

Synthesis of 1-azagalactofagomine, a potent galactosidase inhibitor

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1-Azagalactofagomine [(+)-(3*R*,4*S*,5*R*)-4,5-Dihydroxy-3-(hydroxymethyl)hexahydropyridazine, **2**] was synthesised from achiral starting materials in a chemoenzymic synthesis. The racemic Diels–Alder adduct (2-hydroxymethyl-8-methyl-1,6,8-triazabicyclo[4.3.0]non-3-ene-7,9-dione, **5**) from addition of pentadienol to 4-methyl-1,2,4-triazoline-3,5-dione was resolved using lipase R-catalysed acetylation. The acetate [(*S*)-2-acetoxymethyl-8-methyl-1,6,8-triazabicyclo[4.3.0]non-3-ene-7,9-dione, **6**] was saponified and treated with MCPBA to give a majority of the *syn* epoxide [(2*R*,3*S*,4*R*)-3,4-epoxy-2-hydroxymethyl-8-methyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione, **7**]. This isomer was subjected to epoxide opening with HI followed by a Woodward reaction-like displacement of the iodide with water and peracetylation to give an all-*syn* triacetate [(2*R*,3*S*,4*R*)-3,4-diacetoxy-2-acetoxymethyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione, **11**]. Finally deacetylation and hydrazinolysis gave **2**. The pK_a of **2** was determined to be 5.7. 1-Azagalactofagomine was found to be a potent competitive galactosidase inhibitor. The inhibition constants, K_i , were 40, 300 and 7800 nM versus β -galactosidase from *Aspergillus oryzae*, *Eschinchia coli* and *Saccharomyces fragilis*, respectively, and 280 nM vs. α -galactosidase from green coffee beans.

Introduction

Aza sugars and imino sugars are subject to intense current interest.^{1–3} Some time ago it was found that a subtle change in the classical imino sugar inhibitor of nojirimycin type, by moving the nitrogen to the pseudo-anomeric position (the position that corresponds to the anomeric position in a monosaccharide), gave a very potent class of glycosidase inhibitors the so-called 1-aza sugars.^{4,5} A member of this class of compounds is 1-azafagomine **1**, a hydrazine, that inhibits both α - and β -glucosidase strongly (Fig. 1).^{6,7} The reason for the biological activity of **1** is perhaps that, in protonated form, it mimics the transition states of α -glucoside (A) and β -glucoside cleavage (B). In order to get more evidence for this theory it was the subject of this study to investigate how general this idea was and whether it could be extended to other glycosidases. Ideally one would wish to investigate the bioactivity of the lyxo isomer **2** as it has been shown that **3** is a very potent β -galactosidase inhibitor, while being weak against α -galactosidase.⁸ Nojirimycin analogue **4**, on the other hand, has the opposite inhibitory profile (Fig. 1).⁹ Thus if **1** was mimicking both transition states one would expect **2** to be potent against either type of galactosidase. In this paper we report the synthesis of **2** and report that it is indeed a potent competitive inhibitor of both α - and β -galactosidase.

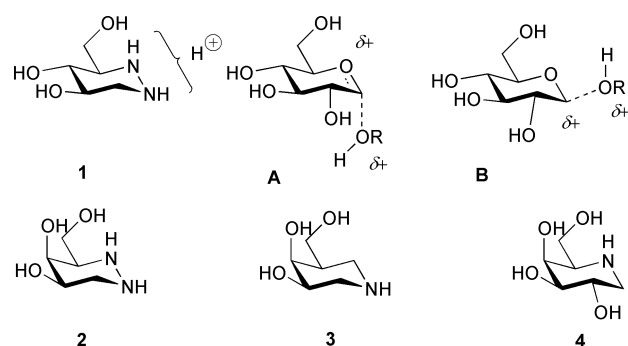
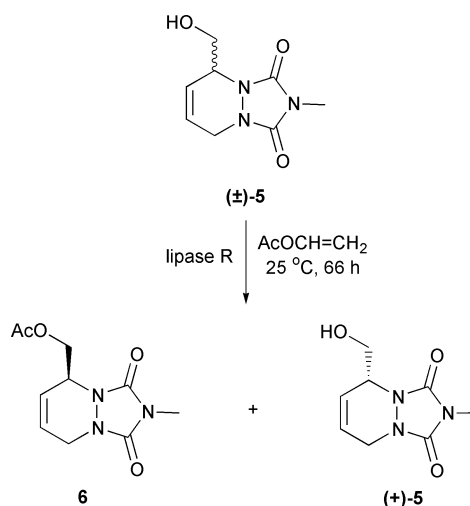


