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PREPARATION OF DICYCLOPROPYLCARBINYL PHOSPHOROCHLORIDATE AND ITS USE IN PHOSPHORYLATION REACTIONS[1]

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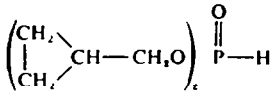
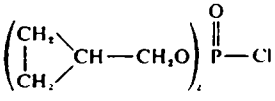
Abstract—Dicyclopropylcarbinyl phosphorochloridate has been synthesized for use in phosphorylation reactions. This reagent and primary or secondary alcohols gave the corresponding esters. It reacted with primary and secondary hydroxyl functions of nucleosides to form nucleotide triesters. Dicyclopropylcarbinyl methyl phosphate was hydrolyzed by aqueous acid to monomenthyl phosphate. The nucleotide triesters gave free nucleotides in amounts reflecting their labilities in acid. Thus, uridine-5′ phosphate was produced in 53% overall yield from 2′,3′-isopropylidene-uridine, whereas thymidine-3′ phosphate was obtained in only 12% yield from 5′-O-tritylthymidine.

SEVERAL phosphorylating agents have been developed for use in preparation of nucleotides during recent years.[2] In addition, the search for better reagents continues.[3] We have reacted dicyclopropylcarbinyl phosphonate (I) with N-chlorosuccinimide to give dicyclopropylcarbinyl phosphorochloridate (II) as an unstable oil which decomposed on attempted distillation. It was characterized by its conversion with ammonia



I



II

to dicyclopropylcarbinyl phosphoramidate (III) and by spectroscopic evidence. The IR spectrum of II showed strong absorption at 7·70 μ, indicative of a free P=O group, and also at 9·65 and 10·10 μ, indicative of the P—O—C group.[4] The NMR spectrum of II showed two multiplets at 0·53 and 1·22 ppm of relative area 4 to 1·1 respectively, characteristic of a monosubstituted cyclopropane.

[1] This investigation was supported by Public Health Service Research Grant No. CA-02857 from the National Cancer Institute.

[2] [a]. H. G. Khorana, *Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest*. J. Wiley, New York (1961); [b]. A. M. Michelson, *The Chemistry of Nucleosides and Nucleotides*. Academic Press, New York and London (1963); [c]. D. M. Brown in *Advances in Organic Chemistry* (Edited by Ralph, Taylor and Wynberg) Vol. III. Interscience, New York and London (1963).

[3] V. M. Clark, D. W. Hutchinson, A. J. Kirby and S. G. Warren, *Angew. Chem., Intern. Ed., Engl.* 3, 678 (1964).

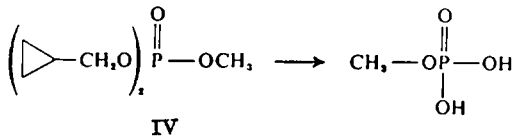
[4] L. Bellamy, *The Infrared Spectra of Complex Molecules* (2nd Edition) J. Wiley, New York (1958).

Dibenzyl phosphorochloridate, a compound closely related to II, was introduced two decades ago.[5] It converts blocked nucleosides having a free 5′-hydroxyl function to nucleotide dibenzyl esters.[6] The benzyl groups are conveniently removed by hydrogenolysis to give free nucleotides. Limitations have been reported in the application of this reagent. For example, it has not been possible to phosphorylate the secondary hydroxyl group of 5′-O-acetyl-2′-deoxyadenosine.[7]

A dialkyl phosphorochloridate might be a more effective reagent than dibenzyl phosphorochloridate, provided that the alkyl groups can be removed under mild conditions after phosphorylation. Cyclopropylcarbinyl esters are apparently unexplored in phosphate ester chemistry. It has been shown that cyclopropylcarbinyl benzenesulfonate undergoes solvolysis in absolute ethanol 500 times faster than ethyl benzenesulfonate and 14 times faster than allyl benzenesulfonate.[8] Solvolysis of cyclopropylcarbinyl chloride in acetic acid and in 50% aqueous ethanol gave similar rate differences.[9] It seemed reasonable to expect that cyclopropylcarbinyl phosphate esters would also undergo rapid solvolyses.

