2-(*p*-Nitrophenyl)-2′-deoxyadenosine, a new type of mutagenic nucleoside

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Received November 28, 1989, Revised and Accepted January 4, 1990

ABSTRACT

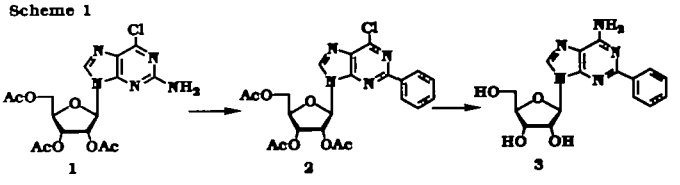
A crude preparation of 2-phenyladenosine was found to be mutagenic in the Ames Salmonella assay. In the purification of this preparation, it was revealed that 2-phenyladenosine itself was nonmutagenic but that 2-(*m*- and *p*-nitrophenyl)-adenosines (5m,p) contaminating the sample were the mutagenic principles. A structure-activity relationship study was carried out, and it was found that 5p, 2-(*p*-nitrophenyl)-adenine (7p), and 2-(*p*-nitrophenyl)-2′-deoxyadenosine (15p) were strongly mutagenic toward *S. typhimurium* TA98 and TA100 without metabolic activation, the potency being in the order 15p > 7p > 5p. The potency of 15p in TA98 was one order of magnitude greater than that of 4-nitroquinoline N-oxide. 15p also showed mutagenicity in the mouse cell line FM3A in culture.

INTRODUCTION

Some nucleosides such as 5-bromodeoxyuridine[2], N^4-hydroxycytidine [3–5], and N^4-aminocytidine[6], and nucleobases such as 2-aminopurine[2] and 2-amino-6-hydroxyaminopurine[7] have been known to be mutagenic. Most of these compounds act actually as promutagens. By cellular metabolism, these compounds are converted into corresponding 2′-deoxynucleoside 5′-triphosphates, which would be incorporated into DNA chains during replication and induce mutations. Elucidation of the mechanism of these phenomena is important for understanding mutagenesis and even carcinogenesis.

We have tested about 100 modified nucleosides for mutagenicity by the reversion of histidine dependent Salmonella system (*Salmonella typhimurium* TA98 for detection of mutagens causing frameshifts and TA100 for those causing base-pair changes)[8] in the absence or presence of rat-liver 9000×g supernatant fraction (S9). Among these nucleoside derivatives, we have found a crude preparation of 2-phenyladenosine (**3**) to be mutagenic to TA98 as well as TA100 without metabolic activation by S9. This nucleoside was synthesized[9] by the route shown in scheme 1. 2-Amino-6-chloropurine riboside (**1**) was treated with an excess of isopentyl nitrite in benzene in the presence of copper(I) oxide to afford a 6-chloro-2-phenylpurine riboside (**2**). The crude product of **2** was then heated with methanolic ammonia in a sealed tube at 70° to give a crude **3**. We describe here the structure determination of the minor mutagenic products in this sample and the results of studies on the structure-activity relationship of the mutagenicity. Preliminary accounts of this work have appeared [10,11].

Scheme 1



RESULTS and DISCUSSION

In order to determine the chemical structure of the mutagenic compounds, the crude sample was purified with high performance liquid chromatography (HPLC) using a reverse phase column (Fig.1). The purified 2-phenyladenosine (**3**; peak 1) showed no mutagenicity in TA98 and TA100. Instead, the mutagenic activity toward both strains was almost entirely concentrated in one of the minor peaks (peak 3), which seemed to consist of more than one compound. From about 200 mg of crude **3**, 0.8 mg of a mixture of the minor nucleosides was isolated by using a semi-preparative column. The ^{1}H-NMR spectra of this sample from peak 3 showed that the mixture consisted of at least two components and the phenyl ring at the 2-position of adenine moiety was mono-substituted (Fig.2). Mass spectral data of this material showed a molecular ion peak at *m/z* 388; the number corresponding to the molecular weight of 2-phenyladenosine minus 1 plus NO_2 (46). From these results, the compounds of peak 3 appeared to be a mixture of isomeric 2-(nitrophenyl)-adenosines.

To determine the position of the nitro group in the phenyl ring, we synthesized 2-(*o*-, *m*- and *p*-nitrophenyl)-adenosines (**5o,m,p**) in an unambiguous manner[12]. Treatment of 4-cyano-5-aminoimidazole riboside (**4**)[11], which was obtained

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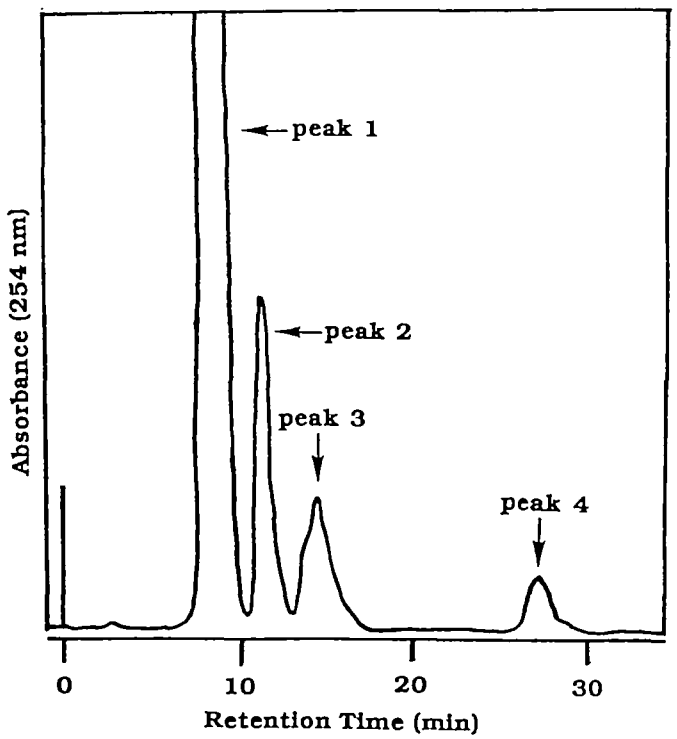
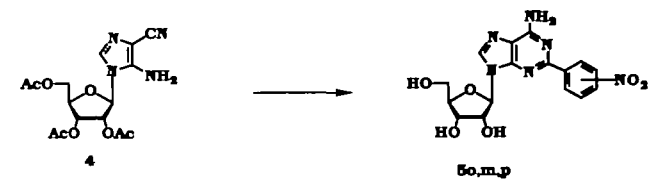


