MedChemComm

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This *Accepted Manuscript* will be replaced by the edited and formatted *Advance Article* as soon as this is available.

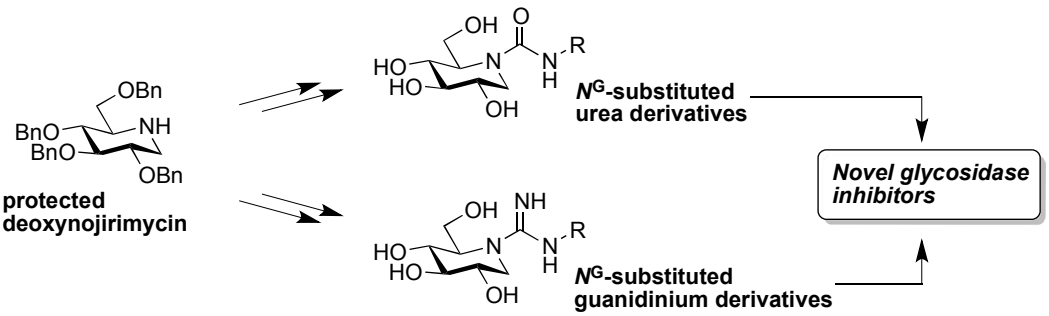
To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about *Accepted Manuscripts* can be found in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard **Terms & Conditions** and the **ethical guidelines** that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these *Accepted Manuscript* manuscripts or any consequences arising from the use of any information contained in them.

RSC Publishing

www.rsc.org/medchemcomm
Registered Charity Number 207890



Novel guanidinium and urea derivatives of 1-deoxynojirimycin (DNJ) were prepared using a concise synthetic protocol. These DNJ derivatives exhibited potent and selective inhibition against a panel of glycosidase enzymes.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Glycosidase inhibition by novel guanidinium and urea iminosugar derivatives

Raymond Kooij, Hilbert M. Branderhorst, Simon Bonte, Sara Wieclawska, Nathaniel I. Martin* and Roland J. Pieters*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Novel guanidinium and urea derivatives of 1-deoxynojirimycin were prepared using a concise synthetic protocol and tested against a panel of glycosidases for their inhibitory properties. Potent and selective inhibition was observed with both classes of compounds. Further investigation involving an expanded series of N^G-substituted guanidinium deoxynojirimycin analogues revealed distinct inhibitory profiles in the inhibition of the glycosidases tested.

Introduction

The potent and selective inhibition of glycosidases is an important goal for the development of therapeutics.[1] Glycosidases cleave the glycosidic bonds in oligosaccharides and glycoconjugates. Their blocking can aid in the treatment of diabetes,[2] viral infections,[3] lysosomal storage diseases,[4] and cancer.[5] Iminosugars have been shown to be highly effective inhibitors but their limited selectivity can lead to side effects when applied therapeutically.[6, 7] It is clear that improving the selectivity of iminosugars as glycosidase inhibitors is an important goal.[6, 8-12] The effectiveness of the iminosugar as a glycosidase inhibitor in many cases depends on its ability to mimic the relevant transition state in the cleavage process.[8] Considering the vast rate enhancements of glycosidases, these enzymes bind the transition state with very high affinity. Consequently, a transition state mimic has the potential to be a very strong inhibitor. The mimicry depends on the complementarity with respect to charge and shape. These issues are intimately linked to the hybridization state of the 'anomeric carbon' and the endocyclic oxygen or in case of derivatives of deoxynojirimycin **1** (Figure 1) the nitrogen. These atoms should have a considerable sp^2 character. We here focused on the hybridization aspect by straightforward derivatisation of the nitrogen of deoxynojirimycin **1** to explore new structural motifs for glycosidase inhibition.

Previous investigations employing sp^2 hybridized inhibitors have yielded promising results. Inhibitors with an sp^2 hybridized C-1 such as D-gluconolactone **2**,[13] showed activity indicating the importance of a somewhat flattened ring, although these compounds were also limited by the lack of charge complementarity. Compounds with both an sp^2 hybridized C-1 and an endocyclic nitrogen that is part of an amidine functional group as in **3**, showed good activities, although poor selectivity. Based on comparisons with less basic derivatives it was concluded that the flattened conformation was more important than the charge.[14] This notion was supported by weakly basic inhibitors with a flattened ring such as **4**.[15]

Nojirimycin derivatives in which only the endocyclic nitrogen was (partially) sp^2 hybridized have also been reported.[16-18] In these compounds the endocylic nitrogen is bridged to the C-6 as in **5** by a guanidinium or a related linkage. Compounds of this type have shown good inhibitory properties and tuneable selectivities as a function of the hydroxyl configurations around the ring. Our own recently developed synthetic methodology to conveniently transform amines into guanidino groups,[19-21] enables access to unconstrained derivatives of deoxynojirimycin. Evaluation of such compounds would delineate the effects of conformation and charge delocalisation due to the introduction of the guanidinium or its neutral urea counterpart, on glycosidase

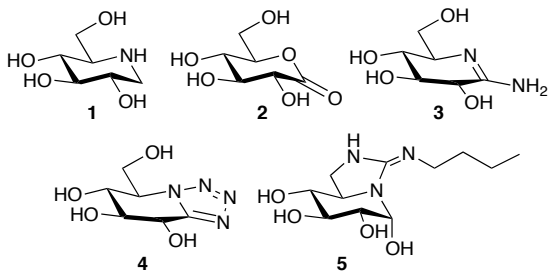


Figure 1. 1-Deoxynojirimycin (**1**) and a series of glycosidase inhibitors with varying degrees of sp^2 hybridization.

*Department of Medicinal Chemistry & Chemical Biology, Utrecht Institute for Pharmaceutical Sciences, University of Utrecht, David de Wied Building, Office: 5.64, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands. E-mail: n.i.martin@uu.nl, r.j.pieters@uu.nl
† Electronic Supplementary Information (ESI) available: Experimental protocols and product characterization. See DOI: 10.1039/b000000x/

inhibition potency and selectivity. Considering the cyclic framework in which the nitrogen resides, the likely reluctance of the nitrogen to adopt a full sp^2 geometry would have an unpredictable effect on its activity. Furthermore, in comparison to **5**, the free movement of the side chain could also lead to additional preferences. Furthermore, as was shown for sialidase inhibition, introducing guanidinium functions can enhance potency.[22]

Results and discussion

Given the unique activities of the compounds described above, we prepared a series of novel analogues of deoxynojirimycin **1** containing alkyl, urea and guanidino substituents at the endocylic nitrogen (Scheme 1). To this end the protected deoxynojirimycin **6** was alkylated by 1-bromohexane and deprotected by hydrogenation to give **8**. The synthesis of the urea counterpart **9** was accomplished by treatment of **6**[23] with *n*-butyl isocyanate followed by hydrogenolysis giving urea **10**.

