Purines. XXXVI.[1] Fission and Reclosure of the Adenine Ring in 3,9-Disubstituted Adenines: Effects of Substituents[2]

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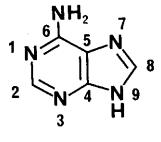
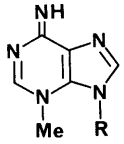
In order to investigate the effects of the N(3)- and N(9)-substituents in 3,9-disubstituted adenines on the stability of the adenine ring, the equilibrium constants and the rates of ring opening and cyclization for the equilibria between the 3,9-disubstituted adenines IVa—l and the *N*-alkylformamidoimidazoles Va—l in H_2O at pH 8.98 and 25 °C have been measured. A bulky substituent at the 3-position of IV has been found to retard the ring opening leading to V, whereas an electron-withdrawing group at the 3- or 9-position accelerates it. A bulky alkyl group on the formamido nitrogen of V markedly retards the cyclization leading to IV, favoring the ring-opened form in the equilibrated mixture. Syntheses of 3-isopropyl-9-methyladenine perchlorate (IVc) and 3-(4-methoxybenzyl)-9-methyladenine perchlorate (IVe), required for the kinetic study as substrates, are also described.

Keywords 3,9-dialkyladenine synthesis; formamido group *N*-alkylation; hydrogenolytic dealkoxylation; amidine formamido cyclization; ring–chain equilibrium; kinetic study; substituent effect; UV spectrophotometry

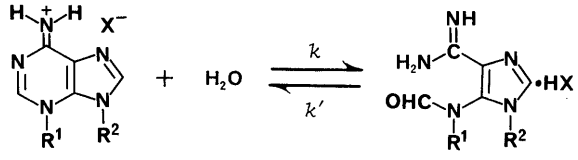
The study of 3,9-disubstitution in the adenine system (I) is acquiring a deeper significance, since 3-methyl-2′-deoxyadenosine (II) is believed to occur as an unstable part structure in deoxyribonucleic acids treated with a variety of methylating agents.[3] Previous papers[4] from our laboratory have described syntheses of II and 3-methyladenosine (III), both in the form of the *p*-toluenesulfonate salt, together with their high susceptibility to hydrolysis leading to 3-methyladenine in aqueous acidic solution and to ring opening of the adenine moiety at the 2-position under basic conditions. The structure of 3-methyladenosine *p*-toluenesulfonate (IVl), especially its exocyclic iminium character, was recently confirmed by us by means of X-ray crystallographic analysis.[5] We also synthesized a series of 3,9-dialkyladenine salts (type IV)[6] and observed their extreme instability under basic conditions.[6b,c] For example,



I



II: R = 2-deoxy-β-D-ribofuranosyl
III: R = β-D-ribofuranosyl



IV V

a: R^1 = Me, R^2 = Me, X = Cl
b: R^1 = Et, R^2 = Me, X = ClO_4
c: R^1 = Me_2CH, R^2 = Me, X = ClO_4
d: R^1 = $PhCH_2$, R^2 = Me, X = ClO_4
e: R^1 = *p*-(MeO)$C_6H_4CH_2$, R^2 = Me, X = ClO_4
f: R^1 = Me, R^2 = Et, X = ClO_4
g: R^1 = Et, R^2 = Et, X = ClO_4
h: R^1 = $PhCH_2$, R^2 = Et, X = ClO_4
i: R^1 = Me, R^2 = $PhCH_2$, X = ClO_4
j: R^1 = Et, R^2 = $PhCH_2$, X = ClO_4
k: R^1 = $PhCH_2$, R^2 = $PhCH_2$, X = ClO_4
l: R^1 = Me, R^2 = β-D-ribofuranosyl, X = *p*-$MeC_6H_4SO_3$

Chart 1

3,9-dimethyladenine hydrochloride (IVa) equilibrated with the ring-opened derivative Va in 0.1 M aqueous $NaHCO_3$ (pH 8.32) at 25 °C, and the reactions in both directions obeyed pseudo-first-order kinetics.[6b,c] Similar reversible ring openings observed for IVd[6b,c] and IVl[4a,c] also exemplified the instability of the 3,9-disubstituted adenine system. In the present work, we tried to extend the scope of the kinetic study to cover many other 3,9-disubstituted adenines with a view to revealing the effects of the N(3)- and N(9)-substituents on the stability of the adenine ring.

