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Studies of Nucleosides and Nucleotides. LVII.[1] Purine Cyclonucleosides. (20).[2] Synthesis and Reactions of 8,5'-S-Cycloadenosine Derivatives

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Starting from 2',3'-isopropylidene-8,5'-anhydro-8-mercaptoinosine (II), synthesized from the corresponding adenosine derivative (I) by deamination with sodium nitrite, various 6-substituted analogs were synthesized. Compound (II) was converted to 6-chloropurine derivative (III) by the reaction with $POCl_3$ and tri-*n*-butylamine at refluxing temperature in 75% yield. Compound (III) was allowed to react either with methylamine, dimethylamine or sodium methylmercaptide to give N^6-methyl (IV), N^6-dimethyl (IX) and 6-methylmercapto (X) derivatives, respectively. Ultraviolet spectrum, circular dichroism and mass spectra of these compounds are listed.

Compound (IV) was alternatively obtained by a Dimroth type rearrangement of N^1-methyl derivative of I by heating in sodium hydroxide solution at pH 8—9. The same type of rearrangement occured also with unprotected 1-methyladenosine-8,5'-S-cyclonucleoside to give compound (VIII).

We have studied synthesis and properties of various purine 8-cyclonucleosides.[4] Among these cyclonucleosides 2',3'-isopropylidene-8,5'-anhydro-8-mercaptoadenosine (I)[5] is especially interesting because its fine three dimensional structure is elucidated by X-ray crystallography[6] and it was found that compound (I) had antiviral activity.[7]

In this paper we describe synthesis of variously substituted derivatives of the purine cyclonucleoside, *i.e.* 6-oxy, 6-chloro, N^6-methyl, N^6-dimethyl and 6-methylmercapto compounds, and their ultraviolet spectrum (UV), circular dichroism (CD) and mass spectra. A Dimroth type rearrangement[8] of N^1-methyl derivative of I is also reported.

The starting material 6-oxy compound (II) has been obtained by the deamination of compound (I) with barium nitrite in acetic acid.[9] However, for completion of the reaction 3—5 days were required. In the present study sodium nitrite was used in 80% acetic acid and after 1 day's reaction the product was extracted with $CHCl_3$ from the reaction mixture. By this procedure 2',3'-isopropylidene-8,5'-anhydro-8-mercaptoinosine (II) was obtained in a yield of 87—90%.

Compound (II) was then treated with $SOCl_2$/dimethyl formamide (DMF) to chlorinate the 6-OH group as in the case of inosine.[10] Although examination of the reaction mixture by thin-layer chromatography (TLC) showed a single spot of the product, isolation of the product in a pure state was failed. The reagent was then changed to $POCl_3$ in tri-*n*-butylamine. After refluxing the reaction mixture for 30 min UV λ_{max} changed from 278 to 290 nm. By

1) Part LVI: M. Ikehara, E. Ohtsuka and S. Uesugi, *Chem. Pharm. Bull.* (Tokyo), **21**, 444 (1973).
2) Part 19: M. Ikehara and Y. Ogiso, *Tetrahedron*, **28**, 3695 (1972).
3) Location: *6-1-1, Toneyama, Toyonaka, Osaka.*
4) M. Ikehara, *Accounts of Chem. Res.*, **2**, 47 (1969) and subsequent papers.
5) M. Ikehara, M. Kaneko and M. Sagai, *Tetrahedron*, **26**, 5757 (1972).
6) K. Tomita, T. Nishida, T. Fujiwara and M. Ikehara, *Biochem. Biophys. Res. Commun.*, **41**, 1043 (1970).
7) Unpublished results.
8) O. Dimroth, *Ann. Chem.*, **373**, 336 (1910).
9) M. Ikehara and M. Muraoka, *Chem. Pharm. Bull.* (Tokyo), **20**, 550 (1972).
10) M. Ikehara, H. Uno and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **12**, 267 (1964); M. Ikehara and H. Uno, *ibid.*, **13**, 221 (1965).

