after various time intervals, the reaction was stopped by the addition of 3.0 ml of 20% (w/v) aqueous trichloroacetic acid. Azo compound remaining was extracted with *n*-butyl alcohol and estimated spectrophotometrically.

Biological Test Methods.—Toxicity determinations were performed using male Swiss mice (22–26 g). The compound, dissolved in saline or suspended in 10% gum acacia, was administered by intraperitoneal injection to groups of 3–6 mice/dose level. Deaths within a 21-day period were recorded and approximate LD_{50} values were estimated graphically from per cent mortality/log dose plots.

Antitumor activities of the compounds against the Murphy-Sturm lymphosarcoma were assessed as follows. The tumor was implanted subcutaneously into male Holtzman rats using a trochar and cannula. Five days later when the tumor had reached a size of about 5 g, the compound was injected intraperitoneally daily for 5 days. Control animals received the vehicle only. On day 12, the volumes of the tumors were calculated from measurements taken by a caliper,[a] and the mean tumor volume of treated rats was compared with the mean tumor volume of control rats (T/C in Table I). Rats were subsequently observed to determine whether complete regression of the tumors occurred.

Synthesis of Fluorinated Pyrimidines and Triazines[1,2]

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The synthesis of fluorinated pyrimidines and triazines as potential thymidylate synthetase inhibitors was accomplished by treating the appropriate carbonyl compound with sulfur tetrafluoride in the presence of hydrofluoric acid. The anomers of 5-trifluoromethyl-6-aza-2′-deoxyuridine were prepared by conventional procedures from 5-trifluoromethyl-6-azauracil. Some preliminary biological results are presented.

One possible approach to the selective control of DNA synthesis and mitosis is by inhibition of the synthesis of thymidine 5′-phosphate (TMP). Thymidylate synthetase[3] catalyzes the conversion of 2′-deoxyuridine 5′-monophosphate (dUMP) to TMP in the presence of the carbon donor, N^5,N^{10}-methylenetetrahydrofolic acid (CH_2THFA). The reaction has been studied in several laboratories[3] and the requirements suggest a sequential reaction of the enzyme with the cofactor (CH_2THFA) followed by reaction of this complex with the substrate dUMP.

Since 5-fluoro-2′-deoxyuridine 5′-monophosphate[3e] (FdURP) is known to be a strong inhibitor of the enzyme, substitution of fluorine for hydrogen in the methyl group of thymine might also confer inhibitory properties. The increase of electronegativity associated with the trifluoromethyl group might be a desirable feature, since Baker in 1960,[4] had postulated that an increased acidity of the N-3 hydrogen could improve the properties of the analogs and allow them to be more strongly bound to the enzyme receptor site.

Thus, 5-trifluoromethyluracil (**2**), 5-difluoromethyluracil (**4a**), and 5-trifluoromethyl-6-azauracil (**6**) and its α- and β-2′-deoxyribosides (**7a** and **b**) were synthesized in an effort to study the requirements of an effective antimetabolite of the thymidylate synthetase sequence of reactions. During the course of this work Heidelberger and co-workers[5] reported the synthesis of 5-trifluoromethyluracil (**2**) and 5-trifluoromethyl-2′-deoxyuridine by another route. The synthesis of 5-trifluoromethyl-6-azauracil (**6**) and the 2′-deoxyriboside anomers (**7**) was communicated[2c,d] jointly with Shen and co-workers.[6]

The initial approaches to the synthesis of **2** *via* primary ring synthesis utilizing ethyl 3,3,3-trifluoropropionate[7] in analogy to Whitehead's[8] synthesis of 5-carbethoxyuracil were unsuccessful. The second approach was based on the aromatic character of C_5 in uracil.[9] Since the trifluoromethyl radical has been reported to add readily to various aromatic systems[10] this procedure was applied to uracil. Photochemical attempts were unsuccessful; thermal decomposition of trifluoromethyl iodide in the presence of mercuric chloride[11] gave uracil-5-carboxylic acid (**1**) indicating that reaction occurred followed by hydrolysis.

Since sulfur tetrafluoride was introduced as a reagent for the conversion of the carboxyl group to the trifluoromethyl group, reports have appeared describing the selective nature of this reagent.[12] Raasch noted the protective effect and Martin, *et al.*,[13] the enhance-

(1) This work was generously supported by grant CA-5639 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) Preliminary communications of portions of this work have appeared: (a) M. P. Mertes and S. E. Saheb, *J. Pharm. Sci.*, **52**, 508 (1963); (b) *J. Med. Chem.*, **6**, 619 (1963); (c) *J. Heterocyclic Chem.*, **2**, 491 (1965); (d) M. P. Mertes, S. E. Saheb, and D. Miller, *ibid.*, 493 (1965). Portions of this work were presented at the Symposium on Newer Concepts of Structure Activity Relationships, 112th Meeting of the American Pharmaceutical Association, Detroit, Mich., March 1965, Abstract A-111. While this paper was in press a similar publication appeared: A. Dipple and C. Heidelberger, *J. Med. Chem.*, **9**, 715 (1966).

(3) (a) A. J. Wahba and M. Friedkin, *J. Biol. Chem.*, **237**, 3794 (1962), and references therein; (b) R. L. Blakley, *ibid.*, **238**, 2113 (1963), and references therein; (c) C. K. Mathews and S. S. Cohen, *ibid.*, 376 (1963), and references therein; (d) E. Jenny and D. M. Greenberg, *ibid.*, 3378 (1963); (e) P. Reyes and C. Heidelberger, *Mol. Pharmacol.*, **1**, 14 (1965), and references therein; (f) P. M. Frearson, S. Kit, and D. R. Dubbs, *Cancer Res.*, **25**, 737 (1965); G. R. Greenberg, R. L. Sommerville, and S. DeWolf, *Proc. Natl. Acad. Sci. U. S.*, **48**, 242 (1962).

(4) B. R. Baker in "Conference on Experimental Clinical Cancer Chemotherapy," B. H. Morrison, Ed., National Cancer Institute Monograph No. 3, August 1960, p 9.

(5) C. Heidelberger, D. G. Parsons, and D. C. Remy, *J. Am. Chem. Soc.*, **84**, 3597 (1962); *J. Med. Chem.*, **7**, 1 (1964).

