

Preparation of 3-, 4-, and 6-deoxyderivatives of guanosine diphosphate-D-mannose

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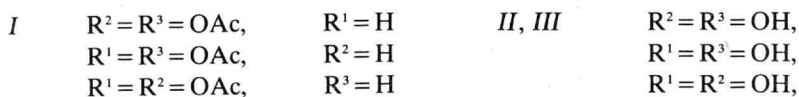
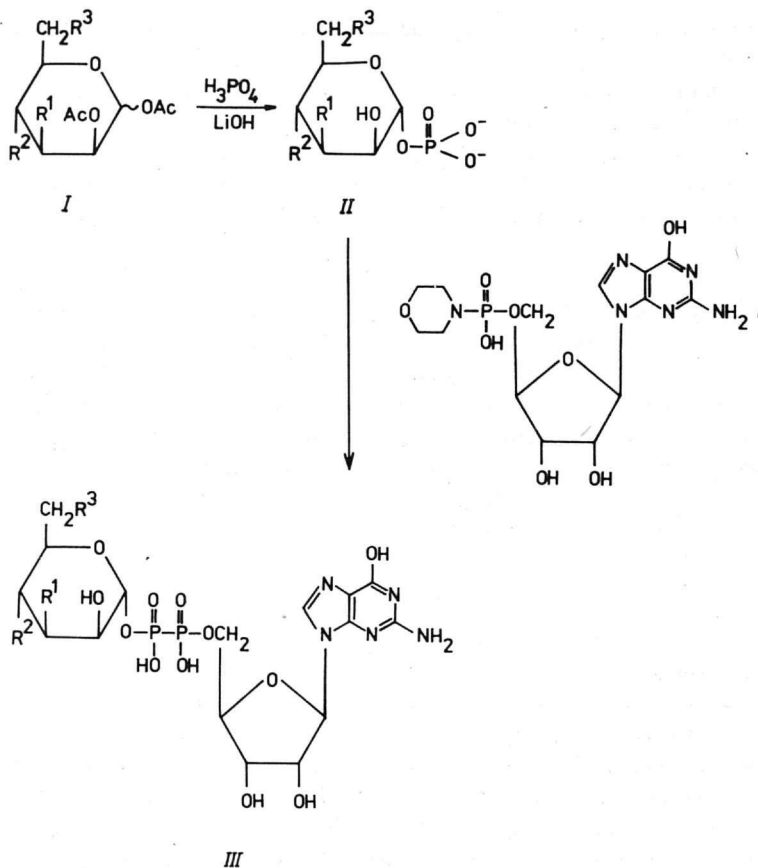
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3-Deoxy- α -D-arabino-hexo-, 4-deoxy- α -D-lyxo-hexo-, and 6-deoxy- α -D-mannopyranosyl phosphates have been prepared by phosphorylation of per-O-acetyl derivatives of corresponding hexoses with crystalline H_3PO_4 . In reactions of the glycosyl phosphates with guanosine 5'-phosphomorpholidate their guanosine diphosphates have been prepared. Synthesis of crystalline 4-deoxy- α -D-lyxo-hexopyranose is also described in the paper.

3-Деокси- α -D-арабино-гексо-, 4-деокси- α -D-ликсо-гексо- и 6-деокси- α -D-маннопиранозилфосфат были приготовлены фосфорилированием пер-O-ацетилированных производных соответствующих гексоз фосфорной кислотой в кристаллическом состоянии. При реакции приведенных гликозилфосфатов с гуанозин 5'-фосфоморфолидатом также приготовили их гуанозин дифосфаты. В работе описан и способ приготовления 4-деокси- α -D-ликсо-гексопиранозы в кристаллическом состоянии.

Research into the deoxyderivatives of nucleoside diphosphate sugars, the analogues of the natural nucleoside diphosphate sugars (NDPS) having the deoxy group in the hexose moiety of the molecule is very important for explaining the effect of the individual hydroxyl groups on the biological activity (substrate specificity) of the whole molecule of NDPS. Absence of the hydroxyl group in the molecule of NDPS causes its acid lability, which insinuates the fact that there has not been yet referred to any case of the synthesis of a deoxyderivative of GDP-mannose in the literature up to now.

