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Isofagomine lactams, synthesis and enzyme inhibition

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Received 9th September 2002, Accepted 12th November 2002
First published as an Advance Article on the web 19th December 2002

The synthesis of isofagomine lactams (2-oxoisofagomines) corresponding to the biologically important hexoses is presented. The D-glucose/D-mannose analogue (3*S*,4*R*,5*R*)-3,4-dihydroxy-5-hydroxymethylpiperidin-2-one (**9**) was synthesised in 9 steps from D-arabinose, the D-galactose analogue (3*S*,4*S*,5*R*)-3,4-dihydroxy-5-hydroxymethylpiperidin-2-one (**10**) was synthesised in 11 steps from D-arabinose and the L-fucose analogue (3*R*,4*R*,5*R*)-3,4-dihydroxy-5-methylpiperidin-2-one (**11**) was synthesised in 12 steps from L-arabinose. The three lactams **9**–**11** were found to be glycosidase inhibitors with micro- to nanomolar inhibition constants. The lactam **10** showed slow onset inhibition of β-galactosidase from *A. Oryzae*. The rate constants for this process were determined to be $k_{on} = 2.55 \times 10^4$ M^{-1} s^{-1} and $k_{off} = 1.7 \times 10^{-3}$ s^{-1}. The activation energies and standard thermodynamic functions were also determined.

Introduction

The most potent glycosidase inhibitors are basic amino- or hydrazinosugars,[1] such as **1**,[2] **2**,[3] **3**[4] or **4**,[5] and can therefore become positively charged (for some new applications of glycosidase inhibitors, see ref. 6). Never the less, neutral glyconolactams, such as **5**,[2,7] have long been known to inhibit glycosidases.[8] The enzyme inhibition by these lactams, and the corresponding lactones, has been interpreted as being caused by the geometric resemblance between the inhibitor and a "flat" transition state. However recent research suggests that glycosidase inhibition by **5** and similar compounds is to a large extent caused by H-bonding to the carbonyl group.[9] Remarkably a new type of glycosidase inhibiting lactam has recently been reported.[10,11] Withers *et al.* found that the lactam **6**, an analogue of xylobiose-type 1-azasugar **7**, is almost as potent (K_i 340 nM) as **7** (K_i 130 nM) against a β-xylosidase, which is surprising since the strong binding of **7** is believed to be associated with salt bridge formation from N.[10] The affinity of **6** was explained with by tautomeric form of the amide being able to act both as an amine and as 2-hydroxy group. Ramana and Vasella have reported that the hydrazinolactam **8** is a much stronger α-mannosidase inhibitor than **4** (**8**: K_i 25 μM, **4**: K_i 3.3 mM) albeit a weaker β-glucosidase inhibitor (**8**: K_i 13 μM, **4**: K_i 0.32 μM).[11] These findings suggest that isofagomine type lactams are good glycosidase inhibitors. In the present paper we have investigated the generality of this idea by synthesising the gluco-, galacto- and L-fuco-lactams, **9**, **10** and **11**, and investigating their glycosidase inhibition (Fig. 1). A preliminary communication reporting on **10** has been made.[12]

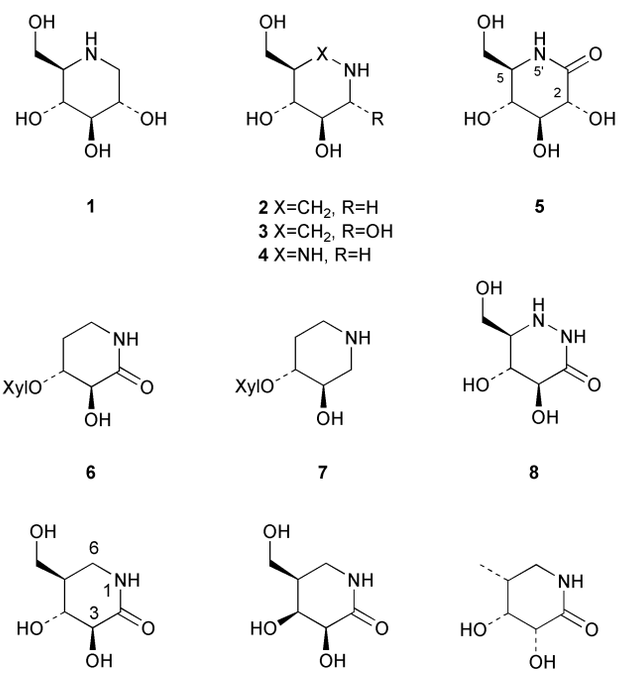


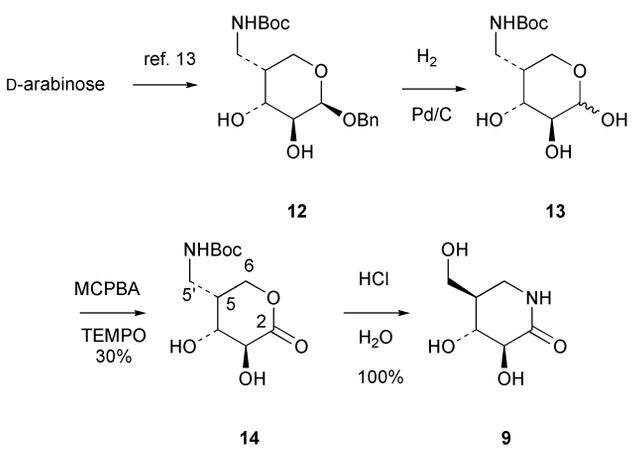
Fig. 1

Results and discussion

Synthesis

Our synthetic route to the three lactams **9**–**11** was based on our recent synthesis of the corresponding hydroxy compounds (noeuromycins, **3** and analogues).[4,13] Indeed it was observed in the synthesis of *galacto*-**3** that the TEMPO oxidation used to obtain the hemi-aminal functionality[4] occasionally caused over-oxidation and resulted in the formation of a lactam. We therefore embarked on the project to synthesise the three lactams **9**–**11** in this way.

