The plotted data indicate that adequate protection was attained when a blood level of 0.01 mM **3** was maintained. Whole-blood ChE could not be correlated with the [**3**] in plasma. One explanation for these results may involve the aging of mice-serum BuChE (see Table II). The correlation between the duration of the pretreatment efficacy and the concentration of **3** in the plasma substantiates our suggestion that protection of AChE provided by **3**, a result of temporary masking of the active site via the formation of a covalent phosphoryl–AChE conjugate, constituted the major antidotal mechanism.

We note that inhibition of mice-brain AChE could not be observed in animals receiving a lethal dose of **3** (450 mg/kg). Thus, AChE activity of mice-brain homogenates (six repetitions) was unchanged after incubation with 0.5 mM 2-PAM for 60 min at 25 °C. These results suggest that **3** does not cross the blood–brain barrier, and its activity is restricted to peripheral tissues.

Conclusions

The results of this study suggest that reasonable in vitro and in vivo protection against soman and paraoxon poisoning is provided by the newly designed cyclic phosphate ester via the reversible masking of AChE by covalent phosphorylation. Effective pretreatment was dependent on subsequent treatment with cholinolytics and TMB-4. The successful use of carbamates[6,8,30] or TEPP[10] as pretreatment against organophosphate poisoning has also been attributed to their ability to form unstable carbamoyl or oxime-reactivable diethylphosphoryl–AChE conjugates. Their protection is also dependent on additional treatment as mentioned above for **3**.[6,8,10,30] However, in contrast to carbamates and tetraethyl pyrophosphate, the acute toxicity of **3** was found to be impressively low (LD_{50} = 444 mg/kg in mice). This low toxicity probably relates to the kinetic parameters for inhibition and spontaneous reactivation of **3**-inhibited AChE. For example, **3** inhibits AChE ~25 times slower than does pyridostigmine,[33] and the inhibited enzyme hydrolyzes 2–3 times faster than the corresponding carbamoyl–AChE conjugate. Thus, it is possible to rationalize the differences between the acute toxicity of **3** and pyridostigmine in kinetic terms. Nevertheless, it should be emphasized that other factors, such as absorption, distribution, excretion, and metabolism, cannot be entirely exluded for the observed differences between the toxicity of the two drugs.

Although **3** was found to relatively stable in experiments close to physiological conditions (serum and phosphate buffer, pH 7.0, 25 °C), the in vivo half-life and duration of its antidotal capacity are relatively short (in mice). It is believed that **3** is a nontoxic potential drug that may be applied for pretreatment of organophosphorus poisoning, in conjunction with cholinolytic and oxime reactivators. In view of the marked species differences in response to protection against soman and other organophosphorus compounds,[6] we are now studying the biological and the pharmacokinetic properties of **3** in monkeys.

Finally, it is worthwhile to mention here that one speculative significance of these findings may be a possible application of the 1,3,2-dioxaphosphorinane 2-oxide moiety as a carrier of biologically active molecules of cholinergic importance, provided that they can serve as convenient leaving groups in reactions where AChE is the attacking nucleophile.

Acknowledgment. We thank Mrs. B. Manisterski for her excellent technical assistance.

Registry No. **3**, 80531-03-9; **4**, 2498-27-3; AChE, 9000-81-1; BuChE, 9001-08-5; paraoxon, 311-45-5; soman, 96-64-0; 2-chloro-1,3,2-dioxaphosphorinane 2-oxide, 872-99-1; 3-(dimethylamino)phenol, 99-07-0; *O*-[3-(dimethylamino)phenyl]-1,3,2-dioxaphosphorinane 2-oxide, 83547-88-0.

Nucleosides. 123. Synthesis of Antiviral Nucleosides: 5-Substituted 1-(2-Deoxy-2-halogeno-β-D-arabinofuranosyl)cytosines and -uracils. Some Structure–Activity Relationships

Kyoichi A. Watanabe, Tsann-Long Su, Robert S. Klein, Chung K. Chu, Akira Matsuda, Moon Woo Chun, Carlos Lopez, and Jack J. Fox*

Laboratories of Organic Chemistry and Herpes Virus Infections, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division of Graduate School of Medical Sciences, Cornell University, New York, New York 10021.
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The syntheses of several 2′-halogeno-5-substituted-arabinofuranosylcytosines and -uracils are described, and relationships of structure to anti herpes virus activity in vitro were examined. Those arabinonucleosides containing the 2′-fluoro function exhibit, generally, more potent anti herpes virus (HSV) activity than do their 2′-chloro or 2′-bromo analogues. The importance of the fluorine in the 2′-"up" (arabino) configuration for enhancement of antiviral effectiveness is demonstrated by the superior activity of 2′-fluoro-5-iodo-*ara*-C [**3a**, FIAC] to that of 2′-fluoro-5-iodo-*ribo*-C. Of all the nucleosides tested herein, FIAC exhibited the most potent in vitro activity against HSV. 2′-Chloro-5-iodo- and -5-methyl-*ara*-C (**3b** and **4b**) were 37 to >500 times *more* effective in vitro against HSV type 2 than against type 1, suggesting that these latter derivatives might serve clinically as useful probes to distinguish between HSV types 1 and 2 in the diagnosis of HSV infections in man.

