Chem. Pharm. Bull. 15(12)1847~1855(1967)

UDC 547.963.3.07

235. Bunji Shimizu, Motoji Asai, and Takuzo Nishimura*1 : Studies on Synthetic Nucleotides. I. A Convenient Synthesis of Ribonucleotides.*2

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A convenient synthetic procedure of pyrimidine and purine nucleotides was described. The fusion of phosphorylated acylhalogenoses with trimethylsilylpyrimidines or -purines and then removal of the protecting groups gave several nucleotides in good yields. The structural confirmation of the nucleotides thus obtained was achieved by chromatographic and spectral analyses, as well as comparison with the authentic samples prepared by the known methods.

(Received October 28, 1966)

Among many synthetic methods now available for the preparation of nucleotides, the phosphorylation of properly protected nucleosides has found the widest application.[1~5]

However, as pointed out previously by Ukita and Hayatsu,[6] this method cannot always be the most convenient one, particularly if the synthesis of the prerequisite nucleosides involves numerous steps which proceed in poor yields. In this connection, they developed a new method in which phosphorylated aldose was condensed with pyrimidine and purine derivatives. They demonstrated also that this method was superior to the previous ones for the preparation of nucleotides containing unnatural bases since no prior synthesis of the corresponding nucleosides was required. However, this direct method[6,7] has neither been extensively explored nor been successfully applied for uracil, hypoxanthine or theophylline.

Our experience in the synthesis of pyrimidine and purine nucleosides[8] led us to examine the direct condensation of the trimethylsilyl derivatives of the bases with phosphorylated acylhalogenosugars as a synthetic method of nucleotides. In fact, as has already been mentioned in a preliminary note,*2 this extension was proved to be a convenient one for the nucleotide synthesis. In this paper we describe in detail the synthesis of several kinds of natural and unnatural nucleotides by this method. Methyl 5-diphenylphosphoryl-D-ribofuranoside,[6] prepared by the phosphorylation of methyl D-ribofuranoside with diphenylphosphorochloridate, was treated with benzoyl chloride to give methyl 5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranoside (I). The product (I) was converted into the corresponding 1-bromo derivative (II) with hydrogen bromide in acetic acid in the usual manner.[6] This bromide (II) was heated with bis(trimethylsilyl)thymine[8b] (IIIa) at 90~100° for 30 min. to give a crude sample of 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)-4-O-trimethylsilylthymine, which on treatment

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*2 A brief report of a part of this work was published as a communication to the Editor in This Bulletin, **13**, 230 (1965).

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with aq. ethanol afforded colorless needles in 48.1% yield. The latter was identified as 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)thymine (IVa) by comparing with an authentic sample prepared by Ukita, *et al.*[6] Catalytic hydrogenation of IVa,

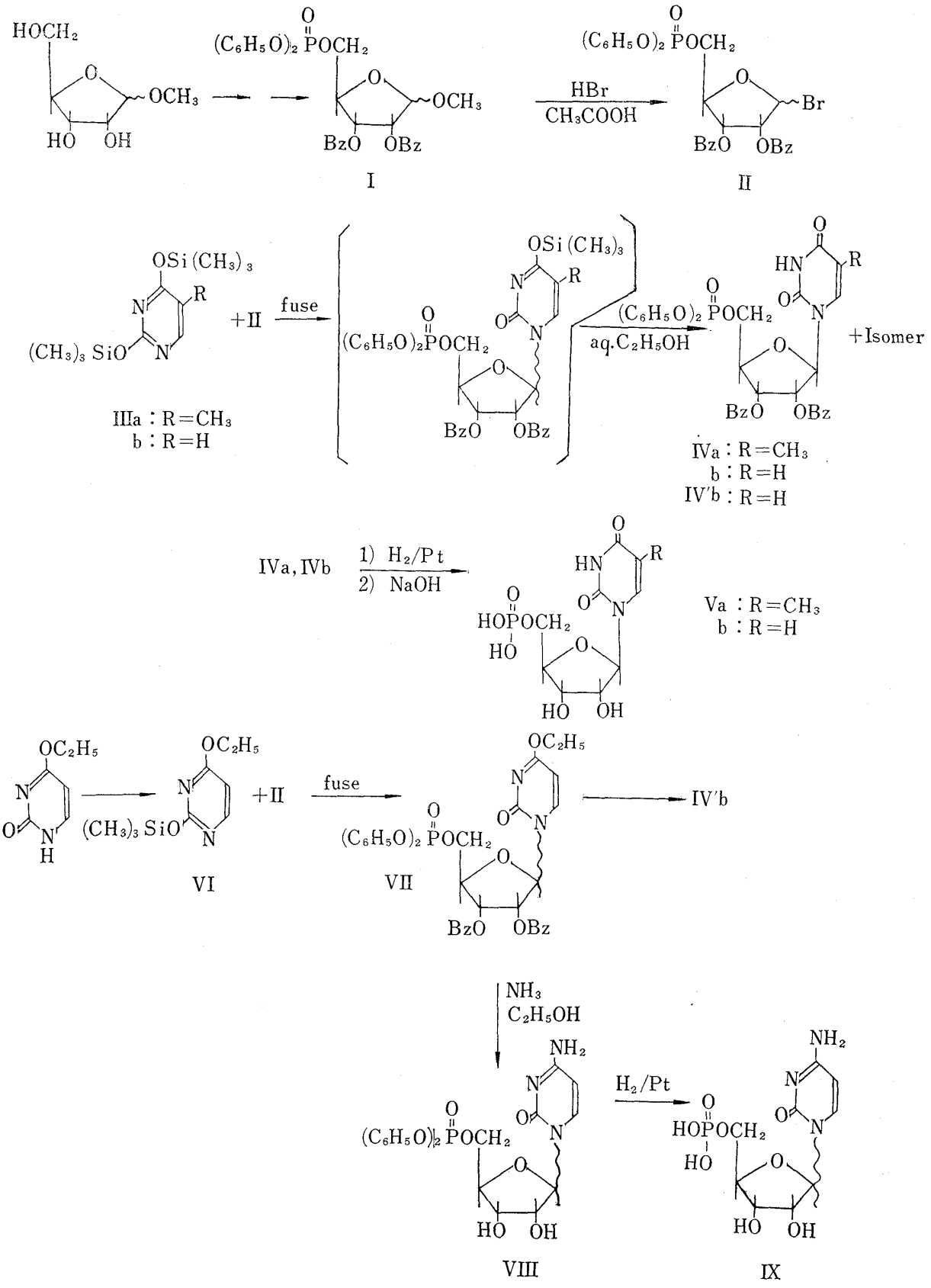

Chart 1.

followed by deacylation with alkali gave 1-β-D-ribofuranosylthymine 5′-phosphate (Va), which was isolated as its barium salt in 33.5% yield.

