Purines. LVII.[1] Regioselective Alkylation of N^6,9-Disubstituted 8-Oxoadenines: Syntheses of the Sea Anemone Purine Caissarone and Some Positional Isomers and Analogues

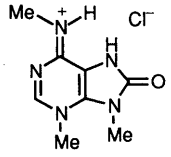
Tohru SAITO, Shigeji MORI, Jun CHIKAZAWA, Tae KANAI, and Tozo FUJII*

Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan. Received April 2, 1993

The first total synthesis of caissarone hydrochloride (1), a constituent of the sea anemone *Bunodosoma caissarum*, has been accomplished *via* a two-step route starting from N^6,9-dimethyl-8-oxoadenine (3), which is obtainable from 9-methyladenine (5) through a four-step route. The key step in the synthesis is the regioselective methylation of 3 at N(3), which has been designed on the basis of a methylation study of N^6-benzyl-9-methyl-8-oxoadenine (11). Some positional isomers (19, 24, and 31) and analogues (8, 26, and 32) of 1 have also been synthesized. A ^{1}H-NMR spectroscopic study has suggested that the free base (23) of caissarone is capable of forming a hetero-base pair (such as 41) with 2′,3′,5′-tri-*O*-acetylguanosine (40) in Me_2SO-d_6.

Keywords caissarone synthesis; sea anemone 8-oxopurine; 8-oxoadenine regioselective alkylation; catalytic hydrogenolysis; 8-oxoadenine acid dissociation constant; hydrogen bonding

Purine alkaloids are a rather small group of natural products characterized primarily by the high content of nitrogen due to their intrinsic purine nucleus, yet the number and kind of those reported have been steadily increasing in recent years.[2,3] In 1986, Zelnik *et al.*[4] reported the isolation of caissarone hydrochloride (**1**), a novel purine alkaloid featuring the 3,9-disubstituted purine skeleton with a methylimino group at C(6) and a carbonyl group at C(8), from the sea anemone *Bunodosoma caissarum* CORREA 1964 (Anthozoa, Actiniaria). The substance was found to induce various anomalies in sea urchin egg development[5] and to increase the motility of isolated mammalian intestine.[6] Its chemical structure (**1**) was established on the basis of spectroscopic measurements and an X-ray crystallographic analysis.[4] Our continuing interest and success in synthesizing purine alkaloids,[7-15] especially the marine sponge base 6-imino-1,9-dimethyl-8-oxopurine (**2**) as well as the N^6,9-dimethyl isomer (**3**),[3,16] led us to design a concise synthetic route to **1** from **3** in the present study; some positional isomers and analogues of **1** were also selected as targets for synthesis. A brief account of a part of the results reported here has been published in a preliminary form.[17]



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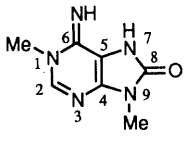

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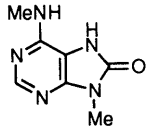

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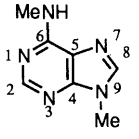

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It has been reported by El'tsov *et al.* that treatment of N^6,9-dimethyladenine (**4**) with MeI in $HCONMe_2$ (DMF) at 100—105 °C (in a sealed vessel) for 10 min resulted in monomethylation on N(1), N(3), and N(7) with a 51 : 30 : 19 selectivity.[18] In view of this second preference for methylation at the 3-position, we first examined the regioselectivity in methylation of the N^6-benzyl analogue **11** in the 8-oxoadenine series. For preparation of **11**, 9-methyl-8-oxoadenine (**6**), obtainable in 84% yield from 9-methyladenine (**5**) according to the previously reported two-step procedure,[3] was benzylated with $PhCH_2Br$ in $AcNMe_2$ at 100 °C for 8 h to give, after basification, the 1-benzylated product (**10**) in 80% yield (Chart 1). On treatment with boiling 1 N aqueous NaOH for 1 h, **10** underwent Dimroth rearrangement, affording the N^6-benzyl isomer **11** in 99% yield. This two-step conversion was analogous to that employed recently by us[3,16] for the synthesis of **3** from **6** through **2**, and the UV spectra of **10** and **11** in various solvents were similar to those of **2** and **3**, respectively.

Treatment of **11** with MeI in $AcNMe_2$ at 38—42 °C for 94 h furnished the 3-methylated product (**7**) in 85% yield, together with the 1-methylated product (**12**) in 7% yield. The 1-methyl structure of the latter product was unequivocally established by its conversion into the hydrochloride salt **13** (97% yield) by the use of Amberlite IRA-402 (Cl^-) and subsequent hydrogenolytic debenzylation [20% $Pd(OH)_2$–C/H_2,[19] H_2O, 1 atm, room temp., 2 h] leading to the known 1,9-dimethyl structure **2**[3] (78% yield). A similar conversion of the major product **7** into the hydrochloride salt **8** (96% yield) and subsequent catalytic hydrogenation [20% $Pd(OH)_2$–C/H_2, 50% (v/v) aqueous AcOH, 1 atm, 65—70 °C, 12 h] did not produce the expected *N*-debenzyl derivative (3,9-dimethyl-8-oxoadenine), but instead gave the monocyclic amidine salt **9** in 62% yield.[20] Characterization of **9** as the 4,5-dihydro-2-imidazolone derivative was readily achieved by elemental analysis and measurements of its IR spectrum [ν_{max}^{Nujol} cm^{-1}: 1710 and 1695 (CO)] and of its ^{1}H-NMR spectrum in Me_2SO-d_6 [selected data: δ 2.65 [3H, s, N(1)-Me], 3.23 [1H, dd, J = 10 and 7.5 Hz, C(5)-H_A], 3.81 [1H, dd, J = 10 Hz each, C(5)-H_B], 4.5—4.6 [1H, m, C(4)-H]].[21] This implied that **8** and hence **7** had the desired N(3)-methyl structure and C(2) as well as N(3) and its attached methyl group in **8** were lost in the course of the hydrogenation effected under acid hydrolytic conditions. The sequence **8**→**14**→**15**→**16**→**17**→**9** as shown in Chart 1 would explain this unexpected transformation. Interestingly, **8** thus obtained is the

