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New Leads for Selective Inhibitors of α-L-Fucosidases. Synthesis and Glycosidase Inhibitory Activities of [(2*R*,3*S*,4*R*)-3,4-Dihydroxypyrrolidin-2-yl]furan Derivatives

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Abstract—Readily derived from D-glucose, 5-[(2*R*,3*S*,4*R*)-3,4-dihydroxypyrrolidin-2-yl]-2-methyl-3-furoic esters and amides are selective and competitive inhibitors ($K_i \geq 3\,\mu M$) of α-L-fucosidase from bovine epididymis and from human placenta.

Inhibitors of glycosidases are useful tools for the study of the biological function of oligosaccharides.[1] They are also potential drugs against diseases where the control of oligosaccharide metabolism can be linked to cellular dysfunction.[2] The control of *N*-linked oligosaccharides biosynthesis to alter cell surface expression in malignant cell[3] or affect viral membrane protein folding and assembly,[4] has therapeutic implications.[5] L-Fucose residue in sialyl Lewis X tetrasaccharide expressed on the surface of leukocyte and some tumor cells is essential for their adhesion to the endothelial-leukocyte adhesion molecules.[6] Fucosidase in invasive human ovarian carcinoma cell mediates degradation of the subendothelial extracellular matrix.[7a] Inhibitors of α-L-fucosidases inhibit the cytopathic effect of HIV and reduces infection.[7b,8] These findings have stimulated the invention of different α-L-fucosidase inhibitors, the most potent, at the moment, 1,5-dideoxy-1,5-imino-L-fucitol (**1**),[9] exhibits K_i values of 3–10 nM. Such remarkable inhibition constants reflect some of the strongest interactions of carbohydrate analogues with proteins known to date. Monosaccharide mimics often lack protein specificity; furthermore, to become a drug a good inhibitor must satisfy a number of conditions[10] such as stability in the stomach and membrane permeability, which often requires the presence of lipophilic moieties that simple sugar mimetics do not have. Any modification of **1** such as methyl side chain extension or removal,[11,12] epimerization at one of the chiral centers, *N*-alkylation[12] or introduction of α-aminomethyl[13] or other alkyl groups[12,13] reduces the inhibitory power significantly.[14] Even L-fucosamidrazone **2** is less active than **1** by at least a factor of 100.[15] More successful is the imine **3** which is almost as active as **1**.[16] Removal of the methyl group of **1** produces the moderate inhibitors **4**[12] and **5**[17] confirming that **1** does not allow much changes to be made to its structure without losing its inhibitory activity significantly. This fact has stimulated the exploration of other structures such as iso-fuco-fagomine **6**[12] which is not better than **4** except if it bears a *gem*-diamine moiety as in **7** and derivatives with the exocyclic amine *N*-acylated.[18] The latter compounds probably lack the necessary chemical stability for potential drug development. More stable fucose mimics is the 5α-carba-α-L-fucopyranosylamine **8**, 200 times less active than **1**. When *N*-conjugated with anhydroaldoses, no better activity was observed.[19] Azepines **9**, **10**[20] and guanidinosugars **11**, **12**[21] have been reported. The results suggest that, contrary to **1**, *N*-alkylation can improve the inhibitory activity of these systems. The thiosugar **13** has a moderate inhibitory activity that can be enhanced upon conjugation (glycosidation) with a lypophilic phenyl system such as **14**[22] or a β-D-galactoside (**15**).[23,24] Aminocyclopentitol **16** that imitates the transition structure of the hydrolysis of an α-D-fucopyranoside is a good inhibitor of α-L-fucosidase.[25] Its potency and selectivity can be improved through *N*-benzylation (see **17**).[26]

A competitive and specific α-L-fucosidase inhibitor could be an L-fucopyranosyl cation mimic to which one

