Phosphorylation of Nucleosides with Phosphorus Oxychloride in Trialkyl Phosphate

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The reaction of guanosine and triethyl phosphate at 50 °C for 15 min produced the guanosine–triethyl phosphate complex in which triethyl phosphate is coordinated to guanosine of *high-anti* form in a 1 : 1 molar ratio. During the conversion of guanosine to guanosine 5′-monophosphate with phosphorus oxychloride, the guanosine–triethyl phosphate complex showed excellent selectivity and high reactivity toward phosphorus oxychloride compared with those of guanosine. The rate of selective phosphorylation of guanosine into guanosine 5′-monophosphate was markedly improved by preheating the mixture of guanosine and triethyl phosphate at 50 °C, followed by adding phosphorus oxychloride to the mixture at 0 °C. Thus, the 5′-phosphorylation of guanosine with phosphorus oxychloride in triethyl phosphate is considered to progress *via* the guanosine–triethyl phosphate complex as the reaction intermediate.

Key words guanosine–triethyl phosphate complex; *high-anti* form; Lewis base; solid state ^{13}C-NMR; reaction mechanism; Hammett's rule

The nucleoside 5′-monophosphates, components of biologically important DNA and RNA, are also important as flavor-enhancing components. Yoshikawa *et al.*[1] reported that the phosphorylation of nucleosides (Nuc) with phosphorus oxychloride ($POCl_3$) in the presence of water in trialkyl phosphates (TAP) gave nucleoside 5′-monophosphates in high yields. However, the reaction mechanism is still unknown. In this paper, we describe the synthesis of the guanosine–triethyl phosphate complex (**2**, Guo–TEP complex) and the reactivity of **2** toward $POCl_3$, and discuss the possible reaction mechanism of the selective phosphorylation of guanosine (**1**, Guo) to guanosine 5′-monophosphate (**3**, 5′-GMP) with $POCl_3$. Further, we present practically useful procedures for the mixed phosphorylation of **1** and inosine (**4**) to **3** and inosine 5′-monophosphate (**8**, 5′-IMP).

Synthesis of Guanosine–Triethyl Phosphate Complex (2) A mixture of **1** and triethyl phosphate (TEP) was heated at 50 °C for 15 min. The mixture was then cooled to 0 °C to give the product **2** in 54% yield. High-pressure liquid chromatography (HPLC) and gas-liquid chromatography (GLC) of compound **2** detected TEP in a ratio of 1 mol per mol of **1**. The molecular formula of **2** was deduced as $C_{16}H_{28}N_5O_9P$ based on the elemental analysis data and the fast atom bombardment mass spectrum (FAB-MS). The infrared (IR) spectra of **2** and a mixture of **1** and TEP showed completely different peaks in the range at 1100, 1260 and 1650 cm^{-1} as shown in Fig. 2. The bands due to the P=O (1260 cm^{-1}) stretching and –C=N– stretching (1650 cm^{-1}) in **2** were shifted to lower wavenumbers compared with those (1280 and 1670 cm^{-1}) in a mixture of **1** and TEP. This result indicates that the

