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Note

On the debenzylation of *O*-benzyl-protected aralkyl thioglucosides with sodium–liquid ammonia

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O-Benzylated 1-thio-D-glucosides have been prepared in this laboratory as models for the initial sugar unit (eventually the reducing residue) in systematic, sequential oligosaccharide synthesis[1]. In the application of this system to the solid-phase mode, a partially protected sugar would be attached to a carrier of high molecular weight, such as polystyrene, via a thioglycoside linkage. The question is whether the sulfur atom of this linkage should be attached directly to an aromatic side chain of the resin, or be separated from the benzene ring by a "spacer" of one or more methylene groups. In seeking to answer this question we have tested the model compounds with sodium–liquid ammonia, which is a candidate reagent for the *O*-debenzylation of a completed oligosaccharide, and possibly for its cleavage from the resin.

In previous work we studied *O*-benzylated phenyl thioglucosides (models for direct attachment) and 3-phenylpropyl thioglucosides (three methylene groups as spacer). The former underwent partial reduction at the anomeric carbon atom, but the thioglucoside groups of the latter were stable to the reagent, thus permitting the selective removal of the *O*-benzyl protecting groups. In the present note we report the synthesis and examination of the intermediate members of the series, the *O*-benzylated benzyl and 2-phenylethyl thioglucosides. The *O*-benzylated normal (*O*) benzyl glucoside was also studied.

Benzyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**4**) was synthesized by the condensation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride (**2**) with α-toluenethiol. Treatment with an excess of sodium in liquid ammonia removed the *O*-benzyl groups and, as expected, also cleaved the *S*-benzyl linkage. The only detectable product was 1-thio-D-glucose. The product of reduction at the anomeric carbon atom, 1,5-anhydro-D-glucitol, was not present. Similarly, the complete debenzylation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (**1**) and benzyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside (**3**) afforded only D-glucose.

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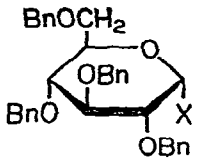
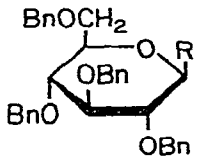
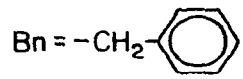
Treatment of **3** and **4** with limited amounts of sodium in liquid ammonia produced mixtures of products. With two g-atoms per mole of compound the principal components were 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose and 2,3,4,6-tetra-*O*-benzyl-1-thio-D-glucopyranose, respectively.



1 X = OH
2 X = Cl



3 R = OBn
4 R = SBn
5 R = SCOEt
6 R = SH
7 R = SCH_2CH_2Ph



To complete the overall synthetic process the thiosugar products of the sodium–liquid ammonia cleavages must be converted into ordinary sugars. This is readily accomplished by hydrolysis in the presence of mercuric chloride, as described in the Experimental part.

The method of preparation of 2-phenylethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**7**) was the same as that for **4**. Treatment with an excess of sodium in liquid ammonia gave 1-thio-D-glucose as the major product. Thus, it appears that a β-elimination of the thioglucose moiety occurred along with the *O*-debenzylation.

The results described here are pertinent to solid-phase operation in which the sugar is attached to the phenyl side-chains of polystyrene through sulfur or oxygen and one or two methylene groups as spacer. If there is one methylene group, the resin–sugar linkages would be benzyl glycoside linkages. The *S*-benzyl bond in the thioglucoside **4** was cleaved more rapidly by sodium–liquid ammonia than bonds to the benzyl ether protecting groups. In solid-phase operation this would mean that the protected sugar would be released and diffuse out of the support before deprotection was complete. If, as has been suggested[2], complete deprotection of the sugar in the internal spaces of the resin would result in its being trapped there, preferential cleavage at the anomeric position is highly desirable. Once exposed to the action of sodium–liquid ammonia the anomeric center does not seem, from the present experiments, to suffer any further change.

