developing improved therapeutic properties.

Experimental Section

Melting points were taken on a Fisher-Johns hot stage and were not corrected. Ultraviolet spectra were run in methanol solution containing some pH 7 buffer on a Cary 11; infrared spectra were run in Nujol on a Perkin-Elmer 137; and optical rotations were measured at the D line on a Perkin-Elmer 141 polarimeter. All evaporations were carried out in spin evaporators at a bath temperature of 45 °C under vacuum (either water aspirator and/or mechanical pump, as required), unless specified otherwise. Anhydrous Na_2SO_4 was used for drying solutions, unless otherwise specified.

Method A. 2-Amino-6-methylthio-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-9*H*-purine (8b). A methanol solution (150 ml) of NaOMe (32 mmol) and MeSH (128 mmol) was stirred with 8.00 g (15.3 mmol) of **3b**[4] for 4 days at room temperature; it was then worked up and evaporated as in the literature procedure[12] for the 3′-deoxy analogue of **8b**. The residue was dissolved in 250 ml of MeOH containing NaOMe (15 mmol) and stirred for 56 h, after which TLC indicated that the conversion of **6b** to **8b** was completed. After neutralization (HOAc) and evaporation, the residue was passed through a 57.5 × 392 mm column of Dowex 1 (OAc) and eluted with water to afford 3.18 g (68%) of **8b**: UV max (MeOH) 220 nm (ε 18800), 245 (15000), 310 (12700). The properties are listed in Table I. In one run, the final treatment with NaOMe was omitted. The product isolated, after columning the residue through Dowex 1 (OAc), was **6b** (53% yield); the properties are given in Table I.

In another run, the reaction was performed at reflux for 3.5 h, without a final NaOMe treatment. The products isolated, after Dowex columning, were **8b** (21% yield) and **5** (12% yield): mp 140–141 °C (lit.[9b] 129–131 °C); $[\alpha]^{21}D$ -20.5 (*c* 0.25, DMF); UV max (MeOH) 248 nm (ε 9300), 280 (8800). Anal. ($C_{11}H_{25}N_5O_4 \cdot 0.5CH_3OH$) C, H, N.

Method B. 2-Amino-6-(6-methyl-2-pyridyl)methylthio-9-(2-deoxy-9-β-D-*erythro*-pentofuranosyl)-9*H*-purine (10b). To 0.56 g (2.0 mmol) of 1b and 3 mmol of NaOMe in 30 ml of methanol was added 0.35 g (2.5 mmol) of 6-methyl-2-(chloromethyl)pyridine.[17] The solution was kept at 50 °C for 20 h, acidified with HOAc, and evaporated to dryness. The residue in 65 ml of methylene chloride was washed with water (225 and 125 ml) and evaporated. The residue was extracted with 300 ml of hot EtOAc, which was concentrated to about 10 ml to afford 0.71 g (84%) of **10b** as white crystals of analytical purity: UV max (MeOH) 245 nm (ε 14000), 311 (13200). See Table I for properties.

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Fluorinated Pyrimidine Nucleosides. 1. Synthesis of a Nitrogen Analogue of the Antitumor Agent 2,2′-Anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine Hydrochloride

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The nitrogen-bridged compound 2,2′-anhydro-1-β-D-arabinofuranosyl-2,4-diamino-5-fluoropyrimidinium chloride (**2**), an analogue of the antitumor agent anhydro-ara-FC (**1**), has been synthesized. 5-Fluorocytidine was converted into 1-β-D-ribofuranosyl-2,4-diamino-5-fluoropyrimidinium chloride (**4**), but cyclization of **4** was not achieved due to a competing side reaction. The nitrogen bridge was therefore introduced by cyclization of 5-fluoroisocytidine (**10**) to give the 2,2′-imino-bridged compound **16**. The latter was converted into **2** by the standard procedure of thiation, S-methylation, and treatment with ammonia. Compound **2**, as well as a number of the synthetic intermediates, was tested for activity against S180 sarcoma in mice. None of the new compounds exhibited any antitumor activity.

