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60926-09-2; ClCH<sub>2</sub>CO<sub>2</sub>C<sub>2</sub>H<sub>5</sub>, 105-39-5; ClCH<sub>2</sub>CONH<sub>2</sub>, 79-07-2; ClCH<sub>2</sub>CONHCH<sub>3</sub>, 96-30-0; ClCH<sub>2</sub>CON(CH<sub>3</sub>)<sub>2</sub>, 2675-89-0; ClC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>CONH<sub>2</sub>, 5875-24-1; ClCH<sub>2</sub>CONHC<sub>6</sub>H<sub>5</sub>, 587-65-5; ClCH<sub>2</sub>CONHCONH<sub>2</sub>, 4791-21-3; ClCH<sub>2</sub>CONHCONHCH<sub>3</sub>, 4791-22-4; ClCH(CH<sub>3</sub>)CONHCONH<sub>2</sub>, 24224-16-6; ClCH<sub>2</sub>CONHCONHC<sub>6</sub>H<sub>5</sub>, 4791-23-5; ClCH<sub>2</sub>CONHCH<sub>2</sub>CONH<sub>2</sub>, 41312-83-8.

## 2'-O-Nitro-1-β-D-arabinofuranosylcytosine. A New Derivative of 1-β-D-Arabinofuranosylcytosine That Resists Enzymatic Deamination and Has Antileukemic Activity

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To overcome the susceptibility of the anticancer drug 1-β-D-arabinofuranosylcytosine (*ara-C*) to enzymatic deamination, and hence deactivation, we prepared the 2'-O-nitro-1-β-D-arabinofuranosylcytosine (termed *nitrara-C*) and evaluated it for biological activity. *Nitrara-C* was resistant to enzymatic deamination and inhibited the proliferation of several strains of human leukemic T and B lymphoblasts grown in culture. Moreover, it substantially extended the life spans of mice with L1210 leukemia. Studies with *ara-C*-resistant human leukemic lymphoblasts deficient in deoxycytidine kinase activity disclosed that the inhibitory activity of the new compound depends on its phosphorylation.

1-β-D-Arabinofuranosylcytosine (*ara-C*), a synthetic nucleoside, is widely used in the treatment of acute myeloblastic and lymphoblastic leukemias.<sup>1-4</sup> The drug is deaminated very rapidly by cytidine deaminase, present in body tissues and some neoplastic cells as well, to 1-β-D-arabinofuranosyluracil (*ara-U*), a metabolite with little or no antitumor activity in most tumor cells.<sup>5-9</sup> Attempts to circumvent the problem of inactivation have included structural modifications of *ara-C*,<sup>10-17</sup> as well as the development of cytidine deaminase inhibitors to be used in combination with the S-phase agent.<sup>18,19</sup> One de-

Table I. Substrate Specificity of Cytidine Deaminase<sup>a</sup>

compd <sup>b</sup>	rel initial rate <sup>c</sup>
cytidine	100 <sup>d</sup>
2'-deoxycytidine	39
<i>ara-C</i>	15
<i>nitrara-C</i>	<1

<sup>a</sup> Cytidine deaminase was isolated and partially purified from mouse kidney acetone powder (Sigma Chemical Co., St. Louis, MO) essentially by the procedure reported in ref 33 and 34. The amount of 1.3 mg of enzyme protein in a final volume of 1.0 mL of phosphate buffer (pH 7.0, 0.05 M) was used per assay. <sup>b</sup> The concentration of each substrate was  $2.5 \times 10^{-4}$  M. <sup>c</sup> Direct spectrophotometric assay was performed at 37 °C by the procedure reported in ref 35 and 36. <sup>d</sup> Deaminase activity is expressed as a proportion of the most active substrate, cytidine, which was arbitrarily assigned a value of 100.

