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236. Motoji Asai, Michiko Miyaki, and Bunji Shimizu*1 : Studies on Synthetic Nucleotides. II.*2 A Direct Synthetic Method of Ribonucleotides.*3

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A direct synthetic method of purine and pyrimidine nucleotides has been described. Condensation of III with 2,3-di-O-benzoyl-5-diphenylphosphoryl-D-ribofuranosyl bromide in dimethylformamide at 40~50°, followed by removal of the protecting groups gave AMP.

Several other purine and pyrimidine nucleotides were also obtained by the same method mentioned above. In those cases, nitromethane, dimethylsulfoxide, acetonitrile and dimethylacetamide were effective for the reaction as solvents.

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The syntheses of nucleotides have involved, as general procedures, the phosphorylation of nucleosides having suitably protected sugar moieties and thus have required prior syntheses of nucleosides.[1)]

In this respect, a number of phosphorylating agents have been investigated and reported in the literature.[1)] However, attempts to synthesize nucleotides by direct condensations of phosphorylated sugars with derivatives of pyrimidine or purine bases are very few[1,2)] and have not been explored extensively.

In Part I*2 of this series, we reported a convenient synthetic method of pyrimidine or purine ribonucleotides starting from 5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl bromide[1)] and trimethylsilyl derivatives of pyrimidine or purines.

In the hope of developing a simplar method for the preparation of both purine and pyrimidine nucleotides, the authors investigated a direct condensation reaction of phenylphosphoryl acylhalogenosugars with the corresponding bases.

The present paper describes the syntheses of ribonucleotides by the application of the direct alkylation method reported by Leonard, *et al.*[3)] for the synthesis of 3-β-D-ribofuranosyl adenine starting from adenine and acylhalogenosugars.

This synthetic method of ribonucleotides allowed us to eliminate the troublesome step of the formation of the metal salt or trimethylsilyl derivatives of purine or pyrimidine bases. Moreover, the condensation was carried out in an organic solvent without any catalyst to give the nucleotides in a good yield. This method seems to be available for the synthesis of purine nucleotides.

Direct alkylation on the 3-position of adenine is preferred,[4~7)] and Leonard[3)] has recently reported a parallel synthesis of 3-ribofuranosyl adenine. Direct alkylation[7~9)] on the 3-position of 6-benzamidopurine has also been shown to be preferred, as in the

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*2 Part I : B. Shimizu, M. Asai, T. Nishimura : This Bulletin, **15**, 1847 (1967).

*3 Brief reports of a part of this work were published as a short communication in Agr. Biol. Chem., **29**, 170 (1965).

1) T. Ukita, H. Hayatsu : J. Am. Cnem. Soc., **84**, 1879 (1962).
2) M. Matsui, A. Nobuhara : Agr. Biol. Chem., **27**, 650 (1963).
3) N.J. Leonard. R.A. Laursen : J. Am. Chem. Soc., **85**, 2026 (1963); *Idem* : Biochemistry, **4**, 354 (1965).
4) N.J. Leonard, T. Fujii : *Ibid.*, **85**, 3719 (1963).
5) B.C. Pal : Biochemistry, **1**, 558 (1962).
6) J.W. Jones, R.K. Robins : J. Am. Chem. Soc., **84**, 1914 (1962).
7) J.A. Montgomery, H.J. Thomas : J. Heterocyclic Chem., **1**, 115 (1964).
8) B. Shimizu, M. Miyaki : Tetrahedron Letters, **1965**, 2059.
9) T. Fujii, T. Itaya, S. Yamada : This Bulletin, **13**, 1017 (1965).

case of adenine. However, the present authors can not report*4 a parallel direct synthesis of 3-β-ribofuranosyl-6-benzamidopurine 5'-phosphate under the following conditions. The condensation of 6-benzamidopurine (Ⅲ) with 5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl bromide (Ⅱ)*2,1) was attempted in various solvents. When 6-benzamidopurine (Ⅲ) was condensed with the bromide (Ⅱ) in dimethylformamide at 40° for 40 hr., 6-benzamido-9-(5-diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)purine (Ⅳ) was obtained in *ca.* 67% yield.

