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Syntheses of 5-Trifluoromethyluracil and 5-Trifluoromethyl-2′-deoxyuridine[1,2]

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5-Trifluoromethyluracil has been synthesized starting with trifluoroacetone, which was converted into the cyanohydrin and then into the cyanohydrin acetate, which was pyrolyzed to trifluoroacrylonitrile. To the latter was added hydrogen bromide in methanol, and β-bromo-α-trifluoromethylpropionamide was obtained, which was condensed with urea or N-acetylurea to give α-trifluoromethyl-β-ureido propionamide or its acetyl derivative. The ureidoamides were cyclized to 5-trifluoromethyl-5,6-dihydrouracil by refluxing in hydrochloric acid. Bromination and dehydrohalogenation of the dihydropyrimidine gave 5-trifluoromethyluracil, which was converted enzymatically into 5-trifluoromethyl-2′-deoxyuridine. The latter compound is incorporated into DNA, is mutagenic to bacteriophage, inhibits (in the nucleotide form) the enzyme, thymidylate synthetase, and is a potent inhibitor of the growth of Adenocarcinoma 755 in mice. 5-Trifluoromethyluracil under mild alkaline conditions is quantitatively converted into 5-carboxyuracil and was condensed with glycine and glycylglycine to give 5-uracoylglycine and 5-uracoylglycylglycine.

Based upon the observation by Rutman, *et al.*,[4] that uracil was utilized to a greater extent for nucleic acid biosynthesis in tumors than in corresponding normal tissues, a new antimetabolite, 5-fluorouracil, was designed and synthesized.[5a,b] This compound turned out to have considerable tumor-inhibitory activity against mouse and human tumors[6a,b] and is detoxified to a lesser extent by tumors than by most normal tissues in mouse and man.[7a,b,c] The compound is incorporated into RNA,[8] but exerts its tumor-inhibitory activity as a result of metabolic conversion into 5-fluoro-2′-deoxyuridine 5′-monophosphate, which is a potent inhibitor of the enzyme, thymidylate synthetase.[9a,b] The nucleoside 5-fluoro-2′-deoxyuridine is more effective than 5-fluorouracil against mouse and human cancers.[10a,b] In view of these interesting biochemical and therapeutic properties, it appeared desirable to determine whether additional fluorinated pyrimidine antimetabolites might be designed and prepared that would have similarly interesting properties.

It has previously been shown that 5-bromouracil and 5-iodouracil are incorporated into DNA in microorganisms,[11a,b] and that 5-bromo-2′-deoxyuridine and 5-iodo-2′-deoxyuridine are similarly incorporated into DNA in mammalian cells.[11c,d] This incorporation, which often has consequences of conferring upon the cells increased mutability[12a] and radiosensitization,[12b] is doubtless made possible by the fact that the bromo and iodo atoms are about the same size as the methyl group in thymine, and these compounds do replace thymine in the DNA. It seemed likely to us that replacement of the methyl group in thymine by a trifluoromethyl group would lead to a fluorinated pyrimidine 5-trifluoromethyluracil (XI), that also might be incorporated into DNA, although the trifluoromethyl group is somewhat larger (van der Waals radius 2.44 Å.) than the methyl group (2.00 Å.). Accordingly, attempts were made to synthesize this compound.

A number of unsuccessful syntheses by a variety of routes were attempted, but the details will not be described here. During the course of this work the

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(2) A preliminary account of this work appeared: C. Heidelberger, D. Parsons, and D. C. Remy, *J. Am. Chem. Soc.*, **84**, 3597 (1962).