(6) T. Y. Shen, W. V. Boyle, and R. L. Bogianesi, *J. Heterocyclic Chem.*, **2**, 495 (1965), and ref 2d.

(7) F. Brown and W. K. R. Musgrave, *J. Chem. Soc.*, 2087 (1953).

(8) C. W. Whitehead, *J. Am. Chem. Soc.*, **74**, 4267 (1952).

(9) D. J. Brown, "The Pyrimidines," Interscience Publishers, Inc., The Netherlands, 1962.

(10) I. M. Whittemore, A. P. Stefani, and M. Szwarc, *J. Am. Chem. Soc.*, **84**, 3799 (1962); E. Hayser and E. Bedard, *J. Org. Chem.*, **29**, 1588 (1964).

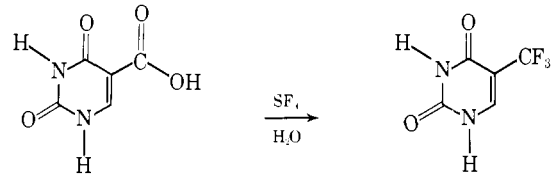
(11) J. Barnus, H. J. Emeleus, and R. N. Haszeldine, *J. Chem. Soc.*, 3041 (1950).

(12) W. R. Hasek, W. C. Smith, and V. A. Engelhart, *J. Am. Chem. Soc.* **82**, 543 (1960).

(13) M. S. Raasch, *J. Org. Chem.*, **27**, 1406 (1962); D. G. MacGn and F. Kagan, *ibid.*, **27**, 3161 (1962); D. G. Martin and J. R. Pike, *ibid.*, **27**, 4085 (1962).

ment of the yield when an excess of hydrogen fluoride was used during fluorination.

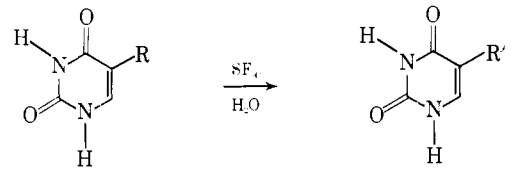
Uracil-5-carboxylic acid (**1**) was recovered unchanged when treated with sulfur tetrafluoride at room temperature and at 50°, with or without hydrofluoric acid. At 100° in the presence of hydrofluoric acid, a 77% yield of 5-trifluoromethyluracil (**2**) was obtained.



1 2

5-Formyluracil (**3a**), the precursor of 5-difluoromethyluracil (**4a**), was prepared by the Reimer–Tiemann reaction on uracil as reported by Wiley and Yamamoto.[14] The substitution of the carbonyl by a difluoro function was achieved with sulfur tetrafluoride; the product **4a** proved to be labile in alkaline media. In neutral media slow decomposition of 5-difluoromethyluracil to the aldehyde **3a** was noted.[15]

5-(1,1-Difluoroethyl)uracil (**4b**) was prepared from 5-acetyluracil[16] (**3b**) by the same method to study the inductive effect of the methyl group on the stabilization of the two α-fluorine atoms. Rapid hydrolysis prevented reliable elemental analysis. The ultraviolet spectrum (hydrochloric acid) and the nmr spectrum



3a, R = CH=O
b, R = $COCH_3$

4a, R′ = CHF_2
b, R′ = CF_2CH_3

(trifluoroacetic acid) showed a λ_{max} at 272 mμ and a triplet due to coupling with the two α fluorines at 1.0 ppm (J = 20 cps). After several minutes a shift was observed in both spectra that was identical with the spectra of the starting material, 5-acetyluracil (**3b**).

The synthesis of 5-monofluoromethyluracil was unsuccessfully attempted by treatment of the alcohol with sulfur fluoride–hydrogen fluoride, displacement of the tosylate with potassium fluoride, and halogen exchange using either 5-chloromethyl- or 5-bromomethyluracil[17] with silver fluoride or antimony trifluoride. In the light of the instability of 5-chloromethyl- and 5-bromomethyluracil,[17] difficulty in isolating 5-fluoromethyluracil would be expected.

The synthesis of 5-carboxy-6-azauracil (**5**) was according to the procedure of Barlow and Welch;[18] the hydrolysis at C_2 was performed in a one-step reaction

(14) R. H. Wiley and Y. Yamamoto, *J. Org. Chem.*, **25**, 1906 (1960).

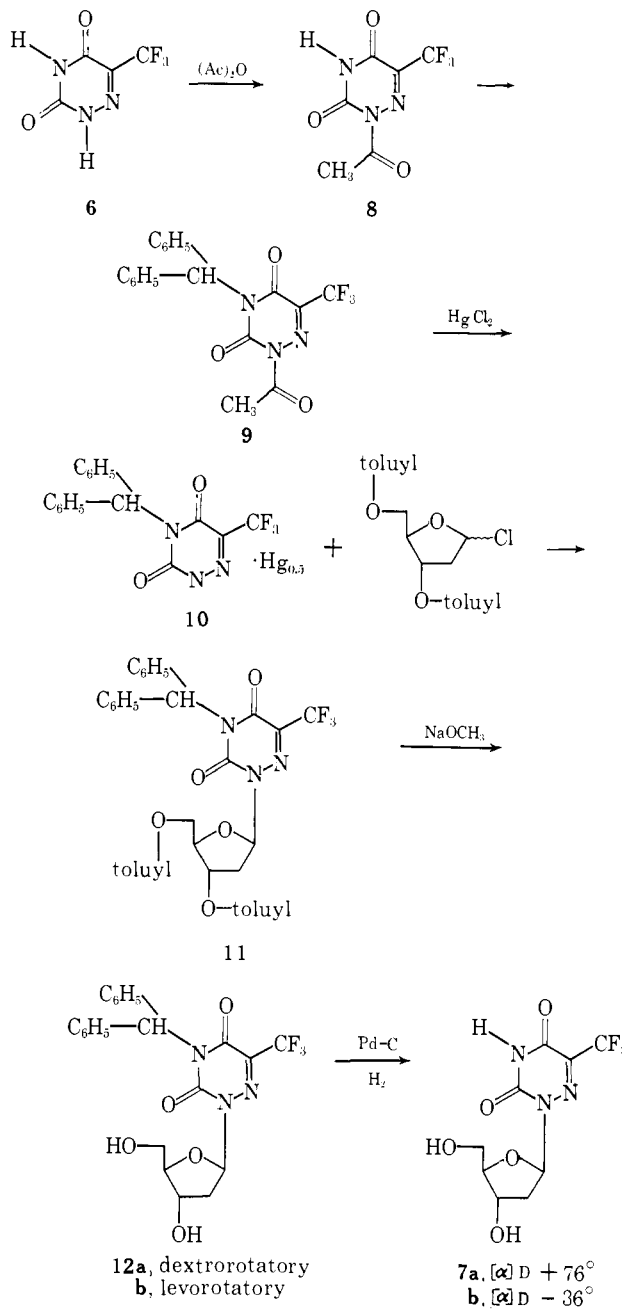
(15) Heidelberger and co-workers noted in a private communication that a sample of 5-difluoromethyluracil submitted for testing was shown to be completely converted to the corresponding aldehyde in 2 min at pH 6.5 in 0.1 *M* phosphate buffer; at pH 4.0 in distilled water, 50% was converted to the aldehyde in 150 min with a shift in pH to 4.3; at pH 1 in HCl, the compound was unchanged in 3 hr.