Figure 1. HPLC chromatogram of the crude sample of 3 Retention times of peak 1, 2, 3, and 4 were 8.2, 11.3, 14.3, and 27.1 min, respectively.

readily from commercially available 5-aminoimidazole-4-carboxamide riboside in two steps, with *o*-, *m*-, or *p*-nitrobenzonitriles in methanolic ammonia using a steel tube at 170°, afforded desired 2-(*o*-, *m*-, or *p*-nitrophenyl)-adenosines (**5o,m,** and **p**) in yields of 20, 51, and 71%, respectively (Scheme 2). The 2-(*m*- and *p*-nitrophenyl)-adenosines (**5m** and **p**) showed retention times (13.7 and 14.0 min, respectively) almost the same as that of peak 3 (14.3 min). The retention time of 2-(*o*-nitrophenyl)-adenosine (**5o**) was found to be very short (4.7 min). ^{1}H-NMR spectral data (the aromatic region) of peak 3 and other derivatives are shown in Fig.2 and it is evident that peak 3 contains 5m and 5p in a ratio of 1:3.

Scheme 2



We next examined the mutagenic activity of these nucleosides. Both **5m** and **5p** showed mutagenic activity with either TA98 and TA100 strains dose-dependently with or without addition of S9 (Fig.3). It is interesting to note that the *m*-nitro analog (**5m**) is selectively active to TA100. In contrast, the *p*-nitro derivative (**5p**) showed potent mutagenicity to TA98 and at higher concentrations, cell killing occurred with TA100. On the other hand, the *o*-nitro derivative (**5o**) showed no activity to either

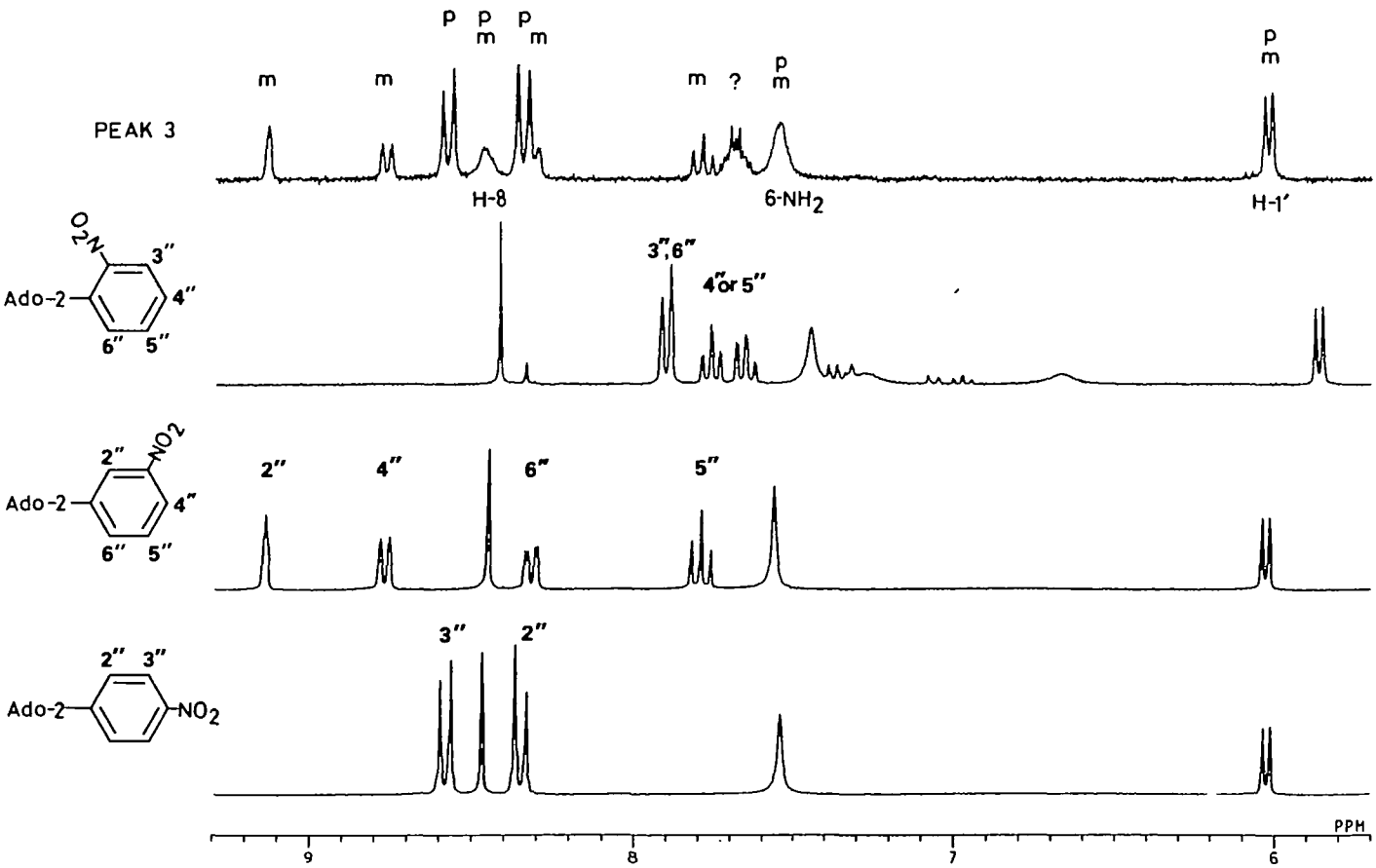


Figure 2. 270 MHz ^{1}H-NMR spectrum of peak 3, 5o, 5m, and 5p in DMSO-d_6.

