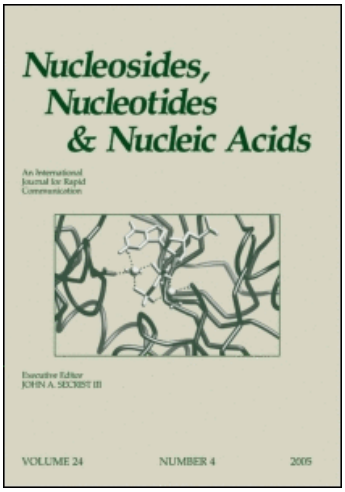

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ELECTROPHILIC AMINATION OF ADENINES. FORMATION AND CHARACTERISTICS OF *N*-AMINOADENINES

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Abstract: Amination of adenine with H_2N-O-SO_3H in alkaline media afforded 1-, 3-, 7- and 9-aminoadenine isomers at a ratio of about 1:1:3:1. In neutral media, the product ratio of the isomers changed to about 3:1:1:0. These results were different from the regioselectivity obtained by methylation of adenine with dimethyl sulfate under similar conditions. Amination of adenine with dinitrophenoxyamine in DMF gave 1-aminoadenine as the main product and this regioselectivity was also different from that of methylation with CH_3I. Chemical characteristics of these *N*-amino adenines are described.

INTRODUCTION

Arylaminating carcinogens such as acetylaminofluorene, 4-nitroquinoline 1-oxide and Trp-P1 are known to arylaminate the base moiety of cellular DNA after they are metabolically converted to an ultimate form, *N*-aryl-*O*-acylhydroxylamine.[1] It is believed that the arylnitrenium ion, the reactive species obtained from the ultimate form, reacts with a base moiety in DNA. The major DNA-carcinogen adduct is C8-arylaminated guanine, however, details of the reaction mechanisms are still unclear. As part of our work on these mechanisms, we have been studying electrophilic amination toward nucleic acid components using simple aminating reagents such as hydroxylamine-*O*-sulfonic acid (HAOS) and 2,4-dinitrophenoxyamine (DNPA).[2~10] Except for adenine, studies of amination reactions toward pyrimidine- and purine-bases and nucleosides are almost complete. The amination of adenine is described in this report.

This paper is dedicated to Professor Emeritus Yoshihisa Mizuno of Hokkaido University on the occasion of his 75th birthday.

RESULTS AND DISCUSSION

Amination of adenine with HAOS and DNPA under various conditions

Electrophilic amination of adenine was carried out under various conditions using HAOS. Reaction of adenine with HAOS in alkaline media gave several *N*-aminated adenines and these were identified as 1-, 3-, 7- and 9-aminoadenine isomers (Fig. 1). Of these, 1-aminoadenine[11,12] and 9-aminoadenine[13] are known compounds. 3-Aminoadenine has only been described as a reaction intermediate[14] and its isolation and characterization had not as yet been reported. 7-Aminoadenine is a new compound which was obtained as the main product under alkaline conditions. The kinetics of formation of *N*-aminated adenines in the reaction of adenine with HAOS (4 mol eq.) was carried out in alkaline media at 30°C and the amounts of the products formed were measured by means of HPLC (Fig. 2A). The pH of the solution was adjusted to 12 at the beginning of the reaction but it gradually dropped to 9 after 30 h. The amount of adenine decreased according to pseudo-first-order kinetics (semi log plotted data were linear, data not shown). After 30 h reaction, adenine was consumed by almost 80% and 7-aminoadenine was concomitantly formed as the main product in a 31% yield. Other products were 1-, 3- and 9-aminoadenines and their yields were 11%, 10% and 8%, respectively. When the regioselectivity of amination by HAOS toward adenine was compared with that of methylation by dimethyl sulfate or other S_N2 type methylating agents under similar alkaline conditions, the amination reaction proceeded more selectively at the N7 position (the product ratio of 1-, 3-, 7- and 9-aminoadenines was about 1:1:3:1), whereas, by methylation, N3 and N9 methylation proceeded selectively (the product ratio of 3-, 7-, and 9-methyladenines is reported to be 5:1:6).[15] The kinetics of formation of *N*-aminated adenines in neutral media were also studied at 30°C using 4 mol eq. of HAOS (Fig. 2B). The pH of the reaction mixture was maintained between 6.7 and 7.4 by the addition of aqueous 1 N KOH solution. Under these conditions, the rate of adenine consumption was slower than in alkaline media. After 48 h reaction, 1-aminoadenine was obtained as the main product (32% yield) and other products were 3- and 7-aminoadenines (10% and 7% yields, respectively). Under these conditions, 9-aminoadenine did not form (the product ratio of 1-, 3-, 7- and 9-aminoadenines was about 3:1:1:0). These results were also different from the methylation of adenine with dimethyl sulfate under neutral conditions, in which 3-methyladenine was formed as the main product followed by 1-methyladenine (the product ratio of 1- and 3-methyladenine is reported to be 1:2).[16]

N^6-Methyladenine was used instead of adenine and the amination reaction in alkaline media was examined. The kinetic curves and the product ratio obtained (Fig. 2C) were almost similar to those of adenine (Fig. 2A), however, the product ratio of the 7-amino

