Synthesis and Interaction with Uridine Phosphorylase of 5′-Deoxy-4′,5-difluorouridine, a New Prodrug of 5-Fluorouracil

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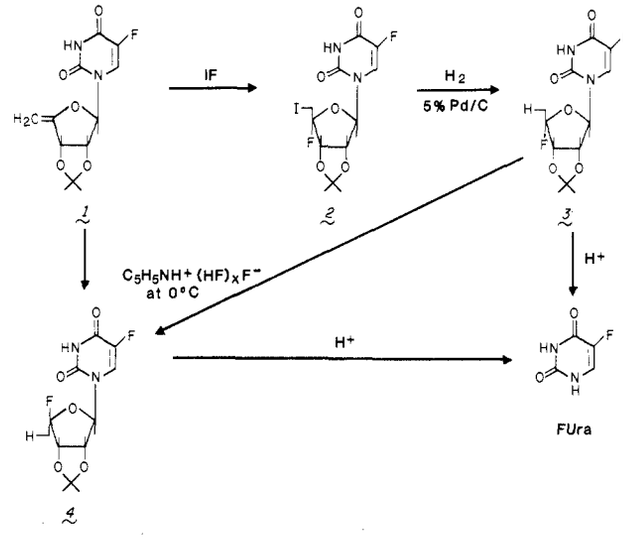
5′-Deoxy-4′,5-difluorouridine (4′-F-5′-dFUrd) (**10**) has been synthesized on the basis of the rationale that the labilization of the glycosidic linkage caused by the 4′-fluoro substituent might allow this compound to be a better prodrug form of the anticancer drug 5-fluorouracil (FUra) than is the widely studied fluoropyrimidine 5′-deoxy-5-fluorouridine (5′-dFUrd). The rate of solvolytic hydrolysis of the glycosidic linkage of 4′-F-5′-dFUrd at pH 1 was about 500-fold faster than that of 5′-dFUrd. Since uridine phosphorylase is thought to be the enzyme that causes degradation of 5′-dFUrd in vivo to generate FUra, we compared the substrate interactions of 5′-dFUrd and 4′-F-5′-dUrd with this enzyme. The V_{max} for hydrolysis of 4′-F-5′-dFUrd to FUra by uridine phosphorylase was about 5-fold greater than that of 5′-dFUrd, whereas the K_m value of 4′-F-5′-dFUrd was 10-fold lower. The combination of these two factors results in 4′-F-5′-dFUrd having a 50-fold higher value of V/K than does 5′-dFUrd. Against L1210 cells in culture, the IC_{50} value for growth inhibition by 4′-F-5′-dFUrd was 3×10^{-7} compared to 3×10^{-6} for 5′-dFUrd.

The most recent fluoropyrimidine to elicit substantial interest among investigators in cancer research is 5′-deoxy-5-fluorouridine (5′-dFUrd). This compound has antitumor activity superior to that of the parent drug 5-fluorouracil (FUra) in a variety of model tumor systems, apparently because of a greater specific toxicity for the tumor compared to potentially susceptible normal host tissues.[1,2] 5′-dFUrd itself is nontoxic because it lacks the 5′-hydroxyl group necessary for its conversion to nucleotides; its cytotoxicity depends on the release of FUra as a result of glycosidic bond cleavage by the enzyme uridine (Urd) phosphorylase.[3-6] A substantial portion of the enhanced tumor-specific toxicity of 5′-dFUrd can be explained on the basis that phosphorylase activity has been found to be considerably higher in tumor tissues than in surrounding normal tissues.[7-9] 5′-dFUrd, however, is a relatively poor substrate for Urd phosphorylase,[10] which is probably reflected in the high doses of this compound that are necessary to obtain a therapeutic effect.[2,5,11]

In a previous study we synthesized a series of 5′-substituted derivatives of 5-fluorouridine in an effort to obtain better substrates for Urd phosphorylase so that we could determine whether an enhanced rate of generation of FUra in tumor tissues would result in improved chemotherapeutic activity.[12] Some of these compounds had slightly better cytotoxic activity than did 5′-dFUrd when compared on a molar basis,[12] but this increase was accounted for by lower K_m values for interaction with Urd phosphorylase. None of the 5′ derivatives of FUrd that we synthesized had V_{max} values for glycosidic bond cleavage appreciably different from that of 5′-dFUrd.[13] Therefore, at saturating levels of these compounds, their rates of degradation to FUra would be similar, and thus their maximal antitumor activities would also be expected to be similar. More desirable for our purposes was a compound with an increased V_{max} rather than a decreased K_m, since this would provide an increased absolute capacity for generation of FUra from the prodrug.

