Nucleosides. XXXVII. 5,6-Substituted 5-Fluorodihydropyrimidines and Their 2′-Deoxyribonucleosides[1,2]

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Addition of hypohalite across the 5,6 double bond of 5-fluorouracil (FU), 5-fluorocytosine (FC), and their deoxyribosides resulted in a series of 5,6-substituted dihydropyrimidines. Proof of structure for the methyl hypobromite adducts was furnished by chemical correlation to the water adduct of FU previously described. 5-Bromo-5-fluoro-6-methoxy-5,6-dihydro-2′-deoxyuridine and its 3′,5′-diacetate (MeOBrFUDR and MeOBr-FUDRdiAc) thus obtained were isolated in two diastereoisomeric forms. The hypobromite adducts of FU and of its deoxyriboside (FUDR) were debrominated to yield the corresponding 5-fluoro-6-hydroxy- (or alkoxy-) 5,6-dihydropyrimidines. From the latter compounds, water or alcohol is eliminated spontaneously whereby the 5,6 double bond and thus the original pyrimidine is regenerated, the alcohol adducts being less stable than the water adducts. The hypobromite (but not hypochlorite) adducts revert, in part, to the original pyrimidine when incubated with reduced glutathione at pH 7 and 35°. Possibly *via* a related reaction *in vivo*, the alkoxybromo derivatives are capable of acting as slow releasers of FU or FUDR. Data presented in Table I show that, in general, a correlation exists between the capacity of the adduct to regenerate the double bond *in vitro* and its effect against mouse leukemia B82A. On a molar basis, the releasers of FU are less potent in this test system than FU and the releasers of FUDR are more potent than FUDR. Thus, these drugs mimic, to a certain extent, the effects which have been reported for slow infusion of FU of FUDR in humans.

Among fluorinated pyrimidines, 5-fluorouracil (FU)[3] and 5-fluoro-2′-deoxyuridine (FUDR)[4] have acquired considerable importance as cancerostatic drugs,[5] while 5-fluorocytosine (FC)[3,6] has shown some promise as a fungistatic agent.[7] The potency (*i.e.*, therapeutic and/or nonspecific cytotoxicity) of FU and FUDR was shown to depend dramatically on the manner of administration.[8] Thus, when given to human cancer patients as single daily intravenous injections, FU and FUDR were found to be about equally potent, on a molar basis. However, upon slow *continuous* intravenous infusion, FU decreased in potency by a factor of two, whereas FUDR increased approximately twentyfold.[8a,b] A similar relationship was observed in mice carrying leukemia B82A.[8c] This phenomenon was explained by invoking the distribution and performance of enzymes regulating the metabolism of these fluorinated pyrimidines.[8a]

In striking contrast, FUDR has been found to be at least 1000 times more effective than FU, when tested *in vitro*, as an inhibitor of formate incorporation into the DNA thymine of Ehrlich ascites cells,[9] or as a growth inhibitor for the chick embryo.[10] In these isolated systems, FUDR (unlike FU) is efficiently converted to the corresponding 5′-phosphate (FUDRP)[11] and it is the latter which is responsible for cytotoxicity. *In vivo*, this anabolic step is largely preempted by the rapid catabolism of FUDR,[15-17] *via* enzymatic cleavage at the nucleoside bond, to FU, catabolic break-

(1) Preliminary accounts of this work were presented: (a) R. Duschinsky, T. Gabriel, W. Tautz, J. O. Malbica, and M. Hoffer, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept 1963, Abstracts, p 12-C; (b) R. Duschinsky, T. Gabriel, J. Purple, and J. H. Burchenal, *Proc. Am. Assoc. Cancer Res., 55th, Chicago, Ill., April 1964*, **5**, 16 (1964).

(2) This investigation was supported in part (to Sloan-Kettering Institute) by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA-08749).

(3) R. Duschinsky, E. Pleven, and C. Heidelberger, *J. Am. Chem. Soc.*, **79**, 4559 (1957).

(4) Synthesis by enzymic transfer of deoxyribose from thymidine to FU: (a) R. Duschinsky, E. Pleven, J. Malbica, and C. Heidelberger, Abstracts, 132nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1957, p 19-C; (b) R. Duschinsky, U. S. Patent 3,168,513 (1965); this patent describes in detail a practical enzymic process for the preparation of FUDR and its isolation *via* the 3′,5′-diacetate. By total synthesis: (c) M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Am. Chem. Soc.*, **81**, 4112 (1959); (d) M. Hoffer, U. S. Patent 2,948,725 (1960).

(5) Reviews: (a) C. Heidelberger and F. J. Ansfield, *Cancer Res.*, **23**, 1226 (1963); (b) C. Heidelberger, *Progr. Nucleic Acid Res. Mol. Biol.*, **4**, 1 (1965).

(6) R. Duschinsky, U. S. Patent 3,040,026 (1962).

(7) (a) J. Malbica, L. Sello, B. Tabenkin, J. Berger, E. Grunberg, J. H. Burchenal, J. J. Fox, I. Wempen, T. Gabriel, and R. Duschinsky, *Federation Proc.*, **21**, 384 (1962); (b) E. Grunberg, E. Titsworth, and M. Bennet, *Antimicrobial Agents Chemotherapy 1962*, 566 (1963); (c) B. A. Koechlin, F. Rubio, S. Palmer, T. Gabriel, and R. Duschinsky, *Biochem. Pharmacol.* **15**, 435 (1966); (d) R. Duschinsky, T. Gabriel, M. Hoffer, J. Berger, E. Titsworth, E. Grunberg, J. H. Burchenal, and J. J. Fox, *J. Med. Chem.*, **9**, 566 (1966).

(8) (a) R. D. Sullivan, C. W. Young, E. Miller, N. Glatstein, D. Clarkson, and J. H. Burchenal, *Cancer Chemotherapy Rept.*, **8**, 77 (1960); (b) C. G. Moertel and R. J. Reitemeier, *Proc. Am. Assoc. Cancer Res., 57th, Denver, Colo., May 1966*, **7**, 50 (1966); (c) Dr. M. S. Lyman, Sloan-Kettering Institute, personal communication.

(9) L. Bosch, E. Harbers, and C. Heidelberger, *Cancer Res.*, **18**, 335 (1958).

(10) D. A. Karnofsky, M. L. Murphy, and C. R. Lacon, *Proc. Am. Assoc. Cancer Res., 49th, Phila., Pa., April 1958*, **4**, 312 (1958).

(11) The nucleotide FUDRP was first synthesized by: (a) W. G. Farkas, L. C. Iacono, and R. Duschinsky, Abstracts, IVth International Congress of Biochemistry, Vienna, Aug 1958, p 9; (b) R. Duschinsky, W. G. Farkas, and C. Heidelberger, U. S. Patent 2,970,139 (1961). Although FUDRP is the actual intracellular inhibitor of thymidylate synthetase[12,13] and held mainly responsible for the antitumor effect produced by the fluorinated pyrimidines, it is less active than FUDR for intact cells.[14] Since cells are impermeable to nucleotides, FUDRP cannot enter cells without prior cleavage.

(12) S. S. Cohen, J. G. Flaks, H. D. Barner, M. R. Loeb, and J. Lichtenstein, *Proc. Natl. Acad. Sci. U. S.*, **44**, 1004 (1958).

(13) K. U. Hartmann and C. Heidelberger, *J. Biol. Chem.*, **236**, 3006 (1961).

(14) K. L. Mukherjee and C. Heidelberger, *Cancer Res.*, **22**, 815 (1962).

(15) (a) K. L. Mukherjee, J. Boohar, D. Wentland, F. J. Ansfield, and C. Heidelberger, *Cancer Res.*, **23**, 49 (1963); (b) B. Clarkson, A. O'Connor, L. R. Winston, and D. J. Hutchison, *Clin. Pharmacol. Therap.*, **5**, 581 (1964). These authors reported a half-life of 10 min in the plasma for FUDR administered by intravenous injection to cancer patients.

(16) G. D. Birnie, H. Kroeger, and C. Heidelberger, *Biochemistry*, **2**, 566 (1963).

(17) (a) N. K. Chaudhuri, K. L. Mukherjee, and C. Heidelberger, *Biochem. Pharmacol.*, **1**, 328 (1959); (b) N. K. Chaudhuri, B. J. Montag, and C. Heidelberger, *Cancer Res.*, **18**, 318 (1958); (c) K. L. Mukherjee and C. Heidelberger, *J. Biol. Chem.*, **235**, 433 (1960).

down of the latter, and elimination of any or all intermediates involved. Furthermore, any 5′-phosphorylation of FUDR which does occur must be rapidly reversed by the ubiquitous extracellular phosphatases.

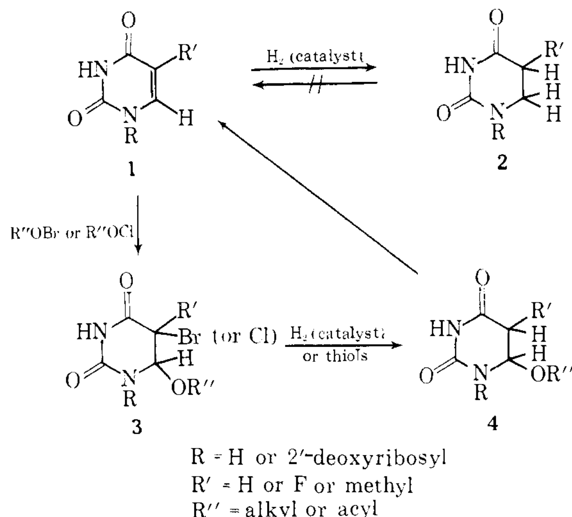
On the other hand, FC is not metabolized either by man or the rat, and is excreted in unchanged form.[7c] The activity of FC in systemic candidiasis in the mouse, however, is improved by administration in divided daily doses, as compared to one single dose.[18]

In view of these observations, it was considered promising to study fluorinated pyrimidine precursors which might be initially resistant to the catabolic attacks enumerated, but which would enter the target cells as such and then be converted gradually to their metabolically active version such as FUDR or its phosphate.

In a prior approach to this problem, FUDR 3′,5′-diacetate[4b] and N^4-*p*-toluoyl-5-fluoro-2′-deoxycytidine[7d] were synthesized. Both nucleosides manifested protection against enzymatic cleavage as compared to their unacylated precursors. Both compounds released FUDR *in vitro*, and, to a certain extent, apparently also *in vivo*.[7d,15a]

Another type of agent which might fulfill the desired role of a gradual releaser of the active substance was conceived to be a class of derivatives of 5-fluorinated pyrimidines in which substituents had been added across the 5,6 double bond. If such dihydro compounds reverted to their unsaturated precursors, they might produce the desired objective. It had been found by Green and Cohen[19] that unsubstituted 5,6-dihydropyrimidine nucleosides (see Chart I: **2**, R = deoxyribose; R′ = H or CH_3) are insensitive to the

Chart I



catabolic enzymes catalyzing nucleoside cleavage and pyrimidine exchange. These dihydropyrimidine nucleosides enter cells unchanged and undergo phosphorylation to nucleotides,[20] thus fulfilling part of the desired requirements for a protected precursor. There is, however, no indication that the 5,6 double bond can be biologically regenerated. Thus, 5,6-dihydro-5-fluorouracil[3,21] is a catabolic degradation product of FU,[17a] the reaction being essentially irreversible.

(18) Dr. H. Scholer, Hoffmann-La Roche, Basle, Switzerland, personal communication.

(19) M. Green and S. S. Cohen, *J. Biol. Chem.*, **225**, 405 (1957).

(20) S. S. Cohen, J. Lichtenstein, H. D. Barner, and M. Green, *ibid.*, **228**, 618 (1957).

We report now on certain substituted dihydropyrimidines, the 5-halo-6-oxy derivatives, which are able to revert under physiological conditions to the unsaturated cytotoxic agent.

The general synthetic approach is outlined in Chart I: addition of hypohalites (*e.g.*, R″OBr or R″OCl) to pyrimidines **1** give 5-halo-5,6-dihydropyrimidines **3**. The halogen thus introduced could be removed, either by catalytic hydrogenation or with such chemical reagents as alkali hydrosulfide and certain thiols such as cysteine or reduced glutathione (GSH), to give compounds of type **4**. The latter compounds are quite sensitive to a variety of conditions and revert spontaneously to the original pyrimidine **1** by elimination of R″OH. Both dehalogenation and elimination could reasonably be expected to occur in a biological milieu, perhaps in concerted fashion, and the regeneration of **1** from **3** (*via* **4**) seemed all the more likely in view of the ubiquitous distribution of glutathione in body fluids and tissues.[22] In point of fact, the expected transformation of compounds of type **3** to **1** was observed both *in vitro* and *in vivo*.