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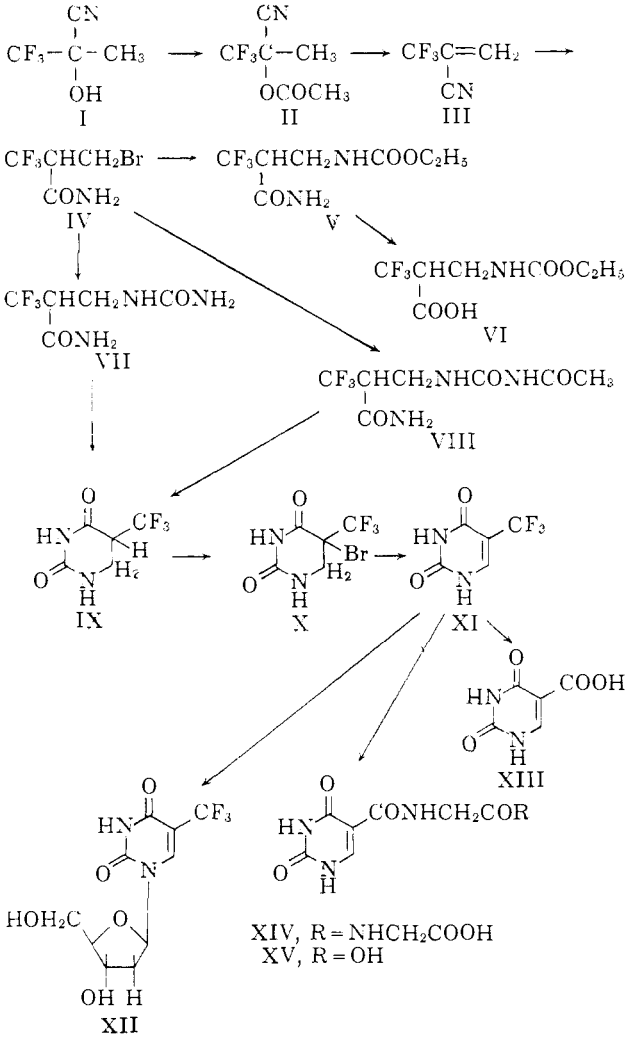
preparations of several other trifluoromethylpyrimidines were described,[13a-e] but none of these compounds had the trifluoromethyl group in the crucial 5-position. After our communication of the original synthesis had been accepted,[1b] Feit[14] described the synthesis of 5-trifluoromethyl-5-hydroxy-5,6-dihydrouracil, but he was unsuccessful in attempts to convert it into 5-trifluoromethyluracil. Very recently, Mertes and Saheb[15] have announced the preparation of 5-trifluoromethyluracil in one step from 5-carboxyuracil by treatment with sulfur tetrafluoride.

The synthetic pathway that was ultimately successful is shown below and is a considerable modification of a very early synthesis of thymine described by Fischer and Roeder.[16] That synthesis involved the condensation of methacrylic acid with urea to give dihydrothymine, which was brominated and dehydrobrominated to give thymine. However, trifluoromethacrylic acid[17] failed to give a reaction with urea from which a product could be isolated. Nevertheless, by making various compounds related to methacrylic acid, the synthesis ultimately was successful.



Trifluoroacetone was used as the starting material and was treated with hydrogen cyanide and converted into cyanohydrin I, which was acetylated to give II.[13] This compound was then pyrolyzed to trifluoromethylacrylonitrile (III).[17] The unsaturated nitrile was then treated with anhydrous hydrogen bromide in the presence of methanol, which saturated the double bond and partially methanolized the nitrile group to give the key intermediate IV. This bromoamide (IV) was allowed to react with urethan, to give V, which led to VI on hydrolysis, but the cyclization of V to a dihydropyrimidine was never accomplished. However, IV was successfully condensed with an excess of urea or with N-acetylurea to give the ureidoamide (VII) or its acetylated derivative VIII, although we are not aware of an analogous alkylation of urea in the literature. Cyclization of either VII or VIII was accomplished under identical conditions by heating with 5 *N* hydrochloric acid, and 5-trifluoromethyl-5,6-dihydrouracil was obtained in reasonable yield; presumably the initial step in this reaction involves the hydrolysis of the amide group to a free carboxyl group, which then reacts with the ureido moiety to give a cyclic product. The dihydropyrimidine was brominated under conditions similar to those described for thymine[18] and dehydrobrominated to give 5-trifluoromethyluracil ("trifluorothymine"). As a result of the substitution of the CF_3 group into the 5-position, the acidity of the proton on N-3 is greatly increased, which has important biological consequences (pK_a thymine = 9.82; pK_a 5-trifluoromethyluracil = 7.35 as determined by the method of Shugar and Fox[19]).

In view of the very low level in mammalian systems of the enzyme that converts thymine into thymidine and the biological ineffectiveness in mammalian systems of 5-bromouracil and 5-iodouracil, we believed that 5-trifluoromethyluracil would have little activity in animal cells, and so it was converted enzymatically to the nucleoside, 5-trifluoromethyl-2'-deoxyuridine ("trifluorothymidine") (XII), by an exchange reaction between thymidine and 5-trifluoromethyluracil.