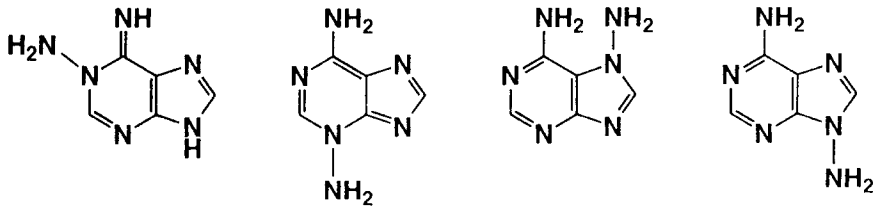

FIG. 1. Structures of the synthesized *N*-aminoadenines.

isomer of N^6-methyladenine increased slightly and the ratio of both 1-amino and 3-amino isomers became slightly smaller than in the case of adenine, respectively, *i.e.*, the yields of 1-, 3-, 7- and 9-amino-N^6-methyladenines after 24 h reaction were 5%, 8%, 40%, and 9%, respectively, and their relative ratio was about 1:2:8:2. On the other hand, amination by HAOS toward N^6,N^6-dimethyladenine under the same alkaline conditions proceeded slowly and the reaction afforded only 3-amino and 9-amino isomers without formation of 1-amino and 7-amino isomers (Fig. 2D). The yields of 1-, 3-, 7- and 9-amino-N^6,N^6-dimethyladenines after 30 h reaction were 0%, 41%, 0% and 21%, respectively, and the relative ratio was about 0:2:0:1.

The amination of adenine with DNPA in DMF afforded 1-aminoadenine as the main product (Fig. 2E), followed by 3-aminoadenine and 3,7-diaminoadenine. The yields of 1-, 3-, 7- and 9-aminoadenines after a 70 h reaction were 54%, 17%, 0% and 0%, respectively, and the ratio was about 3:1:0:0. It is interesting that 3,7-diaminoadenine was formed in a 15% yield. 3,7-Diaminoadenine is considered to be produced *via* the amination of 3-aminoadenine formed prior to the second amination since the N7 position of 3-substituted adenine is known to be an active site for electrophiles.[17] Actually, treatment of 3-aminoadenine with DNPA (4 eq. mol) in DMF afforded 3,7-diaminoadenine (Fig. 2F). Even if the yields of 3-aminoadenine and 3,7-diaminoadenine were combined, the quantity of 1-aminoadenine produced was greater than those of the 3-aminoadenines. This regioselectivity was also different from that of methylation by MeI in DMF in which 3-methyladenine is the main product.[16]

With regard to the difference in regioselectivity between amination and methylation of adenines found in the present study, the following speculation may be made. By comparing aminating rates at the N7- and N9-positions, which are more nucleophilic than the other N's due to deprotonation of the imidazole ring in alkaline media, it appears that the 6-amino group may stabilize the transition state for aminating the N7-position, probably by means of hydrogen bonding or electrostatic interaction with the rather acidic