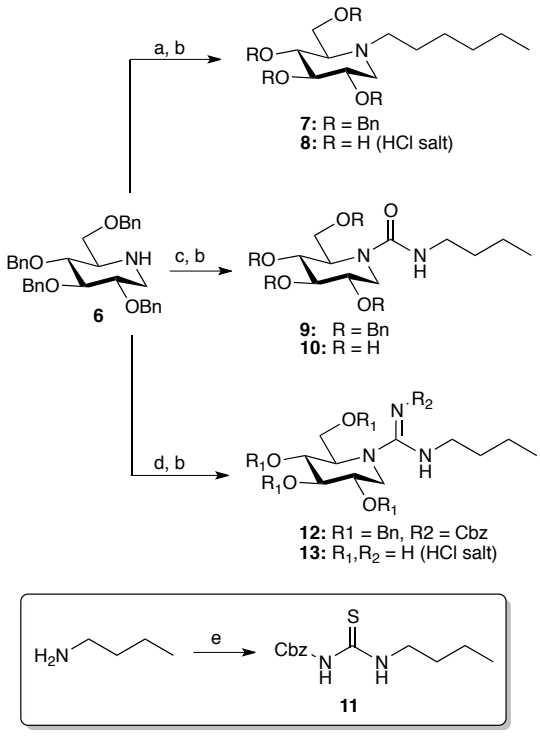
The inhibitory activities of **8, 10** and **13** towards a broad panel of 7 glycosidases were determined. The panel consisted of an α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, α-mannosidase, β-mannosidase and a naringinase. The IC_{50} values are summarized in Table 1. Deoxynojirimycin **1** was employed as a reference compound. Inhibition assays were performed in either phosphate or acetate buffer at the optimum pH for each enzyme. Determination of IC_{50} values was carried out by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases of the appropriate *p*-nitrophenyl glycoside substrate in the presence of a varying concentrations of each iminosugar derivative.[24] In this assay, the alkylated compound **8** showed good inhibition of naringinase with an IC_{50} of 6 μM. This compound also showed inhibition of β-glucosidase and α-galactosidase, with IC_{50} values of 59 and 251 μM respectively. The neutral urea derivative **10** displayed selective inhibition of β-galactosidase with an IC_{50} of 27 μM (Figure 2), while none of the other glycosidases were inhibited by this compound. Incorporation of the guanidinium group in **13** proved to have a significant effect. With the exception of naringinase, **13** displayed more potent inhibition of the glycosidases tested relative to the alkylated analogue **8**. Of particular note is the observation that both α- and β-galactosidase are effectively inhibited by **13** with IC_{50} values in the low micromolar range.

While the potencies of the guanidinium derivative **13** were promising, structural variation of the guanidinium substituent was introduced to study the influence on selectivity. The series of derivatives **14-19** (Figure 3), were synthesized using the same method as for **13** and include alkyl, aryl hydrophilic groups along

Table 1. Glycosidase inhibition values obtained for deoxynojirimycin derivatives.

Enzyme	1	8	10	13
α-galactosidase (green coffee beans)	23 ± 3	251 ± 38	>1000	18 ± 8
β-galactosidase (bovine liver)	>1000	>1000	27 ± 2	15 ± 1
α-glucosidase (baker's yeast)	167 ± 27	134 ± 19	>1000	20 ± 10
β-glucosidase (almonds)	312 ± 62	59 ± 2	>1000	41 ± 5
α-mannosidase (Jack beans)	>1000	>1000	>1000	113 ± 11
β-mannosidase (*Helix pomatia*)	>1000	>1000	>1000	408 ± 45
Naringinase (*Penicilium decumbens*)	33 ± 4	6 ± 3	>1000	>1000

IC_{50} values are reported in μM and are averages obtained from triplicate analysis for each compound.



Scheme 1. Synthesis of *N*-alkylated, urea, guanidinium derivatives of deoxynojirimycin 1. **Reagents and conditions:** (a) **6**, 1-bromohexane, K_2CO_3, DMF, 62%; (b) H_2, Pd/C, EtOH, HCl (1M); (c) **6**, n-butyl isocyanate, dimethoxyethane, 70%; (d) **11**, EDCI, NEt_3, CH_2Cl_2, 64%; (e) CbzNCS, CH_2Cl_2, 68%.

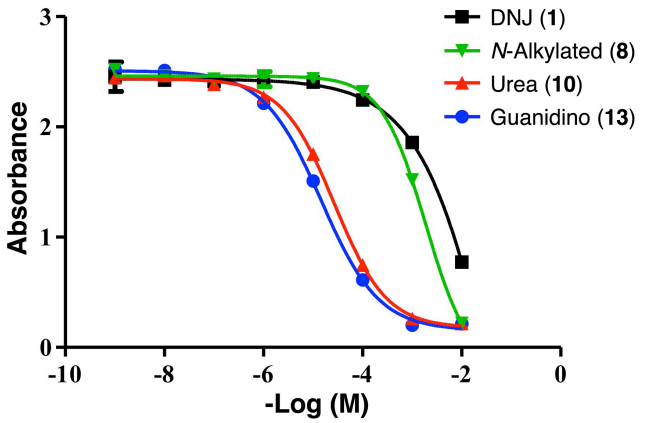


Figure 2. Representative data and fitted curves for the inhibition of β-galactosidase (from bovine liver) by compounds **1**, **8**, **10**, and **13** (for complete inhibition data for all glycosidases tested see Supporting Information section).