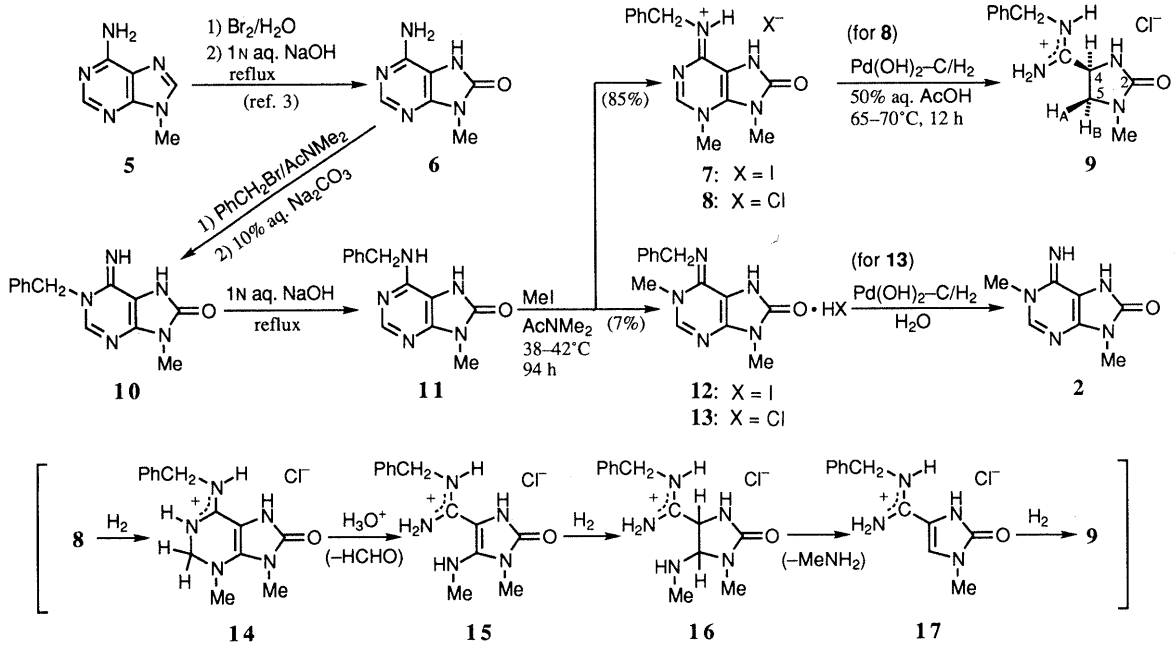


Chart 1

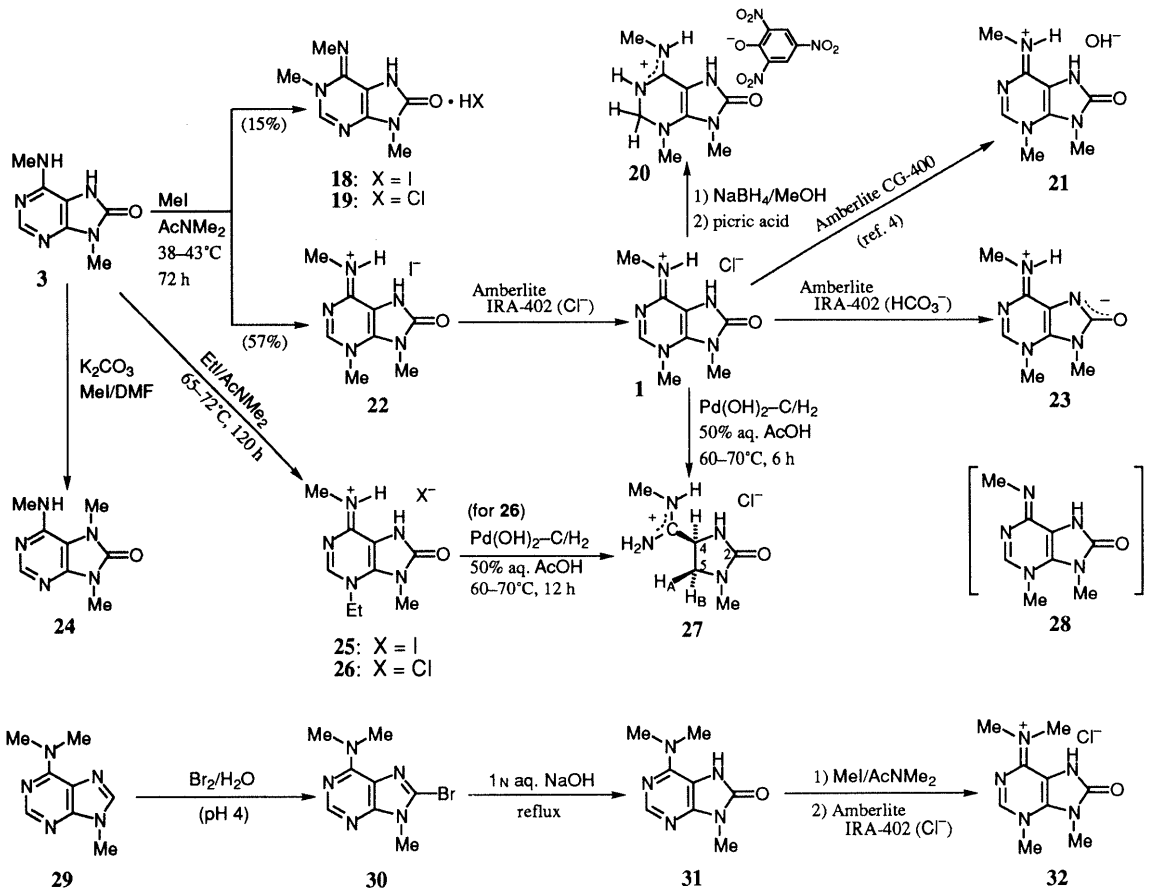


Chart 2

N^6-benzyl analogue of caissarone hydrochloride (**1**).

Encouraged by the above finding of a high N(3)-selectivity in methylation of **11**, we next carried out a similar methylation of the N^6-methyl analogue **3**. On treatment with MeI in $AcNMe_2$ at 38—43 °C for 72 h, **3** furnished the 3-methylated product **22** (57% yield) and the 1-methylated product **18** (15% yield) (Chart 2). The structure assignments of both products were made by analogy with the above methylation of **11** and by comparison of the UV spectra with those of **7** and **12**. Treatment of **22** with Amberlite IRA-402 (Cl^-) in H_2O gave the hydrochloride salt **1** in 98% yield. The synthetic **1** proved identical with a natural sample

of caissarone hydrochloride upon direct comparison. Furthermore, the synthetic **1** was reduced with $NaBH_4$ in MeOH at room temperature for 21.5 h, and the resulting 1,2-dihydro derivative was isolated in the form of the picrate **20** in 74% yield. This picrate was identical with that prepared from natural **1** in a similar manner according to the literature procedure.[4]

The minor product (**18**) from the above methylation of **3** was then treated with Amberlite IRA-402 (Cl^-) in H_2O to provide the hydrochloride salt **19**, a positional isomer of **1** and the N^6-methyl derivative of the marine sponge base **2**, in quantitative yield. Another positional isomer (**24**) was obtained from **3** in 79% yield by methylation with MeI in the presence of anhydrous K_2CO_3 in DMF at room temperature for 3 h. On treatment with 10% ethanolic HCl, **24** gave the hydrochloride **24**·HCl in 95% yield. Yet another positional isomer bearing two of the three methyl groups at N^6 and N(9) would be **31**. For synthesis of **31**, N^6,N^6,9-trimethyladenine (**29**)[22] was brominated with Br_2 in 0.5 M acetate buffer (pH 4) in a manner similar to that[3b] reported for the bromination of **5**, producing the 8-bromo derivative **30** in 68% yield. On treatment with boiling 1 N aqueous NaOH for 7 h, **30** furnished the desired isomer **31** in 80% yield. Methylation of **31** with a large excess of MeI in $AcNMe_2$ at 40—43 °C for 216 h and treatment of the product with Amberlite IRA-402 (Cl^-) provided the N(3)-methylated derivative **32** in 44% yield, paralleling the known N(3)-methylation[23] of N^6,N^6,9-trimethyladenine (**29**) under similar reaction conditions. The UV spectra of **32** in H_2O at various pH's were similar to those of **1**.

Ethylation of **3** with EtI in $AcNMe_2$ (at 65—72 °C for 120 h) was sluggish, but afforded the N(3)-ethylated product **25** in 26% yield. In this case, however, we were unable to isolate the N(1)-ethylated product, if any, from the reaction mixture. Treatment of **25** with Amberlite IRA-402 (Cl^-) in H_2O gave the N(3)-ethyl analogue **26** of caissarone hydrochloride (**1**). Catalytic hydrogenation of **26** [20% $Pd(OH)_2$–C/H_2, 50% (v/v) aqueous AcOH, 1 atm, 60—70 °C, 12 h] provided the monocyclic amidine salt **27** in 26% yield (from **25**). The same monocycle (**27**) was also obtained from **1** in 77% yield by a similar catalytic hydrogenation. These results clearly indicate that N(3) and its attached alkyl group were lost during the hydrogenation, suggesting the existence of the methylamine-eliminating process [**16**→**17** (Chart 1)] in the above-mentioned conversion of the N^6-benzyl analogue **8** into **9**.

