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SYNTHESIS AND PROPERTIES OF 2′-DEOXY-8,2′-METHYLENE-CYCLOADENOSINE

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Abstract—A method was developed to synthesize 2′-deoxy-8,2′-methylene-cycloadenosine (**9**) which was a new carbon-bridged cycloadenosine fixed in a high-antitorsional angle region. 3′,5′-Di-O-acetyl-8-methanesulfonyl-2′-O-tosyladenosine (**5**) was cyclized with carbanions of malonic esters, followed by hydrolysis of the ester (**7**) and decarboxylation to afford **9**. Compound **9** showed a positive CD band at 258 nm and was a substrate for adenosine deaminase with a K_m of 3.2×10^{-4} M and a V_{max} of 2% of that of adenosine.

One of the interesting subjects on the conformation of enzyme-bound nucleosides and nucleotides is concerned with the orientation of the base moiety to the sugar moiety around the glycosyl linkage. Adenosine deaminases[1–3] and polynucleotide phosphorylase[4] recognize only the anti-conformer of the substrates. Kapuler and Reich[5] predicted that ribonucleoside 5′-triphosphates would bind to RNA polymerases as the syn-form and then conformational change of the substrate to the anti-form would be necessary to form the internucleotidic phosphodiester linkages. At the active site of RNase T_1, the conformation of substrates, guanylic acids, around the glycosyl bond was reported to change from the anti to the syn-form.[6] Bovine heart protein kinases interact with ATP at the high-anti glycosyl torsional angle region.[7]

We have been studying the synthesis of carbon-bridged cyclopurine nucleosides and nucleotides with fixed conformations around the glycosyl bond[8] and some of them have been used as probes to study conformational aspects of enzyme–substrate interactions.[9] The anti-fixed 8,5′-cyclo-5′-deoxyguanosine 2′,3′-cyclic phosphate was found to be a substrate of RNase T_1.[9] Also, 8,5′-cycloadenosines were good examples of such studies.[2,8,10,11]

There have been limited synthetic methods available for the carbon-bridged cyclopurine nucleosides including mainly the radical process. Alkyl radicals at the 5′-position of the sugar moiety of purine nucleosides, generated by photo-irradiation or by chemical homolysis, add intramolecularly to the 8-position to afford 8,5′-cyclopurine nucleosides.[8] Intramolecular coupling of biradicals at the 5′- and 8-positions gives rise to the cycloadenosine compounds.[8b,12] Since the 5′-carbon atoms constitute the bridge-head carbons of cyclonucleosides in these cases, these methods are limited to 8,5′-cyclopurine nucleosides. We need other conformationally fixed cyclopurine nucleosides, which are fixed in different torsional angles around the gycosyl linkages, such as 8,2′- and 8,3′-cyclonucleosides, for further investigations in this field. We therefore report herein a new method for the synthesis of 2′-deoxy-8,2′-methylene-cycloadenosine (**9**). Optical properties of **9** and its behavior to adenosine deaminase, compared with those of the anti-fixed carbon bridged cycloadenosines (**10–13**)[8a,c,d] are also included.

It has been described that 8-bromo-2′-O-tosyladenosine (**1**) reacted with sodium hydrosulfide to afford 2′-deoxy-8,2′-S-cycloadenosine[13] and that treatment of 2′-O-tosyladenosine 8-carboxamide in aqueous pyridine gave 1-β-D-arabinofuranosyl-adenine.[14] We have found that a methanesulfonyl group at the 8-position of adenosine was superior to the 8-bromo derivative in displacement reactions with carbon nucleophiles.[16] These findings led us to investigate reactions of 8-methanesulfonyl-2′-O-tosyladenosine (**3**) with carbanions of active methylene compounds as a one-carbon synthon of bridge-head position of the cycloadenosine.

