ported by the Auckland Division of the Cancer Society of New Zealand and by the Medical Research Council of New Zealand.

Registry No. 5, 59292-04-5; 6, 30087-31-1; 7, 118537-75-0; 8, 35614-21-2; 9, 88521-78-2; 10, 117570-56-6; 11, 117570-57-7; 12, 118537-76-1; 13, 117570-45-3; 14, 117570-46-4; 15, 117570-58-8; 16, 118575-12-5; 17, 118537-77-2; 18, 118537-78-3; 19, 117570-59-9; 20, 118537-79-4; 21, 117570-47-5; 22, 117570-48-6; 23, 117570-49-7; 24, 117570-64-6; 25, 117570-63-5; 26, 117570-50-0; 27, 117570-51-1; 28, 117570-52-2; 29, 117570-70-4; 30, 117570-71-5; 31, 117570-72-6; 32, 117570-74-8; 33, 117570-75-9; 34, 117570-73-7; 35, 117570-76-0; 36, 117570-77-1; 37, 117570-78-2; 38, 117570-83-9; 39, 107517-48-6; 40, 117571-26-3; 41, 117571-27-4; 42, 118537-80-7; 43, 43160-03-8; 44, 117570-84-0; 45, 117570-85-1; 46, 117570-86-2; 47, 117570-89-5; 48, 117570-90-8; 49, 69200-01-7; 50, 117571-23-0; 51, 117570-91-9; 52, 69199-67-3; 53, 117570-92-0; 54, 59292-09-0; 55, 59292-12-5; 56, 54160-09-7; 57, 30087-35-5; 58, 118537-81-8; 59, 118537-82-9; 60, 26539-21-9; 61, 54182-77-3; 62, 55950-72-6; 63, 117570-95-3; 64, 117570-96-4; 65, 117571-25-2; 66, 117571-28-5; 67, 117571-29-6; 68, 43160-04-9; 69, 43160-06-1; 70, 117570-97-5; 71, 117570-98-6; 72, 117570-99-7; 73, 117571-00-3; 74, 117571-01-4; 75, 117571-02-5; 76, 117571-03-6; 77, 117571-31-0; 78, 117571-32-1; 79, 15128-43-5; 80, 117570-80-6; 81, 117570-81-7; 82, 117570-82-8; 83, 117571-12-7; 84, 117571-13-8; 85, 117571-14-9; 86, 117571-15-0; 87, 117571-16-1; 88, 117571-17-2; VI (R^2 = 5′-CH_3), 117571-38-7; VI (R^2 = 3′-CH_3), 118537-92-1; VI (R^2 = H), 117571-22-9; VI (R = 4-CH_3), 38692-77-2; VIII (R = 4′-OCH_3), 118537-90-9; VIII (R = 4′-Cl), 118537-91-0; XII (R = 4-CH_3), 117571-33-2; XIII (R = 4-CH_3), 117571-34-3; XIV (R = 4-CH_3), 117571-35-4; XV (R = 4-CH_3), 117571-36-5; XVI (R = 4-cH_3), 117571-37-6; sodium 2,4-dichlorobenzoate, 38402-11-8; sodium 2-methylphenolate, 4549-72-8; sodium o-chlorobenzoate, 17264-74-3; sodium 2-chloro-3-methoxybenzoate, 118537-83-0; sodium 2,3-dichlorobenzoate, 118537-84-1; sodium 2-chloro-3-nitrobenzoate, 118537-85-2; sodium 2-chloro-4-methylbenzoate, 118537-86-3; sodium 2-chloro-4-methoxybenzoate, 118537-87-4; sodium 2-chloro-5-methylbenzoate, 118537-88-5; sodium 2-chloro-5-methoxybenzoate, 118537-89-6; sodium 2,5-dichlorobenzoate, 63891-98-5; sodium 2-chloro-5-nitrobenzoate, 14667-59-5; sodium 5-chloro-2-methylphenoate, 40495-68-9; sodium 3-chloro-2-methylphenolate, 118537-93-2; sodium o-methylphenolate, 4549-72-8; potassium 2-iodo-5-methylbenzoate, 117571-21-8; 2-chloro-4-nitrobenzoic acid, 99-60-5; 5,5′-dinitrobiphenol-2,2′-dicarboxylic acid, 92159-34-7; potassium 2-chlorobenzoate, 16463-38-0; sodium 2-allyl-4-methylphenolate, 118537-94-3; 2-(2-allyl-4-methylphenoxy)benzoic acid, 117571-24-1; 4-methylsalicylic acid, 50-85-1; benzyl bromide, 100-39-0; 6-methylsalicylic acid, 567-61-3; 6-methyl-2-(phenylmethoxy)benzoic acid, 118537-95-4; [6-methyl-2-(phenylmethoxy)phenyl]acetic acid, 118537-96-5; (2-hydroxy-6-methylphenyl)acetic acid, 38692-76-1.

2′-Fluorinated Isonucleosides. 1. Synthesis and Biological Activity of Some Methyl 2′-Deoxy-2′-fluoro-2′-pyrimidinyl-D-arabinopyranosides

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New reactions of methyl 2,2-difluoro glycosides are described that were utilized for synthesis of some novel nucleoside derivatives. Thus, treatment of methyl 2-deoxy-2,2-difluoro-3,4-O-isopropylidene-α(β)-D-*erythro*-pyranoside (**2**) with anhydrous HCl resulted in selective displacement of one fluorine atom with chlorine to give a 2-deoxy-2-chloro-2-fluoro glycoside **3**. Reaction of **3** with silylated uracil in the presence of $SnCl_4$ provided a 2-deoxy-2-fluoro-2-uracil-substituted glycoside **4**. 2-Fluoro-2-deoxy glycosides substituted with other pyrimidines at C-2 were prepared similarly by the reaction of acylated 2,2-difluoro or 2-fluoro-2-bromo derivatives (**5** and **6**, respectively) with silylated pyrimidines. The resulting 2′-fluorinated isonucleosides were evaluated for their antitumor and antiviral activities. Compounds **7a,b**, **8a,b**, and **10a,b** demonstrated 50% tumor cell growth inhibition in vitro (IC_{50}) at 10^{-4}–10^{-5} M. At similar concentrations no antiviral activity was observed in vitro. Therapeutic activity was obtained with **7a,b** and **8a,b** in DBA/2 mice with L1210 leukemia. Administration of **7a,b** at 500 mg/kg, ip daily, for 5 consecutive days, resulted in a 55% increase in life span (% ILS) while administration of **8a,b** in the same manner at 200 mg/kg caused a 29% ILS. Treatment with **7a,b** to mice with drug-resistant L1210 sublines (5-FU and araC) resulted in 22 and 57% increases in life span, respectively. Lewis lung carcinoma and M5076 sarcoma in mice also responded to the administration of **7a,b** with reductions in tumor growth for both tumors and significant increases in life span in mice with Lewis lung carcinoma. Although the mechanism of action of **7a,b** is not known, it has been found to be a relatively fast-acting, cell-cycle nonspecific cytotoxic agent that decreases [^{3}H]deoxyuridine incorporation, blocks L1210 cells at the G_2 phase of the cell cycle, and is not reversed by exogenous thymidine. These 2′-fluorinated isonucleosides have demonstrated biological activity and may have potential as antitumor drugs.

As part of our investigation of *gem*-difluoro monosaccharides,[1] we recently reported a selective nucleophilic displacement of fluorine in methyl 2,2-difluoro glycosides.[2,3] Various nucleophiles, including C-substituents and heterocyclic bases can be introduced at C-2 of methyl glycosides, the products of this reaction being novel carbohydrate derivatives substituted, at C-2, with fluorine and the entering substituent. In this paper, we describe the synthesis and the results of initial antiviral and antitumor evaluation of some 2′-fluoro analogues of methyl 2′-pyrimidinylarabinopyranosides.

