

Highly Methylated Purines and Purinium Salts as Analogues of Heteromines

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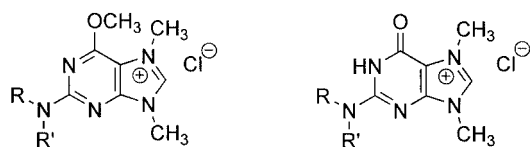
Three new unsubstituted or *N*-methylated derivatives of 2-amino-6,7,9-trimethylpurinium iodides were prepared by quaternization of the corresponding 2-amino-6,7-dimethylpurines. These intermediates were synthesized either by Pd-catalysed cross-coupling of 2-amino-6-chloro-9-methylpurine with trimethylaluminium or by regioselective Fe-catalysed cross-coupling of 2,6-dichloro-9-methylpurine with

methylmagnesium chloride, followed by amination. Both the title purinium salts and the purine intermediates were studied by ¹⁵N NMR, and one example of the purinium salts also by X-ray diffraction. None of the compounds exerted significant cytostatic activity.

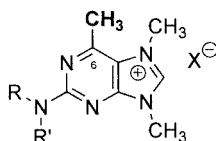
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Introduction

Heteromines are quaternary purinium alkaloids from *Heterostemma brownii*,^[1] used in Taiwanese traditional medicine for treatment of tumours. Heteromines A, B and C (Scheme 1) were isolated and found to exhibit significant cytostatic activity,^[2] and total syntheses of heteromines A^[3] and C^[4] were developed very shortly after their isolation. So far, however, neither have synthetic analogues of these bioactive natural products been reported, nor has the mechanism of action been solved.



Heteromine A: R = R' = H Heteromine D: R = R' = CH₃
 Heteromine B: R = H, R' = CH₃ Heteromine E: R = H, R' = CH₃
 Heteromine C: R = R' = CH₃



Scheme 1. Structures of heteromines and 6-methyl analogues under study.

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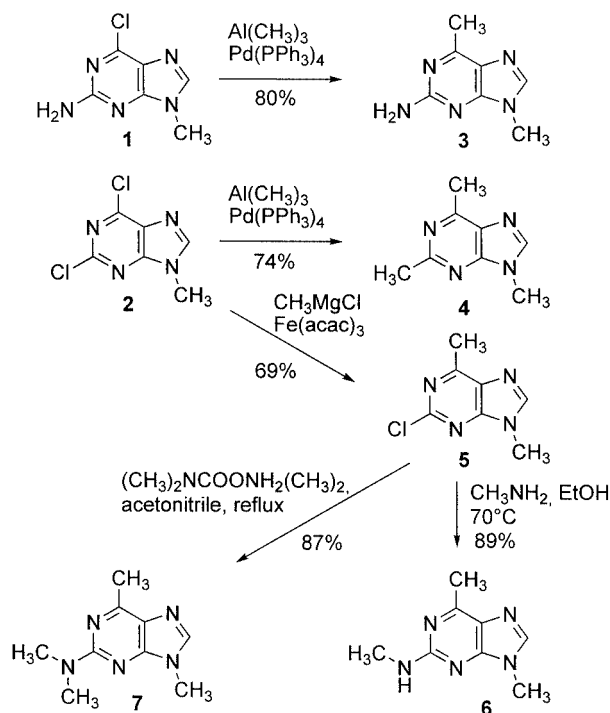
Several types of purines bearing C-substituents in their 6-positions are biologically active. 6-Aryl,^[5] 6-trifluoromethyl^[6] and 6-(hydroxymethyl)purine^[7] ribonucleosides display significant cytostatic activities. 6-Methylpurine is highly cytotoxic,^[8] its liberation from 2'-deoxyribonucleoside by purine nucleoside phosphorylases being used for detection of mycoplasma in cell cultures.^[9] It is highly potent and toxic both to nonproliferating and to proliferating tumour cells, and the use of cytotoxic 6-methylpurine base liberated by purine nucleoside phosphorylases from its non-toxic deoxyribonucleoside has recently been proposed as a novel principle in the gene therapy of cancer.^[10]

In this paper we report on the synthesis of novel compounds combining the unique structural features of cytostatic heteromines and 6-methylpurines. In these novel heteromine analogues, the original methoxy or oxo groups in the 6-positions were replaced by methyl units (Scheme 1). This structural modification should not only test the importance of the oxygen in the 6-positions of the parent compounds but it may also increase stability towards catabolism, due to the presence of a stable C–C bond.

Results and Discussion

The key intermediates in the two total syntheses of heteromines, 2-dimethylamino- or 2-amino-6-chloro-9-methylpurines, were originally prepared either in several steps from acyclic precursors^[3] or in the latter case in two steps from guanine.^[4] In the both syntheses the 6-chloropurines were methoxylated with sodium methoxide and quaternized with iodomethane, and the counteranion was exchanged for chloride. In our synthesis of the corresponding 6-methyl derivatives we used two approaches (Scheme 2): (i) introduction of the methyl group into 2-amino-6-chloropurine (**1**) by cross-coupling, or (ii) regioselective cross-coupling of 2,6-

dichloro-9-methylpurine (**2**) with methylmagnesium chloride, followed by amination in the 2-position.



