The Synthesis and Determination of Acidic Ionization Constants of Certain 5-Substituted 2-Aminopyrrolo[2,3-*d*]pyrimidin-4-ones and Methylated Analogs

Geoffrey C. Hoops, Julie Park, George A. Garcia, and Leroy B. Townsend*

Interdepartmental Graduate Program in Medicinal Chemistry, College of Pharmacy,
University of Michigan, Ann Arbor, Michigan 48109-1065

Received November 27, 1995

Dedicated to the memory of Professor Nicholas Alexandrou

The acidic ionization constants were determined for a series of 5-substituted 2-aminopyrrolo[2,3-*d*]-pyrimidin-4-ones and N-3- and N-7-methylated analogs thereof. The syntheses of the methylated analogs are also described.

J. Heterocyclic Chem., **33**, 767 (1996).

Introduction.

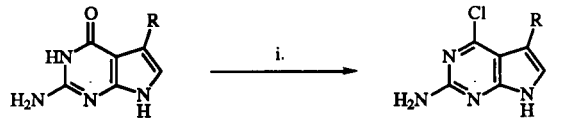
Queuine (2-amino-5-(4,5-*cis*-dihydroxy-1-cyclopenten-3-*trans*-ylaminomethyl)pyrrolo[2,3-*d*]pyrimidin-4-one) is a hypermodified base that is found in transfer RNA in all species with the exception of yeast and archea [1]. Queuine was the first example of a modified base where the basic (parent) ring system (purine or pyrimidine) has been modified. A number of other pyrrolo[2,3-*d*]pyrimidine nucleosides are known in nature (*i.e.*, toyocamycin, cadeguomycin, sangivamycin, *etc.*). However, queuine appears to be unique in that it is biosynthesized as the free base, not as a ribonucleoside. tRNA-guanine transglycosylase (TGT) catalyzes the incorporation of queuine into tRNA by exchanging queuine for the guanine of guanosine-34 [2].

In our investigations of the tRNA-guanine transglycosylase from *Escherichia coli* we have studied a series of 5-substituted 2-aminopyrrolo[2,3-*d*]-pyrimidin-4-ones as analogs of the physiological substrate, 2-amino-5-(aminomethyl)pyrrolo[2,3-*d*]pyrimidin-4-one (preQ$_1$) [3]. These compounds were examined both as competitive inhibitors of TGT-catalyzed guanine incorporation into tRNA and as substrates for TGT-catalyzed exchange into tRNA. Those substrates having an electron-withdrawing substituent at the 5-position exhibited higher V_{max}s than analogs with electron-donating substituents at the 5-position. This would suggest that the N-7 proton must be deprotonated prior to subsequent glycosidic bond formation and that this step may be partially rate-determining in the TGT reaction. Therefore, we have undertaken a detailed study of the ionization constants of these compounds. The ionization constants of these compounds in water were determined by potentiometric titration, but these results were decidedly ambiguous. The pK_a values determined were very similar for all compounds in the series. Comparison with literature values for the pK_a's of guanine suggested that ionization of N-3 of the pyrrolopyrimidine rather than N-7 was being observed. In order to differentiate between the acidic properties of the ring nitrogens of these compounds in aqueous solution, a series of methylated analogs of 5-substituted 2-aminopyrrolo[2,3-*d*]pyrimidin-4-ones were synthesized and the ionzation constants were determined.

Results and Discussion.

2-Amino-5-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**1**) [4] and 2-amino-5-(*N*,*N*-dimethylaminomethyl)pyrrolo[2,3-*d*]-pyrimidin-4-one (**2**) [5] were individually added to phosphorus oxychloride in the presence of a tertiary amine and the resulting solutions heated at reflux temperature to afford 2-amino-4-chloro-5-methylpyrrolo[2,3-*d*]pyrimidine (**3**) and 2-amino-4-chloro-5-(*N*,*N*-dimethylaminomethyl)pyrrolo-[2,3-*d*]pyrimidine (**4**), respectively (Scheme 1).

Scheme 1
Chlorination of 2-Aminopyrrolo[2,3-*d*]pyrimidine-4-ones



1, R = -CH_3
2, R = -$CH_2N(CH_3)_2$

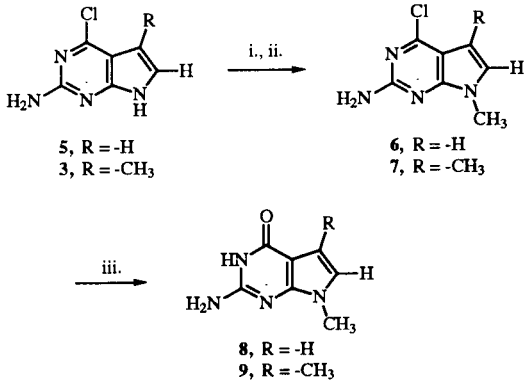
3, R = -CH_3
4, R = -$CH_2N(CH_3)_2$

i. $POCl_3$/DEA/Δ_x.

Regioselective glycosylation of the 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidin-4-ones at N-7 has been accomplished [6] *via* 1) deprotonation of the pyrrole-ring nitrogen by base, followed by 2) treatment with a glycosyl halide. Thus, 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine (**5**) [7], produced for this work by the procedure of Shih and Gossett [8], was treated with sodium hydride (NaH) in dimethylformamide (DMF) followed by methyl iodide at ambient temperature to give 2-amino-4-chloro-7-methylpyrrolo[2,3-*d*]pyrimidine (**6**) (Scheme 2). Treatment of 2-amino-4-chloro-5-methylpyrrolo[2,3-*d*]-pyrimidine (**3**) under similar conditions gave 2-amino-4-chloro-5,7-dimethylpyrrolo[2,3-*d*]pyrimidine (**7**). Hydrolysis of **6** and **7** under basic conditions gave 2-amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**8**) and

2-amino-5,7-dimethylpyrrolo[2,3-*d*]pyrimidin-4-one (**9**), respectively.

Scheme 2
Synthesis of 2-Amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one and 2-Amino-5,7-dimethylpyrrolo[2,3-*d*]pyrimidine-4-one

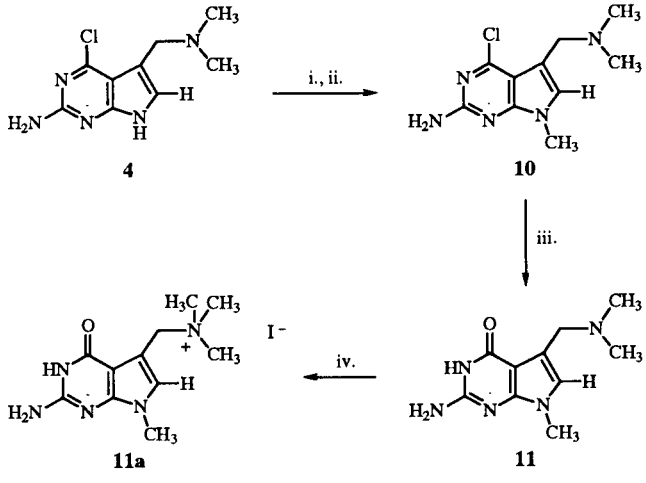


5, R = -H
3, R = -CH_3
6, R = -H
7, R = -CH_3
8, R = -H
9, R = -CH_3

i. NaH/DMF; ii. CH_3I; iii. 1 *M* NaOH/Δ_x.

Methylation of **4**, as for compounds **3** and **5**, gave 2-amino-4-chloro-5(*N*,*N*-dimethylaminomethyl)-7-methylpyrrolo[2,3-*d*]pyrimidine (**10**) (Scheme 3). Basic hydrolysis of **10** gave 2-amino-5-(*N*,*N*-dimethylaminomethyl)-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**11**). Selective methylation of the exocyclic aliphatic amine of **11** by methyl iodide in dimethyl sulfoxide [9] produced 2-amino-7-methyl-5-(*N*,*N*,*N*-trimethylammoniummethyl)-pyrrolo[2,3-*d*]pyrimidin-4-one iodide (**11a**), the methiodide salt of **11**.

Scheme 3
Synthesis of 2-Amino-5-(*N*,*N*-dimethylaminomethyl)-7-methylpyrrolo-[2,3-*d*]pyrimidin-4-one and its Methiodide Salt

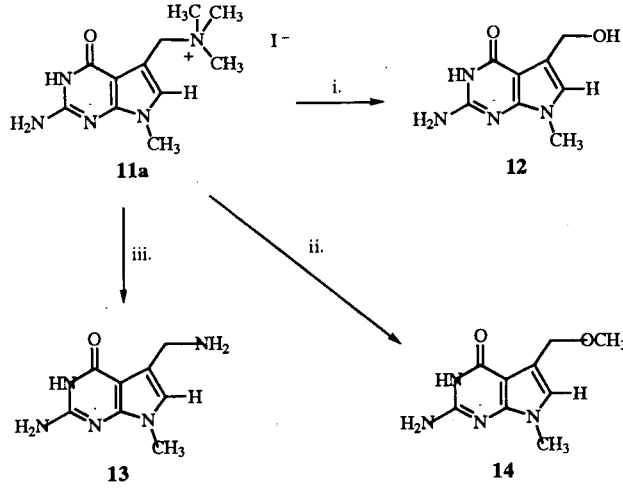


4
10
11a
11

i. NaH/DMF; ii. CH_3I; iii. aq. NaOH/Δ_x; iv. CH_3I/DMSO

Displacement of trimethylamine from **11a** as proposed by Benghiat and Crooks [9] by a variety of nucleophiles was found to proceed smoothly (Scheme 4). Thus, treatment of **11a** with aqueous sodium hydroxide gave 2-amino-5-hydroxymethyl-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**12**). Similarly, treatment of **11a** with sodium methoxide in methanol at reflux temperature gave 2-amino-5-methoxymethyl-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**14**) while treatment of **11a** with methanolic ammonia gave the N-7-methylated analog of preQ$_1$, 2-amino-5-aminomethyl-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**13**).

Scheme 4
Displacement of Trimethylamine from 2-Amino-7-methyl-5-(*N*,*N*,*N*-trimethylammoniummethyl)pyrrolo[2,3-*d*]pyrimidin-4-one by a Variety of Nucleophiles

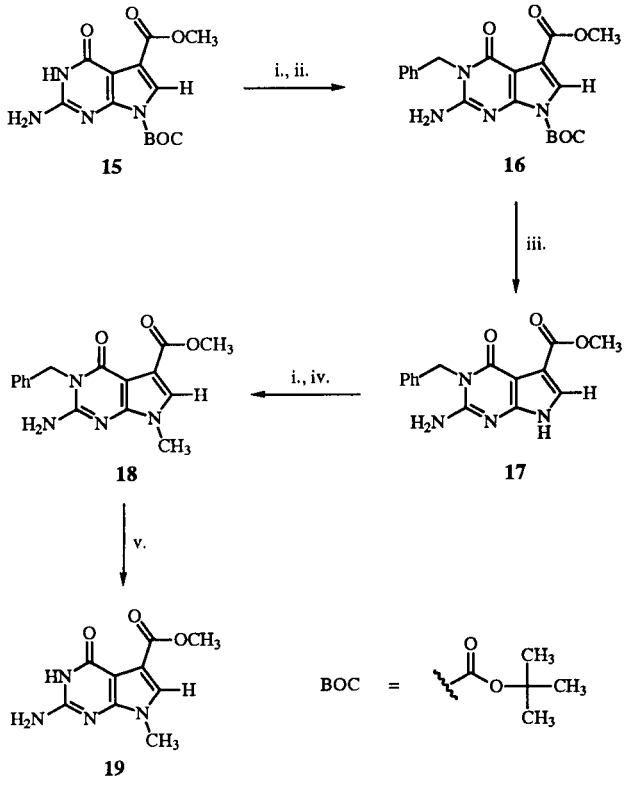


11a
12
13
14

i. aq NaOH/50°C/1 hour; ii. $NaOCH_3/CH_3OH/\Delta_x$/1 hour; iii. NH_3/CH_3OH/50°C/4 hours.

