Fluorinated Pyrimidines. XXXIII. Synthesis of Methylated 5-Fluoro-2′-deoxyuridine Derivatives[1a]

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Methylation of 5′-O-trityl-5-fluoro-2′-deoxyuridine (I) with Me_3I–Ag_2O in 10% methanolic dioxane gave four different products, 1-(2-deoxy-5-O-trityl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (II), 1-(2-deoxy-3-O-methyl-5-O-trityl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (III), 5′-O-trityl-3-N-methyl-5-fluoro-2′-deoxyuridine (IV), and 5′-O-trityl-3-N,3′-O-dimethyl-5-fluoro-2′-deoxyuridine (V), which were separated by adsorption column chromatography and detritylated to give 1-(2-deoxy-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (VI), 1-(2-deoxy-3-O-methyl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (VII), 3-N-methyl-5-fluoro-2′-deoxyuridine (VIII), and 3-N,3′-O-dimethyl-5-fluoro-2′-deoxyuridine (IX), respectively. Compounds II and IV could not be methylated to the corresponding dimethylated derivatives III and V. Methylation of I with MeI-Ag_2O in MeOH gave a 40:60 ratio of O-methylated (II + III) to N-methylated (IV + V) products, whereas in a less polar solvent (dioxane) the ratio of the products was shifted to 54:46. Compound VI on treatment with dilute aqueous alkali or acid gave 5-fluoro-2′-deoxyuridine (FUDR). On heating with methanolic NH_3 under pressure, VI provided a new synthesis of 5-fluoro-2′-deoxycytidine. By similar treatments, VII yielded the first reported syntheses of 3′-O-methylated 2′-deoxyribonucleosides: 3′-O-methyl-5-fluoro-2′-deoxyuridine (X) and 3′-O-methyl-5-fluoro-2′-deoxycytidine (XIII). Compound X was more resistant to acid hydrolysis than the parent compound FUDR. Compound X was phosphorylated with β-cyanoethyl phosphate and DCI, followed by an alkaline treatment to give 3′-O-methyl-5-fluoro-2′-deoxyuridine 5′-phosphate (XI). 5′-O-Methyl-5-fluoro-2′-deoxyuridine (XVII) was prepared from I *via* 5′-O-trityl-3′-O-acetyl-FUDR (XIV), 3′-O-acetyl-FUDR (XV), and 5′-O-mesyl-3′-O-acetyl-FUDR (XVI). Preparations of ^3H- and ^{14}C-labeled methylated FUDR derivatives are also described.

The synthesis and study of fluorinated pyrimidines and their nucleosides have been a major concern of this laboratory for some time.[2-5] As a result of these researches, 5-fluorouracil (FU)[2b] and 5-fluoro-2′-deoxyuridine (FUDR)[3] have demonstrated considerable clinical utility in the palliation of patients with advanced cancer. Although FUDR is very much more active in biochemical systems than is FU,[2a] it is only slightly more effective in the clinic. As stated previously,[2a] two factors prevent more successful chemotherapeutic efficacy of FUDR: (a) its cleavage to FU by nucleoside phosphorylase,[2a] which prevents the direct formation of 5-fluoro-2′-deoxyuridine 5′-phosphate (FUDRP), the active drug;[6] and (b) the emergence of cellular resistance, which has been shown to result from the loss of thymidine kinase, the enzyme that catalyzes the conversion of FUDR to FUDRP.[7] Both of these enzymes have been a subject of study in this laboratory.

It appeared to us that a systematic study of structure–activity relationships among the various possible methylated derivatives might give some valuable insight into the specific structural requirements for the interaction of substrates and analogs with these two enzymes, and that such insight might be helpful in drug design in this series of compounds. Accordingly, we describe in this paper the synthesis of all the possible methylated derivatives of FUDR.

A survey of the literature revealed that the syntheses of deoxyribonucleosides (both purine and pyrimidine) methylated only in the base moieties have been reported. It was only recently that a synthesis of 5′-O-methylthymidine by the displacement of a 5′-tosylate of thymidine with methanolic NaOMe has been described.[8] No synthesis of a 3′-O-methylated deoxyribonucleoside has been reported.

In our syntheses (Chart I) 5′-O-trityl-5-fluoro-2′-deoxyuridine (I)[9] was treated with MeI in the presence of Ag_2O in 10% MeOH in dioxane at room temperature for 18 hr; under these conditions the starting material (I) was completely methylated. The reaction was separated by adsorption column chromatography to give four major products: two monomethylated derivatives, 1-(2-deoxy-5-O-trityl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (II) and 5′-O-trityl-3-N-methyl-5-fluoro-2′-deoxyuridine (IV), and two dimethylated derivatives, 1-(2-deoxy-3-O-methyl-5-O-trityl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (III) and 5′-O-trityl-3-N,3′-O-dimethyl-5-fluoro-2′-deoxyuridine (V). These compounds were detritylated with ice-cold formic acid to 1-(2-deoxy-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (VI), 3-N-methyl-5-fluoro-2′-deoxyuridine (VIII), 1-(2-deoxy-3-O-methyl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (VII), and 3-N,3′-O-dimethyl-5-fluoro-2′-deoxyuridine (IX), respectively. Compound VII on mild alkaline treatment provided 3′-O-methyl-5-fluoro-2′-deoxyuridine (X). The 3′-O-methylated FUDR derivatives were assigned their structures on the following basis: IV and V had a uv absorption maximum at 270 mμ at pH 2, which did not shift when the pH was changed to 12. Acid detritylation of IV gave VIII as shown by its uv absorption spectrum, nmr spectrum, and chromatographic (tlc systems A–D) comparison

(1) (a) This work was supported in part by Grant CA-07175 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service. (b) American Cancer Society Professor of Oncology.