In this reaction, nitromethane, dimethylacetamide, dimethylsulfoxide and acetonitrile were also used: dimethylformamide and nitromethane were found to be most satisfactory for the synthesis of 6-benzamidopurine ribonucleotide. These solvents were chosen as the reaction media since most natural purines are rather soluble in these polar solvents.

Product (Ⅳ) obtained was identical with the authentic specimen prepared according to the silyl method reported in Part I*2 by comparison of its physical properties.

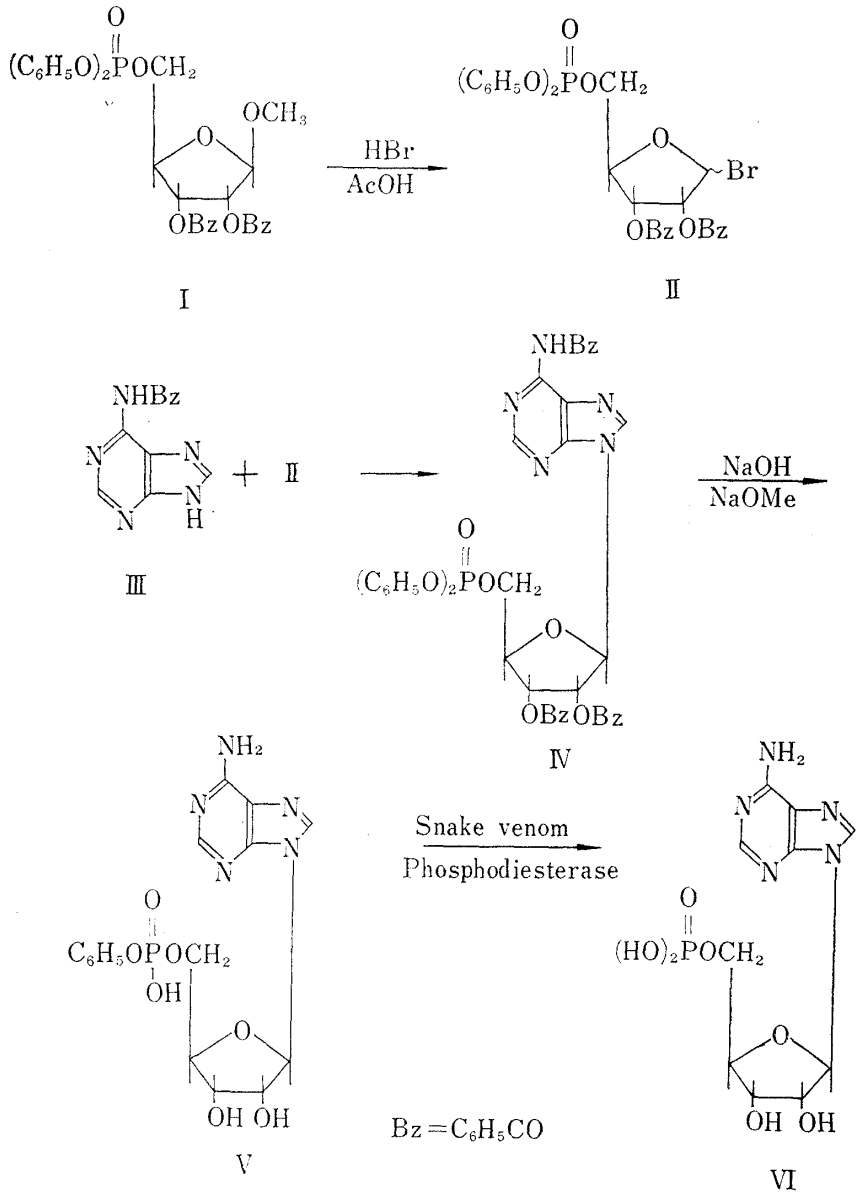


Chart 1.

*4 Under the milder conditions, the 3-ribosyl derivatives could be isolated: B. Shimizu, M. Miyaki: Chem. Indust. (London), **1966**, 664.

