

CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 11 No. 8

August 1963

[Chem. Pharm. Bull]
11 (8) 961 ~ 967

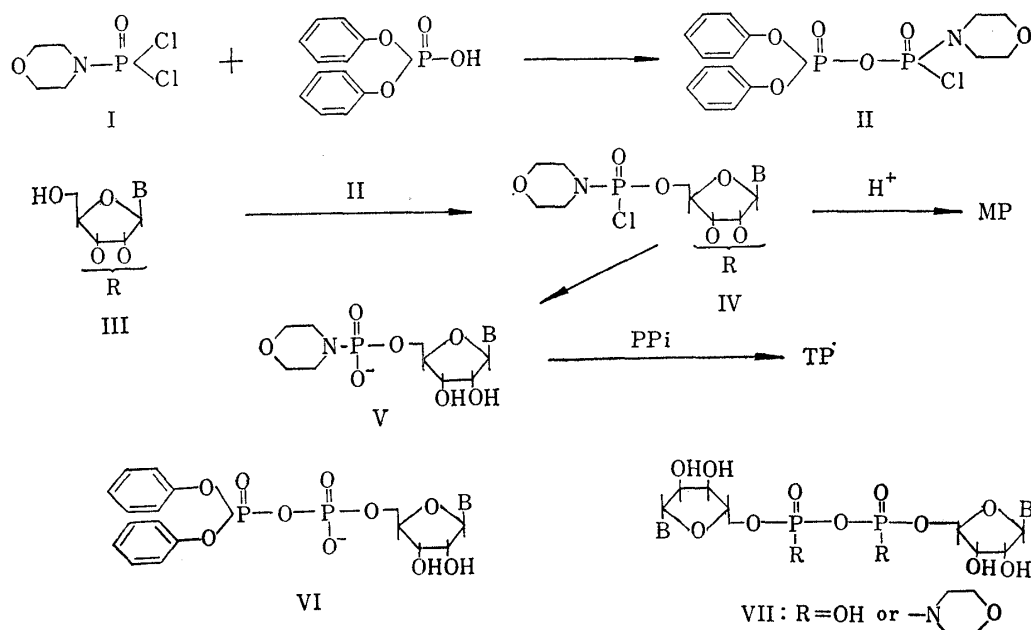
UDC 577.15 : 542.958[546.185]

165. Morio Ikehara and Eiko Ohtsuka : Studies on Coenzyme Analogs.
XV.*¹ A Novel Phosphorylating Agent, P-Diphenyl-,
P'-Morpholino Pyrophosphorochloridate.*²

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Recently, we have reported¹⁾ the synthesis of morpholino phosphorodichloridate and its use in the synthesis of 5'-mono- and tri-phosphates of several nucleosides. After phosphorylation, the morpholidate group of the reagent was easily removed by mild acidic treatment and the group could be used for the further reaction with inorganic phosphate salt. The phosphorylating power of reagent (I) was, however, weak and raise of the reaction temperature lead to bifunctional reaction.²⁾ This behavior of this reagent caused some difficulty for the purification of resulting polyphosphate.

With this in mind, we have synthesized P-diphenyl P'-morpholino pyrophosphorochloridate (II), a phosphoric anhydride type reagent, which proved to be a powerful phosphorylating agent as well known in the case of O-benzylphosphorous O,O-diphenyl-



*¹ Part XIV. M. Ikehara, E. Ohtsuka : This Bulletin, 11, 435 (1963).

*² A preliminary account of the results was reported in this Bulletin, 10, 997 (1962).

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1) M. Ikehara, E. Ohtsuka : This Bulletin, 10, 536, 539 (1962).

2) H.G. Khorana : "The Nucleic Acid" Vol. III, p. 105 (1950), Academic Press Inc., New York.