(16) L. Claisen, *Ann.*, **297**, 1 (1897); W. Bergmann and T. B. Johnson, *Ber.*, **66**, 1492 (1933).

(17) W. A. Skinner, M. G. M. Schelstrate, and B. R. Baker, *J. Org. Chem.*, **25**, 149 (1960); J. H. Burkhalter, R. J. Seiwald, and H. C. Scarborough, *J. Am. Chem. Soc.*, **82**, 991 (1960); J. H. Carbon, *J. Org. Chem.*, **25**, 1731 (1960).

(18) R. B. Barlow and A. D. Welch, *J. Am. Chem. Soc.*, **78**, 1258 (1956).

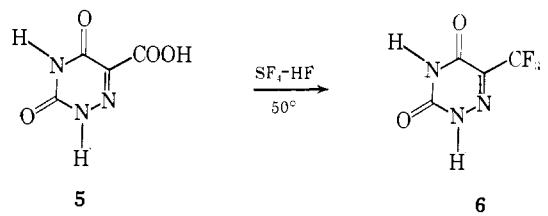
SCHEME I



12a, dextrorotatory
b, levorotatory

7a, $[\alpha]_D$ + 76°
b, $[\alpha]_D$ − 36°

using chloroacetic acid. Conversion of the carboxyl at C_5 to the trifluoromethyl group was successful at 50°, giving 5-trifluoromethyl-6-azauracil (**6**).



5 6

In general, ribosidation and deoxyribosidation of the natural pyrimidines and purines is performed by the Hilbert–Johnson procedure.[19] In contrast to the pyrimidines, azapyrimidines form a mercury complex at both N_1 and N_3. Acetic anhydride, reported[20] to

(19) J. J. Fox and I. Wempen, *Advan. Carbohydrate Chem.*, **14**, 283 (1959); J. A. Montgomery and H. J. Thomas, *ibid.*, **17**, 301 (1962).

(20) M. Prystas and F. Šorm, *Collection Czech. Chem. Commun.*, **30**, 81 (1965).

TABLE I

POSITION OF PROTON LOSS ON FORMATION OF THE MONOANION AND DIANION IN PYRIMIDINES AND TRIAZINES

Compd	pH	λ_{max}, mμ	pH	λ_{max}, mμ	pH	λ_{max}, mμ	Site of deprotonation to give monoanion
Uracil[a]	4.3	260	11.2	284	14	275	N_1
6-Chlorouracil[b]	1.0	262	10	283	14	275	N_1
6-Trifluoromethyluracil[c]	3.7	259	8.3	291	14	282	N_1
5-Chlorouracil[b]	5.0	275	11	300	14	289	N_1
5-Bromouracil[b]	5.0	277	11	302	14	291	N_1
5-Fluorouracil[b]	5.0	268	11	270	14	286	N_3
5-Trifluoromethyluracil (**2**)	4.0	257	9.3	281	13.4	273	N_1
6-Azauracil[a]	5.2	259	9.6	251	14	288	N_3
5-Trifluoromethyl-6-azauracil (**6**)	1.0	262	8.5	257	12.2	292	N_3

[a] Reference 24. [b] Reference 25. [c] Reference 26.

acylate specifically on N_1 in the great majority of pyrimidines, gave 1-acetyl-5-trifluoromethyl-6-azauracil (**8**). The alkylation at N_3[21] with diphenyldiazomethane[22] was followed by the displacement of the acetyl group in **9** to give **10** (Scheme I).

3,5-Di-O-toluyl-D-2′-deoxyribofuranosyl chloride[23] was added to a toluene solution of **10** which after purification on alumina gave the protected deoxynucleoside (**11**) as a pale yellow glass. The integration and assignments in the nmr spectrum verified structure **11**. Methanolysis and chromatography of the product gave 1-(2′-deoxy-D-ribofuranosyl)-3-diphenylmethyl-5-trifluoromethyl-6-azauracil (**12**). Hydrogenolysis of the diphenylmethyl derivative **12** gave a quantitative yield of diphenylmethane, and the product **7** was isolated as the mixed calcium and sodium salt. Conversion to the acid form was effected by washing through a cation-exchange resin to give 5-trifluoromethyl-6-aza-2′-deoxyuridine (**7**).

A partial separation of the anomers of the diphenylmethane derivative **12** was accomplished on silica. The first fraction (dextrorotatory) was reduced to give the (+) anomer **7a**, $[\alpha]^{24}D$ +76°; the levorotatory anomer **12b** was reduced to give **7b**, $[\alpha]^{24}D$ −36°. The synthesis of **7** was concurrently announced[2d] with a similar report from Shen and co-workers,[6] who synthesized the β anomer by utilization of the 3,5-bistrimethylsilyloxy derivative of **6**. Their structural assignment, based on optical rotatory dispersion and nmr data, suggests, in accord with Hudson's isorotation rules, that the levorotatory isomer **7b** is the β anomer.