An interesting facet of the chemistry of 5-trifluoromethyluracil is the extreme lability of the CF_3 group toward alkaline conditions. For example, in 0.1 *N* sodium bicarbonate at room temperature, XI is quantitatively converted in 24 hr. into 5-carboxyuracil (XIII). Because of this extreme alkali lability of the trifluoromethyl group, it appears unlikely that 5-mono- or difluoromethyluracil could exist at physiological PH, and we have not attempted to prepare these compounds.[19a]

Cohen, Thom, and Bendich have recently reported[20] that 6-trichloromethylpurine is readily hydrolyzed by dilute alkali to 6-carboxypurine and also reacts with the amino groups of amino acids and peptides to give N-purinoyl derivatives. Since this reeptivity appeared to be quite analogous to that of 5-trifluoromethyluracil,

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(19a) NOTE ADDED IN PROOF.—Mertes and Saheb (*J. Med. Chem.*, **6**, 619 (1963)) have very recently reported the synthesis of 5-difluoromethyluracil. However, we have found (unpublished) that this compound is completely converted into the corresponding aldehyde in less than 2 min. at pH 6.5, and hence cannot exist at physiological pH, although it is stable below pH 4.0.

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we have prepared the corresponding uracoyl derivatives of glycylglycine (XIV) and glycine (XV). We do not anticipate that these latter two compounds will have any significant biological properties.

5-Trifluoromethyluracil-2-C^{14} and 5-trifluoromethyl-2′-deoxyuridine-2-C^{14} of high specific activity were synthesized from 100 mc. of barium carbonate-C^{14} for biological and biochemical investigations.

The expectation that these compounds might have been interesting biological properties has been borne out. 5-Trifluoromethyl-2′-deoxyuridine is incorporated into the DNA of bacteriophage T4B, resulting in a greatly increased mutability.[21a,b] It is also incorporated into the DNA of mammalian cells grown *in vitro*, thereby producing a significant radiosensitization.[22] The nucleoside is a competitive inhibitor of the cleavage of 5-fluoro-2′-deoxyuridine by the enzyme nucleoside phosphorylase,[21a,23] and in the nucleotide form it inhibits the enzyme, thymidylate synthetase.[21a] 5-Trifluoromethyl-2′-deoxyuridine is also a powerful inhibitor of the growth of Adenocarcinoma 755 in mice.[24]

Experimental

Melting points are corrected. Microanalyses by Spang Microanalytical Laboratories, Ann Arbor, Mich.

2-Cyano-2-hydroxy-1,1,1-trifluoropropane (Trifluoroacetone Cyanohydrin) (I).—A solution containing 115 g. (2.30 moles) of sodium cyanide in 500 ml. of water was cooled to 0°, and 250 g. (2.22 moles) of 1,1,1-trifluoroacetone (Peninsular Chemical Corp., Gainesville, Florida) was added slowly with stirring, and the temperature was kept below 15°. To this solution was added 810 ml. of 6 *N* sulfuric acid dropwise over a period of 3 hr. while the temperature was maintained at 5–10°. A heavy precipitate of sodium sulfate formed, and the mixture was allowed to stand overnight. The darkly colored lower phase was separated, and the aqueous layer was extracted three times with 300 ml. of ether. The combined ether extracts and lower phase were washed with water and dried over anhydrous magnesium sulfate. The ether was distilled at atmospheric pressure and the crude cyanohydrin was distilled to give a yield of 305 g. (99%) b.p. 60–80° (25 mm.).

2-Acetoxy-2-cyano-1,1,1-trifluoropropane (II).—A mixture of 250 g. of I and 250 ml. of acetic anhydride containing 0.5 ml. of concentrated sulfuric acid was refluxed for 1 hr. The excess acetic anhydride was destroyed by the addition of 20 ml. of water, and the mixture was cooled and poured on 1500 ml. of ice and water. The lower layer of ester was separated, and the aqueous phase was extracted twice with ether, the combined extracts were dried over anhydrous magnesium sulfate, and fractionally distilled. The fraction boiling from 145–154° was collected, and 255 g. (78%) was obtained.

2-Cyano-1,1,1-trifluoropropene (Trifluoromethylacrylonitrile) (III).—2-Acetoxy-2-cyano-1,1,1-trifluoropropane (II, 150 g.) was pyrolyzed in a vertical borosilicate glass tube, inside diameter 1.7 cm. and packed with glass tubes 1 cm. × 0.2 mm. for a length of 40 cm. The tube was heated in a 32-cm. furnace and maintained thermostatically at 500°, and the ester was passed through the tube over a period of 4 hr. in an atmosphere of nitrogen. The products were collected in a trap cooled in ice and salt, and 64 g. of III (64%) was collected by fractional distillation at 72–79°.