Chemistry. Methyl 3,4-O-isopropylidene-β-D-arabinopyranoside[4,5] was oxidized by a chromium trioxide–pyridine–acetic anhydride complex[6] to give methyl 3,4-O-isopropylidene-β-D-*erythro*-pentopyranosid-2-ulose (**1**, Scheme I) in 84% yield. While compound **1** was previously prepared by similar oxidation using different reagents,[6–8] the present method gave improved yields and was found better suitable for large-scale preparations. Fluorination

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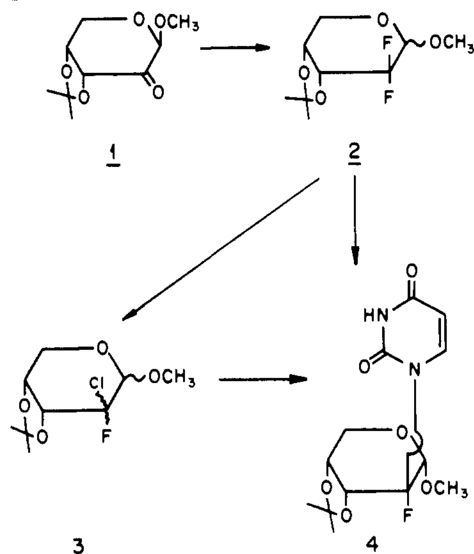
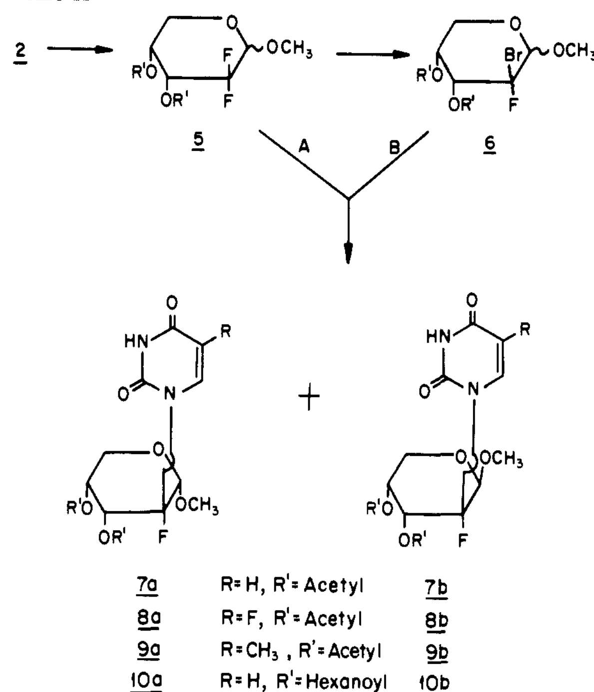
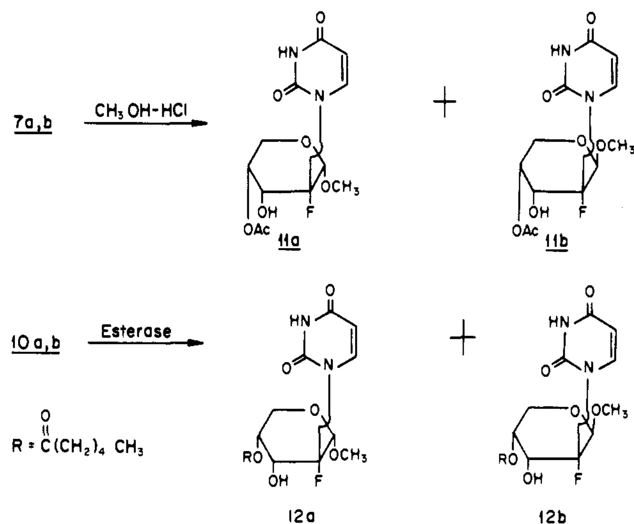
Scheme I



Scheme II



Scheme III



of 1, utilizing (diethylamido)sulfur trifluoride (DAST),[9] proceeded under mild conditions with isomerization about the anomeric carbon to produce methyl 2-deoxy-2,2-difluoro-3,4-*O*-isopropylidene-$\alpha(\beta)$-D-*erythro*-pentopyranoside (**2**) in 78% yield. The presence of the α and β anomers in an approximately 2:1 ratio was detected by NMR; however, the anomers could not be separated by chromatography. Compounds **2**, which could readily be purified by distillation without decomposition, was very labile and susceptible to a variety of substitution reactions in the presence of strong acids. Treatment of **2** with anhydrous HCl in methylene chloride at low temperature gave 2-chloro-2-fluoro glycosides **3**. The formation of **3** could be monitored by TLC where it appeared as a single spot; however, proton and fluorine NMR spectra showed complex patterns of signals, indicating the presence of the α and β anomers and possibly, 2*R* and 2*S* isomers as well. Both compounds **3** and **6** were found unstable and could not be resolved by chromatography. Condensation of **3** with trimethylsilylated uracil in the presence of $SnCl_4$ gave methyl 2-deoxy-3,4-*O*-isopropylidene-2(*R*)-(1,3-dihydro-2,4-dioxo-1-pyrimidinyl)-2-fluoro-α-D-arabinopyranoside (**4**), which was the single major sugar product formed (~ 24%). Four minor byproducts (<5% total) were also present in the reaction mixture; however, they were not investigated. TLC chromatography of the reaction mixture showed no spot corresponding to starting material **3**.

For the preparation of nucleosides protected with the acyl groups, **2** was treated with 95% formic acid followed by evaporation with ethanol, which effected the hydrolysis of the isopropylidene group. Due to the sensitivity of the *gem*-difluoro group of **2** to acids, this hydrolytic step presented some difficulty. To carry out the hydrolysis successfully, it was important to maintain the right combination of the reaction temperature and time, as longer periods of time and/or higher temperature led to removal of fluorine and complex reaction mixtures. The somewhat labile free methyl glycosides **5** (R' = H) (Scheme II) were acetylated with acetic anhydride–pyridine to furnish methyl 3,4-di-*O*-acetyl-2-deoxy-2,2-difluoro-$\alpha(\beta)$-D-*erythro*-pentopyranosides **5** (R' = Ac) or they were treated with hexanoyl chloride in pyridine to give **5** (R' = hexanoyl). Compounds **5** were readily converted to the corresponding 2-bromo-2-fluoro glycosides **6** by treatment with anhydrous HBr. While attempted condensation of **6** (R' = acetyl) with silylated pyrimidines in the presence of $SnCl_4$ led to decomposed material, condensation in the presence of HgO–$HgBr_2$[10] (method A) provided the protected nucleosides **7a,b–10a,b**.

Alternatively, the 2,2-difluoro methyl glycoside **5** was condensed with silylated pyrimidines (method B) in the presence of $BF_3{\cdot}Et_2O$ to afford mixtures of nucleosides **7a,b–10a,b** in low yield and unchanged starting material **5**. The protected nucleosides **4** and **7–10** are relatively stable and can be stored without decomposition at room temperature for at least several months. In aqueous solutions, they remained unchanged when kept at 4 °C for 1 week. Hydrolysis of the acetyl groups in **7a,b** in the presence of basic catalysts was accompanied by substantial decomposition. With use of HCl in methanol at 22 °C, only the 3'-*O*-acetyl group was removed. Acid hydrolysis under more severe conditions, such as heating in HCl–MeOH, led to complex mixtures. Similarly, hydrolysis of the hexanoyl groups in **10a,b** utilizing liver esterase[11,12] as

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the catalyst gave the 3′-deprotected products **12a,b**.

Structure-Determination Studies. In the ^{1}H NMR spectra of nucleosides **7a,b–10a,b**, the C-4′ protons gave rise to signals at δ 5.43–5.49 (Table I), indicating the presence of *O*-acyl groups at C-4′.[13,14] The signals from the C-4 protons in acetylated aldopentofuranosyl derivatives appear at significantly higher fields (δ 4.34–4.63).[15] The geminal C-5′ protons showed distinct chemical shifts, with a typical range of 9.86–10.83 Hz, suggesting conformationally restricted protons. Thus, both the NMR data and the identity of products prepared by different synthetic methods (methods A and B) indicated the pyranosyl structures for **7a,b–10a,b**, the alternative furanosyl ones that might have been produced by method A, would not be expected to be formed by method B. Acyl migration accompanied by a ring contraction has previously been shown to occur by the effects of HBr on methyl triacetyl- or methyl tribenzoylarabinopyranosides.[16,17]

The configuration of the anomeric protons in **4** and **7a,b–12a,b** was determined by the coupling constants (Table II) between H-1′ and F-2′. The larger ones (5.64–12.47) were assigned to the trans α isomers and the smaller ones (0–4.80 Hz) to the cis β isomers. However, these coupling constants of the partially deacetylated nucleosides **11a** and **11b** showed a much smaller difference (0.15 Hz), and, therefore, in this case the assignment of the anomeric configuration should be considered tentative.

The coupling constants between H-3′ and F-2′ showed the range of 17.19–19.50 Hz, which indicated a trans-diaxial configuration[18-21] for H-3′ and F-2′.

The coupling constant between H-3′ and F-2′ (12.47 Hz) in the 3′,4′-*O*-isopropylidene nucleoside **4** was at the lower margin for the trans-diaxial relationship, possibly reflecting the distortion of the chair conformation due to the fused 3′,4′-isopropylidene ring. Splitting patterns of the signals produced by H-5 (pyrimidine ring) of **7a,b** and **4** showed two doublets due to a long-range coupling (^5J)[22,23] between H-5 and F-2′.

The assigned anomeric configuration of all new nucleosides was in good agreement with the signs of optical rotation (Table IV), the α-anomers showing large (+) values whereas the β anomers exhibited large (−) values.

