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Studies of Nucleosides and Nucleotides. LI. Purine Cyclonucleosides. (16). Synthesis of Cyclonucleosides derived from N^6-Dimethyladenosine[1]

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N^6-Dimethyladenosine was derived to 8-bromo-2′- and 3′-TPS compounds by bromination with bromine-water at pH 4, followed by the reaction with NaH and TPS-Cl in DMF. From 2′-TPS compound 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyl-N^6-dimethyladenine (8,2′-S-cyclonucleoside) and from 3′-TPS compound 8,3′-anhydro-8-mercapto-9-β-D-xylofuranosyl-N^6-dimethyladenine (8,3′-S-cyclonucleoside) were obtained. Structure of these cyclonucleosides were determined by ultraviolet (UV), nuclear magnetic resonance (NMR), circular dichroism (CD) and mass spectra. Desulfurization with Raney nickel gave 2′-deoxy and 3′-deoxy-N^6-dimethyladenosine, respectively. Treatment of 2′- and 3′-TPS compound with NaOAc in acetic acid-acetic anhydride mixture, followed by cyclization with ammonia-methanol gave 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyl-(8, 2′-O-cyclonucleoside) and 8,3′-anhydro-8-oxy-9-β-D-xylofuranosyl-N^6-dimethyladenine (8,3′-O-cyclonucleoside), respectively. The structure of these cyclonucleoside was determined by UV, NMR, CD and mass spectra.

N^6-Dimethyladenine is found in nature as the component of RNA[3] and as 3′-deoxy-3′-amino nucleoside in antibiotic puromaycin.[4] We have previously reported the synthesis of N^6-dimethyladenosine 5′-monophosphate,[5] 5′-triphosphate[6] and trinucleotides, N^6-dimethyl-ApApA and N^6-dimethyl-ApCpC.[7] The latter compounds were tested as the messenger coding for lysine and threonine in the stimulation of binding of aminoacyl-tRNA's with ribosomes.[8] On the other hands, purine cyclonucleosides were found to be useful intermediates for nucleoside transformations and for the model of nucleosides in which the base and the sugar moieties were fixed by anhydro linkages.[9] We, therefore, synthesized cyclonucleosides involving 8,2′- and 8,3′-S or O linkages derived from N^6-dimethyladenosine.

N^6-Dimethyladenosine (II) was synthesized from 2′,3′,5′-tri-O-acetylinosine (I) by the treatment with DMF-thionyl chloride complex in chloroform,[10] followed by the reaction with dimethylamine for replacement of 6-chloro to dimethylamino group and deprotection in the sugar moiety. By this procedure N^6-dimethyladenosine was obtained in a yield of 58% and this specimen was identical with an authentic sample.[11] N^6-Dimethyladenosine (II) was then brominated with bromine-water in 0.5 M sodium acetate buffer of pH 4 at room temperature.[12] As compared to the case of adenosine,[13] rate of the reaction and yield of

1) Part L: M. Ikehara, Y. Nakahara, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **19**, 538 (1971).
2) Location: 6—5, *Toneyama, Toyonaka, Osaka.*
3) D.B. Dunn, *Biochim. Biophys. Acta*, **34**, 286 (1959).
4) B.R. Baker, R.E. Schaub, and J.H. Williams, *J. Am. Chem. Soc.*, **77**, 7 (1955).
5) M. Ikehara, E. Ohtsuka, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **9**, 173 (1961).
6) M. Ikehara, E. Ohtsuka, S. Kitagawa, K. Yagi, and Y. Tonomura, *J. Am. Chem. Soc.*, **83**, 2679 (1961).
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8) M. Ikehara and E. Ohtsuka, *Biochem. Biophys. Res. Commun.*, **21**, 257 (1965).
9) M. Ikehara, *Accounts of Chem. Res.*, **2**, 47 (1969).
10) M. Ikehara, H. Uno, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **12**, 267 (1964).
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12) M. Ikehara, S. Uesugi, and M. Kaneko, *Chem. Commun.*, **17** (1967); M. Ikehara and S. Uesugi, *Chem. Pharm. Bull.* (Tokyo), **17**, 348 (1969).
13) M. Ikehara and M. Kaneko, *Tetrahedron*, **26**, 4251 (1970).

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8-bromo-N^6-dimethyladenosine (III) were fairly high. This is presumably due to increased electron density at C-8 by the introduction of N^6-dimethyl group. The structure of compound (III) was confirmed by elemental analysis and ultraviolet (UV) absorption spectra which are slightly shifted bathochromically from those of 8-bromoadenosine.

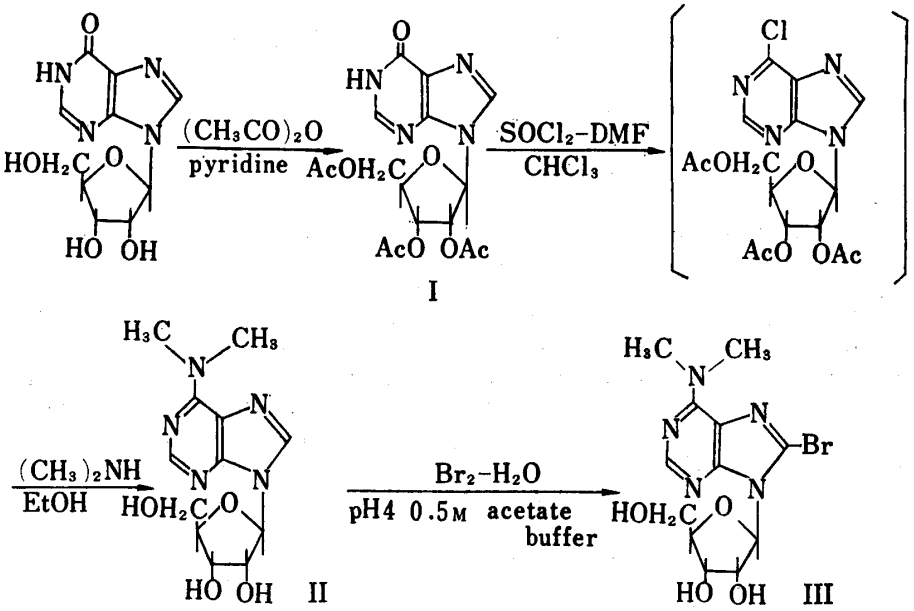

Chart 1

In order to introduce an arylsulfonyl group either to 2′ or 3′-OH, the method developed in the case of adenosine[13] was employed. Compound III was dissolved in dimethyl formamide (DMF) and treated with 1.2 equivalent of sodium hydride at −15° to −20°. After ten minutes at this temperature, triisopropylbenzenesulfonyl chloride (TPS-Cl) was added. Ratio of the occurrence of 2′-(IV) and 3′-TPS compound was 4.5:5.4 as estimated by thin-layer chromatography (TLC). Separation of compounds (IV and V) was attempted by silica