The addition of hypohalous acids to the 5,6 double bond of a pyrimidine was first carried out by Behrend.[23] The assignment of structure to the resulting addition products was based on the fact that 5,5-dibromo- (or dichloro-) 6-hydroxy-6-methylhydrouracil was obtained by Behrend from the reaction of HOBr (or HOCl) with 5-bromo- (or 5-chloro-) 6-methyluracil. These *gem*-dihalo derivatives were oxidized to 5,5-dibromo- (or dichloro-) barbituric acid with fuming nitric acid,[23b] thus proving unequivocally the entry of the added halogen at C-5 rather than at C-6.

Later, a number of bromo- (or chloro-) hydroxy-, alkoxy-, and acetoxypyrimidine addition products were prepared *via* analogous procedures by Johnson and his collaborators,[24] and by others.[25] New interest in substituted 5,6-dihydropyrimidines arose[26] with Sinsheimer's finding[27] that it is possible to add water across the 5,6 double bond of uracil, uridine, and uridylic acid in a photochemical reaction. Cytosine also undergoes this photohydration, whereas thymine, in frozen solution, produces mostly a dimer.[28] Attempts to isolate the extremely labile hydrate of thymine have been reported.[29]

(21) R. Duschinsky, E. Pleven, and W. Oberhansli, *Acta Unio Intern. Contra Cancrum*, **16**, 603 (1960).

(22) Blood contains 34 mg of glutathione/100 ml: W. S. Spector, "Handbook of Biological Data," W. B. Saunders Co., Philadelphia, Pa., 1956, p 52.

(23) (a) R. Behrend, *Ann. Chem.*, **229**, 18 (1885); (b) *ibid.*, **236**, 57 (1886).

(24) (a) H. L. Wheeler and T. B. Johnson, *J. Biol. Chem.*, **3**, 183 (1907); (b) T. B. Johnson, *Am. Chem. J.*, **40**, 19 (1908); (c) T. B. Johnson and J. M. Sprague, *J. Am. Chem. Soc.*, **59**, 2436 (1937); (d) T. B. Johnson and J. C. Ambelang, *ibid.*, **60**, 2941 (1938); (e) M. Fyfelson and T. B. Johnson, *ibid.*, **64**, 306 (1942); (f) T. B. Johnson, *ibid.*, **65**, 1218 (1943).

(25) (a) O. Baudisch and D. Davidson, *Ber.*, **58**, 1680 (1925); (b) Jones, *Z. Physiol. Chem.*, **29**, 20 (1900).

(26) (a) S. Y. Wang, M. Apicella, and B. R. Stone, *J. Am. Chem. Soc.*, **78**, 4180 (1956); (b) S. Y. Wang, *ibid.*, **80**, 6196 (1958); (c) *ibid.*, **80**, 6199 (1958); (d) *J. Org. Chem.*, **24**, 11 (1959); (e) W. E. Cohn, *Biochem. J.*, **64**, 28P (1956); (f) A. M. Moore, *Can. J. Chem.*, **36**, 781 (1958); (g) W. H. Prusoff, *Biochim. Biophys. Acta*, **58**, 588 (1962); (h) Review by A. Wacker, *Progr. Nucleic Acid Res.*, **1**, 369 (1963).

(27) (a) R. L. Sinsheimer and R. Hastings, *Science*, **110**, 505 (1949); (b) R. L. Sinsheimer, *Radiation Res.*, **1**, 505 (1954).

(28) (a) A. Wacker, H. Dellweg, and D. Weinblum, *Naturwiss.*, **47**, 477 (1960); (b) R. Beukers and W. Berends, *Biochim. Biophys. Acta*, **49**, 181 (1961); (c) M. Daniels and A. Grimison, *Nature*, **197**, 484 (1963).

(29) (a) R. Kleber, E. Fahr, and E. Boebinger, *Naturwiss.*, **52**, 513 (1965); (b) C. Nofre, A. Cier, R. Chapurlat, and J. P. Mareschi, *Bull. Soc. Chim. France*, 332 (1965).

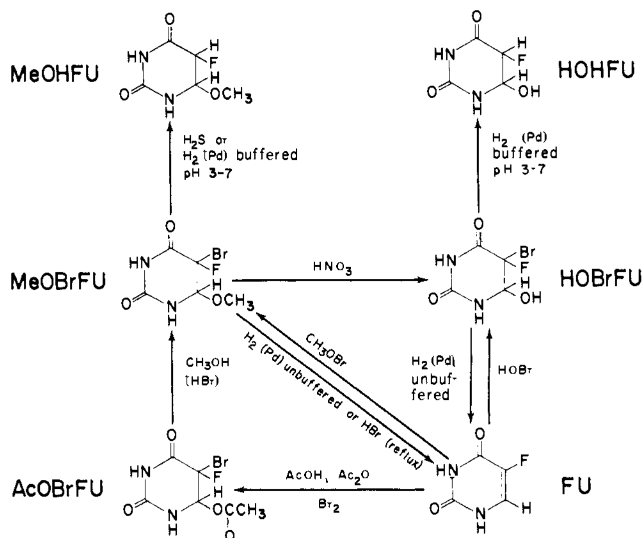
TABLE I
BIOAUTOGRAPHY, REGENERABILITY, AND ANTILEUKEMIC ACTIVITY

Compd	R_f in system[a] 1	R_f in system[a] 2	Antimicrob activity,[a] %	Regeneration of double bond[a]	Activity against leukemia B82A: Dose, mg/kg/day	Therap response[b]	Molar potency[c]
FU	0.58–0.66	0.60–0.66	50	. . .	6.25–25	+	1
FUDR	0.68–0.73	0.66–0.75	100	. . .	6.2–50	+	1
FUDRdiAc		0.78–0.81	0.5	. . .	4.1–16.5	+	2.0
dl-HOBrFU	0.75	0.76, 0.77	0	?[d]	100–200	±	<0.23
dl-HOHFU	0.47–0.52	0.56–0.58	1	5	29–44	−	<0.34
dl-MeOBrFU	0.86	0.84	2	37	50	+	0.48
dl-MeOHFU	0.69		5	85	40–80	+	0.20
dl-EtOBrFU	0.89	0.86	20	32	50–200	Inactive against Sarcoma 180 and Ehrlich solid and ascites	
dl-BuOBrFU	0.92	0.90	30	32	100–250	Inactive against Sarcoma 180 and Ehrlich ascites	
dl-AcOBrFU	0.88	0.78		27	108–216	±	<0.12
dl-HOClFU	0.78	0.80	1	0	20–62.5	Inactive against Sarcoma 180 and Ehrlich solid and ascites	
d-HOBrFUDR	0.72		<0.1	0	50–100	−	<0.34
d-HOHFUDR	0.53		0.1	8	150–250	+	0.13
d-MeOBrFUDR	0.85	0.85–0.88	10	23	2.5–10.8	+	3.6
d-MeOHFUDR	0.66	0.85	10	67	7–27	+	3.9
l-MeOBrFUDR	0.85	0.87	10	20	12.5–25	+	2.8
d-MeOBrFUDRdiAc		0.87		22	8.12–16.25	+	2.3
l-MeOBrFUDRdiAc	0.86–0.89		0	21	3.8–19.2	+	2.9
d-EtOBrFUDR	0.84	0.84	5	18	4.6–18.5	+	3.8
d-EtOHFUDR	0.74			High	6.25–12.5	+	3.6
t-BuOBrFUDR		0.87		10	100–200	−	<0.18
AcOBrFUDRdiAc	0.91		0.05		100	±	<0.47
d-MeOClFUDR		0.84		0	100–200	+	0.16
l-MeOClFUDR		0.83		0	100–200	±	<0.16
t-BuOClFUDR	0.91	0.86	0	0	100–200	−	<0.18
d-MeOBrTDR	0.82–0.90		0	78	100	−	0

[a] See Experimental Section for details. [b] A survival time, T/C, of 1-1.25 = −, 1.25–1.5 = ±, >1.5 = +. [c] Molar ratio FU (or FUDR)/drug when given at the optimal therapeutic dosage or maximal ineffective dosage. [d] The spectrum, produced after reaction with GSH, presented no real FU peak but a shoulder at about 300 mμ, which indicated the presence of another product, so that the FU content could not be calculated.

More recently, Lozeron, *et al.*,[30] investigated the photochemical addition of water across the 5,6 double bond of FU and demonstrated the formation of 5-fluoro-6-hydroxy-5,6-dihydrouracil (HOHFU, Chart II). The structure of HOHFU was reliably established by nmr spectroscopy. Since the same substance was previously obtained in our laboratories[1a] from the reductive debromination of HOBrFU (the addition product of hypobromous acid with FU), the location of the bromine at C-5 in the latter compound is also established. Besides HOBr, we have now added HOCl, alkyl hypochlorite, and alkyl and acetyl hypobromite to the double bond of fluorinated pyrimidines. These addition products and some debrominated derivatives are listed in Table I, together with certain physical and biological properties.

CHART II



Further structural correlations are outlined in Chart II. The addition products of FU and methyl hypobromite (MeOBrFU) and acetyl hypobromite (AcOBrFU) were linked with HOHFU by the reactions depicted: AcOBrFU was converted to MeOBrFU upon refluxing with methanolic HBr. (Heating MeOBrFU with *n*-butyl alcohol and HBr gave the analog, BuOBrFU.) Rather surprisingly, heating MeOBrFU with fuming nitric acid gave HOBrFU rather than 5-bromo-5-fluorobarbituric acid, an expected product of a subsequent oxidation.[23b]

The 5-fluoro-6-oxydihydropyrimidines resulting from reductive debromination of the hypobromite addition products differ substantially in their stability. Thus, the elimination of alcohol from 5-fluoro-6-methoxyhydrouracil (MeOHFU) with regeneration of the double bond proceeds considerably faster than the elimination

(30) H. A. Lozeron, M. P. Gordon, T. Gabriel, W. Tautz, and R. Duschinsky, *Biochemistry*, **3**, 1844 (1964).

of water (*vide infra*). The acetic acid addition product (AcOHFU) appears to be still more unstable: hydrogenation of 5-bromo-6-acetoxy-5-fluorohydrouracil (AcOBrFU) gave a high yield of FU; no AcOHFU was isolated. Similarly, the hydrogenation products derived from the adducts of MeOBr and EtOBr to FC appear to be quite unstable (*vide infra*) and were not isolated.[31]

The mechanism of these addition reactions appears to result in products with fluorine at C-5 *trans* to the hydrogen at C-6.[32] The course of the hydrogenolytic debromination of the addition products based on this assignment is less easily interpreted: in the presence of sodium acetate (pH 3–7), products are obtained which retain the dihydrofluoropyrimidine structure and are believed to possess hydrogen at C-5 and OR at C-6 in the *cis* positions.[30] This must have occurred as the result of a Walden inversion in the hydrogenolysis. Unbuffered hydrogenation regenerated FU quantitatively, presumably since the hydrogenolysis under these acid conditions resulted (*via* retention) in the *trans* isomer, which is susceptible to an elimination reaction. A similar type of observation and deduction was made by Wang[26b] for 1,3-dimethyl-6-hydroxyhydrouracil.

We next turned our attention to the corresponding addition products of pyrimidine glycosides. Addition of hypobromite across the double bond of (natural) pyrimidine nucleosides has been reported,[26e] but the resulting adducts have not been isolated. Our procedure consisted of treating the nucleoside with the appropriate hypohalite, preferably in the absence of undesirable free hydrogen halide. Such hypohalites were generated by the addition of silver carbonate or acetate to a reaction mixture consisting of nucleoside and a solution of halogen in water or the appropriate alcohol. Alternately, in aqueous medium an anion-exchange resin could be used for scavenging hydrogen halide. In most instances, the reaction medium was maintained not only neutral but also anhydrous in order to prevent undesired attacks upon the carbohydrate component of the nucleoside. The progress of the reaction was monitored by the disappearance of the ultraviolet absorption at 280 mμ (in 0.1 *N* HCl), since the dihydro compounds show only end absorption starting at this wavelength.

The presence of molecular asymmetry in the sugar portion of the molecule was expected to lead to diastereoisomerism in the nucleoside adducts. In point of fact, two diastereoisomeric products, in approximately equal parts, were observed. Occasionally, both species could be isolated in crystalline form but in some instances only one isomer crystallized, the other remaining in the mother liquors. Some nucleoside mixtures could not be separated at all. Thus, addition of HOBr to FUDR gave (after passage through a Dowex 1 acetate column followed by chromatography with silica gel) crystalline *d*-HOBrFUDR in about 27% yield. The *levo* diastereoisomer was isolated only as crude amorphous material.

According to Moore and Anderson,[33] 5,5-dibromo-6-hydroxyhydrouracil acts as a brominating agent for uracil, producing 5-bromouracil. We tried to determine if HOBrFUDR would brominate deoxyuridine since this reaction would shift the ultraviolet absorption maximum toward longer wavelength due to formation of FUDR and 5-bromo-2′-deoxyuridine. However, no such interaction between HOBrFUDR and UDR was observed under various reaction conditions.

