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4-*C*-Me-DAB and 4-*C*-Me-LAB – enantiomeric alkyl-branched pyrrolidine iminosugars – are specific and potent α-glucosidase inhibitors; acetone as the sole protecting group

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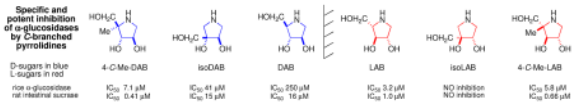
Abstract



The syntheses of 4-*C*-Me-DAB [1,4-dideoxy-1,4-imino-4-*C*-methyl-D-arabinitol] from L-erythronolactone and of 4-*C*-Me-LAB [from D-erythronolactone] require only a single acetonide protecting group. The effect of pH on the NMR spectra of 4-*C*-Me-DAB [pK_a of the salt around 8.4] is discussed and illustrates the need for care in analysis of both coupling constants and chemical shift. 4-*C*-Me-DAB (for rat intestinal sucrase K_i 0.89 μM, IC_{50} 0.41 μM) is a competitive - whereas 4-*C*-Me-LAB (for rat intestinal sucrase K_i 0.95 μM, IC_{50} 0.66 μM) is a non-competitive - specific and potent α-glucosidase inhibitor. A rationale for the α-glucosidase inhibition by DAB, LAB, 4-*C*-Me-DAB, 4-*C*-Me-LAB, and isoDAB – but *not* isoLAB – is provided. Both are inhibitors of endoplasmic reticulum (ER) resident α-glucosidase I and II.

This paper describes the synthesis of the enantiomers 4-*C*-Me-DAB **1D** [1,4-dideoxy-1,4-imino-4-*C*-methyl-D-arabinitol] and 4-*C*-Me-LAB **1L** with only a single acetonide needed as a protecting group, both of which are micromolar inhibitors of some α-glucosidases; in accord with Asano's hypothesis,[i] the D-iminosugar **1D** is a competitive inhibitor, whereas the enantiomer **1L** is a non-competitive inhibitor. Synthetic enantiomers of natural iminosugars

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are frequently powerful glycosidase inhibitors.[ii] Natural and synthetic iminosugars comprise a major family of glycosidase inhibitors.[iii] The introduction of an alkyl substituent into a sugar mimic usually removes any significant glycosidase inhibition,[iv] however, introduction of a C6 methyl branch into the piperidine ring of L-swainsonine increases the inhibition of naringinase by an order of magnitude[v] in comparison to the parent indolizidine, L-swainsonine.[vi] The natural product DAB **3D** is a good – but the enantiomer LAB **3L** is a more potent and more specific – inhibitor of α-glucosidases.[vii] The isomer isoDAB **2D** is also a very good inhibitor of α-glucosidases but the enantiomer **2L** shows no inhibition of any glycosidase. This paper provides a rationale for isoLAB **2L** being the only one of the six simple pyrrolidines **1, 2**, and **3** which does not inhibit α-glucosidases; isoLAB **2L** is the only one of the sugar mimics which partially rescues the defective F508del-CFTR function in cystic fibrosis.[viii] *N*-Alkylation of monocyclic iminosugars can enhance glycosidase inhibition by several orders of magnitude;[ix] such modification of the alkyl-branched parent structures may access a series of new bioactive compounds.

The enantiomeric azidolactones **4L** and **4D** are key intermediates in the synthesis of 4-*C*-methyl pyrrolidines **1L** and **1D** [Scheme 1]. The protected 2-*C*-methyl *arabinono*-lactone **5D** can be prepared by a Kiliani reaction[x] on a protected 1-deoxyribulose[xi] derived from D-erythronolactone **6D; 5D** is a useful chiron for the preparation of 2′-*C*-methyl nucleosides[xii] and carbon-branched ketoses.[xiii] Reaction of **5D** with triflic anhydride, followed by treatment with sodium azide, results in nucleophilic displacement at a tertiary center with inversion to give the azidolactone **4D**.[xiv] The enantiomeric azido-2-*C*-methyl-L-arabinonolactone **4L** was prepared from L-erythronolactone **6L** by similar procedures.

