Reaction of 9-Substituted 1-Aminoadenine with Hydrazine

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Reaction of 9-substituted (methyl or benzyl) 1-aminoadenines **1** with hydrazine afforded 9-substituted 6-hydrazinopurines **2** and 1-substituted 5-amino-4-(4-amino-1,2,4-triazol-3-yl)imidazole (**4**). The product ratio of **2** to **4** rose with increasing amounts of methanol used as the solvent. When the same reaction was carried out using 1,9-dimethyladenine instead of **1**, compounds **2** and **4** were also obtained with N^6,9-dimethyladenine. A possible mechanism for formation of **2** and **4** is discussed.

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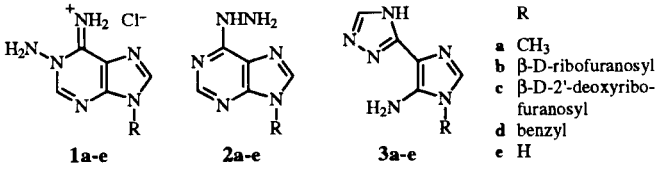
Introduction.

We have carried out extensive studies on a simple electrophilic amination toward nucleic acid components in connection with the chemical carcinogenesis of arylamines and arylhydroxylamines. As a result, we obtained various *N*-aminated nucleic acid base analogues and described their unique chemical characteristics [1-9]. For example, heating 9-substituted 1-aminoadenines **1a-c** in aqueous alkaline solution did not yield 9-substituted 6-hydrazinopurines **2a-c**, so called Dimroth-type rearranged products, but did generate 1-substituted 5-amino-4-(1,2,4-triazol-3-yl)imidazole **3a-c** [2,9] (Chart 1).

Chart 1



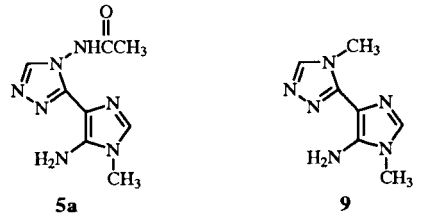
1a-e **2a-e** **3a-e**

Hosmane *et al.* reported that treatment of 1-amino-9-benzyladenine (**1d**) with an excess of hydrazine monohydrate in methanol gave only 9-benzyl-6-hydrazinopurine (**2d**). In order to clarify the mechanism involved, we carried out a similar reaction using 1-amino-9-methyladenine (**1a**) and hydrazine monohydrate without solvent, and obtained 5-amino-1-methyl-4-(4-amino-1,2,4-triazol-3-yl)imidazole (**4a**) which was accompanied by the corresponding 6-hydrazino-9-methylpurine (**2a**) (Scheme 1). In this paper, we characterize the structure of compound **4** and describe possible mechanisms for formation of **2** and **4**.

Results and Discussion.

Hosmane *et al.* reported that treatment of 1-amino-9-benzyladenine (**1d**) with 20 molar equivalents of hydrazine monohydrate in methanol gave only 9-benzyl-6-hydrazinopurine (**2d**) [10]. However, when we carried out a similar reaction using 1-amino-9-methyladenine hydrochloride (**1a**) [2] and 40 molar equivalents of hydrazine monohydrate without a solvent, compound **4a** (39%) was obtained as the main product with a lesser yield of the corresponding 6-hydrazino-9-methylpurine (**2a**, 13% yield) (Scheme 1). The ^{1}H nmr chemical shifts of **4a** were similar to those of 5-amino-1-methyl-4-(1,2,4-triazol-3-yl)imidazole (**3a**) except for the appearance of an amino signal (6.47 ppm) and the disappearance of an NH signal. The uv spectra of **4a** in aqueous acidic and neutral solution resembled those of **3a**. The mass spectrum of **4a** showed M^+ 179 which corresponded to a mass number of (M^+ of **3a**) + 15. From these spectral data, the structure of **4a** was inferred to be 5-amino-1-methyl-4-(*N*-amino-1,2,4-triazol-3-yl)imidazole, however, the position of the *N*-amino group in the triazole ring could not be determined. For X-ray diffraction analysis, the crystallization of **4a** was attempted but was unsuccessful. Acetylation of **4a** afforded 5-amino-1-methyl-4-(4-acetylamino-1,2,4-triazol-3-yl)imidazole (**5a**) (Chart 2) which gave needles suitable for X-ray analysis. X-ray diffraction analysis of **5a** revealed that the acetylamino group was at the N4 position of the triazole moiety.

Chart 2



5a **9**

An ORTEP [11] plot of **5a** is shown in Chart 3 and the relevant crystallographic data are given in the Experimental. Atomic parameters are shown in Table 1, and the bond lengths and angles are in Table 2. From these results, the structure of **4a** was determined to be 5-amino-1-methyl-4-(4-amino-1,2,4-triazol-3-yl)imidazole.

