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Further Studies on the Reaction of Guanosine Derivatives with Phosphorus Trichloride[1)]

TOKUMI MARUYAMA,[a] YOSHIKO SATO,[a] MIKIO HONJO,*,[a] HIROBUMI OISHI,[b] KEIZO OGAWA,[b] TAKAJI FUJIWARA,[b] and KEN-ICHI TOMITA[b]

School of Pharmacy, Tokushima Bunri University,[a] Yamashiro-cho, Tokushima 770, Japan and Faculty of Pharmaceutical Sciences, Osaka University,[b] Yamadaoka, Suita, Osaka 565, Japan

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Reaction of 2′,3′-di- or 2′,3′,5′-tri-*O*-protected guanosine (I, IX) with phosphorus trichloride in ethyl methyl ketone, cyclohexanone or benzaldehyde afforded the corresponding N^2-(1-phosphono)alkylguanosine derivative (IIa—c). The structure of the aglycon (IIIa) was determined definitely by the X-ray diffraction method. A similar reaction in dioxane resulted in no modification of the guanine moiety, while the reaction in tetrahydrofuran gave 4-(inosin-2-yl)amino-4-phosphonobutyl hydrogen phosphonate (VII). None of the reactions in acetylacetone, benzoquinone, naphthoquinone and anthraquinone modified the guanine base. An alternative preparation of guanosine 5′-monophosphate was accomplished by the reaction of isopropylideneguanosine (IX) with phosphorus trichloride in anthraquinone in a stream of oxygen, followed by hydrolysis and deblocking.

Keywords——guanosine derivative; phosphorus trichloride; oxaphosphirane; alkylphosphonation; 5′-GMP; X-ray diffraction

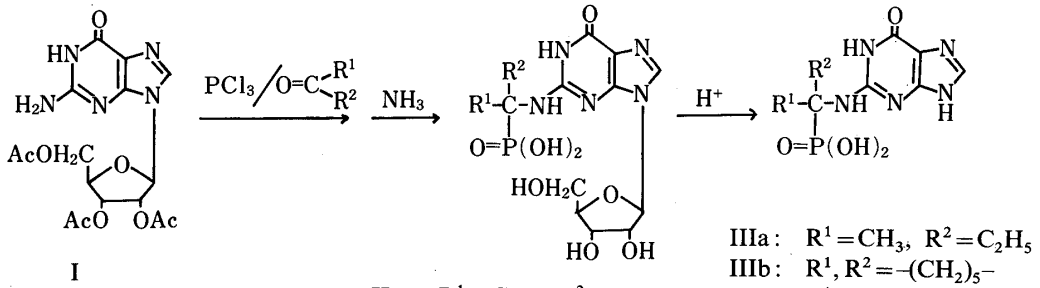
In a previous paper,[2)] we reported the novel reaction of 2′,3′-di- or 2′,3′,5′-tri-*O*-protected guanosine with phosphorus trichloride in acetone to give an N^2-(1-methyl-1-phosphono)ethylguanosine. The present work was undertaken to develop further the reaction in some solvents other than acetone.

Reaction of 2′,3′,5′-Tri-*O*-acetylguanosine (I) with Phosphorus Trichloride in Monoketone or Aldehyde

Compound I was allowed to react with phosphorus trichloride in an excess of ethyl methyl ketone in a stoppered vessel at 0—5 °C for 2.5 h. The reaction mixture, after successive treatments such as hydrolysis, deblocking, purification through a Dowex-1 (formate) column and addition of barium acetate, afforded the barium salt of IIa as a pale yellow powder in high yield. Formation of crude IIa by the above reaction, followed by hydrolysis of the N-glycosidic bond of IIa with hydrochloric acid yielded white prisms (IIIa) ($C_9H_{14}N_5O_4P \cdot 2.5\ H_2O$, after recrystallization from water). The structure of IIIa was determined by the X-ray diffraction method to be N^2-(1-methyl-1-phosphono)propylguanine, and the parent compound (IIa) was thus assigned as the corresponding ribonucleoside. This result provides further confirmation of the correctness of the proposed structure of the reaction product in acetone,[2)] and indicates that an analogous reaction proceeded in ethyl methyl ketone. An analogous reaction of I with phosphorus trichloride in cyclohexanone gave the corresponding ribonucleoside (IIb). Formation of crude IIb by the same reaction, followed by fission of the N-glycosidic bond of IIb yielded the aglycon (IIIb).

Reaction of I with phosphorus trichloride in a large excess of benzaldehyde resulted in no modification of the guanine moiety, while the reaction in less than one mol eq of benzaldehyde with respect to phosphorus trichloride gave an analogously modified ribonucleoside (IIc).

Hydrolysis of the glycosidic bond of IIc afforded the corresponding aglycon (IIIc). This difference can be explained as follows. Phosphorus trichloride reacts with an excess of benzaldehyde to form an inactive polymer $(POCl)_n$,[3] which is not capable of reacting with the guanine moiety. On the other hand, phosphorus trichloride reacts with one mol eq of benzaldehyde to form a reactive oxaphosphirane,[4] which is subject to nucleophilic attack of the 2-amino group of the guanine moiety[2] (Chart 1).



