# Synthesis and Structure-Activity Relationships of 2-Substituted-6-(dimethylamino)-9-(4-methylbenzyl)-9*H*-purines with Antirhinovirus Activity

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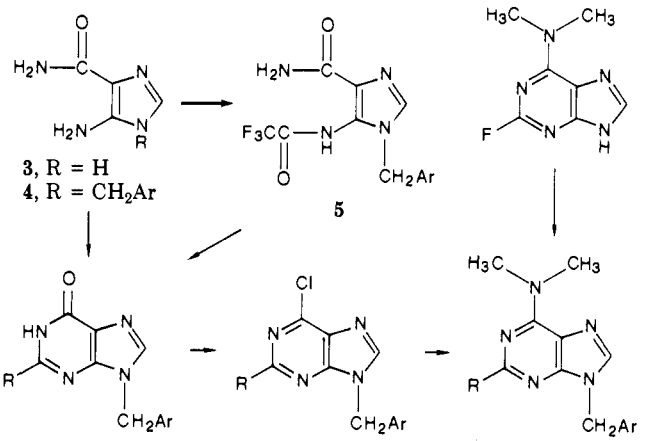
A series of 2-substituted-6-(dimethylamino)-9-(4-methylbenzyl)-9*H*-purines where the 2-substituent was H, F, Cl, $CF_3$, $CH_3$, $CH_2CH_3$, $NH_2$, $NHCH_3$, $N(CH_3)_2$, $SCH_3$, or $SO_2CH_3$ was synthesized and tested for antirhinovirus activity to evaluate the effect of 2-substituents on antiviral activity. Intuitive and quantitative structure–activity relationship (QSAR) analysis showed that optimum antirhinovirus serotype 1B activity was associated with 9-benzylpurines that contained a C-2 lipophilic, electron-withdrawing substituent. The most active compound, 6-(dimethylamino)-9-(4-methylbenzyl)-2-(trifluoromethyl)-9*H*-purine (**14**), had an $IC_{50}$ = 0.03 μM against serotype 1B, but its activity against 18 other serotypes was not uniform; the $IC_{50}$s ranged over 260-fold.

The rhinoviruses are the most important causative agents of the common cold.[1] Development of an adequate vaccine against rhinoviruses has been precluded by the large number of serotypes.[2] Despite many reports of in vitro antirhinovirus activity,[3] no agent has shown significant clinical efficacy.[4] We recently reported a new class of antirhinovirus agents, the 9-benzyl-2-chloro-9*H*-purines.[5] Although these compounds had potent in vitro activity against rhinovirus serotype 1B, most other serotypes were less sensitive. We have extended these studies to a set of 2-substituted-9-benzylpurines (Table I) in an effort to develop an agent with a broader spectrum of activity. We report the synthesis and quantitative structure–activity relationships (QSAR) of these 2-substituted-9-benzylpurines.

**Chemistry.** The 2-substituted purines **14–16** were prepared in four synthetic steps from 5-aminoimidazole-4-carboxamide (**3**) (Scheme I). The sodium salt of **3** was alkylated with 4-methylbenzyl bromide to give **4** in 17% yield. That **4** was the correct isomer was confirmed by condensation of **4** with ethyl formate[6] to give the 6-oxopurine **7**, which had an ultraviolet spectrum similar to that of inosine.[7] Chlorination of **7** gave 6-chloropurine **11**, which was identical with the sample prepared by an unequivocal route.[8] The 2-methyl- (**8**) and 2-ethylpurines (**9**) were isolated when **4** was heated with ethyl acetate or ethyl propionate, respectively. However, when **4** was reacted with trifluoroacetic anhydride in trifluoracetic acid, the intermediate amide **5** was isolated. This trifluoroacetamide was cyclized in refluxing dimethylformamide with sodium methylate to give **6** in fair yield. A similar synthesis of the 9-benzyl analogue of **6** was reported recently.[9] The oxopurines **6**, **8**, and **9** were chlorinated with thionyl chloride–dimethylformamide reagent to give the 6-chloropurines **10**, **12**, and **13**, respectively, which were treated with ethanolic dimethylamine to give **14–16**. The 2-fluoropurine **17** was prepared by alkylation of the sodium salt of 2-fluoro-6-dimethylamino-9*H*-purine[10] with 4-methylbenzyl bromide to give only **17**. Amination of **17** with dimethylamine to give **22** confirmed that **17** was indeed the 9-isomer.

Compounds **20–22** (Scheme II) were prepared by amination of 2-chloropurine **1**[5] or 6-chloropurine **19**. Alkylation of **18** with 4-methylbenzyl bromide gave a mixture of **19** and the 7-isomer, which were separated by flash chromatography to give **19** in low yield. Amination of **19** with dimethylamine gave the 2-aminopurine **20** in 66% yield. That **19** was the 9-isomer was confirmed by the similarity of its UV spectrum with that of the 9-benzyl analogue.[11]

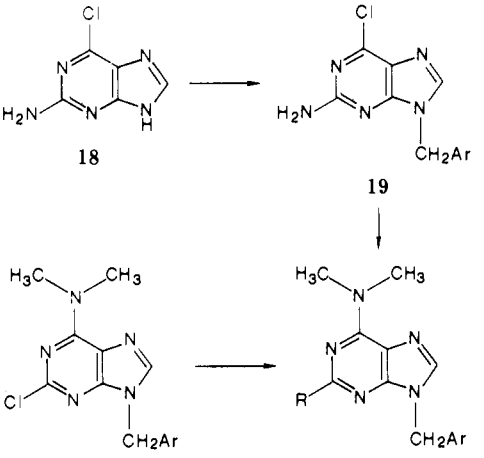
**Scheme I**[a]



**3**, R = H
**4**, R = $CH_2Ar$
**5**
**6**, R = $CF_3$
**7**, R = H
**8**, R = $CH_3$
**9**, R = $CH_2CH_3$
**10**, R = $CF_3$
**11**, R = H
**12**, R = $CH_3$
**13**, R = $CH_2CH_3$
**14**, R = $CF_3$
**15**, R = $CH_3$
**16**, R = $CH_2CH_3$
**17**, R = F

[a] Ar = $C_6H_4CH_3$-4.

**Scheme II**[a]



**18**
**19**
**1**
**20**, R = $NH_2$
**21**, R = $NHCH_3$
**22**, R = $N(CH_3)_2$

[a] Ar = $C_6H_4CH_3$-4.

The 2-(alkylamino)purines **21** and **22** were prepared in good yield from **1** by amination at 100 °C.

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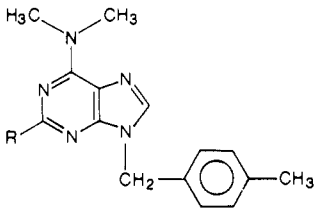
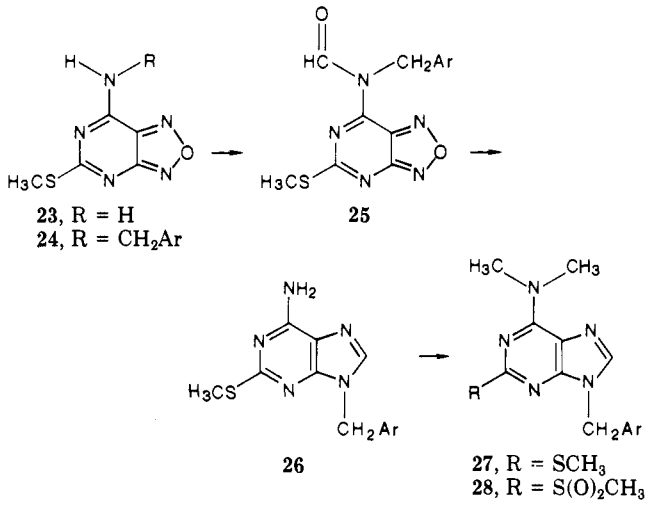
**Table I.** Antirhinovirus Type 1B Activity and Physicochemical Parameters Used To Derive Eq 1–7 for 2-Substituted Analogues of 6-(Dimethylamino)-9-(4-methylbenzyl)-9*H*-purine



