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

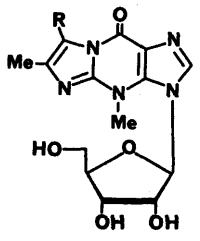
Taisuke ITAYA* and Tsunehiro HARADA

Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan. *Received November 28, 1988*

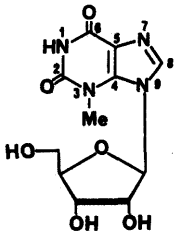
An improved synthesis of 3,9-dimethylxanthine (2a) was achieved *via* the reaction of 1-methyl-5-(methylamino)-imidazole-4-carboxamide (3a) with EtOCOCl in acetate buffer (pH 5) followed by treatment with aqueous NaOH. This method was successfully applied to the synthesis of 3-methylxanthosine (2b), whose *N*-glycosidic bond proved to be remarkably sensitive to acidic hydrolysis: 2b underwent hydrolysis at a rate more than 1000 times faster than that of xanthosine (10) in 1.0 N aqueous HCl at 25 °C.

Keywords 3-methylxanthosine synthesis; 3,9-disubstituted xanthine; imidazolylcarbamic acid ester; base-catalyzed cyclization; amide carbamate cyclization; *N*-glycosidic bond hydrolysis; carbamate *cis–trans* isomerism; NMR; kinetic study

Unusual susceptibility to acid hydrolysis at the *N*-glycosidic bond is a salient feature of wyosine,[1] 7-methylwyosine,[2] and wybutosine,[3] the fluorescent nucleosides isolated from transfer ribonucleic acids. The putative structures **1a**—**c** for them have been synthesized[4] and their glycosidic bonds were indeed highly labile to acid.[4,5] The structures **1** are unique in that they comprise 3-methyl-9-β-D-ribofuranosylpurine as a partial structure. As an approach to elucidation of the relationship between the instability of the glycosidic bonds and the chemical structures, we have synthesized 3-methylguanosine,[4b,c,5,6] 3-methylinosine,[7] and 3-methylisoguanosine[8] as typical 3-methyl-9-β-D-ribofuranosylpurines and determined the rates of their acidic hydrolysis. Synthesis and hydrolysis of 3-methyladenosine have been reported by Saito and Fujii.[9] In connection with these studies, this paper presents a detailed account of the synthesis of an additional example of 3-methyl-9-β-D-ribofuranosylpurines, 3-methylxanthosine (**2b**).[10]



1



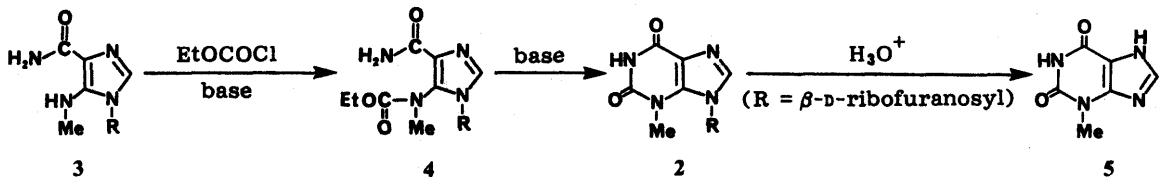
2b

a: R = H
b: R = Me
c: R = $CH_2CH_2C(CO_2Me)(H)NHCO_2Me$

Evidence for the formation of a small amount of **2b** by the reaction of xanthosine with CH_2N_2 was reported by Adler and Gutman in 1964.[11] To our knowledge, this is the earliest instance of a derivative of 3-methyl-9-β-D-ribofuranosylpurine on record. However, they did not isolate **2b** in a pure form. Although 3,9-dimethylxanthine (**2a**), a prototype of **2b**, had been synthesized by Pfleiderer and Nübel,[12] their method seemed too drastic to apply to the synthesis at the nucleoside level. We reported a milder synthesis of **2a** from **3a** through **4a**.[13] In the present study, we utilized this method for the first practical synthesis of **2b**.

Compound **4a** had been obtained in 39% yield by heating **3a** with EtOCOCl in dioxane in the presence of K_2CO_3.[13] We found that the reaction took place smoothly at room temperature in H_2O in the presence of a base. Unfortunately, the yield of **4a** was not improved when the reaction was carried out using $NaHCO_3$ as a base, owing to the concomitant formation of by-products. Although the structures **6a, b** were assignable to them on the basis of proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, the alternatives **8a,b** could not be ruled out at that time. Compound **8** might be formed through the Bamberger fission[14] of **4a** followed by recyclization of the resulting **7**. Compounds **6c, d**, benzyloxy analogs of **6a, b**, were obtained besides **4e** by similar reaction of **3a** with $PhCH_2OCOCl$ and they both regenerated **3a** on hydrogenolysis over Pd–C, confirming the correctness of the structures **6**. When the reaction of **3a** and EtOCOCl was conducted in acetate buffer (pH 5), neither **6a** nor **6b** was formed and **4a** was obtained in 70% yield. Although the ethoxycarbonylation of **3c** took place much more slowly under similar conditions, prolonged reaction afforded **4c** in 36% yield.