Table II. Cytotoxicity of *Nitrara-C* and *Ara-C* against Various Human Leukemic Lymphoblastoid Cell Lines

cell line	IC <sub>50</sub> <sup>a</sup>		ref <sup>b</sup>
	<i>nitrara-C</i>	<i>ara-C</i>	
CCRF-CEM	$6 \times 10^{-7}$	$2 \times 10^{-8}$	37
MOLT-4	$4 \times 10^{-7}$	$1 \times 10^{-8}$	38
RPMI-6410	$6 \times 10^{-6}$	$6 \times 10^{-8}$	39
CCRF-CEM/ <i>ara-C</i> <sup>c</sup>	$1 \times 10^{-3}$	$1 \times 10^{-4}$	40

<sup>a</sup> Molar concentration for 50% inhibition of cell growth.

<sup>b</sup> Culture conditions used were essentially similar to those reported in the references. <sup>c</sup> Mutant deficient in deoxycytidine kinase.

aminase-resistant derivative of *ara-C* is 2,2'-anhydro-1-β-D-arabinofuranosylcytosine (cyclo-C),<sup>20-22</sup> which, although not effective against *ara-C*-resistant tumor lines,<sup>22,23</sup> is more

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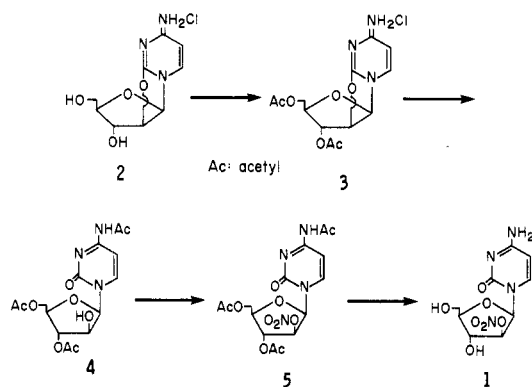
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## Scheme I



effective than *ara-C* in animal models. In man, however, the use of cyclo-C may be limited because of peculiar toxic effects not seen with *ara-C*.<sup>24-26</sup> Substitutions at the "up" 2'-position of the arabinofuranosyl moiety of *ara-C* not only have altered susceptibility to deamination<sup>14-17</sup> but have modified antitumor activity as well.<sup>15,17</sup>

To obtain new analogues of this type, we introduced a neutral nitrate ester group at the "up" 2'-position of *ara-C* to give 2'-*O*-nitro-1- $\beta$ -D-arabinofuranosylcytosine (1, *nitrara-C*). We report here the synthesis of *nitrara-C* and a preliminary biological evaluation of this new compound.

**Chemistry.** Treatment of a suspension of 2,2'-anhydro-1-(3,5-di-*O*-acetyl- $\beta$ -D-arabinofuranosyl)cytosine hydrochloride (3),<sup>27</sup> which can be readily prepared (over 85% yield) from commercially available 2,2'-anhydro-1- $\beta$ -D-arabinofuranosylcytosine hydrochloride (2), in tetrahydrofuran (THF) with acetic anhydride and triethylamine resulted in 1-(3,5-di-*O*-acetyl- $\beta$ -D-arabinofuranosyl)-*N*<sup>4</sup>-acetylcytosine (4), a compound with physical and spectroscopic properties similar to those previously reported (Scheme I).<sup>28-30</sup> Nitration of the latter compound, followed by removal of acetyl groups according to the general procedure recently reported,<sup>31</sup> yielded the target compound, termed *nitrara-C* (1). The UV absorption maxima of 1, in both acidic and basic media, showed a blue shift of approximately 5 nm relative to those of *ara-C*. Similar hypsochromic shifts have been observed for 2'-*O*-acyl derivatives of *ara-C*.<sup>32</sup> As expected, the signals of the proton attached to the carbon atom bearing the *O*-nitrate group shifted by about 1 ppm to a lower magnetic field after *O*-nitration.<sup>31</sup>

## Biological Results

As desired, *nitrara-C* was substantially more resistant than *ara-C* to deamination by cytidine deaminase partially purified from mouse kidney (Table I). Under conditions

