Aldoheptoses in the O-Antigenic Lipopolysaccharide of *Salmonella typhimurium* and Other Gram-Negative Bacteria. I. Chemical Synthesis of L-Glycero-D-mannoheptose and β-L-Glycero-D-mannoheptopyranosyl 1-Phosphate*

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ABSTRACT: L-Glycero-D-mannoheptose is a component of the O-antigenic lipopolysaccharide of *Salmonella typhimurium* and other gram-negative bacteria. Its biosynthetic pathway is unknown and its chemical synthesis has not been described. In order to facilitate studies on this subject, this aldose and its β-1-phosphate ester have been synthesized.

D-Galacturonic acid was converted into L-galactono-1,4-lactone which was subsequently reduced with sodium borohydride to L-galactose. L-Glycero-D-mannoheptose was prepared by condensation of crystalline L-galactose with nitromethane. From the resulting reaction mixture 1-deoxy-1-nitro-L-glycero-D-mannoheptitol was isolated in pure form by differential crystallization and converted into crystalline L-glycero-D-mannoheptose by the Nef reaction. Fusion of the hexaacetate with crystalline phosphoric acid produced β-L-glycero-D-mannoheptopyranosyl 1-phosphate which was isolated as the crystalline biscyclohexylammonium salt. Its observed specific optical rotation ($[\alpha]_D^{26}$ +32°) is in good agreement with the data reported for α-D-mannopyranosyl 1-phosphate.

Aldoheptoses are commonly found in gram-negative bacteria as constituents of the cell wall lipopolysaccharides. In *Salmonella* and *Escherichia* species, L-glycero-D-mannoheptose is present as a component of the backbone or inner core region of the lipopolysaccharide (Cherniak and Osborn, 1966; Lüderitz *et al.*, 1966). The configuration of the heptose was first identified unequivocally by Weidel (1955) who isolated it from *Escherichia coli* cell walls. Although the chemical synthesis of the enantiomorph, D-glycero-L-mannoheptose, was described (see Sowden and Strobach, 1960), the chemical synthesis of the L-glycero-D-manno enantiomorph has not been previously reported. Furthermore, nothing is known about its biosynthesis. As a preliminary to investigations on the biosynthesis and metabolism of this heptose, we have undertaken the chemical synthesis of this aldoheptose and its β-1-phosphate ester using conventional methods. The route of synthesis is summarized in Figure 1.

The steric configuration of carbon atoms 2–7 in L-glycero-D-mannoheptose (VI) is identical with the configuration of L-galactose (IV) (see Figure 1). Therefore, this hexose was used as the starting material for the condensation with nitromethane in order to obtain the desired 7-carbon sugar. L-Galactose (IV) was synthesized by reduction of L-galactono-1,4-lactone (III) with sodium borohydride. Under carefully controlled conditions, we were able to obtain L-galactose routinely in 40% yield. L-Galactose was isolated from the reaction mixture as its 1-methyl-1-phenylhydrazone derivative.

Use of nitromethane (Sowden and Strobach, 1960) for the condensation led to a mixture of crystalline 1-deoxy-1-nitro-L-glycero-D-mannoheptitol (Va) and 1-deoxy-1-nitro-L-glycero-D-glucoheptitol (Vb) which was separated by differential crystallization. Pure 1-deoxy-1-nitro-L-glycero-D-mannoheptitol was obtained in a yield of 21.9%. L-Glycero-D-mannoheptose (VI) was generated from the nitroheptitol by the Nef reaction (Nef, 1894) in 80% yield. Acetylation of the heptose and subsequent treatment with crystalline phosphoric acid (MacDonald, 1962) produced β-L-glycero-D-mannoheptopyranosyl 1-phosphate as its crystalline biscyclohexylammonium salt (VIII).