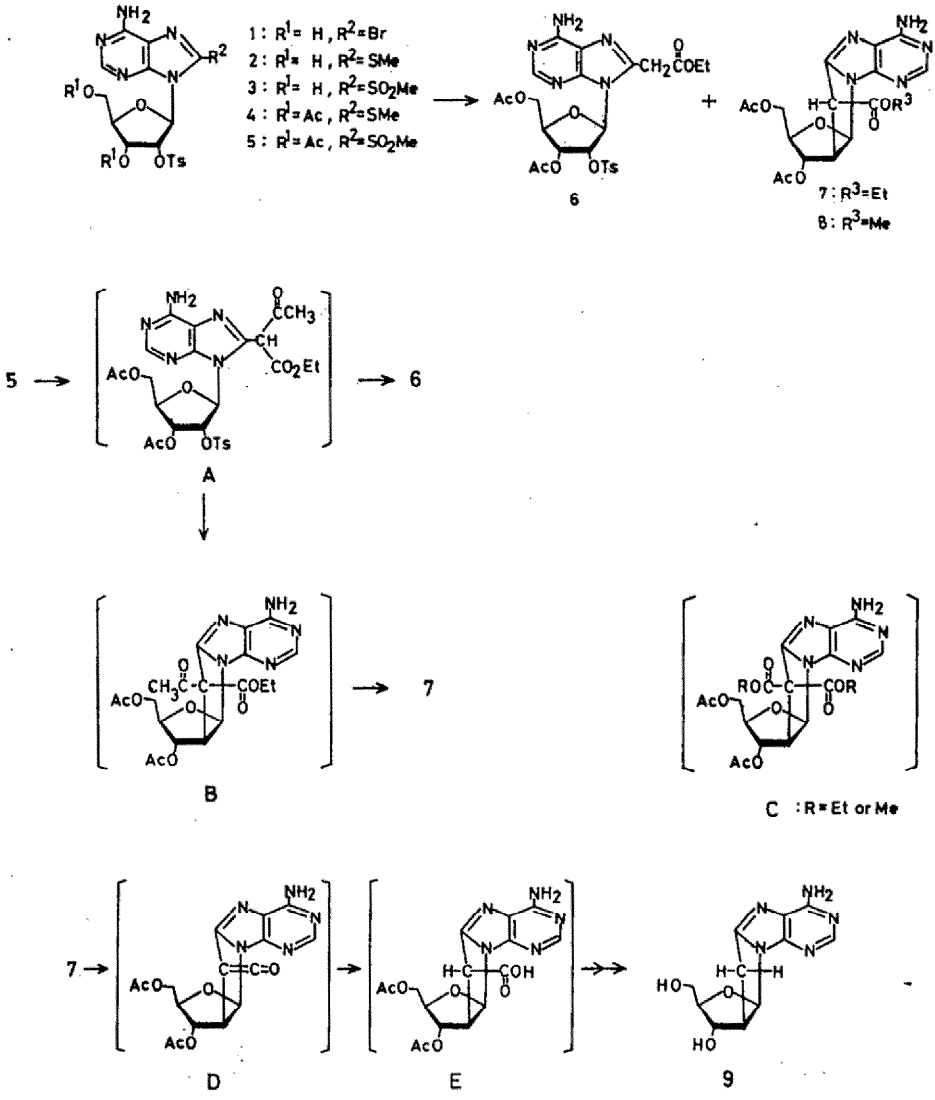
Substitution of 8-bromo-2′-O-tosyladenosine[17] (**1**) with sodium methanethiolate was carried out in aqueous N,N-dimethylformamide (DMF) at room temperature to give 8-methylthio-2′-O-tosyladenosine (**2**) in 90% yield. Oxidation of the sulfur function of **2** was achieved with potassium permanganate in acetic acid to afford 8-methanesulfonyl-2′-O-tosyladenosine (**3**) in 81% yield. An attempt to react **3** with a carbanion of ethylacetoacetate in DMF at room temperature was unsuccessful, the adenine base derivative being released (data not shown). The lability of 2′-O-tosyladenosine in basic conditions has previously been observed.[18] In order to increase the solubility to aprotic solvents such as tetrahydrofuran (THF) and to avoid undesired glycosyl bond cleavage, free hydroxyl groups in **3** should be blocked.[15,19]