| | | plaque reduction | | | | parameters[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| cmpd no. | R | $IC_{50}$, μM[b] | $pI_{50}$ obsd[c] | $pI_{50}$ calcd[c,d] | $\Delta pI_{50}$ | $\pi_m^-$ | $\pi$ | $\sigma_m$ | ***F*** | MR[e] |
| **1** | Cl | 0.08 | 7.10 | 6.91 | 0.19 | 1.04 | 0.71 | 0.37 | 0.41 | 0.60 |
| **2** | H | 8.6 | 5.07 | 5.54 | −0.48 | 0 | 0 | 0 | 0 | 0.10 |
| **14** | $CF_3$ | 0.03 | 7.52 | 7.31 | 0.21 | 1.49 | 0.88 | 0.43 | 0.38 | 0.50 |
| **15** | $CH_3$ | 0.8 | 6.10 | 5.98 | 0.11 | 0.5 | 0.56 | −0.07 | −0.04 | 0.56 |
| **16** | $C_2H_5$ | 2.3 | 5.64 | 6.39 | −0.76 | 0.94 | 1.02 | −0.07 | −0.05 | 1.03 |
| **17** | F | 0.34 | 6.47 | 6.38 | 0.09 | 0.47 | 0.14 | 0.34 | 0.43 | 0.09 |
| **20** | $NH_2$ | 31 | 4.51 | 4.33 | 0.18 | −1.29 | −1.23 | −0.16 | 0.02 | 0.54 |
| **21** | $NHCH_3$ | 4.7 | 5.33 | 4.87 | 0.46 | −0.6[f] | −0.47 | −0.3 | −0.11 | 1.03 |
| **22** | $N(CH_3)_2$ | 1.1 | 5.96 | 5.73 | 0.23 | 0.1 | 0.18 | −0.15 | 0.1 | 1.56 |
| **27** | $SCH_3$ | 0.34 | 6.47 | 6.25 | 0.22 | 0.55 | 0.61 | 0.15 | 0.2 | 1.38 |
| **28** | $SO_2CH_3$ | 25[g] | 4.60 | 5.06 | −0.46 | −1.02 | −1.63 | 0.60 | 0.54 | 1.35 |
| BW683C[h] | | 0.007 | | | | | | | | |

[a] Values for all parameters are from ref 17–19. [b] The $IC_{50}$ is the 50% inhibitory concentration measured as described in ref 15. [c] The $pI_{50}$ values are the log ($1000/IC_{50}$). [d] The calculated $pI_{50}$ values were calculated from eq 7 in Table III. [e] The MR values are scaled by 0.1. [f] Value estimated by interpolation using values for **20** and **22**. [g] Value estimated by extrapolation. Compound inhibited plaque formation by 24% at 8 μM. [h] 4′,6-Dichloroflavan, see ref 14.

**Scheme III**[a]



**23**, R = H
**24**, R = $CH_2Ar$

**25**

**26**

**27**, R = $SCH_3$
**28**, R = $S(O)_2CH_3$

[a] Ar = $C_6H_4CH_3$-4.

The furazanopyrimidine method for synthesis of 9-substituted adenines[12] was used for preparation of **27** and **28** (Scheme III). The amino group of **23** was readily displaced by 4-methylbenzylamine in dimethylformamide to give **24**, which was formylated with acetic–formic anhydride to provide the formamide **25**. The oxadiazole ring was reduced with zinc powder in refluxing acetic acid; in situ cyclization led to formation of adenine **26**. Compound **26** was dimethylated by using sodium hydride and excess methyl iodide[13] to give **27**. The sulfone **28** was prepared by oxidation of **27** with hydrogen peroxide in acetic anhydride.

## Biological Results and Discussion

**Antiviral Activity.** The compounds in Table I were tested initially in a plaque inhibition assay using monolayers of M-HeLa cells. All compounds except **20** and **28** were active at 50 μg per disk. The 50% inhibition concentrations of all compounds except **28** were measured with the plaque reduction assay under conditions where BW 683C[14] had an $IC_{50}$ = 0.007 μM. Compound **28** gave 24% inhibition at 8 μM. The plaque inhibition and plaque reduction assays were performed as described previously.[15]

After discovery of the potent rhinovirus type 1B activity of the 2-chloro-9-benzylpurines,[5] we initiated a synthesis program to investigate the striking effect of this 2-chloro substituent on antiviral activity. Several 9-(substituted-benzyl)purines with a 2-chloro substituent were from 40- to 100-fold more active against rhinovirus type 1B than the 2-deschloro parent compounds. The series of 2-substituted analogues in Table I were tested for activity against rhinovirus type 1B to extend this study. The *p*-methyl derivative **1** was selected as the prototype, because it was nontoxic in acute toxicity studies in rodents, in contrast with the unsubstituted 9-benzyl derivative.

The parent 2-H benzylpurine **2** had an $IC_{50}$ = 8.6 μM. Substitution at the 2-position with a 2-Cl (**1**) or 2-$CF_3$ (**14**)

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**Table II.** Activity of 14 and 22 against 19 Rhinovirus Serotypes[a]

| no. | 1A | 1B | 2 | 3 | 4 | 5 | 8 | 9 | 12 | 13 | 14 | 15 | 16 | 18 | 19 | 21 | 29 | 30 | 31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | 0.1 | 0.03 | 5.7 | (43)[b] | (41)[b] | (18)[c] | (41)[b] | 0.35 | 2.6 | 2.6 | 1.2 | 1.0 | (12)[d] | 1.8 | 6.7 | (40)[c] | 4.2 | 2.2 | 1.2 |
| 22 | <1.2 | 1.1 | 3.1 | >10 | >10 | 17.7 | 14 | 3.5 | N.T. | 13 | 7.9 | >10 | >20 | 8.3 | >20 | >20 | 4.9 | 2.9 | 15.1 |

[a] The numbers are the 50% inhibitory concentration ($IC_{50}$) measured as described in ref 14. In several cases the exact $IC_{50}$ was not determined and is denoted as greater than (>) or less than (<) the concentration. N.T. = not tested. [b] Percent inhibition of plaque formation at 2.5 μM. [c] Percent inhibition of plaque formation at 8 μM. [d] Percent inhibition of plaque formation at 5 μM.

**Table III.** Development of Equations Correlating Antirhinovirus 1B Activity and Physicochemical Parameters

| no. | equation | $n$ | $r$ | $F$ | $s$ |
|---|---|---|---|---|---|
| 1 | $pI_{50} = 2.99\ (\pm0.77)\sigma_m + 5.84$ | 9 | 0.83 | 15.2 | 0.58 |
| 2 | $pI_{50} = 3.95\ (\pm0.65)\sigma_m + 1.02\ (\pm0.37)MR + 5.11$ | 9 | 0.93 | 18.7 | 0.41 |
| 3 | $pI_{50} = 2.18\ (\pm0.76)\sigma_m + 0.57\ (\pm0.28)\pi + 5.76$ | 9 | 0.90 | 12.8 | 0.48 |
| 4 | $pI_{50} = 0.90\ (\pm0.23)\pi + 5.82$ | 11 | 0.80 | 15.6 | 0.62 |
| 5 | $pI_{50} = 1.98\ (\pm0.57)\boldsymbol{F} + 0.96\ (\pm0.15)\pi + 5.48$ | 11 | 0.92 | 23.3 | 0.42 |
| 6 | $pI_{50} = 1.00\ (\pm0.17)\pi_m^- + 5.69$ | 11 | 0.90 | 36.2 | 0.46 |
| 7 | $pI_{50} = 0.91\ (\pm0.59)\boldsymbol{F} + 0.96\ (\pm0.19)\pi_m^- + 5.54$ | 11 | 0.92 | 22.1 | 0.43 |

group led to 100- and 280-fold increases in activity. Both Cl and $CF_3$ are electron-withdrawing, lipophilic groups. Analogues containing lipophilic but electron-donating substituents such as $CH_3$ (**15**) and $CH_2CH_3$ (**16**) had moderate antirhinovirus activity. The $CH_3$ compound **15** was 10-fold more active than **2**, but **16** was only 3-fold more active. The polar, electron-donating 2-$NH_2$ substituent of **20** imparted a 4-fold loss in activity. These findings suggested that lipophilic, electron-withdrawing substituents were required for optimum activity. The steric effect of substituents, as judged by the good activity exhibited by **22** (R = $N(CH_3)_2$) and **27** (R = $SCH_3$), did not appear to be a significant factor. The poor activity of **28** (R = $SO_2CH_3$), which contains an electron-withdrawing, polar substituent, strongly pointed to the importance of lipophilicity ($+\pi$) as a significant 2-substituent physicochemical parameter.