Experimental Section

General Methods. Paper chromatography was performed on Whatman No. 40 paper (acid washed), paper electrophoresis on Whatman No. 3. Melting points were determined with a Fisher-Jones apparatus. The optical rotations were measured with a 2-dm tube in a Rudolph polarimeter. Inorganic phosphate, acid-labile phosphate, and total phosphate were estimated according

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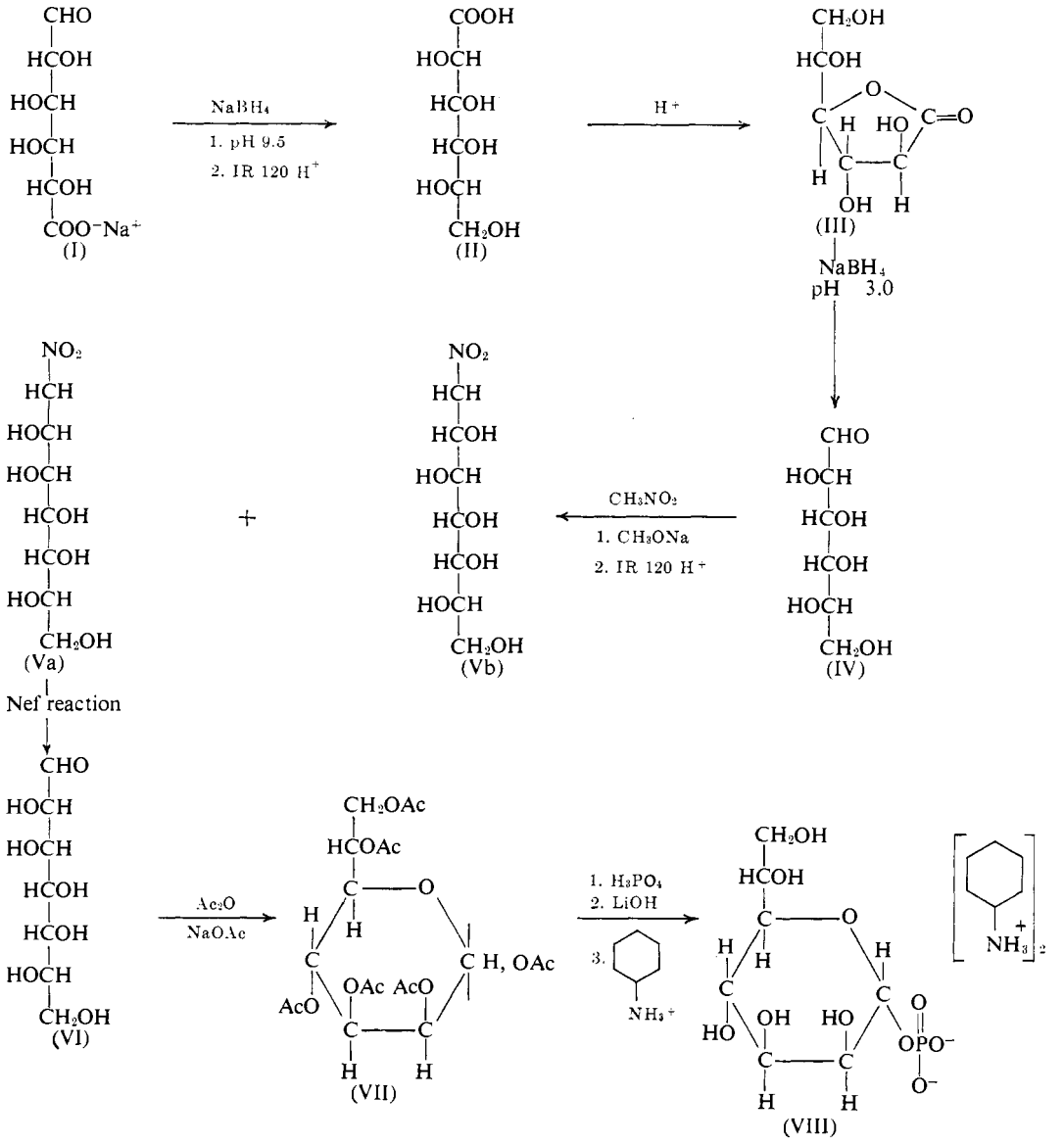

