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Synthesis of 6-Thioguanine and 2,6-Diaminopurine Nucleosides and Nucleotides from Adenine Counterparts *via* a Facile Rearrangement in the Base Portion (Nucleosides and Nucleotides. XIX[1])

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A method of conversion of an adenine moiety to 6-thioguanine (and guanine) and 2,6-diaminopurine moiety in the nucleoside and nucleotide levels were presented. The action of cyanogen bromide with adenosine N^1-oxide afforded an 1,2,4-oxadiazolo(3,2-f)-purine riboside (**2a**), which was in a pH dependent equilibrium with N^6-cyanoadenosine N^1-oxide (**3a**). Methylation followed by alkaline treatment of **3a** resulted in a rearrangement leading to give 2-amino-N^6-methoxyadenosine (**5a**). Catalytic hydrogenation of **5a** gave 2,6-diaminopurine riboside (**6a**). Sulfhydrolysis of **5a** gave 6-thioguanosine (**7a**).

By a similar reaction sequence 2′-deoxyadenosine was converted to 2′-deoxy-6-thioguanosine (**7b**) and 2,6-diaminopurine 2′-deoxyriboside, respectively. Starting from the N^1-oxides of adenosine 5′-phosphate (AMP), 2′-deoxyadenosine 5′-phosphate (dAMP) and 9-β-D-arabinofuranosyladenine 5′-phosphate (araAMP), the corresponding 6-thioguanine nucleotides were likewise prepared.

Keywords——Thiopurine and aminopurine; nucleosides and nucleotides; rearrangement; sulfhydrolysis; anti-tumor agents; UV

6-Thioguanine has been known as an effective anti-cancer agent.[3] A drawback of this thio-base as the chemotherapeutic agent is its high toxicity against bone marrow cells,[4] which may be due, in part, to its extremely low solubility, hence slow excretion from the host. In the form of nucleosides or nucleotides the solubility problem will be solved and the efficiency of the utilization in the nucleotide metabolism will also be enhanced.

6-Thioguanosine is readily prepared by the thiation of 2′,3′,5′-tri-*O*-benzoylguanosine with phosphorus pentasulfide in pyridine followed by deprotection.[5] This method, however, has not been applicable for the synthesis of 2′-deoxy-6-thioguanosine, since an extensive cleavage of the glycosylic linkage of 2′-deoxyguanosine was inevitable under the reaction condition of the thiation. The synthesis of 2′-deoxy-6-thioguanosine, therefore, involved the condensation of a 6-chloro-2-aminopurine and a properly protected 2-deoxyribosyl halide, with a concomitant formation of the α-anomer.[6]

During the studies on chemical conversions of naturally occurring nucleosides to the derivatives of biochemical utilities, we have encountered a new rearragement of a derivative of adenosine N^1-oxide which made the conversion of adenine to 2,6-diaminopurine and 6-thioguanine possible in the nucleoside level. A preliminary result dealing mainly with the conversion of adenosine to guanosine derivative has appeared.[7]

Recent finding of Hamana and co-workers[8] involving treatment of quinoline N-oxide with cyanogen bromide to afford certain aminoquinoline derivatives prompted us to carry

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2) Address: *Kita-12, Nishi-6, Kita-ku Sapporo, 060, Japan.*
3) G.B. Elion and G.H. Hitchings, *J. Am. Chem. Soc.*, **77**, 1676 (1955).
4) D.A. Clarke, F.S. Phillips, S.S. Sternberg, and C.C. Stock, *Ann. N.Y. Acad. Sci.*, **60**, 235 (1954).
5) J.J. Fox, I. Wempen, A. Hampton, and I.L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958).
6) *a*) R.H. Iwamoto, E.M. Acton, and L. Goodman, *Nature*, 198 (1963); *idem*, *J. Med. Chem.*, **6**, 684 (1963); *b*) W.W. Lee, A.P. Martines, L. Goodman, and D.W. Henry, *J. Org. Chem.*, **37**, 2923 (1972).
7) K. Miura, T. Kasai, and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **23**, 464 (1975).
8) M. Hamana and S. Kumadaki, *Chem. Pharm. Bull.* (Tokyo), **21**, 800 (1973).