Compounds **14** and **22** were tested for activity against 18 other rhinovirus serotypes (Table II). The $IC_{50}$'s for **14** ranged from 0.03 for type 1B, the most sensitive, to >8 μM for several other serotypes. The $IC_{50}$'s for **22** were much higher, ranging from 0.94 μM to >20 μM. Thus although these two agents had potent to good activity against serotype 1B, other serotypes were less sensitive.

**Quantitative Structure–Activity Relationships (QSAR).** QSAR analysis was performed on the compounds in Table I to better understand the effects of different 2-substituents on antirhinovirus 1B activity. Computer-assisted multiple regression analyses were performed on PROPHET with the "Fit Multiple" program,[16] which used a stepwise inclusion of the most significant parameters. The most significant equations are listed in Table III; Table IV is a correlation matrix of the five physicochemical parameters that appear in eq 1–7. In these equations the $pI_{50}$ is the log $(1000/IC_{50})$; the $IC_{50}$ is the micromolar concentration of compound that inhibits plaque formation by 50%.

The physicochemical parameters that appear in the equations are (1) the Hammett $\sigma$ ($\sigma_m$) constant for the electronic effect of a meta substituent, (2) the hydrophobic parameter $\pi$, which is a measure of the lipophilic properties of substituents, (3) the hydrophobic parameter $\pi_m^-$, which describes the partition coefficient of compounds having meta electron-donating side chains; (4) the $\boldsymbol{F}$ parameter, which is the Swain and Lupton electronic field (inductive) parameter; and (5) MR, the molar refractivity, which is a measure of the "bulk" of substituents. MR was multiplied by 0.1 to place it on a scale similar to the other parameters. The number of compound data points used to derive the equation is $n$, $r$ is the correlation coefficient, $s$ is the standard deviation, and $F$ is a significance test. In addition to the parameters in Table I, we evaluated the following parameters in deriving eq 1–7: $\sigma_p$, $\boldsymbol{R}$, $\pi_p$, $(\pi_m)^2$, $\pi^2$, $\sigma_m^2$, $\boldsymbol{F}^2$, and molecular weight (MW). Values for all parameters were taken from Hansch et al.,[17] Hansch and Leo,[18] or Norrington et al.[19]

**Table IV.** Correlation Matrix for the Variables in Eq 1–5

| | $\pi_m^-$ | $\pi$ | $\sigma_m$ | $\boldsymbol{F}$ | MR |
|---|---|---|---|---|---|
| $\pi_m^-$ | 1.00 | | | | |
| $\pi$ | 0.95 | 1.00 | | | |
| $\sigma_m$ | 0.28 | −0.03 | 1.00 | | |
| $\boldsymbol{F}$ | 0.18 | −0.12 | 0.95 | 1.00 | |
| MR | −0.19 | −0.14 | −0.11 | −0.03 | 1.00 |

In the initial phase of this research nine compounds (**1**, **2**, **14–17**, and **20–22**) were prepared, tested for antiviral activity, and subjected to QSAR using seven parameters ($\pi$, $\sigma_m$, $\sigma_p$, $\boldsymbol{F}$, $\boldsymbol{R}$, MR, and MW). The most significant single parameter equation was eq 1 (Table III); $\sigma_m$ gave the best correlation with $r = 0.83$. The correlation for these nine compounds was improved when a second parameter (MR) was included in eq 2. Replacement of MR with $\pi$ gave eq 3 with an $r$ value of 0.90. For this set of substituents, the covariance between MR and $\pi$ is low (Table IV), so they are independent variables ($r = -0.14$), but it is difficult to distinguish which parameter is more important on the basis of eq 2 and 3.

Equation 2 indicates that a compound with an electronegative, bulky 2-substituent should have more potent rhinovirus 1B activity. The lipophilicity of the substituent does not enter into the equation. However, eq 3, which is only slightly less significant, indicates that a substituent with a positive $\pi$ value as well as electron-withdrawing properties should lead to compounds with improved activity. Compound **28** was prepared and tested to determine which equation was correct. The $SO_2CH_3$ group in **28** is a polar, electron-withdrawing substituent. If eq 2 was correct, then activity could be sustained or improved with a compound that contained a nonlipophilic substituent. Lowering of log $P$ was deemed desirable because although

(16) PROPHET is a time-sharing computer system administered by Bolt Beranek and Newman Inc. under contract with the Biotechnology Resources Program of the Division of Research Resources of the National Institutes of Health.

(17) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207.
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14 was very active, it was highly lipophilic (log $P > 4$), which was considered undesirable from the pharmacokinetic perspective.[20]

For the set of 11 2-substituted compounds, regression equation 4, which contained $\pi$, gave a good correlation ($r = 0.80$) when only one parameter was allowed. The correlation was improved when the electronic inductive parameter $F$ was added to give eq 5 with $r = 0.92$. The correlation coefficient of the single parameter equation was further improved when $\pi_m^-$ was used instead of $\pi$ (eq 6). This lipophilicity parameter better describes the partition properties of compounds with meta electron-donating substituents such as the 6-$N(CH_3)_2$ group in this series.[19] The two-parameter equation containing both $F$ and $\pi_m^-$ (eq 7) was also significant. Equations 3, 5, and 7 are similar since all contain electronic and lipophilic parameters that are highly covariant with $r = 0.95$ (Table IV). This QSAR analysis showed that a substituent with lipophilic, electron-withdrawing properties in the 2-position of the 9-benzyl-6-(dimethylamino)purines is a predominant factor for potent antirhinovirus 1B activity.

## Conclusion

The effect of various 2-substituents on the antirhinovirus 1B activity of 2-substituted-9-(4-methylbenzyl)purines was investigated through the synthesis and antiviral evaluation of the compounds in Table I. Both intuitive and QSAR regression analysis of the structure–activity data led to the conclusion that optimum antirhinovirus serotype 1B activity was associated with 9-benzylpurines containing a lipophilic, electron-withdrawing substituent. Although 14 had potent activity against serotype 1B, its activity against 19 serotypes of rhinovirus was not uniform; the $IC_{50}$s ranged over 260-fold. Thus, although these compounds do not have an optimum profile of serotype activity, 14 represents an excellent lead for development of an agent with a broader spectrum of antirhinovirus activity.

## Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block or a Thomas-Hoover Unimelt and were uncorrected. UV spectra were measured on a Unicam SP 800 or Cary 118 UV–vis spectrophotometer. NMR data were recorded on a Varian XL-200, a Varian XL-100-15-FT, a Varian FT-80A, or a Hitachi Perkin-Elmer R-24 spectrometer with $Me_4Si$ as an internal standard. The mass spectra were obtained from Oneida Research Services, Whitesboro, NY, on a Finnegan 4500 TFQ mass spectrometer. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on TLC. TLC's were developed on Whatman 200-$\mu$m MK6F plates of silica gel (SG) with fluorescent indicator. Preparative flash chromatography[21] was performed on silica gel 60 (40–63 $\mu$m, E. Merck No. 9385). All compounds were analyzed for C, H, N and gave combustion values within 0.4% of theoretical. Elemental analyses were performed by Atlantic Microlab, Inc. The 5-aminoimidazole-4-carboxamide hydrochloride was purchased from Chemalog.