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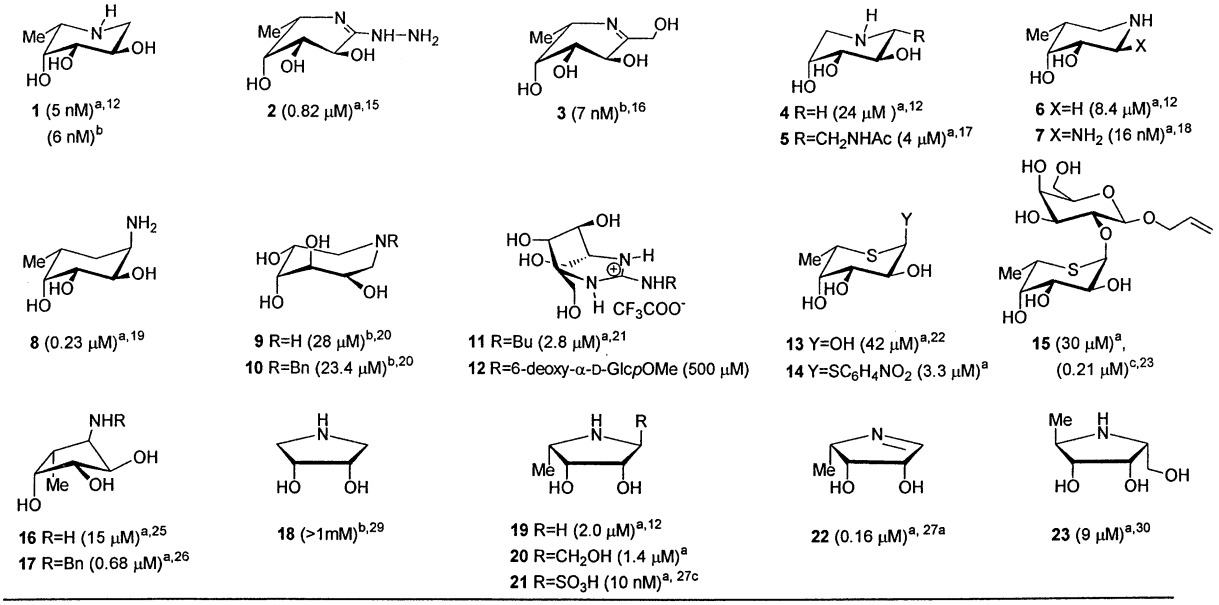
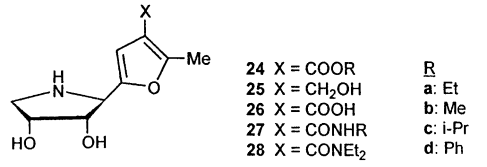
Table 1. Examples of α-L-fucosidase inhibitors (K_i optimal pH)[a,b,c]

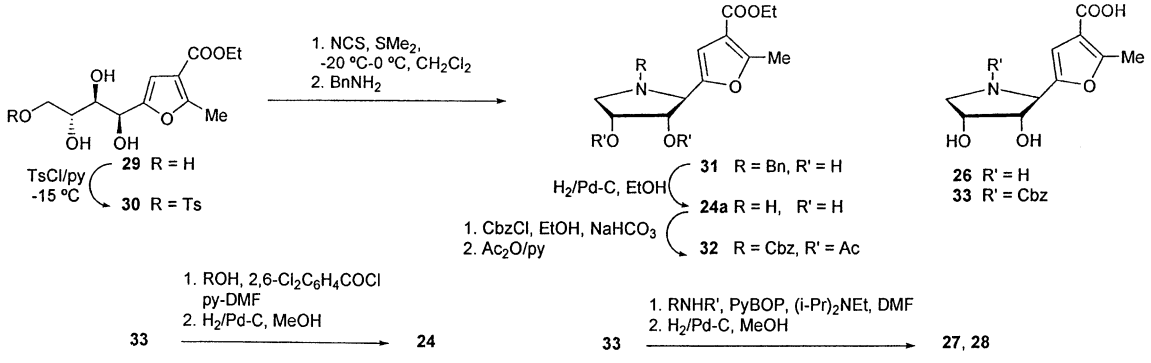


[a]α-L-fucosidase from bovine kidney [b]α-L-fucosidase from bovine epididymis [c]α-L-fucosidase from *bacillus* sp. K40T

attaches substituents able to recognize the rim of the enzyme active site through its shape (bulk that repels water molecules), specific electrostatic (e.g., H-bonds) or/and lypophilic interactions. Since the piperidine analogues of L-fucose do not permit wide modifications without losing inhibitory activity (the exception being perhaps **3**[16]) we envisioned to use a more flexible *cis*-3,4-dihydroxypyrrolidine base[27] and to attach to it adequate groups.[28] The data reported for **18**–**23** in Table 1 do not contradict this plan.[12,27,30] We disclose preliminary results showing that this concept applies for the inhibition of α-L-fucosidases by 5-[(2*R*,3*S*,4*R*)-3,4-dihydroxypyrrolidin-2-yl]-furan derivatives **24**–**28**.