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CHART I

with an authentic sample.[10] This confirms structure IV. Compound V gave a correct elemental analysis for a trityl dimethylated FUDR derivative; on acid detritylation it gave a product, which on acid hydrolysis, like VIII, gave 3-N-methyl-5-fluorouracil[11] as indicated by the identity of its uv absorption spectrum and tlc comparison with an authentic sample. This product was assigned structure IX because it has a higher R_f in tlc systems A–D than VIII.

Compound II gave a correct elemental analysis for a monomethylated trityl FUDR derivative and, like III, it had a uv absorption maximum at 290 mμ. On acid detritylation II gave 1-(2-deoxy-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (VI). Compound VI had a uv absorption maximum at 285 mμ (unchanged by the addition of acid or alkali) and its nmr spectrum showed a three-proton singlet centered at δ 3.95 corresponding to the methoxy group (the N-Me group of VIII is at δ 3.35).[10] VI on treatment with 30% aqueous formic acid (3 days, room temperature) or with 1 *N* aqueous NaOH (40 min, room temperature) gave FUDR as the only uv-absorbing product, as ascertained by tlc (systems A–D) and uv absorption spectra. On heating with methanolic NH_3 under pressure followed by acid detritylation, II gave 5-fluoro-2′-deoxycytidine (FCDR) in over 80% yield.[12] This, along with elemental analyses, confirms structures II and VI. Compound III on detritylation yielded a product (VII) which gave an analysis for a dimethylated FUDR derivative. The product, like VI, had a uv absorption maximum at 285 mμ (at pH 2 and 12). By analogy with VI, VII on treatment with dilute acid or alkali gave X, which, like FUDR, had a UV absorption maximum at 269 mμ. Likewise on strong acid hydrolysis X gave FU as the only uv-absorbing product, but its nmr spectrum showed a three-proton singlet at δ 3.32, indicating the presence of a methyl group. Since this methyl group could only be at the 3′ position (5′ was blocked during methylation), VII and X must be 1-(2-deoxy-3-O-methyl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone and 3′-O-methyl-5-fluoro-2′-deoxyuridine, respectively. As in the synthesis of FCDR described above, III on treatment with methanolic NH_3 followed by detritylation gave 3′-O-methyl-5-fluoro-2′-deoxycytidine (XIII). This constitutes the first synthesis of a series of 3′-O-methylated deoxyribonucleosides.

It is of interest to note that both II and IV could not be converted to the corresponding dimethylated derivatives III and V, in spite of using more vigorous conditions (heating under reflux) and repeated methylations with MeI in the presence of Ag_2O. The methylation of I is a slow process and takes more than 10 hr for completion; short-term methylation of I (2 hr at room temperature) followed by detritylation of the products gave a uv-absorbing component corresponding to X. This suggests that 3′-O-methyl-5′-O-trityl-5-fluoro-2′-deoxyuridine may be one of the first methylation products and is the precursor of III and V. Thus, in the case of pyrimidine 2′-deoxyribonucleosides, base methylation (N-3 or O-4) seems to decrease the reactivity of the free 3′-hydroxyl group in such a way that it is not susceptible to the methylating agent.[13]

Methylation of I with MeI–Ag_2O in DMF gave IV and V as the only products. Further studies with the same methylating agent using different solvents, as shown in Table I, demonstrated that the percentage of

TABLE I
THE PERCENTAGE COMPOSITION OF VARIOUS PRODUCTS OBTAINED BY THE METHYLATION OF I IN DIFFERENT SOLVENTS

	% composition			
Compd	MeOH	10% MeOH–Me_2CO	10% MeOH–dioxane	DMF
II	19	30	32	
III	21	23	22	
IV	44	27	29	63
V	16	20	17	37

N-3 and O-4 methylation of the base varies and depends upon the polarity of the solvent used. Thus in MeOH we obtained a 40:60 ratio of O-methylated (II and III) to N-methylated (IV and V) products, whereas in 10% MeOH in dioxane a 54:46 ratio was observed. This indicates that probably in a polar solvent the mesomeric anion of FUDR exists preponderantly as the ketonate extreme, whereas in a nonpolar solvent the ratio is shifted in the favor of the enolate form. Such solvent-induced tautomeric shifts have also been observed by Shugar's group in the case of the FU anion.[14]

It has been shown that methylation of the 2′-hydroxyl group of a ribonucleoside increases its stability to acid hydrolysis[15,16] and that the corresponding 5′-phosphates are not substrates for bull semen or snake venom 5′-nucleotidases and several phosphorylases.[17,18]

(10) Compound VIII has been prepared by the action of CH_2N_2 on FUDR: T. A. Khwaja and C. Heidelberger, unpublished results.

