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SYNTHESIS OF 3,9-DIALKYLGUANINES AND THEIR CONVERSION INTO 3-ALKYLWYES, MODELS FOR THE FLUORESCENT NUCLEOSIDES FROM PHENYLALANINE TRANSFER RIBONUCLEIC ACIDS

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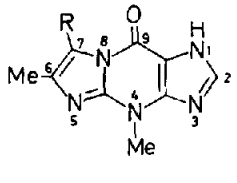
Abstract—Synthesis of 3,9-dialkylguanines **5** has been accomplished by *N*-cyanation of 1-alkyl-5-(alkylamino)imidazole-4-carboxamides **3** followed by base-catalysed cyclisation. Cyclocondensation of 9-alkyl-3-methylguanines **5a, d, f** with $MeCOCH_2Br$ gave 3-alkylwyes **6**, model compounds of the most probable structure for wyosine from *Torulopsis utilis* $tRNA^{Phe}$.

The structures of the fluorescent bases from $tRNAs^{Phe}$ have been elucidated as **1**.[1] Syntheses of **1a**[1a,b] and (±)-**1b**[1d] have been achieved by cyclisation of 3-methylguanine with appropriate α-bromoketones. Wyosine[1b,2] from *Torulopsis utilis* $tRNA^{Phe}$ and wybutosine[3] from yeast $tRNA^{Phe}$ have been proposed to be 3-β-D-ribofuranosides of **1a** (**2a**)[1b,4] and **1b** (**2b**),[3b] respectively. 3-Methylguanosine (type **5**: $R^1 = \beta$-D-ribofuranosyl; R^2 = Me) thus appears to be a good synthetic intermediate for these nucleosides **2**. As a model for the synthesis of 3-methylguanosine we undertook to develop a synthetic method of the so far unknown 3,9-dialkylguanines **5**. This paper describes a general synthesis of **5** and conversion of them into 3-alkylwyes (3-alkyl-3,4-dihydro-4,6-dimethyl-9*H*-imidazol[1,2*a*]purin-9-ones) **6**.†

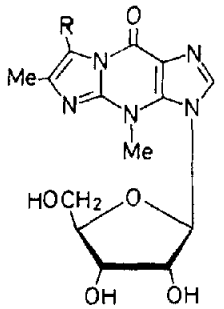
3,5′-Cycloguanosines are examples of 3,9-disubstituted guanines and have been synthesised by intramolecular alkylation at the 3-position of guanosine derivatives.[5,6] However, alkylation of either 9-alkyl-[7] or 3-methylguanine[8] has been reported to occur at the 7-position. Probably the most promising means of obtaining **5** is the cyclisation of appropriately substituted imidazole or pyrimidine derivatives. 1-Methyl-5-(methylamino)imidazole-4-carboxamide **3a**[9] and its dibenzyl analogue[10] seemed to be good starting materials for this approach. We have improved the procedure for preparing these compounds and we have established a general synthesis of 1-alkyl-5-(alkylamino)imidazole-4-carboxamides **3**.[11]

Yamazaki *et al.*[12] have synthesised guanine by the reaction of 5(4)-aminoimidazole-4(5)-carboxamide (AICA) (type **3**: $R^1 = R^2 = H$) with benzoyl isothiocyanate followed by methylation, ammonolysis, and treatment with boiling NaOH aq. Guanosine has been synthesised similarly.[12] However, 3,5′-cycloguanosine has been reported to be hydrolysed to 3,5′-cycloxanthosine[6] under reaction conditions similar to those employed in the last step for the synthesis of guanine or guanosine.[12] This knowledge discouraged us from utilising their method for the synthesis of **5**. Although these authors

†A part of this work was reported in preliminary form.[4b]



1a–d



2a,b

a: R = H

b–d: R = $CH_2CHC(R')(CO_2Me)(NHCO_2Me)$

(b: R′ = H; c: R′ = OH; d: R′ = OOH)

Scheme 1.

described that AICA did not give the cyanamide by treatment with CNBr under various conditions,[12,13] the simplicity and straightforwardness of this approach led us to focus our efforts on the cyanation of **3**.

