Studies on Nucleosides and Nucleotides. VII.[1] Preparation of Pyrimidine Nucleoside 5′-Phosphates and N^3,5′-Purine Cyclonucleosides by Selective Activation of the 5′-Hydroxyl Group

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The reaction of thymidine or uridine with 1.5 molar equivalents each of dibenzyl hydrogenphosphate, diethyl azodicarboxylate, and triphenylphosphine in HMPT at room temperature for 1 day, followed by debenzylation, afforded dpT and pU in 73 and 78% yields, respectively. Neither nucleoside 3′-phosphate nor 3′,5′-diphosphate was formed. 5′-*O*-Benzoylthymidine 3′-(2-cyanoethyl)phosphate also reacted smoothly with thymidine at room temperature giving d-bzT3′p(CNEt)5′T in a 54% yield. Adenosine and Guanosine gave the corresponding N^3,5′-cyclonucleosides as main products, less than 1% pA and pG being formed.

In relation to biosynthesis and genetic control, there is a need for oligo- and polynucleotides of definite and specific sequences. For the synthesis of oligonucleotides, suitably protected nucleosides and/or nucleotides were condensed with each other to form 3′—5′ phosphodiester bonds.[2] While many organic dehydrating reagents have been developed so far, only a few are effective in nucleotides and oligonucleotides syntheses.[3] Since the condensation of nucleosides with phosphate esters and with nucleotides by these reagents involves initial activation of phosphate components, a side reaction occurs to produce pyrophosphate which in turn afford undesirable by-products.[4] In some cases, undesirable side reactions also take place under the conditions necessary for deprotection.[5] A procedure for selective phosphorylation of unprotected nucleosides would thus be desirable.

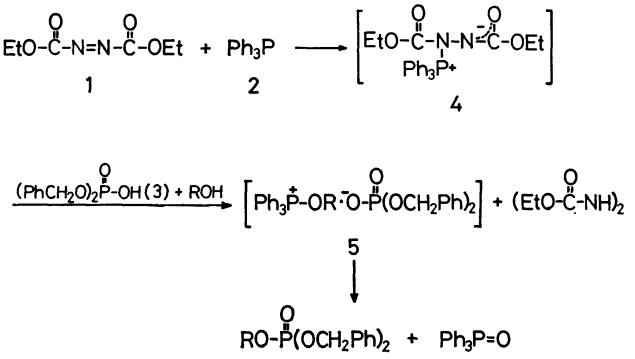
Recently, the triester method has been reported to have some advantages over the diester one.[2,6] Because of the lower reactivity of phosphate diesters as compared to phosphate monoesters, only arenesulfonyl chlorides and their derivatives can be utilized in the triester method. In the triester method, however, these reagents, except for arylsulfonyltetrazoles,[7] are slow in completing the condensation reactions.[8]

An alternative route for the preparation of nucleotides is also known in which nucleosides are converted into activated forms at the first stage of the reaction followed by the reaction with phosphate esters. In this case, pyrophosphate formation can, at least in principle, be avoided. Several attempts have been made to synthesize nucleotides by this procedure, the reaction conditions being so drastic as to cause side reactions such as 3′—2′ phosphoryl group migration.[9]

In this paper, we wish to report selective formation of pyrimidine nucleoside 5′-phosphates and cyclization of purine nucleosides by the use of diethyl azodicarboxylate (**1**) and triphenylphosphine (**2**). The reaction proceeds through initial activation of the 5′-hydroxyl group of nucleosides under mild conditions. At the outset of our work,[10] there was only one procedure available for predominant formation of 3′—5′ internucleotidic phosphate linkage from unprotected nucleosides and protected nucleotides.[11] The present method has been worked out on the basis of the following facts.

1) Because of the steric hindrance of three bulky aryl group, the reaction of triarylmethyl chlorides with nucleosides mainly gave 5′-*O*-triarylmethylnucleosides.[12]

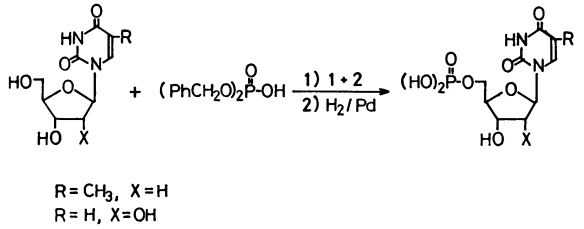
2) The reaction of an alcohol with dibenzyl hydrogenphosphate (**3**) in the presence of **1** and **2** at room temperature, followed by debenzylation, resulted in the formation of the corresponding alkyl dihydrogenphosphate in an excellent yield. It was assumed that the 1 : 1 adduct (**4**) of **1** and **2** initially formed was converted into an alkoxyphosphonium salt (**5**) which collapsed to the phosphate triester as shown in Scheme 1.[13]



Scheme 1.

