3-Methylisoguanosine: Synthesis and Acidic Hydrolysis of the Glycosyl Bond

Taisuke ITAYA* and Tsunehiro HARADA

Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan. *Received April 23, 1990*

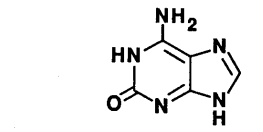
Dehydration of 5-(cyanomethylamino)-1-methyl-1*H*-imidazole-4-carboxamide (14a) with a combination of phosphorus oxychloride and triethylamine afforded the nitrile 17a. This compound underwent selective hydration at the cyanamide moiety to furnish the urea 18a followed by cyclization to 3,9-dimethylisoguaninae (19a) under alkaline conditions. Similar dehydration of the nucleoside analog 14b followed by treatment with 0.1 N aqueous sodium hydroxide led to the first access to 3-methylisoguanosine (19c).

Although 3-methylisoguanosine (19c) proved to undergo hydrolysis at the *N*-glycosidic bond most slowly among the known 3-methyl-9-β-D-ribofuranosylpurines in 0.1 N hydrochloric acid at 25 °C, the rate was 650 times faster than that for the unmethylated isoguanosine (3).

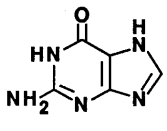
Keywords 3-methylisoguanosine; 3,9-dimethylisoguanine; 4,5-disubstituted imidazole; amide dehydration; cyanamide hydration; nitrile urea cyclization; ring–chain tautomer; nucleoside hydrolysis; kinetic study

Isoguanine (**1**) has attracted less attention than its positional isomer guanine (**2**), because it has been isolated only from butterfly wings.[1] The 9-β-D-ribofuranoside of **1**, isoguanosine (crotonoside) (**3**), had long been known in nature only as a constituent of the croton bean, *Croton tiglium* L.[2] until its recent isolation from an animal, the marine nudibranch mollusk *Diaulua sandiegensis*.[3] Isoguanosine (**3**) was first synthesized in 1951 and since then several syntheses of **3**[4] or its 2′,3′-*O*-isopropylidene derivative [4c,5] have been reported. Although various modified nucleosides have been found in nucleic acids,[6] neither isoguanosine itself nor its methylated derivatives have been isolated from nucleic acids. The first isoguanosine methylated at the base moiety was spongosine (**4**), isolated from the Caribbean sponge *Cryptotethia crypta* in 1956,[7] and its O^2-methylisoguanosine structure was confirmed by chemical synthesis.[8] Later on, N^6-methylisoguanosine (**5**),[4c,9] some N^6-alkyl homologues of **5**,[4c,f,9] and N^6,N^6-dimethylisoguanosine (**6**)[4c] were synthesized. In 1980, 1-methylisoguanosine (**7**), possessing potent muscle-relaxant activity, as well as exhibiting cardiovascular and antiinflammatory properties, was isolated from the marine sponge *Tedania digitata*[10] and from the marine nudibranch *Anisodoris nobilis*.[11] In view of the interest in this compound, **7** itself[10,11b,12] and a variety of its analogs[13,14] have been synthesized so that the structure–activity relationship could be explored. Because spongosine (**4**), a positional isomer of **7**, was also shown to have similar pharmacological properties to those of **7**,[13] the biological activities of the other isomers, 3- and 7-methylisoguanosines (**19c** and **8**), are of considerable interest. Nevertheless, neither **19c** nor **8** was known at the time when the present study was undertaken, though the syntheses of the corresponding *N*-methyl derivatives of other typical purine nucleosides, *e.g.*, adenosine,[15,16] guanosine,[17,18] inosine,[18b,19] and xanthosine,[18b,20] had already been reported. In connection with our continuing efforts to synthesize the tricyclic nucleosides **9**,[17c,d,21,22] the putative structures for the minor nucleosides from transfer ribonucleic acids, as well as related compounds,[17b–d,19,20] we investigated the synthesis and chemical properties of 3-methylisoguanosine (**19c**). Preliminary results from this work have already been published.[23]

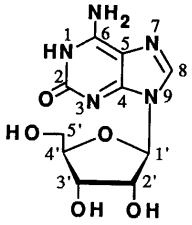
The most direct approach to 3-methylisoguanosine (**19c**) would be methylation of isoguanosine (**3**). Treatment of the silver salt of **3** with methyl iodide, however, was reported to provide mainly the starting material **3** along with three minor products, one of which was O^2-methylisoguanosine