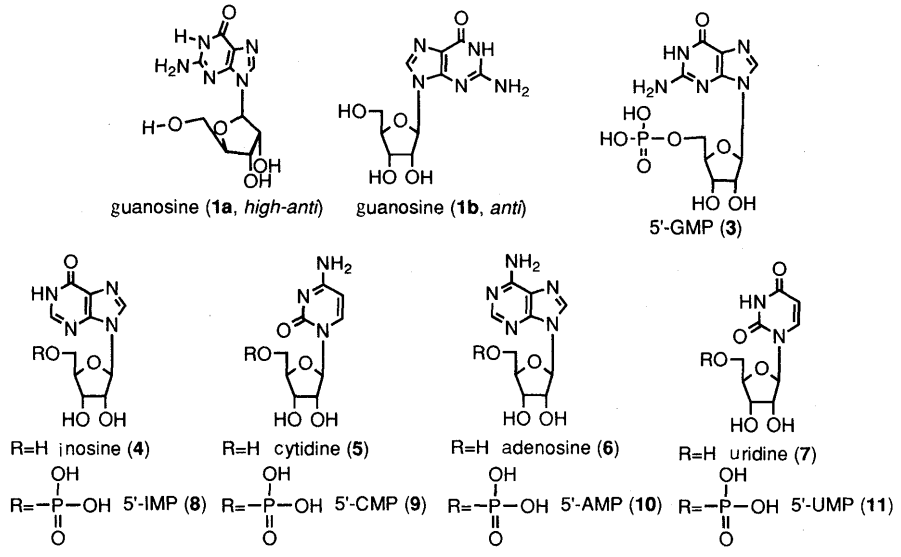

Fig. 1. Structures of Nucleosides and Nucleoside 5′-Monophosphates

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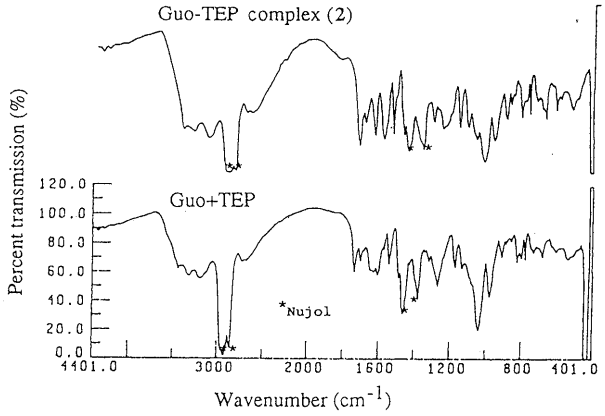


Fig. 2. Infrared Spectra of **2** and a Mixture of **1** and TEP

TABLE I. ^{13}C-NMR Spectral Data for **1** and **2** (δ)

	Compound				
	1[a] (*anti*)	**1**[b] (*anti*)	**1**[b] (*high-anti*)	**2**[a]	**2**[b]
C-5′	62.15	59.6	64.2	62.69	64.3
C-3′	71.50	69.8	73.6	71.67	73.7
C-2′	74.85	77.3	73.6	74.98	73.7
C-4′	86.35	84.3	84.3	86.44	88.7
C-1′	87.35	89.1	89.1	87.66	90.2
C-5	117.00	115.1	118.2	117.91	118.9
C-8	136.85	134.8	139.7	136.78	138.9
C-4	152.30	149.5	149.5	152.45	149.8
C-2	154.55	153.8	153.8	154.80	154.7
C-6	157.75	157.5	157.5	157.91	159.3
TEP-CH_3				17.24	16.4
TEP-CH_2				64.39	51.9

a) In DMSO-d_6[12] (corrected in response to the suggestion of Mantsch and Smith[13] that the assignments of C-2′ and C-3′ should be reversed). *b*) Measured by solid-state NMR.

P=O group of TEP in **2** may have some interaction with N-7 (–C=N–) of **1**.

Thewalt *et al.*[2] demonstrated that **1** which had been crystallized from warm water exists in two forms, *anti*[3] and *high-anti*, by X-ray analysis. Although the ordinary solution ^{13}C-NMR spectrum of **1** or **2** in dimethyl sulfoxide-d_6 (DMSO-d_6) showed the presence of the signals due to only the *anti* form, the solid-state ^{13}C-NMR spectrum of **1** showed the presence of the signals due to both the *anti* and *high-anti* forms, as shown in Table I. The solid-state^{13}C-NMR data of **1** were assigned with reference to the solution ^{13}C-NMR data of **1** obtained in DMSO-d_6. The differences in the peak values of C-2′, C-3′, C-5′, C-5 and C-8 between the *anti* and *high-anti* forms can be explained as follows. It is well known that the sugar moieties in *anti* and *high-anti* forms exist as $_1$T^2 and ^{2}E forms, respectively. This fact may explain the differences between the peak values of C-2′ and C-3′ in the *anti* and *high-anti* forms. The downfield shift $\Delta\delta$=4.6 of the C-5′ peak value in the *high-anti* form compared with that in the *anti* form may be attributable to the ring current effect of the guanine unit. Similarly, the downfield shifts $\Delta\delta$ =3. 1 and 4. 9, respectively, of C-5 and C-8 may be attributable to bond distortion between C-1′ and N-9 caused by the nearer approach of the sugar and the guanine unit in the *high-anti* form. On the other hand, only the signals due to *high-anti* form were observed in the solid-state ^{13}C-NMR spectrum of **2**.

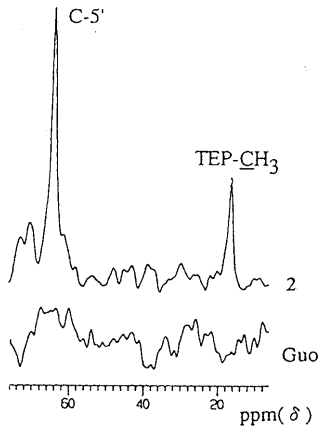
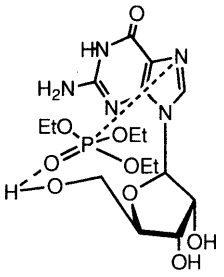


Fig. 3. Dipolar Diphase of **1** and **2**



Guo-TEP complex (**2**, *high-anti*)

Fig. 4. Proposed Structure of **2**

Next, the dipolar diphase[4] was determined from the solid-state ^{13}C-NMR data. As shown in Fig. 3, the signal of C-5′ could be clearly assigned by the dipolar diphase using **2**, while it could not be assigned using **1**. Since the carbons involved in active molecular movement (other than quaternary carbon) can be assigned by this method, it is evident that **2** differs from **1** in the state of C-5′ molecular movement.