FIGURE 1: Scheme of synthesis of β-L-glycero-D-mannoheptopyranosyl 1-phosphate (biscyclohexylammonium salt). (I) Sodium D-galacturonate, (II) L-galactonic acid, (III) L-galactono-1,4-lactone, (IV) L-galactose, (Va) 1-deoxy-1-nitro-L-glycero-D-mannoheptitol, (Vb) 1-deoxy-1-nitro-L-glycero-D-glucoheptitol, (VI) L-glycero-D-mannoheptose, (VII) L-glycero-D-mannoheptose hexaacetate, and (VIII) biscyclohexylammonium β-L-glycero-D-mannoheptopyranosyl 1-phosphate.

to Chen *et al.* (1956) and Ames and Dubin (1960). Reducing sugar was evaluated by the Nelson–Somogyi procedure with D-mannose as standard (Somogyi, 1952). All chemicals were of the highest purity available.

L-Galactono-1,4-lactone (Frush and Isbell, 1962). D-Galacturonic acid monohydrate (Pfanstiehl) (106 g, 500 mmoles) was suspended in 350 ml of water. NaOH pellets were slowly added to the cooled suspension under stirring until the pH reached 8.5. Sodium borohydride (10 g in 150 ml of H_2O) was added, and the solution was kept overnight at 5°. The reaction mixture was stirred with 100 ml of IR 120 (H^+) ion exchanger to destroy excess $NaBH_4$ and passed over a column of 500 ml of IR 120 (H^+) to remove sodium ions, and the column was washed with 300 ml of H_2O. The combined effluents were concentrated *in vacuo* at 40° to a thick residue which was successively evaporated five times with absolute methanol to remove boric acid.[1] The residual syrup was dissolved in 100 ml of hot 1 N HCl and lactonization of the L-galactonic acid was completed by evaporation *in vacuo* at 40°. Traces of remaining HCl were removed *in vacuo* overnight at room temperature. The residue was extracted with 500 ml of boiling absolute ethanol and filtered to remove the insoluble material (~5 g), and the clear solution which crystallized spontaneously was left overnight at room temperature. After 2 additional days at 4°, the supernatant was decanted from the solid crystalline cake. The cake was washed with ice-cold absolute ethanol and dried *in vacuo*. The crystals (49.6 g) of L-galactono-1,4-lactone were used

[1] To test for the complete removal of boric acid, a small aliquot of the methanol distillate is burned on a watch glass. A green flame is a positive test for the presence of methyl borate.

without recrystallization for the reduction to L-galactose. Several more crops of crystalline L-galactono-1,4-lactone were obtained by successive reductions of the volume of the mother liquor by boiling; total yield 74.9 g (412 mmoles) (82.3%); mp 133–134° uncor (lit. (Richtmeyer *et al.*, 1939) mp 134°); $[\alpha]_D^{26}$ +77.2° (*c* 3.87, H_2O) (lit. (Richtmeyer *et al.*, 1939) $[\alpha]_D^{26}$ +78.4°). *Anal.*[2] Calcd for $C_6H_{10}O_6$: C, 40.50; H, 5.66. Found: C, 39.78; H, 5.64.

Reduction of L-*Galactono-1,4-lactone to* L-*Galactose by Sodium Borohydride.* Crystalline L-galactono-1,4-lactone (20 g, 111 mmoles) was dissolved in 35 ml of H_2O in a 500-ml beaker and cooled to 0°. Sodium borohydride (3.4 g, 90 mmoles) was dissolved in 20 ml of cold water. The beaker containing the lactone solution was placed in an ice–salt mixture and the electrode of a calibrated pH meter was used to monitor the pH value during the reduction procedure. The reaction temperature was maintained between 0 and 3°. The reaction was started by slow, dropwise addition of the sodium borohydride to the slowly stirred lactone and the pH was kept between 2.8 and 3.0 (important!) with cold 1.5 N H_2SO_4 during the course of the reduction by adding about 3 drops of H_2SO_4/drop sodium borohydride simultaneously. The addition of sodium borohydride took 2.5 hr. After the final aliquot of sodium borohydride had been added, the mixture was stirred for an additional 20 min and the pH was then brought to 2.0 with 1.5 N H_2SO_4. The amount of L-galactose in this mixture was determined by the phenol–sulfuric acid reaction (Dubois *et al.*, 1956) using D-galactose as a standard; yield 8.1 g (40.5%). The sulfate ions were removed by precipitation with $BaCO_3$ followed by filtration, and the sodium ions by passing the filtrate over a column of 100 ml of IR 120 (H^+). The effluents and washings were evaporated *in vacuo* and boric acid was removed by evaporating five times with absolute methanol. The remaining syrup was dissolved in 100 ml of H_2O, the pH was adjusted to 5.5 with 6.5 ml of concentrated NH_4OH, and the volume was made up to 150 ml with H_2O. This solution was used for the preparation of the methylphenylhydrazone of L-galactose.

