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The 'mirror-image' postulate as a guide to the selection and evaluation of pyrrolidines as α-L-fucosidase inhibitors

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ABSTRACT

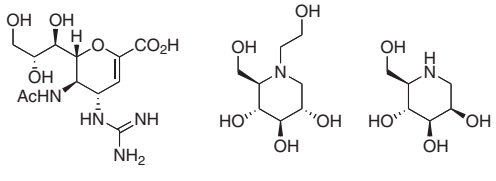
The ability of a series of pyrrolidines to inhibit several glycosidases was investigated. Using Fleet's 'mirror-image postulate', it was proposed that enantiomeric derivatives of 1,4-dideoxy-1,4-imino-D-lyxitol (a known α-D-galactosidase inhibitor) would show inhibitory activity against α-L-fucosidases. Some modest α-L-fucosidase inhibitory activity was observed for selected compounds (particularly an aminomethyl pyrrolidine) and it was proposed that better activity could be obtained by modifying the C-2 side chain of the pyrrolidine core. The D-*galacto* carbamate scaffold also exhibited somewhat selective, albeit modest, α-L-fucosidase inhibitory activity and may prove to be an interesting scaffold for further development.

1. Introduction

Glycosidases are involved in a wide range of important biological processes and accordingly, glycosidase inhibitors have much potential in the treatment of diseases such as viral infection, cancer and genetic disorders.[1] While some effective glycosidase inhibitors, including the anti-influenza drug Zanamivir (**1**)[2] and the α-glucosidase anti-diabetic drug Miglitol (**2**)[3] (Fig. 1), have been developed by considering the stereochemistry of the enzyme's native carbohydrate substrate, mimicking the native substrate is not an all-encompassing strategy. Indeed, a number of glycosidase inhibitors with striking activity against seemingly unrelated enzymes have been discovered, including the piperidine deoxymannojirimycin (DMJ) (**3**), which is a more potent inhibitor of α-L-fucosidase than of α-D-mannosidase.[4] Five-membered iminosugars, or pyrrolidines, also exhibit potent glycosidase activity, and in some cases, have given higher inhibition than their six-membered ring counterparts.[5]

One approach that has been considered in the development of glycosidase inhibitors is that of the mirror-image enzyme postulate.[6–8] There are several examples of mirror-image enzyme active sites in nature[9] and Fleet has proposed that this may be a general structural theme for many carbohydrate-processing enzymes. Despite a lack of knowledge about the 3D structure of rhamnosidases, Fleet successfully predicted that L-swainsonine (L-**4**) (Fig. 2) would be a powerful inhibitor of L-rhamnosidase on the basis of D-swainsonine (D-**4**) being a strong inhibitor of D-mannosidase.[6] Similarly, polyhydroxylated pyrrolidine L-**5**, the enantiomer of known D-mannosidase inhibitor D-**5**, was found to exhibit good and selective inhibition of L-rhamnosidase.[6] This 'mirror-image' postulate has been further supported by Fleet,[7] and more recently by Davis and co-workers who illustrated that racemic iminothreitol azasugars typically showed good competitive inhibition of α-D-mannosidase and α-L-rhamnosidase, while racemic iminoerythritol azasugars (e.g., D/L-**6**) showed good inhibition of α-D-galactosidase and α-L-fucosidase.[10] In all instances, however, fine-tuning of the chiral scaffold was required for optimal inhibitory activity.

Given the difficulties in predicting the glycosidase inhibition activity of any given iminosugar, we became interested in further exploring Fleet's mirror-image postulate, and in particular, the idea that the good (μM) α-galactosidase activity of 1,4-dideoxy-1,4-imino-D-lyxitol (**7**)[11] (Fig. 2) suggests that derivatives of the corresponding enantiomer 1,4-dideoxy-1,4-imino-L-lyxitol (**8**) may have good inhibitory activity against α-L-fucosidase. Indeed, others have applied a similar logic to justify the development of methodology



Zanamivir (**1**) Miglitol (**2**) DMJ (**3**)

Figure 1. Representative glycosidase inhibitors.