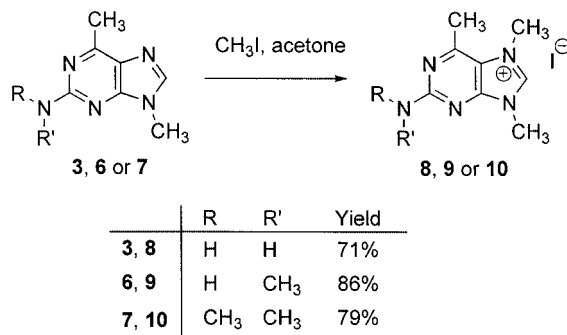
Scheme 2. Synthesis of 2-substituted 6-methylpurines.

Cross-coupling reactions are a general tool^[11] for introduction of C-substituents into purine moieties. Of the variety of possible organometallics, trimethylaluminum^[12] (with Pd catalysis) and methylmagnesium chloride (Fe catalysis)^[13] were the most efficient reagents for the methylation of 6-halopurines and so were the reagents of choice in this study. Thus, the known 2-amino-6-chloro-9-methylpurine (**1**)^[4] reacted smoothly with trimethylaluminum under Pd(PPh₃)₄ catalysis conditions in THF to give the corresponding 6-methyl derivative **3** in a good preparative yield of 80%. In our previous studies we had found that this highly reactive reagent could not be applied for regioselective monomethylation of dihalopurines^[13] but was very efficient in di- and trimethylations of di- and trihalopurines.^[14] Here we applied it in the reaction with 2,6-dichloro-9-methylpurine (**2**)^[15] under the same conditions to afford 2,6,9-trimethylpurine (**4**) in 74% yield.

Fe-catalysed cross-coupling reactions^[16] of dihalopurines with one equivalent of methylmagnesium chloride had previously been shown^[13,14] to be reasonably regioselective.^[17] In 2,6-dichloropurines the substitution preferentially occurred in the 6-position,^[13] while in 6,8-dichloropurines the reaction took place in the 8-position.^[14] Here, treatment of dichloropurine **2** with 1.5 equivalent of MeMgCl in the presence of Fe(acac)₃ in a THF/NMP mixture proceeded regioselectively to give the desired 2-chloro-6,9-dimethylpurine (**5**) in 69% isolated yield accompanied by unreacted starting compound **2** (27%), which was recovered and re-used. The chlorine in the 2-position of the intermediate **5** was substituted with a methylamino group on treatment

with ethanolic methylamine in a sealed tube to give the corresponding 6-methyl-2-methylaminopurine **6** in excellent yield. Dimethylammonium dimethylcarbamate^[18] for analogous nucleophilic substitution. Its reaction with **5** in refluxing acetonitrile proceeded nearly quantitatively to give the 2-dimethylamino derivative **7** in high preparative yield.

The quaternization of the 2-substituted 6,9-dimethylpurines **3–7** (Scheme 3) was performed analogously to the published procedures for the syntheses of heteromines by treatment with excess of iodomethane in acetone at ambient temperature. The reactions were very sluggish, taking 1–2 weeks to reach nearly complete conversion. While the quaternizations of the 2-aminopurines **3**, **6** and **7** proceeded regioselectively at the 7-positions and the desired purinium salts **8**, **9** and **10** crystallized from the reaction mixtures in pure form in good yields, in the cases of the 2-methyl and 2-chloro derivatives **4** and **5** the quaternization occurred unselectively on several N-atoms in the purine rings and the resulting mixtures were not separable and neither were any of the components crystallizable from the reaction mixtures. The quaternization of **4** provided an inseparable 2:1 mixture of 7- and 1-quaternized products (combination of ¹H and ¹H, ¹⁵N-HMBC NMR experiments with the crude mixture).



Scheme 3. Quaternization of purines.

As we suppose that the counteranion does not influence the biological activity, due to dissociation and large excesses of chloride anions in aqueous solutions under physiological conditions, we did not exchange the anions, but used the purinium iodides **8–10** directly for cytostatic activity screening. The intermediate 2-substituted 6,9-dimethylpurines **3–7** were also subjected to cytostatic activity screening for comparison. In vitro cytostatic activity tests (inhibition of cell growth) were performed with cultures of mouse leukemia L1210 cells (ATCC CCL 219), human promyelocytic leukemia HL60 cells (ATCC CCL 240), human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2) and the human T lymphoblastoid CCRF-CEM cell line (ATCC CCL 119). None of the tested compounds showed any significant cytostatic effect in any of these assays (inhibition at 10 μM was lower than 30%, so no IC₅₀ values were determined). The absence of activity in these analogues suggests that the 6-methoxy group in heteromines is important either for inter-

action with the target cellular system (enzyme) or for transport of heteromines through cell membranes.