For the synthesis of 4-*C*-Me-DAB **1D**, it was necessary to invert the configuration at C4 of the azidolactone **4L** [Scheme 2]. Hydrolysis of the isopropylidene protecting group in the 1,5-lactone **4L** by trifluoroacetic acid in aqueous 1,4-dioxane gave the 1,4-lactone **7L**, {m.p. 76-78 °C; $[\alpha]_D^{25}$ -132.9 (*c*, 1.06 in CH_3OH)} in 87% yield. Selective esterification of the primary hydroxy group in **7L** with tosyl chloride in pyridine afforded the tosylate **8L** {m.p. 110-112 °C; $[\alpha]_D^{21}$ -107.7 (*c*, 1.07 in CH_3CN)} in 57% yield. Reaction of L-ribonolactone **8L** with potassium hydroxide in aqueous 1,4-dioxane, followed by treatment with an acidic ion exchange resin, resulted in inversion of configuration at C4 to form the D-lyxonolactone **9D** {oil; $[\alpha]_D^{21}$ +48.4 (*c*, 0.74 in MeOH)} in 87% yield. Esterification of **9D** with tosyl chloride in pyridine yielded the primary tosylate **10D** {oil; $[\alpha]_D^{20}$ +13.2 (*c*, 0.85 in CH_3CN)} (66% yield) which on reduction with sodium borohydride in methanol formed the azidotriol **11D**, (oil, 66% yield); it was thus possible to reduce the hindered lactone **10D** to the triol **11D** without any competing formation of epoxide. Hydrogenation of the unprotected azide **11D** in the presence of palladium on carbon in 1,4-dioxane gave the corresponding amine which spontaneously cyclized to form 4-*C*-Me-DAB **1D**, in quantitative yield. The tosylate salt of **1D** could be converted into the free base **1D** by ion exchange chromatography with Dowex 1-X2 (OH^- form) with water as eluent {free base: oil, $[\alpha]_D^{25}$ -20.1 (*c*, 0.57 in H_2O); hydrochloride salt: $[\alpha]_D^{25}$ -5.22 (*c*, 1.07 in H_2O)}. The enantiomer 4-*C*-Me-LAB **1L** was prepared by an identical route from D-erythronolactone {free base: oil, $[\alpha]_D^{25}$ +21.3 (*c*, 0.71 in H_2O); hydrochloride salt: $[\alpha]_D^{25}$ +4.10 (*c*, 1.0 in H_2O)}.[xv] It is noteworthy that only one isopropylidene group is used throughout this sequence.

Since the pK_a of the salts of imino sugars is around 7-8, the ^{1}H NMR spectrum is strongly dependent on the pH. This is illustrated for 4-*C*-Me-DAB **1D** at six different pH values with CH_3CN as the internal standard [Figure 1]. The pK_a of the hydrochloride salt of **1D** was determined as 8.38 from the change in the chemical shift of the *C*-methyl group with pH [Figure 2]. The full resonance assignments of the salt at pH 2.4 and of the free base at pH 11.1 of **1D** are given in Tables 1 and 2, respectively.

LAB **3L** is a more potent and specific inhibitor of α-glucosidases than its naturally occurring enantiomer DAB **3D** [Table 3],[xvi] whilst isoDAB **2D** is a more potent inhibitor of α-glucosidases than DAB **3D** and is completely specific; in contrast, isoLAB **2L** does not show any inhibition of these enzymes.[viii] 4-*C*-Me-DAB **1D** and 4-*C*-Me-LAB **1L** were both found to be specific and potent (μM) inhibitors of α-glucosidases. In particular, these compounds showed strong inhibition of rat intestinal sucrase with IC_{50} values of 0.41 and 0.66 μM, respectively. The kinetic analysis showed that 4-*C*-Me-DAB **1D** is a competitive inhibitor of rat intestinal sucrase with a K_i value of 0.89 μM, whereas the enantiomer 4-*C*-Me-LAB **1L** is a non-competitive inhibitor (K_i value of 0.95 μM) of the enzyme. Although DAB **3D** has been reported to be a good inhibitor of glycogen phosphorylase and has been investigated as a potential therapeutic agent for the treatment of diabetes,[xvii] LAB **3L**, isoDAB **2D**, isoLAB **2L**, 4-*C*-Me-DAB **1D**, and 4-*C*-Me-LAB **1L** showed no significant inhibition of this enzyme.

