Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF *O*-β-D-GALACTOPYRANOSYL-(1→3)-*O*-β-D-GALACTOPYRANOSYL-(1→4)-D-GLUCOSE*

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(Received February 20th, 1967)

INTRODUCTION

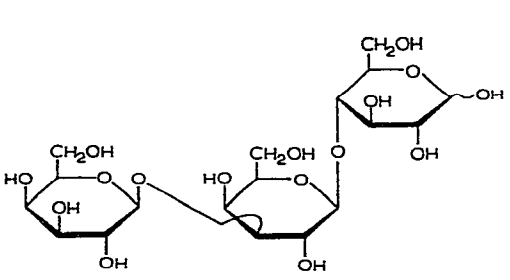
During the course of a study of the chemistry of complex glycosphingolipids containing the sequence 4-*O*-β-D-galactopyranosyl-D-glucose[1], we have investigated the possibility of synthesizing oligosaccharides involving the glycosidation of lactose at positions 3 or 4 of the galactose moiety. Very little work has been published on the selective substitution of lactose. An isopropylidene acetal has been described[2], but its suggested structure was later refuted[3]. An attempt, in our laboratory, to prepare trityl ethers of benzyl β-lactoside was unsuccessful. However, conditions were found for the preparation of an isopropylidene derivative of unambiguous structure which was readily converted into a lactoside with free hydroxyl groups at C-3 and C-4 of the galactopyranose ring. The synthesis of benzyl 2,6-di-*O*-acetyl-β-D-galactopyranoside has been recently described[4]. Treatment of this compound with acylglycosylhalides led to the formation of benzyl 3-*O*-glycosyl-β-D-galactopyranosides[5]. It was therefore expected that an analogous derivative of lactose would react similarly and provide a means of synthesizing *O*-glycosyl-(1→3)-*O*-galactopyranosyl-(1→4)-D-glucose derivatives. The synthesis of the trisaccharide *O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose (**1**), described in this paper, illustrates the validity of this approach, and suggests a route to the preparation of other trisaccharides of biological interest having similar structures.

DISCUSSION

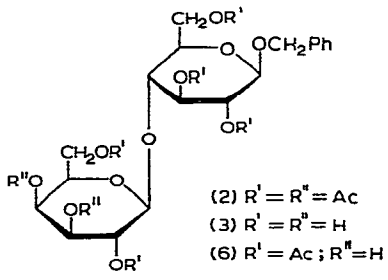
Catalytic deacetylation of benzyl hepta-*O*-acetyl-β-lactoside[6] (**2**) afforded benzyl β-lactoside (**3**) which was treated with acetone in the presence of acid catalysts to give crystalline benzyl 4-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**). The use of anhydrous cupric sulfate as catalyst[7] failed to catalyze any reaction, whereas sulfuric acid[8] led to a moderate yield (about 40%). A much higher yield (76%) was obtained, however, with *p*-toluenesulfonic acid[8]. Excessive

*This paper represents part of a thesis submitted by D. Beith-Halahmi in partial fulfilment of the requirements for the degree of Doctor of Philosophy from The Weizmann Institute of Science, Rehovoth, Israel, 1967.

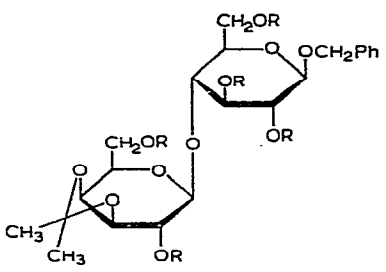
prolongation of the time of the reaction and a large excess of *p*-toluenesulfonic acid led to the cleavage of the disaccharide and formation of considerable amounts of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose and benzyl β-D-glucopyranoside. The acetal (**4**) consumed 1 mole of periodate per mole at 20°, in agreement with the