Synthesis of the glucolactam **9** was carried out from intermediate **13**, which is made in 6 steps from D-arabinose (Scheme 1).[13] Oxidation of **13** using TEMPO and MCPBA as a cooxidant was however a tricky reaction giving side reactions.

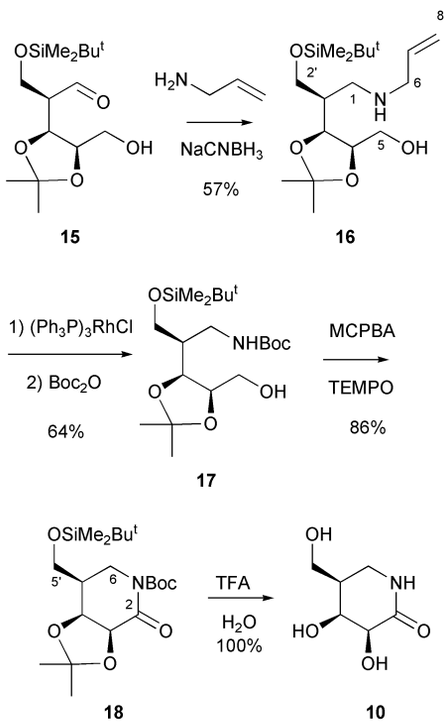


Scheme 1

DOI: 10.1039/b208784g

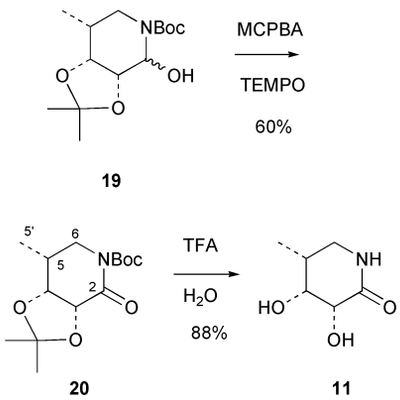
However, the lactone **14**, the product from selective 1-OH oxidation of the hemiacetal, could be isolated in 30% yield. This relatively poor result probably occurs because **13** is unprotected and oxidation may occur at several positions. Evidence for the lactone form of **13** is provided by the typical chemical shift of C6 (66.8 ppm), while the corresponding chemical shift in lactam **9** is 59.7 ppm. Treatment of **14** with hydrochloric acid removed the Boc-group and resulted in spontaneous rearrangement to the lactam **9**, which was obtained in quantitative yield.

Synthesis of the *galacto*-lactam **10** was more efficient due to the availability of the partially protected derivative **15**, which can be made from D-arabinose in 6 steps (Scheme 2).[14] Reductive amination of **15** with allylamine and $NaCNBH_3$ gave the *N*-allyl derivative **16** in 57% yield. Deallylation was carried out with $(Ph_3P)_3RhCl$ to give an amine that was immediately converted to the Boc derivative with Boc_2O giving **17** in 64% yield. Oxidation of **17** with the TEMPO–MCPBA system is much more efficient than the oxidation of **13** because there is only one functionality that can be oxidised and also because the first formed aldehyde spontaneously forms a hemiaminal that is further oxidised to lactam **18**. This product is therefore obtained in 86% yield. Deprotection of **18** with aqueous TFA gave **10** in quantitative yield.



Scheme 2

The *fuco*-lactam **11** was prepared in a similar fashion. The hemiaminal **19**, obtained from L-arabinose,[4] was oxidised with TEMPO–MCPBA to give the lactam **20** in 60% yield. Deprotection with aqueous TFA gave **11** in 88% yield.



Scheme 3

Enzyme inhibition

Gluco analogue **9** was investigated for inhibition of glucosidases and mannosidases (Table 1). The compound competitively inhibited almond β-glucosidase and snail β-mannosidase while no inhibition of α-glucosidases and α-mannosidase was observed. The compound is a poor inhibitor compared with the basic analogue **3**. The inhibition profile of **9** is not surprisingly very similar to **8** except that the latter inhibits α-mannosidase. This difference may be caused by the ability of **8** and not **9** to accept charge at the pseudo ring-oxygen position though it should be noted that **8** is quite a weak base ($pK_a < 3$).[11] Compound **9** is a slightly better inhibitor than gluconolactam **5**, and its β-mannosidase inhibition is similar to that of mannonolactam **21**. The optimized structure of **9** by PM3/MOPAC shows the compound in a slightly distorted halfchair conformation that has a C3–C2–N1–C6 angle of 21°. When this structure is superimposed on the optimised conformation of **5**[7] (Fig. 2) the N1, C2, C3, C4 and C5 atoms of **9** overlap with the C1, C2, C3, C4 and C5 atoms of **5**. The notable difference in the structures is clearly the position of C6 in **9** and N5′ in **5** (Fig. 2). However this position appears to be of limited significance for the following reason: the only difference between **8** and **9** is the replacement of NH with CH_2 in that position and **8** and **9** have essentially the same inhibitory strength. Therefore this position must be relatively unimportant. It is therefore understandable that **5** and **9** have similar biological activity since three OH groups can adopt identical positions. At the same time it is likely that N1 is involved in important H-bond interactions.