Compound **3** was acetylated to give **4**, which was oxidized to afford 3′,5′-di-O-acetyl-8-methanesulfonyl-2′-O-tosyladenosine (**5**) in 96% yield. Then, **5** was treated with the sodium salt of ethyl acetoacetate in THF at reflux temperature for 24 h. A mixture of two nucleosidic products was separated on a silica gel column. The compound eluted first from the column was assigned as the uncyclized 8-ethoxycarbonylmethyl-2′-O-tosyladenosine derivative (**6**, 37%). The structure of **6** was evident from its PMR spectrum, in which methyl protons (δ 2.36) of the tosyl group at the 2′-O-position appeared as a singlet and its

aromatic protons (δ 7.08, 7.53) as two sets of doublets. Additionally, the presence of ethyl and methylene protons (singlet, δ 3.99) shows that the methanesulfonyl group was displaced by the carbanion, but the desired internal cyclization to the 2′-position did not occur. The second product which is more polar than **6** was found to be the desired 2′-deoxy-2″-ethoxycarbonyl-8,2′-methylene-cycloadenosine (**7**, 51%). Assignment of the cyclized structure (**7**) rests on its PMR spectrum which showed the absence of the tosyl protons, the characteristic upfield shift of the 2′-proton signal around δ 4.0 as a multiplet, and also the presence of ethyl protons. In addition, a doublet ($J_{2',2''}$ = 3.4 Hz) at δ 4.47 due to the bridge-head proton at C-2″ was confirmed by the decoupling experiment, which was consistent with the stereochemistry of the *R* configuration at C-2″. These data, together with the mass spectroscopic data (M^+, *m/z* 419, 54%), confirm the structure of **7**.

The most likely pathway for the formation of **6** and **7** from **5** is shown in the scheme. Nucleophilic displacement by the carbanion at the C-8 of **5** would afford the intermediate **A**. The carbanion easily derivable from **A** attacks the 2′-position intramolecularly producing **B**, whose base-promoted deacetylation affords **7**. However, undesirable deacetylation from **A** can compete with intramolecular displacements leading to **B**. Treatment of uncyclized nucleoside **6** with an excess of sodium hydride in THF at reflux temperature for 24 hr afforded only a trace amount of **7** as detected by thin layer chromatography (TLC). Thus, **6** could not be an important intermediate in the formation of **7** from **5**.

In order to improve the yield of **7**, use of malonic esters would be a better choice from above mechanistic considerations. Treatment of **5** with the sodium salt of diethyl malonate in THF at reflux temperature for 8 hr afforded, as expected, the desired cyclized nucleoside (**7**) in 79% yield as a foam. This product had identical TLC and PMR characteristics with an authentic sample prepared above. In a similar manner, treatment of **5** with an anion of dimethyl malonate gave the methyl ester **8** in 75% yield. In both cases, diester intermediate **C** was not isolated under these conditions. It is interesting to note that, in a neutral or acidic medium, **7** as well as **8** showed a UV maximum at 264 nm, but at