Methanol reacted with II in ether in the presence of pyridine to give dicyclopropylcarbinyl methyl phosphate (IV, 88%). 2-Propanol reacted with II to give dicyclopropylcarbinyl 2-propyl phosphate (V, 70%). Dicyclopropylcarbinyl phenyl phosphate (VI, 89%) was prepared from phenyl phosphorodichloridate and cyclopropylcarbinol. Distillations of IV–VI were complicated by rearrangement to the corresponding cyclobutyl and 3-butenyl esters. Thus, the main fraction from distillation (0·04 mm) of VI was di-3-butenyl phenyl phosphate. In a separate experiment, VI was heated to 165° for 15 min. NMR analysis showed the oil to contain cyclopropylcarbinyl, cyclobutyl, and 3-butenyl esters in the ratio 1:4·6:1·9. Distillation of V generally gave up to 50% rearranged esters, whereas IV gave up to 20% rearranged esters. The percentage of rearranged material could be minimized by distilling IV and V in small amounts and by heating the distillation pot rapidly. Undistilled esters were used in the hydrolysis experiments.

Treatment of IV with 80% aqueous formic acid for 30 min at reflux gave methyl phosphate (87%). NMR analysis of the product showed a doublet at 3·78 ppm and a broad singlet at 8·57 ppm having a relative area ratio 2·8:2·0. Hydrolysis of VI with



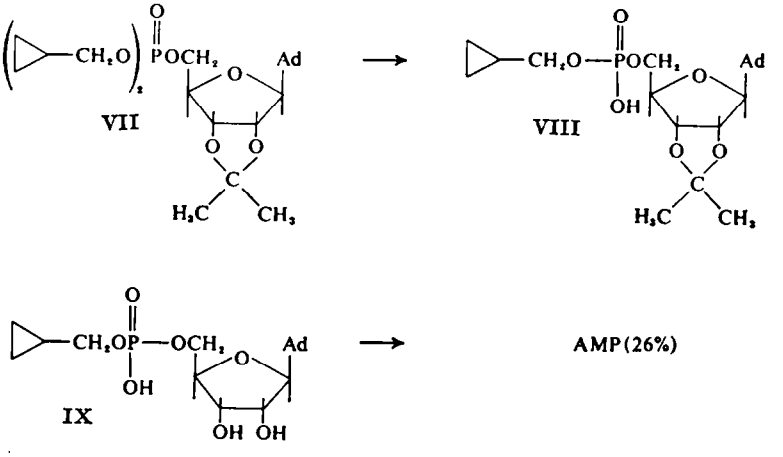
IV

80% aqueous formic acid for 2 hr gave phenyl phosphate (92%). The high yields of monosubstituted phosphates obtained under the hydrolysis conditions used were as

[5] L. Zervas, *Naturwiss.* **27**, 317 (1939); F. R. Atherton, H. T. Openshaw and A. R. Todd, *J. Chem. Soc.* 382 (1945).
[6] J. Baddiley and A. R. Todd, *J. Chem. Soc.* 648 (1947) and later papers in series.
[7] D. H. Hayes, A. M. Michelson and A. R. Todd, *J. Chem. Soc.* 808 (1955).
[8] C. G. Bergstrom and S. Siegel, *J. Amer. Chem. Soc.* **74**, 145, 254 (1952); E. Tommila and M. Lindholm, *Acta Chem. Scand.* **5**, 647 (1951).
[9] J. D. Roberts and R. H. Mazur, *J. Amer. Chem. Soc.* **73**, 2509 (1951).