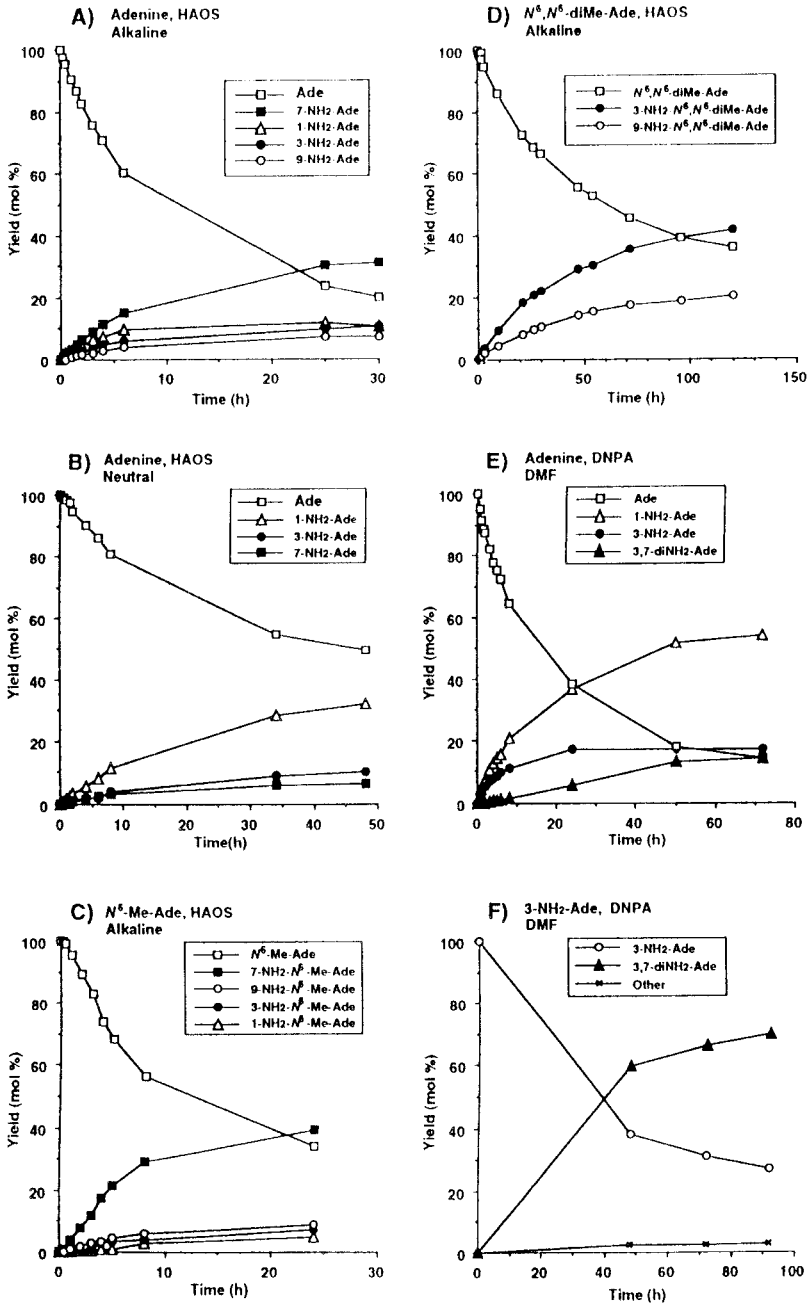

FIG. 2. Kinetics of formation of *N*-aminoadenine derivatives with 4 mol equivalents of HAOS or DNPA at 30°C. (A) Reaction of adenine with HAOS under alkaline conditions. (B) Reaction of adenine with HAOS under neutral conditions. (C) Reaction of N^6-methyladenine with HAOS under alkaline conditions. (D) Reaction of N^6,N^6-dimethyladenine with HAOS under alkaline conditions. (E) Reaction of adenine with DNPA in DMF. (F) Reaction of 3-aminoadenine with DNPA in DMF.

NH hydrogen of the aminating agent. In fact, the more basic 6-methylamino group promoted preferential amination at the N7 position, although the 6-dimethylamino substituent decreased the rate of N7-attack, probably because of its steric hindrance. Therefore, amination occurred more readily at the N3- and N9-positions, similar to methylation reactions. In neutral or organic media, the 6-amino group may assist the aminating agent in attacking the N1-position which may be more nucleophilic than the N7-position in the non-deprotonated form. Methylating agents without such an energetically favorable interaction with the 6-amino group are thought to attack at the N3-position which may be more nucleophilic in nature. For elucidation of the reaction mechanisms involved, further study is required.

With the preparations of these *N*-aminoadenines, the syntheses of all ring-nitrogen monoaminated isomers of adenine, guanine,[5] cytosine,[7] thymine[7] and uracil[7] have been completed.

Synthesis of 7-aminoadenine by other methods

It is known that alkylation of adenine in organic media occurs mainly at the N3 position[16] whereas the alkylation site of 3-alkyladenine is at the N7 position.[17] In order to obtain N7-alkyladenine, 3-benzyladenine is commonly used as a substrate to obtain 3,7-dialkylated adenine which is then debenzylated by catalytic hydrogenation to yield the desired product. However, to obtain N7-aminoadenine by a similar procedure, the N3-benzyladenine is not a suitable starting material since the *N*-amino group of the 7-amino-3-benzyladenine formed is unstable under reducing conditions. The pivaloyloxymethyl group, which can be removed by mild alkali treatment, was then introduced at the N3-position. Amination of 3-pivaloyloxymethyladenine with DNPA in DMF afforded 7-amino-3-pivaloyloxymethyladenine as a single product which was subsequently converted to 7-aminoadenine by alkali treatment (Fig. 3). When methylation of 3-pivaloyloxymethyladenine with CH_3I in DMF was carried out, 7- and 9-methyl derivatives were obtained at a ratio of about 1:1.[31] These results support the concept that the amination is more selective at the N7-position of adenine.

7-Aminoadenine was alternatively prepared starting from 6-ethoxypurine, which was allowed to react with HAOS in alkaline media. The products formed were 7-amino-6-ethoxypurine and 9-amino-6-ethoxypurine at a ratio of about 1:1. After these products were separated by column chromatography, ammonolyses of these compounds were carried out yielding 7-aminoadenine and 9-aminoadenine, respectively.

Characteristics of *N*-aminoadenines

UV absorption and p*K*a values of these *N*-aminoadenines were measured and the results were compared with those obtained for the corresponding *N*-methyladenines (Table

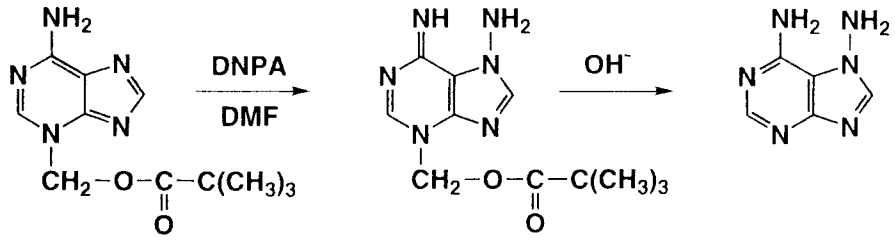

FIG. 3. Synthesis of 7-aminoadenine *via* 3-pivaloyloxymethyladenine.

1). As we have already reported for the aminated pyrimidine derivatives[7] and guanines,[5] the *N*-aminoadenines showed UV absorption profiles and p*K*a's similar to those of the corresponding *N*-methyladenines. The λmax values of *N*-hydroxyadenines are also shown in Table 1. Since 1- and 3-hydroxyadenines can form an N–OH structure in acidic solution and an N^+–O^- structure in neutral solution, only in acidic solution are their λmax's similar to those of the corresponding *N*-methyl and *N*-aminoadenines. On the other hand, since both 7- and 9-hydroxyadenines can form the N–OH structure in both acidic and neutral solutions, their λmax's in acidic and neutral solutions are similar to those of the corresponding *N*-methyl and *N*-aminoadenines.