When an unprotected nucleoside, instead of an alcohol, is used in this reaction, the nucleophilic attack of the 5′-hydroxyl group of the nucleoside on the sterically crowded phosphorus cation of **4** would be expected to be more favourable than that of 3′- and/or 2′-hydroxyl ones affording the corresponding nucleoside 5′-phosphate. In fact, the reagent formed by combination of **1** and **2** has been found to be effective for the condensation of 5′-hydroxyl group of unprotected thymidine and uridine with **3**.

Thymidine was allowed to react with 1.5 molar equivalents each of **1**, **2**, and **3** in tetrahydrofuran (THF) at room temperature for 1 day, the benzyl group being removed by hydrogenolysis. Examination of the resulting solution by paper chromatography revealed the presence of dpT (47%) and thymidine, with no

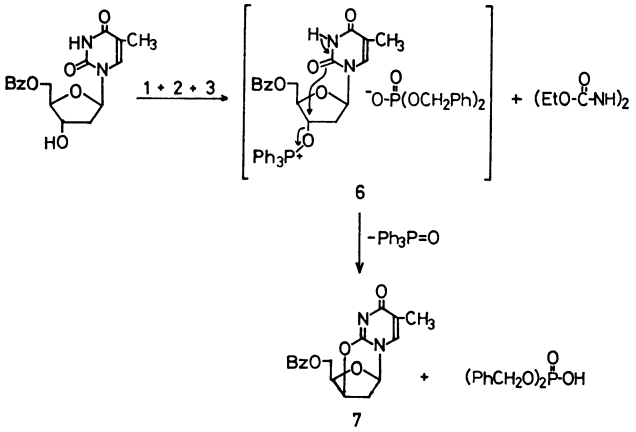


Scheme 2.

formation of dTp and dpTp.[14,15] When the reaction was carried out in dioxane (60 °C) and in hexamethylphosphoric triamide (HMPT; room temperature) for 1 day, followed by debenzylation, dpT was formed in 62 and 73% yields, respectively. Under the same conditions, uridine also reacted smoothly with **3** in dioxane (60 °C) and in HMPT (room temperature) to give pU in 68 and 78% yields, respectively. The analysis of the crude reaction solution obtained after debenzylation was performed by paper chromatography to indicate the absence of by-products derived from the nucleoside (Scheme 2). On the other hand, when thymidine was allowed to react with 3 molar equivalents each of **1**, **2**, and **3** in HMPT at room temperature, followed by hydrogenolysis, the formation of a small amount of a by-product was detected by paper chromatography. The by-product gave a similar UV absorption spectrum, as eluted from paper chromatogram, to that of thymidine, but with twice the electrophretic mobility of dpT at pH 3.2. The by-product was tentatively assigned to be dpTp or 1-(2-deoxy-3,5-bis-*O*-dihydroxyphosphinyl-β-D-xylofuranosyl)thymine. The reaction in pyridine made the solution turn dark, giving dpT in a 19% yield along with several by-products. This would be the result of nucleophilic attack of the solvent to phosphonium salts.[16] The by-products were not characterized.

In order to examine the reactivity of 3′-hydroxyl group of nucleosides, 5′-*O*-benzoylthymidine (dbzT) was allowed to react with **1**, **2**, and **3** in THF at room temperature, a white precipitate being separated from the solution as the reaction proceeded. The precipitate was found to be 5′-*O*-benzoyl-O^2,3′-cyclothymidine (**7**, 46%), 36% of dbzT being recovered.[17] This result indicates that the 3′-hydroxyl group of dbzT can also enter into the reaction to form the corresponding phosphonium salt (**6**), in which an intramolecular displacement leading to **7** takes place more readily than intermolecular phosphate attack on 3′-carbon atom (Scheme 3). The results are summarized in Table 1.