Compound (Ⅳ) was converted to the monophenyl derivative (Ⅴ) *via* the 6-benzamido-monophenyl derivative by treating with alkali according to the procedure reported by Ukita and Hayatsu. The structures of these products were assigned from elemental analysis and ultraviolet spectra. After incubation of Ⅴ with phosphodiesterase prepared from *Trimeresurus flavoviridis* (Hallowell), the nucleotide (Ⅵ) was isolated as its barium salt.

The successful condensation led us to extend this reaction to other purine bases. Treatment of hypoxanthine with Ⅱ gave an amorphous product (Ⅷ) in a yield of 36.6% (DMSO), which was identical with the authentic sample of 9-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)hypoxanthine prepared by the method reported in Part I,*[2] by comparison of its physical properties. Product (Ⅷ) did not consume hydrogen on catalytic hydrogenation. The aqueous alkaline treatment of Ⅷ gave 9-(5-phenylphosphoryl-D-ribofuranosyl)hypoxanthine (Ⅸ), which was isolated in the amorphous state. Its absorption spectrum was similar to that of IMP. The monophenyl derivative (Ⅸ) was enzymatically dephenylated by incubation with the phosphodiesterase to afford the nucleotide (Ⅹ), and it was also identified with authentic inosinic acid by comparison of its physical properties.

Furthermore, theophylline (Ⅺ) reacted with Ⅱ in the same procedure, to give needles of 7-(5-diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)theophylline (Ⅻ) in 77% yield, which was identical with the sample prepared by a reported method,*[2] Compound Ⅻ was hydrolyzed with 0.5*N* sodium hydroxide to give an amorphous powdery product (ⅩⅢ). Its absorption spectrum was similar to that of 7-β-D-ribofuranosyltheophylline. On hydrogenation of ⅩⅢ with Adams platinum oxide, no dephenylated product was