Compounds structurally analogous to caissarone hydrochloride (**1**) include the 3,9-disubstituted adenine salts (type **33**) and N^6,N^6,3,9-tetrasubstituted adenine salts (type **37**). It has been shown that the pK_a value of **33** [for **33**⇌**36** + H^+ (Chart 3)] is considerably high[24,25] and **33**[24,25] as well as **37**[23,26] is unstable under basic conditions. For example, treatment of 3,9-dimethyladenine hydrochloride (**33**: $R^1 = R^2 = Me$; X = Cl) with Amberlite IRA-402 (HCO_3^-) in H_2O did not give the corresponding free base, but afforded its bicarbonate salt (**33**: $R^1 = R^2 = Me$; X = HCO_3) in 97% yield[24]; replacement of the ion-exchange resin by Amberlite CG-400 (OH^-) in that neutralization reaction resulted in the formation of the monocyclic amidine (**35**: $R^1 = R^2 = Me$) in 61% yield[24]; and **33** was equilibrated with the ring-opened derivative **34** in H_2O at pH 8.32 and 25 °C.[24] The high pK_a value of **33** and easy nucleophilic attack of hydroxide ion at C(2) have been explained[25] by the structural analogy between **33** and the "*para*-quinonoidal" resonance structure (**38**) of protonated 1,4-dihydro-4-imino-1-methylpyrimidine whose pK_a [for **38**⇌**39** + H^+ (Chart 3)] is 12.22.[27]

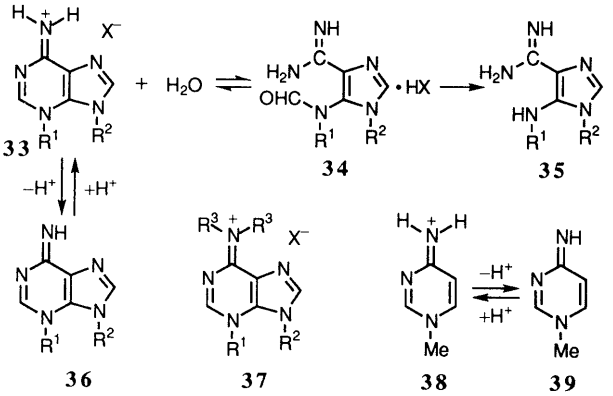
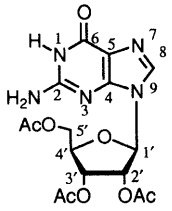


Chart 3

In contrast to these chemical properties of **33**, Zelnik *et al.* reported the successful preparation of a trihydrate of the free base of caissarone from its hydrochloride salt (**1**) by the use of Amberlite CG-400 in H_2O and assigned the structure **21**·$2H_2O$ to the trihydrate.[4] In our hands, treatment of **1** with Amberlite IRA-402 (HCO_3^-) in H_2O also gave the free base, but we were able to obtain an anhydrous sample. We prefer the zwitterionic form **23** (Chart 2) rather than the usual neutral form **28** for the free base on the basis of the following considerations. The acid dissociation constants determined UV spectrophotometrically for **1** and **32** were 6.78 and 6.73, respectively, and the second pK_a of **1** was estimated to be very high. This indicates that the pK_a value 6.78 is for dissociation of H^+ from N(7)-H in **1** and the second, higher one is for that from N^6-H. Interestingly, these acidic pK_a values of **1** and **32** are lower than that (pK_a 8.7)[28] of 8-oxoadenosine by *ca.* 2 units. Such increases in acidity of N(7)-H may be attributable to the electron-withdrawing effect of the exocyclic iminium group at C(6), which should maintain its positively charged structure even at higher pH's. The zwitterionic structure **23** of our anhydrous sample of the caissarone free base was also supported by its IR spectrum lacking a C=O absorption band in the 1755—1680 cm^{-1} region, unlike that of **1**, and by its ^{1}H-NMR spectrum in Me_2SO-d_6, which did not show a **28**-type N(7)-H peak in the δ 11—12 region, but displayed an unresolved doublet at δ 3.01 (N^6-Me). On addition of D_2O, this broad signal turned into a sharp singlet, suggesting the attachment of a proton to N^6. The observed stability of the ring of **1** and hence that of the corresponding free base (**23**) under basic conditions, relative to that of **33**, is interpretable in terms of the negative change in the zwitterionic structure **23** in the alkaline region, which would make C(2) less susceptible to nucleophilic attack as a result of resonance in the zwitterion. However, **1** was still rather unstable in 1 N aqueous NaOH at 55 °C.

Finally, the unique zwitterionic structure of the caissarone free base **23** led us to test its ability to form a hetero-base pair[29] through hydrogen bonding. Thus, **23**, 2′,3′,5′-tri-*O*-acetylguanosine (**40**),[30] and an equimolar mixture of **23** and **40** were each dissolved in Me_2SO-d_6 at 2.5 mM concentration with respect to the individual compounds,



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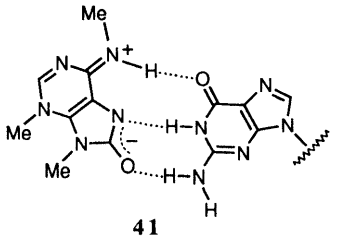

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TABLE I. Association of Caissarone (**23**) with 2′,3′,5′-Tri-*O*-acetylguanosine (**40**) as Detected by ^{1}H-NMR Spectroscopy

Proton	Chemical shift (δ)[a] in Me_2SO-d_6			$\delta_{23+40}-\delta_{23}$ or $\delta_{23+40}-\delta_{40}$
	23	**40**	**23+40**	
N^6-Me	3.01 (d)[b]	—	3.02 (d)[b]	+0.01
N(9)-Me	3.57 (s)	—	3.58 (s)	+0.01
N(3)-Me	4.10 (s)	—	4.10 (s)	0.00
N^6-H	7.78 (br)	—	7.92 (br)	+0.14
C(2)-H	8.27 (s)	—	8.29 (s)	+0.02
O-Ac	—	2.04 (s)	2.04 (s)	0.00
O-Ac	—	2.04 (s)	2.04 (s)	0.00
O-Ac	—	2.11 (s)	2.11 (s)	0.00
C(5′)-H	—	4.26 (dd)[c]	4.26 (dd)[c]	0.00
C(4′)-H	—	4.32 (ddd)[d]	4.32 (ddd)[d]	0.00
C(5′)-H	—	4.38 (dd)[e]	4.38 (dd)[e]	0.00
C(3′)-H	—	5.49 (dd)[f]	5.49 (dd)[f]	0.00
C(2′)-H	—	5.79 (dd)[g]	5.79 (dd)[g]	0.00
C(1′)-H	—	5.98 (d)[h]	5.98 (d)[h]	0.00
C(2)-NH_2	—	6.53 (br)	6.66 (br)	+0.13
C(8)-H	—	7.93 (s)	7.94 (s)	+0.01
N(1)-H	—	10.73 (br s)	11.03 (br s)	+0.30

a) Measured at 27 °C at 2.5 mM concentrations with respect to the individual compounds and expressed in ppm downfield from internal Me_4Si. The letter(s) in parentheses designate(s) the multiplicity or shape of the signal; the abbreviations are given in Experimental. *b*) Unresolved doublet. *c*) With J=5.5 and 11 Hz. *d*) Unresolved doublet-of-doublets-of-doublets. *e*) With J=3.5 and 11 Hz. *f*) Unresolved doublet-of-doublets. *g*) With J=6 Hz each. *h*) With J=6 Hz.

and the ^{1}H-NMR spectra of the resulting solutions were measured at 27 °C. It may be seen from Table I that most of the signals arising from the mixture of **23** and **40** appeared at almost the same fields as did the corresponding signals of **23** or **40** alone. However, the N^6-H, C(2)-NH_2, and N(1)-H protons of the mixture resonated at lower fields by 0.13—0.30 ppm than the corresponding proton(s) of **23** or **40** alone, suggesting that the protons of the former three groups are those involved in hydrogen bonds.[31] Although similar ^{1}H-NMR spectroscopic measurements at higher concentrations were not made because of the low solubility of **23** in Me_2SO-d_6, the data in Table I thus suggest the existence of a hetero-base pair association such as **41** in a solution of **23** and **40** in Me_2SO-d_6.

In conclusion, the above two-step synthesis of **1** from **3** through **22** formally concludes a six-step synthesis of caissarone hydrochloride (**1**) from 9-methyladenine (**5**) in 32% overall yield, since **3** is obtainable from **5** *via* a four-step route in 57% overall yield.[3] The key step in the present synthesis is the regioselective methylation of **3** to give **22**, which, together with the results from ethylation of **3** and from methylation of the N^6-benzyl analogue **11**, indicates that the N(3) atom of N^6-alkyl-9-methyl-8-oxoadenines (type **3** or **11**) is the most favored site of alkylation among the nitrogens in the neutral species. Interestingly, the above ^{1}H-NMR spectroscopic study suggests that the caissarone free base **23** is able to form a hetero-base pair with **40**.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 or a Büchi model 530 capillary melting point apparatus and are corrected. See refs. 1, 12*b*, and 15 for details of chromatographies, instrumentation, and measurements. For the ^{1}H-NMR spectroscopic study on association of the caissarone free base **23** with 2′,3′,5′-tri-*O*-acetylguanosine (**40**),[30] a JEOL JNM-GSX-500 (^{1}H 500 MHz) instrument was used. Chemical shifts are reported in δ values relative to Me_4Si (for Me_2SO-d_6 solutions) or to sodium 3-(trimethylsilyl)-1-propanesulfonate (for D_2O solutions). For the measurements of pH values, a Toa HM-18ET pH meter equipped with a Toa type GST-155C glass electrode was utilized, and pK_a values of **1** and **32** at 25 °C and ionic strength 1.0 were determined in a manner similar to that described previously.[32] Elemental analyses and MS measurements were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, ddd = doublet-of-doublets-of-doublets, m = multiplet, q = quartet, s = singlet, sh = shoulder, t = triplet.

