Synthesis and in Vivo Antitumor Activity of Potential 5-Fluorouracil Prodrugs†

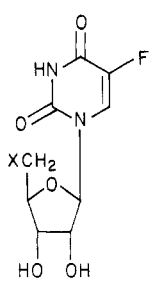
A. Rosowsky,* S.-H. Kim, D. Trites, and M. Wick

Sidney Farber Cancer Institute and Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115.
Received February 12, 1982

Analogues of 5′-deoxy-5-fluorouridine (DFUR, 1) were synthesized with the aim of evaluating the effect of structural modification of the 5′ region of the ribofuranose moiety on the activity of these potential prodrug derivatives of 5-fluorouracil (FU). Reaction of the 2′,3′-*O*-isopropylidene-5′-*O*-*p*-toluenesulfonyl derivative **9** of 5-fluorouridine (FUR, **2**) with lithium dimethylcuprate and acidolysis of the isopropylidene group with 90% trifluoroacetic acid gave the ribohexofuranoside **3**. Azide displacement of the tosylate group from **9**, followed by reduction with triphenylphosphine and acidolysis with trifluoroacetic acid, led to the 5′-amino compound **4**. Use of *n*-butanethiol as the nucleophile provided the thioether **5**. Glycosylation of O^2,O^6-bis(trimethylsilyl)-5-fluorouracil with D-erythrose triacetate and stannic chloride in acetonitrile, followed by deblocking with NaOMe, yielded the erythrofuranoside **8**, which may be viewed as a hybrid of 1 and the FU prodrug Ftorafur. Air oxidation of the 5′-OH group in **2** in the presence of platinum catalyst afforded the 5′-uronic acid **6**. Compounds **3**, **6**, and **8** were active against P-388 leukemia in mice, giving median increases in life span (ILS) of +63, +63, and +75% at doses of 600, 300, and 640 mg/kg (ip, qd × 4). On the same schedule against this tumor, FU gave a +73% ILS at 15 mg/kg and DFUR gave a +54% ILS at 320 mg/kg. As expected, FUR was much more potent than any of the other nucleosides, with a +75% ILS at the optimal nontoxic dose of 4 mg/kg. The cytotoxicity of the 5′-modified DFUR analogues toward L1210 mouse leukemia cells in culture was much lower than that of FU, the difference in ID_{50} values being as great as 1000-fold in the case of the 5′-uronic acid **6**. The activity of these compounds in vivo, coupled with their low activity in vitro, was consistent with a prodrug mechanism and suggested that further studies on the effect of 5′-modification may be of interest as part of a broader search for improved prodrug derivatives of FU.

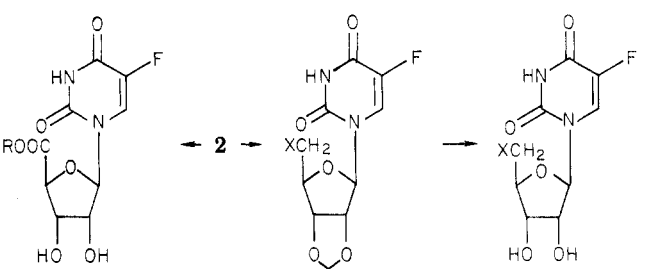
5′-Deoxy-5-fluorouridine (DFUR, 1)[1,2] is a recently developed structural analogue of 5-fluorouridine (FUR, **2**) with promising antineoplastic activity and low host toxicity in several experimental animal tumor models.[3-7] Its favorable therapeutic index[3,4] is considered to reflect preferential bioactivation in tumor cells as opposed to normal proliferative tissues, such as the bone marrow, intestinal epithelium, liver, and spleen.[6] Activation results from cleavage of 1 to 5-fluorouracil (FU). Thus, while 1 is superficially a structural analogue of **2**, its mode of action is actually that of a prodrug of FU. Biochemical studies[7] have demonstrated that the affinity of 1 for uridine phosphorylase in Ehrlich ascites cells is comparable to that of 2′-deoxy-5-fluorouridine (FUdR), but about tenfold lower than that of **2**. The principal acid-soluble metabolites detected in mice following treatment with 1 were 5′-phosphate esters of **2**. Some FUdR 5′-monophosphate also was probably generated, as indicated by evidence of decreased thymidylate synthesis in cultured cells. 5′-Nucleotides of FUR and FUdR cannot form directly from 1, which lacks a 5′-OH group, and must presumably arise via the sequential enzymatic action of phosphoribosyl pyrophosphate transferase, pyrimidine nucleotide kinase, and ribonucleotide reductase on the initially formed FU.[8]

In view of the myelosuppression frequently encountered during FU chemotherapy in man,[9] studies on compounds related in structure and mechanism of action to 1 seemed worthwhile. Prior work[10] showed that deletion of a 2′-OH and 3′-OH substituent from the sugar moiety of 1 results in loss of substrate activity toward uridine phosphorylase, giving products that are inactive in vivo against FU-sensitive tumors, such as S180 mouse sarcoma. When both the 2′-OH and 3′-OH groups are deleted, on the other hand, some activity is observed, although it is unclear at present whether this is a consequence of direct uridine phosphorylase cleavage or of a more complex bioactivation pathway involving a hepatic microsomal enzyme system, as has been proposed for the cleavage of the closely related



1, X = H
2, X = OH

Scheme I



6, R = H
7, R = Et

9, X = *p*-$MeC_6H_4SO_2$
10, X = Me
11, X = N_3
12, X = *n*-BuS

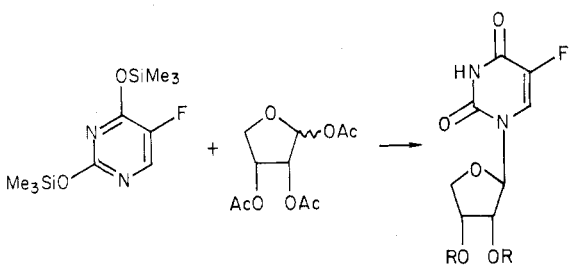
3, X = Me
4, X = NH_2
5, X = *n*-BuS

† This work has been presented in part. See "Abstracts of Papers", 183rd National Meeting of the American Chemical Society, Las Vegas, NV, Mar 28–Apr 2, 1982; American Chemical Society: Washington, DC, 1982; Abstr MEDI 77.

(1) Hrebabecky, H.; Beranek, J. *Collect. Czech. Chem. Commun.* **1978**, *43*, 3268.
(2) Cook, A. F.; Holman, M. J.; Kramer, M. J.; Trown, P. W. *J. Med. Chem.* **1979**, *22*, 1330.
(3) Bollag, W.; Hartmann, H. R. *Eur. J. Cancer* **1980**, *16*, 427.
(4) Ishitsuka, H.; Miwa, M.; Takemoto, K.; Fukuoka, K.; Itoga, A.; Murayama, H. *Gann* **1980**, *71*, 112.
(5) Ohta, Y.; Sueki, K.; Kitta, K.; Takemoto, K.; Ishitsuka, H.; Yagi, Y. *Gann* **1980**, *71*, 190.
(6) Suzuki, S.; Hongo, Y.; Fukuzawa, H.; Ishikara, S.; Shimizu, H. *Gann* **1980**, *71*, 238.
(7) Armstrong, R. D.; Diasio, R. B. *Cancer Res.* **1980**, *40*, 3333.
(8) Heidelberger, C. in "Antineoplastic and Immunosuppressive Agents"; Sartorelli, A.; Johns, D. G., Eds.; Springer Verlag: New York, 1975; Part II, pp 193–231.
(9) Livingston, R. B.; Carter, S. K. "Single Agents in Cancer Chemotherapy"; IFI/Plenum: New York, 1970; pp 195–226.
(10) Cook, A. F.; Holman, M. J.; Kramer, M. J. *J. Med. Chem.* **1980**, *23*, 852.

