$C{\equiv}CCO_2CH_3$, 111-12-6; $PhC{\equiv}CCH_3$, 673-32-5; $PhC{\equiv}CC_3H_7$, 4250-81-1; $PhC{\equiv}CCO_2CH_3$, 4891-38-7; PhC≡CPh, 501-65-5; $Hg(OAc)_2$, 1600-27-7; $CH_3C(OAc){=}C(HgOAc)(CH_2)_3CH_3$, 104114-79-6; $CH_3C(HgOAc){=}C(OAc)(CH_2)_3CH_3$, 104114-80-9; $CH_3C(OAc){=}C(HgOAc)(CH_2)_5CH_3$, 104114-81-0; $CH_3C(HgOAc){=}C(OAc)(CH_2)_5CH_3$, 104114-82-1; $PhC(OAc){=}C(HgOAc)CO_2CH_3$, 104114-83-2; $PhC(HgOAc){=}C(OAc)CO_2CH_3$, 104114-84-3.

Supplementary Material Available: Kinetic data and graphs of kinetic runs of 1,4-diacetoxy-2-butyne with mercuric acetate in acetic acid at 25 °C and 1-phenyl-1-pentyne with mercuric acetate in acetic acid at 25 °C (6 pages). Ordering information is given on any current masthead page.

α-D-Ribofuranosyl 1,2-Cyclic Monophosphate. Isolation, NMR Spectroscopic Properties, and Rates and Mechanism of Acid and Alkaline Hydrolysis

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Received April 3, 1986

The five-membered ring cyclic phosphate α-D-ribofuranosyl 1,2-cyclic phosphate has been synthesized, isolated, and characterized both spectroscopically and with respect to acid and alkaline lability. The ^{31}P NMR chemical shift (17 ppm downfield from internal HPO_4^{2-} at pH 8.0) is similar to that observed for 2′,3′-cAMP and considerably deshielded compared to that of the glucopyranosyl analogue (8.0 ppm). Alkaline hydrolysis of α-D-ribofuranosyl 1,2-cyclic phosphate and of α-D-glucopyranosyl 1,2-cyclic phosphate under identical conditions proceeded at very similar rates and in the former was demonstrated to result in hydroxide attack at the P atom (by employing a specifically ^{18}O-labeled substrate) and led to the formation of both sugar 1- and 2-phosphates. The fast rates of alkaline hydrolysis were similar to those reported for ethylene phosphate (Kumamoto, J.; Cox, J. R.; Westheimer, F. H. *J. Am. Chem. Soc.* **1956**, *78*, 3423), presumably due to the presence of a strained cyclic phosphate ring. Acid hydrolysis of α-D-ribofuranosyl 1,2-cyclic phosphate led to the exclusive formation of the ribose 2-phosphates, presumably by the scission of the anomeric C–O bond.

Two cyclic monophosphate esters of ribofuranose have been found to possess important biological function: the 3′,5′-monophosphates of nucleosides are second hormonal messengers,[1] the 2′,3′-monophosphates of nucleosides are intermediates in the ribonuclease-catalyzed reactions of ribonucleic acids.[2a,b] Although the synthesis of another class, the α-D-ribofuranosyl 1,2-cyclic phosphate was reported by Khorana and associates,[3] no purification, isolation, or spectral or chemical properties were reported. The well-documented behavior of the parent compound ethylene phosphate suggests that such five-membered ring phosphate esters may be both kinetically[4] and thermodynamically[5] unstable to hydrolysis compared to their acyclic analogues. Our interest in the 1,2-cyclic phosphates of ribose stems from the fact that enzymes on the purine salvage pathway employ as substrates α-D-ribofuranosyl 1-phosphate and 5-phospho-α-D-ribofuranosyl-1-pyrophosphate in phosphate (PO_4) and pyrophosphate transfer, respectively, and the related 1,2-cyclic phosphates may act as electrophilic traps for nucleophiles on these enzymes. In fact, we have preliminary evidence that indicates irreversible inhibition of purine nucleoside phosphorylase (EC 2.4.2.1) by α-D-ribofuranosyl 1,2-cyclic monophosphate.[6] Here we report improved synthesis, NMR spectroscopic properties and rates and mechanism of specific acid and base-catalyzed hydrolyses of this compound. In both its spectroscopic properties and alkaline hydrolytic lability the title compound resembles the 2,3-isomer.

