STUDIES ON THE KOENIGS–KNORR REACTION

PART I. SYNTHESIS OF 6-*O*-α-D-GLUCOPYRANOSYL-D-GALACTOSE AND 3-*O*-α-D-GLUCOPYRANOSYL-D-GALACTOSE

H. M. FLOWERS
Department of Biophysics, Weizmann Institute of Science, Rehovoth (Israel)
(Received November 10th, 1970; accepted for publication February 5th, 1971)

ABSTRACT

Two α-D-linked disaccharides, 6-*O*-α-D-glucopyranosyl-D-galactose and 3-*O*-α-D-glucopyranosyl-D-galactose were synthesized by reaction of 2-*O*-benzyl-3,4,6-tri-*O*-*p*-nitrobenzoyl-α(or β)-D-glucopyranosyl bromide with 1,2:3,4-di-*O*-isopropylidene-α-D-galactose and 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-α-D-galactose, respectively, and subsequent removal of the protecting groups. No β-D-linked disaccharide was detected in the reaction mixture, irrespective of the anomeric configuration of the sugar halide used.

INTRODUCTION

The Koenigs–Knorr reaction of substituted glycosyl halides with hydroxyl functions in the presence of an "acid acceptor" gives glycosides or oligosaccharides[1–4]. Generally, "*trans*" glycosides are formed, *i.e.* compounds in which the incoming nucleophile is stereospecifically directed *trans* to the acyl group at C-2, thus giving a ready means of preparing, for example, β-D-gluco- or galacto-pyranosides and α-D-mannopyranosides. Occasionally, "*cis*"-anomers have been isolated, *e.g.* α-D-glucopyranosides[5], α-D-galactopyranosides[6], and α-L-fucopyranosides[7,8].

Use of the reaction for the preparation of *cis*-anomers, many of which are of great interest biologically, has however met with considerable difficulty. The configuration of the product may be determined by a number of factors, including the structure of the reacting halide, the ionic strength of the solvent, and the nature of the acid acceptor.

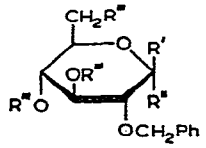
Recently, crystalline D-glucopyranosyl bromides having a non-participating group at C-2 and both α and β configurations at C-1 have been described[9]. We have prepared disaccharides from both the α and β anomers of one of these bromides in an attempt to establish both the effect of replacing a participating acyl group by a benzyl ether and of the anomeric configuration of the reacting bromide on the structure of the product. Two α-D-glucopyranosyl disaccharides of D-galactose have been prepared, one using a condensation with a relatively reactive, primary hydroxyl group, and the other with a more hindered, secondary hydroxyl group. The products were optically pure, and their anomeric configuration was independent of that of the halide employed.

RESULTS AND DISCUSSION

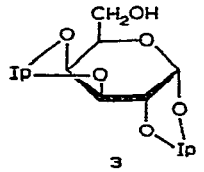
The disaccharide 6-*O*-α-D-glucopyranosyl-D-galactose (**7**) has been prepared by partial acid hydrolysis of polysaccharides obtained from *Salmonella spp.*[10,11]. It was synthesized[12] from the β-D-anomer which had been obtained chemically by means of the Koenigs–Knorr reaction. The peracetate was anomerized and the equilibrium mixture deacetylated. The contaminating β-anomer was removed by selective, enzymic hydrolysis to leave the α-D-linked disaccharide, which was isolated as a strongly dextrorotatory, amorphous solid.

The lipopolysaccharide of many Gram-negative bacteria contains the 3-*O*-α-D-glucopyranosyl-D-galactose (**12**) moiety[10,11]. Although its β-D-anomer has been synthesized, there is little information available on the properties of the α-D-linked compound.

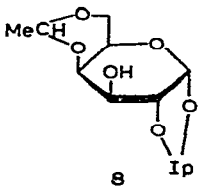
The bromides **1** and **2**, used in the condensation reaction, were prepared from 2-*O*-benzyl-α-D-glucose and purified by recrystallization[9]. Reaction of the α-D halide **1** with either aglycon, 1,2:3,4-di-*O*-isopropylidene-α-D-galactose (**3**) or 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-α-D-galactose (**8**), was more sluggish than was that of the β-D halide **2**. A similar difference in the rates of methanolysis of these bromides has been reported[9].