β-Bromo-α-trifluoromethylpropionamide (IV).—A mixture of 34.8 g. (0.288 mole) of III and 12.1 ml. (0.298 mole) of absolute methanol (dried over calcium hydride) was cooled in an ice–salt bath, and hydrogen bromide was slowly bubbled into the solution until the theoretical amount was absorbed (2–3 hr.) (*cf.* ref. 25). The viscous reaction mixture was then stored in the refrigerator for 36 hr., and then evacuated (water pump) at room temperature until all bubbling ceased. The semisolid reaction mixture was then heated under vacuum to 100° until bubbling stopped. The melt was poured into a large mortar, cooled, and ground with water. The solid was filtered, dried over KOH in a vacuum desiccator, and 47.3 g. (82%) was obtained of a product sufficiently pure for the subsequent condensation reaction. The product was recrystallized from water to give an analytical sample, m.p. 106–107°.

Anal. Calcd. for $C_4H_5BrF_3NO$: C, 21.9; H, 2.29; N, 6.36: F, 25.9. Found: C, 22.0; H, 2.39; N, 6.52; F, 25.8.

β-Ethoxycarbonylamino-α-trifluoromethylpropionamide (V).—A mixture of 7.0 g. of IV and 4.0 g. of urethan was dissolved in 18 ml. of redistilled dimethylformamide and cautiously heated to 120°, when the reaction started and the temperature spontaneously increased to 130°. The mixture was heated at 140° for 2 hr. until the evolution of hydrogen bromide had ceased, during which time the product started to crystallize. The dimethylformamide was distilled *in vacuo*, and the resultant solid residue was crystallized from water to give 6.0 g. (82%), m.p. 179–180°.

Anal. Calcd. for $C_7H_{11}F_3N_2O_3$: C, 36.8; H, 4.85; F, 24.9; N, 12.3. Found: C, 36.8; H, 4.69; F, 23.4; N, 12.7.

β-Ethoxycarbonylamino-α-trifluoromethylpropionic acid (VI).—Amide V (4.0 g.) was heated under reflux in 20 ml. of *N* hydrochloric acid for 4 hr. On cooling, 1.6 g. of a crystalline product was obtained and was recrystallized from water to give glistening plates, m.p. 80°.

Anal. Calcd. for $C_7H_{10}F_3NO_4$: C, 36.7; H, 4.40. Found: C, 36.3; H, 4.73.

α-Trifluoromethyl-β-ureidopropionamide (VII).—A solution of 4.49 g. (0.204 mole) of IV in 15 ml. of hot water was added dropwise to a solution of 2.4 g. (0.40 mole) of urea in 10 ml. of water for 40 min. at 60°. The temperature was raised to 100° for 30 min., after which time a bromide determination on the solution showed that the release of hydrogen bromide was complete. The solution was then evaporated *in vacuo* at 35° to give a sirup that was redissolved in ethanol, which was evaporated under vacuum at 30° to leave a solid residue. The crude product was recrystallized from water (charcoal) to give 1.15 g. (28%) of white needles, m.p. 170–172°. A sample was recrystallized for analysis from ethanol, m.p. 172–173°.

Anal. Calcd. for $C_5H_8F_3N_3O_2 \cdot C_2H_5OH$: C, 34.3; H, 5.72; N, 17.14; F, 23.2. Found: C, 33.9; H, 5.18; N, 17.3; F, 23.6.

When equivalent amounts of the bromo-amide and urea were condensed, the product obtained in largest yield melted at 246°. This compound was most probably the disubstituted urea, but was not further characterized.

β-(N-Acetylureido)-α-trifluoromethylpropionamide (VIII).—A mixture of 10 g. (0.454 mole) of IV and 7.5 g. (0.735 mole) of N-acetylurea in 35 ml. of dimethylformamide was heated to 120° for 3 hr. The orange-red solution was evaporated under vacuum at 50° to give a solid residue, which was crystallized from 250 ml. of hot water with charcoal, to form needles, m.p. 206–208°, yield 6.3 g. (57%). The analytical sample was recrystallized from ethanol, m.p. 209–210°.

Anal. Calcd. for $C_7H_{10}F_3N_3O_3$: C, 34.9; H, 4.17; F, 23.6; N, 17.4. Found: C, 35.0; H, 4.20; F, 23.5; N, 17.7.