2,2′-Anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine hydrochloride (anhydro-ara-FC, **1**, Figure 1) is a promising new antitumor agent originally synthesized by Fox and co-workers[1] and shown in clinical studies by Burchenal et al.[2] and Gee et al.[3] to be active against acute myeloblastic leukemia. Although the remission rate achieved with **1** was

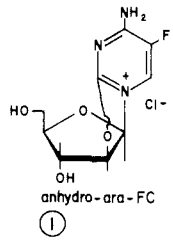
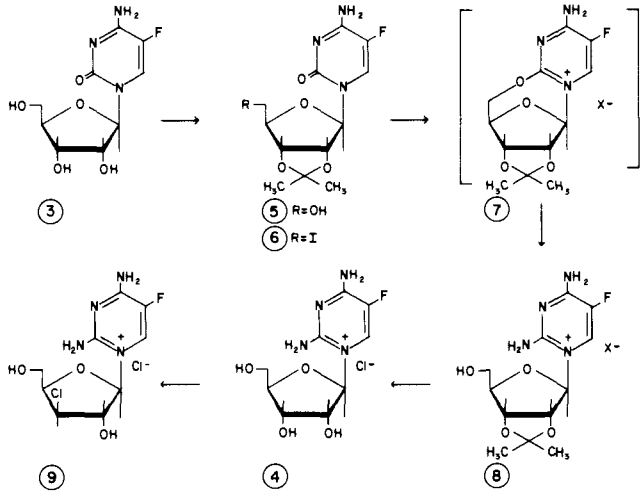
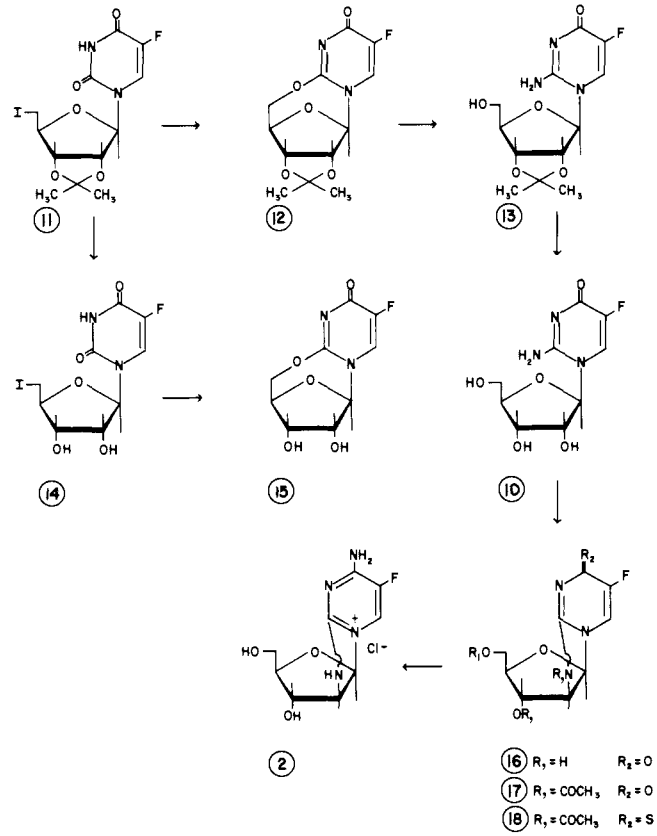


Figure 1. Anhydro-ara-FC.

Scheme I



Scheme II



reported to be no higher than with the widely used antileukemic agent ara-C, remissions were achieved more rapidly, thus minimizing the risk of possible complications. As part of a program in the area of fluorinated pyrimidine nucleosides of potential antitumor activity, we have synthesized the nitrogen analogue **2**, and this synthesis, together with the results of antitumor testing, is described herein.

Results and Discussion

The introduction of the 2,2′-anhydro linkage into pyrimidine nucleosides has generally been achieved by cyclization reactions involving a 2-keto substituent on the pyrimidine moiety and a suitable leaving group on the 2′ (down) position of the carbohydrate ring.[4-7] The formation of a 2,3′-nitrogen bridge has been similarly achieved by a cyclization reaction involving a 2-aminopyrimidine moiety with a mesyl leaving group on the 3′ position.[8] The same kind of approach has been followed in this work in order to synthesize the 2,2′-imino-bridged compound **2**. 5-Fluorocytidine (**3**, Scheme I) was employed as the starting material, and the initial approach involved its conversion into the 2-amino nucleoside **4**, followed by an attempted cyclization of this compound. 5-Fluorocytidine was converted into its 2′,3′-isopropylidene derivative **5**, and the 5′-iodo derivative **6** was obtained by reaction with the iodinating agent triphenyl phosphite methiodide.[9] Reaction of the 5′-iodo derivative **6** with silver acetate in glacial acetic acid rapidly produced the 2,5′-anhydro derivative **7**. This material appeared to be relatively unstable and was not isolated in pure form, since evidence of decomposition was detected during attempts to isolate a crystalline salt. The crude material was therefore directly treated with methanolic ammonia to give the diamino nucleoside **8**, which was isolated as the picrate salt. Removal of the isopropylidene protecting group from **8** was carried out using 80% acetic acid for 35 min at 100 °C, and **4** was obtained in crystalline form as the hydrochloride.

