PERKIN 1

Synthesis and investigation of L-fuco- and D-glucurono-azafagomine

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Received (in Cambridge, UK) 23rd January 2002, Accepted 21st March 2002
First published as an Advance Article on the web 12th April 2002

The new azasugars (3*S*,4*R*,5*S*)-4,5-dihydroxy-3-methylhexahydropyridazine (**3**, azafucofagomine) and (3*S*,4*R*,5*R*)-4,5-dihydroxyhexahydropyridazine-3-carboxylic acid (**4**, azaglucuronofagomine) were synthesised. Azafucofagomine (**3**) was made from D-ribose in ten steps in a synthesis that involved partial 2,3-protection, deoxygenation of the 5-OH, reductive amination with *tert*-butyl carbazate, mesylation, cyclisation and deprotection. Compound **4** was made from L-xylose in 12 steps in a related way starting with 2,3,5-protection, reductive amination with *tert*-butyl carbazate, mesylation and cyclisation. The key step in this synthesis is selective debenzylation of a primary benzyl ether with acetyl bromide to produce a partially benzylated hexahydropyridazine that was oxidised to the acid and deprotected. The 3-, 4- and 6-deoxy analogues of azafagomine [**1**, (3*R*,4*R*,5*R*)-4,5-dihydroxy-3-hydroxymethylhexahydropyridazine] were also made. Compounds **3** and **4** were shown to be potent α-fucosidase and β-glucuronidase inhibitors, respectively.

Introduction

Glycosidases and related enzymes are crucial in many biological processes. Potent and selective inhibitors of these enzymes are important, because they can be used to interfere with such processes. Thus glycosidase inhibitors may be potential agents against diabetes,[1] cancer, AIDS,[2] hepatitis,[3] Gaucher's disease[3] and influenza.[4] A particularly effective way of procuring enzyme inhibitors is to design transition state analogues. Azasugar inhibitors,[5] in particular, are subject to interest in this respect because of their ability to mimic charge in a glycosidase transition state. Positive charge can develop on the ring oxygen as depicted in the transition state **B**, or on the anomeric carbon as in **A** (Fig. 1). Which of the two transition states **A** and **B** is the

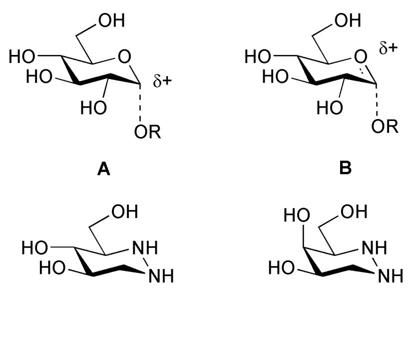
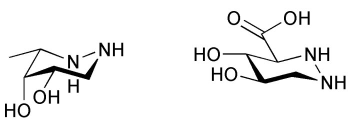


Fig. 1

more important appears to depend on the enzyme studied, but most enzyme transition states are likely to have a component of each.[6–8]

1-Azafagomine (**1**) is a compound that is able to mimic both transition states **A** and **B** and as such appears to be an ideal mimic of the charge in the transition state. Compound **1** is indeed a very good inhibitor of many glucosidases.[9] The galacto-analogue **2** has also been prepared, and this compound is a good galactosidase inhibitor.[10] In the present study we wished to complete the investigation of azafagomines by synthesising the azafagomine analogues of L-fucose and D-glucuronic acid, **3** and **4** (Fig. 2).



3 4

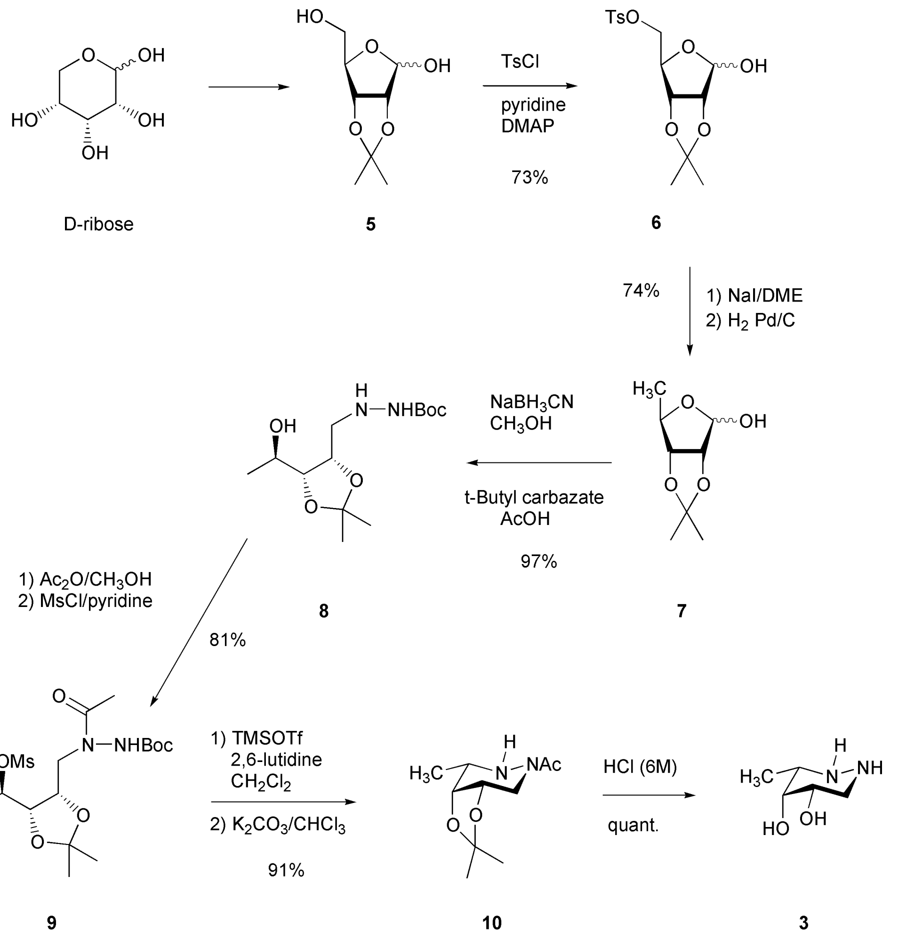
Fig. 2

Two different routes to optically active 1-azafagomines have been established through the synthesis of **1**. This compound can either be prepared from a carbohydrate[11] or be made by a hetero Diels–Alder reaction sequence linked to a lipase catalysed resolution of a racemic intermediate.[12] The former general strategy is probably to be preferred, because it is efficient and provides the highest possible optical purity, but can only be applied to the synthesis of stereoisomers where the pentose starting material is reasonably priced. While **1** can be prepared from relatively inexpensive L-xylose, synthesis of **2** by this method would require expensive L-ribose. For this reason **2** has been prepared by the chemoenzymatic route.[10] Compound **3**, on the other hand, is basically the mirror image of **2** but lacks the 3′-hydroxy group. This means that it might be possible to prepare **3** from inexpensive D-ribose. Compound **4** has the same stereochemistry as **1**, which means that it could potentially be made from L-xylose as well. We here report the synthesis **3** and **4**, an investigation of their glycosidase inhibition, and an evaluation of the importance of the individual hydroxy groups in **1**.

Results and discussion

D-Ribose contains the correctly configured hydroxy groups present in the 4- and 5-positions of the target compound **3**. Similarly to the synthesis of **1** from L-xylose the 4-hydroxy group of D-ribose must be replaced by hydrazine with inversion. However, the 5-hydroxy group of D-ribose also needs to be removed. It was conceived that both objectives could conveniently be carried out by using the isopropylidene derivative **5**

 DOI: 10.1039/b200884j



Scheme 1

as a starting material. Acetonide **5** was obtained in one step from D-ribose by treatment with acetone and acid. If the 5-OH of **5** could be deoxygenated to **7** without affecting the 1-OH, the route would be paved for obtaining **3**, similarly to the synthesis of **1** from L-xylose. In fact **7** is a known compound that has been obtained from D-ribono-1,4-lactone, which avoids potential chemoselectivity problems.[13] However, synthesis from **5** should be more efficient.

