Synthesis of (±)-isofagomine and its stereoisomers from arecoline

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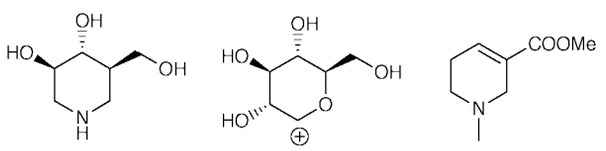
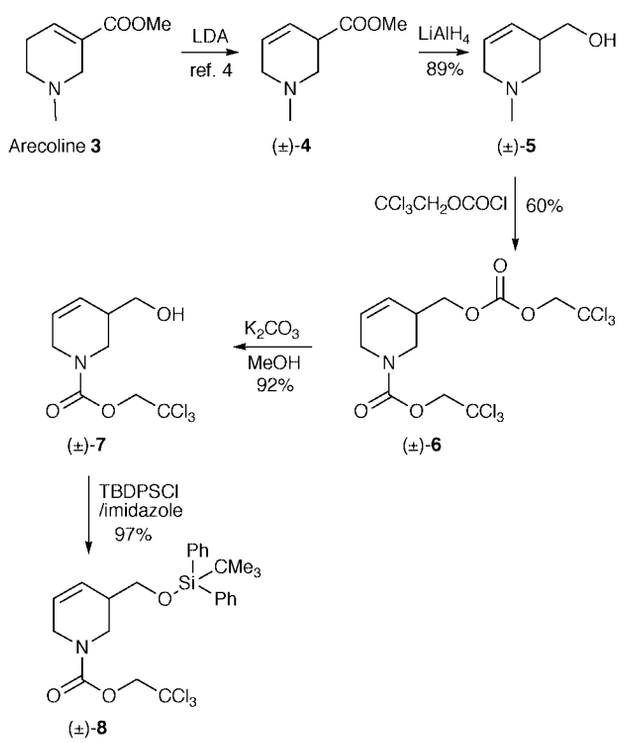
(±)-Isofagomine (**1**) and all its stereoisomers were prepared in a short synthesis from arecoline. The double bond of arecoline was isomerised to be no longer conjugated to the carboxy, and the ester reduced to (hydroxymethyl)tetrahydropyridine **5** which after dihydroxylation of the alkene, separation and *N*-demethylation gave **1** and its isomers.

Introduction

It is becoming increasingly evident that sugar derivatives, in which the anomeric carbon has been replaced with nitrogen, are transition state analogues of enzymatic glycoside cleavage, as seen by the fact that these compounds are strong inhibitors of glycosidases and related enzymes.[1] Thus isofagomine (**1**) is the



strongest known inhibitor of β-glucosidase,[2] possibly because it resembles the cation **2**. Isofagomine and its stereoisomers are therefore important building blocks for rationally designed inhibitors. The synthesis of isofagomine analogues is however relatively long, and an alternative shorter synthesis is of interest. Arecoline (**3**), a natural product that is isolated from palm trees and is commercially available, resembles these compounds and could therefore potentially be a useful starting material for the synthesis of isofagomine and its analogues. In the present paper we report a very short synthesis of all the isofagomine stereoisomers, in racemic form, starting from arecoline.



Scheme 1 Synthesis of tetrahydropyridine **6** from arecoline.

Results and discussion

It is known that arecoline (**3**) can be demethylated in high yields with various reagents including α-chloroethyl chloroformate[3] and 2,2,2-trichloroethyl chloroformate (TrocCl).[4] It is also known that the double bond in arecoline can be isomerised.[4,5] It therefore seemed straightforward to perform these two steps and reduce the ester, thereby obtaining a potential precursor to isofagomine and its *galacto* analogue. Arecoline could be isomerised by following the known procedure[4] to give a 20 : 1 ratio of **4** and arecoline (**3**) in a quantitative yield (Scheme 1). The isomerized product **4** could not be separated chromatographically from the 5% content of arecoline (**3**) and was therefore used as such in the next step.

Demethylation of **4** was tried with 2,2,2-trichloroethyl chloroformate (TrocCl) in toluene at 80 °C, but gave a poor yield (20%) of the corresponding *N*-2,2,2-trichloroethylcarbamate apparently due to the occurence of side reactions. In general, the success of demethylation of the piperidine derivatives was found to be very structure-dependent as has been seen by others.[6] Thus arecoline (**3**) itself could be demethylated in good yield with TrocCl;[4] however that reaction was not useful in the present case, because the double-bond isomerisation failed on the *N*-trichloroethylcarbamate, as the Troc group is base-labile.

