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Synthesis of the First 1-Azaanalogues of L-sugars.

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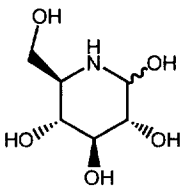
Abstract: Two 1-azaanalogues of L-sugars were prepared. 2-*C*-methyl-1,2,5-trideoxy-1,5-imino-L-ribitol (**1**) or 1-aza-2-deoxy-L-carbafucose was synthesized from L-arabinose in 9 steps. L-Arabinose was converted to the benzyl glycoside, and then to the 3,4-acetonide. Oxidation of the 2-hydroxygroup followed by Wittig methylenation gave benzyl 2-deoxy-3,4-*O*-isopropylidene-2-methylene-β-L-*erythro*-pentopyranoside (**6**). Stereoselective hydrogenation followed by reductive debenzylation and reduction led to 2-deoxy-3,4-O-isopropylidene-2-*C*-methyl-L-ribitol (**9**), which was ditosylated and treated with benzylamine to give a piperidine. Finally deprotection gave **1**. 1,3,5-Trideoxy-2-*C*-hydroxymethyl-1,5-imino-D-*erythro*-pentitol (**2**) was prepared from lactose in 7 steps. 2,2'-*O*-isopropylidene-5-*O*-(4-methylbenzenesulphonyl)-D-isosaccharino-1,4-lactone (**14**) was reacted with ammonia to give the 1,5-lactam. Deprotection and reduction gave **2**. Azasugar **1** was found to be a potent inhibitor of α-fucosidase.

INTRODUCTION

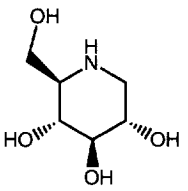
A number of hydroxylated piperidines and pyrrolidines occur in nature in plants and microorganisms[1]. These natural products, which have been called the "sugar-shaped" alkaloids from plants"[2], are reversible, competitive inhibitors of glycosidases. The purpose of these natural products is possibly to inhibit the carbohydrate metabolism and consequently the growth of plant consuming pests. Since selective glycosidase inhibitors have a large number of interesting potential applications including treatment of AIDS[3], diabetes[4], and tumor metastasis[5] they have received a considerable attention.

The "sugar-shaped" alkaloids closely resemble monosaccharides by being analogues of these where the ring oxygen has been exchanged with a nitrogen-atom. Thus nojirimycin[6], 1-deoxynojirimycin[7], homonojirimycin[8], fagomine[9] and castanospermin[10] are all analogues of D-glucose, and they are all glucosidase inhibitors (fig 1). Similarly 1-deoxymannonojirimycin[11], galactostatin[12], and 1-deoxyfuconojirimycin[13] (the latter two are synthetic compounds) are analogues of D-mannose, D-galactose and L-fucose respectively, and they are inhibitors of mannosidase, galactosidase and fucosidase, respectively. The pyrrolidines 2,5-dideoxy-2,5-imino-D-mannitol and 1,4-imino-D-arabinitol resemble D-fructofuranose and D-arabinofuranose, and they inhibit invertase catalysed cleavage of sucrose. All these compounds resemble the substrate of the enzyme they inhibit with the important difference that the basic nitrogen atom can and will be protonated in the active site of the enzyme. The protonated inhibitor resembles in terms of polarity an oxocarbenium ion, which can be expected to be a transition state in the glycoside bond cleavage. Therefore the discovery of these natural

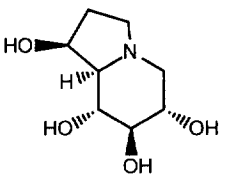
product led scientists to reconsider the catalytic mechanism of glycosidases and suggest ring oxygen protonation to occur at least for some glycosidases[14,15].