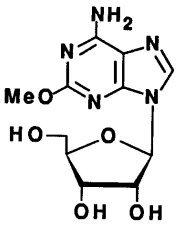
1



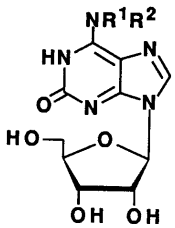
2



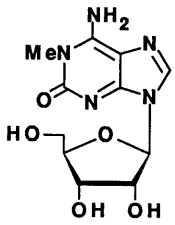
3



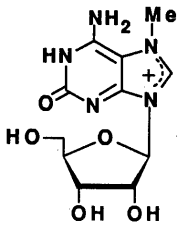
4



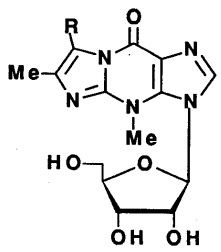
5: R^1 = H; R^2 = Me
6: R^1 = R^2 = Me



7



8



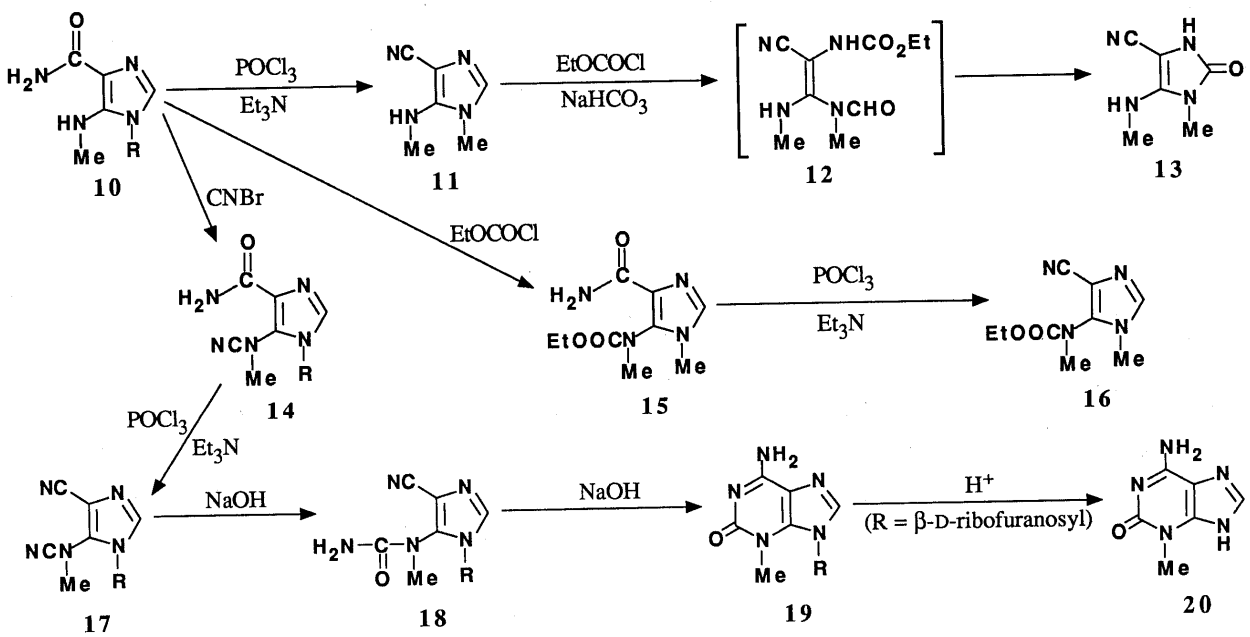
a: R = H
b: R = Me
c: R = CH_2CH_2–C(H, CO_2Me, $NHCO_2Me$)

9

(**4**).[8] Reaction of **3** with methyl iodide in the presence of potassium carbonate was reported to afford **4** and 1-methylisoguanosine (**7**) in 52% and 49% yields, respectively.[10b] Formation of **7** was also recognized in the methylation in the absence of base.[11b] These results were discouraging and this approach seemed unsuitable for the synthesis of 3-methylisoguanosine (**19c**). Synthesis of 3,9-dimethylisoguanine (**19a**), a prototype of **19c**, was first reported by Okano *et al.*, by treatment of isoguanine (**1**) with dimethyl sulfate in *N*,*N*-dimethylacetamide.[24] Kazimierczuk and Shugar, however, claimed that this procedure gave a mixture of products too complex to allow identification of the presence of **19a** and that the ultraviolet (UV) spectra reported for the presumed **19a**[24] were different from those of their own sample of **19a** prepared by ammonolysis of 3,9-dimethyl-6-methylthio-2-oxopurine.[25] Compound **19a** was alternatively obtained from 1-methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide (**10a**) through methyl *N'*-benzoyl-*N*-(4-cyano-1-methyl-1*H*-imidazol-5-yl)-*N*-methylcarbamidothioate.[26] We felt that the reaction conditions (heating with methanolic or aqueous ammonia) employed in the last step of each synthesis[25,26] would be too drastic for the synthesis of **19c**, which we assumed to have a labile *N*-glycosidic bond by analogy with known 3-methyl-9-β-D-ribofuranosylpurines.[15,17b-d,19,20] We therefore planned to develop a new synthesis of **19a** as a model experiment. The intermediate for the synthesis of **19a** that we envisioned was the nitrile-urea **18a**, a monocyclic tautomer of **19a**. This compound **18a** would be accessible starting from **10a**,[27] which had been utilized as a common precursor in syntheses of 3,9-dimethyl derivatives of guanine,[17b,26,28] hypoxanthine[29] and xanthine.[20,28]

We first attempted to prepare **18a** through the nitrile-carbamate **16**. Treatment of the nitrile-amine **11**, available by dehydration of the amide-amine **10a** with phosphorus oxychloride,[26,27b] with ethyl chloroformate in water in the presence of sodium bicarbonate, however, gave the imidazolone **13**. This compound was supposed to be formed through **12**, the Bamberger fission[30] product, followed by recyclization.[31]

The objective nitrile-carbamate **16** was obtained by dehydration of the amide-carbamate **15**, which in turn had been produced by ethoxycarbonylation of the amide-amine **10a**.[20,28] Unfortunately, several attempts to convert **16** into either **18a** or **19a** failed. The nitrile-urea **18a** could be prepared from the nitrile-cyanamide **17a**. We first attempted to prepare this compound **17a** by cyanation of the nitrile-amine **11** with cyanogen bromide in acetate buffer according to the method used in the synthesis of the amide-cyanamides **14**.[17b,28] However, **11** was inert under these conditions. The nitrile-cyanamide **17a** was obtained by treatment of **14a** with phosphorus oxychloride in chloroform in the presence of triethylamine in 78% yield. Brief treatment of **17a** with 0.1 N aqueous sodium hydroxide at room temperature afforded a mixture of the nitrile-urea **18a** and 3,9-dimethylisoguanine (**19a**). When the hydration was performed in 0.5 M carbonate buffer of pH 9.5, **18a** was obtained as a sole product. We followed the changes of concentrations of the components **17a**, **18a**, and **19a** through the transformation of **17a** into **19a** at pH 10.96 and 25 °C, and found that **17a** underwent rapid hydration [pseudo-first-order rate constant (k_{obsd}) $2.5 \times 10^{-2}\,min^{-1}$] to **18a** followed by slow cyclization (k_{obsd} $5.5 \times 10^{-4}\,min^{-1}$) to **19a**. Figure 1 shows that the concentrations of the three components, calculated for the consecutive reactions **17a**→**18a**→**19a** using these values for the rate constants, match well the observed concentrations. This means that **18a** is indeed the intermediate in the transformation of **17a** into **19a**. For the conversion of **17a** into **18a**, we could



a: R = Me
b: R = 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl
c: R = β-D-ribofuranosyl