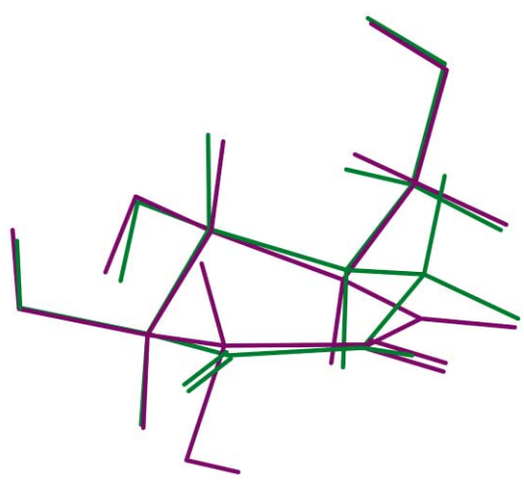
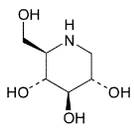
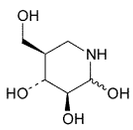
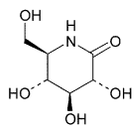
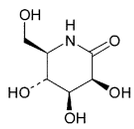
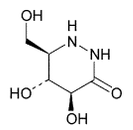
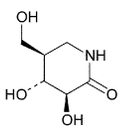


Fig. 2 Molecular model of **9** (green) superimposed on the model of **5** (pink). The model shows a high degree of overlap of all atoms except C6 in **9** and N5′ in **5**. Models were prepared in Chem3D Ultra 6.0 and is optimised for low energy conformations using MOPAC/PM3. From the optimisation of 10–20 different models a number of local minima were obtained for each structure. For each model the minimum with the lowest energy is shown. The model of **5** is consistent with the published X-ray structure.[7]

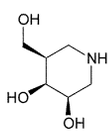
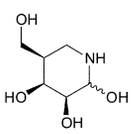
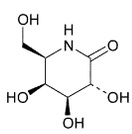
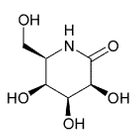
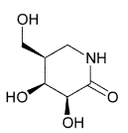
The lactam **10** was found to be a remarkably potent inhibitor of β-galactosidase from *A. Oryzae* (Table 2). When Michaelis–Menten steady state kinetics was used the inhibition was found to be competitive with K_i's of about 18 nM being obtained. However since slow onset inhibition was observed in this case it was more appropriate to use a non-steady state method of determining the kinetic constants. We therefore used the β-method[18] to determine the slow binding followed the simple

Table 1 K_i values (in μM) of **9** and analogous compounds towards glucosidases and related glycosidases[a]

Enzyme	 **1**	 **3**	 **5**	 **21**	 **8**	 **9**
β-Glucosidase (Almonds)	47[b]	0.069	51[b,c]	0.51[b,c]	13[b]	29
α-Glucosidase (Yeast)	13[b]	0.022	>1000[b,d]	>1000[b,d]	>1000[b]	>250
Isomaltase (Yeast)	11[b]	0.025	—	—	—	>250
β-Mannosidase (Almonds)	—	—	>1000[b]	9[b]	6[b,e]	9
α-Mannosidase (Jack bean)	—	—	>1000[b,e]	68[b,e]	25[b,e]	>250

[a] — means that the value has not been measured or reported. All data were measured at pH 6.8 and 25 °C except for β-mannosidase, which was measured at pH 4.0 and 25 °C, unless otherwise noted. The data for **1** is from ref. 15, the data for **3** is from ref. 4, the data for **5** and **21** is from ref. 7 while the data for **8** is from ref. 11. [b] 37 °C. [c] pH 5.0. [d] pH 6.3. [e] pH 4.5.

Table 2 K_i values (in μM) of **10** and analogous compounds towards galactosidases and related glycosidases[a]

Enzyme	 **22**	 **23**	 **24**	 **25**	 **10**
β-Galactosidase (*A. oryzae*)	0.004	0.035	—	—	0.067[b]
β-Galactosidase (*S. fragilis*)	0.33	0.091	—	—	9
β-Galactosidase (*A. Niger*)	—	—	4.5[c,d]	15[c,d]	—
α-Galactosidase (Coffee bean)	50	0.742	>1000[c,d]	>1000[c,d]	5.2
β-Glucosidase (Almond)	0.08	—	85[c,e]	120[c,e]	3

[a] — means that the value has not been measured or reported. All data were measured at pH 6.8 and 25 °C unless otherwise noted. The data for **22** are from ref. 3, the data for **23** are from ref. 4, the data for **24** and **25** are from ref. 7. [b] Slow onset inhibition. [c] 37 °C. [d] pH 4.0. [e] pH 5.0.

model *i.e.* the slow step is the binding of the inhibitor. We then used the GEPASI method[19,22–24] to determine the rate of binding and dissociation of the inhibitor giving a k_{on} of 2.55×10^4 M^{-1} s^{-1}, a k_{off} of 1.7×10^{-3} s^{-1} and consequently a K_i of 67 nM (Table 2). This is therefore a case where classical enzyme kinetics does not give an accurate K_i value.

We also determined the thermodynamic functions of binding by determining k_{on} and k_{off} at 5, 15 and 35 °C as well. The Arrhenius plots of the k_{on} and k_{off} are shown as Figs. 3 and 4,

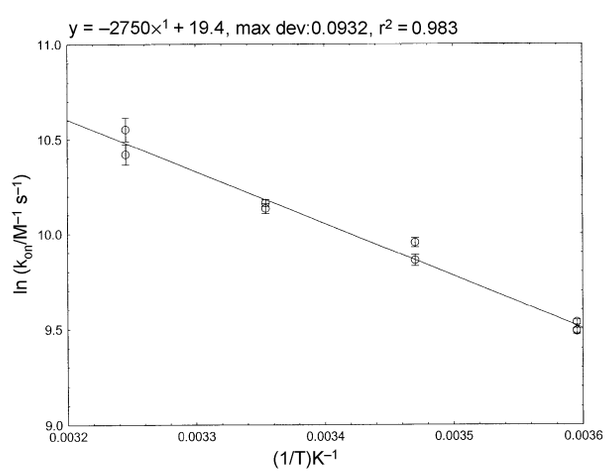


Fig. 3 Arrhenius plot showing ln (k_{on}) *versus* $1/T$, where k_{on} is the rate constant for the binding of **10** to β-galactosidase from *A. Oryzae* at pH 6.8.