In previous reports,[1-3] we described the syntheses and potent anti herpes virus activity of several 5-substituted 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosines and -uracils. Of the newly synthesized compounds, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (2′-

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Table I. Antiherpetic Activity of Some 2′-Halogeno-5-substituted-pyrimidine Nucleosides[a]

compd	X	R	ED_{50},[a] μM HSV-1	ED_{50},[a] μM HSV-2	ID_{50},[b] μM	anal.[c]
1A	F	H	0.12	0.3	0.6	ref 2
1B	Cl	H	0.11	3.8	0.006	ref 11
1C	Br	H	3.2	4.4	0.2	$C_9H_{12}BrN_3O_4 \cdot HCl$
2A	F	Br	0.19	0.02	5.0	ref 2
2B	Cl	Br	>100	>100	>100	$C_9H_{11}BrClN_3O_4 \cdot HCl$
2C	Br	Br	>100	>100	4.5	$C_9H_{11}Br_2N_3O_4 \cdot HCl$
3A	F	I (FIAC)	0.01	0.01	8.6	ref 2
3B	Cl	I	3.4	0.09	3.5	$C_9H_{11}ClIN_3O_4$
3C	Br	I	9.5	2.2	0.2	$C_9H_{11}BrIN_3O_4 \cdot HCl$
4A	F	Me (FMAC)	0.64	0.8	0.8	ref 2
4B	Cl	Me	>100	0.22	20.0	ref 2
4C	Br	Me	>100	26.2	34.0	$C_{10}H_{14}BrN_3O_4 \cdot HCl$
5A	F	H	0.8	6.0	>100	ref 2
6A	F	Br	0.11	0.07	0.9	ref 2
7A	F	I	0.025	0.055	3.3	ref 2
8A	F	Me(FMAU)	0.013	0.013	1.0	$C_{10}H_{13}FN_2O_5$
8B	Cl	Me	>100	>100	>100	$C_{10}H_{13}ClN_2O_5$
8C	Br	Me	>100	>100	8.0	$C_{10}H_{13}BrN_2O_5$
9 (2′-F-5-I-*ribo*-C)			17.9	27.4	1.90	$C_9H_{11}FIN_3O_4$

[a] ED_{50} indicates the effective dose to suppress viral replication by 50% (HSV-1, strain 2391; HSV-2, strain G). [b] ID_{50} indicates the concentration necessary for 50% inhibition of growth of normal human lymphocytic cells. [c] Chemical formulas are given for new compounds that were analyzed for all the elements except oxygen, and analytical results were within ±0.4% of the theoretical value.

fluoro-5-iodo-*ara*-C or FIAC) was consistently the most potent, demonstrating >90% reduction of herpes simplex virus types 1 and 2 (HSV-1, -2) replication in Vero cell monolayers at concentrations as low as 0.01 μM. The 5-bromocytosine analogue (FBrAC) of FIAC and 2′-fluoro-5-methyl-*ara*-U (FMAU) were almost as potent as FIAC in vitro. In addition, the cytotoxicity of these three nucleosides against uninfected Vero cells was found to be minimal.[2,3] We have found[1] that a 2′-fluoro substituent in the sugar moiety of these arabinonucleosides confers better anti herpes virus activity than does a 2′-hydroxy or a 2′-hydrogen substituent. It is apparent from our studies, therefore, that the nature of substituents at the C-5 and C-2′ positions plays an important role in determining antiviral and cytotoxic activity.

Our studies with FIAC indicate that its mechanism of antiviral activity depends, at least in part, on the viral-specified thymidine kinase.[2] Thus, FIAC was found to be about 8000-fold more active against the wild-type HSV-1 than against a mutant lacking this viral enzyme. In contrast, the cytotoxicity of FIAC appears to depend on a cellular deoxycytidine kinase, since this biological activity of FIAC can be reversed by the natural substrate for this enzyme.[2] The antiviral activity is only reversed by high concentrations of thymidine but not by deoxycytidine, again indicating that, in this activity, it acts as an analogue of thymidine and not deoxycytidine.

