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Studies on Compounds Related to Antitumor Agents. Syntheses of 8-Substituted N^6,N^6-Dimethyladenosine Derivatives[1)]

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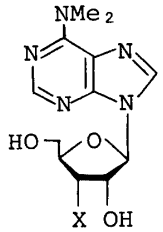
N^6,N^6-Dimethyl-8-methylsulfonyladenosine, obtained from N^6,N^6-dimethyl-8-methylthioadenosine by highly selective oxidation with $KMnO_4$, was treated with cyanide ion to give 8-cyano-N^6,N^6-dimethyladenosine. The conversion of the cyano group to methyl imidate, methoxycarbonyl, carbamoyl, carbothioamide, and carboxylic acid moieties was achieved. These 8-substituted N^6,N^6-dimethyladenosines may possess resonances structures involving positions 6 and 8 in adenosine, as indicated by the spectroscopic data. They showed no antitumor activity.

Keywords——N^6,N^6-dimethyladenosine; puromycin; 8-substituted N^6,N^6-dimethyladenosine; permanganate oxidation; electron-withdrawing group; electron-donating group; antitumor activity

N^6,N^6-Dimethyladenosine (**1a**) is a component of transfer ribonucleic acid (t-RNA) and the antibiotic puromycin (**1b**).[2)] The nucleoside (**1b**) is present in 16S and 18S ribosomal RNA, which is believed to be responsible for the binding of the antibiotic kasugamycin.[3)] One of the authors has already reported the synthesis of a "reduced" analogue of puromycin (**1c**).[4)] We next wished to synthesize 8-substituted N^6,N^6-dimethyladenosines, in order to investigate the structure–activity relationships of 3′- and 8-substituted derivatives.



1a–c

1a : X = OH

1b : X = NHCOCHCH$_2$(NH$_2$)–C$_6$H$_4$–OMe

1c : X = NHCH$_2$CHCH$_2$(NH$_2$)–C$_6$H$_5$

Chart 1

Recently, we reported the permanganate oxidation of N^6,N^6,8-trisubstituted 2′,3′,5′-tri-*O*-acetyladenosines.[5)] We have now established a method for the synthesis of N^6,N^6-dimethyl-8-methylsulfonyladenosine (**13**) from N^6,N^6-dimethyl-8-methylthioadenosine (**9**)[6)] by highly selective oxidation with $KMnO_4$. Because the methylsulfonyl group is known to be a good leaving group in reactions with nucleophiles,[7)] compound **13** may be a useful intermediate for the preparation of 8-substituted N^6,N^6-dimethyladenosines. Attempts to displace the corresponding 8-bromo group with carbanions such as cyanide, alkoxy or nitromethyl anion have been unsuccessful.

We found that N^6,N^6-dimethyl-8-methylsulfonyladenosines reacted readily with cyanide

ion to give 8-cyano-N^6,N^6-dimethyladenosines. The displacement of the 8-cyano group, the resonance structures and the antitumor activities of 8-substituted N^6,N^6-dimethyladenosines obtained in the present study are also described here.

In a previous study,[5] we did not obtain the target compound, 2′,3′,5′-tri-*O*-acetyl-N^6,N^6-dimethyl-8-methylsulfonyladenosine (**3**), by oxidation of 2′,3′,5′-tri-*O*-acetyl-N^6,N^6-dimethyl-8-methylthioadenosine (**2**), but obtained 2′,3′,5′-tri-*O*-acetyl-N^6-methyl-8-methylsulfonyladenosine (**4**) along with 2′,3′,5′-tri-*O*-acetyl-8-methylsulfonyladenosine (**5**).[7] Attempts to oxidize selectively the 8-methylthio group in N^6,N^6-dimethyladenosine with other oxidizing reagents such as acetic acid (AcOH)–H_2O_2, *N*-bromosuccinimide, dimethylsulfoxide (DMSO)–dicyclohexylcarbodiimide,[8] bromine, and *m*-chloroperbenzoic acid have failed.

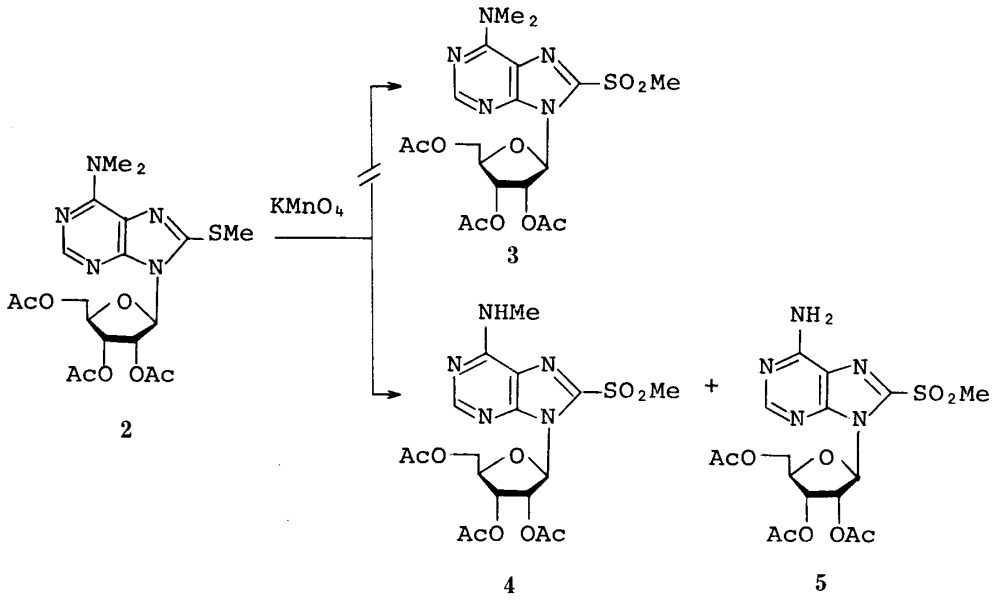
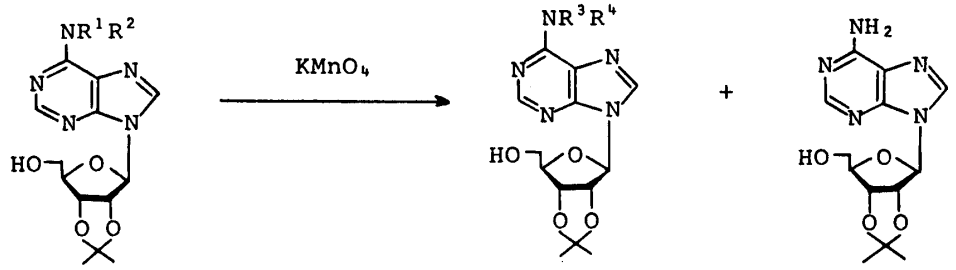