Selective tosylation of **5** has previously been reported.[14] We did the reaction under slightly different conditions obtaining monotosylate **6** in 73% yield (Scheme 1). Treatment of **6** with NaI in dimethoxyethane gave the 5-iodo derivative, which was hydrogenated at 1 atm in the presence Pd–C to give **7** in 74% yield. Reductive amination of **7** using *tert*-butyl carbazate, $NaCNBH_3$ and acetic acid gave the hydrazine **8** in 97% yield. Reaction of **8** with acetic anhydride in methanol gave the *N*-acetyl compound that, without purification, was subjected to mesylation using mesyl chloride and pyridine. This gave *O*-mesylate **9** in 81% yield. Then, treatment of **9** with TMSOTf–lutidine, a reagent selective for removal of Boc groups in the presence of acetonide groups, gave a crude monohydrazide that was subjected to cyclisation by being refluxed for 24 h in $CHCl_3$ in the presence K_2CO_3. This gave **10** in 91% yield. Compound **10** gave a poorly resolved NMR spectrum, but as it was crystalline an X-ray crystal structure was used to confirm the structure (Fig. 3). The structure shows the compound in the anticipated chair conformation. Finally deprotection of **10** was carried out by hydrolysis with refluxing 6 M hydrochloric acid giving **3** in quantitative yield.

The pK_a of **3** was measured as 6.3 by titration. This value was compared to the predicted value obtained by a recently published empirical method as a confirmation of the latter.[15] According to this method each of the nitrogens in a hexahydropyridazine have their pK_a calculated using the formula 7.3 − $\Sigma\sigma_s$ where σ_s is the substituent contribution. In the present case the pK_a of N1 is predicted to be 6.3 and pK_a of N2 is predicted to be 5.9. The overall pK_a becomes 6.4 from the equation pK_a = $\log(1/K_{aN1} + 1/K_{aN2})$,[15] and is thus reasonably well predicted by this method.

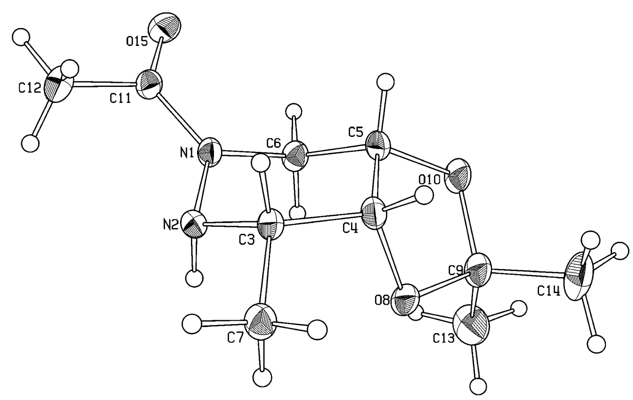
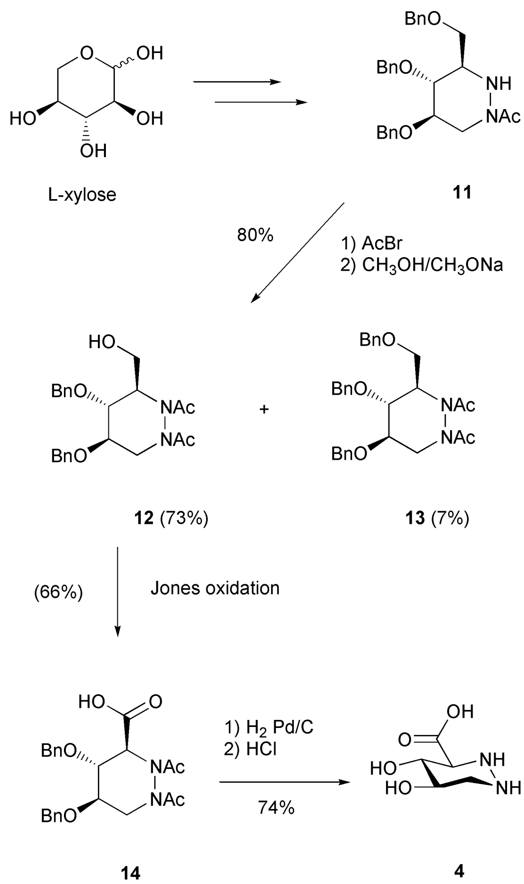


Fig. 3 X-Ray structure of compound **10**.

The target compound **4** has the same stereochemistry as **1** and might therefore be made from L-xylose. It was conceived that the intermediate **11**, which is made from L-xylose in 8 steps,[11] might be a potential starting material for **4** provided a selective debenzylation of the primary benzyl ether could be carried out (Scheme 2). In order to achieve this, **11** was treated with neat acetyl bromide for 7 h at 25 °C. The hydrazine was quickly acetylated, followed by a slower acetylation–debenzylation reaction of the primary OH-group, affording a triacetyl compound. The crude product was *O*-deacetylated using NaOMe in MeOH to give diacetyl compound **12** in 73% yield. A 7% yield of the fully benzylated diacetyl compound **13**



Scheme 2

was also obtained. Then oxidation of **12** using Jones reagent gave a 66% yield of the acid **14**. Finally hydrogenolysis at 1 atm using Pd–C catalyst followed by acidic hydrolysis with 6 M hydrochloric acid at 100 °C gave the target **4** in 74% yield.

The conformational behaviour of **4** is unusual. Compound **4** was observed to be predominantly in the $^1C_4$ conformation when the nitrogen was protonated regardless of whether the carboxylate was protonated or not. The unprotonated compound was, however, in the expected $^4C_1$ conformation (Fig. 4).

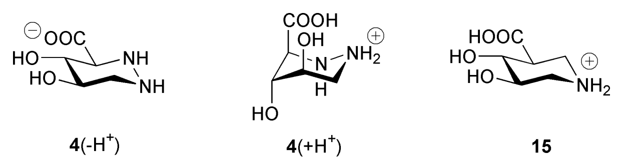


Fig. 4

This was seen by the unusually small $J_{3,4}$ and $J_{4,5}$ at neutral and low pH. The compound had an estimated conformer ratio in water of 9 : 1 between $^4C_1$ and $^1C_4$ at pH 11 and 1 : 4 at pH 1. The known very similar piperidine **15** is however predominantly in the $^4C_1$ conformation as the hydrochloride.[16]

This behaviour can be explained by stereoelectronic substituent effects, which causes the $^1C_4$ conformer to be more basic than the $^4C_1$ conformer.[15] This makes the $^1C_4$ conformer favoured in acidic solution. The reason why only **4** and not also **15** flips predominantly to the $^1C_4$ conformation must be because the steric hindrance between axial substituents in **15** in a $^1C_4$ conformation is larger than in **4**. In particular, the 1,3-steric interactions between the additional CH_2 group and the 4-OH in **15** must make the $^1C_4$ conformation less favourable for this compound than it is for **4** where the CH_2 group has been replaced by an NH-group.

Another aspect required to complete the investigation of **1** was the evaluation of the effect of the biological activity of the different OH groups in **1**. It was therefore necessary to obtain

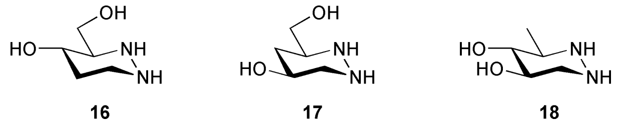
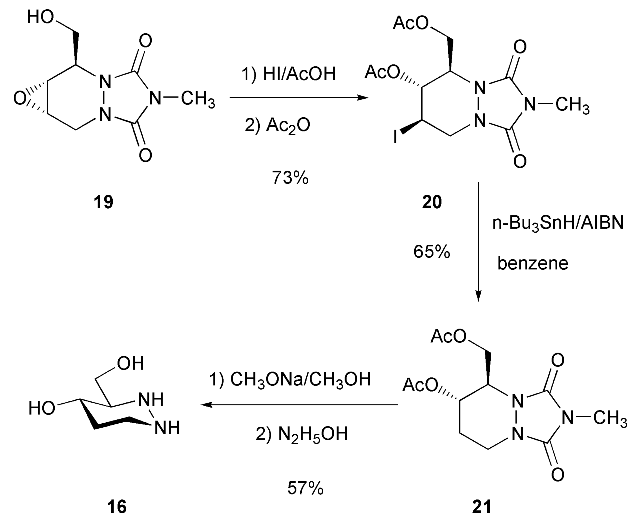