Several of the N-7-methylated analogs bearing an electron-withdrawing 5-substituent were produced from methyl 2-amino-7-(*N*-*t*-butoxycarbonyl)pyrrolo[2,3-*d*]-pyrimidin-4-one-5-carboxylate (**15**) [6]. We opted to try a direct alkylation of N-3 of the N-7-protected **15** by deprotonation with sodium hydride in dimethylformamide followed by treatment with benzyl bromide. The possibility that alkylation would occur at N-3 was supported by the fact that deprotonation followed by treatment with methyl iodide did not result in an alkylation of the exocyclic 2-amino group of compounds **3**, **4**, and **5**. Furthermore, alkylation at N-3, rather than N-1- or *O*-alkylation, has been achieved for several N-7-protected 2-methylthiopyrrolo[2,3-*d*]pyrimidin-4-ones [10]. Thus, **15** was benzylated by deprotonation at N-3 with sodium hydride/dimethylformamide followed by treatment with benzyl bromide at N-3 to give 5-methyl 2-amino-3-benzyl-7-(*N*-*t*-butoxycarbonyl)pyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**16**) (Scheme 5). The BOC protecting group was removed with methanolic ammonia under mild (50°) conditions to give methyl 2-amino-3-benzylpyrrolo[2,3-*d*]-pyrimidin-4-one-5-carboxylate (**17**). Methylation, as before, gave methyl 2-amino-3-benzyl-7-methylpyrrolo-[2,3-*d*]pyrimidin-4-one-5-carboxylate (**18**). Debenzylation in sodium/ammonia azide gave methyl 2-amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**19**).

Scheme 5
Synthesis of 5-Methyl 2-Aminopyrrolo[2,3-*d*]-pyrimidin-4-one-5-carboxylic Acid

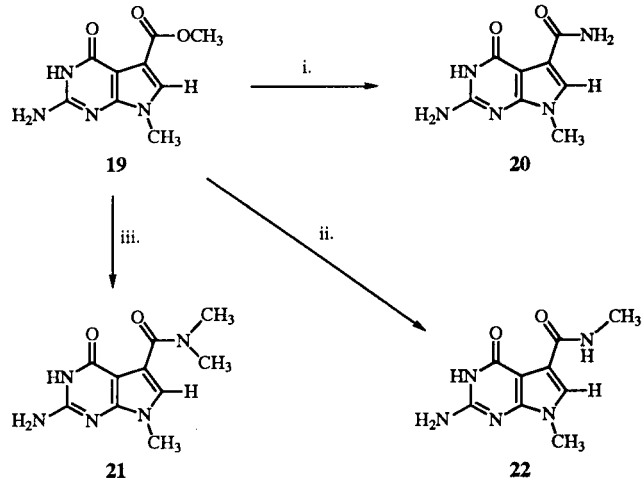


15 16 17 18 19

i. NaH/DMF; ii. $PhCH_2Br$; iii. $NH_3/CH_3OH/50°C/12$ hours; iv. CH_3I; v. Na/NH_3

The N-7-methylated ester **19** served as the starting material for the production of the corresponding N-7-methylated amides **20-22** (Scheme 6). Thus treatment of **19** with ethanolic ammonia, methanolic methylamine, and ethanolic dimethylamine at elevated temperatures gave

Scheme 6
Synthesis of *N*-Methylated 2-Amino-7-methylpyrrolo-[2,3-*d*]pyrimidin-4-one-5-carboxamides



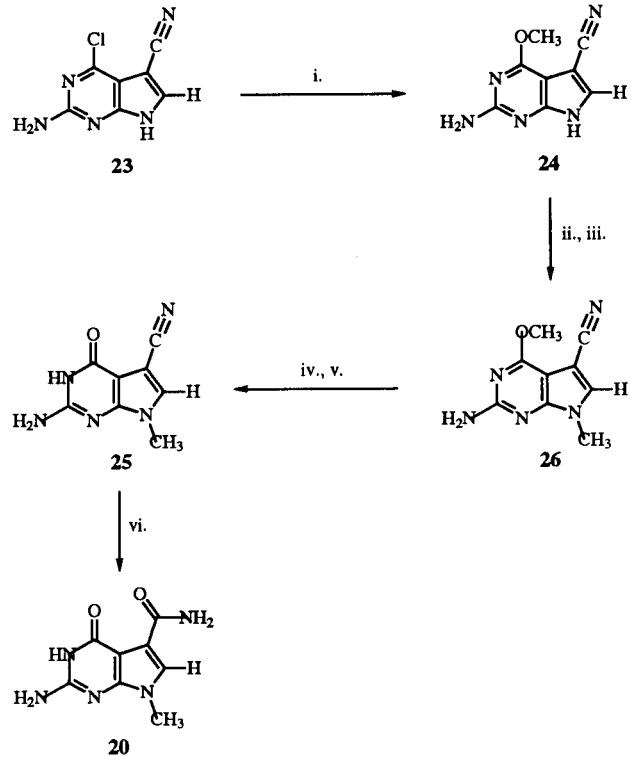
19 20 21 22

i. $NH_3/MeOH$; ii. $H_2NCH_3/EtOH$; iii. $HN(CH_3)_2/EtOH$.

2-amino-7-methylpyrrolo[2,3-d]pyrimidin-4-one-5-carboxamide (**20**), 2-amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-(*N*-methylcarboxamide) (**22**), and 2-amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-(*N,N*-dimethylcarboxamide) (**21**), respectively.

Synthesis of the N-7-methylated analog of $preQ_0$ (a putative intermediate in the *E, coli* quenine pathway and a known substrate [3]) is illustrated in Scheme 7. 2-Amino-4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine (**23**) [6] was treated with sodium methoxide in methanol at reflux temperature to give 2-amino-5-cyano-4-methoxypyrrolo[2,3-*d*]-pyrimidine (**24**). Methylation, as before, gave 2-amino-5-cyano-4-methoxy-7-methylpyrrolo[2,3-*d*]pyrimidine (**26**) in 77% yield. The 4-methoxy group of **26** was then converted back to the keto form without a reaction at the nitrile *via* treatment of **26** with trimethylsilyl iodide to afford 2-amino-5-cyano-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**25**). Compound **25** was found to serve as an alternate route to the carboxamide **20**, by treatment of **25** with hydrogen peroxide in concentrated aqueous ammonia [11].

Scheme 7
Synthesis of *N*-7-Methylated $preQ_0$



23 24 25 26 20

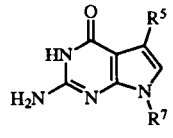
i. $NaOCH_3/CH_3OH/\Delta_x$; ii. NaH/DMF; iii. CH_3I; iv. TMS-I/CH_3CN/Δ_x; v. H_2O; vi. NH_4OH/H_2O_2.

The ^1H nmr signal for H-6 is a singlet in the spectrum of each of the N-7-methylated, 5-substituted 2-amino-pyrrolo[2,3-*d*]pyrimidin-4-ones **8**, **9**, **11-14**, **19-22**, and **25**. This implies that the site of methylation is N-7, as a

doublet for H-6 might be expected if N-7 was protonated. However, the signal for H-6 does not consistently appear as a doublet in the unmethylated or N-3-methylated analogs. The uv spectra (see Table 1) of these compounds are nearly identical to their corresponding unmethylated analogs. Methylation at N-1 or *O*-alkylation to give the corresponding 4-methoxy analogs would be expected [12] to produce a significant (>10 nm) bathochromic shift in the uv spectra of these analogs. In the case of the 2-aminopyrrolo[2,3-*d*]pyrimidin-4-ones, however, the difference between alkylation at N-3 versus N-7 cannot be determined on the basis of uv spectroscopy.

Table 1

Ultraviolet Spectral Data for Unmethylated and *N*-7-Methylated 2-Aminopyrrolo[2,3-*d*]pyrimidin-4-ones



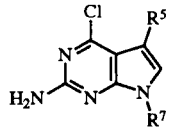
Compound #/[ref]	R^5	R^7	λ_{max} (nm) [a]
[22]	-H	-H	258
8	-H	$-CH_3$	259
1	$-CH_3$	-H	265
9	$-CH_3$	$-CH_3$	267
[3]	$-CH_2OCH_3$	-H	262
14	$-CH_2OCH_3$	$-CH_3$	264
[3]	$-CH_2OH$	-H	265
12	$-CH_2OH$	$-CH_3$	267
2	$-CH_2N(CH_3)_2$	-H	263
11	$-CH_2N(CH_3)_2$	$-CH_3$	263
[5]	$-CH_2NH_2$	-H	262
13	$-CH_2NH_2$	$-CH_3$	263
[6]	$-CONH_2$	-H	296
20	$-CONH_2$	$-CH_3$	296
22	$-CONHCH_3$	$-CH_3$	296
21	$-CON(CH_3)_2$	$-CH_3$	297
[6]	$-CO_2CH_3$	-H	293
19	$-CO_2CH_3$	$-CH_3$	293
[23]	-CN	-H	263, 289
25	-CN	$-CH_3$	263, 290

[a] The uv spectra for compounds listed in Table 1 were determined as 100 μ*M* solutions in ethanol.

The uv spectra of the methylated 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidines (Table 2) also closely resemble their unmethylated analogs. This result would only be expected [12] upon alkylation of N-7. Thus, the site of methylation was established as N-7 in compounds **6**, **7**, and **10** (direct precursors to compounds **8**, **9**, and **11**) and therefore also for compounds **8**, **9**, and **11**. Compounds **12**, **13**, **14**, which are derived from **11**, are also methylated at N-7. Rigorous proof of N-7 as the site of methylation (as opposed to N-3) for compounds **19-22** and **25** was still lacking. The chemical shifts determined by broad-band decoupled ^{13}C nmr for selected 2-amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-ones are given in Table 3.

Table 2

Ultraviolet Spectral Data for 2-Amino-4-chloropyrrolo[2,3-*d*]pyrimidines



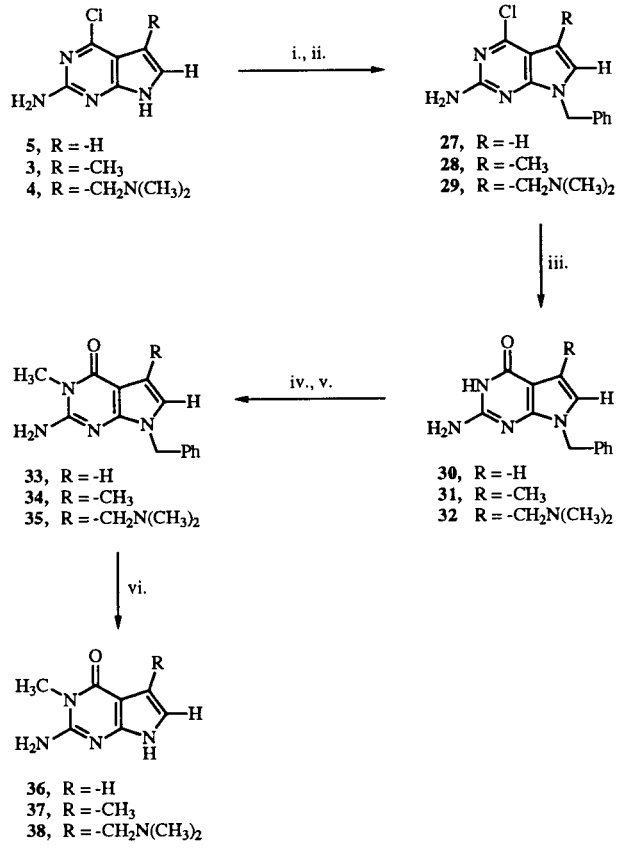
Compound #	R^5	R^7	λ_{max} (nm) [a]
5	-H	-H	260, 318
6	-H	$-CH_3$	262, 318
3	$-CH_3$	-H	265, 318
7	$-CH_3$	$-CH_3$	266, 318
4	$-CH_2N(CH_3)_2$	-H	265, 318
10	$-CH_2N(CH_3)_2$	$-CH_3$	265, 318

[a] The uv spectra for compounds listed in Table 2 were determined as 100 μ*M* solutions in ethanol.

Further proof of N-7 as the site of methylation in this series of compounds was obtained by observing 3-bond heteronuclear coupling in a ^{13}C nmr experiment employing a gated pulse [13]. The site of methylation was established as N-7 by the 3-bond ^{13}C-^{1}H couplings given in Table 4. Thus 3-bond couplings of C-6 and C-7a to the

Scheme 8

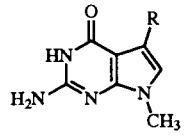
Synthesis of *N*-3-Methylated Analogs



i. NaH/DMF; ii. $PhCH_2Br$; iii. 1 *M* NaOH/Δ_x; iv. NaH/DMF; v. CH_3I; vi. Na/NH_3

Table 3

^{13}C-nmr Data for *N*-7-Methylated 2-Aminopyrrolo[2,3-*d*]pyrimidin-4-ones [a]



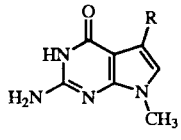
Compound #	R	C-2	C-4	C-4a	C-5	C-6	C-7a	N-CH_3	Other C's
8	-H	151.9	158.5	99.8	101.3	118.1	151.3	30.5	
9	$-CH_3$	152.0	159.4	99.5	112.8	115.0	151.2	30.7	15.1
11	$-CH_2N(CH_3)_2$	151.8	158.0	102.1	115.2	120.1	151.3	30.5	44.1 53.8
19	$-CO_2CH_3$	153.2	157.4	97.5	109.9	126.7	153.5	30.9	50.7 163.5
25	-CN	153.8	157.6	98.8	85.7	135.6	152.1	30.9	127.8

[a] All resonances in ppm downfield from internal TMS in DMSO-d_6.

exocyclic methyl protons were observed, as expected for N-7 methylation. No long range coupling of C-2 or C-4 to the methyl protons was observed. The site of methylation as N-7 was assigned for compounds **20-22** based upon their common synthetic precursor **19**.

