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α-(1 → 2)-, α-(1 → 3)-, and α-(1 → 6)-Linked thioglycosidic disaccharides: syntheses and anti-HIV testing of thiokojibiose octaacetate, thionigerose, and thioisomaltose

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Abstract

The syntheses of several α-linked thioglycosidic disaccharides are described, including thiokojibiose octaacetate (**1**), thionigerose (**2**), and thioisomaltose (**3**). The title compounds were synthesized by coupling 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose (**4**) with either 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethylsulfonyl-β-D-mannopyranose (**7**), 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethylsulfonyl-α-D-allofuranose (**15**), or methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-α-D-glucopyranoside (**17**), respectively. Thiokojibiose octaacetate in turn was converted to 3,4,6-tri-*O*-acetyl-2-*S*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-2-thio-α-D-glucopyranosyl bromide (**9**), which was used to obtain several related disaccharides and one trisaccharide. All of the compounds, including thiomaltose and thiotrehalose, which were resynthesized by known methods, were tested for their anti-HIV activity in either CEM or MT-2 cells. Anti-HIV activity was noted only with thiokojibiose octaacetate and its 1-thio analogue (**14**), which had IC_{50} values of 51 and 48 μg/mL in CEM cells, respectively.

Keywords: Thioglycoside; Thioglycosyl disaccharide; Thiokojibiose; Thionigerose; Thioisomaltose

1. Introduction

The chemical literature of the late 1980's and early 1990's reveals a heightened interest in compounds, of both synthetic and natural origin, with the potential to

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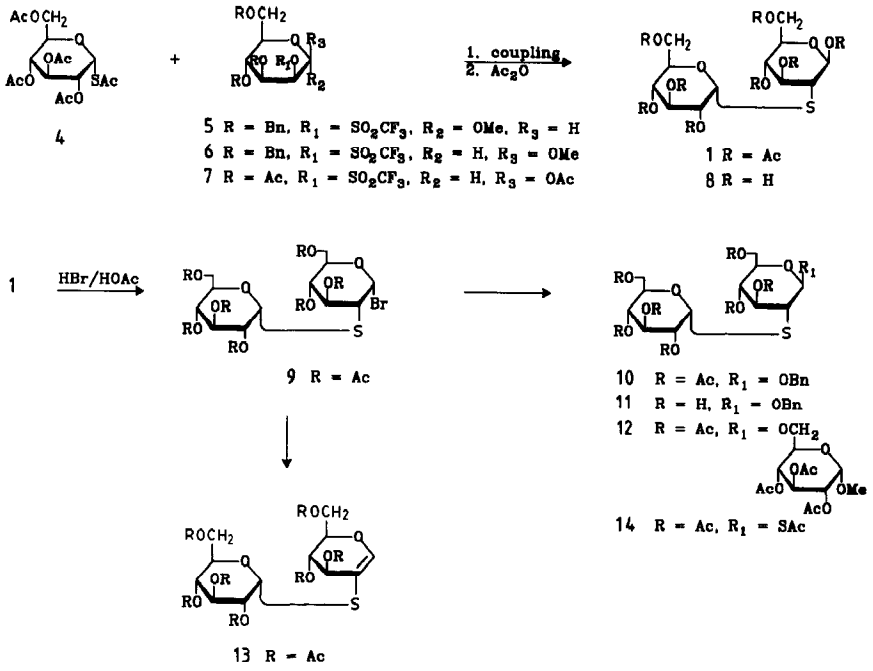
serve as inhibitors of the glycoside-cleaving enzymes. These compounds have demonstrated potential in the treatment of acquired immunodeficiency syndrome [1], diabetes [2], and tumor metastases [3], and have served as valuable tools for studying the complex set of reactions associated with producing mature glycoproteins [4,5]. Most of the glycosidase inhibitors that have demonstrated potent anti-HIV activity, generally azacyclic sugars or amino cyclitol compounds with a strong resemblance to glucose (e.g., *N*-butyldeoxynojirimycin [6] and valiolamine [7]), have proven to be tight-binding, active-site directed inhibitors of the α-glucosidases I and II enzymes.

Thioglycosidic saccharides, because of their resistance to enzymatic digestion, have also been utilized for studying enzyme activity [8]. They have served as useful substrate analogues for studying enzyme induction [9], mechanisms of action [10], and purification by affinity chromatography [11]. The resistance to enzymatic hydrolysis is critical for these types of investigations, and it was this potential to serve as nonmetabolizable substrates that caused our interest in such compounds as candidate α-glucosidase inhibitors that might possess anti-HIV activity. Herein we describe the syntheses of 2-*S*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1,3,4,6-tetra-*O*-acetyl-2-thio-β-D-glucopyranose (**1**, thiokojibiose octaacetate), 3-*S*-(α-D-glucopyranosyl)-3-thio-D-glucose (**2a,b**, thionigerose), methyl 6-*S*-(α-D-glucopyranosyl)-6-thio-α-D-glucopyranoside (**3**, thioisomaltose, methyl glycoside), some related derivatives, and their evaluation as potential HIV inhibitors.