Reaction of **4** with an excess of 2-acetoxyisobutyryl chloride[4] in acetonitrile under reflux did not produce any of the desired imino-bridged derivative but, instead, produced after hydrolysis a chloro nucleoside which was tentatively formulated as **9**. Elemental analysis indicated the presence of two atoms of chlorine in the molecule, one covalent and the other of ionic character. Mechanistically, the formation of this product can be rationalized by the initial formation of a 2′,3′-acetoxonium ion intermediate, followed by discharge of the latter by chloride ion to give a *trans*-chloroacetate; alkaline hydrolysis would then give the chlorohydrin **9**. Steric considerations would suggest that attack of chloride ion on the 3′ position would be preferred, although 2′ attack cannot be ruled out. The susceptibility of **9** toward sodium methoxide, with elimination of chloride ion, gave an indication of the trans orientation of the chlorohydrin. The intramolecular cyclization is presumably less favored due to the relatively poor nucleophilicity of the 2-amino substituent as compared with chloride ion. Russell and co-workers[10] have demonstrated that reaction of 2-acetoxyisobutyryl chloride with adenosine yielded in similar fashion a 3′-chloro-3′-deoxyxylofuranosyladenine derivative. Reaction of **4** with 2-acetoxyisobutyryl chloride in the presence of either triethylamine or dicyclohexylamine similarly did not produce any cyclization, but decomposition was evident; the alkaline lability of 2-amino-4-imino-1-substituted pyrimidines has been previously reported.[11] Hampton and Nichol[5] have shown that 2,2′-anhydro nucleosides can be produced by a reaction involving diphenyl carbonate in DMF, but application of this method also failed to produce any of the desired *N*-anhydro compound.

At this stage the strategy was redesigned so as to introduce the *N*-anhydro bridge by a cyclization reaction involving 5-fluoroisocytidine (**10**, Scheme II); the 2-amino substituent, not having any partial positive charge as is presumably the case with **4**, might be expected to be sufficiently nucleophilic for intramolecular cyclization to

be favored. Accordingly, the preparation of 5-fluoroisocytidine was undertaken using the 5′-iodo compound **6** as starting material. Deamination of **6** using sodium nitrite and acetic acid produced the 5′-iodo-5-fluorouridine derivative **11**, which was smoothly converted into the 2,5′-anhydro compound **12** by treatment with silver acetate in methanol. Reaction of **12** with methanolic ammonia cleaved the anhydro linkage to produce 2′,3′-isopropylidene-5-fluoroisocytidine (**13**), which was then deprotected using 90% trifluoroacetic acid to give 5-fluoroisocytidine (**10**). This synthesis of 5-fluoroisocytidine closely resembles the synthesis of isocytidine as described by Brown et al.;[12] in both cases a 2,5′-anhydro compound is the key intermediate.

Compound **11** was also deprotected using 90% trifluoroacetic acid to give 5′-iodo-5-fluorouridine **14**, which was then cyclized as described for **11** to give 2,5′-anhydro-5-fluorouridine **15**. Both **14** and **15** were required for antitumor testing. Formation of the *N*-anhydro linkage was carried out by treatment of **10** with either 2-acetoxyisobutyryl chloride in acetonitrile or diphenyl carbonate in DMF. In practice the latter method was found to be more efficient, since it provided higher yields and gave the unprotected *N*-anhydro compound **16** directly. The cyclization of **10** by the diphenyl carbonate method was more efficient than for uridine as reported by Hampton and Nichol,[5] and our own experiments have shown that cyclization of 5-fluorocytidine (**3**) by this method is extremely sluggish. These results presumably reflect the greater nucleophilicity of the 2-amino group in **10** as compared with the 2-keto group in uridine and in 5-fluorocytidine; steric factors would not be expected to be of importance since the C–O and C–N bond lengths are quite similar.

For conversion of the 4-keto group of **16** into an amino function, the standard method of thiation and amination was employed.[13] Reaction of the triacetyl derivative **17** with a slight excess of phosphorus pentasulfide in dioxane produced an almost quantitative yield of the acetylated thione **18** under relatively mild conditions (60 °C for 30 min). This material was isolated as a yellow crystalline solid, and the introduction of the 4-thione substituent was evidenced by the appearance of a long wavelength absorption maximum (336 nm) in the UV spectrum. The NMR spectrum indicated a $C_5(F)$–$C_6(H)$ coupling of only 1 Hz, in contrast to the 6–7 Hz coupling as observed in all the other fluoropyrimidine nucleosides described herein. Thione **18** was treated with methyl iodide and sodium hydroxide to give an *S*-methyl derivative which was not isolated but treated directly with anhydrous ammonia under reflux, yielding the required *N*-anhydro compound **2**. Since as expected from the work of Brown and Teitei[11] the free base of **2** gave indications of instability, it was converted into a crystalline hydrochloride salt.