Fig. 1 Structure of azafagomine (**1**), azagalactofagomine (**2**), isogalactofagomine (**3**) and galactostatin (**4**).

Results and discussion

Optically pure **1** can be synthesised both from chiral pool⁷ or achiral starting materials.¹⁰ However, extension of these methods to the synthesis of **2** was not trivial. In order to obtain **2** by the chiral pool synthesis it would require that expensive L-ribose be used as starting material. The chemoenzymic synthesis would require that the double bond in **5** be *cis*-dihydroxylated *syn* to the α -hydroxymethyl group (Scheme 1).



Scheme 1 Enzymic resolution of alkene **5**.

However OsO₄-catalysed dihydroxylation was known to give exclusively the *anti* product.⁶

As a consequence we searched for a procedure that would lead to *cis*-dihydroxylation *syn* to the α -hydroxymethyl group.¹¹ One of the few available methods was that of Woodward and Brucher, in which an alkene is treated with I₂ and AgOAc in wet acetic acid.¹² This results in formation of a iodo acetate C, with iodine at the less hindered face, which subsequently forms an acetoxonium ion D (Fig. 2). Spontaneous hydrolysis of this ion by the water present gave a partially acetylated compound

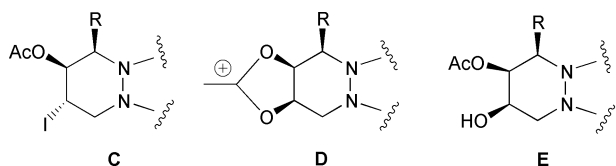
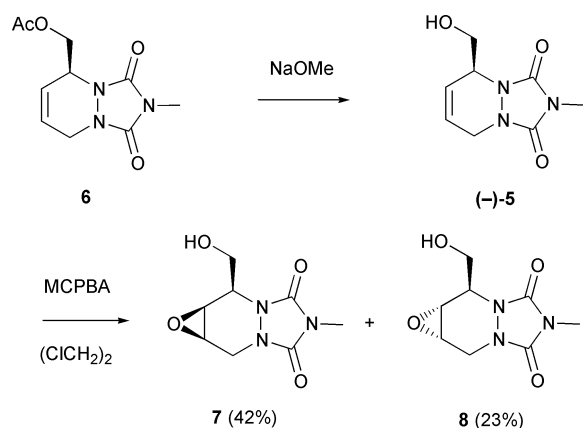


Fig. 2 Some of the intermediates from the Woodward reaction: **C** shows one of the iodides expected to be formed by addition of AgOAc and I₂ to an alkene, **D** shows the subsequent acetoxonium ion and **E** one of the monoacetates formed from hydrolysis of **D**.

E, which by deacetylation gave the *syn*-diol at the more hindered face.¹²

When the Woodward and Brucher protocol was attempted on substrate **5** no significant reaction was observed. Presumably the alkene is too unreactive, as shown by the previous observation that epoxidation of **5** can only occur with the most reactive reagents or under forcing conditions.⁶ However the idea was conceived that if the intermediate iodo acetate **C** could be obtained by a different route the acetoxonium chemistry could be relied upon to give the desired product **E**. An obvious source for iodo acetate **C** was the epoxide **7**, a previously unwanted by-product from the epoxidation of **5**.¹⁰ It was expected, based on previous work,⁶ that epoxidation of **5** with *m*-chloroperbenzoic acid (MCPBA) would give **7** as the major product.