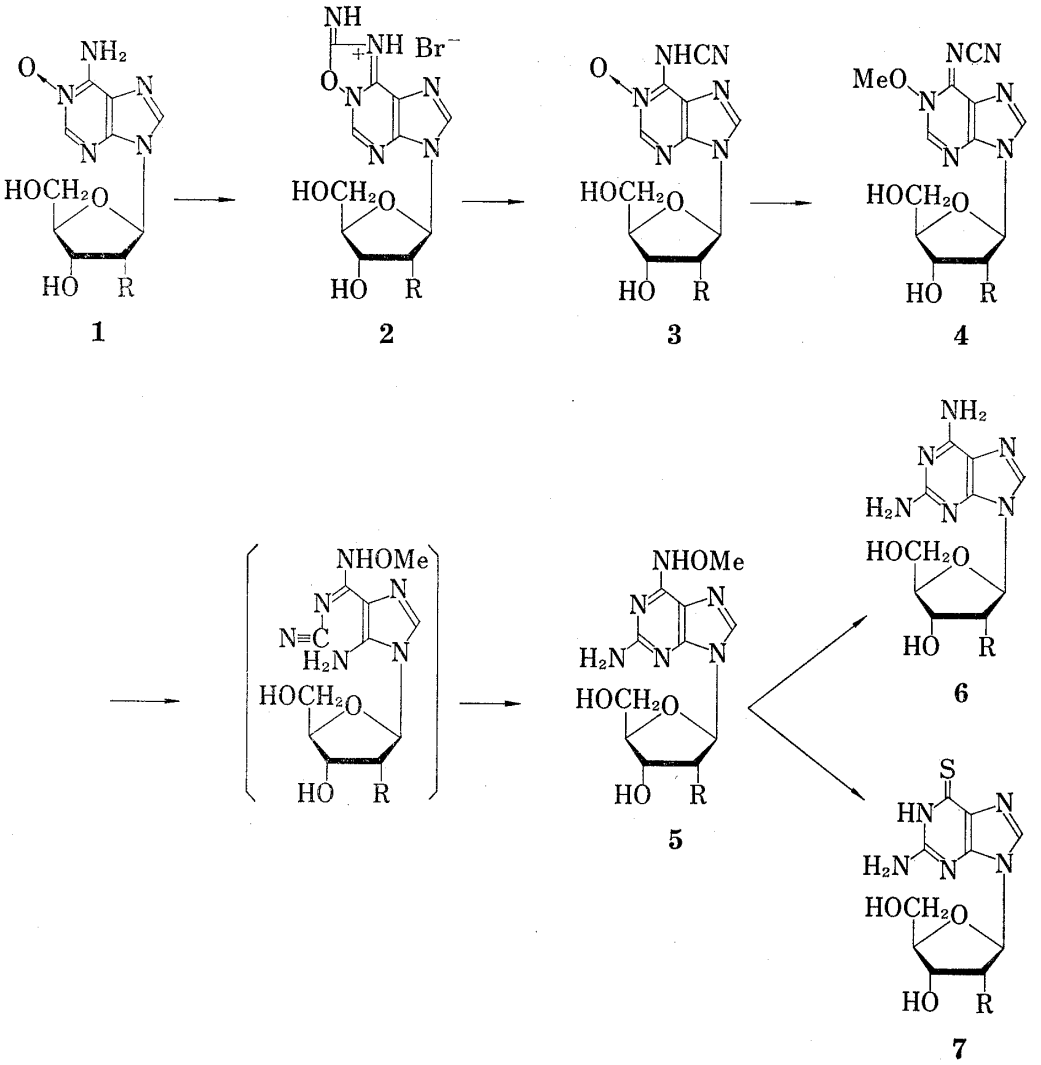

Chart 1 **a** series, R=OH
b series, R=H

out similar reaction of adenosine N^1-oxide, expecting the formation of 2,6-diaminopurine riboside.

Treatment of adenosine N^1-oxide (**1a**)[9] with cyanogen bromide in methanol afforded a product (**2a**) in high yield. The structure of **2a** was determined as follows: elemental analysis showed that **2a** is an adduct of **1a** and cyanogen bromide. Infrared (IR) spectra of **2a** showed absence of a cyano group, instead, an absorption probably due to an azomethine group (1710 cm^{-1}). The nuclear magnetic resonance (NMR) spectra showed that the signals of both protons of 2 and 8 positions of the adenine moiety shifted to the downfield (10.1 and 9.07 ppm, respectively) as compared with those of adenosine. This is explained by assuming the protonation or quarternization of the purine ring in **2a**. From these facts the structure of **2a** was determined as 2-imino-6-β-D-ribofuranosyl-[1,2,4-oxadiazolo(3,2-f)purine] hydrobromide (**2a**). In order to isolate **2a** as the free form, it was treated with methanolic ammonia which turned out to give N^6-cyanoadenosine-N^1-oxide (**3a**). Compound **3a** showed a strong IR absorption at 2180 cm^{-1} showing the presence of a cyano group. The ultraviolet (UV) absorption spectra of **3a** in neutral solution are identical with those of **2a** in an alkaline solution, while the spectra of **3a** in acidic solution were identical with those of **2a** in water. It is thus apparent that the structure **2a** and **3a** are in pH-dependent equilibrium. Hamana and

9) M.A. Stevens, D.I. Magrath, H.W. Smith, and G.B. Brown, *J. Am. Chem. Soc.*, **80**, 2755 (1958).

co-workers also reported[10] the formation of a product similar to **2** from the reaction of 2-aminoquinoline-N-oxide with cyanogen bromide.