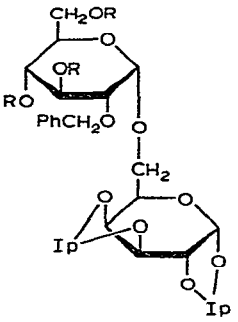
1 R′ = H; R″ = Br; R‴ = $OCC_6H_4NO_2(p)$
2 R′ = Br; R″ = H; R‴ = $OCC_6H_4NO_2(p)$



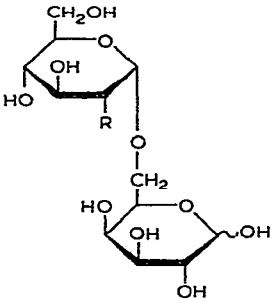
3



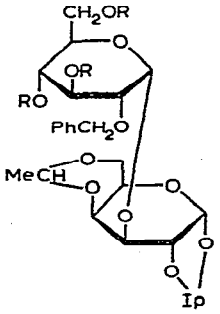
8



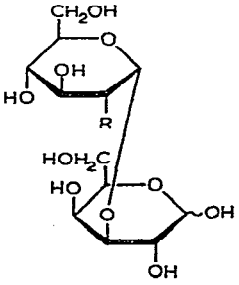
4 R = $OCC_6H_4NO_2(p)$
5 R = H



6 R = OCH_2Ph
7 R = OH



9 R = $COC_6H_4NO_2(p)$
10 R = H



11 R = OCH_2Ph
12 R = OH

After reaction of compound **3** with the β-D bromide **2** in nitromethane–benzene solution in the presence of mercuric cyanide, a good yield of substituted disaccharide **4** was isolated, after chromatography on silica gel, as a syrup which could not be crystallized. The product was shown to be homogeneous by t.l.c. examination, and it was the only disaccharide fraction present in the reaction mixture; its n.m.r. spectrum showed the expected ratio of substituent groups: one benzyl ether, three *p*-nitrobenzoate, and two isopropylidene groups.

Catalytic deacylation of **4** afforded impure 6-*O*-(2-*O*-benzyl-α-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**5**) as an amorphous solid in almost quantitative yield. The optical rotation of **5** and its n.m.r. spectrum indicated the presence of a considerable proportion of α-linked disaccharide. Selective hydrolysis by mild acid treatment gave impure 6-*O*-(2-*O*-benzyl-α-D-glucopyranosyl)-D-galactose (**6**) in reasonable yield. The impurities in the hydrolysis mixture were identified as traces of 2-*O*-benzyl-D-glucose and D-galactose, and small amounts of disaccharide still containing one or two acetal groups.

Catalytic hydrogenolysis gave a product which was homogeneous on t.l.c. and paper chromatography. It could not be separated from the β-D anomer, prepared by reaction of **3** with tetra-*O*-acetyl-α-D-glucopyranosyl bromide by a method similar to that described by Goldstein and Whelan[12]. The optical rotation, after crystallization, was in agreement with that reported for the pure α-D disaccharide and very different from that of its β-anomer[12]. In order to distinguish between the two anomers, they were converted into the per(trimethylsilyl) ether of the corresponding alcohol. Crude **7** gave a single product which had a different retention-time on g.l.c. from that obtained from 6-*O*-β-D-glucopyranosyl-D-galactose. The disaccharide **7** had been prepared and isolated by a procedure that did not involve the removal of secondary products, and the final product was, before crystallization, optically pure and much more dextrorotatory than the β anomer. The Koenigs–Knorr reaction employed had thus been stereospecific and produced only a compound in which the nucleophile had attacked the original glycosyl halide *cis* to the substituent at C-2.

The 3-*O*-substituted D-galactose disaccharide **12** was obtained in lower yield from 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-α-D-galactopyranose (**8**). The protected disaccharide **9** was isolated as a syrup after chromatographic separation from the mixture resulting from the reaction of **2** with **8**. Catalytic deacylation removed the *p*-nitrobenzoate group to give **10** in 25% overall yield from **8**. Attempts to remove the acetal groups from **10** by treatment with hot, aqueous acetic acid resulted in partial acetylation: the 6-hydroxyl group in glucose and galactose has been shown to be acetylated with great facility[13]. However, successful selective hydrolysis of the acetal groups was achieved by use of sulfuric acid in aqueous *p*-dioxane.

