[CONTRIBUTION FROM THE NUTRITION AND PHYSIOLOGY DEPARTMENT, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

Nucleotides of 2-(2′-Deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5(2,4)-dione (Azathymidine)

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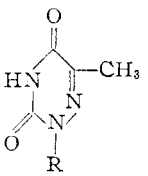
A large scale preparation of 2-(2′-deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5(2,4)-dione is described. This compound was phosphorylated to yield two mono- and one diphosphate. The two mononucleotides were distinguished by the action of a specific 5′-nucleotidase. The effectiveness of these compounds, as inhibitors of DNA synthesis, was compared using a rabbit bone marrow system.

Much interest has been centered recently on the evolvement of specific antagonists of deoxyribonucleic acid (DNA) synthesis because in rapidly proliferating tissue, such as tumors, DNA synthesis is far more rapid than in normal tissues. Such a specific antagonist of DNA biosynthesis is the thymine analog, 6-methyl-*asym*-triazine-3,5-(2,4)-dione (Azathymine) (I), which has been extensively investigated by Welch and Prusoff, and collaborators.[2] They have shown that the deoxyriboside of 6-methyl-*asym*-triazine-3,5(2,4)-dione (II) is a much more potent thymine (or thymidine) antagonist than the free base, through studies with bacteria[3] and mammalian tissue.[4] These studies emphasize the value of nucleoside analogs as antimetabolites over the corresponding free bases.[5] In view of this "enhancement" of antagonism it was of interest to extend the investigation to the corresponding nucleotides. This paper describes a large scale preparation of compound II, adapted from the original enzymatic synthesis of Prusoff,[6] and the phosphorylation of this nucleoside. The relative effectiveness of the free base, nucleoside and nucleotides as antagonists of DNA synthesis was compared using a rabbit bone marrow system.



I, R = H
II, R = 2-deoxy-D-ribose

2-(2′-Deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5(2,4)-dione (II) was synthesized enzymatically according to equation 1 by incubating the

thymidine + "azathymine" (I) ⟶ "azathymidine" (II) + thymine (1)
(1 mole) (100 moles)

reactants with washed resting cells of *Streptococcus faecalis* (ATCC 8043) in a phosphate buffer. After incubation and removal of the cells and inorganic ions the problem became one of isolation of a small quantity of compound II from larger amounts of unreacted I, thymine and uracil.[7] The latter compound may have arisen because of enzymatic demethylation of thymine or it may have been extracted from the cells. No trace of thymidine was found after incubation. The bulk of the 6-methyl-*asym*-triazine-3,5(2,4)-dione crystallized from a concentrated aqueous solution leaving the very water-soluble deoxyriboside in solution.[8] It was possible to separate the four components of the mixture at this stage by ion-exchange chromatography (Fig. 1), but incomplete separation of 6-methyl-*asym*-triazine-3,5(2,4)-dione and its deoxyriboside resulted on scaling up. However, a preliminary distribution of the mixture in a Craig countercurrent machine between ethyl acetate and 3% acetic acid separated all of compound I from the other three compounds, which were then easily separated on a Dowex-1 column. Compound II is a strong chelating agent so it was necessary to saturate the concentrated eluate with hydrogen sulfide and filter off sulfides before isolating the product as a very hygroscopic lyophilized glass (yield was 61% based on thymidine). For the subsequent phosphorylation a sample of compound II was lyophilized directly in the reaction flask.

Structure II is written with deoxyribose attached to position 2 of the asymmetric triazine. The enzymatic reaction *per se* does not establish this position as the point of attachment of the glycosidic linkage. The following paper[9] establishes, chemically, the structures of the 4-D-ribofuranoside and the 2-D-ribofuranoside of 6-methyl-*asym*-triazine-3,5(2,4)-dione.[9] The ultraviolet spectrum of compound II is identical with that of the 2-ribosyl isomer at *p*H values of 1, 7 and 13.[9] This fact coupled with the fact that II is relatively stable to acid or alkaline hydrolysis (which tends to eliminate consideration of an O-glycoside) is reasonable evidence that structure II is correct.