Mass spectra of compounds **7–12** showed corresponding fragments. Compound **7** gave characteristic fragment ions at 360 (M^+), 249 (sugar), 155 (base, uracil-CF=CH), and 112 (uracil). Compound **8** gave characteristic fragment ions at 378 (M^+), 249 (sugar), 173 (5-FU-CF=CH), and 130 (base, 5-FU). Compound **9** exhibited characteristic fragment ions at 374 (M^+), 249 (sugar), 169 (thymine-CF=CH), and 126 (base, thymine), and compound **10** also

Table I. ^{1}H NMR Chemical Shifts of **4** and **7a,b–12a,b** in Ppm Downfield from TMS

protons	compounds (solvent)												
	7a	**7b**	**8a**	**8b**	**9a**	**9b**	**10a** ($CDCl_3$)	**10b** ($CDCl_3$)	**4**	**11a**[a]	**11b**[a]	**12a**[b]	**12b**[b]
H-5	5.76 (dd)	5.76 (dd)					5.75 (d)	5.76 (d)	5.77 (dd)	5.67 (d)	5.67 (d)	5.74 (d)	5.72 (d)
H-6	7.43 (d)	7.55 (d)	7.52 (d)	7.62 (d)	7.26 (s)	7.36 (s)	7.44 (d)	7.56 (d)	7.50 (dd)	7.53 (d)	7.54 (d)	7.65 (d)	7.64 (d)
H-1′	5.77 (d)	5.82 (d)	5.75 (d)	5.81 (s)	5.75 (d)	5.82 (d)	5.74 (d)	5.81 (d)	5.66 (d)	5.75 (d)	5.75 (d)	5.82 (d)	5.87 (d)
H-3′	5.37 (dd)	5.43 (dd)	5.40 (dd)	5.43 (m)	5.38 (dd)	5.39 (dd)	5.38 (dd)	5.43 (dd)	4.73 (dd)	4.40 (dd)	4.39 (dd)	4.54 (dd)	4.51 (t)
H-4′	5.49 (m)	5.49 (m)	5.48 (m)	5.43 (m)	5.49 (m)	5.49 (m)	5.48 (m)	5.51 (m)	4.89 (ddd)	5.30 (dt)	5.30 (d)	5.41 (dt)	5.42 (t)
H-5′	4.36 (dd)	4.39 (dd)	4.36 (dd)	4.40 (dd)	4.36 (dd)	4.41 (dd)	4.36 (dd)	4.38 (dd)	4.22 (m)	4.26 (dd)	4.27 (dd)	4.30 (dd)	4.38 (dd)
H-5″	4.22 (dd)	4.26 (dd)	4.23 (dd)	4.27 (d)	4.21 (dd)	4.32 (d)	4.19 (d)	4.24 (d)	4.22 (m)	4.10 (d)	4.10 (d)	4.06 (dd)	4.11 (d)
NH	8.75 (bs)	8.51 (bs)	9.59 (bs)	9.52 (bs)	8.54 (bs)	8.86 (bs)	8.83 (bs)	8.62 (bs)	9.44 (bs)				
OCH_3	3.41 (s)	3.45 (s)	3.45 (s)	3.47 (s)	3.40 (s)	3.44 (s)	3.39 (s)	3.45 (s)	3.46 (s)	3.39 (s)	3.39 (s)	3.45 (s)	3.42 (s)
OAc	2.11 (s)	2.11 (s)	2.10 (s)	2.11 (s)	2.11 (s)	2.11 (s)				2.06 (s)	2.06 (s)		
OAc	2.12 (s)	2.14 (s)	2.17 (s)	2.15 (s)	2.12 (s)	2.12 (s)							
CH_3-5					1.92 (s)	1.94 (s)							
$2CH_2CO$							2.35 (m)	2.18 (m)					
$2CH_2CH_2CO$								1.63 (m)	1.62 (m)				
$4CH_2$							1.29 (m)	1.30 (m)					
$2CH_3$							0.91 (m)	0.90 (m)					
$CH_3(Me_2CH)$									1.57 (s)				
$CH_3(Me_2CH)$									1.38 (s)				
CH_2CO												2.39 (t)	2.38 (t)
$3CH_2$												1.44 (m)	1.48 (m)
CH_3												0.88 (t)	0.88 (t)

[a] CD_3CN/D_2O. [b] $(CD_3)_2CO/D_2O$.

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Table II. Coupling Constants (Hertz) of 4 and **7a,b–12a,b**

atoms	4	7a	7b	8a	8b	9a	9b	10a	10b	11a	11b	12a	12b
H-5 and H-6	8.17	8.17	8.20					8.04	8.28	8.32	8.07	8.34	8.13
F-5 and H-6				5.87	6.04								
F-2′ and H-5	2.27	2.13	2.15										
F-2′ and H-6	1.63												
H-1′ and F-2′	12.47	9.80	2.98	7.53	0	10.72	4.80	10.67	2.53	4.74	4.59	5.64	3.44
H-3′ and F-2′	12.47	19.25	17.19	19.50		19.11	19.21	19.30	19.23	20.61	20.39	20.17	20.42
H-3′ and H-4′	6.73	5.45	6.13	6.08		6.13	6.04	6.15	6.04	5.67	5.49	6.02	5.13
H-5′ and H-5′		10.59	10.64	10.70	10.67	10.80	9.86	10.83	10.69	10.55	10.84	10.66	10.72
H-4′ and H-5′	5.15	5.45	4.83	5.56	6.08	4.78	6.23	5.38	5.70	5.43	5.49	6.02	5.13
H-4′ and H-5′	2.91	1.64	1.55	0	0	1.07	0	0	0	0.89		1.09	

Table III. ^{19}F NMR Chemical Shifts and Coupling Constants of 2, 4, and **7a,b–12a,b**

compd	solvent	shift, ppm ($CFCl_3$)	coupling, Hz
2	$CDCl_3$	−123.8 (F-2)	m, $J_{F,CH}$ = 1.35
		−143.5 (F-2′)	m
4	$CDCl_3$	−127.6 (F-2′)	t, $J_{F,3'} = J_{F,1'}$ = 12.5
7a	$CDCl_3$	−125.8 (F-2′)	dd, $J_{F,3'}$ = 18.0, $J_{F,1'}$ = 9.3
7b	$CDCl_3$	−122.8 (F-2′)	dd, $J_{F,3'}$ = 18.1, $J_{F,1'}$ = 3.3
8a	$CDCl_3$	−125.0 (F-2′)	dd, $J_{F,3'}$ = 16.1, $J_{F,1'}$ = 7.0
		−164.9 (F-5)	d, $J_{F,6}$ = 5.1
8b	$CDCl_3$	−122.2 (F-2′)	dd, $J_{F,3'}$ = 18.4, $J_{F,1'}$ = 2.7
		−165.0 (F-5)	d, $J_{F,3'}$ = 5.6
10a	$CDCl_3$	−126.83 (F-2′)	dd, $J_{F,3'}$ = 18.4, $J_{F,1'}$ = 11.2
10b	$CDCl_3$	−122.10 (F-2′)	dd, $J_{F,3'}$ = 16.2, $J_{F,11'}$ = 3.4
11a	CH_3OD	−125.5 (F-2′)	d, $J_{F,3'}$ = 20.0, $J_{F,1'}$ = 5.4
11b	CH_3OD	−124.7 (F-2′)	d, $J_{F,3'}$ = 20.3, $J_{F,1'}$ = 4.3
12a	acetone-d_6	−124.12 (F-2′)	dd, $J_{F,3'}$ = 20.2, $J_{F,1'}$ = 5.1
12b	acetone-d_6	−123.95 (F-2′)	dd, $J_{F,3'}$ = 20.7, $J_{F,1'}$ = 3.2

Table IV. Optical Rotations and Melting Points of 1, 4, and **7a,b–10a,b**

compd	$[\alpha]^{20}_D$, deg	mp, °C	concn (%, $CHCl_3$)	anal.[a]
1	−155.8	87–88	0.69	ref 7
4	+66.0	187–188 dec	0.46	$C_{13}H_{17}FN_2O_6$
7a	+88.6	170–171 dec	0.13	$C_{14}H_{17}FN_2O_8\cdot{}^1/_4Me_2CHOH$
7b	−93.3	164–165 dec	0.05	$C_{14}H_{17}FN_2O_8\cdot{}^1/_4Me_2CHOH$
8a	+129.0	188–189 dec	0.10	$C_{14}H_{16}F_2N_2O_8\cdot{}^1/_2H_2O$
8b	−32.1	179–180 dec	0.10	$C_{14}H_{16}F_2N_2O_8\cdot{}^1/_2H_2O$
9a	+50.0	203–205 dec	0.15	$C_{15}H_{19}FN_2O_8$
9b	ND	192–194 dec	0.05	$C_{15}H_{19}FN_2O_8$
10a	+71.7	syrup	0.46	$C_{22}H_{33}FN_2O_8\cdot{}^1/_2EtOAc$[b]
10b	−26.1	syrup	0.53	$C_{22}H_{33}FN_2O_8\cdot{}^1/_2EtOAc$[b]

[a] Chemical formulas are given for new compounds that were analyzed for C, H, N, F. [b] The presence of the solvents was observed in the NMR spectra.

showed characteristic fragment ions at 472 (M^+), 361 (sugar), 155 (base, uracil-CF=CH), and 112 (uracil). The fragmentations of the sugar portions and base portions (pyrimidines) well matched those of the previous report.[24]

Inhibition of Tumor Cell Growth in Vitro. The series of pyrimidine nucleoside analogues **4** and **7a,b–10a,b** having a glycosidic linkage between the 2′-carbon of the sugar moiety (in pyranose configuration) and base were evaluated for their growth-inhibitory action on L1210 leukemia cells maintained in tissue culture. Compounds were dissolved either in water, methanol, or 0.5% Tween 80 in saline prior to addition to tissue culture media. Drug concentrations that inhibited cell growth by 50% (IC_{50}) are listed for each compound in Table V.

