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Studies of Nucleosides and Nucleotides. LXXXI.[1] Synthesis and Characterization of 8-Methyladenosine

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8-Methyladenosine (X) was synthesized by two ways starting from 2′,3′-O-isopropylidene-2-methylthioinosine (I). The compound (I) was methylated with *t*-butyl hydroperoxide in acidic media in the presence of ferrous ion to give 8-methyl compound (II) in a yield of 46%. Raney nickel dethiolation of II and acetylation at 5′-OH followed by chlorination using $SOCl_2$/DMF gave 6-chloro-8-methylpurine derivative (V). The compound (V) was treated with liq. NH_3 and deprotected with trifluoroacetic acid to give 8-methyladenosine (X). Alternatively II was acetylated at 5′-OH, chlorinated with Vilsmeyer–Haack reagent and treated with liq. NH_3 to give 2′,3′-O-isopropylidene-2-methylthio-8-methyladenosine (IX). The compound (IX) was deacetonized and dethiolated with Raney nickel to give X. The physical properties of X was elucidated by ultraviolet, circular dichroism and nuclear magnetic resonance spectra. A syn type conformation was assigned to 8-methyladenosine.

Keywords——radical reaction; *t*-butyl hydroperoxide; syn conformation; UV spectra; CD spectra; ^{1}H–NMR; ^{13}C–NMR

Previously we have synthesized a variety of 8-substituted purine nucleosides and nucleotides.[3–9] Especially, 8-bromoadenosine is valuable as a starting material for other 8-substituted adenosine derivatives and interesting as having syn conformation in crystals[10] as well as in solution.[11] In the circular dichroism (CD) study of 8-bromoadenosine,[11] it gave a positive Cotton band around ultraviolet (UV) absorption maximum in contrast to the ordinary purine nucleotides which show negative Cotton bands. However, as reported by Eyring and coworkers,[12] substitution at C-8 may alter transition moments in the adenine base from the unsubstituted one and may lead to a different coupling mode with magnetic moment(s) of the carbohydrate moiety. To circumvent this uncertainty it may be worthwhile to synthesize 8-methyladenosine, because of similar size and reversed electronic nature of the methyl group and the bromo atom. In fact a X-ray crystallographical study[13] showed thet 2-ethylthio-8-methylinosin[14] existed in a syn conformation. In the literature[15] 8-methyladenosine was synthesized directly from adenosine by a radical attack using *t*-butyl hydroperoxide in acidic media catalyzed by ferrous ion. However, the reaction proceeded rather sluggishly and the yield of 8-methyladenosine was extremely low. It seems to be rather

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13) N. Nagashima and K. Wakabayashi, *Acta Cryst.*, **B30** 1094 (1974).
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difficult to obtain a substantial amount of 8-methyladenosine by this method and its characterization has not been made satisfactorily.

In this paper we wish to report a versatile method for synthesizing 8-methyladenosine in quantity and some physical properties of this nucleoside as studied by UV, CD and nuclear magnetic resonance (NMR) spectra. We chose 2′,3′-O-isopropylidene-2-methylthioinosine (I) which was easily obtainable from aminoimidazolecarboxamide riboside by a method described by Yamazaki, *et al.*,[16] as the starting material. This is because in the radical methylation reaction[15] guanosine underwent methylation in good yield presumably due to an optimum susceptibility of the 8-carbon due to electronic properties of 2 and 6-substituents on the purine ring.