Table I. Ascending Paper[a] Chromatography of Relevant Compounds

compound	solvent A[b]	solvent B[c]
α-D-ribofuranosyl 1-phosphate	0.05	0.33
α-D-ribofuranosyl 1,2-cyclic phosphate	0.56	0.73
α-D-glucopyranosyl 1-phosphate	0.09	0.28
α-D-glucopyranosyl 1,2-cyclic phosphate	0.38	0.65

[a] Whatman No. 31 E/T (0.53 mm, fast flow rate). [b] Isopropyl alcohol/NH_3/H_2O (7:1:2 v/v). [c] *n*-Propyl alcohol/NH_3/H_2O (6:1:3 v/v).

Table II. ^{31}P NMR Chemical Shifts of Cyclic Phosphates

compound	δ
α-D-ribofuranosyl 1,2-cyclic phosphate	17.0
5-phospho-α-D-ribofuranosyl 1,2-cyclic phosphate[b]	17.0
α-D-glucopyranosyl 1,2-cyclic phosphate	8.2
6-phospho-α-D-glucopyranosyl 1,2-cyclic phosphate[b]	8.0
2′,3′-cyclic AMP	17.5
3′,5′-cyclic AMP	−4.0

[a] Measured at 25 °C, pH 8.0, in the presence of 10 mM EGTA; positive sign represents downfield and a negative sign upfield from internal HPO_4^{2-}. [b] Synthesis and properties to be published.

Results and Discussion

Isolation and Purification of α-D-Ribofuranosyl 1,2-Cyclic Phosphate (1) and α-D-Glucopyranosyl 1,2-Cyclic Phosphate (2). The synthetic protocol enabled us to isolate larger amounts of 1, which then could be

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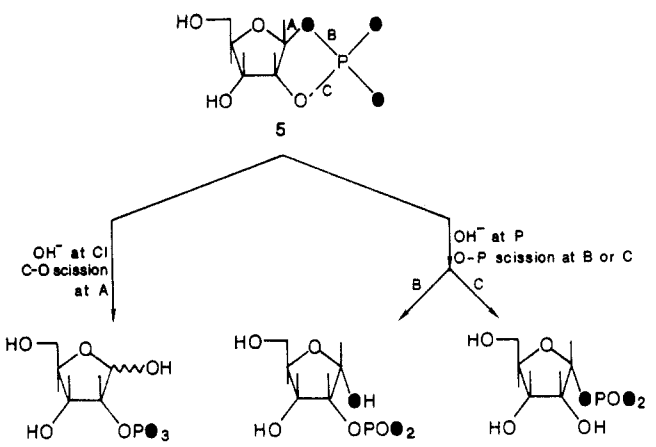
Scheme I



characterized both chemically and physically. α-D-Glucopyranosyl 1,2-cyclic phosphate was synthesized by a method different from that reported by Zmudzka and Shugar.[7] Recent interest in **2** as a potential inhibitor for phosphorylase[8] makes our much improved synthesis timely. The R_f values in two solvent systems are listed for the relevant compounds in Table I. Both solvents were capable of resolving the 1,2-cyclic phosphate from the sugar 1-phosphate.

Multinuclear NMR on α-D-Ribofuranosyl 1,2-Cyclic Phosphate. The ^1H and ^{13}C NMR spectra were assigned (see Experimental Section for assignments) by homonuclear decoupling of the former and specific heteronuclear decoupling experiments of the latter. Perhaps the most surprising (though precedented[9]) result of the ^{13}C assignments is the fact that while C3 is coupled to P with a significant size $J_{C3-C2-O-P}$ (6.3 Hz), C2 is not! C1 is coupled to P with a J_{C1-O-P} of 4.1 Hz.