With mixtures of the α and β anomers, **7a,b** inhibited 50% of cell growth at 5.5 × 10^{-5} M. Blocking the 3′- and 4′-carbons with an isopropylidene group (**4**) resulted in the

Table V. 1210 Leukemia Cell Growth Inhibition by 2′-Fluoro 2′-Aglycon Nucleosides[a]

compd	IC_{50}, M	compd	IC_{50}, M
7a,b	5.5 × 10^{-5}	**8a,b**	3.4 × 10^{-5}
4	>10^{-4}	**9a,b**	>10^{-4}
10a,b	4.0 × 10^{-5}	5-FdUrd	3 × 10^{-9}

[a] Murine leukemic L1210 cells were maintained in complete RPMI 1640 medium. Celllar growth inhibition was determined by incubating 5 × 10^4 cells/mL for 48 h in 2 mL of media containing varying concentrations of drugs. Cell growth inhibition (50% growth inhibitory concentration, IC_{50}) was determined. These results are the average of at least two separate determinations performed with duplicate cultures.

loss of biological activity. Substituting hexanoyl (**10a,b**) for the acetyl groups did not significantly alter growth-inhibitory activity. The 5-fluorouracil analogue (**8a,b**) demonstrated somewhat greater biological activity as compared with that of **7a,b**, while the thymine derivative (**9a,b**) was inactive. For comparison, 5-FdUrd (5-fluorodeoxyuridine) demonstrated an IC_{50} of approximately 3 × 10^{-9} M.

In a similar series of experiments using a variety of other cell lines (murine FM3A/0 mammary carcinoma and L1210/BdUrd leukemia, human A121 ovarian and colo-205 colon carcinomas, and human Raji/0 B-lymphoblast and Molt/4FT-lymphoblast leukemias), the same general pattern of growth inhibition was noted as observed with the L1210 cell line. For comparison, 5-fluorodeoxyuridine (5-FdUrd) demonstrated far greater growth-inhibitory activity in all cell lines with the exception of the deoxythymidine kinase deficient line (L1210/BdUrd) as expected.

Time Dependence of Drug Action. The time dependency of drug action on L1210 leukemia cell growth inhibition was studied with the uracil derivative **7a,b**. L1210 leukemia cells incubated with various concentrations of **7a,b** for either 1, 6, 12, 18, 24, or 48 h demonstrated a time-dependent decrease in IC_{50}. L1210 leukemia cells incubated for 48 h with **7a,b** demonstrated an IC_{50} of 8.4 × 10^{-5} M (30 μg/mL), slightly higher than the previous finding of 5.5 × 10^{-5} M. Decreasing the time of drug exposure resulted in an increased drug concentration necessary to reduce tumor cell growth by 50%. A 24-h exposure required 1.16 × 10^{-4} M while a 1-h exposure required 2.1 × 10^{-4} M to reduce cell growth by 50%. Shorter drug exposures of 5 and 15 min at 5 × 10^{-4} M reduced cell growth to 80 and 60% control, respectively. These results demonstrated a small but significant time dependence for drug action. However, a much greater time-dependent effect was noted for 5-FdUrd (data not shown). Up to a 1-h exposure of L1210 cells to 5 × 10^{-8} M 5-FdUrd resulted in little effect on cell growth. At least 6 h of exposure was necessary to cause any decrease in cell growth measured 48 h later. Other studies demonstrated that L1210 cells in the plateau phase of growth were equally as sensitive to a 48-h exposure to **7a,b** as cells in

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Table VI. Therapeutic Activity of **7a,b** in DBA/2 Mice with L1210 Leukemia[a]

dosage (mkd × 5)	animals per group	mean animal wt,[b] g	survival parameters[c] range, days	median, days	mean ± SD, days	% ILS	p values[d]
0	20	22.5	6–8	7	6.7 ± 0.1		
50	5	23.4	7–8	7	7.2 ± 0.2	7	NS
100	5	24.0	8–8	8	8.0 ± 0	19	NS
200	10	22.0	8–10	9	8.8 ± 0.2	31	$p < 0.01$
400	10	18.8	9–11	10	9.9 ± 0.2	48	$p < 0.01$
500	5	17.3	10–11	10	10.4 ± 0.2	55	$p < 0.01$
600	5	16.8	7–11	10	9.4 ± 0.8	40	$p < 0.01$
700	5	16.6	4–7	7	6.0 ± 0.6	−10	NS
800	5	19.5	2	2	2.0 ± 0	−70	

[a] DBA/2 female mice (20 g) were inoculated ip with 10^6 L1210 leukemia cells on day 0. Mice were weighed and inspected for tumor growth and survival daily. Drug (**7a,b**) was dissolved in 0.5% Tween 80 in saline (0.2 mL) and injected ip daily on days 1–5. Dosages varied from 1 to 800 mg/kg per day (mkd). [b] Mean weight of mice on the day of death. [c] Survival time is measured in days after tumor inoculation. % ILS (increase in life span) is based on mean survival. Parameters were calculated with a software program for an Apple IIe computer. Statistcal significance is based on the Dunn test. NS = not statistically significant. [d] Determined by the Cox–Mantel test.[36]

the exponential phase of growth, indicating the cytotoxic action of **7a,b** is not dependent on cell proliferation. In contrast, exponential phase cells were much more sensitive to the S-phase active drug, 5-FdUrd, ($IC_{50} = 3 \times 10^{-9}$ M) than were plateau phase cells ($IC_{50} > 5 \times 10^{-8}$ M). Finally, analysis of drug-induced L1210 cell cycle growth arrest by flow cytometry demonstrated that **7a,b** blocks L1210 cells in the G_2 phase of the cell cycle while 5-FdUrd causes cells to accumulate in the S phase (data not shown).

The effect of **7a,b** on L1210 leukemia cell colony formation in soft agar was also found to be concentration dependent. A 1-h exposure of L1210 cells to 1.7×10^{-4} M **7a,b** resulted in a 50% decrease in colony formation. These results are in accord with the previous findings on cell-growth inhibition.

Drug Effects on Macromolecular Biosynthesis. Following 12 h of exposure to high concentrations of either **7a,b** or **8a,b**, L1210 leukemia cells demonstrated statistically significant dose-dependent decreases in the utilization of [^{3}H]deoxyuridine, [^{3}H]mannose, and [^{3}H]leucine. This was somewhat different from the inhibition of precursor incorporation observed for cells exposed to 5-FdUrd of the same period of time. In 5-FdUrd-treated cells, [^{3}H]deoxyuridine and [^{3}H]uridine incorporation were substantially reduced (>25%) at very low drug concentrations (1×10^{-9} M). Less inhibitory effect was noted with 5-FdUrd on [^{3}H]mannose and [^{3}H]leucine incorporation as compared with **7a,b** or **8a,b** (data not shown).

Nucleoside Reversal and Pool Size Studies. Competition or reversal of the growth-inhibitory effects of **7a,b**, **8a,b**, and 5-FdUrd was attempted with the addition of exogenous pyrimidine or purine nucleosides. Since 5-FdUrd is known to inhibit de novo thymidine biosynthesis, thymidine addition to cell cultures reversed 5-FdUrd growth inhibition. The addition of 1×10^{-5} M exogenous thymidine to cultures of L1210 cells increased the IC_{50} of 5-FdUrd from $<3 \times 10^{-10}$ M to $>1 \times 10^{-8}$ M, indicating at least a 2 log (>100-fold) reversal in cell growth inhibitory action of 5-FdUrd.