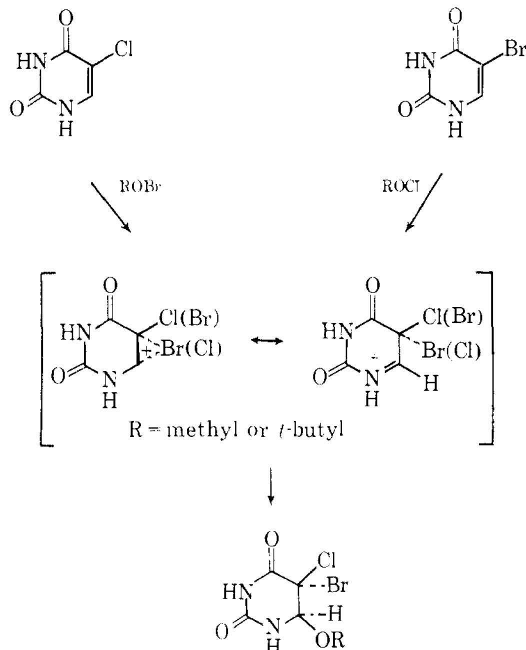
Catalytic hydrogenation of *d*-HOBrFUDR in the presence of sodium acetate proceeded smoothly and gave (after chromatography through an anion-exchange resin followed by treatment with a cation exchanger) an amorphous, but apparently fairly pure, *d*-HOHFUDR. This compound may be identical with an ultraviolet irradiation product of FUDR obtained by Prusoff.[26g]

Addition of MeOBr to FUDR (see Chart III) gave crystalline *d*-MeOBrFUDR in fair yield (*ca.* 40%) by direct crystallization, whereas *l*-MeOBrFUDR was difficult to crystallize and was obtained only in low yield from the mother liquor. On the other hand, FUDR 3′,5′-diacetate[4b] gave, upon treatment with MeOBr, a levorotatory product by direct crystallization and a dextrorotatory isomer by further treatment of the mother liquor. The structural relationships of these diacetates (*d*- and *l*-MeOBrFUDRdiAc) with the corresponding unacetylated MeOBrFUDR were established by acetylation of *d*-MeOBrFUDR to *d*-MeOBrFUDRdiAc.

Oxidation of *d*- and *l*-MeOBrFUDR with fuming nitric acid destroyed the deoxyribosyl moiety and removed the methyl group in each case, giving *d* and *l* enantiomers of HOBrFU, respectively. An artificial mixture of equal amounts of these enantiomorphs gave the racemate identical with the *dl*-HOBrFU pre-

(31) This corroborates the observation that the water addition product of cytosine is less stable than the one of uracil.[29a]

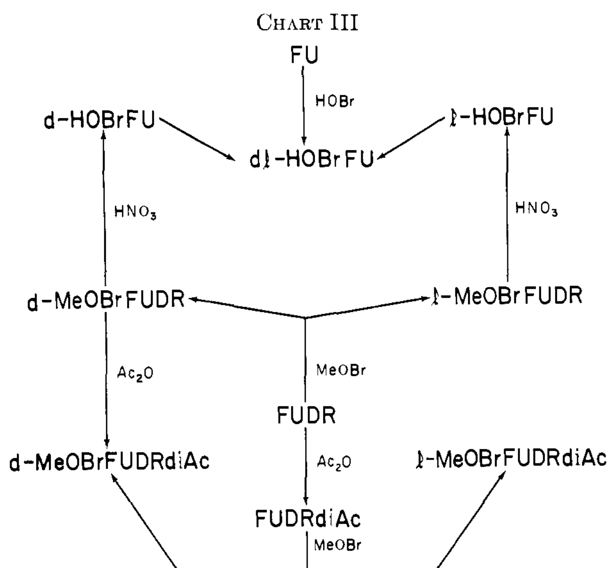
(32) This might not necessarily be the result of straightforward transaddition of the elements of ROBr or ROCl [S. Winstein and R. B. Henderson, *J. Am. Chem. Soc.*, **64**, 2196 (1942); A. Roedig, "Methoden der Organischen Chemie," Houben–Weyl, Vol. V/4, E. Müller, Ed., Georg Thieme Verlag, Stuttgart, 1960, p 144]. We have, in fact, some evidence that the addition may proceed in the following manner. The same compound[24c] is obtained from



either starting material. Stereochemical assignments are tentative. Similar observations, where R = H, have already been made by Johnson.[24b] The resulting postulated *trans* stereochemistry would therefore be directed by the bulkier halogen at C-5, rather than the entering electrophile, as observed with simple alkenes. Both mechanisms, however, may be operative in the various specific instances investigated.

(33) A. M. Moore and S. M. Anderson, *Can. J. Chem.*, **37**, 590 (1959).

CHART III



pared by direct addition of HOBr to FU. It is clear from these experiments that the configurations of the aglycones in *d*- and *l*-MeOBrFUDR are identical with those of the respective *d* and *l* isomers of HOBrFU and demonstrate that addition of MeOBr to the nucleoside, FUDR, followed the same course as did addition of this reagent to FU itself. Addition of MeOBr to 5-fluoro-2′-deoxyuridylate (FUDRP) gave a mixture (not separated) of MeOBrFUDRP isomers which was needed for enzymatic experiments (*vide infra*).

Addition of EtOBr to FUDR and hydrogenation of the resulting *d*-EtOBrFUDR (yield 37%) to *d*-EtOH-FUDR proceeded in a manner analogous to the preparation of the lower homolog. The *l* diastereoisomers were not isolated in the ethyl series. *t*-BuOBrFUDR was obtained only as a mixture of diastereoisomers, as was 5-bromo-5-fluoro-6-acetoxy-5,6-dihydro-2′-deoxyuridine 3′,5′-diacetate when FUDR 3′,5′-diacetate was treated with bromine in acetic acid and acetic anhydride in the presence of silver acetate.

While hypobromite addition to FUDR proceeded readily in the cold or at room temperature using equimolar amounts of reagent, methyl hypochlorite reacted only when a great excess was used and the medium was acidic; *d*-MeOClFUDR was obtained in crystalline form. *t*-Butyl hypochlorite did not react with FUDR at room temperature. When the mixture was refluxed, a product of uncertain steric homogeneity was obtained.

Addition of MeOBr (20% excess) to 5-fluoro-2′-deoxycytidine (FCDR) gave the expected MeOBrFCDR as a mixture of diastereoisomers. A more acidic by-product which contained electropositive bromine was isolated by ion-exchange chromatography. The analysis was consistent with a combination of MeOBrFC-DR + HOBr. The product may be MeOBrFCDR whose exocyclic amino group carries a bromine atom and which contains a molecule of water.[34]

Addition of MeOBr to thymidine gave an 81% yield of crystalline *d*-bromo-6-methoxy-5,6-dihydrothymidine (*d*-MeOBrTDR) which upon hydrogenation did not yield MeOHTDR but reverted mostly to TDR. This corroborates the lability of the thymine photohydration product reported in the literature.[29a,b]

(34) An N-chloroaminopyrimidine was reported in the literature.[24c]

We now describe the stability of these addition products. At elevated temperatures, such as being heated above their melting points, they decompose reverting to the original 5-fluoropyrimidines. This reversal, however, is far from quantitative for most hypohalite addition products. In contrast, the water or alcohol addition products similarly revert in excellent yield.

Behavior toward aqueous acid and base is more complicated. The bromo compounds derived from FU and FUDR are stable at room temperature and at pH 7 and below, exhibiting only end absorption in the ultraviolet. In alkali, however, these compounds decompose slowly, as evidenced by the gradual decrease of their absorption maximum at *ca.* 240 mμ. MeOBrFU gave, upon standing for 20 hr in 1 *N* NaOH, 100% of free bromide ion and was partially transformed into FU. On the other hand, the corresponding deoxyribonucleoside appeared to be more stable; no change of its ultraviolet absorption intensity at the maximum of 247 mμ was observed when its alkaline solution was monitored for prolonged periods.

The water and alcohol addition products are unstable in aqueous solutions at any pH, the latter even more so than the former. Table I shows the amounts of FU and FUDR regenerated when these compounds were incubated in phosphate buffer at pH 7 for 24 hr at 35°. At alkaline pH, more deep-seated changes are observed: HOHFU undergoes, to a large extent, ring opening and cleavage to fluoromalonaldehydic acid.[30] In contrast, MeOHFU reverts quantitatively to FU. The corresponding nucleosides show similar behavior: MeOH-FUDR reverts to FUDR upon treatment with alkali, whereas HOHFUDR reverts only to a small extent and gives rise to an absorption peak at 260 mμ resembling the spectrum of fluoromalonaldehydic acid. The reaction product was not further identified.

Finally, the stability of the hypohalite addition products toward chemical reducing agents was investigated. Treatment with 2–4 moles of reduced glutathione (see Experimental Section) regenerated the unsaturated pyrimidine, as evidenced by the appearance of the characteristic ultraviolet absorption spectrum and confirmed by paper chromatography. The figures in Table I show the percentage of regenerated pyrimidine obtained in 24 hr. No significant increase in conversion after this time was observed, nor did additional amounts of glutathione increase the yield of pyrimidine. The data indicate that a side reaction also occurs but this aspect was not further investigated.[35] Only the alkyl hypobromite addition products gave an appreciable percentage of regenerated pyrimidine, whereas HOBr as well as HOCl or alkyl hypochlorite addition products regenerated little or no pyrimidine under these conditions. *d*-MeOBrTDR, when treated with 2 moles of GSH, gave 78% of thymidine; *dl*-MeOBrFC and *dl*-EtOBrFC were converted almost quantitatively into FC (see Experimental Section). These results corroborate the assumption that an intermediate debrominated derivative is highly unstable and undergoes loss of alcohol with regeneration of the pyrimidine

(35) Bromine is not released as ion, since HNO_3 and $AgNO_3$ give no immediate precipitation of AgBr at room temperature; AgBr is formed after short heating on the water bath. This may be explained by a labile fixation of Br to glutathione or to some other reaction product. The hypobromite addition products themselves yield no AgBr upon short heating with HNO_3 and $AgNO_3$.

double bond without by-product formation. The behavior of HOBrFC toward GSH appeared to be more complex; the formation of FC could not be ascertained.

Biological Aspects

Biochemistry.—None of the dihydronucleosides were cleaved by *Escherichia coli* nucleosidase, as shown by the absence of free deoxyribose after 20 hr of incubation, whereas FUDR was cleaved to the extent of 45%.[36] Nucleoside phosphorylase, prepared from Ehrlich ascites cells which degrades FUDR readily to FU, did not produce FU from *d*-MeOBrFUDR or *d*-MeOHFUDR.[37] Heidelberger, *et al.*,[38] found that these two dihydronucleosides are approximately as effective as FUDR at inhibiting the incorporation of formate into DNA thymine of Ehrlich ascites cells, *providing* they were preincubated for 1 hr with the cells prior to addition of formate. The activity of MeOBrFUDR and MeOHFUDR could be explained either by their conversion into FUDRP *via* FUDR or by their direct conversion into a nucleotide which may furnish FUDRP.

Attempts were made to phosphorylate *d*-MeOBrFUDR, *d*-MeOHFUDR, *d*-MeOBrTDR, *d*-HOBrFUDR, and *t*-BuOClFUDR by use of wheat germ phosphotransferase prepared according to Barner and Cohen,[39] but in no case were we successful. These results are especially surprising since, by this method, we were able to phosphorylate FUDR, FCDR, and its derivatives which are alkylated in the exocyclic amino group,[7b] as well as such "unnatural" nucleosides as α-FUDR and FU carrying deoxyribose in the 3 position.[40a] The failure to obtain nucleotides with this relatively unselective enzyme from the 5,6-disubstituted nucleosides is not associated with an inhibitory effect of these compounds on the enzyme, since FUDR could be phosphorylated to the same extent in the absence of and in the presence of equimolecular amounts of MeOBrFUDR.[40b] These results do not, however, exclude the possibility of phosphorylation of MeOBrFUDR by other enzymes such as thymidine kinase, or its action as an inhibitor of this enzyme. This question deserves further study. However, MeOBrFUDRP, prepared from FUDRP, proved to be a potent inhibitor of thymidylate synthetase. When incubated with the enzyme prior to the addition of deoxyuridylate, the activity of MeOBrFUDRP was of the same magnitude as FUDRP but the kinetics of its inhibition differed from that observed with FUDRP.[41]

Microbiology.—The antimicrobial activity *in vitro* of the FU and FUDR derivatives was evaluated by bioautography, using *Sarcina lutea* as the test organism (see Table I and Experimental Section). Since this method requires the separation of the derivatives from the parent compounds by paper chromatography prior to exposure to the microorganisms, and therefore discerns between inherent antimicrobial activity and activity due to the presence of FU and FUDR, bioautography also permitted the determination of the purity of the compounds in so far as contamination by starting materials is concerned. This contamination was never more than 1%. The inherent activity of the dihydro derivatives which was maximally 30% of the parent compound probably reflects to a certain extent the capacity of the microorganism to regenerate original pyrimidine.