Since it is well known that N^1-alkyl and N^1-alkoxy derivatives of 9-substituted adenines undergo Dimroth rearrangement in alkaline conditions to furnish N^6-alkyl and N^6-alkoxy adenine derivatives,[11,12] compound, **3a** is expected to be a suitable intermediate leading to 2,6-diaminopurine derivatives. Thus, N^1-methoxy-N^6-cyanoadenosine (**4a**) which may be accessible by the methylation of **3a**, would be cleaved and deformylated by alkaline treatment to give an aminoimidazole carboxamidoxime intermediate in which the attack of the 5-amino group to the cyano carbon is highly expected, and would result in a formation of 2-amino-6-methoxyaminopurine riboside (**5a**). This assumption was in fact found to be the case.

Treatment of **3a** with methyl iodide in dimethylformamide afforded N^1-methoxy derivative, **4a**, in high yield, which was isolated as a neutral form. Dissolution of **4a** in dilute sodium hydroxide solution and successive heating in aqueous ethanol after neutralization brought about the rearrangement to give 2-amino-6-methoxyaminopurine derivative (**5a**). Catalytic hydrogenation of **5a** with Raney Ni afforded 2,6-diaminopurine riboside (**6a**).[13] Treatment of **5a** with liquid hydrogen sulfide in aqueous pyridine at 70° for 86 hours in a sealed steel tube afforded 6-thioguanosine (**7a**) in an overall yield of 63% from **3a**. The sulfhydrolysis of 6-methoxyamino group has already been observed in 6-methoxyadenosine to 6-thioinosine.[14] Since the conversion of 6-thioguanosine to guanosine by treatment with hydrogen peroxide is known,[15] the present sequence establishes the conversion of adenine to guanine in the nucleoside level.

Although 6-thioguanosine is readily accessible from guanosine as described,[5] the present method may be especially valid for the synthesis of 2′-deoxy-6-thioguanosine and its phosphates. The action of cyanogen bromide with 2′-deoxyadenosine N^1-oxide (**1b**)[16] afforded the oxadiazolopurine derivative (**2b**) which was converted to the 1-methoxy-6-cyano-2′-deoxyadenosine (**4b**) by the similar treatment as for **4a**. Treatment of **4b** with sodium hydroxide solution and successive heating in ethanol afforded the unstable 2-amino-6-methoxyamino compound (**5b**), which was hydrogenated to give 2,6-diaminopurine 2′-deoxyribofuranoside (**6b**). The sulfhydrolysis of **5b** required milder conditions in terms of heating temperature (40—45°) to avoid the cleavage of the glycosylic linkage of **5b** and/or **7b**. The overall yield of 2′-deoxy-6-thioguanosine (**7b**) from **4b** was 34.6%.

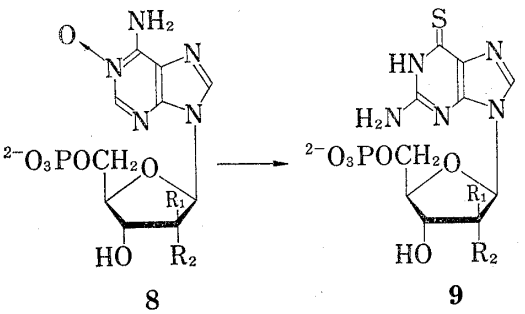

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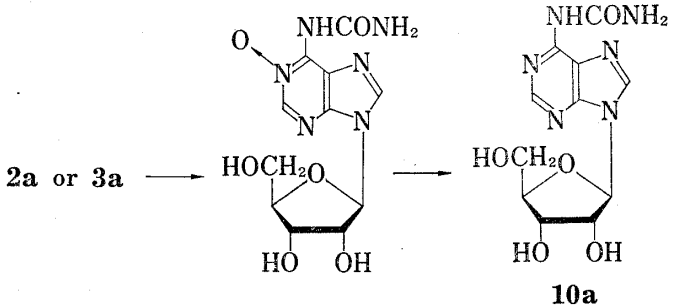
Chart 2 **a**: R_1=H, R_2=OH
b: R_1=R_2=H
c: R_1=OH, R_2=H



10a

Chart 3

10) M. Hamana and S. Kumadaki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1506 (1974).
11) P. Brookes, A.D. Dipple, and P.D. Lawley, *J. Chem. Soc.* (*C*), **1968**, 2026.
12) T. Fujii, C.C. Wu, T. Itaya, S. Moro, and T. Saito, *Chem. Pharm. Bull.* (Tokyo), **21**, 1676 (1973).
13) J. Davoll and B.A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951): T. Naito, K. Ueno, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **12**, 951 (1964).
14) K. Miura and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **23**, 2064 (1975).
15) T.A. Khwaja and R.K. Robins, *J. Am. Chem. Soc.*, **88**, 3640 (1966).
16) H. Klenow and S. Frederiksen, *Biochim. Biophys. Acta*, **52**, 384 (1961).