Table 4

^{13}C-nmr Three Bond Coupling Data Table



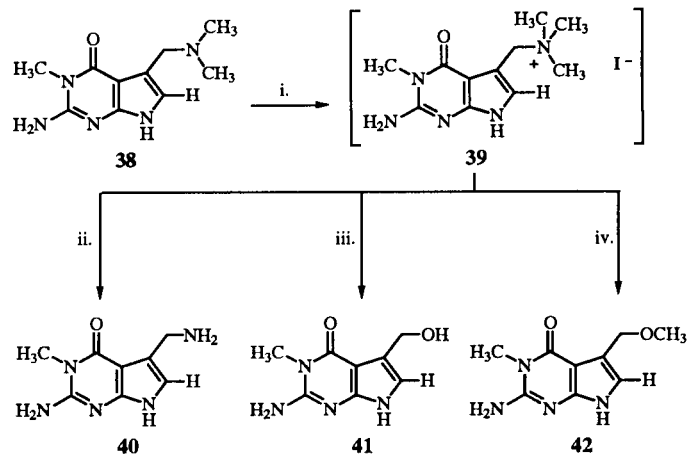
Compound #	R	C-7a $^3J_{CH_3}$	C-6 $^3J_{CH_3}$
8	-H	7.1	7.6
9	$-CH_3$	7.1	7.7
11	$-CH_2N(CH_3)_2$	7.2	7.6
19	$-CO_2CH_3$	7.1	7.9
25	-CN	7.3	7.0

The 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidines **5**, **3**, and **4** were benzylated with benzyl bromide under conditions similar to those employed for methylation to afford 2-amino-7-benzyl-4-chloropyrrolo[2,3-*d*]pyrimidine (**27**), 2-amino-7-benzyl-4-chloro-5-methylpyrrolo[2,3-*d*]pyrimidine (**28**), and 2-amino-7-benzyl-4-chloro-5-(*N*,*N*-dimethylaminomethyl)pyrrolo[2,3-*d*]pyrimidine (**29**), respectively (Scheme 8). The 4-chloro groups of **27**, **28**, and **29** were then removed by basic hydrolysis in sodium hydroxide, as before, to afford 2-amino-7-benzylpyrrolo[2,3-*d*]pyrimidin-4-one (**30**) [14], 2-amino-7-benzyl-5-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**31**), and 2-amino-7-benzyl-5-(*N*,*N*-dimethylaminomethyl)pyrrolo-[2,3-*d*]pyrimidin-4-one (**32**), respectively. Methylation as before afforded 2-amino-7-benzyl-3-methylpyrrolo-[2,3-*d*]pyrimidin-4-one (**33**), 2-amino-7-benzyl-3,5-dimethylpyrrolo[2,3-*d*]pyrimidin-4-one (**34**), and 2-amino-7-benzyl-5-(*N*,*N*-dimethylaminomethyl)-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**35**). The N-7 benzyl protecting groups of **33-35** were then removed under Birch reduction conditions to afford 2-amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**36**), 2-amino-3,5-dimethylpyrrolo[2,3-*d*]pyrimidin-4-one (**37**), and 2-amino-5-(*N*,*N*-dimethylaminomethyl)-3-methylpyrrolo-[2,3-*d*]pyrimidin-4-one (**38**), respectively.

Analogous to **11**, the N-3-methylated heterocycle, **38** served as the starting material for a variety of compounds (Scheme 9). Thus, **38** was methylated, as before, to generate the methiodide salt **39** *in situ*, which was added to methanol (saturated with ammonia at 0°) to afford 2-amino-5-(aminomethyl)-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**40**). Addition of the methiodide **39** to aqueous sodium hydroxide afforded 2-amino-5-(hydroxymethyl)-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**41**). Addition of the methiodide **39** to sodium methoxide in methanol afforded 2-amino-5-(methoxymethyl)-3-methylpyrrolo-[2,3-*d*]pyrimidin-4-one (**42**).

Scheme 9

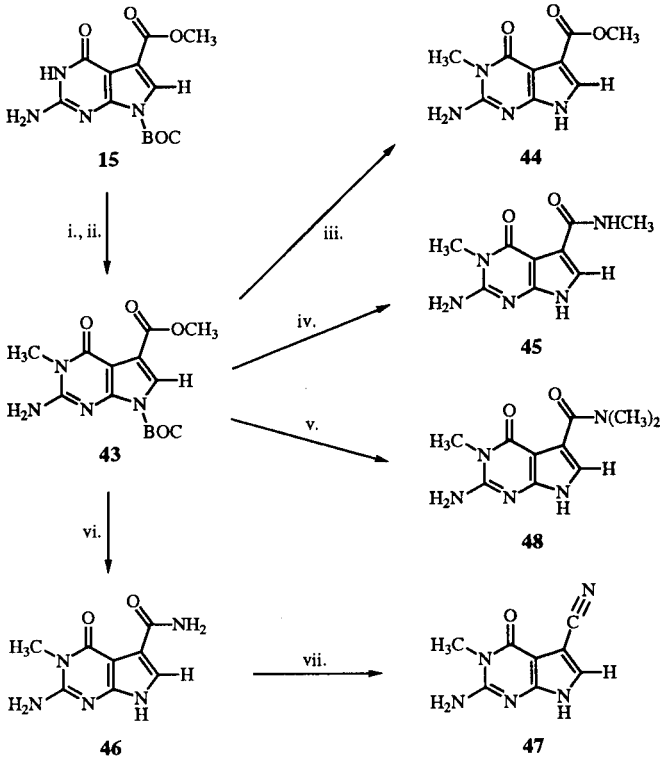
Synthesis of *N*-3-Methylated Analogs (continued)



i. CH_3/DMSO; ii. NH_3/CH_3OH/50°C/4 hours; iii. aq. NaOH/50°C/1 hour; iv. $NaOCH_3/CH_3OH$/room T/2 hours.

Methylation of **15** afforded methyl 2-amino-7-(*N*-*t*-butoxycarbonyl)-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**43**), which served as a common starting material for several 2-amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-ones bearing an electron-withdrawing 5-substituent (Scheme 10). Thus, treatment of **43** with methanolic ammonia under mild conditions (50°) afforded methyl 2-amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**44**). Treatment of **43** with ethanolic methylamine, and ethanolic dimethylamine at 100° afforded 2-amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-(*N*-methyl)carboxamide (**45**) and 2-amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-(*N*,*N*-dimethyl)carboxamide (**48**), respectively. Treatment of **43** with methanolic ammonia under more stringent conditions (120°) afforded 2-amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxamide (**46**). Lastly, 2-amino-5-cyano-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**47**) was produced by dehydration of the exocyclic primary amide of **46** with phosphorus oxychloride under reflux.

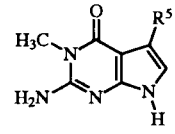
Scheme 10
Synthesis of *N*-3-Methylated 2-Aminopyrrolo[2,3-*d*]pyrimidin-4-ones Bearing an e^--Withdrawing 5-Substituent



i. NaH/DMF; ii. CH_3I; iii. $NH_3/CH_3OH/50°C$; iv. $H_2NCH_3/EtOH/100°C$; v. $HN(CH_3)_2/EtOH/100°C$; vi. $NH_3/CH_3OH/120°C$; vii. $POCl_3/\Delta_x$.

N-3 was supported as the site of methylation for compounds **36-38**, **40-42**, **44-48** on the basis of their uv spectra in methanol (Table 5). These data are nearly identical to the spectral data for the corresponding unmethylated analogs (Table 1). Alkylation at N-1 or *O*-alkylation would be expected to produce large (>10 nm) bathochromic shifts of λ_{max} [12]. As mentioned previously, such spectral studies do not differentiate between alkylation at N-7 versus N-3 in 2-aminopyrrolo[2,3-*d*]pyrimidin-4-ones.

Table 5
Ultraviolet Spectral Data for *N*-3-Methylated 2-Aminopyrrolo-[2,3-*d*]pyrimidin-4-ones



Compound #	R^5	λ_{max} (nm) [a]
36	-H	260
37	$-CH_3$	264
42	$-CH_2OCH_3$	267
41	$-CH_2OH$	267
38	$-CH_2N(CH_3)_2$	264
40	$-CH_2NH_2$	265
46	$-CONH_2$	299
45	$-CONHCH_3$	299
48	$-CON(CH_3)_2$	298
44	$-CO_2CH_3$	295
47	-CN	266, 295

[a] The uv spectra for compounds listed in Table 5 were determined as 100 μ*M* solutions in ethanol.

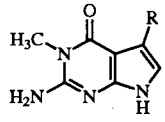
Methylation at the exocyclic 2-NH_2 group was ruled out due to the integration (2H) of the deuterium oxide-exchangeable protons in the ^1H nmr spectra of these compounds in dimethyl sulfoxide.

The broad-band decoupled ^{13}C nmr spectra for the N-3-methylated compounds **36**, **37**, **38**, **44**, and **47** are given in Table 6. The site of methylation for these compounds was established as N-3 rather than N-7 on the basis of 3-bond heteronuclear coupling between the protons on the exocyclic N-3 methyl group and both C-2 and C-4. No long-range coupling of C-7a or C-6 to the methyl protons was observed. As seen in Table 7, long-range heteronuclear coupling of C-2 and C-4 to the methyl protons on the N-3-methyl group in these compounds was observed. Thus, the combination of the uv, ^1H nmr, and ^{13}C nmr spectra for compounds **36**, **37**, **38**, **44**, and **47** unambiguously established N-3 as the site of methylation of the heterocycle. The site for methylation was established as N-3 for compounds **40-42** based upon the structure of their synthetic precursor, compound **39**. Likewise, N-3 methylation for compounds **44-48** was assigned based upon the assignments of **44** and **47**, since all of these compounds are derived from a common N-3-methylated synthetic precursor (**44**).

The N-3, N-7-dimethylated analog of $preQ_0$, 2-amino-5-cyano-3,7-dimethylpyrrolo[2,3-*d*]pyrimidin-4-one (**49**), was produced by methylation of the corresponding N-3-methylated analog **47** (Scheme 11).

Table 6

^{13}C-nmr Data for Pyrrolo[2,3-*d*]pyrimidines [a]

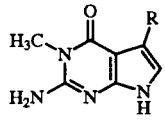


Compound #	R	C-2	C-4	C-4a	C-5	C-6	C-7a	N-CH_3	Other C's
36	-H	151.9	158.3	99.9	101.3	116.2	150.8	35.2	
37	-CH_3	153.1	161.2	100.1	112.5	113.5	150.9	35.1	12.2
38	-$CH_2N(CH_3)_2$	153.0	159.5	102.5	115.0	118.8	150.9	35.1	43.9 53.4
44	-CO_2CH_3	153.1	158.2	97.5	109.8	125.0	152.9	35.4	50.7 163.4
47	-CN	154.1	157.9	98.8	85.7	130.6	152.1	30.9	127.4

[a] All resonances in ppm downfield from internal TMS in DMSO-d_6.

Table 7

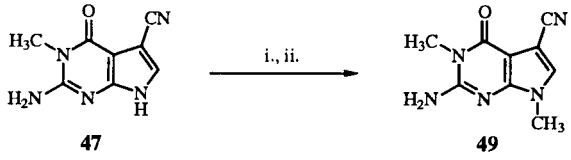
^{13}C-nmr Three Bond Coupling Data Table



Compound #	R	$^3J_{CH_3}$ for C-2	$^3J_{CH_3}$ for C-4
36	-H	6.1	5.2
37	-CH_3	6.2	5.6
38	-$CH_2N(CH_3)_2$	6.4	5.4
44	-CO_2CH_3	6.2	5.7
47	-CN	6.1	5.3

Scheme 11

Synthesis of a 3,7-Dimethylated Analog



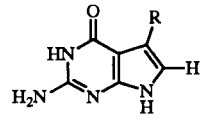
47 **49**

i. NaH/DMF; ii. MeI.