From these analytical results, the structure of **2** was considered to be the Guo–TEP complex, in which **1** of *high-anti* form was complexed with TEP. The site of complexation between **1** and TEP was assigned as follows. The active molecular movement of the 5′ carbon atom suggests a hydrogen bond between the oxygen atom of the P=O group of TEP and the proton of the 5′-hydroxyl group in **1**, while N-7 is assumed to be interacting with the phosphorus atom for the following reasons. I) The order of protonation of the nitrogen atoms in **1** is N-7≫N-1 and N-3,[5] and N-7 is the preferential site of metal ion complexation.[6] II) The N-7 is sterically closest to the 5′-hydroxy group of **1** in the *high-anti* form. As shown in Fig. 4, it is speculated that TEP acts as a Lewis base in such a manner that the oxygen atom of the P=O group is bound to the 5′-hydroxy group *via* a hydrogen bond, while the phosphorus atom of the P=O group interacts with N-7. Consequently, the structure of the Guo–TEP complex was deduced to be **2**.

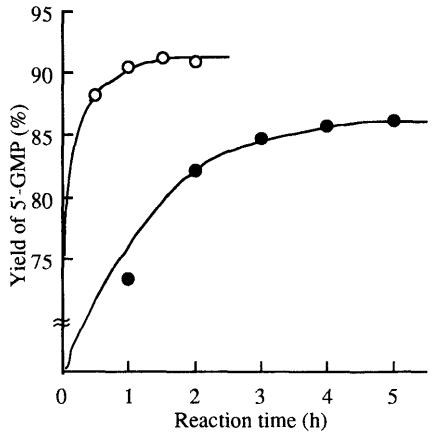


Fig. 5. Yield Curve of 5'-GMP (**3**)

○, **2**; ●, **1**. Conditions: 1) **1** (30 mmol), $POCl_3$ (90 mmol), H_2O (33 mmol), TEP (504 mmol) at 0 °C; 2) **2** (30 mmol), $POCl_3$ (90 mmol), H_2O (24 mmol) and TEP (502 mmol) at 0 °C.

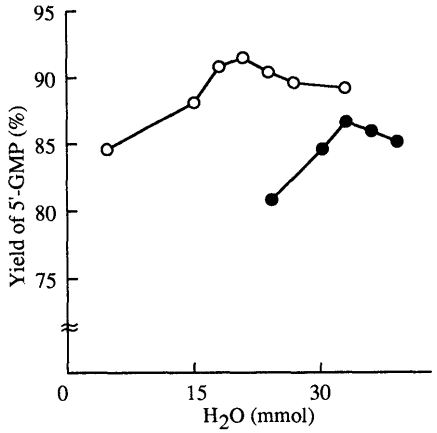


Fig. 6. Effect of Water

○, **2**; ●, **1**. Conditions: 1) **1** (30 mmol), $POCl_3$ (90 mmol), H_2O (as indicated), TEP (504 mmol) at 0 °C; 2) **2** (30 mmol), $POCl_3$ (90 mmol), H_2O (as indicated) and TEP (502 mmol) at 0 °C.

Reactivity and Mechanism of Phosphorylation of Guo–TEP Complex (2) First, **2** was allowed to react at 0 °C using the method reported by Yoshikawa *et al.*[1] As shown in Fig. 5, **2** was converted into **3** in a significantly shorter time, in comparison with **1**. Yoshikawa *et al.* reported that the synthesis of **3** from **1** with $POCl_3$ in TEP is markedly promoted by the addition of about a half amount of water per mol of $POCl_3$ to the reaction mixture. As shown in Fig. 6, the 5'-selective phosphorylation of **2** to **3** was promoted by the addition of a reduced amount of water in comparison with the optimum amount of water reported by Yoshikawa *et al.* This seems to show that **2** is more reactive than **1**.[7] Although **1** gave **3** in 86% yield by using 3 eq of $POCl_3$, **2** gave **3** in 91% yield by using 1.5 eq of $POCl_3$, as shown in Fig. 7. These results demonstrate that the 5'-selective phosphorylation of **2** into **3** with $POCl_3$ offers improved 5'-selectivity and reactivity and shortens the reaction time to one-third in comparison with that for **1**.

