Pergamon

*Tetrahedron*, Vol. 53, No. 27, pp. 9357-9364, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00588-7

# Synthetic Studies of Fluorinated Analogues of 1-Azafagomine: Remarkable Nucleophilic Substitution of Fluorine by Hydrazine.

Ib Thomsen, Bettina V. Ernholt and Mikael Bols*

Department of Chemistry, University of Aarhus, Aarhus C

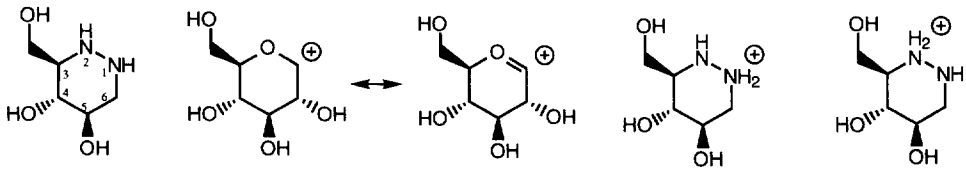
DK-8000, Denmark

***Abstract:*** (±)-5-fluoro-4-hydroxy-3-hydroxymethylhexahydropyridazine (7) and some other azafagomine analogues were synthesised with the aim of comparing their glycosidase inhibition to that of (±)-4,5-dihydroxy-3-hydroxymethylhexahydropyridazine (1-azafagomine). Both in the synthesis of 7 and in the attempted synthesis of 4,5-dihydroxy-3-fluoromethylhexahydropyridazine, a remarkable degree of nucleophilic substitution of fluorine by hydrazine was observed in the final deprotection step involving hydrazinolysis. Fluorine analogue 7 was a considerably weaker glycosidase inhibitor than **1**, suggesting that the 3-OH of **1** play a role in its binding by acting as a hydrogen bond donator. © 1997 Elsevier Science Ltd.

## INTRODUCTION

Potent inhibitors of glycosyl cleaving enzymes offer the opportunity to modulate selectively the crucial metabolism of carbohydrates, and hence opens a large number of potential applications, which includes 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 largely unexploited.

Recently we completed the synthesis of a new potent glycosidase inhibitor (±)-1-azafagomine (**1**).[5] This compound inhibits yeast α-glucosidase and almond β-glucosidase with Ki's of 3.9 and 0.65 μM, respectively. The basis of the compound's inhibition is closely connected to its ability to accept a proton. Hydrazine **1** is a weak base (pKa = 3.9) that when protonated is a mixture of hydrazinium ions **1a** and **1b**. These ammonium ions electronically and sterically resemble the two resonance forms **2a** and **2b** of the transition state of glycoside cleavage and might on this basis be expected to fit well into the active site of the



**1** **2a** **2b** **1a** **1b**

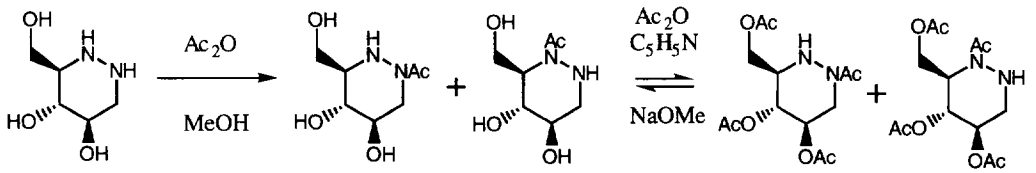
**Fig 1**

enzyme. Evidence suggests that resonance form **2b** may play the major role in glycoside cleavage by β-glucosidase action, while resonance form **2a** is the more important in α-glucosidase action.[5,6] The inhibition by **1** of both enzymes is thus likely to be due to its ability to resemble both **2a** and **2b**.

In this paper we have endeavored to investigate the role of the hydroxygroups of **1** in its binding. This we have tried to do by exchanging hydroxy groups in **1** with the fluorine atom. It is well known that fluorine functions as a bioisostere of the hydroxy group with a similar polarity and shape.[7] Fluorine can also behave similarly to the hydroxy group function as a hydrogen bond acceptor but not as a hydrogen donor. Thus it is possible to obtain information about the role of the hydroxy groups by studying deoxyfluoro analogues of the inhibitor.