We report herein the synthesis and anti-HSV activity of several 2′-deoxy-2′-halogeno-*ara*-C and -*ara*-U derivatives substituted at C-5 of the aglycon with bromine or iodine. In addition, we include a large-scale preparation of FIAC and a practical synthetic procedure for FMAU. Our studies were aimed at further evaluating the structural features essential for enhanced anti-HSV-1 and -HSV-2 activity. The 5-methyl analogues of some 2′-halogeno-*ara*-C and -*ara*-U were prepared in order to determine whether size or electronegativity of the C-5 substituent plays a role in biological activity. In addition, 2′-deoxy-2′-fluoro-5-iodocytidine (the ribo isomer of FIAC) was prepared in order to determine the importance of the configuration of the 2′-fluoro substituent at C-2′ on the anti herpes virus activity.

The cytosine nucleosides (**1A–4A**, Table I) were synthesized by condensation of trimethylsilylated cytosine bases (**1–4**, Scheme I) with suitably protected 2-fluoro-,[4] 2-chloro-,[5,6] or 2-bromoarabinosyl bromide[6,7] (**A**, **B** or **C**, respectively) in methylene chloride without catalyst, followed by saponification of the products. The uracil nucleosides **5A–7A** were prepared according to the procedures reported previously.[1] 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (**8A**, 2′-fluoro-5-methyl-*ara*-U or FMAU) was prepared by hydrolytic deamination of the corresponding 5-methylcytosine nucleoside **4A**. 2′-Chloro- and 2′-bromoarabinosylthymines (**8B** and **8C**) were synthesized by direct condensation of bis(trimethylsilyl)thymine (**8**) with the corresponding sugar halides (**B** and **C**, respectively). 2′-Deoxy-2′-fluoro-5-iodocytidine (**9**, the ribo isomer of FIAC) was prepared by iodination of 2′-deoxy-2′-fluorocytidine.[8]

A wide variety of biological activities was found with the nucleosides listed in Table I. Clearly, FIAC exhibits the most potent and selective antiviral activity. However, FMAU also showed excellent activity as well as minimal cytotoxicity. The data also show that, in general, the fluoro function in the 2′-"up" (arabino) position offered better antiviral activity than did the corresponding 2′-chloro or 2′-bromo (arabino) congeners. The only exception noted thus far is 2′-chloro-5-methyl-*ara*-C (**4B**), which (although essentially inactive against HSV-1) is more active than the corresponding 2′-fluoro analogue (**4A**) against HSV-2. The fact that FIAC (**3A**) is at least 10^3 times more effective in suppression of HSV replication than is the isomeric 2′-fluoro-*ribo*-C (**9**, 2′-"down" configuration) demonstrates clearly that the 2′-fluoro substituent in the up (arabino) configuration is an essential feature for the enhanced antiviral activity.

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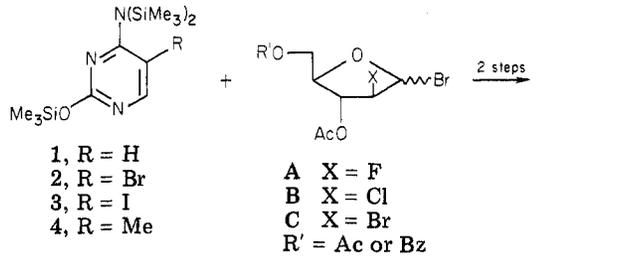
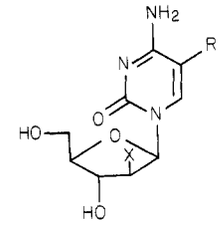
Scheme I

