Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 18, pp. 2169-2174, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00377-0

SYNTHESIS OF L-FUCOPYRANOSYL-4-THIODISACCHARIDES FROM LEVOGLUCOSENONE AND THEIR INHIBITORY ACTIVITY ON α-L-FUCOSIDASE.

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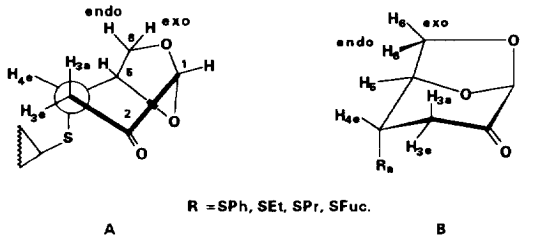
Abstract: A new method of stereoselective synthesis of α-(1-4) linked thiodisaccharides: 4-S-(α-L-fucopyranosyl)-4-thio-3-deoxy-α-D-glucopyranose **6** and 2-acetamido-2,3-dideoxy-4-thio-(α-L-fucopyranosyl)-4-thio-α-D-glucopyranose **7**, by Michael addition of 1-thio-α-L-fucopyranoside to levoglucosenone is described. The inhibition study of **6** and **7**, against α-L-fucosidase (from bovine kidney) indicates the importance of 3-deoxy position of glucosamine moiety for total inhibitory effect K_i=4.0mM and K_i=2.4mM respectivelly.

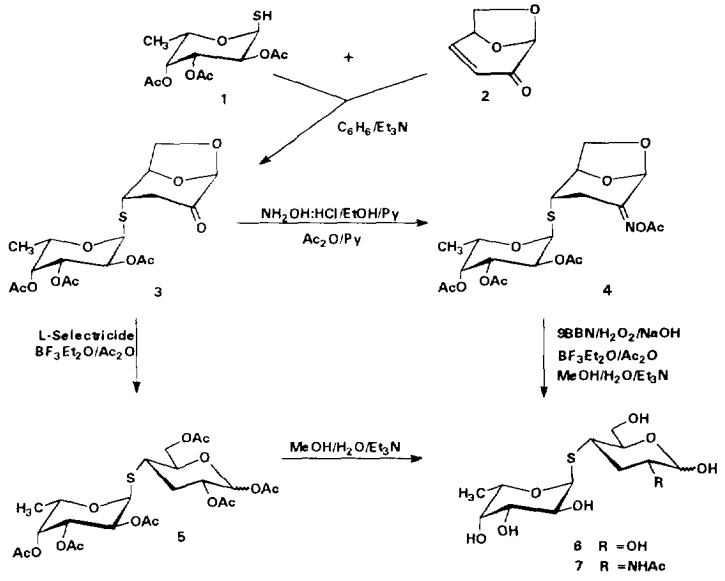
As part of our continuous interest in thiosugars[1,2] as enzyme inhibitors[3] and components of sugar antibiotics,[4] we turned our attention to a new method of synthesis of α- and β-(1-4)-linked thiosugars[3] containing biologically important sugar moieties such as galactose, glucose, mannose and L-fucose. Conventional methods are generally multistep and low overall yield approaches. The biological importance of the α-L-fucopyranosyl moiety is well defined in the literature.[5] Specific inhibitors of α-L-fucosidase are potential anticancer agents especially against metastatic tumors.[6] Recently, Hashimoto's group[7] reported the synthesis and specific inhibitory activity of a few sulfur linked thiodisaccharides with 1-6, 1-4 and 1-3 linkages containing the α-L-fucopyranosyl moiety.



That report prompted us to test the inhibitory activity of earlier and presently synthesized (1-4)-S-thiodisaccharides[8] in our laboratory. Our new approach to (1-4)-S-thiodisaccharides is based on the stereoselective Michael addition of 1-thio-sugars to the α,β- conjugated system of a convenient new chiral synthon levoglucosenone.[9]