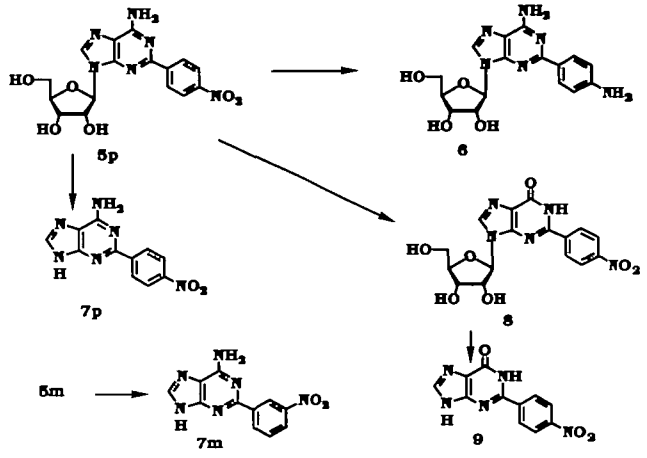
strains. From these results, it is clear that during the diazotization of 1 in benzene with isopentyl nitrite, the intermediate(s) was nitrated to produce 2-(*m*- and *p*-nitrophenyl)-adenosines as minor components, which caused the mutagenicity from crude **3**.

Some aromatic nitro compounds are mutagenic. For example, 4-nitroquinoline N-oxide and dinitropyrenes have been shown to have potent mutagenicity and carcinogenicity. Nitropyrene and dinitropyrenes especially are known to be common mutagenic environmental contaminants. Thus, it is interesting to study whether the mutagenicity of **5m** and **5p** arises from the nucleosidic character or whether they are behaving just as aromatic nitro compounds.

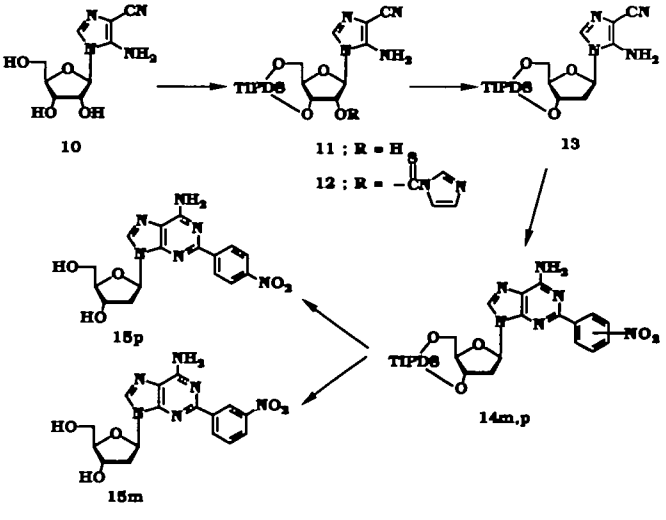
To clarify further the structural requirements for the mutagenicity, several nucleoside and base analogs were prepared. 2-(*p*-Nitrophenyl)-adenosine (**5p**) was deaminated with sodium nitrite in aqueous acetic acid at room temperature for 4 days to give the inosine derivative (**8**) in 71% yield. The nitro group in compound **5p** was reduced by catalytic hydrogenation with 10% palladium on carbon, and the anilino adenosine (**6**) was obtained in 50% yield. Nucleosides **5m, 5p,** and **8** were treated with 2N hydrochloric acid to afford the corresponding 2-(*m*- and *p*-nitrophenyl)-adenines (**7m** and **7p**) and 2-(nitrophenyl)-hypoxanthine (9) (Scheme 3). As sugar analogs of **5m** and **5p**, 2-(*m*- and *p*-nitrophenyl)-2′-deoxyadenosines (**15m** and **15p**) were next synthesized. 5-Amino-4-cyanoimidazole riboside (**10**) was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) in *N,N*-dimethylformamide (DMF) to give the corresponding 3′,5′,*O*-tetraisopropyldisiloxanyl (TIPDS) derivative (**11**) in 74% yield after purification over a silica gel column[13]. Compound **11** was then treated with *N,N*-thiocarbonyldiimidazole in DMF to afford the 2′-thiocarbonyl derivative (**12**) in a crystalline form (72% yield). **12** was subjected to radical-deoxygenation with tributyltin hydride[14] in toluene, giving the desired 2′-deoxynucleoside (**13**) exclusively. Compound **13** was then cyclized with *m*- and *p*-nitrobenzonitriles in ethanolic ammonia to afford the 2-(*m*- and *p*-nitrophenyl)-deoxyadenosine derivatives (**14m** and **14p**) in 48% and 84% yield, respectively. The nucleosides were deblocked with tetrabutylammonium fluoride in tetrahydrofuran to furnish 2-(*m*- and *p*-nitrophenyl)-2′-deoxyadenosines (**15m** and **15p**), respectively, in good yields. In contrast, when the same radical deoxygenation was applied to the 2′-thiocarbonyl-2-(*p*-nitrophenyl)-adenosine derivative, only a complex mixture was obtained (data not shown).