Although **7a,b** and **8a,b** decreased [^{3}H]deoxyuridine incorporation in L1210 cells, suggesting a mechanism of action similar to 5-FdUrd, the addition of thymidine (5×10^{-5} M) to L1210 cell cultures did not significantly increase the IC_{50} value of **7a,b** and only elevated the IC_{50} value of **8a,b** 3-fold [$(2.1–6.4) \times 10^{-5}$ M IC_{50}]. Exogenous uridine (1×10^{-4} M) was also unsuccessful in reversing the toxicity of **7a,b** and **8a,b**. Neither deoxyuridine (1×10^{-6} M) nor the purine ribonucleosides adenosine and guanosine (1×10^{-6} M) were able to decrease the growth inhibition induced by **7a,b**.

Similarly, there was little effect of a 24-h exposure of **7a,b** on L1210 leukemia cell ribonucleotide pools. Two concentrations (10^{-5} and 10^{-4} M) of **7a,b** were evaluated. Growth of cells was reduced during that period to 87% and 54% at 10^{-5} and 10^{-4} M, respectively, while the cellular content of ribonucleotides was relatively unchanged on a per cell or normalized basis (data not shown). A small dose-dependent decrease was noted for UTP, GDP, and ADP; however, these differences were not statistically significant.

Therapeutic Activity of Nucleoside Analogues in Mice with Tumors. DBA/2 female mice (20 g) inoculated ip with 1×10^6 L1210 leukemia cells on day 0 were weighed and inspected for tumor growth and survival daily. Drug (**7a,b**), dissolved in 0.5% Tween 80 in saline (0.2 mL), was injected ip daily on days 1–5. Dosages varied from 1 to 800 mg/kg per day (mkd). The control mice, injected with a 0.2-mL suspension of 0.5% Tween 80 in saline, died from tumor on day 7 (Table VI). Maximal therapeutic efficacy (55% ILS) for **7a,b** using a 5-day ip injection schedule (1 dose/day on days 1–5) was achieved at 500 mg/kg per day × 5. Dosages above 500 mkd resulted in decreased % ILS and increased host toxicity, as evidenced by increased weight loss and eventual mortality due to drug toxicity at 800 mkd (Table VI).

The therapeutic activity of **8a,b** was compared with that of 5-fluorouracil (5-FU) and arabinocytidine (araC) in DBA/2 mice with L1210 leukemia. By comparison, **8a,b** demonstrated less therapeutic activity than araC. 5-FU at dosages of 50, 100, and 200 mkd × 5 was extremely toxic to mice, causing severe weight loss (to 13–14 g). Dosages of 5 or 25 mkd × 3, administered on days 1, 3, and 6, were more effective, increasing the life span of leukemic mice to 41% and 88%, respectively. AraC administered ip on a schedule of 50 or 100 mg/kg × 5 days was most effective, causing greater than a doubling of life span (104% and 126% ILS, respectively).

The therapeutic activity of **7a,b** also was evaluated in DBA/2 female mice inoculated ip with either 10^6 L1210 leukemia cells resistant to araC (L1210/araC) or resistant to 5-FU (L1210/5-FU). By comparison, **7a,b** (500 mg/kg × 5) displayed a significant (57% ILS) increase in the life span of mice with L1210/araC leukemia, while araC administration demonstrated no therapeutic effect. Similarly, **7a,b** at dosages of 100 and 200 mkd × 5 demonstrated small (22% and 11% ILS) therapeutic effects in mice with L1210/5-FU leukemia while 5-FU administration (25 mkd × 3) had no effect.

The therapeutic activity of **7a,b** was evaluated in C57/BL6 female mice inoculated sc with either Lewis lung carcinoma or M5076 sarcoma. Dosages of 400 mg/kg per day administered ip once daily for 5 days on days 3, 4, 5, 6, and 7 to mice inoculated sc with Lewis lung carcinoma resulted in a 40% ILS and a statistically significant de-

crease in tumor size as noted by an increase in time to reach half-maximal tumor size by 4 days (from day 19 to 23). Similarly, **7a,b** administered to mice with M5076 tumors caused a small ILS of 14% and a statistically significant decrease in tumor size; half-maximal tumor size increased from day 32 to 38.

Antiviral Activity. None of the 2′-fluoromethyl glycoside nucleosides demonstrated any appreciable activity against vesicular stomatitus virus, Coxsackie virus B4, or polio virus-1 in HeLa cells. Likewise, no specific antiviral activity was found against various herpes simplex virus type 1 or 2 strains, vaccinia virus, or vesicular stomatitis virus, assayed in primary rabbit kidney cells.

Discussion

Several novel arabinopyranosyl nucleoside derivatives, having the heterocyclic moiety and fluorine at the 2′-position of the sugar ring, inhibited tumor cell growth in vitro at concentrations between 10^{-5} and 10^{-4} M. The α-5-FU derivative **8a** appeared to be the most active compound, demonstrating the growth-inhibitory activity against a large number of different tumor cell lines, including the murine leukemia deoxythymidine kinase deficient L1210/BdUrd cell line. The uracil derivative **7a,b** demonstrated slightly less growth- and colony-inhibitory activity although it would appear that these two agents, **7a,b** and **8a,b**, share a rather similar mechanism of action, which is not dependent on deoxythymidine kinase activity.

The thymine derivative **9a,b** was relatively inactive against murine L1210 leukemia cells ($IC_{50} > 10^{-4}$ M, Table V) but demonstrated tumor cell growth inhibition against the L1210/BdUrd leukemia line and other tumor cell lines including FM3A/0, Raji/0, and Molt/4F. The appearance of growth-inhibitory activity for **9a,b**, the thymine derivative, against the L1210/BdUrd line again suggests a mechanism of drug action for this class of growth-inhibitory agents not dependent on kinase activity. Exchanging the *O*-acetyl groups with *O*-hexanoyl (**10a,b**) resulted in altered antitumor activity; however, blocking the 3′- and 4′-positions on the sugar with the isopropylidine group (**4**) resulted in a complete loss of activity.

The novel nucleoside derivatives (**7a,b**, **8a,b**, **10a,b**) containing esterified substituents on the sugar moiety are lipophilic compounds. This lipophilicity probably allows these compounds to freely enter tumor cells by passive diffusion. A study of the time dependence of growth inhibition demonstrated that **7a,b** was growth inhibitory following a relatively short incubation period (less than 1 h). This is in contrast to the bioactivity of 5-FdUrd, which is a highly potent agent ($IC_{50} < 10^{-9}$ M) when incubated with L1210 cells for 48 h, but requires long exposure times (>6 h) to demonstrate any growth-inhibitory effect on L1210 leukemia cells.

There were other apparent differences noted in the mechanism of drug action between the 2′-fluoro nucleoside derivatives and 5-FdUrd. Inhibition of precursor incorporation by **8a,b** and **7a,b** was not similar to that by 5-FdUrd. No differential sensitivity to **7a,b** was observed between log-phase and plateau-phase L1210 cells while exponential-phase cells exhibited greater sensitivity to 5-FdUrd than plateau phase cells. Additionally, a G_2 L1210 cell cycle block was observed with **7a,b** while 5-FdUrd caused an S-phase block. Exogenous thymidine reversed 5-FdUrd while it had little effect on **7a,b**. The mechanism of action for these novel nucleosides is unknown at present and is under current investigation. It is of interest that G_2 block is characteristic for DNA damaging agents which have similar cytotoxicity for exponential- and plateau-phase cultures.[25-27] G_2 arrest in the cell cycle reflects DNA and chromosome damage by drugs.[28]

Antitumor activity for **7a,b** in mice with tumors was modest. Administration of 100–500 mg/kg per day × 5 to mice with various tumors resulted in decreased tumor sizes and increased mouse life spans. These results suggest that these agents may have therapeutic potential as antitumor agents.

Experimental Section

Thin-layer chromatography was performed on EM Science silica gel 60. The column chromatography was carried out with silica gel (230–400 mesh) from E. Merck Industries Co. All melting points were taken on a Mel-Temp capillary point block and are not corrected. The optical rotations were measured on a Perkin-Elmer polarimeter 241 MC. The nuclear magnetic resonance spectra were determined on a Varian XL100 and Bruker (200 MHz) spectrometers using tetramethylsilane (TMS) or $CFCl_3$ as internal standards. Mass spectral fragmentation was carried out by a Finnigan 4000 GC–MS system 70 (eV) with intermediate resolution. Elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, TN, and were within ±0.4% of the calculated values.

