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Cystic fibrosis and diabetes: isoLAB and isoDAB, enantiomeric carbon-branched pyrrolidine iminosugars

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ABSTRACT

Acetonides are the only protecting groups used in the syntheses of isoDAB from D-ribose and of isoLAB from D-tagatose. isoDAB is a potent and highly specific competitive α-glucosidase inhibitor (for rice α-glucosidase, K_i = 4 μM for isoDAB compared to K_i 14 μM for DAB). isoDAB is not an—whereas DAB is a potent—inhibitor of glycogen phosphorylase. This is the first example of any potent inhibition of glycosidases by a carbon-branched iminosugar pyrrolidine. Although isoLAB shows no inhibition of any glycosidase, preliminary experiments suggest that isoLAB partially rescues the defective F508del-CFTR function and so may have a role in the study of cystic fibrosis.

Around 200 natural products are known that may be described as carbohydrate mimics in which the ring oxygen of a sugar is replaced by nitrogen[1]—but all of them have linear carbon chains; modifications of the analogous iminosugar have been developed to give sub-nanomolar inhibition of fucosidases,[2] hexosaminidases[3] and glucosidases[4]—but there are *no* reports of any significant inhibition of glycosidases by any pyrrolidine bearing a *branched* carbon chain (i.e., a carbon-substituted sugar backbone). DAB **2D**, isolated from *Arachniodes standishii* and *Angylocalyx boutiqueanus*,[5] shows strong inhibition of α-glucosidases and weaker inhibition of several other glycosidases. Synthetic enantiomers of natural iminosugars are frequently powerful glycosidase inhibitors;[6] the unnatural enantiomer LAB **2L** is a more potent and more specific inhibitor of α-glucosidases than DAB **2D**.[7] This Letter describes an efficient synthesis of isoDAB [1,4-dideoxy-2-*C*-hydroxymethyl-1,4-imino-D-threitol] (**1D**) from the acetonide of ribose **9** in an overall yield of 37% over eight steps (Scheme 1) with a sole isopropylidene-protecting group. The enantiomer isoLAB [1,4-dideoxy-2-hydroxymethyl-1,4-imino-L-threitol] (**1L**) was prepared from D-tagatose. isoDAB **1D** is a potent and specific inhibitor of a number of α-glucosidases (for rice α-glucosidase, K_i = 4 μM for isoDAB **1D** compared to K_i = 14 μM for DAB **2D**). isoDAB provides the first example of a carbon-branched iminosugar pyrrolidine showing significant glycosidase inhibition. In contrast, isoLAB **1L** shows no significant inhibition of any glycosidase. DAB **2D** is an excellent inhibitor of glycogen phosphorylase[8] and a moderate inhibitor of glycoprotein processing glucosidases; neither **1D** nor **1L** showed any inhibition of these enzymes.[9] The combination of potency and specificity of isoDAB **1D** as an α-glucosidase inhibitor may provide a useful agent in the study of diabetes. Two *N*-alkyl derivatives of deoxynojirimycin (DNJ), a naturally occurring α-glucosidase inhibitor, are drugs and have potential for the treatment of a number of diseases.[10] In particular, NB-DNJ (miglustat, Zavesca) **3** partially rescues the defective F508del-CFTR function in CF-KM4 cells[11] and thus may have potential for the chemotherapeutic treatment of cystic fibrosis (CF);[12] calnexin may be a therapeutic target for miglustat in CF.[13] Although isoLAB **1L** showed no significant inhibition of any glycosidase, in preliminary experiments isoLAB **1L** also rescued CFTR function and thus is likely to be of value in the investigation of CF. This may be the first example of an imino sugar that apparently acts as a chaperone but is not a glycosidase inhibitor.