1-Amino-9-benzyladenine hydrochloride (**1d**), which was used in the reaction described by Hosmane *et al.*, was

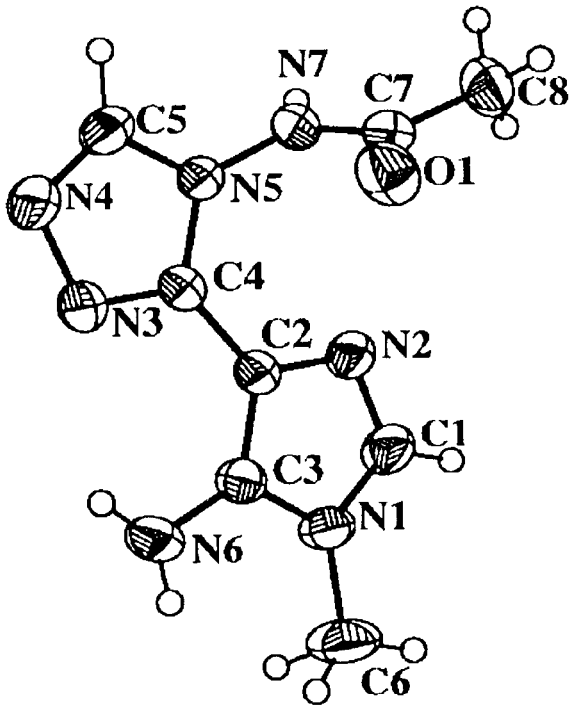


Chart 3. Crystal structure of compound **5a**.

Table 1

Fractional Coordinates and Equivalent Isotropic Temperature Factors with Estimated Standard Deviations in Parentheses

Atom	x	y	z	U_{eq}(Å^2) [a]
O1	0.0357(2)	-0.3426(3)	0.9207(2)	0.0590(13)
N1	0.1089(2)	0.1408(3)	0.9840(2)	0.0457(14)
N2	0.1593(2)	-0.0586(3)	0.9711(2)	0.0452(15)
N3	0.1521(2)	-0.1640(3)	1.2161(2)	0.053(2)
N4	0.1679(3)	-0.2931(3)	1.2612(3)	0.063(2)
N5	0.1781(2)	-0.2999(3)	1.1149(2)	0.0405(13)
N6	0.0913(3)	0.1137(4)	1.1399(3)	0.052(2)
N7	0.1838(2)	-0.3525(3)	1.0291(2)	0.0414(14)
C1	0.1385(3)	0.0624(4)	0.9303(3)	0.050(2)
C2	0.1427(2)	-0.0589(3)	1.0567(3)	0.0366(15)
C3	0.1119(2)	0.0649(3)	1.0652(3)	0.0377(15)
C4	0.1576(2)	-0.1706(3)	1.1275(3)	0.0376(15)
C5	0.1828(3)	-0.3691(4)	1.1995(3)	0.053(2)
C6	0.0857(5)	0.2838(5)	0.9632(6)	0.075(3)
C7	0.1077(3)	-0.3645(3)	0.9304(3)	0.044(2)
C8	0.1241(4)	-0.4058(6)	0.8416(4)	0.057(2)
HN6A	0.050(3)	0.175(5)	1.117(4)	0.02(2) [b]
HN6B	0.085(3)	0.054(5)	1.186(4)	0.08(2)
HN7	0.239(3)	-0.365(4)	1.038(3)	0.05(2)
H1	0.148(2)	0.094(4)	0.870(3)	0.10(2)
H5	0.199(2)	-0.466(4)	1.208(3)	0.058(15)
H6A	0.125(5)	0.333(7)	1.035(7)	0 05(3)
H6B	0.088(4)	0.312(6)	0.895(5)	0.33(5)
H6C	0.034(5)	0.313(6)	0.981 (5)	0.13(4)
H8A	0.172(5)	-0.476(8)	0.858(5)	0.06(3)
H8B	0.075(4)	-0.454(6)	0.788(5)	0.10(3)
H8C	0.145(4)	-0.343(8)	0.825(4)	0.19(4)

[a] $U_{eq} = 1/3\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* (a_i \cdot a_j)$. [b] H atom thermal parameters, U_{iso}.