The alkoxydihydro-FC derivatives were found to be active against *Candida albicans in vitro* and *in vivo* (in mice) according to tests performed by Dr. H. Scholer (Hoffmann-La Roche, Basel); these results will be reported elsewhere.

Antineoplastic Activity. Although the FU and FUDR dihydro derivatives were tested against a variety of experimental solid tumors and mouse leukemias, our present report is limited to results obtained with leukemia B82A. The technique used in this ascitic form of leukemia was described by Burchenal, *et al.*[42] Treatment involves ten daily intraperitoneal injections of the drug, and evaluation is done by measuring the survival time (see Table I). The dose–response curves offer broad peaks, the optimal dose presenting a balance of drug toxicity and therapeutic activity. The average survival time of 8–10 days of the control animals is rarely more than doubled by drug treatment. The potency of the compounds was compared with FU and FUDR which are about equally effective on a molar basis.

It can be seen that all derivatives of FU tested against B82A are less effective than FU itself, the most efficacious compound being MeOBrFU, with half the molar potency of FU.

d-MeOBrFUDR and EtOBrFUDR, their 3′,5′-diacetates, and the corresponding debrominated compounds are two to four times as potent as FUDR. *d*-HOBrFUDR and its reduction product (*d*-HOHFUDR) are ineffective or effective only at a high dosage. The same situation exists for *t*-BuOBrFUDR and AcOBrFUDRdiAc and all the dihydrochloro compounds. As expected, *d*-MeOBrTDR showed no antineoplastic activity.

d-MeOBrFUDR, because of its relative availability, was chosen for further studies. It has also undergone clinical trials in cancer patients.[43]

Discussion and Conclusion

We have postulated that the 5,6-substituted 5-fluorodihydropyrimidines act as releasers of the original pyrimidines in biological systems either by spontaneous regeneration of the double bond (Chart I, **3** → **1**) or by reaction with a reducing agent such as glutathione (Chart I, **3** → **4** → **1**). It should, therefore, be possible to demonstrate a correlation between the regeneration of the double bond observed in the test tube and the

(36) For methodology, see L. Warren, *J. Biol. Chem.*, **234**, 1971 (1959). We are indebted to Mrs. L. H. Sello (Hoffmann-La Roche) for this experiment.

(37) An experiment performed by the method of C. Heidelberger, G. D. Birnie, J. Boohar, and D. Wentland, *Biochim. Biophys. Acta*, **76**, 315 (1963), degraded to FU (expressed as micromoles per milligram of protein) in 20 min: FUDR (0.833), MeOBrFUDR (0.002), MeOHFUDR (0.006). We are indebted for these data to Professor Heidelberger, University of Wisconsin.

(38) C. Heidelberger, J. Boohar, and G. D. Birnie, *Biochim. Biophys. Acta*, **91**, 636 (1964).

(39) R. D. Barner and S. S. Cohen, *J. Biol. Chem.*, **234**, 2988 (1959).

(40) (a) R. Duschinsky, J. J. Fox, L. Kaplan, G. Keller, E. Pleven, J. Malbica, and M. Hoffer, *Federation Proc.*, **21**, 383 (1962). (b) The authors are indebted to J. O. Malbica (Hoffmann-La Roche, Inc.) for these experiments.

(41) P. Reyes and C. Heidelberger, *Biochim. Biophys. Acta*, **103**, 177 (1965).

(42) J. H. Burchenal, J. R. Burchenal, M. N. Kushida, S. F. Johnston, and B. S. Williams, *Cancer*, **2**, 113 (1949).

(43) J. Van Dyk, I. Krakoff, B. Clarkson, M. Sykes, and R. Duschinsky, *Proc. Am. Assoc. Cancer Res., 57th, Denver, Colo., May 1966*, **7**, 73 (1966).

biological effects exerted by these drugs. The biological effects will, of course, depend on various factors, such as the extent and speed of the regenerative process, the metabolic fate of the drugs, and their distribution in the organism. If the drugs regenerate little or no FU or FUDR, or if they release these compounds too slowly, they should have little potency. On the other hand, if the release of FU or FUDR is too rapid, there might be little difference between the precursor and the active pyrimidine. Our premise was that appropriate releasers of FU would be less potent than FU and releasers of FUDR would be more potent than FUDR itself. Protection of the glycosyl linkage in the precursor against enzymatic cleavage with formation of FU was deemed necessary so that the nucleoside could exert its role as substrate for conversion into an active nucleotide. The biochemical behavior of MeOBrFUDR and MeOHFUDR seems to fill that requirement.

The data in Table I show that, in general, a correlation exists between the capacity of the drug to regenerate the double bond *in vitro* with the formation of FU or FUDR and the antileukemic effect. Those drugs which regenerate 5-fluorouracil show, as expected, a lower potency than fluorouracil itself in this test system. In this regard these FU-releasing drugs mimic the effects which have been reported[8a] for slow infusion of FU in humans. On the other hand, those fluorinated dihydronucleosides which regenerate FUDR *in vitro* are more potent on a molar basis against leukemia B82A than is FUDR, thus apparently mimicking to some extent the increase in potency obtained by slow infusion of FUDR. It is noteworthy that the half-life of FUDR in the plasma of cancer patients treated with *d*-MeOBrFUDR was 10 times longer than when FUDR itself was administered.[43] This result would indicate that *d*-MeOBrFUDR acts *in vivo* as a reservoir for the release of FUDR.

Experimental Section[44]

Chromatography and Bioautography (See Table I).—Paper chromatograms were run in the descending manner on Whatman No. 1 paper (liquid front 40–45 cm) using either 1-butanol–acetic acid–water (5:2:3) (system 1) or ethanol–water (85:15) (system 2). The bromo and chloro compounds were fairly well visible upon inspection with ultraviolet light since they exhibit sufficient end absorption. Deoxyribosides were rendered visible by spraying with cysteine–sulfuric acid.[45] The dehalogenated water or alcohol addition products, which exhibit insufficient end absorption to be located on the chromatograms, could be rendered visible by spraying with alkali or, better, by exposure to NH_3 vapor. This treatment regenerates the pyrimidines or their strongly absorbing breakdown products.

For bioautography,[46] the dried and well-aerated developed paper chromatogram strips were applied to 23 × 45 cm rectangular plastic pans containing 300 ml of nutrient agar at pH 6.2 which had been seeded with *Sarcina lutea* (PCI 1001).[47] Strips were allowed to remain in contact with the seeded and hardened agar for about 12 min, then were carefully removed. The agar pans were incubated overnight (18–20 hr) at 35°. The growth inhibition zones which indicate the presence of an active antibacterial substance were shadowgraphed, measured, and matched with standard series of FU and FUDR chromatographed simultaneously. This method permits detection of less than 1 μg of FU and FUDR.

The figures given in Table I present the molar percentage of FUDR which gave an inhibitory zone matching the one produced by the compound examined.

Stability Experiments[48] (Regeneration of Double Bond, Table I).—The compound (0.1 mmole) was dissolved in 2 ml of 0.06 *M* phosphate buffer (pH 7). Less soluble compounds such as MeOBrFU and *d*-MeOBrFUDRdiAc were dissolved in 2 ml of buffer and 18 ml of water; *l*-MeOBrFUDRdiAc was soluble only in 10 ml of buffer, 72 ml of H_2O, and 18 ml of MeOH. To one-half of these solutions was added 61.4 mg of glutathione (0.2 mmole). Both solutions were incubated for specified periods, at 35°, unless specified otherwise. Spectra were taken after dilution with 0.1 *N* HCl to obtain a 0.1 m*M* solution, usually after 19 hr and again after 24 hr. Data recorded in Table I refer to the 24-hr readings. In some instances, an increment of 15–20% was observed between these readings. Controls without GSH were taken at the start and after 24 hr. The hypohalite addition products showed no significant change of absorption in these controls. The spontaneous regeneration of the double bond of MeOHFUDR actually appeared to be slightly delayed by the presence of GSH in the beginning, but the end result was not very different; the figures recorded in Table I for dehalogenated compounds were obtained without GSH. In control runs, which were monitored spectrophotometrically, FU, FC, FUDR, and FUDRdiAc did not undergo significant changes by incubation with GSH.

The ultraviolet data of FU and FUDR are as follows: FU, $\lambda_{max}^{0.1N\,HCl}$ 266 mμ (ε 7330), $\lambda_{max}^{0.1N\,KOH}$ 282–283 mμ (ε 5420), $\lambda_{max}^{1N\,KOH}$ 284 mμ (ε 7050); FUDR, $\lambda_{max}^{0.1N\,HCl}$ 268–269 mμ (ε 8980), $\lambda_{max}^{0.1N\,KOH}$ 268–269 mμ (ε 6960), $\lambda_{max}^{1N\,KOH}$ 269 mμ (ε 7090).

***dl*-5-Chloro-5-fluoro-6-hydroxyhydrouracil (HOClFU).**—To a suspension of 13 g of FU (0.10 mole) in 40 ml of concentrated HCl was added, gradually with shaking and cooling (temperature kept below 55°), 10 ml of 30% H_2O_2. After the exothermic reaction had subsided, another 10 ml of H_2O_2 was added as described above, whereupon a clear solution was obtained. After 5–12 hr, the product (12 g) crystallized at 0°; additional material (2 g) was obtained by extraction of the mother liquor with ether (200 ml). The crude material melted at 189°. Recrystallization by solution in 25 ml of hot ethyl acetate, chilling, and addition of 36 ml of ether raised the melting point to 196°; yield, 12 g (66%).

Anal. Calcd for $C_4H_4ClFN_2O_3$: Cl, 19.45; N, 15.35. Found: Cl, 19.58; N, 15.12.

***dl*-5-Bromo-5-fluoro-6-methoxyhydrouracil (MeOBrFU).**—To a suspension of 52 g of FU (0.4 mole) in 1600 ml of methanol, stirred under reflux, 64 g of Br_2 (0.4 mole) was added dropwise. After refluxing for 30 min, the solution was evaporated *in vacuo*, and the residue was repeatedly leached with methanol, and again concentrated to dryness. The final residue was slurried with 500 ml of H_2O and collected by filtration; yield 78 g (82%), mp 208–210° dec. Recrystallization from ethyl acetate raised the melting point to 214–215° dec.

Anal. Calcd for $C_5H_6BrFN_2O_3$: C, 24.92; H, 2.51; Br, 33.76. Found: C, 25.29; H, 2.33; Br, 33.20.

When MeOBrFU was heated in DMF at 140–145° for 45 min, only a very faint release of Br^- was observed. Upon evaporation, a sirup was obtained from which about 50% of starting material was recovered.

In 0.1 *N* HCl, the compound showed only end absorption commencing at 280 mμ ($\epsilon_{280}^{0.1N\,HCl}$ <60) and no spectral changes occurred within a period in 24 hr. Heating in 0.1 *N* HCl (or H_2SO_4) on the steam bath for 2 hr did not produce any change in the spectrum, and the analysis of the reaction mixture showed no release of Br^-, but there was partial loss of the methoxy group (decrease of OCH_3 from 13.79 to 9.59%). When 241 mg of MeOBrFU (0.001 mole) was refluxed for 15 min in 10 ml of concentrated HBr, the reaction mixture was evaporated to dryness and the residue was treated with 20 ml of ether; 116 mg of FU (90%) was isolated.

In an attempt to oxidize the product to 5-bromo-5-fluorobarbituric acid, a mixture of 1.1 g of MeOBrFU (0.0466 mole) and 10 ml of red fuming HNO_3 was heated on the steam bath for 20 min. After dilution with 50 ml of water, the solution was evaporated to

(44) Melting points were taken on a Thomas–Hoover apparatus and are uncorrected. Ultraviolet spectra were measured on a Cary Model 14M spectrophotometer or, in part, on a Beckman DB instrument. Infrared measurements were carried out on a Beckman IR5 instrument in potassium bromide pellets, unless otherwise indicated.

(45) I. G. Buchanan, *Nature*, **168**, 1091 (1951).

(46) We are indebted to Mr. E. LaSala (Hoffman-La Roche) for these tests.

(47) An amount of 1 ml of a 24-hr shake-flask growth of *S. lutea* in 300 ml of agar is used.