L-*Galactose 1-Methyl-1-phenylhydrazone.* The solution, containing 8.1 g (45 mmoles) of L-galactose, was mixed with 100 ml of absolute ethanol containing 20 g (163 mmoles) of 1-methyl-1-phenylhydrazine and 1.5 ml of glacial acetic acid. The reaction mixture was kept overnight at 37° and subsequently at 4° for 24 hr. The white crystals of L-galactose 1-methyl-1-phenylhydrazone were collected by filtration, washed with a small amount of ice-cold absolute ethanol, and dried at 100° for 30 min; yield 12.0 g (42 mmoles) (93.3%); mp 186–188° uncor after recrystallization from 50% ethanol (lit. (Frush and Isbell, 1962) mp 189°). *Anal.* Calcd for $C_{13}H_{20}N_2O_5$: C, 54.90; H, 7.06; N, 9.86. Found: C, 54.83; H, 7.25; N, 9.43.

L-*Galactose.* L-Galactose 1-methyl 1-phenylhydrazone (38.45 g, 135 mmoles), obtained from three separate preparations, was suspended in 350 ml of water, and 30 ml of benzaldehyde in 150 ml of absolute ethanol was added. The mixture was refluxed for 1 hr, cooled in ice for 1 hr, and cleared of benzaldehyde methylphenylhydrazone by filtration. The filtrate was extracted three times with ether, and the residual ether in the aqueous phase was removed with a stream of cold air. The phenol–sulfuric acid reaction of the filtrate indicated 21.6 g of L-galactose (89%). The mixture was evaporated *in vacuo* to about 150 ml, treated with charcoal, filtered, and evaporated to a thick, slightly yellow syrup. H_2O (10 ml) and absolute methanol (15 ml) were added, and the solution was stirred overnight at 4° while crystallization occurred. The white, fine crystals were collected by filtration, washed with cold absolute methanol, and dried *in vacuo;* 13.5 g. The mother liquor was combined with the methanolic wash fluid and treated as above to yield another 6.1 g of crystallized L-galactose: total yield 19.6 g (80.5%); mp 153–161° uncor with browning decomposition; $[\alpha]_D^{26}$ −76° (*c* 3.23, H_2O) (lit. (Frush and Isbell, 1962) $[\alpha]_D^{26}$ −80°). Paper chromatography in butanol–pyridine–water (6:4:3) showed only one silver nitrate positive component, indistinguishable from D-galactose. *Anal.* Calcd for $C_6H_{12}O_6 \cdot 0.5\ H_2O$: C, 38.20; H, 6.90. Found: C, 38.24; H, 6.67.