Thus (±)-**5**¹⁰ was subjected to enzymic resolution by acetylation with lipase R and vinyl acetate to give acetate **6** and alcohol (+)-**5** (Scheme 1). This was essentially performed as previously described¹⁰ except that only one enzyme was used. Acetate **6** and alcohol (+)-**5** were obtained in 88% and 92% ee, respectively. After deacetylation of **6** with NaOMe–MeOH and recrystallisation the optically pure alcohol (–)-**5** was obtained. Now treatment of this alkene with MCPBA at 80 °C in (ClCH₂)₂ for 18 h, necessary to promote reaction, gave a mixture of the epoxides **7** and **8** in the ratio 2 : 1 (Scheme 2). These



Scheme 2 Deacetylation and epoxidation of acetate **6**.

two isomers were separated by chromatography and isolated pure in 42% and 23% yield, respectively.

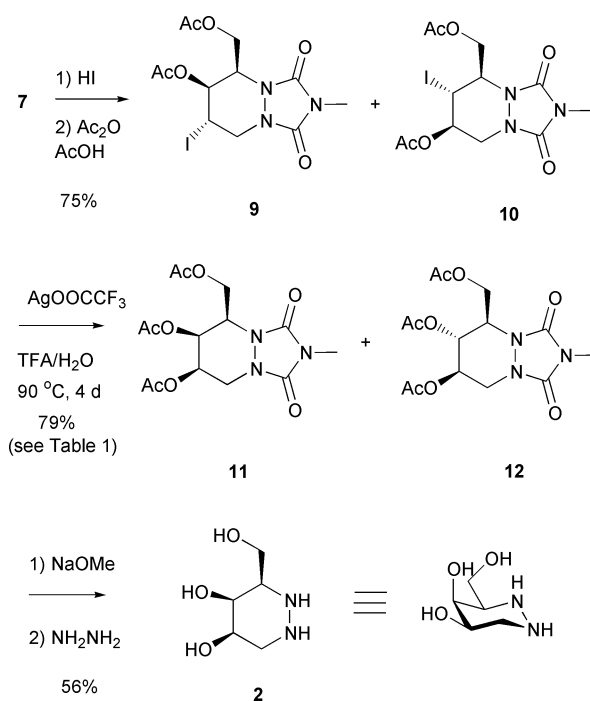
The epoxide **7** was now subjected to treatment with 57% aq. HI in AcOH followed by *in situ* acetylation by addition of Ac₂O, which gave a mixture of the acetylated iodides **9** and **10** in the ratio 1 : 3 and a yield of 75%. The preferential opening in the 3 position of epoxide **7** in this reaction is in contrast to the opening of the corresponding *anti* epoxide **8**, which is hydrolysed very selectively with attack at the 4 position.¹⁰ While the selective hydrolysis of **8** undoubtedly is caused by steric hindrance from the 2-hydroxymethyl group, steric reasons cannot play a role in the preferential formation of iodide **10**. One explanation could be that geometrical constraints in the bicyclic system favor 3*R*,4*R*-di-axial opening regardless of the stereochemistry of the epoxide. The regiochemistry of the

Table 1 Results from various reaction conditions employed in the transformation of iodides **9** and **10** to triacetates **11** and **12** (Scheme 3)

Entry	Reagent	Solvent	% Water	Time/d	Ratio 11/12
1	AgOAc	AcOH	6	2	70 : 30
2	AgOSO ₂ CF ₃	AcOH	6	2	70 : 30
3	AgO ₂ CCF ₃	AcOH	6	2	91 : 9
4	AgO ₂ CCF ₃	AcOH	50	1	87 : 13
5	AgO ₂ CCF ₃	CF ₃ CO ₂ H	6	4	96 : 4

isomers **9** and **10** was determined from a COSY spectrum. The major isomer **10** has a low-field (δ 5.17) quartet from a proton (H-4) that couples with the two protons on C-5 and H-3. Therefore this proton must be next to a acetoxy group.

