1-Azafagomine: A Hydroxyhexahydropyridazine that Potently Inhibits Enzymatic Glycoside Cleavage

Mikael Bols,* Rita G. Hazell, and Ib B. Thomsen

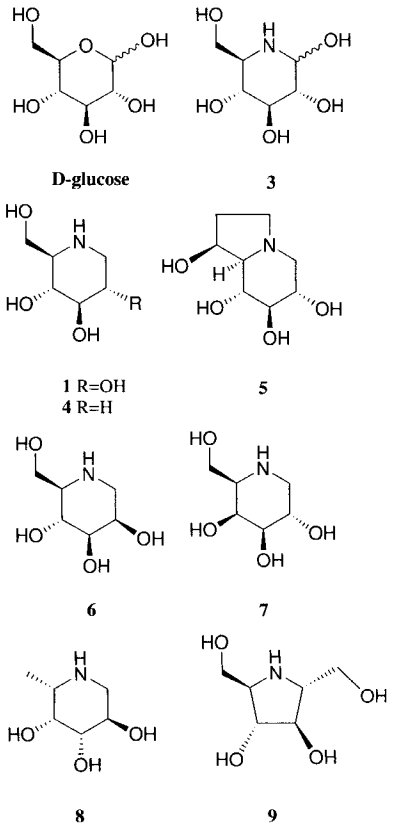
Abstract: (3,4-*trans*-4,5-*trans*)-4,5-dihydroxy-3-hydroxymethylhexahydropyridazine (**16**) was synthesized in four steps from 2,4-pentadienol (**22**) and 4-phenyltriazolin-3,5-dione (**18**) in an overall yield of 32%. In the first step a Diels–Alder reaction between **18** and **22** gave (±)-2-hydroxymethyl-8-phenyl-1,6,8-triazabicyclo[4.3.0]non-3-ene-7,9-dione (**23c**) in 88% yield. Epoxidation of **23c** with trifluoromethyl(methyl)dioxirane, generated in situ, gave the *trans* epoxide **24c** in 62% yield. Hydrolysis of the epoxide with perchloric acid gave stereoselectively (2,3-*trans*-3,4-*trans*)-3,4-dihydroxy-2-hydroxymethyl-8-phenyl-1,6,8-triazabicyclo[4.3.0]-nonane-7,9-dione (**26**) in 73% yield. In the fourth and final step, hydrazinolysis of **26** gave **16** in 84% yield. Pyridazine **16** was found to be a potent inhibitor of α- and β-glucosidase, isomaltase and glycogen phosphorylase, while galactosidases and α-mannosidase were not inhibited. The inhibition of β-glucosidase is independent of pH, and was found to be due to unprotonated **16**.

Keywords
chemoselectivity · enzyme inhibitors · glycosidases · kinetics · pyridazines

Introduction

Inhibitors of glycosyl-cleaving enzymes offer the opportunity of modulating the metabolism of carbohydrates, thus opening up a number of potential applications. These include treatment of AIDS,[1] diabetes,[2] and tumor metastasis,[3] as well as crop protection. Even though a variety of different glycosidase inhibitors are known,[4] the potential uses of such compounds are relatively unexploited as only one such compound, acarbose, has reached the market as an antidiabetic. There is a need for new classes of potent and selective glycosidase inhibitors so that research can progress in this area. Potent inhibitors of other glycosyl-cleaving enzymes are also needed; they are virtually nonexistent at present.

An important class of glycosidase inhibitors is made up of the hydroxylated piperidines and pyrrolidines that occur in plants and microorganisms[5] and have been dubbed the "sugar-shaped alkaloids from plants".[6] They are reversible competitive inhibitors of glycosidases. One example of these compounds is 1-deoxynojirimycin[7] (**1**), which closely resembles glucose: the ring oxygen has been exchanged for a nitrogen atom. In terms of polarity, the *N*-protonated **1** resembles the oxocarbenium ion **2**, which is generally expected to be a transition state in the process of enzymatic glycoside bond cleavage. This resemblance might be the basis of its inhibition. Other examples are nojirimycin[8] (**3**), fagomine[9] (**4**), and castanospermin[10] (**5**), which resemble D-glucose, and 1-deoxymannonojirimycin[11] (**6**), galactostatin[12] (**7**), 1-deoxyfuconojirimycin[13] (**8**), and 2,5-dideoxy-2,5-imino-D-mannitol[14] (**9**), which resemble D-mannose, D-galactose, L-fucose, and D-fructofuranose, respectively (Scheme 1). The probable purpose of these natural products is the inhibition of the carbohydrate metabolism and consequently the growth of plant-consuming pests.



Scheme 1. Nojirimycin and analogues.

Recently we discovered that a synthetic isomer of fagomine (**4**), isofagomine (**10**)—in which a nitrogen replaces the anomeric carbon instead of the ring oxygen—was a much more potent glycosidase inhibitor than **4**.[15–17] It was particularly potent in its inhibition of almond β-glucosidase, and, though yeast α-glucosidase was inhibited, β-glucosidase inhibition was 780 times stronger.

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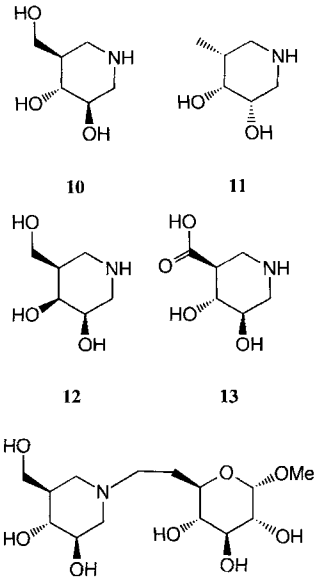
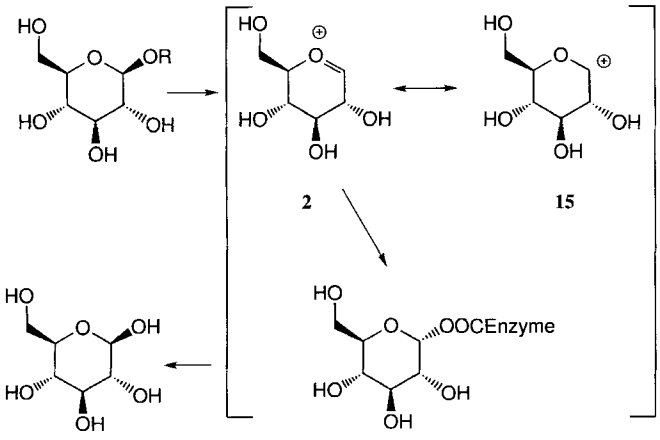


Scheme 2. Isofagomine and analogues.

Subsequently it has been found that the corresponding fucose analogue (**11**), the galactose analogue isogalactofagomine (**12**), and the glucuronic acid analogue (**13**) are potent inhibitors of human placenta α-fucosidase,[18] β-galactosidase,[19] and β-glucuronidase,[20] respectively (Scheme 2). Furthermore, an isomaltose analogue (**14**) of isofagomine has been found to be a very potent inhibitor of glucoamylase, an enzyme that does not accept substrates smaller than disaccharides.[17] Another feature of these compounds is the ability to inhibit glycosyl phosphorylases, not displayed by compounds **1**–**8**.[21]

Isofagomine and its analogues, when *N*-protonated, do not resemble the oxocarbenium ion, but rather the resonance form, the anomeric carbocation **15**. As a resonance form of **2**, the ion **15** is also a reasonable intermediate in glycosyl cleavage of enzymes (Scheme 3).

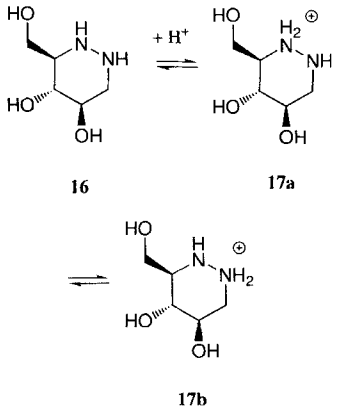


Scheme 3. Simplified mechanism for glycoside cleavage by β-glycosidase.

