Purines. XL.[1] Preparation of 9-(ω-Carboxyalkyl)-3-methyladenines

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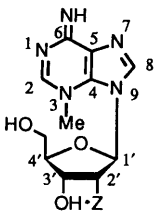
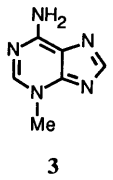
With a view to supplying haptens to be connected to carrier proteins for raising antibodies to 3-methyl-2′-deoxyadenosine (1) and/or 3-methyladenine (3), (3-methyl-9-adenyl)acetic acid hydrochloride (15a) and 4-(3-methyl-9-adenyl)butyric acid hydrochloride (15b) have been prepared from 1-alkoxy-9-(ω-carboxyalkyl)adenine salts (7a and 7b) through the intermediates 11a,b, 12a,b, 13a,b, and 14a,b.

Keywords 3,9-disubstituted adenine; 9-substituted adenine 1-oxide; *N*-oxide *O*-methylation; 9-substituted 1-alkoxyadenine; adenine ring fission; formamido group *N*-methylation; alkaline hydrolysis; hydrogenolytic dealkoxylation; amidine formamido cyclization

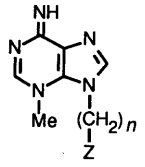
Recent advances in the characterization of deoxyribonucleic acid (DNA) components structurally modified by chemical carcinogens are remarkable.[2] In particular, the modifications produced in DNA by the alkylating *N*-nitroso carcinogens have mostly been identified.[3] 3-Methyl-2′-deoxyadenosine (**1**)[4] is believed to occur as an unstable part structure in methylated DNA molecules.[5] Although loss of 3-methyladenine (**3**) from methylated DNA *in vivo* could be explained in terms of chemical depurinylation alone, active enzymatic excision has also been suggested.[5b] This suggestion led to the isolations of 3-methyladenine-DNA glycosylase in partially purified form from both bacterial and mammalian sources.[5d,e,k,6] The detection of the presence of **1** or **3** could therefore be used to monitor a previous exposure of cells, tissues, and individuals to methylating carcinogens, and it could be quantitatively done by producing antibodies to **1** and/or **3** and setting up radioimmunoassays for them. In the preparation of the antigens, application of the most widely used sugar-cleavage oxidation method[3] to 3-methyladenosine (**2**)[4b,7] would be inadequate because of the extraordinary instability of this nucleoside. The title compounds, 9-(ω-carboxyalkyl)-3-methyladenines (**4**), or 9-(ω-aminoalkyl)-3-methyladenines (**5**) would then provide a practical alternative source of haptens to be connected to carrier proteins[3] for raising antibodies to **1** and/or **3**. In the present study, we synthesized (3-methyl-9-adenyl)acetic acid hydrochloride (**15a**) and 4-(3-methyl-9-adenyl)butyric acid hydrochloride (**15b**), both belonging to type **4**, in response to a request[8] for such potential haptens.



