Purines. LIII.[1] Deamination of 1-(ω-Hydroxyalkyl)adenine Derivatives by Nucleophiles[2]

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On treatment with an excess of imidazole in boiling *N,N*-dimethylformamide (DMF) for 30 min, 9-ethyl-1-(2-hydroxyethyl)adenine hydrobromide (4a) afforded the corresponding 1-[2-(1*H*-imidazol-1-yl)ethyl]hypoxanthine derivative (13a) in 52% yield. The 1-(3-hydroxypropyl) homologue (4b) and 1-(2-hydroxyethyl)adenosine perchlorate (4c) reacted similarly with imidazole, giving the corresponding deaminated products (13b and 13c). Treatment of 4a with pyridine or thiophenol in boiling DMF also caused a similar deamination, furnishing the corresponding hypoxanthine derivative (16 or 17) with replacement of the hydroxy group by the nucleophile. The reaction of 4a with sodium ethoxide in boiling EtOH failed to cause deamination but gave the rearranged product (6a) in 95% yield. The free base (15) of 4a did not give the deaminated product (13a) when treated with imidazole in boiling DMF, and 4a alone was stable in boiling DMF for at least 30 min. On the basis of these results, a probable mechanism is proposed for the deamination.

Keywords 1-(ω-hydroxyalkyl)adenine; nucleophile; deamination; hypoxanthine 1,9-disubstituted; neighboring group participation; tetrahedral intermediate; addition–elimination mechanism; Dimroth rearrangement

Hydrolytic deamination of adenosine (**1**) to inosine (**3**) is an important reaction in the metabolism of the former nucleoside. The reaction is catalyzed by adenosine deaminase (adenosine aminohydrolase), which has a widespread distribution in various organisms.[3] Although the details of the enzyme mechanism,[4] including the amino acid residues involved in the catalytic process,[4,5] remain obscure, the enzyme is believed to operate *via* an addition–elimination type mechanism with attack of water on the substrate to form a tetrahedral intermediate (**2**) (Chart 1). Several studies have suggested that the mechanism involves protonation of **1** at N(1) by a sulfhydryl group, coupled with hydration[4] (through general-base assistance by a histidine residue) or covalent adduct formation (possibly with a sulfhydryl residue) at C(6),[4g] and subsequent ammonia release from the resulting tetrahedral intermediate (type **2**). In a previous paper from this laboratory,[6] we have reported that in hot H_2O at near-neutrality the 1-(ω-hydroxyalkyl)adenine derivatives **4a—c** undergo hydrolytic deamination to give the 1-(ω-hydroxyalkyl)hypoxanthine derivatives **5a—c**, in competition with the usual Dimroth rearrangement to produce the N^6-(ω-hydroxyalkyl)adenine derivatives **6a—c** (Chart 2). The observed deamination is of particular interest since it is also assumed to proceed through the tetrahedral intermediate **7** or **11** by an addition–elimination mechanism (Chart 3 where $Nu{=}OH^-$) somewhat similar to that proposed for the enzymatic deamination, involving intramolecular participation of the neighboring hydroxy group in nucleophilic (**4**→**7**→**8**→**9**→**5**) or general-base catalysis (**10**→**11**→**12**→**5**). To reach a better understanding of the role of the ω-hydroxyalkyl group at the 1-position, we investigated the reactions of the 1-(ω-hydroxyalkyl)-adenine derivatives **4a—c** with several nucleophiles in nonaqueous media in the present study.

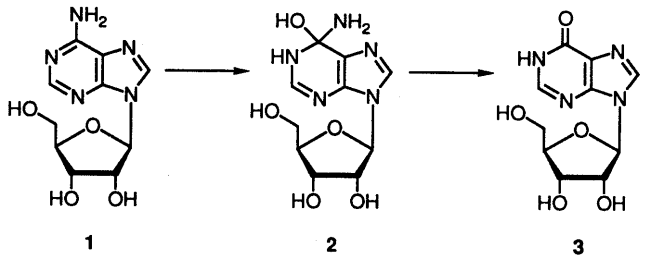
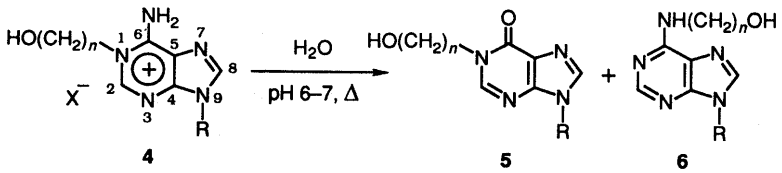


