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Note

Synthesis of benzyl 6-*O*-α-D-mannopyranosyl-1-thio-α-D-mannopyranoside and benzyl 2-*O*-α-D-mannopyranosyl-1-thio-α- and -β-D-mannopyranoside

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In a previous Note[1], we reported the synthesis of 4-*O*-α-D-mannopyranosyl-D-mannopyranose and its benzyl 1-thio-α-glycoside. Benzyl 1-thioglycosides of disaccharides of D-mannose containing the more common, naturally occurring linkages were required for biological evaluation as part of a study of carbohydrate derivatives that interact with fat-cell surface-membranes to mimic[2] or inhibit the actions of insulin. Also of interest, therefore, were the benzyl 1-thioglycosides of 6-*O*-α-D-mannopyranosyl-D-mannopyranose and 2-*O*-α-D-mannopyranosyl-D-mannopyranose. The present work describes the synthesis of these disaccharide derivatives.

1,2,3,4-Tetra-*O*-acetyl-6-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose (**3**) was prepared by condensation of 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose[3] (**1**) with tetra-*O*-acetyl-α-D-mannopyranosyl bromide[4] (**2**) in the presence of mercuric cyanide. This disaccharide octaacetate (**3**) had been synthesized by Evans and co-workers[5] by employing standard Koenigs–Knorr conditions, in, however, lower yield than that obtained in the present study. These workers had also incorrectly assigned[5] the β-D configuration to the (1→6)-interglycosidic linkage, but later re-examination by Gorin and Perlin[6] revealed that the disaccharide possesses the anticipated α-D-linkage. Treatment of **3** with hydrogen bromide–acetic acid gave the peracetylated glycosyl bromide **4**, which was allowed to react with potassium α-toluenethioxide to afford benzyl 2,3,4-tri-*O*-acetyl-6-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside (**5**). The α-D configuration at the potentially reducing end of **5** was confirmed by comparison of (*a*) its 300-MHz n.m.r. spectrum in benzene-d_6 (see Experimental part) with that of benzyl tetra-*O*-acetyl-1-thio-α-D-mannopyranoside[7] (**6**), and (*b*) its molecular rotation (+995°) with the sum of the molecular rotations of the constituents [**6**, +700° (ref. 7); methyl tetra-*O*-acetyl-α-D-mannopyranoside, +178° (sum = +878°)]. Catalytic deacetylation of **5** gave the desired benzyl 6-*O*-α-D-mannopyranosyl-1-thio-α-D-mannopyranoside (**7**).

The synthesis of 1,3,4,6-tetra-*O*-acetyl-2-*O*-(tetra-*O*-acetyl-α-D-mannopyrano-

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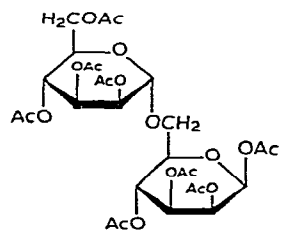
syl)-β-D-mannopyranose (**9**) by condensation of **2** with 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose[8] (**8**) was reported by Bahl and co-workers[9]; however, no physical data were given for characterization. Proof of structure was based[9] on a comparison of the optical rotation of the deacetylated disaccharide with that of 2-*O*-α-D-mannopyranosyl-D-mannopyranose obtained from a mannan produced by *Saccharomyces rouxii*[10]. We have now obtained octaacetate **9** crystalline without chromatography, by using an equivalent amount of tetra-*O*-acetyl-α-D-mannopyranosyl bromide[4]. The α-D-(1→2) configuration was indicated by (*a*) the 300-MHz, n.m.r.-spectral data for **9** in benzene-d_6 (see Experimental part) and (*b*) comparison of its molecular rotation (+81°) with the sum of the molecular rotations of the constituents (β-D-mannopyranose pentaacetate, −99°; methyl tetra-*O*-acetyl-α-D-mannopyranoside, +178°) (sum = +79°). Benzyl 1-thioglycosidation of **9** was achieved *via* the isothiouronium bromide. T.l.c. then revealed the presence of two products which were identified, on the basis of comparison of (*a*) their 300-MHz n.m.r. spectra in benzene-d_6 with those of **6** and benzyl tetra-*O*-acetyl-1-thio-β-D-mannopyranoside[7] and (*b*) molecular rotations, as being benzyl 3,4,6-tri-*O*-acetyl-2-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-1-thio-α- and -β-D-mannopyranoside (**10** and **11**, respectively) (the α anomer being the more-mobile component in t.l.c.). The formation of an anomeric mixture of 1-thioglycosides may be ascribed to the presence of a nonparticipating, neighboring group on C-2 of disaccharide **9**. Catalytic deacetylation of **10** and **11** afforded benzyl 2-*O*-α-D-mannopyranosyl-1-thio-α- and -β-D-mannopyranoside (**12** and **13**, respectively).