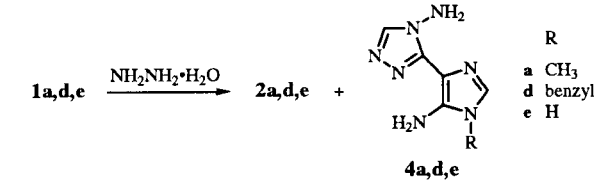
Table 2

Bond Lengths (Å) and Angles (°) for Non-H Atoms with Estimated Standard Deviations in Parentheses

Bond	Length	Bond	Length
O1 - C7	1.215(5)	N1 - C1	1.363(6)
N1 - C3	1.361(5)	N1 - C6	1.469(10)
N2 - C1	1.308(6)	N2 - C2	1.388(5)
N3 - N4	1.401(6)	N3 - C4	1.316(5)
N4 - C5	1.282(7)	N5 - N7	1.379(5)
N5 - C4	1.372(5)	N5 - C5	1.355(6)
N6 - C3	1.372(6)	N7 - C7	1.377(6)
C2 - C3	1.374(5)	C2 - C4	1.435(5)
C7 - C8	1.489(8)		
Bond	**Angle**	**Bond**	**Angle**
C1 - N1 - C3	107.2(3)	C1 - N1 - C6	125.8(5)
C3 - N1 - C6	126.8(5)	C1 - N2 - C2	105.0(3)
N4 - N3 - C4	107.6(4)	N3 - N4 - C5	106.7(4)
N7 - N5 - C4	127.7(3)	N7 - N5 - C5	126.7(4)
C4 - N5 - C5	105.3(3)	N5 - N7 - C7	118.6(4)
N1 - C1 - N2	112.1(4)	N2 - C2 - C3	109.8(3)
N2 - C2 - C4	125.2(3)	C3 - C2 - C4	125.0(4)
N1 - C3 - N6	123.4(4)	N1 - C3 - C2	105.8(3)
N6 - C3 - C2	130.7(4)	N3 - C4 - N5	108.8(3)
N3 - C4 - C2	124.2(4)	N5 - C4 - C2	127.0(3)
N4 - C5 - N5	111.6(4)	O1 - C7 - N7	121.2(4)
O1 - C7 - C8	125.5(5)	N7 - C7 - C8	113.3(4)

synthesized by *N*-amination of 9-benzyladenine [12] with 2,4-dinitrophenoxyamine, and reaction of **1d** with hydrazine monohydrate in the absence of solvent under the same reaction conditions as used for **1a** was carried out. The products isolated were **2d** and **4d** in 35% and 33% yields, respectively (Scheme 1). The higher yield of **2d** (35%) compared to that of **2a** (13%) in the reaction with **1a** may be attributed to the easier separation of **2d**. These results suggested that the structure of the substituent (methyl or benzyl) at the N9 position of **1** has little effect on formation of compound **4**, but the presence of methanol in the reaction mixture may be crucial. In order to examine the effect of methanol, the reaction of **1a** with hydrazine monohydrate was carried out by

Scheme 1. Reaction of 9-substituted 1-aminoadenines with hydrazine monohydrate.



changing the amount of methanol, and the products formed were analysed by hplc (Table 3). The product ratio changed with the amount of solvent used, *i.e.*, an increase in the amount of methanol resulted in an increase in the formation of **2a** (the ratio of **2a** to **4a** increased from 0.94 to 6.63 with the addition of 0.5 to 2.0 ml of methanol).

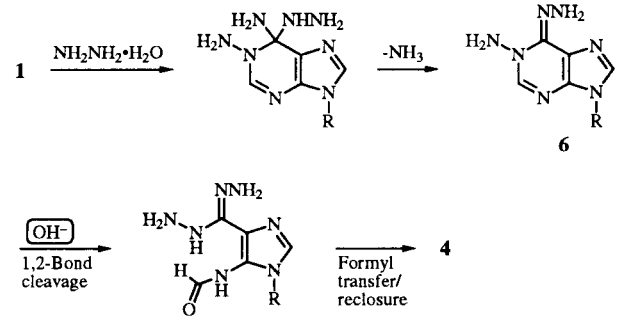
Table 3

Effect of Methanol on the Product Ratio of **2a** to **4a** in the Reaction of **1a** with Hydrazine Monohydrate

Methanol [a] (ml)	Products (%) **2a**	**4a**	Ratio (**2a**/**4a**)
0.5	31	33	0.94
1.0	47	23	2.04
2.0	73	11	6.63

[a] Without methanol, precipitation of **2a** occurred and therefore quantification was not carried out.

Scheme 2. Proposed mechanism for formation of **4** by hydroxide ion.