The 3,9-disubstituted adenines selected as the substrates were IVa—l,[4a,c,6] which included two new compounds, IVc and IVe. The two were prepared in the following manner by application of our general method[6b,c] for the synthesis of 3,9-dialkyladenines. Treatment of the formamidoimidazole VII,[7] prepared easily from 1-methoxy-9-methyladenine (VI)[8] according to the previously reported procedure,[7a] with anhydrous K_2CO_3 and isopropyl iodide (48 h) or *p*-methoxybenzyl chloride (3 h) in $HCONMe_2$ at room temperature gave the *N*-isopropylformamido derivative VIIIc or the *N*-(*p*-methoxybenzyl)formamido derivative VIIIe in 72% or 84% yield, respectively.[9] Hydrogenolyses of VIIIc and VIIIe were separately effected at room temperature with hydrogen and Raney Ni catalyst in H_2O containing one molar eq of HCl, furnishing the amidine hydrochlorides IXc and IXe in 66% and 52% yields, respectively.[9] On treatment with a little Et_3N in boiling EtOH for 8 h and subsequent addition of 70% aqueous $HClO_4$, IXc cyclized to give IVc in 56% yield. A similar cyclization of IXe but at 30 °C for 7 d afforded IVe in 24% yield. In an attempt to cyclize IXe by the previous alternative method,[6b,c] IXe was treated with 70% aqueous $HClO_4$ in boiling EtOH for 36 h. However, the formation of 9-methyladenine perchlorate was suggested by thin-layer chromatographic analysis of the product, reflecting the instability of the *p*-methoxybenzyl group linked to a positively charged adenine ring.[10] The correctness of the structures IVc and IVe was supported by the way in which they were generated, microanalytical data, and ultraviolet (UV) and nuclear magnetic resonance (NMR) spectral features.

In the first phase of the kinetic study, the reversible ring opening of 3,9-dimethyladenine hydrochloride (IVa) (Chart 1) at various pH's was investigated by UV spectrophotom-

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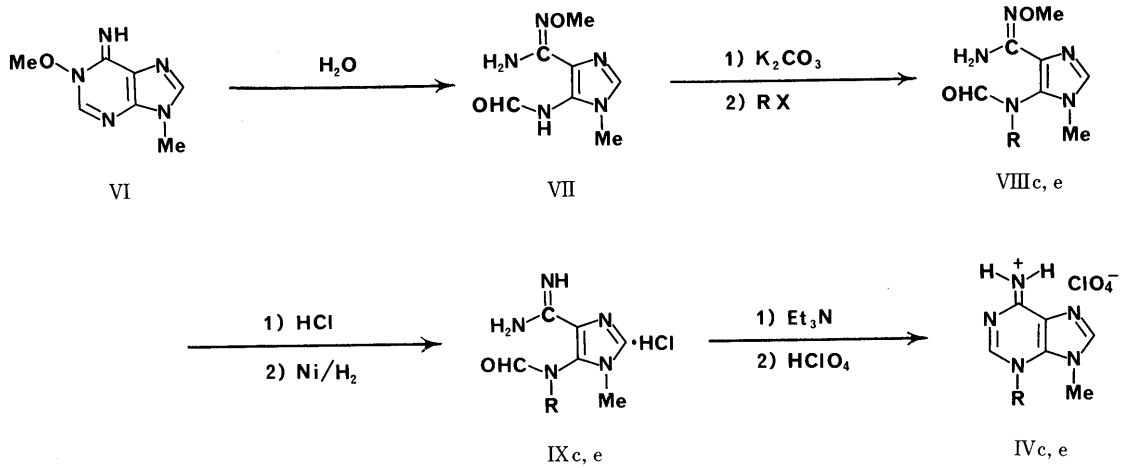

c : R=Me_2CH e : R=*p*-(MeO)$C_6H_4CH_2$

Chart 2

TABLE I. Rate and Equilibrium Constants, k, k', and K, for Ring Opening of IVa and Cyclization of Va in H_2O at Various pH's and Ionic Strength 0.5 at 25 °C