*1-Deoxy-1-nitro-*L-*glycero-*D-*mannoheptitol.* To a stirred suspension of crystalline L-galactose hemihydrate (20.6 g, 109 mmoles) in 40 ml of absolute methanol and 52 ml of nitromethane was added a cold solution containing 12 g of sodium methoxide in 120 ml of absolute methanol. The mixture was stirred for 20 hr at room temperature, and then kept for 5 hr at −18° to precipitate the sodium *aci* salts of the nitroheptitols. The precipitate was filtered, washed with cold (−18°) absolute methanol, dissolved in 200 ml of ice-cold H_2O, and passed immediately over a previously chilled column of 100 ml of IR 120 (H^+) ion exchanger. The column was washed with 200 ml of cold H_2O and the combined effluents were evaporated *in vacuo* at 40°. The first crop of crystals, which separated on evaporation to about 150 ml and cooling the mixture overnight at 4°, was collected by filtration, washed with cold absolute ethanol, and dried *in vacuo* (fraction I, 7.3 g). This material was dissolved in 50 ml of hot water and left at 4° for 2 days. Long, white needles of 1-deoxy-1-nitro-L-glycero-D-mannoheptitol monohydrate formed and were separated and washed as above (fraction IA, 5.8 g). The aqueous mother liquor of fraction I and the alcoholic wash fluids were combined and evaporated to a volume of about 50 ml. The crystalline material (fraction II, 4.8 g) which separated at 4° overnight was collected and washed as above and the filtrate (F) was saved. The solid material (fraction II) was dissolved in 50 ml of hot water and after several days at 4°, 0.6 g (fraction IIA) of almost pure 1-deoxy-1-nitro-L-glycero-D-mannoheptitol monohydrate crystallized in long, white needles. Fraction IIA yielded 0.4 g of the pure substance after another recrystallization from 10 ml of water: total yield 6.2 g (21.9%), mp 159–160° uncor. On recrystallization of an analytical sample from hot acetone, the anhydrous form was obtained: mp 168° uncor; $[\alpha]_D^{26}$ −6.6° (*c* 3.23, H_2O). *Anal.* Calcd for $C_7H_{15}NO_8$:

[2] All elemental analysis were performed by Schwarzkopf Microanalytical Laboratories, Long Island City, N. Y.

C, 34.87; H, 6.28; N, 5.80. Found: C, 34.56; H, 6.36; N, 5.81.

*1-Deoxy-1-nitro-*L*-glycero-*D*-glucoheptitol.* The filtrate (F), on evaporation and treatment with 100 ml of 50% ethanol in the cold, yielded 1.6 g of pure 1-deoxy-1-nitro-L-glycero-D-glucoheptitol. The mother liquor of fraction IIA deposited 1.9 g of pure material under the same conditions; total yield 3.5 g (13.3%); mp 152–153° uncor; $[\alpha]_D^{26}$ −8.8° (*c* 4.0, H_2O). *Anal.* Calcd for $C_7H_{15}NO_8$: C, 34.87; H, 6.28; N, 5.80. Found: C, 35.12; H, 5.84; N, 6.31.

L-*Glycero-*D-*mannoheptose.* To a well-stirred suspension of crystalline 1-deoxy-1-nitro-L-glycero-D-mannoheptitol monohydrate (6.2 g, 23.9 mmoles) in 30 ml of ice-cold water was added dropwise a cold solution of 1.2 g of sodium hydroxide in 7 ml of cold water. The crystals dissolved within 10 min at −1°. This solution was immediately added dropwise under rapid stirring to a cold (−15°) mixture of 4.5 ml of concentrated sulfuric acid and 6 ml of H_2O. The temperature of this solution was allowed to rise slowly to 0° and the reaction mixture was stirred at 0° for 2 hr until the slow evolution of NO_2 had stopped. The volume of the mixture was adjusted to 100 ml with cold H_2O and passed over a previously chilled column of 50 ml of IR 120 (H^+) ion exchanger to remove sodium ions. The sulfate ions of the effluent and washings were precipitated by $BaCO_3$. The neutral filtrate was concentrated *in vacuo* to a pale yellow syrup which on evaporation from absolute methanol yielded 3.95 g of white, hydroscopic material: $[\alpha]_D^{26}$ +15.2° (*c* 2.67, H_2O) (lit. (Hann *et al.*, 1935) value for D-glycero-L-mannoheptose $[\alpha]_D^{26}$ −15.3°). Paper chromatography in butanol–pyridine–water showed only trace amounts of unhydrolyzed nitroheptitol in addition to the heptose.

The syrupy heptose (2.5 g) was dissolved in 10 ml of 80% ethanol and left at 4° for 3 weeks. During this time most of the unhydrolyzed nitroheptitol crystallized out and was removed by filtration. A small aliquot of the filtrate was treated with water to give a final concentration of 50% ethanol. This solution was left *in vacuo* overnight while spontaneous partial crystallization occurred. The main fraction was seeded with this material and crystallization was completed in 3 hr at 4°. The material was recrystallized from ethanol–water according to Hann *et al.* (1935); yield 2.28 g (83.5%); mp 179–181°. *Anal.* Calcd for $C_7H_{14}O_7 \cdot H_2O$: C, 36.82; H, 7.07. Found: C, 37.01; H, 7.11.