(48) We wish to thank Dr. V. Toome of these laboratories for carrying out a large part of the kinetic experiments.

a syrup which was slurried with 50 ml of ether. The resulting suspension of crystals was refluxed for 1 min and chilled in a Dry Ice bath. Filtration and washing with ether and petroleum ether (bp 30–60°) yielded 0.576 g (54%) of product, mp 171–174°. The latter was dissolved in 5 ml of ethyl acetate, and the solution was clarified by filtration and reprecipitated in crystalline form by adding 8 ml of petroleum ether: yield 308 mg (dried at 100°), mp 179–180°. The compound proved to be identical by mixture melting point and infrared spectrum with anhydrous HOBrFU obtained by direct addition of HOBr to FU.[30]

Alkali Treatment of MeOBrFU.—When a solution of 241 mg of MeOBrFU (1 mmole) in 5 ml of 1 *N* NaOH was allowed to stand at room temperature for 20 hr, 100% of the bromine became titrable with $AgNO_3$. The spectrum of the solution exhibited $\lambda_{max}^{1 N NaOH}$ 284 mμ (ε 2810) and $\lambda_{max}^{0.1 N HCl}$ 266 mμ (ε 3110), which indicated a 40% conversion into FU. The presence of FU was confirmed by paper chromatography (ethyl alcohol–water = 85:15, R_f 0.60). A second spot (R_f 0.23) became visible upon inspection with ultraviolet light, only after spraying the chromatogram with 0.1 *N* NaOH. The spot corresponding to MeOBrFU (R_f 0.84) had disappeared.

dl-**5-Bromo-6-ethoxy-5-fluorohydrouracil (EtOBrFU)** was obtained from FU (52 g), 800 ml of ethanol, and 1 mole of Br_2 by the procedure used for the preparation of MeOBrFU: yield 82 g (80%). The product was recrystallized from toluene. It melted at 211° with gas evolution, whereby FU was formed almost quantitatively.

Anal. Calcd for $C_6H_8BrFN_2O_3$: C, 28.25; H, 3.16; Br, 31.33. Found: C, 28.40; H, 2.91; Br, 31.14.

dl-**5-Bromo-6-butoxy-5-fluorohydrouracil (BuOBrFU).**—A solution of 82 g of MeOBrFU (0.34 mole) in 240 ml of 1-butanol and 2 ml of concentrated HBr was subjected to slow fractional distillation. After 2 hr, 100 ml of distillate consisting of butanol and methanol was collected. After addition of 100 ml of butanol to the reaction mixture, the distillation procedure was repeated. The residual butanol solution was washed with water and then evaporated to a syrup which, upon treatment with heptane, became crystalline. The product was recrystallized from aqueous methanol: yield 71 g (78%), mp 167°.

Anal. Calcd for $C_8H_{12}BrFN_2O_3$: C, 33.94; H, 4.27; Br, 28.23. Found: C, 33.94; H, 4.23; Br, 28.28.

dl-**6-Acetoxy-5-bromo-5-fluorohydrouracil (AcOBrFU).**—To a suspension of 20 g (0.154 mole) of FU in 300 ml of acetic anhydride, which was cooled to 5° and continuously stirred, was added 20 ml of bromine (0.39 mole). The solution obtained was allowed to stand at room temperature for 24 hr, after which time it was evaporated *in vacuo*, and the resulting syrup was crystallized from a mixture of 5 ml of acetic anhydride and 20 ml of chloroform. The product was filtered and washed with the same mixture and then with chloroform: yield 31.24 g (75%), mp 163.5–165.5°. Recrystallization from a mixture of 95 ml of acetic anhydride and 300 ml of chloroform gave 25.6 g of product melting at 167–168°. A second recrystallization raised the melting point to 167.5–168.5°. The product showed only end absorption starting at 280 mμ. Bioautography excluded the presence of more than 1% FU; λ_{max} (cm⁻¹) doublet at 3230 (NH of equal very strong intensity), 1748 and 1695 (ester, imido C, urea C=O (s)), strong bands at 1464, 1370 (CH_3 def), 1245, 1205 (COC) of ester, 1170, 1015, 943.

Anal. Calcd for $C_6H_6BrFN_2O_4$: F, 7.06; N, 10.41; Br, 29.70. Found: F, 7.33; N, 10.21; Br, 30.06.

Conversion of AcOBrFU into MeOBrFU.—A solution of 270 mg of AcOBrFU (1 mmole) in 50 ml of methanol and 0.1 ml of concentrated HBr was refluxed for 2 hr. After addition of 1 g of Ag_2CO_3, the mixture was stirred until it became neutral, then filtered and evaporated *in vacuo* to give a white solid (270 mg) which was recrystallized from water: yield 70 mg (20%), mp 208.5–209°. The compound was identical with MeOBrFU prepared directly from FU as judged by mixture melting point.

dl-**5-Fluoro-6-methoxyhydrouracil (MeOHFU). A. KSH Method.**—To a solution of KSH prepared by dissolving 22.5 g of KOH (0.4 mole) in 500 ml of methanol and saturating at 0° with H_2S (13.5–14 g), MeOBrFU (78 g, 324 mmoles) was added gradually, the temperature being held at 0° by outside cooling. The reaction, as evidenced by heat evolution and yellow coloration, took place immediately. The mixture was then refluxed for 20 min, allowed to cool, and filtered from solid material. The filtrate was evaporated *in vacuo* to a crystalline mass, which was dried at 50–60° and then slurried with 50 ml of water, to dissolve potassium salts. The remaining solid was collected by filtration and dissolved in 120 ml of boiling water. The solution was clarified by filtration through a heated suction funnel and yielded, upon cooling, 27 g of yellowish plates. Recrystallization from 15 vol of methanol and evaporation of the mother liquors gave 24.8 g (47%) of product melting at 195° with gas evolution and formation of FU, which then melted at 285° dec.

Anal. Calcd for $C_5H_7FN_2O_3$: C, 37.04; H, 4.35; CH_3O, 19.14. Found: C, 37.15; H, 4.75; CH_3O, 18.82.

B. Catalytic Hydrogenation.—MeOBrFU (960 mg, 3.98 mmoles) was dissolved in 15 ml of methanol and hydrogenated at atmospheric pressure and at 22°, in the presence of 300 mg of prehydrogenated 10% Pd–C catalyst. The uptake stopped in 30 min at the theoretical volume (96 ml) of hydrogen. After separation of the catalyst by filtration, 149 mg of FU (mp 281–283°) was crystallized by addition of 10 ml of ether and 25 ml of petroleum ether. Evaporation of the mother liquor and crystallization from ethanol, ether, and petroleum ether yielded additional 84 mg of FU (total 45%). Extraction of the catalyst with 20 ml of boiling methanol gave a solution containing 14,700 optical density units at $\lambda_{max}^{0.1 N HCl}$ 266 mμ which account for another 2.1 mmoles of FU (53%) and thus for a practically quantitative formation of the compound.

When 964 mg of MeOBrFU (4 mmoles) dissolved in 20 ml of methanol containing 328 mg (4 mmoles) of sodium acetate was hydrogenated in the presence of 200 mg of the catalyst, the uptake was completed in 9 min. The filtrate from the catalyst, when examined spectrophotometrically in 0.1 *N* HCl and in 0.1 *N* NaOH, showed $E_{265}^{0.1 N HCl}$ 8230 corresponding to 1.18 mmoles of FU (29.5%) and $E_{282}^{0.1 N NaOH}$ 15,620 for a total of 3.03 mmoles of FU (76%). Since the increase of absorption in 0.1 NaOH is due to transformation of MeOHFU to FU (*vide infra*), a yield of 46% of MeOHFU could be calculated. The methanolic solution gave, upon evaporation *in vacuo* and recrystallization of the residue from 4 ml of hot water, 243 mg (38%) of crude MeOHFU melting with gas evolution at 191° (followed by resolidification), showing no depression of the melting point on admixture with the product prepared by method A.

dl-**6-Ethoxy-5-fluorohydrouracil (EtOHFU).**—EtOBrFU (100 g, 0.4 mole) was allowed to react with NaSH prepared by dissolving 9.2 g of sodium (0.4 g-atom) in 500 ml of ethanol and saturating it with H_2S as described for the preparation of MeOBrFU. The product was crystallized from 200 ml of water: yield 27 g (41%), mp 195° with gas evolution and formation of FU.

Anal. Calcd for $C_6H_9FN_2O_3$: C, 37.04; H, 4.35; F, 10.79. Found: C, 37.51; H, 4.75; F, 10.90.

dl-**5-Bromo-5-fluoro-6-hydroxyhydrocytosine (HOBrFC).**—To a well-stirred suspension of 2.85 g of FC (0.02 mole) in 100 ml of ice-cold water was added dropwise 1.1 ml of Br_2 (0.022 mole). A clear yellow solution having a residual absorbance of $E_{280}^{0.1 N HCl}$ 2340 was obtained. The reaction mixture was submitted to chromatography on a Dowex 1-X4 (acetate) column 2.2 × 18 cm at a flow rate of 1000 ml/hr, using water as the eluent. After passage of 100 ml showing no ultraviolet absorption, a fraction of 200 ml exhibiting end absorption was collected. Upon evaporation *in vacuo* in the cold to about 5 ml, crystals formed. The product was filtered and washed with water, ethanol, and finally with ether: yield 1.13 g (25%), mp 158° with gas evolution, followed by resolidification and decomposition at about 210°. The ultraviolet spectrum taken in 0.1 *N* HCl showed end absorption starting at 280 mμ. The product proved to be unstable upon storage at room temperature.

Anal. Calcd for $C_4H_5BrFN_3O_2$: C, 21.26; H, 2.23; Br, 35.36; F, 8.41; N, 18.59. Found: C, 21.46; H, 2.41; Br, 35.16; F, 8.30; N, 18.28.

dl-**5-Bromo-5-fluoro-6-methoxyhydrocytosine (MeOBrFC).**—To a solution of 5.5 ml of Br_2 (0.107 mole) in 125 ml methanol was added, with stirring and cooling, 59 g of Ag_2CO_3 (0.215 mole). After 30 min of continuous stirring the reaction mixture was filtered and the filtrate was added, with stirring and cooling to 0°, to a suspension of 12.9 g of FC (0.1 mole) in 250 ml of methanol. The mixture became almost homogeneous within 20 min, then crystals began to separate. The mixture was then allowed to reach room temperature, and, after 3 hr, cooled in ice. The crystals were collected by filtration and washed with methanol and then with ether: yield 16 g. A second crop of 5.8 g was obtained by evaporation of the mother liquor and recrystallization of the residue from 75 ml of methanol. The combined crops gave, upon recrystallization from 220 ml of methanol, 18.5 g

(68%) of product, mp 172–173° (gas evolution). When dried at room temperature, the compound contained 1 mole of methanol. It showed only end absorption starting at 280 mμ, in 0.1 *N* HCl; λ_{max} (cm^{-1}) inflection at 3330, medium band at 3175 (bonded NH, NH_2), strong band multiple at 1680, 1631 (urea C=O), medium doublet at 1443 and 1403, strong 1271, medium 1150, doublet at 1095 and 1080, 1025.

Anal. Calcd for $C_5H_7BrFN_3O_2 \cdot CH_3OH$: F, 6.98; OCH_3, 22.81. Found: F, 6.67; OCH_3, 23.04.

Upon prolonged drying *in vacuo* the compound lost the methanol of crystallization; λ_{max}^{EtOH} 245 mμ (ε 7600).

Anal. Calcd for $C_5H_7BrFN_3O_2$: C, 25.02; H, 2.94; N, 17.51; OCH_3, 11.41. Found: C, 25.24; H, 3.13; N, 17.56; OCH_3, 11.40.

Chemical regeneration of FC with glutathione[48] under the standard conditions was virtually quantitative.

dl-**5-Bromo-6-ethoxy-5-fluorohydrocytosine (EtOBrFC).**—To a solution of 2.6 ml of Br_2 (0.051 mole) in 100 ml of ethanol, cooled to −15°, was added 27.5 g of Ag_2CO_3 (0.1 mole). After stirring the mixture at −15° for 30 min it was filtered and the filtrate was added with stirring and cooling to a suspension of 5 g of FC in 200 ml of ethanol. Since stirring in the cold for 30 min brought about no apparent changes, the reaction was initiated by addition of 5 drops of 2.5 *N* HBr in butanol. After stirring for 30 min at room temperature a solution was obtained which showed 272,000 OD units at λ_{max}^{EtOH} 240 mμ. Upon evaporation to 100 ml, crystals formed which were collected by filtration and washed with ethanol and ether; yield 3.8 g. A second crop of 2.77 g was obtained from the mother liquor by evaporation and crystallization from 50 ml of ethanol; total yield 51%, mp 175–177° dec, λ_{max}^{EtOH} 240 mμ (ε 6480). For analysis, a sample was recrystallized from ethanol, mp 178° dec.

Anal. Calcd for $C_6H_9BrFN_3O_2$: Br, 31.45; F, 7.48; N, 16.54; OC_2H_5, 17.74. Found: Br, 31.65; F, 7.34; N, 16.75; OC_2H_5, 17.64.