The ionization constants determined by potentiometric titration for a series of substrate analogs are given in Table 8. In the case of the acidic ionization constants determined, the site of deprotonation on the pyrrolo-[2,3-*d*]pyrimidine ring system is somewhat ambiguous. From the precedent set by guanine, the most acidic proton in these substrates should be that on N-3. The free base guanine exhibits two acidic ionizations in the range of potentiometric titration of pK_a 9.32 and 12.6 [15]. These ionizations were assigned as deprotonation of N-1 followed by deprotonation of an imidazole ring nitrogen (most likely N-7) based upon the synthesis of *N*-methylated analogs of guanine. It should be noted that the acidic pK_a of isocytosine was found to be 9.42 [16] and 9.59 [17] in two separate determinations, in good agreement with the value of 9.32 assigned to N-1 of guanine. Thus, deprotonation of N-1 of guanine (corresponding to N-3 of the pyrrolo[2,3-*d*]pyrimidine ring system) appears to involve very little influence from the fused imidazole ring. A similar "compartmentalization" of the individual rings of fused ring systems has been noted [18] in the pyrrolo[2,3-*d*]pyrimidine ring system.

Table 8

Ionization Constants for Unmethylated Analogs



Compound #/[ref]	R	Basic pK_a	Acidic pK_a
[22]	-H	2.8 (0.3)	11.8 (0.3)
1	-CH_3	2.8 (0.3)	11.9 (0.1)
[3]	-CH_2OH	3.1 (0.2)	12.0 (0.3)
[5]	-CH_2NH_2	1.8 (0.8), 9.8 (0.3)	12.1 (0.5)
[6]	$CONH_2$	<1	10.5 (0.1)
[23]	-CN	<1	10.7 (0.1)
[15]	Guanine	3.0	9.3, 12.6

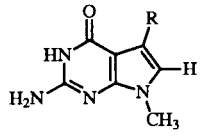
Ionization constants were determined by potentiometric titration at 25° as described in the Experimental. The ionization constants shown for guanine were previously determined and were confirmed in our laboratory.

The 2-aminopyrrolo[2,3-*d*]pyrimidin-4-ones are 7-deaza derivatives of guanine. By analogy with guanine one would expect that the most acidic proton in these compounds would be the proton residing on N-3 (which corresponds to N-1 of guanine). The second most acidic proton on these compounds would then be the proton on N-7 in the pyrrole ring. In the case of 2-aminopyrrolo-[2,3-*d*]pyrimidin-4-one (*i.e.*, 7-deazaguanine) itself, this second deprotonation would be expected to occur with a pK_a higher than the corresponding deprotonation in the imidazole ring of guanine according to the acidic pK_a's determined [19] for pyrrole (pK_a 17.51) vs. imidazole (pK_a 14.17).

The acidic ionization constants (as determined by potentiometric titration with potassium hydroxide) for the series of N-7-methylated 2-aminopyrrolo[2,3-*d*]pyrimidin-4-ones with various substituents (R) on the pyrrole ring are given in Table 9. Comparison of these pK_a values with those obtained for the unmethylated analogs in Table 8 indicates that the proton at N-3 is indeed the most acidic in the unmethylated compounds. It should also be noted that the nature of the pyrrole ring substituent (R) (electron-donating *vs.* electron-withdrawing) appears to have only a very slight influence upon deprotonation of the pyrimidine ring.

Table 9

Acidic Ionization Constants for *N*-7-Methylated Analogs



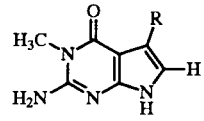
Compound #	R	Acidic pK_a
8	-H	11.8 (0.3)
9	$-CH_3$	11.8 (0.3)
14	$-CH_2OCH_3$	11.6 (0.3)
12	$-CH_2OH$	11.9 (0.4)
11	$-CH_2N(CH_3)_2$	12.4 (0.4)
13	$-CH_2NH_2$	12.1 (0.4)
21	$-CON(CH_3)_2$	11.7 (0.3)
19	$-CO_2CH_3$	11.4 (0.3)
25	-CN	11.0 (0.2)

Ionization constants were determined by potentiometric titration at 25° as described in the Experimental.

The acidic ionization constants for the series of N-3-methylated 2-aminopyrrolo[2,3-*d*]pyrimidin-4-ones with various substituents on the pyrrole ring (R) are given in Table 10. The pK_a values for **44-48** (where R = an electron-withdrawing group) were determined by potentiometric titration. Comparison of the pK_a of the exocyclic tertiary amide **48** with those for the corresponding secondary (**45**) and primary (**46**) amides indicates that these latter values are associated with deprotonation of N-7 rather than deprotonation of the exocyclic amide. The acidic ionization constants for compounds **36**, **37**, **42**, **41**, **38**, and **40**, bearing electron-donating substituents on the pyrrole ring, lie outside the range of determination by potentiometric titration. The acidic ionization constants for compounds **36**, **37**, and **40** were determined by uv spectroscopy in concentrated (1-14 molar) aqueous potassium hydroxide using the *H*-scale determined by Yagil [20]. The ionization constants listed in Table 10 reflect the isolated deprotonation of N-7, as may be the case in the active site of TGT, where close and specific interactions between enzymic residues and the pyrimidine ring of these substrate analogs may also result in selective deprotonation at N-7. It should be noted that the acidic ionization constants for **44-48** lie within 1 pK unit of those determined for the corresponding N-3-methylated and unmethylated analogs.

Table 10

Acidic Ionization Constants for *N*-3-Methylated Analogs



Compound #	R	Acidic pK_a
36	-H	14.5 (0.8) [a]
37	$-CH_3$	14.6 (0.9) [a]
42	$-CH_2OCH_3$	>13 [b]
41	$-CH_2OH$	>13 [b]
38	$-CH_2N(CH_3)_2$	>13 [b]
40	$-CH_2NH_2$	15.2 (1.1) [a]
46	$-CONH_2$	12.1 (0.2) [c]
45	$-CONHCH_3$	12.2 (0.2) [c]
48	$-CON(CH_3)_2$	12.1 (0.3) [c]
44	$-CO_2CH_3$	11.5 (0.6) [c]
47	-CN	12.2 (0.4) [c]

[a] Ionization constants determined by a uv spectrophotometry method as described in the Experimental. [b] Ionization constants were estimated to be >13 due to a lack of inflection of the potentiometric titration. These compounds were not investigated by the uv spectrophotometry method. [c] Ionization constants were determined by potentiometric titration at 25° as described in the Experimental.

In order to discern whether or not deprotonation of the 2-amino group was contributing to the observed ionization constants, the 3,7-dimethylated analog **49** of $preQ_0$ was synthesized. Compound **49** exhibited no ionization within the range of potentiometric titration (up to *p*H 13). Furthermore the uv spectrum of **49** showed little to no change in increasing concentrations of potassium hydroxide (up to 5 molar). The increased acidity of ring nitrogens N-3 and N-7 over the 2-amino group was thus firmly established.

EXPERIMENTAL

Definition of Acidic Ionization Constants.

All ionization constants reported are in the form of acidic ionization constants as suggested by Albert and Serjeant in their laboratory manual [21]. The acidic ionization constant for an acid A is defined as the equilibrium constant (K_a in Equation 2) for the ionization process described in Equation 1. These values are reported as acidic pK_a (the negative logarithm of the ionization constant K_a) in Tables 8-11.

$$HA \rightleftharpoons H^+ + A^- \qquad \text{Equation 1}$$

$$K_a = \frac{[H^+][A^-]}{[HA]} \qquad \text{Equation 2}$$

The acidic ionization constant for a base B is defined as the equilibrium constant (K_a in Equation 4) for the ionization process described in Equation 3. These values are reported as basic pK_a in Table 8.

$$BH^+ \rightleftharpoons H^+ + B \qquad \text{Equation 3}$$

$$K_a = \frac{[H^+][B]}{[HB^+]} \qquad \text{Equation 4}$$

Determination of Ionization Constants by Potentiometric Titration.

Ionization constants were determined in aqueous solutions (2 mmolar) at 25° by potentiometric titration employing an Orion *p*H electrode with an Orion *p*H/millivolt meter. The *p*H electrode was calibrated with commercial (Orion) *p*H standards of *p*H 4.0 and 7.0 for determinations of *p*H <7 and *p*H standards of *p*H 7.0 and 10.0 for determinations of *p*H >7. Due to poor solubility in water, many of the methylated and unmethylated 5-substituted 2-aminopyrrolo[2,3-*d*]pyrimidin-4-ones were dissolved in dimethyl sulfide and then diluted into water to give 2 mmolar aqueous solutions. Acidic ionization constants were determined by titration with aqueous potassium hydroxide (0.10 molar or 1.0 molar) or aqueous hydrochloric acid (0.10 molar or 1.0 molar).

Carbonate-free aqueous potassium hydroxide solutions were prepared as previously described [21]. Thus, aqueous potassium hydroxide solutions were prepared and barium hydroxide was added. The precipitated barium carbonate was removed by filtration and barium removed by passing through a cation exchange column (Amberlite IR, K^+ form). The carbonate-free potassium hydroxide solution was then standardized against potassium hydrogen phthalate (predried for 1 hour at 120°) using phenolphthalein as an indicator and diluting with water to give a solution of the exact desired concentration. These solutions were stored at room temperature in sealed containers under argon.

Aqueous hydrochloric acid solutions were prepared from concentrated hydrochloric acid and standardized against the potassium hydroxide solutions described above. The solutions were diluted with water to give a solution of the exact desired concentration.

Values of pK_a were calculated by the tabular method of Albert and Serjeant [21] with corrections for hydroxide or hydrogen ion concentration at extreme *p*H (<4 or >10).

Determination of Ionization Constants by Spectrophotometry.

The acidic ionization constants for some N-3-methylated compounds were not determinable by potentiometric titration (pK_a > 13). The acidic ionization constants for compounds **36**, **37**, and **40** (listed in Table 10) were determined by the uv spectrophotometric method [19,21] in highly concentrated (up to 15 molar) aqueous potassium hydroxide solutions employing the *H* scale determined [20] for aqueous potassium hydroxide solutions at 25°. Background-subtracted uv spectra were obtained in semimicro quartz cells (Fisher Scientific) on a RESPONSE™ UV-VIS spectrometer from Gilford at 25°.

General Synthetic Procedures.

Melting points were determined on a MEL-TEMP™ apparatus from Laboratory Devices. Background-subtracted uv spectra were obtained in semimicro quartz cells (Fisher Scientific) on a RESPONSE™ uv-vis spectrometer from Gilford. Infrared spectra were obtained from potassium bromide pellets on a 5DXB FT-IR spectrometer from Nicolet. Nuclear magnetic resonance spectra were obtained on a AM 360 MHz spectrometer from Bruker. Methyl iodide was from Lancaster.

All other chemicals were purchased from Aldrich unless otherwise specified. Removal of the solvent *in vacuo* was performed on a rotatory evaporator at 30° and 15 mm Hg (aspirator) or 1 mm Hg (pump) unless otherwise specified. Drying of solids *in vacuo* were performed at 50° and 10 mm Hg (aspirator) to constant mass. Reactions performed in a dry atmosphere were accomplished in flasks equipped with a drying tube filled with Drierite™ (W. A. Hammond). Thin-layer chromatography (tlc) was performed on UNIPLATE™ glass plates from Analtech. Solvent systems used for the development of tlc plates were solvent system A = chloroform/methanol, (9:1) (v:v), solvent system B = ethyl acetate/ethanol/acetone/water, (20:2:2:1) (v:v:v:v), and solvent system C = isopropyl alcohol/water/concentrated aqueous ammonium hydroxide, (86:14:5) (v:v:v). Compounds on developed tlc plates were detected with a Mineralight™ ultraviolet lamp (254 nm) from uv products. Samples were prepared for column chromatography by stirring a suspension of packing material and crude product in methanol at 25° for 1 hour followed by a removal of solvent *in vacuo*. Preparative chromatographic separations were performed in glass columns (5 cm x 20 cm) dry-packed with Silica Gel 60 (40-63 μm) from Malinckrodt under forced air flow (10 ml/minute). Fractions (15 ml) were collected with a CYGNET™ fraction collector from ISCO. The fractions containing product were identified by tlc, pooled, and the solvent removed *in vacuo* unless otherwise specified.

2-Amino-4-chloro-5-methylpyrrolo[2,3-*d*]pyrimidine (**3**).

