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Azasugar and Glycal Inhibitors of α-L-Fucosidase

David P. Dumas, Tetsuya Kajimoto, Kevin K.-C. Liu, and Chi-Huey Wong*
Department of Chemistry, The Scripps Research Institute
La Jolla, CA 92037

David B. Berkowitz and Samuel J. Danishefsky
Department of Chemistry, Yale University
New Haven, CT 06511

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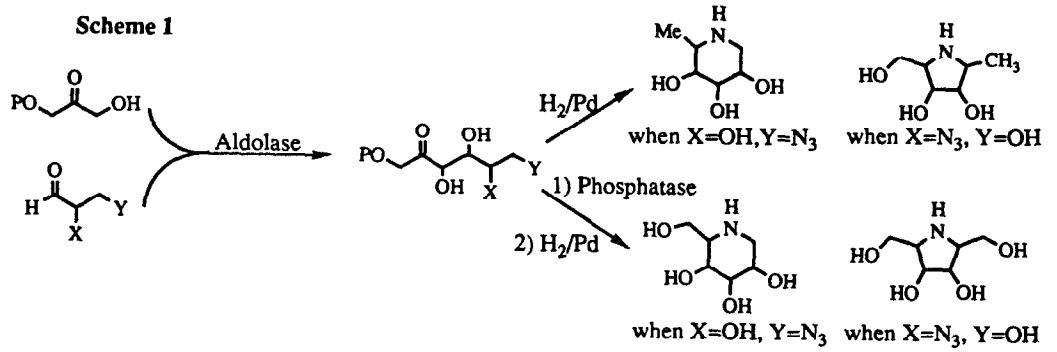
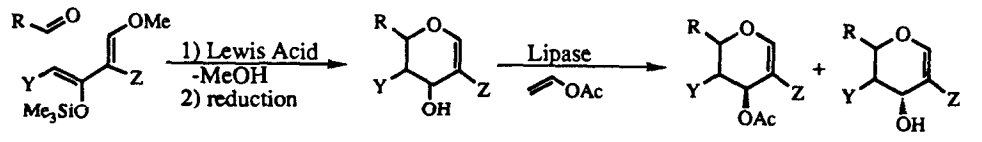
Abstract: Several azasugar and glycal inhibitors were studied to map the active site of α-L-fucosidase enzyme.

Inhibitors of glycosidases are of interest as potential anti-viral,[1] anti-neoplastic agents,[2] and as tools for studying oligosaccharide metabolic pathways.[3] Among the glycosidases, α-L-fucosidase is particularly interesting as a target for inhibitor development. The deficiency of fucosidase in mammals leads to the lysosomal storage disease fucosidosis.[4] Inhibitors for fucosidase might therefore prove useful in the development of animal and cell culture models for the study of this disease. Additionally, the development of inhibitors for fucosidase might provide important clues to the importance of fucosylation in tumor cells.[5] Winchester *et al.* have described the inhibition of human liver fucosidase with the azasugar deoxyfuconojirimycin and some analogs of this compound.[6] This report further explores the use of azasugars synthesized via aldolase reactions (**scheme 1**)[7] as inhibitors of fucosidase as a means of mapping the active site of the bovine kidney enzyme. These studies also include by the analysis of several glycal inhibitors synthesized by a Lewis acid catalyzed diene-aldehyde cyclocondensation followed by lipase-catalyzed resolution as shown in **scheme 2**.[8]

Scheme 1

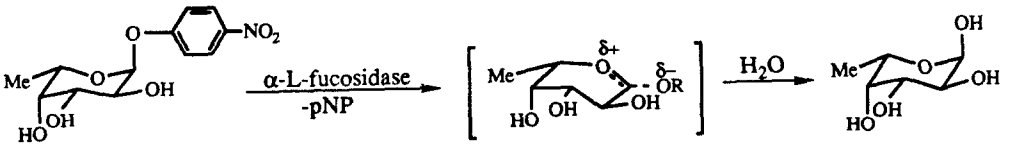


Scheme 2



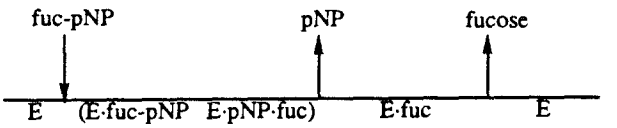
Previous studies with fucosidase have implicated the involvement of two carboxylate catalytic groups in the hydrolysis of *p*-nitrophenyl-α-L-fucoside (fuc-pNP) with a general acid-base mechanism.[9] Hydrolysis occurs with retention of configuration,[10] suggesting a double displacement mechanism or a directed hydroxyl attack with a oxocarbonium ion mechanism as has been proposed for many of the other stereochemistry retaining glycosidases (**scheme 3**).[11]