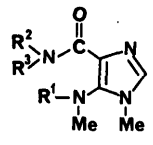
Cis–trans isomerism of amides caused by hindered rota-



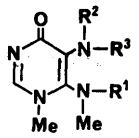
3 **4** **2** **5**

a: R = Me
b: R = β-D-ribofuranosyl
c: R = 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl
d: R = $PhCH_2$

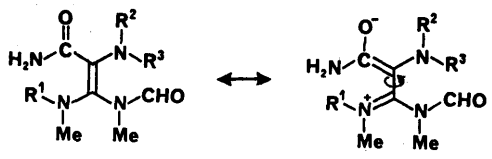
Chart 1



6

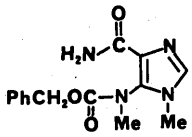


8

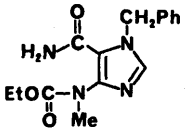


7

a: $R^1=R^2=CO_2Et$, $R^3=H$
b: $R^1=R^2=R^3=CO_2Et$
c: $R^1=R^2=CO_2CH_2Ph$, $R^3=H$
d: $R^1=R^2=R^3=CO_2CH_2Ph$



4e

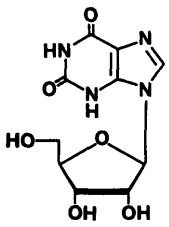


9

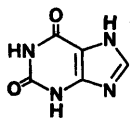
tion about the carbonyl-to-nitrogen bond has been observed by ^{1}H-NMR spectroscopy.[7,15] Although carbamates have rotational barriers lower than those of the corresponding amides,[15a,b] the isomerism is detectable even at room temperature in some cases.[16] The ^{1}H-NMR spectrum of **4a** obtained in $(CD_3)_2SO$ at 25 °C exhibited two signal sets for the ester *C*-methyl and the exocyclic *N*-methyl groups with an intensity ratio of approximate 3:1 (see Experimental). They coalesced at higher temperature, suggesting that the observed complexity was a result of *cis–trans* isomerism of the carbamate. The ^{1}H-NMR spectrum of an analogous carbamate **9**[17] showed no evidence of such isomerism but only slight broadening for the *O*-ethyl signal in contrast to that of **4a**. In order to compare the spectrum of **9** with that of a more closely related compound, we prepared **4d**, a positional isomer of **9**. With **4d**, two sets of signals were observed for the benzylic methylene besides those for the *O*-ethyl and *N*-methyl groups. Furthermore, the benzylic methylene group was observed as AB-type doublets, while the one in **9** appeared as a singlet. Since the carbamate group of **4** is placed between two adjacent substituents at the imidazole ring, **4** may be expected to have higher rotational barriers than **9** for both the carbamate carbonyl-to-nitrogen and the imidazole-to-nitrogen bonds. The slow rotation about the carbonyl-to-nitrogen bond is probably responsible for the isomerism observed in **4a,d**. Non-equivalency observed for the benzylic methylene protons in **4d** is probably a result of the hindered rotation about the imidazole-to-nitrogen bond, by analogy with that observed for *ortho*-substituted anilides.[15a] The ester methylene signal of **4a** exhibited a more complicated pattern than that expected for two quartets, suggesting that the methylene protons were nonequivalent. This may also be due to the slow rotation of the imidazole-to-nitrogen bond of **4a**. Two sets of signals were also observed for most of the different species of protons in the benzyloxycarbonyl analog **4e**. Since the nucleoside **4c** has an asymmetric ribofuranosyl group, hindered rotation about the imidazole-to-nitrogen bond may produce diastereomers[15a] for each *cis–trans* isomer. Three of the four possible isomers of **4c** were detected: three triplets for the ester *C*-methyl and three singlets for the *N*-methyl group.

Cyclization of **4c** was first attempted by treatment with NaH in $HCONMe_2$ according to the reported procedure for **4a**[13] to give a mixture too complex to allow recognition of the presence of **2c**. Re-examination of the cyclization of **4a** disclosed that either NaOH in H_2O or NaOEt in EtOH also catalyzed the reaction effectively. Compound **4c** was thus converted into **2b**[18] by treatment with 1 N NaOH in 69% yield. The correctness of the structure of **2b** was supported by the ultraviolet (UV) and NMR spectral similarity to **2a**[12,13] and was confirmed by transformation of **2b** into 3-methylxanthine (**5**)[19] by treatment with 0.1 N aqueous HCl at room temperature.