1,6,7,9-Tetrahydro-6-imino-9-methyl-1-(phenylmethyl)-8*H*-purin-8-one (10) A mixture of 9-methyl-8-oxoadenine (**6**)[33] (8.26 g, 50 mmol) and $PhCH_2Br$ (17.10 g, 100 mmol) in $AcNMe_2$ (100 ml) was stirred at 100 °C for 8 h. After cooling, the reaction mixture was concentrated *in vacuo*, and the residue was triturated with acetone (40 ml). The resulting mixture was kept at room temperature overnight, and the colorless precipitate that deposited was filtered off and then dissolved in warm H_2O (300 ml). The aqueous solution was brought to pH 9 by addition of 10% aqueous Na_2CO_3 and kept in a refrigerator for 2 h. The colorless crystals that resulted were filtered off, washed with H_2O, and dried to give **10**·H_2O (10.90 g, 80%), mp 213—214 °C (dec.). Recrystallization from EtOH and drying over P_2O_5 at 3 mmHg and room temperature for 18 h yielded an analytical sample of **10**·H_2O as colorless prisms, mp 214—215 °C (dec.); MS *m/z*: 255 (M^+); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 293.5 nm (ε 13200); $\lambda_{max}^{H_2O}$ (pH 1) 275 (11600); $\lambda_{max}^{H_2O}$ (pH 7) 286 (13700); $\lambda_{max}^{H_2O}$ (pH 13) 282 (15600), 315 (sh) (4400); IR ν_{max}^{Nujol} cm^{-1}: 3400 and 3250 (NH, H_2O), 1690 (CO); ^{1}H-NMR (Me_2SO-d_6) δ: 3.21 [3H, s, N(9)-Me], 5.33 (2H, s, C$\underline{H}_2$Ph), 7.31 (5H, m, $CH_2\underline{Ph}$), 8.26 [1H, s, C(2)-H]. *Anal.* Calcd for $C_{13}N_{13}N_5O\cdot H_2O$: C, 57.13; H, 5.53; N, 25.63. Found: C, 57.19; H, 5.43; N, 25.62.

7,9-Dihydro-9-methyl-6-[(phenylmethyl)amino]-8*H*-purin-8-one (11) A stirred mixture of **10**·H_2O (10.21 g, 37 mmol) and 1 N aqueous NaOH (200 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized by addition of 10% aqueous HCl and kept in a refrigerator overnight. The colorless crystals that deposited were filtered off, washed with H_2O, and dried to afford **11** (9.44 g, 99%), mp 205.5—207 °C. Recrystallization from EtOH gave an analytical sample as colorless prisms, mp 207.5—208.5 °C; MS *m/z*: 255 (M^+); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 276 nm (ε 20300); $\lambda_{max}^{H_2O}$ (pH 1) 285.5 (16900); $\lambda_{max}^{H_2O}$ (pH 7) 276 (20900); $\lambda_{max}^{H_2O}$ (pH 13) 286 (21000); IR ν_{max}^{Nujol} cm^{-1}: 3350, 3225, and 3160 (NH), 1695 (CO); ^{1}H-NMR (Me_2SO-d_6) δ: 3.23 [3H, s, N(9)-Me], 4.66 (2H, d, J=5.5 Hz, C$\underline{H}_2$Ph), 6.88 (1H, t, J=5.5 Hz, N$\underline{H}$CH$_2$Ph), 7.34 (5H, m, $CH_2\underline{Ph}$), 8.12 [1H, s, C(2)-H], 10.05 [1H, s, N(7)-H]. *Anal.* Calcd for $C_{13}H_{13}N_5O$: C, 61.17; H, 5.13; N, 27.43. Found: C, 61.31; H, 5.07; N, 27.38.

Methylation of 11 Leading to 3,6,7,9-Tetrahydro-3,9-dimethyl-6-[(phenylmethyl)imino]-8*H*-purin-8-one Hydriodide (7) and 1,6,7,9-Tetrahydro-1,9-dimethyl-6-[(phenylmethyl)imino]-8*H*-purin-8-one Hydriodide (12) A mixture of **11** (5.11 g, 20 mmol) and MeI (28.39 g, 200 mmol) in $AcNMe_2$ (30 ml) was stirred at 38—42 °C for 94 h. After cooling, the colorless crystals that deposited were filtered off, washed with EtOH, and dried to give a first crop (3.56 g, 45%) of **7**, mp 227.5—228.5 °C (dec.). Concentration of the filtrate under reduced pressure and recrystallization of the residue from EtOH yielded a second crop (3.22 g, 40%) of **7** as colorless crystals, mp 222—224 °C (dec.). The total yield of **7** was 6.78 g (85%). The crude **7** was recrystallized from H_2O to provide an analytical sample of **7** as colorless prisms, mp 232—233 °C (dec.); MS *m/z*: 269 (M^+ − HI); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 221 nm (ε 28100), 305 (15800); $\lambda_{max}^{H_2O}$ (pH 1) 226.5 (33000), 298.5 (25400); $\lambda_{max}^{H_2O}$ (pH 7) 226.5 (33300), 308 (18800); $\lambda_{max}^{H_2O}$ (pH 13) 226.5 (33600), 312 (19800); IR ν_{max}^{Nujol} cm^{-1}: 3190 and 3155 (NH), 1750 (CO), 1660 (C=N$^+$); ^{1}H-NMR (Me_2SO-d_6) δ: 3.62 [3H, s, N(9)-Me], 4.15 [3H, s, N(3)-Me], 4.79 (2H, d, J=5.5 Hz, NHC$\underline{H}_2$Ph), 7.36 (5H, m, $CH_2\underline{Ph}$), 8.35 (1H, t, J=5.5 Hz, N$\underline{H}$CH$_2$Ph), 8.59 [1H, s, C(2)-H], 11.02 [1H, br, N(7)-H]. *Anal.* Calcd for $C_{14}H_{15}N_5O\cdot HI$: C, 42.33; H, 4.06; N, 17.63. Found: C, 42.39; H, 4.05; N, 17.73.

The ethanolic filtrate, obtained when the second crop of **7** was isolated, was then concentrated *in vacuo*, and the residue was recrystallized first from EtOH and then from H_2O to give **12**·H_2O (588 mg, 7%) as yellowish

prisms, mp 208—209 °C (dec.). Further recrystallization from H_2O and drying over P_2O_5 at 2 mmHg and room temperature for 24 h furnished an analytical sample of **12**·H_2O as colorless pillars, mp 214.5—215 °C (dec.); MS *m/z*: 269 ($M^+ - HI$); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 220 nm (ε 39900), 300 (13000); $\lambda_{max}^{H_2O}$ (pH 1) 226 (40700), 277 (sh) (9200), 296 (11700); $\lambda_{max}^{H_2O}$ (pH 7) 224 (39900), 297 (13700); $\lambda_{max}^{H_2O}$ (pH 13) 222 (34800), 287 (14700); IR ν_{max}^{Nujol} cm^{-1}: 3420, 3360, and 3260 (NH's, H_2O), 1735 (CO); ^{1}H-NMR (Me_2SO-d_6) δ: 3.31 [3H, s, N(9)-Me], 3.87 [3H, s, N(1)-Me], 4.87 (2H, s, C$\underline{H}_2$Ph), 7.36 (5H, m, CH$_2$$\underline{Ph}$), 8.5—9.1 (1H, br, N$\underline{H}CH_2$Ph), 8.76 [1H, s, C(2)-H], 11.3—12.2 [1H, br, N(7)-H]. *Anal.* Calcd for $C_{14}H_{15}N_5O$·HI·H_2O: C, 40.50; H, 4.37; N, 16.87. Found: C, 40.32; H, 4.27; N, 16.91.

