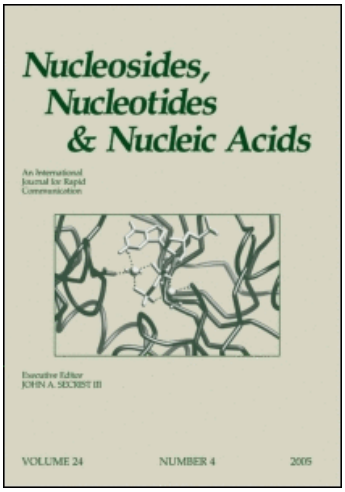

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CERTAIN 3,9-DIDEAZAPURINES AS INHIBITORS OF PURINE NUCLEOSIDE PHOSPHORYLASE

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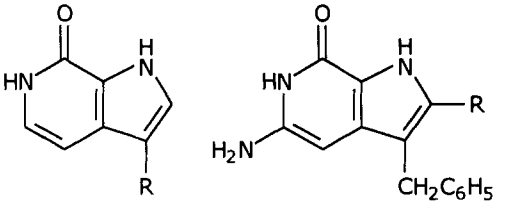
ABSTRACT: The synthesis of four 3,9-dideazapurines is described. In order to achieve the desired substitution pattern, a new approach to the pyrrolo[2,3-*c*]pyridine ring system was devised utilizing the Gassman reaction as the key step for its construction. The four target heterocycles were all evaluated for their ability to inhibit human erythrocytic purine nucleoside phosphorylase.

Introduction

A variety of 9-deaza-9-substituted guanines are potent inhibitors of purine nucleoside phosphorylase (PNP).[1-3] The potency of these compounds is a result of two features: the hydrophobic nature of the 9-substituent and the hydrogen attached to N-7 (purine numbering is used throughout). A study of the binding of these compounds to PNP by X-ray crystallographic analysis did not reveal any important interactions of N-3 with the enzyme, although substitution of a carbon for N-3 would have a significant effect on the electronic distribution around the heterocyclic ring. Because the effect of these shifts on binding to PNP is not easy to predict, we decided to investigate the synthesis of 3,9-dideazapurines **1-4** for evaluation as PNP inhibitors.



1, R=H
2, R=SC_6H_5

3, R=H
4, R=NH_2

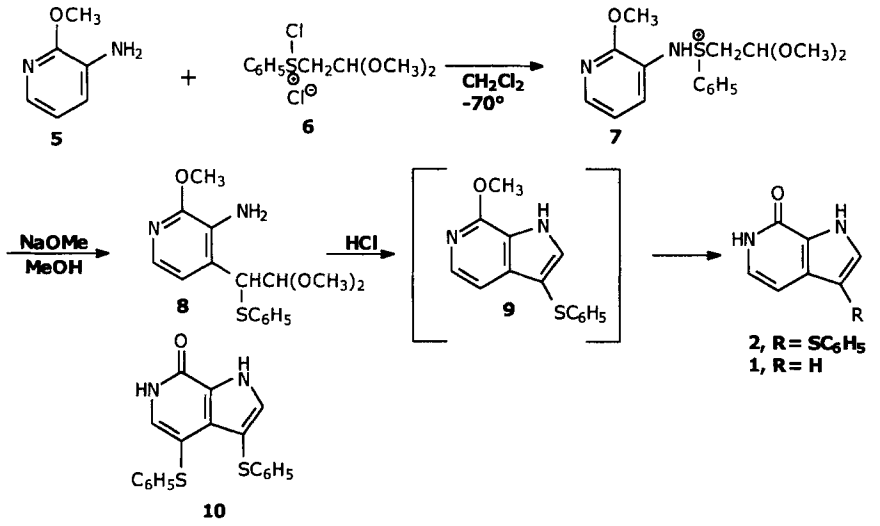
Chemistry

The target compounds are properly designated as pyrrolo[2,3-*c*]pyridines, but have often been referred to in the literature as 6-azaindoles. Typically, these compounds have been made by a cyclization route forming the pyrrole ring from an appropriately substituted aminopyridine derivative.[4-11] More recent synthetic approaches have included palladium-catalyzed heteroannulation reactions to form the pyrrole ring[12,13] and even a cyclization to incorporate the pyridine ring onto a pyrrole precursor.[14] The substitution pattern of interest to us, derived from typical purine nucleosides, was not easily amenable to any of these synthetic methodologies. For that reason we chose to pursue the application of the Gassman reaction for ortho-alkylation of amines[15,16] to the synthesis of our target compounds. This rearrangement has been used successfully on certain 2-aminopyridines,[17] providing some encouragement to us.

Typically, the primary amino group of an aromatic amine is halogenated, most conveniently with *tert*-butyl hypochlorite, to give a haloamine, Ar-NH-X (X = Cl or Br). Reaction with a dialkyl sulfide gives an intermediate azasulfonium salt, which, when treated with a strong base undergoes abstraction of a proton followed by an internal rearrangement whose net effect is migration of the alkyl moiety to a position *ortho* to the amine and regeneration of the primary amino group. If the alkyl group is suitably functionalized with an aldehyde protected as its dimethyl acetal, subsequent reaction with the amino group and attendant ring closure could lead to a variety of indoles or azaindoles, depending on the reactants chosen. The ability to specifically generate a 2,3,4-tri- or 2,3,4,6-tetrasubstituted pyridine by this means ideally suited our needs.

A limitation of the original Gassman procedure was that when the aromatic amine was substituted in the ring with certain electron-releasing groups, particularly methoxy, the chloroamines obtained upon reaction with *tert*-butyl hypochlorite were extremely unstable, and yields of intended product were zero or nearly zero. Fortunately, a modified procedure[15] was developed that provides satisfactory yields of product. Application of this modified procedure led to target 3,9-dideazahypoxanthines **1** and **2** as shown in Scheme 1.