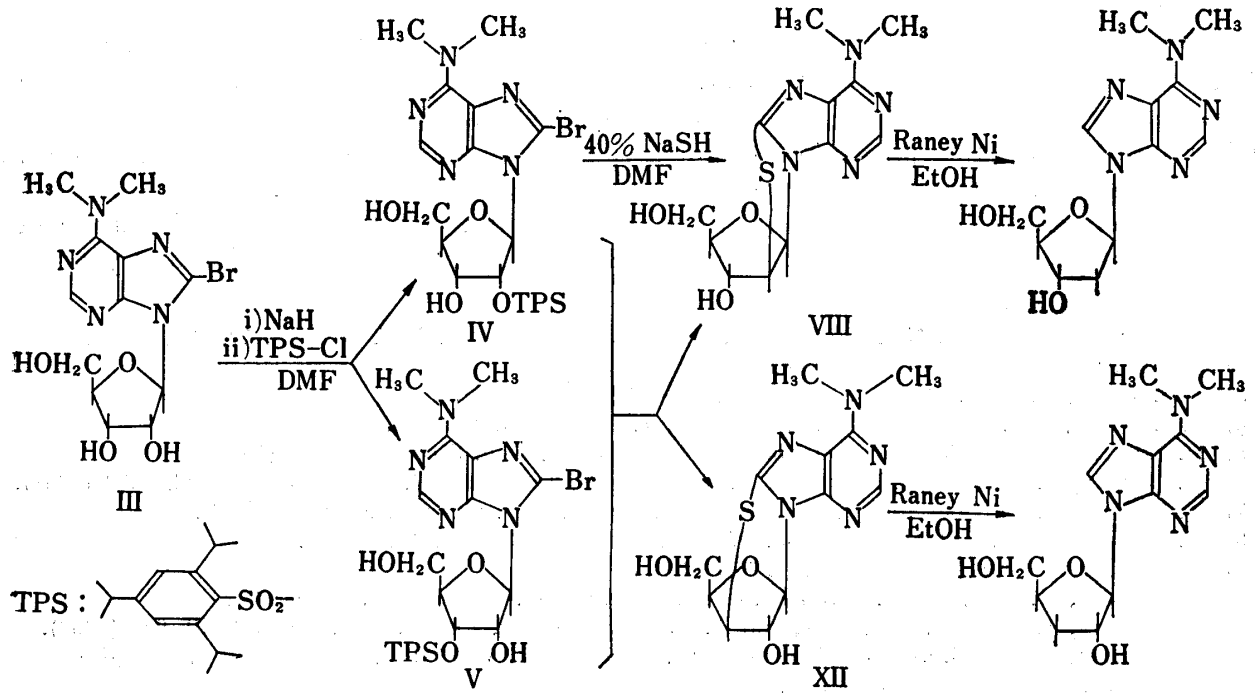

Chart 2

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gel column chromatography, recrystallization and preparative TLC. By the last method we could obtain 2′-TPS compound (IV) in a pure crystalline form having mp 152°. The structure of IV was confirmed by further transformation to 8,2′-S-cyclonucleoside as described later. Although by the above method pure 3′-TPS compound (V) could not be isolated, separation of 2′-(VI) and 3′-TPS (VII) compounds was achieved as described later by introducing trityl group at 5′-OH.

8-Bromo-2′-TPS compound (IV) was then dissolved in DMF and heated with 40% sodium hydrogen sulfide at 80° for 12 hr. By this procedure 8-bromo atom converted to SH, which cyclized to give 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyl-N^6-dimethyladenine (VIII) in a yield of 55%. Compound VIII showed UV absorption properties having λ_{max} 285—288 nm, which are slightly shifted bathochromically from those of 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine.[14] Nuclear magnetic resonance (NMR) of compound VIII showed peaks at 3.38 (N-CH_3), 8.13 (C_2-H) and 6.52 δ ($C_{1'}$-H) with $J_{1'-2'}$=6.5 Hz. As was found in adenosine cyclonucleosides,[9] the coupling constant 5—6 Hz would support the 8,2′-cyclonucleoside structure of VIII. This assumption was further confirmed by mass spectra of compound VIII. As shown in Fig. 1, molecular ion peak (m/e=309) appeared strongly. This is due to the stable molecular structure as being S-cyclonucleoside.[15] Fragmentation occurred as shown in the Chart 1. M-29 ion corresponding to 6-methyl derivative (IX), which have been found in the spectrum of N^6-dimethyladenosine,[16] further gave a peak of m/e=249 corresponding to compound (X). Analogously M^+ ion gave a peak (M-15) and a peak of m/e=246. In addition to this, peak corresponding to 8-mercapto-6-methylpurine (m/e=166) also appeared. All these features were strong supports of the cyclonucleoside structure of compound VIII. Further support of 8,2′-rather than 8,3′-cyclonucleoside structure was obtained by the appearance of peak, m/e=232. This might be due to the elimination of 5′-CH_2OH and 3′-OH to form a specific ion (XI). This ion could not easily be formed in spectra of 8,3′-cyclonucleosides[15] and might be therefore the proof of 8,2′-cyclonucleoside structure of VIII.

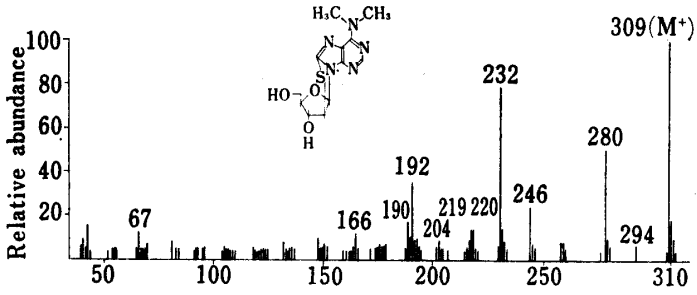

Fig. 1

Circular dichroism (CD) spectrum of the compound VIII as shown in Fig. 2 had large positive Cotton bands at 283 nm, which was characteristic to S-cyclonucleosides.[9] The magnitude of this band was +13500 and it was smaller than that of 8,3′-counterpart. These properties also suggested that compound VIII must be 8,2-S-cyclonucleoside. The final proof of the structure of VIII was given by desulfurization of VIII to a known compound N^6-dimethyl-2′-deoxyadenosine.[17] The structure of its 2′-deoxyfuranose was confirmed by compairing *Rf* values and color developed with cystein-sulfuric acid reagent.[18] As shown in Table I, a pink color having λ_{max} 414 and 491 nm and *Rf* values found in chromatographies in five solvent systems, showed 2′-deoxypentose structure.