3,6,7,9-Tetrahydro-3,9-dimethyl-6-[(phenylmethyl)imino]-8*H*-purin-8-one Hydrochloride (8) The hydriodide **7** (1.99 g, 5 mmol) was dissolved in warm H_2O (300 ml). After cooling, the aqueous solution was passed through a column packed with Amberlite IRA-402 (Cl^-) (12.5 ml), and the column was eluted with H_2O (100 ml). The aqueous eluates were combined and concentrated to dryness *in vacuo*, leaving **8**·1/2H_2O (1.52 g, 96%) as a colorless solid, mp 248—249.5 °C (dec.). Recrystallization from H_2O and drying over P_2O_5 at 2 mmHg and room temperature for 24 h produced an analytical sample of **8**·1/2H_2O as colorless pillars, mp 249—250 °C (dec.); MS *m/z*: 269 ($M^+ - HCl$); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 229 nm (ε 22300), 304 (19400); $\lambda_{max}^{H_2O}$ (pH 1) 227 (20600), 298.5 (24300); $\lambda_{max}^{H_2O}$ (pH 7) 226 (20500), 307 (17800); $\lambda_{max}^{H_2O}$ (pH 13) 226 (20500), 312 (18500); IR ν_{max}^{Nujol} cm^{-1}: 3230—3080 (NH), 1725 (CO), 1670 (C=N$^+$); ^{1}H-NMR (Me_2SO-d_6) δ: 3.60 [3H, s, N(9)-Me], 4.12 [3H, s, N(3)-Me], 4.77 (2H, d, J=6 Hz, NHC$\underline{H}_2$Ph), 7.34 (5H, m, CH$_2$$\underline{Ph}$), 8.55 [1H, s, C(2)-H], 9.47 (1H, t, J=6 Hz, N$\underline{H}$CH$_2$Ph), 11.94 [1H, br, N(7)-H]. *Anal.* Calcd for $C_{14}H_{15}N_5O$·HCl·1/2H_2O: C, 53.42; H, 5.44; N, 22.25. Found: C, 53.26; H, 5.09; N, 22.32.

1,6,7,9-Tetrahydro-1,9-dimethyl-6-[(phenylmethyl)imino]-8*H*-purin-8-one Hydrochloride (13) The hydriodide monohydrate **12**·H_2O (40 mg, 0.096 mmol) was dissolved in warm H_2O (7 ml). After cooling, the aqueous solution was passed through a column of Amberlite IRA-402 (Cl^-) (1 ml), and the column was eluted with H_2O (20 ml). The aqueous eluates were combined and concentrated to dryness *in vacuo* to leave a colorless solid. The solid was dried over P_2O_5 at 2 mmHg and room temperature for 24 h and then exposed to air until a constant weight was reached, giving **13**·2H_2O (32 mg, 97%), mp 218—219 °C (dec.). Recrystallization from H_2O and drying followed by moisturizing in the same manner as described above afforded an analytical sample of **13**·2H_2O as colorless prisms, mp 217—218 °C (dec.); MS *m/z*: 269 ($M^+ - HCl$); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 227 nm (ε 24200), 301 (12000); $\lambda_{max}^{H_2O}$ (pH 1) 226 (29000), 276 (sh) (9700), 297 (12500); $\lambda_{max}^{H_2O}$ (pH 7) 219 (28100), 297 (14200); $\lambda_{max}^{H_2O}$ (pH 13) 287 (15300); IR ν_{max}^{Nujol} cm^{-1}: 3400, 3165, and 3115 (NH, H_2O), 1725 (CO); ^{1}H-NMR (Me_2SO-d_6) δ: 3.29 [3H, s, N(9)-Me], 3.92 [3H, s, N(1)-Me], 4.90 (2H, s, C$\underline{H}_2$Ph), 7.35 (5H, m, CH$_2$$\underline{Ph}$), 8.76 [1H, s, C(2)-H], 8.8—11.6 (2H, br, two NH's). *Anal.* Calcd for $C_{14}H_{15}N_5O$·HCl·2H_2O: C, 49.20; H, 5.90; N, 20.49. Found: C, 49.37; H, 5.87; N, 20.71.

Conversion of 8 into 1-Methyl-2-oxo-*N*-(phenylmethyl)-4-imidazolidinecarboximidamide Hydrochloride (9) A solution of **8**·1/2H_2O (611 mg, 1.94 mmol) in 50% (v/v) aqueous AcOH (35 ml) was hydrogenated over 20% Pd(OH)$_2$–C catalyst (611 mg) at atmospheric pressure and 65—70 °C for 6 h. The reaction was so slow that more catalyst (611 mg) was added at this stage, and hydrogenation was continued under similar conditions for a further 6 h. The catalyst was removed by filtration and washed with H_2O (150 ml). The filtrate and washings were combined and concentrated *in vacuo* to leave a yellow oil, which was triturated with EtOH (5 ml). The insoluble solid that resulted was filtered off, washed with a little EtOH, and dried to furnish **9** (324 mg, 62%), mp 203—206 °C (dec.). Recrystallizations from acetone (1 ml) containing H_2O (1 drop) yielded an analytical sample as colorless prisms, mp 218.5—219.5 °C (dec.); MS *m/z*: 232 ($M^+ - HCl$); IR ν_{max}^{Nujol} cm^{-1}: 3230 and 3150 (NH), 1710 and 1695 (CO); ^{1}H-NMR (Me_2SO-d_6) δ: 2.65 [3H, s, N(1)-Me], 3.23 [1H, dd, J=10 and 7.5 Hz, C(5)-H$_A$],[21] 3.81 [1H, dd, J=10 Hz each, C(5)-H$_B$],[21] 4.54 (2H, m, NHC$\underline{H}_2$Ph), 4.5—4.6 [1H, m, C(4)-H], 6.93 [1H, d, J=3 Hz, N(3)-H], 7.37 (5H, m, CH$_2$$\underline{Ph}$), 9.28 and 9.38 (1H each, br, =N$\underline{H}_2^+$), 10.10 (1H, dull t, J=5.5 Hz, N$\underline{H}$CH$_2$Ph). *Anal.* Calcd for $C_{12}H_{16}N_4O$·HCl: C, 53.63; H, 6.38; N, 20.85. Found: C, 53.47; H, 6.34; N, 20.87.

Conversion of 13 into 1,6,7,9-Tetrahydro-6-imino-1,9-dimethyl-8*H*-purin-8-one (2) A solution of **13**·2H_2O (98 mg, 0.29 mmol) in H_2O (6 ml) was hydrogenated over 20% Pd(OH)$_2$–C catalyst (100 mg) at atmospheric pressure and room temperature for 2 h. The catalyst was filtered off and washed with H_2O (8 ml). The filtrate and washings were combined and concentrated *in vacuo* to leave a colorless solid (60 mg), which was dissolved in warm H_2O (8 ml). The aqueous solution was brought to pH 9 by addition of 10% aqueous Na_2CO_3 and then kept in a refrigerator. The colorless needles that deposited were filtered off, washed with H_2O, and dried to give **2** (40 mg, 78%), mp >300 °C. This sample was identical (by comparison of the MS and UV, IR, and ^{1}H-NMR spectra) with authentic **2**.[3]

Methylation of 7,9-Dihydro-9-methyl-6-(methylamino)-8*H*-purin-8-one (3) Leading to 3,6,7,9-Tetrahydro-3,9-dimethyl-6-(methylimino)-8*H*-purin-8-one Hydriodide (Caissarone Hydriodide) (22) and 1,6,7,9-Tetrahydro-1,9-dimethyl-6-(methylimino)-8*H*-purine-8-one Hydriodide (18) A mixture of N^6,9-dimethyl-8-oxoadenine (**3**)[3] (2.69 g, 15 mmol) and MeI (21.3 g, 150 mmol) in $AcNMe_2$ (15 ml) was stirred at 38—43 °C for 72 h. After cooling, the colorless solid that deposited was filtered off, washed with EtOH (20 ml), and recrystallized from H_2O (40 ml) to give **22** (2.74 g, 57%) as colorless prisms, mp 264—266 °C (dec.). Further recrystallization from EtOH–H_2O (12 : 1, v/v) yielded an analytical sample of **22** as colorless prisms, mp 266—267 °C (dec.); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 224 nm (ε 30800), 301 (16300); $\lambda_{max}^{H_2O}$ (pH 1) 226 (31800), 296 (21300); $\lambda_{max}^{H_2O}$ (pH 7) 226 (32600), 304 (16200); $\lambda_{max}^{H_2O}$ (pH 13) 226 (32300), 310 (16900); IR ν_{max}^{Nujol} cm^{-1}: 3190, 3165, and 3090 (NH), 1730 (CO), 1673 (C=N$^+$); ^{1}H-NMR (Me_2SO-d_6) δ: 3.08 [3H, d, J=5 Hz, NH$\underline{Me}$], 3.61 [3H, s, N(9)-Me], 4.14 [3H, s, N(3)-Me], 7.89 (1H, br, N$\underline{H}$Me), 8.57 [1H, s, C(2)-H], 11.04 [1H, dull s, N(7)-H]. *Anal.* Calcd for $C_8H_{11}N_5O$·HI: C, 29.92; H, 3.77; N, 21.81. Found: C, 29.86; H, 3.82; N, 22.11.