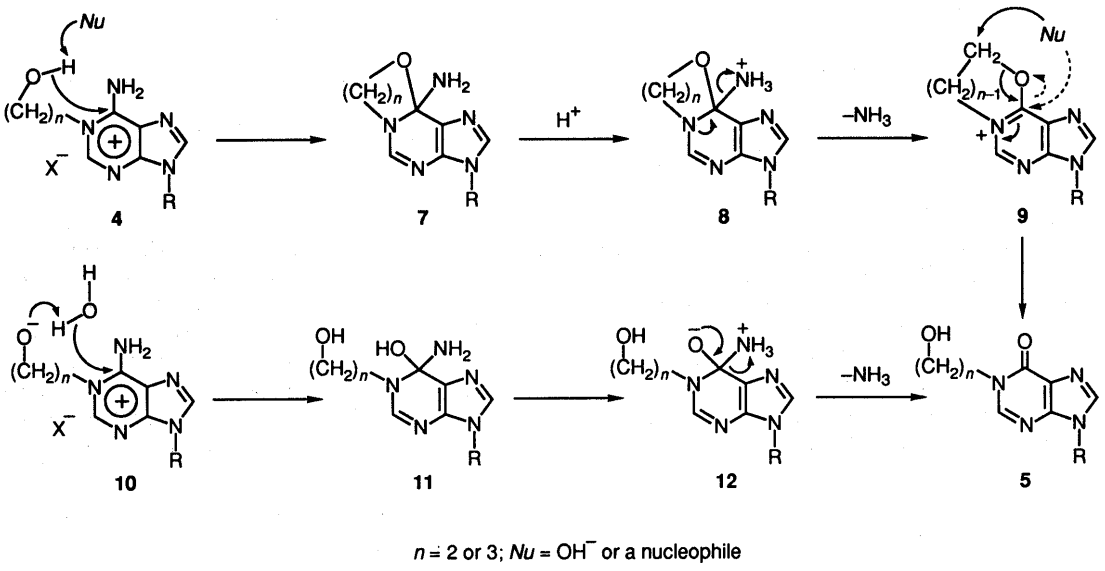
Chart 1

The nucleophile selected first for the substrates **4a—c** was imidazole. On treatment with an excess amount (5 molar eq) of imidazole in boiling *N,N*-dimethylformamide (DMF) for 30 min, **4a** gave 9-ethyl-1-[2-(1*H*-imidazol-l-yl)ethyl]hypoxanthine (**13a**) in 52% yield. Characterization of **13a** as the hypoxanthine derivative was readily achieved by elemental analysis and measurements of its proton nuclear magnetic resonance (^{1}H-NMR) spectrum in Me_2SO-d_6 [δ 4.36 (4H, s, two CH_2's)] and of its ultraviolet (UV) spectra in acid, neutral, and basic madia [which were similar to those[6] of 9-ethyl-1-(2-hydroxyethyl)hypoxanthine (**5a**)], as well as by direct comparison with an authentic sample of the candidate structure. The candidate structure was synthesized from **5a** (Chart 4): tosylation of **5a**[6] with



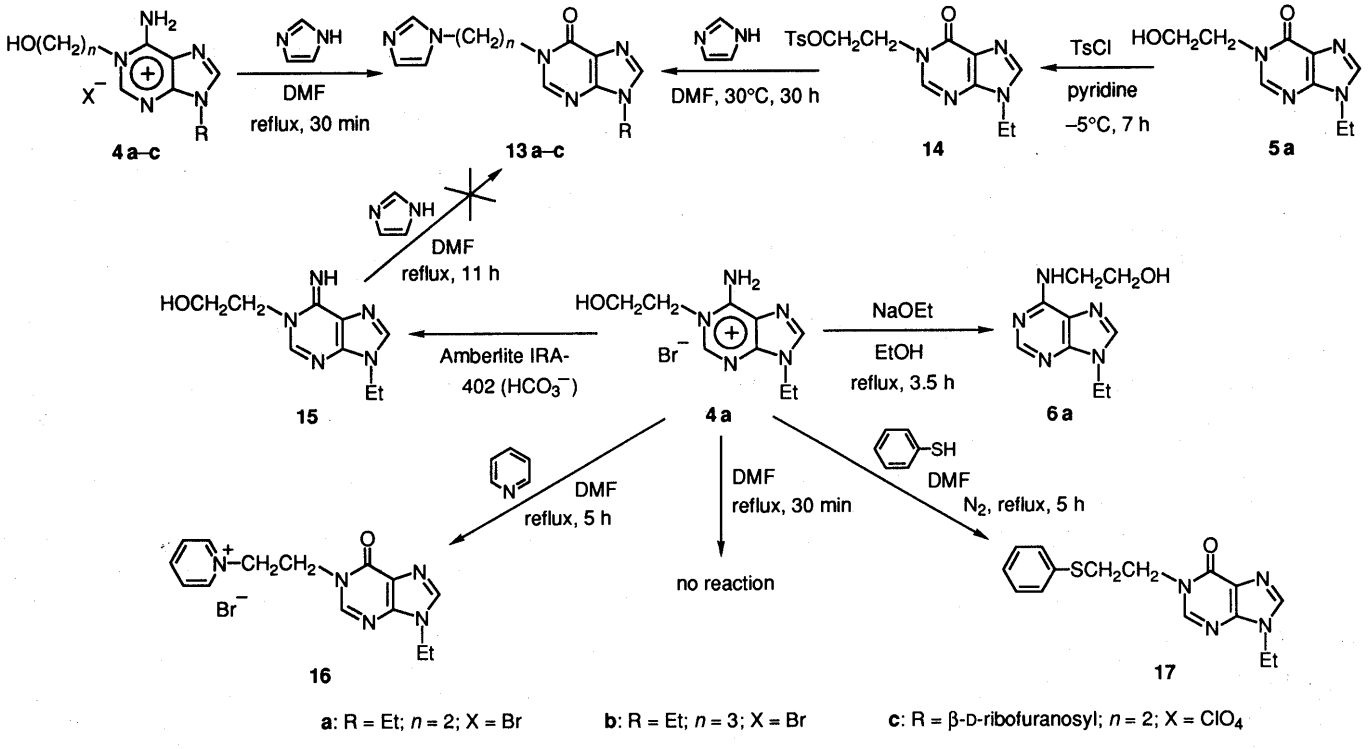
a: R = Et; *n* = 2; X = Br
b: R = Et; *n* = 3; X = Br
c: R = β-D-ribofuranosyl; *n* = 2; X = ClO_4

