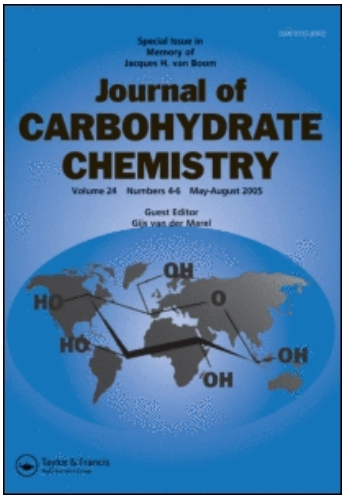


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# SYNTHESIS OF 5-THIO-L-FUCOSE AND ITS INHIBITORY EFFECT ON FUCOSIDASE

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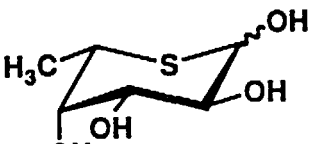
## ABSTRACT

5-Thio-L-fucose was synthesized for the first time from D-glucose in 16 steps and found to have a remarkable inhibitory effect on fucosidases from bovine epididymis (Ki $4.2 \times 10^{-5}$ M) and kidney (Ki $8.4 \times 10^{-5}$ M).

## INTRODUCTION

Since the synthesis of 5-thio-D-glucose[1] and findings of some biological activities of this sugar,[2] many kinds of 5-thio analogs of natural sugars have been synthesized; e.g., 5-thio-D-fructose,[3] 5-thio-L-rhamnose,[4] 2-acetamido-2-deoxy-5-thio-D-glucose,[5] 5-thio-D-galactose,[6] 5-thio-D-mannose.[7] On the other hand, 5-thio-L-fucose has not been synthesized in spite of the abundance of L-fucose in the oligosaccharides of such glycoconjugates as blood group substances. We now report the first synthesis of 5-thio-L-fucose (1) and the inhibitory effect of this sugar on fucosidase.

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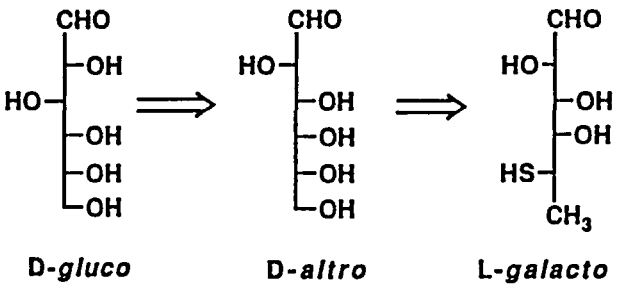
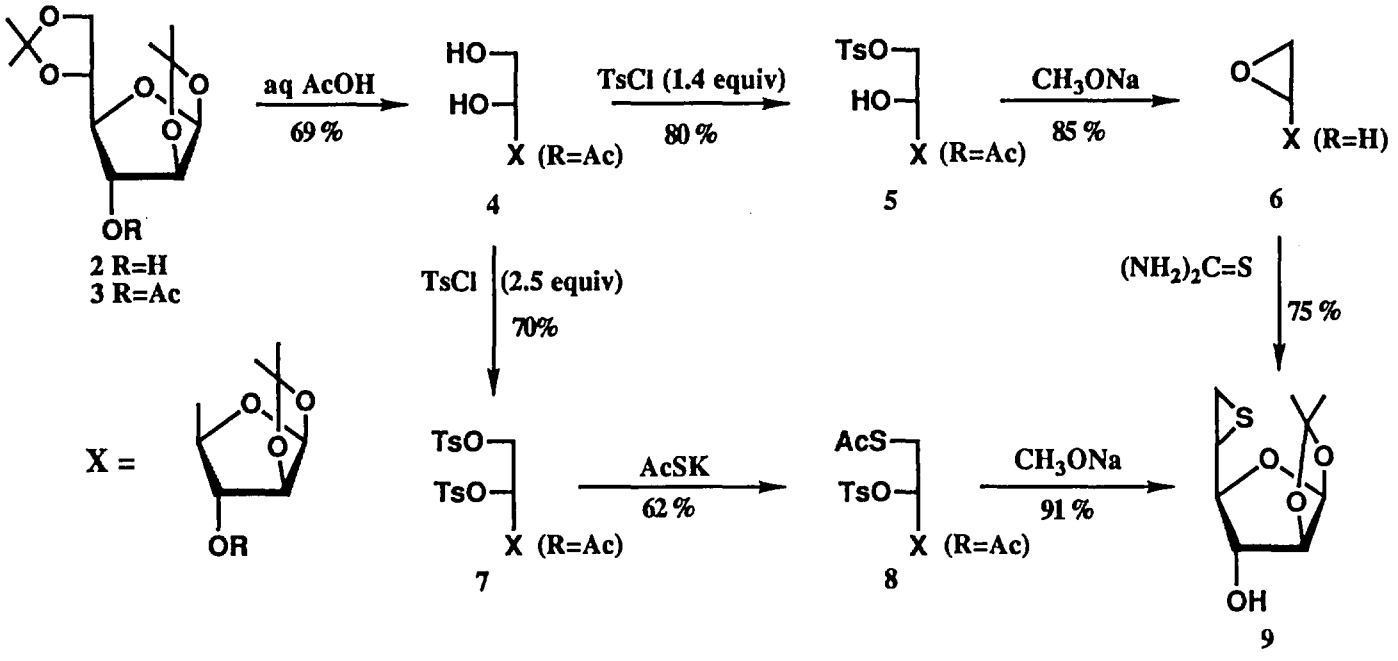