Now treatment of the mixture of iodo acetates **9** and **10** with AgOAc in acetic acid containing 6% of water gave a mixture of partially acetylated compounds that were immediately acetylated with acetic anhydride and Et₃N to give a 7 : 3 mixture of the triacetates **11** and **12** (Scheme 3, Table 1). Not only was the



Scheme 3 Opening of epoxide **7** with HI and subsequent solvolysis.

stereoselectivity in this reaction disappointing, but it was also remarkable that the *arabino* isomer **12** was formed in relatively large amounts. Formation of an *arabino* isomer can occur by substitution, with retention of configuration, of iodide **10** (Scheme 4). Alternatively **12** could be imagined to be formed from intermediate **13** by S_N2 substitution with acetate. This is, however, unlikely because if nucleophilic attack of **12** by acetate were occurring, a *xylo* isomer should also be formed, and this was not observed. The desired *lyxo* isomer **11**, on the other hand, could be formed both through the planned hydrolysis of acetoxonium ion **13** and by direct substitution of **9** and **10** with inversion of configuration (Scheme 4). The large amounts of **12** formed showed that nucleophilic substitution of the iodide **10** was a major reaction pathway. In order to suppress this undesired pathway a series of different reaction conditions were investigated (Table 1). We attempted to replace AgOAc with AgO₂CCF₃ and AgOSO₂CF₃, which have less nucleophilic counter-ions. As seen from Table 1 the least nucleophilic reagent AgOSO₂CF₃ gave unchanged stereoselectivity, while AgO₂CCF₃ actually improved the selectivity considerably

towards **11**. It was possible that, in these reactions, acetate ions from the acetic acid medium were still acting as nucleophiles, leading to formation of **12** by substitution with retention. It was therefore decided to investigate the medium as well. First the water content was investigated. Woodward and Brutcher used as little as 1 mol equiv. of water in their procedure;¹² however, decreasing the water content in this case decreased the reaction rate too much to be practical. Increasing the water content to as much as 50% increased the reaction rate, and gave virtually unchanged stereoselectivity (Table 1). Finally, acetic acid was substituted with trifluoroacetic acid (TFA). As seen from Table 1, when AgO₂CCF₃ in TFA containing 6% water was used the side reaction was suppressed giving a stereoselectivity of 96 : 4 and a 79% yield (Scheme 3). These experi-

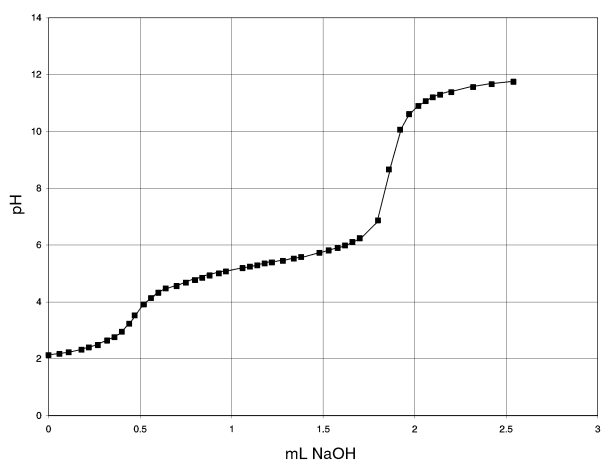
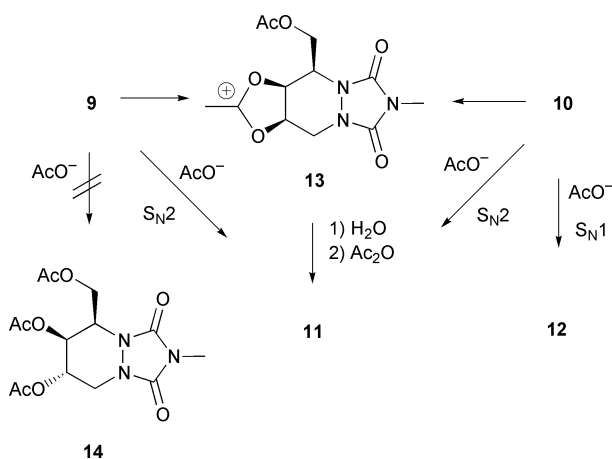


Fig. 3 Titration curve for **2**. Titration performed with 0.099 M NaOH on **2** in water, with 1.13 M HCl added. pK_a determined to be 5.7.