A similar condensation reaction of II with bis(trimethylsilyl)uracil[8b] (IIIb) afforded a compound IV′b, m.p. 193°, in 25.1% yield; the compound was found not to be 1-(5-diphenyl-2,3-di-O-benzoyl-D-ribofuranosyl)uracil (IVb), although its elemental analysis was identical with that of IVb. The structure of IV′b is now under investigation. After removing IV′b the mother liquor was chromatographed on silica gel and phosphorous-containing fractions were collected. From the fractions, an amorphous product was obtained in 10.2% yield. To this product was assigned the structure IVb on the basis of its elemental analysis and the following observations. Catalytic hydrogenation of the product IVb over Adams platinum oxide yielded an amorphous product (A) after absorbing *ca.* 14 mole equivalents of hydrogen. Behavior of A in the paper chromatography and paper electrophoresis revealed that the phenyl groups attached to the phosphoryl residue of IVb had been completely removed. Furthermore, the absence of an absorption at 230 mμ indicated that the benzene rings of the benzoyl residues in the 2′- and 3′-positions of IVb had also been hydrogenated. Deacylation of A with ethanolic sodium ethoxide gave 1-β-D-ribofuranosyluracil 5′-phosphate (Vb). The brucine salt of Vb showed no depression on admixture with that of 5′-UMP from natural source. In addition to IVb, a small amount of IV′b was obtained (5.1% yield).

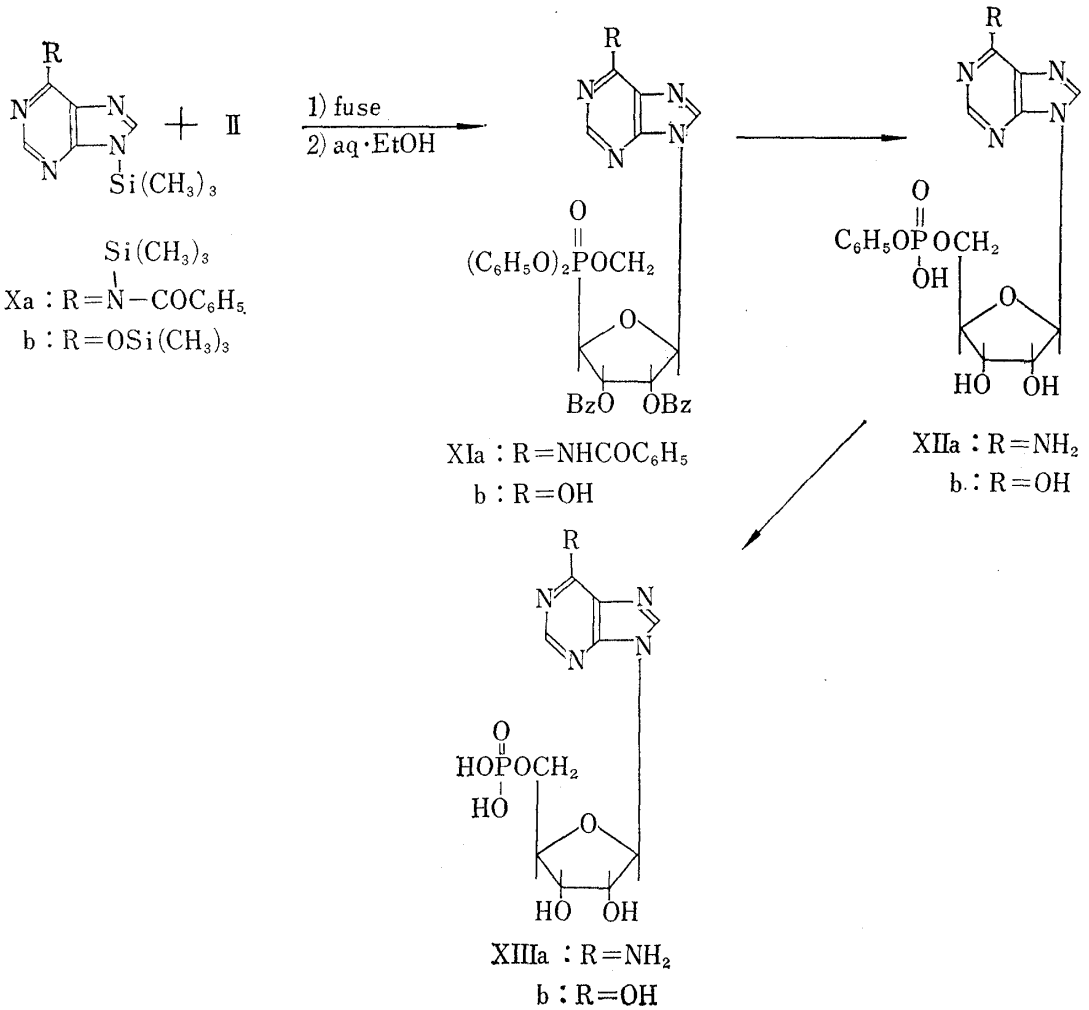

Chart 2.