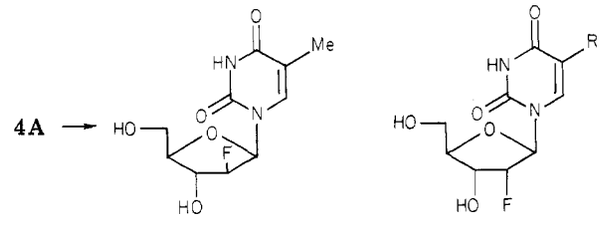


1, R = H
2, R = Br
3, R = I
4, R = Me

A X = F
B X = Cl
C X = Br
R' = Ac or Bz

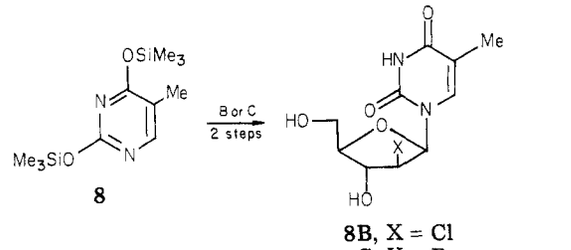


1A-C
2A-C
3A-C
4A-C



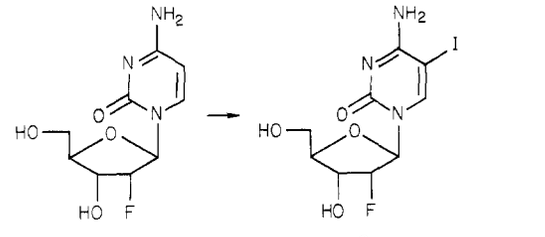
8A

5A, R = H
6A, R = Br
7A, R = I



8

8B, X = Cl
C, X = Br



9

Compounds **2A**, **3B**, and **4B** showed much more activity against HSV-2 than against HSV-1. This selective activity against HSV-2 may be a reflection of differences in virus-specified thymidine kinases, which may differ in structural requirements for substrates.

It is noteworthy that 2′-chloro-*ara*-C (**1B**) (as observed previously in L5178Y and P815 murine leukemic cells)[5] is also very toxic against normal human lymphocyte cells. The 2′-bromo analogue **1C** also exhibits marked toxicity against normal lymphocytic cells. Compounds **1B** and **1C** are more cytotoxic against these normal cells than against HSV-1 and HSV-2 infected Vero cells, suggesting that these nucleosides may be better substrates for deoxycytidine kinase of normal human lymphocytes than for viral-specified thymidine kinase(s). As expected, the α anomer of FIAC was nontoxic toward normal human lymphocytes and was at least 100 times less active than β-FIAC (**3a**) in the plaque-reduction assay with HSV-1 and HSV-2, indicating that α-FIAC is a poor substrate for cellular or virus-specified nucleoside kinases.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. TLC was performed on Uniplates purchased from Analtech Co. and column chromatography on silica gel G-60 (70–230 mesh, ASTM, Merck). Elemental analyses were performed by Galbraith Laboratories, Inc., or Spang Microanalytical Laboratory. ^{1}H NMR spectra (Table II) were recorded on a JEOL PFT-100 spectrometer, and Me_4Si was the internal standard for organic solvents and DSS for deuterium oxide; chemical shifts are reported in parts per million (δ). Values given for coupling constants are first order.

5-Iodocytosine.[9,10] To a solution of cytosine (22.2 g, 0.2 mol) in water (300 mL) and AcOH (500 mL) were added CCl_4 (200 mL), I_2 (20.4 g, 0.16 mol), and HIO_3 (7.04 g, 0.04 mol), and the mixture was stirred vigorously at 50 °C overnight. The mixture was evaporated in vacuo, and the residue was boiled with water (500 mL) and filtered while hot. The dark solid was washed well with Me_2CO to remove unreacted I_2 to give 5-iodocytosine (41 g, 86%) as a slightly tan solid, mp 248 °C dec (lit.[9] mp 225–245 °C dec). The IR spectrum of this sample was identical with that of an authentic sample prepared according to Johnson and Johns.[9]

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (3A, 2′-Fluoro-5-iodo-*ara*-C or FIAC). A solution of 1,3-di-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranose[4] (33.4 g, 0.01 mol) in dry CH_2Cl_2 (140 mL) was chilled in an ice bath, and HBr was bubbled in for 20 min. The mixture was kept at 4 °C overnight, and then the solvent was removed in vacuo below 35 °C. Traces of AcOH were removed by several coevaporations with C_6H_6, and the residue was dissolved in CH_2Cl_2 (50 mL).

The above solution was added to tris(trimethylsilyl)-5-iodocytosine (**3**) [freshly prepared by refluxing 5-iodocytosine (46.5 g, 0.2 mol) in $(Me_3Si)_2NH$ (120 mL) in the presence of 50 mg of $(NH_4)_2SO_4$ until a clear solution was obtained, and then the excess $(Me_3Si)_2NH$ was removed by evaporation in vacuo], and the mixture was stirred at room temperature for 7 days. The reaction mixture was diluted with CH_2Cl_2 (500 mL), and the well-stirred solution was treated dropwise with MeOH (20 mL). The suspension was filtered through a Celite pad, and the filtrate was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was crystallized from Me_2CO to give 16 g (32%) of 1-(3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine, mp 183–184 °C dec, which was stirred at room temperature in NH_3/MeOH (500 mL) for 24 h and then evaporated in vacuo. The residue was triturated with CH_2Cl_2, and the solid was recrystallized from water to give FIAC (**3a**) as colorless needles (9.4 g, 82%), mp 211–213 °C (efferv). One more recrystallization did not alter the melting point. The ^{1}H NMR data of **3A** are given in Table II.

