n.m.r. of the resulting ethylidenediphosphonic acid solution showed a quartet centered at −19.8 p.p.m. with coupling constant *ca.* 17 c.p.s.

Diethyl 1-(Diethoxyphosphinyl)-ethyl Phosphate.—(a) A reaction mixture identical to the above was held at 50° (by initial cooling) for 30 hr. Periodic n.m.r. spectra showed a gradual decrease in area of the reactant peaks and growth of the diphosphonate peak along with a small phosphate peak from the rearrangement product. The phosphonate peak of the rearrangement product was hidden in the large diphosphonate peak. Distillation gave reactants in the forerun, an intermediate fraction (8.1 g., 130° (0.07 mm.)) containing rearrangement product with some diethyl phosphonate, and a fraction (2.3 g., 137° (0.08)) containing nearly pure rearrangement product (−19.9 and +1.9 p.p.m.). All fractions contained a little phosphate ester at *ca.* +1 p.p.m. (b) A mixture of 648 g. (3.6 moles) of diethyl acetylphosphonate and 600 g. (4.4 moles) of diethyl phosphonate was treated with sodium ethoxide catalyst. The temperature rose to 65° and was held at 105° for 4 hr. Distillation gave 260 g. (24%) of rearrangement product having equal n.m.r. peaks at −21.1 and +1.7 p.p.m.; b.p. 130–136° (0.1 mm.) mostly 132°; $n^{25}D$ 1.4275. Comparable values for supposed diphosphonate[1,2]: b.p. 140–150° (0.07 mm.) and 125° (0.06 mm.); $n^{20}D$ 1.4350. Calcd. for $C_{10}H_{24}P_2O_7$: C, 37.74; H, 7.60; P, 19.47. Found: C, 37.24; H, 7.65; P, 18.28.

Diethyl 1-(Diethoxyphosphinyl)-benzyl Phosphate.—A mixture of 242 g. (1 mole) of diethyl benzoylphosphonate and 138 g. (1 mole) of diethyl phosphonate was treated as in part b above. Distillation gave 210 g. (55%) of rearrangement product having equal n.m.r. peaks at −16.2 and +1.4 p.p.m. with impurity at −17.3 p.p.m.; b.p. 171–174° (0.1 mm.), $n^{25}D$ 1.4776. Comparable values for supposed diphosphonate[2]: b.p. 149° (0.07 mm.), $n^{25}D$ 1.4798. Calcd. for $C_{15}H_{26}P_2O_7$: C, 47.37; H, 6.90; P, 16.29. Found: C, 45.46; H, 6.82; P, 16.15. Five attempts to prepare the diphosphonate in this system showed that rearrangement is rapid at the lowest usable reaction temperatures (about 50°) and slow at room temperature; that repeated additions of ethoxide catalyst are necessary because of its reaction with the benzoylphosphonate (demonstrated independently); and that distillation *increases* the amount of impurity at −17 p.p.m. Therefore, the H^1 n.m.r. spectrum discussed in the text was run on an undistilled reaction product containing a little diethyl phosphonate, but no other impurities visible in P^{31} n.m.r.

Chlorination of Diethyl 1-(Diethylphosphinyl)-ethyl Phosphate.—This phosphate was heated with an excess of PCl_5 in CCl_4 and hydrolyzed. The hydrolysate showed 1-chloroethylphosphonic acid (−20.1 p.p.m.) and H_3PO_4 (0 p.p.m.).

Reactions of 1-Hydroxydecylphosphonic Acid and Its Ester with Base.—Diethyl 1-hydroxydecylphosphonate (m.p. 46°, from hexane; n.m.r., −25.5 p.p.m.) was prepared by the ethoxide-catalyzed addition of diethyl phosphonate to an equimolar amount of freshly distilled decylaldehyde. A mixture of 2.1 g. of this ester with 19 g. of 6 *N* NaOH was heated at 97° for 1 hr. On cooling, a yellow semi-solid upper layer of condensed aldehyde formed, and the lower layer showed only the sodium phosphite doublet at −21.3 and + 13.6 p.p.m.

A portion of the ester was hydrolyzed in concd. HCl and purified by water and hexane washes. Calcd. for $C_{10}H_{24}PO_4$: C, 50.41; H, 9.73; P, 13.00; neut. equiv., 238. Found: C, 50.08; H, 9.53; P, 13.15; neut. equiv., 234 g./equiv. N.m.r.: −23.0 p.p.m. in ethanol; m.p. 155° with decomposition.

A mixture of 3.0 g. of the acid and 26 g. of 6 *N* NaOH was heated at 97° for 19 hr. No phase separation occurred on cooling, and the free acid was precipitated with HCl, filtered and dried; recovery: 2.9 g. (97%), identified by m.p. and mixed m.p.

Acknowledgments.—The authors wish to thank Mr. J. T. Yoder for some of the n.m.r. spectra, Mr. D. Bisno for part of the synthetic work, Mr. G. F. Brautigam for some of infrared spectra, and Drs. C. F. Callis, R. R. Irani and M. M. Crutchfield for helpful discussions. This work was supported in part by the Air Research and Development Command, United States Air Force, under Contract AF 33(616)-6950 monitored by the Materials Laboratory, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio.

[CONTRIBUTION FROM THE FACULTY OF PHARMACEUTICAL SCIENCES, THE UNIVERSITY OF TOKYO, TOKYO, JAPAN]

Organic Phosphates. XVII.[1] Syntheses of Nucleotides by Condensation of Phosphorylated Sugar and Bases

BY TYUNOSIN UKITA AND HIKOYA HAYATSU

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Crystalline 2,3,4-tri-*O*-acetyl-6-di-*p*-nitrophenylphosphoryl- (VIII) and 2,3,4-tri-*O*-acetyl-6-diphenylphosphoryl-α-D-glucopyranosyl bromides (IX) were obtained. On condensation of these bromides with dithyminylmercury, VIII afforded an *O*-glucoside which contained two glucosyl residues attached on one thyminyl residue, while the similar condensation of IX with dithyminyl- or *N*-acetylcytosine-mercury gave the respective *N*-glucosides, 1-(2′,3′,4′-tri-*O*-acetyl-6′-diphenylphosphoryl-β-D-glucopyranosyl)-thymine and 1-(2′,3′,4′-tri-*O*-acetyl-6′-diphenylphosphoryl-β-D-glucopyranosyl)-4-acetamido-2(1H)-pyrimidinone. The former product was converted to 1-β-D-glucopyranosylthymine-6′ phosphate by catalytic hydrogenation and subsequent deacetylation. The 2,3-di-*O*-benzoyl-5-diphenylphosphoryl-D-ribofuranosyl bromide was condensed with dithyminylmercury (A) as well as with chloromercuri-6-benzamidopurine (B). The product obtained from A, 1-(2′,3′-di-*O*-benzoyl-5′-diphenylphosphoryl-β-D-ribofuranosyl)-thymine, was converted to 1-β-D-ribofuranosylthymine-5′ phosphate after removal of phenyl groups by catalytic hydrogenation and subsequent deacylation. The condensation product of B, 6-benzamido-9-(2′,3′-di-*O*-benzoyl-5′-diphenylphosphoryl-β-D-ribofuranosyl)-purine, gave adenosine-5′ phosphate on its successive treatment with 0.5 *N* sodium hydroxide and methanolic sodium methoxide to remove one phenyl and three benzoyl groups and subsequent removal of a phenyl group attached at phosphoryl residue with phosphodiesterase from Russel's viper.

Recent progress in nucleotide chemistry has included the total syntheses of several naturally occurring as well as some unnatural nucleotides. These syntheses involve, as general procedures, the phosphorylation of nucleosides having suitably protected sugar moieties and thus require prior syntheses of the nucleosides.[2]

However, when the synthesis of a desired nucleoside requires numerous steps[3,4] or proceeds in

(1) Part XVI of this series: T. Ukita and R. Takeshita, *Chem. Pharm. Bull. (Tokyo)*, **9**, 606 (1961).

(2) (a) J. Baddiley in "The Nucleic Acids," Vol. I, E. Chargaff and J. N. Davidson, Editors, Academic Press, Inc., New York, N. Y., 1955, p. 137. (b) A. R. Todd in "Methods in Enzymology," Vol. III, S. P. Colowick and N. O. Kaplan, Editors, Academic Press, Inc., New York, N. Y., 1957, p. 811. (c) F. Cramer, *Angew. Chem.*, **72**, 236 (1960); (d) H. G. Khorana, *Federation Proc.*, **19**, 931 (1960); (e) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951); (f) J. J. Fox and I. Wempen, *Adv. in Carbohydrate Chem.*, **14**, 283 (1959)

poor yield,[4] or when a series of nucleotide analogs containing several different bases attached to a common phosphoryl sugar moiety is required,[5] the above general procedure is not always the most convenient method.

In order to overcome these difficulties, we have attempted to develop a new synthetic route which involves a prior synthesis of the phosphorylated aldose and subsequent condensation of the product with several pyrimidine and purine bases.[6]

The present paper deals with the preparation of nucleotides by this new route and the total syntheses of an unnatural new nucleotide, 1-β-D-glucopyranosylthymine-6′ phosphate, and the natural nucleotides, 1-β-D-ribofuranosylthymine-5′ phosphate and adenosine-5′ phosphate.

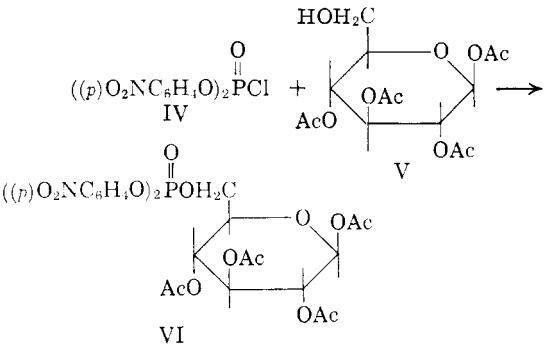
The first and most important problem in this series of syntheses was to find a suitable residue to protect the two phosphoryl dissociations. The protective groups must be stable during halogenation of the phosphorylated sugar and, after subsequent condensation with base, removable under mild conditions.

Among such protective groups, phenyl[7,8] and *p*-nitrophenyl[9] residues were convenient for our purpose, since the former is known to be stable under halogenation conditions[10,11] and the latter is expected to exhibit similar behavior. Benzyl[12] or *p*-substituted benzyl residues[13] seem unsuitable because of instability to nucleophilic reagents such as chloride or iodide ion.[14,15]

In order to confirm the relative stability of these protective residues under halogenation conditions, diphenylethyl (I),[16] di-*p*-nitrophenylethyl (II)[17] and dibenzylethyl phosphates (III)[12] were dissolved in glacial acetic acid containing 16% of dry hydrogen bromide and kept at room temperature. About 20% of I was decomposed to give diphenyl phosphate after 25 minutes, and no decomposition was observed for II even after 90 minutes incubation, while III was completely converted to ethyl phosphate within 5 minutes. On keeping these three phosphotriesters at −1 to −3° in anhydrous ether previously saturated with dry hydrogen chloride at 0°, Compound I was found stable for 100 hours while III was completely degraded to give benzylethyl phosphate and ethyl phosphate after 20 hours.