0022-2623/82/1825-1034$01.25/0

Scheme II



13, R = Ac
8, R = H

prodrug 5-fluoro-1-(tetrahydrofuran-yl)uracil (Ftorafor, FT-206).[11-14] In this paper, we report the chemical synthesis and preliminary antitumor evaluation of several analogues of **2** that retain 2′,3′-dihydroxy substitution on the sugar ring but are *modified in the 5′ region.* The bulk as well as polar character of the groups attached to the 5′ carbon were varied, in the hope that this might bring about an improvement in therapeutic index based on either an increase in substrate specificity for the uridine phosphorylase in tumor cells or a more favorable distribution of prodrug to the tumor as opposed to other host tissues. Specific compounds studied were the analogues **3**–**8** (Schemes I and II), in which the CH_3 moiety of **1** is replaced by CH_3CH_2, NH_2CH_2, *n*-BuSCH$_2$, COOH, COOEt, and H, respectively. Compound **8** may be viewed as either a demethyl analogue of **1** or a dihydroxy derivative of Ftorafur, and is thus an interesting hybrid of these two structures.

Chemistry. 5-Fluorouridine (**2**) was prepared in 72% overall yield by $SnCl_4$-catalyzed condensation of the O^2,O^6-bis(trimethylsilyl) derivative of FU with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose in acetonitrile, followed by debenzoylation with NaOMe.[15] The 2′,3′-*O*-isopropylidene derivative of **2**, obtained in 70% yield on treatment with 2,2-dimethoxypropane in dry acetone containing 0.1 molar equiv of bis(*p*-nitrophenyl) phosphate,[16] was converted to the heretofore undescribed 5′-*O*-tosylate ester **9** (92% yield). Reaction of the latter with lithium dimethylcuprate[17] in a mixture of Et_2O and 1,2-dimethoxyethane at 0 °C overnight afforded the 5′,6′-dideoxy-2′,3′-*O*-isopropylidene-β-D-*ribo*-hexofuranoside **10** (85%), a noncrystalline substance which on acidolysis with 90% trifluoroacetic acid for 1 h at room temperature yielded compound **3** (85%). The UV absorption spectrum of this simple homologue of **1** showed the expected maximum at 270 nm in EtOH, and the NMR spectrum in D_2O confirmed the presence of a CH_3CH_2 group in the sugar moiety. The CH_2 protons gave rise to two overlapping quartets centered at δ 1.6 ($J_{5',6'}$ = 7 Hz, $J_{4',5'}$ = 2 Hz), indicating diastereotopic nonequivalence, and the CH_3 protons were seen as a triplet at δ 0.9 (J = 7 Hz). The β configuration of the glycoside bond was apparent from the anomeric proton signal at δ 5.8 (d, J = 4 Hz). Comparison of the TLC mobility of **3** and **1** (silica gel; 7:2:1 *i*-PrOH–H_2O–concentrated NH_4OH) gave similar R_f values of 0.65 and 0.61, respectively. The slightly higher R_f observed for compound **3** was consistent with our expectation that this molecule would be more lipophilic than **1**. The smooth reaction of the tosylate ester **9** with lithium dimethylcuprate prompted us to attempt extension of this alkylation procedure using other lithium dialkylcuprates. A reaction between **9** and lithium di-*n*-butylcuprate in a 1:1 mixture of Et_2O and 1,2-dimethoxyethane was attempted, but unfortunately none of the desired product could be isolated. Instead, a very complex mixture was obtained, in which the major component was a compound whose NMR spectrum in $CDCl_3$ solution showed complete disappearance of both the H_6 and $H_{1'}$ protons. This finding and the absence of UV absorption at 270 nm, indicating probable destruction of the pyrimidine chromophore, suggested that conjugate addition to the fluorine-activated double had occurred. The propensity of pyrimidine nucleosides to undergo conjugate addition by organometallic reagents has been discussed recently.[18]

Further use of the tosylate ester **9** was made in the preparation of the 5′-amino-5′-deoxynucleoside **4**. Treatment of **9** with 2 equiv of lithium azide in DMF at 70–75 °C for 2 h[19] effected smooth displacement of the tosylate group to form the protected azide **11** (75%), which on reaction with triphenylphosphine in pyridine, followed by addition of concentrated ammonia to decompose the intermediate phosphinimine,[20] yielded the 2′,3′-*O*-isopropylidene derivative of **4**. Deprotection was achieved directly by acidolysis of the crude ketal with 90% trifluoroacetic acid, and the final product was purified by chromatography on a Dowex 50W (H^+) ion-exchange column with dilute ammonia as the eluent. Since the combined yield for these two reactions (22%) was lower than had been reported for the corresponding FUdR analogue,[19] we attempted to reduce the azide group by catalytic hydrogenolysis over 5% Pd/$BaSO_4$. However, while reduction of the azide took place, the NMR spectrum of the crude product revealed substantial dehalogenation, which discouraged further use of this method.

When **9** was allowed to react with sodium *n*-butyl mercaptide in DMF at 60–70 °C, the tosylate group was again displaced to afford the thioether **12** in 56% yield. A companion substance was also formed in 13% yield, which was identified as 2,5′-anhydro-5-fluoro-2′,3′-*O*-isopropylideneuridine on the basis of its UV spectrum, which showed a maximum in EtOH at 247 nm instead of the usual 268–270 nm absorbance typical of all the open-chain compounds in the series. In addition, acidolysis with 90% trifluoroacetic acid at room temperature for 1.5 h converted this minor product into **2**, thus establishing its 2,5′-anhydronucleoside structure. Comparison with an authentic sample synthesized from 5′-deoxy-5-fluoro-5′-iodo-2′,3′-*O*-isopropylideneuridine and silver acetate[21] provided final proof of the structure. When the iodo compound was used in place of **9**, and *n*-butanethiol and K_2CO_3 were substituted for the more strongly basic sodium *n*-butyl mercaptide, **12** was obtained in comparable 52% yield. However, there was still TLC evidence of 2,5′-anhydronucleoside formation, which thus appears to be a side reaction even when a relatively weak base, such as K_2CO_3, is employed. The absence of 2,5′-anhydro-

(11) Wu, A. T.; Au, J. L.; Sadee, W. *Cancer Res.* **1978**, *38*, 210.
(12) Benvenuto, J. A.; Lu, K.; Hall, S. W.; Benjamin, R. S.; Loo, T. L. *Cancer Res.* **1978**, *38*, 3867.
(13) Benvenuto, J. A.; Liehr, J. G.; Winkler, T.; Farquhar, D.; Caprioli, R. M.; Loo, T. L. *Cancer Res.* **1979**, *39*, 3199.
(14) Lin, A. J.; Benjamin, R. S.; Rao, P. N.; Loo, T. L. *J. Med. Chem.* **1979**, *22*, 1096.
(15) Saneyoshi, M.; Inomata, M.; Fukuoka, F. *Chem. Pharm. Bull.* **1978**, *26*, 2990.
(16) Bloch, A.; Fleysher, M. H.; Thedford, R.; Maue, R. J.; Hall, R. H. *J. Med. Chem.* **1966**, *9*, 886.
(17) Johnson, C. R.; Dutra, G. A. *J. Am. Chem. Soc.* **1973**, *95*, 7777.
(18) Rosenthal, A.; Dodd, R. H. *Carbohydr. Res.* **1980**, *85*, 15.
(19) Lin, T.-S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 106.
(20) Mungall, M. S.; Greene, G. L.; Heavner, G. A.; Letsinger, R. *J. Org. Chem.* **1975**, *40*, 1695.
(21) Cook, A. F. *J. Med. Chem.* **1977**, *20*, 344.