2. Results and discussion

Chemistry.—The thio-linked glucopyranosyl glucopyranosides that have previously been reported include the thioglycosidic analogues of trehalose (1α,1′α- [12], 1α,1′β- [12] and 1β,1′β-linked [13]), sophorose (β1,2-linked [14]), cellobiose (β1,4-linked [14]), maltose (α1,4-linked [15]), and gentiobiose (β1,6-linked [16]). Both linear [17] and branched [18] trisaccharides containing an α-Glc(1 → 6)Glc thio-linked disaccharide have been reported. The syntheses of these disaccharides have generally been effected by one of two methods: (*1*) reaction of a 1-thioglucose with a suitably protected 1-, 2-, 4-, or 6-halo or trifluoromethylsulfonylglucoside, or (*2*) reaction of a glycosyl halide with a 1-, 2-, 4-, or 6-thiolate anion of glucose. Most of these syntheses have relied on the nucleophilic enhancement of thiolate in polar aprotic solvents such as hexamethylphosphorictriamide (HMPA) [10,12], although phase-transfer catalysis has also been used [14]. We based the syntheses of the α-(1 → 2)-, α-(1 → 3)-, and α-(1 → 6)-thio-linked glycosyl glycosides **1**, **2**, and **3**, on the first of these two strategies.

Our original approach to the synthesis of thiokojibiose involved coupling the known 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose (**4**) [15] with methyl 3,4,5-tri-*O*-benzyl-2-trifluoromethylsulfonyl-α-D-mannopyranoside (**5**) [19] (Scheme 1). Compound **4** was reacted with sodium methoxide, and the resultant 1-thiolate was coupled in HMPA with **5**. Little to no disaccharide was ever isolated, an observation consistent with the poor reactivity at C-2 in α-mannopyranosides during nucleophilic substitution [19]. Although the more reactive



β-methyl glycoside **6** has been reported [19], we turned to the more readily obtainable, 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethylsulfonyl-β-D-mannopyranose (**7**) [20], which had been used in the synthesis of thiosophorose [14]. Compound **4** was again converted to its 1-thiolate and reacted in HMPA with **7** to provide, after workup, thiokojibiose octaacetate (**1**) in 43% crystallized yield.

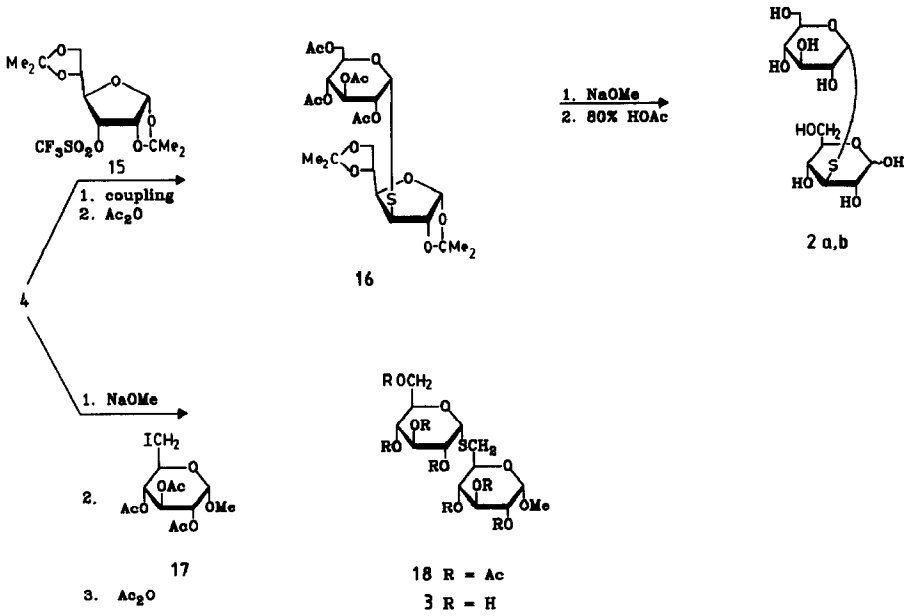
Simple deacetylation (Zemplén) of **1** was surprisingly problematic in light of the reported [14] successful deacetylation of thiosophorose octaacetate with sodium methoxide. Compound **1** was treated with sodium methoxide in methanol for 1 h, neutralized with Dowex 50 (H^+) resin, filtered, and the product(s) were isolated. Chromatographic analysis of the product mixture showed two major bands. After column chromatography, the least polar product(s) was found to have a molecular weight of 340, consistent with a loss of water from the expected product **8**. The more polar product(s) had a molecular weight of 372, consistent with the methyl glycoside of the desired product. Mass spectral evidence also indicated that some of **8** was in the more polar fraction. The ^1H NMR spectrum of each fraction was very complex and showed the presence of at least two components in each group. A portion of the original deacetylated material (unchromatographed) was reacetylated in pyridine–acetic anhydride, and a four-component mixture by TLC (1:1 EtOAc–cyclohexane) was obtained. The mass spectrum of this material after workup showed the presence of **1** (MW 694), a methyl glycoside of **1** (MW 666), the methyl glycoside with one double bond (MW 606), and a product with two double bonds and one free hydroxyl group (MW 532).