pH value	Pseudo-first-order rate constant		Equilibrium constant
	k (min^{-1})	k' (min^{-1})	$K=k/k'$
7.50	5.07×10^{-4}	1.57×10^{-3}	0.32
8.32[a]	2.88×10^{-3}	9.63×10^{-3}	0.30
8.98	1.20×10^{-2}	3.80×10^{-2}	0.32
9.62	4.57×10^{-2}	1.50×10^{-1}	0.30
10.08	9.76×10^{-2}	3.21×10^{-1}	0.30

a) Taken from ref. 6*c*.

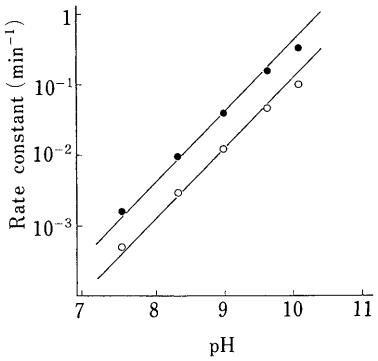

Fig. 1. pH–Rate Profiles for Ring Opening of IVa (—○—) and Cyclization of Va (—●—) in H_2O at 25 °C and Ionic Strength 0.5

etry. It may be seen from Table I and Fig. 1 that in the pH range of 7.50—10.08 both the ring opening and the cyclization proceeded at rates proportional to hydroxide ion concentration, attaining equilibrium when IVa and Va existed in an invariable ratio of *ca.* 10 : 3. The reactions in more alkaline regions were complicated by the concomitant deformylation of Va. Although we were unable to determine the acid dissociation constant of IVa because of its instability under basic conditions, the basicity of the free base of IVa has been estimated to be considerably high ($pK_a \gtrapprox 12$).[4c,6b,c] Therefore, it is reasonable to assume that in the above ring opening of IVa, attack of hydroxide ion on the protonated species at the 2-position is dominant in the pH range investigated.

We next likewise determined the rate constants and equilibrium constants for the reversible reactions between IVb—l and Vb—l in H_2O at pH 8.98 and 25 °C (Chart 1), and Table II lists the results. It may be seen that in the ring opening of IV higher alkyl groups at the 3-position retard attack by hydroxide ion at the 2-position. Since there could be little difference in electron-donating properties among such alkyl groups,[11] the observed retardation is probably owing to their steric bulk. The rate enhancement observed to some extent for IVd and IVh, both carrying the electron-withdrawing (relative to an alkyl group) benzyl group at the 3-position, presents a great contrast to the severe retardation observed for the 3-isopropyl analogue IVc, suggesting that the electronic properties of an N(3)-substituent affect the hydroxide ion attack at the 2-position more significantly than its steric bulk. The slight retardation observed for the ring opening of the 3-(*p*-methoxybenzyl) analogue IVe, relative to the corresponding reaction of the 3-benzyl analogue IVd, may support this view.

It is also noteworthy that the benzyl group and the β-D-ribofuranosyl group at the 9-position accelerate the ring opening of IV, as seen in the cases of IVi—l. This may be attributed to the electron-withdrawing nature, relative to an alkyl group, of these two groups.[11,12] On the other hand, a bulky substituent on the formamido nitrogen of V markedly retards the cyclization leading to IV, favoring the ring-opened form (V) in the equilibrated mixture. A bulky N(9)-substituent seems to exert a similar retardation effect. As a result of the above effects, bulky substituents at both the 3- and 9-positions tend to displace the balance of the interconversion reactions in favor of the ring-opened form (V).