## RESULTS AND DISCUSSION

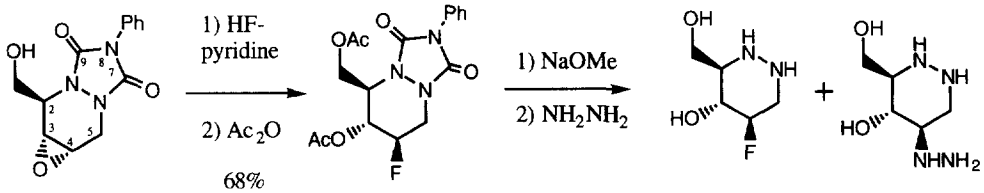
Since we were interested in investigating the importance of the ability of the hydrazine to accept a proton for its inhibitory potency we decided to convert it to an amide. Compound (±)-**1** was N-acetylated by




**1** **3** **4** **3a** **4a**

**Scheme 1**

treatment with acetic anhydride in methanol. This gave two products: The 1-N-acetyl derivative **3** and the 2-N-acetyl derivative **4** were obtained in ratio 4:1. The two compounds could not be separated even after peracetylation to the tetraacetates **3a** and **4a**. The identity of **3** and **4** was determined by NMR: The presence of the acetyl groups on nitrogen could be seen from $^{13}$C-NMR (table 2) as the methyl groups of **3** and **4** were at 26-27 ppm; the presence of the acetyl group at N-1 in **3** could be seen from the low field $^{1}$H-NMR shift of the H-6eq compared to H-6eq in **4**.

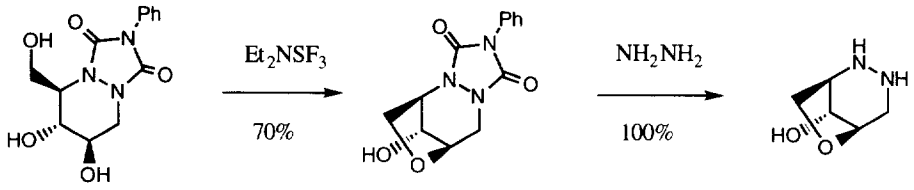



**5** **6** **7** 28% **8** 50%

**Scheme 2**

The 5-hydroxy group of **1** was conveniently displaced with fluorine by using the synthetic intermediate epoxide (±)-**5**.[5] It was known that **5** undergoes selective epoxide opening in the 5-position with water during acidic hydrolysis and could thus be expected to undergo reaction with HF with a similar selectivity. Indeed reaction of (±)-**5** with HF in pyridine, 70% gave the 4-fluoride, which was peracetylated in situ to facilitate isolation to give diacetate **6** in 68% yield. Deacetylation of **6** with NaOMe solution followed by hydrazinolysis with neat hydrazine-hydrate at 100 °C for 18h proceeded to give the 5-deoxy-5-fluoro analogue (±)-**7** in 28% yield together with, surprisingly, a non-fluorinated byproduct **8**, which was obtained in 50% yield. The structure of **8** was elucidated to be a 5-hydrazino derivative from its mass spectrum, $^{1}$H

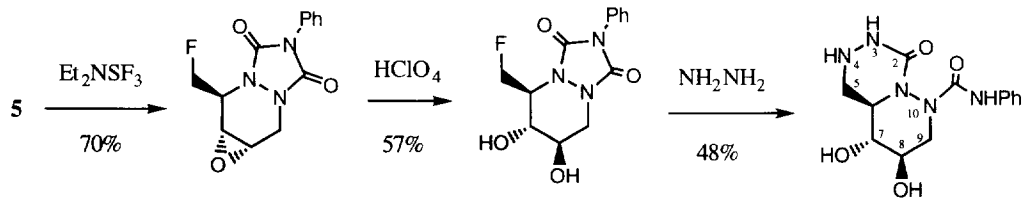
and $^{13}C$ NMR spectra. The chemical shift of C-5 and H-5 had moved upfield, compared to **1**,[5] with 4 and 0.7 ppm, respectively, consistant with the displacement of the 5-hydroxy group with a nitrogen substituent. The proton couplings $J_{45}$, $J_{56ax}$ and $J_{56eq}$ were almost identical to those of **1**, which suggested an identical stereochemistry. The surprising conclusion, which was also confirmed in the chemistry below, was that fluorine atom acted as a leaving group under the conditions of the hydrazinolysis. In this reaction the fluoride must have been substituted by the 4-hydroxy group to give a 4,5 epoxide, which then reacted with hydrazine to give the dihydrazino derivative with retention of stereochemistry at C-5.