As the *p*-nitrophenyl group seemed to be the most suitable for the protection of phosphoryl dissociation, 1,2,3,4-tetra-*O*-acetyl-6-(di-*p*-nitrophenylphosphoryl)-β-D-glucopyranose (VI) was synthesized and used in subsequent halogenation and glycosidation.

CHART I



Di-*p*-nitrophenyl hydrogen phosphate was allowed to react with phosphorus pentachloride to furnish di-*p*-nitrophenyl phosphorochloridate (IV)[18] in an excellent yield. On treatment of IV with sodium ethoxide, it gave II in 68% yield. Compound IV was treated with 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (V)[19] in the presence of 2,6-lutidine to furnish the desired crystalline condensation product, 1,2,3,4-tetra-*O*-acetyl-6-(di-*p*-nitrophenylphosphoryl)-β-D-glucopyranose (VI) in good yield.[20]

The bromination of VI by keeping its solution in glacial acetic acid containing 30% of hydrogen bromide at room temperature afforded crystalline 2,3,4-tri-*O*-acetyl-6-(di-*p*-nitrophenylphosphoryl)-α-D-glucopyranosyl bromide (VIII) as expected in a yield of 92%.

(3) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, *J. Am. Chem. Soc.*, **78**, 2117 (1956).

(4) R. Funakoshi, M. Irie and T. Ukita, *Chem. Pharm. Bull. (Tokyo)*, **9**, 406 (1961).

(5) T. Ukita and M. Irie, *ibid.*, **9**, 217 (1961).

(6) Some successful attempts in condensation of an acylated sugar halogenide having a phthalimido (H. M. Kissman and M. J. Weiss, *J. Am. Chem. Soc.*, **80**, 2575 (1958)), fluoro (H. M. Kissman and M. J. Weiss, *ibid.*, **80**, 5559 (1958)), or an iodo substitution (T. Kanazawa, H. Tamura, Y. Nozoe and T. Sato, *Nippon Kagaku Zasshi*, **79**, 698 (1958), T. Kanazawa and T. Sato, *ibid.*, **80**, 200 (1959)) in place of one of its acyloxy groups, with suitable bases have been reported. However, no report on an application of phosphorylated sugar halogenide in the condensation has appeared except the related case of Parikh, *et al.* (J. R. Parikh, M. E. Wolff and A. Burger, *J. Am. Chem. Soc.*, **79**, 2778 (1957)) who treated 2,3-di-*O*-acetyl-5-deoxy-5-diethylphosphono-D-ribofuranosyl chloride with silver theophylline which did not give the desired nucleotide analog.

(7) (a) H. Bredereck, E. Berger and J. Ehrenberg, *Ber.*, **73**, 269 (1940); (b) J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 1610 (1953).

(8) P. Brigl and H. Müller, *Ber.*, **72**, 2121 (1939).

(9) (a) R. W. Chambers, J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3747 (1957); (b) A. Hampton and M. H. Maguire, *ibid.*, **83**, 150 (1961).

(10) G. M. Tener and H. G. Khorana, *ibid.*, **80**, 1999 (1958).

(11) E. Baer in "Biochemical Preparations," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1949, p. 50.

(12) F. R. Atherton, H. T. Openshaw and A. R. Todd, *J. Chem. Soc.*, 382 (1945).

(13) M. Miyano and S. Funahashi, *J. Am. Chem. Soc.*, **77**, 3522 (1955).

(14) (a) V. M. Clark and A. R. Todd, *J. Chem. Soc.*, 2030 (1950); (b) J. Lecocq and A. R. Todd, *ibid.*, 2381 (1954); (c) R. J. W. Cremlyn, G. W. Kenner, J. Mather and A. R. Todd, *ibid.*, 528 (1958).

(15) M. Miyano, *J. Am. Chem. Soc.*, **77**, 3524 (1955).

(16) M. Lora Tamayo and G. Ramon, *Anales real soc. espan. fis. y quim.*, **45B**, 1123 (1949); *C. A.*, **44**, 8882*i* (1950). This time, Compound I was synthesized by a modified method of Todd, *et al.*[14b]

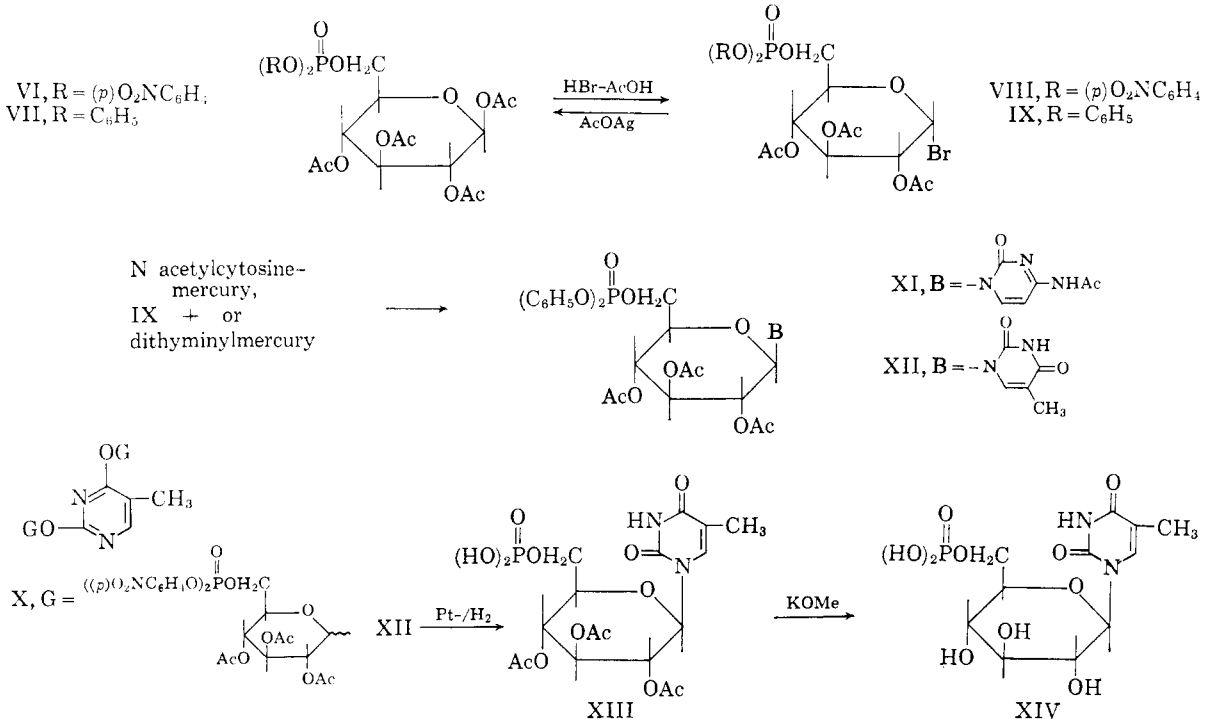
(17) J. A. A. Ketelaar and H. R. Gersmann, *J. Am. Chem. Soc.*, **72**, 5777 (1950).

(18) Excellent results in phosphorylation of the primary hydroxyl group of a nucleoside by use of tetra-*p*-nitrophenyl pyrophosphate have been reported by Chambers, *et al.*[9a] In our synthesis, however, di-*p*-nitrophenyl phosphorochloridate was preferentially used because it is more conveniently prepared than the above reagent. Moreover, one of the two phosphoryl moieties of the latter is not concerned with the phosphorylation. This new chloridate was found to be an excellent reagent for phosphorylation of nucleosides; thus, 2′,3′-isopropylidene-uridine, when treated with IV, gave its 5′-di-*p*-nitrophenylphosphoryl derivative in high yield (unpublished observation).

(19) D. D. Reynolds and W. L. Evans in "Organic Syntheses," Vol. XXII, John Wiley and Sons, Inc., New York, N. Y., 1942, p. 56.

(20) This compound has been synthesized by Hashizume, *et al.* (T. Hashizume, K. Fujimoto, H. Unuma, K. Takanami and K. Morimoto, *Bull. Inst. Chem. Research, Kyoto Univ.*, **38**, 70 (1960)) by phosphorylation of the compound V with tetra-*p*-nitrophenyl pyrophosphate.

CHART II



By similar bromination of 1,2,3,4-tetra-*O*-acetyl-6-diphenylphosphoryl-β-D-glucopyranose (VII),[21] 2,3,4 - tri - *O* - acetyl - 6 - diphenylphosphoryl-α-D-glucopyranosyl bromide (IX) was also obtained as crystals. Thus phenyl groups attached to the phosphoryl residue of VII proved to be more stable to bromination than those in I.

The remarkable dextrorotatory property of these two sugar bromides VIII and IX revealed that their bromines have the α-configuration as in acetobromoglucose. On treatment with silver acetate, they gave the respective parent compound, VI and VII.

Following the method of nucleoside synthesis reported by Fox, *et al.*,[3] VIII was condensed with dithyminylmercury[3] and the product was isolated as an amorphous powder. This product (X), however, was not the desired nucleotide derivative, but a thymine di-*O*-glucoside type compound: thus, analysis showed X to be constituted of phosphoryl sugar and thymine moieties in a ratio of 2:1. It also lacked, in its infrared spectrum, the absorption at 1620–1730 cm.$^{-1}$ due to amide carbonyl group, and readily liberated thymine by treatment with dilute alkali. From these properties together with elemental analyses a structure of 2,6-di-[2′,3′,4′-tri-*O*-acetyl-6′-(di-*p*-nitrophenylphosphoryl) - D - glucopyranosyloxy] - 5-methylpyrimidine[22] was proposed for X.

In a similar reaction of VIII with *N*-acetylcytosinemercury,[23] however, neither an *N*- nor an *O*-glucoside condensation product was obtained.

Contrary to this failure to obtain the desired *N*-glucoside by condensation of VIII with pyrimidine base, successful results were achieved when IX was used. Thus from the reaction mixture of IX and *N*-acetylcytosinemercury, a crystalline condensation product, XI, was isolated in a yield of 57%.

The analyses of the product coincided with those for 1-(2′,3′,4′-tri-*O*-acetyl-6′-diphenylphosphoryl-β-D-glucopyranosyl) - 4 - acetamido - 2(1H)-pyrimidinone and the similarity in absorption (Table I) of this product in the ultraviolet and infrared regions to those of 1-(tetra-*O*-acetyl-β-D-glucopyranosyl) - 4 - acetamido - 2(1H) - pyrimidinone[23] supports the assignment of a structure which contains an *N*-glucosidic linkage between phosphoryl sugar and base for this product.

This successful condensation led us to extend this reaction to another pyrimidine base. Thus dithyminylmercury was similarly treated with IX and gave smoothly 1-(2′,3′,4′-tri-*O*-acetyl-6′-diphenylphosphoryl - β - D - glucopyranosyl) - thymine (XII) as needles in a yield of 41%.

As is shown in Table I, the absorption maxima of XII in the ultraviolet and infrared regions are entirely similar to those of 1-(tetra-*O*-acetyl-β-D-glucopyranosyl)-thymine.[3]

On hydrogenation of XII with Adams platinum oxide, the dephenylated product, 1-(2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyl)-thymine-6′ phosphate (XIII), was obtained in an excellent yield. That no reduction occurred in the pyrimidine moiety of the parent compound XII by this treatment was demonstrated by the similarity in ultraviolet absorption shown by XIII and XII.[24]

(21) H. A. Lardy and H. O. L. Fischer in "Biochemical Preparations," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1952, p. 39.