Instead, reduction of **4** was carried out using lithium aluminium hydride giving the alcohol **5** in 89% yield (Scheme 1). Demethylation of **5** with 2.1 equivalents of TrocCl in toluene at 80 °C was then successful, giving a 60% yield of the *N*,*O*-bis(Troc)-protected derivative **6**. The carbonate could selectively be saponified with K_2CO_3 in water–MeOH, giving the mono *N*-Troc derivative **7** in 92% yield. The primary alcohol functionality in **7** was protected with a *tert*-butyldiphenylsilyl (TBDPS) group by treatment with *tert*-butyldiphenylsilyl chloride (TBDPSCl) and imidazole to give the silyl ether **8** in 97% yield.

Recently Altenbach and Blanda have reported the synthesis of the *N*-phenylcarbamate analogue of **7**, using methyl *N*-benzyl-4-oxopiperidine-3-carboxylate as the starting material.[7] They were also able to enzymatically resolve this analogue of **7** using enantioselective esterification with a lipase. Our strategy provides an alternative high yielding route

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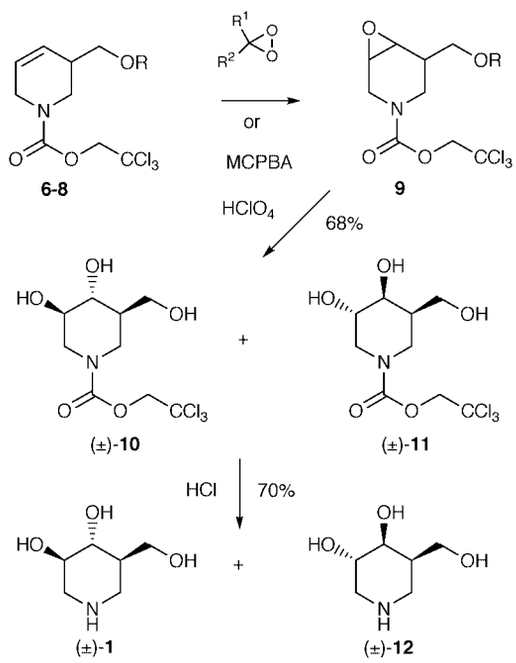
Table 1 Results of epoxidation or dihydroxylation of **6–8**. **6**: R = $COOCH_2Cl_3$, **7**: R = H, **8**: R = TBDPS

R-group	Reagent	Yield (%)	*anti*:*syn* ratio
H	$CF_3(CH_3)CO_2$	91	1:1
CCl_3CH_2OCO	3-$ClC_6H_4CO_3H$	72	2:1
CCl_3CH_2OCO	$CF_3(CH_3)CO_2$	90	3:2
CCl_3CH_2OCO	$(CH_3)_3C(CH_3)CO_2$	42	5:2
CCl_3CH_2OCO	Fructosedioxirane[a]	54	3:1
$(CH_3)_3CPh_2Si$	3-$ClC_6H_4CO_3H$	82	1:1
$(CH_3)_3CPh_2Si$	$CF_3(CH_3)CO_2$	Quantitative	2:1
CCl_3CH_2OCO	OsO_4/NMO	86[b]	1:1

[a] Dioxirane generated from Oxone and 1,2:4,5-di-*O*-isopropylidene-D-*arabino*-hexo-2,6-pyran-2,3-diulose according to ref. 9. [b] Yield of corresponding *syn*-diols.

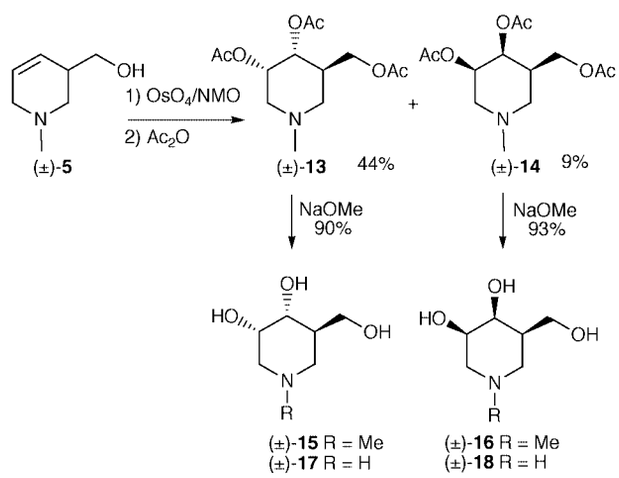


Scheme 2 Epoxidation of alkenes **6–8**. **6**: R = $COOCH_2Cl_3$, **7**: R = H, **8**: R = TBDPS. R^1 or R^2 = Me, CF_3, *t*-Bu or diisopropylidenefructose.