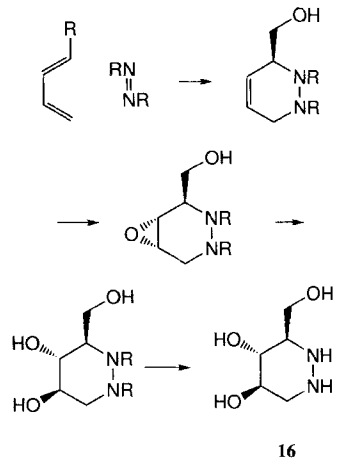
We anticipated that an improved class of glycosidase inhibitor would be obtained if a molecule could mimic both **2** and **15**. We expected that 1-azafagomine (**16**), a compound with a hydrazine incorporated into the ring, would be capable of this. Protonation of the hydrazine could occur on either of the two nitrogens to give the ions **17a** and **17b** (Scheme 4), and thus electronically resemble either resonance form **2** or **15**. In this paper we report an efficient stereoselective synthesis of the new hexahydropyridazine **16** and analogues, and report on our investigations regarding the biological activity of these compounds.

Results and Discussion

A Diels–Alder strategy to reach **16** seemed attractive, because it would be an extremely short route. However, the double bond would have to be *trans*-dihydroxylated in such a way as to obtain 3,4-*trans*-4,5-*trans* stereochemistry. We expected this to be possible if an azadienophile was reacted with a butadiene with a hydroxymethyl or masked hydroxymethyl substituent (Scheme 5). Epoxidation of the adduct could be expected to occur at the less hindered side to give an epoxide *trans* to the hydroxymethyl group. We anticipated that the preferential attack of water in the subsequent hydrolysis of the epoxide would occur at the less hindered position. We then needed to remove the nitrogen protection to obtain racemic **16**. 4-Phenyl-1,2,4-triazoline-3,5-dione (**18**) seemed to us a more suitable azodienophile than the azodicarboxylates due to its extreme reactivity, and because the adduct would be more conformationally restrained. This, we anticipated, would favor a regioselective epoxide opening.



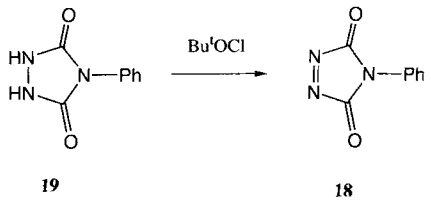
Scheme 4. Protonation of 1-azafagomine (**16**).



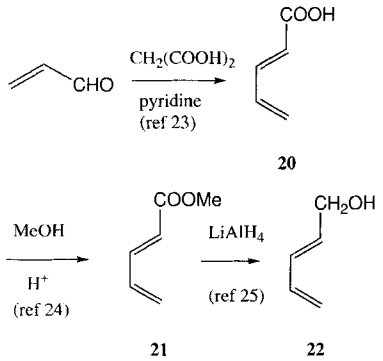
Scheme 5. Strategy for the synthesis of 1-azafagomine (**16**).

Paulsen and Steinert have already synthesized a dihydroxyhexahydropyridazine starting from carbohydrates.[22] However, this compound does not have the required stereochemistry to resemble a naturally occurring monosaccharide.

4-Phenyl-1,2,4-triazoline-3,5-dione (**18**) was easily prepared by *tert*-butylhypochlorite oxidation[23] of 4-phenylurazol (**19**), and was prepared and used in situ (Scheme 6). The most readily available diene with a masked hydroxymethyl substituent was 2,4-pentadienoic acid (**20**), which was commercially available, but expensive. For large-scale synthesis it is better to prepare **20** by means of the Knoevenagel condensation between malonic acid and acrolein.[24] Esterification of **20** with methanol gave **21**,[25] which was reduced to 2,4-pentadienol (**22**) with

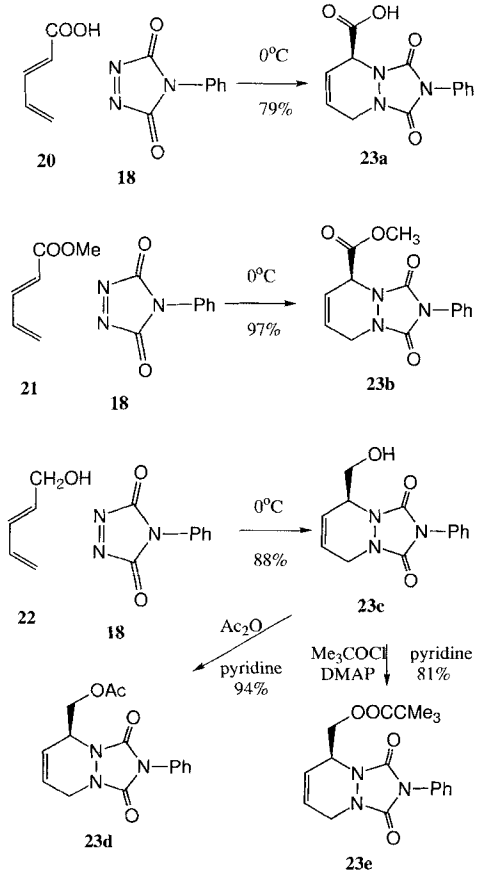


Scheme 6. Preparation of the dienophile.



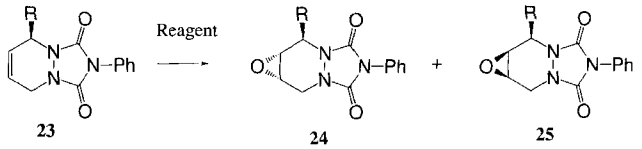
Scheme 7. Synthesis of dienes **20**–**22**.

$LiAlH_4$[26] (Scheme 7). All of these dienes were reacted with **18** (Scheme 8). The reaction between **18** and **20**–**22** was fast at 0 °C and could be followed by the disappearance of the red **18**. Reaction of **20** with **18** gave the Diels–Alder adduct **23a** in 79% yield. Reaction of **21** and **18** gave the corresponding ester **23b** in 97% yield, while reaction of **22** and **18** gave the hydroxymethyl adduct **23c** in 88% yield. Any of the adducts **23a–c** could be potentially employed in the synthesis, because the acid of **23a** or the ester of **23b** could be reduced to a hydroxymethyl group at a later stage. Epoxidation of all the adducts was then investigated in order to obtain the best possible stereoselectivity (Table 1). Two ester derivatives of **23c** were also studied. Acetate **23d** was obtained in 94% yield by standard acetylation of **23c**, while



Scheme 8. Reaction of dienes with **18**.

Table 1. Stereoselectivity of epoxidations.



R	Reactant	Reagent	Yield	Products	Ratio
COOH	**23a**	MCPBA	No reaction	–	–
COOH	**23a**	Me_2CO_2	No reaction	–	–
COOMe	**23b**	Me_2CO_2	96%	**24b**:**25b**	3:1
CH_2OH	**23b**	MCPBA	84%	**24b**:**25b**	1:2
CH_2OH	**23c**	Me_2CO_2	100%	**24c**:**25c**	1:1
CH_2OH	**23c**	$CF_3(CH_3)CO_2$	79–100%	**24c**:**25c**	3:1
CH_2OH	**23c**	$Et(Me)CO_2$	66%	**24c**:**25c**	1:1
CH_2OAc	**23d**	MCPBA	96%	**24d**:**25d**	2:1
CH_2OAc	**23d**	Me_2CO_2	85%	**24d**:**25d**	1:1
CH_2OPiv	**23e**	MCPBA	42%	**24e**:**25e**	3:2

pivalate ester **23e** was obtained in 81% yield by reaction of **23c** with pivaloyl chloride, pyridine, and DMAP.

All the adducts turned out to be surprisingly difficult to epoxidize, and many common epoxidation methods failed. Little information was available from the literature because there were no reports on the epoxidation of a 1,2,3,6-tetrahydropyridazine. The only successful epoxidizing reagents were *m*-chloroperbenzoic acid (MCPBA), under forcing conditions, and dioxiranes. The acid **23a** was the most unreactive. Neither MCPBA nor even dimethyl dioxirane was able to convert **23a** to an epoxide. Methyl ester **23b** was somewhat more reactive: it reacted slowly with dimethyldioxirane to give a 3:1 mixture of the *trans* and *cis* epoxides **24b** and **25b** in 96% yield. Hydroxymethyl derivatives **23c–e** were more reactive: they underwent epoxidation by MCPBA in refluxing dichloroethane. The *cis* and *trans* epoxides **24c–e** and **25c–e** were obtained in good yield (Table 1), but with varying stereoselectivity. The MCPBA oxidation of **23c** gave a 1:2 *trans*/*cis* mixture. Although unwanted, this stereochemical behavior was to be expected as hydroxy groups are known to be able to direct MCPBA addition to the *syn* face. Protection of the hydroxy group had a favorable influence on the selectivity; the acetate **23d** reacted with MCPBA to give **24d** and **25d** in a 2:1 ratio. The pivalate **23e** gave a 3:2 ratio of **24d** and **25d** under these conditions. Selectivity was, however, still not satisfactory.