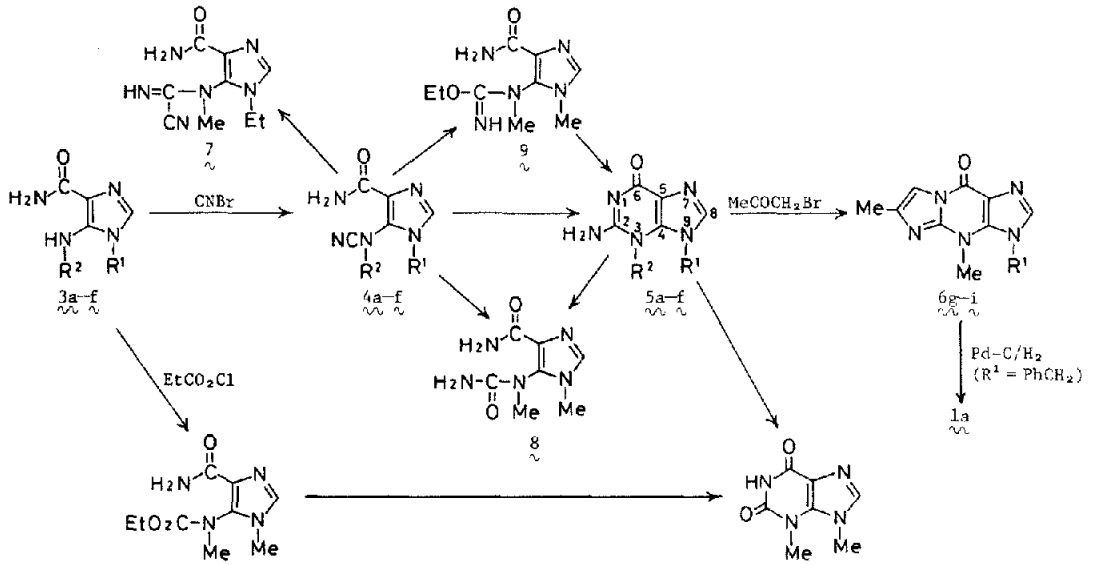
Reaction of 1-ethyl-5-(methylamino)imidazole-4-carboxamide **3d**[11c] with CNBr in CH_2Cl_2 in the presence of NEt_3 took place only slowly at 30°. In aqueous solution at pH 7 **3d** reacted smoothly with CNBr at room temp. However, the main product was not the desired 5-(cyanomethylamino)-1-ethylimidazole-4-carboxamide **4d**, but 5-[(cyanoiminomethyl)methylamino]-1-ethylimidazole-4-carboxamide **7**. Compound **7** was supposed to result from nucleophilic attack of CN^- on the initially formed **4d** and the reaction may be regarded as analogous to that of a secondary amine with $(CN)_2$.[14] The reaction at pH 5 successfully afforded **4d** in 33% yield. This compound was characterised by elemental analysis and the following spectral data. The IR spectrum showed a sharp absorption band at 2225 cm^{-1} due to the C≡N group. The mass spectrum exhibited the M^+ peak at *m/e* 193 and the base peak at *m/e* 176 which might be due to the elimination of NH_3 from the $CONH_2$ group in an analogous fashion to the fragmentation of *o*-substituted benzamides.[15] The NMR spectrum taken in $(CD_3)_2SO$ showed a sharp singlet at 3.24 ppm due to the NMe group and no NH signal other than that of the $CONH_2$ group, whereas the spectrum of **3d**[11c] showed a doublet at 2.83 ppm and a quartet at 5.75 ppm due to the HNMe group.

The reaction rate of **3d** with CNBr at room temp increased as the pH of the reaction medium was increased between pH 2 and pH 5 without any formation of **7**, whereas **7** was formed besides **4d** at pH 6 and above. The reactions at various temperatures between 0° and 30° at pH 5 resulted in no meaningful improvement in the yield of **4d**. Addition of MeOH to the reaction mixture retarded the reaction.

5-(Cyanomethylamino)-1-methyl **4a**, 5-cyanoethylamino)-1-methyl **4b**, and 5-(cyanoethylamino)-1-ethylimidazole-4-carboxamide **4e** were synthesised in a similar manner to that described for **4d**. 5-(Benzylcyanoamino)-1-methyl **4c** and 1-benzyl-5-(cyanomethylamino)imidazole-4-carboxamide **4f** were obtained from water-insoluble **3c**, **f**[11c] by prolonged reaction in a mixed solvent of acetate buffer (pH 5) and MeOH. The results are summarised in Table 1. The structures of these compounds were confirmed by spectral similarity to **4d** (Table 2).

Cyclisation of **4a** to 3,9-dimethylguanine **5a** was effected by various means. On dissolution in 0.1 *N* NaOH aq at room temp, **4a** changed into **5a** (48% yield) and 1-methyl-5-(1-methylureido)imidazole-4-carboxamide **8** rapidly. Even in plain water at reflux, **4a** cyclised to **5a** in 65% yield. Treatment of **4a** with NaOEt in EtOH at room temp gave **5a** in 41% yield together with a 5-(2-ethyl-1-methyl-1-isoureido)-1-methylimidazole-4-carboxamide **9**. Compound **9** was transformed into **5a** by heating with NaOEt in EtOH. Thus, **5a** was obtained more effectively (86% yield) by direct heating of **4a** with NaOEt in EtOH. The highest yield (93%) of **5a** was achieved by treatment of **4a** with NaH in Me_2NCHO at room temp.

Correct analyses were obtained for **5a** as the monohydrate. The 3,9-dimethylguanine structure was supported by UV spectral similarity to 3,5′-cycloguanosine[6] or its 2′,3′-*O*-isopropylidene derivative.[5] Compound **5a** was found to be unstable under alkaline conditions and treatment of **5a** with boiling 1 *N* KOH aq gave 3,9-



Scheme 2.

Table 1. Cyanation of 1-alkyl-5-(alkylamino)imidazole-4-carboxamides 3

Product	Reaction conditions CNBr[a]	Solvent[b]	Time (hr)	Yield (%)	Appearance (Recrystn. solvent)	m.p. (°C)	Formula	Analysis (%) Found (Calcd.) C	H	N
4a	5	A	1	31	Colorless pillars (MeOH)	233–235 (dec)	$C_7H_8N_4O$	46.97 (46.92	5.18 5.06	38.88 39.09)
	10	B	18	25						
4b	5	A	2	43	Colorless plates (EtOH)	174–175	$C_8H_{10}N_4O$	49.83 (49.73	5.81 5.74	36.15 36.25)
4c	10	B	65	51	Colorless needles (EtOH)	212–214 (dec)	$C_{13}H_{12}N_4O$	61.27 (61.16	5.13 5.13	27.49 27.44)
4d	5	A	4	33	Colorless plates (EtOH)	190–191	$C_8H_{10}N_4O$	49.67 (49.73	5.88 5.74	36.29 36.25)
4e	5	A	2	44	Colorless plates (EtOH)	156–158	$C_9H_{12}N_4O$	52.16 (52.16	6.57 6.32	33.63 33.80)
4f	10	B	95	56	Colorless needles (EtOH)	153 (dec)	$C_{13}H_{12}N_4O$	61.05 (61.16	5.09 5.13	27.48 27.44)

[a] The figures denote the molar equivalents.