Chart 1

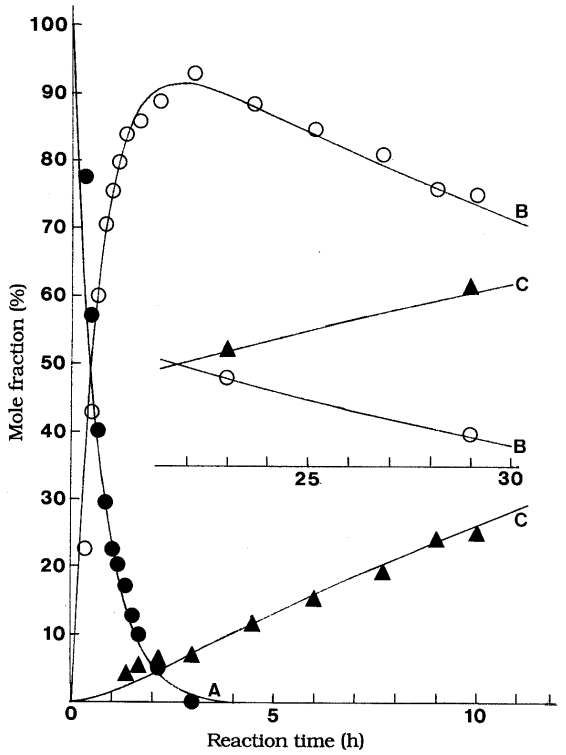


Fig. 1. Variation of the Concentrations of **17a** (●), **18a** (○), and **19a** (▲) in the Reactions of **17a**→**18a**→**19a** at pH 10.96 and 25°C

Solid lines A, B, and C represent mole fractions (%) for the three components calculated for the consecutive reactions using the rate constants 2.5×10^{-2} and $5.5 \times 10^{-4}\,min^{-1}$ for each step of the reactions.

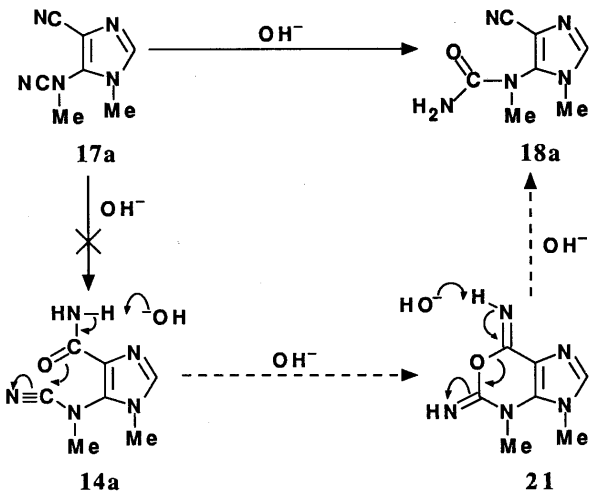
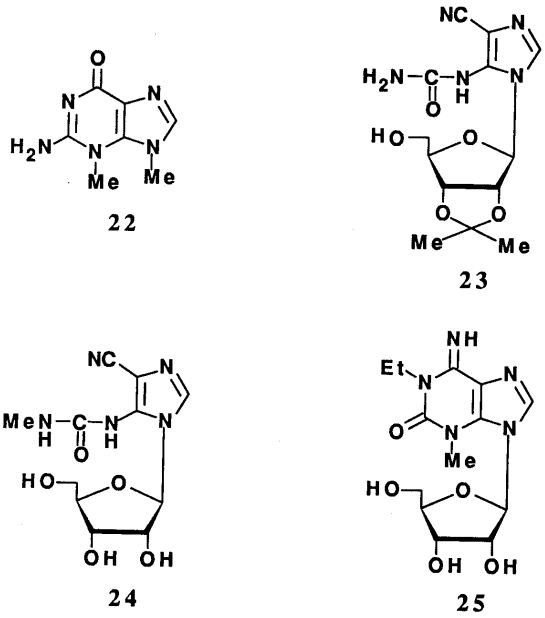


Chart 2

envision two routes as depicted in Chart 2. Selective base-catalyzed hydration of **17a** at the cyanamide moiety would lead to **18a**. Alternatively, hydration of the cyano group at the 4-position of **17a** would give **14a**, which might cyclize to **21**. Base-catalyzed ring opening of **21** would then afford **18a**. The formation of the nitrile-ureas **23** and **24** through such an oxazine ring system as **21** had been postulated.[5b,c,12] In our case, however, **14a** was known to afford 3,9-dimethylguanine (**22**) under similar conditions.[28] This fact rules out the latter mechanism for the formation of **18a**. The structure of **19a** thus obtained was supported by consistent analysis values and a reasonable proton nuclear



magnetic resonance (^{1}H-NMR) spectrum. Furthermore, the UV spectra matched well those reported by Kazimierczuk and Shugar.[25]