A mixture of 2-amino-5-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**1**) [4] (10.0 g, 60.9 mmoles), *N,N*-diethylaniline (9.7 ml, 61 mmoles), and phosphorus oxychloride (200 ml) was heated at reflux with stirring in an anhydrous atmosphere for 4 hours. The dark orange solution was allowed to cool to room temperature and concentrated *in vacuo*. Water (20 ml) was then added to the residue at 0° with vigorous stirring to give an exothermic reaction. Concentrated aqueous ammonium hydroxide was added to *p*H 5 to give a precipitate, which was collected by filtration, washed with water (3 x 5 ml), and dried *in vacuo*. The crude product was purified by silica gel column chromatography in solvent system B. Recrystallization from methanol afforded 5.68 g (31.1 mmoles, 51%) of **3**, mp 225-228°; R_f (in solvent system A) 0.47, (in solvent system B) 0.65; ^{1}H nmr (DMSO-d_6): δ 2.14 (s, 3H, -CH_3), 6.5 (br s, 2H, 2-NH_2), 7.15 (s, 1H, H-6), 12.0 (br s, 1H, 7-NH).

Anal. Calcd. for $C_7H_7N_4Cl$: C, 46.03; H, 3.87; N, 30.68. Found: C, 46.00; H, 3.92; N, 30.65.

2-Amino-4-chloro-5-(*N,N*-dimethylaminomethyl)pyrrolo[2,3-*d*]-pyrimidine (**4**).

Compound **4** was prepared from **2** [5] (10.0 g, 48.2 mmoles) by the method described for **3** to yield 5.38 g (23.8 mmoles, 49%) of **4**, mp 245-250°; R_f (in solvent system A) 0.17, (in solvent system B) 0.42; ^{1}H nmr (DMSO-d_6): δ 3.15 (s, 6H, -$CH_2N(CH_3)_2$), 4.42 (s, 3H, -$CH_2N(CH_3)_2$), 7.25 (br s, 2H, 2-NH_2), 12.2 (br s, 1H, 7-NH).

Anal. Calcd. for $C_9H_{12}N_5Cl$: C, 47.89; H, 5.37; N, 31.04. Found: C, 47.91; H, 5.44; N, 31.00.

2-Amino-4-chloro-7-methylpyrrolo[2,3-*d*]pyrimidine (**6**).

2-Amino-4-chloropyrrolo[2,3-*d*]pyrimidine (**5**) [7] (1.00 g, 5.93 mmoles) and sodium hydride (80% dispersion in oil, 178 mg, 5.93 mmoles) were added to a 100 ml flask equipped with a magnetic stirrer. The flask was fitted with a rubber septum and purged with argon. Anhydrous dimethylformamide (50 ml) was added *via* syringe followed by methyl iodide (Lancaster, 370 µl, 5.93 mmoles). The mixture was stirred at 25° for 24 hours to give a clear yellow solution. The solution was concentrated to 10 ml *in vacuo* and 30 ml of water was added to give a dull yellow precipitate, which was collected by filtration and dried *in vacuo*. Recrystallization from methanol/water afforded pale yellow needles (488 mg, 2.67 mmoles, 45%) mp 174-177°; R_f (in solvent system A) 0.68, (in solvent system B) 0.75; ^{1}H-nmr (DMSO-d_6): δ 3.60 (s, 3H, N-CH_3), 6.26 (d, 3.6 Hz, 1H, H-5), 6.67 (br s, 2H, 2-NH_2), 7.11 (d, 3.6 Hz, 1H, H-6).

Anal. Calcd. for $C_7H_7N_4Cl$: C, 46.03; H, 3.87; N, 30.68. Found: C, 46.02; H, 3.91; N, 30.67.

2-Amino-4-chloro-5,7-dimethylpyrrolo[2,3-*d*]pyrimidine (**7**).

Compound **7** was prepared from **3** (183 mg, 1.00 mmole) by the method described for **6** to yield 162 mg (0.822 mmole, 82%) of **7**, mp 182-185°; R_f (in solvent system A) 0.76, (in solvent system B) 0.88; ^{1}H nmr (DMSO-d_6): δ 2.11 (s, 3H, 5-CH_3), 3.21 (s, 3H, N-CH_3), 6.8 (br s, 2H, 2-NH_2), 7.23 (s, 1H, H-6).

Anal. Calcd. for $C_8H_9N_4Cl$: C, 48.86; H, 4.62; N, 28.49. Found: C, 48.88; H, 4.71; N, 28.50.

2-Amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**8**).

2-Amino-4-chloro-7-methylpyrrolo[2,3-*d*]pyrimidine (**6**, 183 mg, 1.0 mmole) in aqueous sodium hydroxide (2 *M*, 25 ml) was heated at reflux for 6 hours. The solution was cooled to room temperature and neutralized with glacial acetic acid. The resulting precipitate was collected by filtration and dried *in vacuo*. The crude product was purified by silica gel column chromatography, eluting with solvent system B. Recrystallization from methanol afforded 125 mg (0.76 mmole, 76%) of **8**, mp 262-264°; R_f (in solvent system A) 0.31, (in solvent system B) 0.55; ^{1}H nmr (DMSO-d_6): δ 3.44 (s, 3H, N-CH_3), 5.9 (br s, 2H, 2-NH_2), 6.21 (d, 3.5 Hz, 1H, H-5), 6.87 (d, 3.5 Hz, 1H, H-6), 10.8 (br s, 1H, 3-NH)

Anal. Calcd. for $C_7H_8N_4O$: C, 51.20; H, 4.92; N, 34.13. Found: C, 51.19; H, 4.95; N, 34.11.

2-Amino-5,7-dimethylpyrrolo[2,3-*d*]pyrimidin-4-one (**9**).

Compound **9** was prepared from **7** (197 mg, 1.00 mmole) by the method described for **8** to yield 128 mg (0.721 mmole, 72%) of **9**, mp 242-246°; R_f (in solvent system A) 0.37, (in solvent system B) 0.62; ^{1}H nmr (DMSO-d_6): δ 2.11 (s, 3H, 5-CH_3), 3.54 (s, 3H, N-CH_3), 5.8 (br s, 2H, 2-NH_2), 7.08 (s, 1H, H-6), 10.9 (br s, 1H, 3-NH).

Anal. Calcd. for $C_8H_{10}N_4O$: C, 53.91; H, 5.67; N, 31.44. Found: C, 53.92; H, 5.65; N, 31.47.

2-Amino-4-chloro-5-(*N,N*-dimethylaminomethyl)-7-methylpyrrolo[2,3-*d*]pyrimidine (**10**).

Compound **10** was prepared from **4** (226 mg, 1.00 mmole) by the method described for **6** to yield 204 mg (0.85 mmole, 85%) of **10**, mp 192-195°; R_f (in solvent system A) 0.51, (in solvent system B) 0.74; ^{1}H nmr (DMSO-d_6): δ 2.30 (s, 6H, -N(CH_3)$_2$), 3.62 (s, 3H, N-CH_3), 3.81 (s, 2H, 5-CH_2N), 6.4 (br s, 2H, 2-NH_2), 7.15 (s, 1H, H-6).

Anal. Calcd. for $C_{10}H_{14}N_5Cl$: C, 50.10; H, 5.90; N, 29.22. Found: C, 50.08; H, 5.88; N, 29.21.

2-Amino-5-(*N,N*-dimethylaminomethyl)-7-methylpyrrolo-[2,3-*d*]pyrimidin-4-one (**11**).

Compound **11** was prepared from **10** (240 mg, 1.00 mmole) by the method described for **8** to yield 139 mg (0.63 mmole, 63%) of **11**, mp 285° dec; R_f (in solvent system A) 0.12, (in solvent system B) 0.22; ^{1}H nmr (DMSO-d_6): δ 2.35 (s, 6H, -N(CH_3)$_2$), 3.64 (s, 3H, 7N-CH_3), 3.73 (s, 2H, 5-CH_2N), 5.85 (br s, 2H, 2-NH_2), 7.13 (s, 1H, H-6), 10.9 (br s, 1H, 3-NH).

Anal. Calcd. for $C_{10}H_{15}N_5O$: C, 54.27; H, 6.85; N, 31.65. Found: C, 54.31; H, 6.79; N, 31.68.

2-Amino-7-methyl-5-(*N,N,N*-trimethylammoniummethyl)-pyrrolo[2,3-*d*]pyrimidin-4-one Iodide (**11a**).

2-Amino-5-(*N,N*-dimethylaminomethyl)-7-methylpyrrolo-[2,3-*d*]pyrimidin-4-one (**11**) (500 mg, 2.26 mmoles) was dissolved in dimethyl sulfide (10 ml) and the solution stirred under argon. Methyl iodide (2.26 mmoles, 1.0 eq) was added and the mixture stirred at 25° for 0.5 hours under argon. Dilution with water (30 ml) was followed by extraction with chloroform (3 x 10 ml) and the aqueous solution was lyophilized to give **11a** (780 mg, 2.17 mmoles, 96%) as a white powder; mp 202-204°; R_f (in solvent system A) 0.05, (in solvent system B) 0.18; ^{1}H nmr (DMSO-d_6): δ 3.26 (s, 9H, -N(CH_3)$_3$), 3.57 (s, 3H, N-CH_3), 4.55 (s, 2H, 5-CH_2N), 5.8 (br s, 2H, 2-NH_2), 7.31 (s, 1H, H-6), 11.2 (br s, 1H, 3-NH).

Anal. Calcd. for $C_{11}H_{16}N_5OI$: C, 36.57; H, 4.47; N, 19.39. Found: C, 36.58; H, 4.45; N, 19.38.

2-Amino-5-hydroxymethyl-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**12**).

2-Amino-7-methyl-5-(*N,N,N*-trimethylammoniummethyl)-pyrrolo[2,3-*d*]pyrimidin-4-one iodide (**11a**) (100 mg, 0.28 mmole) was dissolved in aqueous sodium hydroxide (1 *M*, 25 ml) and the mixture stirred at 50° for 1 hour. The solution was extracted with chloroform (3 x 10 ml) and neutralized by the addition of glacial acetic acid. Lyophilization gave an off-white powder, which was purified by silica gel column chromatography, eluting with solvent system B. Recrystallization from methanol afforded 30 mg of **12** (0.16 mmole, 56%), mp 292° dec; R_f (in solvent system A) 0.07, (in solvent system B) 0.22; ^{1}H nmr (DMSO-d_6): δ 3.58 (s, 3H, N-CH_3), 4.85 (d, 2H, 5-CH_2OH), 5.1 (br s, 1H, 5-CH_2O*H*), 6.7 (br s, 2H, 2-NH_2), 7.15 (s, 1H, H-6), 10.8 (br s, 1H, 3-NH).

Anal. Calcd. for $C_8H_{10}N_4O_2$: C, 49.47; H, 5.20; N, 28.85. Found: C, 49.48; H, 5.25; N, 28.86.

2-Amino-5-aminomethyl-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**13**).

2-Amino-7-methyl-5-(*N,N,N*-trimethylammoniummethyl)-pyrrolo[2,3-*d*]pyrimidin-4-one iodide (**11a**) (300 mg, 0.84 mmole) and methanol (saturated with ammonia at 0°, 50 ml) were added to a high pressure reaction vessel. The vessel was sealed and heated to 50° for 4 hours. The reaction mixture was cooled to -78° and the steel vessel carefully opened. The methanolic solution was diluted with diethyl ether (100 ml) and incubated for 12 hours at -20° to deposit a precipitate, which was collected by filtration and dried *in vacuo* at room temperature in a dessicator over phosphorus pentoxide to afford 159 mg (0.82 mmole, 98%) of **13**, mp 245° dec; R_f (in solvent system A) 0.0, (in solvent system B) 0.12; ^{1}H nmr (DMSO-d_6): δ 3.06 (s,

3H, N-CH_3), 4.32 (t, 4.4 Hz, 2H, -CH_2NH_2), 5.1 (br s, 2H, -CH_2NH_2), 6.2 (br s, 2H, 2-NH_2), 7.05 (d, 3.7 Hz, 1H, H-6), 10.3 (br s, 1H, 3-NH).

Anal. Calcd. for $C_8H_{11}N_5O$: C, 49.72; H, 5.75; N, 36.25. Found: C, 49.72; H, 5.76; N, 36.19.

2-Amino-5-methoxymethyl-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**14**).

2-Amino-7-methyl-5-(*N,N,N*-trimethylammoniummethyl)-pyrrolo[2,3-*d*]pyrimidin-4-one iodide (**11a**) (100 mg, 0.28 mmole) and sodium methoxide (160 mg, 3.0 mmoles) in methanol (25 ml) were heated at reflux with stirring for 1 hour. The solution was cooled to room temperature and the solvent removed *in vacuo*. The residual solid was resuspended in water (10 ml) and the aqueous solution neutralized with glacial acetic acid. The resulting precipitate was collected by filtration and dried *in vacuo*. Purification by silica gel column chromatography and recrystallization from ethanol afforded 40 mg (0.19 mmole, 68%) of **14**, mp 188-191°; R_f (in solvent system A) 0.15, (in solvent system B) 0.34; ^1H nmr (DMSO-d_6): δ 3.04 (s, 3H, -NCH_3), 3.15 (s, 3H, -OCH_3), 4.75 (s, 2H, CH_2OCH_3), 6.8 (br s, 2H, 2-NH_2), 7.09 (s, 1H, H-6), 10.4 (br s, 1H, 3-NH).