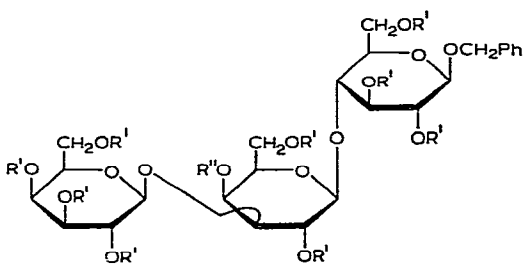
(1)



(2) R' = R'' = Ac
(3) R' = R'' = H
(6) R' = Ac ; R'' = H



(4) R = H
(5) R = Ac



(8) R' = Ac ; R'' = H
(9) R' = R'' = Ac
(10) R' = R'' = H

structure postulated. Acetylation of **4** gave the pentaacetate **5**, which was converted into benzyl 4-*O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**6**) by treatment with hot aqueous acetic acid. Compounds **5** and **6** did not crystallize, but were purified by chromatography on silica gel, and gave satisfactory elementary analyses and the expected n.m.r. spectra. The periodate consumption of **6** (1 mole per mole of **6** at 20°) was in agreement with the structure assigned.

Treatment of **6** with tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**7**) in the presence of mercuric cyanide in nitromethane–benzene solution[7], followed by chromatography on silica gel G, afforded an amorphous product which was formulated as benzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-*O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**8**). This assignment was based on the expected greater reactivity at C-3, rather than at C-4 of the galactopyranoside ring, as shown previously in our laboratory[5]. Acetylation of **8** gave the decaacetate **9**, and catalytic deacetylation of **8** and **9** afforded benzyl *O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**10**), which was purified by chromatography on Sephadex G-15. Periodate oxidation con-

firmed the structure of **10**, and hence, that of **8** and **9**. Catalytic hydrogenolysis of **10** gave the desired trisaccharide (**1**) as an amorphous, hygroscopic solid. The overall yield of **1** from benzyl β-lactoside (**3**) was 19%. Acetylation of **1** with hot acetic anhydride–pyridine gave a crystalline undecaacetate.

EXPERIMENTAL

Melting points were taken between glass slides on a Fisher–Johns apparatus and are corrected. Optical rotations were determined in semimicro tubes with a Perkin–Elmer No. 141 polarimeter. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer, with tetramethylsilane as the internal standard and chloroform-*d* as solvent. "Silica gel" refers to silica gel Davison, grade 950, 60–200 mesh, used without pretreatment; the flowing method was used and elution was stepwise, in order of increasing polarity of the eluants. The proportion of weight of substance to weight of adsorbent added to the column was 1 to 50–100. The volume of the fractions eluted was 2 ml per g of silica gel. Thin-layer chromatograms (and column separations where specified in the text) were performed on silica gel G (Kieselgel G, E. Merck, Darmstadt). Analyses were made in the Institute's Microanalytical Laboratory under the direction of Mr. R. Heller. Periodate oxidations were performed with an adaptation of the spectrophotometric method[9]. A sample of the substance to be examined (about 5 mg) was dissolved in 0.01M sodium metaperiodate solution (10 ml). This solution and the standard solutions were kept in the dark at 20°. Aliquots from both solutions (0.5 ml) were withdrawn and diluted to 50 ml. The relative absorbances of the two solutions were determined at 223 mμ with a Perkin–Elmer 137 u.v. spectrophotometer. The absorbance was recorded as the depth of the peak below the reading at 300 mμ which was taken as base line. The difference in relative absorbances at 223 mμ of the two solutions gave a measure of the periodate consumption. A correction was applied for the absorbance of sodium iodate measured under these conditions (about 13% of that of an equimolar sodium metaperiodate solution).