264 and 342 nm in alkaline solution, absorbance of the latter being decreased rapidly to zero within a few minutes (see Experimental). In contrast, the uncyclized nucleoside **6** showed only a maximum at 263 nm in alkaline solution. The maximum at 342 nm in **7** would correspond to the conjugated ketene **D**, which would be then hydrated to **E**. The red shift of the 8-substituent conjugated to the adenine moiety was similarly observed in 2',3'-O-isopropylidene-5'-oxo-8,5'-cycloadenosine.[8c] In addition, mass spectra of both **7** and **8** showed base ion peaks at 373 (*m/z*, $M^+ - EtOH$ for **7** or $M^+ - MeOH$ for **8**, respectively) which would also correspond to structure **D**. These data suggest that abstraction of the most acidic proton in **7** would yield the ketene **D** and EtOH or MeOH prior to hydrolysis of the ester function in alkaline solution. Although an attempt to measure a pK_a value of the methine proton in **7** failed because of the instability of **D**, this experiment suggested that a weak base treatment of **7** would lead to **E**. Ester **7** was heated under reflux in 85% aqueous pyridine for 2 days, followed by deacetylation with NH_4OH. The product obtained as a crystalline form in 65% yield was assigned as 2'-deoxy-8,2'-methylene-cycloadenosine (**9**) on the basis of the following data. The mass spectrum of **9** showed a molecular ion peak at 263 (*m/z*, 100%), a characteristic 8-methyladeninium ion peak at 149 (*m/z*, 40%),[8a] and the correct elemental analysis ($C_{11}H_{13}N_5O_3$). Two sets of double doublets at δ 2.96 and δ 3.19 in its PMR spectra are assigned as bridged methylene protons at C-2''. This means that the hydrolysis of the ester group in **7** to form **E** is followed by simultaneous decarboxylation to give **9**. In a conventional way, **9** was obtained from **7** by saponification and deacetylation with base followed by decarboxylation with acid treatment. Thus, the synthesis of 2'-deoxy-8,2'-methylene-cycloadenosine (**9**) was accomplished by the reaction of doubly activated adenosine with malonic ester which serves as a one-carbon synthon of a bridge-head position of **9**.

The CD spectrum of **9** showed a positive band at 258 nm (shown in Fig. 1) and the direction of the sign is consistent with those of 8,2'-S- and 8,2'-O-cycloadenosines,[20] respectively, but it is opposite to that of 5'-deoxy-8,5'-cycloadenosine (**10**).[8a,c] Compound **9** is deaminated at about 2% of the rate observed with adenosine by adenosine deaminase (K_m of **9** was 3.2×10^{-4} M while that of adenosine was 2.3×10^{-5}). In contrast to the 8,2'-cycloadenosines,[3] 8,5'-cycloadenosine (**10**–**13**)[8a,c,d] tested (Fig. 2) were found not to be substrates for the deaminase. These findings together with a previous study[21] are in conflict with a previous report[2] that both of the diastereoisomeric 8,5'-cycloadenosines (**11**) were deaminated by adenosine deaminase at a different rate.

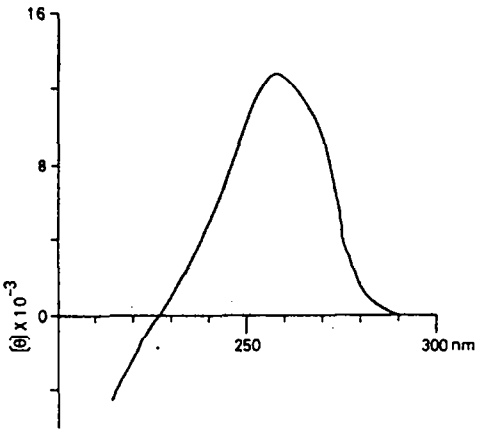


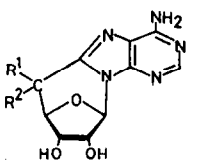
Fig. 1. CD spectrum of 2'-deoxy-8,2'-methylene-cycloadenosine (**9**).

EXPERIMENTAL

M.ps were determined with a Yanaco MP-3 m.p. apparatus and are uncorrected. PMR spectra were recorded on a JEOL JNM-FX 100 spectrometer using TMS as the internal standard. Chemical shifts are reported in ppm (δ) and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), dt (double triplet), and br (broad). Values given for coupling constants are first order. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer. CD spectra were taken on a JASCO J-500 spectrophotometer with DP-500N. MS spectra were measured with a JEOL JMS-D-300 spectrometer. TLC was carried out with Merk TLC plates (silica gel $60F_{254}$, precoated). Silica gel column chromatography was done using Wako gel C-200.