Anal. Calcd. for $C_9H_{12}N_4O_2$: C, 51.90; H, 5.82; N, 26.91. Found: C, 51.87; H, 5.82; N, 26.93.

Methyl 2-Amino-3-benzyl-7-(*N-t*-butoxycarbonyl)pyrrolo-[2,3-*d*]pyrimidin-4-one-5-carboxylate (**16**).

Methyl 2-amino-7-(*N-t*-butoxycarbonyl)pyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**15**) (5.00 g, 16.2 mmoles) and sodium hydride (60% dispersion in oil, 427 mg, 17.8 mmoles) were added to a flask, which was sealed with a rubber septum. The flask was purged with argon and anhydrous dimethylformamide (50 ml) was added *via* syringe. The mixture was stirred at 25° for 0.5 hours under argon to produce a clear yellow solution after the evolution of hydrogen. Benzyl bromide (1.93 ml, 16.2 mmoles) was added *via* syringe and the mixture was stirred at 25° in the dark under argon for 12 hours to give a dull orange solution. The solvent was removed *in vacuo* and the residue purified by column chromatography. Recrystallization from methanol afforded 4.19 g (10.5 mmoles, 65%) of **16**, mp 284-288°; R_f (in solvent system A) 0.44, (in solvent system B) 0.76; ^1H nmr (DMSO-d_6): δ 1.56 (s, 9H, -C(CH_3)$_3$), 3.70 (s, 3H, -CO_2CH_3), 5.25 (s, 2H, -CH_2Ph), 6.6 (br s, 2H, 2-NH_2), 7.1-7.4 (m, 5H, Ph-H), 7.65 (s, 1H, H-6).

Anal. Calcd. for $C_{20}H_{22}N_4O_5$: C, 60.28; H, 5.58; N, 14.06. Found: C, 60.27; H, 5.61; N, 14.08.

Methyl 2-Amino-3-benzylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**17**).

Methyl 2-amino-3-benzyl-7-(*N-t*-butoxycarbonyl)pyrrolo-[2,3-*d*]pyrimidin4-one-5-carboxylate (**16**) (4.00 g, 10.0 mmoles) and methanol (saturated with NH_3 at 0°, 200 ml) were added to a high pressure reaction vessel at 0°, which was then sealed. The reaction mixture was heated to 75° and mechanically stirred for 12 hours. The product mixture was then cooled to 0° and the reaction vessel opened carefully. The solvent was removed *in vacuo* and the product recrystallized from methanol/water to afford 2.60 g (8.71 mmoles, 87%) of **17**, mp 310° dec; R_f (in solvent system A) 0.28, (in solvent system B) 0.65; ^1H nmr (DMSO-d_6): δ 3.68 (s, 3H, -CO_2CH_3), 5.24 (s, 2H, -CH_2Ph), 6.6 (br s, 2H, 2-NH_2), 7.1-7.4 (m, 5H, Ph-H), 7.52 (d, 3.2 Hz, 1H, H-6), 10.8 (br s, 1H, 7-NH).

Anal. Calcd. for $C_{15}H_{14}N_4O_3$: C, 60.39; H, 4.74; N, 18.78. Found: C, 60.41; H, 4.76; N, 18.82.

Methyl 2-Amino-3-benzyl-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**18**).

Compound **18** was prepared from **17** (2.50 g, 8.00 mmoles) by the method described for **6** to yield 2.30 g (7.36 mmoles, 92%) of **17**, mp 204-206°; R_f (in solvent system A) 0.47, (in solvent system B) 0.59; ^1H nmr (DMSO-d_6): δ 3.55 (s, 3H, -NCH_3), 3.72 (s, 3H, -CO_2CH_3), 5.14 (s, 2H, -CH_2Ph), 6.4 (br s, 2H, 2-NH_2), 7.2-7.5 (m, 5H, Ph-H), 7.64 (s, 1H, H-6).

Anal. Calcd. for $C_{16}H_{16}N_4O_3$: C, 61.52; H, 5.17; N, 17.94. Found: C, 61.50; H, 5.13; N, 17.90.

Methyl 2-Amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**19**).

Methyl 2-amino-3-benzyl-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**18**) (2.00 g, 6.40 mmoles) was dissolved in dimethylformamide (10 ml) and treated with sodium hydride (60% dispersion in oil, 240 mg, 10.0 mmoles) with stirring under argon for 0.5 hours at 25°. The mixture was then cooled to -78° and anhydrous ammonia was added to a total volume of 50 ml. Sodium metal (460 mg, 20.0 mmoles) was added and the mixture stirred at -78° for 0.5 hours to give a deep blue color. Ammonium chloride (3.21 g, 60.0 mmoles) was added and the mixture was warmed gradually to room temperature with stirring, allowing ammonia gas to evolve through a bubbler. The solvent was then removed *in vacuo* and the residue purified by silica gel column chromatography eluting with solvent system B. Recrystallization from methanol gave 682 mg (3.07 mmoles, 48%) of **19**, mp 320° dec; R_f (in solvent system A) 0.15, (in solvent system B) 0.32; ^1H nmr (DMSO-d_6): δ 3.55 (s, 3H, -NCH_3), 3.72 (s, 3H, -CO_2CH_3), 6.5 (br s, 2H, 2-NH_2) 7.65 (s, 1H, H-6), 11.1 (br s, 1H, 3-NH).

Anal. Calcd. for $C_9H_{10}N_4O_3$: C, 48.64; H, 4.54; N, 25.22. Found: C, 48.68; H, 4.55; N, 25.23.

2-Amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxamide (**20**).

Methyl 2-amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**19**) (100 mg, 0.45 mmole) and methanol (saturated at 0° with ammonia, 30 ml) were added to a high pressure reaction vessel. The vessel was sealed and the mixture heated with stirring at 120° for 24 hours. The reaction mixture was then cooled to -78° and the vessel carefully opened. The solvent was removed *in vacuo* and the residual solid dried *in vacuo* to afford 91 mg (0.44 mmole, 98%) of **20**, mp 325° dec; R_f (in solvent system A) 0.04, (in solvent system B) 0.19; ^1H nmr (DMSO-d_6): δ 3.75 (s, 3H, N-CH_3), 6.4 (br s, 2H, 2-NH_2), 7.35 (s, 1H, H-6), 9.6 (br s, 1H, -CONH_2), 10.8 (br s, 1H, -CONH_2), 11.6 (br s, 1H, 3-NH).

Anal. Calcd. for $C_8H_9N_5O_2$: C, 46.37; H, 4.39; N, 33.80. Found: C, 46.37; H, 4.41; N, 33.83.

2-Amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-(*N,N*-dimethyl)carboxamide (**21**).

Methyl 2-amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**19**) (100 mg, 0.45 mmole) and ethanolic dimethylamine (Fluka, 30 ml) were added to a high pressure reaction vessel. The vessel was sealed and the mixture was heated with stirring at 100° for 12 hours. The reaction mixture was then cooled to -78° and the vessel was carefully opened. The solvent was

removed *in vacuo*. The residual solid was dissolved in 1 *M* aqueous potassium hydroxide (10 ml). The basic aqueous solution was treated with charcoal, filtered, and neutralized with glacial acetic acid to give a precipitate, which was collected by filtration and dried *in vacuo* to give 92 mg (0.39 mmole, 87%) of **21**, mp 298-301°; R_f (in solvent system A) 0. 17, (in solvent system B) 0.34; ^1H nmr (DMSO-d_6): δ 3.62 (s, 3H, N-CH_3), 3.71 (s, 3H, N-CH_3), 3.85 (s, 3H, N-CH_3), 6.4 (br s, 2H, 2-NH_2), 7.52 (s, 1H, H-6), 11.0 (br s, 1H, 3-NH).

Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C, 51.05; H, 5.58; N, 29.77. Found: C, 51.03; H, 5.53; N, 29.78.

2-Amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-(*N*-methyl)-carboxamide (**22**).

Methyl 2-amino-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**19**) (100 mg, 0.45 mmole) and ethanolic methylamine (Fluka, 30 ml) were added to a high pressure reaction vessel. The vessel was sealed and the mixture heated with stirring at 100° for 12 hours. The reaction mixture was then cooled to -78° and the vessel carefully opened. The solvent was removed *in vacuo*. The residual solid was dissolved in 1 *M* aqueous potassium hydroxide (10 ml). The basic aqueous solution was treated with charcoal, filtered, and neutralized with glacial acetic acid to give a precipitate, which was collected by filtration and dried *in vacuo* to give 79 mg (0.38 mmole, 85%) of **22**, mp 310° dec; R_f (in solvent system A) 0.07, (in solvent system B) 0.35; ^1H nmr (DMSO-d_6): δ 2.82 (d, 4.1 Hz, 3H, -CONHCH_3), 3.65 (s, 3H, 7-NCH_3), 6.6 (br s, 2H, 2-NH_2), 7.64 (s, 1H, H-6), 10.0 (br s, 1H, -CON*H*CH_3), 11.2 (br s, 1H, 3-NH).

Anal. Calcd. for $C_9H_{11}N_5O_2$: C, 48.85; H, 5.02; N, 31.66. Found: C, 48.87; H, 5.01; N, 31.69.

2-Amino-5-cyano-4-methoxypyrrolo[2,3-*d*]pyrimidine (**24**).

2-Amino-4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine (**23**) (5.00 g, 25.8 mmoles) and sodium methoxide (1.41 g, 26 mmoles) were suspended in anhydrous methanol (200 ml). The mixture was heated at reflux with stirring for 4 hours and then cooled to room temperature. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography in solvent system B. Recrystallization from methanol gave 2.39 g (12.7 mmoles, 49%) of **24**, mp 300° dec; R_f (in solvent system A) 0.15, (in solvent system B) 0.37; ^1H nmr (DMSO-d_6): δ 4.10 (s, 3H, 4-OCH_3), 6.4 (br s, 2H, 2-NH_2), 7.60 (s, 1H, H-6), 11.8 (br s, 1H, 7-NH).

Anal. Calcd. for $C_8H_7N_5O$: C, 50.78; H, 3.74; N, 37.02. Found: C, 50.72; H, 3.78; N, 37.10.

2-Amino-5-cyano-4-methoxy-7-methylpyrrolo[2,3-*d*]pyrimidine (**26**).

Compound **26** was prepared from **24** (2.00 g, 10.6 mmoles) by the method described for **6** to yield 2.02 g (9.96 mmoles, 94%) of **26**, mp 224-228°; R_f (in solvent system A) 0.29, (in solvent system B) 0.78; ^1H nmr (DMSO-d_6): δ 3.81 (s, 3H, N-CH_3), 4.15 (s, 3H, 4-OCH_3), 6.8 (br s, 2H, 2-NH_2), 8.07 (s, 1H, H-6).

Anal. Calcd. for $C_9H_9N_5O$: C, 53.19; H, 4.47; N, 34.47. Found: C, 53.19; H, 4.45; N, 34.51.

2-Amino-5-cyano-7-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**25**).

2-Amino-5-cyano-4-methoxy-7-methylpyrrolo[2,3-*d*]pyrimidine (**26**) (1.00 g, 4.92 mmoles) was suspended in anhydrous acetonitrile (100 ml). Trimethylsilyl iodide (850 mg, 5.0 mmoles) was added and the mixture heated at reflux under argon for 12 hours. The product mixture was cooled to room temperature and the solvent removed *in vacuo*. The residue was purified by silica gel column chromatography with solvent system B. Recrystallization from methanol gave 354 mg (1.87 mmoles, 38%) of **25**, mp 307° dec; R_f (in solvent system A) 0.21, (in solvent system B) 0.52; ^1H nmr (DMSO-d_6): δ 3.82 (s, 3H, N-CH_3), 6.7 (br s, 2H, 2-NH_2), 7.75 (s, 1H, H-6), 11.6 (br s, 1H, 3-NH).

Anal. Calcd. for $C_8H_7N_5O$: C, 50.78; H, 3.74; N, 37.02. Found: C, 50.75; H, 3.75; N, 37.03.

2-Amino-7-benzyl-4-chloropyrrolo[2,3-*d*]pyrimidine (**27**).