[b] The letters A and B refer to 1 M acetate buffer (pH 5) and a mixed solvent of 1 M acetate buffer (pH 5) and MeOH (3:4, v/v), respectively.

dimethylxanthine **11**, **8**, and **3a**, further supporting the structure of **5a**. The structure of **11** was established by direct comparison with a sample derived from **3a** through 5 - [(ethoxycarbonyl)methylamino] - 1 - methylimidazole - 4-carboxamide **10**. An alternative isoguanine structure† for **5a** was ruled out by a marked difference between **5a** and 3,9-dimethylisoguanine[16] in UV spectrum.

Treatment of **4b–f** with NaH in a manner similar to the cyclisation of **4a** gave 3-ethyl-9-methyl **5b**, 3-benzyl-9-methyl **5c**, 9-ethyl-3-methyl **5d**, 3,9-diethyl **5e**, and 9-benzyl-3-methylguanine **5f** as shown in Table 3. The structures of these compounds were confirmed by comparison of the UV and NMR spectra with those of **5a** (Table 4).

Ienaga and Pfleiderer[4a] have synthesised **5a** according to the method of Yamazaki *et al.*[12] Since we have found that 3-methylguanosine decomposes completely‡ under the reaction conditions employed in the final step of their synthesis, it seems difficult to prepare 3-methylguanosine by their method.

When **5f** was treated with $MeCOCH_2Br$ in Me_2SO in the presence of K_2CO_3, a fluorescent product was obtained in 37% yield. This compound was characterised as 3-benzylwye **6f** on the basis of its catalytic hydrogenolysis with Pd on C which gave **1a**. The structure of **1a** was confirmed by direct comparison with an authentic sample synthesised according to the literature.[1b] Similar

†Yamazaki *et al.*[13] reported the formation of isoguanine by alkaline treatment of 5(4)-(cyanoamino)imidazole-4(5)-carboxamide.

‡Details will be published elsewhere.

Table 2. UV and NMR spectra of 1-alkyl-5-(alkylcyanoamino)imidazole-4-carboxamides 4

Compound	UV spectra[a] 95% EtOH aq λshoulder (nm)	ε x 10⁻³	H_2O (pH 1) λshoulder (nm)	ε x 10⁻³	H_2O (pH 7) λmax (nm)	ε x 10⁻³	NMR spectra δ (ppm) N-Alkyl	1-Alkyl	NH_2	2-H
4a	234	7.4	235	6.3	242	7.3	3.24 (s)	3.62 (s)	7.26 (br) 7.39 (br)	7.70 (s)
4b	237	7.7	236	6.3	243	7.3	1.20 (t) 3.53 (q)	3.61 (s)	7.25 (br) 7.38 (br)	7.71 (s)
4c	238	7.8	240	6.2	244	7.7	4.67 (s) 7.35 (s)	3.36 (s)	——[b]	7.62 (s)
4d	234	7.7	235	6.2	241	7.5	3.24 (s)	1.37 (t) 3.97 (q)	7.22 (br) 7.34 (br)	7.71 (s)
4e	236	7.5	239	5.6	242	7.4	1.22 (t) 3.53 (q)	1.37 (t) 3.99 (q)	7.25 (br) 7.37 (br)	7.80 (s)
4f	234	8.4	236	7.2	236[c]	8.5	2.96 (s)	5.23 (s) 7.2–7.5 (m)	——[b]	7.93 (s)

[a] The spectra in 0.1 N NaOH aq changed rapidly.

[b] overlpped with a signal due to the phenyl protons

[c] shoulder

Table 3. Cyclisation of 1-alkyl-5-(alkylcyanoamino)imidazole-4-carboxamides **4** with NaH

Product	Amount of NaH (Molar equivalent)	Reaction time (hr)	Yield (%)	Appearance (Recrystn. solvent)	m.p. (°C)	Formula	Analysis (%) Found (Calcd.) C	H	N
5a	0.2	0.5	93	Colorless needles (H_2O)	>300	$C_7H_9N_5O \cdot H_2O$[a]	42.53 (42.63)	5.51 5.62	35.46 35.52)
5b	0.6	20	88	Colorless needles (90% MeOH aq)	>300	$C_8H_{11}N_5O$	49.48 (49.73)	5.80 5.74	36.20 36.25)
5c	1.2	1	77	Colorless needles (MeOH)	280–282 (dec)	$C_{13}H_{19}N_5O \cdot H_2O$[a]	57.03 (57.13)	5.56 5.53	25.72 25.63)
5d	0.2	1	91	Colorless needles (50% MeOH aq)	>300	$C_9H_{11}N_5O \cdot H_2O$[a]	45.57 (45.49	6.27 6.20	33.01 33.16)
5e	1.0	0.5	82	Colorless prisms (H_2O)	270–280 (dec)	$C_9H_{13}N_5O \cdot H_2O$[a]	48.04 (47.99	6.90 6.71	30.82 31.09)
5f	1.0	overnight	83	Colorless pillars (MeOH)	>300	$C_{13}H_{15}N_5O$	61.03 (61.16	5.21 5.13	27.64 27.44)

[a] The sample was dried at 110° and 2 mmHg for several hours followed by exposure to air until a constant weight was reached.