Scheme 1

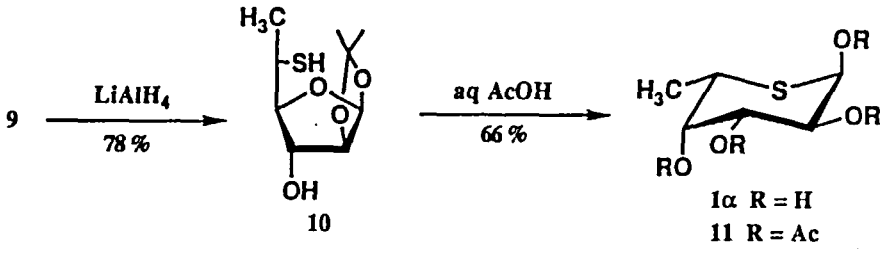
## RESULTS AND DISCUSSION

5-Thio-L-fucose was synthesized from D-glucose via introduction of sulfur with inversion of configuration at C-5 of a D-altrose derivative and deoxygenation at C-6 (Scheme 1). A similar route for 6-deoxy-5-thio-L-idose through episulfide formation with inversion of configuration at C-5 of D-glucofuranose followed by hydrogenation at C-6 has been developed by Creighton and Owen.[8] Thus, 1,2;5,6-di-O-isopropylidene-β-D-altrofuranose (2) (available from D-glucose in 9 steps)[9] was the choice as the starting material.

The diacetal 2 was acetylated and the 5,6-O-isopropylidene group of 3 was selectively hydrolyzed with 10% aqueous acetic acid to give 3-O-acetyl-1,2-O-isopropylidene-β-D-altrofuranose (4) in 69% yield. Selective tosylation of 4 at the primary hydroxyl group with 1.4 equiv of p-toluenesulfonyl chloride gave a monotosylate 5 in 80% yield. By the same reaction with 2.5 equiv of the chloride, a ditosylate 7 was obtained in 70% yield. Both the monotosylate 5 and the ditosylate 7 might be precursors for an episulfide 9. Actually, as shown in Scheme 2, 9 was derived via an epoxide 6 which was obtained in 85% yield by treatment of the monotosylate 5 with sodium methoxide, and also via a 6-thio derivative 8 which was obtained in 62%




**Scheme 2**




Scheme 3

yield by treatment of the ditosylate 7 with potassium thioacetate.

The episulfide 9 was reduced with lithium aluminum hydride to give 6-deoxy-1,2-O-isopropylidene-5-thio-α-L-galactofuranose (10) in 78% yield, and 10 was hydrolyzed with 50% aqueous acetic acid to give 5-thio-α-L-fucopyranose (1α) in 66% yield (Scheme 3). Coupling constants of the tetraacetate 11, i.e., $J_{1,2}$ (2.8 Hz), $J_{2,3}$ (11.2 Hz), $J_{3,4}$ (3.0 Hz) and $J_{4,5}$ (1.5 Hz) support the L-galacto configuration.