Having established a novel synthesis of 3,9-dimethylisoguanine (**19a**), we next started with the protected nucleoside **14b**[17b,d] to construct 3-methylisoguanosine (**19c**) according to this method. Dehydration of **14b** with phosphorus oxychloride in the presence of triethylamine afforded **17b** as a glass in 88% yield. Compound **17b** was then treated with 0.1 N aqueous sodium hydroxide to afford 3-methylisoguanosine (**19c**) as the monohydrate in 41% yield based on **14b**, mp 175—178 °C (dec.). The correctness of the structure **19c** was supported by the UV spectral similarity to **19a** and by transformation into 3-methylisoguanine hydrochloride (**20**·HCl) by treatment with 0.1 N hydrochloric acid. As had been observed with other 3-methyl-9-β-D-ribofuranosylpurines,[15,17c,d,19,20] 3-methylisoguanosine (**19c**) thus obtained also proved to be highly sensitive to acidic hydrolysis at the glycosidic bond [k_{obsd} $1.7 \times 10^{-2}\,min^{-1}$ (half life 41 min) and $4.3 \times 10^{-2}\,min^{-1}$ (ionic strength 1.0) in 0.1 N hydrochloric acid at 25 °C]. The rate studies for the hydrolysis of these nucleosides have revealed that the glycosidic bonds undergo cleavage at rates increasing in the order of **19c**, 3-methyladenosine,[15] 3-methylxanthosine,[20] 3-methylinosine,[19] and 3-methylguanosine[17c,d] in 0.1 N hydrochloric acid at 25 °C. In order to estimate the effect of introduction of the methyl group at the 3-position of isoguanosine (**3**) on the hydrolysis rate, we next investigated the hydrolysis of **3**[4h] itself. Because **3** underwent hydrolysis at the glycosyl bond in 0.1 N hydrochloric acid at 25 °C too slowly to permit convenient analysis, the rate constant (k_{obsd} $7.5 \times 10^{-4}\,min^{-1}$) was determined in 1 N hydrochloric acid at 25 °C. Under these conditions, hydrolysis of **19c** proceeded 650 times faster (k_{obsd} $4.9 \times 10^{-1}\,min^{-1}$) than that of **3**. These results provide an additional example of the marked enhancing effect of introduction of a methyl group at the 3-position of 9-β-D-ribofuranosylpurines on the rates of hydrolysis of the glycosyl bonds, although the extent observed in **3** was somewhat less than those in other purine nucleo-

sides.[15,17c,d,19,20] Interestingly, Kim *et al.* recently reported that the rate-promoting effect of the methyl group at the 3-position was diminished to a large extent by further substitution at the 1-position: 1-ethyl-3-methylisoguanosine (**25**) underwent glycosidic hydrolysis *ca.* 100 times more slowly than **19c** in 0.1 N hydrochloric acid at 25 °C.[14a]

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. Spectra reported herein were recorded in a JASCO IRA-2 or A-202 infrared (IR) spectrophotometer, a Hitachi 320 or 323 UV spectrophotometer using solutions in 95% aqueous ethanol, 0.1 N hydrochloric acid (pH 1), 0.01 N hydrochloric acid (pH 2), 0.005 M phosphate buffer (pH 7), and 0.1 N aqueous sodium hydroxide (pH 13), or a JEOL JNM-FX-100 NMR spectrometer at 25 °C with tetramethylsilane as an internal standard. Optical rotations were measured with a JASCO DIP-181 polarimeter using a 1-dm sample tube. The liquid chromatographic system was a Waters Model 204 ALC which included a 6000A pump, a U6K injector, and a Model 440 absorbance detector. pH's were measured with a Toa HM-18ET pH meter. 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, q = quartet, s = singlet, sh = shoulder, t = triplet.

2,3-Dihydro-1-methyl-5-(methylamino)-2-oxo-1*H*-imidazole-4-carbonitrile (13) A mixture of **11a**[26,27b] (408 mg, 3 mmol), sodium bicarbonate (1.5 g), and ethyl chloroformate (1.5 ml) in water (150 ml) was stirred at room temperature for 2.5 h and the resulting solution was concentrated *in vacuo* to *ca.* 30 ml. The precipitate that separated was collected by filtration to afford **13** as a colorless solid (226 mg), mp 234—239 °C (dec.). The filtrate was concentrated *in vacuo* and the residue was washed successively with chloroform (7 ml) and a little water. The insoluble solid was recrystallized from water to afford a second crop of **13** (29 mg, the total yield was 56%). Recrystallization from ethanol gave an analytical sample of **13** as colorless pillars, mp 240—241 °C (dec.); UV λ_{max} (95% EtOH) nm (ε): 221 (3500), 271 (12000); λ_{max} (H_2O, pH 1) nm (ε): 225 and 270 (unstable); λ_{max} (H_2O, pH 7) nm (ε): 225 (4100), 270 (12200); λ_{max} (H_2O, pH 13) nm (ε): 276 (unstable); IR γ_{max}^{Nujol} cm^{-1}: 2190 (C≡N), 1750 (C=O); ^{1}H-NMR (Me_2SO-d_6) δ: 2.87 (3H, d, J = 3.5 Hz, N^5-Me), 2.97 [3H, s, N(1)-Me], 6.60 (1H, d, J = 3.5 Hz, N^5-H), 9.86 [1H, br, N(3)-H]; MS m/z: 152 (M^+). *Anal.* Calcd for $C_6H_8N_4O$: C, 47.36; H, 5.30; N, 36.82. Found: C, 47.41; H, 5.30; N, 36.77.

5-[(Ethoxycarbonyl)methylamino]-1-methyl-1*H*-imidazole-4-carbonitrile (16) Triethylamine (1.5 ml) and phosphorus oxychloride (0.61 g, 4 mmol) were added to a solution of **15**[20,28] (339 mg, 1.5 mmol) in chloroform (25 ml) in this order and the resulting mixture was stirred at room temperature for 1 h and then concentrated *in vacuo*. The residue was neutralized with 10% aqueous sodium hydroxide and extracted with chloroform (4 × 10 ml). The organic layers were combined, dried over magnesium sulfate, and concentrated *in vacuo* to leave an oil. This was purified on a silica gel column [chloroform–methanol (10 : 1, v/v)] to afford **16** (230 mg, 74%) as a colorless oil, IR γ_{max}^{film} cm^{-1}: 2218 (C≡N), 1732 (C=O); ^{1}H-NMR (Me_2SO-d_6) δ: 1.17 (3H, br t,[32] J = 7 Hz, $CH_2\underline{Me}$), 3.20 (3H, s, N^5-Me), 3.51 [3H, s, N(1)-Me], 4.14 (2H, br q,[32] J = 7 Hz, CH_2), 7.84 [1H, s, C(2)-H].

