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Note

Synthesis of 4-*O*-α-D-mannopyranosyl-D-mannopyranose and its benzyl 1-thio-α-D-glycoside

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As part of a study of carbohydrate derivatives having insulin-like[1] or insulin-antagonistic properties, 1-thioglycosides of 4-*O*-α-D-mannopyranosyl-D-mannopyranose (**6**) were needed for biological evaluation. Surprisingly, inspection of the literature revealed that no direct synthesis of disaccharide **6** from monosaccharide precursors had been reported. The disaccharide had previously been obtained[2] in low yield (after carbon-column and paper chromatography) by melt-polymerization of 1,2,3,4-tetra-*O*-acetyl-α-D-mannopyranose. However, a more facile route to **6** was desirable in order to have available sufficient amounts for further chemical transformation.

Because of the observed low reactivity toward glycosidation of an equatorially disposed, 4-hydroxyl group in D-glycopyranosides in the $^4C_1$(D) conformation, particularly when the adjacent 3-hydroxyl group is acylated[3], 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose[4] (**2**) (readily available by acetonation of the pyrolyzate from ivory-nut mannan[4]), which has the 4-hydroxyl group in *exo*-axial orientation, was chosen for the disaccharide synthesis. Condensation of **2** with tetra-*O*-acetyl-α-D-mannopyranosyl bromide[5] (**1**), employing Helferich conditions for the Koenigs–Knorr reaction, afforded crystalline 1,6-anhydro-2,3-*O*-isopropylidene-4-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose (**3**). The α-D configuration of the (1 → 4)-linkage in **3** was indicated by its 300-MHz, n.m.r. spectrum in benzene-d_6 (see Experimental section)**, as well as by a comparison of its molecular rotation with the sum of the molecular rotations of the constituents. Hydrolysis of the isopropylidene group gave crystalline 1,6-anhydro-4-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose (**4**), and subsequent acetolysis afforded 1,2,3,6-tetra-*O*-acetyl-4-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranose (**5**) as a syrup that could not be induced to crystallize. The α-D configuration of the reducing moiety of **5** was indicated by comparison of its molecular rotation

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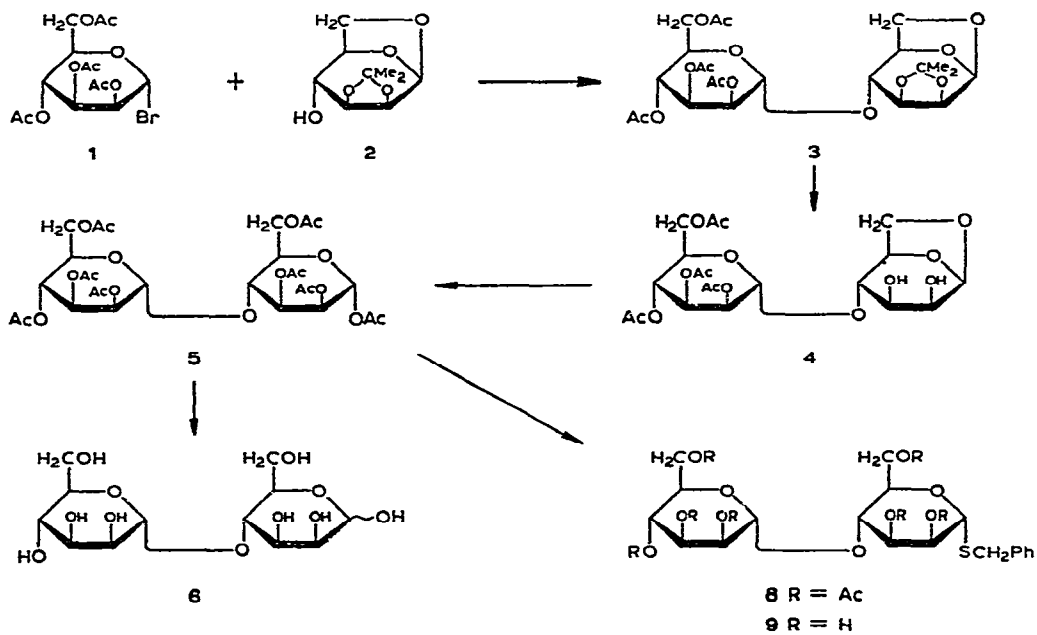
**The ring positions of the pyranosyl group (at the nonreducing end) of the disaccharide are designated with primed numbers.