phosphoric anhydride,³⁾ tetra-*p*-nitrophenylpyrophosphate⁴⁾ and tetrachloropyrophosphate.⁵⁾ Reagent (II) was synthesized by the direct reaction of equimolar morpholino phosphorodichloridate and diphenyl phosphate in the presence of excess 2,6-lutidine as acid acceptor. Because of low reactivity of second chloridate residue in I, the reaction did not proceed to the formation of bis-type compound. The structure of reagent (II) was strongly supported by following evidences, though it was not unequivocally established. First, the precipitate of 2,6-lutidine hydrochloride weighed to be almost equimolar to the reagent. Second, as stated by Khorana⁶⁾ in the case of morpholidate situated on the pyrophosphate, chloridate residue of fully protected pyrophosphate is relatively inert for nucleophilic attack. Third, as described below, the yield of nucleoside morpholino phosphorochloridate increased from 50~60% with reagent (I) to almost quantitative with II. Furthermore, with the use of limited amount of diphenyl phosphate, fairly high yield of phosphorylation was observed. These facts clearly shows the existence of P-diphenyl P'-morpholino pyrophosphorochloridate as true phosphorylating species. Paper electrophoresis of reagent (II) revealed only three spots corresponding to diphenyl phosphate, morpholino phosphate and inorganic phosphate, thus indicating rapid hydrolysis of pyrophosphate linkage prior to the cleavage of morpholidate residue.

The phosphorylation of primary hydroxyl group⁷⁾ of appropriately protected nucleoside with this reagent was then investigated. 2',3'-O-Isopropylideneadenosine and 2',3'-O-isopropylideneuridine were phosphorylated in dioxane solution with two equivalents of reagent (II). Forty hours' reaction at 20° converted quantitatively nucleoside (III) to 5'-morpholino phosphorochloridate (IV). It was revealed by paper electrophoresis as a non-migrating, phosphorus-containing spot,⁸⁾ with another spot of known 5'-phosphoromorpholidate,⁹⁾ which appeared by hydrolysis of chloridate residue during the electrophoretic migration. The possibility of nucleophilic attack on phosphorus atom attached to diphenyloxy group by hydroxyl of nucleoside might be excluded, since morpholino-chlorophosphate seems to be much weaker acid than diphenyl phosphate.³⁾

Nucleoside 5'-morpholino phosphorochloridate, thus obtained, was hydrolyzed by the addition of water at pH 2.0. A large excess of water and rapid hydrolysis were necessary to avoid the pyrophosphate formation.⁹⁾ Extraction of diphenyl phosphate and amines at appropriate pH followed by precipitation with barium acetate and alcohol gave AMP and UMP*⁴ in the yield of 60 and 57%, respectively. The characteristics of these compounds were summarized in Table I. 2',3'-O-Isopropylidene-9- β -D-ribofuranosyl-6-dimethylaminopurine¹⁰⁾ and 9-(4'-hydroxybutyl)adenine¹¹⁾ were phosphorylated by reagent (II) in the similar manner described above. The structure of monophosphates of these compounds were established by the direct comparison with the authentic specimen^{11,12)} by paper electrophoresis and paper chromatography (Table I).

*⁴ Abbreviations : AMP, adenosine 5'-monophosphate; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; UMP, uridine 5'-monophosphate.

- 3) N. S. Corby, G. W. Kenner, A. R. Todd : J. Chem. Soc., **1952**, 3669, 3675.
- 4) R. W. Chambers, J. G. Moffatt, H. G. Khorana : J. Am. Chem. Soc., **79**, 3747 (1957).
- 5) H. Grunze, W. Koransky : Angew. Chem., **71**, 407 (1959).
- 6) J. G. Moffatt, H. G. Khorana : J. Am. Chem. Soc., **83**, 649 (1961).
- 7) When the unprotected nucleoside was phosphorylated with reagent (II), 10~20% phosphorylation of primary hydroxyl group was observed (M. Ikehara, E. Ohtsuka : prepared for publication in this Bulletin).
- 8) This was superposed with the spot of nucleoside and assumed to be AMP-morpholino-amidate in reference,³⁾ page 1. Now, it is clarified that it contained also morpholinochloridate, which had mainly converted to morpholidate at the reaction.
- 9) V. M. Clark, G. W. Kirby, A. R. Todd : J. Chem. Soc., **1957**, 1497.
- 10) M. Ikehara, T. Ueda, S. Horikawa, A. Yamazaki : This Bulletin, **10**, 665 (1962).
- 11) M. Ikehara, E. Ohtsuka, S. Kitagawa, K. Yagi, Y. Tonomura : J. Am. Chem. Soc., **83**, 2679 (1961).
- 12) M. Ikehara, E. Ohtsuka, F. Isikawa : This Bulletin, **9**, 173 (1961).

