+58.8 ± 1.0° (water); $\lambda_{max}^{pH\ 7,\ 14}$ mμ (ε) 216 (25,000), 256 (8850), 280 (9,910).

Anal. Calcd. for $C_{10}H_{14}N_6O_3 \cdot 2H_2O$: C, 39.7; H, 6.00; N, 27.8. Found: C, 39.8; H, 5.25; N, 28.0.

2,6-Diamino-9-(2-deoxy-β-D-ribofuranosyl)purine (β-XI) was prepared by the same procedure and purified from a similar contaminant by recrystallization from methanol and from carbon tetrachloride (10% yield), m.p. 176° dec.; R_f 0.26; $[\alpha]^{21}D$ −29.1 ± 0.5° (water); $\lambda_{max}^{pH\ 7,\ 14}$ mμ (ε), 216 (24,500), 256 (8890), 280 (9910).

Anal. Found: C, 39.4; H, 4.94; N, 27.7.

Chloromercuri-2-amino-6-chloropurine was prepared by the procedure[13] for chloromercuri-6-chloropurine in 96% yield. The transformation was characterized by loss of infrared bands at 6.1 and 7.91 μ due to I and appearance of new bands at 6.2 (strong, broad) and 8.03 μ (medium, sharp).

Anal. Calcd. for $C_5H_3Cl_2HgN_5$: C, 14.8; H, 0.76; Cl, 17.5; N, 17.3. Found: C, 14.9; H, 1.28; Cl, 17.1; N, 16.7.

Nucleoside Preparations Attempted with Chloromercuri-2-amino-6-chloropurine.—Coupling of the chlorosugar VII with chloromercuri-2-amino-6-chloropurine in refluxing benzene by the usual[10] procedure afforded a sirupy nucleoside (23% yield) with strong ester bands in the infrared at 5.80, 7.85, and 9.05 μ, and bands at 6.19 (m) and 6.35 and 6.50 μ (w) due to the purine moiety; λ_{max}^{EtOH} mμ (ε), 227 (52,100), 241 (62,400), 310 (7,000). Elemental analyses after alumina chromatography indicated two sugar moieties were attached to the purine.

Anal. Calcd. for $C_{47}H_{49}ClN_5O_{16}$: C, 64.6; H, 5.07; Cl, 4.06; N, 8.01. Found: C, 63.3; H, 4.80; Cl, 3.92; N, 7.87.

A coupling product obtained from VII and chloromercuri-2-amino-6-chloropurine in dimethyl sulfoxide[12] upon diluting the reaction mixture with water and extracting with benzene was purified by the usual[10] procedure (45% yield). The infrared spectrum differed from that of the product prepared in refluxing benzene only in the prominence of the purine bands at 6.21 (s) and 6.40 μ (m). Elemental analyses suggested half the product contained two sugar groups and half contained one.

Anal. Calcd. for $C_{26}H_{32}ClN_5O_{13}$: Cl, 6.79; N, 13.4. Found: Cl, 5.50; N, 9.90.

Deacylation[13] of the dimethyl sulfoxide product afforded a sirup which crystallized slowly from ethanol (16% yield), and then was recrystallized from methanol (7%), m.p. 167–169° dec.; $\lambda_{max}^{pH\ 1}$ mμ (ε), 219 (23,900), 244 (5,620), 313 (6,690); $\lambda_{max}^{pH\ 14}$ mμ (ε), 246 (6,440), 300 (7,660).

Anal. Calcd. for $C_{20}H_{22}ClN_5O_8 \cdot H_2O$: C, 39.6; H, 4.65; Cl, 11.7; N, 23.1. Found: C, 39.9; H, 4.95; Cl, 11.1; N, 22.8.

Acknowledgment.—The authors are indebted to Dr. Peter Lim for infrared interpretations, to his staff for optical rotations and paper chromatographic data, and to Mr. O. P. Crews for preparation of intermediates.

Pyrimidines. I. The Synthesis of 6-Fluorocytosine and Related Compounds[1,2]

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Syntheses of 6-fluorocytosine and 6-fluoroisocytosine from 2,4,6-trifluoropyrimidine and the preparation of a number of mono- and difluoropyrimidine intermediates are described. 5-Chlorocytosine and 5-chloroisocytosine were obtained from cytosine or isocytosine by use of N-chlorosuccinimide in acetic acid. The relative effects of a 5- and a 6-halogeno atom on the ultraviolet absorption spectra and apparent pK_a values of cytosine and isocytosine are presented.

The importance of 5-fluorouracil and certain other 5-fluorinated pyrimidines and their nucleosides as antitumor agents[3] prompted an investigation of analogous 6-fluorinated pyrimidine derivatives.

A considerable amount of information is available[4,5] concerning the attack of various nucleophilic reagents on 2,4,6-trichloropyrimidine leading to the formation of 6-chloro-2,4-disubstituted pyrimidine derivatives. Therefore, an ideal intermediate for the proposed syntheses of the 6-fluoropyrimidines was provided by the reported preparation of 2,4,6-trifluoropyrimidine[6,7] obtained by treatment of 2,4,6-trichloropyrimidine with silver fluoride.

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 03190-07).

(2) A preliminary report of this work has appeared in the Abstracts of the 144th National Meeting of the American Chemical Society, Los Angeles, California, April, 1963, p. 26L.

(3) (a) For a partial list of published studies on the antitumor activity of 5-fluorinated pyrimidines and their nucleosides, see ref. 8–11 and 13 in the following paper; (b) I. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4755 (1961).

(4) E. Buttner, *Ber.*, **36**, 2227 (1903).

(5) W. Winkelmann, *J. Prakt. Chem.*, [2] **115**, 292 (1929).