Scheme 3



Fucose is a competitive inhibitor versus fuc-pNP (K_i=0.30±0.01mM) and phenol, used as an analog of *p*-nitrophenol, is a noncompetitive inhibitor (K_{ii}=112±16mM and K_{is}=0.18±0.02mM). The kinetic mechanism implicated from these experiments is uni-bi sequential ordered with *p*-nitrophenol being released first and fucose released second (**scheme 4**).

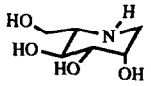
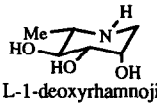
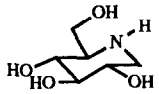
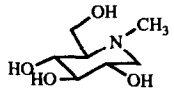
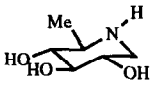
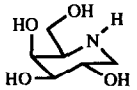
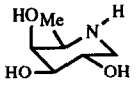
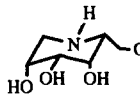
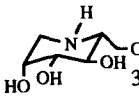
Scheme 4

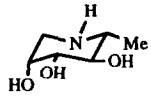
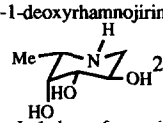
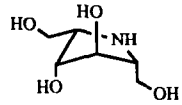
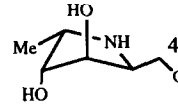
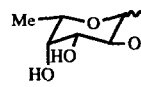
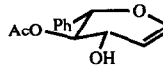
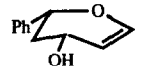
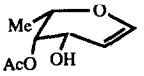
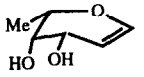


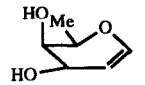
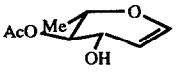
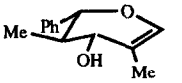
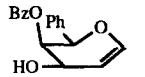
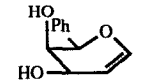
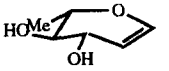
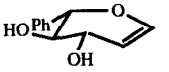
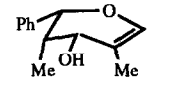
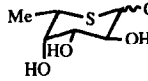
All of the azasugars and most of the glycal inhibitors studied proved to be competitive inhibitors versus fuc-pNP.[12] A comparison of the L-deoxymannojirimycin (**1**) to the 1,6-dideoxyazasugar analog (**2**) shows a 90-fold difference in K_i's. The difference is not as pronounced with the D-azasugars, but in all cases except **8**, binding is diminished with hydroxylation at the 6 position (**3** compared with **5** and **6** compared with **7**). The hydroxyl groups at C-4 and C-3 do not affect binding significantly in the D-series deoxyazasugars (compare **4** with **6** and **5** with **7**). The C-4 OH group is, however, important for the L-series glycals (**18** versus **24**). Adjustment of the C-2 hydroxyl of mannojirimycin from axial to equatorial to give 1-deoxynojirimycin (**3**) essentially abolishes all inhibitory activity. The D-talose-type azasugar, **8**, has about the same inhibition as deoxymannojirimycin (**9**) and 5-thio-L-fucose (**28**).[13] Removal of the 6-hydroxy group of mannojirimycin to give **10** improves the inhibition slightly, but does not reach the level for fuconojirimycin (**11**) inhibition. The pyrrolidine inhibitor **12** mimics the half-chair-like fucosyl cation. The 6-deoxy analog **13** combines the advantages of the half-chair-like conformation of the 5-member ring, a positive charge character upon binding, and the advantages gained by dehydroxylation at the six position as is the trend observed for the pyranose azasugars. This compound is the most potent inhibitor developed in this study.