I

IIa: $R^1=CH_3$, $R^2=C_2H_5$
IIb: $R^1, R^2=-(CH_2)_5-$
IIc: $R^1=C_6H_5$, $R^2=H$

IIIa: $R^1=CH_3$, $R^2=C_2H_5$
IIIb: $R^1, R^2=-(CH_2)_5-$
IIIc: $R^1=C_6H_5$, $R^2=H$

Chart 1

X-Ray Structure Determination of IIIa

The molecular structure of IIIa is shown in Fig. 1, and bond lengths and angles are given in Fig. 2 with the atomic numberings. Tables I and II give the atomic parameters for non-hydrogen and hydrogen atoms, respectively. There are no significant deviations from the standard values of bond lengths and angles in the purine base moiety. The protonation at the N_7-position was confirmed by the extra electron density peak on a difference Fourier map. On the other hand, no peak was observed around either O_1 or O_3, and the P–O_1 and P–O_3 bond distances are apparently shorter than that of P–O_2. Moreover, the bond angle of C_5–N_7–C_8 (107 °) is slightly larger than that generally found in the non-protonated case (104 °). These results indicate that one proton of the phosphate group migrates to the N_7-position and the molecule exists as a zwitterion with a positive charge on the purine ring and a negative charge on the phosphate group. The purine ring is approximately planar, and the exocyclic group attached to the C_2 atom possesses *gauche*, *trans* and *cis* conformations around the C_2–N_2, N_2–C_{10} and C_{10}–C_{12} bonds, respectively. An intramolecular hydrogen bond between N_3 and O_2 may stabilize the molecular conformation (Fig. 1).

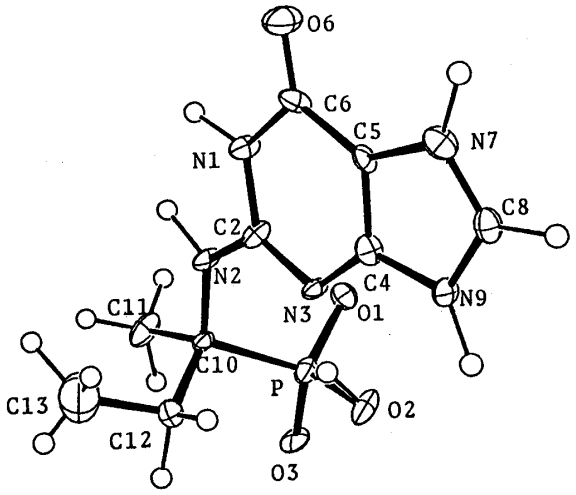


Fig. 1. ORTEP Plots of Compound IIIa

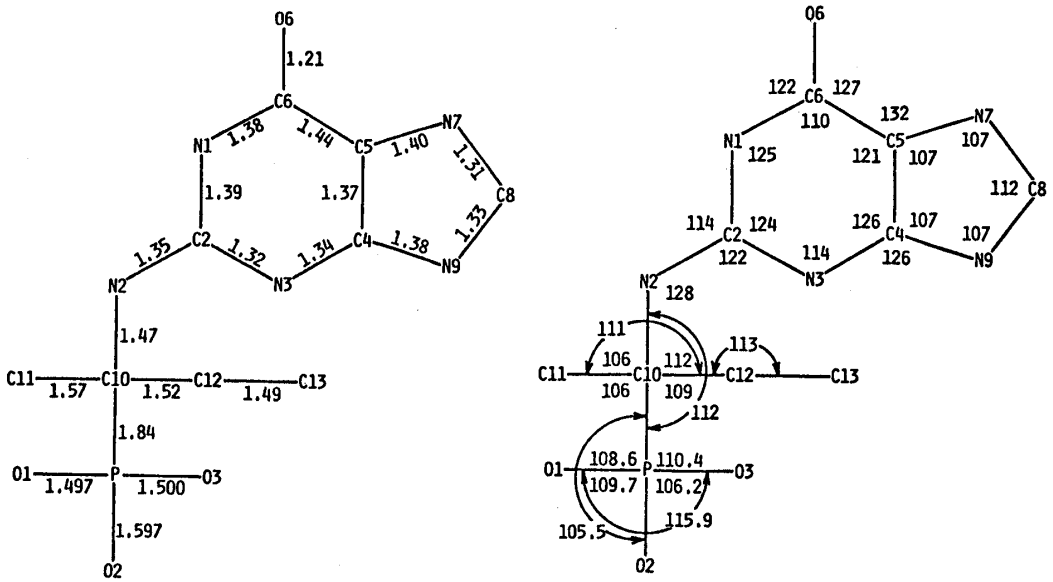


Fig. 2. Bond Distances and Angles

TABLE I. Atomic Coordinates and Equivalent Isotropic Thermal Parameters for the Non-hydrogen Atoms with Estimated Standard Deviations in Parentheses