Chart 2

2′,3′-*O*-Isopropylidene-N^6,N^6-dialkyladenosines (**6a—e**)[9] prepared according to Hampton,[10] were oxidized with $KMnO_4$ in 50% AcOH to give both the monodealkylated compounds (**7a—e**)[9] (identified by deblocking of the isopropylidene group in formic acid to give the corresponding authentic samples[11]) and didealkylated compound, 2′,3′-*O*-isopropylideneadenosine,[12] respectively. However, we found that by introducing the isopropylidene group at the 2′,3′-*cis* vicinal glycol moiety of **6a—e**, dealkylation of the N^6,N^6-dialkyl group by oxidation with $KMnO_4$ was less than with the corresponding 2′,3′,5′-tri-*O*-acetyl-N^6,N^6-dialkyladenosines (Table I).

We now wish to report the synthesis of N^6,N^6-dimethyl-8-methylsulfonyladenosine (**13**) using a novel selective procedure. Acetonation of N^6,N^6-dimethyl-8-methylthioadenosine (**9**) in the presence of 70% $HClO_4$ gave 2′,3′-*O*-isopropylidene-N^6,N^6-dimethyl-8-methylthioadenosine (**10**) in 82% yield. Compound **10** was readily oxidized with 3 eq of $KMnO_4$ at 4 °C for 5 min to deposit 2′,3′-*O*-isopropylidene-N^6,N^6-dimethyl-8-methylsulfonyladenosine (**11**) in 70% yield along with a small amount (2.9%) of the monodemethylated compound (**12**) as crystals. The structures of both **11** and **12** were determined by analysis of the nuclear magnetic resonance (NMR), ultraviolet (UV), infrared (IR) and mass spectra (MS) as well as thin layer chromatography (TLC) on silica gel and elemental analyses. The deblocking of the isopropylidene group of **11** to produce **13** was achieved by treatment with 70% formic acid for 12 h at room temperature.

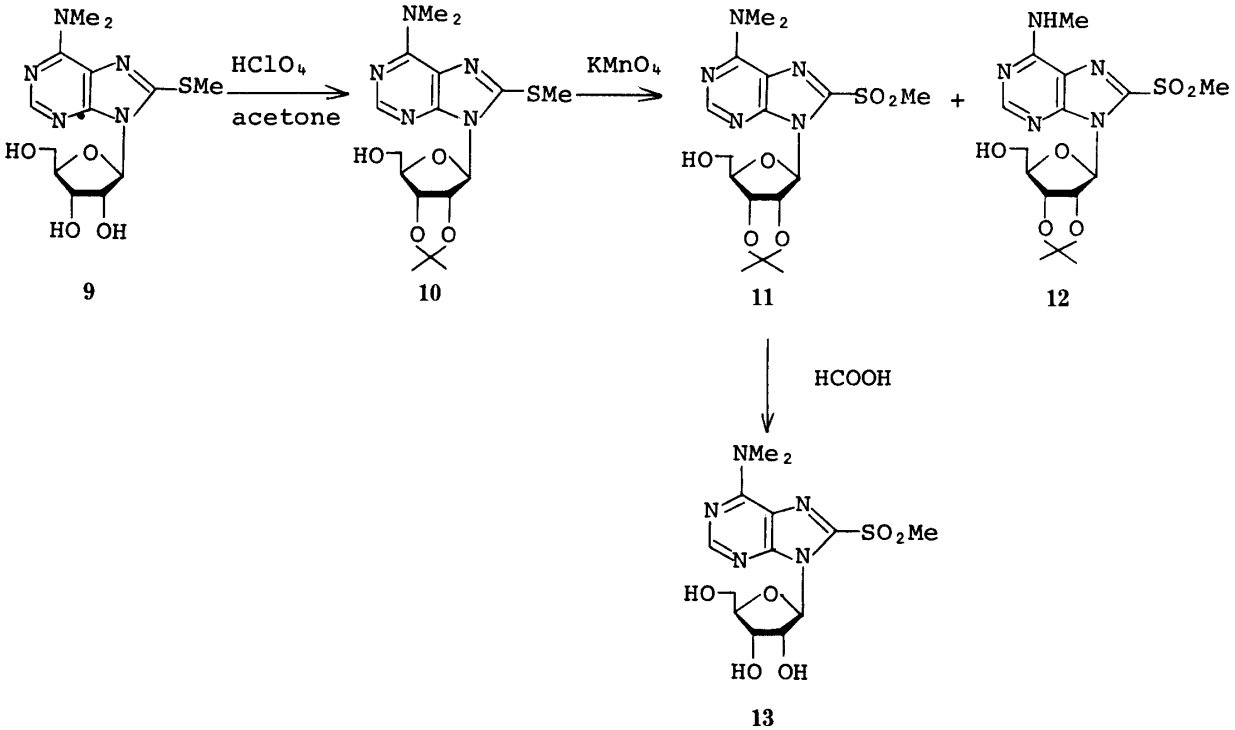
TABLE I. Results of Oxidation of 2′,3′-*O*-Isopropylidene-N^6,N^6-dialkyladenosines (**6a—e**) with $KMnO_4$ under Various Conditions