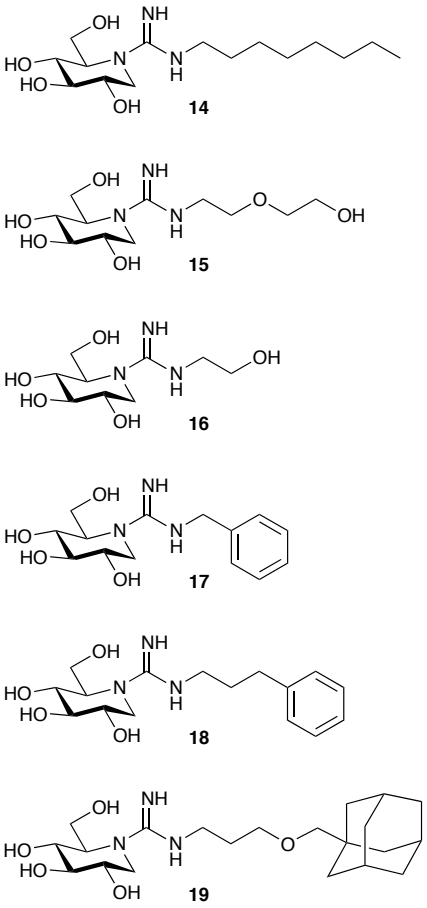


Figure 3. N^G-substituted guanidinium deoxynojirimycin analogues prepared to examine the effect of various N^G-substituents on glycoside inhibtion activity (all HCl-salts).

with the bulky adamantyl group. Evaluation of the series of compounds as inhibitors of a panel of glycosidases revealed interesting variations. For the α-galactosidase inhibition only the saturated alkyl chain-containing **13** and **14** showed good inhibition. The same was also true for α-glucosidase and to a lesser extent for α-mannosidase. In contrast most compounds were able to inhibit β-galactosidase, while the non-guanidino compounds **1** and **8** were not. Looking at the individual compounds it is noticeable that a few compounds such as **14**, **18** and **19** had a preference for one of the tested glycosidases, indicating that the guanidino substituents can be used to direct the selectivity.

Conclusions

In summary, we have successfully devised a straightforward route to a new class of iminosugar derivatives and investigated their ability to inhibit a diverse set of glycosidases. First an alkylated, a urea, and a guanidinium derivative were prepared and their screening against a series of glycosidases showed striking inhibition profiles. Interestingly, the neutral urea derivative **10** proved to be a very selective β-galactosidase inhibitor. Incorporation of the guanidine moiety in **13** led to enhanced potency against several glycosidases. In order to introduce more selectivity the arginine substituent was varied in a second series of compounds. From this series it was shown that selective compounds such as **14**, **18** or **19** could be obtained. This further indicates the promise of the guanidinium motif for the creation of therapeutic glycosidase inhibitors. The guanidinium incorporation seems to alter both the steric and electronic properties of the iminosugar. Its charge is likely more delocalized than in the case of the *N*-alkylated **8**. Furthermore, due to the degree of sp^2 hybridization 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. Future efforts will be aimed at exploring the incorporation of the guanidine centre in other iminosugar structures as a means of further enhancing both the potency and selectivity of glycoside inhibition. In addition, future investigations will explore the use of these guanidinium and urea modified iminosugars for inhibiting enzymes of medicinal interest. Targets may include trehalase enzymes for development of antifungal or antibiotic agents,[25] α-glucosidases located in the endoplasmic reticulum towards anti-cancer agents,[17, 26] and targets related to hepatitis,[6] diabetes,[6] and Gaucher disease.[27]

Table 2. Glycosidase inhibition values obtained for N^G-substituted guanidinium deoxynojirimycin analogues.

Enzyme	**1**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
α-galactosidase (green coffee beans)	23 ± 3	18 ± 8	23 ± 8	>1000	540 ± 46	>1000	>1000	>1000
β-galactosidase (bovine liver)	>1000	15 ± 1	2.6 ± 0.4	>1000	>1000	5.1 ± 0.3	6.2 ± 0.7	5.4 ± 0.4
α-glucosidase (baker's yeast)	167 ± 27	20 ± 10	31 ± 10	251 ± 230	133 ± 34	>1000	>1000	131 ± 23
β-glucosidase (almonds)	312 ± 62	41 ± 5	25 ± 2	>1000	457 ± 42	4.3 ± 0.2	67 ± 3	489 ± 29
α-mannosidase (Jack beans)	>1000	113 ± 11	55 ± 14	>1000	>1000	>1000	>1000	>1000
Naringinase (*Penicilium decumbens*)	33 ± 4	>1000	67 ± 13	172 ± 96	645 ±498	27 ± 3	33 ± 6	22 ± 11

*IC_{50} values are reported in μM and are averages obtained from triplicate analysis for each compound. For ease of comparison, the IC_{50} values obtained for compounds **1** and **13** shown in Table 1 are again included here.*

Experimental Section

General remarks

Reagents, solvents and solutions. 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**,[28] and Cbz-NCS[19] were prepared as previously described. The preparation of compounds **15**, **16**, and **19** each required access to non-commercial amine building blocks that were prepared according to established literature procedures.[29-31 28]

Purification Techniques. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using plates with a UV fluorescent indicator (normal SiO_2, Merck 60 F_{254}). One or more of the following methods were used for visualization: 10% H_2SO_4 in MeOH or ninhydrine. Flash chromatography was performed according to the method of Still *et al*. using Merck type 60, 230-400 mesh silica gel. Removal of solvent was performed under reduced pressure using a rotary evaporator.

Analytical HPLC runs were performed on a Shimadzu automated HPLC system with a reversed phase column (Alltima, C8, 90Å, 5 µm, 250x4.6 mm) equipped with an evaporative light scattering detector (PL-ELS 1000, Polymer Laboratories) and a UV/VIS detector operating at 22 and 254 nm. Elution was effected using a gradient of 5% MeCN and 0.1% TFA in H_2O to 5% H_2O and 0.1% TFA in MeCN.

Instrumentation for Compound Characterization. ^{1}H NMR spectra were recorded at 300 MHz with chemical shifts reported in parts per million (ppm) downfield relative to tetramethylsilane (Me_4Si) or H_2O (δ 4.8). ^{1}H 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. ^{13}C NMR spectra were recorded at 75.5 MHz with chemical shifts reported relative to $CDCl_3$ δ 77.0. ^{13}C NMR spectra were recorded using the attached proton test (APT) sequence. All literature compounds had ^{1}H NMR, and mass spectra consistent with the assigned structures.