The conversion of adenine moiety to 6-thioguanine moiety was found to be adoptable in the nucleotides. The N^1-oxides (**8a—c**) of adenosine 5′-phosphate (AMP), 2′-deoxyadenosine 5′-phosphate (dAMP) and 9-β-D-arabinofuranosyladenine 5′-phosphate (araAMP) were converted to the respective 6-thioguanine nucleotides (**9a—c**) by the similar reaction sequences as described, with chromatographic purifications during appropriate steps. Under proper conditions for the sulfhydrolysis of the respective amino-methoxyaminopurine nucleotides, no dephosphorylation or glycosylic bond cleavage was observed (for detail, see experimental). Oxidative desulfurization of 9-β-D-arabinofuranosyl-6-thioguanine 5′-phosphate (**9c**) afforded the corresponding guanine nucleotide (araGMP).

The additional reaction leading to give an interesting adenosine analog is to be described here. Hydrogenation of **2** with palladium catalyst afforded N^6-carbamoyladenosine (**10**).[17] Treatment of **2** or **3** with acetic acid gave, probably, the N^1-oxide of **10** which was also converted to **10** by catalytic hydrogenation. Similar reaction was observed with the 2′-deoxyriboside (**2b**).

The results of the screening test for anti-tumor activities of 6-thioguanine derivatives synthesized in the present work was carried out[18] which revealed that compound **7b** and **9c** were highly active for mice leukemia L 1210 as well as NF-sarcoma and Sarcoma 180 (as) in the doses of 3 to 10 mg/kg.

Experimental

The UV spectra were measured by Shimadzu D-40 and UV-300 spectrophotometers. IR spectra were measured by Hitachi 215 spectrometer as KBr tablets. NMR spectra were recorded on a Hitachi R-24 NMR spectrometer using tetramethylsilane as an internal standard. Melting points were measured by Yamato MP-1 melting point apparatus and are uncorrected. Paper chromatography was run on Toyo Roshi No. 51A filter paper and following solvents were used: A, H_2O adjusted pH 10 with NH_4OH; B, iPrOH–NH_4OH–H_2O (7: 1: 2), C; EtOH–1 M NH_4OAc (pH 8.3).

2-Imino-6-β-D-ribofuranosyl-[1,2,4-oxadiazolo(3,2-*f*)purine] Hydrobromide (2a)——To the suspension of 5 g of **1** in 400 ml of MeOH was added 2 g of cyanogen bromide and stirred for 1 hr. After evaporation of the solvent the residue was dissolved in 300 ml of MeOH without heating and EtOAc was added until the crystals were separated (*ca.* 300 ml). The mixture was set aside overnight in a refrigerator. The separated colorless needles were collected and dried to give 6.3 g (92%) of **2a**, mp 165—169° (dec.). *Anal.* Calcd. for $C_{11}H_{13}BrN_6O_5 \cdot 1/3H_2O$: C, 33.43; H, 3.49; Br, 20.22 N, 21.27;. Found: C, 33.54; H, 3.58; N, 21.46; Br, 20.27. UV $\lambda_{max}^{H_2O}$ nm (ε): 224 (22400), 283 (20200); $\lambda_{max}^{0.1N\ HCl}$ 224 (25100), 283 (20800); $\lambda_{max}^{0.1N\ NaOH}$ 247 (30200), 293.5 (16700). NMR (DMSO-d_6) δ, ppm: 10.6 (bs, 1, C=NH), 10.1 (s, 1, H-2), 9.07 (s, 1, H-8), 6.09 (d, 1, H-1′, $J_{1',2'}$=5.2 Hz). IR (KBr) 1710 cm^{-1} (C=NH).