Ultraviolet and pK_a Studies.—Based on spectrophotometric studies (Table I) of substituted uracils it has been demonstrated that formation of the monoanion at N_1 (3-methyluracil) corresponds to a bathochromic shift in contrast to the hyposchromic shift observed for the monoanion at N_3 (1-methyluracil).[24] The shifts in absorption maxima for uracil, a bathochromic shift in the monoanion and a hyposchromic shift in the dianion, suggested initial ionization of the N_1 proton. Wempen and Fox[25] observed a bathochromic shift which indicated that 6-halouracils formed the monoanion by loss of the proton from N_1, while the 5-halouracils gave mixed dissociation; 5-chlorouracil gave predominantly ionization at N_1, while 5-fluorouracil gave mainly ionization at N_3.

Giner-Sorolla and Bendich[26] noted a 32-mμ bathochromic shift in the formation of the monoanion of 6-trifluoromethyluracil and proposed ionization initially at N_1. The ultraviolet spectra of 5-trifluoromethyluracil (**2**) at varying pH are shown in Figure 1. The maximum at 257 mμ (pH 6 and below) shifts to 281 mμ (pH 9.28 and 10.6) for the monoanion and, in analogy with the bathochromic shift in uracil, is proposed to ionize initially at N_1. At a pH of 13.4 the ultraviolet spectrum of a fresh solution of **2** displayed a maximum at 273 mμ that rapidly shifted to 289 mμ indicating hydrolysis to 5-carboxyuracil (**1**).[5] The initial bathochromic shift followed by a hyposchromic shift on increasing the pH indicates that 5-trifluoromethyluracil (**2**) ionizes initially at N_1 followed by loss of the N_3 proton to the dianion.

Jonas and Gut[24] also examined the spectral shift pattern in substituted 6-azauracils. They found that loss of the proton from N_1 (3-methyl-6-azauracil) caused a bathochromic shift, and loss of the N_3 proton (1-methyl-6-azauracil) gave a hypsochromic shift which corresponds to the shifts observed in 3- and 1-methyluracils. 6-Azauracil is reported to undergo a hypsochromic shift of 8 mμ suggesting, in contrast to uracil, initial loss of the N_3 proton. The dianion showed a bathochromic shift of 37 mμ from the monoanion absorption maximum.

5-Trifluoromethyl-6-azauracil (**6**) showed maximum absorption peaks at 262 (pH 1), 257 (pH 7.5–9.0), and 292 mμ (pH above 11.7) (Figure 2). Thus, the pattern of shifts on proceeding from the undissociated form to the monoanion and finally to the dianion suggests, in analogy to 6-azauracil and 5-fluorouracil, loss of the proton initially from N_3 to give the monoanion. The formation of the N^1-deoxyriboside (**7**) is also supported by the ultraviolet studies. The change in the maximum absorption peak from 269 mμ at pH 1 to 264 mμ at pH 12.2 is indicative of ionization of the N_3 proton in structure **7** in formation of the monoanion.

The pK_a (7.35)[5] of 5-trifluoromethyluracil (**2**) represents a considerable increase in the acidity of the ring when compared to thymine (pK_a = 9.8). Chang[27] has reported the pK_a values of substituted 6-azauracils and has found that substitution of a nitrogen for the 6 carbon of uracil (6-azauracil) increases the acidity of the ring by 2.5 pK_a units. The pK_a of 5-methyl-6-

(21) M. Prystas and F. Sorm, *Collection Czech. Chem. Commun.*, **27**, 1578 (1962).

(22) L. I. Smith and K. L. Howard, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p 351.

(23) M. Hoffer, *Chem. Ber.*, **93**, 2777 (1960).

(24) J. Jonas and J. Gut, *Collection Czech. Chem. Commun.*, **26**, 2155 (1961); K. Y. Zee-Cheng and C. C. Cheng, *J. Org. Chem.*, **27**, 976 (1962).

(25) I. Wempen and J. J. Fox, *J. Am. Chem. Soc.*, **86**, 2747 (1964).

(26) A. Giner-Sorolla and A. Bendich, *ibid.*, **80**, 5744 (1958).

(27) P. K. Chang, *J. Org. Chem.*, **26**, 1118 (1961).

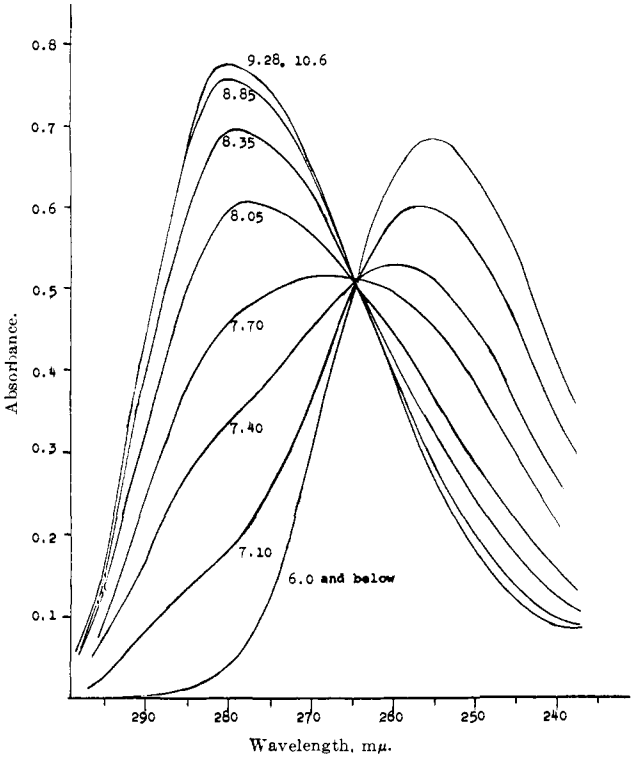


Figure 1.—Ultraviolet spectrum of 5-trifluoromethyluracil at varying pH.

azauracil (azathymine) was found to be 7.3 (by titration). The pK_a of 5-trifluoromethyl-6-azauracil (**6**) was determined by plotting the absorbance at 282 mμ against pH (Figure 2). The apparent pK_a obtained from the plot is approximately 5.8, whereas titration showed a half-neutralization point (pK_a) at pH 5.4, a correction of the reported value of 5.9.[2c] The pK_{a_2} of 5-trifluoromethyl-6-azauracil, determined spectrophotometrically, was found to be about 10.8 (Figure 2).