Scheme 4 Possible reaction routes in the transformation of iodides **9** and **10** to triacetates **11** and **12**.

ments show that a large fraction of **12** is formed by attack of acetate ions, and removing acetate improves stereoselectivity tremendously. However, since a small amount of **12** is obtained even when no acetate is present it appears that water substitutes the iodide with retention to some extent.

Compounds **11** and **12** could not be separated. Therefore **11**, containing 4% **12**, was deacetylated with NaOMe and the product subjected to hydrazinolysis with aq. NH₂NH₂ at 100 °C. This gave a product that could be purified to give the target compound **2** stereochemically pure in 56% yield over the two steps (Scheme 3).

The basicity of **2** was determined by measuring the pK_a of the compound. The titration curve for titration of the hydrochloride of **2** with NaOH is shown in Fig. 3. From the curve the pK_a was found to be 5.7. As this value was in total contrast to the pK_a-value of the *arabino*-isomer **1**, which was previously found to be 3.9,⁶ it was decided to measure the pK_a of **1** again. A titration curve for the titration of the hydrochloride of **1** is shown in Fig. 4. From the curve the pK_a of **1** was found to be 5.3, which means that our previous determination of pK_a was incorrect. To confirm this, NMR spectra of **1** were measured at different pH-values. They showed that **1** was 71% protonated at pH 4.8 and 15% protonated at pH 5.5. Therefore **1** should be approximately 50% protonated at pH 5.3, and this thus confirmed the new pK_a-value.

Azagalactofagomine **2** was tested for inhibition of a series of glycosidases. Not surprisingly, **2** was a poor inhibitor of α-glucosidase, but a strong inhibitor of β-glucosidase and a series of galactosidases. It is noteworthy that compound **2** is a slightly weaker inhibitor of β-galactosidase and β-glucosidase than isogalactofagomine **3**, but more potent than *galacto*-deoxynojirimycin **4**. In contrast, **2** is a stronger inhibitor of α-galactosidase than is **3**, but weaker than **4** (Table 2). These observations are similar to what has been found for the corresponding *arabino*/*gluco*-isomers of **2**, **3** and **4**,⁶ and as such support the transition-state theory outlined in the Introduction and Fig. 1. One may speculate as to why **2** is a significantly weaker inhibitor of α-galactosidase than is **4** and the explanation is

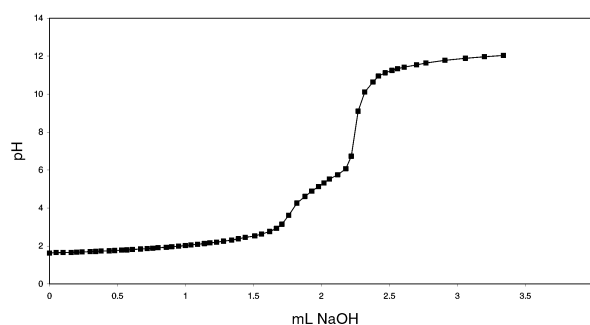


Fig. 4 Titration curve for **1**. Titration performed with 0.099 M NaOH on 6.5 mg **1** in 5 ml of water, with 0.2 ml of 1.13 M HCl added. pK_a determined to be 5.3.

Table 2 K_i-values in μM for inhibition of glycosidases by **2**, **3** and **4** at pH 6.8 and 25 °C

Enzyme	2	3	4
α-Glucosidase (baker's yeast)	570	>2000 ^a	
β-Glucosidase (almonds)	0.13	0.097 ^b	540 ^c
β-Galactosidase (<i>Aspergillus Oryzae</i>)	0.04	0.004 ^c	
β-Galactosidase (<i>E. coli</i>)	0.30	0.2 ^d	12.5 ^c
β-Galactosidase (<i>Saccharomyces fragilis</i>)	7.8	0.33	81
α-Galactosidase (green coffee beans)	0.28	50	0.0016 ^c

^a An IC₅₀-value taken from ref. 5. ^b Taken from ref. 13. ^c Taken from ref. 5. ^d Measured on racemic inhibitor. ^e Taken from ref. 9.

probably that **2** lacks a (carbohydrate) C-2 hydroxy group. It is more potent than **3** against this enzyme because it has a second ring nitrogen atom. The reason why **2** is more potent against the β -glycosidases than is **4** is presumably due to the presence and lack of an anomeric N-atom, respectively. The fact that **2** is slightly weaker than **3** towards the β -glycosidases is probably explained by the lower basicity of **2**.