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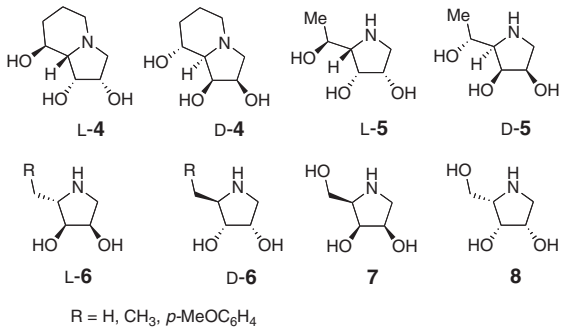
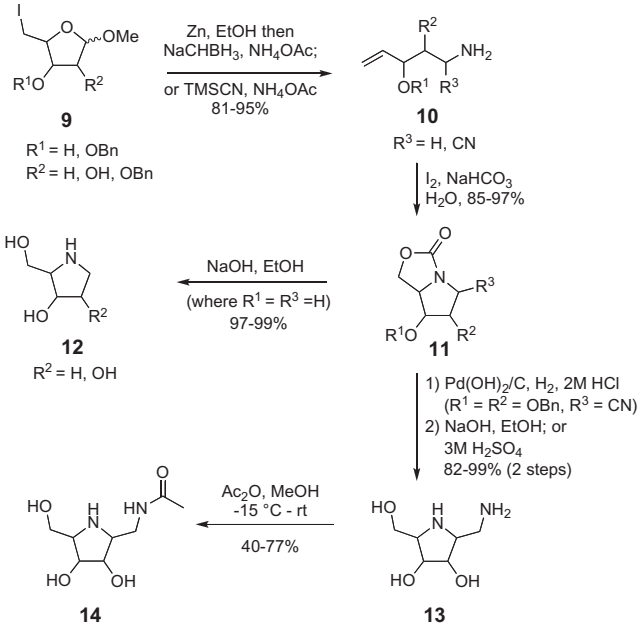


Figure 2. Known and proposed glycosidase inhibitors.



Scheme 1. Synthesis of pyrrolidines.

for the synthesis of L-piperidines given that their D-counterparts have good α-galactosidase (or other) inhibitory activity.[12,13] That said, the mirror-image framework is not all encompassing for although D-lyxitol (**7**) is a good α-D-galactosidase inhibitor,[8,11] it also exhibits modest α-L-fucosidase inhibitory activity.[8] Our interest, however, lies in using the postulate as a guide to the selection of potential α-L-fucosidase inhibitors. The aberrant distribution of α-L-fucosidase is relevant to inflammation,[14] cancer[15,16] and cystic fibrosis,[17] and the generation of selective α-L-fucosidase inhibitors therefore provides an important tool by which to study enzymatic function when developing potential therapeutics.[16,18] To date, C1 or C5-substituted fuconojirimycin (FNJ) derivatives have been found to be particularly effective inhibitors of α-L-fucosidase with K_i values in the picomolar range being observed for several inhibitors containing 1-amidomethyl substituents.[18,19] Accordingly, exploring the α-L-fucosidase inhibitory activities of 1-amino or amidomethyl fuctionalised pyrrolidines may provide further inhibitors of merit.

2. Results and discussion

To prepare our target pyrrolidine inhibitors, we employed our recently developed I_2-mediated carbamate annulation methodology[20–22] as a key step (Scheme 1). As previously described, the pyrrolidines could be prepared from readily available iodo-furanosides **9**, which were subjected to a Vasella reaction and subsequent reductive amination[23] or asymmetric Strecker reaction[24] to give alkenylamines **10**. The highly 4,5-*cis* stereoselective I_2-mediated carbamate annulation then followed (**10** → **11**). Where $R^1 = R^3 = H$, R^2 = H, OH, base-mediated hydrolysis then gave the hydroxy-pyrrolidines **12** in good (48–57%) overall yield and this five-step route was achieved without the need for protecting groups. To prepare the aminomethyl-pyrrolidines **13**, the benzyl protecting groups in carbamate **11** (R^1 = Bn, R^2 = OBn, R^3 = CN) were removed and the nitrile simultaneously reduced under the agency of H_2 and catalytic $Pd(OH)_2/C$. Acid or base hydrolysis then gave the amino-imino-hexitols **13** in 36–39% overall yield. Finally, treatment of **13** with acetic anhydride in MeOH gave the corresponding acetamides **14**.[25]

