Synthesis of 3-substituted isofagomine analogues using an unusual *syn* hydrogenation reaction

Anders Lohse, Henrik H. Jensen, Peter Bach and Mikael Bols*

Department of Chemistry, University of Aarhus, Langelandsgade 140, DK-8000 Aarhus, Denmark. Tel +45 89 42 39 64, Fax +45 86 19 61 99, e-mail mb@kemi.aau.dk

Received (in Lund, Sweden) 14th October 1999, Accepted 12th January 2000
Published on the Web 8th February 2000

Isofagomine (3,4-dihydroxy-5-(hydroxymethyl)piperidine, **1**) and analogues are found to be strong inhibitors of glycosidases, and are therefore of potential interest in treatment of various disorders. Starting from cheap and readily available materials we have developed a new diastereoselective synthesis of 3,4,5-trisubstituted piperidines of the isofagomine type. (±)-3-Amino-3-deoxyisofagomine (**2**) and a series of 11 closely related structures were synthesized *via* three key intermediates **5**–**7** in relatively few and high yielding steps. The biological activity of compounds **2**, **8**–**18** was investigated towards several enzymes, and new inhibitors of glycosidases were found.

Introduction

Inhibitors of glycoside-processing enzymes have in recent years been the subjects of increasing interest due to their potential application as drugs or as biochemical tools for investigating glycobiology.[1] Several glycosidase inhibitors have found use in treatment of various disorders like diabetes[2] and, more recently, also influenza.[3] The most potent and selective inhibitors of carbohydrate-processing enzymes on the monosaccharide level are the azasugars, and thus this type of compound can be anticipated to lead to promising drug candidates in the future.

In the ongoing process of designing new inhibitors, we were interested in investigating the effect of substituting the hydroxy groups of our strong glycosidase inhibitor isofagomine[4] **1**. By substituting a hydroxy group with another polar group, we were hoping to gain knowledge about the hydrogen bond donor/acceptor relations for that particular group. It is possible to imagine that the amino derivative **2** of isofagomine **1** might bind to the an enzyme pocket in a very similar manner to isofagomine **1** itself (Fig. 1).

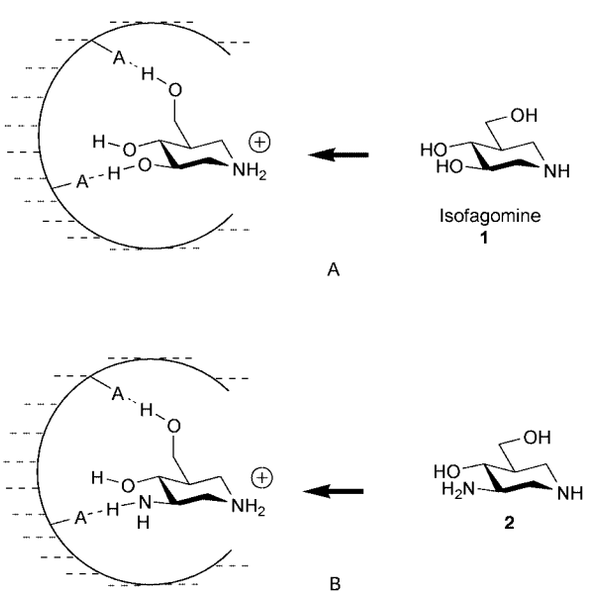


Fig. 1 A possible representation of the binding of isofagomine **1** to an enzyme pocket **A**, and a similar representation for the 3-amino-3-deoxy derivative **2 B**.

Since glycoside-processing enzymes generally are known to be highly stereospecific enzymes, we were also interested in investigating the importance of the stereochemistry for the binding of inhibitors, in particular how various epimeric forms affected binding affinities.

In this work the 3-hydroxy group of **1** was chosen for detailed study. Removal or fluoro substitution of the 3-hydroxy group in nojirimycin and 1-deoxynojirimycin systems has been found to lead to decreased binding.[5] Furthermore 3-deoxy-3-fluoro-1-azafagomine (**24**) was recently found to be a poor inhibitor.[6] This suggested that the 3-hydroxy group in this compound acted a hydrogen-bond donor, which is in line with results from 1-deoxynojirimycin. In general, attempts to improve inhibition, by substituting hydroxy groups in azasugars, have met with little success,[7] but it has been found that substitution of a hydroxy group with an amino group in neuraminic acid derivatives increased binding to neuraminidase strongly.[3a] Thus a key compound we wished to investigate was the 3-amino-3-deoxy derivative of isofagomine, compound **2**.

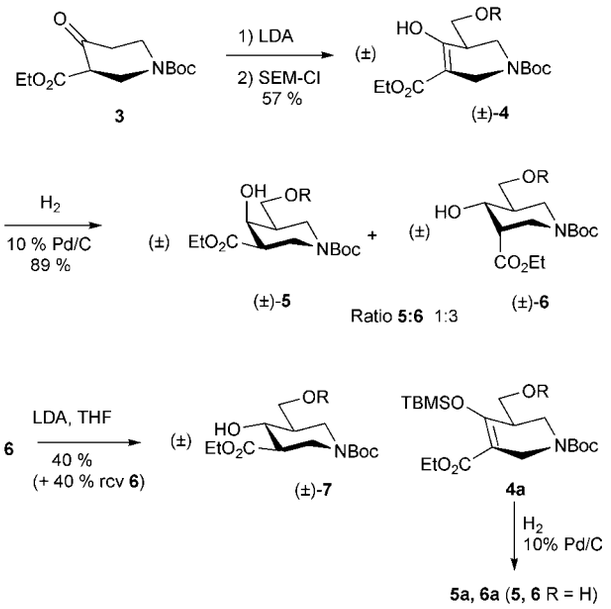
Results and discussion

Our synthetic plan relied on using 4-hydroxy-5-hydroxymethylpiperidine-3-carboxylic acids with proper stereochemistry as key intermediates, as we have recently discovered an efficient way of synthesizing these compounds.[8] This synthesis relied on selective hydroxymethylation of 4-oxopiperidine-3-carboxylic esters followed by hydrogenation of the enol double bond.

Synthesis of the three key intermediates **5**–**7** is outlined in Scheme 1. The starting material for the synthesis was the commercial available ethyl 4-oxopiperidine-3-carboxylate hydrochloride which was protected with di-*tert*-butyl pyrocarbonate by a known procedure[9] to give **3** in almost quantitative yield. Treatment of **3** with 2.5 mol equiv. of LDA generated the di-anion[10] which could be selectively alkylated with 2-(trimethylsilyl)ethoxymethyl chloride (SEM-Cl) in the more reactive 5-position to give **4** in a yield of 57%.[8] SEM-Cl was chosen as a synthon for a hydroxymethyl group with an easily (50% TFA–CH_2Cl_2; 30 min; 0 °C) removable protecting group. Attempts to optimize the yield in the alkylating step were not successful. In particular it was found that in reactions where the concentration of the di-anion exceeded 0.05 M, reaction times greatly increased and yields dropped to 10–15%.

Even though the literature regarding the stereochemistry of catalytic hydrogenation of β-keto esters is relatively sparse, we expected that catalytic hydrogenation of the enol form of

DOI: 10.1039/a908340e

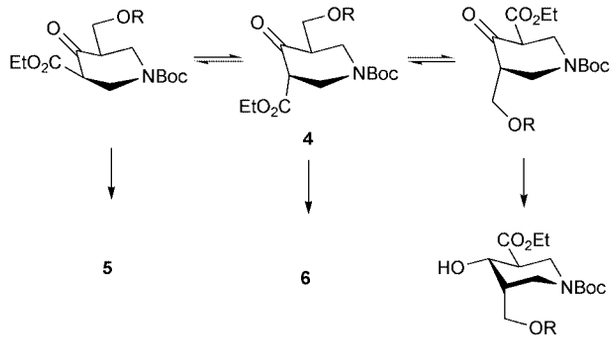


Scheme 1 Synthesis of the three key intermediates **5**–**7** (R = CH_2CH_2-TMS).