Benzyl 4-O-β-D-galactopyranosyl-β-D-glucopyranoside (**3**). — A solution of benzyl hepta-*O*-acetyl-β-lactoside[6] (**2**, 9.0 g) in methanol (10 ml) containing a catalytic amount of sodium methoxide, was kept overnight at room temperature, and was then neutralized with a few drops of glacial acetic acid. The solution was evaporated *in vacuo* and the residue dissolved in water. The aqueous solution was passed through a column of Dowex-50 (H^+) and evaporated *in vacuo*. The dry residue crystallized from hot absolute ethanol as needles (4.5 g, 84%), m.p. 180°; it was homogeneous on t.l.c. in 4:5:1 butyl alcohol–acetone–water, $R_{lactose}$ 2.6; $[\alpha]_D^{25}$ −18.1° (*c* 1.32, water).

Anal. Calc. for $C_{19}H_{28}O_{11}\cdot H_2O$: C, 50.7; H, 6.67. Found: C, 50.6; H, 6.85. Loss in weight at 100° in high vacuum over P_2O_5, 4%, corresponding to 1 molecule of water.

Benzyl 4-O-(3,4-O-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**). — (*a*) A suspension of **3** (200 mg, dried over P_2O_5 in high vacuum overnight at 100°) in anhydrous acetone (60 ml) containing sulfuric acid (0.4 ml) was stirred for 5 h at room temperature. The resulting clear solution was neutralized with anhydrous sodium

carbonate and filtered. The filtrate was evaporated *in vacuo*, and the residue was crystallized from acetone–methanol; yield 82 mg (37%); m.p. 196–197°.

(*b*) A stirred mixture of **3** (3.0 g), anhydrous *p*-toluenesulfonic acid (0.5 g), and pure, anhydrous acetone (1500 ml) was boiled under reflux for 3 h. After being cooled, the colorless solution was neutralized with anhydrous sodium carbonate and filtered. The clear solution was evaporated *in vacuo*, and the product was crystallized from acetone–methanol; yield 2.5 g (76%), m.p. 196–197°, $[\alpha]_D^{25}$ −1.0° (*c* 0.73, pyridine). N.m.r.: τ 2.70 (5H, C_6H_5), τ 8.44 and 8.62 [6H, $C(Me)_2$]. T.l.c. in 65:25:4 chloroform–methanol–water: $R_{lactose}$ 2.75, identical with that of the product described under (*a*). Periodate oxidation showed the consumption of 0.98 mole of sodium metaperiodate per mole of compound **4** during 48 h at 20°; no further consumption during an additional 10 h was observed

Anal. Calc. for $C_{22}H_{32}O_{11}$: C, 55.9; H, 6.78. Found: C, 55.9; H, 6.70.

Benzyl 4-O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**5**). — A solution of **4** (2.0 g) in pyridine (10 ml) and acetic anhydride (15 ml) was kept overnight at room temperature. The solution was concentrated *in vacuo* to a syrup from which traces of pyridine and acetic anhydride were removed by co-evaporation *in vacuo* with toluene. The syrupy residue was dissolved in benzene, and the solution was chromatographed on silica gel. Benzene–ether (1:1, v/v) eluted fractions (2.2 g, 84%) which were homogeneous on t.l.c. in 14:1 benzene–methanol, $R_{Compound\ 2}$ 0.94; $[\alpha]_D^{25}$ −10.7° (*c* 0.61, chloroform). N.m.r.: τ 2.70 (5 H, C_6H_5), τ 7.88–8.02 (15H, 5 OAc groups), and τ 8.46 and 8.66 [6H, C $(Me)_2$].

Anal. Calc. for $C_{32}H_{42}O_{16}$: C, 63.8; H, 6.99. Found: C, 63.8; H, 6.95.

Treatment of compound **3** *with acetone in the presence of a large excess of* p-*toluenesulfonic acid.* — A stirred mixture of **3** (5.0 g), *p*-toluenesulfonic acid (10 g), and acetone (1500 ml) was boiled for 3 h under reflux. The cooled solution was processed as previously described, and the residue was acetylated with acetic anhydride–pyridine. The product was chromatographed on silica gel. Benzene–ether (12:1) eluted benzyl tetra-*O*-acetyl-β-D-glucopyranoside (2.45 g) which crystallized from ether–hexane; m.p. 101–102° (reported[10] m.p. 101–104°).

