Iminosugars as glucosylceramide processing enzymes inhibitors: design, synthesis and evaluation

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1 General Introduction

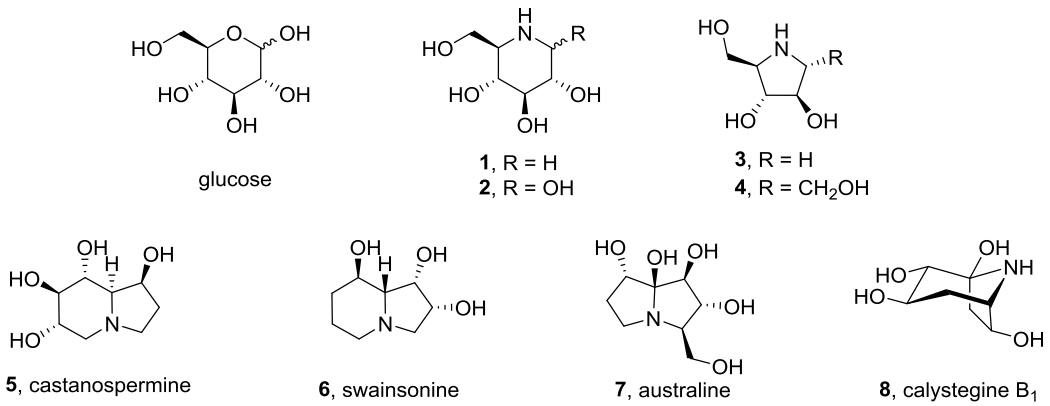
Introduction

Iminosugars, carbohydrate mimetics that feature a nitrogen atom substituting the furanose/pyranose ring oxygen in the parent sugar they emulate (Figure 1), are widely spread in nature.[1] Iminosugars are highly sought-after commodities because of their potential to inhibit glycoprocessing enzymes, with potential application in buffering diet polysaccharide degradation and in modulating glycoconjugate metabolism.[2] Today, iminosugars are in clinical use, or are considered as suitable leads for clinical development, for a range of human disorders including lysosomal storage disorders, type 2 diabetes, cancer, bacterial infections and viral infections.[3]

Natural occurrence and biological activities

The naturally occurring iminosugars can be classified in several structural classes, including pyrrolidines, piperidines, indolizidines, pyrrolizidines and nortropanes (Figure 1). The first iminosugar discovered from natural sources is nojirimycin (NJ, **2**), which was isolated in 1966.[4] Nojirimycin is a close glucopyranose analogue that only differs from glucose in the nature of the heteroatom within the ring: nitrogen instead of oxygen (Figure 1). Nojirimycin is a potent inhibitor of various glucosidases, however the highly acid-labile hemi-aminal moiety that characterizes nojirimycin also renders the compound relatively unstable in physiological conditions. 1-Deoxynojirimycin (DNJ, **1**) is a comparably much more stable compound that however possesses very similar biological properties. DNJ was synthesized in 1968[5] and isolated from plants in 1976,[6] and is a potent inhibitor of a large number of α-glucosidases as well as β-glucosidases.[7, 8] 1,4-Dideoxy-1,4-imino-D-arabinitol (DAB, **3**), and 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxy-pyrrolidine (DMDP, **4**) are representative examples of naturally occurring pyrrolidine iminosugars and have been isolated from tropical and temperate plants.[9] Pyrrolidine **3** is a potent inhibitor of α-glucosidases, β-glucosidases and α-mannosidases,[10] and its close structural analogue **4** inhibits a wide range of glycosidases as well.[11]

***Figure 1**: Structures of different classes of iminosugars*



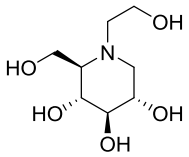
Bicyclic iminosugars abound in nature as well and are also often quite potent glycosidase inhibitors. The archetypal indolizidine iminosugar, castanospermine (**5**), isolated from Leguminosae, is an inhibitor of both α-glucosidases and β-glucosidases, thus resembling the activity profile of DNJ **1**.[12-16] Swainsoine (**6**), isolated from leaves, stems and seeds of various plants,[17, 18] is another widely studied indolizidine iminosugar and is a potent α-mannosidase

inhibitor.[19] A relevant pyrrolizidine iminosugar comprises australine (**7**), an inhibitor of both α-glucosidases and β-glucosidases that is found in *Leguminosae* seeds and leaves.[20] Calystegine B_1 (**8**) is a tetra-hydroxyl nortropanes alkaloids isolated from *Convolvulaceae*, and found to be a strong inhibitor of glucocerebrosidase.[21]

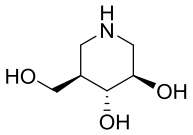
Clinical relevance

Iminosugars have long been regarded as promising starting points for drug development and today several iminosugars are in clinical use for the treatment of a number of human diseases. One early example of the therapeutic use of iminosugar-containing material comprises the application of mulberry leaves and bark, in Asia many years ago, for the treatment of diabetes. Several decades ago, both NJ and DNJ were isolated from mulberry leaves, and subsequently shown to inhibit of intestinal digestive glycosidases, thus affecting the digestion and absorption of carbohydrates. On the basis of DNJ, the antidiabetic agent, Miglitol (**9**), which has a better activity and selectivity compared with DNJ, was developed. Today, Miglitol is in clinical use for the oral treatment of type II diabetes.