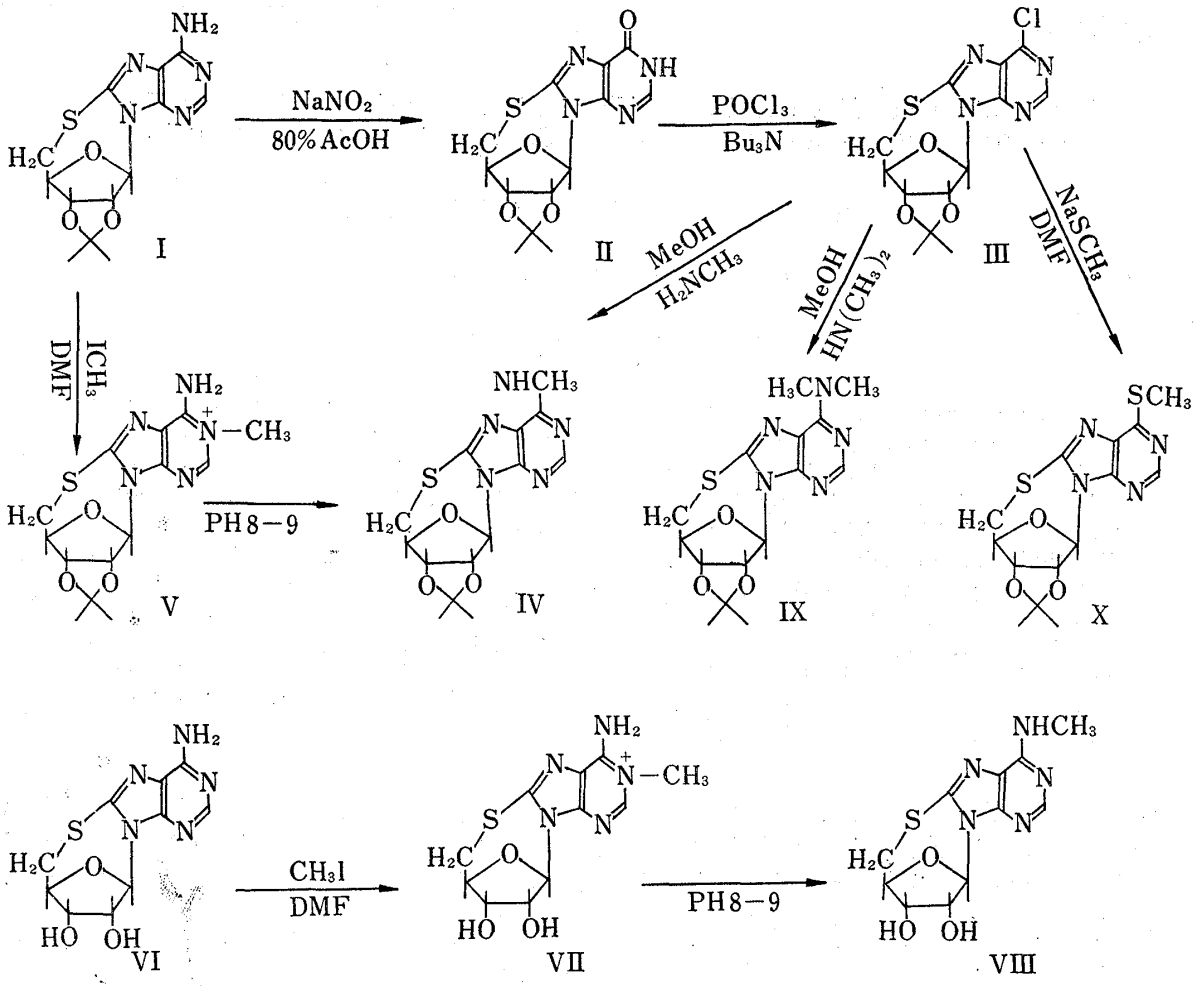

Chart 1

the usual work-up 2′,3′-O-isopropylidene-8,5′-anhydro-6-chloro-8-mercapto-9-β-D-ribofuranosylpurine (III) was obtained in 75—80% yield. The structure of III was confirmed by elemental analysis, UV absorption, CD and mass spectra. The UV absorption spectra closely resembled those of 6-chloropurine 8,2′-S-cyclonucleoside.[9] As shown in Fig. 1, CD spectrum of compound III has peaks at 290 and 300 nm, and troughs at 240 and 259 nm. These features suggest that the compound (III) had a cyclonucleoside structure as found for authentic cyclonucleosides.[11] The mass spectrum of compound (III) (Table II) showed a molecular ion peak at *m/e* 324 as the highest peak (100%) accompanied with a peak at *m/e* 326 corresponding to ^{37}Cl compound (41%). These features are characteristic for the chlorine-containing 8,5′-cyclonucleoside, because adenine-8-cyclonucleosides are known to give the strongest molecular ion peak[12] and the natural abundance of ^{37}Cl is 25% of ^{35}Cl. Other fragment ion peaks were also consistent with those found in other cyclonucleosides.[9,13]