4, would give mainly the *anti* stereoisomer (**5**) arising from addition of hydrogen from the least hindered side of the double bond. Surprisingly this was not the case, and repeated hydrogenation of **4** always gave ratios of **5**:**6** of approx. 1:3. The relative stereochemistries of **5** and **6** were determined based on (a) their subsequent conversion to a symmetrical or an asymmetrical derivative upon reduction (see below) and (b) their NMR spectra. H-4 of **5** gave a broad singlet at low field (δ 4.48), which fitted an equatorial proton with small couplings to its neighbors (a typical pattern for H-4 in galactose derivatives). In compound **6** the H-4 signal is at almost 0.5 ppm to higher field (δ 3.99), suggesting that it is axial. Similarly the H-3 signal is at δ 2.75 in **6** and at δ 2.5 in **5**, suggesting that H-3 is equatorial in **6** and axial in **5**, fitting a 3,4-*cis* and 4,5-*trans* stereochemistry for **6**.

Hydrogenation *syn* to polar groups has previously been reported, and the directional effect of the polar groups has been named the *haptophilic effect*. Thus a polar group, like a hydroxymethyl group, was shown to have a large haptophilic effect directing hydrogen to its side of a neighboring double bond.[11] It therefore seemed reasonable to assume that the stereoselectivity observed in the hydrogenation of **4** was caused by the haptophilic effect. However, varying the R-group of **4** from R = $CH_2CH_2SiMe_3$ to R = H or the bulky R = TBDMS did not change the ratio of the two hydrogenation products **5** and **6**, suggesting that the polarity of the R group was irrelevant for the stereoselectivity in this case. This is somewhat confirmed in the information that the haptophilic effect of our most polar 5-substituent CH_2OH has been found to be small when EtOH was the solvent.[11]

Without the haptophilic effect it is difficult to explain why the enol form of **4** should be hydrogenated to give predominantly **6**. As an alternative explanation it was suggested that the hydrogenation could occur on the keto form of **4**. The hydrogenation reaction was done in ethanol where the keto form of **4** was expected[12] to be present. Consequently hydrogenation of this species might take place, since simple cyclohexanones are readily reduced to cyclohexanols by hydrogenation with palladium catalyst.[13] The keto form of **4** would be expected to be a mixture of two diastereomeric forms (Scheme 2). The *cis* form would be expected to be hydrogenated to **5** for steric reasons thereby accounting for the formation of that product. The *trans* form may adopt two different conformations (Scheme 2), each of which has an axial substituent that sterically may hinder hydrogenation from its face. Hydrogenation of the conformer with the ester group axial should result in formation of **6**, while the one with the ester group equatorial should give a stereoisomer not observed. So the stereoselectivity observed could be explained by a predominance of the former conformer in the mixture of ketones. This is not unreasonable, because one would expect the conformer in which the sp^3-substituted carbon substituent (the alkoxymethyl group) is equatorial to be predominant. That substituent is more sterically demanding than the ester group.



Scheme 2 Hydrogenation of **4**.

To distinguish between the two possibilities we prepared protected enol ether **4a** (R = Bn) by silylation of **4** (R = Bn)[8] with TBDMSCl and DMAP, and subjected it to hydrogenation. This gave the usual 1:3 mixture of diastereomers **5a** and **6a** (R = H). Thus the stereoselectivity of the hydrogenations reported here is consistent with the enol ether being the reacting species, but the haptophilic effect does not convincingly explain the selectivity.

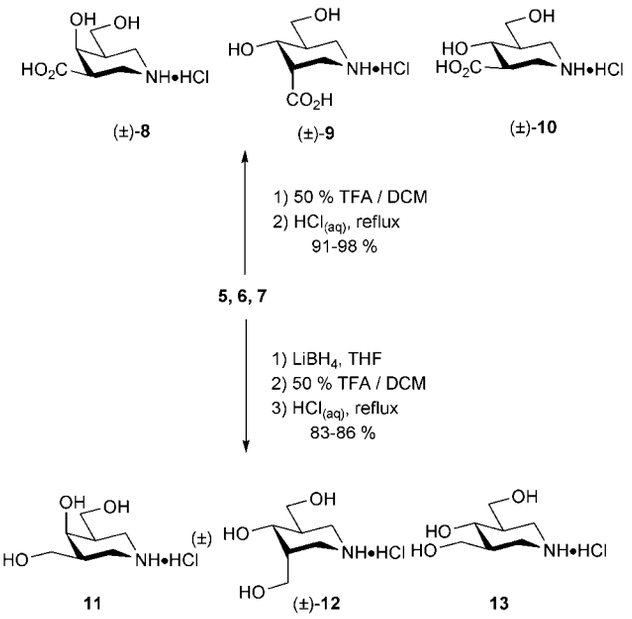
Treatment of **6** with LDA followed by aqueous work-up gave a 1:1 mixture of recovered **6** and **7** which could easily be separated by chromatography to give pure **7** in a recovered yield of 80%. The stereochemistry of **7** was clearly seen from the H-4 signal, which was a triplet with large couplings at relatively high field (δ 3.85). This perfectly fits an axial proton with two neighboring axial protons (as seen for H-4 in glucose derivatives). Having the three key intermediates **5**–**7** at hand we were now able to synthesize a series of 12 new compounds in relatively few, and high yielding, steps.

The most obvious transformation was a complete removal of the protecting groups to give compounds **8**–**10**. This was easily accomplished by first treating **5**–**7** with 50% TFA in CH_2Cl_2 followed by aqueous hydrolysis in 4 M hydrochloric acid, and gave the unprotected amino acids **8**–**10** in excellent yields of 50–54 mg (91–98%) (Scheme 3).

A second series of derivatives were made by first reducing the ester function of **5**–**7** with $LiBH_4$[14] followed by removal of protecting groups, and gave **11**–**13** in yields ranging from 60–62 mg (83–86%). The three bishydroxymethyl compounds **11**–**13** also served as proof for the correct assignment of the configuration of the key intermediates (**5**–**7**). NMR spectroscopy easily revealed the symmetry[15] of **11** and **13**, thereby confirming the original configuration of **5** and **7**. Since **7** was derived from **6** by an epimerization reaction this was also further proof for the configuration of **6** and **12**.

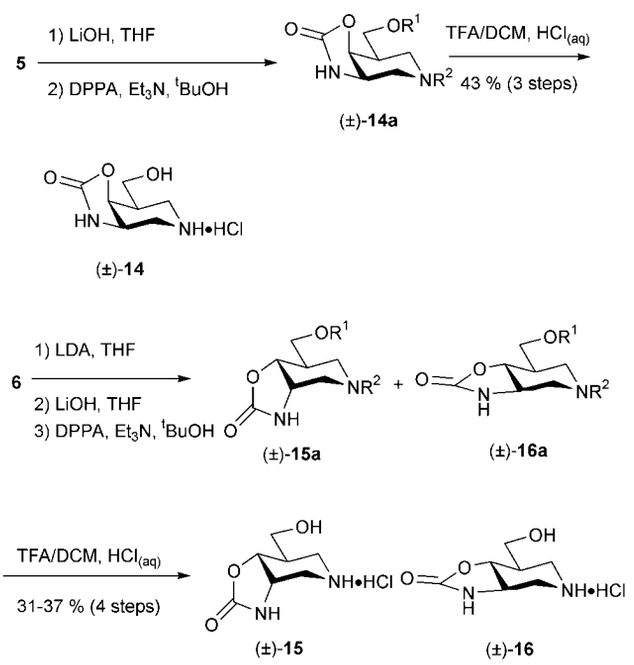
One of the goals of this work was to more deeply investigate hydrogen bond donor/acceptor relations for the binding between glycosidase inhibitor isofagomine **1** and enzymes. Substituting one of the hydroxy groups in isofagomine **1** with another polar group might reveal whether the current group was acting as a hydrogen-bond donor or acceptor. One such group could be an amino group, and we realized that the carboxylic groups of **5**–**7** could be transformed quite easily into amino groups *via* a Curtius rearrangement.[16]

First the ester function of intermediate **5** was hydrolyzed with LiOH in THF to give the free acid, which upon reaction



Scheme 3 Synthesis of compounds **8**–**13** from the three key intermediates **5**–**7**.

