

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 MeI-Ag₂O in 10% methanolic dioxane gave four different products, 1-(2-deoxy-5-O-trityl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidine (II), 1-(2-deoxy-3-O-methyl-5-O-trityl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidine (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)-pyrimidine (VI), 1-(2-deoxy-3-O-methyl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidine (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-Ag₂O 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 NH₃ 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 DCl, 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 ¹⁴C-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 Ag₂O 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)-pyrimidine (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)-pyrimidine (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)-pyrimidine (VI), 3-N-methyl-5-fluoro-2'-deoxyuridine (VIII), 1-(2-deoxy-3-O-methyl-β-D-ribofuranosyl)-4-methoxy-5-fluoro-2(1H)-pyrimidine (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

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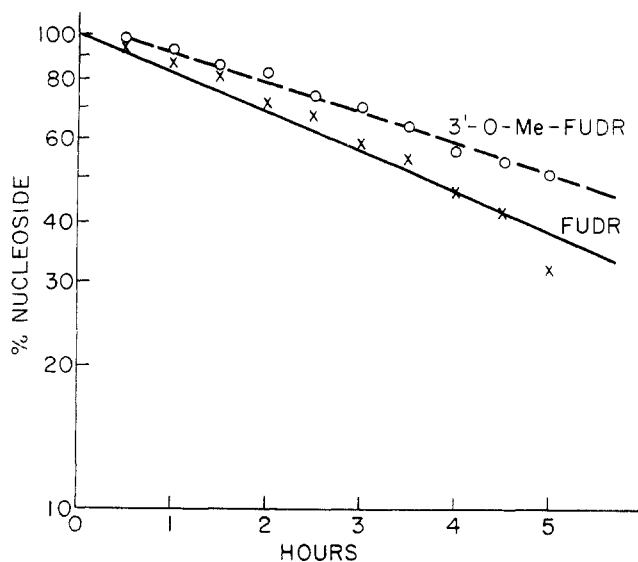


Figure 1.

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 NaOCH₃, with concomitant deacetylation, gave the required compound (XVII) in over 33% yield. The structure of XVII was confirmed by its uv absorption spectrum ($\lambda_{\text{max}}^{\text{pH } 2 \text{ or } 12}$ 269 m μ), 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 ¹⁴C-labeled compounds were prepared: 5-fluoro-2'-deoxyuridine-6-³H derivatives, and those in which FUDR was methylated with ¹⁴CH₃I 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 ¹⁴C-labeled compounds can

be used as double-labeled substrates in various enzyme studies; loss of ¹⁴C 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 μ G/ML (VALUES TAKEN AT 2 HR)

Compound	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 Heidelberg.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 D₂O. The analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. The work 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-H₂O (4:1:2, v/v); B, MeOH-C₆H₆ (1:3, v/v); C, Me₂CO-cyclohexane (1:1, v/v); D, *i*-PrOH-NH₄OH-H₂O (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), Ag₂O (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 Me₂CO 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 CHCl₃ (5 ml) and adsorbed on a silicic acid (100 mesh, Mallinckrodt) column (5.5 \times 30 cm) which was eluted with CHCl₃ 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, $\lambda_{\text{max}}^{\text{MeOH}}$ 270 m μ .

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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

	<i>R_f</i>			
	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), $\lambda_{\max}^{\text{MeOH}}$ 243.5 μ (ϵ 7590). *Anal.* (C₃₀H₂₃FN₂O₅) 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 ($\lambda_{\max}^{\text{MeOH}}$ 269 μ). The silicic acid column was next eluted with 5% MeOH in CHCl₃ 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 CHCl₃-MeOH to give 2.1 g (21%) of colorless crystalline material:

mp 186-188°; uv absorption, $\lambda_{\max}^{\text{MeOH}}$ 290 μ (ϵ 7270). *Anal.* (C₂₉H₂₇FN₂O₅) 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 CHCl₃, 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, $\lambda_{\max}^{\text{MeOH}}$ 290 μ (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 Et₂O. Finally, the residue was extracted with 10 ml of warm H₂O, the insoluble triphenylcarbinol was filtered, and the filtrate was evaporated *in vacuo*. The residual gum was dissolved in EtOH (1 ml), dry Et₂O (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, $\lambda_{\max}^{\text{pH } 2}$ 285 μ (ϵ 6210), $\lambda_{\min}^{\text{pH } 2}$ 241 μ (ϵ 956), $\lambda_{\max}^{\text{pH } 12}$ 285 μ (ϵ 5880), $\lambda_{\min}^{\text{pH } 12}$ 246 μ (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.* (C₁₀H₁₃FN₂O₅) C, H, N.