6a—e **7a—e** **8**

Starting material					Products					
	R^1	R^2	Amount of $KMnO_4$ (eq)	Temp. (°C)	Time (h)		R^3	R^4	Yield[a] (%)	Yield[a] (%)
6a	CH_3	CH_3	1	r.t.	1.5	**7a**	CH_3	H	20.3 (26.3)	**8** None (none)
6a	CH_3	CH_3	3	40	1	**7a**	CH_3	H	25.2 (32.3)	**8** Trace (48.1)
6b	C_2H_5	C_2H_5	3	r.t.	0.5	**7b**	C_2H_5	$COCH_3$	29.0 (42.5)	**8** Trace (37.3)
6b	C_2H_5	C_2H_5	3	r.t.	1	**7b**	C_2H_5	$COCH_3$	28.2 (38.9)	**8** Trace (43.7)
7b	C_2H_5	$COCH_3$	3	r.t.	0.5					**8** 53.3 (83.2)
6c	CH_2-Ph	CH_2-Ph	3	r.t.	0.67	**7c**	CH_2-Ph	H	13.5 (23.3)	**8** Trace (28.4)
6d	$(CH_2)_4$		3	40	1	**7d**	$(CH_2)_3CO$		21.7 (48.2)	**8** Trace (4.3)
6e	$(CH_2)_5$		3	r.t.	0.17	**7e**	$(CH_2)_4COOH$	H	28.5 (31.1)	**8** Trace (38.2)

a) The values in parentheses are % oxidation of 2′,3′,5′-tri-*O*-acetyl-N^6,N^6-dialkyladenosines with $KMnO_4$ (see ref. 5). r.t. = room temperature.



9 **10** **11** **12**

13

Chart 3

Ueda and co-workers[7] have reported the synthesis of 8-cyanoadenosine, and also used this procedure for the preparation of 8-cyano-N^6,N^6-dimethyladenosine (**15**). Treatment of **11** with sodium cyanide in dimethylformamide (DMF) at room temperature for 5 h gave 8-cyano-2′,3′-*O*-isopropylidene-N^6,N^6-dimethyladenosine (**14**) as crystals in quantitative yield. The structure of **14** was confirmed by the detection of a cyano group absorption in the IR spectrum (2250 cm^{-1}), and by elemental and mass analyses. It is of interest that **14** exhibits a characteristic light-blue fluorescence when irradiated with UV light (245 μm). The fluorescence allowed easy detection of **14** on paper chromatography.

The conversion of the 8-cyano group to a carboxy group was performed *via* the methyl imidate as an intermediate. Treatment of **14** with methanolic ammonia at room temperature for 20 h afforded methyl 2′,3′-*O*-isopropylidene-N^6,N^6-dimethyladenosine-8-carboximidate (**16**) in 76% yield. The structure of **16** was confirmed by the disappearance of the cyano group absorption in the IR spectrum, and the appearance of methoxy and imino proton signals (4.03 and 9.38 ppm) in the NMR spectrum. Compound **16** was readily hydrolyzed in an acidic medium at 4 °C for 2 h to give methyl 2′,3′-*O*-isopropylidene-N^6,N^6-dimethyladenosine-8-carboxylate (**18**) in 72% yield, and this was converted to 2′,3′-*O*-isopropylidene-N^6,N^6-dimethyladenosine-8-carboxamide (**20**) by treatment with methanolic ammonia in a sealed tube. Subsequent alkaline hydrolysis of **16** furnished 2′,3′-*O*-isopropylidene-N^6,N^6-dimethyladenosine-8-carboxylic acid (**22**). The free acid (**22**) was unstable and decarboxylation occurred readily in a solution of DMSO, or even in the crystalline state.

Treatment of **14** with hydrogen sulfide in pyridine at 0 °C afforded 2′,3′-*O*-isopropylidene-N^6,N^6-dimethyladenosine-8-carbothioamide (**24**), which was transformed to **14** in boiling pyridine.

Compounds **14**, **16**, **18**, **20**, **22** and **24** were deacetonated in 70% formic acid at room temperature to give the corresponding free nucleosides (**15**, **17**, **19**, **21**, **23** and **25**) in quantitative yields.

The introduction of a carbon unit at the 8-position of N^6,N^6-dimethyladenosine was achieved by means of the reaction described above.

Some spectroscopic characteristics of the 8-substituted N^6,N^6-dimethyladenosines obtained in the present study are noteworthy. The most distinct spectral difference in the 100 MHz NMR spectra between compounds **3**, **11**, and **13**—**25** and compounds **1a**, **9** and **10**, 8-amino-N^6,N^6-dimethyladenosine (**26**),[6] and 8-bromo-N^6,N^6-dimethyladenosine (**27**)[13] are the signal patterns and the chemical shifts of the N^6,N^6-dimethyl group: a sharp singlet was observed at near 3.40 ppm in the latter group, while each N^6-methyl group in the former group was observed as a multiplet or a broad singlet shifted to lower fieled. The observations are similar to those for N^6,N^6,3-trimethyladenosine iodide derivatives,[14] and suggest a quaternary character of the exocyclic nitrogen atom at position 6 in the purine ring. This may be accounted for by three factors: i) hyperconjugation and the inductive effect of the N^6-methyl group, ii) the strongly electronegative character of the pyrimidine portion of the purine ring, and iii) the mesomeric effect of the purine ring. The substituent effects at the C-8 carbon atom of the imidazole ring in **3**, **11**, and **13**—**25**, and in **1a**, **9**, **10**, **26**, and **27**, are consistent with the presence of electron-withdrawing groups in the former and electron-donating groups in the latter. The large downfield shifts of the anomeric protons of these compounds may be due to the deshielding effect of the carboxy group in **14**, **15**, and **18**—**23**, and an imino and a thione goup in **16** and **17**, and **24** and **25**, respectively. The C–H bond polarization of the anomeric protons caused by an electric field effect of the 8-substituent may be due to the electron-withdrawing effect. The UV absorption maxima of **3**, **11**, and **13**—**25** exhibited large bathochromic shifts to the 299—333 nm region, as compared with those of **1a**, **9**, **10**, **26**, and **27** in 274 nm, respectively, and this provides additional support for the conjugation structure between the purine ring and the 8-substituent.