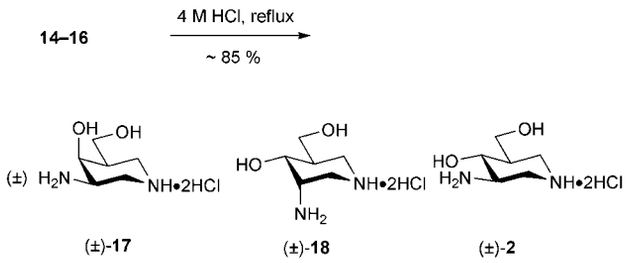
with diphenylphosphoryl azide (DPPA) rearranged to the highly reactive isocyanate, which reacted intramolecularly to give the cyclic carbamate **14a**. Removal of protecting groups gave **14** in an overall yield of 43% for the three steps (see Scheme 4). The presence of the carbamate was clearly seen from a ^{13}C NMR signal at δ_C 159.2, while the transformation of the acid to an amine was witnessed by the appearance of a new signal at δ_C 46.6.



Scheme 4 Synthesis of **14**–**16** (**a**: $R^1 = CH_2CH_2TMS$, R^2 = Boc).

Alternatively **6** was epimerized with LDA, to give a 1 : 1 mixture of **6** and **7**. At this stage we decided to continue with the mixture of two stereoisomers until the final deprotection. In this way it was possible to save an additional purification step which otherwise had to be introduced at this stage. So without further purification this mixture was subjected to basic hydrolysis with LiOH in THF, followed by treatment with DPPA to form the highly reactive isocyanate which reacted intramolecularly to give the protected carbamates **15a** and **16a**. The two stereoisomers were now separated by column chromatography, and final removal of the protecting groups with TFA gave **15** and **16** in yields of 31% and 37% respectively, for the 4 steps.

Finally the carbamates **14**–**16** were hydrolyzed by refluxing them in 4 M hydrochloric acid for 24 h and gave the 3-amino-4-hydroxy-5-(hydroxymethyl)piperidines (**2**, **17**, **18**) as their dihydrochloride salts in yields of approximately 85% (Scheme 5).



Scheme 5 Hydrolysis of the carbamates **14**–**16**.

At this point we were ready to investigate the biological activity. We set up a series of enzyme experiments allowing us to determine the K_i-values for the synthesized compounds (**2**, **8**–**18**).[17] The results from the enzyme assays are summarized in Table 1.

The enzyme experiments revealed some interesting results. First of all we were surprised to find that compound **17** did not show any inhibition of β-galactosidase from *E. coli*. *galacto*-Isofagomine **19** (Chart 1), which **17** greatly resembles,

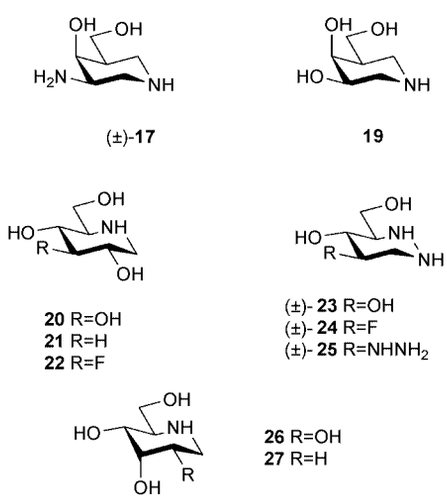
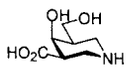
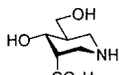
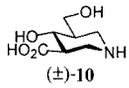
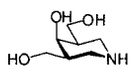
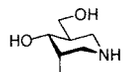
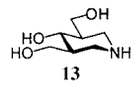


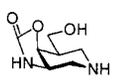
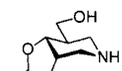
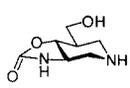
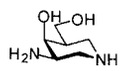
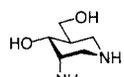
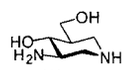
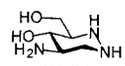
Chart 1 Some known glycosidase inhibitors.

has an inhibitory constant (K_i) towards β-galactosidase from *Aspergillus oryzae* of 4 nM.[18] Even though the variation may in part be caused by the different enzyme source, we still found the loss of activity remarkable. However, compound **2** also showed a substantial reduction in activity towards β-glucosidase compared with isofagomine **1**, which has a K_i-value of 0.11 μM.[4]

To get more information about the role of the 3-amino group we investigated the effect of pH changes on the β-glucosidase inhibition by the three amino-derivatives **2**, **17**, **18**. Running the enzyme experiments at higher pH showed a clear increase in inhibition towards β-glucosidase for all three derivatives. Titration of **2** with NaOH gave an estimate of the two pK_a-values:[19] The ring nitrogen had a pK_a of 8.8 and the exocyclic amine a pK_a of 6.2. This revealed that the exocyclic amino group was mostly deprotonated at both pH 6.8 and 7.5. For the endocyclic amino group, however, the change in pH from 6.8 to 7.5 results in an aproximately 5-fold increase in the amount of free amine. This fits the generally accepted view that an azasugar

Table 1 Inhibition constants (K_i) in μM measured at 25 °C

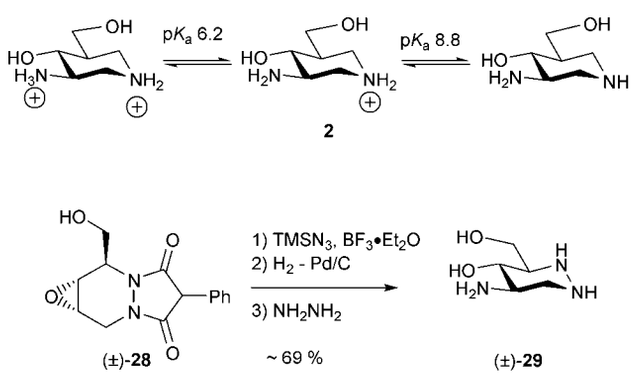
Enzymes	 (±)-**8**	 (±)-**9**	 (±)-**10**	 **11**	 (±)-**12**	 **13**
α-Glucosidase (Bakers' yeast, pH 6.8)	>1000	>1000	>1000	>1000	>1000	>1000
β-Glucosidase (Almonds, pH 6.8)	>1000	>1000	>1000	>1000	47	>1000
β-Glucosidase (Almonds, pH 7.5)					28	
Isomaltase (Bakers' yeast, pH 6.8)	>1000	>1000	>1000	>1000	>1000	>1000
α-Galactosidase (Gr. Coffee beans, pH 6.8)	>1000			>1000		
β-Galactosidase (*E. coli*, pH 6.8)	>1000	>1000	>1000	>1000	>1000	>1000

Enzymes	 (±)-**14**	 (±)-**15**	 (±)-**16**	 (±)-**17**	 (±)-**18**	 (±)-**2**	 (±)-**29**
α-Glucosidase	>1000	>1000	>1000	>1000	>1000	>1000	340
β-Glucosidase (pH 5.7)							46
β-Glucosidase (pH 6.8)	>1000	>1000	140[a]	>1000	26	209	46
β-Glucosidase (pH 7.5)				186	15	106	
Isomaltase	>1000	>1000	>1000	>1000	>1000	>1000	95
α-Galactosidase	>1000			>1000			
β-Galactosidase	>1000	>1000	>1000	>1000	>1000	>1000	

[a] Uncompetitive inhibition. Where no value is given, no determination was made.

inhibitor binds strongest to the enzyme when the ring nitrogen is deprotonated. On the other hand this also meant that these experiments gave us little information about the effect of protonation of the 3-amino group on binding because it was not possible to protonate it without also protonating N-1 and destroying binding.