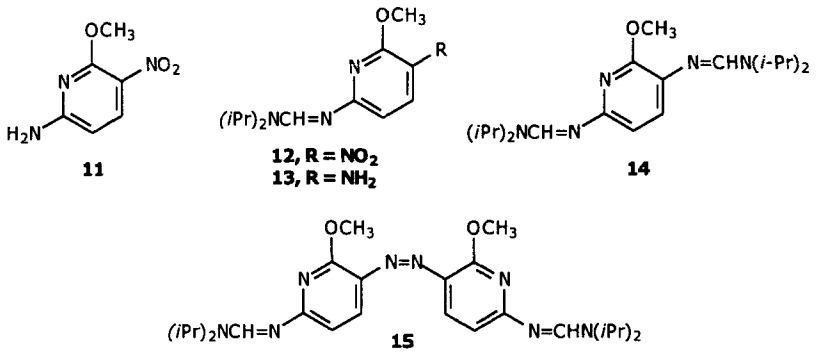
Reaction of 2-(phenylthio)acetaldehyde dimethyl acetal[18] with liquid chlorine in CH_2Cl_2 solution at -70°C gave the chlorosulfonium chloride intermediate **6**, which was transferred to a cold solution of **5**, available by known procedures,[19,20] in CH_2Cl_2. The resulting intermediate **7** was immediately treated with excess NaOMe/MeOH, to effect



Scheme 1

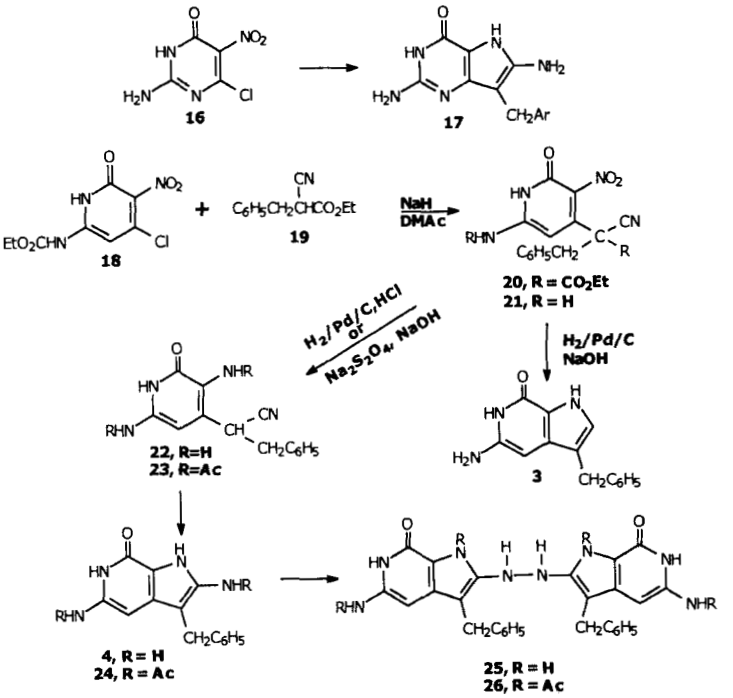
the base-induced rearrangement to **8.** Our first attempt to hydrolyze the acetal and effect cyclization of **8** to **9** under mild conditions (two-phase mixture of **8** in chloroform solution stirred with 1N HCl) was only partially successful; after 3 days **8** was still the main constituent. Finally, briefly heating **8** at reflux with conc. HCl not only effected cyclization but also hydrolyzed the 2-methoxy group as well, thus giving target compound **2** in a single step. A trace of compound **1** was present, as well as a by-product that was assigned structure **10** based on NMR evidence. Upon purification, this by-product was found to comprise <1% of the crude sample. Although the acid hydrolysis had produced a trace of the target compound **1**, a larger quantity of it was obtained by refluxing a solution of **2** in EtOH with Raney nickel saturated with hydrogen; however, 72 hours was required for completion of this reaction, and in retrospect, it may have been easier to just prolong the acid hydrolysis.

The potentially more interesting 3,9-dideazaguanine targets **3** and **4** were initially pursued by extension of the successful methodology of Scheme 1 to a different aminopyridine substrate (**11**). Reaction of **11**, available through an unusual variation of the Chichibabin reaction,[21] with N,N-diisopropylformamide dimethyl acetal[22] gave a nearly quantitative yield of **12**. The diisopropylaminomethylene protective group was introduced to prevent the 6-amino group from participating in the Gassman reaction.



Sodium dithionite reduction of the nitro group of **12**[23] gave the key intermediate **13** in 50% yield. During the reaction workup of **13** a by-product was isolated that was tentatively assigned structure **14**. The yield was only 3%, but the fact that such a product could form at all under the mildly basic workup conditions employed indicates enough lability of the protective group to be troublesome in the more stringent Gassman conditions. A second troublesome observation was that solutions of **13** discolored rapidly, becoming first a pale green and finally a dark, reddish-brown on standing at room temperature.

A few small-scale experiments aimed at finding a more stable protective group were unsuccessful. Therefore, we proceeded with the Gassman reaction using **13** as the starting material. Reaction of **6** (Scheme 1) with **13**, under the same conditions as for **5**, gave the expected dark, red-brown color, and the addition of NaOMe / MeOH and subsequent workup paralleled the procedure used in Scheme 1. Mass spectral analysis of the crude reaction product showed none of the desired rearranged product, either with or without the amino-protective group. Instead, there was a very prominent mass peak corresponding to M=496 and a complex array of unidentified peaks in the 530–929 mass range, several of which were confirmed as probable molecular ions by addition of a trace of LiCl and observing $(M + Li)^+$ cluster peaks. TLC showed that the crude product was indeed a complex mixture of at least eight UV-active components: one was starting material **13**; the others were all intensely colored spots ranging from fluorescent yellows and oranges to reds and deep violets. Structure **15** fits the mass 496 requirement for the most prominent peak in the mass spectrum. Such an assignment is obviously very tentative, but if correct, it could account for both the intense colors and the high molecular weights. If some of the diisopropylaminomethylene protective groups were



Scheme 2

lost, as expected, and both the 3- and the 6-amino groups of **14** became involved in azo-coupling, then several arrangements of molecules containing multiple pyridine units are possible. Because no evidence of the desired product could be found, this work was abandoned in favor of the alternative route to targets **3** and **4** shown in Scheme 2.