Chart 2



n = 2 or 3; Nu = OH^- or a nucleophile

Chart 3



a: R = Et; n = 2; X = Br **b**: R = Et; n = 3; X = Br **c**: R = β-D-ribofuranosyl; n = 2; X = ClO_4

Ts = p-toluenesulfonyl

Chart 4

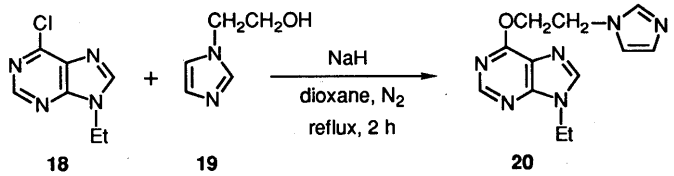


Chart 5

p-toluenesulfonyl chloride (TsCl) in pyridine at −5 °C for 7 h gave the 1-(2-tosyloxyethyl) derivative **14** in 87% yield, and **14** was treated with an excess of imidazole in DMF at 30 °C for 30 h to afford **13a** (53% yield), which was identical with the deaminated product from **4a**. In addition, we confirmed the deaminated product to be different from the isomeric structure **20**, which was prepared in 66% yield from 6-chloro-9-ethylpurine (**18**) by condensation with 1-(2-hydroxyethyl)imidazole (**19**) (Chart 5). Imidazole was further found to react with the 1-(3-hydroxypropyl) homologue **4b** in a similar manner, giving the corresponding deaminated product **13b**, which was isolated in 34% yield in the form of the perchlorate salt. A similar treatment of 1-(2-hydroxyethyl)adenosine perchlorate (**4c**) with imidazole afforded the inosine derivative **13c** in 36% yield.

Pyridine and thiophenol were also separately found to effect a similar deamination of **4a**. On treatment with a large excess of pyridine in boiling DMF for 5h, **4a** furnished

the hypoxanthine derivative **16** in 39% yield. Treatment of **4a** with 5 molar eq of thiophenol in boiling DMF for 5 h produced the 1-(2-phenylthioethyl)hypoxanthine derivative **17** in 31% yield. However, sodium ethoxide, a strongly basic nucleophile, failed to effect a similar deamination when it was boiled with **4a** in EtOH for 3.5 h: the product isolated in 95% yield was 9-ethyl-N^6-(2-hydroxyethyl)adenine (**6a**), a Dimroth rearrangement product.

It may deserve particular mention that the substrate **4a** alone was stable in boiling DMF for at least 30 min and that the corresponding free base **15**, prepared from **4a** by the use of Amberlite IRA-402 (HCO_3^-) in 56% yield, did not give the deaminated product **13a** when treated with 5 molar eq of imidazole in boiling DMF for 30 min. These results suggest that both the protonated form (type **4**) of the substrates and less basic nucleophiles are required for deamination of this type. The reaction is most likely to proceed through the tetrahedral intermediates **7** and **8** and the oxazolinium intermediate **9** (Chart 3 where *Nu* = imidazole, pyridine, or thiophenol) by the addition–elimination mechanism in which the mode of intramolecular participation of the side-chain hydroxy group is nucleophilic. The ring opening of fused oxazolinium rings by attack of a nucleophile at the sp^3 carbon adjacent to the oxygen atom, as in the case of **9**, has been accepted in many instances.[7] A similar mechanism (Chart 3 where $Nu = OH^-$) may be operative in the previously reported[6] hydrolytic deamination of **4a**—**c** in hot H_2O at near-neutrality.

