system, which may include fused, bridged or spiro ring systems, and the nitrogen, phosphorus, carbon, oxygen or sulfur atoms in the heterocyclic ring radical may be optionally oxidized to various oxidation states. In addition, the nitrogen atom may be optionally quaternized; and the ring radical may be partially or fully saturated (i.e., heteroaromatic or heteroaryl aromatic). Examples of such heterocyclic ring radicals include, but are not limited to, azetidiny, acridiny, benzodioxoly, benzodioxany, benzofurnyl, carbazoly, cinnoliny, dioxolany, indoliziny, naphthyridiny, perhydroazepiny, phenaziny, phenothiaziny, phenoxaziny, phthalaziny, pyridyl, pteridiny, puriny, quinazoliny, quinoxaliny, quinoliny, isoquinoliny, tetrazoly, imidazolyl, tetrahydroisouinolyl, piperidiny, piperaziny, 2-oxopiperaziny, 2-oxopiperidiny, 2-oxopyrrolidiny, 2-oxoazepiny, azepiny, pyrroly, 4-piperidony, pyrrolidiny, pyraziny, pyrimidiny, pyridaziny, oxazolyl, oxazoliny, oxasolidiny, triazolyl, indanyl, isoxazolyl, isoxasolidiny, morpholiny, thiazolyl, thiazoliny, thiazolidiny, isothiazolyl, quinuclidiny, isothiazolidiny, indolyl, isoindolyl, indoliny, isoindoliny, octahydroindolyl, octahydroisoindolyl, quinolyl, isoquinolyl, decahydroisoquinolyl, benzimidazolyl, thiadiazolyl, benzopyrany, benzothiazolyl, benzooxazolyl, furyl, tetrahydrofurtyl, tetrahydropyrany, thienyl, benzothienyl, thiamorpholiny, thiamorpholiny sulfoxide thiamorpholiny sulfone, dioxaphospholany, oxadiazolyl, chromany, isochromany.

[43] The heterocyclic ring radical may be attached to the main structure at any heteroatom or carbon atom that results in the creation of a stable structure. The term "heteroaryl" refers to a heterocyclic ring wherein the ring is aromatic.

[44] The term "heteroarylalkyl" refers to heteroaryl ring radical as defined above directly bonded to alkyl group. The heteroarylalkyl radical may be attached to the main structure at any carbon atom from alkyl group that results in the creation of a stable structure.

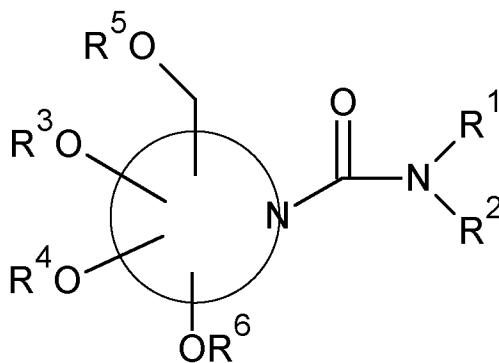
[45] The term "heterocyclyl" refers to a heterocyclic ring radical as defined above. The heterocyclyl ring radical may be attached to the main structure at any heteroatom or carbon

atom that results in the creation of a stable structure. The term "heterocyclalkyl" refers to a heterocyclic ring radical as defined above directly bonded to alkyl group. The heterocyclalkyl radical may be attached to the main structure at carbon atom in the alkyl group that results in the creation of a stable structure.

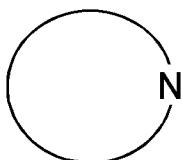
- 5 **[46]** The substituents in the 'substituted alkyl', 'substituted alkenyl', 'substituted alkynyl', 'substituted cycloalkyl', 'substituted cycloalkalkyl', 'substituted cycloalkenyl', 'substituted arylalkyl', 'substituted aryl', 'substituted heterocyclic ring', 'substituted heteroaryl ring', 'substituted heteroarylalkyl', or 'substituted heterocyclalkyl ring', may be the same or different with one or more selected from the groups hydrogen, hydroxy, halogen, carboxyl, cyano, amino, nitro, oxo (=O), thio (=S), or optionally substituted groups selected from alkyl, alkoxy, alkenyl, alkynyl, aryl, arylalkyl, cycloalkyl, aryl, heteroaryl, heteroarylalkyl, heterocyclic ring, -COOR^x, -C(O)R^x, -C(S)R^x, -C(O)NR^xR^y, -C(O)ONR^xR^y, -NR^xCONR^yR^z, -N(R^x)SOR^y, -N(R^x)SO₂R^y, -(=N-N(R^x)R^y), -NR^xC(O)OR^y, -NR^xR^y, -NR^xC(O)R^y, -NR^xC(S)R^y, -NR^xC(S)NR^yR^z, -SONR^xR^y, -SO₂NR^xR^y, -OR^x, -OR^xC(O)NR^yR^z, -OR^xC(O)OR^y, -OC(O)R^x, -OC(O)NR^xR^y, -R^xNR^yR^z, -R^xR^yR^z, -R^xCF₃, -R^xNR^yC(O)R^z, -R^xOR^y, -R^xC(O)OR^y, -R^xC(O)NR^yR^z, -R^xC(O)R^x, -R^xOC(O)R^y, -SR^x, -SOR^x, -SO₂R^x, -ONO₂, wherein R^x, R^y and R^z in each of the above groups can be hydrogen atom, substituted or unsubstituted alkyl, haloalkyl, substituted or unsubstituted arylalkyl, substituted or unsubstituted aryl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkalkyl substituted or unsubstituted heterocyclic ring, substituted or unsubstituted heterocyclalkyl, substituted or unsubstituted heteroaryl or substituted or unsubstituted heteroarylalkyl.

[47] The term "halogen" refers to radicals of fluorine, chlorine, bromine and iodine.

[48] In another aspect of the invention, a method is provided for the synthesis of the compounds having the general formula (X=O):



wherein



is a 5, 6, 7 or 8-membered (saturated) nitrogen-containing cyclic compound;

wherein R^1 is H or a straight or branched alkyl, cycloalkyl, alkenyl, alkynyl, alkoxyalkyl or aminoalkyl containing 1-20 carbon atoms, an aryl, alkylaryl, heteroaryl, heteroalkyl, alkoxy(het)aryl containing 5-12 ring atoms, wherein R^1 is optionally substituted with one or more $-OH$, $-COOH$, $-Cl$, $-F$, $-CF_3$, $-OCF_3$, $-O-C(=O)N-(alkyl)_2$;

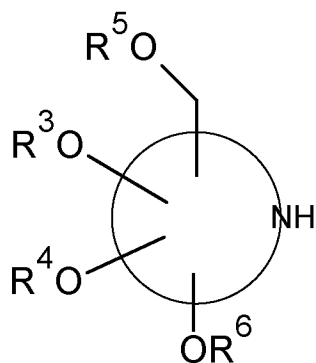
5 wherein R^2 is H or a straight or branched alkyl, cycloalkyl, alkenyl, alkynyl, alkoxyalkyl or aminoalkyl containing 1-12 carbon atoms, an aryl, alkylaryl, heteroaryl, heteroalkyl, alkoxy(het)aryl containing 5-12 ring atoms, wherein R^1 is optionally substituted with one or more $-OH$, $-COOH$, $-Cl$, $-F$, $-CF_3$, $-OCF_3$, $-O-C(=O)N-(alkyl)_2$;

wherein R^3 , R^4 , R^5 , R^6 are independently H or a hydroxyl protecting group,

10 wherein R^7 is H, C1-C6 alkyl;

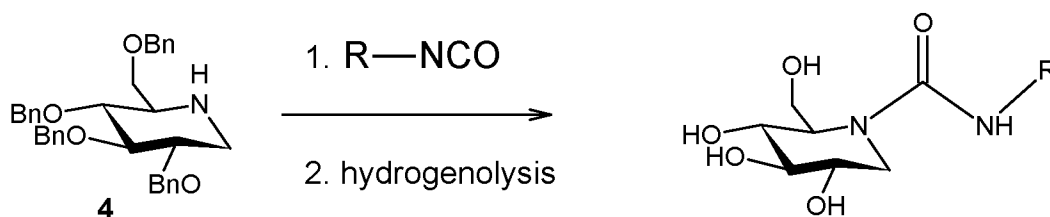
[49] The method starts with the preparation of the suitably protected iminosugar using conventional technologies (Wennekes, T.; Lang, B.; Leeman, M.; van der Marel, G.A.; Smits, E.; Weber, M.; van Wiltenburg, J.; Wolberg, M.; Aerts, J.M.F.G.; Overkleeft, H.S. *J. Org. Chem.* **2007**, 72, 1088-1097. The preferred protecting group is benzyl.

15 **[50]** The urea derivatives of the present invention are synthesised from the protected iminosugar having the general formula

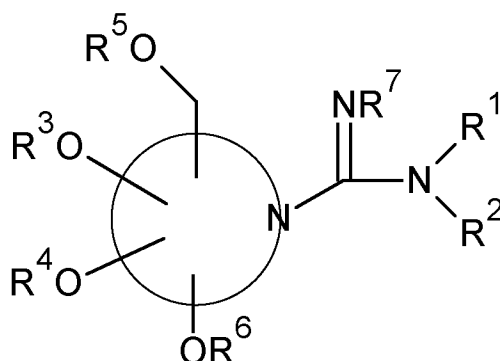


with an isocyanate carrying the desired R-group ($R-NCO$). Hydrogenolysis of the intermediate provides the urea derivative (Riegel's Handbook of Industrial Chemistry, 2003, page 740). An exemplary reaction for a 1-DNJ derivative is shown below.

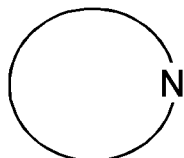
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[51] In another aspect of the invention, a method is provided for the synthesis of the compounds having the general formula



wherein



5

is a 5, 6, 7 or 8-membered (saturated) nitrogen-containing cyclic compound;

wherein R¹ is H or a straight or branched alkyl, cycloalkyl, alkenyl, alkynyl, alkoxyalkyl or aminoalkyl containing 1-20 carbon atoms, an aryl, alkylaryl, heteroaryl, heteroalkyl, alkoxy(het)aryl containing 5-12 ring atoms, wherein R¹ is optionally substituted with one or more -OH, -COOH, -Cl, -F, -CF₃, -OCF₃, -O-C(=O)N-(alkyl)₂;

10

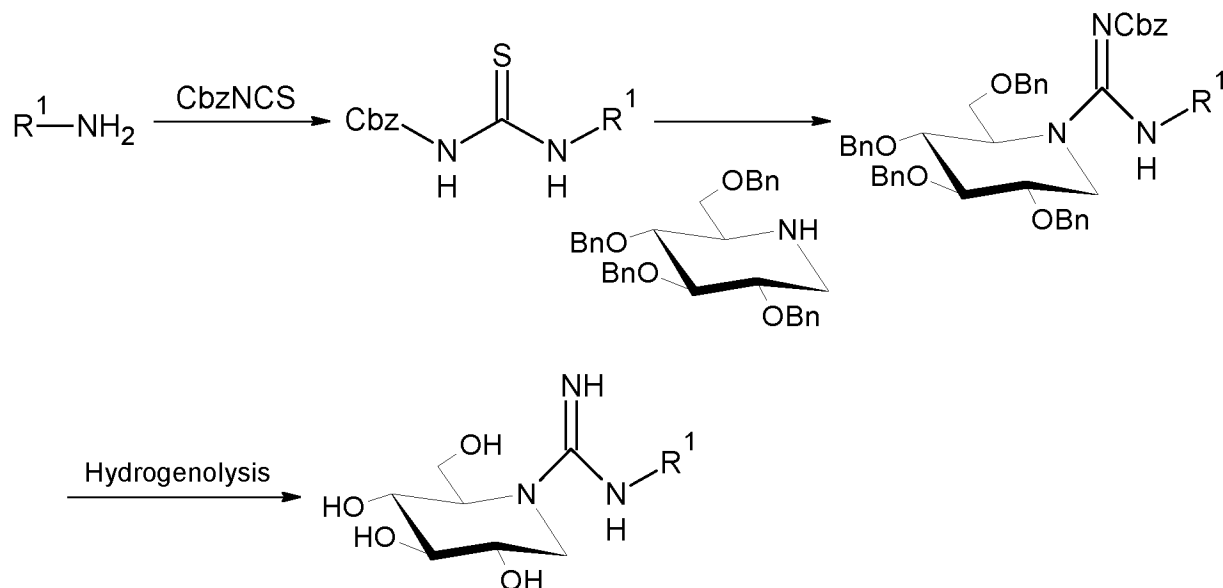
wherein R² is H or a straight or branched alkyl, cycloalkyl, alkenyl, alkynyl, alkoxyalkyl or aminoalkyl containing 1-12 carbon atoms, an aryl, alkylaryl, heteroaryl, heteroalkyl, alkoxy(het)aryl containing 5-12 ring atoms, wherein R² is optionally substituted with one or more -OH, -COOH, -Cl, -F, -CF₃, -OCF₃, -O-C(=O)N-(alkyl)₂;

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wherein R³, R⁴, R⁵, R⁶ are independently H or a hydroxyl protecting group, wherein R⁷ is H, C1-C6 alkyl.

