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Synthesis of heteroanalogues of disaccharides as potential inhibitors of the processing mannosidase Class I enzymes

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Abstract

Three disaccharide analogues of α-D-Man-(1→2)-α-D-Man-OMe in which the ring and/or glycosidic oxygen atoms have been replaced by sulfur have been synthesized as potential inhibitors of Class I mannosidase enzymes. Glycosylation of appropriately protected methyl α-D-mannopyranosides, having free alcohol or thiol functional groups at the 2-position, with 2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-mannopyranosyl trichloroacetimidate was selective for the synthesis of α-linked disaccharides in the case of the 2-alcohols, but yielded an α/β mixture in the case of the 2-thiol glycosyl acceptors. A disaccharide containing a single sulfur in the linkage was synthesized by nucleophilic substitution of the 2-triflate of a methyl α-D-glucopyranoside derivative by the thiolate anion of 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranose. Protecting groups were removed by standard methods to give the free heterosubstituted disaccharides.

Keywords: 5-Thio-α-D-mannopyranosyl trichloroacetimidate; Thio sugars; Disaccharides, heteroatom analogues; Mannosidase inhibitors

1. Introduction

The major biosynthetic pathway of complex *N*-linked oligosaccharides in higher eukaryotes occurs by processing of oligosaccharides via the trimming pathway [1,2]. The trimming of the high mannose structure, $Glc_3Man_9(GlcNAc)_2$ by α-glucosidases I and II removes the three glucose residues, and a collection of processing mannosidases then removes the four α-1,2-mannose residues to yield $Man_5(GlcNAc)_2$. The GlcNAc transferase I, which initiates branching of complex oligosaccharides, then adds GlcNAc, and mannosidase II then removes one α-1,3 and one α-1,6 linked mannose residue to yield $GlcNAcMan_3(GlcNAc)_2$. This oligosaccharide product is extended by branching GlcNAc transferases to give bi-, tri-, and tetra-antennary structures [2]. Distal sugars which include Gal, GalNAc, GlcNAc, Fuc, and sialic acid residues, are finally added by the corresponding

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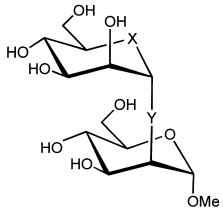
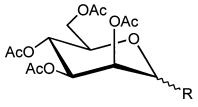
glycosyltransferases to yield the wide variety of structures found in mature glycoproteins [1,3]. Interference with the trimming process by glucosidase or mannosidase inhibitors blocks maturation of oligosaccharides to complex structures [4]. The display of immature or "aberrant" structures can inhibit tumor growth and metastasis, and the infectivity of viruses such as HIV [5]. Inhibition of oligosaccharide processing by glycosidase inhibitors might constitute, therefore, an effective therapeutic strategy.

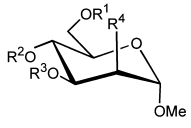
Thus far, we have reported the synthesis of heteroanalogues of disaccharides, α-Glc-(1→2)-Glc-OR, in which the ring and/or glycosidic oxygen atoms have been O/S [6], S/O [7], S/N [8] and S/S [9], as potential inhibitors of Glucosidase I. We now describe the synthesis of heteroanalogues of disaccharides, α-D-Man-(1→2)-(α-D-Man-OMe (**1**–**3**), in which the ring and/or glycosidic oxygen atoms have been replaced by sulfur for evaluation as inhibitors of the Class I mannosidases.



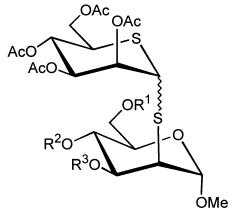
	X	Y
1	S	S
2	S	O
3	O	S



	R
4	α/β: OH
5	α: $OCNHCCl_3$
6	α: SH



	R^1	R^2	R^3	R^4
7	C_6H_5CH		Ac	SAc
8	C_6H_5CH		Ac	SH
9	C_6H_5CH		Bz	OH
10	Bn	Bn	Bn	OH



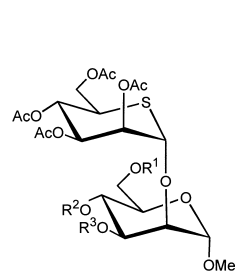
	R^1	R^2	R^3
11	α' isomer	C_6H_5CH	Ac
12	β' isomer	C_6H_5CH	Ac
13	α' isomer	Ac	Ac	Ac

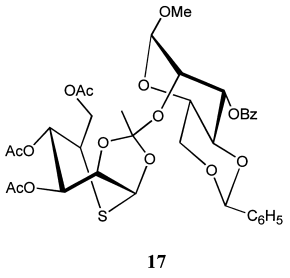
2. Results and discussion

5-Thio-α-D-mannopyranose pentaacetate [10] was converted to the hemiacetal derivative **4** by selective hydrazinolysis of the anomeric acetate. Compound **4** was reacted with trichloroacetonitrile and potassium carbonate in CH_2Cl_2 to give the trichloroacetimidate glycosyl donor **5** that was of sufficient purity for direct use in glycosylation reactions. A suitably blocked 2-thio-mannopyranoside acceptor was prepared by selective *S*-acetate hydrazinolysis of methyl 3-*O*-acetyl-2-*S*-acetyl-4,6-*O*-benzylidene-2-thio-α-D-mannopyranoside (**7**) [11] to give the free thiol (**8**). Although this compound appeared to be stable toward air oxidation or acetate migration reactions at room temperature, it was generally prepared immediately prior to use. Glycosylation of **8** with **5** was performed at low temperature in CH_2Cl_2 using triethylsilyl triflate catalysis, conditions that we have previously used [6,8,12] to prepare heteroatom-linked disaccharides having 5-thio-α-D-glucopyranose as the non-reducing monosaccharide unit. In the present case, two products were formed that were separated by column chromatography. The less polar product (**11**) and the more polar product (**12**) were isolated as crystalline solids, in a ratio of 1:1.2, respectively, with an overall yield of 83%. The assignment of α-5-thiomannopyranosyl stereochemistry to compound **11** and β-5-thiomannopyranosyl stereochemistry to compound **12** rests on evidence from both ^{13}C and ^{1}H NMR chemical shifts and coupling constants. The equatorial H-1′ of the α-isomer **11** (δ 4.24) is deshielded with respect to the axial H-1′ of the β-isomer **12** (δ 4.06), in accord with the general trends observed in glucopyranosides [13]. The observation of a larger C1′–H1′ coupling constant (153 Hz) for isomer **11** compared to that for **12** (146 Hz) is also diagnostic [14]. The typical values of ~170 and ~160 Hz observed for C1′–H1′ coupling in α- and β-5-*O*-glycopyranosides, respectively, are somewhat smaller for 5-*S*-glycopyranosides [15] and, in certain cases, the differences are too small to provide reliable evidence for assignment of anomeric configuration. In the present case, however, the difference of 7 Hz may be considered significant. Further corroboration is provided by the ^{13}C chemical shifts observed for C-3′ and C-5′, which are 70.09 and 40.59 ppm, respectively, for **11** and 73.30 and 44.91 ppm, respectively, for **12**. These results are in accord with the expected γ-gauche upfield shift [16] for C-3 and C-5 of α-thioglycosides relative to β-thioglycosides for which the γ-anti effect at C-3 and C-5 is expected to be negligible [15]. Compound **11** was converted to the heptaacetate **13** by aqueous acetic acid hydrolysis of the 4,6-*O*-benzylidene acetal, followed by acetylation with acetic anhydride in pyridine. Zemplén deacetylation