The mutagenicity of the nucleosides and bases toward *S. typhimurium* TA98 and TA100 in the absence or presence of S9 is summarized in Table 1. Data for known mutagens, N^4-aminocytidine, 4-nitroquinoline N-oxide (4NQO) and benzo[a]pyrene (BP), are also shown for comparison. Among

Scheme 3



Scheme 4



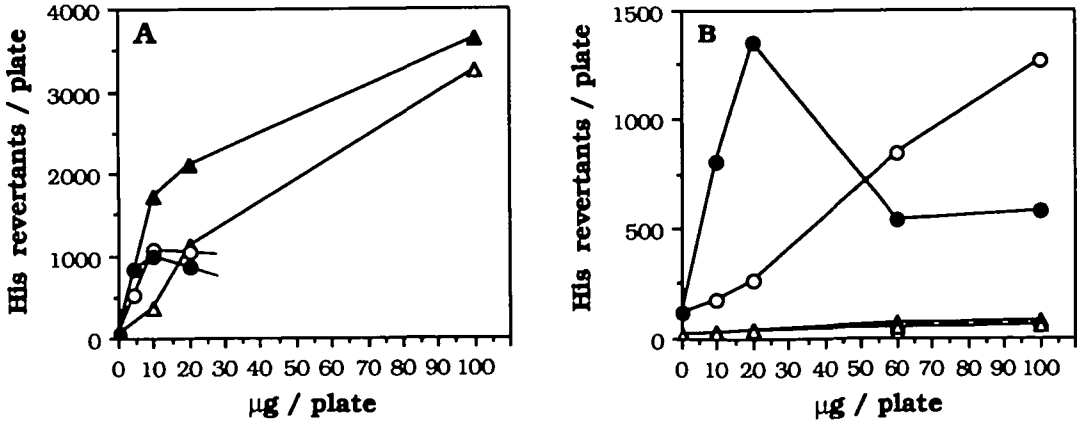


Figure 3. Mutagenicity of 5p (A) and 5m (B) on *S. typhimurium* TA98 and TA100. Circle symbols, TA100; triangle symbols, TA98. Solid symbols, −S9: open symbols, +S9.

Table 1. Mutagenicity of 2-(nitrophenyl)-adenines on *S. typhimurium*[a]

Bacteria	TA98		TA100	
Compds	+S9	−S9	+S9	−S9
5p	820	1700	940	870
5m	4	7	51	680
5o	0	0	0	0
6	79	24	0	8
8	193	546	224	575
7p	1000	30000	1100	40000
7m	0	0	100	900
9	40	1000	0	1300
15p	67000	117000	16000	42000
15m	58	200	180	200
N^4-aminocytidine	0	0	3400	1300
4NQO	0	18000	0	80000
BP	1200	0	2400	0

[a]Net number of revertants/10 μg of compound per plate

the *p*-, *m*-, or *o*-nitrophenyladenosine derivatives (**5p,m,o**), the *p*-nitrophenyl derivative (**5p**) is the most potent. The *m*-nitrophenyl derivative (**5m**) is active only to TA100 without metabolic activation. On the other hand, *o*-nitrophenyl derivative (**5o**) is inactive with either strains. The *o*-nitrophenyl ring cannot be coplanar with the adenine ring because of steric repulsion between the *o*-nitro group and the pyrimidine moiety of adenine ring. This is probably one reason why **5o** did not show mutagenic activity. The anilino derivative (**6**) is almost inactive. The inosine analog 8 shows weaker activity than the corresponding adenosine analog **5p**. 2-(*p*-Nitrophenyl)-adenine (**7p**) is found to be much more potent than the nucleoside, whereas the 2-(*m*-nitrophenyl)-adenine (**7m**) is less mutagenic but selective toward TA100. Again, the hypoxanthine derivative (9) is, as expected, less potent than the adenine derivative. Surprisingly, 2-(*p*-nitrophenyl)-2′-deoxyadenosine (**15p**) is the most potent among 2-nitrophenyladenines in the reversion of TA98 and TA100, and the activity toward TA98 is one order of magnitude greater than that of 4-nitroquinoline N-oxide. Consistent with the weak mutagenicity of **5m, 15m** shows only weak activity. These data indicate that the *p*-nitrophenyl analog is the most potent and the adenine analogs are always more active than the hypoxanthine analogs. The order of mutagenicity is the 2′-deoxynucleosides, the bases and the ribonucleosides.

It is important to elucidate the role of the nitro group for the mutagenicity. When **5p** was tested towards nitroreductase deficient strains (TA98/1,8-DNP and TA98NR), none of the strains (without addition of S9) showed significant number of his^+ revertants (82 and 54 revertants at the concentration of 20 μg/plates, respectively) while TA98 gave 2200 revertants under the same conditions. Compound **7p** (1 μg/plate) showed a low activity to TA100/1,8-DNP (570 revertants) and TA100NR (160 revertants) while TA100 gave 3230 revertants. Although **15p** did not show any activity toward TA100/1,8 DNP and TA100NR at 0.5 μg/plate. TA100 formed 1550 revertants. Thus, it is obvious that the nitro group is essential for the mutagenicity.

The mutagenic activity of **15p** to FM3A cells was also investigated by measuring mutations to ouabain resistance. As shown in Table 2, **15p** exhibited mutagenicity with a potency close to that of N^4-aminocytidine[15]. Since EC_{50} of **15p** for FM3A cells was 3.2×10^{-5} M, this reagent showed no cytotoxicity at the concentrations tested for the mutagenicity.