Atom	$B_{eq}=4/3\sum_i\sum_j\beta_{ij}\boldsymbol{a}_i\boldsymbol{a}_j$			
	X	Y	Z	B_{eq}
N1	0.2862 (8)	0.0030 (7)	0.3023 (8)	2.18 (10)
C2	0.2482 (10)	0.0624 (8)	0.3803 (9)	1.97 (11)
N3	0.2784 (8)	0.0372 (7)	0.5057 (7)	1.87 (10)
C4	0.3552 (10)	−0.0485 (9)	0.5414 (10)	2.24 (10)
C5	0.4003 (9)	−0.1091 (8)	0.4660 (9)	1.96 (10)
C6	0.3640 (10)	−0.0860 (9)	0.3339 (10)	2.32 (11)
N7	0.4788 (8)	−0.1883 (7)	0.5430 (9)	2.88 (9)
C8	0.4798 (10)	−0.1724 (9)	0.6575 (10)	2.50 (11)
N9	0.4059 (8)	−0.0904 (7)	0.6615 (8)	2.27 (9)
P	0.2446 (3)	0.2974 (2)	0.5299 (3)	2.35 (3)
C10	0.1236 (9)	0.2287 (8)	0.3976 (8)	1.95 (10)
C11	0.0604 (12)	0.3186 (10)	0.2979 (10)	3.58 (15)
C12	0.0292 (9)	0.1756 (10)	0.4455 (10)	2.87 (12)
C13	−0.0778 (12)	0.1249 (16)	0.3432 (15)	7.11 (23)
O1	0.3554 (10)	0.3205 (6)	0.4928 (7)	2.59 (7)
O2	0.2836 (15)	0.2128 (6)	0.6437 (6)	3.32 (9)
O3	0.1896 (6)	0.3930 (6)	0.5727 (7)	3.15 (9)
O6	0.3958 (7)	−0.1335 (7)	0.2574 (7)	4.22 (11)
N2	0.1789 (7)	0.1506 (7)	0.3350 (7)	2.08 (10)
OW1	0.3654 (8)	0.5170 (8)	0.3642 (10)	5.76 (14)
OW2	0.2457 (8)	0.6442 (12)	0.4910 (11)	10.40 (21)
OW3	0.0425 (10)	0.5601 (16)	0.4591 (22)	6.58 (31)

Reaction of I or 2′,3′-*O*-Isopropylideneguanosine (IX) with Phosphorus Trichloride in Ether

Compound I or IX was allowed to react with phosphorus trichloride in an excess of dioxane in a stream of oxygen. The reaction mixture was worked up to obtain guanosine or a mixture of guanosine 5′-phosphonate and guanosine 5′-monophosphate (5′-GMP) in a ratio of 3 : 2, respectively.

TABLE II. Atomic Coordinates for the Hydrogen Atoms with Estimated Standard Deviations in Parentheses

Atom	X	Y	Z
C2H1	−0.023 (13)	0.221 (11)	0.491 (11)
C2H2	0.066 (18)	0.104 (10)	0.504 (11)
N9H	0.393 (11)	−0.060 (10)	0.746 (11)
C8H	0.534 (10)	−0.220 (10)	0.737 (11)
N7H	0.530 (11)	−0.251 (10)	0.513 (11)
N2H	0.164 (11)	0.166 (9)	0.236 (11)
N1H	0.252 (11)	0.028 (10)	0.204 (11)
C3H2	−0.150 (11)	0.128 (10)	0.350 (12)
C3H3	−0.102 (11)	0.134 (10)	0.233 (11)
C1H2	−0.012 (11)	0.287 (10)	0.226 (11)
C1H3	0.043 (11)	0.393 (10)	0.350 (11)
C1H1	0.126 (11)	0.346 (10)	0.256 (11)
C3H1	−0.051 (11)	0.041 (10)	0.356 (11)
O2H	0.252 (11)	0.153 (9)	0.578 (11)
W1H1	0.456 (11)	0.541 (10)	0.355 (11)
W1H2	0.345 (11)	0.441 (10)	0.409 (11)
W2H1	0.300 (11)	0.672 (10)	0.544 (11)
W2H2	0.298 (11)	0.596 (9)	0.421 (11)

Treatment of I with phosphorus trichloride in an excess of tetrahydrofuran (THF) in a stoppered vessel gave a product, which was presumed to be *N*-(inosin-2-yl)amidophosphonic acid on the basis of its behavior on the paper electrogram, ultraviolet (UV) absorption spectra and easy cleavage to guanosine in the course of column chromatography. A similar reaction in a stream of oxygen, however, yielded white needles (VII). Its formula, $C_{14}H_{23}N_5O_{11}P_2 \cdot 1.5\ H_2O$, was confirmed by elemental analysis and fast atom bombardment (FAB) mass spectrometry ($m/z = 500$ ($M^+ + 1$), 368 (aglycon$^+ + 1$)). The product migrated faster toward the anode than 5′-GMP on paper electrophoresis, and showed a UV absorption spectrum similar to that of N^2-alkylguanosine. Hydrolysis of the glycosidic bond of VII with hydrochloric acid, followed by Dowex-1 (chloride) column chromatography, afforded a pure aglycon (VIII).