To obtain this information we therefore synthesized 1-azafagomine analogue **29** (Scheme 6). The pK_a of the hydrazino



Scheme 6 Acid–base properties of **2** and synthesis of **29**.

moity in 1-azafagomine is 3.9,[6] and it would therefore be possible to protonate the exocyclic amino group without affecting the general binding affinity of the ring nitrogen atoms. Compound **29** was synthesized from the known epoxide **28**[6] by epoxide opening with $TMSN_3$–BF_3[20] to form the azidohydrin derivative with regioselectivity. This was converted to the amine by hydrogenolysis, which was deprotected with hydrazine hydrate to give **29** in 69% overall yield from **28**.

Inhibition studies of **29** showed that the degree of protonation of the 3-amino group is irrelevant for β-glucosidase inhibition. Inhibition was unchanged when pH was lowered from 6.8 to 5.7, which should transform the exocyclic amine from mostly unprotonated to highly protonated. This suggest that the 3-amino group in **29**, and **2**, does not act as a hydrogen acceptor and its hydrogen donor ability is unaffected by protonation. In both cases the results suggest that the amino group is acting as a poor hydrogen-bond donor compared with the hydroxy group. Whether it acts as a hydrogen donor at all may be questioned; however, since **29** is a more potent inhibitor than fluoro analogue **24** this seems to be the case. In general **29** was a more potent inhibitor than **2** in the enzymes tested (Table 1). For the isomaltase and α-glucosidase inhibition this can be explained with the presence of the extra ring nitrogen, since 1-azafagomine **23** is a more potent inhibitor of these enzymes than is isofagomine **1**. For β-glucosidase the greater inhibition of **29** is probably due to the greater degree of protonation of the very basic ring nitrogen of **2**.

The results also clearly show that substitution of the 3-hydroxy group of both isofagomine **1** and *galacto*-isofagomine (**19**) with a hydroxymethyl group (**13** and **11**) or a carboxylic acid (**10** and **8**) decreases binding tremendously. This shows that the 3-hydroxy group is crucial for the binding to enzymes. These results are supported by previously reported modifications of 1-deoxynojirimycin (**20**), where either a complete removal of the 3-hydroxy group (**21**) or a substitution with fluorine (**22**) results in almost total loss of inhibitory power.[5ab,7] Likewise modifications of the corresponding hydroxy group of the potent inhibitor 1-azafagomine (**23**) with either fluorine (**24**) or hydrazine (**25**) reduces the activity of the compound.[6] Given the magnitude of the difference in inhibition of β-glucosidase between isofagomine (**1**) and **10** or **13** (more than 10.000) it may even suggest that the hydroxymethyl or carboxylate groups cannot fit into the active site.

In this light the lack of inhibition of the cyclic carbamates (**14**–**16**) was unsurprising. The weak inhibition of β-glucosidase by **16** can be explained as follows: The 4-OH is known to be relatively unimportant for substrate recognition by this enzyme, and therefore protection of this group is not crucial. The activity of **16** is thus comparable with that of other 3-amino derivatives such as **2**.

Perhaps the most remarkable observation from the enzyme experiments was the relatively strong activity of compounds **12** and **18** towards β-glucosidase. Both these compounds are C-3 epimers compared with all the known strong inhibitors of β-glucosidase, and we expected the compounds to be poor inhibitors. There can be several explanations for this result; one, however, seems to be supported by the literature. The two natural products 1-deoxyallonojirimycin (**26**) and 1,2-dideoxyallonojirimycin (**27**), are each reported to be a weak inhibitor of

β-glucosidase.[5c,5i] Despite the relatively weak inhibition of **26** and **27**, it still seems that some binding is gained by the 3-hydroxy group, compared with the 3-deoxy derivative **21** which is reported to have no activity.[7] Together with our own results this indicates that there might be, in the active site of β-glucosidase, an extra hydrogen-bond acceptor positioned in the vicinity of the 3-position somewhere beneath the sugar ring.

In this paper we describe a synthetic method that, by taking advantage of a unusual hydrogenation, allows easy synthesis of different stereoisomers of 3-substituted isofagomine derivatives. It is probable that the hydrogenation reaction occurs on the enol ether though the reason for the unusual stereoselectivity is still unexplained. We also found that an axial polar group in the 3-position of an inhibitor increased binding to β-glucosidase. This suggests that a compound with the basic structure **12**, but with an additional equatorial hydroxy group in the 3-position included, may be more potent that isofagomine **1** itself. We plan to investigate this in due course.

Experimental

General

All reactions were carried out under inert atmosphere in preheated glass equipment. Solvents were distilled under anhydrous conditions. Thus THF was distilled from sodium–benzophenone and used directly. All reagents were used as purchased without further purification. Columns were packed with silica gel 60 (230–400 mesh) as the stationary phase. TLC plates (Merck, 60, F_{254}) were visualized by spraying with ninhydrin (2% in butan-1-ol) and heating till colored spots appeared. All enzymes and substrates for the enzyme assays were purchased from Sigma. Petroleum spirit refers to the fraction with boiling point approximately 40 °C.

(±)-*N*-*tert*-Butoxycarbonyl-4-oxo-5-{[2-(trimethylsilyl)ethoxy]-methyl}piperidine-3-carboxylic acid ethyl ester 4

To a solution of freshly prepared LDA (4.2 mmol) in THF (50 mL) at 0 °C was added a solution of the Boc-protected piperidone **3** (0.50 g, 1.8 mmol). After stirring of the mixture for 30 min, SEM-Cl (0.31 g, 1.8 mmol) was added and stirring was continued for another 30 min before saturated aq. $KHSO_4$ (50 mL) was added. The two layers were separated and the water phase was extracted with EtOAc (2 × 50 mL). The combined organic phases were washed with brine (1 × 100 mL), dried over $MgSO_4$, and evaporated to dryness. The resulting yellow oil was purified by column chromatography on silica gel using EtOAc–petroleum spirit (1 : 12) as eluent (R_f 0.41). The yield was 0.42 g (57%) of a thick clear oil, δ_H ($CDCl_3$) 12.1 (br s, 1H, enol OH), 3.32–4.38 (m, 10H, H_2-2, H_2-5′, H_2-6, OCH_2CH_3, OCH_2CH_2), 2.67 (m, 1H, H-5), 1.50 [s, 9H, $C(CH_3)_3$], 1.32 (t, 3H, *J* 7.0 Hz, OCH_2CH_3), 0.97 (t, 2H, *J* 8.0 Hz, CH_2CH_2TMS), 0.00 [s, 9H, $Si(CH_3)_3$]; δ_C ($CDCl_3$) 170.9 (CO_2Et), 169.3 (br, C-4), 154.9 (N*C*O′Bu), 97.4 (C-3), 80.0 [O*C*$(CH_3)_3$], 68.6, 68.3, 60.8 (C-5′, O*C*H_2CH_3, O*C*H_2CH_2), 42.4, 40.4, 40.2 (C-2, -5, -6), 28.5 [C(*C*$H_3)_3$], 18.3, 14.3 (CH_2CH_3, CH_2CH_2TMS), −1.2 [$Si(CH_3)_3$]; *m*/*z* (ES) 424.2145 (M + Na^+). Calc. for $C_{19}H_{35}NO_6Si + Na^+$: *m*/*z*, 424.2132.

Hydrogenation of 4

The starting material **4** (0.80 g, 2.0 mmol) was dissolved in absolute ethanol (60 mL) and diisopropylethylamine (DIEA) (0.26 g, 2.0 mmol) was added. A flow of nitrogen was bubbled through the solution for 5 min before 10% Pd on carbon (400 mg) was added. The mixture was hydrogenated overnight (50 atm; 50 °C) before filtering through a bed of Celite® and evaporating to dryness. Column chromatography on silica gel with 14% EtOAc in petroleum spirit as eluent gave the two stereoisomers described below in an overall yield of 710 mg (89%).