The Michael addition of thiols to levoglucosenone was previously reported.[10-11] The shielding effect of the 1,6-anhydro bridge in levoglucosenone effectively prevents the formation of the 4-equatorial (*e*) product, and only the 4-axial (*a*) product was routinely obtained as a single addition product. This important fact

prompted us to explore the synthetic utility of levoglucosenone for the stereoselective introduction of a sulphur bridge connecting two sugar rings at C-(1-4). Indeed, the ^{1}H-NMR spin-spin coupling constants of the addition products indicate the C-4 as an axial position. The Newman projection (see A) of the adducts fully explains the geometry of the molecules. The most direct way to prove the correct stereochemistry of the 1,4-adduct is to measure the coupling constants, $J_{3ax,4}$ and $J_{3e,4}$ (see B), which range from 5.0-8.0 Hz and 1.0-1.5 Hz respectively. Lack of coupling between H-4 and H-5 indicates that the pyranose rings of the adduct is in the $^1C_4$ conformation, slightly distorted due to the presence of a carbonyl function at C-2 with an axial substituent at the 4-position. Due to long range couplings of 1-2 Hz, the complex multiplicity of the signals for H-3e is consistent with "W" planar arrangement of H-1, H-3, as well as H-5. This particular rule is highly predictable and has been observed by other authors[12] during the course of the Michael conjugate addition, as well as during



SCHEME 1

the base-catalyzed oligomerization of levoglucosenone.[13] The addition reaction of thiol **1**[14] was performed in polar solvent systems, preferably benzene or chloroform ($CHCl_3$), in the presence of a catalytic amount of triethylamine to produce **3**[15] in 91% yields. The carbonyl function of **3** was stereoselectively reduced with L-Selectride[16] followed by opening of the 1,6-anhydro ring through acid catalyzed acetolysis proceeding with the formation of exclusively D-ribo isomer **5**[17] in 85% yields. The final deprotection by O-deacetylation ($MeOH/Et_3N/H_2O$) produced a mixture of anomers **6**[18] in a 62% overall yield. The oximation of **3** with hydroxylamine hydrochloride in an ethanol/pyridine solution, followed by a conventional acetylation, produced acetoxime **4**[19] in a 68% yield. The stereoselective reduction of acetoxime **4** with borane followed by acetylation with acetic anhydride in pyridine and deprotection by deacetylation, produced the *gluco-* isomer **7**[20] in an overall 79% yield. Only traces of the *manno-* isomer were detected by ^{1}H NMR.

This new approach constitutes a highly efficient and stereoselective methodology for the introduction of

the 1-4-thio bridge between two sugar units. Further efforts to extend the methodology to the generalization of various thio-sugar units by coupling with levoglucosenone are currently under extensive investigation in our laboratory. The replacement of the glucosidic linkage by a sulfur bridge will create a longer C-S bond a favorable attribute in an glycosidase inhibitor that mimics the transition state, which should have a "stretched" glycosidic C-O bond.[21]

The activity of **6** and **7** as inhibitors of the α-L-fucosidase from bovine kidney (EC3.2.1.51) are reported in Fig. 1 (Lineweaver-Burk plots) and in the comparison of K_i with the literature data[5c,5d,5e,7] in Table 1. (K_i values [in mM] and the type of inhibitions).