All the new compounds were fully characterized. It is well known that ^{15}N chemical shifts of purines are extremely sensitive to electronic changes at nitrogen,^[19] so ^{15}N NMR spectra of all the compounds were also measured and chemical shifts were assigned with the aid of ^1H – ^{15}N multiple-bond chemical shift correlation experiments optimized for long-range couplings of 4 Hz. Table 1 shows comparisons of ^{15}N chemical shifts of 9-methylated purines **3**, **6** and **7** and the corresponding quaternized 7,9-dimethylpurinium iodides **8**, **9** and **10**. The dramatic N-7 upfield shift (≈ 87 ppm) observed upon quaternization is associated with strong shielding of the ^{15}N nucleus. In the 7,9-dimethylpurinium iodides **8**, **9** and **10** the ^{15}N chemical shifts of N-7 and N-9 are virtually the same, indicating delocalization of the positive charge. The quaternization has also long-range effects on the N-1 and NRR' chemical shifts, which are less shielded than in the parent 9-methylated purines.

Table 1. ^{15}N NMR chemical shifts^[a] of 9-methylated purines **3**, **6** and **7** and the corresponding quaternized iodides **8**, **9** and **10** in $[\text{D}_6]\text{DMSO}$ at 300 K.

	R	R'	N-1	N-3	N-7	N-9	NRR'
3	H	H	241.9	198.3	241.2	146.8	80.4
6	H	CH_3	241.1	— ^[b]	241.4	147.0	74.0
7 ^[c]	CH_3	CH_3	238.7	196.1	237.7	145.2	68.1
8	H	H	254.4	195.8	153.1	152.4	88.3
9	H	CH_3	256.1	190.6	153.4	153.4	83.1
10	CH_3	CH_3	251.8	194.2	152.9	152.5	76.9

[a] With reference to the signal of liquid nitromethane ($\delta = 381.7$ ppm).^[19] [b] Not detected. [c] In CDCl_3 .

2-(Dimethylamino)-6,7,9-trimethylpurinium iodide (**10**) crystallized directly from the reaction mixture as crystals suitable for X-ray diffraction. Its molecular structure, with selected bond lengths, is given in Figure 1. The organic part is fairly planar, the largest distances of non-hydrogen atoms of substituents from best least-squares plane defined by nine atoms of the purine moiety being 0.193(5) Å for C(13). Planarity, together with the rather short C(2)–N(11) distance (see Figure 1, C(2)–N(11) = 1.347(4)), attest to the contribution of the lone electron pair of N(11) into the purine π -system, a finding consistent with crystal structures of other 2- or 6-(dimethylamino)purines described in the literature.^[20,21] The most closely related known structure, though, is 6-amino-2-(dimethylamino)-7-methylpurine (BIFFAX),^[20] in which the corresponding distance was significantly longer (C(2)–N(11) = 1.381 Å). At the same time, the N(7)–C(8) and C(8)–N(9) bond lengths in **10** are significantly closer to each other than in BIFFAX, confirming delocalization of electrons in the charged purine moiety (N(7)–C(8) = 1.325(4), 1.354; C(8)–N(9) = 1.343(4), 1.311 Å for **10** and BIFFAX, respectively). The N(7)–C(15) and N(9)–C(14) bond lengths are also virtually identical and the methyl groups are coplanar with the purine moiety, confirming the delocalization of the positive charge.

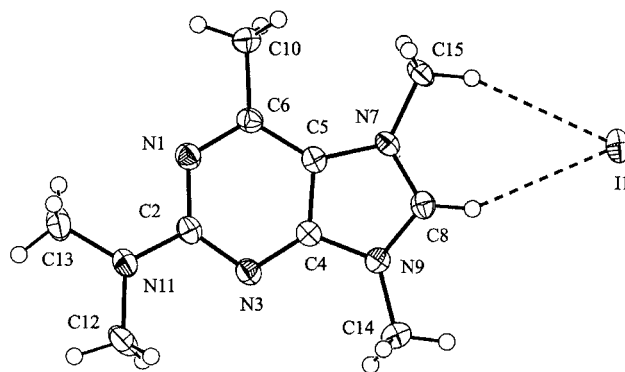


Figure 1. Molecular structure of **10** with selected bond lengths [Å]: N(1)–C(2) = 1.375(4), C(2)–N(3) = 1.350(4), C(2)–N(11) = 1.347(4), N(7)–C(8) = 1.325(4), C(8)–N(9) = 1.343(4), N(7)–C(15) = 1.465(3), N(9)–C(14) = 1.463(4), C(8)–I(1) = 3.843(3), C(8)–H(8)–I(1) = 149°. Displacement ellipsoids are drawn at the 50% probability level.

In conclusion, 2-amino-6,9-dimethylpurines were efficiently prepared either by regioselective Fe-catalysed cross-coupling reactions between 2,6-dichloro-9-methylpurine and methylmagnesium chloride followed by (di)methylamination of the intermediate 2-chloro-6-methylpurine or by Pd-catalysed cross-coupling of 2-amino-6-chloro-9-methylpurine with trimethylaluminium. Quaternization of the 2-amino-6,9-dimethylpurines with iodomethane proceeded slowly but selectively and efficiently to form the desired 2-amino-6,7,9-trimethylpurinium salts as analogues of heteromines. On the other hand, quaternizations of 2-methyl- or 2-chloropurine derivatives were unselective. ^{15}N NMR spectra and crystal structure of these novel purinium derivatives are described for the first time, showing delocalization of the positive charge on the purine moiety.