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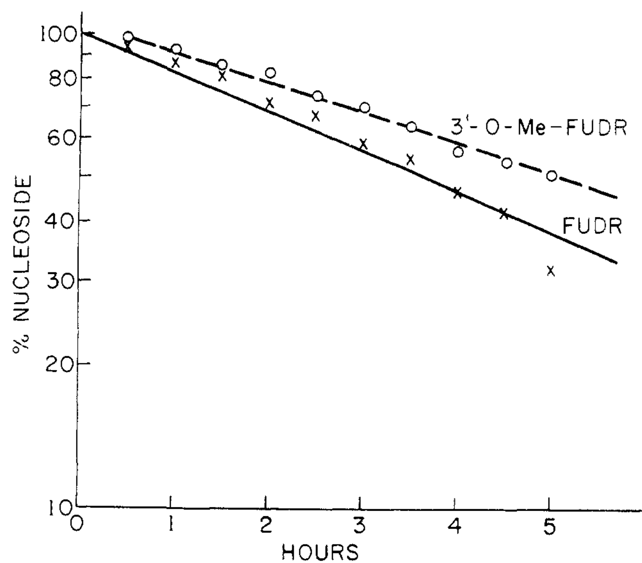

Figure 1.

Figure 1 shows that 3′-O-methyl-5-fluoro-2′-deoxyuridine (X) when heated with 0.1 *N* HCl at 100° had a half-life of 4.8 hr as compared to 3.6 hr for FUDR under similar conditions. This demonstrates that 3′-O-methylation of a 2′-deoxyribonucleoside also strengthens the glycosidic linkage. Compound X was converted into 3′-O-methyl-5-fluoro-2′-deoxyuridine 5′-phosphate (XI) by the method of Tener.[19] However, like FUDRP, XI is a substrate for prostatic phosphomonoesterase. This shows that a free 3′-hydroxyl group of a deoxyribonucleotide is not required for the activity of this enzyme.

In order to prepare 5′-O-methyl-5-fluoro-2′-deoxyuridine (XVII), 3′-O-acetyl-5-fluoro-2′-deoxyuridine (XV) was treated with mesyl chloride in dry pyridine at 0° to give 3′-O-acetyl-5′-O-mesyl-5-fluoro-2′-deoxyuridine (XVI); displacement of the mesyl group with methanolic $NaOCH_3$, with concomitant deacetylation, gave the required compound (XVII) in over 33% yield. The structure of XVII was confirmed by its uv absorption spectrum ($\lambda_{max}^{pH\ 2\ or\ 12}$ 269 mμ), acid hydrolysis to FU, and by the method of synthesis. Its nmr spectrum showed the 5′-methyl group as a three-proton singlet centered at δ 3.35.

It was considered that any biological activity associated with a methylated FUDR derivative might result from its enzymic or metabolic demethylation to the biologically potent parent compound FUDR. In order to investigate this possibility, two series of ^3H- and of ^{14}C-labeled compounds were prepared: 5-fluoro-2′-deoxyuridine-6-^3H derivatives, and those in which FUDR was methylated with $^{14}CH_3I$ by slight modifications of the methods already described. All labeled methylated compounds were obtained with satisfactory specific activities, except 5′-O-methyl-5-fluoro-2′-deoxyuridine-6-^3H, where an appreciable loss of ^3H resulted in the final step of the synthesis. This loss of ^3H from the 6 position is explained by the very recent report of Cushley, *et al.*,[20] on the alkali-induced exchange of the 6-H of various derivatives of FU.

Mixtures of the ^3H- and ^{14}C-labeled compounds can be used as double-labeled substrates in various enzyme studies: loss of ^{14}C activity during an enzyme-catalyzed reaction would indicate the presence of a demethylating enzyme.

Growth Inhibitory Studies. The effects of some of these compounds on the viability of *E. coli* B are shown in Table II. Over the 2 hr studied, FUDR was a powerful killer, whereas its methylated derivatives produced only a minor growth inhibition.

TABLE II
EFFECTS OF METHYLATED NUCLEOSIDES ON THE VIABILITY OF *E. coli* B IN MINIMAL M9 MEDIUM AT 250 μG/ML (VALUES TAKEN AT 2 HR)

Compd	Initial count	Final count	% of control[a]
Control	5.5 × 10^7	2.3 × 10^8	100
FUDR	5.5 × 10^7	1.5 × 10^6	−350
5′-O-Me-FUDR (XVII)	5.5 × 10^7	8.5 × 10^7	30
4-O-Me-FUDR (VI)	5.5 × 10^7	1.3 × 10^8	60
3′-O-Me-FUDR (X)	5.5 × 10^7	1.6 × 10^8	75
4,3′-Di-O-Me-FUDR (VII)	5.5 × 10^7	1.2 × 10^8	54

[a] Calculated from the logarithms of increase (or decrease) in number, as explained in ref 21.

On the other hand, as shown in Table III, some of the methylated derivatives exhibited significant inhibitory activity on the growth of HeLa, L5178Y, and L5178BF cells in culture, according to the conditions described by Umeda and Heidelberger.[21]

However, none of the derivatives was as active as FUDR or FCDR. The N-methyl-FUDR had no activity against HeLa cells, nor did the di-O-methyl compound. It is not clear from these experiments whether the moderate inhibitory effects of the mono-O-methyl derivatives result from demethylation or whether the compounds are intrinsically active.

The interaction of these compounds with enzymes to give structure–activity relationships will be described separately.