Methyl 3,4-*O*-Isopropylidene-β-D-*erythro*-pentopyranosid-2-ulose (1). CrO_3 (129.2 g, 1.29 mol) was added in small portions to a stirred mixture of methylene chloride (1.9 L) and pyridine (209.2 mL) followed by the dropwise addition of a solution of methyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside[4,5] (66 g, 0.32 mol) in methylene chloride (500 mL) in a period of 30 min. Acetic anhydride (122.2 mL, 1.29 mol) was added to this mixture and the reaction was completed after stirring at room temperature for 2 h. The mixture was concentrated (~400 mL) and was poured into ethyl acetate (2 L). The slurry mixture was applied to a silica gel column (8 × 60 cm), which was eluted with ethyl acetate. The total effluent (4 L) was collected, evaporated, and dried in vacuo to a crystalline residue (54.7 g, 83.7%), which was recrystallized from ethyl acetate: Ir (KBr) 1740 (C=O), no OH (no hydrate form);[5] ^{1}H NMR ($CDCl_3$) δ 4.68 (1, s, H-1), 4.65 (1, d, $J_{3,4}$ = 5 Hz, H-3), 4.52 (1, dd, $J_{4,3}$ = 5 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 4.23 (1, dd, $J_{5,5'}$ = 12 Hz, $J_{5,4}$ = 1.5 Hz, H-5), 4.04 (1, d, $J_{5',5}$ = 12 Hz, H-5′), 3.49 (3, s, OCH_3), 1.44 and 1.39 (6, 2 s, isopropyl); ^{13}C NMR ($CDCl_3$ + TMS) δ 198.5 (C-2), 110.3 (=CMe_2), 100.8 (C-1), 77.6 (C-3), 75.3 (C-4) 58.3 (C-5), 55.6 (OCH_3), 27.1 and 26.1 (2 CH_3).

Methyl 2-Deoxy-2,2-difluoro-3,4-*O*-isopropylidene-α(β)-D-*erythro*-pentopyranoside (2). To an ice-cold solution of methyl 3,4-*O*-isopropylidene-β-D-*erythro*-pentopyranosid-2-ulose (**1**, 45.16 g, 0.22 mol) in dry benzene (400 mL) was added dropwise a solution of (diethylamido)sulfur trifluoride (DAST)[9] (54 g, 0.33 mol) in dry benzene (100 mL) in a period of 30 min. The reaction mixture was stirred at room temperature for 18 h, diluted with ether (150 mL), and filtered through glass wool. Ice/water (200 mL) was slowly added to the cooled filtrate, and the separated organic layer was washed with saturated $NaHCO_3$ solution (200 mL × 3) and water (200 mL × 3) and dried (Na_2SO_4). The organic solvent was removed by evaporation and the residue was distilled to give clear yellowish liquid: bp 104–104.5 °C (0.2 mmHg); yield 38.5 g (78.1%); MS m/z = 209 (M^+ – Me); ^{1}H NMR ($CDCl_3$) δ 5.2 (1, m, H-1), 4.84 (2, m, H-3, H-4), 4.23 (2, m, H-5,5′), 3.64 (d, $J_{F,Me}$ = 1.35 Hz, OCH_3, α anomer), 3.62 (d, $J_{F,Me}$ = 1.31 Hz, OCH_3, β anomer).

Methyl 3,4-Di-*O*-acetyl-2-deoxy-2,2-difluoro-α(β)-D-*erythro*-pentopyranoside (5). Compound **2** (4 mL, 0.021 mol) was shaken vigorously with 15 mL of 95% formic acid at room temperature (22 °C) for 8.5 min. The mixture was immediately evaporated to a syrup under reduced pressure (bath temperature <30 °C) and the residue was dried in vacuo (0.2 mmHg) for 10

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min. The residue was evaporated with ethanol (10 mL × 2) and toluene (10 mL × 2) and dried in vacuo for 15 min. The residual hydrolyzed sugar was treated with pyridine (30 mL) and acetic anhydride (15 mL) at room temperature for 16 h. The mixture was treated with ice/water (30 mL) and evaporated to a syrup, which was coevaporated with toluene (10 mL × 3) and dried in vacuo to give a clear yellow syrup, which was used without further purification.

Methyl 3,4-Di-*O*-acetyl-2-deoxy-2(*R*)-(1,3-dihydro-2,4-dioxo-1-pyrimidinyl)-2-fluoro-α(β)-D-arabinopyranoside (7a,b). Method A. Dry HBr was introduced slowly for 10 min to a cooled (~0 °C) solution of compound **5** (~0.021 mol in dry methylene chloride (30 mL)). The reaction mixture was stirred at 0 °C for 20 min and at room temperature for 15 min, evaporated under reduced pressure (bath temperature <30 °C), and dried in vacuo for 20 min to give a dark brownish syrup, methyl 3,4-di-*O*-acetyl-2-bromo-2-fluoro-2-deoxy-D-arabinopyranoside (**6**), which was used without further purification in the next step.

To an ice-cold solution of trimethylsilylated uracil (0.054 mol) in dry methylene chloride (80 mL) was added dropwise a solution of the freshly prepared compound **6** (~0.021 mol) in dry methylene chloride (80 mL) in a period of 20 min followed by addition of dry HgO (4.8 g, 0.022 mol) and $HgBr_2$ (1.6 g, 0.0044 mol). The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 16 h. The mixture was diluted with methylene chloride (250 mL) and filtered through a Celite (300 g) bed. The filtrate was washed with a 15% KI solution (150 mL × 2), 2% acetic acid (150 mL × 2), and water (150 mL × 2), dried (Na_2SO_4), and filtered. The filtrate was evaporated under reduced pressure (bath temperature <30 °C) to a foamy material, which was dissolved in a minimum amount of an ethyl acetate/toluene (2:1) mixture and applied to a silica gel column (2.5 × 80 cm). The column was eluted with ethyl acetate/toluene (2:1). The appropriate fractions were pooled and evaporated to a white foamy residue: yield 1.7 g (22.5%); R_f = 0.31 (silica gel, ethyl acetate/toluene (2:1)); MS m/z = 360 (M^+).

Method B. To an ice-cold solution of trimethylsilylated uracil (0.027 mol) in dry methylene chloride (20 mL) were added a solution of methyl 3,4-di-*O*-acetyl-2-deoxy-2,2-difluoro-α(β)-D-*erythro*-pentopyranoside (**5**) (0.011 mol) in dry methylene chloride (20 mL) and molecular sieves (3A, 8–12 mesh, 3 g) followed by a solution of $BF_3 \cdot Et_2O$ (0.68 mL) in methylene chloride (10 mL). The mixture was stirred at 0 °C for 3 h and at room temperature for 16 h. A solution of $BF_3 \cdot Et_2O$ (0.5 mL) in dry methylene chloride (5 mL) was added to the mixture and the mixture was stirred at room temperature for 16 h. The mixture was diluted with methylene chloride (150 mL) and filtered through a Celite (300 g) bed. The filtrate was washed with a saturated $NaHCO_3$ solution (100 mL × 2) and water (150 mL × 3), dried (Na_2SO_4), and filtered. The filtrate was evaporated to a foamy material, which was purified by column chromatography as described in method A to give a white foamy solid: yield 456 mg (11.5%) (a mixture of α and β isomers) and the starting material (**5**) (750 mg, 25.4%).

Separation of the α and β Isomers (7a and 7b). The mixture of **7a** and **7b** (175 mg) was dissolved in a minimum amount of chloroform 2-propanol (20:1) and applied to a dry silica gel column (1.2 × 70 cm, 230–400 mesh). The column was eluted with chloroform 2-propanol (20:1). The appropriate fractions (α isomer, R_f = 0.32; β isomer, R_f = 0.39; silica gel; chloroform 2-propanol (20:1)) were collected, evaporated, and dried in vacuo to give a foamy solid: α isomer, 60%; β isomer, 30%. Compounds **8a,b–9a,b** were prepared similarly by using method A or B.

Methyl 3,4-Di-*O*-acetyl-2-deoxy-2(*R*)-(1,3-dihydro-2,4-dioxo-5-fluoro-1-pyrimidinyl)-2-fluoro-α-D-arabinopyranoside (8a). Methyl 3,4-Di-*O*-acetyl-2-deoxy-2(*R*)-(1,3-dihydro-2,4-dioxo-5-fluoro-1-pyrimidinyl)-2-fluoro-β-D-arabinopyranoside (8b). Methyl 3,4-Di-*O*-acetyl-2-deoxy-2(*R*)-(1,3-dihydro-2,4-dioxo-5-methyl-1-pyrimidinyl)-2-fluoro-α-D-arabinopyranoside (9a). Methyl 3,4-Di-*O*-acetyl-2-deoxy-2(*R*)-(1,3-dihydro-2,4-dioxo-5-methyl-1-pyrimidinyl)-2-fluoro-β-D-arabinopyranoside (9b).