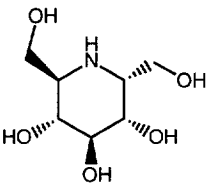
Nojirimycin



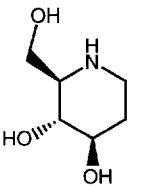
Deoxynojirimycin



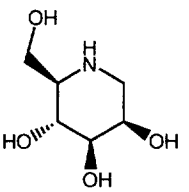
Castanospermine



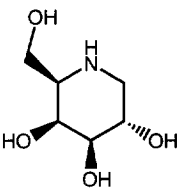
Homonojirimycin



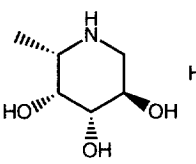
Fagomine



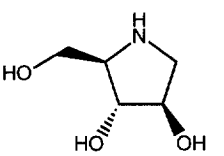
Deoxy-*manno*-nojirimycin



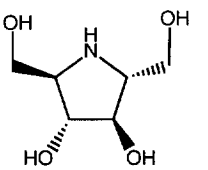
Galactostatin



Deoxy-*fuco*-nojirimycin



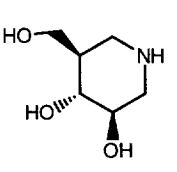
1,4-dideoxy-1,4-imino-D-arabinitol



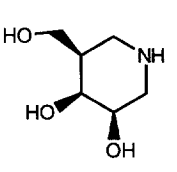
2,5-dideoxy-2,5-D-mannitol

Fig 1

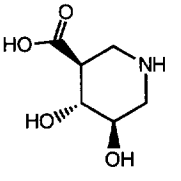
Recently we made the surprising discovery that a synthetic isomer of fagomine, isofagomine, having the nitrogen in place of anomeric carbon and not ring-oxygen was a much more potent glycosidase-inhibitor than fagomine itself[16-18]. Isofagomine was particularly potent in its inhibition of β-glucosidase, and though α-glucosidase was inhibited, β-glucosidase inhibition was 780 times stronger. Subsequently it has been found that the corresponding galactose-analogue (isogalactofagomine) and glucuronic acid-analogue are potent inhibitors of β-galactosidase[19] and β-glucuronidase[20] respectively (fig 2). Isogalactofagomine's inhibition of α-galactosidase was more than 10.000 times weaker. Furthermore an isomaltose analogue of isofagomine (fig 2) has been found to be a very potent inhibitor of glucoamylase, an enzyme that does not accept substrates smaller than disaccharides[18].



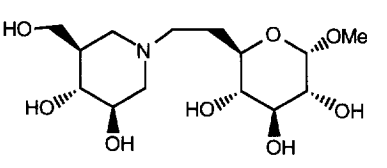
Isofagomine



Iso-*galacto*-fagomine



Glucuronic acid analogue

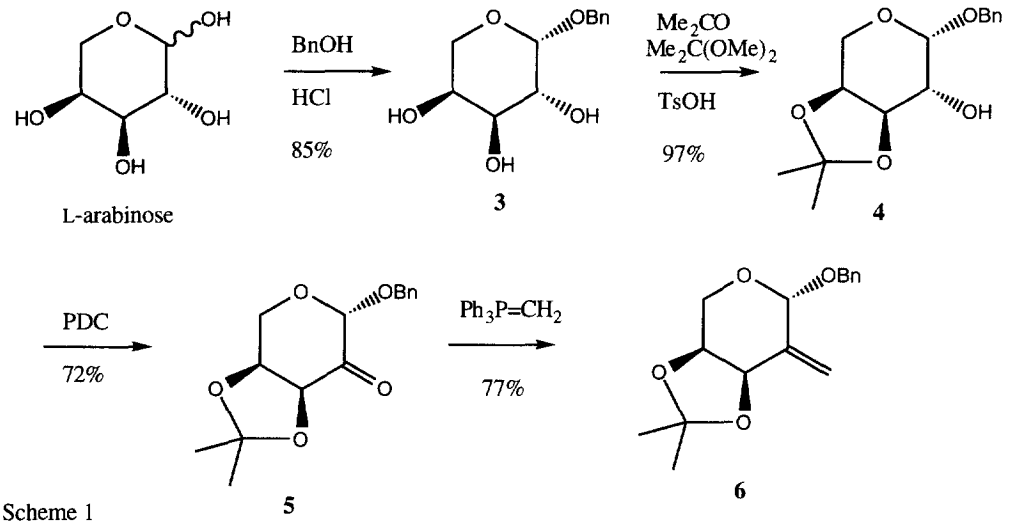


Isomaltose analogue

Fig 2

In these synthetic compounds the protonated inhibitor does not resemble an oxocarbenium ion, but an anomeric carbocation, which however also can be expected to be a transitionstate in the glycoside bond cleavage. All the isofagomine analogues investigated so far have been analogues of D-sugars. However, there

are some glycosidases that hydrolyse glycosides of L-sugars, and α-fucosidase, which is involved in glycoprotein processing, is the most interesting. In this paper we have investigated whether some L-sugar analogues of isofagomine would selectively inhibit α-fucosidase[21].