The partial structure, $HP(O)(OH)OCH_2CH_2CH_2CHP(O)(OH)_2$–NH–C(–NH–)=N–, of VII was established from the proton nuclear magnetic resonance (^{1}H-NMR) spectrum (NaOD), ^{13}C-NMR spectrum (NaOD) and ^{31}P-NMR spectrum (NaOD). Chemical shifts and *J* values in the ^{31}P-NMR spectrum were measured by means of proton noise decoupling and proton non-decoupling, respectively. The two ^{31}P signals of VII were shifted downfield with respect to that of phosphoric acid (an internal reference), suggesting that both phosphorus atoms are pentavalent. The structure of VII was thus confirmed to be 4-(inosin-2-yl)amino-4-phosphonobutyl hydrogen phosphonate, and VIII was assigned as 4-hydroxy-1-(hypoxanthin-2-yl)aminobutylphosphonic acid.

A plausible mechanism for the formation of VII involves the following three steps. (1) An equimolar mixture[5] of phosphorus trichloride and oxygen oxidizes THF to yield 4-hydroxybutanal (V)[6] *via* 2-hydroxy-THF (IV).[7] (2) Compound V reacts with phosphorus trichloride to afford the oxaphosphirane (VI).[4] (3) A subsequent nucleophilic attack of the 2-amino group of I on C-1 of VI is synchronized with fission of the C–O bond and elimination of the chloride ion[2] (Chart 2).

A similar treatment of I in a stoppered vessel with phosphoryl chloride instead of phosphorus trichloride resulted in recovery of guanosine. The possibility is thus ruled out that phosphorus trichloride might be oxidized to phosphoryl chloride, which in turn would react

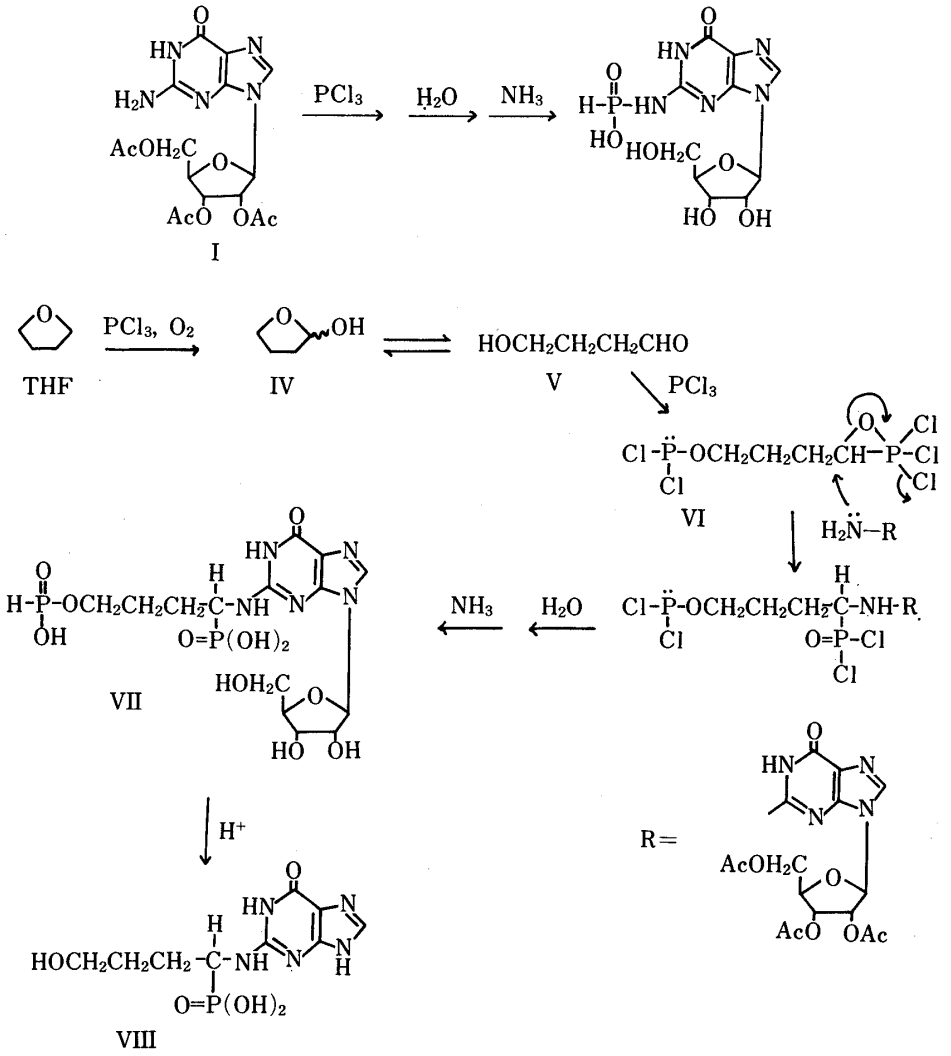


Chart 2

with I in THF to give VII.