From these studies, one can speculate that 2-(*p*-nitrophenyl)-adenine (7p) and the 2′-deoxy analog (**15p**), like other nitro aromatic compounds, would be reduced to the hydroxylamino derivatives by the action of nitroreductase, and that these activated bases would react with DNA to form adducts. Also, the hydroxylamino derivatives may be further activated by acylation and/or sulfation for the reaction with DNA. On the other hand, in the case of the 2′-deoxy compound (**15p**), it might be phosphorylated and incorporated into DNA after or before activation of the nitro group. The detailed mechanism of the mutagenic action of these compounds is a subject of further studies.

Table 2. Mutation assay in FM3A cells

Concentration [M]	No. of ouabain resistant mutants scored/total No. of original cells	Mutation frequency ($oua^s \Rightarrow oua^r$)[a]	Colony-forming ability (%) ±S.D (n=15)
2-(*p*-Nitrophenyl)-2′-deoxyadenosine (15p)			
Control:0	$0/1.5\times10^7$	0	84.9 ± 9.8
5.4×10^{-6}	$31/1.4\times10^7$	2.5×10^{-6}	90.3 ± 8.8
1.0×10^{-5}	$68/1.5\times10^7$	5.3×10^{-6}	85.1 ± 12.4
N^4-Aminocytidine[b]			
3.0×10^{-5}	$87/9.5\times10^6$	1.1×10^{-5}	86

[a](Number of mutants) / (number of original cells×colony-forming ability)
[b]Data taken from ref. 15.

EXPERIMENTAL

Melting points were determined on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on a JEOL FT100FT or FX-270FT spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Electron impact mass spectra (Mass) were measured on a JEOL D-300 spectrometer. TLC was performed on Merck Kieselgel F254 precoated plates. Silica gel used for column chromatography was YMC gel 60A (70−230 mesh). HPLC analyses were performed on a JASCO TRI ROTAR-V system using Shodex ODS pak F-411 (4.6×150 mm) with 40% aqueous methanol as an eluting solvent at a flow rate of 1 ml/min.

2-(p-Nitrophenyl)-adenosine (**5p**). A mixture of 2′,3′,5′-tri-*O*-acetyl-5-amino-4-cyanoimidazole riboside (**4**; 2.0 g, 5.5 mmol) and *p*-nitrobenzonitrile (2.0 g, 13.5 mmol) in methanolic ammonia (saturated at 0°C, 30 ml) was heated at 170°C in a sealed steel tube. After 6 h, the tube was cooled to room temperature, opened, and the resulting precipitates were collected by filtration (**5p**; 1.5 g, 71%). Analytical sample was obtained by recrystallization from aqueous ethanol, mp 252−253°C. Mass *m/z*: 388 (M^+). ^{1}H-NMR (DMSO-d_6): δ 3.62 (m, 2H, 5′,5″-H), 3.97 (m, 1H, 4′-H), 4.21 (m, 1H, 3′-H), 4.68 (m, 1H, 2′-H), 5.03 (t, 1H, 5′-OH), 5.25 (d, 1H, 2′- or 3′- OH), 5.50 (d, 1H, 3′- or 2′-OH), 6.02 (d, 1H, 1′-H, $J_{1',2'}$ = 5.9 Hz), 7.54 (brs, 2H, 6-NH_2), 8.35 (d, 2H, 2″ and 6″, $J_{2'',3''} = J_{5'',6''}$ = 8.8 Hz), 8.47 (s, 1H, 8-H), 8.58 (d, 2H, 3″ and 5″H). *Anal.* Calcd for $C_{16}H_{16}N_6O_6$: C, 49.49; H, 4.15; N, 21.64. Found: C, 49.34; H, 4.09; N, 21.58.

2-(m-Nitrophenyl)-adenosine (**5m**). A mixture of compound **4** (1.0 g, 2.7 mmol) and *m*-nitrobenzonitrile (1.0 g, 6.8 mmol) in methanolic ammonia (30 ml) in a sealed steal tube was heated at 170°C for 8 h. The resulting solution was evaporated to dryness

and the residue was dissolved in methanol. Silica gel was added to the methanol solution, and the mixture was evaporated to dryness. The residue was placed on a silica gel column, which was eluted with chloroform containing 5% ethanol then eluted with chloroform containing 10% ethanol. From the latter fractions, the desired nucleoside was obtained and the residue was crystallized from aqueous ethanol to give **5m** (541 mg, 51%); mp 235−236°C (sintering from 223°C). Mass *m/z*: 388 (M^+). ^{1}H-NMR (DMSO-d_6): δ 3.60 (m, 2H, 5′, 5′′-H), 3.98 (m, 1H, 4′-H), 4.21 (m, 1H, 3′-H), 4.68 (m, 1H, 2′-H), 5.03 (t, 1H, 5′-OH), 5.25 (d, 1H, 2′- or 3′-OH), 5.50 (d, 1H, 3′- or 2′-OH), 6.03 (d, 1H, 1′-H, $J_{1',2'}$ = 6.3 Hz), 7.57 (brs, 2H, 6-NH_2), 7.79 (t, 1H, 5′′-H, $J_{4'',5''}$ = 8.1 Hz), 8.32 (dd, 1H, 6′′-H, $J_{4'',6''}$ = 1.1 Hz, $J_{5'',6''}$ = 8.1 Hz), 8.45 (s, 1H, 8-H), 8.77 (dd, 1H, 4′′-H, $J_{2'',4''}$ = 1.5 Hz, $J_{4'',5''}$ = 8.1 Hz), 9.14 (t, 1H, 2′′-H). *Anal.* Calcd for $C_{16}H_{16}N_6O_6$: C, 49.49; H, 4.15; N, 21.64. Found: C, 49.28; H, 4.30; N, 21.41.