(4-Cyano-1-methyl-1*H*-imidazol-5-yl)methylcyanamide (17a) Triethylamine (6 ml) and phosphorus oxychloride (1.1 ml, 12 mmol) were added to a suspension of **14a**[17b,28] (358 mg, 2 mmol) in chloroform (13 ml) in that order under cooling with ice and the mixture was stirred at room temperature for 1.5 h to give a clear solution. This was mixed with a suspension of sodium bicarbonate (3 g) in water (30 ml). The aqueous layer was extracted with chloroform (4 × 20 ml). The combined organic layers were dried over magnesium sulfate and then concentraed *in vacuo* to leave a yellowish semisolid. Flash chromatography[33] [chloroform–methanol (15 : 1, v/v)] afforded **17a** (252 mg, 78%), mp 55—57 °C. Recrystallization of this compound failed. IR ν_{max}^{Nujol} cm^{-1}: 2215 (C≡N); ^{1}H-NMR ($CDCl_3$) δ: 3.49 (3H, s, N^5-Me), 3.73 [3H, d, J = 0.2 Hz, N(1)-Me], 7.40 [1H, br s, C(2)-H]; (Me_2SO-d_6) δ: 3.39 (3H, s, N^5-Me), 3.67 [3H, s, N(1)-Me], 7.87 [1H, s, C(2)-H]; MS m/z: 161.0702 (M^+, $C_7H_7N_5$ requires 161.0701).

[4-Cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-imidazol-5-yl]methylcyanamide (17b) Phosphorus oxychloride (0.2 ml) was added to a solution of **14b**[17d] (423 mg, 1 mmol) and triethylamine (3 ml) in chloroform (20 ml), and the mixture was stirred at room temperature for 1.5 h. It was then concentrated *in vacuo* and the residue was neutralized with saturated aqueous sodium bicarbonate under cooling with ice water. The whole was extracted with chloroform (50 ml). The chloroform solution was dried over magnesium sulfate and concentrated *in vacuo* to leave a brown oil. This was purified on a silica gel column [chloroform–methanol (20 : 1, v/v)] to afford **17b** (355 mg, 88%) as a colorless glass, (IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2232 (C≡N), 1750 (Ac); ^{1}H-NMR (Me_2SO-d_6) δ: 2.03, 2.07, and 2.11 (3H each, s, three Ac's), 3.36 (3H, s, NMe), 4.32 [3H, m, C(4')-H and C (5')-H_2], 5.39 [1H, dd, J = 6 Hz each, C(3')-H], 5.61 [1H, dd, J = 6 Hz each, C(2')-H], 6.00 [1H, d, J = 6 Hz, C(1')-H], 8.28 [1H, s, C(2)-H].

***N*-(4-Cyano-1-methyl-1*H*-imidazol-5-yl)-*N*-methylurea (18a)** i) Hydration in Aqueous Sodium Hydroxide: The nitrile-cyanamide **17a** (67 mg, 0.42 mmol) was quickly dissolved in 0.1 N aqueous sodium hydroxide at room temperature followed by neutralization with 10% hydrochloric acid with as little delay as possible. The mixture was concentrated *in vacuo* to *ca.* 1 ml and kept in a refrigerator for 2 d. The solid was collected by filtration and washed with a little water to afford **19a** (20 mg, 27%), mp > 300 °C, identical (IR spectroscopy and chromatographic behavior) with an analytical sample described below. The combined filtrate and washings were concentrated *in vacuo* and the residue was purified by layer chromatography on silica gel [chloroform–methanol (5 : 1, v/v)] to afford **18a** (45 mg, 61%) as a colorless solid, mp 186—187 °C. Recrystallization from ethanol gave an analytical sample of **18a** as colorless plates, mp 187—188 °C; UV λ_{max} (95% EtOH) nm (ε): 230 (9100); λ_{max} (H_2O, pH 1) nm (ε): 230 (8500); λ_{max} (H_2O, pH 7) nm (ε): 230.5 (8800); λ_{max} (H_2O, pH 13): unstable; IR ν_{max}^{Nujol} cm^{-1}: 2240 (C≡N), 1680 (C=O); ^{1}H-NMR (Me_2SO-d_6) δ: 3.10 (3H, s, N^5-Me), 3.46 [3H, s, N(1)-Me], 6.44 (2H, br, NH_2), 7.80 [1H, s, C(2)-H]. *Anal.* Calcd for $C_7H_9N_5O$: C, 46.92; H, 5.06; N, 39.08. Found: C, 46.92; H, 4.97; N, 39.31.

ii) Hydration at pH 9.5: A solution of **17a** (161 mg, 1 mmol) in 0.5 M carbonate buffer (prepared from sodium bicarbonate and sodium carbonate) of pH 9.50 (10 ml) was kept at 25 °C for 16.5 h, neutralized with 10% aqueous phosphoric acid, and extracted with dichloromethane using a continuous extractor. The extracts, from which **18a** precipitated, were concentrated *in vacuo*. The solid residue was dissolved in ethanol and a trace of an insoluble solid was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was dried over phosphorus pentoxide under reduced pressure to afford **18a** (167 mg, 93%) as a slightly yellow solid, mp 185—187 °C.

6-Amino-3,9-dihydro-3,9-dimethyl-2*H*-purin-2-one (3,9-Dimethylisoguanine) (19a) i) From **17a**: A solution of **17a** (100 mg, 0.62 mmol) in 0.1 N aqueous sodium hydroxide (10 ml) was allowed to stand at room temperature for 3 h, then neutralized with 10% hydrochloric acid, concentrated to half the initial volume under reduced pressure, and kept in a refrigerator for 6 h. The resulting precipitate was collected by filtration, washed with a little water, and dried to afford a colorless solid (104 mg, 94%), mp > 300 °C. Recrystallization from water gave an analytical sample as colorless needles, mp > 300 °C; UV λ_{max} (H_2O, pH 1) nm (ε): 237 (5700), 287 (14100); λ_{max} (H_2O, pH 7) nm (ε): 238 (sh) (9000), 280 (12100); λ_{max} (H_2O, pH 13) nm (ε): 238 (sh) (9000), 280 (12100); ^{1}H-NMR (Me_2SO-d_6) δ: 3.64 [3H, s, N(3)-Me], 3.94 [3H, s, N(9)-Me], 7.37 (2H, br, NH_2), 7.64 [1H, s, C(8)-H]. *Anal.* Calcd for $C_7H_9N_5O$: C, 46.92; H, 5.06; N, 39.08. Found: C, 46.67; H, 4.95; N, 39.01.