**5-Amino-1-(4-methylbenzyl)imidazole-4-carboxamide (4).** To a magnetically stirred dispersion of pentane-washed sodium hydride (60.2% in mineral oil) (4.90 g, 123 mmol) in dimethylformamide (150 mL) was added 5-aminoimidazole-4-carboxamide hydrochloride (10.0 g, 61.5 mmol) in portions during 0.5 h. After 6 h 4-methylbenzyl bromide (9.45 g, 67.6 mmol) was added, and the reaction was stirred at ambient temperature for 16 h. The reaction mixture was poured into ice water (300 mL), and the solids were collected on a Buchner funnel. The solids were redispersed in ice water (1000 mL) and collected. These solids were dispersed in methanol (200 mL) and collected on a Buchner funnel. Recrystallization from ethanol (Norite) gave 2.48 g (17%) of 4: mp 256–261 °C dec; TLC-SG, $MeOH/CH_2Cl_2$ (10:90), one spot with $R_f = 0.45$; UV (pH 7) $\lambda_{max}$ 267.5 ($\epsilon$ 13 700), $\lambda_{min}$ 230 (5200), sh 217 (11 900) nm; $^1H$ NMR (80 MHz, DMSO-$d_6$) $\delta$ 7.14 (s, 4 H + 1 H, ArH + C-2), 6.66 (br s, 2 H, $CONH_2$), 5.79 (s, 2 H, $NH_2$), 5.01 (s, 2 H, $CH_2$), 2.28 (s, 3 H, $CH_3$); MS, $m/e$ 230 ($M^+$), 105 ($C_8H_9^+$). Anal. ($C_{12}H_{14}N_4O$) C, H, N.

**1-(4-Methylbenzyl)-5-(trifluoroacetamido)imidazole-4-carboxamide (5).** A solution of 4 (3.00 g, 13.0 mmol), trifluoroacetic acid (20 mL), and trifluoroacetic anhydride (9.2 mL) was refluxed with stirring for 16 h. The reaction was spin-evaporated in vacuo. The residue was triturated with ice water, and the pH of the resultant mixture was adjusted to 6.5 with saturated aqueous sodium bicarbonate. The precipitate was slurried in water, collected on a Buchner funnel, and washed with water to give 4.0 g (100%) of product. Recrystallization of 2.5 g of product from ethanol gave 1.07 g (42%) of 5: mp 194–196 °C; TLC-SG, $MeOH/CH_2Cl_2$ (10:90), one spot with $R_f = 0.33$; UV (pH 7) $\lambda_{max}$ 260 ($\epsilon$ 8400) nm; $^1H$ NMR (60 MHz, DMSO-$d_6$) $\delta$ 11.30 (s, 1 H, NH), 7.89 (s, 1 H, C-2), 7.30 (s, 2 H, $NH_2$), 7.14 (s, 4 H, ArH), 5.04 (s, 2 H, $CH_2$), 2.27 (s, 3 H, $CH_3$); MS, $m/e$ 326 ($M^+$), 309 ($M^+ - NH_3$), 105 ($C_8H_{10}^+$). Anal. ($C_{14}H_{13}F_3N_4O_2$) C, H, N.

**1,9-Dihydro-9-(4-methylbenzyl)-2-(trifluoromethyl)-6*H*-purin-6-one (6).** A mixture of 5 (0.742 g, 2.27 mmol), sodium methylate (0.613 g), and dimethylformamide (10 mL) was refluxed with stirring for 24 h. The cooled reaction mixture was poured over ice, and the pH was adjusted to 5 with acetic acid. The aqueous mixture was extracted with ether (3 × 50 mL). The combined extracts were diluted with 5 mL of ethyl acetate and washed once with brine. The ether solution was evaporated, and the residue was recrystallized from ethanol to give 0.171 g (24%) of 6: mp 238.5–240 °C; TLC-SG, $MeOH/CH_2Cl_2$ (10:90), one spot with $R_f = 0.42$; UV (pH 7) $\lambda_{max}$ 255.5 ($\epsilon$ 11 300), $\lambda_{min}$ 231 (3800) nm; $^1H$ NMR (100 MHz, DMSO-$d_6$) $\delta$ 8.48 (s, 1 H, C-8), 7.20 (m, 4 H, ArH), 5.40 (s, 2 H, $CH_2$), 2.27 (s, 3 H, $CH_3$); MS, $m/e$ 308 ($M^+$), 105 ($C_8H_9^+$). Anal. ($C_{14}H_{11}F_3N_4O$) C, H, N.

**1,9-Dihydro-2-methyl-9-(4-methylbenzyl)-6*H*-purin-6-one (8).** Ethanol (50 mL) was added dropwise, through a water-cooled condenser, to stirred, pentane-washed sodium hydride (60.2% in mineral oil) (2.61 g, 65.5 mmol). After effervescence had ceased, 4 (3.02 g, 13.1 mmol) was added, and the mixture was refluxed for 1 h. Ethyl acetate (4.04 g, 45.8 mmol) and ethanol (5 mL) were added, and the reaction was refluxed with stirring for 48 h. The reaction mixture was poured into ice water (500 mL), and the pH was adjusted to 6 with acetic acid. The solids were collected by suction filtration and recrystallized from methanol (Norite) and then from ethanol to give 2.31 g (69%) of 8: mp 279–280 °C; TLC-SG, $MeOH/CHCl_3$ (10:90), one spot with $R_f = 0.45$; UV (pH 7) $\lambda_{max}$ 251 ($\epsilon$ 13 800), $\lambda_{min}$ 230 (6000) nm; $^1H$ NMR (60 MHz, DMSO-$d_6$): $\delta$ 12.14 (br s, 1 H, NH), 8.07 (s, 1 H, C-8), 7.17 (s, 4 H, Ar H), 5.29 (s, 2 H, $CH_2$), 2.37 (s, 3 H, 2-$CH_3$), 2.25 (s, 3 H, $ArCH_3$); MS, $m/e$ 254 ($M^+$), 105 ($C_8H_9^+$). Anal. ($C_{14}H_{14}N_4O$) C, H, N.

**1,9-Dihydro-2-ethyl-9-(4-methylbenzyl)-6*H*-purin-6-one (9).** This compound was prepared from 4 and ethyl propionate on a 10.3-mmol scale by the method for the preparation of 8. Recrystallization from ethanol gave 2.10 g (76%) of 9: mp 272–274 °C; TLC-SG, $MeOH/CH_2Cl_2$ (10:90 v/v), one spot with $R_f = 0.52$; UV (pH 7) $\lambda_{max}$ 251 ($\epsilon$ 13 900), $\lambda_{min}$ 230 (6100) nm; $^1H$ NMR (60 MHz, DMSO-$d_6$) $\delta$ 12.04 (br s, 1 H, NH), 8.08 (s, 1 H, C-8), 7.21 (s, 4 H, ArH), 5.29 (s, 2 H, $CH_2$), 2.65 (q, 2 H, $J = 8.0$ Hz, $CH_2$), 2.27 (s, 3 H, $ArCH_3$), 1.23 (t, 3 H, $J = 6.8$ Hz, $CH_3$); MS, $m/e$ 268 ($M^+$), 105 ($C_8H_9^+$). Anal. ($C_{15}H_{16}N_4O$) C, H, N.