Cyclization reaction of 3′-TPS-8-bromo-N^6-dimethyladenosine (V) was performed analogously with a starting material containing 20—30% 2′-TPS compound. Extent of the reaction was followed by TLC and it was found that replacement of 8-bromo with SH is slower

14) M. Ikehara and H. Tada, *Chem. Pharm. Bull.* (Tokyo), **15**, 94 (1967).
15) M. Ikeda, Y. Tamura, and M. Ikehara, *J. Heterocycl. Chem.*, **7**, 1377 (1970).
16) S.T. Shaw, D.M. Desiderio, K. Tsuboyama, and J.A. McClosky, *J. Am. Chem. Soc.*, **92**, 2510 (1970).
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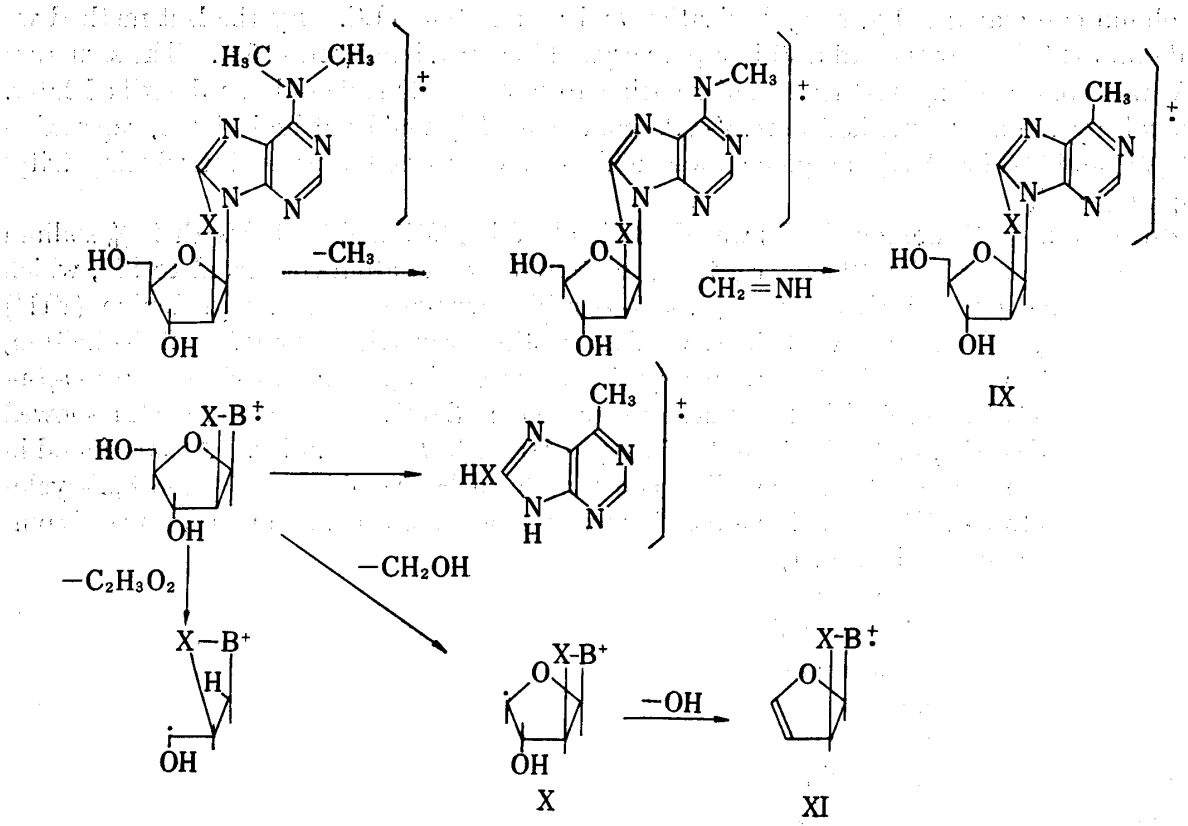

Chart 3

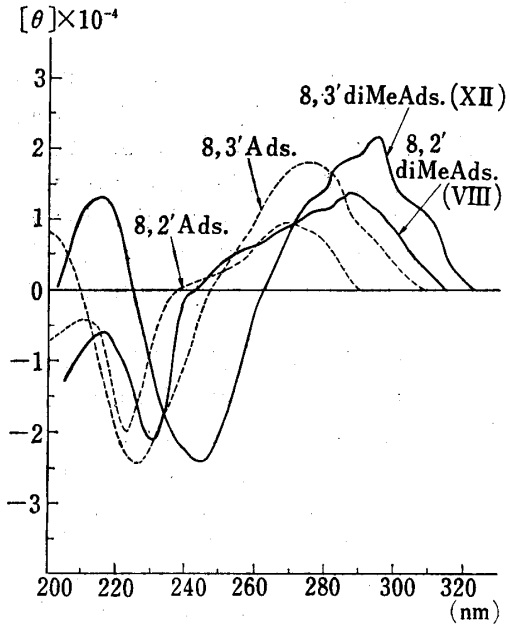

Fig. 2

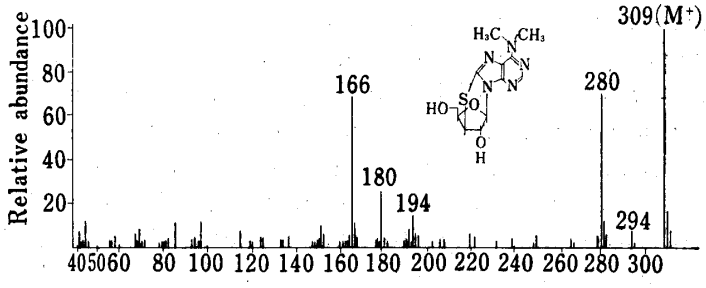

Fig. 3

in 3'- than in 2'-TPS compound. Furthermore, in the case of 3'-TPS compound, substantial amount of 8-mercapto-N^6-dimethyladenine and its disulfide were obtained. Recrystallization of the product mixture from methanol gave 8,3'-anhydro-8-mercapto-9-β-D-xylofuranosyl-N^6-dimethyladenine (XII). The structure of compound XII was confirmed by elemental analysis, UV absorption spectra, NMR and mass spectra. Slight bathochromic shift of λ_{max}'s, small coupling constant $J_{1'-2'}$, and large relative abundance of M^+ peak was found (Fig. 3). These physical properties were well consistent with those observed in case of 8,3'-S-cycloadenosine.[14] The consistency was also observed in CD spectra. As shown in Fig. 2, CD of compound XII showed a large $[\theta]$ than that of 8,2'-counterpart (VIII) and the magnitude of Cotton effect was comparable to that of 8,3'-S-cycloadenosine[17,19] The final proof of the

19) M. Ikehara, S. Uesugi, and M. Kaneko, *Chem. Pharm. Bull.* (Tokyo), **19**, 1381 (1971).