Sircar, *et al.*[24] have reported the conversion in several steps of the substituted chloropyrimidinone **16** into a series of 8-amino-9-substituted- 9-deazaguanines illustrated by structure **17** in Scheme 2.

The displacement of the chlorine of **18**[25] was more difficult than that of **16**, as expected. Several trial reactions of **18** with the anion generated from ethyl 2-cyano-3-phenylpropionate (**19**)[26] by NaH in which reaction time, temperature, reactant ratios, number of additions, *etc.*, were varied, failed to significantly improve the initial yield (~20%) of **20**. Treatment of **20** with 1N NaOH for 6 h followed by acidification achieved both decarboxylation and hydrolysis of the ethyl carbamate to give **21** in 72% yield.

Shorter reaction times were sufficient for decarboxylation, but they resulted in mixtures of the ethyl carbamate and the corresponding amine.

Reduction of **21** with $Na_2S_2O_4$ in 1N NaOH and treatment of the crude product with conc. HCl gave a mixture of **4, 22,** and **25**. The isolation of **4** and **22** was complicated by the rapid cyclization and oxidation of **22** to the dimeric compound **25**. A portion of a freshly precipitated sample of **4** was utilized for the enzyme assay. The rest of the sample was acetylated to prevent the formation of **25** and to provide a stable derivative of **4** for characterization. Chromatographic purification of the product yielded pure material, which was identified as the diacetyl derivative **24**. This purification procedure also provided a smaller amount of a second compound (**26**), a tetraacetyl derivative of **25**. The simplicity of the NMR spectrum of **26** supports a symmetrical structure as depicted. In a series of small-scale experiments, intermediate **21** was subjected to catalytic hydrogenation in EtOH containing added conc. HCl. The ratios of acid to **21** varied from 2:1 to 7:1, and reaction times varied from 16 h to 3 days, but the outcomes were essentially the same. In each case mass spectral analysis of the crude product showed a peak corresponding to the desired product **3**, and a stronger peak corresponding to **22**. There were several trace components, and in common with the series leading to **4**, reaction products and their solutions darkened rapidly. In the free base form, solutions of the two major products deposited tarry brown to black material within a few hours. Based only on TLC observation, the desired product **3** was never more than 25% of the crude product mixture. In an alternative trial, hydrogenation of **21** in EtOH containing exactly one equivalent of 1N NaOH gave the best yield of **3** as judged by TLC. Purification of pooled samples containing **3** on a silica gel column gave a pure sample as the free base. As expected, this sample darkened rapidly, but recolumning and reprecipitation of the hydrochloride salt gave sufficient **3** • HCl for analysis and biological evaluation.

Biological Results

The IC_{50} values against human erythrocytic PNP[27] for compounds **1-4** as well as three related 9-deazapurines are presented in Table 1. As expected, **1**, with no hydrophobic group at C-9 of the dideazapurine, was an extremely poor inhibitor of the enzyme. Surprisingly, **3** was about 100-fold less potent an inhibitor than 9-benzyl-9-deazaguanine. The addition of an amino group at C-8 (**4**) made little difference.

Table 1. PNP Inhibition Data[‡]

Compound	IC_{50} (μM)
1	240
2	0.47
3	4.7
4	6.0
9-Benzyl-9-deazahypoxanthine	0.12[#]
9-Phenylthio-9-deazahypoxanthine[∞]	0.11[#]
9-Benzyl-9-deazaguanine	0.042[#]

[‡] Human erythrocytic PNP. For the experimental procedure, see ref. 27.
[#] S. Bantia, unpublished data, BioCryst Pharmaceuticals, Inc.
[∞] This compound was synthesized at BioCryst by Dr. Philip Morris.

Inhibitory data for 9-benzyl-9-deazahypoxanthine and 9-phenylthio-9-deazahypoxanthine are comparable, as shown in Table 1. In the 3,9-dideazapurine series, the 9-phenylthio compound (**2**) is only slightly less potent than its 9-deazapurine analog. We have no explanation for these unexpected results.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Evaporations were carried out *in vacuo* on a rotary evaporator or under high vacuum by short-path distillation into a glass trap cooled in dry ice/acetone. Samples were dried under high vacuum over P_2O_5 at room temperature unless an elevated temperature is specified. Elemental analyses were performed at Southern Research Institute or by Atlantic Microlabs, Atlanta, Georgia. Analtech precoated (250 μm) silica gel G (F) plates were used for thin-layer chromatography (TLC), and spots were detected by irradiation with a UV-254 light or by absorption of iodine vapor. Column chromatography in either the gravity or flash column mode was performed with silica gel 60 (230-400 mesh) using the slurry method of column packing. Mass spectra were obtained on a Varian-MAT 311A mass spectrometer in the fast-atom bombardment (FAB) mode. Infrared spectra were recorded on a Nicolet FT IR spectrophotometer, model 10DX, using pressed KBr discs. Ultraviolet spectra were determined in 0.1N HCl (pH 1), pH 7 buffer, and 0.1N NaOH (pH 13) with a Perkin-Elmer UV-visible-near

infrared spectrophotometer Model Lambda 9; maxima are reported in the format λ_{max} in nanometers ($\varepsilon \times 10^{-3}$). ^{1}H-NMR spectra were recorded on a Nicolet/GE NT300NB spectrometer operating at 300.635 MHz. Chemical shifts (δ) are reported in ppm downfield from internal tetramethylsilane. Chemical shifts of multiplets are measured from the approximate center of the multiplet, and coupling constants (*J*) are reported in Hz. When solvents are included in elemental analyses, their presence was confirmed by ^{1}H NMR. In the ^{1}H NMR spectral data for compounds **1**, **2**, **3**, **4**, **24**, **25**, and **26**, proton locations are designated according to pyrrolo[2,3-*c*]pyridine numbering, which is used in the Experimental Section only.