2-(o-Nitrophenyl)-adenosine (**5o**). A mixture of compound **4** (1.0 g, 2.7 mmol) and *o*-nitrobenzonitrile (1.0 g, 6.8 mmol) in methanolic ammonia (30 ml) in a sealed steel tube was heated at 170°C for 8 H. The resulting solution was vaporated to dryness and the residue was dissolved in methanol. Silica gel was added to the methanol solution, and the mixture was evaporated to dryness. The residue was placed on a silica gel column, which was eluted with chloroform containing 5% ehtanol then eluted with chloroform containing 10% ethanol. From the latter fractions, the desired nucleoside (**5o**) was obtained as a foam (214 mg, 20%). Mass *m/z*: 388 (M^+). ^{1}H-NMR (DMSO-d_6): δ 3.60 (m, 2H, 5′,5′′-H), 3.93 (m, 1H, 4′-H), 4.13 (m, 1H, 3′-H), 4.60 (m, 1H,2′-H), 5.08 (t, 1H, 5′-OH), 5.17 (d, 1H, 2′- or 3′-OH), 5.42 (d, 1H, 3′- or 2′-OH), 5.86 (d, 1H, 1′-H, $J_{1',2'}$ = 5.9 Hz), 7.45 (brs, 2H, 6-NH_2), 7.67 (dt, 1H, 4′′- or 5′′-H, $J_{3'',4''}$ = $J_{4'',5''}$ = $J_{5'',6''}$ = 7.7 Hz, $J_{3'',5''}$ = $J_{4'',6''}$ = 1.5 Hz), 7.76 (dt, 1H, 5′′- or 4′′-H), 7.90 (d, 2H, 3′′- and 6′′-H), 8.42 (s, 1H, 8-H).

2-(p-Aminophenyl)-adenosine (**6**). 10% Palladium on carbon (30 mg) was added to a solution of 5p (250 mg) in a mixture of ethanol (10 ml) and 50% aqueous acetic acid (20 ml). The mixture was stirred at room temperature under H_2 atmosphere, until a theoretical amount of H_2 was absorbed. The reaction mixture was passed through a celite pad and the eluent was evaporated to dryness. The residue was crystallized from water to give **6** (115 mg, 50%); mp 229−231°C. Mass *m/z*: 358 (M^+). ^{1}H-NMR (DMSO-d_6 + D_2O): δ 3.55 (dd, 1H, 5′-H, $J_{4',5'}$ = 4.0 Hz, $J_{5',5''}$ = 11.7 Hz), 3.67 (dd, 1H, 5′′-H, $J_{4',5''}$ = 4.0 Hz), 3.93 (dd, 1H, 4′-H), 4.20 (dd, 1H, 3′-H), 4.70 (dd, 1H, 2′-H), 5.94 (d, 1H, 1′-H, $J_{1',2'}$ = 6.2 Hz), 6.60 (d, 2H, phenyl, J = 8.4 Hz), 8.04 (d, 2H, phenyl, J = 8.4 Hz), 8.28 (s, 1H, 8-H). *Anal.* Calcd for $C_{16}H_{18}N_6O_4$: C, 53.63; H, 5.06; N, 23.45. Found: C, 53.73; H, 5.16; N, 23.16.

2-(p-Nitrophenyl)-inosine (**8**). Sodium nitrite (280 mg, 4.1 mmol) was added to a suspension of **5p** (250 mg, 0.64 mmol) in 50% aqueous acetic acid at 0°C. The reaction mixture was stirred for 4 days at room temperature under a tight sealing. The resulting precipitate was collected by filtration and the solid was crystallized from 50% aqueous ethanol to afford **8** (142 mg, 71%); mp 180−183°C. Mass *m/z*: 257 $(B+H)^+$. ^{1}H-NMR (DMSO-d_6 + D_2O): δ 3.56 (dd, 1H, 5′-H, $J_{4',5'}$ = 3.8 Hz, $J_{5',5''}$ = 11.5 Hz), 3.66 (dd, 1H, 5′′-H, $J_{4',5''}$ = 3.8 Hz), 3.96 (dd, 1H, 4′-H), 4.17 (dd, 1H, 3′-H), 4.57 (dd, 1H, 2′-H), 5.98 (d, 1H, 1′-H, $J_{1',2'}$ = 6.0 Hz), 8.37 (m, 4H, phenyl), 8.42 (s, 1H, 8-H). *Anal.* Calcd for $C_{16}H_{15}N_5O_7 \cdot 0.5\ H_2O$: C, 48.24; H, 4.05; N, 17.58. Found: C, 48.54; H, 3.90; N, 17.45.

2-(p-Nitrophenyl)-adenine (**7p**). A suspension of **5p** (250 mg) in 2N HCl (20 ml) was heated at 60°C for 1 day with stirring. The resulting crystals were collected by filtration (**7p**; 154 mg, 87% as hydrate); mp >300°C. Mass *m/z*: 256 (M^+). *Anal.* Calcd for $C_{11}H_8N_6O_2 \cdot H_2O$: C, 48.18; H, 3.68; N, 30.64. Found: C, 47.81; H, 3.36; N, 30.34.

2-(m-Nitrophenyl)-adenine (**7m**). A suspension of **5m** (120 mg) in 2N HCl (10 ml) was heated at 60°C for 1 day with stirring. Ethanol (15 ml) was added to the mixture, which was heated until the insoluble materials were dissolved. The resulting solution was filtered and from the filtrate crystalline materials were obtained (**7m**; 75 mg, 95%); mp >300°C. Mass *m/z*: 256 (M^+). *Anal.* Calcd for $C_{11}H_8N_6O_2$: C, 51.56; H, 3.15; N, 32.80. Found: C, 51.53; H, 3.08; N, 32.83.