Table 4. Physical properties of 3,9-dialkylguanines **5**

Compound	pKa	UV spectra 95% EtOH aq λmax (nm)	ε x 10⁻⁴	H_2O (pH 1) λmax (nm)	ε x 10⁻⁴	H_2O (pH 7) λmax (nm)	ε x 10⁻⁴	H_2O (pH 13) λmax (nm)	ε x 10⁻⁴	NMR spectra δ (ppm) 3-Alkyl	9-Alkyl	NH_2	8-H
5a	4.62 ± 0.08	261	1.12	248	1.25	216 3.01 247 0.86 266 1.14		247 0.88 266 1.10		3.67 (s)	3.91 (s)	6.6–7.1 (br)	7.53 (s)
5b	4.64 ± 0.06	262	1.09	250	1.21	218 2.64 249 0.80 267 1.09		249 0.82 267 1.06		1.26 (t) 4.14 (q)	3.90 (s)	6.9 (br)	7.54 (s)
5c	4.12 ± 0.07	248 1.01 262 1.13		248	1.30	218 4.21 247 0.84 267 1.16		247[a] 0.90 266 1.04		5.43 (s) 6.9–7.4 (m)	3.61 (s)	——[b]	——[b]
5d	4.65 ± 0.07	262	1.14	249	1.24	216 2.86 248 0.84 267 1.16		248 0.86 267 1.11		3.64 (s)	1.38 (t) 4.31 (q)	7.1 (br)	7.64 (s)
5e	4.64 ± 0.07	263	1.14	251	1.27	218 2.84 250[a] 0.84 267 1.16		250[a] 0.85 267 1.12		1.24 (t) 4.08 (q)	1.39 (t) 4.22 (q)	6.9 (br)	7.62 (s)
5f	4.21 ± 0.03	262	1.23	250	1.31	214 3.00 249 0.90 267 1.19		249 0.92 267 1.15		3.37 (s)	5.62 (s) 6.9–7.1 (m) 7.3–7.5 (m)	6.8 (br)	7.73 (s)

[a] shoulder

[b] overlapped with a signal due to the phenyl protons

treatment of **5a** and **5d** with $MeCOCH_2Br$ gave 3-methyl **6g** and 3-ethylwye **6h**, respectively, consistent with the synthesis of **6g** by Ienaga and Pfleiderer.[4a] The close UV and NMR spectral similarity of **6g** and **6h** to **6i** established the correctness of their structures. The UV spectra of **6g**, **h**, **i** at pH 7 are very similar in shape to the reported spectrum of wyosine,[1b,2] supporting that wyosine is a 3-substituted wye.

In conclusion, the present work provides a simple and mild synthesis of 3,9-dialkylguanines **5**. This method has been utilised for the facile synthesis[17] of 3-β-D-ribofuranosylwye **2a**, the most probable structure for wyosine, through 3-methylguanosine, both of which are very unstable nucleosides.†

†Syntheses of **2a** and 3-methylguanosine were first reported by Nakatsuka *et al.*[18]

EXPERIMENTAL

General

All m.ps are corrected. Spectra reported herein were determined with a Hitachi 323 UV spectrophotometer, a JASCO IRA-2 IR spectrophotometer, a JEOL JNM-PS-100 or a JEOL JNM-FX 100 NMR spectrometer, or a JEOL JMS-01SG mass spectrometer. Unless otherwise stated, NMR spectra were measured in $(CD_3)_2SO$ at 23–25° using Me_4Si as an internal standard. $Me_3Si(CH_2)_3SO_3Na$ was used for D_2O solns instead of Me_4Si. UV spectra were determined using solns in 95% EtOH aq, 0.1 *N* HCl aq (pH 1), 0.005 *M* phosphate buffer (pH 7), and 0.1 *N* NaOH aq (pH 13). p*K*a's were measured spectrophotometrically[19] at 20° and ionic strength 1.00.

1-*Alkyl*-5-(*alkylcyanoamino*)*imidazole*-4-*carboxamides* **4**

The procedure for the synthesis of **4d** will be described as a typical example. The other compounds were synthesised similarly as specified in Table 1.

5-(Cyanomethylamino)-1-ethylimidazole-4-carboxamide **4d**. A suspension of **3d** (5.00 g, 29.7 mmol)[11c] and CNBr (16 g, 151 mmol) in 1 *M* AcOH–AcONa aq (pH 5.0, 75 ml) was stirred at room temp for 4 h. The mixture was saturated with NaCl and then extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried over $MgSO_4$ and evaporated to dryness. The residue was washed successively with AcOEt (30 ml) and EtOH (4 ml) to give a dark solid. This was dissolved in MeOH–$CHCl_3$ (1:1, v/v) and the soln was treated with charcoal. Removal of the solvent by evaporation left a colourless solid (1.24 g), m.p. 186–194°. The combined AcOEt and EtOH washings were concentrated to a small volume and purified by column chromatography [SiO_2 (100 g), $CHCl_3$–EtOH (8:1, v/v)] to furnish a second crop (0.67 g). For analysis the product was recrystallised from EtOH (Tables 1 and 2). MS *m/e*: 193 (M^+), 176 (M^+–NH_3), 150 (M^+–NH_3–CN); IR (Nujol) cm^{-1}: 3370, 3190, and 3080 (NH), 2225 (C≡N), 1677 (C=O).