^1H and ^{13}C NMR spectra of these *N*-aminoadenines were taken in Me_2SO and their chemical shifts were compared with those of *N*-methyladenines (Table 2). Since 1-methyladenine was not sufficiently soluble in Me_2SO to obtain ^{13}C NMR spectra, its spectrum was taken in D_2O. The 8-H protons of N^7- and N^9-substituted adenines and the 2-H protons of 3-substituted adenines were assigned by deuterium exchange in which the compounds were heated at 70°C in D_2O.[18] Assignments of 2-C and 8-C carbons were performed by ^1H-^{13}C COSY spectrum. Assignments of 4-C, 5-C, 6-C carbons were done by ^1H-^{13}C HMBC spectrum. The ^1H and ^{13}C chemical shifts of *N*-aminoadenine were almost the same as those of the corresponding *N*-methyladenines except for 4-C of 7-methyl- and 7-aminoadenines and for 5-C of 9-methyl- and 9-aminoadenines. These results indicate that the *N*-amino group gave the effect on chemical shifts similar to that of the *N*-methyl group. A difference in chemical shift was observed only in the 4-C carbon of 7-aminoadenine which resonated at 3.4 ppm higher field than that of 7-methyladenine and in the 5-C carbon of 9-aminoadenine which resonated at 1.4 ppm higher field than that of 9-methyladenine. When the chemical shifts of carbons of the *N*-substituted adenines were compared with the those of adenine, the 2-C carbon of 1- and 3-substituted adenines

TABLE 1. Comparison of UV Spectra (λmax nm) and p*K*a Values of *N*-NH_2-Adenines, *N*-CH_3-Adenines and *N*-OH-Adenines

	1-NH_2-Ade[a]	1-CH_3-Ade	1-OH-Ade[c]
+1[b]	257	259	258
0	268	270	231, 262.5
-1			233, 275
p*K*a	7.0	7.2	2.6, 9.0
	3-NH_2-Ade	**3-CH_3-Ade**	**3-OH-Ade[d]**
+1	274	274	222, 277
0	273	273	229, 293
-1			251, 278, 290
p*K*a	6.2	6.1	2.87, 6.91
	7-NH_2-Ade	**7-CH_3-Ade**	**7-OH-Ade[e]**
+1	273	273	274
0	269, 280 (sh)	270, 280 (sh)	243, 269
-1			235, 284
p*K*a	3.7	3.6	3.4, 5.75
	9-NH_2-Ade	**9-CH_3-Ade**	**9-OH-Ade[f]**
+1	257	258	215, 261
0	259	260	245, 259
-1			234, 262
p*K*a	3.7	3.9	3.59, 5.7

[a] Ade, Adenine, [b] +1, cationic; 0, neutral; -1, anionic, [c] ref. 26, [d] ref. 27, [e] ref. 28, [f] ref. 29.

TABLE 2. Carbon and Proton Chemical Shifts in *N*-Aminoadenines and *N*-Methyladenines[a]

	Ade	1AA	1MA	3AA	3MA	7AA	7MA	9AA	9MA
2-C	152.3	142.8	146.7[b]	143.5	143.6	152.2	152.1	152.3	152.3
4-C	151.2	157.2	157.4[b]	150.5	150.2	156.1	159.5	149.2	149.8
5-C	117.6	118.4	121.3[b]	120.7	120.2	111.3	111.6	117.1	118.5
6-C	155.3	149.2	151.7[b]	154.3	154.8	151.6	151.7	155.8	155.8
8-C	139.2	154.4	155.2[b]	152.2	152.3	145.1	145.8	141.8	141.3
CH_3	—	—	39.7[b]	—	35.7	—	33.6	—	29.2
N-CH_3	—	—	3.70	—	3.90	—	3.91	—	3.72
N-NH_2	—	6.31	—	6.54	—	6.49	—	6.00	—
C-NH_2	7.12	[c]	[c]	7.82	7.83	6.92	6.90	7.15	7.17
2-H	8.14	8.13	8.15	8.24	8.30	8.14	8.16	8.15	8.15
8-H	8.12	7.85	7.84	7.78	7.77	8.08	8.16	7.99	8.09
NH	12.8	—	—	—	—	—	—	—	—

[a] Chemical shifts are in ppm with respect to TMS. The spectra were recorded in Me_2SO-d_6 at 27℃. Ade, adenine; AA, aminoadenine; MA, methyladenine.

[b] Spectra were taken in D_2O.

[c] Peaks were not detected.

resonated at higher field (6~10 ppm) and the 8-C carbon at lower field (13~16 ppm). For 7-substituted adenine, the 4-C and 8-C carbons resonated at lower field (5~8 ppm and 5~7 ppm, respectively) and the 5C carbon at higher field (~6 ppm). No remarkable differences were observed with respect to 9-substituted adenine.

By comparing the chemical shifts of the NH_2 protons of *N*-aminoadenine and the corresponding *N*-methyladenines, it was obvious that *C*-amino protons of both compounds resonated at an almost similar field and that *N*-amino protons resonated at higher field than those of C-amino protons.

All NMR data shown in Table 2 were obtained in the present study. Among them, the chemical shifts of carbons of 7-methyladenine[19] and 9-methyadenine[19] and the chemical shifts of protons of 1-aminoadenine[12] and 9-aminoadenine[13] are reported. During the preparation of this manuscript, the assignments of carbon and proton chemical shifts of other *N*-methyladenines were reported.[20] These data support our results.