(3,4-*cis*,4,5-*cis*)-*N*-*tert*-Butoxycarbonyl-4-hydroxy-5-{[2-(trimethylsilyl)ethoxy]methyl}piperidine-3-carboxylic acid ethyl ester 5. Yield 180 mg (23%); R_f 0.23; δ_H ($CDCl_3$) 4.48 (br s, 1H, H-4), 4.18 (m, 3H, H^a-5′, OCH_2CH_3), 3.88 (dd, 1H, $J_{5'a,5'b}$ 4.0 Hz, $J_{5'a,5'b}$ 13.2 Hz, H^b-5′), 3.49 (m, 4H, H^{eq}-2, H^{eq}-6, OCH_2CH_2), 3.21 (t, 1H, *J* 12.6 Hz, H^{ax}-2 or H^{ax}-6), 3.01 (t, 1H, *J* 12.4 Hz, H^{ax}-6 or H^{ax}-2), 2.50 (m, 1H, H-3), 1.77 (m, 1H, H-5), 1.45 [s, 9H, $C(CH_3)_3$], 1.26 (t, 3H, *J* 7.0 Hz, OCH_2CH_3), 0.91 (t, 2H, *J* 8.0 Hz, CH_2CH_2TMS), 0.00 [s, 9H, $Si(CH_3)_3$]; δ_C ($CDCl_3$) 172.3 (CO_2Et), 155.0 (N*C*O′Bu), 80.0 [O*C*$(CH_3)_3$], 71.0, 69.1, 67.5, 61.0 (C-4, -5′, O*C*H_2CH_3, O*C*H_2CH_2), 46.8, 2 × 40.7, 39.5 (C-2, -3, -5, -6), 28.6 [C(*C*$H_3)_3$], 18.3, 14.3 (CH_2CH_3, CH_2CH_2TMS), −1.2 [$Si(CH_3)_3$]; *m*/*z* (ES) 426.2276 (M + Na^+). Calc. for $C_{19}H_{37}NO_6Si + Na^+$: *m*/*z*, 426.2288.

(3,4-*cis*,4,5-*trans*)-*N*-*tert*-Butoxycarbonyl-4-hydroxy-5-{[2-(trimethylsilyl)ethoxy]methyl}piperidine-3-carboxylic acid ethyl ester 6. Yield 0.53 g (66%); R_f 0.13; δ_H ($CDCl_3$) 4.16 (q, 2H, *J* 7.7 Hz, OCH_2CH_3), 3.99 (dd, 1H, $J_{3,4}$ 3.8 Hz, $J_{4,5}$ 6.9 Hz, H-4), 3.64 (m, 1H, H^a-5′), 3.23–3.55 (m, 5H, H^{eq}-2, H^{eq}-6, H^b-5′b, OCH_2CH_2), 3.03 (m, 2H, H^{ax}-2, H^{ax}-6), 2.75 (m, 1H, H-3), 2.20 (m, 1H, H-5), 1.44 [s, 9H, $C(CH_3)_3$], 1.27 (t, 3H, *J* 7.7 Hz, OCH_2CH_3), 0.91 (t, 2H, *J* 8.7 Hz, CH_2CH_2TMS), 0.00 [s, 9H, $Si(CH_3)_3$]; δ_C ($CDCl_3$) 176.0 (CO_2Et), 154.8 (N*C*O′Bu), 79.9 [O*C*$(CH_3)_3$], 69.8, 2 × 68.8, 61.1 (C-4, -5′, O*C*H_2CH_3, O*C*H_2CH_2), 44.1, 42.8, 42.2, 40.3 (C-2, -3, -5, -6), 28.5 [C(*C*$H_3)_3$], 18.3, 14.3 (CH_2CH_3, CH_2CH_2TMS), −1.2 [$Si(CH_3)_3$]; *m*/*z* (ES) 426.2261 (M + Na^+).

(3,4-*trans*,4,5-*trans*)-*N*-*tert*-Butoxycarbonyl-4-hydroxy-5-{[2-(trimethylsilyl)ethoxy]methyl}piperidine-3-carboxylic acid ethyl ester 7. The protected ester **6** (0.45 g, 1.1 mmol) was added to a solution of freshly prepared LDA (2.2 mmol) in THF (15 mL) and the mixture was stirred for 1 h at 0 °C. The reaction was stopped by adding saturated aq. $KHSO_4$ (20 mL), and the mixture was worked up by extraction with EtOAc (3 × 20 mL). The combined organic phases were washed with brine (1 × 50 mL), dried over $MgSO_4$, and evaporated to give a clear yellow oil. The crude product was purified on a silica gel column with 14% EtOAc in petroleum spirit as eluent. From the column it was possible to isolate the desired product in an yield of 180 mg (40%) together with 180 mg of starting material, leading to a recovered yield of 80%. δ_H ($CDCl_3$) 4.20 (m, 4H, H_2-5′, OCH_2CH_3), 3.85 (t, 1H, *J* 10.1 Hz, H-4), 3.51 (m, 4H, H^{eq}-2, H^{eq}-6, OCH_2CH_2), 2.73 (m, 1H, H^{ax}-2), 2.47 (m, 2H, H-3, H^{ax}-6), 1.80 (m, 1H, H-5), 1.48 [s, 9H, $C(CH_3)_3$], 1.28 (t, 3H, *J* 7.0 Hz, OCH_2CH_3), 0.92 (t, 2H, *J* 8.0 Hz, CH_2CH_2TMS), 0.00 [s, 9H, $Si(CH_3)_3$]; δ_C ($CDCl_3$) 173.3 (CO_2Et), 155.2 (N*C*O′Bu), 81.0 [O*C*$(CH_3)_3$], 74.1, 72.0, 69.7, 61.8 (C-4, -5′, O*C*H_2CH_3, O*C*H_2CH_2), 50.2, 2 × 45.1, 42.7 (C-2, -3, -5, -6), 29.1 [C(*C*$H_3)_3$], 19.0, 14.9 (CH_2CH_3, CH_2CH_2TMS), −0.6 [$Si(CH_3)_3$]; *m*/*z* (ES) 426.2282 (M + Na^+).

General procedure for the deprotection of piperidinecarboxylic acids 5–7

To a solution of TFA in CH_2Cl_2 (50%; 10 mL) at 0 °C was added a fully protected piperidinecarboxylic acid **5**–**7** (0.11 mg, 0.37 mmol). The mixture were stirred for 2 h and evaporated to dryness, the residue was re-dissolved in hydrochloric acid (5 M; 10 mL) and the mixture refluxed for 2 h. Final removal of the solvent gave the product as a white solid in a yield of 54 mg (98%).

(3,4-*cis*,4,5-*cis*)-4-Hydroxy-5-(hydroxymethyl)piperidine-3-carboxylic acid hydrochloride 8. δ_H (D_2O) 4.28 (br s, 1H, H-4), 3.44 (dd, 1H, $J_{5'a,5}$ 6.6 Hz, $J_{5'a,5'b}$ 11.4 Hz, H^a-5′), 3.31 (dd, 1 H, $J_{5,5'b}$ 7.4 Hz, H^b-5′), 3.28 (dd, 1H, $J_{2ax,2eq}$ 13.0 Hz, $J_{2eq,3}$ 4.5 Hz,

H^{eq}-2), 3.17 (d, 1H, $J_{6ax,6eq}$ 12.8 Hz, H^{eq}-6), 3.02 (t, 1H, $J_{2ax,3}$ 12.6 Hz, H^{ax}-2), 2.80 (ddd, 1H, $J_{3,4}$ 2.3 Hz, H-3), 2.67 (d, 1H, H^{ax}-6), 1.91 (m, 1H, H-5); δ_C (D_2O) 172.1 (CO_2H), 62.2 (C-4), 59.0 (C-5′), 42.7, 2 × 38.8, 37.6 (C-2, -3, -5, -6); *m*/*z* (ES) 198.0737 (M + Na^+). Calc. for $C_7H_{13}NO_4$ + Na^+: *m*/*z*, 198.0742.