8-Methylthio-2'-O-tosyladenosine (**2**). To a soln of 8-bromo-2'-O-tosyladenosine (**1**, 20 g, 40 mmol) in DMF (150 ml) was added 15% NaSMe aq soln (40 ml). The reaction mixture was stirred for 2 hr at room temp and then neutralized with 1 N HCl under bubbling of N_2 and evaporated to dryness *in vacuo*. The resulting yellowish cake was crystallized from hot H_2O to give 16.8 g (90%) of **2**, m.p. 216–219°. PMR (DMSO-d_6): δ 2.27 (3H, s, tosyl-Me), 2.71 (3H, s, SMe), 3.55 (2H, m, H-5', 5''), 4.06 (1H, m, H-4'), 4.33 (1H, dt, H-3', after D_2O addition, dd, $J_{2',3'} = 4.9$ Hz, $J_{3',4'} = 1.0$ Hz), 5.59 (1H, dd, H-2', $J_{1',2'} = 7.3$ Hz, $J_{2',3'} = 4.9$ Hz), 5.79 (1H, t, 5'-OH, exchangeable), 5.85 (1H, d, H-1', $J_{1',2'} = 7.3$ Hz), 6.05 (1H, d, 3'-OH, exchangeable), 6.97 (2H, d, tosyl-H), 7.28 (2H, brs, 6-NH_2, exchangeable), 7.34 (2H, d, tosyl-H), 7.88 (1H, s, H-2). (Found: C, 46.45; H, 4.53; N, 15.26. Calc for $C_{18}H_{21}N_5O_6S_2$: C, 46.24; H, 4.53; N, 14.98%.)

8-Methanesulfonyl-2'-O-tosyladenosine (**3**). Compound **2** (7 g, 15 mmol) was dissolved in hot 70% aq AcOH (100 ml) and the soln was cooled to 0°. A fine powdered $KMnO_4$ (6 g, 38 mmol) was added to the soln with stirring. After 3 hr, the mixture was diluted with H_2O (50 ml) and 30% H_2O_2 was added until the suspension became colorless. The resulting crystalline ppts were collected by filtration and washed well with H_2O to afford 6.1 g (81%) of **3**, m.p. 203–205°. PMR (DMSO-d_6): δ 2.27 (3H, s, tosyl-Me), 3.58 (3H, s, SO_2Me), 3.63 (2H, m, H-5', 5''), 4.09 (1H, m, H-4'), 4.37 (1H, dt, H-3', after D_2O addition, dd, $J_{2',3'} = 4.9$ Hz, $J_{3',4'} = 1.0$ Hz), 5.58 (1H, t, 5'-OH, exchangeable), 5.67 (1H, dd, H-2', $J_{1',2'} = 7.8$ Hz, $J_{2',3'} = 4.9$ Hz), 6.07 (1H, d, 3'-OH, exchangeable), 6.55 (1H, d, H-1', $J_{1',2'} = 7.8$ Hz), 6.95 (2H, d, tosyl-H), 7.38 (2H, d, tosyl-H), 7.91 (2H, brs, 6-NH_2, exchangeable), 8.06 (1H, s, H-2). (Found: C, 42.97; H, 4.18; N, 14.40. Calc for $C_{18}H_{21}N_5O_8S_2$: C, 43.28; H, 4.24; N, 14.02%.)



10: R^1, R^2 = H, H
11: " = H, OH
12: " = H, CH_2OH
13: " = H CH_2CH_2OH

Fig. 2.

