1-Azafagomine, Part 2[+]

Enantiospecific Synthesis of 1-Azafagomine

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Dedicated to Professor Pierre Sinaÿ on the occasion of his 60th birthday

Abstract: For the first time the two enantiomeric forms of the glycosidase inhibitor 1-azafagomine have been synthesised starting from D- and L-xylose. D-Xylose was converted to the 2,3,5-tribenzylfuranose, which upon reductive amination with *tert*-butyl carbazate gave the protected 1-hydrazino-1-deoxypentitol in high yield. *N*-acetylation, mesylation of the 4-OH, removal of the Boc group, cyclisation and deprotection gave (+)-1-azafagomine (**(+)-1**). By a similar sequence of reactions, L-xylose was converted to (−)-1-azafagomine (**(−)-1**). Enzymatic and other routes to optically pure 1-azafagomine were also studied. Compound **(−)-1** is a potent competitive glycosidase inhibitor, while **(+)-1** has no biological activity. The inhibition of almond β-glucosidase by **(−)-1** was found to be slow owing to a slow binding step of inhibitor to enzyme, with no subsequent conformational rearrangement. The rate constants for binding and release were found to be $3.3 \times 10^4\,\text{M}^{-1}\,\text{s}^{-1}$ and $0.011\ \text{s}^{-1}$, respectively, yielding $K_i = 0.33\ \mu\text{M}$.

Keywords: azasugars · enzyme inhibitors · glycosidases · iminosugars · lipases

Introduction

A great number of biochemical processes in human biology involve glycoside-cleaving enzymes.[1] There is therefore a considerable drug potential in selective inhibitors of glycosidases and similar enzymes. So far glycosidase inhibitors have been investigated as antidiabetic, antiviral or antimetastatic agents, work which has resulted in the antidiabetes drug acarbose[2] and the new antiinfluenza drug Zanamivir.[3] There is, however, a far greater potential in inhibition of these enzymes, provided the required potent, selective inhibitors can be designed and synthesised. An exciting development is the increased understanding of the elements required for glycosidase inhibition that is now being obtained,[4] which may allow future chemists to predict inhibitor structure to a greater extent.

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[+] Part 1: Ref. [8].

For some time it has been known that replacing the ring oxygen of a saccharide with nitrogen creates a glycosidase inhibitor.[5] Subsequently it was found that replacing the anomeric carbon of a saccharide with nitrogen also creates a powerful glycosidase inhibitor.[6] The latter type of inhibitor, isofagomines, were predominantly β-glycosidase inhibitors,[7] while the former type, nojirimycins, more predominantly inhibited α-glycosidases. An explanation for this has been suggested.[8]

It was recently found that the hybrid compound (±)-1-azafagomine (**(±)-1**) is a strong inhibitor of both β-glucosidase and α-glucosidase (Figure 1).[8] It was anticipated that the active enantiomer was the one resembling D-glucose and this then supported the hypothesis given in reference [8]. It may be argued, however, that since L-nojirimycin is an inhibitor[9] of glycosidases the racemic **1** gives an unclear picture in such a study, because some of the activity may be

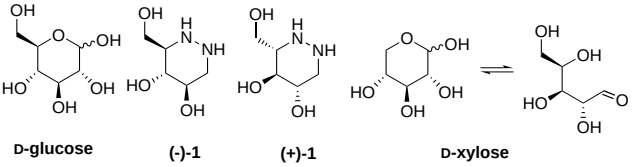

Figure 1. The stereochemical resemblance of 1-azafagomine (**1**) to glucose and xylose.

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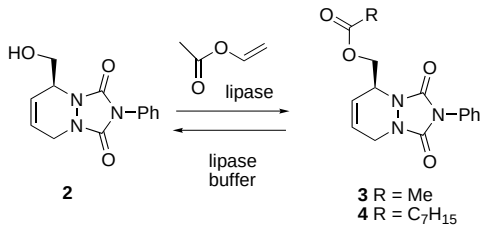
caused by the enantiomer resembling L-nojirimycin. It is therefore important to investigate the individual activities of the compounds, and in the present paper we describe the synthesis of both enantiomers of **1** and the investigation of their biological activities.

Results and Discussion

Synthesis of optically pure **1** was attempted by three different routes, with the third ultimately being successful. The first approach took advantage of the attractive short synthesis of racemic **1**.[8] A key intermediate in this synthesis was the alcohol **(±)-2**, which seemed an obvious candidate for enzymatic resolution using lipases. This could be performed in two ways, either by enzyme-catalysed esterification of **2** or by enzymatic hydrolysis of an ester of **2** (Scheme 1). Half-a-dozen enzymes were investigated for catalysis of both reactions. In general the esterification process gave the better conversion especially when carried out with vinyl acetate in organic solvent. The hydrolysis was extremely slow when the acetate **3**[8] was the substrate, but somewhat better when the octanoate **4** was used.



Scheme 1. Enzymatic resolution of **(±)-2**.

The results of the experiments yielding significant conversion are shown in Table 1. The enantiomeric excess of the products was determined by HPLC on an amylose acetate column. The immobilised *Mucor Mihei* lipase (*MM*L) from NOVO (Bagsvaerd, Denmark; Lipozyme) gave some of the best selectivity in catalysing esterification. Interestingly the selectivity with this enzyme varied widely with solvent. The highest *E* value (*E* = enantiomeric ratio; that is the enzyme's selectivity towards one enantiomer over the other), 3.8 in favor of conversion of the *R* enantiomer, was obtained in dimethoxymethane, while in benzene and acetonitrile no selectivity was observed. In chlorinated solvents the selectivity even reversed so that the *S* enantiomer was preferentially converted. The most selective enzyme was the *Humicola Thermo* lipase (*HT*L) which gave a selectivity of 5.6. The *Candida Antarctica* (*CA*L, Novozym) and the *Humicola Langinosa* (*HL*L) lipases were somewhat less efficient. *Aspergillus Niger* (*AN*L) and *Pseudomonas Cepacia* (*PC*L) lipases could be used to hydrolyse the octanoyl ester **4** to **(*R*)-2**, the latter with a selectivity comparable to the mucor-catalysed reaction. To summarise, the highest selectivity obtained was 5.6 for the *R* enantiomer, and this was not enough for the reaction to be useful for synthesis of the pure enantiomers of **1**.

During the chromatographic purification of enantiomerically enriched batches of both **2** and **3**, the phenomenon of enantiomeric enrichment on an achiral column[10] was observed. On chromatography of a 51% *ee* batch of acetate **(*R*)-3**, the early fractions of the column contained a higher content of *R* enantiomer (59% *ee*) and the late fractions had an *ee* of 38%.

The absolute configuration of the main product **(*R*)-3** was determined by converting a batch of **3**, obtained by Mucor-catalysed acetylation and having an enantiomeric excess of 50%, into 1-azafagomine (**1**) by deacetylation with NaOMe in MeOH to **2** followed by the previously published three-step procedure[8] of epoxidation, hydrolysis and hydrazinolysis. The rotation of this product $[\alpha]_D^{25}$ was measured as -5.4 corresponding to **1** with an enantiomeric excess of **(−)-1** of 54%. The rotation and configuration of pure **(−)-1** was determined by synthesis from L-xylose, as described later in this paper.

Since enzymatic resolution of **2** was too inefficient we turned our attention to total synthesis of **1** from a carbohydrate. Inspection of the structure of D-xylose (Figure 1) revealed that a nucleophilic substitution of the 4-OH with

Table 1. Enantioselectivity of the enzyme-catalysed acylation/deacylation reactions.