(+393°) with the sum of the molecular rotations of the constituents (α-D-mannopyranose pentaacetate, +214°; methyl tetra-*O*-acetyl-α-D-mannopyranoside, +178°) (sum = +392°), and by analysis of its 300-MHz n.m.r. spectrum (see Experimental section). In particular, the chemical shift for H-1 of **5** in chloroform-*d* (δ 6.08) was in excellent agreement with that reported[6] for H-1 of α-D-mannopyranose pentaacetate in the same solvent (δ 6.09).

Zemplén deacetylation of **5** gave the desired disaccharide, 4-*O*-α-D-mannopyranosyl-D-mannopyranose (**6**), as a chromatographically homogeneous, amorphous glass having an optical rotation in good agreement with that reported for the same disaccharide obtained by (*a*) hydrolysis of ivory-nut mannan[7], and (*b*) acetolysis of acetylated glucomannan from larch hemicellulose[8]. Reacetylation of **6** at room temperature with acetic anhydride–pyridine regenerated the α-octaacetate **5**. Without any spectroscopic evidence, O'Colla *et al.* assigned[2] the β configuration to the crystalline octaacetate that they obtained by acetylation of **6** with acetic anhydride–sodium acetate. However, comparisons of molecular rotations indicate that their octaacetate[2] also had the α-D configuration at the reducing end of the disaccharide.



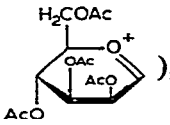
The benzyl 1-thio-α-glycoside (**9**) of **6** was synthesized by way of reaction of the hepta-*O*-acetylglycosyl bromide (**7**) with the potassium salt of α-toluenethiol, which gave benzyl 2,3,6-tri-*O*-acetyl-4-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside (**8**). The α-D configuration at the reducing end of **8** was indicated by comparison of (*a*) its 300-MHz, n.m.r. spectrum in benzene-d_6 (see Experimental section) with that of benzyl tetra-*O*-acetyl-1-thio-α-D-mannopyranoside[9], and (*b*) its

molecular rotation (+914°) with the sum of the molecular rotations of the constituents [methyl tetra-*O*-acetyl-α-D-mannopyranoside, +178°; benzyl tetra-*O*-acetyl-1-thio-α-D-mannopyranoside, +700° (ref. 9)] (sum = +878°). Crystalline benzyl 4-*O*-α-D-mannopyranosyl-1-thio-α-D-mannopyranoside (**9**) was obtained by Zemplén deacetylation of **8**.

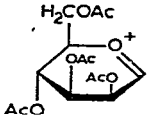
EXPERIMENTAL

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 an LKB Model 9000 spectrometer. N.m.r. spectra were recorded at 60, 100, and 300 MHz with Varian T-60, HA-100, and SC-300 n.m.r. spectrometers, respectively. Chemical shifts are on the δ scale. Unless otherwise stated, spectra were measured at room temperature for solutions in benzene-d_6, with tetramethylsilane ($\delta = 0.00$) as the internal standard. Spectra were analyzed on a first-order basis. 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 (E. Merck; 70–230 mesh). Petroleum ether refers to a fraction having b.p. 35–60°.

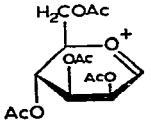
Preparation of 1,6-anhydro-2,3-O-isopropylidene-4-O-(tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose (**3**). — A mixture of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose[4] (**2**; 2.0 g, 9.9 mmol), mercuric cyanide (3.3 g), and anhydrous calcium sulfate (5 g) in dry nitromethane (40 mL) was stirred for 3 h at room temperature, with exclusion of moisture. A solution of tetra-*O*-acetyl-α-D-mannopyranosyl bromide[5] (**1**; 5.4 g, 13.1 mmol) in dry nitromethane (25 mL) was then added, and the mixture was stirred for 20 h at room temperature. Additional bromide (2 g) in nitromethane (20 mL) and mercuric cyanide (1 g) were added, and the mixture was stirred for a further 20 h; this process was repeated with the bromide (1 g) and mercuric cyanide (1 g). The final mixture was filtered through Celite, and the filtrate evaporated. The residue was dissolved in dichloromethane, and the solution washed 4 times with M aqueous potassium bromide and once with cold water, dried (sodium sulfate), and evaporated to a residue that was applied to a column of silica gel and eluted with 7:1 chloroform–ethyl acetate. The fractions containing the fastest-moving component were combined, and evaporated to a syrup that crystallized upon standing. Recrystallization from ethanol gave pure, protected disaccharide **3**; yield 4.4 g (84%), m.p. 153–154°, $[\alpha]_D^{27}$ +1.9 ±1.0° (*c* 1, chloroform); n.m.r. data: δ 5.80 (dd, $J_{2',3'}$ 2.6 Hz, $J_{3',4'}$ 9.6 Hz, H-3'), 5.73 (t, $J_{4',5'}$ 9.6 Hz, H-4'), 5.61 (dd, $J_{1',2'}$ 1.8 Hz, H-2'), 5.28 (d, $J_{1,2}$ 3 Hz, H-1), 4.82 (d, H-1'), 4.60 (bd, H-2), 4.50 (t of d, $J_{5',6'a}$ 2 Hz, $J_{5',6'b}$ 6.2 Hz, H-5'), 4.46 (dd, $J_{6'a,6'b}$ 12 Hz, H-6'a), 4.32 (dd, H-6'b), 4.09 (bd, $J_{6en,6ex}$ 7.4 Hz, H-6en), 3.74 (bd, $J_{5,6ex}$ 7.4 Hz, H-5), 3.69 (dd, $J_{2,3}$ 6 Hz, H-2), 3.60 (s, H-4), 3.42 (t, H-6ex), 1.73, 1.72, 1.64, and 1.64 (3-proton