Preparation of nucleotides of II was by direct phosphorylation followed by ion-exchange separation. This was preferred to an extensive procedure of blocking and subsequent removal of blocking groups in order to direct the entry of the phosphate group. The simple phosphorylation procedure of

(1) To whom inquiries should be addressed. This paper was presented before the Division of Biological Chemistry at the New York Meeting of the American Chemical Society, September 9, 1957.

(2) An excellent review by Arnold D. Welch dealing with 6-methyl-*asym*-triazine-3,5(2,4)-dione and its deoxyriboside, and on the general subject, "Interference with Nucleic Acid Metabolism" appears in the "Henry Ford Hospital International Symposium, Enzymes: Units of Biological Structure and Functions," Academic Press, Inc., New York, N. Y., 1956, p. 547.

(3) W. H. Prusoff and Arnold D. Welch, *J. Biol. Chem.*, **218**, 929 (1956).

(4) W. H. Prusoff, L. G. Lajtha and Arnold D. Welch, *Biochim. et Biophys. Acta*, **20**, 209 (1956).

(5) This concept is also supported by studies on 6-azauracil and its riboside by R. E. Handschumacher, *Federation Proc.*, **16**, 191 (1957), and Arnold D. Welch and R. Schindler, *Science*, **125**, 548 (1957).

(6) W. H. Prusoff, *J. Biol. Chem.*, **215**, 809 (1955).

(7) W. H. Prusoff also noted the presence of uracil in the incubation mixtures; personal communication.

(8) The whole process is not wasteful of material as the recovered 6-methyl-*asym*-triazine-3,5(2,4)-dione can be recycled through the synthesis.

(9) Ross H. Hall, THIS JOURNAL, **80**, 1145 (1958).

Hall and Khorana[10] using a mixture of phosphorus pentoxide and phosphoric acid, although effective for uridine and cytidine, was too vigorous for compound II. The nucleoside was phosphorylated with dibenzyl phosphorochloridate according to the method of Baddiley and Todd.[11] The reaction mixture was hydrogenated directly to give a 95% over-all yield of nucleotides which were easily separated on a Dowex-1 resin column. The three major nucleotides accounting for 78.5% of the total, were the 5′-phosphate (18.5%), 3′-phosphate (36%) and 3′,5′-diphosphate (24%) of 2-(2′-deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5(2,4)-dione.

The first eluted mononucleotide could be dephosphorylated by crude snake venom (*Crotalus adamanteus*),[12] whereas the second eluted mononucleotide could not. Since the monophosphatase in rattlesnake venom is specific for 5′-nucleotides,[13] this configuration was assigned to the first eluted mononucleotide. It follows that the second eluted mononucleotide is the 3′-isomer. A study of the elution patterns of the natural mononucleotides from Dowex-1 resin columns shows that the 5′-mononucleotide is eluted before other isomers,[14] which is added support for the above structure assignment. The nucleotides were isolated as their cyclohexylamine salts, but as cyclohexylamine proved to be very toxic for the biological systems, the nucleotides were converted to their neutral sodium salts before use.

Biological Studies.—A convenient system for study of DNA biosynthesis is a fresh preparation of bone marrow (rabbit).[4,15] We have followed Friedkin's procedure whereby incorporation of 2-C^{14} labeled thymidine into the DNA of bone marrow cells is measured.[16] Addition of compounds that interfere with this incorporation are presumed to be inhibiting DNA synthesis and, as is pointed out by Friedkin, this system thus may serve as a test for possible carcinolytic agents. Table II shows the comparative inhibitory effects of 6-methyl-*asym*-triazine-3,5(2,4)-dione, its deoxyriboside and deoxyribonucleotides. As expected, the nucleoside was an inhibitor of DNA biosynthesis in this system while the free base was not. This observation supports that of Prusoff[4] who studied the effect of these two compounds on incorporation of C^{14}-labeled formate into a bone marrow system. With data in only one biological system, conclusions regarding the comparative inhibition of the nucleotides cannot be drawn. Roll, *et al.*,[17] have pointed out the possibility that dephosphorylation is involved in transport of nucleotides through a cell membrane.

