A New Route to Deoxythiosugars Based on Aldolases

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Abstract: A new route to deoxythiosugars based on aldolases has been developed. Representative syntheses of several thioketoses and 1-deoxy-5-thio-D-glucose, 1-deoxy-5-thio-D-galactose, 1-deoxy-5-thio-L-altrose, 1-deoxy-5-thio-D-mannose, 1-deoxy-5-thio-L-mannose, and 2-deoxy-5-thio-D-ribose are illustrated with the use of fructose 1,6-diphosphate aldolase, fuculose 1-phosphate aldolase, rhamnulose 1-phosphate aldolase, and 2-deoxyribose 5-phosphate aldolase in reaction with an appropriate thioaldehyde, followed by reduction with triethylsilane in the presence of BF_3·Et_2O.

Introduction

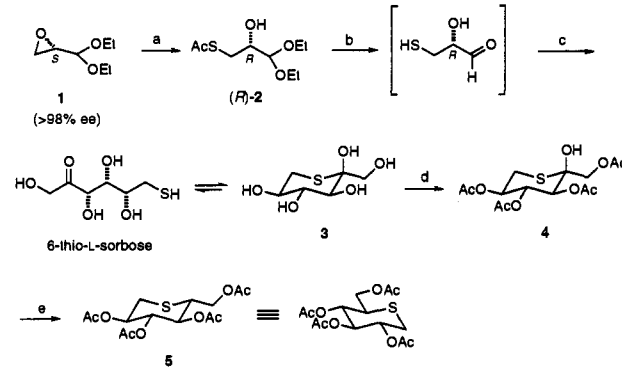
Thiosugars with sulfur in the ring possess interesting biological activities: they interfere with enzymes or proteins involved in the recognition of their natural counterparts.[1-6] 5-Thio-D-glucose, for example, inhibits α-glucosidase[2] and D-glucose transport.[1] It is also a suicide inhibitor of glycolysis,[3] reversibly inhibits spermatogenesis, and selectively enhances hyperthermic killing of tumor cells under hypoxic conditions.[4] 5-Thio-L-fucose is a strong inhibitor of α-fucosidase (K_i = 42 μM).[5a] 5-Thio-D-mannose is a potential mannosidase inhibitor and has been isolated from the marine sponge *Clathriapyramida*.[5c] These interesting properties may be attributed to the different size and electronic properties of the sulfur and oxygen groups. The electron density of the S atom is more dispersed, the C–S bond is longer (*ca.* 1.8 Å), and the C–S–C angle (*ca.* 95–100°) is smaller than the corresponding oxygen-containing structure. 5-Thio-D-glucose is indeed slightly puckered.[6]

Thiosugars are generally prepared from naturally occurring sugars[7] through differential protection/deprotection and replacement. We report here a new general route to this class of molecules based on an enzymatic aldol addition reaction using aldolases.[8,9] More than 20 aldolases have been isolated, and several of them have been shown to exhibit broad acceptor specificity.[10] The use of thioaldehydes as acceptors for the following four different aldolases demonstrates the new synthetic utility of aldolases.

Results and Discussion

A representative synthesis of 6-thio-L-sorbose is depicted in Scheme 1. The required substrate (*R*)-3-thioglyceraldehyde was generated *in situ* from (*R*)-**2**, which was readily obtained by regioselective epoxide ring opening of (*S*)-glycidaldehyde diethyl acetal[11] **1** with thiolacetic acid and its potasium salt. Condensation of the aldehyde from (*R*)-**2** with dihydroxyacetone phosphate

Scheme 1[a]



[a] Reagents and conditions: (a) AcSK (0.5 equiv)/AcSH (5 equiv), 23 °C, 52%; (b) aqueous HCl (7.6 mL), pH 1, 50 °C, 4 h; (c) (i) dihydroxyacetone phosphate (0.5 equiv, 0.39 mmol), RAMA (178 u), pH 6.7, 23 °C, 27 h, (ii) phosphatase (645 u), pH 4.7, 39 °C, 12 h, 85%; (d) Ac_2O/pyridine, 100%; (e) Et_3SiH (2 equiv), BF_3·Et_2O (1 equiv), CH_2Cl_2, 23 °C, 12 h, 58%.

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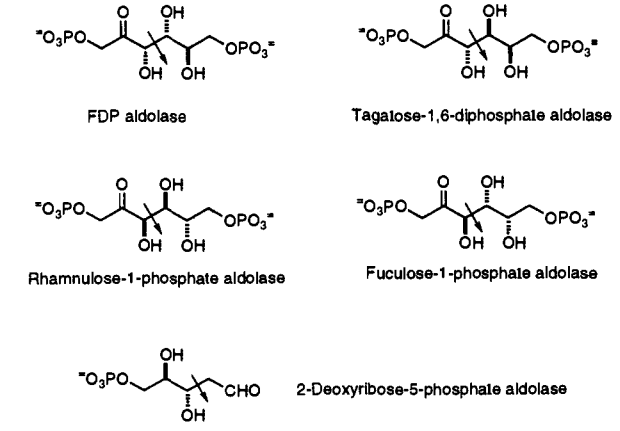