(22) The configuration of the glycosidic linkage of X is not yet confirmed, though the β-type may presumably be assigned by analogy of this type of condensation reaction in syntheses of usual β-*O*-glucosides from α-bromoglycopyranose derivatives (L. J. Haynes and F. H. Newth, *Adv. in Carbohydrate Chem.*, **10**, 207 (1955)).

(23) J. J. Fox, N. Yung, I. Wempen and I. L. Doerr, *J. Am. Chem. Soc.*, **79**, 5060 (1957).

TABLE I

SPECTRAL DATA

	Ultraviolet[a] (EtOH), mμ		Infrared (KBr)
	λ_{max} (ϵ)	λ_{min} (ϵ)	$-\overset{O}{\overset{\|}{C}}-NH-$, cm.$^{-1}$
XI	250 (15600)	227 (2940)	1670(s)
	300 (6150)	277 (3900)	1625(m)
1-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-acetamido-2(1H)-pyrimidinone	249		1670(s)
	298	275	1630(m)
XII	261 (8980)	231 (1680)	1690(s)
			1647(sh)
1-(Tetra-*O*-acetyl-β-D-glucopyranosyl)-thymine	262.5	232.5	1690(s)
			1655(sh)

[a] Measured by a Cary model 11 recording spectrophotometer.

Compound XIII was deacetylated to give 1-β-D-glucopyranosylthymine-6′ phosphate (XIV) which was isolated as its barium salt in a yield of 78% and gave ultraviolet absorption spectra at both *p*H 2 and 12 closely resembling those of ribothymidine-2′(3′) phosphate.[5,25] Furthermore, XIV consumed 2 molar equivalent amounts of periodate indicating the presence of a glucopyranosyl residue which has free hydroxylic groups at its 2′, 3′- and 4′-positions.

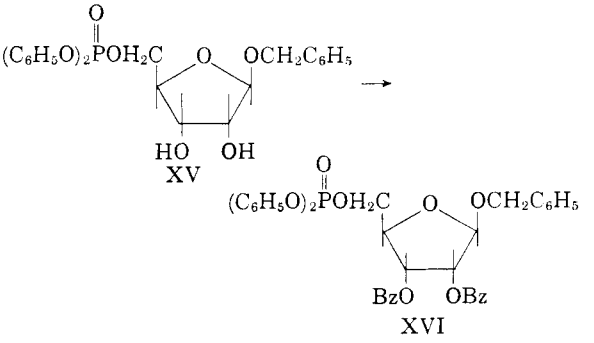
Incubation of XIV with intestinal phosphomonoesterase[26] afforded 1-β-D-glucopyranosyl-thymine.[27] Crude snake venom did not split the phosphoryl group of XIV.

In order to extend this reaction to the synthesis of ribonucleoside-5′ phosphates, an attempt to synthesize benzyl 2,3-di-*O*-benzoyl-5-diphenylphosphoryl-β-D-ribofuranoside (XVI) as a starting material by benzoylation of benzyl 5-diphenylphosphoryl-β-D-ribofuranoside (XV)[28] was made. A crystalline product was obtained.

The bromination of this compound (XVI) and subsequent condensation with dithyminylmercury, however, did not furnish the desired condensation product.[29]

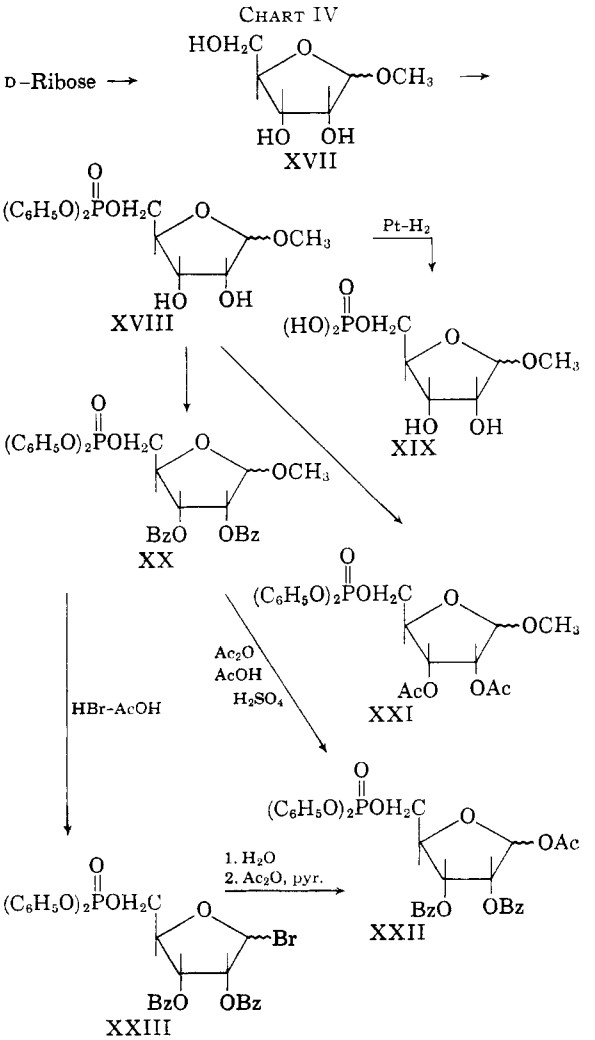
In a second attempt, methyl D-ribofuranoside (XVII) was condensed with diphenyl phosphorochloridate and the product was treated with aqueous alkali according to the procedure reported by Khorana, *et al.*,[28] to separate the 2- or 3-phosphorylated derivatives from the desired methyl 5-diphenylphosphoryl-D-ribofuranoside (XVIII).

CHART III



The latter was isolated as a sirupy material. Catalytic hydrogenation of XVIII with Adams platinum oxide gave the dephenylated compound, methyl D-ribofuranoside-5 phosphate (XIX), which was identified paper chromatographically with a product prepared by phosphorylation of methyl D-ribofuranoside by another method.[30]

CHART IV



(24) Thymine, when involved in a nucleoside as its basic group, is known to resist catalytic hydrogenation (W. Bergmann and R. J. Feeney, *J. Org. Chem.*, **16**, 981 (1951)). The removal of the phenyl groups by catalytic hydrogenation without simultaneous reduction of the base moiety, however, cannot always be attained when the basic group involved in the nucleotide is other than thymine (see refs. 2c and 2d).

(25) F. F. Davis, A. F. Carlucci and I. F. Roubein, *J. Biol. Chem.*, **234**, 1525 (1959).

(26) The preparation of the enzyme was offered by the courtesy of Dr. T. Hashimoto of the Department of Physiological Chemistry and Nutrition, Faculty of Medicine, the University of Tokyo.

(27) It was thus conclusively confirmed that the basic residue in the nucleotide XIV is in the β-configuration.

(28) G. M. Tener and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 1999 (1958).

(29) Although the failure of the condensation was attributed in part to the incomplete removal of benzyl bromide from the bromination product of XVI, even after repeated removal of volatile components from the bromination product by codistillation with xylene, no evidence of reaction was observed. No further research on this problem was undertaken, because the poor total yield (*ca.* 15%) of the benzyl riboside (XVI) from D-ribose precluded the use of this compound as a starting material.

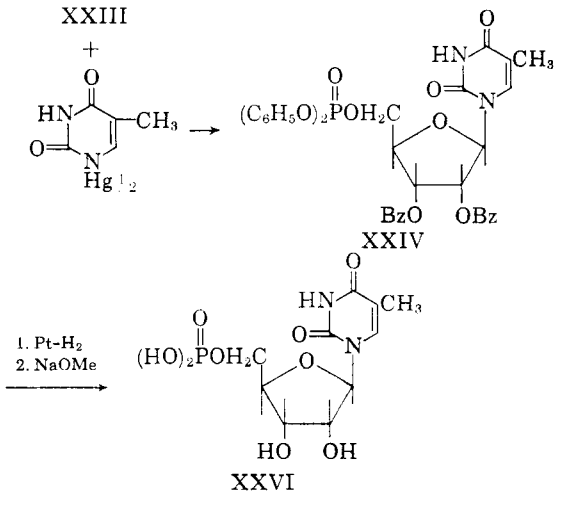
(30) N. Imura, K. Nagasawa and T. Ukita, unpublished.

The crystalline methyl 2,3-di-*O*-benzoyl-5-diphenylphosphoryl-D-ribofuranoside (XX) was obtained by benzoylation of XVIII in an over-all yield of 36% from D-ribose. The acetyl derivative of XVIII, methyl 2,3-di-*O*-acetyl-5-diphenylphosphoryl-D-ribofuranoside (XXI), was also obtained as colorless prisms by treatment of XVIII with acetic anhydride and pyridine.

According to the general stepwise conversion[31] of a methyl glycoside *via* its C_1-acetate into its chloride, known to be the most suitable halogenide in subsequent condensation with base,[3] XX was first acetolyzed with acetic anhydride and glacial acetic acid in the presence of concd. sulfuric acid to give 1-*O*-acetyl-2,3-di-*O*-benzoyl-5-diphenylphosphoryl-D-ribofuranose (XXII). This method was originally reported by Recondo and Rinderknecht[32] in the similar reaction for methyl 2,3,5-tri-*O*-benzoyl-D-ribofuranoside. The maximum yield (31%) of XXII from XX, however, was so poor that this procedure could not be used for our purpose. Although the yield was increased to 58% when acetylation was performed after successive bromination of XX to a bromide (XXIII) and subsequent hydrolysis of the latter,[33] it was still insufficient for further work.

The difficulty of obtaining the acetate XXII in sufficient yield was overcome by submitting the bromide XXIII directly to the following condensation reaction with base. When XXIII was treated with dithyminylmercury, the desired product, 1-(2′,3′-di-*O*-benzoyl-5′-diphenylphosphoryl-β-D-ribofuranosyl)-thymine (XXIV), was obtained as colorless needles and its structure was assigned from its elemental analyses and ultraviolet spectra.

CHART V



Catalytic hydrogenation of XXIV over Adams platinum oxide yielded a crystalline product (XXV) after total consumption of *ca.* 14 mole equivalents of hydrogen. Behavior of XXV in paper chromatography and paper electrophoresis revealed the complete removal of phenyl groups attached to the phosphoryl residue of XXIV, and the loss of absorption at 230 mμ indicated that the benzene ring of benzoyl residues at 2′- and 3′-positions of XXIV was also hydrogenated. Consequently, XXV is represented by 1-(2′,3′-di-*O*-hexahydrobenzoyl-β-D-ribofuranosyl)-thymine-5′ phosphate.