In this paper we have reported synthesis of the potent galactosidase inhibitor **2** for the first time. A new modified version of Woodward's reaction was used in this synthesis to introduce the lyxo stereochemistry. This method should be useful for *de novo* synthesis of other galactose mimics.

Experimental

General

^{13}C NMR and ^1H NMR spectra were recorded on a Varian Gemini 2000 (200 MHz) instrument. D_2O was used as solvent with DHO (^1H NMR: δ_{H} 4.79) and acetone (^1H NMR: δ_{H} 2.05; ^{13}C NMR: δ_{C} 29.8) as reference. With CHCl_3 as solvent, SiMe_4 (TMS) and CHCl_3 (^{13}C NMR: δ_{C} 76.93) were used as references. Mass spectra were obtained on a Micromass LCT instrument. Concentrations were performed on a rotary evaporator at a temperature below 40 °C.

(*R*)-2-Hydroxymethyl-8-methyl-1,6,8-triazabicyclo[4.3.0]non-3-ene-7,9-dione (+)-**5** and (*S*)-2-hydroxymethyl-8-methyl-1,6,8-triazabicyclo[4.3.0]non-3-ene-7,9-dione (–)-**5**

A mixture of (\pm)-**5** (3.040 g, 15.4 mmol) and lipase R (*Penicillium Roqueforti*, 4.0 g) was stirred in vinyl acetate (200 mL) at room temperature. The reaction was monitored by taking samples for NMR analysis. $\approx 50\%$ Conversion was reached after 66 h, when the solution was filtered and the enzyme cake was washed thoroughly with AcOEt. The solution was concentrated *in vacuo* and the residue underwent flash chromatography (first CHCl_3 –AcOEt 1 : 1, then AcOEt) which gave (+)-**5** (1.314 g, 43%, 92% ee) as a colorless solid, and then acetate **6** which appeared as a colorless oil. The ester was deacetylated by dissolving the compound in 70 mL of methanol containing a catalytical amount of NaOCH_3 . After total conversion (TLC control, AcOEt) a piece of solid CO_2 was added to neutralise the solution, which was evaporated to dryness and filtered through silica gel (AcOEt) to obtain the desired alcohol (–)-**5** as a colorless solid (1.256 g, 41%, 88% ee). Recrystallisation of each enantiomer from AcOEt–hexane gave enantiopure alcohols (+)-**5** and (–)-**5**. The NMR spectra of isomers **5** were identical with those previously reported.¹⁰ The ees were determined by HPLC (Daicel AD, hexane– Pr^iOH 80 : 20, flow rate 1.0 mL min^{-1} , UV detection at 210 nm), t_{r} = 11.1 min [(–) enantiomer], t_{r} = 16.8 min [(+) enantiomer].

(–)-(2*R*,3*S*,4*R*)- and (–)-(2*R*,3*R*,4*S*)-3,4-Epoxy-2-hydroxy-methyl-8-methyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione **7** and **8**

Alkenol (–)-**5** (774 mg, 3.9 mmol) was dissolved in 1,2-dichloroethane (10 mL) and MCPBA (2 g) was added. The solution was stirred for 18 h at 80 °C after which the solvent was removed under reduced pressure. The residue was put directly on a column of silica gel and eluted (first CHCl_3 , then AcOEt), which consequently gave epoxides **7** and **8** in an isolated yield of 347 mg (42%) and 190 mg (23%), respectively. R_{f} (**7**) 0.22 in AcOEt, R_{f} (**8**) 0.13 in AcOEt. The NMR spectra of **7** and **8** were identical with those previously reported.¹⁰

(2*R*,3*S*,4*S*)-3-Acetoxy-2-acetoxymethyl-4-iodo-8-methyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione and (2*R*,3*R*,4*R*)-4-acetoxy-2-acetoxymethyl-3-iodo-8-methyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione **9** and **10**