**9** **10** **11**

**Scheme 3**

Displacement of the 3'-hydroxy group of **1** with fluorine we decided to carry out with $Et_2NSF_3$ (DAST) because it was known as a powerful reagent for direct displacement of hydroxy groups with fluorine and has been used to directly displace the 6-hydroxy group of unprotected methyl glucosides.[8] Thus we attempted direct fluorination of synthetic intermediate[5] (±)-**9**. However reaction of **9** with DAST gave one non-fluorinated major product **10** in 70% yield, which was found by NMR and MS to be the 2',4-anhydride. The formation of the anhydride was particularly clear from the down field shift of all carbons of the tetrahydrofurane ring. Formation of **10** can be rationalised by intramolecular substitution of a DAST-derived 2'-leaving group by the 4-OH. Compound **10** was hydrazinolysed with hydrazine-hydrate at 100 °C to give (±)-**11**.




**12** **13** **14**

**Scheme 4**

To avoid the problem of anhydride formation, reaction of the epoxide (±)-**5** with DAST was carried out. This gave the expected fluorinated product **12** in 70% yield. Acidic hydrolysis of **12** with aqueous perchloric acid gave, as have also been observed for **5**, regioselective epoxide opening to give **13** in 57% yield. Even more pronounced defluorination was observed when deprotection of **13** was attempted. Reaction of **13** with hydrazine hydrate at 100°C for 18 h gave one product, the 2'-hydrazino analogue (±)-**14**, which was isolated in 48 % yield. It was clearly seen from $^{13}C$-NMR that the fluorine atom had been displaced with hydrazine. Furthermore the aromatic signals in **14** was different from those of **6**, **10**, **12** and **13** and consistent with those of a N-phenylcarbamoyl group showing that the 2'-hydrazine after formation had reacted with the 9-carbonyl group. This was confirmed by the $^1H$-NMR chemical shift of H-9eq and H-9ax. The difference in chemical shift between these two signals was remarkable large (1.5 ppm) compared to the usual 0.5 ppm difference in the bicyclononane system. This large difference was also seen in **3** and was probably caused by shielding of H-9ax and deshielding H-9eq as a result of magnetic anisotropy from the amide carbonyl group

(fig 3). Such effects could occur if the carbonyl group, when not conformationally restricted in a ring, adopted a conformation where the equatorial proton was close to and in the plane of the carbonyl group, while the axial proton was above the plane of the carbonyl group. The EI mass spectrum of **14** gave a major peak at m/z 249, which corresponds to a loss of NHNHCO from the molecule.

Again this was an example of remarkable leaving group ability of the fluoride atom since it was selectively substituted in preference of hydrazinolysis of the urazole; subsequently the urazole reacted with the hydrazino-group. There are, to our knowledge, very few recorded examples in the literature of direct nucleophilic substitution of a fluoride atom. These consist of reaction of simple alkyl fluorides such as benzyl fluoride[9] or 2-fluoroethanol[10] with strong base to give either substitution or formation of an epoxide. There are however many cases where low yields were obtained in deprotection of deoxyfluoro carbohydrate derivatives and our results suggest an explanation for such phenomenae. *The fluorine atom should not be assumed to be inert to basic or nucleophilic conditions.*

The compounds **3/4**, **7**, **8**, and **11** were tested for inhibition of almond β-glucosidase and baker's yeast α-glucosidase. All the compounds were weak inhibitors or not inhibitors at all. Poor inhibition of the enzymes by the mixture **3/4** was anticipated but the very low level of potency (3 orders of magnitude) compared to **1** was striking and emphasises the importance of the basic hydrazine for the biological activity of these molecules.

| Enzyme/Compound | **1** | **3/4** | **7** | **8** | **11** |
|---|---|---|---|---|---|
| α-glucosidase (bakers yeast) | 3.9 | > 7000 | > 1000 | 1541 | > 1000 |
| β- glucosidase (almonds) | 0.65 | 3440 | 78.8 | 153.2 | 890 |

**Table 1:** Dissociation constants (Ki) in μM between compounds **1**, **3/4**, **7**, **8**, **11**, **14** and glycosidases. pH 6.8, 26°C.