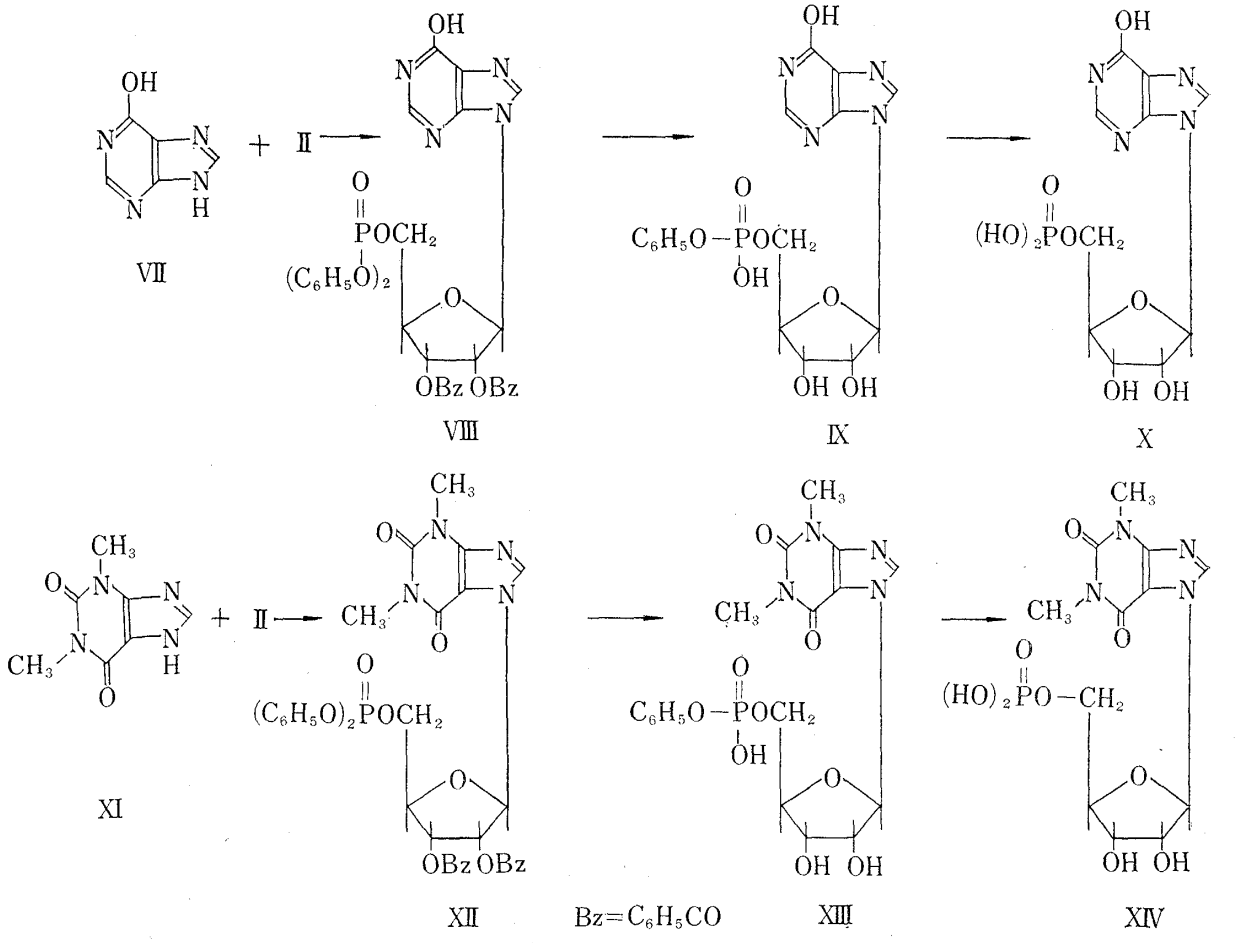


Chart 2.

obtained. In order to remove the remaining phenyl group in the phosphoryl residue of XIII, it was incubated with phosphodiesterase to give 7-β-D-ribofuranosyltheophylline 5′-phosphate (XIV) which was identified with an authentic specimen by comparison of its physical properties.

The successful synthesis of the ribonucleotides of these purines by this route prompted us to synthesize pyrimidine nucleotides by a similar procedure. However an

