# Phosphorylated Sugars. Part XIV.[1] Synthesis of D-*glycero*-L-*manno*-Heptose 7-(Dihydrogen Phosphate)

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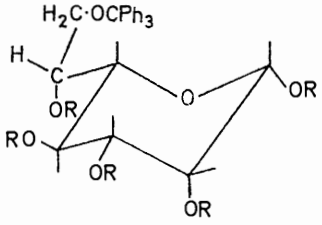
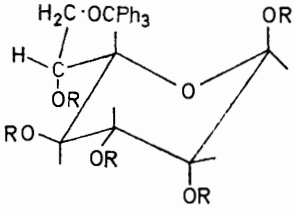
Detritylation of 1,2,3,4,6-penta-*O*-benzoyl-7-*O*-trityl-β-D-*glycero*-L-*manno*-heptose with hydrobromic acid in acetic acid, followed by phosphorylation and removal of the protecting groups, affords D-*glycero*-L-*manno*-heptose 7-phosphate. A similar sequence of reactions starting with 1,2,3,4,6-penta-*O*-acetyl-7-*O*-trityl-D-*glycero*-L-*manno*-heptose gave the same phosphate, showing that detritylation of this heptose derivative is not accompanied by an acid-catalysed acetyl migration similar to that observed previously in detritylation of 1,2,3,4,6-penta-*O*-acetyl-7-*O*-trityl-D-*glycero*-D-*galacto*-heptose.

SEVERAL aldoheptoses have been identified as components of cell wall lipopolysaccharides of Gram-negative bacteria. An acid stable, phosphorylated L-*glycero*-D-*manno*-heptose was isolated from *Shigella flexneri* [2] but the position of the phosphate group was not determined. That the heptose of the lipopolysaccharide core of the Salmonellae is phosphorylated is fairly well established.[3] Except for the acid-labile 1-phosphate of L-*glycero*-D-*manno*-heptose,[4] the only heptose phosphate that has been synthesised to date is D-*glycero*-D-*galacto*-heptose 6-phosphate.[5] Little is known of the behaviour of heptose phosphates in the various reactions (hydrolysis, methylation, acetolysis, *etc.*) used to determine the structures of lipopolysaccharides. With the aim of studying these reactions, we have undertaken the synthesis of several phosphorylated heptoses. That of D-*glycero*-L-*manno*-heptose 7-phosphate is reported here. This sugar is the enantiomorph of the naturally occuring L-*glycero*-D-*manno*-heptose.

It is necessary to comment on the nomenclature of the anomers of derivatives of D-*glycero*-L-*manno*-heptose. According to the British–American Rules of Carbohydrate Nomenclature,[6] the anomeric prefix should be related to the configuration at C-5, which is the reference carbon in this case (rule 22). The absolute configuration of the α-anomer in relation to C-5 should thus be *cis* and that of the β-anomer *trans* (rule 15). If the empirical optical rotation rule for designating the anomers is applied, since D-*glycero*-L-*manno*-heptose is considered as belonging to the L-series, the β-anomer will be the more, and the α-anomer the less, dextrorotatory. However, even in fairly recent literature, D-*glycero*-L-*manno*-heptose seems to have been considered as belonging to the D-series for the purposes of anomeric assignments. Thus, the more dextrorotatory anomers of the hexa-acetates and hexabenzoates of this sugar [7] have been designated D-*glycero*-α-L-*manno*, and the more laevorotatory D-*glycero*-β-L-*manno*, which is in contradiction to the aforementioned rules. To our knowledge, the absolute configurations of these compounds have not been determined. In the present work, it has been shown that for the anomers of 1,2,3,4,6-penta-*O*-acetyl-7-O-trityl- and of 1,2,3,4,6-penta-*O*-benzoyl-7-*O*-trityl-D-*glycero*-L-*manno*-heptose, there is concordance between optical rotation data and absolute configurations as determined by n.m.r. The more dextrorotatory anomer in each case has structure (I; R = Ac or Bz) and should be named D-*glycero*-β-L-*manno*; the more laevorotatory anomers have structure (II; R = Ac or Bz) and should be designated D-*glycero*-α-L-*manno*. The same relations between optical rotation and absolute configuration probably hold true for the aforementioned hexa-acetates