Experimental Section

All melting points are corrected (A. H. Thomas capillary apparatus). The uv absorption spectra were run on a Cary spectrophotometer Model 15. The nmr spectra were determined on a Varian A 60 instrument and all were done in D_2O. The analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Tlc was done on plastic plates coated with silica gel (used with systems B and C) or cellulose (used with systems A and D). The following solvent systems were used: A, EtOH–*n*-PrOH–H_2O (4:1:2, v/v); B, MeOH–C_6H_6 (1:3, v/v); C, Me_2CO–cyclohexane (1:1, v/v); D, *i*-PrOH–NH_4OH–H_2O (7:1:2, v/v). The R_f values of the various compounds are given in Table IV.

Methylation of 5′-O-Trityl-5-fluoro-2′-deoxyuridine (I).—Compound I (9.76 g, 20 mmoles) was dissolved in 12% MeOH in dioxane (680 ml), Ag_2O (40 g) and MeI (200 ml) were added, and the stoppered reaction mixture was kept at room temperature (18 hr) with vigorous stirring. Then the reaction mixture was filtered through a pad of Celite (60 ml of hot Me_2CO was used as the wash liquid) and the filtrate and washings were collected and evaporated to a gum *in vacuo*. The gum was dissolved in $CHCl_3$ (5 ml) and adsorbed on a silicic acid (100 mesh, Mallinckrodt) column (5.5 × 30 cm) which was eluted with $CHCl_3$ and 5-ml fractions were collected. Fractions 40–50 containing the first uv absorbing peak were collected and evaporated to a gum. The gum (after standing 6 weeks in a refrigerator) was triturated with EtOH to give V as colorless crystals, which were recrystallized from EtOH; mp 193–194°; uv absorption, λ_{max}^{MeOH} 270 mμ

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TABLE III
EFFECTS OF METHYLATED NUCLEOSIDES ON THE GROWTH OF HeLa, L5178Y, AND L5178BF CELLS IN CULTURE[a]

Compd	% of control					
	10^{-8} *M*	10^{-7} *M*	10^{-6} *M*	10^{-5} *M*	10^{-4} *M*	10^{-3} *M*
		HeLa Cells				
FUDR		50	9	−20	−57	−82
5′-O-Me-FUDR (XVII)		104	93	66	22	13
4-O-Me-FUDR (VI)				27	0	−28
3′-O-Me-FUDR (X)			98	65	10	−6
4,3′-Di-O-Me-FUDR (VII)		100	100	93	45	−5
3-N-Me-FUDR (VIII)				100	100	15
FCDR		26	6	−58	−67	−81
3′-O-Me-FCDR (XIII)		96	74	38	−23	−66
		L5178Y Cells				
FUDR	−42	−56	−59	−59	−59	
5′-O-Me-FUDR (XVII)	61	−2	−21	−28	−32	
4-O-Me-FUDR (VI)	72	11	−59	−89		
3′-O-Me-FUDR (X)	100	53	−12	−19	−35	
4,3′-Di-O-Me-FUDR (VII)	100	100	100	11	−4	
FCDR	−59	−59	−59	−59	−59	
3′-O-Me-FCDR (XIII)	−13	−50	−69			
		L5178BF Cells (Resistant to FUDR)				
FUDR	112	112	38	−27	−42	
5′-O-Me-FUDR (XVII)	102	93	98	96	35	
4-O-Me-FUDR (VI)	100	100	100	100		
3′-O-Me-FUDR (X)	100	100	100	100		
4,3′-Di-O-Me-FUDR (VII)	100	100	100	100		
FCDR	−9	−20				
3′-O-Me-FCDR (XIII)	93	43	−9			

[a] The methods of culture are described in ref 21, and the calculation of the per cent of controls is explained in ref 21.

TABLE IV
TLC OF COMPOUNDS IN FOUR SOLVENT SYSTEMS[a]

	R_f			
	A	B	C	D
FU	0.65	0.28	0.17	0.44
Relative FU	1.00	1.00	1.00	1.00
FUDR	1.14	0.79	0.65	1.07
5′-O-Tr-FUDR (I)		1.65	2.47	
5′-O-Tr-4-O-Me-FUDR (II)		1.90	2.53	
5′-O-Tr-4,3′-di-O-Me-FUDR (III)		2.18	3.47	
5′-O-Tr-3-N-Me-FUDR (IV)		2.12	3.53	
5′-O-Tr-3-N,3′-O-diMe-FUDR (V)		2.36	4.07	
4-O-Me-FUDR (VI)	1.32	1.15	0.70	2.04
4,3′-Di-O-Me-FUDR (VII)	1.38	1.66	1.88	2.14
3-N-Me-FUDR (VIII)	1.32	1.62	1.77	2.04
3-N-Me-3′-O-Me-FUDR (IX)	1.34	1.71	2.82	2.05
3′-O-Me-FUDR (X)	1.25	1.26	1.59	1.36
3′-O-Me-FUDR-5′-phosphate (XI)	0.76	1.30	0.00	0.14
5′-O-Tr-3′-O-Ac-FUDR (XIV)		1.75	2.41	
3′-O-Ac-FUDR (XV)		1.54	2.00	
5′-O-Ms-3′-O-Ac-FUDR (XVI)		1.50	1.76	
5′-O-Me-FUDR (XVII)	1.32	1.40	1.24	1.54
FCDR		0.50	0.00	
3′-O-Me-FCDR (XIII)		0.80	0.00	

[a] The values are expressed as R_f relative to FU.