In conclusion, the present results reveal that the ω-hydroxyalkyl group at the 1-position of 9-substituted adenines makes deamination possible with a less basic nucleophile, such as imidazole, pyridine, or thiophenol, in boiling DMF. Deamination of this type is of particular interest in connection with the mechanism proposed[4,5] for hydrolytic deamination of adenosine (**1**) by adenosine deaminase. It would become an alternative to the classical direct deamination of adenine derivatives by means of nitrous acid[8] if introduction of an ω-hydroxyalkyl group into 9-substituted adenines could be made more efficient and removal of the control synthon from the deaminated product (type **13**, **16**, or **17**) were feasible.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. Paper chromatography (PPC) was done on Toyo Roshi No. 51 filter paper by the ascending method with solvent system A [1-butanol–H_2O–AcOH (75:20:5, v/v)], solvent system B [1-butanol–28% aqueous NH_3–H_2O (4:1:1, v/v)], or solvent system C [2-propanol–1% aqueous $(NH_4)_2SO_4$ (2:1, v/v)]. Thin-layer chromatography (TLC) was performed on Merck silica gel GF_{254} (type 60) plates, Merck aluminum oxide GF_{254} (type E) plates, or Funakoshi Avicel SF-2020F plates. In both PPC and TLC, spots were detected by means of UV absorbance (at 254 nm) and/or by spraying with the standard I_2–KI reagent. Spectra reported herein were recorded on a Hitachi model 323 UV spectrophotometer [for solutions in 95% (v/v) aqueous EtOH, 0.1 N aqueous HCl (pH 1), 0.005 M phosphate buffer (pH 7), and 0.1 N aqueous NaOH (pH 13)], a JASCO IRA-2 infrared (IR) spectrophotometer, a JEOL JMS-01SG mass spectrometer, or either a JEOL JNM-PS-100 (^{1}H 100 MHz) or a JEOL JNM-EX-270 (^{1}H 270 MHz) NMR spectrometer, and chemical shifts are reported in ppm downfield from internal Me_4Si. 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.

9-Ethyl-1-[2-(1*H*-imidazol-1-yl)ethyl]hypoxanthine (13a) i) From **4a**: A stirred mixture of **4a**[6] (432 mg, 1.5 mmol) and imidazole (511 mg, 7.5 mmol) in dry DMF (7.5 ml) was heated under reflux for 30 min. The resulting yellow solution was concentrated *in vacuo* to leave a yellow oil, which was washed with boiling ether (3 × 5 ml) in order to remove excess imidazole and then dissolved in H_2O (1 ml). The aqueous solution was passed through a column packed with Amberlite IRA-402 (HCO_3^-) (15 ml), and the column was eluted with H_2O (250 ml). The aqueous eluate was concentrated to dryness *in vacuo* to leave a yellow solid. Recrystallization of the solid, after having been dried over conc. H_2SO_4 at 18 mmHg and room temperature overnight, from AcOEt gave **13a** (203 mg, 52%) as a colorless solid, mp 160—163 °C. Further recrystallization from AcOEt provided an analytical sample as colorless prisms, mp 166—167 °C; MS *m/z*: 258 (M^+); UV $\lambda_{max}^{95\%aq.EtOH}$ 248 nm (sh) (ε 8500), 254 (8900); $\lambda_{max}^{H_2O}$ (pH 1) 252 (9200); $\lambda_{max}^{H_2O}$ (pH 7) 253 (9500); $\lambda_{max}^{H_2O}$ (pH 13) 253 (9100) (slightly unstable); ^{1}H-NMR (Me_2SO-d_6) δ[9]: 1.38 [3H, t, J = 7 Hz, N(9)-CH_2Me], 4.14 [2H, q, J = 7 Hz, N(9)-CH_2], 4.36 [4H, s, N(1)-CH_2CH_2-N(1″)], 6.88, 7.14, and 7.50 (1H each, br s, imidazole protons), 7.82 and 8.43 (1H each, s, purine protons). *Anal.* Calcd for $C_{12}H_{14}N_6O$: C, 55.80; H, 5.46; N, 32.54. Found: C, 55.52; H, 5.63; N, 32.57.

ii) From **14**: A stirred mixture of **14** (*vide infra*) (362 mg, 1 mmol) and imidazole (205 mg, 3 mmol) in dry DMF (2 ml) was kept at 30 °C for 30 h. The resulting yellow solution was concentrated *in vacuo* to leave a yellow oil, which was washed with ether (3 × 5 ml) and then dissolved in a little H_2O. The aqueous solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (4 ml), the column was eluted with H_2O (500 ml), and the eluate was concentrated to dryness *in vacuo* to leave a yellow oil. The oil was dried over conc. H_2SO_4 at 18 mmHg and room temperature for 2 d and then extracted with boiling AcOEt (3 × 10 ml). The AcOEt extracts were concentrated to a volume of *ca.* 5 ml and kept at room temperature for 9 h to deposit **13a** (138 mg, 53%) as a colorless solid, mp 158—164 °C. Recrystallization of the solid from AcOEt gave a pure sample (106 mg) as colorless prisms, mp 163—165.5 °C. This product was identical (by comparison of the PPC and TLC mobilities and IR spectrum) with the one prepared from **4a**.