(10) R. H. Hall and H. G. Khorana, THIS JOURNAL, **77**, 1871 (1955).
(11) J. Baddiley and A. R. Todd, *J. Chem. Soc.*, 648 (1947).
(12) Ross Allen's Reptile Institute, Silver Springs, Fla.
(13) G. Schmidt, "The Nucleic Acids," edited by Chargaff and Davidson, Academic Press, Inc., New York, N. Y., 1955, p. 591.
(14) A. Michelson and A. R. Todd, *J. Chem. Soc.*, 951 (1953); see also the chapter by W. Cohn in ref. 13.
(15) M. Friedkin and H. Wood, IV, *J. Biol. Chem.*, **220**, 639 (1956).
(16) We are indebted to Dr. Friedkin for a generous supply of 2-C^{14}-labeled thymidine used in these studies.
(17) P. Roll, H. Weinfeld, E. Carroll and G. B. Brown, *J. Biol. Chem.*, **220**, 439 (1956).

Graph 1.

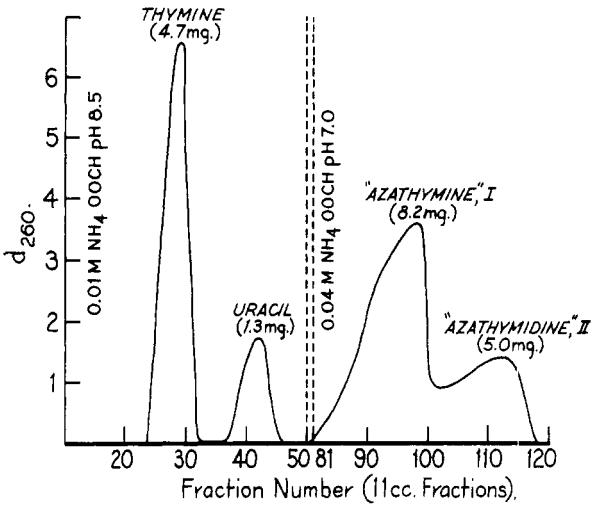


Fig. 1.—Separation of the four main products of the enzymatic reaction mixture. An aliquot of solution A containing 20 mg. of solids was absorbed on Dowex-1 × 4 (200–400 mesh, formate), column size 0.8 cm.² × 28 cm. Flow rate was 0.5 cc. per minute. The second buffer solution was started at fraction 51.

Experimental

Preparation of Cells: *S. faecalis* (ATCC 8043).—The cells were grown in a 100-gal. fermentation tank on a basal medium[18] supplemented with thymine (2 mg./l.) according to the procedure of Prusoff.[19] The cells were harvested in a large size Sharples centrifuge and frozen until needed. Average harvest per fermentation was 700 g. (wet weight).

Enzymatic Preparation of 2-(2′-Deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5(2,4)-dione (II).—One thousand and forty grams (8.2 moles) of compound I[20] was dissolved in 12 l. of water containing 313 g. (7.84 moles) of sodium hydroxide and 400 cc. of 2 *N* phosphoric acid (final *p*H was 8.3). After filtration, 4.21 l. of 0.5 *M* phosphate buffer (840 g. of $Na_2HPO_4 \cdot 12H_2O$ and 20 g. KH_2PO_4 per 5 liters) was added followed by enough distilled water to make a final volume of 42 l. The freshly thawed microörganisms (2,870 g., wet weight) were added followed by 20.4 g. (0.084 mole) of thymidine. The final *p*H of the incubation mixture was 7.9 g. This mixture was maintained at 37° for 4 hours with periodic gentle stirring.