L-*Glycero-*D-*mannoheptose Diethyl Dithioacetal.* Syrupy L-glycero-D-mannoheptose (100 mg) was dissolved in 0.25 ml of concentrated HCl, and 0.25 ml of ethanethiol was added, mixed, and left at 0°. The mixture turned into a white, crystalline mass within a few minutes. Cold water was added (1 ml) and the mixture kept at 4° for 24 hr. The crystals were collected by filtration, washed twice with 1-ml portions of cold water and twice with ethyl ether, and dried *in vacuo* over $CaCl_2$; yield 120 mg (88.5%). The compound is readily recrystallized from hot water: mp 202–203° uncor; $[\alpha]_D^{26}$ +10.2° (*c* 1.19, pyridine). *Anal.* Calcd for $C_{11}H_{24}O_6S_2$: C, 41.75; H, 7.65; S, 20.30. Found: C, 41.97; H, 7.88; S, 20.16. The diethyl dithioacetal of the L-glycero-D-mannoheptose isolated from *E. coli* cell walls had a melting point of 201–202° and a rotation of +9.9° (Weidel, 1955).

L-*Glycero-*D-*mannoheptose 1-Phosphate* (MacDonald, 1962). L-Glycero-D-mannoheptose, 1.75 g of dried syrup. was mixed with 1 g of anhydrous sodium acetate and 14 ml of acetic anhydride. The mixture was refluxed for 1 hr, poured on ice, stirred for 30 min, and extracted with 100 ml of $CHCl_3$. The chloroform layer was extracted three times with a saturated sodium bicarbonate solution and twice with H_2O and dried over $CaSO_4$. Upon evaporation to dryness *in vacuo* at 40°, the chloroform solution yielded 2.9 g of a brown syrup after drying *in vacuo* overnight. Crystalline phosphoric acid (5 g), which was previously dried *in vacuo* over magnesium perchlorate, was added to this syrup, melted at 50°, and stirred *in vacuo* at 50° for 3 hr. The reaction flask was cooled, 102 ml of cold 2 N lithium hydroxide was added, and the flask was shaken vigorously for 5 min during which time the mixture warmed up to about 30° and lithium phosphate precipitated. The mixture was left at room temperature for 6 hr to complete saponification. Lithium phosphate was filtered and washed with 0.001 N LiOH, and the combined filtrates were cooled and passed over a previously chilled column of 50 ml of IR 120 (H^+). The percolate was allowed to drop directly into a stirred solution of 5 ml of cyclohexylamine in 25 ml of H_2O. The column was washed with water until the pH reached 4.5. The percolate was adjusted to pH 7.0 with glacial acetic acid and evaporated *in vacuo* at 40° to a semisolid mass. The residue was extracted with 100 ml of isopropyl alcohol to remove cyclohexylammonium acetate. The insoluble material was collected by filtration and washed with two 20-ml portions of isopropyl alcohol. The light brown precipitate was dissolved in 20 ml of H_2O, treated with charcoal, and filtered, and the filtrate was evaporated to dryness. Inorganic phosphate was removed by dissolving the residue in 20 ml of 1.5 N ammonium hydroxide and adding 0.25 g of magnesium acetate. The precipitated magnesium ammonium phosphate was removed by filtration and the filtrate was passed over a small column of IR 120 H^+ in the cold to remove cations. Cyclohexylamine (1.0 ml) was added to the percolate. The mixture was evaporated to dryness, dissolved in hot ethanol with the addition of a minimum amount of water, and placed in the refrigerator. More absolute ethanol was added during the next several days until crystallization occurred. The crystals were collected by filtration, washed with cold, absolute ethanol, and dried *in vacuo* (yield 400 mg). This material was recrystallized three times by dissolving it in 0.5 ml of water, adding 10 ml of absolute ethanol, and storing overnight at 4°; final yield 200 mg of fine needles; mp 140–142°; $[\alpha]_D^{26}$ +32° (*c* 5.0, H_2O). *Anal.* Calcd for $C_{19}H_{41}N_2O_{10}P \cdot 2H_2O$: C, 43.52; H, 8.64; N, 5.16; P, 5.90. Found: C, 43.56; H, 8.74; N, 5 38; P, 5.74. Paper electrophoresis at pH 3.5 (pyridine–acetic acid–water, 1:10:89) showed only one phosphorus-containing spot which migrated like glucose 6-phosphate and did not stain with alkaline silver nitrate. On cellulose thin-layer sheets (MN Polygram) with methanol–ammonium hydroxide–water (60:10:30) as solvent, the substance was homogeneous and had an R_F value of

