(80%) of **3a** as yellow crystals, 95–97 °C.

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Registry No. 1 (R_2 = H), 5323-58-0; 1 (R_2 = 4-CH_3), 81358-81-8; 1 (R_2 = 3-CH_3), 108191-07-7; 1 (R_2 = 2-CH_3), 108191-08-8; 1 (R_2 = 2,4-$(CH_3)_2$), 108191-09-9; **3a**, 81358-67-0; **3b**, 81358-68-1; **3c**, 81358-71-6; **3d**, 81358-76-1; **3e**, 108190-29-0; **3f**, 81358-87-4; **3g**, 81359-03-7; **3h**, 81359-08-2; **3i**, 108190-30-3; **3j**, 108190-31-4; **3k**, 81358-82-9; **3l**, 81358-93-2; **3m**, 81358-98-7; **3n**, 108190-32-5; **3o**, 108190-33-6; **3p**, 108190-34-7; **3q**, 108190-35-8; **3r**, 81359-13-9; **3s**, 108190-36-9; **3t**, 108190-37-0; **3u**, 108214-79-5; **3v**, 108190-38-1; **3w**, 108190-39-2; **3x**, 108190-40-5; **3y**, 108190-41-6; **3z**, 108190-42-7; **3aa**, 108190-43-8; **3bb**, 108190-44-9; **3cc**, 108190-45-0; **3dd**, 108190-46-1; **3ee**, 108190-47-2; **3ff**, 108190-48-3; **4a**, 64992-85-4; **4b**, 81358-69-2; **4c**, 81358-72-7; **4d**, 81358-77-2; **4e**, 108190-49-4; **4f**, 81358-88-5; **4g**, 81359-04-8; **4h**, 81359-09-3; **4i**, 108190-50-7; **4j**, 108190-51-8; **4k**, 81358-83-0; **4l**, 81358-94-3; **4m**, 81358-99-8; **4n**, 108190-52-9; **4o**, 108214-80-8; **4p**, 108190-53-0; **4q**, 108190-54-1; **4r**, 81359-14-0; **4s**, 108190-55-2; **4t**, 108190-56-3; **4u**, 108190-57-4; **4v**, 108190-58-5; **4w**, 108190-59-6; **4x**, 108190-60-9; **4y**, 108190-61-0; **4z**, 108190-62-1; **4aa**, 108190-63-2; **4bb**, 108190-64-3; **4cc**, 108190-65-4; **4dd**, 108190-66-5; **4ee**, 108190-67-6; **4ff**, 108190-68-7; **5a**, 59353-62-7; **5b**, 63460-47-9; **6a**, 64992-87-6; **6b**, 108214-81-9; **6c**, 81358-73-8; **6d**, 81358-78-3; **6e**, 108190-69-8; **6f**, 81358-89-6; **6g**, 81359-05-9; **6h**, 81359-10-6; **6i**, 108190-70-1; **6j**, 108190-71-2; **6k**, 81358-84-1; **6l**, 81358-95-4; **6m**, 81359-00-4; **6n**, 108190-72-3; **6o**, 108190-73-4; **6p**, 108190-74-5; **6q**, 108190-75-6; **6r**, 81359-15-1; **6s**, 108190-76-7; **6t**, 108190-77-8; **6u**, 108190-78-9; **6v**, 108190-79-0; **6w**, 108190-80-3; **6x**, 108190-81-4; **6y**, 108190-82-5; **6z**, 108190-83-6; **6aa**, 108190-84-7; **6bb**, 108190-85-8; **6cc**, 108214-82-0; **6dd**, 108190-86-9; **6ee**, 108190-87-0; **6ff**, 108190-88-1; **7a**, 64992-84-3; **7a**·fumarate, 64992-88-7; **7b**, 81444-45-3; **7b**·fumarate, 81444-46-4; **7c**, 81358-74-9; **7c**·fumarate, 81358-75-0; **7d**, 81358-79-4; **7d**·fumarate, 81358-80-7; **7e**, 108190-09-6; **7e**·fumarate, 108190-89-2; **7f**, 81358-90-9; **7f**·fumarate, 81358-91-0; **7g**, 81359-06-0; **7g**·fumarate, 81359-07-1; **7h**, 81359-11-7; **7h**·fumarate, 81359-12-8; **7i**, 108190-10-9; **7i**·fumarate, 108190-90-5; **7j**, 108190-11-0; **7j**·fumarate, 108214-83-1; **7k**, 81358-85-2; **7k**·fumarate, 81358-86-3; **7l**, 81358-96-5; **7l**·fumarate, 81358-97-6; **7m**, 81359-01-5; **7m**·fumarate, 81359-02-6; **7n**, 108190-12-1; **7n**·fumarate, 108190-91-6; **7o**, 108190-13-2; **7o**·fumarate, 108190-92-7; **7p**, 108190-14-3; **7p**·fumarate, 108190-93-8; **7q**, 108190-15-4; **7q**·fumarate, 108190-94-9; **7r**, 81359-16-2; **7r**·fumarate, 81359-17-3; **7s**, 108190-16-5; **7s**·fumarate, 81358-97-6; **7t**, 108190-17-6; **7t**·fumarate, 108190-95-0; **7u**, 108190-18-7; **7u**·fumarate, 108214-85-3; **7v**, 108190-96-1; **7v**·fumarate, 108190-97-2; **7w**, 108190-19-8; **7w**·fumarate, 108190-98-3; **7x**, 108190-20-1; **7x**·fumarate, 108190-99-4; **7y**, 108190-21-2; **7y**·fumarate, 108214-86-4; **7z**, 108190-22-3; **7z**·fumarate, 108191-00-0; **7aa**, 108190-23-4; **7aa**·fumarate, 108191-01-1; **7bb**, 108190-24-5; **7bb**·fumarate, 108191-02-2; **7cc**, 108190-25-6; **7cc**·fumarate, 108191-03-3; **7dd**, 108190-26-7; **7dd**·fumarate, 108191-04-4; **7ee**, 108190-27-8; **7ee**·fumarate, 108191-05-5; **7ff**, 108190-28-9; **7ff**·fumarate, 108191-06-6; 7 (R_1 = CH_3, R_2 = 4-CH_3), 64992-94-5; **8a**, 64992-86-5; **8b**, 5347-15-9; CF_3CH_2OH, 75-89-8; *n*-C_3H_7Br, 106-94-5; *n*-C_4H_9Br, 109-65-9; *n*-$C_5H_{11}I$, 628-17-1; *n*-$C_6H_{13}Br$, 111-25-1; *n*-$C_8H_{17}Br$, 111-83-1; *n*-$C_{10}H_{21}I$, 2050-77-3; *n*-$C_5H_{11}Br$, 110-53-2; *n*$C_7H_{15}I$, 4282-40-0; *n*-$C_9H_{19}Br$, 693-58-3; *n*-$C_{11}H_{23}Br$, 693-67-4; *n*-$C_{12}H_{25}Br$, 143-15-7; 2-$C_6H_{13}Br$, 3377-86-4; 3-$C_6H_{13}Br$, 3377-87-5; $C_6H_5O(CH_2)_4Br$, 1200-03-9; $C_6H_5O(CH_2)_6Br$, 51795-97-2; $C_6H_5O(CH_2)_8Br$, 52176-61-1; $C_6H_{11}CH_2Br$, 2550-36-9; CH_2=$CH(CH_2)_2Br$, 5162-44-7; $CH_3(CH_2)_3O(CH_2)_2Br$, 6550-99-8; $CH_3O(CH_2)_6Br$, 50592-87-5; $C_6H_5CH_2O(CH_2)_6Br$, 54247-27-7; $C_7H_{15}Br$, 629-04-9; primaquine, 90-34-6; 2-methylprimaquine, 57695-10-0; 3-methylprimaquine, 64992-44-5; 4-methylprimaquine, 57514-29-1; cyclopentyl bromide, 137-43-9.