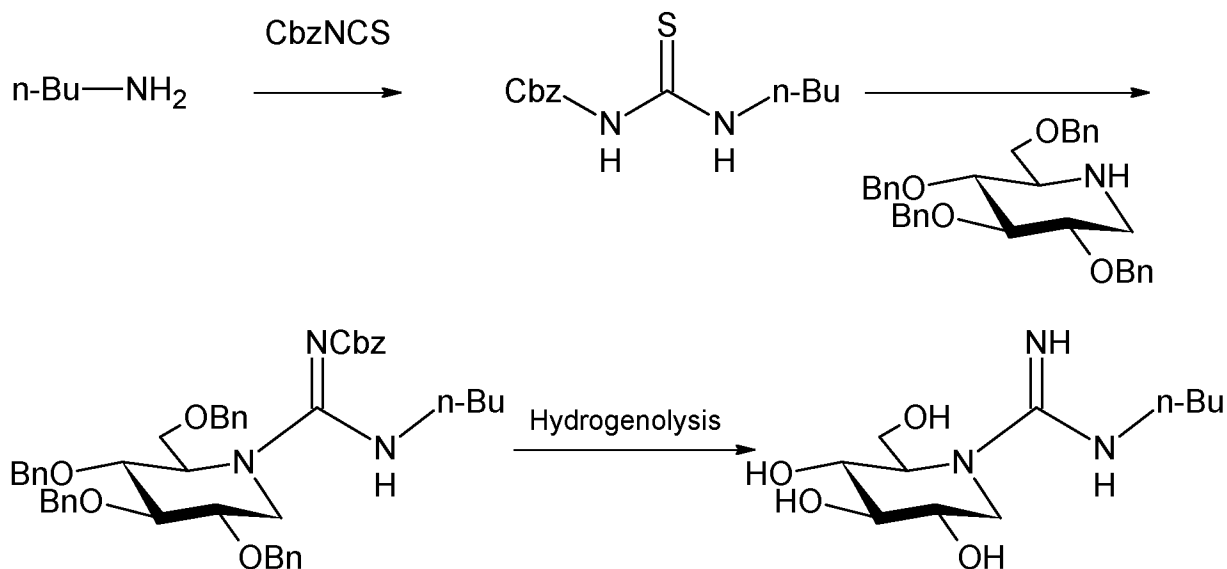
[52] A thiourea intermediate is prepared from a substituted amine (R¹R²NH), preferably a substituted primary amine R¹NH₂ and Cbz-NCS. Cbz-NCS is prepared according to Martin, N.I. *et al.* (2006) Organic Letters, 8, 4035-4036. Alternative reagents are Fmoc-NCS and Pbf-NCS.

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[53] The thiourea intermediate is reacted in the presence of an activating agent with the protected iminosugars leading to a protected guanidinium compound which can subsequently be hydrogenolysed, for instance using hydrogen with Pd/C in the presence of trace amount of HCl to provide the hydrochloride salt of the guanidinium derivative of the iminosugar. The hydrochloride salt of the guanidinium derivative of the iminosugar can be converted to yield other amine salts using conventional techniques such as described in Stahl et al. 'Handbook of pharmaceutical salts, properties, selection and use', Wiley-VCH, 2008.

[54] The strategy can be exemplified by the synthesis of the n-butyl derivative (R^1 is n-butyl)



[55] The protons on the guanidinium nitrogens can be, independently further derivatized, for instance alkylated, using conventional technology.

[56] The invention further relates to a method for modifying (increasing, reducing) glycosidase activity by contacting the glycosidase with an effective amount of one of the compounds of the invention. The glycosidase can be any glycosidase but is preferably

selected from the group consisting of alpha-galactosidase, beta-galactosidase, alpha-glucosidase, beta glucosidase, alpha-mannosidase, beta-mannosidase, naringinase, sialidase, and neuraminidase. In certain embodiments, the glycosidase is a purified or recombinant functional glycosidase enzyme. The contacting of the glycosidase can be in vitro or *in vivo*, depending on the circumstances.

[57] The compounds of the invention display activity, preferably an inhibiting activity *against various glycosidases*. Having an inhibiting activity of a compound vis-a-vis an enzyme is usually expressed as the IC₅₀ value. To compare compounds the IC₅₀ values can be taken relatively. The relative inhibiting activity of compounds towards a certain enzyme can then be expressed in %. The compounds of the present preferably express an inhibitory activity that is significantly higher than currently known compounds towards specific glycosidase enzymes, preferably at least 5, 10, 15, 20, 25 or 50 % higher than currently known compounds such as DNJ at given concentration.

[58] The invention further relates to a method for the treatment of glycosidase-activity related ailments of which non-limiting examples are Pompe's disease, a glycolipid storage disease such as Gaucher's disease, Tay Sachs disease, Sandhoff disease, Fabry disease, GM1 gangliosidosis, and fucosidosis, lysosomal storage disorders, diabetes, viral infections, HIV, Human hepatitis C (HCV), dengue virus, tuberculosis, Niemann-Pick diseases and cancer comprising administering to an individual in need thereof an effective amount of a compound according to the invention and to a pharmaceutical composition comprising a compound according to the invention and a pharmaceutically acceptable carrier.

Examples

Reagents, solvents and solutions.

[59] Unless stated otherwise, chemicals were obtained from commercial sources and used without further purification. All solvents were purchased from Biosolve (Valkenswaard, The Netherlands) and were stored on molecular sieves (4Å). 2,3,4,6-tetra-O-benzyl-D-glucopyranose was obtained from Carbosynth Limited (MT06691). 2,3,4,6-tetra-O-benzyl-1-deoxynojirimycin (**6**) (Wennekes, T.; Lang, B.; Leeman, M.; van der Marel, G.A.; Smits, E.; Weber, M.; van Wiltenburg, J.; Wolberg, M.; Aerts, J.M.F.G.; Overkleeft, H.S. *J. Org. Chem.* 2007, 72, 1088-1097) and Cbz-NCS (Martin, N. I.; Liskamp, R.M.J. *Org. Lett.* 2006, 18, 4035-4038) were prepared as previously described. Removal of solvent was performed under reduced pressure using a rotary evaporator.

Purification Techniques.

[60] All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using plates with a UV fluorescent indicator (normal SiO₂, Merck 60 F₂₅₄). One or more of the following methods were used for visualization: 10% H₂SO₄ in MeOH or ninhydrine.

Instrumentation for Compound Characterization.

[61] ¹H NMR spectra were recorded at 300 MHz with chemical shifts reported in parts per million (ppm) downfield relative to tetramethylsilane (Me₄Si) or H₂O (δ 4.8). ¹H NMR data are reported in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet) and coupling constant (J) in Hertz (Hz). When appropriate, the multiplicity is preceded by br, indicating that the signal was broad. ¹³C NMR spectra were recorded at 75.5 MHz with chemical shifts reported relative to CDCl₃ δ 77.0. ¹³C NMR spectra were recorded using the attached proton test (APT) sequence. All literature compounds had ¹H NMR, and mass spectra consistent with the assigned structures.

Experimental Procedures and Data for Compounds

N-Hexyl-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin (**7**):

[62] To a solution of benzyl-protected 1-DNJ (**6**) (500 mg, 0.95 mmol) in DMF (5 mL) was added K₂CO₃ (400 mg, 2.86 mmol, 3 eq.) and 1-bromohexane (200 μL, 1.43 mmol, 1.5 eq.). The resulting reaction mixture was refluxed overnight at 85 °C. The reaction mixture was warmed up to rt, filtered, concentrated *in vacuo* and the product was isolated via flash chromatography (SiO₂, 1:5 EtOAc/hexane) to yield **7** (363 mg, 62%) of a colorless oil. δ_H (300 MHz; CDCl₃; Me₄Si) 0.87 (3 H, t, CH₃, *J* = 6.74 Hz), 1.11 – 1.40 (8 H, m, CH₂), 2.19 – 2.31 (2 H, m, CH₂NH), 2.51 – 2.68 (2 H, m), 3.09 (1 H, m), 3.43 – 3.70 (5 H, m), 4.41 – 4.98 (8 H, m, CH₂C_{arom}), 7.12 – 7.34 (20 H, m, CH_{arom}). δ_C (75.5 MHz; CDCl₃; Me₄Si) 14.0 (CH₃), 22.5, 23.4, 27.1, 31.6 (CH₂), 52.3, 54.4 (CH₂NH), 72.6, 73.3, 75.1, 75.2 (CH₂), 63.6, 78.5, 87.3 (C-2, C-3, C-4, C-5), 127.3, 127.4, 127.5, 127.7, 128.2 (CH_{arom}), 137.7, 138.5, 139.0 (C_q). *R*_f = 0.38 (EtOAc/hexane 1:5). HRMS Calcd for C₄₀H₄₉NO₄ [M+H]⁺, 608.3740, found 608.3729.

N-Hexyl-1-deoxynojirimycin (**8**).

[63] A suspension of **7** (100 mg, 0.164 mmol) and Pd/C (10%) (100 mg) in EtOH (5 mL) was adjusted to pH = 1 with aqueous HCl (1M). The reaction mixture was stirred vigorously under a H₂ (g) atmosphere for 18 h at rt. The reaction mixture was next filtered over celite, washed with EtOH and concentrated to dryness. **8** was obtained after lyophilization as the HCl-salt (47 mg, quant.). δ_C (75.5 MHz; D₂O) 13.4 (CH₃), 22.0, 22.6, 25.8, 30.7 (CH₂), 52.9,

53.7, 55.0, 65.3, 67.0, 68.1, 76.7 (CH₂NH), C-2, C-3, C-4, C-5, C-6). HRMS for C₁₂H₂₅NO₄ [M+H]⁺, 248.1862, found 248.1857.

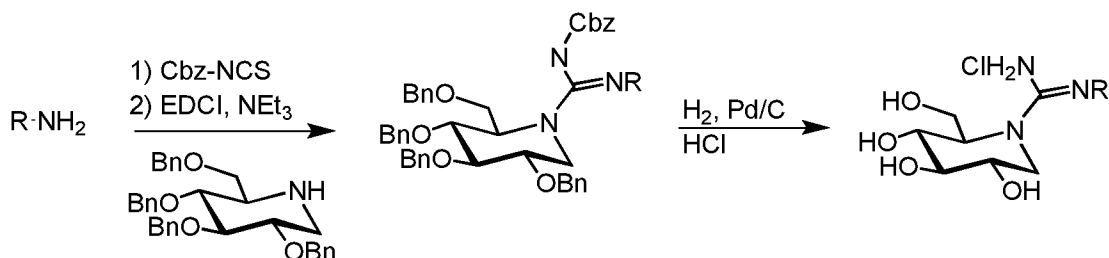
N-1-butylurea-2,3,4,6-tetra-O-benzyl-1-deoxynojirimycin (9):

- 5 **[64]** To a solution of benzyl-protected 1-DNJ (**6**) (200 mg, 0.38 mmol, 1 eq.) in dimethoxyethane (3 mL) was added n-butyl isocyanate (86 μ L, 0.76 mmol, 2 eq.). The resulting reaction mixture was refluxed overnight at 90 °C. The reaction mixture was warmed up to rt, concentrated *in vacuo* and the product was isolated via flash chromatography (SiO₂, 2:5 EtOAc/hexane) to yield **9** (168 mg, 70%) as a colorless oil. δ_{H} (300 MHz; CDCl₃; Me₄Si) 0.82 (3 H, t, CH₃, $J = 7.01$ Hz), 1.17 – 1.30 (4 H, m, CH₂), 3.06 – 3.14 (2 H, m, CH₂NH), 3.29 (1 H, dd, $J_{\text{A}} = 3.71$ Hz, $J_{\text{B}} = 10.18$ Hz), 3.53 – 3.76 (5 H, m), 3.92 (1 H, dd, $J_{\text{A}} = 4.68$ Hz, $J_{\text{B}} = 9.35$ Hz), 3.99 – 4.05 (1 H, m), 4.44 (2 H, d, CH₂C_{arom}, $J = 2.75$ Hz), 4.47 (1 H, s, CH₂C_{arom}), 4.52 (1 H, d, $J = 2.75$ Hz), 4.56 (1 H, s, CH₂C_{arom}), 4.62 (1 H, s, CH₂C_{arom}), 4.70 and 4.74 (2 x 1 H, 2 x d, CH₂C_{arom}), 5.47 (1 H, t, $J = 5.23$ Hz), 7.20 – 7.36 (20H, m, CH_{arom}). δ_{C} (75.5 MHz; CDCl₃; Me₄Si) 13.7 (CH₃), 19.9, 31.8 (CH₂), 40.5, 40.6 (CH₂NH), 70.8, 71.0, 72.8, 73.3 (CH₂), 56.9, 75.8, 78.8, 82.1 (C-2, C-3, C-4, C-5), 127.6, 127.7, 127.9, 128.2 (CH_{arom}), 137.4, 137.8, 137.9, 138.0 (C_q), 159.2 (NHC(O)). $R_{\text{f}} = 0.40$ (EtOAc/hexane 2:1). HRMS Calcd for C₃₉H₄₆N₂O₅ [M+H]⁺, 623.3485, found 623.3471.
- 10
- 15

20 **N-1-butylurea-1-deoxynojirimycin (10)**

- [65]** A suspension of **9** (168 mg, 0.27 mmol) and Pd/C (10%) (168 mg) in EtOH (5 mL) was adjusted to pH = 1 with aqueous HCl (2M). The reaction mixture was stirred vigorously under a H₂ (g) atmosphere for 18 h at rt. The reaction mixture was next filtered over celite, washed with EtOH and concentrated *in vacuo*. **10** was obtained after lyophilization. (82 mg, quant.).
- 25 δ_{H} (300 MHz; D₂O) 0.89 (3 H, t, CH₃), 1.35 (2 H, m, CH₂), 1.48 (2 H, m, CH₂), 3.14 – 3.21 (2 H, m, CH₂NH), 3.41 (1 H, d, $J = 12.1$ Hz), 3.61 (1 H, br s), 3.72 – 3.86 (6 H, m). δ_{C} (75.5 MHz; D₂O) 13.3 (CH₃), 19.6, 31.6 (CH₂), 40.4, 44.2 (CH₂NH), 60.0, 68.7, 71.1, 74.0 (C-2, C-3, C-4, C-5, C-6), 160.5 (NHC(O)). HRMS Calcd for C₁₁H₂₂N₂O₅ [M+H]⁺, 263.1607, 263.1583.

