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Supplementary Material Available: Bond distances, bond angles, fractional coordinates, and temperature factors of (−)-3-bromocyproheptadine (**5**) and circular dichroism spectral features of the (+)-cyproheptadine atropisomers (6 pages). Ordering information is given on any current masthead page.

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Synthesis and Antitumor Activity of 5-Azacytosine Arabinoside[1]

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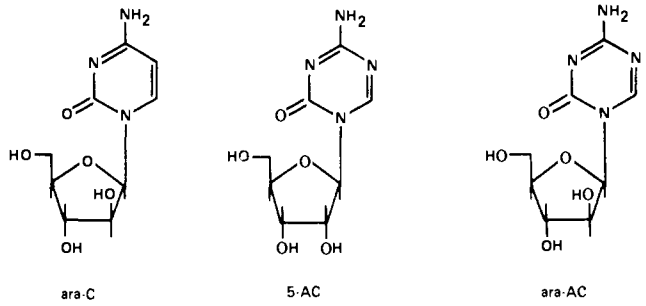
5-Azacytosine arabinoside (*ara*-AC) can be considered a combination of structural elements derived from the antitumor nucleosides cytosine arabinoside (*ara*-C) and 5-azacytidine (5-AC). The synthesis of *ara*-AC, for which standard methods were inadequate, was accomplished using the stable dihydro derivative as a synthetic intermediate. A novel dehydrogenation of the latter through the application of a trimethylsilylation–oxidation procedure gave *ara*-AC in good yield. Using murine L1210 leukemia as a test system, *ara*-AC was evaluated for antitumor properties in parallel determinations with 5-AC and *ara*-C. Although higher dose levels were necessary, *ara*-AC demonstrated a reproducibly greater efficacy in the L1210 system (% ILS = 144–148) than that shown by 5-AC (% ILS = 126–124) or *ara*-C (% ILS = 127–121). Moreover, initial data suggest that *ara*-AC exhibits less host toxicity than either 5-AC or *ara*-C. Although *ara*-AC can equally be considered an analogue of either 5-AC or *ara*-C, preliminary results indicate that *ara*-AC is chemically similar to 5-AC but biologically more closely related to *ara*-C.

The subtlety of a stereochemical inversion at the 2′ position of cytidine results in cytosine arabinoside (*ara*-C), which is an important agent in the treatment of leukemia.[3]

Bioisosteric replacement of the carbon-5 in cytidine with



nitrogen results in 5-azacytidine (5-AC), which is a nucleoside antimetabolite having established clinical antileukemic activity.[4] Although facile hydrolysis in aqueous formulations makes clinical administration of 5-AC somewhat complicated,[5] the hydrolytic instability, on the other hand, apparently contributes to the 5-AC antitumor effects as a consequence of the biosynthesis of less stable nucleic acid leading to disruption of secondary structure and chromosomal breakage.[6] Moreover, the prolonged antileukemic effect in mice shown by 5-AC[7] has been linked to a hydrolysis product.[8]

As part of our continuing interest in cytotoxic cytidine analogues,[9] we set out to construct a nucleoside having the structural subtlety provided by the arabinosyl group combined with the instability afforded by the symmetrical triazine nucleus. The present report describes the synthesis and antitumor properties of the novel 5-azacytosine arabinoside (*ara*-AC) which is at the same time an analogue of *ara*-C as well as 5-AC.

Chemistry. Although the presence of the labile triazine ring system was attractive from a biological point of view, the instability with respect to aqueous acid and base, nucleophiles, and reducing conditions placed severe chemical restrictions on synthetic routes to *ara*-AC. At some point in most synthetic schemes one might envision, blocking groups would have to be removed from the arabinosyl moiety, but, given the above limitations, the number of possible deblocking reagents is not great. As an example of the synthetic difficulties encountered with the triazine nucleosides, Winkley and Robins[10] in an early attempt to synthesize *ara*-AC condensed 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl chloride with the trimethylsilyl derivative of 5-azacytosine to give compound **1**, which is the tri-*O*-benzyl derivative of *ara*-AC. However, attempted debenzylation by hydrogenolysis resulted in destruction of the triazine ring.

