Purines. XXXIV.[1] 3-Methyladenosine and 3-Methyl-2′-deoxyadenosine: Their Synthesis, Glycosidic Hydrolysis, and Ring Fission

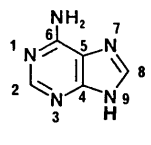
Tozo FUJII,* Tohru SAITO, and Tsuyoshi NAKASAKA

Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan. *Received March 8, 1989*

The first syntheses of 3-methyladenosine (3a) and 3-methyl-2′-deoxyadenosine (3b), both in the form of the *p*-toluenesulfonate salt, have been achieved through two parallel 6-step routes starting from adenosine (5a) and 2′-deoxyadenosine (5b), which are based on the "fission and reclosure" technology for modification of the adenine ring. These 3-methyladenine nucleosides (3a, b·TsOH) were very unstable under not only acidic but also alkaline conditions. At pH 1 and 25 °C, 3a·TsOH (half-life 17 min) underwent glycosidic hydrolysis (depurinylation) some thousand times faster than did adenosine (5a) itself. At pH 3.34 and 25 °C, the 2-deoxyribosyl analogue 3b·TsOH (half-life 2.7 min) was depurinylated 370 times more rapidly than the ribosyl analogue 3a·TsOH (half-life 1010 min). The glycosidic bond of the imidazole 2-deoxyriboside 11b, an intermediate for the present synthesis of 3b·TsOH, was also hydrolyzed easily at pH 1 and room temperature. In H_2O at pH 8.32 and 25 °C, 3a·TsOH readily underwent ring opening in the pyrimidine moiety and came to equilibrium with the (*N*-methylformamido)imidazole derivative 10a, which was further deformylated under more alkaline conditions. The ring opening was *ca.* 30 times as fast as that of 1-methyladenosine (17). In H_2O at pH 8.98 and 25 °C, the 2-deoxyribosyl analogue 3b·TsOH underwent not only similar ring opening but also glycosidic hydrolysis competitively. The possible factors responsible for the high reactivity of 3a·TsOH are discussed on the basis of its X-ray crystal structure.

Keywords 3-methyladenine nucleoside; ring fission–reclosure synthesis; hydrogenolysis; amidine formamido cyclization; glycosidic hydrolysis; depurinylation; alkaline hydrolysis; ring–chain equilibrium; kinetic study; high-performance liquid chromatography

Among the 11 possible types of N^x,N^y-disubstitution in the adenine system (**1**) is 3,9-disubstitution, which is exemplified by 3,9-dialkyladenines (type **2**),[2] 3-substituted adenosines (type **3a**), 3-methyl-2′-deoxyadenosine (**3b**), and so forth. The 3-substituted adenosine family constitutes one of the four possible groups of positional isomers of N^x-monosubstituted adenosines, and they have been prepared as modified cyclonucleoside derivatives with[3] or without[3c,4] extra N^6-substituents (*e.g.*, **4**) or as N^6,N^6-dimethyl derivatives[5] modified in the sugar moiety.[6] Although 3-methyladenosine (**3a**) is the simplest member in this family, it was unknown at the time when the present study was undertaken, whereas the other three N^x-methyladenosines [$x=1$ (**17**), 6 (**19**) or 7][7,8] had already been synthesized. The third exemplar, 3-methyl-2′-deoxyadenosine (**3b**), has been assumed to occur as a part structure in methylated deoxyribonucleic acid (DNA) molecules.[9] As far as DNA sequencing by the original Maxam–Gilbert method[10] is concerned, dimethyl sulfate methylates the 2′-deoxyguanosines in DNA at the 7-position and the 2′-deoxyadenosines at the 3-position, rendering the glycosidic bond of the methylated families labile to hydrolysis on heating at neutral pH. Whereas the methylation of the latter is considerably slower than that of the former, release of the methylated purine base by hydrolysis is considerably faster from the 3-methyl-2′-deoxyadenosines in methylated DNA than from the 7-methyl-2′-deoxyguanosines. This forms a basis for distinguishing between the adenines and guanines in DNA.[9,10] Because of such extraordinary instability of the glycosidic bond in the 3-methyl-2′-deoxyadenosine structure (**3b**) at the polynucleotide level, it is of prime importance to study this part structure at the nucleoside level. We thus investigated the syntheses and stability of 3-methyladenosine (**3a**) and 3-methyl-2′-deoxyadenosine (**3b**).



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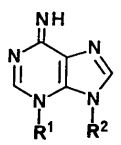

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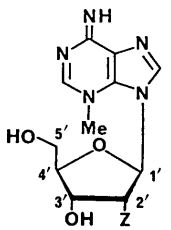
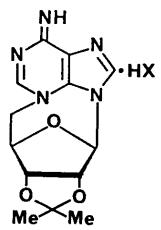


3a: Z=OH
3b: Z=H



4: X= *p*-MeC$_6$H$_4$SO$_3$, I or ClO$_4$

Brief accounts of the results recorded here have been published in preliminary form.[11]

Synthetic Routes

The target 3-methyladenine nucleosides **3a** and **3b** would be most directly accessible from adenosine (**5a**) and 2′-deoxyadenosine (**5b**) by methylation if the sugar group at the 9-position could orient the incoming methyl group to the 3-position, as in the cases of N^6,N^6-dimethyladenosine[5a] and its 2′,3′,5′-tri-*O*-benzoyl or 2′,3′,5′-tri-*O*-benzyl derivative[5a] as well as the 2′-deoxyadenosines in DNA. However, such a one-step route is not feasible since methylation of **5a** (or its 2′,3′-*O*-isopropylidene derivative) and **5b** themselves occurs preferentially at the 1-position.[7,12] For the synthesis of the desired compounds **3a** and **3b**, therefore, a circuitous route had to be arranged, and we tried to extend our previous general synthetic route[2b,c] to 3,9-dialkyladenine (**2**) from 9-alkyladenine to

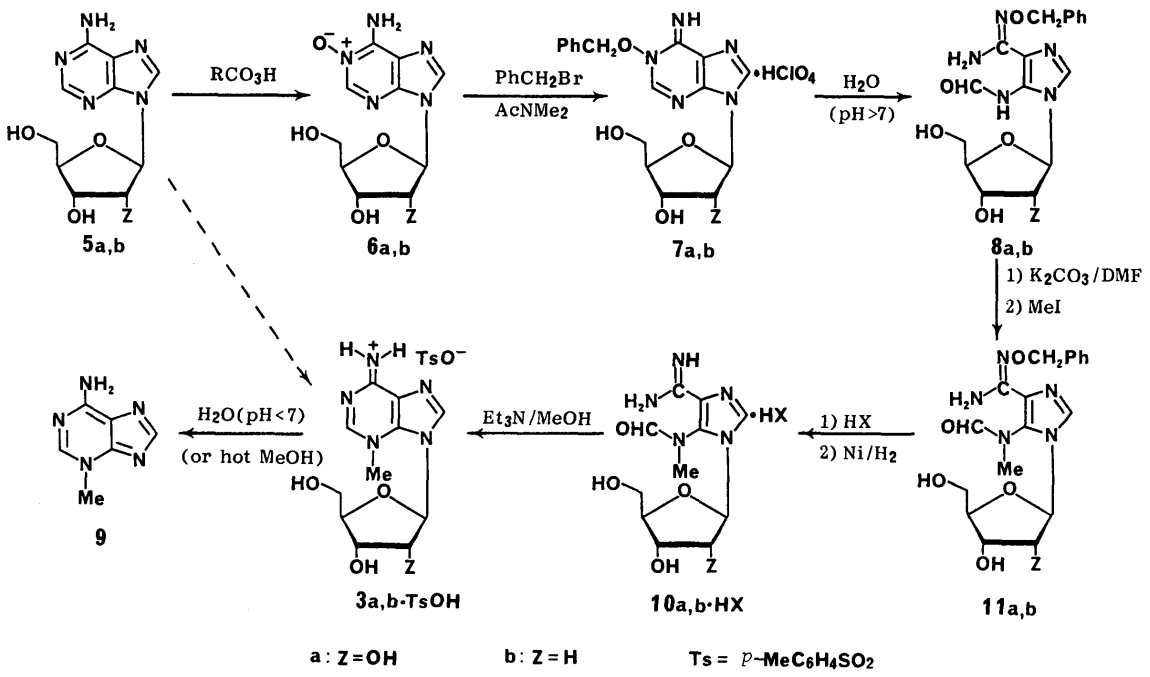


Chart 1

cover the nucleoside level.