L-arabinose **3** **4**

5 **6**

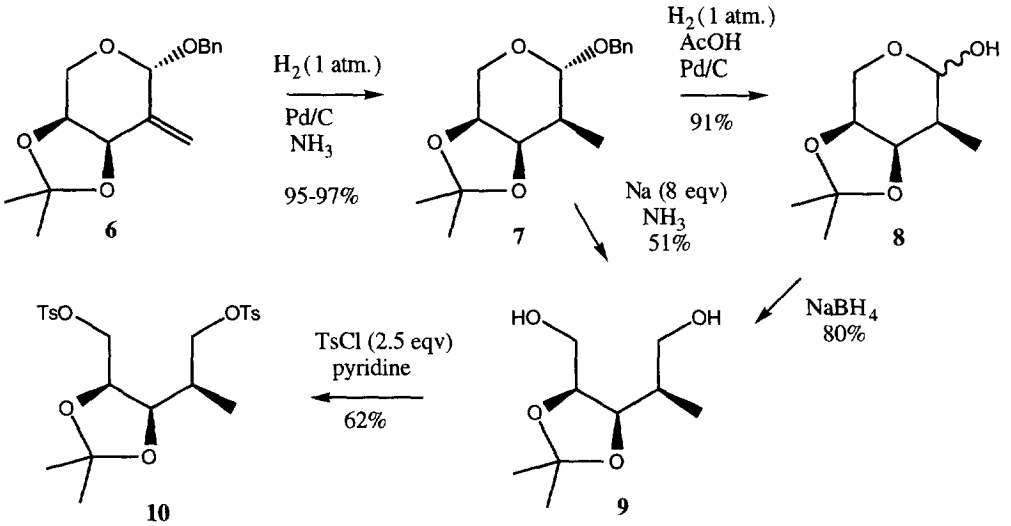
Scheme 1

RESULTS AND DISCUSSION

We decided to prepare two targets: The L-fucose analogue of isofagomine (scheme 3, **1**), because that compound could be expected to be a fucosidase inhibitor in direct analogy with the compounds described above, and the hydroxypiperidine **2** (scheme 4), because that compound could be expected to be readily available from isosaccharinic acid, and does have the stereochemistry of an L-sugar.

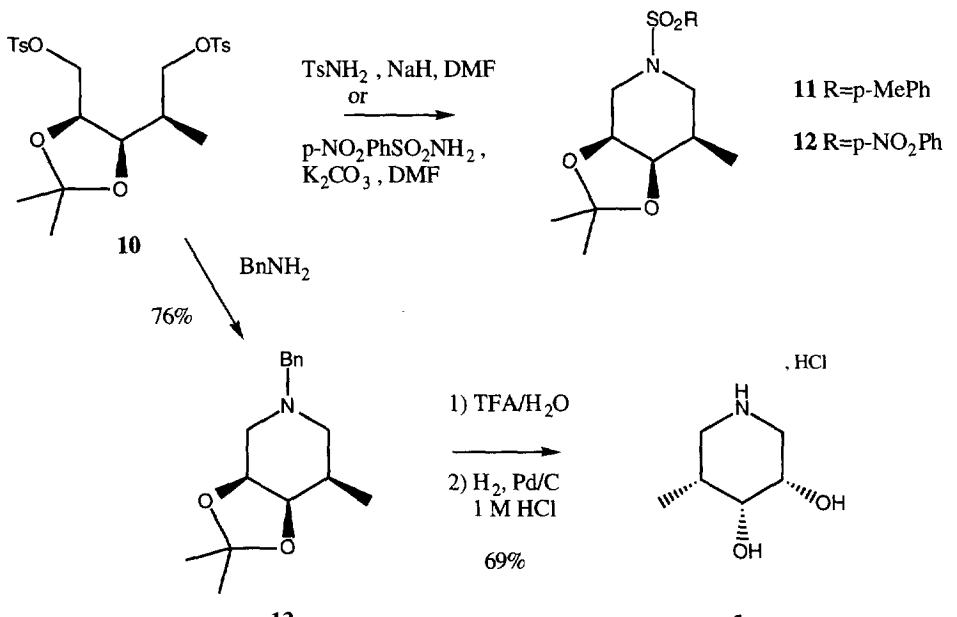
Compound **1** was prepared from L-arabinose through the known intermediate methylene derivative **6**[22] (scheme 1). We made some improving modifications to the synthesis of **6** (scheme 1). L-Arabinose was converted to the benzyl arabinoside **3** in the usual manner using HCl in benzyl alcohol[23], but with the difference that benzyl alcohol was removed by precipitation of **3** with ether[24]. This gave 85% of **3**. The procedure avoids difficult removal of the remaining benzyl alcohol by distillation in the subsequent step. Treatment of **3** with acetone, dimethoxypropane and TsOH gave 97% of the crystalline acetonide **4**[23]. Oxidation of **4** has previously been carried out by either Swern[23] or PDC-oxidation[22]. We investigated both and found the latter method gave a somewhat lower (72%) yield, but was easier to carry out. The Swern oxidation was also potentially undesirable, because traces of sulfur in the product could make the later catalytic hydrogenation step troublesome. Wittig methylenation of ketone **5** gave **6**[22] in 77% yield.

We now expected catalytic hydrogenation of the exocyclic double bond of **6** to occur stereoselectively with addition of hydrogen from the opposite side of the acetonide-grouping, because methyl lithium has been found to add from that face to ketone **5**[23]. Indeed treatment of **6** with 5% palladium on carbon under an atmosphere of hydrogen, and with ammonia present, gave a single stereoisomer **7** in 97% yield (scheme 2). If ammonia was not added partial hydrogenolysis of the benzyl-group occured. A proton-coupling of 7.5 Hz between H-1 and H-2 in the ^{1}H-NMR spectrum of **7** showed that the methyl-group was equatorial and thus had the desired stereochemistry.



Scheme 2

The benzyl-group could be removed by hydrogenolysis with hydrogen and palladium on carbon in the presence of acetic acid to give the hemiacetal **8** in 91% yield. This compound could be reduced with $NaBH_4$ to give diol **9** in 80% yield. Alternatively **7** was reduced directly to diol **9** with sodium in liquid ammonia, which gave a 51% yield. Finaly ditosylation of **9** with 2.5 equiv. of tosyl chloride in pyridine gave a 62% yield of **10**.