In order to rationalize the glucosidase inhibition results, it was assumed that these inhibitors mimic the glucoside substrate. The six pyrrolidines were overlayed with glucose to: (i) position the ring nitrogen close to the ring oxygen of glucose so that, in the protonated form, the positive charge can mimic the partial positive charge of the transition state during hydrolysis, and (ii) match as many hydroxy positions as possible, to retain any specific binding interactions in the active sites.xviii The different glucosidases will recognise the terminal glucose residue of the substrates in different ways. Thus it is not surprising that these inhibitors show slightly different patterns of inhibition for the different glucosidases, however, some general principles can be suggested.

The best match between DAB **3D** and glucose placed the C2 and C3 hydroxy (OH) groups of **3D** to overlay the C3 and C4 OHs of glucose, respectively [Figure 3(a)]. This positioned the CH_2OH group of **3D** close to the C6 OH of glucose and the ring nitrogen of **3D** close to the ring oxygen of glucose. In this orientation the inhibitor mimicked three of the glucose OH groups. 4-*C*-Me-DAB **1D** was overlayed with glucose in the same way as **3D**, with the inhibitor methyl group in the position of the glucose C5 axial proton [Figure 3(b)]. IsoDAB **2D** could be overlayed with glucose in the same way, with the inhibitor CH_2OH group in the position of the glucose C4 axial proton [Figure 3(c)]. This CH_2OH group in **2D** might be able to interact with groups which normally bind to the glucose C6 OH, depending on the glucose C5-C6 torsion angle in the bound form. In contrast, the best match between LAB **3L** and glucose places the C2 and C3 OHs of **3L** overlaying the C2 and C3 OHs of glucose respectively [Figure 3(d)]. This placed the CH_2OH group of **3L** close to the C4 OH of glucose and the ring nitrogen of **3L** close to the ring oxygen of glucose. Again, in this orientation **3L** mimicked three of the glucose OH groups. 4-*C*-Methyl-LAB **1L** was overlayed with glucose in the same way as **3L**, with the methyl group of **1L** in the position of the glucose C4 axial proton [Figure 3(e)]. If the inactive isoLAB **2L** is overlayed in the same manner as **3L**, then the CH_2OH group in **2L** would be in the position of the glucose C3 axial proton, on the C2OH/C4OH face of glucose. For a terminal α-linked glucose, the rest of the glycan was on the C2OH/C4OH face of the glucose residue (indicated by the large spheres in Figure 3). Alternatively, **2L** could be overlayed with the C2 and C3 OHs of **2L** overlaying the C3 and C2 OHs of glucose respectively, with the CH_2OH group in **2L** in the position of the glucose C2 axial proton, on the opposite face [Figure 3(f)]. In either case, the inhibitor could only mimic *two* of the four glucose OH groups.

There are several possible explanations for the absence of activity of isoLAB **2L** compared to the other five pyrrolidines, including: (i) the inhibitor must mimic *at least three* of the glucose hydroxy groups for effective binding, or (ii) a hydroxy group near the position of the glucose C4 hydroxy is required for binding, or (iii) an additional axial group at the positions of C2 or C3 in glucose cannot be tolerated, whereas it can at positions C4 or C5.

The differential inhibition of different α-glucosidases by the various pyrrolidines showed that the remainder of the substrates can significantly affect the potency of inhibition. Whatever the explanation, it is remarkable to find five such structurally simple pyrrolidines with potent and specific α-glucosidase inhibition.

The effect of **1D** and **1L** on cellular endoplasmic reticulum (ER) resident α-glucosidase I and II activity in cells was studied using a free oligosaccharide assay.[xix] HL-60 cells were treated at concentrations of **1D** and **1L** as indicated for 24 hours [Figure 4] and were compared with the effect of 1-deoxynojirimycin (DNJ); free oligosaccharides (FOS) were isolated from cells, fluorescently labelled, and followed by separation using NP-HPLC. Peak areas for $Glc_1Man_4GlcNAc_1$ and $Man_4GlcNAc_1$ (non-glucosylated control species) were determined and expressed as a ratio. The presence of $Glc_1Man_4GlcNAc_1$ indicated inhibition of α-glucosidase II; at 500 μM DNJ is five times a more potent inhibitor than **1L** and forty times more potent than **1D**. The decline in $Glc_1Man_4GlcNAc_1$ at concentrations of DNJ higher than 500 μM is due to inhibition of α-glucosidase I and therefore production of $Glc_3Man_5GlcNAc_1$ species at the expense of $Glc_1Man_4GlcNAc_1$ species. In the presence of 2.5 mM branched imino sugars, cells do not produce any $Glc_3Man_5GlcNAc_1$ indicating that compounds are either unable to inhibit this enzyme, i.e. are specific α-glucosidase II inhibitors, or most likely, insufficient concentrations have been reached to inhibit the α-glucosidase I step in the pathway. In summary, both 4-*C*-methyl branched imino sugars of **1D** and **1L** are weak inhibitors of α-glucosidase II in cells; this is in contrast to the behaviour of the hydroxymethyl branched pyrrolidines **2D** and **2L**, neither of which showed any inhibition of ER glucosidases.[8] Both DAB **3D** and LAB **3L** are presumed inhibitors of processing glucosidases; their *N*-butyl analogues are weak inhibitors (IC50, 319 μM and 769 μM, respectively) of α-glucosidase I using an *in vitro* assay and are consequently ineffective at inhibiting glucosidase activity in cellular assays at concentrations of 1 mM or less.[xx]