5 - [(Cyanoiminomethyl)methylamino] - 1 - ethylimidazole - 4 - carboxamide **7**

A mixture of **3d** (1.01 g, 6 mmol),[11c] CNBr (0.95 g, *ca.* 9 mmol), and H_2O (30 ml) was stirred at 29° for 4 h, being kept at pH 6.9–7.6 by occasional addition of $NaHCO_3$. The mixture was evaporated to dryness and the resulting residue was purified by column chromatography [SiO_2 (50 g), $CHCl_3$–EtOH (8:1, v/v)]. As a less polar material **3d** (0.07 g, 7%) was recovered. Crude **4d** (0.11 g), m.p. 155–167°, was obtained as a more polar material. Further elution afforded **7** (0.32 g, 24% based on **3d**), m.p. 205–208° (dec). Recrystallisations from EtOH gave colourless needles, m.p. 214–215° (dec) (Found: C, 49.34; H, 5.49; N, 37.86. $C_9H_{12}N_6O$ requires: C, 49.08; H, 5.49; N, 38.16%); MS *m/e*: 220 (M^+), 203 (M^+–NH_3); NMR δ: 1.33 (3H, t, J = 7 Hz, Ch_2CH_3), 3.07 (3H, s, NCH_3), 3.87 (2H, q, J = 7 Hz, CH_2CH_3), 7.10 and 7.28 (1H each, br, NH_2), 7.75 (1H, s, 2-H), 9.90 (1H, br, NH); UV $\lambda_{max}^{95\%\ EtOH}$ 222 nm (ε 12,700); $\lambda_{max}^{H_2O}$ (pH 1) unstable; $\lambda_{max}^{H_2O}$ (pH 7) 232 (11,900); $\lambda_{max}^{H_2O}$ (pH 13) unstable.

3,9-Dialkylguanines **5**

The procedures employed for cyclisation of **4a** will be described below. The identity of the products **5a** obtained by different methods was estalished by comparison of the IR spectra. The other 3,9-dialkylguanines **5b–f** were synthesised similarly according to method (i) (Table 3).

3,9-Dimethylguanine **5a**. (i) A mixture of **4a** (1.97 g, 11 mmol) and 50% NaH (0.11 g, 2.3 mmol) in anhyd Me_2NCHO (40 ml) was stirred at room temp for 30 min. The mixture was evaporated to dryness *in vacuo* and the resulting solid was neutralised with 50% AcOH aq. Insoluble solid was filtered off, washed successively with cold H_2O (10 ml) and EtOH (20 ml), and dried over P_2O_5 *in vacuo*. The product was then exposed to air until a constant weight was reached to give **5a** (2.02 g) as monohydrate, m.p. > 300° (Tables 3 and 4).

(ii) To a soln of Na (77 mg, 3.3 matom) in abs EtOH (25 ml) was added **4a** (500 mg, 2.79 mmol) and the mixture was stirred at room temp for 2 h. The solvent was removed by evaporation and the residue was washed with cold H_2O (6 ml) and dried to give **5a** · H_2O (205 mg). The washings were neutralised with 10% HCl aq and evaporated to dryness *in vacuo*. The residue was washed with cold H_2O (2 ml) to give a second crop of **5a** · H_2O (22 mg, total yield 41%). To the aqueous washings was added Al_2O_3 (20 g) and the mixture was evaporated to dryness *in vacuo*. The resulting solid was eluted with $CHCl_3$–EtOH (6:1, v/v). The eluate was evaporated to dryness and the residue was washed with AcOEt–hexane (1:1, v/v) then dried over P_2O_5 at 2 mmHg and 30° for 10 h to afford **9** (212 mg, 34%), m.p. 160–163°. Although this sample was different from an analytically pure **9** in m.p. and IR spectrum, identity was established by NMR spectrum. Recrystallisation from AcOEt and drying over P_2O_5 at 2 mmHg and 30° for 10 h gave colourless needles **9**, m.p. 154–155° (Found: C, 47.82; H, 6.93; N, 31.05. $C_9H_{15}N_5O_2$ requires: C, 47.99; H, 6.71; N, 31.09%); NMR δ: 1.10 (3H, t, J = 7 Hz, CH_2CH_3), 3.05 (3H, s, NCH_3), 3.40 (3H, s, 1-CH_3), 3.99 (2H, q, J = 7 Hz, CH_2CH_3), 5.62 (1H, br, NH), 6.94 and 7.10 (1H each, br, NH_2), 7.54 (1H, s, 2-H); UV $\lambda_{shoulder}^{95\%\ EtOH}$ 235 nm (ε 8100); $\lambda_{shoulder}^{H_2O}$ (pH 1) 231 (8100); $\lambda_{max}^{H_2O}$ (pH 7) 237 (8500); $\lambda_{max}^{H_2O}$ (pH 13) 240 (7800).

(iii) Compound **9** (50 mg, 0.22 mmol) was dissolved in a soln of Na (6 mg, 0.26 matom) in abs EtOH (3 ml) and the soln was refluxed for 5 h. The mixture was then evaporated to dryness to leave a solid, which was neutralised with 10% HCl aq, washed with H_2O (1 ml), and dried to give **5a** · H_2O (34 mg, 77%).