**6-Chloro-9-(4-methylbenzyl)-2-(trifluoromethyl)-9*H*-purine (10).** A mixture of 6 (1.00 g, 3.24 mmol), chloroform (20 mL), dimethylformamide (3 mL), and the solution prepared from dimethylformamide (0.640 g) and thionyl chloride (0.65 mL) was refluxed with stirring for 4 h. The cooled reaction solution was poured over crushed ice, and the pH was adjusted to 6.5 with saturated aqueous sodium bicarbonate. The chloroform layer was separated, washed with water, and added to silica gel 60 (20 g). This mixture was spin-evaporated in vacuo, and the residual solids were introduced on a column (4.5 cm × 17 cm) of silica gel 60 wetted with ethyl acetate/hexane (1:1). The column was eluted with ethyl acetate/hexane (1:1) by using the flash chromatography technique. The appropriate fractions were combined and spin-

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evaporated in vacuo to give 0.574 g (54%) of **10**, which was used without purification in the next step. The TLC, UV, and NMR data were the same as that for **10** prepared from 6-chloro-2-(trifluoromethyl)purine.[22]

**6-Chloro-9-(4-methylbenzyl)-9*H*-purine (11).** A mixture of **4** (0.500 g, 2.17 mmol), sodium methylate (0.702 g, 13 mmol), and absolute ethanol (10 mL) was brought to reflux. Ethyl formate (0.804 g, 10.8 mmol) was added to the refluxing solution. After 2 h the reaction was poured over ice and acidified with acetic acid. The precipitate was collected, washed with water, and dried to give 0.421 g (81%) of crude **7**: mp 285–289 °C dec (lit.[23] mp 292–295 °C); TLC-SG, $MeOH/CH_2Cl_2$ (5:95), one spot with $R_f$ = 0.29; UV (pH 1) $\lambda_{max}$ 250 (ε 11 200), $\lambda_{min}$ 229 (5700) nm; (pH 7) $\lambda_{max}$ 250 (ε 11 900), $\lambda_{min}$ 230 (5500) nm; (pH 13) $\lambda_{max}$ 255 (ε 12 500), $\lambda_{min}$ 229 (2900) nm; $^1$H NMR (200 MHz, DMSO-$d_6$) δ 12.2 (br s, 1 H, NH), 8.15 (s, 1 H, H-2 or H-8), 8.01 (s, 1 H, H-2 or H-8), 7.16 (AB q, 4 H, ArH), 5.29 (s, 2 H, $CH_2$), 2.24 (s, 3 H, $CH_3$); MS, *m/e* 240 ($M^+$), 105 ($C_8H_9^+$).

A mixture of **7** (0.200 g, 0.832 mmol), chloroform (5 mL), and dimethylformamide (5 mL) was brought to reflux. A solution prepared from dimethylformamide (0.154 g, 2.12 mmol) and thionyl chloride (0.272 g, 2.28 mmol) was added, and the reaction was refluxed for 20 min. The reaction was poured into ice, and the pH was adjusted to 7 with saturated sodium bicarbonate. This mixture was extracted with dichloromethane (2 × 20 mL), and the extract was filtered through a 2-cm pad of silica gel. The pad was rinsed with an equal volume of dichloromethane. The combined filtrates were spin-evaporated in vacuo, and the residual oil was triturated with water to give a yellow solid. The solid was collected and recrystallized from cyclohexane to give 0.102 g (47%) of **11** (mp 133–133.5 °C), which was identical by TLC and mixture melting point with **11** prepared by an unambiguous route.[8]

**6-Chloro-2-methyl-9-(4-methylbenzyl)-9*H*-purine (12).** A solution prepared from dimethylformamide (0.365 g) and thionyl chloride (0.40 mL) was added to a refluxing solution of **8** (0.500 g, 1.97 mmol), dimethylformamide (7 mL), and chloroform (10 mL). After 0.5 h the cooled reaction was poured over ice water (50 mL), and the pH was adjusted to 6.8 with saturated aqueous sodium bicarbonate. The mixture was extracted with ether (2 × 40 mL), and the combined extracts were washed with water (2 × 25 mL). The ether solution was concentrated under reduced pressure, and the residue was recrystallized from cyclohexane to give 0.343 g (64%) of **12**: mp 143.5–144 °C; TLC-SG, EtOAc/cyclohexane (3:2), one spot with $R_f$ = 0.49; UV (pH 1) $\lambda_{max}$ 270 (ε 9900), $\lambda_{min}$ 233 (2900) nm; (pH 7) $\lambda_{max}$ 271 (ε 9300), $\lambda_{min}$ 232 (1900) nm; (pH 13) $\lambda_{max}$ 270 (ε 8800), $\lambda_{min}$ 231 (2200) nm; $^1$H NMR (200 MHz, DMSO-$d_6$) δ 8.66 (s, 1 H, C-8), 7.17 (AB q, 4 H, ArH), 5.41 (s, 2 H, $CH_2$), 2.66 (s, 3 H, 2-$CH_3$), 2.24 (s, 3 H, Ar$CH_3$); MS, *m/e* 272 ($M^+$), 257 ($M^+ - CH_3$), 237 ($M^+ - Cl$), 105 ($C_8H_9^+$). Anal. ($C_{14}H_{13}ClN_4$) C, H, N.

**6-Chloro-2-ethyl-9-(4-methylbenzyl)-9*H*-purine (13).** This compound was prepared from **9** on a 1.8-mmol scale by the method for the preparation of **12**. Recrystallization from cyclohexane gave 0.327 g (61%) of **13**: mp 94–95 °C; TLC-SG, EtOAc/hexane (1:1), one spot with $R_f$ = 0.72; UV (pH 1) $\lambda_{max}$ 270 (ε 11 500), $\lambda_{min}$ 234 (4200) nm; (pH 7) $\lambda_{max}$ 271 (ε 11 400), $\lambda_{min}$ 235 (3900) nm; (pH 13) $\lambda_{max}$ 269 (ε 10 200), $\lambda_{min}$ 230 (2400) nm; $^1$H NMR (200 MHz, DMSO-$d_6$) δ 8.68 (s, 1 H, C-8), 7.19 (AB q, 4 H, ArH), 5.41 (s, 2 H, $CH_2$), 2.94 (q, 2 H, $CH_2$), 2.24 (s, 3 H, Ar$CH_3$), 1.29 (t, 3 H, $CH_3$); MS, *m/e* 287 (M + 1), 271 ($M^+ - CH_3$), 251 ($M^+ - Cl$), 182 ($M^+ - C_8H_9$), 105 ($C_8H_9^+$). Anal. ($C_{15}H_{15}ClN_4$) C, H, N.

**6-(Dimethylamino)-9-(4-methylbenzyl)-2-(trifluoromethyl)-9*H*-purine (14).** A solution of **10** (0.548 g, 1.76 mmol) and 2.2 M ethanolic dimethylamine (30 mL) was stirred at ambient temperature for 16 h. The reaction solution was spin-evaporated in vacuo to remove the volatiles. The residue was dissolved in dichloromethane (50 mL), washed with water (4 × 10 mL), filtered through glass wool, and spin-evaporated in vacuo. The residue was recrystallized from pentane to give 0.208 g (35%) of **14**: mp 101–102 °C; TLC-SG, EtOAc/cyclohexane (3:2), one spot with $R_f$ = 0.66; UV (pH 1) $\lambda_{max}$ 277 (ε 15 500), $\lambda_{min}$ 242 (3600) nm; (pH 7) $\lambda_{max}$ 273 (ε 16 100), $\lambda_{min}$ 241 (3000) nm; (pH 13) $\lambda_{max}$ 272 (ε 16 000), $\lambda_{min}$ 241 (2500) nm; $^1$H NMR (100 MHz, DMSO-$d_6$) δ 8.43 (s, 1 H, C-8) 7.18 (s, 4 H, ArH), 5.38 (s, 2 H, $CH_2$), 3.31 (s, 6 H, $N(CH_3)_2$), 2.26 (s, 3 H, $CH_3$); MS, *m/e* 335 ($M^+$), 306 ($M^+ - 29$), 230 ($M^+ - C_8H_9$), 105 ($C_8H_9^+$). Anal. ($C_{16}H_{16}F_3N_5$) C, H, N.

