Organic & Biomolecular Chemistry

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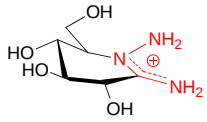
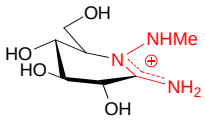


α\β-glucosidase inhibitor



selective α-glucosidase inhibitor

We present a new type of glycosidase inhibitors including a unique hydrazide imide moiety, which display inhibition in the low micromolar range.

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Sugar hydrazide imides: a new family of glycosidase inhibitors

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

The preparation of a novel type of iminosugars including a hydrazide imide moiety is described. The sugar hydrazide imides (3*S*,4*S*,5*R*,6*R*)-1-amino-3,4,5-trihydroxy-6-(hydroxymethyl)-2-iminopiperidine acetate and (3*S*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)-1-(methylamino)-2-iminopiperidine acetate presented behave as inhibitors of α/β-glucosidases in the low micromolar concentration range. The former inhibitor displays a pH-dependent inhibition of β-glucosidase. The *N*-methylated counterpart behaves as an anomer-selective competitive micromolar inhibitor of α-glucosidase.

Introduction

Iminosugars, exemplified by naturally occurring 1-deoxynojirimycin (**1**) (Figure 1),[1] represent carbohydrate mimetics in which the endocyclic oxygen atom has been replaced by a nitrogen atom. This group of compounds resembles carbohydrates and can therefore interact with carbohydrate-processing enzymes, however, they are dissimilar enough from carbohydrates in order not to undergo metabolization mediated processing by such enzymes. These properties among others have highlighted iminosugars as lead compounds in the hunt for new pharmaceuticals.[2] Indeed, many iminosugars inhibit carbohydrate-handling enzymes,[3] which make them attractive candidates for treatment of diseases such as diabetes,[4] cancer,[5] HIV,[6] Alzheimer's disease,[7] and lysosomal storage disorders.[8] Whereby, iminosugars represent attractive synthetic targets.[2b,8,9]

It has been found that the transition state of glycosidase catalysed cleavage of glycosidic bonds resembles both the shape and charge of the oxocarbenium ion **2** (Figure 1).[10] Indeed, such intermediate has recently been observed by NMR upon treatment of per-*O*-acetylated 2-deoxy and 2-bromoglucopyranose with super acids.[11] Thus, a compound that mimics the oxocarbenium ion **2** should also behave as a potent glycosidase inhibitor.[12] In its *N*-protonated state, 1-deoxynojirimycin (**1**) constitutes a charged analogue of the oxocarbenium ion **2**.[12] However, it is anticipated to be in a chair conformation rather than in the flatten anomeric conformation of the oxocarbenium ion **2**.[13] At the same time, a pH dependence analysis of $1/K_i$ for binding of 1-deoxynoririmycin (**1**) to β-glucosidase (almonds) could not conclude whether 1-deoxynojirimycin (**1**) binds to the enzyme in its protonated or unprotonated form.[14] In fact, a basic fluorescence labelled iminosugar has been demonstrated to bind β-glucosidase in its unprotonated form.[15] However, in this context, it should be mentioned that compound **1** behaves as a ca 10^4 times more potent inhibitor of β-glucosidase than does glucose,[14] strongly indicating that the basic nitrogen atom of **1** participates in favourable interactions with the enzyme.[12,14] D-Gluconolactone (**3**) (Figure 1) also displays significant glycosidase inhibitory activity in spite of the absence of a formal positive charge. Its activity was attributed to its shape analogy to the oxocarbenium ion **2**.[16] Pioneering work by Ganem and co-workers, brought the charge resemblance from compound **1** and the shape resemblance from lactone **3** with the oxocarbenium ion **2** into one single species, namely amidine **4** (Figure 1).[17] Indeed, amidine **4** was found to behave as a potent inhibitor in the low micromolar range of a wide range of glycosidases. The innovative work by Ganem and co-workers encouraged others to engage in the synthesis of sugar amidines and evaluate them as glycosidase inhibitors.[18] However, to the best of our knowledge, there are no examples in the literature in which the endocyclic nitrogen of amidines of type **4** has been armed with

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† Electronic Supplementary Information (ESI) available: Synthetic details for the preparation of compounds and their NMR spectra. See DOI: 10.1039/x0xx00000x.
Financial supports from the University of Stavanger, Dirección General de Investigación of Spain (CTQ2016-78703-P), the Junta de Andalucía (FQM134), and the European Regional Development Fund (FEDER) are gratefully acknowledged. Thanks are also due to Dr. Bjarte Holmelid, University of Bergen, for conducting MS analysis, and Servicio de Resonancia Magnética Nuclear, CITIUS (University of Seville) for the performance of some NMR spectra.