5,6-Dihydro-5-trifluoromethyluracil (IX). **A.**—The ureidoamide VII (0.90 g.) was heated under reflux in 5 ml. of 5 *N* hydrochloric acid for 1 hr. The reaction was cooled and the hydrochloric acid removed by evaporation *in vacuo* at 35°. The residue was shaken with 10 ml. of ethanol, and again evaporated to dryness *in vacuo*. The solid obtained was crystallized from water to give 0.48 g. (58%) of small white prisms, m.p. 203–205° dec.

Anal. Calcd. for $C_5H_5F_3N_2O_2$: C, 33.0; H, 2.77; F, 31.3; N, 15.4. Found: C, 33.1; H, 2.81; F, 31.3; N, 15.2.

B.—The N-acetyl-ureidoamide VIII (6.3 g.) was refluxed in 25 ml. of 5 *N* hydrochloric acid for 90 min. The reaction was filtered, the filtrate evaporated to dryness under vacuum at 40°, 25 ml. of water was added, and the mixture evaporated. Finally ethanol was added and the mixture was re-evaporated to remove all traces of HCl. The residue was recrystallized from water

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(22) W. Szybalski, N. Cohn, and C. Heidelberger, *Federation Proc.*, **22**, 532 (1963).

(23) C. Heidelberger, G. D. Birnie, J. Boohar, and D. Wentland, *Biochim. Biophys. Acta*, **76**, 315 (1963).

(24) C. Heidelberger and S. W. Anderson, unpublished.

(25) S. M. McElvain and C. L. Stevens, *J. Am. Chem. Soc.*, **69**, 2663 (1947).

(charcoal) to give a product identical with that obtained by method **A**, yield, 3.3 g. (69%).

5-Bromo-5-trifluoromethyl-6-hydrouracil (X).—A solution of 0.80 g. (4.4 mmoles) of IX in 8 ml. of glacial acetic acid was heated under gentle reflux, and 0.80 g. (5.0 mmoles) of bromine in 10 ml. of acetic acid was added dropwise. After addition of the bromine the solution was refluxed for 3 hr. to give a pale yellow solution, which was evaporated to dryness, and the residue was dissolved in ethanol and re-evaporated to dryness *in vacuo*. The crude product weighed 0.98 g. (85%), and was recrystallized from aqueous ethanol to give thin plates, m.p. 224–228° dec.

Anal. Calcd. for $C_5H_4BrF_3N_2O_3$: C, 23.0; H, 1.55. Found: C, 23.1; H, 1.61.

5-Trifluoromethyluracil (XI).—A solution of 0.81 g. of X was dissolved in 8 ml. of redistilled dimethylformamide and heated at 140° for 75 min., and the solvent evaporated *in vacuo* at 55°, the last traces being removed by coevaporation with ethanol.[26] The residue was crystallized from water (charcoal) to give 0.447 g. (80%) of fine white needles, m.p. 239–241° dec. The analytical sample after two recrystallizations from water had m.p. 245–248° dec.

Anal. Calcd. for $C_5H_3F_3N_2O_2$: C, 33.4; H, 1.68; N, 15.6; F, 31.7. Found: C, 33.8; H, 1.92; N, 15.4; F, 32.0.

Ultraviolet absorption spectra, in 0.1 *N* hydrochloric acid, λ_{max} 257 mμ (ε 7930); in pH 7.0 buffer, λ_{max} 257 mμ (ε 7060); in pH 8.1 buffer, λ_{max} 278 mμ (ε 7150).

Paper chromatographic behavior: butanol–water, 86:14, ascending, R_f 0.79; butanol–glacial acetic acid–water, 5:2:3, descending, R_f 0.80; ethyl acetate–methanol–water–heptane, 10:6:5:3, upper phase, descending, R_f 0.76.[27]

5-Trifluoromethyl-2′-deoxyuridine (XII).—The bacterial enzyme was prepared as follows. Three liters of medium containing 5.0 g./l. of sodium chloride and 10 g./l. of Bactotryptone (Difco) were inoculated with a culture of *Escherichia coli* B and incubated at 37° for 10 hr. The cells were harvested and washed once with 0.067 *M* potassium hydrogen phosphate buffer (pH 6.45). The cells were resuspended in 50 ml. of the phosphate buffer and were passed twice through a French hydraulic press. The resulting mixture was centrifuged at 9000*g* for 15 min., and the supernatant fraction was decanted carefully from the cellular debris. This crude enzyme preparation retained its activity for more than 3 months when stored in a frozen state.