yielded methyl 2-*S*-(5-thio-α-D-mannopyranosyl)-2-thio-α-D-mannopyranoside (**1**). The ^{1}H and ^{13}C NMR spectra of **1** showed the upfield chemical shifts (H-1′, δ 4.30 and C-1′, δ 57.70) typical of S,S acetals compared to O,O acetals.

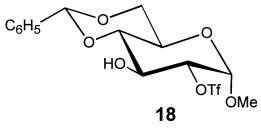
With dithiodisaccharide **1** in hand, we were encouraged to apply the same approach to the synthesis of the analogous 5-thio-α-D-mannopyranosyl disaccharide with oxygen in the linkage. Reaction of **5** with methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**9**) [17] using conditions similar to those previously successful for the preparation of **11**, did not result in the expected disaccharide **14** as a major product. Instead, **14** was produced as a minor product and the orthoester derivative **17** was produced as the major product (ratio of **17:14** = 5:2) We have previously observed that, in related glycosylations using the trichloroacetimidate of 5-thio-α-D-glucose, orthoesters may predominate if the reactions are quenched at low temperature [7,12]. Suitable conditions for the separation of **14** and **17** by column chromatography could not be found, but a sample of pure **17** was obtained by fractional crystallization. ^{1}H NMR analysis indicated that the configuration of the orthoester moiety of **17** was *exo* (orthoester CH_3, δ 1.65), with the non-reducing monosaccharide portion adopting a boat conformation ($^{3}J_{H1',H2'} = 8.1$ Hz). The remaining mixture of **14** and **17** (ratio ~1:1) was subjected to treatment with catalytic amounts of triethysilyl triflate in dichloromethane at room temperature in order to ascertain whether rearrangement of **17** to **14** was feasible. Although **17** was consumed, the low yield of **14** obtained by this procedure prompted us to consider methyl 3,4,6-tri-*O*-benzyl-*a*-D-mannopyranoside (**10**) [18] as an alternative glycosyl acceptor. Reaction of **5** with **10** using triethylsilyl triflate catalysis as before, except that the reaction mixture was allowed to warm to room temperature prior to quenching with triethylamine, yielded the disaccharide **15** in moderate yield. Because of the resistance of sulfur-containing molecules to palladium-catalyzed hydrogenolysis due to catalyst poisoning, deprotection of **15** was effected by a dissolving metal reduction using sodium in a mixed solvent system consisting of tetrahydrofuran and liquid ammonia. Acetylation of the crude product yielded the crystalline heptaacetate **16**. Zemplén deacetylation then gave methyl 2-*O*-(5-thio-α-D-mannopyranosyl)-α-D-mannopyranoside (**2**).

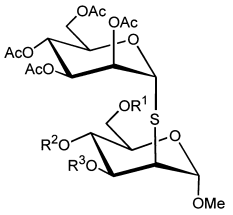




17

	R^1	R^2	R^3
14	C_6H_5CH		Bz
15	Bn	Bn	Bn
16	Ac	Ac	Ac





18

	R^1	R^2	R^3
19	C_6H_5CH		H
20	Ac	Ac	Ac

Our approach to the mannose disaccharide **3** containing sulfur in the glycosidic linkage was based on the use of 1-thiolates in nucleophilic displacement reactions. Such an approach has already been used with some success in the synthesis of *S*-linked glucose disaccharides [19]. For the present purposes, the direct displacement method, leading to a mannose disaccharide, required a nucleophilic displacement reaction at the unreactive 2-position of a glucose derivative. Fortunately, compound **18** [20], which has a triflate at the 2-position of a methyl α-D-glucopyranoside derivative, is readily available and was found to react with the thiolate anion of 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (**6**) [21] in *N*,*N*-dimethylformamide, to give the disaccharide **19**, albeit in modest yield. Deprotection by sequential acid-catalyzed hydrolysis of the benzylidene acetal and base-catalyzed methanolysis of the esters yielded methyl 2-*S*- (α-D-mannopyranosyl)-2-thio-α-D-mannopyranoside (**3**), which was further characterized as the heptaacetate **20**.

3. Experimental

General methods.—TLC was performed using aluminum plates, precoated with Merck silica gel 60-F254, using appropriate mixtures of hexanes–EtOAc or toluene–EtOAc for development. Visualization was achieved by exposure of the dried

plates to UV light or by spraying with a solution of 1% ceric sulfate and 1.5% molybdic acid in 10% aqueous H_2SO_4 and heating. Column chromatography was performed using silica gel 60 (Merck, 230–400 mesh).

Optical rotations were measured at 21 °C with a Rudolph Research Autopol II polarimeter. Melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ unless otherwise stated, using a Bruker AMX-400 spectrometer operating at 400.13 and 100.6 MHz for proton and carbon, respectively. All assignments were confirmed with the aid of two-dimensional $^1H,^1H$ (COSYDFTP) or $^1H,^{13}C$ (INVBTP) correlation experiments using standard Bruker pulse programs with inverse-detection. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra.