Table III. Chemotherapy of L1210:<sup>a</sup> *Nitrara-C* or *Ara-C* Administered Daily for 7 Consecutive Days<sup>b</sup>

dosage, <sup>c</sup> (mg/kg)/day	postinoculation life span, days <sup>d</sup>	
	<i>nitrara-C</i>	<i>ara-C</i>
34	7.40 $\pm$ 0.89 (17%) <sup>e</sup>	12.80 $\pm$ 2.05 (102%) <sup>e</sup>
56	10.40 $\pm$ 2.70 (64%) <sup>e</sup>	13.00 $\pm$ 3.00 (105%) <sup>e</sup>
93	10.80 $\pm$ 1.48 (71%) <sup>e</sup>	14.00 $\pm$ 2.30 (128%) <sup>e</sup>
156	13.80 $\pm$ 3.42 (118%) <sup>e</sup>	16.25 $\pm$ 0.50 (157%) <sup>e</sup>
208	16.60 $\pm$ 4.78 (162%) <sup>e</sup>	16.60 $\pm$ 0.55 (162%) <sup>e</sup>

<sup>a</sup> Female B6D2F<sub>1</sub>/J mice (about 20 g each) were inoculated ip on day 0 with ascites fluid containing a million cells of the murine leukemia, L1210. For general procedures, see Avery, T. L.; Roberts, D. *Eur. J. Cancer* 1974, 10, 425. <sup>b</sup> Treatment by the ip route was begun 24 h after leukemic cell inoculation. Hydrochloride salts of the drugs were used. A volume of 0.01 mL of drug solution in water (neutralized with 1 N NaOH when necessary) was administered per gram of mouse weight. <sup>c</sup> Five mice were treated at each dosage level. <sup>d</sup> Mean  $\pm$  1 SD. Six untreated control mice lived 6.33  $\pm$  0.52. <sup>e</sup> Mean increased life span, calculated as (T/C - 1)  $\times$  100.

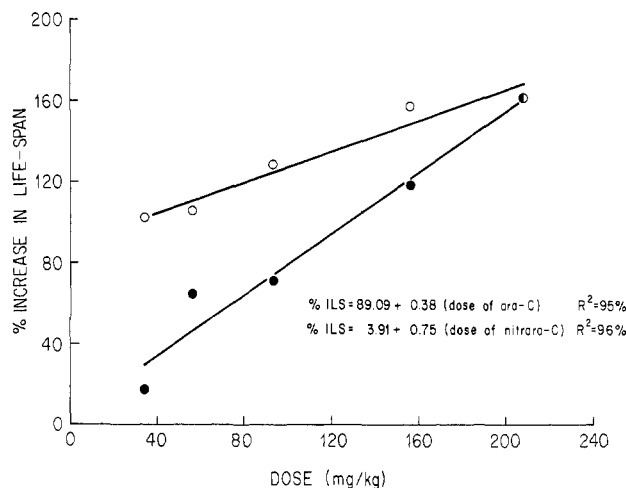
in which *ara-C* was deaminated by 50% in 50 min, *nitrara-C* was not deaminated to any appreciable degree in 7 h and, hence, should be much more stable than *ara-C* in biological systems with high levels of cytidine deaminase. In preliminary experiments (data not shown), the growth-inhibitory effect of *nitrara-C* (IC<sub>50</sub> = 6  $\times$  10<sup>-7</sup> M) on human leukemic lymphoblasts (CCRF-CEM) in culture was not altered when the compound was preincubated with cytidine deaminase partially purified from mouse kidney; under similar conditions, the IC<sub>50</sub> value for *ara-C* was increased by at least 10-fold. Thus, unlike its parent compound, *nitrara-C* was not deactivated by partially purified cytidine deaminase.

Table II shows that this new derivative of *ara-C* was highly cytotoxic to several human leukemia cell lines maintained in culture. *Ara-C*-resistant human leukemic lymphoblasts (CCRF-CEM/*ara-C*), deficient in deoxycytidine kinase, were cross-resistant to *nitrara-C*, suggesting the compound's requirement for activation, presumably to its triphosphate.