The Os-catalysed dihydroxylation, which would give the *syn*-diol, was also attempted (Table 1). Dihydroxylation of **6** gave a 1:1 mixture of *syn*:*anti* products that, as in the case of the epoxides **9**, was inseparable.

Since good selectivity could not be obtained, a 1:1 mixture of the epoxides **9** was hydrolysed with a 2.3% $HClO_4$ solution at 100 °C. This gave an inseparable mixture of triols **10** and **11** in 68% yield. These were subjected to HCl hydrolysis to give a mixture of racemic isofagomine (**1**) and its *gulo* isomer **12**. These could be separated chromatographically to give **1** and **12** in 14 and 44% yield, respectively. Only the D-enantiomer of isofagomine[2] and the L-enantiomer of **12**[10] are known.

The lack of selectivity in the addition reactions to the alkenes **6–8** was puzzling, since the literature contains a number of examples where alkenes of related structure are dihydroxylated with high *anti* selectivity.[11] Thus 3-methylcyclohexene gives only the *anti* diastereomer when reacted with OsO_4.[11b] Since the main difference between substrates **6–8** and cyclohexene is the geometry at the amide nitrogen, it was suggested that dihydroxylation of **5** might give a higher selectivity. Indeed, treatment of **5** with OsO_4 and NMO gave, after peracetylation, a 5:1 mixture of the *anti* and *syn* triacetates **13** and **14**. The diastereomers could be separated to give pure **13** and **14** in 44 and 9% yield, respectively, thus a combined yield of 53% (Scheme 3).



Scheme 3 Dihydroxylation of **5**.

to similar 3-hydroxymethyl-1,2,3,6-tetrahydropyridine derivatives, which also should be good substrates for enzymatic resolution.

Methods for dihydroxylation of the piperidine alkenes were investigated next (Scheme 2, Table 1). If epoxidation could be carried out from the less hindered side, the resulting *trans* oriented epoxide would be expected to give the *gluco*-type *trans*, *trans*-diol upon subsequent hydrolysis. Epoxidation of **6** was carried out with either trifluoromethylmethyldioxirane[8] or *m*-chloroperbenzoic acid (MCPBA), however the diastereoselectivity was 1.5 or less. The *anti* selectivity could be improved to 3 by using dioxiranes prepared from the more sterically hindered ketones *tert*-butyl methyl ketone and 1,2:4,5-di-*O*-isopropylidene-D-*arabino*-hexo-2,6-pyran-2,3-diulose,[9] but the yields of these reactions were considerably lower. When the most reactive of these reagents, trifluoromethylmethyldioxirane, was used on **7**, a lower selectivity was obtained relative to epoxidation of **6** (*anti*:*syn* 1:1) with the same reagent. In contrast, similar epoxidation of **8** gave a somewhat higher *anti* selectivity (*anti*:*syn* 2:1, Table 1). These results fit well with the relative sterical hindrance of the allylic substituent in the substrates. The diastereomers of **9** could not be separated chromatographically. The diastereomeric ratios were determined by NMR.

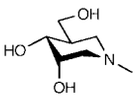
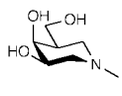
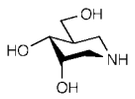
Deacetylation of **13** was performed with NaOMe in MeOH to provide **15** in 90% yield. Similar deacetylation of **14** gave **16** in 93% yield.

The isomers **13** and **14** were also demethylated. This was done by treatment of each compound with 1-chloroethyl chloroformate in dichloroethane, giving a 1-chloroethyl carbamate that was deprotected with MeOH and NaOMe to obtain either *allo*-isofagomine **17** or *galacto*-isofagomine **18** both in 81% yield.

The compounds **12** (one enantiomer of **12** is known[10]) and **15–17** are new and were therefore tested for inhibition of glycosidases (Table 2). Particularly noteworthy was the observation that (±)-**16** is a relatively poor β-galactosidase inhibitor compared with *galacto*-isofagomine (**18**), which has been reported to be extremely potent.[12] The fact that **16** is racemic and **18** sterically pure does not affect this observation, since even if one antipode of **16** is completely inactive the other will only be twice as potent (which is still much less than the potency of **18**). It was also noteworthy that (±)-**17** was a rather potent inhibitor of galactosidase, and that its *N*-methyl analogue (±)-**15** is a poor inhibitor. It suggests that N-methylation decreases glycosidase inhibition.