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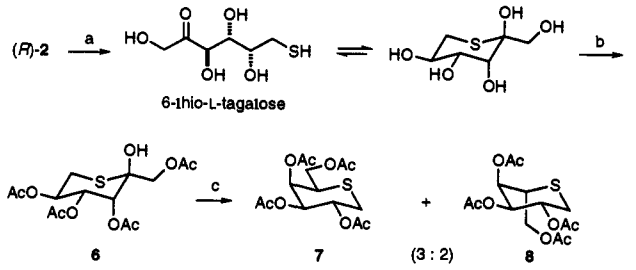
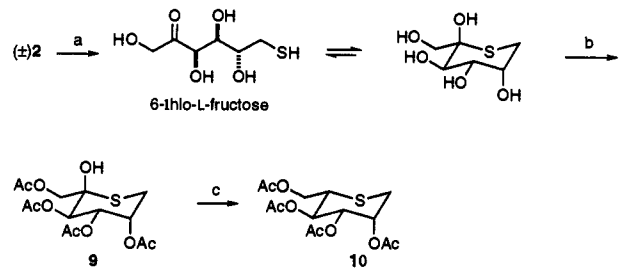
Scheme 2[a]



[a] Reagents and conditions: (a) (i) aqueous HCl (6 mL), pH 1, 50 °C, 4 h, (ii) dihydroxyacetone phosphate (0.5 equiv, 0.32 mmol), Fuc-1-P aldolase (53 u), pH 6.7, 23 °C, 26 h, (iii) phosphatase (430 u), pH 4.7, 39 °C, 22 h, 78%; (b) Ac_2O/pyridine, 100%; (c) Et_3SiH (2 equiv), $BF_3{\cdot}Et_2O$ (1 equiv), CH_2Cl_2, 23 °C, 12 h, 64% (2 isomers, ratio 3:2).

Scheme 3[a]



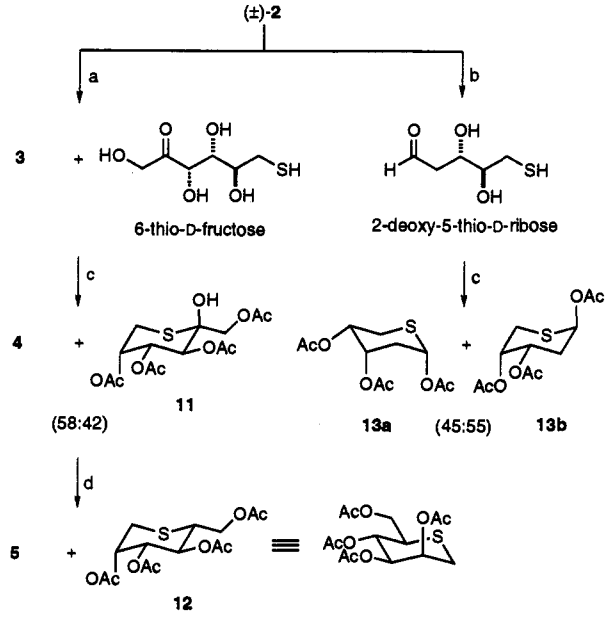
[a] Reagents and conditions: (a) (i) aqueous HCl (10 mL), pH 1, 50 °C, 4 h, (ii) dihydroxyacetone phosphate (0.5 equiv, 0.5 mmol), Rha-1-P aldolase (10 u, 10 mL), pH 6.7, 23 °C, 22 h, (iii) phosphatase (300 u), pH 4.7, 39 °C, 23 h, 81%; (b) Ac_2O/pyridine, 100%; (c) Et_3SiH (2 equiv), $BF_3{\cdot}Et_2O$ (1 equiv), CH_2Cl_2, 23 °C, 16 h, 68%.

(DHAP) catalyzed by fructose 1,6-diphosphate aldolase from rabbit muscle (RAMA, Sigma Co.), followed by removal of the phosphate group using acid phosphatase, gave 85% yield of the desired thiosorbose **3**, which existed as a β-pyranose form. Acetylation of **3** gave the tetraacetate **4**, which was subsequently reduced[12] with triethylsilane in the presence of $BF_3{\cdot}Et_2O$ to afford an optically active thiane **5** (1-deoxy-5-thio-D-glucopyranose peracetate). The sterochemical outcome was consistent with an axial attack of hydride at a thianium intermediate.

By a similar procedure (Scheme 2), reaction of the aldehyde from (*R*)-**2** and DHAP catalyzed by fuculose 1-phosphate (Fuc-1-P) aldolase[13] gave an α/β (1:9) mixture of 6-thio-L-tagatose in 78% yield. On the other hand, when racemic aldehyde was used as the acceptor, only the (*R*)-aldehyde was its substrate and gave the same result with high stereospecificity. Upon acetylation, the β-isomer **6** was obtained and reduced with $Et_3SiH/BF_3{\cdot}Et_2O$ to give 1-deoxy-5-thio-D-galactopyranose peracetate (**7**) and 1-deoxy-5-thio-L-altropyranose peracetate (**8**) in a 3:2 mixture.

By a similar procedure (Scheme 3), reaction of the aldehyde from racemic **2** and DHAP catalyzed by rhamnulose 1-phosphate (Rha-1-P) aldolase[14] gave 6-thio-L-fructose, indicating that the

Scheme 4[a]



[a] Reagents and conditions: (a) (i) aqueous HCl (9 mL), pH 1, 50 °C, 4 h, (ii) dihydroxyacetone phosphate (0.5 equiv, 0.46 mmol), RAMA (198 u), pH 6.7, 23 °C, 27 h, (iii) phosphatase (645 u), pH 4.7, 39 °C, 12 h, 85%; (b) (i) aqueous HCl (5 mL), pH 1, 50 °C, 4 h, (ii) acetaldehyde (3 equiv, 1.5 mmol), DERA (200 u), pH 7.3, 23 °C, 48 h, 33%; (c) Ac_2O/pyridine, 100%; (d) Et_3SiH (2 equiv), $BF_3{\cdot}Et_2O$ (1 equiv), CH_2Cl_2, 23 °C, 12 h, 58%.