Table I. Cytotoxicity of 5′-Modified Analogues of 5-Fluorouridine (FUR) against L1210 Mouse Leukemia Cells in Culture

compd	ID_{50}, μM
1 (DFUR)	140
2 (FUR)	0.0024
3	19
4	38
5	270
6	>1000
7	>1000
8	130
FU	1.0

nucleoside formation during the preparation of the azide **11** from **9** was presumably due to the absence of any added base.

For the preparation of the uronic acid **6**, a slight modification of the previously described[22,23] catalytic oxidation of FUdR was employed, in which a stream of air passed through 50% NaOH to remove CO_2 was substituted for pure oxygen. The nonoptimized yield of **6** following ion-exchange chromatography on Dowex 1-X2 with 1 N formic acid as the eluent was 35% (some unchanged starting material was recovered by first washing the column with a large volume of deionized water). The identity of the product as a uronic acid was confirmed by its high-yield conversion to an ethyl ester (**7**) on treatment at 0 °C with EtOH containing a catalytic amount of $SOCl_2$. The ester was characterized by the expected IR peak at 1740 cm^{-1} and by appropriate NMR signals for the ethyl group. A UV absorption maximum at 268 nm excluded the possibility that **7** was a 2,5′-anhydrolactone, since the latter would be expected to have a spectrum similar to the 2,5′-anhydronucleoside of **2** (i.e., a maximum at about 247 nm).

Lastly, the demethyl analogue (**8**) of **1** was synthesized in straightforward fashion from FU by O^2,O^6-bis(trimethylsilylation), followed by condensation with D-erythrose triacetate[24] and $SnCl_4$ in dry acetonitrile at 50 °C for 3 h. The combined yield of the 2′,3′-di-*O*-acetate **14**, based on FU, was 28% (nonoptimized). The NMR spectrum of **14** showed the expected pair of singlets at δ 2.15 and 2.10 for nonequivalent acetyl groups at the 2′ and 3′ position. Deprotection with NaOMe (room temperature, 2 h) afforded a 76% yield of the nucleoside **8**, which could be purified by crystallization from MeOH–EtOAc without chromatography. As expected, TLC analysis (silica gel; 7:2:1 *i*-PrOH–H_2O–concentrated NH_4OH) showed **8** to be somewhat more polar than the alkyl-substituted analogues **1** and **3**, with an R_f of 0.51 as opposed to 0.61 and 0.65, respectively. Other physical properties (UV, NMR) were likewise consistent with the structure.

Biological Activity. The antitumor effect of compounds **1**–**6** and **8** was tested in vivo against P-388 leukemia in $B6D2F_1J$ mice according to a typical NCI protocol.[25] All the nucleosides, with the exception of the lipophilic 5′-*n*-butyl thioether **5**, dissolved well in water and were therefore administered in sterile 0.9% NaCl solution; compound **5** was given in 10% Tween 80, in solution or suspension depending on the dose. Assays were conducted on a qd × 4 schedule, with drugs being injected ip starting one day after ip implantation of 10^6 tumor cells. The results are presented in Table I.

Significant activity was observed with the 5′,6′-dideoxyribohexofuranoside **3** in two separate experiments, with increases in life span (ILS) of 25–65% over the dose range 150–700 mg/kg. While a small weight loss (<10%) was noted at the highest tolerated dose, this compound appeared to be somewhat better tolerated than DFUR (**1**), which in our hands produced a 20% weight loss at 640 mg/kg and a 5% weight loss at 320 mg/kg on the qd × 4 schedule. Other investigators[4] have reported that P-388 leukemic mice treated ip with 1000 mg/kg of **1** on an intermittent schedule (q4d × 3), instead of daily, showed a +135% ILS with <10% weight loss. It thus appears that intermittent administration of **3** would perhaps have produced better therapeutic results than daily treatment, since it might allow a larger *total* dose of drug to be given over the period of treatment.

The 5′-amino-5′-deoxy analogue **4** was tested at doses of up to 32 mg/kg and was found to be clearly less toxic and less tumor inhibitory than FUR (**2**). Whereas **2** produced a median ILS of +91% at 8 mg/kg on the qd × 4 schedule, this dose caused >20% weight loss. In contrast, an equimolar dose of **4** was completely nontoxic and, in fact, appeared to produce some weight gain relative to untreated tumor-bearing controls. Heidelberger and coworkers[26] have reported that the highest tolerated daily dose for **2** against an array of murine tumors is about 5 mg/kg when the drug is given for 7–10 days. The preparation of larger quantities of **4** for continued antitumor evaluation would seem worthwhile, especially since the 2′-deoxy analogue of **4** has been reported to be cytotoxic in vitro (100% inhibition of growth of S180 mouse sarcoma cells at 1 μg/mL).[19]

The 5′-*n*-butyl thioether **5** displayed no therapeutic activity at doses up to 300 mg/kg and became markedly toxic at 600 mg/kg. Whether the inactivity of this compound in the tolerated dose range (75–300 mg/kg) was due to lack of uridine phosphorylase cleavage, inefficient uptake into tumor cells, or unfavorable pharmacokinetics is unknown. The toxicity of **5** at 600 mg/kg suggests that, at this high level, a toxic metabolite may be formed, possibly via an enzymic process involving the sulfur atom.

The uronic acid **6** gave good antitumor activity over a broad dose range, with a median ILS ranging from +27% at 25 mg/kg to +63% at 300 mg/kg. This compound thus appears to be as effective against the P-388 tumor as DFUR (**1**) in terms of both prolongation of survival and margin of safety (no toxicity over a tenfold dose range). The fact that replacement of a hydrogen atom on the 5′ carbon of **1** by a CH_3 group to give **3** or a COOH group to give **6** did not diminish the therapeutic effect suggests that the polarity of the substituent in the 5′ region of the sugar may be relatively unimportant insofar as substrate specificity toward uridine phosphorylase in tumor cells is concerned. The COOH group in **6**, incidentally, offers an attractive opportunity for further structural modification, including, for example, attachment to macromolecular carriers.[27]

As in the case of the dideoxy-β-D-*ribo*-hexofuranoside **3** and uronic acid **6**, positive antitumor activity was observed with the erythrofuranoside **8**. Increased median survival ranged from +25% at 160 mg/kg to +75% at 640

(22) Tsou, K. C.; Santora, N. J.; Miller, E. E. *J. Med. Chem.* **1969**, *12*, 173.
(23) Schinazi, R. F.; Chen, M. S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 1141.
(24) Murray, D. H.; Prokop, J. *J. Pharm. Sci.* **1967**, *56*, 865.
(25) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 1.
(26) Heidelberger, C.; Griesbach, L.; Cruz, O.; Schnitzer, R. J.; Grunberg, E. *Proc. Soc. Exp. Med.* **1958**, *97*, 470.
(27) Pischel, H.; Holy, A.; Wagner, G. *Collect. Czech. Chem. Commun.* **1980**, *45*, 606; cf. also earlier references cited.

mg/kg, and there was no evidence of toxicity as judged by weight loss or other signs. Moreover, at the highest tested dose of 640 mg/kg there was clearly less weight loss and fewer early deaths than with DFUR.