Dioxiranes seemed at first to be even less stereoselective (Table 1). Dimethyldioxirane[27] gave a 1:1 *trans*/*cis* mixture of epoxides when reacted in acetone solution with either **23c** or **23d**. The more sterically hindered ethylmethyldioxirane also gave a 1:1 ratio of **24c** and **25c** when reacted with **23c**. However, trifluoromethyl(methyl)dioxirane, which was generated in situ according to a recent procedure,[28] reacted with **23c** to give a 3:1 mixture of **24c** and **25c**. This was surprising, as we expected this more reactive dioxirane to be less selective. Perhaps the electronegative trifluoromethyl group and the hydroxy group repelled each other, thus directing the reagent to enter *trans*. Despite the relatively modest stereoselectivity, this procedure turned out to be the most useful, because **24c** was crystalline and could be crystallized readily and reproducibly from the reaction mixture in 62% yield from **23c**.

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Stereochemical assignment of these epoxides needs some comment. Compounds **24b–e** and **25b–e** adopted flattened chair conformations, so that the relative configurations were not readily determined from the ^{1}H NMR coupling constants. We therefore determined the structure of **24c** by X-ray structural analysis (Figure 1). The configuration of the other epoxides was then assigned by comparison of the NMR spectra. The ^{13}C NMR chemical shifts of C2 and C5 were lower in **24c** than in **25c**, and the same was the case for **24d** and **25d**. In order to confirm this, pure **24c** was acetylated to give **24d**. Acetate **24d** and pivalate **24e** had almost identical ^{1}H NMR spectra, which secured the configuration of **24e**.

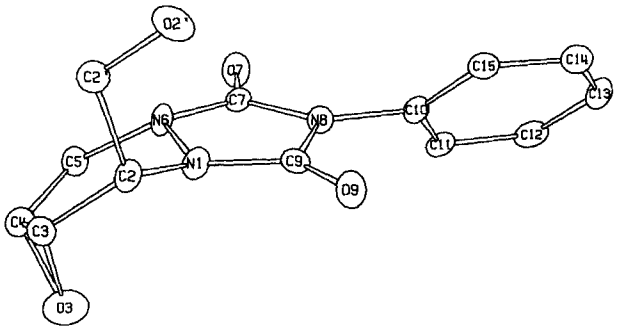
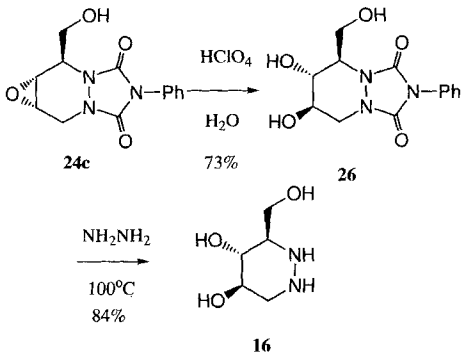


Figure 1. X-ray structure of **24c**.

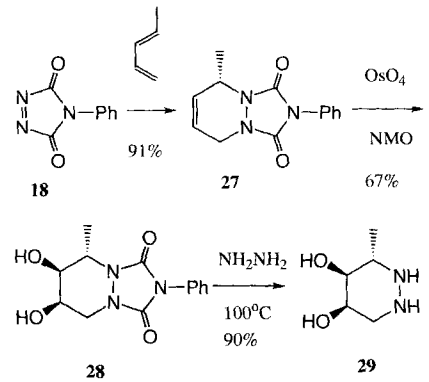
The X-ray structure of epoxide **24c** also indicated that the exocyclic hydroxymethyl group exerted considerable steric hindrance towards epoxide opening at the 7 position. This turned out to be the case. Hydrolysis of **24c** with dilute $HClO_4$ was highly stereoselective to give triol **26** in 73% isolated yield (Scheme 9). Thus, attack by water occurred preferentially at the 5 position. The determination of the configuration by NMR was easier for **26** than for the epoxides. The ^{1}H–^{1}H coupling constants between H2, H3, H4, and H5$_{ax}$ were rather large (6–7 Hz), which is consistent with almost *trans* diaxial orientation. This became even more evident after removal of the *N*-protecting group in the next step.



Scheme 9. Stereoselective synthesis of **26** and its hydrazinolysis to 1-azafagomine (**16**).

Hydrazinolysis of **26** in neat hydrazine hydrate at 100 °C for 18 h gave hexahydropyridazine **16** in 84% yield (Scheme 9). The ^{1}H NMR spectrum of **16** showed large coupling constants (9.4 to 10.5 Hz) between H3, H4, H5, and H6$_{ax}$, indicating that all these protons were axial. This proved beyond doubt that **16** had a chair conformation, and a configuration with all substituents in equatorial positions.

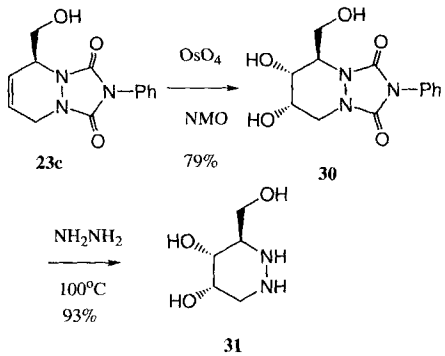
Synthesis of a racemic fucose (6-deoxygalactose) analogue was also investigated. This required that the 6-hydroxymethyl group be exchanged for a methyl group, and the double bond be *syn* dihydroxylated *cis* to the methyl group. Osmium-catalyzed dihydroxylation of tetrahydropyridazines has already been reported.[29] Therefore, we carried out the Diels–Alder reaction of 2,4-pentadiene with **18**. This gave the adduct **27** in 91% yield (Scheme 10). Dihydroxylation of **27** with a catalytic amount of OsO_4 and *N*-methylmorpholine *N*-oxide as a cooxidant gave a single diol **28** as a crystalline product in 67% yield. The mother liquor contained mainly **28** together with smaller amounts of alkene **27** and another compound, possibly the other stereoisomer. The very high stereoselectivity in the dihydroxylation indicated that **28** was actually the 3,4-*trans* stereoisomer, as OsO_4 is known to prefer the less hindered side.



Scheme 10. Three-step synthesis of **29**.

Hydrazinolysis of **28** in neat NH_2NH_2/H_2O gave the dihydroxyhexahydropyridazine **29** in 90% yield. The ^{1}H NMR spectrum of **29** confirmed that the configuration was 3,4-*trans*. The coupling constant J(3,4) was large (9.6 Hz), while both $J(5,6_{ax})$ and $J(5,6_{eq})$ were small (2 and 4.5 Hz), which indicates that H3 and H4 were axial, and H5 was equatorial. Thus, the relative stereochemistry was 3,4-*trans*-4,5-*cis* and not the fucose stereochemistry (3,4-*cis*-4,5-*cis*).

Osmium-catalyzed dihydroxylation of **23c** was also possible, and was likewise highly stereoselective. A single triol (**30**) was obtained as crystals in 79% yield, and no other stereoisomer was observed (Scheme 11). As in the synthesis of **28**, the high



Scheme 11. Synthesis of **31**.

stereoselectivity indicated that the 2,3-*trans* isomer had been formed. This was confirmed by an X-ray structure determination of **30** (Figure 2). Somewhat surprisingly, both the hydroxymethyl group and the 3-hydroxy group were axial, while the 4-hydroxy group was equatorial. The ^{1}H NMR spectrum

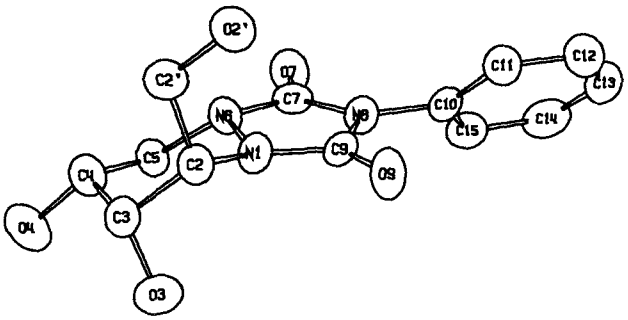


Figure 2. X-ray structure of **30**.

showed that that conformation also prevailed in solution as $J(4,5_{ax})$ was large and $J(3,4)$ was small. Why this conformation is favored is not clear. Hydrazinolysis of **30** gave hexahydropyridazine **31** in 93% yield. In the ^{1}H NMR spectrum both H6's gave small coupling constants to H5, indicating that the latter was equatorial, while H4 had one large coupling (9.3 Hz), indicating that this proton was axial and thus H3 was also axial. The relative stereochemistry of the product was therefore confirmed to be 3,4-*trans*-4,5-*cis*.