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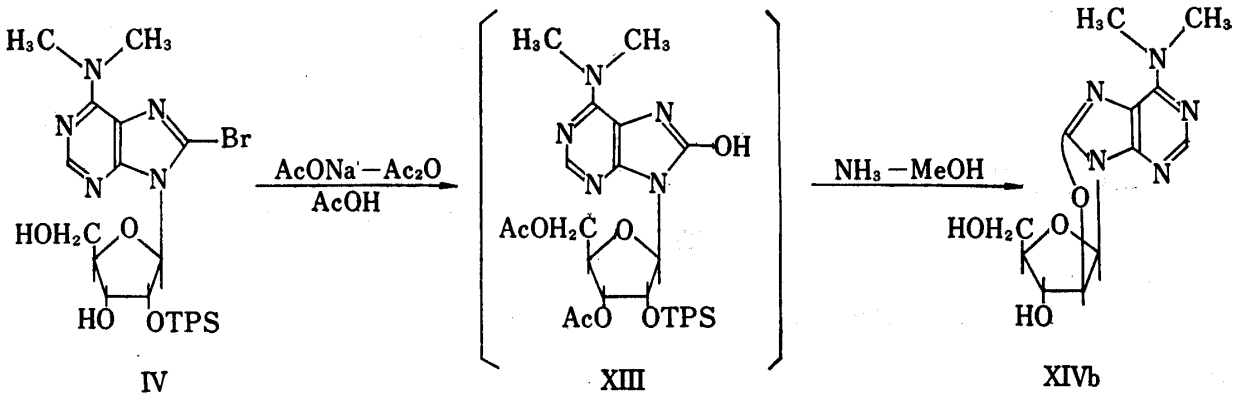
Table I. Identification of Deoxy-Sugars[a]

Solvent	Deoxy-Sugars[a] from			
	2′-Deoxy-Ads.	2′-Deoxy-N^6-dimethyl-Ads.	3′-Deoxy-Ads.	3′-Deoxy-N^6-dimethyl-Ads.
A	0.58	0.57	0.62	0.63
B	0.30	0.31	0.36	0.36
C	0.64	0.64	0.69	0.69
D	0.86	0.89	0.59	0.57
E	0.72	0.71	0.80	0.79
λ_{max} (nm)[b]	414, (491)	415. (493)	498	498

solv. A: BuOH–EtOH–H_2O (4:1:5) B: *n*-BuOH–H_2O (84:16) C: iso-PrOH–NH_4OH–H_2O (7:1:2)
D: *n*-BuOH–AcOH–H_2O (5:2:3) E: EtOH–1M NH_4OAc (7:3)
a) hydrolysis: 1N HCl 90° 30′ *b*) 5% cysteine–75% H_2SO_4

structure was done by Raney nickel desulfurization of XII to afford 3′-deoxy-N^6-dimethyladenosine (N^6-dimethylcordycepin[20]). The comparison of deoxysugars obtained by acid hydrolysis of XII and 3′-deoxyadenosine (cordycepin) supported the structure of compound XII to be correct.

Synthesis of O-cyclonucleosides from N^6-dimethyladenosine was then attempted. As shown in the case of adenosine,[13] the starting material should be the same TPS compound IV and V. When 2′-TPS-8-bromo derivative (IV) was refluxed with excess sodium acetate in acetic acid and acetic anhydride[21] for 4 hr, compound IV converted partially to 8-oxy derivative (XIII). When the reaction mixture was refluxed further for completion of the hydroxylation reaction, a cyclonucleoside (XIVa) appeared with XIII. Deprotection of compound XIVa afforded 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyl-N^6-dimethyladenine (XIVb).



IV XIII XIVb

Chart 4

Since this type of cyclization was thought to occur either with alkaline treatment or with attack of powerful nucleophiles in neutral aprotic solvent, the cyclization in acetic acid was rather unusual. The ease of cyclization of compound IV relative to 8-oxyadenosine[22] could be explained by the increase of electron density at C-8 of compound IV due to the introduction of dimethyl function to N^6-amino group. The structure of cyclonucleoside XIVb was comfirmed by elemental analysis, UV absorption properties having λ_{max} around 271—273 nm, coupling constant of anomeric proton ($J_{1'-2'}$=5.5 Hz) in NMR spectra. As shown in Fig. 4, CD spectrum of compound XIVb showed a large positive Cotton effect in B-band region

20) E. Walton, F.W. Holly, G.E. Boxer, K.F. Nutt, and S.R. Jenkins, *J. Med. Chem.*, 8, 659 (1965).
21) M. Ikehara, H. Tada, and M. Kaneko, *Chem. Pharm. Bull.* (Tokyo), 13, 1140 (1968).
22) M. Ikehara and M. Kaneko, *Chem. Pharm. Bull.* (Tokyo), 18, 2401 (1970).

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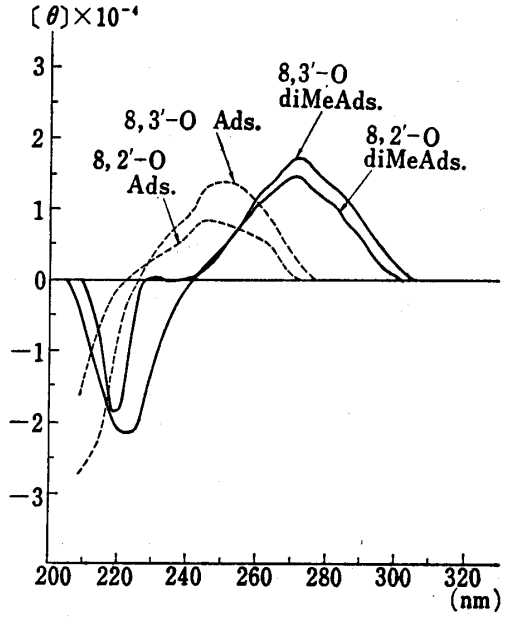

Fig. 4

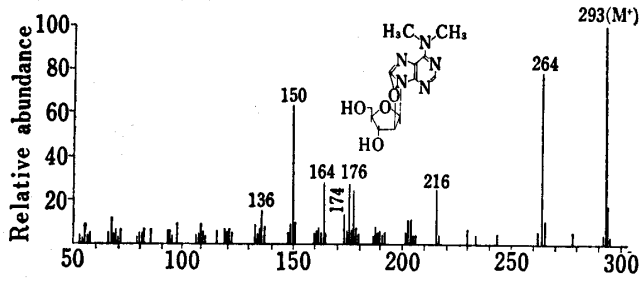

Fig. 5

and this amplitude was +13000. These features are consistent with those observed in 8,2'-O-cycloadenosine.[22,23] Mass spectrum of compound XIVb showed a relatively intense molecular ion peak together with M-15 and M-48 peaks (see Fig. 5). The latter peak appeared in relative abundance of 30%. This type of fragmentation would suggest that the compound XIV had 8,2'-cyclonucleoside structure.