2,3,4,6-Tetra-O-*acetyl-5-thio*-α-D-*mannopyranose trichloroacetimidate* (**5**).—A solution of 1,2,3,4,6-penta-*O*-acetyl-5-thio-α-D-mannopyranose [10] (410 mg, 1.01 mmol) in *N*,*N*-dimethylformamide (6 mL) was stirred with hydrazine acetate (205 mg, 2.23 mmol) at room temperature for 1.5 h. The mixture was diluted with EtOAc (100 mL) and washed with half-satd aq NaCl solution (2×10 mL), dried over anhydrous Na_2SO_4 and concentrated to give crude hemiacetal (**4**) as a colorless oil. Compound **4** was dissolved in CH_2Cl_2 (20 mL) and stirred with anhydrous K_2CO_3 (1.2 g) and trichloroacetonitrile (1.1 mL, 11 mmol) for 20 h at room temperature. The mixture was diluted with an equal volume of diethyl ether and filtered through Celite. Solvent removal gave the trichloroacetimidate (**5**) quantitatively and of sufficient purity for use in glycosylation reactions. Chromatography on silica gel (2:1 Et_2O–hexanes) gave pure **5** as a colorless foam (478 mg, 93%); $[\alpha]_D$ +140° (*c* 1.00, $CHCl_3$); ^1H NMR: δ 8.81 (bs, 1H, NH), 6.05 (d, 1H, $J_{1,2}$ 4.1 Hz, H-1), 5.58 (dd, 1H, $J_{2,3}$ 3.1 Hz, H-2), 5.54 (t, 1H, $J_{3,4}=J_{4,5}$ 10.5 Hz, H-4), 5.36 (dd, 1H, H-3), 4.31 (dd, 1H, $J_{5,6a}$ 5.7, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.10 (dd, 1H, $J_{5,6b}$ 3.5 Hz, H-6b), 3.62 (ddd, 1H, H-5), 2.21, 2.07, 2.05, 2.00 (4s, each 3H, 4 $COCH_3$); ^{13}C NMR: δ 170.46, 169.71, 169.67, 169.58 (4 $COCH_3$), 159.58 (*C*NH), 90.51 (CCl_3), 77.61 (C-1), 70.14 (C-2), 69.37 (C-4), 68.54 (C-3), 61.59 (C-6), 40.68 (C-5), 20.91, 20.63, 20.58, 20.55 (4 $COCH_3$). Anal. Calcd for $C_{16}H_{20}Cl_3NO_9S$: C, 37.77, H, 3.96; N, 2.75. Found: C, 37.67; H, 3.90; N, 2.74.

Methyl 3-O-*Acetyl-4,6*-O-*benzylidene-2-thio*-α-D-*mannopyranoside* (**8**).—The thiolacetate **7** [11] (382 mg, 1.00 mmol) was dissolved in dry pyridine (2 mL) and placed under a nitrogen atmosphere. Hydrazine hydrate (0.052 mL, 1.07 mmol) was added via syringe and the mixture stirred at room temperature for 2 h. Volatile material was removed in vacuo to give a cloudy syrup, which was applied to a silica gel column with a minimum amount of EtOAc and eluted with 3:1 hexanes–EtOAc to yield the thiol **8** as a colorless glass (281 mg, 82%); $[\alpha]_D$ +7.0° (*c* 2.3, $CHCl_3$); ^1H δ 7.5–7.3 (m, 5H, Ph), 5.57 (s, 1H, PhC*H*), 5.38 (dd, 1H, $J_{2,3}$ 4.9, $J_{3,4}$ 10.1 Hz, H-3), 4.82 (d, 1H, $J_{1,2}$ 0.9 Hz, H-1), 4.27 (dd, 1H, $J_{5,6a}$ 4.6, $J_{6a,6b}$ 10.1 Hz, H-6a), 4.10 (dd, $J_{4,5}$ 9.8 Hz, H-4), 3.97 (ddd, 1H, $J_{5,6b}$ 10.1 Hz, H-5), 3.84 (dd, 1H, H-6b), 3.76 (ddd, 1H, $J_{2,SH}$ 8.7 Hz, H-2), 3.39 (s, 3H, OCH_3), 2.11 (s, 3H, $COCH_3$), 1.74 (d, 1H, S*H*); ^{13}C NMR: δ 169.92 ($COCH_3$), 137.27, 129.06, 128.23, 126.26 (Ph), 103.40 (C-1), 102.06 (Ph*C*H), 76.13 (C-4), 69.12 (C-3), 68.87 (C-6), 64.26 (C-5), 55.16 (OCH_3), 42.98 (C-2), 20.94 ($COCH_3$).

Methyl 3-O-*acetyl-4,6*-O-*benzylidene-2*-S-*(2,3,4,6-tetra*-O-*acetyl-5-thio*-α-D-*mannopyranosyl)-2-thio*-α-D-*mannopyranoside* (**11**) *and Methyl 3*-O-*acetyl-4,6*-O-*benzylidene-2*-S-*(2,3,4,6-tetra*-O-*acetyl-5-thio*-β-D-*mannopyranosyl)-2-thio*-α-D-*mannopyranoside* (**12**). The trichloroacetimidate **5** (508 mg, 1.00 mmol) and thiol **8** (279 mg, 0.820 mmol) were dissolved in CH_2Cl_2 (4 mL). The mixture was cooled to −30 °C and stirred under a nitrogen atmosphere while triethylsilyl triflate (0.020 mL, 0.088 mmol) was added via syringe. An immediate reaction to produce a precipitate ensued. After 0.5 h, triethylamine (0.020 mL) was added to quench the reaction and the mixture was warmed to room temperature. Dilution with CH_2Cl_2 (50 mL), filtration, washing with water (2×10 mL), drying ($MgSO_4$), and concentration gave a colorless syrup. Analysis by TLC (2:1 hexanes–EtOAc) indicated that two products had formed. Separation by chromatography on silica gel (3:2 hexanes–EtOAc) gave the faster-eluting α-isomer (**11**) (209 mg, 37%) as a colorless syrup which crystallized on standing: mp 111–113 °C; $[\alpha]_D$ +96.7° (*c* 1.08, $CHCl_3$); ^1H NMR: δ 7.5–7.3 (m, 5H, Ph), 5.57 (s, 1H, PhC*H*), 5.54 (dd, 1H, $J_{2,3}$ 4.6, $J_{3,4}$ 9.8 Hz, H-3), 5.45 (t, 1H, $J_{3',4'}=J_{4',5'}=9.9$ Hz, H-4′), 5.38 (dd, 1H, $J_{1'2'}$ 4.1, $J_{2',3'}$ 3.0 Hz, H-2′), 5.25 (dd, 1H, H-3′), 4.88 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1), 4.33 (dd, 1H, $J_{5',6a'}$ 6.0, $J_{6a',6b'}$ 12.0 Hz, H-6a′), 4.26 (dd,

1H, $J_{5,6}$ 4.5, $J_{6a,6b}$ 9.9 Hz, H-6a), 4.24 (d, 1H, H-1′), 4.13 (dd, 1H, $J_{5',6b'}$ 4.1 Hz, H-6b′), 3.99 (t, 1H, $J_{4,5}$ 9.6 Hz, H-4), 3.93 (ddd, 1H, $J_{5,6b}$ 9.9 Hz, H-5), 3.81 (t, 1H, H-6b), 3.75 (dd, 1H, H-2), 3.67 (ddd, 1H, H-5′), 3.41 (s, 3H, OCH_3), 2.18, 2.09(2), 2.06, 2.02 (5 COCH_3); ^{13}C NMR: δ 170.27, 170.16, 169.79, 169.51, 169.30 (5 *C*OCH$_3$), 137.2, 128.97, 128.15, 126.17 (Ph), 102.84 (C-1), 101.92 (Ph*C*H), 77.70 (C-4), 71.73 (C-2′), 70.09 (C-3′), 69.54, 69.41 (C-3, C-4′), 68.74 (C-6), 64.00 (C-5), 61.86 (C-6′), 55.12 (O*C*H$_3$), 51.66 (C-1′, $J_{C1',H1'}$ 153 Hz), 51.30 (C-2), 40.59 (C-5′), 20.80, 20.74, 20.49 (CO*C*H$_3$). Anal. Calcd for $C_{30}H_{38}O_{14}S_2$: C, 52.47; H, 5.58. Found: C, 52.40; H, 5.55.