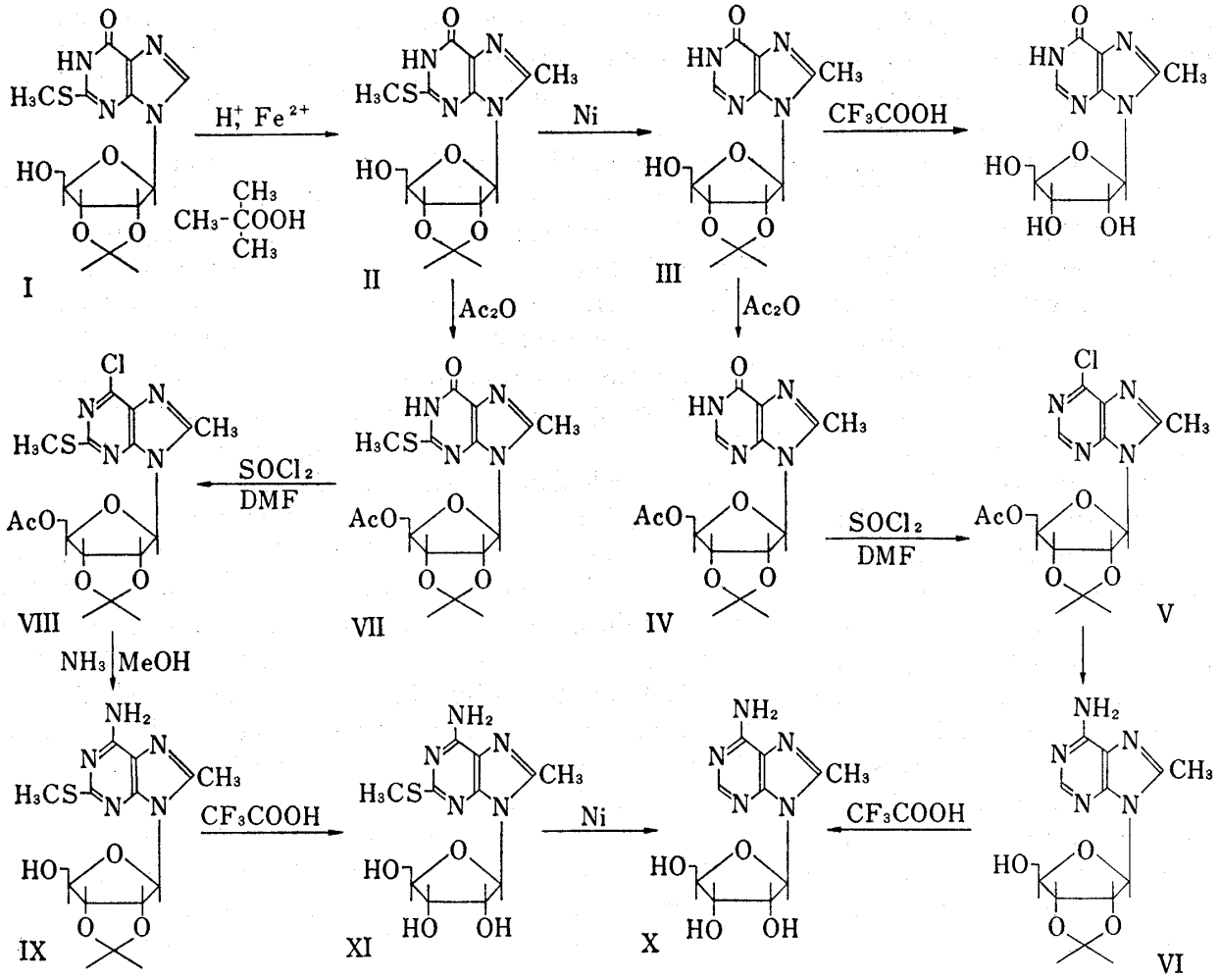

Chart 1

The compound (I) was dissolved in 1 N H_2SO_4 and 2 fold excess of *t*-butyl hydroperoxide was added dropwise in 1.5 hr at 0° in the presence of ferrous sulfate as a catalyst. By this procedure 2′,3′-O-isopropylidene-2-methylthio-8-methylinosine (II) was obtained in a yield of 46% as crystals of mp 234—236°. Although UV absorption of II did not change significantly from that of I, a NMR signal of 8-H at 8.09 ppm disappeared and a new signal at 2.65 ppm corresponding to 8-CH_3 appeared. Elemental analysis also supported the structure. The compound (II) was then subjected to the Raney nickel dethiolation to give 2′,3′-O-isopropylidene-8-methylinosine (III), mp 235—240° in a yield of 87.5%. By UV absorption showing $\lambda_{max}^{H_2O}$ 250 nm, a NMR signal at 8.36 ppm corresponding to 2-H and elemental analysis

16) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.* **32** 3032 (1967).

the structure of III was confirmed. Treatment of III with 90% trifluoroacetic acid gave 8-methylinosine, which is shown to be identical with an authentic sample.[14]