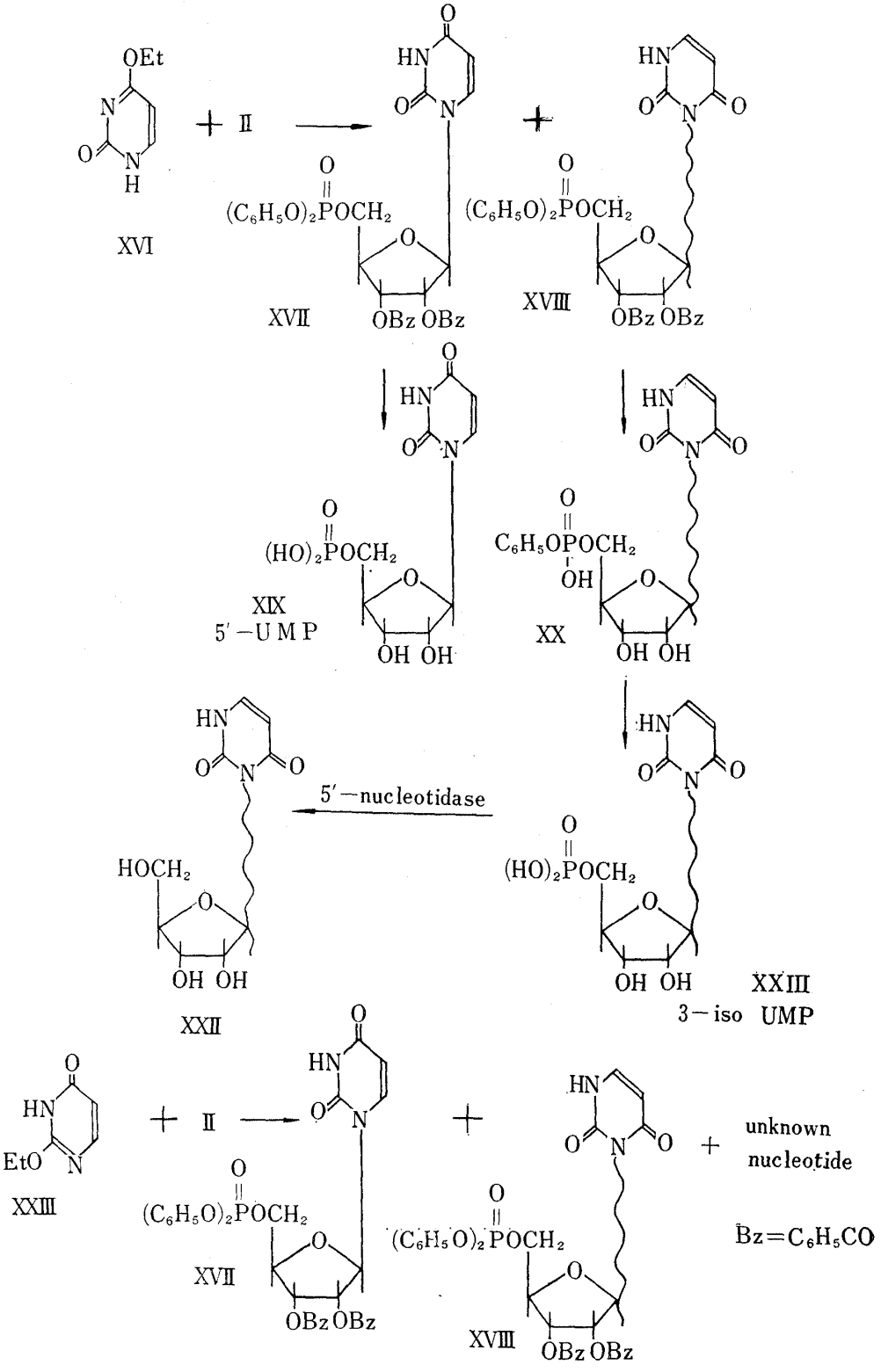


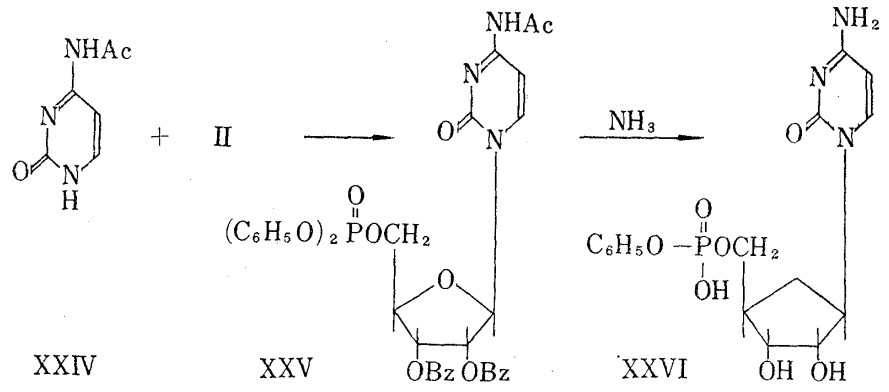
Chart 3.

attempt to prepare uracil or thymine nucleotide by this method using bromide II and corresponding bases failed.

On the other hand, compound II was treated with 4-ethoxy-2-(1*H*)-pyrimidinone (XVI)[10] in dry acetonitrile to give amorphous 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)uracil (XVII), which was identified with an authentic specimen, and 3-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)uracil (XVIII). However, 4-ethoxy-2-(1*H*)-pyrimidinone derivatives were hardly detected. On hydrogenation of XVII with Adams platinum oxide, the dephenylated product, 1-(5-phosphoryl-2,3-di-O-hexahydrobenzoyl-β-D-ribofuranosyl)uracil, was obtained in a good yield. This compound was deacylated to give 1-β-D-ribofuranosyluracil 5′-phosphate (XIX), which was identified with an authentic specimen. Deacylation of XVIII with 0.5*N* sodium hydroxide gave XX, the structure of which was assigned from its elemental analyses and ultraviolet spectra. On incubation with crude snake venom, XX was converted to 3-D-ribofuranosyluracil (XXII), which was identified with an authentic specimen by comparison of its physical properties.

Similarly, condensation of 2-ethoxy-4(3*H*)-pyrimidinone (XXIII)[10] with bromide II gave two products (XVII and XVIII), which were identified with the authentic samples obtained above.