Alkaline treatment of 1-aminoadenine

We previously reported that 1-aminoadenosine and 1-amino-9-methyladenine were converted to 5-amino-1-(ß-D-ribofuranosyl)-4-(1,2,4-triazol-3-yl)imidazole and 5-amino-1-methyl-4-(1,2,4-triazol-3yl)imidazole, respectively, by non-Dimroth-type rearrangement[3,10] carried out by heating at 70°C in aqueous alkaline solution (pH 11) for 2 h. However, when 1-aminoadenine was treated with alkali under the same conditions, the reaction proceeded very slowly and was therefore allowed to continue. After 24 h, the reaction was almost complete, yielding 5-amino-4-(1,2,4-triazol-3-yl)imidazole[21] and 6-hydrazinopurine[22] at a ratio of about 1:1 (Fig. 4). This product ratio did not change even when the reactions were carried out at a pH between 9 to 11. In contrast, the reaction at pH 12 gave only 6-hydrazinopurine by Dimroth rearrangement. With respect to 1-aminoadenosine and 1-amino-9-methyladenine, alkaline treatment gave only triazolylimidazole derivatives and never 6-hydrazinopurine derivatives. It is worth noting that substitution at the 9-position of adenine, which blocks deprotonation of 1-aminoadenine in alkaline media (p*K*a is approximately 11 to 12 in this experiment), may play a role in facilitating the attack of OH^- as well as in yielding selective products in the reaction.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on EX 270, GSX 400 and ALPHA 500 spectrometers (JEOL) and chemical shifts were expressed in parts per million downward from internal tetramethylsilane. Mass spectra were obtained with AX 505HA and SX

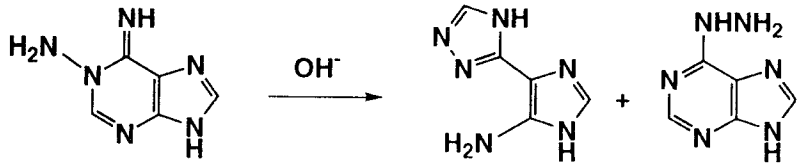

FIG. 4. Alkali treatment of 1-aminoadenine.

102A spectrometers (JEOL). UV spectra were recorded on a Shimadzu UV-2100 spectrophotometer and p*K*a values were estimated from pH dependent UV changes. HPLC analyses were carried out using a Shimadzu LC-10AD apparatus equipped with a photodiode array UV detector SPD-M6A. Melting points were measured with a Yanagimoto micro-melting point apparatus and are uncorrected. Hydroxylamine-*O*-sulfonic acid (HAOS) was purchased from Tokyo Chemical Industry Co. 2,4-Dinitrophenoxyamine (DNPA) was prepared by a previously reported method.[23] 1-Methyladenine and 3-methyladenine were purchased from Sigma Chemical Co. 5-Amino-4-(1,2,4-triazol-3-yl)imidazole,[21] 6-hydrazinopurine,[22] 7-methyladenine[24] and 9-methyladenine[25] were prepared by a previously reported method.

***N*-Aminoadenines.** Adenine (1.35 g, 10 mmol) and KOH (2.24 g, 40 mmol) were dissolved in 100 mL of H_2O. HAOS (4.52 g, 40 mmol, 4 eq. mol) dissolved in water and neutralized with $NaHCO_3$ was added to the adenine solution and the mixture was allowed to stand at room temperature for 30 h. Yields of adenine, 1-aminoadenine, 3-aminoadenine, 7-aminoadenine and 9-aminoadenine were 21%, 11%, 10%, 31% and 8%, respectively, as measured by HPLC. The mixture was then neutralized with AcOH and the solvent was removed by evaporation. Products were extracted from the residue with hot EtOH (50 mL x 3). After the solvent was removed by evaporation, the products were separated by column chromatography (silica gel, MERCK 7734, $CHCl_3$-MeOH = 9:1). Adenine, 9-aminoadenine, 3-aminoadenine, 7-aminoadenine and 1-aminoadenine were eluted in that order.

3-Aminoadenine: Recrystallization from H_2O-MeOH yielded columns. mp 324-326°C (dec); ^1H NMR (Me_2SO-*d*6) δ 6.54 (s, 2H, N-NH_2), 7.78 (s, 1H, 8-H), 7.82 (br s, 2H, C-NH_2), 8.24 (s, 1H, 2-H); ^{13}C NMR (Me_2SO-*d*6) δ 120.7 (5-C), 143.5 (2-C), 150.5 (4-C), 152.2 (8-C), 154.3 (6-C); UV λmax nm (ε), pH 1 274 (16400), pH 8 273 (13200), pH 12 273 (13000); p*K*a 6.2; MS *m/z* 150 (M^+). Anal. Calcd for $C_5H_6N_6$: C, 40.00; H, 4.03; N, 55.97. Found: C, 40.22; H, 4.08; N, 56.42.

7-Aminoadenine: Recrystallization from MeOH yielded needles. mp 249-251°C (dec); ^{1}H NMR (Me_2SO-d_6) δ 6.49 (s, 2H, N-NH_2), 6.92 (br s, 2H, C-NH_2), 8.08 (s, 1H, 8-H), 8.14 (s, 1H, 2-H); ^{13}C NMR (Me_2SO-d_6) δ 111.3 (5-C), 145.1 (8-C), 151.6 (6-C), 152.2 (2-C), 156.1 (4-C); UV λmax nm (ε), pH 1 273 (12300), H_2O and pH 12 269 (9700), 280 (sh) (6500); p*K*a 3.7; MS *m/z* 150 (M^+). Anal. Calcd for $C_5H_6N_6$: C, 40.00; H, 4.03; N, 55.97. Found: C, 39.83; H, 4.15; N, 55.74.