TABLE I. Monophosphate Barium Salt obtained from Various Nucleosides

Starting material	Product	Electrophoretic mobility (cm.)	Rf ^{a)}		Isolated yield (%)	Purity ^{c)} (%)	ϵ_{260}^{pH7}
			A	B			
isp.-Adenosine	AMP-Ba	10.7	0.41	0.30	60	81	1.5×10^4
isp.-Uridine	UMP-Ba	10.2	0.44	0.30	57	71	1.0×10^4
isp.-(CH ₃) ₂ -Adenosine	(CH ₃) ₂ -AMP-Ba	9.5	0.30		33 ^{b)}	—	1.8×10^4
9-(4'-OH-Butyl)adenine	9-(4'-OH-butyl)adenine-MP-Ba	10.7	0.44		47 ^{b)}	—	1.5×10^4

a) Solvent system was as indicated in the experimental

b) The yield was estimated in the form of solution

c) Calculated photometrically on the weight basis from ϵ indicated in the last column

As described in the preceding paper¹⁾ of this series, nucleoside 5'-morpholino phosphorochloridate (IV) or 5'-phosphoromorpholidate (V), will form 5'-polyphosphate by the reaction with inorganic phosphate salt. In order to avoid the concomitant cleavage of the morpholidate residue, acetyl group was chosen as the protective group, which would be removed by alkaline treatment. 2',3',-Di-O-acetyladenosine was phosphorylated with reagent (II) by the slightly modified method involving the change in molar ratio of reactants from 1:2:4 to 1:2:6 for nucleoside-reagent-2,6-lutidine.¹³⁾ In spite of the 2,6-lutidine existing in the reaction mixture, the second chloridate residue did not react further with diphenyl phosphate. It might be attributed to electrostatic deactivation of chlorine atom and steric repulsion by bulky nucleoside. As in the case of isopropylideneadenosine, the quantitative conversion of nucleoside to 5'-morpholino phosphorochloridate was shown by electrophoresis.

Adenosine 5'-morpholidate (V) was obtained by rapid hydrolytic cleavage of chloridate residue, which was followed by the deacetylation with ammonia-methanol solution containing equivalent amount of water. This compound was identified with an authentic sample synthesized according to Khorana⁶⁾ by paper electrophoresis. In the following reaction with pyrophosphate, solvent was replaced by anhydrous pyridine,¹⁴⁾ in order to obtain one-phase mixture. The extent of the reaction was analyzed either by electrophoresis or by ion-exchanger column chromatography. Results were listed in Tables II and III and results obtained in phosphorylation of 3'-deoxythymidine¹⁵⁾ were also tabulated in Table III. The higher yield of triphosphate was obtained by 24 hours' reaction.¹⁶⁾

TABLE II.

	Nucleoside + X ^{a)} (%) ^{b)}	AMP-morpholidate	AMP	ADP	ATP
5 hr.	13	34	3	18	29
24 "	15	30	5	18	32
50 "	25	25	7.5	27	25
5 days	17	15	23	27	19

a) Unidentified phosphate superposed on nucleoside, presumably be AMP-(morpholino)phosphoroamidate and (morpholino)phosphorochloridate

b) Estimated photometrically from the extract of UV absorbing spot cut out from the paper of electrophoresis

13) In the latter reaction additional 2 moles of lutidine was used to prevent hydrolysis of chloridate of resulting product.

14) H.G. Khorana, M. Smith : J. Am. Chem. Soc., **80**, 1141 (1958).

15) A.M. Michelson, A.R. Todd : J. Chem. Soc., **1955**, 816.

16) This is somewhat longer than the reaction carried out with Khorana's morpholidate salt of 4-morpholine N,N'-dicyclohexylcarboxamidine.⁶⁾ The difference may be attributed to the use of salt of different base and the existence of trace amount of water, which could not be removed absolutely by the co-distillation with anhydrous pyridine. However, undesired disproportionation of triphosphate was also much slower than in the former case.

TABLE III.