The compound (III) was then acetylated at 5′-OH with acetic anhydride in pyridine to give 5′-O-acetyl-2′,3′-O-isopropylidene-8-methylinosine (IV), mp 239—241° in a yield of 90%. In order to convert the 6-oxy function of IV to amino group, the compound IV was heated with Vilsmeyer-Haack reagent, which was prepared from thionyl chloride and N,N-dimethylformamide (DMF),[17] at refluxing temperature of $CHCl_3$ as the solvent. The 6-chloro compound (V), thus obtained, showed a single spot on thin-layer chromatography (TLC) and was used without further purification in the next step. The compound (V) was dissolved in anhydrous methanol and liquid ammonia was added under cooling with dry ice–acetone. The sealed tube was kept at room temperature for 18 hr and worked up as usual. 2′,3′-O-Isopropylidene-8-methyladenosine (VI) was obtained in a yield of 25% as colorless prisms of mp 240—243°. UV absorption maximum was found at 260 nm in 50% ethanol and in NMR spectrum a signal at 6.56 ppm corresponding to NH_2 group appeared. Elemental analysis showed correctness of the structure.

In order to improve the yield in the final amination step, an alternate route was investigated. 2′,3′-O-isopropylidene-2-methylthio-8-methylinosine (II) was first acetylated as usual to give 5′-O-acetyl derivative (VII), which showed a single spot on TLC, in almost quantitative yield. The compound (VII) was then subjected to the chlorination reaction by refluxing in chloroform with Vilsmeyer-Haack reagent. After conversion of 6-oxy to chloro function was confirmed by TLC which showed a single spot having UV absorption maxima at 233, 266 and 306 nm, the 6-chloro compound (VIII) was heated in anhydrous 9 N methanolic ammonia at 52° for 17 hr and then 70° for 1 hr. Work up of the reaction mixture and recrystallization from ethanol gave 2′,3′-O-isopropylidene-2-methylthio-8-methyladenosine (IX) as colorless crystals, mp 214—217° in a yield of 41.7% calculated from III. UV absorption properties showing $\lambda_{max}^{H_2O}$ 231 and 276.5 nm and elemental analysis supported the structure.

Deprotection of the compound(VI) was conducted by treating with 90% trifluoroacetic acid at room temperature for 2 hr. 8-Methyladenosine (X) mp 130—133° was obtained in a yield of 68%. The compound (X) was obtained also by an alternative way: *i.e.*, deprotection of the compound (IX) with 90% trifluoroacetic acid at room temperature for 1.5 hr to afford 2-methylthio-8-methyladenosine (XI), mp 126—132°, in a yield of 78%, followed by Raney nickel dethiolation to give 8-methyladenosine (X) in a yield of 44%. The structure of 8-methyladenosine was confirmed by elemental analysis, UV, CD and NMR spectra.

8-Methyladenosine (X) showed UV absorption properties having $\lambda_{max}^{H_2O}$ 261 nm (ε 15000). These values were as expected from the alkyl substitution at C-8 position, which might not affect largely transition moments of the adenine base. In contrast to our expectation the CD spectra as shown in Fig. 1 has a trough at 260 nm ([θ]=−3800) and a peak at 222 nm ([θ]=4600). This spectrum resembled to that of adenosine, but not to 8-bromoadenosine

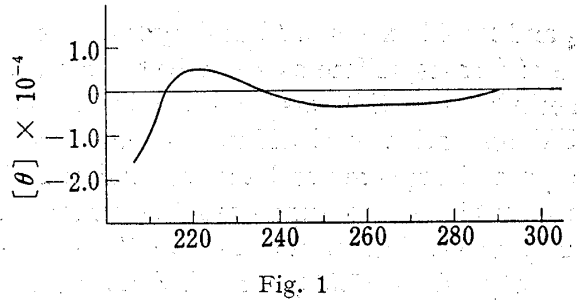

Fig. 1

Table I

	m^8Ad_0 (δ)		Ad_0 (δ)	Δδ
2-H	8.07	(s)	8.15	0.08
6-H	7.21	(s)	7.28	0.07
1′-H	5.84	(d)	5.89	0.05
2′-H	4.85	(q)	4.61	−0.16
3′-H	4.20	(s)	4.18	−0.02
4′-H	4.03	(s)	3.99	−0.04
8-CH_3	2.58	(s)		
8-H			8.32	