2-Amino-4-chloropyrrolo[2,3-*d*]pyrimidine (**5**) [7] (1.90 g, 11.3 mmoles) and sodium hydride (451 mg of 60% oil dispersion, 11.3 mmoles, 1.0 eq) were stirred at 25° under argon in anhydrous dimethylformamide (50 ml) to give a clear yellow solution following evolution of hydrogen. Benzyl bromide (Aldrich, 1.34 ml, 11.3 mmoles, 1.0 eq) was added and stirring was continued at 25° for 24 hours in the absence of light. Analysis (tlc) of the product mixture revealed a complete conversion of starting material to product(s). Water (100 ml) was added dropwise to the stirring mixture to give the gradual precipitation of a solid, which was collected by filtration, washed with water (2 x 50 ml), and dried *in vacuo*. Recrystallization from ethanol gave 2.49 g (9.61 mmoles, 85%) of **27**, mp 228-230°; R_f (in solvent system A) 0.72, (in solvent system B) 0.84; ^1H nmr (DMSO-d_6): δ 5.25 (s, 2H, -CH_2Ph), 6.32 (d, 3.7 Hz, 1H, H-5), 6.7 (br s, 2H, 2-NH_2), 7.15-7.33 (m, 6H, H-6 and Ph-H).

Anal. Calcd. for $C_{13}H_{11}N_4Cl$: C, 60.34; H, 4.29; N, 21.66. Found: C, 60.41; H, 4.35; N, 21.67.

2-Amino-7-benzyl-4-chloro-5-methylpyrrolo[2,3-*d*]pyrimidine (**28**).

Compound **28** was prepared from **3** (5.00 g, 27.4 mmoles) by the method described for **27** to yield 6.80 g (24.9 mmoles, 91%) of **28**, mp 211-213°; R_f (in solvent system A) 0.75, (in solvent system B) 0.86; ^1H nmr (DMSO-d_6): δ 2.15 (s, 3H, 5-CH_3), 5.18 (s, 2H, -CH_2Ph), 6.7 (br s, 2H, 2-NH_2), 7.1-7.6 (m, 6H, H-6 and Ph-H).

Anal. Calcd. for $C_{14}H_{13}N_4Cl$: 61.64; H, 4.81; N, 20.55. Found: C, 61.64; H, 4.85; N, 20.53.

2-Amino-7-benzyl-4-chloro-5-(*N*,*N*-dimethylaminomethyl)-pyrrolo[2,3-*d*]pyrimidine (**29**).

Compound **29** was prepared from **4** (6.50 g, 28.8 mmoles) by the method described for **27** to yield 8.55 g (27.1 mmoles, 94%) of **29**, mp 184-186°; R_f (in solvent system A) 0.69, (in solvent system B) 0.82; ^1H nmr (DMSO-d_6): δ 2.35 (s, 6H, -N(CH_3)$_2$), 3.72 (s, 2H, -CH_2N(CH_3)$_2$, 5.15 (s, 2H, -CH_2Ph), 6.4 (br s, 2H, 2-NH_2), 7.1-7.5 (m, 6H, H-6 and Ph-H).

Anal. Calcd. for $C_{16}H_{18}N_5Cl$: C, 60.84; H, 5.76; N, 22.18. Found: C, 60.85; H, 5.75; N, 22.15.

2-Amino-7-benzylpyrrolo[2,3-*d*]pyrimidin-4-one (**30**).

Compound **30** [14] was prepared from **27** (2.00 g, 7.73 mmoles) by the method described for **8** to yield 1.15 g (4.79 mmoles, 62%) of **33**, mp 322° dec; R_f (in solvent system A) 0.22, (in solvent system B) 0.51; ^1H nmr (DMSO-d_6): δ 5.12 (s, 2H, -CH_2Ph), 6.0 (br s, 1H, 2-NH_2), 6.30 (d, 3.6 Hz, H-5), 7.1-7.4 (m, 6H, H-6 and Ph-H), 11.1 (br s, 1H, 3-NH); ir (cm^{-1}); uv (*p*H 1): λ_{max} in nm (ε) 224 (18,800), 263 (11,000); (*p*H 11): 266 (11,400), lit [14]; (*p*H 1): 224 (19,000), 263 (11,000); (*p*H

11): 266 (11,500).

Anal. Calcd. for $C_{13}H_{12}N_4O$: C, 64.98; H, 5.04; N, 23.32. Found: C, 64.92; H, 5.10; N, 23.41.

2-Amino-7-benzyl-5-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**31**).

Compound **31** was prepared from **28** (5.50 g, 20.2 mmoles) by the method described for **8** to yield 2.72 g (10.7 mmoles, 53%) of **31**, mp 285° dec; R_f (in solvent system A) 0.28, (in solvent system B) 0.45; ^1H nmr (DMSO-d_6): δ 2.15 (s, 3H, 5-CH_3), 5.23 (s, 2H, -CH_2Ph), 6.1 (br s, 1H, 2-NH_2), 7.1-7.5 (m, 6H, H-6 and Ph-H), 11.0 (br s, 1H, 3-NH).

Anal. Calcd. for $C_{14}H_{14}N_4O$: C, 66.11; H, 5.56; N, 22.04. Found: C, 66.12; H, 5.61; N, 22.06.

2-Amino-7-benzyl-5-(*N,N*-dimethylaminomethyl)pyrrolo-[2,3-*d*]pyrimidin-4-one (**32**).

Compound **32** was prepared from **29** (7.00 g, 22.2 mmoles) by the method described for **8** to yield 2.89 g (9.75 mmoles, 44%) of **32**, mp 340° dec; R_f (in solvent system A) 0.15, (in solvent system B) 0.52; ^1H nmr (DMSO-d_6): δ 2.32 (s, 6H, -$CH_2N(CH_3)_2$), 3.65 (s, 2H, -$CH_2N(CH_3)_2$), 5.31 (s, 2H, -CH_2Ph), 6.2 (br s, 2H, 2-NH_2), 7.1-7.5 (m, 6H, H-6 and Ph-H), 10.5 (br s, 1H, 3-NH).

Anal. Calcd. for $C_{16}H_{19}N_5O$: C, 64.61; H, 6.45; N, 23.55. Found: C, 64.65; H, 6.47; N, 23.51.

2-Amino-7-benzyl-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**33**).

Compound **33** was prepared from **30** (1.00 g, 4.16 mmoles) by the method described for **6** to yield 783 mg (3.08 mmoles, 74%) of **33**, mp 241-243°; R_f (in solvent system A) 0.47, (in solvent system B) 0.72; ^1H nmr (DMSO-d_6): δ 3.30 (s, 3H, N-CH_3), 5.23 (s, 2H, -CH_2Ph), 5.75 (br s, 1H, 2-NH_2), 6.21 (d, 3.7 Hz, 1H, H-5), 7.1-7.4 (m, 6H, H-6 and Ph-H).

Anal. Calcd. for $C_{14}H_{14}N_4O$: C, 66.11; H, 5.56; N, 22.04. Found: C, 66.15; H, 5.62; N, 22.05.

2-Amino-7-benzyl-3,5-dimethylpyrrolo[2,3-*d*]pyrimidin-4-one (**34**).

Compound **34** was prepared from **31** (2.50 g, 9.83 mmoles) by the method described for **6** to yield 1.87 g (6.98 mmoles, 71%) of **34**, mp 192-193°; R_f (in solvent system A) 0.35, (in solvent system B) 0.77; ^1H nmr (DMSO-d_6): δ 2.18 (s, 3H, 5-CH_3), 3.28 (s, 3H, N-CH_3), 5.20 (s, 2H, -CH_2Ph), 6.1 (br s, 2H, 2-NH_2), 7.1-7.4 (m, 6H, H-6 and Ph-H).

Anal. Calcd. for $C_{15}H_{16}N_4O$: C, 67.13; H, 6.02; N, 20.88. Found: C, 67.15; H, 6.05; N, 20.86.

2-Amino-7-benzyl-5-(*N,N*-dimethylaminomethyl)-3-methyl-pyrrolo[2,3-*d*]pyrimidin-4-one (**35**).

Compound **35** was prepared from **32** (2.50 g, 8.41 mmoles) by the method described for **6** to yield 1.73 g (5.55 mmoles, 66%) of **35**, mp 238-240°; R_f (in solvent system A) 0.21, (in solvent system B) 0.74; ^1H nmr (DMSO-d_6): δ 2.28 (s, 6H, -$CH_2N(CH_3)_2$), 3.27 (s, 3H, 3-NCH_3), 3.62 (s, 2H, -$CH_2N(CH_3)_2$), 5.31 (s, 2H, -CH_2Ph), 6.3 (br s, 2H, 2-NH_2), 7.1-7.5 (m, 6H, H-6 and Ph-H).

Anal. Calcd. for $C_{17}H_{21}N_5O$: C, 65.56; H, 6.81; N, 22.49. Found: 65.63; H, 6.84; N, 22.47.

2-Amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**36**).

Compound **36** was prepared from **33** (600 mg, 2.36 mmoles) by the method described for **19** to yield 213 mg (1.30 mmoles, 55%) of **36**, mp 285° dec; R_f (in solvent system A) 0.21, (in solvent system B) 0.44; ^1H nmr (DMSO-d_6): δ 3.25 (s, 3H, N-CH_3), 6.0 (br s, 2H, 2-NH_2), 6.25 (d, 3.6 Hz, 1H, H-5), 6.94 (d, 3.6 Hz, 1H, H-6), 10.9 (br s, 1H, 7-NH).

Anal. Calcd. for $C_7H_8N_4O$: C, 51.20; H, 4.92; N, 34.13. Found: C, 51.25; H, 4.95; N, 34.15.

2-Amino-3,5-dimethylpyrrolo[2,3-*d*]pyrimidin-4-one (**37**).

Compound **37** was prepared from **34** (1.50 g, 5.59 mmoles) by the method described for **19** to yield 628 mg (3.52 mmoles, 63%) of **37**, mp 275° dec; R_f (in solvent system A) 0.25, (in solvent system B) 0.47; ^1H nmr (DMSO-d_6): δ 2.14 (s, 3H, 5-CH_3), 3.27 (s, 3H, N-CH_3), 6.0 (br s, 2H, 2-NH_2), 6.41 (d, 1.8 Hz, 1H, H-6), 10.7 (br s, 1H, 7-NH).

Anal. Calcd. for $C_8H_{10}N_4O$: C, 53.91; H, 5.67; N, 31.44. Found: C, 53.90; H, 5.65; N, 31.48.

2-Amino-5-(*N,N*-dimethylaminomethyl)-3-methylpyrrolo-[2,3-*d*]pyrimidin-4-one (**38**).

Compound **38** was prepared from **35** (1.50 g, 4.82 mmoles) by the method described for **19** to yield 554 mg (2.50 mmoles, 52%) of **38**, mp 245° dec; R_f (in solvent system A) 0.15, (in solvent system B) 0.33; ^1H nmr (DMSO-d_6): δ 2.34 (s, 6H, -$CH_2N(CH_3)_2$), 3.30 (s, 3H, 3-NCH_3), 3.65 (s, 2H, -$CH_2N(CH_3)_2$), 6.3 (br s, 2H, 2-NH_2), 7.12 (d, 3.6 Hz, 1H, H-6), 10.8 (br s, 1H, 7-NH).

Anal. Calcd. for $C_{10}H_{15}N_5O$: C, 54.27; H, 6.85; N, 31.65. Found: C, 54.30; H, 6.91; N, 31.71.

2-Amino-5-(aminomethyl)-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**40**).

2-Amino-5-(*N,N*-dimethylaminomethyl)-3-methylpyrrolo-[2,3-*d*]pyrimidin-4-one (**38**) (100 mg, 0.45 mmole) was dissolved in dimethyl sulfide (2 ml). Methyl iodide (0.45 mmole) was added and the mixture stirred at 25° for 0.5 hours under argon to give the methiodide salt **39**. The solution of **39** was added to methanol (saturated with ammonia at 0°, 50 ml) in a high pressure reaction vessel. The vessel was sealed and heated to 50° for 4 hours. The reaction mixture was cooled to -78° and the steel vessel carefully opened. Diethyl ether (100 ml) was added and the resulting product mixture was incubated for 12 hours at -20° to deposit a precipitate, which was collected by filtration and dried *in vacuo* at room temperature in a dessicator over phosphorus pentoxide to afford 80 mg (0.41 mmole, 92%) of **40**, mp 220° dec; R_f (in solvent system A) 0.04, (in solvent system B) 0.15; ^1H nmr (DMSO-d_6): δ 3.29 (s, 3H, N-CH_3), 4.22 (t, 4.5 Hz, 2H, -CH_2NH_2), 4.9 (br s, 2H, -CH_2NH_2), 6.2 (br s, 2H, 2-NH_2), 7.12 (d, 3.4 Hz, 1H, H-6), 10.9 (br s, 1H, 7-NH).