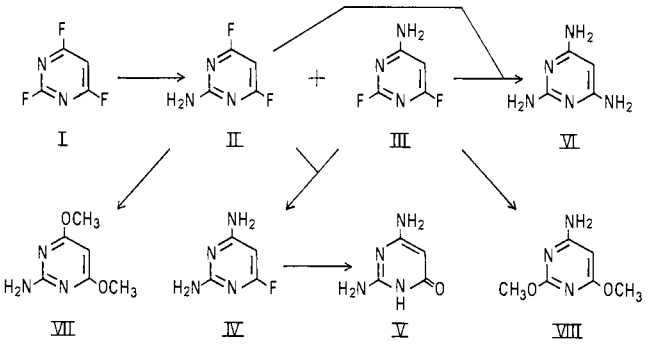
(6) H. Schroeder, *J. Am. Chem. Soc.*, **82**, 4115 (1960).

(7) H. Schroeder, E. Kober, H. Ulrich, R. Rätz, H. Agabigian, and C. Grundmann, *J. Org. Chem.*, **27**, 2580 (1962).

In the preparation of monosubstituted derivatives of 2,4,6-trifluoropyrimidine, the possibility of obtaining mixtures of isomers must be considered. It has been reported[4] that the reaction of 2,4,6-trichloropyrimidine with ammonia gave a mixture of the 2- and 4-aminodichloropyrimidines in the relative proportions of 2:1, respectively. If an analogous mixture of isomers could be obtained by amination of the trifluoropyrimidine, then separation of isomers followed by controlled hydrolysis of *one* of the remaining fluorine atoms should lead to 6-fluorocytosine, 6-fluoroisocytosine, and, conceivably though less likely, by analogy with the relative activities of the 2-chloro *vs.* the 6-chloro analogs, 4-amino-2-fluoro-6-hydroxypyrimidine. Deamination of the former two isomers could be expected to yield 6-fluorouracil.

In our hands, treatment of 2,4,6-trifluoropyrimidine (I) with alcoholic ammonia (see Chart I) gave a crystalline material which at first seemed homogeneous. There appeared to be no marked solubility differentiation; the melting point was not meaningful since sublimation was heavy and complete over a range somewhat dependent on the rate of heating. Paper chromatography showed only a single spot in several systems.

CHART I



A separation was finally accomplished by the use of steam distillation. The steam volatile isomer, which was isolated (66%) in a crystalline state, was proved to be 2-amino-4,6-difluoropyrimidine (II). Unfortunately, the nonvolatile isomer, 4-amino-2,6-difluoropyrimidine (III), was isolated in very poor yields (<10%) due, in part, to its greater instability to the steam distillation. Even repeated recrystallizations failed to give material of the same analytical purity as II. The two isomers could now be differentiated readily by their infrared and ultraviolet absorption spectra, and the degree of purity could be ascertained by examining the ultraviolet spectral ratios.

Proof of structure for these isomers was obtained by converting each to a monoaminodimethoxypyrimidine and comparing the properties of the derivatives thus obtained with those reported in the literature. Thus, it was established that the steam volatile isomer was 2-amino-4,6-difluoropyrimidine (II) since on treatment with sodium methoxide, it was converted to the known 2-amino-4,6-dimethoxypyrimidine (VII)[8,9]; likewise, the nonvolatile isomer was converted to 4-amino-2,6-d.methoxypyrimidine (VIII)[10]; therefore the structure of the material not steam-distillable must be 4-amino-2,6-difluoropyrimidine (III).

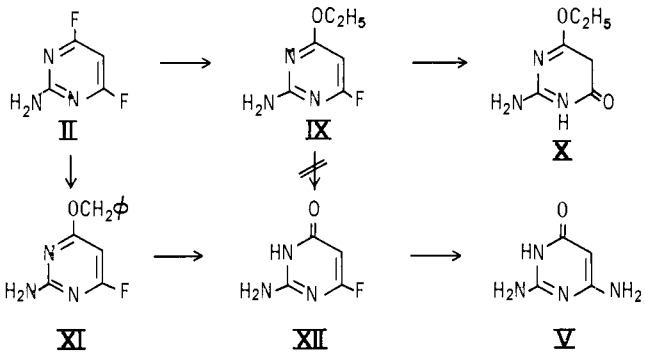
The mixture of isomeric monoaminodifluoropyrimidines, on treatment with alcoholic ammonia in a sealed tube at 105°, gave an analytically pure diaminomonofluoropyrimidine (IV). Alkaline hydrolysis of IV yielded V, which was identified by a comparison of its physical properties (melting point[11] and ultraviolet spectra[12]) with those reported for 2,4-diamino-6-hydroxypyrimidine. Therefore, IV must be 2,4-diamino-6-fluoropyrimidine.

If the ammonia treatment of the mixture of II and III was carried out at much higher temperatures (158°), a product containing no fluorine and having an analysis agreeing with a triaminopyrimidine (VI) was obtained and shown to be identical with 2,4,6-triaminopyrimidine.[13] Conversion of I to VI is further confirmation of the positions of the fluorine atoms in I.

The proposed controlled hydrolysis of one of the fluorine atoms in II was unsuccessful. Even under carefully controlled conditions wherein an excess of base was avoided, there was a loss of at least 20% of the second fluorine as well. This approach was therefore abandoned.

Treatment of II with 1 mole of sodium ethoxide (see Chart II) yielded 2-amino-4-ethoxy-6-fluoropyrimidine

CHART II



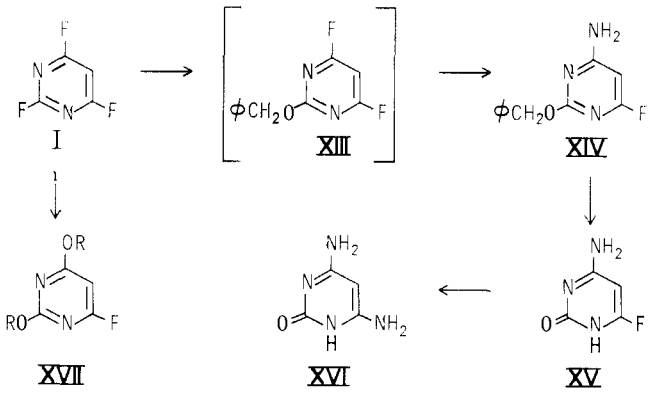
(IX). All attempts to cleave the ether under even the mildest conditions resulted in the isolation of a substance containing no fluorine and analyzing for an aminoethoxyhydroxypyrimidine (X) which, to the best of our knowledge, has not been previously reported. From these data, it is apparent that the 6-fluorine atom is very susceptible to both base- and acid-catalyzed hydrolysis. The susceptibility of an electronegative substituent *ortho* or *para* to a heterocyclic nitrogen to acid-catalyzed nucleophilic displacement has been noted by several investigators.[14,15]