Experimental Section

Melting points were determined on a Kofler block and are uncorrected. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer. NMR spectra were recorded on Bruker Avance 500 (^1H at 500 MHz, ^{13}C at 125.8 MHz) and Bruker Avance 400 (^1H at 400 MHz, ^{13}C at 100.6 MHz, ^{15}N at 40.6 MHz) instruments. ^1H and ^{13}C NMR spectra were with reference either to the signal of TMS or to the residual solvent signal. ^1H , ^{13}C -HMBC experiments were performed for complete assignment of all signals. ^{15}N NMR chemical shifts were obtained by ^1H , ^{15}N -g-HMBC measurements with reference to the signal of liquid nitromethane^[22] (a coaxial 2 mm capillary tube in a 5 mm NMR tube, 381.7 ppm). THF was dried and distilled from sodium/benzophenone. Starting purines **1**^[4] and **2**^[15] were prepared by known procedures.

2-Amino-6,9-dimethylpurine (3): Trimethylaluminium (2 M solution in toluene, 3 mL, 6 mmol) was added dropwise, under argon at 20 °C through a septum, to a stirred solution of 2-amino-6-chloropurine **1** (183 mg, 1 mmol) and $\text{Pd}(\text{PPh}_3)_4$ (60 mg, 0.05 mmol) in THF (20 mL). The mixture was then stirred at 75 °C for 8 h (TLC analysis showed complete conversion) and after cooling to room temp. was poured onto a mixture of crushed ice (ca. 100 mL), ammonium chloride (1 g) and Na_2EDTA (250 mg). The mixture was then extracted with chloroform (4 \times 100 mL). The collected organic

layers were dried with MgSO_4 and the solvent was evaporated in vacuo. The residue was chromatographed on a column of silica gel (100 g, ethyl acetate/hexanes 1:2 \rightarrow ethyl acetate \rightarrow ethyl acetate/methanol 9:1) to give the title compound **3** as a solid, which was crystallized from methanol/toluene/heptane. Yield 131 mg (80%), white crystals, m.p. 222–225 °C. ^1H NMR (400 MHz, $[\text{D}_6]\text{DMSO}$): δ = 2.46 (s, 3 H, 6- CH_3), 3.60 (s, 3 H, 9- CH_3), 6.37 (br. s, 2 H, NH_2), 7.92 (s, 1 H, 8-H) ppm. ^{13}C NMR (100.6 MHz, $[\text{D}_6]\text{DMSO}$): δ = 19.01 (6- CH_3), 29.13 (9- CH_3), 125.79 (C-5), 142.05 (CH-8), 152.89 (C-4), 158.48 (C-6), 160.28 (C-2) ppm. ^{15}N NMR (40.6 MHz, $[\text{D}_6]\text{DMSO}$): δ = 80.4 (NH_2), 146.8 (N-9), 198.3 (N-3), 241.2 (N-7), 241.9 (N-1) ppm. IR: $\tilde{\nu}$ = 3431, 3331, 3205, 1642, 1601, 1525, 1467, 1397, 1331 cm^{-1} . EI MS, m/z (rel. %): 163 (100) $[M]^+$, 148 (16). Exact mass (EI HR MS): 163.0862; calcd. for $\text{C}_7\text{H}_9\text{N}_5$: 163.0858. $\text{C}_7\text{H}_9\text{N}_5$ (163.2): C 51.52, H 5.56, N 42.92; found C 51.16, H 5.49, N 42.56.

2,6,9-Trimethylpurine (4): This compound was prepared in the same way as described for compound **3**, from **2** (203 mg, 1 mmol) and Me_3Al (2 M, 3 mL, 6 mmol). Compound **4** was obtained as colourless crystals (from CH_2Cl_2 /heptane). Yield 120 mg (75%), m.p. 116–118 °C. ^1H NMR (400 MHz, CDCl_3): δ = 2.80 (s, 3 H, 2- CH_3), 2.82 (s, 3 H, 6- CH_3), 3.87 (s, 3 H, 9- CH_3), 7.92 (s, 1 H, 8-H) ppm. ^{13}C NMR (100.6 MHz, CDCl_3): δ = 19.39 (6- CH_3), 25.98 (2- CH_3), 29.64 (9- CH_3), 130.75 (C-5), 143.64 (CH-8), 151.48 (C-4), 158.74 (C-6), 161.97 (C-2) ppm. ^{15}N NMR (40.6 MHz, CDCl_3): δ = 147.6 (N-9), 238.6 (N-7), 242.0 (N-3), 276.2 (N-1) ppm. IR: $\tilde{\nu}$ = 1597, 1515, 1398, 1342 cm^{-1} . EI MS, m/z (rel. %): 162 (100) $[M]^+$, 147 (8). Exact mass (EI HR MS): 162.0901; calcd. for $\text{C}_8\text{H}_{10}\text{N}_4$: 162.0905. $\text{C}_8\text{H}_{10}\text{N}_4$ (162.1): C 59.24, H 6.21, N 34.54; found C 59.07, H 6.29, N 34.20.