The resulting 3-*O*-(2-*O*-benzyl-α-D-glucopyranosyl)-D-galactose (**11**) was isolated as an amorphous solid showing a high-positive optical rotation. Hydrogenolysis afforded an amorphous product (**12**) which was compared with an authentic specimen of 3-*O*-β-D-glucopyranosyl-D-galactose[14]. Their paper and thin-layer chromatographic mobilities were identical, but their optical rotations were markedly different,

compound **12** being much more dextrorotatory. The two disaccharides were separately reduced with sodium borohydride in buffered solutions to prevent excess alkalinity, and converted into the per(trimethylsilyl) ethers. Clear separation was obtained on g.l.c., and the presence of a single peak in the product from **12**, uncontaminated with material obtained from the β-isomer, showed its optical purity. A comparison of the optical rotations shows that **12** was, in fact, the α- anomer.

Repetition of the series of reactions just described, using the α-D halide **1** in place of the β-D halide **2** with compounds **3** and **8**, afforded disaccharides in somewhat lower yields, after longer reaction times. The products were indistinguishable from **7** and **12**, respectively, in thin-layer and paper chromatographic properties, optical rotations, and retention times on g.l.c. of the trimethylsilyl ethers of the corresponding sugar alcohols. They contained no measurable proportion of β-D linked isomers.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns apparatus and were corrected. Rotations were determined in semimicro tubes, using a Perkin–Elmer No. 141 polarimeter. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer and, if not otherwise specified, using chloroform-d_6 as solvent and tetramethylsilane as internal standard. "Silica gel" refers to silica gel Merck, 0.05–0.2 mm, 70–325 mesh, used without pretreatment. Thin-layer chromatograms were prepared on silica gel G (E. Merck, Darmstadt), coated onto microslides by dipping into a slurry of the silica in chloroform and leaving the solvent to evaporate at room temperature. The solvents used for chromatography included: (A) 8:2:1 ethyl acetate–pyridine–water, (B) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water, and (C) 7:1:2 propyl alcohol–ethyl acetate–water. R_G refers to the rate of migration of a spot relative to D-glucose. Gas-liquid chromatography (g.l.c.) was carried out with a Varian Aerograph 1200 instrument using a copper column (1.2 m × 4 mm) packed with 3% SE-30 on Gas Chrom P at 240°, with helium as the carrier gas and a flame-ionization detector. T_S refers to the retention time of a compound relative to that of the per(trimethylsilyl) derivative of sucrose. Per(trimethylsilyl) derivatives were prepared by adding a vial of Sil-Prep (Applied Science Lab., Inc., Pa.) to *ca.* 5 mg of disaccharide alcohol or sucrose and keeping the stoppered mixture for at least 2 h prior to injection into the chromatograph. I.r. spectra were examined on a Perkin–Elmer No. 137 i.r. spectrophotometer in chloroform solution. Analyses were performed in the Institute's Microanalytical Laboratory under the direction of Mr. R. Heller.

6-O-(2-O-Benzyl-α-D-glucopyranosyl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**5**). — A stirred solution of 1,2:3,4-di-*O*-isopropylidene-α-D-galactose[15] (**3**, 0.53 g, 2.4 mmoles) in 1:1 nitromethane–benzene (70 ml) was evaporated until approximately 20 ml of the solvent mixture had distilled, and then it was cooled to 40°. Mercuric cyanide (0.61 g, 2.4 mmoles) and 2-*O*-benzyl-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-glucopyranosyl bromide[9] (**2**, 1.9 g, 2.4 mmoles) were added, and the stirred mixture was kept for 18 h at 40° with exclusion of moisture. The course of the reaction was monitored by t.l.c. in 4:1 benzene–ether. The spot corresponding to **3** had

declined considerably while that of **2** was replaced with a new, slower-migrating material. The solution was cooled, diluted with benzene, washed successively with sodium hydrogen carbonate solution and water, dried (sodium sulfate), and concentrated *in vacuo*. The residue (2.4 g) was dissolved in benzene and chromatographed on a column of silica gel. Benzene–ether (9:1) eluted fractions which were homogeneous on t.l.c. Evaporation of the solvent *in vacuo* left a syrup (**4**, 1.4 g, 63%); i.r. data: $\nu_{max}^{CHCl_3}$ 1720, 1740 (ester), 1610 (aromatic), 1360 cm^{-1} (*C*-methyl); n.m.r. data: τ 1.75 and 1.85 (*p*-nitrobenzoate-12H), 2.58–2.72 (Ph-5H), 8.5–8.7 (C-Me 12H).

Ether–ethyl acetate (1:1) eluted a homogeneous syrup (0.16 g, 30%) that was indistinguishable from **3** on t.l.c. and gave an identical n.m.r. spectrum. There was no indication of the presence of any other material containing both aromatic and isopropylidene groups.