N^6-Cyanoadenosine N^1-Oxide (3a)——Compound **2a** (2 g) was dissolved in 100 ml of MeOH and 20 ml of methanolic ammonia was added. The solution was kept for 1 hr at room temperature. On evaporation of the solution under reduced pressure without heating to a half of the volume the precipitation effected. The precipitates were collected after setting overnight in a refrigerator to give 1.2 g (68%) of **3a**, mp 182—186°. *Anal.* Calcd. for $C_{11}H_{12}N_6O_5 \cdot NH_3 \cdot 1/2H_2O$: C, 39.52; H, 4.49; N, 29.34. Found: C, 39.66; H, 4.73; N, 29.44. UV $\lambda_{max}^{H_2O,pH11}$ nm; 247, 293. $\lambda_{max}^{0.1N\ HCl}$ 224, 283. NMR (D_2O) δ, ppm: 8.53 (s, 1, H-2), 8.38 (s, 1, H-8), 6.08 (d, 1, $J_{1',2'}$= 5.2 Hz, H-1′), 4.1—5.0 (m, 3, H-2′,3′,4′), 3.93 (d, 2, H-5′). IR (KBr); 2160 cm^{-1} (N–C≡N), 1210 cm^{-1} (N–O).

N^1-Methoxy-N^6-cyanoadenosine (4a)——Compound **3a** (3.89 g) and Et_3N (3 ml) were dissolved in 50 ml of DMF, 3 ml of MeI was added, and stirred for 3 hr at room temperature. After evaporation of the solvent the residue was crystallized from hot H_2O to give 2.4 g (74%) of **4a**, mp 107°. *Anal.* Calcd. for $C_{12}H_{14}N_6O_5 \cdot 2/3H_2O$: C, 43.12; H, 4.42; N, 25.14. Found: C, 43.17; H, 4.61; N, 24.98. UV $\lambda_{max}^{H_2O}$ nm (ε): 220 (17800), 286.5 (21000), $\lambda_{max}^{0.1N\ HCl}$ 220.5 (17100), 287 (19900), $\lambda_{max}^{0.1N\ NaOH}$ 244.5 (19200). NMR (DMSO-d_6) δ, ppm: 8.88 (s, 1, H-8), 8.59 (s, 1, H-2), 5.90 (d, 1, H-1′, $J_{1',2'}$=5.2 Hz), 4.11 (s, 3, OCH_3). IR (KBr) 2180 cm^{-1} (N–C≡N). *Rf* 0.80 (solvent B).

2-Amino-N^6-methoxyadenosine (5a)——Compound **4a** (0.5 g) was dissolved in 50 ml of H_2O, 2 ml of 1 N NaOH was added, and kept for 30 min at room temperature while the UV spectral change (λ_{max} 286.5 to 244 nm) was completed. After neutralization of the solution with Dowex 50(H^+) resin and successive addition of EtOH (50 ml) the solution was heated to 70—90° for 3 hr. Evaporation of the solvent afforded slightly yellow solid, which was used for the next step without further purification. *Rf* 0.72 (solvent B);

17) A.M. Serebryanyi and R.M. Mnatsakanyan, *FEBS Letters*, **28**, 191 (1972).
18) The screening tests were carried out at the National Cancer Center, Tokyo, by Drs. F. Fukuoka, and A. Hoshi, which is gratefully acknowledged.

UV $\lambda_{max}^{H_2O}$ nm; 280, $\lambda_{max}^{H^+}$ 256, 294; $\lambda_{max}^{OH^-}$ 284; NMR (DMSO-d_6) δ, ppm: 9.93 (bs, 1, NH–O), 7.79 (s, 1, H-8), 6.54 (bs, 2, NH_2), 5.68 (d, 1, H-1′, $J_{1',2'}$=6 Hz), 3.75 (s, 3, N–CH_3). The same compound was obtained by the treatment of **4a** with triethylamine or DBU in EtOH under reflux for 4 or 1.5 hr, respectively.

2,6-Diamino-9-β-D-ribofuranosylpurine (6a)——Compound **5a** obtained as described above was hydrogenated with Raney Ni in 50% EtOH under atmospheric pressure. After filtration and evaporation of the filtrate, the residue was crystallized from hot H_2O to give 265 mg (60.5% from **4a**) of **6a** as the colorless prisms, mp 234—235.5° (lit.[13] 238—240°). The analytical and UV data were identical with those reported.[13]

6-Thioguanosine (7a)——Compound **5a** (0.5 g) was dissolved in H_2O–pyridine–liquid H_2S (1 : 1 : 3, 50 ml) and heated at 60—80° for 50—70 hr in a steel tube. After vaporization and evaporation of the solvent the residue was taken in hot H_2O, filtered, and decolorized with active charcoal. On cooling the crystalline **7a** (285 mg, 67%) was separated, mp 231—233° (dec.) (lit.[5] 224—227° (dec.)). The analytical values (as the hemi hydrate) and UV spectral characteristics were consonant with the structure **7a**. Compound **7a** was converted to guanosine by treatment with H_2O_2 in 20% NH_4OH as reported.[15]