Inhibitory activity of 5-thio-α-L-fucopyranose (1α) toward some glycosidases was then examined. No inhibitory effect of 1α at a concentration of 200 mM toward α-glucosidase from brewers yeast, β-glucosidase from almonds, α-galactosidase from green coffee beans, β-galactosidase from Aspergillus niger and α-mannosidase from Jack beans was observed. However, a marked competitive inhibitory effect of 1α (Ki: $4.2 \times 10^{-5}$ and $8.4 \times 10^{-5}$ M, respectively) was observed toward α-fucosidases from bovine epididymis and bovine kidney (Fig. 1). Such relatively strong inhibitory effect of 5-thiohexose on glycosidase has not been reported as far as we know.

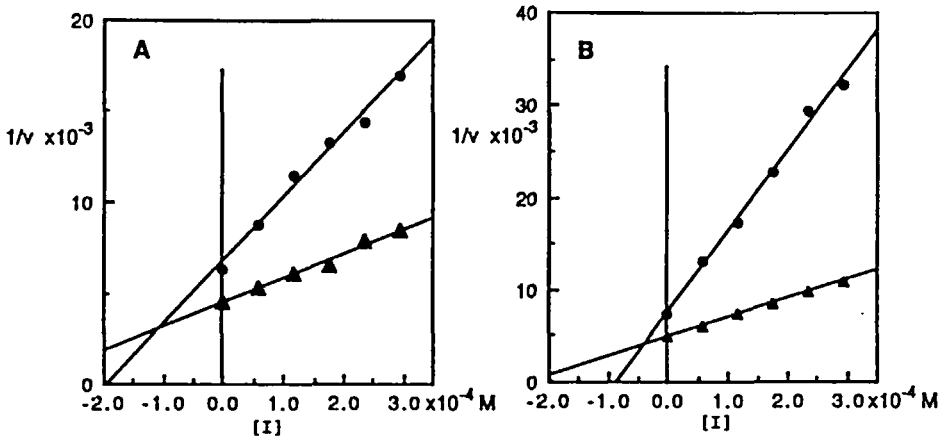



**Fig. 1** Dixon plots showing inhibition of α-L-fucosidases (A; from bovine epididymis, B; from bovine kidney) with 5-thio-α-L-fucopyranose (1α). Concentrations of the substrate: (•) 2.5 x10$^{-4}$ M, (▲) 7.4 x10$^{-4}$ M.



## EXPERIMENTAL

**General Procedures.** Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a JASCO DIP-4 polarimeter. $^{1}H$ NMR spectra were recorded with a JEOL JNM-PS100 spectrometer, and $^{13}C$ NMR spectra were recorded with a JEOL JNM-FX90Q spectrometer. Column chromatography was performed on silica gel (Merck Kieselgel 7734 or Wakogel C-300) with the solvent system specified. Evaporations were conducted *in vacuo*.

**Enzyme Procedures.** All enzymes were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A) and all substrates were purchased from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). Enzyme assays were performed with the essentially same method as that of Evans et al.[10] Assay buffers (600 μL) including 5-thio-L-fucose (final concd range of $5.9 \times 10^{-6}$-$5.9 \times 10^{-3}$ M) were incubated at 25 °C for appropriate time. After addition of 50 mM glycine buffer

(pH 10.1, 1 mL), UV absorptions at 400 nm were read. Ki Values were calculated with Dixon plot.

α-Glucosidase from brewers yeast (Sigma G-4634, 2.8 μg/mL), $4.8 \times 10^{-4}$ M p-nitrophenyl α-D-glucopyranoside, 17 mM citrate buffer (pH 6.0), incubated 12 min.

β-Glucosidase from almonds (Sigma G-8625, 0.33 μg/mL), $6.6 \times 10^{-4}$ M p-nitrophenyl β-D-glucopyranoside, 17 mM citrate buffer (pH 4.8), incubated 12 min.