The poor inhibition by **11** could also be anticipated, because this compound was forced to adopt a different conformation than the substrate.

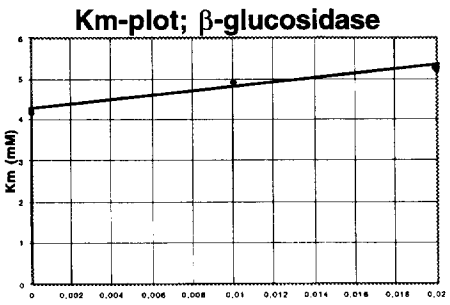



**Fig 2.** Plot of Km' vs [7]. The slope is equal to Km/Ki.

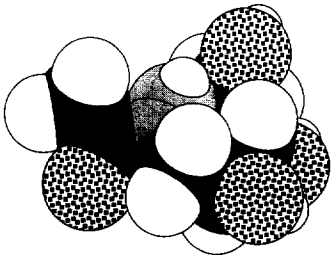
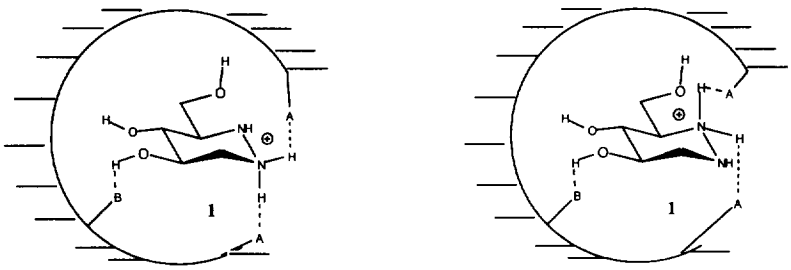


**Fig 3.** Conformation of **3** where magnetic anisotropy of the NAc-carbonyl could influence the chemical shift of the neighboring protons.

The most interesting result was that the fluoride **7** was a relatively poor inhibitor of both α- and β-glucosidase (fig 2). This suggests that **1** is bound to both enzymes in a manner where the action of the 3-OH as a hydrogen bond donor (fig 4) is an important contribution to the binding, because this is the only type of binding interaction **1** but not **7** can make. This result accords with the results of Lee *et al.* on fluorinated 1-deoxynojirimycin derivatives: 1,3-dideoxy-3-fluoro-nojirimycin was also a less potent glycosidase inhibitor than 1-deoxynojirimycin.[11]

In summary a number of conclusions can be drawn from the work in this paper. First of all it has been shown that fluorinated sugar analogues are quite labile to hydrazine and can be almost quantitatively

substituted. Lability to this and other nucleophiles must be anticipated in future synthesis planning. Secondly it has been found that both the basicity of the hydrazine and hydrogen donation of the 3-OH are very important for the binding of 1-azafagomine to α- and β-glucosidase.



β-glucosidase α-glucosidase

**Fig 4**

## EXPERIMENTAL SECTION

| | Solvent | C-3 | C-3' | C-4 | C-5 | C-6 | C=O | C=O | Ac's | Phe |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | $D_2O$ | 62.2* | 65.5* | 74.3$^{\Delta}$ | 73.8$^{\Delta}$ | 54.1 | - | - | - | - |
| **3** | $D_2O$ | 69.3* | 66.2* | 78.7$^{\Delta}$ | 76.5$^{\Delta}$ | 54.1 | - | - | 183.0, 26.8 | - |
| **4** | $D_2O$ | 68.8* | 66.7* | 78.7$^{\Delta}$ | 77.1$^{\Delta}$ | 57.3 | - | - | 26.5 | - |
| **6**$^{\dagger}$ | $CDCl_3$ | 53.9 | 58.5<br>58.3 | 63.7<br>63.2 | 83.8<br>81.3 | 44.5<br>44.3 | 151.1 | 152.9 | 19.6-8,<br>168.0-169.6 | 124.7, 127.5,<br>128.2, 129.9 |
| **7** | $D_2O$ | 69.7<br>69.6 | 66.0 | 77.0<br>76.8 | 100.2<br>97.8 | 56.3<br>56.0 | - | - | - | - |
| **8** | $D_2O$ | 69.7* | 66.4* | 76.0 | 69.8* | 55.5 | - | - | - | - |
| **10**$^{\dagger}$ | $CDCl_3$ | 56.9 | 70.6* | 72.1* | 68.2* | 44.8 | 151.5 | 150.6 | - | 129.8, 129.0,<br>126.3 |
| **11** | $D_2O$ | 63.7 | 74.3* | 79.4* | 75.8* | 51.7 | - | - | - | - |
| **12**$^{\dagger}$ | $CDCl_3$ | 53.4<br>53.2 | 83.0<br>80.7 | 50.4<br>50.3 | 48.5 | 41.7 | 152.2 | 151.5 | - | 129.8, 129.8,<br>126.1 |
| **13**$^{\dagger}$ | $CD_3CN$ | 61.7<br>61.5 | 83.0<br>80.7 | 68.7<br>68.6 | 69.1 | 48.6 | 155.7 | 154.2 | - | 131.1, 130.3,<br>128.5 |
| **14**$^{\dagger\dagger}$ | $D_2O$ | 61.8 | 52.1* | 78.4$^{\Delta}$ | 74.7$^{\Delta}$ | 53.0* | 166.8 | 164.6 | - | 145.0, 136.2,<br>132.3, 127.0 |