The rate of hydrolysis of the glycosidic bond of **2b** in 0.1 N aqueous HCl (ionic strength 1.0) at 25 °C [pseudo-first-order rate constant, k_{obs} 2.8×10^{-1} min^{-1} (half life, $t_{1/2}$ 2.5 min)] proved to be of the same order of magnitude as those of 3-methylguanosine,[4b,c] 3-methylinosine,[7] and **1**.[4b,c,f,g] Since the hydrolysis of the glycosidic bond of xanthosine (**10**) proceeds so slowly under similar conditions, the rates for **10** and **2b** were determined in 1.0 N aqueous HCl at 25 °C for comparison: **2b** underwent hydrolysis at a rate [k_{obs} 8.2×10^{-1} min^{-1} ($t_{1/2}$ 51 s)] 1200 times faster than that [k_{obs} 6.8×10^{-4} min^{-1} ($t_{1/2}$ 17 h)] of **10**. Thus, the present results have provided an additional example of the fact that the introduction of a methyl group into 9-β-D-ribofuranosylpurines at the 3-position remarkably weakens the glycosidic bonds.[4,5,7-9] It is interesting to note, however, that the glycosidic bond of **2b** was fairly stable in 0.1 N aqueous NaOH at 50 °C in contrast to the instability of **1a** and 3-methylguanosine under basic conditions.[4c,5]



10



11

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 on a Hitachi 323 or a Hitachi 320 UV spectrophotometer using solutions in 95% aqueous EtOH, 0.1 N aqueous HCl (pH 1), 0.01 N aqueous HCl (pH 2), 0.005 M phosphate buffer (pH 7), and 0.1 N aqueous NaOH (pH 13), a JASCO IRA-2 or a JASCO A-202 infrared (IR) spectrophotometer using a Nujol mull, or a JEOL JNM-FX-100 NMR spectrometer using a solution in $(CD_3)_2SO$ at 25 °C with Me_4Si as an internal standard. pH's were masured 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, m = multiplet, q = quartet, s = singlet, sh = shoulder, t = triplet.

5-[(Ethoxycarbonyl)methylamino]-1-methylimidazole-4-carboxamide

(4a) i) EtOCOCl (2.0 ml, 21 mmol) was added to a solution of **3a**[15d,20] (308 mg, 2.0 mmol) in 1 M aqueous AcONa–AcOH (pH 5, 20 ml) and the mixture was stirred at room temperature for 30 min. The solution (pH 3.8) was adjusted to pH 5.3 with 10% aqueous NaOH and stirring was continued for a further 30 min. It was then brought to pH 8 by addition of 10% aqueous NaOH and saturated with NaCl. The mixture was extracted with $CHCl_3$ (6 × 20 ml). The combined $CHCl_3$ extracts were dried over $MgSO_4$ and concentrated *in vacuo* to leave a solid residue. This was washed with EtOH (3 ml) and dried to afford **4a** (317 mg, 70%), mp 203—205 °C. Recrystallization from EtOH gave colorless prisms, mp 204—206 °C, identical with an analytical sample,[13] IR ν_{max} cm^{-1}: 3350, 3150 (NH_2), 1711 (carbamate CO), 1679 (amide CO); ^{1}H-NMR δ: 1.05 and 1.26 [3/4 × 3H and 1/4 × 3H, t each, J = 7 Hz, coalesced into 1.12 (t) at 100 °C, $\underline{Me}CH_2$], 3.09 and 3.14 [3/4 × 3H and 1/4 × 3H, s each, coalesced into 3.11 (s) at 100 °C, N^5-Me], 3.45 [3H, s, N(1)-Me], 3.8—4.2 [2H, m, coalesced into 4.05 (q, J = 7 Hz) at 100 °C, CH_2], 7.05 and 7.22 [1H each, br, coalesced into 6.85 (2H, br) at 100 °C, NH_2], 7.63 [1H, s, C(2)-H].

