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# THIO-SUGARS IV: DESIGN AND SYNTHESIS OF S-LINKED FUCOSIDE ANALOGS AS A NEW CLASS OF α-L-FUCOSIDASE INHIBITORS[1]

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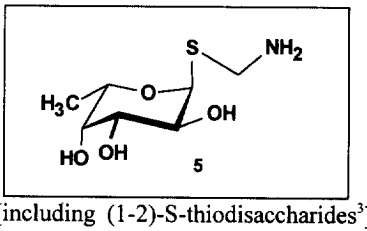
**Abstract:** α-1-Thio-L-fucose derivative **4** and **5** as new α-fucosidase inhibitors ($K_i$ = 4.6, and 5.9 μM) have been synthesized in three steps by base catalyzed coupling with bromonitromethane followed by reduction of the nitro group with sodium borohydride/cobalt chloride complex and acetylation. 

Thiosugars, a class of unnatural carbohydrate mimics including S-thiodisaccharides,[2] are potential glycosidase inhibitors for the treatment of metabolic diseases. In response to our continuous interest in thiosugars, we developed new approaches for the synthesis of new analogs of α-L-fucosidase inhibitors.[3] Natural as well synthetic inhibitors of α-fucosidase have been the focus of much interest,[4] since some (such as nojiramycin) have been shown to have potential therapeutic applications as anticancer drugs. The aza-analogs of L-fucose are well known fucosidase inhibitors.[5] Developing highly selective inhibitors of α-fucosidase could be extremely valuable in the treatment of a number of diseases such as AIDS[6] and metastatic cancer,[7] where affecting glycoprotein processing is essential. Additionally, L-fucose as a component of sialyl Lewis$^x$ (SLe$^x$) and as an essential functional sugar moiety (with all three hydroxyls involved in E-selectin binding), plays an important role in the adhesion of leukocytes to activated endothelial cells[8] involved in inflammation. Therefore, the search for novel fucose mimetics has focused on simpler structures with a high affinity to E-selectin and resistance to α-fucosidases. Because of their different levels of selectivity and response, synthesizing and testing the inhibitory response of these newly designed compounds against fucosidases from all available sources (bovine epididymis, bovine kidney and human placenta) is crucial.



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The approach developed in our laboratory (Scheme 1) supports ongoing studies of the utility of the thiosugar derivative (**5**) as an effective enzyme inhibitor. In designing this type of inhibitor, we took into account the sulfur bridge and methyl amino group as sites for further functionalization. In contrast to existing methods of thiosugar [including (1-2)-S-thiodisaccharides[3]] synthesis, our method does not require a multistep procedure or special protection of functional groups. The choice of 1-thiofucose (**1**) as a starting material is based upon its direct suitability to extend a link at the anomeric position up to a carbon connected to the amino function.

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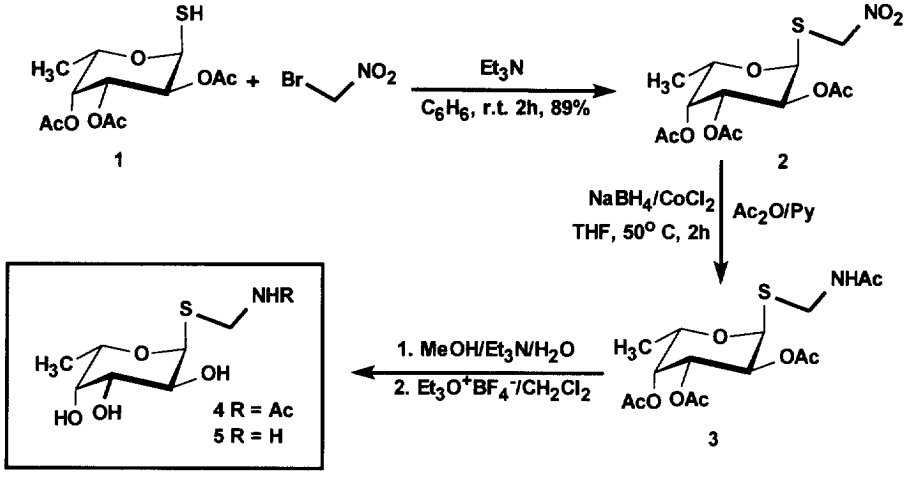
Condensation of 2,3,4,-tri-O-acetyl-1-thiofucose[9] with bromonitromethane in the presence of a catalytic amount of triethylamine produced, as expected, thiosugar **2**[10] in good (73%) yield. The reduction of the terminal nitro group was especially challenging, but was achieved efficiently with sodium borohydride/cobalt chloride complex.