2-(p-Nitrophenyl)-hypoxanthine (**9**). A suspension of 8 (68 mg) in 2N HCl (10 ml) was heated at 60°C for 1 day with stirring. The resulting crystals were collected by filtration to give **9** (36 mg, as a hemihydrate); mp >300°C. Mass *m/z*: 257 (M^+). *Anal.* Calcd for $C_{11}H_7N_5O_3 \cdot 0.5\ H_2O$: C, 49.62; H, 3.03; N, 26.31. Found: C, 49.24; H, 3.07; N, 26.01.

5-Amino-1-(3′,5′-O-TIPDS-β-D-ribofuranosyl)-imidazole-4-carbonitrile (**11**). $TIPDSCl_2$ (6.92 g, 1.1 equiv.) was added to a solution of **10** (4.8 g, 20 mmol) in dry pyridine (50 ml) at 0°C. The mixture was stirred for 4.5 h at room temperature. Ice water (ca. 5 ml) was added to the mixture and the mixture was concentrated by evaporation. This residue was coevaporated several times with aqueous ethanol. The residue was dissolved in ethyl acetate (ca. 100 ml) and the mixture was washed with water (20 ml×3). The organic layer was dried (Na_2SO_4) and evaporated to dryness to leave a powder, which was crystallized from hexane to afford **11** (7.25 g, 75%); mp 163−164°C. Mass *m/z*: 482 (M^+). ^{1}H-NMR ($CDCl_3$ + D_2O): δ 1.09 (m, 28H, isopropyl), 4.06 (m, 4H, 3′,4′,5′,5′′-H), 5.55 (d, 1H, 1′-H), 7.16 (s, 1H, 2-H). *Anal.* Calcd for $C_{21}H_{38}N_4O_5Si_2$: C, 52.25; H, 7.93; N, 11.61. Found: C, 52.20; H, 7.94; N, 11.54.

5-Amino-1-(3′,5′-O-TPIDS-2′-O-thiocarbonylimidazoyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**12**). A solution of compound **11** (1.45 g, 3 mmol) and *N,N′*-thiocarbonyldiimidazole (90% pure; 1.19 g, 2 equiv.) in dry DMF (15 ml) for 22 h at room temperature. The mixture was diluted with ethyl acetate (100 ml) and was washed with water (20 ml×4). The separated organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was crystallized from ethanol to give **12** (1.29 g, 72%); mp 219−220°C. Mass *m/z*: 592 (M^+). ^{1}H-NMR ($CDCl_3$): δ 1.11 (m, 28H, isopropyl), 4.13 (m, 3H, 4′,5′,5′′-H), 4.49 (brs, 2H, 5-NH_2), 4.98 (dd, 1H, 3′-H), 5.82 (m, 2H, 1′,2′-H), 7.30 (s, 1H, 2-H), 7.10, 7.65, 8.35 (each m, each 1H, imidazole protons). *Anal.* Calcd for $C_{25}H_{40}N_6O_5SSi_2$: C, 50.65; H, 6.80; N, 14.17. Found: C, 50.37; H, 6.71; N, 14.25.

5-Amino-1-(2′-deoxy-3′,5′-O-TIPDS-β-D-ribofuranosyl)-imidazole-4-carbonitrile (**13**). Tributyltin hydride (0.87 ml, 1.5 equiv.) was added to suspension of **12** (1.28 g, 2.16 mmol) and azobisisobutylonitrile (50 mg) in dry toluene (20 ml). The mixture was heated under reflux for 40 min and evaporated to dryness. The residue was purified by a silica gel column which was eluted with chloroform containing 1% ethanol. From this fraction, the desired nucleoside (**13**) was obtained (1.0 g, 99%; crystallized from hexane); mp 95−96°C. Mass *m/z*: 466 (M^+). ^{1}H-NMR ($CDCl_3$): δ 1.06 (m, 28H, isopropyl), 2.52 (m, 2H, 2′,2′′-H), 3.82 (m, 1H, 4′-H), 3.97 (m, 2H, 5′,5′′-H), 4.40 (brs, 2H, 5-NH_2), 4.75 (m, 1H, 3′-H), 5.82 (dd, 1H, 1′-H), 7.09 (s, 1H,

2-H). *Anal.* Calcd for $C_{21}H_{38}N_4O_4Si_2$: C, 54.04; H, 8.21; N, 12.00. Found: C, 54.15; H, 8.31; N, 11.86.

2'-Deoxy-3',5'-O-TIPDS-2-(p-nitrophenyl)-adenosine (**14p**). A mixture of **13** (760 mg, 1.6 mmol) and *p*-nitrobenzonitrile (1.4 g, 9.5 mmol) in ethanolic ammonia (saturated at 0°C, 30 ml) was heated at 170°C for 14 h in a sealed tube. The mixture was evaporated to dryness and the residue was suspended in chloroform. The insoluble material was removed by filtration with a celite pad and the filtrate was evaporated. The residue was crystallized from ethanol to give **14p** (845 mg, 84%); mp 216–218°C. Mass *m/z*: 614 (M^+). ^{1}H-NMR (DMSO-d_6): δ 2.61 (m, 1H, 2'-H), 3.10 (m, 1H, 2''-H), 3.87 (m, 3H, 4',5',5''-H), 4.95 (dd, 1H, 3'-H), 6.42 (dd, 1H, 1'-H, $J_{1',2'}$ = 2.9 Hz, $J_{1',2''}$ = 8.1 Hz), 7.56 (brs, 2H, 6-NH_2), 8.32 (d, 2H, 3'',5''-H, $J_{2'',3''}$ = $J_{5'',6''}$ = 8.8 Hz), 8.33 (s, 1H, 8-H), 8.54 (d, 1H, 2'',6''-H). *Anal.* Calcd for $C_{28}H_{42}N_6O_6Si_2$: C, 54.70; H, 6.89; N, 13.67. Found: C, 54.27; H, 7.01; N, 13.54.