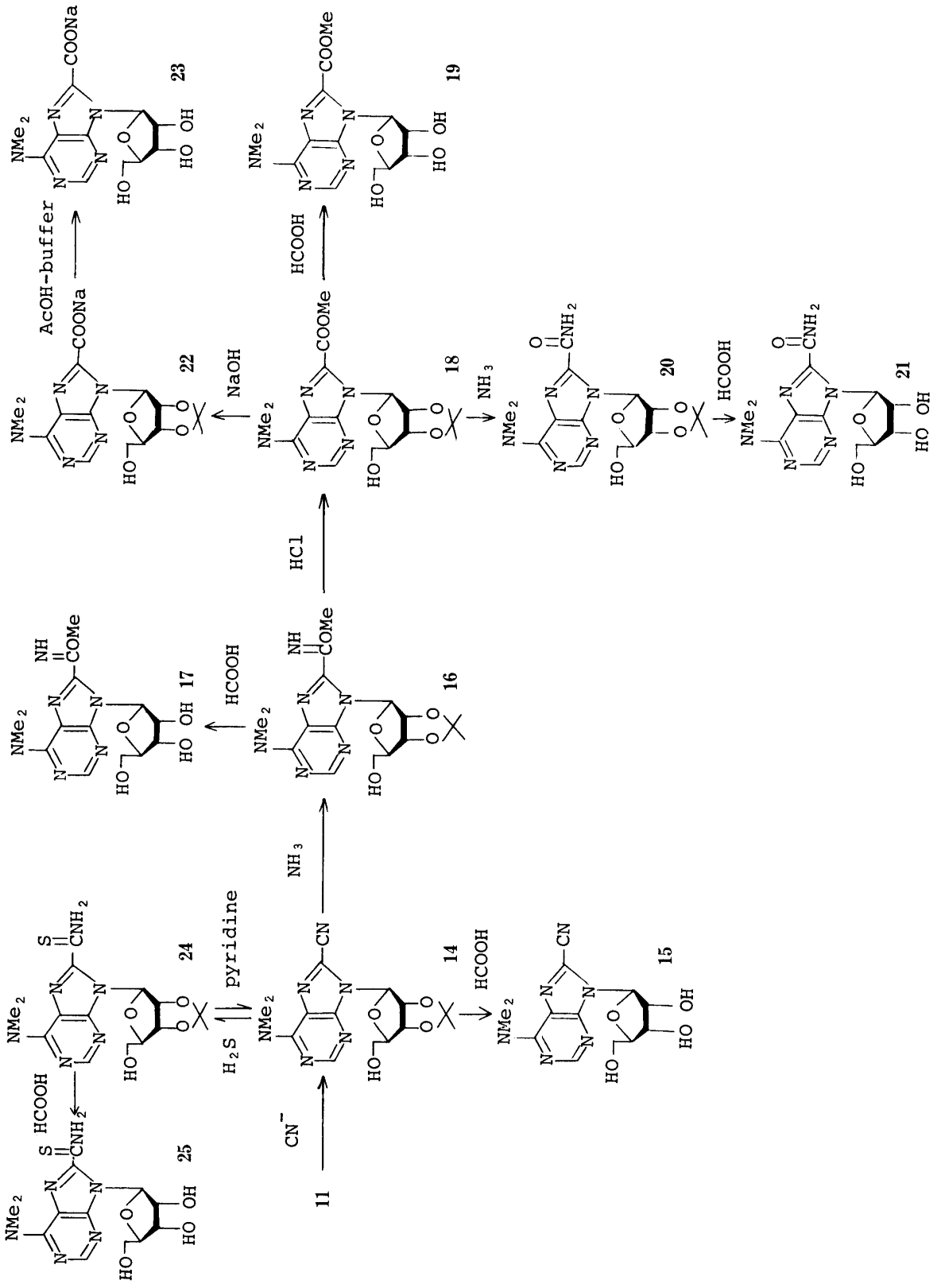
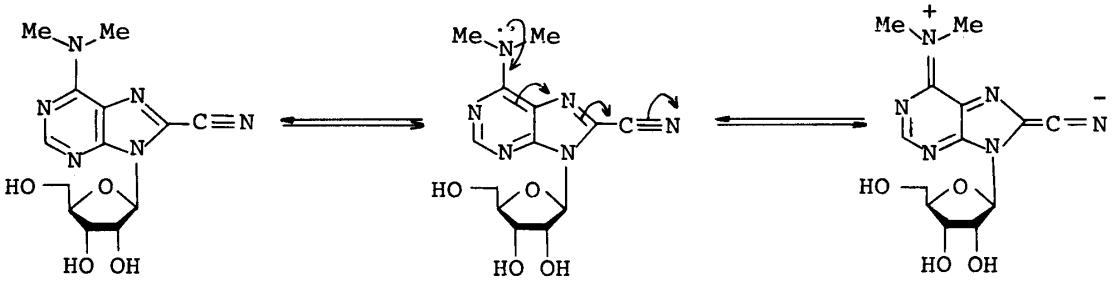


Chart 4

The electronegative nature is presumably related to the rather wide distribution of charge; various resonance structures which may contribute can be drawn. Some of the more probable resonances of **15** are illustrated in Chart 5.



15

Chart 5

Compound **15** exhibited properties which are indicative of a general deficiency of electrons throughout the pyrimidine ring and a general excess of electrons in the imidazole ring. Thus, the nature of the nitrogen atom at position 6 was cationic. Multiple splitting of the signals of the N^6,N^6-dimethyl group was observed in the NMR spectra.

It is noteworthy that the introduction of an electron-withdrawing group at position 8 in adenosine derivatives may cause partial electron transfer from the pyrimidine to the imidazole ring, indicating the existence of resonance structures involving positions 6 and 8.

The antitumor activities of **14**, **15**, **18**—**21**, **24**, and **25** against L1210 leukemia cultured cells and Ehrlich ascites tumor were investigated *in vitro* and *in vivo*, but these compounds were found to be inactive (Table II).