1,6-Dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (3,9-dideazahypoxanthine, **1**). Raney nickel (20 g weighed wet) was stirred in a hydrogen atmosphere for 30 min at normal pressure. A lukewarm solution of **2** (660 mg, 2.72 mmol) in EtOH (80 mL) was added to the catalyst, and the mixture was stirred under H_2 at room temperature for 18 h. Only a trace of product was detected by TLC, so the mixture was refluxed under N_2 for 3 days, after which TLC showed only a trace of starting material. The mixture was filtered through a thin Celite pad under N_2 pressure, and the catalyst was washed with hot EtOH (4x50 mL). Evaporation of the filtrate gave a green solid (315 mg, 86%). Column chromatography with $CHCl_3$ /MeOH (95:5) as eluent gave 87 mg (24%) of **1** with a trace contaminant. The chromatographically homogenous fractions were pooled and evaporated, and during the evaporation, the product began to crystallize in white plates. After thorough cooling, the solid was collected by filtration, washed with cold CH_2Cl_2 and dried *in vacuo* over P_2O_5 at room temperature overnight and at 82 °C for 8 h; yield 180 mg (49%); mp 304-305 °C dec. with prior charring. MS: m/z 135 $(M + H)^+$, 269 $(2M + H)^+$, 403 $(3M + H)^+$. UV: 0.1 N HCl- 224 (27.41), 255 (5.68), 263 (5.39), 289 (7.21); pH 7 buffer-224 (27.98), 255 (5.43), 264 (5.25), 288 (7.12); 0.1 NaOH-224 (27.97), 255 (5.08), 264 (4.87), 288 (6.88). ^{1}H NMR (DMSO-d_6): δ 6.30 (m, 1, H-3), 6.45 (d, 1, H-4, J = 6.6 Hz), 6.86 (m, 1, H-5), 7.27 (m, 1, H-2), 10.88 (br s , 1, 1-N*H*), 11.94 (br s, 1, 6-N*H*). *Anal.* Calcd for $C_7H_6N_2O$: C, 62.68; H, 4.51; N, 20.65. Found: C, 62.41; H, 4.35; N, 20.65.

1,6-Dihydro-3-(phenylthio)-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (9-phenylthio-3,9-dideazahypoxanthine, **2**). A solution of **8** (6.10 g, 19.0 mmol) in $CHCl_3$ (200 mL) was

stirred with conc. HCl (25mL) for 3 days. Evaporation of solvents and reevaporation of the residue with EtOH and toluene gave 4.4 g of green foam shown by MS and TLC to be a mixture of **9** and a smaller quantity of **2**. A solution of the foam in conc. HCl (800 mL) was refluxed for 2 h, and after thorough cooling, the deposit of sand-colored solid was collected by filtration, washed with cold HCl, and dried *in vacuo* over P_2O_5 and NaOH pellets; yield 3.18 g (60% as **2**•HCl). Small-scale recrystallization experiments with 1N HCl and H_2O/EtOH (9:1) gave crystalline material, but without significant improvement in purity as judged by TLC. A solution of the crude sample in $CHCl_3$ /MeOH (1:1) was evaporated with silica gel (~ 50 g), and the mixture was layered onto a silica gel column and eluted with $CHCl_3$ /MeOH (95:5) to give homogenous fractions of **2** totaling 1.77 g (38.5%) and an additional 0.67 g (14.6%) of slightly less pure product. The homogenous fractions were pooled by slurrying in CH_2Cl_2, and the white crystalline solid was collected by filtration and dried *in vacuo* over P_2O_5 at 82° for 24 h; yield 1.73 g (38%); mp 284-286° C dec. MS: m/z 243 $(M + H)^+$, 242 $(M)^+$. UV: 0.1N HCl-224 (31.8), 250 (sh), 290 (sh); pH 7 buffer -224 (31.8), 250 (sh), 290 (sh); 0.1N NaOH - 233 (28.1). ^{1}H-NMR (DMSO-d_6): δ 6.27 (d, 1, H-4, $J_{4,5}$= 7.0 Hz), 6.94 (t,1,H-5), 7.01, 7.08, 7.22 (m, 5, C_6H_5), 7.64 (s, 1, H-2), 11.11 (br s, 1, 6-NH), 12.55 (br s, 1, 1-N*H*). *Anal.* Calcd for $C_{13}H_{10}N_2OS$: C, 64.44; H, 4.16: N, 11.56. Found: C, 64.41; H, 4.21; N, 11.55.

5-Amino-1,6-dihydro-3-(phenylmethyl)-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (9-benzyl-3,9-dideazaguanine, **3**). A suspension of **21** (300 mg, 1.06 mmol) in EtOH (50 mL) was treated with conc. HCl (0.58 mL, 7 mmol), and the mixture was hydrogenated by shaking in a Parr apparatus at 50 lb/in H_2 pressure for 16 h over 10% Pd/C catalyst (300 mg).[28] Shaking was interrupted after 2 h, but the yellow color of **21** was still evident. After 16 h the colorless mixture was filtered through a Celite pad under N_2 pressure, and the pad was washed with warm EtOH (3x25 mL). The filtrate was evaporated; the residue was evaporated with CH_2Cl_2 (2x20 mL) and dried *in vacuo*, to give a beige solid (300 mg). TLC showed a new product spot and a stronger spot that was identified as **22**, as expected. MS showed mass peaks at 240 $(M+H)^+$, corresponding to **22**. Desired product **3** was the minor component.

A number of small-scale hydrogenations were tried in an effort to maximize formation of **3**. The mmol ratio of acid to **21** was varied from 7:1 (above) to 2:1. Reaction times were varied from as short as 3 h to as long as 3 days. Some reactions

were stopped after 16 h; fresh catalyst was added, and the reaction was continued. A different catalyst (30% Pd/C) was also tried. In one case the crude product was heated under N_2 in MeOH and 6N HCl. Finally, hydrogenation of **21** with one equivalent of NaOH present gave a yield of **3**, along with **22**, comparable to any of the acid procedures. None of these efforts succeeded in converting any **22** was present in the initial reaction product to more **3**. It was also observed that samples of **3** and **22** darkened rapidly, particularly in the free base form.