Clearly, simple deacetylation is not straightforward with either **1** or thiosophorose. In his synthesis of thiosophorose, Hamacher [14] provides no mass spectral or NMR data in support of his final deblocked product. We were able to resynthesize a very small amount of thiosophorose from 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose [21] using the Hamacher procedure. In our hands, the TLC of thiosophorose (1:4:7 EtOAc-water–1-propanol) (Hamacher's system) revealed a two-component mixture. Chromatographic analysis in 2:1 $CHCl_3$–MeOH showed a multicomponent mixture with a considerable amount of material more polar than the apparent α/β mixture of desired products. Interestingly, no such complications are reported in the simple deacetylation reactions of sophorose [22] and kojibiose [23].

We tried a number of other methods to deblock compound **1**, including Na_2CO_3–MeOH, KCN–MeOH [24], porcine liver esterase (which would not work because of solubility problems), ammonia in ethanol, hydroxide in water–MeCN, $Ba(OMe)_2$–MeOH, and selective removal of the 1-*O*-acetyl group with ammonia–MeCN [25], followed by Zemplén deacetylation. All of these deblocking attempts were plagued with various complications. To obtain a deblocked form of compound **1** suitable for biological testing, we first treated **1** with HBr–HOAc to obtain the glycosyl bromide **9**, which in turn was reacted with benzyl alcohol to give the benzyl β-glycoside **10**. Simple deacetylation of **10** provided **11** without any complications. Intermediate **9** was also used to synthesize several other thiosaccharides. Compound **9** was coupled to methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside [26] to give trisaccharide **12** in 24% yield, treated with diethylamine to provide the dehydrohalogenated product **13** in 67% yield, and reacted with the trioctylammonium salt of thiolacetic acid to give the 1-β-thioacetyl derivative **14** in 33% yield.

Thionigerose (**2a,b**) was synthesized straightforwardly from the peracetylated 1-thiol **4** by first treating **4** with sodium methoxide and reacting the resultant 1-thiolate with 1,2 : 5,6-di-*O*-isopropylidene-3-*O*-trifluoromethylsulfonyl-α-D-allofuranose (**15**) [27] to give 1,2:5,6-di-*O*-isopropylidene-3-*S*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-3-thio-α-D-glucofuranose (**16**) in 60% crystalline yield (Scheme 2). Treatment of compound **16** with sodium methoxide in methanol overnight, followed by heating in 80% acetic acid at 60°C for 18 h, provided α,β-thionigerose (**2a,b**) as a white powder in 60% yield from **16**. When the latter reaction was followed by TLC, complete hydrolysis of the 5,6-isopropylidene group was noticed in 2 h. Attempts to shorten the hydrolysis time of the 1,2-isopropylidene group by raising the reaction temperature, however, increased the observed thioglycosidic bond cleavage. Prolonged heating at 60°C in 80% HOAc provided the optimum results. The α-linked 1,6-disaccharide **3** (thioisomaltose) was obtained in a similar fashion. Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-α-D-glucopyranoside (**17**) [28] was prepared and reacted in HMPA with the thiolate derivative of **4** to provide compound **18** in 69% yield. Simple deacetylaton with methoxide in MeOH gave **3** as a white powder in 83% yield.

Biological data.—Thiomaltose [15] and 1α,1′α-thiotrehalose [12] were also resynthesized by published methods and, along with thiokojibiose octaacetate (**1**), thionigerose (**2a,b**), thioisomaltose (**3**), and compounds **10–14**, and **18**, were



evaluated [29] for their abilities to inhibit HIV-induced cell killing and virus production in CEM or MT-2 cells. All of the compounds were inactive with the exception of thiokojibiose octaacetate (**1**) and its 1-thio analogue **14**. Thiokojibiose octaacetate was active in both cell lines with IC_{50} values of 51.4 and 8.0 μg/mL in CEM and MT-2 cells, respectively [IC_{50} is defined as the drug concentration (μg/mL) that inhibits the viral cytopathic effect (CPE) by 50% calculated by using a regression analysis program for semilog curve fitting]. Compound **14** was not evaluated in MT-2 cells, but had an IC_{50} of 48 μg/mL in the CEM cell line. All of the compounds were nontoxic up to the highest dose tested (100 μg/mL). None of these compounds was evaluated for glucosidase I or II activity.

3. Experimental

General methods.—Melting points were determined on a Mel-Temp apparatus and are uncorrected. NMR spectra (internal Me_4Si) were recorded with a Nicolet NMC 300 NB spectrometer operating at 300.635 MHz for ^1H and at 75 MHz for ^{13}C. In the NMR data sections, protons in the nonreducing portion of the disaccharides are designated with a prime ('), and in the trisaccharides, the third sugar residue has a double prime ("). Mass spectra were recorded with a Varian MAT 311A mass spectrometer in the EI or FAB (samples dissolved in glycerol or 3-nitrobenzyl alcohol) mode. Microanalyses were performed by the Molecular Spectroscopy Section of the Organic Chemistry Research Department of the Southern Research Institute.