**Table 2.** $^{13}$C-NMR (75MHz) chemical shift. * and $^{\Delta}$ marked shifts may have the opposite assignment. $^{\dagger}$ Subtract 1 from atom nr. $^{\dagger\dagger}$ Add 3 to atom nr.

$^{13}$C-NMR and $^{1}$H-NMR spectra were recorded on Varian instrument Gemini 300. $D_2O$ was used as solvent with DHO ($^{1}$H-NMR: δ 4.7 ppm) and acetone ($^{1}$H-NMR: δ 2.05 ppm; $^{13}$C-NMR: δ 29.8 ppm) as reference. With $CDCl_3$ as solvent TMS or $CDCl_3$ ($^{13}$C-NMR: δ 76.93 ppm) were used as references. Mass spectra were obtained on a VG TRIO-2 instrument. Melting points are uncorrected. Concentrations were performed on a rotary evaporator at a temperature below 40 °C.

*(3,4-trans-4,5-trans)-1-acetyl-4,5-dihydroxy-3-hydroxymethylhexahydropyridazine* (**3**) *and (3,4-trans-4,5-trans)-2-acetyl-4,5-dihydroxy-3-hydroxymethylhexahydropyridazine* (**4**). 1-Azafagomine (**1**, 100 mg) was dissolved in MeOH (8 mL) and acetic anhydride (0.3 mL) was added. The solution was stirred at 50°C for 30 min., and then evaporated. Pyridine (1 mL) and acetic anhydride (0.5 mL) was added, and the mixture was kept overnigth. Then $CHCl_3$ (10 mL) was added, and the solution was washed with aqueous HCl (10 mL), water (10 mL), $NaHCO_3$-solution (10 mL) and again water (10 mL). Finaly the solution was dried ($MgSO_4$), concentrated and subjected to flash-chromatography in EtOAc-pentane 1:1 to give a oily residue (63 mg). This was dissolved in EtOH (5 mL), and a NaOEt-solution made from Na (10 mg) and EtOH (5 mL) was added. After 1 h at room-temperature the solution was concentrated and redissolved in water (50 mL). It was then eluted through a ionexchange column (Amberlyst 15, $H^+$, 5 mL). The eluate was concentrated to give a sirupy residue consisting of **3** and **4** (38 mg, 30 %, ratio 4:1). $^1$H-NMR ($D_2O$), **3**: δ 4.5 (dd, 1H, $J_{6ax6eq}$ 12.9 and $J_{56eq}$ 5.5 Hz, H-6eq), 3.85 (dd, 1H, $J_{3'a3'b}$ 12.3 and $J_{33'a}$ 2.3 Hz, H-3'a), 3.6 (dd, 1H, $J_{33'b}$ 4.5 Hz, H-3'b), 3.5 (ddd, 1H, $J_{56ax}$ 10.3 and $J_{45}$ 9.4 Hz, H-5), 3.35 (t, 1H, $J_{34}$ 9.4 Hz, H-4), 2.7 (m, 2H, H-3, H-6ax), 2.15 (s, 3H, Ac). **4**: δ 4.1 (dd, 1H, $J_{6ax6eq}$ 12.9 and $J_{56eq}$ 5.5 Hz, H-6eq), 3.9 (m, 1H), 3.4 (m, 1H) 3.15 (dd, 1H, $J_{56ax}$ 10.7 Hz, H-6ax), 2.1 (s, 3H, Ac). MS(EI): m/z 190 ($M^+$).