The biological activity of the hydroxyhexahydropyridazines was then investigated (Table 2). As anticipated, racemic **16** was found to be a competitive inhibitor of glucosidases. Almond β-glucosidase was inhibited at pH 6.8 with a K_i of 0.65 μM, while bakers' yeast α-glucosidase was inhibited with a K_i of 3.9 μM. Yeast isomaltase, an α-glucosidase with a preference for 1,6-linkages, was inhibited with a K_i of 1.06 μM. The other glycosidases α- and β-galactosidase and α-mannosidase were not significantly inhibited. Thus glucosidase inhibition by **16** was selective. It was more potent in its inhibition of α-glucosidase and isomaltase than both **1** and **10**, but six times less potent than **10** in its inhibition of β-glucosidase. However, compound **16** was racemic, and as it is quite unlikely that the stereoisomer resembling L-glucose is a strong inhibitor, the K_i value of the inhibiting stereoisomer ((3*R*,4*R*,5*R*), depicted in Scheme 4) is probably half the value of the racemate. The difference in β-glucosidase inhibition of **10** and (3*R*,4*R*,5*R*)-**16** is then probably only threefold.

It was also interesting to study the influence of pH on the inhibition profile of **16**. The inhibition of β-glucosidase by both **1** and **10** declined drastically with pH. However, inhibition of β-glucosidase by **16** was found to be independent of pH (Table 2), and thus equally potent over the enzyme's entire working pH range.

In order to try to explain this effect, we measured the pK_a of protonated **16** by titrating the aqueous acidic solution with NaOH; it was found to be 3.9. Thus, the hydrazine is such a weak base that it is still largely unprotonated even at pH 5. The entity inhibiting β-glucosidase was therefore unprotonated **16**, and not **17a** or **17b**. The unprotonated amine is also the inhibitor in **1** and **10**, and because these compounds are stronger bases, the fraction of free amine, and consequently inhibition, decreases with pH.

Table 2. Inhibition constants (K_i) in μM measured at 26 °C.

Enzyme	**16**	**1** [15]	**10** [15]	**29**	**31**
α-glucosidase (yeast, pH 6.8)	3.9	25	86	>1000	>1000
β-glucosidase (almonds, pH 6.8)	0.65	47	0.11	41	137
β-glucosidase (almonds, pH 5.0)	0.76	330	–	–	–
β-glucosidase (almonds, pH 7.5)	1.09	–	–	–	–
Isomaltase (bakers' yeast, pH 6.8)	1.06	11	7.2	>3000	3080
α-galactosidase (*E. Coli*, pH 6.8)	934	>1000	–	–	–
β-galactosidase (*E. Coli*, pH 6.8)	702	>1000	–	>1000	149
α-mannosidase (jack bean, pH 5.0)	3306	270 [a]	770 [a]	185	323
phosphorylase A (pH 6.8)	13.5 [b]	55000	–	–	–

[a] pH 4.5. [b] IC_{50}.

The NMR spectrum of **16** at pH 1 shows the protonated hexahydropyridazine. Both ^{1}H and ^{13}C NMR exhibited similar changes in the chemical shifts of H3 and H6 or C3 and C6, respectively, indicating that a mixture of **17a** and **17b** was present and both nitrogens were approximately equal in base strength.

It is interesting to observe that **16** combines strong α-glucosidase inhibition of **1** and strong β-glucosidase inhibition of **10**. This may be explained by the stereoelectronics of glycoside cleavage (Figure 3). Cleavage of an α-glucoside can occur directly with assistance from the *trans*-diaxial lone pair of oxygen, leading to an immediate positive charge at oxygen. This is not possible for the β-glucoside, in which cleavage has to take place without assistance from the oxygen lone pairs, and thus development of a positive charge at the anomeric carbon occurs. For this reason we propose that α-glucosidase favors positive charge development at the ring oxygen, while β-glucosidase favors positive charge development at the anomeric carbon. As a consequence, glucose analogues that can accommodate a positive charge at the position of the ring oxygen inhibit the former and those that accommodate positive charge at the anomeric carbon the latter. Compound **16** is capable of both and thus inhibits both enzymes potently.

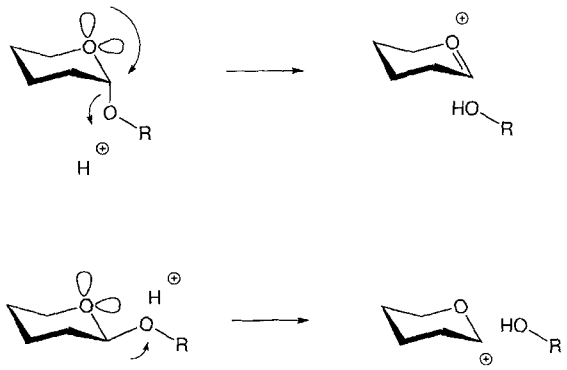


Figure 3. Stereoelectronics of the cleavage of α- and β-glucosides.

Compound **16** also strongly inhibited glycogen phosphorylase from rabbit muscle (Table 2). The difference in inhibition of this enzyme by **16** and **1** was particularly striking. Inhibition of

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a glycosyl phosphorylase should not be very different conceptually from that of a glycosidase, since the only difference in the reaction they catalyze is the external nucleophile, and the inhibition of both enzymes by **16** seemed to confirm this.

The di- and trihydroxyhexahydropyridazines **29** and **31** did not have a configuration at the chiral carbon atoms similar to any natural sugar. Nevertheless, both **29** and **31** were moderately potent competitive inhibitors of β-glucosidase and α-mannosidase, and **31** also inhibited β-galactosidase to a moderate extent (Table 2). The enzyme β-glucosidase is known to be relatively unspecific towards the stereochemistry of the glycon of its substrates; this might explain why the hexahydropyridazines with the "wrong" configuration bound relatively tightly. The inhibition of α-mannosidase and β-galactosidase by these compounds was quite interesting, because it was higher than the inhibition caused by **16**, **1**, or **10**. The *syn*-diol moiety was probably important for the binding of **29** and **31** to these enzymes, as it could mimic the 2,3-diol of mannose or the 3,4-diol of galactose.

In this paper we have shown that hydroxyhexahydropyridazines constitute a new class of potent competitive inhibitors of enzymatic hydrolysis or phosphorolysis of glycosidic bonds. Azafagomine has a potency that equals the corresponding hydroxypiperidines, but in contrast to these, acts potently on both α- and β-glucosidases. It is proposed that this is due to its ability to mimic positive charge at both the anomeric carbon and the ring oxygen. Another difference between **16** and hydroxypyridazines is that inhibition was independent of pH; this is caused by its low basicity. The hydroxyhexahydropyridazines can be produced in a highly efficient manner by the short synthetic route described in this paper.

Experimental Section

^{13}C NMR and ^{1}H NMR spectra were recorded on Varian 200 and 300 MHz Gemini instruments. When $CDCl_3$ was used as the solvent, TMS and $CDCl_3$ (^{13}C NMR: $\delta = 76.93$) were used as references. Melting points are uncorrected. Concentrations were performed on a rotary evaporator at temperatures below 40 °C. An asterisk (*) indicates that D_2O was added as an aid to dissolution.