1: Z=H
2: Z=OH

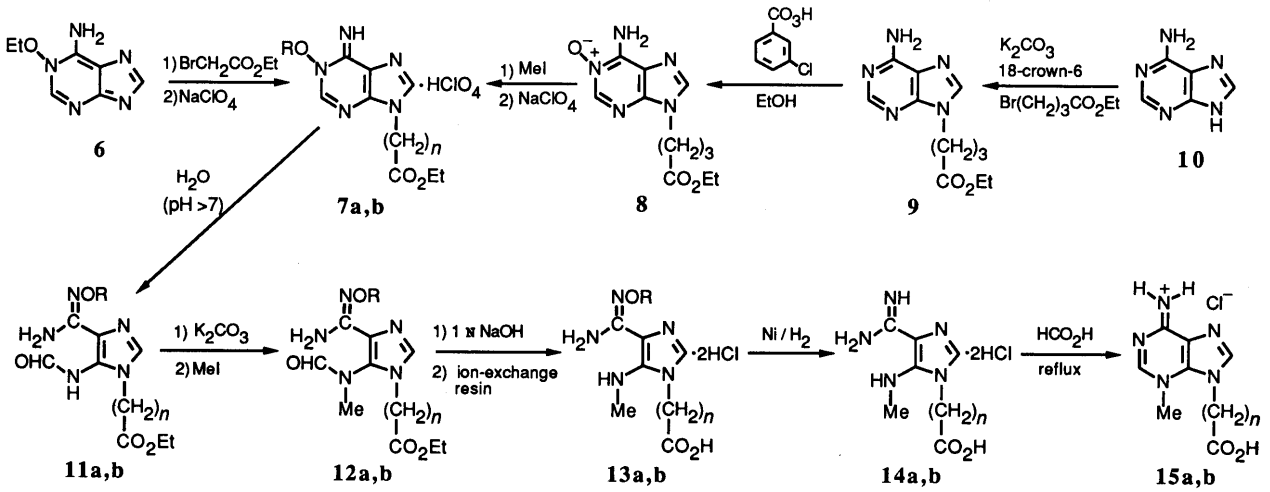


3



4: Z=CO_2H; $n \geq 1$
5: Z=CH_2NH_2; $n \geq 1$

The synthesis of the target compounds **15a** and **15b** was so designed that it becomes a 9-(ω-carboxyalkyl) version of our previous general synthesis[9] of 3,9-dialkyladenine salts, as shown in Chart 1. Treatment of 1-ethoxyadenine (**6**)[10] with ethyl bromoacetate in $AcNMe_2$ at 30 °C for 24 h produced 1-ethoxy-9-[(ethoxycarbonyl)methyl]adenine hydrobromide, which was isolated in the form of the perchlorate **7a** in 56% overall yield (from **6**). This result was in general agreement with those of our previous alkylations of 1-alkoxyadenines at the 9-position.[10] The perchlorate **7a** was converted into the free base by the use of Amberlite IRA-402 (HCO_3^-), and the base was treated with H_2O at 40 °C for 7 h to furnish the formamidoimidazole **11a** in 35% yield. Such a ready ring-opening under mild condi-



6 **7a,b** **8** **9** **10** **11a,b** **12a,b** **13a,b** **14a,b** **15a,b**

series **a**: R = Et; $n = 1$ series **b**: R = Me; $n = 3$

Chart 1

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tions had already been observed by us for 1-alkoxy-9-alkyladenines.[11] On methylation with MeI and anhydrous K_2CO_3 in $HCONMe_2$ at room temperature for 2 h, **11a** afforded the *N*-methylformamido derivative **12a** in 84% yield. Hydrolysis of **12a** was effected in boiling 1 N aqueous NaOH for 15 min, and the product was isolated in 72% yield in the form of the dihydrochloride **13a**. Hydrogenolysis of **13a** using hydrogen and Raney Ni catalyst in H_2O at 1 atm and room temperature for 3 h gave crude **14a**, which was then cyclized with boiling formic acid for 5 h, affording the target compound **15a** in 42% overall yield (from **13a**). A similar cyclization of 1-methyl-5-(methylamino)-1*H*-imidazole-4-carboxamidine hydrochloride (type **14a**: Me in place of CH_2CO_2H) to give 3,9-dimethyladenine hydrochloride had previously been achieved by the use of diethoxymethyl acetate in $HCONMe_2$.[9b]

For the synthesis of the other target compound (**15b**), adenine (**10**) was first treated with ethyl 4-bromobutyrate in $AcNMe_2$ in the presence of anhydrous K_2CO_3 and 18-crown-6 at 30 °C for 24 h, giving ethyl 4-(9-adenyl)butyrate (**9**) in 76% yield.[12] Oxidation of **9** with *m*-chloroperbenzoic acid in EtOH at room temperature furnished the 1-oxide **8** in 81% yield. Methylation of **8** with MeI was effected in $AcNMe_2$ at room temperature for 20 h, and the *O*-methylated product was isolated as the perchlorate **7b** in 82% yield (from **8**). The synthesis of **7b** from **10** through **9** and **8** was 9-[ω-(ethoxycarbonyl)alkyl] version of our well-established general synthesis of 1-alkoxy-9-alkyladenine salts from **10**.[13,14] The succeeding steps beyond **7b** were parallel to those described above for the **a**-series, producing **11b** (63% yield), **12b** (86%), **13b** (crude), **14b** (crude), and **15b** [20% (from **12b**)].