*(2,3-trans-3,4-trans)-3-acetoxy-2-acetoxymethyl-4-fluoro-8-phenyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione* (**6**). The epoxide **5** (100 mg) was dissolved in HF/pyridine (70 %, 2 ml) and kept at 25 °C for 18 h. Then acetic anhydride (0.5 ml) was added, and the solution was kept at 25 °C for 24 h. Then water (10 ml) was added, and the mixture was extracted with $CHCl_3$ (20 ml). The organic layer was washed with aqueous HCl (1 M, 10 ml), $NaHCO_3$-solution (sat., 10 ml), and water (10 ml), dried with $MgSO_4$, filtered and concentrated to give a syrup. Flash chromatography in EtOAc-pentane 1:1 gave **6** (92 mg, 68%). $^1$H-NMR ($CDCl_3$): δ 7.4 (m, 5H, Ar), 5.2 (bd, 1H, $J_{3F}$ 7 Hz, H-3), 4.8 (bdd, 1H, $J_{4F}$ 43 and $J_{34}$ 2.4 Hz, H-4), 4.6 (bdd, 1H, $J_{22'a}$ 9.4 and $J_{22'b}$ 4.7 Hz, H-2), 4.5 (ddd, 1H, $J_{2'a2'b}$ 11.8 and $J_{2'aF}$ 2.4 Hz, H-2'a), 4.25 (m, 2H, H-2'b, H-5eq), 3.5 (ddd, 1H, $J_{5axF}$ 40, $J_{5ax5eq}$ 14 and $J_{45ax}$ 1.4 Hz, H-5ax), 2.1 (2s, 6H, Ac's). MS(EI): m/z 379 ($M^+$).

*(3,4-trans-4,5-trans)-5-fluoro-4-hydroxy-3-hydroxymethylhexahydropyridazine* (**7**). Compound **6** (100 mg) was dissolved in absolute EtOH (5 ml). A few drops of a solution of Na in EtOH was added until the mixture tested strongly alkaline. The solution was kept for 18 h and a small lump of solid $CO_2$ (0.2 g) was added. The mixture was concentrated. To the residue was added hydrazine-hydrate (2 ml), and the resulting solution was stirred for 18 h at 100 °C. It was then concentrated, the residue was dissolved in water (100 ml) and poured through an ionexchange coloumn. Eluting with 5% $NH_4OH$ and concentration gave a residue (59 mg) containing two compounds. Flash chromatography in EtOH-$NH_4OH$, 25 % 20:1 gave first fluoride **7** (15 mg, 28 %) followed by dihydrazine **8** (29 mg, 50%). $^1$H-NMR ($D_2O$), **7**: δ 4.5 (dddd, 1H, $J_{5F}$ 47, $J_{56ax}$ 8.5, $J_{45}$ 8.5 and $J_{56eq}$ 6 Hz, H-5), 3.7 (m, 3H), 3.3 (bd, 1H, $J_{3'a3'b}$ 12 and $J_{33'b}$ 4.5 Hz, H-3'b), 2.7 (m, 2H). **8**: δ 3.8 (dd, 1H, $J_{3'a3'b}$ 12 and $J_{33'a}$ 3.0 Hz, H-3'a), 3.6 (dd, 1H, $J_{3'a3'b}$ 12 and $J_{33'b}$ 6 Hz, H-3'b), 3.4 (t, 1H, $J_{34}$ and $J_{45}$ 9.5 Hz, H-4), 3.3 (dd, 1H, $J_{6ax6eq}$ 12 and $J_{56eq}$ 4.3 Hz, H-6eq), 2.8 (ddd, 1H, $J_{56ax}$ 10 Hz, H-5), 2.7 (ddd, 1H, H-3), 2.5 (dd, 1H, H-6ax). MS(EI), **7**: m/z 150 ($M^+$), 119 (M - $CH_3O$). **8**: m/z 162 ($M^+$), 131 (M - $CH_3O$).