One of the samples (220 mg) containing **3** and **22** was chromatographed on a silica gel flash column using $CHCl_3$/MeOH/HOAc (95:5:1) as eluent to give 25 mg of *slightly* impure **3** in its free base form. MS: m/z 239$(M)^+$, 240$(M+H)^+$. UV: 0.1 N HCl 239(7.35), 308(8.57); pH 7 buffer 239(20.96), 264(sh), 323(5.42); 0.1 N NaOH – 233(19.58), 319(5.47). ^{1}H NMR (DMSO-d_6): δ 3.78 (s, 2, CH_2Ph), 4.89 (s, 2, NH_2), 5.27 (s, 1, H-4), 6.93 (d, 1, H-2), 7.21 (m, 5, phenyl C*H*), 10.06 (br s, 1, 6-N*H*), 11.09 (s, 1, pyrrole N*H*).

In order to obtain a sample of **3** for biological testing, four very dark samples from the pilot experiments containing **3** and **22** had been chromatographed as described above to give ~85 mg of impure **3**. This material was recolumned using $CHCl_3$/MeOH (95:5) as eluent to give 43 mg of **3** of improved quality. This material was recolumned (same conditions) taking extraordinary measures to protect the emerging product band. Fractions were collected under subdued light in screw-cap vials flushed with N_2. Pooling of fractions was done in a N_2-filled bag. After evaporations, N_2 was admitted rather than air. A solution of the product (~26 mg) in EtOH (5 mL) was treated with conc. HCl (0.2 mL) and precipitated as the hydrochloride salt by dropwise addition of Et_2O (50 mL). A second precipitation gave 19 mg of **3** • HCl as a nearly colorless solid that was dried *in vacuo* over P_2O_5 for 24 hours. MS: m/z 240 $(M+H)^+$, 479$(2M+H)^+$. UV: duplicate of the spectrum of the free base, above. The ^{1}H NMR spectrum in DMSO-d_6 on 3 mg of sample was very weak, as expected. Visible peaks were shifted downfield from their free-base positions reported above, and NH's were broadened. In lieu of a complete elemental analysis because of limited sample, an exact mass determination (positive FAB mode) was obtained from Analytical Instrument Group, Inc., Raleigh, NC 27616. Calculated mass of $(M+H)^+$ = 240.113472; Found: 240.11348, matching formula $C_{14}H_{14}N_3O$ $(M+H)^+$. Compound **3** is $C_{14}H_{13}N_3O$. A 10 mg portion of this sample was used for biological testing.

2,5-Diamino-1,6-dihydro-3-(phenylmethyl)-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (8-amino-9-benzyl-3,9-dideazaguanine, **4) and 2-[3,6-Diamino-2(1H) oxopyridin-4-yl]-2-(phenylmethyl) acetonitrile (22).** Solid sodium hydrosulfite (6.0 g, 29.3 mmol as the commercial 85% reagent) was added in one portion to a partial solution of **21** (1.50 g, 5.28 mmol) in 1N NaOH (70 mL). Under a slow N_2 stream, the mixture was placed into a water bath at 85 °C, forming an almost clear dark solution before solid began to separate. After 30 min in the 85° bath, an additional portion (1.0 g, 4.9 mmol) of $Na_2S_2O_4$ was added, and stirring was continued for 20 min. The slurry of pale yellow solid was cooled to near 0 °C in an ice bath, and the mixture was adjusted to pH 1 by addition of conc. HCl. After 15 min, the solid was collected by filtration under N_2, washed with ice-cold H_2O (25 mL) and liberally with Et_2O. A suspension of the crude product (~1.2 g) in conc. HCl (25 mL) was stirred under N_2 at room temperature for 2 h and then heated in a 60° water bath for 1 h. After cooling to 0°, the lemon-yellow suspension was filtered under N_2 pressure. A solution of the filter cake in EtOH (25 mL) was filtered under N_2 and evaporated. A stirred solution of the residual yellow foam in absolute EtOH (25 mL) was diluted dropwise with Et_2O (300 mL) to precipitate a pale yellow solid, which was collected under N_2, washed with Et_2O, and dried *in vacuo* over P_2O_5; yield 365 mg. The aqueous HCl filtrate from above was evaporated to dryness, and the residue was dissolved in EtOH (25 mL), filtered, and precipitated with Et_2O (300 mL) to give 707 mg of yellow solid. The 365 mg crop was shown by TLC to be a mixture of two main products although MS showed only a single strong $(M+H)^+$ at 255; thus the 365 mg crop was believed to be a mixture of **22** and **4**, both of which have a molecular weight of 254. The 707 mg crop was mostly one or the other, but which one was unknown. The remaining 340 mg of the 365 mg crop was stirred under N_2 with conc. HCl (10 mL) at room temperature for 2 h, warmed to 50° (water bath) for 1 h, and finally the yellow slurry was heated to nearly boiling to give a clear solution. Cooling slowly deposited a pale yellow solid, which was collected under N_2, washed with conc. HCl, and dried *in vacuo* over P_2O_5 and KOH pellets; yield 63 mg; mp 168-170 °C dec with prior sintering This material was the faster-moving (TLC) of the two product components of mass 254 and was identified by ^{1}H-NMR data as **22** by the coupling of the non-equivalent benzyl CH_2 protons to the adjacent –C*H*– proton. MS: m/z 255 $(M+H)^+$, 509$(2M+H)^+$. UV: 0.1 N HCl – 253, 364; pH 7 buffer – 241, 299, 374; 0.1 N NaOH – 240, 298. ^{1}H-NMR

(DMSO-d_6): δ 3.02 (dd, 1, -CH_aPh, J=14.1, J=7.3), 3.23 (dd, 1, -CH_bC$_6$H$_5$, J=5.0, J=14.1), 3.72 (s, 2, NH_2 ?), 3.88 (m, 1, -CH(CN)CH$_2$Ph, J=7.3, J=5.0), 5.66 (s, 1, Pyr-H5), 7.20 (m, 7, aromatic plus NH), 10.21 (s, 1, NH), 11.90 (br, 1, NH).