1,3,4,6-Tetra-O-acetyl-2-S-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-2-thio-β-D-glucopyranose (**1**).—Compound **4** [15] (459 mg, 1.13 mmol) and NaOMe (61 mg, 1.1 mmol) were added to deoxygenated anhyd MeOH (14 mL), and the solution was stirred for 18 h at room temperature under N_2. After the MeOH was removed and the residue was dried in vacuo for 6 h, the residue and compound **7** [20] (543 mg, 1.13 mmol) were placed in HMPA (5 mL) and stirred for 18 h at room temperature under N_2. Acetic anhydride (3.6 mL) and pyridine (2.7 mL) were added, and stirring was continued for 18 h. The mixture was then poured into cold water (30 mL) and extracted with Et_2O (3 × 30 mL). The product precipitated both in the separating funnel and in the ether extracts during extraction. The heterogeneous ether extracts were washed with water (2 × 15 mL), and $CHCl_3$ was added to dissolve the suspended product. This solution was dried over $MgSO_4$, filtered, and evaporated to dryness to give the major portion of material. The precipitate in the separating funnel was filtered, and the collected solids were washed with H_2O, dried in vacuo, and combined with the major portion above. The combined products were chromatographed on silica gel (7 × 1.9 cm column, 230–400 mesh) by eluting first with $CHCl_3$ (100 mL) and then with 99:1 $CHCl_3$–MeOH. The product-containing fractions were combined, the solvent was removed in vacuo, and the residue was triturated with Et_2O. Filtering and drying gave 240 mg (31% [1]) of **1** as white crystals, mp 207–208°C. FABMS: m/z 695 (M + 1). ^{1}H NMR ($CDCl_3$): δ 1.95–2.25 (8 s, 24 H, OAc), 3.15 (t, $J_{1,2}$ 10, $J_{2,3}$ 9.5 Hz, 1 H, H-2), 3.81 (m, 1 H, H-5′), 4.06 (dd, $J_{6'a,6'b}$ 13, $J_{5',6'b}$ 2 Hz, 1 H, H-6′b), 4.18 (dd, $J_{6'a,6'b}$ 12, $J_{5',6'a}$ 1 Hz, 1 H, H-6′a), 4.24–4.36 (m, 3 H, H-5, H-6a, H-6b), 4.94 (dd, 1 H, H-2′), 5.03 (m, 3 H, H-4′, H-3, H-4), 5.33 (t, $J_{2',3'}$ 10, $J_{3',4'}$ 8 Hz, 1 H, H-3′), 5.68 (d, $J_{1,2}$ 10 Hz, 1 H, H-1), 5.98 (d, $J_{1',2'}$ 6 Hz, 1 H, H-1′). Anal. Calcd for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51. Found: C, 48.19; H, 5.46.

3-S-(α-D-Glucopyranosyl)-3-thio-α-D-glucopyranose (**2a**) *and 3-S-(α-D-glucopyranosyl)-3-thio-β-D-glucopyranose* (**2b**).—A solution containing compound **16** (0.6 g, 1 mmol) and NaOMe (20 mg, 0.3 mmol) in MeOH (20 mL) was stirred overnight at room temperature. The solution was neutralized with Dowex 50W-X4 (H^+) resin, filtered through Celite, and concentrated in vacuo to give a white foam (400 mg, 92%). A portion of this foam (200 mg) was placed in 80% aq AcOH (10 mL) and heated at 58°C for 18 h. (Note: The 5,6-isopropylidene group was removed in ca. 2 h.) The mixture was concentrated in vacuo and purified chromatographically on silica gel (230–400 mesh) eluting with 3:1 $CHCl_3$–MeOH to give **2a,b** as a foam. This foam was dissolved in a small amount of dry MeOH and added dropwise to a large volume of dry Et_2O to precipitate **2a,b** which was then collected and dried in vacuo to give 107 mg (65% yield), mp > 160°C (dec). FABMS: m/z 359 (M + 1). ^{1}H NMR (D_2O, referenced to internal Bu^{t}OD): δ 2.74 (t, $J_{2\beta,3\beta} \approx 11$, $J_{3\beta,4} \approx 10$ Hz, 0.54 H, H-3β), 2.98 (t, $J_{2\alpha,3\alpha} \approx J_{3\alpha,4\alpha} = 11$ Hz, 0.46 H, H-3α), 3.2 (m, $J_{1\beta,2\beta}$ 7.8, $J_{2\beta,3\beta} \approx 11$ Hz, 0.54 H, H-2β), 3.27–3.86 (complex m, 9.46 H, H-2α, H-2′α,β, H-3′α,β, H-4α,β, H-4′α,β, H-5α,β, H-6α,β,

[1] Note: A larger reaction using 7.09 mmol of both **4** and **7** yielded 43% of **1**.