In a similar reaction of bromide II with N-acetyl cytosine (XXIV), 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)-4-acetamido-2(1*H*)-pyrimidinone (XXV) was obtained as colorless needles in a yield of 30.2% and its structure was assigned from its elemental analyses and ultraviolet spectrum. Deacylation of XXV with methanolic ammonia in



XXIV XXV XXVI

Ac=CH_3CO

Chart 4.

a sealed tube gave an amorphous powdery product (XXVI). As this product gave an ultraviolet absorption spectrum similar to that of cytidine 5′-phosphate, the acetyl residue at the C_6-amino-group on the pyrimidine seemed to have been removed, and lack of absorption at 230 mμ indicated the loss of the benzoyl residue on the hydroxyl groups of the ribosyl moiety. From these properties together with the analysis of phosphorous content, XXVI is assigned the structure 1-D-ribofuranosylcytosine 5′-phenylhydrogenphosphate.

Experimental

Methods——Paper chromatography was carried out on Toyo Roshi No. 51, using the ascending technique. The following solvent systems were employed: (1) *n*-BuOH–AcOH–H_2O (4:1:5); (2) methylethylketone–*tert*-BuOH–AcOH–H_2O (5:5:6:4). Thin-layer chromatography was carried out on silicagel G (by Merck)

10) H. Hilbert: J. Am. Chem. Soc., **57**, 552 (1935).

and the following solvent systems were employed: (TLC-1) benzene-acetone (8:2); (TLC-2) benzene-AcOEt (3:7).

6-Benzamido-9-(5-diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)purine (IV)——6-benzamidopurine (III) was suspended in the solution of 5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl bromide (II), which was prepared from 3.0 g. of I by the method of Ukita and Hayatsu,[1] in 10 ml. of dimethylformamide (DMF). The suspension was heated at 40° for 40 hr. under stirring. Evaporating the reaction mixture gave a resin which was taken up with the mixture of 50 ml. of $CHCl_3$ and 20 ml. of 3%-NH_4OH. After being shaken, the mixture was filtered (0.3 g. of unreacted benzamidopurine was recovered). The $CHCl_3$ layer was separated, washed with H_2O and evaporated to dryness. The benzene solution of the residue was chromatographed on silica gel (50 g.). The column, which was washed with 500 ml. of benzene and 500 ml. of benzene-acetone (95:5), was developed with benzene-acetone (90:10). Effluent (*ca.* 500 ml.) containing nucleotide derivative was evaporated to yield 2.0 g. of amorphous IVb (67% yield based on the reacted benzamidopurine). UV λ_{max}^{EtOH} mμ: 230, 261 (shoulder), 277, 280, $[\alpha]_D^{26}$ −47.6° (c=0.51, $CHCl_3$). *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, 63.33; H, 4.28; N, 8.25; P, 3.41. The nucleotide derivative thus obtained was identical on thin-layer chromatogram (TLC-1) with the sample prepared by the 'silyl method' (Part I).*2

Adenosine 5'-phosphate (5'-AMP) (VI)——Removal of the protecting groups of IV was carried out according to the same method described in Part I.*2 From 500 mg. of IV, 91 mg. of crude 9-(5-phenylphosphoryl-β-D-ribofuranosyl)adenine (V) was obtained. On this stage, the product was characterized by paper chromatography with the sample prepared by the 'silyl method' (Part I)*2; Rf=0.22 (Solvent 1), UV λ_{max}^{pH7} 260 mμ, λ_{min}^{pH7} 230 mμ, λ_{max}^{pH1} 257 mμ, λ_{min}^{pH1} 230 mμ. The remaining phenyl group of V (70 mg.) was enzymically removed to yield adenosine 5'-phosphate, which was identical in IR and UV spectra and paper chromatogram (λ_{max}^{pH1} 260 mμ, Rf=0.19 solvent 2) with the natural specimen. No depression in m.p. was observed in admixture with an authentic sample.