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a substituent that is not synchronously connected to the exocyclic nitrogen atom. The absence of that type of compounds appears surprising since the functionalization of the nitrogen atom in compound **1** with alkyl groups has provided two approved drugs for the treatment of Gaucher's disease[2a] and type II diabetes;[19] namely, miglustat (*N*-butyl-deoxynojirimycin) and miglitol (*N*-(2-hydroxyethyl)deoxynojirimycin), respectively. In addition, a vast array of derivatives of 1-deoxynojirimycin (**1**) carrying various types of *N*-substituents have been prepared and found to display interesting inhibitory activities towards carbohydrate-processing enzymes.[8,13,20]

Thus, in this paper, we address an empty entry of glycosidase inhibitor science by presenting the arming of the endocyclic nitrogen atom of amidine **4** with an amino- and methylamino-group to obtain the hitherto unknown hydrazide imides **5** and **6** (Figure 1), respectively. In addition, as a sugar amidrazone that is electronically similar to our addressed compounds **5** and **6** possesses a pK_a of 8.7,[21] we envisage a similar p*K*a value of **5** and **6**. Thereby, according to our design, compounds **5** and **6** should have the potential to form strong ionic interactions with the enzyme. We also present the screening of compounds **5** and **6** as inhibitors against a panel of glycosidases.

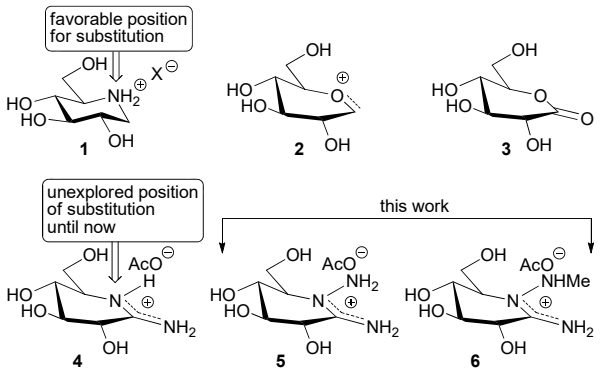

Figure 1 Structure of known inhibitors **1**, **3**, and **4** along with the oxocarbenium ion **2** and the hydrazide imides **5** and **6** described herein.

Synthesis

The synthesis of target hydrazide imides **5** and **6** commenced from known ketonitrile **7** (Scheme 1), which can be generated from methyl α-D-glucopyranoside in five steps following a literature procedure.[22] Thus, compound **7** underwent reductive hydrazidation with *t*-butyl carbazate using sodium cyanoborohydride as the reducing reagent to provide hydrazide epimers **8** and **9** in 31% and 20% yield, respectively. In a similar manner, epimers **10** (38%) and **11** (14%) were obtained when *t*-butyl 2-methylcarbazate was used as the hydrazide source. In the subsequent step, hydrazides **8** and **9** cyclized smoothly into hydrazide imide **12** and **13**, respectively, upon acidic removal of the Boc-protection group. In order to obtain hydrazide imides **14** and **15** on the other hand, the cyclization was significantly slower and it was beneficial to follow the progress of the reaction by ^{1}H-NMR using $CDCl_3$ as solvent. In this context, it is worth mentioning that when the reaction scale exceeded 20 mg of **10** or **11** yield was lowered and the by-products formed were problematic to remove upon silica gel column chromatography. In the final step, the protected sugar hydrazide imides **12**-**15** underwent global debenzylation upon palladium-catalysed hydrogenolysis to provide the unprotected counterparts **5**, **6**, **16**, and **17** as the acetate salts after silica gel column chromatography (eluent; MeCN:25% aqueous AcOH).