The slower-eluting β-isomer **12** (258 mg, 46%) was isolated as a colorless syrup which slowly crystallized on standing: mp 109–112 °C; $[\alpha]_D$ −34.8° (*c* 1.15, $CHCl_3$); ^{1}H NMR: δ 7.5–7.3 (m, 5H, Ph), 5.70 (dd, 1H, $J_{1'2'}$ 1.9, $J_{2',3'}$ 2.9 Hz, H-2′), 5.52 (s, 1H, PhC*H*), 5.43 (dd, 1H, $J_{2,3}$ 4.7, $J_{3,4}$ 10.3 Hz, H-3), 5.39 (t, 1H, $J_{3',4'} = J_{4',5'} = 10.3$ Hz, H-4′), 4.89 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1), 4.83 (dd, 1H, H-3′), 4.22 (dd, 1H, $J_{5,6a}$ 4.6, $J_{6a,6b}$ 10.2 Hz, H-6a), 4.18 (dd, 1H, $J_{5',6a'}$ 4.4, $J_{6a',6b'}$ 11.8 Hz, H-6a′), 4.14 (dd, 1H, $J_{5',6b'}$ 6.3 Hz, H-6b′), 4.06 (d, 1H, H-1′), 3.89 (ddd, 1H, $J_{4,5}$ 9.7, $J_{5,6b}$ 10.3 Hz, H-5), 3.76 (t, 1H, H-6b), 3.73 (dd, 1H, H-4), 3.67 (dd, 1H, H-2), 3.41 (s, 3H, OCH_3), 3.27 (ddd, 1H, H-5′), 2.22, 2.17, 2.08, 2.03 1.98 (5 COCH_3); ^{13}C NMR: δ 170.31(2C), 170.11, 169.78, 169.62, (5 *C*OCH$_3$), 137.19, 129.06, 128.21, 126.21 (Ph), 102.72 (C-1), 101.96 (Ph*C*H), 77.11 (C-4), 73.30 (C-3′), 71.85 (C-2′), 68.91 (C-3), 68.73 (C-6), 68.55 (C-4′), 64.04 (C-5), 62.04 (C-6′), 55.14 (O*C*H$_3$), 52.48 (C-2), 50.10 (C-1′, $J_{C1',H1'}$ 146 Hz), 44.91 (C-5′), 21.29, 20.95, 20.71, 20.55, 20.44 (5 CO*C*H$_3$). Anal. Calcd for $C_{30}H_{38}O_{14}S_2$: C, 52.47; H, 5.58. Found: C, 52.21; H, 5.53.

Methyl 3,4,6-tri-O-*acetyl-2*-S-*(2,3,4,6-tetra*-O-*acetyl-5-thio*-α-D-*mannopyranosyl)-2-thio-a*-D-*mannopyranoside* (**13**).—A solution of the disaccharide **11** (167 mg, 0.243 mmol) in 80% aq HOAc (10 mL) was stirred at room temperature for 2 h and at 45 °C for 5 h. The solvent and benzaldehyde were removed in vacuo and the residue was acetylated by treatment with Ac_2O (1.5 mL) and 4-dimethylaminopyridine (0.020 g) in pyridine (4 mL) at room temperature for 4 h. Volatile material was removed in vacuo and the residue was purified by column chromatography (2:3 hexanes–EtOAc,) to yield **13** (152 mg, 92%) as an amorphous solid: $[\alpha]_D$ +131.7° (*c* 1.04, $CHCl_3$); ^{1}H NMR: δ 5.51 (dd, 1H, $J_{2,3}$ 4.5, $J_{3,4}$ 9.5 Hz, H-3), 5.43 (t, 1H, $J_{3',4'} = J_{4',5'} =$ 9.5 Hz, H-4′), 5.37 (dd, 1H, $J_{1',2}$ 4.3, $J_{2',3'}$ 3.1 Hz, H-2′), 5.28 (dd, 1H, H-3′), 5.25 (dd, 1H, $J_{4,5}$ 9.7 Hz, H-4), 4.89 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 4.33 (dd, 1H, $J_{5',6a'}$ 6.0, $J_{6a',6b'}$ 11.9 Hz, H-6a′), 4.25 (d, 1H, H-1′), 4.20 (dd, 1H, $J_{5,6a}$ 4.7, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.13 (dd, 1H, $J_{5,6}$ 2.5 Hz, H-6b), 4.12 (dd, 1H, $J_{5',6b'}$ 4.5 Hz, H-6b′), 3.92 (ddd, 1H, H-5), 3.63 (ddd, 1H, H-5′), 3.62 (dd, 1H, H-2), 3.41 (s, 3H, OCH_3), 2.17, 2.12, 2.08, 2.07, 2.05, 2.04, 2.01 (7s, each 3H, COCH_3); ^{13}C NMR: δ 170.49, 170.31, 169.91, 169.71, 169.47, 169.31(2C), (7 *C*OCH$_3$), 101.57 (C-1), 71.53 (C-2′), 70.41 (C-3), 69.84 (C-3′), 69.42 (C-4′), 68.51 (C-5), 67.17 (C-4), 62.26 (C-6), 61.84 (C-6′), 55.35 (O*C*H$_3$), 50.83 (C-1′), 50.19 (C-2), 40.51 (C-5′), 20.96, 20.79(2C), 20.65(3C), 20.58, (7 CO*C*H$_3$) Anal. Calcd for $C_{27}H_{38}O_{16}S_2$: C, 47.50; H, 5.61. Found: C, 47.71 ; H, 5.68.