(ϵ 15,820), λ_{max}^{MeOH} 243.5 mμ (ϵ 7590). *Anal.* ($C_{30}H_{29}FN_2O_5$) C, H, N.

The second peak (50–65) contained mainly II with traces of triphenylcarbinol and V as indicated by tlc (system C). Further elution of the column gave compound IV in fractions 80–140, which were collected and evaporated to a gum. The gum was chilled (6 weeks in freezer) and then triturated with EtOH to give IV as a white powder (λ_{max}^{MeOH} 269 mμ). The silicic acid column was next eluted with 5% MeOH in $CHCl_3$ and a fourth peak (fractions 175–183) containing II was obtained. These fractions were combined and evaporated to a gum, which was triturated with MeOH to give II as a white crystalline solid with traces of IV as an impurity. II was recrystallized from $CHCl_3$–MeOH to give 2.1 g (21%) of colorless crystalline material: mp 186–188°; uv absorption, λ_{max}^{MeOH} 290 mμ (ϵ 7270). *Anal.* ($C_{29}H_{27}FN_2O_5$) C, H, N.

Fractions 50–65, corresponding to the second peak (containing impure III), were collected and evaporated to a gum, which was adsorbed on a Florisil (60–100 mesh, 1200°F Act., Floridin Co., Tallahassee, Fla.) column (5 × 30 cm). The column was eluted with $CHCl_3$, and three uv absorbing peaks were obtained. The first small peak contained traces of triphenylcarbinol, the second peak corresponded to V, whereas the last major peak contained pure III. The fractions of the last peak were combined and evaporated to give III as a pale powder, yield 1.35 g (13%). It was shown to be homogeneous by tlc (systems B and C); uv absorption, λ_{max}^{MeOH} 290 mμ (ϵ 7270). Compound III failed to crystallize and was used as such for further reactions.

1-(2-Deoxy-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (VI).—Compound II (200 mg, 0.4 mmole) was treated with 3 ml of cold formic acid (97+%) for 3 min and then evaporated with an oil pump at room temperature. The residual gum was evaporated twice from dioxane (two 5-ml portions), followed by evaporations from EtOH and Et_2O. Finally, the residue was extracted with 10 ml of warm H_2O, the insoluble triphenylcarbinol was filtered, and the filtrate was evaporated *in vacuo*. The residual gum was dissolved in EtOH (1 ml), dry Et_2O (20 ml) was added, and the product was precipitated with petroleum ether (30–60°, 10 ml) (the gummy precipitate was chilled and scratched to induce crystallization). Recrystallization from the same solvent system gave fine needles of VI: mp 132–134°; uv absorption, $\lambda_{max}^{pH\ 2}$ 285 mμ (ϵ 6210), $\lambda_{min}^{pH\ 2}$ 241 mμ (ϵ 956), $\lambda_{max}^{pH\ 12}$ 285 mμ (ϵ 5880), $\lambda_{min}^{pH\ 12}$ 246 mμ (ϵ 2630). The nmr spectrum showed a three-proton singlet centered at δ 3.95. The anomeric proton was a triplet centered at δ 6.1. VI on treatment with dilute acid or alkali gave FUDR as the sole uv-absorbing product as shown by tlc in systems A–D. *Anal.* ($C_{10}H_{13}FN_2O_5$) C, H, N.

5-Fluoro-2′-deoxycytidine (FCDR).—Compound II (200 mg, 0.4 mmole) was dissolved in methanolic NH_3 (50 ml) and the solution was heated in a pressure bottle on a steam bath for 18 hr. Then the contents of the flask were evaporated and the residual gum was detritylated with cold formic acid (as described above). After removal of the formic acid the residue was extracted with warm H_2O (30 ml) and the triphenylcarbinol was filtered. The filtrate was evaporated and the residual gum was adsorbed (aqueous solution) on a Dowex 1-X8 (OH form) column (2 × 5 cm). The column was eluted with 40% aqueous

MeOH, and FCDR emerged as a sharp single peak. The fractions corresponding to the peak were combined and evaporated to a gum, which was twice evaporated from EtOH (two 5-ml portions) and then dissolved in EtOH (2 ml). Anhydrous Et_2O (20 ml) was added and the precipitated FCDR was left overnight in the cold. It was then filtered to give 79 mg (81%) of FCDR, mp 195–196°. Tlc in systems A–D showed it to be a single uv-absorbing component. Its nmr and ir spectra were superimposable on those obtained from authentic FCDR.[12]

1-(2-Deoxy-3-O-methyl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone (VII).—Compound III (775 mg, 1.5 mmoles) was detritylated with 10 ml of cold formic acid as described earlier. After the removal of the acid the residue was extracted with hot H_2O (25 ml). The precipitated triphenylcarbinol was filtered and the filtrate was evaporated *in vacuo*. The residual gum on tlc (systems A–D) showed only one uv-absorbing component. It was crystallized from EtOH–Et_2O–petroleum ether (30-60°) to give colorless needles: mp 124–126°; yield 290 mg (70.5%); uv absorption, $\lambda_{max}^{pH\ 2}$ 285 mμ (ε 6740), $\lambda_{min}^{pH\ 2}$ 241 mμ (ε 1065), $\lambda_{max}^{pH\ 12}$ 285 mμ (ε 6760), $\lambda_{min}^{pH\ 12}$ 246 mμ (ε 2920). *Anal.* ($C_{11}H_{15}FN_2O_5$) C, H, N.