The present paper describes preparation of three deoxyderivatives of GDP-mannose containing 3-deoxy-D-arabino-hexose, 4-deoxy-D-lyxo-hexose, and 6-deoxy-D-mannose in their sugar moiety. In their synthesis a procedure described already by Kochetkov *et al.* [1] based on interaction of nucleoside 5'-phosphomorpholidate (triethylammonium salts) and glycosyl phosphate in anhydrous dimethyl sulfoxide was applied (Scheme 1). Glycosyl phosphates of 3-deoxy-D-



Scheme 1

-*arabino*-hexose, 4-deoxy-D-*lyxo*-hexose, and 6-deoxy-D-mannose as compounds unknown in the literature have been prepared by action of cryst H_3PO_4 on the per-*O*-acetylated derivatives of the corresponding saccharides [2, 3].

Experimental

Chromatography of glycosyl phosphates was performed on Whatman No. 1 paper in 2-propanol—ammonia—water (7:1:2) (solvent A) and electrophoresis in 0.05 M triethylamine—hydrogen carbonate buffer, pH 7.5 (solvent B) on the same paper. The molybdate

reagent for phosphates [4] was used for detection in both cases, nucleotides were visualized under an ultraviolet lamp.

Estimation of the amount of reducing sugars in the molecule of the nucleotide was done by the *Park—Johnson* procedure [5].

Optical rotation was measured with a Perkin—Elmer 141 polarimeter. Melting points were determined on a Kofler hot-stage. ¹H-n.m.r. spectra of glycosyl phosphates (in D₂O) were measured with a Tesla BS 483 B (80 MHz) equipment using DSS as an internal standard.

3-Deoxy-D-*ribo*-hexose was prepared as described by *Rembarz* [6]; m.p. 141—142°C, $[\alpha]_D^{20} + 54^\circ$ (c 1.0, water). Ref. [6] gives m.p. 141—142°C and $[\alpha]_D^{20} + 53.1^\circ$ (c 2.49, water). 6-Deoxy-D-mannose was prepared by a simplified method [7]; m.p. 89—91°C and $[\alpha]_D^{20} - 8.2^\circ$ (c 1.0, water). Ref. [7] gives m.p. 90—91°C and $[\alpha]_D^{20} - 8.2^\circ$ (c 1.0, water). 1,6-Anhydro-4-deoxy-2-*O*-tosyl- β -D-*lyxo*-hexopyranose was prepared according to [8]. The per-*O*-acetylated derivatives of 3-deoxy-D-*arabino*-hexose, 4-deoxy-D-*lyxo*-hexose, and 6-deoxy-D-mannose were prepared by acetylation with acetic anhydride under a catalytic effect of anhydrous sodium acetate. Sirups obtained after acetylation were used in the preparation of glycosyl phosphates.

1,6-Anhydro-4-deoxy- β -D-*lyxo*-hexopyranose

A cooled solution (-50°C) of 1,6-anhydro-4-deoxy-2-*O*-tosyl- β -D-*lyxo*-hexopyranose (0.5 g) in anhydrous tetrahydrofuran (5 ml) was mixed with 0.3 M solution of sodium naphthalene in THF (100 ml) for 10 min. After deionization with Amberlit IR-120 (H⁺), the filtrate was concentrated and the sirup obtained was poured into water (100 ml) and the solution was extracted with chloroform (3 \times 25 ml). The aqueous portion was concentrated and three times evaporated with anhydrous ethanol. Yield 0.12 g (55.4%) of 1,6-anhydro-4-deoxy- β -D-*lyxo*-hexopyranose in sirup, $[\alpha]_D^{23} - 85.3^\circ$ (c 1.05, water). According to [8] $[\alpha]_D - 87^\circ$ (c 0.66, water).

