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Bicyclic isoureas derived from 1-deoxynojirimycin are potent inhibitors of β-glucocerebrosidase†

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A series of bicyclic isourea derivatives were prepared from 1-deoxynojirimycin using a concise synthetic protocol proceeding *via* a guanidino intermediate. Inhibition assays with a panel of glycosidases revealed that these deoxynojirimycin-derived bicyclic isoureas display very potent inhibition against human recombinant β-glucocerebrosidase with IC_{50} values in the low nanomolar range.

Glycosidases are an important class of enzymes capable of cleaving glycosidic bonds.[1] As such they have a strong effect on the glycan decoration of biomolecules and the numerous biological effects that this controls.[2] Iminosugars are a naturally occurring group of carbohydrate analogues and serve as a major source of inspiration for glycosidase inhibitor development.[3] To date, a number of iminosugars have been identified as highly potent and selective inhibitors of glycosidases.[4,5] The effectiveness of iminosugars as glycosidase inhibitors depends largely on their ability to mimic the relevant transition state in the hydrolysis process.[6,7] In the case of iminosugar-based inhibitors, the geometry and hybridization state of the "pseudo-anomeric" carbon and/or the endocyclic nitrogen atom are important points of consideration. Glycosidases can bind with very high affinities to compounds that mimic the relevant transition state such as the naturally occurring 1-deoxynojirimycin (DNJ, **1**) and its derivatives (**2** and **3**), as well as castanospermine (**4**, Fig. 1A).[8] However, these simple iminosugars generally lack selectivity towards specific glycosidases, which can lead to off-target binding and side effects.

Synthetic modification of iminosugars holds much promise for the tuning and optimization of their properties. For example, fused guanidine or isourea systems such as compounds **5–9** were found to be good inhibitors of various glycosidases.[9–13] Given these findings, we recently described the application of a method developed in our group for the convenient conversion of amines into substituted guanidines.[14] Specifically, we prepared novel DNJ derivatives in which the endocyclic amine was converted into a substituted guanidine group (compounds **I**, Fig. 1B).[15] Introduction of an exocyclic guanidine group was envisioned as a means of probing both the effects of altered iminosugar ring conformation and charge delocalization in relation to glycosidase inhibition. These compounds showed a distinct inhibition pattern relative to that of the DNJ parent compound, with respect to both potency and selectivity.

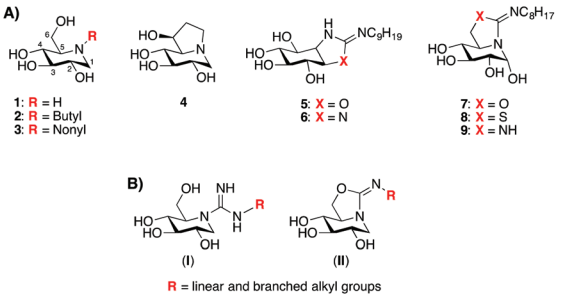

Fig. 1 (A) Chemical structures of selected iminosugar-based glycosidase inhibitors. (B) General structures of guanidine (I) and bicyclic isourea (II) DNJ derivatives prepared in this work.

We here describe the continuation of our investigations with a broader panel of DNJ-derived compounds and their evaluation as glycosidase inhibitors. While such guanidinylated DNJ analogues could be prepared and screened as glycosidase inhibitors (compounds **13a–e**, Scheme 1), we found that they were prone to spontaneous cyclization to generate the corresponding bicyclic isoureas (compounds **14a–e**). Gratifyingly, these isoureas were found to be very stable and exhibited potent β-specific glycosidase inhibition with a strong preference for the human lysosomal β-glycosidase, β-glucocerebrosidase (GBA, EC 3.2.1.45).