Methyl 2-S-*(5-thio*-α-D-*mannopyranosyl)-2-thio*-α-D-*mannopyranoside* (**1**).—To a solution of disaccharide **13** (122 mg, 0.179 mmol) in anhyd MeOH was added a 1 M solution of NaOMe in MeOH (1.0 mL), and the mixture was kept at room temperature for 1.5 h. The solution was neutralized with Rexyn 101 H^+ ion-exchange resin, filtered and concentrated to a syrup. Anhydrous toluene (20 mL) was added and removed in vacuo to give **1** (65 mg, 94%) as a colorless glass: $[\alpha]_D$ +218° (*c* 0.87, H_2O); ^{1}H NMR (D_2O): δ 4.99 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1), 4.29 (d, 1H, $J_{1',2'}$ 3.6 Hz, H-1′), 4.25 (dd, 1H, $J_{2',3'}$ 2.9 Hz, H-2′), 4.13 (dd, 1H, $J_{2,3}$ 4.7, $J_{3,4}$ 9.7 Hz, H-3), 3.94 (dd, 1H, $J_{5',6a'}$ 3.3, $J_{6a',6b'}$ 12.0 Hz, H-6a′), 3.82 (dd, 1H, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 12.0 Hz, H-6a), 3.78 (dd, 1H, $J_{5',6b'}$ 7.1 Hz, H-6b′), 3.76 (dd, 1H, $J_{2',3'}$ 2.8, $J_{4',5'}$ 9.9 Hz, H-4′), 3.69 (dd, 1H, $J_{5,6}$ 5.8 Hz, H-6b), 3.67 (dd, 1H, $J_{3',4'}$ 9.5 Hz, H-3′), 3.58 (ddd, 1H, H-5), 3.49 (t, 1H, $J_{4,5}$ 9.7 Hz, H-4), 3.46 (dd, 1H, H-2), 3.36 (s, 3H, OCH_3), 3.25 (ddd, 1H, H-5′); ^{13}C NMR (D_2O): δ 104.53 (C-1), 75.41 (C-5), 74.97 (C-2′), 74.73 (C-3′), 72.59 (C-4′), 72.21 (C-3), 70.68 (C-4), 63.39 (C-6), 62.94 (C-6′), 57.70 (C-1′), 57.52 (O*C*H$_3$), 55.94 (C-2), 47.72 (C-5′) Anal. Calcd for $C_{13}H_{24}O_9S_2$: C, 40.20; H, 6.23. Found: C, 40.31; H, 6.21.

Methyl 3-O-*benzoyl-4,6*-O-*benzylidene-2*-O-*(2,3,4,6-tetra*-O-*acetyl-5-thio*-α-D-*mannopyranosyl)*-α-D-*mannopyranoside* (**14**) *and 3,4,6-Tri*-O-*Acetyl-1,2-(methyl 3*-O-*benzoyl-4,6*-O-*benzylidene*-α-D-*mannopyranos-2-yl)-5-thio*-β-D-*mannopyranose Orthoacetate* (**17**).—Crude trichloroacetimidate **5** (prepared from 5-thio-α-D-mannopyranose pentaacetate (408 mg, 1.00 mmol)) and methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside

(**9**) [17] (446 mg, 1.15 mmol) were dissolved in CH_2Cl_2 and stirred with 4 Å molecular sieves (~1 g) for 20 min at room temperature under N_2. The solution was cooled in a −40 °C bath and triethylsilyl triflate (0.020 mL, 0.088 mmol) was added via syringe. After 0.5 h, during which the temperature had risen to −35 °C, the reaction was quenched by the addition of triethylamine (0.02 mL) and the mixture was allowed to warm to room temperature. Dilution with CH_2Cl_2 (50 mL), filtration, washing with satd aqueous $NaHCO_3$ and water (10 mL), drying ($MgSO_4$), and concentration gave a colorless foam. Analysis by TLC (hexanes–EtOAc or toluene–EtOAc mixtures) showed only a single major product spot. This product was isolated by column chromatography (1:1 hexanes–EtOAc) as a colorless foam (516 mg). The ^{1}H NMR spectrum of this product, however, clearly showed a mixture of two compounds in an approximate ratio of 5:2. One of the major components of the mixture, orthoester derivative (**17**) (258 mg, 35%), was isolated by selective crystallization from ether–hexanes as colorless needles: mp 154–155 °C; $[\alpha]_D$ −63.7° (*c* 1.6, $CHCl_3$); ^{1}H NMR: δ 8.1–7.2 (m, 10H, Ph), 5.60 (s, 1H, PhC*H*), 5.52 (dd, 1H, $J_{2,3}$ 3.5, $J_{3,4}$ 10.5 Hz, H-3), 5.43 (d, 1H, $J_{1',2'}$ 8.1 Hz, H-1′), 5.30 (dd, 1H, $J_{3',4'}$ 10.4, $J_{4',5'}$ 6.0 Hz, H-4′), 5.17 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-3′), 4.88 (dd, 1H, H-2′), 4.65 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1), 4.36 (dd, 1H, $J_{5',6a'}$ 8.0, $J_{6a',6b'}$ 11.3 Hz, H-6a′), 4.30 (dd, 1H, H-2), 4.27 (dd, 1H, $J_{5,6a}$ 4.2, $J_{6a,6b}$ 9.7 Hz, H-6a), 4.21 (dd, 1H, $J_{4,5}$ 9.2 Hz, H-4), 4.08 (dd, 1H, $J_{5',6b'}$ 6.8 Hz, H-6b′), 3.93 (ddd, 1H, $J_{5,6b}$ 9.9 Hz, H-5), 3.85 (t, 1H, H-6b), 3.23 (ddd, 1H, H-5′), 2.06, 2.02 2.00 (3s, each 3H, COC*H*$_3$), 1.65 (s, 3H, orthoester C*H*$_3$); ^{13}C NMR: δ 170.33, 169.78, 169.08, (3 *C*OCH$_3$), 165.93 (*C*OPh), 137.35, 133.20, 129.92, 129.70, 128.88, 128.47, 128.14, 126.13 (Ph), 122.05 (orthoester *C*CH$_3$), 101.75 (Ph*C*H), 100.75 (C-1), 78.74 (C-1′), 76.03 (C-4), 74.95 (C-2′), 71.49 (C-2), 70.81 (C-3′), 70.07 (C-3), 68.87 (C-6), 66.15, 66.11 (C-4′, C-6′), 64.04 (C-5), (55.10 (O*C*H$_3$), 43.73 (C-5′), 21.11, 20.71, 20.60(2C) (3 CO*C*H$_3$, 1 orthoester C*C*H$_3$) Anal. Calcd for $C_{35}H_{40}O_{15}S$: C, 57.37; H, 5.50. Found: C, 57.40; H, 5.55.

The crystallization mother liquors were concentrated in vacuo to give a syrup (250 mg) consisting of **14** and **17** in an approximately 1:1 ratio. This syrup was dissolved in CH_2Cl_2 (10 mL) and stirred with 4 Å molecular sieves (~1 g) at room temperature. Triethylsilyl triflate (0.020 mL, 0.088 mmol) was added and the reaction mixture was monitored by TLC. After 0.5 h, the formation of several minor decomposition products was noted, but the major spot corresponding to the mixture of **14** and **17** remained invariant. Triethylamine (0.04 mL) was added and the mixture was processed as before. Purification by column chromatography (1:1 hexanes–EtOAc) yielded disaccharide **14** (105 mg, 14%) as an amorphous solid.