In conclusion, the present results confirm that the easy, reversible ring opening of the adenine ring at the 2-position under basic conditions is a general and characteristic reaction of the 3,9-disubstituted adenine system. The observed substituent effects on fission and reclosure in this system (Chart 1) will prove to be useful for a better understanding of the nature of the biochemically important 3-alkyl-2′-deoxyadenosine structure (type II) at the nucle-

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TABLE II. Rate Constants and Equilibrium Constants for the Reversible Reactions between IVa—l and Va—l in H_2O at pH 8.98 and Ionic Strength 0.5 at 25 °C

Compound			Pseudo-first-order rate constant				Equilibrium constant $K=k/k'$
			Ring opening		Cyclization		
No.	R^1	R^2	$k\,(min^{-1})\times10^3$	k_{rel} [a]	$k'\,(min^{-1})\times10^3$	k'_{rel} [b]	
IVa	Me	Me	12.0	1.0	38.0	1.0	0.32
IVb	Et	Me	3.96	0.33	6.47	0.17	0.61
IVc	Me_2CH	Me	1.58	0.13	1.32	0.03	1.20
IVd	$PhCH_2$	Me	12.7	1.06	2.78	0.07	4.57
IVe	p-(MeO)$C_6H_4CH_2$	Me	11.1	0.93	1.49	0.04	7.45
IVf	Me	Et	7.63	0.64	15.1	0.40	0.51
IVg	Et	Et	4.24	0.35	3.20	0.08	1.33
IVh	$PhCH_2$	Et	12.1	1.01	1.21	0.03	10.0
IVi	Me	$PhCH_2$	12.8	1.07	10.7	0.28	1.20
IVj	Et	$PhCH_2$	7.69	0.64	2.42	0.06	3.18
IVk	$PhCH_2$	$PhCH_2$	21.1	1.76	1.24	0.03	17.0
IVl	Me	β-D-ribofuranosyl	26.8	2.23	21.0	0.55	1.28

a) Relative to the rate constant for the ring opening of IVa. *b*) Relative to the rate constant for the cyclization of Va.

oside, nucleotide, and polynucleotide levels.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. See ref. 6*c* for details of 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, m = multiplet, s = singlet, sh = shoulder.

Materials The known compounds selected as the substrates for the kinetic study were taken from stocks which had been prepared according to published procedures: 3,9-dimethyladenine hydrochloride (IVa)[6c]; 3-ethyl-9-methyladenine perchlorate (IVb)[6c]; 3-benzyl-9-methyladenine perchlorate (IVd)[6c]; 9-ethyl-3-methyladenine perchlorate (IVf)[6c]; 3,9-diethyladenine perchlorate (IVg)[6c]; 3-benzyl-9-ethyladenine perchlorate (IVh)[6c]; 9-benzyl-3-methyladenine perchlorate (IVi)[6c]; 9-benzyl-3-ethyladenine perchlorate (IVj)[6c]; 3,9-dibenzyladenine perchlorate (IVk)[6c]; 3-methyladenosine p-toluenesulfonate (IVl)[4c]; 1-benzyl-5-(*N*-methylformamido)-1*H*-imidazole-4-carboxamidine hydrochloride (Vi, X = Cl instead of ClO_4)[6c]; 1-benzyl-5-(*N*-benzylformamido)-1*H*-imidazole-4-carboxamidine hydrochloride (Vk, X = Cl instead of ClO_4)[6c]; 5-(*N*-methylformamido)-1-β-D-ribofuranosyl-1*H*-imidazole-4-carboxamidine p-toluenesulfonate (Vl).[4c] Other compounds were obtained as described below.