(3,4-*cis*,4,5-*trans*)-4-Hydroxy-5-(hydroxymethyl)piperidine-3-carboxylic acid hydrochloride 9. δ_H (D_2O) 4.11 (dd, 1H, $J_{3,4}$ 4.2 Hz, $J_{4,5}$ 7.7 Hz, H-4), 3.71 (dd, 1H, $J_{5,5'a}$ 4.8 Hz, $J_{5'a,5'b}$ 11.5 Hz, H^a-5′), 3.62 (dd, 1 H, $J_{5,5'b}$ 5.8 Hz, H^b-5′), 3.45 (dd, 1H, $J_{2ax,2eq}$ 13.1 Hz, $J_{2eq,3}$ 5.8 Hz, H^{eq}-2), 3.37 (dd, 1H, $J_{6ax,6eq}$ 13.1 Hz, $J_{5,6eq}$ 4.6 Hz, H^{eq}-6), 3.20 (dd, 1H, $J_{2ax,3}$ 3.8 Hz, H^{ax}-2), 3.12 (m, 1H, H-3), 3.02 (dd, 1H, $J_{5,6ax}$ 7.7 Hz, H^{ax}-6), 2.13 (m, 1H, H-5); δ_C (D_2O) 173.3 (CO_2H), 64.2 (C-4), 58.6 (C-5′), 41.6, 40.6, 40,4, 37.6 (C-2, -3, -5, -6); *m*/*z* (ES) 198.0743 (M + Na^+).

(3,4-*trans*,4,5-*trans*)-4-Hydroxy-5-(hydroxymethyl)piperidine-3-carboxylic acid hydrochloride 10. δ_H (D_2O) 4.0–5.0 (m), 3.15-3.68 (m, 3H), 1.74 (m, 1H); δ_C (D_2O) 172.5 (CO_2H), 66.2 (C-4), 57.7 (C-5′), 46.1, 43.7, 42.6, 40.6 (C-2, -3, -5, -6).

General procedure for the preparation of the 4-hydroxy-3,5-bis-(hydroxymethyl)piperidine hydrochlorides 11–13

An ester **5**–**7** (0.18 g, 0.45 mmol) was dissolved in dry THF (10 mL) and $LiBH_4$ (6.9 mg, 0.31 mmol) was added. The mixture was heated to reflux for 10 min, then cooled to 0 °C before saturated $KHSO_4$ (10 mL) was slowly added. The two layers were separated and the water phase was extracted with EtOAc (2 × 10 mL). The combined organic phases were washed with brine (1 × 20 mL), dried over $MgSO_4$, and evaporated to dryness. The resulting oil was treated with a 50% solution of TFA in dichloromethane (10 mL) at 0 °C for 1 h and evaporated to dryness, giving the product as the TFA salt. To obtain the hydrochloride salt the product was dissolved in hydrochloric acid (5 mL) and the solution boiled for 10 min. Final removal of the solvent gave the product as a white solid, yield 62 mg (86%).

(3,4-*cis*,4,5-*cis*)-4-Hydroxy-3,5-bis(hydroxymethyl)piperidine hydrochloride 11. δ_H (D_2O) 3.93 (br s, 1H), 3.46 (dd, 2H), 3.31 (dd, 2H), 3.07 (dd, 2H), 2.72 (t, 2H), 1.75–1.95 (m, 2H); δ_C (D_2O) 64.4 (C-4), 59.3 (2C, C-3′, -5′), 39.3, 39.2 (2 × 2C, C-2, -3, -5, -6); *m*/*z* (ES) 162.1128 (M + H^+). Calc. for $C_7H_{15}NO_3$ + H^+: *m*/*z*, 162.1130.

(3,4-*cis*,4,5-*trans*)-4-Hydroxy-3,5-bis(hydroxymethyl)piperidine hydrochloride 12. δ_H (D_2O) 3.79 (dd, 1H, $J_{3,4}$ 3.8 Hz, $J_{4,5}$ 6.2 Hz, H-4), 3.74–3.98 (m, 4H, H_2-3′, H_2-5′), 3.16 (dd, 1 H, $J_{5,6eq}$ 4.4 Hz, $J_{6ax,6eq}$ 13.2 Hz, H^{eq}-6), 2.86–3.04 (m, 3H, H_2-2, H^{ax}-6), 2.32–2.42 (m, 2H, H-3, -5); δ_C (D_2O) 63.9 (C-4), 58.9, 58.3 (C-3′, -5′), 2 × 40.9 (C-2, -6), 38.3, 35.9 (C-3, -5); *m*/*z* (ES) 162.1123 (M + H^+); 184.0948 (M + Na^+). Calc. for $C_7H_{15}NO_3$ + Na^+: *m*/*z*, 184.0950.

(3,4-*trans*,4,5-*trans*)-4-Hydroxy-3,5-bis(hydroxymethyl)piperidine hydrochloride 13. δ_H (D_2O) 3.58 (dd, 2H, $J_{3,3'a}$ 3.6 Hz, $J_{3'a,3'b}$ 11.8 Hz, H^a-3′, H^a-5′), 3.47 (dd, 2H, $J_{3,3'b}$ 6.0 Hz, H-3′, H-5′), 3.32 (t, 1 H, $J_{3,4}$ 10.2 Hz, H-4), 3.30 (dd, 2H, $J_{2eq,3}$ 4.4 Hz, $J_{2ax,2eq}$ 13.0 Hz, H^{eq}-2, H^{eq}-6), 2.66 (t, 2H, H^{ax}-2, H^{ax}-6), 1.70 (m, 2H, H-3, -5); δ_C (D_2O) 65.1 (C-4), 58.2 (C-3′, -5′), 44.0, 41.3 (C-2, -3, -5, -6); *m*/*z* (ES) 184.0948 (M + Na^+).

(3,4-*cis*,4,5-*cis*)-3-Amino-3-*N*,4-*O*-carbonyl-4-hydroxy-5-(hydroxymethyl)piperidine hydrochloride 14†

The ester **5** (0.58 g, 1.4 mmol) was dissolved in a mixture of THF (40 mL) and LiOH (2 M; 40 mL) and the solution was stirred for 2 h. The volume was reduced to 40 mL and the remaining solution was covered with a layer of diethyl ether (40 mL). The water phase was acidified by addition of solid $KHSO_4$ and extracted with diethyl ether (3 × 25 mL). The combined ether phases were washed with brine (25 mL), dried over $MgSO_4$, and evaporated to dryness to give the corresponding acid (0.53 g, 98%). The free acid was sufficiently pure for further reaction and was used without purification.

To a solution of the free acid (0.36 g, 0.96 mmol) in *tert*-butyl alcohol (8 mL) were added triethylamine (0.13 mL, 0.96 mmol) and diphenylphosphoryl azide (0.21 mL, 0.96 mmol) and the mixture was refluxed overnight. Saturated aq. $NaHCO_3$ (20 mL) was added and the aqueous phase was extracted with diethyl ether (3 × 10 mL). The combined organic phases were washed with brine (10 mL) and dried over $MgSO_4$. The solvent was removed, and the crude product purified by flash chromatography on silica gel with diethyl ether as eluent (R_f 0.1) giving the protected carbamate **14a** as a white crystalline solid.