Next, the conditions of the formation of **3** were investigated. As shown in Fig. 8, when a mixture of **1** and TEP was kept for 15 min at a temperature below 30 °C, the formation of **2** did not completely proceed. However,

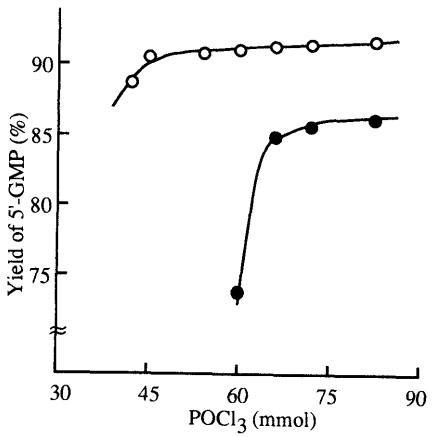


Fig. 7. Effect of $POCl_3$

○, preheating; ●, no preheating. Conditions: **1** (20 mmol), H_2O (10 mmol) and TEP (100 ml) at 0 °C.

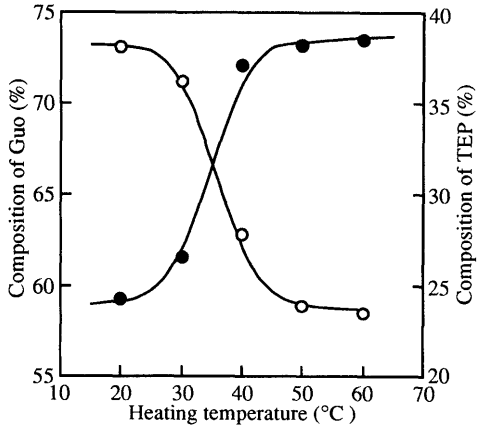


Fig. 8. Effect of Heating Temperature (°C)

○, **1**; ●, TEP. Conditions: **1** (20 mmol) and TEP (100 ml), heating time (15 min).

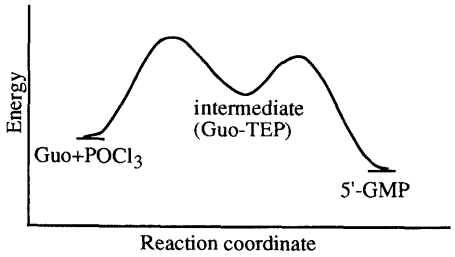


Fig. 9. Energy Profile Diagram of Phosphorylation of **1** with $POCl_3$ in TEP

when the reaction mixture was heated at 40 °C, **2** was rapidly formed. The reaction of **1** or **2** with $POCl_3$ is exothermic, whereas the reaction which yields the probable reaction intermediate (**2**) is endothermic. Therefore, the reaction of **1** with $POCl_3$ is assumed to have the energy profile shown in Fig. 9.

From these results, the 5'-selective phosphorylation of **1** to **3** with $POCl_3$ in TEP is considered to proceed in two steps, as shown in Chart 1. Step 1, which yields **2**, is the rate-determining step. The stability of **2** was confirmed by the powder X-ray analysis of a mixture of crystalline **1** and TEP, of the same composition as **2**. This reveals the conversion of **1** into **2** (noncrystalline) with a gradual