The ^{31}P NMR chemical shifts show some unexpected features (Table II): the chemical shifts of α-D-ribofuranosyl 1,2-cyclic phosphate and 2′,3′-cyclic AMP are similar and those of α-D-glucopyranosyl 1,2-cyclic phosphate vastly different. For sake of comparison 3′,5′-cyclic AMP is included, clearly showing that five-membered and six-membered phosphate esters have very different chemical shifts as reported before by others.[10] The findings on **1** and **2** are more surprising in view of the very similar rates of alkaline hydrolysis found for the two compounds (vide infra). One must conclude that the ^{31}P NMR chemical shift is very strongly environment dependent, in addition to being dependent on both the charge and the bond angle deformation around the phosphorus atom.[10] The different environments in compounds **1** and **2** are the result of the variety of conformations that constitute the equilibrium mixture of the ribofuranosyl and glucopyranosyl rings.[9] A similarly large range of ^{31}P chemical shifts had been reported for a variety of five-membered ring derivatives of phosphorus.[11,12]

Rates and Regiospecificity of Alkaline Hydrolysis. Alkaline hydrolysis (between 0.1 and 0.001 N OH^-) of **1** always led to formation of both ribose 2-phosphates and of α-D-ribofuranosyl 1-phosphate in a ratio of ca. 4 to 1. In the present study we determined the regiospecificity

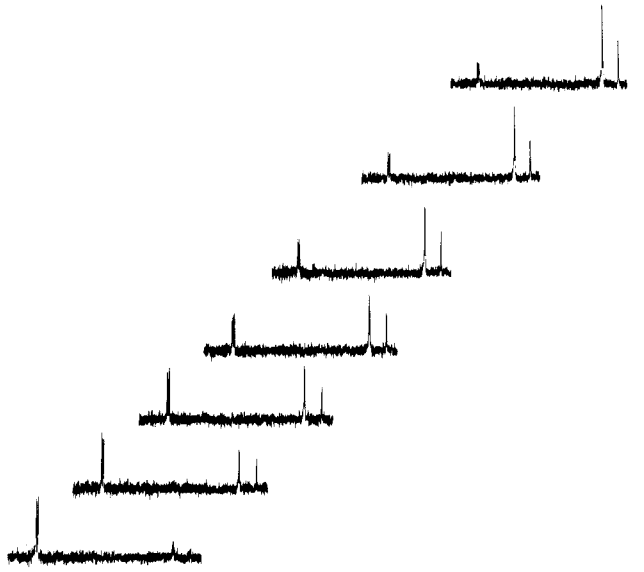
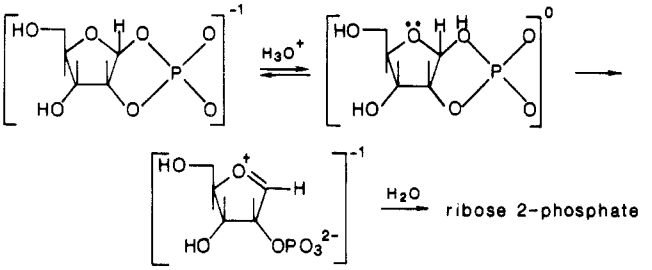


Figure 1. The time course of alkaline hydrolysis of α-D-ribofuranosyl 1,2-cyclic phosphate at pH 12.5, 21 °C, monitored by ^{31}P NMR. The assignments from lower to higher field are α-D-ribofuranosyl 1,2-cyclic phosphate, ribose 2-phosphate, and α-D-ribofuranosyl 1-phosphate.

Scheme II



and the scissile bonds during alkaline hydrolysis of **1** by following the working hypothesis in Scheme I and employing the specifically ^{18}O-labeled analogue of the title compound (**5**).

The products could be distinguished by ^{31}P NMR, according to the ^{18}O-induced upfield chemical shift.[13-15] Figure 1 depicts a typical time course of product formation during alkaline hydrolysis of **1**. C–O bond scission as an exclusive pathway is ruled out by the formation of both ribose 1- and 2-phosphates.