In the initial synthetic plans we designed a series of guanidine-modified DNJ analogues incorporating different N^G-substituents comprised of simple alkyl chains ranging from eight to fourteen carbon atoms in length as well as a bis-lipidated

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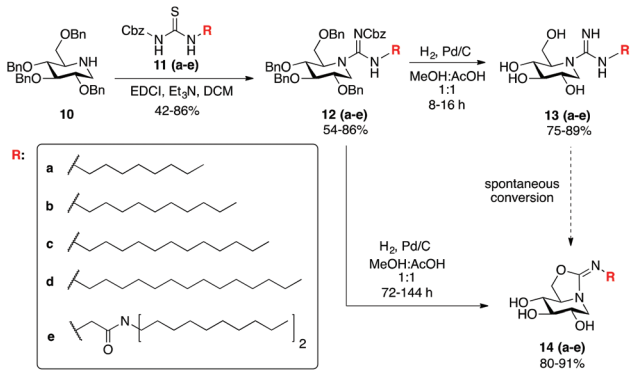
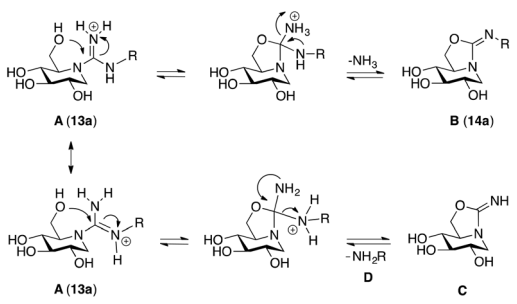
†Electronic supplementary information (ESI) available: Synthetic procedures and analytical data for all new compounds. Supporting figures for, pK_a determination, enzymatic, and biological assays. See DOI: 10.1039/c6ob01735e



Scheme 1 Synthetic route employed in preparing deoxynojirimycin analogues with N^G-substituted guanidine and bicyclic isoureas analogues.

species (Scheme 1). The synthetic approach used in preparing the initially pursued guanidine analogues of DNJ is outlined in Scheme 1. The benzyl protected DNJ species **10** was synthesized on multi-gram scale as previously described[16] and served as a common starting material in the preparation of the protected N^G-substituted guanidine analogues (**12a–e**). For this conversion, a series of Cbz-protected thioureas (**11a–e**) was also needed and generated as previously described by treatment of the corresponding amine with CbzNCS.[17,18] Activation of the thioureas with EDCI followed by addition of benzyl protected DNJ species **10** led to clean formation of protected guanidines **12a–e**. Removal of benzyl and Cbz groups was achieved *via* hydrogenation to yield the guanidine products **13a–e**. Somewhat surprisingly, it was found that upon deprotection, the guanidines were prone to spontaneous cyclization, rearranging to form the bicyclic isourea species **14a–e**. Isolation of guanidines **13a–e** was possible by limiting the time of the final deprotection step followed by immediate HPLC purification and lyophilization. However, over the time course of the subsequent enzyme inhibition assays we found that partial cyclization of the guanidines to the corresponding isoureas was unavoidable. The cyclization can follow two possible pathways, with one involving elimination of ammonia and the other the alkylated amine (Scheme 2 and Fig. 2). While these pathways compete, formation of the lipidated isoureas **14a–e** was found to be generally favored.



Scheme 2 Spontaneous conversion of guanidine-modified iminosugars **A** to bicyclic isourea products **B** and **C**.

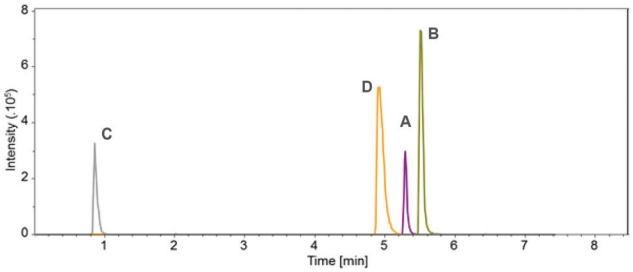


Fig. 2 Extracted ion chromatogram for compound **A** (**13a**) obtained with the optimized UHPLC-MS method of a 1 mM solution (pH 7.0), degraded at 21 °C for 2 days. The following compounds were identified by their *m*/*z* value: (A) *m*/*z* 318 for compound **13a**; (B) *m*/*z* 301 for compound **14a**; (C) *m*/*z* 189 for isourea free amine after alkylamine elimination; (D) *m*/*z* 130 for alkylamine.

The kinetics of the cyclization process were also studied in detail and revealed a pH dependence: at lower pH the guanidines cyclized more slowly than at neutral pH (ESI Table S1†). In preparing quantities of isoureas **14a–e** suitable for enzyme assays, we found it most convenient to perform the deprotection of intermediates **12a–e** over an extended period of 72–144 hours allowing for complete removal of the benzyl groups and cyclization to the isoureas (Scheme 1). Isoureas **14a–e** were found to be stable at both neutral (pH 7) and slightly acidic pH (pH 5) and displayed potent GBA inhibition (Table 1).