The solution was centrifuged in the Sharples centrifuge and 800 g. of barium hydroxide added to the supernatant with stirring. The solution was centrifuged again through the Sharples machine and 4 kg. of Dowex-50 resin (H^+) (50% moisture) added. The *p*H of the solution fell to 4. The resin was filtered off and washed with water. The solution (45 l.) was concentrated *in vacuo* to 6 l. and the precipitate of 723 g. of unreacted I was filtered off. Concentration was continued until a volume of 800 cc. was reached. After cooling overnight, 187 g. of I crystallized out. The filtrate (solution A) was analyzed on a small ion-exchange column (see Fig. 1) and found to contain 12.3 g. of thymine, 3.4 g. of uracil, 22 g. of 6-methyl-*asym*-triazine-3,5(2,4)-dione and 13.4 g. of the desired deoxyriboside. This later figure was confirmed by analysis for deoxyriboside.[21] The Stumpf analysis also showed that the above precipitates contained only 0.5 g. of coprecipitated II.

This solution was diluted to 1 l., made 3% with respect to acetic acid, and placed in the first tube of a 1-l. per phase per tube Craig countercurrent machine. The stationary phase was 3% acetic acid and the mobile phase

(18) H. A. Lepper, "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," Washington, 7th Edition, 1950, p. 784.
(19) W. H. Prusoff, *Proc. Soc. Exp. Biol. Med.*, **85**, 564 (1954).
(20) E. A. Falco, E. Pappas and G. H. Hitchings, THIS JOURNAL, **78**, 1938 (1956).
(21) P. K. Stumpf, *J. Biol. Chem.*, **169**, 367 (1947).

TABLE I

Buffer, *p*H	Total vol., cc.	No. fractions prior to elution of compounds	Compound eluted	Orig. u.v. absorbing material, %
0.02 *M* NH_4OOCH, 7.0	1300	..	Compound II	1
.01 *M* HCOOH + 0.05 *M* NH_4OOCH, 4.45	935[a]	55	5′-Phosphate	17.7
.1 *M* HCOOH + 0.5 *M* NH_4OOCH, 4.2	1770[a]	32	3′-Phosphate	34.2
2.0 *M* HCOOH + 1.0 *M* NH_4OOCH, 3.2	1500	10	3′,5′-Diphosphate	23.0

[a] Both these buffer solutions eluted small amounts of unidentified nucleotides as well as the main product.

ethyl acetate. The fastest moving component was compound I and after 40 transfers it had completely separated from the other three compounds. Compound II, thymine and uracil did not separate from one another and the fractions containing this mixture were combined and evaporated *in vacuo* to near dryness. Water was added and the evaporation repeated. This procedure was repeated a number of times in order to reduce the concentration of acetic acid.

The concentrated solution (500 cc.) was neutralized to *p*H 10 with ammonia and run on a column of Dowex-1 × 4 resin (200–400 mesh, formate, size 80 cm.^{2} (4″) × 57 cm.) previously equilibrated by passing 20 l. of 0.01 *M* ammonium formate solution (*p*H 10.5) through it. The column was washed first with 4 l. of the above buffer and then with 110 l. of 0.02 *M* ammonium formate (*p*H 7) solution. This buffer eluted uracil and thymine. Compound II was eluted with 25 l. of 0.02 *M* formic acid. The effluent was concentrated to a small volume, saturated with hydrogen sulfide, filtered and lyophilized; yield 12.34 g. of chromatographically pure 2-(2′-deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5(2,4)-dione (II); ultraviolet absorption spectra: λ_{max} (*p*H 1) 262 mμ, ε 6,020; λ_{max} (*p*H 7) 262 mμ, ε 6,020; λ_{max} (*p*H 12.5) 251 mμ, ε 6,400. Prusoff[5] reports for λ_{max} in acid and alkali values of 265 and 252 mμ, respectively. The lyophilized glass was analyzed directly.

Anal. Calcd. for $C_9H_{13}N_3O_5$: C, 44.44; H, 5.39; N, 17.28. Found: C, 44.72; H, 5.50; N, 17.10.

Acid and Alkali Stability of Compound II.—Small quantities of II were dissolved in these various solutions and boiled for the specified time. Aliquots were then applied to paper strips which were eluted in the ethyl acetate–3% acetic acid system. In 0.1 *N* hydrochloric acid for two hours approximately 5% of the starting sample was degraded to azathymine. In 1 *N* hydrochloric acid for 15 minutes, 95% of the starting sample was degraded to the free base. Normal sodium hydroxide for two hours had no effect on the deoxyriboside.