Reaction of IX with Phosphorus Trichloride in β-Diketone or Quinone (An Alternative Preparation of 5′-GMP)

Treatment of I with phosphorus trichloride in an excess of 9,10-anthraquinone in a stream of oxygen recovered guanosine. This result was thus applied to an alternative preparation of 5′-GMP. Reaction[8] of IX with phosphorus trichloride in 9,10-anthraquinone, after a treatment including purification by diethylaminoethyl (DEAE) cellulose (formate) column chromatography, afforded a pale brown syrup in *ca.* 51% yield. The product was proved to be 5′-GMP on the basis of its behavior on the paper electrogram, UV absorption

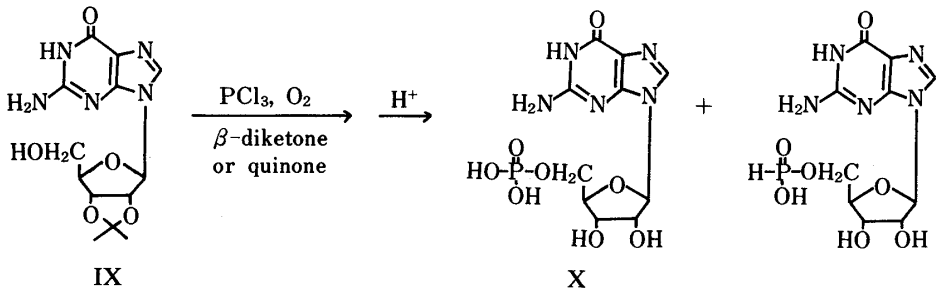


Chart 3

spectrum and enzymatic hydrolysis to guanosine with snake venom (*Trimeresurus flavoriridis* HALLOWELL) 5′-nucleotidase.

A similar treatment of IX with phosphorus trichloride in acetylacetone or 1,4-benzoquinone gave 5′-GMP as a main product, while the reaction in 1,2- or 1,4-naphthoquinone yielded guanosine 5′-phosphonate as a major product and 5′-GMP as a minor product (Chart 3).

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus (hot stage type) and are uncorrected. The UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. The ^{1}H-NMR spectra were recorded with a Nihondenshi GX-400 (400 MHz) spectrometer in D_2O (or NaOD in D_2O) with sodium 3-(trimethylsilyl)propionate as an internal standard. Paper electrophoresis (PE) was carried out at 22 V/cm using 0.01 M phosphate buffer (pH 7.5).

N^2-(1-Methyl-1-phosphono)propylguanosine (IIa)·Ba Salt——Phosphorus trichloride (15 ml) was added dropwise to a cooled (0—5 °C) suspension of tri-*O*-acetylguanosine (I) (1.27 g, 3.10 mmol) in ethyl methyl ketone (50 ml), and the solution was stirred at 0—5 °C for 2.5 h. The reaction mixture was poured into ice-water (1 l), adjusted to pH 7 with 5 N NaOH and passed through a column of activated charcoal (10 g). The column was washed with water and eluted with a mixture of EtOH–H_2O–conc. NH_4OH (10:9:1). The eluate was concentrated to 10 ml and chromatographed on a column of Dowex 1 × 8 (formate, 2.5 × 15 cm) with a gradient of H_2O (1 l)–2 N HCOOH (1 l). The eluate was evaporated to dryness *in vacuo*, the resulting syrup was dissolved in 0.5 M $Ba(OAc)_2$ (6 ml) and the solution was triturated with EtOH to give a white powder (1.49 g, 81%). mp >300 °C. *Anal.* Calcd for $C_{14}H_{20}BaN_5O_8P\cdot 2H_2O$: C, 28.46; H, 4.10; N, 11.85. Found: C, 28.91; H, 4.13; N, 11.45. UV $\lambda_{max}^{0.1\,N\,HCl}$ nm (ε): 262 (15900), 285 (sh), (8200), $\lambda_{max}^{H_2O}$ nm (ε): 259 (18200), 275 (sh) (12800), $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 261 (16100), 275 (sh) (12600). ^{1}H-NMR (D_2O) δ: 8.02 (1H, d, H-8), 5.90 (1H, d-like, $J_{1',2'}$ = 5.49 Hz, H-1′), 4.72 (1H, m, H-2′), 4.40 (1H, m, H-3′), 4.21 (1H, m, H-4′), 3.89 (1H, sestet, H-5′a), 3.81 (1H, q, H-5′b), 2.25 and 1.82 (each 1H, sestet, $(DO)_2(O)PC(CH_3)(C\underline{H}_2CH_3)$–), 1.48 (3H, d-like, J_{HCCP} = 13.56 Hz, $(DO)_2(O)PC(C\underline{H}_3)(CH_2CH_3)$–), 0.89 (3H, sestet, $(DO)_2(O)PC(CH_3)(CH_2C\underline{H}_3)$–).

N^2-(1-Phosphono)cyclohexylguanosine (IIb)·Ba Salt——Phosphorus trichloride (15 ml) was added to a cold suspension of I (1.27 g, 3.10 mmol) in cyclohexanone (50 ml), and the solution was stirred at 0—5 °C for 18 h. The reaction mixture was worked up in a manner similar to that described in the preceding section to give a white powder (1.62 g). mp >300 °C. UV $\lambda_{max}^{0.05\,N\,HCl}$ nm: 262.5, 290 (sh), $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 262.5, 280 (sh). ^{1}H-NMR (D_2O) δ: 7.99 (1H, s, H-8), 5.92 (1H, d, $J_{1',2'}$ = 5.86 Hz, H-1′), 4.74 (1H, t, $J_{2',3'}$ = 5.50 Hz, H-2′), 4.40 (1H, t, $J_{3',4'}$ = 3.66 Hz, H-3′), 4.25 (1H, q, H-4′), 3.91 (1H, q, $J_{5'a,4'}$ = 2.93 Hz, $J_{5'a,5'b}$ = 12.82 Hz, H-5′a), 3.82 (1H, q, $J_{5'b,4'}$ = 4.03 Hz, H-5′b), 1.2—2.2 (10H, m, $-(CH_2)_5-$).