Compound (III) was then allowed to react with excess methylamine at 70—80° for 3 hr. The work-up of the reaction mixture gave N^6-methyl derivative (IV) as white needles of mp 280—283° in a yield of 71%. UV absorption of compound (IV) had λ_{max} around 292 nm in acidic and neutral and 287 nm in alkaline condition. The spectrum resembled that found for N^6-dimethyl derivative described below but slightly shifted towards blue suggesting

11) M. Ikehara, M. Kaneko, Y. Nakahara, S. Yamada and S. Uesugi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1381 (1971).
12) M. Ikeda, Y. Tamura and M. Ikehara, *J. Heterocyclic Chem.*, **7**, 1377 (1970).
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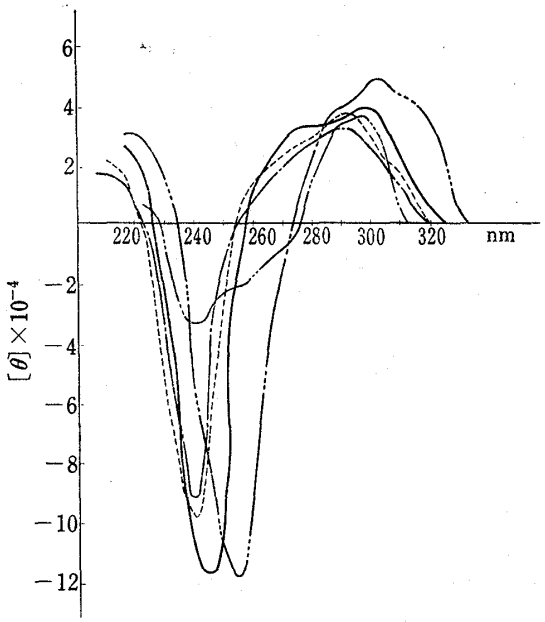


Fig. 1. Circular Dichroism Spectra of 8,5′-S-Cycloadenosines

——: III ----: IV ——: IX
——: X —·—: VIII

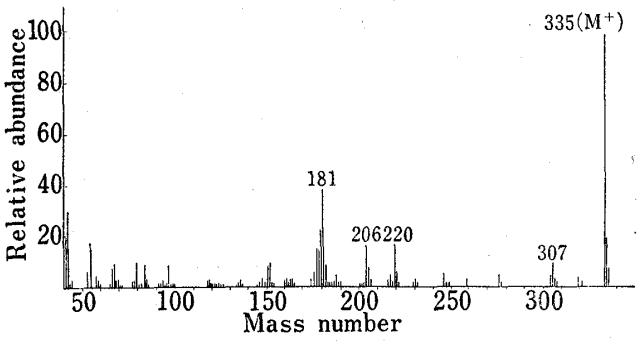


Fig. 2. Mass Spectrum of 2′,3′-O-Isopropylidene-N^6-methyl-8,5′-anhydro-8-mercaptoadenosine

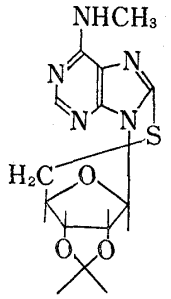


Table I. Mass Number and Abundance of Major Peaks in Mass Spectra of 8,5′-S-Cyclonucleosides