O–P bond scission is confirmed by the magnitude of the ^{18}O-induced shift on the ^{31}P chemical shift when **5** is hydrolyzed under alkaline conditions. The proton-decoupled spectra of ^{18}O-labeled compound **5** and of the "authentic" ^{16}O-labeled material are shown in Figure 2a; the 6-Hz chemical shift difference is consistent with the presence of two nonbridging and one bridging ^{18}O atom attached to P. The products of alkaline hydrolysis under proton decoupled conditions are shown in Figure 2b. The chemical shift difference of ^{16}O- and ^{18}O-labeled ribose 2-phosphates (group on left-hand side) is 4.0 Hz (two nonbridging ^{18}O atoms attached to P) and that of ^{16}O- and ^{18}O-labeled α-D-ribofuranosyl 1-phosphate is 5.1 Hz (consistent with two nonbridging and one bridging ^{18}O atom attached to P—predicted to give a ca. 6-Hz upfield shift). The combined evidence demonstrates exclusive attack by

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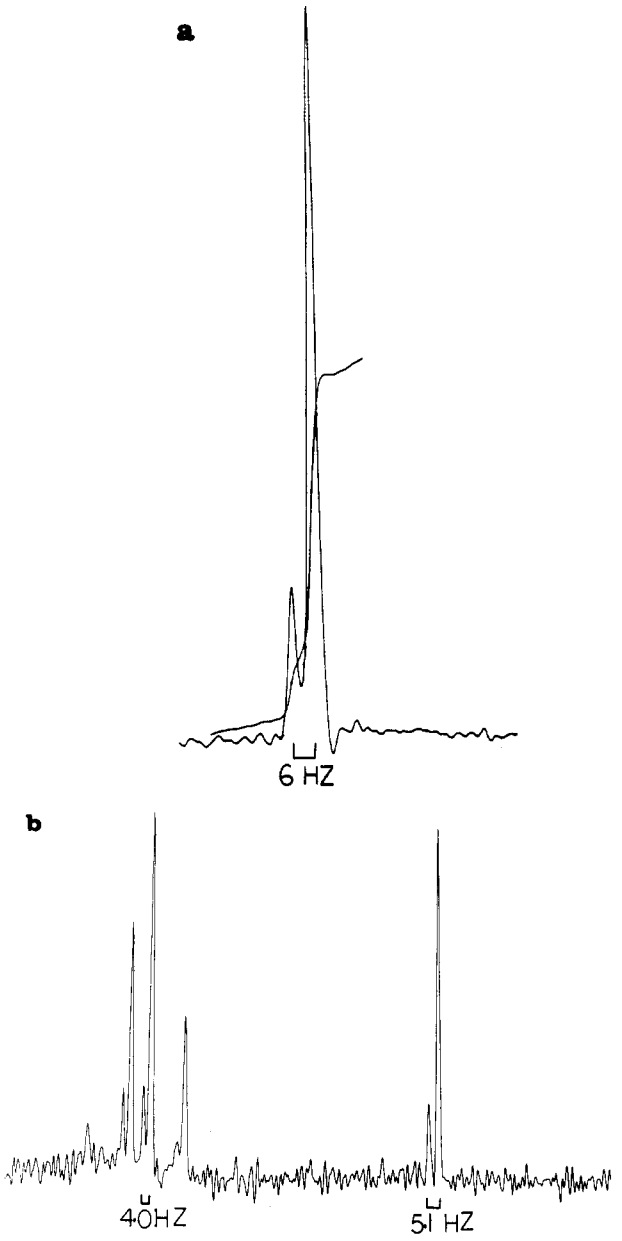

Figure 2. (a) Broad band decoupled ^{31}P NMR spectrum of $P-^{18}O_3$ enriched α-D-ribofuranosyl 1,2-cyclic phosphate at pH 8.0. The small downfield peak is added totally ^{16}O-labeled material for chemical shift reference. (b) Mixture of products resulting from the alkaline hydrolysis of α-D-ribofuranosyl 1,2-cyclic phosphate (shown in a) under broad band proton decoupled conditions. The downfield group represents ribose 2-phosphates, and the upfield pair represent α-D-ribofuranosyl 1-phosphate. The small peaks to the left of the larger ones are from totally ^{16}O-labeled material added for chemical shift comparison with the ^{18}O-enriched materials represented by the larger peaks.

OH^- at the P atom (Scheme I).

The rates of hydrolysis of **1** and **2** were compared under similar conditions. At pH 12.5 and 21 °C the half-life for hydrolysis of **1** was 34 min and that of **2** was 48 min (average of triplicate determinations). The alkaline hydrolysis was accompanied by anomerization of ribose 2-phosphate.