The mother liquor of crystallization of the protected FIAC [containing protected FIAC and its α anomer as the major components as judged by TLC ($CHCl_3$/MeOH, 20:1)] was evaporated to dryness in vacuo, and the residue was chromatographed over a silica gel column (30 × 5 cm; $CHCl_3$/MeOH, 30:1). The fractions (50 mL each, checked by TLC) rich in the α-nucleoside (slightly more polar than the β counterpart) were collected and evaporated, and the residue (5.5 g) was rechromatographed. From the nucleosidic fractions free of the β isomer, the protected α-FIAC (1.2 g) was obtained as a foam: ^{1}H NMR (Me_2SO-d_6) δ 2.05 (3 H, s, OAc), 4.47 (2 H, apparent d, spacing 4.4 Hz, H-5′, 5″), 5.02 (1 H, apparent q, spacing 4.0 Hz, H-4′), 5.39 (1 H, dm, H-3′, $J_{3',F}$ = 24.1 Hz), 5.53 (1 H, dm, H-2′, $J_{2',F}$ = 51.0 Hz), 6.06 (1 H, d, H-1′, $J_{1',F}$ = 15.0 Hz), 6.79 (1 H, br s, part of NH_2), 7.4–8.3 (7 H, m, H-6

(9) An alternate iodination procedure was reported by Johnson, T. B.; Johns, C. O. *J. Biol. Chem.* **1906**, *1*, 305, but the method was found to be unsuitable for large-scale preparations.

(10) This procedure has been used in iodination of 2′-deoxycytidine by Chang, P. K.; Welch, A. D. *Biochem. Pharmacol.* **1961**, *8*, 327; *J. Med. Chem.* **1963**, *6*, 428.

Table II. ^{1}H NMR Parameters of 2′,5-Disubstituted Pyrimidine Nucleosides[a]

compd	chemical shift (δ)							coupling constants, Hz						
	H-1′	H-2′	H-3′	H-4′	H-5′,5″	Me	H-6	exchangeable	$J_{1',2'}$	$J_{1',F}$	$J_{2',3'}$	$J_{2',F}$	$J_{3',4'}$	$J_{3',F}$	solvent
3A	6.25 (dd)	5.27 (dt)	4.43 (dq)		3.80–4.10 (m)		8.47 (s)		3.8	15.2	3.8	51.9	5.0	19.3	D_2O
4A	6.07 (dd)	4.97 (dq)	4.20 (dq)		3.60–3.94 (m)	1.84 (s)	7.52 (s)		3.7	18.0	2.2	53.0	4.0	18.0	Me_2SO-d_6
2B	6.14 (d)	4.56 (t)	4.17 (t)		3.54–3.83 (m)		8.19 (s)	7.97, 7.14 (NH)	5.2		4.9		5.3		Me_2SO-d_6
3B	6.19 (d)	4.67 (t)	4.18 (t)		3.54–3.84 (m)		8.66 (s)	9.35 (NH)[b]	5.8		6.3		6.5		Me_2SO-d_6
4B	6.20 (d)	4.67 (t)	4.20 (t)		3.41–3.84 (m)	1.98 (s)	8.17 (s)	9.17, 9.38 (NH)[b]	5.8		6.0		6.2		Me_2SO-d_6
1C	6.27 (d)	4.74 (t)	4.49 (t)		3.81–4.11 (m)		8.10 (d)[c]		5.8		5.6		5.6		D_2O
2C	6.10 (d)	4.66 (t)	4.30 (t)		3.72–3.80 (m)		8.42 (s)	8.42, 8.64 (NH)	5.5		5.6		5.6		Me_2SO-d_6
3C	6.10 (d)	4.65 (t)	4.37 (t)		3.69–3.79 (m)		8.45 (s)	7.89, 8.67 (NH)	5.5		5.5		5.6		Me_2SO-d_6
4C	6.15 (d)	4.71 (t)	4.31 (t)		3.74–3.83 (m)	2.00 (s)	8.18 (s)	9.33, 9.51 (NH)	6.2		6.3		6.4		Me_2SO-d_6
8A	6.10 (dd)	5.04 (dt)	4.25 (dm)		3.31–3.78 (m)	1.79 (s)	7.60 (s)	11.4 (NH)[b]	3.9	15.5	3.9	53.1	5.2	10.6	Me_2SO-d_6
8B	6.20 (d)	4.60 (t)	4.20 (m)		3.69–3.72 (m)	1.78 (s)	7.76 (s)	11.4 (NH)[b]	6.1		6.1				Me_2SO-d_6
8C	6.14 (d)	4.64 (t)	4.30 (m)		3.67–3.73 (m)	1.79 (s)	7.75 (s)	11.4 (NH)[b]	6.1		6.1				Me_2SO-d_6
9	6.01 (d)	5.08 (dd)	4.57 (dq)	4.20 (d)	3.93 (m)[d]		8.65 (s)		0	17.0	3.6	53.0	8.8	22.1	D_2O