2-Chloro-6,9-dimethylpurine (5): Methylmagnesium chloride (3 M solution in THF, 2 mL, 6 mmol) was added dropwise under argon at 20 °C to a stirred solution of dichloropurine **2** (820 mg, 4 mmol) and $\text{Fe}(\text{acac})_3$ (400 mg, 1.1 mmol) in NMP (1 mL) and THF (20 mL). The mixture was stirred for 2 h and allowed to stand overnight at ambient temperature. It was then poured onto a mixture of crushed ice (ca. 300 mL), ammonium chloride (2 g) and Na_2EDTA (500 mg). The mixture was then extracted with chloroform (4×200 mL). The collected organic layers were dried with MgSO_4 and the solvent was evaporated in vacuo. The residue was chromatographed on a column of silica gel (200 g, ethyl acetate/hexanes 1:2 \rightarrow ethyl acetate). Unreacted compound **2** (220 mg, 27%) was eluted first, followed by the desired 6-methyl derivative **5**, which was crystallized from CH_2Cl_2 /heptane to provide colourless crystals (504 mg, 69%), m.p. 153–156 °C. ^1H NMR (400 MHz, CDCl_3): δ = 2.85 (s, 3 H, 6- CH_3), 3.89 (s, 3 H, 9- CH_3), 8.00 (s, 1 H, 8-H) ppm. ^{13}C NMR (100.6 MHz, CDCl_3): δ = 19.42 (CH₃-6), 30.00 (CH₃-9), 132.03 (C-5), 144.89 (CH-8), 152.54 (C-4), 153.91 (C-2), 161.67 (C-6) ppm. ^{15}N NMR (40.6 MHz, CDCl_3): δ = 149.4 (N-9), 240.8 (N-7), 274.2 (N-1), N-3 not observed. IR: $\tilde{\nu}$ = 1598, 1589, 1511, 1397, 1364, 1330, 1303 cm^{-1} . EI MS, m/z (rel. %): 182 (100) $[M]^+$, 147 (14). Exact mass (EI HR MS): 182.0366; calcd. for $\text{C}_7\text{H}_7\text{ClN}_4$: 182.0359. $\text{C}_7\text{H}_7\text{ClN}_4$ (182.6): C 46.04, H 3.86, N 30.68; found C 45.88, H 3.82, N 30.35.

2-(Methylamino)-6,9-dimethylpurine (6): A mixture of 2-chloro-6-methylpurine **5** (225 mg, 1.24 mmol) and ethanolic CH_3NH_2 (33%, 20 mL) was stirred in a sealed 35 mL Ace[®] pressure tube at 80 °C for 8 h. The solvent was then evaporated and the residue was chromatographed on a column of silica gel (100 g, ethyl acetate/hexanes 1:1 \rightarrow ethyl acetate \rightarrow ethyl acetate/methanol 9:1) to give the title compound **6** as a solid, which was crystallized from methanol/toluene/heptane. Yield 195 mg (89%) of colourless crystals, m.p. 157–

160 °C. ^1H NMR (400 MHz, $[\text{D}_6]\text{DMSO}$): δ = 2.48 (s, 3 H, 6- CH_3), 2.82 (d, 3 H, $J_{\text{CH}_3,\text{NH}}$ = 4.8, $\text{CH}_3\text{-NH}$), 3.62 (s, 3 H, 9- CH_3), 6.84 (q, 1 H, $J_{\text{NH},\text{CH}_3}$ = 4.8, NH), 7.92 (s, 1 H, 8-H) ppm. ^{13}C NMR (100.6 MHz, $[\text{D}_6]\text{DMSO}$): δ = 19.16 (6- CH_3), 28.46 ($\text{CH}_3\text{-NH}$), 29.07 (9- CH_3), 125.55 (C-5), 141.83 (CH-8), 152.81 (C-4), 158.36 (C-6), 159.99 (C-2) ppm. ^{15}N NMR (40.6 MHz, $[\text{D}_6]\text{DMSO}$): δ = 74.0 (HN- CH_3), 147.0 (N-9), 241.1 (N-1), 241.4 (N-7) ppm, N-3 not observed. IR: $\tilde{\nu}$ = 3268, 3075, 2945, 2901, 1610, 15981570, 1519, 1477, 1395, 1322 cm^{-1} . EI MS, m/z (rel. %): 177 (100) $[M]^+$, 148 (55). Exact mass (EI HR MS): 177.1011; calcd. for $\text{C}_8\text{H}_{11}\text{N}_5$: 177.1014. $\text{C}_8\text{H}_{11}\text{N}_5$ (177.2): C 54.22, H 6.26, N 39.52; found C 53.98, H 6.28, N 39.19.