In an earlier study,[9a] we demonstrated that saturation of the 5,6-imino double bond of 5-AC gave a dihydro derivative which showed a greatly improved stability over a broad pH range. Therefore, it occurred to us that reduction of the 5,6 double bond of a similar nucleoside, but in the arabinoside series, might provide an intermediate which would be more amenable to deblocking methods. In order to test this idea, Robins' arabinosyl intermediate (**1**) was prepared and reduced with sodium borohydride to provide the 5,6-dihydro derivative, which readily formed a stable hydrochloride salt (**4**) (Scheme I). Hydrogenolysis, catalyzed by palladium on charcoal, smoothly debenzylated **4** without further reduction of the triazine nucleus and without ring scission. The resulting dihydro compound was isolated from the reaction mixture as the hydrochloride salt **2**. The free base **3** was liberated through the agency of an anion-exchange resin.

Subsequent to the stepwise synthesis of **2** from **1** via the intermediacy of **4**, it was found that the reduction steps could be combined in one reaction. Thus, hydrogenation of **1** in absolute ethanol containing hydrogen chloride gave

Scheme I

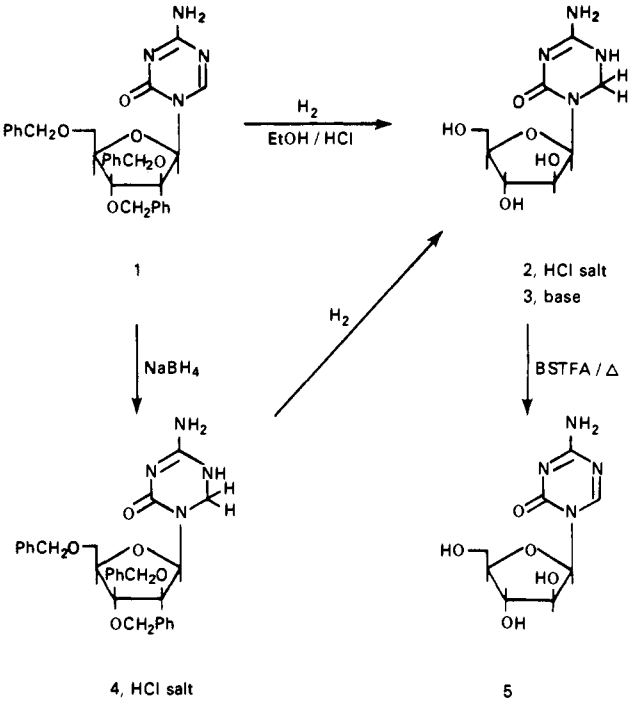
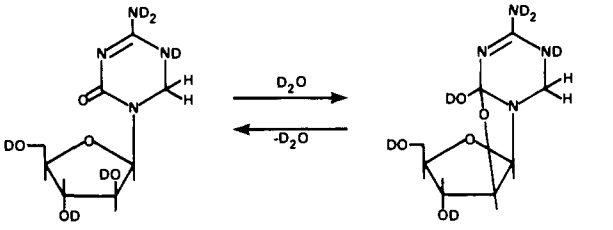


Table I. Changes in the NMR Spectrum of **2** in D_2O Solution with Increasing Time[a]



proton	$t = 0$	$t > 24$ h
C_6 H	4.81 (2, br s)	4.86 (**2**, d, $J = 2$)
$C_{1'}$H	5.98 (1, d, $J = 6$)	5.98 (1, d, $J = 6$)
$C_{2'}$H	4.36 (1, t, $J = 6$)	5.05 (1, d, $J = 6$)
$C_{3'}$H	4.60 (1, br s)	4.49 (1, d, $J = 2$)
$C_{4'}$H	4.04 (1, m)	4.24 (1, td, $J = 6$, $J = 2$)
$C_{5'}$H	3.83 (2, br s)	3.58 (2, d, $J = 6$)

[a] Chemical shifts are reported in δ units downfield from $(CH_3)_3SiCD_2CD_2CO_2Na$. Decoupling experiments were carried out to verify chemical-shift assignments. J values are recorded in hertz. Abbreviations used are: br, broad; s, singlet; d, doublet; m, multiplet; td, triplet of doublets.