Table 3 Thermodynamic functions (P) for the binding of **10** to β-galactosidase from *A. Oryzae* at pH 6.8. The values have been calculated from the slope and intercept of the Arrhenius plots shown in Figs. 3 and 4

Enzyme	ΔP°	$\Delta P^\ddagger_{on}$	$\Delta P^\ddagger_{off}$
G/kJ mol^{-1}	−40.5	47.8	88.3
H/kJ mol^{-1}	−22.6	20.4	43.0
S/J k^{-1} mol^{-1}	59.9	−91.9	−151.8

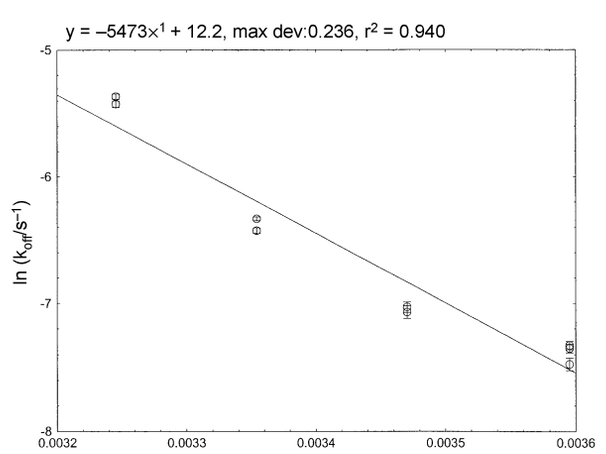
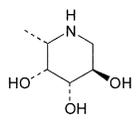
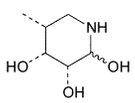
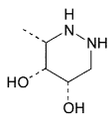
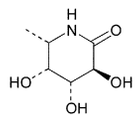
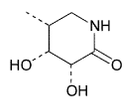


Fig. 4 Arrhenius plot showing ln (k_{off}) *versus* $1/T$, where k_{off} is the rate constant for the dissociation of **10** from β-galactosidase from *A. Oryzae* at pH 6.8.

respectively. From the plots $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were calculated and hence $\Delta G^\ddagger$, ΔG°, ΔH° and ΔS° (Table 3). The standard thermodynamic functions are, unlike other slow binding inhibitors we have studied,[20] relatively predictable: H° of binding is negative and contributes considerably to the driving force of binding, and S° is not excessively large. This means that the binding is caused to a considerable extend by increased bond formation. On the other hand this inhibitor resembles the β-glucosidase inhibitors we have previously studied[19–21] in one respect: the standard entropy increases on binding because $\Delta S^\ddagger_{off}$ is large

Table 4 K_i values (in μM) of **11** and analogous compounds towards fucosidases[a]

Enzyme	 **26**	 **27**	 **28**	 **29**	 **11**
α-Fucosidase (Human placenta)	0.0013	0.0032	0.63	400[b]	18
α-Fucosidase (Bovine epididymis)	—	—	—	—	64
α-Fucosidase (Bovine kidney)	0.029	0.0047	0.81	300	26

[a] — means that the value has not been measured or reported. All data were measured at pH 6.8 and 25 °C unless otherwise noted. The data for **26** are from ref. 1, the data for **27** are from ref. 4, the data for **28** are from ref. 16 and the data for **29** from ref. 17. [b] Obtained on fucosidase from human liver.

and negative. This means that more order is created on release of the inhibitor, which may appear contradictory. This can however be explained by the binding of several water molecules to the enzyme, when the inhibitor is released.

Compound **10** is compared with related galactosidase inhibitors in Table 2. It is surpassed in potency by the corresponding charged inhibitors **22** and **23**, but is nevertheless strong. The lactam **10** is much less potent against the three other enzymes, but it is still a good inhibitor with K_i in the low micromolar range. Interestingly **22** and **23** are also slightly more potent than **10** against these enzymes as well with the exception of the poor inhibition of α-galactosidase by **22**. Compound **10** is more potent than the classical "nojirimycin-like" glycono-lactams **24** and **25**.

It is particularly remarkable that **10** is so potent against β-galactosidase from *A. Oryzae*, because this enzyme appears to be the least sensitive to the presence of a 2-hydroxy group in the inhibitor. In fact noeuromycin **23**, which has a 2-hydroxy group is weaker than its 2-deoxy analogue and also weaker than **10**. It therefore appears not to make sense that the lactam carbonyl group should mimic a hydroxy group in this case. In contrast β-galactosidase from *S. Fragilis*, which in fact appears to be sensitive to the presence of a 2-hydroxy group since **23** is more potent than **22** against this enzyme, is relatively weakly inhibited by **10**. However these observations may be explained by **10** having the 2-carbonyl group better positioned for H-bonding to some enzymes than does **23** (only α-anomer present) and *vice versa*, depending on the geometry of the transition state in a particular enzyme. That an iminol tautomer of **10** should be the binding species to the *Aspergillus Oryzae* enzyme does not appear likely, because if so **23** would be expected to be a more potent inhibitor than **10**, since it is less costly to twist **23** from a chair into a half-chair conformation (~ 20 kJ mol^{-1})[25] than to convert an amide into an iminol (~ 45 kJ mol^{-1}).[10] It should also be noted that the halfchair geometry of these lactams may cause the carbonyl group to be well positioned for hydrogen bonding.

The L-*fuco* lactam **11** was found to be a relatively poor fucosidase inhibitor (Table 4) compared to the strong inhibitors **26**–**28**. It is however clearly stronger than the fuconolactam **29**, which is remarkably weak.