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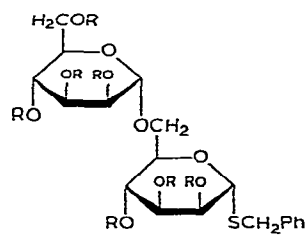

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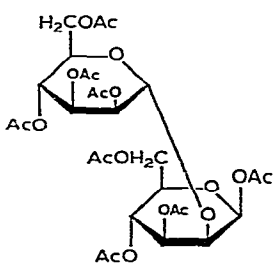
7 R = H



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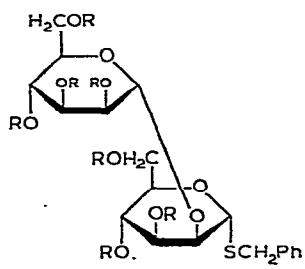

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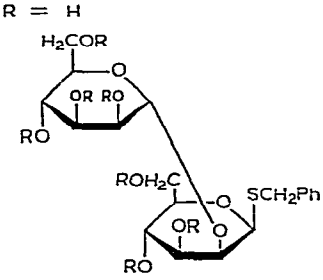


11 R = Ac
13 R = H

Benzyl 6-*O*-α-D-mannopyranosyl-1-thio-α-D-mannopyranoside (**7**) has been found to inhibit the binding of insulin–Sepharose to receptors on fat-cell surface-

membranes in the affinity buoyant density assay, and also to antagonize insulin-stimulated oxidation of D-[^{14}C]glucose to $^{14}CO_2$ in rat adipocytes *in vitro*[11].

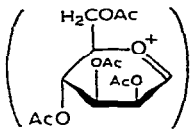
EXPERIMENTAL

General methods. — Solutions were evaporated below 50° under diminished pressure. Melting points were determined with a Thomas–Hoover "Unimelt" apparatus and are uncorrected. Optical rotations were measured with either a Zeiss or a Perkin–Elmer Model 241 polarimeter. Mass spectra were recorded with either an LKB Model 9000 spectrometer (electron impact) or a Varian Model 731 spectrometer (field desorption). N.m.r. spectra were recorded at 300 MHz with a Varian SC-300 n.m.r. spectrometer; unless otherwise stated, spectra were recorded at ambient temperature for solutions in benzene-d_6, with tetramethylsilane (δ = 0.00) as the internal standard. Chemical shifts are given on the δ scale. Spectra were analyzed on a first-order basis. The carbon atoms of the pyranosyl group (nonreducing) of the disaccharides are designated with primed numbers. T.l.c. was performed on plates (250 μm) of Silica Gel GF_{254} (Analtech), and indication was effected with a ceric sulfate (1%)–sulfuric acid (10%) spray. Column chromatography was conducted with silica gel No. 7734 (70–230 mesh; E. Merck). Petroleum ether refers to a fraction having b.p. 35–60°.

*Preparation of 1,2,3,4-tetra-*O*-acetyl-β-*D*-mannopyranose* (**1**). — Compound **1** was prepared from D-mannose by following a modification[3] of the procedure of Helferich and Leete[12]; m.p. 135–136° (lit.[3,12] m.p. 135.5–136.5°); n.m.r. data (after D_2O exchange): δ 5.66 (d, $J_{1,2}$ ~0 Hz, $J_{2,3}$ 3.2 Hz, H-2), 5.62 (s, H-1), 5.60 (t, $J_{3,4}$ 10.4 Hz, H-4), 5.17 (dd, H-3), 3.62 (dd, H-6a), 3.45 (dd, H-6b), 3.13 (dq, H-5), 1.54, 1.58, 1.70 and 1.71 (4 s, 12 H, 4 OAc).