The synthesis of the first target **3a** started with methylation of the formamidoimidazole **8a**,[13] which was prepared from adenosine (**5a**) through the *N*-oxide **6a**[14] and 1-benzyloxyadenosine perchlorate (**7a**)[15] according to the previously reported procedures. The methylation of **8a** was effected with MeI in $HCONMe_2$ in the presence of anhydrous K_2CO_3 at room temperature for 9 h, giving the *N*-methylformamido derivative **11a** in 86% yield. The succeeding steps were removal of the *N'*-benzyloxy group from **11a** and cyclization of the resulting amidine derivative to reach **3a**. In the previous synthesis of 3,9-dimethyladenine hydrochloride (**2**·HCl, $R^1=R^2=Me$),[2b,c] the corresponding steps were catalytic hydrogenolysis in H_2O containing 1 molar eq. of HCl (Raney Ni/H_2, 1 atm, room temp.) and subsequent treatment of the product with Et_3N in EtOH. When similar reaction conditions were applied to **11a**, the desired conversions seemed to have occurred, but the cyclized product **3a**·HCl could not be isolated in crystalline form. This difficulty was overcome by the use of *p*-toluenesulfonic acid (TsOH) instead of HCl for the hydrogenolysis step. Thus, **11a** was hydrogenolyzed with Raney Ni catalyst and hydrogen (1 atm, room temp., 70 min) in H_2O containing 1 molar eq. of TsOH, and crude **10a**·TsOH that resulted was treated with a little Et_3N in MeOH at room temperature for 48 h, producing the target nucleoside **3a** in the form of the crystalline salt **3a**·TsOH in 53% yield (from **11a**). The correctness of the assigned structure **3a**·TsOH was supported by elemental analysis, the above self-consistent reaction sequence through which it was formed, and its ultraviolet (UV) and nuclear magnetic resonance (NMR) spectra. The UV spectra in 95% aqueous EtOH [λ_{max} 272 nm (ε 16500)] and in H_2O at pH's 1 [λ_{max} 270 (17400) (slightly unstable)], 7 [λ_{max} 270 (17400)], and 13 (unstable) were similar to those[2b,c] of 3,9-dimethyladenine hydrochloride (**2**·HCl, $R^1=R^2=Me$) or perchlorate (**2**·$HClO_4$, $R^1=R^2=Me$). The ^{1}H-NMR spectrum in Me_2SO-d_6 exhibited a three-proton singlet at δ 4.19 [N(3)-Me], two one-proton singlets at 8.59 and 8.74 (purine protons), and two one-proton singlets at 9.12 and 9.21 ($=NH_2^+$), which resembled those observed[2c] for 3,9-dimethyladenine perchlorate (**2**·$HClO_4$, $R^1=R^2=Me$), besides signals attributable to the protons in the ribosyl and *p*-toluenesulfonate moieties. Finally, the whole structure of **3a**·TsOH, especially its exocyclic iminium character, has recently been confirmed by means of X-ray crystallographic analysis.[16]

The second target 3-methyl-2'-deoxyadenosine (**3b**) was then prepared in the form of the *p*-toluenesulfonate salt **3b**·TsOH through a parallel sequence of conversions starting from 2'-deoxyadenosine (**5b**). Oxidation of **5b** with monoperphthalic acid to give the *N*-oxide **6b**[17] was carried out according to the procedure reported by Klenow and Frederiksen.[17a] In general agreement with previous results,[13a,15,17b] the reaction of **6b** with $PhCH_2Br$ in $AcNMe_2$ (room temp., 4 h) and treatment of the benzylated product with $NaClO_4$ gave the 1-benzyloxy derivative **7b** in 85% yield. The perchlorate **7b** was converted into the free base by the use of Amberlite IRA-402 (HCO_3^-), and the base was treated with H_2O at 3—4 °C for 8 d to afford the formamidoimidazole **8b** in 70% yield. On methylation with MeI and anhydrous K_2CO_3 in $HCONMe_2$ at room temperature for 5 h, **8b** furnished the *N*-methylformamido derivative **11b** (70% yield), which was hydrogenolyzed in H_2O with Raney Ni catalyst and hydrogen at room temperature in the presence of 1 molar eq. of TsOH. The crude **10b**·TsOH that formed was then treated with a little Et_3N in MeOH at −18 °C for 3—9 d to give the desired compound **3b**·TsOH in 22% yield (from **11b**). The UV spectrum of **3b**·TsOH in H_2O at pH 7 [λ_{max} 271 nm (ε 16900) (slightly unstable)] was closely similar to that of **3a**·TsOH, but those in H_2O at pH's 1 and 13 rapidly and that in 95% aqueous EtOH slowly changed the shape of their curves during measurement. The ^{1}H-NMR spectrum in Me_2SO-d_6

[selected peaks: δ 2.28 (3H, s, $MeC_6H_4SO_3^-$), 4.19 [3H, s, N(3)-Me], 8.63 and 8.71 (1H each, s, purine protons), 9.15 and 9.23 (1H each, br s, $=NH_2^+$)] was also similar to that of **3a**·TsOH, supporting the correctness of the assigned structure **3b**·TsOH.

Glycosidic Hydrolysis of 3-Methyladenine Nucleosides

Remarkable was the instability of the 3-methyladenine nucleosides thus synthesized. On treatment with 0.1 N aqueous HCl (pH *ca.* 1) at 27 °C for 1 h, **3a**·TsOH underwent glycosidic hydrolysis (depurinylation) to give 3-methyladenine (**9**) in 92% yield (Chart 1). We monitored this depurinylation at 25 °C, determining the unaltered nucleoside by means of high-performance liquid chromatography (HPLC), and obtained a pseudo-first-order rate constant of $4.00 \times 10^{-2}\,min^{-1}$ and a half-life of 17 min (Table I). Adenosine (**5a**) itself was virtually stable under the same reaction conditions for at least 3 h, but the rate constant for its depurinylation at pH 1 and 37 °C was estimated to be $2.16 \times 10^{-5}\,min^{-1}$ (half-life 22 d) using data reported by Venner.[18] It thus became clear that the introduction of a methyl group into adenosine (**5a**) at the 3-position made glycosidic hydrolysis some thousand times faster under acidic conditions. The 2-deoxyribosyl analogue **3b**·TsOH was even more susceptible to solvolysis: it afforded **9** in 60% yield on treatment with H_2O at pH 3.34 and 20 °C for 45 min and in 99% yield on treatment with boiling MeOH for 30 min.

The kinetic study was then extended to include the glycosidic hydrolyses of both 3-methyladenine nucleosides **3a**·TsOH and **3b**·TsOH in H_2O at various pH's with a view to comparing their stabilities. It may be seen from Table I that the 2-deoxyribosyl analogue **3b**·TsOH is very unstable even at pH 7.00 and 37 °C, and its rate of hydrolysis at low pH increases with increasing hydronium ion concentration: at pH 3.34 and 25 °C it hydrolyzes 370 times more rapidly than does the ribosyl analogue **3a**·TsOH, being in general agreement with the fact that 2′-deoxyadenosine (**5b**) itself

TABLE I. Rate Constants for the Glycosidic Hydrolyses of Adenine Nucleosides and for the Release of 3-Methyladenine (**9**) from Methylated DNA in H_2O

Substrate	Reaction conditions		Pseudo-first-order rate constant (min^{-1})	Half-life (min)
	pH	Temp. (°C)		
Methylated DNA[a]	7.0	37	4.81×10^{-4}	1440
	5.0	37	1.01×10^{-3}	686
3-Methyl-2′-deoxyadenosine *p*-toluenesulfonate (**3b**·TsOH)	7.00	37	1.99×10^{-2}	35
	5.00	37	3.91×10^{-2}	18
	3.34	25	2.54×10^{-1}	2.7
3-Methyladenosine *p*-toluenesulfonate (**3a**·TsOH)	3.34	25	6.87×10^{-4}	1010
	1[b]	25	4.00×10^{-2}	17
Adenosine (**5a**)[c]	1	37	2.16×10^{-5}	32300

a) Taken from ref. 24. *b*) Data from the hydrolysis in 0.1 N aqueous HCl. *c*) Estimated by using data from ref. 18.