Scheme I



The studies of Jenkins et al.[14] and Owen et al.[15] on the synthesis of nucleocidin derivatives provided a novel approach toward a prodrug of FUra that might have a high V_{max} for glycosidic bond cleavage by Urd phosphorylase. Nucleocidin is a unique derivative of adenosine bearing a fluorine atom at the 4′-position of the ribose ring. During the course of developing syntheses of nucleocidin and some of its derivatives, these workers discovered that the presence of the fluorine atom at the 4′-position made the glycosidic linkage of the nucleoside unusually acid-labile.[15] We reasoned, therefore, that if glycosidic bond cleavage by Urd phosphorylase involves at some point an acid-catalyzed step, 5′-deoxy-4′,5-difluorouridine (4′-F-5′-dFUrd) might be an exceptionally good substrate for this enzyme and thereby possibly a more rapid source of FUra at locations rich in Urd phosphorylase.

In this paper, we describe the synthesis of 4′-F-5′-dFUrd, present evidence that it is a considerably better substrate for Urd phosphorylase isolated from Lewis lung carcinoma

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Table I. 500-MHz Proton NMR Chemical Shifts (ppm)

compd	solvent[a]	$C_{1'}H$	$C_{2'}H$	$C_{3'}H$	$C_{4'}H$	$C_{5'}H_a$	$C_{5'}H_b$	C_6H	other
2	C	5.64 (s)	5.04 (d)	5.1 (dd)			3.47 (m)	7.27 (d)	1.3 and 1.6 (s, 3, $C(Me)_2$)
3	C	5.57 (br s)	4.98 (d)	4.82 (dd)			1.62 (d)	7.22 (d)	1.35 and 1.56 (s, 3, $C(Me)_2$)
4	C	5.98 (d)	4.91 (d)	4.62 (dd)			1.65 (d)	7.51 (d)	1.25 and 1.45 (s, 3, $C(Me)_2$)
6	C	5.82 (d)	4.61 (m)	4.27 (dd)	4.1 (m)		3.85 (m)	7.73 (d)	7.34 (m, 10, Ph), 4.7 (m, 4, CH_2)
7	C	5.83 (d)	4.68 (m)	4.47 (m)	4.37 (m)		3.65 (m)	7.46 (d)	7.3 (m, 10, Ph), 4.68 (m, 4, CH_2), 3.42 (s, 3, CH_3SO_2)
8	C	6.20 (d)	4.45 (m)	4.70 (m)		4.18 (d)	4.30 (m)	7.0 (d)	7.34 (m, 10, Ph), 4.7 (m, 4, CH_2)
9	C	5.35 (br s)	4.14 (d)	4.07 (dd)			1.60 (d)	6.48 (d)	7.3 (m, 10, Ph), 4.6–4.8 (m, 4, CH_2)
10	A	6.7 (dd)	4.66 (dd)	4.13 (dd)			1.61 (d)	7.62 (d)	

[a] C = $CDCl_3$; A = acetone-d_6

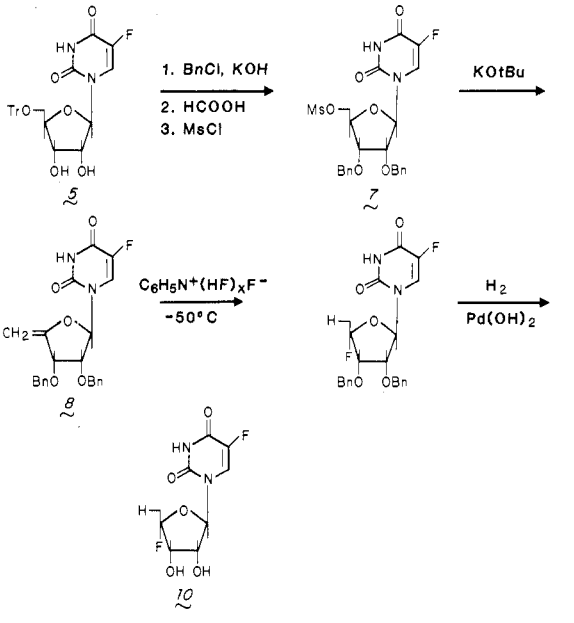
than is 5'-dFUrd, and present preliminary data on its cytotoxic effects on L1210 cells.