Anhydro-ara-FC (**1**) has been shown to be extremely labile in alkaline media[14] with complete hydrolysis after ca. 1 h at pH 10.6. In contrast, the nitrogen analogue **2** was found to be relatively stable; treatment with 1 M aqueous sodium hydroxide for 18 h at room temperature was necessary for the complete destruction of starting material, and a number of fluorescent decomposition products were formed. The 4-oxo analogue **16** was even more stable; on treatment with 1 M aqueous sodium hydroxide at room temperature, no hydrolysis was detected after 48 h. Doerr et al.[8] similarly reported that a 2,3′-imino-bridged nucleoside was particularly stable as compared with its oxygen analogue.

An x-ray crystallographic analysis of **2** as the hydrochloride salt (Figure 2) confirmed the designation of the

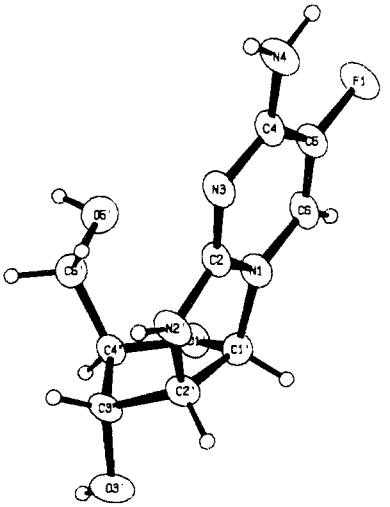


Figure 2. A projection drawing of **2** showing its conformation in the crystalline state.

nitrogen bridge as 2,2′, and its conformation bore a close resemblance to that of 2,2′-anhydro-ara-C as reported by Brennan and Sundaralingam.[15] The glycosyl torsion angle, which describes the orientation of the base with respect to the sugar, was found to be 294° for **2** and reported to be 299° for 2,2′-anhydro-ara-C. Thus the substitution of nitrogen for oxygen does not lead to significant distortion of the anhydro linkage. X-Ray crystallography also indicated the presence of an amino, rather than an imino group on C_4 as observed for anhydro-ara-C by Brennan and Sundaralingam.

The NMR spectrum of **2** was compared with that of anhydro-ara-FC (**1**). The general features of the spectra were quite similar except for $H_{2'}$, which in **2** showed a comparatively large upfield shift of δ 0.65. This relatively large shift can be accounted for by the greater electronegativity of oxygen as compared with nitrogen, a phenomenon which has been previously observed by Doerr et al.[8] in the spectra of a number of 2,3′-N-bridged thymidine derivatives.

Biological Testing. Compounds **2–4**, **10**, and **14–16** were tested for antitumor activity against a transplantable S180 sarcoma in mice. The inactivity of the 2-aminofluoropyrimidine ribonucleoside **4** (50 mg/kg)[16] and 5-fluoroisocytidine (**10**, 50 mg/kg) is in marked contrast to that of 5-fluorocytidine (**3**) (active at 1, 2.5, and 5 mg/kg); thus substitution of an amino for a keto substituent, or reversal of these substituents, leads to loss of antitumor activity. 5′-Deoxy-5′-iodo-5-fluorouridine (**14**) and 2,5′-anhydro-5-fluorouridine (**15**) were synthesized as potential depot forms of the active antitumor agent 5-fluorouridine, a compound reported to be in clinical trial for use against solid tumors.[17] The lack of antitumor activity for **14** (50 mg/kg) and **15** (100 mg/kg) as compared to 5-fluorouridine (active at 5 mg/kg) is an indication that the conversion of **14** and **15** to 5-fluorouridine in vivo is relatively inefficient. The activity of anhydro-ara-FC (**1**) has been attributed to the fact that it acts as a depot for the active compound 1-β-D-arabinofuranosyl-5-fluorocytosine (ara-FC) which is liberated in vivo by chemical hydrolysis.[18] (Burchenal et al.,[2] however, have indicated that in some mammalian cell cultures anhydro-ara-FC might be active per se). The *N*-anhydro compounds **2** and **16**, in contrast, were both inactive (at 100 and 50 mg/kg, respectively). These compounds are both stable at physiological pH and thus cannot function as depot forms for any potentially active arabino nucleosides.