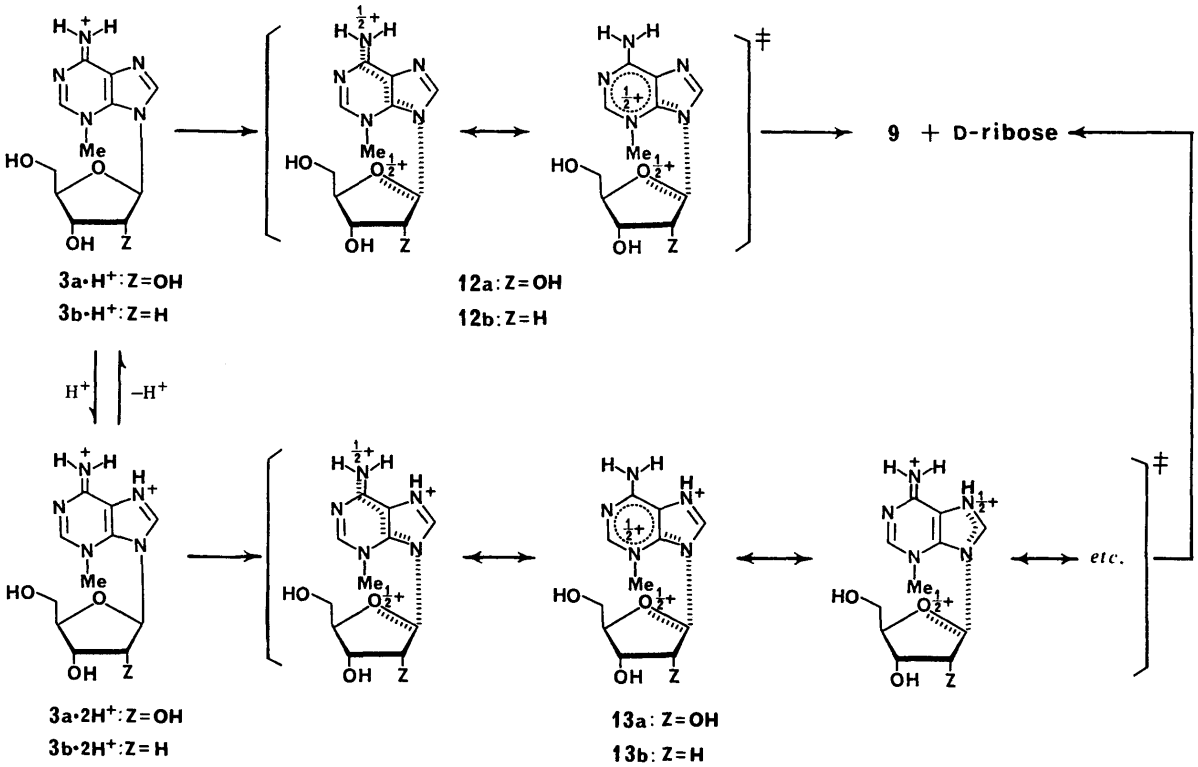


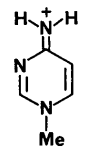
Chart 2

undergoes glycosidic hydrolysis about 1000 times faster than does adenosine (**5a**) itself.[19]

Why do the 3-methyladenine nucleosides **3a**·TsOH and **3b**·TsOH undergo depurinylation so rapidly? A key to the solution of this question may be obtained from the recently reported X-ray crystal structure of **3a**·TsOH.[16] In the crystal, this salt has an N^6-protonated structure in which the exocyclic iminium structure, as shown in formula **3a**·TsOH (Chart 1), is a very important contributor to the possible resonance hybrid. The adenine moiety is almost planar, and the N(9)–C(1′) bond is almost coplanar with the adenine ring. However, the N(3)-methyl group is displaced rather significantly from planarity, and the endocyclic and exocyclic angles at N(3), C(4), and N(9) notably depart from those of the usual adenosine systems, being in favor of keeping the N(3)-methyl and N(9)-ribosyl groups away from each other. The ribose moiety is in the C(2′)-*endo* (^{2}E) puckering conformation and in the *high-anti* (part of *syn*) conformation [with the torsion angle O(1′)–C(1′)–N(9)–C(4), $\chi = -72.3°$] with respect to the adenine moiety. Assuming **3a**·TsOH maintains its exocyclic iminium structure even in solution and an A-1 mechanism[19,20] for solvolyses of nucleosides is operating in its hydrolysis, transition structures **12a** and **13a** derived from the mono- (**3a**·H^+) and diprotonated (**3a**·$2H^+$) forms may be considered, as shown in Chart 2. Since the basicity of 3,9-dimethyladenine (**2**, $R^1 = R^2 = Me$) has been shown to be considerably high,[2b,c] **3a**·TsOH probably exists almost completely in the monoprotonated form (**3a**·H^+) even at pH 7. This view may be supported by the structural analogy between **3a**·H^+ and the "*para*-quinonoidal" resonance structure (**14**) of protonated 1,4-dihydro-4-imino-1-methylpyrimidine whose pK_a is 12.22.[21] The above X-ray structure suggests that the N(3)-methyl group does not exert much of a steric repulsion with the ribosyl moiety, being in agreement with the recent result of molecular mechanics calculations by Czarnik's group.[22] However, the unusual geometry of the adenine moiety described above may cause the free energy of the starting structure (**3a**·H^+) to be raised, whereas the transition structure (**12a**) may be stabilized by resonance, thus permitting the activation energy to be lower.

At low pH's the diprotonated species **3a**·$2H^+$ should be favored with increasing hydronium ion concentration. The UV spectra of 3,9-dimethyladenine hydrochloride (**2**·HCl, $R^1 = R^2 = Me$)[2b,c] or perchlorate (**2**·$HClO_4$, $R^1 = R^2 = Me$)[2] in H_2O at pH 7 [where it is considered to exist almost completely in the monoprotonated form (*vide supra*)] and at pH 1 are very similar, but not superimposable on each other. This is also the case for **3a**·TsOH, suggesting the existence of the diprotonated species (**3a**·$2H^+$) at low pH's. The second site of protonation is considered to be N(7), as proposed for the acid-catalyzed solvolysis of protonated adenine nucleosides,[19a,23] since the nitrogens in the pyrimidine moiety must be less basic owing to the exocyclic iminium structure. Assuming the geometry of the adenine moiety in **3a**·$2H^+$ to be similar to that in **3a**·H^+, the accelerated depurinylation of **3a**·TsOH at low pH's may be explained as in the case of the monoprotonated species.