Enzyme[a]	Substrate	Major product	Solvent	Reactant	Conversion [%]	*ee* [%]	*E*
*CA*L	**(±)-2**	**(*R*)-3**	dioxane	$AcOCH{=}CH_2$	31	41	2.8
*CA*L	**(±)-2**	**(*R*)-3**	$AcOCH{=}CH_2$	$AcOCH{=}CH_2$	36	39	2.8
*CA*L	**(±)-2**	**(*R*)-4**	dioxane	$C_7H_{15}COOMe$	17	41	2.6
*CA*L	**(±)-4**	**(*R*)-2**	dioxane	MeOH	14	30	2.0
*MM*L	**(±)-2**	**(*R*)-3**	DME	$AcOCH{=}CH_2$	30	51	3.8
*MM*L	**(±)-2**	**(*R*)-3**	dioxane	$AcOCH{=}CH_2$	46	37	2.9
*MM*L	**(±)-2**	**(*R*)-3**	MIBK	$AcOCH{=}CH_2$	12	27	1.8
*MM*L	**(±)-2**	**(*S*)-3**	TCE	$AcOCH{=}CH_2$	55	29	2.5
*MM*L	**(±)-2**	**(*S*)-3**	$CHCl_3$	$AcOCH{=}CH_2$	28	22	1.7
*MM*L	**(±)-2**	**(±)-3**	C_6H_6	$AcOCH{=}CH_2$	50	0	1
*MM*L	**(±)-2**	**(±)-3**	MeCN	$AcOCH{=}CH_2$	25	0	1
*HT*L	**(±)-2**	**(*R*)-3**	$AcOCH{=}CH_2$	$AcOCH{=}CH_2$	26	48	3.4
*HL*L	**(±)-2**	**(*R*)-3**	$AcOCH{=}CH_2$	$AcOCH{=}CH_2$	35	61	5.6
*AN*L	**(±)-4**	**(*R*)-2**	aq. $CHCl_3$	H_2O	10	20	1.5
*PC*L	**(±)-2**	**(*S*)-3**	$AcOCH{=}CH_2$	$AcOCH{=}CH_2$	28	21	1.7
*PC*L	**(±)-4**	**(*R*)-2**	aq. $CHCl_3$	H_2O	41	47	3.8

[a] *CA*L: *Candida Antarctica* lipase; *MM*L: *Mucor Mihei* lipase; *HT*L: *Humicola Thermo* lipase; *HL*L: *Humicola Langinosa* lipase; *AN*L: *Aspergillus Niger* lipase; *PC*L: *Pseudomonas Cepacia* lipase.

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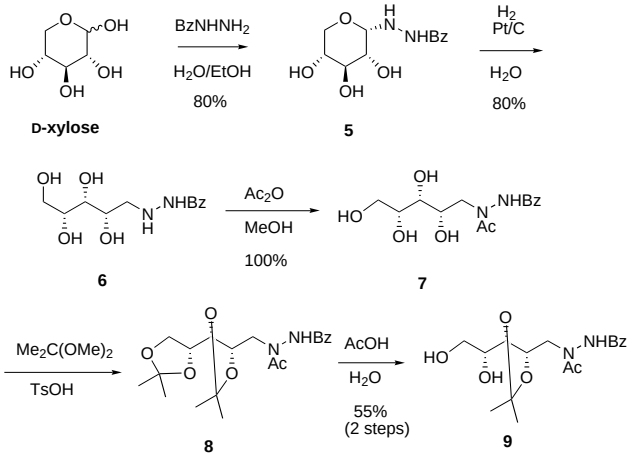
hydrazine with inversion of stereochemistry followed by reductive ring-closure would give **(+)-1**. As **(+)-1** was expected to be the less active enantiomer, preparation and investigation of that stereomer would in all likelihood give the most information about the inhibitory potency of the enantiomers. Since D-xylose is also readily available, this plan was attractive.

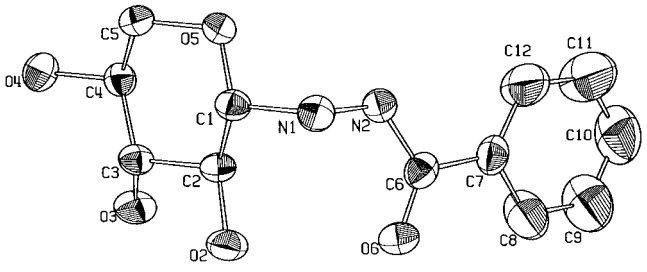
Our first plan for synthesis of **(+)-1** from D-xylose followed this scheme: Reductive amination of the aldehyde of open-chain xylose with a hydrazine derivative would lead to a 1-hydrazinopentitol. By tosylation of the primary alcohol followed by base treatment a 4,5-epoxide would be formed that upon nucleophilic ring-opening would give **1** with the desired stereochemistry. Thus D-xylose was treated with benzoylhydrazide in aqueous EtOH to give an adduct in 80% yield that turned out to be the cyclic hydrazide **5** (Scheme 2). The structure was confirmed by X-ray crystal



Scheme 2. Synthesis based on direct functionalisation of xylose.

structure determination (Figure 2). The structure of **5** contains two independent molecules, but they are practically identical, with only small differences in torsion angles. All bond lengths and angles are as could be expected. Compound **5** is probably formed by attack by the 4-OH on an initially formed hydrazone. The formation of cyclic hydrazide **5** was unexpected, but not unprecedented.[11] It did not impede the synthesis, however, because **5** could be hydrogenated with platinum catalyst to the acyclic hydrazine **6** in 80% yield. The structure of **6** was also confirmed by X-ray crystallographic structure analysis (Figure 2). Treatment of **6** with acetic anhydride in methanol gave a quantitative yield of the *N*-acetate **7**. The structure of **7** was proposed based on fitting the molecular ion in the MS spectrum and the appearance of an acetyl group in the NMR spectrum.

According to the plan mentioned above, an epoxide had to be introduced at C-4 and C-5 through the selective conversion of the primary alcohol to a leaving group. Therefore selective tosylation, mesylation and similar reactions were studied on *N*-acetate **7**, but in no case was it possible to isolate the desired monosulfonylated product. In general the reactions seemed to give several products, according to TLC and NMR, which initially seemed surprising. However a search of the literature



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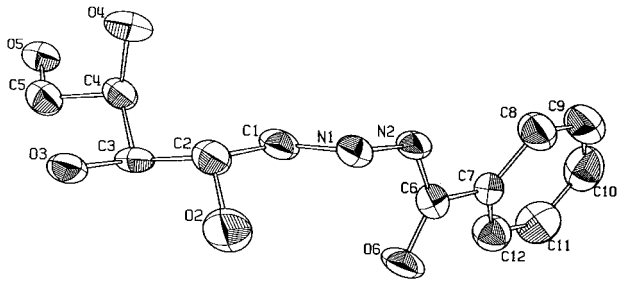

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Figure 2. X-ray structures of **5** and **6**.

revealed that tetrols with *xylo* stereochemistry on monotosylation of the primary alcohol undergo very rapid attack by the δ-OH to form a tetrahydrofuran.[12] Interestingly other polyol tosylates with different stereochemistry such as the corresponding *arabino* compounds do not undergo this cyclisation as readily, and the monotosylates can be isolated without problems.[13] Compounds with *xylo* stereochemistry have a pronounced tendency to undergo this cyclisation. In this light the problems with monosulfonylation of **7** are quite understandable. The 2-OH group could attack the initially formed sulfonate ester, and indeed ^{13}C NMR of the crude reaction product showed signals at low field ($\delta = 80-85$) suggesting that tetrahydrofurans had been formed.

To avoid tetrahydrofuran formation, we decided to protect the 2-OH. This was done by treatment of **7** with dimethoxypropane and TsOH to give the diacetonide **8** followed by selective hydrolysis of the primary acetonide with aqueous acetic acid to form **9** in 55% yield from **7** (Scheme 2). The structure of **8** was determined on the basis of a mass spectrum showing the expected molecular weight, and four peaks from $\delta = 25-30$ in the ^{13}C NMR spectrum showing the presence of two isopropylidene groups. The structure of **9** was proposed based on the mass spectrum showing the expected molecular weight, the ^{1}H NMR spectrum showing only six protons at $\delta = 1.3$ and thus implying the existence of only one isopropylidine group, and the fact that **9** was formed by selective hydrolysis of **8**. It may be argued that the isopropylidene group could be located in a different position, but its position was confirmed by subsequent transformations. The primary alcohol was now converted to a leaving group by treatment of

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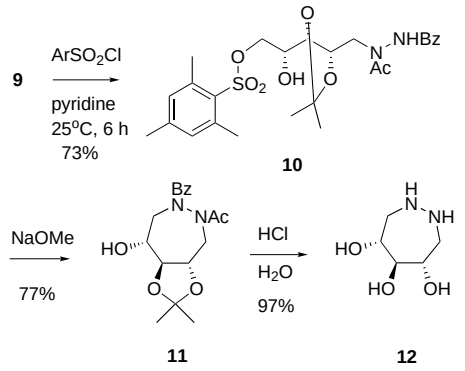
9 with mesitylenesulfonyl chloride in pyridine, which gave **10** in 73% yield (Scheme 3). The presence of the sulfonate was clearly seen from the NMR spectrum by the presence of a new aryl group. When **10** was treated with base under relatively mild conditions, K_2CO_3 in acetone or NaOMe in MeOH, with

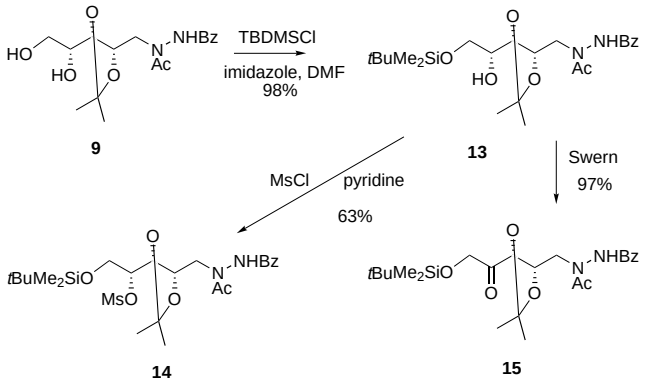


Scheme 3. Synthesis of diazepine **12**.

the intention of forming an epoxide, the diazepine **11** was formed in good yield. Only one product was observed. The NMR spectrum of **11** showed the presence of 3–4 different amide rotamers and were very difficult to interprete. The structure determination therefore had to rely on the mass spectrum and subsequent conversion of **11** into **12** by hydrolysis with aqueous hydrochloric acid. Diazapine **12**, formed in 97% yield, was easily identified by its symmetry.