Concentration of the aqueous mother liquor, obtained from the above first recrystallization of the crude product, under reduced pressure left a slightly yellowish solid. Recrystallization of the solid from EtOH–H_2O (4 : 1, v/v) (15 ml) afforded **18** (734 mg, 15%) as colorless prisms, mp 260—262 °C (dec.). Further recrystallization from EtOH–H_2O (10 : 1, v/v) provided an analytical sample of **18** as colorless prisms, mp 262.5—263.5 °C (dec.); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 223 nm (ε 36300), 296 (10800); $\lambda_{max}^{H_2O}$ (pH 1) 226 (40100), 273 (9600), 292 (9300); $\lambda_{max}^{H_2O}$ (pH 7) 225 (37900), 290 (11500); $\lambda_{max}^{H_2O}$ (pH 13) 224 (33500), 286 (13100); IR ν_{max}^{Nujol} cm^{-1}: 3190, 3140, and 3080 (NH), 1709 (CO); ^{1}H-NMR (Me_2SO-d_6) δ: 3.23 (3H, dull s, NH$\underline{Me}$), 3.33 [3H, s, N(9)-Me], 3.74 [3H, s, N(1)-Me], 8.34 (1H, br, N$\underline{H}$Me), 8.65 [1H, s, C(2)-H], 11.79 [1H, dull s, N(7)-H]. *Anal.* Calcd for $C_8H_{11}N_5O$·HI: C, 29.92; H, 3.77; N, 21.81. Found: C, 29.95; H, 3.82; N, 21.82.

1,6,7,9-Tetrahydro-1,9-dimethyl-6-(methylimino)-8*H*-purin-8-one Hydrochloride (19) A solution of **18** (96 mg, 0.3 mmol) in H_2O (8 ml) was passed through a column of Amberlite IRA-402 (Cl^-) (1 ml), and the column was eluted with H_2O (25 ml). The aqueous eluates were combined and concentrated *in vacuo* to leave a colorless solid. The solid was dried over P_2O_5 at 2 mmHg and 100 °C for 3 h and then exposed to air until a constant weight was reached, giving **19**·2H_2O (80 mg, 100%), mp 246.5—248.5 °C (dec.). Recrystallization from EtOH–H_2O (5 : 1, v/v) and drying followed by moisturizing in the same manner as described above yielded an analytical sample of **19**·2H_2O as colorless needles, mp 247.5—249.5 °C (dec.); UV λ_{max}^{MeOH} 229 nm (ε 25400), 276 (8700), 299 (9400); $\lambda_{max}^{95\%\ aq.\ EtOH}$ 228 (24200), 276 (sh) (7700), 296 (10500); $\lambda_{max}^{H_2O}$ (pH 1) 225.5 (26900), 273 (9600), 292 (9300); $\lambda_{max}^{H_2O}$ (pH 7) 224 (25000), 290 (11600); $\lambda_{max}^{H_2O}$ (pH 13) 286 (13200); IR ν_{max}^{Nujol} cm^{-1}: 3465—3080 (NH, H_2O), 1723 (CO); ^{1}H-NMR (D_2O) δ: 3.36 (3H, s, NH$\underline{Me}$), 3.44 [3H, s, N(9)-Me], 3.83 [3H, s, N(1)-Me], 8.44 [1H, s, C(2)-H]; ^{1}H-NMR (Me_2SO-d_6) δ: 3.23 (3H, s, N^6-Me), 3.33 [N(9)-Me and H_2O], 3.79 [3H, s, N(1)-Me], 8.4—9.2 (1H, br, N$\underline{H}$Me), 8.66 [1H, s, C(2)-H], 11.76 [1H, br, N(7)-H]. *Anal.* Calcd for $C_8H_{11}N_5O$·HCl·2H_2O: C, 36.16; H, 6.07; N, 26.36. Found: C, 36.05; H, 6.16; N, 26.19.

3,6,7,9-Tetrahydro-3,9-dimethyl-6-(methylimino)-8*H*-purin-8-one Hydrochloride (Caissarone Hydrochloride) (1) The hydriodide **22** (642 mg, 2 mmol) was dissolved in warm H_2O (90 ml). After cooling, the aqueous solution was passed through a column of Amberlite IRA-402 (Cl^-) (5 ml), and the column was eluted with H_2O (50 ml). The eluates were combined and concentrated *in vacuo*, leaving a colorless solid, which was dried to give **1** (450 mg, 98%), mp 276.5—278 °C (dec.). Recrystallization from EtOH–H_2O (5 : 1, v/v) yielded an analytical sample of **1** as colorless needles, mp 278.5—279.5 °C (dec.); MS *m/z*: 193 ($M^+ - HCl$); pK_a 6.78±0.05 (at 25 °C); UV λ_{max}^{MeOH} 227.5 nm (ε 19800), 299 (20500); $\lambda_{max}^{95\%\ aq.\ EtOH}$ 228 (19600), 300 (17300); $\lambda_{max}^{H_2O}$ (pH 1) 225 (18900), 296 (21000); $\lambda_{max}^{H_2O}$ (pH 7) 225 (19300), 304 (15900); $\lambda_{max}^{H_2O}$ (pH 13) 225 (19100), 310 (16600); IR ν_{max}^{Nujol} cm^{-1}: 3245—3100 (NH), 1755 (CO), 1680 (C=N$^+$); ^{1}H-NMR (D_2O) δ: 3.14 (3H, s, N^6-Me), 3.71 [3H, s, N(9)-Me], 4.19 [3H, s, N(3)-Me], 8.34 [1H, s, C(2)-H]; ^{1}H-NMR (Me_2SO-d_6) δ: 3.05 (3H, d, J=5 Hz, NH$\underline{Me}$), 3.60 [3H, s, N(9)-Me], 4.13 [3H, s, N(3)-Me], 8.55 [1H, s, C(2)-H], 8.90 (1H, q, J=5 Hz, N$\underline{H}$Me), 11.83 [1H, dull s, N(7)-H]. *Anal.* Calcd for $C_8H_{11}N_5O$·HCl: C, 41.84; H, 5.27; N, 30.49. Found: C, 41.74; H, 5.29; N, 30.46. The synthetic **1** was identical [by mixture melting point test and

comparison of the UV, IR, and ^{1}H-NMR spectra and TLC mobility (in two solvent systems)] with a natural sample, mp 278.5—279.5 °C (dec.).

In a separate experiment, **1** was treated with 1 N aqueous NaOH at 55 °C for 1.5 h. The TLC analysis of the reaction mixture indicated the disappearance of **1** and formation of at least four substances.