The correctness of the structures of **15a** and **15b** was supported by the way in which they had been generated, microanalytical data, and comparison of their ultraviolet (UV) and nuclear magnetic resonance (NMR) spectra with those of known 3,9-dialkyladenine salts.[9b-d] According to Professor A. E. Pegg (The Pennsylvania State University, U.S.A.),[15] his research group has not been able to obtain antibodies to 3-methyladenine (**3**) by using conjugates synthesized with the above precursors, **15a** and **15b**. Their lack of success in the antibody production may be attributable to the instability of the hapten moiety in the conjugates; facile ring-opening would have occurred under mild alkaline conditions because of the 3,9-disubstituted adenine structure.[9b-d]

Experimental

General Notes All melting points were taken on a Yamato MP-1 capillary melting point apparatus and are corrected. See ref. 9*b* for details of instrumentation and measurements. 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.

6-Amino-9*H*-purine-9-butanoic Acid Ethyl Ester (9) A mixture of adenine (**10**) (405 mg, 3 mmol), anhydrous K_2CO_3 (415 mg, 3 mmol), 18-crown-6 (79 mg, 0.3 mmol), and ethyl 4-bromobutyrate (1.17 g, 6 mmol) in $AcNMe_2$ (12 ml) was stirred at 30 °C for 24 h. The reaction mixture was concentrated to dryness *in vacuo*. The residue was washed with two 5-ml portions of hexane and the washings were removed by decantation. The insoluble solid was then extracted with hot benzene (2 × 10 ml, 4 × 5 ml). The benzene extracts were combined and concentrated to dryness *in vacuo*. The residue was chromatographed on a column packed with silica gel (35 g), and fractions eluted with CH_2Cl_2–EtOH (10 : 1, v/v) gave **9** (565 mg, 76%)[12] as a colorless solid, mp 104—107 °C. Recrystallization from benzene–hexane (2 : 1, v/v) yielded colorless needles, mp 110—111 °C (lit. mp 110—111 °C;[13b] mp 108—109 °C[16]). This sample was identical (by comparison of the IR spectrum and thin-layer chromatographic mobility) with authentic **9**.[13b]

6-Amino-9*H*-purine-9-butanoic Acid Ethyl Ester 1-Oxide (8) *m*-Chloroperbenzoic acid (80% purity) (32.36 g, 150 mmol) was added portionwise to a stirred solution of **9** (18.70 g, 75 mmol) in EtOH (600 ml), and the mixture was stirred at room temperature for 3 h. The precipitate that resulted was filtered off and then dissolved in 50% aqueous EtOH (1200 ml). The resulting solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (360 ml), and the column was eluted with H_2O (1000 ml). The eluate was concentrated to dryness *in vacuo* to leave **8** (16.20 g, 81%) as a colorless solid, mp 208—209 °C. Recrystallization from AcOEt furnished an analytical sample as colorless needles, mp 211—212 °C; MS *m/z*: 265 (M^+); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 235 nm (ε 41400), 263 (7200), 300 (1800); $\lambda_{max}^{H_2O}$ (pH 1) 214 (27800), 259 (11600); $\lambda_{max}^{H_2O}$ (pH 7) 232 (40000), 262 (7100), 292 (1600); $\lambda_{max}^{H_2O}$ (pH 13) 232 (29800), 267 (7900), 302 (3200); IR ν_{max}^{Nujol} 1730 cm^{-1} (CO_2Et); NMR (Me_2SO-d_6) δ: 1.14 (3H, t, *J* = 7 Hz, $CO_2CH_2\underline{Me}$), 2.12 [2H, m, N(9)-$CH_2\underline{CH_2}$], 2.30 (2H, m, $\underline{CH_2}CO_2Et$), 4.00 (2H, q, *J* = 7 Hz, $CO_2\underline{CH_2}Me$), 4.21 [2H, t, *J* = 7 Hz, N(9)-$\underline{CH_2}$], 8.24 (2H, br, NH_2), 8.29 [1H, s, C(8)-H], 8.61 [1H, s, C(2)-H]. *Anal.* Calcd for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.70; N, 26.40. Found: C, 49.86; H, 5.49; N, 26.23.