ii) EtOCOCl (2.0 ml) was added dropwise to a stirred solution of **3a**[15d,20] (304 mg, 2.0 mmol) and $NaHCO_3$ (2.00 g) in H_2O (20 ml) over a period of 5 min. Stirring was continued for a further 25 min and then the mixture was extracted with $CHCl_3$ (20 ml). The $CHCl_3$ extract was dried over $MgSO_4$ and concentrated *in vacuo* to leave a slightly yellow semisolid (0.25 g). This was purified on a silica gel (25 g) column [benzene–EtOH (10 : 1 and then 5 : 1, v/v)]. Earlier fractions were further purified on a silica gel layer [benzene–EtOH (5 : 1, v/v)] to afford **6b** (26 mg, 3.6%) as a colorless oil, ^{1}H-NMR δ: 1.0—1.3 (9H, m, three $\underline{Me}CH_2$'s), 3.10 (3H, s, N^5-Me), 3.52 [3H, s, N(1)-Me], 3.9—4.3 (6H, m, three $MeC\underline{H}_2$'s), 7.77 [1H, s, C(2)-H]. From the successive eluate, crude **6a** was obtained. This was further purified by layer chromatography as described above to give **6a** (32 mg, 5.4%) as a colorless oil, ^{1}H-NMR δ: 1.0—1.3 (6H, m, two $\underline{Me}CH_2$'s), 3.10 and 3.14 (a total of 3H, s each, N^5-Me), 3.50 [3H, s, N(1)-Me], 3.9—4.3 (4H, m, two $MeC\underline{H}_2$'s), 7.78 [1H, s, C(2)-H], 9.50 (1H, br, NH). Further elution of the column gave **4a** (90 mg) as a colorless solid, mp 198—202 °C.

The aqueous solution which had been extracted with $CHCl_3$ was further extracted with $CHCl_3$ (4 × 20 ml). The combined $CHCl_3$ extracts were concentrated after having been dried over $MgSO_4$ and the residue was recrystallized from EtOH to afford a second crop of **4a** (60 mg, the total yield was 34%) as colorless prisms, mp 203—205 °C.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-5-[(ethoxycarbonyl)methylamino]imidazole-4-carboxamide (4c) Compound **3c**[7a,21] (1.51 g, 3.79 mmol) was dissolved in 1 M aqueous AcOH–AcONa (pH 5) (60 ml). EtOCOCl (20 ml, 0.21 mol) was added dropwise to this solution over a period of 4 h at a rate such that the reaction temperature did not exceed 30 °C with vigorous stirring, and the mixture was stirred for a further 1 h. During this time the reaction mixture was kept at pH 4.9—5.0 by occasional addition of 10% aqueous NaOH. The resulting mixture was brought to pH 7.0 with 10% aqueous NaOH and then extracted with $CHCl_3$ (2 × 20 ml). The combined $CHCl_3$ extracts were dried over $MgSO_4$ and concentrated *in vacuo* to leave a crystalline residue, which was recrystallized from EtOH (1 ml) to give **4c** (447 mg) as colorless prisms, mp 119—125 °C. The mother liquor was concentrated to a small volume and purified on a silica gel (30 g) column [$CHCl_3$–MeOH (10 : 1, v/v)] to give a second crop of **4c** (200 mg, the total yield was 36%), mp 147—148 °C. For analysis, crude **4c** was recrystallized from EtOH to give colorless prisms, mp 150—151 °C; $[\alpha]_D^{22}$ −15.4 ± 0.2° (c = 1.01, MeOH); UV $\lambda_{max}^{95\%EtOH}$ 229 nm (sh) (ε 9500); $\lambda_{max}^{H_2O}$ (pH 1) 212 (10600), 229 (sh) (9000); $\lambda_{max}^{H_2O}$ (pH 7) 230 (sh) (9100); $\lambda_{max}^{H_2O}$ (pH 13) unstable with isosbestic points at 228 (8800) and 244 (8100); IR ν_{max} cm^{-1}: 3475, 3360, 3335 (NH_2), 1744, 1731 (ester CO), 1706 (carbamate CO), 1673 (amide CO); ^{1}H-NMR δ: 1.02, 1.06, and 1.26 [a total of 3H, t each, J = 7 Hz, coalesced into 1.02 (t) at 140 °C, $\underline{Me}CH_2$], 2.03, 2.06, and 2.09 (a total of 9H, three Ac's), 3.02, 3.06, and 3.11 [a total of 3H, s each, coalesced into 3.07 (s) at 140 °C, NMe], 3.96 and 3.99 [a total of 2H, q each, J = 7 Hz, coalesced into 4.02 (q, J = 7 Hz) at 140 °C, $MeC\underline{H}_2$], 4.32 [3H, m, C(4')-H and C(5')-H_2], 5.12—5.76 [3H, m, C(1')-H, C(2')-H, and C(3')-H], 7.16 and 7.36 (2H, br, NH_2), 7.96 and 8.03 [a total of 1H, s each, coalesced into 7.82 (br s) at 140 °C, C(2)-H]. *Anal.* Calcd for $C_{19}H_{26}N_4O_{10}$: C, 48.51; H, 5.57; N, 11.91. Found: C, 48.35; H, 5.56; N, 11.69.