Contrary to the case of pyrimidine nucleosides, purine nucleosides were scarcely converted into 5′-phosphates, the main products being N^3,5′-cyclonucleo-



Scheme 3.

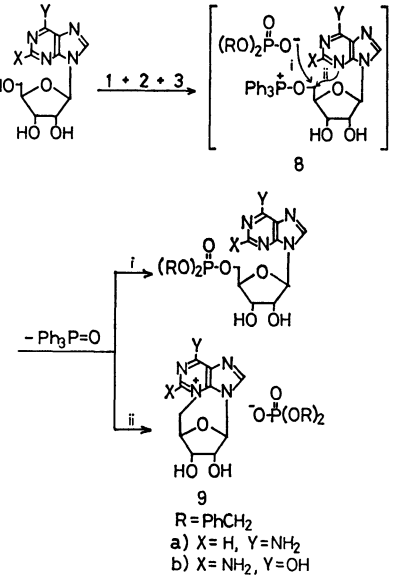
Table 1. Reaction of nucleosides with **1**, **2**, and **3**[a]

Nucleoside	Solvent	Product	Yield, %	Recovered nucleoside, %
dT	THF	dpT	47	50
dT	Dioxane[b]	dpT	62	28
dT	HMPT	dpT	73 (77)[c]	25 (8)[c]
dT	Pyridine	dpT	19	22
dbzT	THF	5′-*O*-Benzoyl-O^2,3′-cyclothymidine	46[d]	36
U	THF	pU	42	50
U	Dioxane[b]	pU	68	27
U	HMPT	pU	78	18
U	Trimethyl phosphate	pU	26	63
A	HMPT	pA	0.5	17
		N^3,5′-Cycloadenosine	67	
A	DMF	pA	0.5	10
		N^3,5′-Cycoladenosine	86	
G	HMPT	pG	0.2	50
		N^3,5′-Cycloguanosine	49	
G	DMF	N^3,5′-Cycloguanosine	24	75

a) Unless otherwise stated, nucleoside was allowed to react with 1.5 molar equivalents each of **1**, **2**, and **3** at room temperature, the yields being determined by paper chromatography. b) The reaction was carried out at 60 °C. c) Three molar equivalents of **1**, **2**, and **3** were used; A by-product was formed in a small amount. d) Isolated yields.

sides (**9**). For example, the reaction of adenosine with **1**, **2**, and **3** in HMPT at room temperature resulted in the formation of pA and N^3,5′-cycloadenosine (**9a**) in 0.5 and 67% yields, respectively. The yield of **9a** increased to 86% when the reaction was carriet out in DMF.[18] Similarly guanosine afforded pG and N^3,5′-cycloguanosine (**9b**) in 0.2 and 49% yields. The results are summarized in Table 1.

The formation of **9** can be explained by assuming intermediacy of a quaternary phosphonium salt (**8**), cyclized by nucleophilic attack of the purine base to 5′-carbon atom (Scheme 4, path ii). The predominant formation of the purine cyclonucleoside would be attributed, at least in part, to electrostatic interaction between the phosphorus cation and purine base of **8** which brought the reaction site (5′-*C* and 3-*N*) so close together as to favor cyclization.[19]

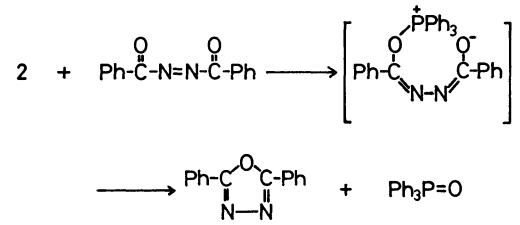


Scheme 4.

Since the intermolecular dehydration described above couples with an oxidation-reduction system,[20] the redox potentials of reagents are expected to play an important role. In order to find the best combination, uridine was allowed to react with **3** in the presence of **2** and various azo compounds for 3 h at room temperature. After hydrogenolysis, the yield of pU was determined by paper chromatography. The results are summarized in Table 2. We see that the combination of **1** and **2** affords the best result. When dibenzoyldiazene was used, 2,5-diphenyl-1,3,5-oxadiazole was isolated in a 50% yield. The formation of the oxadiazole could be explained in terms of the deoxygenation-cyclization process *via* *O*-phosphonium salt (**10**) as shown in Scheme 5.[21] The oxadiazole was also isolated by the reaction of dibenzoyldiazene with **2** in nearly quantitative yield.