17) M. Ikehara, A. Uno, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **12**, 267 (1964).

which showed a positive band at around 264 nm.[11] As shown in Table I, ^{1}H–NMR spectrum of 8-methyladenosine showed base proton signals shifted slightly toward higher field relative to those of adenosine, indicating that a weak electron-releasing property of the 8-CH_3 group. In the carbohydrate moiety protons other than 1′ shifted to lower field and, especially for 2′-H, $\Delta\delta$ value is unusually large. The cause of this shift is not clearly understood yet, but conformational influence of N^3 atom to the magnetic circumstance of H-2′ may be accounted for this phenomenon. In ^{13}C-NMR this is much clearly indicated. As shown in Table II,

Table II

	m^8Ad_0 (δ)	Ad_0 (δ)	$\Delta\delta$
C-2	111.66	112.73	1.07
C-4	110.04	109.49	0.55
C-5	78.51	79.75	−1.24
C-6	115.79	116.51	0.72
C-8	109.38	100.32	−9.06
C-1′	49.02	48.39	−0.63
C-2′	32.48	33.88	0.90
C-3′	31.40	31.06	−0.34
C-4′	47.05	46.29	−0.76
C-5′	22.59	22.13	−0.46
CH_3-8	−25.23		

signal of the 8-methyl carbon appeared at −25.23 ppm and C-8 at 109.38 ppm. Other signals of base carbons shifted fluctually. In the carbohydrate moiety all carbon except for C-2′ shifted towards low field. By contrast C-2′ signal appeared at higher field by 0.90 ppm than that of adenosine. Again C-2′ is shown to be strongly affected by the base N^3. This type of shift of C-2′ was observed in all syn type nucleosides.[18,19]

From these studies it may be concluded that 8-methyladenosine takes a variable conformation more favorable in syn type than in anti. The sterical distortion of 8-methyl group as found for 2-ethylthio-8-methylinosine, which is known to take the syn conformation at least in crystal[13] may be responsible for this conformation. In NMR study 8-methyladenosine showed unusual shifts of C-2′ signals either in ^{1}H or ^{13}C spctra, which are corresponding to a syn conformation at least in time-average means. However, in CD study 8-methyladenosine showed a curve with negative Cotton band at around 260 nm same as observed for adenosine and in contrast to 8-bromoadenosine. This may mean that, though the 8-methyl group of 8-methyladenosine is acting as a sterical barrier for complete rotation of the base around glycosidic linkage, it will allow to take a "near-*anti*" conformation for 8-methyladenosine. Miles, *et al.*[20] reported that if adenosine takes a conformation having $\phi_{CN}=-140°$, $[\theta]$ of the B_{2u} Cotton effect will reach a value nearly −4000. Therefore, if we assume such a conformation for 8-methyladenosine, in which transition moments are not largely different from those of adenosine, this CD property could reasonably be interpreted. Although the cause to take this conformation is not clearly understood, a hydrophobic nature of 8-methyl group[21] may be responsible for it. When a CPK model of 8-methyladenosine was examined, 8-methyl group can situate close to the 2′-carbon atom and some specific interaction may assist to take this conformation. A recent experiment[22] that 8-methyladenosine 5′-diphosphate could be easily polymerized by the catalysis of polynucleotide phospholylase in contrast to its 8-bromo

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20) D.N. Miles, S.J. Hahn, R.K. Robins, M.J. Robins, and H. Eyring, *J. Phys. Chem.*, **72** 1483 (1968).
21) Y. Nozaki and C. Tanford, *J. Biol. Chem.*, **246**, 2211 (1971).
22) W. Limn, unpublished experiments.

counterpart[23] may be consonant with this view. The exact conformation of 8-methyladenosine must await further studies by NMR spectroscopy using shift reagent and X-ray crystallography. Synthesis of a variety of nucleotides and oligonucleotides of 8-methyladenosine is now in progress in our laboratory.