A solution of a portion of the combined disaccharide fractions (1.3 g) was prepared in 1:1 chloroform–methanol (50 ml) and a solution of M sodium methanolate in methanol (5 ml) was added. The reaction was monitored by t.l.c., and was complete after 1 h, a single spot being obtained on charring the plate with sulfuric acid. The solution was acidified with a drop of acetic acid and concentrated *in vacuo*. A solution of the residue in chloroform was washed several times with water and concentrated *in vacuo*. The residue (1.3 g), containing methyl *p*-nitrobenzoate, was dissolved in ethyl acetate, and the solution passed through a column of silica gel. Ethyl acetate eluted methyl *p*-nitrobenzoate and 9:1 ethyl acetate–acetone homogeneous fractions that gave a syrup on removal of the solvent (0.65 g, 97%), $[\alpha]_D^{25}$ +29.2° (*c* 0.515, chloroform); n.m.r. data: τ 2.68 (Ph, 5H), 4.5 (doublet, *J* 5 Hz, 1H: attributed to H-1 of di-*O*-isopropylidene-α-D-galactopyranose moiety), 5.15 (doublet, *J* 3 Hz, 1H), 8.5–8.7 (C–Me, 12H).

Anal. Calc. for $C_{22}H_{32}O_{11}$; C, 55.92; H, 6.82. Found: C, 56.20; H, 6.87.

6-O-(2-O-Benzyl-α-D-glucopyranosyl)-D-galactose (**6**). — A solution of **5** (500 mg) in aqueous acetic acid (50 ml, 60%) was kept for 90 min at 95–100°, the reaction being monitored by t.l.c. The solution was concentrated to a small volume *in vacuo*, diluted with water, and extracted with ether. The ether solution contained a compound similar to unchanged **5** and a compound showing a slightly slower-migrating spot (t.l.c. in 1:1 benzene–methanol), whereas the aqueous solution contained an almost completely homogeneous material (R_5 0.4). Concentration of the aqueous solution *in vacuo* gave a syrup (320 mg) which was dissolved in 6:6:1 chloroform–methanol–water and purified on a column of silica gel. A total of 290 mg (65%) of an amorphous solid was eluted with the same solvent mixture: $[\alpha]_D^{25}$ +68° (*c* 0.96, water).

Anal. Calc. for $C_{19}H_{28}O_{11}$: C, 52.77; H, 6.53. Found: C, 52.90; H, 6.75.

6-O-α-D-Glucopyranosyl-D-galactose (**7**). — (*a*). A solution of **6** (250 mg) was prepared in 90% ethanol (100 ml), and 10% palladium-on-charcoal (50 mg) was added. The suspension was shaken with hydrogen at 3 atm for 24 h at room temperature, the catalyst was removed by filtration, and the solution was concentrated *in vacuo* to an amorphous solid (150 mg, 76%); it was shown to be homogeneous by

t.l.c. in 4:5:1 butyl alcohol–ethanol–water and 3:3:2 ethyl acetate–2-propanol–water and on paper chromatography in solvent A (R_G 0.88), B (R_G 0.50), and C (R_G 0.25). A portion (5 mg) was dissolved in water (5 ml), and sodium borohydride (10 mg) was added portionwise. The solution was kept overnight, acidified with acetic acid, and passed through a small column of Amberlite IR-120 (H^+) resin. The column was washed with water (15 ml), the eluate evaporated in *vacuo*, and the residue dissolved in methanol. Several portions of methanol were added to the residue and evaporated to ensure complete removal of boric acid. The dry residue was converted to the per-(trimethylsilyl) ether and analyzed by g.l.c. A single, sharp peak was obtained at 240° with T_S 2.00, clearly distinguished from that of a sample prepared from an authentic specimen of 6-*O*-β-D-glucopyranosyl-D-galactose (T_S 1.85).

Another portion of the disaccharide (100 mg) crystallized from ethanol–water in needles (80 mg), m.p. 106–108°. After being dried thoroughly *in vacuo* at 80° over phosphorus pentoxide the needles collapsed to an amorphous solid, $[\alpha]_D^{25}$ +123° (*c* 1.00, water); lit.[12]: $[\alpha]_D$ +125°.

Anal. Calc. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 42.28; H, 6.41.