In CD_3OD, the D-*gluco*-configuration of **14** was concluded by ^{1}H-NMR analysis, which gave the coupling constants $J_{3,4}$ 7.6 Hz, $J_{4,5}$ 4.0 Hz, and $J_{5,6}$ 2.9 Hz. The small coupling constants $J_{4,5}$ and $J_{5,6}$ along with a larger coupling constant $J_{3,4}$ mirror a D-*gluco*-configuration in a $^{3,6}B$-conformation[24] for **14**. In DMSO-d_6, ^{1}H-NMR analysis of **15** provided the large coupling constants $J_{3,4}$ 7.4 Hz and $J_{4,5}$ 9.1, which indicates a $^5H_4$-conformation.[24] In addition, the small coupling constant $J_{5,6}$ 5.6 Hz suggests the L-*ido*-configuration of compound **15**. ^{1}H-NMR analysis of **5** in D_2O provided the coupling constants $J_{3,4}$ 9.8 Hz, $J_{4,5}$ 9.8 Hz, and $J_{5,6}$ 7.8 Hz, indicating a D-*gluco*-configuration in a $^5H_4$-conformation.[24] The coupling constants $J_{3,4}$ and $J_{4,5}$ for the 6-epimer of **5,** namely **16,** remained large (8.1 Hz and 10.0 Hz, respectively). However, the coupling constant $J_{5,6}$ decreased to 5.6 Hz, providing support for the L-*ido*-configuration of compound **16** in a $^5H_4$-conformation. Compound **5** was found to be stable in D_2O for days at room temperature. However, the compound decomposed to a complex mixture of products upon treatment with aqueous NaOH (0.5 M).

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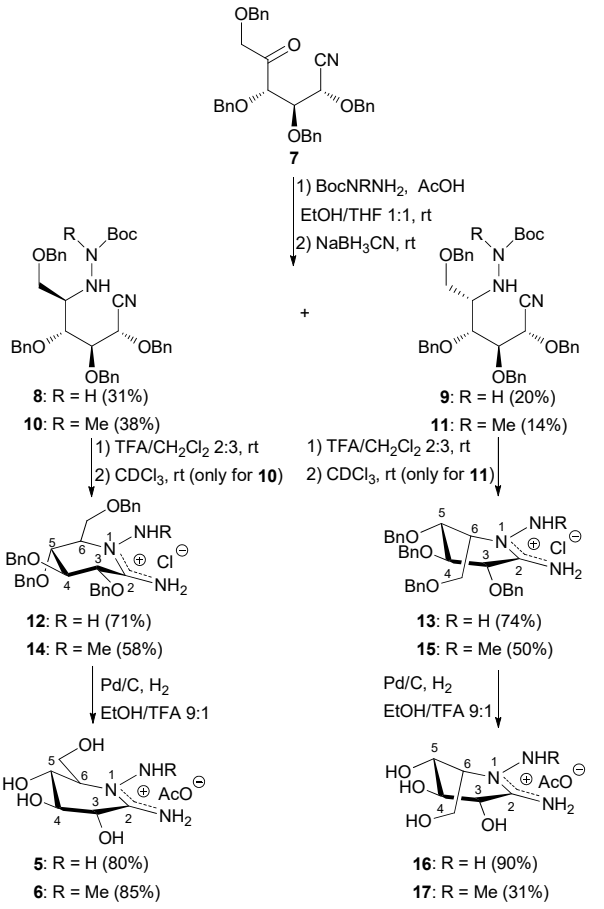

Scheme 1 Synthetic pathway for hydrazide imides **5**, **6**, **16**, and **17**.

Inhibition studies

Hydrazide imides **5**, **6**, **16**, and **17** were evaluated as glycosidase inhibitors against a panel of seven commercially available glycosidases (Table 1). Many of the enzymes in our panel are routinely used in order to evaluate the inhibition profile of iminosugars at early stages of the hunt for new drug candidates. Lineaweaver-Burk (double reciprocal) plots provided the kinetic parameters that afforded the inhibition constants (K_i's); as an example, Figure S1 (in supporting information) depicts such a plot for the inhibition of β-glucosidase by hydrazide imide **5** at pH 8.0. In case Lineaweaver-Burk did not provide undoubtedly the inhibition mode, the double Corsnih-Bowden plot (1/V *vs.* [I] and [S]/V *vs.* [I]) was used (see Figure S2 for the inhibition of β-glucosidase by **5** at pH 8.0).

Compounds **5**, **6**, **16**, and **17** were found to display very distinct inhibition profiles of which hydrazide imide **5** proved to be the most potent glycosidase inhibitor of the series. This compound behaved as a competitive and mixed-type inhibitor of glucosidases and mannosidases, respectively, with a predominantly competitive component ($K_{ia} < K_{ib}$) for mannosidases. Remarkably, compound **5** was found to be a good inhibitor of α-glucosidase, with K_{ia} value (interaction with the free enzyme) within the low micromolar range (9.9 μM) at pH 6.8; the potency was maintained at higher pH, with no relevant change in the K_{ia} value at pH 8.0.