(*R*)-aldehyde is the preferred substrate. Upon acetylation, the β-isomer **9** obtained was reduced to give 1-deoxy-5-thio-L-mannose peracetate (**10**). Interestingly, when tagatose 1,6-diphosphate aldolase was used as the catalyst, a very slow reaction was observed and the product was not isolated.

When racemic 3-thioglyceraldehyde was used as the acceptor (Scheme 4) in the RAMA-catalyzed reaction, a 58:42 mixture of 6-thio-L-sorbose and 6-thio-D-fructose was obtained and characterized as the acetate forms **4** and **11**. Reduction of **11** ($Et_3SiH/BF_3{\cdot}Et_2O$) gave thiane **12** (1-deoxy-5-thio-D-mannopyranose peracetate), the enantiomer of compound **10**.

In another aldolase reaction, a 2-deoxyribose 5-phosphate aldolase (DERA)-catalyzed reaction of acetaldehyde and racemic 3-thioglyceraldehyde gave 2-deoxy-5-thio-D-ribose. Acetylation of 2-deoxy-5-thio-D-ribose yielded the separable anomers **13a,b** (45:55). Their structures as six-membered thiacycles were supported by the ^1H and ^{13}C NMR spectral analysis.

The (*S*)-enantiomer of 3-thioglyceraldehyde (D-configuration) was selectively reacted in the DERA reaction.[15] On the other hand, a poor enantioselectivity was observed in the RAMA reaction, though (*R*)-3-thioglyceraldehyde (L-configuration) was a preferred substrate in the Fuc-1-P aldolase[16] and Rha-1-P aldolase reactions.

The stereospecificity in the reduction of compounds **4**, **9**, and **11** may be due to stabilization of the thianium intermediate by the anomeric effect, favoring hydride attack from the axial position (Figure 1).[12,17] However, the low stereoselectivity in the reduction of compound **6** may be due to the presence of the C-3 axial acetoxyl group.

In summary, we have demonstrated the utility of aldolases for a short and efficient synthesis of deoxythiosugars. The stereo-

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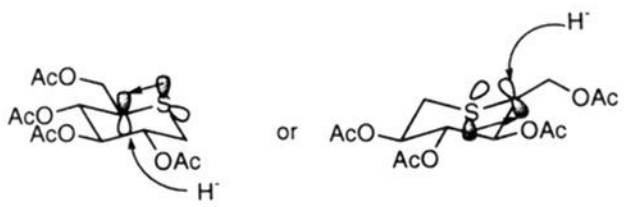

Figure 1. Stereochemical course of the hydride reduction of thianium intermediate.

selectivity of these aldolase-catalyzed carbon–carbon bond forming reactions illustrated in these syntheses is determined by the enzymes and is the same as that in reactions with their natural substrates,[8] providing a convenient and predictable source of sulfur-containing polyfunctional compounds. The general strategy should be applicable to the synthesis of many other thiosugars with different aldolases.

Experimental Section

General Procedure. Brucker AMX-400 and AMX-500 NMR spectrometers were used for 400- and 500-MHz spectra, respectively. High-resolution mass spectra (HRMS) were obtained on a VG ZAB-ZSE mass spectrometer in fast atom bombardment (FAB). HPLC was carried out on a Pirkle DL-phenylglycine column (25 × 0.45 cm) by elution with the indicated solution at a flow rate of 1 mL/min. RAMA (E.C. 4.1.2.13) and acid phosphatase (E.C. 3.1.3.2) were purchased from Sigma and used as received. Recombinant DERA (E.C. 4.1.2.4) was prepared and partially purified from *E. coli* (ATCC 86963) as previously described.[14] Recombinant *E. coli* strains for Rha-1-P aldolase (ATCC # 86983), Fuc-1-P aldolase (ATCC # 86984), and tagatose 1,6-diphosphate (TDP) aldolase (ATCC # 87025) were prepared in this laboratory. Details for cloning, overexpression, and enzyme isolation will be published separately. In brief, Rha-1-P aldolase from *E. coli* K12 was overexpressed in *E. coli* XL1-Blue through PCR and restriction enzyme (Bam HI and Hind III) manipulation of DNA and vector pTrcHis. Approximately 200 U of enzyme activities per liter can be obtained. Fuc-1-P aldolase gene from *E. coli* was cloned into pTrcHis vector through PCR and restriction enzyme manipulation (with EcoRI and PstI) and overexpressed in *E. coli* XL1-Blue (*ca.* 430 U/L). For the preparation of TDP aldolase, the enzyme gene from *Lactococcus lactis* was cloned into pRSET vector via PCR and restriction enzyme (EcoRI and XhoI) manipulation and overexpressed in *E. coli* JM109. For preparation of an aldolase for synthesis, the *E. coli* cells in a phosphate buffer (50 mM, 20 mM $MgCl_2$, pH 7.5) were disrupted by a French press at 16 000 lb/in.^{2} and centrifuged at 23 000*g* for 60 min. The supernatant was collected, and ammonium sulfate (40–75%) was added. After dialysis of the precipitate against the same buffer at 4 °C overnight, the enzyme solution was ready for use in organic synthesis.