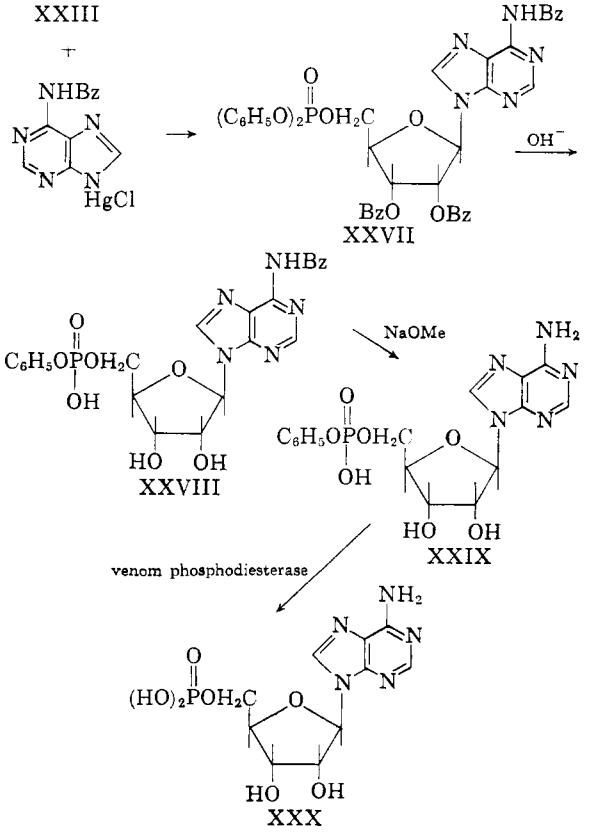
Deacylation of XXV with methanolic sodium methoxide gave 1-β-D-ribofuranosylthymine-5′ phosphate (XXVI) which was isolated as its barium salt in 82% yield.

The 1-β-D-ribofuranosylthymine-5′ phosphate thus obtained gave single spots on paper chromatograms run in four different solvent systems and its mobility in paper electrophoresis was similar to those of glucothymidine-6′ phosphate (XIV) and uridine-5′ phosphate. The ultraviolet absorption spectra of XXVI closely resembled that reported for ribothymidine-2′ or -3′ phosphate.[5,25]

On incubation with crude snake venom, XXVI was converted to 1-β-D-ribofuranosyl-thymine which was isolated and identified with an authentic specimen.[3]

The successful synthesis of 1-β-D-ribofuranosylthymine-5′ phosphate by this route prompted us to synthesize adenosine-5′ phosphate by a similar procedure. Compound XXIII was treated with chloromercuri-6-benzamidopurine[34] in dry xylene to give glassy 6-benzamido-9-(2′,3′-di-*O*-benzoyl-5′-diphenylphosphoryl-β-D-ribofuranosyl)-purine (XXVII) smoothly with a yield of 65%.

CHART VI



(31) H. M. Kissman, C. Pidacks and B. R. Baker, *J. Am. Chem. Soc.*, **77**, 18 (1955).

(32) E. F. Recondo and H. Rinderknecht, *Helv. Chim. Acta*, **42**, 1171 (1959).

(33) *Cf.* R. K. Ness, H. W. Diehl and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **76**, 763 (1954).

(34) This salt was prepared by a modified method of Baker, *et al.* (B. R. Baker, K. Hewson, H. J. Thomas and J. A. Johnson, Jr., *J. Org. Chem.*, **22**, 954 (1957)) applied for the preparation of chloromercuri-6-chloropurine. See Experimental.

The product gave two absorption maxima at 230 (benzoyl group) and 280 mμ in the ultraviolet spectrum, the latter of which resembled with that of 6 - benzamido - 9 - (tetra - *O* - acetyl - β - D-glucopyranosyl)-purine.[35] Contrary to the case of XXIV, XXVII did not consume hydrogen on catalytic hydrogenation with Adams platinum oxide.

Compound XXVII was hydrolyzed with 0.5 *N* sodium hydroxide to give an amorphous powdery product (XXVIII) in 97% yield. As this product gave an ultraviolet absorption spectra similar to that of 6-benzamido-9-(tetra-*O*-acetyl-β-D-glucopyranosyl)-purine, the benzoyl residue at the C_6-amino group on the purine moiety seemed to have remained substituted, and lack of absorption at 230 mμ indicated the loss of the benzoyl residue on the hydroxyl groups of the ribosyl moiety. Furthermore, on periodate oxidation, XXVIII consumed one mole equivalent of the reagent and on paper electrophoresis it showed a mobility corresponding to that for phosphodiester. From these properties together with the analysis of phosphorus content, XXVIII is assigned the structure 6 - benzamido - 9 - β - D - ribofuranosylpurine-5′ phenyl hydrogen phosphate.

Because of the lability of the *N*-benzoyl group of this compound, it was not further purified and was treated with methanolic sodium methoxide to remove the *N*-benzoyl group. The product, adenosine-5′ phenylhydrogenphosphate (XXIX), was obtained as colorless crystals. Its absorption spectrum was similar to that of adenosine. Neither XXVIII nor XXIX consumed hydrogen on catalytic hydrogenation.

In order to remove the remaining phenyl group in the phosphoryl residue of XXIX, it was boiled with alkali,[7b] but even after treatment for one hour of XXIX with 2 *N* lithium hydroxide at 100°, the reaction mixture did not give the paper chromatographic spot corresponding to adenosine-5′ phosphate.

On incubation of XXIX with phosphodiesterase prepared from Russel's viper,[36] the nucleotide XXX was isolated as its barium salt.

The crystalline free nucleotide obtained from the barium salt was identified with authentic adenosine-5′ phosphate[37] by comparison of its physical properties and paper chromatographic behavior.

XXIX was enzymatically dephosphorylated by incubation with crude venom and the nucleoside thus produced was also identified with authentic adenosine.

When the phosphodiester XXVIII was incubated with crude venom, the reaction mixture revealed on paper chromatography a new spot which did not contain phosphorus and gave an ultraviolet absorption similar to that of the parent compound XXVIII. Thus the structure 6-benzamido-9-β-D-ribofuranosylpurine[38] was proposed for this product.

(35) This compound was obtained as a glassy product by condensation of the above[14] chloromercuri-6-benzamidopurine with acetobromoglucose according to the method reported by Davoll, *et al.*[26]

(36) The enzyme preparation was obtained through the courtesy of Dr. A. Ohsaka of the National Institute of Health, Japan.

(37) The authentic specimen used was purchased from Schwarz Laboratories, Inc., Mount Vernon, N. Y., U. S. A.

Acknowledgments.—The authors are indebted to Dr. T. Ueda of the University of Hokkaido for his valuable discussions and to Takeda Chemical Industries for their gift of the starting materials for the present syntheses. Thanks are also due to Misses E. Ninomiya and M. Tanaka for measurement of the infrared spectra and to Mr. D. Ohata of the Sasaki Institute as well as to the members of the Central Analysis Room of this Faculty for carrying out the microanalyses.

Experimental[39]

Methods.—Paper chromatography was carried out on Toyo Roshi No. 53, using the ascending technique. The following solvent systems were employed: (1) isopropyl alcohol–ammonia–water (7:2:1); (2) isobutyric acid–0.5 *N* ammonium hydroxide (10:6); (3) methyl ethyl ketone–*t*-butyl alcohol–acetic acid–water–hydrochloric acid (22.5:22.5:35:19:1); (4) *n*-butyl alcohol–acetic acid–water (4:1:5); (5) *n*-butyl alcohol–water (86:14); (6) isopropyl alcohol–1% ammonium sulfate solution (2:1); (7) *n*-propyl alcohol–ammonia–water (20:10:3); (8) isopropyl alcohol–ammonia–water (7:1:2); (9) methyl alcohol–hydrochloric acid–water (7:2:1), and the R_f value obtained for each solvent is represented by R_{f_1}, R_{f_2} etc.

Paper electrophoresis was performed on Toyo Roshi No. 53 at 700 v./16 cm. for 40 minutes, using, unless otherwise mentioned, a buffer solution composed of *n*-butyl alcohol–pyridine–acetic acid–water (20:10:2:968, *p*H 5.9). The mobility is represented by M_{U5P} and M_{A5P}, taking that for uridine-5′ phosphate and adenosine-5′ phosphate as standard, respectively.

Spots on paper chromatograms were detected by visual observation of their ultraviolet absorption and by spraying the chromatograms with the molybdate–perchloric acid[40] and subsequent irradiation with ultraviolet light.[41] Total phosphorus determinations were carried out by Allen's method.[42]

Stability of Phosphotriesters under Halogenation Conditions.—The reaction conditions tested were as follows: The compound was kept: (A) in a 1:1 mixture of methylene dichloride and 32% solution of anhydrous hydrogen bromide in glacial acetic acid at 20°; (B) in an ethereal solution previously saturated with anhydrous hydrogen chloride at 0°, at −1∼−3°.

Diphenylethyl Phosphate (I).[16]—Compound I was treated under condition (A). After keeping the test solution for 25 minutes, the solvent was rapidly removed *in vacuo*, and anhydrous toluene which was added to the residue was removed under vacuum three times. Subsequent paper electrophoresis of the residual sirup revealed that Compound I was slightly degraded to a phosphodiester. Quantitative determination of the phosphorus content of the cuttings of the spots showed that 18% of I was converted to the diester which was identified with diphenyl phosphate by the following preparative experiment.

A mixture of 300 mg. of I, 7 ml. of methylene dichloride and 7 ml. of 32% hydrogen bromide in glacial acetic acid was kept at room temperature for 50 hours. The solvent was removed *in vacuo* and codistillation with dry toluene was repeated twice. Upon scratching, the residual sirup solidified. The solid weighed 260 mg. (96%) and melted at 60–65°. Subsequent treatment of the solid with water gave crystalline material which melted at 50–51° and which was identified with authentic monohydrate of diphenyl phosphate by mixed fusion.

When the triester I was treated under condition B, no degradation was observed after 100 hours.

Di-*p*-nitrophenylethyl phosphate (II)[17] was completely stable after 90 minutes under condition A.

(38) Recently the substrate specificity of venom 5′ nucleotidase was reported by Mizuno, *et al.* (T. Mizuno, M. Ikehara, T. Ueda, A. Nomura, E. Ohtsuka, F. Ishikawa and Y. Kanai, *Chem. Pharm. Bull. (Tokyo)*, **9**, 338 (1961).

(39) All melting points are uncorrected.

(40) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).

(41) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(42) R. J. L. Allen, *Biochem. J.*, **34**, 858 (1940).

Dibenzylethyl Phosphate (III).[12]—Under condition A, III was degraded rapidly and after 5 minutes was completely decomposed to a phosphomonoester and a trace amount of phosphodiester. On paper chromatography with standard samples, the monoester and diester produced were, respectively, identified with ethyl phosphate[12] and benzylethyl phosphate.[14c]

On treatment under condition B, III was degraded completely to ethyl phosphate and benzylethyl phosphate after 19 hours.

Di-p-nitrophenyl Phosphorochloridate (IV).—A suspension of 4.0 g. of di-*p*-nitrophenyl hydrogen phosphate[42a] in 10 ml. of dry chloroform was cooled with ice, protected from moisture, and treated with stirring with 2.8 g. (15% excess) of phosphorus pentachloride in one portion. During further stirring, the reagents gradually disappeared with simultaneous evolution of hydrogen chloride. After stirring for 2 hours to the clear reaction mixture was added 30 ml. of dry petroleum ether (b.p. 60–70°) to precipitate an oily material, which upon scratching solidified to a crystalline mass. The crude crystalline chloridate thus obtained was filtered, then thoroughly washed with dry petroleum ether. The dried crude product melted at 90–92°, and weighed 3.7 g. From the combined filtrate and washings, an additional 0.1 g. of crystalline IV was obtained, total yield 91%. Recrystallization from chloroform–petroleum ether afforded fine prisms melting at 97–97.5°.[18] When stored with adequate protection from moisture, the chloridate is stable for weeks.