The synthesis of **24**–**28** starts from the known furan derivative obtained in one single step from D-glucose and ethyl acetylacetate.[31] Selective tosylation of its primary alcohol (TsCl/pyridine, −15 °C, 2 h) provided tosylate **30** in 57% yield. Treatment of **30** with *N*-chlorosuccinimide and dimethylsulfide[32] (CH_2Cl_2, −20 °C) gave a mixture of chlorides that reacted with benzylamine to give a unique pyrrolidine **31** in 48% yield. After debenzylation (H_2/Pd–C, EtOH) pure **24a** was obtained in 97% yield. *N*-Protection with CbzCl and acetylation gave **32** in quantitative yield. Its structure was established by its spectral data and confirmed by NOE experiments when comparing with its epimer on C-2′[33] that showed a NOE between proton pairs H-2′/H-3′ that is not observed in compound **32**. Reduction of **24a** with $LiAlH_4$ in THF furnished alcohol **25** (90%). Saponification of **32** gave the furoic acid **33** that was converted into a small library of esters **24** (R = Me, *i*-Pr), amides **27** (R = Et, *i*-Pr, Ph) and **28** after hydrogenolysis. Direct esterification and amidification of **26** (obtained by saponification of **24a**) led to polymer formation. The *N*-protected derivative **33** was used to prepare esters **24** (+ ROH activation with 2,6-dichlorobenzoyl chloride, pyridine in DMF), amides **27** (+ RNH_2), **28** (Et_2NH; activation with PyBOP/DIPEA



Scheme 1.

in DMF). Deprotection of the pyrrolidine moiety was done by hydrogenolysis (10% Pd/charcoal, MeOH) (Scheme 1).

We have tested compounds **24a**,**b**,**c**, **25**, **26**, **27a**,**c**,**d** and **28** for their inhibitory activities toward 25 commercially available glycosidases. The data are summarized in Table 2 for two α-L-fucosidases, one α-L-galactosidase, three β-D-galactosidases, two amyloglucosidases and two α-mannosidases. These compounds did not show any inhibitory activity at 1 mM concentration toward the following enzymes: α-galactosidases from *Aspergillus niger* and from *E. coli*; β-galactosisades from *E. coli*, from *Aspergillus orizae*; maltase from yeast, from rice; isolmaltase from baker yeast; β-glucosidase from almonds, from *Caldocellum saccharolyticum*; β-mannosidase from *Helix pomatia*, α-*N*-acetylgalactosaminidase from chicken liver; β-*N*-acetylglucosaminidase from jack bean, from bovine epididymis A and B. As shown in Table 2, esters **24** as well as amides **27a**,**c** and **28** are good inhibitors of α-L-fucosidases from bovine epididymis and human placenta. The inhibitory activity is

Table 2. Inhibitory activities of pyrrolidine derivatives. Percentage of inhibition at 1 mM, IC_{50} and K_i in μM, when measured. Optimal pH, 35 °C[a,b]

Enzyme/Compound	**24a**	**24b**	**24c**	**25**	**26**	**27a**	**27c**	**17d**	**28**
α-L-Fucosidase									
Bovine epididymis	84% $IC_{50}=200$ $K_i=9$	86% $IC_{50}=100$ $K_i=6.5$	91% $IC_{50}=50$ $K_i=4.9$	NI	NI	91% $IC_{50}=40$ $K_i=3.2$	94% $IC_{50}=40$ $K_i3.0$	< 10%	85% $IC_{50}=110$ $K_i=9.1$
Human placenta	76% $IC_{50}=300$ $K_i=15$	88% $IC_{50}=150$ $K_i=13.8$	92% $IC_{50}=160$ $K_i=8.6$	NI	NI	92% $IC_{50}=80$ $K_i=4.8$	93% $IC_{50}=80$ $K_i=5.3$	51% $IC_{50}=1000$	86% $IC_{50}=220$ $K_i=20.1$
α-Galactosidase									
Coffee bean	NI	42%	29%	NI	NI	NI	NI	NI	NI
β-Galactosidase									
Bovine liver	57% $IC_{50}=850$	54% $IC_{50}=800$	57% $IC_{50}=640$	50%	30%	52%	38%	39%	58%
Aspergillus niger	80% $IC_{50}=370$ $K_i=340$[c]	NI	NI	NI	NI	NI	NI	NI	NI
Jack bean	42%	NI	NI	NI	NI	NI	NI	ND	NI
Amyloglucosidase									
Aspergillus niger	NI	23%	27%	NI	NI	NI	23%	ND	NI
Rhizopus mold	25%	33%	44%	NI	NI	26%	33%	ND	25%
α-Mannosidase									
Jack bean	38%	23%	78% $IC_{50}=250$ $K_i=85$	NI	NI	29%	NI	NI	27%
Almonds	NI	36%	57%	NI	NI	32%	29%	NI	31%