In order to obtain 8,3'-O-cyclonucleoside, we first attempted to cyclize 3'-TPS-8-bromo compound (V) slightly contaminated with 2'-TPS compound (IV) obtained as above. However, as shown in the case of adenosine,[22] compound IV was found to cyclize very rapidly and the resulting 8,3'-cyclonucleoside was decomposed easily. We, therefore, attempted to isolate pure 3'-TPS compound by introducing trityl group at 5'-OH, because it was shown in

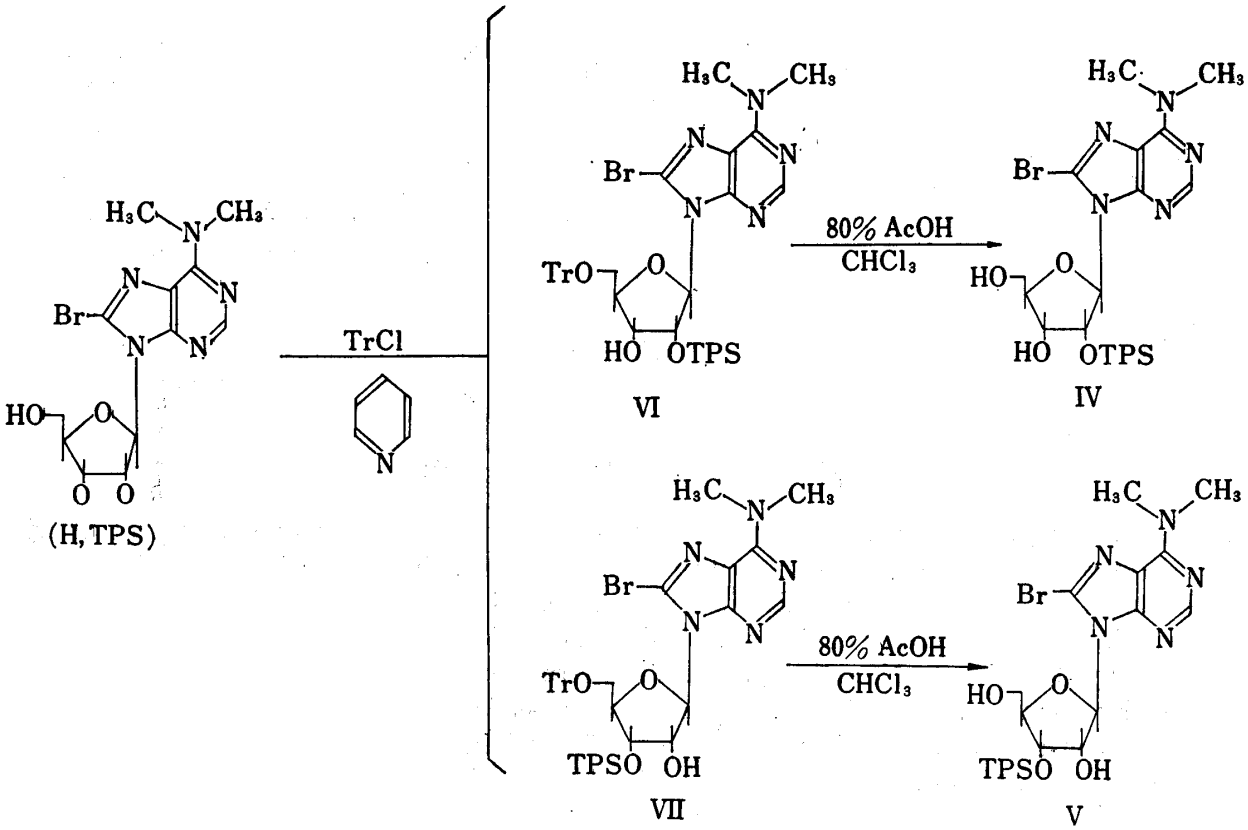

Chart 5

23) M. Ikehara, H. Tada, and M. Kaneko, *Tetrahedron*, **24**, 3489 (1968).

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the case of adenosine that 2′- and 3′-TPS compounds had fairly different migratory behavior in TLC.[13] Tritylation of the mixture of IV and V (3:7) was performed at 70° for 4 hr. The separation of trityl derivatives (VI and VII) was achieved by preparative TLC using repeated development with ethanol-chloroform mixture. From compound VI, as well as VII, the trityl group was safely removed by refluxing in 80% acetic acid.

A pure sample of V, thus obtained. was then acetylated as usual, treated with sodium acetate in acetic acid (and acetic anhydride), and heated with sodium acetate in DMF for 3.5 min. The crude reaction product was treated directly with ammonia-methanol for 12 hr to give 8,3′-anhydro-9-β-D-xylofuranosyl-N^6-dimethyladenine (XV) in a moderate yield. Compound XV showed UV absorption properties closely resembled those of 8,2′-O-cyclonucleoside (XIVb) and the coupling constant of anomeric proton in NMR was 2.8 Hz, which was as expected for 8,3′-cyclonucleoside structure. Correctness of the structure was further supported by CD (Fig. 4) showing a larger amplitude of Cotton effect relative to that of 8,2′-counterpart (XIVb). The elemental analysis also supported the structure to be correct.