The observed 370-fold rate acceleration of the 2-deoxyribosyl analogue **3b**·TsOH compared to the ribosyl analogue **3a**·TsOH is attributable to the absence of the C(2′)-OH group, which in a parallel A-1 pathway (Chart 2) inhibits by its inductive effect the slow N(9)–C(1′) bond cleavage of a protonated nucleoside that results in a C(1′) carbocation.[19,20a] Interestingly, the 3,5′-cyclonucleoside **15** has recently been found to undergo glycosidic hydrolysis 29000 times more slowly than does **3a**·TsOH at 25 °C in 0.1 N aqueous HCl.[22] This surprising stability has been explained in terms of (1) the presence of an electron-withdrawing immonium group in **15** at the 5′-position and (2) the generation of an "anti-Bredt transition structure".[22]



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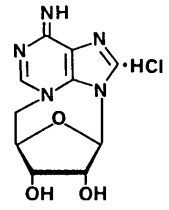

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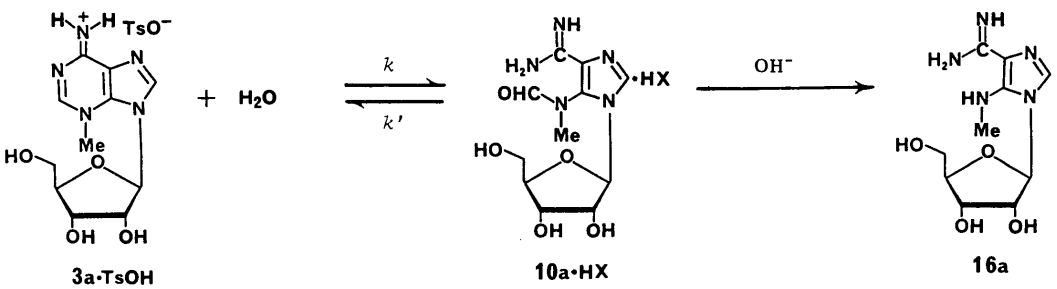
Further interest in the glycosidic hydrolysis stems from the comparison of the rate of depurinylation of **3b**·TsOH with that[24] of methylated DNA which includes **3b** as a part structure. It may be seen from Table I that at pH 5.0 and 7.0 methylated DNA releases 3-methyladenine (**9**) *ca.* 40 times more slowly than does 3-methyl-2′-deoxyadenosine *p*-toluenesulfonate (**3b**·TsOH). This order of the depurinylation rate is coincident with that reported[25] for the release of 7-methylguanine in the 7-methyl-2′-deoxyguanosine series [the nucleoside level (half-life at pH 7.4 and 37 °C, 5.9 h)[25b] > the nucleotide level (23 h)[25b] > the DNA level (69 h)[25b]]. It is suggested that the effect of the negative charge in the phosphate moiety[25a,26] and higher-order structure of the molecule on the glycosidic hydrolysis should be taken into account at the DNA level.

Ring Fission of 3-Methyladenine Nucleosides

Another notable feature of the chemical behavior of **3a**·TsOH and **3b**·TsOH was that they were also unstable



3a·TsOH **10a·HX** **16a**

Chart 3

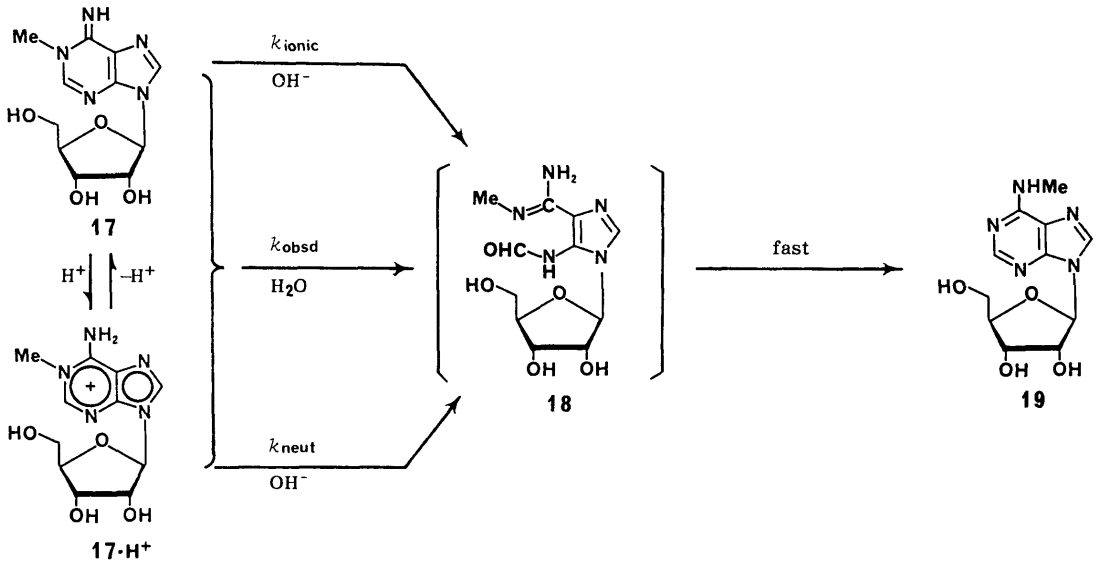


Chart 4

under basic conditions, as in the cases of 3,9-dialkyladenine salts (**2**·HX),[2b,c] 3,5′-cycloadenosine derivatives (*e.g.*, **4**),[3a,c,22,27] and N^6,N^6,3-trimethyladenosine derivatives.[5] On treatment with Amberlite CG-400 (OH^-) in H_2O at room temperature, **3a**·TsOH underwent ring opening in the adenine moiety, and the methylaminoimidazole **16a** was isolated in the form of the dihydrochloride (**16a**·2HCl) (87% yield), paralleling the previous results[2b,c] from a similar treatment of 3,9-dimethyladenine hydrochloride (**2**·HCl, $R^1=R^2=Me$). In view of the low electron density at the 2-position of **3a**·TsOH, the observed conversion may be rationalized in terms of an initial nucleophilic attack of hydroxide ion at the 2-position and succeeding steps proceeding through ring opening to form **10a** and deformylation, as delineated in Chart 3. In 0.1 M aqueous $NaHCO_3$, the UV spectra of both **3a**·TsOH and **10a**·TsOH separately changed with time through the same isosbestic point at 253 nm, converging on an identical spectrum. This indicated the existence of equilibrium between the purine **3a** and the imidazole **10a** under weakly alkaline conditions, as in the cases of 3,9-dialkyladenines (**2**).[2b,c] We then followed UV spectrophotometrically the time-courses of the ring opening of **3a**·TsOH and of cyclization of **10a**·TsOH in 0.1 M aqueous $NaHCO_3$ (pH 8.32) at 25 °C, and the pseudo-first-order rate constants and equilibrium constant for the reversible reaction system (Chart 3) were determined to be $k=6.62\times10^{-3}\,min^{-1}$, $k'=6.88\times10^{-3}\,min^{-1}$, and $K=k/k'=0.96$.

Isomeric with **3a** is 1-methyladenosine (**17**), which is known to give N^6-methyladenosine (**19**) under basic conditions through Dimroth rearrangement.[7,12] The rate (k_{obsd}) of this rearrangement in H_2O at pH 8.32 and 25 °C is calculated to be $2.11\times10^{-4}\,min^{-1}$ on the basis of the rate law reported by Macon and Wolfenden.[28] In view of its reaction mechanism involving a rate-determining initial ring opening (**17**→**18**←**17**·H^+) and a subsequent fast ring closure (**18**→**19**) (Chart 4),[12b,28,29] the rate of the ring opening corresponding to that of **3a**·TsOH may be regarded as that (k_{obsd}) of the rearrangement itself. This leads to the conclusion that ring opening of **3a**·TsOH in H_2O at pH 8.32 and 25 °C is *ca.* 30 times as fast as

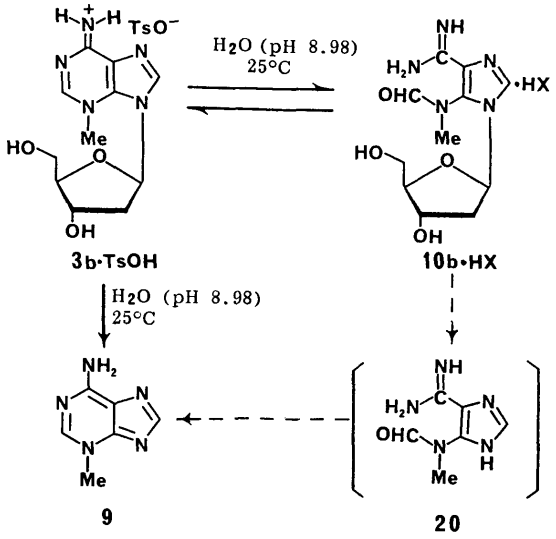


Chart 5

that of the 1-methyl isomer **17**.

