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p-NITROPHENYL 1,5-DITHIO-α-L-FUCOPYRANOSIDE: A NOVEL SULFUR BASED FUCOSIDASE INHIBITOR

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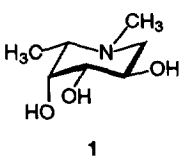
Abstract: Five 5-thio-α-L-fucopyranosyl derivatives were prepared and examined as inhibitors of the α-L-fucosidase from bovine epididymis. The inhibitory activities of the fucosides strongly depend on the hydrophobicity of the aglycon. The best inhibition (K_i = 3.3 μM) was obtained with *p*-nitrophenyl 1,5-dithio-α-L-fucopyranoside. Copyright © 1996 Elsevier Science Ltd

Aza-sugar glycosidase inhibitors have been investigated as potential anti-cancer and anti-human immunodeficiency virus (HIV) agents.[1] In addition to the fact that the aza-sugars which inhibit the processing glycosidases retard glycoprotein syntheses, it has also been suggested that inhibition of the lysosomal glycosidases by aza-sugars hampers the growth of malignant or infected cell by decreasing turnover of glycoproteins.[2] Furthermore, aza-sugars are capable of blocking tumor cell invasion probably through inhibition of the secreted glycosidases that help the invasion by degrading glycoconjugates in the extracellular matrix.[3] Thus the inhibitors of lysosomal glycosidases such as fucosidases, as well as those of processing glycosidases, are attractive candidates for drug development. Indeed, *N*-methyl deoxyfuconojirimycin (**1**) has an anti-HIV activity, presumably through inhibition of human α-L-fucosidase.[4]



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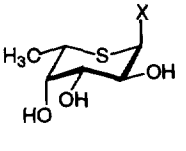


2 X = OH; **3** X = OMe; **4** X = SMe

5 X = O-⬡ ; **6** X = O-⬡-NO_2

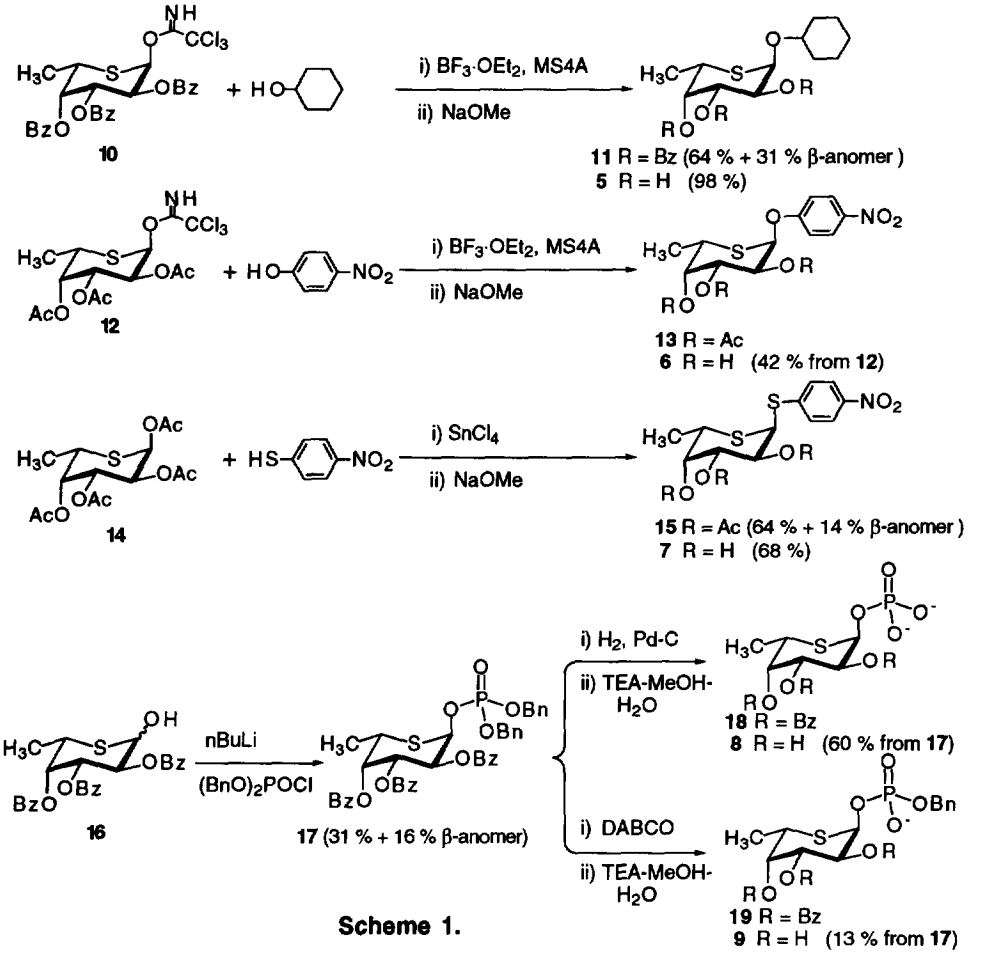
7 X = S-⬡-NO_2 ; **8** X = O-P(=O)(O⁻)-O⁻ $2Na^+$