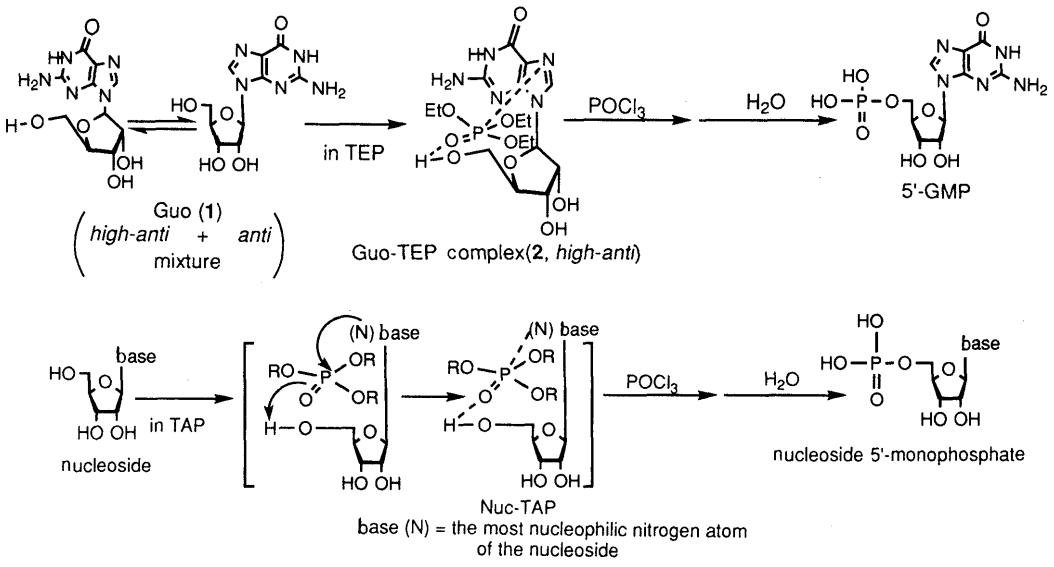


Chart 1. Phosphorylation of Nucleosides in TAP

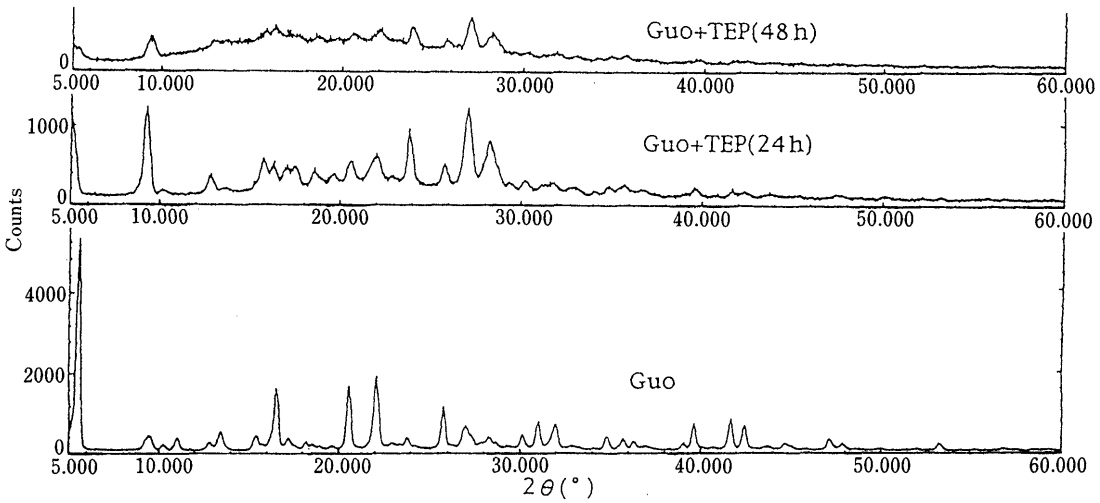


Fig. 10. Conversion of a Mixture of **1** and TEP to **2** Evaluated by Powder X-Ray Analysis

reduction in all peaks of **1** at room temperature, as shown in Fig. 10. In step 2, the 5′-hydroxyl group selectively reacts with $POCl_3$, probably because the oxygen atom of the 5′-hydroxyl group is activated upon the formation of **2**. The above reaction mechanism was also supported by the following experiments.

As seen in Table II, when **1** was phosphorylated with $POCl_3$ in the presence of water in various TAPs under the conditions of the two methods, the reaction rate and the yield of **2** decreased in proportion to the size of the alkyl group as TAP increased. This may be attributable to inhibition of the formation of **2** due to the steric hindrance between **1** and the bulky alkyl group, such as tri-*n*-butyl phosphate. These findings indicate that the selective phosphorylation of **1** into **3** with $POCl_3$ in TEP proceeds *via* by the reaction mechanism depicted in Chart 1.

Conventional Selective Phosphorylation of Nucleosides into Nucleoside 5′-Monophosphates To confirm the applicability of the above reaction mechanism for the selective phosphorylation of other nucleosides, we examined the selective phosphorylation of various nucleosides to nucleoside 5′-monophosphates (**8**—**11**) by methods A and B, as shown in Table III.

TABLE II. Effect of Alkyl Group of Trialkyl Phosphate on 5′-Phosphorylation of **1** with $POCl_3$

Trialkyl phosphate $(RO)_3P=O$ (mmol)	Rate constants (min^{-1})		Product yield (%)		
	a)	*b*)		*a*)	*b*)
R=Me 504	0.0139	0.0448	**3**	88	90
R=Et 504	0.0064	0.0424	**3**	86	91
R=*n*-Bu 504	0.0069	0.0071	**3**	16	17

a) A mixture of **1** (30 mmol), H_2O (15 mmol) and $POCl_3$ (60 mmol) in $(RO)_3P=O$ was allowed to react at 0 °C. *b*) A mixture of **1** (30 mmol) and H_2O (15 mmol) in $(RO)_3P=O$ was heated at 50 °C for 15 min, and $POCl_3$ (60 mmol) was added to the reaction mixture at 0 °C.

A mixture of nucleoside and water in TEP was phosphorylated with $POCl_3$ at 0 °C (method A) . The order of the reaction rates was inosine (**4**, Ino) > cytidine (**5**,

Cyd) > adenosine (**6**, Ado) > **1** > uridine (**7**, Urd). A mixture of nucleoside and water in TEP was heated at 50 °C for 15 min, and the mixture was phosphorylated with $POCl_3$ at 0 °C (method B). The order of the reaction rates was **4** > **1** > **6** > **5** > **7**, which differs from the results for method A. This is consistent with the order of highest occupied molecular orbital energy of nucleobases (guanine > adenine > cytosine > uracil).[8-10] Hence, we assumed that the reaction rate might be decided by the reactivity of the 5′-hydroxyl group and nucleobases. With this in mind, the reaction rate was correlated to pK, as expressed by Eq. 1. Since no data are available for pK values of nucleosides in TEP, the pK values in water were used, since pK values in organic solvents are known to be closely related to the pK values of the same compounds in water. Also, k is a constant for the rate of phosphorylation of the nucleosides.