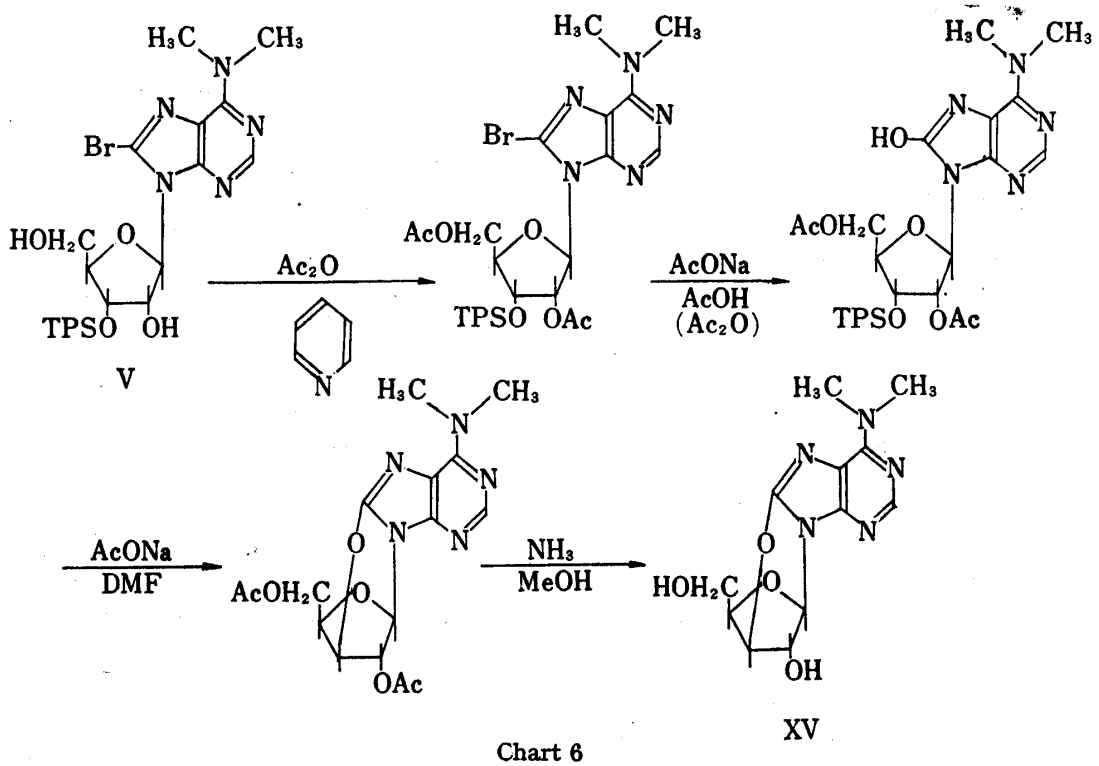

Chart 6

As shown in Fig. 6, mass spectrum of compound XV showed a relatively intense molecular ion peak as expected. Peaks corresponding to M-15 and M-29 also appeared. These features suggested that the structure of XV was correct. However, an unusual fragmentation was recorded for compound XV. The peak having m/e =216 corresponding to the the elimination of CH_2OH and OH, as shown above, from the furanose ring appeared in relative abundance of 25%. This is contrary to the expectation that M-48 peak would appear only from 8,2′-but not from 8,3′-cyclonucleoside fragmentation pattern.[15] From NMR spectrum of the 8,3′-

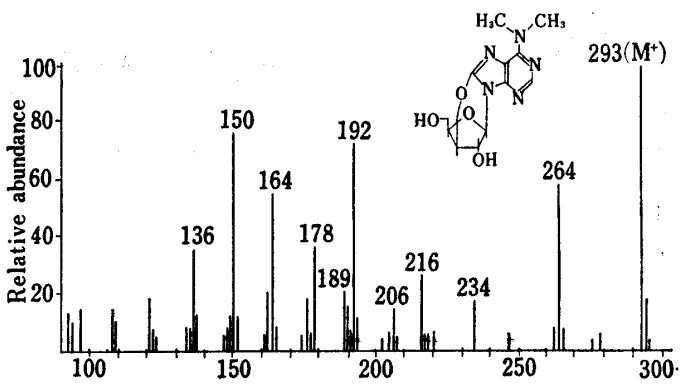

Fig. 6

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cyclonucleoside (XV), a contamination with the 8,2′-cyclonucleoside was excluded. Although this point shoud be clarified by further investigation with other cyclonucleosides, easier migration of 8,3′-anhydro bond to 8,2′-position or formation of cyclopropane ring involving $C_{2'}$, $C_{3'}$ ond $C_{4'}$ atoms in the fragmentation condition may be the cause. A peculiar peak (m/e =192), which is corresponding to N^6-dimethyl-8-oxy-9-methylene ion may suggest an easier fragmentation in the carbohydrate moiety of compound XV.

Experimental[24]

N^6-Dimethyladenosine——Freshly distilled thionyl chloride (3.6 g, 30 mmoles) and DMF (1 ml, 12 mmoles) were added in anhydrous chloroform (30 ml). The mixture was shaken for 30 min under exclusion of moisture. After evolution of heat was seazed, 2′,3′,5′-tri-O-acetylinosine (3.9 g, 10 mmoles) was added into the mixture. After refluxing for 4 hr with exclusion of the moisture, chloroform was evaporated *in vacuo*. The residue was dissolved in chloroform and washed throughly with ice–water. Drying over sodium sulfate and evaporation of chloroform gave a yellow hard oil. UV: λ_{max}^{EtOH} 264.5 nm, *Rf* (B) 0.90. The oil was dissolved in ethanol (15—20 ml) and 40% aqueous dimethylamine (80 ml) was added. The mixture was heated in a glasstube at 90—100° for 4 hr. Ethanol was evaporated *in vacuo* to a small volume. Methanol (*ca.* 5 ml) was added into this solution and the whole was kept in a refrigerator. White crystals which precipitated was collected by filtration, mp 184—185°. Yield was 1.53 g, 58%. UV: $\lambda_{max}^{H^+}$ 268 nm, $\lambda_{max}^{H_2O}$ 275.5 nm, $\lambda_{max}^{OH^-}$ 276 nm. PPC: *Rf* (B) 0.58, *Rf* (C) 0.78. This specimen was identical with an authentic sample.

8-Bromo-N^6-dimethyladenosine——N^6-Dimethyladenosine (1.18g, 4 mmoles) was dissolved in a buffer (pH 4) of 0.5 M sodium acetate and water (20 ml) with slight warming. Into this solution was added saturated bromine-water (20 ml) at room temperature. Excess bromine was removed by bubbling through with nitrogen. Bromo derivative precipitated partially as crystals, which were collected by filtration. Washings and filtrate was combined and evaporated to give further crops. Yield was 1.41 g, 89%. A sample for elemental analysis was recrystallized from methanol or water. 8-Bromo-N^6-dimethyladenosine melted once at 145°, solidified and decomposed at 223°. *Anal.* Calcd. for $C_{12}H_{16}O_4N_5Br$: C, 38.51; H, 4.31; N, 18.72; Br, 21.36. Found: C,38.44; H. 4.45; N, 18.19; Br, 21.56. UV : $\lambda_{max}^{H^+}$ 280.5 nm (ε 21500), $\lambda_{max}^{H_2O}$ 280 nm (ε 21500), $\lambda_{max}^{OH^-}$ 273.5 nm (ε 22800). PPC : *Rf* (A) 0.57, *Rf* (B) 0.72, *Rf* (C) 0.89.

2′- and 3′-Triisopropylbenzenesulfonyl-8-bromo-N^6-dimethyladenosine——8-Bromo-N^6-dimethyladenosine (1.122 g, 3 mmoles), which was dried over phosphorus pentoxide at 70° for 3 hr under 5 mm/Hg, was dissolved in freshly distilled DMF (20 ml). The solution was cooled in an ice-salt bath at −15° to −20°. Into this solution was added sodium hydride (180 mg, containing 50 % mineral oil and washed with dry benzene) suspended in DMF (5 ml) with stirring. Raise of temperature was carefully checked to maintain below −15°. After stirring was continued at this temperature for 10 min, triisopropylbenzenesulfonyl chloride (1.086g, 3.5 mmole, finely divided) was added. Stirring was continued for 2—3 hr. In this while temperature raised to about 0°. The reaction mixture was added dropwise with stirring into ice–water containing 5% sodium bicarbonate. White amorphous powder, thus obtained, was 1.9 g(quantitative) and containing 2′- and 3′-TPS derivatives in ratio of 4.5: 5.5 as checked by TLC and paper chromatography. The product was dissolved in benzene, cyclohexane was added, and seeded with crystal of 2′-TPS compound, which had been separated in pure state by TLC. 2′-TPS compound crystallized out first (yield 400 mg) as needles having mp 152°. UV: $\lambda_{max}^{H^+}$ 279 nm, $\lambda_{max}^{H_2O}$ 279.5 nm, $\lambda_{max}^{OH^-}$ 279.5 nm. IR: ν_{max}^{KBr} 1180 cm^{-1} (aryl sulfonate).