H-6′α,β), 4.07 (m, 1 H, H-5′α,β), 4.59 (d, 0.54 H, $J_{1\beta,2}$ 7.8 Hz, H-1β), 5.16 (d, 0.46 H, $J_{1\beta,2\alpha}$ 4 Hz, H-1α), 5.6 (d, 1 H, $J_{1',2'}$ 5.5 Hz, H-1′α,β). ^{13}C NMR (D_2O, referenced to internal dioxane at 67.4 ppm): 51.30 (C-3α), 54.56 (C-3β), 61.25, 61.34, 61.60, 61.76 (C-6α,β, C-6′α,β), 69.51, 70.37, 70.46, 70.54, 70.73, 71.93, 71.98, 72.09, 73.18, 73.22, 74.39, 79.26 (other unassigned carbons), 85.73 (C-1′α,β, $J_{C,H}$ 162.5 Hz), 92.26 (C-1α, $J_{C,H}$ 170.6 Hz), 97.94 (C-1β, $J_{C,H}$ 162.0 Hz). Anal. Calcd for $C_{12}H_{22}O_{10}S \cdot H_2O$: C, 38.29; H, 6.43. Found: C, 38.39; H, 6.31.

*Methyl 6-*S*-(α-*D*-glucopyranosyl)-6-thio-α-*D*-glucopyranoside* (**3**).—Sodium (6 mg, 0.26 mmol) was dissolved in MeOH (3 mL), compound **18** (77 mg, 0.12 mmol) was added, and the resulting solution was stirred overnight (18 h). The mixture was neutralized with Dowex 50 × 8 (H^+) resin, filtered through Celite, and evaporated to dryness. The residue was dissolved in a small amount of MeOH and added dropwise to dry Et_2O (30 mL) which, precipitated **3** as a white powder (37 mg, 83%), mp > 91°C (dec, with softening from 58–62°C). FABMS: *m/z* 373 (M + 1). ^{1}H NMR (D_2O, 60°C, referenced to internal acetone): δ 2.66 (dd, 1 H, H-6a), 3.02 (dd, 1 H, H-6b), 3.22–3.38 (m, 2 H, H-4, H-4′), 3.33 (s, 3 H, OCH_3), 3.42–3.58 (m, 3 H, H-2, H-3, H-3′), 3.62–3.8 (m, 4 H, H-5, H-2′, H-6′a, H-6′b), 3.86–3.94 (m, 1 H, H-5′), 4.67 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.35 (d, 1 H, $J_{1',2'}$ 5.5 Hz, H-1′). Anal. Calcd for $C_{13}H_{24}O_{10}S \cdot 0.6H_2O$: C, 40.75; H, 6.62. Found: C, 40.65; H, 6.64.

*3,4,6-Tri-*O*-acetyl-2-*S*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-2-thio-α-*D*-glucopyranosyl bromide* (**9**).—A slurry of compound **1** (1.5 g, 2.2 mmol) in glacial AcOH (11 mL) was cooled in an ice bath, HBr–AcOH (39%, 17 mL) was added, and the resulting mixture was frozen overnight (18 h). Upon thawing, the yellow mixture was poured onto ice–$CHCl_3$. The organic layer was separated and washed with satd $NaHCO_3$ (2 × 25 mL). The $CHCl_3$ layer was dried over Na_2SO_4, filtered, and evaporated to dryness. Crystallization (two crops) of the residue from dry Et_2O gave 0.99g (64%) of **9** as a white solid, mp 134–35°C (dec). FABMS: *m/z* 715 (M + 1). ^{1}H NMR ($CDCl_3$): δ 2.0–2.2 (7 s, 21 H, OAc), 3.25 and 3.28 (dd, $J_{1,2}$ 3.5, $J_{2,3}$ 11.1 Hz, 1 H, H-2), 4.0–4.2 (m, 2 H, H-6a, H-6′a), 4.2–4.4 (m, 4 H, H-5, H-5′, H-6b, H-6′b), 4.93–5.04 (dd, 1 H, H-2′ and t, 1 H, H-4′), 5.09 (t, $J_{4,5}$ 9.5, $J_{3,4}$ 9.5 Hz, 1 H, H-4), 5.29 (t, $J_{2',3'}$ 10, $J_{3',4'}$ 9.7 Hz, 1 H, H-3′), 5.39 (dd, $J_{2,3}$ 11.1, $J_{3,4}$ 9.1 Hz, 1 H, H-3), 5.76 (d, $J_{1',2'}$ 5.8 Hz, 1 H, H-1′), 6.38 (d, $J_{1,2}$ 3.4 Hz, 1 H, H-1).

*Benzyl 2,3,6-tri-*O*-acetyl-2-*S*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-2-thio-β-*D*-glucopyranoside* (**10**).—Silver carbonate (0.08g, 0.29 mmol) and silver perchlorate (catalytic amount) were stirred in the dark in dry CH_2Cl_2 in the presence of 4A molecular sieves. Anhydrous benzyl alcohol (0.05 mL, 0.48 mmol) was added, followed by solid glycosyl bromide **9** (0.2 g, 0.28 mmol), and the mixture was stirred for 2 h at room temperature, and then stored in the freezer overnight (18 h). The mixture was filtered and the filtrate was dried over Na_2SO_4, filtered, and then evaporated to a colorless oil. The product **10** crystallized from Et_2O in two crops (0.13 g, 62%) as white needles, mp 148–152°C. FABMS: *m/z* 743 (M + 1). ^{1}H NMR ($CDCl_3$): δ 1.9–2.2 (7 s, 21 H, OAc), 3.14 (dd, $J_{2,3}$ 9 Hz, 1 H, H-2), 3.61 (m, 1 H, H-5), 4.1–4.4 (m, 5 H, H-6a, H-6b, H-6′a, H6′b, H-5′), 4.48 (d, $J_{1,2}$ 8 Hz, 1 H, H-1), 4.67 and 4.84 (ABq, *J* 12 Hz, 2 H, PhCH_2), 4.96 (m, 3 H, H-4′, H-3, H-4), 5.10 (dd, $J_{2',3'}$ 10 Hz, 1 H, H-2′), 5.34 (t, $J_{3',4'}$ 9 Hz, 1 H, H-3′), 6.03 (d, $J_{1',2'}$ 6 Hz, 1