Epoxide **7** (340 mg, 1.60 mmol) was dissolved in AcOH (4.5 mL) and aq. HI (57%; 0.42 mL, 3.2 mmol) was added at room temperature. After 5 h all starting material had disappeared (TLC control, AcOEt), Ac_2O was added (5 mL), and the mixture was stirred at room temperature overnight. To quench the reaction water (10 mL) was carefully added and allowed to react for 1 h. The reaction mixture was then extracted with AcOEt (3×30 mL) and the combined organic phases were washed with saturated aq. of NaHCO_3 and saturated aq. Na_2SO_3 (each 10 mL). After the solution had been dried over anhydrous MgSO_4 , filtered and evaporated, the residue underwent column chromatography (first AcOEt–pentane 1 : 2, then AcOEt–pentane 1 : 1) which resulted in 516 mg (75%) of iodides **9** and **10** (R_{f} 0.38 in AcOEt–pentane 1 : 1) in the ratio 1 : 3. The combination of iodides appeared as a colorless solid. HRMS(ES) Calc. for $\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_6\text{I} + \text{Na}$: m/z 447.9983. Found: m/z , 447.9984; δ_{H} (CHCl_3). **9**: 5.12 (m, 1H, H-3), 4.17–4.67 (m, 5H, H-2'a, H-2'b, H-4, H-5a), 3.57 (dd, J 11.4, 13.6 Hz, 1H, H-5b), 3.00 (s, 3H, NCH_3), 2.13 and 1.95 (each s, 3H, CH_3). **10**: 5.17 (q, 1H, J 2.4 Hz, H-4), 4.54 (dt, $J_{2,3}$ 1.6, $J_{2,2'}$ 7.0 Hz, 1H, H-2), 4.43 (br s, 1H, H-3), 4.33 (d, 2H, H_2 -2'), 3.95 (br dd, 1H, H-5a), 3.86 (dd, $J_{5a,5b}$ 13.2 Hz, 1H, H-5b), 3.04 (s, 3H, NCH_3), 2.05 and 1.98 (each s, 3H, CH_3).

The 3,4-elimination product was also isolated in a yield of 24 mg (6%, R_{f} 0.2 in AcOEt–pentane 1 : 1).

(2*R*,3*S*,4*R*)-3,4-Diacetoxy-2-acetoxymethyl-1,6,8-triazabicyclo[4.3.0]nonane-7,9-dione **11**

A mixture of iodides **9** and **10** (180 mg, 0.42 mmol) was dissolved in wet TFA (4 mL containing 6% water). To the solution was added AgO_2CCF_3 (186 mg, 0.85 mmol). The reaction vessel was sealed and heated in the dark to 90 °C for 4 days. The reaction mixture was then cooled and NaCl (50 mg, 0.85 mmol) was added. Filtration and washing with CH_3OH removed the precipitate. The solvent was then removed under reduced pressure and the residue underwent acetylation by stirring it in CHCl_3 (3 mL) with Ac_2O (1 mL) and Et_3N (1 mL) for 5 h at room temperature. After the reaction was complete, excess of Ac_2O was destroyed by slowly adding water (5 mL) and stirring of the mixture for 30 min. The two phases were then separated and the aqueous phase was extracted with CHCl_3 (4×5 mL). The combined organic phases were washed first with saturated aq. NaHCO_3 (10 mL), then brine (10 mL), and dried over anhydrous MgSO_4 . The solvent was removed and the remaining oil underwent flash chromatography using AcOEt–pentane 1 : 1 as eluent (R_{f} 0.26) to afford 120 mg (79%) of the desired product as a colorless oil containing 4% of the 3-epimer (NMR analysis); δ_{H} (CDCl_3) 5.29 (q, J 3.4 Hz, 1H, H-4), 5.17 (dd, $J_{3,4}$ 3.4, $J_{2,3}$ 5.2 Hz, 1H, H-3), 4.67 (dd, $J_{2,2'a}$ 9.0, $J_{2'a,2'b}$ 11.6, 1H, H-2'a), 4.42–4.56 (m, 1H, H-2), 4.32 (dd, $J_{2,2'b}$ 3.4 Hz, 1H, H-2'b), 3.92 (dd, $J_{4,5a}$ 12.8 Hz, 1H, H-5a), 3.39 (dd, 1H, H-5b), 3.00 (s, 3H, NCH_3), 2.09, 2.05 and 1.96 (each s, 3H, CH_3); δ_{C} (CDCl_3) 170.9, 169.6 and 169.3 [$\text{C}(\text{O})\text{CH}_3$], 155.0 and 153.2 [$\text{NC}(\text{O})\text{N}$], 67.2, 65.4 (C-3, C-4), 60.0 (C-2'), 54.4 (C-2), 46.9 (C-5), 25.5 (NCH_3), 21.1, 20.9, 20.8 ($\text{C}(\text{O})\text{CH}_3$). HRMS(ES) Calc. for $\text{C}_{14}\text{H}_{18}\text{O}_8\text{N}_3 + \text{Na}$: 380.1070. Found: 380.1072.