TABLE II. Antitumor Activity of 8-Substituted N^6,N^6-Dimethyladenosines *in Vitro* and *in Vivo*

Agents	*In vitro* IC_{50}[a] (μg/ml)	*In vivo* (T/C[b] %) 250	100	(mg/kg/d)
DMAP[c]	>5.0			
1a	0.5			
14	5.0			
15	>5.0			
18	>5.0	126.9	106.5	
19	>5.0	118.8	115.7	
20	3.0	125.0	89.6	
21	1.0	60.4	108.3	
24	5.0	118.3	88.2	
25	>5.0	125.6	112.3	

a) IC_{50} is the concentration of an agent required for 50% inhibition of cell growth. *b*) Tumor growth relative to the control. *c*) 6-Dimethylaminopurine.

Experimental

Melting points were determined with a Yanagimoto melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi grating infrared spectrophotometer EP1-G2. NMR spectra were run on a JEOL JNM-MH-100 spectrometer using tetramethylsilane as an internal standard. MS were measured on Hitachi M-52 and JEOL JMS-DX 300 spectrometers. UV spectra were recorded on a Hitachi 124 spectrophotometer and Shimadzu UV-240 UV-visible recording spectrophotometer.

Oxidation with $KMnO_4$ of 2′,3′-*O*-Isopropylidene-N^6,N^6-dialkyladenosines (6a—e)——A solution of 3 mmol of **6a—e** in 50 ml of 50% AcOH was treated with 1—3 eq of $KMnO_4$, and the mixture was stirred at room temperature or 40 °C for 1—3 h. The resulting colored suspension was treated with 35% H_2O_2 until it became colorless, then extracted with $CHCl_3$. The organic layer was washed with $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel ($CHCl_3$–MeOH) to give **7a—e** and **8**.

2′,3′-*O*-Isopropylidene-N^6,N^6-dimethyl-8-methylthioadenosine (10)——A solution of 10 g (29 mmol) of **9** in 400 ml of acetone was treated dropwise with 8.5 g (0.12 mol) of 70% $HClO_4$, and the mixture was stirred for 2 h at room temperature. Then 20 g of $NaHCO_3$ was added, and the suspension was stirred for 30 min. The insoluble material was filtered off, and washed with acetone. The filtrate and washing were combined, and the solvent was evaporated off. The residual semi-crystalline material was chromatographed on silica gel with $CHCl_3$–MeOH (98 : 2). The 1st eluate gave a syrup, which was kept at room temperature for 16 h to crystallize. Colorless needles, mp 85—86.5 °C, yield 8.5 g (76%). *Anal.* Calcd for $C_{16}H_{23}N_5O_4S$: C, 50.38; H, 6.08; N, 18.36. Found: C, 50.13; H, 5.78; N, 18.09. MS *m/z*: 381 (M^+). NMR ($CDCl_3$) δ: 1.39, 1.68 (s, s, 3, 3, CMe_2), 2.72 (s, 3, SMe), 3.52 (s, 6, NMe_2), 3.83, 3.93 (d, d, 1, 1, 5′-H), 4.53 (br s, 1, 4′-H), 5.06 (d, 1, 3′-H), 5.28 (t, 1, 2′-H), 5.99 (d, 1, J=6 Hz, 1′-H), 6.88 (br s, 1, exchangeable with D_2O, 5′-OH), 8.19 (s, 1, 2-H). UV λ_{max}^{MeOH} nm (ε): 229 (17800), 288 (19400).

Oxidation of 10 with $KMnO_4$ to Give 2′,3′-*O*-Isopropylidene-N^6,N^6-dimethyl-8-methylsulfonyladenosine (11) and 2′,3′-*O*-Isopropylidene-N^6-methyl-8-methylsulfonyladenosine (12)——$KMnO_4$ (6.16 g, 39 mmol) was added to a solution of 5 g (13 mmol) of **10** in 110 ml of 50% AcOH at 4 °C, and the suspension was vigorously stirred for 5 min. Then 35% H_2O_2 was added until the mixture became colorless. The crystals that deposited were collected by filtration, washed with cold MeOH, and recrystallized from MeOH to give 3.36 g (62%) of **11**. The filtrate was evaporated to dryness to give crystals, which were chromatographed on a silica gel column with $CHCl_3$–MeOH (9 : 1). The 1st eluate gave 0.43 g (8%) of **11**, and the 2nd eluate gave 0.15 g (2.9%) of **12**. **11**: mp 206—207 °C. *Anal.* Calcd for $C_{16}H_{23}N_5O_6S \cdot 1/3H_2O$: C, 45.81; H, 5.69; N, 16.70. Found: C, 45.59; H, 5.22; N, 16.62. MS *m/z*: 413 (M^+). NMR (pyridine-d_5) δ: 1.41, 1.68 (s, s, 3, 3, CMe_2), 3.38 (br s, 6, NMe_2), 3.37 (s, 3, SO_2Me), 4.12 (br s, 2, 5′-H), 4.68 (m, 1, 4′-H), 5.42 (dd, 1, 3′-H), 5.84 (dd, 1, 2′-H), 6.58 (br s, 1, exchangeable with D_2O, 5′-OH), 7.22 (d, 1, J=3 Hz, 1′-H), 8.46 (s, 1, 2-H). UV λ_{max}^{MeOH} nm (ε): 226 (18600), 274 (sh) (15600), 299 (21800). **12**: mp 194—195 °C. *Anal.* Calcd for $C_{15}H_{21}N_5O_6S$: C, 45.10; H, 5.30; N, 17.53. Found: C, 45.14; H, 5.34; N, 17.70. MS *m/z*: 399 (M^+). NMR (DMSO-d_6) δ: 1.35, 1.56 (s, s, 3, 3, CMe_2), 3.01 (d, 3, exchangeable with D_2O to s, NHMe), 3.54 (br s, 2, 5′-H), 3.57 (s, 3, SO_2Me), 4.23 (m, 1, 4′-H), 5.07 (dd, 1, 3′-H), 5.18 (br s, 1, exchangeable with D_2O, 5′-OH), 5.66 (dd, 1, 2′-H), 6.69 (d, 1, J=2 Hz, 1′-H), 8.41 (s, 1, 2-H), 8.50 (br s, 1, exchangeable with D_2O, NH). UV λ_{max}^{MeOH} nm (ε): 222 (22300), 266 (sh) (13600), 292 (20500).