The conc. HCl filtrate from the 63 mg crop was evaporated, and the residue was stirred for 3 days under N_2 with 5 mL of conc. HCl. The suspension of light yellow solid was filtered under N_2, washed with conc. HCl, and dried *in vacuo* over P_2O_5 and KOH pellets; yield 111 mg. TLC indicated that this material was mainly the slower moving of the two products of mass 254 mixed with the by-product **25** (<10%) of mass 506. ^{1}H-NMR supported structure **4** as the main component as a hydrochloride salt. MS: m/z 255(M+H)$^+$, 506(M)$^+$, 506(M+H)$^+$. UV: 0.1 N HCl – 236, 375; pH7 buffer – 236, 292 (sh), ~400; 0.1 N NaOH – no maximum. ^{1}H-NMR (DMSO-d_6): δ 3.10 (d, 1, - CH_aPh), 3.32 (d, 1, -CH$_b$Ph, $J_{a,b}$ = 16.5 Hz), 3.72 (s, ?, NH(?)), 6.58 (d, 2, NH_2, J=6.7), 7.10 (m, ~7, aromatic CH + ring proton H-4 + NH), 10.28 (s,1, NH), 11.55 (s, 1, NH). This sample of **4** from the 707 mg crop was dissolved in EtOH (5 mL); the filtered solution was diluted dropwise with Et_2O (50 mL) to precipitate **4** as its hydrochloride salt. Of the 94 mg of dried, pale yellow solid obtained, a portion was submitted for PNP inhibition assay; MS, UV, and NMR data were similar to that reported for the 111 mg crop described above.

The original 707 mg crop of **4** plus the 111 mg obtained later represents *μ ca.* 47% yield of **4**, calculated as a dihydrochloride salt.

2-(3-Amino-2-methoxypyridin-4-yl)-2-(phenylthio)acetaldehyde Dimethylacetal (8). The apparatus for collection of the liquid chlorine consisted of a dry graduated centrifuge tube fitted with a rubber septum equipped with a gas inlet and outlet tube vented through a gas bubbler filled with mineral oil. The centrifuge tube was cooled in a dry ice/acetone bath contained in a clear glass beaker so that the graduations could be read. Chlorine from a cylinder was passed in until 2.5 mL (~ 7.8 g, 110 mmol) of liquid was collected. Dry CH_2Cl_2 (10 mL) that had been precooled in the same bath was added by hypodermic syringe, and the solution was transferred with the aid of a hollow stainless steel needle and N_2 pressure to a 3-necked flask containing 100 mL of dry CH_2Cl_2 precooled to –75 °C in a dry ice/acetone bath. The flask was equipped with a pressure-equalized addition funnel, a thermometer, a nitrogen inlet to maintain a positive pressure, and vented through a gas bubbler. To this solution at –75 °C was added a solution of 2-

(phenylthio)acetaldehyde dimethyl acetal[18] (24.34 g, 123 mmol) in CH_2Cl_2 (25 mL) at such a rate that the temperature was kept below –65 °C. The solution was stirred for 10 min more, and then a solution of 3-amino-2-methoxypyridine **5**[19,20] (6.10 g, 49.1 mmol) and dry triethylamine (4.97 g, 49.1 mmol) in CH_2Cl_2 (50 mL) was added dropwise. The resulting deep red-brown mixture was stirred at –70 °C for 4 h, and then a solution of sodium methoxide in methanol (69.0 mL of 2.14 N; 148 mmol) was added rapidly dropwise. External cooling was discontinued, and the dark mixture was stirred at room temperature for 18 h under N_2. The reaction mixture was shaken with 10% aqueous NaOH (200 mL). The aqueous layer was back-extracted with CH_2Cl_2 (2x100 mL), and the combined organic layers were dried (Na_2SO_4) and evaporated to give a dark odorous oil (33 g). Column chromatography on silica gel with $CHCl_3$ /CH_3OH (99:1) as eluent gave a rough separation of product 14 from starting acetal. Careful recolumning of the product-containing band (~ 10 g) with $CHCl_3$ as eluent gave **8** as a clear, viscous, pale green oil; yield 7.0 g (45%). MS: m/z 321$(M+H)^+$, 320$(M)^+$, 179(320-PhS-$CH_3OH)^+$, 75$[CH(OMe)_2]^+$. UV: 0.1N HCl - 247(7.78), 320(6.27); pH 7 buffer- 240(sh), 298(5.80); 0.1 N NaOH-240(sh), 298(5.77). ^{1}H-NMR ($CDCl_3$) δ 3.37, 3.47 [2s, 3 each, $CH(OCH_3)_2$], 3.96 (s, 3, 2-*OCH_3*), 4.18 (s, 2, NH_2], 4.54 [d, 1,-*CH*(SPh)*CH*$(OMe)_2$], (*J* = 6.1 Hz), 4.75 (d , 1, *CH*$(OMe)_2$), 6.77 (d , 1, H-5), 7.17, 7.25 (m, 5, C_6H_5), 7.47 (d, 1, H-6, $J_{5,6}$ = 5.4 Hz).

6-[[(Diisopropylamino)methylene]amino]-2-methoxy-3-nitropyridine (12). Under an atmosphere of dry N_2, a suspension of **11**[21] (9.52 g, 56.3 mmol) in N,N-diisopropylformamide dimethyl acetal[23] (23.20 g, 132 mmol, 2.3 eq.) was stirred at room temperature for 1 h, becoming a clear red-orange solution in ~20 min. Volatiles were evaporated at rt under high vacuum using a glass lyophilizer trap cooled in dry ice/acetone. The yellow solid residue was dried *in vacuo* over P_2O_5 ; yield 15.77 g (99.9%). This material was used in the next step without purification. In a previous pilot run that also gave a nearly quantitative crude yield, the sample was chromatographed on a short silica gel column with $CHCl_3$ as eluent. During evaporation of the pooled column fractions, the product **12** crystallized in bright yellow rosettes. The yield from 500 mg of **11** was 502 mg (61%); mp 82-83 °C. MS: m/z 281 $(M + H)^+$. UV: 0.1N HCl 216 (13.32), 253 (13.91), 344 (19.00); pH 7 buffer 232 (8.50), 270 (sh), 3997 (27.23); 0.1N NaOH 232 (8.73), 270 (sh), 396 (27.76). ^{1}H NMR ($CDCl_3$) δ 1.37, 1.39[2d, 6 each, *J* =

6.7 Hz for each doublet, -CH(CH_3)$_2$], 3.73, 4.92 [2m, 1 each, -C*H*(CH_3)$_2$], 4.08 (s, 3, OCH_3), 6.49 (d, 1, $J_{4,5}$ = 8.8, H-5), 8.27 (d, 1, H-4), 8.87 (s, 1, N=C*H*-N). *Anal.*Calcd for $C_{13}H_{20}N_4O_3$: C, 55.70; H, 7.19; N, 19.99. Found: C, 55.61; H, 7.25; N, 20.05.