NI: no inhibition at 1 mM; ND: not determined.
[a]For the conditions of measurements see ref 34.
[b]The mode of inhibition for which K_i given is competitive.
[c]Mixed type of inhibition.

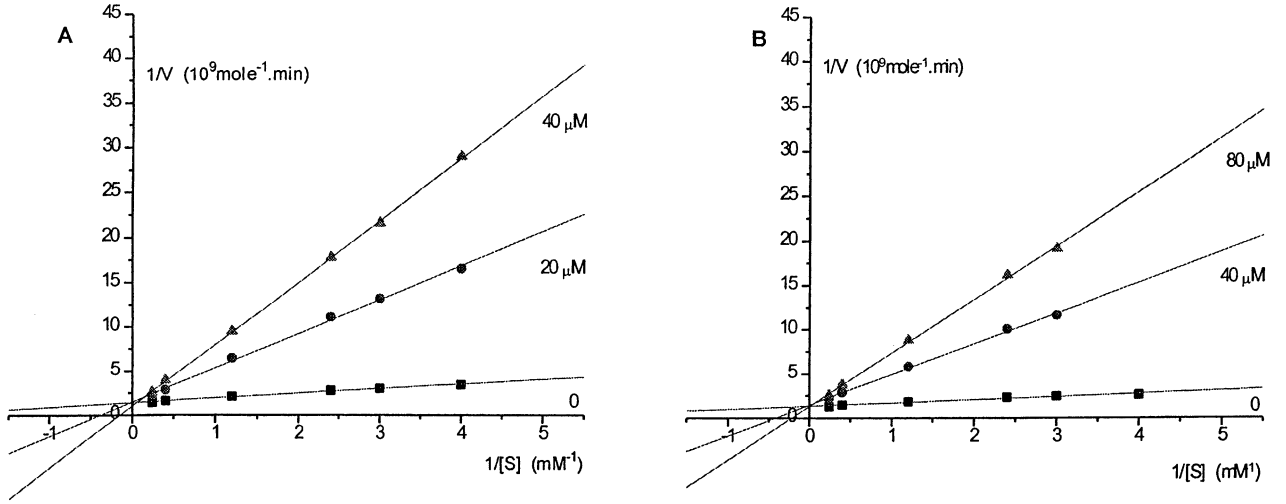


Figure 1. Effect of *p*-nitrophenyl α-fucopyranoside (S) concentration on α-L-fucosidase inhibition by various concentrations of **27a**. The data were plotted according to the Lineweaver–Burk method. (A) bovine epididymis, (B) human placenta.

much weaker for the phenyl amide **27d**, suggesting size restriction for the groups that can be attached to the carboxylic moiety of these systems. Interestingly, both the alcohol **25** and the corresponding carboxylic acid **26** are inactive. For compounds showing more than 50% inhibition at 1 mM, IC_{50} and/or K_i values have been measured.[34] Except for inhibition of β-galactosidase from *Aspergillus niger* by ester **24a** that showed a mixed type of inhibition, all our new α-L-fucosidases inhibitors are competitive (see Fig. 1) with *p*-nitrophenyl α-L-fucopyranoside, thus confirming that they recognize the active site of the enzymes as designed initially. Some of our α-L-fucosidase inhibitors inhibit other glycosidases weakly (Table 2). Nevertheless it appears that amides **27a** and **27c** that are our most potent inhibitors are also the most selective.

The pyrrolidine derivatives **27** are new leads as α-L-fucosidases inhibitors. They are obtained very readily from D-glucose and can be modified widely, as required for the development of drugs based on the inhibition of α-L-fucosidases.

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