Anal. Calc. for $C_{21}H_{26}O_{10}$: C, 57.5; H, 5.90. Found: C, 57.9; H, 5.90.

Benzene–ether (9:1) eluted 6-*O*-acetyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (3.27 g) identical with an authentic specimen[11]; no acetal (**4**) could be isolated from the reaction mixture.

Benzyl 4-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**6**). — A solution of **5** (5.0 g) in 80% acetic acid (50 ml) was kept for 3 h at 100°. The acetic acid was removed by codistillation with water, and the residue was dried by addition of toluene and evaporation *in vacuo*. The dry residue was dissolved in dichloromethane and chromatographed on silica gel. Dichloromethane–ether (1:1) eluted fractions (4.0 g, 85%) which were homogeneous on t.l.c. in 4:1 benzene–methanol, $R_{Compound\ 2}$ 0.51. N.m.r.: τ 2.7 (5H, C_6H_5), τ 7.80–7.99 (15H, 5 OAc groups); the signals at τ 8.46 and 8.66 (CMe_2) present in **5** were absent in **6**; $[\alpha]_D^{25}$ −23.2° (*c* 0.60, chloroform). Periodate oxidation showed the consumption of 0.98 mole of

sodium metaperiodate per mole of **6** during 24 h at 20°; no further consumption was observed on prolongation of the reaction for an additional 30 h.

Anal. Calc. for $C_{29}H_{38}O_{16}$: C, 62.0; H, 6.76. Found: C, 61.8; H, 6.79.

*Benzyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→3)-*O*-(2,6-di-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**8**). — A stirred solution of **6** (300 mg, 0.468 mmole) in 1:1 nitromethane–benzene (50 ml) was boiled until approx. 20 ml of the solvent mixture had distilled to ensure complete dehydration, and was then cooled to 60°. Mercuric cyanide (110 mg, 0.468 mmole) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**7**, 0.266 mg, 0.468 mmole) were added, and the reaction was continued for 24 h at 60–70°, additional portions of mercuric cyanide (0.234 mmole) and of the bromide (**7**, 0.234 mmole) being added after 18 h. The mixture was cooled, diluted with benzene (20 ml), washed successively with a cold solution of sodium hydrogen carbonate (5%) and water, dried with sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in dichloromethane and chromatographed on silica gel G. Dichloromethane–ether (1:1) eluted fractions which were homogeneous on t.l.c. in 4:1 benzene-methanol; $R_{Compound\ 6}$ 1.25; yield 260 mg (50%), $[\alpha]_D^{25}$ −5.2° (*c* 0.81, chloroform). N.m.r.: τ 2.70 (5H, C_6H_5), τ 7.82–8.01 (27H, 9 OAc groups).

*Benzyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**9**). — Syrup **8** (240 mg) was acetylated with pyridine–acetic anhydride at room temperature. The product (240 mg, 99%) did not crystallize, but was homogeneous on t.l.c. in 4:1 benzene–methanol, $R_{Compound\ 8}$ 1.15; $[\alpha]_D^{20}$ +3.6°(*c* 0.99, chloroform). N.m.r.: τ 2.70 (5H, C_6H_5), τ 7.85–8.02 (3 OH, 10 OAc groups).

Anal. Calc. for $C_{45}H_{58}O_{26}$: C, 53.2; H, 5.71. Found: C, 52.9; H, 6.05.