Results and Discussion

In our initial attempt to synthesize 4'-F-5'-dFUrd, we followed a synthetic route analogous to that used by Owen et al.[15] in their synthesis of 4'-fluorouridine derivatives, which involved treatment of the 4',5'-unsaturated nucleoside with iodine monofluoride to introduce stereoselectively the fluorine substituent at the 4'-position of the ribofuranose ring (Scheme I). Accordingly, 1-(5-deoxy-2,3-*O*-isopropylidene-D-*erythro*-pent-4-enofuranosyl)-5-fluorouracil (1) was prepared as previously described[16] and treated with iodine monofluoride to give **2** in moderate yield. The structure of **2** was confirmed by the characteristic vicinal, $J_{3',F}$ = 13 Hz, coupling observed in both ^1H and ^{19}F NMR.[15] Catalytic hydrogenation of **2** afforded **3**, which exhibited $J_{5',F}$ = 16.8 Hz, thereby confirming that the fluorine is at the 4'-position. At this point, we also experimented with pyridinium poly(hydrogen fluoride) as a fluorinating agent that would give the desired product **3** in one step and thereby simplify the synthesis. This reagent has been reported to be stable, convenient, and selective for adding the elements of hydrogen fluoride across olefins.[17] When **1** was treated with pyridinium poly(hydrogen fluoride) in tetrahydrofuran at 0 °C, a compound (**4**) was obtained in 90% yield that had an elemental analysis similar to that of **3** but with an appreciably different fluorine–hydrogen coupling in its NMR spectrum. For compound **4**, $J_{3',F}$ was 6.3 Hz compared to $J_{3',F}$ = 13 Hz for compound **3**, and $J_{1',F}$ was 4 Hz whereas **3** exhibited no appreciable coupling of these atoms. From a comparison of these observations with the NMR data presented by Owen et al.[15] for their derivatives, we conclude that **4** is an isomer of **3** with the opposite configuration at the 4'-position (i.e., the *lyxo* configuration). Further experiments showed that as the temperature in the fluorination reaction mixture was lowered, the ratio of the *ribo* isomer **3** to the *lyxo* isomer **4** progressively increased, and if the reaction was carried out at −50 °C, the amount of **3** was about 90% of the total product. The ratios of the two compounds could be easily followed by TLC, since **3** migrated slightly faster than **4** on silica gel plates.

When the isolated *ribo* isomer **3** was placed back into the pyridinium poly(hydrogen fluoride) reagent at 0 °C for a period of time, the compound that was reisolated was the *lyxo* isomer **4** in quantitative yield. This observation, showing that the compounds **3** and **4** can be interconverted, means either that HF was eliminated from the *ribo* isomer and then readded to form the *lyxo* isomer or, more likely, that the compound underwent a ribose ring opening

Scheme II



reaction followed by re-formation of the ribose with the opposite stereochemistry at the 4'-position. If the latter is true, it would suggest that the *ribo* compound **3** is formed first as the kinetically favored product, whereas the *lyxo* is the thermodynamically most stable product.

After successfully isolating compound **3**, we found that the isopropylidene blocking group was unsuitable because of the acid lability of the glycosidic linkage. Although Owen et al.[15] reported that deblocking of isopropylidene-protected 4'-fluorouridine with 90% formic acid gives a 25% yield of the free nucleoside, attempts to deblock either **3** or **4** under a variety of conditions resulted only in the isolation of the pyrimidine base FUra. Since 4'-fluorouridine was also reported to be unstable under basic conditions,[15] the benzyl group appeared to be the best alternative because its removal by hydrogenolysis under neutral conditions would be less likely to disrupt the glycosidic linkage. Thus, 5'-*O*-trityl-5-fluorouridine[18] was converted to the 2',3'-dibenzyloxy derivative **6** by the method of Michelson and Todd,[19] which upon treatment with methanesulfonyl chloride gave the 5'-*O*-methanesulfonyl derivative **7** (Scheme II). Treatment of **7** in dry dioxane with solid potassium *tert*-butoxide at room temperature gave 1-(5-deoxy-2,3-bis(benzyloxy)-β-D-*erythro*-pent-4-enofuranosyl)-5-fluorouracil (**8**) in 67% yield. The reaction of **8** with pyridinium poly(hydrogen fluoride) followed the same pattern as that of **2**. That is, at 0 °C, a 1:1 mixture of products was obtained, which on the basis of the previously established NMR assignments, we

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Table II. First-Order Coupling Constants (Hz)

compd	$J_{1',2'}$	$J_{1',F}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'b}$	$J_{5'a,5'b}$	$J_{5,6}$	other
2	0		7.2	13	*a*	*a*	0	6.7	
3	0		6.4	14	16.8	16.8	*a*	6.6	
4	0	4	6.3	6.3	20	20		6.7	
6	4.5		4.5	1.2				7.2	
7	5.5		5.5	1.3				7.5	
8	5.5		5.5				3	6.7	
9	0		8	18	20	20		6.8	
10	6		4.5	9	19.5	19.5		7.5	2.3 ($J_{1',5}$)

[a] Unresolved.