(I)




(II)

and hexabenzoates of D-*glycero*-L-*manno*-heptose and the nomenclature used for these anomers should probably be reversed. Since these data are entered as such in the literature,[8,9] in order to avoid confusion which may arise in conforming to the British–American rules, we have adopted the IUPAC Tentative Rules for Carbohydrate Nomenclature [10] in this paper. According to these rules, compound (I) should be named α-D-*glycero*-L-*manno* and compound (II) β-D-*glycero*-L-*manno* (Rule Carb-21).

Strobach *et al.*[5] found that detritylation of 1,2,3,4,6-penta-*O*-acetyl-7-*O*-trityl-D-*glycero*-D-*galacto*-heptose yielded a penta-acetate having the 6- and not the expected 7-hydroxy-group free; they concluded that acetyl migration from position 6 to 7 had occurred. A similar acetyl migration also occurs during detritylation of 2,3,3′,4,4′-penta-*O*-acetyl-1′,6,6′-tri-*O*-tritylsucrose.[11]

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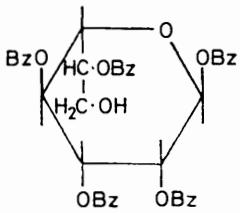
No acyl migration occurred, however, during detritylation of the corresponding pentabenzoyl-tritritylsucrose.[12] For the synthesis of D-*glycero*-L-*manno*-heptose 7-phosphate we therefore chose as starting material 1,2,3,4,6-penta-*O*-benzoyl-7-*O*-trityl-D-*glycero*-L-*manno*-heptose.

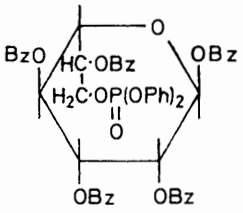
D-*glycero*-L-*manno*-Heptose [13] was converted, by successive treatments with triphenylmethyl chloride and benzoyl chloride, into a mixture of the pentabenzoyl-7-tritylheptoses, from which the pure-β anomer (II; R = Bz) crystallised readily. The α-anomer (I; R = Bz) was isolated from the mother liquors by preparative t.l.c. The structures of these compounds were derived from $^1$H n.m.r. data. The anomeric assignments were made on the basis of the chemical shifts of H-1 [14,15] and confirmed by the relative positions of the signals for H-5.[14] The small coupling constant $J_{1,2}$ (1·5 Hz) showed the β-anomer (II) to be in the $^1C_4$ conformation. The conformation of the α-anomer could not be deduced from its spectrum, but it was assumed to have the $^1C_4$ conformation. Detritylation of the β-anomer gave a crystalline pentabenzoate which could be retritylated to give the starting compound, and which was hence the 1,2,3,4,6-penta-*O*-benzoate. (Tritylation of the penta-benzoate requires more vigorous conditions than does that of the free sugar, probably because of steric hindrance by the benzoyl groups.) Phosphorylation of this pentabenzoate with diphenyl phosphorochloridate yielded a crystalline diphenyl phosphate which, after removal of the phenyl groups by hydrogenolysis and of the benzoyl groups with sodium methoxide, gave the heptose 7-phosphate. This phosphate reduced 5 mol. equiv. of periodate.

A similar sequence of reactions was then carried out with acetyl instead of benzoyl groups for protection. The anomeric assignments of the penta-*O*-acetyl-7-*O*-tritylheptoses were again made on the basis of the $^1$H n.m.r. spectra. The penta-acetate obtained by detritylation of the β-anomer did not crystallise, so it was phosphorylated immediately after detritylation. The heptose phosphate isolated after hydrogenolysis of the oily diphenyl phosphate and deacetylation was identical with the 7-phosphate obtained *via* the benzoate. An additional proof that the phosphate was at the 7-position was obtained by showing that only glycol phosphate was present after periodate oxidation of the heptose phosphate and reduction of the products.[16] (We have shown that as little as 5% of glycerol phosphate can be revealed in the presence of glycol phosphate by this technique.)