In conclusion we have reported a short synthesis of three of the four possible diastereomers of isofagomine starting from

Table 2 Glycosidase inhibition by **12**, **15**, **16** and **17**. Figures show K_i values in μM

	K_i/μM			
				
Compound	(±)-**12**	(±)-**15**	(±)-**16**	(±)-**17**
β-Glucosidase	22	>1000	235	76
α-Glucosidase	>1000	>1000	>1000	>1000
β-Galactosidase	405[a]	530	77	9
α-Galactosidase	>1000	>1000	74	37

[a] Non-competitive (or mixed) inhibition.

arecoline, and also the synthesis of the *N*-methyl analogue of the fourth diastereomer. Arecoline has been found to be a useful source for the synthesis of these compounds in a racemic form. The method may also be extended to the synthesis of the enantiomerically pure compounds, since the enzymatic resolution method reported by Altenbach and Blanda[7] probably can be applied to substrates **5** and **7**.

Experimental

General

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, $60F_{254}$) were visualised either by spraying with ninhydrin (2% in butanol) or CEMOL (1% ceric sulfate and 1.5% ammonium molybdate in 10% aqueous H_2SO_4) and heating until coloured spots appeared. ^{13}C and ^1H NMR spectra were recorded on a Varian Gemini 200 instrument. Mass spectra were obtained on a Micromass LCT-QTOF instrument. Products were concentrated on a rotary evaporator below 40 °C.

(±)-3-Hydroxymethyl-1-methyl-1,2,3,6-tetrahydropyridine (5)

A solution of 9.50 g (61.2 mmol) of **4** (containing 5% of **3**) in 25 mL of THF was added during 60 min to 2.32 g (61.2 mmol) of $LiAlH_4$ in 35 mL of THF at 0 °C. After stirring for another 90 min at room temperature, the mixture was quenched by the careful addition of water (50 mL). Extraction with EtOAc (8 × 50 mL), drying and evaporation left **5** as a yellow oil. Yield: 6.90 g (89%). This substance contained however a 5% impurity of **4**. This could be removed by purification with flash chromatography in EtOAc–MeOH 5:1. ^1H NMR ($CDCl_3$): δ 5.79 (m, 1H, H5, $J_{5,4}$ 10.3 Hz), 5.64 (m, 1H, H4), 4.46 (br s, 1H, 3′-OH), 3.76 (dd, 1H, H3′a, $J_{3'a,3'b}$ 10.3, $J_{3'a,3}$ 3.7 Hz), 3.58 (ddd, 1H, H3′b, $J_{3,3'b}$ 3.7 Hz, $J_{3'b,3'OH}$ 1.5 Hz), 3.06 (m, 1H, H6eq, $J_{6eq,6ax}$ 16.1, $J_{6eq,5}$ 4.0, $J_{6eq,4}$ 1.1 Hz), 2.65 (m, 2H, H2ax, H6ax), 2.49 (dd, 1H, H2eq, $J_{2eq,2ax}$ 11.0, $J_{2eq,3}$ 4.0 Hz), 2.27 (m, 1H, H3), 2.26 (s, 3H, 1-Me). ^{13}C NMR ($CDCl_3$): δ 127.5, 126.5 (C4, C5), 66.7 (C3′), 57.1, 54.8 (C2, C6), 46.0 (C1′), 37.7 (C3). HRMS (ES) *m*/*z*: 150.0889 (M + Na^+), calcd for $C_7H_{13}NO + Na^+$: 150.0895.

(±)-3-(2,2,2-Trichloroethoxycarbonyloxymethyl)-1-(2,2,2-trichloroethoxycarbonyl)-1,2,3,6-tetrahydropyridine (6)