A similar reaction of **5** with **9**, in which the mixture was allowed to warm to room temperature for 1 h prior to quenching, gave **14** (57% yield) in approximately 90% purity after column chromatography. Pure **14** was obtained as colorless needles by crystallization and recrystallization (ether–hexanes): mp 147–149 °C; $[\alpha]_D$ +38.6 ° (*c* 1.1, $CHCl_3$); ^{1}H NMR: δ 8.1–7.2 (m, 10H, Ph), 4.88 (dd, 1H, $J_{1',2'}$ 3.9, $J_{2',3'}$ 2.6 Hz, H-2′), 5.70 (dd, 1H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.3 Hz, H-3), 5.64 (s, 1H, PhCH), 5.46 (t, 1H, $J_{3',4'} = J_{4',5'} = 10.1$ Hz, H-4′), 5.41 (dd, 1H, H-3′), 4.78 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 4.66 (d, 1H, H-1′), 4.33–4.21 (m, 4H, H-2, H-4, H-6a, H-6a′), 4.11 (dd, 1H, $J_{5',6b'}$ 4.0, $J_{6a',6b'}$ 11.9 Hz, H-6b′), 4.00–3.90 (m, 2H, H-5, H-6b), 3.50 (ddd, 1H, $J_{5,6a}$ 5.8 Hz H-5′), 3.45 (s, 3H, OC*H*$_3$), 2.08, 2.07, 2.06, 2.03 (4s, each 3H, COC*H*$_3$); ^{13}C NMR: δ 170.45, 169.78, 169.73, 169.51 (4 *C*OCH$_3$), 165.43 (*C*OPh), 137.22, 133.28, 129.92, 129.29, 128.89, 128.51, 128.13, 126.17 (Ph), 101.77 (PhC*H*), 100.78 (C-1), 83.76 (C-1′), 76.56 (C-4), 76.43 (C-2), 70.40 (C-2′), 70.34(2C) (C-3, C-4′), 69.37 (C-3′), 68.76 (C-6), 63.95 (C-5), 62.00 (C-6′), 55.11 (O*C*H$_3$), 38.62 (C-5′), 20.99, 20.84, 20.65, 20.62 (4 CO*C*H$_3$) Anal. Calcd for $C_{35}H_{40}O_{15}S$: C, 57.37; H, 5.50. Found: C, 57.30; H, 5.60.

Methyl 3,4,6-tri-O-*benzyl-2*-O-*(2,3,4,6-tetra*-O-*acetyl-5-thio*-α-D-*mannopyranosyl)*-α-D-*mannopyranoside* (**15**).—A solution of the trichloroacetimidate **5** (149 mg, 0.293 mmol) and methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**10**) [18] (224 mg, 0.482 mmol) in CH_2Cl_2 (4 mL) was stirred at room temperature with freshly activated, crushed 4Å molecular sieves (~1 g) for 15 min and then cooled in a dry-ice/acetone bath. Triethylsilyl triflate (0.020 mL, 0.088 mmol) was added via syringe and, after 10 min, the cooling bath was removed, thus allowing the reaction mixture to warm to room temperature. After 1.5 h, the mixture was re-cooled to −30 °C and quenched by the addition of excess triethylamine (0.050 mL). Dilution with CH_2Cl_2 (100 mL), washing with 2 N aq HCl (10 mL), satd aq $NaHCO_3$ (10 mL) and water (10 mL) followed by drying (anhyd $MgSO_4$) and concentration gave

a yellow oil which was purified by chromatography on silica gel (3:1 toluene–EtOAc) to yield **15** as a colorless syrup (185 mg, 76%). This disaccharide slowly oxidized and hydrolyzed upon storage with exposure to moist air: Evidence for the formation of benzaldehyde was obtained by ^{1}H NMR. [α]D +116.7° (*c* 1.52, $CHCl_3$); ^{1}H NMR: δ 7.4–7.1 (m, 15H, Ph), 5.65 (dd, 1H, $J_{1',2'}$ 3.9, $J_{2',3'}$ 3.0 Hz, H-2′), 5.43 (t, 1H, $J_{3',4'} = J_{4',5'} = 10.3$ Hz, H-4′), 5.37 (dd, 1H, H-3′), 4.85 (d, 1H, H-1′), 4.80 and 4.50 (2d, 2H, J_{AB} 10.8 Hz, PhCH_2), 4.76 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1), 4.72 and 4.64 (2d, 2H, J_{AB} 11.9 Hz, PhCH_2), 4.62 and 4.59 (2d, 2H, J_{AB} 12.2 Hz, PhCH_2), 4.31 (dd, 1H, $J_{5',6a'}$ 5.3, $J_{6a',6b'}$ 11.9 Hz, H-6a′), 4.04 (dd, 1H, $J_{5',6b'}$ 3.7 Hz, H-6b′),.4.01 (dd, 1H, $J_{2,3}$ 2.7 Hz, H-2), 3.91 (dd, 1H, $J_{3,4}$ 9.1 Hz, H-3), 3.85 (t, 1H, $J_{4,5}$ 9.1 Hz, H-4), 3.79–3.69 (m, 3H, H-5, H-6a, H-6b), 3.50 (ddd, 1H, H-5′), 3.37 (s, 3H, OCH_3) 2.12, 2.08, 2.02, 1.98 (4s, each 3H, COCH_3); ^{13}C NMR: δ 170.41, 169.74, 169.55, 169.24, (4 *C*OCH$_3$), 138.59, 138.45, 138.33, (3 C_{ipso} Ph), 129.03, 128.42, 128.28, 128.04, 127.69, 127.58, 127.53, 127.45, 127.37 (Ph), 99.89 (C-1), 82.98 (C-1′), 80.12 (C-3), 75.51, 75.43 (C-2, C-4), 75.20, 73.29, 72.90 (3 *C*H$_2$Ph), 71.90 (C-5), 70.93 (C-2′), 70.48 (C-3′), 69.59 (C-4′), 69.52 (C-6), 62.14 (C-6′), 54.74 (O*C*H$_3$), 39.49 (C-5′), 20.93, 20.56, (CO*C*H$_3$).