9-Ethyl-1-[3-(1*H*-imidazol-1-yl)propyl]hypoxanthine (13b) A stirred mixture of **4b**[6] (1.00 g, 3.3 mmol) and imidazole (1.12 g, 16.5 mmol) in dry DMF (20 ml) was heated under reflux for 30 min. The reaction mixture was worked up in a manner similar to that described above for **13a**, giving crude **13b** (*ca.* 1.1 g) as a colorless solid. The solid was dissolved in EtOH (1 ml), and a solution of 70% aqueous $HClO_4$ (1.35 g) in EtOH (1 ml) was added. The colorless prisms (806 mg) that deposited were filtered off, washed with EtOH, and recrystallized twice from MeOH to provide **13b**·$HClO_4$ (417 mg, 34%) as colorless prisms, mp 235.5—239 °C. Further recrystallizations from MeOH furnished an analytical sample of **13b**·$HClO_4$, mp 239—241 °C; UV $\lambda_{max}^{95\%aq.EtOH}$ 247 nm (sh) (ε 9100), 253 (9600); $\lambda_{max}^{H_2O}$ (pH 1) 253 (9800); $\lambda_{max}^{H_2O}$ (pH 7) 253.5 (9600); $\lambda_{max}^{H_2O}$ (pH 13) 253.5 (9600) (slightly unstable); ^{1}H-NMR (Me_2SO-d_6) δ[9]: 1.40 [3H, t, J = 7 Hz, N(9)-CH_2Me], 2.27 [2H, quintet, J = 7 Hz, N(1)-$CH_2CH_2CH_2$], 4.06 [2H, t, J = 7 Hz, N(1)-CH_2 or N(1″)-CH_2], 4.17 [2H, q, J = 7 Hz, N(9)-CH_2], 4.25 [2H, t, J = 7 Hz, N(1″)-CH_2 or N(1)-CH_2], 7.68 and 7.80 [1H each, br s, C(4″)-H and C(5″)-H], 8.14 and 8.38 (1H each, s, purine protons), 9.11 [1H, br s, C(2″)-H], 14.17 (1H, br, NH). *Anal.* Calcd for $C_{13}H_{16}N_6O$·$HClO_4$: C, 41.89; H, 4.60; N, 22.54. Found: C, 41.93; H, 4.62; N, 22.56.

1-[2-(1*H*-Imidazol-1-yl)ethyl]inosine (13c) A stirred solution of **4c**[10] (412 mg, 1 mmol) and imidazole (340 mg, 5 mmol) in dry DMF (5 ml) was heated under reflux for 30 min. The reaction mixture was concentrated *in vacuo* to leave a brown oil, which was dissolved in H_2O (1 ml). The aqueous solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (10 ml), and the column was eluted with H_2O (120 ml). The eluate was evaporated to dryness *in vacuo*, and the brown oily residue was dried over conc. H_2SO_4 at 18 mmHg and room temperature overnight and then purified by means of column chromatography [Whatman cellulose powder CC31 (50 g), 1-butanol–28% aqueous NH_3–H_2O (4:1:1, v/v)], yielding a colorless solid (246 mg). The solid was recrystallized from EtOH to give **13c** (132 mg, 36%) as slightly brownish prisms, mp 202.5—215 °C. Further recrystallizations from EtOH provided an analytical sample as colorless prisms, mp 208—212 °C; UV $\lambda_{max}^{95\%aq.EtOH}$ 246 nm (sh) (ε 9200), 252 (9600), 268 (sh) (5300); $\lambda_{max}^{H_2O}$ (pH 1) 251 (10000); $\lambda_{max}^{H_2O}$ (pH 7) 251 (10200); $\lambda_{max}^{H_2O}$ (pH 13) 251 (9300) (slightly unstable); ^{1}H-NMR (Me_2SO-d_6) δ[9]: 3.58 [2H, m, C(5′)-H′s], 3.93 [1H, m, C(4′)-H], 4.11 [1H, m, C(3′)-H], 4.36 [4H, s, N(1)-CH_2CH_2-N(1″)], 4.44 [1H, m, C(2′)-H], 4.7—5.3 [2H, br, C(3′)-OH and C(5′)-OH], 5.44 [1H, br, C(2′)-OH], 5.82 [1H, d, J = 5.5 Hz, C(1′)-H], 6.90, 7.15, and 7.54 (1H each, br s, imidazole protons), 7.88 and 8.36 (1H each, s, purine protons). *Anal.* Calcd for $C_{15}H_{18}N_6O_5$: C, 49.72; H, 5.01; N, 23.19. Found: C, 49.59; H, 5.39; N, 23.03.

9-Ethyl-1-[2-(*p*-toluenesulfonyloxy)ethyl]hypoxanthine (14) The monohydrate **5a**·H_2O[6] (638 mg, 2.82 mmol) was dried over P_2O_5 at 2 mmHg and 110 °C for 3 h and then dissolved in dry pyridine (7 ml) with application of heat. The resulting solution was stirred, keeping the temperature below −5 °C by cooling, and *p*-toluenesulfonyl chloride (1.66 g, 8.71 mmol) was added. After stirring had been continued at the same temperature for 7 h, cold H_2O (13 ml) was added dropwise at such a rate that the inner temperature did not exceed 5 °C. The reaction mixture was then extracted with $CHCl_3$ (2×35 ml). The $CHCl_3$ extracts were combined, washed successively with 10% aqueous H_2SO_4 (13 ml), H_2O (20 ml), saturated aqueous $NaHCO_3$ (10 ml), and H_2O (20 ml), all of which had been ice-cooled, and dried over anhydrous Na_2SO_4 overnight in a refrigerator. The dried $CHCl_3$ solution was concentrated to dryness *in vacuo* below room temperature, leaving **14** (890 mg, 87%) as colorless prisms, mp 223—300 °C (dec.). Recrystallization of **14** by dissolving it in $CHCl_3$ and adding hexane to the resulting solution furnished an analytical sample as colorlss prisms, mp 217—300 °C (dec.); UV $\lambda_{max}^{95\%aq.EtOH}$ 253.5 nm (ε 8900); ^{1}H-NMR (Me_2SO-d_6) δ[9]: 1.52 [3H, t, *J*=7.5 Hz, N(9)-CH_2Me], 2.33 [3H, s, C(4″)-Me], 4.19 [2H, q, *J*=7.5 Hz, N(9)-C$\underline{H}_2$Me], 4.29 [4H, s, N(1)-C$\underline{H}_2$C$\underline{H}_2$O], 7.12 [2H, d, *J*=8 Hz, C(3″)-H and C(5″)-H], 7.56 [2H, d, *J*=8 Hz, C(2″)-H and C(6″)-H], 7.72 and 7.88 (1H each, s, purine protons). *Anal.* Calcd for $C_{16}H_{18}N_4O_4S$: C, 53.03; H, 5.01; N, 15.46. Found: C, 52.83; H, 5.02; N, 15.38.