The formation of compound **11** could occur by direct substitution of the mesitylenesulfonate by the amide anion. However, it is likely that the intended epoxide intermediate is formed in this reaction, but opens rapidly and selectively by attack in the 5-position, for the following reasons: The 4,5-di-*O*-mesylate of **9** (data not shown) was rather unreactive towards base and no diazepine was formed when that reaction was pushed towards completion; only elimination occurred. The *endo* ring-opening of the epoxide is supposedly not so favorable, but in its favor is the fact that the 5-position is primary and that *exo* attack would lead to a six-membered ring with a *trans*-fused acetonide. The diazapine **11** is probably less strained.

To force the formation of the six-membered ring, we protected the 5-OH of **9** with a *tert*-butyldimethylsilyl (TBDMS) group by treatment of **9** with TBDMSCl and imidazole in DMF (Scheme 4). This gave the silyl ether **13** in 98% yield, as witnessed by the appearance of a tertiary butyl signal in the ^{1}H NMR spectrum. This compound was mesylated with mesyl chloride in pyridine to give the mesylate **14** in 63% yield. Structural assignment of **14** relied on the appearance of a ^{1}H NMR signal at $\delta = 3.2$ corresponding to the mesyl group, and the downfield shift of a single proton from $\delta = 4.25$ to 4.65, corresponding to the deshielding effect of the mesyl group on H-4. However, treatment of **14** with various bases (LDA, NaH, KO*t*Bu) did not lead to the desired pyridazine. A comparison of these results with the facile formation of **11** suggests that the *trans* acetonide makes six-membered ring formation very difficult. Nevertheless, formation of a six-membered ring can occur when a *trans*-fused acetonide is present, but in those cases the substitution occurred at a primary center and not α to the acetonide.[14]

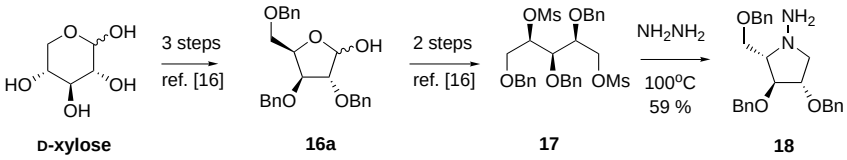


Scheme 4. Functionalisation of diol **9**.

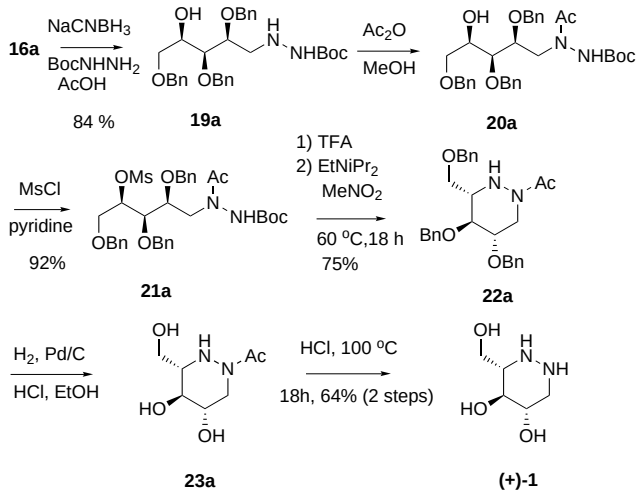
As an alternative, a reductive amidation ring-closure was attempted.[15],[16] Alcohol **13** was converted to the ketone **15** in 97% yield by a Swern oxidation. Treatment of **15** with hydrogen and palladium on carbon under high pressure and temperature did not give conversion, however.

It was now clear that a major problem in the synthesis was the competing attack from the 2-OH. It was therefore necessary to protect this alcohol, but the presence of a *trans* 2,3-acetonide impeded the formation of a six-membered ring. It was consequently necessary to develop a strategy where noncyclic protection groups could be used. Our attention was called to the benzylated xylose derivatives **16a** and **17**, both available from D-xylose (Scheme 5).[17] In these molecules the 2-, 3- and 5-positions are already substituted with benzyl groups.

Obviously direct disubstitution of the dimesylate **17** was tempting, though five-membered ring formation could be expected to be favored. Indeed, treatment of **17** with neat hydrazine hydrate at 100 °C led to a single product **18** that was isolated in 59% yield (Scheme 5). The downfield ^{13}C chemical shifts of the ring carbon atoms clearly showed that a pyrrolidine and not a pyridazine had been formed. Particularly the peaks at $\delta = 84.9$ and 80.3 were inconsistent with a six-membered ring. The MS spectrum showed a peak at 418 consistent with the proposed formula. To avoid pyrrolidine formation it was necessary to control the protection of the two hydrazine nitrogen atoms in a manner similar to the strategy of Schemes 2 and 5. So in a similar way **16a** was subjected to reductive amination with *tert*-butyl carbazate in the presence of $NaCNBH_3$ and acetic acid (Scheme 6). This gave the hydrazide **19a** in 84% yield; the structural assignment relied



Scheme 5. Synthesis of pyrrolidine **18**.



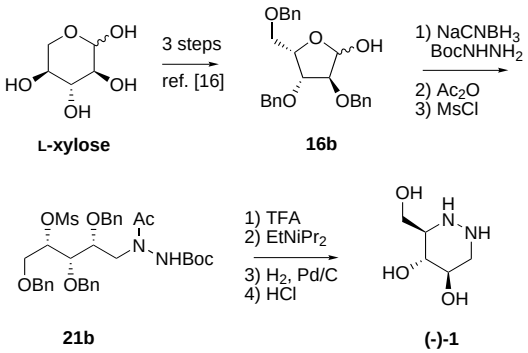
Scheme 6. Synthesis of **(+)-1**.

on the disappearance, in the ^{13}C NMR spectrum, of an anomeric carbon and the appearance of two protons at $\delta = 3.2$ and 3.1 in the ^{1}H NMR spectrum, corresponding to the reduced C-1. The secondary amine was now protected as the *N*-acetate with acetic anhydride in methanol to give **20a**, as seen from the appearance of an *N*-acetyl group in the NMR spectrum. This compound was treated with methanesulfonyl chloride in pyridine to give **21a** in 92% yield (from **19a**). The presence of the mesyl group was clearly seen by the appearance of a mesyl signal at $\delta = 2.95$ in the ^{1}H NMR spectrum and the downfield shift of H-4 to $\delta = 5.0$.

Treatment of **21a** with trifluoroacetic acid removed the Boc group. The resulting amine did not undergo spontaneous intramolecular substitution when treated with base, but overnight treatment with ethyldiisopropylamine in nitromethane at 60 °C gave the desired pyridazine **22a** in 75% overall yield. The structure determination of this compound relied on a molecular ion obtained from mass spectrometry and the presence of two signals at $\delta = 44.7$ and 60.4 in the ^{13}C NMR spectrum, corresponding to the two nitrogen-substituted carbons C-3 and C-6. Note (see Experimental Section) that the ^{13}C NMR chemical shift values in general are much lower than those of **18**; this shows that this compound is a six-membered ring. After hydrogenolysis in hydrochlorous ethanol to **23a** and acidic hydrolysis with refluxing 6N hydrochloric acid, 1-azafagomine was obtained in 64% yield from **22a**. The product, **(+)-1**, had all the physical and spectroscopic characteristics of the racemic compound, except a specific rotation $[\alpha]_D^{25}$ of +10.[8] On the basis of this rotation the configurational preference in the enzymatic reaction of **2** could now be determined.