2-(Dimethylamino)-6,9-dimethylpurine (7): A mixture of 2-chloro-6-methylpurine **5** (240 mg, 1.32 mmol) and dimethylammonium dimethylcarbamate (1 mL, 7.8 mmol) in acetonitrile (20 mL) was heated at reflux for 12 h (TLC analysis showed complete conversion). The solvents were then evaporated and the product was isolated in the same way as for compound **6**. Yield of compound **7**: 220 mg (87%) of yellowish crystals from CH_2Cl_2 /heptane, m.p. 100–103 °C. ^1H NMR (400 MHz, CDCl_3): δ = 2.66 (s, 3 H, 6- CH_3), 3.24 (s, 6 H, $(\text{CH}_3)_2\text{N}$), 3.70 (s, 3 H, 9- CH_3), 7.60 (s, 1 H, 8-H) ppm. ^{13}C NMR (100.6 MHz, CDCl_3): δ = 19.58 (6- CH_3), 29.06 (9- CH_3), 37.43 ($(\text{CH}_3)_2\text{N}$), 125.35 (C-5), 140.54 (CH-8), 152.86 (C-4), 158.99 (C-6), 159.79 (C-2) ppm. ^{15}N NMR (40.6 MHz, CDCl_3): δ = 68.1 ($\text{N}(\text{CH}_3)_2$), 145.2 (N-9), 196.1 (N-3), 237.7 (N-7), 238.7 (N-1) ppm. IR: $\tilde{\nu}$ = 1614, 1587, 1547, 1503, 1411, 1392 cm^{-1} . EI MS, m/z (rel. %): 191 (100) $[M]^+$, 176 (76), 162 (50), 148 (43). Exact mass (EI HR MS): 191.1164; calcd. for $\text{C}_9\text{H}_{13}\text{N}_5$: 191.1172. $\text{C}_9\text{H}_{13}\text{N}_5$ (191.2): C 56.53, H 6.85, N 36.62; found C 56.24, H 6.68, N 36.28.

Quaternization of 2,6-Disubstituted 9-Methylpurines – General Procedure: A solution of 9-methylpurine **3–7** (0.5–0.7 mmol) in acetone (20 mL) was stirred at 20 °C while iodomethane (1 mL, 16 mmol) was added dropwise. The stirring was continued for several days until the product had crystallized from the reaction mixture. In the cases of the unsubstituted or substituted 2-aminopurine compounds **3**, **6** and **7** the desired products **8**, **9** and **10** crystallized in pure form and were just filtered off and washed with diethyl ether and dried. In the cases of 2-methyl- and 2-chloropurines **4** and **5**, the quaternization/methylation occurred unselectively in several positions of the purine ring (NMR analysis of crude mixtures of products), the resulting mixtures were not separable, and neither did any of the components crystallize in pure form.

2-Amino-6,7,9-trimethylpurinium Iodide (8): This compound was prepared from compound **3** (80 mg, 0.49 mmol) in 14 days. Yield 106 mg (71%) of yellowish crystals, m.p. 279–281 °C. ^1H NMR (400 MHz, $[\text{D}_6]\text{DMSO}$): δ = 2.73 (s, 3 H, 6- CH_3), 3.77 (s, 3 H, 9- CH_3), 4.13 (s, 3 H, 7- CH_3), 7.32 (s, 2 H, NH_2), 9.39 (s, 1 H, 8-H) ppm. ^{13}C NMR (100.6 MHz, $[\text{D}_6]\text{DMSO}$): δ = 21.22 (6- CH_3), 31.09 (9- CH_3), 36.38 (7- CH_3), 115.52 (C-5), 141.86 (CH-8), 150.70 (C-4), 157.39 (C-6), 161.87 (C-2) ppm. ^{15}N NMR (40.6 MHz, $[\text{D}_6]\text{DMSO}$): δ = 88.3 (NH_2), 152.4 (N-9), 153.1 (N-7), 195.8 (N-3), 254.4 (N-1) ppm. IR: $\tilde{\nu}$ = 3402, 3313, 3189, 3055, 2986, 1639, 1621, 1599, 1574, 1502, 1455, 1403, 1384, 1374, 1324 cm^{-1} . FAB MS, m/z (rel. %): 178 (100) $[M]^+$ (cation). Exact mass (FAB HR MS): 178.1096; calcd. for $\text{C}_8\text{H}_{12}\text{N}_5$: 178.1093. $\text{C}_8\text{H}_{12}\text{IN}_5$ (305.1): C 31.49, H 3.96, N 22.95; found C 31.43, H 3.91, N 22.67.

2-(Methylamino)-6,7,9-trimethylpurinium Iodide (9): This compound was prepared from compound **6** (119 mg, 0.67 mmol) in 10 days. Yield 184 mg (86%) of yellowish crystals, m.p. 249–252 °C. ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$): δ = 2.73 (s, 3 H, 6- CH_3), 2.87 (bd, $J_{\text{CH}_3,\text{NH}}$ = 4.3 Hz, 3 H, $\text{CH}_3\text{-NH}$), 3.80 (br. s, 3 H, 9- CH_3), 4.14 (s, 3 H, 7- CH_3), 7.84 (br. s, 1 H, NH), 9.39 (s, 1 H, 8-H) ppm.

^{13}C NMR (125.8 MHz, $[\text{D}_6]\text{DMSO}$): δ = 21.17 (6- CH_3), 28.11 ($\text{CH}_3\text{-NH}$), 30.98 (9- CH_3), 36.38 (7- CH_3), 115.18 (C-5), 141.40 (CH-8), 150.60 (C-4), 157.15 (C-6), 161.27 (C-2) ppm. ^{15}N NMR (40.6 MHz, $[\text{D}_6]\text{DMSO}$): δ = 83.1 (NHCH_3), 153.4 (N-7 and N-9), 190.6 (N-3), 256.1 (N-1) ppm. IR: $\tilde{\nu}$ = 3249, 3006, 2986, 2793, 1627, 1592, 1558, 1483, 1463, 1411, 1386, 1376, 1322 cm^{-1} . FAB MS, m/z (rel. %): 192 (100) $[M]^+$ (cation). Exact mass (FAB HR MS): 192.1250; calcd. for $\text{C}_9\text{H}_{14}\text{N}_5$: 192.1249. $\text{C}_9\text{H}_{14}\text{N}_5$ (319.1): C 33.87, H 4.42, N 21.94; found C 33.78, H 4.41, N 21.65.