Again, quantitative conversion to FC was observed upon standard glutathione reduction.[48]

d-**5-Bromo-5-fluoro-6-hydroxy-5,6-dihydro-2′-deoxyuridine (HOBrFUDR).**—To an ice-cooled solution of FUDR (81.3 mmoles) in 250 ml of water was added within 30 min, 5.2 ml of Br_2 (100 mmoles), dropwise, with vigorous stirring. After 1 additional hr, the reaction mixture (monitored spectrophotometrically) contained $E_{280}^{0.1N\,HCl}$ 14,000 corresponding to a maximum of 21.5 mmoles of FUDR[49] (26.5% of starting material). Since at this time the drop of ultraviolet absorption had come to a standstill, the solution was passed through Dowex 1-X4 (acetate) column, 3.8 × 30 cm (200 g of resin), and washed with water. After passage of a 400-ml fraction (1) showing no ultraviolet absorption, an effluent fraction (2) of 685 ml ($E_{260}^{0.1N\,HCl}$ 25,200, $E_{280}^{0.1N\,HCl}$ 3900) and fraction (3) 770 ml ($E_{260}^{0.1N\,HCl}$ 6360, $E_{280}^{0.1N\,HCl}$ 1665) were collected and followed by an ultraviolet nonabsorbing eluate. Fraction 2 was lyophilized to yield 10.07 g of white solid. Fraction 3 yielded 4.42 g of solid and was not further investigated. Thin layer chromatography of fraction 2 on silica gel (3.3 vol of methanol, 96.7 vol of ethyl acetate) showed two spots and the analysis indicated deficiency of Br and F.

A solution of 16 g (81% of original) in 30 ml of acetone was mixed with 10 g of silica gel (0.2–0.5 mμ) and the mixture was evaporated *in vacuo* to a sticky solid. This was put on top of a silica gel (0.2–0.5 mμ) column 4.5 × 29 cm (240 g) and eluted with ethyl acetate. Fractions were monitored by tlc (10% methanol in ethyl acetate) and the tlc plates were sprayed with cysteine sulfuric acid[45] for producing a pink spot characteristic for deoxyribosides. A peak fraction (R_f 0.7) was eluted after three to four column volumes of effluent had been collected. Concentration gave 6.15 g of sticky white solid. This was followed by tail fractions of gradually decreasing intensity which were combined and gave an additional 3 g. A second peak appeared upon elution with a mixture of 10 vol of methanol and 90 vol of ethyl acetate which gave upon evaporation 2.7 g of solid. Finally, a third peak was eluted yielding 0.57 g. These latter two fractions were not further investigated. The first peak fraction (6.15 g) was dissolved in 150 ml of boiling ethyl acetate and filtered from a trace of insoluble material. The solution crystallized after allowing it to stand overnight below 0°. The crystals were filtered and washed with ether; yield 2.17 g, mp 118° dec, $[\alpha]^{28}D$ +34.9° (*c* 1, ethyl acetate).

(49) FUDR has $\epsilon_{280}^{1N\,HCl}$ 6500.

Anal. Calcd for $C_9H_{12}BrFN_2O_6$: C, 31.50; H, 3.53; Br, 23.29; F, 5.57; N, 8.16. Found: C, 31.61; H, 3.52; Br, 23.04; F, 5.56; N, 8.27.

Addition of petroleum ether to the mother liquor afforded a second crop, 1.14 g, mp 118.5° dec, $[\alpha]^{24}D$ +32.40°.

The tail fraction of 3 g gave, upon recrystallization from 75 ml of ethyl acetate, 1.20 g, mp 118.5° dec, $[\alpha]^{24}D$ +36.3° (*c* 1, ethyl acetate), and addition of petroleum ether to the mother liquor gave 1.7 g of product, mp 120° dec, $[\alpha]^{24}D$ +28.1° (*c* 1, ethyl acetate).

Thus, the total amount of isolated HOBrFUDR of varying degrees of purity was 6.21 g (27%, if corrected for unused crude material). The compound exhibited only end absorption starting at 280 mμ when examined in water, in 0.1 *N* HCl (ϵ_{220} *ca.* 5000), and in 1 *N* NaOH, and the spectra of these solutions did not undergo any significant change within a period of 24 hr.

The final mother liquor of the peak fraction was slightly levorotatory. It was evaporated to dryness and dissolved in 5 ml of boiling ethyl acetate to which was added chloroform until the beginning of turbidity. Cooling produced precipitation of an oil which became crystalline upon scratching; yield 0.23 g, mp 119° dec. The mother liquor of this material gave, upon evaporation to dryness, 1.5 g of solid $[\alpha]^{27}D$ −19.06° (*c* 15, ethyl acetate).

According to the Br and F analysis this material is crude 1-HOBrFUDR of about 80% purity.

Anal. Calcd for $C_9H_{12}BrFN_2O_6$: Br, 23.29; F, 5.57; N, 8.16. Found: Br, 20.60; 20.77; F, 4.84, 4.88; N, 7.56, 7.63.

Interaction of *d*-HOBrFUDR and 2′-Deoxyuridine (UDR).—Equal parts of 0.01 *M* solutions of *d*-HOBrFUDR and of UDR were mixed and diluted 1:50 with water or 1 *N* HCl. The ultraviolet spectra were taken at zero time. The water solution was examined after 5 and 24 hr of incubation at 35° and after 6.5 hr on the steam bath; the solution in 1 *N* HCl was examined after 2.5 hr on the steam bath. No significant changes of the initial spectra were observed; in particular, there was no observation of a shift of the location of the UDR maximum from about 260 toward 268 mμ, which would be expected from formation of FUDR.

d-**5-Fluoro-6-hydroxy-5,6-dihydro-2′-deoxyuridine (HOHFUDR).**—A solution of 2 g of *d*-HOBrFUDR (5.83 mmoles) in 90 ml of water containing 513 mg of sodium acetate was hydrogenated at room temperature and atmospheric pressure in the presence of 233 mg of prehydrogenated 10% Pd–C catalyst. The hydrogen uptake (141 ml, 99% of theory) came to a standstill after 15 min. The catalyst was filtered. The filtrate (96 ml) contained 93% of the Br in titratable form (5 ml removed for analysis) and showed $E_{280}^{0.1N\,HCl}$ 2360 corresponding to 0.36 mmole of FUDR (6.2%) and $E_{260}^{1N\,NaOH}$ 54,800.[50] The solution was neutralized to pH 7.0 with 1 *N* NaOH and fractionated on a Dowex 1-X4 (acetate) column, 2.5 × 41 cm, using water as an eluent. The procedure was monitored by taking spectrophotometric readings at 280 mμ in 0.1 *N* HCl and at 260 mμ in 1 *N* NaOH. After passage of 191 ml of nonabsorbing eluate, a fraction of 271 ml containing $E_{280}^{0.1N\,HCl}$ 195 and $E_{260}^{1N\,NaOH}$ 48,700 was collected. Elution with 1 *N* acetic acid then removed a second fraction of $E_{280}^{0.1N\,HCl}$ 1800 and $E_{260}^{1N\,NaOH}$ 2100 from the column. This fraction (FUDR) was discarded. The first fraction was slurried with 20 g of Dowex X8 (H^+ form) in order to remove Na^+, and the resin was separated by filtration. The filtrate was lyophilized, yielding 1.31 g of a fluffy powder (84% yield, calculated as monohydrate). Rotations were taken in 5.2% aqueous solution at 25° at four different wavelengths, the values for [α] being +6.4° (578 mμ), +7.2° (546 mμ), +16.0° (436 mμ), +36.8° (346 mμ).

Anal. Calcd for $C_9H_{13}FN_2O_6 \cdot H_2O$: C, 38.30; H, 4.64; F, 6.73; N, 9.93. Found: C, 38.54, 38.75; H, 4.95, 5.15; F, 6.67; N, 9.83, 10.01.

d-**5-Bromo-5-fluoro-6-methoxy-5,6-dihydro-2′-deoxyuridine (*d*-MeOBrFUDR).**—To a freshly prepared solution of 14 ml (43.6 g) of Br_2 (0.55 mole) in 1000 ml of methanol, which was cooled to about −15°, Ag_2CO_3 (170 g, 0.62 mole) was added with vigorous stirring. After continuous stirring and cooling for 1 hr, the initially red solution had become light yellow. The methyl hypobromite solution was filtered directly into an ice-cold solution of 50 g of FUDR (0.203 mole), in 750 ml of methanol. Stirring and cooling at 0° was maintained for 15 min, then the

(50) This reading was taken *ca.* 15 min after adding the alkali and accounts (besides for the FUDR present) for a decomposition product of HOHFUDR having $\lambda_{max}^{1N\,NaOH}$ 260–261 mμ (ε 8300).

temperature was allowed to ambient gradually, and the solution was kept there for 90 min. The optical density measured at 280 mμ in ethanol was 44,000, constituting some 6.7 mmoles of unreacted FUDR (3.3%). The yellow solution was filtered through Celite and evaporated *in vacuo* to a yellowish froth which was dissolved in 200 ml of ether. Cooling in the refrigerator produced crystals, which were filtered, washed with cold ether, and dried at 60° *in vacuo*; yield 36.6 g, mp 165–167° (then decomposed at 172°). Addition of 200 ml of ether to the combined mother liquor and washing precipitated 1 g of additional product, mp 164–166° dec, total yield 52%.

The resulting mother liquor was again evaporated and the residue was triturated twice with 15 ml of ether to give 5.5 g of crystals, mp 152–156°, $[\alpha]^{25}D$ +46.4° (*c* 1, methanol). The mother liquor (A) of this product was set aside for isolation of the levorotatory diastereoisomer (*vide infra*).

The first crop was recrystallized from 420 ml of butyl acetate, yielding 17.3 g of pure *dextro* isomer melting at 170–171°. Addition of 200 ml of petroleum ether produced an additional amount of 11.85 g, mp 167.5–169°, which, after recrystallization from 130 ml of butyl acetate, afforded 6.81 g, mp 170–171°. The combined two batches (24.11 g), $[\alpha]^{25}D$ +56.6° (*c* 2, water), were analyzed.

Anal. Calcd for $C_{10}H_{14}BrFN_2O_6$: C, 33.63; H, 3.95; Br, 22.38. Found: C, 33.94; H, 4.03; Br, 22.44.

Additional amounts of *d*-MeOBrFUDR were recovered from the second to fourth crops as well as from the recrystallization mother liquors, thus resulting in a 40–45% yield of pure product. The compound exhibited end absorption starting at about 280 mμ in 0.1 *N* HCl and no spectral changes were observed within 24 hr, $\lambda_{max}^{0.1 N NaOH}$ 247 mμ (ε 6780), $\lambda_{min}^{0.1 N NaOH}$ 219 mμ (ε 5890). Refluxing for 2 hr in 2 *N* H_2SO_4 or HNO_3 did not release Br^-; λ_{max} (cm^{-1}) medium 3450 (bonded OH), 3250 (NH), strong doublet at 1755 and 1705 (imide, urea C=O (s)), medium strong 1435, 1235, 1090, 1050.

***l*-5-Bromo-5-fluoro-6-methoxy-5,6-dihydro-2′-deoxyuridine (MeOBrFUDR).**—Addition of 100 ml of petroleum ether to the be the mother liquor (A) produced a gummy precipitate which came solid upon trituration with 50 ml of ether. It was filtered and washed well with ether; yield 6.27 g, mp 139–143° $[\alpha]^{25}D$ −45° (*c* 1, methanol). The product was dissolved in 15 ml of boiling ethanol, the solution was cooled to −10° and separated from a few crystals by filtration. The filtrate gave, upon addition of 12 ml of petroleum ether, 1.76 g of crystals which sintered at 143° and melted at 145–147°, $[\alpha]^{25}D$ −52.8° (*c* 0.5, methanol). The mother liquor gave, upon standing, a second crop of 1.65 g, mp 144–145°. Thus, the total yield of fairly pure *l*-HOBrFUDR was 4.7%. Two recrystallizations from ethanol and petroleum ether raised the melting point to 146.5–148.5° and $[\alpha]^{25}D$ to −56.9° (*c* 0.5 methanol); λ_{max} (cm^{-1}) medium strong doublet at 3415 and 3270, very strong doublet at 1745 and 1709 (C=O), strong bands at 1464, 1380, 1255, very strong triplet at 1105, 1087, and 1075.

Anal. Calcd for $C_{10}H_{14}BrFN_2O_6$: Br, 22.38; F, 5.32; N, 7.84. Found: Br, 22.64; F, 5.28; N, 7.88.

***d*-5-Bromo-5-fluoro-6-methoxy-5,6-dihydro-2′-deoxyuridine 3′,5′-Diacetate (MeOBrFUDRdiAc).**—A suspension of 1.785 g (5.0 mmoles) of *d*-MeOBrFUDR in 10 ml of acetic anhydride to which 0.1 ml of pyridine was added was stirred at room temperature. A clear solution was formed in about 30 min. After letting the mixture stand overnight, it was poured with vigorous stirring into 75 ml of ice–water. An oily suspension formed, which was extracted three times with 25 ml of chloroform. The chloroform extract was dried (Na_2SO_4) and evaporated to leave an oil which became crystalline when leached with methanol, ethyl acetate, and petroleum ether and concentrating the combination. The product was crystallized from a mixture of methanol and petroleum ether: yield 0.47 g, mp 119–120°, $[\alpha]^{25}D$ +44.6° (*c* 4, methanol). An additional 0.81 g of material with the same melting point was obtained from the mother liquor. The total yield was 1.28 g (58%), λ_{max} (cm^{-1}) weak 3330 (NH), strong doublet at 1745 and 1715 (C=O), strong band at 1235 (COC), medium 1095.