2 in 79% yield, which was identical in every way with the material given by the stepwise route. Analysis of **2** with NMR revealed some unusual properties associated with aqueous solutions of this nucleoside. Although the anticipated spectrum of **2** was obtained when Me_2SO-d_6 was used as a solvent, a D_2O solution of **2** gave a spectrum which was gradually replaced over 24 h with a second spectrum. The NMR data are given in Table I. Lyophilization of the NMR solution, followed by addition of fresh D_2O, gave the first spectrum once more, which again changed with time to give the second spectrum as originally observed. As a result of this solution effect, the $C_{2'}$ proton experienced the greatest change in chemical shift ($\Delta\delta$ 0.7), whereas C_6 H and $C_{1'}$ H showed little or no change. The data recorded for **2** in Table I can be explained by the occurrence of an internal condensation of the $C_{2'}$ hydroxyl with the C_2 carbonyl of the triazine ring to form a hemiketal linkage. Apparently, an aqueous solvent is uniquely important for the formation and stabilization of the

Table II. Comparison of *ara*-AC with 5-AC and *ara*-C against L1210 Leukemia[a]

dose, mg/kg	% ILS (T - C)					
	5-AC		*ara*-AC		*ara*-C	
	test no. 1	test no. 2	test no. 1	test no. 2	test no. 1	test no. 2
200			19 (-2.8)	89 (-1.5)		T
100			80 (-4.0)	148 (-1.2)		T
80					92 (-1.8)	
50			144 (-3.3)	92 (-1.2)		28 (-1.8)
40					123 (-1.0)	
25			118 (-4.0)	58 (-0.4)		121 (0)
20					127 (-0.3)	
12.5	T	T	111 (-3.7)	58 (-0.4)		91 (-0.4)
10					84 (-0.2)	
6.25	1 (-6.1)	T	94 (-2.5)	41 (0.4)		34 (0.3)
5					47 (0.4)	
3.12	33 (-5.3)	124 (-1.6)	76 (-3.3)	28 (0.7)		28 (0.3)
1.56	126 (-1.3)	66 (-1.3)	66 (-1.3)			
0.78	66 (-2.0)	26 (-1.0)	39 (-1.2)			

[a] Mice (CDF_1) were given 10^5 L1210 cells intraperitoneally (ip) on day 0. Test compounds in 0.9% saline were administered ip daily beginning on day 1 and continuing through day 9 (nine injections). Mean increase in life span of the test animals beyond the survival time of the untreated control animals expressed as a percentage (% ILS) was used to evaluate antitumor activity. Activity is defined for the present study as a percent ILS of $\geq$ 25%. Values in parentheses give the difference of the average body weight change in grams of the test group (T) and the control group (C) measured on day 5. The appearance of the letter "T" in the table indicates that deaths occurred due to drug-related toxicity.

hemiketal, since other solvents (e.g., Me_2SO and MeOH) did not initiate the internal condensation and lyophilization of aqueous solutions resulted in a retrocondensation. Bond formation involving the $C_{2'}$ hydroxyl and the triazine ring is consistent with the large change in δ observed for the $C_{2'}$ proton. The rigidity imposed upon the molecule by the formation of the tricyclic ring system causes the C_6 methylene protons to change from nuclear equivalence (A_2 spin system) to the threshold of an AB system, wherein only the central doublet of the AB quartet is evident. The UV spectrum of **2** in pH 8 buffer initially exhibited a maximum at 232 nm (ϵ 5300). Similarly, 5,6-dihydro-5-azacytidine, the ribosyl analogue of **2**, gave λ_{max} 233 nm (ϵ 4980) in the same buffer.[9b] However, **2**, as well as its free base **3**, differed from the ribosyl analogue in that a hypochromic shift was observed for the arabinosyl compounds with increasing time. A 20-h-old solution of **2** retained 30% of the initial absorbance at 233 nm and, after approximately 30 h, solutions of **2** and **3** were transparent in the UV. The UV behavior is in accord with the NMR observations and the proposed internal condensation in aqueous solutions.

Successful completion of the *ara*-AC synthesis required that the 5,6 double bond be regenerated from **2** with a suitable dehydrogenation procedure. An appropriate procedure was suggested by our chemical studies on dihydro-5-azacytidine.[11] In preparation for mass spectral analysis, dihydro-5-azacytidine was per(trimethylsilylated) with bis(trimethylsilyl)trifluoroacetamide (BSTFA) in acetonitrile solution. It was observed that if the trimethylsilylation solution was heated in air for prolonged periods of time a formal loss of $(CH_3)_3SiH$ (MS analysis) occurred with the regeneration of the imine double bond. After removal of the trimethylsilyl groups by methanolysis, a good yield of 5-AC was obtained. Application of this unusual reaction to the dehydrogenation of **2** permitted an efficient synthesis of *ara*-AC. Accordingly, a solution of BSTFA and **2** in acetonitrile was refluxed in air under anhydrous conditions for 53 h. The progress of the reaction was conveniently followed by gas chromatography similar to the method reported earlier.[11] Following methanolysis, *ara*-AC (**5**) was obtained in 82% yield. The NMR spectrum of **5** showed a singlet due to one proton at δ 8.22, which is in agreement with the presence of a triazine aromatic proton.