It was desirable, therefore, to convert II to a monoether which could be cleaved mildly under neutral conditions. For this purpose, 2-amino-4-benzyloxy-6-fluoropyrimidine (XI) was synthesized by treatment of II with sodium benzylate. Hydrogenation of XI using palladium–charcoal catalyst proceeded smoothly and afforded the desired 6-fluoroisocytosine (XII). Amination of XII at elevated temperatures (158°) yielded the same diaminopyrimidine (V) obtained previously by alkaline hydrolysis of IV (see Chart I), thus proving the structure of XII to be correct.

Use of the 4-amino-2,6-difluoropyrimidine as starting material for an analogous series of reactions leading to 6-fluorocytosine was impracticable because of the relatively small quantity of this isomer available. Another approach to the synthesis of 6-fluorocytosine (see Chart III) appeared to be utilization of a benzyl ether intermediate XIII prepared by treatment of I with one mole of sodium benzyloxide. The resulting crude oil (XIII) obtained from this reaction was subjected to amination at room temperature and the resulting solid carefully purified. Only the 2-benzyloxy-4-amino-6-fluoropyrimidine (XIV) was isolated.[16] Catalytic hydrogenation of XIV yielded the desired 6-

(8) H. J. Fischer and T. B. Johnson, *J. Am. Chem. Soc.*, **54**, 727 (1932).
(9) F. L. Rose and G. A. P. Tuey, *J. Chem. Soc.*, 81 (1946).
(10) W. Klotzer and H. Bretschneider, *Monatsh. Chem.*, **87**, 136 (1956).
(11) W. Traube, *Ber.*, **33**, 1371 (1900); *ibid.*, **46**, 3839 (1913).
(12) A. A. Plentl and R. Schoenheimer, *J. Biol. Chem.*, **153**, 203 (1944).
(13) S. Gabriel, *Ber.*, **34**, 3362 (1901). A commercial sample was purchased from Mann Research Laboratories, Inc., New York, N. Y.
(14) J. P. Horwitz and A. J. Tomson, *J. Org. Chem.*, **26**, 3392 (1961).
(15) (a) C. K. Banks, *J. Am. Chem. Soc.*, **66**, 1127 (1944); (b) B. Lythgoe, *Quart. Rev.*, **3**, 198 (1949); (c) S. B. Greenbaum and W. L. Holmes, *J. Am. Chem. Soc.*, **76**, 2899 (1954); (d) this behavior has been summarized (see ref. 14 and 15) as due to the formation of an "ammonium" ion on a ring nitrogen in acid solution which induces a positive charge on the *ortho* and *para* positions thus facilitating subsequent attack by a nucleophilic reagent.
(16) It should be noted that treatment of 2,4,6-trichloropyrimidine with 1 mole of sodium alkoxide led to isolation of the 4-alkoxy-2,6-dichloropyrimidines.[5,17] Since the over-all yield of XIV is rather poor, it is conceivable that the 2-benzyloxydifluoro isomer is the minor component, but under the mild conditions used, it is the only isomer which aminates in the subsequent reaction to XIV.
(17) S. Gabriel and J. Colman, *Ber.*, **36**, 3379 (1903).

CHART III



fluorocytosine (XV). The proof that structure XV is 6-fluorocytosine was obtained by further amination of XV at elevated temperatures to yield the known 4,6-diamino-2-hydroxypyrimidine (XVI).[18]

Attempts to deaminate the 6-fluorocytosine or 6-fluoroisocytosine to 6-fluorouracil led to the formation of barbituric acid as the sole isolable product. Another possible precursor of 6-fluorouracil, 2,4-dimethoxy-6-fluoropyrimidine (XVII, R = CH_3), was prepared by treatment of trifluoropyrimidine with 2 moles of sodium methoxide. During the course of this investigation, Horwitz and Tomson[14] reported the synthesis of 2,4-dimethoxy-6-fluoropyrimidine by a different route. As they reported, we also found that attempts to hydrolyze the ether bonds and obtain 6-fluorouracil led to the formation of barbituric acid only.[19, 19a]

The syntheses of 5-*chloro*cytosine and -isocytosine, which surprisingly have not been reported previously, were carried out to obtain these compounds for spectral studies. Direct chlorination of cytosine was reported[20] to yield only 5,5-dichloro-5,6-dihydro-2,4,6-trihydroxypyrimidine. The desired compounds, however, were obtained by treatment of cytosine or isocytosine with N-chlorosuccinimide in glacial acetic acid by a procedure analogous to that of Nishiwaki[21] for the bromination of pyrimidines with N-bromosuccinimide.

Spectral Properties.—The ultraviolet spectral patterns of 6-fluorocytosine (XV) and 6-fluoroisocytosine (XII) (see Experimental) are completely unlike those exhibited by the unsubstituted cytosine[22] or isocytosine,[23] or by the 5-halogenocytosines and -isocytosines. On the other hand, the 6-fluoro derivatives XV and XII do exhibit the same spectral patterns as 6-chlorocytosine and 6-chloroisocytosine, respectively. In addition, the extinction coefficients of all of these 6-halogeno-substituted cytosines are in general considerably higher than those of their parent substances.