expected, in light of kinetic studies in acidic media of trimethyl phosphate.[10] The first order rate constant for the hydrolysis of trimethyl phosphate in 0·2 M perchloric acid was only $0{\cdot}373 \times 10^{-4}$ sec^{-1} at 100°. The rate remained nearly the same for acid concentrations ranging up to 3·0 M perchloric acid. Thus, refluxing trimethyl phosphate in 80% aqueous formic acid for 30 min produced no detectable change in its NMR spectrum. Other monoalkyl and monoaryl phosphates might also be synthesized using II, especially those compounds containing functions which are sensitive to the hydrogenolysis conditions used for removal of phenyl or benzyl blocking groups.

2′,3′-O-Isopropylideneadenosine (1 mole) reacted with II (2 moles) in pyridine solution between −50° and −55° to give 2′,3′-O-isopropylideneadenosine-5′ dicyclopropylcarbinyl phosphate (VII). Formation of VII was usually accompanied by a smaller amount of less soluble material. A similar residue, obtained from the preparation of 2′,3′-O-benzylideneadenosine-5′ dibenzyl phosphate, was designated N-phosphorylated isomer.[11] The NMR spectrum of VII showed two multiplets at 0·42 and 1·08 ppm of relative area 4 to 1.1 indicating that the ester contained cyclopropylcarbinyl groups. Hydrolysis of VII in 10% aqueous acetic acid at reflux for 5 hr proceeded stepwise as outlined below.



The initial step was removal of one cyclopropylcarbinyl group to give 2′,3′-O-isopropylideneadenosine-5′ monocyclopropylcarbinyl phosphate (VIII), followed by conversion to adenosine-5′ phosphate monocyclopropylcarbinyl ester (IX). Neither VIII nor IX was isolated. Evidence is based on data from paper chromatography (Experimental). Spots assigned to VII and VIII gave negative benzidine-periodic acid spray tests[12] for vicinal diols. Those of IX and adenosine-5′ monophosphate (AMP) gave positive tests. In addition, VII could be hydrolyzed exclusively to VIII by refluxing in 0·1N NaOH for 2 hr. After refluxing VII for 5 hr in 10% aqueous acetic acid, it was estimated that the reaction mixture contained AMP (50%), IX (30%), adenine

[10] P. W. C. Barnard, C. A. Bunton, D. R. Llewellyn, C. A. Vernon and V. A. Welch, *J. Chem. Soc.* 2670 (1961).

[11] A. M. Michelson and A. R. Todd, *J. Chem. Soc.* 2476 (1949).

[12] *Chromatographic and Electrophoretic Techniques* (Edited by Ivor Smith) Vol. I, Interscience, New York (1960).

(20%), and no VII or VIII. AMP was isolated as the barium salt in 26% yield. Longer reflux periods gave more hydrolysis to adenine without increasing the yield of AMP.

The efficient production of AMP, then, is limited by the susceptibility of the bond joining the sugar and base to acid catalyzed cleavage and by the slow hydrolysis of IX to AMP. It has been reported[11] that AMP gave 20% cleavage to adenine by heating at 100° with 0·1N H_2SO_4 for 5 hr. In a recent study, a linear free energy relationship was proposed in which a constant L, characteristic of the leaving group, was compared