ii) From **18a**: A solution of **18a** (18 mg, 0.1 mmol) in 0.1 N aqueous sodium hydroxide (5 ml) was allowed to stand at room temperature for 2 h, neutralized with 10% hydrochloric acid, and concentrated *in vacuo* to leave a solid residue. This was washed with a little water and dried to afford **19a** (13 mg, 72%), mp > 300 °C, identical (IR spectroscopy and chromatographic behavior) with the analytical sample described under item (i).

2,3-Dihydro-3-methyl-2-oxoadenosine (3-Methylisoguanosine) (19c) Compound **17b**, obtained from **14b** (423 mg, 1 mmol) in the same way as described above, was dissolved in 0.1 N aqueous sodium hydroxide and the solution was allowed to stand at room temperature for 4 h. It was neutralized with 10% hydrochloric acid, followed by concentration *in vacuo* to *ca.* 3 ml. The residue was kept in a refrigerator overnight, and the resulting precipitate was collected by filtration, washed with a little water, and dried to afford **19c**·H_2O (16 mg), mp 160—168 °C (dec.). The combined filtrate and washings were concentrated *in vacuo* and the residue was purified by reversed-phase chromatography [Merck Lobar column, LiChroprep RP-8, size 310 × 25 mm; H_2O–MeOH (90 : 10, v/v)] to afford a second crop of **19c**·H_2O (112 mg, the total yield was 41%), mp 160—168 °C (dec.). Recrystallization from water gave colorless needles.

These were dried over phosphorus pentoxide at 2 mmHg and 50°C for 17 h to give an analytical sample as a monohydrate, mp 175—178 °C (dec.); $[\alpha]_D^{17}$ −44° (c=0.129, H_2O); pK_a 4.57±0.03 (ionic strength 1.0, 25 °C);[34] UV λ_{max} (95% EtOH) nm (ε): 223 (21000), 240 (sh) (10300), 281 (11000); λ_{max} (H_2O, pH 1): unstable; λ_{max} (H_2O, pH 2) nm (ε): 238 (7100), 286 (13700); λ_{max} (H_2O, pH 7) nm (ε): 220 (21500), 238 (sh) (9300), 279 (11900); λ_{max} (H_2O, pH 13) nm (ε): 239 (sh) (8700), 279 (12000); ^{1}H-NMR (Me_2SO-d_6) δ: 3.62 [5H, s, NMe and C(5′)-H_2], 3.96 [1H, m, C(4′)-H], 4.09 [1H, m, C(3′)-H], 4.39 [1H, m, C(2′)-H], 5.09 [1H, br t, J=6 Hz, C(5′)-OH], 5.27 [1H, br, C(3′)-OH], 5.62 [1H, br, C(2′)-OH], 6.01 [1H, d, J=5 Hz, C(1′)-H], 7.37 (2H, br, NH_2), 8.08 [1H, s, C(8)-H]. *Anal.* Calcd for $C_{11}H_{15}N_5O_5 \cdot H_2O$: C, 41.91; H, 5.43; N, 22.21. Found: C, 41.70; H, 5.39; N, 22.30.

6-Amino-3,9-dihydro-3-methyl-2*H*-purin-2-one Hydrochloride (20·HCl) A solution of **19c**·H_2O (95 mg, 0.3 mmol) in 0.1 N hydrochloric acid (10 ml) was allowed to stand at room temperature for 15 h, then neutralized with 10% aqueous sodium hydroxide. Removal of water by evaporation afforded crude **20** as a colorless solid. Because recrystallization of the free base of **20** was difficult, the residue was dissolved in 10% methanolic hydrogen chloride (25 ml). A small amount of insoluble material was filtered off and the filtrate was concentrated *in vacuo* to leave a colorless solid. This was washed successively with water (2 ml) and ethanol (1 ml), and dried to give **20**·HCl (37 mg, 61%), mp > 300 °C. Recrystallization from methanol gave an analytical sample as colorless needles, mp > 300°C; UV λ_{max} (H_2O, pH 1) nm (ε): 288.5 (12800); λ_{max} (H_2O, pH 7) nm (ε): 239 (7800), 283 (11200); λ_{max} (H_2O, pH 13) nm (ε): 237 (sh) (5600), 285 (13100); ^{1}H-NMR (Me_2SO-d_6) δ: 3.44 (3H, s, Me), 8.32 [1H, s, C(8)-H], 8.73 and 9.84 (1H each, br, two NH's), 13.27 (2H, br, two NH's). *Anal.* Calcd for $C_6H_7N_5O \cdot HCl$: C, 35.74; H, 4.00; N, 34.74. Found: C, 35.82; H, 3.96; N, 34.94. The UV spectra of this sample matched those reported for 3-methylisoguanine monohydrate.[25]

Kinetic Procedure i) Cyclization of **17a** through **18a**: Compound **17a** was dissolved in 0.5 M phosphate buffer (prepared from sodium dihydrogen phosphate and disodium hydrogen phosphate, pH 10.96 at 25 °C) kept at 25±0.05 °C in a thermoregulated constant-temperature bath at a concentration of 2.31×10^{-4} M. At intervals, aliquots (2 ml) of the solution were withdrawn and diluted with 0.5 M aqueous potassium dihydrogen phosphate (2 ml) to quench the reaction. Small portions (20 μl) of the diluted solutions were then analyzed by means of high-performance liquid chromatography on a μBondapak C_{18} column (3.9×300 mm) (0.05 M aqueous potassium dihydrogen phosphate, 1.8 ml/min), and the peak areas of the components were determined by using a UV absorbance detector operated at 254 nm and a Takeda Riken TR-2217 automatic integrator. Concentrations of **17a**, **18a**, and **19a** in the reaction mixture were then estimated from calibration curves which had been obtained with solutions of these compounds of known concentration. Both the decrease of concentration of **17a** and that of **18a** after complete consumption of **17a** obeyed good pseudo-first-order kinetics. The results are summarized in the text and Fig. 1.