The protecting groups were removed by stirring of the product in a 50% solution of TFA in dichloromethane (20 mL) at 0 °C for 2 h. The solvents were removed and the product was taken up in diethyl ether (10 mL) and extracted with hydrochloric acid (2 M; 3 × 10 mL). Evaporation of the water gave a white solid (87 mg, 43% for 3 steps), δ_H (D_2O) 5.06 (dd, 1H, $J_{3,4}$ 2.6 Hz, $J_{4,5}$ 9.2 Hz, H-4), 4.34–4.41 (m, 1H, H-3), 3.69 (dd, 1 H, $J_{5,5'a}$ 7.3 Hz, $J_{5'a,5'b}$ 11.0 Hz, H^a-5′), 3.58 (dd, 1H, $J_{5,5'b}$ 7.3 Hz, H^b-5′), 3.30–3.44 (m, 2H, H^{eq}-2, H^{eq}-6), 3.17 (dd, 1H, $J_{2ax,3}$ 4.0 Hz, $J_{2ax,2eq}$ 13.9 Hz, H^{ax}-2), 2.96 (t, 1H, *J* 12.8 Hz, H^{ax}-6), 2.34–2.54 (m, 1H, H-5); δ_C (D_2O) 159.2 (NCOO), 71.4 (C-4), 58.2 (C-5′), 46.6, 39.8, 36.3, 33.3 (C-2, -3, -5, -6); *m*/*z* (ES) 173.0876 (M + H^+); 195.0721 (M + Na^+). Calc. for $C_7H_{12}N_2O_3$ + H^+: *m*/*z*, 173.0926; for $C_7H_{12}N_2O_3$ + Na^+: *m*/*z*, 195.0746.

(3,4-*cis*,4,5-*trans*)-3-Amino-3-*N*,4-*O*-carbonyl-4-hydroxy-5-(hydroxymethyl)piperidine hydrochloride 15† and (3,4-*trans*,4,5-*trans*)-3-amino-3-*N*,4-*O*-carbonyl-4-hydroxy-5-(hydroxymethyl)-piperidine hydrochloride 16†

The ester **6** (3.88 g, 9.6 mmol) was epimerized by the procedure described for compound **7** to give a 1 : 1 mixture of **6** and **7**, and without further purification the procedure for the preparation of **14** was followed until the purification step. The two isomeric products (**15a** and **16a**) were separated using flash chromatography on silica gel with EtOAc–pentane (1 : 2) as eluent. R_f **16a** 0.33, R_f **15a** 0.18. Both products were found to be white crystalline compounds.

The two products were deprotected using a mixture of dichloromethane (40 mL) and TFA (20 mL) at 0 °C for 2 h. Evaporation of the solvents followed by addition of diethyl ether (50 mL) and extraction with hydrochloric acid (2 M; 3 × 20 mL) and final removal of the water gave the two products in an overall yield of 68% for the four steps. **15**: Yield 0.63 g (31% for 4 steps) of a white powder, δ_H (D_2O) 4.55 (together with DHO signal) (H-4), 4.17–4.27 (m, 1H, H-3), 3.57 (dd, 1 H, $J_{5,5'a}$ 4.0 Hz, $J_{5'a,5'b}$ 11.8 Hz, H^a-5′), 3.48 (dd, 1H, $J_{5,5'b}$ 5.4 Hz, H^b-5′), 3.40 (dd, 1H, $J_{2eq,3}$ 2.2 Hz, $J_{2ax,2eq}$ 14.0 Hz, H^{eq}-2), 3.28 (ddd, 1H, $J_{xx,6eq}$ 1.4 Hz, $J_{5,6eq}$ 4.4 Hz, $J_{6ax,6eq}$ 13.2 Hz, H^{eq}-6), 3.12 (dd, 1H, $J_{2ax,3}$ 4.0 Hz, H^{ax}-2), 2.73 (t, 1H, *J* 12.8 Hz, H^{ax}-6), 1.95-2.15 (m, 1H, H-5); δ_C (D_2O) 159.6 (NCOO), 71.8 (C-4), 58.7 (C-5′), 47.9, 41.2, 41,0, 36.0 (C-2, -3, -5, -6); *m*/*z* (ES) 195.0742 (M + Na^+). Calc. for $C_7H_{12}N_2O_3$ + Na^+: *m*/*z*, 195.0746. **16**: Yield 0.74 g (37% for 4 steps) of a white powder, δ_H (D_2O) 4.03 (t, 1H, *J* 11.4 Hz, H-4), 3.48–3.70 (m, 4H, H^{eq}-2, H-3, H^b-5′), 3.41 (dd, 1 H, $J_{5,6eq}$ 4.4 Hz, $J_{6ax,6eq}$ 13.6 Hz, H^{eq}-6), 3.03 (t, 1H, $J_{2ax,2eq}$ 11.0 Hz, H^{ax}-2), 2.85 (dd, 1H, $J_{6ax,6eq}$ 13.2 Hz, $J_{5,6eq}$ 11.7 Hz, H^{ax}-6), 2.27–2.40 (m, 1H, H-5); δ_C (D_2O) 160.4 (NCOO), 78.8 (C-4), 57.7, 53.6 (C-3, -5′), 44.7, 43.7, 38.1 (C-2, -5, -6); *m*/*z* (ES) 173.0937 (M + H^+). Calc. for $C_7H_{12}N_2O_3$ + H^+: *m*/*z*, 173.0926.

† The IUPAC name for compounds **14**, **15** and **16** is 7-(hydroxymethyl)hexahydro[1,3]oxazolo[4,5-*c*]pyridin-2(3*H*)-one.

General procedure for the hydrolysis of the carbamates 14–16 to give free amines 2, 17, 18

A carbamate **14**–**16** (0.23 g) was dissolved in aq. HCl (4 M; 8 mL) and the solution refluxed for 24 h. Evaporation of the solution yielded 0.20 g (86%) of the dihydrochloride as a highly hygroscopic solid.

(3,4-*cis*,4,5-*cis*)-3-Amino-4-hydroxy-5-(hydroxymethyl)piperidine dihydrochloride 17. δ_H (D_2O) 4.06 (m, 1H, H-4), 3.22–3.59 (m, 4H, H-3, H_2-5′, H^{eq}-6), 3.10 (dd, 1H, $J_{2eq,3}$ 5.9 Hz, $J_{2ax,2eq}$ 12.5 Hz, H^{eq}-2), 3.05 (t, 1H, H^{ax}-2), 2.79 (t, 1H, J 12.8 Hz, H^{ax}-6), 1.85–2.05 (m, 1H, H-5); δ_C (D_2O) 60.8 (C-4), 58.4 (C-5′), 46.8, 38.5, 2 × 38.0 (C-2, -3, -5, -6); *m*/*z* (ES) 147.1205 (M + H^+). Calc. for $C_6H_{14}N_2O_2$ + H^+: *m*/*z*, 147.1133.

(3,4-*cis*,4,5-*trans*)-3-Amino-4-hydroxy-5-(hydroxymethyl)piperidine dihydrochloride 18. δ_H (D_2O) 3.97 (dd, 1H $J_{3,4}$ 3.7 Hz, $J_{4,5}$ 4.4 Hz, H-4), 3.65–3.76 (m, 1H, H-3), 3.61 (dd, 1H, $J_{5,5'a}$ 5.3 Hz, $J_{5'a,5'b}$ 11.5 Hz, H^a-5′), 3.51 (dd, 1H, $J_{5,5'b}$ 4.9 Hz, H^b-5′), 3.00–3.30 (m, 4H, H_2-2, H_2-6), 2.02 (m, 1H, H-5); δ_C (D_2O) 62.7 (C-4), 59.4 (C-5′), 45.3, 40.2, 38.9, 37.7 (C-2, -3, -5, -6); *m*/*z* (ES) 147.1100 (M + H^+).

(3,4-*trans*,4,5-*trans*)-3-Amino-4-hydroxy-5-(hydroxymethyl)-piperidine dihydrochloride 19. δ_H (D_2O) 3.18–3.60 (m, 6H, H^{eq}-2, H-3, -4, H_2-5′, H^{eq}-6), 2.93 (t, 1H, J 12.4 Hz, H^{ax}-2), 2.80 (t, 1H, J 13.0 Hz, H^{ax}-6), 1.70–1.90 (m, 1H, H-5); δ_C (D_2O) 66.1 (C-4), 57.2 (C-5′), 49.4, 43.8, 42.4, 40.3 (C-2, -3, -5, -6); *m*/*z* (ES) 147.1133 (M + H^+).

