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Looking-Glass Synergistic Pharmacological Chaperones: DGJ and L-DGJ from the Enantiomers of Tagatose

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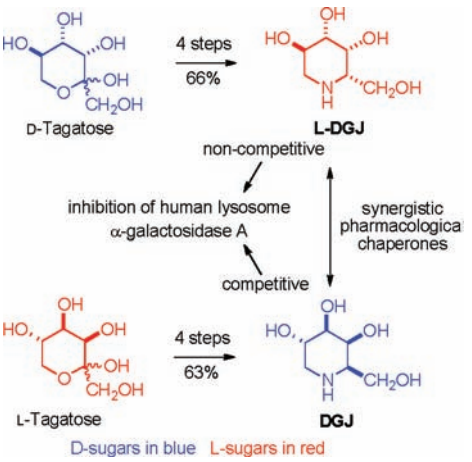
ABSTRACT



The enantiomers of tagatose are converted to L-DGJ [a noncompetitive inhibitor of human lysosome α-galactosidase A (α-Gal A), K_i 38.5 μM] and DGJ [a competitive inhibitor of α-Gal A, K_i 15.1 nM] in 66% yield. L-DGJ and DGJ provide the first examples of pharmacological chaperones that (a) are enantiomeric iminosugars and (b) have synergistic activity with implications for the treatment of lysosomal storage disorders and other protein deficiencies.

This paper reports concise syntheses of L-DGJ **1L** and DGJ **1D** from the enantiomers of tagatose in an overall yield of 66%. L-DGJ **1L** and DGJ **1D** provide the first example of both enantiomers of an iminosugar DGJ **1** acting as pharmacological chaperones; enantiomeric glycosidase inhibitors are well-established.

DGJ **1D** is a competitive nanomolar- and L-DGJ **1L** a noncompetitive micromolar-inhibitor of a number of α-D-galactosidases;[1] similarly, DFJ **2L** (the 6-deoxy analogue of L-DGJ) is a competitive nanomolar, and its enantiomer **2D** a noncompetitive micromolar, inhibitor of α-L-fucosidases.[2]

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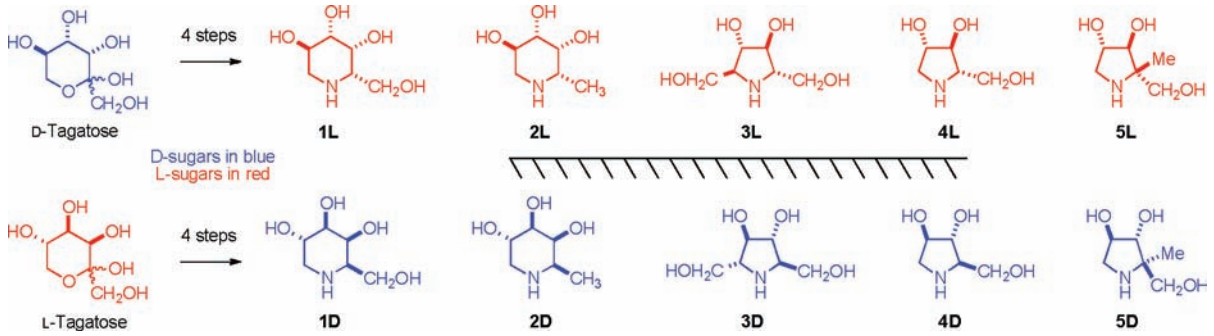

Figure 1. Enantiomeric iminosugar inhibitors of the *same* enzymes.

Many enantiomeric pairs of iminosugars inhibit the same glycosidases; the natural products DMDP **3D** and DAB **4D**, and 4-*C*-methyl DAB **5D** are competitive inhibitors of D-glucosidases, whereas their L-enantiomers L-DMDP **3L**,[3] LAB **4L**[4] and 4-*C*-methyl-LAB **5L**[5] are more potent noncompetitive inhibitors of the *same* enzymes (Figure 1).

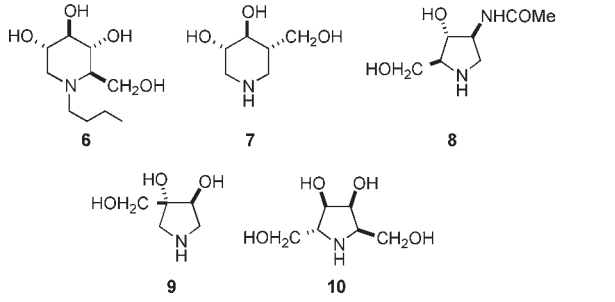

Figure 2. Examples of iminosugars as pharmacological chaperones.