(iv) The reaction mixture described under method (ii) was refluxed for 7 h. It was then worked up in a manner similar to that described under method (iii) to give **5a** · H_2O (473 mg, 86%).

(v) A soln of **4a** (500 mg, 2.79 mmol) in 0.1 *N* NaOH aq (100 ml) was allowed to stand at room temp for 10 min then brought to pH 8 with 10% HCl aq. The mixture was evaporated to dryness *in vacuo* and the residual solid was washed successively with H_2O (7 ml) and EtOH (2 ml) and dried to give **5a** · H_2O (263 mg, 48%). The combined washings were evaporated after addition of Al_2O_3 (10 g) and the resulting solid was eluted with $CHCl_3$–EtOH (6:1, v/v). Removal of the solvent from the eluate by evaporation left **8** (140 mg, 25%) as a colourless solid, m.p. 266–268° (dec). Recrystallisations from MeOH–H_2O (1:1, v/v) gave colourless prisms, m.p. 269–271° (dec) (Found: C, 42.85; H, 5.74; N, 35.70. $C_7H_{11}N_5O_2$ requires: C, 42.63; H, 5.62; N, 35.52%); MS *m/e*: 197 (M^+), 180 (M^+–NH_3); NMR δ: 3.03 (3H, s, NCH_3), 3.42 (3H, s, 1-CH_3), 5.96 (2H, br, ureido NH_2), 6.99 and 7.16 (1H each, br, amido NH_2), 7.61 (1H, s, 2-H); UV $\lambda_{max}^{95\%\ EtOH}$ 236 nm (ε 8400); $\lambda_{max}^{H_2O}$ (pH 1) 212 (11,500); $\lambda_{max}^{H_2O}$ (pH 7 and pH 13) 239 (8500).

(vi) A soln of **4a** (100 mg, 0.558 mmol) in H_2O (100 ml) was refluxed for 4 h. It was then evaporated to dryness and recrystallisation of the resulting solid from H_2O gave **5a** · H_2O (71 mg, 65%).

3,9-Dimethylguanine hemipicrate was prepared as yellow prisms, m.p. 294° (dec), by adding an excess of a sat soln of picric acid in H_2O to a soln of **5a** in hot H_2O followed by recrystallisations from H_2O (Found: C, 40.94; H, 3.67; N, 30.98. $C_7H_9N_5O \cdot 1/2C_6H_3N_3O_7$ requires: C, 40.89; H, 3.60; N, 31.00%).

Hydrochlorides of 3,9-dialkylguanines **5**

To a hot soln of the free base **5** in H_2O or MeOH aq was added an excess of 1 *N* HCl aq and the soln was evaporated to dryness. The resulting solid was recrystallised from an appropriate solvent. The UV spectra (pH 1, pH 7, and pH 13) of the hydrochlorides were superimposable on the corresponding ones of the free bases **5** (Table 4).

3,9-Dimethylguanine hydrochloride **5a** · HCl. Recrystallisations from H_2O gave colourless needles, m.p. 283–284° (dec) (Found: C, 39.13; H, 4.73; N, 32.65. $C_7H_9N_5O \cdot HCl$ requires: C, 38.98; H, 4.67; N, 32.48%); NMR (D_2O at 76°) δ: 3.90 (3H, s, 3-CH_3), 4.05 (3H, s, 9-CH_3), 7.76 (1H, s, 8-H).

3-Ethyl-9-methylguanine hydrochloride **5b** · HCl. This was obtained as colourless needles, m.p. 269–270° (dec), after recrystallisations from 50% EtOH aq (Found: C, 41.62; H, 5.31; N, 30.37. $C_8H_{11}N_5O \cdot HCl$ requires: C, 41.83; H, 5.27; N, 30.50%); NMR (D_2O) δ: 1.48 (3H, t, J = 7 Hz, CH_2CH_3), 4.03 (3H, s, 9-CH_3), 4.30 (2H, q, J = 7 Hz, CH_2CH_3), 7.72 (1H, s, 8-H).

3-Benzyl-9-methylguanine hemihydrochloride **5c** · 1/2HCl. Recrystallisations from H_2O gave the hemihydrochloride as colourless prisms, m.p. 210–211° (dec) (Found: C, 57.12; H, 5.15; N, 25.84. $C_{13}H_{13}N_5O \cdot 1/2HCl$ requires: C, 57.08; H, 4.98; N, 25.61%); NMR δ: 3.63 (3H, s, 9-CH_3), 5.52 (2H, s, CH_2), 7.13–7.47 (5H, m, C_6H_5), 7.66 (1H, s, 8-H).

9-Ethyl-3-methylguanine hydrochloride **5d** · HCl. After recrystallisations from 50% MeOH aq the sample was dried over P_2O_5 at 2 mmHg and 110° for 10 h then exposed to air until a constant weight was reached, colourless plates, m.p. 238–240° (sinter) (Found: C, 38.99; H, 5.62; N, 28.19. $C_8H_{11}N_5O \cdot HCl \cdot H_2O$ requires: C, 38.79; H, 5.70; N, 28.28%); NMR δ: 1.45 (3H, t, J = 7 Hz, CH_2CH_3), 3.77 (3H, s, 3-CH_3), 4.43 (2H, q, J = 7 Hz, CH_2CH_3), 7.97 (1H, s, 8-H).