2-Deoxy-2-(p-nitrophenyl)-adenosine (**15p**). A tetrahydrofuran solution of tetrabutylammonium fluoride (1M, 0.8 ml) was added to a solution of **14p** (221 mg, 0.36 mmol) in dry tetrahydrofuran (10 ml). The mixture was stirred for 30 min at room temperature and then neutralized with acetic acid. The solution was evaporated to dryness and the residue was crystallized from ethanol to afford **15p** (95 mg, 71%); mp >300°C. Mass *m/z*: 372 (M^+). ^{1}H-NMR(DMSO-d_6): δ 2.31–2.38 (m, 1H, 2'-H), 2.77–2.81 (m, 1H, 2''-H), 3.60 (m, 2H, 5',5''-H), 3.90 (m, 1H, 4'-H), 4.47 (m, 1H, 3'-H), 4.94 (t, 1H, 5'-OH), 5.36 (d, 1H, 3'-OH), 6.47 (t, 1H, 1'-H, $J_{1',2'}$ = 7.0 Hz), 7.52 (brs, 2H, 6-NH_2), 8.35 (d, 2H, 2'', 6''-H, $J_{2'',3''}$ = $J_{5'',6''}$ = 8.8 Hz), 8.44 (s, 1H, 8-H), 8.58 (d, 2H, 3'',5''-H). *Anal.* Calcd for $C_{16}H_{16}N_6O_5$: C, 51.61; H, 4.33; N, 22.57. Found: C, 51.60; H, 4.34; N, 22.30.

2'-Deoxy-2-(m-nitrophenyl)-adenosine (**15m**). A mixture of **13** (200 mg, 0.43 mmol) and *m*-nitrobenzonitrile (400 mg, 2.7 mmol) in ethanolic ammonia (saturated at 0°C, 20 ml) in a sealed tube was heated at 170°C for 7 h. The mixture was evaporated to dryness and the residue was purified over a silica gel column eluted with chloroform containing 1% ethanol to give **14m** (127 mg, 48% as a foam), mass *m/z*: 614 (M^+). Compound **14m** (120 mg, 0.195 mmol) was dissolved in dry tetrahydrofuran (5 ml), which was treated with 1M tetrahydrofuran solution of tetrabutylammonium fluoride (0.5 ml) for 30 min at room temperature. The mixture was neutralized with acetic acid and evaporated to dryness. The residue was crystallized from aqueous methanol to afford **15m** (70 mg, 92% as a hydrate); mp. 150–153°C. Mass *m/z*: 372 (M^+). ^{1}H-NMR (DMSO-d_6 + D_2O): δ 2.35 (ddd, 1H, 2'-H, $J_{1',2'}$ = 6.0 Hz, $J_{2',3'}$ = 3.3 Hz, $J_{2',2'}$ = 13.2 Hz), 2.80 (ddd, 1H, 2''-H, $J_{1',2''}$ = 7.1 Hz, $J_{2'',3'}$ = 6.1 Hz), 3.53 (dd, 1H, 5'-H, $J_{4',5'}$ = 4.9 Hz, $J_{5',5''}$ = 11.5 Hz), 3.63 (dd, 1H, 5''-H, $J_{4',5''}$ = 4.9 Hz), 3.90 (m, 1H, 4'-H), 4.46 (dd, 1H, 3'-H), 6.47 (dd,1H, 1'-H), 7.79 (t, 1H, 5''-H, $J_{4'',5''}$ = $J_{5'',6''}$ = 8.2 Hz), 8.30 (m, 1H, 6''-H), 8.42 (s, 1H, 8-H), 8.76 (d, 1H, 4''-H), 9.13 (t, 1H, 2''-H). *Anal.* Calcd for $C_{16}H_{16}N_6O_5 \cdot H_2O$: C, 49.23; H, 4.65; N, 21.52. Found: C, 49.36; H, 4.57; N, 21.32.

Mutation assay

The Ames assay was performed with the preincubation technique[16]. The 9000×g supernatant of liver homogenate (S9) was prepared from rats induced with polychlorinated biphenyl (PCB-54, Tokyo Kasei Chemicals; Cl content, ca. 54%). For metabolic activation, S9 mix containing 50 μl (per plate) of this S9 and supplementary coenzymes was used. In all the mutation assays, each datum represents a mean in two plates. For each value, the background revertant score had been subtracted.

Assay for mutagenic activity in FM3A cells

Mouse mammary carcinoma cell line FM3A, subclone F28–7, was maintained at 37°C under 5% CO_2 in suspension culture in ES medium (Nissui Pharmaceutical Co., Ltd., Tokyo) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Mutation to ouabain resistance was measured. Logarithmically growing cells were inoculated into the medium at a density of 5×10^4 cells/ml. The culture was incubated at 37°C for 1 day; then 2-(*p*-nitrophenyl)-2'-deoxyadenosine (15p) was added and the cell suspension was incubated for 24 h. The medium was changed, and the cells were cultured for additional 3 days for expression. Then the cells were inoculated into plates of soft-agarose consisting of 0.375% Nobel agar (Difco Laboratories, Detroit, Mich.) and 0.125% Agarose 1600 (Wako Pure Chemical Industries, Ltd., Osaka) in ES medium supplemented with 10% FBS. The number of cells inoculated into each dish was 1×10^6. Ouabain had been added in a 2 mM concentration to the agar-agarose layers on the plates. Colony-forming ability was measured as follows. The cells, which had been cultured for additional 3 days for expression, were diluted with fresh ES medium to give a final density of 500 cells/ml. Two hundred μl of the cell suspension was inoculated onto the surface of agar-agarose plate that did not contain ouabain. The plates were cultured for 10 days, and the colonies formed were counted.

ED_{50} value refers to the concentration of drug necessary to inhibit the growth rate of cells b 50% of the control, as determined by the procedure described before[17].

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