Similarly, the condensation of trimethylsilyl-4-ethoxy-2(1*H*)-pyrimidinone (Ⅵ)*[3] with the bromide (Ⅱ) at 80~90° gave 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)-4-ethoxy-2(1*H*)pyrimidinone (Ⅶ). In addition to Ⅶ, the product (Ⅳ'b) was obtained in 7.5% yield. Amination of Ⅶ with ethanolic ammonia in a sealed tube at 100° afforded 1-(5-diphenylphosphoryl-D-ribofuranosyl)cytosine (Ⅷ). Catalytic hydrogenation of Ⅷ over Adams platinum oxide yielded 1-D-ribofuranosylcytosine 5'-phosphate (Ⅸ) which was isolated as its barium salt in 27.4% yield (based on Ⅶ).

The successful synthesis of 1-β-D-ribofuranosylpyrimidine 5'-phosphates prompted us to synthesize purine nucleotides by the same method. Condensation of the bromide (Ⅱ) with bis(trimethylsilyl)-6-benzamidopurine[8b] (Xa) gave 6-benzamido-9-(5-diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)purine (XIa) as a glassy solid in a yield of 37.6%. The ultraviolet absorption spectrum of this product (XIa) was identical with that of an authentic sample. The compound (XIa) was hydrolyzed with 0.5*N* sodium hydroxide to give an amorphous powder. That the ultraviolet absorption spectrum of this product showed an absorption maximum at 280 mμ indicated that the benzoyl residue on the C_6 amino group still remained. On the other hand, lack of absorption at 230 mμ indicated the absence of the benzoyl residue on the ribosyl moiety. Because of the unstability of the product, it was treated, without further purification, with methanolic sodium methoxide to give 9-(5-phenylphosphoryl-β-D-ribofuranosyl)adenine (XIIa). The absorption spectrum of XIIa was similar to that of adenosine. On incubation of XIIa with phosphodiesterase[9] prepared from *Trimeresurus flavoviridis* (Hallowell) the nucleotide (XIIIa) was isolated as its barium salt. The free nucleotide liberated from the barium salt was identical with an authentic adenosine 5'-phosphate by the criteria of its physical properties and paper chromatographic behavior.

Similarly, the condensation of bis(trimethylsilyl)hypoxanthine[8b] (Xb) with the compound (Ⅱ) gave as an amorphous solid 9-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)hypoxanthine (XIb) in 59.2% yield. The structure was assigned on the basis of the elemental analysis and ultraviolet spectrum. Deacylation of XIb with 1*N* sodium hydroxide gave an amorphous product (XIIb). The absence of absorption band at 5.8 μ in the IR spectrum of XIIb indicated that the benzoyl residues in the 2'- and 3'-positions of XIb had been completely removed. Dephenylation of XIIb with the phosphodiesterase gave 9-β-D-ribofuranosylhypoxanthine 5'-phosphate (XIIIb) (39.4%). Consequently, XIIb should be represented by 9-(5-phenylphosphoryl-β-D-ribofuranosyl)hypoxanthine (XIIb).

Similarly, the condensation of trimethylsilyltheophylline (XIV)*[4] with the bromide (Ⅱ) gave 7-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)theophylline (XV) in 73.3% yield. Deacylation of XV with 1*N* sodium hydroxide gave 7-(5-phenylphosphoryl-β-D-ribofuranosyl)theophylline (XVI), which, on incubation with the phosphodiesterase, liberated the remaining phenyl group to yield 7-β-D-ribofuranosyltheophylline 5'-phosphate (XVII) in 39.2% yield. The structure of XVII was confirmed by comparison with an authentic sample.[10]

*[3] The structure of this product (Ⅵ) was verified as to be shown in Chart 1 because an absorption band at 1660 cm^{-1} in IR spectrum was not observed but a new band appeared at 1070 cm^{-1} caused by O-Si stretching vibrations. Furthermore, the UV absorption spectrum of Ⅵ in dioxane solution was almost identical with that of 2,4-diethoxypyrimidine.

*[4] Characterization of XIV as 7-substituted theophylline (see Chart 3) was achieved in comparison with 7-ribosyltheophylline in UV spectrum of dioxane solution. This structural assignment was supported by the fact that coupling of XIV with the bromide (Ⅱ) gave 7-substituted derivative (XV).

9) *a*) The enzyme preparation was obtained through courtesy of Dr. H. Ikezawa of the National Institute of Health, Japan. *b*) E.J. Williams, S. Sung, M. Laskowski : J. Biol. Chem., **236**, 1130 (1961).

10) *a*) T. Kanazawa, H. Tamura, T. Sato : Nippon Kagaku Zasshi, **79**, 393 (1958). *b*) T. Kanazawa : *Ibid.*, **81**, 809 (1960).

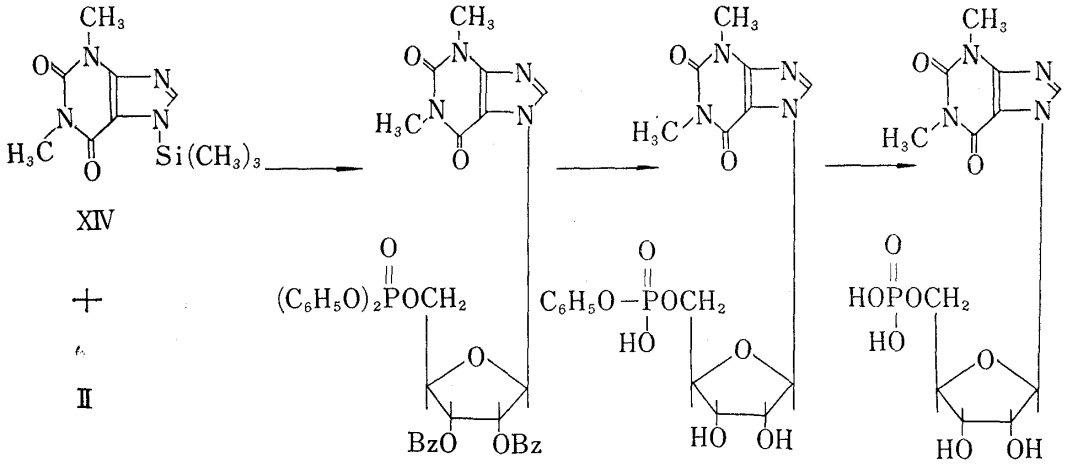

Chart 3.