singlets, OAc), and 1.15 and 1.62 (3-proton singlets, CMe_2); *m/e* 532 (M), 517 ($M-CH_3$), 457 (517−AcOH), 415 (457−CH_2CO), 331 (), and 229 (331−AcOH−CH_2CO).

Anal. Calc. for $C_{23}H_{32}O_{14}$: C, 51.88; H, 6.06. Found: C, 51.89; H, 6.13.

*1,6-Anhydro-4-*O*-(tetra-*O*-acetyl-α-*D*-mannopyranosyl)-β-*D*-mannopyranose* (**4**). — A solution of **3** (1.9 g) in chloroform (90 mL) was treated with trifluoroacetic acid (10 mL) containing 1% of water, kept for 24 h at room temperature, and evaporated. Traces of acid were removed by several co-evaporations with toluene. [The residue was sufficiently pure (t.l.c.) for use in the acetolysis reaction described.] An analytical sample was obtained by chromatography on a column of silica gel with 25:1 chloroform–methanol as the eluant. Crystallization was achieved with ethanol–petroleum ether. Recrystallization from ethanol gave pure **4**; m.p. 75–77°, $[\alpha]_D^{27}$ −17.7 ±1.0° (*c* 1, chloroform); *m/e* 331 (), 289 (331−CH_2CO), 271 (331−AcOH), 229 (331−AcOH−CH_2CO), and 211 (271−AcOH).

Anal. Calc. for $C_{20}H_{28}O_{14}\cdot 0.5H_2O$: C, 47.91; H, 5.83. Found: C, 47.68; H, 5.83.

*1,2,3,6-Tetra-*O*-acetyl-4-*O*-(tetra-*O*-acetyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranose* (**5**). — The residue from the previous reaction was dissolved in 35:15:1 (v/v) acetic anhydride–acetic acid–concentrated sulfuric acid (30 mL). The mixture was kept for 3.5 h at room temperature, poured into ice–water, and extracted with dichloromethane (4 × 50 mL); the extracts were combined, washed successively with cold water, cold saturated aqueous sodium hydrogencarbonate, and cold water, dried (sodium sulfate), and evaporated. The residue (2.3 g, 95%) (only slightly contaminated with a slower-moving component) was purified by chromatography on a column of silica gel, with 18:1 chloroform–ethyl acetate as the eluant. The fractions containing the faster-moving component were combined, and evaporated, to give octaacetate **5** as a chromatographically homogeneous syrup that could not be induced to crystallize; yield 1.5 g (62%, based on **3**), $[\alpha]_D^{27}$ +57.9 ±0.8° (*c* 0.63, chloroform); n.m.r. data: δ 6.34 (bs, H-1), 5.75 (t, $J_{3',4'} = J_{4',5'} = 10$ Hz, H-4'), 5.69–5.63 (m, 3 H, H-2,3,3'), 5.57 (t, $J_{1',2'} = J_{2',3'} = 2$ Hz, H-2'), 5.23 (d, H-1'), 4.55 (dd, $J_{5,6a}$ 2 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.50–4.24 (m, 5 H, H-4,5,6b,6'a,6'b), 4.01 (8-line pattern, H-5'), 2.13, 1.84, 1.71, and 1.44 (3-proton singlets, OAc), and 1.63 and 1.54 (6-proton singlets, OAc); $CDCl_3$: δ 6.08 (H-1); *m/e* 619 (M−OAc), 559 (619−AcOH), 331 (), and 289 (331−CH_2CO).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.94; H, 5.58.