30 **Synthesis of the protected compounds, general procedure**



- [66]** An excess of the amine (1.25 mmol) was dissolved in DCM (6 mL) and CbzNCS (1.0 mmol, 2 mL, 0.5M in DCM) was added. The resulting mixture was stirred for 0.5 h at r.t. The

mixture was then washed with 0.2 M HCl (6 mL) to remove the excess of amine. The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was dissolved in DCM (6 mL) and EDCI (1.0 mmol, 192 mg), benzyl protected 1-DNJ **6** (0.5 mmol, 250 mg) and Et₃N (1.0 mmol, 140 µL) were added. The mixture was stirred overnight at r.t. The product was obtained after a silica gel column chromatography with appropriate hexanes/EtOAc mixtures.

[67] Using the above procedure, the following compounds were prepared, analysed and their structure confirmed:

N-(benzyloxycarbonyl)-*N'*-(*n*-butyl) 2,3,4,6-tetra-*O*-benzyl-deoxynojirimycin (**12**);

Benzyl ((octylamino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (**14**);

Benzyl (((2-(2-(benzyloxy)ethoxy)ethyl)amino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (**16**);

Benzyl (((2-(benzyloxy)ethyl)amino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (**18**);

Benzyl (((3-phenylpropyl)amino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (**20**);

Benzyl ((benzylamino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (**22**);

Benzyl (((3-((1*R*,2*S*,5*S*)-adamantan-2-ylmethoxy)propyl)amino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (**24**).

Deprotection, general procedure

[68] A suspension of the protected compound (0.1 mmol) and Pd/C (10%) (75 mg) in EtOH (5 mL) was adjusted to pH = 1 with aqueous HCl (1M). The reaction mixture was stirred vigorously under a H₂ (g) atmosphere for 18 h at r.t. The reaction mixture was filtered over celite, washed with EtOH and concentrated *in vacuo*. After lyophilization the HCl-salts were obtained.

[69] Using the above procedure, the following compounds were prepared, analysed and their structure confirmed:

N-1-butylguanidine-1-deoxynojirimycin hydrochloride (**13**);

((2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide hydrochloride (**15**);

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-(2-hydroxyethoxy)ethyl)-2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride (**17**) ;

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-hydroxyethyl)-2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride (**19**) ;

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-(3-phenylpropyl)piperidine-1-carboximidamide hydrochloride (**21**);

(2*R*, 3*R*, 4*R*, 5*S*)-*N*-benzyl-3,4,5-trihydroxy- 2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride (**23**) ;

5 (2*R*, 3*R*, 4*R*, 5*S*)-*N*-(3-(adamantylmethoxy)propyl)-3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide hydrochloride (**25**) .

***N*-(benzyloxycarbonyl) *N'*-(*n*-butyl) thiourea (**11**).**

10 **[70]** A solution of *n*-butylamine (309 μ L, 3.12 mmol) in CH_2Cl_2 (10 mL) was treated with a solution of Cbz-NCS (5 mL, 0.5M in CH_2Cl_2). The resulting reaction mixture was stirred for 30 min at rt. The reaction mixture was concentrated *in vacuo* and the product was isolated via flash chromatography (SiO_2 , EtOAc/hexane 1:6) to yield **11** (456 mg, 68%) as a white powder. δ_{H} (300 MHz; CDCl_3 ; Me_4Si) 0.93 (3 H, t, CH_3), 1.26 – 1.41 (2 H, m, CH_2), 1.44 – 1.66 (2 H, m, CH_2), 3.62 (2 H, q, NHCH_2), 5.13 (2 H, s, $\text{CH}_2\text{C}_{\text{arom}}$), 7.26 – 7.40 (5 H, m, CH_{arom}), 8.80 (1 H, br s, NH), 9.66 (1 H, br s, NH). δ_{C} (75.5 MHz; CDCl_3 ; Me_4Si) 13.5 (CH_3), 19.8, 30.0 (CH_2), 45.1 (NHCH_2), 67.6 ($\text{CH}_2\text{C}_{\text{arom}}$), 127.5, 127.8, 128.1, 128.4, 128.7, 128.8 (CH_{arom}), 134.4 (C_q), 152.5 (NHC(S)NH), 178.7 (NHC(O)). R_f = 0.55 (EtOAc/hexane 1:5). HRMS Calcd for $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$, 267.1167, found 267.1155.

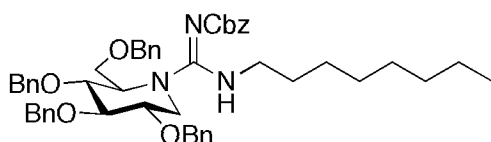
20 ***N*-(benzyloxycarbonyl) *N'*-(*n*-butyl) 2,3,4,6-tetra-*O*-benzyl-deoxynojirimycin (**12**).**

[71] Thiourea **11** (305 mg, 1.14 mmol, 2 eq.) in CH_2Cl_2 (10 mL) was treated with benzyl protected 1-DNJ (**6**) (300 mg, 0.57 mmol, 1 eq.), EDCI (220 mg, 1.14 mmol, 2 eq.) and Et_3N (160 μ L, 1.14 mmol, 2 eq.). The resulting reaction mixture was stirred for 18 h at rt. The reaction mixture was concentrated *in vacuo* and the product was isolated via flash chromatography (SiO_2 , EtOAc/hexane 1:3 $\rightarrow$ 1:2 $\rightarrow$ 1:1 $\rightarrow$ 2:1) to yield **12** (270 mg, 64%) as a colorless oil. δ_{H} (300 MHz; CDCl_3 ; Me_4Si) 0.77 (3 H, t, CH_3 , J = 7.15 Hz), 1.08 – 1.32 (4 H, m, CH_2), 2.93 – 3.04 and 3.06 – 3.14 (2 x 1 H, 2 x m, CH_2NH), 3.43 (1 H, dd, J_A = 3.2 Hz, J_B = 10.4 Hz), 3.59 – 3.79 (6 H, m), 3.98 – 4.03 (1 H, m), 4.40 (2 H, s, $\text{CH}_2\text{C}_{\text{arom}}$), 4.47 and 4.51 (2 x 1 H, 2 x d, $\text{CH}_2\text{C}_{\text{arom}}$, J = 6.85 Hz), 4.61 (2 H, s, $\text{CH}_2\text{C}_{\text{arom}}$), 4.64 and 4.68 (2 x 1 H, 2 x d, $\text{CH}_2\text{C}_{\text{arom}}$), 5.09 and 5.16 (2 x 1 H, 2 x d, $\text{CH}_2\text{C}_{\text{arom}}$, J = 12.65 Hz), 7.20 – 7.42 (25H, m, CH_{arom}). δ_{C} (75.5 MHz; CDCl_3 ; Me_4Si) 13.5 (CH_3), 19.7, 31.5 (CH_2), 43.5, 44.3 (CH_2NH), 66.4, 69.7, 71.1, 72.6, 72.9, 73.2 (CH_2), 58.5, 75.2, 77.6, 80.4 (C-2, C-3, C-4, C-5), 127.2, 127.6, 127.8, 128.0, 128.3 (CH_{arom}), 137.4, 137.7, 137.9 (C_q), 160.8 (NHC(O)), 162.9 (NHC(N)). R_f = 0.59 (EtOAc/hexane 1:1). HRMS Calcd for $\text{C}_{47}\text{H}_{53}\text{N}_3\text{O}_6$ $[\text{M}+\text{H}]^+$, 756.4013, found 756.3996.

N-1-butylguanidine-1-deoxynojirimycin hydrochloride (13):

[72] A suspension of **12** (75 mg, 0.1 mmol) and Pd/C (10%) (75 mg) in EtOH (5 mL) was adjusted to pH = 1 with aqueous HCl (1M). The reaction mixture was stirred vigorously under a H₂ (g) atmosphere for 18 h at rt. The reaction mixture was next filtered over celite, washed with EtOH and concentrated *in vacuo*. **13** was obtained after lyophilization as the HCl-salt (30 mg, quant.) δ_{H} (300 MHz; D₂O) 0.93 (3 H, t, CH₃, *J* = 7.1 Hz), 1.35 – 1.43 (2 H, m, CH₂), 1.60 – 1.64 (2 H, m, CH₂), 3.28 – 3.33 (2 H, m, NCH₂), 3.60 and 3.68 (2 x 2 H, 2 x s), 3.85 – 3.99 (4 H, m). δ_{C} (75.5 MHz; D₂O) 12.7 (CH₃), 30.0, 42.0, 45.2 (CH₂), 59.2 (CH₂), 63.7, 68.0, 70.4, 72.9 (C-2, C-3, C-4, C-5). HRMS Calcd for C₁₁H₂₃N₃O₄ [M+H]⁺, 262.1767, found 262.1771.

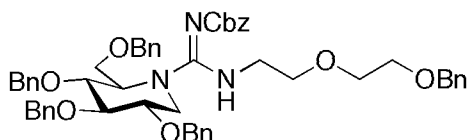
Benzyl ((octylamino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (14)



[73] ¹H NMR (300 MHz, CDCl₃) δ 7.45 – 7.23 (m, 25H), 5.15 (q, 2H), 4.70 – 4.63 (m, 4H), 4.52 (dd, 2H), 4.43 (s, 2H), 4.05 (q, 1H), 3.81 – 3.62 (m, 7H), 3.44 (dd, 1H), 3.17 – 2.97 (m, 2H), 1.28 – 1.17 (m, 14H), 0.90 (t, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 163.3, 161.2, 138.3, 138.2, 138.1, 137.8, 128.7, 128.4, 128.2, 127.6, 81.0, 78.1, 75.7, 73.7, 73.4, 73.0, 71.6, 70.1, 66.8, 59.0, 44.7, 44.3, 32.0, 29.9, 29.4, 29.4, 27.0, 22.9, 14.4. HRMS: calcd. for C₅₁H₆₁N₃O₆: 812.4633, found 812.4637.

((2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide hydrochloride (15)

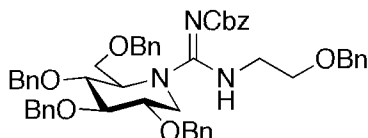
Benzyl (((2-(2-(benzyloxy)ethoxy)ethyl)amino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate(16)



[74] ¹H NMR (300 MHz, CDCl₃) δ 7.43 – 7.18 (m, 30H), 5.14 (q, 2H), 4.62 (t, 4H), 4.50 – 4.47 (m, 4H), 4.41 (s, 2H), 4.11 – 4.06 (m, 1H), 3.78 – 3.71 (m, 4H), 3.66 – 3.59 (m, 3H), 3.55 – 3.23 (m, 10H). ¹³C NMR (75 MHz, CDCl₃) δ 162.9, 160.7, 138.4, 138.3, 138.2, 138.0, 128.6, 128.5, 128.2, 128.0, 80.9, 78.0, 75.4, 73.5, 73.4, 73.3, 72.9, 71.5, 70.4, 69.8, 69.7, 69.6, 66.9, 58.8, 44.6, 43.6. HRMS: calcd. for C₅₄H₅₉N₃O₈: 878.4375, found 878.4413.

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-N-(2-(2-hydroxyethoxy)ethyl)-2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride (17))

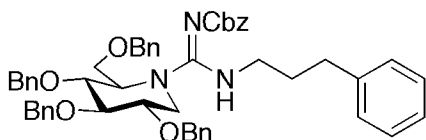
Benzyl (((2-(benzyloxy)ethyl)amino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (18))



[75] ^1H NMR (300 MHz, CDCl_3) δ 7.38 – 7.14 (m, 30H), 5.13 (q, 2H), 4.63 – 4.56 (m, 4H), 4.45 (dd, 2H), 4.36 (d, 4H), 4.05 (q, 1H), 3.78 – 3.56 (m, 6H), 3.44 – 3.28 (m, 5H). ^{13}C NMR (75 MHz, CDCl_3) δ 162.9, 160.8, 138.3, 138.1, 138.1, 137.9, 128.6, 128.4, 128.2, 128.1, 128.0, 80.9, 76.9, 75.5, 73.5, 73.3, 73.0, 72.9, 71.5, 69.8, 68.8, 66.9, 58.9, 44.5, 43.7. HRMS: calcd. for $\text{C}_{52}\text{H}_{55}\text{N}_3\text{O}_7$: 834,4113, found 834,4135.