Methyl 2-Deoxy-2(*R*)-(1,3-dihydro-2,4-dioxo-1-pyrimidinyl)-2-fluoro-3,4-di-*O*-hexanoyl-α(β)-D-arabinopyranoside (10a + 10b). To an ice-cold solution of methyl 2-bromo-2-fluoro-3,4-di-*O*-hexanoyl-D-arabinopyranoside (**6**, R' = hexanoyl, 0.009 mol) in methylene chloride (80 mL), prepared similarly to **6** (R' = acetyl) except hexanoyl chloride was used in place of acetic anhydride, was added a solution of trimethylsilylated uracil (0.054 mol) in methylene chloride (80 mL) followed by dry HgO (4.8 g) and $HgBr_2$ (1.6 g). The mixture was stirred at 0 °C for 2 h and at room temperature for 16 h and was then worked up according to method A. The residue was purified by silica gel column chromatography (230–400 mesh, 2.5 × 75 cm), eluting with ethyl acetate/toluene (1:1). The appropriate fractions were pooled, evaporated to a syrup, and dried in vacuo: yield 1.23 g (29.0%) of a mixture of α/β isomers, 2:1 ratio; R_f = 0.53 (silica gel, ethyl acetate/toluene (2:1)); m/z = 472 (M^+). The mixture was separated by silica gel column chromatography (2.5 × 86 cm, 230–400 mesh). The column was eluted with a methylene chloride/ethyl acetate (3:1) mixture. α isomer: R_f = 0.24. β isomer: R_f = 0.34 (silica gel, methylene chloride/ethyl acetate 3:1).

Methyl 2-Deoxy-3,4-*O*-isopropylidene-2(*R*)-(1,3-dihydro-2,4-dioxo-1-pyrimidinyl)-2-fluoro-α-D-arabinopyranoside (4). To an ice-cold mixture of **3** (0.0045 mol) (prepared from **2** similarly to **6**, except dry HCl was used in place of HBr) and trimethylsilylated uracil (0.009 mol) was added dropwise a solution of $SnCl_4$ (0.52 mL) in 1,2-dichloroethane (10 mL) for a period of 30 min. The mixture was stirred at 0 °C for 1.5 h and at room temperature for 16 h. The reaction mixture was diluted with 1,2-dichloroethane (150 mL) and filtered through a Celite (300 g) bed. The filtrate was washed with a saturated $NaHCO_3$ solution (150 mL × 2) and water (150 mL × 3), dried over Na_2SO_4, and filtered. The filtrate was evaporated under reduced pressure (bath <30 °C) to a foamy material. The residue was chromatographed on a column of silica gel (230–400 mesh, 1.5 × 80 cm). The column was eluted with ethyl acetate/toluene (2:1) mixture. The appropriate tubes were pooled and evaporated to a white foamy material: yield 0.332 g (23.3%); R_f = 0.32 (silica gel, ethyl acetate/toluene (2:1) mixture); MS m/z = 316 (M^+).

Hydrolysis of Methyl 3,4-Di-*O*-acetyl-2-deoxy-2(*R*)-(1,3-dihydro-2,4-dioxo-1-pyrimidinyl)-2-fluoro-D-arabinopyranoside (7a,b). A mixture of compounds **7a,b** (20 mg) was dissolved in absolute methanol (5 mL) and treated with methanolic hydrogen chloride saturated at 0 °C (0.5 mL). After stirring at room temperature for 5 h, the reaction mixture was evaporated at 40 °C under reduced pressure and coevaporated with toluene (5 mL × 3) to dryness. The residue was dissolved in the minimum amount of methanol and applied to a prepared TLC (E.M. Science, silica gel $60F_{254}$, 0.25 mm, 10 × 20 cm). The TLC was developed with chloroform 2-propanol (9:1). Three major new bands were detected: bands A, B, and C with R_f values 0.62, 0.48, and 0.36, respectively. The R_f value of the starting material was 0.80.

The appropriate bands A, B, and C were scraped out and extracted with ethyl acetate (10 mL × 2), and the corresponding mixtures were filtered through an ultrafine fritted disk filter (maximum pore size was 0.9–1.4 μm). Each filtrate was evaporated, coevaporated with toluene (5 mL × 3), and dried in vacuo to give a solid. Compound A was discarded. Its highest mass spectrum fragment (284) indicated that hydrolysis in this case was accompanied by other reaction(s) and, therefore, this byproduct was not investigated.

Compound B was a partial hydrolysis product, namely methyl 4-*O*-acetyl-2-deoxy-2(*R*)-(1,3-dihydro-2,4-dioxo-1-pyrimidinyl)-2-fluoro-α-D-arabinopyranoside (**11a**) and its mass fragments were 318 (M^+), 207 (sugar), 155 (uracil-CF=C^+H), and 112 (uracil). Compound C was an isomer of B, methyl 4-*O*-acetyl-2-deoxy-2-(*R*)-(1,3-dihydro-2,4-dioxo-1-pyrimidinyl)-2-fluoro-β-D-arabinopyranoside (**11b**) and its mass fragments were 318 (M^+), 207 (sugar), 155 (uracil-CF=CH), and 112 (uracil).

Hydrolysis of Methyl 2-Deoxy-2(*R*)-(1,3-dihydro-2,4-dioxo-1-pyrimidinyl)-2-fluoro-3,4-di-*O*-hexanoyl-α(β)-D-arabinopyranoside (10a,b). **10a,b** (120 mg) was dissolved in methanol (2 mL) and water (480 mL). This solution was treated with 1 mL of esterase (E 3128 from porcin liver suspension in 3.2 M $(NH_4)_2SO_4$ solution, pH 8.0, Sigma). After stirring at room temperature for 3 days, the reaction mixture was concentrated to about 150 mL at 40 °C under reduced pressure and lyophilized. The residue was dissolved in the minimum amount of ethyl acetate and applied to a silica gel column (230–400 mesh, 1 × 30 cm). The column was eluted with ethyl acetate. The appropriate fractions

were collected and pooled. Fractions 14–16, 2 mL each (R_f = 0.59, silica gel, ethyl acetate), were pooled and evaporated to dryness to give **12b**. Fractions 21–25, 2 mL each (R_f = 0.50 silica gel, ethyl acetate), were pooled and evaporated to dryness to give **12a**. Compounds **12a** and **12b** were further purified by the prepared TLC plates (E.M. Science silica gel $60F_{254}$, 0.25 mm, 10 × 20 cm) with ethyl acetate/dichloromethane (3:1) as the eluant. The appropriate bands were scraped out, extracted with acetone (5 mL × 4), evaporated to dryness, coevaporated with toluene (5 mL × 3), and dried in vacuo.

Tumor Cell Growth and Colony Formation in Vitro. Murine leukemic L1210 cells were maintained in complete medium consisting of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (HIFCS) and 20 mM *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.35 (Grand Island Biological Co., Grand Island, NY). Cell cultures were routinely screened for mycoplasma contamination by using a nucleic acid hybridization assay supplied by Gen-Probe, San Diego, CA. For determinations of cellular growth inhibition, exponential phase cells were diluted to 5 × 10^4 cells/mL and were incubated for 48 h at 37 °C in 2 mL of complete medium containing increasing concentrations of the various nucleoside analogues. Forty-eight hours later, aliquots of cells were removed from these cultures and counted with a Coulter electronic cell counter. Percentage of control growth was calculated and the concentration of agent inhibiting cell growth by 50% (IC_{50}) was obtained graphically.[29]

Growth-inhibitory activity of certain nucleoside analogues were evaluated in the presence of exogenous nucleosides. Both purine ribonucleosides (adenosine and guanosine) and pyrimidine ribonucleosides (uridine, thymidine, and deoxyuridine) were evaluated as potential competitive antagonists of the nucleoside analogues. For comparison, growth inhibition caused by 5-fluorodeoxyuridine (FdUrd) was evaluated in the presence and absence of exogenous thymidine. In addition to the parental murine L1210 leukemia cell line described above, a L1210/BdUrd line deficient in deoxythymidine kinase, an A-121 human ovarian cell line, the colo 205 human colon carcinoma cell line, the FM3A murine mammary-carcinoma cell line, the Raji human B lymphoblast cell line, and the Molt/4F human T lymphoblast cell line also were used for the evaluation of the growth-inhibitory properties of these nucleoside derivatives. Methods for the propagation of these cell lines have been previously described[30,31] and IC_{50} values were obtained in a similar fashion to that outlined above.

Survival of L1210 cells treated for 1 h with drug was determined by measuring colony formation in complete RPMI 1640 medium containing sea plaque agarose (FMC Bioproducts, Rockland, ME) at a final concentration of 0.25% (w/v). Control and drug-treated cells were centrifuged and resuspended in drug-free medium. Equal volumes of cells and cloning medium were mixed, and 1-mL aliquots were plated in 35 × 100 mm tissue culture dishes in triplicate and allowed to gel. Seeded cells were incubated in a humidified, 5% CO_2 incubator at 37 °C for 4 days. Colonies were stained overnight with 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride, 50 μg/mL, and counted on the fifth day.