**6-(Dimethylamino)-2-methyl-9-(4-methylbenzyl)-9*H*-purine (15).** This compound was prepared from **12** on a 1.1-mmol scale by the method for the preparation of **14**. Recrystallization from cyclohexane–pentane gave 0.17 g (54%) of **15**: mp 89–90 °C; TLC-SG, $EtOH/CHCl_3$ (5:95), one spot with $R_f$ = 0.50; UV (pH 7) $\lambda_{max}$ 278.5 (ε 20 100), $\lambda_{min}$ 238.5 (2700) nm; $^1$H NMR (80 MHz, DMSO-$d_6$) δ 8.11 (s, 1 H, C-8), 7.15 (s, 4 H, ArH), 5.29 (s, 2 H, $CH_2$), 3.43 (s, 6 H, $N(CH_3)_2$), 2.42 (s, 3 H, 2-$CH_3$), 2.26 (s, 3 H, Ar$CH_3$); MS, *m/e* 281 ($M^+$), 252 ($M^+ - 29$), 176 ($M^+ - C_8H_9$), 105 ($C_8H_9^+$). Anal. ($C_{16}H_{19}N_5$) C, H, N.

**6-(Dimethylamino)-2-ethyl-9-(4-methylbenzyl)-9*H*-purine (16).** This compound was prepared from **13** on a 1.0-mmol scale by the method for the preparation of **14**. Recrystallization from pentane gave 0.175 g (56%) of **16**: mp 75.5–76.5 °C; TLC-SG, $EtOH/CHCl_3$ (5:95), one spot with $R_f$ = 0.49; UV (pH 7) $\lambda_{max}$ 279 (ε 20 300), $\lambda_{min}$ 239 (3100) nm; $^1$H NMR (80 MHz, DMSO-$d_6$) δ 8.12 (s, 1 H, C-8), 7.17 (s, 4 H, ArH), 5.29 (s, 2 H, $CH_2$), 3.44 (s, 6 H, $N(CH_3)_2$), 2.70 (q, 2 H, $J$ = 7.0 Hz, $CH_2$), 2.26 (s, 3 H, Ar$CH_3$), 1.25 (t, 3 H, $J$ = 7.6 Hz, $CH_3$); MS, *m/e* 295 ($M^+$), 280 ($M^+ - CH_3$), 266 ($M^+ - 29$), 252 ($M^+ - CH_2{=}NCH_3$), 190 ($M^+ - C_8H_9$). Anal. ($C_{17}H_{21}N_5$) C, H, N.

**6-(Dimethylamino)-2-fluoro-9-(4-methylbenzyl)-9*H*-purine (17).** To a stirred dispersion of sodium hydride (60.2% in mineral oil) (0.45 g, 11.2 mmol) in dimethyl sulfoxide (50 mL) was added 6-(dimethylamino)-2-fluoro-9*H*-purine[10] (1.81 g, 10 mmol). After 4 h, 4-methylbenzyl bromide (1.85 g, 10 mmol) was added, and the resultant solution was stirred at ambient temperature for 18 h. The solution was poured into ice water (200 mL) and extracted with dichloromethane (3 × 50 mL). The combined extracts were washed with water (6 × 40 mL), filtered through glass wool, and spin-evaporated in vacuo. The residual solid was dissolved in ethyl acetate/cyclohexane (1:1) (60 mL) and added to silica gel 60 (10 g). This mixture was spin-evaporated in vacuo, and the residual solids were introduced on a column (5 cm × 15 cm) of silica gel 60 wetted with ethyl acetate/cyclohexane (1:2). The column was eluted with ethyl acetate/cyclohexane (1:2) (400 mL) and ethyl acetate/cyclohexane (1:1) by using the flash chromatography technique. The appropriate fractions were combined and spin-evaporated in vacuo. The resulting solid was recrystallized from cyclohexane to give 1.70 g (59%) of **17**: mp 124–125 °C; TLC-SG, EtOAc/cyclohexane (3:2), one spot; UV (pH 7) $\lambda_{max}$ 282.5 (ε 19 000), 273.5 (22 600), $\lambda_{min}$ 280 (18 500), 233.5 (2700) nm; $^1$H NMR (60 MHz, DMSO-$d_6$) δ 8.20 (s, 1 H, H-8), 7.18 (m, 4 H, ArH), 5.28 (s, 2 H, $CH_2$), 3.45 (br s, 6 H, $N(CH_3)_2$), 2.26 (s, 3 H, $CH_3$); MS, *m/e* 285 ($M^+$), 256 ($M^+ - 29$), 180 ($M^+ - C_8H_9$), 105 ($C_8H_9^+$). Anal. ($C_{15}H_{16}FN_5$) C, H, N.

**2-Amino-6-chloro-9-(4-methylbenzyl)-9*H*-purine (19).** A mixture of 2-amino-6-chloro-9*H*-purine (**18**) (5.00 g, 29.5 mmol), potassium carbonate (4.48 g, 32.4 mmol), and dimethyl sulfoxide (50 mL) was stirred for 15 min. 4-Methylbenzyl bromide (4.63 mL, 32.4 mmol) was added, and the reaction was stirred for 4 h. The reaction mixture was poured over ice, and the pH was adjusted to 5 with acetic acid. The mixture was extracted with dichloromethane (2 × 400 mL), and the combined extracts were washed with brine to remove the emulsion. The dichloromethane extracts were washed with water (5 × 600 mL) and then spin-evaporated in vacuo to a yellow solid. This material was adsorbed to silica gel 60 and chromatographed on silica gel 60 by using methanol/dichloromethane (5:95) to give 1.97 g (24%) of pure 9-isomer. A portion (0.80 g) of this material was recrystallized from ethanol to give analytically pure **19**: yield 0.273 g, mp 185–195 °C dec; TLC-SG, $MeOH/CH_2Cl_2$ (10:90), one spot with $R_f$ = 0.59; UV (pH 7) $\lambda_{max}$ 308 (ε 4100), $\lambda_{min}$ 266 (580) nm; $^1$H NMR (60 MHz, DMSO-$d_6$) δ 8.22 (s, 1 H, C-8), 7.19 (s, 4 H, ArH), 6.92 (br s, 2 H, $NH_2$), 5.26 (s, 2 H, $CH_2$), 2.26 (s, 3 H, $CH_3$); MS, *m/e* 273 ($M^+$), 105 ($C_8H_9^+$). Anal. ($C_{13}H_{12}ClN_5$) C, H, N.

**2-Amino-6-(dimethylamino)-9-(4-methylbenzyl)-9*H*-purine (20).** A mixture of **19** (0.470 g, 1.72 mmol) and 2.2 M ethanolic dimethylamine (35 mL) was heated in a stainless steel reaction vessel at 116 °C for 65 h. The cooled mixture was spin-evaporated in vacuo, the residue was triturated with water, and the solid was collected by suction filtration. The solid was recrystallized from

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ethyl acetate–cyclohexane to give 0.321 g (66%) of **20**: mp 175.5–176.5 °C; TLC-SG, MeOH/$CH_2Cl_2$ (10:90), one spot with $R_f$ = 0.53; UV (pH 7) $\lambda_{max}$ 285 (ε 15600), $\lambda_{min}$ 247 (7400), sh 263.5 (9900) nm; $^1$H NMR (60 MHz, DMSO-$d_6$) δ 7.75 (s, 1 H, C-8), 7.13 (s, 4 H, ArH), 5.78 (br s, 2 H, $NH_2$), 5.16 (s, 2 H, $CH_2$), 3.37 (s, 6 H, $N(CH_3)_2$), 2.24 (s, 3 H, $CH_3$); MS, *m/e* 282 ($M^+$), 253 ($M^+ - 29$), 177 ($M^+ - C_8H_9$). Anal. ($C_{15}H_{18}N_6$) C, H, N.