Table III. First-Order Rates of Hydrolysis of 4'-F-5'dFUrd and 5'-dFUrd to FUra at 37 °C

compd	pH	$t_{1/2}$, h
4'-F-5'-dFUrd[a]	1	0.5
	7.5	12
5'-dFUrd[b]	1	250
	7.5	*c*

[a] Hydrolysis measured spectrophotometrically as A280. [b] Hydrolysis followed by thin-layer chromatography on silica gel plates with $CHCl_3$–MeOH, 5:1, as the elution solvent. [c] No detectable hydrolysis after 7 days.

identified as the β-D-ribo isomer **9** and its α-L-*lyxo* isomer.[20] The isomeric products could be separated by TLC on silica gel with chloroform–acetone, 19:1. However, when the reaction was performed at −50 °C, the β-D-ribo isomer **9** was obtained as the sole product in an overall 80% yield.

Catalytic hydrogenation in the presence of either palladium on carbon or palladium chloride is commonly used to remove benzyl groups,[21] but problems were encountered with both of these reagents. The benzyl groups of **9** were unusually inert to palladium on carbon, whereas hydrogenation of **9** in the presence of palladium chloride at atmospheric pressure for 30 min led to the formation of two products, neither of which was 4'-F-5'-dFUrd. One of these products was identified as FUra. A ^{19}F NMR spectrum of the other product showed an absence of the $C_{4'}$ fluorine atom. An examination of the ^1H NMR spectrum led us to identify this compound tentatively as the 5'-deoxy-4'-methoxy-5-fluorouridine. Recently, the cleavage of *O*-benzyl ethers has been accomplished by the use of neutral, nonpyrophoric 20% palladium hydroxide on carbon (Pearlman's catalyst).[22] Hydrogenation of **9** with Pearlman's catalyst in dry dioxane at 40 psi for 10 h at room temperature gave 4'-F-5'-dFUrd in 20–30% yield as an amorphous powder that we were unable to crystallize.

It is interesting to note from a comparison of the NMR spectra that the coupling constant $J_{4',5'}$ did not change when the benzyl-protected nucleoside **9** was deblocked to give 4'-F-5'-dFUrd, whereas the $J_{3',4'}$ and $J_{2',3'}$ values decreased considerably (Table II). The previously observed dependence of the $J_{HH'}$ (vicinal) and J_{HF} (vicinal) on molecular geometry suggests that the geometrical relationship of the fluorine to the $C_{2'}$ and $C_{3'}$ protons is considerably different in the protected and the unprotected nucleosides.

Kinetics of Hydrolysis. The first-order rates of hydrolysis of 4'-F-5'-dFUrd and 5'-dFUrd to FUra are given in Table III. These data show that the glycosidic linkage of 4'-F-5'-dFUrd is hydrolyzed about 500-fold more rapidly than that of 5'-dFURd under acidic conditions. However, at neutral pH the 4'-fluoro analogue is reasonably stable and so would not be expected to decompose before reaching the target tissue if it were used to treat tumors in vivo.

Table IV. Kinetic Parameters for Interaction with Urd Phosphorylase[a]

compd	K_m, mM	relative V_{max}
4'-F-5'-dFUrd	0.6 ± 0.2	5 ± 1
5'-dFUrd	6.0 ± 2.5	1 ± 0.2

[a] Obtained from double-reciprocal plots of velocity versus substrate concentration (not shown). The values are an average of two determinations.

Table V. Inhibition of Growth of L1210 Mouse Leukemia Cells in Culture by 4'-F-5'-dFUrd and 5'-dFUrd[a]

compd	IC_{50},[b] M
5'-dFUrd	$3 \pm 0.5 \times 10^{-6}$
4'-F-5'-dFUrd	$3 \pm 1 \times 10^{-7}$
FUra	$3 \pm 1 \times 10^{-7}$

[a] Aliquots of 10^5 mycoplasma-free L1210 cells were inoculated into flasks containing RPMI 1640 medium supplemented with 10% fetal calf serum and the compounds to be tested to give a final volume of 2.0 mL. The cells were allowed to grow for 48 h and then were counted. [b] The concentration of drug required to inhibit cell growth by 50%. The IC_{50} values are an average of two determinations.