Antitumor Evaluation. Comparative studies of 5-azacytosine arabinoside (*ara*-AC, **5**) with 5-azacytidine (5-AC) and cytosine arabinoside (*ara*-C) using the murine L1210 leukemia test system were carried out according to protocols[12] devised by the Division of Cancer Treatment, National Cancer Institute. Dose–response assays were conducted for each of the three nucleosides using a chronic administration schedule wherein the animals were treated daily for 9 consecutive days (QD 1–9). The results of the comparative evaluation, obtained from duplicate experiments, are recorded in Table II. Although higher dose levels were necessary, Table II shows that *ara*-AC gave reproducible higher percent ILS values than either 5-AC or *ara*-C on this schedule. Active indications (% ILS $\geq$ 25) were given by *ara*-AC from 0.78 mg/kg up to a dose level 200-fold higher. The thresholds of antitumor activity and toxicity are separated by a much more narrow dose-level range in the case of 5-AC and *ara*-C. Although substantial weight losses were observed with *ara*-AC in one experiment, no drug-related deaths occurred in either experiment over the dose ranges tested.

Discussion

In terms of molecular structure, *ara*-AC can be considered an analogue of 5-AC equally as well as *ara*-C. Therefore, it was of interest to enquire experimentally whether *ara*-AC is best considered an analogue of 5-AC or *ara*-C in terms of biological properties. With respect to chemical behavior, *ara*-AC clearly resembles 5-AC. As shown by liquid chromatography (LC) analysis, aqueous solutions of *ara*-AC gave chromatographic profiles which were similar to those recorded for 5-AC. Therefore, similar hydrolysis pathways for 5-AC and *ara*-AC are indicated. As reported earlier,[8] the decomposition of 5-AC in water solution is conveniently followed with LC using a reverse-phase column eluted with water. The 5-AC disappearance at 25 °C was approximated by a first-order rate equation, which showed that 15% of the 5-AC was lost after 5.5 h and that the half-life was reached after 47 h. Similarly, the concentration of *ara*-AC in water solution at 25 °C was found by LC analysis to be decreased by 15% after 4 h; the half-life occurred at 34 h. As observed for 5-AC,[8] the behavior of aqueous solutions of *ara*-AC in the UV is characterized by increases in optical density with time due to the formation of the ring-opened *N*-formyl

hydrolysis product. As further hydrolysis of the *N*-formyl product occurs to give UV-transparent products, a plateau is reached, followed by a gradual decrease in optical density.

In terms of antitumor effect in the L1210 system, 5-AC seems to be relatively independent of administration schedule in our experience.[9b] For example, percent ILS values of ca. 125 were obtained with 5-AC whether administered on days 1, 5, and 9 post-tumor-implantation[1] or daily for 9 consecutive days (Table II). By contrast, the maximum percent ILS obtainable against L1210 leukemia with *ara*-C has a well-documented schedule dependency.[3] Our initial evaluations of *ara*-AC were done using a day 1, 5, 9 schedule,[1] which gave maximum percent ILS values in the range of 75–100. The data resulting from a chronic schedule (QD 1–9) is reported in Table II. Maximum percent ILS values were in the range of 144–148. Therefore, the antitumor activity of *ara*-AC exhibits an apparent schedule dependency in the L1210 system similar to *ara*-C.