(*S*)-Acetyl-3-thioglyceraldehyde Diethyl Acetal (2). To a solution of thiolacetic acid (5.6 mL, 75.2 mmol) and potassium thiolacetate (962 mg, 8.3 mmol) in CH_2Cl_2 (20 mL) was added glycidaldehyde diethyl acetal (2.32 g, 15.9 mmol) slowly at –78 °C under Ar. The reaction mixture was stirred for 1 h at –78 °C and 38 h at room temperature (23 °C). After removal of the solid by filtration, the reaction mixture was poured into ice, then the aqueous layer was extracted with CH_2Cl_2. The organic layers were combined and washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and purified by flash column chromatography (silica gel, Et_2O/hexane, 1:3 to 1:2) to give **2** (2.66 g, 75%) as a liquid, TLC R_f = 0.35 (EtOAc/hexane, 1:2). By a similar procedure, (*R*)-**2**, $[\alpha]^{22}D$ = –32.5° (*c* 0.55, $CHCl_3$), was prepared from the reaction of thiolacetic acid/potassium thiolacetate and (*S*)-glycidaldehyde diethyl acetal (>98% ee): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (t, *J* = 7.1 Hz, 6 H), 2.36 (s, 3 H), 2.45 (br s, 1 H, OH), 3.01 (dd, *J* = 8.0, 14.0 Hz, 1 H), 3.27 (dd, *J* = 3.6, 14.0 Hz, 1 H), 3.56–3.62 (m, 2 H), 3.71–3.80 (m, 3 H), 4.38 (d, *J* = 5.4 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.24 (q, two CH_3), 30.42 (q, CH_3COS), 31.20 (t, CH_2S), 63.58 (t, two CH_2Me), 70,97 (d), 103.46 (d), 196.14 (s); HRMS for $C_9H_{18}O_4SNa$ (M + Na) calcd 245.0824, found 245.0829.

6-Thio-β-L-sorbopyranose (3).[9] An aqueous solution of (*R*)-**2** (172 mg, 0.78 mmol) was adjusted to pH 1.0 with HCl (total volume of 7.6 mL) and heated to 50 °C for 4 h. The pH of this solution was adjusted to 6.7 with 2 N NaOH, followed by the addition of DHAP (0.39 mmol) and RAMA (56 u). After addition, the pH was again adjusted to 6.7 and stirred at 23 °C under Ar for 4 h. At this time, another protion of the aldolase (122 u) was added and stirring continued for an additional 23 h. The pH of the solution was adjusted to 4.7 with 2 N HCl followed by the addition of acid phosphatase (from sweet potatoes, type XA, 645 U) and incubated at 39 °C for 12 h. The reaction mixture was adjusted to pH 7.0 with 2 N NaOH and lyophilized to give a crude solid. Extraction with MeOH followed by concentration under reduced pressure afforded a solid which was chromatographed on silica gel (MeOH/$CHCl_3$, 1:2), giving **3** (65 mg, 85%): white solid, mp 160 °C (darkness, decomposed), lit.[9] 160–161 °C; TLC R_f = 0.27 (MeOH/$CHCl_3$, 1:2); $[\alpha]^{22}D$ = –30.6° (*c* 1.78, H_2O), lit.[9] $[\alpha]D$ = –103.7° (*c* 0.8, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.65 (dd, *J* = 4.5, 13.3 Hz, 1 H, H-6e), 2.84 (dd, *J* = 11.4, 13.3 Hz, 1 H, H-6a), 3.58 (dd, *J* = 9.3, 9.3 Hz, 1 H, H-4), 3.66 (d, *J* = 11.8 Hz, 1 H, H-1), 3.70 (d, *J* = 9.3 Hz, 1 H, H-3), 3.71 (ddd, *J* = 4.5, 9.3, 11.4 Hz, 1 H, H-5), 3.77 (d, *J* = 11.8 Hz, 1 H, H-1); ^{13}C NMR (100 MHz, D_2O) δ 28.04 (t, C-6), 65.39 (t, C-1), 73.46 (d), 75.02 (d), 75.14 (d), 84.07 (s, C-2).

1,3,4,5-Tetra-*O*-acetyl-6-thio-β-L-sorbopyranose (4). Compound **3** (120 mg, 0.61 mmol) was treated with Ac_2O (1 mL) and pyridine (3 mL) at 23 °C for 22 h to give **4** (222 mg) in quantitative yield: TLC R_f = 0.28 (EtOAc/hexane, 3:2); $[\alpha]^{22}D$ = –49.0° (*c* 0.39, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s, 3 H), 2.04 (s, 3 H), 2.07 (s, 3H), 2.13 (s, 3 H), 2.81 (dd, *J* = 4.6, 13.1 Hz, 1 H, H-6e), 3.07 (dd, *J* = 11.3, 13.1 Hz, 1 H, H-6a), 3.52 (d, *J* = 1.2 Hz, 1 H, OH), 4.17 (d, *J* = 11.9 Hz, 1 H, H-1), 4.24 (d, *J* = 11.9 Hz, 1 H, H-1), 5.10 (ddd, *J* = 4.6, 9.8, 11.3 Hz, 1 H, H-5), 5.35 (dd, *J* = 1.2, 9.8 Hz, 1 H, H-3), 5.52 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.50 (q), 20.54 (q), 20.61 (q), 20.81 (q), 25.91 (t, C-6), 67.40 (t), 71.03 (d), 72.86 (d), 74.46 (d), 81.82 (s, C-2), 169.67 (s), 169.70 (s), 169.90 (s), 171.27 (s); HRMS for $C_{14}H_{20}O_9SCs$ (M + Cs) calcd 496.9882, found 496.9887.