Compound 8 was of interest because of its structural relationship to Ftorafur and the ring-hydroxylated metabolites thereof.[14] Ishitsuka and co-workers[4] reported that Ftorafur produced median survival increases in P-388 leukemic mice of +39 and +61% at doses of 125 and 250 mg/kg, respectively, when these were give qd × 3; at 500 mg/kg, however, there was appreciable toxicity. Thus, compound 8 appears to have antitumor activity comparable to Ftorafur but may be somewhat less toxic at the upper end of the dose range. An attractive feature of 8 as a prodrug of FU would be its excellent water solubility. A further advantage over DHFR is that 8 can be synthesized *directly from FU*, whereas both the currently available syntheses of DFUR start with FUR[1,2] and are therefore lengthier and costlier.

An important point that had to be established with regard to the in vivo activity of the compounds obtained in this work was that the observed therapeutic effects could not be due to adventitious contamination of the bioassay specimen by a small amount of FU and especially FUR, which is highly cytotoxic. An effort to address this question was made by (a) comparing the in vivo activity of each compound with its cytotoxicity in culture and (b) monitoring the purity of each bioassay sample via an HPLC assay sensitive enough to detect FU or FUR levels as low as 1 part in 10 000 (0.01%). Both these methods were used recently by us in connection with another potential FU prodrug, the acyclonucleoside 1-(2-hydroxyethoxy)-5-fluorouracil.[28]

Cytotoxicity determinations were made for compounds 1–8 against L1210 mouse leukemia cells in culture (Table I). The 50% growth-inhibitory dose (ID_{50}) for compound 3 was approximately sevenfold lower than that of DFUR (2). However, this effect appears not to be related to the increased lipophilicity of the sugar moiety, since the 5′-amino analogue 4, which would be expected to be protonated at neutral pH, was nearly as active on a molar basis as 3 and approximately fourfold more toxic than 2. The very lipophilic 5′-*n*-butyl thioether 5, on the other hand, was about 14-fold less cytotoxic than 3, while the erythrofuranoside 8, which lacks a 5′ carbon altogether, had the same activity as 3. Neither the 5′-uronic acid 6 nor the corresponding ethyl ester 7 showed activity below 1 mM. All the members of the series were at least 20-fold less active than FU, and the difference relative to FUR was even more pronounced, ranging from a factor of 8000 in the case of 3 to >40 000 for 6.

The very low cytotoxicity of compounds 3, 6, and 8 in culture is significant in light of the therapeutic effect these analogues display in vivo. For example, the 8000-fold difference in molar ID_{50} values between 3 and FUR can be interpreted to mean that if the cytotoxicity of the bioassay sample of 3 were due solely to a trace of FUR being carried all the way through the multiple purifications inherent in a four-step synthesis, the level of contamination by FUR would have to be on the order of 0.01 mol %. Translated to the in vivo setting, this means that animals treated with 600 mg/kg of 3 would be receiving about 0.06 mg/kg of FUR, a dose which is clearly too low to account for the observed therapeutic effect. A similar argument can be made for compounds 6 and 8, whose ID_{50} relative to FUR and FU, respectively, indicate that these species cannot be present in amounts sufficient to account for the in vivo activity. Supporting evidence for the absence of significant levels of FUR in the bioassay specimens of 3 and 6 was obtained by analytical HPLC, which revealed the FUR content in a 1 mM solution of 3 to be ca. 0.2 μM (i.e., 0.02 mol %). In 6, the concentration of FUR by this assay was below the detection limit of 0.01 mol %. When the erythrofuranoside 8 was analyzed by HPLC, the content of FU (the starting material in this instance) was 0.7%. While this level of contamination was higher than the amount of FUR in 3 or 6, it was still less than would be needed to account for the in vivo effect.[29]

In summary, the results of this study indicate that structural modification of the 5′ region of DFUR can be made without appreciable loss of in vivo antitumor activity. Such changes include replacement of CH_3 by CH_3CH_2, COOH, and H to give *ribo*-hexofuranoside, uronic acid, and erythrofuranoside analogues, respectively. In experimental antitumor assays against P-388 leukemia in mice, these compounds behave very similarly to DFUR, in that they exhibit therapeutic activity over a tenfold dose range and produce much less host toxicity than would be obtained with equimolar doses of FU on the same schedule. In culture, on the other hand, these compounds are essentially inactive. These characteristics are consistent with a prodrug mechanism, with FU being formed in vivo via cleavage of the glycoside bond. Further studies to delineate the mechanism of prodrug action of these compounds and to assess their effect on tumors other than P-388 leukemia would be of interest as part of an overall search for new FU derivatives with increased clinical scope and efficacy.

Experimental Section

Infrared (IR) spectra were obtained on a Perkin-Elmer Model 137B double-beam recording spectrophotometer, ultraviolet (UV) absorbance spectra were measured on a Cary Model 15 instrument, and nuclear magnetic resonance (NMR) spectra were recorded on a Varian T60A instrument with Me_4Si as the reference. TLC analysis was performed on Eastman 13181 silica gel or Eastman 13254 cellulose sheets containing a fluorescent indicator, and spots were visualized under UV light at 254 nm. Conventional column chromatography was carried out on Baker silica gel (60–200 mesh). Woelm activity grade III/30 silica gel (ICN Nutritional Biochemicals, Cleveland, OH) was employed for dry-column chromatography. Melting points were measured in open Pyrex capillary tubes in a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. Microchemical analyses were performed by Galbraith Laboratories, Knoxville, TN, and were within ±0.4% of theoretical values unless indicated otherwise.

5-Fluoro-2′,3′-*O*-isopropylidene-5′-*O*-*p*-toluenesulfonyluridine (9). Purified *p*-toluenesulfonyl chloride (1.1 g, 5.2 mmol) was added to a solution of 5-fluoro-2′,3′-*O*-isopropylideneuridine (0.8 g, 2.6 mmol) in dry pyridine (10 mL) that was cooled to 0 °C, and the mixture was kept at this temperature overnight. Excess MeOH (3 mL) was added, and, after 30 min of stirring, most of the solvent was removed by vacuum evaporation. Residual pyridine was coevaporated repeatedly with toluene (3 × 10 mL), and the residue was treated with ice–H_2O (20 mL). Extraction with $CHCl_3$ (3 × 50 mL), washing of the combined organic layers with ice–H_2O, drying (Na_2SO_4), and rotary evaporation yielded a noncrystalline product, which was purified by column chromatography on silica gel (50 g) with 9:1 $CHCl_3$–Me_2CO as the

(28) Rosowsky, A.; Kim, S.-H.; Wick, M. *J. Med. Chem.* **1981**, *24*, 1177.