Acetyl group migration during detritylation of penta-acetyl-7-tritylheptoses is therefore not a general reaction of heptoses. The reaction observed by Strobach *et al.*[5] is probably an extreme but not an isolated case. We have observed that detritylation of benzyl-2,3,4,6-tetra-*O*-acetyl-7-*O*-trityl-β-D-*glycero*-D-*gulo*-heptopyranoside [17] under the same conditions gave a syrup showing two spots on t.l.c., probably corresponding to two tetra-acetates. This reaction is being further investigated.



(III)



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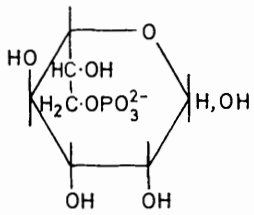

(V)

The synthesis of D-*glycero*-L-*manno*-heptose 7-phosphate *via* the benzoate is the more convenient of the two. The β-anomer of the pentabenzoyl-7-tritylheptose can be obtained more easily and in higher yield than can that of the corresponding penta-acetate, and crystalline products are obtained in the two subsequent steps.

The rates of hydrolysis of the phosphate group of this sugar phosphate have been determined under three different sets of conditions and compared with those of methyl-β-D-*glycero*-L-*manno*-heptopyranoside 7-phosphate [17] under the same conditions.

## EXPERIMENTAL

All evaporations were carried out under reduced pressure below 40°. M.p.s were taken on a Kofler hot-stage apparatus. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. $^1$H N.m.r. spectra were obtained at 60 MHz for solutions in $CDCl_3$ with trimethylsilane as internal standard. T.l.c. was performed on silica gel Schleicher and Schüll plastic-coated plates with (A) ethyl acetate–hexane (1 : 2 v/v) and (B) ethyl acetate–benzene (1 : 4 v/v) as solvents; plates were sprayed with sulphuric acid in ethanol (5% v/v) and heated at 120°. Preparative t.l.c. was carried out on glass plates coated with Merck silica gel 60 F254 (1·5 mm). Paper electrophoresis of phosphate esters was performed on Whatman 3MM paper in 0·2M-pyridine–acetic acid buffer (pH 5) at 2900 V for 1 h; phosphates were revealed by the Hanes–Isherwood reagent [18] and reducing sugars by aniline hydrogen phthalate. Periodate was estimated by the method of Avigad; [19] aqueous solutions $10^{-3}$M with respect to sugar phosphate and $10^{-2}$M with respect to sodium periodate were used. After acetylations, benzoylations, and phosphorylations in pyridine, a

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few drops of water were added to the cooled reaction mixtures which, after 1 h, were poured into ice–water. The precipitates were filtered off and washed with water. When appropriate, solutions of the precipitates in chloroform were washed successively with iced water, cold sulphuric acid (1% v/v), iced water, a cold saturated solution of sodium hydrogen carbonate, and twice with iced water, and dried ($Na_2SO_4$).

1,2,3,4,6-*Penta*-O-*benzoyl*-7-O-*trityl*-D-glycero-L-manno-*heptose* [(I) and (II); R = Bz].—D-*glycero*-L-*manno*-Heptose monohydrate [13] (3 g) was dissolved in anhydrous pyridine (70 ml) and the pyridine was removed. This process was repeated once. The heptose was dissolved in anhydrous pyridine (70 ml), triphenylmethyl chloride (5·17 g) was added, and the mixture was left for 90 h at room temperature. Benzoyl chloride (10 ml) was added dropwise to the stirred solution cooled in ice and the mixture was stirred for 24 h at room temperature. The precipitate obtained after work-up was dissolved in chloroform, the solution was washed and dried as usual, and the chloroform was removed. Crystallisation of the residue from a mixture of ethyl acetate–ethanol gave the pure β-*anomer* (II; R = Bz) (6·7 g, 52%), m.p. 235—236°, $[\alpha]_D^{24}$ −21° (*c* 2 in $CHCl_3$), δ 6·62 (1H, d, $J_{1,2}$ 1·5 Hz, 1-H), 6·0 (3H, m, 2-, 3-, and 4-H), 5·43 (1H, sext, $J_{5,6}$ 1·5, $J_{6,7} = J_{6,7'} = 7$ Hz, 6-H), 4·76 (1H, m, 5-H), and 3·60 and 3·35 (2H, octet, $J_{7,6} = J_{7',6} = 7$, $J_{7,7'}$ 9 Hz, 7- and 7′-H (Found: C, 75·3; H, 5·0. $C_{61}H_{48}O_{12}$ requires C, 75·3; H, 4·9%).