To 3.02 g (23.7 mmol) of **5** in 30 mL toluene was added 4.13 mL (23.7 mmol) of diisopropylethylamine. Over a period of 15 min a solution of 10.54 g (49.8 mmol) 2,2,2-trichloroethyl chloroformate in 10 mL toluene was added. The mixture was heated to 80 °C for 20 hours. A solid precipitate formed on cooling to room temperature. The mixture was filtered, the filtrate washed with HCl (1 M, 50 mL), NaOH (1 M, 50 mL), water (50 mL), dried and evaporated to leave a crude product. Flash chromatography in CH_2Cl_2–pentane (1:1) gave **6**. Yield: 6.55 g (60%). ^1H NMR ($CDCl_3$): δ 5.79 (m, 2H, H4, H5), 4.73 (m, 4H, CH_2-CCl_3's), 4.2–3.5 (m, 6H, H3′, H2, H6), 2.69 (m, 1H, H3). ^{13}C NMR ($CDCl_3$): δ 154.4, 154.2, 154.0 (rotamer C=O's), 127.7, 126.9, 125.0, 124.6 (rot. C4, rot. C5), 96.0, 94.8 (CH_2CCl_3's), 78.2 (CH_2CCl_3), 75.6, 75.4 (rot. CH_2CCl_3), 69.5, 69.0 (rot. C3′), 44.3, 43.9, 42.9, 42.8 (rot. C2, rot. C6), 35.3, 35.1 (rot. C3). HRMS (ES): *m*/*z* 483.8815 (M + Na^+), calcd for $C_{12}H_{13}NO_5Cl_6 + Na^+$: 483.8823.

(±)-3-Hydroxymethyl-1-(2,2,2-trichloroethoxycarbonyl)-1,2,3,6-tetrahydropyridine (7)

To 1.93 g (4.16 mmol) of **6** was added 10 mL MeOH and a solution of K_2CO_3 (5 g) in 10 mL water. After stirring for 1 hour the mixture was extracted with ether (2 × 20 mL). The combined organic phases were washed with HCl (1 M, 10 mL), NaOH (1 M, 2 × 10 mL), water (10 mL), dried and evaporated to leave **7** as an oil. Yield: 1.11 g (92%). ^1H NMR ($CDCl_3$): δ 5.75 (m, 2H, H4, H5), 4.73 (m, 2H, CH_2CCl_3), 4.1–3.4 (m, 6H, H3's, H2's, H6's), 2.47 (m, 1H, H3). ^{13}C NMR ($CDCl_3$): δ 155.0, 154.1 (rot. C=O), 126.8, 126.5, 126.3, 125.7 (rot. C4, rot. C5), 96.1 (CCl_3), 75.6, 75.5 (rot. CH_2CCl_3), 63.8, 63.5 (rot. C3′), 44.5, 44.3, 43.1, 42.9 (rot. C2, rot. C6), 38.5, 38.4 (rot. C3). HRMS (ES): *m*/*z* 309.9776 (M + Na^+), calcd for $C_9H_{12}NO_3Cl_3 + Na^+$: 309.9781.

(±)-3-(*tert*-Butyldiphenylsiloxy)methyl-1-*N*-(2,2,2-trichloroethoxycarbonyl)-1,2,3,6-tetrahydropyridine (8)

To 0.560 g (1.94 mmol) of **7** in 2 mL DMF was added 0.246 g (3.88 mmol) of imidazole and 510 μL (1.96 mmol) of *tert*-butyldiphenylsilyl chloride. The solution was stirred for 16 h under nitrogen. Quenching with water (10 mL) was followed by extraction with pentane (2 × 10 mL), drying ($MgSO_4$) and evaporation, leaving **8** as an oil. Yield: 0.995 g (97%). ^1H NMR ($CDCl_3$): δ 7.20 (m, 10H, phenyl), 5.58 (m, 2H, H4, H5), 4.62 (m, 2H, CH_2CCl_3), 4.2–3.2 (m, 6H, H3's, H2's, H6's), 2.42 (m, 1H, H3), 0.98 (s, 9H, *tert*-butyl). ^{13}C NMR ($CDCl_3$): δ 153.3, 153.2 (rot. C=O), 135.0, 132.3, 129.2, 127.1 (phenyl), 126.2, 125.7, 124.5, 124.0 (rot. C4, rot. C5), 95.1 (CCl_3), 74.5 (CH_2CCl_3), 64.6, 64.2 (rot. C3′), 43.4, 43.0, 42.3 (rot. C2, rot. C6), 37.6, 37.2 (rot. C3), 26.1 (*tert*-butyl). HRMS (ES): *m*/*z* 548.0964 (M + Na^+), calcd for $C_{25}H_{30}NO_3SiCl_3 + Na^+$: 548.0958.

