Note

Phosphorylation of the hemiacetal hydroxyl group: Synthesis of β-D-mannopyranosyl phosphate*

H. S. PRIHAR AND E. J. BEHRMAN

Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210 (U. S. A.)

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Glycosyl phosphates have usually been synthesized either by reaction of an acetylated glycosyl halide with a silver phosphate derivative or by fusion of the totally acetylated sugar with anhydrous phosphoric acid[1]. It is frequently difficult, by these techniques, to obtain the phosphate having a particular anomeric configuration. Antia and Watson[2] have discussed the factors that influence the steric course of the reactions of acetylated glycosyl halides and silver phosphate derivatives. The structure of the silver phosphate derivative, the C-2 neighboring-group effect, and the C-5 substituent are all contributory factors. The mechanism of the MacDonald fusion procedure with phosphoric acid is less well understood, but it is clear that synthesis of the thermodynamically less stable anomer of the glycosyl phosphate can be achieved only if its rate of formation is fast relative to its rate of conversion into the more stable anomer under the fusion conditions[3].

We report the successful application of a method based on phosphorylation of the hemiacetal hydroxyl group by *o*-phenylene phosphorochloridate, the reagent developed by Khwaja and Reese[4]. Previous attempts to phosphorylate the hemiacetal hydroxyl group had either been unsuccessful[5], or were reported without full experimental details[6]. In agreement with Cori, *et al.*[5] we were unable to observe any significant reaction between phosphorus oxychloride and the hemiacetal hydroxyl group over a period of days. However, *o*-phenylene phosphorochloridate reacted rapidly at room temperature with the hemiacetal hydroxyl group to give good yields of the expected phosphate diester, from which the glycosyl phosphate could be obtained by oxidation with bromine and subsequent deacetylation.

The extraordinary reactivity of *o*-phenylene phosphorochloridate may be rationalized on the basis of the well-documented rate-enhancement toward nucleophilic attack of five-membered cyclic phosphates as compared with their open-chain analogs[7]. This increase in reactivity is of the order of 6×10^6.

We report here the synthesis of β-D-mannopyranosyl phosphate (**4**), which was characterized by the following evidence. The di(cyclohexylammonium) salt of **4**

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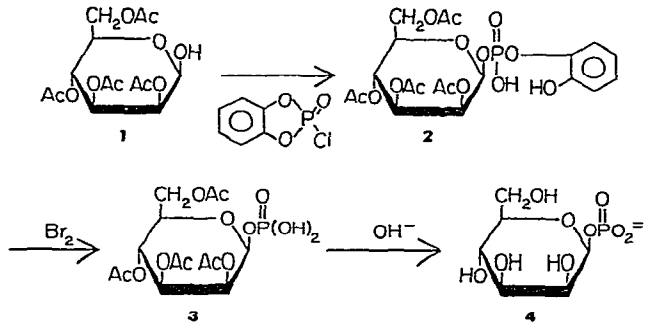
showed $[\alpha]_D^{25}$ −6.5° as compared* with the corresponding salt of the α-anomer[8], which had $[\alpha]_D^{25}$ +28.7°. Compound **4** gave single spots, having R_F values indistinguishable from the α-anomer, in the following paper-chromatographic systems: 5:3:2 butyl alcohol–acetic acid–water, 7:1:2 isopropyl alcohol–ammonia (sp. gr.



0.88)–water, 7:3 ethanol–ammonium acetate (M, pH 7.5). Compound **4** resembled the α-anomer in reacting slowly with alkaline silver nitrate. The Benedict test was negative. Compound **4** consumed 1.90 moles of periodate per mole of sugar phosphate, and one mole of formic acid was produced. Hydrolysis of **4** in 0.1M hydrochloric acid for 15 min at 100° gave quantitative yields of both mannose and inorganic phosphate, in equimolar ratio. Reaction of **4** with *N*,*N*′-dicylohexylcarbodiimide gave the expected cyclic phosphate, together with the *N*-phosphorylurea[12]. Under identical conditions, the α-anomer did not react. The rate of hydrolysis was measured for both anomeric phosphates at 37° in 0.955M hydrochloric acid. The pseudo-first-order rate constants (±5%) were 37.5×10^{-3} min^{-1} (**4**, β-anomer) and 10.6×10^{-3} min^{-1} (α-anomer). The n.m.r. spectrum of **4** in deuterium oxide with tetramethylsilane as an external standard confirmed the β-D-configuration and established the conformation as C1(D). The H-1 signal appeared as a quartet at τ 4.70 with $J_{1,2}$ 1.0 Hz, indicating axial–equatorial coupling of the protons on C-1 and C-2, as H-2 is in an antiperiplanar relationship with the ring oxygen atom[13]. The corresponding data for the α-anomer are τ 4.1 and $J_{1,2}$ 1.5 Hz, indicating an equatorial–equatorial relationship[14].