When most of the β-anomer had crystallised, the α-*anomer* (I; R = Bz) was isolated from the mother liquors by multiple development (5—6 runs) preparative t.l.c. (ethyl acetate–hexane, 1 : 3); m.p. 117—119°, $[\alpha]_D^{25}$ + 77·5° (*c* 2 in $CHCl_3$), δ 6·29 (1H, d, $J_{1,2}$ 1 Hz, 1-H), 5·3—6·1 (4H, m, 2-, 3-, 4-, and 6-H), 4·38 (1H, m, 5-H), and 3·54 (2H, m, 7- and 7′-H) (Found: C, 74·9; H, 4·85%).

1,2,3,4,6-*Penta*-O-*acetyl*-7-O-*trityl*-D-glycero-L-manno-*heptose* [(I) and (II); R = Ac).—D-*glycero*-L-*manno*-Heptose monohydrate [13] (1 g) was dehydrated and treated with triphenylmethyl chloride (1·6 g) in anhydrous pyridine (25 ml) as above. Acetic anhydride (6 ml) was added to the cooled (−10°) solution which was then left for 24 h at room temperature. After the usual work-up, the precipitate was dried *in vacuo* ($P_2O_5$; KOH) and dissolved in the minimum amount (*ca.* 50 ml) of ethanol. The mixture of α- and β-anomers (2·47 g, 85%) which crystallised was separated by fractional crystallisation from ethanol, the β-*anomer* (II; R = Ac) being the more insoluble. It had m.p. 209—210°, $[\alpha]_D^{24}$ −49·5° (*c* 2 in $CHCl_3$), δ 6·12 (1H, d, $J_{1,2}$ 1·5 Hz, 1-H), 4·95—5·45 (4H, m, 2-, 3-, 4-, and 6-H), 4·3 (1H, m, 5-H), 3·35 and 3·10 (2H, sept, $J_{7,7'}$ 9 Hz, 7- and 7′-H), and 2·13, 2·07, 2·05, 2·00, and 1·85 (15H, 5Ac) (Found: C, 64·9; H, 5·8. $C_{36}H_{38}O_{12}$ requires C, 65·25; H, 5·8%).

The α-*anomer* (I; R = Ac) had m.p. 107—109°, $[\alpha]_D^{24}$ +17·8°, δ 5·77 (1H, d, $J_{1,2}$ 1 Hz, 1-H), 5·0—5·5 (4H, m, 2-, 3-, 4-, and 6-H), 3·88 (1H, m, 5-H), 3·35 (2H, m, 7- and 7′-H), 2·18 (3H, s, Ac), 2·05 (9H, s, 3 Ac), and 1·96 (3H, s, Ac) (Found: C, 64·3; H, 5·85%).

1,2,3,4,6-*Penta*-O-*benzoyl*-β-D-glycero-L-manno-*heptose* (III).—The trityl benzoate (II; R = Bz) (3·7 g) was dissolved in boiling acetic acid (50 ml) and the solution was cooled in ice (*ca.* 10°). To the shaken solution dry hydrogen bromide in acetic acid (1 ml; 40% v/v) was added. Shaking was continued for 90 s after a precipitate started to form. The mixture was worked up in the usual way.[5] The chloroform was removed, the residue was dissolved in the minimum amount of ethyl acetate, and light petroleum (b.p. 48—60°) was added to slight turbidity. The crystalline *pentabenzoate* (1·65 g, 59%) was filtered off, washed with ethyl acetate–light petroleum (1 : 1) and dried. It had m.p. 185—186° and was practically pure on t.l.c. [solvent (A)]. A sample recrystallised from the same solvents had m.p. 188—190° (previous softening), $[\alpha]_D^{22}$ −14° (*c* 2 in $CHCl_3$) (Found: C, 69·1; H, 4·7. $C_{42}H_{34}O_{12}$ requires C, 69·0; H, 4·7%).