*(2,3-trans-3,4-trans)-3-hydroxy-4,2-oxymethyleno-8-phenyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione* (**10**). Compound **9** (100 mg) was suspended in $CH_2Cl_2$ (2 ml) and cooled to -40 °C. Diethylaminosulphur trifluoride (0.25 ml) was added, and the solution was kept at room temperature for 60 min. The solution was cooled to -10 °C and methanol (2 ml) was added. The solvents were evaporated, and the residue was subjected to flash chromatography in EtOAc to give the anhydride **10** (55 mg, 59%) as an amorpous white solid. $^1$H-NMR ($CDCl_3$): δ 7.4 (m, 5H, Ar), 4.7 (t, 1H, *J* 3.6 Hz, H-4), 4.4 (m, 1H, H-3), 4.35 (dd, 1H, *J* 5.5 and 3.6 Hz, H-2), 4.0 (m, 3H, H-2'a, H-2'b, H-5a), 3.6 (d, 1H, $J_{5a5b}$ 12 Hz, H-5b), 2.9 (bs, 1H, OH). MS(EI): m/z 275 ($M^+$)).

*(3,4-trans-4,5-trans)-4-hydroxy-5,3-oxymethylenohexahydropyridazine* (**11**). Compound **10** (22 mg) was dissolved in hydrazine-hydrate (2 mL) and heated for 2 days at 100 °C. The reagent was removed by

evaporation, and the residue was redissolved in water and eluted through an ion exchange chromatography column (Amberlyst 15, $H^+$). By eluting with 5% $NH_4OH$ and concentrating the eluates compound **11** was obtained (10 mg, quantitative) as syrup. $^1$H-NMR ($D_2O$): δ 4.0-4.2 (m, 4H, H-3, H-3'a, H-4, H-5), 3.3 (bd, 1H, $J_{3'a3'b}$ 12.3 and $J_{33'b}$ 4.5 Hz, H-3'b), 3.1 (dd, 1H, $J_{6ax6eq}$ 14 and $J_{56eq}$ 2 Hz, H-6eq), 2.5 (bd, 1H, H-6ax). MS(EI): m/z 130 ($M^+$).

*(2,3-trans-3,4-cis)-3,4-epoxy-2-fluoromethyl-8-phenyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione* (**12**). Compound **5** (100 mg) was dissolved in $CH_2Cl_2$ (2 ml) and cooled to -80 °C. Diethylaminosulphur trifluoride (0.5 ml) was added and the solution was left at room temperature for 30 min. The solution was cooled to -20 °C and methanol (2 ml) was added. The solvents were evaporated and the residue was subjected to flash chromatography in EtOAc to give the epoxyfluoride **12** (71 mg, 70%) as an amorpous white solid. $^1$H-NMR ($CDCl_3$): δ 7.4 (m, 5H, Ar), 4.9 (ddd, 1H, $J_{2'aF}$ 47, $J_{2'a2'b}$ 10 and $J_{22'a}$ 3.8 Hz, H-2'a), 4.7 (ddd, 1H, $J_{2'bF}$ 45 and $J_{22'b}$ 2.5 Hz, H-2'b), 4.6 (bdd, 1H, $J_{2F}$ 25, H-2), 4.3 (d, 1H, $J_{5a5b}$ 14 Hz, H-5a), 3.8 (d, 1H, H-5b), 3.6 (m, 2H, H-3, H-4).

*(2,3-trans-3,4-trans)-3,4-dihydroxy-2-fluoromethyl-8-phenyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione* (**13**). Epoxyfluoride **12** (71 mg) was dissolved in water (7 ml) and $HClO_4$ (0.3 g, 70%) was added. The solution was heated to 100 °C and kept at that temperature for 4 h. Then it was neutralised with $KHCO_3$ (0.21 g), and water was removed by evaporation. The residue was extracted with EtOAc and subjected to Flash-chomatography in EtOAc. This gave diol **12** as a colourless oil (43 mg, 57%). $^1$H-NMR ($CDCl_3$): δ 7.7 (m, 5H, Ar), 5.2 (ddd, 1H, $J_{2'aF}$ 48, $J_{2'a2'b}$ 10.2 and $J_{22'a}$ 6.4 Hz, H-2'a), 5.1 (ddd, 1H, $J_{2'bF}$ 46 and $J_{22'b}$ 3.5 Hz, H-2'b), 4.5 (ddt, 1H, $J_{2F}$ 19 and $J_{23}$ Hz, H-2), 4.3 (d, 1H, $J_{5a5b}$ 14 Hz, H-5a), 3.8 (d, 1H, H-5b), 3.6 (m, 2H, H-3, H-4).