**SCHEME 1**

For the purpose of purification, acetylation of the reduction product was used to produce an acetamido derivative **3**[11] in 66% overall yield. In order to produce the target compounds **4** and **5**, the cleavage of ester groups of **3** was carried out with an aqueous/methanolic solution of triethylamine to give **4**[12] in excellent 93% yield. Removal of the acetamido function of **4** was performed with triethyloxonium fluoroborate according to the literature method[13] and produced free amino derivative **5**[14] in 68% yield.

Compounds **4** and **5** were tested for the inhibition of α-L-fucosidase from all available fucosidases catalyzing the hydrolysis of 4-nitrophenyl α-L-fucopyranoside at 25 °C (37 °C for fucosidase from human placenta). At pH 6.5 compound **4** showed mixed/competitive inhibition against all fucosidases with the dissociation constant $K_i$ of 4.4, 5.6, 3.9 μM respectively,(Table 1). Similarly, at pH 6.5 compound **5** showed mixed/competitive inhibition against all fucosidases with the dissociation constant $K_i$ of 5.9, 6.8, and 6.1 μM, respectively (Table 1). This suggests that the free amino function does not have significance on the inhibitory activity against all the fucosidases

This simple sequence will prove useful for synthesizing a wider range of 1-thiosugars with various linkages at the anomeric sulfur. The thiomethyleneamino- function attached at the anomeric position of fucose creates new leads in designing this novel class of functionalized analogs of fucosidase inhibitors. Their comparative inhibition studies are currently under intense investigation in our laboratory. The synthesis of second-generation mimetics is also under investigation and the results will be forthcoming.

**Table 1.** Inhibitory activity of **4** and **5** against α-L-fucosidase[a].

| Compound | $K_i$ values (μM) | Inhibition type | Enzyme source |
|---|---|---|---|
| **4** | 4.6 | mixed | bovine kidney |
| **4** | 5.6 | competitive | bovine epididymis |
| **4** | 3.9 | mixed | human placenta |
| **5** | 5.9 | mixed | bovine kidney |
| **5** | 6.8 | competitive | bovine epididymis |
| **5** | 6.1 | mixed | human placenta |
| 5-thio-α-L-fucose[4e] | 0.042 (mM) | competitive | bovine kidney |
| deoxyfuconojirimycin[4f] | 6.2 (ηM) | competitive | bovine epididymis |
| α-L-homofuconojirimycin[4f] | 5.8 (ηM) | competitive | bovine epididymis |

[a] α-L-Fucosidase from bovine kidney (EC 3.2.1.51), bovine epididymis and human placenta were purchased from Sigma Chemical Co. The enzyme assay was performed[15] with the same method as that of Evans et.al[16] . The inhibition modes were determined by Lineweaver- Burk plots. The constant $K_m$ was calculated with Enzyme Kinetics (Windows Chem. Software). The $K_i$ values were calculated by plotting the apparent $K_m$ values versus the inhibitor concentration.