The *erythro* epimer of isoDAB **4** is a weak inhibitor of purine nucleoside phosphorylase (PNP); neither **4** nor its enantiomer shows any significant inhibition of glycosidases (Scheme 2).[14] Both the branched pyrrolidine, DADMe-immucillinH **5** and its enantiomer are nanomolar inhibitors of PNP.[15] Carbon branching of imino sugars usually removes the glycosidase inhibition properties;[16] an exception is that a C6 methyl group branch in L-swainsonine **6** increases the inhibition of naringinase by an order of magnitude in comparison with the parent indolizidine, L-swainsonine.[17] In contrast to

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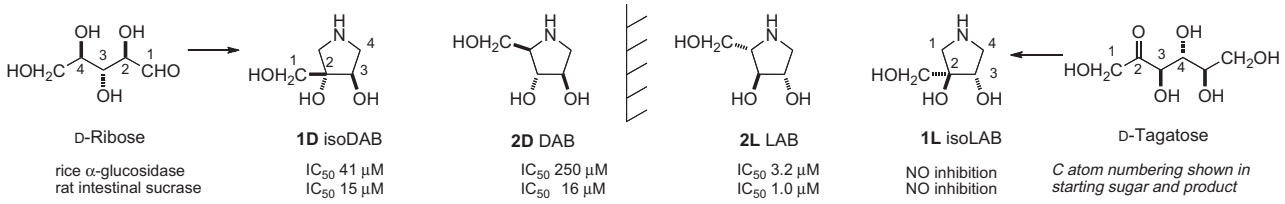
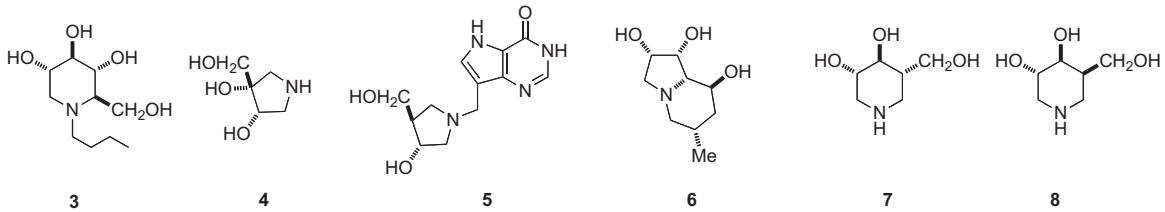


Scheme 1. Structure, synthesis and α-glucosidase inhibition of isoDAB **1D** and isoLAB **1L**.

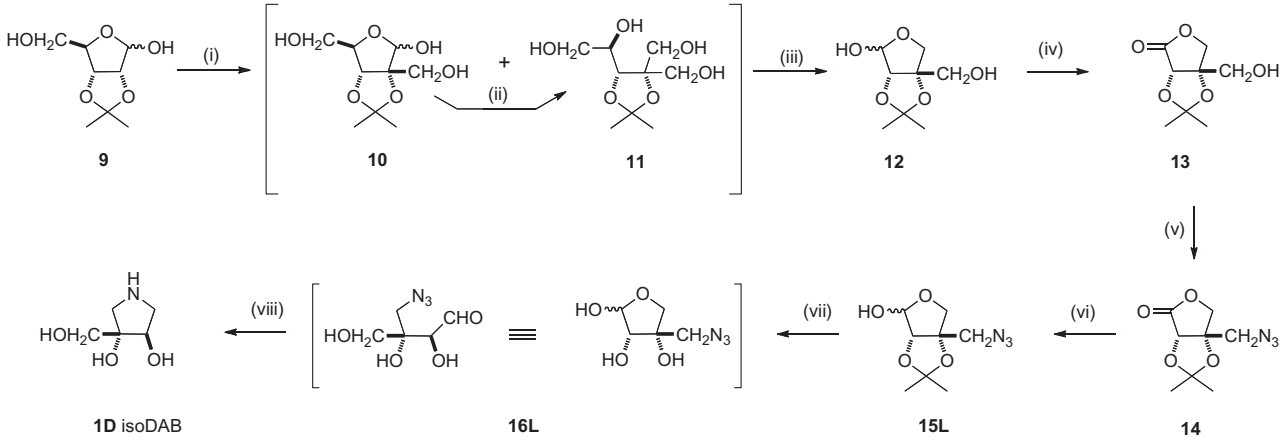


Scheme 2. NB-DNJ **3** and some carbon branched imino sugars.