A second indication that *ara*-AC might be considered more a biological analogue of *ara*-C than of 5-AC resulted from a comparison of the antitumor properties of the dihydro derivatives of 5-AC and *ara*-AC. Reduced 5-AC (5,6-dihydro-5-azacytidine) has a substantial antitumor activity against L1210, which is comparable to that observed for the parent.[9b] However, the dihydro derivative of *ara*-AC (**2**) was completely inactive in the L1210 system when tested according to a dose-response assay (400–6.25 mg/kg) using a chronic administration schedule (QD 1–9). In three separate experiments no toxicity was observed, as shown by the absence of drug-related deaths and excessive weight loss with respect to the untreated control animals. Although the reduced *ara*-AC (**2**) was freshly formulated in saline daily and administered to the test animals within 15 min, it, nevertheless, can still be argued that the inactivity results not from a lack of biological similarity with triazine nucleosides but rather from the internal condensation of **2** in aqueous milieu to form a hemiketal structure. It is interesting to note that the hemiketal (Table I) bears certain structural similarities to tetrodotoxin.[14] Both have a guanidine function incorporated into a ring, as well as a tertiary hydroxyl group located on a carbon atom which bears additional electronegative substituents. The proton from the tertiary hydroxyl group of tetrodotoxin is sufficiently acidic to be donated to the guanidine moiety to form an inner salt. The cyclized form of **2** might similarly form an inner salt, which could be not only the driving force for the internal condensation but also a contributing factor for the absence of biological activity. However, the formation of the hemiketal is not instantaneous; the noncyclized form (**2**) when dissolved in water has a half-life in the range of 10–15 h at room temperature. Therefore, if **2** had antitumor activity, however weak, one would have expected to detect it at the high dose levels employed (e.g., 400 mg/kg) when fresh solutions were administered daily over 9 days.

Preliminary results suggest that *ara*-AC is chemically similar to 5-AC but biologically more closely related to *ara*-C. Future work in this area will seek to clarify and strengthen the latter relationship.

Experimental Section

Gas chromatography was carried out with a Varian Aerograph 2740 equipped with a 5 ft × 0.125 in. stainless-steel column packed with 3% SE-30 on 120-mesh Variport Q and operated isothermally at 220 °C. Chromatograms were interpreted in terms of isothermal retention indices[13] (IRI). Liquid chromatography analyses were done with a Waters Associates Model ALC/GPC-244 equipped with a fixed-wavelength (254 nm) UV detector and a 7 (i.d.) × 300 mm μBondapak/C_{18} column, which was eluted with distilled water at 4 mL/min. A Cary Model 15 spectrophotometer was used to obtain UV spectra, and a Perkin-Elmer Model 621 was used to record infrared spectra. Proton NMR spectra were recorded with a Varian HA-100D spectrometer in $CDCl_3$ or Me_2SO-d_6 using Me_4Si as an internal standard. Mass spectra were determined with a DuPont 21-492 mass spectrometer with a 75-eV ionizing voltage. Specific rotations were measured in a 1-dm cell with a Perkin-Elmer Model 141 polarimeter. Melting points were taken with a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by the Section on Microanalytical Services and Instrumentation, NIAMDD, NIH, and by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are reported only by the element symbols, results were within ±0.4% of the theoretical values.

4-Amino-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-1,3,5-triazin-2(1*H*)-one (1). The method of Winkley and Robins[10] was used to prepare **1** in 47% yield: mp 144–146 °C, lit.[10] mp 142–143 °C; $[\alpha]^{23}_D$ 92° (*c* 1.0, CH_2Cl_2). The NMR spectral properties of **1** in $CDCl_3$ solution agreed with those previously reported in ref 10.

4-Amino-5,6-dihydro-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-1,3,5-triazin-2(1*H*)-one Hydrochloride (4). To a stirred solution of **1** (5.14 g, 10 mmol) in 30 mL of HMPA was added 0.76 g (20 mmol) of sodium borohydride over 15 min. After the addition, the reaction solution was heated at 50 °C for 1 h, cooled, and left at room temperature for 3 h. The solution was treated with water (10 mL) and methanol (10 mL), and the pH was adjusted to 7 with dilute hydrochloric acid. The deposited syrup was washed with water and partitioned between chloroform (150 mL) and water. The organic layer was dried (Na_2SO_4), concentrated to 70 mL, treated with 50 mL of chloroform which had been saturated with hydrogen chloride at −10 °C, and evaporated under vacuum after standing at room temperature for 1 h. The resulting syrup was coevaporated with benzene (three times) and then triturated with ether to give 5.22 g (94%) of **4** as a white solid: mp 120–132 °C dec. Recrystallization from dry benzene gave **4** (4.85 g, 88%) in two crops: mp 156–157 °C; $[\alpha]^{23}_D$ −33° (*c* 1.0, CH_2Cl_2); NMR (Me_2SO-d_6) δ 7.32 (s, 15, phenyl), 5.96 (d, *J* = 6 Hz, 1, $C_{1'}$ H), 4.68 and 4.66 (pair of singlets, 2, C_6 H), 4.56 (s, 2, benzyl), 4.53 (s, 4, benzyl). Anal. ($C_{29}H_{32}N_4O_5$·HCl, 553.04) C, H, N, Cl.