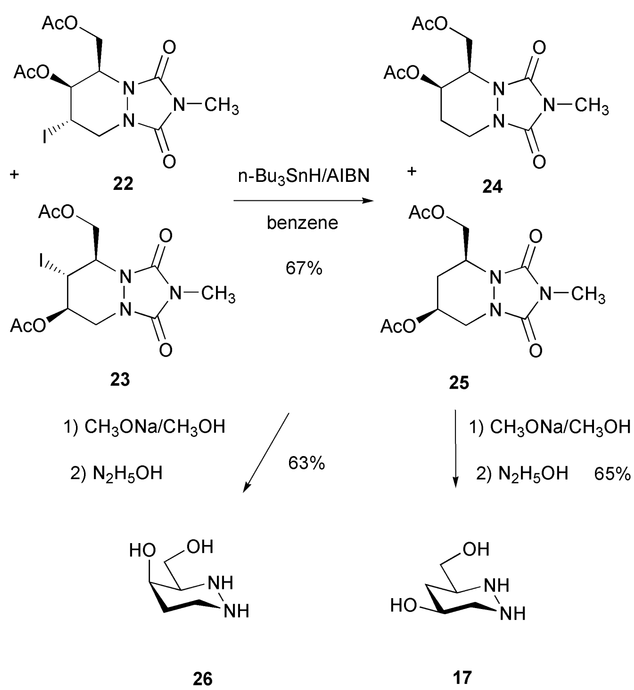
Fig. 5

the three deoxygenated analogues **16**–**18** in optically pure form (Fig. 5). The 5-deoxy analogue **16** was obtained by a modification of the chemoenzymatic synthesis of **1** (Scheme 3).[12] In this synthesis the optically active epoxide **19** was an intermediate which, upon acidic hydrolysis, gave selective attack of water at C-4. It was found that treatment of **19** with HI in acetic acid gave the same selectivity. After acetylation the iodide **20** was obtained in 73% yield. This iodide was subjected to radical reduction using Bu_3SnH–AIBN to give the 4-deoxy derivative **21** in 65% yield. Finally deacetylation with NaOMe–MeOH followed by hydrazinolysis with neat hydrazine at 100 °C for 24 h gave **16** in 57% yield.

The 4-deoxy analogue **17** was made by a modification of the synthesis of **2**.[10] In this synthesis the iodides **22** and **23** were intermediates (Scheme 4). This inseparable 1 : 3 mixture of



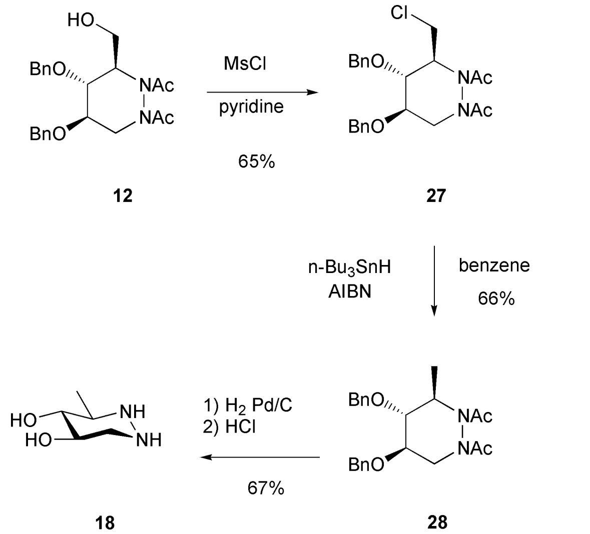
Scheme 3



Scheme 4

iodides was obtained from the *cis* diastereomer of epoxide **19** by reaction with HI followed by acetylation similar to the transformation **19** into **20**, and both isomers were converted into **2**. However, the major isomer **23** should be source for **17**. Therefore the mixture of **22** and **23** was reduced with Bu_3SnH–AIBN to give a mixture of de-iodo compounds **24** and **25**. This mixture was separated by chromatography to give 46% of **25** and 21% of **24**. Both compounds were deprotected with NaOMe–MeOH followed by hydrazine hydrate at 100 °C, which afforded **17** in 65% yield and **26** in 63% yield.

The 3′-deoxy analogue **18** was made by a modification of the synthesis of **4**, since the intermediate **12** was ideal for this purpose (Scheme 5). Compound **12** was chlorinated using methanesulfonyl chloride in pyridine at 80 °C in the presence of CsCl. This procedure gave the 3′-chloro derivative **27** in 65% yield. This compound was reduced with Bu_3SnH–AIBN to give a 3′-deoxy compound **28** in 66% yield. Finally, hydrogenolysis at 1 atm in the presence of HCl and Pd–C followed by acidic hydrolysis using 6 M hydrochloric acid at 100 °C gave 67% of **18**.

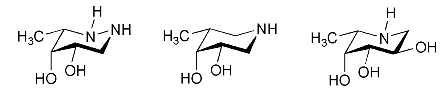


Scheme 5

The target compounds **3**, **4**, **16**–**18** were tested for inhibition of a series of glycosidases. The known[15] compound (±)-**29** was included in the evaluation since it was of interest as a nor-hydroxymethyl analogue of **1**. The two known[15] deoxyisofagomine analogues (±)-**30** and (±)-**31** were also investigated as being useful to compare with **16** and **17**. The unintended product **26** was also investigated, but a racemic sample of **26** was used due to insufficient optically active material. (±)-**26** was made in the same way, but starting with (±)-**22** and (±)-**23**.[10] Inhibition of the enzymes was measured at 25 °C and pH 6.8 in a sodium phosphate buffer, with the exception of β-glucuronidase which was measured at 37 °C and pH 4.6. K_i values were obtained by determination of K_m with and without the presence of an inhibitor and plotting the results in a Hanes plot. In all cases competitive inhibition (or no inhibition) was observed.

Compound **3** was found to be a submicromolar inhibitor of two α-fucosidases (Table 1[17–19]). The compound was 5–10 times more potent than the corresponding isofagomine **32**[16,17] yet 250–500 times weaker than the deoxynojirimycin analogue **33**.[5] This shows that (a) the price in inhibition potency of not having a 2-OH is high and (b) a nitrogen in place of endocyclic oxygen increases inhibition somewhat. This is similar to what has previously been seen for galactosidase inhibitors.[10] For α-galactosidases the inhibition order is 1-deoxynojirimycin > azafagomine > isofagomine. For α-glucosidase inhibition, on the other hand, the order is azafagomine > 1-deoxynojirimycin > isofagomine.[8] The results suggest that an important drawback of **3** and other azafagomines as α-glycosidase inhibitors is their lack of a 2-hydroxy group. The results also suggest that the hydrazine moiety is a better transition state mimic than an amine in either position. It would be interesting to compare the inhibition of **3** with the 2-deoxy analogue of **33** to confirm this, but that compound has not been reported. This suggests that a 2-hydroxy analogue of **3** would be a better α-fucosidase inhibitor than **33** or the 2-hydroxy analogue of **32**. Such a compound would, however, probably be very unstable and form a hydrazone.

Table 1 K_i values in μM at pH 6.8, 25 °C (—, not tested)



	3	**32**	**33**
α-Fucosidase (human placenta)	0.63	6.4[a]	—
α-Fucosidase (bovine kidney)	0.81	4[b]	0.0013[c]

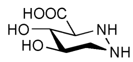
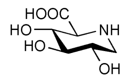
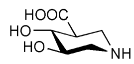
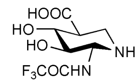
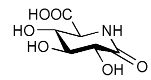
[a] Value from ref. 17. [b] Value from ref. 18. [c] Value from ref. 19.

The K_i value of **4** for the inhibition of β-glucuronidase is shown in Table 2[20–23] together with a series of glucuronidase inhibitors from the literature. Compound **4** is a weaker inhibitor than the corresponding isofagomine **15**, but stronger than the deoxynojrimycin **34**. The order of inhibition isofagomine > azafagomine > deoxynojirimycin has also been found for inhibition of other β-glycosidases.[8,11] The difference in potency between the azafagomine and isofagomine has, however, never been seen as large as in this case. One effect that could contribute to the decreased inhibition of **4** compared to the isofagomine **15** is the conformational behaviour discussed above. Compound **4** probably binds in protonated form, but protonated **4** is predominantly in the undesirable $^1C_4$ conformation, which decreases the concentration of desired $^4C_1$ conformation and thereby the observed inhibition.