7-Aminoadenine prepared by the amination of 3-pivaloyloxymethyladenine.

3-Pivaloyloxymethyladenine: Adenine (1.7 g, 12.6 mmol) was dissolved in 50 mL of DMF. Chloromethyl pivalate (97%, 4.5 ml, 30.3 mmol) was added to the solution and the mixture was allowed to stand at room temperature for 5 days. After insoluble materials in the reaction mixture were removed by filtration, the solvent was removed by evaporation. Products were separated by column chromatography (silica gel, $CHCl_3$-MeOH = 39:1, then 19:1) to yield 250 mg (8%) of 3-pivaloyloxymethyladenine. Recrystallization from MeOH yielded colorless needles. mp 227-229°C; ^{1}H NMR (Me_2SO-d_6) δ 1.01 (s, 9H, CH_3 x 3), 6.21 (s, 2H, CH_2), 7.76 (s, 1H, 2-H or 8-H), 8.13 (br s, 2H, NH_2), 8.47 (s, 1H, 2-H or 8-H); UV λmax nm (ε), pH 1 275 (14900), H_2O 276 (11400), pH 12 decomposed; MS *m/z* 249 (M^+). Anal. Calcd for $C_{11}H_{15}N_5O_2 \cdot 4/5H_2O$: C, 50.11; H, 6.34; N, 26.56. Found: C, 50.15; H, 6.10; N, 26.35.

7-Aminoadenine: 3-Pivaloyloxymethyladenine (100 mg, 0.40 mmol) and DNPA (240 mg, 1.2 mmol, 3 eq. mol) were dissolved in 20 mL of DMF and the mixture was heated at 70°C for 4 h. After the solvent was removed by evaporation, the residue was dissolved in 20 mL of aqueous 1 N HCl solution. This aqueous solution was washed twice with AcOEt and then evaporated to dryness. After the residue was dissolved in a small amount of MeOH, 5 mL of aqueous 25% NH_4OH was added and the mixture left to stand at room temperature for 1 h. After the solvent was removed, 7-aminoadenine was separated by PLC (silica gel, $CHCl_3$-MeOH = 7:3) in a 34 mg (54%) yield.

7-Aminoadenine prepared by the amination of 6-ethoxypurine. 6-Ethoxypurine (100 mg, 0.61 mmol) and K_2CO_3 (1.26 g, 9.1 mmol) were dissolved in 20 mL of water. HAOS (688 mg, 6.1 mmol, 10 eq. mol) was added gradually to the solution with stirring and the mixture was left standing at room temperature for 48 h. After the solvent was removed by evaporation to dryness, products were extracted from the residue with hot MeOH. Product ratio of 7-amino- and 9-amino-6-ethoxypurines was 10:13 by

the measurement of NMR spectroscopy. Products were purified by column chromatography (silica gel, 25 x 200 mm, $CHCl_3$-MeOH = 29:1). 9-Amino-6-ethoxypurine (13.1 mg, 12% yield), 6-ethoxypurine (29.6 mg, 29.6%) and 7-amino-6-ethoxypurine (4.6 mg, 4.2%) were eluted in that order.

7-Amino-6-ethoxypurine: ^1H NMR (Me_2SO-d_6) δ 1.43 (t, 3H, J = 7.3 Hz, CH_3), 4.57 (q, 2H, CH_2), 6.36 (s, 2H, NH_2), 8.29 (s, 1H, 2-H or 8-H), 8.46 (s, 1H, 2-H or 8-H); UV λmax nm, pH 1 258, H_2O and pH 12 259; MS *m/z* 179 (M^+).

9-Amino-6-ethoxypurine: ^1H NMR (Me_2SO-d_6) δ 1.41 (t, 3H, J = 7.3 Hz, CH_3), 4.58 (q, 2H, CH_2), 6.19 (s, 2H, NH_2), 8.23 (s, 1H, 2-H or 8-H), 8.51 (s, 1H, 2-H or 8-H); UV λmax nm, pH 1, H_2O and pH 12 251; MS *m/z* 179 (M^+).

7-Amino- and 9-aminoadenines: The prepared 7-amino-6-ethoxypurine and 9-amino-6-ethoxypurine were each (25 mg) dissolved in MeOH (3 mL) in stainless steel reaction tubes, and ammonolysis was carried out at 100°C at 35 kg/cm^2 for 20 h. After removal of the solvent, the residues were recrystallized from MeOH to yield 7-aminoadenine and 9-aminoadenine, each in about an 80% yield.

***N*-Amino-N^6-methyladenines.** N^6-Methyladenine (597 mg, 4 mmol) and KOH (673 mg, 12 mmol) were dissolved in 20 mL of H_2O. HAOS (1.36 g, 12 mmol, 3 eq. mol) dissolved in 10 mL of water and neutralized with $NaHCO_3$ was added to the N^6-methyladenine solution and the mixture was allowed to stand at room temperature for 48 h. After the mixture was neutralized with aqueous 1 N HCl, the solvent was removed by evaporation. Products were separated by column chromatography (silica gel, 0 to 10% stepwise gradiation of MeOH in $CHCl_3$). N^6-Methyladenine, 9-amino-N^6-methyladenine (21 mg, 3% yield), 3-amino-N^6-methyladenine (21 mg, 3%) and 7-amino-N^6-methyladenine (200 mg, 31%) were eluted in that order.