Methyl 3,4,6-tri-O-*acetyl-2*-O-*(2,3,4,6-tetra*-O-*acetyl-5-thio*-α-D-*mannopyranosyl)*-α-D-*mannopyranoside* (**16**).—A solution of disaccharide **15** (242 mg, 0.298 mmol) in anhydrous tetrahydrofuran (10 mL) was cooled in a −60 °C bath. Ammonia (~20 mL) was condensed into the flask and small pieces of sodium were added until a dark-blue solution formed. When the blue color had persisted for 15 min, it was discharged by the addition of a few drops of methanol. The cooling bath was removed and the ammonia evaporated by gentle warming in a warm water bath. The tetrahydrofuran solvent was removed in vacuo and the residue was acetylated by treatment with Ac_2O (7 mL) and 4-dimethylaminopyridine (0.050 g) in pyridine (10 mL) at room temperature for 1.5 h. Volatile material was removed in vacuo and the residue was partitioned between CH_2Cl_2 (80 mL) and satd aq $NaHCO_3$ solution (15 mL). The organic phase was washed with 2 N HCl solution (10 mL), satd aq $NaHCO_3$ solution (10 mL) and water (10 mL). After drying (anhyd $MgSO_4$), the solvent was evaporated to give a pale yellow syrup (181 mg) which slowly began to crystallize. The product was purified by column chromatography (2:3 hexanes–EtOAc,) to yield **16** (136 mg, 68%) as a crystalline solid: mp 141–143 °C; $[\alpha]_D$ +67.3° (*c* 1.04, $CHCl_3$); ^{1}H NMR: δ 5.47 (t, 1H, $J_{3',4'} = J_{4',5'} = 9.9$ Hz, H-4′), 5.42 (dd, 1H, $J_{2',3'}$ 2.8 Hz, H-3′), 5.38 (dd, 1H, $J_{1',2'}$ 3.9 Hz, H-2′), 5.34 (t, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.28 (dd, 1H, $J_{2,3}$ 2.9 Hz, H-3), 4.79 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 4.25 (d, 1H, H-1′), 4.30 (dd, 1H, $J_{5',6a'}$ 5.5, $J_{6a',6b'}$ 11.9 Hz, H-6a′), 4.25 (dd, 1H, $J_{5,6a}$ 4.7, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.17 (dd, 1H, $J_{5,6}$ 2.4 Hz, H-6b), 4.08 (dd, 1H, $J_{5',6b'}$ 3.8 Hz, H-6b′), 4.04 (dd, 1H, H-2), 3.91 (ddd, 1H, H-5), 3.51 (ddd, 1H, H-5′), 3.41 (s, 3H, O*C*H$_3$), 2.16, 2.14, 2.09, 2.07, 2.03, 2.02, 2.01 (7s, each 3H, COCH_3); ^{13}C NMR: δ 170.91, 170.45, 170.36, 169.75, 169.67, 169.33, 168.98 (7 *C*OCH$_3$), 99.75 (C-1), 83.11 (C-1′), 76.29 (C-2), 71.59 (C-2′), 70.72 (C-3), 69.61 (C-4′), 69.53 (C-3′), 68.45 (C-5), 66.64 (C-4), 62.26 (C-6), 61.98 (C-6′), 55.16 (O*C*H$_3$), 39.58 (C-5′), 20.92, 20.59(6C), (7 CO*C*H$_3$) Anal. Calcd for $C_{27}H_{38}O_{17}S$: C, 48.65; H, 5.75. Found: C, 48.54; H, 5.68.

Methyl 2-O-*(5-thio*-α-D-*mannopyranosyl)*-α-D-*mannopyranoside* (**2**).—To a solution of disaccharide **16** (136 mg, 0.204 mmol) in anhyd MeOH was added a 1 M solution of NaOMe in MeOH (1.0 mL), and the mixture was kept at room temperature for 4 h. The solution was neutralized with Rexyn 101 H^+ ion-exchange resin, filtered and concentrated to a syrup. Anhydrous toluene (20 mL) was added and removed in vacuo to give **2** (72 mg, 95%) as a pale-yellow glass: $[\alpha]_D$ +146° (*c* 0.63, H_2O); ^{1}H NMR (D_2O): δ 4.96 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 4.84 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.30 (dd, 1H, $J_{2',3'}$ 2.1 Hz, H-2′), 4.01 (dd, 1H, $J_{2,3}$ 3.4 Hz, H-2), 3.91 (dd, 1H, $J_{5',6a'}$ 3.2, $J_{6a',6b'}$ 11.9 Hz, H-6a′), 3.86 (dd, 1H, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.85 (dd, 1H, $J_{3,4}$ 9.4 H-3), 3.77 (dd, 1H, $J_{5',6b'}$ 6.9 Hz, H-6b′), 3.76–3.71 (2H, m, H-3′ and H-4′), 3.72 (dd, 1H, $J_{5,6}$ 6.0 Hz, H-6b), 3.64 (t, 1H, $J_{4,5}$ 9.8 Hz, H-4), 3.56 (ddd, 1H, H-5), 3.37 (s, 3H, OCH_3), 3.05 (ddd, 1H, H-5′); ^{13}C NMR (D_2O): δ 102.33 (C-1), 89.06 (C-1′), 79.91 (C-2), 75.32 (C-5), 74.39, 74.36 (C-2′ and C-4′), 73.21 (C-3), 72.24 (C-3′), 69.70 (C-4), 63.53 (C-6), 63.17 (C-6′), 57.44 (O*C*H$_3$), 46.88 (C-5′) Anal. Calcd for $C_{13}H_{24}O_{10}S$: C, 41.93; H, 6.50. Found: C, 42.05; H, 6.61.

Methyl 4,6-O-*benzylidene-2*-S-*(2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl)-2-thio*-α-D-*mannopyranoside* (**19**).—A solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranose (**6**) [21] (3.66 g, 10.1 mmol) in dry *N*,*N*-dimethylformamide (20 mL) was cooled in an ice bath and stirred with 4 Å

molecular sieves (2 g). Sodium hydride, 60% in oil (0.40 g, 10.0 mmol) and, after 10 min, methyl 4,6-*O*-benzylidene-2-*O*-trifluoromethanesulfonyl-α-D-mannopyranoside (**18**) [20] (4.14 g, 10.0 mmol) were added. The cooling bath was removed and the mixture stirred at room temperature for 2 h. Methylene chloride (200 mL) was used to transfer the solution, with filtration, into a separatory funnel for washing with satd. brine (50 ml). After drying (anhydr Na_2SO_4), the solvents were removed, first with aspirator vacuum and then with high vacuum. The residue was purified by column chromatography (1:1 hexanes–EtOAc) to yield **19** (2.89 g, 46%) as a pale yellow syrup which slowly crystallized on standing: mp 116–117 °C; $[\alpha]_D$ +92.2° (*c* 1.22, $CHCl_3$); ^{1}H NMR: δ 7.5–7.3 (m, 5H, Ph), 5.59 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 5.58 (s, 1H, PhC*H*), 5.44 (dd, 1H, $J_{2',3'}$ 2.8 Hz, H-2′), 5.28 (t, 1H, $J_{3',4'} = J_{4',5'} = 10.0$ Hz, H-4′), 5.26 (dd, 1H, H-3′), 4.87 (d, 1H, $J_{1,2}$ 0.8 Hz, H-1), 4.41 (dt, 1H, $J_{3,4}$ 9.4, $J_{3,OH} = J_{2,3} = 4.6$ Hz, H-3), 4.37 (ddd, 1H, $J_{5',6a'}$ 6.2, $J_{5',6b'}$ 2.4 Hz, H-5′), 4.27 (dd, 1H, $J_{6a',6b'}$ 12.1 Hz, H-6a′), 4.25 (m, 1H, H-6a), 4.13 (dd, 1H, , H-6b′), 3.87 (m, 1H, H-4), 3.79 (m, 2H, H-5, H-6b), 3.55 (dd, 1H, H-2), 3.38 (s, 3H, OC*H*$_3$), 2.47(d, 1H, O*H*), 2.15, 2.10, 2.06, 1.99 (4s, each 3H, COC*H*$_3$); ^{13}C NMR: δ 170.46, 169.64(3C), (4 *C*OCH$_3$), 137.24, 129.20, 128.30, 126.28 (Ph), 102.67 (C-1), 102.33 (PhC*H*), 83.54 (C-1′), 80.29 (C-4), 70.65 (C-2′), 69.50 (C-3′), 69.34 (C-5′), 68.80 (C-6), 68.22 (C-3), 66.73 (C-4′), 63.75 (C-5), 62.73 (C-6′), 55.03 (O*C*H$_3$), 51.11 (C-2), 20.75, 20.60, 20.52(2C), (4 CO*C*H$_3$) Anal. Calcd for $C_{28}H_{36}O_{14}S$: C, 53.50; H, 5.77. Found: C, 53.44; H, 5.85.