2′-Fluorinated Arabinonucleosides of 5-(2-Haloalkyl)uracil: Synthesis and Antiviral Activity

Herfried Griengl,*† Erich Wanek,† Wolfgang Schwarz,† Wolfgang Streicher,‡ Brigitte Rosenwirth,*‡ and Erik De Clercq§

Institute of Organic Chemistry, Technical University Graz, A-8010 Graz, Austria, Sandoz Forschungsinstitut, A-1235 Vienna, Austria, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium.
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The synthesis of 5-(2-fluoroethyl)-2′-deoxyuridine (FEDU, **4b**), its 2′-fluoro analogue 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(2-fluoroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (FEFAU, **4k**), and the 2′-fluoro analogue of the potent antiherpes virus compound 5-(2-chloroethyl)-2′-deoxyuridine (CEDU), 5-(2-chloroethyl)-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (CEFAU, **4i**), is described. The antiviral activities of these compounds were determined in cell culture against herpes simplex virus (HSV) types 1 and 2 and varicella zoster virus (VZV). All compounds were shown to possess significant and selective antiviral activity. FEDU proved less potent than CEDU against VZV replication; however, it was more active against HSV-2. CEFAU showed marked activity against HSV-1, HSV-2, and VZV. The compound containing fluorine at both positions, FEFAU, exhibited the strongest antiviral potency against HSV-1, HSV-2, and VZV. It inhibited HSV-1 at a concentration of 0.03–0.2 μg/mL, HSV-2 at 0.1–0.3 μg/mL, and VZV at 0.03 μg/mL. Neither FEDU nor CEFAU or FEFAU exerted a significant inhibitory effect on cell proliferation at a concentration of 100 μg/mL. Thus, the cytotoxicity of these compounds is as low as that of CEDU and compares favorably to that of previously described 2′-fluoroarabinosyl nucleoside analogues.

5-(2-Bromovinyl)-2′-deoxyuridine (BVDU)[1] and the recently described 5-(2-chloroethyl)-2′-deoxyuridine (CEDU)[2] are two of the most potent and selective antiviral representatives of the large class of 5-substituted pyri-

† Technical University Graz.
‡ Sandoz Forschungsinstitut.
§ Katholieke Universiteit Leuven.

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midine nucleoside analogues.[3] Both compounds effectively inhibit herpes simples type 1 virus (HSV-1) and varicella zoster virus (VZV) replication in vitro.[1,2,4-6] Herpes simplex type 2 virus (HSV-2) replication is affected only at considerably higher concentrations of BVDU or CEDU. The antiviral indexes of CEDU and BVDU, as determined by the ratio of the minimum toxic dose for the normal host cell to the minimum inhibitory dose for HSV-1, are about 2000 and 5000, respectively. In vivo CEDU is effective against systemic HSV-1 infection at a dose that is about 10-fold lower than those required for BVDU and the reference compound acyclovir (ACV), whereas in vitro BVDU is active at about $^1/_{10}$ the concentration of CEDU.[2,5,6]

Our studies on the structure–activity relationship of 5-(2-haloethyl)-2′-deoxyuridines[2] have demonstrated the importance of the halogen substituent for antiherpetic activity: i.e., the bromo and iodo derivatives were 30–100-fold less potent than CEDU. Therefore, we were interested in examining the biological activity of the remaining member of this series, 5-(2-fluoroethyl)-2′-deoxyuridine (FEDU).