Dissociation Constants.—The effect on the basic dissociation of a fluorine atom in position 5 of cytosine and some of its nucleosides has been discussed previously.[3b] With the availability of a number of 5- and 6-halogenocytosines and -isocytosines, an opportunity was afforded to compare the relative effects which both the position and degree of electronegativity of the substituent exert on the apparent pK_a values of cytosine and isocytosine (see Table I). The presence of halogeno atoms in the 5- or 6-positions of cytosine or isocytosine exerts a general, expected, base-weakening effect. It is noted that with the 5-halogenoisocytosines, the differences in their basic pK_a values are not significant. Even with the 5-halogenocytosines, though the base-weakening effect is of the order F > Cl > Br, the magnitude of the increments is quite small. With the 6-substituted halogeno derivatives, the difference in the basic pK_a values between the fluoro and chloro derivatives is great.

This effect of the 6-fluoro *vs.* the 5-fluoro substituent on the basic dissociation is somewhat analogous to the observations of Brown and McDaniel[28] on the relative base strengths of the 2- and 3-monohalopyridines. They noted that 2-fluoropyridine was a much weaker base than the 3-fluoropyridine and explained this difference on the predominance of the inductive effect, as opposed to the resonance effect, of the 2-fluorine atom *vs.* the 3-fluorine atom in their system. However, the fact that 6-chlorocytosine has the highest pK_a value of all the halopyrimidines in Table I cannot be explained

TABLE I

THE EFFECT OF HALOGEN SUBSTITUTION ON THE "APPARENT" pK_a VALUES[a]

	Basic dissociation		Acidic dissociation	
	pK_{a_1}	ΔpK_{a_1}	pK_{a_2}	ΔpK_{a_2}
Cytosine	4.61[b]		12.2	
5-Fluoro-[c]	2.83	−1.78	10.87	−1.33
5-Chloro-	2.94	−1.67	10.36	−1.84
5-Bromo-	3.04	−1.43	10.33	−1.87
6-Fluoro-	1.50	−3.11	9.05	−3.15
6-Chloro-[c]	3.26	−1.35	8.59	−3.61
Isocytosine	4.01[d]		9.42[d]	
5-Fluoro-[e]	2.57	−1.44	8.04	−1.38
5-Chloro-	2.53	−1.48	7.97	−1.45
5-Bromo-	2.56	−1.45	8.05	−1.37
6-Fluoro-	<0[f]	> −4	8.38	−1.04
6-Chloro-[g]	0.50	−3.51	8.06	−1.36

[a] All values except those otherwise indicated were determined spectrophotometrically. [b] Value taken from ref. 3b. [c] Value taken from ref. 22. [d] See ref. 24. [e] See ref. 25. [f] Values taken from ref. 26. [g] In the spectra of XII the curves at pH 0.50 and pH 1 are identical. The second limiting curve for the basic dissociation must lie in theory at least four pH units below pH 1 and could not be determined; therefore, the pK_{a_1} is estimated to lie *ca.* two pH units below pH 1. [h] See ref. 27.

(18) A. Bendich, J. Tinker, and G. B. Brown, *J. Am. Chem. Soc.*, **70**, 3109 (1948). The authors thank Dr. A. Bendich of this Institute for a sample of 4,6-diamino-2-hydroxypyrimidine.

(19) Still another approach to 6-fluorouracil through the intermediate, 2,4-dibenzyloxy-6-fluoropyrimidine (XVII, R = $CH_2C_6H_5$), followed by hydrogenation under neutral conditions is presently under investigation in our laboratory.

(19a) NOTE ADDED IN PROOF.—The synthesis of 6-fluorouracil by this procedure has been achieved recently in this Laboratory (I. Wempen and J. Fox, to be published).

(20) T. B. Johnson, *J. Am. Chem. Soc.*, **65**, 1218 (1943).

(21) T. Nishiwaki, *Chem. Pharm. Bull.* (Tokyo), **9**, 38 (1961).

(22) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).

(23) R. B. Angier and W. V. Curran, *J. Org. Chem.*, **26**, 1891 (1961).

(24) The authors wish to thank Dr. Robert Duschinsky of Hoffmann-La Roche, Inc., Nutley, New Jersey, for the samples of 5-fluorocytosine and 5-fluoroisocytosine used in this study.

(25) H. L. Wheeler and G. S. Jamieson, *Am. Chem. J.*, **32**, 348 (1904); W. Pfleiderer and H. Fink, *Ann. Chem.*, **657**, 149 (1962). The authors wish to thank Dr. W. Pfleiderer of the Institut für Organische Chemie und Org.-Chem. Technologie, Stuttgart, West Germany, for a sample of 6-chlorocytosine.

(26) P. A. Levene, L. W. Bass, and H. S. Simms, *J. Biol. Chem.*, **70**, 229 (1926).

(27) H. S. Forrest, R. Holl, H. J. Rodda, and A. R. Todd, *J. Chem. Soc.*, 3 (1951). The authors wish to thank Mr. L. B. Holum of the Southern Research Institute, Birmingham, Alabama, for the sample of 6-chlorocytosine.

(28) H. C. Brown and H. McDaniel, *J. Am. Chem. Soc.*, **77**, 3752 (1955).

in this way. (Unfortunately, neither 6-bromocytosine nor -isocytosine is available for comparison.)

The presence of a halogen substituent in either the 5- or 6-position exerts the usual acid-strengthening effect on the acidic pK_a values of cytosine and isocytosine. Again, the greatest effect is exhibited by the 6-substituted derivatives. The order of relative magnitude is similar to that observed in the comparison of *o*- and *m*-monohalogenated phenols.[29] The greater acid-strengthening effect of an electronegative substituent in position 6 *vs.* position 5 is also demonstrated by a comparison of the pK_{a_1} values for 5- and 6-trifluoromethyluracils, 7.35[30] and 5.7,[31] respectively.