DNJ[xxi] [an α-glucosidase inhibitor] and isoLAB **2L**[8] [which has no α-glucosidase inhibition] both show significant rescue of the defective F508del-CFTR function and thus might have potential in the treatment of cystic fibrosis.[xxii] Neither of the 4-*C*-methyl pyrrolidines **1D** and **1L** had any corrector effect on CFTR function in CF-KM4 cells,[xxiii] as assessed by single-cell fluorescence imaging;[xxiv] thus, isoLAB **2L** – the only one of the DAB analogues that is not an α-glucosidase inhibitor – is the sole pyrrolidine to have chloride channel rescue properties.

In summary, only a single acetonide protecting group is used in the synthesis of the first examples of potent glycosidase inhibitors by *C*-alkyl branching of the carbon chain of iminosugars. Of the three pairs of enantiomers **1, 2** and **3**, five structurally simple pyrrolidines are potent (μM) and specific α-glucosidase inhibitors; isoLAB **2L** does not inhibit any glycosidase but is the only one to exhibit significant rescue of the defective F508del-CFTR function. Only DAB **3D** shows any inhibition of glycogen phosphorylase.

Acknowledgments

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xv. Data for free base **1D**: $[\alpha]_D^{25}$ -20.1 (*c* 0.57, H_2O); δ_H (400 MHz, D_2O) 1.01 (3H, s, CH_3), 2.65 (1H, dd, H1, $J_{1,1'}$ 12.1, $J_{1,2}$ 6.2), 3.17 (1H, dd, H1′, $J_{1',1}$ 12.1, $J_{1',2}$ 7.3), 3.44 (1H, d, H5, $J_{5,5'}$ 11.6), 3.49 (1H, d, H5′, $J_{5',5}$ 11.6), 3.80 (1H, d, H3, $J_{3,4}$ 6.0), 4.18 (1H, ddd, H2, $J_{2,1'}$ 7.3, $J_{2,1}$ 6.2, $J_{2,3}$ 6.0); δ_C (100.6 MHz, D_2O) 17.6 (C4′), 48.4 (C1), 63.4 (C4), 67.0 (C5), 77.6 (C2), 80.7 (C3). Data for HCl salt of **1D**: $[\alpha]_D^{25}$ -5.22 (*c* 1.07, H_2O); ν_{max} (thin film, Ge): 3356 (br s, OH, NH); δ_H (400 MHz, D_2O) 1.33 (3H, s, CH_3), 3.20 (1H, dd, H1, $J_{1,1'}$ 12.7, $J_{1,2}$ 5.0), 3.66 (1H, dd, H1′, $J_{1',1}$ 12.7, $J_{1',2}$ 6.8), 3.69 (1H, d, H5, $J_{5,5'}$ 12.3), 3.80 (1H, d, H5′, $J_{5',5}$ 12.3), 4.01 (1H, d, H3, $J_{3,4}$ 5.0), 4.40 (1H, ddd, H2, $J_{2,1'}$ 6.8, $J_{2,1}$ 5.0, $J_{2,3}$ 5.0); δ_C (100.6 MHz, D_2O) 15.1 (C4′), 47.8 (C1), 63.7 (C5), 69.5 (C4), 74.3 (C2), 77.4 (C3); HRMS (FI^+) Calcd. for $C_6H_{13}NO_3$ [$M^{\bullet}$]: 147.0895. Found: 147.0895.

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xviii. Molecular modelling was performed on a Silicon Graphics Fuel workstation, using the programs InsightII and Discover (Accelrys Inc., San Diego, USA).