In summary this work shows that lactams analogous of isofagomines are glycosidase inhibitors of intermediate potency and in unique cases may be very potent. Compared to glyconolactam these new lactams are generally stronger inhibitors.

Experimental

General

^{1}H- (200 or 400 MHz) and ^{13}C-NMR (50 MHz) spectra were recorded on a Varian Gemini 200 or a Varian 400 spectrometer using the signal of the solvent as reference. Chemical shifts are expressed in δ values (ppm). Chemical shift assignment marked with * or ** may be reversed. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Optical rotations are given in units of 10^{-1} deg cm^2 g^{-1}. The mass spectra and the high-resolution mass spectra were measured with a Micromass LC-TOF. For K_i determination a Milton Roy Genesys 5 spectrophotometer was used. Unless otherwise stated, all reactions were performed under normal atmosphere and pressure. THF was distilled prior to use from sodium and benzophenone, and CH_2Cl_2 was distilled with CaH_2. The acetone used was HPLC grade, and DMF was dried using 4 Å molecular sieves. Other solvents and reagents from stock or purchased were used as received. When dry glassware was necessary it was heated in an oven at a temperature above 125 °C for at least 6 h, then quickly assembled and cooled under a stream of dry inert gas. Flash column chromatography was performed on silica gel (Merck silica gel 60, 230–400 mesh). TLC plates were viewed under an ultraviolet lamp or treated with one of the reagents listed here;: i) 3 g of $KMnO_4$, 20 g of K_2CO_3, 5 ml of NaOH (5%, aq.) and 300 ml of H_2O; ii) 10 g of Ce(IV) sulfate, 15 g of $(NH_4)_2MoO_4$ in 1 L 10% H_2SO_4. Ion-exchange chromatography was performed with Amberlite IR-120, H^+ and eluted with 2.5% NH_3(aq.). Rotary evaporation was performed under reduced pressure (min. 25 mbar) and a maximum temperature of 40 °C.

Measurement of glycosidase inhibition

***K_i* determination.** Each glycosidase assay was performed by preparing fourteen 2 ml samples in cuvettes containing 1 ml sodium phosphate buffer (0.1 M) of pH 6.8 or acetate buffer of pH 4.0, along with 0.05 ml to 0.70 ml of different substrates. The concentration of the substrate was in the range of $0.25K_m$ to $5K_m$. The substrates used were 2-nitrophenyl-β-D-galactopyranoside, 4-nitrophenyl-α-D-galactopyranoside, 4-nitrophenyl-β-D-glucopyranoside, 4-nitrophenyl-α-D-glucopyranoside or 4-nitrophenyl-β-D-mannopyranoside. Also added was 0.025–0.1 ml of a solution of either the inhibitor or water, and finally each cuvette was filled up to a total volume of 1.9 ml with distilled water. Seven of the samples contained the inhibitor at a fixed concentration but with varying concentrations of nitrophenyl glycoside. The other seven samples contained no inhibitor, but also varying concentrations of nitrophenyl glycoside. Finally the reaction was started by adding 0.1 ml of a diluted enzyme solution. The formation of 4- or 2-nitrophenol was monitored for 2 min at 25 °C by measurement of the absorbance at 400 nm. In the case of the β-mannosidase assay the velocity of substrate hydrolysis was measured by quenching a 200 μL solution with 1800 μL borate buffer (1.0 M, pH 9) every 30 seconds over 3 minutes and then measuring the absorbance at 400 nm. Initial velocities for each reaction were calculated from the slopes and used to construct two Hanes plots ([S]/*v vs.* [S]), one with and one without the inhibitor. From the two Michaelis–Menten constants, K_m and K_m', thus obtained, the inhibition constant, K_i, was calculated.

Slow onset inhibition of β-galactosidase from *A. Oryzae* by **10** was carried out at pH 6.8 in 0.1 M phosphate buffer and otherwise performed as described in refs. 18, 19 and 21.

(3*S*,4*R*,5*R*)-5-(*tert*-Butoxycarbonylaminomethyl)-3,4-dihydroxy-3,4,5,6-tetrahydro-2*H*-pyran-2-one (14).

A solution of **12**[13] (74 mg, 0.21 mmol) in dry EtOH (100 ml) was hydrogenated over Pd/C (10%) under normal pressure at room temperature for about 16 h. The catalyst was removed by filtration and the solvent evaporated to obtain **13** (55 mg). Now TEMPO (4 mg, 0.026 mmol) and $Bu_4N^+Br^-$ (4 mg, 0.012 mmol) in CH_2Cl_2 (9 ml) were added. Then MCPBA (58–85%, 80 mg, 0.27–0.39 mmol) was added in portions until the starting material disappeared. The reaction mixture was concentrated *in vacuo*. The residue was purified by chromatography ($CHCl_3$–MeOH 20 : 1) to give the desired product, which was contaminated by some MCPBA. This product was further purified by chromatography (pentane–ethyl acetate 2 : 1) to furnish **14** (16 mg, 30%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.12 (t, 1H, *J* 6.0 Hz, NH), 4.32 (t, 1H, $J_{5,6ax} = J_{6ax,6eq}$ 11.0 Hz, H-6ax), 4.31 (d, 1H, $J_{3,4}$ 5.8 Hz, H-3), 4.20 (dd, 1H, $J_{5,6eq}$ 4.6 Hz, H-6eq), 4.03 (t, 1H, $J_{4,5}$ 5.8 Hz, H-4), 3.60–3.35 (m, 2H, OH, H-5′a), 3.10 (dt, 1H, $J_{NH,5'b} = J_{5,5b'}$ 4.6, $J_{5'a,5'b}$ 14.4 Hz, H-5′b), 2.32 (m, 1H, H-5), 1.43 [s, 9H, $(CH_3)_3C$]. ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.6 (C-2), 155.6 (C=O), 80.7 [$(CH_3)_3C$], 72.9 (C-3), 70.2 (C-4), 66.8 (C-6), 40.8 (C-5′), 37.2 (C-5), 28.5 [$(CH_3)_3C$]. HRMS (ES): calcd for $C_{11}H_{19}NO_6$ + Na^+ 284.1110, found 284.1110.