2-(Dimethylamino)-6,7,9-trimethylpurinium Iodide (10): This compound was prepared from compound 7 (113 mg, 0.59 mmol) in 7 days. Yield 155 mg (79%) of yellowish crystals, m.p. 268–270 °C. ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$): δ = 2.78 (s, 3 H 6- CH_3), 3.21 (br.s, 6 H, $(\text{CH}_3)_2\text{N}$), 3.81 (s, 3 H, 9- CH_3), 4.15 (s, 3 H, 7- CH_3), 9.42 (s, 1 H, 8-H) ppm. ^{13}C NMR (125.8 MHz, $[\text{D}_6]\text{DMSO}$): δ = 21.65 (6- CH_3), 30.91 (9- CH_3), 36.39 (7- CH_3), 37.23 ($(\text{CH}_3)_2\text{N}$), 114.99 (C-5), 142.16 (CH-8), 150.61 (C-4), 156.78 (C-6), 159.97 (C-2) ppm. ^{15}N NMR (40.6 MHz, $[\text{D}_6]\text{DMSO}$): δ = 76.9 ($\text{N}(\text{CH}_3)_2$), 152.5 (N-9), 152.9 (N-7), 194.2 (N-3), 251.8 (N-1) ppm. IR: $\tilde{\nu}$ = 3027, 2990, 1629, 1581, 1560, 1462, 1415, 1402, 1386, 1368, 1332 cm^{-1} . FAB MS, m/z (rel. %): 206 (100) $[M]^+$ (cation). Exact mass (FAB HR MS): 206.1407; calcd. for $\text{C}_{10}\text{H}_{16}\text{N}_5$: 206.1406. $\text{C}_{10}\text{H}_{16}\text{N}_5$ (333.2): C 36.05, H 4.84, N 21.02; found C 36.03, H 4.93, N 20.76.

X-ray Crystallographic Study for 10: The diffraction data were collected on NoniusKappaCCD diffractometer at 150 K, Mo- K_α radiation, λ = 0.71073, graphite monochromator, ω -scans. Data were corrected for LP factor and absorption by Gaussian methods.^[23] The structure was solved by direct methods (SIR)^[24] and refined by full-matrix, least-squares methods based on F^2 with all measured reflections, all non-hydrogen atoms are refined anisotropically (SHELXL).^[25] The positions of the hydrogen atoms were calculated into idealized positions and refined as riding on their pivot atom with assigned temperature factors $\text{H}_{\text{iso}}(\text{H}) = 1.2 \text{ U}_{\text{eq}}(\text{C}(8) \text{ atom})$ and $\text{H}_{\text{iso}}(\text{H}) = 1.5 \text{ U}_{\text{eq}}(\text{pivot atom})$ for methyl moieties.

Crystal Data for 10: The colourless crystal of $\text{C}_{10}\text{H}_{16}\text{N}_5\text{I}$ ($0.27 \times 0.25 \times 0.025 \text{ mm}$) was triclinic, space group $P-1$ (No.2), a = 6.5390(2), b = 7.7630(2), c = 13.4670(5) Å, α = 94.066(2), β = 99.003(2), γ = 99.418(2)°, V = 662.82(4) Å³, Z = 2, M = 333.18, d_{calc} = 1.669 g cm^{-3} , μ = 2.400 mm^{-1} , T_{min} = 0.563, T_{max} = 0.908, 11048 reflections (θ_{max} = 27.5°) were collected, of which 3025 were unique [R_{int} = 0.033]. No. of parameters = 150, R_1 = 0.024 for 2825 reflections [$I > 2\sigma(I)$], wR_2 = 0.067 (all, 3025 refl), $\Delta\rho_{\text{max}}$ = 0.609 $\text{e} \cdot \text{Å}^{-3}$, $\Delta\rho_{\text{min}}$ = -0.679 $\text{e} \cdot \text{Å}^{-3}$.

CCDC-265258 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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[1] Y.-L. Lin, H.-P. Lee, J.-C. Ou, K.-H. Kuo, *Heterocycles* **1996**, *43*, 115–122.