Iminosugars[6] have a wide range of chemotherapeutic applications,[7] including their ability to act as small molecule pharmacological chaperones to assist protein folding and stabilization of their native conformation.[8] Several iminosugars, including Miglustat **6** and isofagomine **7**,[9] are being tested as chaperones for Gaucher's disease (Figure 2);[10,11] **6** and LABNAc **8** have potential as chaperones for late-onset Tay-Sachs disease.[12] Also, **6** and isoLAB **9**[13] partially rescue the defective F508del-CFTR function in CF-KM4 cells[14] and may provide a strategy for the chemotherapy of cystic fibrosis.[15]

In 1999, Fan put forward the concept of active site-specific chaperone-mediated therapy for lysosomal storage diseases, using iminosugars to prevent misfolding and premature degradation of mutant enzymes in the endoplasmic reticulum.[16] DGJ [Amigal] **1D** is a chaperone for the treatment of Fabry's disease, a lysosomal storage disorder caused by an X-linked inherited deficiency of α-galactosidase A (α-Gal A; EC 3.2.1.22). Deficiency of α-Gal A activity results in the progressive accumulation of globotriaosylceramide (Gb3) in the lysosomes of vascular endothelial cells.[17] The severity of the disease is well correlated

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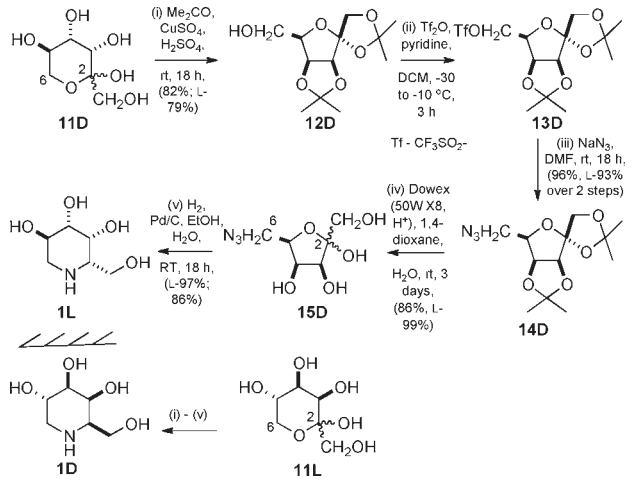
with the residual enzyme activity. Residual α-Gal A activity in lymphoblasts derived from Fabry patients and in tissues of R301Q or Q279E α-Gal A transgenic mice was enhanced by treatment with DGJ [Amigal] **1D**, a competitive inhibitor of α-Gal A.[18] DGJ acts as a pharmacological chaperone that stabilizes the native folding state in the endoplasmic reticulum (ER) by occupying the active site of the mutant enzyme, thus allowing its maturation and trafficking to the lysosome.[19] Clinical trials of Amigal in patients with Fabry disease are encouraging; other piperidine[20] and pyrrolidine inhibitors of α-Gal A, such as **10**,[21] are also being evaluated. This paper reports that L-DGJ **1L** shows activity as a pharmacological chaperone and provides the first example of both enantiomers of an iminosugar acting as chaperones, and that its mode of action is synergistic with that of DGJ. Concise syntheses of **1L** and **1D** from the enantiomers of tagatose are also described

Scheme 1. Enantiomers of DGJ **1** from Tagatose **11**



The synthesis of L-DGJ **1L** from D-tagatose **11D** in 66% overall yield requires introduction of an azide at C-6 followed by an intramolecular reductive amination with the carbonyl at C-2 (Scheme 1). D-Tagatose, until recently a rare and expensive sugar, is now readily available from galactose[22] as a food substitute in soft drinks and ready to eat cereals;[23] it has been used as a chiron[24] for the synthesis of more complex targets.[25] L-DGJ **1L** has previously been prepared by multistep syntheses from Garner's aldehyde.[1,26]

Reaction of D-tagatose **11D** with acetone, copper(II) sulfate and catalytic sulfuric acid gave the diacetonide **12D** [mp 55−58 °C; $[\alpha]_D^{22}$ +66.3 (*c*, 1.04)][27] in 82% yield. Esterification of the primary hydroxyl group in **12D** with triflic anhydride in dichloromethane in the presence of pyridine gave the crystalline triflate **13D** [mp 44−46 °C; $[\alpha]_D^{22}$ +43.6 (*c*, 0.89)] which, on treatment with sodium azide in DMF, afforded the azide **14D** [oil, $[\alpha]_D^{22}$ +44.5 (*c*, 1.01)] in 96% yield for the two steps. Removal of the acetonide protecting groups in **14D** with Dowex resin (50W X8, H^+) in water proceeded slowly to give 6-azidotagatose **15D** [oil $[\alpha]_D^{21}$ +15.6 (*c*, 1.0 in MeOH)] as a 3:1 mixture of anomers after 3 days in 86% yield; heating the reaction mixture led to extensive decomposition. Hydrogenation of the azide gave the corresponding amine which underwent a highly stereoselective intramolecular reductive amination to afford L-DGJ **1L** $[\alpha]_D^{25}$ −9.2 (*c*, 0.43 in H_2O) in 97% yield; for **1L** as the HCl salt $[\alpha]_D^{25}$ −54.8 (*c*, 0.16 in H_2O).[28] The enantiomer, DGJ **1D**, {$[\alpha]_D^{25}$ +9.0 (*c*, 0.16 in H_2O); **1D** as HCl salt[29] $[\alpha]_D$ +53.7 (*c*, 0.1 in H_2O)} was synthesized by an identical route from L-tagatose **11L**.[30] L-Tagatose, a less available hexose than its enantiomer **11D**, is accessible by green biotechnology from L-psicose,[31] L-sorbose,[32] or galactitol.[33]