(3,4-*trans*,4,5-*trans*)-5-Amino-4-hydroxy-3-(hydroxymethyl)-hexahydropyridazine 29

The epoxide **28** (100 mg, 0.36 mmol) was dissolved in trimethylsilyl azide (1 mL), and freshly distilled BF_3·Et_2O (0.1 mL) was added. The mixture was stirred for 2 h at 25 °C. The reaction was quenched by addition of MeOH (5 mL) and HCl in MeOH (1 M; 0.05 mL). After storage at 25 °C for 30 min the solution was concentrated to give the crude azidohydrin (R_f 0.65 in EtOAc).

This compound was hydrogenated in MeOH (25 mL) in the presence of Pd/C (100 mg) at a H_2 pressure of 1 atm. After consumption of the expected amount of H_2 the mixture was filtered through Celite© and concentrated to give the crude amine (108 mg).

This amine was dissolved in hydrazine hydrate (5 mL), kept at 100 °C for 18 h, and the solution concentrated to give crude **29**. This compound was bound to an ion-exchange column (Amberlite IR-120, H^+), which was washed with water and eluted with dil. NH_4OH (1 M). The basic eluate was concentrated, and then subjected to flash chromatography in 4:1 EtOH–NH_4OH (25%) to give pure **29** (37 mg, 69%), δ_H (D_2O) 3.47 (m, 2H), 3.38 (dd, 1H, J 12.5 and 5 Hz), 3.24 (dd, 1H, J 13 and 5 Hz), 3.08 (dt, 1H, J 10.5 and 5 Hz), 2.8 (m, 2H); δ_C (D_2O) 74.6 (C-4), 65.9 (C-3′), 61.8, 56.3, 54.4 (C-6) [Found: (EI) 147.1007 (M^+). Calc. for $C_5H_{13}N_3O_2$: *M*, 147.1007].

Acknowledgements

We thank the Forskningsstyrelsen for financial support through the THOR program. We also thank Mr Ib Thomsen for synthesis of compound **29**.

References and notes

1 (*a*) M. Bols, *Acc. Chem. Res.*, 1998, **31**, 1; (*b*) B. Ganem, *Acc. Chem. Res.*, 1996, **29**, 340; (*c*) T. D. Heightman and A. T. Vasella, *Angew. Chem., Int. Ed.*, 1999, **38**, 750; (*d*) C.-H. Wong, *Acc. Chem. Res.*, 1999, **32**, 376; (*e*) R. V. Stick, *Top. Curr. Chem.*, 1997, **187**, 187.
2 (*a*) S. P. Clissold and C. Edwards, *Drugs*, 1988, **35**, 214; (*b*) J. A. Balfour and D. McTavish, *Drugs*, 1993, **46**, 1025.
3 (*a*) M. von Itzstein, W.-Y. Wu, G. B. Kok, M. S. Pegg, J. C. Dyason, B. Jin, T. V. Phan, M. L. Smythe, H. F. White, S. W. Oliver, P. M. Colman, J. N. Varghese, D. M. Ryan, J. M. Woods, R. C. Bethell, V. J. Hotham, J. M. Cameron and C. R. Penn, *Nature*, 1993, **363**, 418; (*b*) C. U. Kim, W. Lew, M. A. Williams, H. Liu, L. Zhang, S. Swaminathan, N. Bischofberger, M. S. Chen, D. B. Mendel, C. Y. Tai, W. G. Laver and R. C. Stevens, *J. Am. Chem. Soc.*, 1997, **119**, 681; (*c*) D. P. Calfee and F. G. Hayden, *Drugs*, 1998, **56**, 537.
4 (*a*) T. M. Jespersen, D. Wenling, M. R. Sierks, T. Skrydstrup, I. Lundt and M. Bols, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1778; (*b*) T. M. Jespersen, M. Bols, M. R. Sierks and T. Skrydstrup, *Tetrahedron*, 1994, **50**, 13449.
5 (*a*) C.-Kuan Lee, H. Jiang, L. L. Koh and Y. Xu, *Carbohydr. Res.*, 1993, **239**, 309; (*b*) S. M. Andersen, M. Ebner, C. W. Ekhart, G. Gradnig, G. Legler, I. Lundt, A. E. Stütz, S. G. Withers and T. Wrodnigg, *Carbohydr. Res.*, 1997, **301**, 155; (*c*) N. Asano, K. Oseki, H. Kizu and K. Matsui, *J. Med. Chem.*, 1994, **37**, 3701; (*d*) X.-D. Wu, S.-K. Khim, X. Zhang, E. M. Cederstrom and P. S. Mariano, *J. Org. Chem.*, 1998, **63**, 841; (*e*) N. Asano, K. Oseki, E. Kaneko and K. Matsui, *Carbohydr. Res.*, 1994, **258**, 255; (*f*) S. Hiraizumi, U. Spohr and R. G. Spiro, *J. Biol. Chem.*, 1993, **268**, 9927; (*g*) L. A. G. M. van den Broek, D. J. Vermaas, B. M. Heskamp, C. A. A. van Boeckel, M. C. A. A. Tan, J. G. M. Bolscher, H. L. Ploegh, F. J. van Kemenade, R. E. Y. de Goede and F. Miedema, *Recl. Trav. Chim. Pays-Bas*, 1993, **112**, 82; (*h*) I. K. Khanna, F. J. Koszyk, M. A. Stealey, R. M. Weier, J. Julien, R. A. Mueller, S. N. Rao and L. Swenton, *J. Carbohydr. Chem.*, 1995, **14**, 843; (*i*) A. Kato, N. Asano, H. Kizu, K. Matsui, A. A. Watson and R. J. Nash, *J. Nat. Prod.*, 1997, **60**, 312.
6 I. Thomsen, B. V. Ernholt and M. Bols, *Tetrahedron*, 1997, **53**, 9357.
7 A. E. Stütz, in *Iminosugars as Glycosidase Inhibitors*, *Nojirimycin and Beyond*, Wiley-VCH, GmbH, Weinheim, 1999.
8 P. Bach, A. Lohse and M. Bols, *Tetrahedron Lett.*, 1999, **40**, 367.
9 M. Moreno-Mañas, M. Pérez and R. Pleixats, *Tetrahedron*, 1994, **50**, 515.
10 (*a*) P.-E. Sum and L. Weiler, *J. Chem. Soc., Chem. Commun.*, 1977, 91; (*b*) M. Giles, M. S. Hadley and T. Gallagher, *J. Chem. Soc., Chem. Commun.*, 1990, 1047.
11 (*a*) H. W. Thompson and J. K. Wong, *J. Org. Chem.*, 1985, **50**, 4270; (*b*) H. W. Thompson, E. McPherson and B. L. Lences, *J. Org. Chem.*, 1976, **41**, 2903.
12 NMR spectra of **3** run in CD_3OD showed an almost complete shift of the equilibrium towards the keto form; however, a similar spectrum of **4** showed mainly the enol tautomer.
13 S. Nishimura, M. Katagiri and Y. Kunikata, *Chem. Lett.*, 1975, 1235.
14 Reduction of esters with $LiBH_4$: H. C. Brown, S. Narasimhan and Y. M. Choi, *J. Org. Chem.*, 1982, **47**, 4702.
15 ^{13}C NMR spectra show only 4 carbon signals.
16 D. Ma and H. Sun, *Tetrahedron Lett.*, 1999, **40**, 3609.
17 The enzyme measurements were performed using the method previously described: M. Bols, R. G. Hazell and I. B. Thomsen, *Chem. Eur. J.*, 1997, **3**, 940, and ref. 6.
18 Y. Ichikawa and Y. Igarashi, *Tetrahedron Lett.*, 1995, **36**, 4585.
19 Titration gave the following pK_a-values: pK_{a1}, 6.2, pK_{a2}, 8.8.
20 S. Tomoda, Y. Matsumoto, Y. Takeuchi and Y. Nomura, *Chem. Lett.*, 1986, 1193.

Paper a908340e