(*b*) Reaction between **3** (0.13 g) and 2-*O*-benzyl-3,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl bromide[9] (**1**, 0.45 g), in the presence of mercuric cyanide as described previously, was continued until all **1** had completely reacted; a total of 66 h was required. The mixture was processed, and the crude syrup was dissolved directly in 1:1 chloroform–methanol and deacylated catalytically. Chromatographic purification gave a product (290 mg, 53%) indistinguishable from **5** on t.l.c. and having an identical n.m.r. spectrum; $[\alpha]_D^{20}$ +70.5° (*c* 1.1, water).

Selective acid hydrolysis of the product (250 mg) afforded an amorphous solid (135 mg) indistinguishable from **6** on t.l.c. This solid was dissolved in 90% ethanol and hydrogenolyzed as described previously (yield 95 mg); $[\alpha]_D^{25}$ +123° (*c* 0.80, water). The product was indistinguishable from the compound **7** previously described on t.l.c. and paper chromatography in the solvent systems previously specified. A portion was reduced with sodium borohydride, trimethylsilylated, and analyzed by g.l.c. It had a T_S identical with that of the ether previously described, and did not show the presence of any of the β-anomer.

6-O-β-D-Glucopyranosyl-D-galactose. — Reaction of **3** (0.44 g, 2 mmoles) with tetra-*O*-acetyl-α-D-glucopyranosyl bromide[16] (2 mmoles) in 1:1 nitromethane–benzene in the presence of mercuric cyanide for 24 h at 40°, followed by the usual procedure of purification, afforded a syrup (0.90 g, 77%) that showed the expected n.m.r. peaks. After catalytic deacetylation and selective acid hydrolysis with 0.1M sulfuric acid in 4:1 *p*-dioxane–water for 30 min at 95–100°, the product was purified by chromatography on silica gel. Chloroform–methanol–water (12:6:1) eluted a total of 200 mg, $[\alpha]_D^{25}$ +19° (*c* 1.00, water); lit.[12]: $[\alpha]_D$ +10° (*c* 1, water); lit.[17]: $[\alpha]_D$ +13.9°. The disaccharide was indistinguishable from **7** on t.l.c. and paper chromatography in the solvent systems previously specified. The per(trimethylsilyl) derivative of the sugar alcohol gave a single peak on g.l.c. clearly separated from that shown by the α-anomer.

4,6-O-Ethylidene-3-O-(2-O-benzyl-α-D-glucopyranosyl)-1,2-O-isopropylidene-α-D-galactopyranose (**10**). — Reaction of 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-α-D-galactopyranose[18] (**8**, 0.50 g, 2.0 mmoles) with **2** as described for **5** required 2 days. After the usual processing, the disaccharide product, which migrated slightly slower than **2** on t.l.c. in 14:14:1 benzene–ether–methanol, was eluted from a column of silica gel, with the same solvent mixture, as a syrup, (0.9 g, 47%); n.m.r. data: τ 1.75–2.0 (*p*-nitrobenzoate, 12H), 2.65–2.75 (Ph, 5H), 8.4–8.8 [C–Me and CH-Me, 8H (should be 9H)]. After catalytic deacylation, the product was separated from methyl *p*-nitrobenzoate by column chromatography to give an amorphous solid(0.27 g, 25% overall yield from **8**); n.m.r. data (dimethyl sulfoxide): τ 2.60 (Ph, 5H), 4.17 (doublet, *J* 4Hz, 1H), 8.54 (doublet, *J* 6Hz, C–Me_2, 6H), 8.80 (doublet, *J* 4.5Hz, CH-Me, 3H).

Anal. Calc. for $C_{23}H_{34}O_{11}$: C, 56.78; H, 7.04. Found: C, 56.65; H, 7.40.

3-O-(2-O-Benzyl-α-D-glucopyranosyl)-D-galactose (**11**). — (*a*) A solution of **10** (150 mg) in 4:1 *p*-dioxane–water containing 0.1M sulfuric acid was kept for 30 min at 95–100°. The solution was cooled, diluted with water, and extracted twice with chloroform. The aqueous layer was treated with an excess of barium carbonate, and the clear filtrate was evaporated. The solid residue, which was almost homogeneous (t.l.c.), was dissolved in 6:6:1 chloroform–methanol–water and purified by column chromatography. The same solvent mixture eluted 120 mg (90%) of an amorphous solid. A portion crystallized from alcohol as needles, m.p. 188–190°, $[\alpha]_D^{25}$ +131° (*c* 0.515, water).

Anal. Calc. for $C_{19}H_{28}O_{11}$: C, 52.77; H, 6.53. Found: C, 52.52; H, 6.64.