3-Amino-N^6-methyladenine: Recrystallization from MeOH yielded colorless needles. mp 273-275°C (dec); ^1H NMR (Me_2SO-d_6) δ 3.01 (s, 3H, CH_3), 6.57 (s, 2H, N-NH_2), 7.76 (s, 1H, 8-H), 8.25 (s, 1H, NH), 8.34 (s, 1H, 2-H); ^{13}C NMR (Me_2SO-d_6) δ 27.3 (CH_3), 143.6 (2-C), 149.4 (4-C), 151.7 (8-C), 153.2 (6-C), 5-C was not detected; UV λmax nm, pH 1 282, pH 8 and pH 12 285; MS *m/z* 164 (M^+). HRMS *m/e* M^+ Calcd for $C_6H_8N_6$: 164.0810. Found: 164.0809.

7-Amino-N^6-methyladenine: Recrystallization from MeOH yielded colorless needles. mp 247-248°C (dec); ^1H NMR (Me_2SO-d_6) δ 3.00 (d, 3H, J = 4.6 Hz, CH_3), 6.52 (br s, 2H, N-NH_2), 7.02 (br d, 1H, NH), 8.05 (s, 1H, 8-H), 8.22 (s, 1H, 2-H); ^{13}C NMR (Me_2SO-d_6) δ 27.0 (CH_3), 111.5 (5-C), 144.3 (8-C), 151.3 (6-C), 152.2 (2-C), 155.0 (4-C); UV λmax nm (ε), pH 1 279 (15000), pH 8 and pH 12 278 (13600), 285 (sh)

(9200); p*K*a 4.5; MS *m/z* 164 (M^+). Anal. Calcd for $C_6H_8N_6 \cdot H_2O$: C, 39.73; H, 5.52; N, 46.00. Found: C, 39.68; H, 5.58; N, 45.93.

9-Amino-*N*^{6}-methyladenine: Recrystallization from MeOH yielded colorless columns. mp 230-231°C (dec); ^{1}H NMR (Me_2SO-d_6) δ 2.96 (br s, 3H, CH_3), 6.01 (s, 2H, N-NH_2), 7.02 (br s, 1H, NH), 7.99 (s, 1H, 8-H), 8.22 (br s, 1H, 2-H); ^{13}C NMR (Me_2SO-d_6) δ 26.9 (CH_3), 117.6 (5-C), 141.4 (8-C), 148.3 (4-C), 152.3 (2-C), 154.8 (6-C); UV λmax nm (ε), pH 1 263 (12200), pH 8 and pH 12 266 (11500); p*K*a 4.0; MS *m/z* 164 (M^+). HRMS *m/e* M^+ Calcd for $C_6H_8N_6$: 164.0810. Found: 164.0813.

1-Amino-*N*^{6}-methyladenine: Since the yield of 1-amino-*N*^{6}-methyladenine was very low in the reaction of *N*^{6}-methyladenine with HAOS, the authentic sample of 1-amino-*N*^{6}-methyladenine (149 mg, 1 mmol) and DNPA (796 mg, 4 mmol) in 20 mL of DMF was heated at 95°C for 2 h. After the reaction, the solvent was removed by evaporation and the desired 1-amino-*N*^{6}-methyladenine was separated by column chromatography (silica gel, stepwise elution with 0, 5, 10% MeOH in $CHCl_3$). Although 1-amino-*N*^{6}-methyladenine was isolated in an 18 mg (11%) yield, other products were obtained as a mixture. ^{1}H NMR (Me_2SO-d_6) δ 3.54 (s, 3H, CH_3), 6.34 (br s, 2H, N-NH_2), 7.85 (s, 1H, 8-H), 8.09 (s, 1H, 2-H), an NH peak was not detected; ^{13}C NMR (Me_2SO-d_6) δ 30.7 (CH_3), 118.4 (5-C), 143.2 (2-C), 148.1 (6-C), 154.2 (8-C), 157.9 (4-C); UV λmax nm, pH 1 260, H_2O 264, pH 12 272; MS *m/z* 164 (M^+).

***N*-Amino-*N*^{6},*N*^{6}-dimethyladenine.** *N*^{6},*N*^{6}-Dimethyladenine (326 mg, 2 mmol) and KOH (1.12 g, 20 mmol) were dissolved in 40 mL of H_2O. HAOS (2.26 g, 20 mmol, 10 eq. mol) was dissolved in 10 mL of water and neutralized with $NaHCO_3$. The HAOS solution was then added to the *N*^{6},*N*^{6}-dimethyladenine solution and the mixture was heated at 100°C for 72 h. After the mixture was neutralized with aqueous 1 N HCl, the solvent was removed by evaporation. Products were separated by column chromatography (silica gel, 0 to 10% stepwise gradiation of MeOH in $CHCl_3$). 3-Amino-*N*^{6},*N*^{6}-dimethyladenine was obtained in a 48 mg (13%) yield. 9-Amino-*N*^{6},*N*^{6}-dimethyladenine was obtained as a mixture (35 mg) containing starting material and its purification was not successful.