Enzyme inhibition studies. With compounds **14a–e** in hand, inhibition studies were performed against a panel of readily available glycosidase enzymes as well as human recombinant GBA and β-galactocerebrosidase (GALC). Notably, in the case of the plant enzymes, relatively strong inhibition was seen for the β-glycosidases (Table 1). No inhibition was observed for the α-specific enzymes. Strikingly, the strongest inhibition was observed for the human lysosomal β-glycosidase GBA with low nanomolar inhibition (IC_{50} 1.5–20 nM). Interestingly, these IC_{50} values are significantly lower than observed for the *N*-alkylated reference compound NN-DNJ (**3**) which was found to have an IC_{50} for GBA of over 500 nM in our assay. Furthermore, despite the observed β-selectivity, our isoureas did not inhibit the lysosomal β-galactosidase GALC, indicating a high degree of selectivity among the human lysosomal enzymes. Also of note is the pH dependence observed for GBA inhibition by compounds **14a–e**; in general, the IC_{50} values measured at pH 7.0 were an order of magnitude lower than those measured at pH 5.2 (Table 1).

To gain insight into the possible binding mode(s) of isoureas **14a–e** within the GBA active site, modeling was performed (see ESI† for detailed description of docking experiments). Fig. 3 depicts the docking of compound **14a** in the GBA active site highlighting the predicted interactions with active site residues. This analysis reveals an ionic bond to Glu_{235} and a cation–pi interaction with Tyr_{313}. Notably, these interactions are not seen in the X-ray structure of GBA when co-crystalized with NN-DNJ[19] and may indicate a higher binding affinity of **14a** relative to NN-DNJ (as reflected in our inhibition data). These additional interactions require that the isourea moiety

Table 1 Glycosidase inhibition values obtained for **14a–e**[a]

Enzyme	14a	14b	14c	14d	14e	NNDNJ
α-Glu[b]	>100 000	>100 000	>100 000	>100 000	>100 000	>100 000
α-Gal[c]	>100 000	>100 000	>100 000	>100 000	>100 000	>100 000
β-Glu[d]	5650 ± 387	2080 ± 95	1650 ± 70	661 ± 30	>100 000	>100 000
β-Gal[e]	420 ± 9	136 ± 4	88 ± 10	138 ± 34	938 ± 91	>100 000
Nar[f]	128 ± 6	117 ± 6	141 ± 7	195 ± 1	518 ± 7	116 ± 5
GBA[g] (pH 7.0)	20.8 ± 1.3	2.6 ± 0.9	1.8 ± 0.1	1.5 ± 0.2	1.7 ± 0.1	562.5 ± 56.6
GBA[g] (pH 5.2)	135.5 ± 3.9	16.7 ± 0.5	12.8 ± 1.2	15.4 ± 1.7	14.0 ± 1.2	1293.0 ± 55.3
GALC[g]	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000

[a] IC_{50} values are reported in nM and are averages obtained from triple independent duplicate analysis of each compound. For ease of comparison, the IC_{50} values obtained for all compounds shown in this table are compared to a reference compound NNDNJ. [b] α-Glucosidase (from baker's yeast, Sigma G5003): 0.05 U mL^{-1}, the activity was determined with *p*-nitrophenyl-α-D-glucopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 7.2). [c] α-Galactosidase (from green coffee beans, Sigma G8507): 0.05 U mL^{-1}; α-galactosidase activity was determined with *p*-nitrophenyl-α-D-galactopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 6.8). [d] β-Glucosidase (from almond, Sigma G4511): 0.05 U mL^{-1}; the activity was determined with *p*-nitrophenyl-β-D-glucopyranoside (0.7 mM final conc. in well) in sodium acetate buffer (100 mM, pH 5.0). [e] β-Galactosidase (from bovine liver, Sigma G1875): 0.05 U mL^{-1}; activity was determined with *p*-nitrophenyl-β-D-galactopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 7.2). [f] Naringinase (from *Penicillium decumbens*, Sigma N1385): 0.06 U mL^{-1}. The activity was determined with *p*-nitrophenyl-β-D-glucopyranoside (0.7 mM final conc. in well) in sodium acetate buffer (100 mM, pH 5.0). [g] β-Glucocerebrosidase (GBA) and β-galactocerebrosidase (GALC) activities were determined using 4-methylumbelliferyl-β-D-glucopyranoside and 4-methylumbelliferyl-β-D-galactopyranoside respectively using assay conditions based on those previously reported.[22]