Biological Studies.—The *in vitro* studies were carried out on enzyme preparations. The isolation of thymidylate synthetase[28] was according to the method of Wahba and Friedkin[3] from *E. coli* B. 5-Trifluoromethyluracil (**2**) was inactive against thymidylate synthetase at a concentration of 1.7×10^{-2} *M* in the assay solution. 5-Trifluoromethyl-6-azauracil (**6**) showed 50% inhibition of thymidylate synthetase at a concentration of 4×10^{-3} *M*. The inhibitor/substrate (dUMP) ratio in the assay solution at this concentration was 95. Both anomers of 5-trifluoromethyl-6-aza-2′-deoxyuridine (**7a** and **7b**) were inactive against the synthetase preparation at a concentration ratio (inhibitor/dUMP) of 17. This is not unexpected, since 5-fluoro and 5-trifluoromethyl analogs of 2′-deoxyuridine are also inactive against this enzyme unless converted to the 5′-phosphate.

Dihydrofolate reductase,[28] purified according to the method of Mathews and Huennekens,[29] was insensitive to **2**, **6**, **7a**, and **7b** at the ratio of inhibitor/DHFA tested: [**7a**]/[DHFA] = 1.0, [**7b**]/[DHFA] = 1.0, [**2**]/[DHFA] = 200, and [**6**]/[DHFA] = 250.

Nucleoside phosphorylase[30] (horse serum) was assayed according to the procedure of Friedkin and

(28) M. P. Mertes and N. R. Patel, *J. Med. Chem.*, **9**, 868 (1966).
(29) C. K. Mathews and F. M. Huennekens, *J. Biol. Chem.*, **238**, 3436 (1963).
(30) Generously supplied by Professor Friedkin.

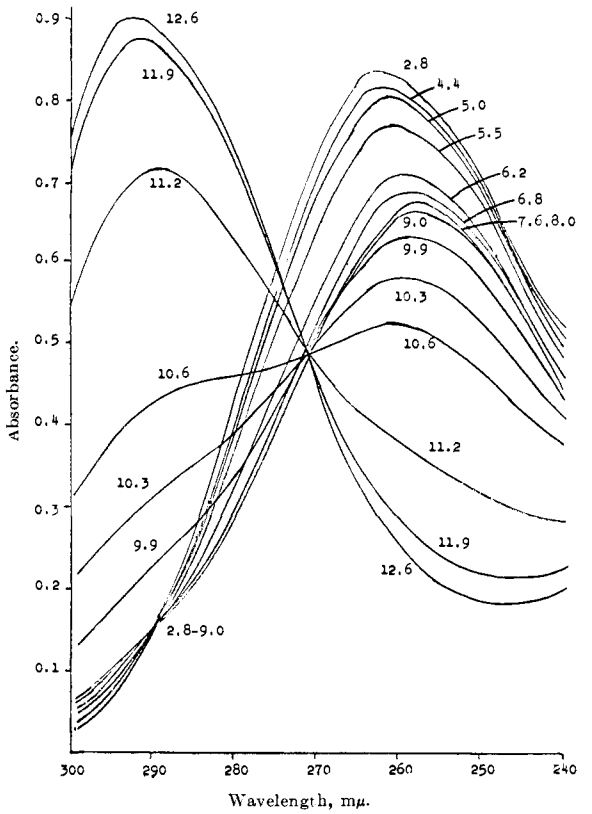


Figure 2.—Ultraviolet spectrum of 5-trifluoromethyl-6-azauracil (**6**) at varying pH.

Roberts[31] and found to be insensitive to **6** at a concentration ratio of [**6**]/[thymidine] of 1.2 in the assay solution.

In vivo tests carried out by the Cancer Chemotherapy National Service Center for compounds **2** (NSC 73757) and **6** (NSC 91365) revealed little activity (Table II). The biological activity of 5-trifluoromethyl-2′-deoxyuridine and its 5′-monophosphate has been extensively investigated by Heidelberger and coworkers.[3e,32] Activity against herpes simplex and many tumor systems was reported in addition to strong inhibition of thymidylate synthetase; $K_i = 2 \times 10^{-7}$ *M* to 6×10^{-8} *M* after preincubation. 5-Difluoromethyluracil (**4a**, NSC 78065) was not examined due to instability in neutral or alkaline media.

TABLE II
In Vivo BIOLOGICAL TESTING BY CCNSC

Compd	NSC no.	Test system	Dose, mg/kg	Tumor wt T/C[a]	Survivors
2	73757	Sarcoma 180	500	520/1472	2/6
			400	615/1472	3/6
			300	1083/1472	5/6
			200	939/1472	5/6
6	91365	Leukemia L1210	250	8.5/8.7	6/6
		KB cells	1 μg/ml		*b*

[a] T/C = test/control. [b] $ED_{50} > 100$ μg/ml.

Experimental Section

Nmr spectra were obtained in $CDCl_3$, CCl_4, or D_2O using a Varian A-60 instrument with tetramethylsilane or sodium 3-(trimethylsilyl)-1-propanesulfonate as an internal standard. A Cary 14, Bausch and Lomb 505, and Beckman DB spectro-

(31) M. Friedkin and D. Roberts, *J. Biol. Chem.*, **207**, 245, 257 (1954).
(32) C. Heidelberger, *Progr. Nucleic Acid Res. Mol. Biol.*, **4**, 1 (1965).

photometers were used to determine ultraviolet absorption. The reported melting points were taken on a Thomas–Hoover capillary melting-point apparatus and are corrected except when otherwise mentioned. Microanalyses were performed by A. Bernhardt, Max Planck Institute, Mulheim, Germany, or Huffman Laboratories, Wheatridge, Colo., unless otherwise noted.