3-Amino-*N*^{6},*N*^{6}-dimethyladenine: Recrystallization from $CHCl_3$ yielded colorless columns. mp 231-233°C (dec); ^{1}H NMR (Me_2SO-d_6) δ 3.28 (br s, 3H, CH_3), 3.82 (br s, 3H, CH_3), 6.54 (s, 2H, N-NH_2), 7.77 (s, 1H, 8-H), 8.31 (s, 1H, 2-H); ^{13}C NMR (Me_2SO-d_6) δ 37~38 (CH_3 x 2), 121.1 (5-C), 142.6 (2-C), 150.7 (4-C), 151.2 (8-C), 152.2 (6-C); UV λmax nm (ε), pH 1 291 (13400), pH 8 and pH 12 293 (10900); p*K*a 5.9; MS *m/z* 178 (M^+). Anal. Calcd for $C_7H_{10}N_6 \cdot 1/3H_2O$: C, 45.68; H, 5.83; N, 45.62. Found: C, 45.53; H, 5.53; N, 45.63.

9-Amino-N^6,N^6-dimethyladenine: ^{1}H NMR (Me_2SO-d_6) δ 3.45 (br s, 6H, CH_3 x 2), 5.99 (s, 2H, N-NH_2), 8.01 (s, 1H, 8-H), 8.22 (s, 1H, 2-H); UV absorption was determined with a photodiode array UV detector after the mixture was separated by HPLC. λ_{max} nm, pH 6.8 276.

Kinetics of the amination. *A) Reaction of HAOS in alkaline media:* Adenine (27 mg, 0.2 mmol), N^6-methyladenine (29.8 mg, 0.2 mmol) and N^6,N^6-dimethyladenine (32.6 mg, 0.2 mmol) were each dissolved in 10 mL water containing KOH (45 mg, 0.8 mmol). HAOS (90.5 mg, 0.8 mmol) was dissolved in another 10 mL of water and the pH of the solution was adjusted to 7 with $NaHCO_3$. After both solutions were mixed, the reaction mixture was kept at 30°C. At an appropriate time, 100 μL of the reaction mixture was taken, neutralized with acetic acid and diluted with 1 mL of water; this solution was used for HPLC analysis. *B) Reaction of HAOS in neutral media:* Adenine (27 mg, 0.2 mmol) was dissolved in 10 mL of water. HAOS (90.5 mg, 0.8 mmol) was dissolved in another 10 mL of water and the pH of the solution was adjusted to 7 with $NaHCO_3$. After both solutions were mixed, the reaction mixture was kept at 30°C. During the reaction, the pH of the solution was adjusted to 7 by addition of aqueous NaOH solution. At an appropriate time, 100 μL of the reaction mixture was taken and diluted with 1 mL of water; this solution was used for HPLC analysis. *C) Reaction of DNPA in DMF:* Adenine (27 mg, 0.2 mmol) was dissolved in 10 mL of DMF. To this solution, 10 mL of DMF containing DNPA (159 mg, 0.8 mmol) was added, and the mixture was kept at 37°C. At an appropriate time, 50 μL of the reaction mixture was taken, 500 μL of $CHCl_3$ and 450 μL of water were added, and the mixture was then stirred vigorously. After it was allowed to stand for a short while, the upper phase was used for HPLC analysis. Identification of 3,7-diaminoadenine was made only on the basis of its short retention time in HPLC and the fact that its UV spectra (λ_{max} 276 nm in the acidic form and 225 nm and 280 nm in the free form) were the same as those of 3,7-dimethyladenine.[30]

Product analysis. A MERCK LiChrospher ODS column (250 x 4 mm) was used. For analysis of *N*-aminoadenines, a solution of 66.7 mM phosphate buffer (pH 6.8) – 1% MeOH was used as an eluent at flow rate of 0.8 mL/min. Retention times of adenine, 1-, 3-, 7- and 9-aminoadenines, and 3,7-diaminoadenine were 23.2 min, 4.0 min, 7.7 min, 10.2 min, 14.6 min and 3.5 min, respectively. Detection of the products was carried out at a wavelength of 265 nm and quantification of the products was performed using their respective ε values. For analysis of *N*-amino-N^6-methyladenines, a solution of 66.7 mM phosphate buffer (pH 6.8) – 8% MeOH was used as an eluent, and detection of the

product was carried out at a wavelength of 270 nm. Retention times of N^6-methyladenine and 1-, 3-, 7- and 9-amino-N^6-methyladenines were 30.0 min, 4.0 min, 8.5 min, 11.9 min and 16.9 min, respectively. Identification of 1-amino-N^6-methyladenine was made only by the comparing its UV spectra with those of 1,N^6-dimethyladenine. For analysis of N-amino-N^6,N^6-dimethyladenines, a solution of 66.7 mM phosphate buffer (pH 6.8) – 20% MeOH was used as an eluent, and detection of the product was carried out at a wavelength of 280 nm. Retention times of N^6,N^6-dimethyladenine, and 3- and 9-amino-N^6,N^6-dimethyladenines were 32.1 min, 12.3 min and 18.2 min, respectively. Identification of 3-amino-N^6,N^6-dimethyladenine was made only by comparing its UV spectra with those of 3,N^6,N^6-trimethyladenine.

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31. A mixture of 3-pivaloyloxymethyladenine (100 mg) and CH_3I (0.5 mL) in 10 mL of MeOH was heated at 60°C for 5 h. After the solvent was removed by evaporation, 1 mL of aqueous 25% NH_4OH was added and the mixture left to stand at room temperature for 2 h. After solvent was removed, product ratio of 7- and 3-methyladenines (1:1) was measured by NMR spectroscopy. Products were separated by PLC (silica gel, $CHCl_3$-MeOH = 7:3). 9-Methyladenine (9 mg, 15% yield), adenine (15 mg, 28%) and 7-methyladenine (11 mg, 18%) were eluted in that order.