The present synthesis of β-D-mannopyranosyl phosphate, our previous note[15] on the synthesis of β-L-rhamnopyranosyl phosphate, and some preliminary results with β-L-fucopyranosyl phosphate and β-D-glucopyranosyl phosphate, suggest the general utility of this procedure which is, in principle, applicable for synthesis of both anomers of any glycosyl phosphate. The synthesis of the thermodynamically stable

*Lemieux and Martin[9] have pointed out that the 2A value for an anomeric pair of D-mannopyranosides is that of the D-*gluco* derivative minus twice the contribution of an asymmetric unit defined by the 1- and 2-substituents in a *gauche* relationship. Following Whiffen[10], Lemieux and Martin used a value of 4500° for this interaction. On the basis of a 2A value of 25,400° for the phosphate group in the *gluco* series, as determined by Putman and Hassid[11], the corresponding value for 2A in the *manno* series is 16,400°. As the cyclohexylammonium salt of α-D-mannopyranosyl phosphate[8] has $[M]_D$ 13,200°, the β-anomer should have $[M]_D$ (13,200° − 16,400°) = −3200°. Our value for $[\alpha]_D^{25}$ yields $[M]_D$ = −3100°.

phosphate requires only the preparation of the suitably protected, stable hemiacetal. As the thermodynamically unstable hemiacetals are, in general, available by displacement with inversion from the acetylated glycosyl halides, the thermodynamically unstable phosphates can be made, provided only that phosphorylation is fast relative to anomerization.

EXPERIMENTAL

2,3,4,6-Tetra-O-acetyl-β-D-mannopyranose (**1**) was prepared according to Bonner in a yield of 70%; m.p. 122°, $[\alpha]_D^{25}$ −14.0° (*c* 1.0, chloroform), (lit.[16], m.p. 124°, $[\alpha]_D^{25}$ −15.5° (*c* 2.0, chloroform).

β-D-Mannopyranosyl di(cyclohexylammonium) phosphate. — A solution of *o*-phenylene phosphorochloridate (0.77 g, 4 mmoles, Aldrich Chemical Co.) in anhydrous tetrahydrofuran (5 ml) was added dropwise with stirring at room temperature with occasional cooling to a solution of **1** (1.39 g, 4 mmoles) in anhydrous tetrahydrofuran (8 ml) containing *sym*-collidine (0.56 ml, 4 mmoles).

Following the final addition, the mixture was stirred for 25 min. The precipitated salts were filtered off and washed with tetrahydrofuran (10 ml). The combined filtrate and washings were treated with *sym*-collidine (0.56 ml, 4 mmoles) and then with water (0.12 ml, 6.6 mmoles). After 30 min, the products were concentrated under diminished pressure to a gum. Paper electrophoresis (0.03M ammonium formate, pH 3.6) of an aliquot showed a u.v.-absorbing, major component (~85%) having $R_{Picrate}$ 0.78; presumably the phosphate diester **2**. Bromine (1 ml) was added dropwise to a vigorously stirred solution of the gum in aqueous triethylammonium hydrogen carbonate buffer, pH 7.5 (150 ml, 0.2M). After 10 min at room temperature, the yellow precipitate was filtered off, and the filtrate was extracted with benzene (2 × 50 ml). During this period the pH dropped from 7 to 2–3. The aqueous layer was readjusted to pH 8 with a freshly prepared solution of lithium hydroxide. The products were centrifuged to remove the precipitate, and the supernatant liquid was decanted, concentrated to about 35 ml, adjusted to pH 10.5 with lithium hydroxide, and kept for 8 h at room temperature. The pH of the solution was lowered to 8.0 with acetic acid, the solution was centrifuged, and the filtrate treated with Norit A. The colorless filtrate was then treated with 0.9 g of barium acetate. The resultant suspension was centrifuged, and the supernatant material was treated with ethanol until a faint turbidity persisted. After several h at 4°, the precipitate was collected by centrifugation, washed with ether, and dried *in vacuo* over phosphorus pentaoxide. The barium salt was dissolved in water (20 ml), centrifuged to remove traces of insoluble matter, and reprecipitated by the addition of ethanol (60 ml). This process was repeated three times; yield 0.63 g, $[\alpha]_D^{25}$ −6° (*c* 1.1, water).