H, H-1'), 7.28–7.49 (m, 5 H, $C_6H_5CH_2$). Anal. Calcd for $C_{33}H_{42}O_{17}S \cdot 0.4H_2O$: C, 52.85; H, 5.75. Found: C, 52.81; H, 6.00.

Benzyl 2-S-(α-D-glucopyranosyl)-2-thio-β-D-glucopyranoside (**11**).—Compound **10** (0.70 g, 90 μmol) was stirred at room temperature for 24 h in the presence of NaOMe (from 4 mg, 174 μmol Na) in dry MeOH (12 mL). The solution was neutralized with Dowex 50 (H^+) resin, filtered through Celite, and evaporated to dryness. The residue was dissolved in dry MeOH (2–3 mL) and added dropwise to dry Et_2O to precipitate the product. The white solid was filtered, washed with Et_2O, and dried to give 41 mg (98% yield) of **11** as a white powder, mp 165°C (dec). FABMS: *m/z* 449 (M + 1). ^1H NMR (D_2O, referenced to internal acetone): δ 2.1 (acetone), 2.64 (m, $J_{1,2} \approx J_{2,3}$ 9 Hz, 1 H, H-2), 3.15–4.1 (m, 11 H, H-3, H-4, H-5, H-6, H-2', H-3', H-4', H-5', H-6'), 4.62 (d, $J_{1,2}$ 9 Hz, 1 H, H-1), 4.7 and 4.85 (ABq, 2 H, *J* 12 Hz, $C_6H_5CH_2$), 5.61 (d, $J_{1',2'}$ 5.5 Hz, 1 H, H-1'), 7.25-7.4 (m, 5 H, Ar). Anal. Calcd for $C_{19}H_{28}O_{10}S \cdot 0.66H_2O$: C, 49.57; H, 6.42. Found: C, 49.56; H, 6.31.

Methyl 2,3,4-tri-O-acetyl-6-O-[3,4,6-tri-O-acetyl-2-S-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-2-thio-β-D-glucopyranosyl]-α-D-glucopyranoside (**12**).—Methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside [26] (0.26 g, 0.8 mmol), silver carbonate (0.17 g, 0.6 mmol), silver perchlorate (0.16 g, 0.8 mmol), and crushed 4A molecular sieves were suspended in dry CH_2Cl_2 (10 mL). The mixture was protected from light and cooled in an ice bath. Solid glycosyl bromide **9** (0.53 g, 0.7 mmol) was added, and the mixture was stirred in the cold for 1 h and then at room temperature for 2.5 h. The gray mixture was filtered, diluted with CH_2Cl_2, and treated with dry pyridine (1.4 mL) and Ac_2O (2 mL). The resulting yellow mixture was stored in the cold for 2.5 days, then poured into ice–water, and extracted with $CHCl_3$ (3 × 25 mL). The combined extracts were washed sequentially with water, dil HCl, 5% $NaHCO_3$, and water, then dried over $MgSO_4$ to give a gum which was chromatographed on silica gel (2 × 20 cm, 270–400 mesh) eluting with 1:1 cyclohexane–EtOAc. The product-containing fractions were collected and evaporated to dryness to give **12** as a colorless glass (0.16 g, 24%), mp 70–80°C (dec). FABMS: *m/z* 955 (M + 1). ^1H NMR ($CDCl_3$): δ 1.95–2.2 (10 s, 30 H, OAc), 2.98 (dd, 1 H, H-2'), 3.44 (s, 3 H, OCH_3), 3.6 (dd, 1 H, H-6b), 3.68 (m, 1 H, H-5'), 3.84 (dd, 1 H, H-6a), 4.04 (td, 1 H, H-5), 4.4–4.08 (m, 5 H, H-5'', H-6''a,b, H-6'a,b), 4.54 (d, $J_{1',2'}$ 8.8 Hz, 1 H, H-1'), 4.8–5.06 (m, 6 H, H-1, H-4', H-4'', H-4, H-3', H-2), 5.14 (dd, 1 H, H-2''), 5.34 (t, $J_{2'',3''}$ 9, $J_{3'',4''}$ 9 Hz, 1 H, H-3''), 5.48 (t, $J_{2,3}$ 10, $J_{3,4}$ 10 Hz, 1 H, H-3), 6.02 (d, $J_{1'',2''}$ 5.8 Hz, 1 H, H-1''). Anal. Calcd for $C_{39}H_{54}O_{25}S \cdot 1.5C_6H_{12}$: C, 53.22; H. 6.71. Found: C, 53.50; H, 6.93.