Anal. Calcd. for $C_{12}H_8O_7N_2PCl$: C, 40.18; H, 2.25; N, 7.81; P, 8.64. Found: C, 39.82; H, 2.15; N, 7.79; P, 8.39.

Di-*p*-nitrophenylethyl Phosphate (II).[17]—To a solution of 360 mg. (1 mmole) of IV in 5 ml. of dry dioxane was added dropwise a solution of 23 mg. (1 mmole) of sodium in 6 ml. of absolute ethanol within 10 minutes at 0°, and the mixture was immediately poured into cold water. The precipitate material was collected and recrystallized from ethanol to give 250 mg. (68%) of II as colorless needles, m.p. 132–136°. Admixture of the product with an authentic specimen showed no depression of the melting point.

1,2,3,4-Tetra-O-acetyl-6-(di-p-nitrophenylphosphoryl)-β-D-glucopyranose (VI).[20]—To a stirred solution of 5.0 g. of di-*p*-nitrophenyl phosphorochloridate in 15 ml. of dry chloroform in an ice-bath with protection from moisture was added dropwise a mixture of 4.7 g. (one mole equivalent) of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose,[19] 3.5 ml. of dry 2,6-lutidine and 5 ml. of dry chloroform. After stirring for 1 hour, the ice-bath was removed and the reaction mixture was kept at room temperature for 24 hours. The mixture was washed successively with water, 1 *N* hydrochloric acid and water. Removal of the solvent *in vacuo* left a sirup, which was triturated with 15 ml. of methanol to afford a crystalline mass, m.p. 139–141°, which was collected and suspended in 50 ml. of methanol. On warming, it turned to voluminous needles, which, after cooling, were collected to give 6.0 g. (72%) of VI melting at 146–147°. The melting point did not alter on recrystallization from methanol (reported[20] melting point 144–145°), $[\alpha]^{33}D$ +28.7° (*c* 2.72, chloroform).

Anal. Calcd. for $C_{26}H_{27}O_{17}N_2P$: C, 46.56; H, 4.06; N, 4.18; P, 4.63. Found: C, 46.73; H, 4.23; N, 4.24; P, 4.39.

2,3,4-Tri-O-acetyl-6-(di-*p*-nitrophenylphosphoryl)-α-D-glucopyranosyl Bromide (VIII).—To 4.0 g. of VI was added 15 ml. of a 30% solution of anhydrous hydrogen bromide in glacial acetic acid. The flask was well stoppered and kept at room temperature with occasional shaking. Within 30 minutes all reactants had gone into solution. After an additional 40 minutes, the reaction mixture was poured dropwise into 150 ml. of cold water with vigorous mechanical stirring. The semi-crystalline mass of crude bromosugar was collected, washed with cold water, and all possible water was pressed out. The crude bromide was treated with 20 ml. of methanol and scratched, and the crystalline solid was collected after chilling to give 4.2 g. (92%) of bromosugar VIII (m.p. 109–110°). Recrystallization from absolute ethanol afforded colorless leaflets melting at 110–111°, $[\alpha]^{33}D$ +167° (*c* 2.00, chloroform). The bromosugar VIII gave a positive Beilstein test and immediately precipitated silver bromide on addition of ethanolic silver nitrate; VIII was stable for weeks when protected from moisture.

Anal. Calcd. for $C_{24}H_{24}O_{15}N_2PBr$: N, 4.05; P, 4.49. Found: N, 4.04; P, 4.83.

To a solution of 300 mg. of VIII in 10 ml. of dry benzene was added 1.0 g. of dry powdered silver acetate. The reaction mixture was shaken for 90 minutes at room temperature and filtered. Evaporation of the filtrate *in vacuo* afforded a sirup, which, on triturating with methanol, gave a crystalline solid, m.p. 139–141°, yield 170 mg. (60%). A mixed melting point of this product with VI (m.p. 146–147°) was 141–143°.

2,6-Di-[2′,3′,4′-tri-O-acetyl-6′-(di-*p*-nitrophenylphosphoryl)-D-glucopyranosyloxy]-5-methyl-pyrimidine (X).—A vigorously stirred suspension of 0.80 g. (1.74 mmoles) of dithyminylmercury[3] in 60 ml. of dry xylene was dried by azeotropic distillation of approximately one-third of the solvent. After addition of 2.40 g. (3.46 mmoles) of the bromosugar VIII to the cool stirred mixture, the mixture was refluxed for 90 minutes and was filtered hot. To the filtrate was added 200 ml. of petroleum ether to give precipitate which was collected after cooling, and dissolved in 40 ml. of chloroform. The chloroform solution was washed successively with 30% aqueous potassium iodide and 5% aqueous sodium bicarbonate until the latter showed no yellow coloration. After final washing with water, the chloroform solution was dried and evaporated *in vacuo* to leave a sirup, which was dissolved in ether. Subsequent evaporation of the solvent *in vacuo* afforded 1.65 g. of colorless powder. In the infrared spectrum of this powder no absorption in the region of 1620–1730 $cm.^{-1}$ was observed. Storage for 2 months in an ice-chest of an ethanolic solution of the powder, after addition of a small amount of acetone, gave 200 mg. (8.6%) of amorphous solid which melted at 100–102°. The melting point was raised to 102–105° by repetition of the above purification procedure. This purified compound also lacked absorption in the region of 1620–1730 $cm.^{-1}$, $[\alpha]^{32}D$ +22.3° (*c* 1.70, chloroform).

Anal. Calcd. for $C_{53}H_{52}O_{32}N_6P_2$: C, 47.26; H, 3.89; N, 6.24; P, 4.61. Found: C, 47.23; H, 3.63; N, 6.37; P, 4.57.

Treatment of X with Alkali.—To a solution of X in dioxane was added an equal volume of 0.5 *N* aqueous lithium hydroxide and the resulting clear solution was kept at room temperature for 1 hour. Paper chromatographic detection revealed that the glucosyl bond was considerably degraded to liberate thymine, the spots of which strongly absorbed ultraviolet light and gave R_f's corresponding to those of thymine: R_{f_4}, 0.71, R_{f_7} 0.74 and R_{f_8} 0.69. The ultraviolet spectrum of an aqueous extract of the spot was also identical with that of thymine.

Phosphorus/Thymine Ratio of X.—A small portion of X was hydrolyzed with 1 *N* lithium hydroxide (50% aq. dioxane) at 100° for 2 hours and an aliquot was taken to determine the phosphorus content by Allen's method.[42] Another aliquot was submitted to paper chromatography (solvent 1), and thymine was completely extracted from the corresponding zone with water. The content of thymine in the extract was determined photometrically. The phosphorus:thymine ratio found was 2:0.96.

2,3,4-Tri-O-acetyl-6-diphenylphosphoryl-α-D-glucopyranosyl Bromide (IX).—A solution of 4.0 g. of 1,2,3,4-tetra-*O*-acetyl-6-diphenylphosphoryl-β-D-glucopyranose[21] in 15 ml. of a 32% solution of anhydrous hydrogen bromide in glacial acetic acid was kept at room temperature for 3 hours. The reaction mixture was poured dropwise into 250 ml. of cold water with stirring to give an oily precipitate, which was extracted with 120 ml. of chloroform. After washing with water, the extract was dried and evaporated *in vacuo* leaving a colorless sirup, which crystallized on trituration with 10 ml. of petroleum ether (b.p. 40–65°). The solid was collected, washed with petroleum ether and dried to give 3.8 g. (92%) of bromosugar IX as colorless crystalline mass melting at 65–68°. Recrystallization from petroleum ether furnished colorless needles melting at 74–75°, $[\alpha]^{33}D$ +153° (*c* 1.51, chloroform). Similar to VIII, IX showed positive reactions both in the Beilstein and ethanolic silver nitrate tests. Even under anhydrous condition, IX turned brown in 1 day.

Anal. Calcd. for $C_{24}H_{26}O_{11}PBr$: P, 5.15. Found: P, 5.28.

(42a) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3741 (1957).

A suspension of 170 mg. of IX and 300 mg. of silver acetate in 3 ml. of dry benzene was shaken for 2 hours at room temperature. The sirupy product obtained on removal of the solvent was crystallized from 3 ml. of isopropyl ether to furnish 70 mg. (43%) of VII, m.p. 61–64°. A mixed melting point with standard sample (m.p. 65–68°) was 61–65°.

1-(2′,3′,4′-Tri-O-acetyl-6′-diphenylphosphoryl-β-D-glucopyranosyl)-thymine (XII).—From a vigorously stirred suspension of 1.80 g. (4 mmoles) of dithyminylmercury[3] in 60 ml. of dry xylene approximately one-third of solvent was azeotropically distilled. To the stirred warm mixture was added 4.80 g. (8 mmoles) of bromosugar IX and 20 ml. of dry xylene. The bath temperature was maintained at 150–160° for 80 minutes, while the reaction mixture once turned clear and became turbid. The mixture was filtered hot and to the filtrate was added 200 ml. of petroleum ether to give a precipitate. After cooling, the precipitate was collected and taken up in 50 ml. of chloroform. The chloroform solution was washed successively with 30% aqueous potassium iodide and water. The dried solution was evaporated *in vacuo* to leave a sirup, from which chloroform was completely removed by codistillation with ethanol. The residual glassy material crystallized on scratching in 5 ml. of ether. After cooling, the crystalline solid was collected and recrystallized from 5 ml. of methanol to afford 1.05 g. (41%) of XII as colorless needles melting at 166–169°. The melting point was raised to 170–172° by repeated recrystallization from methanol; $[\alpha]^{33}D$ +48.0° (*c* 2.08, chloroform).

Anal. Calcd. for $C_{29}H_{31}O_{13}N_2P$: C, 53.87; H, 4.79; N, 4.34; P, 4.80. Found: C, 53.95; H, 4.69; N, 3.99; P, 4.87.

1-(2′,3′,4′-Tri-*O*-acetyl-β-D-gluocopyranosyl)-thymine-6′ Phosphate (XIII).—A solution of 760 mg. of XII in 15 ml. of absolute methanol was hydrogenated over 100 mg. of Adams platinum oxide. Hydrogen uptake reached completion after 1 hour. After removal of the catalyst and subsequent evaporation of the solvent, 3 ml. of petroleum ether was added to the residual sirup and the sirup was solidified by addition of several drops of ethanol to give 576 mg. (99%) of XIII as colorless crystals, m.p. 230° dec. The product was very soluble in water, soluble in methanol or ethanol and insoluble in acetone, chloroform, ethyl acetate, ether or benzene; $[\alpha]^{29}D$ +17.7° (*c* 2.59, water), R_{f_1}: 0.30, M_{U5P} 0.89 (in this case a buffer solution of *p*H 9.0, which was prepared by adding ammonia to the standard buffer of *p*H 5.9, was used); ultraviolet absorption: λ_{max}^{EtOH} 262 (9050), λ_{min}^{EtOH} 231 (1730).

Anal. Calcd. for $C_{17}H_{23}O_{13}N_2P$: C, 41.30; H, 4.69; N, 5.66; P, 6.27. Found: C, 41.11; H, 4.76; N, 5.68; P, 6.29.