We next monitored the reaction of the 2-deoxyribosyl analogue **3b**·TsOH in H_2O at pH 8.98 and 25 °C by means of HPLC. The results revealed that **3b**·TsOH was slowly converted into 3-methyladenine (**9**) in 45 h, during which time the temporary formation of the monocycle **10b** was observed. The appearance of **10b** during the reaction is attributable to the attainment of equilibrium with the starting material, being in agreement with the ring opening of the ribosyl analogue **3a**·TsOH described above. The observed glycosidic cleavage in alkaline solution to produce 3-methyladenine (**9**) was quite notable since nucleosides are generally very resistant to alkaline hydrolysis.[29b,30] In view of the unique structure of **3b**·TsOH in which the base moiety is protonated even at pH 8.98, the formation of **9** is assumed to be a result of the glycosidic hydrolysis of **3b**·TsOH, and the possibility of the alternative pathway **10b**→**20**→**9** (Chart 5) may be excluded.

Although loss of 3-methyladenine (**9**) from methylated DNA *in vivo* could be explained in terms of chemical depurinylation alone, active enzymatic excision has also

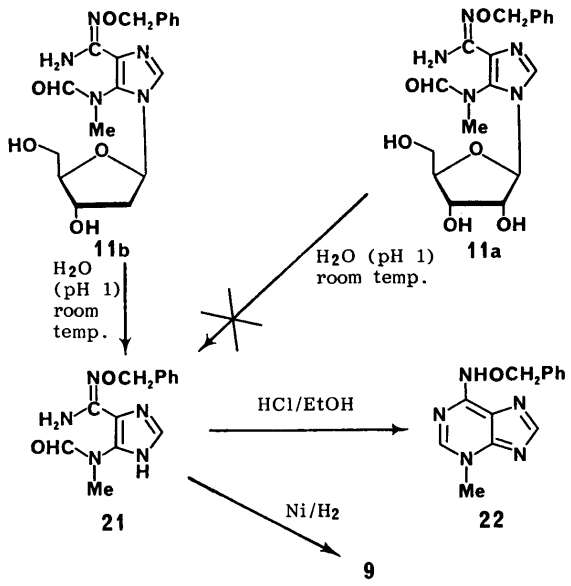


Chart 6

been suggested.[24] This led to the isolations of 3-methyladenine-DNA glycosylase in partially purified form from both bacterial and mammalian sources.[31] The enzymatic release of 3-methyladenine (**9**) from methylated DNA has been reported[31b,f] to be markedly dependent on the secondary structure of the DNA: double-stranded DNA is an effective substrate, whereas single-stranded DNA is only attacked at a very slow rate, but a short alkali-treatment has usually been applied to cause separation of the methylated DNA strands.[31b,f] Considering the instability of the 3-methyl-2′-deoxyadenosine part structure in the DNA under alkaline conditions as inferred from the observations at the nucleoside level, the latter half of the above conclusion appears to be unsound.

Glycosidic Hydrolysis of Imidazole Nucleosides

The glycosidic bond of the imidazole 2-deoxyriboside **11b** was also unstable in aqueous acidic solution. On treatment with 0.1 N aqueous HCl at room temperature for 3.5 h, **11b** furnished the aglycone **21** in 61% yield. The ribosyl analogue **11a** was found to be stable under the same conditions for at least 12 h. The structure of **21**[32] was confirmed by its cyclization in ethanolic HCl at room temperature to produce N^6-benzyloxy-3-methyladenine (**22**)[33] and by its hydrogenolysis (Raney Ni/H_2, 50% aqueous EtOH, 1 atm, 30°C, 3 h) and spontaneous cyclization to yield 3-methyladenine (**9**). It has been shown that the glycosidic bond of 1-β-D-ribofuranosylimidazole is resistant to acid hydrolysis, but introduction of an amino group into the 5-position facilitates the hydrolysis.[23a,30a] The ready glycosidic hydrolysis observed for **11b** may be attributed to electron-withdrawing effects of the formamido and the N'-benzyloxyamidine groups in the protonated form and to the 2-deoxyribosyl structure.

Conclusion

The syntheses of the 3-methyladenine nucleosides **3a**·TsOH and **3b**·TsOH have now become possible through two parallel six-step routes starting from adenosine (**5a**) and 2′-deoxyadenosine (**5b**), respectively, as shown in Chart 1. This emphasizes the synthetic utility of our "fission and reclosure" technology[34] for modification of the adenine ring. These new nucleosides are very unstable under not only acidic but also basic conditions. At pH 1 and 25 °C, 3-methyladenosine *p*-toluenesulfonate (**3a**·TsOH) (half-life 17 min) undergoes glycosidic hydrolysis (depurinylation) some thousand times faster than does adenosine (**5a**) itself. This accelerated depurinylation may be attributed to the N^6-protonated structure (even in the weakly alkaline region) of **3a**·TsOH in which the exocyclic iminium structure is a very important contributor to the possible resonance hybrid and to the unusual geometry of the adenine moiety, as indicated by its X-ray crystal structure.[16] The 2-deoxyribosyl analogue **3b**·TsOH is depurinylated at pH 3.34 and 25 °C (half-life 2.7 min) 370 times more rapidly than the ribosyl analogue **3a**·TsOH and at pH 5.0 or 7.0 at 37 °C *ca.* 40 times more rapidly than the corresponding structure in methylated DNA. The glycosidic bond of the imidazole 2-deoxyriboside **11b**, an intermediate for the present synthesis of **3b**·TsOH, is also hydrolyzed easily at pH 1 and room temperature.

In H_2O at pH 8.32 and 25 °C, the ribosyl analogue **3a**·TsOH easily opens its adenine ring and comes to equilibrium with the (*N*-methylformamido)imidazole **10a**, which is deformylated under more basic conditions to afford **16a**. The ring opening is *ca.* 30 times as fast as that (**17**→**18**) of 1-methyladenosine (**17**). The 2-deoxyribosyl analogue **3b**·TsOH undergoes not only similar ring opening in H_2O at pH 8.98 and 25 °C, equilibrating with the monocycle **10b**, but also competitive glycosidic hydrolysis to give 3-methyladenine (**9**) in 45 h.

It is hoped that the present findings concerning the extraordinary instability of the 3-methyladenine nucleosides (**3a,b**·TsOH), especially that of the 3-methyl-2′-deoxyadenosine structure (**3b**) under alkaline conditions, will be of help in reaching a better understanding of the nature of the corresponding structure at the polynucleotide level when taken into account in chemical and biochemical studies of methylated DNA.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. Paper partition chromatographies (PPC) were developed as described in ref. 2*c*. High-performance liquid chromatographic (HPLC) analyses were carried out on a Waters ALC/GPC 204 liquid chromatograph. See refs. 1 and 2*c* for details of other instrumentation and measurements. Elemental analyses were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, m = multiplet, s = singlet, sh = shoulder, t = triplet.

1-Benzyloxy-2′-deoxyadenosine Perchlorate (7b) A mixture of 2′-deoxyadenosine 1-oxide (**6b**)[17] (5.89 g, 22 mmol) and $PhCH_2Br$ (18.8 g, 110 mmol) in $AcNMe_2$ (50 ml) was stirred at room temperature for 4 h. The resulting solution was diluted with ether (300 ml) to deposit an oil, which was separated from the ethereal layer by decantation, washed with ether (2 × 60 ml), and then dissolved in warm H_2O (80 ml). The aqueous solution was washed with benzene (2 × 40 ml) and kept, after addition of a solution of $NaClO_4$ (13.47 g, 110 mmol) in H_2O (20 ml), in a refrigerator overnight. The precipitate that resulted was filtered off, washed with a little H_2O, and dried to give **7b** (8.61 g, 85%), mp 143.5—144.5 °C (dec.). Recrystallization from EtOH yielded an analytical sample as colorless minute prisms, mp 143.5—144.5 °C (dec.); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 258 nm (ε 12700), 263 (sh) (11900); $\lambda_{max}^{H_2O}$ (pH 1) 259 (13200); $\lambda_{max}^{H_2O}$ (pH 7) 259 (13100); $\lambda_{max}^{H_2O}$ (pH 13) 257.5 (13200), 262 (sh) (12300); NMR (Me_2SO-d_6) δ: 5.41 (2H, s, OCH_2Ph), 6.38 [1H, dd, J = 6.6 Hz each, C(1′)-H], 7.40—7.70 (5H,

br m, OCH_2Ph), 8.76 and 8.94 (1H each, s, purine protons). *Anal.* Calcd for $C_{17}H_{19}N_5O_4 \cdot HClO_4$: C, 44.60; H, 4.40; N, 15.30. Found: C, 44.36; H, 4.31; N, 15.32.