1-Benzyl-5-[(ethoxycarbonyl)methylamino]imidazole-4-carboxamide (4d) Compound **3d**[15d,20b–d] (461 mg, 2.0 mmol) was allowed to react with EtOCOCl in the same way as described under method (i) for **4a**. The reaction mixture was neutralized with 10% aqueous NaOH and the resulting precipitate was collected by filtration and dried to afford **4d** (141 mg, 23%) as colorless needles. Recrystallizations from EtOH gave an analytical sample, mp 188—189 °C; IR ν_{max} cm^{-1}: 3360, 3220, 3185 (NH_2), 1707 (carbamate CO), 1655 (amide CO); ^{1}H-NMR δ: 0.96 and 1.25 [5/7 × 3H and 2/7 × 3H, t each, J = 7 Hz, coalesced into 1.05 (br) at 80 °C, $\underline{Me}CH_2$], 2.72 and 2.77 [2/7 × 3H and 5/7 × 3H, s each, coalesced into 2.81 (s) at 80 °C, NMe], 3.87 and 4.12 [5/7 × 2H and 2/7 × 2H, q, J = 7 Hz, coalesced into 3.95 (br) at 80 °C, $MeC\underline{H}_2$], 4.83 and 5.21 (a total of 2/7 × 2H), and 4.96 and 5.16 (a total of 5/7 × 2H) [d each, J = 15 Hz, coalesced into 4.93 and 5.13 (d each, J = 15 Hz) at 80 °C, $PhC\underline{H}_2$], 7.0—7.4 (7H, m, NH_2 and Ph), 7.82 [1H, s, C(2)-H]. *Anal.* Calcd for $C_{15}H_{18}N_4O_3$: C, 59.59; H, 6.00; N, 18.53. Found: C, 59.74; H, 5.84; N, 18.36.

5-[(Benzyloxycarbonyl)methylamino]-1-methylimidazole-4-carboxamide (4e) A mixture of **3a**[15d,20] (771 mg, 5.0 mmol), $PhCH_2OCOCl$ (1.71 g, 10 mmol), $NaHCO_3$ (1.00 g, 11.9 mmol), and H_2O (50 ml) was stirred vigorously at room temperature for 1.5 h. The mixture was extracted with CH_2Cl_2 (2 × 30 ml). The combined CH_2Cl_2 extracts were dried over $MgSO_4$ and concentrated *in vacuo* to a small volume. The residue was purified by flash chromatography[22] [column diameter, 40 mm; eluant, hexane–AcOEt (120 : 360 ml). AcOEt (400 ml), and then AcOEt–EtOH (600 : 60 ml)] to give **6d** (491 mg, 18%) as a colorless heavy oil, ^{1}H-NMR δ: 2.96 with a small peak at 3.02 (a total of 3H, s each, N^5-Me), 3.45 [3H, br s, N(1)-Me], 4.92 and 5.07 [1H each, d, J = 12.5 Hz, N^5-$CO_2C\underline{H}_2Ph$], 5.16 [4H, s, 4-$CON(CO_2C\underline{H}_2Ph)_2$], 7.26 (15 H, m, three Ph's), 7.70 [1H, s, C(2)-H]. Compound **6c** (96 mg, 4.5%) was obtained as the more polar substance, as a colorless glass, ^{1}H-NMR δ: 3.14 with a small peak at 3.20 (a total of 3H, s each, N^5-Me), 3.45 and 3.48 [a total of 3H, s each, N(1)-Me], 5.06 with a small peak at 5.13 [a total of 2H, s each, N^5-$CO_2C\underline{H}_2Ph$], 5.19 (2H, s, 4-$CONCO_2C\underline{H}_2Ph$), 7.37 (10H, m, two Ph's), 7.79 [1H, s, C(2)-H], 9.77 (1H, s, NH). Compound **4e** (229 mg, 16%) was obtained as the most polar product, mp 185—188 °C. Recrystallizations from EtOH gave an analytical sample as colorless plates, mp 188—189 °C; IR ν_{max} cm^{-1}: 3330, 3180 (NH_2), 1713 (carbamate CO), 1687 (amide CO); ^{1}H-NMR δ: 3.12 and 3.19 [5/7 × 3H and 2/7 × 3H, s each, coalesced into 3.15 (s) at 80 °C, N^5-Me], 3.39 and 3.44 [5/7 × 3H and 2/7 × 3H, s each, coalesced into 3.40 (s) at 80 °C, N(1)-Me], 5.02 and 5.08 (5/7H each, d, J = 14 Hz), and 5.19 (2/7 × 2H, s) [these two sets of signals coalesced into 5.08 (2H, br s) at 80 °C, CH_2], 7.0—7.2 [7H, m, changed into 6.91 (2H, br, NH_2) and 7.29 (5H, br s, Ph) at 80 °C], 7.62 [1H, s with sh at 7.64, coalesced into 7.57 (s) at 80 °C, C(2)-H]. *Anal.* Calcd for $C_{14}H_{16}N_4O_2$: C, 58.32; H, 5.59; N, 19.44. Found: C, 58.19; H, 5.62; N, 19.46.