(*b*) Attempted selective hydrolysis of **10** (100 mg) with 80% acetic acid at 95–100° for 3 h resulted in the formation of two products which were separated by column chromatography: about one-third (slower-migrating on t.l.c.) of the mixture was identical with **11**, whereas the remainder was a diacetyl derivative [n.m.r. (D_2O) peaks at τ 7.88, 7.94].

3-O-α-D-Glucopyranosyl-D-galactose (**12**). — (*a*) Amorphous **11** (100 mg) was hydrogenolyzed in the usual fashion with the addition of a drop of acetic acid to the solution. The resulting product was homogeneous on t.l.c. in 4:5:3 butyl alcohol–acetone–water and 12:6:1 chloroform–methanol–water, and on paper chromatography in solvents A, B, and C. It could not be crystallized, $[\alpha]_D^{25}$ +138° (*c* 1.29, water).

Anal. Calc. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 42.25; H, 6.70.

A portion (5 mg) of **11**, dissolved in water (5 ml), was reduced with sodium borohydride (25 mg added portionwise over 3 h) in the presence of boric acid (50 mg) as a buffer to prevent excess alkalinity. The solution was kept overnight at room temperature and processed in the usual fashion. The per(trimethylsilyl) derivative was analyzed by g.l.c. and its T_S (1.96) compared with that of the reduction product obtained from 3-*O*-β-D-glucopyranosyl-D-galactose. It was found that $T_\alpha/T_\beta = 1.07$, where α and β refer to the respective α or β-D-linked disaccharide derivatives.

(*b*) Reaction of **8** with the α-halide **1** was prolonged for 6 days at 40°. After the usual processing, the crude syrup was deacylated catalytically and the product

separated from methyl *p*-nitrobenzoate. Deacetalation was achieved with sulfuric acid in *p*-dioxane–water, and the disaccharide was separated from small amounts of (mainly) monosaccharide impurities by chromatography. After hydrogenolysis and removal of the catalyst and solvent, an amorphous solid was obtained in 15% overall yield from **8**; $[\alpha]_D^{25}$ +136° (*c* 0.74, water). A portion was converted to the per(trimethylsilyl) derivative of the sugar alcohol and analyzed by g.l.c. One peak (T_S 1.96) was obtained.

REFERENCES

1 W. KOENIGS AND E. KNORR, *Ber.*, 34 (1901) 957.
2 W. L. EVANS, D. D. REYNOLDS, AND E. A. TALLEY, *Adv. Carbohyd. Chem.*, 6 (1951) 27.
3 G. ZEMPLÉN AND A. GERECS, *Ber.*, 63 (1930) 2720.
4 B. HELFERICH AND K. F. WEDEMEYER, *Ann. Chem.*, 563 (1949) 139.
5 B. HELFERICH AND J. ZIRNER, *Ber.*, 95 (1962) 2604.
6 J. LEHMANN AND D. BECK, *Ann. Chem.*, 630 (1960) 56.
7 H. M. FLOWERS, A. LEVY, AND N. SHARON, *Carbohyd. Res.*, 4 (1967) 189.
8 A. LEVY, H. M. FLOWERS, AND N. SHARON, *Carbohyd. Res.*, 4 (1967) 305.
9 T. ISHIKAWA AND H. G. FLETCHER, JR., *J. Org. Chem.*, 34 (1969) 563.
10 B. A. D. STOCKER, A. M. STAUB, R. TINELLI, AND B. KOPACKA, *Ann. Inst. Pasteur*, 98 (1960) 505.
11 O. LÜDERITZ, K. JANN, AND R. WHEAT, in M. FLORKIN AND E. H. STOTZ (Eds.), *Comprehensive Biochemistry*, Vol. 26 A, Elsevier, Amsterdam, 1968, p. 105.
12 I. J. GOLDSTEIN AND W. J. WHELAN, *J. Chem. Soc.*, (1963) 4264.
13 R. B. DUFF, *J. Chem. Soc.*, (1957) 4730.
14 H. M. FLOWERS, *Carbohyd. Res.*, 4 (1967) 312.
15 O. SCHMIDT, *Methods Carbohyd. Chem.*, 2 (1963) 324.
16 H. E. CARTER, *Biochem. Prepn.*, 1 (1949) 34.
17 K. FREUDENBERG, A. WOLF, E. KNOPF, AND S. A. ZAHEER, *Ber.*, 61 (1928) 1743.
18 D. H. BALL, *J. Org. Chem.*, 31 (1966) 220.