α-Galactosidase from green coffee beans (Sigma G-8507, 0.50 μg/mL), $1.5 \times 10^{-3}$ M p-nitrophenyl α-D-galactopyranoside, 17 mM citrate buffer (pH 4.0), incubated 10 min.

β-Galactosidase from Aspergillus niger (Sigma G-9132, 1.9 μg/mL), $7.1 \times 10^{-4}$ M o-nitrophenyl β-D-galactopyranoside, 17 mM citrate buffer (pH 4.0), incubated 10 min.

α-Mannosidase from Jack beans (Sigma M-7257, 0.83 μg/mL), $1.0 \times 10^{-3}$ M p-nitrophenyl α-D-mannopyranoside, 17 mM citrate buffer (pH 4.5), incubated 12 min.

α-Fucosidase from bovine epididymis (Sigma F-7753, 1.7 μg/mL), $2.5 \times 10^{-4}$ and $7.4 \times 10^{-4}$ M p-nitrophenyl α-L-fucopyranoside, 17 mM citrate buffer (pH 5.8), incubated 10 min.

α-Fucosidase from bovine kidney (Sigma F-5884, 1.3 μg/mL), $2.5 \times 10^{-4}$ and $7.4 \times 10^{-4}$ M p-nitrophenyl α-L-fucopyranoside, 17 mM citrate buffer (pH 5.5), incubated 10 min.

**3-O-Acetyl-1,2;5,6-di-O-isopropylidene-β-D-altrofuranose (3).** The diacetal 2 (300 mg, 1.15 mmol) was acetylated with acetic anhydride (3 mL) and pyridine (3 mL) in the usual manner and purified on a column of silica gel (hexane-ethyl acetate 3:1) to give crystalline 3 (299 mg, 86%). Compound 3 was recrystallized from diethyl ether-petroleum ether: mp 94 °C, $[\alpha]_D^{26}$ +16.0° (c 1.0, chloroform), $^1$H NMR ($CDCl_3$) δ 5.81 (d, 1H, $J_{1,2}$=4.0 Hz, H-1),

5.28 (s, 1H, H-3), 4.53 (d, 1H, H-2), 3.82-4.40 (m, 4H, H-4, H-5, H-6a and H-6b), 2.09 (s, 3H, Ac), 1.55, 1.41, 1.35 and 1.31 (4s, each 3H, 2Isp).

Anal. Calcd for $C_{14}H_{22}O_7$: C, 55.62; H, 7.33. Found: C, 55.89; H, 7.50.

**3-O-Acetyl-1,2-O-isopropylidene-β-D-altrofuranose (4).** A mixture of 3 (299 mg, 1.00 mmol) and 10% aqueous acetic acid (5 mL) was stirred for 30 h at room temperature. The reaction mixture was concentrated and co-evaporated with toluene. The residue was purified on a column of silica gel (hexane-ethyl acetate 1:2) to give syrupy 4 (208 mg, 80%): $[\alpha]_D^{27}$ -8.8° (c 1.0, chloroform), $^1$H NMR ($CDCl_3$) δ 5.86 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 5.29 (s, 1H, H-3), 4.55 (d, 1H, H-2), 4.38-3.52 (m, H-4, H-5, H-6a and H-6b), 3.44 (bs, 2H, 2OH), 2.10 (s, 3H, Ac), 1.32 and 1.52 (2s, each 3H, Isp).

Anal. Calcd for $C_{11}H_{18}O_7$: C, 50.03; H, 6.92. Found: C, 50.03; H, 7.34.

**3-O-Acetyl-1,2-O-isopropylidene-6-O-(p-toluenesulfonyl)-β-D-altrofuranose (5).** To a stirred solution of 4 (778 mg, 2.97 mmol) in pyridine (20 mL) was slowly added p-toluenesulfonyl chloride (792 mg, 4.15 mmol) at 0 ℃ and the reaction mixture was gradually warmed to room temperature. After 44 h, the reaction mixture was mixed with water, and extracted with chloroform. After evaporation of the organic layer, the residue was purified on a column of silica gel (hexane-ethyl acetate 2:1) to give syrupy 5 (988 mg, 80%): $[\alpha]_D^{23}$ +25.8° (c 1.0, chloroform), $^1$H NMR ($CDCl_3$) δ 7.77 and 7.30 (2d, each 2H, J=9.0 Hz, aromatic protons), 5.85 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 5.29 (s, 1H, H-3), 4.56 (d, 1H, H-2), 4.40-3.88 (m, 4H, H-4, H-5, H-6a and H-6b), 3.02 (bs, 1H, OH), 2.45 (s, 3H, $C_6H_4\underline{CH_3}$), 2.09 (s, 3H, Ac), 1.45 and 1.24 (2s, each 3H, Isp).