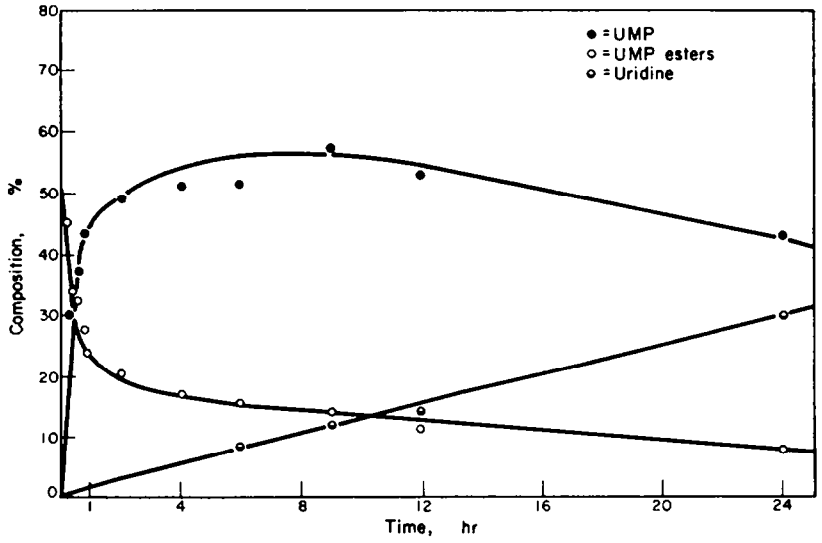


FIG. 1. Hydrolysis of 2′,3′-O-Isopropylideneuridine-5′ dicyclopropylcarbinyl phosphate (X) in refluxing 10% aqueous acetic acid.

roughly with the pK_a of the acid formed in both S_N1 and S_N2 reactions.[13] While phosphates were not included in this study, it follows that alkyl dicyclopropylcarbinyl phosphate esters should solvolyze easily, since the pK_a of the acid formed is in the range 1 to 2.[14] The pK_a of the acid formed upon removal of the second cyclopropylcarbinyl group is 6 to 6·5, which leads to the prediction that this reaction should proceed slower than solvolysis of cyclopropylcarbinyl acetate (pK_a acetic acid = 4·72). Indeed, it is well known that secondary phosphate esters are hydrolysed very slowly under mild conditions.[15]

Perhaps more important is the possibility of conversion of VII to less reactive esters by internal return. Cyclopropylcarbinyl chloride is known to isomerize to 3-butenyl chloride and cyclobutyl chloride under solvolysis conditions.[9] Since pyrimidine nucleotides are less labile in acid than purine ribonucleotides,[11,16] the hydrolysis of 2′,3′-O-isopropylideneuridine-5′ dicyclopropylcarbinyl phosphate (X) with 10% aqueous acetic acid was studied. The amounts of uridine-5′ monophosphate (UMP), UMP monoester (XI), and uridine which were formed over a 24 hr period, are summarized graphically in Fig. 1. It should be observed that even though only 34% XI

[13] C. G. Swain and K. H. Lohmann, in *Solvolysis Mechanisms* (Edited by E. R. Thornton) pp. 164–166. Ronald Press, New York (1964).

[14] See Ref. 2*b*, p. 141.

[15] J. R. Cox and O. B. Ramsay, *Chem. Rev.* **64**, 317 (1964).

[16] A. M. Michelson, *Tetrahedron* **2**, 333 (1956).

remained after 30 min hydrolysis 8% was still present after 24 hr. In light of the previous work[17] it is likely that the monocyclopropylcarbinyl ester was converted to the less reactive monocyclobutyl and mono-3-butenyl esters during hydrolysis. Preliminary solvolysis experiments in aqueous media show that simple cyclopropylcarbinyl esters can also give products due to internal return. Figure 1 also shows that the maximum yield of UMP is obtained using a 6 to 10 hr hydrolysis period. Hydrolysis to uridine becomes limiting for periods longer than 10 hr.

Compound X was isolated as an oil (93%) which was hydrolyzed to UMP (54%) by refluxing in 10% aqueous acetic acid for 6 hr. Direct conversion of 2',3'-O-isopropylideneuridine to UMP without isolation of X gave a similar yield. Cytidine-5' monophosphate (CMP) was prepared in 33% yield, without isolating the intermediate ester, 2',3'-O-isopropylidenecytidine-5' dicyclopropylcarbinyl phosphate (XII).