9-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)hypoxanthine (VIII)——The condensation reaction was carried out using 1.3 g. of hypoxanthine (VII) and II (from 6.0 g. of I); the procedure was similar to that for IV except that 20 ml. of dimethylsulfoxide was used in place of dimethylformamide. Unreacted hypoxanthine (0.46 g.) was recovered. The crude product obtained was chromatographed on a column of silica gel (100 g.), after being washed with 500 ml. of $CHCl_3$ and 100 ml. of $CHCl_3$-MeOH (99:1), the column was developed with a solvent ($CHCl_3$-MeOH; 98:2). After 500 ml. of the solvent passed down, the desired nucleotide derivative was eluted in total 500 ml. of effluent. Evaporation of the solvent gave 1.6 g. of amorphous VIII (yield; 36.6% based on the reacted hypoxanthine). It was identical in UV and IR spectra with an authentic sample prepared by the 'silyl method.' $[\alpha]_D^{26}$ −50.0° (c=0.52, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{29}O_{10}N_4P$: N, 7.91; P, 4.38. Found: N, 8.11; P, 4.19.

Inosine 5'-Phosphate (5'-IMP) (X)——Removal of the protecting groups of IX was carried out by the same method described in Part I.*2

From 0.6 g. of IX, 0.11 g. of the crude inosine 5'-phosphate (X) was obtained, UV λ_{max}^{pH6} 248 mμ (ε=12,200). On paper chromatogram (solvent 2), two spots (Rf=0.12, 0.15) were visualized under ultraviolet light. The former is same in Rf value as natural 5'-IMP.

7-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)theophylline (XII)——To a solution of II (from 2.0 g. of I) in 10 ml. of acetonitril was added 0.6 g. of dry theophylline (XI) and the mixture heated at 40° for 40 hr. with stirring. After evaporation of the solvent, *in vacuo*, the mixture of 30 ml. of $CHCl_3$ and 10 ml. of 3% NH_4OH was added to the residue, and vigorously shaken. Unreacted theophylline (0.35 g.) was recovered by filtration. The separated $CHCl_3$ layer was washed with H_2O and evaporated to give a thick syrup. The syrup was chromatographed on a column of silica gel (40 g.). After passing 500 ml. of benzene and 500 ml. of benzene-acetone (98:2), the solvent was changed to benzene-acetone (95:5). The desired product appeared in the effluent which was occasionally cheked by thin-layer chromatography (TLC-2). Evaporation of the solvent gave 0.8 g. (77% based on the reacted XI) of XII. The pure product recrystallized from EtOH melted at 154°, $[\alpha]_D^{26}$ −30.2° (c=0.52, $CHCl_3$). It was identical in UV and IR spectra with an authentic sample prepared by the 'silyl method.' *Anal.* Calcd. for $C_{38}H_{33}O_{11}N_4P$: C, 60.64; H, 4.39; P, 4.12. Found: C, 60.64; H, 4.42; P, 4.01.

7-β-D-Ribofuranosyltheophylline 5'-Phosphate (XIV)——Removal of the protecting groups of XII was carried out using 3.5 ml. of dioxane, 3.5 ml. of 1*N* NaOH and 290 mg. of XII; the procedure was similar to that for IX. The product (XIII 155 mg.) thus obtained was enzymically dephenylated as in the foregoing experiment. The desired product (60 mg.) was identified with the sample prepared by the 'silyl method.'*2

Reaction of 4-Ethoxy-2(1*H*)-pyrimidinone (XVI) with 5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl Bromide (II)——The condensation reaction was carried out using 0.5 g. of 4-ethoxy-2(1*H*)-pyrimidinone (XVI) and II (from 3.0 g. of I); the procedure was similar to that for IV except that 10 ml. of acetonitril was used in place of dimethylformamide. The crude product thus obtained was chromatographed on a column of silica gel (50. g.).

After being washed with 300 ml. of benzene and 400 ml. of benzene-acetone (9:1), the column was developed with the same solvent. Effluent (*ca.* 500 ml.) containing nucleotide derivative was evaporated to yield

0.23 g. of amorphous 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-β-D-ribofuranosyl)uracil (XVII) (15% yield based on the reacted (XVI). $[\alpha]_D^{26}$ −53° (c=0.51, $CHCl_3$). *Anal.* Calcd. for $C_{35}H_{29}O_{11}N_2P$: C, 61.40; H, 4.24; N, 4.09; P, 4.53. Found: C, 61.38; H, 4.30; N, 4.16; P, 4.46.