Compound	Principal mass number (Abundance %)							
III	341(20.9)	339(51)	326(41)	324(100)	285(18)	283(45)	188(11)	186(31)
IV	335(100)	307(7)	278(3.8)	220(16)	206(16)	181(39)		
VIII	295(93)	206(18)	181(100)	153(37)				
IX	349(100)	334(20)	320(59)	194(29)	192(29)	165(39)		
X	353(100)	199(26)	198(38)	197(82)				

that N^6 atom was substituted with one methyl group. CD spectrum of IV (Fig. 1) had a maximum at 290 nm and a large trough at 240 nm in agreement with the proposed cyclonucleoside structure.[11] In the mass spectrum (Fig. 2 and Table I), a large peak corresponding to the molecular ion (*m/e* 333) was observed. In contrast to the previous observation[13,14] that an ion missing CH_3 group and CH_2=NH (M-29) appeared as a principal peak from N^6-dimethyladenosine, in the present case it appeared as a minor peak. Furthermore, a peak of *m/e* 181 corresponding to 8-mercapto-6-methylaminopurine appeared in 39% abundance. This fragmentation pattern suggests that 6-$NHCH_3$ group in compound (IV) is fairly stable and peaks other than the molecular ion peak appeared rather poorly.

In order to test the possibility of obtaining N^6-methyl derivative *via* a Dimroth rearrangement, we first methylated 2′,3′-isopropylidene-8,5′-S-cycloadenosine (I) with excess methyl iodide in DMF at room temperature. After 24 hr I was converted to a substance (V) having lower *Rf* on paper chromatogram and faster migration in paper electrophoresis. UV λ_{max} changed to 284 nm at neutral condition, which resembled the spectrum of I in acidic condi-

14) S.T. Shaw, D.M. Desederio, K. Tsuboyama and J.A. McClosky, *J. Am. Chem. Soc.*, **92**, 2510 (1970).

tion. These facts suggest that the methylation occurred at N^1-position. Compound (V) was then refluxed in water pf pH 8—9 according to the condition of the rearrangement of N^1-methyladenosine.[15] After refluxing for 5 hr, a single spot was observed on TLC. White needles obtained after work-up were shown to be identical in every respects with the N^6-methyl derivative (IV) obtained as above. The overall yield from compound (I) was 68%. This rearrangement reaction was found to occur also in 1N NaOH at room temperature for 10 hr. Since it has been found[16] that 2′,3′-O-isopropylidene group influenced the ease of 8,5′-S cyclization probably by the sterical factor, the influence of isopropylidene group to the stability of the cyclonucleosides during the rearrangement was investigated using unprotected 8,5′-S-cycloadenosine (VI).[5] When compound VI was methylated with excess ICH_3 as above, powdery N^1-methyl derivative (VII) was obtained. Treatment of VII in water of pH 8—9 for 5 hr at refluxing temperature gave a crystalline compound, mp 280—283°. This was confirmed as 8,5′-anhydro-8-mercapto-N^6-methyladenosine (VIII) by elemental analysis, UV and mass spectra. The UV spectra closely resembled those of compound IV. In the mass spectrum a molecular ion peak (*m/e* 294) appeared in 93% abundance and a peak corresponding to 8-mercapto-N^6-methyladenine (*m/e* 181) appeared in 100% abundance. In CD spectrum compound VIII showed a positive Cotton effect at the B band region. These results clearly demonstrate that a rearrangement of Dimroth type could occur on 8,5′-S-cycloadenosine without any cleavage of the cyclonucleoside bond.

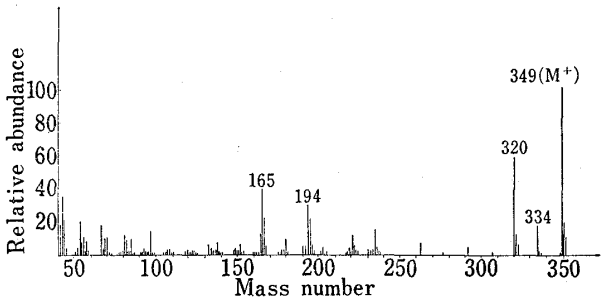
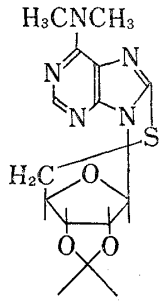