Bromination of 1,2,3,4-tetra-O-acetyl-6-bis(*p*-nitrophenyl)phosphoryl-β-D-glucopyranose with 30% hydrogen bromide solution in glacial acetic acid gave crystalline 2,3,4-tri-O-acetyl-6-bis(*p*-nitrophenyl)phosphoryl-α-D-glucopyranosyl bromide (XVIII).[6] Treatment of XVIII with bis(trimethylsilyl)uracil (IIIb) at 110~120° yielded a colorless amorphous substance, which was confirmed to be 1-[6-bis(*p*-nitrophenyl)phosphoryl-2,3,4-tri-O-acetyl-D-glucopyranosyl]uracil (XIX) on the basis of its elemental analysis and ultraviolet spectrum. As in the case of IVa hydrogenolysis of XIX over platinum oxide afforded the dephenylated product, 1-D-glucopyranosyluracil 6'-phosphate (XX) in good yield. Similarity between the ultraviolet spectra of XX and XIX suggested that no reduction of the pyrimidine nucleus of XIX had occurred during this treatment. Further work on the configurational assignment of this product XX is now in progress.

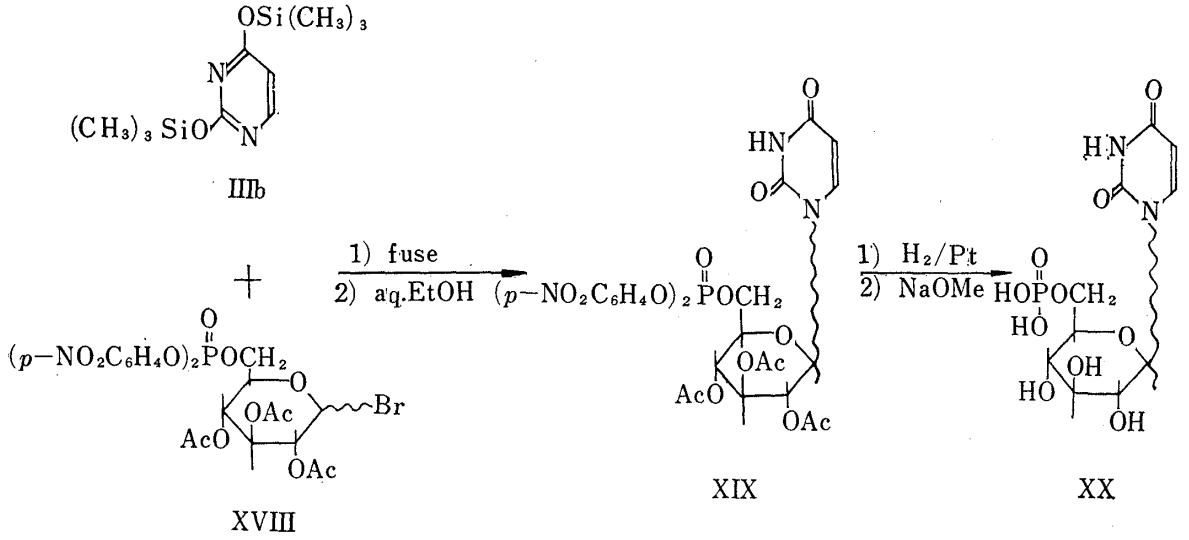

Chart 4.

Except isomeric uridine 5'-phosphate (IV'b) and isomeric cytidine 5'-phosphate (IX), all the natural and unnatural nucleotides described in this paper had previously been reported[1,3,4,6,10] and their physical properties were in good accordance with those reported.

Experimental

Methods——Paper chromatography was carried out on Toyo Roshi No. 51, using the ascending technique.

1-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)thymine (IVa)——Bis(trimethylsilyl)thymine (IIIa, 1.4 g.) and II which was prepared by treating 3.0 g. of methyl 5-diphenylphosphoryl-2,3-di-O-benzoyl-

D-ribofuranoside (I) with 14 ml. of 32% solution of anhydrous hydrogen bromide in glacial acetic acid, were dissolved in 80 ml. of dry toluene. After evaporation of the solvent *in vacuo*, the residue was heated at 100~110° for 40 min. under reduced pressure (100~80 mm Hg). After cooling, the reaction mixture was dissolved in aq. EtOH, and the solvent evaporated *in vacuo* to a gum. The gum was dissolved in 60 ml. of hot xylene. Insoluble thymine (180 mg.) was filtered off. To the filtrate was added a large amount of petroleum ether. After cooling in the refrigerator, a supernatant was removed by decantation. The oily precipitate was dissolved in 30 ml. of EtOH and again concentrated under reduced pressure to a thick sirup. The sirup was redissolved in 40 ml. of hot EtOH and concentrated to a volume of approximately 20 ml. The solution on standing in the cold overnight gave 1.25 g. (48.1%) of 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)thymine (IVa) as clusters melting at 138~140°. Recrystallization was repeated twice from EtOH to furnish colorless needles, melting at 142~143°; $[\alpha]_D^{25}$ −51.3° (c=1.2, $CHCl_3$), UV : λ_{max}^{EtOH} 229, 261 mμ (ε= 33,000, 12,000), λ_{min}^{EtOH} 218, 250 mμ. A mixed fusion of this product with an authentic sample[11] showed no depression. *Anal.* Calcd. for $C_{36}H_{31}O_{11}N_2P$: N, 4.01; P, 4.44. Found : N, 4.36; P, 4.80.