1-Methyl-5-(methylamino)imidazole-4-carboxamide (3a) i) Compound **6c** (86 mg, 0.204 mmol) was hydrogenated over 10% Pd–C (30 mg) in EtOH (10 ml) at room temperature and atmospheric pressure for 9 h. The catalyst was filtered off and washed with EtOH (10 ml). The combined filtrate and washings were concentrated *in vacuo* to leave crude **3a** (25 mg, 66%), mp 190—198 °C (dec.). Recrystallization from EtOH gave colorless prisms, mp 211—212 °C (dec.), identical (IR spectroscopy and thin-layer chromatographic mobility) with an authentic sample.[20c]

ii) Compound **6d** (57 mg, 0.102 mmol) was hydrogenated in a manner similar to that described above to afford **3a** (14 mg, 88%), mp 196—200 °C. Recrystallization from EtOH gave colorless prisms, mp 211—213 °C, identical with an authentic sample.[20c]

3,9-Dimethylxanthine (2a) i) Na (470 mg) was dissolved in anhydrous EtOH (20 ml). Compound **4a** (66 mg, 0.29 mmol) was suspended in this solution (3 ml) and the mixture was stirred at room temperature for 1 h to give a clear solution. H_2O (1 ml) was added to the solution and the mixture was concentrated *in vacuo* to *ca.* 1 ml. The solution was brought to pH 7 with 10% aqueous HCl. The resulting precipitate was filtered off, washed with a little H_2O, and dried to afford **2a** (43 mg, 81%) as colorless needles mp > 300 °C. This sample was identical (IR spectroscopy and chromatographic behavior) with an authentic sample.[13]

ii) Compound **4a** (66 mg, 0.29 mmol) was treated with 1 N aqueous NaOH (3 ml) at room temperature for 1 h. The solution was neutralized with 10% aqueous HCl. The precipitate that separated was collected by filtration, washed with a little H_2O, and dried to give **2a**[13] (44 mg, 83%) as colorless needles, mp > 300 °C.

3-Methylxanthosine (2b) A suspension of **4c** (1.41 g, 3.0 mmol) in 1 N aqueous NaOH (30 ml) was stirred at room temperature for 3 h. The resulting solution was brought to pH 7 with 1 N aqueous HCl. The precipitate that separated was collected by filtration, washed with H_2O (1 ml), and dried to give **2b** · 1/3H_2O (626 mg, 69%), mp 190—200 °C (dec.). Recrystallizations from boiling H_2O gave colorless needles, which were dried over P_2O_5 at 2 mmHg and 50 °C for 10 h and then exposed to air until constant weight was reached to afford an analytical sample, mp *ca.* 200 °C (dec.); UV $\lambda_{max}^{95\%\ EtOH}$ 238 nm (ε 10000), 265 (9500); $\lambda_{max}^{H_2O}$ (pH 2) unstable; $\lambda_{max}^{H_2O}$ (pH 7) 238 (10400), 268 (10500); $\lambda_{max}^{H_2O}$ (pH 13) 247 (8300),

268 (11300); ^{1}H-NMR δ: 3.62 [5H, s, NMe and C(5′)-H_2], 3.95 [1H, m, C(4′)-H], 4.10 [1H, m, C(3′)-H], 4.38 [1H, m, C(2′)-H], 5.10 [1H, t, J=5 Hz, C(5′)-OH], 5.28 [1H, d, J=5 Hz, C(3′)-OH], 5.64 [1H, d, J=6 Hz, C(2′)-OH], 6.00 [1H, d, J=5 Hz, C(1′)-H], 8.12 [1H, s, C(8)-H], 11.18 [1H, br s, NH or OH].[18] *Anal.* Calcd for $C_{11}H_{14}N_4O_6 \cdot 1/3H_2O$: C, 43.42; H, 4.86; N, 18.41. Found: C, 43.30; H, 4.82; N, 18.25.