1,2,3,6,7,9-Hexahydro-3,9-dimethyl-6-(methylimino)-8*H*-purin-8-one Picrate (1,2-Dihydrocaissarone Picrate) (20) A suspension of **1** (100 mg, 0.44 mmol) in MeOH (5 ml) was stirred at room temperature, and $NaBH_4$ (33 mg, 0.87 mmol) was added portionwise. Stirring was continued at room temperature for 2.5 h, for a further 2 h after another addition of $NaBH_4$ (33 mg, 0.87 mmol), and for a further 17 h after yet another addition of $NaBH_4$ (33 mg, 0.87 mmol). The reaction mixture was then combined with a saturated solution (4 ml) of picric acid in EtOH and kept in a refrigerator for 7 h. The yellow precipitate that deposited was filtered off, washed with EtOH, and recrystallized from 50% (v/v) aqueous EtOH to give **20** (137 mg, 74%), mp 227—229 °C (dec.). Further recrystallization from 50% (v/v) aqueous EtOH provided an analytical sample of **20** as yellow needles, mp 226—227.5 °C (dec.); IR ν_{max}^{Nujol} cm^{-1}: 3240 (NH), 1715 (CO); ^{1}H-NMR (Me_2SO-d_6) δ: 2.88 (3H, d, J=5 Hz, N^6-Me), 2.98 and 3.22 (3H each, s, two NMe's), 4.58 [2H, d, J=4 Hz, C(2)-H's], 8.06 (1H, q, J=5 Hz, N^6-H), 8.48 [1H, m, N(1)-H], 8.58 (2H, s, aromatic protons), 9.93 [1H, s, N(7)-H]. *Anal.* Calcd for $C_{14}H_{16}N_8O_8$: C, 39.63; H, 3.80; N, 26.41. Found: C, 39.36; H, 3.77; N, 26.21. This sample was identical (by comparison of the IR and ^{1}H-NMR spectra and TLC mobility) with the one prepared from natural **1** according to the literature procedure.[4]

3,6,7,9-Tetrahydro-3,9-dimethyl-6-(methylimino)-8-oxo-8*H*-purin-7-ide (Caissarone) (23) The hydrochloride **1** (459 mg, 2 mmol) was dissolved in warm H_2O (15 ml). After cooling, the solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (5 ml), and the column was eluted with H_2O (100 ml). The eluates were combined and concentrated *in vacuo*, and the residue was first dried over P_2O_5 at 2 mmHg and room temperature for 2 d and then exposed to air until a constant weight was reached, leaving a colorless solid (480 mg), mp 250—251 °C (dec.). Three recrystallizations of the solid from MeOH, followed by drying over P_2O_5 at 2 mmHg and room temperature for 12 h, furnished **23** as colorless prisms, mp 260—267 °C (dec.); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 227 nm (ε 19400), 320 (15600); $\lambda_{max}^{H_2O}$ (pH 1) 225 (19300), 296 (21200); $\lambda_{max}^{H_2O}$ (pH 7) 225 (19300), 304 (16100); $\lambda_{max}^{H_2O}$ (pH 13) 225 (19100), 310 (16600); IR ν_{max}^{Nujol} cm^{-1}: 3265 (NH), 1660 (C=N$^+$); ^{1}H-NMR (D_2O) δ: 3.07 (3H, s, N^6-Me), 3.63 [3H, s, N(9)-Me], 4.13 [3H, s, N(3)-Me], 8.04 [1H, s, C(2)-H]; ^{1}H-NMR (Me_2SO-d_6) (see Table I). *Anal.* Calcd for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25. Found: C, 49.65; H, 5.79; N, 36.06.

3-Ethyl-3,6,7,9-tetrahydro-9-methyl-6-(methylimino)-8*H*-purin-8-one Hydriodide (25) A mixture of **3**[3] (358 mg, 2 mmol) and EtI (3.12 g, 20 mmol) in $AcNMe_2$ (5 ml) was stirred at 65—72 °C for 120 h. The reaction mixture was concentrated *in vacuo*, and the residue was triturated with EtOH (15 ml). The insoluble solid that resulted was filtered off, washed with EtOH (2 ml), and dried to give the crude product (327 mg), mp 229.5—231 °C (dec.). Recrystallization of the crude product from EtOH (70 ml) yielded **25** (171 mg, 26%) as slightly yellowish needles, mp 255.5—258 °C (dec.). Three more recrystallizations in a similar manner gave an analytical sample of **25** as colorless needles, mp 262—263 °C (dec.); MS m/z: 207 (M$^+$ − HI); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 224 nm (ε 31600), 301 (17200); $\lambda_{max}^{H_2O}$ (pH 1) 226 (33400), 296 (21000); $\lambda_{max}^{H_2O}$ (pH 7) 226 (33600), 304 (15700); $\lambda_{max}^{H_2O}$ (pH 13) 226 (33700), 310 (16200); IR ν_{max}^{Nujol} cm^{-1}: 3210 (NH), 1730 (CO), 1665 (C=N$^+$); ^{1}H-NMR (Me_2SO-d_6) δ: 1.47 [3H, t, J=7 Hz, N(3)-CH_2<u>Me</u>], 3.09 (3H, d, J=5 Hz, N^6-Me), 3.59 [3H, s, N(9)-Me], 4.53 [2H, q, J=7 Hz, N(3)-C<u>H</u>$_2$Me], 7.7—8.1 (1H, br, N^6-H), 8.67 [1H, s, C(2)-H], 11.09 [1H, br s, N(7)-H]. *Anal.* Calcd for $C_9H_{13}N_5O$·HI: C, 32.25; H, 4.21; N, 20.90. Found: C, 32.00; H, 4.31; N, 20.86.

3-Ethyl-3,6,7,9-tetrahydro-9-methyl-6-(methylimino)-8*H*-purin-8-one Hydrochloride (26) The hydriodide **25** (168 mg, 0.5 mmol) was dissolved in warm H_2O (10 ml). After cooling, the solution was passed through a column of Amberlite IRA-402 (Cl^-) (1.5 ml), and the column was eluted with H_2O (20 ml). The eluates were combined and concentrated *in vacuo* to leave a colorless solid. This solid was dried and recrystallized from MeOH to give **26** (93 mg) as colorless prisms, mp 259—260 °C (dec.). This sample was directly used in the following hydrogenation experiment.

***N*,1-Dimethyl-2-oxo-4-imidazolidinecarboximidamide Hydrochloride (27)** i) From **1**: A solution of **1** (459 mg, 2 mmol) in 50% (v/v) aqueous AcOH (30 ml) was hydrogenated over 20% $Pd(OH)_2$–C catalyst (459 mg) at atmospheric pressure and 60—70 °C for 6 h. The catalyst was removed by filtration and washed with H_2O (100 ml). The filtrate and washings were combined and concentrated *in vacuo* to leave a colorless oil. The oil was triturated with EtOH (5 ml), and the mixture was kept in a refrigerator overnight. The colorless solid that deposited was filtered off, washed with EtOH (2 ml), and dried to furnish a first crop (241 mg, 63%) of **27**, mp 222—223 °C (dec.). The ethanolic filtrate and washings were combined and concentrated *in vacuo*, and trituration of the residual solid with EtOH (2 ml) yielded a second crop (57 mg), mp 219—220 °C (dec.). The total yield of **27** was 298 mg (77%). Recrystallization of the crude **27** from EtOH provided an analytical sample as colorless needles, mp 222—223 °C (dec.); MS m/z: 156 (M$^+$ − HCl); IR ν_{max}^{Nujol} cm^{-1}: 3220 (NH), 1710 and 1690 (CO); ^{1}H-NMR (Me_2SO-d_6) δ: 2.64 [3H, s, N(1)-Me], 2.84 (3H, d, J=1.5 Hz, NH<u>Me</u>), 3.21 [1H, dd, J=9 and 7.5 Hz, C(5)-H_A],[33] 3.77 [1H, dd, J=10 and 9 Hz, C(5)-H_B],[34] 4.4—4.6 [1H, m, C(4)-H], 6.88 [1H, d, J=3 Hz, N(3)-H], 8.96 and 9.33 (1H each, br, =NH$_2^+$), 9.62 (1H, br, N<u>H</u>Me). *Anal.* Calcd for $C_6H_{12}N_4O$·HCl: C, 37.41; H, 6.80; N, 29.08. Found: C, 37.22; H, 6.64; N, 29.18.

ii) From **26**: A portion (80 mg) of the recrystallized sample of **26** (*vide supra*) was dissolved in 50% (v/v) aqueous AcOH (6 ml), and the solution was hydrogenated over 20% $Pd(OH)_2$–C catalyst (80 mg) at atmospheric pressure and 60—70 °C for 6 h. At this stage, more catalyst (80 mg) was added, and hydrogenation was continued under similar conditions for a further 6 h. The reaction mixture was worked up in a manner similar to that described above under item (i) (**1**→**27**), giving **27** (22 mg, 26% overall yield from **25**) as colorless needles, mp 217—218 °C (dec.). This sample was identical (by comparison of the IR spectrum) with the one prepared from **1** by method (i).