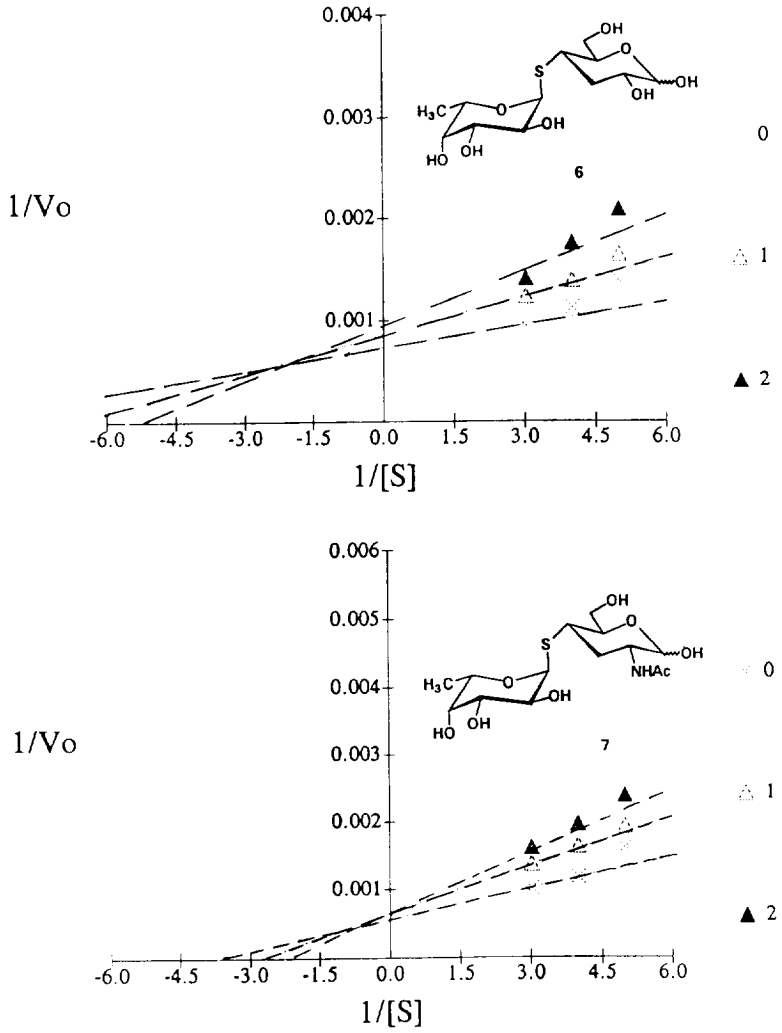


Figure 1. Lineweaver-Burk plots for the inhibition of α-L-fucosidase by compounds **6** and **7** at concentration 0 mM, 1 mM, and 2 mM of the inhibitor.

The preliminary enzyme inhibition assays of synthesized dithiasaccharides were determined by double -reciprocal plots (1/V vs. 1/S) for the hydrolysis of standard p-nitrophenyl-α-L-fucopyranoside against α-L-fucosidase (bovine kidney). The observed inhibitory activity for compounds **6** and **7**, which are 3-deoxy analogs of glucose and glucosamine as compared to the data reported by Hashimoto et al.[7] suggests the importance of the above position particularly in the glucosamine moiety of compound **7**. Comparative inhibition studies of various analogs of **6** and **7** are under investigation in our laboratory.

Table 1. Inhibitory activities of α-(1-4)-thiodisaccharides **6** and **7** against α-L-fucosidase.[a]

Compound	K_i values (mM)	Inhibition Type	Enzyme Source
6	4.0	mixed	
7	2.4	mixed	
Allyl 2-acetamido-2-deoxy-4-S-(α-L-fucopyranosyl)-4-thio-β-D-glucopyranoside[7]	4.9	mixed	
L-fucose[5c]	0.30	compettitive	
5-thio-α-L-fucose[5d]	0.042	compettitive	
	0.084	compettitive	bovine epididymis
1,5-dideoxy-1,5-imino-L-talitol[5e]	0.007	compettitive	
	0.011	competitive	bovine epididymis
α-L-homofuconojirimycin[5f]	5.8 (nM)	compettitive	bovine epididymis
deoxyfuconojirimycin[5f]	6.2 (nM)	competitive	bovine epididymis

[a] α-L-Fucosidase from bovine kidney (EC3.2.1.51) was purchased from Sigma Chemical Co. The enzyme assay was performed[22] by essentially the same method as that of Evans et al.[23] The inhibition modes were determined by Lineweaver-Burk plots. The constant K_m was calculated with the program Enzyme Kinetics (Windows Chem. Software). The K_i, values were calculated by plotting the apparent K_m values versus the inhibitor concentration.

ACKNOWLEDGMENT. Financial support from the University of Connecticut Research Foundation and State of Connecticut under the "Windows of Opportunity" grants program is gratefully acknowledged.