[a] Signals (in parentheses) are expressed as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; dt, double triplet; dq, double quartet; dm, double multiplet. Values given for coupling constants are first order. [b] HCl salt. [c] $J_{5,6}$ = 8.0 Hz (H-5, 6.26). [d] Octet, $J_{5',5''}$ = 13.1 Hz, $J_{4',5'}$ = 1.5 Hz, $J_{4',5''}$ = 2.8 Hz.

and part of NH_2). The protected α-FIAC (1.0 g) was treated with saturated NH_3/MeOH for 3 days. After the solvent was removed in vacuo, the residue was triturated with CH_2Cl_2, and the solid was recrystallized twice from EtOH to give free α-FIAC (400 mg, 56%): mp 189.5–190 °C dec; ^{1}H NMR (Me_2SO-d_6–D_2O) δ 3.49 (2 H, d, H-5′, 5″), 4.22 (1 H, d, H-3′, $J_{3',F}$ = 16.2 Hz), 4.30 (1 H, m, H-4′), 5.11 (1 H, d, H-2′, $J_{2',F}$ = 51.3 Hz), 5.89 (1 H, d, H-1′, $J_{1',F}$ = 15.0 Hz), 7.93 (1 H, s, H-6).

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methylcytosine (4A, 2′-Fluoro-5-methyl-*ara*-C or FMAC). From the 2-fluoroarabinose (20.4 g, 0.06 mol) and 5-methylcytosine (15 g, 0.12 mol), with a method similar to that described above, 13.7 g (56%) of the blocked nucleoside, mp 219–220 °C, was obtained. After deprotection with NH_3/MeOH, FMAC (**4A**) was obtained as colorless cyrstals: mp 207–208 °C; yield 17.7 g (88%, recrystallized from EtOH–Et_2O). For the ^{1}H NMR data of **4A**, see Table II.

1-(2-Chloro-2-deoxy-β-D-arabinofuranosyl)-5-bromocytosine Hydrochloride (2B). A solution of 1.20 g (4.07 mmol) of 1,3,5-tri-*O*-acetyl-2-chloro-2-deoxy-α-D-arabinofuranose[5,6] in 50 mL of dry CH_2Cl_2 was treated at 0 °C with dry gaseous HBr for its conversion to the halogenose. The reaction was conducted, and the product was isolated as described above. The bromo sugar (**B**) thus obtained as a syrup was treated with the trimethylsilylated 5-bromocytosine (**2**) [from 1.24 g (8 mmol) of 5-bromocytosine in 15 mL of $(Me_3Si)_2NH$ and 5 mg of $(NH_4)_2SO_4$], and the reaction mixture was worked up as usual after 16 h. The major product of condensation was crystallized from a mixture of CH_2Cl_2–Et_2O to give 1.02 g (61%) of the pure 3′,5′-di-*O*-acetyl derivative of **2B**, mp 203–204 °C dec. Removal of the acetyl groups by treatment with 3% HCl in MeOH for 20 h at room temperature, followed by evaporation to dryness and crystallization of the residue from EtOH–Et_2O, afforded **2B** (553 mg, 61%), mp 174–175 °C.

In a similar manner, the 5-iodocytosine nucleoside **3B**, mp 196–197 °C dec, and the 5-methylcytosine derivative **4B**, mp 209–210 °C, were synthesized. The ^{1}H NMR data for these compounds are given in Table II.