(29) A reviewer has suggested that compounds such as 3 and 8 could act in vivo by potentiating the effect of small contaminating amounts of FUR or FU, respectively. Such potentiation could come about, for example, if 3 were an inhibitor of FUR degradation by uridine phosphorylase or if 8 blocked the catabolic breakdown of FU. While these possibilities seem rather remote, they cannot be ruled out at this time.

eluent to obtain a colorless solid (1.1 g, 92%): mp 92–99 °C (prior shrinking); R_f 0.35 (silica gel, 3:1 $CHCl_3$–Me_2CO); NMR ($CDCl_3$) δ 7.74 (d, 2, J = 4 Hz, aromatic protons), 7.35 (d, 2, J = 3 Hz, aromatic protons), 5.76 (s, 1, $H_{1'}$), 4.83 and 4.33 (br s, 5, CH_2OSO_2, $H_{2'}$, $H_{3'}$, and $H_{4'}$), 2.45 (s, 3, aromatic Me), 1.56 and 1.33 [s, 6, $(CH_3)_2C$]. Anal. ($C_{19}H_{21}FN_2O_8S$·0.1H_2O) C, H, N, S.

5′,6′-Dideoxy-5-fluoro-2′,3′-*O*-isopropylidene-1-β-D-*ribo*-hexofuranosyluracil (10). Methyllithium (14 mL, 1.6 M solution in Et_2O) was added dropwise at 0 °C under N_2 to a magnetically stirred suspension of vacuum-dried CuI (2.1 g, 11 mmol) in anhydrous Et_2O (20 mL). When all the solid had dissolved and the yellow color of Me_2Cu had disappeared, the temperature was lowered to −78 °C by means of a dry ice/acetone bath, and a solution of the tosylate ester **9** (1.0 g, 2.2 mmol) in a mixture of Et_2O (15 mL) and 1,2-dimethoxyethane (5 mL) was added dropwise with stirring. The temperature was then allowed to rise gradually to 0 °C, and the mixture was left to stir overnight with continued cooling. Saturated aqueous NH_4Cl (10 mL) was added dropwise to terminate the reaction, the organic layer was filtered, the filter cake was washed several times with EtOAc until the washings changed from blue to colorless, and the combined filtrates were dried (Na_2SO_4) and evaporated. The oily residue was chromatographed on a column of silica gel (50 g), which was eluted with $CHCl_3$. Individual volumes of 5–10 mL were monitored by TLC, and appropriate fractions were collected and evaporated under reduced pressure to obtain a colorless foam (0.56 g, 85% yield): R_f 0.58 (silica gel; 9:1 $CHCl_3$–EtOH); NMR ($CDCl_3$) δ 7.38 (d, 1, J = 6 Hz, H_6), 5.73 (d, 1, J = 3 Hz, $H_{1'}$), 4.90 (q, 1, $J_{2',3'}$ = 3 Hz, $J_{1',2'}$ = 1 Hz, $H_{2'}$), 4.55 (q, 1, $J_{2',3'}$ = 4 Hz, $J_{3',4'}$ = 2 Hz, $H_{3'}$), 4.0 (hex, 1, $J_{4',5'}$ = 7 Hz, $J_{3',4'}$ = 2 Hz, $H_{4'}$), 2.0–1.5 (m, 4, CH_3CH_2, partially obscured), 1.62 and 1.35 [singlets, 6, $(CH_3)_2C$], 1.0 (t, 3, J = 7 Hz, CH_3CH_2). Anal. ($C_{11}H_{17}FN_2O_5$·0.5H_2O) C, H, N.

5′,6′-Dideoxy-5-fluoro-1-β-D-*ribo*-hexofuranosyluracil (3). The preceding 2′,3′-*O*-isopropylidene derivative **10** (0.70 g, 2.3 mmol) was dissolved in 90% aqueous trifluoroacetic acid (5 mL) at room temperature, and after 1 h the solution was evaporated to dryness under reduced pressure. The final traces of acid were removed by repeated coevaporation with EtOH, and the residue was triturated with Et_2O, filtered, and dried to obtain a colorless solid (0.46 g, 85%): mp 178–180 °C; R_f 0.65 (silica gel; 7:2:1 *i*-PrOH–H_2O–concentrated NH_4OH); UV (EtOH) λ_{max} 270 nm; NMR (D_2O) δ 7.75 (d, 1, J = 6 Hz, H_6), 5.8 (d, 1, J = 4 Hz, $H_{1'}$), 4.4–3.7 (m, 3, $H_{2'}$, $H_{3'}$, and $H_{4'}$), 1.6 (two overlapping quartets, 2, $J_{5',6'}$ = 6 Hz, $J_{4',5'}$ = 2 Hz, CH_3CH_2), 0.9 (t, 3, J = 7 Hz, CH_3CH_2). Anal. ($C_{10}H_{13}FN_2O_5$·0.1H_2O) C, H, F, N.

5′-Azido-5′-deoxy-5-fluoro-2′,3′-*O*-isopropylideneuridine (11). A mixture of the tosylate ester **9** (1 g, 2.2 mmol) and LiN_3 (0.22 g, 4.4 mmol) in dry DMF (10 mL) was heated at 70–75 °C (bath temperature) for 2 h. Vacuum evaporation, removal of the final traces of DMF by repeated coevaporation with EtOH, trituration of the residue with H_2O, acidification with glacial AcOH, extraction with $CHCl_3$, washing of the $CHCl_3$ layer successively with 5% $NaHCO_3$ and H_2O, drying (Na_2SO_4), and vacuum evaporation left a colorless solid (0.51 g, 73%): mp 160–163 °C dec; R_f 0.51 (silica gel; 19:1 $CHCl_3$–MeOH); IR ($CHCl_3$) ν 2101 (azide), 1706 and 1669 (amide C=O) cm^{-1}. Anal. ($C_{12}H_{14}FN_5O_5$·0.2H_2O) C, H, N.

5′-Amino-5′-deoxy-5-fluorouridine (4). The 5′-azido compound **11** (0.46 g, 1.4 mmol) and triphenylphosphine (0.75 g, 2.8 mmol) were dissolved in dry pyridine (4 mL), and the solution was kept at room temperature for 1 h. Concentrated NH_4OH (0.5 mL) was then added, and after being stirred for 2 h the reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved directly in 90% trifluoroacetic acid (5 mL) and the solution was left to stir at room temperature for 40 min. After vacuum evaporation, cold H_2O (10 mL) was added, and the precipitated triphenylphosphine oxide was removed by extraction with $CHCl_3$ (3 × 20 mL). The aqueous layer was concentrated to a volume of 20 mL and applied onto a Dowex 50W (H^+) ion-exchange column, which was eluted successively with distilled H_2O (100 mL) and 1 M NH_4OH. Individual 5- to 10-mL volumes were monitored by TLC, and homogeneous UV-absorbing fractions were pooled and evaporated to a pale-yellow powder, which was washed thoroughly with EtOH and Et_2O and then left to stand in concentrated NH_4OH (2 mL) overnight. After dilution with H_2O, the solution was extracted with Et_2O to remove the final traces of triphenylphosphine oxide contaminant. Freeze–drying afforded the product as a fluffy pale-yellow solid (79 mg, 22%): mp 140 °C dec (with prior darkening); R_f 0.42 (silica gel; 7:2:1 *i*-PrOH–H_2O–concentrated NH_4OH), 0.63 (cellulose, H_2O); NMR (Me_2SO-d_6) δ 8.33 (d, 1, J = 7 Hz, H_6), 5.7 (m, 1, $H_{1'}$). Anal. ($C_9H_{12}FN_3O_5$·0.75H_2O) C, H, N.