(±)-8-Phenyl-1,6,8-triazabicyclo[4.3.0]non-3-ene-7,9-dione-2-carboxylic acid (23a): 4-Phenylurazole (**2**) (1.1 g, 6.2 mmol) was suspended in EtOAc (3 mL) at 0 °C, and *tert*-butylhypochlorite (0.7 g) was added to give a red, homogeneous solution. Penta-2,4-dienoic acid (**20**) (0.7 g, 7.1 mmol) was added after 5 min, and the mixture was allowed to reach RT over a period of 30 min and kept thus for 18 h. The precipitated adduct **23a** was isolated by filtration and washed with pentane (15 mL). Yield: 1.33 g (79%); ^{1}H NMR (CD_3OD): $\delta = 7.25$ (m, 5H, Ar), 5.95 (s, 2H, H3, H4), 4.95 (s, 1H, H2), 4.15 (d, 1H, $H5_{eq}$, $J(5_{ax},5_{eq}) = 16.7$ Hz), 3.85 (d, 1H, $H5_{ax}$); MS (EI): *m/z*: 273.075 [M^+], calcd for $C_{13}H_{11}N_3O_4$: 273.0749.

(±)-2-Methoxycarbonyl-8-phenyl-1,6,8-triazabicyclo[4.3.0]non-3-ene-7,9-dione (23b): 4-Phenylurazole (**2**) (2.2 g, 12.4 mmol) was suspended in EtOAc (6 mL) at 0 °C and *tert*-butylhypochlorite (1.5 g) was added to give a red, homogeneous solution. Methyl penta-2,4-dienoate (**21**) (1.5 g, 13.4 mmol) was added after 5 min, and the mixture was allowed to reach RT over a period of 30 min. The precipitate was isolated by filtration and recrystallized from $CHCl_3$ to give **23b**. Yield: 3.45 g (97%); ^{1}H NMR ($CDCl_3$): $\delta = 7.4$–7.5 (m, 5H, Ar), 6.1 (s, 2H, H3, H4), 5.1 (s, 1H, H2), 4.4 (d, 1H, $H5_{eq}$, $J(5_{ax},5_{eq}) = 16.6$ Hz), 4.0 (d, 1H, $H5_{ax}$), 3.8 (s, 3H, OMe); MS (EI): *m/z*: 287.090 [M^+], calcd for $C_{14}H_{13}N_3O_4$: 287.0906.

(±)-2-Hydroxymethyl-8-phenyl-1,6,8-triazabicyclo[4.3.0]non-3-ene-7,9-dione (23c): 4-Phenylurazole (**19**) (12.4 g) was suspended in EtOAc (40 mL) at 0 °C and *tert*-butylhypochlorite (7.63 g) was added to give a red, homogeneous solution. Penta-2,4-dien-1-ol (**22**) (6.8 g) was added after 5 min, and the mixture was allowed to reach RT over a period of 30 min, then left at RT for 18 h. The solution was concentrated, redissolved in $CHCl_3$ (700 mL), filtered, and concentrated to give **23c** (15.95 g, 88%). M.p. 150 °C; ^{13}C NMR (CD_3CN): $\delta = 128.5$, 127.6, 125.7 (Ar), 122.6, 121.5 (C3, C4), 61.7 (C2′), 55.7 (C2), 42.8 (C5); ^{1}H NMR ($CDCl_3$): $\delta = 7.4$–7.5 (m, 5H, Ar), 6.1 (d, 1H, H4, $J(3,4) = 11$ Hz), 5.85 (dd, 1H, H3, $J(2,3) = 8.3$ Hz), 4.6 (ddd, 1H, H2, $J(2,2'_b) = 6.2$, $J(2,2'_a) = 3.3$ Hz), 4.25 (d, 1H, $H5_{ax}$, $J(5_{ax},5_{eq}) = 16.5$ Hz), 4.15 (d, 1H, $H5_{eq}$), 3.95 (d, 1H, $H2'_a$, $J(2'_a,2'_b) = 12.4$ Hz), 3.90 (d, 1H, $H2'_b$); MS (EI): *m/z*: 259.096 [M^+], calcd for $C_{13}H_{13}N_3O_3$: 259.0957.

(±)-2-Acetoxymethyl-8-phenyl-1,6,8-triazabicyclo[4.3.0]non-3-ene-7,9-dione (23d): Pyridine (1 mL) and acetic anhydride (1 mL) were added to alcohol **23c** (518 mg, 2 mmol) dissolved in CH_2Cl_2 (10 mL). The solution was kept at RT for 18 h. The solution was washed with water (10 mL), hydrochloric acid (1 M, 10 mL), and $NaHCO_3$ (5%, 10 mL), dried with $MgSO_4$, filtered, and concentrated to give **23d**. Yield: 563 mg (94%). M.p. 118–124 °C; ^{13}C NMR ($CDCl_3$): $\delta = 125$–129 (Ar), 123.1, 121.5 (C3, C4), 61.8 (C2′), 52.6 (C2), 43.7 (C5), 20.6 (Me); MS (EI): *m/z*: 301.1061 [M^+], calcd for $C_{15}H_{15}N_3O_4$: 301.1062.

(±)-8-Phenyl-2-(2,2,2-trimethylacetoxy)methyl-1,6,8-triazabicyclo[4.3.0]non-3-ene-7,9-dione (23e): Pyridine (2 mL), then pivaloyl chloride (965 mg, 8 mmol) and 4-dimethylaminopyridine (10 mg) were added to alcohol **23c** (1.04 g, 4 mmol) dissolved in CH_2Cl_2 (20 mL). The solution was kept at RT for 3 d. More CH_2Cl_2 (20 mL) was added, and the solution was washed with water (20 mL), hydrochloric acid (1 M, 20 mL), and $NaHCO_3$ (5%, 20 mL), dried with $MgSO_4$, filtered, and concentrated. Ether was added, and the precipitate of **23e** collected. Yield: 1.11 g (81%). M.p. 97–98 °C.

(2,3-*trans*-3,4-*cis*) and (2,3-*cis*-3,4-*cis*)-2-Carboxymethyl-3,4-epoxy-8-phenyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione (24b and 25b): Methyl ester **23b** (112 mg, 0.39 mmol) was dissolved in CH_2Cl_2 (1 mL), and a solution of dimethyldioxirane in acetone[27] (0.092 M, 18.5 mL, 1.7 mmol) was added. After 3 d the solution was concentrated, giving a 3:1 mixture of the epoxides **24b** and **25b** (113 mg, 96%). Flash chromatography in EtOAc/pentane 1:1 gave first **25b** (9 mg, 8%) and then **24b** (78 mg, 66%).

Epoxide **24b**: ^{13}C NMR ($CDCl_3$): $\delta = 166.7$ (C2′), 129.9, 129.1, 126.4 (Ar), 55.4, 53.9 (C3, C4), 50.8, 49.5 (C2, OMe), 43.6 (C5); ^{1}H NMR ($CDCl_3$): $\delta = 7.4$ (m, 5H, Ar), 5.03 (d, 1H, H2, $J(2,3) = 5.4$ Hz), 4.44 (dd, 1H, $H5_{eq}$, $J(5_{ax},5_{eq}) = 13.5$, $J(4,5_{eq}) = 1.8$ Hz)), 3.85 (dd, 1H, $H5_{ax}$, $J(4,5_{ax}) = 10.8$ Hz), 3.8 (s, 3H, OMe), 3.58 (m, 2H, H3, H4).

Epoxide **25b**: ^{13}C NMR ($CDCl_3$): $\delta = 166.7$ (C2′), 129.9, 129.1, 126.4 (Ar), 56.3, 54.8, 54.0 (C2, C3, C4), 49.6 (OMe), 42.6 (C5); ^{1}H NMR ($CDCl_3$): $\delta = 7.4$ (m, 5H, Ar), 5.2 (s, 1H, H2), 4.2 (dd, 1H, $H5_{eq}$, $J(5_{ax},5_{eq}) = 13.5$, $J(4,5_{eq}) = 3.9$ Hz), 3.9 (d, 1H, $H5_{ax}$), 3.8 (s, 3H, OMe), 3.8 (s, 1H, H3), 3.6 (s, 1H, H4); MS (EI): *m/z*: 303.0854 [M^+], calcd for $C_{14}H_{13}N_3O_5$: 303.0855.