N^2-(α-Phosphono)benzylguanosine (IIc)·Ba Salt——Benzaldehyde (15 ml, 148 mmol) was added dropwise over a period of 30 min to a cold solution of I (1.23 g, 3.0 mmol) in phosphorus trichloride (25 ml, 286 mmol). The mixture was stirred at 0—5 °C for 6 h, poured into ice-water (1 l), washed with $CHCl_3$, adjusted to pH 7 with 5 N NaOH and passed through a column of activated charcoal. The eluate was concentrated to 10 ml and chromatographed on a column of DEAE cellulose (bicarbonate, 2.7 × 25 cm) with a gradient of H_2O (600 ml)–0.1 M triethylammonium bicarbonate (TEAB) (600 ml). The eluate was evaporated to dryness *in vacuo* to give a syrup, which was dissolved in 0.5 M $Ba(OAc)_2$ (3 ml). The solution was triturated with EtOH to yield a white powder (811 mg, 47%). mp >300 °C. *Anal.* Calcd for $C_{17}H_{18}BaN_5O_8P\cdot 5H_2O$: C, 30.08; H, 4.15; N, 10.31. Found: C, 30.18; H, 3.80; N, 9.87. UV $\lambda_{max}^{0.1\,N\,HCl}$ nm (ε): 259.5 (15400), 280 (sh) (9400), $\lambda_{max}^{H_2O}$ nm (ε): 255 (15800), 277 (sh) (10600), $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 260 (13200), 275 (sh) (10800). ^{1}H-NMR (D_2O) δ: 7.72 (1H, s, H-8), 7.25—7.60 (5H, m, $C_6\underline{H}_5$–), 5.73 (1H, d, $J_{1',2'}$ = 5.12 Hz, H-1′), 5.06 (1H, t, J_{HCP} = 19.66 Hz, $C_6H_5C\underline{H}P(O)(OD)_2$–), 4.60 (1H, t, $J_{2',3'}$ = 5.54 Hz, H-2′), 4.33 (1H, t, $J_{3',4'}$ = 4.72, H-3′), 4.14 (1H, q, H-4′), 3.92 (1H, q, $J_{5'a,4'}$ = 3.42 Hz, $J_{5'a,5'b}$ = 12.39 Hz, H-5′a), 3.82 (1H, q, $J_{5'b,4'}$ = 5.78 Hz, H-5′b).

N^2-(1-Methyl-1-phosphono)propylguanine (IIIa)——Phosphorus trichloride (9 ml) was added to a cold suspension of I (945 mg, 2.31 mmol) in ethyl methyl ketone (30 ml), and the solution was stirred at 0—5 °C for 2.5 h. The reaction mixture was poured into ice-water (1 l), refluxed for 2 h and passed through a column of activated charcoal. The eluate was concentrated to 10 ml and chromatographed on a column of Dowex 1 × 8 (chloride, 2.5 × 15 cm) with 0.05 N HCl (500 ml). The eluate was concentrated to give colorless prisms (355 mg, 48%). mp 264—266 °C. *Anal.* Calcd for $C_9H_{14}N_5O_4P\cdot 2H_2O$: C, 33.44; H, 5.61; N, 21.67. Found: C, 33.01; H, 5.17; N, 21.21. UV: $\lambda_{max}^{0.1\,N\,HCl}$ nm (ε): 253 (14700), 280 (sh) (6700), $\lambda_{max}^{H_2O}$ nm (ε): 251.5 (13300), 275 (sh) (8500), $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 261 (10300), 280 (sh) (9000). ^{1}H-NMR (1 N NaOD) δ: 7.76 (1H, s, H-8), 2.68 and 2.22 (each 1H, m, $(DO)_2(O)PC(CH_3)(C\underline{H}_2CH_3)$–), 1.86 (3H, d, J_{HCCP} = 12.0 Hz, $(DO)_2(O)PC(C\underline{H}_3)(CH_2CH_3)$–), 1.26 (3H, t, $(DO)_2(O)PC(CH_3)(CH_2C\underline{H}_3)$–).