Experimental Procedures and Data for Compounds 7-19

***N*-hexyl-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin (7)**. To a solution of 1-DNJ (**6**) (500 mg, 0.95 mmol) in DMF (5 mL) was added K_2CO_3 (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 (SiO_2, 1:5 EtOAc/hexane) to yield **7** (363 mg, 62%) of a colorless oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 0.87 (t, CH_3, *J* = 6.74 Hz, 3H), 1.11 – 1.40 (m, CH_2, 8H), 2.19 – 2.31 (m, CH_2NH, 2H), 2.51 – 2.68 (m, 2H), 3.09 (m, 1H), 3.43 – 3.70 (m, 5H), 4.41 – 4.98 (m, CH_2C$_{arom}$, 8H), 7.12 – 7.34 (m, CH_{arom}, 20H). δ_C (75.5 MHz; $CDCl_3$; Me_4Si) 14.0 (*C*H_3), 22.5, 23.4, 27.1, 31.6 (*C*H_2), 52.3, 54.4 (*C*H_2NH), 72.6, 73.3, 75.1, 75.2 (*C*H_2), 63.6, 78.5, 87.3 (*C*-2, *C*-3, *C*-4, *C*-5), 127.3, 127.4, 127.5, 127.7, 128.2 (*C*H$_{arom}$), 137.7, 138.5, 139.0 (C_q). R_f = 0.38 (EtOAc/hexane 1:5). HRMS Calcd for $C_{40}H_{49}NO_4$ $[M+H]^+$, 608.3740, found 608.3729.

***N*-hexyl-1-deoxynojirimycin (8)**. 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 H_2 (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_H (300 MHz; D_2O) 0.75 (br s, CH_3, 3H), 1.20 (br m, CH_2, 6H), 1.55 (br s, CH_2, 2H), 2.73 – 2.84 (m, 2H), 2.95 (s, 1H), 3.06 (s, 1H), 3.32 (m, 2H), 3.46 (m, 1H), 3.51 (br s, 1H), 3.62 – 3.93 (m, 2H). δ_C (75.5 MHz; D_2O) 13.4 (*C*H_3), 22.0, 22.6, 25.8, 30.7 (*C*H_2), 52.9, 53.7, 55.0, 65.3, 67.0, 68.1, 76.7 (*C*H_2NH), *C*-2, *C*-3, *C*-4, *C*-5, *C*-6). HRMS for $C_{12}H_{25}NO_4$ $[M+H]^+$, 248.1862, found 248.1857.

***N*-1-butylurea-2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin (9)**. To a solution of 1-DNJ (**6**) (200 mg, 0.38 mmol, 1 eq.) in dimethoxyethane (3 mL) was added n-butyl isocyanate (86 µL, 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 (SiO_2, 2:5 EtOAc/hexane) to yield **9** (168 mg, 70%) as a colorless oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 0.82 (t, CH_3, *J* = 7.01 Hz, 3H), 1.17 – 1.30 (m, CH_2, 4H), 3.06 – 3.14 (m, CH_2NH, 2H), 3.29 (dd, J_A = 3.71 Hz, J_B = 10.18 Hz, 1H), 3.53 – 3.76 (m, 5H), 3.92 (dd, J_A = 4.68 Hz, J_B = 9.35 Hz, 1H), 3.99 – 4.05 (m, 1H), 4.44 (d, CH_2C$_{arom}$, *J* = 2.75 Hz, 2H), 4.47 (s, CH_2C$_{arom}$), 1H), 4.52 (d, *J* = 2.75 Hz, 1H), 4.56 (s, CH_2C$_{arom}$, 1H), 4.62 (s, CH_2C$_{arom}$, 1H), 4.70 and 4.74 (2 x d, CH_2C$_{arom}$, 2 x 1H), 5.47 (t, *J* = 5.23 Hz, 1H), 7.20 – 7.36 (m, CH_{arom}, 20H). δ_C (75.5 MHz; $CDCl_3$; Me_4Si) 13.7 (*C*H_3), 19.9, 31.8 (*C*H_2), 40.5, 40.6 (*C*H_2NH), 70.8, 71.0, 72.8, 73.3 (*C*H_2), 56.9, 75.8, 78.8, 82.1 (*C*-2, *C*-3, *C*-4, *C*-5), 127.6, 127.7, 127.9, 128.2 (*C*H$_{arom}$), 137.4, 137.8, 137.9, 138.0 (C_q), 159.2 (NH*C*(O)). R_f = 0.40 (EtOAc/hexane 2:1). HRMS Calcd for $C_{39}H_{46}N_2O_5$ $[M+H]^+$, 623.3485, found 623.3471.

***N*-1-butylurea-1-deoxynojirimycin (10)**. 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 H_2 (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 as the HCl-salt (81 mg, quant.). δ_H (300 MHz; D_2O) 0.89 (t, CH_3, 3H), 1.35 (m, CH_2, 2H), 1.48 (m, CH_2, 2H), 3.14 – 3.21 (m, CH_2NH, 2H), 3.41 (d, *J* = 12.1 Hz, 1H), 3.61 (br s, 1H), 3.72 – 3.86 (m, 6H). δ_C (75.5 MHz; D_2O) 13.3 (*C*H_3), 19.6, 31.6 (*C*H_2), 40.4, 44.2 (*C*H_2NH), 60.0, 68.7, 71.1, 74.0 (*C*-2, *C*-3, *C*-4, *C*-5, *C*-6), 160.5 (NH*C*(O)). HRMS Calcd for

$C_{11}H_{22}N_2O_5$ $[M+H]^+$, 263.1607, 263.1583.

***N*-(benzyloxycarbonyl) *N*'-(butyl) thiourea (11)**. A solution of n-butylamine (309 µL, 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 (t, CH_3, 3H), 1.26 – 1.41 (m, CH_2, 2H), 1.44 – 1.66 (m, CH_2, 2H), 3.62 (q, NHCH_2, 2H), 5.13 (s, CH_2C_{arom}, 2H), 7.26 – 7.40 (m, CH_{arom}, 5H), 8.80 (br s, N*H*, 1H), 9.66 (br s, N*H*, 1H). δ_C (75.5 MHz; $CDCl_3$; Me_4Si) 13.5 (*C*H_3), 19.8, 30.0 (*C*H_2), 45.1 (NH*C*H_2), 67.6 (*C*H_2C_{arom}), 127.5, 127.8, 128.1, 128.4, 128.7, 128.8 (*C*H_{arom}), 134.4 (C_q), 152.5 (NH*C*(S)NH), 178.7 (NH*C*(O)). R_f = 0.55 (EtOAc/hexane 1:5). HRMS Calcd for $C_{13}H_{18}N_2O_2S$ $[M+H]^+$, 267.1167, found 267.1155.