5-(*N*-Isopropylformamido)-*N'*-methoxy-1-methyl-1*H*-imidazole-4-carboxamidine (VIIIc) A mixture of 5-formamido-*N'*-methoxy-1-methyl-1*H*-imidazole-4-carboxamidine (VII)[7] (2.96 g, 15 mmol), anhydrous K_2CO_3 (3.11 g, 22.5 mmol), and $HCONMe_2$ (60 ml) was stirred at room temperature for 1 h, and then isopropyl iodide (3.06 g, 18 mmol) was added. After having been stirred at room temperature for 48 h, the reaction mixture was concentrated *in vacuo* to leave a yellowish brown jelly, which was extracted with boiling benzene (4 × 60 ml). The benzene extracts were combined and concentrated *in vacuo*, and the dark reddish residue was purified by column chromatography [silica gel (180 g), AcOEt–EtOH (10 : 1, v/v)] to give VIIIc (2.59 g, 72%) as a colorless solid, mp 126.5—127.5 °C. Recrystallization from benzene yielded an analytical sample as colorless prisms, mp 127—128 °C; MS *m/z*: 239 (M^+); UV $\lambda_{max}^{95\%\,EtOH}$ 250 nm (sh) (ε 5200); $\lambda_{max}^{H_2O}$ (pH 1) 247 (6950); $\lambda_{max}^{H_2O}$ (pH 7) 250 (sh) (5300); $\lambda_{max}^{H_2O}$ (pH 13) 250 (sh) (5300); NMR (Me_2SO-d_6) δ: 0.95—1.50 (6H, br m, $CHMe_2$), 3.39 (0.7H) and 3.50 (2.3H) [s each, N(1)-Me], 3.59 (2.3H) and 3.65 (0.7H) (s each, OMe), 3.85—4.45 (1H, br m, $CHMe_2$), 5.64 and 5.58 (sh) (2H, br, NH_2), 7.70 (0.23H) and 7.74 (0.77H) [s each, C(2)-H], 7.93 (0.77H) and 8.45 (0.23H) (s each, NCHO).[13] *Anal.* Calcd for $C_{10}H_{17}N_5O_2$: C, 50.20; H, 7.16; N, 29.27. Found: C, 50.26; H, 7.29; N, 29.52.

***N'*-Methoxy-5-[*N*-(4-methoxybenzyl)formamido]-1-methyl-1*H*-imidazole-4-carboxamidine (VIIIe)** A mixture of VII[7] (2.96 g, 15 mmol), anhydrous K_2CO_3 (3.11 g, 22.5 mmol), and $HCONMe_2$ (75 ml) was stirred at room temperature for 1 h, and then p-methoxybenzyl chloride (2.35 g, 15 mmol) was added. After having been stirred at 30 °C for 3 h, the reaction mixture was filtered in order to remove an insoluble solid. The solid was washed with benzene (3 × 10 ml), and the combined filtrate and washings were concentrated *in vacuo* to leave a viscous brown oil. Purification of the oil by column chromatography [silica gel (150 g), AcOEt–EtOH (10 : 1, v/v)] furnished VIIIe (4.01 g, 84%) as a colorless solid, mp 85—86 °C. Recrystallization from benzene afforded an analytical sample as colorless prisms, mp 90.5—91.5 °C; MS *m/z*: 317 (M^+); UV $\lambda_{max}^{95\%\,EtOH}$ 250 nm (sh) (ε 6700), 280 (sh) (3000); $\lambda_{max}^{H_2O}$ (pH 1) 250 (sh) (7600); $\lambda_{max}^{H_2O}$ (pH 7) 250 (sh) (6300); $\lambda_{max}^{H_2O}$ (pH 13) 250 (sh) (6250); IR ν_{max}^{Nujol} cm^{-1}: 3430, 3315 (NH_2), 1692 (HCON); NMR (Me_2SO-d_6) δ: 2.82 (0.9H) and 3.04 (2.1H) [s each, N(1)-Me], 3.66 (2.1H) and 3.74 (0.9H) (s each, NOMe), 3.71 (3H, s, ArOMe), 4.30—5.15 (2H, br m, NCH_2Ar), 5.65 (major) and 5.70 (minor) (2H, br, NH_2), 6.70—6.90 [2H, m, C(3')-H and C(5')-H],[14] 7.00—7.20 [2H, m, C(2')-H and C(6')-H],[14] 7.53 (0.3H) and 7.58 (0.7H) [s each, C(2)-H], 8.12 (0.7H) and 8.54 (0.3H) (s each, NCHO).[13] *Anal.* Calcd for $C_{15}H_{19}N_5O_3$: C, 56.77; H, 6.03; N, 22.07. Found: C, 56.60; H, 6.24; N, 22.18.