*Tritylation of the Penta*-O-*benzoylheptose* (III).—The foregoing pentabenzoate (100 mg) was dissolved in pyridine (1 ml) and triphenylmethyl chloride (70 mg) was added. The solution was left for 4 days at room temperature and heated for 48 h at 50°. At this stage, t.l.c. [solvent (A)] showed that no starting material remained. The trityl benzoate (100 mg, 75%) obtained after the usual work-up had m.p. and mixed m.p. 235—236°, $[\alpha]_D^{22}$ −20·5° (*c* 2 in $CHCl_3$).

1,2,3,4,6-*Penta*-O-*benzoyl*-β-D-glycero-L-manno-*heptose* 7-(*Diphenyl Phosphate*) (IV).—Diphenyl phosphorochloridate (1·08 g) was added to a solution of the pentabenzoate (III) (2·38 g) in dry pyridine (10 ml) cooled in salt–ice. The mixture was left for 5 days in the dark at room temperature. The solid (2·95 g) obtained after work-up was dried *in vacuo* ($P_2O_5$) and dissolved in hot ethanol (35 ml). The solution was cooled with shaking in ice until the oil which formed solidified, and was then left at 4° overnight. The precipitate (2·5 g) was filtered off, washed with cold ethanol, and dried. It was virtually pure on t.l.c. [solvent (B)]. This process was repeated with 30 ml of ethanol. The *diphenyl phosphate* (2·2 g) had m.p. 77—79°, $[\alpha]_D^{23}$ +22·8° (*c* 2 in $CHCl_3$) (Found: C, 67·1; H, 4·5; P, 3·0. $C_{54}H_{43}PO_{15}$ requires C, 67·4; H, 4·5; P, 3·2%).

D-glycero-L-manno-*Heptose* 7-(*Dilithium Phosphate*) (V).—(a) *From* 1,2,3,4,6-*penta*-O-*benzoyl*-β-D-glycero-L-manno-*heptose* 7-(*diphenyl phosphate*) (IV). The diphenyl phosphate (1 g) was dissolved in glacial acetic acid (25 ml) and hydrogenated over platinum oxide (0·3 g). The bulk of the acetic acid was removed below 35° and the rest by lyophilisation. The residue was dissolved in anhydrous methanol (20 ml) and methanolic 0·87N-sodium methoxide (20 ml) was added. The mixture was left for 24 h at room temperature and poured slowly onto a slight excess of IR 120 ($H^+$) resin in iced water. The resin was filtered off and the filtrate was neutralised (pH 6·9) with lithium hydroxide solution and concentrated to dryness. Ethanol was added to the residue and removed. More ethanol was added; the residue (300 mg; 86%) was triturated until it solidified, left in the cold overnight, centrifuged off, washed thoroughly with ethanol and finally with acetone, and dried in air. The *lithium salt* had $[\alpha]_D^{23}$ −7·1° [*c* 1·78 in $H_2O$ (calc. for anhydrous salt)] (Found: C, 24·7; H, 4·6; P, 8·9. $C_7H_{13}Li_2O_{10}P,2H_2O$ requires C, 24·85; H, 5·0; P, 9·2%).