3′-O-Methyl-5-fluoro-2′-deoxyuridine (X). Method 1.—Compound VII (100 mg) was dissolved in 30% aqueous formic acid (5 ml) and allowed to stand at room temperature for 3 days. Then the solution was evaporated *in vacuo* (bath temperature 30°). Tlc of the residue (system B) showed no uv-absorbing component corresponding to the starting material; X was the major product with traces of 5-fluorouracil. The residue was purified by preparative tlc (silicic acid plates, system B). The product was crystallized from EtOH–Et_2O to give 30 mg (32%) of fine colorless needles. It was recrystallized from H_2O: mp 146°, uv absorption, $\lambda_{max}^{pH\ 2}$ 269 mμ (ε 8880), $\lambda_{min}^{pH\ 2}$ 234.5 mμ (ε 1780), $\lambda_{max}^{pH\ 12}$ 269 mμ (ε 6920), $\lambda_{min}^{pH\ 12}$ 249 mμ (ε 4860). The nmr spectrum showed a three-proton singlet centered at δ 3.32. The anomeric proton was a triplet located at δ 6.12. *Anal.* ($C_{10}H_{13}FN_2O_5$) C, H, N.

Method 2.—Compound VII (100 mg) was dissolved in 1 *N* NaOH (3 ml) and left at room temperature for 40 min. Then the solution was neutalized with Amberlite IR-120 (H^+) and filtered, and the filtrate was evaporated and crystallized from EtOH–Et_2O to give 70 mg (74%) of X. The compound was shown to be identical with that obtained by method 1 by tlc (systems A and D) and by its uv absorption spectrum.

3′-O-Methyl-5-fluoro-2′-deoxycytidine (XIII).—Compound III (330 mg, 0.64 mmole) was dissolved in saturated methanolic NH_3 (60 ml) in a pressure bottle. The solution was heated on a steam bath overnight and evaporated, and the residue was detritylated with cold 98% formic acid (15 ml). After the removal of the acid the residue was extracted with hot H_2O (15 ml), which on tlc (systems B and C) revealed a single uv-absorbing component with a higher R_f than 5-fluorocytosine or FCDR. The solution was evaporated and the residue was dissolved in EtOH (5 ml). Then methanolic HCl (5 ml) was added, followed by dry Et_2O (40 ml). XIII was precipitated as its hydrochloride and filtered to give a pale solid, yield 120 mg (67%). It was recrystallized from MeOH: mp 157.5° dec; uv absorption, $\lambda_{max}^{pH\ 2}$ 288 mμ (ε 10,300), $\lambda_{min}^{pH\ 2}$ 250.5 mμ (ε 2820), $\lambda_{max}^{pH\ 12}$ 281 mμ (ε 8280) and 237.5 mμ (ε 8140), $\lambda_{min}^{pH\ 12}$ 260 mμ (ε 5670) and 227 mμ (7710). The nmr spectrum showed a three-proton singlet at δ 3.32 corresponding to the 3′-O-methyl group; the anomeric proton was a triplet located at δ 6.1. *Anal.* ($C_{10}H_{14}FN_3O_4 \cdot HCl$) C, H, N.

3′-O-Methyl-5-fluoro-2′-deoxyuridine 5′-Phosphate (XI).—Compound X (52 mg, 0.2 mmol) was phosphorylated with β-cyanoethyl phosphate (136 mg of Ba salt, 0.4 mmole) and DCI (412.6 mg, 2 mmoles) according to the method of Tener.[19] After removal of the precipitated urea, the filtrate and washings (dry pyridine, 1 ml) were collected and evaporated to a gum. The gum on tlc in systems A and D showed a single uv-absorbing component (β-cyanoethyl ester of X) which gave a positive test with molybdate spray. This gum was treated with NaOH (0.1 *N*, 10 ml) and refluxed at 100° for 30 min. Then the contents were cooled and neutralized with Amberlite IR-120 (H^+) and filtered. The acidic filtrate was neutralized with aqueous NH_4OH and evaporated to a gum *in vacuo*. The aqueous solution of the gum (1 ml) was absorbed on a Dowex 1 (formate) column (2 × 8 cm) and the column was eluted with H_2O (15-ml fractions were collected). Fractions 3–9 gave a small uv-absorbing peak (shown to be unphosphorylated X). After 21 fractions the column was eluted with 0.05 *M* ammonium formate (pH 6.5); no uv-absorbing fraction was removed. Then (after fraction 35) the column was eluted with 0.5 *M* ammonium formate (pH 6.5) and XI gave a strong uv-absorbing peak (fractions 40–47). These fractions were combined, evaporated and desalted with the help of a charcoal column. The filtrate containing the product was evaporated to a gum and then dissolved in EtOH; Et_2O (40 ml) was added to give XI as a white precipitate of its diammonium salt. The precipitate was chilled in a freezer overnight and then centrifuged. It was purified by repeated precipitation from EtOH–Et_2O, yield 20 mg (27%). The product moved as a single homogenous uv-absorbing, phosphate-positive component on tlc in systems A and D. The structure of XI was ascertained by its dephosphorylation with prostatic phosphomonoesterase, which gave X (tlc systems A–D) as the only uv-absorbing product.