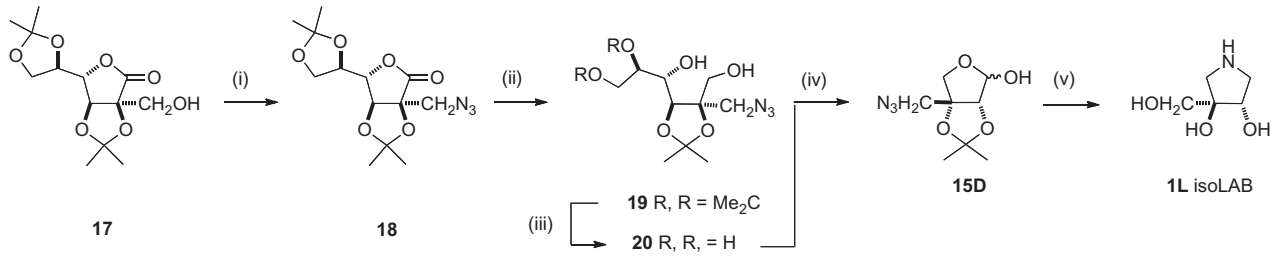
the α-glucosidase inhibition by DNJ and its alkyl derivatives, the branched analogue isofagomine **7** is a potent β-glucosidase inhibitor[18] whereas the *galacto*-analogue **8** inhibits β-galactosidases.[19]

The protected azido-L-apiose **15L** was the key intermediate for the synthesis of isoDAB **1D** (Scheme 3). Reaction of the D-ribose acetonide **9** with formaldehyde in the presence of potassium carbonate introduced the branching hydroxymethyl group via a Ho crossed aldol reaction[20] to give a mixture of the protected D-hamamelose **10** together with the tetraol **11**, resulting from a crossed Cannizzaro reaction; the mixture was treated with sodium borohydride in water to afford pure **11**. Oxidative cleavage of the 1,2-diol in the tetraol **11** with sodium periodate gave L-apiose acetonide **12**[21] [mp 70–72 °C; $[\alpha]_D^{19}$ +37.8 (*c*, 1.7). lit.[22] mp 74 °C; $[\alpha]_D^{rt}$ +39 (*c*, 1.7)] in 84% yield from **9**. Only chromatography was the final purification of **12**, which may conveniently be prepared from **9** on a multigram scale. Treatment of the lactol **12** with bromine water gave the L-apionolactone **13** [mp 90–92 °C; $[\alpha]_D^{22}$ +70.5 (*c*, 0.95)][23] in 90% yield. Esterification of the free alcohol in **13** with triflic (trifluoromethanesulfonic) anhydride in dichloromethane in the presence of pyridine gave the corresponding triflate which on reaction with sodium azide in DMF gave the L-azidolactone **14** [mp 62–64 °C; $[\alpha]_D^{17}$ +104.5 (*c*, 1.2)] in 67% yield. Reduction of the lactone **14** with diisobutylaluminum hydride (DIBALH) in dichloromethane afforded the L-azidolactol **15L** [oil; $[\alpha]_D^{21}$ +109.9 (*c*, 0.94)] in 93% yield. The isopropylidene-protecting group in **15L** was removed quantitatively by hydrolysis with acidic Dowex ion exchange resin to give 3-*C*-azidomethyl-L-erythrose (**16L**) [oil; $[\alpha]_D^{18}$ −1.9 (*c* 1.37, MeOH)] as a mixture of furanose anomers. Hydrogenation of the azidolactol **16L** in the presence of palladium (10% on carbon) caused reduction of the azide to the corresponding amine followed by an intramolecular reductive amination to give the target isoDAB **1D**[24] [oil; $[\alpha]_D^{25}$ −39.7 (*c* 0.17, H_2O)] in 71% yield. The overall yield of isoDAB **1D** from the acetonide **9** was 37% over eight steps.