(2,3-*trans*-3,4-*cis*)- and (2,3-*cis*-3,4-*cis*)-3,4-Epoxy-2-hydroxymethyl-8-phenyl-1,6,8-triaza-bicyclo[4.3.0]nonane-7,9-dione (24c and 25c):

By the trifluoromethyl(methyl)dioxirane method:[28] Alcohol **23c** (1.00 g, 3.86 mmol) was dissolved in MeCN (30 mL) and water (20 mL) in a flask fitted with a funnel and a dry ice/acetone condenser. The solution was cooled to 0 °C in an ice bath, and 1,1,1-trifluoroacetone (4 mL) and $NaHCO_3$ (2.6 g) was added followed by oxone (12.3 g) in small portions over a period of 5 min. The mixture was stirred at RT for 18 h. Another charge of $NaHCO_3$ (1.3 g) and oxone (6.15 g) was added, and after 2 h the reaction was worked up by the addition of water (200 mL) and extraction with $CHCl_3$ (8 × 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give a solid mixture of epoxides **24c** and **25c** (1.09 g) in a 3:1 ratio. On addition of $CHCl_3$ (40 mL) pure **24c** crystallized out (656 mg, 62%). M.p. 182–184 °C.

Epoxide **24c**: ^{13}C NMR ($CDCl_3$): $\delta = 128.3$, 127.5, 124.6 (Ar), 61.1 (C2′), 54.2, 50.2 (C3, C4), 47.6 (C2), 41.0 (C5); ^{1}H NMR ($CDCl_3$): $\delta = 7.4$ (m, 5H, Ar), 4.55 (dd, 1H, H2, $J(2,2'_b) = 4.7$, $J(2,2'_a) = 4.1$ Hz), 4.39 (dd, 1H, $H5_{eq}$, $J(5_{ax},5_{eq}) = 13.2$, $J(4,5_{eq}) = 1.0$ Hz), 4.18 (dd, 1H, $H2'_a$, $J(2'_a,2'_b) = 12.2$ Hz), 4.04 (dd, 1H, $H2'_b$), 3.88 (dd, 1H, $H5_{ax}$, $J(4,5_{ax}) = 1.4$ Hz), 3.6 (ddd, 1H, H4, $J(3,4) = 4.0$ Hz), 3.53 (d, 1H, H3).

Epoxide **25c**: ^{13}C NMR ($CDCl_3$): $\delta = 128.3$, 127.5, 124.6 (Ar), 60.6 (C2′), 55.0, 50.8 (C3, C4), 48.4 (C2), 42.8 (C5); ^{1}H NMR ($CDCl_3$): $\delta = 7.4$ (m, 5H,

Ar), 4.23 (ddd, 1H, H2, $J(2,2'_b)$ = 6.1, $J(2,2'_a)$ = 5.5, $J(2,3)$ = 3.7 Hz), 4.01 (dd, 1H, $H5_{eq}$, $J(5_{ax},5_{eq})$ = 13.4, $J(4,5_{eq})$ = 0.6 Hz), 3.91 (dd, 1H, $H2'_a$, $J(2'_a,2'_b)$ = 11 Hz), 3.76 (dd, 1H, $H2'_b$), 3.59 (dd, 1H, $H5_{ax}$, $J(4,5_{ax})$ = 1.8 Hz), 3.52 (dd, 1H, H3, $J(3,4)$ = 3.7 Hz), 3.45 (ddd, 1H, H4); MS (EI): *m/z*: 275.100 [M^+], calcd for $C_{13}H_{13}N_3O_4$: 275.0906.

By the MCPBA method: Alcohol **23c** (518 mg, 2 mmol) and *m*-chloroperbenzoic acid (1.00 g) were dissolved in 1,2-dichloroethane (5 mL) and heated at 80 °C for 3 h. After cooling to RT, the mixture was filtered, and the precipitate was washed with CH_2Cl_2 (10 mL). The combined filtrates were washed with Na_2CO_3 solution (10%, 10 mL), dried with $MgSO_4$, and concentrated to give the epoxides **24c** and **25c** (463 mg, 84%) in a 1:2 ratio.

By the dimethyldioxirane method: Alcohol **23c** (112 mg, 0.39 mmol) was dissolved in $CHCl_3$ (1 mL), and a solution of dimethyldioxirane in acetone[27] (0.092 M, 13.5 mL, 1.24 mmol) was added. After 24 h the solution was concentrated to the epoxides **24c** and **25c** (119 mg, 100%) in a 1:1 ratio.

By the ethylmethyldioxirane method: A solution of ethylmethyldioxirane in 2-butanone[27] (16 mL) was added to alcohol **23c** (100 mg, 0.39 mmol). After 24 h the solution was concentrated to give a 1:1:1 mixture of epoxides **24c** and **25c** and starting material **23c**.

(2,3-*trans*-3,4-*cis*)- and (2,3-*cis*-3,4-*cis*)-2-Acetoxymethyl-3,4-epoxy-8-phenyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione (24d and 25d): Acetate **23d** (100 mg, 0.33 mmol) was dissolved in CH_2Cl_2 (1 mL), and a solution of dimethyldioxirane in acetone[27] (0.092 M, 10 mL, 0.92 mmol) was added. After 3 d the solution was concentrated to a 1:1 mixture of epoxides **24d** and **25d** (119 mg).

Epoxide **24d**: ^{13}C NMR ($CDCl_3$): δ = 130.0, 129.1, 126.3 (Ar), 61.9 (C2'), 52.5, 50.7 (C3, C4), 48.5 (C2), 42.7 (C5), 21.4 (Ac); ^{1}H NMR ($CDCl_3$): δ = 7.4–7.5 (m, 5H, Ar), 4.8 (ddd, 1H, H2, $J(2,2'_b)$ = 5.3, $J(2,2'_a)$ = 4.2, $J(2,3)$ = 1.3 Hz), 4.62 (dd, 1H, $H2'_a$, $J(2'_a,2'_b)$ = 11.8 Hz), 4.3 (dd, 1H, $H2'_b$), 4.15 (d, 1H, $H5_{eq}$, $J(5_{ax},5_{eq})$ = 13.2 Hz), 3.95 (dd, 1H, $H5_{ax}$, $J(4,5_{ax})$ = 2.7 Hz), 3.56 (ddd, 1H, H4, $J(3,4)$ = 3.8 Hz), 3.54 (d, 1H, H3), 2.0 (s, 3H, Ac).

Epoxide **25d**: ^{13}C NMR ($CDCl_3$): δ = 130.0, 129.1, 126.3 (Ar), 60.7 (C2'), 51.0, 50.4 (C3, C4), 50.0 (C2), 45.2 (C5), 21.4 (Ac); ^{1}H NMR ($CDCl_3$): δ = 7.4-5 (m, 5H, Ar), 3.65 (m, 1H, H3), 3.48 (m, 1H, H4), 2.0 (s, 3H, Ac).

By the MCPBA method: Acetate **23d** (101 mg, 0.33 mmol) and *m*-chloroperbenzoic acid (168 mg) were dissolved in 1,2-dichloroethane (3 mL) and heated at 80 °C for 3 h. After cooling to RT the mixture was filtered, and the filter cake was washed with CH_2Cl_2 (10 mL). The combined filtrates were washed with Na_2CO_3 solution (10%, 10 mL), dried with $MgSO_4$, and concentrated to give the epoxides **24d** and **25d** (102 mg, 96%) in a 2:1 ratio.

From the epoxide alcohol: Alcohol **24c** (50 mg) was dissolved in pyridine (1 mL) and acetic anhydride (0.5 mL) and kept for 1 h. Toluene (2 mL) was added, and the mixture was concentrated. NMR of the residue identified the product as **24d**.

(2,3-*trans*-3,4-*cis*)- and (2,3-*cis*-3,4-*cis*)-3,4-Epoxy-8-phenyl-2-(2,2,2-trimethylacetoxy)methyl-1,6,8-triazabicyclo[4.3.0]nonane-2,4-dione (24e and 25e): Pivalate **23e** (79 mg, 0.23 mmol) and *m*-chloroperbenzoic acid (200 mg) were dissolved in 1,2-dichloroethane (3 mL) and heated at 80 °C for 18 h. After cooling to RT, the mixture was filtered, and the filter cake was washed with CH_2Cl_2 (10 mL). The combined filtrates were washed with Na_2CO_3 solution (10%, 10 mL), dried with $MgSO_4$, and concentrated to give a residue containing the epoxides **24e** and **25e** (98 mg) in a 3:2 ratio. Further purification by flash chromatography in EtOAc gave 35 mg (42%).