5-Trifluoromethyl-2,4(1H,3H)-pyrimidinedione (5-Trifluoromethyluracil (2)).—Uracil-5-carboxylic acid (1.0 g, 0.006 mole) was put in a 300-ml high-pressure reaction vessel,[33] 0.5 ml of water was added, and the vessel was sealed and immersed in Dry Ice–acetone for 2 hr. Approximately 45 g (0.41 mole) of SF_4 was admitted (copper tubing), and the vessel was sealed, and allowed to come slowly to room temperature. After 16 hr, the vessel was vented, and the gases were passed through a stirred solution of 10% KOH. The residue was crystallized from water and identified by melting point as the starting material.

When this reaction was repeated as described above except that the reaction vessel was heated to 100°, feathery needles were obtained by recrystallization of the residue from water (0.88 g, 77%); mp 247–249° dec, lit.[5] mp 239–241° dec.

Anal. Calcd for $C_5H_3F_3N_2O_2$: C, 33.36; H, 1.68; F, 31.65; N, 15.56. Found: C, 33.49; H, 1.61; F, 31.87; N, 15.69.[34]

The ultraviolet spectra recorded were (undissociated) $\lambda_{max}^{0.1\ N\ HCl}$ 257 mμ (ε 8150), $\lambda_{max}^{pH\ 9.3}$ (monoanion) 281 mμ (ε 9200), $\lambda_{max}^{pH\ 13.4}$ (dianion), 273 mμ (ε 7900); lit.[5] $\lambda_{max}^{0.1\ N\ HCl}$ 257 mμ (ε 7050), $\lambda_{max}^{pH\ 7.0}$ 257 mμ (ε 6830), $\lambda_{max}^{pH\ 9.6}$ 279 (ε 6900)).

5-Difluoromethyl-2,4(1H,3H)-pyrimidinedione (5-Difluoromethyluracil, 4a).—5-Formyluracil[14] (0.71 g, 0.005 mole) and 0.5 ml of H_2O were placed in a high-pressure reaction vessel, and the vessel was sealed. After cooling for 2 hr in a Dry Ice–acetone bath, 35 g (0.32 mole) of SF_4 was admitted. After warming gradually to room temperature the vessel was heated to 50°, agitated for 15 hr, and finally maintained at 100° for 10 hr. After cooling the vessel in air, it was vented and the gases were passed through a 20% KOH solution. The residue in the bomb was removed and protected from moisture. On sublimation at 165–175° (0.1–0.01 mm), 0.49 g (60%) of a white powder was obtained, decomposing at 285–300°.

Anal. Calcd for $C_5H_4F_2N_2O_2$: C, 37.03; H, 2.46; F, 23.46; N, 17.28. Found: C, 37.34; H, 2.50; F, 23.20; N, 17.40.

Chromatography on Whatman No. 1 paper showed one spot with an R_f value of 0.69 (ascending butanol–acetic acid–water, 5:2:3). The following values in the ultraviolet spectrum were recorded: $\lambda_{max}^{pH\ 1}$ 263 mμ (ε 7450), $\lambda_{max}^{pH\ 7}$ 265 mμ (ε 7340). The shift of the ultraviolet absorption spectrum in acid and in neutral media indicated a slow decomposition. The ultraviolet spectrum of the basic solution (pH 8.1) was superimposible on that of the starting aldehyde, and formation of the latter was confirmed by paper chromatography.

6-Trifluoromethyl-*as*-triazine-3,5(2H,4H)-dione (5-Trifluoromethyl-6-azauracil, 6).—5-Carboxy-6-azauracil (**5**)[18] (31 g, 0.2 mole) and 14.4 ml of water (0.8 mole) in a 300-ml bomb were cooled in Dry Ice–acetone for 2 hr, the bomb was evacuated, and 190 g (2 moles) of SF_4 was admitted. The sealed vessel was allowed to warm to room temperature, warmed gradually, and kept at 50° overnight. After cooling, excess SF_4, thionyl fluoride, and HF (formed *in situ*) were passed into a stirred, 5% KOH solution. The material at the bottom of the vessel was placed under vacuum until dry. Washing with $CHCl_3$ gave 20.1 g (56%). Sublimation of a small sample gave a white powder that melted at 153°. The R_f values in butanol–acetic acid–water (5:2:3) on Whatman No. 1 were: ascending, 0.85; descending, 0.86, as compared to 0.34 and 0.38 for the acid. The ultraviolet showed $\lambda_{max}^{0.1\ N\ HCl}$ (undissociated) 263 mμ (ε 6500), $\lambda_{max}^{pH\ 7.8}$ (monoanion) 258 mμ (ε 5200), λ_{max} (in phosphate buffer at pH 12.6) (dianion) 292 mμ (ε 6950). The λ_{max} of a solution of **6** at pH 12.6 did not change over a period of 24 hr, indicating that **6** is more stable to alkali than 5-trifluoromethyluracil (**2**).[5]

Anal. Calcd for $C_4H_2F_3N_3O_2$: C, 26.51; H, 1.11; F, 31.48; N, 23.21. Found: C, 26.62; H, 1.15; F, 31.23; N, 23.04.

2-Acetyl-6-trifluoromethyl-*as*-triazine-3,5(2H,4H)-dione (1-Acetyl-5-trifluoromethyl-6-azauracil, 8).—A solution of **6** (2.5 g, 0.014 mole) in 15 ml of freshly distilled acetic anhydride was refluxed for 2 hr. The reaction mixture was concentrated to one-fifth its original volume and allowed to cool at room temperature for 5 hr. When a solid did not form, the process was repeated with another 15 ml of acetic anhydride. The dark, crystalline mass which precipitated was filtered, washed with CCl_4, and recrystallized from CCl_4 to give 1.5 g (48%) of a hygroscopic, white solid which melted at 118°. The yield was increased to 95%, when the reaction mixture was concentrated almost to dryness, left to cool, and then washed with small amounts of CCl_4.