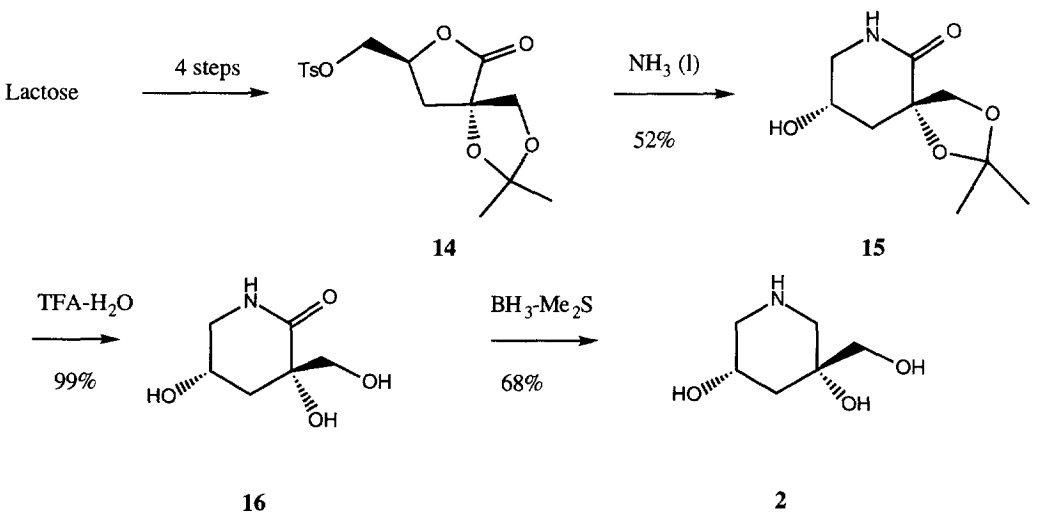
Scheme 3

Ring closure of the ditosylate **10** by disubstitution with an azanucleophile initially caused some problems (scheme 3). Compound **10** was completely unreactive towards ammonia. Disubstitution with

sulphonamide anions was possible. Thus reaction of **10** with tosyl amide and NaH in DMF gave tosyl piperidide **11** in 56% yield. We were however unable to remove the tosyl-group either by sodium in ammonia or sodium naphthalide treatment. Therefore we replaced tosyl amide with 4-nitrobenzenesulphonyl amide relying on a recently published procedure for mild nucleophilic cleavage of 4-nitrobenzenesulphonyl amides[25]. Reaction of **10** with 4-nitrobenzenesulphonyl amide and K_2CO_3 in DMF gave **12** in 64% yield. We were however not able to cleave the sulphonyl amide in **12** by any of the recommended procedures[25].

The solution to the problem was reaction of **10** with neat benzylamine at 40°C according to a recent procedure that has been employed to convert polyol 1,5-ditosylates to piperidines[26]. The reaction gave benzyl piperidine **13** in 76% yield. Deprotection of **13** using 90% aqueous trifluoroacetic acid for deacetonation followed by hydrogenation in dilute HCl using palladium on carbon as catalyst gave **1** in 69% yield.

Compound **2** was prepared from lactose in 7 steps taking advantage of the facile synthesis of isosaccharinic acid by base-treatment of lactose[27], and subsequent conversion to the known[28] lactone **14**. We have previously shown that 5-bromo-1,4-lactones are readily converted to 1,5-lactams using ammonia[29,17]. Similar treatment of **14** with liquid ammonia gave the 1,5-lactam **15** in 52% yield. The acetonide was hydrolysed using 90% aqueous trifluoroacetic acid to give deprotected lactam **16**, which was reduced with borane-dimethylsulfide to the piperidine **2**.



Scheme 4

The hydroxypiperidines **1** and **2** and the lactam **16** was tested for inhibition of a number of glycosidases (table 1). Iso-*fuco*-fagomine **1** was a potent competitive inhibitor of α-fucosidase with an

Enzyme/Compound	**1**	**16**	**2**
α-glucosidase (bakers yeast)	>1000[a]	2300[b]	>1000[a]
β-glucosidase (almonds)	121[a]	>1000[b]	222[c]
α-fucosidase (human placenta)	6.4[a]	-	>1000[c]
β-galactosidase (E. Coli)	>1000[d]	-	>1000[d]

Table 1: Dissociation constants (Ki) in μM between compounds **1**, **2**, **16** and glycosidases. a) pH 7.5, 26°C. b) pH 6.8, 22°C. c) pH 7.5, 27°C. d) pH 6.8, 26°C.

inhibition constant of 6.4 μM. Inhibition was selective as neither α-glucosidase nor β-galactosidase was inhibited, and β-glucosidase was only weakly inhibited. Piperidine **2** showed also weak inhibition of β-glucosidase, but did not inhibit any of the other glycosidases.