Hydrolytic Cleavage of the Glycosidic Bond of 3-Methylxanthosine (2b) When a solution of **2b** in H_2O was heated under reflux, **5** was found by thin-layer chromatography (TLC) 10 min after the start of the reaction. However, the UV absorbance of the spot corresponding to **5** was very weak in comparison with that of **2b** even after heating for a further 50 min. In 0.1 N aqueous NaOH at 50 °C for 3 h, very little **5** was found to be formed from **2b** by TLC. Thus **2b** proved to be fairly stable in a neutral or a higher pH region. However, **5** was rapidly formed as a sole UV-absorbing product (checked by TLC) under acidic conditions and the absorbance (A_∞) of a reaction mixture on completion of the reaction agreed with that of an equimolar solution of pure **5** in every kinetic run described below.

i) Compound **2b**·1/3H_2O (100 mg, 0.33 mmol) was dissolved in 0.1 N aqueous HCl (5 ml) and the solution was allowed to stand at room temperature. The reaction was completed in 30 min. The precipitate that separated was dissolved by addition of H_2O (12 ml) and the solution was neutralized with concentrated aqueous NH_3. The resulting precipitate was filtered off, washed with a little H_2O, and dried to afford **5** (46 mg, 84%), mp > 300 °C (lit.[19] mp > 300 °C); UV $\lambda_{max}^{H_2O}$ (pH 1) 270 nm (ε 10300); $\lambda_{max}^{H_2O}$ (pH 7) 270.5 (10600); $\lambda_{max}^{H_2O}$ (pH 13) 274.5 (12300); ^{1}H-NMR δ: 3.37 (3H, s, NMe), 7.99 [1H, s, C(8)-H], 11.06 (br s) and 13.45 (br) (1H each, NH's). *Anal.* Calcd for $C_6H_6N_4O_2$: C, 43.37; H, 3.64; N, 33.73. Found: 43.48; H, 3.47; N, 33.64.

ii) Cleavage Rate at pH 2 and 25 °C: A 7.3—7.6 $\times 10^{-5}$ M solution in 1.0 M HCl–KCl buffer (pH 2.00 at 25 °C) was kept at 25 °C (accurate to ±0.05 °C). At intervals, aliquots were removed from the mixture and the optical densities (A_t's) were determined at 238 nm. A plot of ln ($A_t - A_\infty$) against time gave a straight line and linear regression analyses for the results obtained in two separate runs afforded the pseudo-first-order rate constant (k_{obs}), $(3.5 \pm 0.1) \times 10^{-2}$ min^{-1}.

iii) Cleavage Rate in 0.1 N Aqueous HCl (Ionic Strength 1.0) at 25 °C: A stock solution of **2b** (2.657 mg) was prepared by dissolving in 1.0 M aqueous KCl (100 ml). A portion of the stock solution (4.5 ml) and 1.0 M aqueous HCl (0.5 ml) was mixed at 25 °C (the observed pH was 1.02) in the same way as reported for the hydrolysis of 3-methylinosine in 0.1 N aqueous HCl[7b] and the absorbance of the mixture was followed at 238 nm in a manner similar to that reported for the hydrolysis of 3-methylguanosine under similar conditions.[4c] For three separate runs k_{obs} $(2.8 \pm 0.1) \times 10^{-1}$ min^{-1} was obtained.

iv) Cleavage Rate in 1 N Aqueous HCl: A stock solution was prepared by dissolving **2b** (1.006 mg) in H_2O (20 ml). The rate analyses for the two kinetic runs were performed using a mixture of the stock solution (2.0 ml) and 2.0 N aqueous HCl (2.0 ml) in the same way as described under method (iii) to give k_{obs} $(8.2 \pm 0.2) \times 10^{-1}$ min^{-1}.

Hydrolysis of Xanthosine (10) to Xanthine (11) Commercial **10** was recrystallized from H_2O and then dried over P_2O_5 at 2 mmHg and 100 °C for 3 h. Exposure of this material to air until constant weight was reached afforded an analytical sample as the sesquihydrate,[23] mp *ca.* 220 °C (dec.). *Anal.* Calcd for $C_{10}H_{11}N_4O_6 \cdot 3/2H_2O$: C, 38.59; H, 4.86; N, 18.00. Found: C, 38.38; H, 4.78; N, 18.13. The rate of hydrolysis of the glycosidic bond of **10** at 25 °C in 1 N aqueous HCl was determined using a 7.02×10^{-5} M solution of the analytical sample of **10** in the same way (analytical wavelength 240 nm) as described for **2b** under method (ii) to give k_{obs} 6.8×10^{-4} min^{-1}. The infinity reading (A_∞) agreed with the absorbance of an equimolar concentration of pure **11**.