*Nitrara-C* also produced antileukemic activity in vivo. Five days after ip inoculation of B6D2F<sub>1</sub>/J mice with 1  $\times$  10<sup>5</sup> cells, the mean number of L1210 leukemia cells recovered from the abdominal compartments of untreated mice was (255  $\pm$  39)  $\times$  10<sup>6</sup>. At that time, other mice were treated with either the drug or saline. Twenty-four hours later, the mean value of L1210 cells in the ip compartments

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**Figure 1.** Linear regression lines showing the changes in percent increase in life span vs. dosages of *nitrara-C* (●) or *ara-C* (○).  $R^2$  is the proportion of the original sum of squared deviations that is removed by fitting the straight line.

had increased by 52% for NaCl-injected control mice, however, for mice treated ip, at the level of 720 mg/kg, either with *nitrara-C* or *ara-C*, cell numbers had decreased, respectively, by 90 and 77%. Hence, under these conditions, *nitrara-C* was more cytotoxic than *ara-C*. This result led to a dose-ranging study in which the new drug was given for 7 consecutive days to mice inoculated ip with  $1 \times 10^6$  L1210 cells. Dose-dependent responses were produced (Table III). The activity of *nitrara-C* was less than that of *ara-C* at lower dosages. At higher dosages, however, the anti-L1210 activity of *nitrara-C* approached that of *ara-C*. With escalating dosages of either drug, the mean life span increased in a linear fashion, with responses to *nitrara-C* being more pronounced. At the highest dosage tested, 208 mg/kg, both drugs extended postinoculation life span by 162%. Lines defined by linear regression equations depict this change (Figure 1). The difference in the two slopes, an indication of the rates of increase, is statistically significant ( $t = 3.63$ ;  $p = 0.01$ ).<sup>41</sup> *Nitrara-C* did not produce limiting toxicity at the dosages studied; neither drug-induced death nor overt morbidity were observed. Based on change in body weight, *nitrara-C* was much less toxic than was *ara-C*. Indeed, the mice treated with 208 mg/kg of *nitrara-C* for 7 consecutive days gained, on the average, 0.2 g in body weight from day 1 (at the start of treatment) to day 8 (24 h after the last treatment); while, under similar conditions, mice treated with 208 mg/kg of *ara-C* showed an average weight loss of 2.8 g, an indication of host toxicity. It appears, therefore, that at even higher dosages *nitrara-C* may be therapeutically superior to *ara-C*. Accordingly, the chemotherapeutic potential of this new compound will be further defined in studies that involve larger dosages of *nitrara-C*, as well as additional schedules and routes of administration.

## Experimental Section

Melting points were determined with a Fisher digital melting point analyzer Model 355 and were not corrected. The thin-layer chromatography plates used were those of Macherey-Nagel & Co., Polygram Sil G/UV<sub>254</sub> with a fluorescent indicator. Almost all evaporations were conducted in vacuo, at 12–20 mm, and at temperatures of 30–40 °C in the bath. Analytical samples were dried at room temperature in vacuo (0.10–0.01 mm) for 10 h. Elemental analyses were performed by Midwest Microlab, Ltd.,

Indianapolis, IN. The UV spectra were measured with a Perkin-Elmer spectrophotometer 555; the IR spectra were recorded with a Beckman spectrophotometer 4230. The <sup>1</sup>H NMR spectra were obtained at ambient temperature on a JEOL spectrometer Model JNM-MH-100. Tetramethylsilane was used as an internal standard, unless stated otherwise. Specific rotations were measured with Rudolph Research Autopol III automatic polarimeter. The regression analysis was performed essentially according to the method of Dunn and Clark.<sup>42</sup>