The inhibition of fucosidase by **1** was expected and complements the previous results with 1-azasugars as outlined above. The lack of biological activity of **2** was also predictable and confirms that to obtain good binding to a glycosidase close stereochemical resemblance to the substrate is nescessary. The weak inhibition of β-glucosidase by both **1** and **2** was probably because this enzyme has a relatively large substrate tolerance and cleave β-glycosides of a number of other monosaccharides.

Though a strong inhibitor of α-fucosidase, iso-*fuco*-fagomine (**1**) seems to be considerable less potent than 1-deoxyfuconojirimycin, which has been found to inhibit α-fucosidases from other sources up to 10^5 times more strongly[30]. A similar trend was observed for the inhibition of α-galactosidase by galactostatin and iso-galacto-fagomine: The former compound is a strong inhibitor[12], while the latter is much weaker[19]. For β-galactosidase the pattern is reversed. There is thus some evidence that α-glycosidases in general are more potently inhibited by hydroxypiperidines of the nojirimycin type while β-glycosidases are more potently inhibited by hydroxypiperidines of the isofagomine type.

ACKNOWLEDGEMENTS

We thank Torben N. Laugesen and Ulrik H. Sørensen for experimental assistance in the preparation of **2**. We thank the Danish Natural Research council for financial support (grant nr. 9502986), and NATO for supporting us with Collaborative Research Grant 930718. We also thank J. Ø. Madsen and B. O. Pedersen for Mass spectra.

EXPERIMENTAL SECTION

	C-1	C-2	C-3	C-4	C-5	C-2'	Bn/Ts	Ph	Ph	Ph	iPr	iPr	iPr
1	44.4*	34.4	68.7$^{\Delta}$	72.2$^{\Delta}$	45.8*	16.5	-	-	-	-	-	-	-
2	51.2	71.9	38.8	66.8	50.9	65.8	-	-	-	-	-	-	-
3	99.0	68.3*	68.4*	68.7*	63.3	-	69.1*	127.4	128.2	138.2	-	-	-
4	96.8	69.8*	75.7$^{\Delta}$	72.7$^{\Delta}$	59.5	-	69.5*	127.8	128.3	137	25.7	27.8	109
5	98.8	198.4	77.5*	77.3*	58.4	-	69.7	127.9	128.4	135.7	25.8	26.9	110.1
6	97.5	140.6	74.6*	74.4*	61.3	118.1	68.9	127.5	128.3	137.5	25.4	26.8	109.5
7	100.8	34.8	74.9*	73.4*	62.3	14.2	69.5	127.4	128.2	138.2	24.8	26.4	108.6
8	95.6	36.0	75.2*	72.9*	62.6	13.9	-	-	-	-	24.9	26.6	108.6
9	61.3	34.0	81.5*	77.8*	67.5	13.4	-	-	-	-	25.7	28.2	108.6
10	67.4	32.0	77.4*	74.1*	72.2*	13.5	21.6	127.8	129.7	132, 145	25.4	27.8	109.0
11	46.0*	31.5	71.7$^{\Delta}$	74.5$^{\Delta}$	46.2*	14.4	21.4	127	144		25.4	27.5	108.8
13	55.5*	32.3	76.2$^{\Delta}$	73.4$^{\Delta}$	56.0*	15.4	63.1	127.6	128.8	129.6	26.9	28.9	108.8
15	176.0	75.6	42.8	73.9	49.7	64.5	-	-	-	-	27.4	28.2	114.7
16	187.0	74.6	39.9	68.8	49.9	64.4	-	-	-	-	-	-	-

Table 2. ^{13}C-NMR chemical shift in $CDCl_3$ (except for **1**, **2**, **15**, **16** which was in D_2O). * and $^{\Delta}$ marked shifts may have the opposite assignment.

^{13}C-NMR and ^{1}H-NMR spectra were recorded on Bruker instruments AC 200, AC 250 and AM 500. D_2O was used as solvent with DHO (^{1}H-NMR: δ 4.7 ppm) and acetone (^{1}H-NMR: δ 2.05 ppm; ^{13}C-NMR: δ 29.8 ppm) as reference. With $CHCl_3$ as solvent TMS and $CHCl_3$ (^{13}C-NMR: δ 76.93 ppm) were used as references. Mass spectra were obtained on a VG TRIO-2 instrument. Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 141 polarimeter. Concentrations were performed on a rotary evaporator at a temperature below 40 °C. Dry tetrahydrofuran and diethyl ether were prepared by distillation from sodium and benzophenone.

*Benzyl β-*L*-arabinopyranoside* (**3**)[23,24].

To a mixture of benzyl alcohol (250 ml) and acetyl chloride (10 ml) at 0°C was added L-arabinose (50 g, 0.33 mmol). The resulting mixture was stirred at 50°C for 22 h. Diethyl ether was added (750 ml) and after 2 days at 5°C the precipitated product **3** was collected and washed with diethyl ether (67.8 g, 85%).

*Benzyl 3,4-*O*-isopropylidene-β-*L*-arabinopyranoside* (**4**)[23].