A solution containing a mixture of 5.42 g. of thymidine (0.0224 mole), 1.05 g. (0.0058 mole) of 5-trifluoromethyluracil, and 60 ml. of the crude enzyme preparation was made up to 600 ml. in 0.067 *M* phosphate buffer (pH 6.7). The solution was incubated at 37° with shaking for 3.5 hr. and then heated in a boiling water bath to coagulate the proteins. After centrifugation, the supernatant solution was evaporated to dryness *in vacuo* (1-octanol was added to minimize foaming). The residue was extracted with eight 75-ml. portions of hot absolute ethanol, and the combined extracts evaporated to dryness *in vacuo* at 30°. The residue was then shaken up with 150 ml. of warm absolute ethanol and allowed to stand at 3° for 3 hr. The insoluble material was filtered and was shown by paper chromatography to consist mainly of thymine with a little thymidine. The alcoholic solution was evaporated to dryness *in vacuo* and redissolved in 50 ml. of water. This was kept refrigerated as a stock solution at pH 7.0.

A 10-ml. portion of this stock solution was chromatographed on a 4 × 30-cm. column of Dowex-1-formate, which had been equilibrated with 0.1 *M* ammonium formate adjusted to pH 7.8. Elution was started with this buffer, which eluted the thymidine, closely followed by thymine. The pH was then changed to 5.5, keeping the formate concentration the same. Under these conditions, 5-trifluoromethyl-2′-deoxyuridine was eluted in a total of 1100 ml. Finally, unchanged 5-trifluoromethyluracil and a little 5-carboxyuracil were eluted with 0.1 *M* formic acid. Fractions 195–288 (Fig. 1) were combined and evaporated to dryness at 40° *in vacuo* and coevaporated twice with absolute ethanol. The dry residue was then extracted with five 30-ml. portions of ethyl acetate, and the combined extracts were evaporated at 40° *in vacuo* to give a colorless oil that solidified on standing. This was redissolved in 20 ml. of ethyl acetate and evaporated to 2 ml., which on standing gave 49.0 mg. (14%) of 5-trifluoromethyl-2′-deoxyuridine (XII), m.p., 172–176°. In some subsequent preparations, the extractions with ethyl acetate were eliminated, and the eluate from the Dowex-1-formate columns were desalted by passage through Dowex-50 H^+ columns. Yields of the nucleoside as high as 24% have been obtained.

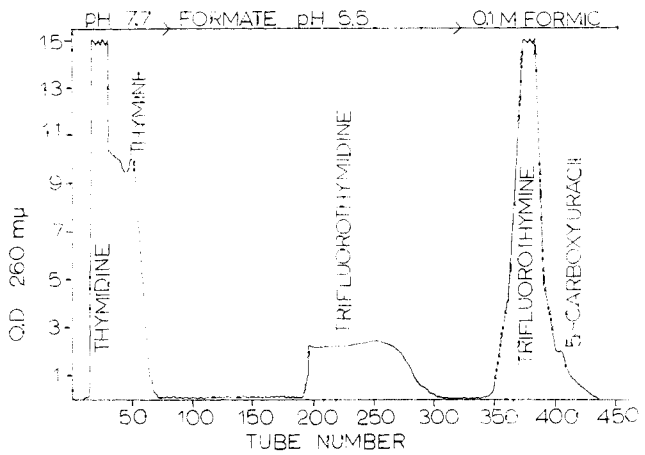

Fig. 1.—Chromatography on Dowex-1-formate column of the reaction mixture from the enzymatic exchange reaction between thymidine and 5-trifluoromethyluracil.

Ultraviolet spectra: in 0.1 *N* hydrochloric acid λ_{max} 260 mμ (ε 9960), 280/260 0.27; in 0.1 *N* NaOH λ_{max} 260 mμ (ε 6590); 280/260 0.25; the R_f descending, in ethyl acetate–methanol–water–heptane, 10:6:5:3, upper phase, 0.60. A crystalline, analytical sample was obtained, m.p. 186–189°.

The best analytical method for the separation and analysis of the pyrimidine base and the deoxyribonucleoside is by paper electrophoresis in 0.05 *M* triethylammonium bicarbonate buffer brought to pH 7.0 with concentrated formic acid. Under these conditions, for 3.5 hr. at 2200 v. and 6 ma. there is a complete separation: 5-trifluoromethyluracil migrates faster than 5-trifluoromethyl-2′-deoxyuridine.

Anal. Calcd. for $C_{10}H_{11}F_3N_2O_5$: C, 40.5; H, 3.74; N, 9.46; F, 19.2. Found: C, 40.1; H, 4.0; F, 18.9; N, 9.51.