	Nucleoside (%) ^c	MP-morpholidate	MP	DP	TP	Higher P
10 hr. ^{a)}	11	26	14	13	29	7
24 " ^{a)}	10	14	17	11	33	10
20 " ^{b)}	35	2	12	27	14	9

a) Reaction of diacetyladenosine

b) Reaction of 3'-deoxythymidine

c) Calculated from the optical density units of separated peak eluted from Dowex-I-X8 (Cl⁻ form) ion-exchanger column (elution with 0.003*N* HCl + 0.35*M* LiCl by gradient elution technique). Each fractions were evaporated and tested for their uniformity by paper chromatography and electrophoresis

Several side reactions would be expected to occur with the proceeding of above reaction. P-Diphenyl P'-adenosine pyrophosphate (VI) may be formed from morpholidate and diphenyl phosphate. But, compound (VI) is a reactive phosphorylating species¹⁷⁾ and would form triphosphate by the reaction with inorganic pyrophosphate existing in large excess. Second possible reaction to form symmetric diadenosine pyrophosphate (VII) having one or two morpholidate residues will be negligible as described in Khorana's report.¹⁸⁾ To avoid contamination of the desired product with diphenylphosphate, it was rapidly extracted from slightly acidic solution in advance to the chromatography

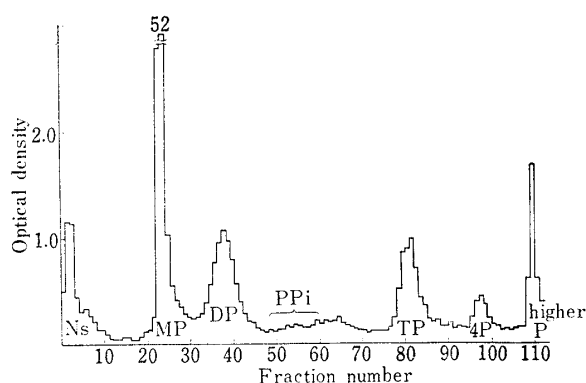


Fig. 1. Eluting Pattern of Ion-exchanger Chromatography of Adenosin-5' TP

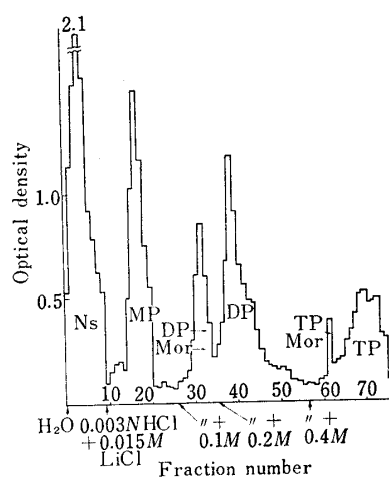


Fig. 2. Eluting Pattern of Ion-exchanger Chromatography of 3'-Deoxythymidine-TP

17) F. Cramer, R. Wittman : Chem. Ber., **94**, 322, 328 (1961).

18) In the experiment of reference 6) compound of the type (VII) has been isolated substantially. But in present condition, a small amount of unreacted reagent would be degraded in the time course of the reaction.

on ion-exchanger column. If necessary,¹⁹⁾ active charcoal treatment was recommended to remove inorganic phosphate. In the case of the reaction with 3'-deoxythymidine the removal of pyrophosphate was necessary, since it was eluted in the same fraction as deoxythymidine triphosphate and was hardly removed by reprecipitation technique (Fig. 1, 2).

Each fraction eluted in a single peak was collected, neutralized and concentrated *in vacuo*. Methanol-acetone precipitation¹⁴⁾ or anhydrous methanol washing technique²¹⁾ gave desired triphosphate as lithium salt. Further purification of the material was performed by reprecipitation from water-ethanol and tested by paper chromatography and electrophoresis.

By the use of P-diphenyl P'-morpholino pyrophosphorochloridate the synthesis of nucleoside polyphosphate is improved in the overall yield and handlings. Various types of triphosphate are now being synthesized in our laboratory and will be investigated as the substrate of actomyosin systems.²²⁾

Experimental

Paper Chromatography—Solvent A, iso-PrOH-1% (NH₄)₂SO₄=3:2; B, iso-PrOH-aq.-NH₃-H₂O=7:1:2; C, EtOH-MNH₄Ac=7:3 (pH 7.5). Descending technique was used except for Solvent C.

Paper Electrophoresis—0.05M triethylammonium bicarbonate, pH 7.5, 20 v./cm., 1 hr. Toyo Filter Paper Co. No. 51 A*⁵ was used.