*1,2,3,4-Tetra-*O*-acetyl-6-*O*-(tetra-*O*-acetyl-α-*D*-mannopyranosyl)-β-*D*-mannopyranose* (**3**). — A mixture of **1** (7.5 g, 21.5 mmol) and mercuric cyanide (7.8 g, 36.9 mmol) in dry 1 : 1 benzene–nitromethane (1.25 liters) was concentrated to 900 ml at atmospheric pressure and then cooled to room temperature. A solution of tetra-*O*-acetyl-α-D-mannopyranosyl bromide[4] (**2**) (11.5 g, 28.0 mmol) in benzene (110 ml) was added, and the mixture was stirred, with exclusion of moisture, for 24 h at room temperature; then, an additional 3.3 g of **2** and 3.0 g of mercuric cyanide were added, and the mixture was stirred for another 24 h, filtered through Celite, and the filtrate evaporated to a residue that was partitioned between chloroform (250 ml) and water. The organic layer was successively washed with M aqueous potassium iodide (4 × 75 ml) and cold water, dried (sodium sulfate), and evaporated, to give a syrup that crystallized from 95% ethanol. Recrystallization from 95% ethanol gave pure **3**, yield 7.2 g (49%), m.p. 147–149°, $[\alpha]_D^{27}$ +19° (*c* 1, chloroform) {lit.[5] m.p. 152–153°, $[\alpha]_D^{25}$ +19.6° (*c* 3.7, chloroform)}; n.m.r. data: δ 5.59–5.76 (m, 5 H, H-2,4,2′,3′,4′), 5.56 (s, $J_{1,2}$ ~0 Hz, H-1), 5.16 (dd, $J_{2,3}$ 2.6 Hz, $J_{3,4}$ 9.8 Hz, H-3), 4.70 (d, $J_{1',2'}$ 1.7 Hz, H-1′), 4.43 (dd, $J_{5',6'a}$ 5.0 Hz, $J_{6'a,6'b}$ 12.6 Hz, H-6′a), 4.25 (dd, $J_{5',6'b}$ 2.4 Hz, H-6′b), 4.20 (m, H-5′), 3.90 (dd, $J_{5,6a}$ 4.8 Hz, $J_{6a,6b}$ 10.8 Hz, H-6a), 3.48 (dd, $J_{5,6b}$

3.3 Hz, H-6b), 3.25 (dq, $J_{4,5}$ 9.6 Hz, H-5), 1.58, 1.60, 1.68, 1.69, 1.70, 1.76, 1.79, and 1.89 (8 s, 24 H, 8 OAc); *m/e* 618 (M — AcOH), 558 (618 — AcOH), 456 (618 — AcOH — CH_2CO), 331



and 289 (331 — CH_2CO).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.18; H, 5.61.

2,3,4-Tri-O-acetyl-6-O-(tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl bromide (**4**). — A solution of **3** (2.0 g) in dry dichloromethane (18 ml) was cooled to 0° and treated with 31% hydrogen bromide in acetic acid (5 ml). The mixture was stirred for 2 h at 0°, diluted with dichloromethane (25 ml), washed 4 times with ice–water, dried (sodium sulfate), and evaporated to a glass (2.0 g) that was used without further purification for the subsequent 1-thioglycosidation reaction.