1-(2-Bromo-2-deoxy-β-D-arabinofuranosyl)-5-bromocytosine Hydrochloride (2C). A solution of 1,3,5-tri-*O*-acetyl-2-bromo-2-deoxy-α-D-arabinofuranose[6,7] (825 mg, 2 mmol) in CH_2Cl_2 (30 mL) was cooled in an ice bath, and HBr was bubbled in for 30 min. The solvent was removed in vacuo, and traces of AcOH were coevaporated several times with toluene. The residue was dissolved in CH_2Cl_2 (30 mL), the solution was added to crude tris(trimethylsilyl)-5-bromocytosine (**2**) [freshly prepared from 5-bromocytosine (760 mg, 4 mmol)], and the mixture was stirred at room temperature for 48 h. MeOH (1 mL) was added to the reaction, and the mixture was filtered through a Celite pad. The filtrate was evaporated in vacuo, and the residue was crystallized from CH_2Cl_2–Et_2O to give the protected nucleoside (268 mg, 28%), mp 188–189 °C. Deacetylation of the protected nucleoside (210 mg, 0.45 mmol) by treatment with 3% HCl/MeOH for 20 h at room temperature, followed by evaporation of the solvent in vacuo and crystallization of the residue from *i*-PrOH, afforded 80 mg (42%) of **2C**, mp 178–179 °C dec.

In a similar manner, 2′-bromo-*ara*-C (**1C**, mp 203–204 °C dec), 2′-bromo-5-iodo-*ara*-C (**3C**, mp 195–196 °C dec), and 2′-bromo-5-methyl-*ara*-C (**4C**, mp 209–210 °C dec) were prepared. The ^{1}H NMR data for these nucleosides are given in Table II.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (8A, 2′-Fluoro-5-methyl-*ara*-U or FMAU). FMAC (**4A**, 5.18 g, 20 mmol) was refluxed with 75% AcOH (100 mL), and the solution was monitored by TLC ($CHCl_3$–MeOH, 7:3, v/v). After 48 h, four spots were detected; after 96 h, two major spots resulted. The mixture was evaporated in vacuo to dryness, and traces of AcOH were removed by several azeotropic distillations with toluene. The residue was dissolved in NH_3/MeOH (250 mL). After 24 h, the solvent was removed in vacuo, and the residue crystallized from MeOH to give 3.75 g (75%) of FMAU (**8A**), mp 184–185 °C. The mother liquor was concentrated, and the residue was chromatographed ($CHCl_3$–MeOH, 9:1, v/v). From the major fraction 0.8 g of FMAU was obtained to give the total yield of 4.55 g (88%).

1-(2-Chloro-2-deoxy-β-D-arabinofuranosyl)thymine (8B). A mixture of the 2-chloroarabinosyl bromide[5,6] (2 mmol) and bis(trimethylsilyl)thymine (**8**; 4 mmol) in CH_2Cl_2 (30 mL) was

stirred at room temperature for 48 h. MeOH (1 mL) was added to the reaction, and the mixture was filtered through a Celite pad. The filtrate was concentrated, and the residue was chromatographed ($CHCl_3$). The nucleoside fractions were collected and concentrated, and the residue was treated with 3% HCl/MeOH for 24 h. After the solvent was removed by evaporation, the residue was crystallized from water to give 107 mg (33%) of **8B**, mp 124–125 °C.

In a similar manner, the 2′-bromo analogue (**8C**), mp 134–136 °C, was obtained via condensation of bis(trimethylsilyl)thymine (**8**) with the bromo sugar (**C**). For the ^1H NMR data of **8B** and **8C**, see Table II.

1-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-5-iodocytosine Hydrochloride (9, 2′-Fluoro-2′-deoxy-5-iodocytidine).[10] 2′-Fluoro-2′-deoxycytidine[8] (390 mg, 1.6 mmol) was dissolved in 3% HCl/MeOH (10 mL), and the solution was concentrated to dryness. The residue was suspended in AcOH (10 mL), and AcCl (2 mL) was added. The mixture was stirred for 30 min at 90–95 °C and then concentrated in vacuo. The residue was dissolved in water (2 mL), and to this solution were added I_2 (194 mg, 0.8 mmol), HIO_3 (56 mg), CCl_4 (2 mL), and AcOH (3 mL), and the mixture was stirred for 24 h at 50–55 °C. The mixture was concentrated in vacuo, and the residue triturated several times with CCl_4 and then crystallized from EtOH to give 150 mg of crude 3′,5′-di-*O*-acetyl-2′-deoxy-2′-fluoro-5-iodocytidine hydrochloride which was dissolved in 10% HCl/MeOH and stirred for 24 h at room temperature. After concentration of the mixture in vacuo, the residue was triturated several times with Et_2O, the insoluble solid was dissolved in water and filtered, and the filtrate was lyophilized to give **9** as a fluffy solid (62 mg). The ^1H NMR spectral data are given in Table II.