Microbiological Studies.[32]—6-Fluorocytosine was tested by the agar disk method against *Escherichia coli* B, *Bacillus subtilis* 6051, and *Bacillus subtilis* 16 (a uracil-requiring mutant). No inhibitory zones were observed at a concentration of 129 γ/disk.[32a] Likewise, no growth inhibition was exhibited against *Clostridium feseri* (ATCC10092) at a concentration of 300 γ/disk.[32b] A concentration of 300 γ/ml. of 6-fluorocytosine is required for 50% inhibition of growth of *Streptococcus faecalis* (ATCC 8043); this is 1000 times more than the comparable inhibitory concentration of 5-fluorocytosine (200 mγ/ml.).[32c]

Since 5-fluorocytosine possesses an appreciable growth inhibitory activity against various fungi,[33] 6-fluorocytosine was tested against *Candida albicans* and *Saccharomyces carlbergensis*. With *C. albicans*, 50% inhibition of growth was obtained at a concentration of 100 γ/ml. and with *S. carlbergensis* at 30 γ/ml. In contrast to this limited activity of 6-fluorocytosine, 5-fluorocytosine shows 50% inhibition of growth of both fungi at < 1 γ/ml. concentration.[32d]

It is noteworthy that neither 5-chlorocytosine nor 5-bromocytosine exhibits any inhibitory activity against *S. faecalis* or the fungi discussed above, in strong contrast to that shown by 5-fluorocytosine.

Experimental[34]

2,4,6-Trichloropyrimidine.—This compound was prepared by the method of Baddiley and Topham[35] with the following modifications. A suspension of barbituric acid (156 g.) in phosphorus oxychloride (460 ml.) was heated to 90° and treated dropwise with diethylaniline (266 ml.). After addition was complete, the reaction mixture was refluxed for 0.5 hr., *ca.* 100 ml. of the phosphorus oxychloride distilled, and the dark red solution poured over ice–ether; the product was isolated and distilled in the usual way. The yields of pure product obtained in this way averaged better than 90%.

2,4,6-Trifluoropyrimidine (I).—Compound I was prepared from 2,4,6-trichloropyrimidine essentially as described.[7] The yields of fluorinated product depended to a great degree on the quality of the silver fluoride[36] which was found to vary widely among various lots. The better grades contained a higher proportion of orange-yellow solids; the completely grayish brown lots were inert in the exchange reaction. It was found possible to purify the silver fluoride in small amounts by taking advantage of its solubility in warm methanol. After removal of the insolubles, the methanol solution was evaporated *in vacuo* until heavy precipitation occurred. The orange precipitate was filtered, washed with ether, and stored in a light-proof container in a refrigerated desiccator. All operations were carried out under red light. The purified AgF was found to be extremely reactive, causing the exothermic exchange reaction with the chloro compound to start at nearly room temperature. Since the quantities of active reagent obtained by this laborious purification process were relatively small, aliquots were used to catalyze the commercial reagent. In our hands, yields of pure I averaged 70%.

It was found more convenient to follow the conversion of the trichloropyrimidine to its trifluoro analog by spectrophotometric methods rather than by index of refraction. A large hypsochromic shift of the ultraviolet absorption maximum from 261 mμ for pure trichloropyrimidine in heptane solution (spectrophotometric grade) to 232 mμ for pure I occurs as the exchange reaction progresses.

Treatment of Trifluoropyrimidine with Ammonia.—A solution of 20.2 g. (0.15 mole) of I in *ca.* 100 ml. absolute ethanol was added slowly to 500 ml. of a well stirred solution of alcohol previously saturated with ammonia at 0°. A fine white precipitate deposited. After addition was complete, the reaction mixture was stirred an additional hour and filtered. The precipitate was washed thoroughly with water and finally given a brief wash with cold ethanol. Yield of mixture (A) was 3.4 g.; the spectral ratio max./max. was 4.16 indicating a preponderance of the 2-amino-4,6-difluoropyrimidine isomer.

The original alcoholic filtrate was evaporated to dryness *in vacuo*, the residue thoroughly triturated with water, filtered, and washed briefly with cold ethanol. Yield of mixture (B), 11.2 g., spectral ratio max./max. was 3.15 indicating a mixture of the 2- and 4-aminodifluoropyrimidines.

Separation of the Isomers.—The mixtures A and B were subjected separately to steam distillation, whereby the volatile isomer II was obtained in the distillate while the nonvolatile isomer III remained in the pot. **From A:** The distillate yielded 3.1 g. of crystalline II, spectral ratio max./max. in water, 5.20. After recrystallization from ethyl acetate, the steam volatile isomer II was obtained in pure form, 2.8 g., sublimes completely by 215°, spectral ratio 225/265 mμ 4.94. The liquor from the pot was filtered hot and chilled. The resultant precipitate was recrystallized repeatedly from ethyl acetate until the spectral ratio of max./max. (in water) approached 2.0; yield, 0.1 g.; m.p. 215–216° with some sublimation from *ca.* 180°.

Mixture B was treated in an identical manner resulting in 7.9 g. of the pure 2-amino isomer II and 1.3 g. of the 4-amino isomer III.

Anal. Calcd. for $C_4H_3F_2N_3$: C, 36.65; H, 2.31; N, 32.05; F, 28.99. Found (2-amino isomer): C, 36.42; H, 2.38; N, 31.82; F, 28.85. Found (4-amino isomer): C, 36.76; H, 2.87; N, 31.43; F, 28.23.

Ultraviolet absorption maxima in water solution for compound II are 225 and 265 mμ, ratio max./max. 4.95; for compound III, maxima are 226 and 252 mμ, ratio max./max. 2.0.

2,4-Diamino-6-fluoropyrimidine (IV).—Ten grams (0.076 mole) of the mixed isomers II and III was heated in a sealed tube for 8 hr. at 105° with a solution of ethanol saturated with ammonia at 0°. The cooled tube was opened and the contents were filtered. The filtrate was evaporated *in vacuo* and the residue leached repeatedly with hot acetone. The ammonium fluoride was filtered, yielding 2.75 g. (98%), m.p. 165–170°. The acetone filtrate was concentrated to dryness *in vacuo* and the residue recrystallized from hot water. The precipitated product, 5.2 g. (54%) had m.p. 195–196°. An additional 2.5 g. of lower melting material was obtained from the mother liquors. A small aliquot was recrystallized again for analysis, m.p. 198–199°.

Anal. Calcd. for $C_4H_5FN_4$: C, 37.50; H, 3.93; N, 43.73; F, 14.83. Found: C, 37.48; H, 4.13; N, 43.98; F, 14.79.