Conversion of 5-Trifluoromethyluracil to 5-Carboxyuracil (XIII).—A solution of 10 mg. of XI in 1.0 ml. of *N* sodium hydroxide was allowed to remain at room temperature for 3 hr., and then the reaction products were put through a Dowex-50 (H^+) column (1 × 5 cm.) and washed with water. All the material absorbing at 260 mμ was eluted in the first 25 ml., and this was concentrated to 2 ml. Chromatography in butanol–formic acid–water, 77:10:13 system showed only one ultraviolet absorbing compound, R_f 0.37, identical with an authentic sample of 5-carboxyuracil run on the same chromatogram. The spectra in acid and alkaline solution and melting points of the hydrolysis product were all identical with those of 5-carboxyuracil. In another experiment it was shown that a solution of 5-trifluoromethyluracil in 0.1 *M* sodium bicarbonate was quantitatively converted to 5-carboxyuracil in 24 hr. at room temperature.

5-Uracoylglycylglycine (XIV).—A solution of 1.3 g. of glycylglycine in 50 ml. of water containing 3.0 g. of sodium bicarbonate was prepared, and to this was added a solution of 1.8 g. of 5-trifluoromethyluracil in 70 ml. of water, and the mixture was stirred at room temperature for 66 hr. during which time the pH changed from 8.0 to 7.4. The mixture was acidified and cooled, and the precipitated material was centrifuged and crystallized from 300 ml. of water, and 1.3 g. of product (48%) was obtained. After crystallization again from water an analytical sample was obtained, m.p. 267–273° dec.

Anal. Calcd. for $C_9H_{10}N_4O_5$: C, 40.0; H, 3.73; N, 20.8. Found: C, 39.8; H, 3.86; N, 20.8.

Ultraviolet absorption spectra: in 0.1 *N* hydrochloric acid, λ_{max} 271 mμ (ε 9220); λ_{min} 242 mμ (ε 2930); λ_{max} 220 mμ (ε 9780); in 0.1 *N* sodium hydroxide, λ_{max} 292 mμ (ε 12,810); λ_{min} 260 mμ (ε 2440); λ_{max} 241 mμ (ε 7880). Paper chromatography: R_f 0.05 in butanol–water, 86:14. R_f 0.35 in butanol–acetic acid–water, 5:3:2.

5-Uracoylglycine (XV).—5-Trifluoromethyluracil (0.90 g.) was dissolved in 50 ml. of warm water, and the solution was cooled to 25°. To this was added a solution of 0.75 g. of glycine and 3.0 g. of sodium bicarbonate in 30 ml. of water. The solution was

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stirred at room temperature for 24 hr., the volume reduced to 20 ml. *in vacuo*, and a yellowish semicrystalline compound was collected after standing for 3 hr. at 3°. This compound was crystallized from water (charcoal), and a yield of 0.35 g. (20%) was obtained, m.p. 295° dec.

Anal. Calcd. for $C_7H_7N_3O_5$: C, 39.4; H, 3.32; N, 19.7. Found: C, 39.2; H, 3.49; N, 19.5.

Ultraviolet absorption spectrum; in 0.1 *N* hydrochloric acid, λ_{max} 271 mμ (ε 10,700); λ_{min} 241 mμ (ε 3160); λ_{max} 220 mμ (ε 11,400); in 0.1 *N* sodium hydroxide, λ_{max} 291 mμ (ε 14,600); λ_{min} 260 mμ (ε 3290); λ_{max} 240 mμ (ε 10,200). Paper chromatography: R_f 0.14 in butanol–water, 86:14; R_f 0.36 in butanol–acetic acid–water, 5:3:2.

Preparation of 5-trifluoromethyluracil-2-C^{14} and 5-Trifluoromethyl-2′-deoxyuridine-2-C^{14}.—Barium carbonate-C^{14} (100 mc.) was diluted with nonradioactive barium carbonate to give a total of 4.5 g. This was converted in two batches into barium cyanamide by heating for hr. at 850° in a slow stream of ammonia gas, and 4.0 g. was obtained.[28] Water (20 ml.) was added to the barium cyanamide, the bigger lumps were crushed, and the suspension was cooled to 5°. Sulfuric acid was added dropwise with stirring until the pH reached 7.0 and the barium sulfate was removed by centrifugation. The supernatant solutions and the washings from the barium sulfate were combined and the aqueous solution was concentrated to a small volume *in vacuo* at 30°. The cyanamide was converted into urea[23] by treatment of 10 ml. of solution with 1.5 ml. of concentrated hydrochloric acid and refluxing for 10 min. The solution was cooled, neutralized with sodium bicarbonate, and evaporated to dryness *in vacuo*. The residue was extracted with five 20-ml. portions of boiling absolute ethanol, and the combined extracts were evaporated to 20 ml. and cooled. The small amount of sodium chloride present was filtered, and the filtrate was evaporated to dryness to give 1.105 g. (81%) of urea. The urea was acetylated with a mixture of 1.85 ml. of acetic anhydride, 0.75 ml. of acetic acid, and 0.02 ml. of concentrated sulfuric acid, which was heated to 130° for 10 min. and allowed to cool. The N-acetylurea crystallized, was dissolved in 30 ml. of water, passed through a 1.5 × 20 cm. column of Dowex-1-formate, and eluted with 80 ml. of water. The solution was evaporated to dryness, and the N-acetylurea was crystallized from water to give 1.615 g. (86%).