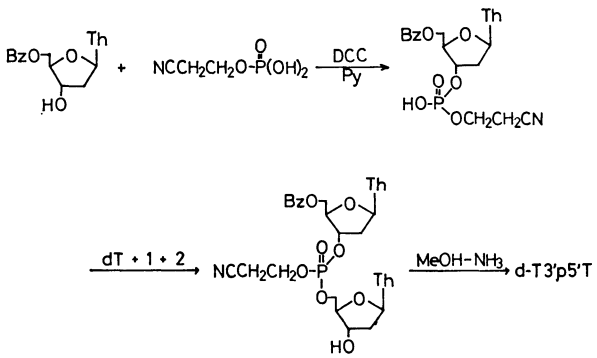
Table 2. Reaction of uridine with **3** in the presence of **2** and azo compounds

R–C(=O)–N=N–C(=O)–R	Yield of pU, %	Recovered U, %
EtO	60	27
Ph	3.5	90
	0	94



Scheme 5.

In order to cofirm that the present system is applicable to the formation of internucleosidic phosphate linkage, the reaction of 5′-*O*-benzoylthymidine 3′-(2-cyanoethyl)-phosphate [dbzTp(CNEt)] with thymidine was carried out. The dbzTp(CNEt) reacted smoothly with thymidine in the presence of **1** and **2** in HMPT at room temperature. After the solution had been kept stirring overnight, d-bzT3′p(CNEt)5′T was isolated in a 54% yield by column chromatography. Removal of the protecting groups afforded d-T3′p5′T which was completely degraded to dT and dpT by snake venom phosphodiesterase (Scheme 6).



Scheme 6.

The results indicate that the condensation reaction by the use of **1** and **2** proceeds through the initial activation of nucleosides. The system is effective in the selective formation of nucleoside 5′-phosphates and of 3′—5′ internucleotidic linkage by the triester method under mild neutral conditions with the following limitation. 1) Pyrimidine nucleosides selectively give the corresponding nucleoside 5′-phosphates, while purine nucleosides mainly affords N^3,5′-cyclonucleosides. 2) Phosphate diesters can also be activated giving pyrophosphates when hindered nucleosides are used.[22] 3) Since the selectivity originates from the difference in steric requirements between 5′- and 3′(or 2′)-hydroxyl groups, the 3′-hydroxyl group can also enter into the reaction giving nucleoside diphosphates and/or O^2,3′-pyrimidine cyclonucleosides. On hydrolysis, the latter compounds are converted into xylofuranosylnucleoside. 2′-Hydroxy groups would also undergo the same reaction affording O^2,2′-cyclonucleosides which are hydrolyzed to arabino epimers.[23] The use of excess condensing reagent should be avoided.

In spite of several limitations, the selective preparation of pyrimidinenucleoside 5'-phosphates by the use of **1** and **2** was successfully applied to the synthesis of d-T3'p5'T. In order to extend the nucleoside activation process, a method of preventing the intramolecular cyclization of nucleosides should be worked out.[24]

Experimental

Diethyl azodicarboxylate (**1**),[25] dibenzoyldiazene,[26] bis(morpholinocarbonyl)diazene,[27] dibenzyl hydrogenphosphate,[28] and 2-cyanoethyl dihydrogenphosphate[29] were prepared by the known procedure. Unless otherwise stated, the reaction was carried out at room temperature. Paper chromatography was performed by ascending technique using Toyoroshi No. 51A paper. Solvent systems: A, 1-propanol–2 M HCl=5: 1; B, 1-propanol–concd NH_3–H_2O=6: 3: 1; C, 1-butanol–acetic acid–H_2O=5: 2: 3; D, 2-propanol–concd NH_3–H_2O=7: 1: 2. The R_f values of different compounds are given in Table 3. Ultraviolet absorption spectra were obtained on a Hitachi EPS-3T recording spectrometer; the extinction coefficients used in calculating yields are given in Table 3. Tetrahydrofuran (THF) was distilled from Na; stored over Na and distilled from CaH_2 immediately before use. Hexamethylphosphoric triamide (HMPT) was distilled from CaH_2 immediately before use. Dioxane was distilled from Na and stored over Na. It is essential that moisture be excluded from all coupling reaction systems.