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-N-(2-hydroxyethyl)-2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride (19)

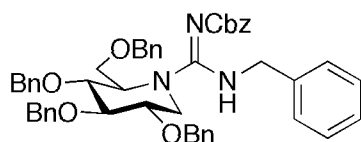
Benzyl (((3-phenylpropyl)amino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (20))



[76] ^1H NMR (300 MHz, CDCl_3) δ 7.55 – 7.08 (m, 30H), 5.18 (q, 2H), 4.59 (q, 4H), 4.47 (d, 2H), 4.43 (d, 2H), 4.34 (d, 2H), 4.28 – 4.25 (m, 2H), 4.05 (q, 1H), 3.77 – 3.59 (m, 6H), 3.45 (dd, 1H). ^{13}C NMR (75 MHz, CDCl_3) δ 162.7, 160.8, 137.9, 137.7, 137.5, 129.0, 128.3, 127.7, 80.1, 75.0, 73.2, 73.0, 72.5, 71.2, 69.5, 66.7, 58.7, 47.8, 44.4. HRMS: calcd. for $\text{C}_{50}\text{H}_{51}\text{N}_3\text{O}_6$: 790,3851, found 790,3839.

(2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-N-(3-phenylpropyl)piperidine-1-carboximidamide hydrochloride (21))

Benzyl ((benzylamino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (22)



5

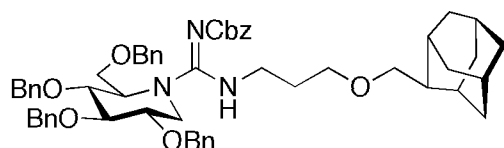
[77] ^1H NMR (300 MHz, CDCl_3) δ 7.45 – 7.01 (m, 30H), 5.12 (q, 2H), 4.63 (q, 4H), 4.48 (dd, 2H), 4.39 (s, 2H), 4.02 (q, 1H), 3.78 – 3.60 (m, 8H), 3.42 (dd, 1H), 3.17 – 2.99 (m, 2H), 2.43 (t, 2H), 1.82 (m, 2H), 1.57 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 163.4, 161.3, 141.6, 138.3, 138.2, 138.1, 137.8, 128.7, 128.5, 128.2, 128.2, 128.0, 127.7, 126.2, 80.6, 78.0, 77.8, 77.4, 77.0, 75.6, 73.7, 73.3, 73.0, 71.6, 70.1, 68.2, 66.9, 59.0, 44.7, 43.8, 33.3, 31.7, 25.9. HRMS: calcd. for $\text{C}_{52}\text{H}_{55}\text{N}_3\text{O}_6$: 818,4164, found 818,4155.

10

(2*R*, 3*R*, 4*R*, 5*S*)-*N*-benzyl-3,4,5-trihydroxy- 2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride (23)

15

Benzyl (((3-((1*R*,2*S*,5*S*)-adamantan-2-ylmethoxy)propyl)amino)((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (24)



[78] ^1H NMR (300 MHz, CDCl_3) δ 7.44 – 7.18 (m, 25H), 5.14 – 5.09 (q, 2H), 4.65 (t, 4H), 4.51 (dd, 2H), 4.41 (s, 2H), 4.06 (q, 1H), 3.80 – 3.62 (m, 6H), 3.48 – 3.41 (m, 1H), 3.30 – 3.15 (m, 4H), 2.87 (s, 2H), 1.93 (s, 3H), 1.72 – 1.53 (m, 10H), 1.49 (d, 6H). ^{13}C NMR (75 MHz, CDCl_3) δ 163.0, 161.0, 138.3, 138.2, 138.2, 138.0, 128.6, 128.1, 82.4, 81.3, 78.2, 75.6, 73.6, 73.4, 73.1, 71.5, 69.8, 69.6, 66.9, 58.8, 44.9, 41.9, 39.9, 37.4, 34.3, 29.9, 28.5. HRMS: calcd. for $\text{C}_{57}\text{H}_{67}\text{N}_3\text{O}_7$: 906,5052, found 906,5039.

25

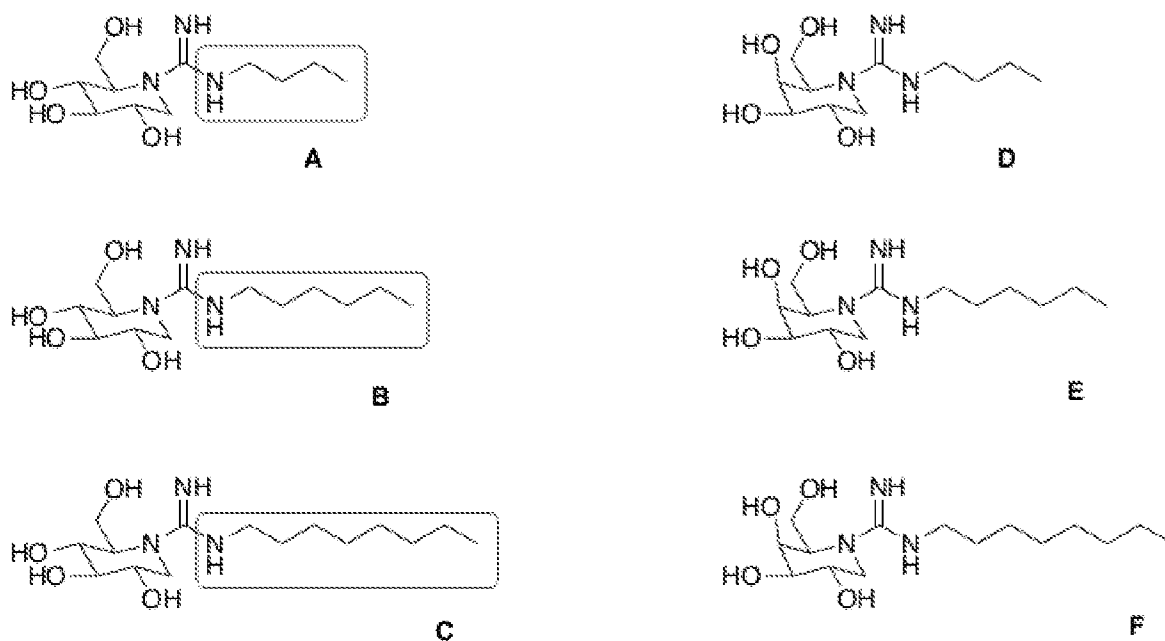
(2*R*, 3*R*, 4*R*, 5*S*)-*N*-(3-(adamantylmethoxy)propyl)-3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide hydrochloride (25)

30

Novel guanidinium derivatives of 1-deoxynojirimycin were prepared using a concise synthetic protocol and will be tested against a panel of glycosidases for their inhibitory properties.

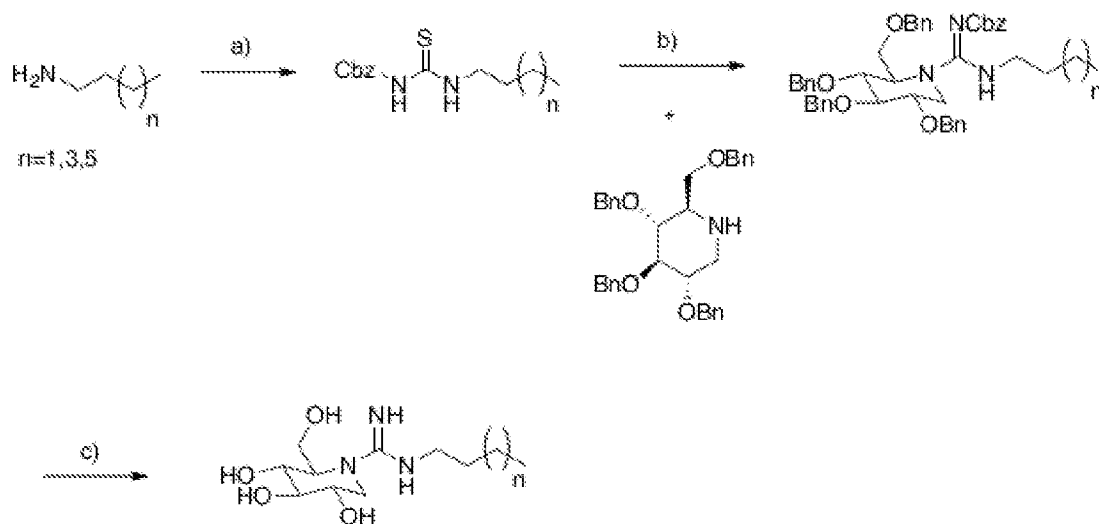
35

Exploring the side chain variations will enlighten how the length of the alkyl group attached to the guanidinium moiety impacts the potency of these inhibitors when evaluated against a panel of glycosidase enzymes. Furthermore, gluco- and galacto- iminosugars were chosen to compare inhibitory differences due to stereochemistry properties.



Procedure and Results:

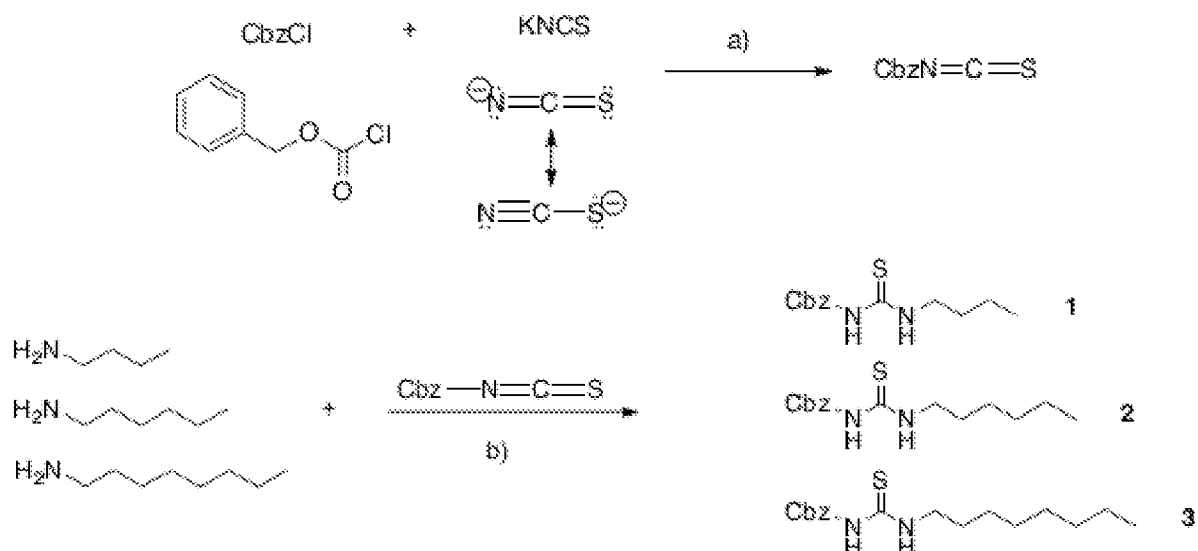
Glucose Unit



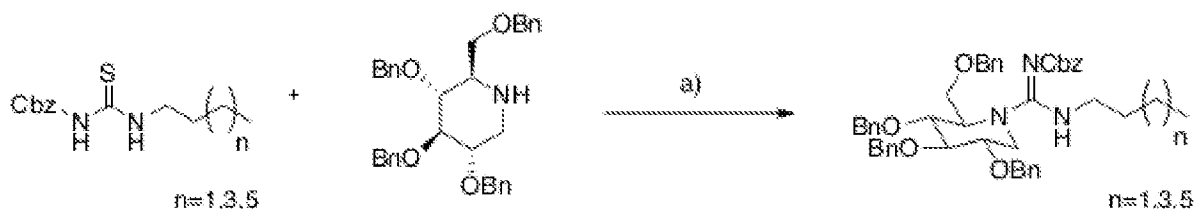
	n	compound
butyl-	1	7
hexyl-	3	8
octyl-	5	9

- 5 Scheme 1. Overview scheme for synthesis of 7, 8 and 9: a) CbzNCS, DCM, RT, 15min; b) EDCI, Et₃N, DCM, RT, over night; c) H₂, Pd/C (10%), MeOH:AcOH = 1:1. The synthesis of the guanidinium analogues x-x started with the preparation of thioureas 1-3 from n-alkyl

amine and Cbz-NCS. Reaction with benzyl chloroformate, dry potassium thiocyanate in DCM and in presence of phase-transfer agent gave 1-3 in 86-92 % yield.