P-Diphenyl P'-Morpholino Pyrophosphorochloridate—Morpholino phosphorochloridate (204 mg., 1.0 mmole) and diphenyl phosphate²³⁾ (250 mg., 1.0 m mole, dried over P₂O₅ at 3 mm./Hg at 80° for 5 hr.) was dissolved in anhydrous dioxane (2 ml., distilled over Na). Into this solution 2,6-lutidine²⁴⁾ (227 μl., 2.0 mmole, dried over KOH) was added. White precipitate began to appear several minutes after the addition of 2,6-lutidine²⁵⁾ and increased gradually during 15 min. at room temperature. This mixture was used *in situ* for further reaction. Examination of the mixture by paper electrophoresis showed three spots corresponding to morpholino phosphate (R_{AMP} 1.22, detected by molybdate spray²⁶⁾), diphenyl phosphate (R_{AMP} 0.85, detected by UV lamp and molybdate spray) and inorganic phosphate (R_{AMP} 1.45, detected by molybdate spray).

Adenosine 5'-Monophosphate—Into a reagent solution obtained by a procedure described above (containing 1.0 mmole of P-diphenyl, P'-morpholino pyrophosphorochloridate) 154 mg. (0.5 mmole) of 2',3'-O-isopropylideneadenosine (dried over P₂O₅ at 5 mm./Hg, at 60° for 5 hr.) was added. The reaction mixture was kept at room temperature for 48 hr. under exclusion of moisture. Addition of 20 ml. of H₂O made the solution turbid. When this was heated at 70°, the turbidity disappeared. After heating at 70° for 1 hr., whole was concentrated *in vacuo* to ca. 10 ml. The solution was extracted with CHCl₃ (5 × 20 ml.), adjusted to pH 8.5 with N LiOH and extracted again with Et₂O (5 × 10 ml.) thoroughly. Two molar solution of Ba(OAc)₂ (1.0 ml.) was added and resulting precipitate was removed by centrifugation. Precipitate was washed with H₂O, washing was combined to the supernatant and concentrated to a small bulk. Addition of 2 volumes of EtOH and storage in a refrigerator overnight gave white fluffy precipitate, which was collected by centrifugation. Washing with EtOH, Me₂CO and Et₂O, and drying at 1 mm./Hg for 5 hr. over P₂O₅ gave 0.3 g. of AMP-Ba. Purity estimated photometrically on the weight basis was 58% (calculated from ε₂₆₀=1.5 × 10⁴). Yield calculated as pure AMP was 75%. Purification by reprecipitation from H₂O-2 volumes of EtOH gave 178 mg. of 81% pure

*⁵ When acid untreated filter paper was used, migrating distance of di- and tri-phosphate fluctuated greatly.

19) In the case of reference 6), ATP was eluted after inorganic pyrophosphate and charcoal absorption was omitted.

20) R. W. Chambers, H. G. Khorana : J. Am. Chem. Soc., **79**, 3752 (1957).

21) R. W. Chambers : *Ibid.*, **18**, 3032 (1959).

22) M. Ikehara, E. Ohtsuka, S. Kitagawa, Y. Tonomura : Biochim. Biophys. Acta., in press.

23) P. Brigl, H. Müller : **72**, 2121 (1939).

24) H. Engel : Ind. Eng. Chem., **40**, 168 (1948).

25) When precipitate began to appear immediately after the addition of 2,6-lutidine, the reagent mixture should not be used. This phenomena was caused by HCl previously formed by the hydrolysis of reagent with contaminating moisture.

26) C. S. Hanes, F. A. Isherwood : Nature, **164**, 1107 (1949).

(photometrically estimated) material. Yield 60% as pure AMP. Paper chromatography showed single spot at R_f 0.30 (solvent B) and 0.41 (solvent A). Paper electrophoresis, R_{AMP} 1.0.

Uridine 5'-Monophosphate—Synthesis of UMP was performed by an analogous procedure as described for AMP. Phosphorylation of 2',3'-O-isopropylideneuridine (0.5 mmole) with 0.6 mmole of reagent (II) showed the extent of 81% after 44 hr. at 25° (migrating distance on paper electrophoresis, 5.2 cm., R_{AMP} 0.59). Further 24 hrs'. reaction gave 0.18 g. (dried over P₂O₅ at 3 mm./Hg for 5 hr.) of UMP-Ba. Purity and chromatographical behaviors were listed in Table I.