1-β-D-Glucopyranosyl-thymine-6′ Phosphate (XIV).—A solution of 580 mg. of XIII in a mixture of 12 ml. of absolute methanol and 8 ml. of 0.6 *N* potassium methoxide in methanol was stored in an ice-chest overnight with protection from moisture. Removal of the solvent *in vacuo* left a gelatinous material, which was dissolved in a small volume of water and evaporated to dryness *in vacuo*. The residue was dissolved in 5 ml. of water and decationized with excess of Dowex-50 (H^+) resin to make *p*H *ca.* 1–2. To the acidic solution thus obtained was added dropwise a saturated aqueous barium hydroxide solution to adjust the *p*H to *ca.* 9. A small amount of insoluble matter was removed by centrifugation and 20 ml. of ethanol was added to precipitate a white product, which was collected and redissolved in 45 ml. of water. The solution was filtered, concentrated *in vacuo* to about 5 ml. and treated with 15 ml. of ethanol. The precipitate was collected by centrifugation. After successive washing with 20 ml. of ethanol (twice), 20 ml. of absolute ethanol (twice) and 20 ml. of absolute ether, the product was dried over phosphorus pentoxide at 95° for 10 hours *in vacuo* to furnish 490 mg. (78%) of barium 1-β-D-glucopyranosyl-thymine-6′ phosphate as white powder, $[\alpha]^{32}D$ −5.28 (*c* 2.08, 0.11 *N* HCl); ultraviolet absorption: $\lambda_{max}^{pH\ 2}$ 263 (8870), $\lambda_{min}^{pH\ 2}$ 232 (2050), $\lambda_{max}^{pH\ 12}$ 264 (6830), $\lambda_{min}^{pH\ 12}$ 246 (5140); XIV was homogeneous both in paper chromatography and paper electrophoresis: R_{f_1} 0.24, R_{f_2} 0.36, R_{f_3}, 0.73, R_{f_4}, 0.29; M_{U5P} = 1.0. Periodate oxidation of XIV was carried out by the procedure of Moffatt and Khorana[43] and showed that 2.03 mole of the oxidant was consumed within 22 hours and did not increase thereafter.

Anal. Calcd. for $C_{11}H_{15}O_{10}N_2PBa\cdot 2H_2O$: C, 24.48; H, 3.55; N, 5.19; P, 5.74; Ba, 25.45. Found: C, 24.21; H, 3.77; N, 4.84; P, 5.72, 5.97; Ba, 25.75.

Enzymatic Dephosphorylation of XIV.—One hundred milligrams of XIV suspended in water was treated with Dowex-50 (H^+) and the acidic solution was made slightly alkaline (*p*H *ca.* 9) by addition of aqueous sodium hydroxide. To this solution was added 2 ml. of 0.2 *M* ammonium chloride–ammonia (*p*H 9.5) buffer, 2 ml. of 0.005 *M* magnesium acetate and 0.35 ml. of a solution of rat intestinal phosphomonoesterase.[44] The mixture was incubated for 64 hours at 36–38°. Paper chromatography revealed that about 90% of XIV was dephosphorylated to 1-β-D-glucopyranosyl-thymine. The product was separated by chromatography on sheets of paper (solvent 1). The cuttings of the paper containing the nucleoside were extracted with water and the extract was evaporated to dryness *in vacuo*. The residue was repeatedly evaporated with ethanol to afford 12 mg. (23%) of crystalline solid whose melting point and mixed melting point with authentic 1-β-D-glucopyranosyl-thymine (m.p. 264–265°) were 264–265°. Infrared and ultraviolet spectra of the product were also identical with those of authentic specimen. Furthermore, paper chromatograms gave identical R_f values for both the product and 1-β-D-glucopyranosyl-thymine: R_{f_1} 0.54, R_{f_2} 0.57, R_{f_5} 0.14, R_{f_6} 0.75.

1-(2′,3′,4′-Tri-*O*-acetyl-6′-diphenylphosphoryl-β-D-glucopyranosyl)-4-acetamido-2(1H)-pyrimidinone (XI).—To an azeotropically dried suspension of 0.70 g. (2 mmoles) of *N*-acetylcytosinemercury[23] in 40 ml. of xylene was added with stirring 1.20 g. (2 mmoles) of bromosugar IX and some dry xylene. After 10 minutes, when the temperature raised to 140°, another 1.20 g. (2 mmoles) of IX was added. Reaction occurred rapidly and within several minutes the mixture became completely clear. The bath temperature was maintained at 135° for additional 20 minutes. After decantation from a small amount of resinous material, the reaction mixture was treated with 200 ml. of petroleum ether. The precipitate was collected and dissolved in 30 ml. of chloroform, then the chloroform solution was treated as usual. Evaporation of the solvent *in vacuo* left a sirup, which was dissolved in ethanol. After removal of a small amount of insoluble matter by filtration, the solvent was removed *in vacuo* and the residue was triturated with 5 ml. of ether to give a crystalline solid, which was collected to furnish 770 mg. (57%) of crude XI. Recrystallization from ethanol afforded 500 mg. of XI as colorless needles melting at 183–185°. Recrystallization was repeated to the constant melting point of 202.5–203°, $[\alpha]^{33}D$ +37.7° (*c* 1.59, chloroform).

Anal. Calcd. for $C_{30}H_{32}O_{13}N_3P$: C, 53.49; H, 4.79; N, 6.23; P, 4.60. Found: C, 53.42; H, 5.18; N, 6.36; P, 4.57.

Benzyl 2,3-Di-*O*-benzoyl-5-diphenylphosphoryl-β-D-ribofuranoside (XVI).—Sirupy benzyl 5-diphenylphosphoryl β-D-ribofuranoside (XV) was prepared from benzyl β-D-ribofuranoside[33] according to Khorana's method[28] in 53% yield. To a solution of 1.15 g. of XV in 10 ml. of dry pyridine cooled in an ice-bath was added dropwise 1.1 ml. of benzoyl chloride with stirring and with adequate protection from moisture. After keeping at 40° for 75 minutes, 50 ml. of methylene dichloride was added to the mixture and the solution was washed successively with cold water, cold 3 *N* sulfuric acid and 5% sodium bicarbonate. From the dried methylene dichloride layer the solvent was removed *in vacuo* to give a sirup, which was dissolved in 1 ml. of isopropyl alcohol with gentle warming. The crystals that appeared on scratching were aged in an ice-chest, then collected; yield 1.25 g. (75% from I), m.p. 90–91°. Recrystallization from isopropyl alcohol gave colorless needles melting at 93.5–94.5°, $[\alpha]^{12}D$ −10.7° (*c* 2.14, chloroform).

Anal. Calcd. for $C_{38}H_{33}O_{10}P$: C, 67.05; H, 4.89; P, 4.55. Found: C, 67.00; H, 4.72; P, 4.28.

(43) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 1194 (1957).

(44) One milliliter of the enzyme solution split 490 γ/min. of orthophosphate from L-α-glycerophosphate.

Methyl 2,3-Di-*O*-benzoyl-5-diphenylphosphoryl-D-ribofuranoside (XX) from D-Ribose.—To a stirred solution of 10 g. of dry D-ribose in 200 ml. of absolute methanol was added 4 ml. of absolute methanol previously saturated with dry hydrogen chloride at 0°. Stirring was continued for 90 minutes at room temperature with protection from moisture, and 22 ml. of dry pyridine was added to the reaction mixture. A sirup obtained on removal of solvent *in vacuo* was dissolved in 40 ml. of dry pyridine. The pyridine solution was stirred and cooled in an ice-bath, then 18.0 g. of diphenyl phosphorochloridate was added dropwise with exclusion of moisture. When about half of the chloridate was added, crystalline pyridine hydrochloride appeared. After the addition, the mixture was stirred for 20 minutes with ice cooling, and left at room temperature overnight. To the solution was added 12 ml. of water and the mixture was concentrated *in vacuo* to a thick sirup. To the sirup was added 50 ml. of 1 *N* aqueous sodium hydroxide and, after vigorous shaking for 20 minutes, the mixture was extracted three times with 100-ml. portions of ether. The ethereal extract was washed twice with each 30 ml. of water and then dried over anhydrous sodium sulfate. Removal of the solvent gave pale yellow sirupy methyl 5-diphenylphosphoryl-D-ribofuranoside (XVIII), which could not be crystallized, in a yield of 14.9 g. (56% from D-ribose).

To a stirred solution of XVIII in 40 ml. of dry pyridine 14 g. of benzoyl chloride was added dropwise with ice cooling and exclusion of moisture. The reaction mixture was kept at room temperature overnight and poured into 400 ml. of cold water with stirring. The separated oil was extracted twice with each 100 ml. of chloroform and the extract was successively washed with 3 *N* sulfuric acid, 5% sodium bicarbonate solution and water. After drying, the solution was evaporated *in vacuo* to leave a sirup which was treated with 40 ml. of ethanol. On shaking the mixture, crystallization occurred at once. After cooling in an ice-chest overnight, the product was collected to give 16.5 g. (41% from D-ribose) of XX as colorless needles which melted at 75–79°. Recrystallization from 40 ml. of ethanol gave a pure product: yield 14.5 g. (36%), m.p. 85–86°, $[\alpha]^{9}D$ +31.5° (*c* 2.03, chloroform).

Anal. Calcd. for $C_{32}H_{29}O_{10}P$: C, 63.57; H, 4.84; P, 5.12. Found: C, 63.66; H, 4.85; P, 5.15.

A portion of XVIII was hydrogenated in methanol with Adams platinum catalyst. On paper chromatogram (solvent 1), the reaction mixture showed a single spot, the R_{f1} value of which was identical with that of standard methyl ribofuranoside-5 phosphate (XIX).[30]

Methyl 2,3-Di-*O*-acetyl-5-diphenylphosphoryl-D-ribofuranoside (XXI).—A solution of 2.0 g. of sirupy methyl 5-diphenylphosphoryl-D-ribofuranoside (XVIII) in a mixture of 7 ml. of dry pyridine and 1.5 ml. of acetic anhydride was kept at room temperature overnight. The mixture was poured into 60 ml. of cold water to precipitate an oil which was extracted with 50 ml. of chloroform. The extract was washed successively with 1 *N* hydrochloric acid, 5% sodium bicarbonate and water, and dried. Removal of chloroform *in vacuo* gave a sirup, which crystallized on standing at room temperature. After trituration with 5 ml. of petroleum ether (b.p. 40–60°), the product was collected to give 1.2 g. (25% from D-ribose) of XXI as colorless crystals, m.p. 48–54°. Recrystallization from ether–petroleum ether afforded fine prisms of pure product that melted at 62–62.5°, $[\alpha]^{10}D$ −8.0° (*c* 2.02, chloroform).

Anal. Calcd. for $C_{22}H_{25}O_{10}P$: C, 55.00; H, 5.25; P, 6.45. Found: C, 54.91; H, 5.11; P, 6.45.