N^2-(1-Phosphono)cyclohexylguanine (IIIb)——Compound I (315 mg, 0.77 mmol) was dissolved in a cold mixture

of cyclohexanone (10 ml) and phosphorus trichloride (3 ml). The solution was stirred at 0—5 °C for 2.5 h, and treated in a manner similar to that described in the preceding section to give colorless prisms (199 mg, 78%). mp 275—278 °C. *Anal.* Calcd for $C_{11}H_{16}N_5O_4P \cdot H_2O$: C, 39.88; H, 5.48; N, 21.14. Found: C, 40.21; H, 5.08; N, 20.75. UV $\lambda_{max}^{0.1\,N\,HCl}$ nm (ε): 254.5 (15200), 280 (sh) (7100), $\lambda_{max}^{H_2O}$ nm (ε): 252 (14000), 275 (sh) (9000), $\lambda_{max}^{0.1\,N\,NaOH}$ nm (ε): 262 (10900), 280 (sh) (9400). ^{1}H-NMR (D_2O) δ: 8.23 (1H, s, H-8), 1.5—3.5 (10H, m, $-(CH_2)_5-$).

N^2-(α-Phosphono)benzylguanine (IIIc)·Ba Salt——A solution of IIc Ba salt (224 mg, 0.38 mmol) in 1 N HCl (2.5 ml) was refluxed for 2 h. The reaction mixture was desalted on a column of activated charcoal, concentrated to 10 ml and chromatographed on a column of Dowex 1×8 (chloride, 1.7×15 cm) with 0.05 N HCl. The eluate was evaporated to dryness *in vacuo*, the resulting syrup was dissolved in 0.5 M $Ba(OAc)_2$ (1 ml) and the solution was triturated with EtOH to give a white powder (70 mg). mp >300 °C. UV $\lambda_{max}^{0.05\,N\,HCl}$ nm: 251.5, 275 (sh), $\lambda_{max}^{H_2O}$ nm: 248, 278, $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 279, 250 (sh). ^{1}H-NMR (1 N NaOD) δ: 7.42 (1H, s, H-8), 7.1—7.6 (5H, m, $C_6\underline{H}_5CHP(O)(OD)_2-$), 4.88 (1H, d, J_{HCP}=21 Hz, $C_6H_5C\underline{H}P(O)(OD)_2-$).

A Mixture of Guanosine 5′-Phosphonate and 5′-GMP——Phosphorus trichloride (0.3 ml) was added to a suspension of isopropylideneguanosine (IX) (25 mg) in dioxane (1 ml). The mixture wa stirred vigorously in a stream of oxygen at room temperature for 18 h and poured into ice-water (16 ml). The solution was adjusted to pH 1.5, heated at 70 °C for 40 min, and desalted with activated charcoal. An aliquot of the solution was analyzed by PE, which showed a major UV-absorbing spot (59% yield) at $M_{5'\text{-GMP}}$=0.63 and a minor spot (36% yield) at $M_{5'\text{-GMP}}$=1.00. Both UV absorption spectra were superimposable on that of 5′-GMP.

4-(Inosin-2-yl)amino-4-phosphonobutyl Hydrogen Phosphonate (VII)——Phosphorus trichloride (15 ml) was added to a cold suspension of I (1.78 g, 4.35 mmol) in THF (50 ml). The solution was stirred in a stream of oxygen at 5—10 °C for 16 h. The reaction mixture was poured into ice-water (1 l), adjusted to pH 7 with 5 N NaOH and passed through a column of activated charcoal. The eluate was concentrated to 10 ml and chromatographed on a column of DEAE cellulose (bicarbonate, 2.7×25 cm) with a gradient formed from H_2O (600 ml) and 0.2 M TEAB (600 ml). The eluate was evaporated to dryness *in vacuo* and the residue was dissolved in a small amount of water. The solution was passed through a column of Amberlite IR 120B (H^+, 1.7×10 cm) and concentrated to give white crystals (674 mg, 29%). mp >300 °C. *Anal.* Calcd for $C_{14}H_{23}N_5O_{11}P_2 \cdot 1.5H_2O$: C, 31.94; H, 4.98; N, 13.31. Found: C, 32.00; H, 4.80; N, 13.03. UV $\lambda_{max}^{0.05\,N\,HCl}$ nm: 261, 285 (sh), $\lambda_{max}^{H_2O}$ nm: 257, 280 (sh), $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 260, 280 (sh). ^{1}H-NMR (2.4 N NaOD) δ: 8.33 (1H, s, H-8), 6.30 (1H, d, $J_{1',2'}$=6 Hz, H-1′), 5.25 (1H, m, H-2′), 4.55—4.85 (2H, m, H-3′ and $-OCH_2CH_2CH_2C\underline{H}P(O)(OD)_2-$), 4.05—4.55 (5H, m, H-4′, H-5′ and $-OC\underline{H}_2CH_2CH_2CHP(O)(OD)_2-$). 1.8—2.7 (4H, $-OCH_2C\underline{H}_2C\underline{H}_2CHP(O)(OD)_2-$). ^{13}C-NMR (1 N NaOD) δ: 159.10 (C-6), 153.72, (d, J_{CNCP}=7.94 Hz, $-CHP(O)(OD)_2-NH-\underline{C}(-NH-)=N-$), 151.48 (C-4), 139.46 (C-8), 116.95 (C-5), 90.51 (C-1′), 84.89 (C-4′), 72.95 (C-2′), 71.02 (C-3′), 65.63 (q, J_{COP}=20.14 Hz, J_{CCCCP}=3.66 Hz, $HP(O)(OD)O\underline{C}H_2CH_2CH_2CHP(O)(OD)_2-$), 62.54 (C-5′), 50.12 (d, J_{CP}=142.8 Hz, $HP(O)(OD)OCH_2CH_2\underline{C}HP(O)(OD)_2-$), 28.0—29.5 (m, $HP(O)(OD)OCH_2\underline{C}H_2\underline{C}H_2-CHP(O)(OD)_2-$)). ^{31}P-NMR (1 N NaOH) δ: 18.87 ($-C\underline{H}P(O)(OD)_2-$), 3.36 (d, J_{PH}=566.4 Hz, $H\underline{P}(O)(OD)OCH_2-$), 3.02 (t, J_{PD}=86.1 Hz, $D\underline{P}(O)(OD)OCH_2-$).