The evaluation of the deoxy analogues **16**–**18** resulted in some interesting observations (Table 3). It is clear from the data that removal of hydroxy groups from **1** always reduces its potency. However, the effect of removal of a hydroxy group is less severe when (a) it is in the 6-position (sugar numbering) and (b) the enzyme is almond β-glucosidase. This shows that all the hydroxy groups of **1** contribute to binding, and together with the observations above show that the best azasugar inhibitor would be one with all the hydroxy groups of the substrate intact. It is remarkable that the primary hydroxy is by far the least important of the hydroxy groups. Removal of this OH reduces binding by a factor of only 10–15 for three glycosidases. However removal of the entire hydroxymethyl group, as in (±)-**29**, reduces inhibition of the same three enzymes a 1000-fold, so the presence of the methyl group is far more important than the OH group. Compound **29** is a more conformationally flexible molecule than **18**, but this cannot explain the tremendous loss of activity. It is likely that the methyl group in **18** displaces a molecule of water from the active site which is important for the overall binding process. This is confirmed by compound **4** also being a stronger glucosidase inhibitor than **29** (Table 2). (±)-**29** is also a surprisingly poor β-xylosidase inhibitor (Table 3).

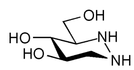
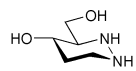
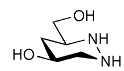
The importance of the 3- and 4-hydroxy groups (carbohydrate numbering) can be confirmed by comparing the K_i values of (±)-**30** and (±)-**31** with those of **37** (Table 4). Both (±)-**30** and (±)-**31** are extremely poor inhibitors of the glycosidases investigated, with the exception of the inhibition of almond β-glucosidase by (±)-**31**. The 4-deoxy analogue (±)-**31** is 50 times less potent than **37**, which is very similar to the difference in inhibition between azafagomine (**1**) and its 4-deoxy analogue **17**. This confirms the relatively low importance of the 4-hydroxy group for the inhibition of almond β-glucosidase,

Table 2 K_i values in µM at pH 6.8, 25 °C (NI, no inhibition; —, not tested)

	 4	 34	 15	 35	 36
β-Glucuronidase (bovine liver)	1	<560 [a]	0.079 [b]	0.065 [c]	0.039 [d]
β-Glucosidase (almond)	7	NI	NI	98 [c]	1200 [e]
α-Glucosidase (yeast)	160	>560 [a]	NI	NI	—

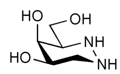
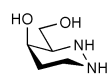
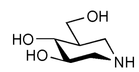
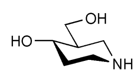
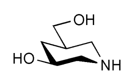
[a] Value from ref. 20. [b] Value from ref. 16. [c] Value from ref. 21. [d] Value from ref. 22. [e] Value from ref. 23.

Table 3 K_i values in µM at pH 6.8, 25 °C (—, not tested)

	 1	 16	 17	 18	 ±29
β-Glucosidase (almond)	0.33 [a]	30	9	3	540
α-Glucosidase (yeast)	6.9 [a]	>3000	>3000	92	3600
Isomaltase (yeast)	0.27 [a]	1300	>3000	4	690
β-Galactosidase (A. oryzae)	702 [b,c]	—	650	—	—
α-Galactosidase (coffee bean)	934 [b,c]	—	>3000	—	—
β-Xylosidase (A. niger)	—	—	—	—	1690

[a] Value from ref. 11. [b] Value from ref. 9. [c] Obtained on racemic inhibitor.

Table 4 K_i values in µM at pH 6.8, 25 °C (—, not tested)

	 2	 (±)-26	 37	 (±)-30	 (±)-31
β-Glucosidase (almond)	0.13 [a]	100	0.11 [b]	2200	5.6
α-Glucosidase (yeast)	570 [a]	—	86 [b]	>3000	>3000
Isomaltase (yeast)	—	25	7.2 [b]	>3000	>3000
β-Galactosidase (A. oryzae)	0.04 [a]	300	—	—	1100
α-Galactosidase (coffee bean)	0.28 [a]	>3000	—	—	—

[a] Value from ref. 10. [b] Value from ref. 8.

which is also reflected by both **1** and **2** inhibiting this enzyme strongly. The only discrepancy between the isofagomine analogues and the azafagomine analogues is the observation that **16** is a moderately good β-glucosidase inhibitor while **30** is extremely poor. The 3-deoxy analogue (carbohydrate numbering) of **2**, **26**, is a 400–10 000 times weaker inhibitor than **2**, which is another example of the importance of this OH-group (Table 4).

In conclusion it has been found that azafagomines are good inhibitors of both α- and β-glucosidases. All the hydroxy groups are important for binding. In fact the general lack of a 2-hydroxy group (carbohydrate numbering) in an azafagomine appears to be an important deficiency in the inhibitor in its binding to α-glycosidases. A 2-hydroxyazafagomine, if sufficiently stable, might indeed be a very strong α-glycosidase inhibitor since 2-hydroxyisofagomine (noeuromycin) is much more potent than isofagomine against α-glycosidases and since azafagomines generally are more potent than isofagomines against these enzymes.

Experimental

General

Solvents were distilled under anhydrous conditions. All reagents were used as purchased without further purification. Pyridine was dried over potassium hydroxide before use. Evaporation was carried out on a rotatory evaporator with the temperature kept below 40 °C. Glassware used for water-free reactions was dried for a minimum of 2 hours at 130 °C before use. Columns were packed with silica gel 60 (230–400 mesh) as the stationary phase. TLC-plates (Merck, 60, F_{254}) were visualized by spraying with cerium sulfate (1%) and molybdic acid (1.5%) in 10% H_2SO_4 and heating till coloured spots appeared. ^{1}H NMR, ^{13}C NMR and COSY were carried out on a Varian Gemini 200 instrument. For samples in water, the water-signal (δ 4.7) was used as the reference. Mass spectra were run on a Micromass LC-TOF instrument. Optical rotations are given in units of 10^{-1} deg cm^2 g^{-1}.

2,3-Di-*O*-isopropylidene-5-*O*-tosyl-D-ribofuranose (6)

Primary alcohol **5** (1.69 g, 8.84 mmol) was dissolved in freshly dried pyridine (10 ml). Dimethylaminopyridine (50 mg) was added together with toluene-*p*-sulfonyl chloride (2.10 g, 11 mmol). The tosyl chloride was added in three portions, 1 hour apart, and the reaction was left to stir for 24 hours at room temperature. After this time, water and dichloromethane (50 ml of each) were added. The phases were separated and the aqueous phase was extracted with dichloromethane (5 × 50 ml). The combined organic extracts were dried ($MgSO_4$) and the solvents were removed. The residue underwent column chromatography using EtOAc–pentane (3 : 7) as eluent. This yielded 2.00 g (73%) of the desired product **6**. NMR spectra were identical to those previously reported.[14]

2,3-Di-*O*-isopropylidene-5-deoxy-D-ribofuranose (7)

Tosyl compound **6** (2.0 g, 6.49 mmol) was dissolved in freshly dried dimethoxyethane (50 ml). Sodium iodide (1.947 g, 13 mmol) was added and the reaction mixture was refluxed for 2 hours. Dichloromethane and water (50 ml of each) were added, whereafter the aqueous phase was extracted with dichloromethane (3 × 50 ml). The combined organic extracts were dried ($MgSO_4$) and the solvents were removed. The residue was dissolved in ethanol (28 ml) and triethylamine (2.1 ml) and Pd–C (10%, 700 mg) were added. Hydrogen pressure was applied (1 atm, room temperature) and the mixture was stirred for 2 days. After this period of time an additional portion of catalyst was added (150 mg) and the mixture was stirred overnight. The reaction mixture was then filtered through a bed of Celite® and concentrated to a residue that underwent chromatography through a short column of silica gel using EtOAc–pentane as eluent (1 : 3). This gave a yield of 832 mg (74%) of the reduced compound **7**. NMR spectra were identical to those previously reported.[13]