**6-(Dimethylamino)-2-(methylamino)-9-(4-methylbenzyl)-9*H*-purine (21).** A mixture of 1[5] (0.500 g, 1.66 mmol), 40% aqueous methylamine (3.88 g, 50 mmol), and absolute ethanol (40 mL) was heated (100 °C) for 16 h in a glass-lined, stainless steel reaction vessel. Additional 40% aqueous methylamine (1.30 g, 17 mmol) was added to the reaction vessel, and the reaction was heated (105 °C) for 16 h. The reaction mixture was spin-evaporated in vacuo, and the residue was covered with ethanol and re-evaporated. The residue was treated with ethanol and evaporated to a 3-mL volume, which afforded a crystalline solid. This mixture was diluted with water (20 mL), and the solids were collected by suction filtration. Recrystallization from pentane–cyclohexane gave 0.276 g (56%) of **21**: mp 108–109 °C; TLC-SG, EtOAc/cyclohexane (3:2), one spot with $R_f$ = 0.49; UV (pH 7) $\lambda_{max}$ 292 (ε 13000), 237.5 (19600), $\lambda_{min}$ 273.5 (8400), sh 263 (10500), 257 (11200) nm; $^1$H NMR (60 MHz, DMSO-$d_6$) δ 7.76 (s, 1 H, C-8), 7.16 (s, 4 H, ArH), 6.20 (m, 1 H, *J* = 4.4 Hz, NH), 5.16 (s, 2 H, $CH_2$), 3.37 (s, 6 H, $N(CH_3)_2$), 2.80 (d, 3 H, *J* = 4.8, HNMe), 2.25 (s, 3 H, $CH_3$); MS, *m/e* 296 ($M^+$), 267 ($M^+ - 29$), 191 ($M^+ - C_8H_9$). Anal. ($C_{16}H_{20}N_6$) C, H, N.

**2,6-Bis(dimethylamino)-9-(4-methylbenzyl)-9*H*-purine (22). Amination of 1.** A mixture of 1[5] (0.50 g, 1.66 mmol) and 0.83 M ethanolic dimethylamine (40 mL) in a glass-lined, stainless steel reaction vessel was heated (100 °C) for 16 h. The cooled reaction mixture was spin-evaporated in vacuo. The residue was triturated with water to give a solid, which was collected and washed with water. The solid was recrystallized from pentane–cyclohexane to give 0.315 g (61%) of **22**: mp 109.5–111 °C; TLC-SG, MeOH/$CH_2Cl_2$ (2:98), one spot with $R_f$ = 0.35; UV (pH 7) $\lambda_{max}$ 297 (ε 11500), 246 (23200), $\lambda_{min}$ 278 (7800), 229 (15000) nm; $^1$H NMR (60 MHz, DMSO-$d_6$) δ 7.79 (s, 1 H, C-8), 7.18 (m, 4 H, ArH), 5.14 (s, 2 H, $CH_2$), 3.37 (s, 6 H, 6-$N(CH_3)_2$), 3.07 (s, 6 H, 2-$N(CH_3)_2$), 2.23 (s, 3 H, $CH_3$); MS, *m/e* 310 ($M^+$), 295 ($M^+ - CH_3$), 281 ($M^+ - 29$), 205 ($M^+ - C_8H_9$). Anal. ($C_{17}H_{22}N_6$) C, H, N.

**Amination of 17.** A mixture of **17** (1.1 g, 4.0 mmol) and 40% aqueous dimethylamine (25 mL) in a glass-lined, stainless steel reaction vessel was heated at 135 °C for 16 h. The volatiles were removed by spin evaporation in vacuo, and the residual solids gave TLC $R_f$ and UV absorbance values identical with those obtained for **22** prepared from **1**.

**7-[(4-Methylbenzyl)amino]-5-(methylthio)[1,2,5]oxadiazolo[3,4-*d*]pyrimidine (24).** A solution of 4-methylbenzylamine (5.19 g, 42.0 mmol), **23**[12] (7.00 g, 38.2 mmol), and dry dimethylformamide (70 mL) was stirred at ambient temperature for 2.5 h when an additional portion of 4-methylbenzylamine (5.19 g, 42.0 mmol) was added. After 16 h the reaction mixture was poured into water (200 mL) and extracted with 12 portions of ether (12 × 400 mL). The combined ether extract was reduced to a volume of 300 mL by spin evaporation in vacuo and then washed with water (4 × 100 mL). The ether phase was evaporated to dryness under reduced pressure, and the residue was triturated with water (200 mL). The solids were collected by suction filtration to give 12.0 g of crude **24**. One gram of crude material was dissolved in ethyl acetate, and the solution was added to silica gel 60 (10 g). This mixture was spin-evaporated in vacuo, and the residual solids were introduced on a column (5 cm × 18 cm) of silica gel 60 wetted with ethyl acetate/hexane (1:8). The column was eluted with ethyl acetate/hexane (1:8), and the appropriate fractions were combined and spin-evaporated in vacuo to give a white solid. Recrystallization of the solid from hexane–ethyl acetate gave 0.468 g (46%) of **24**: mp 143–144 °C; TLC-SG, EtOAc/cyclohexane (1:6), one spot with $R_f$ = 0.42; $^1$H NMR (80 MHz, DMSO-$d_6$) δ 10.4 (br s, 1 H, NH), 7.19 (AB q, 4 H, ArH), 4.67 (s, 2 H, $CH_2$), 2.47 (s, 3 H, $SCH_3$), 2.25 (s, 3 H, $ArCH_3$); MS, *m/e* 287 ($M^+$), 270 ($M^+ - OH$), 105 ($C_8H_9^+$). Anal. ($C_{13}H_{13}N_5OS$) C, H, N.

**7-[*N*-(Methylbenzyl)formamido]-5-(methylthio)[1,2,5]oxadiazolo[3,4-*d*]pyrimidine (25).** A mixture of 98% formic acid (63 mL, 1.64 mol) and acetic anhydride (157 mL, 1.66 mol) was magnetically stirred at 0 °C for 1 h after which **24** (11.0 g, 38.3 mmol) was added. The reaction was stirred for 16 h. The volatiles were removed by spin evaporation in vacuo, and the solids were stirred with water (250 mL). The solids were collected on a Büchner funnel, washed with water (25 mL), and suction-dried to give 8.40 g (69%) of **25** as a yellow solid. Recrystallization of 1.0 g from ethanol–water gave the analytical sample: mp 155–156 °C; TLC-SG, EtOAc/cyclohexane (1:5), one spot with $R_f$ = 0.62; $^1$H NMR (80 MHz, DMSO-$d_6$) δ 10.07 (s, 1 H, CHO), 7.20 (AB q, 4 H, ArH), 5.22 (s, 2 H, $CH_2$), 2.60 (s, 3 H, $SCH_3$), 2.26 (s, 3 H, $ArCH_3$); MS, *m/e* 315 ($M^+$), 286 ($M^+ - CHO$), 257 ($M^+ - CO - NO$), 105 ($C_8H_9^+$). Anal. ($C_{14}H_{13}N_5O_2S$) C, H, N.

**6-Amino-9-(4-methylbenzyl)-2-(methylthio)-9*H*-purine Hydrochloride (26).** A mixture of **25** (10.8 g, 34.2 mmol) and glacial acetic acid (150 mL) was stirred at ambient temperature on a water bath. Powdered zinc (22 g) was added in 0.5-g increments over the course of 20 min, and the mixture was then refluxed with stirring for 3 h. The reaction was cooled, and the liquid portion was decanted through a sintered glass funnel. The solid residue was refluxed in acetic acid (100 mL) for 15 min and then filtered hot through a sintered glass funnel. The combined filtrates were spin-evaporated in vacuo, and the yellow solid was triturated with water (150 mL). The solids were collected on a Büchner funnel, washed with water (50 mL), suction-dried for 2 h, and then dried in vacuo at 95 °C for 4 h to give 6.80 g (70%) of crude product. The product was dispersed in 2-propanol-concentrated hydrochloric acid and then spin-evaporated in vacuo. The residual paste was dispersed in 2-propanol, and the solids were collected by suction filtration. Recrystallization from ethanol gave an analytical sample of **26** as its hydrochloride: mp 240–243 °C; TLC-SG, EtOAc/cyclohexane (3:2), one spot with $R_f$ = 0.48; UV (pH 1) $\lambda_{max}$ 270 (ε 14200), $\lambda_{min}$ 243 (6800) nm; (pH 7) $\lambda_{max}$ 276 (ε 13200), $\lambda_{min}$ 251 (7100) nm; (pH 13) $\lambda_{max}$ 276 (ε 13100), $\lambda_{min}$ 251 (7100) nm; $^1$H NMR (80 MHz, DMSO-$d_6$) δ 8.65 (s, 1 H, H-8), 7.24 (AB q, 4 H, ArH), 5.35 (s, 2 H, $CH_2$), 5.12 (br s, 3 H, $NH_3^+$), 2.53 (s, 3 H, $SCH_3$), 2.27 (s, 3 H, $ArCH_3$); MS, *m/e* 285 ($M^+$), 238 ($M^+ - SMe$), 180 ($M^+ - C_8H_9$), 105 ($C_8H_9^+$). Anal. ($C_{14}H_{15}N_5S$·HCl) C, H, N.