2′-Deoxyadenosine N¹-Oxide (1b)——2′-Deoxyadenosine (5.0 g) and *m*-chloroperbenzoic acid (2 eq.) were dissolved in 500 ml of 30% aqueous dioxane and stirred for 3 hr at room temperature in the darkness. The excess peracid was degraded by the addition of Pd-charcoal and the filtered solution was evaporated to leave solids. *m*-Chlorobenzoic acid was extracted with ether and the residue was crystallized from H_2O–EtOH to give 3.9 g (74.4%) of **1b**.[16]

2-Imino-6-β-D-2′-deoxyribofuranosyl[1,2,4-oxadiazolo-(3,2-*f*)purine] Hydrobromide (2b)——Treatment of **1b** (267 mg) with 1.1 eq. of cyanogen bromide in 25 ml of MeOH for 2 hr at room temperature afforded **2b** (337 mg, 90.4%) after similar work-up as described in the synthesis of **2a**, mp 159—160° (dec.). *Anal.* Calcd. for $C_{11}H_{13}BrN_6O_4$: C, 35.41; H, 3.51; Br, 21.41 N, 22.52;. Found: C, 35.38; H, 3.53; N, 22.46; Br, 21.62. UV $\lambda_{max}^{H_2O}$ nm, 224, 283. IR (KBr), 1710 cm^{-1} (C=NH).

1-Methoxy-N⁶-cyano-2′-deoxyadenosine (4b)——A mixture of **2b** (1 g) and Et_3N (1 ml) in 5 ml of DMF was stirred for 40 min and 0.5 ml of MeI was added. The stirring was continued for 3.5 hr. After evaporation of the solvent the residue was taken in 100 ml of H_2O, applied to a column of Amberlite XAD-2 (3.5×43 cm), and eluted with the following solvents; H_2O, 5% MeOH, 10% MeOH, 20% MeOH and 30% MeOH (1.0 l, each). The eluates of 20 and 30% MeOH fractions were collected and evaporated to leave the solids and crystallized from hot H_2O to give 620 mg (75.6%) of **4b**, mp 94—97°. *Anal.* Calcd. for $C_{12}H_{14}N_6O_4$: C, 47.05; H, 4.61; N, 27.44. Found: C, 46.77; H, 4.63; N, 27.34. UV $\lambda_{max}^{H_2O}$ nm, 220, 287.5; $\lambda_{max}^{OH^-}$ 243 nm.

2-Amino-N⁶-methoxy-2′-deoxyadenosine (5b)——Compound **4b** (1.0 g) was dissolved in 80 ml of 0.05 N NaOH and kept for 30 min at room temperature. After neutralization of the solution with 1 N HCl the equal volume of EtOH was added, heated at 60° for 4 hr, and evaporated to leave **5b**, *Rf* 0.60 (solvent A). UV $\lambda_{max}^{H_2O}$ 278 nm; $\lambda_{max}^{H^+}$ 255, 294 nm; $\lambda_{max}^{OH^-}$ 287 nm. This compound was used for further reactions without purifications. Treatment of **4b** with Et_3N or DBU in refluxing EtOH resulted in a partial cleavage of the glycosylic linkage of **4b**.

2,6-Diamino-2′-deoxy-9-β-D-ribofuranosylpurine (6b)——Catalytic hydrogenation of **5b** (200 mg) with Raney Ni in 50% EtOH followed by the work-up as described in the preparation of **6a** afforded **6b** (97 mg, 54.6%), crystallized from hot H_2O, mp 146—148° (lit.[6b] 176°, dec.). *Anal.* Calcd. for $C_{11}H_{14}N_6O_3 \cdot 1/3H_2O$: C, 44.11; H, 5.43; N, 30.87. Found: C, 43.99; H, 5.45; N, 30.75. UV spectra of **6b** were identical with those reported[19] ($\lambda_{max}^{H_2O}$ 215, 256, 280 nm).

2′-Deoxy-6-thioguanosine (7b)——Compound **5b** obtained from 1 g of **4b** was taken in a solution of H_2O (15 ml)–pyridine (20 ml)–liquid H_2S (30 ml) and kept for 184 hr at 40—45° in a steel tube. After vaporization and evaporation of the solvent the residue was crystallized from hot H_2O to give crude **7b** (500 mg) which was recrystallized from H_2O to furnish 308 mg (34.6%) of **7b**, mp>190°, *Rf* 0.28 (solvent B): *Anal.* Calcd. for $C_{10}H_{13}N_5O_3S \cdot 1/2H_2O$: C, 41.00; H, 4.79; N, 23.97; S, 10.96. Found: C, 40.83; H, 4.70; N, 23.73; S, 10.73. UV $\lambda_{max}^{H_2O}$ 223 (sh), 255, 341 nm. The survey of the residue of the reaction with paper chromatography revealed a presence of trace amount of 6-thioguanine (*Rf* 0.15, solvent B) together with unreacted **5b** in ~30%.