Interaction with Urd Phosphorylase. The kinetic parameters for the interaction of 5'-dFUrd and 4'-F-5'-dFUrd with Urd phosphorylase isolated from Lewis lung carcinoma are shown in Table IV. In contrast to the large ratio of hydrolytic rates in solution, the V_{max} for hydrolysis of 4'-F-5'-dFUrd to FUra was only 5-fold greater than that of 5'-dFUrd. On the other hand, the K_m value of 4'-F-5'-dFUrd was expected to be about the same as that of 5'-dFUrd but turned out to be 10-fold smaller. Thus, the V_{max}/K_m ratio, which at lower than saturating substrate levels can be considered a more valid indicator of "substrate ability" than either K_m or V_{max} separately, is 50-fold greater for 4'-F-5'-dFUrd than for 5'-dFUrd.

In Vitro Toxicity. The data in Table V show that the growth inhibitory effect of 4'-F-5'-dFUrd on L1210 cells in culture is about 10-fold greater than that of 5'-dFUrd and identical with that of FUra. This result is consistent with the above data showing that 4'-F-5'-dFUrd is a more rapid source of FUra than is 5'-dFURd.

Experimental Section

General Procedures. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was done with 0.2 mm thickness silica gel F plates obtained from E. Merck. The preparative separations were carried out on Analtech 1- or 2-mm (20 × 20) silica gel F glass plates. Altex high-performance liquid chromatography (HPLC) equipped with Hewlett-Packard 3380A integrator was used to check the purity of the samples. UV spectra were obtained with a Beckman 25 spectrophotometer, NMR spectra were obtained with a Bruker WM-500 spectrophotometer,

(20) A ^1H NMR spectrum of the putative *lyxo* isomer of **9** in $CDCl_3$ showed δ 7.17 (d, 1, $J_{5,6}$ = 6.8 Hz, CHCF), 6.44 (d, 1, $J_{1',2'}$ = 1 Hz, $J_{1',F}$ = 6 Hz, $C_{1'}H$), 4.9 (d, 1, $J_{2',3'}$ = 9 Hz, $C_{2'}H$), 4.7 (m, 5, $J_{3',4'}$ = 11 Hz, CH_2 of methylene and $C_{3'}H$), 1.61 (d, 3, $J_{4',5'}$ = 20 Hz, 5'-CH_3); ^{19}F ($CDCl_3$) −90.2 and −167.3 ppm relative to $CFCl_3$.

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and mass spectral data were obtained from with a Hewlett-Packard GC/MS 5985A equipped with a dual EI/CI source at 70 eV. The elemental analyses were performed by Galbraith Laboratory, Inc., Knoxville, TN. Fluorination reactions were conducted in a polyolefin bottles, and plastic pipets were used to measure pyridinium poly(hydrogen fluoride).

5'-Deoxy-4',5-difluoro-5'-iodo-2',3'-isopropylideneuridine (2). To a stirred solution of 1[16] (400 mg, 1.40 mmol) in methylene chloride (40 mL) containing freshly powdered silver fluoride (1 g, 7.88 mmol) was added a solution of iodine (800 mg, 3.15 mmol) in methylene chloride (20 mL) dropwise over 30 min. An aqueous solution containing 5% sodium bicarbonate (10 mL) and 5% sodium thiosulfate (10 mL) was then added. The mixture was shaken vigorously and then filtered through Celite, which was washed with chloroform (25 mL). The aqueous phase was extracted with chloroform (2 × 50 mL), and the combined organic phase was washed successively with 5% sodium bicarbonate, 5% sodium thiosulfate solution, and water, dried over sodium sulfate, and evaporated to dryness. The residue was purified by preparative TLC with chloroform–acetone (19:1) to give 365 mg (60%) of **2** as a homogeneous foam: ^{19}F NMR ($CDCl_3$) −107.1 and −168.8 ppm relative to $CFCl_3$. Anal. ($C_{12}H_{13}F_2IN_2O_5$) C, H, N.

5'-Deoxy-4',5-difluoro-2',3'-isopropylideneuridine (3). A mixture of **2** (250 mg, 0.58 mmol), methanol (15 mL), triethylamine (0.16 mL), and 5% palladium on carbon (150 mg) was treated for 1.5 h with hydrogen at atmospheric pressure. The mixture was then filtered through Celite, and the filtrate was evaporated to dryness. The residue was partitioned between ethyl acetate (25 mL) and water (15 mL). The ethyl acetate layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by preparative TLC with chloroform–methanol (95:5). Elution of the major band gave **3** as a foam: ^{19}F NMR ($CDCl_3$) −106.5 and −167.5 ppm relative to $CFCl_3$. Anal. ($C_{12}H_{14}F_2N_2O_5$) C, H, N.