ii) Glycosidic Hydrolysis of **19c** in 0.1 N Hydrochloric Acid and in 0.1 N Hydrochloric Acid of Ionic Strength 1.0: The reaction solutions were prepared by dissolving the substrate at a concentration of 9×10^{-5} M in 0.1 N hydrochloric acid or 0.1 N hydrochloric acid, whose ionic strength was adjusted at 1.0 with potassium chloride. The solutions were kept at 25±0.05 °C and the reaction was followed spectrophotometrically using 242 nm as an analytical wavelength. The results are given in the text.

iii) Glycosidic Hydrolysis of **19c** in 1N Hydrochloric Acid: A stock solution of **19c** was prepared by dissolving it in water at a concentration of 3.3×10^{-4} M. The reaction solution was prepared by mixing the stock solution (2 ml) and 2 N hydrochloric acid (2 ml) in the same way as reported for the hydrolysis of 3-methylinosine in 0.1 N hydrochloric acid,[19b] and the reaction was followed spectrophotometrically at 244 nm in the same way as reported for the hydrolysis of 3-methylguanosine in 0.1 N hydrochloric acid at 25 °C.[17d] For three separate runs, k_{obsd} $(4.9 \pm 0.2) \times 10^{-1}$ min^{-1} was obtained.

iv) Glycosidic Hydrolysis of **3** in 1 N Hydrochloric Acid: An analytical sample of **3**·1/2H_2O was prepared according to the reported procedure[4h] and dried over phosphorus pentoxide at 2 mmHg and 55 °C for 14 h followed by exposure to air until constant weight was reached, mp 237—241 °C (dec.) [lit.[4h] mp 240 °C (dec.)]. *Anal.* Calcd for $C_{10}H_{13}N_5O_5 \cdot 1/2H_2O$: C, 41.10; H, 4.83; N, 23.96. Found: C, 41.31; H, 4.73; N, 23.85. This sample was dissolved in 1 N hydrochloric acid at a concentration of 6.66×10^{-5} or 6.73×10^{-5} M and the solution was kept at 25±0.05 °C. The reaction was followed spectrophotometrically at 282 nm for 22 h. The infinity value was calculated from the absorbance of a solution of isoguanine hemisulfate[35] in 1N hydrochloric acid of known concentration. This value was in good accord with the infinity reading (200 h after the start of the reaction) of the reaction mixture. We also confirmed that isoguanine was stable under those conditions and the whole UV spectrum of the reaction mixture at completion of the reaction could be superimposed on that of isoguanine of equimolar concentration. For two separate runs, k_{obsd} 7.5×10^{-4} min^{-1} was obtained.