3′-O-Acetyl-5′-O-mesyl-5-fluoro-2′-deoxyuridine (XVI).—Compound I (1.63 g, 3.34 mmoles) was dissolved in dry pyridine (5 ml), freshly distilled Ac_2O (8 ml) was added, and the solution was kept overnight at room temperature. The reaction was then evaporated *in vacuo* and the residual gum was detritylated with cold formic acid (98%, 3 ml). The acid was removed and the residue was extracted with hot H_2O (40 ml). The solution was concentrated, allowed to cool, and gave colorless crystalline 3′-O-acetyl-5-fluoro-2′-deoxyuridine (XV), yield 609 mg (60%), mp 201°. The compound was identical with an authentic sample.[22]

Compound XV (576 mg, 2 mmoles) was twice evaporated from dry pyridine (two 5-ml portions) and dissolved in dry pyridine (10 ml). The stoppered solution was cooled to 0° and cold $MeSO_2Cl$ (0.16 ml) was added. The reaction was protected from moisture and kept overnight in a refrigerator. Then EtOH (0.2 ml) was added, the solution was maintained at 0° for 1 hr and treated with 5 ml of H_2O, and the solution was evaporated. The residual gum was crystallized from EtOH to give large colorless crystals of XVI, mp 134–134.5°, yield 510 mg (70% based on XV). The ir spectrum of XVI showed a sharp band at 1170 cm^{-1} due to the presence of the mesyl group; uv absorption, λ_{max}^{MeOH} 269 mμ (ε 11,300). *Anal.* ($C_{12}H_{15}FN_2O_8S$) C, H, N.

5′-O-Methyl-5-fluoro-2′-deoxyuridine (XVII).—Compound XVI (250 mg, 0.68 mmole) was dissolved in MeOH (dry, 4 ml), 1 ml of 3 *N* NaOMe in MeOH was added, and the solution was heated under reflux (absence of moisture) for 2 hr. Then the solution was filtered and absorbed on a Dowex 1 (OH) column (2 × 7.5 cm), which was eluted with 60% aqueous MeOH (20-ml fractions) until no more uv-absorbing material appeared (up to fraction 24). Then the column was treated with 0.1 *M* ammonium formate and XVII was eluted in fractions 36–68. These fractions were combined, evaporated, and desalted with a charcoal column. The filtrate containing XVII was evaporated to a gum and crystallized from EtOH to give colorless needles: mp 163°; yield 60 mg (33%); uv absorption, $\lambda_{max}^{pH\ 2}$ 269 mμ (ε 8890), $\lambda_{min}^{pH\ 2}$ 234.5 mμ (ε 1590), $\lambda_{max}^{pH\ 12}$ 269 mμ (ε 7410), $\lambda_{min}^{pH\ 12}$ 249 mμ (ε 5300). The nmr spectrum showed a three-proton singlet corresponding to the methyl group at δ 3.35. A triplet corresponding to the anomeric proton was centered at δ 6.16. *Anal.* ($C_{10}H_{13}FN_2O_5$) C, H, N.

Acid Hydrolysis of FUDR and 3′-O-Methyl-5-fluoro-2′-deoxyuridine (X).—FUDR (2.46 mg) and X (2.6 mg) were separately dissolved in 0.25 ml of 0.1 *N* HCl. The solutions were heated under reflux on an oil bath (bath temperature 100°). At fixed intervals of time an aliquot of each was examined on tlc (system D) and uv-absorbing spots corresponding to the starting material and 5-fluorouracil were cut and eluted with a known volume of 0.1 *N* HCl. The percentage proportion of each component at a given time was calculated from their optical densities and molar extinction coefficients. As shown in Figure 1 the half-life of X was 4.8 hr as compared to 3.6 hr for FUDR.

Methylation of 5′-O-Trityl-5-fluoro-2′-deoxyuridine (I) with MeI and Ag_2O in Different Solvents.—The methylation of I was carried out in four different solvents (MeOH, 10% methanolic Me_2CO, 10% methanolic dioxane, and DMF). In each case 244 mg (0.5 mmole) of I was dissolved in 17 ml of solvent and methylated with 5 ml of MeI and 1 g of Ag_2O at room temperature with vigorous stirring. Next morning the reaction mixture was filtered and washed with hot Me_2CO (30 ml), and the filtrate and washings were collected and evaporated to a gum. In the DMF reaction, the gum was dissolved in $CHCl_3$ and repeatedly extracted with aqueous $Na_2S_2O_3$. Then the $CHCl_3$ layer was dried

(22) D. C. Remy, A. V. Sunthankar, and C. Heidelberger, *J. Org. Chem.*, **27**, 2491 (1962).

over Mg_2SO_4 and evaporated, and the residual gum was used for detritylation. Each product was detritylated as described earlier and the resulting nucleosides were extracted from the gum with hot H_2O (25 ml). The aqueous solutions of the products were used for paper chromatography (system C). In each case there were spots corresponding to the four major products, VI-IX (in the case of MeOH and methanolic Me_2CO, 10% of the starting material was recovered as FUDR; there were also some minor slow-moving products). The spots of the products were cut and eluted with H_2O and the relative percentages of each were calculated. The results are presented in Table I.