Of the glycal compounds, L-fucal (**18**, K_i=0.05±0.01 mM) was the best inhibitor. There is little change in the inhibition constant for this compound when the 4-hydroxy group is acylated (**17**), but inversion of stereochemistry at C-4 (**24**) results in weak uncompetitive inhibition (K_i=23±5 mM). Five of the glycals, **19, 20, 23, 24,** and **25**, showed uncompetitive inhibition indicating these compounds have a much greater affinity for the ES complex than for the free enzyme. None of the

Table 1: α-Fucosidase Inhibitors Versus *p*-Nitrophenyl-α-L-Fucoside, pH 5.5, 25°C

Compound	K_i (mM)
1  L-1-deoxymannojirimycin	90±50
2  L-1-deoxyrhamnojirimycin	1.00±0.07
3 	>100
4 	5.3±0.4
5 	1.7±0.3
6 	34±7
7 	7.9±0.9
8  D-1-deoxytalonojirimycin	$53x10^{-3}$±$6x10^{-3}$
9  D-1-deoxymannojirimycin	$30x10^{-3}$±$10x10^{-3}$

Compound	K_i (mM)
10  D-1-deoxyrhamnojirimycin	$11x10^{-3}$±$1x10^{-3}$
11  L-1-deoxyfuconojirimycin	$2.9x10^{-5}$±$0.1x10^{-5}$
12 	0.91±0.02
13 	4 x 10^{-3}±2 x 10^{-3}[a]
14  L-fucose	0.30±0.01
15 	>100[b]
16 	>100[b]
17 	0.09±0.02[b]
18  L-fucal	0.05±0.01

Compound	K_i (mM)
19  D-fucal	UC: 35±3[b]
20 	UC: 26±3[b, c]
21 	>100[b]
22 	>100[b]
23 	UC: 83±20[b, c]
24 	UC: 23±5[c]
25 	UC: 22±2[b, c]
26 	>100[b]
27 	NC: K_{ii}=112±16 K_{is}=0.18±0.02[c]
28 	0.08[d]

[a] The ratio of pseudo-axial to pseudo-equatorial for the methyl group is 6. [b] Reactions in 10% MeOH.
[c] UC = uncompetitive inhibition, NC = noncompetitive inhibition. [d] Hashimoto *et al.* (1990).

D-glycals bind to any significant degree to the free enzyme form with D-fucal (**19**) and **23** being uncompetitive inhibitors with K_{ii}=35±3 and 83±20 mM, respectively.

In summary, compounds that mimic the L-fucosyl cation (the oxonium ion) by possessing a half-chair-like conformation and a positive charge are potent inhibitors of L-α-fucosidase. It appears that the 5-membered ring pyrrolidines fit these requirements and are exceptional inhibitors -- findings which are consistent with our previous studies on other glycosidases.

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11. Sinnott, M.L. *Chem. Rev.* **1990**, *90*, 1171.
12. Bovine kidney α-L-fucosidase and fuc-pNP were purchased from Sigma Chemicals. Deoxymannojirimycin was purchased from Boehringer Mannheim. The remaining azasugars were synthesized as previously described[7] as were the glycals.[8] 1-Deoxyfuconojirimycin was synthesized as described in ref. 6. Enzymatic activity was measured by incubating the enzyme with fuc-pNP in 50 mM Acetate buffer, pH 5.5 (total volume 0.4 mL) at 25°C in the presence and absence of the various inhibitors for 20 minutes (this time was determined empirically to be within the linear region of the enzymatic reaction). After incubation, the reaction was quenched by addition of 0.8 mL 2M glycine buffer, pH 10.5. The optical density at 400 nm was measured to determine the amount of liberated p-nitophenol (ε_{400}= 18.4 $mM^{-1}cm^{-1}$). Inhibition data was fit by multidimensional nonlinear regression analysis to the following appropriate equations using Sigmaplot 4.0 by Jandel Scientific.
competitive inhibition: $v = V_{max}S/[S + K_m(1+I/K_{is})]$
noncompetitive inhibition: $v = V_{max}S/[S(1+I/K_{ii}) + K_m(1+I/K_{is})]$
uncompetitive inhibition: $v = V_{max}S/[S(1+I/K_{ii}) + K_m]$
K_{is} is the slope inhibition constant and K_{ii} is the intercept inhibition constant.
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