Benzyl 2,3,4-tri-O-acetyl-6-O-(tetra-O-acetyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside (**5**). — To a solution of the bromide **4** (1.9 g, 2.7 mmol) in dry acetone (10 ml) were added α-toluenethiol (0.34 ml, 2.9 mmol) and then a solution of potassium hydroxide (0.16 g, 2.9 mmol) in water (2 ml). The mixture was stirred for 24 h at room temperature and evaporated, and the residue was partitioned between dichloromethane and water; the organic layer was successively washed 3 times with 5% aqueous sodium hydroxide and once with cold water, dried (magnesium sulfate), and evaporated to a residue that was treated overnight with acetic anhydride (5 ml) and pyridine (10 ml). The excess of reagents was removed by evaporation under diminished pressure followed by several co-evaporations with toluene, the residue applied to a column of silica gel, and the product eluted with 20:1 chloroform–ethyl acetate. Pure **5** was obtained as a chromatographically homogeneous syrup that could not be induced to crystallize; yield 1.0 g (50%), $[\alpha]_D^{27}$ +134° (*c* 0.9, chloroform); n.m.r. data: δ 5.62–5.83 (m, 6 H, H-2,3,4,2',3',4'), 5.25 (s, $J_{1,2}$ ~0 Hz, H-1), 4.73 (d, $J_{1',2'}$ 1.6 Hz, H-1'), 4.27–4.42 (m, 4 H, H-5,5',6'a,6'b), 3.88 (dd, $J_{5,6a}$ 6.4 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 3.79 (d, 1 H, *J* 13.6 Hz, SCH_2Ph), 3.63 (d, 1 H, SCH_2Ph), 3.43 (dd, $J_{5,6b}$ 2.2 Hz, H-6b), 1.63, 1.64, 1.70, 1.73, and 1.80 (5 s, 15 H, 5 OAc), and 1.66 (s, 6 H, OAc); *m/e* 742 (M) and 619 (742 — SCH_2Ph).

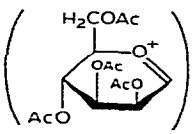
Anal. Calc. for $C_{33}H_{42}O_{17}S$: C, 53.36; H, 5.70; S, 4.32. Found: C, 53.18; H, 5.83; S, 4.49.

Benzyl 6-O-α-D-mannopyranosyl-1-thio-α-D-mannopyranoside (**7**). — To a solution of **5** (0.40 g) in dry methanol (10 ml) was added 0.1M methanolic sodium methoxide (0.5 ml). The mixture was kept overnight at room temperature, made neutral with Bio-Rad AG50W-X4 (H^+) ion-exchange resin, filtered, and evaporated, to give **7** as a chromatographically homogeneous syrup; yield 0.23 g (93%), $[\alpha]_D^{27}$ +258° (*c* 1, methanol).

Anal. Calc. for $C_{19}H_{28}O_{10}S \cdot 0.5H_2O$: C, 49.88; H, 6.39; S, 7.01. Found: C, 49.85; H, 6.44; S, 6.99.

1,3,4,6-Tetra-O-acetyl-β-D-mannopyranose (**8**). — Compound **8** was prepared by direct acetylation of D-mannose according to the procedure of Deferrari *et al.*[8]; m.p. 162–164° (lit.[8] m.p. 164–165°); n.m.r. data (after D_2O exchange): δ 5.67 (t, $J_{3,4}$ 10.2 Hz, $J_{4,5}$ 10 Hz, H-4), 5.50 (s, $J_{1,2}$ ~0 Hz, H-1), 4.92 (dd, $J_{2,3}$ 3.4 Hz, H-3), 4.28 (dd, $J_{5,6a}$ 4.8 Hz, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.06 (dd, $J_{5,6b}$ 2.4 Hz, H-6b), 3.88 (d, H-2), 3.26 (dq, H-5), 1.50 and 1.64 (2 s, 6 H, 2 OAc), and 1.66 (s, 6 H, 2 OAc).