1-(2-*tert*-Butyloxycarbonylhydrazino)-1,5-dideoxy-2,3-di-*O*-isopropylidene-D-ribitol (8)

Hemiacetal **7** (680 mg, 3.91 mmol) was dissolved in methanol (17 ml) and *tert*-butyl carbazate (1.033 g, 7.82 mmol) was added together with sodium cyanoborohydride (982 mg, 15.6 mmol). Acetic acid was added until pH ~ 5 was reached. The reaction mixture was stirred at room temperature for 42 hours whereafter a saturated aqueous solution of $NaHCO_3$ (10 ml) was added. The methanol was then removed under reduced pressure whereafter the aqueous phase was extracted with dichloromethane (3 × 20 ml). The organic extracts were dried ($MgSO_4$), the solvent removed, and the residue filtered through a short column of silica gel using EtOAc–pentane (1 : 1) as eluent (R_f = 0.38). This resulted in 1.096 g (97%) of the Boc-protected hydrazine **8**, which appeared as a colourless oil. $[\alpha]_D^{25}$ 2.75 (*c* 2, $CHCl_3$); $\delta_H(CDCl_3)$ 7.15 (br s, 1H, *H*NBoc), 4.70 (br s, 2H, O*H*, N*H*NHBoc), 4.26 (quintet, 1H, *J* 4.8 Hz, H4), 3.76–3.90 (m, 2H, H2, H3), 3.10 (dd, 1H, $J_{1a,2}$ 9.2 Hz, $J_{1a,1b}$ 12.4 Hz, H1a), 3.00 (dd, 1H, $J_{1b,2}$ 4.2 Hz, H1b), 1.41 [s, 9H, $C(CH_3)_3$], 1.25–1.34 [m, 9H, $OC(CH_3)_2O$, H5]; $\delta_C(CDCl_3)$ 156.9 ($NCO_2{}^tBu$), 108.3 [$C(OCH_3)_2$], 82.4, 80.9, 74.6, 65.2, 51.8 [C1, C2, C3, C4, $OC(CH_3)_3$], 28.4 [$OC(CH_3)_3$], 28.2, 25.6 [$OC(CH_3)_2O$], 20.5 (C5). HRMS(ES): Calcd. for $C_{13}H_{26}N_2O_5$ + Na: 313.1739, found 313.1737.

1-(1-Acetyl-2-*tert*-butoxycarbonylhydrazino)-1,5-dideoxy-2,3-di-*O*-isopropylidene-4-*O*-methylsulfonyl-D-ribitol (9)

Secondary alcohol **8** (1.066 g, 3.68 mmol) was dissolved in methanol (65 ml) and acetic anhydride (6.5 ml) was added. The mixture was stirred at room temperature for 2 hours whereafter a saturated aqueous solution of $NaHCO_3$ (40 ml) was added. The mixture was stirred for 15 min, whereafter the methanol was removed under reduced pressure. The aqueous phase was extracted with CH_2Cl_2 (5 × 40 ml) and the organic extract washed with brine (40 ml) before being dried ($MgSO_4$). The residue after evaporation was dissolved in pyridine (6.6 ml) and methanesulfonyl chloride (0.43 ml, 5.51 mmol) was added in two portions (1 equiv. then 0.5 equiv.) separated by an 18 hour interval. The reaction was finished according to TLC (AcOEt–pentane 1 : 1) after 24 hours. Water (20 ml) and CH_2Cl_2 (20 ml) were added, the phases separated and the aqueous phase was extracted with CH_2Cl_2 (5 × 20 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated. The remaining oil underwent column chromatography on silica gel (eluent: first AcOEt–pentane 1 : 2, then 1 : 1). This resulted in 1.225 g (81%) of the desired compound **9**, which appeared as a white foam. R_f(AcOEt–pentane 1 : 1) = 0.29. $[\alpha]_D^{25}$ −15.2 (*c* 2, $CHCl_3$); $\delta_H(CDCl_3)$ 6.9–7.1 (br s, 1H, N*H*), 4.79 (qv, 1H, *J* 6.2 Hz, H4), 4.30–4.55 (m, 2H, H1a, H2), 4.05 (t, 1H, *J* 6.2 Hz, H3), 3.07 (s, 3H, SO_2CH_3), 2.8–3.0 (br s, 1H, H1b), 2.05 [s, 3H, $NC(O)CH_3$], 1.50 (d, 3H, H5), 1.44 [s, 12H, $C(CH_3)_3$, CH_3], 1.31 (s, 3H, CH_3). Cross-peaks corresponding to *J*-coupling between the signals at 4.30–4.55 and 2.8–3.0 were observed in the COSY spectrum; $\delta_C(C_6D_6)$ 173.7 [$NC(O)CH_3$], 154.7 [$NC(O)O^tBu$], 109.3 [$OC(CH_3)_2O$], 81.4, 78.4, 76.7, 75.7 [C2, C3, C4, $OC(CH_3)_3$], 47.6 (C1), 38.9 (SO_2CH_3), 28.2 [$OC(CH_3)_3$], 27.9, 25.7 [$OC(CH_3)_2O$], 20.5, 18.8 [C5, $C(O)CH_3$]. HRMS(ES): Calcd. for $C_{16}H_{30}N_2O_8$ + Na: 433.1621, found 433.1619.

(3*S*,4*R*,5*S*)-1-Acetyl-4,5-isopropylidenedioxy-3-methylhexahydropyridazine (10)

Mesylate **9** (652 mg, 1.59 mmol) was dissolved in freshly distilled CH_2Cl_2 (30 ml) and 2,6-lutidine (1.48 ml, 12.7 mmol) and TMSOTf (1.44 ml, 7.95 mmol) were added at 0 °C. The reaction was allowed to reach room temperature over 1 hour and stirred additionally for 6 hours at this temperature. A solution of aqueous Na_2CO_3 (10%, 20 ml) was added and the aqueous phase extracted with $CHCl_3$ (15 × 20 ml). The volume of organic solvent was reduced to *ca.* 250 ml, whereafter anhydrous K_2CO_3 (3.5 g) was added and the mixture refluxed for 24 hours. The mixture was filtered and the residue concentrated to a pale oil. This underwent filtration through silica gel (eluent: first AcOEt–pentane 1 : 1, then AcOEt), which gave 310 mg (91%) of the cyclised product **10** that appeared as colourless crystals. R_f(AcOEt) = 0.39; mp(uncorr.) 143–144 °C; $[\alpha]_D^{25}$ −21.0 (*c* 1, $CHCl_3$); $\delta_H(CDCl_3)$ 1.35, 1.50 [s, 6H, $C(OCH_3)_2$], 1.80 (d, 3H, *J* 6.6 Hz, H3′), 2.14 [s, 3H, $NC(O)CH_3$], 2.75 (dd, 1H, $J_{5,6ax}$ 8.0 Hz, $J_{6ax,6eq}$ 13.6 Hz, H6ax), 2.98–3.11 (m, 1H, H3), 3.36 (d, 1H, *J* 13.0 Hz, N*H*), 3.96 (dd, 1H, $J_{3,4}$ 2.0 Hz, $J_{4,5}$ 5.2 Hz, H4), 4.23 (m, 1H, H5), 4.43 (dd, 1H, H6eq); $\delta_C(CDCl_3)$ 172.8 [$NC(O)CH_3$], 109.1 [$OC(CH_3)_2O$], 73.3, 70.3, 53.6, 42.6 (C3, C4, C5, C6), 28.3, 26.3 [$OC(CH_3)_2O$], 20.9, 15.3 [C3′, $NC(O)CH_3$]; HRMS(ES): Calcd. for $C_{10}H_{18}N_2O_3$ + Na: 237.1215, found 237.1237.