1-β-D-Ribofuranosylthymine 5'-Phosphate (Va)——Deblocking of IVa was carried out according to the method of Ukita and Hayatsu.[6] From 1.93 g. of IVa, crystalline 1-(5-phosphoryl-2,3-di-O-hexahydrobenzoyl-β-D-ribofuranosyl)thymine (1.48 g.) was obtained. It was treated with ethanolic sodium ethoxide to give 1-β-D-ribofuranosylthymine 5'-phosphate (Va) which isolated as colorless barium salt, 0.49 g. (33.5%) $[\alpha]_D^{26}$ −12.3° (c=2.0, 0.1*N* HCl), UV : $\lambda_{max}^{pH\,2}$ 267 mμ (ε=8,780). The single spot (Rf=0.19) was observed on paper chromatogram (solvent : *n*-BuOH-AcOH-H_2O=4:1:5). *Anal.* Calcd. for $C_{10}H_{13}O_9N_2PBa\cdot 3H_2O$: N, 5.31; P, 5.78. Found : N, 4.73; P, 6.30.

1-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)uracil (IVb)——A mixture of 2.6 g. of bis(trimethylsilyl)uracil (IIIb) and II, which was prepared from 6.0 g. of I by the similar procedure described above, was heated at 113° for 35 minutes and successively at 115° for 15 minutes under reduced pressure (100~80 mm.Hg). After cooling, the reaction mixture was dissolved in hot EtOH to remove trimethylsilyl group and the solvent evaporated to dryness *in vacuo*. The residue was dissolved in 80 ml. of hot xylene. Insoluble uracil (0.4 g.) was filtered and washed three times with each 10 ml. of $CHCl_3$. The filtrate and the washings were collected and the solvent was removed *in vacuo*. The gummy residue was dissolved in a minimum amount of benzene and then the solution cooled. Filtration gave unidentified crystalline product (IV'b) which was recrystallized from EtOH to give 0.62 g. of colorless needles (m.p. 190~193°). Further crop of the needles was obtained by chromatographic purification. After removal of the unidentified by-product, the filtrate was applied to the top of a column packed with silica gel (3.5 cm. *i.d.* × 30 cm. length). After 1L. of benzene was passed through the column, elution was begun using the mixture of benzene and ethyl acetate (8:2). The effluent was then collected in 100 ml. of fractions. In fraction 1~3, unidentified sugar fragment (0.45 g.) was eluted. Fraction 4~5 was evaporated to give a crystalline product which was recrystallized from benzene to yield 1.80 g. of colorless needles (m.p. 185~187°). From its elemental analysis (*Anal.* Calcd. for $C_{23}H_{19}O_7N_2Br$: C, 53.59; H, 3.69; N, 5.44. Found : C, 53.26; H, 3.48; N, 5.50) it has been suggested to be bromo-deoxy-dibenzoylribosyluracil. The determination of the structure of this product is in progress. Fraction 6~8 were collected and stripping the solvent gave 0.89 g. of unidentified resinous product. From 9~13, 0.46 g. of desired 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl) uracil (IVb) was obtained in 10.2% yield, as amorphous solid $[\alpha]_D^{26}$ −51.0° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{35}H_{29}N_2O_{11}P$: C, 61.40; H, 4.24; N, 4.09. Found : C, 61.24; H, 4.57; N, 4.17. Evaporation of fraction 16~35 gave 0.51 g. of crystalline product. It was recrystallized from EtOH to give 0.422 g. of needles which were identical with the crystals obtained directly from the benzene solution of the reaction mixture. *Anal.* Calcd. for $C_{35}H_{29}O_{11}N_2P$: N, 4.09; P, 4.53. Found : N, 4.39; P, 4.59, it was shown to be an isomer to IVb. Determination of its structure will be reported.

1-β-D-Ribofuranosyluracil 5'-Phosphate (Dibrucine salt) (Vb-dibrucinate)——A solution of 450 mg. of IVb in 50 ml. of CH_3OH was hydrogenated under atmospheric pressure in presence of 50 mg. of Adams platinum oxide for 10 hr. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The UV spectrum of the residue was measured; UV : λ_{max}^{EtOH} 262 mμ, λ_{min}^{EtOH} 247 mμ. To a solution of the residue in 12 ml. of absolute EtOH was added 8.5 ml. of 1*N* EtONa in EtOH. The solution was refluxed for 1 hr. and then concentrated to dryness *in vacuo*. The solution of the residue in *ca.* 6~8 ml. of H_2O was decationized with wet Dowex-50 resin (H^+). After removal of the resin, the aqueous solution was washed three times with $CHCl_3$ and evaporated. The aqueous solution of the residue was neutralized with NH_4OH and then applied to the top of a column (2.3 cm. *i.d.* × 13 cm. length) packed with Dowex-1 resine (Cl^- form, 200~400 mesh). After eluting the column with dilute hydrochloric acid (35 ml., pH 2.7; 765 ml., pH 2.5; 1215 ml., pH 2.3), pH was lowered to 2.0 and effluent was collected in 15 ml. fractions. Fraction 104~144, which showed intensively ultraviolet absorbing properties (λ_{max} 263 mμ), were evaporated to dryness under 40°. The residue was taken up in a small amount of H_2O and the solution evaporated dryness. This procedure was repeated twice for removal of the last trace of HCl. The nucleotide thus obtained was converted to its sodium

11) We should like to thank Prof. T. Ukita of the University of Tokyo for his generous gift of the authentic sample.

salt which gave the same NMR spectrum as that of natural 5′-UMP. An aqueous solution of the sodium salt was treated with Dowex-50 resin (H^+) and then neutralized with methanolic brucine solution. Evaporation of the solvent and recrystallization of the residue from 95% EtOH gave 332 mg. of dibrucine 1-β-D-ribofuranosyluracil 5′-phosphate. It softened at 170°, completely decomposed at 229~232° and showed no depression on admixture with the brucine salt of natural 5′-UMP. *Anal.* Calcd. for $C_{55}H_{65}O_{17}N_6P \cdot 3/2H_2O$: C, 57.94; H, 5.97; N, 7.37; P, 2.72. Found : C, 58.02; H, 6.05; N, 7.43; P, 2.24.

