Synthesis and Biological Evaluation of 6-Amino-1*H*-pyrrolo[3,2-*c*]pyridin-4(5*H*)-one (3,7-Dideazaguanine)

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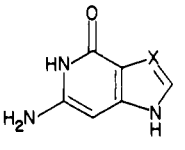
The synthesis of 6-amino-1*H*-pyrrolo[3,2-c]pyridin-4(5*H*)-one (3,7-dideazaguanine, **2**) has been accomplished from 3-(ethoxycarbonyl)pyrrole-2-acetonitrile. In contrast to 3-deazaguanine, compound **2** did not show any antitumor, antiviral, or antibacterial properties. Furthermore, it was not a substrate for hypoxanthine-guanine phosphoribosyltransferase or purine nucleoside phosphorylase.

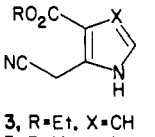
3-Deazaguanine (**1**)[1] has been shown to possess antitumor,[2] antiviral,[3] and antibacterial[4] properties. In an effort to determine the structural features of **1** that are required for its biological properties,[5-7] 3,7-dideazaguanine (6-amino-1*H*-pyrrolo[3,2-*c*]pyridin-4(5*H*)-one, **2**) arose as a useful target compound. The synthesis of **2**[8] and its biological properties are described herein.



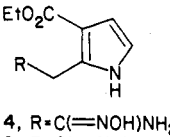
1, X = N
2, X = CH

Chemistry. Initially, the simplest means to **2** seemed to be either (i) via reaction of the readily available 4,6-diaminopyridin-2(1*H*)-one[9] with chloroacetaldehyde[10] (Scheme I) or (ii) by treatment of 3-(ethoxycarbonyl)-pyrrole-2-acetonitrile (**3**)[11] with anhydrous ammonia.[12] However, method (i) produced polymeric products and method (ii) led to recovery of starting material.

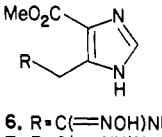
Efforts then turned to treating **3** with hydroxylamine, which gave ethyl 2-[2-amino-2-(hydroxyimino)ethyl]-pyrrolo-3-carboxylate (**4**) in a manner similar to the first step (**5** → **6**) in a reported[13] preparation of **1**. Subjecting **4** to a variety of Raney nickel catalyzed hydrogenolysis conditions, including those described[13] for transforming **6** into **1** via **7**, led not to the expected **8** (by TLC) but instead resulted in either hydrolysis to 3-(ethoxycarbonyl)pyrrole-2-acetamide (**9**)[14] from aqueous reaction systems or recovery of starting material under anhydrous circumstances.



3, R = Et, X = CH
5, R = Me, X = N



4, R = C(=NOH)NH$_2$
8, R = C(=NH)NH$_2$
9, R = CONH$_2$



6, R = C(=NOH)NH$_2$
7, R = C(=NH)NH$_2$

Even though this latter method failed to produce **2**, the ease of formation of **4** from **3** indicated that the nitrile functionality of **3** was capable of reacting with nucleophiles and that the failure of ammonia to convert **3** into **2** was due to the diminished nucleophilicity of ammonia (relative to hydroxylamine). To overcome this problem, acidic conditions were used to enhance the electrophilicity of the nitrile carbon of **3** and, as a consequence, improve its reactivity with ammonia. Thus, treatment of **3** with an-

Scheme I

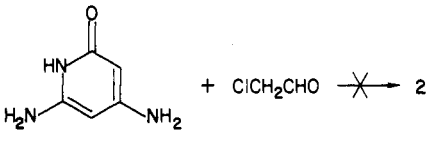


Table I. Effect of **2** on Mice Inoculated Intraperitoneally with P388 Lymphocytic Leukemia[a]

drug	dose, mg/kg	wt differences,[b] g	control body wt change, g[c]	T/C, %	median life span, days postimplant
		Experiment 1			
untreated controls			1.4		10.8
2	200	−1.6		114	12.4
	100	−0.6		113	12.3
	50	−0.2		108	11.7
		Experiment 2			
untreated controls			0.9		12.9
2	400	−0.7		126	16.3

[a] P388 tumor cells (10^6) were implanted ip in groups of 6 CDF_1 mice (experiment 1) or BDF_1 mice (experiment 2) (30 mice in untreated control groups) 24 h before initiation of therapy. A saline solution of **2** was administered ip on days 1–5. Characteristics of this ip model have been described previously.[24] [b] Average animal body weight change of test group minus that of control animals. The average weight changes for each group were obtained by subtracting the average weight on day 1 from that on day 5. [c] Average weight of the control animals on day 5 minus the average weight on day 1.