1-Ethoxy-1,6-dihydro-6-imino-9*H*-purine-9-acetic Acid Ethyl Ester Perchlorate (7a) A mixture of 1-ethoxyadenine (**6**)[10] (21.50 g, 0.12 mol) and ethyl bromoacetate (100.2 g, 0.6 mol) in $AcNMe_2$ (370 ml) was stirred at 30 °C for 24 h. The reaction mixture was concentrated *in vacuo*, and the residual oil was triturated with ether (2 × 200 ml). The insoluble solid that resulted was filtered off, washed with ether, and dissolved in warm H_2O (80 ml), and then a solution of $NaClO_4$ (22.04 g, 0.18 mol) in H_2O (40 ml) was added. The precipitate that deposited was filtered off, washed with H_2O, and recrystallized from 80% aqueous EtOH to yield a first crop (20.89 g, 48%) of **7a**, mp 215—217 °C (dec.). A further crop from the mother liquor of the recrystallization raised the yield of **7a** to 24.78 g (56%). Recrystallization of the first crop of crystals from H_2O gave an analytical sample as colorless prisms, mp 212—213 °C (dec.); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 258 nm (ε 12300); $\lambda_{max}^{H_2O}$ (pH 1) 258 (12400); $\lambda_{max}^{H_2O}$ (pH 7) 258 (12300); $\lambda_{max}^{H_2O}$ (pH 13) (unstable) 258 (12700), 265 (sh) (11400); IR ν_{max}^{Nujol} 1756 cm^{-1} (CO_2Et); NMR (Me_2SO-d_6) δ: 1.24 (3H, t, *J* = 7 Hz, $CO_2CH_2\underline{Me}$), 1.43 [3H, t, *J* = 7 Hz, N(1)-$OCH_2\underline{Me}$], 4.21 (2H, q, *J* = 7 Hz, $CO_2\underline{CH_2}Me$), 4.44 [2H, q, *J* = 7 Hz, N(1)-$O\underline{CH_2}Me$], 5.25 [2H, s, N(9)-CH_2], 8.53 and 9.13 (1H each, s, purine protons), 9.65 and 10.36 (1H each, br, $=NH_2^+$ or 2 × NH). *Anal.* Calcd for $C_{11}H_{15}N_5O_3 \cdot HClO_4$: C, 36.13; H, 4.41; N, 19.15. Found: C, 36.15; H, 4.46; N, 19.05.

1,6-Dihydro-6-imino-1-methoxy-9*H*-purine-9-butanoic Acid Ethyl Ester Perchlorate (7b) A mixture of **8** (3.18 g, 12 mmol) and MeI (4.26 g, 30 mmol) in $AcNMe_2$ (24 ml) was stirred at room temperature for 20 h. The reaction mixture was concentrated *in vacuo*, and the residue was triturated with ether (2 × 20 ml). The insoluble solid that resulted was separated from the ethereal layer by decantation and dissolved in warm H_2O (5 ml), and then a solution of $NaClO_4$ (2.20 g, 18 mmol) in H_2O (3 ml) was added. The crystals that deposited were filtered off, washed with H_2O, and dried to give crude **7b** (3.71 g, 82%), mp 138—141 °C. Recrystallization from H_2O afforded an analytical sample as colorless prisms, mp 153.5—154.5 °C; UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 259 nm (ε 12500); $\lambda_{max}^{H_2O}$ (pH 1) 260 (12500); $\lambda_{max}^{H_2O}$ (pH 7) 260 (12500); $\lambda_{max}^{H_2O}$ (pH 13) (unstable) 258 (12900), 265 (sh) (11500); IR ν_{max}^{Nujol} 1735 cm^{-1} (CO_2Et); NMR (Me_2SO-d_6) δ: 1.16 (3H, t, *J* = 7 Hz, $CO_2CH_2\underline{Me}$), 2.11 [2H, m, N(9)-$CH_2\underline{CH_2}$], 2.31 (2H, m, $\underline{CH_2}CO_2Et$), 4.03 (2H, q, *J* = 7 Hz, $CO_2\underline{CH_2}Me$), 4.17 [3H, s, N(1)-OMe], 4.30 [2H, t, *J* = 6.5 Hz, N(9)-CH_2], 8.56 and 9.15 (1H each, s, purine protons), 9.69 and 10.30 (1H each, br, $=NH_2^+$ or 2 × NH). *Anal.* Calcd for $C_{12}H_{17}N_5O_3 \cdot HClO_4$: C, 37.95; H, 4.78; N, 18.44. Found: C, 37.90; H, 4.93; N, 18.47.