Preparation of N¹-Oxides of Adenine Nucleotides (General Procedures)——Adenine nucleotide was dissolved in 50% EtOH as the neutralized sodium salt. To this solution was added 2 eq. of *m*-chloroperbenzoic acid and it was kept for 3 days at room temperature while pH of the solution was maintained at 7. After evaporation of the solvent the residue was taken in H_2O, filtered, and extracted with ether. The aqueous layer was concentrated to a small volume and hot EtOH was added to effect slight turbidity, and kept overnight in a refrigerator. Compound **8a** (2.63 g, 50%) was obtained from 5 g of AMP as the crystalline form. Compound **8b** (722 mg, 70%) was obtained from dAMP (1 g). The N¹-oxide (**8c**) of araAMP was also prepared by this method from araAMP[19] as a solid form.

6-Thioguanosine 5′-Phosphate (9a)——Compound **8a** (2 Na salt, 1.2 g) was treated with BrCN (990 mg) in 40 ml of 50% MeOH at room temperature for 4 hr. The solution was concentrated to a half of its volume,

19) Prepared by the method of Ikehara and co-workers: M. Ikehara, and M. Kaneko, *Tetrahedron*, **26**, 4251 (1970) and *Chem. Pharm. Bull.* (Tokyo), **18**, 2401 (1970), M. Ikehara and S. Uesugi, *Tetrahedron*, **28**, 3687 (1972).

added 2 ml of Et_3N, and evaporated to dryness. The residue was dissolved in H_2O–DMF (1: 1, 40 ml) to which was added Et_3N (1 ml) and MeI (1.5 ml) and stirred for 22 hr at room temperature. The concentrated sirup of the above mixture was taken in 100 ml of H_2O and applied to a column of Amberlite XAD-4 (3.7×28 cm). The column was washed with H_2O (2.0 l), 5% MeOH (400 ml), and eluted the N^1-methoxy derivative with 10% MeOH (1.0 l), 30% MeOH (500 ml), and 50% MeOH (500 ml), successively. The aqueous MeOH fractions were combined, concentrated to a sirup, and dissolved in 35 ml of 0.14 N NaOH (pH 11.7). After 30 min the solution was neutralized with 1 N HCl, added 40 ml of EtOH, and kept for 1 hr at 80°. The solution was concentrated to leave sirupy material which was taken in H_2O–pyridine–liquid H_2S (1: 1: 3, 50 ml) and heated at 70° for 75 hr in a steel tube. After vaporization and evaporation of the solvent the residue was taken in H_2O, and applied to a column of DEAE–cellulose (HCO_3^-, 3.5×29 cm). The product (**9a**) was obtained by a linear gradient elution with H_2O (2.0 l) and 0.3 M NH_4HCO_3 (2.0 l) at the fraction numbers 133—181 collected 18 ml per tube, OD unit at 341 nm; 18000. The combined eluates were concentrated to a small volume and lyophillized to leave 268 mg (27.3%) of **9a** as ammonium salt, UV $\lambda_{max}^{H_2O}$ 258, 341 nm. Treatment of **9a** with H_2O_2–NH_4OH at room temperature for 3 hr gave guanosine 5′-phosphate in 67% yield as checked by DEAE-cellulose chromatography and paper electrophoresis.

2′-Deoxy-6-thioguanosine 5′-Phosphate (9b)——Compound **8b** (2Na salt, 1.11 g) was treated with BrCN (1.0 g) in 50% MeOH at room temperature for 1 hr. The solution was neutralized by addition of Et_3N (2 ml), concentrated, and the residue was treated with MeI (1 ml with 0.5 ml of Et_3N) in aq. DMF (30 ml) overnight at room temperature. The concentrate of this solution was taken in H_2O and applied to a column of DEAE–cellulose (HCO_3^-, 2×40 cm) and eluted with a linear gradient of H_2O (2000 ml) and 0.3 M Et_3NH^+-HCO_3^- (2000 ml). The fractions containing 1-methoxy-N^6-cyano-dAMP (No. 101—180, one fraction 15 ml) were combined, concentrated, and taken in 0.5 M $Et_3NH^+HCO_3^-$ (50 ml) and EtOH (50 ml). After setting for 2 days the solution was concentrated and the residue was kept for 8 days at 40° in H_2O–pyridine–liquid H_2S (1: 2: 2, 50 ml) under sealed condition. After removal of the solvent the residue was dissolved in 30 ml of H_2O and applied to a column of DEAE-cellulose (HCO_3^-, 2.0×40 cm). The column was washed with H_2O (1000 ml) and eluted with a linear gradient of H_2O (1000 ml) and 0.3 M NH_4HCO_3 (1000 ml) into a fractions of 10 ml. Fractions of the main peak (No. 70—109, O.D. unit at 341 nm, 13800) were combined, concentrated, and lyophilyzed to leave 276 mg (22.7%) of **9b**, UV $\lambda_{max}^{H_2O}$ 258, 341 nm. *Anal.* Calcd. for $C_{10}H_{14}N_5O_7PS \cdot NH_3 \cdot 3H_2O$: C, 27.65; H, 5.34; N, 19.35. Found: C, 27.94; H, 5.02; N, 18.99. Oxidative hydrolysis of **9b** by H_2O_2 followed by separation with DEAE-cellulose chromatography afforded 2′-deoxyguanosine 5′-phosphate in 57% yield. *Rf* 0.16 (solvent C).