1-*O*-Acetyl-2,3-di-*O*-benzoyl-5-diphenylphosphoryl-D-ribofuranose (XXII). (A) From XX *via* 2,3-Di-*O*-benzoyl-5-diphenylphosphoryl-D-ribofuranosyl Bromide.—A solution of 500 mg. of XX in 2 ml. of glacial acetic acid containing anhydrous hydrogen bromide in 32% amount was kept at room temperature for 45 minutes in a well-stoppered flask. After removal of solvent *in vacuo*, the volatile components were codistilled with 10 ml. of benzene under diminished pressure. The residual sirup was dissolved in 3 ml. of methylene dichloride and the solution was poured dropwise into a stirred mixture of 6 ml. of acetone, 0.6 ml. of water and 700 mg. of silver carbonate under ice cooling. After additional stirring for 1 hour at this temperature, the insoluble salts were removed by filtration. From the filtrate, which gave no more precipitate on addition of ethanolic silver nitrate, solvent was removed *in vacuo*. To the residue, a few milliliters of ethanol was added and again evaporated *in vacuo* to leave a pale yellow transparent sirup. The sirup was dissolved in 5 ml. of methylene dichloride and, after drying, the solvent was removed *in vacuo* to furnish the anhydrous sirup. To the dry sirup dissolved in 3 ml. of dry pyridine was added 1.5 ml. of acetic anhydride at 0°. The mixture was kept at room temperature for 15 minutes, then at 40° for 1 hour, then diluted with 20 ml. of methylene dichloride and the resulting solution was washed successively with each 30 ml. of cold water, cold 3 *N* sulfuric acid and 5% sodium bicarbonate. Removal of the solvent *in vacuo* from the dried methylene dichloride layer left a thick sirup, which crystallized upon trituration with 5 ml. of isopropyl alcohol. After aging in an ice-chest overnight, the crystalline material was collected, m.p. 103–106°, yield 305 mg. (58%). Recrystallization from isopropyl alcohol gave colorless needles which melted at 107–111°, $[\alpha]^{10}D$ +27.4° (*c* 2.01, chloroform).

Anal. Calcd. for $C_{33}H_{29}O_{11}P$: C, 62.66; H, 4.62; P, 4.90. Found: C, 62.62; H, 4.69; P, 4.96.

(B) By Direct Acetolysis of XX.—To a solution of 500 mg. of XX in 1.4 ml. of acetic anhydride and 0.6 ml. of glacial acetic acid was added dropwise 0.2 ml. of concentrated sulfuric acid at 0°. After keeping at room temperature overnight, the clear solution was poured into cold water. The oil that separated was extracted with chloroform and the extract was washed with 5% sodium bicarbonate and water successively, and then dried. Removal of solvent *in vacuo* left a sirup, which was dissolved in 1 ml. of ethanol and stored in an ice-chest for weeks. The precipitated crystalline mass melted at 105–110° and weighed 165 mg. (31%). Recrystallization from isopropyl alcohol gave colorless needles melting at 107–111°. A mixed fusion of this product with that synthesized by method A showed no depression.

1-(2′,3′-Di-*O*-benzoyl-5′-diphenylphosphoryl-β-D-ribofuranosyl)-thymine (XXIV).—To a solution of 1.70 g. (2.8 mmoles) of XX in 7 ml. of dry methylene dichloride and 0.25 ml. of acetic anhydride was added 7 ml. of 32% solution of anhydrous hydrogen bromide in glacial acetic acid and the solution was kept for 40 minutes at room temperature. The reaction mixture was evaporated *in vacuo* with protection from moisture and the residue was codistilled with 20 ml. of anhydrous toluene *in vacuo*. The codistillation with toluene was repeated until the sirup was completely free from acidic odor. The sirupy bromosugar XXIII thus obtained was dissolved in 20 ml. of dry xylene and immediately used for the following condensation reaction.

A suspension of 0.63 g. (1.4 mmoles) of dithyminylmercury[3] in 40 ml. of dry xylene was azeotropically dried by distillation of approximately one-third of the solvent with vigorous stirring. To the cool stirred suspension was added the xylene solution of bromosugar and the mixture was refluxed for 90 minutes. The turbid mixture was filtered hot and to the filtrate was added 200 ml. of petroleum ether. After cooling in a cold room, the oily precipitate was taken up in 30 ml. of chloroform and the chloroform solution was treated as usual. The removal of chloroform *in vacuo* gave a sirup which was dissolved in 20 ml. of ethanol and again concentrated under vacuum to a brown sirup. The sirup was redissolved in 30 ml. of ethanol, decolorized with Norit, and concentrated to a volume of approximately 15 ml. Upon scratching, crystals precipitated. After crystallization was completed the yellowish needles were collected to give 0.49 g. (25%) of desired product, sufficiently pure for subsequent use, m.p. 137–140°. Recrystallization was repeated twice from ethanol to furnish colorless needles melting at 142–142.5°, $[\alpha]^{10}D$ −59.5° (*c* 1.39, chloroform); ultraviolet absorption: λ_{max}^{EtOH} 229 (34500), 261 (12300); $\lambda_{m,n}^{EtOH}$ 218 (26300), 250 (10600).

Anal. Calcd. for $C_{36}H_{31}O_{11}N_2P$: C, 61.89; H, 4.47; N, 4.01; P, 4.43. Found: C, 61.82; H, 4.45; N, 4.14; P, 4.60.

1-(2′,3′-Di-*O*-hexahydrobenzoyl-β-D-ribofuranosyl)-thymine-5′ Phosphate (XXV).—A solution of 450 mg. of XXIV in 35 ml. of methanol was shaken in hydrogen atmosphere over 50 mg. of Adams platinum oxide. Approximately 240 ml. (14 mole equiv.) of hydrogen was consumed during 21-hour shaking. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to a sirup, which on codistillation with ethanol gave a colorless

crystalline mass; yield 360 mg. (100%), m.p. 209–211° dec. (sintering 188–190°), $[\alpha]^{19}D$ −19.5° (*c* 1.54, ethanol), R_{f_1} 0.64, M_{U5P} = 0.68[45]; ultraviolet absorption: λ_{max}^{EtOH} 264 (9020), λ_{min}^{EtOH} 233 (2220).

Anal. Calcd. for $C_{24}H_{35}O_{11}N_2P$: N, 5.01; P, 5.54. Found: N, 4.83; P, 5.25.

1-β-D-Ribofuranosylthymine-5′ Phosphate (XXVI).—To a solution of 250 mg. of XXV in 10 ml. of absolute ethanol was added 7 ml. of 1 *N* sodium ethoxide in ethanol. The mixture, in which a gelatinous sodium salt of XXVI began to appear within several minutes, was refluxed for 1 hour and concentrated *in vacuo* to dryness. To the residual powder was added 5 ml. of water and decationized with excess of Dowex-50 (H^+). After removal of the resin, the aqueous solution was washed twice with each 5 ml. of chloroform and concentrated *in vacuo* to *ca.* 2 ml. The *p*H of the solution was adjusted to 9 by cautious addition of saturated aqueous barium hydroxide. A small amount of insoluble matter was centrifuged off and to the supernatant was added three volumes of ethanol to give a white precipitate, which was collected by centrifugation and washed successively with 10 ml. of ethanol, 8 ml. of absolute ethanol and 10 ml. of dry ether. Drying *in vacuo* over calcium chloride overnight afforded 194 mg. (82%) of white powdery barium 1-β-D-ribofuranosylthymine-5′ phosphate, $[\alpha]^{8}D$ −15.6° (*c* 2.36, 0.1 *N* HCl); ultraviolet absorption: $\lambda_{max}^{pH\,2}$ 267 (8920), $\lambda_{min}^{pH\,2}$ 235 (2190); $\lambda_{max}^{pH\,12}$ 267 (7430), $\lambda_{min}^{pH\,12}$ 245 (5120). The single spot that was observed on paper chromatograms gave R_f values of R_{f_1} 0.08, R_{f_2} 0.43, R_{f_3} 0.62 and R_{f_4} 0.19; M_{U5P} = 1.0. On periodate oxidation of this product according to the procedure of Moffatt and Khorana,[43] the theoretical uptake (1.08 moles) of the reagent was observed within 14 minutes, without further consumption of the oxidant.

Anal. Calcd. for $C_{10}H_{13}O_9N_2PBa\cdot 3H_2O$: C, 22.76; H, 3.63; N, 5.31; P, 5.87. Found: C, 22.63; H, 3.30; N, 5.11; P, 6.21.

Enzymatic Dephosphorylation of XXVI.—One hundred and fifty milligrams of barium 1-β-D-ribofuranosylthymine-5′ phosphate was treated with Dowex-50 (H^+) in 10 ml. of water, and the acidic solution thus obtained was neutralized with 1 *N* sodium hydroxide. To the solution was added 3 ml. of 2 *M* glycine–sodium chloride–sodium hydroxide buffer (*p*H 8.5), 5 ml. of 0.06 *M* magnesium chloride and 50 mg. of crude venom of "Habu,"[46] and the mixture was incubated at 37° for 2 hours. Paper chromatography revealed that XXVI was completely dephosphorylated to 1-β-D-ribofuranosyl-thymine.[3] The reaction mixture was heated at 100° for 2 minutes and to the cooled mixture an equal volume of ethanol was added and centrifuged to remove the denaturated enzyme protein. The supernatant was again concentrated *in vacuo* to *ca.* 5 ml., which, on addition of 20 ml. of ethanol, precipitated glycine and inorganic salts.[47] The supernatant obtained after removal of these precipitates by centrifugation was concentrated to dryness. After the trituration of the residue with 10 ml. of hot ethanol and subsequent removal of the insoluble matter, the ethanolic solution was evaporated to dryness under diminished pressure. The residue was dissolved in 5 ml. of water and successively deionized with Dowex-50 (H^+) and IR-4B (OH^-). The deionized solution was evaporated *in vacuo* to give a sirup, which was repeatedly evaporated five times with added ethanol. The residual sirup gave a crystalline product on trituration with 2 ml. of warm ethanol and subsequent scratching. This was collected by filtration to afford 30 mg. (41%) of 1-β-D-ribofuranosyl-thymine which showed melting point and mixed melting point with an authentic sample[3] (m.p. 182°) of 182°. Both infrared and ultraviolet spectra of the product were identical with those of an authentic sample, respectively. The R_f values were also identical with those of 1-β-D-ribofuranosyl-thymine R_{f_1} 0.60 and R_{f_2} 0.56.

Chloromercuri-6-benzamidopurine.[34]—To a solution of 1.36 g. (5 mmoles) of mercuric chloride in 50 ml. of 50% aqueous ethanol was added 1.20 g. (5 mmoles) of 6-benzamidopurine[48] and the suspension was stirred and refluxed. To the mixture was added dropwise 10 ml. of 0.5 *N* sodium hydroxide within 30 minutes. After cooling, the precipitated chloromercuri salt was collected and washed successively with water, ethanol and ether, and dried *in vacuo* over phosphorus pentoxide at 100° for 5 hours to give 2.37 g. (100%) of chloromercuri-6-benzamidopurine as a white powder.

6-Benzamido-9-(2′,3′-di-*O*-benzoyl-5′-diphenylphosphoryl-β-D-ribofuranosyl)-purine (XXVII).—A solution of the bromosugar XXIII prepared from 604 mg. (1 mmole) of XX in 10 ml. of dry xylene was added with stirring to an azeotropically dried warm suspension of 474 mg. (1 mmole) of chloromercuri-6-benzamidopurine in 30 ml. of xylene. The bath temperature was raised to 120–125°; within several minutes the reaction mixture became completely transparent. After warming for an additional 30 minutes at this temperature, the mixture was treated with 200 ml. of petroleum ether to give a precipitate, which was separated by filtration and dissolved in 30 ml. of chloroform. The chloroform solution was treated as usual and removal of solvent *in vacuo* afforded a sirup, which was dissolved in acetone and again evaporated *in vacuo* to leave 530 mg. (65%) of yellowish powder that showed no tendency to crystallize; ultraviolet absorption: λ_{max}^{EtOH} 230, 261 (shoulder), 280; λ_{min}^{EtOH} 255. The product gave a single spot with R_{f_1} 0.99 in paper chromatography and did not consume hydrogen on catalytic hydrogenation over Adams platinum catalyst at *ca.* 40°, even when the reaction was performed in acidic media.