The *O*-benzyl glucoside **3** behaved exactly as the *S*-glucoside **4**, but gave normal (OH on C-1) products. Here the additional step of sulfur removal was not required. Hence, if cleavage from a support and deprotection in a single step proves feasible, an *O*-benzyl resin–sugar linkage will be preferable to a thioglycoside linkage, provided the *O*-glycoside structure is equally readily constructed in the first place.

Judging from the behavior of compound **7**, a phenylethyl thioglycoside structure in a solid-phase system would also undergo cleavage with sodium–liquid ammonia. Whether this cleavage would be more rapid than *O*-debenzylation was not determined. In any case, a phenylethyl thioglycoside structure would be more difficult to generate on a resin than a benzyl-type structure.

EXPERIMENTAL

General methods. — Thin-layer chromatography was performed on silica gel G (E. Merck) and column chromatography on E. Merck's silica gel, 0.05–0.2 mm (70–325 mesh). For t.l.c. the following solvent systems were used: *A*, 35:14:1 chloroform–methanol–acetic acid; *B*, 99:1 chloroform–ethyl acetate; and *C*, 19:1 chloroform–ethyl acetate. Sephadex LH-20 chromatography was performed according to Holick and DeLuca[3]. Optical rotations were recorded with a Perkin–Elmer Model 141 polarimeter and p.m.r. spectra with a Varian T-60 spectrometer, with internal tetramethylsilane as a reference standard. I.r. spectra were recorded with a Beckman IR-5 spectrometer. The number of benzyl (or phenylethyl) groups in each compound was checked by ultraviolet spectrometry with a Cary-15 spectrophotometer. The value of 186 was taken[4] as the expected molar absorbancy per benzyl group at 258 nm. Elemental analyses were performed by Galbraith Laboratories.

Materials. — 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl chloride (**2**) was prepared from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (**1**) as previously described[1]. Benzyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside (**3**) was made by the procedure of Tate and Bishop[4]. Potassium ethylxanthate was obtained as pale yellow crystals by adding ethanol with stirring to a mixture of carbon disulfide and aqueous potassium hydroxide at 0°. The sodium salt dihydrate of 1-thio-β-D-glucopyranose was obtained by the deesterification of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl ethylxanthate with sodium methoxide[5]. Immediately before use the salt was converted into the free thiol with Amberlite IR-120 (H^+) ion exchange resin.

*Benzyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside* (**4**). — Crude glucosyl chloride **2** (0.57 g, 1.0 mmole) was dissolved in benzene (2.8 ml) and added to a stirred solution of potassium hydroxide (0.48 g, 8.6 mmole) and α-toluenethiol (1.24 g, 10 mmole) in 1-propanol (4.5 ml). The reaction mixture was stirred for 24 h at room temperature. Benzene (5 ml) was added, and the organic layer was washed thrice with 2M potassium hydroxide (5 ml), and then with water, dried (sodium sulfate), filtered, and concentrated under diminished pressure to an oil. The oil was purified on a column of silica gel (2.5 cm diameter, 140 g, 0.6 ml/min). The residual thiol was washed out by benzene, whereupon 99:1 benzene–acetone was used to elute the product. Crystallization from ethanol gave 0.480 g (74%) of **4**; m.p. 75.5–76°, $[\alpha]_D^{25}$ −40.2°, $[\alpha]_{436}^{25}$ −83.5° (*c* 1, chloroform)*; λ_{max}^{EtOH} 258 nm (ε 940); p.m.r. (C_6D_6)

*The c.d. spectra of this compound showed strong concentration dependence, believed to be due to the interaction of the benzyl groups.

τ 5.00 (d, 1, *J* 6.0 Hz, H-1). Found: C, 75.94; H, 6.56; S, 5.09. $C_{41}H_{42}O_5S$ (646.81) requires: C, 76.13; H, 6.55; S, 4.96.