(3*S*,4*R*,5*S*)-4,5-Dihydroxy-3-methylhexahydropyridazine (3)

Acetohydrazide **10** (304 mg, 1.42 mmol) was dissolved in hydrochloric acid (6 M, 25 ml) and heated to 100 °C overnight in a sealed flask. The solvent was removed, and the residue underwent ion-exchange chromatography (Amberlite IR-120, H^+). The product was released from the resin with 2.5% NH_4OH. This gave a quantitative yield (185 mg) of the desired hexahydropyridazine **3**, which appeared as a colourless powder. Mp 155 °C (decomp.); $[\alpha]_D^{25}$ −28.0 (*c* 1, H_2O); $\delta_H(D_2O)$ 3.65–3.70 (m, 2H, H4, H5), 2.55–2.8 (m, 3H, H3, H6ax, H6eq), 0.98 (d, 3H, *J* 6.8 Hz, H3′); $\delta_C(D_2O)$ 68.8, 67.3 (C4, C5), 54.4, 45.2 (C3, C6), 14.1 (C3′); HRMS(ES): Calcd. for $C_5H_{12}N_2O_2$ + Na: 155.0796, found 155.0793.

(3*R*,4*R*,5*R*)-1,2-Diacetyl-4,5-dibenzyloxy-3-hydroxymethylhexahydropyridazine (12)

To monoacetyl compound **11** (521 mg, 1.15 mmol) freshly distilled acetyl bromide (8 ml) was added at 0 °C under an atmosphere of nitrogen. The ice bath was removed and the mixture was stirred for 7 hours at room temperature. It was then diluted with chloroform (10 ml) and carefully poured into an ice-bath cooled flask containing chloroform (10 ml) and saturated aqueous Na_2CO_3 solution (20 ml). The solution was further neutralised by adding saturated $NaHCO_3$ solution. The aqueous phase was then extracted three times with chloroform and the combined organic phases were dried over anhydrous $MgSO_4$. The drying agent was removed by filtration, and the organic solvent was removed under reduced pressure. The remaining oil was immediately deacetylated by being stirred in methanol (10 ml) containing a catalytic amount of sodium methoxide. After 1 hour a small lump of dry ice was added, and the methanol was removed by evaporation under reduced pressure. The remaining oil underwent flash chromatography in AcOEt–pentane 1 : 1, resulting in 40 mg (7%) of **13** (R_f = 0.5) and 340 mg (73%) of **12** (R_f = 0.15), which both appeared as colourless oils. δ_H($CDCl_3$) **12**, 2.00–2.35 (m, 6H), 3.08 (d, 1H), 3.34–4.05 (m, 6H), 4.32–4.95 (m, 4.5H), 5.12 (t, 0.25H, *J* 6.0 Hz), 5.26 (dd, 0.25H, *J* 4.0 Hz, *J* 11.4 Hz), 7.20–7.40 (m, 10H); **13**, 1.98–2.20 (m, 6H), 3.08 (d, 1H, *J* 13.2 Hz), 3.40–3.94 (m, 4H), 4.26–4.89 (m, 7.25H), 5.35 (dd, 0.75H, *J* 4.4 Hz, *J* 9.4 Hz), 7.05–7.40 (m, 15H); MS(ES) **13**, Calcd. for $C_{30}H_{34}N_2O_5$ + Na: 525.2, found: 525.0; HRMS(ES) **12**, Calcd. for $C_{23}H_{28}N_2O_5$ + Na: 435.1898, found: 435.1899.

(3*S*,4*R*,5*R*)-1,2-Diacetyl-4,5-dibenzyloxyhexahydropyridazine-3-carboxylic acid (14)

To a solution of **12** (191 mg, 0.46 mmol) in acetone (8 ml) at 0 °C, Jones reagent (*ca.* 0.3 ml; Jones reagent: 2.67 g of CrO_3 was added to 2.3 ml of concentrated sulfuric acid and then diluted to 10 ml) was added in three portions over 30 min. All starting material had disappeared after 2 hours (TLC monitoring). *i*-PrOH (1 ml) and water (10 ml) were then added, and the acetone was removed under reduced pressure. The remaining water was extracted five times with AcOEt, and the combined organic phases were dried over $MgSO_4$, filtered and concentrated. The remaining oil underwent flash chromatography in AcOEt–pentane 1 : 1 containing 1% HCO_2H (R_f = 0.45) resulting in 130 mg (66%) of **14**. The compound appeared as a colourless powder, mp 180–183 °C (uncorr.); δ_H($CDCl_3$) 1.97–2.24 (m, 6H), 3.48–3.51 (m, 1H), 3.70–3.72 (m, 1H), 3.99–4.83 (m, 6H), 5.70 (t, 1H, *J* 3.4 Hz), 7.00–7.40 (m, 10H); HRMS(ES) Calcd. for $C_{23}H_{26}N_2O_6$ + Na: 449.1689, found: 449.1689.

(3*S*,4*R*,5*R*)-4,5-Dihydroxyhexahydropyridazine-3-carboxylic acid (4)

Carboxylic acid **14** (105 mg, 0.25 mmol) was dissolved in methanol (12 ml) and 10% Pd–C (50 mg) was added. Hydrogen pressure (1 atm) was applied and 2 drops of concentrated hydrochloric acid were added. The mixture was stirred at room temperature for 2 hours after which the catalyst was removed by filtration through Celite®. The methanol was removed by evaporation and to the remaining oil hydrochloric acid (6 M, 20 ml) was added. The flask was sealed and heated to 100 °C for 24 hours. The aqueous acid was then removed by evaporation and the residue loaded onto a column of ion-exchange resin (Amberlite IR-120, H^+), which was then carefully washed. The compound was released with 5% NH_4OH and subjected to column chromatography eluting with 7 : 2 : 1 *i*-PrOH–water–conc. NH_4OH (R_f = 0.3). This gave 30 mg (74%) of **4**. $[a]_D^{25}$ −159.2 (*c* 0.18, H_2O); δ_H(D_2O, neutral) 2.93 (dd, 1H, $J_{5,6ax}$ 7.0 Hz, $J_{6ax,6eq}$ 13.2 Hz, H6ax), 3.42 (dd, 1H, $J_{5,6eq}$ 3.8 Hz, H6eq), 3.44 (d, 1H, $J_{3,4}$ 6.6 Hz, H3), 3.78 (dt, 1H, H5), 3.87 (t, 1H, H4); δ_H(D_2O, acidified with DCl to pH ~ 1) 3.19 (dd, 1H, $J_{5,6ax}$ 4.4 Hz, $J_{6ax,6eq}$ 13.2 Hz, H6ax), 3.60 (dd, 1H, $J_{5,6eq}$ 2.4 Hz, H6eq), 3.88 (d, 1H, $J_{3,4}$ 4.0 Hz, H3), 4.00 (dt, 1H, H5), 4.18 (t, 1H, H4); δ_H(D_2O, basified with Na_2CO_3 to pH ~ 10) 2.56 (dd, 1H, $J_{5,6ax}$ 10.0 Hz, $J_{6ax,6eq}$ 13.0 Hz, H6ax), 3.08 (d, 1H, $J_{3,4}$ 9.2 Hz, H3), 3.16 (dd, 1H, $J_{5,6eq}$ 5.0 Hz, H6eq), 3.47 (t, 1H, H4), 3.56 (dt, 1H, H5); δ_C(D_2O, neutral) 174.2 (*C*OOH), 70.3, 67.3, 63.3 (C3, C4, C5), 48.7 (C6). HRMS(ES) Calcd. for $C_5H_{10}NO_4$ + Na: 185.0538, found 185.0539.