1-(5-Deoxy-4-fluoro-2,3-isopropylidene-α-L-lyxofuranosyl)-5-fluorouracil (4). A solution of 1[2] (1 g, 3.5 mmol) in dry tetrahydrofuran (5 mL) was added dropwise over 10 min to a 0 °C precooled stirred solution of pyridinium poly(hydrogen fluoride) (4 mL) in a polyolefin bottle, and the mixture was stirred at 0 °C for 1 h. The reaction mixture was quenched with ice–water (15 mL), poured with vigorous stirring into a cold saturated solution of sodium bicarbonate (30 mL), and partitioned between methylene chloride (75 mL). The organic layer was washed with saturated sodium bicarbonate solution and water, dried over sodium sulfate, and evaporated to dryness to give 0.9 g (85%) of a white crystalline product. An analytical sample was crystallized from ethanol: mp 130–132 °C dec; ^{19}F NMR ($CDCl_3$) −93.9 and −169.4 ppm relative to $CFCl_3$. Anal. ($C_{12}H_{14}F_2N_2O_5$) C, H, N.

2',3'-Bis(benzyloxy)-5-fluorouridine (6). The mixture of 5'-*O*-trityl-5-fluorouridine[8] (**5**) (12 g, 23.8 mmol), dry benzene (75 mL), dry dioxane (30 mL), benzyl chloride (17 mL, 0.15 mol), and dry potassium hydroxide (35 g, 0.625 mol) was refluxed for 4.5 h. The solvent was decanted, and the residual potassium hydroxide was washed with benzene. The combined benzene layers were washed with water, dried over sodium sulfate, and evaporated to dryness. The gummy residue was washed with petroleum ether (bp 40–60 °C) and dissolved in 90% formic acid. The stirred solution was refluxed for 15 min and evaporated to dryness. The residue was washed thoroughly with hot hexane and then was applied onto a column of silica gel (500 g). The column was eluted with hexane followed by chloroform–methanol (95:5). The column fractions were evaporated to dryness to give **6** (7.15 g, 68%) as a syrup. Anal. ($C_{23}H_{23}FN_2O_6$) C, H, N.

5'-*O*-Mesyl-2',3'-bis(benzyloxy)-5-fluorouridine (7). A solution of **6** (6 g, 13.54 mmol) in dry pyridine (60 mL) was treated at 0 °C with methanesulfonyl chloride (5 mL) and then stirred for 40 min. The solution was then concentrated and partitioned between cold water (100 mL) and ethyl acetate (150 mL). The aqueous layer was extracted with ethyl acetate (2 × 100 mL), and the combined organic extracts were washed with water, dried over sodium sulfate, and evaporated to dryness to give **7** (6.25 g, 88%) as a foam. Anal. ($C_{24}H_{25}FN_2O_8S$) C, H, N, F.

1-(5-Deoxy-2,3-bis(benzyloxy)-β-D-*erythro*-pent-4-enofuranosyl)-5-fluorouracil (8). To a stirred solution of **7** (4 g, 7.67 mmol) in dry dioxane (100 mL) was added solid potassium *tert*-butoxide (1.1 g, 9.8 mmol) over 30 min. To the reaction mixture was added 5% aqueous sodium dihydrogen phosphate (50 mL), and the mixture was then partitioned between water (50 mL) and ethyl acetate (100 mL). The aqueous layer was further extracted with ethyl acetate (4 × 75 mL). The combined organic extracts was dried over sodium sulfate and evaporated to dryness. The residue was applied to a column of silica gel (100 g). The column was eluted with chloroform–methanol (95:5). Fractions containing **8** were collected and evaporated to dryness to give **8** as a foam (2.2 g, 67%). Anal. ($C_{23}H_{21}FN_2O_5$) C, H, N.

5'-Deoxy-4',5-difluoro-2',3'-bis(benzyloxy)uridine (9). A solution of **8** (5 g, 11.8 mmol) in dry tetrahydrofuran (20 mL) was added over a period of 10 min to a −50 °C precooled, stirred solution of pyridinium poly(hydrogen fluoride) (20 mL) in a polyolefin bottle, and the mixture was stirred at −50 °C for 1 h. The reaction was quenched with ice–water (50 mL), and methylene chloride (50 mL) was added. The mixture was poured into a cold, stirred solution of saturated sodium bicarbonate (100 mL) and was partitioned between methylene chloride (100 mL). The organic layer was washed with saturated sodium bicarbonate and water, dried over sodium sulfate, and evaporated to dryness to give 4.5 g (86%) of **9** as a foam: ^{19}F NMR ($CDCl_3$) −111.9 and 169.9 ppm relative to $CFCl_3$. Anal. ($C_{23}H_{22}F_2N_2O_5$) C, H, N.