3-Amino-6-[[(Diisopropylamino)methylene]amino]-2-methoxypyridine (13). In a 2L, 3-necked flask fitted with a magnetic stirrer and N_2 inlet and outlet tubes, a solution of **12** (15.19 g, 54.2 mmol) in tetrahydrofuran (600 mL) was added to a solution of sodium hydrosulfite (56.61 g, 355.1 mmol, 6 eq, Aldrich 85%) in 600 mL of 0.1M phosphate buffer (13.61 g of KH_2PO_4 per liter of water adjusted to pH 7.1 with KOH pellets).[24] The resulting 2-phase mixture was stirred vigorously under N_2; a mild exotherm was noted (~40° maximum). After 2 h the mixture was evaporated *in vacuo* ; the residue was triturated with cold water (100 mL), and the cream-colored solid was collected by filtration under N_2 pressure and washed with cold water (2x25 mL); yield ~100% as the free amine; 92% as the bisulfite salt. The crude product was stirred under N_2 for 15 min with a solution of Na_2CO_3 (4.74 g, 45 mmol) in water (125 mL); the solid was collected under N_2 pressure, washed with cold water (3 x 50 mL) and dried *in vacuo* over P_2O_5 overnight; yield 10.29 g (76%). Column chromatography on silica gel with $CHCl_3$/MeOH (85:15) as eluent gave 6.78 g (50%) of **13,** mp 126-128 °C dec. Special care (subdued light, N_2 atmosphere where possible) was taken during workup of fractions, but the solid product discolored rapidly from nearly colorless to gray. This material was characterized only by MS and NMR spectra; the rest was used immediately in the next step, the failed Gassman reaction described in the text. MS: m/z 251 (M + H)$^+$. ^{1}H NMR ($CDCl_3$): δ 1.28[br d, 12, CH(C*H*$_3$)$_2$] 3.48 (br s, 2, N*H*$_2$), 3.64, 4.95[2br s, 1 each, -C*H*(CH_3)$_2$], 3.97 (s, 3, *OCH*$_3$), 6.46 (d, 1, H-4), 6.89 (d, 1, H-5, $J_{4,5}$~9Hz), 8.58 (s, 1, N=C*H*-N).

α-Ethoxycarbonyl-α-[6-(ethoxycarbonylamino)-3-nitro-2(1*H*)-oxopyridin-4-yl]-benzenepropanenitrile (20). Under an atmosphere of dry N_2 and with external cooling in an ice water bath, a solution of freshly distilled ethyl 2-cyano-3-phenylpropionate (**19**, 28.20 g, 138.8 mmol)[26] in dry N,N-dimethylacetamide (30 mL) was added dropwise in 5 min with magnetic stirring to a 60% dispersion of NaH in mineral oil (3.34 g; ~2.0 g, 83.4 mmol of NaH), accompanied by vigorous H_2 evolution and formation of a thick white slurry. The cooling bath was removed, and a solution of ethyl 4-chloro-5-nitro-6(1*H*)-oxo-2-pyridinecarbamate **18**[25] (4.63 g, 17.7 mmol) in

DMAc (40 mL) preheated to 80° C was added rapidly to the white slurry. The resulting red-orange solution was heated in a 90° oil bath for 5 h, and heating was continued overnight at 65° C. The cooled solution was adjusted to pH 6.5 (meter) with 1N HCl and solvents were evaporated *in vacuo*. The gummy residue was washed by trituration and decantation with hexane (4x100 mL). A turbid solution of the residue in EtOAc (500 mL) was filtered and evaporated. Column chromatography of the residual oil with $CHCl_3$ /MeOH/HOAc (98:2:1) as eluent gave partial purification. Fractions containing the desired product (4.8 g) were rechromatographed using (99:1:1) as eluent, and fractions containing the purified product were pooled, evap., and reevaporated 2x with toluene to aid removal of residual acetic acid. The dried yellow solid weighed 2.58 g (34%). A small portion of **20** was recrystallized from EtOH to give an analytical sample; 97 mg, mp 233-234°C dec. MS: m/z 429 $(M + H)^+$, 91 $(PhCH_2)^+$. UV: 0.1N HCl-345 (sh); pH 7 buffer-234 (sh), 264(8.17); 0.1N NaOH-234 (sh). ^{1}H-NMR (DMSO-d_6): δ 1.12, 1.26(t, t; 3 each; CH_3 of EtO), 3.62(s, 2, PhCH_2), 4.18(m, 4, CH_2 of EtO), 7.20–7.33(m, 6, Ph, PyrH-5), 10.70, 12.62 (very broad, NH). *Anal.* Calcd for $C_{20}H_{20}N_4O_7$: C, 56.07; H, 4.71; N, 13.08. Found: C, 55.90; H, 4.64; N, 13.09.

α-(6-Amino-3-nitro-2-oxo(1H)-pyridin-4-yl)-benzenepropanenitrile (21). A solution of **20** (4.54 g, 10.6 mmol) in 1N NaOH (300 ml) was stirred at room temperature for 6 h, depositing an orange solid after ~1.5 h. The slurry was adjusted to pH 2 with conc. HCl and as stirred at pH 2 for 30 min; then it was chilled to ~5 °C and readjusted to pH 6 with NaOH. The solid was collected, washed with cold H_2O, and dried *in vacuo* over P_2O_5; yield 2.91 g (97%). This crude sample was stirred with MeOH (200 mL), collected, and washed with CH_2Cl_2 (2x100 mL) to remove an unknown minor contaminant. The dried yellow solid **21** (1.85 g, 61%) was suitable for use as an intermediate without further purification. The filtrate from the solvent wash was evaporated. The residue (0.9 g) was chromatographed on silica gel using $CHCl_3$/MeOH/HOAc (90:10:0.1) as eluent to give an additional 377 mg (12%) of **21.** A homogeneous (TLC) fraction was taken for analysis; 129 mg; mp ~255 °C dec. with prior charring. MS: m/z 285 $(M+H)^+$. UV: 0.1N HCl-304 (2.97), 395 (13.77); pH 7 buffer-303 (3.17), 396 (13.26); 0.1N NaOH-271 (6.35). ^{1}H NMR (DMSO-d_6): δ 2.94, 2.99 (d, d, 1, -CH_aPh), 3.23, 3.28 (d, d, 1, -CH_bPh), 4.79, 4.82 (d, d, 1, -C*H*-CH_2Ph), 5.69 (s, 1, Pyr-H5), 7.32 (m, 5, phenyl), 7.64 (br s, 2, NH_2), 11.99 (br s, 1, 1-NH).