Some Results on the Acid-Catalyzed Hydrolysis of α-D-Ribofuranosyl 1,2-Cyclic Phosphate. On acid hydrolysis of **1**, the exclusive products observed by ^{31}P NMR are ribose 2-phosphates (Figure 3, the half-life at pH 1.5 and 21 °C was 15 min—the average of triplicate determinations). This is consistent with formation of an intermediate ribosylium cation (Scheme II), previously observed for the hydrolysis of sugar phosphates located on

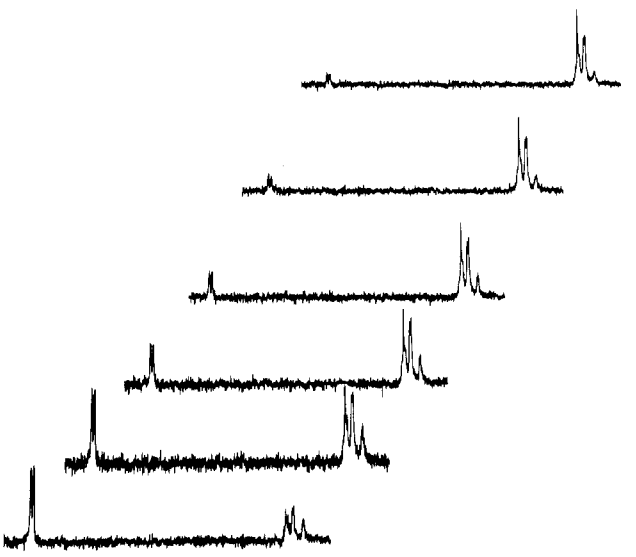

Figure 3. The time course of acid hydrolysis of α-D-ribofuranosyl 1,2-cyclic phosphate at pH 1.50, 21 °C, monitored by ^{31}P NMR. The lower field peak represents cyclic phosphate and the higher field set ribose 2-phosphates.

the anomeric carbon.[16] An alternative mechanism leading to anomerization and furanose–pyranose interconversion cannot be ruled out in view of the formation of all four ribose 2-phosphates (i.e., pyranoses and α- and β-furanoses) under the reaction conditions.

An inspection of all the spectra shows that anomerization and pyranose–furanose interconversion result under both acidic and alkaline conditions since several sets of ^{31}P signals result for ribose 2-phosphate even under proton decoupled conditions.

Finally, the relative rates of alkaline hydrolysis of **1** and **2** deserve mention. Both hydrolyze at rather similar rates under the same conditions of alkalinity and temperature. The observed rates are also similar to those determined for ethylene phosphate (at 25 °C and 0.14 N KOH we estimate a first order half-life of about 170 min from data in ref 4) and orders of magnitude faster than those estimated for dimethyl phosphate, the acyclic analogue.[4,17-19] The facile alkaline hydrolysis of **1** and **2** presumably reflects the presence of highly strained cyclic phosphate esters in these compounds, similar to those found in ethylene phosphate and in 2′,3′-cyclic nucleotides.

Experimental Section

General. All nuclear magnetic resonance spectra were recorded on an IBM WP 200-SY spectrometer operating in the Fourier transform mode: proton at 200.13 MHz [chemical shifts in D_2O recorded in ppm downfield from internal 4,4-dimethyl-4-silapentane-1-sulfonic acid, sodium salt (DSS)]; phosphorus at 81.026 MHz [chemical shifts (in 90/10 v/v H_2O/D_2O containing 10 mM EGTA) in ppm from internal HPO_4^{2-} at pH 8.0]; carbon at 50.31 MHz [chemical shifts in D_2O in ppm downfield from an external capillary of tetramethylsilane (Me_4Si)].

Paper chromatography was used routinely to monitor the homogeneity of the products and was carried out on Whatman 31 E/T paper (0.53 mm, fast flow rate) with solvent A (7:1:2 2-propanol/NH_3/H_2O) or solvent B (6:1:3 1-propanol/NH_3/H_2O). Prior to developing the chromatograms the paper was first washed with 1 N HCl and then with distilled water. The phosphate