5'-O-Tritylthymidine reacted with II to give 5'-O-tritylthymidine-3' dicyclopropylcarbinyl phosphate (XIII). Hydrolysis of XIII with 10% aqueous acetic acid for 2 hr gave 23% thymidine-3' phosphate (3'TMP). The phosphorylation of the secondary hydroxyl group of this nucleoside proceeded in fair yield but the hydrolysis step gave a low yield of 3'TMP, in agreement with the greater lability of deoxyibonucleotides than ribonucleotides in aqueous acid.[16] A summary of compounds hydrolyzed and yields of nucleotides obtained is given in Table 1.

Although nucleotide preparations from II were accomplished in modest yields compared with other methods, this method gives neutral nucleotide ester intermediates from which all blocking groups can be removed under mild acidic conditions in a single step.

TABLE 1. DICYCLOPROPYLCARBINYL NUCLEOTIDE ESTERS AND THEIR NUCLEOTIDE HYDROLYSIS PRODUCTS

Nucleoside	Ester (% yield)	Nucleotide	Reflux Period hr	Yield of Nucleotide (%)[a] From Ester	From Nucleoside[b]
2',3'-O-Isopropylideneadenosine	VII (60)	AMP	5	26	18
2',3'-O-Isopropylideneuridine	X (93)	UMP	5	54	53
2',3'-O-Isopropylidenecytidine	XII (—)	CMP	5	—	33
5'-O-Tritylthymidine	XIII (59)	3'TMP	2	23	12

[a] Yields were calculated by measuring intensity of UV absorption at λ max of the barium salts.
[b] These values are for runs where esters were not isolated.

EXPERIMENTAL

NMR spectra were obtained using a Varian A60 spectrometer. CCl_4 was used as solvent unless otherwise noted. All peak positions are in ppm from internal tetramethylsilane reference.

Pyridine was dried over calcium hydride before use. All evaporations were performed under vacuum below 40°.

Dicyclopropylcarbinyl phosphonate (I). A solution of cyclopropylcarbinol (7·2 g, 0·10 mole) and pyridine (8·71 g, 0·11 mole) was added dropwise to a stirred solution of PCl_3 (6·87 g, 0·05 mole) in ether (100 ml) at 0° over a 30 min period. After another 15 min a second portion of cyclopropylcarbinol (3·6 g, 0·05 mole) was added. The mixture was warmed to room temp and stirred for 12 hr. After filtration of the pyridine hydrochloride, the solution was evaporated to give a clear oil (9·1 g, 96%). This oil was sufficiently pure for subsequent syntheses. The analytical sample distilled at 90–94° (0·24 mm). (Found: C, 50·49; H, 8·15; P, 16·55; $C_8H_{15}O_3P$ requires: C, 50·52; H, 7·95; P, 16·29%.)

IR spectrum: 4·07 μ (P—H); 7·94 (P=O); 9·64, 10·32 (P—O—CH_2).

[17] M. C. Caserio, W. H. Graham and J. D. Roberts, *Tetrahedron* **11**, 171 (1960).

Dicyclopropylcarbinyl phosphorochloridate (II). Dicyclopropylcarbinyl phosphonate (2·85 g, 0·015 mole) in benzene (50 ml) was treated with N-chlorosuccinimide (2·0 g, 0·015 mole). The mixture, which became warm at first, was allowed to stand at room temp for 2 hr. The precipitated succinimide was collected by filtration and the solution evaporated to give an oil (3·1 g, 91%). Attempted distillation resulted in decomposition. IR spectrum: 7·70 μ (P=O); 9·65, 10·10 (P—O—CH_3). NMR spectrum: 0·53 (multiplet) (area 8·0); 1·22 (multiplet) (2·2); 4·0 (2 doublets) (4·0).