Fig. 3. Mass Spectrum of 2′,3′-O-Isopropylidene-N^6-dimethyl-8,5′-anhydro-8-mercapotoadenosine



The reaction of compound (III) with excess dimethylamine easily afforded 2′,3′-isopropylidene-8,5′-anhydro-8-mercapto-N^6-dimethyladenosine (IX), mp 144—145°, in a yield of 90%. UV absorption properties closely resembled those of 8,2′- or 8,3′-S-cyclo-N^6-dimethyladenosine synthesized previously.[13] Elemental analysis, CD and mass spectra also supported this structure. In the mass spectrum (Fig. 3) M^+ appeared as the highest peak and M-29 ion appeared at 59% abundance. Other fragmentation patterns were as observed in the case of dimethyladenosine cyclonucleosides.[13] Thus three cyclonucleosides having 8,2′-, 8,3′- and 8,5′-anhydro bonds of N^6-dimethyladenosine were synthesized.

Compound (III) was finally allowed to react with sodium methyl mercaptide. As reported for the substitution of 8-bromo atom of adenosine derivatives,[17] $NaSCH_3$ in DMF was an excellent reagent and afforded 6-methylmercapto derivative (X) at room temperature of 1.5 hr in a yield of 62%. The UV spectrum of X (λ_{max} 254, 302 and 313 nm) suggested the 6,8-dialkylthiopurine structure. Elemental analysis, mass and CD spectra proved the structure to be correct. In the mass spectrum a relatively simple fragmentation pattern involving a molecular ion (100%) and an ion corresponding to 6-methylmercapto-8-mercapto purine was observed. The biological activity of these cyclonucleoside derivatives will be reported elsewhere.

15) P. Brooks, A. Dipple and P.D. Lawley, *J. Chem. Soc.*, **1968**, 2026.
16) M. Ikehara, M. Kaneko and M. Sagai, *Chem. Pharm. Bull.* (Tokyo), **16**, 1151 (1968).
17) M. Ikehara, E. Ohtsuka and S. Uesugi, *Chem. Pharm. Bull.* (Tokyo), **21**, 444 (1973).

Experimental[18]

2′,3′-O-Isopropylidene-8,5′-anhydro-8-mercaptoinosine (II)——2′,3′-O-Isopropylidene-8,5′-anhydro-8-mercaptoadenosine (I) (321 mg, 1 mmole) was dissolved in 80% acetic acid (20 ml). To this solution was added sodium nitrite (365 mg, 5 mmole) and the mixture was kept at room temperature for 1 day in a stoppered flask. After the reaction extent was examined by TLC ($CHCl_3$–EtOH, 15: 1), acetic acid was evaporated *in vacuo*. Residue was extracted with chloroform and the extracts were recrystallized from benzene. Amorphous powder (280—289 mg) was obtained in yields of 87—90%. A small amount of this sample was treated with 80% acetic acid to afford 8,5′-anhydro-8-mercaptoinosine, which shows identical *Rf* value and UV absorption with an authentic sample.[9] TLC ($CHCl_3$–EtOH, 15: 1): *Rf* 0.25.

Table II. UV Absorption Properties of 8,5′-S-Cyclonucleosides

Compound	$\lambda_{max}^{pH\ 7}$ nm (ε)	$\lambda_{max}^{pH\ 11}$ nm (ε)	$\lambda_{max}^{pH\ 1}$ nm (ε)
III[a]	190(19600)	290(19500)	290(19300)
	296(18400)sh[b]	296(17900)sh	296(18100)sh
	237(5900)	237(7300)	237(6300)
IV	292(21200)	292(20900)	287(24700)
	285(19700)sh	285(19500)sh	285(19500)
	303(14300)sh	303(14300)sh	
	239(10100)	239(10300)	235(6800)
VIII	292(19100)	292(20800)	287(24100)
	295(18100)sh	295(19100)sh	
	303(13100)sh	303(13900)sh	
	240(9000)	240(9600)	235(6900)
IX	298(22100)	298(21100)	289(24200)
	291(20500)sh	291(20300)sh	
	313(13600)sh	313(13600)sh	
	243(11700)	243(11900)	239(6500)
X[a]	302(28100)	302(27000)	302(27100)
	313(25800)	313(25600)	313(24900)
	254(11600)	254(12100)	254(13100)