9-Ethyl-1-(2-hydroxyethyl)adenine (15) A solution of **4a**[6] (700 mg, 2.43 mmol) in H_2O (1 ml) was passed through a column packed with Amberlite IRA-402 (HCO_3^-) (9.7 ml), and the column was eluted with H_2O (350 ml). Concentration of the eluate under vacuum and drying of the residue gave crude **15** (508 mg) as a slightly brown solid, which was shown to be contaminated with a small amount of the rearranged product **6a** on PPC and TLC analyses. Two recrystallizations of the solid from acetone yielded a chromatographically pure sample of **15** (282 mg, 56%) as colorless prisms, mp 169.5—173.5 °C (dec.). Further recrystallizations from acetone furnished an analytical sample, mp 185—186.5 °C (dec.); UV $\lambda_{max}^{95\%aq.EtOH}$ 260.5 nm (ε 12800); $\lambda_{max}^{H_2O}$ (pH 1) 261 (12300); $\lambda_{max}^{H_2O}$ (pH 7) 261 (12400); $\lambda_{max}^{H_2O}$ (pH 13) 260.5 (13300); ^{1}H-NMR (Me_2SO-d_6) δ: 1.36 [3H, t, *J*=7 Hz, N(9)-CH_2Me], 3.43—4.36 (6H, m, three CH_2's), 5.10—6.53 (2H, br, OH and NH), 7.86 (2H, s, purine protons). *Anal.* Calcd for $C_9H_{13}N_5O$: C, 52.16; H, 6.32; N, 33.79. Found: C, 51.86; H, 6.35; N, 33.59.

In a separate experiment, a stirred mixture of **15** (10 mg, 0.048 mmol) and imidazole (164 mg, 0.241 mmol) in dry DMF (0.24 ml) was heated under reflux for 11 h. At intervals, aliquots of the reaction mixture were analyzed by means of TLC. However, the detection of a spot corresponding to that of **13a** was difficult because of the complicated pattern of the chromatogram.

Stability of 4a in Boiling DMF A stirred suspension of **4a**[6] (144 mg, 0.5 mmol) in dry DMF (2.5 ml) was heated under reflux for 30 min. The reaction mixture, showing only one spot corresponding to that of **4a** on PPC and TLC analyses, was allowed to cool, and the crystals that deposited were filtered off, washed with EtOH, and dried to recover a first crop (53 mg, 37%) of **4a**, mp 254—255 °C (dec.). The filtrate and washings were combined and concentrated to dryness *in vacuo* to leave a solid, which was recrystallized from EtOH, giving a second crop (60 mg, 42%) of **4a**, mp 246—247 °C (dec.). The total recovery of **4a** was 113 mg (78%). Each of the two samples was identical (by mixture melting point test and comparison of the PPC and TLC mobilities and IR spectrum) with authentic **4a**.[6]

1-[2-(9-Ethyl-6-oxo-1*H*-purin-1-yl)ethyl]pyridinium Bromide (16) A stirred mixture of **4a**[6] (864 mg, 3 mmol) and dry pyridine (6 ml) in dry DMF (15 ml) was heated under reflux for 5 h and then cooled in an ice bath. The fine precipitate that resulted was filtered off, washed with a little EtOH, and dried to give **16** (409 mg, 39%) as a slightly brownish solid, mp 273—273.5 °C (dec.). Recrystallizations from MeOH produced an analytical sample as slightly brownish prisms, mp 273—274.5 °C (dec.); UV $\lambda_{max}^{95\%aq.EtOH}$ 254 nm (ε 12500); $\lambda_{max}^{H_2O}$ (pH 1) 253.5 (13100); $\lambda_{max}^{H_2O}$ (pH 7) 253.5 (13100); $\lambda_{max}^{H_2O}$ (pH 13) unstable; ^{1}H-NMR (Me_2SO-d_6) δ: 1.40 [3H, t, *J*=7 Hz, N(9)-CH_2Me], 4.13 [2H, q, *J*=7 Hz, N(9)-C$\underline{H}_2$Me], 4.37—5.20 [4H, m, N(1)-C$\underline{H}_2$C$\underline{H}_2$], 7.83—9.19 (7H, m, purine and pyridine protons). *Anal.* Calcd for $C_{14}H_{16}BrN_5O$: C, 48.01; H, 4.60; N, 20.00. Found: C, 48.03; H, 4.74; N, 20.21.