3,4,6-Tri-O-acetyl-2-S-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-1-deoxy-2-thio-D-arabinohex-1-enopyranose (**13**).—Compound **9** (0.12 g, 0.17 mmol), diethylamine (0.05 mL, 0.48 mmol), and Bu_4NBr (0.06 g, 0.17 mmol) were dissolved in dry MeCN (9 mL), and the resulting solution was stirred at room temperature for 1 h. The mixture was evaporated to dryness and the solid residue was partitioned between water and toluene. The organic layer was separated, washed with water, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was triturated with Et_2O and filtered to give **13** as a white solid (0.071 g, 67%), mp 186.5–188.5°C.

FABMS: m/z 635 (M + 1). ^{1}H NMR ($CDCl_3$): δ 1.96–2.15 (7 s, 21 H, OAc), 4.06 (dd, 1 H, H-6b), 4.17 (dd, $J_{5',6'a} \approx J_{5',6'b} = 2$, $J_{6'a,6'b}$ 12 Hz, 1 H, H-6'b), 4.24 (dd, $J_{5,6a} \approx J_{5,6b} = 2$, $J_{6a,6b}$ 12 Hz, 1 H, H-6a), 4.3–4.45 (m, 3 H, H-5, H-5', H-6'a), 5.02 (t, $J_{3',4'}$ 10, $J_{4',5'}$ 9 Hz, 1 H, H-4'), 5.1 (dd, $J_{2',3'}$ 11 Hz, 1 H, H-2'), 5.22 (dd, 1 H, H-4), 5.27 (t, $J_{2',3'}$ 11, $J_{3',4'}$ 10 Hz, 1 H, H-3'), 5.47 (d, $J_{3,4}$ 5 Hz, 1 H, H-3) 5.48 (d, $J_{1',2'}$ 5 Hz, 1 H, H-1'), 6.74 (s, 1 H, H-1). Anal. Calcd for $C_{26}H_{34}O_{16}S$: C, 49.21; H, 5.40. Found: C, 49.42; H, 5.68.

3,4,6-Tri-O-acetyl-2-S-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-1-S-acetyl-2-thio-β-D-glucopyranose (**14**).—Solid compound **9** (0.06 g, 0.084 mmol) was added to a solution of trioctylamine (0.037 mL, 0.085 mmol) and thiolacetic acid (0.006 mL, 0.084 mmol) in dry, degassed CH_2Cl_2 (5 mL). After overnight stirring (18 h), the pale yellow mixture was diluted with $CHCl_3$ (10 mL) and washed with water (2 × 20 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and evaporated to a pale yellow gum. This gum was dissolved in $CHCl_3$, preabsorbed onto silica gel (230–400 mesh) and applied to a silica gel column (2 × 20 cm). The column was eluted with 3:2 cyclohexane–EtOAc and the product-containing fractions were combined and evaporated to give a white solid. Trituration with Et_2O, centrifuging, removal of the supernate, and drying gave **14** as a crystalline solid (20 mg, 33%), mp 208–211°C. FABMS: m/z 711 (M + 1). ^{1}H NMR ($CDCl_3$): 1.9–2.08 (7 s, 21 H, OAc), 2.21 (s, 3 H, SAc), 3.12 (t, 1 H, H-2), 3.80 (m, 1 H, H-5), 4.05 (dd, 1 H, H-6b), 4.13 (d, $J_{6'a,6'b}$ 12 Hz, 1 H, H-6'b), 4.0-4.15 (m, $J_{5',6'} = J_{5,6} = 2$ Hz, 3 H, H-6a, H-6'a, H-5'), 4.93–5.07 (m, 3 H, H-2', H-4, H-4'), 5.12 (dd, $J_{2,3}$ 11, $J_{3,4}$ 9.1 Hz, 1 H, H-3), 5.26 (d, $J_{1,2}$ 11.2 Hz, 1 H, H-1), 5.31 (dd, $J_{2',3'} = J_{3',4'} = 10$ Hz, 1 H, H-3'), 6.02 (d, $J_{1',2'}$ 5.9 Hz, 1 H, H-1'). Anal. Calcd for $C_{28}H_{38}O_{17}S_2 \cdot 0.5C_4H_{10}O$: C, 48.18; H, 5.80. Found: C, 48.19; H, 5.75.