4-Amino-5,6-dihydro-1-(β-D-arabinofuranosyl)-1,3,5-triazin-2(1*H*)-one Hydrochloride (2). Method A. From Compound 1. To a solution of **1** (3.0 g, 5.8 mmol) in 100 mL of absolute ethanol containing 10 mL of ethanolic hydrogen chloride (saturated at 0 °C) was added 3.0 g of 10% palladium on charcoal catalyst, and the mixture was hydrogenated (50 psi initial pressure) at room temperature for 24 h. The catalyst was removed by filtration through a Celite pad, which subsequently was washed (2 × 50 mL) with absolute ethanol. The combined filtrate and washings were concentrated under vacuum (25 °C bath) to ca. 20 mL, then 20 mL of acetone was added, and the mixture was cooled to 4 °C and allowed to crystallize. White crystals of **2** (1.3 g, 79%) were obtained: mp 166–169 °C dec; $[\alpha]^{24}_D$ −26° (*c* 1.0, H_2O, *t* = 0); $[\alpha]^{24}_D$ −62° (*c* 1.0, H_2O, *t* > 4 days); UV λ_{max} (H_2O) end absorption; λ_{max} (pH 8 buffer) 232 nm ($\epsilon^{t=0}$ 5300, $\epsilon^{t=1h}$ 3800, $\epsilon^{t=20h}$ 1600); IR (Nujol) 1717, 1681, 1241, 1157, 1127, 1070, 778 cm^{-1}; NMR (Me_2SO-d_6) δ 5.77 (d, *J* = 5 Hz, 1, $C_{1'}$ H), 4.70 (br s, 2, C_6 H), 3.57 (br s, 2, $C_{5'}$ H). Anal. ($C_8H_{14}N_4O_5$·HCl, 282.7) C, H, N, Cl.

Method B. From Compound 4. A solution of **4** (4.0 g, 7.2 mmol) in 100 mL of absolute ethanol containing 3 drops of ethanolic hydrogen chloride (saturated at 0 °C) was combined with 4.9 g of 10% palladium on charcoal catalyst and hydrogenated (50 psi initial pressure) for 20 min. The reaction workup was as described above for method A, which gave 1.8 g (88%) of **2**, mp 166–168 °C dec. Recrystallization from ethanol–acetone (1:1) gave 1.32 g of **4**, mp 168–170 °C dec. Mixture melting point with the material obtained from method A above showed no depression, and the UV and NMR spectra were superimposable.

4-Amino-5,6-dihydro-1-(β-D-arabinofuranosyl)-1,3,5-triazin-2(1*H*)-one (3). A methanol solution of the hydrochloride salt **2** was slowly passed through a Dowex 1-X2 column (OH^- form), the eluates were evaporated, and the residue was recrystallized from ethanol to give the free base **3**: mp 179–180 °C

dec; UV λ_{max} (MeOH) 233 nm (ϵ 4900); λ_{max} (pH 8 buffer) 232 nm ($\epsilon^{t=0}$ 5600). Anal. ($C_8H_{14}N_4O_5$, 246.2) C, H, N.

The free base can also be liberated by dissolving the hydrochloride **2** in ethanolic ammonia. Evaporation, after standing several hours at room temperature, and recrystallization of the residue from ethanol gave **3**, mp 179–180 °C dec.