TABLE III. Constants for Rate of 5′-Phosphorylation of Nucleosides in TEP

Compound	k (min^{-1})		pK[a]
	[b]	[c]	
4	0.0536	0.0541	1.20
1	0.0064	0.0424	2.10
6	0.0163	0.0250	3.55
5	0.0170	0.0180	4.19
7	0.0043	0.0049	

Conditions: Nuc (30 mmol), $POCl_3$ (60 mmol), H_2O (15 mmol) and TEP (100 ml) at 0 °C. *a*) Taken from "Basic Principles in Nucleic Acid Chemistry."[4] *b*) Reacted by method A described in Experimental. *c*) Reacted by method B described in Experimental.

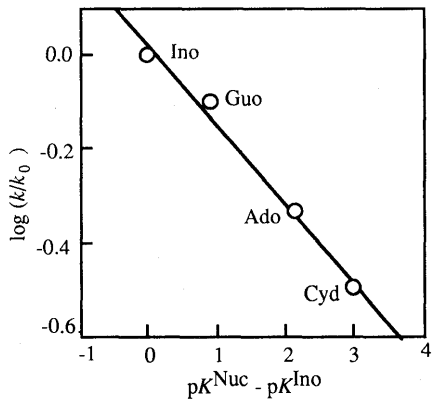


Fig. 11. Relation between pK and Reaction Rate

$$k = 0.0682 - 0.0121 \times pK \quad (\gamma = -1.000) \tag{1}$$

To demonstrate the close relation between the reaction rate of the 5′-hydroxyl group and nucleobases, Equation 2 (Hammett's rule) showing the relation between reactivity and substituents, was applied. A correlation expressed by Eq. 3 was demonstrated:

$$\log(k/k_0) = \rho \times \alpha \tag{2}$$

$$\log(k/k_0) = 0.0178 - 0.159 \times (pK^{Nuc} - pK^{Ino}) \quad (\gamma = -0.994) \tag{3}$$

where k_0 is the rate constant for the phosphorylation of **4**, ρ is a proportionality constant, and α is a constant showing the nucleophilicity of the nucleobase.

From the present experimental results on the reaction rate and pK, we speculated that a nucleoside is converted into the nucleoside 5′-monophosphate by $POCl_3$ in TAP *via* a 2-step reaction mechanism, as illustrated in Chart 1. Namely, in step 1, the most nucleophilic nitrogen atom of the nucleoside[5] and the hydrogen atom of the 5′-hydroxyl group of the nucleoside form a complex (nucleoside–trialkyl phosphate complex (Nuc–TAP complex)) with TAP and then, in step 2, the oxygen atom of the 5′-hydroxyl group thus activated selectively reacts with $POCl_3$. The activation of the 5′-hydroxyl group by TAP may be controlled by the relative nucleophilicity of the nitrogen of the nucleosides and the stereochemistry of the nucleosides. The role of TAP is also consistent with the fact that the phosphorylation of nucleosides with $POCl_3$ in other solvents gave unsatisfactory results.[11] Although Guo–TEP complex-like compounds could not be isolated by the reaction of nucleosides (**4**—**7**) with TEP at 50 °C for 15 min, the phosphorylation of nucleosides (**4**—**7**) under the conditions of method B gave the corresponding nucleoside 5′-monophosphates (**8**—**11**) in good yields, as shown in Table IV. However, the interaction site of the phosphorus atom of the P=O group with the corresponding nucleobase has not yet been elucidated.

On the basis of these findings, a mixture of **3** and **8**, which are found in important foods, could be obtained by the phosphorylation of **1** and **4**. When the phosphorylation of a mixture of **1** and **4** was conducted under the conditions of method A, most of **1** was phosphorylated to **3**, together with the formation of **8** and diphosphate (PP) produced from **4**. On the other hand, when a mixture of **1** and **4** was phosphorylated under the conditions of