Conclusions

Compound **2**, a nitrogen analogue of the antitumor agent 2,2′-anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine (**1**), has been synthesized from 5-fluorocytidine. Since attempts at cyclization of the 2,4-diamino nucleoside **4** were unsuccessful, the nitrogen bridge was introduced by cyclization of 5-fluoroisocytidine (**10**) which was prepared from **3** in six steps. Compound **10** was converted into **2** by thiation, S-methylation, and ammonia treatment.

The nitrogen analogue **2** as well as a number of synthetic intermediates was tested for antitumor activity against an S180 sarcoma in mice. None of the compounds exhibited any antitumor activity.

Experimental Section

General. Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. Proton magnetic resonance spectra were obtained using either a Varian XL-100 or HA-100 spectrometer and IR spectra with a Perkin-Elmer 621 or a Beckman IR-9 instrument. UV spectra were obtained using a Cary Model 14 recording spectrometer.

2′,3′-*O*-Isopropylidene-5-fluorocytidine (5). A suspension of 5-fluorocytidine (100 g, 383 mmol) in acetone (1.5 l.) and 2,2-dimethoxypropane (187 ml) was treated with *p*-toluenesulfonic acid monohydrate (78.5 g, 413 mmol) and stirred at room temperature for 2 h. Excess sodium bicarbonate was added, and the solution was stirred until neutral, filtered through Celite, and evaporated to dryness. The residue was triturated with ethyl acetate (1 l.) and stirred for 1 h, and the solid was collected, washed with ethyl acetate, and dried in vacuo: yield 108.9 g (95%). An analytical sample was obtained from methanol–ethyl acetate: mp 182–184 °C; NMR (Me_2SO-d_6) δ 8.04 (d, 1, J = 6 Hz, CHCF), 1.28, 1.47 [s, 3, $(CH_3)_2C$], 3.59 (m, 2, CH_2O). Anal. ($C_{12}H_{16}FN_3O_5$) C, H, N, F.

5′-Deoxy-2′,3′-*O*-isopropylidene-5′-iodo-5-fluorocytidine (6). A solution of **5** (40 g, 133 mmol) in DMF (375 ml) was treated with triphenyl phosphite methiodide[9] (75 g, 166 mmol) for 2.5 h and then treated with methanol (100 ml). After 1 h the solution was evaporated to dryness, and the residual oil was partitioned between ethyl acetate (750 ml) and aqueous sodium thiosulfate (5%, 750 ml). The ethyl acetate layer was washed with aqueous thiosulfate (1 × 750 ml) and water (2 × 750 ml) and evaporated to dryness. The product was dissolved in ethyl acetate (200 ml) and hexane was added to the hot solution until crystallization commenced. On cooling the crystals were collected, washed with hexane, and dried in vacuo: yield 40.2 g (74%); mp 192–194 °C; NMR (Me_2SO-d_6) δ 8.0 (d, 1, J = 7 Hz, CHCF), 1.28, 1.47 [s, 3, $(CH_3)_2C$], 3.45 (m, 2, CH_2I). Anal. ($C_{12}H_{15}FIN_3O_4$) C, H, N, I.

1-(2′,3′-*O*-Isopropylidene-β-D-ribofuranosyl)-2,4-diamino-5-fluoropyrimidinium Picrate (8). A solution of **6** (10 g, 24.3 mmol) in glacial acetic acid (300 ml) was stirred with silver acetate (20 g) for 4 h at room temperature. The product was treated with hydrogen sulfide to precipitate dissolved silver salts, and the silver sulfide was removed by filtration through Celite. The filtrate was evaporated to a gum and treated with methanolic ammonia (200 ml, saturated) overnight at room temperature. After filtration through Celite, the solution was evaporated to a foam and treated with aqueous picric acid (450 ml, saturated). A small amount of gummy material was removed by filtration through Celite, and the filtrate was stirred at 0 °C until crystals were deposited. The crystals were collected, washed briefly with cold water, and dried in vacuo to give **8**: 6.36 g (49%); mp 169–170 °C; NMR (Me_2SO-d_6) δ 8.31 (d, 1, CHCF), 1.31, 1.54 [s, 3, $(CH_3)_2C$]. Anal. ($C_{18}H_{20}FN_7O_{11}$) C, H, F, N.