References and Notes

1) S. Takemura, H. Kasai, and M. Goto, *J. Biochem.* (Tokyo), **75**, 1169 (1974).
2) J. A. McCloskey, P. F. Crain, C. G. Edmonds, R. Gupta, T. Hashizume, D. W. Phillipson, and K. O. Stetter, *Nucleic Acids Res.*, **15**, 683 (1987).
3) *a*) U. L. RajBhandary, R. D. Faulkner, and A. Stuart, *J. Biol. Chem.*, **243**, 575 (1968); *b*) R. Thiebe and H. G. Zachau, *Eur. J. Biochem.*, **5**, 546 (1968); *c*) S. H. Blobstein, R. Gebert, D. Grunberger, K. Nakanishi, and I. B. Weinstein, *Arch. Biochem. Biophys.*, **167**, 668 (1975).
4) *a*) S. Nakatsuka, T. Ohgi, and T. Goto, *Tetrahedron Lett.*, **1978**, 2579; *b*) T. Itaya, T. Watanabe, and H. Matsumoto, *J. Chem. Soc., Chem. Commun.*, **1980**, 1158; *c*) T. Itaya, H. Matsumoto, T. Watanabe, and T. Harada, *Chem. Pharm. Bull.*, **33**, 2339 (1985); *d*) B. Golankiewicz and W. Folkman, *Nucleic Acids Res.*, **11**, 5243 (1983); *e*) H. Bazin, X-X. Zhou, C. Glemarec, and J. Chattopadhyaya, *Tetrahedron Lett.*, **28**, 3275 (1987); *f*) T. Itaya, *Chem. Pharm. Bull.*, **35**, 4372 (1987); *g*) T. Itaya, M. Shimomichi, and M. Ozasa, *Tetrahedron Lett.*, **29**, 4129 (1988).
5) T. Itaya and T. Harada, *J. Chem. Soc., Chem. Commun.*, **1984**, 858.
6) T. Itaya and K. Ogawa, *Tetrahedron Lett.*, **1978**, 2907.
7) *a*) T. Itaya and H. Matsumoto, *Tetrahedron Lett.*, **1978**, 4047; *b*) *Idem, Chem. Pharm. Bull.*, **33**, 2213 (1985).
8) T. Itaya and T. Harada, *Tetrahedron Lett.*, **23**, 2203 (1982).
9) T. Saito and T. Fujii, *J. Chem. Soc., Chem. Commun.*, **1979**, 135.
10) A preliminary report of this work has been published: T. Itaya and T. Harada, *Heterocycles*, **19**, 687 (1982).
11) M. Adler and A. B. Gutman, *Nature* (London), **204**, 681 (1964).
12) W. Pfleiderer and G. Nübel, *Justus Liebigs Ann. Chem.*, **647**, 155 (1961).
13) T. Itaya and K. Ogawa, *Tetrahedron*, **38**, 1767 (1982).
14) *a*) E. Bamberger and B. Berlé, *Justus Liebigs Ann. Chem.*, **273**, 342 (1893); *b*) O. Gerngross, *Ber. Dtsch. Chem. Ges.*, **46**, 1913 (1913).
15) *a*) W. E. Stewart and T. H. Siddall, III, *Chem. Rev.*, **70**, 517 (1970); *b*) L. M. Jackman, "Dynamic Nuclear Magnetic Resonance Spectroscopy," ed. by L. M. Jackman and F. A. Cotton, Academic Press, New York, 1975, pp. 204—215; *c*) T. Itaya and K. Ogawa, *Chem. Pharm. Bull.*, **33**, 1906 (1985); *d*) T. Fujii, T. Itaya, T. Saito, K. Mohri, M. Kawanishi, and T. Nakasaka, *ibid.*, in press; *e*) References cited in ref. 15*d*.
16) *a*) E. Lustig, W. R. Benson, and N. Duy, *J. Org. Chem.*, **32**, 851 (1967); *b*) S. van der Werf, T. Olijnsma, and J. B. F. N. Engberts, *Tetrahedron Lett.*, **1967**, 689; *c*) S. van der Werf and J. B. F. N. Engberts, *ibid.*, **1968**, 3311; *d*) L. H. Keith and A. L. Alford, *J. Ass. Offic. Anal. Chem.*, **53**, 157 (1970).
17) Prepared by T. Fujii *et al.*: unpublished work.
18) We prefer not to commit ourselves as to the tautomeric form of the base moiety.
19) L. B. Townsend and R. K. Robins, *J. Am. Chem. Soc.*, **84**, 3008 (1962).
20) *a*) J. W. Marsico and L. Goldman, *J. Org. Chem.*, **30**, 3597 (1965); *b*) T. Itaya and K. Ogawa, *Heterocycles.*, **6**, 965 (1977); *c*) T. Itaya, K. Ogawa, H. Matsumoto, and T. Watanabe, *Chem. Pharm. Bull.*, **28**, 2819 (1980); *d*) T. Fujii, T. Saito, and M. Kawanishi, *Tetrahedron Lett.*, **1978**, 5007.
21) T. Itaya, H. Matsumoto, and T. Watanabe, *Chem. Pharm. Bull.*, **30**, 86 (1982).
22) W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
23) The hemihydrate was reported: G. A. Howard, A. C. McLean, G. T. Newbold, F. S. Spring, and A. R. Todd, *J. Chem. Soc.*, **1949**, 232.