1,3,4,6-Tetra-O-acetyl-2-O-(tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose (**9**). — To a solution of **8** (3.0 g, 8.6 mmol) in dry acetonitrile (10 ml) were added mercuric cyanide (1.0 g), mercuric bromide (1.5 g), and then a solution of tetra-*O*-acetyl-α-D-mannopyranosyl bromide[4] (3.6 g, 8.8 mmol) in acetonitrile (10 ml). The mixture was stirred overnight at room temperature with exclusion of moisture, and evaporated, and the residue was partitioned between chloroform and 0.5M aqueous potassium bromide. The organic layer was successively washed twice with 0.5M aqueous potassium bromide and twice with cold water, dried (sodium sulfate), and evaporated to a syrup that crystallized from absolute ethanol. Two recrystallizations from ethanol gave pure **9**; yield 2.9 g (49%), m.p. 90–95° (softens at 85°), $[\alpha]_D^{27}$ +1.2° (*c* 1, chloroform); n.m.r. data: δ 5.92 (dd, $J_{2',3'}$ 3.4 Hz, $J_{3',4'}$ 10.4 Hz, H-3'), 5.86 (t, $J_{4',5'}$ 9 Hz, H-4'), 5.64 (t, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 5.62 (t, $J_{1',2'}$ 1.8 Hz, H-2'), 5.52 (s, $J_{1,2}$ ~0 Hz, H-1), 5.14 (d, H-1'), 5.03 (dd, $J_{2,3}$ 3 Hz, H-3), 4.62 (dt, $J_{5',6'a}$ 3.4 Hz, $J_{5',6'b}$ 2.6 Hz, H-5'), 4.54 (dd, $J_{5,6a}$ 4 Hz, $J_{6a,6b}$ 11.6 Hz, H-6a), 4.32 (dd, $J_{6'a,6'b}$ 12.8 Hz, H-6'a), 4.26 (dd, $J_{5,6b}$ 2.4 Hz, H-6b), 4.09 (dd, H-6'b), 3.58 (d, H-2), 3.30 (dq, H-5), 1.70, 1.80, 2.00, and 2.01 (4 s, 12 H, 4 OAc), and 1.62 and 1.64 (2 s, 12 H, 4 OAc); *m/e* 619 (M — OAc), 331



and 289 (331 — CH_2CO).

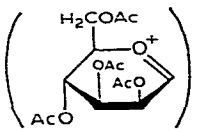
Anal. Calc. for $C_{28}H_{38}O_{19} \cdot 0.5H_2O$: C, 48.91; H, 5.72. Found: C, 48.77; H, 5.69.

Benzyl 3,4,6-tri-O-acetyl-2-O-(tetra-O-acetyl-α-D-mannopyranosyl)-1-thio-α- (**10**) *and -β-D-mannopyranoside* (**11**). — To a solution of the octaacetate **9** (2.0 g, 2.9 mmol) in dry dichloromethane (22 ml) cooled to −10° was added 31% hydrogen bromide in acetic acid (6 ml). The mixture was stirred for 60 min at −10° and then processed as for bromide **4**. To a solution of the resulting syrup in dry acetone (25 ml) was added thiourea (0.34 g, 4.5 mmol); the mixture was boiled under reflux for 16 h with stirring, cooled, and evaporated, and the residue was partitioned between water (12 ml) and diethyl ether (10 ml). The aqueous layer was washed with diethyl ether (2 × 10 ml), and then treated with potassium carbonate (0.40 g, 2.9 mmol), potassium pyrosulfite ($K_2S_2O_5$; 0.80 g, 3.6 mmol), and, finally, a solution of α-bromotoluene (0.50 g, 2.9 mmol) in acetone (10 ml). The mixture was stirred overnight at room temperature, the acetone removed by evaporation, the aqueous residue extracted with dichloromethane, and the combined extracts washed with water,

and evaporated. The residue was applied to a column of silica gel, and the products were eluted with 2:1 and then 3:1 ether–petroleum ether. The fractions containing the faster-moving component were combined and evaporated, to give the α anomer (**10**) as a chromatographically homogeneous syrup; yield 331 mg (15%), $[\alpha]_D^{27}$ +112° (*c* 0.9, chloroform), $[M]_D$ +832° (sum of molecular rotations of constituents = +878°); n.m.r. data: δ 5.68—5.87 (m, 3 H, H-4,3′,4′), 5.54 (dd, $J_{2,3}$ 2.6 Hz, H-3), 5.52 (t, $J_{1',2'}$ 1.7 Hz, H-2′), 5.47 (d, $J_{1,2}$ 1.6 Hz, H-1), 4.68 (d, H-1′), 4.26–4.46 (m, 4 H, H-5,6a,6b,5′), 4.08–4.12 (m, 2 H, H-2,6′a), 3.99 (dd, $J_{5',6'b}$ 1.4 Hz, $J_{6'a,6'b}$ 11 Hz, H-6′b), 3.58 (d, 1 H, *J* 13.8 Hz, S*CH*$_2$Ph), 3.44 (d, 1 H, S*CH*$_2$Ph), 1.62, 1.65, 1.79, 1.82 and 2.06 (5 s, 15 H, 5 OAc), and 1.60 (s, 6 H, 2 OAc); *m/e* 742 (M) and 619 (742 — SCH_2Ph).