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xxiv. CFTR Cl^- channel activity was assayed by single-cell fluorescence imaging using the potential-sensitive probe, bis-(1,3-diethylthiobarbituric acid)trimethine oxonol. For experimental details, see reference 21 (a).

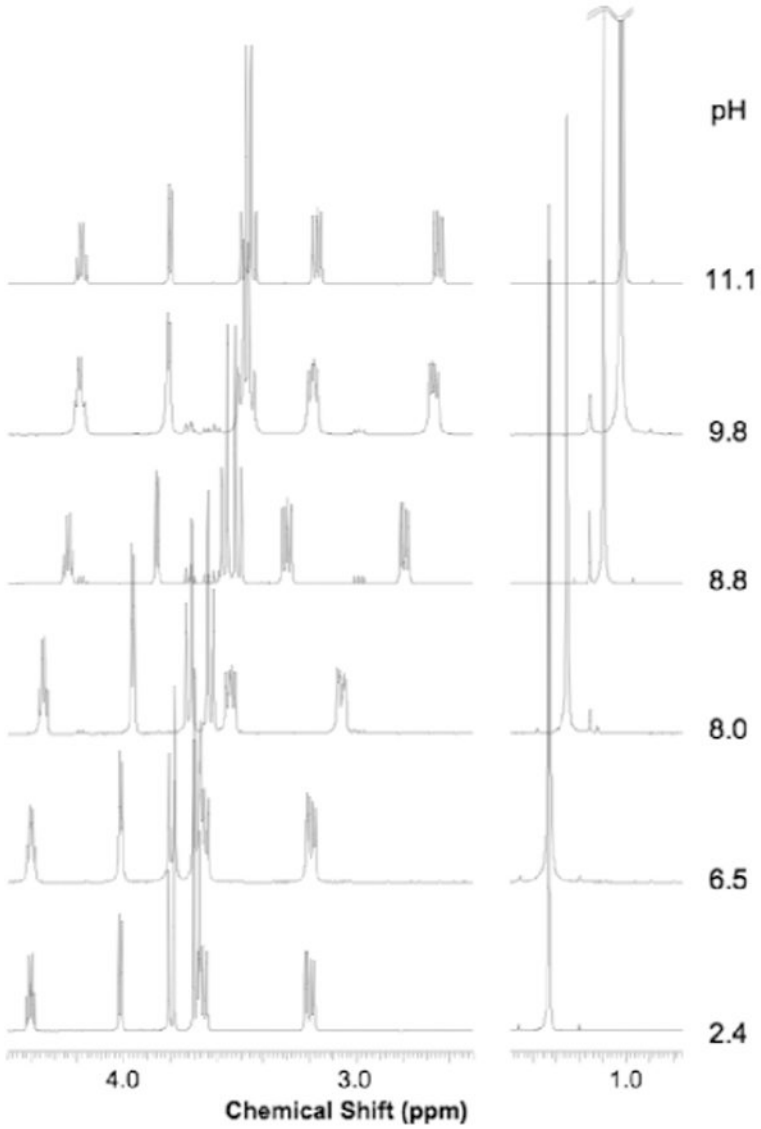


Figure 1.
^{1}H NMR (500 MHz) of 4-*C*-Me-DAB **1D** in D_2O at different pHs.

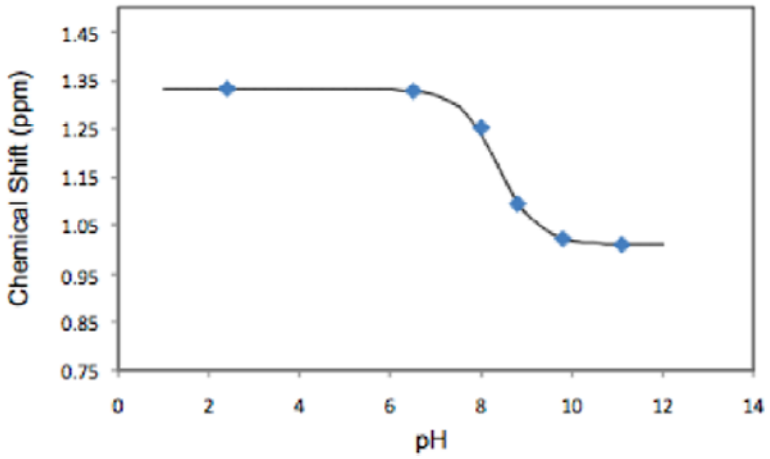


Figure 2.
Chemical shift of the methyl peak in **1D** versus pH. The points are the experimental values; the line is the fitted curve, pK_a of 8.38.