1-β-D-Ribofuranosyl-2,4-diamino-5-fluoropyrimidinium Chloride (4). A solution of **8** (6.36 g, 12 mmol) in 80% aqueous acetic acid (200 ml) was heated on a steam bath for 50 min and then cooled and evaporated to dryness. Water (125 ml) was added to the residue, and the insoluble material was removed by filtration. The filtrate was evaporated to dryness, dissolved in methanol–water (1:1, 200 ml), and stirred with an excess of Dowex 1 (chloride) resin until the solution was colorless. The resin was filtered off and the filtrate evaporated to dryness. After dissolution in methanol (40 ml), filtration, and evaporation of the filtrate, the residue was dissolved in ethanol (25 ml), and crystallization rapidly commenced. After storage at 0 °C overnight the crystals were collected, washed briefly with cold ethanol, and dried in vacuo. A second crop was obtained by addition of ethyl acetate: yield 2.23 g (62%); NMR (Me_2SO-d_6) δ 8.67 (d, 1, J = 7 Hz, CHCF); UV (MeOH) λ_{max} 209 nm (ε 17 070), 243 (11 420), sh 275 (6880). Anal. ($C_9H_{14}ClFN_4O_4$) C, H, N, F.

Reaction of 4 with 2-Acetoxyisobutyryl Chloride. A suspension of **4** (297 mg, 1 mmol) in acetonitrile (5 ml) was stirred with 2-acetoxyisobutyryl chloride[4] (0.58 ml, 4 mmol) under reflux for 1 h. The solution was filtered and addition of ether (100 ml) to the filtrate gave a precipitate which was collected and washed with ether. This precipitate was treated with hydrogen chloride in methanol (0.5 N, 5 ml) for 4 days and then evaporated to dryness. The residue was crystallized from ethanol–ether to give a compound tentatively formulated as **9**: 100 mg (30%); mp 133–142 °C (indef, dec); NMR (Me_2SO-d_6) δ 8.30 (d, 1, J = 7 Hz, CHCF); UV λ_{max} 203–204 nm (ε 19 650), 236 (11 820), 274–275 (7460). Anal. ($C_9H_{13}Cl_2FN_4O_3$·0.5MeOH) C, H, N, Cl, Cl^-.

5′-Deoxy-5′-iodo-2′,3′-*O*-isopropylidene-5-fluorouridine (11). A 0 °C solution of **6** (35 g, 85 mmol) in 50% aqueous acetic acid (700 ml) was stirred with sodium nitrite (175 g). After 4 h a second portion of sodium nitrite (175 g) was added, and the mixture was stirred overnight and allowed to gradually warm to room temperature. Glacial acetic acid (700 ml) was added, and the mixture was stirred until evolution of gases had ceased. After evaporation to dryness the residue was partitioned between ethyl acetate (1.5 l.) and water (1.5 l.). The ethyl acetate layer was washed with water (3 × 1 l.), evaporated to dryness, and crystallized from ethyl acetate–hexane: yield 30.2 g (86%); mp 202–203.5 °C; NMR (Me_2SO-d_6) δ 8.10 (d, 1, J = 6 Hz, CHCF), 1.30, 1.49 [s, 3, $(CH_3)_2C$], 3.43 (m, 2, CH_2I); UV (MeOH) λ_{max} 263 nm (ε 9320). Anal. ($C_{12}H_{14}FIN_2O_5$) C, H, N.

2,5′-Anhydro-2′,3′-*O*-isopropylidene-5-fluorouridine (12). A solution of **11** (30 g, 73 mmol) in methanol (500 ml) was stirred under reflux in the presence of silver acetate (60 g) for 30 min and then filtered through Celite. Hydrogen sulfide was bubbled through the filtrate in order to precipitate silver salts which were subsequently removed by filtration through Celite. The filtrate was evaporated to dryness and dissolved in hot ethyl acetate (900 ml), and addition of hexane (1.5 l.) slowly with stirring precipitated **12**: 17.7 g (86%); mp 213–215 °C; NMR (Me_2SO-d_6) δ 8.34 (d, 1, CHCF), 1.29, 1.42 [s, 3, $(CH_3)_2C$]; UV (MeOH) λ_{max} 246 nm (ε 11 720). Anal. ($C_{12}H_{13}FN_2O_5$) C, H, N, F.

2′,3′-*O*-Isopropylidene-5-fluoroisocytidine (13). Compound **12** was treated with methanolic ammonia (300 ml) for 2 days at room temperature, and after filtration through Celite the solution was evaporated to dryness and triturated with boiling ethyl acetate (400 ml) to induce crystallization of **13**: 19.3 g (88%); mp 208–209 °C; NMR (Me_2SO-d_6) δ 7.85 (d, 1, J = 7 Hz, CHCF), 1.30, 1.54 [s, 3, $(CH_3)_2C$]; UV (MeOH) λ_{max} 202–203 nm (ε 24 000), sh 255 (7200). Anal. ($C_{12}H_{16}FN_3O_5$) C, H, N, F.