Phosphorylation of Compound II.—To 2.15 g. (8.22 mmoles) of dibenzyl phosphite[22] dissolved in 25 cc. of absolute benzene was added 1.11 g. (8.32 mmoles) of N-chlorosuccinimide. After two hours, the precipitated succinimide was filtered off and the filtrate concentrated *in vacuo* to the consistency of a light oil. The material was added with the aid of a minimal amount of dry pyridine to a solution of 0.37 g. (1.52 mmoles) of II in 5 cc. of dry pyridine chilled to −30°. The solution was maintained at a temperature just above the freezing point for 6 hours, then left at room temperature overnight. All the above operations were performed under a positive pressure of dry nitrogen.

The brown reaction mixture was filtered and the crystals washed with a small quantity of pyridine. The filtrate was diluted with 25 cc. of water and evaporated to a gum. This operation was repeated twice. The residue was dissolved in 20 cc. of 50% ethanol and the *p*H lowered to 2 with 2 *N* hydrochloric acid. After addition of 0.32 g. of 10% palladium-on-charcoal catalyst, the mixture was hydrogenated in a Parr hydrogenation apparatus at 15-lb. pressure for 2 hours. A total of 17 mmoles of hydrogen was taken up during this time. The solution was filtered and evaporated to near dryness. Water was added and the solution was re-evaporated to remove traces of alcohol. The residue was dissolved in 15 cc. of water and ammonia was added until the *p*H rose to 10.

The above solution was run onto a column of Dowex-1 × 4 resin (200–400 mesh, formate, size 3.14 cm.^{2} × 27 cm.), previously equilibrated with 0.01 *M* ammonium formate solution (*p*H 10). After washing the column with 65 cc. of this buffer, the nucleotides were eluted by the buffers listed in Table I. The effluent was collected in 10-cc. fractions and the ultraviolet absorption was measured at 260 mμ.

The combined fractions containing each of the three desired nucleotides were lyophilized. After all the water had sublimed the flasks were warmed in a water-bath at 40° to remove ammonium formate. Final traces of ammonium formate were removed by dissolving the residue in a small quantity of water and repeating the lyophilization.

2-(2′-Deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5-(2,4)-dione-5′-phosphate.—The lyophilized material (0.135 g.) was dissolved in 2 cc. of 1 *M* cyclohexylamine (in methanol), filtered and diluted with ether until crystallization commenced. After chilling several hours, 0.155 g. (78% of theory) of crystals was recovered, m.p. 136–139° (all m.p.'s uncorrected). Recrystallization from an ethanol–acetone mixture rasied the m.p. to 142–143°.

Anal. Calcd. for $C_{21}H_{40}N_5O_8P$: C, 48.37; H, 7.74; N, 13.43; P, 5.95. Found: C, 48.01; H, 7.60; N, 12.70; P, 6.21.

2-(2′-Deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5-(2,4)-dione-3′-phosphate.—The lyophilized solid (0.173 g.) was dissolved in 2 cc. of 1 *M* cyclohexylamine in methanol, filtered and concentrated to 1 cc. After addition of 4 cc. of ether and refrigeration for several hours, 0.227 g. (81% recovery) of crystals was obtained, m.p. 217–220°. Recrystallization from an ethanol–acetone mixture raised the melting point to 226–227°.

Anal. Calcd. for $C_{21}H_{40}N_5O_8P$: C, 48.37; H, 7.74; N, 13.43; P, 5.95. Found: C, 48.54; H, 8.00; N, 12.96; P, 6.26.

2-(2′-Deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5-(2,4)-dione-3′,5′-diphosphate.—The lyophilized solid was dissolved in 1 cc. of 2 *M* cyclohexylamine in methanol and filtered. Absolute ether was added until incipient cloudiness, then the solution was chilled overnight. Crystals weighing 0.071 g. were filtered off, m.p. 180–190°. Recrystallization from an ethanol–acetone mixture raised the m.p. to 201–203°.