With the synthesis of **(+)-1** secured it was now an easy task to synthesise the enantiomer **(−)-1** from L-xylose (Scheme 7). Though L-xylose is an unnatural sugar, it is relatively inexpensive. Thus L-xylose was converted to **16b**, the enantiomer of **16a**, by the same sequence of reactions.[17] From **16b** reductive amination, acetylation and mesylation gave **21b**. TFA treatment, base-promoted cyclisation and deprotection gave **(−)-1**, which had a specific rotation of −9.8.

The two enantiomeric forms of 1-azafagomine were then investigated for glycosidase inhibition (Table 2). This was



Scheme 7. Synthesis of **(−)-1**.

Table 2. K_i values [μM] for the inhibition of glycosidase by **1** and **12**.

Enzyme	(+)-1	(±)-1[8]	(−)-1	12
almond β-glucosidase	> 10000	0.65	0.32	63.7
yeast α-glucosidase	2900	3.9	6.9	42
isomaltase	871	1.06	0.27	31

done in the usual way, by adding the compound to the enzyme-catalysed hydrolysis of 4-nitrophenyl glycosides and following the formation of nitrophenol spectrophotometrically. The (+) form was found to be a very poor inhibitor. It follows that the activity of the racemic form[8] had to be caused by the (−) form; this was confirmed. Thus **(−)-1** competitively inhibited almond β-glucosidase with a K_i of 0.33 μM, which is about half of the value of 0.65 μM measured for **(±)-1**. Similarly α-glucosidase and isomaltase were inhibited competitively and strongly by the **(−)-1** form.

The diazepine **12** was found to inhibit β-glucosidase with a K_i of 64 μM, while α-glucosidase and isomaltase were inhibited at 30–40 μM. Though this is not weak inhibition, it is much weaker than azafagomine. It suggests that **12** may fit into the active site and bind to some of the functional groups that azafagomine binds to, but that some interactions are prevented.

These results show a remarkably large difference in biological activity between two enantiomers. Against β-glucosidase, the inhibition differed by a factor of more than 30000. This shows that close resemblance to the substrate or transition state is essential for binding, and suggests that **(−)-1** binds in a mode resembling either one.

Close inspection of the progress curves for the β-glucosidase-catalysed formation of 4-nitrophenol from 4-nitrophenyl β-glucoside in the presence of **(−)-1** revealed that the curves at the beginning of the reaction were slightly convex and not linear as one would expect (progress curves with and without the inhibitor **(−)-1** are shown in Figure 3). Linearity was, however, reached after a few minutes of reaction. Even more pronounced when **(−)-1** was allowed to stand with the enzyme before addition of 4-nitrophenyl β-glucoside, a clearly concave progress curve was seen. Again a linear curve was reached in a few minutes.

These observations mean that inhibition of the enzyme is a relatively slow process that requires measurable time to run to completion. This is remarkable because normally host–guest binding is a very fast, almost diffusion-controlled process. The

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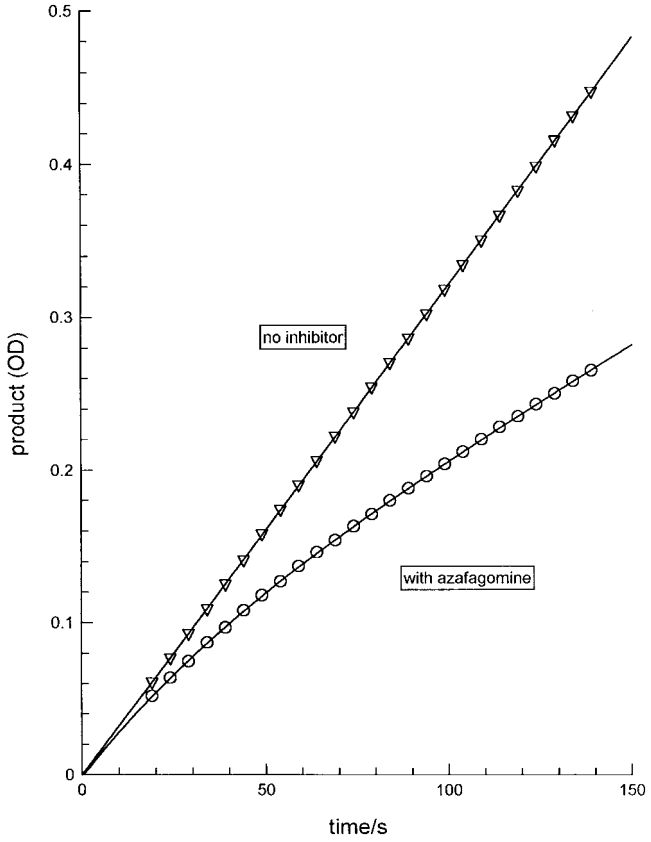
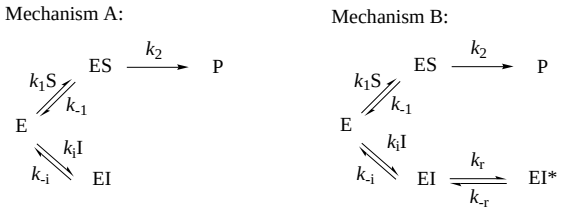


Figure 3. Control experiment demonstrating that the time-dependent hydrolysis rate observed is due solely to the presence of inhibitor. The enzyme was preincubated without inhibitor for 45 min, and the reaction was then initiated by the addition of substrate and buffer (upper curve) and, in addition, the inhibitor **(−)-1** (1.5 μM). The substrate concentration was 8 mM, corresponding to 2 K_m. The continuous curves are fits of Equation (1) to the data by nonlinear regression. The temperature was 25 °C.

phenomenon is called slow inhibition. The occurrence of slow-binding enzyme inhibitors is known[18] for a number of different enzymes including glycosidases;[19–23] however, little is known about when or why it occurs. Two possible mechanisms may be considered for the slow inhibition phenomenon (Scheme 8). Binding between enzyme and



Scheme 8. Alternative kinetic schemes for the inhibition of β-glucosidase by **(−)-1**.

inhibitor may either involve the usual single step, but having very slow on and off rates (k_iI and k_{-i} being small, Scheme 8, mechanism A). Alternatively, an initial fast binding step is followed by a slow reversible transformation of the enzyme–inhibitor complex (EI) into a more stable complex (EI*, Scheme 8, mechanism B).[18] The latter more "complex" model B seems to have become the accepted model, probably because it is linked to a reasonable explanation for the phenomenon: The slow transformation of EI to EI* may involve a large conformational change of the enzyme, which could well be a time-consuming process.

To get a more detailed insight into the binding mode of **1** we used a kinetic analysis of the progress curves to distinguish between the two mechanisms A and B. The two models have been analysed before.[18, 24–26] The progress curve for product formation for both models may be written as in Equation (1), where V_{SS} is the steady state rate attained as $t \to \infty$, A and C are constants, the latter of which depends on the initial conditions, and β is the observed rate constant. V_{SS}, A and β are functions of the substrate and inhibitor concentrations.

$$P(t) = V_{SS}t + AC(\exp(-\beta t) - 1)/\beta \quad (1)$$

A study of β as a function of inhibitor concentration allows us to distinguish between models A and B. For mechanism A we have $\beta = k_i[I]K_m/(K_m + [S]) + k_{-i}$, that is, β is a linear function of the inhibitor concentration. In contrast, for mechanism B, $\beta = k_r[I]K_m/(K_mK_i + K_i[S] + K_m[I]) + k_{-r}$, which describes a hyperbola. In both cases a numerical analysis of β allows determination of the relevant kinetic constants. Therefore we determined β for almond β-glucosidase at a number of different concentrations of **(−)-1** by fitting an exponential function to the corresponding progress curves using the curve-fitting software package Simfit.[27] In Figure 4, β is plotted against [I]. It is seen that the data are consistent with a straight line and not with a hyperbolic relationship. It must therefore be concluded that the binding follows mechanism A.