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Table II. Antiviral Activity of **2** and Related Compounds

	minimal inhibitory concentration,[a] μg/mL											
	in primary rabbit kidney cells				in Vero cells					in HeLa cells		
compd	HSV-1 (KOS)[b]	HSV-2 (G)[b]	vaccinia virus	VSV[b]	measles virus	reovirus type I	parainfluenza virus type 3	Sindbis virus	Coxsackie virus type B4	VSV[b]	Coxsackie virus type B4	polio virus type 1
3,7-dide-azaguanine (**2**)	>400	>400	400	>400	400	>400	>400	>400	>400	>400	>400	>400
3-deazaguanine (**1**)	4	150	0.1	15	2 (10)[c]	2 (10)	2 (10)	10	0.2	2	2	1
ribavirin	>400	>400	40	40	20	40	10	40	200	4	40	20
(*S*)-DHPA[b]	>400	>400	10	7	20	40	4	>400	70	40	>400	>400

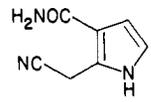
[a] Required to reduce virus-induced cytopathogenicity by 50%. Cytopathogenicity was recorded as soon as it reached completion in the control (virus-infected, untreated) cell cultures. [b] Abbreviations: HSV-1 (KOS), herpes simplex virus type 1 (strain KOS); HSV-2 (G), herpes simplex virus type 2 (strain G); VSV, vesicular stomatitis virus; (*S*)-DHPA, (*S*)-9-(2,3-dihydroxypropyl)adenine. [c] In parentheses: minimal cytotoxic concentration (μg/mL) causing a microscopically detectable alteration of normal cell morphology. For (*S*)-DHPA and ribavirin no such alteration was observed at the highest concentration tested (400 μg/mL). For 3-deazaguanine, cytotoxicity was only evident beginning with the fourth day.

Table III. Comparative Growth Inhibition of CHO Cells and Two Mutant CHO Cell Lines by 3-Deazaguanine (**1**) and 3,7-Dideazaguanine (**2**)

cell line	enzyme deficiency	minimum inhib concn, mM	
		1	**2**
CHO	none	0.01	1.0
AA^R-6[a]	APRTase	0.50	1.0
TG^R-1[b]	HGPRTase	1.50	1.0

[a] A mutant cell line of CHO that is resistant to 8-azaadenine and lacks adenine phosphoribosyltransferase. [b] A mutant cell line of CHO that is resistant to 6-thioguanine and lacks hypoxanthine-guanine phosphoribosyltransferase.

hydrous ammonia containing ammonium chloride in a sealed reaction vessel gave **2** in 67% yield. Whether the ammonia actually reacted at the nitrile center to give **8** as envisioned above could not be proven since no evidence for **8** could be obtained upon interruption of the reaction. Therefore the alternative amidation of **3** to **10** which could also have, subsequently, ring closed to **2** cannot be ruled out.



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Biological Results

The biological importance of the N-7 atom (by purine numbering) of **1** was indicated when compound **2** was found to possess weak, but unconfirmed, antitumor properties in the P388 lymphocytic leukemia screening program of the National Cancer Institute (Table I),[15] to lack antiviral potential (Table II), and to have no significant inhibitory effect on the growth of *Escherichia coli* B3 or CHO cells and two pertinent enzyme-deficient cell lines derived from the latter (Table III). It was also found that **2** was neither a substrate for nor an inhibitor of human erythrocytic hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) and human erythrocytic purine nucleoside phosphorylase (PNP) and did not inhibit CHO cell HGPRTase. These data reconfirm[16] the importance of a nitrogen atom at a position analogous to N-7 in purines in order for purine derivatives to interact with these enzymes.

One final property of **2** was its apparent conversion to the biologically inactive 3,7-dideazaxanthine[14] by mammalian (rat liver) guanase.[17] Since 3-deazaguanine is also a substrate for rat liver guanase,[18] it is unlikely that the reaction of **2** with guanase would account for its lack of antitumor, antiviral, and antibacterial properties.

Experimental Section

All melting points were obtained on a Thomas-Hoover or a Mel-Temp melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman AccuLab 3 spectrophotometer. The ^1H NMR spectra were determined at 60 MHz with a Varian EM-360 spectrometer and are reported in parts per million downfield from Me_4Si as an internal standard. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), and q (quartet). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. The microanalyses are indicated only by symbols of the elements, and for those elements so designated, the analytical results obtained were within ± 0.4% of the theoretical values.