4-Amino-1-(β-D-arabinofuranosyl)-1,3,5-triazin-2(1*H*)-one (5). A mixture of BSTFA (40 mL) and dry acetonitrile (40 mL) was gently refluxed with **2** (2.0 g, 7.1 mmol) under anhydrous conditions. As the reaction proceeded, the single peak in the gas chromatogram due to **3**·TMS_5 (IRI 2538) was accompanied by increasing concentrations of a second compound having a longer retention time. By mass spectral analysis it was shown to be **5**·TMS_4 (IRI 2650). After refluxing for 53 h, the latter compound produced the only peak in the chromatogram, indicating a complete thermal conversion: MS (**5**·TMS_4) *m/e* (rel intensity) 532 (1.7), 517 (6.0), 442 (1.7), 387 (2.4), 349 (0.7), 315 (19.8), 258 (44.8), 243 (24.3), 217 (61.6), 169 (17.4), 147 (21.4), 73 (100). The solvent was removed under vacuum and the residue taken up in 100 mL of methanol. Methanolysis of the trimethylsilyl groups was accomplished by slow distillation of the methanol solution during 1.5 h. The concentrate was diluted to 100 mL with a fresh portion of methanol, and the distillation was continued for another 1.5 h. After renewing the solution volume a second time with methanol, heat was removed and the solution was allowed to come to room temperature, causing 1.4 g (82%) of white crystals of **5** to separate: mp 223–225 °C dec; $[\alpha]^{24}_D$ 122° (*c* 1.0, H_2O, *t* = 0); $[\alpha]^{24}_D$ 63° (*c* 1.0, H_2O, *t* = 5 h); UV λ_{max} (H_2O, *t* = 0) 243 nm (ϵ 6800); λ_{max} (H_2O, *t* = 1 h) 242 nm (ϵ 7200); λ_{max} (H_2O, *t* = 3 h) 241 nm (ϵ 7800); λ_{max} (pH 2 buffer, *t* = 0) 252 nm (ϵ 3100); λ_{max} (pH 8 buffer, *t* = 0) 242 nm (ϵ 7400); IR (Nujol) 1702, 1665, 1616, 1165, 1082, 822, 799 cm^{-1}; NMR (Me_2SO-d_6) δ 8.22 (s, 1, C_6 H), 6.03 (d, *J* = 4 Hz, 1, C_1' H). Anal. ($C_8H_{12}N_4O_5$, 244.2) C, H, N.

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References and Notes

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Potential Antitumor Agents. 31. Quantitative Structure–Activity Relationships for the Antileukemic Bis(guanylhydrazones)

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Certain L1210-active bis(guanylhydrazones) have structural and biological properties in common with the DNA minor groove binding, antileukemic, bisquaternary ammonium heterocycles. Monitoring of the DNA binding of the bis(guanylhydrazones), by fluorimetric quantitation of drug displacement of DNA-bound ethidium, shows that, like the bisquaternary salts, these agents bind more strongly to poly[d(A-T)] than poly[d(G-C)]. The drug concentrations necessary to inhibit L1210 DNA-dependent DNA polymerase in vitro by 50% (IC_{50}) are linearly related to measures of drug–DNA binding with no preference for a particular primary sequence of DNA being evident. Mammalian toxicity of the bis(guanylhydrazones) is effectively modeled by a regression equation containing binomial terms in R_m values, used as a measure of agent lipophilic–hydrophilic balance, and the logarithms of the IC_{50} values.

Development of high-activity clinical candidates, from the initial lead of a L1210 leukemia active bisquaternary ammonium heterocycle (BQAH),[1] first relied on the development of qualitative structure–activity relationships.[2] These qualitative relationships demonstrated a need for (1) two strongly basic centers separated by an aromatic framework providing a charge separation of greater than 18Å, (2) a capacity to fit to a slotlike annular site, and (3) agent lipophilic–hydrophilic balance within a critical range.[2] When it was later appreciated that these agents act by interference with DNA-template activity of tumor cells,[3,4] it proved possible to develop QSAR by utilizing measures of agent–DNA interactions as an indicator of site fit.[3] The measure of DNA interaction employed was derived from competition studies between agents and the fluorochrome ethidium for DNA sites.[3,5] A C_{50} value for the drug–DNA interaction was defined as the micromolar drug concentration necessary to displace 50% of DNA-bound ethidium, as monitored by fluorimetry. It can be demonstrated that such C_{50} values are inversely related to drug–DNA association constants.[5] This micromethod, which employs milligrams of drug and only micrograms of DNA, permitted an examination of the importance of drug binding to different DNA primary sequences for antitumor activity.[3] The BQAH agents bind more strongly to adenine–thymine (A-T) rich DNAs than to their guanine–cytosine (G-C) containing counterparts, and there was limited covariance between the C_{50} values observed for agent binding to poly[d(A-T)] and poly[d(G-C)]. Equations from multiple regression analysis demonstrated that the C_{50} values for drug interaction with poly[d(A-T)] could incorporate significantly more of the variance in the biologic data than those obtained from poly(dA)·poly(dT) or poly[d(G-C)]. Interaction of these agents with DNA can

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