***N*-(benzyloxycarbonyl) *N*'-(butyl) 2,3,4,6-tetra-*O*-benzyl-deoxynojirimycin (12)**. Thiourea **11** (305 mg, 1.14 mmol, 2 eq.) in CH_2Cl_2 (10 mL) was treated with 1-DNJ (**6**) (300 mg, 0.57 mmol, 1 eq.), EDCI (220 mg, 1.14 mmol, 2 eq.) and Et_3N (160 µL, 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 → 1:2 → 1:1 → 2:1) to yield **12** (270 mg, 64%) as a colorless oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 0.77 (t, CH_3, J = 7.15 Hz, 3H), 1.08 – 1.32 (m, CH_2, 4H), 2.93 – 3.04 and 3.06 – 3.14 (2 x m, CH_2NH, 2 x 1H), 3.43 (dd, J_A = 3.2 Hz, J_B = 10.4 Hz, 1H), 3.59 – 3.79 (m, 6H), 3.98 – 4.03 (m, 1H), 4.40 (s, CH_2C_{arom}, 2H, 4.47 and 4.51 (2 x d, CH_2C_{arom}, J = 6.85 Hz, 2 x 1H), 4.61 (s, CH_2C_{arom}, 2H), 4.64 and 4.68 (2 x d, CH_2C_{arom}, 2 x 1H), 5.09 and 5.16 (2 x d, CH_2C_{arom}, J = 12.65 Hz, 2 x 1H), 7.20 – 7.42 (m, CH_{arom}, 25H). δ_C (75.5 MHz; $CDCl_3$; Me_4Si) 13.5 (*C*H_3), 19.7, 31.5 (*C*H_2), 43.5, 44.3 (*C*H_2NH), 66.4, 69.7, 71.1, 72.6, 72.9, 73.2 (*C*H_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 (*C*H_{arom}), 137.4, 137.7, 137.9 (C_q), 160.8 (NH*C*(O)), 162.9 (NH*C*(N). R_f = 0.59 (EtOAc/hexane 1:1). HRMS Calcd for $C_{47}H_{53}N_3O_6$ $[M+H]^+$, 756.4013, found 756.3996.

***N*-1-butylguanidine-1-deoxynojirimycin (13)**. 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 H_2 (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; D_2O) 0.93 (t, CH_3, J = 7.1 Hz, 3H), 1.35 – 1.43 (m, CH_2, 2H), 1.60 – 1.64 (m, CH_2, 2H), 3.28 – 3.33 (m, NCH_2, 2H), 3.60 and 3.68 (2 x s, 2 x 2H), 3.85 – 3.99 (m, 4H). δ_C (75.5 MHz; D_2O) 12.7 (*C*H_3), 30.0, 42.0, 45.2 (*C*H_2), 59.2 (*C*H_2), 63.7, 68.0, 70.4, 72.9 (*C*-2, *C*-3, *C*-4, *C*-5). HRMS Calcd for $C_{11}H_{23}N_3O_4$ $[M+H]^+$, 262.1767, found 262.1771.

General one-pot procedure for the preparation of N^G-substituted guanidine modified DNJ derivatives 14-19. In the preparation of compounds **14-19** a one-pot procedure was employed to generate the fully protected N^G-substituted guanidine modified DNJ derivatives. In this procedure, the primary amine of interest was first converted to the corresponding thiourea by treatment with an equimolar quantity Cbz-NCS. Formation of the thiourea is generally complete within minutes and can be followed via TLC. A two-fold excess of the thiourea was then directly activated with EDCI/NEt_3 and treated with DNJ building block **6** to yield protected N^G-substituted guanidine modified DNJ derivatives **14a-19a**. Global deprotection of the intermediate protected compounds was achieved via hydrogenation over 10% Pd/C in slightly acidic media to yield final products **14-19**. As an example, the preparation of compounds **14a** and **14** are provided below.

***N*-(benzyloxycarbonyl) *N*'-(octyl) 2,3,4,6-tetra-*O*-benzyl-deoxynojirimycin (14a)**. A solution of Octylamine (198 mL, 1.2 mmol) in CH_2Cl_2 (10 mL) was treated with a equimolar quantity of Cbz-NCS (delivered as 2.4 mL of a 0.5 M solution in CH_2Cl_2). The resulting reaction mixture was stirred for 30 min at RT at which point TLC indicated complete conversion to the thiourea. To the crude thiourea was added the protected DNJ building block **6** (314 mg, 0.6 mmol, 1 eq.), EDCI (230 mg, 1.2 mmol, 2 eq.) and Et_3N (167 µL, 1.2 mmol, 2 eq.). The resulting reaction mixture was stirred for 18 h at rt. The reaction mixture was concentrated *in vacuo* and the residue applied directly to a silica column. The product was isolated by eluting with a gradient of EtOAc/hexane (1:2 → 1:1 → 2:1) to yield the fully protected **14a** (255 mg, 52%) as a colorless oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 0.90 (t, J = 6.9 Hz, 3H), 1.06 – 1.37 (m, 12H), 2.93 – 3.19 (m, 2H), 3.45 (dd, J_A = 3.2 Hz, J_B = 13.5 Hz, 2H), 3.59 – 3.83 (m, 6H), 4.04 (q, J = 5.1 Hz, 1H), 4.43 (s, 2H), 4.50 (d, J = 6.3 Hz, 1H), 4.54 (d, J = 6.2 Hz, 1H), 4.61 – 4.71 (m, 4H), 5.08 – 5.22 (m, 2H), 7.22 – 7.46 (m, 25H). δ_C (75.5 MHz; $CDCl_3$; Me_4Si) 14.4, 22.9, 27.0, 29.4, 29.9, 32.0, 44.3, 44.7, 59.0, 66.8, 70.1, 71.6, 73.0, 73.4, 73.7, 75.7, 78.1, 81.0, 127.6, 128.2, 128.4, 128.6, 137.8, 138.1, 138.2, 138.3, 161.2, 163.3. HRMS Calcd for $C_{51}H_{61}N_3O_6$ $[M+H]^+$, 812.4633, found 812.4637.

***N*-1-octylguanidine-1-deoxynojirimycin (14)**. A suspension of fully protected **14a** (103 mg, 123 µmol) 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 H_2 (g) atmosphere for 18 h at rt. The reaction mixture was next filtered over celite, washed with EtOH and concentrated *in vacuo*. **14** was obtained after lyophilization as the HCl-salt (44 mg, quant.). δ_H (500 MHz; D_2O) 0.74 – 0.86 (t, 3H), 1.16 – 1.34 (m, 12H), 1.50 – 1.62 (m, 2H), 3.17 – 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). δ_{HSQC} (500 MHz; D_2O) 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 $C_{15}H_{32}N_3O_4$ $[M+H]^+$, 318.24, found 318.50.