2-Acetyl-4-diphenylmethyl-6-trifluoromethyl-*as*-triazine-3,5-(2H,4H)-dione (1-Acetyl-3-diphenylmethyl-5-trifluoromethyl-6-azauracil, 9).—Diphenyldiazomethane[22] (0.75 g, 0.004 mole) in 10 ml of dry dioxane was added portionwise to a stirred 60° solution of 0.56 g (0.0025 mole) of **8** in 10 ml of dry dioxane during 6 hr. After another 6 hr, 1 ml of 5 *N* HCl and 6 ml of ethanol were added, and the solution was refluxed for 30 min. The mixture was evaporated to a yellow gum which was dissolved in $CHCl_3$. The $CHCl_3$ solution was extracted with 5% NaOH. The alkaline extract was washed with $CHCl_3$, acidified with 5 *N* HCl, and again extracted with $CHCl_3$. The final $CHCl_3$ extract was dried and evaporated *in vacuo* to yield a white solid which was further purified on a 15-g silica column (Merck AG., silica gel, 0.05–0.2 μ), using a mixture of $CHCl_3$–ethyl acetate (30:1). The second band was collected and dried *in a stream of air* to yield 0.57 g (58%) of a white solid, mp 85°, $\lambda_{max}^{5\%\ NaOH}$ 308 mμ. It was rechromatographed to obtain an analytical sample.

Anal. Calcd for $C_{18}H_{14}F_3N_3O_3$: C, 58.61; H, 3.58; F, 14.65; N, 10.79. Found: C, 58.47; H, 3.73; F, 14.33; N, 10.51.

Mercury Salt of 4-Diphenylmethyl-6-trifluoromethyl-*as*-triazine-3,5(2H,4H)-dione (Mercury Salt of 3-Diphenylmethyl-5-trifluoromethyl-6-azauracil, 10).—A solution of **9** (1.94 g, 0.005 mole) in 5 ml of 1 *N* NaOH and 5 ml of ethanol was added dropwise during 30 min to a vigorously stirred solution of mercuric chloride (0.69 g, 0.0025 mole) in 25% ethanol. After stirring overnight, the solid was filtered and dried thoroughly over P_2O_5 to give a quantitative yield of pure material.

Anal. Calcd for $C_{36}H_{22}F_6N_6O_6Hg$: C, 45.71; H, 2.48. Found: C, 45.96; H, 2.62.

2-(3',5'-Di-O-toluyl-D-2'deoxyribofuranosyl)-4-diphenylmethyl-6-trifluoromethyl-*as*-triazine-3,5(2H,4H)-dione (11).—A solution of the mercuric salt **10** (0.89 g, 0.001 mole) in toluene (170 ml) was kept boiling for 5 min, and 70 ml of toluene was slowly distilled to remove water. 3,5-Di-O-toluyl-D-2'-deoxyribofuranosyl chloride[23] (0.62 g, 0.0016 mole) in toluene (4 ml) was added (gentle stirring). The mixture was left overnight at room temperature, concentrated to 50 ml, and extracted three times with a total volume of 10 ml of water. The toluene solution was dried and evaporated to dryness *in vacuo* to yield a yellow semisolid. This was chromatographed on 50 g of alumina (Woelm grade III) with benzene as eluent, giving one band which was collected and evaporated to dryness *in vacuo* to yield a pale glass.

Anal. Calcd for $C_{38}H_{32}F_3N_3O_7$: C, 65.23; H, 4.57; F, 8.15. Found: C, 65.38; H, 4.72; F, 9.16.

The nmr of **11** in CCl_4 supports the assigned structure: a doublet at δ 7.85 (4 H, *ortho*-protons of the tolyl groups), a singlet with two shoulders at 7.3 (14 H, diphenyl and *meta* protons of the tolyl groups), a sharp singlet at 5.20 (1 H, benzylic proton), and a sharp singlet at 2.40 (6 H, *p*-methyl protons). The protons on the sugar portion of the molecule integrate correctly and are assigned as follows: H_1 and H_3 as a multiplet at δ 6.3, H_4 at 5.25, H_5 at 4.45, and H_2 as a broad multiplet centering at 2.5.

2-(2'-Deoxy-D-ribofuranosyl)-4-diphenylmethyl-6-trifluoromethyl-*as*-triazine-3,5(2H,4H)-dione (12).—A solution of **11**, the entire fraction from the alumina column, in about 20 ml of absolute methanol was added in one portion to about 20 ml of absolute methanol in which a chip of sodium had been dissolved. After 12 hr, the solution was neutralized with Dowex 50 W, H^+ form, 50–100 mesh, to pH 7.2. The resin was filtered, and the solution was evaporated to dryness *in vacuo* at room temperature and purified by chromatography on 15 g of silica (Merck AG., silica gel, 0.05–0.2 μ) using ether (1500 ml) as the eluent. The third band was collected, evaporated, and dried overnight under vacuum to yield a white solid (approximately 120 mg) melting at 47–60° (over-all yield from the mercuric salt, about 24%); $\lambda_{max}^{H_2O}$ 270 mμ (273 mμ in 10% HCl).

Anal. Calcd for $C_{32}H_{26}F_3N_3O_5 \cdot CH_3OH$: C, 55.75; H, 4.88; F, 11.50; N, 8.48. Found: C, 56.05; H, 5.23; F, 11.52; N, 8.78.

The nmr spectrum of **12** in $CDCl_3$ shows much the same pattern as **10**. The assignments made for the sugar are as follows.

(33) AutoClave Engineers Inc., Erie, Pa.

(34) These results were obtained from Dr. Earl M. Chamberlin, Merck and Co., Rahway, N. J.

The hydroxyl protons appear as a broad band at δ 2.7, H_1 is a multiplet at 6.5, H_2 at 2.3, H_3 at 4.5, H_4 at 4.0, and H_5 at 3.7.

It was found that the α and β anomers of **12** could be separated by repeated chromatography on silica gel using $CHCl_3$ and $CHCl_3$–methanol as the solvents. The first fraction was **12a**, $[\alpha]^{24}D$ +45° (*c* 10.6, CH_3OH), and the second fraction was **12b**, $[\alpha]^{24}D$ −10° (*c* 4.26, CH_3OH).