The effect of water in the reaction of **1a** with hydrazine monohydrate in methanol on formation of **4a** was examined (Scheme 2) because we thought that water in the reaction mixture may play a role in the formation of **4a** through 1,2-bond cleavage of **6**, followed by formyl transfer and reclosure, *via* the same mechanism reported for formation of **3a-c** from **1a-c** in aqueous alkaline solution [2,9]. Therefore, water was added to the reaction mixture and the products formed were analyzed by hplc. However, no remarkable increase in the formation of **4** was observed, as shown in Table 4. These results indicate that the process of 1,2-bond cleavage by hydroxide ion and subsequent formyl transfer may not be involved in the formation of **4** (Scheme 2).

1,9-Dimethyladenine hydroiodide (**7**), a methyl analogue of **1a**, was treated with hydrazine monohydrate in methanol and the products formed were analyzed by hplc.

Table 4

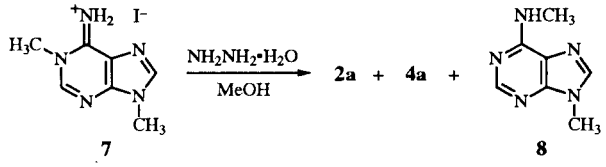
Effect of Water on the Product Ratio of **2a** to **4a** in the Reaction of **1a** with Hydrazine Monohydrate [a]

Water [b] (molar equivalent for reagent)	Products (%) **2a**	**4a**	Ratio (**2a**/**4a**)
0	73	13	5.62
1	58	11	5.27
2	56	9	6.22
4	59	7	8.43

[a] Under conditions of 2 ml of methanol. [b] Water added exogenously.

We expected the formation of two products in this reaction, *i.e.*, a Dimroth-type rearranged product, N^6,9-dimethyladenine (**8**) [13], and a compound corresponding to compound **4**, 5-amino-1-methyl-4-(4-methyl-1,2,4-triazol-3-yl)imidazole (**9**) (Chart 2). However, the reaction unexpectedly yielded **2a** (43%) and **4a** (30%) as the main products instead of compound **8** (16%) which was present as only a minor product (Scheme 3). Since no other peaks with a uv absorption pattern similar to that of compound **4** were observed in the region of the more slowly eluted

Scheme 3. Reaction of 1,9-dimethyladenine with hydrazine monohydrate in methanol.



products, we concluded that compound **9** may not have been produced. The possibility of formation of **2a** *via* the reaction of product **8** with hydrazine monohydrate under these conditions was discounted by the experimental evidence. The fact that there was a smaller yield of **8** (16%) than of **2a** (43%) in the reaction suggested that the methylamino group was quite easily replaced by the hydrazino group in certain reaction steps. Formation of **2a** and **4a** from **7** indicated that the *N*-amino group on the triazole ring of **4** had not necessarily originated from the *N*-amino group of **1** but may have come from hydrazine. The effect of the amount of methanol in the reaction mixture on formation of **2a**, **4a** and **8** was also examined and it was found that the product ratio of **2a** to **4a** increased with increasing amounts of methanol (Table 5), similar to the result obtained for 1-amino-9-methyladenine (**1a**) as already described (Table 3).

Table 5

Effect of Methanol on the Product Ratio of **2a** to **4a** in the Reaction of **7** with Hydrazine Monohydrate

Methanol (ml)	Products (%) **2a**	**4a**	**8**	Ratio (**2a**/**4a**)
0.5	29	41	13	0.71
1.0	43	30	16	1.43
2.0	49	14	17	3.50

Heating 9-substituted 1-aminoadenines (**1a-c**) in aqueous alkaline solution (*p*H 9 to 11) afforded only the non-Dimroth-type rearranged product, 5-amino-1-methyl-4-(1,2,4-triazol-3-yl)imidazoles **3a-c** [2,9] (Chart 1). However, as we reported quite recently, heating 1-aminoadenine (**1e**) under the same alkaline conditions gave both

6-hydrazinopurine (**2e**) and 5-amino-4-(1,2,4-triazol-3-yl)imidazole (**3e**) in a 1:1 ratio, but at *p*H 12, the reaction gave only 6-hydrazinopurine (**2e**) [14] (Chart 1). The reaction of **1e** with hydrazine was then examined. Reaction of **1e** with hydrazine monohydrate (40 molar equivalents) at 50° for 2 days without solvent yielded 5-amino-4-(4-amino-1,2,4-triazol-3-yl)imidazole (**4e**) as a major product in a 44% yield. This result was different from that obtained in the reaction of **1e** in aqueous alkaline solution. The reaction using 1-methyladenine with hydrazine monohydrate (40 molar equivalents) also afforded **4e** in a 44% yield. When analysis was carried out, including the effect of methanol in the reaction of **1e** with hydrazine monohydrate as determined by hplc, the reaction with 0.5 ml methanol afforded **4e** as the main product with a smaller yield of **2e** (Table 6). Other products formed remained unidentified including decomposed products. An increase in the amount of methanol to 2 ml resulted in a steep decrease in the yield of **4e**.