***N*-(benzyloxycarbonyl) *N*'-(2-2-(benzyloxy)ethoxy)ethyl) 2,3,4,6-tetra-*O*-benzyl-deoxynojirimycin (15a)**. As described above for compound **14a**, working at a 0.6 mmol scale (relative to protected DNJ building block **6**), **15a** (499 mg, 95%) was obtained as a colorless oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 3.20 – 3.47 (m, 5H), 3.49 (s, 4H), 3.58 – 3.68 (m, 2H), 3.69 – 3.82 (m,

4H), 4.05 – 4.17 (m, 1H), 4.42 (s, 2H), 4.45 – 4.52 (m, 4H), 4.58 (s, 2H), 4.64 (d, J = 11.6 Hz, 1H), 5.14 (d, J = 7.2 Hz, 2H), 7.18 – 7.47 (m, 30H). δ_C (75.5 MHz; $CDCl_3$; Me_4Si) 43.7, 44.5, 58.9, 66.9, 68.8, 69.8, 71.5, 72.9, 73.0, 73.3, 73.5, 75.5, 78.0, 80.9, 127.9, 128.0, 128.1, 128.2, 128.4, 128.6, 137.9, 138.1, 138.3, 160.7, 162.9. HRMS Calcd for $C_{54}H_{59}N_3O_8$ $[M+H]^+$, 878.4375, found 878.4413.

***N*-1-(2-(2-hydroxyethoxy)ethyl)guanidine-1-deoxynojirimycin (15)**. **15a** (103 mg, 117 µmol) was deprotected as described above for the preparation of **14** to yield **15** as the HCl-salt (39 mg, quant.). δ_H (500 MHz; D_2O) 3.46 (t, 2H), 3.53 – 3.58 (m, 2H), 3.58 – 3.64 (m, 4H), 3.64 – 3.73 (m, 4H), 3.78 – 3.85 (m, 2H), 3.85 – 3.92 (m, 2H). δ_{HSQC} (500 MHz; D_2O) 57.1, 60.0, 73.8, 74.9, 78.6, 82.6, 83.8, 85.0, 86.4, 87.5. LRMS Calcd for $C_{11}H_{24}N_3O_6$ $[M+H]^+$, 294.17, found 294.35.

***N*-(benzyloxycarbonyl) *N*'-(2-(benzyloxy)ethyl) 2,3,4,6-tetra-*O*-benzyl-deoxynojirimycin (16a)**. As described above for compound **14a**, working at a 0.6 mmol scale (relative to protected DNJ building block **6**), **16a** (403 mg, 80%) was obtained as an yellowish oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 3.21 – 3.34 (m, 1H), 3.36 – 3.49 (m, 4H), 3.58 – 3.82 (m, 6H), 4.04 – 4.19 (m, 1H), 4.39 (d, J = 4.2 Hz, 2H), 4.48 (dd, J_A = 3.6 Hz, J_B = 11.6, 2H), 4.60 (d, J = 4.5 Hz, 2H), 4.62 – 4.69 (m, 2H), 5.10 – 5.24 (m, 2H), 7.14 – 7.51 (m, 30H). δ_C (75.5 MHz; $CDCl_3$; Me_4Si) 43.6, 44.6, 58.8, 66.9, 69.6, 69.8, 70.4, 71.4, 72.9, 73.3, 73.4, 73.5, 75.4, 78.0, 80.9, 128.0, 128.1, 128.2, 128.4, 128.5, 128.6, 138.0, 138.1, 138.3, 138.4, 160.7, 162.9. HRMS Calcd for $C_{52}H_{55}N_3O_7$ $[M+H]^+$, 834.4113, found 834.4135.

***N*-1-(2-hydroxyethyl)guanidine-1-deoxynojirimycin (16)**. The protected intermediated **16a** (102 mg, 123 µmol) was deprotected as described above for the preparation of **14**. Compound **16** was thus obtained as the HCl-salt (35 mg, quant.). δ_H (500 MHz; D_2O) 3.36 – 3.42 (m, 2H), 3.50 – 3.58 (m, 2H), 3.58 – 3.65 (m, 2H), 3.65 – 3.75 (m, 2H), 3.77 – 3.93 (m, 4H). δ_{HSQC} (500 MHz; D_2O) 59.2, 59.9, 73.8, 74.7, 78.5, 82.6, 84.9, 87.4. LRMS Calcd for $C_9H_{20}N_3O_5$ $[M+H]^+$, 250.14, found 250.35.

***N*-(benzyloxycarbonyl) *N*'-(benzyl) 2,3,4,6-tetra-*O*-benzyl-deoxynojirimycin (17a)**. As described above for compound **14a**, working at a 0.6 mmol scale (relative to protected DNJ building block **6**), **17a** (433 mg, 91%) was obtained as a colorless oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 3.44 – 3.53 (m, 1H), 3.61 – 3.70 (m, 2H), 3.74 (m, 2H), 3.78 (d, J = 4.9 Hz, 2H), 4.04 – 4.12 (m, 1H), 4.20 – 4.34 (m, 2H), 4.37 (d, J = 2.0 Hz, 2H), 4.48 (dd, J_A = 2.3 Hz, J_B = 11.6 Hz, 2H), 4.55 – 4.69 (m, 4H), 5.09 – 5.25 (m, 2H), 7.06 – 7.56 (m, 30H). δ_C (75.5 MHz; $CDCl_3$; Me_4Si) 44.8, 48.1, 59.0, 67.0, 69.8, 71.6, 72.9, 73.3, 73.5, 75.4, 80.4, 128.0, 128.6, 137.8, 138.0, 138.2, 161.1, 163.0. HRMS Calcd for $C_{50}H_{51}N_3O_6$ $[M+H]^+$, 790.3851, found 790.3839.