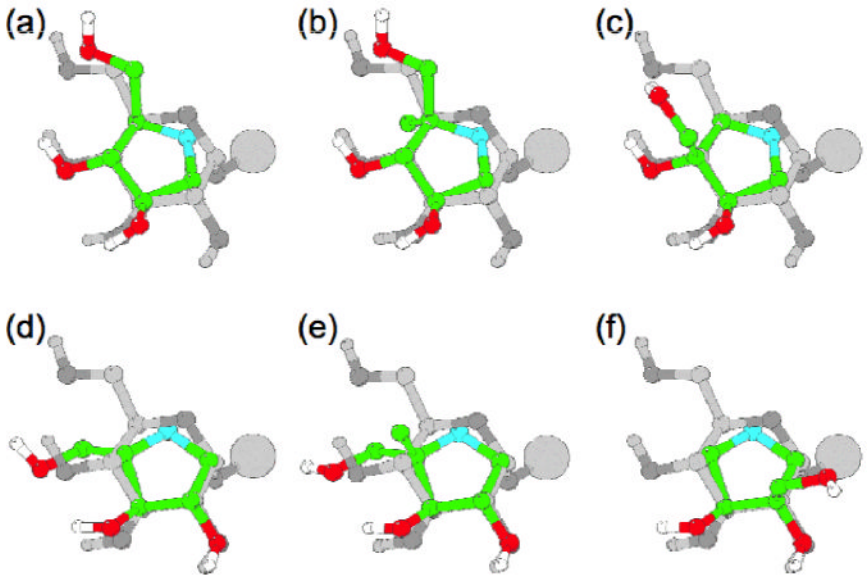


Figure 3.

Overlay of the six inhibitors, (a) DAB **3D**, (b) 4-*C*-Me-DAB **1D**, (c) isoDAB **2D**, (d) LAB **3L**, (e) 4-*C*-Me-LAB **1L** and (f) isoLAB **2L**, with an α-glucoside. The glucose residues are in grey, with the oxygens in slightly darker grey, and the inhibitors are in colour. Only the hydroxy protons are shown, for clarity. The large sphere shows the position of the rest of the glycan.

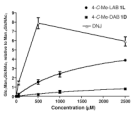
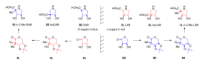
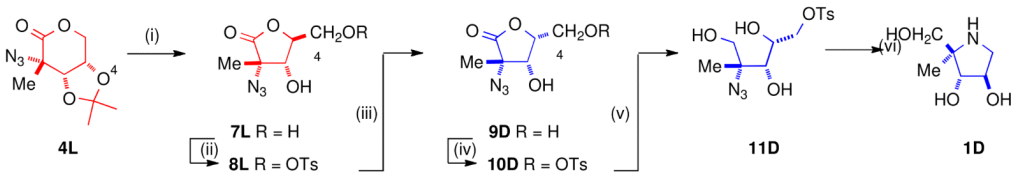


Figure 4.
$Glc_1Man_4GlcNAc_1$ following α-glucosidase inhibition in FOS assay.



Scheme 1. Structural relationship and synthesis of 4-*C*-methyl branched pyrrolidines



Scheme 2. (i) CF_3COOH:H_2O:1,4-dioxane, 4:1:1, 87% (ii) TsCl, pyridine, 57% (iii) KOH, 1,4-dioxane; then Dowex, 87% (iv) TsCl, pyridine, 66% (v) $NaBH_4$, MeOH, 66% (vi) H_2, Pd/C, 1,4-dioxane: H_2O (2:1), 100%

Table 1

NMR [^{1}H (500 MHz) ^{13}C (125 MHz) D_2O] assignments of the HCl salt of **1D**, pH 2.4.