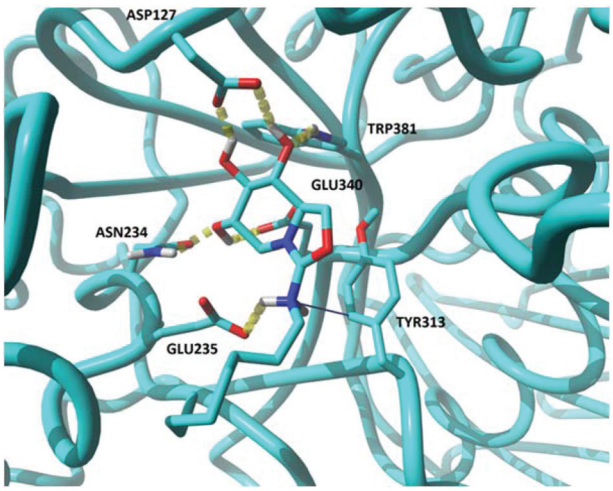


Fig. 3 GBA (PDB-ID 2V3E) with the docked pose of **14a**, showing ionic hydrogen bonding and cation–pi interactions.

be protonated in the bound state. In this regard, we also measured the pK_a of **14a** to be 9.30, indicating that this is indeed likely to be the case.

The DNJ-derived bicyclic isourea analogues described in this study are a novel class of glycosidase inhibitors. While we initially set out to explore exocyclic guanidine analogues of DNJ it was found that such compounds are prone to intramolecular cyclization yielding instead the corresponding bicyclic isoureas. Our data indicate that compounds **14a–e** are among the most potent GBA inhibitors reported to date. Of note is a closely related nojirimycin analogue 7 that was also previously reported as a GBA inhibitor.[11] While compound 7 was prepared in 8 steps in *ca.* 20% overall yield, the corresponding DNJ analogue **14a** can be prepared in 6 steps with a near 50% overall yield. Also of note is the striking difference observed for these compounds in their capacity to inhibit GBA. Using similar assay conditions, we measured a near 200-fold lower IC_{50} value for compound **14a** (20.8 nM) relative to that reported for 7 (3.8 μM).[11] Also of note are the similar IC_{50} values measured for NN-DNJ (**3**) which was used as a reference inhibitor in both studies suggesting the values here measured for compound **14a** can legitimately be compared to that reported for 7. The difference in GBA inhibition between **14a** and 7 is likely attributable to the pseudoanomeric hydroxyl group present in 7. A derivative of nojirimycin, compound 7 retains the aminal functionality of the parent compound. This structural feature may lead to less stable compounds as derivatives of 7 were also reported to rearrange at lower pH[20] and to anomerize upon binding to GBA.[21] An additional factor that might contribute to the difference in GBA inhibition by compounds 7 and **14a** are their different pK_a values (7.0 reported for 7,[20] *vs.* 9.3 determined for **14a**). The enhanced basicity of **14a** may facilitate the formation of the ionic interactions as identified by the modeling.

In summary, we here describe the preparation of a novel class of DNJ-derived bicyclic isoureas and evaluation of their inhibitory effects on a series of glycosidases. These investigations revealed compounds **14a–e** to be among the most potent known inhibitors of GBA, a human lysosomal β-glycosidase. It is known that mutations in GBA can lead to serious lysosomal storage disorders such as Gaucher's disease. Interestingly, in some cases GBA inhibitors can serve to counteract the loss of GBA activity by functioning as pharmacological chaperones. Our findings suggest that further investigations into the potential for DNJ-derived bicyclic isoureas like compounds **14a–e** may be warranted. Preliminary data from experiments in our group using Gaucher patient derived fibroblasts bearing the N370S GBA mutation indicate that **14a** possesses a chaperone activity on par with that of the known chaperone NN-DNJ **3** (see ESI†). More comprehensive studies

into the full potential of compounds **14a–e** to serve as pharmacological chaperones will be reported in due course.

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