Dicyclopropylcarbinyl phosphoramidate (III). A stream of ammonia gas was passed through a solution of II (1·69 g, 0·0075 mole) in CCl_4 (75 ml) at 0° for 5 min. The mixture was allowed to stand at room temp for 6 hr, then heated to boiling and filtered hot. Ligroin (*d*, 0·64; 50 ml) was added to the hot filtrate and the solution stored at 0° for 12 hr. Filtration gave the white fluffy product (1·3 g, 85%) m.p. 75·5–77·5°. Recrystallization from benzene-cyclohexane gave the analytical sample, m.p. 79–80° (corrected). (Found: C, 46·84; H, 7·71; N, 6·82; P, 14·63; $C_8H_{16}NO_3P$ requires: C, 46·82; H, 7·86; N, 6·83; P, 15·10%.) NMR spectrum: 0·47 (multiplet) (area 8·0); 1·18 (multiplet) (2·2); 3·88 (multiplet) (6·0).

Dicyclopropylcarbinyl methyl phosphate (IV). Dicyclopropylcarbinyl phosphorochloridate (6·7 g, 0·03 mole) in ether (50 ml) was added to a stirred solution of MeOH (0·96 g, 0·03 mole) and pyridine (3·56 g, 0·045 mole) in ether (100 ml) at 0° over a 30 min period. After another 30 min, the mixture was allowed to stand at room temp for 12 hr. Pyridine hydrochloride was removed by filtration. The solution was evaporated to give a clear liquid (5·8 g, 88%) which was of sufficient purity to be used for hydrolysis studies. The analytical sample distilled at 95–97° (0·15 mm). (Found: C, 49·50; H, 8·09; P, 14·25; $C_9H_{17}O_4P$ requires: C, 49·09; H, 7·78; P, 14·07%.) NMR spectrum: 0·47 (multiplet) (area 8·0); 1·13 (multiplet) (2·1); 3·79 (3 doublets) (6·9).

Dicyclopropylcarbinyl 2-propyl phosphate (V). Dicyclopropylcarbinyl phosphorochloridate (3·37g, 0·015 mole) in ether (50 ml) when added to a stirred solution of 2-propanol (0·90 g, 0·015 mole) and pyridine (1·82 g, 0·023 mole) in ether (100 ml.), as in the preparation of IV, gave an oil which was dissolved in $CHCl_3$ (75 ml). The solution was washed with 1N HCl (75 ml), 1N NaOH (75 ml) and twice with water (75 ml each). The organic layer was dried (Na_2SO_4) and the solvent evaporated to give a colourless oil (2·5 g, 67%). The analytical sample distilled at 121–125° (1·0 mm). Some isomerized material was obtained from this distillation. (Found: C, 53·00; H, 8·83; P. 11·98; $C_{11}H_{21}O_4P$ requires: C, 53·21; H, 8·53; P, 12·48%.) NMR spectrum: 0·47 (multiplet) (area 8·0); 1·29 (doublet) (4·8); 3·82 (2 doublets) (4·0); 4·61 (heptet) (1·1).

Dicyclpropylcarbinyl phenyl phosphate (VI). Phenyl phosphorodichloridate (5·25 g, 0·25 mole) was added dropwise to a stirred solution of cyclopropylcarbinol (3·60 g, 0·05 mole) and pyridine (5·93 g, 0·075 mole) in ether (150 ml) at 0° over a 30 min period. The mixture was stirred at room temp for 18 hr and filtered. The solution was evaporated to give an oil which was redissolved in ether and washed with 1N HCl (100 ml), 1N NaOH (100 ml) and twice with water (100 ml each). The ether layer was dried (Na_2SO_4) and evaporated to give a pale yellow oil (6·3 g, 89%). NMR spectrum: 0·44 (multiplet) (area 8·0); 1·18 (multiplet) (2·25); 3·93 (2 doublets) (4·0); 7·24 (singlet) (5·25).