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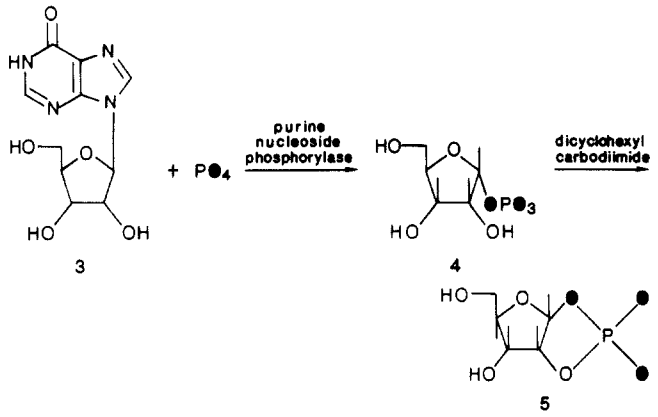
Scheme III



compounds were detected by spraying with ammonium molybdate reagent.[20]

α-D-Ribofuranosyl 1,2-Cyclic Phosphate (1). To a stirred solution of α-D-ribofuranosyl 1-phosphate dicyclohexylammonium salt (0.150 g, 0.35 mmol) in 2.5 mL of formamide was added dicyclohexylcarbodiimide (0.300 g, 1.45 mmol) dissolved in 8 mL of *tert*-butyl alcohol, followed by 2.5 mL of 2 N ammonium hydroxide. The suspension was warmed to effect dissolution, and the resulting homogeneous solution was heated under reflux. The progress of the cyclization was monitored by paper chromatography using solvent system A. The conversion was completed in 2 h. The reaction was stopped after 2.7 h, and the solution was allowed to cool to room temperature. The *tert*-butyl alcohol was removed at a rotary evaporator. The residual solution was mixed with an equal amount of water and extracted with ether (3 × 5 mL). The dicyclohexylurea was removed after the first extraction. The aqueous solution was concentrated under a vacuum (0.02 torr) to remove the formamide. The residue was dissolved in methanol (3 mL) and applied to a Dowex 50 (cyclohexylammonium form) column (20 × 0.9 cm). The column was eluted with methanol (15 mL). The methanol was removed, and a cold solution of ethanol/acetone (1:4, 30 mL) was added to the residue. The monocyclohexylammonium salt of α-D-ribofuranosyl 1,2-cyclic phosphate precipitated out. The precipitate was washed four times with cold ethanol/acetone (10 mL), yielding 1 as a hygroscopic white powder (0.088 g, 81%); R_f (solvent A) 0.56, (solvent B) 0.73; ^{1}H NMR (D_2O/DSS, pH_{app} 8.0) 5.91 ppm (dd, 1 H, J = 4.1, 16.98 Hz, C1-H), 4.85 (m, 1 H, C2H), 3.94 (m, 1 H, C3H), 4.09 (m, 2 H, C5H's), 3.72 (m, 1 H, C4H); ^{13}C NMR (broad band decoupled, 90/10 H_2O/D_2O, pH 8.0, external Me_4Si) 101.1 ppm (d, J = 4.1 Hz, C1), 80.3 (s, C4), 78.4 (s, C2), 69.7 (d, J = 6.3 Hz, C3), 59.7 (s, C5); ^{31}P NMR (proton coupled, 90/10 H_2O/D_2O, pH 8.0, internal HPO_4^{2-}) 17.38 ppm (ddd, J = 1.4, 4.6, 17.00 Hz). Anal. Calcd for $C_5H_8PO_7C_6H_{14}N{\cdot}0.5H_2O$: C, 41.25; H, 6.92; N 4.37; P, 9.67. Found: C, 41.26; H, 7.11; N, 4.46, P, 9.41.