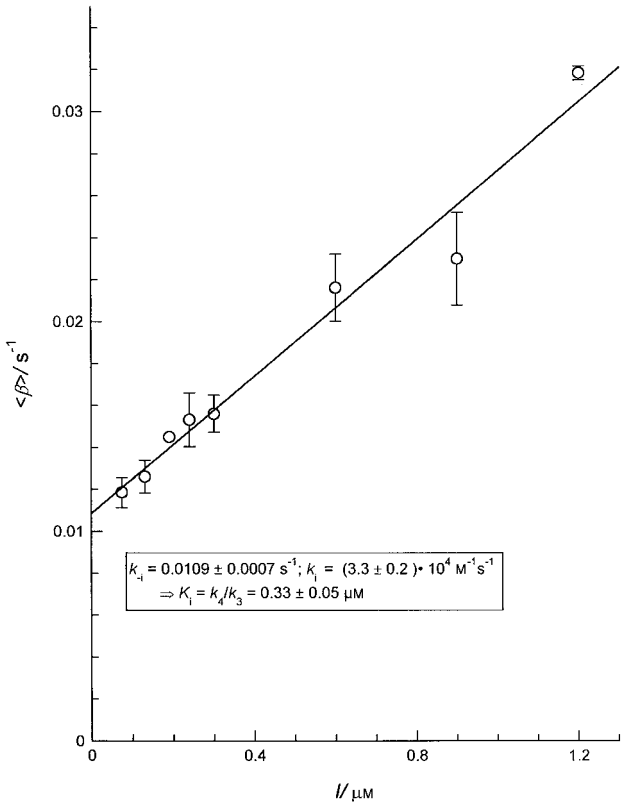


Figure 4. Plots of the observed rate constant β at 25 °C vs. concentration of inhibitor **(−)-1**. Each point is a mean of four seperate determinations.

From this plot the kinetic binding constants of the inhibitor could be determined as $k_i = (3.3 \pm 0.2) \times 10^4\,M^{-1}s^{-1}$, $k_{-i} = (1.1 \pm 0.2) \times 10^{-2}\,s^{-1}$ and $K_i = 0.32 \pm 0.02\,\mu M$. The association constant k_i is relatively small, showing that the rate of binding of the inhibitor is much smaller than diffusion-controlled binding ($10^8 - 10^9\,M^{-1}s^{-1}$). The reason why binding is so slow is not clear. It may be that the entrance and arrangement of the inhibitor in the active site is a difficult and time-consuming process. Given the high degree of resemblance of inhibitor and substrate one would, however, then also expect the substrate and enzyme to associate at an equally slow rate. From the present set of experiments it is not possible to see whether they do, because the substrate concentration is so high that binding on the time scale of the experiment will be fast in any case.

Another explanation is that the inhibitor and the substrate bind to the enzyme in different fashions. Given the fact that a small deviation from glucose stereochemistry in the pattern and stereochemistry of the hydroxyl groups on the inhibitor results in a large decrease in inhibition, it seems likely that this different binding mode is a mode resembling the transition state.

Conclusion

In this study we have synthesised the two enantiomers of glycosidase inhibitor 1-azafagomine (**1**) and found that only the stereoform resembling D-glucose causes significant inhibition. This supports the belief that the inhibitor binds glycosidases in a mode resembling the substrate or the transition state.[28]

Experimental Section

General: ^{13}C NMR and ^1H NMR spectra were recorded on a Varian Gemini 200 instrument with DHO (^1H NMR: $\delta = 4.7$) or acetone (^1H NMR: $\delta = 2.05$; ^{13}C NMR: $\delta = 29.8$) as reference. FAB mass spectra were obtained on a Kratos MS50RF mass spectrometer with EB geometry. The resolving power of the spectrometer was set to 3000 (10% valley definition). The accelerating voltage was 8 kV, and the post-accelerating detector potential was 9 kV. Ions were generated by fast atom bombardment (FAB) of the compounds dissolved in 3-nitrobenzylalcohol, glycerol or sulfolane matrices. The FAB gun employed xenon gas, 99.99%, and was operated at 9 kV. The instrument mass scale was externally calibrated using CsI, and the mass-to-charge ratio (m/z) of observed signals was corrected using the coobserved signals of known glycerol cluster ions, whereby an estimated mass accuracy of 100 ppm was achieved. The electrospray mass spectra (ESMS) were obtained by means of a Finnigan TSQ 700 triple quadropole instrument equipped with a nanospray source (Protana A/S, Odense, DK). Source conditions were: spray potential 800 V; heated (150 °C) capillary: 20 V; tube lens: 70 V. Water solutions were approximately 1 mM in the analyte and 1 mM in glycerol. Again the mass-to-charge ratios of the observed $M+1$ signals were corrected using the coobserved signals of known cluster ions.

Lipase-catalysed transesterification of (±)-2: These reactions were performed in two ways. Either a mixture of **(±)-2** (20 mg) and a lipase (2 mg) in vinyl acetate (2 mL) was stirred at 25 °C for several days, or **(±)-2** (20 mg) and the lipase (10 mg) was reacted with vinyl acetate (0.08 mL) in a solvent (0.2–10 mL, Table 1) at 40 °C for a number of hours. In both cases the reaction was stopped when TLC showed a desirable conversion. For work-up, the lipase was filtered off and the solution concentrated in vacuo. The residue was separated by flash chromatography (EtOAc, R_f(**2**): 0.31, R_f(**3**): 0.63) to give **2** and **3**. The *ee* of **2** and **3** was determined by HPLC (column: Daicel Chiralcel AD; UV: 220 nm; flow rate: 0.5 mL min^{-1}; **(*S*)-2**: 53 min and **(*R*)-2**: 58 min, **(*R*)-3**: 39 min and **(*S*)-3**: 58 min, *n*-hexane/isopropanol 9:1).

1-(2-Benzoyl)hydrazino-1-deoxy-α-D-xylopyranoside (5): D-xylose (4.53 g, 30 mmol) was dissolved in water (10 mL), and a solution of benzoyl hydrazide (4.08 g, 30 mmol) in ethanol (10 mL) was added. The solution was refluxed for 18 h, and then concentrated to a volume of 10 mL that gave white crystals of **5** (5.85 g) upon cooling. Further concentration and precipitation from the mother liquor gave an additional 0.58 g for a total yield of 6.43 g (80%). M.p. 170–171 °C. $[\alpha]_D^{25} = -40.3$ ($c = 0.6$, H_2O); MS(EI): m/z: 269 [$M+1$]; MS(FAB): m/z: 269.11; calcd for $C_{12}H_{16}N_2O_5$ + H: 269.11; ^{13}C NMR ([D$_6$]DMSO, 50 MHz): $\delta = 166.2$ (COPh), 132.8, 131.8, 128.6, 127.6 (Ph), 92.2 (C-1), 76.8, 71.6, 70.1 (C-2, C-3, C-4), 67.2 (C-5); ^1H NMR ([D$_6$]DMSO, 200 MHz): $\delta = 10.19$ (d, 1H, BzN*H*, $J_{NH,NH} = 5.5$ Hz), 7.8 (dd, 2H, Ph*H*, $J_{o,m} = 8.1$, $J_{o,p} = 1.5$ Hz), 7.43–7.55 (m, 3H, Ph*H*), 5.94 (dd, 1H, N*H*, $J_{NH,NH} = 5.5$, $J_{NH,1} = 3.0$ Hz), 5.24 (d, 1H, O*H*, $J = 4.3$ Hz), 5.04 (d, 1H, O*H*, $J = 4.3$ Hz), 4.99 (d, 1H, O*H*, $J = 4.8$ Hz), 3.86 (dd, 1H, H-1, $J_{1,2} = 8.4$, $J_{NH,1} = 3.0$ Hz), 3.69–3.75 (dd, 1H, H-5a, $J_{5a,5b} = 11.0$, $J_{4,5a} = 6.2$ Hz), 2.97–3.37 (m, 4H, H-2, H-3, H-4, H-5b).

1-(2-Benzoyl)hydrazino-1-deoxy-D-xylitol (6): As above, D-xylose (4.53 g, 30 mmol) was dissolved in water (10 mL), and a solution of benzoylhydrazide (4.08 g, 30 mmol) in ethanol (10 mL) was added. The solution was refluxed for 18 h. Water (30 mL) and platinum on carbon (600 mg, 5%) were added, and the mixture was hydrogenated for 18 h at 40 atm and 35 °C. The mixture was diluted to 200 mL with warm water and filtered through Celite. The filtrate was concentrated to a crystalline residue that was recrystallised from methanol. Yield: 5.2 g (64%). M.p. 161–162 °C. $[\alpha]_D = -17.9$ ($c = 0.6$, H_2O); MS(EI): m/z: 271 [$M+1$], 149 [$M - C_4H_9O_4$], 121 [M – NHNHBz], 105 (Bz); MS(FAB): m/z: 271.13; calcd for $C_{12}H_{18}N_2O_5$+H: 271.13; ^{13}C NMR ([D$_6$]DMSO, 50 MHz): $\delta = 165.3$ (*C*OPh), 133.3, 131.5, 128.6, 127.2 (Ph), 72.2, 71.8, 69.8 (C-2, C-3, C-4), 63.0 (C-5), 54.6 (C-1); ^1H NMR ([D$_6$]DMSO, 200 MHz): $\delta = 10.07$ (d, 1H, BzN*H*, $J_{NH,NH} = 6.2$ Hz), 7.82 (dd, 2H, Ph*H*, $J_{o,m} = 6.0$, $J_{o,p} = 1.8$ Hz), 7.42–7.50 (m, 3H, Ph*H*), 5.29 (dd, 1H, N*H*, $J_{NH,NH} = 6.2$, $J_{NH,1} = 5.6$ Hz), 4.70 (d, 1H, O*H*, $J = 4.8$ Hz) 4.42–4.51 (m, 2H, O*H*), 4.27 (d, 1H, O*H*, $J = 6.2$ Hz), 3.67 (dt, 1H), 3.55 (m, 1H), 3.43 (m, 1H, H-2, H-3, H-4), 2.78–2.91 (m, 2H, H-5a, H-5b), 2.50 (m, 2H, H-1a, H-1b).