4-Deoxy-D-*lyxo*-hexopyranose

1,6-Anhydro-4-deoxy- β -D-*lyxo*-hexopyranose (0.5 g) was dissolved in water (40 ml), 1 g of Amberlit IR-120 (H⁺) was added and the mixture was kept at 90°C on the water bath (course of the hydrolysis followed by paper chromatography in the solvent, 2-butanon saturated with water). After finishing the hydrolysis (about 5 h) Amberlit was filtered off, the filtrate was decolourized with charcoal and evaporated three times with anhydrous ethanol. Thus obtained 4-deoxy-D-*lyxo*-hexopyranose (0.42 g), m.p. 68—70°C, $[\alpha]_D + 6.4^\circ$ (c 0.62, water). According to [8] $[\alpha]_D + 3^\circ$ (c 0.57, water).

Glycosyl phosphates

A solution of cryst H₃PO₄ (441 mg), dried in a desiccator over Mg(ClO₄)₂ in a minimal volume of anhydrous THF was mixed with a solution of the per-*O*-acetylated derivatives of

a corresponding deoxysugar (130 mg). THF was removed by distillation at ca. 0.133 kPa. The residue was kept further at this pressure at room temperature for 90 min and at 50°C for another 90 min. The cooled reaction mixture was dissolved in a minimal volume of THF (about 3 ml), 1 M-LiOH (15 ml) was added and stirred for 18 h. The precipitant formed was filtered off, washed twice with 0.1 M-LiOH (3 ml) and the filtrate was applied to a column (2 × 20 cm) of Dowex 50 (H⁺). The eluate obtained was neutralized with cyclohexylamine, concentrated to dryness and dissolved in 0.05 M-TEAB (200 ml) and passed through a column (3.2 × 60 cm) of Dowex 1 × 8 (HCO₃⁻). The column had been washed with 0.05 M-TEAB and the separation was performed in a linear gradient of 0.05 M-TEAB (2 l) and 0.3 M-TEAB (2 l). The fractions collected (15 ml, 1 ml/min) were analyzed on acid-labile phosphorus [4] and those containing the α anomer of a deoxyglycosyl phosphate were pooled and concentrated under a diminished pressure and purified from the TEAB by repeated evaporation with water. Yields of the phosphorylation reaction and the values of optical rotation are presented in Table 1.

Configuration of the glycosyl phosphates was determined using ¹H-n.m.r. spectroscopy. The value of $J_{1,2}$ for all the glycosyl phosphates prepared is 1.5 Hz, which corresponds to the α anomers.

Table 1

Yields and the values of optical rotation of the α anomers of triethylammonium salts of the glycosyl phosphates

α -D-Glycopyranosyl phosphate	Yield %	$[\alpha]_D$	c (water)
3-Deoxy-D- <i>arabino</i> -hexose	21.5	+ 53.3°	0.13
4-Deoxy-D- <i>lyxo</i> -hexose	22.8	+ 29.4°	0.5
6-Deoxy-D-mannose	27.4	+ 23.0°	0.78

Deoxyanalogues of GDP-mannose

Triethylammonium guanosine 5'-phosphomorpholidate (0.4 mmole) and triethylammonium salt of the corresponding glycosyl phosphate were dried separately by repeated azeotropic distillation of ethanol—benzene mixture (1:1, v/v 4 × 3 ml), then anhydrous dimethyl sulfoxide (1 ml) was added and the solution was stored for 48 h at room temperature. The reaction was monitored by paper electrophoresis. Water (500 ml) was added and the solution was applied to a column (2 × 20 cm) of Dowex 1 × 2 (Cl⁻). Elution was performed with the NaCl solution in a gradually increasing molarity from 0.02 M up to 0.08 M and monitored on a Uvicord. Fractions containing the deoxyderivatives of GDP-mannose were desalted using a column (3 × 200 cm) of Sephadex G-10. The structure was proved by estimating the ratio of acid-labile phosphate : total phosphate : reducing sugar. The results and yields are presented in Table 2.