9-Ethyl-1-(2-phenylthioethyl)hypoxanthine (17) A stirred mixture of **4a**[6] (2.02 g, 7 mmol) and thiophenol (3.86 g, 35 mmol) in dry DMF (70 ml) was heated under reflux in an atmosphere of N_2 for 5 h. The reaction mixture was concentrated to dryness *in vacuo* to leave a greenish oil. The oil was dissolved in EtOH (14 ml), and a solution of 70% aqueous $HClO_4$ (1.54 g) in EtOH (9 ml) was added. The resulting mixture was kept in a refrigerator overnight, and the colorless prisms (**17**·$HClO_4$) that deposited were filtered off, washed with cold EtOH (3×5 ml), and then dissolved in hot H_2O (100 ml). The aqueous solution was brought to pH 8 by addition of 10% aqueous Na_2CO_3 and kept in a refrigerator overnight. The colorless prisms that deposited were filtered off, washed with cold EtOH, and dried to yield the free base **17** (659 mg, 31%), mp 102.5—103 °C. Recrystallization from 80% (v/v) aqueous EtOH gave an analytical sample as colorless prisms, mp 103 °C; positive to a test for detection of sulfur by the sodium fusion method[11]; UV $\lambda_{max}^{95\%aq.EtOH}$ 253.5 nm (ε 15900); $\lambda_{max}^{H_2O}$ (pH 1) 251 (13400); $\lambda_{max}^{H_2O}$ (pH 7) 254 (13200); $\lambda_{max}^{H_2O}$ (pH 13) 254 (13200); ^{1}H-NMR (Me_2SO-d_6) δ: 1.39 [3H, t, *J*=7 Hz, N(9)-CH_2Me], 3.35 (2H, t, *J*=7 Hz, PhS-C$\underline{H}_2$$CH_2$), 4.15 [2H, q, *J*=7 Hz, N(9)-C$\underline{H}_2$Me], 4.22 [2H, t, *J*=7 Hz, N(1)-C$\underline{H}_2$$CH_2$], 7.0—7.4 (5H, m, PhS), 8.10 and 8.28 (1H each, s, purine protons). *Anal.* Calcd for $C_{15}H_{16}N_4OS$: C, 59.98; H, 5.37; N, 18.65. Found: C, 60.00; H, 5.49; N, 18.69.

9-Ethyl-N^6-(2-hydroxyethyl)adenine (6a) Metallic sodium (of 98% purity) (120 mg, 5 mg.-atom) was dissolved in abs. EtOH (5 ml), and **4a**[6] (288 mg, 1 mmol) was added to the resulting solution. The mixture was heated under reflux for 3.5 h with stirring and then concentrated *in vacuo* to leave an oily solid, which was dissolved in H_2O (1 ml). The aqueous solution was brought to pH 7 by addition of 10% aqueous HCl and, if necessary, 28% aqueous NH_3, and concentrated to dryness *in vacuo*. After having been dried over conc. H_2SO_4 at 18 mmHg and room temperature overnight, the residue was extracted with boiling benzene (6×10 ml). The benzene extracts were combined and concentrated *in vacuo* to leave **6a** (197 mg, 95%) as a slightly brownish solid, mp 133—135.5 °C. Recrystallization from AcOEt gave a pure sample as colorless needles, mp 136.5—138.5 °C. This product was identical (by comparison of the PPC and TLC mobilities and IR spectrum) with anthentic **6a**.[6]

9-Ethyl-6-[2-(1*H*-imidazol-1-yl)ethoxy]purine (20) A mixture of 1-(2-hydroxyethyl)imidazole (**19**)[12] (336 mg, 3 mmol) and an oil dispersion (144 mg) containing 50% NaH (3 mmol) in dry dioxane (12 ml) was stirred at room temperature in an atmosphere of N_2 for 2 h, and then 6-chloro-9-ethylpurine (**18**)[13] (274 mg, 1.5 mmol) was added. The resulting mixture was heated under reflux for 2 h with stirring. Concentration of the reaction mixture under reduced pressure left a yellowish semisolid, which was extracted with boiling benzene (4×5 ml). The benzene extracts were combined and concentrated *in vacuo* to leave a slightly yellow oil. Purification of the oil by means of column chromatography [alumina (30 g), $CHCl_3$–EtOH (20:1, v/v)] and recrystallization of the resulting yellowish solid (mp 83—85 °C) from benzene–hexane (3:1, v/v) furnished **20** (259 mg, 66% yield from **18**) as colorless needles, mp 100.5—103 °C. Further recrystallization in a similar manner and drying over P_2O_5 at 2 mmHg and room temperature for 30 h yielded an analytical sample, mp 99.5—103 °C; MS *m/z*: 258 (M^+); UV $\lambda_{max}^{95\%aq.EtOH}$ 251 nm (ε 11500); $\lambda_{max}^{H_2O}$ (pH 1) 254.5 (10500); $\lambda_{max}^{H_2O}$ (pH 7) 255 (11000); $\lambda_{max}^{H_2O}$ (pH 13) 255 (11100); ^{1}H-NMR (Me_2SO-d_6) δ[9]: 1.43 [3H, t, *J*=7 Hz, N(9)-CH_2Me], 4.26 [2H, q, *J*=7 Hz, N(9)-C$\underline{H}_2$Me], 4.26—4.96 [4H, m, N(1″)-C$\underline{H}_2$C$\underline{H}_2$O], 6.84, 7.20, and 7.63 (1H each, br s, imidazole protons), 8.36 and 8.43 (1H each, s, purine protons). *Anal.* Calcd for $C_{12}H_{14}N_6O \cdot 1/5H_2O$: C, 55.04; H, 5.54; N, 32.09. Found: C, 55.08; H, 5.47; N, 32.05.