**6-(Dimethylamino)-9-(4-methylbenzyl)-2-(methylthio)-9*H*-purine (27).** To a stirred dispersion of pentane-washed sodium hydride (60.2% in mineral oil) (1.10 g, 27.5 mmol) in dimethylformamide (35 mL) was added **26**·HCl (3.56 g, 12.5 mmol) in several portions. After 1 h methyl iodide (1.71 mL, 27.5 mmol) was added, and the reaction was stirred for 16 h. The reaction was poured into an ice-water slurry (100 mL) and extracted with ether (3 × 100 mL). The combined ether extract was washed with water (200 mL) and brine (150 mL) and dried (sodium sulfate). The solution was spin-evaporated in vacuo to give 2.56 g (65%) of a yellow solid. The solids were dissolved in warm ethyl acetate, and the solution was added to silica gel 60 (20 g). This mixture was spin-evaporated in vacuo, and the residual solid was introduced on a column (3.5 cm × 18 cm) of silica gel 60 wetted with ethyl acetate/hexane (1:2). The column was eluted with ethyl acetate/hexane (1:2), and the appropriate fractions were combined and spin-evaporated in vacuo to give 0.92 g (24%) of **27**. Recrystallization of 0.20 g of this material from hexane gave the analytical sample: mp 110.5–111 °C; TLC-SG, EtOAc/cyclohexane (1:2), one spot with $R_f$ = 0.48; UV (pH 1) $\lambda_{max}$ 276 (ε 18300), $\lambda_{min}$ 245 (14100) nm; (pH 7) $\lambda_{max}$ 287 (ε 20100), 247 (25500), $\lambda_{min}$ 263 (12600), 229 (13200) nm; (pH 13) $\lambda_{max}$ 287 (ε 20000), 247 (25200), $\lambda_{min}$ 262 (12300), 228 (11700) nm; $^1$H NMR (80 MHz, DMSO-$d_6$) δ 8.10 (s, 1 H, H-8), 7.18 (complex multiplet, 4 H, ArH), 5.27 (s, 2 H, $CH_2$), 3.42 (br s, 6 H, $N(CH_3)_2$), 2.49 (s, 3 H, $SCH_3$), 2.26 (s, 3 H, $ArCH_3$); MS, *m/e* 313 ($M^+$), 284 ($M^+ - 29$), 208 ($M^+ - C_8H_9$), 105 ($C_8H_9^+$). Anal. ($C_{16}H_{19}N_5S$) C, H, N.

**6-(Dimethylamino)-9-(4-methylbenzyl)-2-(methylsulfonyl)-9*H*-purine (28).** To a solution of **27** (0.400 g, 1.28 mmol) in glacial acetic acid (10 mL) was added acetic anhydride (2.52 mL, 25.6 mmol) and 30% hydrogen peroxide (2.12 mL, 25.6 mmol). The solution was stirred for 18 h. Saturated sodium sulfite (40 mL) was added to the reaction, and the mixture was extracted with ethyl acetate (3 × 100 mL). The combined ethyl acetate extract was washed with water (2 × 200 mL) and saturated brine (100 mL) and then dried (sodium sulfate). The volatiles were spin-evaporated in vacuo to give an oil that was coevaporated with

ethanol several times to give 0.388 g of a white solid. Recrystallization from ethyl acetate–hexane gave 0.293 g (66%) of analytically pure **28**: mp 135.5–136.5 °C; TLC-SG, ethyl acetate/cyclohexane (1:1), one spot with $R_f$ = 0.35; UV (pH 1) $\lambda_{max}$ 276 (ε 13 700), $\lambda_{min}$ 246 (3500) nm; (pH 7) $\lambda_{max}$ 274 (ε 14 400), $\lambda_{min}$ 246 (3800) nm; (pH 13) $\lambda_{max}$ 274 (ε 14 600), $\lambda_{min}$ 246 (4000) nm; $^1$H NMR (80 MHz, DMSO-$d_6$) δ 8.44 (s, 1 H, H-8), 7.19 (AB q, 4 H, Ar H), 5.38 (s, 2 H, $CH_2$), 3.40 (br s, 6 H, $N(CH_3)_2$), 3.34 (s, 3 H, $SO_2CH_3$), 2.26 (s, 3 H, $CH_3$); MS, *m/e* 345 ($M^+$), 316 ($M^+ - 29$), 266 ($M^+ - SO_2CH_3$), 240 ($M^+ - C_8H_9$), 105 ($C_8H_9^+$). Anal. ($C_{16}H_{19}N_5O_2S$) C, H, N.

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# Synthesis and Cytostatic and Antiviral Activities of 1-β-D-Ribofuranosyl-5-alkylcytosine (5-Alkylcytidine) Cyclic 3′,5′-Monophosphates

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A series of 5-alkylcytidines and their 5′-monophosphates and cyclic 3′,5′-monophosphates have been synthesized and evaluated for antiviral and antitumor activity. The 5-alkyl cyclic nucleotides were not cytostatic ($ID_{50}$ > 200 μg/mL) against leukemia L1210 cells and a deoxycytidine kinase-deficient subline thereof. Certain of the corresponding nucleosides and their 5′-monophosphates did show activity within the range of 35–162 μg/mL, as did the unsubstituted cytidine cyclic 3′,5′-monophosphate. No antiviral activity was found for any of the compounds at 400 μg/mL. A drug design rationale for utilization of 5-alkylcytidines based on their potential conversion to biologically active 5-alkyl-2′-deoxyuridines is not supported by these experimental findings.

Nucleoside cyclic 3′,5′-monophosphates have been of interest in recent years because of the implicated role of cAMP in the malignant transformation process.[1] Moreover, it has been suggested that cyclic 3′,5′-monophosphates could be the metabolites responsible for the antitumor and antiviral activities of certain nucleosides.[2] The potent in vivo antitumor and antiviral activities of cyclic nucleotides derived from araA,[3] araC,[4] and the 6-mercapto- and 6-(methylthio)-9-β-D-ribofuranosylpurines[5] have been known for some time, as has the antiviral capacity of the cyclic 3′,5′-monophosphate of ribavirin.[6] More recently we have examined a large number of cyclic 3′,5′-monophosphates derived from 5-alkyl-,[7] 5-halo-,[8] and 5-(trifluoromethyl)-2′-deoxyuridines,[8] 5-halocytidines,[9] and 5-halouridines.[10] Potent antiviral and cytostatic activities were found for a number of these compounds, although the cyclic diesters were normally 10–100 times less active than the corresponding nucleosides or 5′-monophosphates. However, significant cytostatic activity of the cyclic diesters against thymidine kinase deficient tumor cell lines was not found. A striking inhibitory effect against thymidine kinase deficient herpes simplex virus type 1 (HSV-1) was noted[11] for certain 5-substituted 2′-deoxyuridine cyclic 3′,5′-monophosphates as well as the corresponding nucleosides and nucleoside 5′-monophosphates which are known to inhibit thymidylate synthase. Active cyclic diesters probably are prodrug forms of the nucleoside or nucleoside monophosphate in most cases; however, studies of the potent antiviral activity of the cyclic phosphate diester of 2′-nordeoxyguanosine (DHPG) suggest a biological role of the cyclic compound itself.[12]

On the basis of the above and the well-known[13,14] striking

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