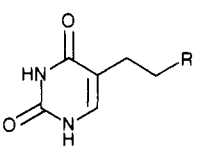
Modification of the sugar moiety of nucleosides has yielded compounds possessing antiviral and anticancer activity, e.g., 9-β-D-arabinofuranosyladenine (araA), 1-β-D-arabinofuranosylcytosine (araC), and 1-β-D-arabinofuranosylthymine (araT). However, these derivatives show little or no selectivity against herpes infections and have proven toxic.[7]

A number of 5-substituted pyrimidine nucleoside analogues containing the 2-deoxy-2-fluoro-β-D-arabinofuranosyl moiety has been synthesized by Fox and collaborators.[8-12] Several of these are potent and selective inhibitors of HSV-1, HSV-2, VZV, and cytomegalovirus (CMV) replication,[8,9,13,14] the most active being 2′-fluoro-5-iodo-araC (FIAC), 2′-fluoro-5-iodo-araU (FIAU), and 2′-fluoro-5-methyl-araU (FMAU). These derivatives show clearly higher cytotoxicity than BVDU, CEDU, or ACV.[15,16] Recently, the 2′-fluoroarabinofuranosyl analogues of BVDU and 5-ethyl-2′-deoxyuridine (EDU) were synthesized and their biological activities were described.[16] The cytotoxicities of these compounds were lower than those of FIAC, FMAU, or FIAU. The 2′-fluoroarabinofuranosyl analogue of BVDU was less potent against HSV-1 than the parent compound; however, unlike BVDU, it showed activity against both types of herpes simplex virus.

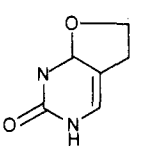
The potent antiherpetic activities of FIAC, FMAU, and FIAU prompted us to synthesize the 2′-fluoroarabinofuranosyl analogues of CEDU and FEDU. Our purpose to increase the anti-HSV-2 activity, while conserving the low cytotoxicity, was achieved as is evident from the biological properties of these derivatives.

Chemistry

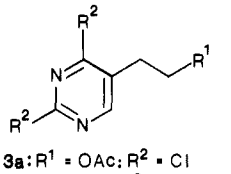
All nucleoside analogues were prepared via glycosylation of the corresponding 5-substituted uracils with reactive pentose derivatives. A straightforward synthesis of 5-(2-fluoroethyl)uracil (**1d**) starting from 5-(2-hydroxyethyl)-uracil (**1a**)[17,18] under a variety of conditions failed. The bicyclic derivative **2**[18] was the main reaction product if **1a** was reacted with (diethylamino)sulfur trifluoride (DAST) or 5-[2-(tosyloxy)ethyl]uracil (**1b**) with KF in DMF in the presence of 18-crown-6. To prevent this undesired ring closure, 2,4-dimethoxy-5-(2-hydroxyethyl)pyrimide (**3b**) was prepared from **1a** via the acetate **1c** and the dichloro derivative **3a**. Compound **3b** could be fluorinated with DAST to give the fluoroethyl compound **3c**. Demethylation with HBr/HOAc gave the aglycon **1d**, which was converted into its 2′-deoxyuridine derivatives **4a** and **4b** (FEDU) following a reaction similar to that described recently for the other 5-(2-haloethyl) nucleoside analogues.[2]



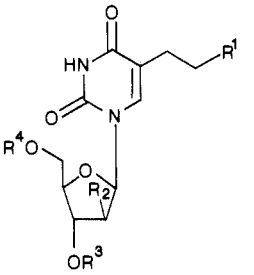
1a: R = OH
b: R = OTs
c: R = OAc
d: R = F
e: R = Cl



2



3a: R^1 = OAc; R^2 = Cl
b: R^1 = OH; R^2 = OCH_3
c: R^1 = F; R^2 = OCH_3



+ α-anomers **5**

4a: R^1 = F; R^2 = H; R^3 = R^4 = p-toluoyl
b: R^1 = F; R^2 = R^3 = R^4 = H
c: R^1 = OH; R^2 = OBn; R^3 = R^4 = Bn
d: R^1 = OH; R^2 = OH; R^3 = R^4 = H
e: R^1 = Cl; R^2 = OH; R^3 = R^4 = H
f: R^1 = Br; R^2 = OH; R^3 = R^4 = H
g: R^1 = Cl; R^2 = F; R^3 = Ac; R^4 = Bz
h: R^1 = R^2 = F; R^3 = Ac; R^4 = Bz
i: R^1 = Cl; R^2 = F; R^3 = R^4 = H
k: R^1 = R^2 = F; R^3 = R^4 = H

5-(2-Hydroxyethyl)uracil (**1a**) after silylation was reacted with 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride[19,20]

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Table I. Antiviral Activities of Test Compounds against HSV-1, HSV-2, and VZV in Different Cell Lines

	minimum inhibitory concentration,[a] μg/mL						
	HSV-1 (Brand)		HSV-1 (KOS),	HSV-2 (K979)		HSV-2 (G),	VZV (Ellen),
compd	Vero	HEp-2	PRK[b]	Vero	HEp2	PRK[b]	HLF-1[b]
CEDU	0.01	0.03	0.14	30	3	2.1	0.01
FEDU (**4b**)	0.1	0.03	0.58	3	1	1.5	3
CEAU (**4e**)	30	30	nd[c]	100	100	nd	nd
CEFAU (**4i**)	0.3	0.1	0.30	10	1	2.0	0.1
FEFAU (**4k**)	0.03	0.03	0.2	0.3	0.1	0.2	0.03
BVDU	0.001	0.01	0.007	30	3	2.3	0.001
IDU	0.1	0.3	0.2	1	0.3	0.91	10
FIAC	0.01	0.01	0.02	0.1	0.03	0.03	0.01
FIAU	0.01	0.01	0.02	0.1	0.1	0.02	0.003
FMAU	0.03	0.3	0.04	0.3	0.1	0.02	0.001
ACV	0.003	1	0.06	1	3	0.04	3
DHPG	0.003	0.01	0.2	0.3	0.3	0.1	3

[a] Required to reduce virus-induced cytopathogenicity by at least 25%. [b] PRK, primary rabbit kidney cells; HLF-1, human lung fibroblast cells. [c] Not determined.