(2*R*,3*S*,4*R*,5*R*)-2-(Acetoxymethyl)-3,4,5-triacetoxythiane (5). To a solution of **4** (38.0 mg, 0.10 mmol) in CH_2Cl_2 (2 mL) were added triethylsilane (53 μL, 0.20 mmol) and BF_3·Et_2O (14 μL, 0.10 mmol) at 23 °C under Ar. The reaction mixture was stirred for 12 h and quenched by addition of saturated $NaHCO_3$ (0.5 mL). After 0.5 h, the aqueous layer was extracted with CH_2Cl_2 and dried over Na_2SO_4. The combined extracts were concentrated and chromatographed on silica gel (EtOAc/hexane, 3:2) to give **5** (21 mg, 58%): TLC R_f = 0.47 (EtOAc/hexane, 3:2); HPLC t_R = 14.6 min (2-propanol/hexane, 1:4); $[\alpha]^{22}D$ = +2.0° (*c* 0.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s, 3 H), 2.02 (s, 3 H), 2.03 (s, 3 H), 2.04 (s, 3 H), 2.67 (dd, J = 10.7, 13.3 Hz, 1 H, H-6a), 2.90 (dd, *J* = 2.6, 13.3 Hz, 1 H, H-6e), 3.17 (ddd, *J* = 3.4, 5.8, 10.6 Hz, 1 H, H-2), 4.14 (dd, *J* = 3.4, 11.9 Hz, 1 H, H-1), 4.25 (dd, *J* = 5.8, 11.9 Hz, 1 H, H-1), 5.03-5.07 (m, 2 H, H-4 and H-5), 5.22 (dd, *J* = 9.3, 10.6 Hz, 1 H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.65 (q, two CH_3), 20.72 (q), 20.79 (q), 29.92 (t, C-6), 44.15 (d, C-2), 61.70 (t), 72.13 (d), 72.57 (d), 74.23 (d), 169.47 (s), 169.74 (s), 169.87 (s), 170.57 (s); HRMS for $C_{14}H_{20}O_8SNa$ (M + Na) calcd 371.0777, found 371.0675.

1,3,4,5-Tetra*O*-acetyl-6-thio-β-L-tagatopyranose (6). (*R*)-**2** (142 mg, 0.64 mmol) was hydrolyzed in HCl solution (6 mL, pH 1.0, 50 °C, 4 h), and the pH of this solution was adjusted to 6.7. The solution was treated with DHAP (0.32 mmol) and Fuc-1-P aldolase (1.5 mL, 53 U) by a procedure similar to that for **3** (pH 6.7, 23 °C, 26.5 h). After cleavage of the phosphate with acid phosphatase (430 u, pH 4.7, 39 °C, 22 h), the reaction mixture was lyophilized (pH 7.0), extracted with MeOH and chromatographed (silica gel, MeOH/$CHCl_3$, 1:2) to give 6-thio-L-tagatose (49 mg, 78%). This product was composed to two anomers (α/β = 1:9) as indicated by the ^{1}H NMR spectrum: white solid, mp 185 °C (darkness, decomposed); TLC R_f = 0.29 (MeOH/$CHCl_3$, 1:2); $[\alpha]^{22}D$ = –10.3° (*c* 0.44, H_2O); ^{1}H NMR (400 MHz, D_2O), for the major β-anomer, δ 2.59 (dd, *J* = 4.5, 13.0 Hz, 1 H, H-6e), 2.78 (dd, *J* = 11.2, 13.0 Hz, 1 H, H-6a), 3.59 (d, *J* = 12.0 Hz, 1 H, H-1), 3.68 (d, *J* = 12.0 Hz, 1 H, H-1), 3.70 (dd, *J* = 3.0, 9.8 Hz, 1 H, H-4), 3.85 (ddd, *J* = 4.5, 9.8, 11.2 Hz, 1 H, H-5), 3.92 (d, *J* = 3.0 Hz, 1 H, H-3); ^{1}H NMR (400 MHz, D_2O), for the minor α-anomer, δ 2.53 (dd, *J* = 7.2, 14.2 Hz, 1 H, H-6), 2.99 (dd, *J* = 3.2, 14.2 Hz, 1 H, H-6), 3.70 (dd, *J* = 3.0, 7.2 Hz, 1 H, H-4), 3.71 (d, *J* = 12.3 Hz, 1 H, H-1), 3.79 (d, *J* = 12.3 Hz, 1 H, H-1), 4.05 (d, *J* = 3.0 Hz, 1 H, H-3), 4.06 (ddd, *J* = 3.2, 7.2, 7.2 Hz, 1. H, H-5); ^{13}C NMR (100 MHz, D_2O), for the β-anomer, δ 30.86 (t, C-6), 68.50 (t, C-1), 71.90 (d), 74.82 (d), 75.73 (d), 87.79 (s, C-2); ^{13}C NMR (100 MHz, D_2O), for α-anomer (*inter alia*), δ 67.64 (t, C-1), 71.60 (d), 75.86 (d). Acetylation of 6-thio-L-tagatose by the standard procedure (Ac_2O/pyridine) gave **6** in quantitative yield: TLC R_f = 0.62 (MeOH/$CHCl_3$, 1:14); $[\alpha]^{22}D$ = +5.12° (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.99 (s, 3 H), 2.04 (s, 3 H), 2.12 (s, 3 H), 2.17 (s, 3 H), 2.17 (s, 3 H), 2.82 (dd, *J* = 4.5, 13.0 Hz, 1 H, H-6e), 3.05 (dd, *J* = 11.0, 13.0 Hz, 1 H, H-6a), 3.30 (br s, 1 H, OH), 4.13 (d, *J* = 12.0 Hz, 1 H, H-1), 4.18 (d, *J* = 12.0 Hz, 1 H, H-1), 5.25 (ddd, *J* = 4.5, 10.5, 11.0 Hz, 1 H, H-5),

5.42 (dd, J = 3.5, 10.5 Hz, 1 H, H-4), 5.48 (d, J = 3.5 Hz, 1 H, H-3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.64 (q), 20.68 (q), 20.87 (q), 20.94 (q), 26.31 (t, C-6), 67.03 (t), 69.15 (d), 69.92 (d), 70.79 (d), 82.77 (s, C-2), 169.76 (s), 169.79 (s), 170.22 (s), 171.30 (s); HRMS for $C_{14}H_{20}O_9$SCs (M + Cs) calcd 496.9882, found 496.9906.