To a suspension of **3** (67.8 g, 0.283 mol) in acetone (1300 ml) was added 2,2-dimethoxypropane (65 ml) and 4-methylbenzenesulfonic acid (15.6 mg). After stirring 19 h at room temperature, the solution was neutralised with ammonia (25%, 0.5 ml) and concentrated by rotary-evaporation. The oily residue dissolved in CH_2Cl_2 (780 ml) and washed twice with saturated $NaHCO_3$ solution (260 ml), dried with $MgSO_4$ and concentrated under pressure to a syrup which slowly crystallised. Yield: 76.3 g (97%).

*Benzyl 3,4-*O*-isopropylidene-β-*L*-erythro-pent-2-ulosylpyranoside* (**5**)[22].

To a solution of **4** (5 g, 17.8 mmol) and pyridinium dichromate (7.48 g, 26.8 mmol) in CH_2Cl_2 (89 ml) was added molecular sieves (3Å, 14.3 g) and glacial acetic acid (1.8 ml). The mixture was kept for 20 h at 25 ° C. Celite (8.9 g) was added and after stirring 20 min. the black mixture was filtered. Concentration and coconcentration with toluene left a dark tar, that was dissolved in diethyl ether, dried with $MgSO_4$, treated with activated carbon, filtered and concentrated. The product was clear syrupy **5** (3.59 g, 72%).

*Benzyl 2-deoxy-3,4-*O*-isopropylidene-2-methylene-β-*L*-erythro-pentopyranoside* (**6**)[22].

To a solution of $CH_3PPh_3^+Br^-$ (8.64 g, 24.2 mmol) in THF (67 ml) at -40°C (under N_2) was dropwise added butyl lithium (16.3 ml, 1.5 M in hexane, 24.5 mmol) to give a yellow solution. After 1 h a solution of **5** (3.58 g, 12.9 mmol) in THF (34 ml) was added and after 30 min. the solution was allowed to reach room temperature at which temperature it was left for 17 h. Diethyl ether was added (154 ml), and the solution was washed with 5 % NH_4Cl (154 ml), twice with water (115 ml) and saturated NaCl-solution (115 ml). Drying with $MgSO_4$, filtration, concentration and flash-chromatography in (hexane-ethyl acetate 10:1, v/v) gave **6** as a syrup (2.75 g, 77%).

*Benzyl 2-deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-β-*L*-ribopyranoside* (**7**).

To a solution of **6** (2.15 g, 7.79 mmol) in EtOAc (237 ml) was added Pd/C (5%, 800 mg) and NH_4OH (25%, 3 ml), and the mixture was hydrogenated at 1 atm. H_2-pressure for 19 h. The solution was filtered through a celite-pad and concentrated to yield crude **7** as a syrup (2.09 g, 97%). This material was pure enough to continue the synthesis. Alternatively flash-chromatography (hexane-ethyl acetate 4:1, v/v) yielded pure **7** as an oil (1.45 g, 70%). ^{1}H-NMR ($CDCl_3$): δ 7.3 (s, 5H, Phe), 4.75 (d, 1H, *J* 12 Hz, Bn), 4.5 (d, 1H, J_{12} 7.5 Hz, H-1), 4.45 (d, 1H, *J* 12 Hz, Bn), 4.2 (dd, 1H, J_{34} 7.0, J_{23} 2.5 Hz, H-3), 4.1 (dt, 1H, J_{45a} 2.5, J_{45b} 2.0 Hz, H-4), 3.7 (dd, 1H, J_{5a5b} 12.5 Hz, H-5a), 3.5 (dd, 1H, H-5b), 1.7 (ddq, 1H, $J_{22'}$ 7 Hz, H-2), 1.4, 1.3 (2s, 6H, Me_2C), 1.1 (d, 3H, H-2'). $[\alpha]_D^{22}$ -104.6° (*c* 0.70; $CHCl_3$). MS(EI): m/z 278 (M^+).

2-Deoxy-3,4-O-isopropylidene-2-C-methyl-β-L-ribopyranose (**8**).

To a solution of **7** (478 mg, 1.72 mmol) in ethyl acetate (70 ml) was added palladium on carbon (5%, 250 mg) and a few drops of glacial acetic acid. The mixture was hydrogenated at 1 atm. of H_2-pressure and room temperature for 18 h. Finaly the mixture was filtered through celite and concentrated at reduced pressure to give **8** as white crystals (0.298 g, 91 %). Mp 55-57°C. $[\alpha]_D^{22}$ +42.4 (*c* 4.2, $CHCl_3$). MS(CI, NH_3) m/z 189 (M + H).

2-Deoxy-3,4-O-isopropylidene-2-C-methyl-L-ribitol (**9**). *From* **7**.