**1-(3,5-Di-O-acetyl-β-D-arabinofuranosyl)-N<sup>4</sup>-acetylcytosine (4).** To a stirred suspension of 2,2'-anhydro-1-(3,5-di-O-acetyl-β-D-arabinofuranosyl)cytosine hydrochloride (**3**; 7.60 g, 22 mmol) in tetrahydrofuran (THF) (400 mL) were added acetic anhydride (3.08 mL, 33 mmol) and triethylamine (3.53 mL, 25 mmol). After being stirred at room temperature for 7 days, the product was collected by filtration and washed with THF, ice-water, and finally with THF again to give 4.45 g (55%) of **4**. Another 895 mg (11%) of **4** was recovered from the THF mother liquors after they had stood at room temperature for 72 h. The pooled crude product was then crystallized from 96% ethanol, resulting in 4.33 g (53%) of pure **4**; mp 233–234.1 °C dec (lit. 225–228,<sup>28</sup> 213–214,<sup>29</sup> 217–220 °C<sup>30</sup>). The UV absorption maxima for **4** were similar to those previously reported:<sup>28–30</sup> UV (0.1 N HCl)  $\lambda_{\max}$  307 and 240 nm ( $\epsilon$  13610 and 9100); UV (0.1 N NaOH)  $\lambda_{\max}$  303 and 276 nm ( $\epsilon$  9270 and 9240); UV (96% ethanol)  $\lambda_{\max}$  299 and 247 nm ( $\epsilon$  8000 and 15490). The <sup>1</sup>H NMR resonances for **4** were in good agreement with those reported by Fromageot and Reese:<sup>29</sup> <sup>1</sup>H NMR (Me<sub>2</sub>SO-*d*<sub>6</sub> + D<sub>2</sub>O)  $\delta$  2.01 (s, 3, NAc), 2.07 (s, 6, OAc), 5.95 (d,  $J = 3.4$  Hz, 1, H-1'), 7.08 (d,  $J = 7.5$  Hz, 1, H-5), 7.80 (d,  $J = 7.5$  Hz, 1, H-6);  $[\alpha]_D^{25} +88.7 \pm 0.7^\circ$  (c 0.2, MeOH). Anal. (C<sub>15</sub>H<sub>18</sub>N<sub>3</sub>O<sub>8</sub>) C, H, N.

**1-(3,5-Di-O-acetyl-2-O-nitro-β-D-arabinofuranosyl)-N<sup>4</sup>-acetylcytosine (5).** To fuming nitric acid (20 mL, 477 mmol) was added gradually, with efficient stirring, acetic anhydride (20 mL, 212 mmol) while the temperature was kept between –30 to –35 °C by external cooling. With continued vigorous stirring, compound **4** (10 g, 27 mmol) was added in batches to the homogeneous mixture that was maintained at about –30 °C. The clear reaction mixture was allowed to warm gradually to 0 °C. After being stirred for 30 min at this temperature, the solution was poured into a mixture of ice and saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution that was stirred until all the ice had melted. The aqueous phase was then extracted with ethyl acetate. The combined extracts were washed with saturated sodium bicarbonate solution and water and then dried (Na<sub>2</sub>SO<sub>4</sub>). Crude nitrate **5** (10.66 g, 95%) was obtained by removal of the ethyl acetate under reduced pressure. The solid residue was crystallized from ethyl acetate to obtain pure **5** (9.65 g, 86%); mp 159.7–160.6 °C; UV  $\lambda_{\max}$  (0.1 N HCl) 300 and 246 nm ( $\epsilon$  9790 and 12190); UV (96% ethanol)  $\lambda_{\max}$  297 and 249 nm ( $\epsilon$  6470 and 15000); <sup>1</sup>H NMR (Me<sub>2</sub>SO-*d*<sub>6</sub> + D<sub>2</sub>O)  $\delta$  2.01 (s, 3, NAc), 2.10 (s, 6, OAc), 5.83–5.93 (m, 1, H-2'), 6.31 (d,  $J = 5$  Hz, 1, H-1'), 7.16 (d,  $J = 7.3$  Hz, 1, H-5), 7.90 (d,  $J = 7.3$  Hz, 1, H-6); IR (KBr) 3260, 1752, 1720, 1670, 1560, 1490, 1320, 1245, 850 cm<sup>–1</sup>;  $[\alpha]_D^{25} +94.2 \pm 0.6^\circ$  (c 1.0, MeOH). Anal. (C<sub>15</sub>H<sub>18</sub>N<sub>3</sub>O<sub>10</sub>) C, H, N.