Anal. Calcd for $C_{14}H_{12}N_4O_3 \cdot 0.2\ H_2O$: C, 58.41; H, 4.34; N, 19.46. Found: C, 58.41; H, 4.40; N, 19.26. Mass spectral analysis of a column fraction containing the by-product mentioned above gave 303$(M+H)^+$ and 309$(M+Li)^+$ with LiCl. Mass 302 corresponds to Pyr-4-CH(Bn)-$CONH_2$, $C_{14}H_{14}N_4O_4$, formed by partial hydrolysis of the nitrile; 463 mg, 14%.

Synthesis of Acetylated Compounds 23, 24, and 26. The rapid conversion in solution of **4** to **25** and the presence of HCl/H_2O had badly degraded the quality of NMR spectra. Preparation of stable acetyl derivatives was intended to halt the conversion process and provide stable compounds in their free base forms for spectral examination.

A 70 mg sample known to contain a lesser quantity of **22** and a greater quantity of **4** was dissolved in pyridine (10 mL), and acetic anhydride (1 mL) was added at room temperature. After 1 h, solvents were evaporated, and the residue was evaporated with MeOH and toluene to aid removal of Ac_2O and HOAc. Column chromatography on silica gel with $CHCl_3$/MeOH/HOAc (95:5:0.1) as eluent gave a 5 mg sample shown by MS and NMR to be a diacetyl derivative of **22**; TLC: R_f 0.45 with $CHCl_3$/MeOH/HOAc 90:10:1. MS: m/z 339 $(M+H)^+$ corresponding to **23**. ^{1}H NMR (DMSO-d_6): δ 2.08, 2.11 (2s, 3 each, Ac C*H*$_3$), 2.99, 30.03 (dd, 1 each, non-equivalent C*H*$_2$Ph, *J*=10, *J*=5), 4.24 (q, 1, Pyr-C*H*(CN)), 7.31 (m, 6, Ph + Pyr ring proton), 9.19 (s, 1, -N*H*Ac), 10.0 – 12.5 (very br, 2, NH). Further elution of the column gave a second band (30 mg, R_f 0.26 with $CHCl_3$/MeOH/HOAc 90:10:1) of product that was shown by MS and NMR to be **24**, the diacetyl derivative of **4**. The exact location of the two Ac groups among the three available (NH) sites is tentative. MS: m/z 339 $(M+H)^+$. ^{1}H NMR (DMSO-d_6): δ 2.00, 2.09 (2s, 3 each, Ac C*H*$_3$), 3.88 (s, 2, C*H*$_2$Ph), 5.97 (s, 1, ring proton H-4), 7.18, 7.26 (m, 5, phenyl), 10.31, (s, 1, NH), 10.42 (s, 1, NH), 11.34 (br s, 2, NH_2).

Some impure samples known to contain both **4** and **25** were combined (199 mg) and reacted with Ac_2O (2 mL) in pyridine (15 mL) for 3 h. Workup was as described above. Column chromatography with $CHCl_3$/MeOH (4:1) as eluent gave two significant product bands. The first was not homogeneous; it was recolumned using $CHCl_3$/MeOH (9:1) to give 36 mg of solid **24** that was identical by TLC [R_f 0.62 with $CHCl_3$/MeOH/HOAc (80:20:1)] to authentic **24** described above. In addition to MS and NMR spectral data that confirmed the data given for **24**, above, this sample had the following characteristics. mp 273-275 °C *dec.* UV: 0.1 N HCl – 253(28.54),

290(11.11); pH 7 buffer – 253(28.13), 290(11.60); 0.1 N NaOH – 255(21.39). *Anal.* Calcd for $C_{18}H_{18}N_4O_3 \cdot 0.5\ H_2O$: C, 62.24; H, 5.51; N, 16.13. Found: C, 62.35; H, 5.34; N, 15.98.

The second product band from the column ($CHCl_3$/MeOH 4:1) gave a solid (28 mg, R_f 0.43 with $CHCl_3$/MeOH/HOAc 80:20:1) that was shown to be **26**, the tetraacetyl derivative of **25**. The simplicity of the NMR spectrum requires a symmetrical arrangement like that drawn for **26**, and the absence of any – N*H*$_2$ resonance requires that two of the four Ac's must be located on the two 2-NH_2 groups. The location of the other two Ac's on the pyrrole nitrogens is probable, but tentative. mp: chars, but does not melt below 380 °C. MS: m/z 675 $(M+H)^+$. UV: sample too insoluble in MeOH or EtOH to get a spectrum; a solution in DMSO run against a DMSO blank gave the following: 0.1 N HCl – 301 (22.48); pH 7 buffer – 301 (22.98); 0.1 N NaOH – no maximum. ^{1}H NMR (DMSO-d_6): δ 1.74, 2.04 (s, s, 3 each, Ac C*H*$_3$), 2.99, 3.52 (d, d, 1 each, -C*H*$_2$Ph, $J_{a,b}$ = 17.0), 6.49, 7.01 (m, m; 2,3; phenyl C*H*), 8.37 (s, 1, ring proton H-4), 10.11 (s, 1, 2-N*H*-), 11.35 (s, 1, 6-N*H*), 11.70 (s, 1, 5- AcN*H*-). *Anal.* Calcd for $C_{36}H_{34}N_8O_6 \cdot 2.5\ H_2O$: C, 60.08; H, 5.46; N, 15.57. Found: C, 59.93; H, 5.35; N, 15.56.

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