Epoxide **24e**: ^{1}H NMR ($CDCl_3$): δ = 7.4–7.5 (m, 5H, Ar), 4.78 (m, 1H, H2, $J(2.2'_b)$ = 4.3, $J(2,2'_a)$ = 3.8 Hz), 4.69 (dd, 1H, $H2'_a$, $J(2'_a,2'_b)$ = 12.2 Hz), 4.34 (dd, 1H, $H2'_b$), 4.12 (d, 1H, $H5_{eq}$, $J(5_{ax},5_{eq})$ = 14.1 Hz), 3.99 (dd, 1H, $H5_{ax}$, $J(4,5_{ax})$ = 3 Hz), 3.59 (m, 1H, H4), 3.5 (m, 1H, H3), 1.25 (s, 9H, Me's).

Epoxide **25e**: ^{1}H NMR ($CDCl_3$): δ = 7.4–7.5 (m, 5H, Ar), 4.7 (m, 1H, H2, $J(2,2'_b)$ = 6.6, $J(2,2'_a)$ = 5.6 Hz), 4.56 (dd, 1H, $H2'_a$, $J(2'_a,2'_b)$ = 11.3 Hz), 4.38 (dd, 1H, $H2'_b$), 4.17 (dd, 1H, $H5_{ax}$, $J(5_{ax},5_{eq})$ = 12.2, $J(4,5_{ax})$ = 3.8 Hz), 3.6–3.7 (m, 2H, H3, H4), 1.25 (s, 9H, Me's).

(2,3-*trans*-3,4-*trans*)-3,4-Dihydroxy-2-hydroxymethyl-8-phenyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione (26): Epoxide **24c** (500 mg) was dissolved in water (50 mL) and $HClO_4$ (70%, 1.25 mL) was added. The solution was refluxed for 5 h, then neutralized with $KHCO_3$ (1.49 g) and concentrated. Flash chromatography in EtOAc gave pure **26** (388 mg, 73%). ^{13}C NMR (D_2O): δ = 136.6, 133.8 (Ar), 66.5, 66.2 (C3, C4), 61.2, 58.5 (C2, C2'), 46.1 (C5); ^{1}H NMR ($CDCl_3$): δ = 7.4 (m, 5H, Ar), 4.21 (dd, 1H, $H2'_a$, $J(2'_a,2'_b)$ = 12.8, $J(2,2'_a)$ = 3.2 Hz), 3.94 (dd, 1H, $H2'_b$, $J(2,2'_b)$ = 5.4 Hz), 3.91 (dd, 1H, $H5_{eq}$, $J(5_{ax},5_{eq})$ = 11.7, $J(4,5_{eq})$ = 2.4 Hz), 3.79 (ddd, 1H, H4, $J(4,5_{ax})$ = 7.4, $J(3,4)$ = 6.4 Hz), 3.71 (ddd, 1H, H2, $J(2,3)$ = 6.4 Hz), 3.64 (dd, 1H, H3), 3.34 (dd, 1H, $H5_{ax}$); MS (EI): *m/z*: 293.101 [M^+], calcd for $C_{13}H_{15}N_3O_5$: 293.1012.

(3,4-*trans*-4,5-*trans*)-4,5-Dihydroxy-3-hydroxymethylhexahydropyridazine (16): Triol **26** (256 mg) was dissolved in hydrazine hydrate (5 mL) and heated at 100 °C for 18 h. The solution was concentrated to give a syrup. This was dissolved in water and put on a column of ion-exchange resin (Amberlyst 15, H^+), washed with water, and eluted with 2.5% NH_4OH. Concentration followed by flash chromatography in EtOH/NH_4OH(25%) 9:1 gave pure **16** (109 mg, 84%). ^{13}C NMR (D_2O, pH 6): δ = 74.3, 73.8 (C4, C5), 65.5, 62.2 (C3, C3'), 54.1 (C6); (pH 1): δ = 72.1, 70.8 (C4, C5), 63.7, 60.9 (C3, C3'), 51.4 (C6); ^{1}H NMR (D_2O, pH 6): δ = 3.8 (dd, 1H, $H3'_a$, $J(3'_a,3'_b)$ = 12.5, $J(3,3'_a)$ = 3.0 Hz), 3.6 (dd, 1H, $H3'_b$, $J(3,3'_b)$ = 6.0 Hz), 3.5 (ddd, 1H, H5, $J(5,6_{ax})$ = 10.5, $J(4,5)$ = 9.4, $J(5,6_{eq})$ = 5.0 Hz), 3.3 (t, 1H, H4, $J(3,4)$ = 9.4 Hz), 3.15 (dd, 1H, $H6_{eq}$, $J(6_{eq},6_{ax})$ = 12.5 Hz), 2.65 (ddd, 1H, H3), 2.55 (dd, 1H, $H6_{ax}$); (pH 1): δ = 3.85 ($H3'_a$), 3.75 ($H3'_b$), 3.7 (H5), 3.5 (H4), 3.5 ($H6_{eq}$), 3.05 (H3), 2.9 ($H6_{ax}$); MS (EI): *m/z*: 148.0847 [M^+], calcd for $C_5H_{12}N_2O_3$: 148.0848.

(±)-2-Methyl-8-phenyl-1,6,8-triazabicyclo[4,3,0]non-3-ene-7,9-dione (27): 4-Phenylurazole (**19**) (1.77 g) was suspended in EtOAc (5 mL) at 0 °C, and *tert*-butylhypochlorite (1.1 g) was added to give a red, homogeneous solution. Penta-2,4-diene (1.5 g) was added after 5 min, which led to immediate discoloration. The mixture was allowed to reach RT over 30 min. The solution was filtered, and the precipitate was washed with EtOAc (10 mL). The combined filtrates were concentrated to give crystalline **27** (2.22 g, 91%). M.p. 122–125 °C. ^{13}C NMR ($CDCl_3$): δ = 129.6, 128.6, 127.9, 125.9, 120.0 (Ar, C3, C4), 50.8 (C2), 44.0 (C5), 18.0 (C2'); ^{1}H NMR ($CDCl_3$): δ = 7.4 (m, 5H, Ar), 5.83 (bs, 2H, H3, H4), 4.5 (m, 1H, H2, $J(2,2')$ = 6 Hz), 4.24 (dd, 1H, $H5_{eq}$, $J(5_{ax},5_{eq})$ = 16, $J(4,5_{eq})$ = 1.4 Hz), 3.97 (dd, 1H, $H5_{ax}$, $J(4,5_{ax})$ = 2.4 Hz), 1.35 (d, 3H, H2'); MS (EI): *m/z*: 243.100 [M^+], calcd for $C_{13}H_{13}N_3O_2$: 243.1008.

(2,3-*trans*-3,4-*cis*)-3,4-Dihydroxy-2-methyl-8-phenyl-1,6,8-triazabicyclo-[4.3.0]nonane-7,9-dione (28): Alkene **27** (564 mg) was dissolved in acetone (1 mL) and water (1 mL) and *N*-methylmorpholine *N*-oxide (400 mg) was added. Finally 1 mL of a solution of OsO_4 in *t*-butanol (10 g L^{-1}) was added, and the mixture was stirred for 4 d at 25 °C. A solution of $Na_2S_2O_5$ (30 mL) was added, and the mixture was extracted with EtOAc (5 × 20 mL). The organic layers were dried ($MgSO_4$), filtered and concentrated. Recrystallization of the residue from EtOAc/ether gave pure **28** (431 mg, 67%). ^{13}C NMR (CD_3CN*): δ = 128.3, 127.5, 125.8 (Ar), 69.3, 63.0 (C3, C4), 54.3 (C2), 43.2 (C5), 12.3 (C2'); ^{1}H NMR (CD_3CN*): δ = 7.46 (m, 5H, Ar), 4.2 (m, 1H, H2, $J(2,2')$ = 6.5, $J(2,3)$ = 3.3 Hz), 4.11 (ddd, 1H, H4, $J(4,5_{ax})$ = 9.8, $J(4,5_{eq})$ = 4.9 ($J(3,4)$ = 2.6 Hz), 3.83 (dd, 1H, $H5_{eq}$, $J(5_{ax},5_{eq})$ = 11.1 Hz), 3.83 (dd, 1H, H3), 3.34 (dd, 1H, $H5_{ax}$), 1.28 (d, 3H, H2'); m.p. 197–199 °C; MS (EI): *m/z*: 277.1062 [M^+], calcd for $C_{13}H_{15}N_3O_4$: 277.1062.