Epoxidation or dihydroxylation of 6–8. General procedure

a) Epoxidations using MCPBA. To 1 mmol of the alkene in 4 mL dichloromethane was added 1.2–1.5 mmol of MCPBA,

and the solution was stirred for 20 h. The organic phase was washed with $NaHSO_3$ (10%), $NaHCO_3$ (sat.), water, dried ($MgSO_4$) and evaporated. The inseparable diastereomeric mixture of epoxides was purified by flash chromatography if necessary.

b) Epoxidations using dioxiranes. To 1 mmol of the alkene in a mixture of water–CH_3CN (2:3, 10 mL) was added 1,1,1-trifluoroacetone (10 mmol) and $NaHCO_3$ (8 mmol). To this mixture at 0 °C was added Oxone (6 mmol) in small portions during 10 min. The solution was stirred at room temperature for 1–20 h. Quenching with water (20 mL), extraction with dichloromethane (2 × 30 mL), drying and evaporation gave the inseparable diastereomeric mixture of epoxides, which could be purified by flash chromatography if necessary.

(3,4-*trans*-4,5-*trans*)-5-Hydroxymethyl-1-(2,2,2-trichloroethoxycarbonyl)-3,4-dihydroxypiperidine (10) and (3,4-*trans*-4,5-*cis*)-5-hydroxymethyl-1-(2,2,2-trichloroethoxycarbonyl)-3,4-dihydroxypiperidine (11)

To 1.062 g (3.69 mmol) of **9** (R = H, *syn*:*anti* ratio 1:1) in 30 mL water was added 1 mL $HClO_4$ (70%). The solution was heated to reflux for 90 min and then brought to a neutral pH by addition of K_2CO_3. Salts were removed by filtration, and the filtrate extracted with EtOAc (5 × 25 mL). Drying and evaporation left a 1:1 ratio of **10** and **11** as a mixture of inseparable diastereomers. Yield: 0.810 g (68%).

(3,4-*trans*-4,5-*trans*)-5-Hydroxymethyl-3,4-dihydroxypiperidine (1)[2a] and (3,4-*trans*-4,5-*cis*)-5-hydroxymethyl-3,4-dihydroxypiperidine (12)[10]

A solution of 8 mL aqueous HCl (3 M) and 0.142 g (0.44 mmol) of the mixture of **10** and **11** (ratio, 1:2) was heated to reflux for 24 h. After evaporation, (±)-isofagomine (**1**) was separated from the *gulo* isomer (**12**) by flash chromatography in EtOH–NH_4OH (1%) (72:28). The yield of pure **1** was 12 mg (14%), while 39 mg of pure **12** (44%) were isolated together with 11 mg of a mixture of **1** and **12**. Total yield: 62 mg (70%). NMR data of **1** were identical to literature values.[2a] Data for the hydrochloride of **12**:[10] ^{1}H NMR (D_2O): δ 3.77 (m, 2H, H3, H4), 3.46 (dd, 1H, H5′a, $J_{5'a,5'b}$ 11.4, $J_{5'a,5}$ 6.6 Hz), 3.32 (dd, 1H, H5′b, $J_{5,5'b}$ 7.0 Hz), 3.03 (m, 3H, H2's, H6eq), 2.75 (t, 1H, H6ax, $J_{6ax,6eq}$ 12.5, $J_{6ax,5}$ 12.5 Hz), 2.14 (m, 1H, H5). ^{13}C NMR (D_2O): δ 64.0, 63.9 (C3, C4), 59.0 (C5′), 43.0, 39.4 (C2, C6), 33.7 (C5). HRMS (ES): *m*/*z* 170.0800 (M + Na^+), calcd for $C_6H_{13}NO_3Na^+$: 170.0793.

(3,4-*cis*-4,5-*trans*)-5-Acetoxymethyl-1-methyl-3,4-diacetoxypiperidine (13) and (3,4-*cis*-4,5-*cis*)-5-acetoxymethyl-1-methyl-3,4-diacetoxypiperidine (14)