Table II. Effect of Test Compounds on the Proliferation of Vero and HEp-2 Cells

	optical density at day 4, percent of control,[a] concn of test compound (μg/mL):					
	Vero			HEp-2		
compd	330	100	30	330	100	30
CEDU	82	91	84	86	99	104
FEDU (**4b**)	71	97	94	60	86	97
CEFAU (4i)	63	74	75	68	107	116
FEFAU (**4k**)	90	87	98	72	105	113
BVDU	55	71	74	31	68	79
IDU	16	34	61	0	20	61
FIAC	24	43	68	13	32	64
FIAU	0	6	37	0	13	49
FMAU	0	6	7	0	3	36
ACV	56	73	77	41	78	89
DHPG	13	69	82	40	84	93

[a] Cells in suspension were seeded at low density into tissue culture plates to allow growth for 4 days before becoming contact inhibited. The test compounds were present in the culture fluid at the indicated concentrations. Propagation of cells was monitored by determination of absorbance, upon staining, as described in the Experimental Section.

to give the protected intermediate **4c**. Deprotection by catalytic hydrogenation to give **4d** and halogenation[2,21,22] gave predominantly the desired 5-(2-haloethyl)arabinofuranosyluracils **4e** and **4f**.

Fluoroarabino analogues were prepared via glycosylation of the aglycons **1e** and **1d** with 3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-deoxy-2-fluoroarabinofuranosyl bromide.[11,23] After separation of small amounts of the corresponding α-isomers, **4g** and **4h** were obtained, respectively. Deprotection of **4g** gave the chloroethyl derivative **4i**. Deprotection of **4h** gave the fluoroethyl derivative **4k**.

Biological Results and Discussion

The antiviral activities of test and reference compounds were determined in three different cell lines and are summarized in Table I. As reported previously,[2,5,6] CEDU inhibits HSV-1 and VZV at lower concentrations than does 5-iodo-2′-deoxyuridine (IDU). Its potency is comparable to that of ACV, FIAC, FIAU, and FMAU, but it is slightly less active than BVDU and dihydroxypropoxymethylguanine (DHPG). CEDU and BVDU are clearly less inhibitory to HSV-2 than to HSV-1, whereas IDU, FIAC, FIAU, FMAU, ACV, and DHPG show similar activities against the replication of the two herpes simplex virus types. The minimum inhibitory concentrations depend largely on the nature of the cell line used, e.g., CEDU and BVDU are more potent against HSV-2 in HEp-2 than in Vero cells and ACV is more active against HSV-1 in Vero than in HEp-2 cells.

The 5-fluoroethyl analogue of CEDU, FEDU, inhibits HSV-1 replication at the same or slightly higher concentration than CEDU. It is, however, much less inhibitory to VZV, while it is more active against HSV-2 than is CEDU. Thus, the nature of the halogen substituent in the ethyl side chain at position 5 of the pyrimidine ring not only influences the antiviral potency, as had been shown previously with regard to the bromo and iodo analogues,[2] but also modulates the antiviral spectrum.

The arabinofuranosyl analogue of CEDU, CEAU, is virtually inactive against HSV-1 and HSV-2, whereas the 2-deoxy-2-fluoroarabinofuranosyl analogue (CEFAU) shows marked antiherpetic activity. Obviously, the introduction of the 2′-"up" fluoro substituent amplifies antiviral activity as was observed previously by Fox and collaborators.[8,9,24] CEFAU is inhibitory to HSV-1 and VZV at a slightly higher concentration than CEDU; however, it clearly has higher potency against HSV-2 than CEDU.

The derivative containing fluorine at both the C-2′ of the arabinofuranosyl moiety and the C-5 side chain of the pyrimidine ring shows remarkably improved antiherpetic activity: FEFAU not only inhibits HSV-1 and VZV rep-

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lication at low concentrations similar to CEDU but is comparable to the reference compounds ACV, DHPG, FIAC, FIAU, and FMAU in potency against HSV-2.

Thus, as observed for the 2′-fluoroarabinosyl derivative of BVDU,[16] the sugar moiety in FIAC, FIAU, and FMAU, obviously, contributes to the anti-HSV-2 activity of these compounds. With the present series of compounds, substitution of 2-fluoroethyl for 2-chloroethyl at C-5 of the pyrimidine ring further increased anti-HSV-2 potency.

Table II summarizes the cytotoxic effects of test and reference compounds at various concentrations. CEDU at 330 μg/mL exerted only a minimal inhibitory effect on the proliferation of Vero and HEp-2 cells, as described previously.[2,5,6] ACV, BVDU, and DHPG at 330 μg/mL reduced Vero cell density by 44%, 45%, and 87%, respectively, and HEp-2 cell density by 59%, 69%, and 60%, respectively, measured at day 4 after seeding of the cells; at 100 μg/mL these compounds cause a minimal inhibition of cell growth. The reference 2′-fluoroarabinosyl analogues exerted a cytotoxic effect at a concentration as low as 30 μg/mL, the most cytotoxic derivative being FMAU followed by FIAU and FIAC.

Neither the 5-fluoroethyl analogue of CEDU, FEDU, nor the 2′-fluoroarabinosyl derivatives, CEFAU and FEFAU, showed an increased cytotoxicity as compared to CEDU: at 330 μg/mL, FEDU, CEFAU, and FEFAU effected a reduction in Vero cell density of only 29%, 37%, and 10%, respectively, and a reduction in HEp-2 cell density of only 40%, 32%, and 28%, respectively.