N'-Benzyloxy-1-(2-deoxy-β-D-ribofuranosyl)-5-formamido-1H-imidazole-4-carboxamidine (8b) A solution of **7b** (4.12 g, 9 mmol) in H_2O (400 ml) was passed through a column of Amberlite IRA-402 (HCO_3^-) (45 ml, 56.3 mmol eq), and the column was eluted with H_2O. The eluate (850 ml) was concentrated *in vacuo* to a volume of *ca.* 80 ml. The residual solution was kept in a refrigerator at 3—4 °C for 8 d and then concentrated to dryness *in vacuo* to leave a colorless glass. Purification of this substance by column chromatography [silica gel (380 g), $CHCl_3$–EtOH (7:1, v/v)], followed by trituration with $CHCl_3$, afforded **8b** (2.41 g, 70%) as a colorless solid, mp 137—139 °C (dec.). The solid was recrystallized from AcOEt and dried over P_2O_5 at 2 mmHg and 50 °C for 20 h to give an analytical sample of **8b**·1/4H_2O as colorless needles, mp 138—139 °C (dec.); MS *m/z*: 375 (M^+); UV $\lambda_{max}^{H_2O}$ (pH 1) 253 nm (ε 8600); $\lambda_{max}^{H_2O}$ (pH 7) 250 (sh) (7100); $\lambda_{max}^{H_2O}$ (pH 13) 253 (12400); NMR (Me_2SO-d_6) δ: 4.87 and 4.93 (2H, s each, OCH_2Ph), 5.50—5.70 (2H, br, NH_2), 5.68 and 5.87 [1H, dd each, *J* = 6 Hz each, C(1')-H], 7.28—7.40 (5H, m, OCH_2Ph), 7.94 [1H, s, C(2)-H], 8.03 [0.5H, d, *J* = 10.7 Hz, HCONH (*trans*)], 8.20 [0.5H, s, HCONH (*cis*)], 9.42 [0.5H, d, *J* = 10.7 Hz, HCONH (*trans*)], 9.66 [0.5H, s, HCONH (*cis*)].[35] *Anal.* Calcd for $C_{17}H_{21}N_5O_5 \cdot 1/4H_2O$: C, 53.75; H, 5.70; N, 18.46. Found: C, 53.79; H, 5.70; N, 18.36.

N'-Benzyloxy-5-(N-methylformamido)-1-β-D-ribofuranosyl-1H-imidazole-4-carboxamidine (11a) A mixture of **8a**[13] (5.87 g, 15 mmol) and anhydrous K_2CO_3 (3.11 g, 22.5 mmol) in $HCONMe_2$ (95 ml) was stirred at room temperature for 1 h. A solution of MeI (2.56 g, 18 mmol) in $HCONMe_2$ (5 ml) was then added, and the resulting mixture was stirred at room temperature for 9 h. The reaction mixture was concentrated *in vacuo* to leave a yellowish brown jelly, which was triturated under cooling after addition of H_2O (20 ml) and saturated aqueous NaCl (80 ml). The solid that resulted was collected by filtration, washed with cold H_2O (15 ml), and dried to give **11a** (4.61 g, 76%), mp 154—156 °C. The filtrate was extracted with AcOEt (3 × 50 ml), and the AcOEt extracts were washed with saturated aqueous NaCl (20 ml), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was crystallized from $CHCl_3$ to afford a second crop of **11a** (594 mg, 10%) as a colorless solid, mp 154—156 °C. The total yield was 5.20 g (86%). For analysis, crude **11a** was recrystallized from AcOEt to furnish colorless prisms, mp 160—161 °C; $[\alpha]_D^{20}$ −37.7° (*c* = 1.01, MeOH); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 250 nm (sh) (ε 6650); $\lambda_{max}^{H_2O}$ (pH 1) 246 (sh) (8100); $\lambda_{max}^{H_2O}$ (pH 7) 250 (sh) (5900); $\lambda_{max}^{H_2O}$ (pH 13) 250 (sh) (6000); NMR (Me_2SO-d_6) δ: 2.93 (2.7H) and 3.08 (0.3H) (s each, HCONMe), 3.54 [2H, m, C(5')-H's], 3.89 [1H, m, C(4')-H], 4.01 [1H, m, C(3')-H], 4.28 [1H, m, C(2')-H], 4.85 and 4.90 (2H, s each, OCH_2Ph), 5.01 [1H, t, *J* = 5 Hz, C(5')-OH], 5.22 [1H, d, *J* = 5 Hz, C(3')-OH], 5.26 [1H, d, *J* = 6 Hz, C(1')-H], 5.48 [1H, d, *J* = 7 Hz, C(2')-OH], 5.79 and 5.72 (sh) (2H, br, NH_2), 7.33 (5H, m, OCH_2Ph), 7.97 (major), 8.13 (minor), and 8.14 (minor) (1H, s each, HCONMe), 8.06 (major), 8.00 (minor), and 8.01 (minor) [1H, s each, C(2)-H].[35,36] *Anal.* Calcd for $C_{18}H_{23}N_5O_6$: C, 53.33; H, 5.72; N, 12.27. Found: C, 53.22; H, 5.76; N, 17.11.

In order to check the stability of **11a** in H_2O at pH 1, a solution of **11a** (20 mg) in 0.1 N aqueous HCl (5 ml) was kept at room temperature. At intervals, small samples of the solution were withdrawn and subjected to thin-layer chromatographic (TLC) [silica gel, $CHCl_3$–EtOH (8:1, v/v)] and PPC analyses, which indicated that **11a** was completely stable over a period of at least 12 h.

N'-Benzyloxy-1-(2-deoxy-β-D-ribofuranosyl)-5-(N-methylformamido)-1H-imidazole-4-carboxamidine (11b) A mixture of **8b** (263 mg, 0.69 mmol) and anhydrous K_2CO_3 (145 mg, 1.05 mmol) in $HCONMe_2$ (5 ml) was stirred at room temperature for 1 h, and then MeI (120 mg, 0.84 mmol) was added. After having been stirred at room temperature for 5 h, the reaction mixture was concentrated *in vacuo* to leave a yellowish brown jelly, which was extracted with boiling AcOEt (3 × 15 ml). Concentration of the AcOEt extracts under reduced pressure left a colorless glass. This was triturated with AcOEt (30 ml) under cooling and kept in a refrigerator overnight. The precipitate that resulted was filtered off, washed with a little AcOEt, and dried to give a first crop (134 mg, 50%) of **11b** as a colorless powder, mp 138—139 °C (dec.). The filtrate and washings were combined and concentrated *in vacuo*, and the residue was purified by column chromatography [silica gel (16 g), $CHCl_3$–EtOH (7:1, v/v)], affording a second crop (54 mg, 20%) of **11b**, mp 139—140 °C (dec.). The total yield was 188 mg (70%). Recrystallization of crude **11b** from AcOEt provided an analytical sample as colorless minute needles, mp 141—142 °C (dec.); MS *m/z*: 389 (M^+); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 250 nm (sh) (ε 5800); $\lambda_{max}^{H_2O}$ (pH 1) 250 (sh) (7400) (slightly unstable); $\lambda_{max}^{H_2O}$ (pH 7) 250 (sh) (5800); $\lambda_{max}^{H_2O}$ (pH 13) 250 (sh) (5800); NMR (Me_2SO-d_6) δ: 2.93 and 3.10 (3H, s each, HCONMe), 4.84 (2H, s, OCH_2Ph), 5.74 [1H, dd, *J* = 6.0 Hz each, C(1')-H], 5.77 (2H, br, NH_2), 7.16—7.36 (5H, m, OCH_2Ph), 7.99 (major, s), 8.02 (major, s), and 8.10 (minor, br) [2H, C(2)-H and HCONMe].[35] *Anal.* Calcd for $C_{18}H_{23}N_5O_5$: C, 55.52; H, 5.95; N, 17.98. Found: C, 55.48; H, 5.75; N, 17.74.