5 - [(Ethoxycarbonyl)methylamino] - 1 - methylimidazole - 4 - carboxamide **10**

A mixture of **3a** (1.00 g, 6.49 mmol),[11c] K_2CO_3 (897 mg, 6.49 mmol), ethyl chloroformate (775 mg, 7.14 mmol), and dioxane (45 ml) was refluxed for 30 min. The resulting mixture was evaporated to dryness *in vacuo* and the residue was extracted with $CHCl_3$. The $CHCl_3$ extracts were evaporated to dryness and

the residue was purified by column chromatography [SiO_2 (126 g), $CHCl_3$–EtOH (8:1, v/v)] to give **10** (578 mg, 39%) as a colourless solid, m.p. 200–205°. Further elution of the column afforded **3a** (260 mg, 26%). Recrystallisations of **10** from EtOH gave colourless prisms, m.p. 204.5–206° (Found: C, 47.90; H, 6.37; N, 24.84. $C_9H_{14}N_4O_3$ requires: C, 47.78; H, 6.24; N, 24.77%); UV $\lambda_{max}^{95\%\ EtOH}$ 236 nm (ϵ 9000); $\lambda_{max}^{H_2O}$ (pH 1) 212 (11,400); $\lambda_{max}^{H_2O}$ (pH 7) 240 (8800); $\lambda_{max}^{H_2O}$ (pH 13) unstable.

3,9-Dimethylxanthine **11**

(i) A mixture of **10** (300 mg, 1.33 mmol) and 50% NaH (130 mg, 2.7 mmol) and Me_2NCHO (6 ml) was stirred at room temp for 6 h. The solvent was removed by evaporation and the solid residue was neutralised with 50% AcOH aq. Insoluble solid was filtered off, washed successively with a little H_2O and EtOH, and dried to afford **11** (237 mg, 99%), m.p. > 300° (lit.[20] m.p. 321–322°) (Found: C, 46.62; H, 4.36; N, 31.37. $C_7H_8N_4O_2$ requires: C, 46.66; H, 4.48; N, 31.10%); NMR δ: 3.61 (3H, s, 3-CH_3),† 3.91 (3H, s, 9-CH_3),† 7.62 (1H, s, 8-H), 11.04 (1H, br, NH or OH); $\lambda_{max}^{95\%EtOH}$ 236 nm (ϵ 9500), 266 (9300); $\lambda_{max}^{H_2O}$ (pH 1) 237 (7900), 266 (9700); $\lambda_{max}^{H_2O}$ (pH 7) 236 (8800), 268 (10,100); $\lambda_{max}^{H_2O}$ (pH 13) 237 (shoulder) (7100), 244 (shoulder) (6600), 269 (10,400).

(ii) Pyrolysis of **10** (200 mg, 0.884 mmol) was conducted at 220° for 10 min. The resulting solid was washed with EtOH (4 ml) and dried to give **11** (109 mg, 69%), identical (IR spectrum) with that from (i).

3,4-Dihydro-3,4,6-trimethyl-9H-imidazo[1,2-a]purin-9-one **6g**

A hot soln of **5a** · H_2O (592 mg, 3 mmol) in Me_2SO (85 ml) was allowed to cool to room temp with stirring to give a suspension of minute crystals. After addition of K_2CO_3 (1.24 g, 9 mmol), $MeCOCH_2Br$ (2.47 g, 18 mmol) was added to the mixture and the mixture was stirred at 30° for 5 h. The mixture was evaporated to dryness *in vacuo* and the residue was extracted with hot MeOH (3 × 20 ml). The combined extracts were evaporated to dryness after adding Al_2O_3 (5 g) and the resulting solid was placed on top of a column packed with Al_2O_3 (50 g). The column was eluted with AcOEt–EtOH (8:1, v/v). The eluate containing a fluorescent material was collected and evaporated to dryness to give a solid, which was washed with EtOH (15 ml) and dried to furnish **6g** (137 mg), m.p. 290–293° (dec). The EtOH washings were evaporated to dryness and the residue was purified by column chromatography [Al_2O_3 (22 g), $CHCl_3$–EtOH (20:1, v/v)] to afford a second crop of **6g** (37 mg, total yield 27%), m.p. 291–292° (dec). Recrystallisations from EtOH gave colourless needles, m.p. 292–294° (dec) (lit.[4a] m.p. 296–297°) (Found: C, 55.02; H, 5.01; N, 32.49. $C_{10}H_{11}N_5O$ requires: C, 55.29; H, 5.10; N, 32.24%); NMR δ: 2.22 (3H, slightly broad owing to long range coupling with 7-H, 6-CH_3), 4.04 and 4.08 (3H each, s, NCH_3), 7.33 (1H, slightly broad, 7-H), 7.74 (1H, s, 2-H); UV $\lambda_{max}^{95\%\ EtOH}$ 233 nm (ϵ 32,900), 293 (7800); $\lambda_{max}^{H_2O}$ (pH 1) 228 (37,200), 277 (11,700); $\lambda_{max}^{H_2O}$ (pH 7 and pH 13) 233 (36,000), 264 (4100), 297 (7800); p*Ka* 3.41 ± 0.02.