(2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-3,4,5-triacetoxythiane (7) and (2*S*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-3,4,5-triacetoxythiane (8). By a procedure similar to that for **5**, compound **6** (41.5 mg, 0.114 mmol) was reduced with Et_3SiH (37 μL, 0.228 mmol) in the presence of $BF_3{\cdot}Et_2O$ (14 μL, 0.114 mmol) to give two isomers **7** and **8** (ratio 3:2) (25.4 mg, 64%), TLC R_f = 0.20 (EtOAc/hexane, 1:2). **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.07 (s, 3 H), 2.10 (s, 3 H), 2.11 (s, 3 H), 2.14 (s, 3 H), 2.76 (dd, J = 5.8, 14.4 Hz, 1 H, H-6), 3.04 (dd, J = 3.0, 14.4 Hz, 1 H, H-6), 3.37–3.43 (m, 1 H, H-2), 4.25 (dd, J = 5.4, 11.8 Hz, 1 H, H-1), 4.29 (dd, J = 6.6, 11.8 Hz, 1 H, H-1), 5.13 (ddd, J = 3.0, 5.8, 8.5 Hz, 1 H, H-5), 5.22 (dd, J = 2.7, 6.1 Hz, 1 H, H-3), 5.41 (dd, J = 2.7, 8.5 Hz, 1 H, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.67 (q), 20.74 (q, two CH_3), 21.00 (q), 27.41 (t, C-6), 39.50 (d, C-2), 62.62 (t), 68.61 (d), 68.77 (d), 69.49 (d), 169.38 (s), 169.82 (s), 170.45 (s), 170.66 (s). **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3 H), 2.05 (s, 3 H), 2.06 (s, 3 H), 2.16 (s, 3 H), 2.72 (dd, J = 10.2, 13.5 Hz, 1 H, H-6a), 2.94 (dd, J = 4.2, 13.5 Hz, 1 H, H-6e), 3.37–3.43 (m, 1 H, H-2), 4.04 (dd, J = 6.8, 11.3 Hz, 1 H, H-1), 4.13 (dd, J = 7.8, 11.3 Hz, 1 H, H-1), 4.91 (dd, J = 3.0, 9.7 Hz, 1 H, H-4), 5.24 (ddd, J = 4.2, 9.7, 10.2 Hz, 1 H, H-5), 5.64 (dd, J = 2.3, 3.0 Hz, 1 H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.77 (q, two CH_3), 20.81 (q), 20.91 (q), 29.68 (t, C-6), 42.84 (d, C-2), 61.45 (t), 68.25 (d, two CH_2), 68.95 (d), 169.85 (s), 169.92 (s), 170.00 (s), 170.22 (s); HRMS for $C_{14}H_{20}O_8$SNa (M + Na) calcd 371.0777, found 371.0771.

1,3,4,5-Tetra-*O*-acetyl-6-thio-β-L-fructopyranose (9). (±)-**2** (221 mg, 1 mmol) was hydrolyzed in HCl solution (10 mL, pH 1.0, 50 °C, 4 h), and the pH of this solution was adjusted to 6.7. The solution was treated with DHAP (0.5 mmol) and Rha-1-P aldolase (10 U) by a procedure similar to that for **3** (pH 6.7, 22 °C, 22 h). After cleavage of the phosphate with acid phosphatase (300 u, pH 4.7, 39 °C, 23 h), the reaction mixture was lyophilized (pH 7.0), extracted with MeOH, and chromatographed (silica gel, MeOH/$CHCl_3$, 1:3) to give 6-thio-L-fructose (79 mg, 81%): TLC R_f = 0.26 (2-propanol/NH_4OH/H_2O, 7:3:1); ^{1}H NMR (400 MHz, D_2O) δ 2.60 (dd, J = 4.2, 14.5 Hz, 1 H, H-6), 3.15 (dd, J = 1.7, 14.5 Hz, 1 H, H-6), 3.63 (d, J = 11.8 Hz, 1 H, H-1), 3.69 (d, J = 11.8 Hz, 1 H, H-1), 3.75 (dd, J = 3.1, 9.8 Hz, 1 H, H-4), 3.89 (d, J = 9.8 Hz, 1 H, H-3), 4.23 (ddd, J = 1.7, 3.1, 4.2 Hz, 1 H, H-5); ^{13}C NMR (100 MHz, D_2O) δ 29.96 (t, C-6), 65.88 (t, C-1), 69.58 (d), 71.30 (d), 71.44 (d), 84.69 (s, C-2). Acetylation of 6-thio-L-fructose by the standard procedure (Ac_2O/pyridine) gave **9** in quantitative yield: TLC R_f = 0.14 (EtOAc/hexane, 2:3); $[\alpha]^{22}$D = +132.1° (c 0.43, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (s, 3 H), 2.10 (s, 3 H), 2.15 (s, 3 H), 2.18 (s, 3 H), 2.79 (dd, J = 4.3, 14.7 Hz, 1 H, H-6), 3.33 (dd, J = 1.7, 14.7 Hz, 1 H, H-6), 3.46 (br s, 1 H, OH), 4.21 (d, J = 11.9 Hz, 1 H, H-1), 4.28 (d, J = 11.9 Hz, 1 H, H-1), 5.39 (dd, J = 3.0, 10.2 Hz, 1 H, H-4), 5.51 (ddd, J = 1.7, 3.0, 4.3 Hz, 1 H, H-5), 5.63 (d, J = 10.2 H, 1 H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.63 (q, three CH_3), 21.06 (q), 27.71 (t, C-6), 68.03 (t), 69.13 (d), 69.39 (d), 71.51 (d), 82.32 (s, C-2), 169.82 (s), 170.00 (s), 170.46 (s), 171.40 (s); HRMS for $C_{14}H_{20}O_9$SCs (M + Cs) calcd 496.9882, found 496.9870.