***N*-1-benzylguanidine-1-deoxynojirimycin (17)**. The protected intermediated **17a** (99 mg, 125 µmol) was deprotected as described above for the preparation of **14.** Compound **17** was thus obtained as the HCl-salt (41 mg, quant.). δ_H (500 MHz; D_2O) 3.29 (s, 1H), 3.51 – 3.60 (m, 2H), 3.60 – 3.67 (m, 2H), 3.74 – 3.81 (m, 1H), 3.83 – 3.94 (m, 2H), 4.42 – 4.54 (m, 2H), 7.30 – 7.42 (m, 5H). δ_{HSQC} (500 MHz; D_2O) 59.8, 60.2, 63.4, 73.7, 78.3, 82.6, 84.7, 87.2, 141.6, 142.6, 143.5. LRMS Calcd for $C_{14}H_{22}N_3O_4$ $[M+H]^+$, 296.16, found 296.10.

***N*-(benzyloxycarbonyl) *N*'-(3-phenylpropyl) 2,3,4,6-tetra-*O*-benzyl-deoxynojirimycin (18a)**. As described above for compound **14a**, working at a 0.6 mmol scale (relative to protected DNJ building block **6**), **18a** (476 mg, 97%) was obtained as a colorless oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.61 (m, 2H), 1.87 (m, 1H), 2.47 (dd, J_A = 6.5 Hz, J_B = 9.0 Hz, 2H), 2.98 – 3.27 (m, 2H), 3.47 (dd, J_A = 3.1 Hz, J_B = 13.5 Hz, 1H), 3.63 – 3.86 (m, 8H), 4.03 – 4.12 (m, 1H), 4.44 (s, 2H), 4.53 (dd, J_A = 5.5 Hz, J_B = 11.5 Hz, 2H), 4.61 – 4.73 (m, 4H), 5.08 – 5.25 (m, 2H), 6.95 – 7.68 (m, 30H). δ_C (75.5 MHz; $CDCl_3$; Me_4Si) 25.9, 31.7, 33.3, 43.8, 44.7, 59.0, 66.9, 68.2, 70.1, 71.6, 73.0, 73.3, 73.7, 75.6, 78.0, 80.6, 126.1, 127.7, 128.0, 128.1, 128.2, 128.5, 128.6, 128.7, 137.8, 138.1, 138.2, 138.3, 141.5, 161.3, 163.3. HRMS Calcd for $C_{52}H_{55}N_3O_6$ $[M+H]^+$, 818.4164, found 818.4155.

***N*-1-(3-phenylpropyl)guanidine-1-deoxynojirimycin (18)**. The protected intermediated **18a** (100 mg, 122 µmol) was deprotected as described above for the preparation of **14.** Compound **18** was thus obtained as the HCl-salt (42 mg, quant.). δ_H (300 MHz; D_2O) 1.89 – 2.05 (m, 2H), 2.73 (t, 2H), 3.24 – 3.39 (m, 2H), 3.52 (d, 2H), 3.60 – 3.69 (m, 2H), 3.75 – 3.98 (m, 4H), 7.25 – 7.43 (m, 5H). δ_C (75.5 MHz; D_2O) 29.4, 32.0, 41.6, 45.1, 48.8, 59.2, 63.7, 68.0, 70.3, 72.8, 126.2, 128.4, 128.7, 141.4, 158.0, 163.2. LRMS Calcd for $C_{16}H_{26}N_3O_4$ $[M+H]^+$, 324.19, found 324.10.

***N*-(benzyloxycarbonyl) *N*'-(3-adamantylpropyl) 2,3,4,6-tetra-*O*-benzyl-deoxynojirimycin (19a)**. As described above for compound **14a**, working at a 0.45 mmol scale (relative to protected DNJ building block **6**), **19a** (379 mg, 94%) was obtained as a colorless oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.87 (s, 2H), 3.11 – 3.23 (m, 2H), 3.24 – 3.33 (m, 2H), 3.44 (dd, J_A = 3.1 Hz, J_B = 13.3 Hz, 1H), 3.59 – 3.81 (m, 6H), 4.01 – 4.11 (m, 1H), 4.41 (s, 2H), 4.51 (dd, J_A = 4.3 Hz, J_B = 11.6 Hz, 2H), 4.59 – 4.71 (m, 4H), 5.07 – 5.20 (m, 2H), 7.19 – 7.45 (m, 25H). δ_C (75.5 MHz; $CDCl_3$; Me_4Si) 28.4, 29.9, 34.4, 37.4, 39.9, 41.9, 66.9, 69.6, 71.5, 73.1, 73.3, 73.6, 75.5, 78.1, 81.2, 82.5, 128.0, 128.1, 128.2, 128.4, 128.5, 128.6, 137.9, 138.2, 138.3, 161.0, 163.0. HRMS Calcd for $C_{57}H_{67}N_3O_7$ $[M+H]^+$, 906.5052, found 906.5039.

***N*-1-(3-adamantylpropyl)guanidine-1-deoxynojirimycin (19).** The protected intermediated **19a** (99 mg, 109 µmol) was deprotected as described above for the preparation of **14.** Compound **19** was thus obtained as the HCl-salt (49 mg, quant.). δ_H (500 MHz; D_2O) 1.21 (t, 1H), 1.46 (s, 6H), 1.54 – 1.70 (m, 6H), 1.77 – 1.86 (m, 2H), 1.88 (s, 3H), 2.98 – 3.02 (s, 2H), 3.25 – 3.41 (m, 2H), 3.44 – 3.53 (m, 2H), 3.52 – 3.57 (m, 2H), 3.62 (s, 2H), 3.75 – 3.84 (m, 2H), 3.84 – 3.92 (m, 2H). δ_{HSQC} (500 MHz; D_2O) 42.6, 42.7, 51.3, 53.7, 53.8, 59.8, 61.2, 63.4, 73.8, 78.5, 82.6, 82.2, 87.7, 84.8, 96.4. LRMS Calcd for $C_{21}H_{38}N_3O_5$ $[M+H]^+$, 412.28, found 412.20.

General Procedure for Enzyme Inhibition Assays

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 glycosides substrates were purchased from Carbosynth Limited.

Inhibition assays were performed in either phosphate or acetate buffer at the optimum pH for each enzyme. Determination of the IC_{50} 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 iminosugar derivatives. The incubation mixture consisted of 15 µL of inhibitor solution in water (0.1 U/mL) and 15 µL 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.

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 addition of 75 µL 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 ^{o}C for 30 min, the reaction was quenched with 0.5M Na_2CO_3 (240 µL) and the absorbance of 4-nitrophenol released from the substrate was read immediately at 405 nm using a BioTek µQuant Microplate Spectrophotometer.

IC_{50} values were determined as a concentration of the iminosugars that inhibits 50% of the enzyme activity under the assay conditions. IC_{50} 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).