TABLE IV. 5′-Phosphorylation of Nucleosides

Compound		Product yield (%)						Reaction time (h)	
		[a]	[b]		[a]	[b]		[a]	[b]	[a]	[b]
5	**5**	2	4	**9**	85	83	Ado-PP	10	11	4.0	5.0
6	**6**	2	3	**10**	92	90	Cyd-PP	3	3	1.5	2.0
1	**1**	1	7	**3**	91	82	Guo-PP	3	5	2.0	6.0
4	**4**	1	1	**8**	92	91	Ino-PP	3	3	2.0	2.0
7	**7**	17	17	**11**	73	72	Urd-PP	3	2	25.0	25.0

a) A mixture of a compound (30 mmol) and H_2O (15 mmol) in TEP (100 ml) was heated at 50 °C for 15 min and $POCl_3$ (60 mmol) was added to the reaction mixture at 0 °C. The reaction mixture was further stirred at 0 °C. *b*) A mixture of a compound (30 mmol), H_2O (15 mmol) and $POCl_3$ (60 mmol) in TEP (100 ml) was stirred at 0 °C.

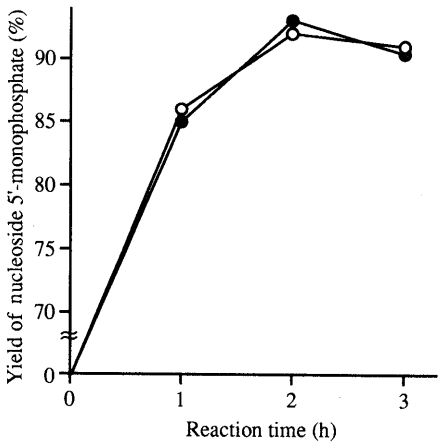


Fig. 12. Phosphorylation of **1** and **4**

○, **8**; ●, **3**. Conditions: **1** (15 mmol), **4** (15 mmol), H_2O (15 mmol), $POCl_3$ (90 mmol) and TEP (100 ml) at 0 °C. Before adding $POCl_3$ the slurry of **1** and **4** in TEP was heated at 50 °C, for 15 min, then cooled to 0 °C.

method B, **1** and **4** were converted to **3** and **8**, respectively, at almost the same rate in good yield, as shown in Fig. 12. The reaction rate of **1** was increased about 7 times by preheating compared with that of **1** with no preheating, while the reaction rate of **4** was not changed by preheating, as shown in Table III. In addition to its high efficiency, this reaction could allow the use of a smaller amount of $POCl_3$, as previously stated.

Experimental

IR spectra were recorded on a JEOL JIR-REX3000 spectrophotometer. Solid-state ^{13}C-NMR spectra were measured on a JEOL JNM-EX270 spectrometer and ^{13}C-NMR spectra were measured on a Hitachi R-90H spectrometer in DMSO-d_6 solution using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were recorded on a JEOL JMS-AX505W spectrometer. Elemental analysis were performed with a Foss/Hereaus CHN analyzer and Hitachi U3210 spectrophotometer. Powder X-ray spectra were measured on a Rigaku powder X-ray diffractometer. HPLC was performed on a Mitsubishi Kasei MLC-GEL-CDR10 column (5 i.d. × 250 mm) with 0.5 M AcOH–$AcONH_4$ at 60 °C. Detection was effected with a Shimadzu SPD-6A spectrophotometric detector at 254 nm. A Shimadzu GC-9A gas chromatograph equipped with a flame ionization detector and a 3 mm i.d. × 1 m glass column packed with GL Sciences PEG 1000 Uniport R 60/80 was used for the analysis. The column and injection temperatures were 130 and 180 °C, respectively. The nitrogen carrier gas flow rate was 20 ml/min.

Purification of Guanosine (1) Commercial guanosine (10 g, Wako Pure Chemical Industries, Ltd.) was dissolved in water (20 ml) at 80 °C, and the solution was cooled to 10 °C to give crystals of **1** (8.2 g) in 82% yield. Composition; Guo, 96.5%; H_2O, 3.0%.

Synthesis of Guo–TEP Complex (2) A suspension of **1** (8.5 g, 30 mmol) in TEP (100 ml) was heated at 50 °C for 15 min and then cooled to 0 °C for more than 180 min. The reaction mixture was centrifuged to separate the product (**2**). Compound **2** was washed three times with 200 ml portions of diethyl ether, followed by overnight drying in a vacuum dryer (110 °C internal temperature, 5 mmHg) to separate **2** (7.5 g, yield 54%). Composition: Calcd: Guo, 60. 86%; TEP, 39. 14%. Found: Guo, 60.81%; TEP, 39.61%. IR (Nujol): 1260 (P=O), 1650 (–C=N–) cm^{-1}. FAB-MS *m/z*: 466 [Guo–TEP complex $(M+H)^+$, 2%], 284 [Guo $(M+H)^+$, 16%], 183 [TEP $(M+H)^+$, 100%]. *Anal.* Calcd for $C_{16}H_{28}N_5O_9P$: C, 41.30; H, 6.06; N, 15.05; P, 6.66. Found: C, 41.16; H, 6.11; N, 15.23; P, 6.52.