***Figure 2**: Structures of Miglitol and isofagomine*



9, Miglitol



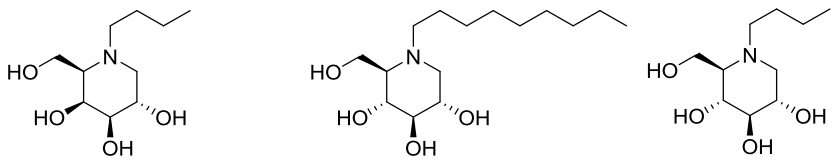
10, isofagomine

Another glycoprocessing enzyme associated with diabetes is hepatic glycogen phosphorylase. Hepatic glucose levels are increased in type II diabetes patients[22] and it is thought that buffering glucose levels can be effected through inhibition of hepatic glycogen phosphorylase. DAB (**3**, Figure 1) proved to be a potent hepatic glycogen phosphorylase inhibitor and by this virtue lowers gluconeogenesis from hepatic glycogen.[23] Isofagomine (**10**) is another potent hepatic glycogen phosphorylase inhibitor, and is also able to partially prevent gluconeogenesis in animal models.[24]

Perhaps the most successful application of iminosugars in therapy is found in the area of lysosomal storage disorders, in particular Gaucher disease. Lysosomal storage disorders (LSD) comprise about 50 inherited diseases, each caused by inherited, genetic mutations in particular lysosomal proteins, most commonly hydrolytic enzymes. Gaucher disease is a relevant example both for the work described in this Thesis and for LSDs in general: it is

perhaps the best studied member of the LSD family and several therapeutic intervention strategies are in clinical practice or are in clinical development. Gaucher disease is characterized by partial or complete (depending on the nature of the mutation) dysfunctional lysosomal glucosylceramidase (GBA1). GBA1 hydrolyses the interglycosidic linkage in glucosylceramide and (partial) deficiency in GBA1 leads to accumulation of this substrate as well as the unusual lysolipid, glucosylsphingosine. Gaucher pathology likely is partially caused by accumulation of these lipids. Gaucher patients (at least those suffering from relatively mild Gaucher as the result of partial, but not complete, impairment in GBA1) can be treated intravenously with recombinant GBA1 in what is termed enzyme replacement therapy (ERT). Alternatively, the enzyme responsible for the synthesis of the primary storage material in Gaucher disease, glucosylceramide synthase (GCS, producing glucosylceramide from UDP-glucose and ceramide) can be partially inhibited by *N*-butyl-deoxynojirimycin (**13**, marketed as Zavesca) in what has become known as substrate reduction therapy (SRT). Not clinical practice yet but an approach receiving considerable attention from academia and pharmaceutical industry alike is termed pharmacological chaperone therapy (PCT), which aims at stabilizing genetically impaired GBA1 to such an extent that (close to) normal glucosylceramide levels are reached. *N*-Nonyl-deoxynojirimycin (**12**) is a competitive GBA1 inhibitor that is under investigation as a pharmacological chaperone.[25] Its mode of action relies on its inhibitory activity: by active site occupation the enzyme 'folds' around the inhibitor and thus retains its active conformation. CMT has in fact reached the clinic for another LSD: Fabry disease (characterized by genetic deficiency in lysosomal alpha-galactosidase). *N*-Butyl-deoxygalactonojirimycin, or migalastat (**11**) is an α-galactosidase inhibitor and patients suffering from Fabry disease[26] can be treated with this compound, in combination with recombinant α-galactosidase (thus, a combined ERT/PCT treatment regime).

***Figure 3**: Structures of migalastat, NN-DNJ and Zavesca*



11, migalastat **12**, NN-DNJ **13**, Zavesca

The anti-viral activity of iminosugars is generally thought to be related to their ability to inhibit endoplasmic reticulum (ER) α-glucosidases. Blocking ER α-glucosidases affects the folding and trafficking of viral envelops glycoproteins.[27] Potent α-glucosidase inhibitors such

as DNJ (**1**), NN-DNJ (**12**) and castanospermine (**5**) have been subjected to clinical studies in relation to HIV infections, though no clinical drug has emerged from these studies.

In a recent publication, the anti-influenza activity of iminosugars was reported. In assays on infected cells both NN-DNJ and NB-DNJ were found to display antiviral activity against human influenza A, with NN-DNJ being the more potent compound. This activity is also thought to be related to ER α-glucosidase inhibition, since, upon treatment with **12** levels of viral hemagglutinin and neuraminidase proteins were found to be reduced.[28] Related studies have shown that iminosugars may block intracellular proliferation of yet another pathogenic virus: hepatitis B virus (HBV).

Outline of the thesis:

The research executed in the context of this Thesis comprises the design and synthesis of focused libraries of unprecedented iminosugars. **Chapter 2** reviews one of the most effective routes of synthesis towards deoxynojirimycin derivatives that is also central to parts of this Thesis: double reductive amination on an appropriately functionalized 5-keto-aldehyde. Application of this strategy in the synthesis of glycosylated DNJ derivatives is presented in **Chapter 3**. In **Chapter 4**, a series of *N*-alkyl iminosugars was designed and synthesized as selective inhibitors of the neutral glucosylceramidase, GBA2. In **Chapter 5**, and based on the dual GCS/GBA2 inhibitor described in the literature, biphenyl-L-*ido*-DNJ, a series of modified biphenyl-L-*ido* DNJ iminosugars are designed and synthesized and evaluated on their activity and selectivity on the three glucosylceramide processing enzymes, GCS, GBA1 and GBA2. In **Chapter 6**, a focused library of iminosugars characterized by the presence of a geminal bis(hydroxymethyl) motif is described, and **Chapter 7** summarizes the results described in this Thesis, presents plans for future research and details some initial steps that have been taken in this direction.

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