1-(1-Acetyl-2-benzoyl)hydrazino-1-deoxy-D-xylitol (7): The hydrazide **6** (5.2 g, 19.3 mmol) was dissolved in methanol (100 mL), and acetic anhydride (9.82 g, 5 mol equiv) was added. The solution was kept for 1 h at 25 °C and then concentrated. The residue was coevaporated several times with water and toluene. Yield: 6.0 g (100%). $[\alpha]_D = -17.1$ ($c = 0.2$, H_2O); MS(FAB): m/z: 313.13; calcd for $C_{14}H_{20}N_2O_6$+H: 313.14; ^{13}C NMR (D_2O, 50 MHz): $\delta = 175.6$ (*C*OMe), 167.8 (*C*OPh), 133.3, 131.7, 129.3, 128.2 (Ph), 73.3, 71.9, 70.1 (C-2, C-3, C-4), 63.9 (C-5), 52.1 (C-1), 21.0 (Ac); ^1H NMR (D_2O, 200 MHz): $\delta = 10.37$ (br s, 1H, BzN*H*), 7.82 (dd, 2H, Ph*H*), 7.30–7.50 (m, 3H, Ph*H*), 3.3–4.2 (m, 7H), 2.05 (s, 3H, CH_3).

+1-(1-acetyl-2-benzoyl)hydrazino-1-deoxy-2,3-*O*-isopropylidene-D-xylitol (9): The *N*-acetate **7** (6.0 g, 19.2 mmol) was dissolved in acetone (130 mL) and 2,2-dimethoxypropane (26 mL). *p*-Toluenesulfonic acid (660 mg, 0.2 mol equiv) was added. The reaction mixture was stirred for 2 h, after which time TLC ($R_f = 0.85$; EtOAc/EtOH 9:1) showed completion of the reaction. The mixture was neutralised with saturated $NaHCO_3$ solution and then concentrated. The residue was redissolved in CH_2Cl_2 (200 mL) and washed with water (3 × 200 mL). The organic phase was dried with $MgSO_4$ and concentrated to give the diacetonide **8** as a colourless syrup: MS(FAB): $m/z = 393.20$; calcd for $C_{12}H_{18}N_2O_5$ + H: 393.20; ^{13}C NMR ($CDCl_3$, 200 MHz): $\delta = 173.3$ (*C*OMe), 166.3 (*C*OPh), 132.6, 131.7, 128.6, 127.2 (Ph), 109.6 (2C, $C(CH_3)_2$), 76.8, 74.7, 69.5 (C-2, C-3, C-4), 65.5 (C-5), 53.9 (C-1), 29.2, 27.1, 26.0, 25.3 ($C(CH_3)_2$), 20.3 (CH_3); ^1H NMR ($CDCl_3$, 200 MHz): $\delta = 9.1$ (s, 1H, BzN*H*), 7.8 (dd, 2H, Ph*H*), 7.40–7.60 (m, 3H, Ph*H*), 3.3–5.5 (m, 7H), 2.0 (s, 3H, Ac), 1.3 (2s, 12H, CH_3's). This syrup was dissolved in 80% acetic acid (30 mL) and stirred at 40 °C for 2 h, after which TLC ($R_f = 0.47$ EtOAc/EtOH 9:1) showed one principal product. The solution was concentrated, and the residue subjected to flash chromatography in EtOAc to give **9** as a syrup (3.72 g, 55%). $[\alpha]_D = -19.3$ ($c = 0.4$, $CHCl_3$); MS(FAB): m/z: 353.17; calcd for $C_{12}H_{18}N_2O_5$+H: 353.17; ^1H NMR ($CDCl_3$, 200 MHz): $\delta = 9.3$ (br s, 1H, BzN*H*), 7.85 (dd, 2H, Ph*H*), 7.40–7.60 (m, 3H, Ph*H*), 4.25 (dt, 1H), 3.9 (m, 3H), 3.7 (m, 3H), 2.1 (s, 3H, Ac), 1.3 (s, 6H, CH_3's).

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1-(1-Acetyl-2-benzoyl)hydrazino-1-deoxy-2,3-*O*-isopropylidene-5-*O*-(2,4,6-trimethylbenzene)sulfonyl-D-xylitol (10): The diol **9** (352 mg, 1 mmol) was dissolved in pyridine (2 mL), and 2,4,6-trimethylbenzenesulfonyl chloride (240 mg, 2.2 equiv) and 4-*N*,*N*-dimethylaminopyridine (7 mg) were added. The reaction mixture was stirred for 18 h, and the mixture was separated between EtOAc (10 mL) and H_2O (10 mL). The organic layer was washed twice with water (10 mL) and then dried (Na_2SO_4) and concentrated. The residue (870 mg) was subjected to flash chromatography (EtOAc/pentane 1:1), which gave first 1-(1-acetyl-2-benzoyl)hydrazino-1-deoxy-4,5-di-*O*-(2,4,6-trimethylbenzene)sulfonyl-2,3-*O*-isopropylidene-D-xylitol (170 mg, 24%) and then **10** (388 mg 73%). $[\alpha]_D = -12.7$ ($c = 2.2$, $CHCl_3$); MS(FAB): *m*/*z*: 535.208; calcd for $C_{26}H_{34}N_2O_8S$+H: 535.211; ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 143.6$, 140.0, 132.8, 131.8, 128.9, 127.3 (Ph's), 78.7, 75.0, 70.4, 67.2 (C-2, C-3, C-4, C-5), 49.0 (C-1), 27.2, 26.7 ($(CH_3)_2$C), 22.6 (ArCH_3), 21.0 (Ac); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 9.0$ (br s, 1H, BzN*H*), 7.8 (d, 2H, Ph*H*, $J_{o,m} = 8.1$ Hz), 7.5 (t, 1H, Ph*H*), 7.4 (t, 2H, Ph*H*), 6.9 (s, 2H, Ph*H*), 4.2 (m, 1H), 3.8–4.0 (m, 3H), 3.75 (d, 1H), 2.9 (m, 2H, H-5a, H-5b), 2.5 (s, 6H, ArCH_3's), 2.25 (s, 3H, ArCH_3), 2.05 (s, 3H, Ac), 1.3 (s, 6H, $(CH_3)_2$C).

(4*S*,5*S*,6*R*)-2-*N*-Acetyl-1-*N*-benzoyl-4,5-*O*-isopropylidene-4,5,6-trihydroxy-1,2-diazapine (11): Compound **10** (245 mg, 0.46 mmol) was dissolved in a solution of Na (35 mg, 1.46 mmol) in MeOH (1 mL) and kept at 25 °C for 30 min. A small lump of solid CO_2 was added to neutralise excess base. The solution was concentrated and subjected to flash chromatography in EtOAc. This gave **11** as a syrup (118 mg, 77%). $[\alpha]_D = +16.1$ ($c = 0.5$, $CHCl_3$); MS(FAB): *m*/*z*: 335.17; calcd for $C_{17}H_{22}N_2O_5$+H: 335.16; ^{13}C NMR ($CDCl_3$) at least two rotamers: $\delta = 171.6$, 171.5 (C=O), 131.7–133.2, 126.4–129.1 (Ph), 111.0, 110.8 (C(CH_3)$_2$), 84.8, 83.7 (C-5), 73.0, 72.5 (C-4), 70.8, 67.8 (C-6), 55.3, 52.6 (C-3), 48.2, 46.7 (C-7), 27.0, 26.7 (*C*(CH_3)$_2$), 20.3, 20.2 (Ac).