N^6,N^6-Dimethyl-8-methylsulfonyladenosine (13)——A solution of 4.13 g (10 mmol) of **11** in 50 ml of 75% formic acid was stirred at room temperature for 16 h, then evaporated to dryness. The residue was dissolved in EtOH and a small amount of formic acid was evaporated off by azeotropic distillation to give crystals, which were recrystallized from EtOH. Colorless needles, mp 190 °C, yield 2.87 g (77%). *Anal.* Calcd for $C_{13}H_{19}N_5O_6S \cdot 1/2H_2O$: C, 40.83; H, 5.27; N, 18.32. Found: C, 40.53; H, 5.01; N, 18.68. MS *m/z*: 373 (M^+). NMR (DMSO-d_6) δ: 3.52 (br s, 9, NMe_2 + SO_2Me), 3.76 (br s, 2, 5′-H), 4.16 (br s, 1, 4′-H), 4.30 (m, 1, 3′-H), 5.00 (m, 2, exchangeable with D_2O to q, 2′-H + OH), 5.20 (d, 1, exchangeable with D_2O, OH), 6.02 (br d, 1, exchangeable with D_2O, OH), 6.49 (d, 1, J=7 Hz, 1′-H), 8.25 (s, 1, 2-H). UV λ_{max}^{MeOH} nm (ε): 226 (17000), 274 (sh) (16000), 299 (21000).

8-Cyano-2′,3′-*O*-isopropylidene-N^6,N^6-dimethyladenosine (14)——A mixture of 2.07 g (5 mmol) of **11**, 0.49 g (10 mmol) of NaCN, and 65 ml of DMF was stirred at room temperature for 16 h. After evaporation of the solvent, ice-water was added to the residue, and the deposited crystal were filtered off and recrystallized from ether–petro. ether (1 : 1). Colorless needles, mp 86—88 °C, yield 1.71 g (95%). *Anal.* Calcd for $C_{16}H_{20}N_6O_4$: C, 53.32; H, 5.59; N, 23.32. Found: C, 53.34; H, 5.36; N, 23.62. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2250 (CN). NMR ($CDCl_3$) δ: 1.36, 1.66 (s, s, 3, 3, CMe_2), 3.31, 3.70 (br s, br s, 3, 3, NMe_2), 3.86 (m, 2, 5′-H), 4.50 (s, 1, 4′-H), 4.97 (d, 1, 3′-H), 5.16 (t, 1, 2′-H), 6.01 (d, 1, J=5 Hz, 1′-H), 6.21 (br d, 1, exchangeable with D_2O, 5′-OH), 8.16 (s, 1, 2-H). UV λ_{max}^{MeOH} nm (ε): 234 (20000), 270 (sh) (5400), 279 (6200), 312 (14600).

8-Cyano-N^6,N^6-dimethyladenosine (15)——A solution of 3.6 g (10 mmol) of **14** in 50 ml of 75% formic acid was kept in a refrigerator for 16 h. After evaporation of the solvent at below 25 °C, the residue was dissolved in EtOH, and a small amount of formic acid was removed by azeotropic distillation to give a syrup, which was chromatographed on a silica gel column with $CHCl_3$–MeOH (93 : 7). The 1st eluate gave a syrup, which was crystallized with $CHCl_3$–petro. ether. Colorless amorphous powder, mp 73—77 °C, yield 2.4 g (75%). *Anal.* Calcd for $C_{13}H_{16}N_6O_4$: C, 48.75; H, 5.04; N, 26.24. Found: C, 48.92; H, 5.26; N, 25.98. NMR ($CDCl_3$ + D_2O) δ: 3.24, 3.64 (br s, br s, 3, 3, NMe_2), 3.80 (m, 2, 5′-H), 4.28 (s, 1, 4′-H), 4.36 (d, 1, 3′-H), 4.87 (t, 1, 2′-H), 5.97 (d, 1, J=7 Hz, 1′-H), 8.06 (s, 1, 2-H). UV λ_{max}^{MeOH} nm (ε): 233 (19700), 270 (sh) (5300), 279 (6000), 315 (14500).

Methyl 2′,3′-*O*-Isopropylidene-N^6,N^6-dimethyladenosine-8-carboximidate (16)——A solution of 1.8 g (5 mmol) of **14** in 300 ml of methanolic ammonia was kept in a refrigerator for 16 h, then evaporated at below 25 °C to *ca.* 20 ml. The crystals that deposited on cooling were filtered off and recrystallized from MeOH. Colorless needles, mp 168—171 °C, yield 1.78 g (91%). *Anal.* Calcd for $C_{17}H_{24}N_6O_5$: C, 52.03; H, 6.16; N, 21.42. Found: C, 51.92; H, 6.33; H, 21.09. NMR ($CDCl_3$) δ: 1.38, 1.64 (s, s, 3, 3, CMe_2), 3.53 (br s, 6, NMe_2), 3.82, 3.90 (dd, dd, 1, 1, 5′-H), 4.03 (s, 3,

OMe), 4.48 (br s, 1, 4′-H), 5.09 (d, 1, 3′-H), 5.31 (t, 1, 2′-H), 5.84 (br s, 1, exchangeable with D_2O, 5′-OH), 6.66 (d, 1, $J=5$ Hz, 1′-H), 8.23 (s, 1, 2-H), 9.38 (br s, 1, exchangeable with D_2O, NH). UV λ_{max}^{MeOH} nm (ε): 226 (22300), 271 (sh) (6800), 278 (7900), 312 (17500).