4-(*N'*-Ethoxyamidino)-5-formamido-1*H*-imidazole-1-acetic Acid Ethyl Ester (11a) A solution of **7a** (20.12 g, 55 mmol) in H_2O (1200 ml) was passed through a column of Amberlite IRA-402 (HCO_3^-) (132 ml), and the column was eluted with H_2O. The eluate (2200 ml) was concentrated *in vacuo* to a volume of *ca.* 400 ml and kept at 40 °C for 7 h. The reaction mixture was concentrated to dryness *in vacuo*, and the residue was purified by column chromatography [silica gel (540 g), CH_2Cl_2–EtOH (10 : 1, v/v)] followed by two recrystallizations from AcOEt, furnishing **11a** (5.43 g, 35%) as colorless minute prisms, mp 111—112 °C. Further recrystallization in the same manner yielded an analytical sample of **11a**, mp 113—114 °C; MS *m/z*: 283 (M^+); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 250 nm (sh) (ε 6700); $\lambda_{max}^{H_2O}$ (pH 1) 253 (8100); $\lambda_{max}^{H_2O}$ (pH 7) 219 (11900), 250 (sh) (6500); $\lambda_{max}^{H_2O}$ (pH 13) 249

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(11100); IR ν_{max}^{Nujol} cm^{-1}: 1738 (CO_2Et), 1704 (CONHAr); NMR (Me_2SO-d_6) δ: 1.0—1.35 (6H, m, $CO_2CH_2\underline{Me}$ and $NOCH_2\underline{Me}$), 3.86 (2H, q, J= 6.8 Hz, $NOC\underline{H}_2Me$), 4.14 (2H, q, J=7.1 Hz, $CO_2C\underline{H}_2Me$), 4.74 and 4.83 [2H, s each, N(1)-CH_2], 5.5—5.7 (2H, br, NH_2), 7.65 (sh) and 7.66 [1H, C(2)-H], 8.01 (0.6H, d, J=10.5 Hz, *trans*-HCONH), 8.17 (0.4H, s, *cis*-HCONH), 9.34 (0.6H, d, J=10.5 Hz, *trans*-HCONH), 9.65 (0.4H, dull s, *cis*-HCONH).[17] *Anal.* Calcd for $C_{11}H_{17}N_5O_4$: C, 46.64; H, 6.05; N, 24.72. Found: C, 46.80; H, 6.04; N, 24.78.