0.69. Paper chromatography of the free sugar after acid hydrolysis (1 N HCl, 10 min at 100°) showed one silver nitrate reducing spot migrating identical with L-glycero-D-mannoheptose in butanol–pyridine–water. The chemical analysis of the heptose 1-phosphate is shown in Table I.

TABLE I: Chemical Composition of β-L-Glycero-D-mannoheptopyranosyl 1-Phosphate.

	Moles[d]
Total phosphate	1.01
Acid-labile phosphate[a]	1.00
Inorganic phosphate[b]	0.08
Reducing sugar[c]	0.006
Reducing sugar after acid hydrolysis[a,c]	0.93

[a] 1 N HCl, 10 min, 100°. [b] Arises from the hydrolysis of heptose 1-phosphate during phosphate determination. [c] With D-mannose as standard. [d] Moles per mole of acid-labile phosphate.

Discussion

The major difficulty in the synthesis of L-glycero-D-mannoheptose was the production of a sufficient quantity of L-galactose since, as was shown in the case of D-galactose (Sowden and Strobach, 1960), the condensation with nitromethane would produce the desired 1-deoxy-1-nitro-L-glycero-D-mannoheptitol in no more than 20% yield. The recently reported reduction (80–100% yield) of free (Giudici and Fluharty, 1967) or fully acetylated (Kohn *et al.*, 1965) 1,4-lactones to the corresponding aldoses using commercial bis(3-methyl-2-butyl)borane (disiamylborane) as reducing agent failed in our hands. With the commercial 1 M solution of disiamylborane in tetrahydrofuran (Ventron Corp.), we were able to reduce no more than 15% of the lactone to L-galactose. The reduction of L-galactono-1,4-lactone with sodium borohydride at pH 3.5–4.0 and 0° (Wolfrom and Wood, 1951) gave similar results. However, when carried out at pH 2.8–3.0 (Dr. L. M. Lerner, personal communication), the reduction with a 0.8–1.5 molar excess of sodium borohydride routinely yielded 40% of L-galactose. A larger excess did not increase the yield significantly, but did increase the difficulty of work-up of the L-galactose.

In order to avoid overreduction of the free galactose to galactitol, every reduction was monitored for the formation of galactose by the phenol–sulfuric acid test (Dubois *et al.*, 1956) after the addition of each 0.5 molar ratio of sodium borohydride. When the yield reached 40% of theory, there was little further increase in galactose with added borohydride and in some cases there was a measurable decrease.