Thermal isomerization of VI *to di-3-butenyl phenyl phosphate.* Dicyclopropylcarbinyl phenyl phosphate was distilled to give as the main fraction (135–136° at 0·04 mm) di-3-butenyl phenyl phosphate. (Found: C, 60·06; H, 7·21; P, 10·97; $C_{14}H_{19}O_4P$ requires: C, 59·57; H, 6·79; P, 10·97%.) NMR spectrum: 2·40 (quartet) (area 4·5); 4·11 (2 triplets) (3·9); 5·08 (multiplet) (4·0); 5·80 (multiplet) (2·0); 7·23 (singlet) (5·0).

Hydrolysis of IV *to methyl phosphate.* Treatment of IV (0·771 g, 0·0035 mole) with 80% aqueous formic acid (5 ml) for 30 min under reflux gave a clear colourless solution, which was evaporated leaving an oil. Final traces of formic acid were removed by passing a stream of N_2 over the oil for 30 min, followed by keeping the oil under vacuum for 4 hr. The oil (0·382 g, 97%) was shown to be 90% pure methyl phosphate by NMR analysis.

NMR spectrum* (dimethyl sulfoxide–d_6): 3·78 (doublet) (area 2·8); 8·57 (broad singlet) (2·0).

Hydrolysis of VI *to phenyl phosphate.* Dicyclopropylcarbinyl phenyl phosphate (2·82 g, 0·010 mole) was treated with 80% aqueous formic acid (25 ml) under reflux for 2 hr to give an amber solution which was evaporated to give an oil. EtOH (50 ml) and benzene (50 ml) were added and the

* The peak position of the acidic protons was dependent on concentration. The solution was prepared by dissolving the entire sample in 0·6 ml DMSO-d_6.

solution evaporated again. The semi-solid residue was triturated with CCl_4 to give crude phenyl phosphate (1·7 g) m.p. 65–75°. Recrystallization from $CHCl_3$–CCl_4 gave 1·6 g solid (92%) m.p. 94–98° (corrected) identical in properties with an authentic sample.

2′,3′-O-Isopropylideneadenosine-5′ dicyclopropylcarbinyl phosphate (VII). 2′,3′-O-Isopropylideneadenosine (2·31 g, 0·0075 mole) which had been recrystallized from 2-propanol and dried 12 hr at 125° was dissolved in pyridine (30 ml) with warming. The solution was cooled rapidly in a 2-propanol bath at −50 to −55°. Dicyclopropylcarbinyl phosphorochloridate, prepared from 2·85 g (0·015 mole) of I was added to the cold solution with shaking. After 4 hr, the bath temperature had risen to −40°. The mixture was stored in a freezer (−17°) for 12 hr and then warmed to room temp before work up. Sodium carbonate (2 g) and water (10 ml) were added and the mixture evaporated to an oil. Chloroform (100 ml) and water (100 ml) were added. The $CHCl_3$-layer was washed twice with 1% $NaHCO_3$aq (100 ml each) and then dried (Na_2SO_4). The solution was filtered and evaporated to give a yellow oil which was twice dissolved in EtOH (50 ml) and evaporated. EtOH (50 ml) was added and the solution filtered to remove unreacted 2′,3′-O-isopropylideneadenosine. Ligroin (*d*, 0·64; 300 ml) was added and the solution cooled at 0° for 1 hr. The supernatant was decanted from a small amount of semi-solid material and left at −17° for 5 days. Seeding helped crystallize the product (2·5 g, 68%) m.p. 129–131°. The analytical sample was recrystallized from CCl_4, m.p. 131–133° (corrected). (Found: C, 51·07; H, 6·03; N, 14·39; P, 6·41; $C_{21}H_{30}N_5O_7P$ requires: C, 50·90; H, 6·10; N, 14·14; P, 6·25%.) IR spectrum: 3·00 and 3·20 μ (NH_2); 7·92 (P=O); 9·65, 9·90 and 10·15 (P—O—CH_2). NMR spectrum ($CDCl_3$): 0·42 (multiplet) (area 8·0); 1·08 (multiplet) (2·25); 1·51 (doublet) (6·5); 3·87 (multiplet) (4·0); 4·40 (multiplet) (3·5); 5·19 (multiplet) (1·25); 5·47 (multiplet) (1·25); 6·20 (doublet) (1·0); 6·54 (broad singlet) (2·0); 8·03 (singlet) (1·0); 8·40 (singlet) (1·0).