Acknowledgements

We would like to thank Cees Versluis and Mirjam Damen of Biomolecular Mass Spectrometry and Proteomics (Utrecht University) for measuring HRMS. We gratefully acknowledge the Dutch Technology Foundation STW, applied science division of NWO and the Technology Program of the Ministry of Economic Affairs, for the financial support.

References

1. N. Asano, *Cell. Mol. Life Sci.*, 2009, **66**, 1479-1492.
2. L. K. Campbell, D. E. Baker and R. K. Campbell, *Ann. Pharmacother.*, 2000, **34**, 1291-1301.
3. M. von Itzstein, *Curr. Opin. Chem. Biol.*, 2008, **12**, 102-108.
4. a) B. E. Smid, J. M. Aerts, R. G. Boot, G. E. Linthorst and C. E. Hollak, *Expert Opin. Investig. Drugs*, 2010, **19**, 1367-1379; b) Goddard-Borger, E. D.; Tropak, M. B.; Yonekawa, S.; Tysoe, C.; Mahuran, D. J.; Withers, S. G. *J. Med. Chem.* **2012**, 55, 2737.
5. D. A. Kuntz, S. Nakayama, K. Shea, H. Hori, Y. Uto, H. Nagasawa and D. R. Rose, *ChemBioChem*, 2010, **11**, 673-680.
6. G. Horne, F. X. Wilson, J. Tinsley, D. H. Williams and R. Storer, *Drug Discov. Today*, 2011, **16**, 107-118.
7. E. Borges de Melo, A. da Silveira Gomes and I. Carvalho, *Tetrahedron*, 2006, **62**, 10277-10302.
8. a) T. M. Gloster and G. J. Davies, *Org. Biomol. Chem.*, 2010, **8**, 305-320; b) Gloster, T. M.; Vocaldo, D. J.; *Nature Chem. Biol.* **2012**, *8,* 683.
9. W. Yu, T. Gill, L. Wang, Y. Du, H. Ye, X. Qu, J.-T. Guo, A. Cuconati, K. Zhao, T. M. Block, X. Xu and J. Chang, *J. Med. Chem.*, 2012, **55**, 6061-6075.
10. J. C. Lee, S. Francis, D. Dutta, V. Gupta, Y. Yang, J.-Y. Zhu, J. S. Tash, E. Sch√∂nbrunn and G. I. Georg, *J. Org. Chem.*, 2012, **77**, 3082-3098.
11. M. Liu, S. Wang, Y.-D. Zhou, T. Xiang, H. Dong, K. Yang and X.-L. Zhang, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 564-570.
12. H. S. Overkleeft, G. H. Renkema, J. Neele, P. Vianello, I. O. Hung, A. Strijland, A. M. van der Burg, G. J. Koomen, U. K. Pandit and J. M. Aerts, *J. Biol. Chem.*, 1998, **273**, 26522-26527.
13. J. Conchie, A. J. Hay, I. Strachan and G. A. Levvy, *Biochem. J.*, 1967, **102**, 929-941.
14. G. Papandreou, M. K. Tong and B. Ganem, *J. Am. Chem. Soc.*, 1993, **115**, 11682-11690.
15. P. Ermert, A. Vasella, M. Weber, K. Rupitz and S. G. Withers, *Carbohydr. Res.*, 1993, **250**, 113-128.
16. M. I. Garcia-Moreno, P. Diaz-Perez, C. O. Mellet and J. M. G. Fernandez, *Chem. Commun.*, 2002, 848-849.
17. E. M. Sanchez-Fernandez, R. Risquez-Cuadro, M. Chasseraud, A. Ahidouch, C. O. Mellet, H. Ouadid-Ahidouch and J. M. G. Fernandez, *Chemical Communications*, 2010, **46**, 5328-5330.
18. M. Aguilar-Moncayo, M. I. Garcia-Moreno, A. Trapero, M. Egido-Gabas, A. Llebaria, J. M. Fernandez and C. O. Mellet, *Org. Biomol. Chem.*, 2011, **9**, 3698-3713.
19. N. I. Martin, J. J. Woodward and M. A. Marletta, *Org. Lett.*, 2006, **8**, 4035-4038.
20. N. I. Martin, W. T. Beeson, J. J. Woodward and M. A. Marletta, *J. Med. Chem.*, 2008, **51**, 924-931.
21. N. I. Martin and R. M. Liskamp, *J. Org. Chem.*, 2008, **73**, 7849-7851.
22. N. R. Taylor and M. von Itzstein, *J. Med.Chem.*, 1994, **37**, 616-624.
23. T. Wennekes, B. Lang, M. Leeman, G. A. v. d. Marel, E. Smits, M. Weber, J. v. Wiltenburg, M. Wolberg, J. M. F. G. Aerts and H. S. Overkleeft, *Org. Process Res. Dev.*, 2008, **12**, 414-423.
24. J. S. Rountree, T. D. Butters, M. R. Wormald, S. D. Boomkamp, R. A. Dwek, N. Asano, K. Ikeda, E. L. Evinson, R. J. Nash and G. W. Fleet, *ChemMedChem*, 2009, **4**, 378-392.
25. D. Bini, F. Cardona, M. Forcella, C. Parmeggiani, P. Parenti, F. Nicotra and L. Cipolla, *Beilstein J. Org. Chem.*, 2012, **8**, 514-521.
26. T. M. Wrodnigg, A. J. Steiner and B. J. Ueberbacher, *Anti-Cancer Agents Med. Chem.*, 2008, **8**, 77-85.
27. J. M. Benito, J. M. Garcia Fernandez and C. O. Mellet, *Expert Opin. Ther. Pat.*, 2011, **21**, 885-903.
28. T. Wennekes, R. J. van den Berg, W. Donker, G. A. van der Marel, A. Strijland, J. M. Aerts and H. S. Overkleeft, *J. Org. Chem.*, 2007, **72**, 1088-1097.
29. A. Diez-Martinez, Z. Gultekin, I. Delso, T. Tejero and P. Merino, *Synthesis*, 2010, 678-688.
30. Y. T. Li, K. M. Mullen, T. D. W. Claridge, P. J. Costa, V. Felix and P. D. Beer, *Chem. Commun.*, 2009, 7134-7136.
31. E. Klein, S. DeBonis, B. Thiede, D. A. Skoufias, F. Kozielski and L. Lebeau, *Bioorg. Med. Chem.*, 2007, **15**, 6474-6488.