9-β-D-Ribofuranosyl-6-dimethylaminopurine 5'-Monophosphate—9(2', 3'-O-Isopropylidene)-β-D-ribofuranosyl-6-dimethylaminopurine (1.5 g., 4.5 mmole) was phosphorylated with reagent (II) (8 mmole) as described above. After usual work-up procedure, N⁶-dimethyl-AMP was isolated as crude Ba-salt. This sample was titrated with N H₂SO₄ (Na Rhodizonate as indicator), BaSO₄ was removed, and the filtrate was estimated photometrically. 1.36 mmole (33%) of N⁶-dimethyl-AMP was obtained. This solution was taken into dryness *in vacuo* and used *in situ* for further reaction. Paper chromatographical and electrophoretic behaviors were listed in Table I.

9-(4'-Hydroxybutyl)-6-aminopurine 4'-Monophosphate—9(4'-Hydroxybutyl)-6-aminopurine (275 mg., 1.33 mmole) was phosphorylated with reagent (II) (2.66 mmole) by an analogous procedure as described in the preceding section. 9(4'-Hydroxybutyl)-6-aminopurine 4'-monophosphate was obtained in a yield of 47% (0.58 mmole). Paper chromatographical and electrophoretic behaviors were listed in Table I.

Phosphorylation of Isopropylideneadenosine by the Use of a limited Amount of Diphenylphosphate—Morpholino phosphorodichloridate (122 mg., 0.6 mmole), diphenylphosphate (75 mg., 0.3 mmole) and 2,6-lutidine (137 μl., 1.2 mmole) was dissolved in 2 ml. of dioxane in this order. After 15 min., 154 mg. (0.5 mmole) of 2',3'-O-isopropylideneadenosine was added. Reaction was carried out at room temperature for 2 days under exclusion of moisture. Paper electrophoresis showed a spot at R_{AMP} 0.57 corresponding to that of 5'-phosphoromorpholidate. Reaction extent was estimated as 70% from UV absorption of the eluant of this spot. Further 4 days' reaction gave 87% phosphorylation.

Adenosine 5'-Triphosphate—Into a dioxane solution (5 ml.) of 2 mmole of reagent (II) (prepared from morpholino phosphorodichloridate (408 mg., 2 mmole), diphenylphosphate (500 mg., 2 mmole) and 2,6-lutidine (681 μl., 6.0 mmole)), 351 mg. (1 mmole) of 2',3'-di-O-acetyladenosine was added. Reaction was carried out at 20° under exclusion of moisture. After 40 hr. reaction was almost complete, which was confirmed by a spot on paper electrophoresis (R_{AMP} 0.58, migratory distance 6.2 cm.). Resulting white precipitate (mainly lutidine hydrochloride) was rapidly filtered and filtrate was combined with MeOH previously saturated with NH₃ containing 4 mmole of H₂O. After 15 min. the main spot converted to R_f 0.62 (solvent A), which corresponded to diacetyl-AMP-morpholidate. Examination of the reaction mixture after standing overnight revealed a spot of R_{AMP} 0.58 on paper electrophoresis and R_f 0.48 (solvent A), 0.45 (solvent B) on paper chromatogram. These values were well coincided with those of an authentic sample.⁶⁾ Solvent was removed under reduced pressure and dried by codistillation with anhyd. pyridine. Residue was taken up in 5 ml. of anhyd. pyridine,⁶⁾ and a pyridine solution (5 ml.) of 5 mmole of *bis*-tributylammonium pyrophosphate was added. At each intervals listed in Tables II and III, an aliquot was extracted and examined either by paper electrophoresis or by ion-exchanger chromatography (Dowex-1X8, Cl⁻ form, eluted with 0.003 N HCl and 0.003 N HCl + 0.35 M LiCl by gradient elution technique). Results were showed in Tables II and III. From these analyses it was concluded that the reaction should not exceed 24 hr. at room temperature. Reaction mixture was diluted with 20 ml. of H₂O and pyridine was removed by Et₂O extraction. H₂O-layer was concentrated to a small bulk under reduced pressure and adjusted to pH 7.5 with N LiOH. This solution was applied to a column of Dowex-1X3 (Cl⁻ form 2 × 8 cm., 200~400 mesh) and eluted with 0.003 N HCl and 0.003 N HCl + 0.35 M LiCl by concave gradient elution technique. ATP-fraction was collected, neutralized with LiOH and evaporated to a small bulk in rotary evaporator at 20~25°. Two volumes of EtOH was added and the clear solution was diluted with 20 volumes of Me₂CO. Storage of the solution in a refrigerator in stoppered flask caused the precipitation of ATP Li-salt. This was collected by centrifugation, washed with Me₂CO, Et₂O and dried over P₂O₅ at 3 mm./Hg. Analysis of this specimen by spectrophotometry showed 68.3% pure ATP·Li₄ (ε₂₆₀ = 1.5 × 10⁴). Yield, 186 mg. (20.8% calculated from diacetyladenosine). Paper electrophoresis: R_{AMP} 1.5. Paper chromatography: R_f 0.18, R_{AMP} 0.46 (solvent A), R_f 0.03, R_{AMP} 0.25 (solvent C). *Anal.* Found: Total P-labile P-adenosine = 2.95: 1.92: 1.00 (theory 3:2:1).