5-Formamido-4-(*N'*-methoxyamidino)-1*H*-imidazole-1-butanoic Acid Ethyl Ester (11b) A solution of **7b** (6.84 g, 18 mmol) in H_2O (300 ml) was passed through a column of Amberlite IRA-402 (HCO_3^-) (45 ml), and the column was eluted with H_2O. The eluate (700 ml) was concentrated *in vacuo* to a volume of *ca.* 200 ml and kept first in a refrigerator for 3 d and then at 30 °C for 48 h. The reaction mixture was worked up in a manner similar to that described above for **11a** except that the eluent for silica gel (55 g) column chromatography was AcOEt, giving **11b** (3.37 g, 63%), mp 125.5—126.5 °C. Recrystallization from AcOEt afforded an analytical sample as colorless prisms, mp 126—127 °C; UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 250 nm (sh) (ε 6750); $\lambda_{max}^{H_2O}$ (pH 1) 254 (8100); $\lambda_{max}^{H_2O}$ (pH 7) 218.5 (11800), 250 (sh) (6700); $\lambda_{max}^{H_2O}$ (pH 13) 254 (10900); IR ν_{max}^{Nujol} cm^{-1}: 1732 (CO_2Et), 1696 (CONHAr); NMR (Me_2SO-d_6) δ: 1.17 (3H, t, J=7 Hz, $CO_2CH_2\underline{Me}$), 1.90 [2H, m, N(1)-$CH_2C\underline{H}_2$], 2.27 (2H, m, $C\underline{H}_2CO_2Et$), 3.63 and 3.67 (3H, s each, NOMe), 3.84 [2H, m, N(1)-CH_2], 4.03 and 4.04 (2H, q each, J=7 Hz, $CO_2C\underline{H}_2Me$), 5.59 and 5.64 (2H, dull s each, NH_2), 7.67 [1H, s, C(2)-H], 8.05 (0.5H, d, J=10 Hz, *trans*-HCONH), 8.22 (0.5H, s, *cis*-HCONH), 9.39 (0.5H, d, J=10 Hz, *trans*-HCONH), 9.63 (0.5H, s, *cis*-HCONH).[17] *Anal.* Calcd for $C_{12}H_{19}N_5O_4$: C, 48.48; H, 6.44; N, 23.56. Found: C, 48.19; H, 6.52; N, 23.26.

4-(*N'*-Ethoxyamidino)-5-(*N*-methylformamido)-1*H*-imidazole-1-acetic Acid Ethyl Ester (12a) A mixture of **11a** (5.13 g, 18.1 mmol) and anhydrous K_2CO_3 (3.76 g, 27.2 mmol) in $HCONMe_2$ (180 ml) was stirred at room temperature for 1 h. A solution of MeI (3.08 g, 21.7 mmol) in $HCONMe_2$ (20 ml) was then added, and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* to leave a brown jelly, which was extracted with boiling benzene (3×180 ml). The benzene extracts were combined and concentrated *in vacuo*, and the residue was recrystallized from hexane–AcOEt (2:1, v/v) to afford a first crop (4.07 g, 76%) of **12a**, mp 101—102 °C. Concentration of the mother liquor from the recrystallization and purification of the residue by column chromatography [silica gel, benzene–EtOH (15:1, v/v)] gave a second crop (0.45 g) of **12a**, mp 99—101 °C. The total yield of **12a** was 4.52 g (84%). Further recrystallizations of crude **12a** from hexane–AcOEt (2:1, v/v) yielded an analytical sample as colorless minute prisms, mp 103—104 °C; MS *m/z*: 297 (M^+); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 250 nm (sh) (ε 5600); $\lambda_{max}^{H_2O}$ (pH 1) 249 (8490); $\lambda_{max}^{H_2O}$ (pH7) 250 (sh) (5470); $\lambda_{max}^{H_2O}$ (pH 13) 250 (sh) (5670); IR ν_{max}^{Nujol} cm^{-1}: 3520, 3410 (NH_2), 1749 (CO_2Et), 1678 (HCON); NMR (Me_2SO-d_6) δ: 1.15 (3H, t, J=7 Hz, $NOCH_2\underline{Me}$ or $CO_2CH_2\underline{Me}$), 1.20 (3H, t, J=7 Hz, $CO_2CH_2\underline{Me}$ or $NOCH_2\underline{Me}$), 2.93 (0.86×3H) and 3.19 (0.14×3H) (s each, NMe), 3.83 (2H, q, J=7 Hz, $NOC\underline{H}_2Me$), 4.16 (2H, q, J=7 Hz, $CO_2C\underline{H}_2Me$), 4.7 (br) and 4.89 (dull s) [2H, N(1)-CH_2], 5.64 (2H, dull s, NH_2), 7.72 (0.14H) and 7.76 (0.86H) [s each, C(2)-H], 7.96 (0.86H) and 8.20 (0.14H) (s each, CHO).[17] *Anal.* Calcd for $C_{12}H_{19}N_5O_4$: C, 48.48; H, 6.44; N, 23.56. Found: C, 48.46; H, 6.48; N, 23.66.