Experimental[24]

2′,3′-O-Isopropylidene-2-methylthio-8-methylinosine (II)——2′,3′-O-Isopropylidene-2-methylthioinosine (3.50 g) (I) was dissolved in 1 N H_2SO_4 (400 ml) containing $FeSO_4$ (11.1 g) at 0°. A solution of *t*-butyl hydroperoxide (3.60 g) dissolved in H_2O (90 ml) was added dropwise in 1.5 hr at 0° with stirring. After the addition, the stirring was maintained for 30 min. The reaction mixture was neutralized with 2 N NaOH to pH 3 and extracted with $CHCl_3$ (170 ml × 5). The $CHCl_3$ layer was dried over Na_2SO_4 and $CHCl_3$ was evaporated *in vacuo*. The residue was recrystallized from EtOH to give colorless needles, mp 234—236°, in a yield of 1.66 g (46%). *Anal.* Calcd. for $C_{15}H_{20}N_4O_5S$: C, 48.90; H, 5.47; N, 15.21; S, 8.70. Found: C, 48.51; H, 5.39; N, 15.11; S, 8.95. UV: $\lambda_{max}^{H_2O}$ 261, $\lambda_{shoulder}^{H_2O}$ 283 nm. *Rf* values are summarized in Table III and IV.

Table III

Compound	Solvent system A	Solvent system B
I	0.73	
II	0.72	0.79
III	0.74	0.74
IV	0.72	0.77
V	0.81	
X	0.72	
XI	0.84	

Table IV

Compound	Solvent system A	Solvent system B
I	0.08	0.44
II	0.07	0.50
III	0.07	0.46
IV	0.14	0.44
V	0.47	
VI	0.19	0.62
VII	0.24	
VIII	0.24	
IX	0.45	0.81
X		0.19
XI	0.05	0.33

2′,3′-O-Isopropylidene-8-methylinosine (III)——2′,3′-O-Isopropylidene-2-methylthio-8-methylinosine (II) (800 mg) dissolved in H_2O (18 ml) and dioxane (50 ml). The solution was refluxed for 4 hr with portional additions of Raney nickel (5.8 ml) under vigorous stirring. The catalyst was removed by filtration and washed with hot H_2O–dioxane. The filtrate and washings were combined and evaporated *in vacuo*. The residue was recrystallized from H_2O to give colorless needles, mp 234—236°, in a yield of 550 mg (87.5%). *Anal.* Calcd. for $C_{14}H_{18}N_4O_5$: 52.17; H, 5.63; N, 17.38. Found: C, 52.06; H, 5.89; N, 21.25. UV $\lambda_{max}^{H_2O}$ 250 nm. *Rf* values were shown in Table III and IV. A small amount of III was dissolved in 95% CF_3COOH (0.5 ml) and kept at room temperature for 1.5 hr. CF_3COOH was evaporated and removed thoroughly by azeotropic distillation several times. The residue was applied to paper chromatography in solvent B. Chromatography with an authentic sample showed the same *Rf* values. *Rf* (A) 0.56.

5′-O-Acetyl-2′,3′-O-isopropylidene-8-methylinosine (IV)——2′,3′-O-Isopropylidene-8-methylinosine (520 mg) was dissolved in pyridine (16 ml) and acetic anhydride (4 ml) were added. After the mixture was kept at room temperature for 10 hr, the solvent was evaporated *in vacuo*. The residue was recrystallized for EtOH to give colorless needles, mp 239—241°, in a yield of 529 mg (90%). *Anal.* Calcd. for $C_{16}H_{20}N_4O_6$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.83; H, 5.75; N, 15.37. UV: $\lambda_{max}^{H_2O}$ 250 nm. *Rf* values were in Table III and IV.

23) M. Ikehara, I. Tagawa, and T. Fukui, *Biochemistry.*, **8**, 736 (1969).