(−)-(2*R*,3*R*,4*R*)-3-Acetoxy-2-acetoxymethyl-4-iodo-8-methyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione (20)

Epoxide **19** (144 mg, 0.68 mmol) was dissolved in acetic acid (2 ml) and a 57% aqueous solution of HI was added (173 mg, 1.35 mmol). The reaction mixture was allowed to stir at room temperature for 1 h. Acetic acid anhydride (5 ml) was added and allowed to react for 3 hours before the reaction was quenched with water (5 ml). After another hour the reaction mixture was extracted with AcOEt (3 × 15 ml) and the combined organic phases washed with saturated solutions of $NaHCO_3$ and Na_2SO_3 (10 ml). After being dried over $MgSO_4$, filtered and evaporated, the residue underwent column chromatography (AcOEt–pentane 1 : 1), which resulted in 210 mg (73%) of iodide **20**, which appeared as a colourless oil. R_f(AcOEt–pentane 1 : 1) = 0.26. $[a]_D^{25}$ −31.0 (*c* 1, $CHCl_3$); δ_H($CDCl_3$) 5.30 (t, 1H, t, $J_{2,3;3,4}$ 6.0 Hz, H3), 4.69 (dd, 1H, $J_{2,2'a}$ 4.4 Hz, $J_{2'a,2'b}$ 12.0 Hz, H2′a), 4.56 (dd, 1H, $J_{2,2'b}$ 5.4 Hz, H2′b), 4.1–4.2 (m, 1H, H2 or H4), 4.0–4.1 (m, 1H, H4 or H2), 4.01 (dd, 1H, $J_{4,5a}$ 4.0 Hz, $J_{5a,5b}$ 12.6 Hz, H5a), 3.79 (dd, 1H, $J_{4,5b}$ 6.0 Hz, H5b), 3.02 (s, 3H, NCH_3), 2.08 (s, 3H, CH_3), 2.02 (s, 3H, CH_3); δ_C($CDCl_3$) 170.6, 169.1 [*C*(O)CH$_3$], 154.0, 153.1 [N*C*(O)N], 69.9 (C3), 60.4 (C2′), 58.8, 50.2 (C2, C5), 25.5 (N*C*H$_3$), 20.9 [double intensity, C(O)*C*H$_3$], 16.9 (C4); HRMS(ES) Calcd. for $C_{12}H_{16}N_3O_6I$ + Na: 447.9983, found: 447.9984.

(−)-(2*R*,3*S*)-3-Acetoxy-2-acetoxymethyl-8-methyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione (21)

Iodide **20** (210 mg, 0.49 mmol) was dissolved in benzene (2.5 ml) and *n*-Bu_3SnH (0.288 mg, 0.99 mmol) and AIBN (0.4 mg) were added. The temperature was raised to 80 °C, and the mixture was stirred for 4.5 hours at this temperature. The solvent was then removed under reduced pressure, and the residue loaded directly onto a column of silica gel and eluted (first $CHCl_3$, then AcOEt–pentane 1 : 1) to give 95 mg (65%) of the reduced product **21**. R_f(AcOEt–pentane 1 : 1) 0.19. $[a]_D^{25}$ −24.8 ($CHCl_3$, *c* 1); δ_H($CDCl_3$) 5.12 (q, 1H, *J* 2.8 Hz, H3), 4.44 (m, 1H, H2), 4.32 (dd, 1H, $J_{2,2'a}$ 6.0 Hz, $J_{2'a,2'b}$ 11.2 Hz, H2′a), 4.16 (dd, 1H, $J_{2,2'b}$ 7.4 Hz, H2′b), 3.8–4.0 (m, 1H, H5a), 3.28 (dt, 1H, $J_{4a,5b}$ 4.8 Hz, $J_{4b,5b;5a,5b}$ 11.8 Hz, H5b), 3.06 (s, 3H, NCH_3), 2.00–2.15 (m, 2H, H4a, H4b), 2.07 (s, 3H, CH_3), 2.02 (s, 3H, CH_3); δ_C($CDCl_3$) 170.6, 169.9 [*C*(O)CH$_3$], 154.9, 153.1 [N*C*(O)N], 64.7 (C3), 60.0 (C2′), 54.8, 40.0 (C2, C5), 25.3, 24.7 (N*C*H$_3$, C4), 21.1, 20.8 [C(O)*C*H$_3$]; HRMS(ES): calcd. for $C_{12}H_{17}O_6N_3$ + Na: 322.1015, found: 322.1020.

(+)-(3*R*,4*S*)-4-Hydroxy-3-hydroxymethylhexahydropyridazine (16)

Diacetate **21** (92 mg, 0.31 mmol) was deacetylated in methanol (4 ml) containing a catalytic amount of sodium methoxide at room temperature. After reaction completion (20 min) the methanol was removed and N_2H_5OH (5 ml) was added. The mixture was refluxed for 24 h. The solvent was then removed and the residue underwent ion exchange (Amberlite IR-120, H^+). The product was released with 5% NH_4OH. Concentration followed by flash chromatography in EtOH–conc. NH_4OH 9 : 1 (R_f = 0.44) produced 23 mg (57%) of **16**. $[a]_D^{25}$ 24.7 (*c* 1, H_2O); δ_H(D_2O) 3.82 (dd, 1H, $J_{3,3'a}$ 3.0 Hz, $J_{3'a,3'b}$ 12.0 Hz, H3′a), 3.61 (dd, 1H, $J_{3,3'b}$ 6.3 Hz, H3′b), 3.54 (ddd, 1H, $J_{4,5eq}$ 5.0 Hz, $J_{3,4}$ 9.5 Hz, $J_{4,5ax}$ 11.0 Hz, H4), 3.10 (ddd, 1H, $J_{5eq,6eq}$ 2.7 Hz, $J_{5ax,6eq}$ 5.0 Hz, $J_{6ax,6eq}$ 13.1 Hz, H6eq), 2.77 (dt, 1H, $J_{5eq,6ax}$ 3.0 Hz, H6ax), 2.61 (ddd, 1H, H3), 2.05 (tdd, 1H, $J_{5ax,5eq}$ 13.1 Hz, H5eq), 1.50 (ddt, 1H, H5ax); δ_C(D_2O) 66.7, 64.5 (C3′, C4), 60.6 (C3), 46.3 (C6), 33.4 (C5).

(−)-(2*S*,4*S*)-4-Acetoxy-2-acetoxymethyl-8-methyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione (25) and (+)-(2*R*,3*R*)-3-acetoxy-2-acetoxymethyl-8-methyl-1,6,8-triazabicyclo[4.3.0]-nonane-7,9-dione (24)

A mixture of iodides **22** and **23** (506 mg, 1.2 mmol) was dissolved in benzene (5 ml) and *n*-Bu_3SnH and AIBN were added according to the procedure for synthesis of compound **3**. Work-up and purification were done as described earlier. This yielded 162 mg (46%) of **25** [R_f(AcOEt–pentane 1 : 1) = 0.12] and 75 mg (21%) of **24** [R_f(AcOEt–pentane 1 : 1) = 0.16]. **25**: $[a]_D^{25}$ = −21.3

(c 1, $CHCl_3$); $\delta_H(CDCl_3)$ 5.13 (qv, 1H, J 3.2 Hz, H4), 4.20–4.52 (m, 3H, H2, H2′a, H2′b), 4.01 (dd, 1H, $J_{5a,5b}$ 13.0 Hz, H5a) 3.26 (dd, 1H, H5b), 3.04 (s, 3H, NCH_3), 2.06 [s, 3H, $C(O)CH_3$], 2.00 [s, 3H, $C(O)CH_3$], 2.00–2.10 (m, 2H, H3a, H3b); $\delta_C(CDCl_3)$:

170.9, 169.9 [$C(O)CH_3$], 155.3, 153.4 [NC(O)N], 64.5, 62.6 (C4, C2′), 50.5, 47.9 (C2, C5), 28.8 (C3), 25.4 (NCH_3), 21.4, 20.9 [$C(O)CH_3$]. HRMS(ES): Calcd. for $C_{12}H_{17}O_6N_3$ + Na: 322.1015, found: 322.1009. **24**: $[\alpha]_D^{25}$ 5.5 ($CHCl_3$, c 1); $\delta_H(CDCl_3)$: 5.10 (td, 1H, J 5.0 Hz, J 10.2 Hz, H3), 4.41–4.62 (m, 3H, H2, H2′a, H2′b), 3.96 (td, 1H, $J_{4a,5a;4b,5a}$ 4.0 Hz, $J_{5a,5b}$ 12.2 Hz, H5a), 3.23 (dt, 1H, $J_{4a,5b}$ 4.0 Hz, H5b), 3.05 (s, 3H, NCH_3), 1.99–2.16 (m, 2H, H4a, H4b), 2.11 [s, 3H, $C(O)CH_3$], 2.00 [s, 3H, $C(O)CH_3$]; $\delta_C(CDCl_3)$ 170.8, 169.7 [$C(O)CH_3$], 154.8, 153.1 [NC(O)N], 67.7, 59.1 (C2′, C3), 53.6, 42.5 (C2, C5), 25.4 (double intensity, C4, NCH_3), 21.1, 20.8 [$C(O)CH_3$]. HRMS(ES): Calcd. for $C_{12}H_{17}O_6N_3$ + Na: 322.1015, found: 322.1014.