To a solution of **7** (2 g, 7.2 mmol) in 1,2-dimethoxyethan (10 ml) at -78°C was added liquid ammonia (60 ml) and sodium (2 g, 12 eqv), and the dark blue solution was kept at -78°C for 1 h. Solid ammonium chloride was added until the solution was colorless, and the ammonia was allowed to evaporate at roomtemperature. Water (60 ml) was added, pH was adjusted to 6 with acetic acid, and the solution was extracted twice with CH_2Cl_2 (60 ml). The organic layers were dried ($MgSO_4$), filtered and concentrated to a syrup. Flash-chromatography (hexane-ethyl acetate 3:1, v/v) yielded **9** as a clear syrup (700 mg, 51%). $[\alpha]_D^{22}$ -60.9° (*c* 1.0, $CHCl_3$). MS(CI, NH_3): m/z 208 ($M+NH_4$).

2-Deoxy-3,4-O-isopropylidene-2-C-methyl-L-ribitol (**9**). *From* **8**.

A solution of **8** (298 mg, 1.6 mmol) in ethanol (20 ml) was cooled to 0°C. $NaBH_4$ (0,4 g, 6.6 mmol, 4.1 eqv.) was added. After 30 min. the reaction was finished and moist acidic ionexchange resin was added (3 ml, Amberlite IR-120, H^+). After 15 min. the solution was neutral and the mixture was filtered and concentrated. The residue was coconcentrated twice with methanol to give diol **9** as a syrup (242 mg, 80 %).

2-Deoxy-1,5-di-O-(4-methylbenzenesulfonyl)-3,4-O-isopropylidene-2-C-methyl-L-ribitol (**10**).

A solution of **9** (224 mg, 1.18 mmol) in dry pyridine (5 ml) was cooled to 0°C and 4-methylbenzenesulfonyl chloride (1.13 g, 5.9 mmol, 2.5 eqv.) was added. The solution was kept at 5°C for 21 h. Then CH_2Cl_2 (12 ml) and water (12 ml) was added, and layers were separated. The waterlayer was extracted with CH_2Cl_2 (12 ml) and the combined organic layers were washed with aqueous HCl (18%, 5 ml) and water (5 ml), then dried with $MgSO_4$, filtered and concentrated to give a yellow syrup. Flash-chromatography (hexane-ethyl acetate 3:1, v/v) yielded **10** as a clear syrup (361 mg, 62%). $[\alpha]_D^{25}$ -16.5 (*c* 1.14, $CHCl_3$). MS(CI, NH_3): m/z 516 ($M+NH_4$).

N-*(4-Methylbenzenesulphonyl)-3,4-O-isopropylidene-2-C-methyl-1,2,5-trideoxy-1,5-imino-L-ribitol* (**11**).

NaH (103 mg, 55% in oil, 1.54 mmol, 2.2 eqv.) and DMF (1 ml) was mixed under N_2, and a solution of 4-methylbenzenesulphonamide (120 mg, 0.7 mmol) in DMF (1 ml) was added. A faint foaming was observed and the mixture became grey. The ditosylate **10** (300 mg, 0.602 mmol), dissolved in DMF (2 ml), was added, and the solution was allowed to stir at 100°C for 1 h. MeOH (3 ml) was added to decompose excess NaH, and the solution was concentrated to remove DMF. Water (10 ml) was added and the mixture was extracted 3 times with EtOAc (10 ml). The solution was dried with $MgSO_4$, concentrated and subjected to flash-chromatography (hexane-ethyl acetate 3:1, v/v) to yield **11** as a clear syrup. Yield: 109 mg (56%). $[\alpha]_D^{22}$ -22.1° (*c* 0.85, $CHCl_3$), MS(EI): m/z 326 (M+H).

N-*(4-Nitrobenzenesulphonyl)-3,4-O-isopropylidene-2-C-methyl-1,2,5-trideoxy-1,5-imino-L-ribitol* (**12**).

4-nitrophenylsulphonyl amide (74 mg, 0.37 mmol) was dissolved in DMF (1 ml), K_2CO_3 (199 mg, 1.44 mmol, 4 eqv.) was added at 0°C (under N_2), and after 10 min. of stirring the ditosylate **10** (200 mg, 0.4 mmol, 1.1 eqv.) dissolved in DMF (2 ml) was added at 0°C. The solution was heated to 100°C for 2 h. Water (20 ml) and EtOAc (10 ml) was added, and the layers were separated. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was subjected to flash-chromatography (hexane-ethyl acetate 2:1, v/v) to give **12** (91 mg, 64%) as a syrup. $[\alpha]_D^{22}$ +6.7° (*c* 0.17, $CHCl_3$), MS(EI): m/z 356 (M^+).

N-*Benzyl 3,4-*O*-isopropylidene-2-*C*-methyl-1,2,5-trideoxy-1,5-imino-*L*-ribitol* (**13**).

The ditosylate **10** (50 mg, 0.10 mmol) was dissolved in benzylamine (1 ml) and kept at 40°C for 2 days. The solution was concentrated by rotary evaporation at 80-90°C and a pressure of 0.1-1 mmHg to give a slightly yellow crystalline residue (129 mg). Flash-chromatography (hexane-ethyl acetate 10:1, v/v followed by 4:1, v/v) yielded **13** as a clear syrup (20 mg, 76%). $[\alpha]_D^{25}$ -25.3° (*c* 0.1, $CHCl_3$). MS(EI): m/z 261 (M^+).