5′-Deoxy-5′-iodo-5-fluorouridine (14). A solution of **11** (4 g, 9.7 mmol) in 90% aqueous trifluoroacetic acid (30 ml) was stored at room temperature for 15 min and then evaporated to dryness. The residue was coevaporated with ethanol (2 × 40 ml) and recrystallized from ethyl acetate (120 ml) to give **14**: 3.15 g (87%); mp 174.5–175.5 °C; NMR (Me_2SO-d_6) δ 8.0 (d, 1, J = 7 Hz, CHCF); UV (MeOH) λ_{max} 267–268 nm (ε 9130). Anal. ($C_9H_{10}FIN_2O_5$) C, H, N, I.

2,5′-Anhydro-5-fluorouridine (15). A solution of **14** (4.6 g, 12.4 mmol) in methanol (100 ml) was stirred under reflux with silver acetate (9 g) for 25 min, and the solid was removed by filtration. The filtrate was treated with hydrogen sulfide for 15 min and filtered through Celite, and the filtrate was evaporated to dryness. The residue was recrystallized from methanol (150 ml)–ether (150 ml) to give **15**: 1.3 g (43%); mp 220.5–223.5 °C; NMR (Me_2SO-d_6) δ 8.42 (d, 1, J = 6 Hz, CHCF); UV (H_2O) λ_{max} 249 nm (ε 11 400). Anal. ($C_9H_9FN_2O_5$) C, H, N, F.

5-Fluoroisocytidine (10). A solution of **13** (16 g, 53.3 mmol) in 90% aqueous trifluoroacetic acid (75 ml) was stored at room temperature for 15 min and then evaporated to dryness. The residue was coevaporated with ethanol (2 × 100 ml), dried, and dissolved in methanol (90 ml). Triethylamine was added to pH 8–9, and crystals were slowly deposited. After storage at 0 °C overnight the crystals were collected and dried in vacuo to give

10: 11.15 g (80%); mp 190–191 °C. NMR (Me_2SO-d_6) δ 7.97 (d, 1, J = 7 Hz, CHCF); UV (H_2O) λ_{max} 202–203 nm (ε 19 200), 262 (6000). Anal. ($C_9H_{12}FN_3O_5$) C, H, N.

2,2′-Anhydro-1-β-D-arabinofuranosyl-5-fluoroisocytosine (16). A solution of 10 (10.1 g, 38.7 mmol) and diphenyl carbonate (10.1 g, 55.5 mmol) in DMF (75 ml) was stirred at 140 °C in the presence of sodium bicarbonate (1 g) for 80 min. After filtration the filtrate was evaporated to dryness and the residue was partitioned between chloroform (100 ml) and water (100 ml). The water layer was washed twice with chloroform and evaporated to dryness, and the residue was triturated with hot methanol. After storage at room temperature overnight the crystals were collected and dried in vacuo to give 16, 7.73 g (82%). An analytically pure sample was obtained by recrystallization from methanol–water (9:1): mp 269–271 °C dec; NMR (Me_2SO-d_6) δ 7.83 (d, 1, J = 6 Hz, CHCF); UV (H_2O) λ_{max} 219 nm (ε 15 420), 270 (3580). Anal. ($C_9H_{10}FN_3O_4$) C, H, N, F.

2,2′-Anhydro-1-(2′,3′,5′-N,O,O-triacetyl-β-D-arabinofuranosyl)-5-fluoroisocytosine (17). A suspension of 16 (7.8 g, 32 mmol) in pyridine (150 ml) and acetic anhydride (40 ml) was stirred overnight at room temperature. The solution was cooled to 0 °C, methanol (50 ml) was added, and after 1 h the solution was evaporated to dryness. The residue was coevaporated with ethanol (50 ml × 2) and triturated with ethanol (50 ml), and after storage overnight the crystals were collected and dried in vacuo: 10.85 g (92%). An analytically pure sample was obtained from ethyl acetate: mp 202.5–204 °C; NMR (Me_2SO-d_6) δ 8.39 (d, 1, J = 6 Hz, CHCF), 1.90, 2.12 (s, 3, CH_3COO), 2.57 (s, 3, CH_3CON); UV (MeOH) λ_{max} 221 nm (ε 27 500), sh 255 (9000). Anal. ($C_{15}H_{16}FN_3O_7$) C, H, F, N.