Benzyl O-β-D-*galactopyranosyl-(1→3)-*O*-β-D-*galactopyranosyl-(1→4)-β-D-glucopyranoside* (**10**). — A solution of **9** (200 mg) in methanol (10 ml) containing a catalytic amount of sodium methoxide was kept overnight at room temperature, and was then neutralized with a few drops of glacial acetic acid. The neutralized solution was concentrated *in vacuo*, and the residue dissolved in water. The aqueous solution was chromatographed on Sephadex G-15. Fractions (1 ml) were eluted with water. The fractions containing saccharides were identified by spot-tests on a microslide coated with silica gel G with anisaldehyde–sulfuric acid as detecting reagent[12]. The fractions which were homogeneous on t.l.c. in 4:5:1 butanol–acetone–water (R_F 0.69) were combined and conc. *in vacuo* to a syrup. Abs. ethanol precipitated a hygroscopic powder (90 mg, 77%); $[\alpha]_D^{20}$ +17.9° (*c* 0.95, water). On periodate oxidation, 3 moles of sodium metaperiodate were consumed per mole of **10** during 60 h at 20°.

Anal. Calc. for $C_{25}H_{38}O_{16} \cdot H_2O$: C, 49.0; H, 6.22. Found: C, 48.6; H, 6.04.

O-β-D-*Galactopyranosyl-(1→3)-*O*-β-D-*galactopyranosyl-(1→4)-D-glucose* (**1**). — A solution of **10** (120 mg) in 80% aqueous ethanol (50 ml) was hydrogenolyzed in presence of palladium–charcoal (10%) for 20 h at 50 lb.in^{-2}. The catalyst was removed by filtration, and the solvent was evaporated *in vacuo*. The resulting syrup was dissolved in ethanol, and the solution was filtered to remove residual traces of charcoal,

and concentrated *in vacuo*. Addition of a few drops of ethanol gave 91 mg (92% yield) of a hygroscopic, white precipitate which was homogeneous on t.l.c. in 4:5:1 butanol–acetone–water, $R_{Compound\ 10}$ 0.54; $[\alpha]_D^{25}$ +26.1° (*c* 0.69, water).

Anal. Calc. for $C_{18}H_{32}O_{16} \cdot H_2O$: C, 41.4; H, 6.56. Found: C, 41.8; H, 6.50.

A solution of **1** (1.1 g) in pyridine (20 ml) and acetic anhydride (20 ml) was kept for 1 h at 100°. After removal of the acetic anhydride and pyridine *in vacuo*, the residue was crystallized from aqueous ethanol to yield 1.5 g (81%) of an undecaacetate, m.p. 108–110°, $[\alpha]_D^{15}$ +17.2° (*c* 0.61, chloroform). The product was homogeneous on t.l.c. in 4:1 benzene–methanol ($R_{Compound\ 2}$ 0.3).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.7; H, 5.63. Found: C, 49.3; H, 5.56.

SUMMARY

O-β-D-Galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose was obtained by selective glycosidation of benzyl 4-*O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside with tetra-*O*-acetyl-α-D-galactopyranosyl bromide.

The disaccharide was prepared from benzyl 4-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside, which was obtained in good yield by treatment of benzyl β-lactoside with acetone and *p*-toluenesulfonic acid.

REFERENCES

1 L. SVENNERHOLM, *J. Lipid Res.*, 5 (1964) 145.
2 J. STANĚK AND J. ŠÁDA, *Collection Czech. Chem. Commun.*, 13 (1948) 540.
3 Z. EL HEWEIHI, *Ber.*, 86 (1953) 862.
4 A. LEVY, H. M. FLOWERS AND N. SHARON, *Carbohyd. Res.*, 4 (1967) 305.
5 H. M. FLOWERS, *Carbohyd. Res.*, 4 (1967) 312.
6 N. K. RICHTMYER, *J. Am. Chem. Soc.*, 68 (1946) 1136.
7 A. N. DE BELDER, *Advan. Carbohydrate Chem.*, 20 (1965) 219.
8 A. HAMPTON, *J. Am. Chem. Soc.*, 83 (1961) 3640.
9 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind.* (London), (1957) 1216.
10 W. J. HICKINBOTTOM, *J. Chem. Soc.*, (1928) 3140.
11 H. OHLE AND G. BEREND, *Ber.*, 58 (1925) 2585.
12 E. STAHL AND U. KALTENBACH, *J. Chromatog.*, 5 (1961) 351.