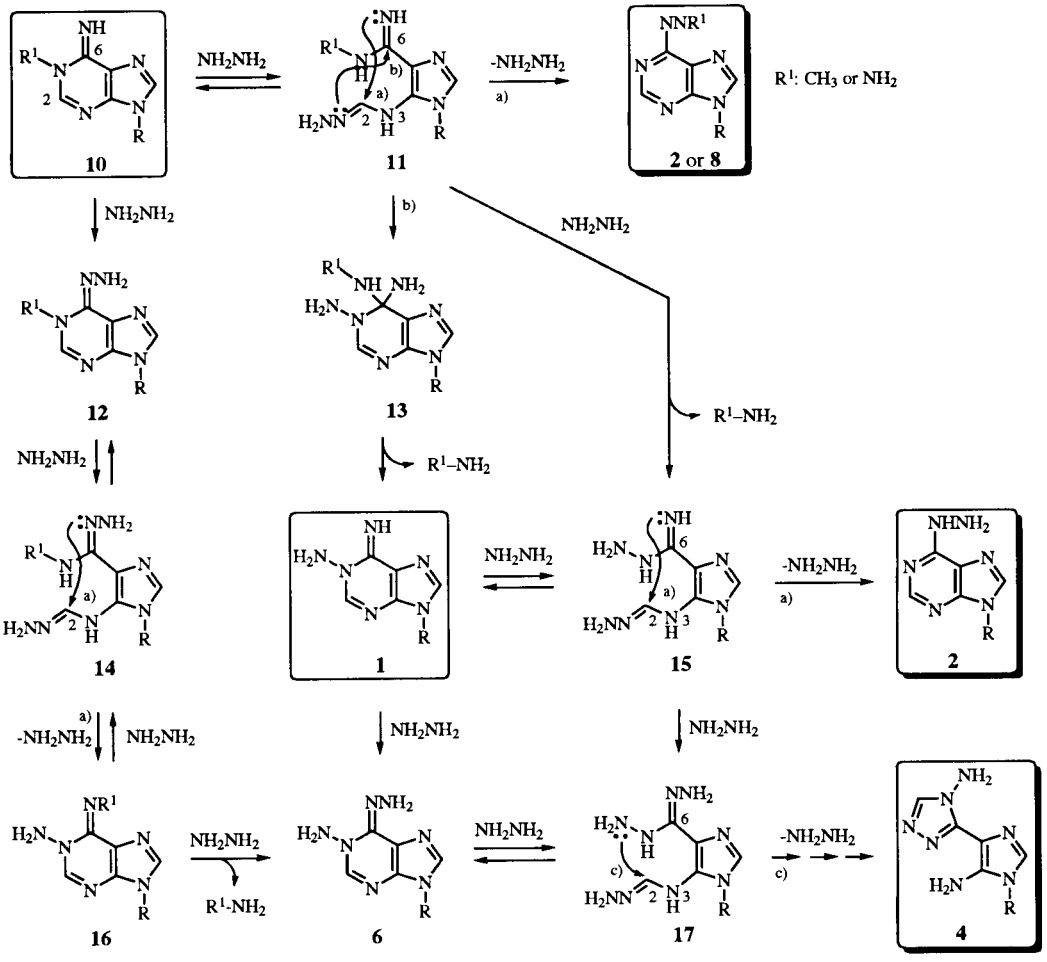
Table 6

Effect of Methanol on the Product Ratio of **2e** to **4e** in the Reaction of **1e** with Hydrazine Monohydrate

Methanol (ml)	Products (%) 2e	4e	Ratio (2e/4e)
0.5	2	34	0.06
1.0	4	34	0.12
2.0	5	10	0.50

The mechanism for formation of **2** and **4** by the reaction of 1,9-disubstituted adenines **10** with hydrazine monohydrate was assumed to be as shown in Scheme 4. There are two possible reaction sites in compound **10** for the initial attack of hydrazine, *i.e.*, attack may occur i) at the C2 carbon of **10** to form the 1,2-bond cleaved compound **11**, or ii) at the C6 carbon of **10**, followed by the release of ammonia to form **12**. Dimroth-type rearrangement of **11** (route a) gives N^6,9-disubstituted adenine **2** or **8**. Replacement of the R^1-NH group of **11** by the hydrazino group forms **15** and subsequent reclosure (route a) gives **2**. Replacement of the imino group of **15** at the C6 position [15] forms **17**, a key intermediate, whose amino moiety of the hydrazino group

Scheme 4. Possible mechanism for formation of compounds **2**, **4** and **8**.