1-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)-4-ethoxy-2(1*H*)-pyrimidinone (VII)——To a suspension of 2.8 g. of dry powdered 4-ethoxy-2-(1*H*)-pyrimidinone and 2.4 g. of trimethylchlorosilane in 20 ml. of dry benzene was added dropwise the solution of 2.2 g. of triethylamine in dry benzene with stirring under anhydrous condition at room temperature. After the stirring was continued overnight, the reaction mixture was filtered. The filtrate was evaporated to yield a liquid which gave 2.8 g. of pure VI by fractional distillation, b.p_6 102°, UV : $\lambda_{max}^{dioxane}$ 259 mμ. *Anal.* Calcd. for $C_9H_{16}O_2N_2Si$: C, 50.95; H, 7.55; N, 13.21; Found : C, 51.35; H, 7.67; N, 12.88. The silyl derivative (VI, 1.1 g.) and II (from 3.0 g. of I) were dissolved in 70 ml. of dry toluene. After evaporation of the solvent *in vacuo*, the residue was heated at 80~90° for 50 min. under reduced pressure (100~80 mm.Hg). By treating the reaction mixture as described for IV, 2.9 g. of crude VII and 0.2 g. of 4-ethoxy-2(1*H*)pyrimidinone were obtained, which was chromatographically purified. Benzene solution of the crude VII was applied to a column (3 cm. *i.d.* × 30 cm. length) packed with silica gel, and the column washed with 560 ml. of benzene and 500 ml. of benbene-$CHCl_3$ (1:1). The solvent was changed to $CHCl_3$ and then the desired product appeared in effluent which was checked by thin-layer chromatography (Silica gel G; AcOEt-benzene=7:3). Evaporation of the eluant containing nucleotide derivative gave a resin which was triturated with ethanol to yield 0.8 g. of crystals. By recrystallization from EtOH, 0.7 g. (29.8%) of pure VII was obtained, m.p. 147~148°, $[\alpha]_D^{26}$ −103° (c=1.0, $CHCl_3$). UV: λ_{max}^{EtOH} 229, 267 mμ (ε=26,800, 9,000). *Anal.* Calcd. for $C_{37}H_{33}O_{11}N_2P$: C, 62.36; H, 4.63; N, 3.93; P, 4.35. Found : C, 62.13; H, 4.77; N, 4.10; P, 4.08. After elution of VII, 0.25 g. of the product, missing the ethyl group on pyrimidine ring, was obtained. It showed no depression on admixture with the unidentified crystals (IV′b).

1-D-Ribofuranosylcytosine 5′-Phosphate (IV)——A solution of 350 mg. of 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)-4-ethoxy-2(1*H*)pyrimidinone (VII) in 5 ml. of absolute EtOH saturated with dry NH_3 at 0° was heated at 100° for 10 hr. in a sealed tube. After cooling, the reaction mixture was evaporated to dryness. The residue was dissolved in 5 ml. of water, and extracted with 30 ml. of $CHCl_3$. The aqueous layer was evaporated to dryness *in vacuo*. The amorphous residue was obtained, UV : $\lambda_{max}^{pH\,1}$ 280 mμ, $\lambda_{max}^{pH\,12}$ 273 mμ, $\lambda_{min}^{pH\,1}$ 240 mμ, $\lambda_{min}^{pH\,12}$252 mμ. The solution of an amorphous product (VIII) in 20 ml. of EtOH was shaken in hydrogen atmosphere over 25 mg. of Adams platinum oxide, for 24 hr. The catalyst was removed by filtration and the filtrate concentrated *in vacuo* to a thick sirup. The residue was worked up as in the case of Va. Thus 69 mg. (27.4% based on VII) of barium salt of IX was obtained in a pure state. The product gave a single spot with Rf value of 0.17 on paper chromatogram (solvent : methylethylketone-*tert*-BuOH-AcOH-H_2O=5:5:6:4) $[\alpha]_D^{27}$ −55.2° (c=0.4, 0.1*N* HCl) UV : $\lambda_{max}^{pH\,2}$278 mμ, $\lambda_{min}^{pH\,2}$ 239 mμ, $\lambda_{max}^{pH\,12}$ 272 mμ, $\lambda_{min}^{pH\,12}$ 248 mμ. *Anal.* Calcd. for $C_9H_{12}O_8N_3PBa \cdot 3H_2O$: P, 6.05. Found : P, 6.49.

6-Benzamido-9-(5-diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)purine (XIa)——Two grams of bis(trimethylsilyl)-6-benzamidopurine (Xa) and II, which was prepared from 3 g. of I by the similar procedure described above were dissolved in 70 ml. of dry toluene. After evaporation of the solvent *in vacuo*, the residue was heated 100~110° for 50 min. By the similar procedure described for the corresponding IVb, amorphous product (3.4 g.) was obtained and 0.3 g. of 6-benzamidopurine was recovered. The crude product was dissolved in 30 ml. of benzene and chromatographed as in the case of IVb. The column was washed with 500 ml. of benzene and then the solvent was changed to 500 ml. of the mixture of $CHCl_3$ and AcOEt (95~80 : 5~20). Fractions containing nucleotide derivative were collected and evaporated *in vacuo*. The yellowish residue (1.2 g., 37.6%) showed no tendency to crystallize. The product gave a single spot on thin-layer chromatogram (benzene-AcOEt=3:7 on silica gel G). UV : λ_{max}^{EtOH} 230, 261 (shoulder), 277, 280 mμ. λ_{min}^{EtOH} 255 mμ. *Anal.* Calcd. for $C_{43}H_{34}O_{10}N_5P$: C, 63.63; H, 4.19; N, 8.63; P, 3.82. Found : C, 63.59; H, 4.43; N, 8.57; P, 3.56.

9-β-D-Ribofuranosyladenine 5′-Phosphate (5′-AMP) (XIIIa)——From 880 mg. of XIa, 460 mg. of amorphous 6-benzamido-9-(5-phenylphosphoryl-β-D-ribofuranosyl)purine[11] was obtained by hydrolysis with NaOH in aq. dioxane according to the method of Ukita and Hayatsu.[6] On paper chromatogram, this material gave major spot with Rf value of 0.82 (iso-PrOH-ammonia-H_2O=7:2:1) along with a small amount of a more slowly traveling spot (monophenyl derivative XIIa). UV : $\lambda_{max}^{H_2O}$ 280 mμ, $\lambda_{min}^{H_2O}$ 244 mμ.