The nucleotide (XVII) thus obtained was identical with the sample prepared by the 'silyl method' (Part I) by comparison of its IR spectrum and thin-layer chromatogram (TLC-1).

According to the method described previously in Part I, the XVII was converted to the nucleotide, which was identical with an authentic uridine 5'-phosphate (XIX).

The column was further developed with benzene–acetone (8:2). After 200 ml. of the solvent passed through, the another nucleotide derivative was eluted in total 300 ml. of effluent. Evaporation of the solvent gave 0.71 g. of amorphous 3-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)uracil (XVIII) (yield: 46.3% based on the reacted (XVI). $[\alpha]_D^{26}$ +16.6° (c=0.51, $CHCl_3$). *Anal.* Calcd. for $C_{35}H_{29}O_{11}N_2P$: C, 61.40; H, 4.24; N, 4.09; P, 4.53. Found: C, 61.22; H, 4.51; N, 4.13; P, 4.36.

Removal of the protecting groups of XVIII was carried out by the similar procedure described above. From 90 mg. of XVIII the amorphous product (XX) (45 mg.) was obtained. UV $\lambda_{max}^{pH\,1}$ 263 mμ, $\lambda_{max}^{pH\,11}$ 293 mμ.

Dephosphorylation by incubation of the product (XX) with crude snake venom was carried out according to the direction of Ukita and Hayatsu. From 45 mg. of XX, 15 mg. of nucleoside (XXII) was obtained. This nucleoside (XXII) was identical in UV spectrum and paper chromatogram ($\lambda_{max}^{pH\,1}$ 264.5 mμ, $\lambda_{max}^{pH\,13}$ 293.5 mμ, $\lambda_{min}^{pH\,1}$ 232 mμ, $\lambda_{min}^{pH\,13}$ 245 mμ; Rf=0.60: solvent 2) with an authentic specimen 3-ribofuranosyluracil prepared by the 'silyl method.'

Reaction of 2-Ethoxy-4(3*H*)-pyrimidinone (XXIII) with II——The condensation reaction was carried out using 0.1g. of XXIII and II (from 0.5 g. of I). The procedure was similar to that for the above example, and the resulting crude products (1- and 3-isomers) were obtained, which were characterized by thin-layer chromatogram (TLC-1) with the sample of above experiment.

1-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)-4-acetamido-2(1*H*)-pyrimidinone (XXV)——To a solution of 0.153 g. of N-acetylcytosine (XXIV) and 3 ml. of acetonitril was added II (obtained from 0.6 g. of I) and the mixture heated at 50° for 70 hr. with stirring. By the similar procedure described above, the crude product obtained. The product was chromatographed on a column of silica gel (10 g.). After passing 200 ml. of benzene, 200 ml. of benzene–acetone (95 : 5) and 300 ml. of benzene–acetone (90:10), the solvent was changed to benzene–acetone (85:15). The desired product appeared in the effluent which was occasionally checked by thin-layer chromatography (TCL-1). Evaporation of the solvent gave 0.09 g. of XXV (30.2% yield based on the XXIV). The pure product recrystallized from EtOH melted at 176~177°. UV λ_{max}^{EtOH} mμ: 232, 284, 299, $[\alpha]_D^{26}$ −56.7° (c=0.5, $CHCl_3$). *Anal.* Calcd. for $C_{17}H_{32}O_{11}N_3P$: C, 61.24; H, 4.41; N, 5.79; P, 4.28. Found: C, 60.86; H, 4.46; N, 6.00; P, 3.89.

Reaction of 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)-4-acetamido-2(1*H*)-pyrimidinone (XXV) with Methanolic Ammonia——A solution of 90 mg. of XXV in 3 ml. of absolute MeOH saturated with dry ammonia 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 a 2 ml. of water, and washed with 20 ml. of $CHCl_3$. The aqueous layer was evaporated to dryness *in vacuo*. An amorphous residue (XXVI) was obtained. UV $\lambda_{max}^{pH\,1}$ 280 mμ, $\lambda_{min}^{pH\,1}$ 240 mμ, $\lambda_{max}^{pH\,12}$ 273 mμ, $\lambda_{min}^{pH\,12}$ 252 mμ.

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