5′-(*n*-Butylthio)-5′-deoxy-5-fluoro-2′,3′-*O*-isopropylideneuridine (12). Method A. 1-Butanethiol (0.64 mL, 98% purity, 6.0 mmol) was added to a suspension of 50% NaH in mineral oil (144 mg, 3.0 mmol) in dry DMF (10 mL) under N_2. After 15 min, a solution of the tosylate ester **9** (1.25 g, 2.54 mmol) in dry DMF (15 mL) was added, and the pale-yellow solution was heated at 60–70 °C (bath temperature) for 2.5 h. The DMF was evaporated under reduced pressure, the residue was taken up in $CHCl_3$ (100 mL), and the solution was acidified by the addition of glacial AcOH. Successive washing of the organic layer with saturated aqueous $NaHCO_3$ and H_2O, drying (Na_2SO_4), and vacuum evaporation yielded an oil, which was chromatographed on a silica gel dry-column (50 g) using $CHCl_3$ as the initial eluent. Individual 5- to 10-mL volumes were monitored by TLC and UV absorption, and appropriate volumes were combined and evaporated to give 0.58 g (56%) of **12** as a colorless viscous oil: R_f 0.77 (silica gel; 9:1 $CHCl_3$–EtOH). Further elution of the column with 9:1 $CHCl_3$–EtOH afforded a second product (0.095 g, 13%): mp ~200 °C (no definite melting) (lit.[2] mp 213–215 °C); R_f 0.66 (silica gel; 9:1 $CHCl_3$–EtOH); UV (EtOH) λ_{max} 247 nm; NMR (Me_2SO-d_6) δ 8.35 (d, 1, J = 6 Hz, H_6), 5.76 (s, 1, $H_{1'}$), 4.98 (s, 2, $H_{5'}$), 5.1–4.2 (m, 3, $H_{2'}$, $H_{3'}$, and $H_{4'}$), 1.38 and 1.25 [singlets, 6, $(CH_3)_2C$]. Anal. ($C_{12}H_{13}FN_2O_5$) C, H, N. Comparison of this material with an authentic specimen of 2,5′-anhydro-5-fluoro-2′,3′-*O*-isopropylideneuridine, prepared via the literature method from 5′-deoxy-5-fluoro-5′-iodo-2′,3′-*O*-isopropylideneuridine and AgOAc,[21] showed that the two products were the same.

Method B. 1-Butanethiol (0.21 mL, 98% purity, 1.9 mmol) was added to a suspension of 5′-deoxy-5-fluoro-5′-iodo-2′,3′-*O*-isopropylideneuridine (0.7 g, 1.7 mmol)[21] and anhydrous K_2CO_3 (0.3 g, 2.2 mmol) in dry DMF (30 mL) under N_2, and the mixture was heated for 2 h at 60–65 °C (bath temperature). The DMF was evaporated under reduced pressure, H_2O (50 mL) was added, and the mixture was acidified with AcOH. Extraction with $CHCl_3$ (5 × 50 mL), successive washing of the combined organic layers with H_2O and 5% $NaHCO_3$, rinsing to neutrality with H_2O, drying (Na_2SO_4), and vacuum evaporation yielded an oil whose TLC (silica gel; 9:1 $CHCl_3$–EtOH) indicated the probable presence of small amounts of the 2,5′-anhydro compound (see above). The crude product was chromatographed on a silica gel dry-column (2.5 × 25 cm) using $CHCl_3$ as the eluent. Individual 5 to 10-mL volumes were monitored as in the preceding experiment, pooled as appropriate, and evaporated to a colorless viscous oil, which was triturated with petroleum ether (bp 30–60 °C) to remove a trace of residual DMF. The petroleum ether was decanted, and the oil was dried in vacuo: yield 0.29 g (52%): R_f 0.77 (silica gel; 9:1 $CHCl_3$–EtOH); UV (EtOH) λ_{max} 268 nm; NMR ($CDCl_3$) δ 7.6 (d, 1, J = 7 Hz, H_6), 5.8 (d, 1, J = 2 Hz, $H_{1'}$), 5.0–4.7 (m, 2, $H_{2'}$ and $H_{3'}$), 4.5–4.2 (m, 1, $H_{4'}$), 3.0–2.5 (m, 4, CH_2S and $H_{5'}$), 1.68 and 1.46 [singlets, 6, $(CH_3)_2C$]. Anal. ($C_{16}H_{23}FN_2O_5S$) C, H, N.

5′-(*n*-Butylthio)-5′-deoxy-5-fluorouridine (5). A solution of the ketal derivative **12** (0.3 g, 0.8 mmol) in 90% trifluoroacetic acid (5 mL) was left to stand at room temperature for 1 h before being evaporated to dryness under reduced pressure. The last traces of acid were removed by repeated coevaporation with EtOH, the residue was triturated with a small volume of EtOAc, and the undissolved solid was filtered. The filtrate was evaporated, the residue was triturated with Et_2O, and the undissolved solid was filtered. Since both solids were identical by TLC, they were combined: yield 0.19 g (71%); mp 155–157 °C; R_f 0.43 (silica gel; 3:1 $CHCl_3$–EtOH); UV (MeOH) λ_{max} 268 nm; NMR (Me_2SO-d_6) δ 7.98 (d, 1, J = 6 Hz, H_6), 5.71 (dd, 1, J = 4 and 1 Hz, $H_{1'}$). Anal. ($C_{13}H_{19}FN_2O_5S$·0.1H_2O) C, H, F, N, S.