9 X = O-P(=O)(O⁻)-O-CH$_2$-⬡ Na^+

We have shown that 5-thio-L-fucose (**2**) was a potent inhibitor of α-L-fucosidase with a K_i value of 42 μM for the enzyme from bovine epididymis.[5] This uncovered a new repertory of fucosidase inhibitors other than aza-sugars. It is desirable to have a lot of repertories in drug development since this is often hampered by a lot of factors such as difficulty in uptake, lack of cytotoxicity, and prolonged storage in body. Thus we prepared five 5-thio-α-L-fucopyranosyl derivatives in hope of getting stronger and versatile inhibitors of fucosidases.

Inhibition of fucosidase by 5-thio-L-fucose **2** has been ascribed to the ring sulfur and the α-oriented anomeric hydroxyl from comparison studies with a ring oxygen analog and some glycoside analogs (**3** and **4**).[6] Accordingly, replacement of 1-OH of 5-thio-L-fucose **2** with methoxyl group or methylthio group led to a decrease of the activity. However, all of the three disaccharide analogs having 5-thio-L-fucose at the non-reducing end showed comparable activity to that of 5-thio-L-fucose **2**.[7] This non-specific inhibition of the three disaccharides might indicate that the aglycon sugar is favorably recognized through a hydrophobic interaction. Thus we elected the cyclohexyl 5-thio-α-L-fucopyranoside (**5**), *p*-nitrophenyl 5-thio-α-L-fucopyranoside (**6**), and *p*-nitrophenyl 1,5-dithio-α-L-fucopyranoside (**7**) to introduce hydrophobic face at the aglycon part.[8] 5-Thio-α-L-fucopyranosyl phosphate (**8**) and 5-thio-α-L-fucopyranosyl monobenzyl phosphate (**9**) were also of choice to see the effect of polar groups at the anomeric position.