at the C6 position targets the C2 carbon (route c), followed by 2,3-bond cleavage and several unidentified processes, including reclosure, to give 1-substituted 5-amino-4-(4-amino-1,2,4-triazol-3yl)imidazole **4**. Alternatively, compound **15** can be formed *via* **13**, and compound **17** can be formed *via* **12** and **13**. By means of similar reactions, as already demonstrated in Scheme 4, compound **11** can form **14**, compound **14** can form **6**, **16** and **17**, compound **15** can form **6**, and compound **13** can form **16** *via* a direct pathway (the arrows seen in Scheme 4 do not reflect these changes). The effect of methanol on the preferential formation of **2** *versus* **4** may be attributed to the ease of formation of **2** from **15** (or **2** from **11**) compared to the formation of **4** from **17** by the solvent effect. Further study is required to clarify this point. With respect to the lower yield of **8** compared to **2a** in the reaction of **7** with hydrazine monohydrate in methanol (Scheme 3, Table 5), it was considered that replacement of the methylamino group by the hydrazino group in hydrazine proceeded efficiently (Scheme 4). With respect to the lack of formation of compounds **3** and **9**, it was speculated that the reaction intermediates **14** and **15** cannot participate in the types of reactions shown for compound **17** (route c) (Scheme 4), and only attack of the sp^2 nitrogens of **14** and **15** at the C2 position could proceed. On the other hand, in compound **17**, reclosure by the nucleophilic sp^2 nitrogen produces hydrazine reactive compound **6** which again forms **17** (Scheme 4). Although the reactivity of the amino moiety of the hydrazino group at the C6 position of **17** is lower than the sp^2 nitrogen, the attack of the amino nitrogen at the C2 carbon (route c) can result in the formation of stable compound **4** after several steps, therefore, compound **4** may be the only product obtained. Further study is required to elucidate the mechanism of formation of **4** as well as the effect of methanol on the preferential formation of **2**.

EXPERIMENTAL

The ^1H nmr spectra were recorded on a JEOL EX 270 or GSX 400 spectrometer and chemical shifts were expressed in parts per million downward from internal tetramethylsilane. The mass spectra were obtained with a JEOL JMS-AX 505 HA spectrometer. The uv spectra were recorded on a Shimadzu UV-2100 spectrophotometer. Melting points were measured with a Yanagimoto micro-melting point apparatus and are uncorrected. The hplc analyses were carried out with a Shimadzu LC-1OA hplc system equipped with a photodiode array uv detector.

Reaction of 1-Amino-9-methyladenine (**1a**) with Hydrazine Monohydrate. Formation of 5-Amino-1-methyl-4-(4-amino-1,2,4-triazol-3-yl)imidazole (**4a**).

A mixture of 1-amino-9-methyladenine hydrochloride [2] (**1a**, 100 mg, 0.5 mmole) and hydrazine monohydrate (1.00 g, 20 mmoles) was heated at 50° for 1 day. The precipitates formed were filtered and washed with a small amount of ether and ethanol to give 11 mg (13% yield) of 6-hydrazino-9-methylpurine (**2a**) [16]. The main product, 5-amino-1-methyl-4-(4-amino-1,2,4triazol-3-yl)imidazole (**4a**), was isolated from the filtrate by means of alumina (chloroform/methanol = 19/1) column chromatography and Sephadex LH20 (methanol) column chromatography and appeared as a light brown paste in a 35 mg (39%) yield. Attempts to crystallize **4a** failed and only a precipitate was obtained; ^1H nmr (dimethyl sulfoxide-d_6): δ 8.25 (s, 1H, triazole CH), 7.31 (s, 1H, imidazole CH), 6.47 (s, 2H, *N*-NH_2), 5.63 (s, 2H, 5-NH_2), 3.49 (s, 3H, CH_3); uv: (*p*H 1): λ max 255 nm (sh) and 263 nm; (*p*H 7 and *p*H 12): λ max 265 nm; ms: m/z 179 (M^+), 163 (M^+-NH_2).

Acetylation of **4a**. Synthesis of 5-Amino-l-methyl-4-(4-acetylamino-1,2,4-triazol-3-yl)imidazole (**5a**).