Antiviral Activity. Antiviral activity was determined for HSV-1 (strain 2931) and HSV-2 (strain G) on monolayers of Vero cells by the plaque-reduction assay.[2] A dose that reduced the number of virus plaques by 50% (ED_{50}) was determined.

Cytotoxicity. In other experiments (data not presented), we have found that inhibition of lymphocytic proliferation in response to the mitogen PHA demonstrated levels of cytotoxicity similar to those obtained with the inhibition of Vero cell proliferation.[2] Lymphocyte proliferation was quantitated by [^3H]thymidine incorporation after a 4-h pulse.[11]

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Registry No. **1A**, 56632-83-8; **1B**, 58461-30-6; **1C**, 67036-66-2; **1C**·HCl, 83966-85-2; **2**, 83966-86-3; **2A**, 69123-93-9; **2B**, 80791-95-3; **2B**·HCl, 83966-87-4; **2B** diacetate, 83966-88-5; **2C**, 83966-89-6; **2C**·HCl, 83966-90-9; **2C** diacetate, 83966-91-0; **3**, 83966-92-1; **3A**, 69123-90-6; **3A** (α isomer), 83999-91-1; **3B**, 80791-94-2; **3C**, 83966-93-2; **3C**·HCl, 83966-94-3; **4A**, 89636-53-0; **4A** 3-*O*-acetate 5-*O*-benzoate, 83966-95-4; **4B**, 80791-96-4; **4C**, 83966-96-5; **4C**·HCl, 83966-97-6; **5A**, 69123-94-0; **6A**, 69123-97-3; **7A**, 69123-98-4; **8**, 7288-28-0; **8A**, 69256-17-3; **8B**, 80791-97-5; **8C**, 83966-98-7; **9**, 80791-93-1; **9** diacetate HCl, 83966-99-8; $(Me_3Si)_2NH$, 32713-31-8; cytosine, 71-30-7; 5-iodocytosine, 1122-44-7; 1,3-di-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranose, 84025-00-3; 1-(3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-fluoro-β-D-arabinofuranosyl)-5-iodocytosine, 83967-00-4; 5-methylcytosine, 554-01-8; 1,3,5-tri-D-acetyl-2-chloro-2-deoxy-α-D-arabinofuranose, 30589-74-3; 5-bromocytosine, 2240-25-7; 1,3,5-tri-D-acetyl-2-bromo-2-deoxy-α-D-arabinofuranose, 83967-01-5; 3,5-di-*O*-acetyl-2-chloro-2-deoxy-D-arabinofuranosyl bromide, 84025-01-4; 3,5-di-*O*-acetyl-2-bromo-2-deoxy-D-arabinofuranosyl bromide, 84025-02-5; 2-fluoro-2′-deoxycytidine, 10212-20-1.

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Carbocyclic Analogues of 5-Substituted Uracil Nucleosides: Synthesis and Antiviral Activity

Y. Fulmer Shealy,* C. Allen O'Dell, William M. Shannon, and Gussie Arnett

Kettering-Meyer Laboratories, Southern Research Institute, Birmingham, Alabama 35255. *Received July 29, 1982*

Carbocyclic analogues of 3′-deoxyuridines, 3′-deoxyuridines, and uridines with substituents at position 5 of the uracil moiety were prepared by direct halogenation (5-bromo and 5-iodo groups) and by displacement of the 5-bromo group by amino and substituted-amino groups. The analogue of 5-(hydroxymethyl)uridine was prepared via reaction of the isopropylidene derivative of the uridine analogue with paraformaldehyde. The carbocyclic analogues of thymidine and of 5-bromo-, 5-iodo-, and 5-(methylamino)-2′-deoxyuridine were highly active in vitro against herpes simplex virus, types 1 and 2. The corresponding analogues of 5-substituted 3′-deoxyuridines and of 5-substituted uridines were not active in this assay.

Several pyrimidine nucleosides, notably 1-β-D-arabinofuranosylcytosine (*ara*-C)[1,2] and 5-fluoro-2′-deoxyuridine,[3,4] have useful anticancer activity. Many pyrimidine nucleosides have antiviral activity;[5-10] among the antiviral pyrimidine nucleosides, a great variety of 5-substituted 2′-deoxyuridines (**1**) inhibit the replication of herpes viruses. Because of the anticancer, antiviral, and other types of biological activity found among pyrimidine nucleosides, there is a continuing interest in this type of structure.

Previously, we have reported the synthesis of carbocyclic analogues of uracil nucleosides[11] (**2a**, **3a**, and **4a**), thymidine nucleosides[12,13] (**2b**, **3b**, and **4b**), 5-fluorouracil nu-

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