3-Ethyl-3,4-dihydro-4,6-dimethyl-9H-imidazo[1,2-a]purin-9-one **6h**

A suspension of **5d** · H_2O (211 mg, 1 mmol) in Me_2SO (25 ml) was treated in a manner similar to that described for the synthesis of **6g** and the resulting mixture was stirred at 30° for 3 h. The orange coloured soln thus obtained was evaporated to dryness *in vacuo* and the solid residue was extracted with hot EtOH (10 ml). The EtOH soln was evaporated to dryness after addition of Al_2O_3 (2 g). The residual solid was placed on top of a column packed with Al_2O_3 (40 g). The column was eluted with $CHCl_3$–EtOH (20:1, v/v). The fluorescent eluate was evaporated to dryness to give a partly crystalline residue, which was washed with EtOH (1 ml) to afford **6h** (69 mg, 30%), m.p. 259–264° (dec). Recrystallisations from EtOH gave colourless pillars, m.p. 266–269° (dec) (Found: C, 57.03; H, 5.62; N, 30.43. $C_{11}H_{13}N_5O$ requires: C, 57.13; H, 5.67; N, 30.29%); NMR δ: 1.46 (3H, t, J = 7 Hz, CH_2CH_3), 2.22 (3H, d, J = 1 Hz, 6-CH_3), 4.05 (3H, s, 4-CH_3), 4.44 (2H, q, J = 7 Hz, CH_2CH_3), 7.35 (1H, q, J = 1 Hz, 7-H), 7.86 (1H, s, 2-H); UV $\lambda_{max}^{95\%\ EtOH}$ 233 nm (ϵ 33,200), 293 (8100); $\lambda_{max}^{H_2O}$ (pH 1) 228 (37,200), 277 (11,700); $\lambda_{max}^{H_2O}$ (pH 7 and pH 13) 233 (35,900), 264 (4200), 297 (7800); p*Ka* 3.41 ± 0.02.

3-Benzyl-3,4-dihydro-4,6-dimethyl-9H-imidazo[1,2-a]purin-9-one **6i**

A mixture of the starting materials containig **5f** (383 mg, 1.5 mmol) was prepared and allowed to react in essentially the same way as described for the synthesis of **6h**. The resulting soln was evaporated to dryness *in vacuo* and the residue was washed with hot EtOH to extract a fluorescent matter. To the EtOH extracts was added SiO_2 (5 g) and the mixture was evaporated to dryness. The solid residue was placed on top of a column (SiO_2, 25 g), which was eluted with AcOEt–EtOH (10:1, v/v) then with AcOEt–EtOH (6:1, v/v). The fluorescent eluate was collected and removal of the solvent by evaporation left a yellowish solid. This was washed with Et_2O (20 ml) and dried to give **6i** · H_2O (172 mg, 37%), m.p. 220°. Recrystallisations from MeOH gave colourless pillars. These were dried over P_2O_5 at 2 mmHg and 100° for 5 h and then exposed to air until a constant weight was reached, m.p. 245–247° (sinter at 220°) (Found: C, 61.99; H, 5.28; N, 22.57. $C_{16}H_{15}N_5O \cdot H_2O$ requires: C, 61.72; H, 5.50; N, 22.50%); NMR δ: 2.19 (3H, d, J = 1.2 Hz, 6-CH_3), 3.79 (3H, s, 4-CH_3), 5.76 (2H, s, $CH_2C_6H_5$), 7.0–7.2 (2H, m, phenyl protons), 7.37 (splitted by 1.2 Hz, 7-H, overlapped with a three-proton multiplet at 7.3–7.5 ppm due to the phenyl protons), 7.97 (1H, s, 2-H); UV $\lambda_{max}^{95\%\ EtOH}$ 234 nm (ϵ 33,900), 294 (8400); $\lambda_{max}^{H_2O}$ (pH 1) 229 (38,000), 277 (12,400); $\lambda_{max}^{H_2O}$(pH 7 and pH 13) 235 (34,700), 297 (7800); p*Ka* 3.27 ± 0.03.

Alkaline hydrolysis of 3,9-dimethylguanine **5a**

A mixture of **5a** · H_2O (200 mg, 1.01 mmol) and 1 *N* KOH aq (10 ml) was refluxed for 30 min, cooled, and brought to pH 8 with 10% HCl aq. The resulting precipitate was filtered off, washed with EtoH (2 ml), and dried to give **11** (39 mg, 21%) as colourless needles, m.p. > 300°. This sample was identical (IR spectrum and paper chromatography) with an authentic sample derived from **10**.

The combined filtrate and washings were evaporated to dryness and the residue was extracted with hot EtOH. The EtOH soln was evaporated to dryness after adding Al_2O_3 (5.0 g) and the solid residue was eluted with $CHCl_3$–EtOH (6:1, v/v). Removal of the solvent from the eluate by evaporation gave a solid. This was washed with EtOH (3 ml) to afford crude **8**. Recrystallisation from H_2O gave **8** (67 mg, 34%) as colourless prisms, m.p. 266–268°, identical (IR spectrum) with an authentic sample derived from **4a**.

The EtOH washings of **8** were evaporated to dryness and the solid residue was washed with AcOEt (3 ml) then dried to give **3a** (5.4 mg, 3.5%), m.p. 195–200°, identical (IR spectrum) with an authentic sample.[11c]

Hydrogenolysis of 3-benzylwye **6i**

A soln of **6i** · H_2O (16 mg, 0.05 mmol) in AcOH (10 ml) was hydrogenated over 10% Pd on C (30 mg) at 1 atm and room temp for 6 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to leave **1a** (10 mg, 96%). Recrystallisation from H_2O gave colourless prisms, m.p. > 300°. The tlc behaviour and IR spectrum of this sample matched with those of authentic **1a**.[1b]

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†Comparison with the spectrum of 3-methylxanthosine[21] enables the assignment.

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