(3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-hydroxymethylpiperidin-2-one (9)

Pyranone **14** (16 mg, 0.061 mmol) was treated with aqueous HCl (1.2 M, 1 ml) for 5 min to give **9** (10 mg, quant.) as a colourless oil. ^{1}H NMR (200 MHz, D_2O): δ 3.99 (d, 1H, $J_{3,4}$ 9.3 Hz, H-3), 3.91–3.60 (m, 3H, H-4, H-5′a, H-5′b), 3.35 (dd, 1H, $J_{5,6eq}$ 5.4, $J_{6eq,6ax}$ 12.0 Hz, H-6eq), 3.12 (t, 1H, $J_{5,6ax}$ 12.0 Hz, H-6ax), 2.13 (m, 1 H, H-5). ^{13}C NMR (50 MHz, D_2O): δ 173.1 (C-2), 70.8 (C-3), 69.8 (C-4), 59.7 (C-5′), 40.8 (C-6), 38.7 (C-5). HRMS (ES): calcd for $C_6H_{11}NO_4$ + Na^+ 184.0586, found 184.0586.

1-Allylamino-2-(*tert*-butyldimethylsiloxymethyl)-1,2-dideoxy-3,4-*O*-isopropylidene-D-ribitol (16).

Compound **15** (46 mg, 0.14 mmol) was dissolved in methanol (6 ml). A solution of allylamine (17 mg, 0.29 mmol) was neutralised to pH 6 using glacial acetic acid, and this solution was added to the solution of aldehyde **15**. The reaction was then treated with $NaCNBH_3$ (2 mg, 0.03 mmol). The transformation was complete within 2 h at room temperature. The solvent was removed, and the reaction was dissolved in chloroform (20 ml), the mixture was washed with aq. 10% Na_2CO_3(15 ml). The aqueous phase was extracted with chloroform (2 × 15 ml), the combined organic phases were dried over $MgSO_4$, and the solvent was removed. The residue was purified by column chromatography (methanol–chloroform 1 : 20) to furnish **16** (28 mg, 57%) as a clear oil. $[\alpha]_D^{20} = -18.3$, (*c* 0.8, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.83 (ddt, 1H, $J_{6a,7} = J_{6b,7}$ 6.0 Hz, $J_{7,8a(cis)}$ 10.2 Hz, $J_{7,8b(trans)}$ 17.2 Hz, H-7), 5.12 (dq, 1H, $J_{6a,8b} = J_{6b,8b} = J_{8a,8b}$ 1.8 Hz, H-8b), 5.04 (dq, 1H, $J_{6a,8a} = J_{6b,8a}$ 1.8 Hz, H-8a), 4.12 (q, 1H, $J_{3,4} = J_{4,5a} = J_{4,5b}$ 5.8 Hz, H-4), 4.04 (dd, 1H, $J_{2,3}$ 8.2 Hz, H-3), 3.65–3.40 (m, 4H, H-5a, H-5b, H-2′a, H-2′b), 3.18 (dt, 2H, H-6a, H-6b), 2.80 (dd, 1H, $J_{1a,1b}$ 12.0 Hz, $J_{1a,2}$ 5,2 Hz, H-1a), 2.51 (dd, 1H, $J_{1b,2}$ 5.2 Hz, H-1b), 2.64–2.46 (1H, OH), 2.08–1.92 (m, 1H, H-2), 1.38/1.25 [s, 6H, $C(CH_3)_2$], 0.90 (s, 9H, *t*-Bu), 0.04 [s, 6H, $Si(CH_3)_2$]. ^{13}C NMR (50 Hz, $CDCl_3$): δ 136.7 (C-7), 116.1 (C-8), 107.5 [$C(CH_3)_2$], 78.4*(C-3), 78.0* (C-4), 64.4 (C-2′), 61.5 (C-5), 52.7 (C-6), 48.9 (C-1), 40.2 (C-2), 28.4** (CH_3C), 26.0 ($C(CH_3)_3$), 25.8** (CH_3C), 18.2 ($C(CH_3)_3$, MeSi signals are below zero. HRMS (ES): *m*/*z* 360.2566 (M + H^+) calcd for $C_{18}H_{37}O_4NSi$ + H^+ 360.2570.

1-(*tert*-Butoxycarbonylamino)-2-(*tert*-butyldimethylsiloxymethyl)-1,2-dideoxy-3,4-*O*-isopropylidene-D-ribitol (17)