2,4,6-Triaminopyrimidine (VI) from II and III.—Five grams (0.038 mole) of a mixture of II and III was heated in a sealed tube with ethanolic ammonia for 12 hr. at 158°. After cooling, the tube was opened, the contents were filtered, and the filtrate

(29) H. C. Brown, D. H. McDaniel, and O. Fafliger in "Determination of Organic Structures by Physical Methods," Vol. I, E. A. Braude and F. C. Nachod, Ed., Academic Press, Inc., New York, N. Y., 1955, p. 589.

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was evaporated to dryness *in vacuo*. The water-soluble residue was dissolved in hot ethanol, decolorized with charcoal, and cooled slowly. The yield of crystalline, water-soluble compound, was 2.75 g. (57%). This material appeared to be still slightly contaminated with salts; therefore, an aliquot was converted to its picrate salt, and recrystallized from a large volume of boiling water. The highly crystalline product, no m.p. <300° was compared with the picrate salt prepared from an authentic sample of 2,4,6-triaminopyrimidine[13] and found to be identical by paper chromatography in several systems and by ultraviolet absorption and infrared spectra. In addition, the picrate of VI gave the correct nitrogen analysis for a monopicrate.

Anal. Calcd. for $C_{10}H_{10}N_8O_7$: N, 31.62. Found: N, 31.82.

Treatment of the Aminodifluoropyrimidine Isomers with Sodium Methoxide. (A) 2-Amino-4,6-dimethoxypyrimidine (VII).—A methanolic solution of II (0.66 g., 0.005 mole) was refluxed for 24 hr. with a solution of 0.23 g. (0.01 mole) of sodium in methanol. Dry xylene (*ca.* 30 ml.) was added and the methanol distilled. The xylene solution was then refluxed an additional 3 hr. After cooling, the solution was filtered and the filtrate evaporated *in vacuo*. The residue was reconcentrated several times with methanol and finally triturated thoroughly with water, filtered, and dried; yield of product, 700 mg. (90%), m.p. 90–94° (if the capillary was cooled and subjected again to melting point determination, m.p. 94.5–96°; lit.[8,9] 95° and 92–93°). A picrate salt of an aliquot of the product was prepared, m.p. 206.5–207.5° dec. (lit.[9] m.p. 208°).

(B) 4-Amino-2,6-dimethoxypyrimidine (VIII).—Treatment of III in the same manner as outlined in A above yielded 480 mg. (61%) of a white solid which shrank at 110°, started to melt at 117°, melting completely at 135°. Cooling the capillary and remelting gave m.p. 128–131° with no prior sintering (lit.[10] m.p. of impure material, 110–140°). After recrystallization from methanol, the product had m.p. 130–139°; after cooling and remelting, m.p. 135–138°.

Anal. Calcd. for $C_6H_9N_3O_2$: C, 46.45; H, 5.85; N, 27.08. Found: C, 46.56; H, 6.06; N, 26.72.

2-Amino-4-ethoxy-6-fluoropyrimidine (IX).—A solution of 0.26 g. (0.011 mole) of sodium in 15 ml. of ethanol diluted with 300 ml. of dry toluene was added to a stirred solution of 1.5 g. (0.011 mole) of II in 150 ml. of anhydrous ethanol, holding the temperature at 55–60°. After the addition was finished, the temperature was kept at 60° for an additional hour. After cooling, the precipitated sodium fluoride was filtered, the filtrate evaporated *in vacuo*, and the gel-like residue taken up in ether, washed with water, and dried over sodium sulfate. After removal of the drying agent, the ethereal solution was evaporated to an oil which was poured into a large volume of petroleum ether and chilled. Microscopic prisms precipitated (1.1 g., 61%), m.p. 117–121°. An aliquot recrystallized for analysis from benzene, gave rosettes of prisms, m.p. 120–123.5° (sl. s. 117°). Further recrystallization did not alter this melting point.

Anal. Calcd. for $C_6H_8FN_3O$: C, 45.86; H, 5.13; N, 26.74; F, 12.09. Found: C, 45.80; H, 4.89; N, 26.61; F, 12.33.

2-Amino-4-ethoxy-6-hydroxypyrimidine (X).—IX (100 mg.) was refluxed for 0.5 hr. with 10 ml. of *N* NaOH. After cooling, the solution was diluted to 20 ml. and neutralized with acetic acid. The gelatinous precipitate was filtered and recrystallized from ethanol yielding 85 mg. (86%) of shiny, micaceous platelets, m.p. 292–294°.

Anal. Calcd. for $C_6H_9N_3O_2$: C, 46.45; H, 5.85; N, 27.08. Found: C, 46.76; H, 6.07; N, 26.82.

2-Amino-4-benzyloxy-6-fluoropyrimidine (XI).—A solution of sodium benzyloxide prepared in 200 ml. of boiling toluene from 0.26 g. (0.011 mole) of sodium and 5 ml. of benzyl alcohol was added rapidly to a refluxing solution of 1.5 g. (0.011 mole) of II in 150 ml. of toluene. After addition was complete, the reaction mixture was allowed to reflux an additional 1.5 hr. After cooling, the suspension was filtered through a Celite pad and the clear filtrate evaporated *in vacuo*. The residue was dissolved in ether, washed with water, and after drying over Na_2SO_4 the ether layer was evaporated *in vacuo* to an oil. On addition of a few milliliters of heptane a solid precipitated (160 mg.) which was identified as starting material. The filtrate was diluted to *ca.* 90 ml. with cold heptane and chilled thoroughly. The resultant precipitate was filtered, 1.6 g. (64%), m.p. 85–90°. After recrystallization from benzene–petroleum ether, the product had m.p. 100.5–102.5°.

Anal. Calcd. for $C_{11}H_{10}FN_3O$: C, 60.26; H, 4.60; N, 19.17; F, 8.67. Found: C, 60.36; H, 4.65; N, 19.46; F, 8.89.