Preparation of Pyrimidinenucleoside 5'-Phosphates. A solution of **1** (261 mg, 1.5 mmol) in THF (1 ml) was added dropwise over a period of 1 h to a suspension of nucleoside (1 mmol), **2** (393 mg, 1.5 mmol) and **3** (417 mg, 1.5 mmol) in THF (1 ml).[30] The nucleoside dissolved on addition of **1**. After the solution had been kept stirring overnight, the solvent was removed under reduced pressure. The residue was dissolved in 75 % ethanol (20 ml) and subjected to hydrogenolysis over PdO (200 mg). After the theoretical amount of hydrogen had been absorbed, the catalyst was removed by filtration, the filtrate being made up to 50 ml. A measured volume of the solution was chromatographed for the determination of the amounts of nucleoside 5'-phosphate and the recovered nucleoside. The spots and appropriate blank areas of the paper were eluted after being cut into short pieces by soaking in standard volume of water for about 12 h, and their concentrations were determined spectrophotometrically.

When the reaction was carried out in dioxane, a solution of **1** was added over a period of 1 h to a suspension of nucleoside, **2**, and **3** in dioxane at 60 °C with stirring. Before the work-up, the mixture was held at 60 °C for 2 h and then kept stirring overnight at room temperature.

Thymidine and uridine (1 mmol) nearly completely dissolved in HMPT (1 ml). Triphenylphosphine oxide which separated was removed by filtration before hydrogenolysis. The results are summarized in Table 1.

Comparison of the Combination of Various Azo Compounds with Triphenylphosphine. A solution of azo compound (1.5 mmol) and **3** (1.5 mmol) in HMPT (2 ml) was added dropwise to a solution of uridine (1 mmol) and **2** (1.5 mmol) in HMPT (1 ml) over a period of 2 h at room temperature. After the solution had been kept stirring for 1 h, followed by hydrogenolysis, the yield of pU was determined by paperchromatography. 5 ml of HMPT was required to dissolve bis(morpholinocarbonyl)diazene. The results are summarized in Table 2.

Isolation of 2,5-Diphenyl-1,3,4-oxadiazole. Uridine (1 mmol) was allowed to react with 1.5 molar equivalents each of **2**, **3**, and dibenzoyldiazene. The mixture was poured into water (15 ml) with continuous shaking and left to stand overnight. The aqueous layer was removed by decantation, the residue being washed with water (10 ml). To the residue was added benzene and 4 % aqueous $NaHCO_3$ solution (5 ml), partitioned, and the aqueous phase was extracted with ethyl acetate. The combined extracts were dried, evaporated, and applied to silica gel plates (Merck PF_{254}, 20 cm×20 cm). 2,5-Diphenyl-1,3,4-oxadiazole was isolated by developing the plates with chloroform, 165 mg, 50%, mp 138 °C (ligroin). MS; *m/e* 222 (M^+), 166, 165. The oxadiazole was identified by comparison of its IR spectrum with a standard chart.

Reaction of Dibenzoyldiazene with Triphenylphosphine. A solution of dibenzoyldiazene (238 mg, 1 mmol) in THF (2 ml) was added dropwise to a solution of **2** (262 mg, 1 mmol) in THF (2 ml). After the reaction solution had been kept stirring for 6 h, the solution was chromatographed on silica gel plates (Merck PF_{254}, 20 cm×20 cm, $CHCl_3$) giving 2,5-diphenyl-1,3,4-oxadiazole (214 mg, 96%, mp 136—137.5 °C) and triphenylphosphine oxide (211 mg, 76 %).

Reaction of Purine Nucleosides with 1, 2, and 3. A solution of **1** (270 mg, 1.55 mmol) and **3** (417 mg, 1.5 mmol) in HMPT (1 ml) was added dropwise over a period of 1 h to a suspension

Table 3. R_f values and extinction coefficients used for the calculation of yields