*Methyl 2-*S*-(α-*D*-mannopyranosyl)-2-thio-α-*D*-mannopyranoside* (**3**).—A solution of disaccharide **19** (2.87 g, 4.56 mmol) in 80% aq HOAc (50 mL) was warmed to 50 °C for 4.5 h. After cooling, the solvents were removed in vacuo and the residue was dried by evaporation of toluene (2×25 mL) and kept under high vacuum at 50 °C until the last traces of benzaldehyde had been removed. The pale yellow foam was dissolved in anhyd MeOH (45 mL) and a 1 M solution of NaOMe in MeOH (5.0 mL) was added. The mixture was kept at room temperature for 19 h, neutralized with Rexyn 101 H^+ ion-exchange resin, filtered and concentrated to a syrup. The crude product was purified by chromatography on silica gel (4:2:0.3 EtOAc–MeOH–H_2O) to give **3** (1.12 g, 66%) as a pale-yellow foam.: $[\alpha]_D$ +133° (*c* 1.0, H_2O); ^{1}H NMR (D_2O): δ 5.32 (d, 1H, $J_{1',2'}$ 1.4 Hz, H-1′), 4.92 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1), 4.10 (dd, 1H, $J_{2,3}$ 4.7, $J_{3,4}$ 9.4 H-3), 4.05 (dd, 1H, $J_{2',3'}$ 3.4 Hz, H-2′), 3.94 (ddd, 1H, $J_{4',5'}$ 9.4, $J_{5',6a'}$ 2.2, $J_{5',6b'}$ 7.1 Hz, H-5′), 3.85 (dd, 1H, $J_{6a',6b'}$ 12.3 Hz, H-6a′), 3.81 (dd, 1H, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 12.3 Hz, H-6a), 3.70 (dd, 1H, $J_{3',4'}$ 9.6 Hz, H-3′), 3.68 (dd, 1H, H-6b′), 3.67 (dd, 1H, $J_{5,6b}$ 5.8 Hz, H-6b), 3.58 (dd, 1H, H-4′), 3.56 (ddd, 1H, $J_{4,5}$ 9.6 Hz, H-5), 3.46 (dd, 1H, H-4), 3.38 (dd, 1H, H-2), 3.32(s, 3H, OC*H*$_3$); ^{13}C NMR (D_2O): δ 104.52 (C-1), 89.73 (C-1′), 76.41 (C-5′), 75.59 (C-5), 74.25 (C-2′), 73.81 (C-3′), 72.26 (C-3), 70.64 (C-4), 70.05 (C-4′), 63.76, 63.63 (C-6 and C-6′), 57.64 (O*C*H$_3$), 54.98 (C-2), Anal. Calcd for $C_{13}H_{24}O_{10}S$: C, 41.93; H, 6.50. Found: C, 41.65; H, 6.68.

*Methyl 3,4,6-tri-*O*-acetyl-2-*S*-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-2-thio-α-D-mannopyranoside* (**20**).—A solution of disaccharide **3** (1.10 g, 2.95 mmol) and 4-dimethylaminopyridine (0.020 g) in pyridine (15 mL) and Ac_2O (7.5 mL) was kept at 40 °C for 1 h. Volatile material was removed in vacuo and the residue was dissolved in CH_2Cl_2 (100 mL) and washed with satd $NaHCO_3$ solution (2×15 mL), 2 N aq HCl (2×15 mL), satd $NaHCO_3$ solution (10 mL) and satd brine (10 mL). The solution was dried over anhyd $MgSO_4$ and concentrated to give **20** (1.84 g, 94%) as a crystalline solid: mp 194–195 °C (EtOH); $[\alpha]_D$ +92.1° (*c* 1.08, $CHCl_3$); ^{1}H NMR: δ 5.50 (dd, 1H, $J_{2,3}$ 4.6, $J_{3,4}$ 9.6 Hz, H-3), 5.36–5.23 (m, 4H, H-2′, H-3′, H-4′, H-4), 5.32 (d, 1H, $J_{1',2'}$ 1.3 Hz, H-1′), 4.88 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1), 4.36 (ddd, 1H, $J_{4',5'}$ 9.5, $J_{5',6a'}$ 6.2, $J_{5',6b'}$ 2.4 Hz, H-5′), 4.26 (dd, 1H, $J_{6a',6b'}$ 12.1 Hz, H-6a′), 4.20 (dd, 1H, $J_{5,6a}$ 4.7, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.12 (dd, 1H, $J_{5,6b}$ 2.5 Hz, H-6b), 4.09 (dd, 1H, H-6b′), 3.91 (ddd, 1H, $J_{4,5}$ 9.9 Hz, H-5), 3.56 (dd, 1H, H-2), 3.38 (s, 3H, OC*H*$_3$), 2.15, 2.12, 2.09, 2.06, 2.05, 2.03, 2.00 (7s, each 3H, COC*H*$_3$); ^{13}C NMR: δ 170.62, 170.42, 170.00, 169.64(2C), 169.54, 169.37 (7 *C*OCH$_3$), 101.33 (C-1), 82.74 (C-1′), 70.85 (C-2′), 70.27 (C-3), 69.37 (C-5), 69.08 (C-5′), 68.70 (C-3′), 66.87 (C-4), 66.56 (C-4′), 62.50 (C-6′), 62.35 (C-6), 55.20 (O*C*H$_3$), 48.56 (C-2), 20.75, 20.68(2C), 20.58(2C), 20.50(2C) (7 CO*C*H$_3$) Anal. Calcd for $C_{27}H_{38}O_{17}S$: C, 48.65; H, 5.75 . Found: C, 48.64; H, 5.72.

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