24) UV absorption spectra were taken with a Hitachi 124 or EPS-3T spectrophotometer, CD spectra were taken with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment, ^{1}H–NMR spectra were taken with a Hitachi R-22 spectrometer operated at 90 MHz with tetramethylsilane as internal standard. ^{13}C–NMR spectra were taken with a Hitachi R-22 spectrometer operated at 22.63 MHz in the Fourier transform mode in correction with a Hitachi-1011 computer using d_6-DMSO as internal standard. Paper chromatography was performed on Toyo filter paper 51A in solvent system: A, iso-PrOH–conc. NH_4OH–H_2O (7:1:2); B, BuOH–AcOH–H_2O (3:2:3). TLC were performed on Kieselgel plate in solvent system: A, $CHCl_3$–EtOH (16:1); B, $CHCl_3$–EtOH (1:5).

5′-O-Acetyl-2′,3′-O-isopropylidene-6-chloro-8-methyl-9-β-D-ribofuranosylpurine (V)——Thionyl chloride (0.96 ml) and DMF (4.48 ml) were added to $CHCl_3$ (14 ml) and kept at room temperature for 30 min under exclusion of moisture. Into the solution 5′-O-acetyl-2′,3′-O-isopropylidene-8-methylinosine (425 mg) was added and the mixture was refluxed for 2 hr. Solvent was removed by evaporation *in vacuo*, the residue taken up in $CHCl_3$ (30 ml), and washed with saturated $NaHCO_3$ and H_2O. The $CHCl_3$ solution was dried over Na_2SO_4 and evaporated *in vacuo*. The residual yellowish glass (594 mg) showed a single spot on TLC [$CHCl_3$–EtOH (6: 1)] at *Rf* 0.49. The starting material migrated at *Rf* 0.14. UV: $\lambda_{max}^{50\%EtOH}$ 262 nm. *Rf* (A) was listed in Table IV.

2′,3′-O-Isopropylidene-8-methyladenosine (VI)——The glass of compound (V) (590 mg) obtained as above, was dissolved in MeOH (0.8 ml) and transferred to a steel tube. Liq. NH_3 (20 ml) was added under cooling with dry ice-acetone bath and the sealed tube was kept at room temperature for 18 hr. NH_3 and MeOH was evaporated carefully and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was dried and evaporated *in vacuo*. The residue was recrystallized from EtOH to give pale yellow prisms, mp 240—243°, in a yield of 92 mg (25% calculated from IV). Sample for elemental analysis was recrystallized further from $CHCl_3$ to give colorless prisms. *Anal.* Calcd. for $C_{14}H_{19}N_5O_4$: C, 52.33; H, 5.96; N, 21.80. Found: C, 52.06; H, 5.89; N, 21.75. UV $\lambda_{max}^{50\%EtOH}$ 260 nm. *Rf* values are shown in Table III and IV.

5′-O-Acetyl-2′,3′-O-isopropylidene-2-methylthio-8-methylinosine (VII)——2′,3′-O-Isopropyridene-2-methylthio 8-methylinosine (2.67 g) was dissolved in pyridine (70 ml) and acetic anhydride (14 ml) was added. The mixture was kept at room temperature overnight and the solvent was evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ (120 ml), washed with saturated $NaHCO_3$ and water, and dried over $Na_2SO_4 \cdot CHCl_3$ was evaporated *in vacuo* and the residue (3.11 g) was obtained. This sample showed a single spot in TLC $CHCl_3$ at *Rf* 0.24 and had $\lambda_{max}^{50\%EtOH}$ at 261 and 283 nm.

5′-O-Acetyl-2′,3′-O-isopropylidene-6-chloro-2-methylthio-9-β-D-ribofuranosylpurine (VIII)——Thionyl chloride (4.89 ml) and DMF (2.25 ml) were dissolved in $CHCl_3$ (69 ml) and the mixture was kept at room temperature for 30 min. Into the solution was added 5′-O-acetyl-2′,3′-O-isopropylidene-2-methylthio-8-methylinosine (3.11 g) and the mixture was refluxed for 2 hr. After cooling the reaction mixture was evaporated *in vacuo*, taken up in $CHCl_3$ (120 ml) and washed with sat. $NaHCO_3$ (100 ml) and H_2O (120 ml × 2), successively. After drying over Na_2SO_4, $CHCl_3$ was removed by evaporation *in vacuo*. The residue showed a single spot in TLC. $\lambda_{max}^{H_2O}$ 233, 266, 306 nm. *Rf* values are shown in Table III and IV.