	λ_{max}/nm (Solvent)	$\varepsilon\times10^{-3}$	R_f value in system[a]			
			A	B	C	D
dT	267 (H_2O)	9.65				0.64
dpT	267 (H_2O)	9.00	0.70			0.17
dTp			0.83			
U	262 (H_2O)	10.1				0.47
pU	262 (H_2O)	10.0		0.22		0.05
Up				0.31		0.12
A	261 (H_2O)	14.9			0.45	
pA	262 (H_2O)	15.0		0.14	0.03	
N^3,5'-Cycloadenosine	273 (0.05 M HCl)	13.6			0.26	
G	256 (0.05 M HCl)	12.3				0.22
pG	256 (0.05 M HCl)	12.2		0.04		
N^3,5'-Cycloguanosine	250 (0.05 M HCl)	11.8				0.11

a) The solvent systems are; A, 1-propanol–2 M HCl=5: 1; B, 1-propanol–concd NH_3–H_2O= 6: 3: 1; C, 1-butanol–acetic acid–H_2O=5: 2: 3; D, 2-propanol–concd NH_3–H_2O=7: 1: 2.

of adenosine (267 mg, 1 mmol) and **2** (393 mg, 1.5 mmol) in HMPT (2 ml). A virtually clear solution was obtained. Precipitation took place shortly afterwards. After the mixture had been kept stirring overnight, triphenylphosphine oxide was removed by filtration and washed with small quantities of HMPT and water successively. The combined filtrate and washing were made up to 25 ml with ethanol and water (solution A). A measured volume was applied to Toyoroshi No. 51A paper which was developed in system C. Two bands corresponding to unchanged adenosine and N^3,5′-cycloadenosine were eluted with water and 0.05 M hydrochloric acid, respectively, and subjected to analysis. The yield of pA was also determined by paper chromatography after the solution A (20 ml) had been subjected to hydrogenolysis over Pd–C (300 mg).

When the reaction was carried out in DMF, no precipitation of triphenylphosphine oxide took place.

In a similar manner, the reactions of guanosine with **1**, **2**, and **3** were carried out in HMPT and in DMF and the yields of products were estimated by paper chromatography. The chromatograms were developed in system D for the estimation of N^3,5′-cycloguanosine and guanosine, and in system B for pG. Respective bands were eluted with 0.05 M HCl. When the reaction was carried out in HMPT, a virtually clear solution was obtained with the progress of reaction. On the other hand, guanosine was hardly soluble in DMF, the undissolved guanosine (65%) being filtered off before analysis.

The results are summarized in Table 1.

Preparation of dbzTp(CNEt). A mixture of 2-cyanoethyl dihydrogenphosphate (10 mmol) and dbzT (692 mg, 2 mmol)[31] was dried by repeated evaporation of added portion of pyridine, dissolved in pyridine (40 ml), and treated with dicyclohexylcarbodiimide (DCC, 30 mmol) for 2 days at room temperature. Water (40 ml) was added and, after allowing the mixture to stand at room temperature for several hours, *N,N′*-dicyclohexylurea was removed by filtration. The filtrate was extracted with petroleum ether (three 40 ml portion) in order to remove DCC. The residual aqueous pyridine solution was passed through a column of Dowex-50 (H^+) ion exchange resine and the column was washed with water. The total effluent was adjusted to pH 7.5 with 0.05 M $Ba(OH)_2$ at 0 °C. The solution was concentrated to *ca.* 5 ml (below 40 °C) and excess 2-cyanoethyl dihydrogenphosphate (barium salt) was removed by precipitation with three volume of ethanol and centrifugation. The supernatant layer was concentrated *in vacuo* (below 30 °C) giving a crystalline product. In order to remove barium ion, the product was applied to Dowex-50 (H^+) ion exchange resin column and effluent was lyophilized affording paper chromatographically homogenious dbzTp(CNEt); 680 mg, 71%, R_f (system C) 0.70.

Preparation of d-bzT3′p(CNEt)5′T. A solution of **1** (174 mg, 1 mmol) in HMPT (1.5 ml) was added dropwise over a period of 1 h to a solution of dbzTp(CNEt) (240 mg, 0.5 mmol), thymidine (242 mg, 1 mmol), and **2** (262 mg, 1 mmol) in HMPT (1.5 ml) at room temperature. After the solution had been kept stirring overnight, triphenylphosphine oxide precipitated was removed by filtration. Solids separated by addition of water (80 ml) to the filtrate. However, on centrifugation followed by removal of the supernatant liquid, the solids became gum which was taken up in THF (3 ml). On addition of ethyl acetate (20 ml), white solids precipitated. They were collected and purified by means of Sephadex LH-20 column (3×40 cm, eluted with THF), giving chromatographically homogenious d-bzT3′p(CNEt)5′T in a 54 % yield; softened at 137 °C, turning a clear melt at 150 °C; R_f (system C) 0.78.