The compounds **5-9** were prepared (Scheme 1)[9] from the previously reported 5-thiofucose derivatives.[7,10] The reaction of the trichloroacetimidate **10** with cyclohexanol in the presence of 0.33 equiv $BF_3 \cdot OEt_2$ mainly gave the cyclohexyl α-glycoside **11** and the corresponding β-glycoside in 2 : 1 ratio. The benzoyl groups of the α-glycoside **11** were removed with sodium methoxide to give the cyclohexyl glycoside **5**. The *p*-nitrophenyl glycoside **6** was prepared by the glycosidation reaction of the trichloroacetimidate **12** with *p*-nitrophenol and subsequent Zemplén deacetylation. Only α-anomer was produced in this case. The reaction of the tetra-acetate **14** with *p*-nitrobenzenethiol in the presence of 1.2 equiv of $SnCl_4$ mainly gave the desired *p*-nitrophenyl α-thioglycoside **15** together with the undesired β-glycoside. Zemplén deacetylation and recrystallization from EtOH gave the crystalline **7**. Treatment of the tribenzoate **16** with n-BuLi followed by dibenzyl phosphorochloridate gave the α-phosphate **17** together with the undesired β-phosphate. The free phosphate **8** was prepared by the usual deprotection of the compound **17** by hydrogenolysis with Pd-C and debenzoylation with triethylamine-methanol-water. Selective mono-debenzylation of the dibenzyl phosphate **17** was accomplished with 1.0 equiv of 1,4-diazabicyclo[2.2.2]octane (DABCO) in refluxing toluene to give the monobenzyl phosphate **19**, which was then debenzoylated to give the monobenzyl phosphate **9** only in 13 % after purification with Bio-Gel P2 chromatography.



Scheme 1.

Table 1. Inhibitory activity of 5-thio-α-L-fucopyranosyl derivatives toward α-L-fucosidase[a]

Compound:	2	3	4	5	6	7	8	9
K_i (μM):	42[b]	690[c]	2300[c]	198	118	3.3	406	33

[a] α-L-Fucosidase from bovine epididymis (EC3.2.1.51) was purchased from Sigma Chemical Co. Enzyme assay was performed[11] by essentially the same method as that of Evans et al.[12] [b] Datum from ref 5. [c] Data from ref 6.

All the compounds tested displayed competitive inhibition. The K_i values for the compounds **5**-**9** are listed in Table 1 together with those obtained previously for 5-thiofucose **2** and the methyl *O*- and *S*-glycosides (**3** and **4**). The compound having a hydrophobic group, especially the aromatic group, at the aglycon part tends to display high affinity for the enzyme. This presumably indicates that the enzyme has a hydrophobic recognition

site. Noteworthy is that the compound **7** displayed better inhibition than 5-thiofucose **2** by as much as one order. This is contrary to what one would expect in comparison with the compounds **3**, **4**, and **6**. Replacement of the glycosidic oxygen of the compound **3** for a sulfur atom resulted in a loss of the affinity probably because of a loss of the hydrogen-bond accepting ability in the glycosidic sulfur of the compound **4**.[6] The fact that the compound **7** has a higher affinity for the enzyme than the compound **6** may be attributed to an increased hydrophobicity at the aglycon part by virtue of the glycosidic sulfur. Even the compound **9**, which has an aromatic group in addition to a large and polar phosphate group at the aglycon part, displayed the affinity comparable to 5-thio-L-fucose **2**.

In conclusion, we discovered a novel sulfur-based fucosidase inhibitor **7**, the strongest so far known aside from aza-sugars. We also demonstrated that some derivatives of 5-thiofucose are worth consideration as a repertory for anti-cancer and anti-HIV drug search.

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8. The nitro group was selected as a modifier of the phenyl aglycon to make the compounds soluble to water.
9. ^{1}H NMR and FAB Mass spectra for the compounds **5**-**9** were in agreement with the assigned structures.
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11. *p*-Nitrophenyl α-L-fucopyranoside (four concentrations between 0.07 and 0.33 mM), the inhibitor **5**, **6**, **7**, **8**, or **9** (four appropriate concentrations), and α-L-fucosidase (1.4 units/mL) were incubated for 30-40 min at 25 °C in 300 μL citrate buffer (20 mM, pH 5.8). After addition of 500 μL glycine buffer (50 mM, pH 10.0), absorption at 400 nm was measured. The K_i values were obtained by Lineweaver-Burk plot. Since the compound **7** has an absorption at 400 nm, the data were corrected with an absorption coefficeint of 2.04 (L· mmol^{-1}· cm^{-1}).
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