To 0.334 g (2.63 mmol) of **5** in acetone–water (1:1, 4 mL) was added 0.886 g (6.56 mmol) 4-methylmorpholine *N*-oxide monohydrate. To this solution was added by syringe 1.67 mL (2.5 mol%) of a 1% solution of OsO_4 in *tert*-butyl alcohol and it was stirred for 5 days. $NaHSO_3$ was added in excess and the mixture was stirred for 30 min. The mixture was purified by ion exchange giving a crude product of inseparable diastereomers. To allow separation, the mixture was acetylated by dissolving in pyridine (3 mL) and acetic anhydride (3 mL), and was stirred for 45 min. After evaporation the two diastereomers **13** and **14** could be separated by flash chromatography in EtOAc. Yield of **13**: 331 mg (44%). ^{1}H NMR ($CDCl_3$): δ 5.31 (dd, 1H, H3, $J_{3,2}$ 5.1, $J_{3,4}$ 3.3 Hz), 4.77 (dd, 1H, H4, $J_{4,5}$ 11.0 Hz), 4.06 (d, 1H, H5′a, $J_{5'a,5}$ 3.7 Hz), 4.04 (d, 1H, H5′b, $J_{5'b,5}$ 5.2 Hz), 3.0–2.1 (m, 5H, H2's, H6's, H3), 2.31 (s, 3H, Me), 2.14, 2.06, 2.03 (s, 3H, Ac's). ^{13}C NMR ($CDCl_3$): δ 170.2, 169.9, 169.6 (C=O), 69.4, 66.7 (C3, C4), 61.9 (C5′), 56.4, 55.9 (C2, C6), 45.0 (Me), 34.9 (C5), 20.4, 20.1 (Ac's). HRMS (ES): *m*/*z* 288.1442 (M + H^+), calcd for $C_{13}H_{21}NO_6$ + H^+: 288.1447. Yield of **14**: 70 mg (9%). ^{1}H NMR ($CDCl_3$): δ 5.41 (m, 1H, H4), 4.98 (ddd, 1H, H3, $J_{3,4}$ 3.3, $J_{3,2eq}$ 4.3, $J_{3,2ax}$ 10.1 Hz), 4.04 (dd, 1H, H5′a, $J_{5'a,5'b}$ 10.3, $J_{5'a,5}$ 7.7 Hz), 3.90 (dd, 1H, H5′b, $J_{5'b,5}$ 6.7 Hz), 2.1–2.7 (m, 5H, H2's, H5, H6's), 2.36 (s, 3H, Me), 2.09, 2.03, 2.00 (3s, 9H, Ac's). ^{13}C NMR ($CDCl_3$): δ 170.2, 169.4, 169.3 (C=O's), 68.6, 65.6 (C3, C4), 61.3 (C5′), 52.4, 51.3 (C2, C6), 44.9 (Me), 37.0 (C5), 20.1 (Ac's). HRMS (ES): *m*/*z* 310.1288 (M + Na^+), calcd for $C_{13}H_{21}NO_6Na^+$: 310.1267.

(3,4-*cis*-4,5-*trans*)-5-Hydroxymethyl-1-methyl-3,4-dihydroxypiperidine (15)

To 70 mg (0.25 mmol) of **13** in 2 mL dry MeOH was added a small piece of sodium. After stirring for 1 h the solution was evaporated. Flash chromatography in EtOH–NH_4OH (25%, 9:1) gave **15**. Yield: 36 mg (90%). ^{1}H NMR (D_2O): δ 3.65 (dd, 1H, H3, $J_{3,4}$ 4.0, $J_{3,2}$ 5.9 Hz), 3.49 (dd, 1H, H4, $J_{4,5}$ 11.4 Hz), 3.33 (dd, 1H, H5′a, $J_{5'a,5'b}$ 11.0, $J_{5'a,5}$ 6.2 Hz), 3.29 (dd, 1H, H5′b, $J_{5'b,5}$ 3.3 Hz), 2.58, 2.1–1.7 (m, 5H, H2's, H6's, H3), 1.97 (s, 3H, Me). ^{13}C NMR (D_2O): δ 68.3, 66.4 (C3, C4), 60.1 (C5′), 56.9, 54.0 (C2, C6), 43.4 (C1′), 37.9 (C5). HRMS (ES): *m*/*z* 162.1143 (M + H^+), calcd for $C_7H_{15}NO_3$ + H^+: 162.1130.

(3,4-*cis*-4,5-*cis*)-5-Hydroxymethyl-1-methyl-3,4-dihydroxypiperidine (16)

To 62 mg (0.22 mmol) of **14** in 2 mL dry MeOH was added a small piece of sodium. After stirring for 1 h the solution was evaporated. Flash chromatography in EtOH–NH_4OH (25%) (9:1) gave **16**. Yield: 33 mg (93%). ^{1}H NMR (D_2O): δ 3.74 (t, 1H, H4, $J_{4,3}$ and $J_{4,5}$ 2.2), 3.49 (ddd, 1H, H3, $J_{3,2ax}$ 11.4 Hz, $J_{3,2eq}$ 5.1 Hz), 3.41 (dd, 1H, H5′a, $J_{5'a,5'b}$ 11.0, $J_{5',5}$ 7.0 Hz), 3.29 (dd, 1H, H5′b, $J_{5,5'b}$ 6.7 Hz), 2.49 (dd, 1H, H2eq, $J_{2eq,2ax}$ 11.0 Hz), 2.38 (dd, 1H, H6eq, $J_{6eq,6ax}$ 11.0, $J_{6eq,5}$ 3.3 Hz), 2.04 (s, 3H, Me), 2.00 (t, 1H, H2ax), 1.78 (t, 1H, H6ax, $J_{6ax,5}$ 11.1 Hz), 1.68 (m, 1H, H5). ^{13}C NMR (D_2O): δ 67.1, 65.4 (C3, C4), 59.6 (C5′), 52.6, 49.0 (C2, C6), 43.1 (Me), 39.8 (C5). HRMS (ES): *m*/*z* 162.1119 (M + H^+), calcd for $C_7H_{15}NO_3$ + H^+: 162.1130.