6-Fluoroisocytosine (XII).—Compound XI (0.5 g., 0.002 mole) was hydrogenated at atmospheric pressure in absolute ethanol with 5% palladium–charcoal catalyst. The charcoal was filtered and the filtrate evaporated *in vacuo* yielding a white solid, 190 mg. (66%). An aliquot, recrystallized from hot water, precipitated as long needles, dec. from *ca.* 280° with residue unmelted at 300°; the product gave only one spot when checked by paper chromatography in several systems. Ultraviolet absorption properties: in *N* HCl (mostly cationic species), maxima at 222 and 270 mμ, ϵ_{max} 8000 and 10,050, respectively; minimum at 242 mμ, ϵ_{min} 2060; at pH 1 to 6.50 (neutral species), maxima at 223 and 270 mμ, ϵ_{max} 7980 and 11,020, respectively; minimum at 242 mμ, ϵ_{min} 1900; at pH 13 (anionic species), maxima at 226 and 259 mμ, ϵ_{max} 7280 and 6510, respectively; minimum at 245 mμ, ϵ_{min} 4050.

Anal. Calcd. for $C_4H_4FN_3O$: C, 37.22; H, 3.12; N, 32.55; F, 14.72. Found: C, 36.97; H, 3.36; N, 32.15; F, 14.71.

2,6-Diamino-4-pyrimidone (V). (A) From 6-Fluoroisocytosine.—XII (80 mg.) was treated with alcoholic ammonia in a sealed tube at 120° for 6 hr. After cooling, the tube was opened and the contents were filtered. The filtrate was evaporated *in vacuo* and the residue recrystallized from water, 50 mg. (63%); m.p. 279–281° dec. A second recrystallization gave needles, m.p. 281–282.5° dec. (lit.[11] for the monohydrate, 280–283° dec.). The ultraviolet absorption data also agreed with that reported.[12]

Anal. Calcd. for $C_4H_6N_4O \cdot H_2O$: C, 33.33; H, 5.59; N, 38.87. Found: C, 33.52; H, 5.63; N, 38.67.

(B) From 2,4-Diamino-6-fluoropyrimidine.—IV (100 mg.) was heated with *N* NaOH for 0.5 hr. By this time the ultraviolet absorption maximum had shifted to that reported for compound V.[12] The solution was neutralized, evaporated *in vacuo*, and the residue recrystallized from hot water, 60 mg. (61%); m.p. 281–283.5° dec. A mixture melting point with the product from A showed no depression.

2-Benzyloxy-4-amino-6-fluoropyrimidine (XIV).—To a suspension of sodium sand (2.3 g., 0.1 mole) prepared in 1 l. of boiling toluene (previously distilled from sodium), 10.8 g. (0.1 mole) of benzyl alcohol was added slowly and the resulting yellow reaction mixture stirred and heated until the sodium disappeared (*ca.* 1.5 hr.). The suspension was then cooled and diluted with an additional 500 ml. of dry toluene. The sodium benzyloxide was added slowly with stirring to a solution of 13.4 g. (0.1 mole) trifluoropyrimidine in 250 ml. of dry toluene cooled to 0° in an ice bath. After the addition was complete, the reaction mixture was stirred for 2 hr. at room temperature and allowed to stand overnight. The gel-like sodium fluoride was filtered through a Celite pad and the colorless filtrate evaporated *in vacuo* to a yellow oil. This crude oil, without further purification, was treated with a solution of ethanol, previously saturated with dry ammonia gas at 0°, in a pressure bottle at room temperature overnight. The precipitated NH_4F was removed and the filtrate evaporated *in vacuo*. The residue was taken up in ether and washed with water to remove remaining NH_4F. The dried ether layer was evaporated *in vacuo* to an oil. The crude oil was treated repeatedly with portions of heptane to remove residual benzyl alcohol and toluene. Finally, on scratching, the oil solidified. Total crude yield was 4.84 g. (33%), m.p. 90–95°. Recrystallization from hot-benzene–heptane gave pure product as rosettes of needles, m.p. 94–95°.

Anal. Calcd. for $C_{11}H_{10}FN_3O$: C, 60.26; H, 4.60; N, 19.17; F, 8.67. Found: C, 60.48; H, 4.51; N, 18.92; F, 8.52.

6-Fluorocytosine (XV).—The benzyloxy derivative XIV (2.50 g., 0.011 mole) was subjected to hydrogenation at atmospheric pressure in absolute ethanol solution using 5% palladium-on-charcoal as catalyst. After reduction was completed, the catalyst was filtered and the filtrate evaporated *in vacuo* to a gel which on trituration with ethanol–ether solidified, 0.49 g. (33%); no m.p. <300°, gradually turning orange in color and appearing to decompose. The charcoal was leached with boiling water and, on cooling, the water solution deposited a further crop of product, 0.50 g. (34%). A third small crop was obtained by further treatment of the charcoal; this crop, however, was contaminated with degradation products and was discarded; total yield, 0.99 g. (67%). The product was not purified further since the 6-fluorine atom was partially hydrolyzed on recrystallization from boiling water. The 6-fluorocytosine demonstrated only one spot when examined by paper chromatography in several systems. Ultraviolet absorption properties: in 3 *N* HCl (cat-

ionic species), maximum at 264 mμ, ϵ_{max} 12020; minimum at 232 mμ, ϵ_{min} 1470; at pH 6.50 (neutral species), maxima at 219 and 272 mμ, ϵ_{max} 7720 and 14890, respectively; minima at 206 and 242 mμ, ϵ_{min} 5940 and 510, respectively; at pH 12 (anionic species) maximum at 265 mμ, ϵ_{max} 8370, shoulder at *ca.* 225 mμ, minimum at 243 mμ, ϵ_{min} 2,280.

Anal. Calcd. for $C_4H_4FN_3O$: C, 37.22; H, 3.12; N, 32.55; F, 14.72. Found: C, 37.35; H, 3.15; N, 32.28; F, 14.70.