Powdery 9-(5-phenylphosphoryl-β-D-ribofuranosyl)adenine (XIIa) (152 mg.) was obtained in 40.2% yield from 6-benzamido purine derivative described above by the treatment with NaOMe according to the reported method.[6] UV : $\lambda_{max}^{H_2O}$ 260 mμ, $\lambda_{min}^{H_2O}$ 230 mμ. Rf=0.22 (*n*-BuOH-AcOH-H_2O=4:1:5).

Sodium salt of XIIa was treated with phosphodiesterase[9] prepared from *Trimeresurus flavoviridis* (Hallowell) in the presence of magnesium ion at pH 9.0.[6] Paper chromatograms for the hydrolysate using two solvent systems revealed that XIIa was almost hydrolyzed to produce adenosine 5′-phosphate (AMP) (XIIIa). UV : $\lambda_{max}^{pH\,7}$ 260 mμ (ε=15,000), $\lambda_{min}^{pH\,7}$ 232 mμ (ε=2,200). $[\alpha]_D^{24}$ = −46.3° (in H_2O). Rf=0.19 (methylethylketone-*tert*-BuOH-AcOH-H_2O=5:5:6:4).

9-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)hypoxanthine (XIb)——Bis(trimethylsilyl)hypoxanthine (Xb) (1.4 g.) and II, prepared from 3.0 g. of I were dissolved in 70 ml. of dry toluene. After evaporation of the solvent *in vacuo*, the residue was heated 100~110° for 50 min. By the similar procedure described for XIa, an amorphous product (2.8 g.) and hypoxanthine (0.25 g.) were obtained. The crude product was dissolved in 30 ml. of benzene and chromatographed as in the case of VII. The column washed with 500 ml. of benzene and 300 ml. of $CHCl_3$ and then developed using the mixture of $CHCl_3$ and AcOEt (80:20). Effluent containing nucleotides derivatives were collected and evaporated *in vacuo* to give 1.3 g. (59.2%) of colorless amorphous product that showed no tendency to crystallize. The product gave a single spot on thin-layer chromatogram (Silica gel G, AcOEt-benzene=7:3). UV : λ_{max}^{EtOH} 230, 265 (shoulder) mμ. $[\alpha]_D^{26}$ −51° (c=0.5 in $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{29}O_{10}N_4P$: N, 7.92; P, 4.38. Found : N, 8.39; P, 4.28

9-β-D-Ribofuranosylhypoxanthine 5'-Phosphate (IMP) (XIIIb)——To a solution of 700 mg. of XIb in 10 ml. of dioxane was added 10 ml. of 1*N* NaOH and the resulting solution was kept at room temperature for 3 hr. To the reaction mixture was added Dowex-50 resin (H^+) to adjust the pH of the mixture to *ca.* 2. The acidic solution was concentrated to dryness *in vacuo* and to the residue 7 ml. of water was added. The resulting aqueous solution was repeatedly extracted with ether, the aqueous layer was evaporated to dryness *in vacuo*. On treatment of the residue with acetone, 370 mg. of amorphous product (XIIb) was obtained. The product did not show a definite m.p. sintering at 70~90°, and slowly decomposed at 140°. The product gave a spot with Rf value of 0.19 on paper chromatogram (solvent : *n*-BuOH-AcOH-H_2O=4:1:5). UV : $\lambda_{max}^{H_2O}$ mμ(pH) : 250 (1.0), 251 (7.0), 257 (12.0).

To a suspension of XIIb in 7 ml. of water was added 1*N* NaOH to adjust the pH to *ca.* 9. To the solution were added 1.7 ml. of 0.005*M* magnesium acetate, 1.1 ml. of 0.1*M* ammonia-ammonium chloride buffer (pH 8.8) and 3 mg. of phosphodiesterase described above and the resulting solution was incubated at 37~40° for 20 hr. Paper chromatography revealed that over *ca.* 95% of XIIb was converted to inosine 5'-phosphate. To the reaction mixture was added Dowex 50 resin (H^+) to adjust the pH of mixture to *ca.* 2. The acidic solution was concentrated *in vacuo* to a volume of *ca.* 3 ml. After the pH of the solution was adjusted to 9.5 by addition of saturated aqueous $Ba(OH)_2$ solution, a small amount of insoluble material was removed by centrifugation. To the supernatant was added three volumes (30 ml.) of EtOH and the resulting precipitate was collected by centrifugation. The crude barium salt of XIIIb thus obtained was decationized with 0.3 ml. of Dowex 50 resin (H^+) in 15 ml. of H_2O. The aqueous solution was concentrated *in vacuo* to *ca.* 1.5 ml. to which acetone was added to turbidity. Upon scratching, colorless crystals appeared, which were collected to give 120 mg. (39.4% based on XIIb) of inosine 5'-phosphate (XIIIb). UV : $\lambda_{max}^{pH\,6}$ 248 mμ (ε=12,200), $\lambda_{min}^{pH\,6}$ 225 mμ (ε=3,500). $[\alpha]_D^{24}$ −18.4° (c=0.9, 2% HCl).

A single spot (Rf=0.12) was observed on paper chromatogram (methylethylketone-*tert*-BuOH-AcOH-H_2O 5:5:6:4), which was identical with that for authentic inosine 5'-phosphate.

7-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)theophylline (XV)——The mixture of 9.0 g. of dried theophylline, 6.0 g. of trimethylchlorosilane and 5.6 g. of triethylamine was treated as in the case of VI. Trimethylsilyltheophylline (XIV) (11.0 g.) was obtained, m.p. 163~165° (decomp.), UV : $\lambda_{max}^{dioxane}$ 277 mμ. *Anal.* Calcd. for $C_{10}H_{16}O_2N_4Si$: C, 47.62; H, 6.35; N, 22.22. Found : C, 47.16; H, 6.35; N, 22.80.