5'-Deoxy-4',5-difluorouridine (10). A solution of **9** (0.500 mg, 1.22 mmol), dry dioxane (10 mL), and 20% palladium hydroxide on carbon[21] (140 mg) was shaken with hydrogen at 45 psi for 10 h. The mixture was then filtered through Celite, which was washed with dry dioxane, and evaporated to dryness. Chromatography of the residue on preparative TLC with methylene chloride–ethanol (85:15) gave 100 mg (31%) of **10** as an amorphous powder: MS, *m/e* 264.1 (M^+), 245.1 ($M^+ - F$); UV (dioxane) λ_{max} 268–269 nm (ε 8720); ^{19}F NMR (acetone-d_6) −90 and −166 ppm relative to $CFCl_3$. Anal. ($C_9H_{10}F_2N_2O_5$) C, H, N.

Partial Purification of Urd Phosphorylase. A small piece of the Lewis lung carcinoma tumor (about 100 mg) was implanted under the skin of DBA/2 mice with a syringe. The tumor was allowed to grow on the mice until the average diameter was about 3 cm. The tumors were then removed after sacrificing the mice by cervical dislocation. Tumor tissue (30 g wet weight) was placed into 50 mL of Tris buffer, pH 7.6, containing 5 mM 2-mercaptoethanol, cut into small pieces, and further homogenized with a Polytron tissue homogenizer until no discrete pieces were visible. The homogenate was then sonicated with a Braunsonic 1510 sonicator for 5 × 15 s. The sonicate was centrifuged on a Beckman J-21C centrifuge at 45 000 rpm for 45 min. The supernatant (about 40 mL) was removed, and ammonium sulfate added to 33% saturation. The solution was centrifuged at 14 000 rpm for 30 min, and the precipitate was dissolved in 10 mL of 25 mM phosphate buffer, pH 7.2, containing 2 mM dithiothreitol. Part of this solution (2 mL) was placed on a column of Sephadex G-100 (1 × 20 cm) and eluted with the same buffer. Fractions of 5 mL were collected. Urd and dThd phosphorylase activities were both found in fractions 5–10. These fractions were pooled and applied to a column of DEAE-cellulose (5 × 15 cm). The column was washed with 50 mL of 25 mM phosphate, pH 7.2, containing 2 mM dithiothreitol. A linear gradient was then applied consisting of 25–500 mM phosphate, pH 7.2, containing 2 mM 2-mercaptoethanol, with 500 mL in each reservoir, and fractions of 4 mL were collected. Fractions 15–25 contained dThd phosphorylase activity, while Urd phosphorylase activity was found in fractions 20–40. Fractions 30–40 were pooled, dialyzed against the 25 mM phosphate buffer, and concentrated by ultrafiltration to a volume of 10 mL. The Urd phosphorylase activity of this solution was 1.0 μmol/min per mL. Enzyme activity was stable indefinitely when stored at 4 °C.

Nucleoside Phosphorylase Activity. The activities of the nucleoside phosphorylases were assayed by separating the substrates and the product (FUra) by thin-layer chromatography. The reaction mixtures contained the enzyme, arsenate (25 mM), and the nucleoside substrate (0.45–4.5 mM), in 0.2 mL of phosphate buffer, pH 7.2. Aliquots of 20 μL were withdrawn at intervals and spotted on a silica gel plate with FUra as a marker. The plates were eluted with EtOH–CH_2Cl_2, 15:85. The spots corresponding to FUra were scraped off, and the silica gel was washed with water to remove the FUra. The amount of FUra eluted from the plate was estimated by HPLC, with a reverse-

phase column eluted with water–MeOH, 95:5. All velocities were determined from at least four time points that were within the initial reaction rate period.

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Registry No. 1, 71609-59-1; **2**, 113548-90-6; **3**, 113548-91-7; 4, 113548-92-8; **5**, 3871-66-7; **6**, 113548-93-9; **7**, 113548-94-0; **8**, 113548-95-1; **9**, 113548-96-2; **10**, 113548-97-3; FUra, 51-21-8; uridine phosphorylase, 9030-22-2.