(3,4-*cis*-4,5-*trans*)-5-Hydroxymethyl-3,4-dihydroxypiperidine (17)

To 78 mg (0.27 mmol) of **13** in 2 mL of dry 1,2-dichloroethane was added 50 μL (0.4 mmol) of 1-chloroethyl chloroformate and 50 μL of ethyldiisopropylamine. The solution was heated to reflux for 3 h. After evaporation the residue was dissolved in 2 mL of dry MeOH and heated to reflux for 1 h. Heating was stopped and a small piece of sodium was added. After stirring for 1 h the solvent was evaporated, and the residue purified by flash chromatography in EtOH–NH_4OH (25%) 9:1. Yield: 32 mg (81%). ^{1}H NMR (D_2O): δ 3.60 (dd, 1H, H3, $J_{3,4}$ 2.9, $J_{3,2}$ 4.8 Hz), 3.40 (m, 3H, H4, H5's), 2.77 (m, 2H, H2eq, H6eq), 2.40 (dd, 1H, H2ax, $J_{2ax,2eq}$ 14.3 Hz), 2.10 (dd, 1H, H6ax, $J_{6ax,6eq}$ 13.2, $J_{6ax,5}$ 11.0 Hz), 1.66 (m, 1H, H5). ^{13}C NMR (D_2O): δ 68.7, 66.3 (C3, C4), 59.8 (C5), 47.5, 44.4 (C2, C6), 38.4 (C5). HRMS (ES): *m*/*z* 170.0786 (M + Na^+), calcd for $C_6H_{13}NO_3Na^+$: 170.0793.

(3,4-*cis*-4,5-*cis*)-5-Hydroxymethyl-3,4-dihydroxypiperidine (18)

To 12 mg (0.042 mmol) of **14** in 1 mL of dry 1,2-dichloroethane was added 20 μL (0.16 mmol) of 1-chloroethyl chloroformate and 20 μL of ethyldiisopropylamine. The solution was heated to reflux for 3 h. After evaporation the residue was dissolved in 1 mL of dry MeOH and heated to reflux for 1 h. The heating was stopped and a small piece of sodium was added. After stirring for 1 h the solvent was evaporated, and the residue purified by flash chromatography in EtOH–NH_4OH (25%) 9:1. Yield: 5 mg (81%). NMR data for **18** were identical to literature values.[12]

Enzymatic assays

The substrates 4-nitrophenyl α- and β-D-glucopyranoside, 4-nitrophenyl α-D-galactopyranoside and 2-nitrophenyl β-D-galactopyranoside, and the enzymes α-glucosidase (yeast, Sigma G 5003), α-galactosidase (Green Coffee Bean), β-galactosidase (Aspergillus Oryzae) and β-glucosidase (sweet almonds, Sigma G 4511) were purchased from Sigma Chemical Co. All assays were carried out in a sodium phosphate buffer (0.05 M, pH 6.8) at 25 °C. Formation of the product, 4-nitrophenol, was measured continually at 400 nm using a Milton Roy Genesys 5 spectrometer. In all kinetic runs less than 1% of the initial substrate was consumed assuring the constancy of the substrate concentration.

K_i determinations were performed as follows. Two thermostatted solutions of 1) 1 mL of 0.1 M buffer, 800 μL substrate in varied concentration ($[S] = 0.25\ K_M$ to $4\ K_M$) and 100 μL water and 2) 100 μL enzyme were mixed, and the reaction was immediately monitored. From 5 experiments with varied substrate concentrations, initial reaction rates were calculated from the slope of the 1st order plot of product absorption *vs* reaction time. K_M and V_{max} were calculated from a Hanes plot. From 5 experiments K_M' was calculated from the Hanes plot and from K_M and K_M', K_i was calculated.

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