2,3,4,6-Tetra-O-benzyl-β-D-glucopyranosyl ethylxanthate (**5**). — Crude glucosyl chloride **2** (0.98 g, 1.75 mmole) was dissolved in benzene (15 ml). Potassium ethylxanthate (0.280 g, 1.75 mmole) dissolved in 15 ml of abs. ethanol was added, and the reaction mixture was stirred for 5 h at room temperature. (An excess of potassium ethylxanthate would be desirable, to the extent permitted by the limited solubility of the compound in the reaction medium used.) The reaction mixture was partitioned between chloroform and water, and the dried (sodium sulfate) organic layer was concentrated under diminished pressure. The resulting syrup was chromatographed on a column of silica gel (2.7 cm diameter, 173 g, 0.5 ml/min, solvent *B*). Crystallization from ethanol produced 0.712 g (63%) of **5**: m.p. 83°, $[\alpha]_D^{25}$ +27.1°, $[\alpha]_{436}^{25}$ +96.5° (*c* 1.6, chloroform); λ_{max}^{EtOH} 272 nm; p.m.r. ($CDCl_3$) τ 4.65 (d, 1, *J* 9.5 Hz, H-1). Found: C, 68.93; H, 6.25; S, 9.93. $C_{37}H_{40}O_6S_2$ (644.82) requires: C, 68.91; H, 6.25; S, 9.95.

2,3,4,6-Tetra-O-benzyl-1-thio-β-D-glucopyranose (**6**). — A suspension of the ethylxanthate **5** (0.120 g, 0.186 mmole) in 7 ml of dry methanol was stirred at room temperature, and sodium (50 mg) was added in increments until the starting material had gone into solution. Dilute aqueous acetic acid at 0° was then added dropwise until the pH of the solution was 7. Water was added to complete the crystallization. After 1 h at 4°, the white crystals were collected to give 0.094 g (91%) of **6**: m.p. 83.5–84°, $[\alpha]_D^{25}$ +18.1°, $[\alpha]_{436}^{25}$ +38.9° (*c* 0.75, chloroform); λ_{max}^{EtOH} 258 nm (ε 750); i.r. (KBr) 2550 cm^{-1} (S-H); p.m.r. (C_6D_6) τ 4.85–5.82 (m, 9, H-1, PhCH_2), and 7.86 (d, 1, *J* 8.0 Hz, S*H*). Found: C, 73.20; H, 6.33; S, 5.76. $C_{34}H_{36}O_5S$ (556.69) requires: C, 73.35; H, 6.52; S, 5.76.

2-Phenylethyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (**7**). — Crude glucosyl chloride **2** (0.95 g, 1.70 mmole) was treated with 2-phenylethanethiol (prepared by acid hydrolysis of its Bunte salt[6]) and potassium hydroxide according to the procedure described for the benzyl thioglucoside **4**. The product was purified on a column of silica gel (2.7 cm diameter, 183 g, 0.6 ml/min, 99:1 benzene–acetone). Crystallization from ethanol gave 0.902 g (80%) of **6**: m.p. 81.5–83°, $[\alpha]_D^{25}$ −2.3°, $[\alpha]_{436}^{25}$ −1.3° (*c* 0.66, $CHCl_3$)*; λ_{max}^{EtOH} 258 nm (ε 960); p.m.r. τ 4.95 (d, 1, *J* 6.0 Hz, H-1). Found: C, 76.43; H, 6.72; S, 4.72. $C_{42}H_{44}O_5S$ (660.84) requires: C, 76.34; H, 6.71; S, 4.85.