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9. Thiosugar **1,** was prepared by the method of Matta K. L.; Girotra, R.N.; Barlow, J.J. *Carbohydr. Res.* **1975**, 43, 101, with the modification at the final stage using method of Joseph, B.; Rollin, P. *J. Carbohydr. Chem.* **1993**, *12*, 719. Syrup $[\alpha]^{23}$-165.° (*c* 1.00 $CHCl_3$).
10. Compound **2**, 2'-nitromethyl 1-thio-2,3,4-tri-*O*-acetyl-β-L-fucopyranose, syrup $[\alpha]^{23}$-152.3° (*c* 1.00 $CHCl_3$); $^1$H NMR (250 MHz, $CDCl_3$) δ 1.14 (3H, d, $J$ = 6.5, **$CH_3$**-H-6), 2.03, 2.06, and 2.18 (each: 3H, s, OAc), 3.83 (2H, m, H-2'), 4.1 (1H, dq, $J$ = 6.0, 1.8 H-5), 4.8 (1H, dd, $J$ = 12, 5.8, H-1), 5.21 (1H, dd, $J$ = 10, 3.1, H-3), 5.29 (1H, m, H-4), 5.36 (1H, dd, $J$ = 10, 5.9, H-2); $^{13}$C NMR: ($CDCl_3$) δ 16.5 (**$CH_3$**-C-6), 20.9, 20.8, 20.6 (**$CH_3$**CO-), 37.4 (C-2'), 67.8 (C-5), 68.0 (C-2), 70.9 (C-3), 72.9 (C-4), 96.6 (C-1), 170.6, 170.3, 170.1 (-CO**$CH_3$**). HRMS $(M)^+$ *m/z*: Calcd for $C_{13}H_{19}NO_9S$: 365.35. Found: 365.07.
11. Compound **3**, 2'-acetamidomethyl 1-thio-2,3,4-tri-*O*-acetyl-β-L-fucopyranose, syrup $[\alpha]^{23}$-165.5° (*c*1.00 $CHCl_3$).$^{13}$C NMR ($CDCl_3$) δ 16.4 (**$CH_3$**-C-6), 20.3, 20.6, 20.9 (**$CH_3$**CO-), 22.6 ($NHCOCH_3$), 39.8 (C-2'), 67.8 (C-5), 68.3 (C-2), 71.6 (C-3), 73.3 (C-4), 98.6 (C-1), 170.6, 170.3, 170.1 (-CO**$CH_3$**). HRMS $(M)^+$ *m/z*: Calcd for $C_{15}H_{23}NO_8S$: 377.40. Found: 377.07.
12. Compound **4**, 2'-acetamidomethyl 1-thio-β-L-fucopyranose, syrup $[\alpha]^{23}$-181.9° (*c* 1.00,$CHCl_3$); $^1$H NMR (250MHz, $D_2O$) δ 1.16 (3H, d, $J$ = 6.6, **$CH_3$**-H-6), 3.74 (d,1H-3, $J$ = 9.9Hz), 3.86 (dd, 1H-4, $J$ = 5Hz,), 4.32 (q, 1H-5, $J_{5,6}$ = 6.0), 4.2- 4.06 (m, 2H, H-2'), 4.83 (d, 1H-1, $J_{1,2}$ =5.4), 5.32 (1H, dd, $J$ = 10, 5.9, H-2), 5.81 (d,1H, $J_{2'NH}$ = 8.9, NH); $^{13}$C NMR ($D_2O$) δ 16.5 (**$CH_3$**-C-6), 40.9 (C-2'), 68.2 (C-5), 69.8 (C-2), 72.1 (C-3), 73.6 (C-4), 99.6 (C-1), 173.8 (NH**CO**$CH_3$). HRMS $(M)^+$ *m/z*: Calcd for $C_9H_{17}NO_5S$: 251.29. Found: 251.08.
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14. Compound **5**, 2'-aminomethyl 1-thio-β-L-fucopyranose, syrup $[\alpha]^{23}$-181.9° (*c* 1.00 $CHCl_3$); $^1$H NMR (250 MHz, $D_2O$) δ 1.16 (3H, d, $J$ = 6.6, **$CH_3$**-H-6), 3.83 (2H, m, H-2'), 4.3 (1H, dq, $J$ = 6.6, 2.0, H-5), 4.8 (1H, dd, $J$ = 12, 5.8, H-1), 5.26 (1H, dd, $J$ = 10, 3.1, H-3), 5.39 (1H, m, H-4), 5.42 (1H, dd, $J$ = 9.8, 6.2, H-2), 8.36 (1H, dd, $J$ = 10, 5.9, NH); $^{13}$C NMR ($D_2O$) δ 16.9 (**$CH_3$**-C-6), 42.9 (C-2'), 69.2 (C-5), 70.8 (C-2), 72.0 (C-3), 73.9 (C-4), 99.6 (C1).
15. The typical enzyme assays were performed at 25 °C and 37 °C for 45 min in 20 mM citrate buffer (pH 5.5 for bovine kidney, 300 mL) which contained the following assay components: *p*-nitrophenyl fucoside (0.07- 033 mM), compound **4** or **5** (each 0- 6.7 mM) α-L-fucosidase (0.8- 1.1 units/mL). Initial velocities (less than 10% substrate consumed) were measured by quenching the reaction after 45 min by the addition of 50 mL of glycine buffer (pH 10.00, 500 μL and determining the released *p*-nitrophenolate ion concentration by visible absorption spectroscopy at 400 nm. (reference, same solution without enzyme).
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