Anal. Calcd. for $C_8H_{11}N_5O$: C, 49.72; H, 5.75; N, 36.25. Found: C, 49.67; H, 5.81; N, 36.31.

2-Amino-5-(hydroxymethyl)-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**41**).

2-Amino-5-(*N,N*-dimethylaminomethyl)-3-methylpyrrolo-[2,3-*d*]pyrimidin-4-one (**38**) (100 mg, 0.45 mmole) was dissolved in dimethyl sulfoxide (2 ml). Methyl iodide (0.45 mmole) was added and the mixture stirred at 25° for 0.5 hours under argon to give the methiodide salt **39**. Aqueous sodium hydroxide (1 *M*, 25 ml) was added and the mixture stirred at 50° for 1 hour. The solution was extracted with chloroform (3 x 10 ml) and neutralized by the addition of glacial acetic acid. Lyophilization gave an off-white powder, which was purified by silica gel column chromatography, eluting with solvent system

B. Recrystallization from methanol afforded 73 mg (0.38 mmole, 84%) of **41**, mp 197° dec; R_f (in solvent system A) 0.04, (in solvent system B) 0.12; ^1H nmr (DMSO-d_6): δ 3.31 (s, 3H, N-CH_3), 4.85 (d, 5.0 Hz, 2H, -CH_2OH), 5.1 (br s, 1H, -CH_2O*H*), 6.4 (br s, 2H, 2-NH_2), 7.06 (d, 3.6 Hz, 1H, H-6), 10.8 (br s, 1H, 7-NH).

Anal. Calcd. for $C_8H_{10}N_4O_2$: C, 49.47; H, 5.20; N, 28.85. Found: C, 49.47; H, 5.25; H, 28.84.

2-Amino-5-(methoxymethyl)-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**42**).

2-Amino-5-(*N,N*-dimethylaminomethyl)-3-methylpyrrolo-[2,3-*d*]pyrimidin-4-one (**38**) (100 mg, 0.45 mmole) was dissolved in dimethyl sulfoxide (2 ml). Methyl iodide (0.45 mmole) was added and the mixture stirred at 25° for 0.5 hours under argon to give the methiodide salt **39**. Methanol (25 ml) and sodium methoxide (160 mg, 3.0 mmoles) were added and the resulting suspension was heated at reflux with stirring for 1 hour. The resulting clear yellow solution was cooled to room temperature and the solvent removed *in vacuo*. The residual solid was resuspended in water (10 ml) and the aqueous solution neutralized with glacial acetic acid. The resulting precipitate was collected by filtration and dried *in vacuo*. Purification by silica gel column chromatography in solvent system B and recrystallization from ethanol afforded 85 mg (0.41 mmole, 91%) of **42**, mp 217° dec; R_f (in solvent system A) 0.11, (in solvent system B) 0.24; ^1H nmr (DMSO-d_6): δ 3.11 (s, 3H, -CH_2OCH_3), 3.30 (s, 3H, N-CH_3), 4.77 (s, 2H, -CH_2OCH_3), 6.2 (br s, 2H, 2-NH_2), 7.07 (d, 3.7 Hz, 1H, H-6), 11.0 (br s, 1H, 7-NH).

Anal. Calcd. for $C_9H_{12}N_4O_2$: C, 51.90; H, 5.82; N, 26.91. Found: C, 51.88; H, 5.85; N, 26.95.

Methyl 2-Amino-7-(*N*-*t*-butoxycarbonyl)-3-methylpyrrolo-[2,3-*d*]pyrimidin-4-one-5-carboxylate (**43**).

Compound **43** was prepared from **15** (5.00 g, 16.2 mmoles) by the method described for **6** to yield 3.71 g (11.5 mmoles, 71%) of **43**, mp 321-323°; R_f (in solvent system A) 0.61, (in solvent system B) 0.82; ^1H nmr (DMSO-d_6): δ 1.55 (s, 9H, -$C(CH_3)_3$), 3.35 (s, 3H, N-CH_3), 3.62 (s, 3H, -OCH_3), 6.6 (br s, 2H, 2-NH_2), 7.35 (d, 2.9 Hz, 1H, H-6).

Anal. Calcd. for $C_{14}H_{18}N_4O_5$: C, 52.16; H, 5.64; N, 17.38. Found: C, 52.11; H, 5.69; N, 17.33.

Methyl 2-Amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxylate (**44**).

Compound **44** was prepared from **43** (100 mg, 0.31 mmole) by the method described for **17** to yield 67 mg (0.30 mmole, 97%) of **44**, mp 278-281°; R_f (in solvent system A) 0.08, (in solvent system B) 0.21; ^1H nmr (DMSO-d_6): δ 3.35 (s, 3H, N-CH_3), 3.67 (s, 3H, -OCH_3), 6.7 (br s, 2H, 2-NH_2), 7.45 (d, 3.0 Hz, 1H, H-6), 10.6 (br s, 1, 7-NH).

Anal. Calcd. for $C_9H_{10}N_4O_3$: C, 48.64; H, 4.54; N, 25.22. Found: C, 48.65; H, 4.55; N, 25.22.

2-Amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-(*N*-methyl)carboxamide (**45**).

Compound **45** was prepared from **43** (100 mg, 0.31 mmole) by the method described for **22** to yield 64 mg (0.29 mmole, 93%) of **45**, mp 307° dec; R_f (in solvent system A) 0.05, (in solvent system B) 0.19; ^1H nmr (DMSO-d_6): δ 2.87 (d, 2.9 Hz, 3H, -CONHC*H*$_3$), 3.37 (s, 3H, 3-NCH_3), 6.5 (br s, 2H, 2-NH_2), 7.21 (s, 1H, H-6), 9.50 (s, 1H, -CON*H*CH_3), 10.6 (br s, 1H, 7-NH).

Anal. Calcd. for $C_9H_{11}N_5O_2$: C, 48.85; H, 5.02; N, 31.66. Found: C, 48.80; H, 5.10; N, 31.69.

2-Amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxamide (**46**).

Compound **46** was prepared from **43** (500 mg, 1.55 mmoles) by the method described for **20** to yield 295 mg (1.43 mmoles, 92%) of **46**, mp 345° dec; R_f (in solvent system A) 0.02, (in solvent system B) 0.15; ^1H nmr (DMSO-d_6): δ 3.28 (s, 3H, N-CH_3), 6.4 (br s, 2H, 2-NH_2), 7.24 (s, 1H, H-6), 7.51 (s, 1H, -$CONH_2$), 9.55 (s, 1H, -$CONH_2$), 10.8 (br s, 1H, 7-NH).

Anal. Calcd. for $C_8H_9N_5O_2$: C, 46.37; H, 4.39; N, 33.80. Found: C, 46.41; H, 4.43; N, 33.85.

2-Amino-5-cyano-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**47**).

A mixture of 2-amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-carboxamide (**46**) (100 mg, 0.48 mmole) and phosphorus oxychloride (25 ml) were heated at reflux with stirring in an anhydrous atmosphere for 4 hours. The dark orange solution was allowed to cool to room temperature and concentrated *in vacuo*. Water (20 ml) was then added to the residue at 0° with vigorous stirring to give an exothermic reaction. Concentrated ammonium hydroxide was added to *p*H 5 to give a precipitate, which was collected by filtration, washed with water (3 x 5 ml), and dried *in vacuo*. The crude product was purified by silica gel column chromatography in solvent system B. Recrystallization from methanol afforded 43 mg (0.23 mmole, 47%) of **47**, mp 286° dec; R_f (in solvent system A) 0.12, (in solvent system B) 0.35; ^1H nmr (DMSO-d_6): δ 3.35 (s, 3H, N-CH_3), 6.4 (br s, 2H, 2-NH_2), 7.64 (s, 1H, H-6), 10.8 (br s, 1H, 7-NH).

Anal. Calcd. for $C_8H_7N_5O$: C, 50.78; H, 3.74; N, 37.02. Found: C, 50.79; H, 3.76; N, 37.04.

2-Amino-3-methylpyrrolo[2,3-*d*]pyrimidin-4-one-5-(*N,N*-dimethyl)carboxamide (**48**).

Compound **48** was prepared from **43** (100 mg, 0.31 mmole) by the method described for **21** to yield 69 mg (0.29 mmole, 95%) of **48**, mp 254-256°; R_f (in solvent system A) 0.11, (in solvent system B) 0.25; ^1H nmr (DMSO-d_6): δ 3.05 (s, 6H, $CON(CH_3)_2$), 3.34 (s, 3H, 3-NCH_3), 6.6 (br s, 2H, 2-NH_2), 7.25 (d, 2.8 Hz, 1H, H-6), 10.7 (br s, 1H, 7-NH).

Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C, 51.05; H, 5.58; N, 29.77. Found: C, 51.07; H, 5.62; N, 29.79.

2-Amino-5-cyano-3,7-dimethylpyrrolo[2,3-*d*]pyrimidin-4-one (**49**).

Compound **49** was prepared from **47** (100 mg, 0.49 mmole) by the method described for **6**. The crude product was purified by silica gel column chromatography in solvent system A to yield 65 mg (0.32 mmole, 65%) of **49**, mp 312-314°; R_f (in solvent system A) 0.21, (in solvent system B) 0.52; ^1H nmr (DMSO-d_6): δ 3.15 (s, 3H, -NCH_3), 3.32 (s, 3H, -NCH_3), 6.5 (br s, 2H, 2-NH_2), 7.55 (s, 1H, H-6).

Anal. Calcd. for $C_9H_9N_5O$: C, 53.19; H, 4.47; N, 34.47. Found: C, 53.18; H, 4.51; N, 34.44.

Acknowledgements.

This work was supported by NIH grants GM45968 and R01-AI33332, an NIH predoctoral traineeship to GCH (GM07767), and the University of Michigan, Horace H. Rackham School of Graduate Studies. We thank Ms. Marina Savic for the preparation of this manuscript.

REFERENCES AND NOTES

[1] S. Nishimura, *Prog. Nucleic Acid Res. Molec. Biol.*, **28**, 49 (1983).

[2] N. Okada and S. Nishimura, *J. Biol. Chem.*, **254**, 3061 (1979).

[3] G. C. Hoops, L. B. Townsend, and G. A. Garcia, *Biochemistry*, **34**,15381 (1995).

[4] J. A. Secrist and P. S. Liu, *J. Org. Chem.*, **43**, 3937 (1978).

[5] H. Akimoto, E. Imamiya, T. Hitaka, H. Nomura, and S. Nishimura, *J. Chem. Soc., Perkin Trans. 1*, 1637 (1988).

[6] K. Ramasamy, R. V. Joshi, R. K. Robins, and G. Revankar, *J. Chem. Soc., Perkin Trans. 1*, 2375 (1989).

[7] F. Seela, A. Kehne, and H.-D. Winkeler, *Liebigs Ann. Chem.*, 137 (1983).

[8] C. Shih and L. S. Gossett, *Heterocycles*, **35**, 825 (1993).

[9] E. Benghiat and P. A. Crooks, *J. Heterocyclic Chem.*, **20**, 1023 (1983).

[10] F. Seela and H.-D. Winkeler, *J. Org. Chem.*, **47**, 226 (1982).

[11] R. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Am. Chem. Soc.*, **91**, 2102 (1969).

[12] R. L. Tolman, G. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Heterocyclic Chem.*, **7**, 799 (1970).

[13] B. L. Cline, P. E. Fagerness, R. P. Panzica, and L. B. Townsend, *J. Chem. Soc., Perkin Trans. 2*, 1586 (1980).

[14] W. C. Noell and R. K. Robins, *J. Heterocyclic Chem.*, **1**, 34 (1964).

[15] W. Pfleiderer, *Liebigs Ann. Chem.*, **647**, 167 (1961).

[16] P. A. Levene, L. W. Bass, and H. S. Simms, *J. Biol. Chem.*, **70**, 229 (1926).

[17] D. J. Brown and N. W. Jacobsen, *J. Chem. Soc.*, 3172 (1962).

[18] K. H. Schram and L. B. Townsend, *J. Chem. Soc., Perkin Trans. 1*, 1253 (1975).

[19] G. Yagil, *Tetrahedron*, **23**, 2855 (1967).

[20] G. Yagil, *J. Phys. Chem.*, **71**, 1034 (1967).

[21] A. Albert and E. P. Serjeant, The Determination of Ionization Constants. A Laboratory Manual, Chapman and Hall, New York, 1984.

[22] J. Davoll, *J. Chem. Soc.*, **131** (1960).

[23] T. Kondo, S. Nakatsuka, and T. Goto, *Chem. Letters*, **559** (1980).