Anal. Calcd for $C_{18}H_{24}O_9S$: C, 51.91; H, 5.81; S, 7.70. Found: C, 51.84; H, 5.70; S, 7.81.

**3-O-Acetyl-1,2-O-isopropylidene-5,6-di-O-(p-toluenesulfonyl)-β-D-altrofuranose (7).** Treatment of **4** (2.36 g, 9.0 mmol) with p-toluenesulfonyl chloride (4.28 g, 22.4 mmol) as described in the preparation of 5 and purification on a column of silica gel (hexane-ethyl acetate 2:1) gave syrupy 7 (750 mg, 70%): $[\alpha]_D^{25}$ +5.4° (c 1.0, chloroform), $^1$H-NMR ($CDCl_3$) δ 7.75, 7.71 (2d, each 2H, J=8.0 Hz each, aromatic protons), 7.30 (d, 4H, aromatic protons), 5.82 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 5.08 (s, 1H, H-3), 4.96 (m, 1H, H-5), 4.46 (d, 1H, H-2), 4.02-4.38 (m, 3H, H-4 and H-6), 2.47 (s, 6H, $2C_6H_4C\underline{H}_3$), 2.04 (s, 3H, Ac), 1.45 and 1.25 (2s, each 3H, Isp).

Anal. Calcd for $C_{25}H_{30}O_{11}S_2$: C, 52.62; H, 5.30; S, 11.24. Found: C, 52.62; H, 5.40; S, 11.02.

**5,6-Anhydro-1,2-O-isopropylidene-β-D-altrofuranose (6).** A solution of 5 (541 mg, 1.30 mmol) in 0.09 M sodium methoxide/methanol (20 mL) was kept at room temperature for 1 h. The reaction mixture was diluted with water and concentrated at room temperature for removal of methanol. The residual solution was extracted with chloroform and the organic layer was concentrated. The residue was purified on a column of silica gel (hexane-ethyl acetate 2:1) to give crystalline 6 (226 mg, 85%). Compound 6 was recrystallized from diethyl ether-petroleum ether: mp 124 °C, $[\alpha]_D^{25}$ +22.0° (c 1.0, chloroform), $^1$H NMR ($CDCl_3$) δ 5.90 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 4.54 (d, 1H, H-2), 4.40 (bs, 1H, H-3), 3.61 (dd, 1H, $J_{3,4}$=1.5, $J_{4,5}$=8.0 Hz, H-4), 3.35 (bs, 1H, OH), 3.24 (ddd, 1H, $J_{5,6a}$=4.0, $J_{5,6b}$=2.5 Hz, H-5), 2.88 (dd, 1H, $J_{6a,6b}$=5.0 Hz, H-6a), 2.69 (dd, 1H, H-6b), 1.53 and 1.34 (2s, each 3H, Isp), $^{13}$C NMR ($CDCl_3$) δ 112.5 (Isp), 106.0 (C-1), 89.0 (C-4), 86.5 (C-2), 76.3 (C-3), 51.2 (C-5), 47.2 (C-6), 26.7 and 25.8 (Isp).

Anal. Calcd for $C_9H_{14}O_5$: C, 53.46; H, 6.98. Found: C, 53.05; H, 6.94.