1,2 : 5,6-Di-O-isopropylidene-3-S-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-3-thio-α-D-glucofuranose (**16**).—Sodium methoxide (0.47 g, 8.70 mmol) was added to a solution of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glycopyranose (**4**) [15] (3.56 mg, 8.77 mmol) in oxygen-free MeOH (100 mL), and the resulting solution was stirred overnight (18 h) at room temperature. The mixture was concentrated to dryness and the residue was dried in vacuo for 6 h. 1,2:5,6-Di-*O*-isopropylidene-3-*O*-trifluoromethylsulfonyl-α-D-allofuranose (**15**) [27] (3.43 g, 8.75 mmol) was dissolved in HMPA (38 mL) and added to the above residue, and the resultant mixture was stirred at room temperature under N_2 overnight (18 h). A 4:3 mixture of Ac_2O–pyridine (48 mL, v/v) was then added, and the mixture was stirred at room temperature for an additional 24 h. The mixture was then poured into ice–water (200 mL) and extracted with Et_2O (3 × 50 mL). The ether extracts were washed successively with H_2O, dil HCl (0.5 M), H_2O, and finally 5% $NaHCO_3$. Drying over Na_2SO_4, filtering, and removal of solvent gave 5.5 g of an oil. Crystallization from cold Et_2O gave 3.2 g (60%) of a white solid. Chromatographic purification [on silica gel, 270–400 mesh, with 99:1 $CHCl_3$–MeOH] of the ether filtrate yielded another 577 mg of **16** for a total yield of 71%. FABMS: m/z 607 (M + 1). ^{1}H NMR ($CDCl_3$): δ 1.2–1.6 (3 s, 12 H, Me_2C), 1.9–2.2 (3 s, 12 H, 2'-, 3'-, 4'-, and 6'-OAc), 3.53 (dd, $J_{3,4}$ 3 Hz, 1 H, H-3), 3.97 (dd, 1 H, H-5), 4.14 (m, 3 H, H-4, H-6a, H-6'a), 4.38 (m, 2 H, H-6b, H-6'b), 4.46 (m, $J_{5',6'a}$ 6, $J_{4',5'}$ 10 Hz, 1 H,

H-5'), 4.69 (br d, $J_{1,2}$ 3 Hz, 1 H, H-2), 5.03 (t, $J_{4',5'}$ 10, $J_{3',4'}$ 9 Hz, 1 H, H-4'), 5.10 (dd, $J_{2',3'} = J_{1',2'} = 10$, $J_{1',2'}$ 5 Hz, 1 H, H-2'), 5.36 (t, $J_{3',4'}$ 9, $J_{2',3'}$ 10 Hz, 1 H, H-3'), 5.79 (d, $J_{1,2}$ 3 Hz, 1 H, H-1), 5.92 (d, $J_{1',2'}$ 5 Hz, 1 H, H-1'). Anal. Calcd for $C_{26}H_{38}O_{14}S \cdot 0.6H_2O$: C, 50.58; H, 6.39. Found: C, 50.64; H, 6.32.

*Methyl 2,3,4-tri-*O*-acetyl-6-*S*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)-6-thio-α-*D*-glucopyranoside* (**18**).—Sodium (12.0 mg, 0.052 mmol) was dissolved in dry, degassed MeOH (5 mL) under N_2, compound **4** [15] (200 mg, 0.493 mmol) was added as a solid all at once, and the mixture was placed back under N_2 and stirred for 18 h. After the MeOH was evaporated and the residue was dried in vacuo, the resulting solid and powdered methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-α-D-glucopyranoside (**17**) [19] (212 mg, 0.493 mmol) were placed in HMPA (7 mL) and stirred under N_2 for 48 h. 4:3 Ac_2O–pyridine (7 mL) was added and the mixture was stirred overnight (24 h). The mixture was poured into ice–water and extracted with Et_2O. The product crystallized from Et_2O (227 mg, 69%); however, repeated crystallizations failed to remove a slightly slower moving impurity (TLC, 99:1 $CHCl_3$–MeOH). The solid was preabsorbed onto silica gel and chromatographed (2 × 16 cm) on silica gel eluting first with 2:1 cyclohexane–EtOAc and then 1:1 cyclohexane–EtOAc. The product-containing fractions were combined and evaporated, and the resulting solid was triturated with ether, filtered, and dried to give **18** as a white solid, mp 151–152°C. FABMS: m/z 667 (M + 1). ^{1}H NMR ($CDCl_3$): δ 1.97–2.15 (7 s, 21 H, OAc), 2.59 (dd, $J_{6a,6b}$ 13, $J_{6a,5'}$ 7 Hz, 1 H, H-6a), 2.78 (dd, $J_{6b,5'}$ 3 Hz, 1 H, H-6b), 3.42 (s, 3 H, OCH_3), 3.94 (m, $J_{4,5}$ 10 Hz, 1 H, H-5), 4.06 (dd, $J_{6'a,6'b}$ 12 Hz, 1 H, H-6'b), 4.27 (dd, $J_{6'a,5'}$ 3 Hz, 1 H, H-6'a), 4.41 (m, $J_{4',5'}$ 10 Hz, 1 H, H-5'), 4.85 (dd, $J_{2,3}$ 9 Hz, 1 H, H-2), 4.90 (d, $J_{1,2}$ 4 Hz, 1 H, H-1), 4.98 (dd, $J_{4,5}$ 10, $J_{3,4}$ 9 Hz, 1 H, H-4), 5.01 (dd, $J_{2',3'}$ 10, $J_{1',2'}$ 6 Hz, 1 H, H-2'), 5.04 (t, $J_{4',5'}$ 10, $J_{3',4'}$ 8 Hz, 1 H, H-4'), 5.38 (t, $J_{2',3'}$ 10 Hz, 1 H, H-3'), 5.45 (t, $J_{3,4}$ 9 Hz, 1 H, H-3), 5.75 (d, $J_{1',2'}$ 6 Hz, 1 H, H-1'). Anal. Calcd for $C_{27}H_{38}O_{17}S$: C, 48.64; H, 5.74. Found: C, 48.56; H, 5.80.

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