The N-acetylurea (0.016 mole) and 2.38 g. (0.011 mole) of IV were dissolved in 15 ml. of redistilled dimethylformamide and heated for 3 hr. at 125°. The solvent was evaporated *in vacuo*, and the residue was crystallized from water (charcoal) to give a yield of 1.02 g. (40%) of labeled VIII. This was then heated under reflux in 15 ml. of 5 *N* hydrochloric acid for 1 hr. and evaporated to dryness *in vacuo*. The residue was crystallized from water (charcoal) to give 290 mg. (38%) of dihydrotrifluoromethyluracil-2-C^{14}. No additional crystalline material could be obtained from the mother liquors. Thus, 290 mg. was dissolved in 10 ml. of glacial acetic acid and brominated with 600 mg. of bromine in 6 ml. of acetic acid at reflux temperature for 90 min. The bromine and acetic acid were evaporated *in vacuo*, and the residue was coevaporated 3 times with absolute ethanol and heated in 10 ml. of dimethylformamide at 130° for 1 hr. After evaporation of the solvent *in vacuo* and coevaporation with water, the residue was passed through a 1.5 × 20 cm. column of Dowex-1-formate, and after elution of impurities with water, the 5-trifluoromethyl-2-C^{14} uracil was eluted with 0.05 *M* formic acid. The solution was evaporated to dryness, coevaporated with water to give 242 mg. (85%) of product that gave only a single radioactive spot in three paper chromatographic systems. The over-all yield, based on barium carbonate, was 5.9%. The specific activity was 4.15 mc./mmole (23 μc./mg.)

The labeled 5-trifluoromethyluracil (234 mg.) was converted to the deoxyribonucleoside with an enzyme obtained from *Lactobacillus Leichmanii*, kindly provided by Dr. Jack Siegel of the Pabst Laboratories, Milwaukee, Wis., and was purified as described above to give 158 mg. (41%) of 5-trifluoromethyl-2′-deoxyuridine-2-C^{14}, which gave only a single radioactive spot in three paper chromatographic systems, and had a specific activity of 4.15 mc./mmole (14 μc./mg.).

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Pyrimidine Derivatives. V. Synthesis of Substituted Pyrimidines from 4-Amino-6-chloro-2-methylthiopyrimidine[1-3]

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The use of 4-amino-6-chloro-2-methylthiopyrimidine as a versatile intermediate for the synthesis of a number of substituted pyrimidines is described. In the course of this work several previously unreported pyrimidines have been prepared. Feigl's iodine–azide reagent has been employed for the rapid detection of mercaptopyrimidines and a sponge nickel catalyst has been found to be quite satisfactory for the facile dethiation of these derivatives. Quantitative ultraviolet absorption spectra are given for all compounds, as well as a summary of growth-inhibitory properties in several *in vitro* and *in vivo* bioassay systems.

For some time a program of synthesis of pyrimidine derivatives for biological evaluation has been in progress in these laboratories[3] and, in connection with this program, the preparation of various pyrimidines as potential cancer chemotherapeutic agents and as precursors of condensed pyrimidine systems has been undertaken. This communication describes the synthesis of a number of substituted pyrimidines starting from the versatile intermediate 4-amino-6-chloro-2-methylthiopyrimidine (III), which was prepared following the method of Baker, *et al.*[4]

4-Amino-6-hydroxy-2-methylthiopyrimidine (II) was obtained by reaction of thiourea and ethyl cyanoacetate, with methylation *in situ* of the anion of 4-amino-6-hydroxy-2-mercaptopyrimidine (I) by means of freshly distilled dimethyl sulfate. The use of aged dimethyl sulfate led to the isolation of I[5] as a by-prod-

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