The enantiomer isoLAB **1L** was synthesized from D-tagatose. The C2 hydroxymethyl group in the diacetonide **17** was introduced into D-tagatose by a Kiliani ascension, followed by acetonation (Scheme 4);[25] substrate **17** has been used as chiron in a number of syntheses.[26] The diacetonide **17** was converted into the corresponding triflate on reaction with triflic anhydride in dichloromethane in the presence of pyridine; subsequent reaction of the triflate with sodium azide in DMF gave the D-*talono*-azidolactone **18** [mp 81–83 °C; $[\alpha]_D^{25}$ −82.9 (*c* 1.0)] in 94% yield. Two-step reduction of the lactone **18** by DIBALH in dichloromethane, followed by sodium borohydride in methanol, afforded the diol **19** [oil; $[\alpha]_D^{25}$ −52.2 (*c* 1.0)] in 90% yield. Selective hydrolysis of the terminal acetonide in **19** formed the tetraol **20** [mp 98 °C; $[\alpha]_D^{25}$ −98.1 (*c* 1.0, $CHCl_3$)]



Scheme 3. (i) CH_2O, K_2CO_3, MeOH, H_2O; (ii) $NaBH_4$, H_2O; (iii) $NaIO_4$, H_2O, 84% (from **9**); (iv) Br_2, $BaCO_3$, H_2O, 0 °C to rt, 90%; (v) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −30 °C; then NaN_3, DMF, 67%; (vi) DIBALH, CH_2Cl_2, −78 °C, 93%; (vii) Dowex (50 W-X8 H^+ form) H_2O/1,4-dioxane, 4:1, 75 °C, 100%; (viii) Pd/C (10%), H_2, H_2O/AcOH, 9:1, 71%.



Scheme 4. (i) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −30 °C; then NaN_3, DMF, 94%; (ii) DIBALH, CH_2Cl_2, −78 °C; then $NaBH_4$, MeOH, 90%; (iii) H_2O/AcOH, 1:1, 83%; (iv) $NaIO_4$, H_2O, 90%; (v) Dowex (50 W-X8 H^+ form) H_2O/1,4-dioxane, 4:1, 75 °C; then Pd/C (10%), H_2, H_2O/AcOH, 9:1, 74%.

(83% yield) which on oxidation with sodium periodate cleaved the C4–C5 and C5–C6 bonds to give the isopropylidene 3-*C*-azidomethyl-D-erythrose **15D** [oil; $[\alpha]_D^{25}$ −102.1 (*c* 1.0), other data were identical to that of **15L** mentioned above] in 90% yield. Hydrolysis of the acetonide **15D** with acidic ion exchange resin afforded the deprotected azidolactol **16D** [oil; $[\alpha]_D^{25}$ +1.7 (*c* 1.3, MeOH)] which on hydrogenation gave isoLAB **1L** [oil; $[\alpha]_D^{25}$ +35.6 (*c* 0.27, H_2O)] in 74% yield. The ^{13}C and ^1H NMR spectra of isoLAB **1L** were identical to those of isoDAB **1D** $[\alpha]_D^{25}$ −39.7 (*c* 0.17, H_2O). The overall yield of isoLAB **1L** from the *talono*-diacetonide **17** was 47%.

Various assays on **1D** and **1L** were conducted to evaluate their biological properties. Glucosidase inhibition exhibited by isoDAB **1D** and isoLAB **1L** was compared with that of DAB **2D** and LAB **2L** (Table 1).[27] As previously reported, DAB **2D** was a good inhibitor of a number of α-glucosidases but also showed weak inhibition of β-glucosidases; LAB **2L** was a more potent and highly specific inhibitor of α-glucosidases. isoDAB **1D** was as potent as an inhibitor of α-glucosidases as DAB **2D** but was completely specific, showing no significant inhibition (less than 50% inhibition at 1000 μM) of any other glycosidases. DAB **2D** and LAB **2L** were good inhibitors of rat intestinal trehalase and weak inhibitors of bovine liver β-galactosidase; neither isoDAB **1D** nor isoLAB **1L** showed any inhibition of these enzymes. None of the pyrrolidines exhibited any inhibition of α-galactosidases (coffee bean and human lysosome), β-galactosidases (rat intestinal lactase), Jack bean α-mannosidase, snail β-mannosidase, *Penicillium decumbens* α-rhamnosidase and bovine epididymis α-mannosidase. DAB **2D** is a potent inhibitor of glycogen phosphorylase and has been investigated as a potential therapeutic agent for the treatment of diabetes;[28] in contrast, neither isoDAB **1D** nor isoLAB **1L** showed any inhibition.