7,9-Dihydro-7,9-dimethyl-6-(methylamino)-8*H*-purin-8-one (24) A mixture of **3**[3] (717 mg, 4 mmol) and anhydrous K_2CO_3 (828 mg, 6 mmol) in DMF (10 ml) was stirred at 90—95 °C for 1.5 h and then cooled to room temperature. After addition of MeI (1.70 g, 12 mmol), the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in H_2O (6 ml). The aqueous solution was neutralized with 10% aqueous HCl and then extracted with AcOEt. The AcOEt extracts were combined, washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to leave **24** (610 mg, 79%) as a colorless solid, mp 217—219 °C. Recrystallization from EtOH yielded an analytical sample as colorless needles, mp 222—223 °C; MS m/z: 193 (M$^+$); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 276.5 nm (ε 16400); $\lambda_{max}^{H_2O}$ (pH 1) 279.5 (14600); $\lambda_{max}^{H_2O}$ (pH 7) 276.5 (17100); $\lambda_{max}^{H_2O}$ (pH 13) 276.5 (17100); IR ν_{max}^{Nujol} cm^{-1}: 3380 (NH), 1690 (CO); ^{1}H-NMR (Me_2SO-d_6) δ: 2.90 (3H, d, J=5 Hz, NH<u>Me</u>), 3.25 and 3.50 (3H each, s, NMe's), 6.59 (1H, br, N<u>H</u>Me), 8.11 [1H, s, C(2)-H]. *Anal.* Calcd for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25. Found: C, 49.69; H, 5.75; N, 36.23.

7,9-Dihydro-7,9-dimethyl-6-(methylamino)-8*H*-purin-8-one Hydrochloride (24·HCl) The free base **24** (193 mg, 1 mmol) was dissolved in warm EtOH (3 ml), and 10% ethanolic HCl (1.24 g, 3.4 mmol) was added. The mixture was kept in a refrigerator overnight, and the colorless crystals that deposited were filtered off, washed with a little EtOH, and dried to afford **24**·HCl (217 mg, 95%), mp 265—267 °C (dec.). Recrystallization from MeOH produced an analytical sample as colorless needles, mp 269—270 °C (dec.); UV λ_{max}^{MeOH} 277 nm (ε 16000); IR ν_{max}^{Nujol} cm^{-1}: 3255 (NH), 1729 (CO); ^{1}H-NMR (D_2O) δ: 3.12 (3H, s, N^6-Me), 3.45 and 3.63 (3H each, s, NMe's), 8.33 [1H, s, C(2)-H]; ^{1}H-NMR (Me_2SO-d_6) δ: 2.97 (3H, s, N^6-Me), 3.29 and 3.53 (3H each, s, NMe's), 6.9 (br, NH's), 8.23 [1H, s, C(2)-H]. *Anal.* Calcd for $C_8H_{11}N_5O$·HCl: C, 41.84; H, 5.27; N, 30.49. Found: C, 41.95; H, 5.36; N, 30.44.

8-Bromo-*N*,*N*,9-trimethyl-9*H*-purin-6-amine (30) N^6,N^6,9-Trimethyladenine (**29**)[22] (4.43 g, 25 mmol) was dissolved in 0.5 M NaOAc–AcOH buffer (pH 4) (250 ml) by application of heat. The resulting solution was cooled to room temperature, and a solution of Br_2 (8.00 g, 50 mmol) in H_2O (500 ml) was added dropwise with stirring over a period of 15 min. After the mixture had been stirred at room temperature for 46 h, the reaction was quenched by adding 10% aqueous $NaHSO_3$ (*ca.* 50 ml). The resulting mixture was brought to pH 9 with 10% aqueous Na_2CO_3 and extracted with AcOEt (5 × 300 ml). The AcOEt extracts were combined, washed with saturated aqueous NaCl, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to leave a colorless solid. Two recrystallizations of the solid from EtOH gave **30** (4.36 g, 68%) as colorless prisms, mp 137—139 °C. Further recrystallization from EtOH yielded an analytical sample as colorless prisms, mp 138.5—139.5 °C; MS m/z: 255 and 257 (M$^+$); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 220 nm (ε 20800), 281 (18900); $\lambda_{max}^{H_2O}$ (pH 1) 274 (20600); $\lambda_{max}^{H_2O}$ (pH 7) 281 (19400); $\lambda_{max}^{H_2O}$ (pH 13) 281 (19400); ^{1}H-NMR (Me_2SO-d_6) δ: 3.40 (6H, s, N^6-Me's), 3.66 [3H, s, N(9)-Me], 8.20 [1H, s, C(2)-H]. *Anal.* Calcd for $C_8H_{10}BrN_5$: C, 37.52; H, 3.94; N, 27.35. Found: C, 37.36; H, 3.85; N, 27.38.

6-(Dimethylamino)-7,9-dihydro-9-methyl-8*H*-purin-8-one (31) A stirred mixture of **30** (2.56 g, 10 mmol) and 1 N aqueous NaOH (150 ml) was heated

under reflux for 7 h. After cooling, the reaction mixture was neutralized with 10% aqueous HCl and then kept in a refrigerator overnight. The colorless crystals that deposited were filtered off, washed with H_2O, and recrystallized from H_2O to give a first crop (755 mg) of **31**, mp 304—306 °C (dec.). The mother liquor, obtained when the crude product was filtered off, was concentrated *in vacuo* to a small volume, and the solid that deposited was filtered off and recrystallized from H_2O to yield a second crop (795 mg) of **31**, mp 304—306 °C (dec.). The total yield of **31** was 1.55 g (80%). For analysis, the crude **31** was recrystallized from H_2O to provide colorless pillars, mp 307—308 °C (dec.); MS *m/z*: 193 (M^+); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 220 nm (ε 24900), 283 (16700); $\lambda_{max}^{H_2O}$ (pH 1) 222 (18300), 279 (12800), 294 (12700); $\lambda_{max}^{H_2O}$ (pH 7) 219 (24500), 282.5 (17500); $\lambda_{max}^{H_2O}$ (pH 13) 222 (21500), 293 (18600); IR ν_{max}^{Nujol} cm^{-1}: 3165 and 3090 (NH), 1710 (CO); ^{1}H-NMR (Me_2SO-d_6) δ: 3.12 (6H, s, N^6-Me's), 3.23 [3H, s, N(9)-Me], 8.08 [1H, s, C(2)-H], 10.80 [1H, br s, N(7)-H]. *Anal.* Calcd for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25. Found: C, 49.43; H, 5.76; N, 36.25.

6-(Dimethyliminio)-3,6,7,9-tetrahydro-3,9-dimethyl-8*H*-purin-8-one Chloride (32) A mixture of **31** (386 mg, 2 mmol) and MeI (2.84 g, 20 mmol) in $AcNMe_2$ (24 ml) was stirred at 40—43 °C for 216 h. The reaction mixture was cooled in an ice bath, and the solid that deposited was filtered off, washed with EtOH, and dried to give a first crop (170 mg) of the crude iodide salt. The filtrate was concentrated *in vacuo* to leave a brown solid, which was triturated with EtOH (10 ml). The insoluble solid that resulted was filtered off, washed with EtOH, and dried to yield a second crop (455 mg) of the crude iodide salt. The first and second crops of the iodide salt were combined and dissolved in warm H_2O (240 ml). After cooling, the aqueous solution was passed through a column of Amberlite IRA-402 (Cl^-) (19 ml), and the column was eluted with H_2O (100 ml). The eluates were combined and concentrated *in vacuo* to leave a colorless solid, which was recrystallized from 90% (v/v) aqueous EtOH, giving **32** (213 mg, 44%) as colorless prisms, mp 307—308.5 °C (dec.). Further recrystallization in the same manner yielded an analytical sample of **32** as colorless prisms, mp 307—308 °C (dec.); MS *m/z*: 207 ($M^+ - HCl$); pK_a 6.73 ± 0.06 (at 25 °C); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 234 nm (ε 16300), 311 (20200); $\lambda_{max}^{H_2O}$ (pH 1) 231 (16100), 305 (22000); $\lambda_{max}^{H_2O}$ (pH 7) 231 (17400), 313 (18200); $\lambda_{max}^{H_2O}$ (pH 13) 232 (17600), 318 (18400); IR ν_{max}^{Nujol} cm^{-1}: 3480 and 3130 (NH), 1740 (CO), 1640 (C=N$^+$); ^{1}H-NMR (Me_2SO-d_6) δ: 3.31 (6H, s, N^6-Me's), 3.64 [3H, s, N(9)-Me], 4.13 [3H, s, N(3)-Me], 8.50 [1H, s, C(2)-H], 11.81 [1H, s, N(7)-H]. *Anal.* Calcd for $C_9H_{13}N_5O \cdot HCl$: C, 44.36; H, 5.79; N, 28.74. Found: C, 44.59; H, 5.88; N, 28.97.

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