Ribonucleotide Pool Size Analysis. Ribonucleotide pool size analysis was performed on L1210 leukemia cells, exposed to nucleoside analogue for 48 **h**, following procedures described by Bernacki et al.[32] Approximately 5 × 10^6 cells were washed twice in RPMI 1640, extracted with 6% perchloric acid, and centrifuged at 800*g* for 2 min. The supernatant material was neutralized with 2 N KOH, and the resulting precipitate was removed by centrifugation. An aliquot of the supernatant material was analyzed for ribonucleotides with a Dupont 830 high-pressure liquid chromatographic system equipped with a 254- and 280-nm detector. An ABX column was eluted with a 2.5 mM to 0.5 M phosphate buffer gradient. The elution profile of the cellular extract was compared with ribonucleotide standards and each peak was automatically integrated by a Spectra Physics Autolab Minigrator.

Incorporation of Radiolabeled Precursors. Incorporation of radiolabeled precursors [[methyl-^{3}H]thymidine (sp act. 20 Ci/mmol), 0.3 μCi/mL; [6-^{3}H]uridine (sp act. 38.9 Ci/mmol), 0.3 μCi/mL; [6-^{3}H]deoxyuridine (21.4 Ci/mmol), 0.08 μCi/mL; [4,5-^{3}H]leucine (50 Ci/mmol), 3,3 μCi/mL; or D-[2-^{3}H]mannose (23 Ci/mmol), 6.6 μCi/mL] was monitored in the presence of nucleoside analogues. All radioisotopes were purchased from New England Nuclear, Boston, MA. Radioisotopes were incubated separately in quadruplicate microtiter wells containing 3.2 × 10^4 L1210 cells/mL in 200 μL of complete medium to which various concentrations of the nucleoside analogues were added. Microtiter plates were incubated for 12 h, and cells were collected on filter paper disks with an automated cell harvester. Cells were washed with 10 volumes of 0.9% NaCl solution (saline). Each washed disk was placed in a scintillation vial with 3 mL of Liquiscint-2 scintillation cocktail (National Diagnostics, Somerville, NJ) and the radioactivity measured with a Beckman LSC-100 scintillation counter. Results were expressed as the percentage of control incorporation. On the basis of previous determinations using 10% trichloroacetic acid precipitation, less than 10% of the measured radioactivity in L1210 cells was acid soluble.[33]

Preclinical Therapeutic Evaluation of Nucleoside Analogues in Mice with Tumor. Leukemias. A line of L1210 leukemia (obtained from Dr. A. Ovejera, DCT Tumor Repository, National Cancer Institute-Frederick Cancer Research Facility, Fredrick, MD) has been maintained in DBA/2 female mice by weekly ip transfer of 10^5 cells. Therapeutic trials were performed with 10^6 cell inocula transplanted ip on day 0 to female DBA/2 mice (19–20 g) obtained from Jackson Laboratories, Bar Harbor, ME. Drugs were routinely administered ip starting on day 1 for 5 consecutive days. Survival of mice was monitored daily. Median and mean survival, percentage of increase in life span (% ILS), and a statistical comparison of drug-treated versus untreated groups were performed. Additionally, lines of L1210 leukemia resistant to arabinocytidine (L1210/AraC) and 5-fluorouracil (L1210/5-FU), obtained from the National Cancer Institute, were maintained in DBA/2 female mice in a manner similar to that described above for the parental (L1210) tumor line and used for the therapeutic evaluation of the nucleoside analogues.

Solid Tumors. Female C57BL/6 mice, 7–10 weeks old, weighing 19–20 g, were obtained from the West Seneca animal facilities of this Institute. Fifty-milligram pieces of nonnecrotic, viable, Lewis lung carcinoma, or M5076 sarcoma, originally provided to this laboratory by the National Cancer Research, DCT Tumor Repository, Frederick Cancer Research Facility, Frederick, MD, were transplanted sc by trochar implant in the abdominal flank of mice. Three days later tumors were palpable and drugs were administered ip once daily for five consecutive days. Tumor growth and animal weight were monitored every 3 days by caliper measurements of two perpendicular diameters, and tumor mass (*M*) was estimated algrebraically by using the equation: $M = 0.4 \times \text{long axis} \times \text{short axis}^2$. Mouse survival was checked daily for at least 60 days. Statistical comparisons were performed with software prepared for an Apple 2e microcomputer.[34]

Antiviral Activity. Antiviral activity measurements were based on the inhibition of viral cytopathogenicity, as described previously.[35]

Acknowledgment. This investigation was supported in part by Grants CA13038 and CA09072 awarded by the

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National Cancer Institute. We thank Dr. O. Frankfurt for performance of flow cytometry.

Registry No. 1, 4096-64-4; α-**2**, 105165-96-6; β-**2**, 105165-97-7; α-**3** (epimer 1), 118716-18-0; α-**3** (epimer 2), 118716-19-1; β-**3** (epimer 1), 118716-20-4; β-**3** (epimer 2), 118716-21-5; **4**, 118716-22-6; α-**5** (R′ = acetyl), 105165-98-8; β-**5** (R′ = acetyl), 105165-99-9; α-**6** (R′ = acetyl, epimer 1), 105166-00-5; α-**6** (R′ = acetyl, epimer 2), 118716-10-2; α-**6** (R′ = hexanoyl, epimer 1), 118716-12-4; α-**6** (R′ = hexanoyl, epimer 2), 118716-13-5; β-**6** (R′ = acetyl, epimer 1), 105182-60-3; β-**6** (R′ = acetyl, epimer 2), 118716-11-3; β-**6** (R′ = hexanoyl, epimer 1), 118716-14-6; β-**6** (R′ = hexanoyl, epimer 2), 118716-15-7; **7a**, 105166-02-7; **7b**, 105166-01-6; **8a**, 105166-08-3; **8b**, 105166-06-1; **9a**, 105166-07-2; **9b**, 105166-05-0; **10a**, 118716-16-8; **10b**, 118716-17-9; **11a**, 118716-23-7; **11b**, 118716-24-8; **12a**, 118722-47-7; **12b**, 118716-25-9; methyl 3,4-O-isopropylidene-β-D-arabinopyranoside, 4594-60-9; bis(trimethylsilyl)uracil, 3442-82-8.

Hydroxyacetophenone-Derived Antagonists of the Peptidoleukotrienes

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Received February 8, 1988

Considerations of the possible similarities between leukotriene D_4 and its prototypical antagonist, FPL 55712, led to the development of a new series of leukotriene antagonists incorporating a hydroxyacetophenone group (e.g., the toluic acids **16** and **18**). Although considerable attention has focused on FPL 55712-derived analogues, only limited investigations into alternatives for the standard 4-acetyl-3-hydroxy-2-propylphenoxy moiety have been reported. Therefore, an extensive study of modifications to the hydroxyacetophenone portion of toluic acid **18** was undertaken. Although no viable alternative to the 3-hydroxy moiety was discovered, replacements for the 2-propyl group (**34**, **37**) and the 4-acetyl functionality (**56**, **59**) yielded potent antagonists. A number of compounds exhibited longer duration of action in vivo than FPL 55712.

Ever since the recognition of slow reacting substance of anaphylaxis (SRS-A) as a potent constrictor of smooth muscle,[1] it has been the focus of considerable interest. Two key developments set the stage for further elucidation of the role of this potential pathophysiological agent—the discovery of FPL 55712,[2] a relatively selective receptor antagonist for SRS-A, and the structural determination that SRS-A was comprised of products from the lipoxygenase pathway of the arachadonic acid cascade, the peptidyl leukotrienes C, D, and E (LTC_4, LTD_4, and LTE_4).[3] Evidence has now accumulated that the leukotrienes are key mediators of immediate hypersensitivity reactions.[4] In particular, their abilities to constrict airway smooth muscle,[5] enhance mucoid secretions,[6] possibly retard mucociliary clearance,[7] and increase vascular permeability[8] have led to the presumption that the leukotrienes play a major role in the etiology of asthma. Indeed, the administration of leukotrienes to human volunteers has evoked asthmatic symptoms.[9] The identification of discrete populations of leukotriene receptors in human lung tissue[10a,c] has generated intense interest in the development of leukotriene antagonists as potential therapeutic agents for asthma.[11] Although Fisons' prototypic antagonist FPL 55712 has done much to shape these endeavors, its poor bioavailability and short half-life[12] have relegated it to the role of an important pharmacological tool.

Biological Evaluation of Leukotriene Antagonists

Biological activity of the leukotriene antagonists presented here was initially determined by their ability to inhibit LTD_4-induced contractions of guinea pig tracheal strips. In the later stages of the work LTE_4 was favored as the agonist because of its demonstrated selectivity for one of the two LTD_4 receptors in the guinea pig trachea[10b] and the apparent pharmacological similarity of this receptor to the single human airway peptido-leukotriene receptor.[10a] A comparison of the two agonists demonstrated that results from inhibition assays were essentially identical for this series of compounds. Details of the assay are presented in the Experimental Section.

Compounds of special interest were subject to additional in vitro evaluation. First, selectivity for antagonism of the leukotriene-induced contraction was determined by comparison with the concentration required to inhibit contractions induced by the nonspecific agonist, barium

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