(−)-(3*S*,5*S*)-5-Hydroxy-3-hydroxymethylhexahydropyridazine (17)

Diacetate **25** (88 mg, 0.29 mmol) was deprotected as described for the synthesis of **16**. This yielded 25 mg (65%) of the desired product **17**. R_f(EtOH–conc. NH_4OH 9 : 1) = 0.4. $[\alpha]_D^{25}$ −5.0 (c 1, H_2O); $\delta_H(D_2O)$ 1.15 (q, 1H, J 11.8 Hz, H4ax), 1.96–2.10 (dm, 1H, H4eq), 2.38 (dd, 1H, $J_{5,6ax}$ 10.8 Hz, $J_{6ax,6ax}$ 12.3 Hz, H6ax), 2.78–2.95 (m, 1H, H3), 3.10 (dd, 1H, $J_{5,6eq}$ 4.8 Hz, H6eq), 3.46 (dd, 1H, $J_{3,3'a}$ 6.2 Hz, $J_{3'a,3'b}$ 11.7 Hz, H3′a), 3.55 (dd, 1H, $J_{3,3'b}$ 4.8 Hz, H3′b), 3.76 (m, 1H, H5); $\delta_C(D_2O)$ 34.6 (C4), 51.8 (C6), 57.2 (C3), 62.8, 65.5 (C3′, C5). HRMS(ES) Calcd. for $C_5H_{12}O_2N_2$ + H: 133.0977, found: 133.0977.

(−)-(3*R*,4*R*)-4-Hydroxy-3-hydroxymethylhexahydropyridazine (26)

Diacetate **24** (75 mg, 0.25 mmol) was deprotected as described for the synthesis of **16**. This yielded 21 mg (63%) of the desired product **26**. R_f(EtOH–$CHCl_3$–conc. NH_4OH 7 : 2 : 1) = 0.26. $[\alpha]_D^{25}$ −10.0 (c 0.25, H_2O); $\delta_H(D_2O)$ 3.99 (q, 1H, J 1.8 Hz, H4), 3.56 (d, 2H, $J_{3,3'}$ 7.0 Hz, H3′a, H3′b), 3.00–3.10 (m, 1H, H6ax), 2.94 (dt, 1H, H3), 2.81 (td, 1H, $J_{5ax,6eq;5eq,6eq}$ 4.0 Hz, $J_{6ax,6eq}$ 13.2 Hz, H6eq), 1.77–1.86 (m, 2H, H5ax, H5eq); $\delta_C(D_2O)$ 63.4 (C4), 61.0 (double intensity, C3, C3′), 41.6 (C6), 30.8 (C5).

(3*S*,4*R*,5*R*)-1,2-Diacetyl-4,5-dibenzyloxy-3-chloromethylhexahydropyridazine (27)

Primary alcohol **12** (53 mg, 0.13 mmol) was dissolved in pyridine (0.8 ml). The mixture was cooled to 0 °C and methanesulfonyl chloride was added (45 μl, 4.5 equiv.). The mesyl chloride was added in 3 portions with 1 hour intervals, and the mixture was left to stir for 4 hours at 80 °C with a pinch of CsCl (approx. 10 mg). After this time the solvents were removed, and the residue underwent column chromatography using EtOAc–pentane (1 : 3) as eluent. This yielded 36 mg (65%) of the desired product **27**. The NMR spectrum of **27** was complex due to extensive rotamer formation. HRMS(ES): Calcd. for $C_{23}H_{27}O_4N_2Cl$ + Na: 453.1557, found: 453.1558.

(3*R*,4*R*,5*R*)-1,2-Diacetyl-4,5-dibenzyloxy-3-methylhexahydropyridazine (28)

Chloride **27** (36 mg, 0.084 mmol) was dissolved in toluene (2.5 ml) and n-Bu_3SnH (42.5 μl, 2.2 equiv.) and AIBN (0.4 mg) were added. The temperature was raised to 110 °C and the mixture was stirred for 1.5 hours at this temperature. The solvent was then removed under reduced pressure and the residue subjected to column chromatography on silica gel in AcOEt–pentane 1 : 7 to give 22 mg (66%) of the reduced product **28**. The NMR spectrum of **28** was complex due to extensive rotamer formation. HRMS(ES): Calcd. for $C_{23}H_{28}O_4N_2$ + Na: 419.1947, found: 419.1947.

(3*R*,4*R*,5*R*)-4,5-Dihydroxy-3-methylhexahydropyridazine (18)

Diacetate **28** (36 mg, 0.09 mmol) was dissolved in methanol (5 ml) and 10% Pd–C (50 mg) was added. Hydrogen pressure (1 atm) was applied and 2 drops of concentrated hydrochloric acid were added. The mixture was stirred at room temperature for 18 hours after which the catalyst was removed by filtration through Celite®. The methanol was removed by evaporation and to the remaining oil hydrochloric acid (6 M, 6 ml) was added. The flask was sealed and heated to 100 °C for 24 hours. The aqueous acid was then removed by evaporation and the residue subjected to column chromatography in 99 : 1 EtOH–conc. NH_4OH. This gave 8 mg (67%) of **18**. $[\alpha]_D^{25}$ −12 (H_2O, c 0.3); $\delta_H(D_2O)$ 3.65 (ddd, 1H, H5, J_{45} 8.8 Hz, J_{56eq} 4.6 Hz, J_{56ax} 10.4 Hz, H5), 3.36 (m, 1H, J_{6ax6eq} 13.2 Hz, H6eq), 3.24 (t, 1H, J_{34} 9.6 Hz, H4), 2.91 (dq, 1H, $J_{3,3'}$ 6.6 Hz, H3), 2.78 (dd, 1H, H6ax), 1.22 (d, 3H, CH_3); $\delta_C(D_2O)$ 76.8, 70.7 (C4, C5), 57.2 (C3), 51.4 (C6), 14.2 (C3′).

X-Ray crystallography †

The crystal structure of **10**, (3*S*,4*R*,5*S*)-1-Acetyl-4,5-isopropylidenedioxy-3-methyl-hexahydropyridazine, $C_{10}H_{18}N_2O_3$, M = 214.27, was solved using data collected at 120 K from a colourless plate on a SIEMENS SMART CCD diffractometer. The crystals are monoclinic, space group $P2_1$, with unit cell: a = 6.436(1) Å, b = 10.547(2) Å, c = 8.763(2) Å, β = 105.899(3)°, V = 572.1(2) Å^3, Z = 2, μ = 0.092, R_{int} = 0.078, for 6253 measured reflections of which 3168 were independent. Direct methods were applied[24] for the structure solution, and the structure refined by least-squares methods to a final R = 0.039, R_w = 0.046, GoF = 1.37 for 2811 reflections with $I > 3\sigma(I)$ and 208 parameters. The structure is held together in chains by hydrogen bonds from N2 to the keto oxygen, O15, in a molecule related by a screw axis.

Enzyme inhibition

The enzyme assays were carried out as described previously.[9] All assays were performed at pH 6.8 and 25 °C except the β-glucuronidase assay which was performed at pH 4.6 and 37 °C. The inhibition constants (K_i) were obtained from the formula $K_i = [I]/(K_M'/K_M^{-1})$, where K_M' and K_M are Michaelis–Menten constants with and without inhibitor present. K_M' and K_M were obtained from a Hanes plot, which also was used to ensure that inhibition was competitive. The following K_M values (without inhibitor) were obtained using 4-nitrophenyl glycosides as substrates and the above conditions: β-glucuronidase (bovine liver), 1 mM; β-glucosidase (almonds), 3.8 mM; α-glucosidase (baker's yeast), 0.25 mM; isomaltase (yeast), 1.3 mM; β-galactosidase (*Aspergillus oryzae*), 1.4 mM; α-galactosidase (green coffee beans), 0.6 mM; α-fucosidase (human placenta), 0.23 mM; α-fucosidase (bovine kidney), 0.24 mM.

† CCDC reference number 178401. See http://www.rsc.org/suppdata/p1/b2/b200884j for crystallographic files in .cif or other electronic format.

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