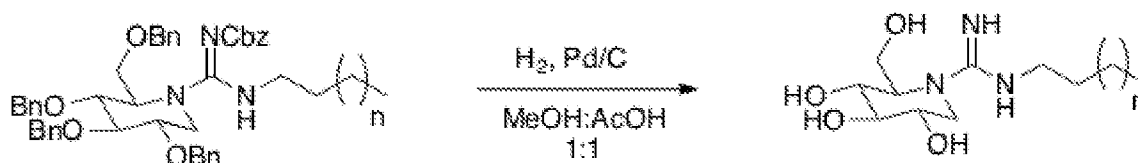


Scheme 2. Synthesis of thiourea analogues 1, 2 and 3: a) Tetrachloroethylene (C_2Cl_4), 18-crown-6 (5%), reflux, over night reaction; b) CbzNCS, DCM, RT, 15min. After reacting with EDCI, NEt_3 , and commercially available DNJ, the benzylated guanidinium compounds 4-6 were obtained.



	n	w (%)	nr.
butyl-	1	85	4
hexyl-	3	51	5
octyl-	5	84	6

Scheme 3. Thioureas 1 (n=1), 2 (n=3) and 3 (n=5) coupled with 1-deoxynojirimycin. a) EDCI, NEt_3 , DCM; room temperature, over night reaction. Afterwards, the featured benzyl groups of 4, 5 and 6 were deprotected with hydrogenation using palladium on activated charcoal (10%) in a 1:1 mixture of methanol and acetic acid. Various deprotection approaches were tried, however presence of acetic acid was preferred in this case



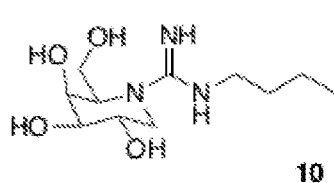
	n	w (%)	nr.
butyl-	1	45	7
hexyl-	3	52	8
octyl-	5	53	9

Scheme 4. Deprotection of gluco-derivatives 4 (n=1), 5 (n=3) and 6 (n=5) with palladium on activated charcoal (10%); room temperature, 3 h reaction. Final products 7-9 were obtained after purification by prep-HPLC in relatively good yields.

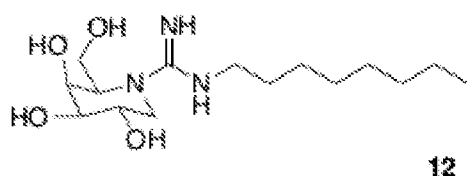
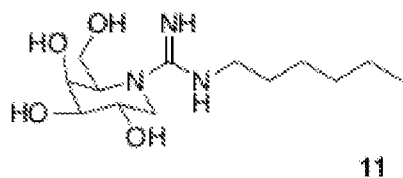
5

Galactose Unit

Same approach was used to prepare galacto- compounds 10, 11 and 12.



	n	w (%)	nr.
butyl-	1	83	10
hexyl-	3	85	11
octyl-	5	86	12



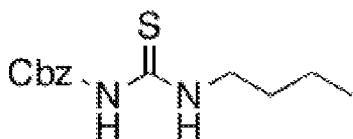
- 10 **10:** (2*R*, 3*S*, 4*R*, 5*S*)-*N*-butyl -3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide;
11: (2*R*, 3*S*, 4*R*, 5*S*)-*N*-hexyl -3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide;
12: (2*R*, 3*S*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide;

15

Scheme 5. Galacto- inhibitors 10-12 to investigate and evaluate stereochemistry Properties.

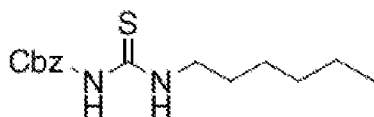
5 **Analytical data**

N-(Benzyloxycarbonyl)-N'-(butyl) thiourea



dH (300 MHz; CDCl₃; Me₄Si) 0.93 (t, CH₃, 3H), 1.26–1.41 (m, CH₂, 2H), 1.44–1.66 (m, CH₂, 2H), 3.62 (q, NHCH₂, 2H), 5.13 (s, CH₂Carom, 2H), 7.26–7.40 (m, CH₂arom, 5H), 8.80(brs,NH, 1H), 9.66(brs,NH,1H). dC (75.5 MHz; CDCl₃; Me₄Si) 13.5 (CH₃), 19.8, 30.0 (CH₂), 45.1 (NHCH₂), 67.6 (CH₂Carom), 127.5, 127.8, 128.1, 128.4, 128.7, 128.8 (CH₂arom), 134.4 (Cq), 152.5 (NHC(S)NH), 178.7 (NHC(O)). HRMS calcd for C₁₃H₁₈N₂O₂S [M + H]⁺, 267.1167, found 267.1155.

15 **N-(Benzyloxycarbonyl)-N'-(hexyl) thiourea**



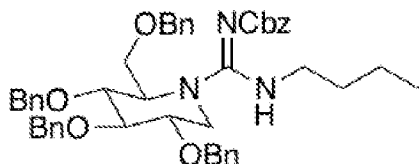
dH (300 MHz; CDCl₃; Me₄Si) 0.895 (t, CH₃, 3H), 1.20–1.41 (m, 3xCH₂, 6H), 1.60–1.79 (m, CH₂, 2H), 3.62 (q, NHCH₂, 2H), 5.17 (s, CH₂Carom, 2H), 7.26–7.40 (m, CH₂arom, 5H), 7.97 (brs,NH, 1H), 9.60 (brs, NH, 1H).

N-(Benzyloxycarbonyl)-N'-(octyl) thiourea



dH (300 MHz; CDCl₃; Me₄Si) 0.88 (t, CH₃, 3H), 1.23–1.41 (m, 6xCH₂, 12H), 1.56–1.70 (m, CH₂, 2H), 3.62 (q, NHCH₂, 2H), 5.18 (s, CH₂Carom, 2H), 7.33–7.38 (m, CH₂arom, 5H), 7.96 (brs, NH, 1H), 9.60 (brs, NH, 1H).

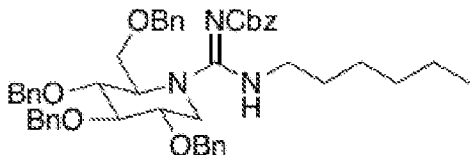
N-(Benzyloxycarbonyl)-N'-(butyl) 2,3,4,6-tetra-O-benzyl-deoxynojirimycin



dH (300 MHz; CDCl₃; Me₄Si) 0.77 (t, CH₃, J = 7.15 Hz, 3H), 1.08–1.32 (m, CH₂, 4H),
 2.93–3.04 and 3.06–3.14 (2xm, CH₂NH, 2x1H), 3.43(dd, J_A=3.2Hz, J_B=10.4Hz, 1H),
 5 3.59–3.79 (m, 6H), 3.98–4.03 (m, 1H), 4.40 (s, CH₂Carom, 2H), 4.47 and 4.51 (2=d,
 CH₂Carom, J=6.85 Hz, 2x1H), 4.61 (s, CH₂Carom, 2H), 4.64 and 4.68 (2xd, CH₂Carom,
 2=1H), 5.09 and 5.16 (2=d, CH₂Carom, J=12.65 Hz, 2x1H), 7.20–7.42 (m, CH_{arom}, 25H).
 dC (75.5 MHz; CDCl₃; Me₄Si) 13.5 (CH₃), 19.7, 31.5 (CH₂), 43.5, 44.3 (CH₂NH),
 66.4, 69.7, 71.1, 72.6, 72.9, 73.2 (CH₂), 58.5, 75.2, 77.6, 80.4 (C-2, C-3, C-4, C-5),
 10 127.2, 127.6, 127.8, 128.0, 128.3 (CH_{arom}), 137.4, 137.7, 137.9 (C_q), 160.8 (NHC(O)),
 162.9 (NHC(N)). HRMS calcd for C₄₇H₅₃N₃O₆ [M + H]⁺, 756.4013, found 756.3996.

The compound is deprotected using the herein described method and tested in an enzymatic
 assay as described herein elsewhere. The activity of the compound against various
 15 galactosidases is noted.

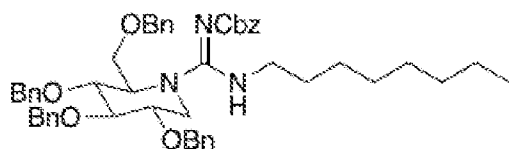
N-(Benzyloxycarbonyl)-N'-(hexyl) 2,3,4,6-tetra-O-benzyl-deoxynojirimycin



dH (300 MHz; CDCl₃; Me₄Si) 0.84 (t, CH₃, J=9.0 Hz, 3H), 1.10–1.28 (m, CH₂, 8H),
 20 2.92–3.10 (m, CH₂NH, 2H), 3.43 (dd, J_A=3.0Hz, J_B=12.0Hz, 1H), 3.62–3.78 (m, 6H),
 3.98–4.03 (m, 1H), 4.40 (s, CH₂Carom, 2H), 4.46–4.52 (2xd, CH₂Carom, J=6.76 Hz,
 2x1H), 4.61 (s, CH₂Carom, 2H), 4.62–4.67 (2xd, CH₂Carom, 2x1H), 5.07–5.18 (2xd,
 CH₂Carom, J=12 Hz, 2x1H), 7.18–7.44 (m, CH_{arom}, 25H)

25 The compound is deprotected using the herein described method and tested in an enzymatic
 assay as described herein elsewhere. The activity of the compound against various
 galactosidases is noted.

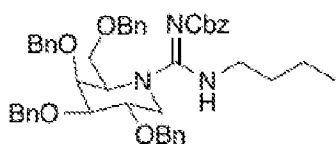
N-(Benzyloxycarbonyl)-N'-(octyl) 2,3,4,6-tetra-O-benzyl-deoxynojirimycin



dH (300 MHz; CDCl₃; Me₄Si) 0.85 (t, CH₃, J=6.0 Hz, 3H), 1.13–1.28 (m, CH₂, 12H), 2.92–3.12 (m, CH₂NH, 2H), 3.45 (dd, J_A=3.0Hz, J_B=12.0Hz, 1H), 3.61–3.76 (m, 6H), 3.98–4.01 (m, 1H), 4.40 (s, CH₂Carom, 2H), 4.46–4.52 (2xd, CH₂Carom, J=6.76 Hz, 2x1H), 4.60 (s, CH₂Carom, 2H), 4.61–4.68 (2xd, CH₂Carom, 2x1H), 5.07–5.18 (2xd, CH₂Carom, J=12 Hz, 2x1H), 7.20–7.42 (m, CH_{arom}, 25H).

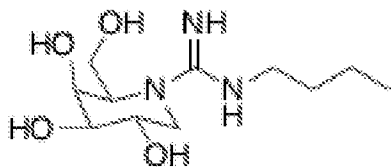
The compound is deprotected using the herein described method and tested in an enzymatic assay as described herein elsewhere. The activity of the compound against various galactosidases is noted.

Benzyl-(Z)-(butylamino)((2R,3S,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)-methyl)piperidin-1-yl)methylene)carbamate



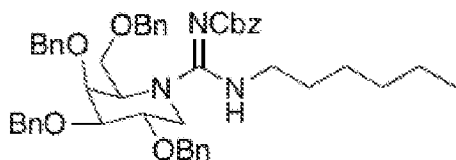
dH (300 MHz; CDCl₃; Me₄Si) 0.77–0.82 (t, CH₃, J=6.0 Hz, 3H), 1.11–1.35 (m, CH₂, 4H), 2.92–3.05 (m, CH₂NH, 2H), 3.15–3.21 (dd, J_A=1.85 Hz, J_B=14.63Hz, 1H), 3.56 (t, 1H), 3.79 (t, 1H), 3.89–4.21 (m, 4H), 4.35 (m, 1H), 4.42–4.74 (2xd, CH₂Carom, 8H), 5.14 (s, CH₂Carom, 2H), 7.18–7.44 (m, CH_{arom}, 25H)

The compound is deprotected using the herein described method to yield



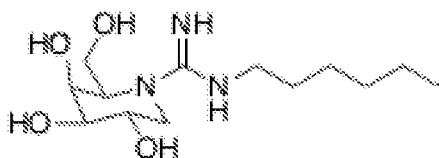
and tested in an enzymatic assay as described herein elsewhere. The activity of the compound against various galactosidases is noted.

Benzyl-(Z)-(hexylamino)((2R,3S,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)-methyl)piperidin-1-yl)methylene)carbamate



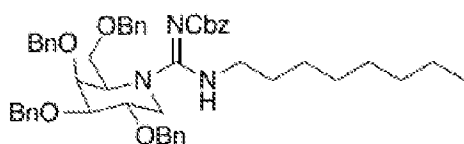
dH (300 MHz; CDCl₃; Me₄Si) 0.82-0.87 (t, CH₃, J=6.78 Hz, 3H), 1.14–1.34 (m, CH₂, 8H), 2.89-3.04 (m, CH₂NH, 2H), 3.15-3.20 (dd, J_A=1.91 Hz, J_B=14.78Hz, 1H), 3.54 (t, 1H), 3.79 (t, 1H), 3.89–4.05 (m, 4H), 4.17-4.22 (m, 1H), 4.32-4.74 (CH₂Carom, 8H), 5.12 (s, CH₂Carom, 2H), 7.18–7.44 (m, CH_{arom}, 25H).

The compound is deprotected using the herein described method to yield



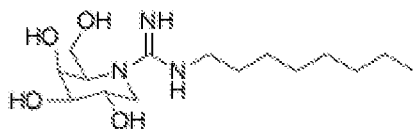
and tested in an enzymatic assay as described herein elsewhere. The activity of the compound against various galactosidases is noted.