In conclusion, modification of the sugar moiety of CEDU to 2′-deoxy-2′fluoroarabinofuranosyl and substitution of chloro by fluoro at the C-5 side chain of the pyrimidine ring lead to compounds with an altered antiviral spectrum, in particular an increased anti-HSV-2 activity. The derivative containing a fluorine at both positions displayed the most potent and broadest antiherpetic activity of this series. In no case was the improvement of antiviral potency associated with increased cytotoxicity: akin to CEDU or BVDU all derivatives inhibited cell proliferation only at very high concentrations.

Experimental Section

Chemistry. Melting points were determined on a Büchi melting point apparatus and are uncorrected. NMR spectra were taken on a Hitachi Perkin-Elmer R-24B (60 MHz) and on a Bruker WH-90 (90 MHz) spectrometer with Me_4Si as internal standard. Me_2SO-d_6 was used as solvent unless otherwise stated. Chemical shifts are reported in parts per million (δ). The spectral data were fully consistent with the structures given. Elemental analyses were carried out by the Analytical Department, Institute of Organic Chemistry, University of Graz. The analytical results obtained for all new compounds were within ±0.3% of the theoretical values (C, H, N).

Column chromatography was performed on silica gel 60, 230–400 mesh, ASTM, Merck, Darmstadt, with the following eluants: (A) *n*-hexane/ethyl acetate (9:1, v/v), (B) *n*-hexane/ethyl acetate (1:1, v/v), (C) $CHCl_3$/acetone (9:1, v/v), (D) $CHCl_3$/methanol (9:1, v/v), (E) $CHCl_3$/methanol (6:1, v/v).

5-(2-Acetoxyethyl)-2,4-dichloropyrimidine (3a). A mixture of 10 g (50.5 mmol) of 5-(2-acetoxyethyl)-1*H*,3*H*-pyrimidine-2,4-dione (**1c**),[18] 100 mL of $POCl_3$ and 10 mL of *N*,*N*-diethylaniline was refluxed for 30 min.[25] All volatile material was evaported in vacuo, the oily residue was dissolved in 100 mL of CCl_4, and the solution was washed twice with cold water. After drying with sodium sulfate and evaporation of the solvent, 11.7 g (98.6%) of **3a** was obtained as a yellowish oil. Anal. $C_8H_8Cl_2N_2O_2$) C, H, N.

2,4-Dimethoxy-5-(2-hydroxyethyl)pyrimidine (3b). A solution of 11.7 g (49.8 mmol) of **3a** in 250 mL of 1 N NaOMe/MeOH was stirred for 20 h. After neutralization with ether, saturated with gaseous HCl at 0 °C, and evaporation to dryness, the residue was stirred in 200 mL of $CHCl_3$ for 1 h and filtered. The filtrate was dried over sodium sulfate and the solvent was distilled off in vacuo, giving 8.8 g (96%) of **3b** as a syrup, which crystallized on chilling, mp 48–49 °C. Anal. ($C_8H_{12}N_2O_3$) C, H, N.

2,4-Dimethoxy-5-(2-fluoroethyl)pyrimidine (3c). A solution of 5.05 g (27.4 mmol) of **3b** in 100 mL of dry dichloromethane was cooled to −40 °C and treated with 14.3 mL (104 mmol) of triethylamine and 4.85 mL (36.4 mmol) of DAST. Within 30 min the temperature was raised to 10 °C. The reaction mixture was poured on ice/sodium hydrogen carbonate solution. The organic layer was washed twice with water, dried with $MgSO_4$, and evaporated to dryness. The residue (6.6 g) was chromatographed with solvent A to give **3c** and with solvent B: 2.65 g recovered **3b**. Yield of **3c** was 2.05 g (40%; 84% based on 3b reacted), mp 33–35 °C (diethyl ether). Anal. ($C_8H_{11}FN_2O_2$) C, H, N.

5-(2-Fluoroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (1d). A solution of 7.2 g (38.5 mmol) of **3c** in 170 mL of HBr (33% in acetic acid) was refluxed for 1 h. After evaporation to dryness and coevaporation with water, the residue was taken up in 150 mL of water and brought to pH 6 with 2 N NaOH. Compound 1a precipitated after cooling to 4 °C to yield 5.33 g of **1d** (91%), mp 254 °C dec. Anal. ($C_6H_7FN_2O_2$) C, H, N.

1-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-5-(2-fluoroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (4a) and 1-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl)-5-(2-fluoroethyl)-1*H*-pyrimidine-2,4-dione (5a). A 600-mg (3.8 mmol) sample of **1d** was refluxed in 15 mL of hexamethyldisilazane (HMDS) in the presence of 0.5 mL of trimethylchlorosilane until a clear solution was obtained. Excess HMDS was removed in vacuo and the residue was dissolved in 30 mL of $CHCl_3$. A 1.6-g (4 mmol) sample of 3,5-di-*O*-*p*-toluoyl-2-deoxy-D-*erythro*-pentofuranosyl chloride[26] and 0.1 mL of trimethylsilyl triflate were added, and the solution was stirred at room temperature for 1 h. After dilution with 50 mL of $CHCl_3$ and extraction with 150 mL of cold saturated $KHCO_3$ solution, the organic phase was separated, dried over sodium sulfate, and evaporated. The oily residue was dissolved in 10 mL of dry ethanol and kept overnight at 4 °C. Pure β-anomer 4a was filtered off. The remaining mixture containing the anomers **4a** and **5a** in a 1:1 ratio was separated by column chromatography (eluant C), yielding 1.4 g (72%) of **4a**, mp 152 °C (ethanol). Anal. ($C_{27}H_{27}FN_2O_7$) C, H, N. Additionally was obtained 0.2 g (10%) of **5a**, mp 129 °C (ethanol). Anal. ($C_{27}H_{27}FN_2O_7$) C, H, N.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(2-fluoroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (4b). A solution of the blocked nucleoside **4a** in dry hot ethanol (20 mL/mmol) was treated with 1 N NaOEt solution (2 mL/mmol). After quantitative reaction (TLC, eluant E), the mixture was neutralized with strongly acidic ion exchange resin (Merck I, H^+) and evaporated to dryness. The oily residue was taken up in water, washed once with ether, and again evaporated. After treatment with acetone/$CHCl_3$ (1:1), 720 mg (95%) of **4b** was obtained, mp 152–153 °C (acetone/$CHCl_3$). Anal. ($C_{11}H_{15}FN_2O_5$) C, H, N.