5-(*N*-Isopropylformamido)-1-methyl-1*H*-imidazole-4-carboxamidine Hydrochloride (IXc) A solution of VIIIc (2.39 g, 10 mmol) in H_2O (150 ml) containing 1 N aqueous HCl (10 ml) was hydrogenated over Raney Ni W-2 catalyst[15] (7.5 ml) at atmospheric pressure and room temperature for 4 h. The catalyst was removed by filtration and washed with H_2O (120 ml). The combined filtrate and washings were concentrated *in vacuo*, and the residue was dried and washed with cold EtOH (4 ml) to leave IXc (1.61 g, 66%) as a colorless solid, mp 235—238 °C (dec.). The solid was recrystallized by dissolving it in MeOH and adding AcOEt to the resulting methanolic solution, giving an analytical sample as colorless prisms, mp 238—239.5 °C (dec.); UV $\lambda_{max}^{95\%\,EtOH}$ 254 nm (ε 7300); $\lambda_{max}^{H_2O}$ (pH 1) 251 (7900); $\lambda_{max}^{H_2O}$ (pH 7) 251 (7900); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 0.92—1.40 (6H, m, $CHMe_2$), 3.48 (major) and 3.54 (minor) [3H, s each, N(1)-Me], 4.00—4.64 (1H, m, $CHMe_2$), 8.06 [1H, s, C(2)-H], 8.22 (minor) and 8.43 (major) (1H, s each, NCHO), 8.26—8.52 (1H, br, NH), 8.82 (1H, br, NH), 9.16 (2H, br, NH_2).[13] *Anal.* Calcd for $C_9H_{15}N_5O\cdot HCl$: C, 43.99; H, 6.56; N, 28.50. Found: C, 43.69; H, 6.86; N, 28.29.

5-[*N*-(4-Methoxybenzyl)formamido]-1-methyl-1*H*-imidazole-4-carboxamidine Hydrochloride (IXe) A solution of VIIIe (952 mg, 3 mmol) in a mixture of H_2O (45 ml) and EtOH (10 ml) containing 1 N aqueous HCl (3 ml) was hydrogenated over Raney Ni W-2 catalyst[15] (3 ml) at atmospheric pressure and room temperature for 5 h. The reaction mixture was then worked up in a manner similar to that described above for IXc, affording IXe (508 mg, 52%) as a colorless solid, mp 179—180 °C. Recrystallization of the solid by dissolving it in MeOH and adding AcOEt to the resulting methanolic solution gave an analytical sample as colorless prisms, mp 181—182 °C; UV $\lambda_{max}^{95\%\,EtOH}$ 261 nm (ε 9700), 282 (sh) (4100); $\lambda_{max}^{H_2O}$ (pH 1) 250 (sh) (8100), 280 (sh) (2100); $\lambda_{max}^{H_2O}$ (pH 7) 250 (sh) (8250), 280 (sh) (2100); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 2.94 (major) and 3.15 (minor) [3H, s each, N(1)-Me], 3.72 (3H, s, OMe), 4.28—5.15 (2H, br m, NCH_2Ar), 6.8—7.2 (4H, m, C_6H_4), 7.90 (major) and 7.93 (minor) [1H, s each, C(2)-H], 8.35 (minor) and 8.62 (major) (1H, s each, NCHO), 8.56 (2H, br, NH_2), 9.16 (2H, br, NH_2^+).[13] *Anal.* Calcd for $C_{14}H_{17}N_5O_2\cdot HCl$: C, 51.93; H, 5.60; N, 21.63. Found: C, 51.64; H, 5.57; N, 21.64.

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3-Isopropyl-9-methyladenine Perchlorate (IVc) A stirred suspension of IXc (246 mg, 1 mmol) in EtOH (10 ml) containing Et_3N (0.02 ml, *ca.* 0.14 mmol) was heated under reflux for 8 h. The reaction mixture was concentrated *in vacuo* to leave a semisolid, which was dissolved in hot EtOH (3 ml). After addition of 70% aqueous $HClO_4$ (218 mg, 1.5 mmol), the hot ethanolic solution was cooled in a refrigerator for 5 h. The precipitate that resulted was filtered off, washed with a little EtOH, and dried to yield IVc (163 mg, 56%) as a slightly yellowish solid, mp 249.5—250 °C (dec.). Recrystallization from H_2O furnished an analytical sample as colorless needles, mp 250.5—251 °C (dec.); UV $\lambda_{max}^{95\%\,EtOH}$ 273 nm (ε 14500); $\lambda_{max}^{H_2O}$ (pH 1) 271 (14600); $\lambda_{max}^{H_2O}$ (pH 7) 271 (15200); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 1.64 (6H, d, J=6.5 Hz, CH$\underline{Me}_2$), 4.09 [3H, s, N(9)-Me], 5.18 [1H, septet, J=6.5 Hz, C$\underline{H}$Me$_2$), 8.34 [1H, s, C(8)-H],[16] 8.83 [1H, s, C(2)-H],[16] 9.12 (2H, br, $=NH_2^+$). *Anal.* Calcd for $C_9H_{13}N_5 \cdot HClO_4$: C, 37.06; H, 4.84; N, 24.01. Found: C, 37.04; H, 4.87; N, 23.93.