The d-bzT3′p(CNEt)5′T thus obtained was treated with methanol saturated with NH_3 overnight at room temperature. The solution was applied to Toyoroshi No. 51A paper and developed in system C. The band with R_f 0.32 was eluted and the effluent was liophilized giving d-T3′p5′T, which was completely degraded to dT and dpT (1:1 ratio) by snake venom phosphodiesterase (tris buffer, pH 8.1 at 37 °C).

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References

1) Part VI: J. Kimura and O. Mitsunobu, *Bull. Chem. Soc. Jpn.*, **51**, 1903 (1978).

2) R. I. Zhdanov and S. M. Zhenodarova, *Synthesis*, **1975**, 222; V. Amarnath and A. D. Broom, *Chem. Rev.*, **77**, 183 (1977); Y. Mizuno, O. Mitsunobu, and T. Hata, "Nukureoshido, Nukureochido no Gosei (Synthesis of Nucleosides and Nucleotides)," Maruzen, Tokyo (1977).

3) T. M. Jacob and H. G. Khorana, *J. Am. Chem. Soc.*, **86**, 1630 (1964); E. Ohtsuka, T. Sugiyama, and M. Ikehara, *Chem. Pharm. Bull.*, **23**, 2257 (1975).

4) E. Ohtsuka, M. W. Moon, and H. G. Khorana, *J. Am. Chem. Soc.*, **87**, 2956 (1965).

5) E. Ohtsuka, *Yuki Gosei Kagaku Kyokai Shi*, **36**, 723 (1978).

6) a) R. L. Letsinger and K. K. Ogilvie, *J. Am. Chem. Soc.*, **91**, 3350 (1969); b) J. C. Catlin and F. Cramer, *J. Org. Chem.*, **38**, 245 (1973).

7) J. Stawinski, T. Hozumi, S. A. Narang, C. P. Bahl, and R. Wu, *Nucleic Acid Res.*, **4**, 353 (1977); T. Hirose, R. Crea, and K. Itakura, *Tetrahedron Lett.*, **1978**, 2449.

8) J. Smrt, *Coll. Czech. Chem. Commun.*, **37**, 1870 (1972).

9) a) E. W. Haeffner, *Biochim. Biophis. Acta*, **212**, 182 (1970); b) J. Zemlicka and J. Smrt, *Tetrahedron Lett.*, **1964**, 2081; c) Y. Mizuno, T. Sasaki, T. Kanai, and H. Igarashi, *J. Org. Chem.*, **30**, 1533 (1965); d) Y. Mizuno and T. Sasaki, *J. Am. Chem. Soc.*, **88**, 863 (1966); e) J. Nagyvary, *Biochemistry*, **5**, 1316 (1966); f) P. C. Srivastava, M. M. Dhar, and K. L. Nagpal, *Indian J. Chem.*, **7**, 1055 (1969), *Chem. Abstr.*, **72**, 13013t (1970).

10) O. Mitsunobu, K. Kato, and J. Kimura, *J. Am. Chem. Soc.*, **91**, 6510 (1969).

11) a) Condensation of d(MeOTr)Tp(CNEt) with thymidine by the use of 2,4,6-triisopropylbenzenesulfonyl chloride afforded d-(MeOTr)T3′p(CNEt)5′T containing 4 % of 3′-3′ linked isomer: R. L. Letsinger and V. Mahadevan, *J. Am. Chem. Soc.*, **87**, 3526 (1965) and Ref. 6a; b) For selective phosphorylation of nucleosides, see J. Hes and M. P. Mertes, *J. Org. Chem.*, **39**, 3767 (1974): Y. Taguchi and Y. Mushika, *Bull. Chem. Soc. Jpn.*, **48**, 1528 (1975); see also Ref 10. By the triester method, fully protected nucleotide monoesters were exclusively condensed with 5′-hydroxyl groups of 2′-*O*-tetrahydropyranylnucleosides or thymidine. T. Neilson and E. S. Werstiuk, *Can. J. Chem.*, **49**, 3004 (1971); M. Sekine and T. Hata, *Tetrahedron Lett.*, **1975**, 1711.

12) a) M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 430 (1962); b) H. U. Blank and W. Pfleiderer, *Tetrahedron Lett.*, **1967**, 869.