8,2′-Anhydro-8-mercapto-9-β-D-arabinofuranosyl-N^6-dimethyladenine——8-Bromo-2′-TPS-N^6-dimethyladenosine (318 mg, 0.5 mmoles) was dissolved in DMF (5 ml) and bubbled through with nitrogen gas for 30 min. Into this solution was added 40% aqueous sodium hydrogen sulfide (0.26 ml, 2 mmoles). Stoppered reaction mixture was heated at 80° in an oil bath for 12 hr. Color of the solution changed from light green to deep green. After it was cooled to room temperature, 1 N HCl was added dropwise under bubbling of nitrogen gas. Addition of HCl was stopped when the color of the solution turned to pink. The reaction

24) UV absorption spectra were taken with a Hitachi EPS-3T spectrophotometer, NMR spectra were taken with a Varian A-60 spectrometer operated at 60 mHz with tetramethylsilane as internal standard, CD spectra were taken with a JASCO ORD/UV-5 spectropolarimeter with CD attachment at concentration 1-2 OD/ml, and mass spectra were taken with a Hitachi mass spectrometer RM-60 at 70 eV.

Paper chromatography (PPC) solvents were: A, water adjusted at pH 10 with ammonia: B *n*-butanol–water (86:14); C, isopropanol–ammonia–water (7:1:2). Ascending technique was employed on Toyo filter paper No. 51A. Thin–layer chromatography (TLC) was performed on Kieselgel HF 254 in chlorofrom ethanol systems (ratio of solvents as indicated in the text).

Melting points were measured with a Yanagimoto hot stage and uncorrected.

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mixture was evaporated *in vacuo*. If the color of the solution changed back to green, pH of the solution was readjusted with HCl. Residue was dissolved in water (3—4 ml) with slight warming, insoluble material was removed by centifugation and supernatant was evaporated in vacuo. Residue was dissolved in methanol with heating and insoluble material was filtered off. Filtrate was stored in a refrigerator to give white needles (65 mg) having mp 250° (decomp.). Further 20 mg of crystals were obtained as the second crop (total yield was 55%). *Anal.* Calcd. for $C_{12}H_{15}O_3N_5S$: C, 46.60; H, 4.89; N, 22.65; S, 10.35. Found: C, 45.80; H, 4.95; N, 22.30; S, 10.06. UV: $\nu^{H^+}_{max}$ 285 nm (ε 23400), 294 nm (sh. ε 21200); $\lambda^{H_2O}_{max}$ 288 nm (ε23700); $\lambda^{OH^-}_{max}$ 289 nm (ε 24000). NMR (d_6-DMSO): 3.38 δ(s, N-CH_3), 8.13 δ(s, C_2-H), 6.52 δ (d, $C_{1'}$-H, $J_{1'/2'}$=6.5 Hz). PPC: *Rf*(A) 0.52, *Rf*(B) 0.67. CD and mass spectra were shown in Fig.1 and Fig.2.

8,3′-Anhydro-8-mercapto-9-β-D-xylofuranosyl-N⁶-dimethyladenine——3′-TPS-8-Bromo-N^6-dimethyladenosine (containing 20—30% 2′-TPS compound, 320 mg) was dissolved in DMF (5 ml) and nitrogen gas bubbled through this solution for 30 min. Aqueous sodium hydrogen sulfide (40%, 0.26 ml) was added to the mixture, which was stoppered and heated in an oil bath (85—90°) for 20 hr. Color of the mixture turned to dark green. The reaction mixture was made acidic with N HCl under bubbling through with nitrogen gas. The mixture was evaporated *in vacuo*, residue dissolved in water (5 ml) by heating, and insoluble material was removed by filtration. Filtrate was evaporated, residue dissolved in methanol, treated with charcoal and filtered. Gradual evaporation of methanol gave insoluble material, which was removed by filtration. Storing in refrigerator of the filtrate gave crystals of 8,3′- and 8,2′-cyclonucleosides, which were recrystallized from methanol to give 8,3′-cyclonucleoside by seeding with pure sample, which was separated in a pure state by TLC. Analytical sample was further recrystallized from methanol. *Anal.* Calcd. for $C_{12}H_{15}O_3N_5S$: C, 46.60; H, 4.89; N, 22.65; S, 10.35. Found: C, 46.44; H, 4.85; N, 22.66; S, 10.39. UV: $\lambda^{H^+}_{max}$ 289.5 nm (ε 25200), 296 nm (sh, ε 23400); $\lambda^{H_2O}_{max}$ 287 nm (sh. ε 22800), 293.5 nm (ε 23600), 303 nm (sh, ε 5900); $\lambda^{OH^-}_{max}$ 289 nm (sh ε 23300), 295 nm (ε 24700), 305 nm (ε 16100). NMR (d_6-DMSO): 3.37 δ (s, N-CH_3), 8.13 δ (s, C_2-H), 5.86 δ (d, $C_{1'}$-H, $J_{1'-2'}$ =2.8 Hz). PPC: *Rf*(A) 0.45, *Rf*(B) 0.72. CD and mass spectra were shown in Fig. 2 and Fig. 3.

2′-Deoxy-N⁶-dimethyladenosine——8,2′-S-Cyclonucleoside (10 mg) was dissolved in 98% ethanol (7 ml) refluxed with Raney nickel (W-2, 15 mg) for 4 hr. Refluxing was further continued for 2 hr with additional nickel (6 mg). After the test by PPC in solvent A showed one spot, nickel was removed by filtration, filtrate and washing (hot methanol) were combined and evaporated *in vacuo*. White powder, thus obtained, was recrystallized from methanol to give 2′-deoxy compound, mp 201—203°. UV: $\lambda^{H^+}_{max}$ 268.5 nm, $\lambda^{H_2O}_{max}$ 276 nm, $\lambda^{OH^-}_{max}$ 277 cm. Cystein-sulfuric acid test: pink (λ_{max} 414, 491 nm). PPC: *Rf* (A) 0.63. These properties were as described in the literature.

3′-Deoxy-N⁶-dimethyladenosine——8,3′-S-Cyclonucleoside (10 mg) was dissolved in 99% ethanol (7 ml) and refluxed with Raney nickel (W-2, 20 mg) until PPC in solvent A showed one spot. Nickel was removed by filtration, filtrate and washing (hot methanol) were combined and evaporatend *in vacuo*.. Residue was recrystallized from ethanol to give white crystals, mp 170—172°. UV: $\lambda^{H^+}_{max}$ 268.5 nm, $\lambda^{H_2O}_{max}$ 275.5 nm, $\lambda^{OH^-}_{max}$ 276 nm. PPC: *Rf* (A) 0.62, *Rf*(C) 0.71. Cystein-sulfuric acid test gave a pink color (λ_{max} 415, 493 nm), which has same λ_{max} with 3′-deoxyribose obtained from 3′-deoxyadenosine (cordycepin).