a) measured in EtOH–H_2O (1: 1) *b*) shoulder

2′,3′-O-Isopropylidene-8,5′-anhydro-6-chloro-8-mercapto-9-β-D-ribofuranosyl Purine (III)——Compound (II) (322 mg, 1 mmole) was dissolved in a mixture of $POCl_3$ (6 ml) and tri-*n*-butylamine (0.4 ml). The mixture was refluxed under exclusion of moisture for 30 min. Examination of the reaction mixture by TLC ($CHCl_3$–EtOH, 15 ml) gave a single spot at *Rf* 0.75 (starting material *Rf* 0.25). The mixture was poured into saturated aqueous sodium carbonate solution with stirring. The pH of the solution was carefully maintained above 7.0 by adding 1N NaOH. After 3—4 hr stirring solid material was collected by filtration and recrystallized from methanol. Yield 255 mg (75%). *Anal.* Calcd. for $C_{13}H_{13}O_3N_4SCl$: C, 45.82; H, 3.85; N, 16.37; S, 9.12; Cl, 10.40. Found: C, 45.74; H, 3.59; N, 16.37; S, 9.12; Cl, 10.44. UV absorption properties are summarized in Table I. CD spectrum was shown in Fig. 1. Major fractions in mass spectrum are summarized in Table II. TLC ($CHCl_3$–EtOH, 15: 1): *Rf* 0.75.

2′,3′-O-Isopropylidene-8,5′-anhydro-8-mercapto-N⁶-methyladenosine (IV)——i) From 6-Chloro Derivative: 6-Chloro-cyclonucleoside (III) (340 mg, 1 mmole) was dissolved in methanol (100 ml) and 20% aqueous methylamine (30 ml). The mixture was heated in a sealed tube at 70—80° for 3 hr. TLC ($CHCl_3$–EtOH, 15: 1) at this point showed a single spot of the product at *Rf* 0.54. The solvent was evaporated *in vacuo* and the residue was recrystallized from water. White needles, mp 144—145° was obtained (301 mg). Yield was 90%. *Anal.* Calcd. for $C_{14}H_{17}O_3N_5S$: C, 49.31; H, 5.07; N, 20.90; S, 9.47. Found: C, 49.52; H, 5.12; N, 20.62; S, 9.55. UV absorption properties are summarized in Table I. CD spectrum was as shown in Fig.

18) UV spectra were taken with a Hitachi EPS-3T or 124 spectrophotometer, IR spectra were taken with a Hitachi EPI-L spectrophotometer, mass spectra were taken with a Hitachi RMU-6E2 mass spectrometer and CD curves were measured with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment. Concentration was adjusted to 1—2 OD/ml. The measurement was performed at 20° in a 10 mm light-path cell. UV absorption properties and peaks in mass spectra are tabulated in Table I and II.

1. Mass spectrum is shown in Fig. 2 and major fragments are summarized in Table II. TLC ($CHCl_3$–EtOH, 15: 1): *Rf* 0.54.

ii) By the Dimroth Rearrangement: 2′,3′-Isopropylidene-8,5′-S-cycloadenosine (I) (321 mg, 1 mmole) was dissolved in DMF (10 ml) and to the solution was added methyl iodide (5 ml). The mixture was kept at room temperature for 1 day in a sealed tube. TLC ($CHCl_3$–EtOH, 15: 1) showed a single spot at *Rf* 0.19. (Starting material *Rf* 0.42). The UV absorption of the material extracted from the spot of *Rf* 0.19 showed $\lambda_{max}^{pH\ 1}$ 284 nm, $\lambda_{max}^{pH\ 7}$ 284 nm and $\lambda_{max}^{pH\ 13}$ 280, 289 nm. This material migrated as having positive charge (R_{AMP}0.95). The reaction mixture was poured into chloroform and yellow precipitates were obtained by decantation. The yellow solid was dissolved in 20 ml of water and the solution was adjusted to pH 8—9 with dil. aqueous sodium hydroxide. After refluxing for 5 hr, the material having *Rf* 0.19 disappeared and a new spot having *Rf* 0.45 appeared. Neutralization of the reaction mixture with 0.1N HCl and evaporation *in vacuo* gave a glassy residue. The residue was recrystallized from water and isopropylidene-8,5′-S-cyclo-N^6-methyladenosine, mp 144—145° (221 mg) was obtained in an overall yield of 68%. This material was identical with a sample obtained above.