13) O. Mitsunobu and M. Eguchi, *Bull. Chem. Soc. Jpn.*, **44**, 3427 (1971).

14) Abbreviations used for nucleosides and their derivatives are mostly in line with the principles set up by IUPAC-IUB Commission on Biochemical Nomenclature; *Biochemistry*, **9**, 4022 (1970).

15) Unless otherwise stated, the yields of products were determined by paper chromatography.

16) The amino group has been reported to react with

quaternary phosphonium salts. A. E. Arbuzov and N. N. Sazonova, *Dokl. Akad. Nauk SSSR*, **115**, 1119 (1957), *Chem. Abstr.*, **52**, 6239 (1958); J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, **37**, 2289 (1972) and references cited therein.

17) 5′-*O*-Benzoyl-O^2,3′-cyclothymidine has the following properties; mp 230—232 °C, λ_{max}^{MeOH} 232, 255 (sh) nm, λ_{min} 215 nm, *cf.* A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, **1955**, 816; J. J. Fox and N. C. Miller *J. Org. Chem.*, **28**, 936 (1963). The formation of cyclonucleosides *via* phosphonium salts has been reported; J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, **35**, 2319 (1970); M. Wada and O. Mitsunobu, *Tetrahedron Lett.*, **1972**, 1279.

18) The facile cyclization of adenosine in DMF has also been observed in the reaction with *p*-nitrobenzoic acid, **1**, and **2**. S. Shimokawa, J. Kimura, and O. Mitsunobu, *Bull. Chem. Soc. Jpn.*, **49**, 3357 (1976).

19) T. Kurihara, Y. Nakajima, and O. Mitsunobu, *Tetrahedron Lett.*, **1976**, 2455.

20) T. Mukaiyama, *Angew. Chem. Int. Ed. Engl.*, **15**, 94 (1976).

21) The formation of 2,5-diphenyl-1,3,4-oxadiazole through the reaction of tributylphosphine with dibenzoyldiazene in the presence of benzoic acid has also been described. I. Kuwajima, Master of Sc. Thesis, Tokyo Institute of Technology, 1963.

22) No attempt was made to detect tetrabenzyl pyrophosphate in the present system. An evidence of the formation of benzoic anhydride was obtained in the formation of benzoate of a sterically crowded alcohol by means of **1** and **2**. O. Mitsunobu, J. Kimura, K. Iizumi, and N. Yanagida, *Bull. Chem. Soc. Jpn.*, **49**, 510 (1976).

23) a) J. J. Fox, *Pure Appl. Chem.*, **18**, 223 (1969); b) K. K. Ogilvie and D. J. Iwacha, *Can. J. Chem.*, **52**, 1787 (1974) and references cited therein. No detectable evidence of the formation of O^2,3′-pyrimidine cyclonucleoside 5′-phosphate was obtained. However, when thymidine was allowed to react with benzoic acid (1.5 molar equivalent) in the presence of 2.5 molar equivalents each of **1** and **2** at room temperature, **7** and 1-(2-deoxy-3,5-dibenzoyl-β-D-xylofuranosyl)thymine were obtained in 45 and 50 % yields, respectively.

24) Attempts to avoid the intramolecular cyclization of nucleosides have been reported. W. Jahn, *Chem. Ber.*, **98**, 1705 (1965); O. Mitsunobu, S. Takizawa, and H. Morimoto, *J. Am. Chem. Soc.*, **98**, 7858 (1976).

25) J. C. Kauer, *Org. Synth.*, Coll. Vol., IV, 411 (1963).

26) T. Imamoto and Y. Yukawa, *Yuki Gosei Kagaku Kyokai Shi*, **30**, 665 (1972).

27) H. Bock and J. Kroner, *Chem. Ber.*, **99**, 2039 (1966).

28) V. M. Clark and A. R. Todd, *J. Chem. Soc.*, **1950**, 2030.

29) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).

30) Solubility of unprotected nucleosides in organic solvents is usually lower than that of protected nucleosides. In order to increase the yield of the desired product and to avoid the undesirable formation of pyrophosphate, a solution of **1** was added over a long period of time. In the all cases, except for the reaction of guanosine in DMF, virtually clear solution were obtained.

31) O. Mitsunobu, J. Kimura, and Y. Fujisawa, *Bull. Chem. Soc. Jpn.*, **45**, 245 (1972).