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14. Thio sugar **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 $CHCl_3$). Lit. [7] $[\alpha]^{23}$ - 162° (c 1.00 $CHCl_3$); For ^{13}C NMR see ref. 7.
15. Compound **3**, 1,6-anhydro-3-deoxy-4-S-(2,3,4,-tri-O-acetyl-α-L-fucopyranosyl)-D-*glycero*-hexo-pyranos-2-ulose, syrup $[\alpha]^{23}$ - 153° (c 1.00 $CHCl_3$) ; ^1H NMR (250MHz,$CDCl_3$) :δ 5.66 (d, 1H, $J_{1',2'}$=5.8Hz, H-1'), 5.16 (s, 1H, H-1), 4.72 (m, 1H, H-5), 5.06 (dd,1H, $J_{2',3'}$= 11.2Hz, $J_{3',4'}$= 3Hz, H-3'), 4.04 (dd, 1H, $J_{5,6e}$=1.6Hz, J_{6a6e}=12Hz, H-6e), 4.23 (q, 1H, $J_{5',6'}$= 6Hz, H-5'), 3.45 (d, $J_{3a,4}$= 8Hz, H-4), 3.12 (dd, 1H, $J_{3a,4}$= 8Hz, H-3a), 2.58 (d, 1H, $J_{3a,3e}$= 16.4Hz, $J_{3a,4}$= 7.9Hz, $J_{3e,4}$= 0.8Hz, H-3e), 2.06, 2.10, 2.12, (3s, 9H, 3Ac); ^{13}C NMR:($CDCl_3$):δ 17.5 (**CH_3**-C-6'), 20.9, 20.8, 20.6 (**CH_3**CO-), 39.8 (C-3), 44.2 (C-4), 67.8 (C-6), 70.9 (C-5'), 74.8 (C-3'), 77.6 (C-5), 77.9 (C-4'), 83.3(C-2'), 98.2 (C-1'), 101.3 (C-1), 170.6, 170.3, 170.1 (-**CO**CH_3), 197.9 (C-2).
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17. Compound **5**, acetyl, 3-deoxy-4-S-(2,3,4,-tri-O-acetyl-α-L-fucopyranosyl)-α,β-3,6-di-O-acetyl-D-glucopyranose, syrup $[\alpha]^{23}$-165° (c1.00 $CHCl_3$). ^1H NMR (250MHz,$CDCl_3$):δ 5.68 (d, 1H, $J_{1',2'}$= 5.6Hz, H-1'), 5.09 (dd, 1H, $J_{2',3'}$= 11.0Hz, $J_{3',4'}$= 3.2Hz, H-3'), 4.68 (m, 1H, H-5), 4.48 (d, 1H, H-1), 4.38 (q 1H, $J_{5',6'}$= 5.8Hz, H-5'), 3.86 (dd, 1H, $J_{5,6a}$= 5Hz, $J_{6a,6e}$= 12Hz, H-6e), 3.78 (d, 1H, $J_{3',4'}$= 3Hz, H-4'), 3.62-3.42 (m, 3H, H-2, 5, 3'), 3.2 (dd, 1H, H-3a), 2.68 (m, 1H, H-3e), 2.01, 2.04, 2.06, 2.08,

2.10, 2.12, (6s, 18H, 6Ac), 1.18 (s, 1H, CH_3-C-6'); ^{13}C NMR ($CDCl_3$):δ 16.1 (**CH_3**-C-6'), 20.4, 20.5, 20.6, 20.7, 20.8, 20.9, (-CO**CH_3**), 39.6 (C-3), 46.2 (C-4), 61.8 (C-6), 66.8 (C-5'), 68.6 (C-3'), 72.2 (C-4'), 74.8 (C-5), 96.2 (C-1'), 101.1 (C-1), 169.8, 170.0, 170.1, 170.4, 170.8 (-**CO**CH_3).

18. The anomeric mixture **6** of 3-deoxy-4-S-(α-L-fucopyranosyl)-α,β-D-glucopyranose was separated by flash column chromatography on silica gel by elution with AcOEt/Hexane 3:1 v/v. ^1H NMR 250MHz,$CDCl_3$): δ 5.42 (d,1H, $J_{1',2'}$ = 5.2Hz, H-1'), 4.83 (q, 1H, $J_{5,6}$=5.8Hz, H-5'), 4.75 (d, 1H, H-1 α-anomer), 4.48 (d,1H, H-1, β-anomer), 4.42 (q, 1H, $J_{5',6'}$ = 6Hz, H-5'), 4.18-4.04 (m, 2H, H-2', H-6a), 3.8 (dd, 1H, $J_{5,6a}$ = 5Hz, $J_{6a,6e}$=12.6Hz, H-6e), 3.72 (d, 1H, $J_{3',4'}$ = 2.8Hz, H-4'), 3.68-3.48 (m, 3H, H-2,5,3'), 3.10 (dd, 1H, H-3a), 2.56 (d, $J_{3a,3e}$=16Hz, $J_{3e,4}$= 7.9Hz, 1H, H-3e), 1.16 (s, 1H, CH_3-C-6'); ^{13}C NMR (D_2O):(α-anomer); δ 15.9 (**CH_3**-C-6'), 46.4 (C-3), 60.4 (C-6), 66.8 (C-5'), 67.6 (C-2'), 69.2 (C-3'), 71.8 (C-4'), 73.6 (C-2), 74.6 (C-3), 74.9 (C-5), 99.1 (C-1'), 100.6 (C-1); (β-anomer): 102.3 (C-1).