- [2] Y.-L. Lin, R.-L. Huang, C.-M. Chang, Y.-H. Kuo, *J. Nat. Prod.* **1997**, *60*, 982–985.
- [3] C. Peinador, J. M. Quintela, M. J. Moreira, *Tetrahedron* **1997**, *53*, 8269–8272.
- [4] E. Jacobsen, L.-L. Gundersen, *Heterocycles* **2000**, *53*, 935–940.
- [5] a) M. Hocek, A. Holý, I. Votruba, H. Dvořáková, *J. Med. Chem.* **2000**, *43*, 1817–1825; b) M. Hocek, A. Holý, I. Votruba, H. Dvořáková, *Collect. Czech. Chem. Commun.* **2001**, *66*, 483–499.
- [6] D. Hocková, M. Hocek, H. Dvořáková, I. Votruba, *Tetrahedron* **1999**, *55*, 11109–11118.
- [7] P. Šilhár, R. Pohl, I. Votruba, M. Hocek, *Org. Lett.* **2004**, *6*, 3225–3228.
- [8] A. Montgomery, K. Hewson, *J. Med. Chem.* **1968**, *11*, 48–52.
- [9] a) G. J. McGarrity, D. A. Carson, *Exp. Cell Res.* **1982**, *139*, 199–205; b) A. M. Whitaker, G. D. Windsor, C. M. Burnett, C. H. Taylor, *Dev. Biol. Stand.* **1987**, *66*, 503–509; c) K. Ishiguro, S. Taira, T. Sasaki, H. Nariuchi, *J. Immunol. Methods* **1988**, *108*, 39–43.
- [10] a) W. B. Parker, S. A. King, P. W. Allan, L. L. Bennett Jr., J. A. Secrist III, J. A. Montgomery, K. S. Gilbert, W. R. Waud, A. H. Wells, G. Y. Gillespie, E. J. Sorscher, *Hum. Gene Ther.* **1997**, *8*, 1637–1644; b) W. B. Parker, P. W. Allan, S. C. Shaddix, L. M. Rose, H. F. Speegle, G. Y. Gillespie, L. L. Bennett Jr., *Biochem. Pharmacol.* **1998**, *55*, 1673–1681.
- [11] a) M. Hocek, *Eur. J. Org. Chem.* **2003**, 245–254; b) L. A. Agrofoglio, I. Gillaizeau, Y. Saito, *Chem. Rev.* **2003**, *103*, 1875–1916; c) M. K. Lakshman, *J. Organomet. Chem.* **2002**, *653*, 234–251.
- [12] a) K. Hirota, Y. Kitade, Y. Kanbe, Y. Maki, *J. Org. Chem.* **1992**, *57*, 5268–5270; b) M. Česnek, M. Hocek, A. Holý, *Collect. Czech. Chem. Commun.* **2000**, *65*, 1357–1373.
- [13] M. Hocek, H. Dvořáková, *J. Org. Chem.* **2003**, *68*, 5773–5776.
- [14] a) M. Hocek, D. Hocková, H. Dvořáková, *Synthesis* **2004**, 889–894; b) M. Hocek, R. Pohl, *Synthesis* **2004**, 2869–2876.
- [15] G. Beaman, R. K. Robins, *J. Org. Chem.* **1963**, *28*, 2310–2313.
- [16] a) A. Fürstner, A. Leitner, *Angew. Chem. Int. Ed.* **2002**, *41*, 609; b) A. Fürstner, A. Leitner, M. Mendez, H. Krause, *J. Am. Chem. Soc.* **2002**, *124*, 13856–13864; c) A. Fürstner, A. Leitner, *Angew. Chem. Int. Ed.* **2003**, *42*, 308.
- [17] Other examples of regioselective cross-couplings of dihalopurines: a) G. Langli, L.-L. Gundersen, F. Rise, *Tetrahedron* **1996**, *52*, 5625–5638; b) J. M. J. Nolsøe, L.-L. Gundersen, F. Rise, *Acta Chem. Scand.* **1999**, *53*, 366–372; c) M. Havelková, D. Dvořák, M. Hocek, *Synthesis* **2001**, 1704–1710; d) M. Hocek, I. Votruba, H. Dvořáková, *Tetrahedron* **2003**, *59*, 607–611; e) M. Hocek, D. Hocková, J. Štambaský, *Collect. Czech. Chem. Commun.* **2003**, *68*, 837–848. For a general review on regioselective cross-coupling reactions of dihaloheterocycles, see: f) S. Schöter, C. Stock, T. Bach, *Tetrahedron* **2005**, *61*, 2245–2267.
- [18] Krečmerová, M. Masojdková, A. Holý, *Collect. Czech. Chem. Commun.* **2004**, *69*, 1889–1913.
- [19] R. Marek, V. Sklenář, *Annu. Rep. NMR Spectrosc.* **2004**, *54*, 201–242.
- [20] N. Vasil’chenko, V. V. Mitkevich, A. A. Moiseenko, V. G. Khomenko, V. A. Chernov, *J. Struct. Chem.* **1982**, *23*, 257–263.
- [21] a) T. Dahl, *Acta Chem. Scand. B.* **1987**, *41*, 379–382; b) M. Kubicki, P. W. Coddington, *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2001**, *57*, o332–o334.
- [22] S. Wishart, C. G. Bigam, J. Yao, F. Abildgaard, H. J. Dyson, E. Oldfield, J. L. Markley, B. D. Sykes, *J. Biomol. NMR* **1995**, *6*, 135–140.
- [23] P. Coppens, *Acta Crystallogr., Sect. A* **1970**, *26*, 71–83.
- [24] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori, M. Camalli, *J. Appl. Crystallogr.* **1994**, *27*, 435–436.
- [25] M. Sheldrick, *SHELXL97*, University of Göttingen, Germany, **1997**.

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