5-(N-Methylformamido)-1-β-D-ribofuranosyl-1H-imidazole-4-carboxamidine *p*-Toluenesulfonate (10a·TsOH) A solution of **11a** (811 mg, 2 mmol) in H_2O (50 ml) containing *p*-toluenesulfonic acid monohydrate ($TsOH \cdot H_2O$) (380 mg, 2 mmol) was hydrogenated over Raney Ni W-2 catalyst[37] (1.5 ml) at atmospheric pressure and room temperature for 70 min. The catalyst was removed by filtration and washed with H_2O (15 ml). The filtrate and washings were combined and concentrated *in vacuo*, and the residue was dried to give crude **10a**·TsOH (930 mg) as a pale bluish glass, UV $\lambda_{max}^{H_2O}$ (pH 1) 250 nm; $\lambda_{max}^{H_2O}$ (pH 7) 250. This material was directly used in the next cyclization step without purification.

1-(2-Deoxy-β-D-ribofuranosyl)-5-(N-methylformamido)-1H-imidazole-4-carboxamidine *p*-Toluenesulfonate (10b·TsOH) Hydrogenolysis of **11b** (779 mg, 2 mmol) was effected in a manner similar to that described above for **10a**·TsOH, and crude **10b**·TsOH (910 mg) was obtained as a pale bluish glass. This sample was directly used in the next cyclization step without purification.

3-Methyladenosine *p*-Toluenesulfonate (3a·TsOH) A solution of crude **10a**·TsOH (930 mg) in MeOH (15 ml) containing Et_3N (0.05 ml, 0.36 mmol) was kept at room temperature for 48 h. The colorless precipitate that resulted was collected by filtration, washed with MeOH (1 ml), and dried to give a first crop (373 mg, 41%) of **3a**·TsOH, mp *ca.* 150 °C (dec.). The filtrate was kept at room temperature for a further 48 h, depositing a second crop (107 mg, 12%) of the solid, mp *ca.* 150 °C (dec.). The total yield was 480 mg (53% from **11a**). Recrystallization of crude **3a**·TsOH from MeOH furnished an analytical sample as colorless plates, mp *ca.* 150 °C (dec.); $[\alpha]_D^{20}$ −28.2° (*c* = 1.00, H_2O); CD (*c* = 5.56 × 10^{-5} M, MeOH) $[\theta]^{21}$ (nm): 0 (315), −100 (310), −1600 (290), −3100 (276) (neg. max.), −1300 (260), 0 (250); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 272 nm (ε 16500); $\lambda_{max}^{H_2O}$ (pH 1) 270 (17400) (slightly unstable); $\lambda_{max}^{H_2O}$ (pH 7) 270 (17400); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 2.28 (3H, s, $MeC_6H_4SO_3^-$), 3.67 [2H, br m, C(5')-H's], 3.9—4.3 [2H, br m, C(3')-H and C(4')-H], 4.19 [3H, s, N(3)-Me], 4.45 [1H, br m, C(2')-H], 4.7—5.5 [2H, br, C(5')-OH and C(3')-OH], 5.5—5.9 [1H, br, C(2')-OH], 6.19 [1H, d, *J* = 4 Hz, C(1')-H], 7.08 and 7.46 (2H each, d, *J* = 8 Hz, $MeC_6H_4SO_3^-$), 8.59 and 8.74 (1H each, s, purine protons), 9.12 and 9.21 (1H each, s, $=NH_2^+$); crystal data and X-ray structure analysis (see ref. 16). *Anal.* Calcd for $C_{11}H_{15}N_5O_4 \cdot CH_3C_6H_4SO_3H$: C, 47.68; H, 5.11; N, 15.44. Found: C, 47.94; H, 5.04; N, 15.53.

2'-Deoxy-3-methyladenosine *p*-Toluenesulfonate (3b·TsOH) A solution of crude **10b**·TsOH (760 mg) in MeOH (1 ml) containing Et_3N (0.05 ml, 0.36 mmol) was kept at −18 °C for 72 h. The colorless precipitate that resulted was filtered off, washed with a little MeOH, and dried to yield a first crop (88 mg) of **3b**·TsOH, mp *ca.* 120 °C (dec.). The filtrate was kept at −18 °C for a further 48 h to deposit a second crop of the solid, which was isolated in a similar manner. Repetition of this process several times raised the yield of **3b**·TsOH to 163 mg (22% from **11b**). For purification, the total amount of crude **3b**·TsOH was dissolved in $HCONMe_2$ (30 ml), and the solution was kept at −18 °C for 3—5 h after addition of AcOEt (50 ml). The precipitate that resulted was filtered off, washed successively with AcOEt (3 × 3 ml) and MeOH (3 × 3 ml), and dried over P_2O_5 at 3 mmHg and room temperature for 48 h, giving an analytical sample as a colorless powder, mp *ca.* 120 °C (dec.); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 272 nm (unstable); $\lambda_{max}^{H_2O}$ (pH 1) unstable; $\lambda_{max}^{H_2O}$ (pH 7) 271 (ε 16900); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 2.28 (3H, s, $MeC_6H_4SO_3^-$), 2.84 [2H, br m, C(2')-H's], 3.52 [2H, br m, C(5')-H's], 3.92 [1H, m, C(4')-H], 4.19 [3H, s, N(3)-Me], 4.44 [1H, m, C(3')-H], 4.86 [1H, t, *J* = 5 Hz, C(5')-OH], 5.45 [1H, d, *J* = 5 Hz, C(3')-OH], 6.57 [1H, dd, *J* = 6 Hz each, C(1')-H], 7.08 and 7.44 (2H each, d, *J* = 8 Hz, $MeC_6H_4SO_3^-$), 8.63 and 8.71 (1H each, s, purine protons), 9.15 and 9.23 (1H each, br s, $=NH_2^+$). *Anal.* Calcd for $C_{11}H_{15}N_5O_3 \cdot CH_3C_6H_4SO_3H$: C, 49.42; H, 5.30; N, 16.01. Found: C, 49.15; H, 5.45; N, 16.17.

Acid Hydrolysis of 3a·TsOH Leading to 3-Methyladenine (9) A solution of **3a**·TsOH (363 mg, 0.8 mmol) in 0.1 N aqueous HCl (80 ml) was allowed to stand at 27 °C for 1 h. The reaction mixture was concentrated to dryness *in vacuo* to leave a solid, which was dissolved in warm H_2O (0.5 ml). The aqueous solution was made alkaline by addition of conc. aqueous NH_3, and the colorless crystals that deposited were filtered off, washed with a small amount of cold H_2O, and dried to give **9** (109 mg, 92%), mp 295—297 °C (dec.). Recrystallization from H_2O yielded a pure sample as colorless needles, mp > 300 °C. This sample was identical [by

mixture melting point test and comparison of the infrared (IR) spectrum and TLC mobility] with authentic **9**.[38]

Acidic Hydrolysis of 3b·TsOH Leading to 3-Methyladenine (9) A solution of **3b**·TsOH (17.0 mg, 0.039 mmol) in 0.2 M acetate buffer (pH 3.34 at 25 °C) (1 ml) was kept at 20 °C for 45 min. The reaction mixture was concentrated to dryness *in vacuo*, and the residue was extracted with hot EtOH (3 × 3 ml). Evaporation of the ethanolic extracts under reduced pressure left a colorless solid, which was purified by preparative TLC [alumina, $CHCl_3$–EtOH (5 : 1, v/v)] to give a colorless solid (3.5 mg, 60%), mp > 300 °C. This sample was identical (by comparison of the IR spectrum and PPC and TLC mobilities) with authentic **9**.[38]