3-(4-Methoxybenzyl)-9-methyladenine Perchlorate (IVe) A solution of IXe (324 mg, 1 mmol) in EtOH (15 ml) containing Et_3N (0.02 ml, *ca.* 0.14 mmol) was stirred at 30 °C for 7 d. The reaction mixture was concentrated *in vacuo* to leave a yellow oil, which was dissolved in H_2O (5 ml). After addition of 70% aqueous $HClO_4$ (1 ml), the aqueous solution was kept in a refrigerator overnight. The precipitate that resulted was filtered off, washed with a little H_2O, and dried to give IVe (87 mg, 24%) as slightly yellowish needles, mp 165—167 °C (dec.). Recrystallization from MeOH produced an analytical sample as colorless needles, mp 166—167 °C (dec.); UV $\lambda_{max}^{95\%\,EtOH}$ 274 nm (ε 15100); $\lambda_{max}^{H_2O}$ (pH 1) 272 (18300); $\lambda_{max}^{H_2O}$ (pH 7) 272 (18100); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 3.75 [3H, s, N(9)-Me or OMe], 3.80 [3H, s, OMe or N(9)-Me], 5.79 [2H, s, N(3)-C$\underline{H}_2$Ar], 6.97 [2H, d, J=8.8 Hz, C(3′)-H and C(5′)-H],[14] 7.19 [2H, d, J=8.8 Hz, C(2′)-H and C(6′)-H],[14] 8.26 [1H, s, C(8)-H],[16] 8.73 [1H, s, C(2)-H],[16] 9.31 and 9.38 (1H each, dull s, $=NH_2^+$). *Anal.* Calcd for $C_{14}H_{15}N_5O \cdot HClO_4$: C, 45.48; H, 4.36; N, 18.94. Found: C, 45.72; H, 4.40; N, 19.09.

Kinetic Procedure i) Equilibrium between IVa and Va at Various pH's: The reversible ring-opening reaction of IVa, shown in Chart 1, in aqueous solution at various pH's and ionic strength 0.5 at 25 °C was followed by UV spectrophotometry in a manner similar to that reported previously[6c] for the same reaction system at pH 8.32. Buffer solutions employed for kinetic runs were 0.1 M KH_2PO_4–Na_2HPO_4 (pH 7.50 at 25 °C) and 0.1 M $NaHCO_3$–Na_2CO_3 (pH 8.98, 9.62, and 10.08 at 25 °C), and were brought to ionic strength 0.5 with KCl. The results are given in Tables I and II and Fig. 1.

ii) Equilibria between IVb—l and Vb—l at pH 8.98: The ring-opening reactions of IVb—l and cyclizations of IXc, IXe, Vi (X=Cl instead of ClO_4), Vk (X=Cl instead of ClO_4), and Vl, shown in Chart 1, in 0.1 M $NaHCO_3$–Na_2CO_3 (adjusted to ionic strength 0.5 by adding KCl) at pH 8.98 and 25 °C were separately followed as described above under item (i). The results are listed in Table II.

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References and Notes

1) Paper XXXV in this series, T. Fujii, T. Itaya, S. Yoshida, and S. Matsubara, *Chem. Pharm. Bull.*, **37**, 3119 (1989).
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13) The observed complexity of the proton signals is interpretable in terms of *cis–trans* isomerism of the formamido moiety, as we have experienced in similar structures.[4c,6c]
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