2′,3′-O-Isopropylidene-2-methylthio-8-methyladenosine (IX)——5′-O-Acetyl-2′,3′-O-isopropylidene-6-chloro-2-methylthio-9-β-D-ribofuranosylpurine (100 mg) was dissolved in 9N methanolic ammonia (170 ml) under cooling with dry ice–acetone. The reaction mixture was sealed in a steel tube and was heated at 52° for 17 hr and at 70° for 1 hr. The reaction mixture was evaporated carefully to remove NH_3 and MeOH. The residue was dissolved in $CHCl_3$ (120 ml) and washed with H_2O (100 ml). After drying over Na_2SO_4 the solvent was evaporated *in vacuo* to give a residue, which was recrystallized from EtOH to give yellow prisms, mp 214—217°, in a yield of 1.11 g (41.7% calculated from II). *Anal.* Calcd. for $C_{15}H_{21}N_5O_4S$: C, 49.03; H, 5.76; N, 19.06, S, 8.73. Found: C, 49.03; H, 5.94; N, 18.85; S, 8.82. UV: $\lambda_{max}^{50\%EtOH}$ 232, 276 nm. *Rf* values are shown in Table IV.

2-Methylthio-8-methyladenosine (XI)——2′,3′-O-Isopropylidene-2-methylthio-8-methyladenosine (220 mg) was dissolved in 90% trifluoroacetic acid (3.5 ml) and kept at room temperature for 1.5 hr. The reaction mixture was evaporated *in vacuo* and the residue was made free from trace of trifluoroacetic acid by azeotropic distillation three times. The residue was dissolved in a small amount of H_2O and neutralized with dil. NH_4OH. MeOH was evaporated *in vacuo* to give crystalline residue, which was recrystallized from H_2O, mp 126—132°. Yield was 152 mg (77.6%). *Anal.* Calcd. for $C_{12}H_{17}N_5O_3S$: C, 44.03; N, 5.23; N, 21.39. Found: C, 44.10; H, 5.04; N, 21.55. UV $\lambda_{max}^{H_2O}$ 231.5, 276.5 nm. *Rf* values are shown in Table III and IV.

8-Methyladenosine (X)——i) 2-Methylthio-8-methyladenosine (138 mg) was dissolved in dioxane–H_2O (3: 1, vol/vol. 10 ml) and refluxed for 6 hr in the presence of Raney nickel (1 ml) with stirring. The catalyst was filtered and washed with hot dioxane–H_2O several times. The filtrate and washings were combined and evaporated *in vacuo* to give a glass. Coevaporations of the residue several times with H_2O gave colorless crystals, which were recrystallized from H_2O, mp 125—130°. Yield was 52 mg (44.2%). *Anal.* Calcd. for $C_{11}H_{15}N_5O_4 \cdot 1/2H_2O$: C, 45.51; H, 5.56; N, 24.13. Found: C, 45.24; H, 5.20; N, 24.31 Drying of this sample over P_2O_5 at 60—70° under 10 mm/Hg gave an analytical value for $C_{11}H_{15}N_5O_4 \cdot 1/3H_2O$: C, 45.99; H, 5.50; N, 24.38. Found: C, 46.00; H, 5.23; N, 24.27. UV: $\lambda_{max}^{H_2O}$ 261 nm (ε 15800), $\lambda_{max}^{pH\,2}$ (16500), $\lambda_{max}^{pH\,12}$ 261 (15300). NMR: signals appeared as summarized in Table I and II. CD: λ_{trough} 262 ($[\theta] = 0.48 \times 10^4$), λ_{peak} 220 (0.56×10^4). *Rf* values are shown in Table III and IV.

ii) 2′,3′-O-Isopropylidene-8-methyladenosine (180 mg) was dissolved in 90% CF_3COOH (5 ml) and kept at room temperature for 2 hr. The reaction mixture was evaporated *in vacuo* to *ca.* 1 ml and applied to a column (1.1 × 23.5 cm) of charcoal. Elution with 50% EtOH containing 5% conc. NH_4OH gave 8-methyladenosine in a yield of 80 mg (68%), mp 130—133°. Cochromatography with the sample obtained in i) showed identical *Rf* values as summarized in Table III and IV.

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