5-Fluoro-2'-deoxycytidine (FCDR).—Compound II (200 mg, 0.4 mmole) was dissolved in methanolic NH₃ (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 H₂O (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 Et₂O (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 H₂O (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–Et₂O–petroleum ether (30–60°) to give colorless needles: mp 124–126°; yield 290 mg (70.5%); uv absorption, $\lambda_{\text{max}}^{\text{pH } 2}$ 285 mμ (ε 6740), $\lambda_{\text{min}}^{\text{pH } 2}$ 241 mμ (ε 1065), $\lambda_{\text{max}}^{\text{pH } 12}$ 285 mμ (ε 6760), $\lambda_{\text{min}}^{\text{pH } 12}$ 246 mμ (ε 2920). *Anal.* (C₁₁H₁₅FN₂O₅) C, H, N.

3'-O-Methyl-5-fluoro-2'-deoxyuridine (X). **Method 1.**—Compound VII (100 mg) was dissolved in 30% aqueous formic acid (3 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–Et₂O to give 30 mg (32%) of fine colorless needles. It was recrystallized from H₂O: mp 146°, uv absorption, $\lambda_{\text{max}}^{\text{pH } 2}$ 269 mμ (ε 8880), $\lambda_{\text{min}}^{\text{pH } 2}$ 234.5 mμ (ε 1780), $\lambda_{\text{max}}^{\text{pH } 12}$ 269 mμ (ε 6920), $\lambda_{\text{min}}^{\text{pH } 12}$ 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.* (C₁₀H₁₃FN₂O₅) 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 neutralized with Amberlite IR-120 (H⁺) and filtered, and the filtrate was evaporated and crystallized from EtOH–Et₂O 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 NH₃ (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 H₂O (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 Et₂O (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, $\lambda_{\text{max}}^{\text{pH } 2}$ 288 mμ (ε 10,300), $\lambda_{\text{min}}^{\text{pH } 2}$ 250.5 mμ (ε 2820), $\lambda_{\text{max}}^{\text{pH } 12}$ 281 mμ (ε 8280) and 237.5 mμ (ε 8140), $\lambda_{\text{min}}^{\text{pH } 12}$ 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.* (C₁₀H₁₃FN₃O₄·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 DCl (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 NH₄OH 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 H₂O (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; Et₂O (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–Et₂O, 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 Ac₂O (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 H₂O (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 MeSO₃Cl (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 H₂O, 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, $\lambda_{\text{max}}^{\text{pH } 2}$ 269 mμ (ε 11,300). *Anal.* (C₁₂H₁₅FN₂O₆S) 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, $\lambda_{\text{max}}^{\text{pH } 2}$ 269 mμ (ε 8890), $\lambda_{\text{min}}^{\text{pH } 2}$ 234.5 mμ (ε 1590), $\lambda_{\text{max}}^{\text{pH } 12}$ 269 mμ (ε 7410), $\lambda_{\text{min}}^{\text{pH } 12}$ 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.* (C₁₀H₁₃FN₂O₅) 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 Ag₂O in Different Solvents.—The methylation of I was carried out in four different solvents (MeOH, 10% methanolic Me₂CO, 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 Ag₂O at room temperature with vigorous stirring. Next morning the reaction mixture was filtered and washed with hot Me₂CO (30 ml), and the filtrate and washings were collected and evaporated to a gum. In the DMF reaction, the gum was dissolved in CHCl₃ and repeatedly extracted with aqueous Na₂S₂O₃. Then the CHCl₃ layer was dried

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over MgSO_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- ^3H .—5-Fluoro-2'-deoxyuridine-6- ^3H (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- ^3H were cut and eluted with MeOH to give 200 mg of the desired product.

Methylated 5-Fluoro-2'-deoxyuridine-6- ^3H Derivatives.—5'-O-Trityl-5-fluoro-2'-deoxyuridine-6- ^3H (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 $0-5^\circ$) 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- ^3H 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)-pyrimidinone-6- ^3H (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- ^3H . It was purified by preparative tlc on silicic acid plates with system B to give 13.8 μCi (sp act. 0.527 $\mu\text{Ci}/\mu\text{mole}$) 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)-pyrimidinone-6- ^3H .

3'-O-Methyl-5-fluoro-2'-deoxyuridine-6- ^3H .—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 1R-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 $\mu\text{Ci}/\mu\text{mole}$).

5'-O-Methyl-5-fluoro-2'-deoxyuridine-6- ^3H .—5'-O-Trityl-5-fluoro-2'-deoxyuridine-6- ^3H (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 1R-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 $\mu\text{Ci}/\mu\text{mole}$).

3'-O-Methyl- ^{14}C -5-fluoro-2'-deoxyuridine-6- ^3H 5'-Phosphate.—3'-O-Methyl-5-fluoro-2'-deoxyuridine-6- ^3H (sp act. 0.247 $\mu\text{Ci}/\mu\text{mole}$) and 3'-O-methyl- ^{14}C -5-fluoro-2'-deoxyuridine (sp act. 0.568 $\mu\text{Ci}/\mu\text{mole}$) 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 $\mu\text{Ci}/\mu\text{mole}$).

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