(3,4-*trans*-4,5-*cis*)-4,5-Dihydroxy-3-methylhexahydropyridazine (29): Diol **28** (176 mg) was dissolved in hydrazine hydrate (10 mL) and heated at 100 °C for 18 h. The solution was concentrated to give a syrup, which was dissolved in water and put on a column of ion-exchange resin (Amberlyst 15, H^+), washed with water, and eluted with 2.5% NH_4OH. Concentration followed by flash chromatography in EtOH/NH_4OH (25%) 50:1 gave pure **29** (76 mg, 90%). ^{13}C NMR (D_2O): δ = 75.7, 69.0 (C4, C5), 55.2, 54.3 (C3, C6), 17.8 (C3'); ^{1}H NMR (D_2O): δ = 3.68 (ddd, 1H, H5, $J(5,6_{eq})$ = 4.5, $J(4,5)$ = 3.0, $J(5,6_{ax})$ = 2 Hz), 3.11 (dd, 1H, H4, $J(3,4)$ = 9.6 Hz), 2.78 (dd, 1H, $H6_{eq}$, $J(6_{eq},6_{ax})$ = 14.5 Hz), 2.7 (m, 1H, H3), 2.68 (dd, 1H, $H6_{ax}$), 0.83 (d, 3H, H3', $J(3,3')$ = 6.2 Hz); MS (EI): *m/z*: 132.0896 [M^+], calcd for $C_5H_{12}N_2O_2$: 132.0899.

(2,3-*trans*-3,4-*cis*)-3,4-Dihydroxy-2-hydroxymethyl-8-phenyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione (30): Alkene **23c** (601 mg) was dissolved in acetone (1 mL) and water (1 mL) and *N*-methylmorpholine *N*-oxide (400 mg) was added. Finally 1 mL of a solution of OsO_4 in *t*-butanol (10 g L^{-1}) was added, and the mixture was stirred for 5 d at 25 °C. Solid $Na_2S_2O_5$ (0.5 g) was added, and the mixture concentrated. The residue was subjected to Soxhlet extrac-

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tion with acetone. The acetone extract was concentrated, and the residue recrystallized from acetone to give pure **30** (537 mg, 79%). ^{13}C NMR (CD_3CN*): δ = 127.6, 124.8, 125.8 (Ar), 63.6, 61.9 (C3, C4), 58.2, 56.3 (C2, C2'), 42.5 (C5); ^{1}H NMR (CD_3CN*): δ = 7.4–7.5 (m, 5H, Ar), 4.18 (ddd, 1H, H2, $J(2,2'_b)$ = 6.5, $J(2,2'_a)$ = 5.4, $J(2,3)$ = 4.1 Hz), 4.14 (ddd, 1H, H4, $J(4,5_{ax})$ = 9.2, $J(4,5_{eq})$ = 4.4, $J(3,4)$ = 2.7 Hz), 4.07 (dd, 1H, H3), 3.87 (dd, 1H, H2'$_a$, $J(2'_a,2'_b)$ = 12.2 Hz), 3.79 (dd, 1H, H5$_{eq}$, $J(5_{ax},5_{eq})$ = 11.6 Hz), 3.74 (dd, 1H, H2'$_b$), 3.44 (dd, 1H, H5$_{ax}$); m.p. 204–206 °C; MS (EI): *m/z*: 293.1010 [M^+], calcd for $C_{13}H_{15}N_3O_5$: 293.1012.

(3,4-*trans*-4,5-*cis*)-4,5-Dihydroxy-3-hydroxymethylhexahydropyridazine (31): Triol **30** (180 mg) was dissolved in hydrazine hydrate (10 mL) and heated at 100 °C for 18 h. The solution was concentrated to give a syrup. This was dissolved in water and put on a column of ion-exchange resin (Amberlyst 15, H^+), washed with water, and eluted with 2.5% NH_4OH. Concentration followed by flash chromatography in EtOH/NH_4OH (25%) 20:1 gave pure **31** (85 mg, 93%). ^{13}C NMR (D_2O): δ = 70.4, 68.9 (C4, C5), 63.0, 60.7 (C3, C3'), 54.1 (C6); ^{1}H NMR (D_2O): δ = 3.8 (ddd, 1H, H5, $J(5,6_{ax})$ = 2.9, $J(4,5)$ = 2.0, $J(5,6_{eq})$ = 1.6 Hz), 3.6 (dd, 1H, H3'$_a$, $J(3'_a,3'_b)$ = 11.3, $J(3,3'_a)$ = 3.1 Hz), 3.4 (dd, 1H, H3'$_b$, $J(3,3'_b)$ = 6.2 Hz), 3.4 (dd, 1H, H4, $J(3,4)$ = 9.3 Hz), 2.8 (dd, 1H, H6$_{eq}$, $J(6_{eq},6_{ax})$ = 14.4 Hz), 2.8 (ddd, 1H, H3), 2.8 (dd, 1H, H6$_{ax}$); MS (EI): *m/z*: 148.0841 [M^+], calcd for $C_5H_{12}N_2O_3$: 148.0848.

Measurements of glycosidase inhibition: Each glycosidase assay was performed by preparing eight 2 mL samples in cuvettes containing 1 mL sodium phosphate buffer (0.1 M) of either pH 6.8 and 7.5 or phosphate citrate buffer (0.1 M) at pH 5.0, along with 0.2 to 0.8 mL of a 5 or 10 mM solution of either 4-nitrophenyl α-D-glucopyranoside, 4-nitrophenyl β-D-glucopyranoside, 4-nitrophenyl α-D-mannopyranoside, 4-nitrophenyl α-D-galactopyranoside or 2-nitrophenyl β-D-galactopyranoside in water, and 0.1 mL of a solution of either the potential inhibitor (**2**, **10**, or **16**) or water, and topped up to a total volume of 1.9 mL with distilled water. Four of the samples contained the potential inhibitor at a fixed concentration but with varying concentrations of nitrophenyl glycoside. The other four samples contained no inhibitor, but also varying concentrations of nitrophenyl glycoside. Finally the reaction was started by adding 0.1 mL of a diluted solution of either α-glucosidase from bakers' yeast (EC 3.2.1.20, Sigma G-5003), isomaltase from bakers' yeast (EC 3.2.1.10, Sigma I-1256), β-glucosidase from almonds (EC 3.2.1.21, Sigma G-0395), α-mannosidase from jack beans (EC 3.2.1.24, Sigma M-7257), α-galactosidase from *E. Coli* (EC 3.2.1.22, Sigma G-6762) or β-galactosidase from *E. Coli* (EC 3.2.1.23, Sigma G-6008). The formation of 4- or 2-nitrophenol was monitored for 2–10 min at 26 °C by measurement of the absorbance at 400 nm. Initial velocities were calculated from the slopes for each of the eight reactions and used to construct two Hanes plots, one with and one without inhibitor. From the two Michaelis–Menten constants (K_m) thus obtained, the inhibition constant (K_i) was calculated.

Glycogen phosphorylase inhibition: A procedure similar to that used by Johnson et al.[30] was used. The glycogen phosphorylase A was from rabbit muscle (EC 2.4.1.1, Sigma P-1261). The substrates used were a D-glucopyranose 1-phosphate (0.1 M) and a 4% glycogen solution. Experiments were carried out in a 0.1 M NaF/HCl buffer, pH 6.8, at 26 °C. The reaction was followed in the direction of glycogen synthesis; phosphate evolution was followed as described by Palmgren et al.[31]

Table 3. Crystallographic data for **24c** and **30**.

Compound	**24c**	**30**
Formula	$C_{13}H_{13}N_3O_4$	$C_{13}H_{15}N_3O_5$
Formula weight	275.27	293.29
a (Å)	10.917(4)	11.204(4)
b (Å)	7.075(3)	7.364(3)
c (Å)	16.814(6)	17.067(6)
β(°)	116.56(2)	115.94(2)
T	120 K	295 K
R	0.050	0.069
GoF	1.294	1.362

Crystallographic data: Both **24c** and **30** crystallized as thin, colorless plates. X-ray data were recorded with a HUBER 4-circle diffractometer in the θ–2θ step scan mode with $Mo_{K\alpha}$ radiation. Both compounds formed monoclinic crystals, space group $P2_1/c$ with $Z = 4$. The structures were solved by means of the SIR 92[32] direct methods package and refined on F by the full-matrix least-squares method, allowing anisotropic displacement parameters for non-hydrogen atoms. Hydrogen atoms were introduced in calculated positions, and were refined in **24c** but not in **30** because of lack of data. Important data are given in Table 3.[33]

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[33] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-100368. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: Int. code +(1223) 336-033; e-mail: deposit@chemcrys.cam.ac.uk).