3'-Deoxythymidine 5'-Triphosphate—The synthesis was carried out by an essentially analogous procedure as described for ATP. 3'-Deoxythymidine¹⁵⁾ (130 mg., 0.56 mmole) was phosphorylated with reagent (II) (1.16 mmole) for 40 hr. at room temperature. Reaction extent estimated photometrically from a spot (R_{AMP} 0.64) on paper of electrophoresis was 75%. Evaporated reaction mixture was caused to react with 2.5 mmole of *bis*-tributylammonium pyrophosphate for 20 hr. at room temperature (see

*⁶ At this stage if crystalline residue hardly soluble in anhydrous pyridine was obtained, it was solubilized by the addition of 1 mole of tributylamine.

Table III). Reaction was stopped by H₂O (20 ml.), extracted with Et₂O, and absorbed on 5 g. of activated charcoal and eluted with 50% EtOH containing 2% of NH₃ (400 ml.). Eluant was evaporated *in vacuo* to a small bulk and adjusted to pH 3.5 with NH₃. Chromatography on Dowex-1×8 (Cl⁻ form, 200~400 mesh, 2×8 cm.) column, which was eluted with HCl-LiCl system by stepwise elution, gave the following results :

Peak	Eluting buffer	Compound	Migratory distance (cm.)	R _{AMP}	Yield (%)
I	H ₂ O	nucleoside	0.7	0.08	35.2
II	0.003NHCl+0.015MLiCl	MP	9.6	1.13	11.5
III	0.003NHCl+0.1MLiCl	DP-morpholidate	—	—	11.0
IV	0.003NHCl+0.2MLiCl	DP	11.5	1.47	16.5
V	0.003NHCl+0.3MLiCl	TP	13.2	1.55	14.0
VI	2NHCl	higher P	13.5	1.59	9.0

Elution pattern was shown in Fig. 2. Triphosphate fraction was collected, neutralized with NLiOH to pH 7.0 and evaporated in a rotary evaporator to a small bulk under reduced pressure at 15~20°. Resulting syrup was taken up in 2 volumes of EtOH and diluted with 20 volumes of Me₂CO to cause the precipitation of TP Li-salt. After keeping it in a refrigerator for 1 hr., resulting white precipitate was collected by centrifugation. Washing with Me₂CO and Et₂O and drying at 3 mm./Hg over P₂O₅ gave 46 mg. of 3'-deoxythymidine 5'-triphosphate Li₄. Analysis by spectrophotometry on the weight basis showed 60.5% purity (calculated as $\epsilon_{265} 8.0 \times 10^3$). *Anal.* Found: total P-labile P-deoxythymidine=2.87:1.95:1.00 (theoretical, 3:2:1).

Authors wish to thank Mr. K. Narita, Miss A. Maeda and T. Tohma of this Faculty for elementary analyses. A part of this work was financially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, which is gratefully acknowledged.

Summary

P-Diphenyl P'-morpholino pyrophosphorochloridate was prepared from morpholino phosphorochloridate and diphenylphosphate and used for the phosphorylation of isopropylidene-adenosine, -uridine, -N⁶-dimethyladenosine and 9-(4'-hydroxybutyl)adenine. AMP, UMP, N⁶-dimethyl-AMP and 9-(4'-hydroxybutyl)adenine 4'-monophosphate was obtained in 33~60% isolated yield. By the reaction of products obtained in the phosphorylation of 2',3'-di-O-acetyladenosine and 3'-deoxythymidine with this reagent, with pyrophosphate salt, ATP and 3'-deoxythymidine 5'-triphosphate were synthesized in good over-all yield.

(Received March 9, 1963)