1-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-5-(2-fluoroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (5b). This compound was prepared as described for 4b from 0.2 g of **5a** to yield 80 mg (75%) of **5b**, mp 128 °C (acetone/$CHCl_3$). Anal. ($C_{11}H_{15}FN_2O_5$) C, H, N.

1-β-D-Arabinofuranosyl-5-(2-hydroxyethyl)-1*H*,3*H*-pyrimidine-2,4-dione (4d). A suspension of 2.93 g (18.75 mmol) of **1a** in 30 mL of HMDS and 4 mL of trimethylchlorosilane was refluxed for 3 h. Excess HMDS was removed in vacuo, and the oily residue was dissolved in 20 mL of dry xylene and evaporated again. To the resulting syrup were added a solution of freshly prepared 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride[19,20] in 50 mL of dry CH_2CL_2 (3.86 g, 8.78 mmol) and 10 g of molecular sieve (4 Å). The mixture was shaken for 2 days, molecular sieve was filtered off, and the solution was washed with CH_2Cl_2. The filtrate was extracted with aqueous $KHCO_3$ solution, and the combined organic layers were evaporated in vacuo. Crude **4c** was

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dissolved in 100 mL of ethanol and added to a suspension of Pd (prepared by hydrogenation of 1 g of $PdCl_2$ and hydrogenated overnight in a Parr apparatus, room temperature, 4 bars). A crystalline precipitate was filtered off, dissolved in hot water, filtered again to remove Pd, evaporated, and recrystallized from water to give 1.15 g of **4d**. The methanolic filtrate from the hydrogenation was neutralized with strongly basic ion exchange resin (Merck II, OH^-), filtered over charcoal, evaporated, and recrystallized from water to give additional **4d** (0.32 g). Overall yield 1.47 g (58%), mp 212–215 °C (water). Anal. ($C_{11}H_{16}N_2O_7$) C, H, N.

1-β-D-**Arabinofuranosyl-5-(2-chloroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (4e)**. To a solution of 400 mg (1.4 mmol) of **4d** in 10 mL of dry DMF were added 0.7 g (2.62 mmol) of triphenylphosphine, 0.3 mL (1.95 mmol) of dry CCl_4, and 0.3 mL of dry pyridine. The mixture was stirred at room temperature until conversion was complete (TlC, eluant D). After addition of 1-butanol (5 mL), the mixture was evaporated to give a syrup, which was separated by column chromatography (eluant D) to yield 180 mg (42%) of **4e**, mp 182–183 °C (ethanol). Anal. ($C_{11}H_{15}ClN_2O_6$) C, H, N.

1-β-D-**Arabinofuranosyl-5-(2-bromoethyl)-1*H*,3*H*-pyrimidine-2,4-dione (4f)**. A solution of 320 mg (1.11 mmol) of **4d**, 650 mg (2.48 mmol) of triphenylphosphine, and 300 mg (1.65 mmol) of *N*-bromosuccinimide in 5 mL of dry DMF was stirred at room temperature for 1.5 h. Five milliliters of 1-butanol was added, and the solvents were distilled off in vacuo. The oily residue was separated by column chromatography (eluant E) to give 177 mg (44%) of **4f**, mp 166–167 °C (acetone/$CHCl_3$). Anal. ($C_{11}H_{15}BrN_2O_6$) C, H, N.

1-(**3-*O*-Acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(2-chloroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (4g)**. A 0.83-g (4.7 mmol) sample of 5-(2-chloroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (**1e**)[2] was silylated with 10 mL of HMDS and 0.3 mL of trimethylsilyl chloride as described for **4a**. The silylated product was dissolved in 25 mL of dry acetonitrile and treated with 1.7 g of 3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide[23] and 0.3 mL of trimethylsilyl triflate. After 5 h of refluxing the solution was diluted with dichloromethane and the organic phase washed with saturated sodium hydrogencarbonate solution and dried with sodium sulfate. By chromatography with solvent B, the lower spot of a double spot was isolated to give **4g** (0.48 g, 21%) as a viscous oil.

1-(**3-*O*-Acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(2-fluoroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (4h)**. A suspension of 17.1 g (30 mmol) of **1f** in 180 mL of HMDS and 5 mL of trimethylsilyl chloride was refluxed for 1 h to give a clear solution, which was evaporated to dryness and coevaported twice with toluene. The residue and 40 g of 3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide[23] were taken up in 300 mL of dry acetonitrile and treated with 21 mL of trimethylsilyl triflate. The mixture was refluxed for 1.5 h. After concentration, 50 mL of methanol and 100 mL of saturated $NaHCO_3$ solution were added. The solution was extracted with dichloromethane, and the extracts were dried with sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed with *n*-hexane/ethyl acetate as the solvent. The lower of a double spot was isolated to give pure β-anomer **4h** (13 g, 25%) as a viscous oil.