Conversion into the di(cyclohexylammonium) salt was effected by passing an aqueous solution of the lithium salt (prepared by treatment of the barium salt with Dowex-50 lithium) through Dowex-50W-X8, 200–400 mesh, in the cyclohexylammonium form. The effluent was concentrated to 10 ml. The product crystallized on

gradual addition of 60 ml of acetone. After 2 days at 4°, the colorless, crystalline salt was separated and dried over phosphorus pentaoxide at 56°; yield 0.25 g (12%), m.p. 180°, $[\alpha]_D^{25}$ (−6.5° (*c* 1, water).

Anal. Calc. for $C_{18}H_{39}N_2O_9P \cdot H_2O$: C, 45.33; H, 8.60; N, 5.88; P, 6.51. Found: C, 45.39; H, 8.60; N, 5.95; P, 6.46.

Reaction of β-D-mannopyranosyl phosphate (**4**) *with N,N'-dicyclohexylcarbodiimide.* — The pyridinium salt of **4** (10 mg) was dissolved in 2.4 ml of pyridine containing 0.4 ml of water. After adding 40 mg of *N,N'*-dicyclohexylcarbodiimide, the homogeneous reaction mixture was kept for 3 h at room temperature. The mixture was then processed according to the procedure of Khorana *et al.*[12]. Subsequent chromatography in 7:1:2 isopropyl alcohol–ammonia–water showed complete conversion of **4** (R_F 0.1) into major and minor products having R_F 0.48 and 0.82, respectively. These corresponded to the hexopyranosyl 1,2-cyclic phosphate and the *N*-phosphorylurea.

REFERENCES

1 E. A. DAVIDSON, *Carbohydrate Chemistry*, Holt Rinehart and Winston, Inc., New York, N. Y., 1967, Chapter 11. D. L. MACDONALD, *Methods Enzymol.*, 8, (1966) 121. Two other approaches have been reported recently: C. L. STEVENS, AND R. E. HARMON, *Carbohyd. Res.*, 11, (1969) 99; M. HALMANN, R. A. SANCHEZ, AND L. E. ORGEL, *J. Org. Chem.*, 34 (1969) 3702.

2 N. J. ANTIA AND R. W. WATSON, *J. Amer. Chem. Soc.*, 80 (1958) 6134.

3 A. K. CHATTERJEE AND D. L. MACDONALD, *Carbohyd. Res.*, 6 (1968) 253; D. L. MACDONALD, *Carbohyd. Res.*, 3 (1966) 117; D. H. LEABACK, E. C. HEATH, AND S. ROSEMAN, *Biochemistry*, 8 (1969) 1351; P. M. BARNA, *Syn. Commun.*, 1 (1971) 207.

4 T. A. KHWAJA AND C. B. REESE, *Tetrahedron*, 27 (1971) 6189; T. A. KHWAJA, C. B. REESE, AND J. C. M. STEWART, *J. Chem. Soc.* (C), (1970) 2092; T. A. KHWAJA AND C. B. REESE, *J. Amer. Chem. Soc.*, 88 (1966) 3446.

5 C. F. CORI, S. P. COLOWICK, AND G. T. CORI, *J. Biol. Chem.*, 121 (1937) 465.

6 T. POSTERNAK, *J. Amer. Chem. Soc.* 72 (1950) 4824.

7 J. R. COX AND O. B. RAMSAY, *Chem. Rev.*, 64 (1965) 317; E. T. KAISER AND K. KUDO, *J. Amer. Chem. Soc.*, 89 (1967) 6725.

8 D. L. HILL AND C. E. BALLOU, *J. Biol. Chem.*, 241 (1966) 895.

9 R. U. LEMIEUX AND J. C. MARTIN, *Carbohyd. Res.*, 13 (1970) 139.

10 D. H. WHIFFEN, *Chem. Ind.* (London), (1956) 964.

11 E. W. PUTMAN AND W. Z. HASSID, *J. Amer. Chem. Soc.*, 79 (1957) 5057.

12 H. G. KHORANA, G. M. TENER, R. S. WRIGHT, AND J. G. MOFFATT, *J. Amer. Chem. Soc.*, 79 (1957) 430.

13 L. D. HALL, J. F. MANVILLE, AND N. S. BHACCA, *Can. J. Chem.*, 47 (1969) 1; L. D. HALL AND J. F. MANVILLE, *ibid.*, 47 (1969) 19; D. HORTON AND W. N. TURNER, *J. Org. Chem.*, 30 (1965) 3387.

14 P. PERCHEMLIDES, T. OSAWA, E. A. DAVIDSON, AND R. W. JEANLOZ, *Carbohyd. Res.*, 3 (1967) 463.

15 H. S. PRIHAR AND E. J. BEHRMAN, *Abstr. Papers Amer. Chem. Soc.*, 162 (1971) BIOL-285.

16 W. A. BONNER, *J. Amer. Chem. Soc.*, 80 (1958) 3372.