Anal. Calc. for $C_{33}H_{42}O_{17}S$: C, 53.36; H, 5.70; S, 4.32. Found: C, 53.21; H, 5.86; S, 4.41.

Intermediate fractions afforded a mixture of the anomers (143 mg, 7%). The fractions containing only the slower-moving component were combined, and evaporated to a syrup that crystallized from ethanol to give pure β anomer (**11**); yield 322 mg (15%), m.p. 149–150.5°, $[\alpha]_D^{27}$ −85.7 ±0.5° (*c* 1, chloroform); $[M]_D$ −637° (sum of molecular rotations of constituents = −545°); n.m.r. data: δ 5.94 (dd, $J_{2',3'}$ 3.6 Hz, $J_{3',4'}$ 10.6 Hz, H-3′), 5.85 (t, $J_{4',5'}$ 9.6 Hz, H-4′), 5.74 (t, $J_{1',2'}$ 1.6 Hz, H-1′), 5.63 (t, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 10 Hz, H-4), 5.08 (d, H-1′), 4.92 (m, 1 H, H-5′), 4.88 (dd, $J_{2,3}$ 3 Hz, H-3), 4.63 (dd, $J_{5,6a}$ 4.2 Hz, $J_{6a,6b}$ 12.6 Hz, H-6a), 4.52 (dd, $J_{5,6b}$ 2.2 Hz, H-6b), 4.33 (dd, $J_{5',6'a}$ 4.2 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6′a), 4.14 (dd, $J_{5',6'b}$ 2.4 Hz, H-6′b), 4.10 (s, $J_{1,2}$ ~0 Hz, H-1), 3.74 (d, 1 H, *J* 13.4 Hz, S*CH*$_2$Ph), 3.64 (d, H-2), 3.50 (d, 1 H, S*CH*$_2$Ph), 3.22 (dq, H-5), 1.60, 1.66, 1.81, 1.98, and 1.99 (5 s, 15 H, 5 OAc), and 1.63 (s, 6 H, 2 OAc); *m/e* 742 (M), 682 (742 — AcOH), 619 (742 — SCH_2Ph), and 331



Anal. Calc. for $C_{33}H_{42}O_{17}S$: C, 53.36; H, 5.70; S, 4.32. Found: C, 53.58; H, 5.71; S, 4.47.

Benzyl 2-O-α-D-mannopyranosyl-1-thio-α-D-mannopyranoside (**12**). — To a solution of **10** (310 mg) in dry methanol (10 ml) was added a catalytic amount of sodium methoxide. The mixture was kept for 5 h at room temperature and then processed in the usual way, to afford **12** as a chromatographically homogeneous syrup; yield 181 mg (95%); $[\alpha]_D^{27}$ +258° (*c* 0.5, methanol).

Anal. Calc. $C_{19}H_{28}O_{10}S \cdot 0.5\ H_2O$: C, 49.88; H, 6.39; S, 7.01. Found: C, 49.77; H, 6.58; S, 7.20.

Benzyl 2-O-α-D-mannopyranosyl-1-thio-β-D-mannopyranoside (**13**). — A mixture of **11** (240 mg) with dry methanol (10 ml) was treated with a catalytic amount of sodium methoxide. Dissolution resulted after stirring for a few min, and, after 4 h, the solid that separated out was filtered off and dried. Recrystallization from

ethanol–methanol afforded pure **13**; yield 130 mg (90%), m.p. 238–240°, $[\alpha]_D^{27}$ −79.7° (*c* 0.5, methanol).

Anal. Calc. for $C_{19}H_{28}O_{10}S$: C, 50.88; H, 6.29; S, 7.15. Found: C, 50.83; H, 6.29; S, 7.27.

ACKNOWLEDGMENTS

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