Anal. Calcd. for $C_{33}H_{67}N_7O_{11}P_2$: C, 49.56; H, 8.45; N, 12.26; P, 7.76. Found: C, 49.21; H, 8.20; N, 11.81; P, 8.02.

Sodium Salts of Nucleotides.—The above nucleotide derivatives were dissolved in water and passed through individual Dowex-50 (H^+) columns (size 1 cm. × 4 cm.). The columns were washed with water until the effluents were neutral. Each effluent was neutralized with sodium hydroxide to *p*H 7 and concentrated so that the final concentration of nucleotide was 50 μ*M*/cc.

Paper Chromatography. 1. Nucleotides.—Solvent, isopropyl alcohol:1% ammonium sulfate solution (2:1); papers were soaked in 1% ammonium sulfate solution and dried before use.

Compound	R_f
Compound II	0.71
5′-Phosphate	.45
3′-Phosphate	.21
3′,5′-Diphosphate	.09

2. Nucleosides and Bases.—Papers were run downward, with ethyl acetate saturated water in bottom of tank; solvent, ethyl acetate saturated with 3% acetic acid.

Compound	R_f
Uracil	0.22
Compound II	.30
Thymine	.40
Compound I	.65

(22) O. M. Friedman, D. L. Klass and A. M. Seligman, THIS JOURNAL, **76**, 916 (1954).

TABLE II

Each tube contained 0.2 ml. of freshly prepared rabbit bone marrow suspension,[15] 0.2 μg. of 2-C^{14}-labeled thymidine,[16] plus 1 μmole of neutralized compound. The tubes were shaken two hours at 37° in a Dubnoff shaker. The DNA was isolated according to Friedkin's procedure[15] and its radioactivity was measured in a Tracerlab windowless flow counter. Each value represents a separate tube.

Compound	C.p.m. of DNA fraction
Saline (10 λ)	2020;1950
6-Methyl-*asym*-triazine-3,5(2,4)-dione	2010;1980
2-(2'-Deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5(2,4)-dione	1100; 900
The 3'-phosphate	1700;1400
The 5'-phosphate	1550;1370
The 3',5'-diphosphate	1560;1400

Action of *Crotalus adamanteus* Venom on 2-(2'-Deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5(2,4)-dione-3'- and 5'-phosphates.—In a test-tube containing 0.1 cc. of 1 *M* glycine buffer (*p*H 8.5), 0.1 cc. of 0.1 *M* magnesium chloride and 0.5 cc. of enzyme solution[23] was added 10 μmoles of nucleotide. The tubes were incubated for one hour at 37° then analyzed for orthophosphate by Gomori's procedure.[24] At the end of this time the tube containing the second eluted mononucleotide gave a blank reading (3'-phosphate). The first eluted mononucleotide gave a reading corresponding to 50% of the theoretical amount of orthophosphate, therefore this was the -5'-nucleotide.

Acknowledgment.—The authors wish to thank Dr. T. H. Jukes for many helpful suggestions concerning this problem. They are particularly indebted to Drs. A. D. Welch and W. H. Prusoff of Yale University for many discussions and help with the transdeoxyribosidation enzyme system of Dr Prusoff. They also wish to thank Dr. J. Clark and staff for the preparation of azathymine and Mr. L. Brancone and staff for the microanalyses.

(23) The enzyme solution was prepared by dissolving 10 mg. of crude lyophilized venom in 1 cc. of water.

(24) G. Gomori, *J. Lab. Clin. Med.*, **27**, 955 (1942).

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE BRITISH COLUMBIA RESEARCH COUNCIL]

Nucleoside Polyphosphates. VI.[1] An Improved and General Method for the Synthesis of Ribo- and Deoxyribonucleoside 5'-Triphosphates

BY MICHAEL SMITH AND H. G. KHORANA

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A generally satisfactory procedure for the synthesis of ribo- and deoxyribonucleoside 5'-triphosphates is described. The method involves the reaction of tri-*n*-butylammonium salts of nucleoside 5'-monophosphates and orthophosphoric acid with dicyclohexylcarbodiimide.