Anal. Calcd for $C_{14}H_{18}BrFN_2O_8$: C, 38.11; H, 4.11; F, 4.31; N, 6.35. Found: C, 37.91; H, 4.22; F, 4.03; N, 6.28.

***l*-5-Bromo-5-fluoro-6-methoxy-5,6-dihydro-2′-deoxyuridine 3′,5′-Diacetate (MeOBrFUDRdiAc).**—To a solution of 9.72 g of FUDR 3′,5′-diacetate (29.4 mmoles) in 300 ml methanol was added 32 mmoles of MeOBr in 130 ml of methanol obtained from 5.2 g of Br_2 and 17 g of Ag_2CO_3. After standing overnight in the cold, the E_{280} had dropped to 5300. Upon evaporation to dryness *in vacuo* in the cold, a crystalline residue was obtained which was triturated with 50 ml of Dry Ice cooled ether, filtered, and washed with cold ether; yield 6.81 g, mp 118–119° (not quite clear). This product was recrystallized by dissolving in 20 ml of hot ethyl acetate, clarifying the solution with Hyflo, adding 35 ml of petroleum ether, and cooling with Dry Ice: yield 5.83 g (45%), mp 118–119°, $[\alpha]^{25}D$ −4.93° (*c* 2, methanol). Recrystallization from ethyl acetate–petroleum ether raised the melting point to 120–121° and $[\alpha]^{25}D$ to −5.99° (*c* 2, methanol).

Anal. Calcd for $C_{14}H_{18}BrFN_2O_8$: Br, 18.11; OCH_3, 7.03. Found: Br, 18.29; OCH_3, 7.18.

The mother liquor of the 5.83 g of *l* compound isolated above gave, upon addition of 50 ml of petroleum ether, 4.83 g of product which was recrystallized from a mixture of 5 ml of methanol and 10 ml of water: yield 3.97 g (31%), mp 118–119°, $[\alpha]^{25}D$ +45.6° (*c* 1.2, methanol). This product was identified by mixture melting point with *d*-MeOBrFUDRdiAc obtained by acetylation from *d*-MeOBrFUDR; λ_{max} (cm^{-1}) 3330, 1740 and 1715, 1230, shallow doublet at 1100 and 1080.

Oxidation of MeOBrFUDR to HOBrFU.—A mixture of 1.05 g (2.94 mmoles) of *d*-MeOBrFUDR and 10 ml of red fuming nitric acid was heated on a steam bath for 20 min. After cooling, 100 ml of water was added. The solution was evaporated *in vitro*, the residue was slurried with ether, and the mixture was chilled in a Dry Ice bath; yield 0.35 g (52.4%), mp 176–178° dec.

Recrystallization from about 7 ml of ethyl acetate (upon chilling with Dry Ice) gave 170 mg, mp 182–183° dec, $[\alpha]^{25}D$ +40.7° (*c* 1.0, ethyl acetate). An admixture of this *d*-HOBrFU with the *dl* compound prepared from FU (mp 179–180°) melted at 173–174°.

Anal. Calcd for $C_4H_4BrFN_2O_3$: C, 21.16; H, 1.78; Br, 35.20; F, 8.37. Found: C, 21.57; H, 1.99; Br, 35.05; F, 8.44.

The ethyl acetate mother liquor gave, upon addition of petroleum ether, a second crop of 165 mg, mp 177–178° dec, $[\alpha]^{25}D$ +32.7° (*c* 1.2, ethyl acetate).

The infrared spectra of *d*- and *dl*-HOBrFU[30] are essentially identical particularly in the CO (1748–1695 cm^{-1}) and the fingerprint regions. The characteristic difference consists in the presence of a distinct OH peak at 3500 cm^{-1} in the *d* compound, whereas only bonded OH and NH are present in the *dl* compound.

An analogous oxidation of crude (amorphous) *l*-MeOBrFUDR furnished 50 mg of crude amorphous *l*-HOBrFU, mp 177–178°, $[\alpha]^{25}D$ −34.2° (*c* 1, ethyl acetate).

To 4 ml of an ethyl acetate solution containing 20 mg of *d*-HOBrFU and 20 mg of *l*-HOBrFU was added 10 ml of petroleum ether. A crystalline precipitate was filtered and washed with petroleum ether, yield 17 mg, mp 173–175°, which on admixture with *dl*-HOBrFU (mp 179–180) melted at 176–177°.

***d*-5-Fluoro-6-methoxy-5,6-dihydro-2′-deoxyuridine (MeOHFUDR).**—A solution of 6.5 g of *d*-MeOBrFUDR (0.0182 mole) in 115 ml of water containing 1.55 g (0.0189 mole) of sodium acetate was hydrogenated in the presence of 0.85 g of 10% prehydrogenated Pd–C catalyst. The hydrogenation came to a standstill after 32 min when the calculated amount of 454 ml had been taken up. After removal of the catalyst by filtration, the optical density was determined in 0.1 *N* HCl and in 1 *N* NaOH: $E_{269}^{0.1 N HCl}$ was 16,800 corresponding to 2.58 mmoles (14.2%) of FUDR; $E_{281}^{0.1 N NaOH}$ was 125,500, corresponding to 17.94 mmoles of FUDR (existing + regenerated by NaOH). The yield of MeOHFUDR was therefore 15.36 mmoles (84.3%). The solution was lyophilized in order to remove acetic acid, the material obtained was dissolved in 76 ml of water and the pH of the solution was adjusted to 6 with a few drops of NH_4OH. It was then absorbed on a column of Dowex 1-4X (acetate), 100–200 mesh, 2.4 × 25 cm. Elution was performed (water for fractions 1–6, 1 *N* acetic acid for fractions 7–10) at a flow rate of 100–200 ml/hr and fractions were taken every 0.5 hr. Each fraction was analyzed for content of MeOHFUDR by taking the spectrum in 0.1 *N* HCl and in 1 *N* NaOH. The bulk of the compound (13.18 mmoles, in addition to 2.02 mmoles of FUDR) was found in fractions 3 and 4 (142 ml). Fractions 9 and 10 (201 ml) contained 1.71 mmoles of pure FUDR.

Fractions 3 and 4 were lyophilized, the dry material was dissolved in 65 ml of water and, after adjustment to pH 6, again chromatographed on a Dowex 1-4X (acetate) column, 2.4 × 24 cm. Elution (flow rate 200–250 ml/hr) yielded in fractions 3 and 4 (150 ml) a total of 13.06 mmoles of MeOHFUDR (purity 90.5%).

Fractions 3 and 4 were lyophilized, the dry material was dissolved in 62 ml of water, the solution was adjusted to pH 6 and chromatographed again on a 4 × 33 cm column of Dowex 1-X4 (acetate) 100–200 mesh, eluting with water (flow rate 200 ml/hr). Fractions 5 and 6 (208 ml) contained 11.25 mmoles of MeOH-FUDR (purity 97%). The material weighed, after lyophilization, 3.43 g. This was dissolved in a boiling mixture of 7 ml of dioxane and 37 ml of butyl acetate. By cooling, 2.58 g (51%) of crystals melting at 136.5–138.5° were obtained, $[\alpha]^{24}D$ +44.8° (*c* 1.0, methanol). According to spectrophotometry (only end absorption, $E_{280}^{0.1 N HCl}$ 113/g) it contained a maximum of 0.4% of FUDR.

Anal. Calcd for $C_{10}H_{15}FN_2O_6$: F, 6.83; CH_3O, 11.15. Found F, 7.00, 7.07; CH_3O, 11.35.

Conversion of MeOHFUDR into FUDR by NaOH.—Crude MeOHFUDR obtained by hydrogenation of 1 mmole MeOBr-FUDR was dissolved in 7 ml of 1 *N* NaOH. The spectrum exhibited $\lambda_{max}^{N NaOH}$ 268 mμ corresponding to 0.91 mmole of FUDR. The solution was fractionated on a Dowex 1-4X (acetate) column, 1.4 × 20 cm. The column was first eluted with 70 ml of water and then with 0.1 *N* acetic acid. A peak (145 ml) was taken up three times with a few milliliters of ethanol and reevaporated. The final residue was dissolved in 10 ml of boiling butyl acetate and the solution was allowed to crystallize in the cold; yield 146 mg (59%), mp 145.5–146°. A mixture with an authentic sample of FUDR (mp 149–150°) melted at 147–149°. Recrystallization gave 93 mg, mp 147–148°, which was further identified by its infrared spectrum and by analysis.

Anal. Calcd for $C_9H_{11}FN_2O_5$: C, 43.91; H, 4.51. Found: C, 44.12; H, 4.43.

***d*-5-Bromo-5-fluoro-6-ethoxy-5,6-dihydro-2′-deoxyuridine (EtOBrFUDR).**—A solution of 10 ml of bromine (31.2 g, 0.39 mole) in 640 ml of ethanol was stirred for 30 min at −5° with 107.5 g of Ag_2CO_3 (0.39 mole). The light yellow solution was filtered rapidly into an ice-cold (partial) solution of 30 g of FUDR (0.122 mole) in 500 ml of ethanol. Continued stirring produced rapid dissolution. After allowing the mixture to stand for 16 hr in the refrigerator, it was concentrated *in vacuo* at 30° to a deep orange-red syrup.

The latter was dissolved in 200 ml of ethanol and the Dry Ice cooled solution was stirred with small amounts of Ag_2CO_3 which were added gradually until the liquid was colorless. After filtration through Celite the solution showed only end absorption (E_{280}^{EtOH} 12,600). Evaporation *in vacuo* gave a pale yellow syrup which became partly crystalline after seeding. It was taken up with 25 ml of butyl acetate and allowed to crystallize in the cold; yield 7.06 g, mp 128–129.5°. The mother liquor gave, upon addition of petroleum ether, a second crop of 10 g, mp 127.5–128.5°; total yield 37.5%. Recrystallization from 1.4 vol of ethyl acetate raised the melting point to 129–130°, $[\alpha]^{26}D$ +60.2° (*c* 2.0, methanol). Bioautography showed absence of FUDR (less than 0.1%).

Anal. Calcd for $C_{12}H_{16}BrFN_2O_6$: Br, 21.51; F, 5.12; C_2H_5O, 12.12. Found: Br, 20.94; F, 5.08; C_2H_5O, 12.22.

***d*-5-Fluoro-6-ethoxy-5,6-dihydro-2′-deoxyuridine (EtOH-FUDR).**—Ten grams (27 mmoles) of *d*-EtOBrFUDR (mp 127.5–128.5°) were hydrogenated in 100 ml water containing 2.25 g of sodium acetate (27.4 mmoles) in the presence of 0.5 g of 10% Pd–C catalyst. The hydrogen uptake was 630 ml (97.5%) within 40 min. The catalyst was filtered, and the filtrate was lyophilized. Chromatography on a Dowex 1-X4 acetate column 3.5 × 32 cm gave, by elution with water, two fractions (320 ml) of only weakly absorbing material. Fraction 3 (94.5 ml), fraction 4 (132 ml), and fraction 5 (132 ml) contained 13.5 (98% pure), 7.02 (91% pure), and 1.39 (86.1% pure) mmoles of *d*-EtOHFUDR, respectively. Total yield was 78.3%. Fraction 3 was lyophilized and the dry material (4.01 g) was crystallized from 96 ml of ethyl acetate to yield 2.79 g (35.3%), mp 146–147°. $E_{280}^{0.1 N HCl}$ was found to be 123/g which corresponds to a maximal FUDR content of 0.46%; $[\alpha]^{26}D$ +37.2° (*c* 2.0, water).

Anal. Calcd for $C_{11}H_{17}FN_2O_6$: C_2H_5O, 15.42; F, 6.50. Found: C_2H_5O, 15.79; F, 5.94.

5-Bromo-5-fluoro-6-*t*-butoxy-5,6-dihydro-2′-deoxyuridine (Mixture of Diastereoisomers) (*t*-BuOBrFUDR).—To a solution of 5 ml (15.6 g) of Br_2 (97 mmoles) in 100 ml of *t*-butyl alcohol was added with stirring 50 g of Ag_2CO_3 (182 mmoles). After 95 min of stirring the mixture was filtered directly into a stirred suspension of 16.5 g of FUDR (25 mmoles) in 150 ml of *t*-butyl alcohol. The reaction mixture became homogeneous within 15 min and the optical density E_{280}^{MeOH} dropped gradually within 2.5 hr from initially 163,600 to 7350.

The mixture was evaporated to a froth which was dissolved in 40 ml of ether. The solution was poured slowly with stirring into 150 ml of petroleum ether to produce a white slightly gummy precipitate, which was dissolved again in 50 ml of ether and reprecipitated with 350 ml of petroleum ether. A white electrostatic solid (7.9 g, 79%) was isolated by filtration and washing with petroleum ether. It gradually became soft upon heating to 65° and then decomposed at 75–95° (gas evolution); $[\alpha]^{25}D$ +36.3° (*c* 1, methanol).