Esters X and XIII were prepared similarly, but X could not be crystallized. Solidification of XIII usually occurred after the second evaporation of ethanol. If XIII was an oil at this stage, it was solidified by triturating with ligroin (*d*, 0·64).

Hydrolysis of VII *to AMP.* Compound VII (0·10 g, 0·20 mmole) was dissolved in 1-propanol (1 ml) with warming and 10% aqueous acetic acid (10 ml) added. This solution was refluxed 5 hr to give a colourless solution which contained three UV absorbing components by paper chromatography; VIII, 30%; AMP, 50%; and adenine, 20%. The solution was treated with conc. NH_4OH (2 ml) and evaporated to small volume (2 ml). This solution was streaked on thick paper* and the chromatogram developed in solvent A. The slower moving band (R_f ·15 to ·40) was cut out and eluted with water (500 ml), filtered, and evaporated to small volume (5 ml). Barium acetate solution (0·5 M, 1 ml) was added and a small amount of residue was removed by filtration. EtOH (10 ml) was added and the mixture stored 1 hr at 0°. The white precipitate was collected by centrifugation, washed with EtOH and ether, and dried 6 hr under vacuum over P_2O_5. AMP barium salt (0·052 g) was 50% pure by UV unalysis using $\varepsilon_{259} = 15{,}400$ at pH 7. The yield calculated as pure AMP was 26%. Paper chromatography of the product showed a single spot. UMP and 3′TMP were prepared similarly from X and XIII respectively. Results of these experiments are recorded in Table 1.

Synthesis of UMP from 2′,3′-O-isopropylideneuridine without isolation of X. 2′,3′-O-Isopropylideneuridine (0·135 g, 0·48 mmole) was dissolved in pyridine (3 ml) and the solution treated with II, prepared from 0·285 g (1·5 mmole) of I at −50 to −55° in a 2-propanol bath. After 5 hr the mixture was placed in the freezer (−17°) for 18 hr and then warmed to room temp. Water (1 ml) was added and the solvent evaporated. EtOH (25 ml) was added to the oil and the solution was again evaporated.

Compound	R_f values		
	Solvent A	Solvent B	Solvent C
VII	0·92	0·88	0·85
VIII	0·77	0·25	—
IX	0·64	0·08	0·69
AMP	0·24	0·01	0·36
Adenine	0·65	0·44	0·58

* Schleicher and Schuell 470A filter paper was used for preparative scale experiments.

The oil was treated with 10% aqueous acetic acid (25 ml) and the solution refluxed for 5 hr. The cooled solution was treated with excess conc NH_4OH (8 ml), evaporated to small volume (3 ml) and streaked on two sheets of thick filter paper which were developed in solvent A. Elution of the slower moving band (R_f 0·15 to 0·35) and isolation of the barium salt as in the preparation of AMP gave UMP (0·083 g, 53%) calculated using $\varepsilon_{262} = 10{,}000$ at pH 7. AMP, CMP and 3'TMP were prepared similarly. Hydrolysis times and yields are given in Table 1.

Paper chromatography of AMP and related compounds. Paper chromatography was carried out using an ascending technique on Whatman 1 MM paper; solvent A, 1-propanol-1% NH_4OHaq, 3:2; solvent B, 1-butanol–water, 86:14, solvent C, EtOH–1M triethylammonium bicarbonate, 5:2. Nucleotides were located by viewing under a UV lamp. Compounds containing vicinal diol functions were detected using a benzidine-periodic acid spray.[11]