General procedure for iodide substitution

A 3 : 1 mixture of iodides **9** and **10** (20 mg) was dissolved in AcOH or TFA containing water (0.45 mL). 2 Mole equivalents of the silver salt (AgOAc , AgOTf or AgO_2CCF_3) was added. The reaction mixture was heated to 90 °C in darkness in a sealed flask for 24–96 hours (TLC control, AcOEt–pentane 1 : 1). The reaction mixture was allowed to cool to room temperature and 2 mole equivalents of NaCl was added. Filtration and washing with CH_3OH removed the precipitate. The solvent was removed under reduced pressure and the residue underwent

acetylation by being stirred in CHCl_3 (0.3 mL) with Ac_2O (0.1 mL) and Et_3N (0.1 mL) for 5 h at room temperature. After the reaction had gone to completion, excess of Ac_2O was destroyed by addition of water (0.5 mL) and stirring of the mixture for 30 min. All the solvent was removed by evaporating several times with toluene. The product was sufficiently pure for NMR analysis from which the ratio between the two epimers was determined by integration.

(+)-(3*R*,4*S*,5*R*)-4,5-Dihydroxy-3-(hydroxymethyl)hexahydro-pyridazine 2

Triacetate **11** (120 mg, 0.34 mmol) was dissolved in methanol (3 mL) containing a catalytic amount of NaOCH_3 . The deacetylation was complete in 30 min, and the solvent was removed. To the residue was added hydrazine hydrate (4 mL) and the mixture was refluxed for 18 h. The solvent was then removed under reduced pressure and the remaining oil underwent ion exchange (Amberlite IR-120, H^+). The product was released from the resin with 2.5% NH_4OH . Concentration followed by chromatography (ethanol–25% NH_4OH 20 : 1, R_f 0.18) gave 28 mg (56% over two steps) of **2** (without any trace of the 4-epimer) as a colorless solid, $[\alpha]_{\text{D}}^{22} + 11.9 \times 10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$ (H_2O); $\delta_{\text{H}}(\text{D}_2\text{O})$ 3.96 (br s, 1H, H-4), 3.69–3.79 (m, 1H, H-5), 3.59 (d, $J_{3,3'}$ 6.6 Hz, 2H, H₂-3'), 2.89 (dt, $J_{3,4}$ 1.6 Hz, 1H, H-3), 2.85 (dd, $J_{5,6\text{eq}}$ 3.2 Hz, 1H, H-6eq), 2.75 (dd, $J_{5,6\text{ax}}$ 10.8, $J_{\text{H6eq,H6ax}}$ 12.8 Hz, 1H, H-6ax); $\delta_{\text{C}}(\text{D}_2\text{O})$ 68.3, 67.1 (C-4, C-5), 61.5, 60.9 (C-3, C-3'), 46.9 (C-6); HRMS(ES) Calc. for $\text{C}_5\text{H}_{12}\text{N}_2\text{O}_3 + \text{Na}$: m/z , 171.0746. Found: m/z , 171.0740.

Acknowledgements

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