*(6,7-cis-7,8-trans)-7,8-dihydroxy-10-phenylaminocarbonyl-1,3,4,10-tetraazabicyclo[4.4.0]decane-2-one* (**14**). Compound **13** (56 mg) was dissolved in hydrazine-hydrate (5 mL) and heated for 16 h at 90-100 °C. The reagent was removed by evaporation. The residue consisted of only one product **14**. After purification by column chromatography (EtOH-25% $NH_4OH$ 9:1) pure **14** (28 mg, 48 %) was obtained. $^1$H-NMR ($D_2O$): δ 7.2-7.5 (m, 5H, Ar), 4.3 (dd, 1H, $J_{9ax9eq}$ 12.3 and $J_{89eq}$ 3.5 Hz, H-9eq), 4.1 (dd, 1H, *J* 9.2 and *J* 6.9 Hz), 3.9 (d, 1H, *J* 9.2 Hz), 3.6 (m, 2H), 3.5 (d, 1H, *J* 9.2 and 9.2 Hz), 2.7 (m, 1H, $J_{9ax9eq}$ 12.3 and $J_{89ax}$ 10.4 Hz, H-9ax). MS(EI): m/z 249 (M-58)).

*Measurements of glycosidase inhibition.* Each glycosidase assay was performed by preparing four 2 ml samples in cuvettes consisting of 1 ml sodium phosphate buffer (0.1 M) of pH 6.8, 0.2 to 0.8 ml of a 1.0 or 10 mM solution of either 4-nitrophenyl α-D-glucopyranoside or 4-nitrophenyl β-D-glucopyranoside, 0.1 ml of a solution of either the potential inhibitor or water, and distilled water to a total volume of 1.9 ml. Two times four of the samples contained the potential inhibitor at two fixed concentrations, but with variant nitrophenyl glycoside concentration. Other four samples contained no inhibitor, but also variant nitrophenyl glycoside concentration. Finaly 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), β-glucosidase from almonds (EC 3.2.1.21, Sigma G-0395), and the formation of 4-nitrophenol was followed for 2 min. at 26°C by measuring absorbance at 400 nm. Initial velocities was calculated from the slopes for each of the eight reactions and used to construct two Hanes plots; one with and without inhibitor. From the two Michaelis-Menten constants (Km) thus obtained the inhibition constant (Ki) was calculated.

## REFERENCES

1. Karpas, A.; Fleet, G. W. J.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Jacob, G. S.; Rademacher, T. W *Proc. Natl. Acad. Sci. U.S.A.* **1988** *85* 9229-33.

2. Robinson, K. M.; Begovic, M. E.; Rhinehart, B. L. Heineke, E. W.; Ducep, J. -B.; Kastner, P. R.; Marshall, F. N.; Danzin, C. *Diabetes* **1991** *40* 825-830.
3. Bernacki, R. J.; Niedbala, M. J.; Korytnyk, W. *Cancer and Metastasis Reviews* **1985** *4* 81-102.
4. Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990** *48* 319-84.
5. Bols, M.; Hazell, R.; Thomsen, I. *Chem. Eur. J.* **1997** (in press).
6. Hansen, A.; Tagmose, T. M.; Bols, M. *Tetrahedron* **1997** *53* 697-706.
7. Tsuchiya, T. *Adv. Carbohydr. Chem. Biochem.* **1990** *48* 91-277.
8. Card, P. *J. Org. Chem.* **1983** *48* 393-395.
9. Bernstein, J.; Roth, J. S.; Miller, W. T. Jr. *J. Am. Chem. Soc.* **1948** *70* 2310-4.
10. Bronnert, D. L. E.; Saunders, B. C. *Tetrahedron* **1960** *10* 160-3.
11. Lee, C. -K.; Jiang, H.; Linden, A.; Scofield, A. *Carbohydrate Letters* **1996** *1* 417-23.

## ACKNOWLEDGEMENTS

We thank the Danish Natural Research council for financial support (grant nr. 9502986). We also thank B. O. Pedersen for Mass spectra.

(*Received in UK* 14 *April* 1997; *revised* 16 *May* 1997; *accepted* 22 *May* 1997)