Benzyl-(Z)-(octylamino)((2R,3S,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)-methyl)piperidin-1-yl)methylene)carbamate

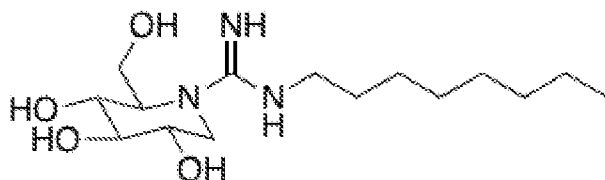


dH (300 MHz; CDCl₃; Me₄Si) 0.85-0.89 (t, CH₃, J=6.74 Hz, 3H), 1.15–1.32 (m, CH₂, 12H), 2.93-3.04 (m, CH₂NH, 2H), 3.15-3.20 (dd, J_A=1.93 Hz, J_B=14.82Hz, 1H), 3.55 (t, 1H), 3.77-3.79 (t, 1H), 3.89–4.05 (m, 4H), 4.11-4.22 (m, 1H), 4.35-4.74 (CH₂Carom, 8H), 5.14 (s, CH₂Carom, 2H), 7.18–7.44 (m, CH_{arom}, 25H).

The compound is deprotected using the herein described method to yield



and tested in an enzymatic assay as described herein elsewhere. The activity of the compound against various galactosidases is noted.

N-1-Octylguanidine-1-deoxynojirimycine

dH (500 MHz; D₂O) 0.74–0.86 (t, 3H), 1.16–1.34 (m, 12H), 1.50–1.62 (m, 2H), 3.17–
5 3.26 (m, 2H), 3.50–3.55 (m, 2H), 3.60 (d, 2H), 3.76–3.82 (m, 2H), 3.82–3.90 (m, 2H).
dHSCQ (500 MHz; D₂O) 28.0, 40.3, 45.6, 36.6, 42.5, 42.8, 56.8, 59.8, 82.6, 87.5, 73.8,
78.3, 85.0. LRMS calcd for C₁₅H₃₂N₃O₄ [M + H]⁺, 318.24, found 31

General Procedure for Enzyme Inhibition Assays

10 **[79]** Glycosidases used in the inhibition studies were purchased from Sigma; α -
galactosidase (from *green coffee beans*; G8507), β -galactosidase (from *bovine liver*; G1875),
 α -glucosidase (from *bakers yeast*; G5003), β -glucosidase (from *almonds*; G4511), α -
mannosidase (from *Jack beans*; M7257), β -mannosidase (from *Helix pomatia*; M9400) and
Naringinase (from *Penicillium decumbens*; N1385). The corresponding *p*-nitrophenyl
15 glycosides substrates were purchased from Carbosynth Limited.

[80] Inhibition assays were performed in either phosphate or acetate buffer at the optimum
pH for each enzyme. Determination of the IC₅₀ values of the iminosugars were carried out by
spectrophotometrically measuring the residual hydrolytic activities of the glycosidases on the
corresponding *p*-nitrophenyl glycosides substrate in the presence of a concentration range of
20 iminosugar derivatives. The incubation mixture consisted of 15 μ L of inhibitor solution in water
(0.1 U/mL) and 15 μ L of enzyme solution. The concentrations of the enzyme were adjusted
so that the reading for the final absorbance was in the range of 0.5 – 1.5 units.

[81] Inhibitor and enzyme solutions were mixed in a disposable 96-well microtiter plate and
then incubated at room temperature for 5 minutes. Next the reactions were initiated by
25 addition of 75 μ L of a solution of the corresponding *p*-nitrophenyl glycosides substrate
solution in the appropriate buffer at the optimum pH for the enzyme. After the reaction mixture
was incubated at 37 °C for 30 min, the reaction was quenched with 0.5M Na₂CO₃ (240 μ L)
and the absorbance of 4-nitrophenol released from the substrate was read immediately at
405 nm using a BioTek μ Quant Microplate Spectrophotometer.

30 **[82]** IC₅₀ values were determined as a concentration of the iminosugars that inhibits 50% of
the enzyme activity under the assay conditions. IC₅₀ values were determined graphically with
GraphPad Prism (version 5.0) by making a plot of percentage inhibition versus the log of
inhibitor concentration, using at least 8 different inhibitor concentrations (triplicate).

[83] The results for various enzymes and iminosugars are presented in the Figures 1-7 and in tables below.

8 : *N*-Hexyl-1-deoxynojirimycin

10 : *N*-1-butylurea-1-deoxynojirimycin

5 **13** : (2*R*, 3*R*, 4*R*, 5*S*)-*N*-butyl-3,4,5-trihydroxy-2-(hydroxymethyl)piperidine-1-carboximidamide hydrochloride salt;

15: (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide hydrochloride

10 **17**: (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-(2-hydroxyethoxy)ethyl) 2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride;

19: (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-hydroxyethyl)-2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride;

23: (2*R*, 3*R*, 4*R*, 5*S*)-*N*-benzyl-3,4,5-trihydroxy- 2-(hydroxymethyl)-piperidine-1-carboximidamide hydrochloride;

15 **21**: (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-(3-phenylpropyl)piperidine-1-carboximidamide hydrochloride;

25: (2*R*, 3*R*, 4*R*, 5*S*)-*N*-(3-(adamantylmethoxy)propyl)-3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide hydrochloride.

Enzyme	15	17	19	23	21	25
Alpha glucosidase (Bakers yeast G5003)	14	506	256	>1000	>1000	>1000
Beta glucosidase (Almonds G4511)	25	>1000	>1000	43	72	138
Alpha galactosidase (green coffee beans; G8507)	23	>1000	149	>1000	116	>1000
Beta galactosidase (Bovine liver G1875)	3	>1000	215	5	6	6
Alpha- mannosidase (Jack Beans M7257)	80	>1000	>1000	>1000	>1000	>1000
Naringinase (<i>Penicillium decimbens</i> N1385)	>1000	12	21	27	43	>1000

5

Enzyme	DNJ	8	10	13
Alpha-glucosidase (Bakers yeast G5003)	167 ± 27	134 ± 19	>1000	20 ± 10
Beta-glucosidase (Almonds G4511)	312 ± 62	59 ± 2	>1000	41 ± 5
Alpha-galactosidase (green coffee beans; G8507)	23 ± 3	251 ± 38	>1000	18 ± 8
Beta-galactosidase (Bovine liver G1875)	>1000	>1000	27 ± 2	15 ± 1
Alpha-mannosidase (Jack Beans M7257)	>1000	>1000	>1000	113 ± 11
Beta-mannosidase (<i>Helix pomatia</i> M9400)	>1000	>1000	>1000	408 ± 45
Naringinase (<i>Penicillium decimbens</i> N1385)	33 ± 4	6 ± 3	>1000	>1000

The other guanidine derivatives of gluco and galacto-based iminosugar compounds described herein are tested in the enzymatic assay under the same protocol and the effect on the activity of the enzymes is observed.

10

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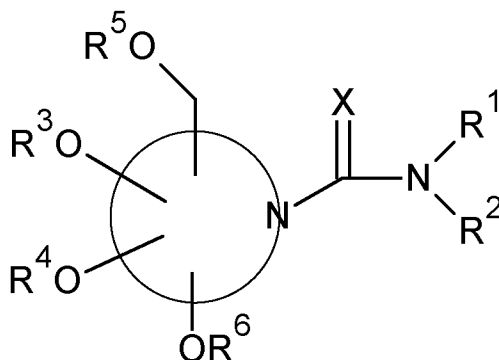
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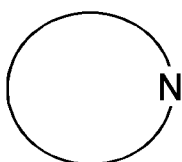
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CLAIMS

1. A compound represented by the formula



- 5 wherein



is a 5, 6, 7 or 8-membered nitrogen-containing cyclic compound;

- 10 wherein X is O or NR⁷;

wherein R¹ is H or a straight or branched alkyl, cycloalkyl, alkenyl, alkynyl, alkoxyalkyl or aminoalkyl containing 1-20 carbon atoms, an aryl, alkylaryl, heteroaryl, heteroalkyl, alkoxy(het)aryl containing 5-12 ring atoms, wherein R¹ is optionally substituted with one or more -OH, -COOH, -Cl, -F, -CF₃, -OCF₃, -O-C(=O)N-(alkyl)₂.

- 15 wherein R² is H or a straight or branched alkyl, cycloalkyl, alkenyl, alkynyl, alkoxyalkyl or aminoalkyl containing 1-12 carbon atoms, an aryl, alkylaryl, heteroaryl, heteroalkyl, alkoxy(het)aryl containing 5-12 ring atoms, wherein R¹ is optionally substituted with one or more -OH, -COOH, -Cl, -F, -CF₃, -OCF₃, -O-C(=O)N-(alkyl)₂.

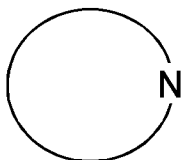
wherein R³, R⁴, R⁵, R⁶ are independently H or a hydroxyl protecting group;

- 20 wherein R⁷ is H, C1-C6 alkyl;

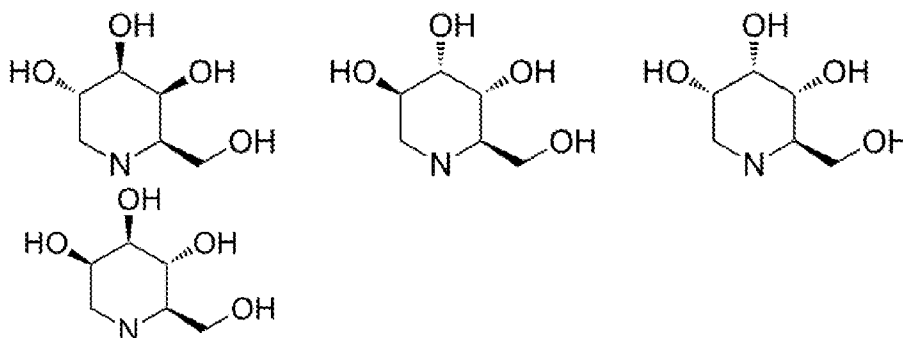
and pharmaceutically acceptable salts, tautomers, enantiomers stereoisomers and/or esters thereof.

2. A compound according to claim 1, wherein R¹ and /or R² is, independently, H or a
25 straight or branched alkyl or cycloalkyl, containing 1-20 carbon atoms, preferably H or a straight alkyl containing 1-12 carbon atoms, more preferably H.

3. A compound according to claim 1, wherein

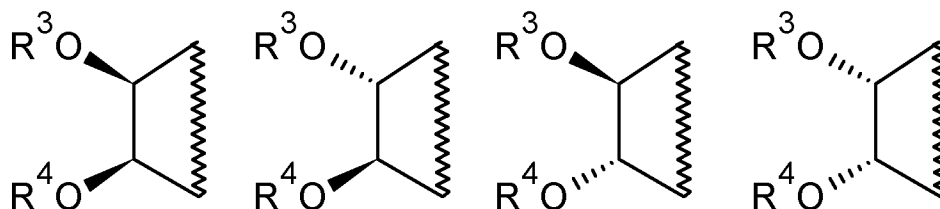


is a piperidine-based iminocyclitols such as 1-deoxymannonojiromycin, 1-deoxyaltronojiromycin, 1-deoxygalactonojirimycin and 1-deoxyallonojiromycin



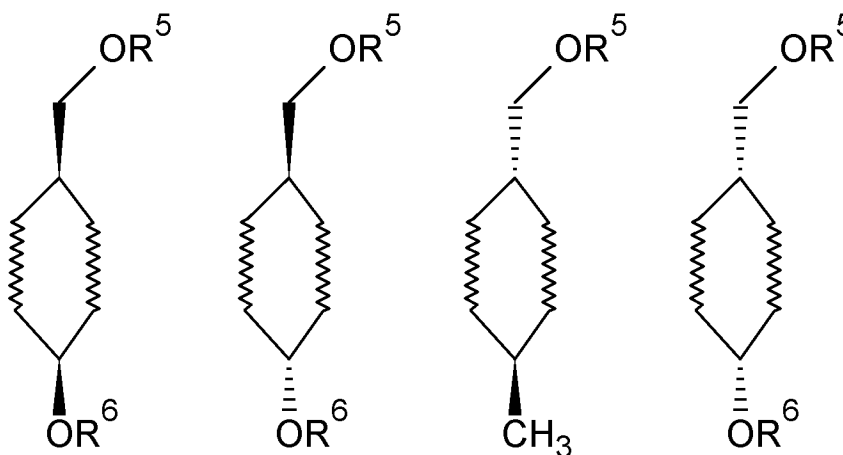
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4. A compound according to claims 1-3, wherein the (substituted) hydroxyl groups on the nitrogen-containing cyclic compound have an orientation selected from the group consisting of



10

5. A compound according to claims 1-4, wherein the R^5 and R^6 substituted hydroxyl groups on the nitrogen-containing cyclic compound have an orientations selected from the group consisting of



15

6. A compound according to claims 1-5, wherein the hydroxyl protecting group is selected from the group consisting of Acetyl (Ac), Benzoyl (Bz), Benzyl (Bn), β -Methoxyethoxymethyl ether (MEM), Dimethoxytrityl, [bis-(4-methoxyphenyl)phenylmethyl] (DMT), Methoxymethyl ether (MOM), Methoxytrityl [(4-methoxyphenyl)diphenylmethyl, MMT), p-Methoxybenzyl ether (PMB), Methylthiomethyl