(b) *From* 1,2,3,4,6-*penta*-O-*acetyl*-7-O-*trityl*-β-D-glycero-L-manno-*heptose* (II; R = Ac). This penta-acetyl-trityl-heptose (2·8 g) was dissolved in hot glacial acetic acid (20 ml), the solution was cooled (*ca.* 10°), and a solution (1 ml) of hydrobromic acid in glacial acetic acid (40% w/v) was added. The mixture was shaken for 1 min and then treated in the usual way.[5] The chloroform was removed and to a solution of the residue in pyridine (15 ml), cooled in salt–ice, diphenyl phosphorochloridate (1·4 g) was added. The mixture was left for 5 days in the dark at room temperature, then cooled, and a few drops of water were added. After 1 h, the pyridine was removed, the residue was dissolved in chloroform and the chloroform layer was washed with cold,

dilute hydrochloric acid until the aqueous layer remained acidic, then with iced water, and dried ($Na_2SO_4$). The chloroform was removed, the residue was dissolved in methanol, and the methanol was evaporated off. This process was repeated once. The residue was dissolved in methanol (100 ml) and hydrogenated over platinum oxide (0·5 g). The solution was neutralised with 0·44N-barium methoxide and dried ($CaSO_4$). More barium methoxide was added (pH 13) and the mixture was left for 24 h at room temperature. It was poured onto a slight excess of IR 120 ($H^+$) resin in iced water. The resin was filtered off and the filtrate neutralised with lithium hydroxide solution. The solution was concentrated to dryness, and ethanol was added to the residue and removed. The residue was triturated with ethanol, centrifuged off, thoroughly washed with ethanol, and air-dried. This lithium salt had $[\alpha]_D^{23}$ −7·1° [*c* 1·76 in $H_2O$ (calc. for anhydrous salt)] (Found: C, 24·0; H, 5·0; P, 9·3. Calc. for $C_7H_{13}Li_2O_{10}P,2{\cdot}5H_2O$: C, 24·2; H, 5·2; P, 8·9%).

The sugar phosphate reduced 4·9 mol. equiv. of periodate in 4—5 h at room temperature.

*Oxidation and Reduction of* D-glycero-L-manno-*heptose 7-Phosphate*.—Sodium periodate (136 mg) in water (4 ml) was added to a solution of the foregoing lithium salt (20 mg) in water (2 ml) and the mixture was left in the dark for 42 h. Sodium borohydride (170 mg) in water (2 ml) was added to the stirred solution, which was then left overnight at room temperature. The solution was treated with IR 120 ($H^+$) resin, neutralised with barium hydroxide solution, and concentrated to dryness. The residue was triturated with water (2 ml) and insoluble material was centrifuged off. This contained no phosphate and was discarded. The filtrate was treated with IR 120 ($H^+$) resin and concentrated to dryness. Methanol was added to the residue and evaporated off five times, and the remaining solid was then dissolved in water (0·5 ml) and neutralised with lithium hydroxide. This solution was shown by paper electrophoresis to contain only one phosphate ester, indistinguishable from glycol phosphate and well separated from glycerol phosphate.

*Acid Hydrolyses of* D-glycero-L-manno-*Heptose 7-Phosphate and of Methyl* α-D-glycero-L-manno-*Heptopyranoside 7-Phosphate*.—The lithium salt of each sugar phosphate (*ca.* 2 mg) was dissolved in each of the following media: (a) 0·1N-$H_2SO_4$; (b) 1N-$H_2SO_4$; (c) trifluoroacetic acid solution (pH 2·43). A sample (0·5 ml) of each solution was taken immediately for the estimation of total phosphorus.[20] Solutions (a) and (b) were heated on a boiling water bath and samples (0·1 ml) were withdrawn at regular intervals over a period of 3 h for estimation of inorganic phosphate.[21] Solution (c) was heated in an oil-bath at 50° and samples were removed over a period of 50 h for estimation of inorganic phosphate. After 3 h, D-*glycero*-L-*manno*-heptose 7-phosphate was hydrolysed to the extent of 3·4% in 0·1N-$H_2SO_4$, and 4·5% in 1N $H_2SO_4$ at 100°. The corresponding figures for the heptoside phosphate were 1·7 and 2·6%. After 50 h at 50° in trifluoroacetic acid, the free sugar phosphate yielded 1·5% of inorganic phosphate and the heptoside phosphate 1·0%.

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