(4,5-*trans*-5,6-*trans*)-4,5,6-Trihydroxy-1,2-diazapine hydrochloride (12): Compound **11** was dissolved in aqueous hydrochloric acid (4 M, 12 mL) and refluxed for 18 h. The solution was concentrated to syrup containing **12** and some benzoic acid. The latter was removed by extracting the residue with diethyl ether (4 × 20 mL). Very pure **12** remained as the hydrochloride. Yield: 141 mg (97%); MS(EI): *m*/*z*: 148 [M^+]; ^{13}C NMR (D_2O): $\delta = 75.7$ (C-5), 68.5 (C-4, C-6), 46.5 (C-3, C-7); ^{1}H NMR (D_2O): $\delta = 3.82$ (dt, 2H, $J_{3b,4} = J_{6,7b} = 6.3$, $J_{4,5} = J_{5,6} = 6.3$, $J_{3a,4} = J_{6,7a} = 4.0$ Hz, H-4, H-6), 3.61 (t, 1H, $J_{4,5} = J_{5,6} = 6.3$ Hz, H-5), 3.24 (d, 2H, $J_{3a,4} = J_{6,7a} = 4.0$ Hz, H-3a, H-7a), 3.23 (d, 2H, $J_{3b,4} = J_{6,7b} = 6.3$ Hz, H-3b, H-7b).

1-(1-Acetyl-2-benzoyl)hydrazino-5-*O*-*tert*-butyldimethylsilyl-1-deoxy-2,3-*O*-isopropylidene-D-xylitol (13): Imidazole (510 mg, 2.5 mol equiv) and *tert*-butyldimethylsilyl chloride (652 mg, 1.2 mol equiv) were added to a solution of **9** (1.06 g, 3 mmol) in DMF (2.2 mL) at 0 °C. The mixture was stirred until TLC showed the disappearance of starting material (20 min) and formation of one product ($R_f = 0.46$, EtOAc/pentane 1:1). Water (100 mL) was added, and the mixture was extracted with ether (3 × 100 mL). The combined organic phase was washed with water (200 mL), dried ($MgSO_4$) and concentrated to give **13** as a syrup (1.38 g, 98%). $[\alpha]_D = +3.1$ ($c = 0.3$, $CHCl_3$); MS(EI): *m*/*z*: 467 [M^+]; ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 8.9$ (s, 1H, BzN*H*), 7.85 (dd, 2H, Ph*H*), 7.40–7.60 (m, 3H, Ph*H*), 4.25 (dt, 1H), 3.6–3.9 (m, 6H), 2.1 (s, 3H, Ac), 1.4 (s, 6H, CH_3's), 0.9 (s, 9H, *t*Bu), 0.05 (s, 6H, SiCH_3).

1-(1-Acetyl-2-benzoyl)hydrazino-5-*O*-*tert*-butyldimethylsilyl-1-deoxy-4-*O*-methanesulfonyl-2,3-*O*-isopropylidene-D-xylitol (14): The alcohol **13** (245 mg, 0.5 mmol) was dissolved in pyridine (1.7 mL) at 0 °C and methanesulfonyl chloride (61 μL, 0.79 mmol, 1.5 equiv) was added. After 2.5 h the reaction was complete, and a small amount of water (0.2 mL) was added and allowed to react for 5 min. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with HCl (10 mL), $NaHCO_3$ (10 mL) and water (10 mL). The organic phase was dried ($MgSO_4$) and concentrated to give the mesylate **14** as a colourless syrup (171 mg, 63%). ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 9.1$ (br s, 1H, BzN*H*), 7.85 (dd, 2H, Ph*H*), 7.40–7.60 (m, 3H, Ph*H*), 4.65 (m, 1H), 3.7–4.3 (m, 6H), 3.2 (s, 3H, CH_3SO_2), 2.1 (s, 3H, Ac), 1.4 (s, 6H, CH_3's), 0.9 (s, 9H, *t*Bu), 0.05 (s, 6H, SiCH_3).

5-(1-Acetyl-2-benzoyl)hydrazino-1-*O*-*tert*-butyldimethylsilyl-5-deoxy-3,4-*O*-isopropylidene-L-thre-2-ulose (15): To a solution of oxalyl chloride (0.36 mL, 4.18 mmol) in dry CH_2Cl_2 (6 mL) at −65 °C was added a solution of DMSO (0.695 mL, 9.76 mmol) in dry CH_2Cl_2 (3 mL). After 5 min at that temperature a solution of **13** (1.3 g, 2.79 mmol) in CH_2Cl_2 (3 mL) was added over 10 min. The reaction mixture was allowed to warm to −50 °C, and Et_3N (2.5 mL, 18.1 mmol) was added. After 30 min in which the mixture was allowed to reach 25 °C, the reaction was complete, and one product was observed on TLC ($R_f = 0.78$, pentane/EtOAc 1:1) The solution was diluted with CH_2Cl_2 (200 mL), and washed with water (2 × 200 mL) and saturated NaCl solution (200 mL). The organic phase was dried ($MgSO_4$) and concentrated to give **15** as a colourless syrup (1.26 g, 97%). ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.9$ (dd, 2H, Ph*H*), 7.40–7.60 (m, 3H, Ph*H*), 4.3–4.7 (m, 5H), 3.1 (q, 1H), 2.1 (s, 3H, Ac), 1.4 (d, 6H, CH_3's), 0.9 (s, 9H, *t*Bu), 0.1 (s, 6H, SiCH_3).

(2*S*,3*S*,4*S*)-1-*N*-Amino-2′,3,4-tri-*O*-benzyl-3,4-dihydroxy-2-hydroxymethylpyrrolidine (18): The dimesylate **17** (106 mg, 0.18 mmol) was dissolved in hydrazine hydrate (1 mL) and heated to 100 °C for 18 h. The solution was concentrated, and the residue was subjected to flash chromatography (EtOAc) to give **18** as a clear syrup (45 mg, 59%). MS(EI): *m*/*z*: 418 [M^+]; ^{13}C NMR ($CDCl_3$): $\delta = 138.2$, 137.9, 127.6–128.4 (Ph), 84.9, 80.3 (C-2, C-3), 74.1, 73.4, 71.6, 71.1, 70.7 (C-4, C-5, 3Bn), 62.9 (C-1).

1-(2-*tert*-Butyloxycarbonyl)hydrazino-1-deoxy-2,3,5-tri-*O*-benzyl-D-xylitol (19a): A solution of **16a** (5.9 g, 14 mmol) in methanol (70 mL) was treated with *tert*-butylcarbazate (3.7 g, 28 mmol), acetic acid (1.47 g, 24.5 mmol) and $NaCNBH_3$ (3.33 g, 53.4 mmol) at 60 °C for 18 h. $NaHCO_3$ solution (satd, 30 mL) was added, then water (100 mL), and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was dried, concentrated and subjected to flash chromatography (EtOAc/CH_2Cl_2, 1:4). This gave **19a** (6.4 g, 84%). MS(FAB): *m*/*z*: 537.296; calcd for $C_{31}H_{40}N_2O_6$+H: 537.297; ^{13}C NMR ($CDCl_3$): $\delta = 138.1$, 127.6–128.4 (Ph), 74.2, 73.2, 72.1, 71.1, 68.2 (C-4, C-5, 3Bn), 28.3 ((*C*H_3)$_3$C); ^{1}H NMR ($CDCl_3$): $\delta = 7.3$ (m, 15H, Ph*H*), 6.2 (br s, 1H, BocN*H*), 4.4–4.7 (m, 6H, Bn's), 4.3 (br s, 2H, OH, NH), 4.0 (t, 1H, $J = 6$ Hz, H-3), 3.7 (m, 2H), 3.55 (dd, 1H, $J = 8.3$ Hz, $J = 6$ Hz, H-5a), 3.45 (dd, 1H, $J = 8.3$ Hz, $J = 7$ Hz, H-5b), 3.2 (dd, 1H, $J = 12$ Hz, $J = 4.3$ Hz, H-1a), 3.1 (dd, 1H, $J = 12$ Hz, $J = 3.2$ Hz, H-1b), 1.4 (s, 9H, CH_3's).

1-(2-*tert*-Butyloxycarbonyl)hydrazino-1-deoxy-2,3,5-tri-*O*-benzyl-L-xylitol (19b): Synthesis carried out as for **19a** above, but starting from **16b**. $[\alpha]_D = +3.7$ ($c = 1.0$, $CHCl_3$).