3',5' - Di - O - *acetyl* - 8 - *methanesulfonyl* - 2' - O - *tosyladenosine* (**5**). To a soln of **2** (30 g, 64 mmol) in dry pyridine (200 ml) was added Ac_2O (28 ml). The mixture was stirred for 5 hr at room temp, H_2O (10 ml) was then added, this was then concentrated to dryness *in vacuo*. Traces of pyridine were removed by several coevaporations with aq EtOH. The residue was dissolved in 70% aq AcOH (350 ml). A fine powdered $KMnO_4$ (22.5 g, 142 mmol) was added with stirring at 0°. After 4 hr, the mixture was diluted with H_2O (100 ml) and 30% H_2O_2 was added until the suspension became colorless. The resulting crystalline ppts were collected by filtration and washed well with H_2O to afford 36 g (90%) of **5**, m.p. 133–140°. Recrystallization of this compound was unsuccessful. PMR ($CDCl_3$): δ 2.03 (3H, s, Ac), 2.13 (3H, s, Ac), 2.37 (3H, s, tosyl-Me), 3.41 (3H, s, SO_2Me), 4.37 (3H, m, H-4', 5', 5''), 5.66 (1H, m, H-3'), 6.21 (1H, dd, H-2', $J_{1',2'}$ = 5.4 Hz, $J_{2',3'}$ = 6.4 Hz), 6.47 (2H, brs, 6-NH_2, exchangeable), 6.78 (1H, d, H-1', $J_{1',2}$ = 5.4 Hz), 7.11 (2H, d, tosyl-H), 7.56 (2H, d, tosyl-H), 8.29 (1H, s, H-2); *m/z* 583 (M^+, 1%). This product was used in the next step without further purification.

Reaction of **5** *with ethyl acetoacetate*. A mixture of ethyl acetoacetate (44 ml) and dry THF (50 ml) was treated with NaH (1.6 g, 60% in mineral oil, 40 mmol). After the evolution of H_2 ceased, **5** (5.83 g, 10 mmol) was added and the mixture was heated under reflux for 24 hr. The resulting suspension was neutralized with 1 N HCl and evaporated to dryness *in vacuo*. The residue was suspended in AcOEt and washed with H_2O. The resulting oil was chromatographed on a silica gel column using 4% EtOH in $CHCl_3$ as eluent. 3',5'-Di-O-*acetyl*-8-*ethoxycarbonylmethyl*-2'-O-*tosyladenosine* (**6**, 2.17 g, 37%) was eluted from the column first (crystallized from EtOH), m.p. 88–90°. 3',5'-*Di*-O-*acetyl*-2'-*deoxy*-2''-*ethoxycarbonyl*-8,2' - *methylene* - *cycloadenosine* (**7**, 2.15 g, 51%) was eluted from the column with 8% EtOH in $CHCl_3$ (obtained as a foam). PMR data for **6** in $CDCl_3$: δ 1.32 (3H, t, $-CH_2CO_2CH_2\underline{CH_3}$), 2.03 (3H, s, Ac), 2.11 (3H, s, Ac), 2.36 (3H, s, tosyl-Me), 3.99 (2H, s, $-\underline{CH_2}CO_2CH_2CH_3$), 4.28 (2H, q, $-CH_2CO_2\underline{CH_2}CH_3$), 4.37 (3H, m, H-4', 5', 5''), 5.62 (2H, brs, 6-NH_2, exchangeable), 5.74 (1H, m, H-3'), 6.07 (2H, m, H-1', 2'), 7.08 (2H, d, tosyl-H), 7.53 (2H, d, tosyl-H), 8.12 (1H, s, H-2); UV $\lambda_{max}^{H_2O}$ 263 nm (ε, 12,300), 230 nm (ε, 12,900), 211 nm (ε, 19,900); $\lambda_{min}^{H_2O}$ 245 nm (ε, 7300), 225 nm (ε, 12,500), 208 nm (ε, 19,700); $\lambda_{max}^{0.5\ N\ HCl}$ 261 nm (ε, 14,200), 231 nm (ε, 13,250); $\lambda_{min}^{0.5\ N\ HCl}$ 245 nm (ε, 8550), 222 nm (ε, 12,250); $\lambda_{max}^{0.05\ N\ NaOH}$ 264 nm (ε, 13,100); $\lambda_{min}^{0.05\ N\ NaOH}$ 244 nm (ε, 7000), sh 230 nm (ε, 12,700). (Found: C, 50.31; H, 4.93; N, 11.83. Calc for $C_{25}H_{29}N_5O_{10}S$: C, 50.76; H, 4.91; N, 11.84%.) For **7** in $CDCl_3$: δ 1.35 (3H, t, $-CO_2CH_2\underline{CH_3}$), 1.81 (3H, s, Ac), 2.16 (3H, s, Ac), 3.99–4.11 (1H, m, H-2', overlapped with H-5', 5''), 4.03 (2H, d, H-5', 5'', J = 3.9 Hz), 4.32 (2H, q, $-CO_2\underline{CH_2}CH_3$), 4.47 (1H, d, H-2'', $J_{2',2''}$ = 3.4 Hz), 4.51 (1H, dt, H-4', $J_{3',4'}$ = $J_{4',5'}$ = 3.9 Hz), 5.13 (1H, dd, H-3', $J_{2',3'}$ = 2.9 Hz, $J_{3',4'}$ = 3.9 Hz), 5.78 (2H, brs, 6-NH_2, exchangeable), 6.55 (1H, d, H-1', $J_{1',2'}$ = 6.4 Hz), 8.35 (1H, s, H-2); *m/z* 419 (M^+, 54%), 373 ($M^+ - 46$, 100%); high resolution MS: found *m/z* 419.1457 (M^+), calc for $[C_{18}H_{21}N_5O_7]^+$ = 419.1441; found *m/z* 373.0998 ($M^+ - 46$), calc for $[C_{16}H_{15}N_5O_6]^+$ = 373.1002 for structure **D** in the scheme. UV $\lambda_{max}^{H_2O}$/264 nm (ε, 15,150), 209 nm (ε, 21,300); $\lambda_{min}^{H_2O}$ 230 nm (ε, 2800); $\lambda_{max}^{0.5\ N\ HCl}$ 260 nm (ε, 15,150); $\lambda_{min}^{0.5\ N\ HCl}$ 230 nm (ε, 3200). In alkaline soln, **7** absorbs 264 and 342 nm. The 342 nm peak decreases to zero within a few minutes concomitant with a slight increase of the 264 nm peak. The absorbance ratio (A 264 nm/342 nm) depends on the pH of the alkaline soln: 10.8 (pH 11.0), 2.99 (pH 11.8), and 2.19 (pH 12.4).