A mixture was made of **4a** (39 mg, 0.2 mmole), pyridine (3 ml) and acetic anhydride (0.32 ml, 3.4 mmoles) which was stirred at room temperature for 1.5 hours. It was then poured into 10 ml of water in an ice bath and stirred at room temperature for 30 minutes. After the mixture was washed with chloroform (20 ml x 2, 10 ml x 1), the aqueous layer was evaporated *in vacuo*. Recrystallization of the product from methanol gave red needles of 5-amino-1-methyl-4-(4-acetylamino-1,2,4-triazol-3-yl)imidazole (**5a**) in a 14 mg (32%) yield. An analytically pure sample could not be obtained even after several recrystallizations, mp 222-225°; ^1H nmr (dimethyl sulfoxide-d_6): δ 11.3 (br s, 1H, NH), 8.35 (s, 1H, triazole CH), 7.20 (s, 1H, imidazole CH), 5.76 (s, 2H, 5-NH_2), 3.46 (s, 3H, *N*-CH_3), 1.99 (s, 3H, CH_3CO); uv: (*p*H 1): λ max 255 (sh) and 270 nm; (*p*H 7): λ max 272 nm; (*p*H 12): λ max 258 nm; hrms: m/z M^+ Calcd. for $C_8H_{11}N_7O$: 221.1024; Found: 221.1025.

Reaction of 1-Amino-9-benzyladenine (**1d**) with Hydrazine Monohydrate.

Synthesis of 1-Amino-9-benzyladenine (**1d**).

A mixture of 9-benzyladenine [12] (225 mg, 1.0 mmole), *N,N*-dimethylformamide (6 ml), ethanol (2 ml) and 2,4-dinitrophenoxyamine (399 mg, 2.0 mmoles) was allowed to react at 40° for 2 days. The reaction mixture was evaporated to one third the original volume and 2 ml of 1*N* hydrochloric acid were then added. The mixture was evaporated *in vacuo* and the residue was suspended in a small amount of ether. The insoluble solid was filtered and washed with ether to give the product **1d**•hydrochloride in a 275 mg (99%) yield. Recrystallization from water-ethanol gave light brown needles, mp 284-290° (lit [10] free form, 170-173°); ^1H nmr (dimethyl sulfoxide-d_6): δ 10.05 and 9.22 (each, br s, 1H, 6-NH_2), 8.67 (s, 1H, 2-H), 8.65 (s, 1H, 8-H), 7.35 (m, 5H, phenyl), 6.67 (s, 2H, *N*-NH_2), 5.49 (s, 2H, CH_2); uv: (*p*H 1 and *p*H 7): λ max 257 nm (ε 12,900); (*p*H 12): λ max 258 nm (ε 13,900), 265 nm (sh) and 290 nm (sh); ms: m/z 240 (M^+), 224 (M^+-NH_2).

Anal. Calcd. for $C_{12}H_{13}ClN_6$: C, 52.08; H, 4.74; N, 30.37. Found: C, 51.84; H, 4.87; N, 30.19.

Reaction of **1d** with Hydrazine Monohydrate. Formation of 5-Amino-1-benzyl-4-(4-amino-1,2,4-triazol-3-yl)imidazole (**4d**).

A mixture of **1d**•hydrochloride (55 mg, 0.2 mmole) and hydrazine monohydrate (0.50 ml, 10 mmoles) was allowed to react at 50° for 3 days. The precipitate which appeared was

filtered and washed with a small amount of ethanol to give 17 mg (35% yield) of 9-benzyl-6-hydrazinopurine (**2d**) [17]. The filtrate was evaporated *in vacuo* to about 0.5 ml and left standing for 3 days at room temperature. The precipitates which appeared were filtered and washed with a small amount of ethanol to give 17 mg (33% yield) of 5-amino-1-benzyl-4-(4-amino-1,2,4-triazol-3-yl)imidazole (**4d**). Recrystallization from ethyl acetate gave brown plates, mp 177-179°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.25 (s, 1H, triazole CH), 7.43 (s, 1H, imidazole CH), 7.40-7.23 (m, 5H, phenyl), 6.45 (s, 2H, *N*-NH_2), 5.72 (s, 2H, 5-NH_2), 5.17 (s, 2H, CH_2); uv: (*p*H 1): λ max 259 nm (sh) and 274 nm (ε 8,500); (*p*H 7): λ max 264 nm (ε 7,100); (*p*H 12): decomposed; ms: m/z 255 (M^+).

Anal. Calcd. for $C_{12}H_{13}N_7$: C, 56.46; H, 5.13; N, 38.41. Found: C, 56.66; H, 5.18; N, 38.26.

Crystal Data for 5-Amino-1-methyl-4-(4-acetylamino-1,2,4-triazol-3-yl)imidazole (**5a**).

Formula, $C_8H_{11}N_7O$; M = 221.23; crystal size, 0.4 x 0.3 x 0.2 mm; crystal system, monoclinic; space group, *C2/c*; cell parameters, a = 17.479(3), b = 9.953(2), c = 14.258(2) Å;β = 119.95(1)°; V = 2149.1(7) Å^3; Z = 8; μ (Cu*K*α) = 0.80 mm^{-1}; Dc = 1.367 Mg m^{-3}; final R = 0.048 for 1300 reflections with $|Fo| > 2\sigma(Fo)$.