Synthesis and Oral Antiallergic Activity of Carboxylic Acids Derived from Imidazo[2,1-*c*][1,4]benzoxazines, Imidazo[1,2-*a*]quinolines, Imidazo[1,2-*a*]quinoxalines, Imidazo[1,2-*a*]quinoxalinones, Pyrrolo[1,2-*a*]quinoxalinones, Pyrrolo[2,3-*a*]quinoxalinones, and Imidazo[2,1-*b*]benzothiazoles

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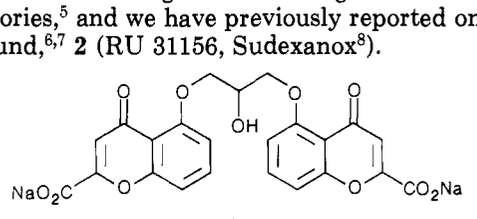
4*H*-Imidazo[2,1-c][1,4]benzoxazine-2-carboxylic acid (**3**) was found to possess potent activity in the IgE-induced rat passive cutaneous anaphylaxis model which may be predictive of clinical antiallergic activity. Compared to disodium cromoglycate (DSCG, **1**), **3** was less active following iv administration but unlike DSCG showed very significant oral activity. To explore the structural requirements for this activity, a range of tricyclic compounds was prepared and their activities were measured. Individual 2-carboxylic acids derived from imidazo[1,2-*a*]quinolines, imidazo[1,2-*a*]quinoxalines, imidazo[1,2-*a*]quinoxalinones, pyrrolo[1,2-*a*]quinoxalinones, pyrrolo[2,3-*a*]quinoxalinones, and imidazo[2,1-*b*]benzothiazoles showed iv activities up to 10^3 times as potent as DSCG and many of them showed significant oral activity. From these, imidazo[1,2-*a*]quinoxaline-2-carboxylic acid **114** has been chosen for further development.

Asthma is a disease of uncertain etiology primarily involving the small bronchi and manifested clinically by intermittent wheezing and dyspnea of varying intensity.[1] Treatment for the condition has involved use of bronchodilators, e.g., β-adrenergic agonists and theophylline and corticosteroids, but a major step forward was made with the introduction of disodium cromoglycate (DSCG, **1**)[2] as a prophylactic agent against the disease. Subsequent clinical trials have shown the efficacy of DSCG in suitable patients,[3,4] but it is not active orally and has to be insufflated as a powder. The discovery of an oral, prophylactic antiasthmatic agent remains a goal of a number of laboratories,[5] and we have previously reported one such compound,[6,7] **2** (RU 31156, Sudexanox[8]).



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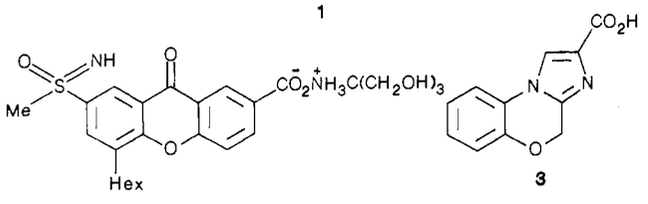

2 **3**

The origin of the work described in this paper was the unexpected rearrangement which gave 4*H*-imidazo[2,1-*c*][1,4]benzoxazines as previously described.[9] 4*H*-Imidazo[2,1-*c*][1,4]benzoxazine-2-carboxylic acid (**3**) was found to possess significant activity in the rat IgE-mediated passive cutaneous anaphylaxis test (ED_{50} = 2.89 mg/kg iv, Table I), a possible but not unequivocally predictive model for clinical efficacy.[5] This result led us to undertake a more systematic examination of structure–activity requirements for PCA activity in related tricyclic heterocyclic systems. Initial approaches retained the imidazole ring of **3** and varied the 4,5-positions to give series **4a–e**, **4h**, **5**, and **6**, and then retaining the quinoxalin-4-(5*H*)-one system, the five-membered ring was varied to give pyrrole **4f**, pyrazole **4g**, and triazole **4i**. Subsequent work produced ring systems **7–10** in order to observe the effects of a wider variety of modifications.

Chemistry

Structures **4a–j** and **5–10** show the variety of ring systems synthesized (Chart I).

(a) Imidazo[2,1-*c*][1,4]benzoxazines (3, 11–39; Table I). The basic method of preparation of this system was described earlier,[9] and the derivatives studied are listed in Table I along with their pharmacological properties.

(b) Imidazo[1,2-*a*]quinolines (46–113; Tables II and III). Treatment of the quinolinium quaternary salt **40** with ammonium acetate in glacial acetic acid heated under reflux was reported[11] to give the imidazolidine **41**, but the

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