Debenzylation. — The benzyl compound (10–100 mg) in 0.1–1 ml of abs. tetrahydrofuran was placed in a flask equipped with a cold-finger type of condenser filled with a Dry Ice–ethanol slurry. Ammonia gas dried by passage over potassium hydroxide pellets was admitted and condensed to a volume of 0.3–3 ml. The reaction mixture was stirred with a glass-coated magnet, and freshly cut sodium was added in small increments. For partial debenzylations 2–7 g-atoms per mole of benzylated

*The c.d. spectra of this compound showed strong concentration dependence, believed to be due to the interaction of the benzyl groups.

sugar were used; for complete debenzylation the addition of sodium was continued until an indigo color persisted for 0.5 h. The ammonia was evaporated, methanol (10 μl per mg of sodium used) was added, and the solution was evaporated to dryness. The residue was taken up in 0.1–1 ml of water. The solution was neutralized with solid carbon dioxide to pH 9 and then extracted thrice with 0.1–1 ml of benzene. The combined aqueous layers were deionized by treatment with Dowex 50-X8 resin in the pyridine form.

The aqueous fraction from the complete debenzylation of benzyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**4**) showed one spot on t.l.c. (system *A*) with the R_F value of 1-thio-D-glucose. Concentration of the solution *in vacuo* gave the amorphous product in 94% yield. Its p.m.r. spectrum was identical with that of authentic 1-thio-D-glucose. After treatment of **4** with 2 g-atoms of sodium per mole, t.l.c. (system *C*) of the benzene layer showed spots having the R_F values of the starting material and 2,3,4,6-tetra-*O*-benzyl-1-thio-D-glucose (**6**). Positive identification of **6** was made by i.r. analysis (S-H, 2550 cm^{-1}), after purification of the reaction mixture from 10 mg of **4** by Sephadex LH-20 chromatography (1.1 cm diameter, 6 g, 0.5 ml/min, 1 ml fractions, 1:1 Skellysolve B-chloroform). The unreacted starting material was eluted first, followed by pure **6**. When 6–7 g-atoms of sodium per mole were used, the t.l.c. showed diminution of the spot for **4** and the presence of two slower-moving products in addition to **6**.

The aqueous fractions from the complete debenzylation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (**1**) and benzyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside (**3**) showed one spot on t.l.c. (system *A*) having the R_F value of D-glucose. Concentration of the solutions gave products in yields of 97 and 93%, respectively, that had p.m.r. spectra corresponding to that of D-glucose. T.l.c. (system *C*) of the benzene layer from the treatment of **3** with 2 g-atoms of sodium per mole showed one major spot, having the mobility of **1**. When the product from 8 mg of **3** was purified by chromatography on Sephadex LH-20 as already described, 5 mg of pure **1** crystallized spontaneously upon evaporation of the solvent. The m.p. was 151–152° (lit.[7] 151–152°), undepressed on admixture with an authentic sample. When 6–7 g-atoms of sodium per mole of **3** were used, two additional, slower-moving spots appeared on the t.l.c. plate.

T.l.c. analysis (system *A*) of the aqueous fraction from the complete debenzylation of 2-phenylethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**7**) indicated a substance having the R_F value of 1-thio-D-glucose. After concentration of the solution, the p.m.r. spectrum of the residue was identical with that of 1-thio-D-glucose.

Hydrolyses with mercuric chloride. — The sulfur compound was dissolved in a solution of mercuric chloride (3 moles per mole of thiosugar) in water or in 1:2 water–tetrahydrofuran. Barium carbonate was added to maintain neutrality. The reaction mixture was stirred at room temperature, the reaction being monitored by t.l.c. (system *A* or *C*). In all cases 5–10% of side products were detected. On completion of the reaction (18 h for 1-thio-D-glucose and compound **6**, 3 days for **4**) the

mixture was filtered through sintered glass and the residue washed thoroughly with water. The *O*-benzylated products from **4** and **6** were extracted from the filtrate with chloroform and concentrated *in vacuo* to yield crystalline tetra-*O*-benzyl-α-D-glucopyranose (**1**) (80%), m.p. 150–152° from **4** and 149.5–152° from **6**. In the case of 1-thio-D-glucose, the aqueous filtrate was concentrated *in vacuo* and deionized with well-washed Amberlite MB-3 (pyridinium, hydrogen carbonate form[1]), and then evaporated to dryness. This gave D-glucose (82%), as shown by t.l.c. (system *A*) and p.m.r.

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