Anal. Calcd for $C_{13}H_{20}BrFN_2O_6$: C, 39.11; H, 5.05; Br, 20.02; N, 7.02. Found: C, 39.32; H, 5.43; Br, 20.03; N, 6.82.

5-Bromo-5-fluoro-6-acetoxy-5,6-dihydro-2′-deoxyuridine 3′,5′-Diacetate (Diastereomeric Mixture) (AcOBrFUDRdiAc).—To an ice-cold solution of 9.89 g of FUDR 3′,5′-diacetate (33.4 mmoles) in a mixture of 75 ml of acetic acid and 75 ml of acetic anhydride, was added with cooling and stirring 5.85 g of silver acetate (35 mmoles) and 1.8 ml of bromine (5.6 g, 35 mmoles). The mixture was allowed to rise to room temperature and was stirred 30 min. AgBr was filtered and washed with acetic acid. The orange-colored filtrate contained neither Ag^+ or Br^- and had E_{280}^{EtOH} 3450. It was evaporated *in vacuo* to a syrup, which was dissolved in 50 ml of ether. The solution was clarified by filtration through Celite and poured with stirring into 700 ml of petroleum ether. After 15 min of stirring, a white powdery precipitate and sticky material adhering to the glass walls was formed. The powdery precipitate (6.69 g) was filtered and washed with petroleum ether. The sticky material was redissolved in 25 ml of ether and reprecipitated with 250 ml of petroleum ether to yield 5.62 g, $[\alpha]^{27}D$ +9.3° (*c* 1.0, $CHCl_3$).

Anal. Calcd for $C_{15}H_{18}BrFN_2O_9$: C, 38.39; H, 3.87; N, 5.97; F, 4.05; Br, 17.03. Found: C, 38.71; H, 3.96; N, 5.81; F, 3.98; Br, 16.71.

The crop of 6.69 g became sticky upon storage. It was dissolved in 10 ml of ether and the solution was poured with stirring into 250 ml of petroleum ether. The gummy precipitate was redissolved in 75 ml of ether and again poured into the original mother liquor. After stirring for 30 min the precipitate was filtered; yield 4.87 g, $[\alpha]^{27}D$ +7.4° (*c* 2.0 in chloroform), total yield 67%.

Anal. Calcd for $C_{15}H_{18}BrFN_2O_9$: C, 38.39; H, 3.87; Br, 17.03. Found: C, 38.31; H, 3.81; Br, 17.16.

***d*-5-Chloro-5-fluoro-6-methoxy-5,6-dihydro-2′-deoxyuridine (MeOClFUDR).**—Chlorine gas was passed into 800 ml of methanol (cooled in an ice bath) until iodometric determination showed a titer of 2.4 mmoles of Cl_2/ml. To 120 ml of this solution (288 mmoles/of Cl_2) was added 79 g of Ag_2CO_3 (287 mmoles) with stirring and cooling below 0° for a period of 30 min. The MeOCl solution thus prepared was filtered directly into a well-cooled solution of 15 g of FUDR (61 mmoles) in 200 ml of methanol. A colorless solution was obtained containing 510,000-optical density units at λ_{max}^{MeOH} 270 mμ. Exposure to room temperature for 45 min followed by storage in the refrigerator produced no drop of the absorbancy. More methanolic chlorine solution (not treated with Ag_2CO_3) was then added gradually and the mixture was allowed to stand at room temperature until a sample showed practically no $E_{280}^{0.1 N HCl}$. This solution (Cl^- content 1.8 equiv) was neutralized to pH 6.8 by addition of 418 ml of 4.3 *N* $NaOCH_3$ in methanol. The precipitated NaCl (92.2 g) was removed by filtration. Evaporation of the filtrate to a syrup and further treatment of the syrup with methanol and ether removed additional 10.91 g of NaCl. The final filtrate gave upon evaporation a partially crystallizing syrup which was mixed with 20 ml of water to produce rectangular plates of *d*-MeOClFUDR which were filtered and washed free of Cl^- with 50 ml of water; yield 4.17 g (22%), mp 169.5–170° (decomposed at 210°). Recrystallization from 25 ml of butyl acetate gave 2.72 g, mp 167–169° (decomposed at 192°), which was chromatographically uniform; rotation in methanol (*c* 1): $[\alpha]^{25}$ +64.6° (578 mμ), +72.3° (546 mμ), +130.1° (436 mμ), +195° (364 mμ).

Anal. Calcd for $C_{10}H_{14}ClFN_2O_6$: C, 38.41; H, 4.51; Cl, 11.34; F, 6.08; OCH_3, 9.93. Found: C, 38.68; H, 4.44; Cl, 11.36; F, 6.28; OCH_3, 9.92.

***l*-5-Chloro-5-fluoro-6-methoxy-5,6-dihydro-2′-deoxyuridine (Crude) (MeOClFUDR).**—The mother liquor of the isolated *d*-MeOClFUDR deposited, upon evaporation and treatment of the residue with methanol, an additional 4.16 g of NaCl (total recovered 99%). The filtrate gave upon evaporation a syrup which was dissolved in 30 ml of water. The slightly acid (pH

3.8) solution was neutralized with a few drops of 1 *N* NaOH. It contained $E_{280}^{0.1 N HCl}$ 332 and 11.3 mequiv of Cl^-. The solution was chromatographed through a Dowex 1-X4 acetate column containing 45 g of resin. Elution with water removed first Na^+; subsequent elution with 0.05 *N* acetic acid gave fractions showing end absorption starting at 270 mμ when measured in 0.1 *N* HCl. Lyophilization of these eluates gave 2.86 g (14.9%) of colorless material which was chromatographically uniform (cysteine–sulfuric acid spray); rotation in methanol (*c* 1): $[\alpha]^{25}$ −7.5° (578 mμ), −9.0° (546 mμ), −19.1° (436 mμ), −39.2° (364 mμ).

Anal. Calcd for $C_9H_{14}ClFN_2O_6$: Cl, 11.34; F, 6.08; N, 8.96; OCH_3, 9.93. Found: Cl, 10.81; F, 6.08; N, 8.75; OCH_3, 9.67.

5-Chloro-5-fluoro-6-*t*-butoxy-5,6-dihydro-2'-deoxyuridine (Diastereomeric Mixture) (*t*-BuOClFUDR).—To a solution (heated to 35°) of 5 g of FUDR (20.3 mmoles) in 100 ml of *t*-butyl alcohol was added 3 ml (2.75 g, 25.2 mmoles) of *t*-butyl hypochlorite. No reaction took place at room temperature. The mixture was refluxed for 80 min, when the E_{270}^{EtOH} had dropped from initially 157,000 to 1740. Evaporation *in vacuo* left 11.25 g of a thin syrup which was dissolved in about 50 ml of benzene. Evaporation gave a spongy mass which was repeatedly dissolved in ether and reevaporated. The residue had now become sparingly soluble in ether. It was triturated with portions of ether. The ether extract was poured into petroleum ether to precipitate the reaction product. A total of about 100 ml of ether and 800 ml of petroleum ether was used. The precipitate was filtered and washed with petroleum ether: yield 4.63 g (65%), mp 59–64°, $[\alpha]_D$ +27° (*c* 1.0 methanol).

Anal. Calcd for $C_{13}H_{20}ClFN_2O_6$: Cl, 9.99; F, 5.36; N, 7.90. Found: Cl, 10.40; F, 5.76; N, 8.12.

A solution of 354 mg of *t*-BuOClFUDR (1 mmole) in 20 ml of water and 2 ml of ethanol containing 88 mg of sodium acetate (1.07 mmoles) was hydrogenated with 40 mg of 10% Pd-C catalyst. Hydrogen uptake was 26.1 ml in 35 min. The solution filtered from the catalyst showed $E_{280}^{0.1 N HCl}$ 1816 corresponding to 0.28 mmole of FUDR and $E_{268}^{0.1 N NaOH}$ 7040 corresponding to 1 mmole of FUDR (existing + regenerated). Hence 0.72 mmole of *t*-BuOHFUDR was formed, which, however, was not isolated.

5-Bromo-5-fluoro-6-methoxy-5,6-dihydro-2'-deoxycytidine (Diastereoisomeric Mixture) (MeOBrFCDR).—To a cold solution of 0.25 ml of bromine (0.78 g, 4.88 mmoles) in 50 ml of methanol was added 1.4 g of Ag_2CO_3 (5.07 mmoles). The mixture was stirred with cooling in an ice bath for 20 min. Then a cold solution of 1 g of 5-fluoro-2'-deoxycytidine (4.08 mmoles) in 100 ml of methanol was added. After stirring and cooling for 10 min the absorption had dropped to $E_{280}^{0.1 N HCl}$ 4000. Filtration gave a colorless solution showing a positive starch–iodine test, indicative of partial bromination of the amino group. Lyophilization gave 1.75 g of a powder which contained excess bromine.

The product was dissolved in 15 ml of water and chromatographed on a Dowex 1-X4 (acetate) column, 1 × 11 cm. Water (59 ml) eluted ultraviolet-absorbing material. The solution was lyophilized to yield 522 mg. A subsequent fraction (76 ml) gave an additional 34 mg: total yield 38.5%; $[\alpha]^{25}_D$ +41.3° (*c* 1.0, methanol): $\lambda_{max}^{0.1 N NaOH}$ 266 mμ (ε 8640), $\lambda_{max}^{pH 7}$ 245 mμ (ε 8060), in 0.1 *N* HCl only end absorption (ϵ_{260} 1770) was found.

Anal. Calcd for $C_{10}H_{15}BrFN_3O_5 \cdot H_2O$: Br, 21.36; F, 5.08; N, 11.23; CH_3O, 8.29. Found: Br, 20.88; F, 5.29; N, 11.36; CH_3O, 8.10.

Subsequent elution with 0.1 *N* acetic acid gave a fraction of 80 ml which was lyophilized to yield 600 mg of powdery product. It gave a positive starch–iodine test, $\lambda_{max}^{pH 7}$ 263–267 mμ (ε 7530). The analysis agrees with a hypobromous acid salt of MeOBrFCDR, or N-bromo derivative containing 1 mole of water.

Anal. Calcd for $C_9H_{15}Br_2N_3O_6$: Br, 35.28; N, 9.27; CH_3O, 6.85. Found: Br, 34.93; N, 9.30; CH_3O, 6.95.

***d*-5-Bromo-6-methoxy-5,6-dihydrothymidine (MeOBrTDR).**—An ice-cold freshly prepared solution of 131 mmoles of methyl hypobromite was treated at +2° by stirring with a suspension of 12.36 g of thymidine (51 mmoles) in 210 ml of methanol. After 15 min complete solution was observed (E_{268}^{EtOH} was 5940). The solution was evaporated *in vacuo* to a syrup and the latter was dissolved in 30 ml of warm butyl acetate. Upon cooling, crystals were obtained which were filtered and washed with cold butyl acetate, and then with ether: yield 14.56 g (81%), mp 101–102° dec. Recrystallization from 46 ml of ethyl acetate gave 10.40 g of product melting at 102–103° dec, $[\alpha]_D$ +77° (*c* 3.0, methanol).

Anal. Calcd for $C_{11}H_{17}BrN_2O_6$: C, 37.41; H, 4.85; N, 7.93; Br, 22.63; CH_3O, 8.79. Found: C, 37.44; H, 5.00; N, 7.90; Br, 22.55; CH_3O, 9.02.

Catalytic hydrogenation in the presence of sodium acetate resulted in 73% reconversion into thymidine.

5-Bromo-5-fluoro-6-methoxy-5,6-dihydro-2'-deoxyuridine 5'-Phosphate (Mixture of Diastereoisomers, MeOBrFUDRP).—A solution of 0.1 ml of Br_2 (2 mmoles) in 10 ml of methanol was treated in the cold with 0.55 g of Ag_2CO_3 (2 mmoles). One milliliter of the MeOBr thus prepared (0.2 mmole) was mixed with an ice-cold solution of 9.5 mg of ammonium 5-fluoro-2'-deoxyuridylate (26.4 μmoles) in 5 ml of methanol. The drop of the ultraviolet absorption came to a stop after 2 hr. The solution was evaporated to dryness, taken up with methanol and reevaporated to yield 16 mg of a white solid. A paper chromatogram (descending system I) showed two spots (R_f 0.41 and 0.61) upon inspection with ultraviolet light. Both gave a deoxyriboside test but only the spot of R_f 0.61 gave a strong phosphate reaction upon treatment with Hanes–Isherwood reagent.[51] The product was dissolved in 1 ml of methanol and streaked across 3 MM Whatman paper which had been previously washed with the solvent system I used for the chromatography and dried. Chromatography with the same system produced a migration of 30 cm of the liquid front in 22.5 hr. Bands were visible upon inspection with ultraviolet light at R_f 0.54 and 0.77. The latter band was excised and the paper was extracted with water in the refrigerator overnight. The extract showed only end absorption and contained 16.8 μmoles of P. After lyophilization 11.3 mg of a white solid was obtained which according to the P content corresponded to 62% of anhydrous free nucleotide.

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(51) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).