Reaction of **5** *with diethyl malonate*. A mixture of diethyl malonate (5 ml) and dry THF (40 ml) was treated with NaH (400 mg, 60% in mineral oil, 10 mmol). After the evolution of H_2 ceased, **5** (1.46 g, 2.5 mmol) in dry THF (10 ml) was added. The mixture was heated under reflux for 8 hr. The resulting suspension was neutralized with 1 N HCl and filtered through a celite pad. The filtrate was concentrated and purified over a silica gel column which was washed with $CHCl_3$ to remove the excess diethyl malonate. Compound **7** (824 mg, 79%) was eluted with 8% EtOH in $CHCl_3$ and obtained as a foam. The PMR spectrum of this sample was identical with that of **7** prepared by cyclization of **5** with ethyl acetoacetate.

Reaction of **5** *with dimethyl malonate*. Compound **5** (5.83 g, 10 mmol) was treated with the sodium salt of dimethyl malonate (prepared from 20 ml of dimethyl malonate and 1.63 g of 60% NaH in 60 ml of dry THF) for 8 hr at reflux temp. The major product was purified by silica gel column chromatography (8% EtOH in $CHCl_3$ as the eluent) to give 3',5' - *di* - O - *acetyl* - 2' - *deoxy* - 2'' - *methoxycarbonyl* - 8,2' - *methylene* - *cycloadenosine* (**8**, 3.05 g, 75%) as a foam. PMR ($CDCl_3$): δ 1.81 (3H, s, Ac), 2.16 (3H, s, Ac), 3.88 (3H, s, $-CO_2\underline{CH_3}$), 4.02–4.14 (1H, m, H-2'), 4.04 (2H, d, H-5', 5'', $J_{4',5'}$ = 4.4 Hz), 4.52 (1H, dt, H-4', $J_{3',4'}$ = 3.4 Hz, $J_{4',5'}$ = 4.4 Hz), 5.12 (1H, dd, H-3', $J_{2',3'}$ = $J_{3',4'}$ = 3.4 Hz), 5.82 (2H, brs, 6-NH_2, exchangeable), 6.56 (1H, d, H-1', $J_{1',2'}$ = 6.4 Hz), 8.35 (1H, s, H-2); *m/z* 405 (M^+, 52%), 373 ($M^+ - 32$, 100%).