Methyl N^6,N^6-Dimethyladenosine-8-carboximidate (17)——A solution of 0.39 g (1 mmol) of **16** in 20 ml of 20% formic acid was kept in a refrigerator for 10 d, then neutralized with Dowex 1 (OH^-) resin. After removal of the resin, the filtrate was concentrated and the residue was crystallized from hot H_2O. Colorless needles, mp 185—188 °C, yield 0.21 g (60%). *Anal.* Calcd for $C_{14}H_{20}N_6O_5$: C, 47.72; H, 5.72; N, 23.85. Found: C, 47.51; H, 6.00; N, 23.58. NMR (DMSO-d_6 + D_2O) δ: 3.56 (br s, 6, NMe_2), 3.74 (m, 2, 5′-H), 3.98 (s, 3, OMe), 4.16 (br s, 1, 4′-H), 4.34 (dd, 1, 3′-H), 5.96 (t, 1, 2′-H), 6.75 (d, 1, $J=7$ Hz, 1′-H). 8.21 (s, 1, 2-H). UV λ_{max}^{MeOH} nm (ε): 226 (24000), 271 (sh) (7000), 278 (8000), 312 (17600).

Methyl 2′,3′-*O*-Isopropylidene-N^6,N^6-dimethyladenosine-8-carboxylate (18)——A solution of 3.92 g (10 mmol) of **16** in 100 ml of MeOH was treated dropwise with 25 ml of 1 N HCl under cooling, and the mixture was stirred for 2 h under cooling, then neutralized with Dowex 1 (OH^-) resin. After removal of the resin, the solution was evaporated to *ca.* 20 ml. The deposited crystals were filtered off and recrystallized from H_2O–EtOH (3 : 1). Colorless small needles, mp 141—142 °C, yield 3.5 g (89%). *Anal.* Calcd for $C_{17}H_{23}N_5O_6$: C, 51.90; H, 5.89; N, 17.80. Found: C, 52.12; H, 5.72; N, 18.03. NMR ($CDCl_3$) δ: 1.38, 1.68 (s, s, 3, 3, CMe_2), 3.54 (br s, 6, NMe_2), 3.75, 3.90 (br s, br s, 1, 1, 5′-H), 3.98 (s, 3, OMe), 4.46 (s, 1, 4′-H), 5.08 (d, 1, 3′-H), 5.32 (t, 1, 2′-H), 7.10 (d, 1, $J=5$ Hz, 1′-H), 8.24 (s, 1, 2-H), 6.30 (br s, 1, exchangeable with D_2O, 5′-OH). UV λ_{max}^{MeOH} nm (ε): 235 (25300), 270 (sh), (7600), 278 (8300), 318 (17800).

Methyl N^6,N^6-Dimethyladenosine -8-carboxylate (19)——A solution of 1.97 g (5 mmol) of **18** in 100 ml of 75% formic acid was kept in a refrigerator for 2 d, and then evaporated to dryness. The residue was dissolved in EtOH and a small amount of formic acid was evaporated off by azeotropic distillation. The crystals obtained were recrystallized from MeOH twice. Colorless needles, mp 204 °C, yield 1.56 g (88%). *Anal.* Calcd for $C_{14}H_{19}N_5O_6$: C, 47.59; H, 5.42; N, 19.82. Found: C, 47.45; H, 5.44; N, 19.74. MS *m/z*: 353 (M^+). NMR (DMSO-d_6 + D_2O) δ: 3.56 (br s, 6, NMe_2), 3.99 (s, 3, OMe), 4.16 (br s, 1, 4′-H), 4.34 (dd, 1, 3′-H), 4.96 (t, 1, 2′-H), 6.80 (d, 1, $J=8$ Hz, 1′-H), 8.21 (s, 1, 2-H). The signal of the proton at position 5′ overlapped with that of DOH. UV λ_{max}^{MeOH} nm (ε): 234 (21500), 270 (sh) (7000), 278 (7800), 315 (15300).

2′,3′-*O*-Isopropylidene-N^6,N^6-dimethyladenosine-8-carboxamide (20)——A suspension of 0.39 g (1 mmol) of **18** in 50 ml of methanolic ammonia in a sealed tube was heated at 100 °C for 15 h. After evaporation of the solvent, the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (9 : 1). The 2nd eluate gave a syrup, which was crystallized from hot H_2O. Colorless fish-scales, mp 200—202 °C, yield 0.3 g (79%). *Anal.* Calcd for $C_{16}H_{22}N_6O_5$: C, 50.79; H, 5.86; N, 22.21. Found: C, 51.01; H, 6.00; N, 22.01. MS *m/z*: 378 (M^+). NMR (DMSO-d_6) δ: 1.38, 1.71 (s, s, 3, 3, CMe_2), 3.47 (br s, 6, NMe_2), 3.67—4.04 (m, 2, 5′-H), 4.50 (br s, 1, 4′-H), 5.11 (dd, 1, 3′-H), 5.38 (t, 1, 2′-H), 6.50 (br s, 1, exchangeable with D_2O, 5′-OH), 7.40 (br s, 2, exchangeable with D_2O, $CONH_2$), 7.50 (d, 1, $J=5$ Hz, 1′-H), 8.28 (s, 1, 2-H).

N^6,N^6-Dimethyladenosine-8-carboxamide (21)——A solution of 0.38 g (1 mmol) of **20** in 30 ml of 75% formic acid was kept in a refrigerator for 16 h, then evaporated to dryness to give crystals, which were recrystallized from H_2O. Colorless needles, mp 251—253 °C, yield 0.28 g (82%). *Anal.* Calcd for $C_{13}H_{18}N_6O_5 \cdot 1/4H_2O$: C, 45.54; H, 5.44; N, 24.52. Found: C, 45.76; H, 5.43; N, 24.30. NMR (DMSO-d_6 + D_2O) δ: 3.54 (br s, 6, NMe_2), 3.70 (q, 2, 5′-H), 4.02 (m, 1, 4′-H), 4.28 (m, 1, 3′-H), 5.00 (t, 1, 2′-H), 7.03 (d, 1, $J=7$ Hz, 1′-H), 8.21 (s, 1, 2-H). UV λ_{max}^{MeOH} nm (ε): 233 (20700), 270 (sh) (6000), 279 (sh) (7500), 310 (13000).