Ethyl 2-[2-Amino-2-(hydroxyimino)ethyl]pyrrole-3-carboxylate (4). To a stirred mixture of 0.41 g (5.9 mmol) of hydroxylamine hydrochloride and 0.34 g (3.2 mmol) of Na_2CO_3

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(15) This data was obtained from the Developmental Therapeutics Program of the Division of Cancer Treatment of the National Cancer Institute.
(16) 7-Deazaguanine is also neither a substrate nor inhibitor of human erythrocytic PNP and HGPRTase (Stoeckler, J. D.; Agarwal, K. C.; Townsend, L. B.; Parks, R. E., Jr., unpublished results) and 7-deazainosine is not a substrate for human erythrocytic PNP (Parks, R. E., Jr.; Stoeckler, J. D.; Cambor, C.; Savarese, T. M.; Crabtree, G. W.; Chu, S.-H. "Molecular Actions and Targets for Cancer Chemotherapeutic Agents"; Sartorelli, A. C., Lazo, J. S., Bertino, J. R.; Eds.; Academic Press: New York, 1981; p 235.
(17) Different spectral shifts were observed for **2** when it was incubated with guanase alone or with guanase plus xanthine oxidase. This indicates that **2** is a substrate for guanase and its deamination product reacts with xanthine oxidase. No spectral change occurred with xanthine oxidase alone.
(18) Stoeckler, J. D.; Townsend, L. B.; Parks, R. E., Jr., unpublished results.

in 2 mL of H_2O at room temperature was added 0.53 g (2.97 mmol) of **3**.[11] This mixture was refluxed for 1 h at which time the solvent was evaporated in vacuo and the residue recrystallized from H_2O (with decolorizing charcoal) to produce 0.45 g (2.13 mmol, 71.5%) of **4** as white needles: mp 159–161 °C dec; IR (KBr) 3500–3100 cm^{-1} (br, OH), 1680 cm^{-1} (C=O); ^1H NMR (Me_2SO-d_6) δ 1.24 (t, 3 H, J = 7 Hz, Me), 3.63 (s, 2 H, CH_2 of side chain), 4.16 (q, 2 H, J = 7 Hz, CH_2 of ester), 5.36 (s, 2 H, NH_2, exchangeable with D_2O), 6.33 (d, 1 H, J = 2 Hz, H-4), 6.6 (d, 1 H, J = 2 Hz, H-5), 8.9 (s, 1 H, OH, exchangeable with D_2O), 11.23 (br s, 1 H, NH, exchangeable with D_2O). Anal. ($C_9H_{13}N_3O_3$) C, H, N.

Attempted Hydrogenolysis of 4 in the Presence of Raney Nickel. A sample of activated Raney nickel (W-2)[19] was washed well with H_2O and weighed wet (1 g). It was then transferred into a pressure hydrogenation bottle containing a solution of **4** (0.48 g, 2.27 mmol) dissolved in 20 mL of hot H_2O. This mixture was hydrogenated at 48–53 °C on a Parr apparatus at 45 psi for 24 h. The reaction mixture was removed from the Parr apparatus and heated to reflux for 15 min and the catalyst then removed by filtration. The solvent was evaporated in vacuo and the residue triturated with petroleum ether to give a dark brown product. Following recrystallization of this material from benzene–petroleum ether, the colorless crystals were identified as **9** by spectral, melting point, and TLC comparisons with an authentic sample.[14]

6-Amino-1*H*-pyrrolo[3,2-*c*]pyridin-4(5*H*)-one (3,7-Dideazaguanine, 2). A mixture of 0.89 g (5 mmol) of **3**,[11] 1.05 g (19.6 mmol) of ammonium chloride, and 2.5 mL of liquid NH_3 in a stainless steel Parr reaction vessel was heated at 125 °C for 24 h. The vessel was then cooled and vented to atmospheric pressure to remove the excess NH_3. The solid residue was extracted with hot acetonitrile. The combined extracts were cooled to room temperature to afford 0.5 g (3.35 mmol, 67%) of **2** as a white solid following recrystallization from acetonitrile: mp 223–225 °C (with sintering); IR (KBr) 3380 and 3220 (NH_2), 1640 cm^{-1} (C=O); ^1H NMR (Me_2SO-d_6) δ 5.22 (s, 2 H, NH_2), 5.40 (s, 1 H, H-7), 6.23 (d of d, 1 H, $J_{2,3}$ = 3 Hz, $J_{1,3}$ = 2 Hz, H-3), 6.67 (d of d, 1 H, $J_{2,3}$ = 3 Hz, $J_{1,2}$ = 2 Hz, H-2), 9.95 (s, 1 H, H-5), 10.67 (s, 1 H, NH). Anal. ($C_7H_7N_3O$) C, H, N.