(2*S*,3*R*,4*S*,5*R*)-2-(Acetoxymethyl)-3,4,5-triacetoxythiane (10). By a procedure similar to that for **5**, compound **9** (33.1 mg, 0.09 mmol) was reduced with Et_3SiH (29 μL, 0.18 mmol) in the presence of $BF_3{\cdot}Et_2O$ (13 μL, 0.09 mmol) to give **10** (21.1 mg, 68%): TLC R_f = 0.28 (EtOAc/hexane, 2:3); $[\alpha]^{22}$D = +51.7° (c 0.33, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 (s, 3 H), 2.08 (s, 3 H), 2.09 (s, 3 H), 2.15 (s, 3 H), 2.84 (dd, J = 2.7, 14.4 Hz, 1 H, H-6e), 2.91 (dd, J = 5.9, 14.5 Hz, 1 H, H-6a), 3.18 (ddd, J = 5.3, 6.8, 8.3 Hz, 1 H, H-2), 4.22 (dd, J = 5.3, 11.7 Hz, 1 H, H-1), 4.30 (dd, J = 6.8, 11.7 Hz, 1 H, H-1), 4.97 (dd, J = 3.0, 8.4 Hz, 1 H, H-4), 5.40 (dd, J = 8.4, 8.4 Hz, 1 H, H-3), 5.42 (ddd, J = 2.7, 3.0, 5.9 Hz, 1 H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.66 (q), 20.72 (q, two CH_3), 21.04 (q), 28.39 (t, C-6), 42.76 (d, C-2), 62.61 (t), 68.63 (d), 69.05 (d), 71.77 (d), 169.63 (s), 169.71 (s), 170.23 (s), 170.60 (s); HRMS for $C_{14}H_{20}O_8$SCs (M + Cs) calcd 480.9933, found 480.9950.

1,3,4,5-Tetra-*O*-acetyl-6-thio-β-D-fructopyranose (11).[7e] (±)-**2** (203 mg, 0.92 mmol) was hydrolyzed in HCl solution (9 mL, pH 1.0, 50 °C, 4 h), and the pH of this solution was adjusted to 6.7. The solution was treated with DHAP (0.46 mmol) and RAMA (198 u) by a procedure similar to that for **3** (pH 6.7, 23 °C, 27 h). After cleavage of the phosphate with acid phosphatase (645 u, pH 4.7, 39 °C, 12 h), the reaction mixture was lyophilized (pH 7.0), extracted with MeOH, and chromatographed (silica gel, MeOH/$CHCl_3$, 1:2) to give 6-thio-L-sorbose and 6-thio-D-fructose (76 mg, 85%) in a ratio of 58:42. The component of 6-thio-D-fructose contained two anomers (α/β = 1:9) as indicated by the ^{1}H NMR spectrum.[7g,9] ^{1}H NMR (400 MHz, D_2O), for the major β-anomer: δ 2.63 (dd, J = 4.2, 14.5 Hz, 1 H, H-6a), 3.20 (dd, J = 1.7, 14.5 Hz, 1 H, H-6e), 3.70 (d, J = 11.8 Hz, 1 H, H-1), 3.75 (d, J = 11.8 Hz, 1 H, H-1), 3.79 (dd, J = 3.1, 9.8 Hz, 1 H, H-4), 3.93 (d, J = 9.8 Hz, 1 H, H-3), 4.27 (ddd, J = 1.7, 3.1, 4.2 Hz, 1 H, H-5). ^{1}H NMR (400 MHz, D_2O), for the minor α-anomer (*inter alia*): δ 2.93 (dd, J = 9.3, 14.0 Hz, 1 H, H-6a), 3.58 (dd, J = 3.3, 5.9 Hz, H-4), 4.07 (d, J = 5.9 Hz, H-3), 4.15 (ddd, J = 2.9, 3.3, 9.3 Hz, H-5). ^{13}C NMR (100 MHz, D_2O, β-anomer): δ 29.94 (t, C-6), 65.89 (t, C-1), 69.55 (d), 71.32 (d), 71.42 (d), 84.58 (s, C-2). The mixture of 6-thio-L-sorbose and 6-thio-D-fructose (58:42) was subjected to the standard acetylation procedure (Ac_2O/pyridine) to give **4** and **11**. Compound **11**: TLC R_f = 0.28 (EtOAc/hexane, 3:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (s, 3 H), 2.10 (s, 3 H), 2.15 (s, 3 H), 2.18 (s, 3 H), 2.79 (dd, J = 4.3, 14.7 H, 1 H, H-6a), 3.33 (dd, J = 1.7, 14.7 Hz, 1 H, H-6e), 3.48 (d, J = 1.2 Hz, 1 H, OH), 4.20 (d, J = 11.9 Hz, 1 H, H-1), 4.27 (d, J = 11.9 Hz, 1 H, H-1), 5.38 (dd, J = 3.1, 10.2 Hz, 1 H, H-4), 5.50–5.52 (m, 1 H, H-5), 5.63 (dd, J = 1.2, 10.2 Hz, 1 H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.61 (q, three CH_3), 21.05 (q), 27.72 (t, C-6), 68.13 (t), 69.07 (d), 69.39 (d), 71.53 (d), 82.34 (s, C-2), 169.80 (s), 169.98 (s), 170.44 (s), 171.39 (s).