**1-(2-O-Nitro-β-D-arabinofuranosyl)cytosine (1).** To a solution of **5** (10 g, 24 mmol) in anhydrous methanol (425 mL) under an atmosphere of dry nitrogen was added 1.9 N barium methoxide (5 mL, 9.5 mequiv) in methanol. After 1 h of stirring at room temperature under an inert atmosphere, the reaction mixture was neutralized with cation-exchange resin (Bio-Rex 70/H<sup>+</sup>). The combined filtrate and washings were evaporated under reduced pressure to obtain the crude **1** (6.60 g, 95%), which was crystallized from H<sub>2</sub>O, yielding pure **1** (5.97 g, 86%); mp 151.2–152.7 °C; UV  $\lambda_{\max}$  (0.1 N HCl) 275 nm ( $\epsilon$  12970); UV (0.1 N NaOH)  $\lambda_{\max}$  268 and 230 nm ( $\epsilon$  9430 and 8380); UV (96% ethanol)  $\lambda_{\max}$  269 and 239 nm ( $\epsilon$  8400 and 7950); UV (H<sub>2</sub>O)  $\lambda_{\max}$  268 and 231 nm ( $\epsilon$  8597 and 8243); <sup>1</sup>H NMR (Me<sub>2</sub>SO-*d*<sub>6</sub> + D<sub>2</sub>O)  $\delta$  5.60 (dd,  $J = 5.6$  Hz, 1, H-2'), 5.77 (d,  $J = 7.6$  Hz, 1, H-5), 6.28 (d,  $J = 5.6$  Hz, 1, H-1'), 7.68 (d,  $J = 7.6$  Hz, 1, H-6); IR (KBr) 3420, 3280, 1650, 1525, 1495, 1285, 835 cm<sup>–1</sup>;  $[\alpha]_D^{25} +116.4 \pm 0.5^\circ$  (c 1.0, H<sub>2</sub>O). Anal. (C<sub>9</sub>H<sub>12</sub>N<sub>4</sub>O<sub>7</sub>) C, H, N.

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We prepared the hydrochloride salt of *nitrara*-C (1) by acidifying a solution of 1 in MeOH with concentrated hydrochloric acid until the pH of the solution was  $\sim 1.0$ . The resulting solid was isolated and crystallized from a mixture of 1:1 absolute ethanol/96% ethanol: yield 65%; mp  $>170^\circ\text{C}$  dec; UV  $\lambda_{\text{max}}$  (0.1 N HCl) 276 nm ( $\epsilon$  13280); UV (0.1 N NaOH)  $\lambda_{\text{max}}$  270 nm ( $\epsilon$  9290); UV (96% ethanol)  $\lambda_{\text{max}}$  273 nm ( $\epsilon$  8730); UV ( $\text{H}_2\text{O}$ )  $\lambda_{\text{max}}$  270 and 236 nm ( $\epsilon$  9446 and 7305);  $^1\text{H}$  NMR ( $\text{Me}_2\text{SO}-d_6 + \text{D}_2\text{O}$ )  $\delta$  5.44 (dd,  $J = 5.6$  Hz, 1, H-2'), 5.89–5.98 (two superimposing d,  $J = 7.6$  and 5.6 Hz, 2, H-5 and H-1'), 7.82 (d,  $J = 7.6$  Hz, 1, H-6); IR (KBr) 3250, 3070, 1715, 1680, 1655, 1285, 1090, 1040, 870  $\text{cm}^{-1}$ ;  $[\alpha]_{\text{D}}^{25} +84.9 \pm 0.5^\circ$  (c 1.0,  $\text{H}_2\text{O}$ ). Anal. ( $\text{C}_9\text{H}_{13}\text{ClN}_4\text{O}_7$ ) C, H, Cl, N.