Antiviral Activity. The antiviral assays were based upon the inhibition of virus-induced cytopathogenicity. The method for measuring this inhibition has been described previously.[20,21] The origin of the viruses was as follows: HSV-1 (KOS) (herpes simplex virus type 1, strain KOS), HSV-2 (G) (herpes simplex virus type 2, strain G), see ref 21; vaccinia virus, vesicular stomatitis virus (VSV), measles virus, Sindbis virus, Coxsackie virus type B4 and polio virus type 1, see ref 20; reovirus type 1 (ATCC VR-230) and parainfluenza virus type 3 (ATCC VR-93) were obtained from the American Type Culture Collection (Rockville, MD). The virus stocks were grown in primary rabbit kidney cells (HSV-1, HSV-2, VSV), Vero cells (measles, reo, parainfluenza, Coxsackie), HeLa cells (polio), chick embryo cells (Sindbis), or chorioallantoic membrane cells (vaccinia). The antiviral assays were run in either primary rabbit kidney cells, Vero cells, or HeLa cells, as indicated in Table II.

Antibacterial Activity. *E. coli* B3 was grown in a minimal medium as described previously[4] that was supplemented with thymine, 20 μg/mL, and casein hydrolysate, 0.4%. Growth curves were carried out in 250-mL nephelometer flasks containing 9 mL of medium and indicated additions to make a final volume of 10 mL. Incubation was at 37 °C in a New Brunswick G-77 waterbath shaker. The media were inoculated with a logarithmic culture such that the absorbance at 540 nm was 0.07–0.15. Thereafter absorption readings were taken every 30 min at 540 nm with a Bausch and Lomb Spectronic 20 colorimeter.

Growth Inhibition of Chinese Hamster Ovary (CHO) Cells. For the determination of a minimum inhibitory drug concentration, logarithmically growing CHO cells were trypsinized and appropriately diluted with McCoy's 5A medium containing dialyzed fetal calf serum as previously described.[6] They were dispensed into a series of 35-mm plastic Petri dishes (approximately 200 cells/dish) and were allowed to form clones in a broad range of drug concentrations: 0.5, 1, 5, 10, 50, 100, 200, 500, 1000, 1500, 2000, and 3000 μM. After incubation for 7 days in humidified CO_2 at 37.5 °C, the media was poured of, and the clones were fixed with 10% formaldehyde for 10 min and stained with 0.1% crystal violet. The minimum inhibitory drug concentration was the lowest tested concentration of drug that resulted in the formation of clones containing fewer than 50 cells.

HGPRTase Assay. Compound **2** was tested as a substrate for partially purified human erythrocytic HGPRTase by using the technique of high-pressure liquid chromatography (HPLC). The reaction mixture containing Tris-*HCl* (pH 8.0) (100 mM), $MgSO_4$ (4 mM), phosphoribosyl pyrophosphate (PRPP) (1 mM), mercaptoethanol (7 mM), **2** or guanine (0.5 mM) and erythrocytic HGPRTase (0.018 unit/mL) was incubated at 37 °C on a shaking water bath. After 30 or 60 min of incubation, perchloric acid extracts were made and analyzed by HPLC for the nucleotides in a manner previously described.[22]

PNP Assay. Human erythrocytic PNP was purified to sp act. 1.5 and assayed as described earlier.[23] The substrate activity of **2** with PNP was tested at room temperature (22 °C) by scanning the spectrum on a Perkin-Elmer 402 spectrophotometer before and after the addition of enzyme. The 3-mL reaction mixture contained 75 μM of **2**, 300 μM of ribose 1-phosphate, and 0.3 unit of PNP in 25 mM Tris-acetate, pH 7.5. Inhibitory activity was tested at 30 °C by observing the effects of **2** at concentrations ranging from 30 to 150 μM on the phosphorolysis of 30 μM inosine (the K_m concentration) as has been described.[23]

Guanase Assay. A crude guanase preparation was isolated from rat liver and assayed for activity as described elsewhere.[23] The substrate activity of **2** with guanase was determined at room temperature (22 °C) by recording the UV spectrum on a Perkin-Elmer 402 spectrophotometer before and after addition of enzyme. The 3.0-mL reaction mixture contained 67 μM of **2** and 0.01 unit of guanase in 80 mM Tris-acetate, pH 8.0. The reaction was monitored in the presence and absence of 0.1 unit of milk xanthine oxidase (type III, Sigma Chemical Co., St. Louis, MO).

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Registry No. **2**, 75938-36-2; **3**, 67464-81-7; **4**, 75938-41-9.

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