19. Compound **4**, 1,6-anhydro-2-acetoximino-2,3,-dideoxy-4-S-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-α,β-D-*glycero*-hexo-pyranose, syrup $[\alpha]^{23}$-159° (c 1.00 $CHCl_3$). ^1H NMR (250MHz, $CDCl_3$):δ 5.59 (d,1H, $J_{1',2'}$ = 5.4Hz, H-1'), 5.16 (s,1H, H-1), 5.1 (dd, 1H, $J_{2',3'}$=11.0Hz, $J_{3,4}$ = 3.2Hz, H-3', 4.76 (m, 1H, H-5), 4.02 (dd, 1H, $J_{5,6a}$ = 1.8Hz, $J_{6a,6e}$=12.0Hz, H-6e), 4.48 (q, 1H, $J_{5',6'}$ = 6Hz, H-1), 3.78 (d,1H, $J_{3',4'}$ = 2Hz, H-4'), 3.12 (dd, 1H, H-3a), 2.54 (d,1H, $J_{3a,3e}$ =16.2Hz, $J_{3a,4}$ =7.8Hz, $J_{3e,4}$ =0.9Hz, H-3e), 2.04, 2.06, 2.08 (3s, 9H, 3Ac), 2.2 (s, 3H, -NOAc), 1.23 (d, 3H,CH_3-6'); ^{13}C NMR: ($CDCl_3$):δ 16.1 (**CH_3**-C-6'), 20.6, 20.8, 20.9, (**CH_3**CO-), 22.6 (NHCO**CH_3**), 39.4 (C-3), 44.6 (C-4), 66.8 (C-5'), 67.8 (C-2'), 68.2 (C-6), 69.2 (C-3'), 72.5 (C-4'), 77.2 (C-5), 98.3 (C-1'), 101.6 (C-1), 187.3 (C-2).

20. Compound **7**, 2-acetamido-2-deoxy-4-S-(α-L-fucopyranosyl)-4-thio-α,β-D-glucopyranoside, syrup $[\alpha]^{23}$ -201° (c 1.00 H_2O); ^1H NMR (250MHz, D_2O):δ 5.8 (d,1H, $J_{2,NH}$= 8.9Hz, NH),5.36 (d,1H, $J_{1',2'}$ = 5.2Hz, H-1'), 4.5 (d, 1H, $J_{1,2}$= 8.4Hz, H-1), 4.32 (q, 1H, $J_{5',6'}$ = 6.0Hz, H-5'), 4.2-4.06 (m, 2H, H-6a, H-2'), 3.86 (dd, 1H, $J_{5,6a}$ = 5Hz, $J_{6a,6e}$=12Hz, H-6e), 3.78 (d, 1H, $J_{3',4'}$ = 2.6Hz, H-4'), 3.71-3.52 (m, 3H, H-2,5,3'), 2.72 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10Hz, H-4), 2.02 (s, 3H, AcO, 1.23 (d, 3H, CH_3-6'); ^{13}C NMR (D_2O): δ 15.2 (**CH_3**-C-6'), 21.8 (CO**CH_3**), 38.2 (C-3), 44.6 (C-4), 56.8 (C-2), 61.4 (C-6), 66.6 (C-5'), 68.2 (C-2'), 68.8 (C-3'), 71.8 (C-4'), 74.6 (C-5), 95.2 (C-1'), 100.1 (C-1), 173.8 (NH**CO**CH_3)

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22. Inhibition assays were performed at 25 ° C for 45 min in 20 mM citrate buffer (pH 5.5 for bovine kidney, 300 μL), which contained the following assay components: p-nitrophenyl-α-fucopyranoside (0.07-0.33 mM), the S-linked disaccharide **6**, **7** (each 0-6.7 mM.), α-L-fucosidase (0.8-1.1.units/mL from bovine kidney. 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 (pH10.00, 500μL and determining the p-nitrophenolate ion concentration from its absorption at 400 nm (reference, same solution without enzyme). The data were expressed by Lineweaver-Burk plots and respective replots of the slope versus the inhibitor concentration and the intercept versus the inhibitor concentration indicate the modes of inhibition and give the K_i values.

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(*Received in USA* 27 *June* 1995; *accepted* 18 *August* 1995)