6-Trifluoromethyl-2-(2'-deoxy-D-ribofuranosyl)-*as*-triazine-3,5(2H,4H)-dione (5-Trifluoromethyl-6-aza-2'-deoxyuridine, 7).—The mixture of anomers **12a** and **12b** (0.10 g, 0.22 mmole), 10 ml of methanol, and approximately 50 mg of prereduced 5% palladium on carbon in methanol (20 ml) was hydrogenated at room temperature and pressure. After the mixture had taken up 10 ml of hydrogen (99% of theory), the reduction was stopped. The mixture was filtered and the filtrate was evaporated to dryness *in vacuo* to yield a transparent, semisolid mass (81 mg) which was chromatographed on silica (Merck AG., silica gel, 0.05–0.2 μ) with $CHCl_3$. The first fraction contained diphenylmethane (identified by nmr). Changing to 15% methanol in $CHCl_3$ gave a fraction containing 54 mg (82%) of the anomers of **7** as a clear glass. After passing an aqueous solution through a Dowex 50 W (H^+ form) column, the fractions absorbing in the ultraviolet were evaporated, and the residue was redissolved in methanol, filtered, and evaporated to give **7** as a glass, softening at 78°. The presence of methanol was confirmed by nmr.

Anal. Calcd for $C_9H_{10}F_3N_3O_5 \cdot CH_3OH$: C, 36.48; H, 4.28; N, 12.76. Found (two samples): C, 36.83, 36.12; H, 3.86, 4.18; N, 13.22.

Similar reduction of the anomer **12a** gave anomer **7a**, purified on silica, $[\alpha]^{24}D$ +76° (*c* 2.3, CH_3OH), λ_{max} (pH 1.0) 269 mμ (ε 5000), (pH 12.4) 264 mμ (ε 5000).

The anomer **12b** gave **7b**, $[\alpha]^{24}D$ −36° (*c* 2.4, CH_3OH), λ_{max} (pH 1.0) 269 mμ (ε 4900), (pH 12.4) 264 mμ (ε 4900), after chromatography with methanol. Compound **7b** gives the following signals in the sugar portion of the molecule: H_1, δ 6.6; H_2, 2.5; H_3, 4.5; H_4, 4.0; and H_5, 3.7. The integral is consistent with the structure.

Inhibition Studies. Dihydrofolate Reductase Assay.—The stock solution of NADPH was prepared by dissolving NADPH (Sigma Chemical Corp.) in 0.02 *M* Tris buffer at a pH of 8.5 to give a final concentration of 0.5 μmole/ml. Dihydrofolic acid, prepared by dithionite reduction of folic acid according to Futterman,[35] was dissolved in 0.005 *M* acetate buffer containing 0.01 *M* mercaptoethanol at a pH of 4.5 to give a final concentration of 0.5 μmole/ml. The assay was that of Friedkin and co-workers[36] and contained 0.2 ml of the NADPH stock solution, 0.1 ml of the dihydrofolic acid stock solution, 0.75 ml of 0.02 *M* mercaptoethanol in 0.1 *M* phosphate buffer at pH of 7.5 and enzyme, inhibitor, or inhibitor solvent, and water to a total of 1.5 ml. Dihydrofolic acid was replaced by 0.005 *M* acetate buffer containing 0.01 *M* mercaptoethanol at a pH of 4.5 in the reference cell.

The change in absorbance at 340 mμ (32°) in a Beckman DB spectrophotometer was recorded with 10 × expansion of the transmission scale and was linear for 10 min. After conversion to absorbance units, 52% of the change in absorbance represents the utilization of dihydrofolic acid, and this change was used in determining the specific activity of the enzyme.

Inhibitors were dissolved in water, alcohol, or 0.02 *M* NaOH to give stock solutions containing 1 μmole/0.1 ml. When base or alcohol was used as the solvent the effect of the solvent on the uninhibited reaction was examined, and these rates were compared to the inhibited reaction.

Thymidylate Synthetase.—The assay solution contained 0.05 μmole of deoxyuridine 5'-monophosphate (Sigma Chemical Corp.), 0.22 μmole of tetrahydrofolic acid (General Biochemicals Corp.), 15 μmoles of formaldehyde, 25 μmoles of $MgCl_2$, 130 μmoles of mercaptoethanol, 0.9 μmole of disodium ethylenediaminetetraacetic acid, 45 μmoles of Tris buffered at a pH of 7.4, and enzyme to a total volume of 1.2 ml. Tetrahydrofolic acid was added to 1 *M* mercaptoethanol and adjusted to pH 7.4 and this solution containing approximately 20 μmoles/ml was divided into small fractions and frozen. Deoxyuridine 5'-monophosphate was dissolved in water to give a stock solution containing approximately 1 μmole/ml and was stored, frozen. The assay stock solution (minus mix) contained 12 mmoles of mercaptoethanol, 27 μmoles of tetrahydrofolic acid, 1.8 mmoles of formaldehyde, and 3 mmoles of $MgCl_2$ in a total volume of 30 ml. The assay sample cell contained 0.25 ml of the minus mix and 0.05 ml of the deoxyuridine 5'-monophosphate stock solution and enzyme, inhibitor solution, or inhibitor solvent and buffer A were added to a total of 1.2 ml. Deoxyuridine 5'-monophosphate was replaced by water in the reference cell.

The change in absorbance at 340 mμ was read at 30° in a Beckman DB spectrophotometer and recorded with 10 × expansion of the transmission scale and converted to absorbance. Under these conditions the change in absorbance usually was linear for the first 10 min.

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1,4,5,6-Tetrahydro-*as*-triazines. I. Sulfuric Acid Catalyzed Condensation of Nitriles and Hydrazino Alcohols[1]

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Variously substituted 1,4,5,6-tetrahydro-*as*-triazines were prepared by treating nitriles with hydrazino alcohols in the presence of concentrated sulfuric acid. The scope and mechanism of this reaction was investigated. The spectral properties and pharmacological activity of the compounds are discussed. *trans*-(−)-3-(*o*-Chlorophenyl)-1,6-dimethyl-5-phenyl-1,4,5,6-tetrahydro-*as*-triazine was found to possess analgetic activity as measured by the inhibition of hydrochloric acid induced writhing and by a modified hot plate procedure. Five other compounds were found to inhibit maximal electroshock seizures in mice indicating anticonvulsant activity.

We observed that N-amino-(−)-ephedrine (I) reacted with benzonitrile in concentrated sulfuric acid at ambient temperature to give *trans*-(+)-1,6-dimethyl-3,5-diphenyl-1,4,5,6-tetrahydro-*as*-triazine (II). A literature search revealed the absence of any reports on the acid-catalyzed condensation of hydrazino alcohols and nitriles. In fact, the only example of a

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