Methanolysis of 3b·TsOH A solution of **3b**·TsOH (22.0 mg, 0.05 mmol) in MeOH (10 ml) was heated under reflux for 30 min. The reaction mixture was concentrated to dryness *in vacuo*, and the residue was purified by column chromatography [alumina, $CHCl_3$–EtOH (5 : 1, v/v)] to give a crystalline solid. Recrystallization of the solid from H_2O provided 3-methyladenine (**9**) (7.5 mg, 99%) as colorless needles, mp > 300 °C. This sample was identical (by comparison of the IR spectrum and PPC and TLC mobilities) with authentic **9**.[38]

Conversion of 3a·TsOH into 5-(Methylamino)-1-β-D-ribofuranosyl-1*H*-imidazole-4-carboxamidine (16a) A solution of **3a**·TsOH (453 mg, 1 mmol) in H_2O (20 ml) was passed through a column of Amberlite CG-400 (OH^-) (13 ml), and the column was eluted with H_2O. The eluate (100 ml) was concentrated to dryness *in vacuo* to leave a colorless glass (274 mg). The glass was dissolved in MeOH (10 ml) and a solution of conc. aqueous HCl (200 mg) in MeOH (2 ml) was added. The resulting mixture was concentrated to dryness *in vacuo* to give **16a**·2HCl·H_2O (316 mg, 87%) as a colorless solid, mp 102 °C (dec.). Recrystallization of the solid was effected by dissolving it in MeOH and adding ether to the methanolic solution, yielding an analytical sample (dried over P_2O_5 at 2 mmHg and room temperature for 48 h) as colorless prisms, mp 99—102 °C (dec.); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 289 nm (ε 7100); $\lambda_{max}^{H_2O}$ (pH 1) 260 (sh) (5300), 288 (8200); $\lambda_{max}^{H_2O}$ (pH 7) 287 (6400); $\lambda_{max}^{H_2O}$ (pH 13) 250 (sh) (7700); NMR (Me_2SO-d_6) δ: 2.74 (3H, s, NHMe), 3.62 [2H, m, C(5′)-H's], 3.9—4.35 [3H, m, C(2′)-H, C(3′)-H, and C(4′)-H], 5.64 [1H, d, J = 5 Hz, C(1′)-H], 6.61 (7H, NHMe, NH^+, OH's, and H_2O), 8.28 [1H, s, C(2)-H], 8.4—9.1 (4H, protonated amidine protons). *Anal.* Calcd for $C_{10}H_{17}N_5O_4 \cdot 2HCl \cdot H_2O$: C, 33.16; H, 5.84; N, 19.33. Found: C, 33.45; H, 5.97; N, 19.48.

Hydrolysis of 3b·TsOH at pH 8.98 Reaction of **3b**·TsOH in 0.1 M carbonate buffer [pH 8.98 and ionic strength 0.5 (KCl)] at 25 °C was monitored by means of HPLC analysis in a manner similar to that described below in the kinetic procedure for acidic hydrolysis of **3b**·TsOH. The results are summarized in the text.

***N′*-Benzyloxy-5-(*N*-methylformamido)-1*H*-imidazole-4-carboxamidine (21)** A solution of **11b** (195 mg, 0.5 mmol) in 0.1 N aqueous HCl (20 ml) was stirred at room temperature for 3.5 h. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$ (5 × 10 ml). The $CHCl_3$ extracts were washed with saturated aqueous NaCl (10 ml), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to leave a glass. The glass was purified by preparative TLC [silica gel, $CHCl_3$–EtOH (8 : 1, v/v)] to give **21**[32] (83 mg, 61%) as a colorless glass, MS *m/z*: 273 (M^+); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 250 nm (sh) (ε 9300); NMR (Me_2SO-d_6) δ: 3.03 (major) and 3.17 (minor) (3H, s each, HCONMe), 4.94 (2H, s, OCH_2Ph), 5,70 (minor) and 5.98 (major) (2H, br, NH_2), 7.30—7.40 (5H, m, OCH_2Ph), 7.63 [1H, s, C(2)-H], 8.16 (1H, s, HCONMe), 12.48 [1H, br, N(1)-H].[35]

Conversion of 21 into N^6-Benzyloxy-3-methyladenine (22) A solution of **21** (10 mg) in 10% (w/w) ethanolic HCl (3 ml) was allowed to stand at room temperature for 72 h. The precipitate that resulted was filtered off and dissolved in H_2O (*ca.* 0.5 ml). The aqueous solution was neutralized with 5% aqueous NH_3 and concentrated to dryness *in vacuo*. Recrystallization of the residue from 30% (v/v) aqueous EtOH gave **22** as colorless prisms, mp 180—181 °C. This sample was identical (by mixture melting point test and comparison of the IR spectrum and PPC and TLC mobilities) with authentic **22**.[33]

Conversion of 21 into 3-Methyladenine (9) A solution of **21** (35 mg, 0.13 mmol) in 50% (v/v) aqueous EtOH (3 ml) was hydrogenated over a small amount of Raney Ni W-2 catalyst[37] at atmospheric pressure and 30 °C for 3 h. The catalyst was removed by filtration and washed with H_2O (3 ml). The combined filtrate and washings were concentrated to dryness *in vacuo* to leave **9** (16 mg, 84%) as a colorless solid, mp 295—296 °C (dec.). Recrystallization from H_2O gave a pure sample as colorless needles, mp > 300 °C. This was identical (by comparison of the IR spectrum and PPC and TLC mobilities) with authentic **9**.[38]

Kinetic Procedure i) Glycosidic Hydrolysis of **3a**·TsOH in 0.1 N Aqueous HCl: The substrate (**3a**·TsOH) was dissolved, at a concentration of 1.3×10^{-3} M, in 0.1 N aqueous HCl kept at 25 ± 0.05 °C in a thermoregulated constant-temperature bath. At intervals, aliquots (1 ml) of the reaction solution were withdrawn and diluted with 0.05 M KH_2PO_4–MeOH (90 : 10, v/v) by a factor of 10. Small portions (14 μl) of the diluted solutions were then analyzed by means of HPLC. The HPLC analyses were carried out on a μBondapak C_{18} column [0.05 M KH_2PO_4–MeOH (90 : 10, v/v), 1150 p.s.i., 1.5 ml/min], and the peak height of the substrate, located by using a UV absorbance detector operated at 254 nm, was determined. Concentration of the unaltered substrate in the reaction mixture was then estimated from a calibration curve which had been obtained with substrate solutions of known concentration, and the decrease of the concentration of the substrate was found to obey good pseudo-first-order kinetics. The results are summarized in the text and Table I.

ii) Glycosidic Hydrolyses of **3a**·TsOH and **3b**·TsOH at pH's 3.34, 5.00, and 7.00: Buffer solutions used for these hydrolyses were 0.2 M HCl–NaOAc (pH 3.34 at 25 °C), 0.2 M AcOH–NaOAc (pH 5.00 at 37 °C), and 0.2 M KH_2PO_4–Na_2HPO_4 (pH 7.00 at 37 °C). The substrates were separately dissolved in one of the buffer solutions at 2.0×10^{-3}—2.5×10^{-3} M concentration, and the resulting solutions were kept at 25 ± 0.05 °C or at 37 ± 0.05 °C in a thermoregulated constant-temperature bath. Decrease of the substrate in each reaction mixture was followed by means of HPLC in a manner similar to that described above under item (i) except that MeOH/0.05 M KH_2PO_4–Na_2HPO_4 (pH 6.0) (10/90, v/v) was used for 10-fold dilution of the reaction mixture and for elution of the HPLC column. In all cases, good pseudo-first-order kinetics were obtained. The results are listed in Table I.

iii) Equilibrium between **3a**·TsOH and **10a**·TsOH at pH 8.32: The ring opening reaction of **3a**·TsOH and cyclization of **10a**·TsOH in 0.1 M aqueous $NaHCO_3$ (pH 8.32) at 25 °C were separately followed by UV spectrophotometry in a manner similar to that reported[2c] previously for the 3,9-dialkyladenine (type **2**) series. The results are given in the text.

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