Using this annulation strategy, a number of potential glycosidase inhibitors were prepared (Fig. 3). These included 1,4-dideoxy-1,4-imino-L-lyxitol (**8**),[21] 1,4-dideoxy-1,4-imino-L-xylitol (**16**),[26] 1,4-dideoxy-1,4-imino-D-xylitol (**18**)[21] and 1,2,4-trideoxy-1,4-imino-L-xylitol (**20**)[27] and their corresponding carbamates (**15**,[21] **17**,[26] **19**,[21] and **21**,[27] respectively). Of the aminomethyl-pyrrolidines, we were interested in those of the D-*galacto* (**22**, **23** and **24**)[25] and L-*altro* (**25**, **26** and **27**)[25] configurations. Based on the mirror-image postulate, these previously undisclosed isomers were anticipated to have good inhibitory activity of α-L-fucosidases.

With our potential inhibitors in hand, the polyhydroxylated pyrrolidines were first tested against α-L-fucosidase, α-D-glucosidase and α-D-mannosidase (Table 1). Enzymes other than α-L-fucosidase were chosen to provide an indication about the selectivity of enzyme inhibition because some L-iminosugars, especially pyrrolidines, can mimic the conformation of natural D-hexose substrates[28] (which is presumably due to the high structural flexibility of the pyrrolidine core). Our results, however, illustrated that the hydroxy pyrrolidines on the whole showed no or poor inhibitory activity against α-L-fucosidase and where α-L-fucosidase inhibitory activity was observed (e.g., **16**) this was not selective. These results were discouraging, however, it was interesting to note that carbamates **15** and **17** had some inhibitory activity against α-D-glucosidase, thus suggesting that further functionalisation of this carbamate scaffold could generate new classes of iminosugar inhibitors. In all instances, active inhibitors were found to bind competitively to the enzymes (see Supplementary data for further details). The less complex 1,2,4-trideoxy-imino xylitol **21** and corresponding carbamate **20**, however, showed no inhibitory activity and thus appear to have little value as glycosidase inhibitors. It is also interesting to compare our studies to the earlier findings by Fleet and co-workers, whereby 1,4-dideoxy-1,4-imino-D-lyxitol (**7**) and 1,4-dideoxy-1,4-imino-L-lyxitol (**8**) showed comparable, albeit modest, inhibition of an α-L-fucosidase from *Bovine epididymis* (IC_{50} = 98 and 80 μM, respectively).[8] This highlights the differences in inhibition of different enzymes of the same family given that **8** in our assay showed no inhibition of α-L-fucosidase from *Thermotoga maritime*. Enantiomer **8** in Fleet's assay, however, exhibited much greater selective inhibition of α-L-fucosidase than D-*lyxitol* **7** and this observation supports the validity of using the mirror image postulate as a guide to the identification of more selective (and therefore better) α-L-fucosidase inhibitors.

Next we turned our attention to the glycosidase inhibitory activities of the aminomethyl pyrrolidines. As illustrated (Table 2), better α-L-fucosidase activity was observed for the aminomethyl pyrrolidines on a whole, and in particular, by the free amine (**25**) and acetamide (**27**) of the L-*altro* series with K_i values of 0.2 and 0.12 mM, respectively. The D-*galacto* aminomethyl pyrrolidine **22** and carbamate **23** exhibited some α-L-fucosidase activity (with K_i