4-Hydroxy-1-(hypoxanthin-2-yl)aminobutylphosphonic Acid (VIII)——VII (256 mg, 0.51 mmol) was dissolved in 1 N HCl (10 ml) and the solution was refluxed for 2 h. After cooling, the reaction mixture was desalted with activated charcoal, concentrated to 3 ml and chromatographed on a column of Dowex 1×8 (chloride, 1.7×12 cm) with a gradient formed from H_2O (500 ml) and 0.2 N HCl (500 ml). The eluate was evaporated *in vacuo* to give a white solid (91 mg). mp >300 °C. PE $M_{5'\text{-GMP}}$[9]=1.00. ^{1}H-NMR (1 N NaOD) δ: 8.12 (1H, s, H-8), 4.52 (1H, q, $HOCH_2CH_2CH_2C\underline{H}P(O)(OH)_2-$), 4.06 (2H, m, $HOC\underline{H}_2CH_2CH_2CHP(O)(OH)_2-$), 2.0—2.8 (4H, br s, $HOCH_2C\underline{H}_2C\underline{H}_2CHP(O)(OH)_2-$).

***N*-(Inosin-2-yl)amidophosphonic Acid**——Phosphorus trichloride (1.5 ml) was added to a suspension of I (178 mg, 0.44 mmol) in THF (5 ml) and the solution was kept at 50 °C for 3 d. The reaction mixture was treated in a usual manner to give a pale brown syrup, which showed a major UV-absorbing spot at $M_{5'\text{-GMP}}$[9]=0.66 by PE. UV $\lambda_{max}^{0.05\,N\,HCl}$ nm: 256, 270 (sh), $\lambda_{max}^{H_2O}$ nm: 255, $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 255.

5′-GMP (X)——Isopropylideneguanosine (IX) (123 mg, 0.38 mmol) and anthraquinone (237 mg, 0.18 mmol) was suspended in dioxane (5 ml) and phosphorus trichloride (1.5 ml) was added to the suspension. The mixture was stirred vigorously in a stream of oxygen at room temperature for 18 h and poured into ice-water (50 ml). Insoluble material was filtered off and the clear solution was adjusted to pH 1.5, heated at 70 °C for 40 min, and applied to a column of activated charcoal (3 g), which was washed with water (50 ml) and eluted with a mixture of EtOH–H_2O–conc. NH_4OH (10 : 9 : 1) (60 ml). The eluate was concentrated to 10 ml and chromatographed over a column of DEAE cellulose (bicarbonate, 1.7×15 cm) with 0—0.1 M TEAB (1 l) to give a syrup (53%), which showed a single UV-absorbing spot at the same migration distance as that of 5′-GMP on PE. The UV spectrum was superimposable upon that of 5′-GMP. Enzymatic hydrolysis of the product with snake venom 5′-nucleotidase (*Trimeresurus flavoviridis* HALLWELL) gave guanosine.

Crystallographic Measurements——Compound IIIa crystallized from aqueous solution as colorless prisms. The cell dimensions were determined by least-squares refinement of the angular values of 25 reflections in the range of $28.76 \leq 2\theta \leq 103.38°$, and the density was measured in C_6H_6–$CHCl_3$ mixture. The intensity data were collected by the 2θ–ω scanning technique using graphite-monochromated CuK_α radiation on a Rigaku automatic four-circle

diffractometer in the range of $2\theta \leq 125°$. In total, 2390 independent reflections from a crystal of $0.11 \times 0.13 \times 0.05$ mm had intensities above the $2\sigma(F)$ level and they were used for the structure determination.

Crystal Data——$C_9H_{14}N_5O_4P \cdot 2.5H_2O$. $M_r = 332.25$. Monoclinic $a = 11.421$ (3), $b = 12.356$ (2), $c = 11.370$ (2) Å, $\beta = 111.11$ (1)°. $D_c = 1.475$ and $D_m = 1.451$ (4) g/cm^3. $Z = 4$. Space group $P2_1/c$.

Determination of the Structure——The structure was solved by direct methods using the program MULTAN 78 and refined by the full-matrix least-squares method with anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms except those of a half-occupied water molecule site were located on a difference Fourier map and included in the further refinement with isotropic temperature factors. The final R-factor was 0.084 ($R_w = 0.090$). All the computations were carried out on an ACOS-900 computer at the Crystallographic Research Center, Institute for Protein Research, Osaka University.

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