20

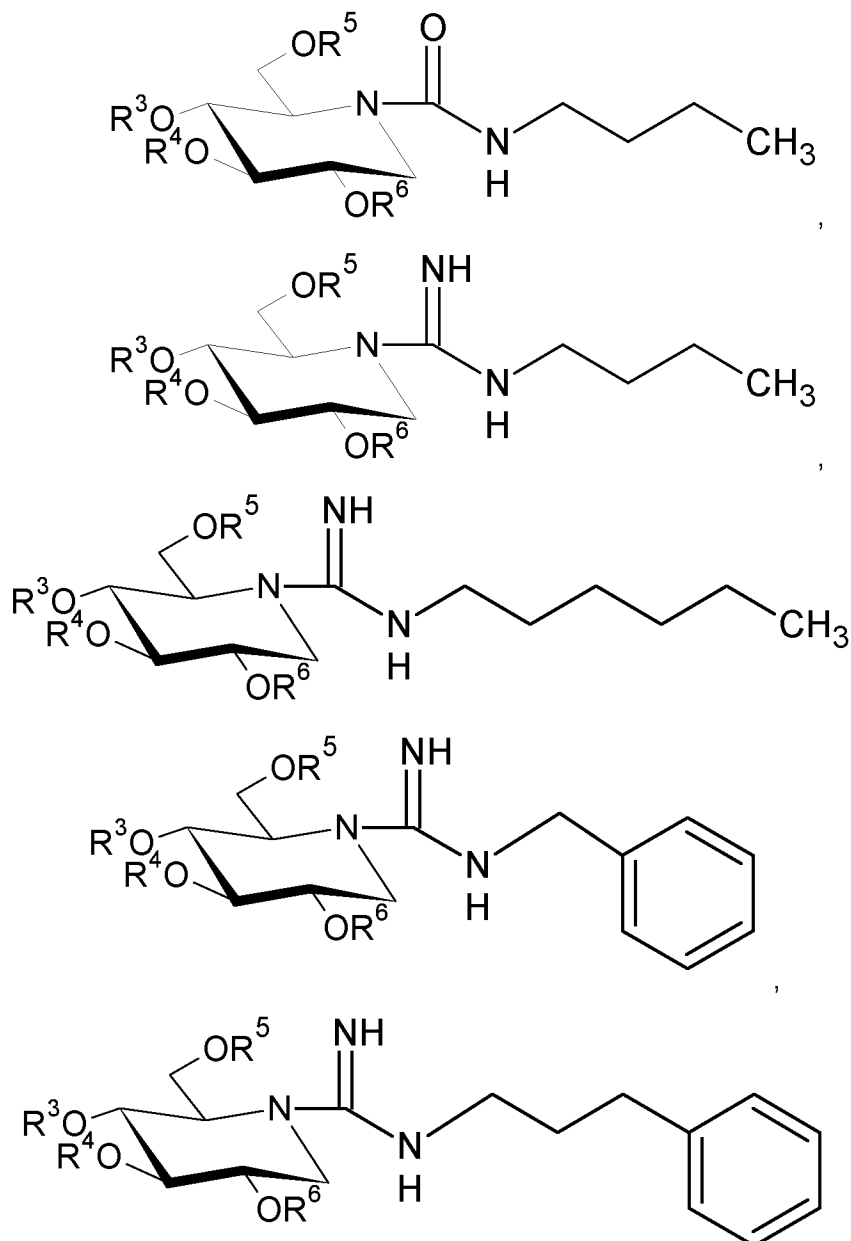
ether, Pivaloyl (Piv), Tetrahydropyranyl (THP), Trityl (Tr), Silyl ether (most popular ones include trimethylsilyl (TMS), tert-butyldimethylsilyl (TBDMS), tri-iso-propylsilyloxymethyl (TOM), and triisopropylsilyl (TIPS) ethers), Methyl Ethers, Ethoxyethyl ethers (EE), preferably a benzyl (Bn) -protecting group.

5

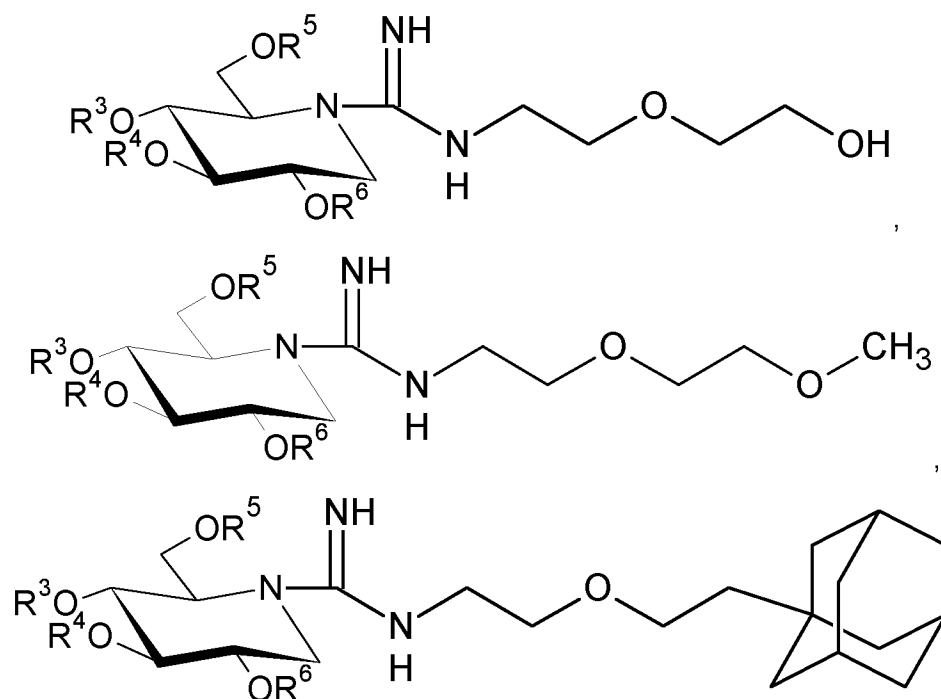
7. A compound according to claim 1-6, wherein X= O.

8. A compound according to claim 1-6, wherein X= NR⁷

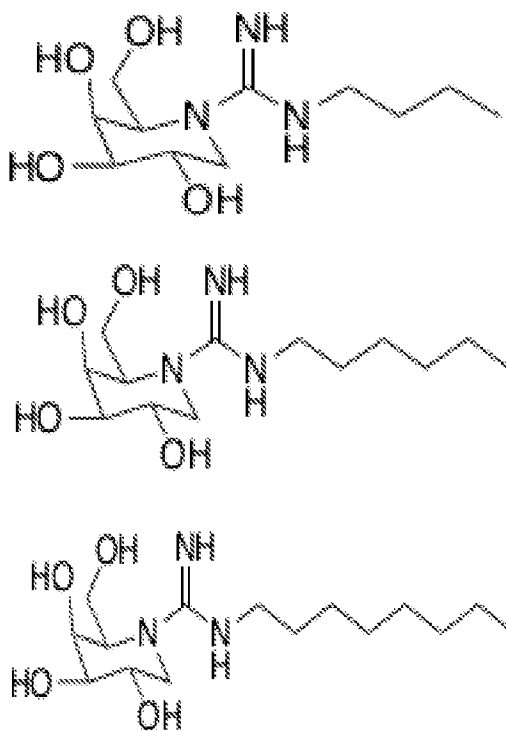
10 9. A compound according to claims 1-8, having the structure



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10. A compound according to claims 1-9, wherein the compound is selected from the group consisting of
N-1-butylurea-1-deoxynojirimycin;

- (2*R*, 3*R*, 4*R*, 5*S*)-*N*-butyl-3,4,5-trihydroxy-2-(hydroxymethyl)piperidine-1-carboximidamide;
- (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide;
- 5 (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-hydroxyethyl)-2-(hydroxymethyl)-piperidine-1-carboximidamide;
- (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-(2-hydroxyethoxy)ethyl) 2-(hydroxymethyl)-piperidine-1-carboximidamide;
- (2*R*, 3*R*, 4*R*, 5*S*)-*N*-benzyl-3,4,5-trihydroxy- 2-(hydroxymethyl)-piperidine-1-
- 10 carboximidamide;
- (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-(3-phenylpropyl)piperidine-1-carboximidamide;
- (2*R*, 3*R*, 4*R*, 5*S*)-*N*-(3-(adamantylmethoxy)propyl)-3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide;
- 15 (2*R*, 3*S*, 4*R*, 5*S*)-*N*-butyl -3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide;
- (2*R*, 3*S*, 4*R*, 5*S*)-*N*-hexyl -3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide;
- (2*R*, 3*S*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-octylpiperidine-1-
- 20 carboximidamide;
11. A compound according to claims 1-10, which is the hydrochloride salt of
- (2*R*, 3*R*, 4*R*, 5*S*)-*N*-butyl-3,4,5-trihydroxy-2-(hydroxymethyl)piperidine-1-
- carboximidamide;
- 25 (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide;
- (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-hydroxyethyl)-2-(hydroxymethyl)-piperidine-1-carboximidamide;
- (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy-*N*-(2-(2-hydroxyethoxy)ethyl) 2-(hydroxymethyl)-
- 30 piperidine-1-carboximidamide;
- (2*R*, 3*R*, 4*R*, 5*S*)-*N*-benzyl-3,4,5-trihydroxy- 2-(hydroxymethyl)-piperidine-1-carboximidamide;
- (2*R*, 3*R*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-(3-phenylpropyl)piperidine-1-carboximidamide;
- 35 (2*R*, 3*R*, 4*R*, 5*S*)-*N*-(3-(adamantylmethoxy)propyl)-3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide;

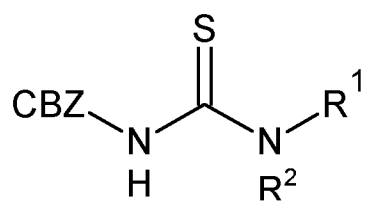
(2*R*, 3*S*, 4*R*, 5*S*)-*N*-butyl -3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide;

(2*R*, 3*S*, 4*R*, 5*S*)-*N*-hexyl -3,4,5-trihydroxy- 2-(hydroxymethyl)piperidine-1-carboximidamide;

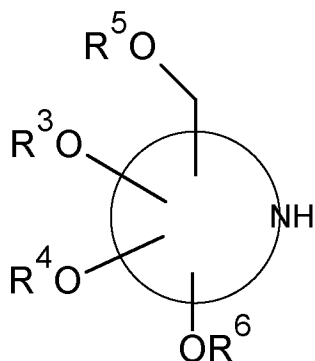
5 (2*R*, 3*S*, 4*R*, 5*S*)-3,4,5-trihydroxy- 2-(hydroxymethyl)-*N*-octylpiperidine-1-carboximidamide.

12. A compound according to claim 1, wherein the pharmaceutical acceptable salt is selected from the group consisting of 1-hydroxy-2-naphthoic acid, 2,2-dichloroacetic acid, 2-hydroxyethanesulfonic acid, 2-oxoglutaric acid, 4-acetamidobenzoic acid, 4-aminosalicylic acid, acetic acid, adipic acid, ascorbic acid (L), aspartic acid (L), benzenesulfonic acid, benzoic acid, camphoric acid (+), camphor-10-sulfonic acid (+), capric acid (decanoic acid), caproic acid (hexanoic acid), caprylic acid (octanoic acid), carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid (D), gluconic acid (D), glucuronic acid (D), glutamic acid, glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, isobutyric acid, lactic acid (DL), lactobionic acid, lauric acid, maleic acid, malic acid (- L), malonic acid, mandelic acid (DL), methanesulfonic acid , naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, nicotinic acid, nitric acid, oleic acid, oxalic acid, palmitic acid, palmoic acid, phosphoric acid, propionic acid, pyroglutamic acid (- L), salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, tartaric acid (+ L), thiocyanic acid, toluenesulfonic acid (p), undecylenic acid, preferably the hydrochloride or hydrobromide.

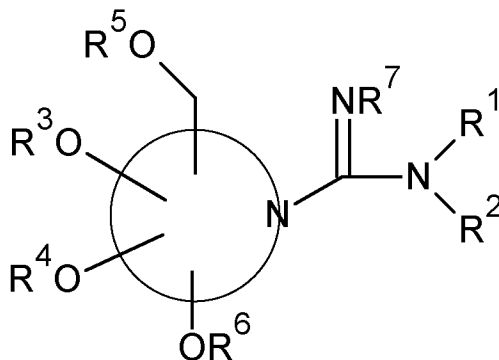
13. Method for the synthesis of compounds as defined in claim 1-12, wherein $X=NR^7$, comprising the steps of
- (a) reacting an amine with a carboxybenzylthiocyanate to provide a corresponding thiourea having the structure



- (b) reacting a 5, 6, 7 or 8-membered nitrogen-containing cyclic compound of the general formula:



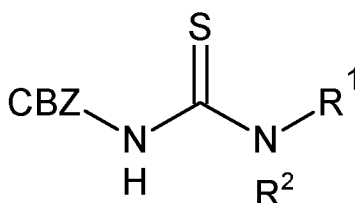
with the thiourea of step (a) followed by hydrogenolysis of the product of to yield



wherein R^1 , R^2 , R^3 , R^4 , R^5 , R^6 and R^7 are as defined in claims 1-8.