α-D-Glucopyranosyl 1,2-Cyclic Phosphate (2). α-D-Glucopyranosyl 1-phosphate disodium salt (150 mg, 0.49 mmol) was converted to the free acid by using Dowex 50 (H^+ form, 12 mL in water). After 10 min the resin was removed by filtration and washed with H_2O (4 × 5 mL), and the combined filtrates were evaporated to dryness under reduced pressure, yielding a colorless residue. Next 2.5 mL of CH_3OH was added to the residue followed by the addition of 0.300 g (1.45 mmol) dicyclohexylcarbodiimide dissolved in 6 mL *tert*-butyl alcohol, 2.5 mL of 2 N ammonium hydroxide, and 2.5 mL formamide. The clear homogeneous solution that resulted upon warming the mixture was heated at reflux for 2.7 h. TLC using solvent system A gave one spot (R_f 0.38). The reaction mixture was allowed to cool to room temperature, and *tert*-butyl alcohol and CH_3OH were removed under reduced pressure. The residue was diluted with an equal volume of water and filtered through a fine sintered-glass filter to remove the dicyclohexyl urea, and the filtrate was extracted with ether (3 × 5 mL). The aqueous layer was concentrated at a rotary evaporator and left overnight under high vacuum. The resultant glassy residue was dissolved in 20 mL water and passed through a Dowex 50 column (cyclohexylammonium form). The aqueous solution was lyophilized, and cold methanol/acetone (1:4, 25 mL) was added to the residue. The α-D-glucopyranosyl 1,2-cyclic phosphate monocyclohexylammonium salt precipitated out and was washed with cold methanol/acetone (4 × 10 mL) and dried over P_2O_5 under a high vacuum, yielding **2** (106.5 mg, 77%): ^{31}P NMR (90/10 H_2O/D_2O, HPO_4^{2-} internal standard, pH 8.06) 8.2 ppm (dd, J = 1.8, 17 Hz). Anal. Calcd for $C_6H_{10}PO_8{\cdot}1.3C_6H_{14}N$: C, 44.63; H, 7.65; N, 4.94. Found: C, 44.56; H, 8.07; N, 5.14.

Synthesis of ^{18}O-Labeled α-D-Ribofuranosyl 1,2-Cyclic Phosphate (5). The reaction scheme in Scheme III was adopted (O = ^{16}O, ● = ^{18}O). Compound **4** was synthesized enzymatically[21] from $H_3P^{18}O_4$ that had been synthesized from PCl_5 and $H_2{}^{18}O$ (ref 22). α-D-Ribofuranosyl [1-$^{18}O_4$]phosphate (**4**) (100 mg, 0.43 mmol) was reacted wth 150 mg dicyclohexylcarbodiimide in 6 mL of *tert*-butyl alcohol for 2.5 h at reflux. After extraction with ether (3 × 3 mL) and removal of solvent, a glassy residue of **5** resulted. ^{31}P NMR at 81.026 MHz gave a chemical shift ca. 6-Hz upfield from totally ^{16}O-labeled material, consistent with the presence of one bridging and two nonbridging ^{18}O atoms at the P atom.[21]

Kinetic Methods. The specific acid and base catalyzed hydrolyses of **1** and **2** were monitored by ^{31}P NMR spectroscopy, taking advantage of the very different ^{31}P chemical shifts of **1** (17 ppm), ribose 2-phosphate (subject to anomerization and pyranose–furanose equilibration giving four sets of signals ca. 2 ppm downfield from P_i), and α-D-ribofuranosyl 1-phosphate (near the chemical shift of internal P_i). Data were collected at prescribed intervals, and electronic integration was employed to determine the fraction of starting material remaining at different time intervals. The pH of the solutions was adjusted on a Radiometer pHm 62 meter equipped with a combination microelectrode.

Acknowledgment. Financial support by NIH-GM 26682, the Rutgers University Busch Fund, the BRSG of NIH, and Hoffmann-LaRoche Inc. Nutley, NJ is gratefully acknowledged, as are the elemental analyses performed by Dr. F. J. Scheidl of Hoffmann-LaRoche Inc. Nutley, NJ.

Registry No. **1**, 90275-35-7; **2**, 64161-84-8; **4**, 103669-03-0; **5**, 103669-04-1; 2′,3′-cyclic AMP, 634-01-5; 3′,5′-cyclic AMP, 60-92-4; α-D-ribofuranosyl 1-phosphate dicyclohexylammonium salt, 103669-02-9; α-D-glucopyranosyl 1-phosphate disodium salt, 56401-20-8; α-D-glucopyranosyl 1-phosphate, 59-56-3; 5-phospho-α-D-ribofuranosyl 1,2-cyclic phosphate, 90275-36-8; 6-phospho-α-D-glucopyranosyl 1,2-cyclic phosphate, 103669-05-2.

Supplementary Material Available: ^{31}P NMR of compound **1** and the first-order rate plots of the alkaline and acid hydrolysis of **1** (Figures 4–6, respectively) (4 pages). Ordering information is given on any current masthead page.

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