2'-Deoxy-8,2'-methylene-cycloadenosine (**9**). (a) A soln of **7** (446 mg, 1.1 mmol) in 85% aq pyridine (7 ml) was heated under reflux for 2 days and then NH_4OH (28%, 2 ml) was added. The mixture was kept overnight at room temp and concentrated to dryness *in vacuo*. The residue was crystallized from MeOH to afford 182 mg (65%) of **9**, m.p. 251–254°. PMR (DMSO-d_6): δ 2.96 (1H, dd, H-2''a, $J_{2''a,2'}$ = 3.9 Hz, $J_{a,b}$ = 17.1 Hz), 3.19 (3H, m, 2''b proton is overlapped with H-5', 5'' signals), 3.39–3.57 (1H, m, H-2'), 3.99 (1H, dt, H-4', $J_{3',4'}$ = 3.9 Hz, $J_{4',5'}$ = 4.9 Hz), 4.08 (1H, dd, H-3', $J_{2',3'}$ = 2.9 Hz, $J_{3',4'}$ = 3.9 Hz), 4.72 (1H, t, 5'-OH, exchangeable), 5.45 (1H, d, 3'-OH, exchangeable), 6.30 (1H, d, H-1', $J_{1',2'}$ = 6.3 Hz), 7.02 (2H, brs, 6-NH_2, exchangeable), 8.06 (1H, s, H-2). UV $\lambda_{max}^{H_2O}$ 262 nm (ε, 16,570), 207 nm (ε, 24,600); $\lambda_{min}^{H_2O}$ 226 nm (ε, 2700); $\lambda_{max}^{0.5\ N\ HCl}$ 259.5 nm (ε, 15,400); $\lambda_{min}^{0.5\ N\ HCl}$ 229 nm (ε, 2750); $\lambda_{max}^{0.5\ N\ NaOH}$ 262 nm (ε, 16,850); $\lambda_{min}^{0.5\ N\ NaOH}$ 231 nm (ε, 3900). CD (H_2O): $(\theta)_{258\ nm}$ +12,850, $(\theta)_{226\ nm}$ 0; *m/z* 263 (M^+, 100%), 245 (17%), 232 (20%), 215 (49%), 204 (20%), 186 (72%), 174 (74%), 162 (43%), 149 (40%). (Found: C, 50.16; H, 5.09; N, 27.00. Calc for $C_{11}H_{13}N_5O_3$: C, 50.19; H, 4.98; N, 26.60%.)

(b) A mixture of **7** (670 mg, 1.6 mmol) in EtOH (20 ml) and 1 N NaOH (2.2 ml) was stirred for 3 hr at room temp. The mixture was adjusted to pH 4 with HCO_2H, kept for 2 hr at 60° with stirring, and concentrated to dryness *in vacuo*. The residue was crystallized from MeOH to give **9** (208 mg, 49%).

Adenosine deaminase assays. Adenosine deaminase [EC.3.5.4.4] from calf intestinal mucosa (type III) was obtained from the Sigma Chemical Co. The enzyme experiments were performed in 0.05 M phosphate buffer (pH 7.5) at 25°. The kinetic parameters were determined by the procedure of Lineweaver and Burk.[23] In the case of compounds **10**–**13**, the assay solns containing about 1×10^{-4} M of the compounds and 15 μg of the enzyme were incubated at 25° for 26 hr. The absorbances at λ_{max} of these compounds were not changed.

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