Since L-galactono-1,4-lactone is readily available, we stopped the reaction at the 40% level. It was not possible to crystallize L-galactose directly from the deionized reaction mixture, due to the difficulty in removing the remaining lactone, L-galactonic acid, and galactitol. In order to isolate the L-galactose, the reaction mixture was freed of boric acid and sodium ions, adjusted to pH 5.5 with concentrated NH_4OH (not NaOH), and treated with 1-methyl-1-phenylhydrazine. L-Galactose was converted into its insoluble, crystalline 1-methyl-1-phenylhydrazone in greater than 90% yield. In order to avoid precipitation of the ammonium salt of L-galactonic acid during the isolation of the L-galactose methylphenylhydrazone, the ethanol concentration was kept at 40% instead of the usual 50%. Upon regeneration of L-galactose from the hydrazone by benzaldehyde, the sugar crystallized readily from methanol–H_2O (Frush and Isbell, 1962). Condensation of L-galactose with nitromethane worked exactly as described by Sowden and Strobach (1960) for D-galactose. The physical constants for 1-deoxy-1-nitro-L-glycero-D-mannoheptitol and 1-deoxy-1-nitro-L-glycero-D-glucoheptitol agree very well within the limits of experimental error with the data reported for the corresponding enantiomers derived from D-galactose as shown in Table II. The generation of L-glycero-D-mannoheptose from the corresponding nitroheptitol by the Nef (1894) reaction was enhanced by working at low temperatures according to Murray and Prokop (1965). Under these conditions the unwanted side reactions caused by dissolving the nitroheptitols in alkali at room temperature could be eliminated. L-Glycero-D-mannoheptose was obtained in crystalline form as its monohydrate. It was characterized as its crystalline diethyl dithioacetal whose optical rotation and melting point are in excellent agreement with the data reported for the natural L-glycero-D-mannoheptose isolated from *E. coli* by Weidel (1955). No efforts were made to crystallize the hexaacetates of the heptose, probably a mixture of the α and β anomers. The dried syrup was treated directly with crystalline phosphoric acid according to MacDonald (1962). As in the case of D-mannose (Hill and Ballou, 1966; Salo *et al.*, 1968) it is not essential to use the crystalline acetate. The crystalline biscyclohexylammonium salt of the heptose 1-phosphate had a specific optical rotation of +32° which proves that the conformation of carbon atoms 1–5 is identical with α-D-mannopyranosyl 1-phosphate. Since this compound is of the L series, it is properly named biscyclohexylammonium β-L-glycero-D-mannoheptopyranosyl 1-phosphate. It should be no problem to synthesize any desired heptose nucleotide from the 1-phosphate by the phosphoromorpholidate procedure of Roseman *et al.* (1961).

Although the biosynthetic pathway of L-glycero-D-mannoheptose and its incorporation into the lipopolysaccharide of gram-negative bacteria remains unknown, a possible source might be sedoheptulose 7-phosphate or sedoheptulose 1,7-diphosphate, from which it can theoretically be derived assuming several conventional enzymatic reaction steps. Jones *et al.* (1962) have suggested that D-glycero-D-mannoheptose, a component of the extracellular polysaccharide of *Azotobacter indicum*, is formed from specifically labeled radioactive hexoses by pathways involving *trans*-aldolase, *trans*-ketolase,

TABLE II: Comparison of the Physical Constants of Substances in the L-Glycero-D-mannoheptose and the D-Glycero-L-mannoheptose Series.

Compound	Mp (°C)	$[\alpha]_D$ (deg)	References
1-Deoxy-1-nitro-L-glycero-D-mannoheptitol monohydrate	159–160	−6.6[a]	
(enantiomorph)	(158–159)	(+6.3)[a]	Sowden and Strobach (1960)
1-Deoxy-1-nitro-L-glycero-D-glucoheptitol	152–153	−8.8	
(enantiomorph)	(152–153)	(+7.8)	Sowden and Strobach(1960)
L-Glycero-D-mannoheptose monohydrate	79–81	+15.2[a]	
(enantiomorph)	(77–78)	−15.3[a]	Hann *et al.* (1935)
L-Glycero-D-mannoheptose diethyl dithioacetal	202–203	+10.2	
(enantiomorph)	(205)	−9.7	Hann and Hudson (1937)
E. coli heptose diethyl dithioacetal	201–202	+9.9	Weidel (1955)
Biscyclohexylammonium β-L-glycero-D-mannoheptopyranosyl 1-phosphate	140–142	+32	
Biscyclohexylammonium α-D-mannopyranosyl 1-phosphate	183–185	+30.8	Salo *et al.* (1968)

[a] Anhydrous form.

and aldolase. Ginsberg *et al.* (1962), who isolated guanosine diphosphate D-glycero-D-mannoheptose from yeast, have proposed a biosynthetic pathway for this substance starting from sedoheptulose 7-phosphate.

Work on the biosynthesis of L-glycero-D-mannoheptose and D-glycero-D-mannoheptose (which was recently reported in *Salmonella* by Adams *et al.* (1967)) and its role in the biosynthesis of the lipopolysaccharide in *Salmonella typhimurium* is in progress in our laboratory.

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