1-(1-Acetyl-2-*tert*-butyloxycarbonyl)hydrazino-1-deoxy-2,3,5-tri-*O*-benzyl-4-*O*-methanesulfonyl-D-xylitol (21a): Compound **19a** (203 mg, 0.38 mmol) was dissolved in methanol (20 mL) and acetic anhydride (2 mL) was added. The mixture was kept for 18 h and then concentrated. The residue was identified by NMR as the acetate **20a** (^{1}H NMR ($CDCl_3$): $\delta = 7.2$ (br s, 15H, Ph*H*), 6.85 (br s, 1H, N*H*), 4.4–4.7 (m, 7H), 3.9 (m, 3H), 3.4–3.8 (m, 3H), 1.95 (s, 3H, Ac), 1.4 (s, 9H, ((*C*H_3)$_3$C)). This compound was dissolved in pyridine (10 mL) and cooled to 0 °C. Methanesulfonyl chloride (50 mg, 0.44 mmol) was added, and the mixture was allowed to reach room temperature over 1 h. Water (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (2 × 10 mL). Finally the organic layer was concentrated and coevaporated with toluene. The residue was subjected to column chromatography (EtOAc/CH_2Cl_2 0:1 to 1:4) to give **21a** (229 mg, 92%). MS(FAB): *m*/*z*: 657.285; calcd for $C_{34}H_{44}N_2O_9S$+H: 657.285; ^{1}H NMR ($CDCl_3$): $\delta = 7.3$ (br s, 15H, Ph*H*), 6.85 (br s, 1H, N*H*), 5.0 (m, 1H), 4.4–4.7 (m, 7H), 3.9 (m, 1H), 3.5–3.8 (m, 3H), 3.25 (m, 1H), 2.95 (s, 3H, Ms), 1.95 (s, 3H, Ac), 1.45 (s, 9H, ((CH_3)$_3$C).

1-(1-Acetyl-2-*tert*-butyloxycarbonyl)hydrazino-1-deoxy-2,3,5-tri-*O*-benzyl-4-*O*-methanesulfonyl-L-xylitol (21b): Synthesis carried out as for **21a** above, but starting from **19b**. $[\alpha]_D = -4.8$ ($c = 1.0$, $CHCl_3$).

(3*S*,4*S*,5*S*)-1-*N*-Acetyl-3′,4,5-tri-*O*-benzyl-4,5-dihydroxy-3-hydroxymethylhexahydropyridazine (22a): The mesylate **21a** (220 mg, 0.33 mmol) was dissolved in TFA (2 mL) and kept for 1 h at room temperature. The TFA was removed by evaporation and $NaHCO_3$ solution (satd, 10 mL) was added to the residue. This mixture was extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated. This residue was dissolved in nitromethane (2 mL), Et*N*iPr_2 (200 μL, 148 mg, 1.15 mmol) was added, and the mixture was heated at 60 °C for 18 h. The solvent was removed by concentration, and the residue was subjected to chromatography (EtOAc/CH_2Cl_2, 0:1 to 1:4) to give **22a** (115 mg, 75%). MS(EI): *m*/*z*: 460 [M^+], 339 [$M - CH_2OBn$]; MS(FAB): *m*/*z*: 461.238; calcd for $C_{28}H_{32}N_2O_4$+H: 461.244; ^{13}C NMR ($CDCl_3$): $\delta = 172.8$, 138.3, 138.0, 137.7, 127.8–128.5 (Ph), 77.8, 74.7, 73.4 (2C), 72.0, 67.0 (3Bn, C-3, C.4, C-5), 60.4 (C-6), 44.7 (C-2), 20.6 (Ac).

(3*R*,4*R*,5*R*)-1-*N*-Acetyl-3′,4,5-tri-*O*-benzyl-4,5-dihydroxy-3-hydroxymethylhexahydropyridazine (22b): Synthesis carried out as for **22a** above, but starting from **21b**. $[\alpha]_D = +8.8$ ($c = 1.1$, $CHCl_3$).

(3*S*,4*S*,5*S*)-4,5-Dihydroxy-3-hydroxymethylhexahydropyridazine, (+)-1-azafagomine ((+)-1): To a solution of **22a** (140 mg, 0.30 mmol) in EtOH (10 mL) was added HCl (1M, 2 mL) and Pd/C (50 mg), and the solution was hydrogenated at 1 atm H_2 until no more hydrogen was absorbed (4–6 h). The mixture was filtered through Celite and concentrated to give a residue containing (3*S*,4*S*,5*S*)-1-*N*-acetyl-4,5-dihydroxy-3-hydroxymethylhexahydropyridazine (**23a**). This was dissolved in HCl (6M, 8 mL) and heated to 100 °C for 18 h. The solution was concentrated and subjected to ion-exchange chromatography using Amberlite IR-120 resin (H^+, 10 mL) and eluting with dilute NH_4OH solution (1M, 100 mL). Concentration of the basic eluate gave **(+)-1** (29 mg, 64%): $[\alpha]_D = +10.0$ ($c = 0.3$, H_2O). The NMR spectra were identical to those of **(±)-1**.[8]

(3*R*,4*R*,5*R*)-4,5-Dihydroxy-3-hydroxymethylhexahydropyridazine (−)-1-azafagomine ((−)-1): Synthesis carried out as for **(+)-1**, but starting from **22b**: m.p.: 157–167 °C (decomp); $[\alpha]_D = -9.8$ ($c = 0.85$, H_2O). The NMR spectra were identical to those of **(±)-1**.[8]

Enzymatic assays

***α*- and *β*-glucosidase**: 4-Nitrophenyl-α- and -β-D-glucopyranoside, α-glucosidase (yeast, Sigma G 5003, 4.5 sigma units mg^{-1}), isomaltase (yeast, Sigma I-1256, 47 sigma units mg^{-1}) and β-glucosidase (sweet almonds, Sigma G 4511, 21 sigma units mg^{-1}) were purchased from Sigma Chemical. The β-glucosidase is a purified enzyme that showed only a single band on an SDS electrophoresis gel at 62 kDa. 4-Nitrophenyl-α- or -β-D-glucopyranoside was used as substrate and α- or β-glucosidase as catalyst in a sodium phosphate buffer (0.05M, pH 6.8) at 25 °C. Formation of the product, 4-nitrophenol, was measured continually at 400 nm by means of a Milton Roy Genesys 5 spectrometer. In all kinetic runs less than 1% of the initial substrate was consumed, assuring the constancy of the substrate concentration.

K_i determinations were performed as follows: Two thermostatted solutions of a) 1 mL of 0.1M buffer, 800 μL substrate in varied concentration ([S] = 0.2 K_m to 5 K_m) and 100 μL water, and b) 100 μL enzyme were mixed, and the reaction was immediately monitored. From eight experiments with varied substrate concentration, initial reaction rates were calculated from the slope of the first-order plot of product absorption vs. reaction time. K_M and V_{max} were calculated from a Hanes plot. From four experiments K'_M was calculated from the Hanes plot, and from K_M and K'_M, K_i was calculated.

Slow inhibition experiments were carried out with and without preincubation of enzyme and inhibitor. Experiments without preincubation were carried out as follows: Two thermostatted solutions of a) 1 mL of 0.1M buffer, 800 μL substrate (40 mM) and 100 μL inhibitor in varied concentrations ([I] = 5–200 K_i) and b) 100 μL enzyme (1 mg mL^{-1}) were mixed, and the reaction was immediately monitored spectrophotometrically. Experiments with preincubation were carried out as follows: Solutions of 25 μL inhibitor ([I] = 20 K_i) and 25 μL enzyme (2 mg mL^{-1}) were mixed and kept at 25 °C for 60 min. A thermostatted mixture of 1 mL of 0.1M buffer, 800 μL substrate (40 mM) and 150 μL of inhibitor in varied concentrations ([I] = 0–130 K_i) was added, and the reaction was immediately monitored spectrophotometrically.

Crystallographic data: Both **5** and **6** grew as thin, colourless needles. X-ray data for **6** were measured on a Huber four-circle diffractometer in the $\theta-2\theta$ step scan mode, whereas those for **5** were collected on a Siemens SMART area detector diffractometer, collecting narrow-frame ω scans. In both cases, $Mo_{K\alpha}$ radiation was used at room temperature. Both structures were solved by means of the SIR92 direct methods package and refined by the full-matrix least-squares method, allowing anisotropic displacement parameters for all but the hydrogen atoms; hydrogen atoms on carbon were kept in calculated positions. Details of the analysis are given in Table 3.

Table 3. X-ray data for the structures of **5** and **6**.

	5	**6**
formula	$C_{12}H_{16}N_2O_5$	$C_{12}H_{19}N_2O_5$
formula weight	268.28	271.30
crystal system	monoclinic	monoclinic
space group	$C2$	$P2_1$
a [Å]	20.003(1)	9.318(2)
b [Å]	8.3239(4)	5.228(1)
c [Å]	16.9126(9)	13.469(2)
β [°]	112.359(3)	89.58(1)
Z	8	2
R	0.058	0.047
R_w	0.065	0.046
GoF	2.19	1.36
reflections used	2404	880
parameters	342	172

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