5

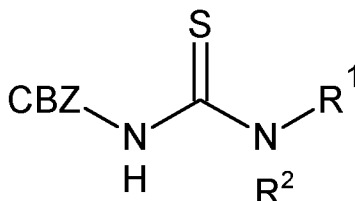
14. Method according claim 13, wherein the amine is a primary amine, R^1NH_2 .
15. Method according claim 13 or 14, wherein the hydrogenolysis is in the presence of a catalyst.
16. Method for the synthesis of a thiourea of the general formula



by reacting a carboxybenzylthiocyanate with an amine R^1R^2NH , preferably a primary amine R^1NH_2 , and wherein R^1 and R^2 are as defined in claims 1-3.

15

17. A compound of the general formula



wherein Cbz is carboxymethyl and R^1 and R^2 is as defined in claims 1-6.

18. Method for modifying (increasing, reducing) glycosidase activity comprising contacting the glycosides with an effective amount of a compound according to claims 1-12.
- 5 19. Method according to claim 18, wherein the glycosidase is selected from the group consisting of alpha-galactosidase, beta-galactosidase, alpha-glucosidase, beta glucosidase, alpha-mannosidase, beta-mannosidase, naringinase.
- 10 20. Method according to claim 18-19, wherein the glycosidase is a purified or recombinant functional glycosidase enzyme.
21. Method according to claim 18-19, wherein the contacting is *in vitro* or *in vivo*.
- 15 22. Method for the treatment of Pompe's disease, a glycolipid storage disease such as Gaucher's disease, Tay Sachs disease, Sandhoff disease, Fabry disease, GM1 gangliosidosis, and fucosidosis, lysosomal storage disorders, diabetes, viral infections, HIV, Human hepatitis C (HCV), dengue virus, tuberculosis, Niemann-Pick diseases and cancer comprising administering to an individual in need thereof an effective amount of a compound according to any of the claims 1-12.
- 20 23. Medicament comprising a compound according to any of the claims 1-12
24. Pharmaceutical composition comprising a compound to any of the claims 1-12 and a pharmaceutically acceptable carrier.

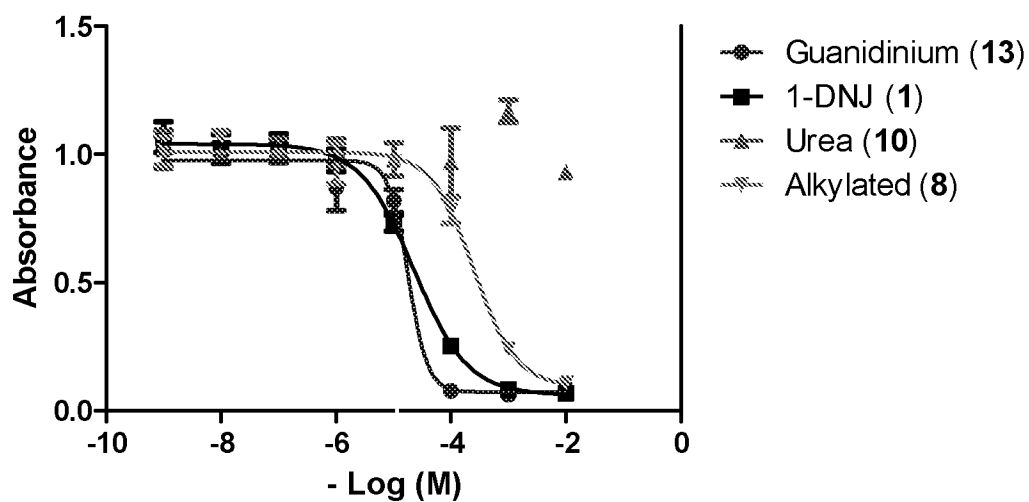


Figure 1.

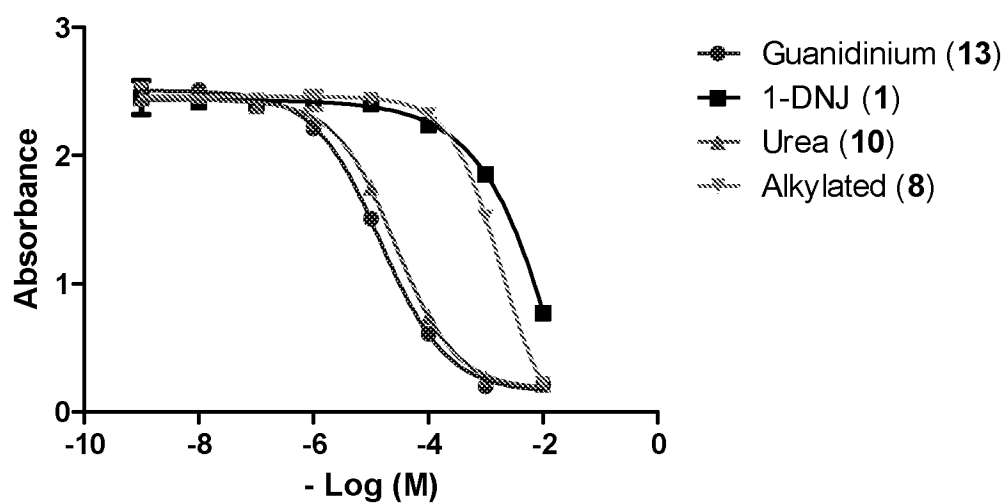


Figure 2.

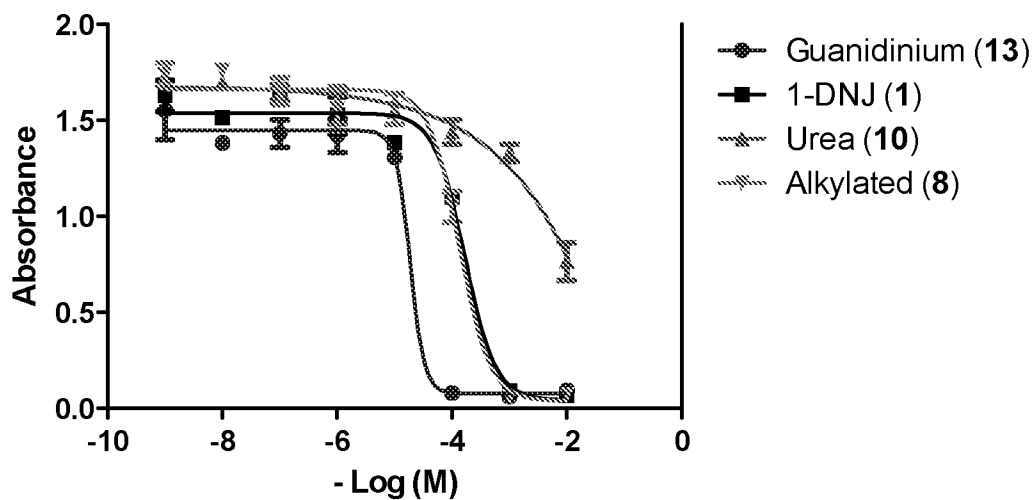


Figure 3.

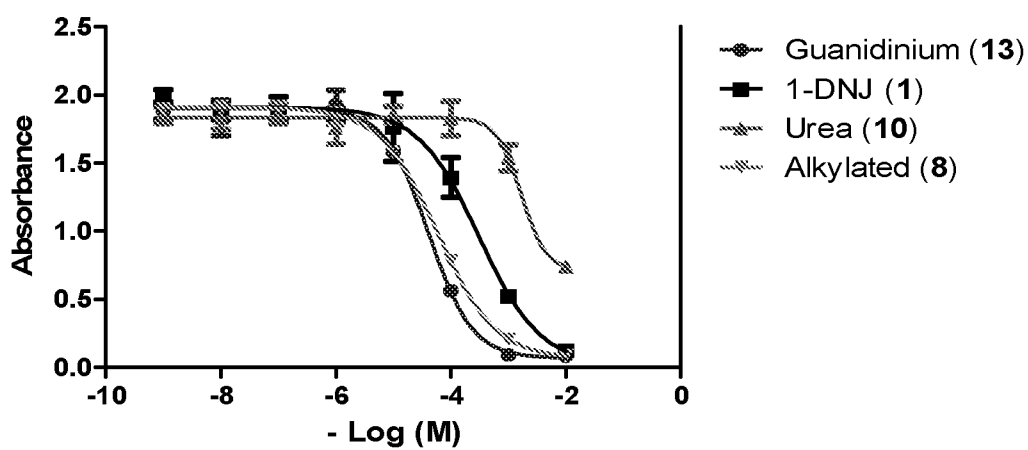


Figure 4.

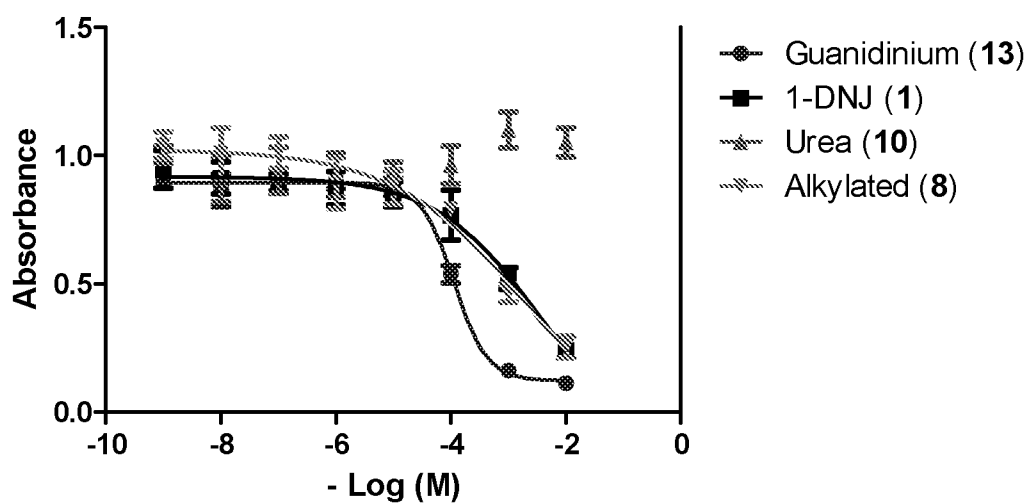


Figure 5.

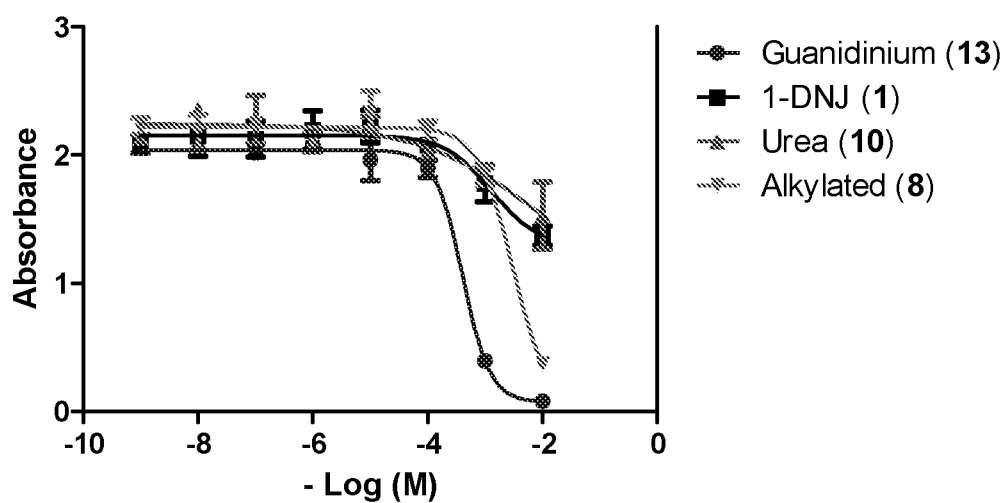


Figure 6.

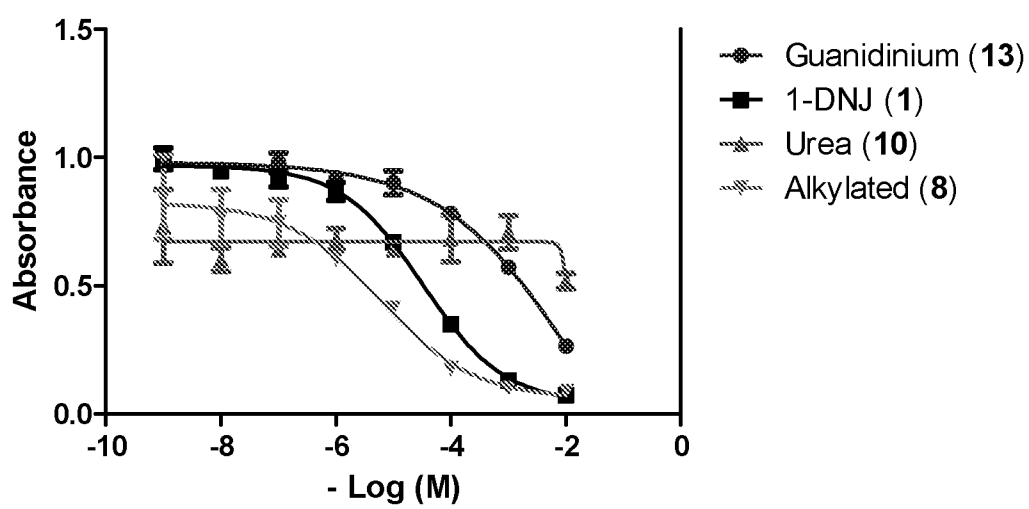


Figure 7.