*2-*C*-Methyl-1,2,5-trideoxy-1,5-imino-*L*-ribitol, hydrochloride* (**1**).

The piperidine **13** (19 mg, 0.073 mmol) was dissolved in a mixture of trifluoroacetic acid-water (9:1 v/v, 10 ml) and kept at 25°C for 2 h and then concentrated. The residue was then dissolved in aqueous HCl (1 M, 10 ml) and palladium on carbon (10%, 25 mg) was added. The mixture was hydrogenolysed at 1 atm. H_2-pressure for 18 h, filtered through celite and concentrated. The residue was dissolved in water (25 ml) and poured through a column of ionexchange resin (Amberlyst strongly acidic, 10 ml). The column was washed with water to neutrality, and the product was eluted with 2.5 % NH_3-solution. Concentration of the eluate, redissolving in MeOH, adding 1 drop of 37% HCl, filtration and concentration of the filtrate gave **1** as a hydrochloride (8.4 mg, 69 %). $[\alpha]_D^{22}$ -6.3° (*c* 0.75, H_2O), MS(EI): m/z 131 (M^+).

*1,3,5-Trideoxy-2-*C*-hydroxymethyl-1,5-imino-*D*-erythro-pentitol* (**2**).

2,2'-*O*-isopropylidene-5-*O*-(4-methylbenzenesulphonyl)-*D*-isosaccharino-1,4-lactone **14** (6.0 g, 16.9 mmol) was dissolved in liquid NH_3 (120 ml) in a cooled (-78°C) pressure vessel that was sealed and kept at room temperature for 7 days. The container was again cooled to -78°C and opened, and the ammonia was allowed to evaporate at room temperature. The residue was dissolved in MeOH, and ammonium tosylate was precipitated by addition of a small amount of EtOAc. The filtrate was concentrated and subjected to flash-chromatography (MeOH-ethyl acetate 1:10, v/v) to give 5-amino-3,5-dideoxy-2-C-hydroxymethyl-2,2'-O-isopropylidene-*D*-erythro-pentono-1,5-lactam (**15**, 3.12 g, 52%) as a syrup.

The lactam **15** (100 mg, 0.5 mmol) was dissolved in a mixture of trifluoroacetic acid-water 9:1 (15 ml) and kept for 15 min., and then concentrated. The residue was purified by flash-chromatography (MeOH-ethyl acetate 1:2, v/v) to give 5-amino-3,5-dideoxy-2-*C*-hydroxymethyl-*D*-*erythro*-pentono-1,5-lactam (**16**, 80 mg, 99%) as a syrup.

The lactam **16** (300 mg, 1.86 mmol) was dissolved in dioxane (5 ml) under N_2, and a solution of borane-dimethyl sulfide (1 ml) in dioxane (3 ml) was added at 0°C. The mixture was refluxed for 4 h, aqueous HCl (1 M, 4.5 ml) was added, and the mixture was refluxed for another 2 h. The mixture was concentrated and coevaporated twice with MeOH. The residue was subjected to flash-chromatography (EtOH-NH_4OH, 25% 20:1, v/v) to give **2** (186 mg, 68%) as a clear syrup. $[\alpha]_D^{22}$ -8.9 ° (*c* 1.4, H_2O), MS(EI): m/z 147 (M^+).

Measurements of glycosidase inhibition.

Each glycosidase assay was performed by preparing eight 2 ml samples in cuvettes consisting of 1 ml sodium phosphate buffer (0.1 M) of either pH 6.8 and 7.5, 0.2 to 0.8 ml of a 5 or 10 mM solution of either 4-nitrophenyl α-*D*-glucopyranoside, 4-nitrophenyl β-*D*-glucopyranoside, 4-nitrophenyl α-*L*-fucopyranoside or 2-nitrophenyl β-*D*-galactopyranoside in water, 0.1 ml of a solution of either the potential inhibitor (**1, 2** or **16**) or water, and distilled water to a total volume of 1.9 ml. Four of the samples contained the potential inhibitor at a fixed concentration but with variant nitrophenyl glycoside concentration. The other four samples contained no inhibitor, but also variant nitrophenyl glycoside concentration. Finaly the reaction was started by adding 0.1 ml of a diluted solution of either α-glucosidase from bakers yeast (EC 3.2.1.20, Sigma G-5003), β-glucosidase from almonds (EC 3.2.1.21, Sigma G-0395), α-fucosidase from human placenta (EC 3.2.1.51, Sigma F-6151) or β-galactosidase from E. Coli (EC 3.2.1.23, Sigma G-6008), and the formation of 4-nitrophenol was followed for 2 to 10 min. at 22-27°C by measuring absorbance at 400 nm. Initial velocities was calculated from the slopes for each of the eight reactions and used to construct two Hanes plots; one with and without inhibitor. From the two Michaelis-Menten constants (Km) thus obtained the inhibition constant (Ki) was calculated.

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