Noteworthy, when the testing was extended to β-glucosidase, compound **5** was found to be a moderate inhibitor at pH 6.8 (K_i= 31.3 μM), and a 4.1-fold increase in activity was observed at pH 8.0, leading to a good inhibition of this enzyme (K_{ia}= 7.7 μM). Taken as a whole, such behaviour strongly contrasts with parent amidine **4**, which was found to be a non-selective inhibitor of β-glucosidase and α-mannosidase (K_i's: 10 and 9 μM, respectively). Furthermore, for the former enzyme, a complete independence of the activity on the assay pH was observed for compound **4**.[17b]

The *R*-stereochemistry in the 6-position of inhibitor **5** was found to be essential for the inhibitory activity, as the 6-epimer of **5**, namely **16**, only displayed modest activity towards the investigated enzymes. In fact, a similar relationship has been found between 1-deoxynojirimycin (**1**) and L-*ido*-1-deoxynojirimycin where the former is a significantly more potent inhibitor.[25] Interestingly, the *N*-methyl sugar hydrazide imide **6** displayed a quite different inhibition profile compared to inhibitor **5** lacking the *N*-methyl group. Indeed, in contrast to hydrazide imide **5**, compound **6** behaved as a competitive inhibitor of α-glucosidase in the micromolar concentration range (K_{ia} 32.6 μM). Apart from the inhibition of α-glucosidase, hydrazide imide **6** acted as a poor inhibitor for the other enzymes in the panel of targets, which label compound **6** as a selective α-glucosidase inhibitor. The significantly stronger inhibition of **5** than **6** against β-glucosidase may be to the additional NH functionality of the former that provide an additional interaction with a hydrogen bond acceptor in the enzyme. In agreement with L-*ido*-hydrazide imide **16**, the *N*-methylated counterpart **17** also behaved as a modest inhibitor of all enzymes included in our biological tests.

Conclusions

In conclusion, we have carried out the synthesis of four unprecedented sugar hydrazide imides **5**, **6**, **16**, and **17**. Compounds **5** and **6** behave as good α- and β-glucosidase inhibitors in the low micromolar concentration range. The type of iminosugars presented herein, carrying a hydrazide imide moiety are unique and thus the work presented has the potential to serve as a platform in the hunt for new pharmaceutical targets. Indeed, in the search for pharmacological chaperones for treatment of lysosomal storage disorders, iminosugars armed with lipophilic *N*-groups have appeared as such candidates.[8] Thus, arming of compound **5** with various lipophilic *N*-groups will provide additional pharmacological chaperone candidates.

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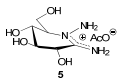
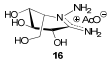
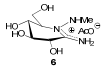
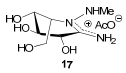
Table 1. Inhibitory constants (K_i, μM) for hydrazide imides **5**, **6**, **16**, and **17** against a panel of glycosidases.

Enzyme	 5	 16	 6	 17
α-Glucosidase (*Saccharomyces cerevisiae*)	9.9±2.2 (12.3)[a] Competitive	3667 Competitive	32±8 (25)[a] Competitive	>250[b]
β-Glucosidase (almonds)	31±4 (15)[a] Competitive[c] 7.7 ±1.8 (7.9)[a] Competitive[d]	>250[b]	542 (537)[a] Competitive	>250[b]
α-Mannosidase (Jack beans)[e]	K_{ia}= 62±27 K_{ib}= 398±27 Mixed	>250[b]	>250[b]	>250[b]
β-Mannosidase (*Helix pomatia*)[e]	K_{ia}= 211 K_{ib}= 1245 Mixed	K_{ib} = 2991 Uncompetitive	>250[b]	>250[b]
α-Galactosidase (green coffee beans)	>250[b]	>250[b]	850	708
β-Galactosidase (*Asp. oryzae*)	>250[b]	>250[b]	>250[b]	>250[b]
β-Galactosidase (*E.coli*)	>250[b]	>250[b]	>250[b]	>250[b]

[a]IC_{50} values (μM); [b]Maximum inhibitor concentration tested; [c]At pH 6.8; [d]At pH 8.0; [e]At pH 5.6

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