Table 2

Yields and the ratio of acid-labile phosphate: total phosphate: reducing sugar in the deoxyanalogues of GDP-mannose

GDP-	Yield %	Acid-labile phosphate: : total phosphate: : reducing sugar
3-Deoxy-D- <i>arabino</i> -hexose	28.4	1:1.98:1.02
4-Deoxy-D- <i>lyxo</i> -hexose	27.5	1:2.04:1.03
6-Deoxy-D-mannose	30.1	1:2.01:1.02

Discussion

The starting deoxysaccharides were prepared following the methods already known with one exception, 4-deoxy-D-*lyxo*-hexose prepared according to [8] and modified in the reductive desulfonation of 1,6-anhydro-4-deoxy-2-*O*-tosyl- β -D-*lyxo*-hexopyranose using sodium naphthalene [9]. The yield of 1,6-anhydro-4-deoxy- β -D-*lyxo*-hexopyranose was lower (about 55%) than that in the original method (about 70%), the preparation was however simpler (the synthesis was shortened about one step). 4-Deoxy-D-*lyxo*-hexopyranose was obtained by hydrolyzing 1,6-anhydro-4-deoxy- β -D-*lyxo*-hexopyranose in crystalline form (in the literature described just as a sirup).

Acetylation of the deoxysugars was carried out with acetic anhydride under a catalytic effect of anhydrous sodium acetate. The crude product prepared in this way containing a mixture of α and β anomers of tetra-*O*-acetylated derivatives of deoxysugars was used without further separation for preparation of glycosyl phosphates. Phosphorylation was carried out with anhydrous crystalline phosphoric acid [10]. Under these conditions the course of phosphorylation depends mainly on the orientation of the substituents in the proximity of the anomeric centre and the thermodynamic conditions of the reaction. The acetyl group at C-2 atom of the studied derivatives of D-mannose is in axial position, therefore the product of the phosphorylation should have an α configuration independent of the thermodynamic conditions of the reaction as well as of the configuration of the substituents at anomeric centre of the starting per-*O*-acetylated derivative of sugar. This presumption was confirmed in preparation of α -D-mannopyranosyl phosphate [11] when the same results were obtained whether a pure crystalline α anomer of per-*O*-acetylated D-mannose or the sirupy mixture of both α and β anomers of peracetylated D-mannose was used as a starting product of the reaction. Similarly, a sirupy mixture of α and β anomers of tetra-*O*-acetyl-L-rhamnose was used for

phosphorylation in preparation of α -L-rhamnosyl phosphate [12]. In our case the sirupy mixtures of α and β anomers of tetra-*O*-acetylated derivatives of 3-deoxy-D-*arabino*-hexose, 4-deoxy-D-*lyxo*-hexose, and 6-deoxy-D-mannose were used as starting materials, and as the products of phosphorylation only the α anomers of 3-deoxy-D-*arabino*-hexosyl phosphate, 4-deoxy-D-*lyxo*-hexosyl phosphate, and 6-deoxy-D-mannosyl phosphate were obtained. The α configuration of the phosphates prepared was confirmed using $^1\text{H-n.m.r.}$ spectroscopy.

Formation of the pyrophosphate linkage is the main problem of the chemical synthesis of a NDPS. The method of *Khorana et al.* [13] based on condensation of a nucleoside 5'-phosphomorpholidate with α -D-glycosyl phosphate is sensitive to the presence of the traces of water in the reaction mixture so that the greater portion of water causes the greater extent of hydrolysis of the starting phosphomorpholidate. Substitution of pyridine by some other aprotic solvents as dimethyl sulfoxide results in retarding the hydrolytic reaction [1]. The method modified in this way proved to be the most suitable for preparation of the deoxyanalogues of GDP-mannose. We succeeded in preparing the GDP-derivatives of 3-deoxy-D-*arabino*-hexose, 4-deoxy-D-*lyxo*-hexose, and 6-deoxy-D-mannose as chromatographically and electrophoretically homogeneous compounds. The ratio of acid-labile phosphate:total phosphate:reducing sugar in the molecule of the nucleotide (Table 2) confirms the composition of the compounds prepared.

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