The carbodiimide method[2] for the condensation of unprotected phosphate esters has been used successfully for the synthesis of a number of nucleoside 5'-polyphosphates,[3,4] nucleotide coenzymes[5] and related compounds of biological interest.[6] However, the experimental conditions for effecting condensation have varied greatly with different nucleotides[7] and the yields of the desired products have often been unsatisfactory. Clearly, the usefulness of the method would be considerably increased if generally applicable procedures for the condensation reactions could be found. The present communication describes one such procedure which leads to improved syntheses of the ribo- and deoxyribonucleoside 5'-triphosphates.

Aqueous pyridine is the only solvent system which has proved satisfactory in the previous synthetic work. Homogeneous solutions were not obtained in this medium because of the contrasting solubility properties, on the one hand, of phosphate esters and, on the other, of DCC,[8a] the reagent which has been used exclusively in the past. The varying degree of partition of different nucleotides in the two phase systems employed precluded standardization of reaction conditions and led in some cases[1] to unsatisfactory yields of the desired products. In seeking to devise a general procedure for nucleoside polyphosphate synthesis, our aim was to develop a one phase reaction system.

In the present work, attempts first were made to obtain homogeneous solutions[8b] in aqueous pyridine by the use of a more polar carbodiimide. The water-soluble reagent IV[9] was prepared according to the route I → IV, and its use in the self-condensa-

(1) Paper V. R. W. Chambers and H. G. Khorana, THIS JOURNAL, **79**, 3752 (1957).

(2) H. G. Khorana, *ibid.*, **76**, 3517 (1954).

(3) R. H. Hall and H. G. Khorana, *ibid.*, **76**, 5056 (1954).

(4) (a) R. L. Potter, S. Schlesinger, V. Buettner-Janusch and L. Thompson *J. Biol. Chem.*, **226**, 381 (1957); (b) A. Kornberg, unpublished work on the synthesis of thymidine 5'-triphosphate; (c) B. L. Horecker, J. Hurwitz and L. A. Heppel, THIS JOURNAL, **79**, 701 (1957).

(5) (a) E. P. Kennedy, *J. Biol. Chem.*, **222**, 185 (1956). (b) F. M. Huennekens and G. L. Kilgour, THIS JOURNAL, **77**, 6716 (1955). (c) G. W. Kenner, A. R. Todd and R. F. Webb, *J. Chem. Soc.*, 2843 (1954). (d) P. Reichard and N. R. Ringertz, THIS JOURNAL, **79**, 2025 (1957).

(6) (a) P. T. Talbert and F. M. Huennekens, *ibid.*, **78**, 4671 (1956). (b) P. Berg, *Federation Proc.*, **16**, 152 (1957).

(7) Compare, for example, the syntheses of uridine and guanosine 5'-polyphosphates.[1]

(8) (a) Abbreviations used in this paper are: dicyclohexyl carbodiimide, DCC; adenosine 5'-monophosphate, AMP; adenosine 5'-diphosphate, ADP; adenosine 5'-triphosphate, ATP; uridine 5'-monophosphate, UMP; uridine 5'-diphosphate, UDP; uridine 5'-triphosphate, UTP; cytidine 5'-phosphate, CMP; cytidine 5'-diphosphate, CDP; cytidine 5'-triphosphate, CTP; guanosine 5'-phosphate, GMP; guanosine 5'-diphosphate, GDP; guanosine 5'-triphosphate, GTP; 2'-deoxynucleoside 5'-phosphates are indicated by adding the prefix "deoxy-" to the above abbreviations. (b) A one-phase system was obtained in one previous case by using free acids in dimethylformamide. The unsatisfactory results then obtained indicated that the presence of a base such as pyridine in the reaction mixture was desirable.

(9) Water-soluble aliphatic carbodiimides of the general type IV were first prepared by E. Schmidt and W. Striewsky, *Ber.*, **74**, 1285 (1941). See also E. Schmidt, W. Striewsky and F. Hitzler, *Ann.*, **560**, 222 (1948). The preparation of some members of this group by completely analogous procedures has recently been recorded by J. C. Sheehan and J. J. Hlavka, *J. Org. Chem.*, **21**, 439 (1956).