Synthesis of Guanosine 5′-Monophosphate (3) from Guo–TEP Complex (2) with $POCl_3$ A mixture of the Guo–TEP complex (14.0 g, 30 mmol) and water (0.43 g, 24 mol) suspended in TEP (91.8 g) was treated with $POCl_3$ (13.8 g, 90 mmol) under cooling at 0 °C, and the entire mixture was further stirred at 0 °C for 1.5 h. The reaction mixture was poured into ice-water (150 ml) and stirred for 1 h at 0 °C to give **3** (10.0 g, yield 92%, as determined by HPLC). The reaction mixture was poured into an activated carbon column (1.6 i.d. × 50 cm), which was eluted with 1% NaOH solution (200 ml). The eluate was adjusted to pH 8. 0 with 10% HCl solution. The product **3** was crystallized from aqueous MeOH. The yield of 5′-GMP·Na_2·7.5H_2O was 15.5 g (80.8% from the reaction mixture).

Synthesis of 3 from Guanosine (1) with $POCl_3$ A mixture of guanosine (8.5 g, 30 mmol) and water (0.65 g, 33 mol) suspended in TEP (92.4 g) was treated with $POCl_3$ (13.8 g, 90 mmol) under cooling at 0 °C, and the entire mixture was further stirred at 0 °C for 5 h. The reaction mixture was poured into ice-water (150 ml) and the whole was stirred for 1 h at 0 °C to give **3** (9.37 g, yield 86%, as determined by HPLC). The reaction mixture was poured into an activated carbon column (1.6 i.d. × 50 cm), which was eluted with 1% NaOH solution (200 ml). The eluate was adjusted to pH 8. 0 with 10% HCl solution. The product **3** was crystallized from aqueous MeOH. The yield of 5′-GMP·Na_2·7.5 H_2O was 14.5 g (81.2% from the reaction mixture).

Method A (Common Procedure) A mixture of nucleoside (30 mmol) and water (33 mol) suspended in TEP (100 ml) was treated with $POCl_3$ (60 mmol) under cooling at 0 °C, and the whole was further stirred at 0 °C, then poured into ice-water (150 ml). The mixture was stirred for 1 h at 0 °C to give nucleoside 5′-monophosphate, which was assayed by HPLC.

Method B (Common Procedure) A mixture of nucleoside (30 mmol) and water (15 mmol) in TEP (100 ml) was heated at 50 °C for 15 min and $POCl_3$ (60 mmol) was added at 0 °C. The reaction mixture was poured into ice-water (150 ml) and the whole was stirred for 1 h at 0 °C to give nucleoside 5′-monophosphate, which was assayed by HPLC.

Common Procedure for Determination of Rate Constant of Phosphorylation A mixture of nucleoside (30 mmol) and water (33 mol) suspended in TEP (100 ml) was treated with $POCl_3$ (90 mmol) under cooling at 0 °C, and the whole was further stirred at 0 °C. A 1 ml sample was taken from the reaction mixture at 5 min intervals. After hydrolysis, each sample was assayed for nucleoside 5′-monophosphate content by HPLC to determine the reaction rate.

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References

1) M. Yoshikawa, T . Kato, T. Takenishi, *Bull. Chem. Soc. Jpn.*, **42**, 3505 (1969) .
2) U. Thewalt, C. E. Bugg, R. Marsch, *Acta Cryst.*, **B26**, 1089 (1970) .
3) W. Saenger, "Principles of Nucleic Acid Structure," Springer–Verlag, New York, 1984, p. 14.
4) S. J. Opella, M. H. Frey, T. A. Cross, *J. Am. Chem. Soc.*, **101**, 5854 (1979).
5) P. O. P. Ts'o, "Basic Principles in Nucleic Acid Chemistry," Vol. 1, Academic Press, New York and London, 1974, p. 461.
6) D. J. Hodgson, *Prog. Inorg. Chem.*, **23**, 211 (1977) .
7) R. F. Hudson, G. Moss, *J. Chem. Soc.*, **1962**, 3599.
8) B. Pullman, A. Pullman, "Quantum Biochemistry," New York, 1963, p. 217.
9) B. Pullman, A. Pullman, "Quantum Biochemistry," New York, 1963, p. 91.
10) V. A. Kuprievich, V. I. Danilov, A. Denis, *Teor. Eksper. Khim.*, **2**, 734 (1963).
11) M. Yoshikawa, T. Kato, T. Takenishi, *Tetrahedron Lett.*, **50**, 5065 (1967).
12) A. J. Jones, M. W. Winkley, D. M. Grant, R. K. Robins, *Proc. Natl. Acad. Sci. U.S.A.*, **65**, 27 (1970).
13) H. H. Mantsch, I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, **46**, 808 (1972).