5-(2-Chloroethyl)-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1*H*,3*H*-pyrimidine-2,4-dione (4i). A 2.3 g (5 mmol) sample of **4g** was dissolved in of 20 mL of methanol cooled to 4 °C and treated with 10 mL of 1 N $NaOCH_3$. After 10 min the solution was neutralized with Amberlite CG-50 (H^+). The oil obtained after filtration and evaporation of the solvent was chromatographed with ethyl acetate as the solvent. Pure fractions were pooled, evaporated to dryness, and taken up in water which as lyophilized to give 1.4 g (90%) of **4i** as a white amorphous powder. Anal. ($C_{11}H_{14}ClFN_2O_5$, C, H, N.

1-(**2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(2-fluoroethyl)-1*H*,3*H*-pyrimidine-2,4-dione (4k)**. A 10.7-g (24.4 mmol) sample of **4h** was dissolved in 100 mL of methanol and treated with 48.8 mL of 1 N $NaOCH_3$. After 1 h at room temperature, the solution was neutralized with Amberlite CG-50 (H^+). After filtration the solvent was distilled off in vacuo. The residue was taken up in 5 mL of methanol and treated with *n*-hexane. The solvent was decanted and the residue taken up again in 5 mL of methanol. This solution was added dropwise with stirring to 200 mL of hexane to give **4k** as a white amorphous hygroscopic powder (6.53 g, 92%). Anal. ($C_{11}H_{14}F_2N_2O_5$) C, H, N.

Biology. Materials. Cells. Vero, HEp-2, and human lung fibroblast (HLF-1) cells were obtained from the American Type Culture Collection, Rockville, MD. PRK refers to primary rabbit kidney cell cultures.

Viruses. Herpes simplex virus type 1 (HSV-1) strain Brand and HSV-2 strain K979 were originally isolated by H. zur Hausen in Erlangen, FRG, and by B. Vestergaard in Copenhagen, Denmark, respectively; they were kindly provided by M. Scriba, Vienna, Austria. HSV-1 strain KOS was originally obtained from Dr. W. E. Rawls, Baylor University College of Medicine, Houston, TX, through the aid of Dr. J. Desmyter of the Rega Institute for Medical Research, Leuven, Belgium. HSV-2 strain G and varicella zoster virus (VZV) strain Ellen were purchased from the American Type Culture Collection, Rockville, MD.

Compounds. The reference compound (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) was synthesized according to Jones et al.,[27] 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, ACV,) according to Schaeffer et al.,[28] and 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG) according to Martin et al.[29] 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU), and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil (FMAU) were obtained from Dr. J. J. Fox, Sloan Kettering Institute for Cancer Research, New York, NY, through the aid of Dr. I. Ghazzouli, Bristol-Myers Co., Syracuse, NY. 5-Iodo-2′-deoxyuridine (IDU) was purchased from Mack, Illertissen, FRG.

Methods. In Vitro Antiviral Assays. HSV-1 and -2. Cytopathic effect (CPE) inhibition assays were performed as follows: Serial 3-fold dilutions of test compound in Eagle's minimum essential medium (E-MEM) were prepared in flat-bottomed microtiter plates. Equal parts of virus in E-MEM and cell suspension in E-MEM plus 15% fetal calf serum were added. Virus infected cultures without compound were included as controls as were uninfected cells treated with compound. The cell input was adjusted so as to give a confluent monolayer after 1–2 days of incubation, and the virus input was so as to cause 90–100% CPE in the infected control cell cultures after 3 days. At this time, the plates were fixed and stained, and the extent of virus-induced CPE in infected controls and in drug treated wells was estimated.

VZV. Frozen VZV-infected HLF-1 cells were added to fresh monolayers; after development of cytopathic effect the cells were scraped off the plates and suspended in culture medium. Total number of 1.5×10^5 of these cells were immediately used to infect HLF-1 monolayers (ca. 3×10^5 cells/well) in 24-well tissue culture dishes. After 2-h adsorption at 37 °C, the medium was replaced by medium containing test compound at various concentrations. The development of cytopathic effect was judged microscopically at days 2, 3, and 4 postinfection and the effect compared to that of the untreated control.

Cellular Toxicity Assay. Cells suspended in culture medium containing test compound at various concentrations were seeded into 24-well tissue culture dishes (Vero, 5×10^4 cells/well; HEp-2, 1×10^5 cells/well); four wells were used per variable. After 1, 2, 3, and 4 days aliquots of the cells were stained with 0.01% neutral red in phosphate-buffered saline (PBS) for 2 h at 37 °C, washed with PBS, and dried. The stained cells were dissolved in 1.5 mL/well ethanol/0.1 M NaH_2PO_4 (1:1), and the optical density of the solution was measured at 546 nm. The absorbance was found to be proportional to the cell number.

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Registry No. 1a, 23956-12-9; 1c, 90301-77-2; 1d, 108008-58-8; 1e, 90301-68-1; 3a, 108008-55-5; 3b, 108008-56-6; 3c, 108008-57-7; 4a, 108008-59-9; 4b, 108008-61-3; 4c, 108008-62-4; 4d, 90301-71-6; 4e, 90301-70-5; 4f, 90301-72-7; 4g, 108034-57-7; 4h, 108008-63-5; 4i, 90301-73-8; 4k, 108008-64-6; 5a, 108008-60-2; 5b, 108034-76-0; 3,5-di-*O*-*p*-toluoyl-2-deoxy-D-*erythro*-pentofuranosyl chloride, 3601-89-6; 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride, 4060-34-8; 3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide, 56632-81-6.