Compound **16** (28 mg, 0.08 mmol) was dissolved in a mixture of acetonitrile and water (86 : 16 v/v, 6 ml) and brought to reflux. A suspension of Wilkinson's catalyst (74 mg, 0.08 mmol) in acetonitrile–water (86 : 16 v/v, 5 ml) was added. The reaction was then allowed to reflux vigorously for 3 h. Then the mixture was cooled to room temperature. The reaction was treated with $NaHCO_3$ (200 mg, 2.38 mmol), and then a solution of $(Boc)_2O$ (124 mg, 0.57 mmol) in CH_2Cl_2 (6 ml) was added. The reaction was complete after 3 h. The solvent was removed, the residue was dissolved in water (15 ml), extracted with chloroform (3 × 20ml) and the combined organic phases were dried with $MgSO_4$. The solvent was removed and the residue chromatographed (chloroform–ethyl acetate 6 : 1) to give compound **17** (21 mg, 64%) as a colourless oil. $[\alpha]_D^{20} = -11.9$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.02 (br s, 1H, NH), 4.12 (dt, 1H, $J_{3,4} = J_{4,5a}$ 5.3 Hz, $J_{4,5b}$ 7.2 Hz, H-4), 4.10 (dd, 1H, $J_{2,3}$ 7.2 Hz, H-3), 3.64 (dd, 1H, $J_{2,2'a}$ 5.0 Hz, $J_{2'a,2'b}$ 10.5 Hz, H-2′a), 3.62 (dd, 1H, $J_{5a,5b}$ 10.5 Hz, H-5), 3.50 (dd, 2H, $J_{2,2'b}$ 7.2 Hz, H-5b, H-2′b), 3.28 (dd, 1H, $J_{1a,2}$ 5.5 Hz, $J_{1a,1b}$ 13.8 Hz, H-1a), 3.16 (dd, 1H, $J_{1a,2}$ 6.2 Hz, H-1b), 2.33 (1H, OH), 2.06–1.91 (m, 1H, H-2), 1.35 (s, 9H, *t*-BuO), 1.35/1.28 (s, 6H, 2 × CH_3), 0.80 (s, 9H, *t*-BuSi), 0.04 [s, 6H, $Si(CH_3)_2$]. ^{13}C NMR (50 MHz, $CDCl_3$): δ 156.2 (–N*C*OO–), 107.6 (O–*C*–O), 79.2 (–COO*C*$(CH_3)_3$), 78.1* (C-3), 77.9* (C-4), 64.1 (C-2′), 61.9 (C-5), 41.1** (C-1), 40.2** (C-2), 28.6 (–COOC($CH_3)_3$), 28.5*** (MeC), 26.1 [–$Si(CH_3)_2C(CH_3)_3$], 25.8*** (MeC), 18.2 [$Si(CH_3)_2C(CH_3)_3$]. HRMS (ES): *m*/*z* 442.2600 (M + Na^+), calcd for $C_{20}H_{41}O_6NSi$ + Na^+ 442.2600.

(3*S*,4*S*,5*R*)-1-(*tert*-Butoxycarbonyl)-5-(*tert*-butyldimethylsiloxymethyl)-3,4-dihydroxy-3,4-*O*-isopropylidenepiperidin-2-one (18)

To a mixture of **17** (38 mg, 0.09 mmol), Bu_4NBr (10 mg, 0.03 mmol) and 0.66 eq. of TEMPO (10 mg, 0.06 mmol) in CH_2Cl_2 (5 ml) was slowly added MCPBA (57–72%, 55 mg, 0.18–0.23 mmol) until the starting material disappeared. The mixture was washed with saturated $NaHCO_3$ (3 × 10 ml) and the organic phase was dried with $MgSO_4$, filtered and concentrated. The residue was chromatographed (ethyl acetate–pentane 1 : 5) to give the product **18** as a colourless oil (32 mg, 86%). ^{1}H NMR(200 MHz, $CDCl_3$): δ 4.46 (s, 2H, H-3, H-4), 3.89 (dd, 1H, $J_{5,6eq}$ 3.6 Hz, $J_{6ax,6eq}$ 12.8 Hz, H-6eq), 3.67 (dd, 1H, $J_{5,5'a}$ 6.3 Hz, $J_{5'a,5'b}$ 9.9 Hz, H-7a), 3.51 (dd, 1H, $J_{5,5'b}$ 7.8 Hz, H-7b), 3.31 (dd, 1H, $J_{5,6ax}$ 11.2 Hz, H-6ax), 2.14–2.00 (m, 1H, H-5), 1.46 (s, 9H, *t*-BuO), 1.40/1.28 (s, 6H, 2 × CH_3), 0.83 (s, 9H, *t*-BuSi), 0.04 [s, 6H, $Si(CH_3)_2$]. ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.7 (C-2), 151.9 [*C*OOC$(CH_3)_3$], 110.7 (O–C–O), 83.7 [COO*C*-$(CH_3)_3$], 76.6* (C-3), 73.4* (C-4), 61.2 (C-5′), 41.9 (C-6), 40.2 (C-5), 28.1 [COOC($CH_3)_3$], 26.4** (CCH_3), 26.0** (CCH_3), 26.1 [$Si(CH_3)_2C(CH_3)_3$], 18.4 [$Si(CH_3)_2C(CH_3)_3$]. HRMS (ES): *m*/*z* 438.2297 (M + Na^+) calcd for $C_{20}H_{37}O_6NSi$ + Na^+ 438.2288.

(3*S*,4*S*,5*R*)-3,4-Dihydroxy-5-hydroxymethylpiperidin-2-one (10)

A solution of **18** (18 mg, 0.04 mmol) in CF_3COOH–H_2O 5 : 1 was stirred for 5 min at room temperature. Concentration of the mixture gave **10**[12] (6 mg) in essentially quantitative yield.

(3*R*,4*R*,5*R*)-1-(*tert*-Butyloxycarbonyl)-3,4-*O*-isopropylidene-3,4-dihydroxy-5-methylpiperidin-2-one (20)

To a solution of **19** (20 mg, 0.070 mmol), TEMPO (5 mg, 0.032 mmol) and $Bu_4N^+Br^-$ (4 mg) in CH_2Cl_2 was added MCPBA (58–85%, 40 mg, 0.13–0.20 mmol) at room temperature. The solution obtained was stirred for 18 h at the same temperature. After the reaction, the solution was washed with a saturated aqueous solution of $NaHCO_3$ and concentrated *in vacuo*. The residue was purified by chromatography (pentane–ethyl acetate 3 : 1) to give **20** (12 mg, 60%) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.45 (d, 1H, $J_{3,4}$ 6.8 Hz, H-3), 4.34 (ddd, 1H,