The biological activity of L-DGJ **1L** was compared with DGJ **1D**. The inhibition constant (K_i) and the mode of inhibition of DGJ and L-DGJ were determined by Lineweaver−Burk plots. Whereas DGJ was a competitive inhibitor of α-Gal A (K_i 15.1 nM), L-DGJ showed noncompetitive inhibition about ∼1000 times weaker (K_i 38.5 μM). L-DGJ showed enhancement of activity in Fabry R301Q fibroblasts. Treatment with L-DGJ for 3 days dose-dependently increased intracellular α-Gal A activity, with maximal increase of 10.8-fold at 10 mM (Figure 3B).

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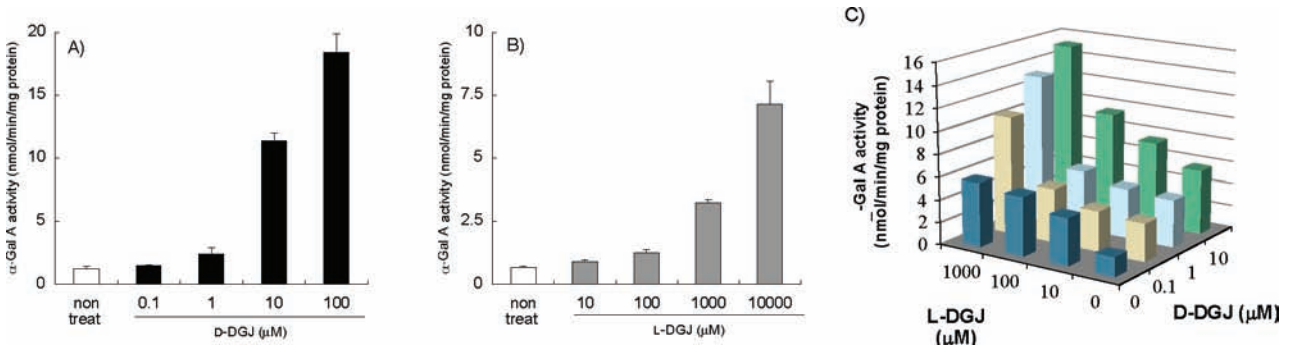

Figure 3. Influence of DGJ **1D** and L-DGJ **1L** on α-Gal A activity in Fabry R301Q fibroblasts. (A) DGJ **1D** was added to the culture medium of R301Q cells at a concentration of 0.1−100 μM. (B) L-DGJ was added to the culture medium of R301Q cells at a concentration of 10−10000 μM. (C) Combination of DGJ (0.1−10 μM) and L-DGJ (10−1000 μM). Cells were subsequently incubated for 3 days; cell growth was not affected by the inclusion of DGJ and L-DGJ.

This enhancement of activity was similar to that observed with 10 μM DGJ (Figure 3A). The *in vitro* inhibitory effect of L-DGJ on human α-Gal A was ~1000 times less than DGJ. We further investigated whether a combination effect was manifested upon Fabry R301Q fibroblasts when DGJ (a competitive inhibitor) and L-DGJ (a noncompetitive inhibitor) were administrated simultaneously. Very interestingly, each enantiomer did not counteract the chaperone effects of the other enantiomer but clearly showed dose−response synergistic effects (Figure 3C). These results indicate that L-DGJ could be of therapeutic benefit to Fabry disease, and that the combination of the competitive inhibitors and noncompetitive inhibitors may be broadly applicable to lysosomal storage disorders and other protein deficiencies.

In summary, this paper describes the concise synthesis of DGJ **1D** and L-DGJ **1L** from L- and D-tagatose respectively in just four steps, and shows the value of the enantiomers of tagatose as chirons. As with other α-galactosidases L-DGJ was found to be an inhibitor of α-Gal A, about 1000-fold weaker than its enantiomer DGJ. L-DGJ was also found to be a chaperone for α-Gal A, again about 1000 times less active than DGJ. Noncompetitive inhibition by **1L** is consistent with binding at a different site than **1D** which shows competitive inhibition and binds at the active site. While the inhibition of the same glycosidase by both enantiomers of an iminosugar is well-known, this is the first example of enantiomeric iminosugars acting as synergistic pharmacological chaperones for misfolded proteins; this result may indicate that binding at the active site of the enzyme is not necessary.

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Supporting Information Available. Experimental procedures and full spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.