9-β-D-Arabinofuranosyl-6-thioguanine 5′-Phosphate (9c)——The crude **8c** prepared from 3 mmol of araAMP was taken in 50 ml of 40% MeOH and BrCN (1.09 g) was added. After similar work-up as described above the product was methylated with MeI and applied on a column of Amberlite XAD-4 (3.5×38 cm), developed with aq. MeOH, and the fractions containing N^1-methoxy derivative were collected, and concentrated. The residue was taken in H_2O (20 ml), made alkaline by 4 ml of 1 N NaOH, and kept for 30 min. The solution was neutralized by 1 N HCl, added equal volume of EtOH, and kept for 40 min at 80—90°. After removal of the solvent the residue was taken in H_2O–pyridine–liquid H_2S (1: 2: 3, 60 ml) and kept for 64 hr at 65° in a steel tube. The evaporated residue was applied on a column of DEAE-cellulose (HCO_3^-, 3.7×33 cm) and eluted with a linear gradient of H_2O (2000 ml) and 0.4 M NH_4HCO_3 (2000 ml) in 18 ml fractions. From the eluents (No. 90—150), after a rechromatography in a same condition, analytically pure **9c** was obtained by the lyophyllization, 180 mg (14.5%), 9300 O.D.U. at 341 nm. *Anal.* Calcd. for $C_{10}H_{14}O_7N_5PS \cdot NH_3 \cdot 1.5H_2O$: C, 28.30; H, 4.75; N, 19.80. Found: C, 28.16; H, 4.78; N, 19.62. UV $\lambda_{max}^{H_2O}$, 256, 340 nm. The separate experiment in which the conditions for the sulfhydrolysis were 70° for 86 hr, resulted in an increase of the yield up to 60%. Oxidative desulfurization of **9c** afforded 9-β-D-arabinofuranosylguanine 5′-phosphate (37%; *Anal.* Calcd. for $C_{10}H_{14}N_5O_8P \cdot NH_3 \cdot H_2O$: C, 30.16; H, 4.81; N, 21.10. Found: C, 30.25; H, 4.80; N, 21.20. UV $\lambda_{max}^{H_2O}$, 252, 270 (sh) nm), after purification through DEAE-cellulose chromatography and isolation by the lyophyllization.

N^6-Carbamoyladenosine (10a)——Compound **2a** (500 mg) in 40 ml of 50% EtOH was hydrogenated over Pd-charcoal under ordinary pressure and temperature overnight. After removal of the catalyst the filtrate was concentrated and the residue was crystallized from H_2O to give 208 mg (53%) of **10a**, mp 169—172°; UV $\lambda_{max}^{H_2O}$ nm (ε): 210 (24300), 267 (21400), 273.5 (17800) sh; $\lambda_{max}^{0.1N\ HCl}$, 211 (23800), 274.5 (21000); $\lambda_{max}^{0.1N\ NaOH}$, 279 (14400) sh, 293 (21300). *Anal.* Calcd. for $C_{11}H_{14}N_6O_5$: C, 42.58; H, 4.55; N, 27.09. Found: C, 42.50; H, 4.59; N, 27.23. Treatment of **2a** or **3a** in 50% EtOH with a few drops of acetic acid for 3 hr gave a product (*Rf* 0.80 in solvent A, UV λ_{max} 240 and 296). Hydrogenation of this solution afforded **10a**. Hydrogenation of **3a** in 50% EtOH over Pd-charcoal afforded, after 3 days, **10a**.

N^6-Carbamoyl-2′-deoxyadenosine (10b)——Compound **2b** (200 mg) in 20 ml of 50% EtOH was hydrogenated over Pd-charcoal as above and colorless crystals were obtained by the similar work-up, mp140—143°, resolidified and decomposed at ~170°, UV $\lambda_{max}^{H_2O}$ 210, 267, 274 (sh) nm.

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