(2*R*,3*S*,4*R*,5*S*)-2-(Acetoxymethyl)-3,4,5-triacetoxythiane (12). By a procedure similar to that for **5**, a mixture of **4** and **11** (58:42, 68.6 mg, 0.19 mmol) was reduced with Et_3SiH (61 μL, 0.38 mmol) in the presence of $BF_3{\cdot}Et_2O$ (27 μL, 0.19 mmol) to give **5** and **12**. A sample was separated by HPLC to give **12**: t_R = 16.7 min (2-propanol/hexane, 1:4); ^{1}H NMR (400 MH, $CDCl_3$) δ 2.04 (s, 3 H), 2.08 (s, 3 H), 2.09 (s, 3 H), 2.15 (s, 3 H), 2.83 (dd, J = 2.7, 14.4 Hz, 1 H, H-6e), 2.91 (dd, J = 6.0, 14.4 Hz, 1 H, H-6a), 3.18 (ddd, J = 5.2, 6.8, 8.4 Hz, 1 H, H-2), 4.22 (dd, J = 5.2, 11.7 Hz, β1 H, H-1), 4.30 (dd, J = 6.8, 11.7 Hz, 1 H, H-1), 4.97 (dd, J = 3.0, 8.4 Hz, 1 H, H-4), 5.40 (dd, J = 8.4, 8.4 Hz, 1 H, H-3), 5.42 (ddd, J = 2.7, 3.0, 6.0 Hz, 1 H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.54 (q), 20.72 (q, two CH_3), 21.03 (q), 28.39 (t, C-6), 42.77 (d, C-2), 62.61 (t), 68.64 (d), 69.06 (d), 71.77 (d), 169.63 (s), 169.71 (s), 170.23 (s), 170.59 (s); HRMS for $C_{14}H_{21}O_8$S (M + H) calcd 349.0957, found 349.0960. (For chemical synthesis, see ref 6c.)

2-Deoxy-5-thio-1,3,4-tri-*O*-acetyl-D-ribopyranose (13a,b). To a solution (5 mL) containing (±)-**2** (100 mM) and acetaldehyde (300 mM) in triethanolamine buffer (100 mM, pH 7.3) were added EDTA (1 mM) and DERA (200 u). The resulting solution was stirred under N_2 in the dark for 2 days and quenched by addition of acetone (10 mL). The mixture was then incubated in ice for 20 min and centrifuged to remove the precipitate. After removal of the solvent under reduced pressure, the residue was subjected to the standard acetylation procedure (Ac_2O/pyridine) to yield a mixture of two products (45.5 mg, 33%) (**13a**/**13b** = 45:55) which were separated by preparative TLC (MeOH/$CHCl_3$/hexane, 1:90:10). **13a**: TLC R_f = 0.27 (MeOH/$CHCl_3$/hexane, 1:90:10); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.06 (s, 3 H), 2.09 (s, 3 H), 2.13 (s, 3 H), 2.29 (ddd, J = 2.8, 3.5, 15.4 Hz, H-2), 2.50 (dd, J = 3.8, 12.8 Hz, 1 H, H-5), 2.63 (ddd, J = 3.5, 4.4, 15.4 Hz, 1 H, H-2), 3.36 (dd, J = 11.1, 12.8 Hz, 1 H, H-5), 5.12 (ddd, J = 2.6, 3.8, 11.1 Hz, 1 H, H-4), 5.22-5.25 (m, 1 H, H-3), 5.79 (dd, J = 3.5, 3.5 Hz, 1 H, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 2087 (q), 20.97 (q), 21.09 (q), 22.73 (t, C-5), 35.87 (t, C-2), 67.39 (d, C-3), 68.84 (d, C-4), 70.18 (d, C-1), 169.25 (s), 169.83 (s), 170.09 (s); HRMS for $C_{11}H_{16}O_6$SNa (M + Na) calcd 299.0565, found 299.0565; HRMS for $C_{11}H_{16}O_6$SCs (M + Cs) calcd 408.9722, found 408.9726. **13b**: TLC R_f = 0.36 (MeOH/$CHCl_3$/hexane, 1:90:10); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.05 (s, 3 H), 2.12 (s, 3 H), 2.08-2.15 (m, 1 H, H-2), 2.16 (s, 3 H), 2.44 (ddd, J = 2.9, 11.3, 13.6 Hz, 1 H, H-2), 2.87 (dd, J = 1.6, 14.6 Hz, 1 H, H-5), 3.26 (dd, J = 1.9, 14.6 Hz, 1 H, H-5), 5.21 (ddd, J = 3.8, 3.8, 11.3 Hz, 1 H, H-3), 5.34 (ddd, J = 1.6, 1.9, 3.8 Hz, 1 H, H-4), 6.03 (br s, 1 H, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.0 (q), 21.1 (q), 21.2 (q), 28.4 (t, C-5), 32.5 (t, C-2), 66.1 (d), 67.3 (d), 72.4 (d, C-1), 169.4 (s), 170.1 (s), 170.4 (s); HRMS for $C_{11}H_{16}O_6$SNa (M + Na) calcd 299.0565, found 299.0577; HRMS for $C_{11}H_{16}O_6$SCs (M + Cs) calcd 408.9722, found 408.9722.

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