$J_{4,5}$ 2.8, $J_{4,6eq}$ 1.5 Hz, H-4), 3.68 (ddd, 1H, $J_{5,6eq}$3.0, $J_{6eq,6ax}$ 12.8 Hz, H-6eq), 3.34 (dd, 1H, $J_{5,6ax}$ 11.0, H-6ax), 1.97 (m, 1H, H-5), 1.47 [s, 9H, $C(CH_3)_3$], 1.40/1.30 (s, 6H, 2 × CCH_3), 1.02 (d, 3H, $J_{5,5'}$ 6.8 Hz, CH_3-5). ^{13}C NMR (50 MHz, $CDCl_3$): C=O signals two weak to be measured, δ 110.5 [$C(CH_3)_2$], 83.7 [$C(CH_3)_3$], 77.5 (C-3), 76.6 (C-4), 45.5 (C-6), 33.1 (C-5), 28.2 [$C(CH_3)_3$], 26.3/24.6 [$C(CH_3)_2$], 14.0 (CH_3-5). HRMS (ES): calcd for $C_{14}H_{23}NO_5 + Na^+$ 308.1474, found 308.1470.

(3*R*,4*R*,5*R*)-3,4-Dihydroxy-5-methylpiperidin-2-one (11)

Compound **20** (11 mg, 0.039 mmol) was dissolved in CF_3COOH–H_2O (1 : 1, 1 ml). The solution obtained was stirred for 10 min and concentrated *in vacuo* to provide **11** (5 mg, 88%) as an oil. ^{1}H NMR (200 MHz, D_2O): δ 4.17 (d, 1H, $J_{3,4}$ 3.0 Hz, H-3), 4.00 (dd, 1H, $J_{4,5}$ 1.5 Hz, H-4), 3.21 (dd, 1H, $J_{5,6eq}$ 6.6, $J_{6eq,6ax}$ 12.4 Hz, H-6eq), 3.34 (dd, 1H, $J_{5,6ax}$ 12.4, H-6ax), 2.20 (m, 1H, H-5), 1.02 (d, 3H, $J_{5,5'}$ 7.0 Hz, CH_3-5). ^{13}C NMR (50 MHz, D_2O): δ 70.9 (C-3), 69.4 (C-4), 43.1 (C-6), 30.3 (C-5), 13.7 (CH_3-5). C-2 signal was to weak to be measured. HRMS (ES): calcd for $C_6H_{11}NO_3 + Na^+$ 168.0637, found 168.0634.

References

1 A. E. Stütz, *Iminosugars as glycosidase inhibitors: nojirimycin and beyond*, Wiley–VCH, Weinheim, 1999).
2 Y. Miyake and M. Ebata, *Agric. Biol. Chem.*, 1988, **52**, 661–666.
3 Y. Ichikawa and Y. Igarashi, *Tetrahedron Lett.*, 1995, **36**, 4585–4586.
4 H. Liu, X. Liang, H. Søhoel, A. Bülow and M. Bols, *J. Am. Chem. Soc.*, 2001, **123**, 5116–5117.
5 H. H. Jensen and M. Bols, *J. Chem. Soc., Perkin Trans. 1*, 2001, 905–909.
6 J. Alper, *Science*, 2001, **291**, 2338–2343.
7 Y. Nishimura, H. Adachi, T. Satoh, E. Shitara, H. Nakamura, F. Kojima and T. Takeuchi, *J. Org. Chem.*, 2000, **65**, 4871–4882.
8 M. P. Dale, H. E. Ensley, K. Kern, K. A. R. Sastry and L. D. Byers, *Biochemistry*, 1985, **24**, 3530–3539.
9 A. T. Vasella and T. D. Heightman, *Angew. Chem., Int. Ed.*, 1999, **38**, 750–770.
10 S. J. Williams, V. Notenboom, J. Wicki, D. R. Rose and S. G. Withers, *J. Am. Chem. Soc.*, 2000, **122**, 4229–4230.
11 C. V. Ramana and A. Vasella, *Helv. Chim. Acta*, 2000, **83**, 1599–1610.
12 H. Søhoel, X. Liang and M. Bols, *J. Chem. Soc., Perkin Trans. 1*, 2001, 1584–1585.
13 J. Andersch and M. Bols, *Chem. Eur. J.*, 2001, **7**, 3744–3747.
14 H. Søhoel, X. Liang and M. Bols, *Synlett.*, 2000, **7**, 347–348.
15 T. M. Jespersen, W. Dong, T. Skrydstrup, M. R. Sierks, I. Lundt and M. Bols, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1778–1779.
16 H. H. Jensen, A. Jensen, R. Hazell and M. Bols, *J. Chem. Soc., Perkin Trans. 1*, 2002, 1190–1198.
17 G. W. J. Fleet, N. G. Ramsden, R. A. Dwek, T. W. Rademacher, L. E. Fellows, R. J. Nash, D. S. C. Green and B. Winchester, *Chem. Commun.*, 1988, 483–485.
18 A. Lohse, T. Hardlei, A. Jensen, I. Plesner and M. Bols, *Biochem. J.*, 2000, **349**, 211–215.
19 I. W. Plesner, A. Bülow and M. Bols, *Anal. Biochem.*, 2001, **295**, 186–193.
20 A. Bülow, I. Plesner and M. Bols, *J. Am. Chem. Soc.*, 2000, **122**, 8567–8568.
21 A. Bülow, I. Plesner and M. Bols, *Biochem. Biophys. Acta*, 2001, **1545**, 207–215.
22 P. Mendes, *Comput. Appl. Biosci.*, 1993, **9**, 563–571.
23 P. Mendes, *Trends Biochem. Sci.*, 1997, **22**, 361–363.
24 P. Mendes and D. B. Kell, *Bioinformatics*, 1998, **14**, 869–883.
25 J. March, *Advanced Organic Chemistry*, 4th edn., J. Wiley & Sons, New York, 1992, p. 143.