Label	**^{1}H**			**^{13}C**
pH 2.24	**δ (ppm)**	**mult**	**$^{3}J_{HH}$ (Hz)**	**δ (ppm)**
C1	3.664 3.202	dd dd	12.7 / 6.8 12.7 / 5.0	47.83
C2	4.401	ddd	6.8 / 5.0 / 5.0	74.26
C3	4.014	d	5.0	77.36
C4	--	--	--	69.48
C5	3.797 3.688	d d	12.3 12.3	63.75
CH_3	1.332	s	na	15.12

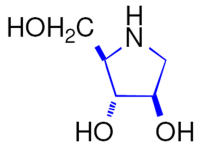
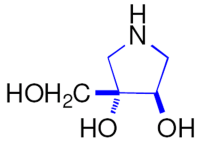
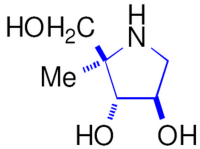
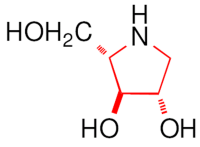
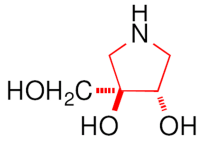
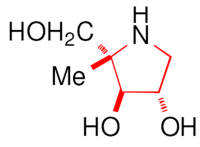
Table 2

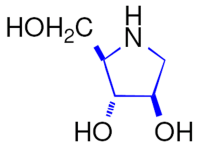
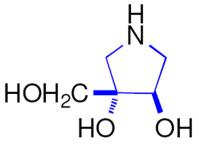
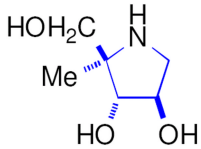
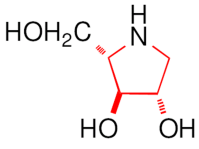
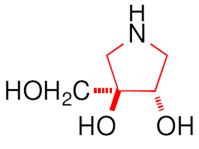
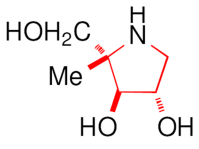
NMR [^{1}H (500 MHz) ^{13}C (125 MHz) D_2O] assignments of the free base of **1D**, pH 11.1.

Label	^{1}H			^{13}C
pH 11.1	**δ (ppm)**	**mult**	**$^{3}J_{HH}$ (Hz)**	**δ (ppm)**
C1	3.170 2.648	dd dd	12.1 / 7.3 12.1 / 6.2	48.39
C2	4.181	ddd	7.3 / 6.2 / 6.0	77.61
C3	3.801	D	6.0	80.67
C4	--	--	--	63.41
C5	3.485 3.443	D d	11.6 11.6	66.98
CH_3	1.010	S	na	17.60

Table 3

Concentration of iminosugars giving 50% inhibition of various glycosidases and glycogen phosphorylase.

	IC_{50} (μM)					
						
Enzyme	**DAB 3D**	**isoDAB 2D**	**4-*C*-Me-DAB 1D**	**LAB 3L**	**isoLAB 2L**	**4-*C*-Me-LAB 1L**
α-Glucosidase						
Rice	250	41	7.1	3.2	NI	5.8
Yeast	0.15	NI	1.9	70	NI	NI
Rat intestinal maltase	55	24	0.74	0.93	NI	2.4
Rat intestinal isomaltase	5.8	20	3.4	0.36	NI	5.1
Rat intestinal sucrase	16	15	0.41	1.0	NI	0.66
β-Glucosidase						
Almond	250	NI	NI	NI	NI	NI
Bovine liver	638	NI	NI	NI	NI	NI
Rat intestinal cellobiase	756	NI	NI	NI	NI	NI
α-Galactosidase						
Coffee beans	NI[a]	NI	NI	NI	NI	NI
Human lysosome	NI	NI	NI	NI	NI	NI
β-Galactosidase						
Bovine liver	NI	NI	NI	NI	NI	NI
Rat intestinal lactase	323	NI	NI	415	NI	NI
α-Mannosidase						
Jack beans	320	NI	NI	NI	NI	NI
β-Mannosidase						
Snail	NI	NI	NI	NI	NI	NI
α-L-Rhamnosidase						
P. decumbens	NI	NI	NI	NI	NI	NI
α-L-Fucosidase						

	IC$_{50}$ (μM)					
						
Enzyme	**DAB 3D**	**isoDAB 2D**	**4-*C*-Me-DAB 1D**	**LAB 3L**	**isoLAB 2L**	**4-*C*-Me-LAB 1L**
Bovine epididymis	NI	NI	NI	NI	NI	NI
Trehalase						
Rat intestinal trehalase	61	NI	38	75	NI	NI
Glycogen phosphorylase						
Rabbit muscle	0.33	NI	NI	NI	NI	NI

[a] NI: No inhibition (less than 50% inhibition at 1000 μM).