Neither isoDAB **1D** nor isoLAB **1L** showed any effect on endoplasmic reticulum (ER) resident α-glucosidase I and II activity in cells at 0.5 mM using a free oligosaccharide assay.[29] At 1 mM, **1D** showed weak inhibition of α-glucosidase II. These data suggest that the lack of significant inhibition of glucosidase processing enzymes is due to the inability to administer sufficient concentrations of these weak inhibitors to cells to observe any effects. Both DAB **2D** and LAB **2L** are presumed inhibitors of processing glucosidases[30] and their *N*-butyl analogues are weak inhibitors (IC_{50}, 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.

Table 1
Concentration of iminosugars giving 50% inhibition of various glucosidases

Enzyme	IC_{50} (μM)			
	2D DAB	**1D** isoDAB	**2L** LAB	**1L** isoLAB
α-Glucosidase				
Rice	250 (K_i 14 μM)	41 (K_i 4 μM)	3.2	NI
Yeast	0.15	NI[a]	70	NI
Rat intestinal maltase	55 (K_i 1 μM)	24 (K_i 6 μM)	0.93	NI
Rat intestinal isomaltase	5.8	20	0.36	NI
Rat intestinal sucrase	16	15	1.0	NI
β-Glucosidase				
Almond	250	NI	NI	NI
Bovine liver	638	NI	NI	NI
Rat intestinal cellobiase	756	NI	NI	NI

NI: no inhibition (less than 50% inhibition at 1000 μM).

Cystic fibrosis is an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The most common mutation, F508del, is observed in more than 90% of patients with CF. Trafficking of F508del-CFTR mutants from the ER to the apical plasma membrane of epithelial cells is extremely inefficient.[32] Incubation of NB-DNJ **3** partially rescues the defective F508del-CFTR function.[33] Rescue of misfolded trafficking defective mutant proteins by pharmacological chaperones emerged with the finding that ligands (agonists and antagonists) increased the efficiency of receptor maturation and restored the function of these proteins.[34] In most cases, however, the mechanism of pharmacological rescue is not clearly understood but it supports a binding of the corrector to an active site of the F508del-CFTR protein. NB-DNJ **3**, an inhibitor of α-glucosidases, is a partial corrector of F508del-CFTR; a hypothesis to explain the mechanism of action of **3** was that inhibition of the α-1,2 glucosidase disturbs the ER quality control, more specifically the interaction between the ER lectin calnexin and F508del-CFTR, and allows the restoration of F508del-CFTR to the plasma membrane; whether the glucosidase inhibition of **3** is associated with the chaperone activity has yet to be established. A host of chaperones, enzymes and regulatory proteins control the folding, complex assembly and ultimately exit of secretory proteins, which can be viewed as the clients of this ER machinery and as potential corrector targets.[35]