5′-Deoxy-5-fluorouridine-5′-carboxylic Acid (6). Platinum oxide (1.1 g) was suspended in glacial AcOH (5 mL) in a Parr apparatus and reduced at room temperature, and after removal of the H_2 by repeated evaporation, the catalyst was filtered, washed thoroughly with H_2O, and air-dried. The prereduced catalyst was added to a solution of 5-fluorouridine (1.5 g, 5.7 mmol) in pH 8.8 sodium bicarbonate buffer (175 mL) in a three-necked

Table II. Antitumor Activity of Sugar-Modified 5-Fluorouridine Analogues against P-388 Leukemia in Mice

compd	dose,[a] mg/kg	no. of mice	av wt on day 1, g/mouse	7-day wt change[b] g/mouse	7-day wt change[b] %	survival[c] range	survival[c] median	% ILS[d]
1	80	5	28.6	+2.2	+7	11-13	13 (11)	+18
	160	5	26.8	+0.4	+2	14-15	15	+36
	320	5	26.2	−1.4	−5	15-21[e]	17	+54
	640	5	27.0	−5.6	−21	9-25	17	+54
2	2	5	26.0	+1.2	+15	18-26	20 (12)	+66
	4	5	26.4	−3.2	−12	18-21	21	+75
	8	5	25.8	−6.0	−23	21-27	23	+91
3	75	5	21.8	+2.0	+9	14-16	15 (11)	+36
	150	5	25.8	+1.8	+7	15-20	17	+54
	300	5	23.2	+0.6	+3	17-24	17	+54
	600	5	23.2	−2.4	−10	17-19	18	+63
	85	5	26.2	+1.2	+5	13-16	13 (11)	+18
	170	5	26.4	+1.6	+6	13-15	14	+27
	340	5	27.8	+1.4	+5	15-21	16	+45
	680	5	25.8	−1.4	−5	16-20	17	+54
4	8	5	25.6	+5.8	+23	11-12	12 (12)	0
	16	5	26.4	+4.4	+16	10-13	12	0
	32[f]	5	26.8	+3.2	+12	11-13	13	+8
5	75	5	26.6	+2.0	+8	11-12	11 (12)	−9
	150	5	27.8	+1.4	+5	10-13	11	−9
	300	5	28.0	−0.4	−1	11-13	11	−9
	600	5	26.4	[g]	[g]	3-4	4	−67
6	25	5	24.6	+2.6	+10	14-15	14 (11)	+27
	50	5	24.2	+1.6	+7	13-17	15	+36
	100	5	23.6	+2.0	+8	15-18	16	+45
	200	5	23.8	−0.8	−3	16-21	16	+45
	300	5	25.0	0.0	0	16-20	18	+63
8	40	5	25.2	+3.0	+12	12-17	12 (12)	0
	80	5	24.2	+2.6	+11	11-17	14	+16
	160	5	24.2	+1.6	+8	14-16	15	+25
	320	5	25.8	+0.2	+1	16-26	17	+41
	640	5	25.6	−2.4	−9	17-23	21	+75
5-FU	15	5 × 5	24.4	+0.4	+2	15-24	19 (11)	+73
	30	5 × 5	24.2	−3.2	−13	11-36	21	+91

[a] Groups of B6D2F1J male mice were inoculated intraperitoneally with 10^6 P-338 cells on day 0, and drugs were injected intraperitoneally on days 1-4. All compounds were given in sterile saline except for **5**, which was administered in 10% Tween 80. [b] The average weight per mouse on day 1 for the multiple groups of untreated controls in these experiments was 27.1 g (25.1-27.8 g). The average 7-day weight gain for the controls was 3.6 g (+13%). [c] Numbers in parentheses are the median survival of the untreated control group for the individual experiment. Control groups consisted of 10-17 animals per experiment. Survival is given in days. [d] ILS = increase in life span. [e] One long-term survivor was sacrificed on day 107. [f] Highest dose tested. [g] All animals died of toxicity before day 7.

flask fitted with a gas inlet tube, reflex condenser, and magnetic stirrer. Compressed air was passed through a gas scrubber containing 50% NaOH solution (w/v) in order to remove the CO_2 and then through the reaction mixture at 90 °C (silicone oil bath) for 24 h. The reaction was allowed to cool to room temperature, and the catalyst was removed by filtration, first through a layer of Celite and then through a Millipore filter (0.45 μm) fitted to the end of a syringe. Evaporation of the solvent under reduced pressure gave a solid (1.3 g), which was redissolved in a small volume of 5% $NaHCO_3$ and applied onto a Dowex 1-X2 ion-exchange column (3 × 25 cm, $HCOO^-$ form), which was eluted first with a large volume of deionized water to remove all unreacted starting material and then with 1 N formic acid. Individual volumes of 5–10 mL were monitored by TLC, and appropriately pooled fractions were concentrated under reduced pressure and finally freeze–dried. Since the residue at this point (0.77 g) appeared to be incompletely neutralized, it was redissolved in H_2O, a small amount of Dowex 50W (H^+) was added, the mixture was stirred and filtered, and the filtrate was lyophilized once more to afford a fluffy colorless powder (0.60 g, 35%): mp 193–195 °C; R_f 0.25 (cellulose; 7:2:1 *i*-PrOH–H_2O–concentrated NH_4OH); IR (KBr) ν 1650–1690 (amide C=O) cm^{-1}; UV (EtOH) λ_{max} 270 nm; NMR (Me_2SO-d_6 + D_2O) δ 8.17 (d, 1, J = 6 Hz, H_6), 5.87 (d, 1, J = 4 Hz, $H_{1'}$), other peaks ($H_{2'}$, $H_{3'}$, and $H_{4'}$) obscured by a large H_2O signal. Anal. ($C_9H_9FN_2O_7 \cdot 1.5H_2O$) C, H, F, N.

Ethyl 5′-Deoxy-5-fluorouridine-5′-carboxylate (7). The foregoing acid **6** (0.57 g, 2.1 mmol) was suspended in absolute EtOH (50 mL), the mixture was cooled to 0 °C, $SOCl_2$ (0.3 mL) was added dropwise, and stirring was continued at room temperature overnight. The colorless precipitate was suction filtered and washed with EtOH, and the combined filtrates were concentrated in vacuo to ca. 10 mL. Addition of cold H_2O (20 mL) containing $NaHCO_3$ (1.5 g) led to precipitation of a white solid, which was collected, washed with cold H_2O and then EtOH, and dried: yield 0.63 g (ca. 100%): mp 212–220 °C dec; R_f 0.69 (cellulose; 7:2:1 *i*-PrOH–H_2O–concentrated NH_4OH); IR (KBr) ν 1740 (ester C=O), 1650–1710 (amide C=O) cm^{-1}; UV (EtOH) λ_{max} 268 nm; NMR (Me_2SO-d_6) δ 8.28 (d, 1, J = 6 Hz, H_6), 5.92 (q, 1, $J_{1',2'}$ = 3 Hz, $J_{1',6}$ = 1 Hz), 4.5–3.9 (m, 5, CH_3CH_2, $H_{2'}$, $H_{3'}$, and $H_{4'}$), 1.23 (t, 3, J = 7 Hz, CH_3CH_2). Anal. ($C_{11}H_{13}FN_2O_7$) C, H, F, N.