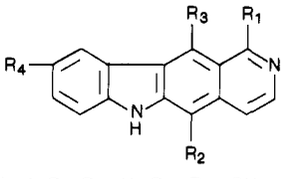
Synthesis and Biological Properties of Some 6*H*-Pyrido[4,3-*b*]carbazoles

Sydney Archer,*† Bruce S. Ross,† Livia Pica-Mattoccia,‡ and Donato Cioli‡

Cogswell Laboratories, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York 12180-3590, and Laboratory for Cell Biology, 18/A Via G. Romagnosi, 00196 Rome, Italy. *Received October 23, 1986*

The effect of methyl substitution on the biological properties of the ellipticines was reexamined. 9-Hydroxy-6*H*-pyrido[4,3-*b*]carbazole was synthesized and shown to be devoid of antitumor activity in murine P388 lymphocytic leukemia in mice. 5-(Hydroxymethyl)-11-methyl-6*H*-pyrido[4,3-*b*]carbazole (**46**) and its *N*-methylcarbamate (**48**) were synthesized and their effect on macromolecular synthesis in HeLa cells and their antitumor properties were compared with those of ellipticine. In contrast to the alkaloid **1** and the hydroxymethyl derivative **46**, which produced partially reversible inhibition of [^{3}H]thymidine incorporation, the carbamate ester irreversibly blocked incorporation of the tritiated pyrimidine. The ester was also a more potent antitumor agent in P388 lymphocytic leukemia than **1** or **46**.

A great deal of interest has been shown in the alkaloids ellipticine (**1**, 5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) and its regioisomer olivacine (**2**, 1,5-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) because of their antitumor properties in animals and humans.[1] Ellipticine has been shown to react with DNA by an intercalation process,[2] which may account for its cytotoxicity. Li and Cowie[3] found that **1** markedly inhibited DNA polymerase but not RNA polymerase. At concentrations of 0.2 and 1.0 μg/mL, the drug inhibited DNA and RNA synthesis as measured by the incorporation of [^{3}H]thymidine and [^{3}H]uridine. At these concentrations there was little effect on protein synthesis. The authors concluded that inhibition of nucleic acid synthesis was an important contribution to the cytotoxic effect of ellipticine. Sethi[4] was able to show that **1** did inhibit RNA polymerase but at concentrations far higher than those of other antitumor agents such as dactinomycin, adriamycin, and daunomycin.

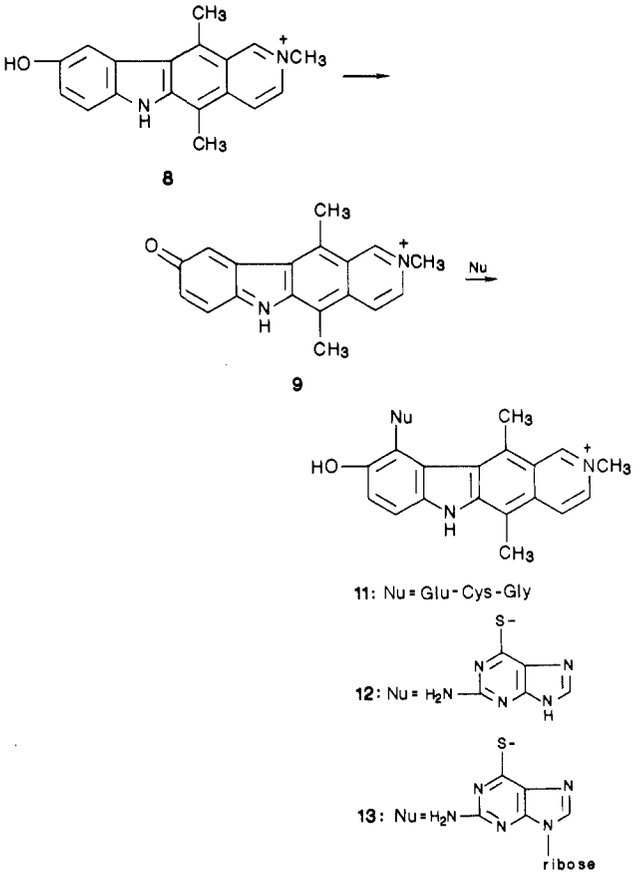


1 (ellipticine): $R_1 = R_4 = H$, $R_2 = R_3 = CH_3$
2 (olivacine): $R_1 = R_2 = CH_3$, $R_3 = R_4 = H$
3 (9-hydroxyellipticine): $R_1 = H$, $R_2 = R_3 = CH_3$, $R_4 = OH$
4: $R_1 = R_3 = R_4 = H$, $R_2 = CH_3$
5: $R_1 = R_2 = R_3 = R_4 = H$
6: $R_1 = R_2 = CH_3$, $R_3 = H$, $R_4 = OH$
7: $R_1 = R_3 = H$, $R_2 = CH_3$, $R_4 = OH$

Ellipticine, 9-hydroxyellipticine (**3**), and its corresponding methoacetate **8** were shown to produce DNA double and single strand breaks in L1210 cells exposed to these drugs.[5,6] The double strand breaks resulting from the interaction with mammalian topoisomerase II were reversible in L1210 cell cultures and in cell-free systems.[6-9]

9-Hydroxyellipticine (**3**) is the major metabolite of ellipticine in the rat.[10,11] LePecq et al.[2] have shown that this metabolite is a better intercalating and a more active antitumor agent than ellipticine. It was also reported that 5-methyl-6*H*-pyrido[4,3-*b*]carbazole (**4**) and 6*H*-pyrido[4,3-*b*]carbazole (**5**) were not cytotoxic in in vitro L1210 systems.[2] 9-Hydroxyolivacine (**6**) was found to be more

Scheme I



8

9

11: Nu = Glu-Cys-Gly

12: Nu = H_2N

13: Nu = H_2N, ribose

† Cogswell Laboratory.
‡ Laboratory for Cell Biology.

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