**3-O-Acetyl-6-S-acetyl-1,2-O-isopropylidene-6-thio-5-O-(p-toluenesulfonyl)-β-D-altrofuranose (8).** A mixture of 7 (106 mg, 0.186 mmol), potassium thioacetate (22 mg, 1.05 mmol) and acetone (10 mL) was refluxed for 4h. The reaction mixture was mixed with water and extracted with chloroform. The organic layer was concentrated and the residue was purified on a column of silica gel (hexane-ethyl acetate 2:1) to give syrupy 8 (55 mg, 62%): $[\alpha]_D^{25}$ +7.2° (c 1.0, chloroform), $^1$H NMR ($CDCl_3$) δ 7.90 and 7.40 (2d, each 2H, J=4.0 Hz, aromatic protons), 5.93 (d, 1H, $J_{1,2}$=4.4 Hz, H-1), 5.20 (d, 1H, $J_{3,4}$=1.5 Hz, H-3), 5.07 (dt, 1H, $J_{4,5}$=9.0, $J_{5,6a}$=$J_{5,6b}$=4.0 Hz, H-5), 4.56 (d, 1H, H-2), 4.17 (dd, 1H, H-4), 3.54 (dd, 1H, $J_{6a,6b}$=15.0 Hz, H-6a), 3.31 (dd, 1H, H-6b), 2.50 (s, 3H, $C_6H_4C\underline{H}_3$), 2.32 (s, 3H, SAc), 2.08 (s, 3H, OAc), 1.57 and 1.29 (2s, each 3H, Isp).

Anal. Calcd for $C_{20}H_{26}O_9S_2$: C, 50.62; H, 5.52; S, 13.51. Found: C, 51.10; H, 5.68; S, 13.61.

**5,6-Dideoxy-5,6-epithio-1,2-O-isopropylidene-α-L-galactofuranose (9).**

(i) From the anhydride 6: A mixture of 6 (250 mg, 1.22 mmol), thiourea (279 mg, 3.67 mmol) and methanol (10 mL) was kept with stirring at room temperature for 19 h. The reaction mixture was mixed with water and extracted with diethyl ether. The organic layer was concentrated and the residue was purified on a column of silica gel (hexane-ethyl acetate2:1) to give crystalline 9 (202 mg, 75%).

(ii) From the 6-thio derivative 8: To a stirred solution of 8 (681 mg, 1.43 mmol) in chloroform (8 mL) was added 1.0 M sodium methoxide/methanol (1.4 mL) at -30 ℃ and the reaction mixture was gradually warmed to 0 ℃. After 3.5 h, the reaction mixture was mixed with water and extracted with chloroform. The organic layer was concentrated and the residue was purified on a column of silica gel (hexane-ethyl acetate 2:1) to give crystalline 9 (286 mg, 91%).

Compound **9** was recrystallized from dichloromethane: mp 152-155 °C, $[\alpha]_D^{25}$ +84.0° (c 1.0, chloroform), $^1$H NMR (methanol-$d_4$) $\delta$ 5.83 (d, 1H, $J_{1,2}$=3.8 Hz, H-1), 4.49 (d, 1H, H-2), 4.09 (bs, 1H, H-3), 3.25 (m, 1H, H-4), 2.64-2.24 (m, 3H, H-5 and H-6), 1.44 and 1.28 (2s, each 3H, Isp), $^{13}$C NMR (DMSO-$d_6$) $\delta$ 111.5 (Isp), 105.3 (C-1), 92.8 (C-4), 86.3 (C-2), 77.3 (C-3), 36.1 (C-5), 26.8 and 25.9 (Isp), 22.2 (C-6).

Anal. Calcd for $C_9H_{14}O_4S$: C, 49.53; H, 6.46; S, 14.69. Found: C, 49.70; H, 6.42; S, 14.21.