References and Notes

1) *a*) R. Purrmann, *Justus Liebigs Ann. Chem.*, **544**, 182 (1940); *b*) G. R. Pettit, R. H. Ode, R. M. Coomes, and S. L. Ode, *Lloydia*, **39**, 363 (1976) [*Chem. Abstr.*, **86**, 136604*h* (1977)].
2) E. Cherbuliez and K. Bernhard, *Helv. Chim. Acta*, **15**, 464 (1932).
3) F. A. Fuhrman, G. J. Fuhrman, R. J. Nachman, and H. S. Mosher, *Science*, **212**, 557 (1981).
4) *a*) J. Davoll, *J. Am. Chem. Soc.*, **73**, 3174 (1951); *b*) F. Cramer and G. Schlingloff, *Tetrahedron Lett.*, **1964**, 3201; *c*) A. Yamazaki, I. Kumashiro, T. Takenishi, and M. Ikehara, *Chem. Pharm. Bull.*, **16**, 2172 (1968); *d*) K. Miura and T. Ueda, *ibid.*, **23**, 2064 (1975); *e*) N. K. Saxena and D. S. Bhakuni, *Indian J. Chem., Sect. B*, **18B**, 344 (1979) [*Chem. Abstr.*, **92**, 129233*g* (1980)]; *f*) D. S. Bhakuni and P. K. Gupta, *ibid.*, **22B**, 48 (1983) [*Chem. Abstr.*, **99**, 22837*z* (1983)]; *g*) V. Nair and D. A. Young, *J. Org. Chem.*, **50**, 406 (1985); *h*) J.-W. Chern, H.-Y. Lee, M. Huang, and F.-J. Shish, *Tetrahedron Lett.*, **28**, 2151 (1987).
5) *a*) J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **33**, 432 (1968); *b*) A. Yamazaki, M. Okutsu, and Y. Yamada, *Nucleic Acids Res.*, **3**, 251 (1976); *c*) C. B. Reese, Y. S. Sanghvi, and R. Kuroda, *J. Chem. Soc., Perkin Trans. 1*, **1987**, 1527.
6) *a*) R. H. Hall, "The Modified Nucleosides in Nucleic Acids," Columbia University Press, New York, 1971; *b*) T. Itaya, *Yuki Gosei Kagaku Kyokai Shi*, **45**, 431 (1987); *c*) G. R. Björk, J. U. Ericson, C. E. D. Gustafsson, T. G. Hagervall, Y. H. Jönsson, and P. M. Wikström, *Annu. Rev. Biochem.*, **56**, 263 (1987).
7) W. Bergmann and D. C. Burke, *J. Org. Chem.*, **21**, 226 (1956).
8) W. Bergmann and M. F. Stempien, Jr., *J. Org. Chem.*, **22**, 1575 (1957).
9) W. Kampe, M. Thiel, K. Stach, W. Schaumann, and K. Dietmann, S. African Patent 6707630 (1968) [*Chem. Abstr.*, **70**, 88212*z* (1969)].
10) *a*) R. J. Quinn, R. P. Gregson, A. F. Cook, and R. T. Bartlett, *Tetrahedron Lett.*, **21**, 567 (1980); *b*) A. F. Cook, R. T. Bartlett, R. P. Gregson, and R. J. Quinn, *J. Org. Chem.*, **45**, 4020 (1980).
11) *a*) F. A. Fuhrman, G. J. Fuhrman, Y. H. Kim, L. A. Pavelka, and H. S. Mosher, *Science*, **207**, 193 (1980); *b*) Y. H. Kim, R. J. Nachman, L. Pavelka, H. S. Mosher, F. A. Fuhrman, and G. J. Fuhrman, *J. Nat. Prod.*, **44**, 206 (1981).
12) J.-W. Chern and L. B. Townsend, *Tetrahedron Lett.*, **26**, 6419 (1985).
13) R. T. Bartlett, A. F. Cook, M. J. Holman, W. W. McComas, E. F. Nowoswait, M. S. Poonian, J. A. Baird-Lambert, B. A. Baldo, and J. F. Marwood, *J. Med. Chem.*, **24**, 947 (1981).
14) *a*) Y. H. Kim, S. J. Lee, and H. S. Mosher, *Heterocycles*, **23**, 21 (1985); *b*) Y. H. Kim, *Stud. Org. Chem.* (Amsterdam), **26**, 211 (1986) [*Chem. Abstr.*, **106**, 214277*b* (1987)].
15) *a*) T. Saito and T. Fujii, *J. Chem. Soc., Chem. Commun.*, **1979**, 135; *b*) T. Fujii, T. Saito, and T. Nakasaka, *Chem. Pharm. Bull.*, **37**, 2601 (1989).
16) *a*) T. Fujii, F. Tanaka, K. Mohri, T. Itaya, and T. Saito, *Tetrahedron Lett.*, **1973**, 4873; *b*) T. Fujii and T. Saito, *Heterocycles*, **17**, 117 (1982); *c*) B. Singer, L. Sun, and H. Fraenkel-Conrat, *Biochemistry*, **13**, 1913 (1974).
17) *a*) S. Nakatsuka, T. Ohgi, and T. Goto, *Tetrahedron Lett.*, **1978**, 2579; *b*) T. Itaya and K. Ogawa, *ibid.*, **1978**, 2907; *c*) T. Itaya, T. Watanabe, and H. Matsumoto, *J. Chem. Soc., Chem. Commun.*, **1980**, 1158; *d*) T. Itaya, H. Matsumoto, T. Watanabe, and T. Harada, *Chem. Pharm. Bull.*, **33**, 2339 (1985); *e*) J. Boryski, W. Folkman, and B. Golankiewicz, *Tetrahedron Lett.*, **29**, 4163 (1988).
18) *a*) J. A. Haines, C. B. Reese, and L. Todd, *J. Chem. Soc.*, **1962**, 5281; *b*) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **85**, 193 (1963).
19) *a*) T. Itaya and H. Matsumoto, *Tetrahedron Lett.*, **1978**, 4047; *b*) *Idem, Chem. Pharm. Bull.*, **33**, 2213 (1985).
20) *a*) T. Itaya and T. Harada, *Heterocycles*, **19**, 687 (1982); *b*) *Idem, Chem. Pharm. Bull.*, **37**, 1235 (1989).
21) *a*) T. Itaya, *Chem. Pharm. Bull.*, **35**, 4372 (1987); *b*) T. Itaya, M. Morisue, M. Takeda, and Y. Kumazawa, *ibid.*, **38**, 2656 (1990).
22) T. Itaya, M. Shimomichi, and M. Ozasa, *Tetrahedron Lett.*, **29**, 4129 (1988).

23) T. Itaya and T. Harada, *Tetrahedron Lett.*, **23**, 2203 (1982).
24) T. Okano, S. Goya, and T. Kaizu, *Yakugaku Zasshi*, **87**, 469 (1967).
25) Z. Kazimierczuk and D. Shugar, *Acta Biochim. Poln.*, **21**, 455 (1974).
26) K. Ienaga and W. Pfleiderer, *Tetrahedron Lett.*, **1978**, 1447.
27) *a*) J. W. Marsico and L. Goldman, *J. Org. Chem.*, **30**, 3597 (1965); *b*) T. Itaya, K. Ogawa, H. Matsumoto, and T. Watanabe, *Chem. Pharm. Bull.*, **28**, 2819 (1980); *c*) T. Fujii, T. Itaya, T. Saito, K. Mohri, M. Kawanishi, and T. Nakasaka, *ibid.*, **37**, 1504 (1989).
28) T. Itaya and K. Ogawa, *Tetrahedron*, **38**, 1767 (1982).
29) *a*) T. Itaya and K. Ogawa, *Heterocycles*, **6**, 965 (1977); *b*) *Idem, Chem. Pharm. Bull.*, **33**, 1906 (1985).
30) *a*) T. Fujii and T. Saito, *Chem. Pharm. Bull.*, **21**, 1954 (1973) and references cited therein; *b*) R. F. Pratt and K. K. Kraus, *Tetrahedron Lett.*, **22**, 2431 (1981).
31) We isolated compound **12** and confirmed that it cyclized to **13**. Details will be reported elsewhere.
32) The broadening of the signal is probably due to slow rotation around the carbon-to-nitrogen bond of the carbamate group.[20b] The signal became sharp at 80 °C.
33) W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
34) Determined spectrophotometrically at 256 nm in a usual manner (A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen & Co., London, 1962, pp. 69—92).
35) Recrystallization of the commercial product of Sigma Chemical Co. from water followed by drying over phosphorus pentoxide at 2 mmHg and 100 °C for 5 h afforded an analytical sample as colorless plates, mp *ca.* 270 °C (dec.). *Anal.* Calcd for $C_5H_5N_5O \cdot 1/2H_2SO_4 \cdot 1/2H_2O$: C, 28.71; H, 3.37; N, 33.48. Found: C, 28.70; H, 3.35; N, 33.64.