1-(2′,3′-Di-*O*-acetyl-β-D-erythrofuranosyl)-5-fluorouracil (13). Acetic anhydride (7 mL) was added dropwise at 0 °C to a stirred solution of D-erythrose (3 g, 85% purity, Aldrich Chemical Co., Milwaukee, WI) in pyridine (20 mL) containing Linde 3A molecular sieves (3 g). The mixture was left to stir at room temperature overnight and evaporated to dryness under reduced pressure. The residue was treated with ice–H_2O (50 mL), and the product was extracted into $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated under reduced pressure. The last traces of pyridine were removed by repeated coevaporation with toluene, leaving the crude triacetate as an amber-colored syrup (5.3 g), which was used without further purification. A mixture of 5-fluorouracil (2.7 g, 0.021 mol) and the crude sugar ester (5.2 g) in dry acetonitrile (60 mL) was treated with *O,N*-bis(trimethylsilyl)acetamide (25 mL) under N_2, and then stirred at 50 °C (bath temperature) for 3 h. After being cooled in an ice bath, the reaction mixture was stirred while $SnCl_4$ (4.6 mL) was added dropwise. The reaction was left at room temperature overnight, and then terminated by being poured into ice-cold H_2O (200 mL) containing $NaHCO_3$ (40 g) and EtOAc (100 mL). The two-phase

mixture was filtered through Celite, and the organic layer was separated. The aqueous layer was extracted again with EtOAc (2 × 300 mL), and the combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was chromatographed on a silica gel column (50 g), which was eluted first with $CHCl_3$ (250 mL) and then with 19:1 $CHCl_3$–EtOH. Evaporation of appropriately pooled 5–10 mL volumes of eluate yielded a straw-colored foam (1.8 g, 28%): R_f 0.60 (silica gel; 19:1 $CHCl_3$–EtOH); NMR ($CDCl_3$) δ 7.45 (d, 1, J = 6 Hz, H_6), 5.95 (d, 1, J = 2 Hz, $H_{1'}$), 5.53 (m, 2, $H_{2'}$ and $H_{3'}$), 4.6–3.8 (m, CH_2O), 2.15 and 2.10 (s, 6, CH_3CO). Anal. ($C_{12}H_{13}FN_2O_7 \cdot 0.6C_2H_5OH \cdot 0.2CHCl_3$) C, H, N.

1-β-D-Erythrofuranosyl-5-fluorouracil (8). The diacetate **13** (1.6 g, 5.1 mmol) was dissolved in MeOH (50 mL), and 1 M NaOMe (16 mL) was added. The resultant clear solution was stirred at room temperature for 2 h. Dowex 50W-X8 (H^+) was then added, stirring was continued for another 15 min, and the resin was removed by filtration. Evaporation of the filtrate under reduced pressure left a straw-colored residue, which was recrystallized from a mixture of MeOH and EtOAc to give a colorless solid (0.89 g, 76%): mp 192–194 °C; R_f 0.51 (silica gel; 7:2:1 *i*-PrOH–H_2O–concentrated NH_4OH), 0.84 (cellulose, H_2O); UV (EtOH) λ_{max} 269 nm; NMR (Me_2SO-d_6) δ 8.03 (d, 1, J = 7 Hz, H_6), 5.66 (d, 1, J = 3 Hz, $H_{1'}$), 5.3–5.0 (m, 2, $H_{2'}$ and $H_{3'}$), 4.3–3.9 (m, 2, CH_2O). Anal. ($C_8H_9FN_2O_5$) C, H, N.

Antitumor Assays. Standard NCI protocols were employed.[25] Groups of five male B6D2F_1J mice (Jackson Laboratories, Bar Harbor, ME) were inoculated ip with 10^6 P-388 leukemic cells on day 0, and drug treatment was begun on day 1 for 4 consecutive days (qd × 4). All compounds except **5** were administered in sterile saline; compound **5** was given in 10% Tween 80. The increase in survival (ILS) was calculated according to the formula % ILS = [(T/C) − 1]100, where T and C are the median survival times in days for the treated and control groups, respectively. All animals were weighed on days 1 and 7, and 7-day weight changes were calculated as a percentage. The results are given in Table I.

Analysis of Nucleoside Purity by HPLC. Compounds **1–3**, **5**, and **6** were analyzed by HPLC on a Waters 10μ C_8 reversed-phase column (RCM 8 radial compression cartridge, Waters Associates, Milford, MA) using 0.01 M sodium phosphate buffer, pH 5.7, as the mobile phase and a flow rate of 3 mL/min. Compound **8** was analyzed on a Waters μBondapak CN column with 15% EtOH in isooctane as the mobile phase and a flow rate of 1 mL/min. 5-Bromo-2′-deoxyuridine was used as an internal standard, with eluting peaks being monitored at 280 nm. Excellent base-line separation between FUR (**2**) and the other nucleosides was obtained, and it was determined by using standard mixtures that as little as 0.01% of **2** could be detected if it were present as a contaminant of **1**, **3**, or **5**. Similarly, **8** and FU were readily separable and it was established that as little as 0.01% FU could be detected as a contaminant of the nucleoside **8**. The following analyses of *maximum* contamination by **2** were obtained: **3**, 0.02%; **5**, 0.01%; other nucleosides, all <0.01%. The maximum possible content of FU in the sample of **8** was determined to be 0.7%.

Cytotoxicity Assays. Cell growth inhibition by the compounds described in this paper was measured as described previously,[30] using L1210 murine leukemia cells in Eagle's minimal essential medium supplemented with 15% fetal calf serum and containing streptomycin (100 μg/mL), penicillin (100 units/mL), and 0.05 mM 2-mercaptoethanol. Cells were counted after 48 h with the aid of a Coulter hemocytometer (Model F). Assays were performed in triplicate and have a standard deviation of ±10%. The results are given in Table II.

Acknowledgment. This work was supported in part by Grants CA 23151 and CA 19589 from the National Cancer Institute (NIH). The authors are indebted to Linda Anderson and Gerda Swedowsky for their help in obtaining in vivo antitumor data and in vitro cytotoxicity data, respectively.

(30) Rosowsky, A.; Kim, S.-H.; Ross, J.; Wick, M. *J. Med. Chem.* **1982**, *25*, 171.

Pyrazolo[3,4-*d*]pyrimidine Ribonucleosides as Anticoccidials. 2. Synthesis and Activity of Some Nucleosides of 4-(Alkylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidines

Janet L. Rideout,*,† Thomas A. Krenitsky,*,† George W. Koszalka,† Naomi K. Cohn,† Esther Y. Chao,† Gertrude B. Elion,† Victoria S. Latter,‡ and Raymond B. Williams§

Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709, Langley Court, Beckenham, Kent BR3 3BS, United Kingdom, and Berkhamsted, Herts HP4 2QE, United Kingdom.
Received February 23, 1982

A series of 4-(alkylamino)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines was synthesized by enzymatic and chemical methods. On the basis of the previous finding that 4-(alkylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines were effective anticoccidial agents, this series was examined for efficacy against *Eimera tenella* in chicks. The most active anticoccidial agent in the present study was the 4-cyclopentylamino derivative (**8**), which cleared chicks of the parasite at 200 ppm in the diet. Some members of this series were toxic to embryonic chick liver cells, mouse cells, and human cells in vitro. The 4-diethylamino derivative (**16**), which was not toxic in vitro, appeared to be toxic in chicks.

In the first paper of this series,[1] it was shown that ribonucleosides of 4-(alkylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidines inhibited the growth of avian coccidia both in vitro and in vivo. The most active compound of that series was the 4-ethylthio derivative. As part of this continuing investigation, a series of 4-(alkylamino)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines was synthesized, and the structure–activity relationships were investigated.

Results and Discussion

Chemistry. Scheme I shows the methods (A–D) used to prepare the compounds. Methods are also indicated in Table II, and examples are provided under Experimental

† Research Triangle Park, NC.
‡ Kent, UK.
§ Herts, UK.

(1) Krenitsky, T. A.; Rideout, J. L.; Koszalka, G. W.; Inmon, R. B.; Chao, E. Y.; Elion, G. B.; Latter, V. S.; Williams, R. B. *J. Med. Chem.* **1982**, *25*, 32.

0022-2623/82/1825-1040$01.25/0