**6-Deoxy-1,2-O-isopropylidene-5-thio-$\alpha$-L-galacto-furanose (10).** A mixture of the episulfide **9** (202 mg, 0.926 mmol), lithium aluminum hydride (106 mg, 2.79 mmol) and diethyl ether (20 mL) was refluxed for 1.5 h. After cooling, the reaction mixture was mixed with ethyl acetate (30 mL) followed by 1N acetic acid (50 mL), and extracted with diethyl ether. The organic layer was washed with aqueous hydrogencarbonate and concentrated. The residue was purified on a column of silica gel (hexane-ethyl acetate 2:1) to give crystalline **10** (159 mg, 78%). Compound **10** was recrystallized from diethyl ether: mp 157-158 °C, $[\alpha]_D^{28}$ +41.7° (c 1.0, dichloromethane), $^1$H NMR ($CDCl_3$) $\delta$ 6.08 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 4.68 (d, 1H, H-2), 4.34 (d, 1H, $J_{3,4}$=3.2 Hz, H-3), 3.83 (dd, 1H, $J_{4,5}$=8.5 Hz, H-4), 3.28 (m, 1H, H-5), 1.59 and 1.38 (2s, each 3H, Isp), 1.41 (d, 3H, $J_{5,6}$=6.0 Hz, H-6), $^{13}$C NMR ($CDCl_3$) $\delta$ 113.1 (Isp), 105.0 (C-1), 91.6 (C-4), 87.5 (C-2), 76.1 (C-3), 36.5 (C-5), 27.2 and 26.4 (Isp), 20.1 (C-6).

Anal. Calcd for $C_9H_{16}O_4S$: C, 49.07; H, 7.32; S, 14.55. Found: C, 49.15; H, 7.59; S, 14.27.

**5-Thio-$\alpha$-L-fucopyranose (1$\alpha$).** A solution of **10** (159 mg, 0.72 mmol) in 50% aqueous acetic acid (1 mL) was heated at 75 °C for 1.5 h. After cooling, the reaction mixture was concentrated and co-evaporated with toluene to give a crystalline residue. The residue was recrystallized from

methanol to give 1$\alpha$ (86 mg, 66%): mp 159-160 °C, $[\alpha]_D^{22}$ -259.1° (5 min) ⟶ $[\alpha]_D^{19}$ -233.0° (equilibrium, 2 days) (c 1.2, water), $^1$H NMR ($D_2O$) $\delta$ 5.43 (d, 1H, $J_{1,2}$=3.0 Hz, H-1), 4.57 (dd, 1H, $J_{3,4}$=3.0, $J_{4,5}$=1.8 Hz,H-4), 4.49 (dd, 1H, $J_{2,3}$=10.8 Hz, H-2), 4.33 (dd, 1H, H-3), 4.04 (dq, 1H, $J_{5,6}$=7.0 Hz, H-5), 1.70 (d, 3H, H-6), $^{13}$C NMR ($D_2O$) $\delta$ 75.6, 75.1, 72.1 and 71.5 (C-1, C-2, C-3 and C-4), 37.4 (C-5), 17.0 (C-6). No signal for the corresponding $\beta$-anomer was observed.

Anal. Calcd for $C_6H_{12}O_4S$: C, 39.99; H, 6.71; S, 17.79. Found: C, 40.16; H, 6.47; S, 17.68.

5-Thio-$\alpha$-L-fucopyranose (1$\alpha$) was acetylated with acetic anhydride and pyridine in the usual manner to give 1,2,3,4-tetra-O-acetyl-5-thio-$\alpha$-L-fucopyranose (11) as crystals: mp 132-133 °C, $[\alpha]_D^{23}$ -269.2° (c 1.0, chloroform), $^1$H NMR ($CDCl_3$) $\delta$ 6.06 (d, 1H, $J_{1,2}$=2.8 Hz, H-1), 5.54 (dd, 1H, $J_{3,4}$=3.0, $J_{4,5}$=1.5 Hz, H-4), 5.43 (dd, 1H, $J_{2,3}$=11.2 Hz, H-2), 5.26 (dd, 1H, H-3), 3.64 (dq, 1H, $J_{5,6}$=7.0 Hz, H-5), 2.18 and 2.14 (2s, each 3H, 2Ac), 1.97 (s, 6H, 2Ac), 1.17 (d, 3H, H-6), $^{13}$C NMR ($CDCl_3$) $\delta$ 170.5, 169.8, 169.7 and 169.1 (C=O), 72.2, 71.9, 69.2 and 69.0 (C-1, C-2, C-3 and C-4), 35.6 (C-5), 20.8 and 20.5 ($\underline{C}H_3CO$), 15.7 (C-6).

Anal. Calcd for $C_{14}H_{20}O_8S$: C, 48.27; H, 5.79; S, 9.20. Found: C, 48.24; H, 5.72; S, 9.31.

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