4-(*N'*-Methoxyamidino)-5-(*N*-methylformamido)-1*H*-imidazole-1-butanoic Acid Ethyl Ester (12b) A mixture of **11b** (2.97 g, 10 mmol) and anhydrous K_2CO_3 (2.07 g, 15 mmol) in $HCONMe_2$ (50 ml) was stirred at room temperature for 1 h. A solution of MeI (1.70 g, 12 mmol) in $HCONMe_2$ (5 ml) was then added, and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo*, and the residue was triturated with H_2O (5 ml). The insoluble solid that resulted was filtered off, washed with H_2O, and dried to give **12b** (2.68 g, 86%), mp 90.5—91.5 °C. Recrystallization from H_2O provided an analytical sample as colorless prisms, mp 90.5—91.5 °C; UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 250 nm (sh) (ε 5560); $\lambda_{max}^{H_2O}$ (pH 1) 251.5 (8120); $\lambda_{max}^{H_2O}$ (pH 7) 250 (sh) (5720); $\lambda_{max}^{H_2O}$ (pH 13) 250 (sh) (5760); IR ν_{max}^{Nujol} cm^{-1}: 3435, 3315 (NH_2), 1730 (CO_2Et), 1692 (HCON); NMR (Me_2SO-d_6) δ: 1.17 (3H, t, J=7 Hz, $CO_2CH_2\underline{Me}$), 1.92 [2H, m, N(1)-$CH_2C\underline{H}_2$], 2.33 (2H, m, $C\underline{H}_2CO_2Et$), 3.01 (0.83×3H) and 3.26 (0.17×3H) (s each, NMe), 3.60 (0.83×3H) and 3.66 (0.17×3H) (s each, NOMe), 3.86 [2H, m, N(1)-CH_2], 4.03 and 4.05 (2H, q each, J=7 Hz, $CO_2C\underline{H}_2Me$), 5.16 (sh) and 5.67 (2H, br, NH_2), 7.74 (0.17H) and 7.78 (0.83H) [s each, C(2)-H], 8.01 (0.83H) and 8.25 (0.17H) (s each, CHO).[17] *Anal.* Calcd for $C_{13}H_{21}N_5O_4$: C, 50.15; H, 6.80; N, 22.49. Found: C, 50.03; H, 7.01; N, 22.56.

4-(*N'*-Ethoxyamidino)-5-(methylamino)-1*H*-imidazole-1-acetic Acid Dihydrochloride (13a) A solution of **12a** (892 mg, 3 mmol) in 1 N aqueous NaOH (15 ml) was heated under reflux for 15 min. After cooling, the reaction mixture was passed through a column of Dowex 50W-X8 (H^+) (40 ml), and the column was washed with H_2O until the eluate became neutral. The column was then eluted with 5% aqueous NH_3 (400 ml), and the ammoniacal eluate was applied to a column of Amberlite IRA-402 (OH^-) (20 ml), which was then eluted successively with H_2O (300 ml) and 2% aqueous HCl (300 ml). The acid eluate was concentrated *in vacuo*, and the residual solid was washed with acetone (10 ml) to give **13a** (680 mg, 72%), mp 172—173 °C (dec.). Recrystallization of crude **13a** by dissolving it in H_2O and adding acetone to the resulting aqueous solution produced an analytical sample as colorless needles, mp 178—180 °C (dec.); IR ν_{max}^{Nujol} 1772 cm^{-1} (CO_2H); NMR (Me_2SO-d_6) δ: 1.26 (3H, t, J= 7 Hz, $NOCH_2\underline{Me}$), 2.70 (3H, s, NMe), 4.02 (2H, q, J=7 Hz, $NOC\underline{H}_2Me$), 4.91 (2H, s, $C\underline{H}_2CO_2H$), 8.17 [1H, s, C(2)-H]. *Anal.* Calcd for $C_9H_{15}N_5O_3\cdot 2HCl$: C, 34.41; H, 5.45; N, 22.29. Found: C, 34.07; H, 5.50; N, 22.06.