References and Notes

1) Paper LII in this series, K. Ogawa, M. Nishii, J. Inagaki, F. Nohara, T. Saito, T. Itaya, and T. Fujii, *Chem. Pharm. Bull.*, **40**, 1315 (1992).
2) A part of this work was presented in a preliminary form: T. Saito, M. Murakami, T. Inada, H. Hayashibara, and T. Fujii, Abstracts of Papers, 18th Congress of Heterocyclic Chemistry, Fukuoka (Japan), October 1986, pp. 197—200.
3) R. P. Agarwal, S. Cha, G. W. Crabtree, and R. E. Parks, Jr., "Chemistry and Biology of Nucleosides and Nucleotides," ed. by R. E. Harmon, R. K. Robins, and L. B. Townsend, Academic Press, New York, 1978, p. 160.
4) For mechanistic work, see, for example, *a*) L. C. Kurz and C. Frieden, *Biochemistry*, **22**, 382 (1983); *b*) P. M. Weiss, P. F. Cook, J. D. Hermes, and W. W. Cleland, *ibid.*, **26**, 7378 (1987); *c*) L. C. Kurz and C. Frieden, *ibid.*, **26**, 8450 (1987); *d*) W. Jones, L. C. Kurz, and R. Wolfenden, *ibid.*, **28**, 1242 (1989); *e*) W. M. Kati and R. Wolfenden, *ibid.*, **28**, 7919 (1989); *f*) M. Orozco, E. I. Canela, and R. Franco, *J. Org. Chem.*, **55**, 2630 (1990); *g*) B. A. Orsi, N. McFerran, A. Hill, and A. Bingham, *Biochemistry*, **11**, 3386 (1972).
5) A. Lucacchini, A. D. Bertolini, G. Ronca, D. Segnini, and C. A. Rossi, *Biochim. Biophys. Acta*, **569**, 220 (1979).
6) T. Fujii, T. Saito, and N. Terahara, *Chem. Pharm. Bull.*, **34**, 1094 (1986).

7) See, for example, *a*) N. J. Leonard, K. Conrow, and R. S. Sauers, *J. Am. Chem. Soc.*, **80**, 5185 (1958); *b*) T. Fujii and S. Yoshifuji, *Chem. Pharm. Bull.*, **20**, 1451 (1972); *c*) T. Fujii, K. Yoshida, M. Ohba, and S. Yoshifuji, *ibid.*, **25**, 2336 (1977); *d*) T. Fujii, M. Ohba, and S. Yoshifuji, *ibid.*, **25**, 3042 (1977); *e*) T. Fujii, M. Ohba, M. Tsuchida, K. Saito, Y. Hirano, and J. Sakaguchi, *ibid.*, **34**, 496 (1986); *f*) C. N. Filer, F. E. Granchelli, P. Perri, and J. L. Neumeyer, *J. Org. Chem.*, **44**, 285 (1979); *g*) J. R. Piper, A. G. Laseter, T. P. Johnston, and J. A. Montgomery, *J. Med. Chem.*, **23**, 1136 (1980); *h*) N. Buckley and T. P. Brent, *J. Am. Chem. Soc.*, **110**, 7520 (1988).

8) See, for example, *a*) P. A. Levene and W. A. Jacobs, *Ber. Dtsch. Chem. Ges.*, **43**, 3150 (1910); *b*) G. B. Elion, *J. Org. Chem.*, **27**, 2478 (1962); *c*) Ref. 6; *d*) K. Ogawa, M. Nishii, F. Nohara, T. Saito, T. Itaya, and T. Fujii, *Chem. Pharm. Bull.*, **40**, 612 (1992).

9) For convenience, each aromatic carbon in the side chain is indicated by a double-primed number.

10) H. G. Windmueller and N. O. Kaplan, *J. Biol. Chem.*, **236**, 2716 (1961).

11) L. F. Fieser, "Experiments in Organic Chemistry," 3rd ed., D. C. Heath and Co., Boston, 1955, pp. 226—228.

12) T. Yoshino, S. Inaba, H. Komura, and Y. Ishido, *J. Chem. Soc., Perkin Trans. 1*, **1977**, 1266.

13) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961).