Anal. Calcd. for $C_{43}H_{34}O_{10}N_5P$: P, 3.82. Found: P, 3.96.

6-Benzamido-9-β-D-ribofuranosyl-purine-5′ Phenyl Hydrogen Phosphate (XXVIII).—To a solution of 220 mg. of XXVII in 3 ml. of dioxane was added 3 ml. of 1 *N* sodium hydroxide and the resulting transparent yellow solution was kept at room temperature for 3 hours. To the reaction mixture was added excess Dowex-50 (H^+) resin to adjust the *p*H of the mixture to *ca.* 1–2. The acidic solution was concentrated *in vacuo*, during which a crystalline solid appeared in the mixture. Concentration was continued to dryness and to the residue a few milliliters of ethanol was added and evaporated *in vacuo* to remove a trace amount of water and dioxane. On treatment of the residual sirup with acetone, a white solid appeared, which was collected to give 138 mg. (97%) of the product. The product, crude XXVIII, did not show a definite melting point, but sintered at 65–70° and slowly decomposed at 145–160°. On paper chromatography, this product gave a main spot of R_{f_1} 0.82 with a trace amount of a more slowly traveling spot of XXIX. The mobility of XXVIII in paper electrophoresis run in a buffer of *p*H 9.0 (prepared by adding ammonia to the standard buffer of *p*H 5.9) was M_{A5P} = 0.65, indicating that XXVIII is a phosphodiester; ultraviolet absorption: $\lambda_{max}^{H_2O}$ 280; $\lambda_{min}^{H_2O}$ 244, shoulder, 232, 250. The ultraviolet absorption of 6-benzamido-9-(tetra-*O*-acetyl-β-D-glucopyranosyl)-purine[12] (λ_{max}^{EtOH} 233, 279; λ_{min}^{EtOH} 226, 245) was very similar to that observed for XXVIII; $[\alpha]^{15}D$ −12.2° (*c* 1.31, water).

Anal. Calcd. for $C_{23}H_{22}O_8N_5P$: P, 5.88. Found: P, 6.18.

Because of the instability of XXVIII, further purification was not attempted; thus, a short treatment of XXVIII in hot methanol gave a considerable amount of debenzoylated product (XXIV). On periodate oxidation,[43] the crude XXVIII consumed 0.86, 0.93 and 1.04 moles of the reagent in 4, 30 minutes and 17 hours, respectively. Similar to XXVII, XXVIII also resisted to be hydrogenated catalytically.

Sodium salt of crude XXVIII was treated with crude venom of "Habu"[46] in the presence of magnesium ion (*p*H 8.6). A paper chromatogram prepared for the hydrolyzate

(45) The unusually high R_f and low M_{U5P} values observed for this phosphomonoester are probably due to its solubility characteristics: XXV was easily soluble in ethanol, pyridine or acetone, but only slightly soluble in water, and almost insoluble in other organic solvents such as chloroform, ether, ethyl acetate or benzene. This property is assumed to be due to the hexahydrobenzoyl substituents at the 2′- and 3′-positions.

(46) The enzyme preparation was offered by the courtesy of Dr. D. Mizuno of the National Institute of Health, Japan.

(47) Although paper chromatography revealed that a considerable amount of 1-β-D-ribofuranosyl-thymine was contained in this precipitate, no further isolation of this nucleoside from the precipitate was performed.

(48) A. Kossel, *Z. physiol. Chem.*, **12**, 241 (1888); J. R. Parikh, M. E. Wolff and A. Burger, *J. Am. Chem. Soc.*, **79**, 2778 (1957).

using solvent system 4 indicated that XXVIII was dephosphorylated to 6-benzamido-9-β-D-ribofuranosylpurine which gave a spot with R_{f_4} 0.82.

Adenosine-5′ Phenyl Hydrogen Phosphate (XXIX).—To a solution of 100 mg. of crude XXVIII in 3.5 ml. of absolute methanol was added 1.5 ml. of methanolic 1 *N* sodium methoxide. After refluxing the mixture for 70 minutes, the *p*H was adjusted to *ca.* 2 by addition of Dowex-50 (H^+). The acidic solution was concentrated *in vacuo* to dryness and trace amounts of water were removed by co-distillation with ethanol *in vacuo*. On addition of acetone and subsequent removal of the solvent *in vacuo*, the residue afforded a crude desired product as a yellowish powder; yield 70 mg. (88%). On paper chromatography, the product gave a main spot of R_{f_1} 0.66 with a trace amount of slowly traveling unidentified contaminant; ultraviolet absorption $\lambda_{max}^{H_2O}$ 230. When the crude XXIX was treated with 4 ml. of water, the almost colorless pure product, m.p. 221–222° dec., was obtained as an insoluble precipitate: yield 24 mg. (30%). This compound showed a single spot (R_{f_1} 0.66) on paper chromatogram. A suspension of this product in a small amount of water was neutralized with 1 *N* sodium hydroxide to give a clear solution which was adjusted to *p*H *ca.* 4 by portionwise addition of Dowex-50 (H^+). To the solution separated from resin, was added several grains of Dowex-50 (H^+) to precipitate colorless crystals (m.p. 222° dec.), which were collected and analyzed; ultraviolet absorption: $\lambda_{max}^{pH\ 8}$ 260 (14700), $\lambda_{min}^{pH\ 8}$ 228 (3100).

Anal. Calcd. for $C_{16}H_{18}O_7N_5P \cdot 1/2H_2O$: C, 44.45; H, 4.43; N, 16.20; P, 7.16. Found: C, 44.21; H, 4.57; N, 16.42; P, 7.42.

The sodium salt of XXIX was treated with crude venom as in the case of XXVIII. Paper chromatograms obtained with three different solvent system revealed that XXIX was completely hydrolyzed to adenosine and orthophosphate. Aqueous extract of the spots, which gave the R_f value of R_{f_1} 0.83, R_{f_4} 0.36 and R_{f_6} 0.79, showed identical ultraviolet spectrum with that of adenosine.

Adenosine-5′ Phosphate (XXX).—To a suspension of 70 mg. of crude powdery XXIX in 3 ml. of water was added 1 *N* sodium hydroxide to adjust the *p*H to *ca.* 9. After the addition of 0.5 ml. of 0.005 *M* magnesium acetate, 3.5 ml. of ammonia–ammonium chloride buffer (*p*H 9.0) and 0.3 ml. of enzyme solution (venom phosphodiesterase of Russel's viper),[36,49] the solution was incubated at 36–38° for 23 hours. Paper chromatography revealed that over 95% of XXIX was converted to adenosine-5′ phosphate. The mixture was decationized with 0.2 ml. of Dowex-50 (H^+) and concentrated *in vacuo* to *ca.* 3 ml. After the *p*H was adjusted to 9.5 with saturated aqueous barium hydroxide solution, a small amount of insoluble material was removed by centrifugation. To the supernatant was added three volumes of ethanol and the resulting precipitate was collected. The crude barium salt of XXX thus obtained was decationized with 0.1 ml. of Dowex-50 (H^+) in 5 ml. of water. The aqueous solution was concentrated *in vacuo* to *ca.* 0.5 ml. to which acetone was added to turbidity. The oily precipitate was seeded with a trace of adenosine-5′ phosphate. Upon scratching, colorless crystals appeared, which were collected to give 24 mg. (42%) of adenosine-5′ phosphate. The melting point of the product was 187–188° (turned brown and bubbled). The standard sample[37] of adenosine-5′ phosphate as well as a mixture of the latter and the product similarly turned brown and bubbled on melting at 187–188°. The each single spot observed for the product on paper chromatograms gave respective R_f values of R_{f_1} 0.08, R_{f_2} 0.57, R_{f_3} 0.56, which was identical with that for authentic adenosine-5′ phosphate. The ultraviolet spectrum in water or 1% sodium bicarbonate was also identical with that of the standard sample.

(49) One milliliter of the enzyme solution contained 40γ of the enzyme protein.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, THE WEIZMANN INSTITUTE OF SCIENCE, REHOVOTH, ISRAEL, AND FROM THE DEPARTMENT OF BIOCHEMISTRY AND THE UNIT FOR RESEARCH IN AGEING, THE ALBERT EINSTEIN COLLEGE OF MEDICINE, YESHIVA UNIVERSITY, BRONX 61, NEW YORK]

Peptide Synthesis *via* Oxidative Activation of Acid Hydrazides[1,2]

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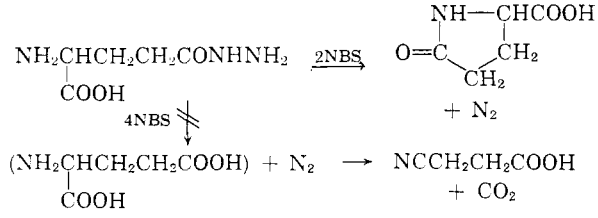
A new method for the synthesis of peptides has been developed based on the oxidative activation of N-acyl-α-amino acid or peptide hydrazides. The method is simple, rapid and gives yields which compare favorably with and occasionally exceed those found with methods in current use. It was found to give little racemization and accordingly, could be applied to the synthesis of peptides containing side chains which are not sensitive to the oxidative conditions employed. Oxidation of a tripeptide hydrazide led to the synthesis of a poly-α-amino-acid with a known sequence.

Curtius[4] first showed that upon oxidation of acyl hydrazides by iodine bis-diacyl hydrazides were obtained. A few years ago Carpino found that

$$2RCONHNH_2 + 2I_2 \longrightarrow RCONHNHOCR + N_2 + 4HI$$

under highly acidic conditions acyl hydrazides may be oxidized by chlorine to the corresponding acyl chlorides.[5] Recently it was found that various acyl hydrazides including benzyloxycarbonylamino acid hydrazides were oxidized by 2 moles of N-bromosuccinimide (NBS) in dilute aqueous solution to the corresponding carboxylic acids with evolution of nitrogen. However, the γ hydrazide of glutamic acid was not oxidized through glutamic acid to β-cyano propionic acid as expected but pyrrolidone carboxylic acid was obtained in quantitative yield.[6] These data suggested the possibility that the intermediate which resulted from acyl hydrazide oxidation was a powerful acylating agent and that this intermediate could be formed



(1) Presented in part before the 28th meeting of the Israel Chemical Society, Rehovoth 1961 (Y. Wolman and P. M. Gallop, *Bull. Research Council Israel*, **10A**, 43 (1961)). A preliminary communication of this work has been reported (Y. Wolman, P. M. Gallop and A. Patchornik, *J. Am. Chem. Soc.*, **83**, 1263 (1961)).

(2) Part of a thesis submitted by Y. Wolman in partial fulfillment of the requirements for the degree of Doctor of Philosophy to the Hebrew University, Jerusalem, May 1961.

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(4) T. Curtius, *J. Prak. Chem.*, **50**, 281 (1894).

(5) L. A. Carpino, *J. Am. Chem. Soc.*, **79**, 96 (1957).

(6) P. M. Gallop, S. Seifter and C. Franzblau, unpublished results.