8,2′-Anhydro-8-oxy-9-β-D-arabinofuranosyl-N⁶-dimethyladenine——8-Bromo-2′-TPS-compound (320 mg, 0.5 mmoles) and sodium acetate (anhydrous, 600 mg, 7.5 mmoles) were dissolved in acetic acid and acetic anhydride mixture (10 ml, 1: 1, v/v). The solution was refluxed in an oil-bath (170°) for 8 hr. Examination with TLC (chloroform–ethanol, 18.5: 1.5) showed three spots. Reaction mixture was evaporated *in vacuo* with repeated addition of ethanol until order of acetic anhydride diminished. Residual glass, thus obtained, was extracted with chloroform (50 ml) and ice–water (50 ml). Organic layer was washed thoroughly with water, dried over sodium sulfate, and evaporated *in vacuo*. The glass, thus obtained, was dissolved in anhydrous methanol (6 ml), which was saturated with ammonia gas at 0°. Stoppered reaction mixture was kept at room temperature for 4 hr and heated at 80° for 8 hr. Methanol was evaporated carefully and residue was distributed by shaking in water (10 ml) and chloroform (10 ml). Water layer was evaporated *in vacuo* and residue was dissolved in methanol. Storing in a refrigerator gave crystalline material (50 mg, 35%). Small amount of starting material was recovered from chloroform layer. Analytical sample was recrystallized further from methanol to give needles, mp 214° (decomp.). *Anal.* Calcd. for $C_{12}H_{15}O_4N_5$: C, 49.14; H, 5.16; N, 23.88. Found: C, 49.12; H, 5.10; N, 23.86. UV: $\lambda^{H^+}_{max}$ 271 nm (ε 17900), 290 nm (sh, ε 10900); $\lambda^{H_2O}_{max}$ 272 nm (ε 20100); $\lambda^{OH^-}_{max}$ 273 nm (ε 19700). NMR (d_6-DMSO): 3.33 δ (s, N-CH_3), 8.12 δ (s, C_2-H), 6.52 δ (d, $C_{1'}$-H, $J_{1'-2'}$=5.5 Hz). PPC: *Rf*(A) 0.58, *Rf*(B) 0.60. CD and mass spectra were shown in Fig. 4 and Fig.5.

8,3′-Anhydro-8-oxy-9-β-D-xylofuranosyl-N⁶-dimethyladenine——i) 8-Bromo-3′-TPS-5′-trityl compound (640 mg) was dissolved in chloroform (10 ml) and 80% acetic acid (30 ml). The solution was refluxed for 30 min and evaporated *in vacuo*. Acetic acid odor was nearly diminished by repeated addition and evaporation of ethanol. Residue was dissolved in pyridine (15 ml) and acetic anhydride (0.6 ml) and kept at room temperature overnight. The reaction mixture was evaporated *in vacuo* with repeated addition of toluene (3 ml × 2). Into the residue was added acetic acid (6 ml), sodium acetate (anhydrous, 770 mg) and acetic anhydride (1 ml). The solution was refluxed in an oil-bath (170°) for 5 hr. Test by TLC at this stage showed one spot. Ethanol was added to the reaction mixture, which was added dropwise into ammonia–water (500 ml, pH 9—10). White precipitate was extracted with chloroform, dried over sodium sulfate and evaporated. Well-dried

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residue was dissolved in DMF (25 ml) containing sodium acetate (750 mg) and calcium carbonate (1.9 g). The mixture was refluxed for 3.5 min and cooled to room temperature. Precipitated salt was removed by filtration, filtrate was evaporated *in vacuo*, and the residue was extracted with chloroform. After washing with water and drying over sodium sulfate, the organic layer was evaporated *in vacuo*. Residue was dissolved in anhydrous methanol (10 ml) and saturated with ammonia gas at 0°. After the mixture was kept at room temperature for 12 hr, it was carefully evaporated and the residue was dissolved in methanol (5 ml). Insoluble material was centrifuged off, supernatant was concentrated slightly, and kept in a refrigerator. 8,3′-O-cyclonucleoside was obtained as colorless crystals, mp 236.5° (decomp.). Yield, 15 mg. Analytical sample was recrystallized again from methanol with charcoal decolorization. *Anal.* Calcd, for $C_{12}H_{15}O_4N_5$: C, 49.14; H, 5.16; N, 23.88. Found: C, 49.34; H, 5.25; N, 24.00. UV: $\lambda_{max}^{H^+}$ 273 nm (ε 17400), 290 nm (sh, 10800); $\lambda_{max}^{H_2O}$ 275 nm (ε 20800); $\lambda_{max}^{OH^-}$ 278 nm (ε 19600). NMR (d_6-DMSO): 3.33 δ(s, N-CH_3), 8.11 δ (s, C_2-H), 5.83 δ (d, C_1-H, $J_{1'-2'}=2.5$ Hz). PPC: *Rf*(A) 0.58, *Rf*(B) 0.65. CD and mass spectra were shown in Fig. 4 and 6.

ii) 8-Bromo-3′-TPS compound (414 mg, 0.7 mmoles) was dissolved in pyridine (12 ml) and acetic anhydride (0.6 ml). The mixture was kept at room temperature for 6 hr. Evaporation of the mixture with occasional addition of ethanol gave a residue, which was dissolved in chloroform (50 ml) and washed thoroughly with water. Organic layer was dried with sodium sulfate and evaporated *in vacuo*. The residue was evaporated again with toluene (9 ml × 2) to remove traces of pyrdine. The residue (464 mg) was dissolved in acetic acid (6 ml) with sodium acetate (anhydrous 770 mg) and acetic anhydride (1 ml). Refluxing of the mixture for 4.5 hr and evaporation of cetic acid gave a syrup, which was dissolved in ethanol. The ethanol solution was added dropwise into ammoniacal water (300 ml, pH 9—10). Rrecipitated powder was extracted with chloroform, dried over sodium sulfate and evaporated. The residue was dissolved in DMF (25 ml) containing sodium acetate (50 mg) and calcium carbonate (1.9 g). Pefluxing for 1 min and cooling gave precipitate, which was removed by filtation. The filtrate was evaporated *in vacuo* and the residue was extracted with chloroform. Drying over sodium sulfate and evaporation of the solvent *in vacuo* gave residue, which was dissolved in methanol (10 ml). Deacetylation with ammonia was carried out as in i). Evaporation and recrystallization of the mixture gave 32 mg of crude 8,3′-O-cyclonucleoside. Recrystallization of this specimen from methanol gave 10 mg of pure material. Properties of this specimen is same with those obtained in i).

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