5'-O-Trityl-5-fluoro-2'-deoxyuridine-6-^{3}H.—5-Fluoro-2'-deoxyuridine-6-^{3}H (1 mCi) (obtained from Schwarz BioResearch, Inc.) was diluted with nonradioactive FUDR to 160 mg and dissolved in dry pyridine (5 ml). The solution was heated under reflux (bath temperature 100°) with trityl chloride (194 mg) for 1 hr. The cooled reaction was poured over ice-water (50 ml) and extracted with $CHCl_3$ (three 20-ml portions). The $CHCl_3$ layers were collected and evaporated, and the residue was subjected to preparative tlc (silicic acid plates, system C). The bands corresponding to 5'-O-trityl-5-fluoro-2'-deoxyuridine-6-^{3}H were cut and eluted with MeOH to give 200 mg of the desired product.

Methylated 5-Fluoro-2'-deoxyuridine-6-^{3}H Derivatives.—5'-O-Trityl-5-fluoro-2'-deoxyuridine-6-^{3}H (146.3 mg, 0.3 mmol) was dissolved in dioxane (1 ml) and treated with Ag_2O (500 mg) and MeI (0.63 ml, 10 mmoles). The stoppered solution was stirred and kept at room temperature for 24 hr. The mixture was filtered (washed with 3 ml of hot Me_2CO) and the filtrate and washings were collected and evaporated to a gum. The gum was detritylated with cold formic acid (0.5 ml, 2 min at 0-5°) as described above. The detritylated residue was extracted with hot H_2O (3 ml), subjected to preparative tlc (silicic acid plates, system C), and developed twice with system C. There were four major bands. The top one corresponded to 3-N,3'-O-dimethyl-5-fluoro-2'-deoxyuridine-6-^{3}H and was removed (several plates) and eluted with MeOH. It was further purified by preparative tlc in system C. The second band (from top) contained 1-(2-deoxy-3-O-methyl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidone-6-^{3}H (purified by preparative tlc on silicic acid plates, system B), and the third band on elution gave 3-N-methyl-5-fluoro-2'-deoxyuridine-6-^{3}H. It was purified by preparative tlc on silicic acid plates with system B to give 13.8 μCi (sp act. 0.527 μCi/μmole) of the product. The lowest uv-absorbing band was cut and eluted to give 1-(2-deoxy-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidione-6-^{3}H.

3'-O-Methyl-5-fluoro-2'-deoxyuridine-6-^{3}H.—The labeled VII obtained in the last step was dissolved in 1 *N* NaOH (2 ml) and kept at room temperature for 1 hr. Then it was neutralized with Amberlite IR-120 (H^+) and filtered, and the filtrate was evaporated to a gum. Preparative tlc (system B) gave 12.8 mg of the required product (yield 13.7 μCi, sp act. 0.247 μCi/μmole).

5'-O-Methyl-5-fluoro-2'-deoxyuridine-6-^{3}H.—5'-O-Trityl-5-fluoro-2'-deoxyuridine-6-^{3}H (54 mg) was dissolved in dry pyridine (0.2 ml) and acetylated with Ac_2O (0.3 ml) as described earlier to give crude labeled XIV which on acid treatment gave 30 mg of radioactive XV, which was mesylated, and the tritiated XVI (obtained as a pale glass, 36.6 mg) was heated under reflux (bath temperature 80°) with 3 *N* methanolic NaOMe for 1 hr. The cooled reaction products were neutralized with Amberlite IR-120 (H^+) and filtered, and the filtrate was evaporated to a gum which was purified by preparative tlc (silicic acid, system B) to give 4.72 mg (0.006 μCi) of product.

Methylation of 5'-O-Trityl-5-fluoro-2'-deoxyuridine with MeI-^{14}C.—Nonradioactive I was methylated with MeI-^{14}C (213 mg, 1.5 mmoles, 1 mCi) (obtained from Amersham-Searle) in dioxane (1 ml) in the presence of Ag_2O (375 mg) by the methods previously described. The reaction products were detritylated and separated by preparative tlc to give the various ^{14}C-methylated products as described above.

3'-O-Methyl-^{14}C-5-fluoro-2'-deoxyuridine was obtained by alkaline treatment of ^{14}C-labeled VII obtained in the previous step. It was purified by preparative tlc methods described earlier, yield 6.44 mg, 13.8 μCi (sp act. 0.568 μCi/μmole).

3'-O-Methyl-^{14}C-5-fluoro-2'-deoxyuridine-6-^{3}H 5'-Phosphate.—3'-O-Methyl-5-fluoro-2'-deoxyuridine-6-^{3}H (sp act. 0.247 μCi/μmole) and 3'-O-methyl-^{14}C-5-fluoro-2'-deoxyuridine (sp act. 0.568 μCi/μmole) were mixed in a ratio of 8:2 (w/w) and then phosphorylated and worked up by the method described for the preparation of the nonradioactive XI.

5'-O-Methyl-^{14}C-5-fluoro-2'-deoxyuridine.—MeOH-^{14}C (1 mCi, obtained from Amersham-Searle) was diluted to 0.1 ml with nonradioactive MeOH, then Na (16 mg) was added to prepare NaOMe-^{14}C. This methanolic solution of NaOMe-^{14}C was treated with unlabeled XVI (36.6 mg, 0.1 mmole) and worked up according to the methods described for the preparation of the corresponding tritiated derivative, yield 8.48 mg, 5.95 μCi (sp act. 0.182 μCi/μmole).

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