Sodium Salt of 2′,3′-*O*-Isopropylidene-N^6,N^6-dimethyladenosine-8-carboxylic Acid (22)——A solution of 0.39 g (1 mmol) of **20** in 10 ml of 0.05 N NaOH was stirred for 1 h at room temperature, then adjusted to pH 4 with 1 N HCl. The deposited crystals were filtered off and dissolved in 10 ml of 1 N NaOH. The solution was evaporated to dryness to give a semi-crystalline material, which was recrystallized from MeOH–H_2O (1 : 1). Colorless amorphous powder, mp 295—298 °C, yield 0.21 g (52%). *Anal.* Calcd for $C_{16}H_{20}N_5NaO_6$: C, 47.88; H, 5.02; N, 17.45. Found: C, 48.17; H, 5.37; N, 17.55. NMR (D_2O) δ: 1.39, 1.69 (s, s, 3, 3, CMe_2), 3.56 (br s, 6, NMe_2), 5.10 (m, 1, 3′-H), 5.49 (dd, 1, 2′-H), 6.99 (d, 1, $J=5$ Hz, 1′-H), 8.07 (s, 1, 2-H). The signals of the protons at positions 5′ and 4′ overlapped with that of DOH. UV λ_{max}^{MeOH} nm (ε): 240 (19000), 274 (6000), 309 (16900).

Sodium Salt of N^6,N^6-Dimethyladenosine-8-carboxylic Acid (23)——A solution of 0.4 g (1 mmol) of the sodium salt of **22** in 30 ml of AcOH buffer (pH 4) was stirred for 20 h, then evaporated to *ca.* 5 ml. The crystals that deposited on cooling were filtered off, and recrystallized from MeOH–H_2O (1 : 1) several times. Colorless amorphous powder (hygroscopic), mp > 300 °C, yield 0.08 g (22%). *Anal.* Calcd for $C_{13}H_{16}N_5NaO_6$: C, 43.22; H, 4.46; N, 19.38. Found: C, 42.86; H, 4.77; N, 19.26. UV $\lambda_{max}^{H_2O}$ nm (ε): 238 (18600), 274 (5600), 309 (17000).

2′,3′-*O*-Isopropylidene-N^6,N^6-dimethyladenosine-8-carbothioamide (24)——H_2S gas (obtained from $NaHSO_3$ and $MgCl_2$) was bubbled through a solution of 1.08 g (3 mmol) of **14** in 10 ml of dry pyridine under cooling, and the mixture was kept at room temperature for 2 h with a stopper. After evaporation of the solvent, the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (94 : 6). The appropriate eluate fractions yielded a syrup, which was crystallized from EtOH. Yellow pillars, mp 215—217 °C, yield 0.92 g (78%). *Anal.* Calcd for $C_{16}H_{22}N_6O_4S$: C, 48.72; H, 5.62; N, 21.31. Found: C, 48.61; H, 5.93; N, 21.66. NMR (DMSO-d_6) δ: 1.38, 1.69 (s, s, 3, 3, CMe_2), 3.32—3.80 (m, 8, 5′-H + NMe_2), 4.18 (m, 1, 4′-H), 5.12 (m, 1, 3′-H), 5.20 (br s, 1, 5′-OH), 5.57 (dd, 1, 2′-H),

7.31 (d, 1, $J=3$ Hz, 1′-H), 8.29 (s, 1, 2-H), 10.02, 10.44 (br s, br s, 1, 1, NH_2). UV λ_{max}^{MeOH} nm (ε): 242 (17900), 274 (sh) (7400), 285 (7800), 333 (11600).

N^6,N^6-Dimethyladenosine-8-carbothioamide (25)——A solution of 0.79 g (2 mmol) of **24** in 50 ml of 75% formic acid was kept in a refrigerator for 2 d, then evaporated. The residue was dissolved in EtOH, and a small amount of formic acid was evaporated off by azeotropic distillation to give a syrup, which was crystallized from ether. Yellow small needles, mp 127—129 °C (sintered), 135—137 °C (melted), yield 0.68 g (90%). *Anal.* Calcd for $C_{13}H_{18}N_6O_4S \cdot 1/3H_2O$: C, 41.26; H, 5.51; N, 22.21. Found: C, 41.45; H, 5.24; N, 22.50. NMR (DMSO-d_6 + D_2O) δ: 3.52 (s, 6, NMe_2), 3.84 (m, 2, 5′-H), 4.18 (m, 1, 4′-H), 5.00 (q, 1, 2′-H), 7.26 (d, 1, $J=7$ Hz, 1′-H), 8.18 (s, 1, 2′-H). The signal of the proton at position 3′ overlapped with that of DOH. UV λ_{max}^{MeOH} nm (ε): 238 (20000), 274 (sh) (9200), 283 (9400), 325 (11500).

The Conversion of 24 to 14——A mixture of 0.39 g (1 mmol) of **24** in 30 ml of dry pyridine was refluxed for 48 h on an oil bath. After evaporation of the solvent, the residue was chromatographed on silica gel with $CHCl_3$–MeOH (95 : 5). The 1st eluate was collected to give a syrup, which was crystallized with ether. The crystals were recrystallized from MeOH. The compound (0.25 g, 69%) was identical with **14** obtained above (comparisons of the spectral and physical data).

Tumor System——For *in vitro* assay, L1210 cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells were plated at a density of 1×10^5 cells/ml in the medium containing drugs and grown in suspension for 4 d. After culture, the number of cells which excluded trypan blue dye was counted. For *in vivo* assay, ICR mice were inoculated i.p. with 1×10^5 cells/mouse of Ehrlich ascites tumor on day 0. The drugs were suspended in 0.5% carboxymethyl cellulose in physiological saline and given i.p. once daily from day 1 through day 5. The evaluation of activity was made in terms of T/C (tumor growth relative to the control).

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