3,6-Dihydro-6-imino-3-methyl-9*H*-purine-9-acetic Acid Hydrochloride (15a) A solution of **13a** (220 mg, 0.7 mmol) in H_2O (15 ml) was hydrogenated over Raney Ni W-2 catalyst[18] (240 mg) at atmospheric pressure and room temperature for 3 h. The catalyst was removed by filtration and washed with H_2O (10 ml). The filtrate and washings were combined and concentrated to dryness *in vacuo* to leave a pale greenish glass (203 mg), presumed to be 4-amidino-5-(methylamino)-1*H*-imidazole-1-acetic acid dihydrochloride (**14a**). The glass was suspended in formic acid (of over 98% purity) (7 ml), and the suspension was heated under reflux for 5 h. The reaction mixture was concentrated *in vacuo*, and the residue was triturated with ether (2×10 ml). The insoluble solid that resulted was separated from the ethereal layer by decantation and recrystallized by dissolving it in warm 5% aqueous HCl (5 ml) and adding acetone (70 ml) to the resulting acid solution, affording **15a** [72 mg, 42% (from **13a**)]. Further recrystallization in a similar manner gave an analytical sample as colorless prisms, mp 264—265 °C (dec.); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 271.5 nm (ε 15900); $\lambda_{max}^{H_2O}$ (pH 1) 270 (16200); $\lambda_{max}^{H_2O}$ (pH 7) 270 (16200); $\lambda_{max}^{H_2O}$ (pH 13) unstable; IR ν_{max}^{Nujol} 1735 cm^{-1} (CO_2H); NMR (Me_2SO-d_6) δ: 4.07 [3H, s, N(3)-Me], 5.51 (2H, s, $C\underline{H}_2CO_2H$), 8.41 [1H, s, C(8)-H], 8.65 [1H, s, C(2)-H], 9.25 and 9.30 (1H each, br s, $=NH_2^+$). *Anal.* Calcd for $C_8H_9N_5O_2\cdot HCl$: C, 39.44; H, 4.14; N, 28.74. Found: C, 39.25; H, 4.12; N, 28.74.

3,6-Dihydro-6-imino-3-methyl-9*H*-purine-9-butanoic Acid Hydrochloride (15b) A suspension of **12b** (2.18 g, 7 mmol) in 1 N aqueous NaOH (35 ml) was heated under reflux for 20 min. After cooling, the reaction mixture was worked up in a manner similar to that described above for **13a**, producing a yellow glass presumed to be 4-(*N'*-methoxyamidino)-5-(methylamino)-1*H*-imidazole-1-butanoic acid dihydrochloride (**13b**). The glass was then hydrogenated in H_2O (70 ml) over Raney Ni W-2 catalyst[18] (1.2 g) for 6 h as described above for **15a**, affording a greenish glass (866 mg), presumed to be 4-amidino-5-(methylamino)-1*H*-imidazole-1-butanoic acid dihydrochloride (**14b**). Cyclization of the total amount of crude **14b** with boiling formic acid (40 ml) for 4 h, work-up of the reaction mixture, and recrystallization of the product **15b**·H_2O [411 mg, 20% (from **12b**)] also followed those described above for **15a**. After drying over P_2O_5 at 3 mmHg and room temperature for 24 h, an analytical sample of **15b**·H_2O was obtained as colorless minute prisms, mp 245—246 °C (dec.); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 271.5 nm (ε 15900); $\lambda_{maz}^{H_2O}$ (pH 1) 271 (16300); $\lambda_{max}^{H_2O}$ (pH 7) 271 (16300); $\lambda_{max}^{H_2O}$ (pH 13) unstable; IR ν_{max}^{Nujol} 1736 cm^{-1} (CO_2H); NMR (Me_2SO-d_6) δ: 2.06 [2H, m, N(9)-$CH_2C\underline{H}_2$], 2.40 (2H, t, J=6 Hz, $C\underline{H}_2CO_2H$), 4.19 [3H, s, N(3)-Me], 4.50 [2H, t, J=7 Hz, N(9)-CH_2], 8.44 [1H, s, C(8)-H], 8.64 [1H, s, C(2)-H], 9.12 and 9.19 (1H each, s, $=NH_2^+$), 12.31 (1H, br, CO_2H). *Anal.* Calcd for $C_{10}H_{13}N_5O_2\cdot HCl\cdot H_2O$: C, 41.46; H, 5.57; N, 24.17. Found: C, 41.39; H, 5.61; N, 24.46.

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