2,2′-Anhydro-1-(2′,3′,5′-N,O,O-triacetyl-β-D-arabinofuranosyl)-5-fluoro-4-thioisocytosine (18). A solution of 17 (3.69 g, 10 mmol) in dioxane (50 ml) was stirred in the presence of phosphorus pentasulfide (2.7 g, 14.1 mmol) at 60 °C for 30 min. The solids were removed by filtration and the filtrate was evaporated and partitioned between ethyl acetate (100 ml) and saturated aqueous sodium bicarbonate (100 ml). The ethyl acetate layer was washed with water (2 × 100 ml), evaporated to dryness, and coevaporated with ethanol (2 × 50 ml) to give 18 as an almost pure orange solid, 3.7 g (96%). An analytical sample was obtained with difficulty by recrystallization from ethanol, since the material showed a tendency to form an oil: mp 83–88 °C; NMR ($CDCl_3$) δ 7.25 (d, 1, J = 1 Hz, CHCF), 1.93, 2.16 (s, 3, CH_3COO), 2.77 (s, 3, CH_3CON); UV (MeOH) λ_{max} 226 nm (ε 16 960), 264 (6590), 336 (20 750). Anal. ($C_{15}H_{16}FN_3O_6S \cdot 0.5C_2H_5OH$) H, N, S, F; C: calcd, 47.06; found, 46.49.

2,2′-Anhydro-1-β-D-arabinofuranosyl-2,4-diamino-5-fluoropyrimidinium Chloride (2). Aqueous sodium hydroxide (1 N, 19.2 ml) was added dropwise over 1 h to a suspension of 18 (7.4 g, 19.2 mmol) and methyl iodide (2.3 ml) in methanol (200 ml). After a further 30 min the solution was evaporated, coevaporated with ethanol (2 × 100 ml), and pumped to dryness. The residue was treated with liquid ammonia (100 ml) under reflux overnight, and after storage at room temperature to remove ammonia, the residue was coevaporated with methanol (2 × 50 ml) and dried in vacuo overnight. This material was dissolved in water (200 ml) and applied to a Dowex 50 column (3.3 × 60 cm, pyridinium form), and after preliminary washes of water (2 l.) and 0.1 M pyridinium formate (pH 4.8, 2 l.), the column was eluted with 0.5 M pyridinium formate (pH 4.8, 4 l.). The eluate was evaporated to dryness, dissolved in hot 2-propanol (200 ml), and filtered to remove some solid material. The filtrate was evaporated to dryness and redissolved in 2-propanol (50 ml), and after storage overnight a second batch of solid was removed by filtration. The filtrate was evaporated to dryness, dissolved in water (200 ml), and applied to a Dowex 1 column (2.3 × 40 cm, chloride form) which was eluted with water (1 l.). The eluate was evaporated to dryness, coevaporated with ethanol (2 × 50 ml), and triturated with 2-propanol (50 ml) to give 2 as an amorphous solid, 1.9 g (36%). Analytically pure material was obtained by crystallization from methanol: mp 275–285 °C dec; NMR (Me_2SO-d_6) δ 8.53 (d, 1, J = 6 Hz, CHCF); UV (H_2O) λ_{max} 205 nm (ε 18 510), 225 (13 710), 284–286 (4120). Anal. ($C_9H_{12}ClFN_4O_3$) C, H, N, F.

Crystals of 2 are monoclinic, space group $P2_1$, with a = 9.490 (5), b = 11.150 (6), and c = 5.746 (4) Å, β = 107.16 (4)°, and d_{calcd} = 1.592 g cm^{-3} for Z = 2. X-Ray crystallographic intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered Cu Kα radiation, θ–2θ scans). The approximate size of the crystal used for data collection was 0.08 × 0.30 × 0.35 mm; no absorption corrections were made. There were 1243 accessible reflections with θ < 76°, of which 1188 were considered observed. The structure was solved by a multiple solution procedure and was refined by full-matrix least squares. The final discrepancy indices were R = 0.048 and R_w = 0.060 for the 1188 observed reflections.

Biological Testing. Small pieces of S180 sarcoma (20–30 mg) were implanted subcutaneously by trocar into the right inguinal region of 18–20-g albino mice. The fragments were obtained from donors bearing firm subcutaneous tumors implanted 7–10 days previously. Compounds were dissolved, or suspended (with sonication), in water for intraperitoneal administration. Treatment was begun on the day of implantation and was continued once daily for a period of 8 days. The animals were then sacrificed and the tumors were excised and weighed. The ratio of the average weight of the tumors from an untreated control group (C) divided by the average weight of the tumors from the treated group (T) was calculated. If the C/T ratio was 2.0 or greater (50% or more inhibition) the compound was considered active at the dose tested.

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