8,5′-Anhydro-8-mercapto-N^6-methyladenosine (VIII)——8,5′-Anhydro-8-mercaptoadenosine (100 mg) was dissolved in DMF (5 ml). After the addition of methyl iodide (2 ml), the mixture was kept at room temperature for 1 day in a stoppered flask. Examination with TLC ($CHCl_3$–EtOH, 1: 1) showed conversion of the starting material (*Rf* 0.51) to a product (*Rf* 0.20). Pouring the reaction mixture into chloroform or ether gave a powder, which had UV absorption maxima at 284 nm in acidic and neutral solution and 280 and 289 nm in alkaline solution. This material was dissolved in water of pH 8—9 (10 ml) and refluxed for 5 hr for completion of the rearrangement. Evaporation of the solvent and recrystallization of the residue from EtOH gave colorless needles, mp 280—283° (74.5 mg), in a yield of 71%. *Anal.* Calcd. for $C_{11}H_{13}O_3N_5S$: C, 44.74; H, 4.44; N, 23.71; S, 10.86. Found: C, 44.46; H, 4.24; N, 23.38; S, 10.88. UV spectra data are summarized in Table I. CD spectrum is shown in Fig. 1. Major peaks in mass spectrum are described in Table II. TLC ($CHCl_3$–EtOH, 15: 1): *Rf* 0.57.

2′,3′-O-Isopropylidene-8,5′-anhydro-N^6-dimethyladenosine (IX)——6-Chloro derivative (III) (340 mg, 1 mmole) was dissolved in methanol (100 ml) and 20% aqueous dimethylamine (20 ml). The mixture was heated at 70—80° in a sealed tube for 3 hr. TLC at this stage showed a spot having *Rf* 0.75 (starting material 0.56). The solvent was evaporated *in vacuo* and the residue was recrystallized from water. White crystals, mp 144—145°, were obtained in a yield of 90%. *Anal.* Calcd. for $C_{15}H_{19}O_3N_5S$: C, 51.74; H, 5.17; N, 20.13; S, 9.19. Found: C, 51.49; H, 5.57; N, 20.06; S, 9.29. UV absorption properties are summarized in Table I. CD spectrum is shown in Fig. 1. Mass spectrum is shown in Fig. 3 and major peaks are listed in Table II.

2′,3′-O-Isopropylidene-8,5′-anhydro-6-methylmercapto-8-mercapto-9-β-D-ribofuranosylpurine (X)——6-Chloro compound (III) (340 mg, 1 mmole) was dissolved in DMF (20 ml) containing $NaSCH_3$ (3 equivalents). The mixture was kept at room temperature for 2 hr. Examination with TLC ($CHCl_3$–EtOH, 15: 1) showed a spot corresponding to the product appeared at *Rf* 0.57 (starting material 0.75). The reaction mixture was neutralized with 1N HCl and N_2 gas was bubbled through to remove methyl mercaptan. Evaporation of the solvent *in vacuo* and recrystallization of the residue from methanol–$CHCl_3$ gave colorless crystals, mp 217—218° in a yield of 62% (218 mg). *Anal.* Calcd. for $C_{14}H_{16}O_3N_4S_2$: C, 47.72; H, 4.58; N, 15.90; S, 18.13. Found: C, 47.77; H, 4.80; N, 15.62; S, 17.96. UV absorption properties are summarized in Table I. CD spectrum is shown in Fig. 1. Major peaks in mass spectrum are listed in Table II. TLC ($CHCl_3$–EtOH, 15: 1): *Rf* 0.57.

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