The silyl derivatives (XIV) (1.3 g.) and II which was prepared from 3.0 g. of I was dissolved in 60 ml. of dry toluene. After evaporation of the solvent *in vacuo*, the residue was heated 100~110° for 40 min. By the similar procedure described as XIa the crude product (XV) was obtained. This material was dissolved in 40 ml. of benzene and insoluble theophylline (0.53 g.) was filtered off. The benzene solution was chromatographed as in the case of XIa. The column washed with 600 ml. of benzene and the solvent was changed to benzene-$CHCl_3$ (98:2). Fractions containing nucleotide derivatives were collected and evaporated *in vacuo*. A residue was treated with EtOH to yield 1.2 g. (73.3%) of crystals, which were recrystallized from EtOH to give 1.0 g. of XV, m.p. 152~154°. $[\alpha]_D^{26}$ −27.2° (c=2.0, $CHCl_3$) UV : λ_{max}^{EtOH} 230, 274 mμ (ε=25,000, 7,300). λ_{min}^{EtOH} 252 mμ. *Anal.* Calcd. for $C_{38}H_{33}O_{11}N_4P$: C, 60.64; H, 4.39; N, 7.45; P, 4.12. Found : C, 60.44; H, 4.42; N, 7.58; P, 4.19.

7-β-D-Ribofuranosyltheophylline 5'-Phosphate (XVII)——To the mixture of 300 mg. of XV, 3.5 ml. of of dioxane and 3.5 ml. 1*N* NaOH, the similar procedure was applied as in the case of XIa. The crude product of XVI (140 mg.) was obtained. UV : $\lambda_{max}^{pH\,1}$ 271 mμ, $\lambda_{min}^{pH\,1}$ 248 mμ, $\lambda_{max}^{pH\,10}$ 272 mμ, $\lambda_{min}^{pH\,10}$ 246 mμ.

The sodium salt of XVI was treated with phosphodiesterase as in the case of XIIIa. Paper chromatograms obtained with two different solvent system revealed that over 90% of XVI was converted to 7-β-D-ribofuranosyltheophylline 5'-phosphate (XVII). To the reaction mixture, the same procedure was applied as in the case of XIIIa. The crude barium salt of XVII thus obtained was decationized with 0.3 ml. of Dowex-50 resin (H^+) in 15 ml. of water. The aqueous solution was concentrated *in vacuo* to *ca.* 1.5 ml. to which acetone was added to turbidity. Upon scratching, colorless precipitate appeared, which was collected to give 61 mg. (39.2% based on XV) of 7-β-D-ribofuranosyltheophylline 5'-phosphate. UV : $\lambda_{max}^{pH\,1}$ 273 mμ, $\lambda_{min}^{pH\,1}$ 248 mμ, $\lambda_{max}^{pH\,11}$ 273 mμ, $\lambda_{min}^{pH\,11}$ 246 mμ. $[\alpha]_D$ +20.0°. Thin-layer chromatography : the product gave a single spot (*tert*-AmOH-AcOH-H_2O=4:3:3 on silica gel. G). *Anal.* Calcd. for $C_{12}H_{17}O_9N_4P$: N, 14.18; P, 8.12. Found : N, 14.41; P, 7.91. This substance was identified with an authentic sample of 7-β-D-ribofuranosyltheophylline 5'-phosphate.[10]

1-[6-Bis(*p*-nitrophenyl)phosphoryl-2,3,4-tri-O-acetyl-glucopyranosyl]uracil (XIX)——The silyl uracil (IIIb) (0.8 g.) and 6-bis(*p*-nitrophenyl)phosphoryl-2,3,4-tri-O-acetyl-*α*-D-glucopyranosyl bromide (XVII)(2.0 g.) was dissolved in 30 ml. of dry toluene. After evaporation of the solvent *in vacuo*, the residue was heated at 110~120° for 40 minutes by the similar procedure described as XIa. The crude material (1.3 g.) was obtained.

The material was dissolved in 15 ml. of benzene and insoluble uracil (0.15 g.) was filtered off. The benzene solution was chromatographed as in the case of XIa. The column washed with 200 ml. of benzene, 200 ml. of $CHCl_3$ and the solvent was changed to $CHCl_3$-AcOEt (8:2). Fractions containing nucleotide derivatives were collected and evaporated *in vacuo*. The pale yellowish residue (375 mg., 16.5%) showed no tendency to crystallize. The product gave a single spot on thin-layer chromatogram (benzene-AcOEt=7:3 on silica gel G), UV : λ_{max}^{EtOH} 259 mμ; λ_{min}^{EtOH} 232 mμ. *Anal.* Calcd. for $C_{28}H_{29}O_{17}N_4P$: P, 4.28. Found : P, 4.64.

1-D-Glucopyranosyluracil 6′-Phosphate (XX)——The solution of 300 mg. of XIX in 10 ml. of CH_3OH was hydrogenated under atmospheric pressure in presence of 40 mg. of Adams platinum oxide for 16 hr. The catalyst was removed by filtration and the filtrate was concentrated to dryness *in vacuo*. The residue was worked up as in the case of Va. Thus 65 mg. (27.1%) of barium salt of XX was obtained in a pure state.

The product gave a single spot with Rf value of 0.17 on paper chromatogram (Solvent : methylethylketon-*tert*-BuOH-AcOH-H_2O=5:5:6:4). UV : $\lambda_{max}^{pH\,1,6}$ 258 mμ, $\lambda_{min}^{pH\,1,6}$ 229 mμ, $\lambda_{max}^{pH\,11}$ 258 mμ, $\lambda_{min}^{pH\,11}$ 233 mμ. *Anal.* Calcd. for $C_{10}H_{13}O_{10}N_2P$-Ba·$5H_2O$: N, 4.83; P, 5.35. Found : N, 4.56; P, 4.82.

The authors wish to thank Mr. M. Matsui and Dr. I. Iwai for their interest in this work, and Mrs. M. Miyaki, Messrs. K. Iwase and A. Saito for their assistance in the experimental work.