The potential corrector effect of isoDAB **1D** and isoLAB **1L** on CFTR function in CF-KM4 cells[36] was assessed using single-cell fluorescence imaging (Fig. 1).[37] The cells were treated for 2 h with 100 μM of isoDAB **1D**, isoLAB **1L** or NB-DNJ **3** and then CFTR proteins were stimulated by a cocktail of forskolin (Fsk) + genistein (Gst). Figure 1A shows an example of the typical trace obtained in untreated or isoLAB-treated CF-KM4 cells. As expected, no variation of the fluorescence was observed after the cocktail stimulation in untreated CF-KM4 bearing the defective F508del-CFTR protein (Fig. 1A). However, following a treatment of CF-KM4 cells with isoLAB **1L**, a strong increase of the recorded fluorescence signal after Fsk + Gst stimulation (Fig. 1A) was observed. The signal was then fully inhibited by the CFTR inhibitor $CFTR_{inh}$-172 (Fig. 1A). These results are in accordance with a restoration of F508del-CFTR protein to the cell surface after 2 h of isoLAB treatment. Similarly, CF-KM4 cells were treated with isoDAB **1D** or NB-DNJ **3**; CFTR

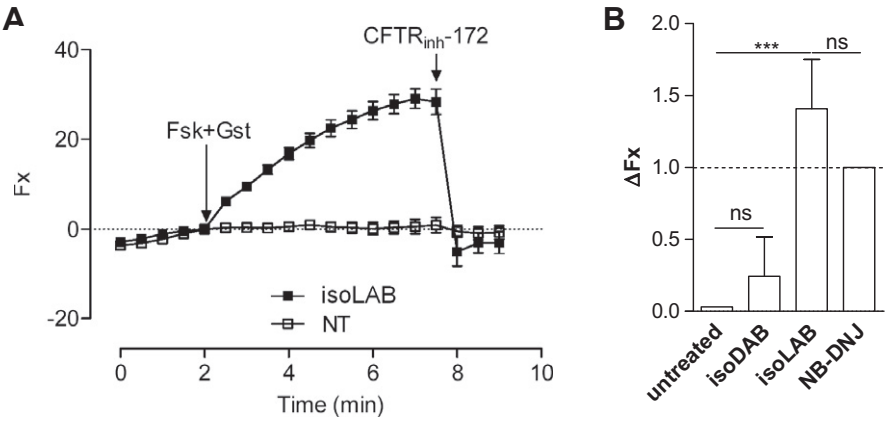


Figure 1. Functional evaluation of F508del-CFTR activity by single-cell fluorescence imaging assay in CF-KM4 cells treated or not with imino-sugar (100 μM, 2 h). (A) Examples of typical time courses obtained with untreated cells or with cells treated with isoLAB **1L**. Data represent the mean (± SEM) of the relative fluorescence collected from all the cells of a field (n = 12). A mixture of forskolin (Fsk, 10 μM) + genistein (Gst, 30 μM) is used to activate CFTR. $CFTR_{inh}$-172 (10 μM) is used to inhibit CFTR. (B) Histograms summarizing the results collected from separate experiments (N = 2; with a total of 24 cells). ***p <0.001; ns, not significant.[31]

activity was then measured. Figure 1B summarizes the results obtained in several separate experiments by stimulation of Fsk + Gst on untreated or treated CF-KM4 cells; NB-DNJ **3** an iminosugar which has already been described as a F508del-CFTR corrector was used as a positive control. The corrector effect of isoLAB **1L** and of NB-DNJ **3** was confirmed, but no effect of isoDAB **1D** was observed (Fig. 1B). isoLAB **1L**—in contrast with NB-DNJ **3**—did not show any inhibition of any glycosidases. It may be that the chaperone effect of iminosugars need not be due to glycosidase inhibition. isoLAB **1L** is likely to have value in studies of the mechanisms of chaperoning the folding of CFTR.

In summary, this Letter reports the efficient syntheses of isoDAB **1D** (in an overall yield of 37% from isopropylidene-ribose **9**) and isoLAB **1L** (in an overall yield of 47% from a diacetonide derived from tagatose **17**) with only acetonides as the protecting groups. isoDAB **1D** was a potent and specific inhibitor of α-glucosidases and is the first example of a carbon-branched pyrrolidine that is a potent glycosidase inhibitor; in contrast, isoLAB **1L** does not inhibit any glycosidases. The rescue of CFTR function in CF-KM4 cells by isoLAB **1L** suggests that there may be other mechanisms, other than glycosidase inhibition, for iminosugars to act as chaperones.

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