4-O-α-D-Mannopyranosyl-D-mannopyranose (**6**). — The octaacetate **5** (1.2 g) was dissolved in dry methanol (20 mL), and treated overnight with a catalytic amount of sodium methoxide. Neutralization with Bio-Rad AG 50W-X4 (H^+) ion-exchange resin, filtration through Celite, and evaporation, gave **6** as a chromatographically homogeneous, amorphous glass; yield 0.55 g (91%), $[\alpha]_D^{27}$ +66.2 ±0.9° (*c* 0.5, water) {lit. $[\alpha]_D^{17}$ +49° (*c* 0.6, H_2O)[7]; $[\alpha]_D^{18}$ +54 ±5° (*c* 0.4, H_2O)[8]; $[\alpha]_D$ +80° (*c* 0.6, H_2O)[2]}.

Anal. Calc. for $C_{12}H_{22}O_{11}\cdot 2/3H_2O$: C, 40.68; H, 6.64. Found: C, 40.54; H, 6.69.

2,3,6-Tri-O-acetyl-4-O-(tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl bromide (**7**). — To a solution of the octaacetate **5** (1.5 g), 2.2 mmol) in dry dichloromethane (15 mL) was added 31% hydrogen bromide in acetic acid (3 mL). The mixture was kept for 8 h at 0°, diluted with dichloromethane (25 mL), and poured into ice–water; the organic layer was washed with 3 portions of ice–water, dried (sodium sulfate), and evaporated, to afford the bromide **7** as a syrup (1.4 g, 91%) that was employed without further purification for the subsequent thioglycosidation.

Benzyl 2,3,6-tri-O-acetyl-4-O-(tetra-O-acetyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside (**8**). — To a solution of the bromide **7** (1.4 g, 2.0 mmol) in dry acetone (10 mL) were added α-toluenethiol (0.25 ml, 2.1 mmol) and then a solution of potassium hydroxide (0.12 g, 2.1 mmol) in water (2 mL). The mixture was stirred for 24 h at room temperature, and evaporated; the residue was partitioned between dichloromethane and water, the organic layer washed twice with 5% aqueous sodium hydroxide, once with ice–water, dried (sodium sulfate), and evaporated, affording a residue that was treated overnight with acetic anhydride (5 mL) and pyridine (10 mL). Excess reagents were removed by evaporation under diminished pressure, followed by several co-evaporations with toluene. The resulting syrup was applied to a column of silica gel, and the product was eluted with 7:1 chloroform–ethyl acetate. Compound **8** was obtained as a chromatographically homogeneous syrup; yield 0.60 g (40%), $[\alpha]_D^{27}$ +123° (*c* 0.9, chloroform); n.m.r. data: δ 5.78–5.62 (m, 4 H, H-2,3,3′,4′), 5.54 (t, $J_{1',2'} = J_{2',3'} = 2$ Hz, H-2′), 5.20 (d, H-1′), 5.12 (d, $J_{1,2}$ 1.4 Hz, H-1), 4.55–4.22 (m, 7 H, H-4,5,6a,6b,5′,6′a,6′b), 3.54 (d, 1 H, *J* 13 Hz, SCH_2Ph), 3.38 (d, 1 H, SCH_2Ph), and 2.11, 1.85, 1.75, 1.66, 1.64, 1.54, and 1.49 (3-proton singlets, 7 OAc).

Anal. Calc. for $C_{33}H_{42}O_{17}S$: C, 53.36; H, 5.70; S, 4.32. Found: C, 53.65; H, 5.62; S, 4.57.

Benzyl 4-O-α-D-mannopyranosyl-1-thio-α-D-mannopyranoside (**9**). — To a solution of the heptaacetate **8** (0.40 g, 0.54 mmol) in dry methanol (5 mL) was added a catalytic amount of sodium methoxide. The mixture was kept overnight at room temperature, made neutral with Bio-Rad AG50W-X4 (H^+) ion-exchange resin, the suspension filtered, and the filtrate evaporated. The product was purified by thick-layer chromatography on plates (1.000 mm) of silica gel GF_{254} (Analtech) with 4:1 ethyl acetate–methanol as the developer, and extraction with methanol. Evaporation

of the extract afforded crystalline **9**; yield 0.19 g (76%), m.p. 187–192° (dec.), $[\alpha]_D^{27}$ +249° (*c* 0.9, methanol).

Anal. Calc. for $C_{19}H_{28}O_{10}S \cdot H_2O$: C, 48.92; H, 6.48; S, 6.87. Found: C, 49.01; H, 6.38; S, 6.89.

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