Reaction of 1-Aminoadenine (**1e**) or 1-Methyladenine with Hydrazine Monohydrate. Formation of 4-Amino-5-(4-amino-1,2,4-triazol-3-yl)imidazole (**4e**).

A mixture of 1-aminoadenine•hydrochloride (**1e**, 93 mg, 0.5 mmole) or 1-methyladenine•HI (139 mg, 0.5 mmole) and hydrazine monohydrate (1.00 g, 20 mmoles) was heated at 50° for 2 days. After the mixture was left standing in a freezer for 12 hours, the white needles which appeared were filtered and washed with ethanol to give 4-amino-5-(4-amino-1,2,4-triazol-3-yl)imidazole (**4e**) in a 36 mg (44%) yield from **1e** and in a 39 mg (44%) yield from 1-methyladenine. The product was recrystallized from water, mp 248-250° dec; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 10.11 (br s, 1H, NH), 8.24 (s, 1H, triazole CH), 7.28 (s, 1H, imidazole CH), 6.42 (br s, 1H, *N*-NH_2), 5.37 (br s, 1H, C-NH_2); uv: (*p*H 1): λ max 273 nm (ε 12,200) and 250 nm (sh); (*p*H 7): λ max 270 nm (ε 12,400); (*p*H 12): 272 nm (ε 12,200); ms: m/z 165 (M^+).

Anal. Calcd. for $C_5H_7N_7$: C, 36.36; H, 4.27; N, 59.37. Found: C, 36.46; H, 4.46; N, 59.21.

Product Analysis by HPLC were:

The HPLC Conditions.

The ODS column (Merck LiChrospher 100 RP-18 (e), 250 x 4 mm) used was eluted with methanol in 1/15 *M* phosphate buffer (*p*H 6.8) at a flow rate of 1 ml/minute under the following conditions: for the reaction products from **1a** these were 0-5 minutes with 1% methanol in buffer and 5-15 minutes with a linear gradient of 1-30% methanol in buffer; for products from **7** these were 0-5 minutes with 5% methanol in buffer and 5-15 minutes with a linear gradient of 5-40% methanol in buffer; and for products from **1e** conditions were 0-5 minutes with 5% methanol in buffer and 5-15 minutes with a linear gradient of 5-30% methanol in buffer. Quantification of the products was carried out using a calibration curve for each authentic sample.

Effect of Methanol.

A mixture of **1a** (20 mg, 0.1 mmole), methanol (0.5, 1.0 or 2.0 ml) and hydrazine monohydrate (0.1 ml, 2 mmoles) was stirred in a tightly capped test tube at room temperature for 36 hours. Aliquots (0.1 ml) of the reaction mixture were diluted with a solution of 1*N* hydrochloric acid and 50 m*M* potassium dihydrogenphosphate-sodium hydroxide buffer (*p*H 6.8) as follows: for the experiment with 0.5 ml of methanol, 0.4 ml of hydrochloric acid plus 0.5 ml of buffer were used; for 1.0 ml of methanol, 0.2 ml of hydrochloric acid plus 0.7 ml of buffer were used; and for 2.0 ml of methanol, 0.1 ml of hydrochloric acid plus 0.8 ml of buffer were used. The sample solution thus obtained was subjected to hplc analysis.

Effect of Water.

A mixture of **1a** (20 mg, 0.1 mmole), methanol (2.0 ml), hydrazine monohydrate (0.1 ml, 2 mmoles) and water (0, 2, 4 or 8 mmoles) was stirred in a tightly capped test tube at room temperature for 36 hours. Aliquots (0.1 ml) of the reaction mixture were diluted with a solution of 1*N* hydrochloric acid (0.1 ml) and 50 m*M* potassium dihydrogenphosphate-sodium hydroxide buffer (*p*H 6.8) (0.8 ml), and each sample was subjected to hplc analysis.

Reaction of 1,9-Dimethyladenine (**7**) with Hydrazine Monohydrate in Methanol.

A mixture of 1,9-dimethyladenine hydroiodide (**7**, 29 mg, 0.1 mmole), methanol (1.0 ml) and hydrazine monohydrate (0.1 ml, 2 mmoles) was stirred in a tightly capped test tube at room temperature for 36 hours. Aliquots (0.1 ml) of the reaction mixture were diluted with a solution of 1*N* hydrochloric acid (0.2 ml) and 50 m*M* potassium dihydrogenphosphate-sodium hydroxide buffer (*p*H 6.8) (0.7 ml) and each sample was subjected to hplc analysis. The effect of methanol (0.5, 1.0 or 2.0 ml) was examined by the same procedure used for **1a**.

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