4,6-Diamino-2-pyrimidone (XVI) from XV.—XV (100 mg.) was heated with alcoholic ammonia in a sealed tube for 6 hr. at 140°. After cooling, the solid was filtered and recrystallized from hot water, 60 mg. (62%), no m.p. <300°. The product demonstrated the same ultraviolet absorption spectra and the same R_f by paper chromatography in several systems when compared with an authentic sample[18] of XVI.

2,4-Dimethoxy-6-fluoropyrimidine.—2,4,6-Trifluoropyrimidine (6.7 g., 0.05 mole), dissolved in a mixture of 50 ml. each of anhydrous methanol and dry benzene, was cooled to 15°, and a solution of sodium (2.3 g., 0.10 mole) in 50 ml. of anhydrous methanol was added, keeping the temperature below 20°. After standing several hours, the suspension was filtered through a Celite Pad and the filtrate evaporated *in vacuo* at *ca.* 30°. The residue was taken up in ether and washed with water; the ether solution was dried over sodium sulfate and evaporated *in vacuo*. The oily residue was purified by dissolving in dioxane, decolorizing the solution with Norit, and reprecipitating by addition of water. The product, 5.8 g., m.p. 51–53°, exhibited ultraviolet absorption maxima in water at 212 and 246 mμ. An aliquot sublimed at 100° (20 mm.) yielded prisms, m.p. 54–55° (lit.[14] m.p. 54–56°, absorption maximum at 245.5 mμ).

Anal. Calcd. for $C_6H_7FN_2O_2$: F, 12.01. Found: F, 12.23.

5-Chlorocytosine.—Cytosine (1 g., 0.009 mole) was dissolved in 20 ml. of warm glacial acetic acid. To the yellow solution was added 1.3 g. (0.01 mole) of N-chlorosuccinimide (NCS) and the temperature held at 105° for 1.5 hr. The NCS dissolved within 5 min.; gradually thereafter, precipitation of product began. After cooling, the suspension was filtered and the precipitate washed with water. The crude product was suspended in water and brought to pH 11 with ammonium hydroxide. The resulting solution was treated with glacial acetic acid and the precipitated product recrystallized from water, 0.9 g. (69%), browns at 285°, m.p. 291–292° dec. Ultraviolet absorption properties: in 0.1 *N* HCl (cationic species), maxima at 217 and 293 mμ, ϵ_{max} 11,340 and 8250, respectively; minimum at 248 mμ, ϵ_{min} 760; at pH 7.6 (neutral species), maxima at 216 and 282 mμ, ϵ_{max} 12,460 and 4940, respectively; minima at 212 and 257 mμ, ϵ_{min} 12,340 and 2850, respectively; at pH 12–14 (anionic species), maximum at 296 mμ, ϵ_{max} 6850; minimum at 256 mμ, ϵ_{min} 920.

Anal. Calcd. for $C_4H_4ClN_3O$: C, 33.01; H, 2.77; N, 28.87; Cl, 24.36. Found: C, 33.15; H, 2.84; N, 28.60; Cl, 24.05.

5-Chloroisocytosine.—Isocytosine was chlorinated by a procedure identical to that used for 5-chlorocytosine. Yield of recrystallized product was 43%, m.p. 306–307° dec. Ultraviolet absorption properties: in 0.1 *N* HCl (cationic species), maxima at 223 and 272 mμ, ϵ_{max} 8370 and 6260, respectively; at pH 5.61 (neutral species), maximum at 299 mμ, ϵ_{max} 4270, shoulder at 270–280 mμ; minimum at 253 mμ, ϵ_{min} 1870; at pH 11 (anionic species), maxima at 232 and 286 mμ, ϵ_{max} 6910 and 5730, respectively; minima at 222 and 256 mμ, ϵ_{min} 6320 and 1480, respectively.

Anal. Calcd. for $C_4H_4ClN_3O$: C, 33.01; H, 2.77; N, 28.87; Cl, 24.36. Found: C, 33.00; H, 2.84; N, 29.09; Cl, 24.62.

Spectrophotometric Studies.—Ultraviolet absorption data were determined with a Cary recording spectrophotometer, Model 15, using buffers and techniques previously described.[22] The apparent pK_a values are accurate to ±0.05 pH unit and were determined spectrophotometrically by methods previously employed.[22,37]

Paper Chromatography.—Chromatographic analyses were performed by the ascending method using Schleicher and Schuell 597 paper in the following systems: (a) butanol–water (86:14); (b) ethanol–water (85:15); (c) 2-propanol–water (70:30). The compounds were visualized on the paper chromatograms under ultraviolet light.

Key to Charts.—The structures of all "hydroxypyrimidines," *e.g.*, V, X, XII, etc., are drawn in the carbonyl (lactam) form. It is understood that such representation need not necessarily reflect the true tautomeric state.

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Synthesis of Possible Cancer Chemotherapeutic Compounds Based on Enzyme Approach. V. Tetrazolium Nitrogen Mustards[1]

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Several tetrazolium salts (I) and formazans (II) that bear a nitrogen mustard group have been synthesized. The introduction of a tetrazolium ring into an aromatic nitrogen mustard drastically reduces its toxicity. The tetrazolium nitrogen mustards can be reduced to the more toxic formazan nitrogen mustards. For certain types of cancer with dehydrogenase activity these tetrazolium salts are of interest as possible chemotherapeutic agents. Furthermore, if *in vivo* reduction did take place beyond the formazan stage, a potent nitrogen mustard, N,N-bis-(2-chloroethyl)-*p*-phenylenediamine, lethal to tumor cells, would be liberated. The synthesis, preliminary toxicity, and antitumor activity in animal screening of these new nitrogen mustards and related tetrazolium salts are discussed.

The synthesis of possible cancer chemotherapeutic compounds based on differences in the distribution of enzymes between normal and cancer cells is one of the promising areas of cancer chemotherapy. Previously, we reported the synthesis of several series of alkylating compounds[2–4] which had been designed to take advantage of the low esterase[5,6] or high phosphoramidase[7] content of tumor cells. The present paper reports our initial attempt to design and synthesize new chemo-

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