**Cell-Kill in Vivo.** Female B6D2F<sub>1</sub> mice were inoculated ip with  $1 \times 10^5$  murine L1210 leukemic cells on day 0. Five days later, leukemic cells were washed from the ip compartments of untreated mice, and base-line counts were made. At that time,

other mice were treated with the drug; control mice received saline. Twenty-four hours later, leukemic cells were washed from the ip compartments of mice. The number of cells from the treated and control mice was determined and expressed as the percentage of the base-line counts.

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## Synthesis of an *N*-Aminopyrazinonium Analogue of Cytidine<sup>1</sup>

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An *N*-aminated pyrazine analogue of cytidine, in which the pyrimidine N(3) ring nitrogen and C(4) amino group were replaced by a C-amino and an *N*-amino function, respectively, was prepared as a potential deaminase-resistant cytidine antimetabolite. The nucleoside 1,2-diamino-4- $\beta$ -D-ribofuranosylpyrazin-2-onium chloride (6) was a mild cytostatic agent but was neither a substrate for nor an inhibitor of mouse kidney cytidine deaminase. It ionized with a lower  $\text{pK}_a$  than expected. The anion did not undergo the dimerization usually observed with *N*-imino heterocyclic ylides but underwent hydrolysis of the 2-amino group to yield a 1-aminopyrazine-2,3-dione nucleoside.

A recurring problem with the use of amino-substituted purine and pyrimidine nucleoside antimetabolites as cancer chemotherapeutic agents is their rapid deamination in vivo to inactive derivatives.<sup>2-4</sup> The concomitant administration of deaminase inhibitors, such as tetrahydrouridine for cytidine deaminase<sup>5,6</sup> and erythro-9-(2-hydroxy-3-nonyl)-adenine<sup>7,8</sup> or 2'-deoxycoformycin<sup>8-10</sup> for adenosine deaminase, has been one approach to alleviating this problem with arabinosylcytosine (*ara*-C) and arabinosyladenine (*ara*-A), respectively. An alternative approach is the synthesis of biosteres that retain chemotherapeutic activity but resist deamination. 2-Fluoro-*ara*-A,<sup>11,12</sup> carbocyclic

*ara*-A,<sup>13</sup> and 2'-amino-*ara*-C<sup>14</sup> are examples of such derivatives. We have investigated the potential of a novel variation of the latter approach. On the premise that an *N*-amino group might be deaminase resistant, we prepared an analogue of cytidine in which the 3-nitrogen has been replaced by an amino substituent, and an *N*-amine function has been substituted for the enzymatically labile 4-amino group.

**Chemical Studies.** 2-Hydroxy-3-carboxamidopyrazine (1, Scheme I), was prepared by condensation of glyoxal sodium bisulfite and aminomalonic acid by an improved procedure that avoids the heavy contamination of salt encountered with the reported<sup>15</sup> synthesis of 1. This was converted to 2-amino-3-hydroxypyrazine (2),<sup>15</sup> which was then reacted with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- $\beta$ -D-ribofuranose in the presence of  $\text{SnCl}_4$ <sup>16</sup> to afford the tribenzoyl derivative of 1- $\beta$ -D-ribofuranosyl-3-aminopyrazin-2-one (3) as an oil. Removal of the benzoyl groups ( $\text{CH}_3\text{OH}/\text{NH}_3$ ) from purified 3 afforded the nucleoside 4 in an 80% yield for the two steps. The position of ribosylation was indicated by the presence of carbonyl absorption ( $1600\text{ cm}^{-1}$ ) in the IR, the absence of an NH resonance in the NMR spectrum, and the similarity of the neutral UV-absorption spectrum to that of the neutral form, rather than the anion, of 2. The  $\beta$  configuration at the anomeric C-1' position of 4 was indicated by a  $\Delta\delta$  of 0.21 ppm between the two methyl groups of the isopropylidene derivative.<sup>17,18</sup>

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